Benzodiazepine-Based Drug Discovery

Farzad Zamani and Esmail Doustkhah
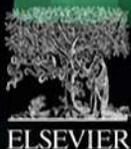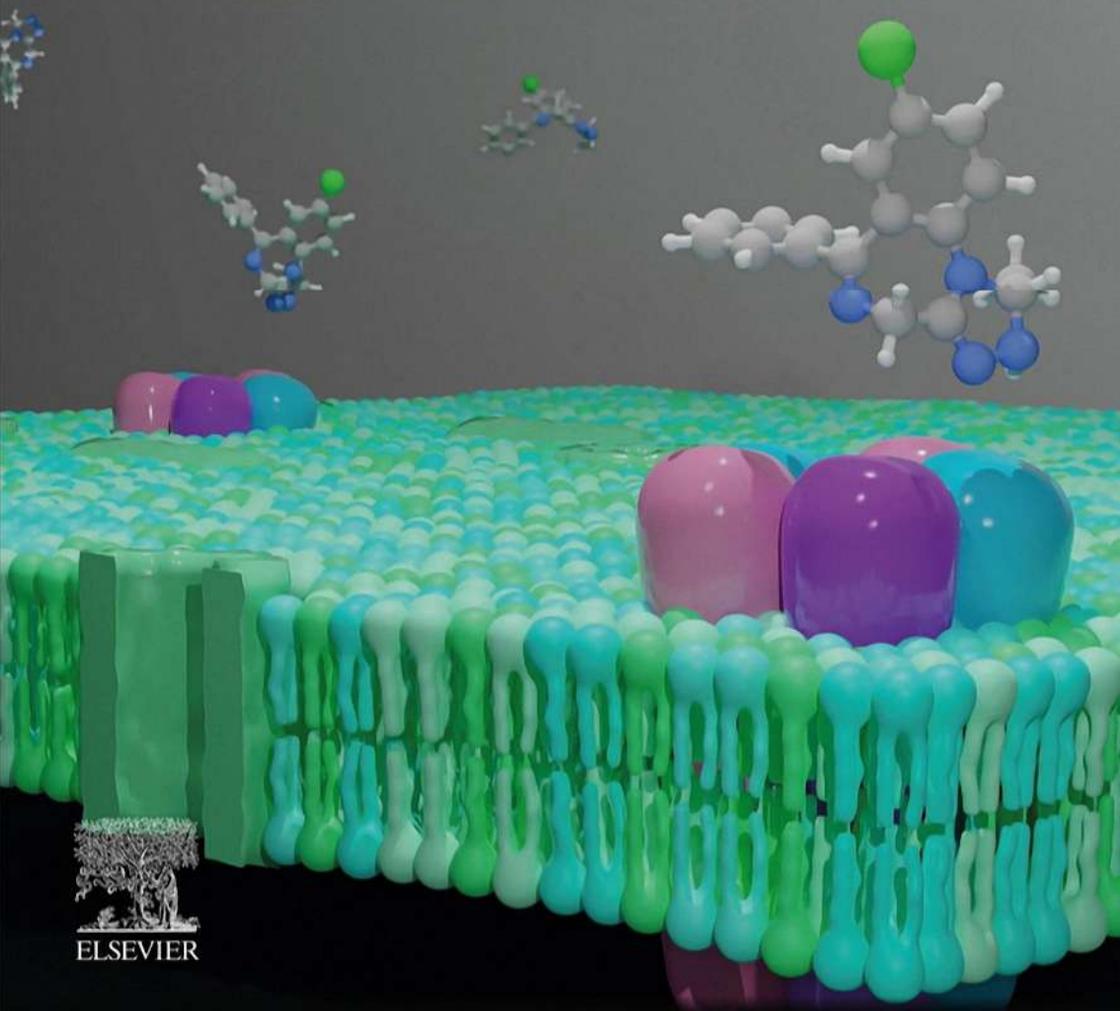
BENZODIAZEPINE-BASED DRUG DISCOVERY

Heterocyclic Drug Discovery Series

BENZODIAZEPINE-BASED DRUG DISCOVERY

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Contents

Preface

Benzodiazepines and benzothiazepines are two pivotal classes of heterocyclic compounds widely used as core structures of various drugs to treat depression, epilepsy, seizures, and muscle spasms. Accordingly, these versatile skeletons are in a continual stream to receive up-to-date advances in medicinal research by synthesizing and screening their analogs for novel applications in drug discovery. Despite the high importance of benzodiazepines and benzothiazepines in psychoactive drugs in the market, there is no updated reference resource with comprehensive coverage and details in the case of benzodiazepine/benzothiazepine-based drugs. Several medical books have been recently written, focusing solely on the adverse effects of benzodiazepines as part of a discussion of the problems with psychotropic drugs. Furthermore, some others have exclusively discussed the pharmaceutical aspects of benzodiazepines. This book is the first to demonstrate detailed chemical–pharmaceutical features of both benzodiazepines and benzothiazepines, including their synthetic procedures, structural features, pharmacokinetics and pharmacodynamics, clinical uses, and adverse effects. We believe that this book would be an all-inclusive resource for scientists interested in the design and development of innovative drugs based on benzodiazepines and benzothiazepines, researchers and undergraduate/graduate students engaged in organic and medicinal chemistry, and R&D experts in the pharmaceutical industry.

First, we wish to express our sincere thanks to Professor Ruben Vardanyan (University of Arizona) for his valuable support and encouragement. Second, we thank all our contributors who helped us in writing this book. Special appreciation also goes to the publishing house, Elsevier, for their exemplary collaboration and support. Last but not least, we would like to thank our families. This book would never have been achieved without their warm encouragement and steady support.

Farzad Zamani
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Acknowledgment

An introduction to benzodiazepines and benzothiazepines

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Chapter outline

1.1 Benzodiazepines

Benzodiazepines as pharmaceutical compounds have emerged as a powerful tool for curing anxiety disorders. According to a national 12-month survey conducted by the US National Comorbidity Survey Replication (NCS-R) and the National Institute of Mental Health (NIMH), approximately 40% of people in the US suffer from generalized anxiety disorder (GAD), panic disorder, post-traumatic stress disorder (PTSD), obsessive-compulsive disorder (OCD), or any other types of mental ailments (Kessler, Chiu, Demler, & Walters, 2005). Traditional clinical treatments for anxiety comprised of consuming general sedatives such as opiates, alcohol, chloral hydrate, and lithium bromide. These anxiolytics however displayed limited efficacy along with several drawbacks such as dizziness, impaired cognitive functions, sexual dysfunction, death, and other adverse effects. In this regard, the development of efficient treatments for anxiety disorders has always been a hot topic in medicinal research.

Benzodiazepines (BDZs) are a family of bicyclic heterocycles consisting of a benzene ring fused to a diazepine unit. According to Hantzsch–Widman systematic chemical nomenclature recommended by The International Union of Pure and Applied Chemistry (IUPAC), the "benzo" prefix accounts for the benzene ring, and the diazepine refers to the seven-membered heterocycle with two nitrogen and five carbon atoms forming
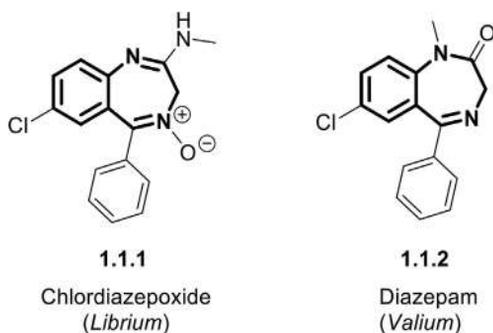
Figure 1.1 Chemical structures of first marketed benzodiazepines, chlordiazepoxide (*Librium*), and diazepam (*Valium*).

the maximum number of contiguous double bonds (Samardzic & Strac, 2016; Schütz, 1982). Changing the position of the nitrogen atoms results in the formation of different classes of these benzo-fused diazepines. In this regard, there are four main groups of BDZs depending upon the nitrogen positions: (1,4)-, (1,5)-, (2,4)-, and (2,5)-BDZs. Among them, the isomers (1,4) and (1,5) are the most common benzodiazepines in both chemical and medicinal research. The imine bond in the BDZs skeleton, especially in 1,4-benzodiazepines, is proved to be a bio-reactive site, which initiates the biological processes to these molecules after metabolism (Kuch, 1979). The first benzodiazepine compound, chlordiazepoxide (**1.1.1**, Fig. 1.1), was serendipitously discovered by an Australian chemist, Dr. Leo Sternbach, and his research group in Hoffmann–La Roche laboratories in 1957, where they were working on the synthesis of some new quinazoline-based tranquilizers (Sternbach, 1979; Sternbach & Reeder, 1961). During this research project, a nicely pure water-soluble crystal was unexpectedly formed as a by-product. It was soon realized that this compound displays outstanding sedative and an-ticonvulsant effects in mice. After this initial discovery, Hoffmann–La Roche initiated clinical studies on the chlordiazepoxide, where a large number of patients were being treated with this drug. These exceptional studies led to introduce the compound **1.1.1** [7-chloro-2-(methylamino)-5-phenyl-3*H*-l,4-benzodiazepine 4-oxidel] as an anxiolytic under the trademark of *Librium* in 1960. Compared to the conventional antidepressants and sedatives, *Librium* proved to be a promising new type of effective anxiolytic with fewer adverse effects in short-term treatment. By pursuing the structural alteration of the chlordiazepoxide in order to improve its biological performance, diazepam (*Valium*, **1.1.2**) was synthesized in Roche's laboratories in 1963 as a more potent benzodiazepine (Fig. 1.1). Installing a carbonyl group on

the 2-position of the quinoxaline ring resulted in significantly improved bioactivity of *Valium*, which quickly became the most frequently prescribed seductive drug for the treatment of not only anxiety but also epilepsy and muscle spasms in the 1970s (Calcaterra & Barrow, 2014), reached a peak of 2.3 billion tablets sold in the US in 1978.

Following the success of *Valium*, more than 20 benzodiazepine analogs were approved for human use under various authorities (Lader, 1991). Since the 1960s, BZDs quickly have gained great prevalence and their consumption as drugs have been dramatically boosting until today. Despite many helpful applications of BZDs in the treatment of anxiety disorders, the extraordinary growth of BDZs usage has raised concerns in different countries due to their high prescription rate and the potential incompensable consequences associated with their long-term consumption such as addiction, cognitive impairments, paradoxical reactions, depression, physical dependence, dementia, and cancer (Penninkilampi & Eslick, 2018; Schmitz, 2016). Benzodiazepines consumption is relatively safe for a maximum duration of two to four weeks, and several physical and psychological health risks may appear in approximately 50% of those patients who use benzodiazepines for more than one month (Lader, 2011). Nevertheless, benzodiazepines have still continued to be one of the highly prescribed drugs of all time in many countries. For example, a recent study disclosed that 30.6 million adults have used benzodiazepines in the US in 2018, including 25.3 million are prescribed and 5.3 million have misuse cases (Agarwal & Landon, 2019).

Although benzodiazepines quickly became one of the top-selling drugs in medical treatment, it took about 15 years to discover their mechanism of action. The earliest report by Roche in 1974 demonstrated that diazepam (**1.1.2**) might alter the behavior of the GABA (gamma-aminobutyric acid) receptors in the spinal cord (Polc et al., 1974). To understand the mechanism of action of benzodiazepines, it is necessary to understand the function of GABA receptors. GABA is one of the main inhibitory neurotransmitters in the nerve cells released by the brain. They play a pivotal role in controlling neuronal excitability via binding to their receptors, $GABA_A$ and $GABA_B$ (Wang et al., 2015). The $GABA_A$ receptors are chloride-permeable channels, which are susceptible to get modified by various ligands through allosteric sites in the receptor. Upon binding the GABA to the receptors, transferring the chloride ions (Cl^-) through neuronal cell membranes is facilitated, causing neuronal inhibition. Disruption of these receptors in an anxiety situation leads to different neurological disorders (Nemeroff, 2003). In this case, benzodiazepines are perfect therapeutic agents that could serve

as positive allosteric regulators of the GABA receptors via having several interactions with the specific sites on the α-γ subunit interface. Upon these interactions, the GABA complex undergoes a conformational modification that increases the chloride ion influx in neurons, leading to hyperpolarizing postsynaptic membranes and subsequently improving the response of the central nervous system (CNS) depression to endogenous GABA (Nutt & Malizia, 2001). These events occurred in the limbic system finally cause anxiolytic effects that end the state of anxiety (Zakusov et al., 1977). Substitution patterns on the core structure of benzodiazepines can generally affect their binding capability to the GABA receptors, modulating their therapeutic and pharmacological effects such as the potency and duration of the effect. For example, the benzo-fused ring with electron–withdrawing groups at the 7-position and/or the pendant phenyl group with either no substitution or *ortho*-halo substitution results in significantly improved anxiolytic performance in benzodiazepines (Sternbach, 1979). Furthermore, removal of the pendant phenyl group from benzodiazepines can change their mechanism of action, in which they become neutral allosteric modulators proved to be valuable radiotracers for GABA receptors (Brogden & Goa, 1988; Lassen et al., 1995).

1.2 Benzothiazepines

Benzothiazepines structurally resemble benzodiazepines, composed of bicyclic heterocycles consisting of a benzene unit fused to a thiazepine ring. Depending on the heteroatom's position on the ring, different nomenclatures can possess. Among them, 1,4-benzothiazepines and 1,5-benzothiazepines are the most common structural isomers for these heterocyclic scaffolds. The last 50 years have witnessed substantial progress in the chemistry of benzothiazepines led to the synthesis of various analogs and the discovery of their biological activities. These versatile skeletons have represented a wide range of therapeutic functions such as CNS depressant, antimicrobial, antifungal, antiplatelet aggregation, anti–HIV, Ca^{+2} antagonist, calmodulin antagonist, and bradykinin receptor antagonist activities (Bariwal et al., 2008; Saha et al., 2015). The first example of benzodiazepines introduced into the pharmaceutical market was thiazesim (*Altinil*, **1.2.1**) as an antipsychotic agent for CNS disorders (Geyer et al., 1970), followed by diltiazem (*Cardizem*, **1.2.2**) developed as a cardiovascular drug of this family (Fig. 1.2) (Nagao et al., 1972). Benzothiazepines, which are bioisosters of
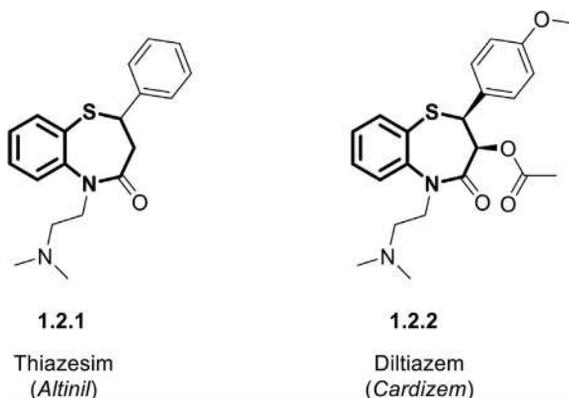
Figure 1.2 Chemical structures of first marketed benzothiazepines, thiazesim (*Altinil*), and diltiazem (*Cardizem*).

benzodiazepines, represent a similar mechanism of action as CNS depressants and anticonvulsant agents (Nikalje et al., 2016; Parjane et al., 2020; Sarro et al., 1995). They act as positive allosteric regulators via binding to the GABA$_A$ receptors, which facilitates the GABA-mediated Cl$^-$ channel opening in the neuronal cell membranes. This phenomenon increases the chloride ions influx in the cells, leading to inducing sedative and hypnotic properties.

The present book aims to evidently unite both synthesis and pharmaceutical aspects of benzodiazepine- and benzothiazepine-based structures. Here, we are providing a comprehensive vision of chemical structural properties, recent and general synthetic routes, mechanism of biological mechanisms, and pharmaceutical effects. Although a large number of efficient synthetic procedures and biological activity profiles for benzodiazepines and benzothiazepine are already demonstrated, we expect that the compiling medicinal research findings along with the recent progress in organic synthesis of benzodiazepine- and benzothiazepine-based compounds will open a window to new aspects of these interesting heterocycles in the drug discovery process.

References

Agarwal, S. D., & Landon, B. E. (2019). Patterns in outpatient benzodiazepine prescribing in the United States. *JAMA Network Open, 2*(1), e187399. https://doi.org/10.1001/jamanetworkopen.2018.7399.

Bariwal, J. B., Upadhyay, K. D., Manvar, A. T., Trivedi, J. C., Singh, J. S., Jain, K. S., & Shah, A. K. (2008). 1,5-Benzothiazepine, a versatile pharmacophore: a review. *European Journal of Medicinal Chemistry, 43*(11), 2279–2290. https://doi.org/10.1016/j.ejmech.2008.05.035.

Brogden, R. N., & Goa, K. L. (1988). Flumazenil. A preliminary review of its benzodiazepine antagonist properties, intrinsic activity and therapeutic use. *Drugs, 35*(4), 448–467. https://doi.org/10.2165/00003495-198835040-00004.

Calcaterra, N. E., & Barrow, J. C. (2014). Classics in chemical neuroscience: Diazepam (valium). *ACS Chemical Neuroscience, 5*(4), 253–260. https://doi.org/10.1021/cn5000056.

Geyer, H. M., Watzman, N., & Buckley, J. P. (1970). Effects of a tranquilizer and two antidepressants on learned and unlearned behaviors. *Journal of Pharmaceutical Sciences, 59*(7), 964–968. https://doi.org/10.1002/jps.2600590709.

Kessler, R. C., Chiu, W. T., Demler, O., & Walters, E. E. (2005). Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication. *Archives of General Psychiatry, 62*(6), 617−627. https://doi.org/10.1001/archpsyc.62.6.617.

Kuch, H. (1979). Clobazam: Chemical aspects of the 1,4- and 1,5-benzodiazepines. *British Journal of Clinical Pharmacology, 7*(1), 17S–21S. https://doi.org/10.1111/j.1365-2125.1979.tb04659.x.

Lader, M. (1991). History of benzodiazepine dependence. *Journal of Substance Abuse Treatment, 8*(1–2), 53–59. https://doi.org/10.1016/0740-5472(91)90027-8.

Lader, M. (2011). Benzodiazepines revisited – will we ever learn? *Addiction, 106*(12), 2086–2109. https://doi.org/10.1111/j.1360-0443.2011.03563.x.

Lassen, N. A., Bartenstein, P. A., Lammertsma, A. A., Prevett, M. C., Turton, D. R., Luthra, S. K., … Patsalos, P. N. (1995). Benzodiazepine receptor quantification in vivo in humans using [^{11}C]flumazenil and PET: application of the steady-state principle. *Journal of Cerebral Blood Flow and Metabolism, 15*(1), 152–165. https://doi.org/10.1038/jcbfm.1995.17.

Nagao, T., Sato, M., Iwasawa, Y., Takada, T., & Ishida, R. (1972). Studies on a new 1,5-benzothiazepine derivative (CRD-401). 3. Effects of optical isomers of CRD-401 on smooth muscle and other pharmacological properties. *Japanese Journal of Pharmacology, 22*(4), 467–478. https://doi.org/10.1254/jjp.22.467.

Nemeroff, C. B. (2003). The role of GABA in the pathophysiology and treatment of anxiety disorders. *Psychopharmacology Bulletin, 37*(4), 133–146.

Nikalje, A. P. G., Ghodke, M. S., Khan, F. A. K., & Sangshetti, J. N. (2016). Microwave assisted facile synthesis and biological evaluation of novel 2-indolyl-1,5-benzothiazepines. *Open Pharmaceutical Sciences Journal, 6*, 117–130. https://doi.org/10.2174/1874844901603010117.

Nutt, D. J., & Malizia, A. L. (2001). New insights into the role of the GABA$_A$-benzodiazepine receptor in psychiatric disorder. *British Journal of Psychiatry, 179*(5), 390–396. https://doi.org/10.1192/bjp.179.5.390.

Parjane, S. K., Kunkulol, R., & Nandal, D. (2020). Synthesis of some novel 1,5-benzothiazepine derivatives biological screening for anticonvulsant activity. *IOSR Journal of Applied Chemistry, 13*(3), 50–55. https://doi.org/10.9790/5736-1303015055.

Penninkilampi, R., & Eslick, G. D. (2018). A systematic review and meta-analysis of the risk of dementia associated with benzodiazepine use, after controlling for protopathic bias. *CNS Drugs, 32*, 485–497. https://doi.org/10.1007/s40263-018-0535-3.

Polc, P., Mohler, H., & Haefely, W. (1974). The effect of diazepam on spinal cord activities: Possible sites and mechanisms of action. *Naunyn-Schmiedeberg's Archives of Pharmacology, 284*, 319–337. https://doi.org/10.1007/BF00504702.

Saha, D., Jain, G., & Sharma, A. (2015). Benzothiazepines: chemistry of a privileged scaffold. *RSC Advances, 5*(86), 70619–70639. https://doi.org/10.1039/C5RA12422K.

Samardzic, J., & Strac, D. S. (2016). Benzodiazepines and anxiety disorders: from laboratory to clinic. In F. Durbano, & B. Marchesi (Eds.), *New developments in anxiety disorders*. London: IntechOpen https://doi.org/10.5772/64959.

Sarro, G. D., Chimirri, A., Sarro, A. D., Gitto, R., Grasso, S., & Zappalà, M. (1995). 5H-[1,2,4]Oxadiazolo[5,4-*d*][1,5]benzothiazepines as anticonvulsant agents in DBA/2 mice. *European Journal of Medicinal Chemistry, 30*(12), 925–929. https://doi.org/10.1016/0223-5234(96)88311-5.

Schmitz, A. (2016). Benzodiazepine use, misuse, and abuse: a review. *Mental Health Clinician, 6*(3), 120–126. https://doi.org/10.9740/mhc.2016.05.120.

Schütz, H. (1982). *Benzodiazepines* (1st ed.). Berlin Heidelberg: Springer-Verlag https://doi.org/10.1007/978-3-642-68426-5.

Sternbach, L. H. (1979). The benzodiazepine story. *Journal of Medicinal Chemistry, 22*(1), 1–7. https://doi.org/10.1021/jm00187a001.

Sternbach, L. H., & Reeder, E. (1961). Quinazolines and 1,4-benzodiazepines. II. Rearrangement of 6-chloro-2-chlormethyl-4-phenylquinazoline 3-oxide into 2-amino derivatives of 7-chloro-5-phenyl-3*H*-1,4-benzodiazepine 4-oxide. *Journal of Organic Chemistry, 26*(4), 1111–1118. https://doi.org/10.1021/jo01063a034.

Wang, P., Eshaq, R. S., Meshul, C. K., Moore, C., Hood, R. L., & Leidenheimer, N. J. (2015). Neuronal gamma-aminobutyric acid (GABA) type A receptors undergo cognate ligand chaperoning in the endoplasmic reticulum by endogenous GABA. *Frontiers in Cellular Neuroscience, 9*, 188. https://doi.org/10.3389/fncel.2015.00188.

Zakusov, V. V., Ostrovskaya, R. U., Kozhechkin, S. N., Markovich, V. V., Molodavkin, G. M., & Voronina, T. A. (1977). Further evidence for GABA-ergic mechanisms in the action of benzodiazepines. *Archives Internationales De Pharmacodynamie Et De Therapie, 229*(2), 313–326.

Non-Print Items

Abstract

This chapter briefly explains the history, mechanism of action, and medical applications of benzodiazepines and benzothiazepines.

Structural features of 1,4-benzodiazepines

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Chapter outline

2.1 Introduction

Benzodiazepines and benzothiazepines are two pharmaceutically important heterocyclic compounds where a benzene ring is fused to either a diazepine or a thiazepine ring, respectively (Saha et al., 2015). The position of heteroatoms in the seven-membered ring determines the type and the chemistry of these cyclo-condensed compounds. According to IUPAC, when a heterocyclic ring is fused with a benzene unit, it has to be indicated by the "benzo" prefix. The numbering of benzodiazepines and benzothiazepines should be started from the immediate heteroatom adjacent to the
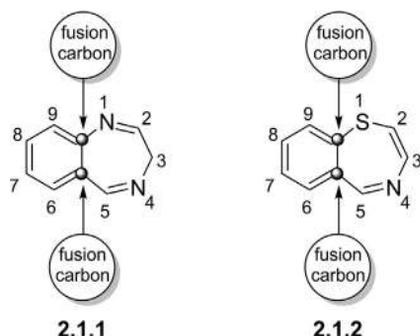
Figure 2.1 The general structures of 1,4-benzodiazepine (**2.1.1**) and 1,4-benzothiazepine (**2.1.2**).

fusion carbon atom with no given number to the fusion atoms (Fig. 2.1). 1,4-Benzodiazepines (1,4-BDZs) **2.1.1** and 1,4-benzothiazepines **2.1.2** are the most common structural isomers of benzodiazepines and benzothiazepines with the heteroatoms at positions 1 and 4 (Fig. 2.1).

From the pharmaceutical viewpoint, 1,4-benzodiazepines can be further divided into two main classes including FDA-approved 1,4-BDZs and designer 1,4-BDZs, also known as synthetic and non-FDA-approved 1,4-BDZs (Greenblatt & Greenblatt, 2019). Designer 1,4-benzodiazepines are newly developed compounds having relatively similar structures to the FDA-approved counterparts with some slight deliberate modifications and with no FDA endorsement yet. Pyrazolam (**2.1.8**) is the first compound of designer 1,4-BDZs family structurally derived from alprazolam (**2.1.3**) and bromazepam (**2.1.4**), which became available in online dispensation on a large scale in mid-2012 with no medical license (Fig. 2.2) (Gilman et al., 1990). Although being of high availability as drugs in many countries occured, pharmacological properties and potential risks for the medical use of most designer 1,4-benzodiazepines have not been fully investigated yet (Moosmann & Auwärter, 2018).

For obtaining 1,4-benzodiazepine- and 1,4-benzothiazepine-based pharmaceutically significant compounds with evolved and better performance, it is highly desirable to recognize the structure-activity relationships (SAR) of these scaffolds from different aspects. This chapter principally illustrates the chemical structural features of 1,4-benzodiazepines and 1,4-benzothiazepines to provide a clear understanding of the structural behavior of these heterocyclic skeletons.

(A) Examples of FDA-approved 1,4-BDZs

2.1.3

alprazolam

2.1.4

bromazepam

2.1.5

clonazepam

2.1.6

triazolam
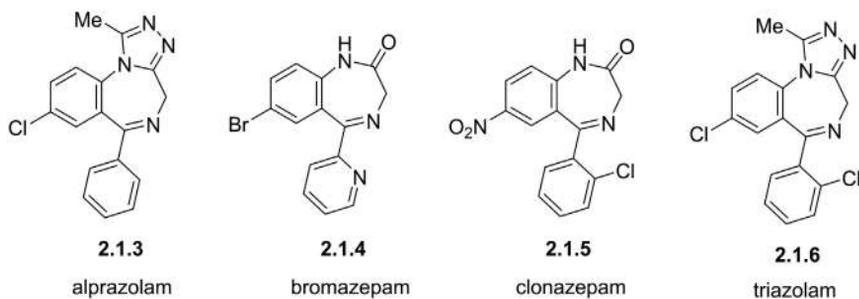
(B) Examples of designer 1,4-BDZs

2.1.7

flubromazepam

2.1.8

pyrazolam

2.1.9

clonazolam
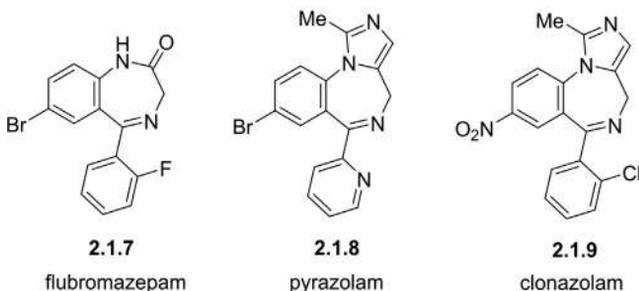
Figure 2.2 Examples of (A) FDA-approved 1,4-BDZs and (B) non-FDA-approved designer 1,4-BDZs.

2.2.1

Figure 2.3 Structural variations of 1,4-benzodiazepines.

2.2 Structural variations of 1,4-benzodiazepines

Looking more closely at the structural variations of 1,4–BDZs (Fig. 2.3), it can be understood that these molecules primarily differ in the substituents at three major positions, namely at 1, 2, and 7 on the core

structure (Borea, 1981; Meguro & Kuwada, 1970). Various functional groups or side chains can be attached to these positions (indicated by R^1, R^2, and R^7) located on the benzene and diazepine rings. Some techniques such as X–ray crystallography can provide a precise molecular structure that affirms the molecular shape and the chemical structure (Chkirate et al., 2019; Hester et al., 1971). The chemistry of substituting in each position of 1,4–benzodiazepines can be elaborated as follows.

1) Position 1 inclines to accept either hydrogen atoms or alkyl groups, in which the anxiolytic activity of such structures is improved by alkylation (Borea, 1981).

2) Position 2 is considered as the key position to add heteroatoms (S or O) to form a (thio)keto group, except for those benzodiazepines that are fused to another ring from this position. The heteroatom linked to position 2 serves as a proton acceptor (Brönsted base), and it is liable to get protonated at physiological pH and consequently, would affect the lipophilicity.

3) Position 3 is generally unfavored to be functionalized by a substituent. As such, the presence of a hydroxyl group in several 1,4-benzodiazepine derivatives, for example, lorazepam, oxazepam, and temazepam, significantly enhances the polarity of these skeletons, leading to more glucuronidation and subsequently faster drug elimination from the body. Furthermore, it has been demonstrated that OH-substituted 1,4–benzodiazepines at position 3 are thermally unstable, undergoing a thermal a Frigerio-type rearrangement (*via* dehydration) to transform 1,4–diazepine ring to the stable 1,3-diazinane unit (Bourcier et al., 2001).

4) Position 4 tends to accept a double bond as an unsaturation at positions 4 and 5 can be observed in all varieties of 1,4-benzodiazepines.

5) Position 5 generally tends to accept a simple aromatic ring as a substituent, wherein all common 1,4-benzodiazepines have a phenyl group at this position. Not only the presence of a phenyl ring at position 5 plays a key role in (bio)chemical activity of the final 1,4-BDZs, but also the substituents present on the phenyl group can improve the activity. In this context, the presence of electron–withdrawing groups at the *ortho* or di-*ortho* positions of the phenyl ring results in an increased potency, such as flubromazepam **2.1.7** and clonazepam **2.1.9** with F and Cl at position 2′, respectively (Fig. 2.3).

6) Position 7 is a critical site to improve the biological activity of 1,4–BDZs, where introducing an electron–withdrawing substituent such as CF_3,
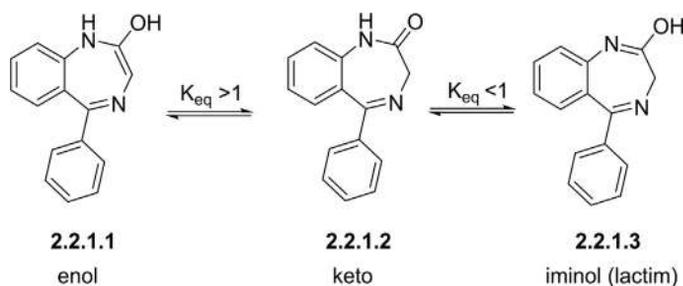
Figure 2.4 Tautomeric equilibrium of 1,4-benzodiazepine-2-one.

NO_2, and halogens (F, Cl, Br, I) would enhance the compound's potency (Sternbach, 1980).

7) Other positions (6, 8, and 9) are barely favorable for substitution, which may reduce the biological activity of benzodiazepine (Sternbach, 1980).
In the following sections, 1,4–benzodiazepines are arranged according to their structural variations.

2.2.1 1,4-Benzodiazepinones

1,4–Benzodiazepine-2-one and 1,4–benzodiazepine-2,5–diones are considered as the most potent members of 1,4–BDZ family, not only due to their wide clinical applications but also for their capability to undergo various modifications to furnish diverse biologically active structures (Boojamra et al., 1997; Cummings et al., 2006; Spencer et al., 2010; Sternbach, 1971). Although structural modifications of 1,4-benzodiazepinones may improve their physicochemical properties and chemical stabilities, these compounds are prone to undergo tautomerization due to the presence of the carbonyl group. In contrast to other types of 1,4-benzodiazepines, 1,4–benzodiazepinones may exist in three forms, namely keto, enol, and iminol in a kinetically favored equilibrium (Fig. 2.4); both iminol and enol isomers are less stable than the corresponding keto form. Computational studies have demonstrated that introducing various aromatic substituents at the C5 position displays minor changes in relative energies between enol/iminol and the parent keto form (Pem & Vrček, 2017). On the other hand, the presence of acyl-type substituents at the C3 center strongly favors the enol tautomer compared to iminol and keto counterparts (Pem & Vrček, 2017). The extent of enolization/iminolization in 1,4-benzodiazepinones by choosing a proper substituent leads to various potential biological
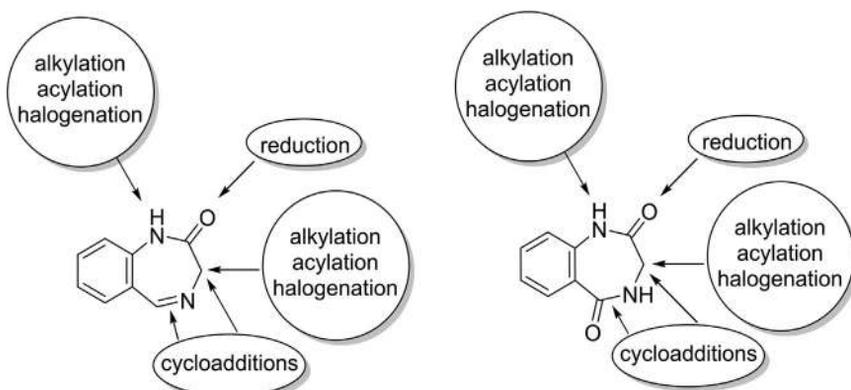
Figure 2.5 The reactivity of the 1,4-benzodiazepine-2-one and
1,4-benzodiazepine-2,5-diones at different sites.

activities of these heterocycles, which opens a new avenue for optimizing
novel 1,4-benzodiazepines with targeted properties. On the downside, the
tautomerization of 1,4-benzodiazepinones results in racemization when they
have a stereocenter at the C3 position, which consequently reduces their
enantiomeric or diastereomeric purity (Aso et al., 1988; Hok et al., 2019;
Yang, 1995).

   1,4-Benzodiazepine-2-one and 1,4-benzodiazepine-2,5-diones have a
strong tendency to participate in various organic transformations at different
reactive sites (Fig. 2.5). The amide carbonyl group at position 1 is susceptible
to the addition of nucleophiles and the reduction *via* a reducing agent (Batlle
et al., 2019; Sharp, 1984). There are two types of N1 and C3 anions present
in 1,4-benzodiazepinones because of the N—H and C—H deprotonations
of amide, respectively. The higher stability of the former versus the latter
discloses the higher acidity of the N1—H proton compared with the
C3—H (Popovic et al., 2003). It is also evident that the N1 center is more
reactive towards electrophiles than the C3 position, in which alkylation and
acylation reactions often occur (Archer & Sternbach, 1968; Khan et al., 2018;
Sternbach, 1971). The excellent regioselectivity of these reactions can be
attributed to the higher nucleophilicity of iminol isomer in comparison
to the keto tautomer. Halogenation or alkylation/acylation of either N1 or
C3 centers readily provide useful scaffolds for further manipulations such as
amination and thiation (Carlier et al., 2006; Sharp, 1984). Formal cycloaddi-
tion reactions can occur at C3 and N4—C5 of 1,4-benzodiazepinones using
various 1,3-dipoles including azomethine ylides and nitrilimines (Fan et al.,
2021; Molteni et al., 2002; Sharp, 1984).
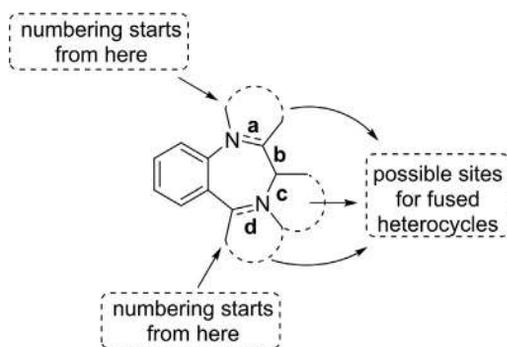
Figure 2.6 Schematic representation of possible sites of 1,4-BDZs for fusing another heterocycle.

2.2.2 Fused 1,4-benzodiazepines

The commercial success and medical significance of 1,4-benzodiazepines have resulted in the development of wide-ranging synthetic studies on these scaffolds (Archer & Sternbach, 1968; Meyer et al., 2017). One approach that has led to the generation of a large number of synthetic reports over the last decades is the syntheses of 1,4-benzodiazepines with an additional bioactive fused heterocyclic ring. These fused heterocyclic systems provide an interesting subclass of 1,4-benzodiazepines possessing extra bioactive heterocyclic rings mainly annulated to the *a, c,* and *d* faces of the core skeleton (Fig. 2.6). Pyrrole, imidazole, triazole, and indole are among the most eminent heterocycles incorporated into the 1,4-benzodiazepine ring system to enhance their chemical and pharmacological properties. Numbering in the fused systems is slightly different from the normal 1,4–BDZs, starting from the heteroatom that is in common with the diazepine ring and the fused ring. Correlating the chemical activity of these compounds to the type and location of substituents is also applicable in the case of fused 1,4–BDZs. The assembly of a new ring on 1,4–benzodiazepines is rather straightforward on their *a* side since these skeletons have a nucleophilic N at position 1, which can be readily functionalized to generate an electrophilic center at position 2 (Sharp, 1984). Accordingly, a large number of ring systems can be fabricated on the *a* side by simply changing the standard synthetic procedures.

Triazine ring is a versatile heterocyclic ring proven to have strong π-interaction abilities and increased ability to get involved in H-bond networks (Mooibroek & Gamez, 2007). Accordingly, the fusion of this unique scaffold to 1,4–BDZs may improve their binding affinity to the $GABA_A$ receptors in the cells, leading to significant enhancement of their biological activities. For
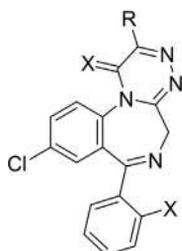
2.2.2.1

R = Me, CH₂NMe₂, CH₂Cl
X = O, H₂

Figure 2.7 An example of triazino-fused 1,4-benzodiazepine.

example, the fused compound **2.2.2.1** has been reported to exhibit excellent anxiolytic activity in animals (Fig. 2.7) (Moffett et al., 1977).

Triazole is a five-membered unsaturated aromatic heterocycle with more than two heteroatoms including two carbons and three nitrogens, where one of the nitrogen atoms is pyrrole type and two others are pyridine type. Triazolo–1,4–benzodiazepines are the most potent agents of the 1,4-benzodiazepine family, commonly used as central nervous system depressants. FDA-approved alprazolam (**2.1.3**) and triazolam (**2.1.6**) are two important members of these structural classes introduced in the drug market to treat panic attacks and insomnia. The annulation of 1,4-benzodiazepine skeleton with an electron–rich triazole ring can enhance the stability and basicity of the fused system, leading to the formation of active molecules with a high binding capability to the benzodiazepine cell receptors. The triazole unit also serves as a proton acceptor that may interact with histidine residues of the GABA$_A$ receptor complex (Ayati et al., 2016). Due to their medicinal importance, the developmental strategies for the synthesis and biological evaluation of triazolo–1,4–benzodiazepine derivatives are incessantly garnering immense interest of organic and medicinal chemists. Common structural isomers of triazolo–fused 1,4–benzodiazepines in drug discovery are illustrated in Fig. 2.8 (Alanis et al., 2020; Donald & Martin, 2011; Narayana et al., 2006; Sudhapriya et al., 2015).

The quinazoline–fused 1,4–BDZs are principally found in naturally oc-curring alkaloids, where the quinazoline ring is mainly fused to the 1,4–BDZ from the N1−C2 site (Fig. 2.9). For example, benzomalvin A-C (**2.2.2.8** to **2.2.2.10**) isolated from *spenicillium* and Sclerotigenin **2.2.2.11** derived from *penicillium sclerotigenum* are two important members of the quinazoline-fused 1,4–BDZs (Joshi et al., 1999; Sun et al., 1994; Tseng et al., 2010).

2.2.2.2

benzo[f][1,2,4]
triazolo[4,3-a][1,4]diazepine

2.2.2.3

benzo[f][1,2,3]
triazolo[1,5-a][1,4]diazepine

2.2.2.4

benzo[e][1,2,3]
triazolo[1,5-a][1,4]diazepine

2.2.2.5

benzo[e][1,2,4]
triazolo[4,3-a][1,4]diazepine

2.2.2.6

benzo[f][1,2,4]
triazolo[1,5-d][1,4]diazepine

2.2.2.7

benzo[f][1,2,3]
triazolo[1,5-d][1,4]diazepine
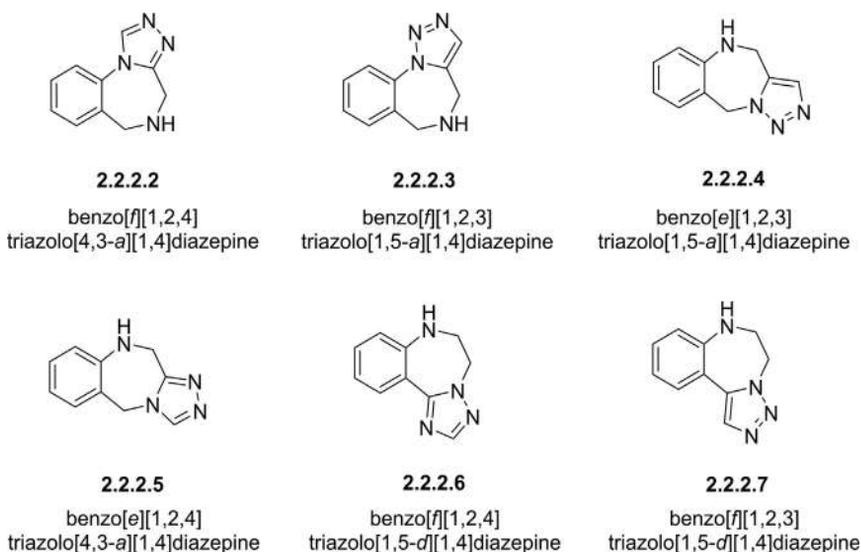
Figure 2.8 General examples of common triazolo-fused 1,4-benzodiazepines.

2.2.2.8

benzomalvin A

2.2.2.9

benzomalvin B

2.2.2.10

benzomalvin C

2.2.2.11

sclertigenin
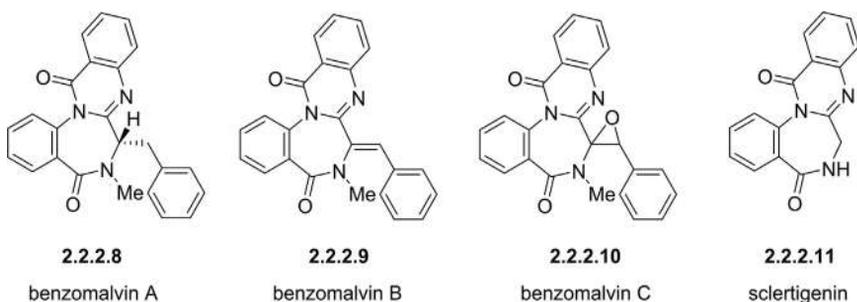
Figure 2.9 Examples of quinazoline-fused 1,4-benzodiazepines found in alkaloids.

1,4–Benzodiazepines can be condensed to oxazole, oxazine, and aziridine rings through the N4−C5 position located on the *d* face (Fig. 2.10). The fusion of these heteroarenes to the diazepine nucleus furnishes the correspondingly unique 1,4-benzodiazepines, displaying good anticonvulsant performance (Cortes et al., 2007; De Sarro et al., 1996). In spite of the various biological activities of these 1,4-benzodiazepine derivatives, fewer research studies have been carried out on the development of these fused molecules relative to other fused 1,4-benzodiazepines, possibly due to their chemical instability leading to problems in structural modifications, and the steric hindrance of the heterocyclic units that restrict their interaction with cellular receptors and diminish their penetration into the central nervous
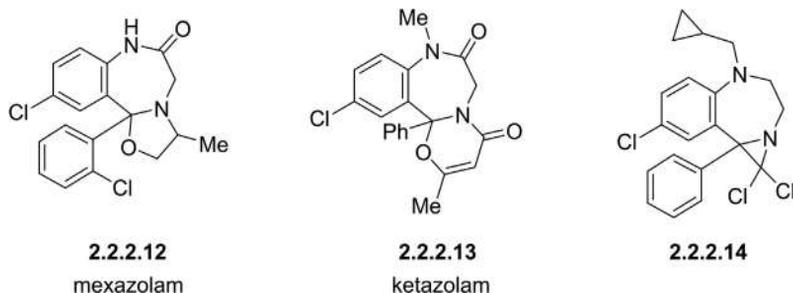
Figure 2.10 Examples of oxazole-, oxazine-, and aziridine-fused 1,4-benzodiazepines.
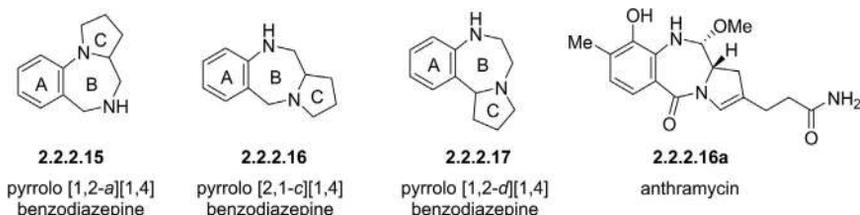
Figure 2.11 Examples of pyrrolo-1,4-benzodiazepines.

system (CNS) through the blood–brain barrier. Mexazolam (**2.2.2.12**) and ketazolam (**2.2.2.13**) are the most popular anxiolytic oxazole- and oxazine-fused 1,4–benzodiazepine analogs (Fernandes & Moreira, 2014; Rickels et al., 1980), respectively, which have been discontinued for human consumption in many countries due to their major adverse effects and tolerance. In another example, the steric hindrance of the azirine unit in **2.2.2.14** constrains adopting the proper conformation of the phenyl group for binding to the neurotransmitter receptors and consequently lowering its anxiolytic activity (De Sarro et al., 1996).

Pyrrole analogs, largely found in natural products, are considered privileged structures possessing a wide variety of biological activities (Joshi et al., 2013). Pyrrolo–1,4–benzodiazepines are fused heterocyclic ring systems comprising a benzene A–ring, a 1-4–diazepine B–ring, and an aromatic or non–aromatic pyrrole C–ring (Fig. 2.11), where the position of substitutions on the A- and C–rings, and the degree and position of unsaturation on the C–ring provided various structural types (Varvounis, 2016). The pyrrolo–1,4–benzodiazepines are classified by three common structural isomers including [1,2-*a*][1,4], [2,1-*c*][1,4], and [1,2-*d*][1,4] (Fig. 2.11). The first analog of these fused ring skeletons has been a pyrrolo[2,1-*c*][1,4]benzodiazepine, anthramycin **2.2.2.16a**, which is isolated from cultures of *Streptomyces* in 1965 (Leimgruber et al., 1965). Anthramycin
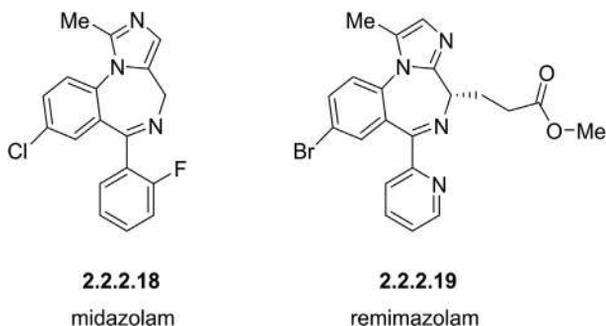
Figure 2.12 FDA-approved midazolam and remimazolam drugs as two examples of imidazo-1,4-benzodiazepines.

is recognized to display high biological activity against breast and gastrointestinal cancers, lymphomas, and sarcomas with low hematological toxicity. However, the dose–limiting cardiotoxicity of anthramycin restricted its clinical use, which is presumably owing to the presence of C9 oxygen (Korman & Tendler, 1965). Since the discovery of anthramycin, the pyrrolo[2,1-c][1,4]benzodiazepines became the most intensively studied isomer among the other structural isomers (Hartley, 2011). Garnering significant attention in the development of pyrrolo[2,1-c][1,4]benzodiazepines is primarily due to their strong ability to interact with DNA. These heterocyclic molecules perfectly attach to the minor groove of DNA *via* the interaction of their right–handed helical conformation with 5′–purine–G–purine sequences, generating a covalent bond with guanine residues (Varvounis, 2016).

Imidazole–fused 1,4–benzodiazepines with an imidazole ring substituted at the *a* face of the diazepine core are classified as powerful benzodiazepines widely used as FDA–approved 1,4–BDZs in clinical anesthesia, such as midazolam (**2.2.2.18**) and remimazolam (**2.2.2.19**) (Fig. 2.12). The presence of basic nitrogen in the imidazole unit enables these benzodiazepines to form water–soluble salts, which improves their pharmacokinetic characteristics, solubility, and bioavailability (Saari et al., 2011). Appropriate modifications of these heterocyclic compounds have been shown to create molecules with different potential pharmacological features (Fustero et al., 2006; Gall et al., 1988).

Indolo–fused 1,4–benzodiazepines comprising indole and benzodiazepine skeletons are pharmaceutically important molecules identified as antihypertensive and anti–allergic agents (Ho et al., 1986) (Ho, 1985). The indole nucleus can be fused to the benzodiazepine core skeleton through *a, c, d* faces of the diazepine ring (Fig. 2.13). Similar to imidazolo–fused 1,4-benzodiazepines, indole containing 1,4-benzodiazepines are susceptible
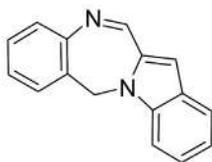
2.2.2.20

benzo[6,7][1,4]diazepino[1,2-*a*]indole
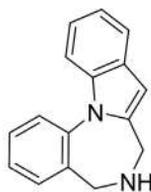
2.2.2.21

benzo[5,6][1,4]diazepino[1,2-*a*]indole
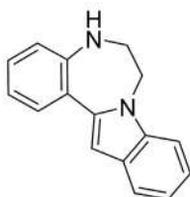
2.2.2.22

benzo[5,6][1,4]diazepino[1,7-*a*]indole
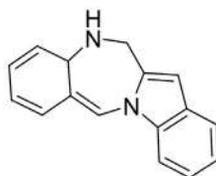
2.2.2.23

benzo[6,7][1,4]diazepino[1,2-*a*]indole

Figure 2.13   General structural isomers of indolo-1,4-benzodiazepines.

to form water-soluble salt with physiologically acceptable acids such as hydrochloric acid, which enhances their pharmacokinetic features (Ho, 1985).

2.3 Conformational studies of 1,4-benzodiazepines

Despite wide-ranging investigations on the synthesis of 1,4-benzodiazepines (see Chapter 3), the mechanism of action and pharmaceutical activity of these important heterocycles have not been fully understood. There are two major factors in the interactive behavior of 1,4–BDZs with the cell receptors; the conformational and the electronic features of 1,4–BDZs. Thus, the conformational status and electronic charge distribution in their biointerface with the receptor/enzyme/protein may provide a reasonable clue towards a better understanding of their action mechanism. From this perspective, altering the conformation of 1,4–BDZs possibly affects their biological properties (Hadjipavlou-Litina & Hansch, 1994; Pertejo et al., 2014). Consequently, there is enduring attention in developing a deeper insight of conformational preferences of 1,4–benzodiazepines that provides more practical control of conformer ratios with an aim to expand their potential
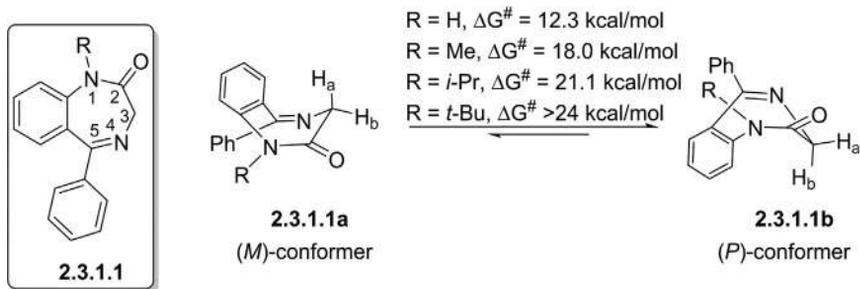
Figure 2.14 General boat shape conformations of 1,4-benzodiazepin-2-ones with the ring inversion.

applications. In general, the conformational behavior of seven–membered ring heterocycles possessing two heteroatoms is significantly different from derivatives having a single heteroatom. The additional heteroatom has a strong effect on the conformational equilibrium of 1,4–benzodiazepines mainly by intramolecular H-bonding and electrostatic interactions (Riddel, 1980). Single crystal X-ray analysis, theoretical calculations, e.g., Hartree-Fock (HF) and density functional theory (DFT), together with experimental spectroscopic methods such as nuclear magnetic resonance (NMR) spectroscopy and circular dichroism (CD) are the most powerful strategies to evaluate the preferred conformations of 1,4–benzodiazepines (Meanwell & Walker, 2008). 1,4–Benzodiazepine structure often exists in a seven–membered boat-like conformation with the hydrogens in axial and equatorial positions at the C3 center, generating two *M* and *P* conformational enantiomers. There are several factors affecting the ratio of these conformers in the reaction mixture. For example, increasing the size of the N1 substituent in the 1,4–diazepine unit enhances the inversion barrier to distinguish these isomers *via* ^{1}H NMR analysis. However, small functionalities on N1 avoid the resolution of the conformers at room temperature due to the lower racemization barrier. In the following sections, several examples of conformational status of 1,4–benzodiazepines are discussed in more detail.

2.3.1 Conformational status of 1,4-benzodiazepin-2-ones

The 2,3–dihydro-1*H*-1,4–benzodiazepin-2-one derivatives (**2.3.1.1**), as the simplest members of 1,4-benzodiazepin-2-ones, exist as an equilibrium of two *M* and *P* boat-like conformers (Fig. 2.14). The interconversion barrier between these conformers highly depends upon the size of the substituent on N1, where H, Me, *i*-Pr, and *t*-Bu groups represent $\Delta G^{\#} = 12.3, 18.0,$
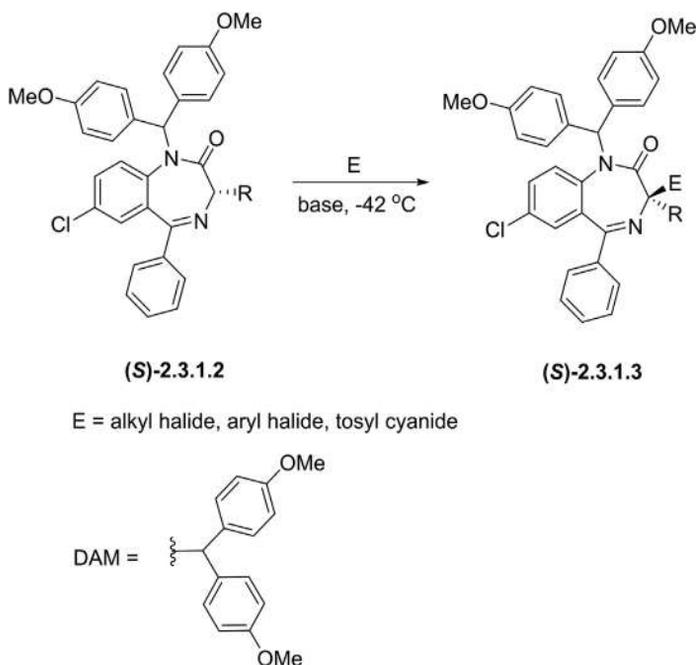
Figure 2.15 The effect of N1-substituent and reaction temperature on the retention of absolute configuration of 7-chloro-(3S)-methyl-1,4-benzodiazepin-2-one **2.3.1.2**.

21.1, and >24 kcal/mol, respectively, as a parameter of escalating non-bonded peri interactions with a possible substituent at the C9 position (Meanwell & Walker, 2008). It has also been shown that those conformers with interconversion barriers of more than 20 kcal/mol can be separated by column chromatography technique, exhibiting different behavior with both human serum albumin and cellular receptors (Paizs & Simonyi, 1999). Bulkier substituents on the C3 stereotypically favor an equatorial position, in which the absolute configuration of 1,4-benzodiazepin-2-ones has a significant effect on the conformational status, as such a (3S)–analog prefers the (M)-conformation (Meanwell & Walker, 2008; Salvadori et al., 1997; Zhao et al., 2005).

The preservation of chirality at the C3 position is mainly dependent on the N1 substituent, the reaction temperature, and the polarity of the solvent. For example, it has been shown that the 7-chloro–(3S)–methyl derivative of 1,4-benzodiazepin-2-one **2.3.1.2**, bearing a di(p-anisyl)methyl (DAM) substituent on the N1 center, exhibits high reactivity toward several electrophiles with retention of configuration (ee > 99%) at –42 °C (Fig. 2.15) (Carlier et al., 2006). The bulky DAM group induces a con-
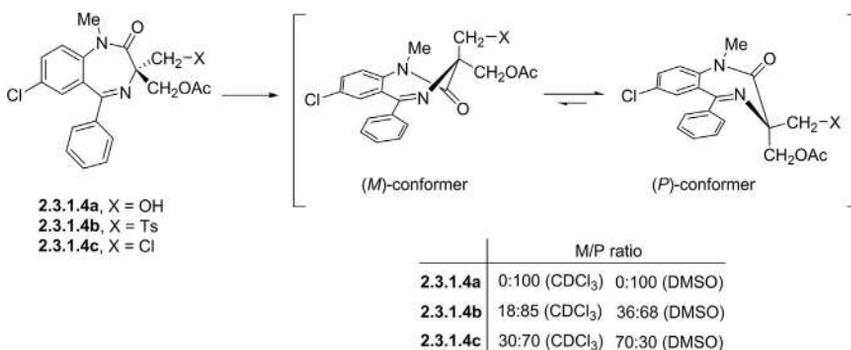
Figure 2.16 The effect solvent polarity on the absolute conformation of enantiomerically pure 3,3-disubstituted 1,4-benzodiazepin-2-one **2.3.1.4**.

siderable barrier to racemization of the conformationally chiral enolate intermediates, retaining the absolute configuration. In another study, Avdagić and co-workers have demonstrated the first example of the solvent effect on the absolute conformation of enantiomerically pure 3,3-disubstituted 1,4-benzodiazepin-2-one **2.3.1.4** (Fig. 2.16) (Avdagić et al., 1999). Based on the ^{1}H NMR analysis, chiral 3,3-disubstituted analog **2.3.1.4a** exists in only (*P*)-conformer in both CDCl$_3$ and DMSO solvents, whereas **2.3.1.4b** and **2.3.1.4c** are present as equilibrium mixtures of two *M/P* conformers in both solvents. The higher ratio of the (*P*)-conformer in a less polar solvent (CDCl$_3$) can be explained by the stronger repulsive interaction of axial substituent with the anisotropic cone of the annulated benzene ring, which can be expunged in NMR analysis as the ^{1}H NMR signal for the axial group is significantly shifted upfield. Furthermore, the complete (*P*)-conformer preference of **2.3.1.4a** is possibly related to the higher stability of the conformer as a result of strong H-bonding between the hydroxyl group of equatorially oriented CH$_2$OH and the amide oxygen.

1,4-Benzodiazepin-2-ones are generally classified as type III peptidomimetics based on their matched spatial topology to their precursor peptides. As illustrated in Fig. 2.17, the substituents on the N1, C3, C6, and C8 positions of **2.3.1.5** topologically fit the Cα substituents of a four-residue β-turn in the peptide **2.3.1.6**, determined by X-ray and NMR analyses (Ripka et al., 1993). Considering that the peptide portion of **2.3.1.5** keeps a similar β-turn conformation in all amino acid cyclic peptides, the 1,4-benzodiazepin-2-one itself probably effectively mimics the β-turn opposite in the ring.

Conformational isomers of clonazepam (**2.1.5**) as a member of FDA-approved 1,4-BDZs are depicted in Fig. 2.18 (Menezes et al., 2012). The [1]H
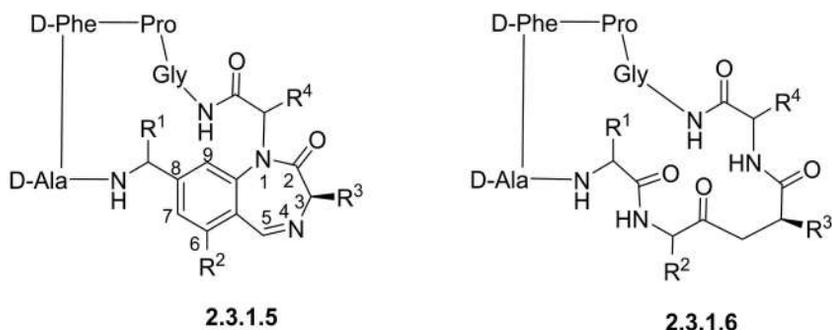
Figure 2.17 The benzodiazepine β-turn peptidomimetic in the model cyclic octapeptide **2.3.1.5**, compared with its antecedent peptide **2.3.1.6**.
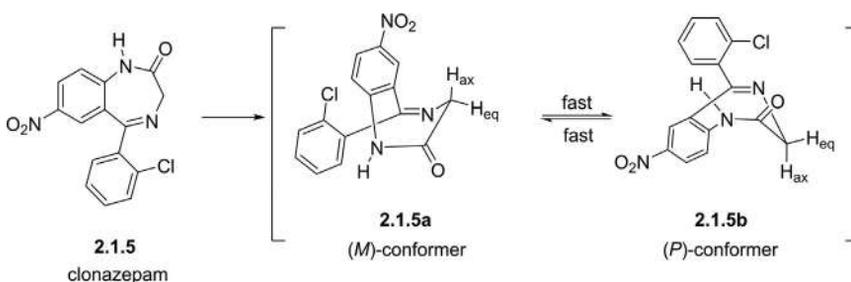
Figure 2.18 Conformational enantiomers for clonazepam.

NMR analysis of clonazepam at room temperature showed the presence of one broad signal at $\delta = 4.31$ ppm attributed to C3–hydrogens (H_{ax} and H_{eq}) due to the low inversion barrier between the conformers (**2.1.5a** and **2.1.5b**), where the quick flipping of diazepine ring leads to the fast interconversion of the seven–membered boat on the NMR time scale. It is known that increasing the activation enthalpy and the subsequent inversion barrier occur through the presence of bulky substituents on the N1 center in the diazepine ring system. By introducing a methylene group on the N1 position of clonazepam, two doublet signals attributed to the equatorial and axial hydrogens at the C3 position are observable in the ^{1}H NMR spectrum, indicating the existence of the conformational enantiomers **2.1.5a** and **2.1.5b** (Menezes et al., 2012).

2.3.2 Conformations of 1,4-benzodiazepine-2,5-diones

1,4–Benzodiazepine–2,5–diones prefer a boat–like conformation with pseudo–axial and pseudo–equatorial substituents at the C3 center
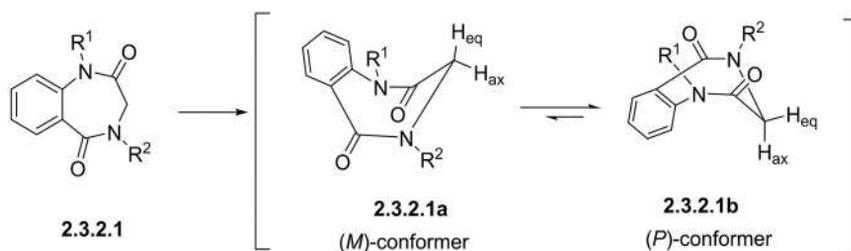
Figure 2.19 Two pseudo-axial and pseudo-equatorial conformers of 1,4-benzodiazepine-2,5-diones.

(Fig. 2.19). The NMR analysis (experimental study) and *ab initio* HF and DFT techniques (theoretical calculations) are efficient strategies for the conformational studies of these molecules, in which the structures of 1,4-benzodiazepine-2,5-diones (**2.3.2.1** and **2.3.2.2**) recommended by theoretical calculations are in agreement with the experimental findings (Jadidi et al., 2004). The energy difference between two pseudo–axial and pseudo–equatorial conformers is negligible, and the presence of sub-stituents on C3 and C4 positions has a minor effect on the energy barrier. Similar to 1,4-benzodiazepin-2-ones, the interconversion barriers between the conformational isomers of 1,4-benzodiazepine-2,5-diones are strongly dependent on the size of the N1 substituent, where the inversion barriers are 17 kcal/mol and >23 kcal/mol for N1−Me and N1−*t*-Bu substituted derivatives, respectively (Blackburn et al., 1997). The combination of an N1−*t*-Bu moiety with a chlorine atom at the C9 position provides stable atropoisomers that can be separated by column chromatography, displaying different binding affinities for the platelet glycoprotein IIb/IIIa receptor (Blackburn et al., 1997).

In an interesting variable-temperature (VT) ^{1}H NMR study of C3-benzyl 1,4-benzodiazepine-2,5-dione analog **2.3.2.2** recorded in DMSO-d_6, the proton on N1 position exhibits two split peaks attributed to its pseudo–axial and pseudo–equatorial conformations at the temperature rang-ing from 296 K (*ca*. 23 °C) to 313 K (*ca*. 40 °C) (Fig. 2.20) (Křemen et al., 2017), suggesting the slow interconversion of two conformers on the NMR time scale. However, as the temperature increases to 353 K (*ca*. 80 °C), the N1 characteristic peak appears as a singlet broad peak, which indicates that the interconversion occurs quickly at the measured temper-atures so that the NMR instrument cannot distinguish two split different peaks.
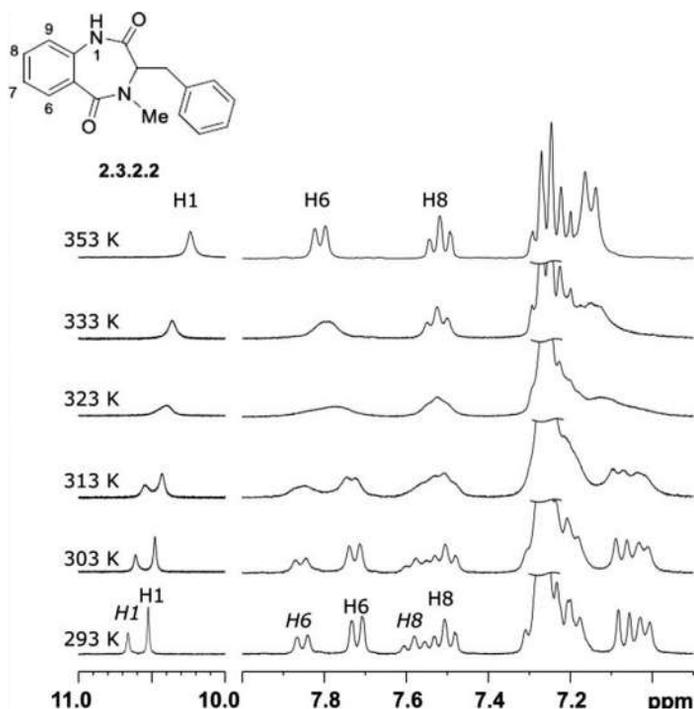
Figure 2.20 ^{1}H NMR analysis of 1,4-benzodiazepine-2,5-diones **2.3.2.2** at various temperatures. *(Reprinted with permission from Křemen, F., Gazvoda, M., Kafka, S., Proisl, K., Srholcová, A., Klásek, A., Urankar, D., & Košmrlj, J. (2017). Synthesis of 1,4-benzodiazepine-2,5-diones by base promoted ring expansion of 3-aminoquinoline-2,4-diones. The Journal of Organic Chemistry, 82(1), 715–722. https://doi.org/10.1021/acs.joc.6b01497.).*

2.4 Polarity and charge distribution of 1,4-benzodiazepines

Regarding the importance of the dipole moments in generating reactive sites in a molecule, investigation of the polarity and charge distribution of 1,4-benzodiazepines is of enormous importance. The relative charge distribution of these compounds is the fundamental element of generating potential sites (hydrophilic/lipophilic regions) for binding to the cellular receptors (Gomes et al., 2011; Loew et al., 1984). The electron affinity and the electronegativity of nitrogen atoms as well as the types and locations of substituents play a key role in determining the dipole moments of these molecules. For instance, 1,4-BDZs possessing an electronegative halogenic substituent at C7 have a higher electron density than those counterparts that

Table 2.1 Charge distributions (e⁻) of several common 1,4-BDZs, calculated by NPO at B3LYP/6–311++G(d,p) level of 1,4-BDZs gas phase.

Compound	C7	N1	C2′
Diazepam (7–Cl–1–Me–2′–H–BDZ)	–0.066	–0.491	–0.195
Flurazepam (7–Cl–1–NEt₃–2′–F–BDZ)	–0.063	–0.495	+0.464
Desalkylflurazepam (7–Cl–2′–F–BZ)	–0.069	–0.654	+0.444
Hydroxyethylflurazepam (7–Cl–1–OH–2′–F–BDZ)	–0.062	–0.496	+0.446
Nitrazepam (7–NO₂–2′–H–BDZ)	+0.045	–0.650	–0.194
Clonazepam (7–NO₂–2′–Cl–BDZ)	+0.046	–0.649	–0.026
Flunitrazepam (7–NO₂–1–Me–2′–F–BDZ)	+0.048	–0.486	+0.447
Desmethylflunitrazepam (7–NO₂–2′–F–BDZ)	+0.045	–0.649	+0.446

carry a nitro group at the same position. 1,4–Benzodiazepines with either a fluorine atom at the C2′ position or a polar substituent on N1 also exhibit an increase in the polarity. The charge distributions of several common 1,4–BDZs are presented in Table 2.1 (Gomes et al., 2011).

The N1 position, as one of the most negative regions of the 1,4–benzodiazepine structure, is more liable to acidic coordination, where its negative charge distribution is mainly due to the inductive effects of substituted sigma electronic density donors such as alkyl chains. Looking closely at the difference of the charge distributions at C7, it is clear that the electron density–withdrawing effect of a nitro group is much greater than a chlorine atom. Therefore, 1,4–BDZs containing a chlorine atom at the C7 position have a slight negative charge or are close to neutral, while those with a nitro group at the same position are slightly electropositive. The sp^2 hybridization of the nitro substitution promotes the flow of the electron density to the oxygen atoms, which results in a negative inductive effect at the C7 center. In general, the substituent on C7 has significant interactions with a lipophilic area of a cellular receptor in the vicinity of glycine and valine residues (Gomes et al., 2011). The chlorine atom is found to be relatively more lipophilic than the nitro group, owing to the π hydrophobic descriptor of 0.71 and –0.28 for the former and the latter, respectively (Meréndez & Avendaño, 2001). This phenomenon indicates that 1,4–benzodiazepines bearing a chlorine atom at the C7 position display a more favorable binding affinity to binding sites in cells. The effect of fluorine substitution on the charge distribution of C2′ position seems to be distinctive compared to hydrogen and chlorine atoms, in which the C–F bond reverses the polarity of the C2′ atom to become completely electropositive. The much higher electronegativity of the fluorine atom, its relatively large van der Waals radius of 1.47 Å, and the similarity of its orbital with the carbon atom, provide a
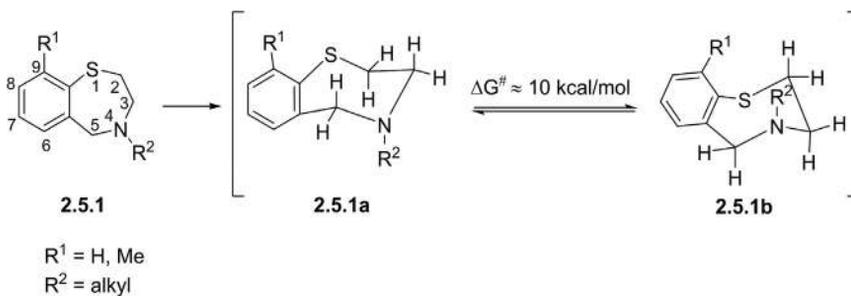
Figure 2.21 Enantiomorphic chair conformations of *N*-substituted 1,4-benzothiazepines.

super-strong C–F bond with a large dipole moment (O'Hagan, 2008). This high dipole moment combined with the electrostatic distribution of a 1,4–benzodiazepine molecule may cause the ability to establish more efficient intermolecular interactions with cellular receptors.

2.5 1,4-Benzothiazepines

1,4-Benzothiazepine is one of the possible isomers of benzo-fused thiazepines, where 2,3,4,5-tetrahydro-l,4-benzothiazepine-3,5-dione was reported as the first example of these scaffolds in 1948 (McClelland et al., 1948). Despite the pharmaceutical significance of 1,4-benzothiazepines (Cale et al., 1989; Garofalo et al., 1993; Neamati et al., 1999; Raghavendra et al., 2014; Shi et al., 2012), these bioactive molecules have received much less attention in both organic synthesis and medicinal chemistry compared to 1,4-benzodiazepines.

N-substituted 1,4-benzothiazepines **2.5.1**, as the most common types of this family, exist in two enantiomorphic chair conformations with interconversion barriers of approximately $\Delta G^{\#} = 10$ kcal/mol in CD_2Cl_2, estimated by the Eyring equation (Fig. 2.21) (Katritzky et al., 2002). The ^1H–^1H COSY and NOESY analyses at low temperature (-94 °C) allow the assignment of all proton signals of **2.5.1** conformers as well as both vicinal and geminal couplings of the hydrogens at positions 2 to 5. For example, the axial hydrogens at positions 2 and 3 can be identified by two large couplings of approximately 10-15 Hz, attributed to one geminal and one axial-axial. The most deshielded methylene protons (CH_2) in **2.5.1** are allocated to C5 in the thiazepine ring, appearing at $\delta = 4$-4.5 ppm based on the R^1 and R^2 substitutions. The ^1H NMR spectra of **2.5.1** at this temperature generally display sharp peaks, which get broadened as the temperature increases to

25 °C. This phenomenon can be explained by the fact that only one conformer exists in a noticeable concentration at a very low temperature (-94 °C). However, the second conformer is formed at higher temperatures (between -94 °C and 25 °C) and it is in equilibrium with the first counterpart with the low inversion barrier (Katritzky et al., 2002). The ^{13}C NMR spectra of **2.5.1** are identical in CD_2Cl_2 at temperatures ranging from -94 °C to 25 °C, indicating that there is the only chair-to-chair interconversion between mirror-image conformers.

2.6 Conclusion

This chapter represents the current knowledge relating to the chemical structures of 1,4-benzodiazepines and 1,4-benzothiazepines to allow better insight into the design of these drug candidates. The different classes of 1,4-benzodiazepinone, fused 1,4-benzodiazepine, or 1,4-benzothiazepine ring systems have displayed their prowess in drug marketing over the last decades. These privileged structures have been either synthetically prepared or can be isolated from natural resources. Although two classes of FDA-approved 1,4-BDZs and designer 1,4-BDZs have been studied extensively, 1,4-benzothiazepines have received much less attention in chemical research and drug discovery. It has been shown that the pharmaceutical performance and conformational behavior of 1,4-benzodiazepine derivatives are highly dependent on the type of substitutions at positions N1, C3, and C7. As is typical of all areas of research, further investigations towards the chemistry of 1,4-benzodiazepines and 1,4-benzothiazepines for gaining a greater understanding of their structure-activity relationship will be undeniably required.

References

Alanis, B. A. V., Iorio, M. T., Silva, L. L., Bampali, K., Ernst, M., Schnürch, M., & Mihovilovic, M. D. (2020). Allosteric GABA$_A$ receptor modulators—A review on the most recent heterocyclic chemotypes and their synthetic accessibility. *Molecules, 25*(4), 999–1046. https://doi.org/10.3390/molecules25040999.

Archer, G. A., & Sternbach, L. H. (1968). Chemistry of benzodiazepines. *Chemical Reviews,, 68*(6), 747–784. https://doi.org/10.1021/cr60256a004.

Aso, Y., Yoshioka, S., Shibazaki, T., & Uchiyama, M. (1988). The kinetics of the racemization of oxazepam in aqueous solution. *Chemical and Pharmaceutical Bulletin, 36*(5), 1834–1840.

Avdagić, A., Lesac, A., & Šunjić, V. (1999). First example of the solvent effect on absolute conformation of chiral 3,3-disubstituted 1,4-benzodiazepin-2-ones. *Tetrahedron, 55*(5), 1407–1416. https://doi.org/10.1016/S0040-4020(98)01113-2.

Ayati, A., Emami, S., & Foroumadi, A. (2016). The importance of triazole scaffold in the development of anticonvulsant agents. *European Journal of Medicinal Chemistry, 109*, 380–392. https://doi.org/10.1016/j.ejmech.2016.01.009.

Batlle, E., Lizano, E., Viñas, M., & Pujol, M. D. (2019). 1,4-Benzodiazepines and new derivatives: Description, analysis, and organic synthesis. In J. Vašková, & L. Vaško (Eds.), *Medicinal Chemistry* (pp. 63–90). London: IntechOpen Limited. https://doi.org/10.5772/intechopen.79879.

Blackburn, B. K., Lee, A., Baier, M., Kohl, B., Olivero, R., Matamoros, R., & McDowell, R. S. (1997). From peptide to non-peptide. 3. Atropisomeric GPIIbIIIa antagonists containing the 3,4-dihydro-1*H*-1,4-benzodiazepine-2,5-dione nucleus. *Journal of Medicinal Chemistry, 40*(5), 717–729. https://doi.org/10.1021/jm960652r.

Boojamra, C. G., Burow, K. M., Thompson, L. A., & Ellman, J. A. (1997). Solid-phase synthesis of 1,4-benzodiazepine-2,5-diones. Library preparation and demonstration of synthesis generality. *The Journal of Organic Chemistry, 62*(5), 1240–1256. https://doi.org/10.1021/jo9622845.

Borea, P. A. (1981). Structure - activity relationships in 1,4-benzodiazepines. *Bollettino Della Societa Italiana Di Biologia Sperimentale, 57*(6), 628–632.

Bourcier, S., Hoppilliard, Y., Kargar-Grisel, T., Pechiné, J. M., & Perez, F. (2001). Do thermally-labile 1,4-benzodiazepines rearrange under electrospray and particle bombardment? *European Journal of Mass Spectrometry, 7*(4–5), 359–371. https://doi.org/10.1255/ejms.446.

Cale, A. D., Gero, T. W., Walker, K. R., Lo, Y. S., Welstead, W. J., Jaques, L. W., & Johnson, D. N. (1989). Benzo- and pyrido-1,4-oxazepin-5-ones and –thiones: Synthesis and structure–activity relationships of a new series of H$_1$ antihistamines. *Journal of Medicinal Chemistry, 32*(9), 2178–2199. https://doi.org/10.1021/jm00129a026.

Carlier, P. R., Zhao, H., MacQuarrie-Hunter, S. L., DeGuzman, J. C., & Hsu, D. C. (2006). Enantioselective synthesis of diversely substituted quaternary 1,4-benzodiazepin–2-ones and 1,4-benzodiazepine-2,5-diones. *Journal of the American Chemical Society, 128*(47), 15215–15220. https://doi.org/10.1021/ja0640142.

Chkirate, K., Kansiz, S., Karrouchi, K., Mague, J. T., Dege, N., & Essassi, E. M. (2019). Crystal structure and Hirshfeld surface analysis of a new benzodiazepine derivative: 4-Dichloromethyl-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one. *Acta Crystallographica Section E: Crystallographic Communications, E75*, 33–37. https://doi.org/10.1107/S205698901801681X.

Cortes, E., Ramírez, E. P., & de Cortés, O. G. M. (2007). Synthesis and spectral determination of new derivatives of 1-[(*p*-substituted) phenyl]-3*a*-[(*o*-and *p*-substituted) phenyl]-5-chloro-9-methylthio-10,3*a*-dihydro-[1,2,4]-oxadiazolo [2,3-*b*][1,4] benzodiazepines. *Journal of Heterocyclic Chemistry, 44*(1), 189–192. https://doi.org/10.1002/jhet.5570440129.

Cummings, M. D., Schubert, C., Parks, D. J., Calvo, R. R., LaFrance, L. V., Lattanze, J., & Lu, T. (2006). Substituted 1,4-benzodiazepine-2,5-diones as α–helix mimetic antagonists of the HDM2-p53 protein-protein interaction. *Chemical Biology and Drug Design, 67*, 201–205. https://doi.org/10.1111/j.1747-0285.2006.00365.x.

De Sarro, G., Chimirri, A., Zappala, M., Guisti, P., Lipartiti, M., & De Sarro, A. (1996). Azirino [1,2-*d*][1,4] benzodiazepine derivatives and related 1,4–benzodiazepines as anticonvulsant agents in DBA/2 mice. *General Pharmacology: The Vascular System, 27*(7), 1155–1162. https://doi.org/10.1016/S0306-3623(96)00049-3.

Donald, J. R., & Martin, S. F. (2011). Synthesis and diversification of 1,2,3-triazole-fused 1,4-benzodiazepine scaffolds. *Organic Letters, 13*(5), 852–855. https://doi.org/10.1021/ol1028404.

Fan, X.-Z., Wu, H.-H., Tang, Z., Zhang, H., Cai, L.-Y., Bi, X.-F., & Zhao, H.-W. (2021). Formal [3 + 2] cycloaddition between *in situ* formed 1,4-benzodiazepin-2-one-based azomethine ylides and azodicarboxylic acid derivatives: Diastereoselective synthesis of spiro-1,4-benzodiazepin-2-ones. *Advanced Synthesis and Catalysis, 363*(5), 1379–1389. https://doi.org/10.1002/adsc.202001486.

Fernandes, H., & Moreira, R. (2014). Mexazolam: Clinical efficacy and tolerability in the treatment of anxiety. *Neurology and Therapy, 3*, 1–14. https://doi.org/10.1007/s40120-014-0016-7.

Fustero, S., González, J., & Del Pozo, C. (2006). 1,4-Benzodiazepine *N*-nitrosoamidines: Useful intermediates in the synthesis of tricyclic benzodiazepines. *Molecules (Basel, Switzerland), 11*(8), 583–588. https://doi.org/10.3390/11080583.

Gall, M., Kamdar, B. V., Lipton, M. F., Chidester, C. G., & Duchamp, D. J. (1988). Mannich reactions of heterocycles with dimethyl (methylene) ammonium chloride: A high yield, one-step conversion of estazolam to adinazolam. *Journal of Heterocyclic Chemistry, 25*(6), 1649–1661. https://doi.org/10.1002/jhet.5570250610.

Garofalo, A., Balconi, G., Botta, M., Corelli, F., & Nacci, V. (1993). Thioanalogues of anti-tumor antibiotics. II. Synthesis and preliminary *in vitro* cytotoxicity evaluation of tricyclic [1,4]benzothiazepine derivatives. *European Journal of Medicinal Chemistry, 28*(3), 213–220. https://doi.org/10.1016/0223-5234(93)90136-3.

Gilman, N. W., Rosen, P., Earley, J. V., Cook, C., & Todaro, L. J. (1990). Atropisomers of 1,4-benzodiazepines. Synthesis and resolution of a diazepam-related 1,4-benzodiazepine. *Journal of the American Chemical Society, 112*(10), 3969–3978. https://doi.org/10.1021/ja00166a038.

Gomes, L. R., Santos, L. M. N. B. F., Beleza, J., & Low, J. N. (2011). Structural and electronic effects of the C2′ substituent in 1,4-benzodiazepines. *European Journal of Chemistry, 2*(1), 1–7. https://doi.org/10.5155/eurjchem.2.1.1-7.322.

Greenblatt, H. K., & Greenblatt, D. J. (2019). Designer benzodiazepines: A review of published data and public health significance. *Clinical Pharmacology in Drug Development, 8*(3), 266–269. https://doi.org/10.1002/cpdd.667.

Hadjipavlou-Litina, D., & Hansch, C. (1994). Quantitative structure-activity relationships of the benzodiazepines. A review and reevaluation. *Chemical Reviews, 94*(6), 1483–1505. https://doi.org/10.1021/cr00030a002.

Hartley, J. A. (2011). The development of pyrrolobenzodiazepines as antitumour agents. *Expert Opinion on Investigational Drugs, 20*(6), 733–744. https://doi.org/10.1517/13543784.2011.573477.

Hester, J. B., Jr, Duchamp, D. J., & Chidester, C. G (1971). A synthetic approach to new 1,4-benzodiazepine derivatives. *Tetrahedron Letters, 12*(20), 1609–1612. https://doi.org/10.1016/S0040-4039(01)87414-1.

Ho, C. Y. (1985). 6H-indolo [2,1-c][1,4] benzodiazepines and 12-oxo derivatives useful as antihypertensives. US Patent, 4529724A.

Ho, C. Y., Hageman, W. E., & Persico, F. J. (1986). Indolo[2,1-*c*][1,4]benzodiazepines: A new class of antiallergic agents. *Journal of Medicinal Chemistry, 29*(6), 1118–1121. https://doi.org/10.1021/jm00156a034.

Hok, L., BoŽičević, L., Sremec, H., Šakić, D., & Vrček, V. (2019). Racemization of oxazepam and chiral 1,4-benzodiazepines. DFT study of the reaction mechanism in aqueous solution. *Organic and Biomolecular Chemistry, 17*(6), 1471–1479. https://doi.org/10.1039/c8ob02991a.

Jadidi, K., Aryan, R., Mehrdad, M., Lügger, T., Hahn, F. E., & Ng, S. W. (2004). Simple synthesis, structure and *ab initio* study of 1,4-benzodiazepine-2,5-diones. *Journal of Molecular Structure, 692*(1–3), 37–42. https://doi.org/10.1016/j.molstruc.2003.12.024.

Joshi, B. K., Gloer, J. B., Wicklow, D. T., & Dowd, P. F. (1999). Sclerotigenin: A new antiinsectan benzodiazepine from the sclerotia of *Penicillium sclerotigenum*. *Journal of Natural Products, 62*(4), 650–652. https://doi.org/10.1021/np980511n.

Joshi, S. D., More, U. A., Kulkarni, V. H., & Aminabhavi, T. M. (2013). Pyrrole: Chemical synthesis, microwave assisted synthesis, reactions and applications: A review. *Current Organic Chemistry, 17*(20), 2279–2304. https://doi.org/10.2174/13852728113179990040.

Katritzky, A. R., Akhmedov, N. G., Ghiviriga, I., & Steel, P. J. (2002). ^{1}H and ^{13}C NMR study of tetrahydro-1,4-benzothiazepine conformations. *Journal of the Chemical Society, Perkin Transactions, 2*(11), 1816–1822. https://doi.org/10.1039/B205768A.

Khan, R., Felix, R., Kemmitt, P. D., Coles, S. J., Tizzard, G. J., & Spencer, J. (2018). N1-Arylation of 1,4-benzodiazepine-2-ones with diaryliodonium salts. *Synlett, 29*(2), 193–198. https://doi.org/10.1055/s-0036-1590920.

Korman, S., & Tendler, M. D. (1965). Clinical investigations of cancer chemotherapeutic agents for neoplastic disease. *The Journal of New Drugs, 5*(5), 275–285. https://doi.org/10.1002/j.1552-4604.1965.tb00247.x.

Křemen, F., Gazvoda, M., Kafka, F., Proisl, K., Srholcová, A., Klásek, A., & Košmrlj, J. (2017). Synthesis of 1,4-benzodiazepine-2,5-diones by base promoted ring expansion of 3-aminoquinoline-2,4-diones. *The Journal of Organic Chemistry, 82*(1), 715–722. https://doi.org/10.1021/acs.joc.6b01497.

Leimgruber, W., Stefanovic, V., & Shenker, F. (1965). Isolation and characterization of anthramycin, a new antitumour antibiotic. *Journal of the American Chemical Society, 87*(24), 5791–5793. https://doi.org/10.1021/ja00952a050.

Loew, G. H., Nienow, J. R., & Poulsen, M. (1984). Theoretical structure-activity studies of benzodiazepine analogues. Requirements for receptor affinity and activity. *Molecular Pharmacology, 26*(1), 19–34.

McClelland, E. W., Rose, M. J., & Stammers, D. W. (1948). The formation of thionaphthens from derivatives of phenylthioacetic acid. *Journal of the Chemical Society*, 81–85. https://doi.org/10.1039/JR9480000081.

Meanwell, N. A., & Walker, M. A. (2008). 1,4-Diazepines. In A. R. Katritzky, C. A. Ramsden, & R. J. K. Taylor (Eds.), *Comprehensive Heterocyclic Chemistry 13*, (pp. 183–235). Amsterdam: Elsevier.

Meguro, K., & Kuwada, Y. (1970). Syntheses and structures of 7-chloro-2-hydrazino-5-phenyl-3*H*-1,4-benzodiazepines and some isomeric 1,4,5-benzotriazocines. *Tetrahedron Letters, 11*(47), 4039–4042. https://doi.org/10.1016/S0040-4039(01)98659-9.

Menezes, C. M. S., Rivera, G., Alves, M. A., do Amaral, D. N., Thibaut, J. P. B., Noël, F., & Lima, L. M. (2012). Synthesis, biological evaluation, and structure–activity relationship of clonazepam, meclonazepam, and 1,4-benzodiazepine compounds with schistosomicidal activity. *Chemical Biology and Drug Design, 79*(6), 943–949. https://doi.org/10.1111/j.1747-0285.2012.01354.x.

Meréndez, J. C., & Avendaño, C. (2001). Optimizacion de un protótipo. *Correlationes cualitativas estrutura química − actividad biológica. Introduccion a la química Farmaceutica* (2nd ed., pp. 87–88). New York: McGraw Hill.

Meyer, A. G., Bissember, A. C., Hyland, C., Smith, J. A., Williams, C. C., Zamani, F., & Abel, S. A. G. (2017). Seven-membered rings. In G. W. Gribble, & J. A. Joule (Eds.), *Progress in Heterocyclic Chemistry* (29, pp. 579–663). Amsterdam: Elsevier. https://doi.org/10.1016/B978-0-08-102310-5.00016-3.

Moffett, R. B., Evenson, G. N., & Voigtlander, P. F. V. (1977). Central nervous system depressants. 14. *As*-triazino-1,4-benzodiazepines. *Journal of Heterocyclic Chemistry, 14*(7), 1231–1244. https://doi.org/10.1002/jhet.5570140720.

Molteni, G., Broggini, G., & Pilati, T. (2002). Diastereoselective nitrilimine cycloaddition to the C−N bond of enantiopure 1,4-benzodiazepinones. *Tetrahedron: Asymmetry, 13*(22), 2491–2495. https://doi.org/10.1016/S0957-4166(02)00663-8.

Mooibroek, T. J., & Gamez, P. (2007). The s-triazine ring, a remarkable unit to generate supramolecular interactions. *Inorganica Chimica Acta, 360*(1), 381–404. https://doi.org/10.1016/j.ica.2006.07.061.

Moosmann, B., & Auwärter, V. (2018). Designer benzodiazepines: Another class of new psychoactive substances, *New Psycoactive Substances* (252, pp. 383–410). Switzerland: Springer. https://doi.org/10.1007/164_2018_154.

Narayana, B., Raj, K. K. V., Ashalatha, B. V., & Kumari, N. S. (2006). Synthesis of some new substituted triazolo [4,3-*a*][1,4] benzodiazepine derivatives as potent anticonvulsants. *European Journal of Medicinal Chemistry, 41*(3), 417–422. https://doi.org/10.1016/j.ejmech.2005.12.003.

Neamati, N., Turpin, J. A., Winslow, H. E., Christensen, J. L., Williamson, K., Orr, A., & Nacci, V. (1999). Thiazolothiazepine inhibitors of HIV-1 integrase. *Journal of Medicinal Chemistry, 42*(17), 3334–3341. https://doi.org/10.1021/jm990047z.

O'Hagan, D. (2008). Understanding organofluorine chemistry. An introduction to the C−F bond. *Chemical Society Reviews, 37*(2), 308–319. https://doi.org/10.1039/b711844a.

Paizs, B., & Simonyi, M. (1999). Ring inversion barrier of diazepam and derivatives: An *ab Initio* study. *Chirality, 11*(8), 651–658. https://doi.org/10.1002/(SICI)1520-636X(1999)11:8≤651::AID-CHIR7≥3.0.CO;2-5.

Pem, B., & Vrček, V. (2017). Substituent effects on the stability of 1,4-benzodiazepin-2-one tautomers. A density functional study. *International Journal of Quantum Chemistry, 118*(8), e25523. https://doi.org/10.1002/qua.25523.

Pertejo, P., García-Valverde, M., Peña, P., Cordero, N. A., Torroba, T., & González-Ortega, A. (2014). Experimental and theoretical studies on the effect of the oxo group in 1,4-benzodiazepines. *Organic and Biomolecular Chemistry, 12*(27), 4905–4916. https://doi.org/10.1039/C4OB00444B.

Popovic, G. V., Sladic, D. M., Stefanovic, V. M., & Pfendt, L. B. (2003). Study on protolytic equilibria of lorazepam and oxazepam by UV and NMR spectroscopy. *Journal of Pharmaceutical and Biomedical Analysis, 31*(4), 693–699. https://doi.org/10.1016/S0731-7085(02)00686-6.

Raghavendra, K., Kumar, K. A., & Shashikanth, S. (2014). Synthesis of novel 1,4-benzothiazepines and *in vitro* screening of their antimicrobial activity. *International Journal of Pharmacy and Pharmaceutical Sciences, 6*(5), 90–93.

Rickels, K., Csanalosi, I., Greisman, P., Mirman, M. J., Morris, R. J., Weise, C. C., & Weiss, G. (1980). Ketazolam and diazepam in anxiety: A controlled study. *Journal of Clinical Pharmacology, 20*(10), 581–589. https://doi.org/10.1002/j.1552-4604.1980.tb01673.x.

Riddel, G. (1980). *The conformational analysis of heterocyclic compounds*. Cambridge, Massachusetts: Academic Press.

Ripka, W. C., De Lucca, G. V., Bach, A. C., Pottorf, R. S., & Blaney, J. M. (1993). Protein β-turn mimetics II: Design, synthesis, and evaluation in the cyclic peptide gramicidin S. *Tetrahedron, 49*(17), 3609–3628. https://doi.org/10.1016/S0040-4020(01)90218-2.

Saari, T. I., Uusi-Oukari, M., Ahonen, J., & Olkkola, K. T. (2011). Enhancement of GABAergic activity: Neuropharmacological effects of benzodiazepines and therapeutic use in anesthesiology. *Pharmacological Reviews, 63*(1), 243–267. https://doi.org/10.1124/pr.110.002717.

Saha, D., Jain, G., & Sharma, A. (2015). Benzothiazepines: Chemistry of a privileged scaffold. *RSC Advances,, 5*(86), 70619–70639. https://doi.org/10.1039/C5RA12422K.

Salvadori, P., Bertucci, C., Ascoli, G., Uccello-Barretta, G., & Rossi, E. (1997). Direct resolution, characterization, and stereospecific binding properties of an atropisomeric 1,4-benzodiazepine. *Chirality, 9*(5–6), 495–505.

Sharp, J. T. (1984). Seven-membered rings with two or more heteroatoms. *Comprehensive Heterocyclic Chemistry* (7, pp. 593–651). Amsterdam: Elsevier. https://doi.org/10.1016/B978-008096519-2.00124-7.

Shi, F., Zeng, X.-N., Cao, X.-D., Zhang, S., Jiang, B., Zheng, W.-F., & Tu, S.-J. (2012). Design and diversity-oriented synthesis of novel 1,4-thiazepan-3-ones fused with bioactive heterocyclic skeletons and evaluation of their antioxidant and cytotoxic activities. *Bioorganic and Medicinal Chemistry Letters, 22*(1), 743–746. https://doi.org/10.1016/j.bmcl.2011.09.081.

Spencer, J., Rathnam, R. P., & Chowdhry, B. Z. (2010). 1,4-Benzodiazepin-2-ones in medicinal chemistry. *Future Medicinal Chemistry, 2*(9), 1441–1449. https://doi.org/10.4155/fmc.10.226.

Sternbach, L. H. (1971). 1,4-Benzodiazepines. Chemistry and some aspects of the structure–activity relationship. *Angewandte Chemie International Edition, 10*(1), 34–43. https://doi.org/10.1002/anie.197100341.

Sternbach, L. H (1980). The benzodiazepine story. In R. G. Priest, U. V. Filho, R. Amrein, & M. Skreta (Eds.), *Benzodiazepines*. Dordrecht: Springer https://doi.org/10.1007/978-94-011-7238-7_3.

Sudhapriya, N., Nandakumar, A., Arun, Y., Perumal, P. T., Balachandran, C., & Emi, N. (2015). An expedient route to highly diversified [1,2,3]triazolo[1,5-*a*][1,4]benzodiazepines and their evaluation for antimicrobial, antiproliferative and in silico studies. *RSC Advances, 5*(81), 66260–66270. https://doi.org/10.1039/c5ra12497b.

Sun, H. H., Barrow, C. J., Sedlock, D. M., Gillum, A. M., & Cooper, R. (1994). Benzomalvins, new substance P inhibitors from a *Penicillium sp. The Journal of Antibiotics, 47*(5), 515–522. https://doi.org/10.7164/antibiotics.47.515.

Tseng, M.-C., Yang, H.-Y., & Chu, Y.-H. (2010). Total synthesis of asperlicin C, circumdatin F, demethylbenzomalvin A, demethoxycircumdatin H, sclerotigenin, and other fused quinazolinones. *Organic and Biomolecular Chemistry, 8*(2), 419–427. https://doi.org/10.1039/B910545J.

Varvounis, G. (2016). An update on the synthesis of pyrrolo [1,4] benzodiazepines. *Molecules (Basel, Switzerland), 21*(2), 154–209. https://doi.org/10.3390/molecules21020154.

Yang, S. K. (1995). Substitution and racemization of 3-hydroxy- and 3-alkoxy-1,4-benzodiazepines in acidic aqueous solutions. *Chirality, 7*(5), 365–375. https://doi.org/10.1002/chir.530070510.

Zhao, H., Hsu, D. C., & Carlier, P. R. (2005). Memory of chirality: an emerging strategy for asymmetric synthesis. *Synthesis, 1*, 1–16. https://doi.org/10.1055/s-2004-834931.

Non-Print Items

Abstract

This chapter discusses the current knowledge of the chemical structures, conformational and electronic features of different types of 1,4–benzodiazepines and 1,4–benzothiazepines.

Synthesis of 1,4-benzodiazepines and 1,4-benzothiazepines

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Chapter outline
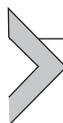
3.1 Introduction

Synthesis of pharmaceutically and biologically active 1,4–benzodiazepine and 1,4–benzothiazepine derivatives has always been of great interest to chemists due to their diverse therapeutic properties, such as anti–tuberculosis, anti–psychotics, anti–HIV, anti–cancer, anti–microbial, anxiolytic, muscle relaxant, anticonvulsant, hypnotic, and anti–in–ammatory activities (Bagal et al., 2013; Hsu et al., 1991; Kaneko, 1994; Krsiak & Sulcova, 1990; Nakamura et al., 2001; Sulcova & Krsiak, 1989; Ye et al., 2013). The medicinal importance of 1,4–benzodiazepines and 1,4–benzothiazepines has led to numerous synthetic studies on related novel molecules expecting to discover agents that would be more potent for the clinical treatment (Meyer et al., 2020, Meyer et al., 2021). Although there is a multitude of methodologies for the synthesis of these privileged compounds, there are four most commonly employed methods, which include: (A) cyclocondensation reactions of 2–halobenzoic acids or 2–halobenzophenones and their analogs with diamines, (B) cyclization

(A)

3.1.1

X = halogen
R¹ = alkyl, aryl, Cl, OH
R² = R³ = alkyl, aryl

3.1.2

3.1.3

(B)

3.1.4

3.1.5

R¹ = alkyl, aryl, Cl, OH
R² = alkyl, aryl
R³ = protecting group
R⁴ = H, OH, OR, R

3.1.6

(C)

3.1.7

X = SH, NH₂
R¹ = R² = R³ = alkyl, aryl
Z = leaving group

3.1.8

3.1.9

Y = S, NH

(D)

3.1.10

X = SH, NH₂

3.1.11

3.1.12
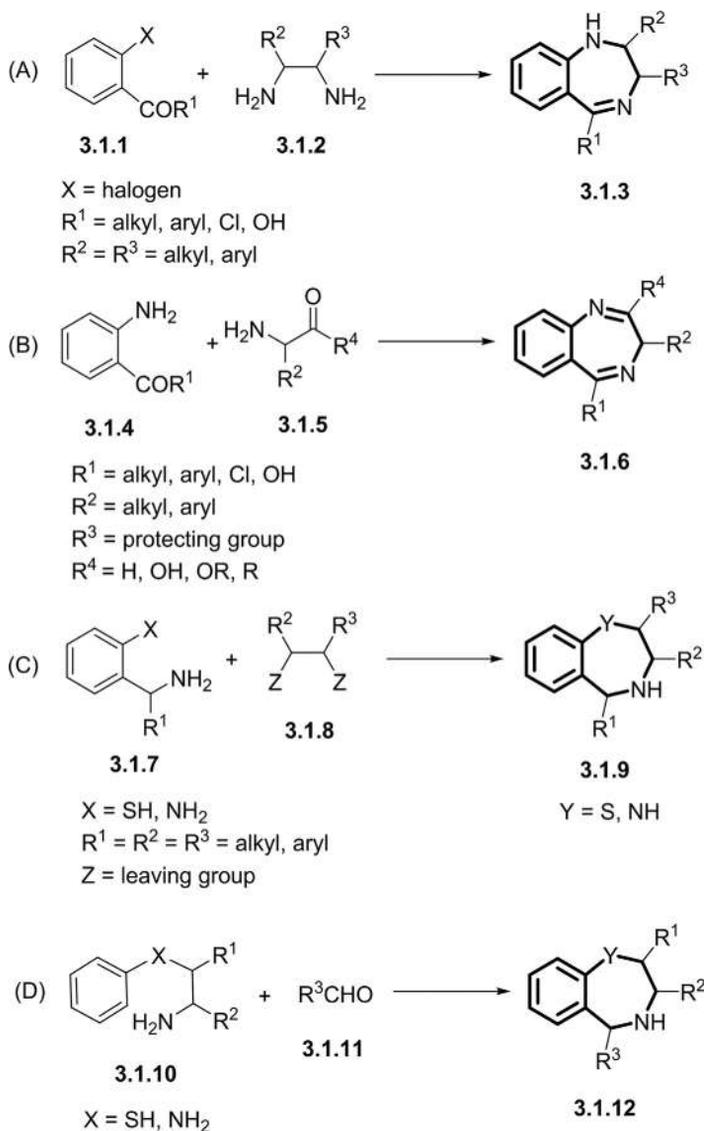
Figure 3.1 General synthetic approaches to 1,4-benzodiazepines and 1,4-benzothiazepines.

reactions of 2-aminobenzoic acids or 2-aminobenzophenones and their derivatives with α-amino carbonyl compounds, (C) the reaction of diamines or aminothiols with bis–electrophiles, (D) Pictet–Spengler-type reaction of an amine with aldehydes (Fig. 3.1) (Al-Awar et al., 2004; Herrero et al., 2003; Katritzky et al., 2002; Kukla et al., 1991; Nadin et al., 2003;
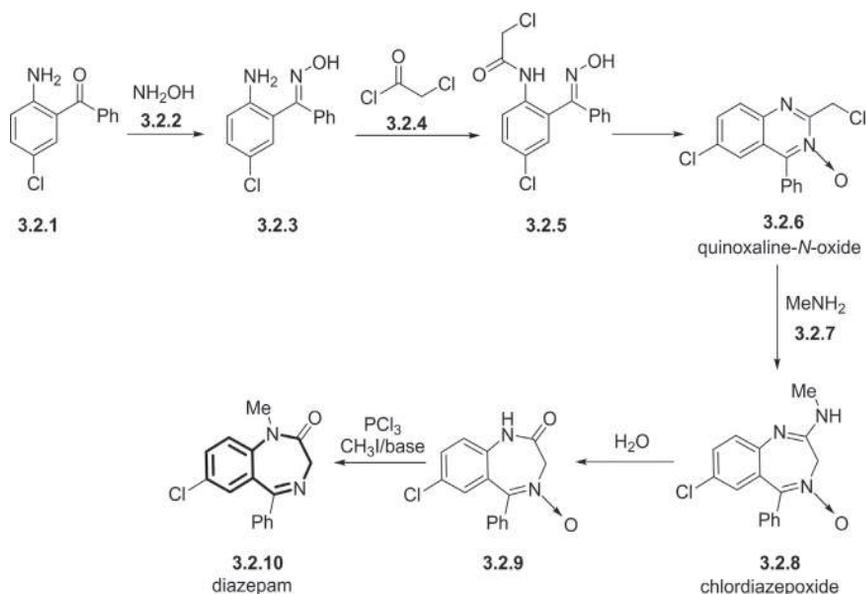
Figure 3.2 Synthesis of chlordiazepoxide and diazepam.

Yar et al., 2009). These protocols often suffer from drawbacks such as low yields, harsh reaction conditions, tedious work–up procedures, and the formation of by–products. Therefore, there is a continuous need to develop more efficient protocols, especially catalytic procedures, for the synthesis of these versatile entities. The main objective of this chapter is to provide an overview of the recent practical advances in synthesis of 1,4-benzodiazepines and 1,4-benzothiazepines using available reactants under mild reaction conditions.

3.2 Synthesis of 1,4-benzodiazepines

The medicinally important 1,4–benzodiazepines, *i.e.*, chlordiazepoxide (**3.2.8**) and diazepam (**3.2.10**), were first synthesized *via* a simple synthetic approach in the 1950s (Fig. 3.2) (Sternbach, 1979). Initially, hydroxylamine **3.2.2** undergoes a nucleophilic addition with 2-aminobenzophenone **3.2.1** to form oxime **3.2.3**, which then reacts with chloroacetyl chloride **3.2.4** to afford the chloroacetamide intermediate **3.2.5**. Intramolecular ring closure reaction of the intermediate **3.2.5** occurs to give quinoxoline-*N*-oxide **3.2.6**. Chlordiazepoxide **3.2.8** is accordingly obtained through the reaction of **3.2.6** with methylamine **3.2.7**. Two successive hydrolysis and reduction
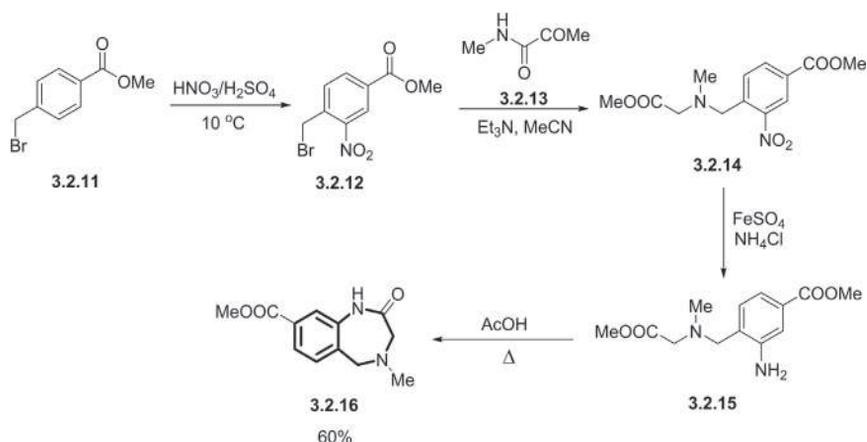
Figure 3.3 Synthesis of carboxylate-containing 1,4-benzodiazepin-2-one.

reactions of **3.2.8** finally yield diazepam (**3.2.10**) (Sternbach & Reeder, 1961).

1,4–Benzodiazepinones containing a carbonyl group on diazepine ring have been demonstrated to be as fundamental cores in most prescribed anxiolytics (Arora et al., 2020; Dinis–Oliveira, 2017; Gill et al., 2014; Taher & Mohammed, 2013). A four–step approach for the synthesis of benzodiazepin–2-one **3.2.16** from readily available starting materials is described in Fig. 3.3. In the first step, methyl 4-bromomethyl benzoate **3.2.11** undergoes the nitration reaction using HNO_3/H_2SO_4 to furnish methyl 4-bromo-3-nitrobenzoate **3.2.12**. This is followed by an S_N2 nucleophilic substitution reaction of **3.2.12** with sarcosine methyl ester **3.2.13**, producing the compound **3.2.14**. The reduction reaction of **3.2.14** in the presence of $FeSO_4$ leads to the formation of the amine **3.2.15**. Two successive reduction/cyclization reactions finally generate the desired product **3.2.16** (Voskressensky et al., 2012).

A catalyst–free approach for the synthesis of various 5-aryl-1,4-benzodiazepin-2-ones **3.2.19** is described (Ghelani & Naliapara, 2016). The condensation reaction of 2-amino benzophenone **3.2.17** with chloroacetyl chloride **3.2.4** first provides the intermediate **3.2.18** (Fig. 3.4), which subsequently undergoes an intramolecular cyclization reaction using a mixture of hexamine and ammonium acetate in EtOH to form the final compound **3.2.19** in good to excellent yields.

In another related approach, 5-aryl-1,4-benzodiazepin-2-ones have been also synthesized *via* three simple steps from readily accessible 2-amino-dibenzophenones **3.2.20** (Fig. 3.5) (Sandra et al., 2012). The intermediate
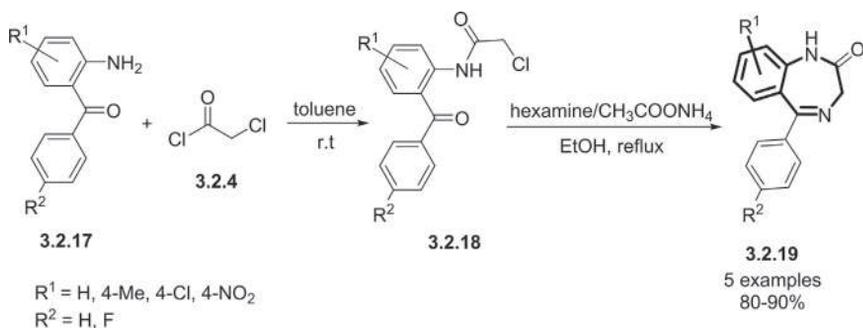
Figure 3.4 Catalyst-free synthesis of various 5-aryl-1,4-benzodiazepin-2-ones.
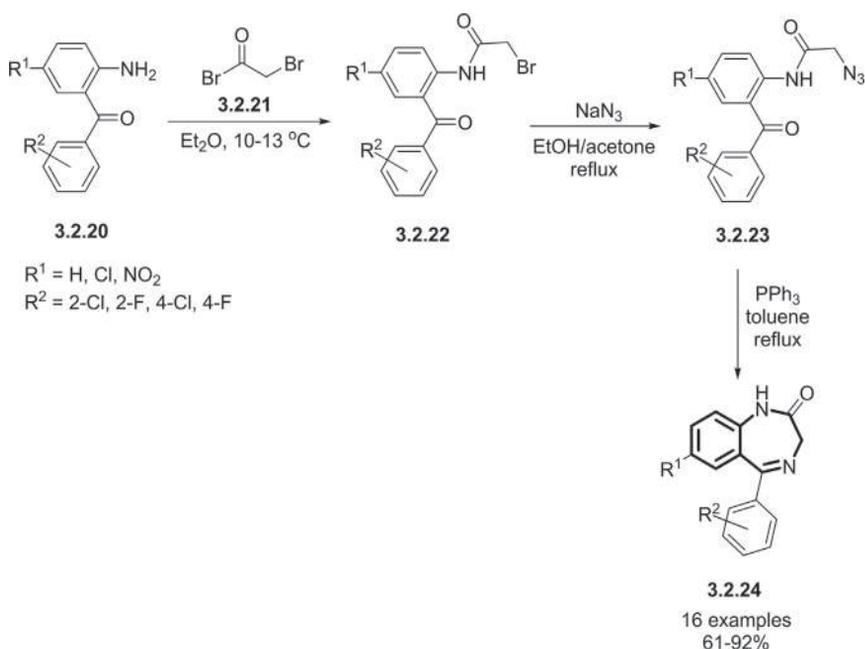
Figure 3.5 Three-step synthesis of 5-aryl-1,4-benzodiazepin-2-ones.

3.2.22 can be obtained through the reaction of 2–amino–dibenzophenones **3.2.20** with bromoacetyl bromide **3.2.21**, followed by the formation of azide **3.2.23** using sodium azide. Finally, an intramolecular aza–Wittig reaction provides the desired 1,4–benzodiazepin–2–ones **3.2.24**.

The regulation of GABA-receptor–related ion channels by benzodi-azepines to provoke anxiolytic effects in the brain significantly depends on both the substitution nature and absolute configuration (Andronati et al., 2010; Farges, 2003). It is also proven that enantiopure benzodiazepines are
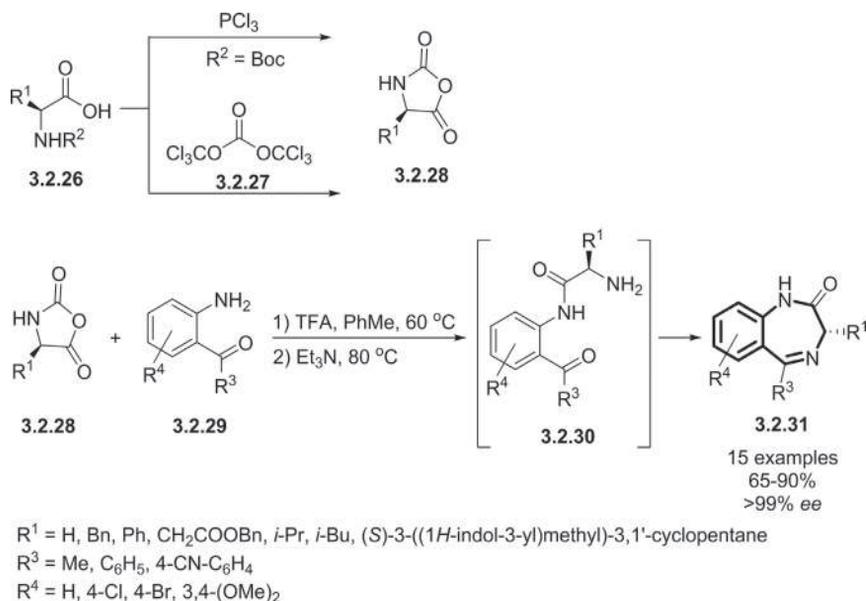
Figure 3.6 One-pot synthesis of enantiopure 5-substituted 1,4-benzodiazepin-2-ones.

needed for the design of pharmacophore/receptor prototypes for BzR subtypes of the GABA receptors (Li et al., 2003). Despite the broad range of 1,4-benzodiazepines' synthetic routes known so far, examples of reactions for the preparing enantiopure 1,4-benzodiazepines containing a quaternary stereogenic center are extremely rare. For example, α-amino acids are commercially inexpensive starting materials that can be used to provide the configuration at the C3 position of 1,4-benzodiazepines. However, these enantiomerically pure skeletons remain largely unexplored due to their easy racemization (Zamani et al., 2017). Therefore, developing robust synthetic methods towards highly enantioenriched benzodiazepines is important. A facile one-step synthesis of several enantiopure 5-substituted 1,4-benzodiazepin-2-ones from readily accessible α-amino acids **3.2.26** and *N*-carboxyanhydrides (NCAs) **3.2.28** has been reported (Fier & Whittaker, 2017). Various NCAs (Leuchs' anhydrides) are first prepared through the cyclization reaction of α-amino acids **3.2.26** using PCl$_3$ or triphosgene **3.2.27** (Fig. 3.6) to form anhydrides **3.2.28**. This is followed by an acid-catalyzed ring-opening reaction of **3.2.28** with *ortho*-ketoanilines **3.2.29** to generate the intermediate **3.2.30**, followed by the dehydration-cyclization sequence to give the corresponding benzodiazepinones **3.2.31**. In this synthetic method, no racemization of the α-amino

acids has been observed, and the products were all synthesized in excellent *ee* of >99%.

Diastereoselective synthesis of various highly enantioenriched 2,3,4-trisubstituted 1,4-benzodiazepin-5-ones (**3.2.39, 3.2.40**) with the formation of a new stereocenter at the C3 position has also been reported (Pertejo et al., 2015). In this stereoselective methodology, commercially available chiral (*S*)-α-methyl benzylamine **3.2.34** is employed in two Ugi reaction pathways by varying one of the components (out of four components) to produce the chiral azide **3.2.37** and the nitro **3.2.38** intermediates (Fig. 3.7). The generated nitro and azide Ugi intermediates then undergo a cyclization reaction *via* a Staudinger/aza–Wittig sequence or a reductive cyclization reaction (with SnCl$_2$/HCl reagent), respectively, to give the corresponding 1,4-benzodiazepin-5-one diastereomers, which can be separated by column chromatography or recrystallization. Interestingly, a complete reversal of diastereoselectivity can be obtained based on the cyclization methods, due to their different reaction mechanisms during the cyclization process. Specifically, a [2 + 2] cycloaddition occurs between the phosphazene and the carbonyl group in the Staudinger/aza–Wittig reaction of **3.2.37**, leading preferentially to α*S*,3*S* configuration (**3.2.39**). However, the addition of the nitrogen to the C3 position of the Ugi adduct (**3.2.38**) in the reduction cyclization route takes place through a nucleophilic/radical process, which results in the formation of α*S*,3*R* configuration (**3.2.40**).

Several new chiral 3-substituted 1,3,4,5-tetrahydrobenzo[*e*][1,4]diazepin-2-ones analogs (**3.2.43**) have been synthesized *via* a domino cyclocondensation reaction between *ortho*-halobenzyl halides (**3.2.41**) and α-amino acid amides (**3.2.42**) in the presence of CuI and K$_2$CO$_3$ (Fig. 3.8) (Lu et al., 2015). A novel and facile protocol through a cascade reaction of K$_2$CO$_3$-promoted S$_N$2 nucleophilic substitution at first, and then, a copper-catalyzed C–N intramolecular coupling reaction occurs. Significantly, the amino acid chirality remains intact during the reaction process, and thus, the chirality of the final product is determined by the amino acids.

Benzynes are versatile intermediates for the practical synthesis of a wide range of fused heterocyclic compounds *via* cycloaddition reactions (Pellissier & Santelli, 2003). While the regioselectivity is a challenge when unsymmetrically substituted benzynes are employed, a facile regiocontrolled cycloaddition of 2-(trimethylsilyl)phenyl triflates **3.2.44** with imidazolidinones **3.2.45** to construct a series of 6-(triflyloxy)-1,4-benzodiazepin-5-ones **3.2.47** has been developed (Kanekoa et al., 2018). The benzyne
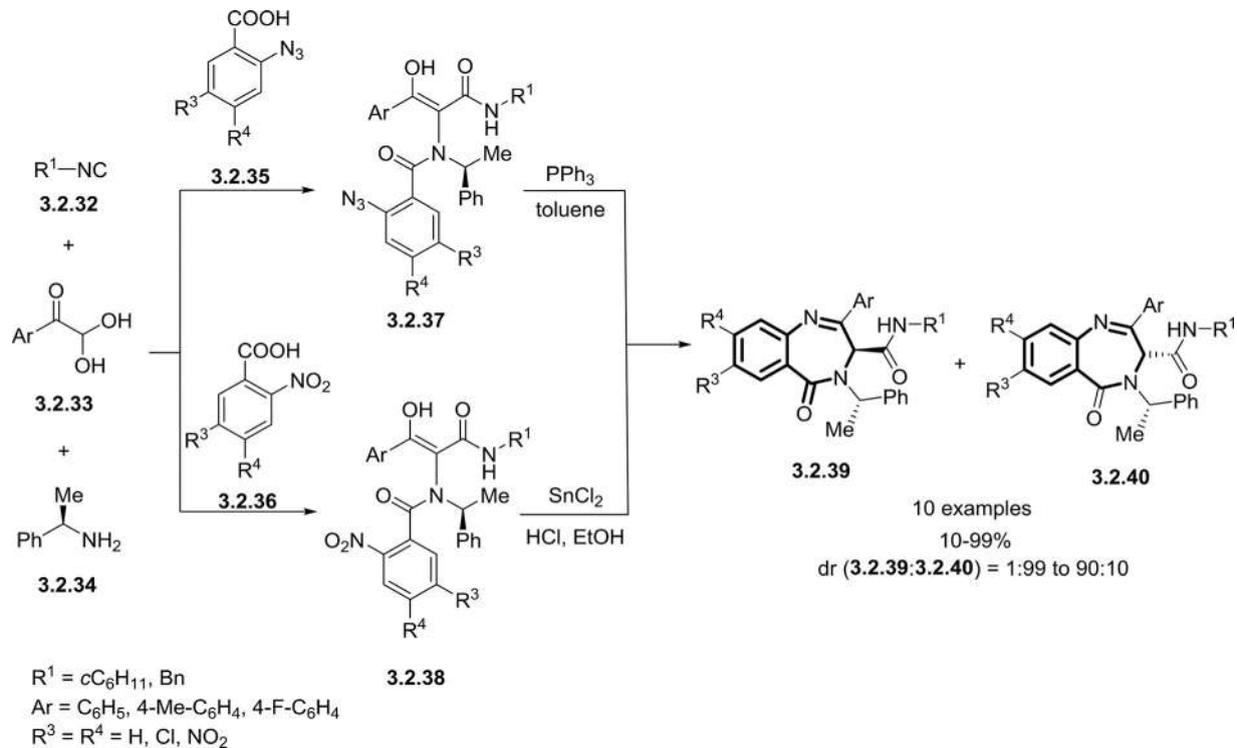
Figure 3.7 Synthesis of highly enantioenriched 2,3,4-trisubstituted 1,4-benzodiazepin-5-ones.
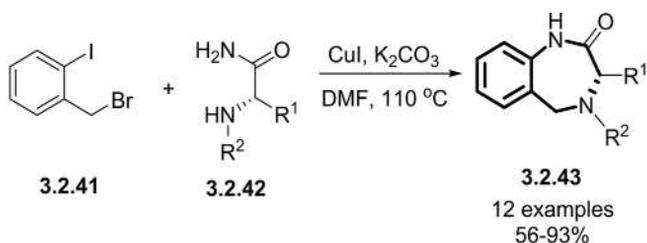
R^1 = Me, Et, i-Pr, i-Bu, -(CH$_2$)$_3$-, Bn, C$_6$H$_5$, (1H-indol-3-yl)CH$_2$-, 4-OH-C$_6$H$_4$CH$_2$-
R^2 = H

Figure 3.8 Synthesis of chiral 3-substituted tetrahydro-1,4-benzodiazepin-2-ones.
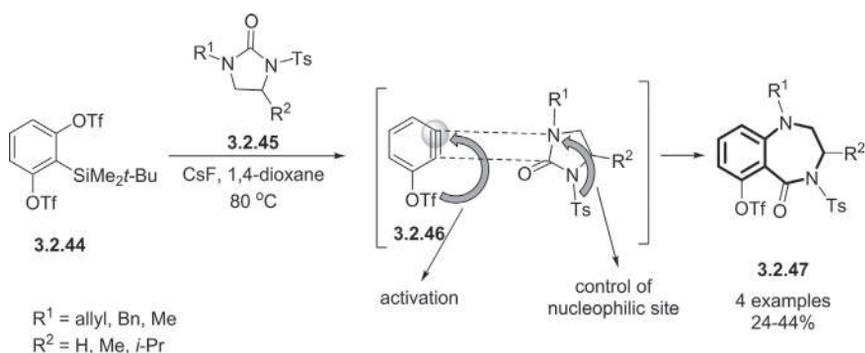
R^1 = allyl, Bn, Me
R^2 = H, Me, i-Pr

Figure 3.9 Regioselective synthesis of 6-(triflyloxy)-1,4-benzodiazepin-5-ones.

intermediate **3.2.46** is first obtained from the reaction of 2-(trimethylsilyl)phenyl triflate **3.2.45** with CsF in 1,4-dioxane, which then reacts with 1-methyl-3-(p-toluenesulfonyl)imidazolidin-2-one **3.2.45** in a highly regioselective manner to give the desired benzodiazepines **3.2.47**. Notably, the regioselectivity of this method is controllable by introducing a triflyloxy group in the 2-(trimethylsilyl)phenyl triflate **3.2.45** and a tosyl group in the imidazolidinone **3.2.44**. Due to the strong electron-withdrawing inductive effect of the triflyloxy group at the C3 position, the C1 position of the benzyne **3.2.46** becomes more electrophilic, resulting in the selective nucleophilic addition of the N-tosylimidazolidin-2-ones **3.2.45** to this position. Furthermore, the inductive effect of the tosyl group can control the nucleophilic site on the adjacent nitrogen (Fig. 3.9).

Chemler *et al.* demonstrated a facile copper-mediated synthesis of 2-aminomethyl functionalized 1,4-benzodiazepin-5-ones **3.2.53** *via* the alkene deamination reaction of several 2-sulfonamido–N-allyl benzamide analogs **3.2.51** (Fig. 3.10) (Karyakarte et al., 2015). Mechanistically, the deamination reaction is initiated by the coordination of Cu(II) to **3.2.51**,

R^1 = Ts, Ms, Ns
R^2 = Bn, C_6H_5, 4-F-C_6H_4, 4-OMe-C_6H_4
R^3 = Ts, Ns, SES, alkyl, aryl
X = H, 8-Cl, 7-OMe
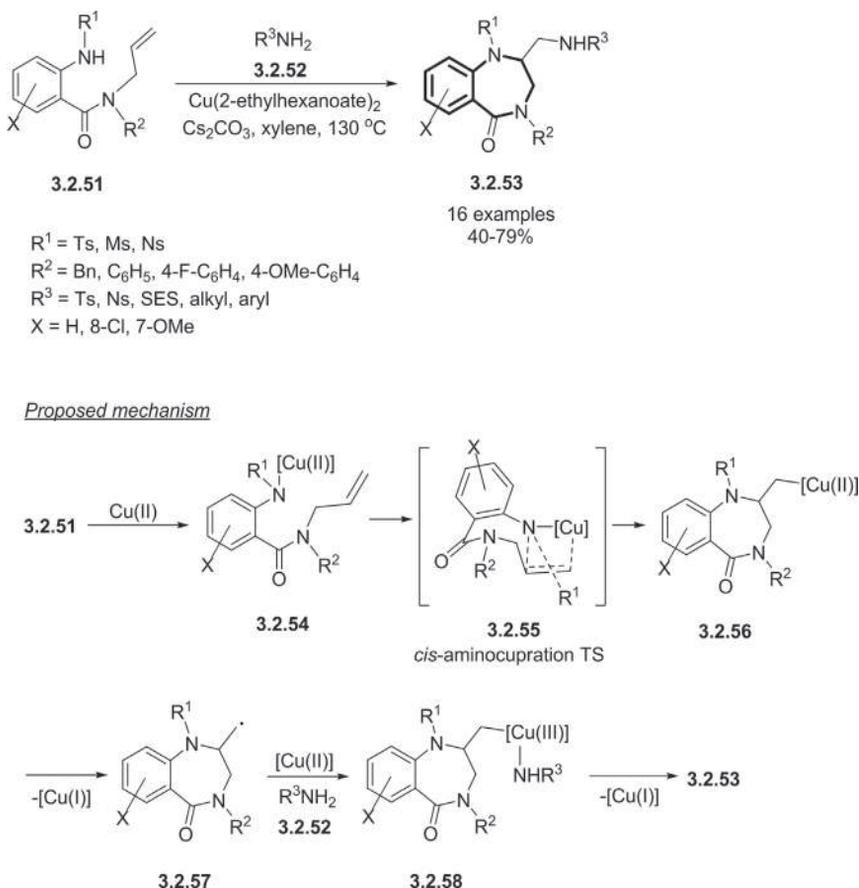
Figure 3.10 Copper-mediated synthesis of 2-aminomethyl functionalized 1,4-benzodiazepin-5-ones *via* the alkene diamination reaction.

producing the organocopper(II) moiety **3.2.56** through a nine–membered ring *cis*-aminocupration transition state (**3.2.55**). This unstable intermediate then generates the radical **3.2.57** that subsequently coordinates to both Cu(II) and R^3NH_2 **3.2.52** and, finally, undergoes a reductive elimination to form the desired 1,4-benzodiazepin-5-one product **3.2.53**.

A straightforward and robust strategy for stereoselective synthesizing a series of the novel (*E*)-2-aryl- (or vinyl)methylidene-1,4-benzodiazepines (**3.3.61**) was demonstrated *via* the treatment of 2-aminotosyl-*N*-methyl-*N*-(prop-2-ynyl)benzamides (**3.2.59**) with aryl/vinyl halides or triflates (**3.2.60**) under palladium–catalyzed reaction conditions (Fig. 3.11) (Kundu et al., 2015). *Trans*-aminopalladation is the key step during the cyclization reaction (7-*exo*-*dig*), leading to the generation of the products **3.2.61** with
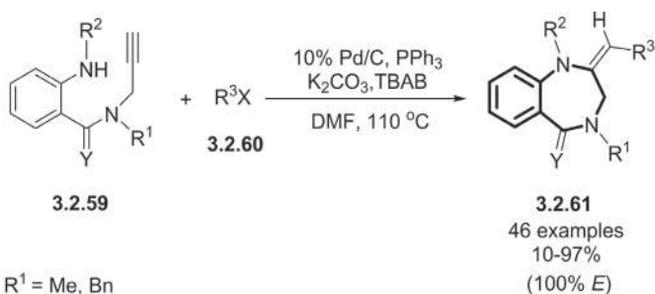
R^1 = Me, Bn

R^2 = Ts, Ns, Ms, Bs

R^3 = C_6H_5, naphthalen-1-yl, 4-F-C_6H_4, 4-CF_3-C_6H_4, 4-Me-C_6H_4, 4-OMe-C_6H_4, 4-COOMe-C_6H_4
   2-CHO-C_6H_4, 4-Br-C_6H_4, 2-COOMe-C_6H_4, 2-Me-C_6H_4, 4-NO_2-C_6H_4, 4-NO_2-2-Me-C_6H_3,
   2-CH_2COMe-C_6H_4, 3-pyrindyl, 2,4-dimethoxypyrimidin-5-yl, pyridin-3-yl, thiophen-2-yl
   1,3-dimethylpyrimidine-2,4(1H,3H)-dione-5-yl, 4-methoxybenzylidene

X = I, Br, OTf

Y = H_2, O

Proposed mechanism
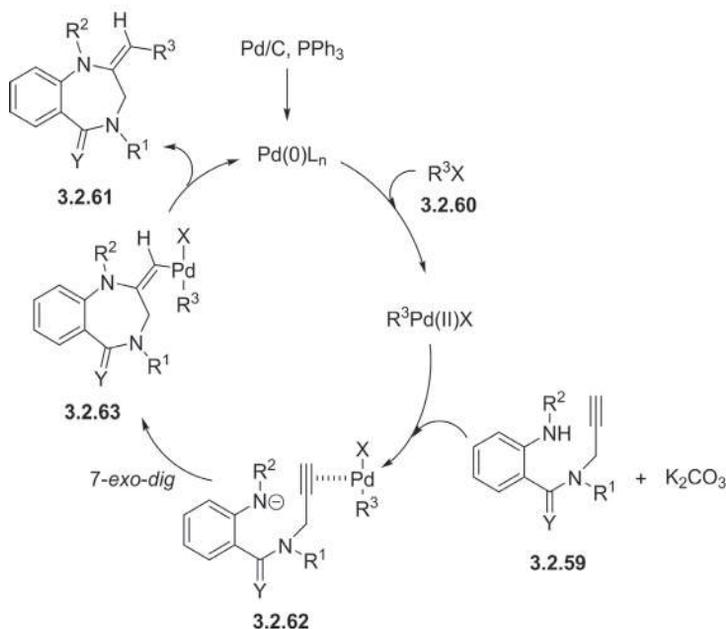
Figure 3.11 Stereoselective synthesis of (*E*)-2-aryl-(or vinyl)methylidene-1,
4-benzodiazepines *via* palladium-catalyzed cyclization of 2-aminotosyl-*N*-methyl-
N-(prop-2-ynyl)benzamides.
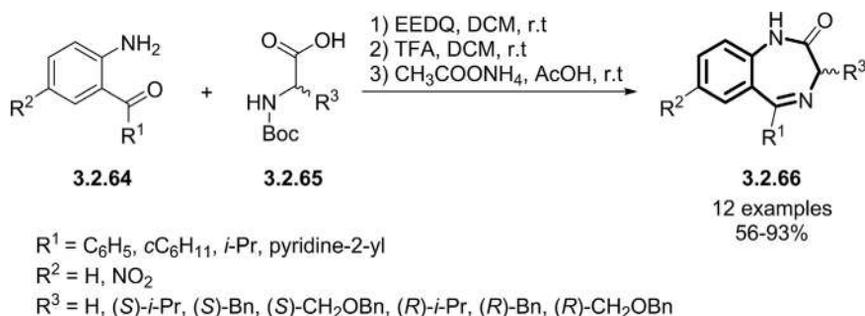
Figure 3.12 Synthesis of 3,5,7-trisubstituted-trisubstituted 1,4-benzodiazepin-2-ones.

an exclusive (*E*)-stereochemistry. Leaching of palladium from the Pd/C surface into the solution upon the interaction with the phosphine ligand first generates the active $Pd(0)L_n$ complex. Oxidative addition of **3.2.60** to the Pd(0) species then forms $R^3Pd(II)X$, which subsequently acts as Lewis acid and activates the triple bond of **3.2.59**. An intramolecular cyclization takes place exclusively through a *trans*-aminopalladation pathway, resulting in the formation of (*E*)-vinyl palladium moiety **3.2.63**, followed by reductive elimination to furnish the final product **3.2.61**. Notably, the low-cost alternative Pd/C was employed instead of relatively expensive palladium catalysts such as $PdCl_2(PPh_3)_2$ and $Pd(OAc)_2$, along with the PPh_3 ligand to give the corresponding 1,4–benzodiazepines derivatives in low to high yields (10–97%).

A wide range of racemic 3,5,7–trisubstituted 1,4–benzodiazepin–2–ones (**3.2.66**) can be synthesized *via* a simple multistep protocol without interme-diates purification (Spencer et al., 2011). 2-Amino phenylketones (**3.2.64**) undergo an imine formation reaction with *N*–Boc protected α–amino acids (**3.2.65**), followed by *N*–Boc deprotection to afford the corresponding 1,4–benzodiazepin–2–ones in moderate to high yields (Fig. 3.12).

Among the family of benzodiazepines, 1,4–benzodiazepine–2,5–dione have demonstrated activity as anticholinesterase inhibitors (Mohamed & El-Yamany, 2012), histone acetylase inhibitors (Loudni et al., 2007), melanocortin agonists (Joseph et al., 2008), and non-peptide peptidomimet-ics (Cabedo et al., 2005; Verdié et al., 2007). Thus, the development of new synthetic methods for these skeletons is important. A series of 3-substituted 1,4–benzodiazepine–2,5–diones (**3.2.70**) were synthesized through a simple two–step strategy starting from isotonic anhydride **3.2.67** and α–amino acid methyl esters **3.2.68** and subsequent use of H_2PtCl_6 (chloroplatinic acid) as an acidic catalyst (Fig. 3.13) (Anil et al., 2019). The ring-opening reaction of
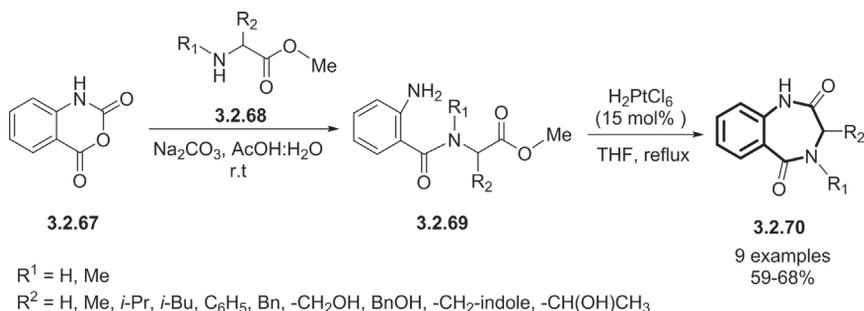
Figure 3.13 Pt-catalyzed synthesis of 1,4-benzodiazepine-2,5-diones from isotonic anhydrides.

isotonic anhydride 3.2.67 with 3.2.68 initially provides the corresponding 2-aminobenzamido methyl esters **3.2.69** under mild reaction conditions, followed by the Pt-catalyzed intramolecular cyclization to afford the respective 1,4-benzodiazepine-2,5-dione products (**3.2.70**) in good yields (59–68%). Note that the 2-aminobenzamido methyl esters bearing −OH group on the R^2 side chain failed to produce the desired products due to suppressing the catalytic performance of H_2PtCl_6 through coordination to the −OH.

A mild and simple four-step procedure for the synthesis of several 1,3,4-trisubstituted 1,4-benzodiazepine-2,5-diones has been reported by the rearrangement of 3-aminoquinoline-2,4-(1*H*,3*H*)-diones **3.2.76** (Křemen et al., 2017). For this synthesis, a multi-step procedure starts from the reaction of anilines **3.2.71** with diethyl malonates **3.2.72** to afford 4-hydroxy-2-(1*H*)-quinolones **3.2.73** (Fig. 3.14). Then, the chlorination of **3.2.73** with sulfuryl chloride (SO_2Cl_2) for generation of 3-chloroquinolin-2,4-(1*H*,3*H*)-diones **3.2.74** occurs, subsequently undergoing a nucleophilic substitution reaction with various primary amines **3.2.75** to furnish 3-aminoquinoline-2,4-(1*H*,3*H*)-diones **3.2.76**. 1,4-Benzodiazepines-2,5-diones **3.2.80** can be finally obtained *via* a molecular rearrangement of **3.2.76** under basic conditions. Mechanistically, an appropriate base such as benzyltrimethylammonium hydroxide (Triton B) enables the intramolecular addition of the adjacent amine to the carbonyl group and the formation of aziridine oxoanion **3.2.78**. Two subsequent ring opening-protonation reactions then occur to give the final product **3.2.80**.

A one-pot intermolecular cyclization protocol for direct access to a wide range of 1,2,4,5-tetrahydro-1,4-benzodiazepine-3-one analogs **3.2.83** under mild reaction conditions has been recently reported (Fig. 3.15) (Sasiambarrena et al., 2019). This one-pot approach is based on a cascade
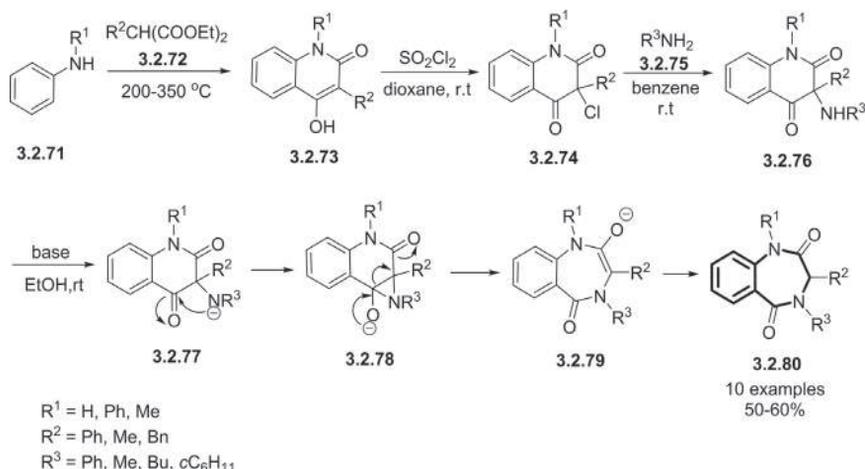
Figure 3.14 Synthesis of 1,3,4-trisubstituted 1,4-benzodiazepine-2,5-diones.
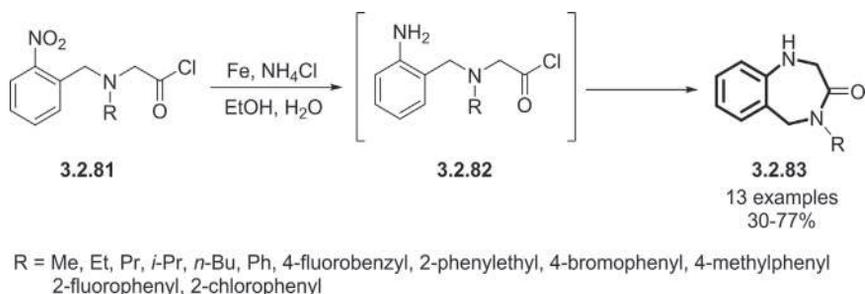
Figure 3.15 Synthesis of 1,2,4,5-tetrahydro-1,4-benzodiazepine-3-ones.

reaction of NH deprotonation and aza–Mannich addition/intramolecular S_N2 nucleophilic addition. The ring-forming reaction is carried out by the reduction of the $-NO_2$ group to generate the intermediate 2–chloro-*N*-(2-aminobenzyl)acetamides (**3.2.82**), and a subsequent intramolecular S_N2 reaction to afford the desired product.

Crescentini and co-workers demonstrated a regioselective synthesis of several 5*H*-1,4-benzodiazepine-3-carboxylates **3.2.87**, starting from 1,2-diaza-1,3-dienes (DDs) **3.2.85** (Attanasi et al., 2011). DDs are versatile building blocks with a highly reactive electrophilic center at the C4 position, which easily react with various nucleophiles to construct diverse heterocyclic skeletons. The initial aza–Michael addition of the benzylic nitrogen of 2-aminobenzylamine **3.2.84** to the terminus carbon of DDs results in the formation of α–aminohydrazones **3.2.86**. A regioselective 7–*exo*–*trig* closure *via* the nucleophilic addition of the nitrogen to the hydrazonic carbon (accompanied by the spontaneous loss of a *tert*-butylcarbazate molecule)
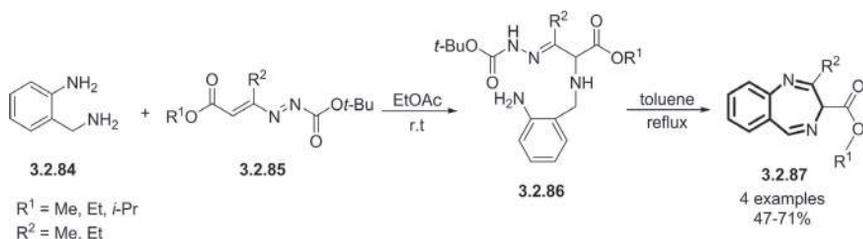
Figure 3.16 Regioselective synthesis of 5H-1,4-benzodiazepine-3-carboxylates.
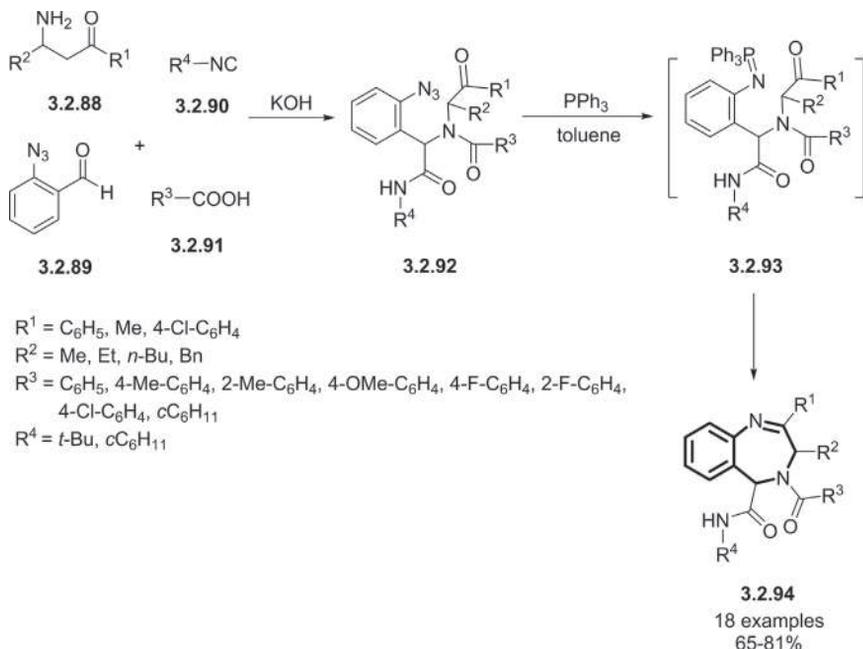
Figure 3.17 Synthesis of 2,3,4,5-tetrasubstituted 1,4-benzodiazepines *via* a sequential Ugi 4CC/Staudinger/aza-Wittig reaction.

affords the desired product **3.2.87** in moderate to good yields (47–71%) (Fig. 3.16).

In another study, a wide range of 2,3,4,5-tetrasubstituted 1,4-benzodiazepines **3.2.94** were synthesized *via* a one-pot four-component sequential Ugi 4CC/Staudinger/aza-Wittig reaction of α-amino ketones **3.2.88**, *ortho*-azidobenzaldehyde **3.2.89**, isocyanides **3.2.90**, and carboxylic acids **3.2.91** (Fig. 3.17) (Wang et al., 2013). It has been shown that conducting the reaction in a stepwise fashion results in the formation of several by-products, while the one-pot four-component reaction under mild reaction conditions affords the corresponding 1,4-benzodiazepines **3.2.94** in good to high yields.
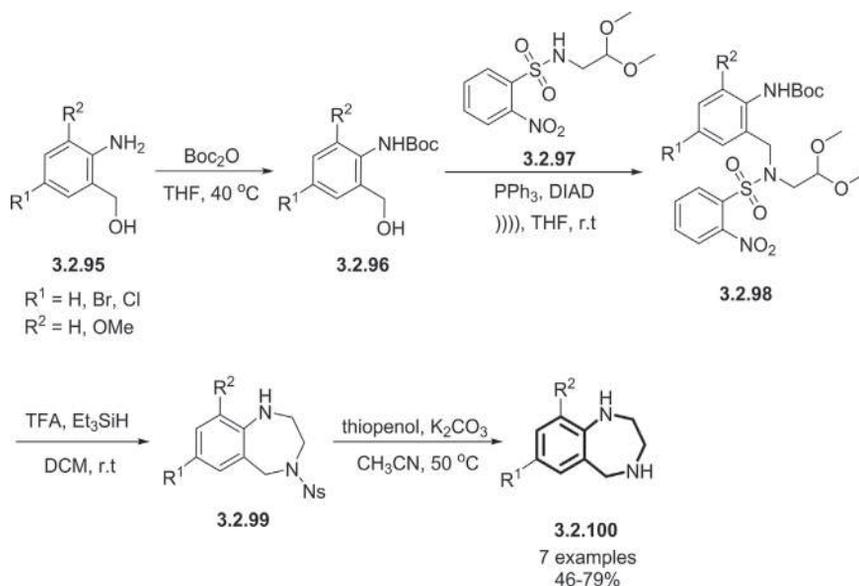
Figure 3.18 Stepwise synthesis of 2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepines.

A mild and efficient strategy for the synthesis of a number of 2,3,4,5–tetrahydro–1*H*–1,4–benzodiazepines **3.2.100** starting from 2-aminobenzyl alcohols (**3.2.95**) has been developed (Popp et al., 2016). The *ortho*–amino–benzylic **3.2.95** is initially protected with a Boc group, followed by an ultrasound-assisted Mitsunobu coupling of *N*–Boc–protected 2–aminobenzyl alcohols **3.2.96** with *N*–Ns–protected 2–aminoacetaldehyde dimethyl acetal **3.2.97** to produce the adduct **3.2.98** (Fig. 3.18). This is followed by a one-pot Boc deprotection-ring closure pathway using a mixture of organosilane and trifluoroacetic acid for *N*–Boc deprotection and reductive amination of acetal, respectively, affording the corresponding 1,4-benzodiazepines **3.2.99**. Removal of the nosyl (Ns) protecting group from the compounds **3.2.99** can be easily achieved using thiophenol and potassium carbonate to provide the 1,4–benzodiazepines **3.2.100**. These scaffolds are highly versatile since the diazepine ring enjoys an unsubstituted and saturated state as well as two free NH groups ready for further modifications.

3.3 Synthesis of fused 1,4-benzodiazepines

Fused 1,4–benzodiazepines are a unique class of conjugated skeletons widely identified as important core units in natural products (Annor-Gyamfi et al., 2018; Hurley et al., 1988). The combination of 1,4–benzodiazepines

with other cyclic systems in a single molecule has been found to exhibit biological activities as pharmaco–active agents such as antitumors (Kaliszczak et al., 2010), antibiotics (Fotso et al., 2009), and anti-HIV agents (Otto, 1992). Thus, the development of some efficient strategies to construct new rings fused to 1,4–benzodiazepines continuously attracts attention from the synthetic and medicinal chemistry community. This is relatively simple on the *a* side of the 1,4-benzodiazepine skeletons because of the presence of nucleophilic N1, which is easily functionalized to produce an electrophilic center at position 2 (See Section 2.2.2, Chapter 2). Fusion of rings on the *b, c, d* faces of the benzodiazepines can also be achieved *via* adaptations of the related synthetic methods. In this section, recent progress on fused 1,4–benzodiazepines syntheses will be discussed.

Pyrrole-fused 1,4-benzodiazepines are tricyclic ring systems exhibiting interesting biological activities (Varvounis, 2016). The first analog of these heterocycles, anthramycin, was first isolated and discovered from *Streptomyces* species in 1965 (Leimgruber et al., 1965). Anthramycin was later found to have outstanding biological activities against breast cancers, sarcomas, and lymphomas. These interesting findings have encouraged synthetic and biological research efforts around pyrrolo–1,4-benzodiazepines. Samanta *et al.* described an efficient one-pot strategy for the synthesis of a series of pyrrolo[1,2-*a*]-1,4-benzodiazepine derivatives **3.3.6** *via* Pictet–Spengler reaction under mild reaction conditions (Fig. 3.19) (Ali et al., 2019). In this synthesis, 3-(3–formylcycloalkenyl)–acrylic esters **3.3.1** initially undergo imine formation with 2-aminobenzylamine **3.3.2**, followed by aza-Michael reaction to give the *N*-benzylamine substituted pyrroles **3.3.3**. The imines **3.3.5** are subsequently generated *via* the reaction of the intermediate **3.3.3** with the carbonyl moiety **3.3.4** in the presence of the catalytic amount of acetic acid/TFA. An intramolecular cyclization-deprotonation sequence finally occurs to provide the corresponding product **3.3.6** in high yields.

A series of novel pyrrolo[2,1-*c*]-1,4-benzodiazepines containing an N10−C11 imine bond are synthesized through a sequential Ugi–cyclization reaction with a reduction-cyclization pathway (P. Pertejo et al., 2017). The reaction of an equimolar amount of 2-nitrobenzylamine **3.3.7**, glyoxal **3.3.8**, 3-bromopropionic acid **3.3.9**, and various alkyl isocyanides **3.3.10** in MeOH provides the Ugi adduct **3.3.11**, which is converted to the pyrrolidinone intermediate **3.3.12** *via* a spontaneous cyclization (Fig. 3.20). In the next step, the reduction of the nitro group of *N*-[2-nitrobenzyl]pyrrolidinone **3.3.12** can be carried out using stannous chloride (SnCl$_2$) under acidic conditions. Finally, the compound **3.3.13** undergoes
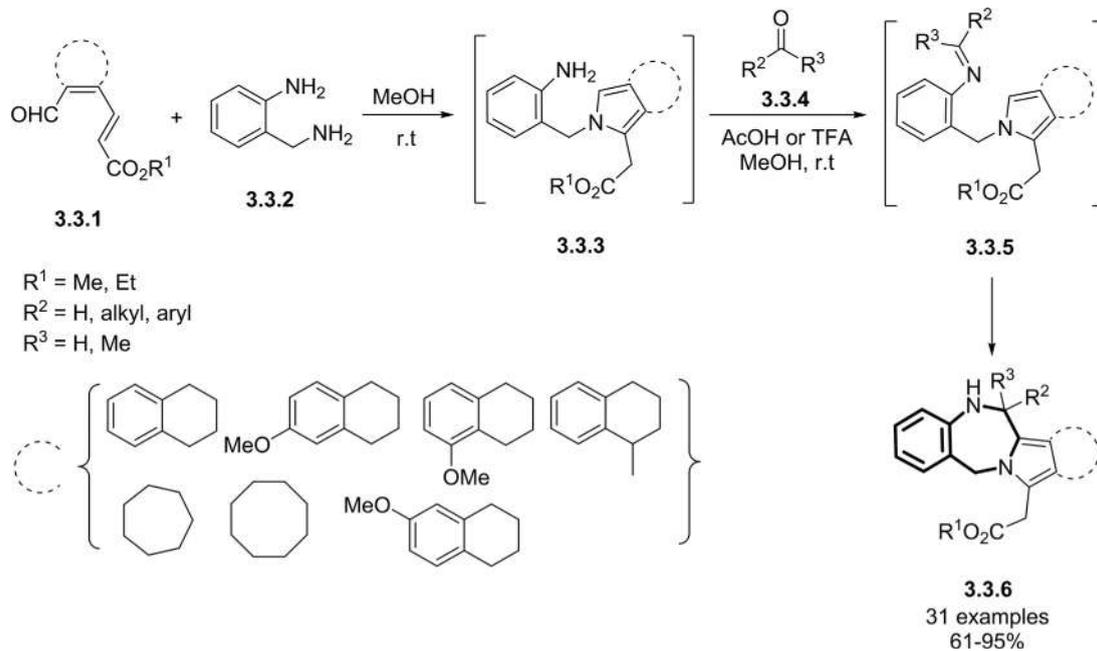
Figure 3.19 Synthesis of pyrrolo[1,2-a]-1,4-benzodiazepines *via* a Pictet–Spengler reaction.
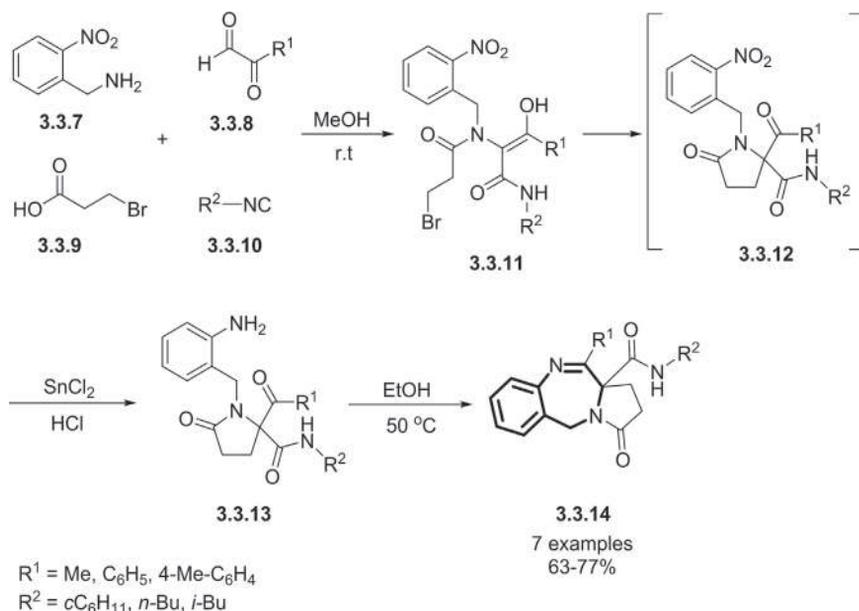
Figure 3.20 Synthesis of pyrrolo[2,1-c]-1,4-benzodiazepines *via* a Ugi-cyclization approach.

an intramolecular cyclization–deprotonation reaction to furnish the corresponding product **3.3.14**.

Shin *et al.* established an efficient metal-free cascade [5 + 2]-[2 + 2] cycloaddition protocol for the synthesis of pentacyclic 1,4-benzodiazepines **3.3.17** under mild reaction conditions (Shin et al., 2017). The synthetically available pyridinium zwitterion **3.3.15** acts as a 1,5 dipole and undergoes [5 + 2] cycloaddition with the benzyne species, *in situ* generated from 2-(trimethylsilyl)phenyl triflate **3.3.16**, to give the desired compounds **3.3.17** (Fig. 3.21). This step is the rate-determining step because of the dearomatization of **3.3.15**. Interestingly, upon the formation of **3.3.18**, a spontaneous quick intermolecular [2 + 2] cycloaddition reaction with a second benzyne molecule occurs, affording the final polycyclic 1,4-benzodiazepine **3.3.17**. This methodology offers one-pot formation of four bonds including one C−N bond and three C−C bonds under metal-catalyst-free mild reaction conditions.

Indole-fused 1,4-benzodiazepines are another pharmaceutically important fused scaffold in drug discovery, with various biological activities (Xu et al., 2018). Xiao and co-workers described a novel one-pot
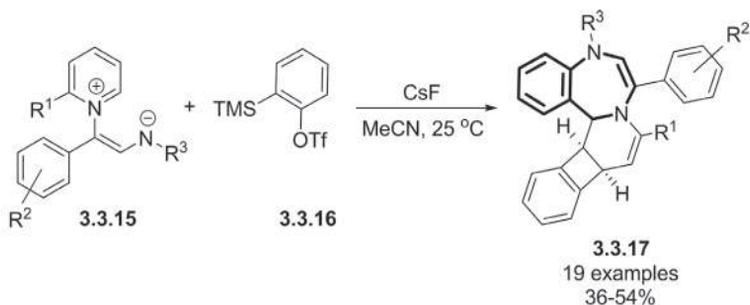
R^1 = C$_6$H$_5$, 4-Me-C$_6$H$_4$, 4-OMe-C$_6$H$_4$, 4-Cl-C$_6$H$_4$, 4-F-C$_6$H$_4$, 3-Me-C$_6$H$_4$, 4-Ac-C$_6$H$_4$, 2-naphtyl
R^2 = H, 4-Me, 4-OMe, 4-t-Bu, 3-Me, 4-CF$_3$, 3-Me, 4-F, 3-F, 4-Br
R^3 = Ts, SO$_2$Ph, SO$_2$(n-Bu), SO$_2$(4-CF$_3$-C$_6$H$_4$)

Proposed mechanism
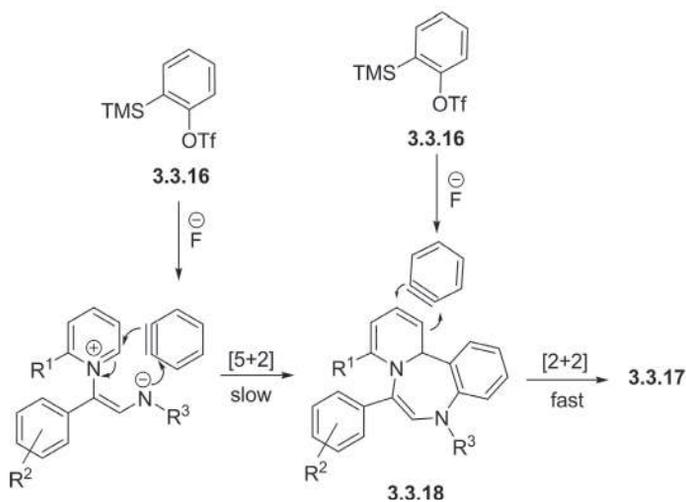
Figure 3.21 Synthesis of polycyclic 1,4-benzodiazepines *via* a metal-free cascade [5 + 2]-[2 + 2] cycloaddition protocol.

redox–neutral [5 + 2] protocol for the synthesis of two classes of indole–1,2– fused 1,4–benzodiazepines (**3.3.22** and **3.3.23**)(Fig. 3.22) (S. Wang et al., 2019). They treated several 3–alkylindole derivatives **3.3.19** with *ortho*–aminobenzaldehydes **3.3.20** or **3.3.21** in the presence of the catalytic amount of 1,1′–binaphthyl–2,2′–diyl hydrogen phosphate (PA) as a Brønsted acid catalyst to generate the desired product **3.3.22** or **3.3.23**, respectively. Mechanistically, *N*–alkylation of 3–alkylindole **3.3.19a** with *ortho*–aminobenzaldehyde **3.3.21a** occurs to give the 1–indolylalkanol **3.3.24**, followed by PA–catalyzed dehydration to yield the intermediate **3.3.25**. A

$n = 0, 1, 2$
R^1 = H, 5-OMe, 5-OBn, 5-Me, 5-Cl, 5-F, 4-F, 6-F, 5-Br
R^2 = Me, i-Pr, (1H-indol-3-yl)methyl
R^3 = H, 4-CF$_3$, 3-Cl, 4-Br, 4-OMe, 3-Br, 2-F
R^4 = H, Me, CO$_2$Me

3.3.22
4 examples
62-80%

3.3.23
18 examples
24-83%
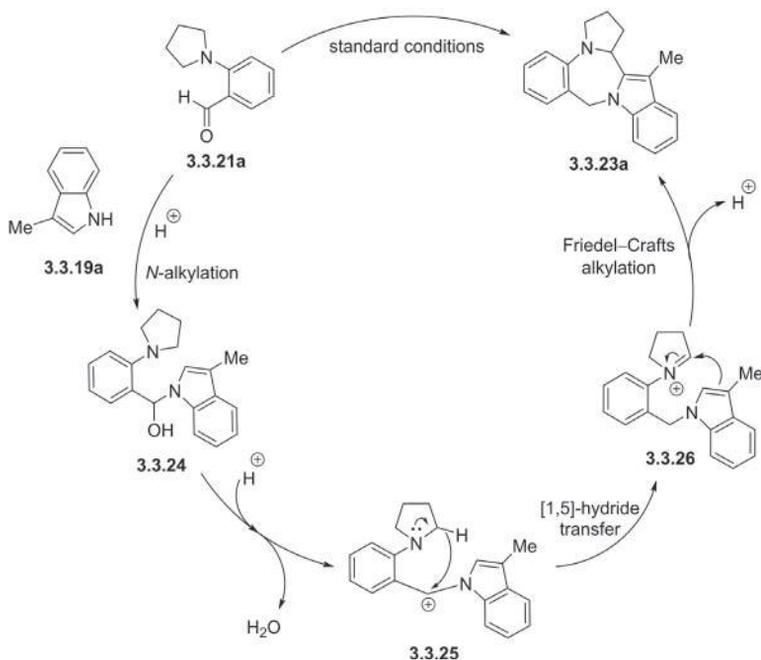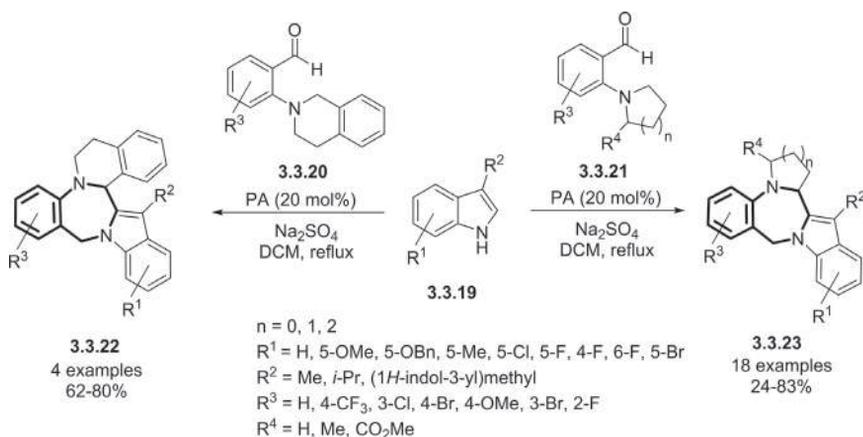
Figure 3.22 Synthesis of two classes of indole-fused 1,4-benzodiazepines *via* a redox-neutral [5 + 2] strategy.

sequence of intramolecular [1,5]–hydride transfer–Friedel–Crafts alkylation finally furnishes the corresponding indole–1,2–fused 1,4–benzodiazepines **3.2.23**. Metal–free reaction conditions, excellent regioselectivity, high step economy, and broad substrate scope can be considered as the main advantages of this methodology.

By using a facile CuI/L-proline catalyzed intramolecular *N*-arylation method, Wang *et al.* performed a two-step synthesis of several indole-fused 1,4-benzodiazepine-3-ones (Wang et al., 2011). For this synthesis, they used a key intermediate of **3.3.29**, obtained by Py-Bop coupling of various *N*-methyl-2-iodobenzylamines **3.3.27** and 1*H*-indole-2-carboxylic acids **3.3.28** under mild reaction conditions (Fig. 3.23). Employing the standard Buchwald catalytic system including CuI/*trans*-*N*,*N′*-dimethyl-1,2-cyclohexanediamine results in an incomplete reaction with low yields of the desired product **3.3.30**. On the other hand, in the presence of CuI (1 mol%) and L-proline (2 mol%) at 95 °C, the intramolecular *N*-arylation of **3.3.29** proceeds smoothly to completion, giving the final compounds **3.3.30** in high yields upon an aqueous workup. It has been also shown that the cyclization of the bromo-derivatives of **3.3.29** requires higher reaction temperature (120 °C) and higher catalyst loading (2 mol% of CuI and 4 mol% of L-proline).

Therapeutic activities of 1,4-benzodiazepine can be significantly enhanced by the incorporation of a triazole ring into the benzodiazepines structure, resulting in the discovery of several clinically and commercially successful drugs such as alprazolam and estazolam. A series of benzimidazotriazolo dual-fused 1,4-benzodiazepine derivatives are readily accessible *via* iodine-catalyzed one-pot tandem sequence reactions of *ortho*-(propargylamine)anilines **3.3.31** and *ortho*-azidobenzaldehydes **3.3.32** (Fig. 3.24) (Kumar et al., 2015). Initially, iodine activates the carbonyl group of the **3.3.32** to form the intermediate **3.3.34**, followed by a cyclocondensation reaction with an amine group of the propargylic reagent **3.3.31** to form the intermediate **3.3.35**. A sequential [3 + 2] cycloaddition-spontaneous aerobic oxidation finally affords the title product **3.3.33**.

A convenient method for the synthesis of several tricyclic compounds incorporating a fusion of the 1,2,3-triazole moiety with 1,4-benzodiazepin-6-ones has been recently described (Chowdhury et al., 2010). The Sonogashira coupling of readily accessible 2-amino-*N*-methyl-*N*-(prop-2-ynyl)benzamide **3.3.37** with aryl iodide followed by *in situ* diazotization, azidation, and concurrent cycloaddition reactions provide the corresponding 1,2,3-triazolo-1,4-benzodiazepin-5-ones **3.3.39** (Fig. 3.25). The amide functionality can be efficiently reduced by LiAlH$_4$ to afford the pharmaceutically important amine derivatives **3.3.40** in good yields. The operational simplicity and easy substrate availability are the main advantages of this methodology.
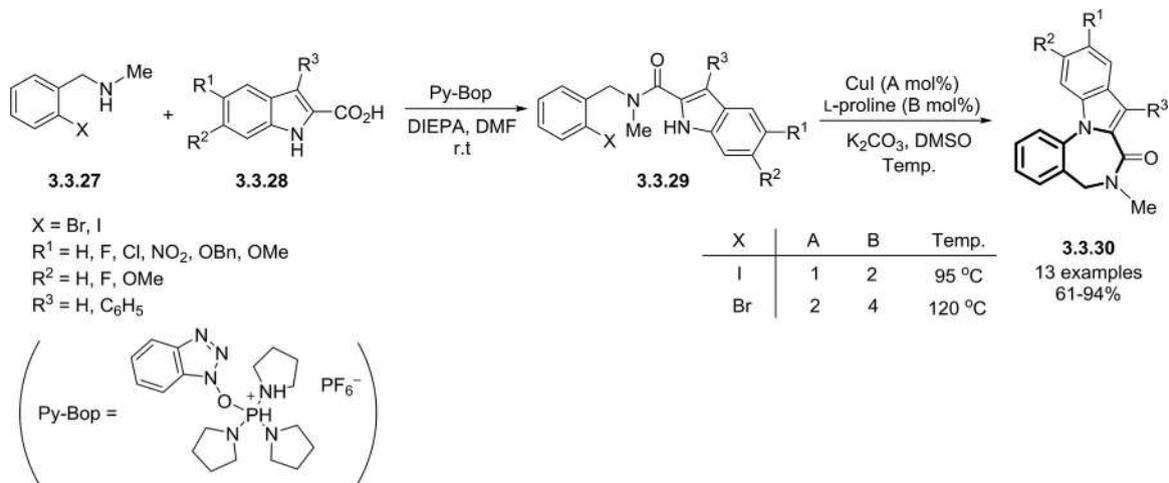
Figure 3.23 CuI/L-proline catalyzed synthesis of indole-fused 1,4-benzodiazepine-3-ones.
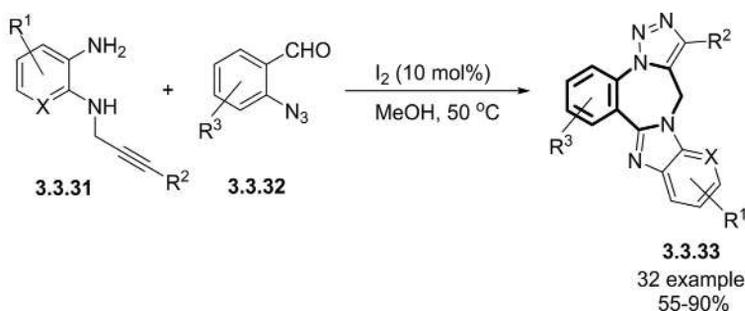
R^1 = H, 4-Cl, 4-F, 4-CF$_3$, 4-CO$_2$Me, 4-Me, 5-Me, 4,5-di-Cl, 4,5-di-Me, 4-(phenyl)methanone
R^2 = H, C$_6$H$_5$, 4-Cl-C$_6$H$_4$, 3,4-di-Cl-C$_6$H$_3$, thiophen-2-yl, [d][1,3]dioxole
R^3 = H, 3-OMe, 4-Cl, 4-OMe
X = N, C

Proposed mechanism
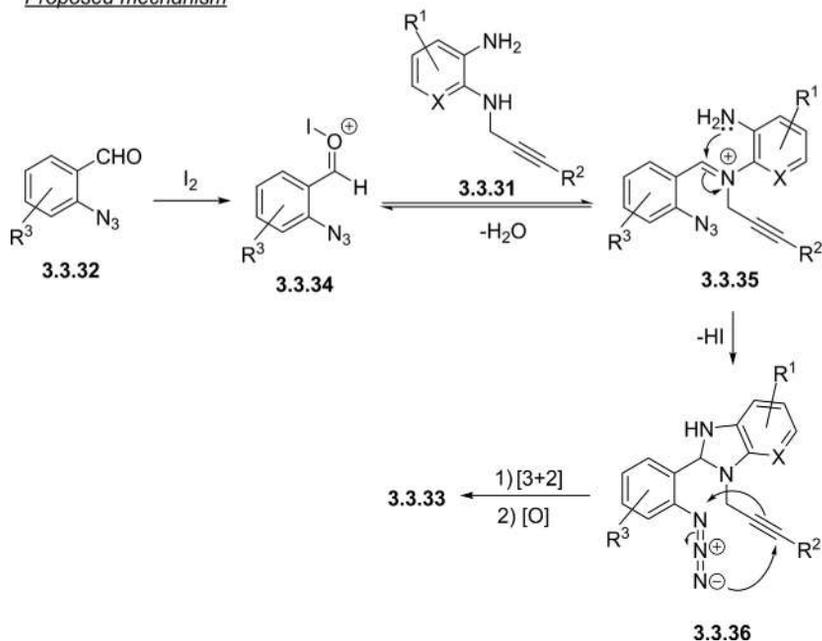
Figure 3.24   Iodine-catalyzed synthesis of benzimidazotriazolo[1,4]-benzodiazepine derivatives.

Majumdar and Ganai reported a copper-catalyzed domino Ullmann C–N coupling/azide-alkyne cycloaddition procedure to synthesize a number of triazolo[1,5-*a*][1,4]benzodiazepines (Majumdar & Ganai, 2013). They treated various *ortho*-azidobenzylbromides **3.3.41** with *N*-propargylated anilines **3.3.42** in presence of CuI (10 mol%) and base (K$_2$CO$_3$) to the
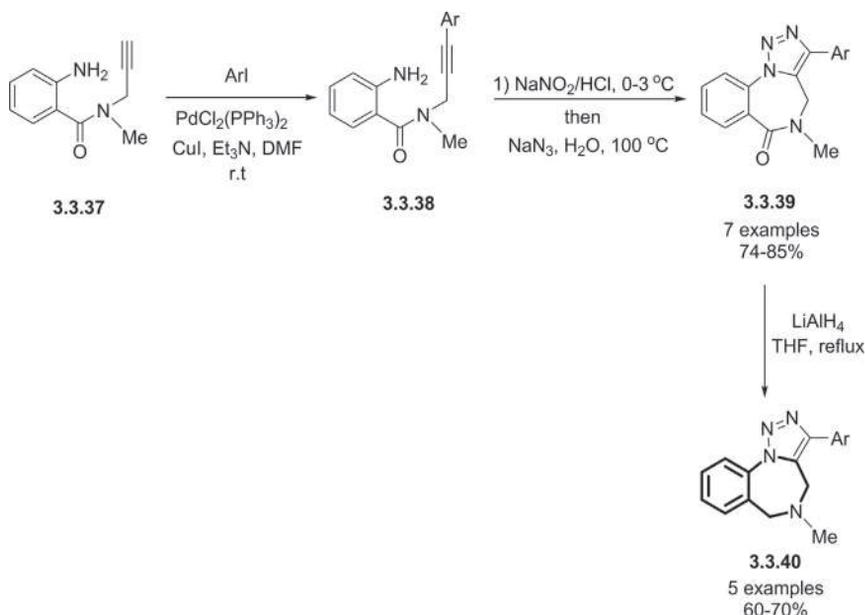
Figure 3.25 Synthesis of 1,2,3-triazolo[1,5-*a*][1,4]benzodiazopin-6-one derivatives.

generate 1,4-benzodiazepines (Fig. 3.26). Mechanistically, the formation of the 1,5-regioisomer **3.3.44** does not occur, and the reaction proceeds through the initial Cu-catalyzed intermolecular C–N coupling of azide **3.3.41** with amine **3.3.42** towards intermediate **3.3.45**. This is followed by the intramolecular azide-alkyne cycloaddition of **3.3.45**, giving the triazole-fused benzodiazepines **3.3.43**. This ligand-free strategy offers several benefits such as step-economy, excellent yields, and operational simplicity.

Imidazole-fused benzodiazepines are prominent pharmacophores proved to be potent central nervous system (CNS) depressants in several marketed drugs such as loprazolam, flumazenil, and midazolam (Welsch et al., 2010). Considering this background, the development of simple and efficient platforms to construct new imidazole-fused benzodiazepines is of interest to chemists. A cascade copper-catalyzed *N*-arylation-condensation strategy for the synthesis of a library of new tetracyclic imidazobenzodiazepines has been developed (Murugesh et al., 2016). The reaction of chiral 1,2-diaminocyclohexanes **3.3.46** and 2-halobenzaldehydes **3.3.47** generates the corresponding benzodiazepines **3.3.48** in moderate to high yields with an enantiomeric excess (*ee*) of >95% (Fig. 3.27), which are then treated with toluenesulfonylmethyl isocyanide (TosMIC) **3.3.50** to furnish the desired chiral imidazobenzodiazepines **3.3.51**. A gram-scale quantity (10 mmol) of
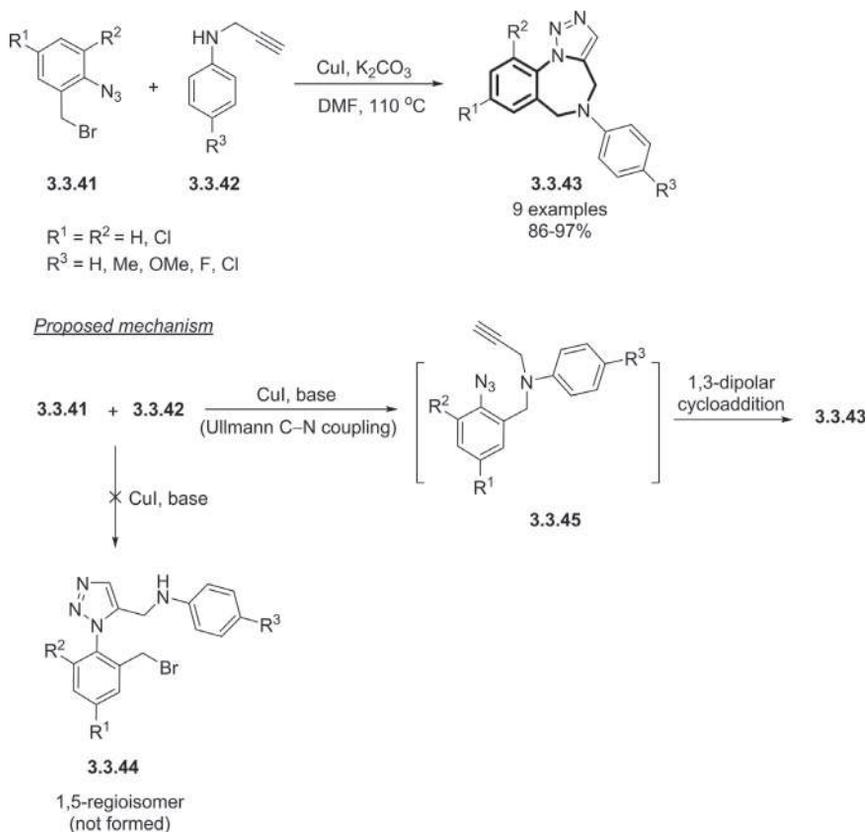
Figure 3.26 Synthesis of triazolo[1,5-a][1,4]benzodiazepine analogs *via* Cu-catalyzed tandem Ullmann C–N coupling/azide-alkyne cycloaddition reactions.

the domino reaction under the standard reaction conditions also gave the desired product **3.3.51** with a good yield of 80%.

In another contribution, Anzini and co-workers synthesized a diverse range of 3,5-di-substituted imidazobenzodiazepines as central benzodi-azepine receptor (CBR) ligands *via* a simple three-step procedure (Anzini et al., 2011). Various benzophenone derivatives **3.3.52** react with either alanine ethyl ester or aspartic acid dimethyl ester hydrochloride (**3.3.53**) in pyridine to furnish the racemic 3-substituted 1,4-benzodiazepinones **3.4.54** (Fig. 3.28). The imidazo annulation of **3.4.54** is accomplished *via* the formation of phosphonate intermediate **3.4.55**, followed by the reaction with isocyanoacetates **3.3.56**, affording the desired imidazoester-fused 1,4-benzodiazepines **3.3.57** in good yields. The presence of imidazoester in the

R¹ = H, Me, Ph, 2-bromophenyl
R² = H, 3-Cl, 4-Cl, 4-Me, 5-F, 5-Br, 5-OMe, 4,5-di-OMe, 6-F, [*d*][1,3]dioxole
X = Cl, Br, I
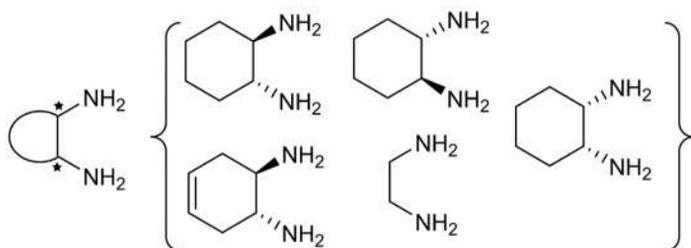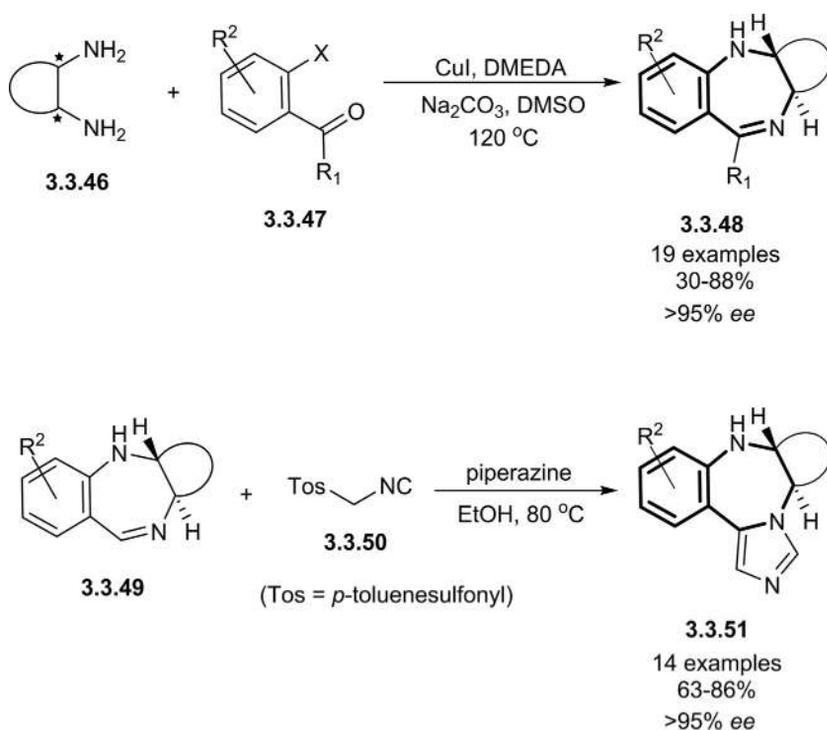
Figure 3.27 Copper-catalyzed synthesis of chiral tetracyclic imidazobenzodiazepines *via* a cascade *N*-arylation-condensation method.

benzodiazepine skeletons significantly increases their inhibitory affinity for CBR with K_i values ranging from 2.3 to 550 nM.

A series of highly condensed fused 1,4–benzodiazepines **3.3.60** have been synthesized through a one-pot FeCl₃-catalyzed condensation reaction of *ortho*–amino–substituted benzaldehydes **3.3.58** and aminomalonic acid ester hydrochlorides **3.3.59**, followed by a tandem intramolecular 1,5-hydride transfer-7-*endo* cyclization reaction sequence (Fig. 3.29) (Liu et al., 2018).
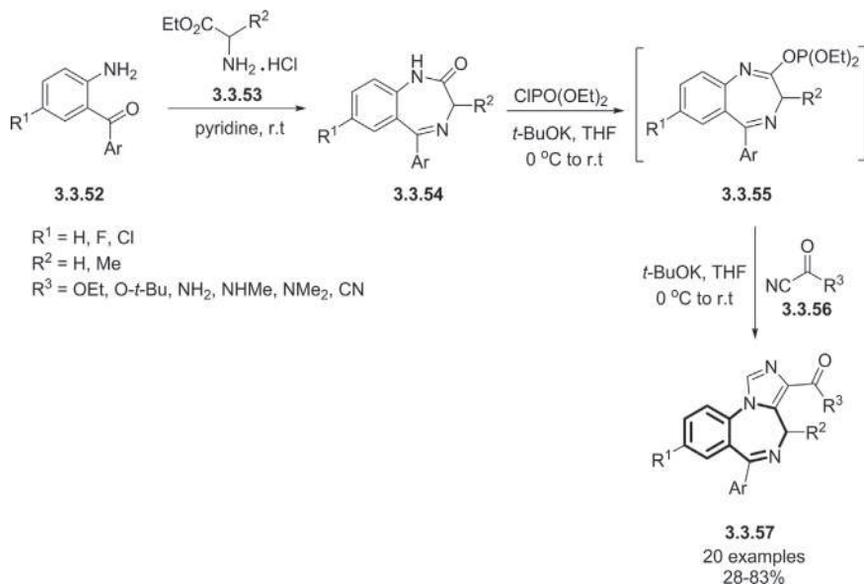
Figure 3.28 Synthesis of 3,5-di-substituted imidazobenzodiazepines.
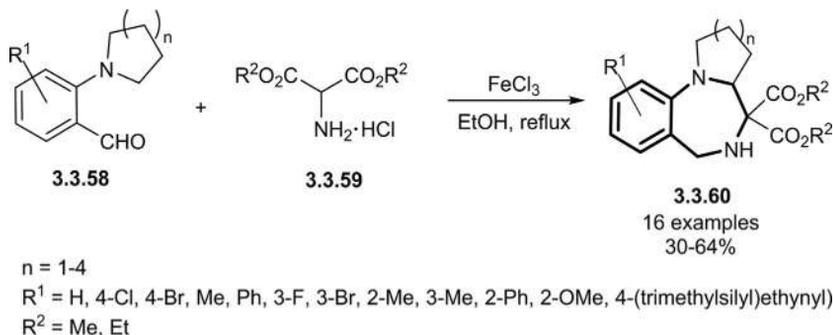
Figure 3.29 FeCl$_3$-catalyzed synthesis of highly condensed fused 1,4-benzodiazepines.

The scope of benzaldehydes **3.3.58** displayed good tolerance of electron-withdrawing and electron–donating substituents to generate the respective 1,4-benzodiazepines **3.3.60** in 30%–64% yields. This strategy provides high step- and atom-economy using an inexpensive catalyst and starting materials, which can also produce the desired benzodiazepines on a gram scale ($n = 1$, $R^1 = H, R^2 = Me$, 8 mmol, 45% yield).

Soural *et al.* demonstrated a stereoselective solid–phase/solution–phase synthesis of various tricyclic benzodiazepines starting from polymer-supported Ser(*t*–Bu)-OH (Králová et al., 2017). They employed several bromoketones **3.3.65** and *ortho*-nitrobenzoic acid **3.3.68** as the commercially available starting materials (Fig. 3.30). The procedure is initiated by
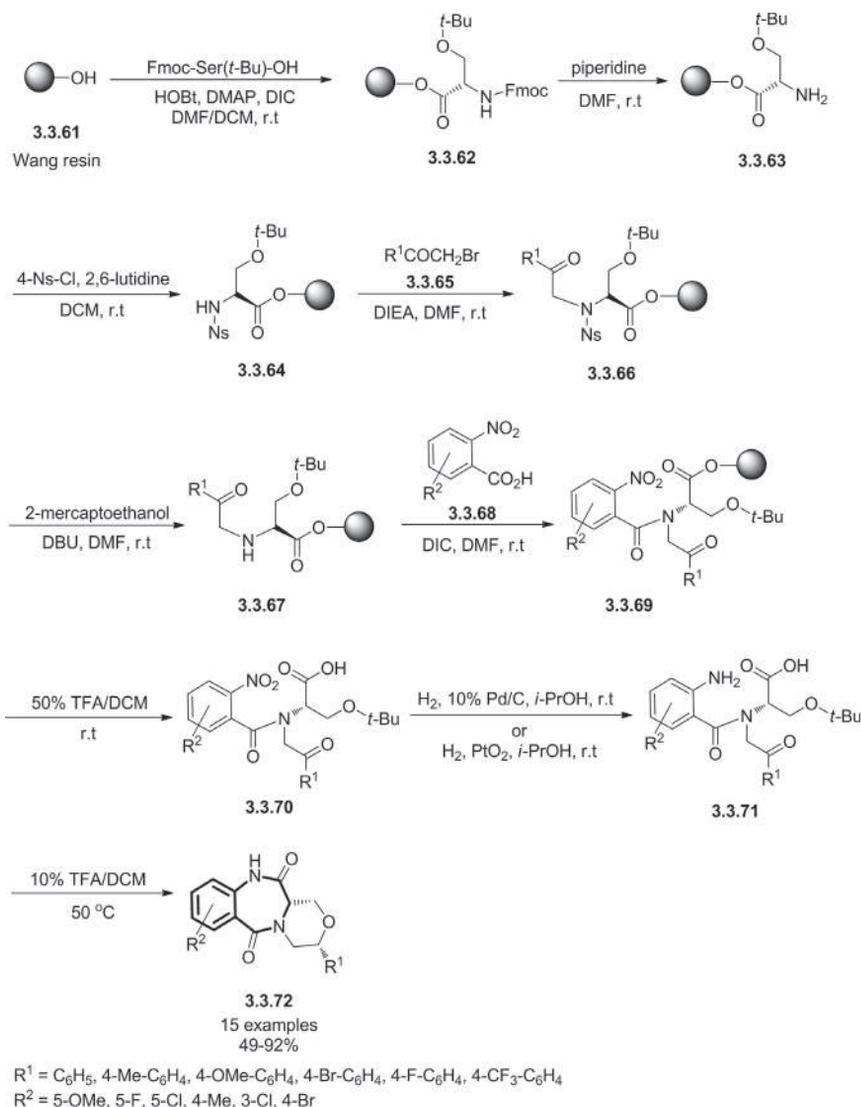
Figure 3.30 Stereoselective solid-phase/solution-phase synthesis of benzo[e][1,4]oxazino[4,3-a][1,4]diazepine-6,12-diones.

the immobilization of Fmoc–Ser(t-Bu)-OH on Wang resin **3.3.61**, followed by the Fmoc–deprotection/Ns–protection sequence to produce the intermediate **3.3.64**. Two successive steps of N-alkylation/Ns-deprotection occur to give the species **3.3.67**, which subsequently undergoes an acylation reaction with 2-nitrobenzoic acid **3.3.68** to generate the adduct
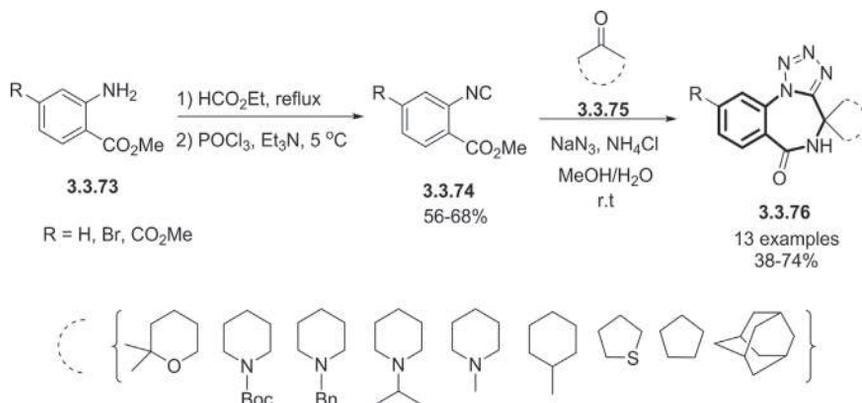
Figure 3.31 Synthesis of tetrazolo[1,5-*a*][1,4]benzodiazepine derivatives *via* a four-component Ugi reaction.

3.3.69. After TFA-facilitated cleavage from the resin, the reduction of the nitro group of the intermediate **3.3.70** provides the corresponding amine compound **3.3.71**. Finally, the TFA-catalyzed intramolecular cyclization reaction of the amino group with the ketone happens to furnish the desired benzo[*e*][1,4]oxazino[4,3-*a*][1,4]diazepine-6,12-diones **3.3.72** as two separable diastereomers with 1:1 to 8:1 ratios. The major diastereomer ($3R,12^aS$) can be isolated by semi-preparative HPLC and the configuration of the stereocenters can be confirmed by 2D NMR analysis. Detailed mechanism and possible intermediates of the reaction from **3.3.71** to **3.3.72** were not provided in this study.

Several novel tetrazolo[1,5-*a*]-fused 1,4-benzodiazepine derivatives have been efficiently synthesized *via* a four-component Ugi reaction approach under mild reaction conditions (Borisov et al., 2012). A formylation reaction of the commercially available methyl 2-aminobenzoates **3.3.73** with a subsequent dehydration initially affords the corresponding methyl 2-isocyanobenzoates **3.3.74** in good yields (Fig. 3.31). The resulting compounds **3.3.74** are then used for the next step with no purification and participate in the four-component Ugi reaction with various aliphatic ketones (**3.3.75**), sodium azide, and ammonium chloride in a mixture of water-methanol (1:3), to give the final 1,4-benzodiazepine products **3.3.76**. It is of note that the aliphatic aldehydes give no desired products due to the self-condensation of the aldehyde molecules. Furthermore, aromatic ketones do not work well in this reaction, which can be explained by the low reactivity of their carbonyl group compared with the aliphatic ketones.
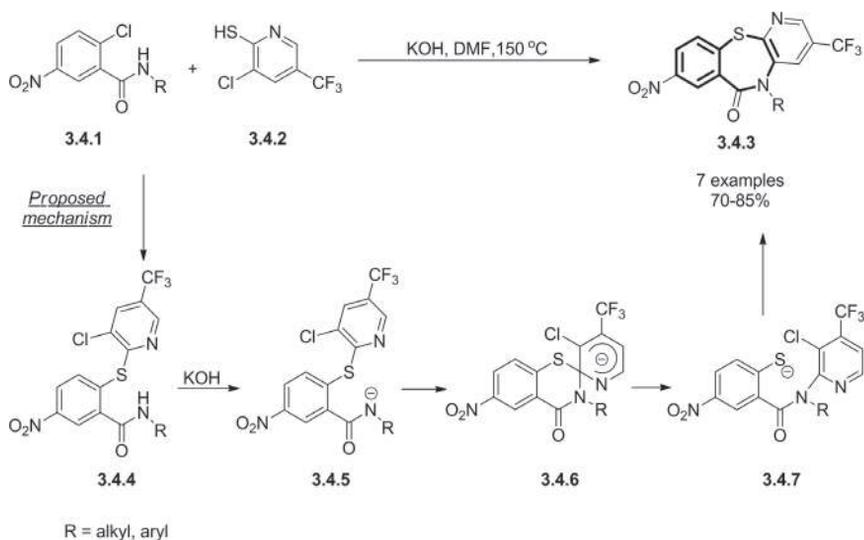
Figure 3.32 One-pot synthesis of benzo[*f*]pyrido[3,2-*b*][1,4]thiazepin-10-(11*H*)-one derivatives under basic metal-free conditions.

3.4 Synthesis of 1,4-benzothiazepines

1,4–Benzothiazepines are one of the important heterocyclic cores for the preparation of many bioactive molecules such as CNS depressants, anxiolytics, anti–HIV agents, antitumors, antiplatelet aggregation inhibitors, and Ca^{2+} and calmodulin antagonists (Garofalo et al., 1993; Geyer et al., 1970; Kawakita et al., 1991; Liao et al., 1999; Neamati et al., 1999; Saha et al., 2015). Despite their biological importance, much less attention has been paid to these versatile scaffolds in both synthetic and medicinal chemistry compared to 1,4-benzodiazpines. In this section, we do not intend to give exhaustive coverage of reported synthetic protocols of 1,4-benzothiazepines, but rather will represent selected recent milestones that occurred in the synthetic tactics for the generation of 1,4-benzothiazepine skeletons.

A simple and efficient route to several benzo[*f*]pyrido[3,2–*b*][1,4] thiazepin-10-(11*H*)-one derivatives **3.4.3** based on a one–pot cyclization re-action of synthetically accessible *N*-substituted 2-chloro-4-nitrobenzamides **3.4.1** with 3-chloro-5-(tri-uoromethyl)pyridine-2-thiol **3.4.2** under basic metal–free conditions has been reported (Fig. 3.32) (Zhao et al., 2013). In this protocol, *N*-protected 2-chloro-5-nitro-benzamide (**3.4.1**) undergoes a nucleophilic addition to 3-chloro-5-(trifluoromethyl)pyridine-2-thiol (**3.4.2**)

to give the intermediate **3.4.4**. Smiles rearrangement then occurs *via* an intramolecular nucleophilic attack to the imido nitrogen of **3.4.4**, followed by a sulfur migration–ring expansion to furnish the desired product **3.4.3**. Note that the steric hindrance on the nitrogen plays a pivotal role in this method as the substrates with bulky protecting groups on nitrogen, *e.g.*, isopropyl or cyclohexyl, do not provide the final products, even at higher temperatures and with longer reaction times. The embedded trifluoromethyl (CF$_3$) substituent within the synthesized benzothiazepinones **3.4.3** can be considered as the major advantage of this research since the CF$_3$ group is an important moiety in medicinal chemistry (Burton et al., 1996; Lin & Jiang, 2000; McClinton & McClinton, 1992; Schlosser, 2006).

Tarasiuk and co–workers developed a multistep synthetic pathway for the preparation of a series of *N*-carboxyalkyl-1,4-benzothiazepine-3(2*H*)-ones (**3.4.16**) bearing 4-alkylcarboxy substituents (Fig. 3.33) (Tarasiuk et al., 2014). For this synthesis, several *N*-(2-chloro-5-nitrobenzyl) amino esters **3.4.13** react with methyl thioglycolate **3.4.14** in DMSO, forming intermediates **3.4.15** that subsequently undergo an intramolecular acylation to yield the final products **3.4.16**. Two different pathways are used to synthesize the amino ester reactants starting from the commercially available aldehyde **3.4.8**. In pathway A, the aldehyde (**3.4.8**) is first condensed with the amino acid hydrochlorides (**3.4.11**) followed by imine bond reduction to form the desired intermediate (**3.4.13**). Pathway B is based on the reduction of the aldehyde **3.4.8** to its corresponding alcohol **3.4.9** with subsequent conversion of the hydroxyl group to chlorine using SOCl$_2$. The coupling reaction of **3.4.10** with the amino acid hydrochlorides (**3.4.11**) finally delivers the related intermediate **3.4.13**. The results have indicated that route B is more efficient due to the reduced formation of unknown and undesired by-products.

A simple method for the synthesis of various 2,3,4,5-tetrahydrobenzo[1,4]thiazepines through cyclization of *N*-acyl-*N*-(2-(phenylthio)ethyl)methaniminium ions has been demonstrated (Deng et al., 2017). 2,3,4,5-Tetrahydrobenzo[1,4]thiazepines are fundamental components in synthesizing commercial JTV-519 and S107 drugs, which belong to cardioprotective agents enhancing RyR-calstabin binding and stabilizing the closed state of the RyR (Andersson & Marks, 2010). These 1,4-benzothiazepine analogs JTV519 and S107 are small–molecule agents that effectively improve RyR–calstabin interaction in both cardiac and neuronal cells and, thus, are identified as promising therapeutic tools for heart failure and myopathies (Lehnart et al., 2008; Tse & Lam, 2001). Several substituted
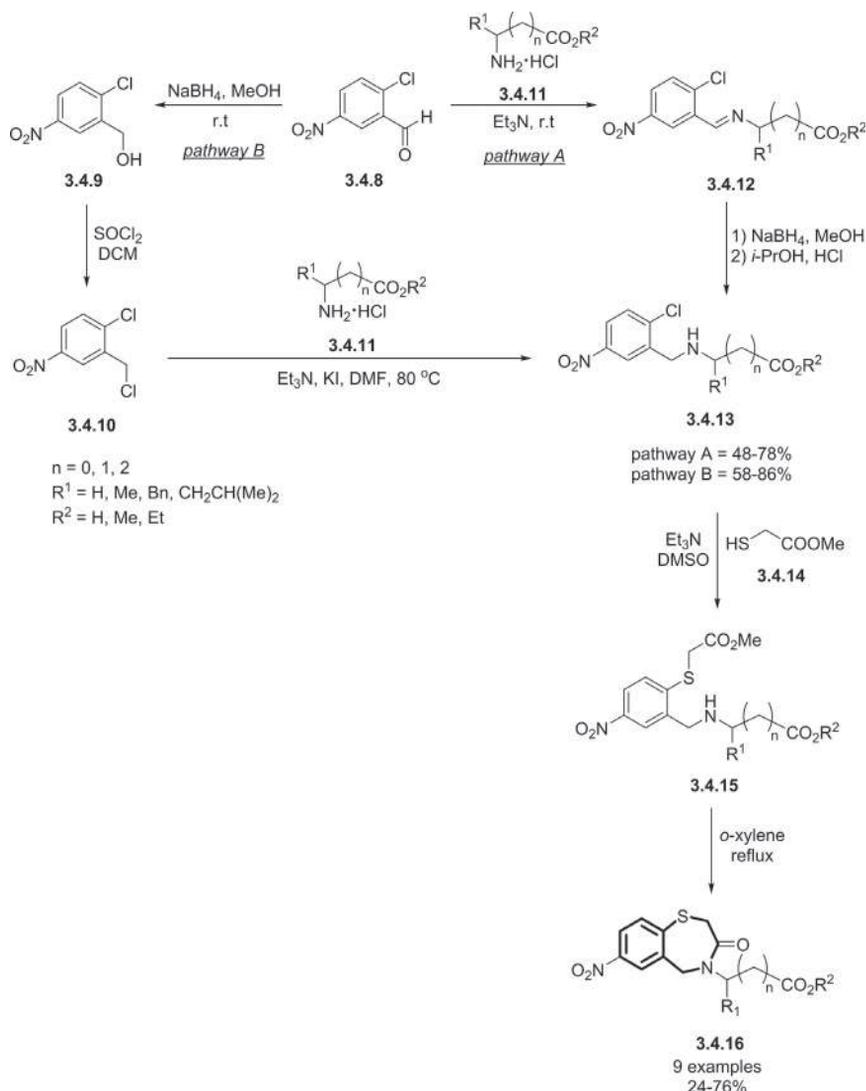
Figure 3.33 Multistep synthesis of *N*-carboxyalkyl-1,4-benzothiazepine-3(2*H*)-one derivatives.

2-(phenylthio)ethan–1-amines (**3.4.19**) can be prepared *via* the nucleophile reaction of a number of commercially available substituted thiophenols (**3.4.17**) and 2-chloroethane-1-amine hydrochloride (**3.4.18**) (Fig. 3.34). The resulting compounds **3.4.19** are subsequently converted to carbamate (Cbz) or acetyl (Ac) derivatives **3.4.21** under mild reaction conditions. Treatment of these intermediates (**3.4.21**) with paraformaldehyde in the
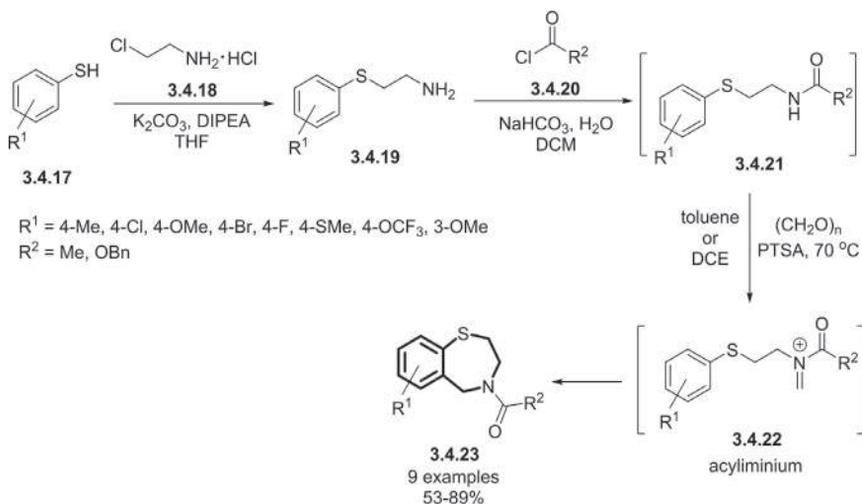
Figure 3.34 Synthesis of 2,3,4,5-tetrahydrobenzo[1,4]thiazepines.

presence of *para*-toluenesulfonic acid (PTSA) leads to the generation of the ring-closed *N*-protected benzothiazepines **3.4.23** through the suggested acyliminium species **3.4.22**. The Cbz group can be easily removed under acidic conditions, affording free amine for further manipulations in organic synthesis.

Mironov's group designed an efficient one-pot four-component Ugi reaction for the synthesis of several 1,4–benzothiazepin–5-one derivatives (Mironov et al., 2004). In their report, the condensation of thiosalicylic acid **3.4.24** with chloroacetone **3.4.25** affords the corresponding 2-(acetoxythio)benzoic acid **3.4.26**. Subsequently, the resulting **3.4.26** is subjected to an intramolecular cyclization with various isocyanides **3.4.27** and amines **3.4.28**, giving the desired products **3.4.29** in good yields after purification by column chromatography (Fig. 3.35). The use of isocyanides **3.4.27** bearing electron–donating substituents results in higher yields of the target compounds **3.4.29**, compared with those with electron–withdrawing counterparts.

For the synthesis of 1,4–benzothiazepin–5-one analogs, a one-pot palladium-catalyzed domino procedure has been described (Zeng & Alper, 2010). A series of readily accessible *N*-tosyl ring-fused aziridines **3.4.31** undergo a palladium-catalyzed ring-opening reaction, followed by an intramolecular carboxamidation with *ortho*-iodothiophenols **3.4.30** in the
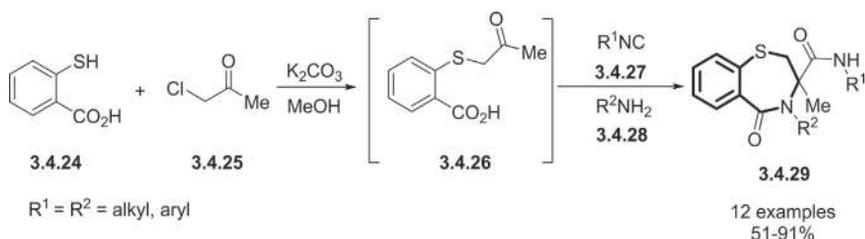
Figure 3.35 One-pot four-component synthesis of 3,3-disubstituted 1,4-benzothiazepin-5-one derivatives.
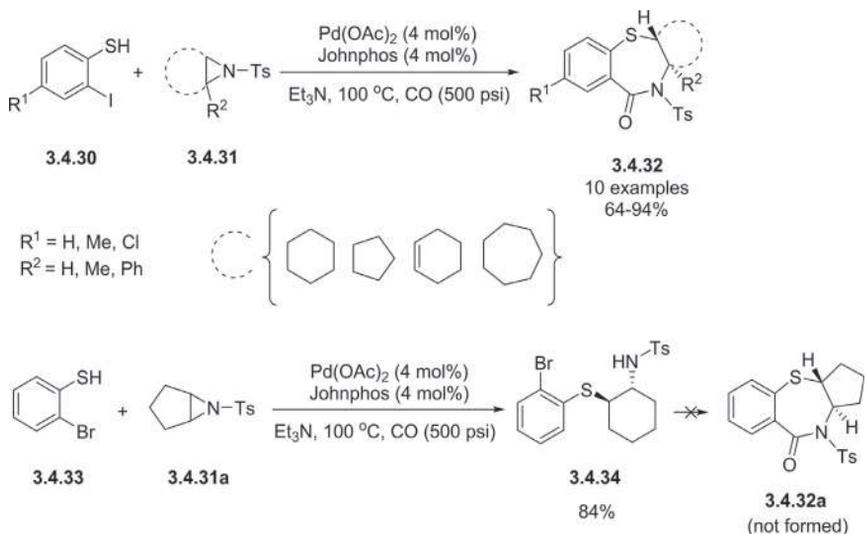
Figure 3.36 One-pot palladium-catalyzed tandem ring-opening/carboxamidation synthesis of 1,4-benzothiazepin-5-one derivatives.

presence of 500 psi of CO at 100 °C to produce various 1,4–benzothiazepin–5–ones **3.4.32** (Fig. 3.36). In this method, the type of halogen plays a pivotal role as the replacement of *ortho*–iodothiophenol with *ortho*–bromothiophenol **3.4.33**, resulted in the desired product **3.4.32a** not being formed – possibly due to the lower rate of oxidative addition of the Pd species into Ar—Br bond (Zeng & Alper, 2010). The *trans* stereochemistry of the products can be determined by measuring the coupling constant of the protons in the ^{1}H NMR spectrum of **3.4.32a**, which can be further confirmed by a NOESY experiment showing no through–space interaction between the protons.

3.5 Conclusion and future directions

This chapter demonstrated the recent progress in the synthesis of functionalized 1,4-benzodiazepines and 1,4-benzothiazepines based on various readily accessible substrates. In many cases, the synthesis occurs regioselectively along with metal-free reaction conditions, high step economy, and broad substrate scopes, which signify their potential synthetic applications in drug design. Furthermore, the majority of structural modifications have been made on the diazepine core with some minor alternations on the benzene ring. Although the preparation of 1,4-benzodiazepines and 1,4-benzothiazepines has been extensively developed during the past decades, asymmetric synthesis of these versatile heterocycles has received very limited attention. The future of 1,4-benzodiazepines and 1,4-benzothiazepines chemistry research will continue to be concentrated on the development of new synthetic strategies, with the main aim of generating novel scaffolds, particularly optically active analogs, for the sustainable production of fine drugs. Research endeavors are also expected to be devoted to employing easily available substrates, and more catalytic and green chemistry procedures.

References

Al-Awar, R. S., Ray, J. E., Hecker, K. A., Joseph, S., Huang, J., Shih, C., … Zhang, F. (2004). Preparation of novel aza-1,7-annulated indoles and their conversion to potent indolocarbazole kinase inhibitors. *Bioorganic & Medicinal Chemistry Letters, 14*(15), 3925–3928. https://doi.org/10.1016/j.bmcl.2004.05.088.

Ali, S. A., Mondal, S. K., Das, T., Manna, S. K., Bera, A., Dafadar, D., … Samanta, S. (2019). One-pot tandem cyclisation to pyrrolo[1,2-*a*][1,4]benzodiazepines: a modified approach to the Pictet–Spengler reaction. *Organic & Biomolecular Chemistry, 17*(18), 4652–4662. https://doi.org/10.1039/c9ob00448c.

Andersson, D. C., & Marks, A. R. (2010). Fixing ryanodine receptor Ca leak - A novel therapeutic strategy for contractile failure in heart and skeletal muscle. *Drug Discovery Today: Disease Mechanisms, 7*(2), e151–e157. https://doi.org/10.1016/j.ddmec.2010.09.009.

Andronati, S., Semenishyna, E., Pavlovsky, V., Simonov, Y., Makan, S., Boyko, I., … Mazepa, A. (2010). Synthesis, structure and affinity of novel 3-alkoxy-1,2-dihydro-3*H*-1,4-benzodiazepin-2-ones for CNS central and peripheral benzodiazepine receptors. *European Journal of Medicinal Chemistry, 45*(4), 1346–1351. https://doi.org/10.1016/j.ejmech.2009.12.027.

Anil, S. M., Shobith, R., Kiran, K. R., Swaroop, T. R., Malleshab, N., & Sadashiva, M. P. (2019). Facile synthesis of 1,4-benzodiazepine-2,5-diones and quinazolinones from amino acids as anti-tubercular agents. *New Journal of Chemistry, 43*(1), 182–187. https://doi.org/10.1039/C8NJ04936J.

Annor-Gyamfi, J. K., Jarrett, J. M., Osazee, J. O., Bialonska, D., Whitted, C., Palau, V. E., & Shilabin, A. G. (2018). Synthesis and biological activity of fused tetracyclic pyrrolo[2,1-*c*][1,4]benzodiazepines. *Heliyon, 4*(2), e00539. https://doi.org/10.1016/j.heliyon.2018.e00539.

Anzini, M., Valenti, S., Braile, C., Cappelli, A., Vomero, S., Alcaro, S., … Biggio, G. (2011). New insight into the central benzodiazepine receptor-ligand interactions: Design, synthesis, biological evaluation, and molecular modeling of 3-substituted 6-phenyl-4*H*-imidazo[1,5-*a*][1,4]benzodiazepines and related compounds. *Journal of Medicinal Chemistry, 54*(16), 5694–5711. https://doi.org/10.1021/jm2001597.

Arora, N., Dhiman, P., Kumar, S., Singh, G., & Monga, V. (2020). Recent advances in synthesis and medicinal chemistry of benzodiazepines. *Bioorganic Chemistry, 97*, 103668. https://doi.org/10.1016/j.bioorg.2020.103668.

Attanasi, O. A., De Crescentini, L., Favi, G., Mantellini, F., & Nicolini, S. (2011). Divergent regioselective synthesis of 2,5,6,7-tetrahydro-1*H*-1,4-diazepin-2-ones and 5*H*-1,4-benzodiazepines. *Journal of Organic Chemistry, 76*(20), 8320–8328. https://doi.org/10.1021/jo201497r.

Bagal, S. K., Brown, A. D., Cox, P. J., Omoto, K., Owen, R. M., Pryde, D. C., … Swain, N. A. (2013). Ion channels as therapeutic targets: a drug discovery perspective. *Journal of Medicinal Chemistry, 56*(3), 593–624. https://doi.org/10.1021/jm3011433.

Borisov, R. S., Polyakov, A. I., Medvedeva, L. A., Guranova, N. I., & Voskressensky, L. G. (2012). Synthesis of tetrazolodiazepines by a five-centered four-component azide Ugi reaction. Scope and limitations. *Russian Chemical Bulletin, 61*, 1609–1615. https://doi.org/10.1007/s11172-012-0214-3.

Burton, D. J., Yang, Z.-Y., & Qiu, W. (1996). Fluorinated ylides and related compounds. *Chemical Reviews, 96*(5), 1641–1716. https://doi.org/10.1021/cr941140s.

Cabedo, N., Pannecoucke, X., & Quirion, J.-C. (2005). An efficient asymmetric synthesis of 2-substituted 1,4-benzodiazepin-3-one as a potential molecular scaffold. *European Journal of Organic Chemistry, 8*, 1590–1596. https://doi.org/10.1002/ejoc.200400682.

Chowdhury, C., Sasmal, A. K., & Achari, B. (2010). An expedient and facile route for the general synthesis of 3-aryl substituted 1,2,3-triazolo[1,5-*a*][1,4]benzodiazepin-6-ones and 1,2,3-triazolo[1,5-*a*][1,5]benzodiazocin-7-ones. *Organic and Biomolecular Chemistry, 8*(21), 4971–4977. https://doi.org/10.1039/C0OB00217H.

Deng, S., Cheng, Z., Ingalls, C., Kontes, F., Yan, J., & Belvedere, S. (2017). Synthesis of 2,3,4,5-tetrahydrobenzo[1,4]thiazepines *via N*-acyliminium cyclization. *Organic Process Research and Development, 21*(11), 1801–1805. https://doi.org/10.1021/acs.oprd.7b00260.

Dinis-Oliveira, R. J. (2017). Metabolic profile of oxazepam and related benzodiazepines: Clinical and forensic aspects. *Drug Metabolism Reviews, 49*(4), 451–463. https://doi.org/10.1080/03602532.2017.1377223.

Farges, R. C. (2003). Peripheral benzodiazepine receptor ligands and their anti-inflammatory effects. *Current Medicinal Chemistry - Anti-Inflammatory and Anti-Allergy Agents, 2*, 409–416. https://doi.org/10.2174/1568014033483680.

Fier, P. S., & Whittaker, A. M. (2017). An atom-economical method to prepare enantiopure benzodiazepines with *N*–carboxyanhydrides. *Organic Letters, 19*(6), 1454–1457. https://doi.org/10.1021/acs.orglett.7b00417.

Fotso, S., Zabriskie, T. M., Proteau, P. J., Flatt, P. M., Santosa, D. A., & Mahmud, T. (2009). Limazepines A–F, pyrrolo[1,4]benzodiazepine Antibiotics from an Indonesian *Micrococcus* sp. *Journal of Natural Products, 72*(4), 690–695. https://doi.org/10.1021/np800827w.

Garofalo, A., Balconi, G., Botta, M., Corelli, F., D'Incalci, M., Fabrizi, G., … Nacci, V. (1993). Thioanalogues of anti-tumor antibiotics. II. Synthesis and preliminary in vitro cytotoxicity evaluation of tricyclic [1,4]benzothiazepine derivatives. *European Journal of Medicinal Chemistry, 28*(3), 213–220. https://doi.org/10.1016/0223-5234(93)90136-3.

Geyer, H. M., Watzman, N., & Buckley, J. P. (1970). Effects of a tranquilizer and two antidepressants on learned and unlearned behaviors. *Journal of Pharmaceutical Sciences, 59*(7), 964–968. https://doi.org/10.1002/jps.2600590709.

Ghelani, S. M., & Naliapara, Y. T. (2016). Design, synthesis, and characterization of 1,3-disubstituted 1,4-benzodiazepine derivatives. *Journal of Heterocyclic Chemistry, 53*(6), 1795–1800. https://doi.org/10.1002/jhet.2486.

Gill, R. K., Kaushik, S. O., Chugh, J., Bansal, S., Shah, A., & Bariwal, J. (2014). Recent development in [1,4]benzodiazepines as potent anticancer agents: A review. *Mini Reviews in Medicinal Chemistry, 14*(3), 229–256. https://doi.org/10.2174/1389557511313990081.

Herrero, S., García-López, M. T., & Herranz, R. (2003). Expedient one-pot synthesis of novel chiral 2-substituted 5-phenyl-1,4-benzodiazepine scaffolds from amino acid–derived amino nitriles. *The Journal of Organic Chemistry, 68*(11), 4582–4585. https://doi.org/10.1021/jo034286c.

Hsu, M. C., Schutt, A. D., Holly, M., Slice, L. W., Sherman, M. I., Richman, D. D., … Volsky, D. J. (1991). Inhibition of HIV replication in acute and chronic infections *in vitro* by a Tat antagonist. *Science (New York), 254*(5039), 1799–1802. https://doi.org/10.1126/science.1763331.

Hurley, L. H., Reck, T., Thurston, D. E., Langley, D. R., Holden, K. G., Hertzberg, R. P., … Mong, S. M. (1988). Pyrrolo[1,4]benzodiazepine antitumor antibiotics: Relationship of DNA alkylation and sequence specificity to the biological activity of natural and synthetic compounds. *Chemical Research in Toxicology, 1*(5), 258–268. https://doi.org/10.1021/tx00005a002.

Joseph, C. G., Wilson, K. R., Wood, M. S., Sorenson, N. B., Phan, D. V., Xiang, Z., … Haskell-Luevano, C. (2008). The 1,4-benzodiazepine-2,5-dione small molecule template results in melanocortin receptor agonists with nanomolar potencies. *Journal of Medicinal Chemistry, 51*(5), 1423–1431. https://doi.org/10.1021/jm701303z.

Kaliszczak, M., Antonow, D., Patel, K. I., Howard, P., Jodrell, D. I., Thurston, D. E., & Guichard, S. M. (2010). Optimization of the antitumor activity of sequence-specific pyrrolobenzodiazepine derivatives based on their affinity for ABC transporters. *The AAPS Journal, 12*(4), 617–627. https://doi.org/10.1208/s12248-010-9225-x.

Kaneko, N. (1994). New 1,4-benzothiazepine derivative, K201, demonstrates cardioprotective effects against sudden cardiac cell death and intracellular calcium blocking action. *Drug Development Research, 33*(4), 429–438. https://doi.org/10.1002/ddr.430330406.

Kanekoa, H., Ikawa, T., Yamamotob, Y., Arulmozhiraja, S., & Akai, H. T. S. (2018). 3-(Triflyloxy)benzynes enable the regiocontrolled cycloaddition of cyclic ureas to synthesize 1,4-benzodiazepine derivatives. *Synlett, 29*(7), 943–948. https://doi.org/10.1055/s-0036-1591924.

Karyakarte, S. D., Sequeira, F. C., Zibreg, G. H., Huang, G., Matthew, J. P., Ferreira, M. M. M., & Chemler, S. R. (2015). Copper-promoted synthesis of 1,4-benzodiazepinones *via* alkene diamination. *Tetrahedron Letters, 56*(23), 3686–3689. https://doi.org/10.1016/j.tetlet.2015.01.171.

Katritzky, A. R., Xua, Y. J., & He, H. Y. (2002). Convenient syntheses of 2,3,4,5-tetrahydro-1,4-benzothiazepines, –1,4-benzoxazepines and –1,4-benzodiazepines. *Journal of the Chemical Society, Perkin Transactions, 1*, 592–598. https://doi.org/10.1039/B111296C.

Kawakita, S., Kinoshita, M., Ishikawa, H., Kagoshima, T., Katori, R., Ishikawa, K., & Hirota, Y. (1991). Efficacy and safety of clentiazem in patients with essential hypertension: Results of an early pilot test. *Clinical Cardiology, 14*(1), 53–60. https://doi.org/10.1002/clc.4960140112.

Králová, P., Maloň, M., & Soural, M. (2017). Stereoselective synthesis of benzo[*e*][1,4]oxazino[4,3-*a*][1,4]diazepine-6,12-diones with two diversity positions. *ACS Combinatorial Science, 19*(12), 770–774. https://doi.org/10.1021/acscombsci.7b00134.

Křemen, F., Gazvoda, M., Kafka, S., Proisl, K., Srholcová, A., Klásek, A., … Košmrlj, J. (2017). Synthesis of 1,4-benzodiazepine-2,5-diones by base promoted ring expansion of 3-aminoquinoline-2,4-diones. *The Journal of Organic Chemistry, 82*(1), 715–722. https://doi.org/10.1021/acs.joc.6b01497.

Krsiak, M., & Sulcova, A. (1990). Differential effects of six structurally related benzodiazepines on some ethological measures of timidity, aggression and locomotion in mice. *Psychopharmacology, 101*(3), 396–402. https://doi.org/10.1007/BF02244060.

Kukla, M. J., Breslin, H. J., Pauwels, R., Fedde, C. L., Miranda, M., Scott, M. K., … Andries, K. (1991). Synthesis and anti–HIV-1 activity of 4,5,6,7-tetrahydro-5-methylimidazo[4,5,1-*jk*][1,4]benzodiazepin-2(1*H*)–one (TIBO) derivatives. *Journal of Medicinal Chemistry, 34*(2), 746–751. https://doi.org/10.1021/jm00106a040.

Kumar, R., Arigela, R. K., Samala, S., & Kundu, B. (2015). Diversity oriented synthesis of indoloazepinobenzimidazole and benzimidazotriazolobenzodiazepine from N^1-alkyne-1,2-diamines. *Chemistry (Weinheim An Der Bergstrasse, Germany), 21*(51), 18828–18833. https://doi.org/10.1002/chem.201502956.

Kundu, P., Mondal, A., Das, B., & Chowdhury, C. (2015). A straightforward approach for the stereoselective synthesis of (*E*)-2-aryl/vinylmethylidene-1,4-benzodiazepines and -1,4-benzodiazepin-5-ones through palladium/charcoal-catalysed reactions. *Advanced Synthesis & Catalysis, 357*(16–17), 3737–3752. https://doi.org/10.1002/adsc.201500661.

Lehnart, S. E., Mongillo, M., Bellinger, A., Lindegger, N., Chen, B.-X., Hsueh, W., … Marks, A. R. (2008). Leaky Ca^{2+} release channel/ryanodine receptor 2 causes seizures and sudden cardiac death in mice. *Journal of Clinical Investigation, 118*(6), 2230–2245. https://doi.org/10.1172/JCI35346.

Leimgruber, W., Stefanović, V., Schenker, F., Karr, A., & Berger, J. (1965). Isolation and characterization of anthramycin, a new antitumor antibiotic. *Journal of the American Chemical Society, 87*(24), 5791–5793. https://doi.org/10.1021/ja00952a050.

Li, X., Cao, H., Zhang, C., Furtmueller, R., Fuchs, K., Huck, S., … Cook, J. M. (2003). Synthesis, *in vitro* affinity, and efficacy of a bis 8-ethynyl-4*H*–imidazo[1,5*a*]- [1,4]benzodiazepine analogue, the first bivalent α5 subtype selective BzR/GABA(A) antagonist. *Journal of Medicinal Chemistry, 46*(26), 5567–5570. https://doi.org/10.1021/jm034164c.

Liao, Y., Venhuis, B. J., Rodenhuis, N., Timmerman, W., Wikström, H., Meier, E., … Sundell, S. (1999). New (sulfonyloxy)piperazinyldibenzazepines as potential atypical antipsychotics: Chemistry and pharmacological evaluation. *Journal of Medicinal Chemistry, 42*(12), 2235–2244. https://doi.org/10.1021/jm991005d.

Lin, P., & Jiang, J. (2000). Synthesis of monotrifluoromethyl–substituted saturated cycles. *Tetrahedron, 56*(23), 3635–3671. https://doi.org/10.1016/S0040-4020(00)00158-7.

Liu, S., Zhao, T., Qu, J., & Wang, B. (2018). Expedient synthesis of 1,4-benzodiazepines *via* a tandem condensation/[1,5]-hydride transfer/cyclization process. *Advanced Synthesis & Catalysis, 360*(21), 4094–4098. https://doi.org/10.1002/adsc.201800781.

Loudni, L., Roche, J., Potiron, V., Clarhaut, J., Bachmann, C., Gesson, J.-P., & Tranoy-Opalinski, I. (2007). Design, synthesis and biological evaluation of 1,4-benzodiazepine-2,5–dione-based HDAC inhibitors. *Bioorganic and Medicinal Chemistry Letters, 17*(17), 4819–4823. https://doi.org/10.1016/j.bmcl.2007.06.067.

Lu, D., Ma, C., Yuan, S., Zhou, L., & Zeng, Q. (2015). Synthesis of chiral 3-substituted 1,3,4,5-tetrahydro-1,4-benzo-diazepin-2-ones *via* a domino copper-catalyzed S_N2/coupling reaction. *Advanced Synthesis and Catalysis, 357*(16–17), 3491–3494. https://doi.org/10.1002/adsc.201500477.

Majumdar, K. C., & Ganai, S. (2013). An expedient approach to substituted triazolo[1,5-*a*][1,4]benzodiazepines *via* Cu-catalyzed tandem Ullmann C–N coupling/azide-alkyne cycloaddition. *Tetrahedron Letters, 54*(46), 6192–6195. https://doi.org/10.1016/j.tetlet.2013.08.125.

McClinton, M. A., & McClinton, D. A. (1992). Trifluoromethylations and related reactions in organic chemistry. *Tetrahedron, 48*(32), 6555–6666. https://doi.org/10.1016/S0040-4020(01)80011-9.

Meyer, A. G., Bissember, A. C., Hyland, C. J. T., Williams, C. C., Szabo, M., Pearsall, M. A., … Olivier, W. J. (2020). Seven-membered rings. In G. W. Gribble, & J. A. Joule (Eds.), *Progress in Heterocyclic Chemistry* (pp. 597–647). Amsterdam: Elsevier Vol. 31. https://doi.org/10.1016/B978-0-12-819962-6.00016-6.

Meyer, A. G., Bissember, A. C., Hyland, C. J. T., Williams, C. C., Szabo, M., Wales, S. M., … Olivier, W. J. (2021). Seven-membered rings. *Progress in Heterocyclic Chemistry* (pp. 565–614). Amsterdam: Elsevier Vol. 32. https://doi.org/10.1016/B978-0-323-89812-6.00016-X.

Mironov, M. A., Ivantsova, M. N., Tokareva, M. I., & Mokrushin, V. S. (2004). New synthesis of 1,4-benzothiazepin-5-ones. *Russian Chemical Bulletin, 53*, 1232–1236. https://doi.org/10.1023/B:RUCB.0000042278.14695.b3.

Mohamed, L. W., & El-Yamany, M. F. (2012). Design and synthesis of novel 1,4-benzodiazepine derivatives and their biological evaluation as cholinesterase inhibitors. *Archives of Pharmacal Research, 35*(8), 1369–1377. https://doi.org/10.1007/s12272-012-0806-3.

Murugesh, V., Harish, B., Adiseshu, M., Nanubolu, J. B., & Suresh, S. (2016). Tandem copper-catalyzed *N*-arylation-condensation and van Leusen reaction: Synthesis of 1,4-benzodiazepines and imidazobenzodiazepines (ImBDs). *Advanced Synthesis and Catalysis, 358*(8), 1309–1321. https://doi.org/10.1002/adsc.201501048.

Nadin, A., López, J. M. S., Owens, A. P., Howells, D. M., Talbot, A. C., & Harrison, T. (2003). New synthesis of 1,3-dihydro-1,4-benzodiazepin-2(2*H*)-ones and 3-amino-1,3-dihydro-1,4-benzodiazepin-2(2*H*)-ones: Pd-catalyzed cross-coupling of imidoyl chlorides with organoboronic acids. *The Journal of Organic Chemistry, 68*(7), 2844–2852. https://doi.org/10.1021/jo026860a.

Nakamura, T., Koizumi, F., Kaneko, N., Tamura, T., Chiwaki, F., Koh, Y., … Nishio, K. (2001). Reversal of cisplatin resistance by the 1,4-benzothiazepine derivative, JTV-519. *Japanese Journal of Cancer Research, 92*(6), 597–602. https://doi.org/10.1111/j.1349-7006.2001.tb01136.x.

Neamati, N., Turpin, J. A., Winslow, H. E., Christensen, J. L., Williamson, K., Orr, A., … Nacci, V. (1999). Thiazolothiazepine inhibitors of HIV-1 integrase. *Journal of Medicinal Chemistry, 42*(17), 3334–3341. https://doi.org/10.1021/jm990047z.

Otto, M. J. (1992). Synthesis and anti-HIV activity of pyrrolo-[1,2-*d*]-(1,4)-benzodiazepin-6-ones. *Bioorganic and Medicinal Chemistry Letters, 7*(12), 1639–1644. https://doi.org/10.1016/S0960-894X(00)80447-3.

Pellissier, H., & Santelli, M. (2003). The use of arynes in organic synthesis. *Tetrahedron, 59*(14), 701–730. https://doi.org/10.1016/S0040-4020(03)00296-5.

Pertejo, P., Corres, N., Torroba, T., & García-Valverde, M. (2015). Reversal of diastereoselectivity in the synthesis of peptidomimetic 3-carboxamide-1,4-benzodiazepin-5-ones. *Organic Letters, 17*(3), 612–615. https://doi.org/10.1021/ol503628r.

Pertejo, P., Pena-Calleja, P., Carreira-Barral, I., Quesada, R., Cordero, N. A., Rodriguez, F. J., & Garcia-Valverde, M. (2017). Novel pyrrolobenzodiazepine and pyrroloquinoline scaffolds synthesized by a simple and highly selective Ugi/cyclization sequence. *Organic and Biomolecular Chemistry, 15*(36), 7549–7557. https://doi.org/10.1039/C7OB01807J.

Popp, T. A., Uhl, E., Ong, D. N., Dittrich, S., & Bracher, F. (2016). A new approach to monoprotected 1,4-benzodiazepines *via* a one-pot *N*-deprotection/reductive cyclization procedure. *Tetrahedron, 72*(13), 1668–1674. https://doi.org/10.1016/j.tet.2016.02.019.

Saha, D., Jaina, G., & Sharma, A. (2015). Benzothiazepines: Chemistry of a privileged scaffold. *RSC Advances, 5*(86), 70619–70639. https://doi.org/10.1039/C5RA12422K.

Sandra, C.-M., Eduardo, C. C., Simon, H.-O., Teresa, R. A., Antonio, N. C., Lijanova, I. V., & Marcos, M.-G. (2012). Anticancer activity and anti-inflammatory studies of 5-aryl-1,4-benzodiazepine derivatives. *Anti-Cancer Agents in Medicinal Chemistry, 12*(6), 611–618. https://doi.org/10.2174/187152012800617713.

Sasiambarrena, L. D., Barri, I. A., Fraga, G. G., Bravo, R. D., & Ponzinibbio, A. (2019). Facile synthesis of 4-substituted 1,2,4,5-tetrahydro-1,4-benzodiazepine-3-ones by reductive cyclization of 2-chloro-*N*-(2-nitrobenzyl)acetamides. *Tetrahedron Letters, 60*(13), 264–267. https://doi.org/10.1016/j.tetlet.2018.12.029.

Schlosser, M. (2006). CF_3-bearing aromatic and heterocyclic building blocks. *Angewandte Chemie International Edition, 45*(33), 5432–5446. https://doi.org/10.1002/anie.200600449.

Shin, J., Lee, J., Ko, D., De, N., & Yoo, E. J. (2017). Synthesis of fused polycyclic 1,4-benzodiazepines *via* metal–free cascade [5 + 2]/[2 + 2] cycloadditions. *Organic Letters, 19*(11), 2901–2904. https://doi.org/10.1021/acs.orglett.7b01137.

Spencer, J., Rathnam, R. P., Harvey, A. L., Clements, C. J., Clark, R. L., Barrett, M. P., … Mackay, S. P. (2011). Synthesis and biological evaluation of 1,4-benzodiazepin-2-ones with antitrypanosomal activity. *Bioorganic and Medicinal Chemistry, 19*(5), 1802–1815. https://doi.org/10.1016/j.bmc.2011.01.010.

Sternbach, L. H. (1979). The benzodiazepine story. *Journal of Medicinal Chemistry, 22*(1), 1–7. https://doi.org/10.1021/jm00187a001.

Sternbach, L. H., & Reeder, E. (1961). Quinazolines and 1,4-benzodiazepines. II. The rearrangement of 6-chloro-2-chloromethyl-4-phenylquinazoline 3-oxide into 2-amino derivatives of 7-chloro-5-phenyl-3*H*-1,4-benzodiazepine 4-oxide. *The Journal of Organic Chemistry, 26*(4), 1111–1118. https://doi.org/10.1021/jo01063a034.

Sulcova, A., & Krsiak, M. (1989). Differences among nine 1,4-benzodiazepines: An ethopharmacological evaluation in mice. *Psychopharmacology, 97*(2), 157–159. https://doi.org/10.1007/BF00442240.

Taher, A. T., & Mohammed, L. W. (2013). Synthesis of new 1,3,4-benzotriazepin-5-one derivatives and their biological evaluation as antitumor agents. *Archives of Pharmacal Research, 36*(6), 684–693. https://doi.org/10.1007/s12272-013-0081-y.

Tarasiuk, T. M., Volovnenko, T. A., Volovenko, Y. M., Medviediev, V. V., & Shishkin, O. V. (2014). Synthesis of *N*-carboxyalkyl-1,4-benzothiazepine-3(2*H*)-one derivatives using esters of *N*-(2-chloro-5-nitrobenzyl)amino acids. *Monatshefte Für Chemie - Chemical Monthly, 145*, 483–489. https://doi.org/10.1007/s00706-013-1133-1.

Tse, H. F., & Lam, W. F. (2001). JTV-519 Japan Tobacco. *Current Opinion in Investigational Drugs, 2*(7), 936–939.

Varvounis, G. (2016). An update on the synthesis of pyrrolo[1,4]benzodiazepines. *Molecules, 21*(2), 154. https://doi.org/10.3390/molecules21020154.

Verdié, P., Subra, G., Feliu, L., Sanchez, P., Bergé, G., Garcin, G., & Martinez, J. (2007). On-line synthesis of pseudopeptide library incorporating a benzodiazepinone turn mimic: Biological evaluation on MC1 receptors. *Journal of Combinatorial Chemistry, 9*(2), 254–262. https://doi.org/10.1021/cc060054q.

Voskressensky, L. G., Borisova, T. N., Babakhanova, M. I., Akbulatov, S. V., Tsarkova, A. S., Titov, A. A., … Varlamova, A. V. (2012). On the reaction of fused benzodiazepines with alkynes containing electron–withdrawing groups. *Russian Chemical Bulletin, 61*, 1220–1230. https://doi.org/10.1007/s11172-012-0166-7.

Wang, H. J., Wang, Y., Camara, F., Paquette, W. D., Csakai, A. J., & Mangette, J. E. (2011). Facile synthesis of 6,12*b*-diaza-dibenzo[*a,h*]azulen-7-ones and benzo[*f*]pyrrolo[1,2-*a*][1,4]diazepin-4-ones *via* CuI/l-proline catalyzed intramolecular *N*-arylation. *Tetrahedron Letters, 52*(4), 541–543. https://doi.org/10.1016/j.tetlet.2010.11.117.

Wang, S., Shen, Y.-B., Li, L.-F., Qiu, B., Yu, L., Liu, Q., & Xiao, J. (2019). Aminobenzaldehydes: Access to indole-1,2-fused 1,4-benzodiazepines. *Organic Letters, 21*(22), 8904–8908. https://doi.org/10.1021/acs.orglett.9b03011.

Wang, Y., Chen, M., & Ding, M.-W. (2013). A simple and one-pot synthesis of 2,3,4,5-tetrasubstituted-4,5-dihydro-3*H*-1,4-benzodiazepines. *Tetrahedron, 69*(43), 9056–9062. https://doi.org/10.1016/j.tet.2013.08.034.

Welsch, M. E., Snyder, S. A., & Stockwell, B. R. (2010). Privileged scaffolds for library design and drug discovery. *Current Opinion in Chemical Biology, 14*(3), 347–361. https://doi.org/10.1016/j.cbpa.2010.02.018.

Xu, Z., Wang, Q., & Zhu, J. (2018). Metamorphosis of cycloalkenes for the divergent total synthesis of polycyclic indole alkaloids. *Chemical Society Reviews, 47*(21), 7882–7898. https://doi.org/10.1039/C8CS00454D.

Yar, M., McGarrigle, E. M., & Aggarwal, V. K. (2009). Bromoethylsulfonium salt–A more effective annulation agent for the synthesis of 6- and 7-membered 1,4-heterocyclic compounds. *Organic Letters, 11*(2), 257–260. https://doi.org/10.1021/ol8023727.

Ye, N., Neumeyer, J. L., Baldessarini, R. J., Zhen, X., & Zhang, A. (2013). Update 1 of: Recent progress in development of dopamine receptor subtype-selective agents: Potential therapeutics for neurological and psychiatric disorders. *Chemical Reviews, 113*(5), PR123–PR178. https://doi.org/10.1021/cr300113a.

Zamani, F., Pyne, S. G., & Hyland, C. J. T (2017). Oxazolidinones and 2,5-dihydrofurans *via* zinc-catalysed regioselective allenylation reactions of L-α-amino aldehydes. *The Journal of Organic Chemistry, 82*(13), 6819–6830. https://doi.org/10.1021/acs.joc.7b00969.

Zeng, F., & Alper, H. (2010). Palladium-catalyzed domino ring-opening/carboxamidation reactions of *N*-tosyl aziridines and 2-iodothiophenols: A facile and efficient approach to 1,4-benzothiazepin-5-ones. *Organic Letters, 12*(23), 5567–5569. https://doi.org/10.1021/ol102394h.

Zhao, Y., Dai, Q., Chen, Z., Zhang, Q., Bai, Y., & Ma, C. (2013). One pot regioselective synthesis of a small library of dibenzo[*b,f*][1,4]thiazepin-11(10*H*)-ones *via* Smiles rearrangement. *ACS Combinatorial Science, 15*(2), 130–134. https://doi.org/10.1021/co300139s.

Non-Print Items

Abstract

This chapter gives an overview of recent developments in the synthesis of 1,4–benzodiazepines and 1,4-benzothiazepines utilizing readily available reactants under practical synthetic procedures.

Biological behavior of 1,4-benzodiazepines and 1,4-benzothiazepines

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Chapter outline

4.1 Introduction

After the discovery of the first benzodiazepine, chlordiazepoxide (1,4–benzodiazepine derivative), conventional sedative drugs such as barbiturates were largely supplanted by benzodiazepines in the 1960s due to their high therapeutic index and minor adverse events. Soon later, 1,4–benzodiazepines

became one of the major FDA-approved medications employed in phar-macological treatments as anxiolytics, anticonvulsants, sedatives/hypnotics, anesthesia, and muscle relaxants (Balon & Starcevic, 2020; Garakani et al., 2020). 1,4-Benzothiazepines have also exhibited a broad range of biological activities in both cardiovascular and central nervous systems (Saha et al., 2015; Pathania et al., 2019; Inagaki et al., 2000). The pharmacologic activity of 1,4-benzodiazepines and 1,4-benzothiazepines is presumed to be exerted through their interaction with specific binding sites within the cells (Möhler & Okada, 1977; Tallman et al., 1980; Olsen, 1982; Hagiwara et al., 1997), which may produce various therapeutic effects. For example, some 1,4-benzodiazepines are more potent than others as anticonvulsants due to their differences in affinity for receptor subtypes. Nonetheless, while these pharmaceutically important heterocycles significantly vary in their basic receptor affinity, the qualitative concept of their drug-receptor interaction is relatively similar. This chapter principally describes the general metabolic mechanisms of 1,4-benzodiazepines and 1,4-benzothiazepines, and their detailed interaction mechanisms with various cellular receptors and channels, although the structures of receptors and channels have received limited reports.

4.2 Metabolism of 1,4-benzodiazepines and 1,4-benzothiazepines

The main pharmacological preferences of 1,4-benzodiazepine and 1,4-benzothiazepine drugs are rapid onset of action, high rates of efficacy, and low toxicity (Riss et al., 2008). The onset of action depends on the drug absorption rate from the gastrointestinal tract, and the clinical efficacy is attributed to the rate and extent of drug distribution to the target tissues and the rate of drug elimination from the body. Following an oral administration, most of these drugs are rapidly absorbed from the digestive tract with high bioavailability ranging from 80% to 100% (see Chapters 5 and 10 for more details). Midazolam is an exception among 1,4-benzodiazepines that displays low oral bioavailability because of its extensive metabolism in intestinal epithelial cells, decreasing approximately 50% of the initial drug dosage to reach the bloodstream (Anderson & Miller, 2002). This phenomenon is the main reason that midazolam is frequently administrated through intravenous and intramuscular routes (See Chapter 5 for more details). After absorption from the gastrointestinal tract, 1,4-benzodiazepines and 1,4-benzothiazepines

bind with plasma proteins such as human serum albumin (HSA) and α-1-acid glycoprotein (AAG) (S. Schmidt et al., 2010; Wanat, 2020). Plasma protein binding is a factor that may affect the overall body distribution, tissue penetration rate, and pharmacokinetics of a drug. Although only the free and unbound portion of the drug is responsible for the effects, protein binding can help to improve the longevity of the effects (Arendt et al., 1987). As a general concept, binding to plasma proteins may facilitate the transportation of hydrophobic medications within the aqueous medium of the human body. However, it is evident that highly protein-bound drugs often cannot cross the blood–brain barrier (BBB) because of the high molecular mass of the drug-protein complex, which leads to a significant reduction of their pharmacological efficacy (Wanat, 2020). Considering the high protein binding capability of benzodiazepines and benzothiazepines, it is expected that the number of unbound benzodiazepines and benzothiazepines is probably more important to cause a pharmacological response. However, several studies have demonstrated that the drug-protein complexes of these heterocycles can easily penetrate BBB to a surprisingly high extent (Wanat, 2020; Jones et al., 1988; Tanaka & Mizojiri, 1999). To explain this phenomenon, it has been suggested that the protein undergoes conformational changes when connecting to capillary walls so that the drug molecule is readily released from the drug-protein complex. Nonetheless, drug passage through BBB is a complex process and further studies are needed. Following the entry of 1,4-benzodiazepines and 1,4-benzothiazepines into the CNS, they attach to specific receptors on the neurons to exerts their clinically relevant impacts (see Section 4.3).

The central nervous system (CNS) basically constitutes the blood–brain barrier (BBB) to protect itself from toxic xenobiotics. BBB, which is composed of blood vessels and endothelial cells, also provides oxygen and nutrients for the brain cells (Wanat, 2020). Anxiolytics, sedatives, and hypnotics with low molecular mass and high lipophilicity can easily cross BBB by diffusion and gradually reach the equilibrium state in the cells. Biotransformation of drugs into water-soluble metabolites is a key mechanism to prevent the accumulation of these molecules in the body, which subsequently can be excreted from the kidneys (Handschin & Meyer, 2003). The metabolic pathway of benzodiazepines and benzothiazepines includes two phases; hepatic oxidation, reduction, or hydrolysis catalyzed by cytochrome P450 (CYP) enzymes (phase I) and glucuronide conjugation (phase II). The cytochrome P450 family has 57 various types of isozymes, playing fundamental roles in drug metabolism and drug pharmacokinetics

(Veith et al., 2009). These enzymes are encoded by their corresponding genes in the liver and small intestine, where they metabolize approximately 80% of marketed drugs in humans (Dasgupta, 2020). CYP3A4 is the most abundant CYP enzyme (30%) that is responsible for the metabolism of more than 50% of known drugs, including benzodiazepines and benzothiazepines, in the human liver. Phase I metabolism converts the drug into more polar metabolites by introducing functional groups such as OH, $-NH_2$, or $-SH$, which leads to either activation or deactivation of the parent molecule. Most 1,4-benzodiazepines and 1,4-benzothiazepines usually undergo metabolic phase I to generate pharmacologically active metabolites, where the elimination half-lives of both drug and its metabolites determine the duration of action in the body. Phase II includes the conjugation of the drug or its metabolites with glutathione, glucuronide, and sulfate. This metabolic step is typically catalyzed by several transferase enzymes such uridine 5′-diphospho(UDP)-glucuronosyltransferases, glutathione S-transferases, sulfotransferases, N-acetyltransferases, to generate conjugated metabolites with larger sizes and better water solubility, which can be easily excreted into the bile and eliminated in the urine and feces (Mandrioli et al., 2008) (see Chapters 5 and 10 for more details). Several 1,4-benzodiazepines, e.g., lorazepam, temazepam, and oxazepam, tend to be directly metabolized through phase II reactions into inactive metabolites. Such benzodiazepines are potentially better candidates for patients with hepatic dysfunction.

4.3 GABA metabolism and GABA receptors

γ-Aminobutyric acid (GABA) is an amino acid ($C_4H_9NO_2$) that acts as the most important inhibitory neurotransmitter in the central nervous system. It binds to post-synaptic GABA receptors, for example, $GABA_A$ and $GABA_B$, and stimulates relaxation in the brain by balancing excitation through regulating ion channels, hyperpolarizing the cells, and inhibiting an action potential (AP) at the synaptic level (Kuffler & Edwards, 1958). GABA is primarily formed in the cytoplasm of the presynaptic neurons called GABAergic *via* the decarboxylation of glutamate catalyzed by glutamate decarboxylase (GAD). The amino acid is then stored into synaptic vesicles by the corresponding vesicular transporter, followed by docking the vesicles into the plasma cell membranes. When an action potential reaches the axon terminals, Ca^{2+} channels open and the ion concentration increases on the outer surface of neurotransmitter vesicles. This phenomenon facilitates the fusion of the vesicles within the plasma membrane where GABA is

finally released into the synaptic cleft and subsequently binds with GABA receptors. To avoid the persistence of GABA activity, it is removed from the synaptic cleft by the astrocytes and transferred to mitochondria where two sequential metabolic pathways occur (Malaspina et al., 2016). The GABA molecules first undergo a transamination reaction catalyzed by mitochondrial GABA transaminase enzyme (GABA-T) to form the corresponding succinic semialdehyde (SSA). This is followed by the oxidation of SSA by succinic semi-aldehyde dehydrogenase to succinate in the tricarboxylic acid cycle (TCA cycle). Dysfunction in GABAergic signaling is attributed to many neurologic and psychiatric disorders such as epilepsy, anxiety, and insomnia, in which regulation of GABA signaling is the primary target of pharmacologic therapies in neurology and psychiatry (Kondziella, 2017). GABA receptors are subdivided into GABA$_A$ and GABA$_B$ subtypes. The GABA$_A$ receptor is a pentameric membrane protein that serves as an ionotropic receptor to inhibit the formation of an action potential. These receptors are mainly concentrated in the limbic system and the retina considered fast synaptic inhibitors. Upon binding GABA to the receptor, chloride ion channels open and the negatively charged ions move across the cell membrane into the positively charged intracellular space, resulting in an inhibitory effect by reducing the resting potential of the cell and inhibiting the neurotransmission (Sigel & Steinmann, 2012). The GABA$_B$ receptor is a G-protein coupled receptor (GPCR) considered a slow synaptic inhibitor, which is mainly located in the thalamic pathways and cerebral cortex. In the presence of GABA, the GABA$_B$ receptor increases postsynaptic conductance of K^+ channels where adenylyl cyclase is subsequently activated, leading to decreasing presynaptic calcium entry and inhibiting the transmission of the action potential (Padgett & Slesinger, 2010).

GABA$_A$ receptors are typically constructed from two α (α1–6), two β (β1-3), and one γ (γ1-3) subunits aligned in a counterclockwise direction of γ-β-α-β-α, which create a sustained margin around the central chloride ion-conducting channel. Each subunit consists of an extracellular N-terminus (NT), four transmembrane α-helices (M1–4), and an extracellular C-terminus (CT), where the NTs are mainly composed of β-sheets furnishing agonist binding sites, and the M domains form the ion channel (Schofield et al., 1987). The two $\alpha\beta$ extracellular N-terminus interfaces create two GABA binding sites consisted of the principal β subunit (+) side and the complementary (−) face of the α subunit. A single extracellular $\alpha+/\gamma-$ interface forms one binding site for benzodiazepines, which is called the benzodiazepine site (Ramerstorfer et al., 2011; Zhu et al., 2018). Figs. 4.1
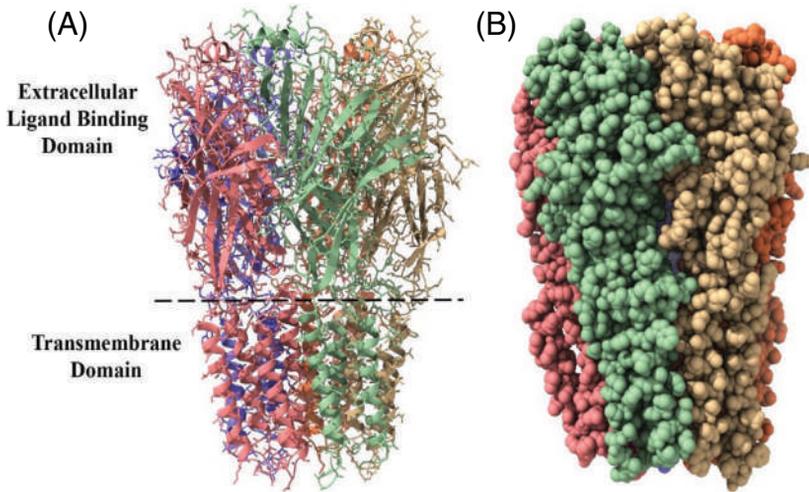
Figure 4.1 (A) Ribbon representation and (B) space-filling models of a GABA$_A$ receptor (side view) (PDB: 6DW0).
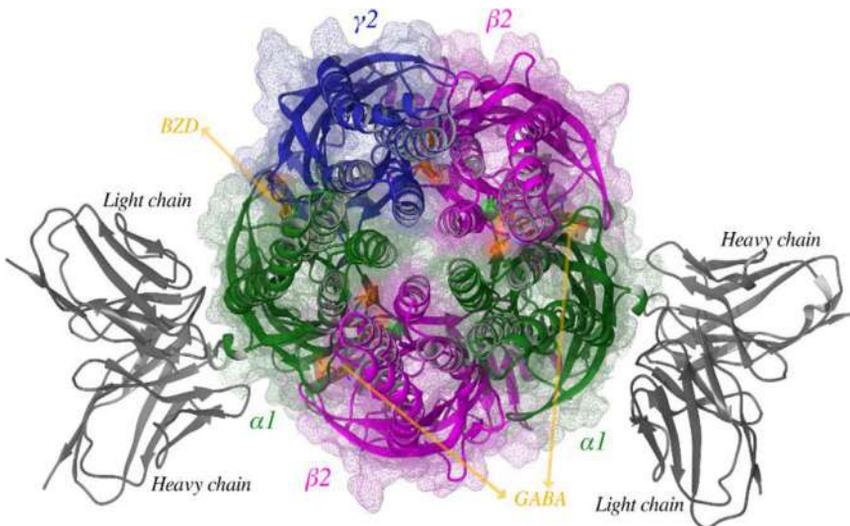
Figure 4.2 Ribbon representation of $\alpha 1\beta 2\gamma 2$ human GABA$_A$ receptor in complex with diazepam (BZD) and GABA (top view) (PDB: 6X3X).

and 4.2 illustrate both ribbon and space-filling representations of the most common $\alpha 1\beta 2\gamma 2$ human GABA$_A$ receptor together with its complex with diazepam and GABA. In general, GABA$_A$ receptors with the only $\alpha 1$, $\alpha 2$, $\alpha 3$, and $\alpha 5$ subunits are sensitive to 1,4-benzodiazepines due to the

presence of an essential histidine residue at the homologous positions of $\alpha 1(101)$, $\alpha 2(101)$, $\alpha 3(126)$, and $\alpha 5(105)$ (Wieland et al., 1992). In contrast, $\alpha 4$ and $\alpha 6$ subunit isoforms in GABA$_A$ receptors have an arginine residue at $\alpha 4(99)$ and $\alpha 6(100)$ positions, which deprive the corresponding receptors of benzodiazepine sensitivity (Korpi & Seeburg, 1993; Yang et al., 1995). For example, diazepam displays more than 600-fold lower binding affinity to $\alpha 6 \beta 2 \gamma 2$ receptor ($K_i > 10000$ nM) than $\alpha 1 \beta 2 \gamma 2$ counterpart ($K_i = 16$ nM) (Lüddens et al., 1990). It has been also shown that a replacement of the arginine residue with histidine in $\alpha 4$ or $\alpha 6$ enables these receptors to become sensitive to diazepam (Wieland et al., 1992). The benzodiazepine-sensitive α subunits exhibit a diverse CNS distribution with various localization patterns and specialized functions (McKernan & Whiting, 1996).

The subunit assembly mechanism, membrane trafficking, and synaptic accumulation of GABA$_A$ receptors are complicated (Jacob et al., 2008). Following the translation of subunits, GABA$_A$ receptors are immediately assembled out of the endoplasmic reticulum, containing amino acid sequences in the N-terminus affecting the subtype of receptors. Receptor trafficking to the plasma membrane then occurs, which is induced by several GABA$_A$ receptor-associated proteins such as PRIP (phospholipase C-related, but catalytically inactive protein), GABARAP (GABA$_A$ receptor-associated protein), BIG2 (brefeldin A–inhibited GDP/GTP exchange factor 2), gephyrin, and radixin. Clathrin-mediated endocytosis (CME) finally takes place to internalize the receptors within cell membranes. The ratio of the GABA$_A$ receptor subunits constantly changes during normal human brain development. In a study developed by Milenkovic and co-workers, the immunohistochemical distribution of four GABA$_A$ receptor subunits including $\alpha 1$, $\alpha 2$, $\alpha 3$, and $\gamma 2$ was investigated in the human hindbrain during normal development in 18 fetuses at 16 weeks pregnancy, five infants, and three adults ranging 59 to 83 years (Stojanovic et al., 2016). While the $\gamma 2$ subunit has been found to have the largest proportion amongst other subunits of GABA$_A$ receptor in adults, α subunits display an age–dependent distribution. The $\alpha 1$ is primarily expressed in the Purkinje cell layer during embryonic development, which is only observed in the granular layer of the cerebellum after birth. The distribution of the $\alpha 2$ subunit gradually increases in the rhombencephalon during brain development, especially in the cerebellar cortex. Although the expression of $\alpha 3$ is relatively weak in the cerebellar cortex through aging, it is extensively found in subcortical brain structures in the developing brain. These observations provide strong evidence for the region– and age–dependent distribution of GABA$_A$ receptor subunits,

Table 4.1 Relative abundance, distribution, and function of common α subunits of GABA$_A$ receptor.

Subunit	Frequency (%)	Major localization	Functions
α1	60	Synaptic	Sedation, amnesia, anticonvulsant, dependence liability
α2	15–20	Synaptic	Anxiolysis, antihyperalgesia, muscle relaxation
α3	10–15	Synaptic	Anxiolysis, antihyperalgesia
α5	<5	Extrasynaptic	Anxiolysis, cognitive impairment, and cognitive improvement

resulting in various inherent pharmacological effects of the drugs during the maturation of the human brain.

Table 4.1 represents an overview of the general abundance, distribution, and known functions of α1, α2, α3, and α5 GABA$_A$ receptor subunits in the mouse brains (Rudolph & Möhler, 2004; Engin et al., 2018). The common GABA$_A$ receptors can be found either synaptically or extrasynaptically. Synaptic GABA$_A$ receptors basically induce fast phasic inhibition in response to high GABA concentrations. On the other hand, extrasynaptic GABA$_A$ receptors are activated by relatively low concentrations of GABA (µM), producing a persistent tonic inhibition (Sieghart & Sperk, 2002; Weir et al., 2017). The α1-containing GABA$_A$ receptors are synaptically located on neuronal cell membranes throughout the entire brain, inducing the sedative, amnesic, and anticonvulsant effects of 1,4-benzodiazepines (Rudolph & Möhler, 2004; McKernan et al., 2000). The expression of α2 subunit widely occurs at synaptic sites of the cortex, hippocampus, hypothalamus, and amygdala, where it is predominantly responsible for mediating anxiety reduction and muscle relaxation potency of 1,4-benzodiazepines (Atack, 2011). The α3 subunit is also widely expressed in the cortex, hippocampus, thalamus, amygdala, and brainstem with the same cellular functions as α2. The α5 subunit is mainly distributed in the hippocampus that seems to be mainly involved in cognitive functions (Collinson et al., 2002; Dawson et al., 2006).

Considering the unique physiology and pharmacology of GABA$_A$ α subunits in the CNS, the α-containing receptors are an attractive target for the treatment of many neurodevelopmental disorders. For example, drug targeting α5-GABA$_A$ receptors has been developed in various mental illnesses such as schizophrenia, depression, and cognitive impairment (Jacob, 2019). Despite the progress in the understanding of the α subunits neurobiology, there are still knowledge gaps between the functional roles of these

receptors and pathologies linked to α-containing GABA$_A$ abnormality in drug discovery.

4.4 Mechanism of action of 1,4-benzodiazepines on GABA$_A$ receptors

An essential prerequisite for the understanding of 1,4–benzodiazepines' mechanism of action is the knowledge about their binding domains on the cell membranes. The first observation on the sedative effect of 1,4–benzodiazepines was made by Zimmermann and co-workers in 1967 when they observed that diazepam displays an inhibitory activity on the spinal column of cats (Schmidt et al., 1967). However, they were unable to explain the action mechanism of the drug *via* identifying its specific binding locus. Following the discovery of the key neurotransmission role of GABA in the central nervous system in 1971 (Bloom & Iversen, 1971), the first clue of the action mechanism of benzodiazepines was disclosed by observing diazepam enhancing neurotransmission in the GABAergic neurons *via* improving GABA binding affinity to its postsynaptic receptors (Costa et al., 1975; Polc et al., 1974). The significance of this discovery became clearer when two independent research studies reported the presence of a saturable and stereospecific binding site for benzodiazepines in synaptic membranes of the human and rat brain cells, which were found to be associated with the GABA activity (Squires & Brastrup, 1977). Further electrophysiological and biochemical analyses have led to today's decent belief that benzodiazepines operate as positive allosteric regulators to enhance GABAergic synaptic conduction (Richards et al., 1991; Sattelle, 1987; Hunkeler et al., 1981; Martin et al., 1995). 1,4–Benzodiazepines exert their clinically relevant impacts by interacting with specific allosteric binding sites on GABA$_A$ receptors (Smith & Olsen, 1995; Haefely et al., 1975). Upon attaching to these binding domains, most 1,4–benzodiazepines modulate the GABA$_A$ receptor's function by increasing the GABA binding to its receptor, resulting in greater recurrence of chloride channel opening and consequently enhancing the inhibitory action of GABA molecules at the synaptic level (Sigel & Steinmann, 2012; Zhu et al., 2018). This indirect activation of the GABA$_A$ receptor by the benzodiazepines can be elucidated by low efficient benzodiazepine–induced conformational modifications to the receptor channel, as well as the presence of only one high–affinity benzodiazepine binding pocket on the receptor. These drugs are known as positive allosteric modulators (PAMs) widely used as anticonvulsants and sedative agents. Another group of 1,4–benzodiazepines

such as flumazenil and sarmazenil is considered as competitive (or neutral) allosteric modulators which have no effect on the GABA affinity and competitively bind to the allosteric site with zero intrinsic efficacy (Braestrup et al., 1983; Brogden & Goa, 1988). These compounds provide a safe mechanism for an effective reversal of benzodiazepine overdoses and careful benzodiazepine withdrawal by blocking receptor binding sites.
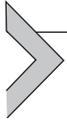
4.5 Molecular effects of chronic 1,4-benzodiazepines exposure

Understanding the molecular basis of drug action provides a great way for improving therapeutic characteristics and creating more potent and selective compounds. 1,4-Benzodiazepines in long-term use can cause neuroplasticity changes in the neural network of the brain, leading to various psychological and physical symptoms such as tolerance, dependence, and withdrawal symptoms (Gravielle, 2016). Patients who are given these drugs on a regular basis grow less susceptible to their effects, which include anticonvulsant, sedative, hypnotic, and myorelaxant properties. In addition, 1,4-benzodiazepine discontinuation can cause withdrawal symptoms char-acterized by increased anxiety, insomnia, and sensory abnormalities. Indeed, tolerance and withdrawal may be two sides of the same mechanism, in which withdrawal occurs when the benzodiazepine action is no longer present (Allison & Pratt, 2003). This concept was supported by a 2-deoxyglucose quantitative autoradiography study on chronically administered diazepam to rats (Pratt et al., 1998). Highly concentrated diazepam treatment (5 mg/kg, intraperitoneal (i.p.) injection daily) resulted in broad reductions in local rates of cerebral glucose utilization (LCGU) across the rat brain. After 3 days of therapy, sensory processing brain regions developed tolerance to diazepam's sedative effects, whereas the Papez circuit of emotion gained tolerance after 28 days of treatment. These findings imply that tolerance to diazepam's effects may be mediated by adaptive alterations in separate neuroanatomical circuits. There were significant increases in glucose uti-lization in the Papez circuit, the nucleus accumbens, and the basolateral amygdala after flumazenil-precipitated diazepam withdrawal. This clearly indicates that the Papez circuit plays an important role in diazepam tolerance and withdrawal, and these phenomena are linked by a single adaptive process. Despite years of academic and clinical research, our understanding of how 1,4-benzodiazepines lose their efficacy over time is very limited. It is gener-ally assumed that long-term benzodiazepine treatment causes compensatory

alterations in the central nervous system. In this regard, the $GABA_A$ receptors may become less sensitive to ongoing acute effects of 1,4-benzodiazepines due to several possible mechanisms including modifications in the $GABA_A$ receptors, intracellular events, or alternations in other neurotransmitters systems such as the glutamatergic system (Vinkers & Olivier, 2012). Although adaptive processes are likely to have a key role, there may be variations between preclinical and clinical development of tolerance. As a result, the simultaneous co-existence of different adaptive mechanisms complicates benzodiazepine tolerance studies. Furthermore, these adaptive modifications may be restricted to one or more brain areas, which makes it extremely difficult to identify a certain unifying process for tolerance development (Vinkers & Olivier, 2012). Before having discussions on various molecular mechanisms that have been suggested to explain 1,4-benzodiazepine tolerance, it is crucial to mention that pharmacokinetic factors are unlikely to have a significant influence on tolerance development (Fernandes et al., 1996). To further support this general conception, the response to acute diazepam delivery was evaluated in 19 chronically alprazolam-treated patients with panic disorder and 23 untreated panic disorder patients (Cowley et al., 1995). No noticeable differences in diazepam effects on plasma cortisol levels, heart rate, short-term memory, saccade latency, and self-rated anxiety between the groups were observed, confirming that 1,4-benzodiazepine tolerance has the least influence on the drug pharmacokinetics. The $GABA_A$ receptor is the most important candidate in inducing the adaptive modifications of cellular and synaptic functions upon long-term benzodiazepine exposure. Therefore, the following sections will explain the current knowledge on neuroadaptive events of $GABA_A$ receptors after prolonged benzodiazepine administration.

4.5.1 Uncoupling of $GABA_A$ receptors

One of the explanations for the reduction of 1,4-benzodiazepine function over time (tolerance) is the loss of $GABA_A$ receptor allosteric coupling. This uncoupling process is characterized as a weakened ability of these drugs to improve GABA-induced inhibitory postsynaptic potentials at the $GABA_A$ receptor, which can be attributed to modifications in $GABA_A$ receptor subunit composition, changes to the $GABA_A$ receptor mechanisms such as phosphorylation, or any process that alters the $GABA_A$ receptor's conformational state (Tietz et al., 1989; Marley & Gallager, 1989). An initial electrophysiological experiment in rats indicated that chronic diazepam-treated animals displayed a reduction in the GABA ability to induce benzodiazepine binding in cerebral cortical membranes with no noticeable

changes in benzodiazepine binding site density or affinity (Gallager et al., 1984). Since then, more confirmations of diminished allosteric coupling have been reported for chronic 1,4-benzodiazepine exposure to GABA$_A$ receptor–expressing transfected cell lines or neurons (Primus et al., 1996; Klein & Harris, 1996; Klein et al., 1995; Itier et al., 1996). GABA receptor composition can be altered when the GABA$_A$ receptor assembly process is changed, either by subunit substitutions or altered receptor expression. Due to decreased GABA$_A$ receptor coupling, the receptors with various functioning may have lower benzodiazepine sensitivity. Despite these extensive studies, the precise role of the uncoupling process in the development of benzodiazepine tolerance is still not fully understood. In an *in vivo* study on mice, the relationship of agonist efficacy of different 1,4-benzodiazepines to alternations in GABA sensitivity and anticonvulsant tolerance upon chronic benzodiazepine exposure was investigated (Hernandez et al., 1989). Their findings support the hypothesis that the tolerance to benzodiazepines requires active interaction with their benzodiazepine binding sites on GABA$_A$ receptors. In other words, the level of GABA sensitivity after long-term treatment with benzodiazepines is highly correlated to the agonist efficacy of the drugs. There is also a close relationship between the degree of the uncoupling process and the magnitude of benzodiazepine tolerance upon continuous drug administration. In another study, Tietz *et al.* evaluated the effect of prolonged benzodiazepine exposure on GABA/benzodiazepine coupling in rat brains (Tietz et al., 1989). Rats were administrated either acutely or chronically (4 weeks) with diazepam and flurazepam, and regional changes in GABA/benzodiazepine coupling were investigated. GABA significantly increased benzodiazepine binding in the cerebellum, medulla, and olfactory bulb. A significant reduction in the GABA efficacy was observed in the cortex of chronically treated rats immediately after the treatment. The experiments have indicated that long-term treatment with benzodiazepines mediates the uncoupling process. However, an acute benzodiazepine treatment has no effect on the coupling of GABA$_A$ receptor allosteric sites, indicating that the chronic treatment may lead to uncoupling of GABA/benzodiazepine site interactions. No obvious changes in benzodiazepine/chloride ion coupling in the cortex or hippocampus of acutely and chronically treated rats were also observed. This study suggests that a functional uncoupling of GABA$_A$ receptor in cortex serves may be involved in the development of drug tolerance.

Another explanation for the GABA$_A$ receptor uncoupling because of chronic benzodiazepine treatment is the phosphorylation process.

Phosphorylation of GABA$_A$ receptors is a post-translational process identified as an important factor to control the activity and subcellular distribution of the receptors (Kittler & Moss, 2003). GABA$_A$ receptor trafficking and function can be divergently regulated by phosphorylation of residues within the intracellular loop of the receptor by various protein kinases, including protein kinase A (PKA), protein kinase C (PKC), protein kinase G (PKG), tyrosine kinase Src, and Ca^{2+}/calmodulin–dependent protein kinase II (CaM KII) (Kittler & Moss, 2003). The precise effects of the phosphorylation process on GABA$_A$ receptor function are complex due to the heterogeneity of GABA$_A$ receptor subunit expression in the central nervous system. Protein kinase A (PKA)-controlled phosphorylation basically causes rapid desensitization of the receptors *via* phosphorylation of Ser409 on the β subunit, leading to a significant reduction of chloride channel opening (Macdonald et al., 1992; MacDonald & Twyman, 1992). However, there is an exception where PKA-dependent phosphorylation in the granule cells of the dentate gyrus increases GABAergic function (Kapur & Macdonald, 1996). Protein kinase C (PKC)–based phosphorylation has also been identified to display several functions such as reducing both chloride channel opening and surface expression of GABA$_A$ channels (Chapell et al., 1998; Connolly et al., 1999; Sigel & Baur, 1988), and inhibiting recycling of GABA$_A$ complexes to the neuronal surface. An electrophysiological analysis performed on *Xenopus* oocytes expressing recombinant GABA$_A$ receptors has shown that the activation of protein kinase C (PKC) by phorbol 12-myristate 13-acetate (PMA) improves the potency of diazepam *via* enhancing the benzodiazepine potentiation of GABA responses (Leidenheimer et al., 1993). Several studies have demonstrated that the changes in the GABA$_A$ receptor because of chronic benzodiazepine exposure are induced by the activation of phosphorylation protein kinases. 2-Day flunitrazepam treatment of rat cerebellar granule cells has been found to induce a temporary downregulation of the protein levels of GABA$_A$ receptor α1 subunit, which is completely reversed by the activation of staurosporine, a protein kinase inhibitor. This report hypothesizes that flunitrazepam and GABA employ the same mechanism to trigger the downregulation of α1 protein and the intracellular protein kinases seem to underlie the process (Brown & Bristow, 1996). Chronic exposure of Sf9 cells expressing recombinant GABA$_A$ to diazepam is also found to cause an increase in the rate of receptor internalization (Ali & Olsen, 2001). While the drug treatment of these cells in the presence of PKA inhibitor H-89 leads to an uncoupling of diazepam and the binding sites, PKA-induced phosphorylation provides coupling and recoupling. It

is suggested that continuous diazepam occupation of the GABA$_A$ receptor causes a conformational change in the receptor that favors internalization and uncoupling (Ali & Olsen, 2001). The GABA$_A$ is then internalized into intracellular vesicles with an acidic environment, in which potentiation by GABA is diminished. All in all, alternations in the phosphorylation state of GABA$_A$ receptors and subsequent changes in GABA$_A$ receptors can be the possible mechanisms of the development of 1,4-benzodiazepine tolerance.

The modification of surface/synaptic GABA$_A$ receptor abundance is highly dependent on the destiny of receptors following endocytosis. In this case, alteration in the endocytosis rates of the GABA$_A$ receptor is also considered as another key process to induce the uncoupling of the GABA$_A$ receptors. Ali and Olsen developed a mechanistic study on the effect of continuous benzodiazepine treatment on recombinant GABA$_A$ receptors expressed in Sf9 cells (Ali & Olsen, 2001). As in rats exposed to flumazenil and diazepam for more than a week, the GABA$_A$ receptors lost the allosteric enhancement of drug binding by GABA agonists, mainly due to benzodiazepine-induced uncoupling *via* the internalization of the receptor. The uncoupling process was reversed by an osmotic shock of membrane homogenates to lyse intracellular sections, and it was replicated in untreated cells by conducting benzodiazepine binding assays in acidic conditions. The results indicate that GABA$_A$ receptors are internalized into an acidic intracellular environment where benzodiazepine binding happens, producing increased receptor endocytosis (Ali & Olsen, 2001).

4.5.2 Downregulation of GABA$_A$ receptors

A broad downregulation of GABA$_A$ receptors throughout the brain would be the most basic explanation for reduced GABA$_A$ receptor sensitivity following prolonged benzodiazepine treatment. As classical 1,4-benzodiazepines attach to GABA$_A$ receptors containing $\alpha1$, $\alpha2$, $\alpha3$, or $\alpha5$ subunits, it is reasonable to assume that the expression of these receptors will be altered. Continuous activation of the GABA molecule at the synaptic level results in the downregulation of GABA$_A$ receptors due to interrupting their life cycle at multiple steps such as reduced subunit mRNA transcription, modifications in the endocytosis, and subunit degradation in the endoplasmic reticulum (Vinkers & Olivier, 2012). For example, chronic diazepam therapy in rats causes neuronal inhibitory GABAergic synapses to be disrupted because of substantial time- and dose-dependent downregulation of GABA$_A$ receptors, which is mediated *via* dynamin-dependent internalization. In a negative feedback loop, diazepam activates

a metabotropic PLCδ/Ca^{2+}/calcineurin signaling cascade, leading to the downregulation of GABA$_A$ receptors in synapses (Nicholson et al., 2018). A receptor autoradiography experiment on the effect of chronic diazepam administration in rat brain revealed that repeated treatment of diazepam induces changes in benzodiazepine and GABA$_A$ receptor binding in an accumbens–habenula circuit, but not necessarily in GABA$_A$ receptor number (Brett & Pratt, 1995). On contrary, chronically administration of lorazepam to mice examined in several brain regions over a period of 14 days showed that the specific benzodiazepine uptake reduced by approximately 50% in each region by day 7 (Miller et al., 1988). However, apparent affinity *in vivo* at the receptor displayed a negligible decrease, suggesting that the drug uptake was because of decreased GABA$_A$ receptor number. Although many experiments have investigated the possible mechanisms of long–term benzodiazepine exposure on GABA$_A$ receptor expression, there is a lack of consistency for subunit changes in various brain regions (Uusi–Oukari & Korpi, 2010; Foitzick et al., 2020). Furthermore, differences in the time and type of chronic treatment appear to be important. Whether diazepam is given as daily systemic injections or *via* osmotic minipumps, subunit mRNA levels of GABA$_A$ receptors can be influenced after prolonged diazepam exposure in mice (Arnot et al., 2001). Binding experiments have also found no alterations in benzodiazepine binding after long-term use (Brett & Pratt, 1995; Heninger & Gallager, 1988; Little et al., 1987). Therefore, there is no consistent downregulation mechanism or a general regiospecific alternation for GABA$_A$ receptor expression upon chronic benzodiazepine administration in the literature. Since molecular results are rarely combined with behavioral clinical tests, a direct link between behavioral tolerance and molecular changes is almost impossible to establish.

4.5.3 Alternations in the GABA$_A$ receptor subunit composition

GABA$_A$ receptor subunit compositions seem to be another key factor for the development of benzodiazepine dependence and tolerance upon their chronic benzodiazepine administration. Many reports have demonstrated that the long-term drug treatments mediate selective alternations in the composition of GABA$_A$ receptor subunits (Uusi–Oukari & Korpi, 2010), which depend on the treatment pattern, the type of subunit, and the brain region. It is generally believed that a specific subunit by itself cannot play a role in the 1,4–benzodiazepine tolerance development after continuous exposure. For example, simultaneous activation of α1 and α5 GABA$_A$

subunits has been found to be necessary for the tolerance to the sedative effects of diazepam (van Rijnsoever et al., 2004). In this study, α1-5 GABA$_A$ subunits were rendered insensitive to diazepam by a histidine–arginine point mutation in mice. Long-term diazepam treatment with mutant α1-GABA$_A$ receptors leads to a constant increased phasic inhibition in those forebrain regions associated with motor control in mice. This repeated phasic signaling can modify the amount of the tonic inhibition generated by the concurrent drug activation of α5-GABA$_A$ receptors in the dentate gyrus, the subicular complex, and the entorhinal cortex. This α1-GABA$_A$ receptor-mediated change in the inhibitory potency as a result of the prolonged diazepam exposure is reflected by a reduction of α5-GABA$_A$ receptors in mice. In another study, persistent administration of diazepam in rats produces a significant reduction in α1 subunit level in the hippocampus, and no change in this subunit level occurs in the cortex or cerebellum (Wu et al., 1994). The relatively same reductions in the levels of α5 and γ2 subunits are also observed in hippocampus and cortex regions. Chronic exposure of hippocampal neurons derived from embryonic day 18 rats to flurazepam shows a noticeable decrease in surface and total levels of α2-containing GABA$_A$ receptors, while a negligible change happens in the corresponding α2-containing receptors. This is possibly due to the increased degradation of α2-containing receptors after endocytosis in the hippocampus that reduces the potency of GABAergic inhibition (Jacob et al., 2012). These observations indicate that 1,4-benzodiazepines in long-term use mediate subtype-selective trafficking modification of GABA$_A$ receptors, leading to alternations in the receptor subunit composition at the cell membrane.

4.5.4 Alternations in glutamatergic neurotransmission

Glutamate is the major excitatory neurotransmitter in the brain that acts on two postsynaptic types of receptors, ionotropic receptors (iGluRs) and metabotropic receptors (mGluRs). Ionotropic glutamate receptors are a type of ligand-gated cation channels subdivided into the NMDA receptor (*N*-methyl–D-aspartate), the AMPA receptor (α-amino–3-hydroxy–5-methyl–4-isoxazole–4-propionic acid), and the kainate receptor, which increase the currents of K$^+$, Na$^+$, or Ca^{2+} ions after glutamate binding (Traynelis et al., 2010). NMDA receptors are composed of two obligatory GluN1 and two regulatory GluN2 and GluN3 subunits, in which each GluN subunit has extracellular loops where glycine or D-serine, and glutamate can bind to GluN1/ GluN2 and GluN2 subunits, respectively (Bard & Groc, 2011). Any

changes in membrane potential can modulate the channel permeability to Na^+, Ca^{2+}, and K^+ ions (Paoletti & Neyton, 2007). AMPA receptors are ligand–gated ion channels consisting of four classes of subunits (GluA1–GluA4) (Nakagawa, 2010). Although glutamate displays a lower affinity for the AMPA receptors than the NMDA receptors, faster desensitization and deactivation kinetics occur at the AMPA receptors. Kainate receptors are assembled from five subunits, e.g., GluR5, GluR6, GluR7, KA1, and KA2, which can be aligned in various ways (Lerma, 2006). These synaptic receptors exhibit a slower rise and decay time than the NMDA and AMPA receptors. Metabotropic receptors are monomeric proteins containing a neurotransmitter binding site on the extracellular domain and a G–protein binding site on the intracellular domain, acting as transducers to mediate longer–term neuromodulatory effects of glutamate (Javitt, 2004; Goudet et al., 2009). G–proteins behave as transducers to connect neurotransmitter binding to postsynaptic ion channels, regulating longer–term neuromodulatory effects of glutamate. Activation of glutamate receptors contributes to basal excitatory synaptic transmission and several types of synaptic plasticity, e.g., long–term potentiation (LTP) and long–term depression (LTD) mechanisms (Pinheiro & Mulle, 2008). Long–term potentiation (LTP) is a process in which synapses are continually strengthened, resulting in a long–lasting increase in signal transmission across nerve cells. In contrast, long–term depression (LTD) is considered as a long–lasting decrease in the efficiency of neuronal synapses following complex signaling cascades. GABA and glutamate are two essential opposing neurotransmitter systems in the central integration process of hypothalamo–pituitary–adrenocortical (HPA) stress responses, modulating synaptic plasticity (Herman et al., 2004). Inhibitory GABA and excitatory glutamate work together to keep the excitability of the brain in balance *via* interacting with parvocellular paraventricular nucleus (PVN) neurons. GABAergic neurons in the bed nucleus of the stria terminalis (BNST), hypothalamus, and preoptic region can directly restrict PVN influx and subsequently reduce adrenocorticotropic hormone (ACTH) release (Herman et al., 2004). On contrary, the HPA axis is activated by glutamate *via* hypothalamic and brainstem projections to the PVN. It is not surprising that chronic activation of the GABAergic system during benzodiazepine administration may disturb glutamatergic transmission, as these two opposing neurotransmitter systems maintain an intricate balance in the brain. Therefore, sensitization of the glutamatergic system could potentially be another factor of benzodiazepine tolerance and withdrawal (C Allison & Pratt, 2003).

Several investigations have shown the compensatory glutamate sensitization theory following long-term benzodiazepine exposure. Coadministration of the NMDA receptor antagonists such as 3-[(+/-)-2-carboxypiperazin-4-yl]–propyl-1-phosphonate (CPP), dizocilpine, and ketamine to rodents inhibit the development of tolerance to the sedative effects of diazepam and chlordiazepoxide (Khanna et al., 1997; File & Fernandes, 1994; Steppuhn & Turski, 1993). Concurrent CPP/lorazepam treatment of mice has also been demonstrated to significantly block the development of tolerance to the anticonvulsant effects of lorazepam, in which the role of CPP modulates the behavioral tolerance is not clearly understood (Koff et al., 1997). In another contribution, mice have been analyzed after administration of diazepam (2 mg/kg) measured by an increase in the time spent in social interaction (Fernandes & File, 1999). Simultaneous dosing of dizocilpine did not prevent the tolerance development of diazepam's anxiolytic effects, suggesting that the mechanism of tolerance to the anxiolytic effects of diazepam may be different from that of tolerance to its sedative effects. The effect of chronic lorazepam treatment on hippocampal glutamatergic neurotransmission was also investigated (Bonavita et al., 2003). A 206% *in vitro* increase in glutamate release together with an increment of 103% in the NMDA-stimulated cGMP influx in chronically treated rats were observed, despite no changes in any of the parameters following a single administration of the medication. These observations indicate that NMDA-related mechanisms play a key role in the development of 1,4-benzodiazepine tolerance. Bonavita *et al.* explored several parameters of the glutamatergic neurotransmission in rats constantly given lorazepam for 21 days (Bonavita et al., 2002). A reduction in the affinity of cortical NMDA receptors for ^{3}H–glutamate in mice ($K_d = 124.4$ nM in mice versus $K_d = 71.6$ nM in controls, $P < 0.05$) along with a decrease in the *in vitro* K^+-triggered cortical glutamate release (59% in mice versus 153% in controls, $P < 0.05$) were observed. The results clearly imply a new hypothesis against the general oppositional model of tolerance to the 1,4-benzodiazepines, as tolerance to the sedative effects of lorazepam upon the chronic administration is associated with a decreased sensitivity for glutamate, rather than an increased sensitivity. GluA subunit-deficient mice rendered tolerant by chronic flurazepam treatment develop less tolerance than their controls for muscle relaxation and sensory functions (Aitta-Aho et al., 2009). The reduced tolerance of the knockout mice can be attributed to the lack of down-scaling of the GABA system due to the defect of the glutamate system. No pharmacological or genetic study about the development of 1,4-benzodiazepine tolerance based on glutamatergic

kainate receptors has been reported so far. Overall, it seems that none of the proposed potential processes can sufficiently explain the precise mechanism of 1,4–benzodiazepine tolerance, suggesting the coexistence of multiple synergistic mechanisms.

4.5.5 Alternations to neurotrophins

Neurotrophins are a class of regulatory proteins that control neuronal survival, function, and plasticity through tyrosine kinase receptors (Trk) and p75 receptors (p75NTRs) in the vertebrate nervous system (E. J. Huang & Reichardt, 2001). Nerve growth factor (NGF), brain–derived neurotrophic factor (BDNF), neurotrophin–3 (NT-3), and neurotrophin-4 (NT-4) are all neurotrophins since they are all derived from the same gene and have comparable sequences and structures. It is evident that adaptations leading to tolerance during long-term benzodiazepine treatment can partially be induced by these neurotrophic proteins, which operate as powerful regulators of rapid synaptic inhibition. For example, postsynaptic activation of Trk receptors by BDNF and subsequent Ca^+ mobilization in the CNS is observed to diminish $GABA_A$ receptor immunoreactivity in the rat hippocampal CA1 region (Tanaka et al., 1997). This lower immunoreactivity is followed by reduced postsynaptic responses to the direct $GABA_A$ receptor agonist muscimol, which is assumed to be produced by a reduction in $GABA_A$ receptor surface expression. BDNF-mediated inhibition of GABAergic signaling is probably due to modified $GABA_A$ receptor composition, reduced subunit synthesis, extended $GABA_A$ receptor phosphorylation process, and enhanced postsynaptic receptor internalization (Henneberger et al., 2002; Brünig et al., 2001). For example, BDNF can control $GABA_A$ receptor–induced activity *via* Trk receptors signaling, resulting in a kinase-dependent short–latency impact and a delayed longer latency effect defined by receptor internalization (Cheng & Yeh, 2003). Furthermore, the activity of $GABA_A$ receptors is regulated by BDNF through an initial increase in the miniature IPSC amplitude that leads to prolonged depression, occurring simultaneously with enhanced PKC-induced phosphorylation and protein phosphatase 2A (PP2A)-mediated dephosphorylation of the $GABA_A$ receptor (Jovanovic et al., 2004). Despite all the reported indications regarding the effect of neurotrophins, particularly BDNF, on the $GABA_A$ receptors, a few studies have investigated the effects of benzodiazepine treatment on neurotrophic expression and functions (Huang & Hung, 2009; Huopaniemi et al., 2004; Licata et al., 2013). For example, Sadri–Vakili *et al.* have investigated the regulation of BDNF through acute or chronic treatment of triazolam and diazepam in mice (Licata et al., 2013). No

Table 4.2 Common physical and psychological symptoms of 1,4-benzodiazepine withdrawal.

Type of symptoms	Signs
Anxiety–related symptoms	Anxiety, panic attacks, hyperventilation, tremor, rebound insomnia, anorexia, muscle spasms, visual disturbance, sweating, dysphoria, weight loss
Perceptual distortions	Hyperacusis, abnormal bodily sensations, depersonalization/derealization
Major events	Grand mal seizure, hallucinations, delusions, and delirium

significant effects were observed in response to chronic 1,4–benzodiazepine administration. Instead, there was a noticeable decrease in BDNF expression in the hippocampus (HIP) after acute benzodiazepine exposure. It has been hypothesized that these benzodiazepine–induced alternations in BDNF protein levels may be involved in stress-related processes across the brain. In other words, acute exposure to stress agents is recognized to be one of the causes of BDNF expression reductions in the hippocampus (Schaaf et al., 1998; Smith et al., 1995), suggesting that the inhibitory drug function in this study may trigger a stress response.

4.5.6 Withdrawal syndrome

Long–term 1,4–benzodiazepine use can result in the development of benzodiazepine dependence because of adaptive modifications in the neuro-chemical events on which these medications work across the brain (Mercier-Guyon et al., 2004; Rickels et al., 1984; Dietch, 1983; MacKinnon & Parker, 1982). Benzodiazepine dependence can be emerged by the onset of a temporary withdrawal syndrome when attempts are made to stop benzo-diazepine administration. The earliest observation of 1,4-benzodiazepine physical dependence in humans was reported by Hollister and co-workers, soon after the introduction of the first 1,4–benzodiazepine derivative into the drug market (Hollister et al., 1961). In this clinical study, chlordiazepoxide was given to 36 hospitalized psychiatric individuals in high doses of 100 to 600 mg/day for periods of 1 to 7 months. A subsequent abrupt change to placebo in a single-blinded manner in 11 cases led to the appearance of withdrawal symptoms such as depression, worsening of psychosis, insomnia, agitation, decreased appetite, seizures, and twitching in 10 patients.

    Withdrawal symptoms are commonly divided into three main groups including anxiety–related symptoms, perceptual distortions, and major events (Table 4.2) (Hood et al., 2014). These withdrawal signs are opposite the direct pharmacological benefits of 1,4–benzodiazepines. It is generally believed

that neural hyperexcitability because of adaptive responses to prolonged administration with these drugs may be the main reason for withdrawal syndrome (MacKinnon & Parker, 1982).

Neural processes underlying 1,4-benzodiazepine withdrawal symptoms linked to drug dependence are not fully known. Despite the fact that 1,4-benzodiazepines directly target GABA$_A$ receptors, investigations on GABAergic system modifications have failed to reach a clear conclusion. In this case, it is suggested that the excitatory glutamatergic synaptic transmission becomes more sensitive to benzodiazepine-induced continuous augmentation of GABAergic inhibition, leading to establishing a modified balance between excitatory and inhibitory neurotransmission (Stephens, 1995). On discontinuation of chronic benzodiazepine treatment, the compensatory enhancement of glutamate neurotransmission is no longer balanced by increased GABAergic activity, which causes unmasking the hyperactive glutamatergic system and subsequently the withdrawal symptoms appear. Alternations of the glutamatergic systems *via* chronic 1,4-benzodiazepine administration are well explored in several brain regions. For example, Costa *et al.* examined the time-course appearance of withdrawal syndrome following termination of a 14-day repeated diazepam administration in the frontal cortex and hippocampus of rats (Izzo et al., 2001). AMPA receptor GluR1 subunit mRNA and protein expression increase by 30% in cortex and hippocampus together with the emergence of dependence signs after 96 h of diazepam withdrawal, indicating that excitatory glutamatergic processes *via* AMPA receptors may be involved in the occurrence of dependence symptoms after the drug withdrawal. Another *in vivo* study demonstrated that cessation of long-term flurazepam treatment in mice results in a significant increase of neurotransmission of AMPA glutamate receptors located in hippocampal CA1 pyramidal neurons, due to enhanced membrane internalization of GluR1 subunit possessing AMPA receptors (Song et al., 2007). The effect of acute discontinuation of chronic flurazepam treatment on NMDA receptors (NMDAR) has also been described in hippocampal CA1 and CA2 pyramidal neurons of mice (Van Sickle et al., 2002). The NMDAR-induced EPSC (excitatory postsynaptic currents) amplitude is found to be reduced by 52% in the neurons, along with a selective decrease in NMDA-R2B subunit mRNA and protein expression in the CA1 and CA2 areas after flurazepam administration. Therefore, it can be concluded that the diminished NR2B subunit-containing NMDA receptors in the CA1 and CA2 parts may be linked to decreased NMDA-induced currents following prolonged benzodiazepine treatment.

To investigate whether benzodiazepine withdrawal-induced anxiety causes alternations in AMPA receptor characteristics, two groups of mice were given a 28-day diazepam treatment regime by either intraperitoneal (i.p.) or subcutaneous (s.c.) injection (Allison & Pratt, 2006). The antagonist radioligand [^{3}H]Ro 48-8587 was also employed to map the distribution of AMPA receptors across the animal's brain. All rats under s.c. treatment showed withdrawal anxiety after acute termination of diazepam, in which their brains displayed enhanced AMPA receptor binding in both thalamus and hippocampus along with reduced GluR1/R2 and GluR1 subunits expression in the amygdala and limbic-associated cortex, respectively. This finding suggests that AMPA receptor properties in these brain areas play a key role in anxiety and aversion expression during the 1,4-benzodiazepine withdrawal. Even though the i.p. administrated group showed no withdrawal anxiety, their AMPA receptor binding, and subunit gene expression were changed. It is speculated that these mice may undergo repeated withdrawal experiences through the intraperitoneal injections, making them more resistant to withdrawal anxiety.

4.5.7 Addiction

Dopamine is an essential neurotransmitter in the central nervous system involved in various brain functions such as motivation, cognition, reward-related incentive learning, and even regulating body movements (Arias-Carrión & Pöppel, 2007). Dopaminergic neurons constitute the primary source of dopamine in the central nervous system, which is located in the ventral tegmental area (VTA), substantia nigra pars compacta (SNpc), and the retrorubral field (RRF) (Chinta & Andersen, 2005). Dopamine is identified as a critical component in the transmission of the rewarding effects of potentially addictive medications, contributing to feelings of pleasure and satisfaction. These drugs seem to enhance the dopamine levels and cause long-lasting synaptic alternations in the mesolimbic reward pathway originating from the VTA, which may eventually lead to pathological addictive behaviors (Di Chiara & Imperato, 1988; Wise, 1980).

Chronic benzodiazepine use has long-term effects on the brain's dopamine regulatory systems, resulting in addiction in vulnerable people. However, the neurological mechanism for benzodiazepine addiction is not well elucidated. Several *in vivo* experiments have shown that benzodiazepines decrease extracellular dopamine concentrations in the nucleus accumbens. For example, both acute and chronic midazolam injections (s.c. route) to rats have shown about a 25% reduction in the amount of extracellular dopamine

in the nucleus accumbens, but with no effect on dopamine concentrations in the striatum (Finlay et al., 1992). This study clearly indicates that the midazolam-mediated decrease of extracellular dopamine in the midbrain is not influenced by repeated drug treatment. Zetterström and Fillenz investigated the effect of flurazepam exposure on extracellular dopamine levels in the anterior striatum and medial nucleus accumbens of mice (Zetterström & Fillenz, 1990). Administrating through perfusion medium (10 μM) for 20 min diminished the dopamine concentrations by 60% in dialysates from the nucleus accumbens, while no effect on levels of dopamine was observed in perfusates from the striatum. Blocking the GABA$_A$ receptor-associated chloride channels with the benzodiazepine antagonist flumazenil prevented the effect of flurazepam on the dopamine concentrations in the nucleus accumbens, indicating that the action of flurazepam on dopamine was affected by the GABA$_A$/benzodiazepine receptor complex. It is obvious that the dopaminergic projection to the nucleus accumbens appears to be more susceptible to benzodiazepine-induced suppression than the projection to the striatum.

Electrophysiological analyses have shown that dopaminergic neurons in the midbrain are sensitive to GABA inhibitory effects of 1,4-benzodiazepines, which may be indirectly impacted by GABAergic neurons (Gale, 1981; Luo & Huang, 2016). For example, intraperitoneal injections of midazolam and diazepam to rats enhance the dopamine levels in the VTA *via* the positive regulation of GABA$_A$ receptors in adjacent interneurons (Tan et al., 2010). It is suggested that the disinhibition of dopamine secretion depends on the benzodiazepine binding sites of α1-containing GABA$_A$ receptors in the VTA, which is strongly supported by miniature inhibitory postsynaptic currents (mIPSCs) measurement (Tan et al., 2010). While GABA$_A$-induced quantal transmission of GABA neurons with increased miniature IPSC amplitude is stronger than that of dopaminergic neurons, the former displays a higher input resistance than the latter counterpart, which enables the same charge transfer to affect the membrane potential of GABA neurons more effectively than dopamine neurons (Tan et al., 2010). Furthermore, the benzodiazepine-mediated increase of each IPSC on dopamine-producing nerve cells provides little suppression of their activity because GABAergic neurons are silent and no longer produce the IPSCs. The enhancement of dopamine levels provokes drug-induced synaptic plasticity in excitatory afferents onto dopaminergic cells and eventually causes drug reward. In addition, intravenously administered diazepam to mice has shown a significant increase in the dopamine-releasing in the VTA, although

no excitation of dopaminergic neurons was observed (O'Brien & White, 1987). This study further confirms that 1,4-benzodiazepines can indirectly disinhibit VTA dopaminergic neurons through the inhibition of GABAergic interneurons.

Several lines of evidence suggest that the alternation of glutamatergic neurotransmission at VTA glutamate synapses is also an essential element in the long-term effects of drugs of abuse on brain function (Schenk & Snow, 1994; Zhang et al., 1997; Hyman & Malenka, 2001; Faleiro et al., 2004). For example, chronic exposure to cocaine, morphine, and ethanol significantly increases the levels of glutamate receptor subunits (GluRl and NMDARl) in the mesolimbic dopamine neurons in mice (Fitzgerald et al., 1996). The repeated drug-induced synaptic plasticity at glutamatergic synapses triggers a chain of cellular events such as activating the VTA dopamine neurons, which may facilitate the development of addiction (Saal et al., 2003). Korpi and coworkers have investigated the effects of single *in vivo* doses (2-20 mg/kg) of diazepam, morphine, and ethanol on glutamatergic transmission in VTA glutamatergic neurons (Heikkinen et al., 2009). Similar to morphine and ethanol, diazepam induces an enhancement in the ratio between AMPA and NMDA receptor-mediated excitatory currents, due to enhanced AMPA receptor-mediated neurotransmission at either existing excitatory synapses or newly formed synapses (Heikkinen et al., 2009). The diazepam administration also increases the frequency of impulsive miniature AMPA receptor-mediated currents in dopamine cells in the VTA with no changes in the amplitudes, which can be attributed to a presynaptic modification (Heikkinen et al., 2009).

4.6　Effect of 1,4-benzodiazepines on cholecystokinin receptors

CCK is the most abundant neuropeptide throughout the body's nervous system, which contributes to the regulation of satiety, anxiety, and analgesia in the CNS as well as motility and gastric emptying in the gastrointestinal (GI) tract (Wank, 1995; Herranz, 2003). Endogenously secreted CCK plays a key role to maintain the normal exocrine function of the pancreas and inducing enzyme secretion. These neuropeptides are also found to coexist with specific midbrain dopamine neurons, suggesting that they may play an equivalent role in the brain's dopaminergic circuits (Hökfelt et al., 1980; Hommer et al., 1986). The biological actions of CCK are regulated by two subtypes of G protein-coupled receptors known as CCK_1 and CCK_2, where binding of CCK occur at either the extracellular surface

aspalicin
(isolated from *Aspergillus alliaceus*)

4.6.1

devazepide

4.6.2

pranazepide

4.6.3
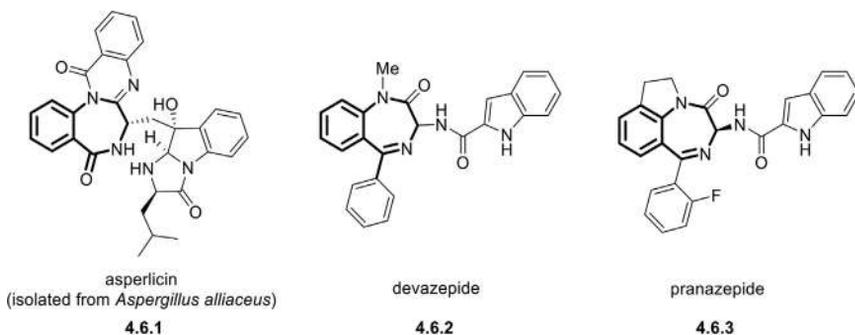
Compound	IC_{50} (nM)	
	$CCK_1{}^a$	$CCK_2{}^b$
asperlicin (**4.6.1**)	1400	$>10^5$
devazepide (**4.6.2**)	0.08	270
pranazepide (**4.6.3**)	0.4 (72^b) (0.06^c)	310

[a]Measured binding affinities in rat pancreas.
[b]Measured binding affinities in guinea pig brain.
[c]Measured binding affinities in guinea pig gallbladder.

Figure 4.3 The first and most potent 1,4-benzodiazepine-derived selective CCK_1 receptor antagonists.

or the helical bundle of the receptors. CCK_1 and CCK_2 receptors share 48% similarity in their amino acid sequences, with the main differences in the intra- and extracellular loops, as well as the transmembrane regions close to the extracellular space (Wank, 1995; Wank, 1998; Kopin et al., 1995; Silvente–Poirot & Wank, 1996). As mentioned earlier in this chapter, 1,4–benzodiazepines typically interact with $GABA_A$ receptors in the CNS to exert their clinically relevant impacts. In 1985, Chang *et al*. identified asperlicin (**4.6.1**, Fig. 4.3), a natural 1,4–benzodiazepine molecule, as a selective CCK_1 receptor antagonist in the pancreatic tissue of rats, displaying inhibition constant (K_i) and half–maximal inhibitory concentration (IC_{50}) of 1400 nM and 0.6 μM, respectively (Chang et al., 1985). Following this discovery, structural alterations of asperlicin (**4.6.1**) have provided several potent antagonists for both CCK_1 and CCK_2 receptor subtypes, which exhibited similar affinity to that of asperlicin (Bock et al., 1986; Evans et al., 1987). Initial research efforts on 1,4–benzodiazepine CCK receptor ligands mostly presumed that these compounds could act at a region overlapping with the endogenous/orthosteric binding site of the natural CCK ligand

(Gao et al., 2008). However, recent studies have revealed that G protein-coupled receptors may also contain allosteric domains, which are topographically separate from the orthosteric binding sites. Accordingly, the corresponding allosteric ligands such as 1,4-benzodiazepines target topographically distinct sites, providing both allosteric and orthosteric ligands to simultaneously bind to the receptors with the possibility of cooperative interactions (Gao et al., 2008). The following sections will highlight the results from both basic and clinical investigations of selective 1,4-benzodiazepine-derived antagonists for CCK_1 and CCK_2 receptors.

4.6.1 1,4-benzodiazepine-derived CCK1 receptor antagonists

Devazepide (also known as MK–329, **4.6.2**, Fig. 4.3) is the first potent, selective, and orally effective 1,4-benzodiazepine-based CCK_1 receptor antagonist developed from the structural optimization of asperlicin (**4.6.1**) to treat several gastrointestinal disorders such as dyspepsia, gastroparesis, and gastric reflux (Evans et al., 1986; Evans et al., 1988; Scarpignato et al., 1993). This ligand with much higher potency for CCK_1 receptors compared with asperlicin (**4.6.1**) has been demonstrated to be highly powerful in gastric emptying and gall bladder contraction assays in several species, e.g., rats, rabbits, and pigs, with no agonistic activity (Freidinger, 1989). Devazepide (**4.6.2**) decreases CCK–induced gall bladder contraction and promotes gastric motility and gastric emptying following meals in healthy people (Cantor et al., 1992; Cantor et al., 1991; Liddle et al., 1989). Despite the high performance of devazepide (**4.6.2**), several adverse effects have been reported for this antagonist such as gallstone toxicity (Iversen et al., 1991) and inducing hyperplasia in the rat's bile ducts and liver (Ohlsson et al., 1996). Among the devazepide derivatives modified as CCK_1 ligands, pranazepide (also known as FK–480, **4.6.3**, Fig. 4.3) is a highly selective and orally active CCK1 receptor antagonist for the treatment of chronic pancreatitis (Satoh et al., 1994), although no data about the investigations of this antagonist in human clinical trials are available in the peer-reviewed literature. Pranazepide (**4.6.3**) inhibits ^{125}I-labeled CCK_8 (a ^{125}I-labeled derivative of the C-terminal octapeptide CCK) binding to mice pancreatic and guinea pig gallbladder membranes with IC_{50} values of 0.40 and 0.06 nM, respectively (Ito et al., 1994). Funakoshi and Miyasaka also investigated the effect of pranazepide on gene expression of CCK and secretin in mice's intestines. The results have suggested that the ingested pranazepide increases the mRNA level of CCK in the intestine with no effect on plasma CCK immunoreactivity or CCK content in the intestinal mucosa (Funakoshi & Miyasaka, 1994). Despite the
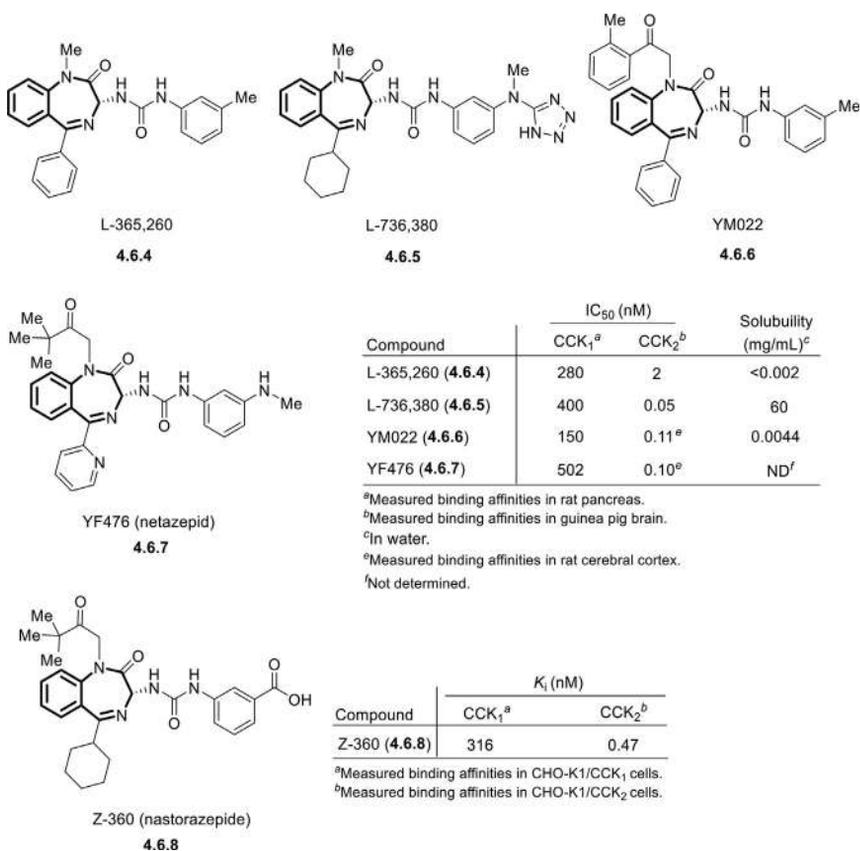
Figure 4.4 The first and most potent 1,4-benzodiazepine-derived selective CCK$_2$ receptor antagonists.

excellent potency of developed 1,4–benzodiazepine–derived selective CCK$_1$ receptor antagonists, their detailed mechanism of action has been poorly characterized.

4.6.2 1,4-benzodiazepine-derived CCK2 receptor antagonists

Merck researchers synthesized the first highly active 1,4–benzodiazepine–derived CCK$_2$ receptor antagonist, known as L–365,260 (**4.6.4**, Fig. 4.4), *via* replacing the indol–2–yl–amide of devazepide (**4.6.2**) with an aryl urea group at the C3 position of the 1,4-benzodiazepine nucleus and changing the stereochemistry at the same position (Bock et al., 1989). L–365,260 (**4.6.4**) exhibits a similar high affinity for both stomach gastrin and brain CCK$_2$ receptors in man, rat, and guinea pig (IC$_{50}$ = 2.0–3.9 nM), which

may be due to a close resemblance of the brain receptors with their gastrin counterparts (Lotti & Chang, 1989). *In vivo* experiments have also shown that orally administered L–365,260 (**4.6.4**) efficiently antagonizes gastrin-induced acid secretion by approximately 80% in rats ($ED_{50} = 0.9$ mg/kg) and guinea pigs ($ED_{50} = 5.1$ mg/kg) (Lotti & Chang, 1989). However, this ligand produces a modest inhibition of gastrin-induced gastric acid output in humans, displaying 50% inhibition when the mean total plasma concentration of the compound reaches about 500 ng/ml (Murphy et al., 1993). Although it has been demonstrated that CCK_2 receptor antagonists can suppress panic-like symptoms in both healthy volunteers and patients (Bourin et al., 1996; van Megen et al., 1996; van Vliet et al., 1997), the clinical trials failed to detect significant differences between L–365,260 and placebo (Kramer et al., 1995; Pande, 1997). Kramer and co-workers have also shown that L–365,260 (**4.6.4**) is an ineffective treatment for panic disorders, where 88 patients received either L–365,260 (30 mg q.i.d.) or placebo for 6 weeks (Kramer et al., 1995). However, this antagonist has been reported to produce a noticeable reduction in the frequency of CCK_4- and lactate-induced panic attacks in panic disorder patients (Bradwejn et al., 1994; van Megen, Westenberg, & den Boer, 1996). Reasons for such inconsistencies are not fully understood, however, certainly include several factors such as animal models, procedures, gender, and scoring technique, and limited oral bioavailability due to low aqueous solubility (< 0.002 mg/mL) (Griebel, 1999; Lin et al., 1996). These discouraging clinical results motivated researchers to search for other 1,4-benzodiazepine derivatives with better oral bioavailability and CNS penetration to successfully control panic and anxiety disorders. Introducing a tetrazol-5-ylamino moiety attached to the phenyl ring of the arylurea group of L–365,260 (**4.6.4**) led to the discovery of L–736,380 (**4.6.5**), which is among the most selective CCK_2 antagonists ever synthesized (Fig. 4.4; Castro et al., 1996). This ligand dose-dependently inhibits pentagastrin–induced gastric acid secretion in anesthetized rats with an ID_{50} value of 0.064 mg/kg as well as high aqueous solubility (60 mg/mL). However, the CNS penetration of L–736,380 (19%, upon intravenous (i.v.) injection of 10 mg/kg) measured by an *ex vivo* binding assay in BKTO mice is significantly less than the parent L–365,260 compound (**4.6.4**) (50%, upon intravenous (i.v.) injection of 13 mg/kg) (Castro et al., 1996). Further structural manipulations of L–365,260 through incorporating bulky substituents at the N1 position of the 1,4-benzodiazepine core enhance the CCK_2 receptor affinity of these antagonists. For example, YM022 (also known as netazepid, **4.6.6**) possessing

a 2-methyl-benzoylmethyl group at the N1 position has displayed high affinity ($IC_{50} = 0.11$ nM) at CCK_2 receptors of the rat brain (Satoh et al., 1995; Nishida et al., 1994). YM022 (**4.6.6**) administered *via* an intravenous dose of 0.03 μM/kg greatly suppresses pentagastrin-induced gastric acid output by 81% with an ED_{50} value of 9.5 nmol/kg (Satoh et al., 1995). However, this potent 1,4-benzodiazepine-derived antagonist suffers from low aqueous solubility (0.0044 mg/mL) and should be formulated as a solid dispersion to obtain optimal oral bioavailability (Yano et al., 1996). The introduction of basic groups such as substituting the 5-phenyl group with a 2-pyridyl substituent and the 3-methyl group of the aryl urea moiety with a methylamino group can lead to the formation of YF476 (**4.6.7**, Fig. 4.4) with greater aqueous solubility and oral bioavailability (Semple et al., 1997; Takinami et al., 1997). This molecule has a similar binding potency at CCK_2 receptors compared with YM022, but a 5-fold higher selectivity for CCK_1 receptors. By both i.v. and oral administration of the drug to rats and Heidenhain pouch dogs, YF476 displays a modest difference in the activity to inhibit pentagastrin-stimulated gastric acid secretion, while YM022 is approximately 70 times less active following the oral route than by the i.v. route. This excellent oral bioavailability of YF476 together with its long duration of action (6 h) nominates this antagonist for the treatment of gastrointestinal disorders (Semple et al., 1997).

Gastrin is a peptide hormone identified to have an important effect on the development of various gastrointestinal cancers due to frequent overexpression of CCK_2 receptors, in which CCK_2 receptor expression is noticeably increased in human cancer cells compared with normal tissue (Rehfeld et al., 1989; Reubi et al., 2003). Several pre-clinical studies have also revealed the role of gastrin and the CCK_2 receptors in human pancreatic cancer, where CCK_2 receptor antagonists may be promising candidates for the treatment of patients with this malignancy (Smith et al., 1995; Smith et al., 1996). Z-360 (also known as nastorazepide, **4.6.8**, Fig. 4.4) is a newly synthesized potent and highly selective CCK_2 receptor antagonist that is derived from YF476 (Grabowska et al., 2008). This molecule significantly decreases the pentagastrin-induced gastric acid secretion in rats and Heidenhain pouch dogs with ID_{50} values of 0.17 mg/kg (i.d.) and 0.28 mg/kg (PO), respectively (Miura et al., 2001). *In vitro* analyses have also shown that Z-360 (**4.6.8**) exhibits affinity for both recombinant human CCK_1 and CCK_2 receptors, with K_i values of 316 and 0.47 nM, respectively (Fig. 4.4). In several mouse pancreatic cancer xenograft models, Z-360 (**4.6.8**) has inhibited pancreatic cancer growth by ranging 42%–80% in

combination with gemcitabine, a first drug for the treatment of pancreatic cancer (Grabowska et al., 2008; Kawasaki et al., 2008; Kobayashi et al., 2010). Most patients with pancreatic cancer have severe, opioid-resistant pain, which drastically affects their daily activities. In a phase Ib/IIa clinical study, co-administration of Z-360 (**4.6.8**) with gemcitabine significantly reduced pain in patients with advanced pancreatic cancer with no toxicity effect (Meyer et al., 2010). In cancer-induced pain, interleukin-1b (IL-1b) production in cancer-infected areas promotes ephrin B1 gene expression in DRGs, which then increases NR2B tyrosine phosphorylation *via* the Eph B receptor in the spinal cord, causing severe pain in patients. Although Z-360 (**4.6.8**) shows a lower affinity for CCK_1 receptors than for CCK_2 receptors, it is assumed that this antagonist alleviates the cancer-induced pain by blocking the pain cascade *via* suppressing IL-1b production, most likely through the CCK_1 receptor blockage (Orikawa et al., 2010). The same also occurs in the case of devazepide (**4.6.2**), a CCK_1 receptor antagonist, as the compound decreases the IL-1b protein level in cancer-inoculated sites, and considerably blocks the ephrin B1 gene expression in DRGs with a similar degree as Z-360 (**4.6.8**) (Yoshinaga et al., 2010). This comparison further confirms the hypothesis that the analgesic effect of Z-360 (**4.6.8**) is attributed to the blockage of CCK_1 receptors.

Despite many basic and clinical investigations of selective 1,4-benzodiazepine-derived antagonists for CCK_1 and CCK_2 receptors, their detailed mechanism of action and binding mode to the gastrin and CCK_1/CCK_2 receptors remain still unclear. Therefore, more pharmacological research is needed to fully evaluate the interaction of 1,4-benzodiazepine-derived ligands with the CCK receptors.

4.7 Effect of 1,4-benzodiazepines on opioid receptors

Opioid receptors (OR) belong to G protein-coupled receptors (GPCRs) that are widely expressed in the CNS (Waldhoer et al., 2004). These receptors are classified into three main subtypes designated as mu (μ), delta (δ), and kappa (κ) opioid receptors. Upon activation by endogenous opioid peptides and their connection to pertussis toxin-sensitive heterotrimeric Gi/o proteins, these receptors dissociate into Gai/o and G$\beta\gamma$ subunits to interact with multiple intracellular effectors systems (Machelska & Celik, 2018). Gai/o interrupts adenylyl cyclases (AC), cyclic adenosine monophosphate (cAMP) formation, and protein kinase A (PKA) activity, resulting in the block of transient receptor potential cation channel subfamily
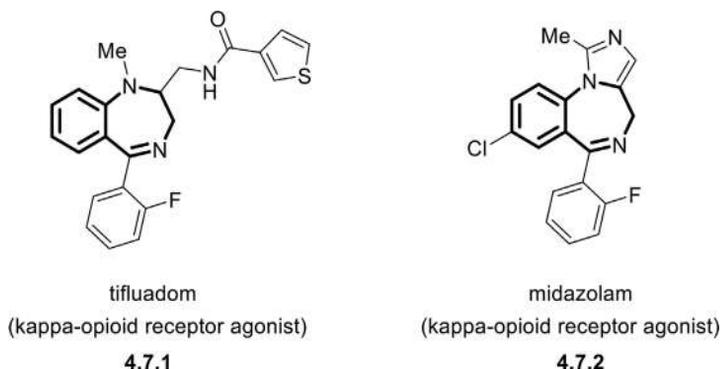
Figure 4.5 The most potent and selective 1,4-benzodiazepine-derived selective κ-opioid receptor agonists.

V member 1 (TRPV1) (Machelska & Celik, 2018). G$\beta\gamma$ species inhibits voltage–gated calcium channels and stimulates several K^+ channels including G protein–coupled inwardly rectifying K^+ (GIRK) channels and adenosine triphosphate–sensitive K^+ (KATP) channels (Machelska & Celik, 2018). These opioid–induced processes finally cause the prevention of excitatory neurotransmitter release, hyperpolarization, and a reduction in neuronal excitability, leading to analgesia and pain relief (Machelska & Celik, 2018).

As mentioned earlier, it is well-established that most of the 1,4–benzodiazepines efficiently bind to specific benzodiazepine binding sites in the CNS, exerting their biological effects by regulating the neuronal transmission of GABA. However, several 1,4–benzodiazepines show only a low affinity for the benzodiazepine receptors. Instead, such compounds exhibit a high binding affinity for opiate receptors and exert their analgesic effects through the modification of these receptors. For example, tifluadom (**4.7.1**, Fig. 4.5) is identified as a selective κ–opioid receptor agonist with potent analgesic performance in animal models. In a comparative binding study between tifluadom and diazepam on rat brain homogenates, tifluadom (**4.7.1**) displaces ^{3}H–naloxone (an opioid receptor radioligand) from opioid receptors with an IC_{50} value of 12 nM, while no effect on the binding of ^{3}H–flunitrazepam (a benzodiazepine binding site radioligand) is observed up to 1 μM concentration (Römer et al., 1982b). On the contrary, diazepam displays no replacement activity for ^{3}H–naloxone–labelled opioid receptors, however, displaces ^{3}H–flunitrazepam from GABA$_A$ receptors with an IC_{50} value of 15 nM (Römer et al., 1982b). Another receptor binding analysis of tifluadom (**4.7.1**) on guinea pig brain riched in κ– and δ–opioid receptors has represented that tifluadom is a selective κ–opioid receptor ligand, which

is less potent than bremazocine (a highly potent κ–opioid receptor agonist) but 25 times more active than morphine (Römer et al., 1982a).

Midazolam is an analgesic in animal and human trials after spinal administration. The mechanism by which this compound causes analgesia after spinal injection is controversial. Midazolam has an indirect influence on pain transmission *via* its effects on $GABA_A$ receptors. For example, midazolam increases GABA-mediated responses in rat substantia gelatinosa neurons, implying an increase in inhibitory neurotransmission (Kohno et al., 2000). In contrast, other studies have shown that midazolam displaces selective opioid radioligands, e.g., ^{3}H-naloxone, ^{3}H-DAGO, ^{3}H-DSTLE, and ^{3}H-EKC, from their corresponding endogenous receptors in rat spinal cord with a rank order of efficiency of kappa > delta > mu (Rattan et al., 1991; Rattan & Tejwani, 1994). An *in vitro* experiment of midazolam on human opioid receptor-transfected Chinese hamster ovary (CHO) cells using ^{3}H-diprenorphine binding assay has also shown that midazolam can inhibit the binding of radioligand to human κ–opioid receptors with an IC_{50} value of 58 μM, indicating that the analgesic efficacy of this 1,4-benzodiazepine derivative may be somewhat attributed to direct interaction with κ–opioid receptors (Cox & Collins, 2001). Debruyne *et al.* investigated the effects of five benzodiazepine-receptor agonists, e.g., alprazolam, clonazepam, flunitrazepam, loprazolam, and zolpidem, on the cell surface modification of μ–opioid receptor stimulated by buprenorphine in different rat brain regions including the amygdala, cortex, hippocampus, hypothalamus, and thalamus (Poisnel et al., 2009). The results have disclosed that the benzodiazepine exposure mediates alternations in μ–opioid binding sites and modifies the cell surface adaptation caused by buprenorphine. Such adaptative modifications include reductions of both cell surface receptors (approximately -40%) and intracellular desensitization that leads to a decrease in the agonist efficacy (Poisnel et al., 2009). These changes are observed more in the thalamus, amygdala, and hippocampus than hypothalamus and cortex, which can be rationalized by the fact that the latter regions contain a lower number of μ–opioid receptors. Taken together, it can be noted that the coadministration of opioids with those 1,4-benzodiazepines inducing significant changes in μ–opioid receptors may cause an enlarged potential of toxicity and addiction.

4.8 Effect of 1,4-benzothiazepines on cellular receptors

The excitation–contraction coupling pathway in the heart includes the muscle membrane depolarization, which stimulates the RyR2 cardiac

JTV519 (also known as K-201)   S107

4.8.1   **4.8.2**
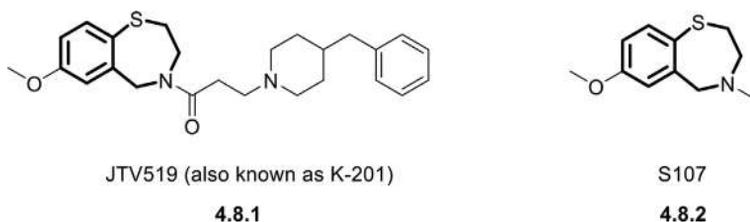
Figure 4.6 The most potent 1,4-benzothiazepine-derived RyR2 receptor modulators.

ryanodine receptor on the sarcoplasmic reticulum to open and release Ca^{2+}. RyR2 possesses the main Ca^{2+} release channel on the sarcoplasmic reticulum in cardiomyocytes that basically provides calcium ions to the cardiac muscle during the excitation–contraction coupling process. The released ion is the signal that produces muscle contraction, followed by pumping back the calcium into the sarcoplasmic reticulum, which causes muscle relaxation (Kalogeris et al., 2012). RyR2 channel function is basically regulated by interaction with calcium channel stabilizing binding protein-2 (calstabin2), which is also known as FK506 binding protein 12.6 (FKBP12.6), and interruption of the RyR2-FKBP12.6 relationships may cause Ca^{2+} leak from the sarcoplasmic reticulum during diastole, enhancing the possibility of ventricular arrhythmias and heart failure (Chelu & Wehrens, 2007; Jiang et al., 2004; Zissimopoulos & Lai, 2005). Therefore, suppressing diastolic Ca^{2+} leak through the RyR2 calcium channels in the sarcoplasmic reticulum has emerged as a promising treatment for heart arrhythmias.

JTV519 (**4.8.1**, Fig. 4.6) (also known as K–201) is the first derivative of 1,4-benzothiazepines originally developed as a cardioprotective agent to suppress cardiac cell damage because of intracellular Ca^{2+} overload (Kaneko, 1994; Kaneko et al., 1997). In this study, the suppressive effect of JTV519 (**4.8.1**) on the myofibrillar over–contraction model of isolated rat heart was compared with propranolol, verapamil, and diltiazem. While propranolol (at 10^{-5} M concentration), verapamil (at 10^{-6} M concentration), and diltiazem (at 10^{-6} M concentration) displayed a significant inhibition effect with myocardial injury score (MIS) of 1.0, 1.5, and 1.5, respectively, JTV519 (**4.8.1**) at much lower concentration of 10^{-7} M demonstrated substantial suppression with MIS of 1.8 (Kaneko, 1994; Kaneko et al., 1997). JTV519 (**4.8.1**) can efficiently suppress the FK506–induced Ca^{2+} leak in both normal and failing sarcoplasmic reticulum of dogs by restoring the conformational state of RyR2 (Kohno et al., 2003). JTV519-treated dogs suffering from pacing-induced heart failure also display an increased amount of FKBP12.6 immunoprecipitated with RyR2 (Yano et al., 2003). JTV519-stimulated

enhancement of FKBP12.6 binding to RyR2 inhibits lethal ventricular arrhythmias in FKBP12.6 deficient mice (Wehrens et al., 2004). A quartz-crystal microbalance experiment has disclosed that JTV519 (**4.8.1**) directly binds to RyR2 within amino acids ranging from 2114 to 2149, correcting the defective interdomain interaction within RyR2 and preventing the calcium leak in heart failure (Yamamoto et al., 2008). Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a genetic disorder identified as potentially life-threatening heart rhythm disturbance, which is induced by impulsive RyR2-regulated calcium release in response to the calcium over-load in sarcoplasmic reticulum during β-adrenergic stimulation (Leenhardt et al., 1995). *In vitro* studies of human RyR2 missense mutations (P2328S, Q4201R, and V4653F) in Finnish families with CPVT have demonstrated that JTV519 (**4.8.1**) can stabilize mutant channel gating by improving the binding of calstabin2 to RyR2, resulting in the normalization of the channel function and prevention of exercise-induced arrhythmias (Lehnart et al., 2004). JTV519 also acts as a RyR2 stabilizer to reduce the frequency of ouabain-induced arrhythmogenic events in ouabain-treated wild-type and RyR2$^{R4496C+/-}$ mouse myocytes with a human CPVT mutation (Sedej et al., 2010). However, this cardioprotective agent has failed to prevent delayed afterdepolarizations (DADs) in RyR2$^{R4496C+/-}$ myocytes and ventricular arrhythmias in RyR2$^{R4496C+/-}$ mice with CPVT (Liu et al., 2006).

S107 (**4.8.2**, Fig. 4.6) is another 1,4-benzothiazepine-derived RyR2-stabilizing compound that can decrease the risk of arrhythmias. For example, it has been shown that S107 improves the binding ability of calstabin2 to the mutant RyR2-R2474S channel to inhibit the channel leak and suppress cardiac arrhythmias in a CPVT mouse model (Lehnart et al., 2008). This calcium channel stabilizer also enhances exercise capacity in wild-type but not in *cal1*$^{-/-}$ mice *via* preserving binding of calstabin1 to RyR1 during exercise (Bellinger et al., 2008). Duchenne muscular dystrophy (DMD) is a progressive dilated cardiomyopathy in males that originated from dystrophin deficiency because of changes in Ca^{2+} homeostasis in skeletal muscle. S107 (**4.8.2**) effectively prevents ventricular arrhythmias in the *mdx* mouse model with Duchenne muscular dystrophy (Fauconnier et al., 2010). S107 (**4.8.2**) treatment of wild-type and RyR2-S2808D$^{+/+}$ mice significantly reduces the RyR2-induced diastolic sarcoplasmic reticulum calcium leak and diminishes heart failure progression (Shan et al., 2010). Despite the remarkable progress in 1,4-benzothiazepine-derived ligands, the detailed mechanisms underlying their interaction with cellular receptors are not yet completely established.

4.9 Conclusion

1,4–Benzodiazepines and 1,4-benzothiazepines are still frequently used in medical practice to treat anxiety, sleep disorders, and cardiovascular diseases. These compounds exert their pharmacologic effects *via* the regulation of various cellular receptors such as GABA_A, CCK, opioids, and RyR2 receptors. However, prolonged administration of 1,4-benzodiazepines causes significant receptor modifications together with an adaptation to continuous enhancement of GABAergic signaling that leads to the development of drug tolerance. Taken inconsistent *in vivo* results on the 1,4-benzodiazepine tolerance mechanisms, the relevance of the structural alternations of GABA_A receptor to develop the tolerance is not well elucidated. The application of genetic approaches that allow for neuron–specific manipulation of wild-type or mutant cellular receptors, together with developments in optogenetic manipulations, may be promising strategies to learn more about the adaptational mechanisms of neurons involved in these drug–induced behaviors.

References

Aitta-Aho, T., Vekovischeva, O. Y., Neuvonen, P. J., & Korpi, E. R. (2009). Reduced benzodiazepine tolerance, but increased flumazenil-precipitated withdrawal in AMPA-receptor GluR–A subunit-deficient mice. *Pharmacology, Biochemistry, and Behavior, 92*(2), 283–290. https://doi.org/10.1016/j.pbb.2008.12.015.

Ali, N. J., & Olsen, R. W. (2001). Chronic benzodiazepine treatment of cells expressing recombinant GABA_A receptors uncouples allosteric binding: Studies on possible mechanisms. *Journal of Neurochemistry, 79*(5), 1100–1108.

Allison, C., & Pratt, J. A. (2003). Neuroadaptive processes in GABAergic and glutamatergic systems in benzodiazepine dependence. *Pharmacology & Therapeutics, 98*(2), 171–195.

Allison, C., & Pratt, J. A. (2006). Differential effects of two chronic diazepam treatment regimes on withdrawal anxiety and AMPA receptor characteristics. *Neuropsychopharmacology,, 31*(3), 602–619.

Anderson, G. D., & Miller, J. W. (2002). Benzodiazepines: Chemistry, biotransformation, and pharmacokinetics. In R. H. Levy, R. H. Mattson, B. S. Meldrum, & E. Peracca (Eds.), *Antiepileptic Drugs* (5th ed.) (pp. 187–205). Philadelphia, PA: Lippincott Williams & Wilkins.

Arendt, R. M., Greenblatt, D. J., Liebisch, D. C., Luu, M. D., & Paul, S. M. (1987). Determinants of benzodiazepine brain uptake: Lipophilicity versus binding affinity. *Psychopharmacology, 93*(1), 72–76.

Arias-Carrión, O., & Pöppel, E. (2007). Dopamine, learning, and reward-seeking behavior. *Acta Neurobiologiae Experimentalis, 67*(4), 481–488.

Arnot, M. I., Davies, M., Martin, I. L., & Bateson, A. N. (2001). GABA_A receptor gene expression in rat cortex: Differential effects of two chronic diazepam treatment regimes. *Journal of Neuroscience Research, 64*(6), 617–625.

Atack, J. R. (2011). GABA_A receptor subtype-selective modulators. I. $\alpha 2/\alpha 3$-selective agonists as non–sedating anxiolytics. *Current Topics in Medicinal Chemistry, 11*(9), 1176–1202.

Balon, R., & Starcevic, V. (2020). Role of benzodiazepines in anxiety disorders. *Advances in Experimental Medicine and Biology, 1191*, 367–388. https://doi.org/10.1007/978-981-32-9705-0_20.

Bard, L., & Groc, L. (2011). Glutamate receptor dynamics and protein interaction: Lessons from the NMDA receptor. *Molecular and Cellular Neurosciences, 48*(4), 298–307. https://doi.org/10.1016/j.mcn.2011.05.009.

Bellinger, A. M., Reiken, S., Dura, M., Murphy, P. W., Deng, S.-X., Landry, D. W., … Marks, A. R. (2008). Remodeling of ryanodine receptor complex causes "leaky" channels: A molecular mechanism for decreased exercise capacity. *Proceedings of the National Academy of Sciences of the United States of America, 105*(6), 2198–2202. https://doi.org/10.1073/pnas.0711074105 .

Bloom, F. E., & Iversen, L. L. (1971). Localizing ^{3}H-GABA in nerve terminals of rat cerebral cortex by electron microscopic autoradiography. *Nature, 229*(5287), 628–630.

Bock, M. G., DiPardo, R. M., Evans, B. E., Rittle, K. E., Whitter, W. L., Veber, D. E., Anderson, P. S., & Freidinger, R. M. (1989). Benzodiazepine gastrin and brain cholecystokinin receptor ligands: L-365,260. *Journal of Medicinal Chemistry, 32*(1), 13–16.

Bock, M. G., DiPardo, R. M., Rittle, K. E., Evans, B. E., Freidinger, R. M., Veber, D. F., Chang, R. S., Chen, T. B., Keegan, M. E., & Lotti, V. J. (1986). Cholecystokinin antagonists. Synthesis of asperlicin analogues with improved potency and water solubility. *Journal of Medicinal Chemistry, 29*(10), 1941–1945.

Bonavita, C. D., Bisagno, V., Bonelli, C. G., Acosta, G. B., Rubio, M. C., & Wikinski, S. I. (2002). Tolerance to the sedative effect of lorazepam correlates with a diminution in cortical release and affinity for glutamate. *Neuropharmacology, 42*(5), 619–625.

Bonavita, C., Ferrero, A., Cereseto, M., Velardez, M., Rubio, M., & Wikinski, S. (2003). Adaptive changes in the rat hippocampal glutamatergic neurotransmission are observed during long-term treatment with lorazepam. *Psychopharmacology, 166*(2), 163–167.

Bourin, M., Malinge, M., Vasar, E., & Bradwejn, J. (1996). Two faces of cholecystokinin: anxiety and schizophrenia. *Fundamental & Clinical Pharmacology, 10*(2), 116–126.

Bradwejn, J., Koszycki, D., Couëtoux du Tertre, A., van Megen, H., den Boer, J., & Westenberg, H. (1994). The panicogenic effects of cholecystokinin-tetrapeptide are antagonized by L-365,260, a central cholecystokinin receptor antagonist, in patients with panic disorder. *Archives of General Psychiatry, 51*(6), 486–493.

Braestrup, C., Nielsen, M., Honoré, T., Jensen, L. H., & Petersen, E. N. (1983). Benzodiazepine receptor ligands with positive and negative efficacy. *Neuropharmacology, 22*(12B), 1451–1457.

Brett, R. R., & Pratt, J. A. (1995). Changes in benzodiazepine-GABA receptor coupling in an accumbens-habenula circuit after chronic diazepam treatment. *British Journal of Pharmacology, 116*(5), 2375–2384.

Brogden, R. N., & Goa, K. L. (1988). Flumazenil. A preliminary review of its benzodiazepine antagonist properties, intrinsic activity and therapeutic use. *Drugs, 35*(4), 448–467.

Brown, M. J., & Bristow, D. R. (1996). Molecular mechanisms of benzodiazepine-induced down–regulation of GABA$_A$ receptor α1 subunit protein in rat cerebellar granule cells. *British Journal of Pharmacology, 118*(5), 1103–1110.

Brünig, I., Penschuck, S., Berninger, B., Benson, J., & Fritschy, J. M. (2001). BDNF reduces miniature inhibitory postsynaptic currents by rapid downregulation of GABA$_A$ receptor surface expression. *European Journal of Neuroscience, 13*(7), 1320–1328.

Cantor, P., Mortensen, P. E., Myhre, J., Gjorup, I., Worning, H., Stahl, E., & Survill, T. T. (1992). The effect of the cholecystokinin receptor antagonist MK-329 on meal-stimulated pancreaticobiliary output in humans. *Gastroenterology, 102*(5), 1742–1751.

Cantor, P., Olsen, O., Gertz, B. J., Gjorup, I., & Worning, H. (1991). Inhibition of cholecystokinin-stimulated pancreaticobiliary output in man by the cholecystokinin receptor antagonist MK-329. *Scandinavian Journal of Gastroenterology, 26*(6), 627–637.

Castro, J. L., Ball, R. G., Broughton, H. B., Russell, M. G., Rathbone, D., Watt, A. P., … Matassa, V. G (1996). Controlled modification of acidity in cholecystokinin B receptor antagonists: *N*-(1,4-benzodiazepin-3-yl)-*N*'-[3-(tetrazol-5-ylamino) phenyl]ureas. *Journal of Medicinal Chemistry, 39*(4), 842–849.

Chang, R. S., Lotti, V. J., Monaghan, R. L., Birnbaum, J., Stapley, E. O., Goetz, M. A., Albers-Schönberg, G., Patchett, A. A., Liesch, J. M., & Hensens, O. D. (1985). A potent nonpeptide cholecystokinin antagonist selective for peripheral tissues isolated from *Aspergillus alliaceus*. *Science (New York, N.Y.), 230*(4722), 177–179.

Chapell, R., Bueno, O. F., Alvarez-Hernandez, X., Robinson, L. C., & Leiden-heimer, N. J. (1998). Activation of protein kinase C induces gamma-aminobutyric acid type A receptor internalization in *Xenopus* oocytes. *Journal of Biological Chemistry, 273*(49), 32595–32601.

Chelu, M. G., & Wehrens, X. H. T. (2007). Sarcoplasmic reticulum calcium leak and cardiac arrhythmias. *Biochemical Society Transactions, 35*(5), 952–956.

Cheng, Q., & Yeh, H. H. (2003). Brain-derived neurotrophic factor attenuates mouse cerebellar granule cell GABA(A) receptor-mediated responses via postsynaptic mechanisms. *Journal of Physiology, 548*(3), 711–721.

Chinta, S. J., & Andersen, J. K. (2005). Dopaminergic neurons. *International Journal of Biochemistry & Cell Biology, 37*(5), 942–946.

Collinson, N., Kuenzi, F. M., Jarolimek, W., Maubach, K. A., Cothliff, R., Sur, C., … Rosahl, T. W. (2002). Enhanced learning and memory and altered GABAergic synaptic transmission in mice lacking the α5 subunit of the GABA$_A$ receptor. *The Journal of Neuroscience, 22*(13), 5572–5580.

Connolly, C. N., Kittler, J. T., Thomas, P., Uren, J. M., Brandon, N. J., Smart, T. G., & Moss, S. J. (1999). Cell surface stability of γ-aminobutyric acid type A receptors. Dependence on protein kinase C activity and subunit composition. *Journal of Biological Chemistry, 274*(51), 36565–36572.

Costa, E., Guidotti, A., Mao, C. C., & Suria, A. (1975). New concepts on the mechanism of action of benzodiazepines. *Life Sciences, 17*(2), 167–185.

Cowley, D. S., Roy-Byrne, P. P., Radant, A., Ritchie, J. C., Greenblatt, D. J., Nemeroff, C. B., & Hommer, D. W. (1995). Benzodiazepine sensitivity in panic disorder: effects of chronic alprazolam treatment. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology, 12*(2), 147–157.

Cox, R. F., & Collins, M. A. (2001). The effects of benzodiazepines on human opioid receptor binding and function. *Anesthesia and Analgesia, 93*(2), 354–358.

Dasgupta, A. (2020). Genetic factors associated with opioid therapy and opioid addiction. *Fighting the opioid epidemic* (1st ed., pp. 61–88). Amsterdam, Netherlands: Elsevier.

Dawson, G. R., Maubach, K. A., Collinson, N., Cobain, M., Everitt, B. J., MacLeod, A. M., … Atack, J. R. (2006). An inverse agonist selective for α5 subunit-containing GABA$_A$ receptors enhances cognition. *Journal of Pharmacology and Experimental Therapeutics, 316*(3), 1335–1345.

Di Chiara, G., & Imperato, A. (1988). Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proceedings of the National Academy of Sciences of the United States of America, 85*(14), 5274–5278.

Dietch, J. (1983). The nature and extent of benzodiazepine abuse: An overview of recent literature. *Hospital & Community Psychiatry, 34*(12), 1139–1145.

Engin, E., Benham, R. S., & Rudolph, U. (2018). An Emerging circuit pharmacology of GABA$_A$ receptors. *Trends in Pharmacological Sciences, 39*(8), 710–732. https://doi.org/10.1016/j.tips.2018.04.003.

Evans, B. E., Bock, M. G., Rittle, K. E., DiPardo, R. M., Whitter, W. L., Veber, D. F., Anderson, P. S., & Freidinger, R. M. (1986). Design of potent, orally effective, nonpeptidal antagonists of the peptide hormone cholecystokinin. *Proceedings of the National Academy of Sciences of the United States of America, 83*(13), 4918–4922.

Evans, B. E., Rittle, K. E., Bock, M. G., DiPardo, R. M., Freidinger, R. M., Whitter, W. L., Lundell, G. F., Veber, D. F., Anderson, P. S., & Chang, R. S. (1988). Methods for drug discovery: development of potent, selective, orally effective cholecystokinin antagonists. *Journal of Medicinal Chemistry, 31*(12), 2235–2246.

Evans, B. E., Rittle, K. E., Bock, M. G., DiPardo, R. M., Freidinger, R. M., Whitter, W. L., … Veber, D. F. (1987). Design of nonpeptidal ligands for a peptide receptor: Cholecystokinin antagonists. *Journal of Medicinal Chemistry, 30*(7), 1229–1239.

Faleiro, L. J., Jones, S., & Kauer, J. A. (2004). Rapid synaptic plasticity of glutamatergic synapses on dopamine neurons in the ventral tegmental area in response to acute amphetamine injection. *Neuropsychopharmacology, 29*(12), 2115–2125.

Fauconnier, J., Thireau, J., Reiken, S., Cassan, C., Richard, S., Matecki, S., Marks, A. R., & Lacampagne, A. (2010). Leaky RyR2 trigger ventricular arrhythmias in Duchenne muscular dystrophy. *Proceedings of the National Academy of Sciences of the United States of America, 107*(4), 1559–1564. https://doi.org/10.1073/pnas.0908540107.

Fernandes, C., & File, S. E. (1999). Dizocilpine does not prevent the development of tolerance to the anxiolytic effects of diazepam in rats. *Brain Research, 815*(2), 431–434.

Fernandes, C., File, S. E., & Berry, D. (1996). Evidence against oppositional and pharmacokinetic mechanisms of tolerance to diazepam's sedative effects. *Brain Research, 734*(1–2), 236–242.

File, S. E., & Fernandes, C. (1994). Dizocilpine prevents the development of tolerance to the sedative effects of diazepam in rats. *Pharmacology, Biochemistry, and Behavior, 47*(4), 823–826.

Finlay, J. M., Damsma, G., & Fibiger, H. C. (1992). Benzodiazepine-induced decreases in extracellular concentrations of dopamine in the nucleus accumbens after acute and repeated administration. *Psychopharmacology, 106*(2), 202–208.

Fitzgerald, L. W., Ortiz, J., Hamedani, A. G., & Nestler, E. J. (1996). Drugs of abuse and stress increase the expression of GluR1 and NMDAR1 glutamate receptor subunits in the rat ventral tegmental area: Common adaptations among cross-sensitizing agents. *The Journal of Neuroscience, 16*(1), 274–282.

Foitzick, M. F., Medina, N. B., Iglesias García, L. C., & Gravielle, M. C. (2020). receptor α1 subunit gene via L-type voltage-gated calcium channel activation in rat cerebrocortical neurons. *Neuroscience Letters, 721*, 134801. https://doi.org/10.1016/j.neulet.2020.134801.

Freidinger, R. M. (1989). Cholecystokinin and gastrin antagonists. *Medicinal Research Reviews, 9*(3), 271–290.

Funakoshi, A., & Miyasaka, K. (1994). Effect of a new cholecystokinin antagonist (FK 480) on gene expression of cholecystokinin and secretin in rat intestine. *Journal of Gastroenterology, 29*(3), 385–387.

Gale, K. (1981). Relationship between the presence of dopaminergic neurons and GABA receptors in substantia nigra: effects of lesions. *Brain Research, 210*(1–2), 401–406.

Gallager, D. W., Lakoski, J. M., Gonsalves, S. F., & Rauch, S. L. (1984). Chronic benzodiazepine treatment decreases postsynaptic GABA sensitivity. *Nature, 308*(5954), 74–77.

Gao, F., Sexton, P. M., Christopoulos, A., & Miller, L. J. (2008). Benzodiazepine ligands can act as allosteric modulators of the Type 1 cholecystokinin receptor. *Bioorganic & Medicinal Chemistry Letters, 18*(15), 4401–4404. https://doi.org/10.1016/j.bmcl.2008.06.053.

Garakani, A., Murrough, J. W., Freire, R. C., Thom, R. P., Larkin, K., Buono, F. D., & Iosifescu, D. V. (2020). Pharmacotherapy of anxiety disorders: current and emerging treatment options. *Frontiers in Psychiatry, 11*, 595584. https://doi.org/10.3389/fpsyt.2020.595584.

Goudet, C., Magnaghi, V., Landry, M., Nagy, F., Gereau, R. W., & Pin, J.-P. (2009). Metabotropic receptors for glutamate and GABA in pain. *Brain Research Reviews, 60*(1), 43–56. https://doi.org/10.1016/j.brainresrev.2008.12.007.

Grabowska, A. M., Morris, T. M., McKenzie, A. J., Kumari, R., Hamano, H., Emori, Y., Yoshinaga, K., & Watson, S. A. (2008). Pre-clinical evaluation of a new orally-active CCK-2R antagonist, Z-360, in gastrointestinal cancer models. *Regulatory Peptides, 146*(1–3), 46–57.

Gravielle, M. C. (2016). Effect of chronic benzodiazepine exposure on GABA$_A$ receptors: Regulation of GABA/benzodiazepine site interactions. In V. R. Preedy (Ed.), *Neuropathology of drug addictions and substance misuse* (1st ed.) (pp. 308–316). Amsterdam, Netherlands: Elsevier. https://doi.org/10.1016/B978-0-12-800634-4.00030-5.

Griebel, G. (1999). Is there a future for neuropeptide receptor ligands in the treatment of anxiety disorders? *Pharmacology & Therapeutics, 82*(1), 1–61.

Haefely, W., Kulcsár, A., & Möhler, H. (1975). Possible involvement of GABA in the central actions of benzodiazepines. *Psychopharmacology Bulletin, 11*(4), 58–59.

Hagiwara, M., Adachi-Akahane, S., & Nagao, T. (1997). High-affinity binding of DTZ323, a novel derivative of diltiazem, to rabbit skeletal muscle L-type Ca^{++} channels. *Journal of Pharmacology and Experimental Therapeutics, 281*(1), 173–179.

Handschin, C., & Meyer, U. A. (2003). Induction of drug metabolism: The role of nuclear receptors. *Pharmacological Reviews, 55*(4), 649–673.

Heikkinen, A. E., Möykkynen, T. P., & Korpi, E. R. (2009). Long-lasting modulation of glutamatergic transmission in VTA dopamine neurons after a single dose of benzodiazepine agonists. *Neuropsychopharmacology, 34*(2), 290–298. https://doi.org/10.1038/npp.2008.89.

Heninger, C., & Gallager, D. W. (1988). Altered γ-aminobutyric acid/benzodiazepine interaction after chronic diazepam exposure. *Neuropharmacology, 27*(10), 1073–1076.

Henneberger, C., Jüttner, R., Rothe, T., & Grantyn, R. (2002). Postsynaptic action of BDNF on GABAergic synaptic transmission in the superficial layers of the mouse superior colliculus. *Journal of Neurophysiology, 88*(2), 595–603.

Herman, J. P., Mueller, N. K., & Figueiredo, H. (2004). Role of GABA and glutamate circuitry in hypothalamo-pituitary-adrenocortical stress integration. *Annals of the New York Academy of Sciences, 1018*, 35–45.

Hernandez, T. D., Heninger, C., Wilson, M. A., & Gallager, D. W. (1989). Relationship of agonist efficacy to changes in GABA sensitivity and anticonvulsant tolerance following chronic benzodiazepine ligand exposure. *European Journal of Pharmacology, 170*(3), 145–155.

Herranz, R. (2003). Cholecystokinin antagonists: Pharmacological and therapeutic potential. *Medicinal Research Reviews, 23*(5), 559–605.

Hökfelt, T., Rehfeld, J. F., Skirboll, L., Ivemark, B., Goldstein, M., & Markey, K. (1980). Evidence for coexistence of dopamine and CCK in meso-limbic neurones. *Nature, 285*(5765), 476–478.

Hollister, L. E., Motzenbecker, F. P., & Degan, R. O. (1961). Withdrawal reactions from chlordiazepoxide ("Librium"). *Psychopharmacologia, 2*, 63–68.

Hommer, D. W., Stoner, G., Crawley, J. N., Paul, S. M., & Skirboll, L. R. (1986). Cholecystokinin-dopamine coexistence: Electrophysiological actions corresponding to cholecystokinin receptor subtype. *The Journal of Neuroscience, 6*(10), 3039–3043.

Hood, S. D., Norman, A., Hince, D. A., Melichar, J. K., & Hulse, G. K. (2014). Benzodiazepine dependence and its treatment with low dose flumazenil. *British Journal of Clinical Pharmacology, 77*(2), 285–294. https://doi.org/10.1111/bcp.12023.

Huang, E. J., & Reichardt, L. F. (2001). Neurotrophins: Roles in neuronal development and function. *Annual Review of Neuroscience, 24*, 677–736.

Huang, T.-L., & Hung, Y.-Y. (2009). Lorazepam reduces the serum brain-derived neurotrophic factor level in schizophrenia patients with catatonia. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 33*(1), 158–159. https://doi.org/10.1016/j.pnpbp.2008.10.016.

Hunkeler, W., Möhler, H., Pieri, L., Polc, P., Bonetti, E. P., Cumin, R., Schaffner, R., & Haefely, W. (1981). Selective antagonists of benzodiazepines. *Nature, 290*(5806), 514–516.

Huopaniemi, L., Keist, R., Randolph, A., Certa, U., & Rudolph, U. (2004). Diazepam-induced adaptive plasticity revealed by alpha1 GABA$_A$ receptor-specific expression profiling. *Journal of Neurochemistry, 88*(5), 1059–1067.

Hyman, S. E., & Malenka, R. C. (2001). Addiction and the brain: The neurobiology of compulsion and its persistence. *Nature Reviews Neuroscience, 2*(10), 695–703.

Inagaki, K., Kihara, Y., Izumi, T., & Sasayama, S. (2000). The cardioprotective effects of a new 1,4–benzothiazepine derivative, JTV519, on ischemia/reperfusion–induced Ca^{2+} overload in isolated rat hearts. *Cardiovascular Drugs and Therapy, 14*(5), 489–495.

Itier, V., Granger, P., Perrault, G., Depoortere, H., Scatton, B., & Avenet, P. (1996). Protracted treatment with diazepam reduces benzodiazepine1 receptor-mediated potentiation of gamma-aminobutyric acid-induced currents in dissociated rat hippocampal neurons. *Journal of Pharmacology and Experimental Therapeutics, 279*(3), 1092–1099.

Ito, H., Sogabe, H., Nakarai, T., Sato, Y., Tomoi, M., Kadowaki, M., Yoshida, K., … Yoshida, K. (1994). Pharmacological profile of FK480, a novel cholecystokinin type-A receptor antagonist: Comparison to loxiglumide. *Journal of Pharmacology and Experimental Therapeutics, 268*(2), 571–575.

Iversen, L. L., Dourish, C. T., & Iversen, S. D. (1991). Cholecystokinin receptors: Synthetic antagonists with selectivity for receptor subtypes and possible clinical applications. *Biochemical Society Transactions, 19*(4), 913–915.

Izzo, E., Auta, J., Impagnatiello, F., Pesold, C., Guidotti, A., & Costa, E. (2001). Glutamic acid decarboxylase and glutamate receptor changes during tolerance and dependence to benzodiazepines. *Proceedings of the National Academy of Sciences of the United States of America, 98*(6), 3483–3488.

Jacob, T. C. (2019). Neurobiology and therapeutic potential of α5-GABA type A receptors. *Frontiers in Molecular Neuroscience, 12*, 179. https://doi.org/10.3389/fnmol.2019.00179.

Jacob, T. C., Michels, G., Silayeva, L., Haydon, J., Succol, F., & Moss, S. J. (2012). Benzodiazepine treatment induces subtype-specific changes in GABA$_A$ receptor trafficking and decreases synaptic inhibition. *Proceedings of the National Academy of Sciences of the United States of America, 109*(45), 18595–18600. https://doi.org/10.1073/pnas.1204994109.

Jacob, T. C., Moss, S. J., & Jurd, R. (2008). GABA$_A$ receptor trafficking and its role in the dynamic modulation of neuronal inhibition. *Nature Reviews Neuroscience, 9*(5), 331–343. https://doi.org/10.1038/nrn2370.

Javitt, D. C. (2004). Glutamate as a therapeutic target in psychiatric disorders. *Molecular Psychiatry, 9*(11), 984–997.

Jiang, D., Xiao, B., Yang, D., Wang, R., Choi, P., Zhang, L., … Chen, S. R. W. (2004). RyR2 mutations linked to ventricular tachycardia and sudden death reduce the threshold for store-overload-induced Ca^{2+} release (SOICR). *Proceedings of the National Academy of Sciences of the United States of America, 101*(35), 13062–13067.

Jones, D. R., Hall, S. D., Jackson, E. K., Branch, R. A., & Wilkinson, G. R. (1988). Brain uptake of benzodiazepines: Effects of lipophilicity and plasma protein binding. *Journal of Pharmacology and Experimental Therapeutics, 245*(3), 816–822.

Jovanovic, J. N., Thomas, P., Kittler, J. T., Smart, T. G., & Moss, S. J. (2004). Brain-derived neurotrophic factor modulates fast synaptic inhibition by regulating GABA$_A$ receptor phosphorylation, activity, and cell-surface stability. *The Journal of Neuroscience, 24*(2), 522–530.

Kalogeris, T., Baines, C. P., Krenz, M., & Korthuis, R. J. (2012). Cell biology of ischemia/reperfusion injury. *International Review of Cell and Molecular Biology, 298*, 229–317. https://doi.org/10.1016/B978-0-12-394309-5.00006-7.

Kaneko, N. (1994). New 1,4-benzothiazepine derivative, K201, demonstrates cardioprotective effects against sudden cardiac cell death and intracellular calcium blocking action. *Drug Development Research, 33*(4), 429–438. https://doi.org/10.1002/ddr.430330406.

Kaneko, N., Ago, H., Matsuda, R., Inagaki, E., & Miyano, M. (1997). Crystal structure of annexin V with its ligand K-201 as a calcium channel activity inhibitor. *Journal of Molecular Biology, 274*(1), 16–20.

Kapur, J., & Macdonald, R. L. (1996). Cyclic AMP-dependent protein kinase enhances hippocampal dentate granule cell GABA$_A$ receptor currents. *Journal of Neurophysiology, 76*(4), 2626–2634.

Kawasaki, D., Emori, Y., Eta, R., Iino, Y., Hamano, H., Yoshinaga, K., Tanaka, T., Takei, M., & Watson, S. A. (2008). Effect of Z-360, a novel orally active CCK-2/gastrin receptor antagonist on tumor growth in human pancreatic adenocarcinoma cell lines in vivo and mode of action determinations in vitro. *Cancer Chemotherapy and Pharmacology, 61*(5), 883–892.

Khanna, J. M., Chau, A., & Shah, G. (1997). Effect of NMDA antagonists on rapid tolerance to benzodiazepines. *Brain Research Bulletin, 42*(2), 99–103.

Kittler, J. T., & Moss, S. J. (2003). Modulation of GABA$_A$ receptor activity by phosphorylation and receptor trafficking: Implications for the efficacy of synaptic inhibition. *Current Opinion in Neurobiology, 13*(3), 341–347.

Klein, R. L., & Harris, R. A. (1996). Regulation of GABA$_A$ receptor structure and function by chronic drug treatments *in vivo* and with stably transfected cells. *Japanese Journal of Pharmacology, 70*(1), 1–15.

Klein, R. L., Mascia, M. P., Harkness, P. C., Hadingham, K. L., Whiting, P. J., & Harris, R. A. (1995). Regulation of allosteric coupling and function of stably expressed gamma-aminobutyric acid (GABA)A receptors by chronic treatment with GABA$_A$ and benzodiazepine agonists. *Journal of Pharmacology and Experimental Therapeutics, 274*(3), 1484–1492.

Kobayashi, N., Seto, K., Orikawa, Y., Hamano, H., Yoshinaga, K., & Takei, M. (2010). Z-360, a novel cholecystokinin-2/gastrin receptor antagonist, inhibits gemcitabine-induced expression of the vascular endothelial growth factor gene in human pancreatic cancer cells. *Biological & Pharmaceutical Bulletin, 33*(2), 216–222.

Koff, J. M., Pritchard, G. A., Greenblatt, D. J., & Miller, L. G. (1997). The NMDA receptor competitive antagonist CPP modulates benzodiazepine tolerance and discontinuation. *Pharmacology, 55*(5), 217–227.

Kohno, M., Yano, M., Kobayashi, S., Doi, M., Oda, T., Tokuhisa, T., Okuda, S., Ohkusa, T., Kohno, M., & Matsuzaki, M. (2003). A new cardioprotective agent, JTV519, improves defective channel gating of ryanodine receptor in heart failure. *American Journal of Physiology. Heart and Circulatory Physiology, 284*(3), H1035–H1042.

Kohno, T., Kumamoto, E., Baba, H., Ataka, T., Okamoto, M., Shimoji, K., & Yoshimura, M. (2000). Actions of midazolam on GABAergic transmission in substantia gelatinosa neurons of adult rat spinal cord slices. *Anesthesiology, 92*(2), 507–515.

Kondziella, D. (2017). The Top 5 Neurotransmitters from a Clinical Neurologist's Perspective. *Neurochemical Research, 42*(6), 1767–1771. https://doi.org/10.1007/s11064-016-2101-z.

Kopin, A. S., McBride, E. W., Quinn, S. M., Kolakowski, L. F., & Beinborn, M. (1995). The role of the cholecystokinin-B/gastrin receptor transmembrane domains in determining affinity for subtype-selective ligands. *Journal of Biological Chemistry, 270*(10), 5019–5023.

Korpi, E. R., & Seeburg, P. H. (1993). Natural mutation of GABA$_A$ receptor α6 subunit alters benzodiazepine affinity but not allosteric GABA effects. *European Journal of Pharmacology, 247*(1), 23–27.

Kramer, M. S., Cutler, N. R., Ballenger, J. C., Patterson, W. M., Mendels, J., Chenault, A., Shrivastava, R., Matzura-Wolfe, D., Lines, C., & Reines, S. (1995). A placebo-controlled trial of L-365,260, a CCKB antagonist, in panic disorder. *Biological Psychiatry, 37*(7), 462–466.

Kuffler, S. W., & Edwards, C. (1958). Mechanism of gamma aminobutyric acid (GABA) action and its relation to synaptic inhibition. *Journal of Neurophysiology, 21*(6), 589–610.

Leenhardt, A., Lucet, V., Denjoy, I., Grau, F., Ngoc, D. D., & Coumel, P. (1995). Catecholaminergic polymorphic ventricular tachycardia in children. A 7-year follow-up of 21 patients. *Circulation, 91*(5), 1512–1519.

Lehnart, S. E., Mongillo, M., Bellinger, A., Lindegger, N., Chen, B.-X., Hsueh, W., … Marks, A. R. (2008). Leaky Ca^{2+} release channel/ryanodine receptor 2 causes seizures

and sudden cardiac death in mice. *Journal of Clinical Investigation, 118*(6), 2230–2245. https://doi.org/10.1172/JCI35346.

Lehnart, S. E., Wehrens, X. H. T., Laitinen, P. J., Reiken, S. R., Deng, S.-X., Cheng, Z., Landry, D. W., Kontula, K., Swan, H., & Marks, A. R. (2004). Sudden death in familial polymorphic ventricular tachycardia associated with calcium release channel (ryanodine receptor) leak. *Circulation, 109*(25), 3208–3214.

Leidenheimer, N. J., Whiting, P. J., & Harris, R. A. (1993). Activation of calcium-phospholipid-dependent protein kinase enhances benzodiazepine and barbiturate potentiation of the GABA$_A$ receptor. *Journal of Neurochemistry, 60*(5), 1972–1975.

Lerma, J. (2006). Kainate receptor physiology. *Current Opinion in Pharmacology, 6*(1), 89–97.

Licata, S. C., Shinday, N. M., Huizenga, M. N., Darnell, S. B., Sangrey, G. R., Rudolph, U., Rowlett, J. K., & Sadri-Vakili, G. (2013). Alterations in brain-derived neurotrophic factor in the mouse hippocampus following acute but not repeated benzodiazepine treatment. *Plos One, 8*(12), e84806. https://doi.org/10.1371/journal.pone.0084806.

Liddle, R. A., Gertz, B. J., Kanayama, S., Beccaria, L., Coker, L. D., Turnbull, T. A., & Morita, E. T. (1989). Effects of a novel cholecystokinin (CCK) receptor antagonist, MK-329, on gallbladder contraction and gastric emptying in humans. Implications for the physiology of CCK. *Journal of Clinical Investigation, 84*(4), 1220–1225.

Lin, J. H., Storey, D. E., Chen, I. W., & Xu, X. (1996). Improved oral absorption of L-365,260, a poorly soluble drug. *Biopharmaceutics & Drug Disposition, 17*(1), 1–15.

Little, H. J., Nutt, D. J., & Taylor, S. C. (1987). Bidirectional effects of chronic treatment with agonists and inverse agonists at the benzodiazepine receptor. *Brain Research Bulletin, 19*(3), 371–378.

Liu, N., Colombi, B., Memmi, M., Zissimopoulos, S., Rizzi, N., Negri, S., … Priori, S. G. (2006). Arrhythmogenesis in catecholaminergic polymorphic ventricular tachycardia: Insights from a RyR2 R4496C knock-in mouse model. *Circulation Research, 99*(3), 292–298.

Lotti, V. J., & Chang, R. S. (1989). A new potent and selective non-peptide gastrin antagonist and brain cholecystokinin receptor (CCK-B) ligand: L-365,260. *European Journal of Pharmacology, 162*(2), 273–280.

Lüddens, H., Pritchett, D. B., Köhler, M., Killisch, I., Keinänen, K., Monyer, H., Sprengel, R., & Seeburg, P. H. (1990). Cerebellar GABA$_A$ receptor selective for a behavioural alcohol antagonist. *Nature, 346*(6285), 648–651.

Luo, S. X., & Huang, E. J. (2016). Dopaminergic neurons and brain reward pathways: From neurogenesis to circuit assembly. *American Journal of Pathology, 186*(3), 478–488. https://doi.org/10.1016/j.ajpath.2015.09.023.

MacDonald, R. L., & Twyman, R. E. (1992). Kinetic properties and regulation of GABA$_A$ receptor channels. *Ion Channels, 3*, 315–343.

Macdonald, R. L., Twyman, R. E., Ryan-Jastrow, T., & Angelotti, T. P. (1992). Regulation of GABA$_A$ receptor channels by anticonvulsant and convulsant drugs and by phosphorylation. *Epilepsy Research Supplement, 9*, 265–277.

Machelska, H., & Celik, M. Ö. (2018). Advances in achieving opioid analgesia without side effects. *Frontiers in Pharmacology, 9*, 1388. https://doi.org/10.3389/fphar.2018.01388.

MacKinnon, G. L., & Parker, W. A. (1982). Benzodiazepine withdrawal syndrome: A literature review and evaluation. *American Journal of Drug and Alcohol Abuse, 9*(1), 19–33.

Malaspina, P., Roullet, J.-B., Pearl, P. L., Ainslie, G. R., Vogel, K. R., & Gibson, K. M. (2016). Succinic semialdehyde dehydrogenase deficiency (SSADHD): Pathophysiological complexity and multifactorial trait associations in a rare monogenic disorder of GABA metabolism. *Neurochemistry International, 99*, 72–84. https://doi.org/10.1016/j.neuint.2016.06.009.

Mandrioli, R., Mercolini, L., & Raggi, M. A. (2008). Benzodiazepine metabolism: An analytical perspective. *Current Drug Metabolism, 9*(8), 827–844.

Marley, R. J., & Gallager, D. W. (1989). Chronic diazepam treatment produces regionally specific changes in GABA-stimulated chloride influx. *European Journal of Pharmacology, 159*(3), 217–223.

Martin, J. R., Jenck, F., & Moreau, J. L. (1995). Comparison of benzodiazepine receptor ligands with partial agonistic, antagonistic or partial inverse agonistic properties in precipitating withdrawal in squirrel monkeys. *Journal of Pharmacology and Experimental Therapeutics, 275*(1), 405–411.

McKernan, R. M., Rosahl, T. W., Reynolds, D. S., Sur, C., Wafford, K. A., Atack, J. R., … Whiting, P. J. (2000). Sedative but not anxiolytic properties of benzodiazepines are mediated by the GABA$_A$ receptor α1 subtype. *Nature Neuroscience, 3*(6), 587–592.

McKernan, R. M., & Whiting, P. J. (1996). Which GABA$_A$-receptor subtypes really occur in the brain? *Trends in Neuroscience, 19*(4), 139–143.

Mercier-Guyon, C., Chabannes, J. P., & Saviuc, P. (2004). The role of captodiamine in the withdrawal from long-term benzodiazepine treatment. *Current Medical Research and Opinion, 20*(9), 1347–1355.

Meyer, T., Caplin, M. E., Palmer, D. H., Valle, J. W., Larvin, M., Waters, J. S., Coxon, F., Borbath, I., Peeters, M., Nagano, E., & Kato, H. (2010). A phase Ib/IIa trial to evaluate the CCK2 receptor antagonist Z-360 in combination with gemcitabine in patients with advanced pancreatic cancer. *European Journal of Cancer, 46*(3), 526–533. https://doi.org/10.1016/j.ejca.2009.11.004.

Miller, L. G., Greenblatt, D. J., Barnhill, J. G., & Shader, R. I. (1988). Chronic benzodiazepine administration. I. Tolerance is associated with benzodiazepine receptor downregulation and decreased γ-aminobutyric acid A receptor function. *Journal of Pharmacology and Experimental Therapeutics, 246*(1), 170–176.

Miura, N., Yoneta, T., Ukawa, H., Fukuda, Y., Eta, R., Mera, Y., Omata, T., Kinomoto, T., Kurimoto, T., & Itoh, Z. (2001). Pharmacological profiles of Z-360, a novel CCKB/gastrin (CCK2) receptor antagonist with excellent oral potency. *Gastroenterology, 5*(120), A311. https://doi.org/10.1016/S0016-5085(08)81544-2.

Möhler, H., & Okada, T. (1977). Benzodiazepine receptor: demonstration in the central nervous system. *Science, 198*(4319), 849–851.

Murphy, M. G., Sytnik, B., Kovacs, T. O., Mertz, H., Ewanik, D., Shingo, S., Lin, J. H., Gertz, B. J., & Walsh, J. H. (1993). The gastrin-receptor antagonist L-365,260 inhibits stimulated acid secretion in humans. *Clinical Pharmacology and Therapeutics, 54*(5), 533–539.

Nakagawa, T. (2010). The biochemistry, ultrastructure, and subunit assembly mechanism of AMPA receptors. *Molecular Neurobiology, 42*(3), 161–184. https://doi.org/10.1007/s12035-010-8149-x.

Nicholson, M. W., Sweeney, A., Pekle, E., Alam, S., Ali, A. B., Duchen, M., & Jovanovic, J. N. (2018). Diazepam-induced loss of inhibitory synapses mediated by PLCδ/Ca^{2+}/calcineurin signalling downstream of GABA$_A$ receptors. *Molecular Psychiatry, 23*(9), 1851–1867. https://doi.org/10.1038/s41380-018-0100-y.

Nishida, A., Miyata, K., Tsutsumi, R., Yuki, H., Akuzawa, S., Kobayashi, A., … Katuyama, Y. (1994). Pharmacological profile of (*R*)-1-[2,3-dihydro-1-(2-methylphenacyl)-2-oxo-5-phenyl-1*H*-1,4-benzodiazepin-3-yl]-3-(3-methylphenyl)-urea (YM022), a new potent and selective gastrin/cholecystokinin-B receptor antagonist, in vitro and in vivo. *Journal of Pharmacology and Experimental Therapeutics, 269*(2), 725–731.

O'Brien, D. P., & White, F. J. (1987). Inhibition of non-dopamine cells in the ventral tegmental area by benzodiazepines: Relationship to A10 dopamine cell activity. *European Journal of Pharmacology, 142*(3), 343–354.

Ohlsson, B., Axelson, J., Rehfeld, J. F., & Ihse, I. (1996). Devazepide-induced hyperplasia in the rat liver and bile ducts. European Surgical Research. Europaische Chirurgische Forschung. *Recherches Chirurgicales Europeennes, 28*(4), 299–305.

Olsen, R. W. (1982). Drug interactions at the GABA receptor-ionophore complex. *Annual Review of Pharmacology and Toxicology, 22*, 245–277.

Orikawa, Y., Kato, H., Seto, K., Kobayashi, N., Yoshinaga, K., Hamano, H., … Takei, M. (2010). Z-360, a novel therapeutic agent for pancreatic cancer, prevents up-regulation of ephrin B1 gene expression and phosphorylation of NR2B via suppression of interleukin-1β production in a cancer-induced pain model in mice. *Molecular Pain, 6*, 72. https://doi.org/10.1186/1744-8069-6-72.

Padgett, C. L., & Slesinger, P. A. (2010). GABA_B receptor coupling to G-proteins and ion channels. *Advances in Pharmacology, 58*, 123–147. https://doi.org/10.1016/S1054-3589(10)58006-2.

Pande, A. C. (1997). Lack of efficacy of a cholecystokinin-B antagonist in anxiety disorders. *Biological Psychiatry, 42*(1), 18S. https://doi.org/10.1016/S0006-3223(97)86929-6.

Paoletti, P., & Neyton, J. (2007). NMDA receptor subunits: Function and pharmacology. *Current Opinion in Pharmacology, 7*(1), 39–47.

Pathania, S., Narang, R. K., & Rawal, R. K. (2019). Role of sulphur-heterocycles in medicinal chemistry: An update. *European Journal of Medicinal Chemistry, 180*, 486–508. https://doi.org/10.1016/j.ejmech.2019.07.043.

Pinheiro, P. S., & Mulle, C. (2008). Presynaptic glutamate receptors: Physiological functions and mechanisms of action. *Nature Reviews Neuroscience, 9*(6), 423–436. https://doi.org/10.1038/nrn2379.

Poisnel, G., Dhilly, M., Le Boisselier, R., Barre, L., & Debruyne, D. (2009). Comparison of five benzodiazepine-receptor agonists on buprenorphine-induced μ-opioid receptor regulation. *Journal of Pharmacological Sciences, 110*(1), 36–46.

Polc, P., Möhler, H., & Haefely, W. (1974). The effect of diazepam on spinal cord activities: Possible sites and mechanisms of action. *Naunyn-Schmiedeberg's Archives of Pharmacology, 284*(4), 319–337.

Pratt, J. A., Brett, R. R., & Laurie, D. J. (1998). Benzodiazepine dependence: From neural circuits to gene expression. *Pharmacology, Biochemistry, and Behavior, 59*(4), 925–934.

Primus, R. J., Yu, J., Xu, J., Hartnett, C., Meyyappan, M., Kostas, C., … Gallager, D. W. (1996). Allosteric uncoupling after chronic benzodiazepine exposure of recombinant gamma-aminobutyric acid (A) receptors expressed in Sf9 cells: Ligand efficacy and subtype selectivity. *Journal of Pharmacology and Experimental Therapeutics, 276*(3), 882–890.

Ramerstorfer, J., Furtmüller, R., Sarto-Jackson, I., Varagic, Z., Sieghart, W., & Ernst, M. (2011). The GABA_A receptor $\alpha+\beta-$ interface: A novel target for subtype selective drugs. *The Journal of Neuroscience, 31*(3), 870–877. https://doi.org/10.1523/JNEUROSCI.5012-10.2011.

Rattan, A. K., McDonald, J. S., & Tejwani, G. A. (1991). Differential effects of intrathecal midazolam on morphine-induced antinociception in the rat: Role of spinal opioid receptors. *Anesthesia and Analgesia, 73*(2), 124–131.

Rattan, A. K., & Tejwani, G. A. (1994). Sodium ions modulate differentially the effect of a benzodiazepine agonist on rat spinal μ-, δ- and κ-opioid receptors. *Pharmacology, 48*(1), 30–40.

Rehfeld, J. F., Bardram, L., & Hilsted, L. (1989). Gastrin in human bronchogenic carcinomas: Constant expression but variable processing of progastrin. *Cancer Research, 49*(11), 2840–2843.

Reubi, J. C., Waser, B., Gugger, M., Friess, H., Kleeff, J., Kayed, H., Büchler, M. W., & Laissue, J. A. (2003). Distribution of CCK1 and CCK2 receptors in normal and diseased human pancreatic tissue. *Gastroenterology, 125*(1), 98–106.

Richards, G., Schoch, P., & Haefely, W. (1991). Benzodiazepine receptors: New vistas. *Seminars in Neuroscience, 3*(3), 191–203. https://doi.org/10.1016/1044-5765(91)90016-H.

Rickels, K., Case, G. W., Winokur, A., & Swenson, C. (1984). Long-term benzodiazepine therapy: Benefits and risks. *Psychopharmacology Bulletin, 20*(4), 608–615.

Riss, J., Cloyd, J., Gates, J., & Collins, S. (2008). Benzodiazepines in epilepsy: Pharmacology and pharmacokinetics. *Acta Neurologica Scandinavica, 118*(2), 69–86. https://doi.org/10.1111/j.1600-0404.2008.01004.x.

Römer, D., Büscher, H. H., Hill, R. C., Maurer, R., Petcher, T. J., Zeugner, H., Benson, W., Finner, E., Milkowski, W., & Thies, P. W. (1982a). Unexpected opioid activity in a known class of drug. *Life Sciences, 31*(12–13), 1217–1220.

Römer, D., Büscher, H. H., Hill, R. C., Maurer, R., Petcher, T. J., Zeugner, H., Benson, W., Finner, E., Milkowski, W., & Thies, P. W. (1982b). An opioid benzodiazepine. *Nature, 298*(5876), 759–760.

Rudolph, U., & Möhler, H. (2004). Analysis of GABA$_A$ receptor function and dissection of the pharmacology of benzodiazepines and general anesthetics through mouse genetics. *Annual Review of Pharmacology and Toxicology, 44*, 475–498.

Saal, D., Dong, Y., Bonci, A., & Malenka, R. C. (2003). Drugs of abuse and stress trigger a common synaptic adaptation in dopamine neurons. *Neuron, 37*(4), 577–582.

Saha, D., Jain, G., & Sharma, A. (2015). Benzothiazepines: Chemistry of a privileged scaffold. *RSC Advances, 5*(86), 70619–70639. https://doi.org/10.1039/C5RA12422K.

Satoh, M., Kondoh, Y., Okamoto, Y., Nishida, A., Miyata, K., Ohta, M., Mase, T., & Murase, K. (1995). New 1,4-benzodiazepin-2-one derivatives as gastrin/cholecystokinin-B antagonists. *Chemical & Pharmaceutical Bulletin, 43*(12), 2159–2167.

Satoh, Y., Matsuo, T., Sogabe, H., Itoh, H., Tada, T., Kinoshita, T., Yoshida, K., & Takaya, T. (1994). Studies on a novel, potent and orally effective cholecystokinin A antagonist, FK-480. Synthesis and structure–activity relationships of FK-480 and related compounds. *Chemical & Pharmaceutical Bulletin, 42*(10), 2071–2083.

Sattelle, D. B. (1987). Benzodiazepine/GABA receptors and chloride channels: Structural and functional properties. *Quarterly Review of Biology, 62*(3), 309. https://doi.org/10.1086/415535.

Scarpignato, C., Varga, G., & Corradi, C. (1993). Effect of CCK and its antagonists on gastric emptying. *Journal of Physiology, 87*(5), 291–300.

Schaaf, M. J., de Jong, J., de Kloet, E. R., & Vreugdenhil, E. (1998). Downregulation of BDNF mRNA and protein in the rat hippocampus by corticosterone. *Brain Research, 813*(1), 112–120.

Schenk, S., & Snow, S. (1994). Sensitization to cocaine's motor activating properties produced by electrical kindling of the medial prefrontal cortex but not of the hippocampus. *Brain Research, 659*(1–2), 17–22.

Schmidt, R. F., Vogel, M. E., & Zimmermann, M. (1967). Effect of diazepam on presynaptic inhibition and other spinal reflexes. *Naunyn-Schmiedebergs Archiv Fur Experimentelle Pathologie Und Pharmakologie, 258*(1), 69–82.

Schmidt, S., Gonzalez, D., & Derendorf, H. (2010). Significance of protein binding in pharmacokinetics and pharmacodynamics. *Journal of Pharmaceutical Sciences, 99*(3), 1107–1122. https://doi.org/10.1002/jps.21916.

Schofield, P. R., Darlison, M. G., Fujita, N., Burt, D. R., Stephenson, F. A., Rodriguez, H., … Glencorse, T. A. (1987). Sequence and functional expression of the GABA$_A$ receptor shows a ligand-gated receptor super-family. *Nature, 328*(6127), 221–227.

Sedej, S., Heinzel, F. R., Walther, S., Dybkova, N., Wakula, P., Groborz, J., … Pieske, B. (2010). Na$^+$-dependent SR Ca^{2+} overload induces arrhythmogenic events in mouse cardiomyocytes with a human CPVT mutation. *Cardiovascular Research, 87*(1), 50–59. https://doi.org/10.1093/cvr/cvq007.

Semple, G., Ryder, H., Rooker, D. P., Batt, A. R., Kendrick, D. A., Szelke, M., … Miyata, K. (1997). (3*R*)-*N*-(1-(tert-butylcarbonylmethyl)-2,3-dihydro-2-oxo-5-(2-pyridyl)-1*H*-1,4-benzodiazepin-3-yl)-*N'*-(3-(methylamino)phenyl)urea (YF476): A potent and orally active gastrin/CCK-B antagonist. *Journal of Medicinal Chemistry, 40*(3), 331–341.

Shan, J., Betzenhauser, M. J., Kushnir, A., Reiken, S., Meli, A. C., Wronska, A., Dura, M., Chen, B.-X., & Marks, A. R. (2010). Role of chronic ryanodine receptor phosphorylation in heart failure and β-adrenergic receptor blockade in mice. *Journal of Clinical Investigation, 120*(12), 4375–4387. https://doi.org/10.1172/JCI37649.

Sieghart, W., & Sperk, G. (2002). Subunit composition, distribution and function of GABA$_A$ receptor subtypes. *Current Topics in Medicinal Chemistry, 2*(8), 795–816.

Sigel, E., & Baur, R. (1988). Activation of protein kinase C differentially modulates neuronal Na$^+$, Ca^{2+}, and gamma-aminobutyrate type A channels. *Proceedings of the National Academy of Sciences of the United States of America, 85*(16), 6192–6196.

Sigel, E., & Steinmann, M. E. (2012). Structure, function, and modulation of GABA$_A$ receptors. *Journal of Biological Chemistry, 287*(48), 40224–40231. https://doi.org/10.1074/jbc.R112.386664.

Silvente-Poirot, S., & Wank, S. A. (1996). A segment of five amino acids in the second extracellular loop of the cholecystokinin–B receptor is essential for selectivity of the peptide agonist gastrin. *Journal of Biological Chemistry, 271*(25), 14698–14706.

Smith, G. B., & Olsen, R. W. (1995). Functional domains of GABA$_A$ receptors. *Trends in Pharmacological Sciences, 16*(5), 162–168.

Smith, J. P., Fantaskey, A. P., Liu, G., & Zagon, I. S. (1995). Identification of gastrin as a growth peptide in human pancreatic cancer. *American Journal of Physiology, 268*(1), R135–R141.

Smith, J. P., Shih, A., Wu, Y., McLaughlin, P. J., & Zagon, I. S. (1996). Gastrin regulates growth of human pancreatic cancer in a tonic and autocrine fashion. *American Journal of Physiology, 270*(5), R1078–R1084.

Smith, M. A., Makino, S., Kvetnansky, R., & Post, R. M. (1995). Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin–3 mRNAs in the hippocampus. *The Journal of Neuroscience, 15*(3), 1768–1777.

Song, J., Shen, G., Greenfield, L. J., & Tietz, E. I. (2007). Benzodiazepine withdrawal-induced glutamatergic plasticity involves up-regulation of GluR1-containing α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors in Hippocampal CA1 neurons. *Journal of Pharmacology and Experimental Therapeutics, 322*(2), 569–581.

Squires, R. F., & Brastrup, C. (1977). Benzodiazepine receptors in rat brain. *Nature, 266*(5604), 732–734.

Stephens, D. N. (1995). A glutamatergic hypothesis of drug dependence: Extrapolations from benzodiazepine receptor ligands. *Behavioural Pharmacology, 6*(5–6), 425–446.

Steppuhn, K. G., & Turski, L. (1993). Diazepam dependence prevented by glutamate antagonists. *Proceedings of the National Academy of Sciences of the United States of America, 90*(14), 6889–6893.

Stojanovic, T., Capo, I., Aronica, E., Adle-Biassette, H., Höger, H., Sieghart, W., Kovacs, G. G., & Milenkovic, I. (2016). The α1, α2, α3, and γ2 subunits of GABA$_A$ receptors show characteristic spatial and temporal expression patterns in rhombencephalic structures during normal human brain development. *Journal of Comparative Neurology, 524*(9), 1805–1824. https://doi.org/10.1002/cne.23923.

Takinami, Y., Yuki, H., Nishida, A., Akuzawa, S., Uchida, A., Takemoto, Y., … Miyata, K. (1997). YF476 is a new potent and selective gastrin/cholecystokinin–B receptor antagonist in vitro and in vivo. *Alimentary Pharmacology & Therapeutics, 11*(1), 113–120.

Tallman, J. F., Paul, S. M., Skolnick, P., & Gallager, D. W. (1980). Receptors for the age of anxiety: Pharmacology of the benzodiazepines. *Science, 207*(4428), 274–281.

Tan, K. R., Brown, M., Labouèbe, G., Yvon, C., Creton, C., Fritschy, J.-M., Rudolph, U., & Lüscher, C. (2010). Neural bases for addictive properties of benzodiazepines. *Nature, 463*(7282), 769–774. https://doi.org/10.1038/nature08758.

Tanaka, H., & Mizojiri, K. (1999). Drug-protein binding and blood-brain barrier permeability. *Journal of Pharmacology and Experimental Therapeutics, 288*(3), 912–918.

Tanaka, T., Saito, H., & Matsuki, N. (1997). Inhibition of GABA$_A$ synaptic responses by brain-derived neurotrophic factor (BDNF) in rat hippocampus. *The Journal of Neuroscience, 17*(9), 2959–2966.

Tietz, E. I., Chiu, T. H., & Rosenberg, H. C. (1989). Regional GABA/benzodiazepine receptor/chloride channel coupling after acute and chronic benzodiazepine treatment. *European Journal of Pharmacology, 167*(1), 57–65.

Traynelis, S. F., Wollmuth, L. P., McBain, C. J., Menniti, F. S., Vance, K. M., Ogden, K. K., … Dingledine, R. (2010). Glutamate receptor ion channels: Structure, regulation, and function. *Pharmacological Reviews, 62*(3), 405–496. https://doi.org/10.1124/pr.109.002451.

Uusi-Oukari, M., & Korpi, E. R. (2010). Regulation of GABA$_A$ receptor subunit expression by pharmacological agents. *Pharmacological Reviews, 62*(1), 97–135. https://doi.org/10.1124/pr.109.002063.

van Megen, H. J., Westenberg, H. G., & den Boer, J. A. (1996). Effect of the cholecystokinin–B receptor antagonist L–365,260 on lactate-induced panic attacks in panic disorder patients. *Biological Psychiatry, 40*(8), 804–806.

van Megen, H. J., Westenberg, H. G., den Boer, J. A., & Kahn, R. S. (1996). Cholecystokinin in anxiety. *European Neuropsychopharmacology, 6*(4), 263–280.

van Rijnsoever, C., Täuber, M., Choulli, M. K., Keist, R., Rudolph, U., Mohler, H., … Crestani, F. (2004). Requirement of α5-GABA$_A$ receptors for the development of tolerance to the sedative action of diazepam in mice. *The Journal of Neuroscience, 24*(30), 6785–6790.

Van Sickle, B. J., Cox, A. S., Schak, K., Greenfield, L. J., & Tietz, E. I. (2002). Chronic benzodiazepine administration alters hippocampal CA1 neuron excitability: NMDA receptor function and expression. *Neuropharmacology, 43*(4), 595–606.

van Vliet, I. M., Westenberg, H. G., Slaap, B. R., den Boer, J. A., & Ho Pian, K. L. (1997). Anxiogenic effects of pentagastrin in patients with social phobia and healthy controls. *Biological Psychiatry, 42*(1), 76–78.

Veith, H., Southall, N., Huang, R., James, T., Fayne, D., Artemenko, N., Shen, M., Inglese, J., Austin, C. P., Lloyd, D. G., & Auld, D. S. (2009). Comprehensive characterization of cytochrome P450 isozyme selectivity across chemical libraries. *Nature Biotechnology, 27*(11), 1050–1055. https://doi.org/10.1038/nbt.1581.

Vinkers, C. H., & Olivier, B. (2012). Mechanisms underlying tolerance after long-term benzodiazepine use: A future for subtype-selective GABA$_A$ receptor modulators? *Advances in Pharmacological Sciences, 2012*, 416864. https://doi.org/10.1155/2012/416864.

Waldhoer, M., Bartlett, S. E., & Whistler, J. L. (2004). Opioid receptors. *Annual Review of Biochemistry, 73*, 953–990.

Wanat, K. (2020). Biological barriers, and the influence of protein binding on the passage of drugs across them. *Molecular Biology Reports, 47*(4), 3221–3231. https://doi.org/10.1007/s11033-020-05361-2.

Wank, S. A. (1995). Cholecystokinin receptors. *American Journal of Physiology, 269*(5), G628–G646.

Wank, S. A. (1998). G protein-coupled receptors in gastrointestinal physiology. I. CCK receptors: An exemplary family. *American Journal of Physiology, 274*(4), G607–G613.

Wehrens, X. H. T., Lehnart, S. E., Reiken, S. R., Deng, S.-X., Vest, J. A., Cervantes, D., Coromilas, J., Landry, D. W., & Marks, A. R. (2004). Protection from cardiac arrhythmia through ryanodine receptor-stabilizing protein calstabin2. *Science, 304*(5668), 292–296.

Weir, C. J., Mitchell, S. J., & Lambert, J. J. (2017). Role of GABA$_A$ receptor subtypes in the behavioural effects of intravenous general anaesthetics. *British Journal of Anaesthesia, 119*(Suppl 1), i167–i175. https://doi.org/10.1093/bja/aex369.

Wieland, H. A., Lüddens, H., & Seeburg, P. H. (1992). A single histidine in GABA$_A$ receptors is essential for benzodiazepine agonist binding. *Journal of Biological Chemistry, 267*(3), 1426–1429.

Wise, R. A. (1980). The dopamine synapse and the notion of 'pleasure centers' in the brain. *Trends in Neuroscience, 3*(4), 91–95. https://doi.org/10.1016/0166-2236(80)90035-1.

Wu, Y., Rosenberg, H. C., Chiu, T. H., & Zhao, T. J. (1994). Subunit- and brain region-specific reduction of $GABA_A$ receptor subunit mRNAs during chronic treatment of rats with diazepam. *Journal of Molecular Neuroscience, 5*(2), 105–120.

Yamamoto, T., Yano, M., Xu, X., Uchinoumi, H., Tateishi, H., Mochizuki, M., Oda, T., Kobayashi, S., Ikemoto, N., & Matsuzaki, M. (2008). Identification of target domains of the cardiac ryanodine receptor to correct channel disorder in failing hearts. *Circulation, 117*(6), 762–772. https://doi.org/10.1161/CIRCULATIONAHA.107.718957.

Yang, W., Drewe, J. A., & Lan, N. C. (1995). Cloning and characterization of the human $GABA_A$ receptor α4 subunit: Identification of a unique diazepam-insensitive binding site. *European Journal of Pharmacology, 291*(3), 319–325.

Yano, K., Kajiyama, A., Yamazaki, S., Matsumura, Y., Watanabe, K., & Yamamoto, K. (1996). In vitro stability and in vivo absorption studies of colloidal particles formed from a solid dispersion system. *Chemical & Pharmaceutical Bulletin, 44*(12), 2309–2313.

Yano, M., Kobayashi, S., Kohno, M., Doi, M., Tokuhisa, T., Okuda, S., Suetsugu, M., Hisaoka, T., Obayashi, M., Ohkusa, T., Kohno, M., & Matsuzaki, M. (2003). FKBP12.6-mediated stabilization of calcium-release channel (ryanodine receptor) as a novel therapeutic strategy against heart failure. *Circulation, 107*(3), 477–484.

Yoshinaga, K., Horii, T., Hamano, H., Eta, R., Ozaki, T., Orikawa, Y., Yoshii, K., Kawabata, Y., Hori, Y., Seto, K., Takei, M., & Kuraishi, Y. (2010). Pharmacological evaluation of analgesic effects of the cholecystokinin2 receptor antagonist Z-360 in mouse models of formalin- and cancer-induced pain. *Biological & Pharmaceutical Bulletin, 33*(2), 244–248.

Zetterström, T., & Fillenz, M. (1990). Local administration of flurazepam has different effects on dopamine release in striatum and nucleus accumbens: A microdialysis study. *Neuropharmacology, 29*(2), 129–134.

Zhang, X. F., Hu, X. T., White, F. J., & Wolf, M. E. (1997). Increased responsiveness of ventral tegmental area dopamine neurons to glutamate after repeated administration of cocaine or amphetamine is transient and selectively involves AMPA receptors. *Journal of Pharmacology and Experimental Therapeutics, 281*(2), 699–706.

Zhu, S., Noviello, C. M., Teng, J., Walsh, R. M., Kim, J. J., & Hibbs, R. E. (2018). Structure of a human synaptic $GABA_A$ receptor. *Nature, 559*(7712), 67–72. https://doi.org/10.1038/s41586-018-0255-3.

Zissimopoulos, S., & Lai, F. A. (2005). Interaction of FKBP12.6 with the cardiac ryanodine receptor C-terminal domain. *Journal of Biological Chemistry, 280*(7), 5475–5485.

Non-Print Items

Abstract

This chapter describes the biological behavior of 1,4–benzodiazepines and 1,4–benzothiazepines through their detailed molecular effects on various cellular receptors.

Pharmaceutical applications of 1,4-benzodiazepines

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Chapter outline

5.1 Introduction

Benzodiazepines are one of the most prescribed medicines in the US alone – for example, approximately 65.9 million office-based physician visits, or 27 per 100 adult patients, resulted in a prescription for benzodiazepines each year between 2014 and 2016 (Santo et al., 2020). Since the emergence of the first 1,4-benzodiazepine, chlordiazepoxide (*Librium*) in 1957, a large number of analogs have been synthesized and evaluated in biology and medicine. The increased clinical efficacy and relatively minor side effects

125

Table 5.1 Overview of FDA-approved short-acting 1,4-benzodiazepine-based drugs.

Generic name	Brand name	FDA-approved indications	Approval date	Manufacturer
Midazolam	Versed	Seizures, sedative	1985	Roche, HLR
Triazolam	Halcion	Insomnia	1982	Pharmacia & Upjohn

over other pharmaceutical agents have rendered 1,4-benzodiazepines the medicine of choice in the treatment of anxiety, insomnia, epilepsy, muscle spasms, alcohol withdrawal, and dementia (Griffin et al., 2013; Atkin et al., 2018; Sachdeva et al., 2015). As discussed in Chapter 4, benzodiazepines decrease neuronal stimulation to give a sedative effect, which is thought to be a result of γ-aminobutyric acid (GABA) receptor binding, enhancing the effect of GABA and increasing cellular chloride influx (Rudolph et al., 1999). While a comprehensive understanding of their pharmacology (including efficacy and side effects) can inform their pharmaceutical applications, 1,4-benzodiazepines and their active metabolites are often grouped on the basis of their duration of action (described by half-life, $t_{1/2}$). In this chapter, the pharmaceutical applications of FDA-approved 1,4-benzodiazepines is discussed, covering short-acting ($t_{1/2} = 1$–8 h), intermediate-acting ($t_{1/2} = 8$–40 h) and long-acting ($t_{1/2} = 40$–200 h) types.

5.2 Short-acting 1,4-benzodiazepines

Short-acting 1,4-benzodiazepines are often used to treat sleep disturbance, taken in low doses three to four times a day. With a half-life of less than 8 h, the risk of overdose is markedly reduced. These medicines are practical for mitigating immediate, one-off cases of stress and anxiety in a PRN (*Pro Re Nata*, or "taken as necessary") fashion (Starcevic, 2012). The risk of dependence to short-acting 1,4-benzodiazepines is significantly high if taken for more than three weeks, where withdrawal symptoms include sleep disturbance, irritability, and difficulty concentrating and can be eased via careful monitoring and management by health professionals. Table 5.1 gives an overview of two commercial short-acting 1,4-benzodiazepine-based drugs, which will be discussed in the following sections.

5.2.1 Midazolam

Introduction: Midazolam, often sold under the brand name *Versed*, belongs is a short-acting 1,4-benzodiazepine. Initially approved by the FDA in 1985,
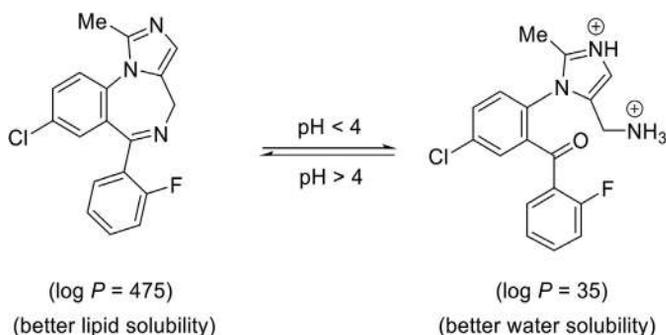
Figure 5.1 Reversible ring-opening of midazolam at different pH conditions.

it can be administered orally, rectally, intranasally, or as an intramuscular (i.m.) or intravenous (i.v.) injection and is used in various clinical settings such as dentistry, cardiac surgery, and endoscopic procedures as a pre-anesthetic and an adjunct to local anesthesia. In late 2018, i.m. administration of this drug was approved by the FDA for the treatment of status epilepticus in adults. In May 2019, the nasal spray of this drug was approved for the acute treatment of distinctive intermittent, and stereotypic seizure episodes in patients whose ages are older than 12 years old. Due to the low risk of dependence and abuse of midazolam, this medication is classified as a schedule IV drug in the United States (Wright et al., 1990).

Midazolam is usually employed for conscious sedation in emergency situations to relieve anxiety and to provide either analgesia or anesthesia during orthopedic or surgical procedures (Nordt & Clark, 1997). Due to its safety profile, rapid onset of clinical effects, and ultra-short length of action (1-3 h), this unique fused heterocycle is also utilized in endoscopy and oral surgeries (Cole et al., 1983; Milgrom et al., 1993). Midazolam is a white to a light yellow crystalline compound that has no solubility in water. The imidazole unit in the midazolam structure is rather basic, which allows the preparation of water-soluble midazolam salt as midazolam hydrochloride ($C_{18}H_{13}ClFN_3 \cdot HCl$). Under acidic conditions (pH < 4), the diazepine ring of midazolam undergoes a ring-opening reaction that leads to improving its water solubility with the partition coefficient between *n*-octanol and phosphate buffer (log *P*) of approximately 35 (Fig. 5.1) (Dundee et al., 1984). This water solubility eliminates organic solvents (e.g., propylene glycol) for the medicine packaging and minimizes both pains on injection and the incidence of thrombophlebitis with this medicine.

Pharmacodynamics: Like other benzodiazepines, midazolam cannot directly activate GABA$_A$ receptors, but synergizing the impact of

neurotransmitter GABA (γ-aminobutyric acid) on the GABA$_A$ receptors which results in neural inhibition. This leads to sedation reduction in anxiety, induction of sleep, muscle relaxation anterograde amnesia, and anticonvulsant effects.

Metabolism: Upon entering the bloodstream, midazolam is totally converted to its ring-closed form at the physiological pH and its lipid solubility significantly increases, showing an *n*-octanol/phosphate buffer partition coefficient (log *P*) of 475. This phenomenon results in the rapid onset of action of midazolam in the body (Gerecke, 1983). Furthermore, the metabolism of midazolam undergoes hepatic CYP450 enzymes and glucuronide conjugation.

Pharmacokinetics and dosage: Regardless of the administration route, midazolam after absorption needs to pass a route to reach the target tissues to release its bioactivity in the patient's body. Accordingly, the drug bioavailability (F) and maximum drug concentration in plasma (C_{max}) are two underlying parameters to evaluate the effectiveness of the drug in terms of pharmacokinetics. In detail, C_{max} means the highest drug concentration after administration, and bioavailability (F) reveals the fraction of administered drug that successfully reaches the systemic circulation while remaining chemically intact. In contrast to diazepam and chlordiazepoxide, which have painful and partial intramuscular absorption, midazolam can be quickly absorbed through an intramuscular injection. Although midazolam can be easily absorbed from the gastrointestinal (GI) tract after oral administration, its bioavailability is limited to around 40% due to the first-pass metabolism, which is affected by the inconsistency of intestinal blood flow. This bioavailability is significantly enhanced up to 90% when it is taken via an intramuscular route (Table 5.2) (Nordt & Clark, 1997; Dundee et al., 1984; Clausen et al., 1988). In this case, it is important to mention that the midazolam bioavailability of nasal administration mainly depends on its formulation. For example, the intranasal bioavailability of midazolam is found to be 60% when a propylene glycol–based solution of midazolam is taken nasally (Table 5.2), however, 73% bioavailability is determined when administered in an aqueous cyclodextrin solution (Loftsson et al., 2001) (Wermeling et al., 2009). Likewise, the maximum plasma concentration (C_{max}) and time to peak (T_{max}) of midazolam are dependent upon the medicine dosage and the type of administration (Malinovsky et al., 1993; Crevoisier et al., 1983; Hung et al., 1996). For example, C_{max} values reach 182 ng/L and 48 ng/L within around 12 and 13 min, respectively, after the nasal and rectal administration of 0.2 mg/kg in children (Malinovsky et al., 1993).

Table 5.2 Dosage and pharmacokinetic profile of midazolam.

Administration	Commercial form	Recommended dose (mg/kg)	Onset of action (min)	Bioavailability (%)
Intravenous	Ampoule (1–5 mg/mL)	0.05–0.1	2–3	100
Intramuscular	Ampoule (1–5 mg/mL)	0.05–0.1	5	$\sim$ 90
Oral	Tablet or syrup (7.5–15 mg)	0.3–0.75	15	$\sim$ 40
Nasal	Spray (5–20 mg/mL)	0.4–1.0	15	$\sim$ 60[a]
Rectal	Ampoule (1–5 mg/mL)	0.2-0.3	10-15	$\sim$ 50

[a] Bioavailability increases to 73% when administered in an aqueous cyclodextrin solution (17 mg/mL).

Midazolam can be administered through intravenous (i.v.), intramuscular (i.m.), nasal, oral, and rectal routes (Table 5.2), with mostly i.v. and i.m. uses in adults. The recommended midazolam dosage for both i.v. and i.m. administration is approximately 0.05-0.1 mg/kg, depending on the nature of the clinical procedure (Kupietzky & Houpt, 1993). A higher dosage ranging between 0.3-0.75 mg/kg is normally required for the oral route to provide similar clinical effects as the i.v. route. Nasal and rectal midazolam are commonly used for children with the optimal sedative doses of 0.4-1 mg/kg and 0.2-0.3 mg/kg for the former and the latter administration, respectively (Kupietzky & Houpt, 1993). Midazolam is commercially available in sterile, nonpyrogenic ampoules with various volumes (1-10 mL) ready for either intramuscular or intravenous injection. Each vial has midazolam hydrochloride equivalent to 1 mg/mL or 5 mg/mL of midazolam, mixed with sodium chloride (0.8%), edetate disodium (0.01%), and benzyl alcohol (1%). Midazolam is also available in tablet or syrup forms for oral administration. While the dosage ranges in tablets are between 7.5 and 15 mg, the syrup contains 2 mg/mL midazolam in the form of midazolam hydrochloride with a pH of 2.8-3.6, adjusted by hydrochloric acid. This medicine shows a rapid onset of action within 2–3 min after intravenous injection and within 15 min following oral, nasal, and rectal administration (Nordt & Clark, 1997).

Adverse effects: The use of midazolam during the pregnancy is unsafe and it can be recommended only when there is no other alternative for the patient. If this medicine is used during pregnancy, or pregnancy occurs while taking this medicine, the patient should be informed of possible side effects that may threaten the health of the fetus (Kronenfeld et al., 2017). Moreover, infants exposed to high doses of midazolam in the last

weeks of pregnancy may face some serious health issues including fetus heart irregularities, hypotension, poor sucking, hypothermia, and respiratory problems. The baby should also be monitored for signs of acute postpartum withdrawal syndrome. The clinical examinations on humans show that benzodiazepine–related teratogenicity and congenital anomalies can occur as its side effect. However, no information has been provided on the adverse effects of midazolam use in the third trimester of pregnancy on fetus brain development. This drug belongs to group C of AU TGA of pregnancy, which means that it is one of the drugs that can be dangerous for the fetus and cause abnormalities for the baby. Moreover, since this drug can be released in breast milk, it is advised to use it with caution (Nordt & Clark, 1997). For example, some experts recommend waiting at least 4 hours after an i.v. injection for breastfeeding. The American Academy of Pediatrics (AAP) has declared that midazolam with prolonged exposure has an unknown effect on infants but may be worrying. In addition, the use of midazolam for people with obesity may have moderate danger by distributing in the fatty tissues of the patient, prolonging the half–life ($t_{1/2}$), and eventually intensifying the side effects of midazolam.

Interactions: Currently, 464 drugs have been reported that can interact with midazolam. In this case, while 34 drugs have major interactions, 391 and 39 drugs display moderate and minor interactions, respectively. Aluminum hydroxide, baclofen, betamethasone, bupropion, calcium carbonate, codeine, darunavir, efavirenz, fluconazole, ketamine, and lonafarnib are some examples that may interact with the midazolam (Ahonen et al., 1997; Dirig & Yaksh, 1995; Ma et al., 2015). Alcohol can also interact with midazolam and therefore, the simultaneous use of them can enhance the feeling of drowsiness, tiredness, and dizziness. There are no major interactions reported for the simultaneous use of midazolam and fruits, except for grapefruit, which may cause some potential side effects (Cheng et al., 2002).

5.2.2 Triazolam

Introduction: Triazolam (as a generic name) under the brand name of *Halcion* and with IUPAC name of 8-chloro-6-(*o*–chlorophenyl)–1–methyl–4*H*–triazolo-[4,3-*a*]-[1,4]-benzodiazepine is a white crystalline powder broadly administered for the oral treatment of severe or chronic insomnia. Triazolam is a chemical analog of alprazolam with the difference of having a chlorine atom in the *ortho* position of the 6-phenyl ring, which was approved by the FDA as a short–term hypnotic agent in 1982 (Abraham & Sheppard, 1999; Cairns et al., 1994).
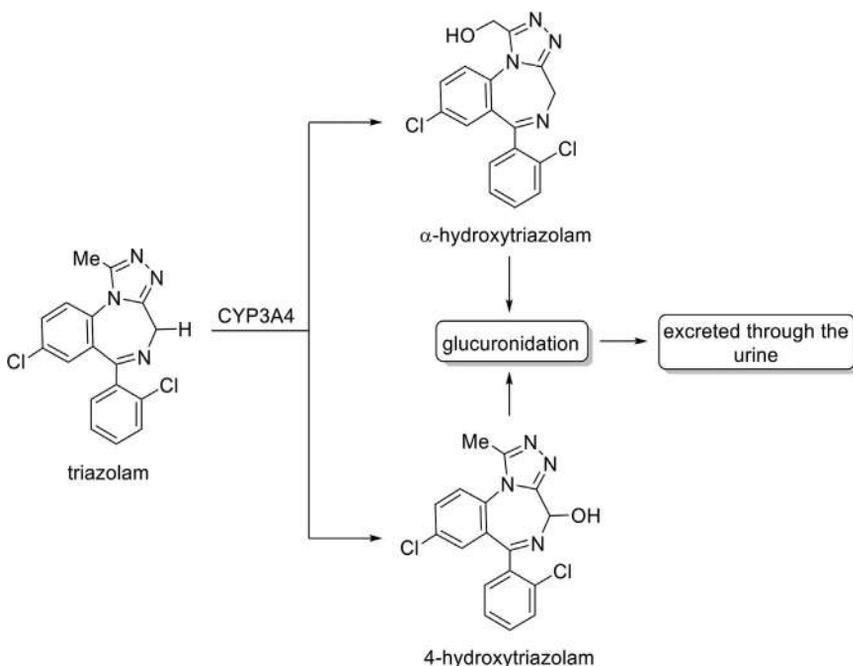
Figure 5.2 Metabolic pathway of triazolam.

Pharmacodynamics: Triazolam is extremely advised by the FDA to be administered at low doses with appropriate care and labeling (Pakes et al., 1981). Similar to most benzodiazepines, triazolam also binds to benzodiazepine receptors, which are classified as BNZ1 receptor being responsible for mediating sleep, and BNZ2 receptor for muscle relaxation, anticonvulsant activity, motor coordination, and memory. Upon the formation of triazolam-receptor complexes, they are coupled to γ-aminobutyric acid A (GABA$_A$) receptors, which eventually enhances the affinity of GABA neurotransmitters to the receptors. Binding the GABA neurotransmitter to the specific site of GABA opens the chloride channel and results in a hyperpolarized cell membrane that prevents further excitation of the cell (Zhang & Jackson, 1993).

Metabolism: Similar to other benzodiazepines, triazolam metabolizes in the human liver by CYP3A4 enzyme into two main metabolites, *i.e.*, α-hydroxytriazolam and 4-hydroxytriazolam. The drug can be excreted through the urinary system, mainly as glucuronide-conjugated α-hydroxy and 4-hydroxy metabolites, which are not assumed as active metabolites (Fig. 5.2) (Eberts et al., 1981).

Table 5.3 Pharmacokinetic parameters (single 0.88 mg dose) of triazolam.

Plasma albumin binding	89%
Maximum plasma concentration (C_{max})	8.8 ng/ml
Time to peak drug (T_{max})	1.3 h
Volume of distribution (V_d)	1.1 L/kg
Elimination half-life ($t_{1/2}$)	2.2–2.7 h

Table 5.4 Administration profile of triazolam.

Insomnia	Adults (< 61 years old): 0.25–0.5 mg/day	Geriatrics (> 61 years old): 0.125–0.25 mg/day

Pharmacokinetics and dosage: Triazolam with the onset time of 15–30 min, followed by a quick elimination with a half-life ($t_{1/2}$) of 2-5 h, is known as one of the short–acting commercially available benzodiazepines (Roth et al., 1983). Upon the oral administration of 0.88 mg triazolam, the drug quickly reaches out to the central nervous system (CNS) due to its high lipophilicity, exhibiting a maximum plasma concentration (C_{max}) of 8.8 ng/mL after 1.3 h (Table 5.3). Triazolam has relatively a shorter half–life in comparison with chlordiazepoxide, diazepam, and flurazepam, where more than 90% of the drug can be excreted from the circulatory system within 72 h. Triazolam dosage as oral tablets for adults and geriatrics are 0.25–0.5 mg/day and 0.125–0.25 mg/day, respectively, with a maximum level of 1.0 mg/day (Table 5.4). On the basis of a minimum administrated dosage, triazolam is significantly more potent than other benzodiazepines in the treatment of insomnia (Pakes et al., 1981). For example, a study of 20 male patients with insomnia prescribed with triazolam and flurazepam revealed that 1.0 mg of triazolam had a significantly superior impact on inducing sleep compared to 30 mg of flurazepam (Wang & Stockdale, 1973).

Adverse effects: Currently, more than 30 hypnotics are available in the market for the treatment of insomnia. Most of them, such as barbiturates, meprobamate, and diphenhydramine, cause several side effects, e.g., high risk of addiction and overdose, and frequent paradoxical effects in the elders (Dawson–Butterworth, 1970; Lamy & Kitler, 1971; Piccione et al., 1980), while triazolam seems to be a promising alternative by exhibiting better tolerance, less toxicity, and lower risk of overdose (Murphy et al., 1982). Additionally, the major side effects of hypnotic benzodiazepines are associated with a daytime hangover due to the remaining drug in the circulatory system during the daytime. However, the short–acting triazolam could be the best choice for the treatment of insomnia in geriatrics because of its quick elimination from the body. On the downside, triazolam has been

associated with numerous reports of retrograde amnesia (Bixler et al., 1991). Triazolam may have some further minor side effects such as drowsiness, headache, dizziness, nervousness, and laziness. Importantly, its interaction with some other drugs such as nefazodone, antibiotics, antifungals, anti–HIV, anti–hepatitis C, and codeine or hydrocodone drugs can cause serious issues such as the risk of severe breathing disorders or coma. Thus, precautions for the safe coadministration of triazolam are highly advised. Another precaution for triazolam use is associated with the pregnancy and lactating period of females. Triazolam is classified as a category X drug by the US FDA for pregnant females since it can trigger fetus abnormalities and serious birth defects. Infants exposed to triazolam via breast milk should be controlled for respiratory depression, sedation, withdrawal symptoms, and feeding problems (van Strien et al., 2013). It is therefore highly offered that a lactating woman should consider pumping and discarding breast milk 28 h after triazolam administration to reduce the risk of drug exposure to her infant.

Interactions: Triazolam, same as other benzodiazepines, is a highly interactive drug with many other medications, in which coadministering with other drugs significantly diminishes the impact of triazolam, generating some mild to serious side effects in patients. Examples of deactivating agents include boceprevir, mifepristone, telaprevir, antidepressants (e.g., nefazodone and fluoxetine), azole antifungals (e.g., itraconazole and ketoconazole), HIV protease inhibitors (e.g., lopinavir and ritonavir), rifamycins (e.g., rifampin, rifabutin). macrolide antibiotics (e.g., erythromycin and clarithromycin) (Yuan et al., 1999; Phillips et al., 1986).
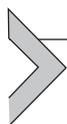
5.3 Intermediate-acting 1,4-benzodiazepines

Intermediate–acting 1,4-benzodiazepines with an elimination half-life of 8-40 h are usually administrated for the treatment of anxiety and insomnia (Leung, 2011). These medications may have some next–day residual effects such as drowsiness, dysarthria, and fatigue when taken for hypnotic purposes, in which the total sleep time is increased via reducing the number of awakenings. Compared with the short-acting benzodiazepines such as triazolam for treating insomnia, the intermediate-acting analogs are more effective for those patients suffering from awaking in the middle of the night and with difficulties of falling asleep again. To be more precise, short-acting benzodiazepines are generally prescribed for initiating sleep insomniacs, while intermediate–acting agents are administered for difficulties with sleep maintenance. 1,4-Benzodiazepines with the intermediate

Table 5.5 Overview of FDA-approved intermediate-acting 1,4-benzodiazepine-based drugs.

Generic name	Brand name	FDA-approved indications	Approval date	Manufacturer
Estazolam	ProSom	Insomnia	1990	Abbott
Lorazepam	Ativan	Anxiety, seizures sedation	1977, 1980	Wyeth, Valeant
Temazepam	Restoril	Insomnia	1981	Tyco Healthcare
Alprazolam	Xanax, Niravam	Anxiety, depression	1981, 2005	Pfizer, Schwarz Pharma

therapeutic acting should preferably be taken about once or twice a day, which is frequently used for long-term treatment. Similar to other 1,4-benzodiazepines, these drugs have continuous popularity in the management of anxiety disorders due to their consistent effectiveness, relative safety in overdose, rapid onset of action, and the possibility of administration on a PRN (as needed) basis (Starcevic, 2012). On the contrary, abrupt withdrawal of intermediate-acting 1,4-benzodiazepines may cause severe withdrawal symptoms, which appeared in both physical and psychological symptoms such as seizures, weakness, muscle tension, panic disorders, agitation, hallucinations, and delirium (Soyka, 2017). Therefore, the withdrawal procedure must be considered to avoid the withdrawal symptoms with a slow and gradual reduction of the drug dosage. Table 5.5 outlines the most common FDA-approved intermediate-acting 1,4-benzodiazepine-based drugs, which will be discussed in this section.

5.3.1 Estazolam

Introduction: Estazolam (as a generic name) with the US brand name of *ProSom* and IUPAC name of 8-chloro-6-phenyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine is a triazole-fused benzodiazepine approved by the FDA for the treatment of insomnia in 1990. In general, estazolam has anxiolytic, anticonvulsant, hypnotic, sedative, and skeletal muscle relaxant properties prescribed for the remedy of insomnia by decreasing the number of awakenings and improving sleep maintenance during a night (Post et al., 1991; Roehrs et al., 1983; Lamphere et al., 1986).

   Pharmacodynamics: Estazolam is a 1,4-benzodiazepine-based drug derived from triazolobenzodiazepines that binds to the benzodiazepine receptors in GABA postsynaptic neurons in several parts of the central nervous system (Braestrup & Squires, 1978). This phenomenon leads to

Table 5.6 Pharmacokinetic parameters of estazolam.

Plasma albumin binding	93%
Time to peak drug (T_{max})	0.5–6 h (average = 2 h)
Elimination half-life ($t_{1/2}$)	10–24 h
Excretion	Urine (> 70% as inactive metabolites), Feces (4%)
Bioavailability	93%
Duration of action	Variable

Table 5.7 Administration profile of estazolam.

Insomnia	Adults (< 61 years old):	Geriatrics (> 61 years old):
	1–2 mg orally at bedtime	1 mg orally at bedtime

increasing the permeability of the neuronal membrane to chloride ions and inducing relaxation, sleep regulation, muscle relaxant, and anticonvulsant. In the case of insomnia treatment, taking estazolam at high doses causes a significant decrease in histamine release in the brain via having an effect on the benzodiazepine-GABA receptor complex, which promotes NREM (non-rapid eye movement) sleep by shutting down the cortex and thalamus (Oishi et al., 1986).

Metabolism: Hepatic metabolism of estazolam extensively occurs in the liver to its 11 metabolites, mainly as 4-hydroxyestazolam, 1-oxo-estazolam, 4′-hydroxyestazolam, which are then excreted in the urine. The main metabolite of estazolam, 4-hydroxyestazolam, is found to be a slightly active metabolite, where its high accumulation in the body may cause drug overdose (El-Haj et al., 2018).

Pharmacokinetics and dosage: Estazolam is commercially available as oral tablets (1 and 2 mg), displaying a rapid onset time of 15–30 min with $t_{1/2}$ = 10–24 h. By using estazolam, total sleep time in patients can increase up to an average of 63 minutes on the first night of therapy, with the peak plasma level ranging 1–6 h. Owing to the relatively long half-life of estazolam, it can be also prescribed for insomniacs who are concomitant with general anxiety disorders. For example, Post and co-workers analyzed 99 randomly selected patients administered either a 2.0 mg dose of estazolam or equivalent placebo for 7 nights. The results indicated that the hypnotic impact of estazolam was statistically remarkable compared to placebo in treating the sleep disorder cases ($p < 0.01$) (Post et al., 1991). Pharmacokinetics parameters and recommended dosages of estazolam are shown in Tables 5.6 and 5.7, respectively (Aoshima et al., 2003; Gustavson & Carrigan, 1990).

Adverse effects: Somnolence, hypokinesia, sleepiness, dizziness, and abnormal balance are the main side effects of estazolam (Pierce et al., 1990). Although the adverse effects of benzodiazepines are generally higher for geriatrics, estazolam is safer and more effective for this group of patients (> 65 years old) in the treatment of insomnia (Vogel & Morris, 1992). While the long- and short-acting benzodiazepines prescribed for insomnia frequently disclose anterograde amnesia (i.e., a loss of memory for recent events) and daytime sedative effects, respectively, neither of them has been observed for estazolam. Nevertheless, estazolam should be administered with carefulness to avoid any possible negative effects. The major concerns of taking estazolam are the risks of drug dependency and addiction, in which abrupt discontinuation of the medication may cause subsequent withdrawal syndrome such as rebound insomnia (Carico et al., 2018). Therefore, it is highly recommended to take estazolam for a short-term treatment with the lowest effective dosage to prevent physical or behavioral dependence. The misuse of estazolam may proceed to exanimation, mydriasis, stunning, or even death. It has been observed in some cases that taking estazolam causes allergies, which is not undeniable. Patients should not use this medicine if they have sleep apnea. Sleep apnea is a condition that the muscles and tissues around the throat relax and block the passage of air, causing the person to stop breathing for 10 seconds or more during sleep. Asthma and chronic obstructive pulmonary disorders are some of the diseases that should not be treated with estazolam. Furthermore, people with liver and kidney problems should not take estazolam for the treatment of insomnia because they can hardly excrete estazolam from the body, leading to an overdose. Estazolam should not also be administrated in the cases of pregnancy and during breastfeeding, where it causes birth defects and inevitable symptoms. In a study carried out by Iqbal and co-workers, it was shown that a fetus fed by a mother using estazolam exhibited several withdrawal symptoms (Iqbal et al., 2002).

Interactions: Estazolam has a potential drug-drug interaction with 28 medications, in which the simultaneous consumption of estazolam with them may deal with several problems such as drowsiness and slow breathing. For example, ritonavir (used for the treatment of HIV/AIDS), itraconazole, and ketoconazole have serious interactions with estazolam, thus, their concurrent administrations should be avoided. The simultaneous use of alcohol may have no serious interactions but can intensify the sedative and hypnotic effects of estazolam during the treatment.

5.3.2 Lorazepam

Introduction: Lorazepam (as a generic name) under the brand name of *Ativan* and IUPAC name of 7-chloro-5-(2-chlorophenyl)-3-hydroxy-1,3-dihydro-1,4-benzodiazepin-2-one, is a white powder that is relatively soluble in water, known as a drug for treating insomnia, anxiety, and epilepsy. It is categorized as a Class II drug that is available both as oral tablets and in injection. Lorazepam was initially developed and marketed by Wyeth Pharmaceuticals under the brand name Ativan and Temesta in the US in 1977, followed by the FDA label approval in 1985 (The US Food and Drug, 2019).

Pharmacodynamics: Lorazepam is an anxiolytic, sedative, hypnotic, anti-amnesia, anticonvulsant, and muscle relaxant medicine (The US Food and Drug, 2019). Similar to other benzodiazepines, lorazepam relaxes the body via binding to the benzodiazepine receptors in GABA postsynaptic neurons in the central nervous system, particularly in the subcortical and limbic areas. This medicine also stimulates GABA receptors in the ascending reticular activating system (ARAS), modulating wakefulness and sleep-wake cycles (Gupta et al., 1990; Siepmann et al., 2007; The US Food and Drug, 2019).

Metabolism: Lorazepam is extensively bound to plasma proteins and metabolized in the liver, in which about 95% of orally administered lorazepam is excreted in the urine and feces within 5 days. Three main metabolites are generally formed 4 h after administration of lorazepam including the lorazepam–glucuronide, metabolites I, and II (Fig. 5.3) (Elliott, 1976). The concentration and types of metabolites highly depend on the animal species.

Pharmacokinetics and dosage: Lorazepam is administered orally, intravenously (i.v.), and intramuscularly (i.m.). The onset time of intravenous, intramuscular, and oral administrations of lorazepam are 1-3 min, 15-30 min, and 20-30 min, respectively (Greenblatt et al., 1982; Greenblatt et al., 1982). Lorazepam can be readily absorbed with an absolute bioavailability of 90% and the mean half-life of unconjugated lorazepam in human plasma is approximately 12 h (Table 5.8). Peak concentrations in plasma happen about 2 h after oral administration. The peak plasma level of lorazepam for the 2 mg dose is approximately 20 ng/mL. Since lorazepam has high lipophilicity, its rectal administration is not practical (Greenblatt et al., 1979). The usual bedtime dose of lorazepam is 2–6 mg/day given in divided doses; however, the daily dosage could vary from 1 to 10 mg daily


lorazepam

metabolite I

lorazepam-glucuronide
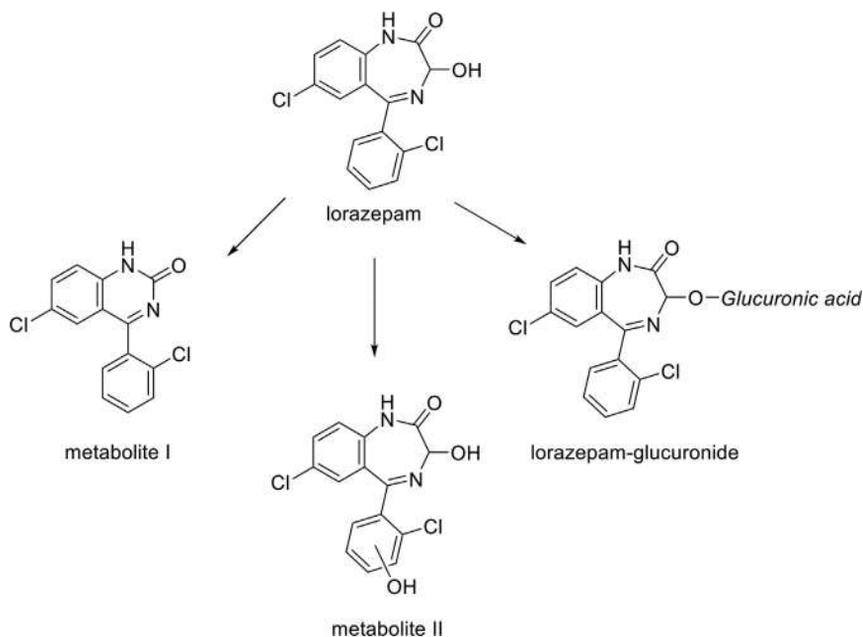
metabolite II

Figure 5.3 Three main metabolites of lorazepam.

Table 5.8 Pharmacokinetic parameters of lorazepam.

Plasma albumin binding	91%
Bioavailability	90%
Time to peak drug ($T_{\max}$)	$\leq$ 3 h (i.m.), $\sim$ 2 h (oral)
Volume of distribution (V_d)	(a) Neonates (i.v.): 0.14–1.3 L/kg (average: 0.76 L/kg); (b) Pediatric (i.v.): for 5 months to < 3 years: 0.67–3.4 L/kg (average: 1.62 L/kg), for 3 to < 13 years: 0.49–3 L/kg (average: 1.5 L/kg), for 13 to < 18 years: 1–1.54 L/kg (average: 1.27 L/kg); (c) Adults: 1.3 L/kg
Elimination half-life ($t_{1/2}$)	(a) Neonates (i.v.): 18–73 h (average: 40 h); (b) Pediatric (i.v.): for 5 months to < 3 years: 6–28 h (average: 16 h), for 3 to < 13 years: 7.5–41 h (average: 17 h), for 13 to < 18 years: 8.2–42 h (average: 18 h); (c) Adults: $\sim$12 h (oral), $\sim$ 14 h (i.v.), $\sim$ 13–18 h (i.m.)
Excretion	Urine ($\sim$ 88%, predominantly as inactive metabolites), Feces ($\sim$ 7%)
Onset of action	Anticonvulsant (i.v.): 10 min; Hypnosis (i.m.): 20–30 min; Sedation (i.v.): 15–20 min; Sedation (oral): 20–30 min
Duration of action	Adults (i.m. and i.v.): 6–8 h

Table 5.9 Administration profile of lorazepam.

Age	Insomnia	Anxiety	Light anesthesia	Status epilepticus
Adults	2 mg/day (oral)	2–3 mg/day (oral)	2 mg (i.m.)	2 mg (i.m.)
Geriatrics	2–4 mg/day (oral)	1–2 mg/day (oral)	–	–
Pediatrics (≥12 years old)	2–4 mg/day (oral)	–	–	–

(Riss et al., 2008). The size and duration of the drug effects in the body are highly dose dependent. Commercially available oral tablets contain 0.5 mg, 1 mg, or 2 mg of lorazepam. Those patients suffering from anxiety should take the initial prescribed dose of 1 mg two to three times daily. Due to transient situational stress or anxiety, insomniacs require a single dose of 2-4 mg at bedtime. For geriatrics, an initial dosage of 1–2 mg/day in divided doses is recommended (Greenblatt et al., 1979). It has been recommended that patients with the experience of alcohol dependence should be carefully administrated with the lowest dosage of lorazepam because of the possibility of several harmful consequences such as hostility, seizure, and confusion (Ramanujam et al., 2015). The pharmacokinetic parameters and recommended dosages of lorazepam are shown in Tables 5.8 and 5.9, respectively.

Adverse effects: Lorazepam is classified as a Class IV drug, according to the Drug Enforcement Administration (DEA), which means that administration of lorazepam has a risk of dependence and abuse. Side effects of lorazepam occur on the CNS (i.e., headache, dizziness, fatigue, weakness, drowsiness, nightmare, ataxia, tremor, and depression), GI (i.e., nausea, vomiting, dry mouth, difficulty in swallowing, constipation, and anorexia), and other symptoms such as rash and skin allergies, bradycardia, vascular collapse, urinary retention, binoculars, blurred vision, nystagmus, and respiratory depression. More importantly, abusing or taking high dosages of lorazepam may increase the possibility of death in humans (Kripke, 2016). According to a study on rats, repeated injection of lorazepam may cause drug resistance due to delaying sodium channel regeneration in the rats' spinal cells (McLean & Macdonald, 1988). Furthermore, it has been shown that a long-term administration of lorazepam can arise the incident of memory loss (Bishop & Curran, 1998). Formulations of lorazepam with 2 and 4 mg/mL concentrations have a significant amount of 830 mg/mL propylene glycol.

Accordingly, propylene glycol poisoning often happens in the case of high-dosage or long-term prescriptions of lorazepam (Riker & Fraser, 2005). The US FDA has classified lorazepam as a group C drug for pregnant women, having a high potential of harmful effects on the fetus. For example, the use of lorazepam during pregnancy or childbirth will cause some babies to be urged for respiratory treatment at birth. It should be noted that glucuronidation of this drug may competitively inhibit bilirubin binding that leads to hyperbilirubinemia in infants (Di Michele et al., 1996). According to the latest announcement of the AAP, this drug may result in alarming risks in babies who are breastfed by lorazepam-contaminated milk for a long period of time (Kronenfeld et al., 2017).

Interactions: Lorazepam has been shown to have interactions with aspirin, vitamin C, vitamin B12, and omega 3. This medication can also interact with alcohol to intensify its side effects, appearing in the forms of dizziness, drowsiness, and difficulty in concentrating (Aranko et al., 1985). Obesity can also extend the half-life of lorazepam due to the high lipophilicity of lorazepam, resulting in the high distribution in the body fat and blood plasma.

5.3.3 Temazepam

Introduction: Temazepam (as a generic name) under the commercial name of *Restoril* with the IUPAC name of 7-chloro-1,3-dihydro-3-hydroxy-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one is a white crystalline compound displaying slight solubility in water and alcohol. This 1,4-benzodiazepine-based medication was first developed in 1964, and soon after it became one of the most widely marketed drugs for the treatment of insomnia (Mitler, 1981; The US Food and Drug Administration, 2016b).

Pharmacodynamics: Temazepam operates by manipulating GABA within the brain, generating anti-anxiety, sedative, anticonvulsant, and muscle relaxant effects. Abnormal activity of hypothalamic–pituitary–adrenocortical (HPA) system in the brain is demonstrated to be eminently involved in the pathogenesis of major stress and depression. In a study conducted on rats, it was shown that temazepam could serve in a sedative manner to normalize the HPA axis activity directly via modulating $GABA_A$ receptors and indirectly by enhancing the intra-hypothalamic concentrations of vasopressin (AVP) (Welt et al., 2006). Like other benzodiazepines, binding temazepam to the $GABA_A$ receptors site increases the neurotransmission effects of this chemical mediator, opening the chloride channel on the receptors and enhances neuron polarization. Temazepam appears to potentiate the
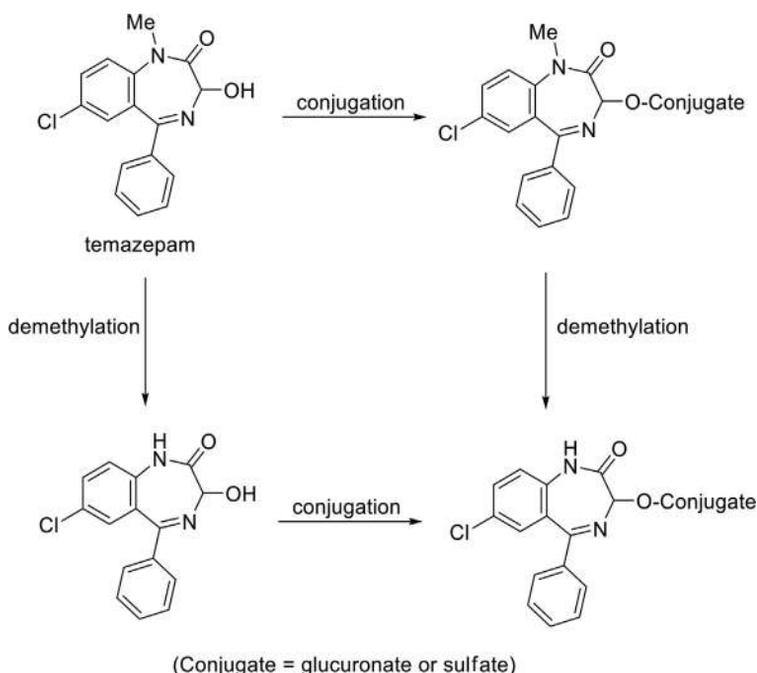
Figure 5.4 Metabolic pathway of temazepam.

inhibitory effect of GABA, which thereby reduces neuronal stimulation in many areas of the brain (Atack, 2003).

Metabolism: Temazepam with 96% plasma protein binding is extensively metabolized in the liver converting to its *O*-conjugates at high concentrations, and only small amounts of the *N*-desmethyl metabolites are found (Fig. 5.4). These four species comprise about 90% of the metabolized temazepam in the blood, which are then completely exerted in the urine and feces (Schwarz, 1979). Note that there is a considerable difference between the metabolic behavior of temazepam in the blood and the urine. For example, the quantity of temazepam in the blood is approximately 36%, while it is reduced to around 2% in the urine. Furthermore, *O*-conjugate and *N*-desmethyl metabolites occur 47% in the blood compared with 96% in the urine. These data strongly suggested that this medication is either conjugated or demethylated before excretion (Schwarz, 1979).

Pharmacokinetics and dosage: Temazepam is pharmaceutically available in either oral capsules or tablets with 7.5–30 mg of the drug. Clinical studies demonstrated that the pharmacokinetics of temazepam depend on the formulations (Salonen et al., 1986; Fuccella, 1979;

Table 5.10 Pharmacokinetic parameters of temazepam after single oral administration of 20 mg.

Oral administration type	C_{max} (ng/mL)	T_{max} (h)	AUG (µg/mL × h)	$t_{1/2}$ (h)
Soft gelatin capsule	0.892	0.83–1.1	3.86–4.23	7–11
Hard gelatin capsule	0.668	1.44–2.8	3.84–4.27	7–11
Tablet	0.284	3.1–4.0	3.95–5.55	7–11

Fuccella et al., 1977). Following ingestion of 20 mg gelatin capsules or tablets by six healthy volunteers, pharmacokinetics parameters of the medication have been compared (Table 5.10). Temazepam is much more rapidly absorbed from the soft gelatin capsule ($T_{max} = 0.83–1.1$ h) than from the tablet ($T_{max} = 3.1–4.0$ h), due to faster gastrointestinal absorption. There is no significant difference observed in the total AUC and elimination half-life of the three temazepam formulations, indicating that only the rate of absorption and distribution of the drug is dependent on the prescribed dosage form. The recommended usual dosage of temazepam for adult insomniacs is 10 to 20 mg half an hour before bedtime. However, 7.5 mg is suggested as the initial dose for individuals aged 65 or above because of the high risk of oversedation, dizziness, and confusion in elderly patients (The US Food and Drug Administration, 2016b). In a sleep assessment of eight healthy male volunteers, the effects of 10-day administration of 30 mg dosage of temazepam were compared with placebo (Ferrillo et al., 1984). Temazepam was found to significantly reduce the number of nocturnal awakenings, phase shifts, as well as a major increase of duration of stage II and sleep efficiency.

Adverse effects: Laziness, motor disorders, low driving ability, faintness, nystagmus, and the dangers of falling in the elderly are among the most common side effects of taking temazepam (The US Food and Drug Administration, 2016b). The tolerance of these effects may vary in people of different species, ages, and gender. It has been evident that temazepam gradually loses its effectiveness after around 14 days of consumption (Kales et al., 1986a). According to the FDA drug categories, this drug falls into category X, which means that temazepam may cause abnormalities to the unborn baby during pregnancy. Therefore, patients should be advised to stop taking the medication before becoming pregnant. The excretion of temazepam into breast milk during its daily consumption as a sleep-inducing drug in the postpartum period was investigated by Lebedevs and co-workers (Lebedevs et al., 1992). Ten infants (< 15 days old) were breastfed by mothers who had administrated 10 or 20 mg of temazepam (0.16 to 0.32 mg/kg)

at bedtime once daily for at least 2 days. None of the infants had any observable adverse reactions. Temazepam can induce physical dependency and addiction same as other benzodiazepines. Removal of temazepam following daily usage also contributes to the withdrawal syndrome. A slow and careful reduction of the drug dosage is recommended to sustain severe withdrawal symptoms (MacKinnon & Parker, 1982). Rare reports of the relationship between sudden removal of temazepam and psychotic states have been recorded (Terao & Tani, 1988).

Interactions: A variety of antidepressants, anti-anxiety medicines, and opioid analgesics intensify the sedative effects of this medicine. The addition of CNS depressants such as barbiturates, opiates, alcohol, non-selective MAO inhibitors, phenothiazines, and different antipsychotics, skeletal muscle relaxants, and antihistamines can disrupt the function of temazepam. However, the pharmacokinetic interactions of this medication with other co-administered drugs seem to be minimal. Since CYP3A enzymes play a key role in the metabolism of 1,4-benzodiazepines, possible interactions with those drugs having inhibitory effects on the enzymes should be considered. In this case, no noticeable interaction of temazepam with the CYP3A system has been reported (The US Food and Drug Administration, 2016b).

5.3.4 Alprazolam

Introduction: Alprazolam (as a generic name) under the brand names of *Xanax* and *Niravam* with the IUPAC name of 8-chloro-1-methyl-6-phenyl-4*H*-triazolo[4,3-*a*][1,4]benzodiazepine is an intermediate-acting triazolo-fused benzodiazepine, which has been approved for the treatment of anxiety, panic disorders, and depression (Ait-Daoud et al., 2018). Alprazolam was approved by the US FDA in 1981 to be prescribed to treat patients suffering from anxiety or panic disorder. This drug is available as a white crystalline powder that is soluble in methanol and ethanol with no significant solubility in water (The US Food and Drug Administration, 2016d), exhibiting low lipophilicity (log $P \approx 2.5$, where P is octanol–water partition coefficient). According to clinical data of 84 research studies (Jonas & Cohon, 1993), alprazolam has been shown to be as effective as or even superior to other benzodiazepines and anti-depressants such as diazepam, lorazepam, and bromazepam, clomipramine, dothiepin, amitriptyline, desipramine, doxepin, and imipramine, for the treatment of anxiety disorders.

Pharmacodynamics: The fused triazole ring in the alprazolam structure is believed to play a key role in its adsorption and metabolism. It has been well-understood that therapeutic agents with a fused triazole or
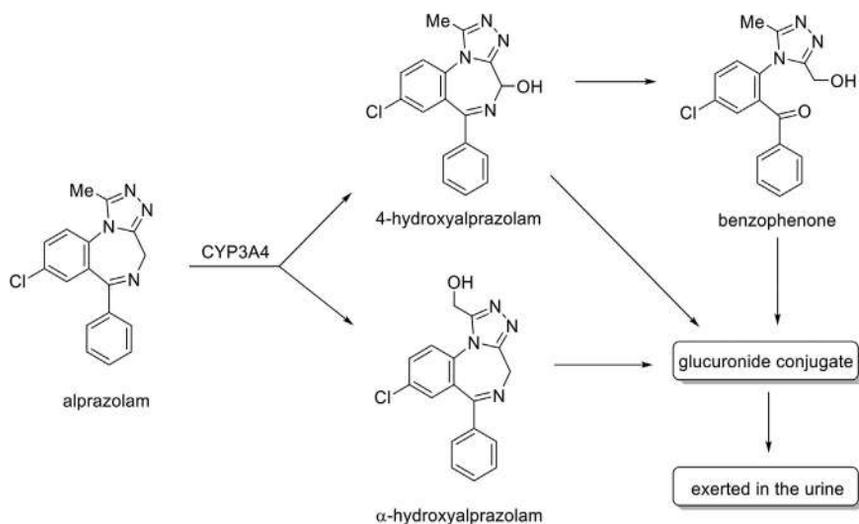
Figure 5.5　Metabolic pathway of alprazolam.

imidazole rings, such as alprazolam and midazolam, are quickly absorbed and metabolized by α-hydroxylation of the methyl group on the triazole or imidazole ring (Ait-Daoud et al., 2018). The presence of the fused triazole ring also provides a strong binding affinity to the $GABA_A$ receptors, enhancing its effectiveness. Alprazolam is also known to be significantly superior to placebo and as effective as tricyclic antidepressants (TCAs) such as clomipramine and imipramine, but with better tolerance and low withdrawal effects (Verster & Volkerts, 2004). Soon enough after alprazolam's medical use approval, it became one of the most widely prescribed psychotropic medications in the US, reporting over 48 million prescriptions in 2013 (Ait-Daoud et al., 2018).

Metabolism: Following oral administration, more than 90% of alprazolam is quickly absorbed from the gastrointestinal tract and binds to plasma albumin proteins, which are mainly metabolized in the liver by the CYP3A4 enzyme. After ingestion of alprazolam, it is converted to two major metabolites, 4-hydroxyalprazolam and α-hydroxyalprazolam, followed by further metabolism of 4-hydroxyalprazolam to its corresponding benzophenone through dissociation of the diazepine ring. Eventually, all metabolites are conjugated with glucuronide and exerted in the urine (Fig. 5.5) (Yasui et al., 1996; von Moltke et al., 1993).

Pharmacokinetics and dosage: Alprazolam's onset time is less than an hour with the elimination half-life ($t_{1/2}$) of 9 to 16 h, depending upon the rate of release of the drug. The duration of action for this drug is approximately

Table 5.11 Pharmacokinetic parameters of alprazolam (single 1.0 mg dose).

Plasma albumin binding	> 90%
Maximum plasma concentration (C_{max})	12–22 ng/L
Time to peak drug (T_{max})	1–2 h
Volume of distribution (V_d)	0.8–1.3 L/kg
Elimination half-life ($t_{1/2}$)	9–16 h
Clearance	0.7–1.5 mL/min/kg

Table 5.12 Administration profile of alprazolam.

	Extended-release tablets		Disintegrating tablets or solution	
Treatment	Adults	Geriatrics	Adults	Geriatrics
Anxiety disorders[a]	0.25–0.5 mg (3 times daily)	0.25 mg (3 times daily)	0.25–0.5 mg (3 times daily)	0.25 mg (3 times daily)
Panic disorders[b]	0.5–1 mg (once daily)	0.5 mg (once daily)	0.5 mg (3 times daily)	0.25 mg (3 times daily)

[a] Maximum level = 4 mg/day.
[b] Maximum level = 10 mg/day.

6 h, where the maximum plasma concentration (C_{max}) reaches 12–22 ng/L within 1–2 h. Pharmacokinetic parameters of alprazolam are highly dose-dependent (Abernethy et al., 1984; Fleishaker & Hulst, 1994), as such, its plasma pharmacokinetic profile after administration of a single 1 mg dose is summarized in Table 5.11. Alprazolam is commercially available either as disintegrating tablets with the dosage ranges from 0.25 mg to 2 mg or an oral solution containing 1 mg/mL, administered in divided doses (The US Food and Drug Administration, 2016d). It is also available in extended-release tablets, enabling the drug to be released in the body gradually after being taken and thereby reducing the frequency of administration. Depending on the type of disorder, age, and the form of medication, various drug dosages are prescribed to the individuals (Table 5.12) (The US Food and Drug Administration, 2016d). Anxiety treatment is often initiated at a low dose of 0.25 mg three times daily for both groups of patients, which may be increased to no more than 4 mg per day based on the drug response (Chouinard et al., 1982). The recommended starting dosages for adults and geriatrics with panic disorders are 0.5 mg/day and 0.25 mg/day, respectively, with a maximum level of 10 mg/day (The US Food and Drug Administration, 2016d; Fawcett & Kravitz, 1982).

Adverse effect: Despite many medical advantages of alprazolam, most addiction specialists have considered alprazolam to have higher misuse liability than other benzodiazepines. In this case, the US National Emergency Department (NED) announced that alprazolam was the second most routine medication and the most common benzodiazepine involved in drug misuse cases (Ait-Daoud et al., 2018). The main reasons for the alprazolam misuse potential may come from its rapid adsorption and metabolism, short half-life ($t_{1/2}$), and high euphoric effects (Verster & Volkerts, 2004), where drug addicts tend to consume the drug for recreational purposes. In terms of medical use, abrupt discontinuation of alprazolam after long-term administration can cause many withdrawal effects such as dizziness, drowsiness, dysarthria, headache, fatigue, memory impairment, and depression. Thus, the drug's daily usage is gradually reduced to zero over a period of several weeks or months to avoid severe quitting consequences (Ait-Daoud et al., 2018). The US FDA has considered alprazolam as a pregnancy category D drug that indicates initial evidence of human fetus risk (The US Food and Drug Administration, 2016d). Approximately one-third of pregnant females are predicted to receive anxiolytics to manage their psychiatric symptoms, which harms the pregnancy (The US Food and Drug Administration, 2016d). Alprazolam and its active metabolite (4-hydroxyalprazolam) can easily pass through the blood–milk barrier into breast milk, displaying similar pharmacokinetics to the drug in the blood plasma of the pregnant mother (Oo et al., 1995). In a MEDLINE search of retrospective studies (1966–2000) investigating pregnancy consequences of females taking alprazolam through the first trimester of pregnancy (Iqbal et al., 2002), those exposed to high doses of alprazolam, e.g., more than 4 mg/day for anxiety and 10 mg/day for panic, exhibited severe growth aberrations and mental disorders in their newborn infants including inguinal hernia, cleft lip, cryptorchidism, hypospadias, tracheoesophageal fistula, strabismus, microcephaly, congenital hip dislocation, and Down's syndrome. Considering the significant potential risks, careful monitoring is vital when prescribing alprazolam for pregnant or breastfeeding females are considered. The use of this drug during pregnancy is recommended only when there is no other alternative for the mother and the benefits of using it outweigh the potential risks. This is a very sensitive and important issue in relation to allergies that willow is considered during the first trimester of pregnancy because its use may be associated with an increased risk of congenital anomalies. However, there are still insufficient studies on this drug in pregnant women and in order to know the possible risks associated with the use of this drug on the fetus

(Ait-Daoud et al., 2018; Gidai et al., 2008). Due to reports on the effects of breastfeeding on infants, it may not be a suitable option for repeated use of the benzodiazepine group during lactation. At this point in time, the use of short–acting benzodiazepines without the active metabolite is preferred (Shyken et al., 2019).

Interactions: Since alprazolam is metabolized by the CYP3A4 enzyme, any drug that inhibits the activity of the CYP3A enzymes can disrupt the metabolism of alprazolam, leading to the accumulation of the drug in the body and intensifying its side effects. Many drugs have been reported to have interactions with alprazolam by inhibiting the CYP3A4 enzyme such as erythromycin, cimetidine, norfluoxetine, fluvoxamine, itraconazole, ketoconazole, propoxyphene, nefazodone, and ritonavir (Fleishaker & Hulst, 1994; Greene et al., 1995; Allqvist et al., 2007). For example, simultaneous usage of the imipramine and desipramine with alprazolam increases an average side effect of 31% and 20%, respectively (Schweizer et al., 1993; von Moltke et al., 1995). Concomitant use of alprazolam with alcohol synergizes the effect of the drug on the body, causing enhanced sedative effects, changes in behavior, and intoxication (Bond & Silveira, 1993).
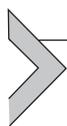
5.4 Long-acting 1,4-benzodiazepines

Long-acting 1,4-benzodiazepines display a longer half-life ($t_{1/2} > 24$ h) with a greater effect on the patient's daily relaxation than other 1,4–benzodiazepines. Upon administration of these drugs, the patient may not feel any improvements on the first night, but on the second or third night. The duration of action for these medications is about 40 to 200 h. However, they contain a risk of accumulation in the elderly and in individuals with severely impaired liver function. On contrary, the severity of rebound effects and withdrawal symptoms of long–acting 1,4-benzodiazepines are lower than those of other types of 1,4–benzodiazepines. Table 5.13 represents the most common FDA–approved long-acting 1,4-benzodiazepine–based drugs along with some details including their brand names, half-life category, and their pharmaceutical applications.

5.4.1 Clorazepate

Introduction: Clorazepate (as a generic name) under the brand name of *Tranxene* (as well as *Tranxene*, *Tranxilium*, *Novo-Clopate*) and with the IUPAC name of 7-chloro-2,3-dihydro-2-oxo-5-phenyl-1

Table 5.13 Overview of FDA-approved long-acting benzodiazepine-based drugs.

Generic name	Brand name	FDA-approved indications	Approval date	Manufacturer
Clorazepate	Tranxene	Anxiety, alcohol withdrawal	1972	Lunbeck
Clonazepam	Klonopin, Rivotril	Anxiety, seizures	1975	Roche
Chlordiazepoxide	Librium	Anxiety, alcohol withdrawal	1960	Valeant
Flurazepam	Dalmane, Dalmadorm	Insomnia	1970	Valeant
Diazepam	Valium, Diastat	Anxiety, alcohol withdrawal, seizures, muscle spasms	1963, 1997	Roche, Valeant
Quazepam	Doral	Insomnia	1985	Questcor

H-1,4-benzodiazepine–3–carboxylic acid, is one of the long-acting 1,4–benzodiazepines widely administered as adjunctive therapy for managing partial seizers and for the treatment of anxiety and acute alcohol withdrawal. An OPPIDUM survey of benzodiazepine uses in France revealed that clorazepate was the second most common benzodiazepine-based drug during the 1990s (Micallef et al., 2016), where clorazepate was mostly used in abuse or dependence situations. Clorazepate is a light–yellow odorless powder that is insoluble in most organic solvents and water. Clorazepate is only stable in its monopotassium or dipotassium salt form, and it has been shown that clorazepate is unstable in aqueous solutions, undergoing rapid decomposition (Tranxene clorazepate dipotassium tablets label, 2010). This instability is originated from the decarboxylation process at position 3 of the diazepine unit, which increases via increasing the solution pH (Clark et al., 1982). Due to the rapid decomposition of clorazepate in the gastrointestinal system to its metabolites, identification of the intact drug in the body fluids is often challenging.

Pharmacodynamics: Clorazepate's mechanism of action is in a similar way to other 1,4-benzodiazepines, activating the frequency of GABA$_A$ receptor and opening chloride (Cl$^-$) channels via binding to the benzodiazepine receptors in GABA postsynaptic neurons at several sites in the CNS such as the limbic system and retinal formation. This increase of GABA–mediated chloride influx across the cell membrane leads to hyperpolarization and synaptic inhibition.
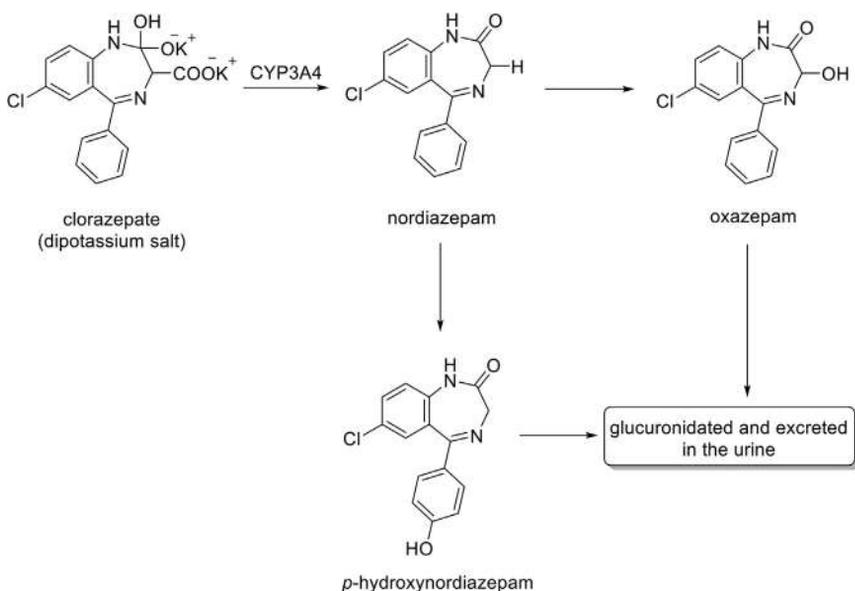
Figure 5.6 Metabolic pathway of clorazepate.

Table 5.14 Pharmacokinetic parameters of clorazepate.

Plasma protein binding	98%
Bioavailability	91%
Volume of distribution (V_d)	0.7–2.2 L/kg
Elimination half-life ($t_{1/2}$)	20–160 h
Time to peak (T_{max})	0.5–2 h
Excretion	Urine (62–67%), feces (15–19%)

Metabolism: Same as other 1,4-benzodiazepines, clorazepate is extensively bound to plasma proteins (98%) and metabolized in the liver, in which about 90% of orally administered clorazepate is excreted in the urine and feces (Fig. 5.6). This drug in the form of dipotassium salt is largely absorbed as *N*-desmethyldiazepam (nordiazepine) upon decarboxylation in the stomach. This major active metabolite, which displays significant anticonvulsant activity at the GABA$_A$ receptor, is further hydroxylated to oxazepam and finally glucuronidated and excreted from the body (Frey & Scherkl, 1988).

Pharmacokinetics and dosage: Although clorazepate has been marketed for decades, little information is available on the pharmacokinetics of this medication. The metabolites of clorazepate reach their peak concentrations within 2 h, which display a long half-life of 20–160 h (Table 5.14)

Table 5.15 Administration profile of clorazepate.

Treatment	Age group	Dosage schedule
Anxiety	Adult	(a) Initial dose: 15 mg, orally at bedtime or in divided doses; (b) Maintenance dose: 15-60 mg orally in divided doses
Alcohol withdrawal syndrome	Adult	Day 1: 30 mg orally followed by 30 to 60 mg in divided doses; Day 2: 45 to 90 mg orally in divided doses; Day 3: 22.5 to 45 mg orally in divided doses; Day 4: 15 to 30 mg orally in divided doses; Day 5: The daily dose should be reduced to 7.5–15 mg (Maximum dose: 90 mg/day)
Seizures	Adult	(a) Initial dose: 7.5 mg orally 3 times a day; (b) Maintenance dose: no more than 7.5 mg orally per week; (c) Maximum dose: 90 mg per day
Seizures	Pediatrics (≥ 12 years)	(a) Initial dose: 7.5 mg orally 3 times a day; (b) Maintenance dose: no more than 7.5 mg orally per week; (c) Maximum dose: 90 mg/day

(Lader et al., 1980; Rey et al., 1979). The recommended initial dosage of clorazepate for the treatment of anxiety, alcohol withdrawal, and seizure in both adults and pediatrics is 15 mg/day, which is administered orally in divided doses. The drug dose should be gradually increased within the range of 15–60 mg in accordance with the response of the patient and with a maximum level of 90 mg per day (Table 5.15). After the patient has been stabilized on a desirable dosage, the frequency of dosing should be reduced to twice or once per day with the main portion taken at night to produce the suitable anxiolytic effect without the appearance of drowsiness or mental impairment (Tranxene clorazepate dipotassium tablets label, 2010). In managing acute alcohol elimination, dosages up to 90 to 120 mg per day are often administrated. In the United States and Canada, clorazepate is commercially available as capsules or tablets with 3.75, 7.5, and 15 mg dosages, while tablets marketed in Europe contain 5 mg, 10 mg, 20 mg, and 50 mg of clorazepate.

Adverse effects: No acute toxicity for the long-term single daily doses of clorazepate has been reported. However, patients with the sudden termination of high doses are accompanied by insomnia, nervousness, diarrhea, or mind failure. Clorazepate is not recommended during pregnancy or lactation. The active clorazepate metabolite, nordiazepam, can easily cross the human placenta and is also excreted in breast milk. There is an increased risk of congenital malformations associated with the use of clorazepate during

the first trimester of pregnancy (McElhatton, 1994). Therefore, one should be advised to consult a physician about the termination of the drug if she intends to become pregnant.

Interactions: As clorazepate is often considered as an adjunctive medication in the maintenance treatment of anxiety, its potential drug-drug interaction with the common antidepressant drugs is highly important. In this case, Kiejna and co-workers investigated the effect of clorazepate on the function of a series of neuroleptics and antidepressant drugs in 36 patients for the management of anxiety and aggression (Kiejna et al., 1997). No destructive interactions between the co-administered clorazepate with the other medications were observed, nominating clorazepate as a safe drug with a low risk of drug-drug interactions. On contrary, simultaneous consumption of illicit drugs such as morphine, methadone, opioids, and marijuana (cannabis) with clorazepate can cause severe side effects (Lelong–Boulouard et al., 2006; Gurvich & Cunningham, 2000).

5.4.2 Clonazepam

Introduction: Clonazepam (as a generic name) under the brand names of *Klonopin* and *Rivotril* with the IUPAC name of 5-(2-chlorophenyl)–1,3-dihydro-7-nitro-2*H*-1,4-benzodiazepine-2-one was initially used as an adjunctive therapy to treat a broad range of primary or secondary generalized seizures (Riss et al., 2008; The US Food and Drug, 2013). It is insoluble in water classified as Class II of the Biopharmaceutical Classification System (BCS), exhibiting high cell-permeability due to its great lipid-solubility (Löbenberg & Amidon, 2000). Due to its slower elimination, clonazepam displays a longer acting than other anti-panic benzodiazepines, e.g., alprazolam and lorazepam, which is why the risk of withdrawal syndromes and rebound anxiety is less in the short-term administration. However, long-term clonazepam use may cause pharmacodynamic tolerance and withdrawal symptoms such as dizziness, headache, seizure, and tremor upon acute termination of clonazepam (Riss et al., 2008). The intensity of these symptoms seems to be affected by several parameters including dosage, the duration of therapy, and the drug-tapering rate. Clonazepam is also effective for treating generalized epilepsies in patients with no seizures. Dreifuss *et al*. made an analysis on 10 epileptic children with absence seizures treated with clonazepam, where 7 patients had a 75% decrease in seizure frequency after 8 weeks of the drug administration (Dreifuss et al., 1975). In another clinical study, it has been shown that clonazepam is the drug of choice in rare childhood epilepsy called Northern epilepsy syndrome (Hirvasniemi et al., 1995).

Pharmacodynamics: Although the exact mechanism of action of clonazepam has not been well understood, it is suggested that this medication may increase the level of serotonin in the brain and also enhance the concentration of the neurotransmitter at synaptic receptor sites (Davidson & Moroz, 1998). In a preliminary study carried out by Battistin and co-workers (Battistin et al., 1984), the effect of clonazepam was investigated on brain GABA levels, glutamate decarboxylase (GAD), and $GABA_T$ activities. The drug exhibited inhibition of GAD activity but had no direct effects on $GABA_T$ and GABA levels. In general, the primary action sites of benzodiazepines are located at the GABAergic synapses, where they increase GABA transmission. Binding benzodiazepines to GABA receptors is associated with changes in permeability to ions, modulating neuronal activity. In this case, the GAD inhibition induced by clonazepam imitates this process in the human brain. Since GABA affects prolactin secretion in the hypothalamus, the potency of three 1,4-benzodiazepines, e.g., clonazepam, diazepam, and chlordiazepoxide, was investigated to inhibit prolactin release correlated to GABA. While the inhibitory activities of diazepam and chlordiazepoxide on the prolactin release have been found to be dose-dependent, all analyzed doses of clonazepam are effective and this medication displays the highest potency compared with the other drugs (Grandison, 1982).

Metabolism: Clonazepam with 86% plasma protein binding is majorly metabolized in the liver by CYP3A4 and CYP3A4 enzymes through the reduction of the nitro group to form the 7-amino clonazepam, which is subsequently converted to 7-acetamido clonazepam by *N*-acetyl transferase 2 (NAT2) (Fig. 5.7). Hydroxylation of the parent molecule and the resulting metabolites at the C3 position as the minor metabolic pathway also occurs to give the corresponding 3-hydroxy metabolites (Tokola & Neuvonen, 1983; Tóth et al., 2016). 7-Amino clonazepam species has the highest concentration in the plasma compared to the other metabolites, even though it is recognized to be pharmacologically inactive. Nevertheless, an *in vivo* study on mice demonstrated that the 7-amino metabolite could compete with the parent drug to occupy benzodiazepine-binding sites, leading to a quick reduction of clonazepam attachment to the GABA receptors after the termination of treatment (Munakata & Tsuchiya, 2008).

Pharmacokinetics and dosage: Clonazepam can be administered through intravenous (i.v.), intramuscular (i.m.), oral, and rectal routes in the treatment of epileptic patients and panic disorders (Berlin & Dahlström, 1975; Browne, 1976). It is highly absorbed after i.m. and oral administration, displaying an average bioavailability of 93% and 90%, respectively. Oral
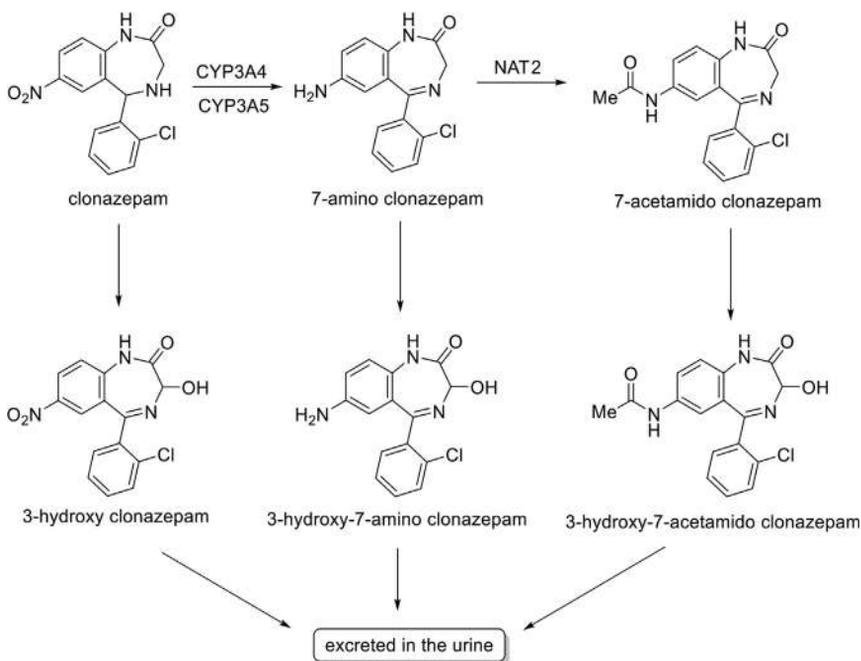
Figure 5.7 Metabolic pathway of clonazepam.

clonazepam therapy is the method of choice for patients with panic disorders, displaying fewer withdrawal–related symptoms and a longer duration of action compared with other anti–panic medications such as alprazolam and lorazepam (Davidson, 1997). The i.m. administration of clonazepam has no privilege over its oral route, which is often prescribed at the same dose as an oral formulation. However, i.m. method may be useful for patients with dysphagia and intense seizures, where the i.v. route is not possible. In the case of prolonged seizures, intravenously administration of anticonvulsants, such as diazepam and clonazepam, is an effective therapy. However, some situations may occur where these drugs cannot be prescribed intravenously. In these cases, rectal administration could be a promising alternative (Klosterskov Jensen et al., 1983). Diazepam is the common medication to be used in this way, which causes respiratory depression. On contrary, clonazepam exhibits much fewer side effects than diazepam, nominating it as a suitable drug for continuing convulsions. The recommended initial dosages of clonazepam for the treatment of seizures and panic disorders in adults are 0.5 mg/day and 0.25 mg/day, respectively, with a maximum dose limit of 20 mg daily. A significantly reduced amount of 0.01 mg/kg/day clonazepam is often

Table 5.16 Administration profile of clonazepam.

Treatment case	Adults (< 65 years old)	Pediatrics (≤ 10 years old)
Seizures	0.5–20.0 mg/day	0.01–0.05 mg/kg/day
Panic disorders	0.25–4.0 mg/day	–

Table 5.17 Pharmacokinetic parameters of clonazepam after single dose of 2 mg.

Administration type	C_{max} (ng/mL)	T_{max} (h)	AUG (ng/mL × h)	$t_{1/2}$ (h)
Intravenous (i.v.)	27.0	< 0.1	613	38
Intramuscular (i.m.)	11.0	3.1	620	43.6
Oral	14.9	1.7	561	39.0
Rectal[a]	18.4–40.5	0.1–2	NR	NR

[a] Administrated 0.1 mg/kg of body weight. (NR = not reported).

administrated for pediatrics with seizures due to their low CYP3A-catalyzed drug metabolism (Table 5.16) (Browne, 1976; The US Food and Drug, 2013).

A clinical study for the investigation of the pharmacokinetics of clonazepam was conducted with 12 healthy volunteers who were given a single dose of 2 mg clonazepam through i.v., i.m., and oral routes (Table 5.17) (Crevoisier et al., 2003). The absorption rates after i.m. and oral prescriptions are considerably different, as the plasma concentration reaches the maximum of 11.0 and 14.9 ng/mL after 3.1 h and 1.7 h, respectively. The elimination half-life of clonazepam can vary from 38 to 44 h based on the administration type. This long half-life may decrease the severity of clonazepam withdrawal syndrome compared to other benzodiazepine drugs. No significant difference is observed between the area under the plasma concentration–time curves (AUC) of the i.m. and oral administration, indicating that almost the same amount of drug comes into the systemic circulation after either i.m. or oral routes. In the case of the rectal route, plasma concentration peaks ranging 18.4–40.5 ng/ml occur in 0.1–2 h after given 0.1 mg/kg clonazepam (Table 5.17) (Rylance et al., 1986).

Adverse effects: If patients are treated with a low dose of clonazepam (< 2.0 mg/day), mild to moderate adverse reactions may occur. A clinical study on 69 patients with anxiety disorder compared the efficiency of clonazepam with lorazepam and alprazolam (Wang et al., 2016), in which the individuals were administrated orally with 0.25, 0.5, and 1 mg/day of clonazepam, lorazepam, and alprazolam, respectively, for a period of 6 weeks. The results showed that all three drugs are equally potent in the

treatment of anxiety. The incidence of adverse events is notably lower in the clonazepam–administrated patients than with the other two medications (clonazepam = 26.7%, alprazolam = 48.4%, and lorazepam = 43.9%), where somnolence (9.3%) and dizziness (6.7%) are the most common adverse reactions for clonazepam. According to the FDA guideline for clonazepam use, 50% of people suffering from seizure disorders treated with clonazepam experience drowsiness (The US Food and Drug, 2013). As mentioned earlier, there are no severe withdrawal symptoms in the short–term administration of clonazepam, as somnolence and depression are the most common adverse events. No comprehensive clinical study is available regarding the risks of clonazepam taken by pregnant women to their unborn babies. A few surveys have reported congenital anomalies, paralytic ileus, and apnea among the infants of mothers with epilepsy who used clonazepam during their pregnancy (Fisher et al., 1985; Czeizel et al., 1992; Haeusler et al., 1995). Taking these adverse effects into mind, clonazepam is recommended to be prescribed during pregnancy only if its clinical benefits to the mother outweigh the risks to the fetus, though the use of the lowest dose for the shortest time is not in the first trimester. Like other benzodiazepines, clonazepam is excreted in human breast milk. Infants exposed to the drug during breastfeeding should be monitored for CNS depression or apnea (The US Food and Drug, 2013). Therefore, extreme caution is advised when clonazepam is administered during lactation.

Interactions: Clonazepam is mainly cleared via oxidative metabolism; therefore, it is susceptible to have interactions with those drugs that modulate oxidative metabolism such as amiodarone (Witt et al., 1993). Furthermore, an interaction may occur between clonazepam and phenytoin (a famous antiepileptic drug), in which the addition of clonazepam significantly reduces phenytoin plasma concentration levels from 24.8 to 16 µg/ml, even at high dosages of phenytoin (Saavedra et al., 1985). Co–consumption of clonazepam and alcohol causes increased drowsiness and depressed breathing as these substances enhance intoxicating effects of each other.

5.4.3 Chlordiazepoxide

Introduction: Chlordiazepoxide (as a generic name) with the IU–PAC name of 7–chloro–2–methylamino–5–phenyl–3H–1,4–benzodiazepin–4–oxide is the first benzodiazepine compound accidentally discovered by Leo Sternbach in 1955 when he was working on the development of tranquilizers (Sternbach, 1972). The first clinical studies were then conducted on schizophrenic patients, where chlordiazepoxide was found to exhibit no

specific antipsychotic effect. Instead, it significantly reduced anxiety in the patients, signifying its potential as an antianxiety agent. Initial animal testing also showed that chlordiazepoxide had substantial sedative, anticonvulsant, and muscle relaxant effects. In 1960, three extensive clinical trials of chlordiazepoxide were carried out on patients with psychoneurotic disorders and chronic depression. Results showed the noticeable therapeutic efficiency of chlordiazepoxide with minor adverse effects on intellectual activity (Harris, 1960). Soon after, Hoffmann–La Roche commercialized it as "*Librium*", which was one of the major revolutions in the pharmacological treatment of anxiety disorders. Prior to the discovery of chlordiazepoxide, meprobamate and barbiturates were the drugs of choice to treat patients with serious anxiety conditions (Wick, 2013). However, these compounds had severe physical dependence and potential withdrawal syndrome. Chlordiazepoxide has also been tested on mice, rats, cats, and dogs, displaying an excellent muscle relaxation effect (López–Muñoz et al., 2011). Chlordiazepoxide is a weak base ($pK_a = 4.8$) with poor aqueous solubility at physiological pH. Therefore, this drug is often formulated in the form of hydrochloride salt (prepared at acidic pH of around 3.0) to ensure its water solubility in the body. The configuration of the nitrone group in chlordiazepoxide makes it unstable in the presence of ultraviolet light or in solution (L. Sternbach et al., 1962). Accordingly, oral dosage forms are supplied in opaque capsules or the drug solution is freshly prepared before use.

Pharmacodynamics: The mechanism of action of chlordiazepoxide is not completely known, however, it is suggested that this medication works through opening the chloride channel located on the GABA receptor, which consequently induces the chlorine ion influx inside the cell membrane and results in hyperpolarization. This phenomenon accordingly has a sedative and anxiolytic effect on the central nervous system (Choi et al., 1977).

Metabolism: The metabolism of chlordiazepoxide in humans is a complex process that consists of biotransformation into several long-acting pharmacologically active metabolites including desoxychlordiazepoxide, demoxepam, desmethylchlordiazepoxide (norchlordiazepoxide), desmethyl-diazepam (nordiazepam), and oxazepam (Fig. 5.8) (Greenblatt, Shader, MacLeod, & Sellers, 1978). The drug is first metabolized to norchlor-diazepoxide and desoxychlordiazepoxide catalyzed by the CYP3A4 enzyme in the liver. Norchlordiazepoxide is then biotransformed to demoxepam, followed by degradation to form nordiazepam, which is also produced from the conversion of desoxychlordiazepoxide (Greenblatt, Shader, MacLeod, Sellers, et al., 1978) (Greenblatt, Shader, Franke, et al., 1978). Note that the
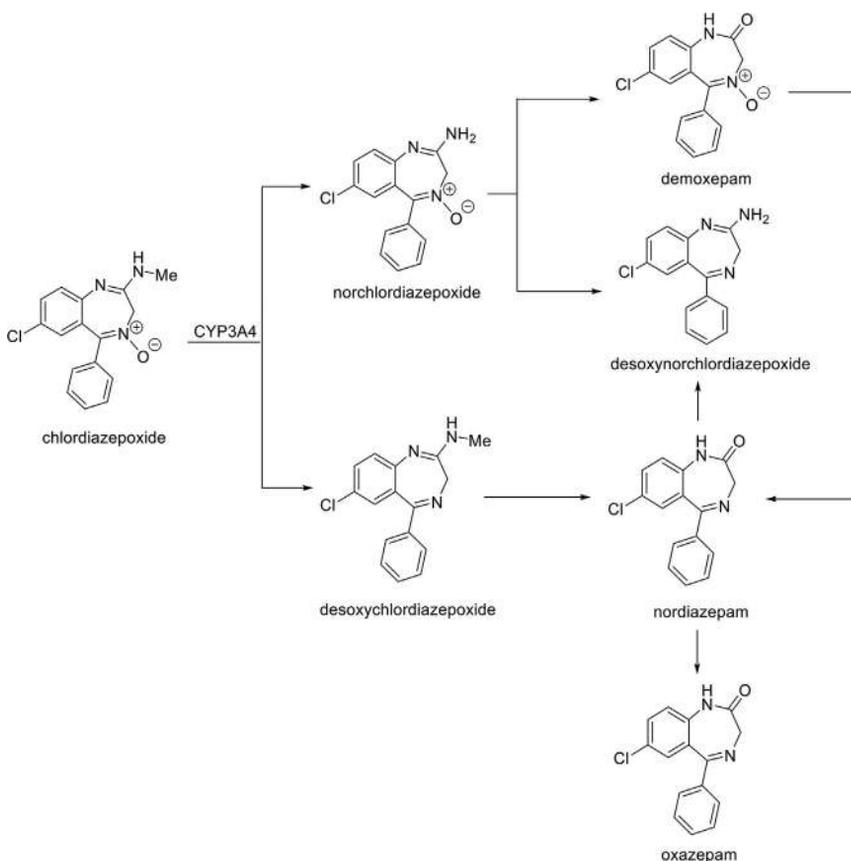
Figure 5.8 Metabolic pathway of chlordiazepoxide.

nordiazepam metabolite can be oxidized to an active oxazepam metabolite. Eventually, all metabolites are conjugated with glucuronide and exerted in the urine.

Pharmacokinetics and dosage: Chlordiazepoxide is pharmaceutically available in opaque capsules with 5 mg, 10 mg, or 25 mg chlordiazepoxide hydrochloride salt. Clinical studies demonstrated that the pharmacokinetics of chlordiazepoxide depend on the administration type (Greenblatt, Shader, MacLeod, Sellers, et al., 1978; Greenblatt, Shader, Franke, et al., 1978; Schwartz et al., 1971). Pharmacokinetics parameters of the medication following three i.v., i.m., and oral administration are compared in Table 5.18. The i.m. chlordiazepoxide is adsorbed more slowly than oral administration due to faster gastrointestinal absorption. There is no significant difference observed between the volume distributions of the three routes, indicating

Table 5.18 Pharmacokinetic parameters of chlordiazepoxide after single dose.

Administration type	C_{max} (µg/mL)	V_d (L/kg)	T_{max} (h)	$t_{1/2}$ (h)[a]
Intravenous (i.v.)[b]	NR	0.26–0.58	NR	5–30
Intramuscular (i.m.)[c]	0.48–1.23	0.25–0.50	2.5–12	5–30
Oral[c]	1.15–2.07	0.25–0.50	1–12	5–30

[a] Elimination half-life of active metabolites = 36–200 h. (NR = not reported).
[b] 30 mg dosage.
[c] 50 mg dosage.

that almost the same amount of drug is distributed into the systemic circulation. Although the parent compound displays a 5-30 h elimination half-life ($t_{1/2}$), its corresponding active metabolites have $t_{1/2} = 36–200$ h. Therefore, chlordiazepoxide is considered as a long-acting 1,4-benzodiazepine prescribed for long-term treatment. Similar to other benzodiazepines, the aging process has a significant effect on the pharmacokinetics of chlordiazepoxide. In a clinical study conducted by Shader and co-workers (Shader et al., 1977), a number of healthy individuals ranging from 30–60 years old were given a single 25 mg oral dose of chlordiazepoxide. The results showed that the average elimination half-life in the elderly cases was remarkably longer than that of the young volunteers. Furthermore, the total clearance of the drug was significantly reduced in the elderly. These observations may be due to the increased sensitivity of aged people to the CNS depressant effects.

The recommended dosages of chlordiazepoxide for the treatment of anxiety or acute alcohol withdrawal syndrome highly depend on the patient's response to the drug. In the case of mild and moderate anxiety disorders in adults, the initial oral dose is 5 mg administrated 3-4 times daily, with a maximum dose limit of 300 mg/day (The US Food and Drug, 2005). People with severe anxiety symptoms are recommended to take 20 mg chlordiazepoxide orally, 3 to 4 times daily. The usual starting dose of the drug for geriatrics is also 5 mg given 2-4 times per day. Chlordiazepoxide is one of the best medications for acute alcohol withdrawal therapy, in which the initial dose of 50 to 100 mg is suggested, followed by repeated doses as required until agitation is managed, with no more than 300 mg daily (The US Food and Drug, 2005).

Adverse effects: The occurrence and severity of the adverse reactions caused by the chlordiazepoxide were much less than those of other benzodiazepines. For example, ataxia, drowsiness, and confusion are some of the minor side effects reported in aged patients (The US Food and Drug, 2005). Agranulocytosis, jaundice, and hepatic dysfunction have also been observed during chlordiazepoxide drug therapy. In this case, periodic blood counts

and liver function analyses are highly recommended, since chlordiazepoxide treatment is protracted (The US Food and Drug, 2005). Chlordiazepoxide is categorized by the Drug Enforcement Administration (DEA) as a Schedule IV controlled drug, representing the least potential for abuse and low risk of dependence (The US Food and Drug, 2005). The intensity of withdrawal symptoms because of abrupt termination of chlordiazepoxide highly depends on the drug dose and the therapy. The more excessive drug doses over an extended time, the more severe withdrawal events can occur. Therefore, after extended treatment, a gradual dosage tapering schedule should be followed. There is an increased incidence of congenital malformations to the human fetus as a result of the use of chlordiazepoxide during the first trimester of pregnancy (Czeizel et al., 2004). Since the drug can be excreted into human milk, it is also recommended not to use chlordiazepoxide by mothers during breastfeeding.

Interactions: The coadministration of chlordiazepoxide and opioids may increase the risk of respiratory depression due to disruptive actions at respiratory-related receptors in the CNS (The US Food and Drug, 2005). Furthermore, there is a high risk of potential drug interactions associated with the concomitant use of chlordiazepoxide with other benzodiazepines, antidepressant agents, and antihistamines such as alprazolam, lorazepam, zolpidem, fluoxetine, fluvoxamine, nefazodone, cimetidine, clozapine, cetirizine, and diphenhydramine (Kurowski & Reim, 1986; Feldman & Smith, 1978; Rodgers et al., 1997). Alcohol and marijuana (cannabis) can also intensify the adverse effects of chlordiazepoxide such as dizziness, drowsiness, impairment in thinking and concentrating (Chan et al., 1982).

5.4.4 Flurazepam

Introduction: Flurazepam (as a generic name) under the brand name of *Dalmane* or *Dalmadorm* and IUPAC name of 7–chloro–1–(2–(diethylamino) ethyl)–5–(*o*–fluorophenyl)–1,3–dihydro–2*H*–1,4–benzodiazepine–2–one is a long–acting 1,4–benzodiazepine known as a hypnotic drug for the treatment of insomniacs with difficulty in falling asleep, frequent nocturnal awakenings, and early morning awakenings (The US Food and Drug, 2007). It was initially introduced to the US as a sleep–inducing agent in 1970 and soon later became popular reaching 6.5 million prescriptions in 1973. Flurazepam is available as a yellow crystalline dihydrochloride salt that is highly soluble in water and alcohol. This medication is more potent than a placebo and displays the same efficiency as other hypnotic drugs in short–term treatments.

However, flurazepam is more effective in long-term dosage therapy than other hypnotics such as barbiturates, glutethimide, chloral hydrate, meprobamate, and methaqualone (Greenblatt et al., 1975a). These medications become ineffective when administrated constantly over two weeks, however, flurazepam persists to provide pharmacologically effects even when ingested repeatedly for up to 4 weeks (Kales et al., 1970). These features signify flurazepam's advantages over other nonbenzodiazepine hypnotics.

Pharmacodynamics: Several studies have described that flurazepam displays more central CNS depression and less anxiety reduction than other benzodiazepines (Randall et al., 1969; Tseng & Wang, 1971), which clarifies flurazepam's usage as a hypnotic rather than as an antianxiety drug. These observations demonstrate that flurazepam acts as a central muscle relaxant via blockade of GABAergic interneurons at supraspinal level with no effects on the spinal cord. Cannizzaro and co-workers investigated the effect of flurazepam on rats after single-dose administration, where the medication resulted in CNS depression but no behavioral disinhibition (Cannizzaro et al., 1972). However, the mice showed tolerance to the depressant effects of flurazepam upon repeated ingestion, followed by the appearance of behavioral disinhibition. The drug has also an inhibitory effect on the direct electrical stimulation of interneurons in the brainstem reticular system, exhibiting no impact on both monosynaptic and polysynaptic reflex behaviors (Greenblatt et al., 1975a). In a study on mice given ataxia-producing doses of several benzodiazepines (Fennessy & Lee, 1972), it was shown that flurazepam decreases the amount of dopamine in their brain, whereas other benzodiazepines including diazepam, nitrazepam, chlordiazepoxide, clonazepam, and medazepam represent either no change or increased the dopamine brain content. Although many studies have been carried out on flurazepam behavior, the clinical connection of these analyses is not known yet.

Metabolism: Flurazepam concentration in the blood after administration is extremely low, which is observable only for a few hours after a single dose (Miller et al., 1988). The metabolic pathway of flurazepam is represented in Fig. 5.9, consisting of the formation of three metabolites. Flurazepam is quickly absorbed from the GI tract (within 30 min) and biotransformed to its metabolic derivatives. Concentrations of two major metabolites, e.g., hydroxyethyl flurazepam and desalkylflurazepam, become significantly high after approximately one hour of administration, where the desalkylflurazepam metabolite stays in the blood for a longer period. Therefore, these pharmacologically active metabolites may cause flurazepam's
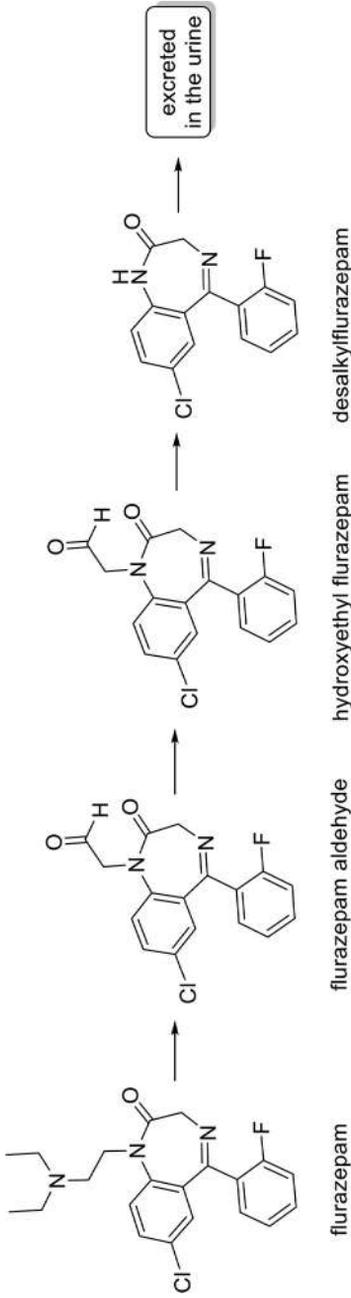
Figure 5.9 Metabolic pathway of flurazepam.

flurazepam

flurazepam aldehyde

hydroxyethyl flurazepam

desalkylflurazepam

Table 5.19 Pharmacokinetic parameters of desalkylflurazepam as the major flurazepam metabolite after single 15 mg oral dose.

Gender (age)	Weight (kg)	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)
Male (19–34)	62–86	5.3–9.6	0.5–16	37–118
Female (20–33)	52–61	11.5–20.0	0.75–73	35–133
Male (66–72)	66–89	8.2–15.1	0.75–96	71–289
Female (61–85)	46–73	10.3–19.1	0.75–24	85–165

Table 5.20 Pharmacokinetic parameters of flurazepam and its metabolites after single 30 mg oral dose.

Species	C_{max} (mean) (ng/mL)	T_{max} (mean) (h)	Total AUC (mean) (ng/ml × h)	$t_{1/2}$ (mean) (h)
Flurazepam	0–10 (2.4)	0.5–2.0 (1.0)	0.5–4.4 (1.8)	0–19.0 (5.3)
Flurazepam aldehyde	3.1–19.3 (7.8)	0.75–2.5 (1.3)	1.3–28.9 (6.9)	7.4–155.7 (48.3)
Hydroxyethyl flurazepam	7.1–23.4 (15.1)	0.75–2.0 (1.1)	0.9–2.4 (1.3)	14.8–65.2 (38.4)
Desalkyl flurazepam	7.4–47.0 (20.4)	1.0–72 (10.2)	34.1–154 (71.4)	911–3084 (1969)

clinical effects in the human body (Miller et al., 1988). Flurazepam and its metabolic derivatives are finally excreted in the urine, where hydroxyethyl flurazepam and desalkylflurazepam consist of 22%–55% and 1% of the dose, respectively.

Pharmacokinetics and dosage: In a clinical study, Greenblatt *et al.* have made an analysis on the pharmacokinetics of desalkylflurazepam as the major metabolite in 26 healthy noninsomniac volunteers aging from 19 to 85 years old after orally given 15 mg single dose of flurazepam (Greenblatt et al., 1981). Maximum desalkylflurazepam plasma concentration ranging from 5.0 to 20.2 ng/mL occurs between 0.5 and 96 h after administration, which is found to be higher in females possibly due to their lower body weights (Table 5.19). The elimination half-life of the metabolite increases with age in both genders as changes in body composition with age may influence the drug uptake of tissues, leading to altered pharmacokinetics. In a similar clinical analysis, the relative contribution of the parent drug and its metabolites in the human blood was investigated (Table 5.20) (Miller et al., 1988). The mean maximum plasma levels of flurazepam and flurazepam aldehydes are 2.4 and 7.8 ng/mL, respectively, which become undetectable within the respective 3 h and 6.9 h after administration. Hydroxyethyl

flurazepam reaches the average concentration level of 15 ng/mL after 1.1 h, becoming unobservable after 8 h. On the contrary, desalkylflurazepam shows the highest plasma peak concentration (20.4 ng/mL) that occurs at 10.2 h after dosing with an average $t_{1/2} = 71.4$ h. This metabolite remains in the blood much longer than other species, which is still detectable after 9 days of administration.

Flurazepam is marketed as capsules containing 15 mg or 30 mg flurazepam hydrochloride (The US Food and Drug, 2007). The recommended dosage for adults is 30 mg before sleeping at night. However, this drug amount is reduced to 15 mg for elderly people (> 65 years old). There have been no clinical analysis of flurazepam in children, which is why this medication is not recommended for use in people under 15 years old.

Adverse effects: Flurazepam has an advantage over other hypnotics as there is no rapid eye movement (REM) rebound observed upon discontinuation of the drug (Isjanovski & Isjanovski, 2019). According to the FDA flurazepam instruction label, hangover, drowsiness, headache, and dry mouth are the most common side effects of flurazepam (The US Food and Drug, 2007). Studies have also shown that this drug has significantly less potential for abuse and displays less toxicity when ingested in excess (Greenblatt et al., 1975a; Greenblatt et al., 1975b). Flurazepam decreases the sleep time in stages 3 and 4, being maintained drug the medication withdrawal (Kales et al., 1971). Flurazepam is categorized as a Class X by the FDA, indicating that it should not be used during pregnancy (The US Food and Drug, 2007). Despite the global extensive administration of flurazepam, there is little clinical information available about its effect on the fetus during pregnancy. A study on the effect of flurazepam during organogenesis of the rat fetus has described that the medication ingestion during the second half of pregnancy may result in severe physical abnormalities (Takzare et al., 2008). The FDA has also reported the presence of high plasma concentration of desalkylflurazepam in the infant due to the transplacental transfer of this long–acting metabolite to the fetal circulation (The US Food and Drug, 2007).

Interactions: Common nonbenzodiazepine hypnotics such as barbiturates and glutethimide are known to modulate the performance of hepatic microsomal enzymes, causing severe interactions with other drugs, especially in the case of oral anticoagulant drugs. In contrast, flurazepam exhibits no significant enzyme induction, where it can be prescribed to patients simultaneously taking oral anticoagulants (Greenblatt et al., 1975b). Several studies have also shown that there is no noticeable interaction of small

doses of flurazepam with alcohol in the human (Greenblatt et al., 1975b). Nevertheless, patients should be advised that flurazepam in high dosages may increase the CNS depression resulted from alcohol and other CNS-depressant drugs.

5.4.5 Diazepam

Introduction: Diazepam (as a generic name**)** under the brand name of *Valium* and IUPAC name of 7–chloro–1,3–dihydro–1–methyl–5–phenyl–2*H*–1,4–benzodiazepin–2–one is the second clinically approved benzodiazepine widely used for the treatment of intensive care sedation, repeated seizures, and short-term anxiety (Dundee & Haslett, 1970; The US Food and Drug Administration, 2016c). Following the discovery of chlordiazepoxide, the first marketed benzodiazepine introduced in 1957, further structural alterations on this compound were carried out to improve its biological performance, leading to the synthesis of diazepam as a more potent benzodiazepine. This drug was first introduced to the market by Hoffman-La Roche in 1963, and soon later it became the top-selling anxiolytic in the US from 1968 to 1982, which may owe to its quick onset of action and high potency with low toxicity (L. H. Sternbach, 1978). This colorless crystalline compound is insoluble in water with a calculated partition coefficient (cLog*P*) and topological polar surface area (TPSA) of 3 and 32.7, respectively, indicating good drug metabolism behavior and CNS penetration (Calcaterra & Barrow, 2014). Due to the well-characterized effects of diazepam in the literature, most laboratory analyses of anxiety on animals often employ diazepam as a control test to establish the validity of the assay (Calcaterra & Barrow, 2014).

    Pharmacodynamics: Diazepam is known as a positive allosteric modulator of the $GABA_A$ receptor complex, which attaches to specific binding sites on the α–γ subunit interfaces. Upon the interaction of the drug with these sites, chloride ion influx in the neurons significantly boosts that leads to hyperpolarized postsynaptic membranes and subsequently increasing CNS depression (Costa et al., 1978). This phenomenon eventually has anxiolytic and antiepileptic effects on neuronal processes in the thalamus, hypothalamus, limbic system, and cerebral cortex. In addition to this mechanism of action, some studies have shown the possibility of diazepam binding to voltage-gated sodium and calcium channels that may be associated with its anticonvulsant effects (Willow et al., 1984; Taft & DeLorenzo, 1984). Diazepam is also found to block stress-induced prolactin release (Grandison, 1982), acetylcholine release (Miller & Richter, 1985), and histamine recycling in mice brains (Oishi et al., 1986). Regardless of these various mechanisms

of action, the majority of the diazepam pharmacological activities seem to be mediated via the positive allosteric modulatory effects of diazepam on the GABA$_A$ chloride channels.

Metabolism: Diazepam is metabolised in the liver by CYP450 enzymes and biotransformed to three main active metabolites, desmethyldiazepam (nordiazepam), temazepam, and oxazepam (Fig. 5.10) (Hillestad, Hansen, & Melsom, 1974; Hillestad, Hansen, Melsom, et al., 1974). Initially, the parent compound is demethylated by CYP2C9, CYP2C19, CYP2B6, CYP3A4, and CYP3A5 to give nordiazepam. In an alternative route, diazepam is also 3′–hydroxylated by CYP3A4 and CYP3A5 enzymes to form temazepam. These metabolites are further hydroxylated or demethylated to afford oxazepam, which is finally glucuronidated and excreted by the urinary system. Typically, a trace amount of intact diazepam can be found in the urine along with approximately the whole original drug dose that is eliminated as metabolites. Nordiazepam constitutes the majority of the circulating dose during daily administration and remains much longer than the other metabolites in the blood, reaching steady-state after three weeks.
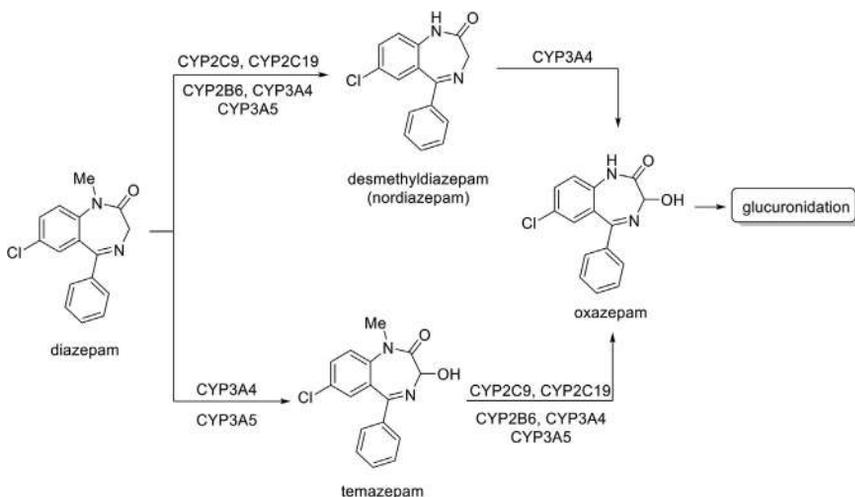
Figure 5.10 Metabolic pathway of diazepam.

Pharmacokinetics and dosage: Diazepam is commercially available as tablets (2 mg, 5 mg, or 10 mg), syrup (5 mg/ml), and ampoules (5 mg/ml). All three diazepam active metabolites have been pharmaceutically marketed as anxiolytics and anticonvulsants due to their potent activity. Temazepam has a short elimination half–life ($t_{1/2} = 8$–20 h) that nominates it for the treatment of insomnia. Oxazepam displays a slow onset of action ($\sim$ 60 min) and no

Table 5.21 Pharmacokinetic parameters of diazepam after single 10 mg dose.

Administration type	C_{max} (ng/mL)	V_d (L/kg)	T_{max} (h)	$t_{1/2}$ (h)
Oral	177	0.8–1.0	90	24–48
Intramuscular (i.m.)	100[a], 149[b]	1.1–2.0	90	24–48
Intravenous (i.v.)	400[c]	1.1–2.0	15	24–48
Rectal	369	1.1–2.0	17	24–48

[a] Injection in buttock.
[b] Injection in thigh.
[c] Reached 500 ng/mL with 20 mg dose.

hepatic oxidation is required for elimination, making it suitable for elderly patients or those with hepatic impairment suffering from anxiety (Calcaterra & Barrow, 2014). Diazepam is a lipid-soluble compound widely distributed across the central nervous system (V_d = 0.8–2.0 L/kg) (Table 5.21). It is extensively bound to plasma protein (96–99%) with a high bioavailability of 90–100%. After a single oral administration, the drug is completely absorbed from the GI tract and peak plasma levels reach 65–145 ng/mL within 30–90 min (Table 5.21). Age is the key factor to affect the drug plasma concentration, in which children and elderly people display the highest and lowest values (Mandelli et al., 1978). Intramuscular administration has a poorer absorption rate and peak plasma levels occur after 90 min of injection. More importantly, the concentration levels are higher in an injection in the thigh, possibly due to a richer blood supply to the thigh than the buttock (Table 5.21). In the emergency control of seizures, diazepam is the drug of choice, where its anticonvulsant activity usually appears at peak plasma levels of 400–500 ng/mL through intravenous injection of 10–20 mg. However, if the intravenous route cannot be achieved in elderly patients with intensive seizures, the best alternative is the rectal administration of diazepam (Seigler, 1990). The drug elimination half-life normally occurs in 24–48 h, increasing in pediatric patients due to decreased albumin levels in their serum.

The initially recommended dosages for the management of anxiety disorders in adults are from 2 to 10 mg administered orally 2–4 times daily (The US Food and Drug Administration, 2016c), with a maximum dose of 30 mg. For the relief of acute alcohol withdrawal symptoms, 10 mg of diazepam is usually required taken orally 3–4 times during the first 24 h, followed by a reduction to 5 mg as needed. Muscle antispasm uses typically start from 2 mg, adjunctively administered orally 3 or 4 times per day. In geriatric patients, it is highly suggested that the drug dosage should be limited to the smallest amount (2–2.5 mg once or twice daily) in order to prevent the development of oversedation, which can be then

increased gradually as needed (The US Food and Drug Administration, 2016c). All four administration routes of diazepam (oral, i.m., i.v., or rectal) are also used adjunctively with other antiseizure medications in the treatment of convulsive disorders, 2–10 mg, 2 to 4 times daily. Diazepam is strictly prohibited for use in pediatric patients under 6 months. Due to various responses to CNS-acting drugs in pediatric patients (< 18 years old), the therapy should be initiated with the lowest doses of 1 mg to 2.5 mg, 3–4 times daily (The US Food and Drug Administration, 2016c).

Adverse effects: Fatal adverse reactions related to diazepam such as respiratory arrest and prolonged seizures are very rare, occurring due to prolonged habitual use and the consequence interaction with other anxiolytics, e.g., opioids or alcohol. The most commonly reported minor side effects of diazepam are fatigue, drowsiness, ataxia, and muscle weakness (The US Food and Drug Administration, 2016c). Diazepam has a lower potential risk for dependence and abuse than other benzodiazepines, which is why it is commonly prescribed for the treatment of withdrawal reactions from alcohol or other benzodiazepines. Nevertheless, acute discontinuation of the long-term treatment may be associated with withdrawal symptoms such as abdominal and muscle cramps, headache, anxiety, tension, confusion, and irritability. Diazepam is subject to Schedule IV control under the Controlled Substances Act of 1970 (The US Food and Drug Administration, 2016c). The drug doses can be gradually tapered off before a complete termination, to preclude the occurrence of dependence and withdrawal effects from chronic prescription, dosages can be slowly tapered off before complete termination. Diazepam is classified in Category D pregnancy drugs, indicating that it has some human fetus risks based on side effects and the drug can be administrated during pregnancy if its potential benefits outweigh its potential risks. The drug and its metabolites can easily cross the placenta and penetrate the fetus's systematic circulation. The teratogenic risk for embryo or fetus in utero, orofacial clefts, cardiovascular malformations are the most common adverse effects of diazepam administrated in high doses during pregnancy (Erkkola et al., 1974). Nevertheless, it has been shown that the short-term drug treatment in usual doses during pregnancy leads to no noticeable teratogenic risk to the fetus (Erkkola et al., 1974).

Interactions: The concomitant use of diazepam with centrally acting agents such as phenothiazines, barbiturates, antipsychotics, and anticonvulsants should be carefully monitored to prevent any potential interactions (The US Food and Drug Administration, 2016c). Simultaneous use of diazepam with alcohol is not advised because of escalating the sedative

effect. Furthermore, compounds that disturb hepatic enzymes function, e.g., ketoconazole, cimetidine, fluoxetine, fluvoxamine, and omeprazole, can affect the pharmacokinetics of diazepam, leading to prolonged sedation. Drinking grape juice should be avoided with diazepam as it may interrupt the liver's ability to properly metabolize the drug and allows it to remain in the body longer (Ozdemir et al., 1998; Greenblatt, Allen, et al., 1978).

5.4.6 Quazepam

Introduction: Quazepam (as a generic name) under the brand name of *Doral* with the IUPAC name of 7–chloro–1–(2,2,2–trifluoroethyl)–5–(*o*–fluorophenyl)–1,3–dihydro–2*H*–1,4–benzodiazepin–2–thione is known as a long–acting 1,4–benzodiazepine hypnotic, which has been demonstrated to be highly efficient in inducing sleep in both healthy people and insomniacs (Freemon et al., 1977; Roth et al., 1979). It is a white crystalline compound having some structural similarities with flurazepam and temazepam, which is soluble in ethanol but with poor solubility in water. Quazepam improves both sleep efficacy and maintenance through a minimal effect on EEG changes in sleeping stages 2 to 4 and REM as well as a noticeable increase in total sleep time and reduction in the number of awakenings. Clinical studies have shown that this drug at dosages of 15–30 mg displays similar efficiency as flurazepam and triazolam with the significantly reduced rebound of insomnia after withdrawal, which often occurs in the case of triazolam (Ankier & Goa, 1988a; Kales, 1990). This advantage is probably associated with carryover hypnotic effects of quazepam after treatment termination because of the same or longer elimination half-lives of active metabolites than the parent drug. Nevertheless, long–term use of quazepam may result in tolerance or dependence, and effects on psychomotor skills (Ankier & Goa, 1988a). Therefore, it can be said that quazepam is the drug of choice for the short- or medium-term treatment of insomnia when withdrawal events are particularly irritating.

Pharmacodynamics: Detailed quazepam mechanism of action has not been fully understood. Selective binding of benzodiazepines such as quazepam with a trifluoroethyl group on position 1 to the benzodiazepine receptor type I (BZ_1) is a common phenomenon, where the lack of ataxia with quazepam observed in several animal studies may be originated from this behavior (Iorio et al., 1984). There are several general hypotheses about the pharmacodynamic behavior of quazepam in the cerebral cortex, substantia nigra, and the cerebellum. Since BZ_1 receptors are attached to

the GABA receptor complex, the binding of quazepam to these receptors could induce GABA transmission (Corda et al., 1986). Furthermore, quazepam seems to reduce cyclic $3',5'$–guanosine monophosphate (*c*GMP) concentrations in the neurons via promoting GABA transmission, leading to pentylenetetrazol–induced convulsions in the brain (Biggio et al., 1977; Ongini et al., 1982). Some animal studies have also described that quazepam may activate sleep-inducing and synchronizing hypnogenic mechanisms at the level of bulbo-pontine reticular neurons in the brain stem (Mariotti & Ongini, 1983; Ongini et al., 1982).

Metabolism: Quazepam is extensively metabolized by the CYP3A4 enzyme in the liver, but at a slower rate than other benzodiazepine hypnotics such as flurazepam and triazolam. The first metabolic derivative of quazepam is 2-oxoquazepam produced from the substitution of sulfur by oxygen. This pharmacologically active metabolite then undergoes either *N*-dealkylation or hydroxylation to form *N*-desalkyl–2-oxoquazepam or 3-hydroxy-2-oxoquazepam, respectively (Fig. 5.11). Conjugation of these metabolites with glucuronic acid finally occurs followed by their excretion in the urine (Zampaglione et al., 1985). Quazepam and 2-oxoquazepam are the main species in plasma that reach their maximum concentrations within the first 2 h after taking the medication. In contrast, *N*-desalkyl–2-oxoquazepam appears in plasma more slowly, reaching its peak concentration within 14 h, and stays longer in the body. Therefore, quazepam and 2-oxoquazepam could be responsible for sleep induction, and *N*-desalkyl–2-oxoquazepam with a longer half–life (Table 5.22) may contribute to the lack of rebound insomnia (Zampaglione et al., 1985).

Pharmacokinetics and dosage: Quazepam is commercially available for oral administration as 15 mg Doral tablets. Following a single 15 mg oral dose of quazepam, a peak plasma concentration of approximately 30 µg/L is reached within 2.5 h (Ankier & Goa, 1988b). It is extensively bound to plasma protein ($> 95\%$) with a rapid onset of action of 30 min. Interestingly, the plasma concentration of the drug is significantly lower when taken in the morning (21 µg/L) compared with bedtime (32.3 µg/L), which is probably due to changes in hemodynamics related to sleep or the supine position at night time (Hilbert et al., 1984). Following the 25 mg oral dose of the medication, the 2-oxoquazepam active metabolite appears in plasma with the maximum plasma concentration of 45 µg/L within 1.6 h, while *N*-desalkyl-2-oxoquazepam is formed more slowly reaching 50 µg/L within 14 h (Table 5.22). The elimination half-life of quazepam varies from 25 to 47 h following a single 15 mg oral dosage in young individuals (27 years
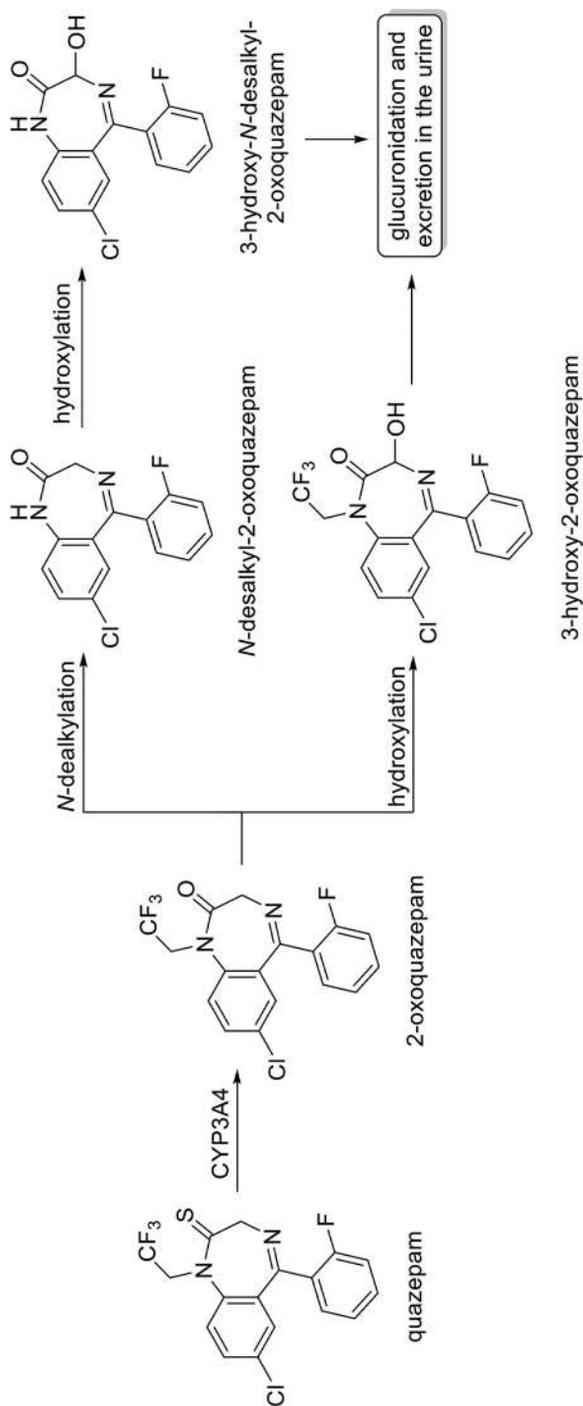
Figure 5.11 Metabolic pathways of quazepam.

Table 5.22 Pharmacokinetic parameters of quazepam and its active metabolites after single 15 mg dose.

Protein albumin binding	> 95%
Onset of action (min)	30
C_{max} (µg/L)	29–31
T_{max} (h)	2.5–2.7
V_d (L/kg)	5–8.6
AUC_{0-28} (µg/L × h)	509
AUC_{0-120} (µg/L × h)	275–320
$t_{1/2}$ (h)	25–53.3
Quazepam[a]	$C_{max} = 148$ µg/L, $T_{max} = 1.5$ h, $t_{1/2} = 39$ h
2-Oxoquazepam metabolite[a]	$C_{max} = 45$ µg/L, $T_{max} = 1.6$ h, $t_{1/2} = 40$ h
N-Desalkyl-2-oxoquazepam metabolite[a]	$C_{max} = 50$ µg/L, $T_{max} = 14$ h, $t_{1/2} = 69$ h

[a] After 25 mg oral dose.

old). In contrast, this value is slightly longer (53.3 h) in the elderly patients that results from their impairment in hepatic oxidative enzymes such as CYP3A4 and less capability in bio-transforming the drug. Quazepam is widely distributed in the body tissues with a large volume of distribution (5-8.6 L/kg), reflecting the high lipophilicity of the drug (log $P = 403$). The recommended initial dose of quazepam is 15 mg prior to bedtime with the possibility to increase to 30 mg, which provides hypnotic effects in adults with chronic or transient insomnia (The US Food and Drug Administration, 2016a). Although the onset of hypnotic effects is often observable after the initial dosage, the 30 mg dose may induce sleep much quicker than the 15 mg dose. In elderly people, the initial quazepam dose of 15 mg is advised to be reduced to 7.5 mg, as this patient group may be more sensitive to the medication (The US Food and Drug Administration, 2016a).

Adverse effects: The most routine adverse effects of quazepam include drowsiness, headache, feeling very tired, dizziness, dry mouth, and upset stomach. This drug is generally well tolerated and side effects such as amnesia and confusion, which are the usual adverse reactions with triazolam and alprazolam, are very rarely with 30 mg quazepam administration and even have not been reported with 15 mg dosage (Ankier & Goa, 1988b). Kales and co-workers have shown that sleepiness induced by 30 mg quazepam is more frequent and intense than 30 mg flurazepam (Kales et al., 1986a). The common adverse events of memory impairment, hyperexcitability, and psychotic syndromes that occurred with the administration of triazolam

and alprazolam, have not been observed with 15 mg dose of quazepam or have been very rare with the 30 mg dosage (Kales et al., 1986a). As mentioned earlier, quazepam causes no withdrawal syndromes of rebound insomnia, which is so common in other benzodiazepines. Quazepam is widely distributed to the placenta and is present in breast milk (Kales et al., 1986a). According to the US FDA, quazepam is classified as Pregnancy Category C medication, which indicates that there are no adequate and well-controlled studies in pregnant women, even though the drug's adverse effects on the animal fetus are documented (The US Food and Drug Administration, 2016a). Administration of quazepam in animal studies prior to or during pregnancy has been shown to result in hypotonia, hypothermia, respiratory depression, and minor skeletal variations in infants. Therefore, it is highly advised that quazepam can be used during pregnancy only if the potential benefits justify the potential risks (Kales et al., 1986a).

Interactions: There is a lack of information regarding the potential quazepam interactions with alcohol or other medications. Nevertheless, according to the US FDA, quazepam may cause enhanced CNS depressant effects when co-administered with alcohol or other CNS depressants such as psychotropic medications, anticonvulsants, and antihistamines (The US Food and Drug Administration, 2016a).

References

Abernethy, D. R., Greenblatt, D. J., Divoll, M., Smith, R. B., & Shader, R. I. (1984). The influence of obesity on the pharmacokinetics of oral alprazolam and triazolam. *Clinical Pharmacokinetics, 9*(2), 177–183.

Abraham, J., & Sheppard, J. (1999). Complacent and conflicting scientific expertise in British and American drug regulation: clinical risk assessment of triazolam. *Social Studies of Science, 29*(6), 803–843.

Ahonen, J., Olkkola, K. T., & Neuvonen, P. J. (1997). Effect of route of administration of fluconazole on the interaction between fluconazole and midazolam. *European Journal of Clinical Pharmacology, 51*(5), 415–419.

Ait-Daoud, N., Hamby, A. S., Sharma, S., & Blevins, D. (2018). A review of alprazolam use, misuse, and withdrawal. *Journal of Addiction Medicine, 12*(1), 4–10. https://doi.org/10.1097/ADM.0000000000000350.

Allqvist, A., Miura, J., Bertilsson, L., & Mirghani, R. A. (2007). Inhibition of CYP3A4 and CYP3A5 catalyzed metabolism of alprazolam and quinine by ketoconazole as racemate and four different enantiomers. *European Journal of Clinical Pharmacology, 63*(2), 173–179.

Ankier, S. I., & Goa, K. L. (1988a). Quazepam. A preliminary review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in insomnia. *Drugs, 35*(1), 42–62.

Ankier, S. I., & Goa, K. L. (1988b). Quazepam. A preliminary review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in insomnia. *Drugs, 35*(1), 42–62.

Aoshima, T., Fukasawa, T., Otsuji, Y., Okuyama, N., Gerstenberg, G., Miura, M., Ohkubo, T., Sugawara, K., & Otani, K. (2003). Effects of the CYP2C19 genotype and cigarette smoking on the single oral dose pharmacokinetics and pharmacodynamics of estazolam. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 27*(3), 535–538.

Aranko, K., Seppälä, T., Pellinen, J., & Mattila, M. J. (1985). Interaction of diazepam or lorazepam with alcohol. Psychomotor effects and bioassayed serum levels after single and repeated doses. *European Journal of Clinical Pharmacology, 28*(5), 559–565.

Atack, J. R. (2003). Anxioselective compounds acting at the GABA$_A$ receptor benzodiazepine binding site. *Current Drug Targets. CNS and Neurological Disorders, 2*(4), 213–232.

Atkin, T., Comai, S., & Gobbi, G. (2018). Drugs for insomnia beyond benzodiazepines: pharmacology, clinical applications, and discovery. *Pharmacological Reviews, 70*(2), 197–245. https://doi.org/10.1124/pr.117.014381.

Battistin, L., Varotto, M., Berlese, G., & Roman, G. (1984). Effects of some anticonvulsant drugs on brain GABA level and GAD and GABA-T activities. *Neurochemical Research, 9*(2), 225–231.

Berlin, A., & Dahlström, H. (1975). Pharmacokinetics of the anticonvulsant drug clonazepam evaluated from single oral and intravenous doses and by repeated oral administration. *European Journal of Clinical Pharmacology, 9*(2–3), 155–159.

Biggio, G., Brodie, B. B., Costa, E., & Guidotti, A. (1977). Mechanisms by which diazepam, muscimol, and other drugs change the content of cGMP in cerebellar cortex. *Proceedings of the National Academy of Sciences of the United States of America, 74*(8), 3592–3596.

Bishop, K. I., & Curran, H. V. (1998). An investigation of the effects of benzodiazepine receptor ligands and of scopolamine on conceptual priming. *Psychopharmacology, 140*(3), 345–353.

Bixler, E. O., Kales, A., Manfredi, R. L., Vgontzas, A. N., Tyson, K. L., & Kales, J. D. (1991). Next-day memory impairment with triazolam use. *Lancet (London, England), 337*(8745), 827–831.

Bond, A. J., & Silveira, J. C. (1993). The combination of alprazolam and alcohol on behavioral aggression. *Journal of Studies on Alcohol Supplement, 11*, 30–39.

Braestrup, C., & Squires, R. F. (1978). Pharmacological characterization of benzodiazepine receptors in the brain. *European Journal of Pharmacology, 48*(3), 263–270.

Browne, T. R. (1976). Clonazepam. A review of a new anticonvulsant drug. *Archives of Neurology, 33*(5), 326–332.

Cairns, E. R., Dent, B. R., Ouwerkerk, J. C., & Porter, L. J. (1994). Quantitative analysis of alprazolam and triazolam in hemolysed whole blood and liver digest by GC/MS/NICI with deuterated internal standards. *Journal of Analytical Toxicology, 18*(1), 1–6.

Calcaterra, N. E., & Barrow, J. C. (2014). Classics in chemical neuroscience: diazepam (valium). *ACS Chemical Neuroscience, 5*(4), 253–260. https://doi.org/10.1021/cn5000056.

Cannizzaro, G., Nigito, S., Provenzano, P. M., & Vitikova, T. (1972). Modification of depressant and disinhibitory action of flurazepam during short term treatment in the rat. *Psychopharmacologia, 26*(2), 173–184.

Carico, R., Zhao, X., Thorpe, C. T., Thorpe, J. M., Sileanu, F. E., Cashy, J. P., Hale, J. A., Mor, M. K., Radomski, T. R., Hausmann, L. R. M., Donohue, J. M., Suda, K. J., Stroupe, K., Hanlon, J. T., Good, C. B., Fine, M. J., & Gellad, W. F. (2018). Receipt of overlapping opioid and benzodiazepine prescriptions among veterans dually enrolled in medicare part D and the department of veterans affairs: A cross-sectional study. *Annals of Internal Medicine, 169*(9), 593–601. https://doi.org/10.7326/M18-0852.

Chan, A. W., Greizerstein, H. B., & Strauss, W. (1982). Alcohol–chlordiazepoxide interaction. *Pharmacology, Biochemistry, and Behavior, 17*(1), 141–145.

Cheng, C., Roemer-Becuwe, C., & Pereira, J. (2002). When midazolam fails. *Journal of Pain and Symptom Management, 23*(3), 256–265.

Choi, D. W., Farb, D. H., & Fischbach, G. D. (1977). Chlordiazepoxide selectively augments GABA action in spinal cord cell cultures. *Nature, 269*(5626), 342–344.

Chouinard, G., Annable, L., Fontaine, R., & Solyom, L. (1982). Alprazolam in the treatment of generalized anxiety and panic disorders: a double-blind placebo-controlled study. *Psychopharmacology, 77*(3), 229–233.

Clark, C. R., Ravis, W. R., Dockens, R., Barksdale, J. M., & Arrington, H. S. (1982). Liquid chromatographic determination of clorazepate decomposition rates. *Journal of Chromatography A, 240*(1), 196–201. https://doi.org/10.1016/S0021-9673(01)84022-X.

Clausen, T. G., Wolff, J., Hansen, P. B., Larsen, F., Rasmussen, S. N., Dixon, J. S., & Crevoisier, C. (1988). Pharmacokinetics of midazolam and α-hydroxy-midazolam following rectal and intravenous administration. *British Journal of Clinical Pharmacology, 25*(4), 457–463.

Cole, S. G., Brozinsky, S., & Isenberg, J. I. (1983). Midazolam, a new more potent benzodiazepine, compared with diazepam: a randomized, double-blind study of preendoscopic sedatives. *Gastrointestinal Endoscopy, 29*(3), 219–222.

Corda, M. G., Sanna, E., Concas, A., Giorgi, O., Ongini, E., Nurchi, V., … Biggio, G. (1986). Enhancement of γ-aminobutyric acid binding by quazepam, a benzodiazepine derivative with preferential affinity for type I benzodiazepine receptors. *Journal of Neurochemistry, 47*(2), 370–374.

Costa, E., Guidotti, A., & Toffano, G. (1978). Molecular mechanisms mediating the action of diazepam on GABA receptors. *The British Journal of Psychiatry: The Journal of Mental Science, 133*, 239–248.

Crevoisier, C., Delisle, M. C., Joseph, I., & Foletti, G. (2003). Comparative single-dose pharmacokinetics of clonazepam following intravenous, intramuscular and oral administration to healthy volunteers. *European Neurology, 49*(3), 173–177.

Crevoisier, C., Ziegler, W. H., Eckert, M., & Heizmann, P. (1983). Relationship between plasma concentration and effect of midazolam after oral and intravenous administration. *British Journal of Clinical Pharmacology, 16*(1), 51S–61S.

Czeizel, A. E., Bod, M., & Halász, P. (1992). Evaluation of anticonvulsant drugs during pregnancy in a population-based Hungarian study. *European Journal of Epidemiology, 8*(1), 122–127.

Czeizel, A. E., Rockenbauer, M., Sørensen, H. T., & Olsen, J. (2004). A population-based case-control study of oral chlordiazepoxide use during pregnancy and risk of congenital abnormalities. *Neurotoxicology and Teratology, 26*(4), 593–598.

Davidson, J. R. T. (1997). Use of benzodiazepines in panic disorder. *Journal of Clinical Psychiatry, 58*(Suppl 2), 29–31 26-8.

Davidson, J. R. T., & Moroz, G. (1998). Pivotal studies of clonazepam in panic disorder. *Psychopharmacology Bulletin, 34*(2), 169–174.

Dawson-Butterworth, K. (1970). The chemopsychotherapeutics of geriatric sedation. *Journal of the American Geriatrics Society, 18*(2), 97–114.

Di Michele, V., Ramenghi, L., & Sabatino, G. (1996). Clozapine and lorazepam administration in pregnancy. *European Psychiatry: The Journal of the Association of European Psychiatrists, 11*(4), 214. https://doi.org/10.1016/0924-9338(96)88396-9.

Dirig, D. M., & Yaksh, T. L. (1995). Intrathecal baclofen and muscimol, but not midazolam, are antinociceptive using the rat-formalin model. *Journal of Pharmacology and Experimental Therapeutics, 275*(1), 219–227.

Dreifuss, F. E., Penry, J. K., Rose, S. W., Kupferberg, H. J., Dyken, P., & Sato, S. (1975). Serum clonazepam concentrations in children with absence seizures. *Neurology, 25*(3), 255–258.

Dundee, J. W., Halliday, N. J., Harper, K. W., & Brogden, R. N. (1984). Midazolam. A review of its pharmacological properties and therapeutic use. *Drugs, 28*(6), 519–543.

Dundee, J. W., & Haslett, W. H. (1970). The benzodiazepines. A review of their actions and uses relative to anaesthetic practice. *British Journal of Anaesthesia, 42*(3), 217–234.

Eberts, F. S., Philopoulos, Y., Reineke, L. M., & Vliek, R. W. (1981). Triazolam disposition. *Clinical Pharmacology and Therapeutics, 29*(1), 81–93.

El-Haj, B. M., Ahmed, S. B. M., Garawi, M. A., & Ali, H. S. (2018). Linking aromatic hydroxy metabolic functionalization of drug molecules to structure and pharmacologic activity. *Molecules (Basel, Switzerland), 23*(9), 2119. https://doi.org/10.3390/molecules23092119.

Elliott, H. W. (1976). Metabolism of lorazepam. *British Journal of Anaesthesia, 48*(10), 1017–1023.

Erkkola, R., Kanto, J., & Sellman, R. (1974). Diazepam in early human pregnancy. *Acta Obstetricia Et Gynecologica Scandinavica, 53*(2), 135–138.

Fawcett, J. A., & Kravitz, H. M. (1982). Alprazolam: pharmacokinetics, clinical efficacy, and mechanism of action. *Pharmacotherapy, 2*(5), 243–254.

Feldman, R. S., & Smith, W. C. (1978). Chlordiazepoxide-fluoxetine interactions on food intake in free-feeding rats. *Pharmacology, Biochemistry, and Behavior, 8*(6), 749–752.

Fennessy, M. R., & Lee, J. R. (1972). The effect of benzodiazepines on brain amines of the mouse. *Archives Internationales De Pharmacodynamie Et De Therapie, 197*(1), 37–44.

Ferrillo, F., Balestra, V., Carta, F., Nuvoli, G., Pintus, C., & Rosadini, G. (1984). Comparison between the central effects of camazepam and temazepam. Computerized analysis of sleep recordings. *Neuropsychobiology, 11*(1), 72–76.

Fisher, J. B., Edgren, B. E., Mammel, M. C., & Coleman, J. M. (1985). Neonatal apnea associated with maternal clonazepam therapy: a case report. *Obstetrics and Gynecology, 66*(3), 34S–35S.

Fleishaker, J. C., & Hulst, L. K. (1994). A pharmacokinetic and pharmacodynamic evaluation of the combined administration of alprazolam and fluvoxamine. *European Journal of Clinical Pharmacology, 46*(1), 35–39.

Freemon, F. R., Al-Marashi, M. S., & Lee, J. C. (1977). The effect of a new benzodiazepine on the polygraphically monitored sleep of normal volunteers. *Journal of Clinical Pharmacology, 17*(7), 398–401.

Frey, H. H., & Scherkl, R. (1988). Clorazepate, correlation between metabolism and anticonvulsant activity. *European Journal of Pharmacology, 158*(3), 213–216.

Fuccella, L. M. (1979). Bioavailability of temazepam in soft gelatin capsules. *British Journal of Clinical Pharmacology, 8*(1), 31S–35S.

Fuccella, L. M., Bolcioni, G., Tamassia, V., Ferrario, L., & Tognoni, G. (1977). Human pharmacokinetics and bioavailability of temazepam administered in soft gelatin capsules. *European Journal of Clinical Pharmacology, 12*(5), 383–386.

Gerecke, M. (1983). Chemical structure and properties of midazolam compared with other benzodiazepines. *British Journal of Clinical Pharmacology, 16*(1), 11S–16S.

Gidai, J., Acs, N., Bánhidy, F., & Czeizel, A. E. (2008). An evaluation of data for 10 children born to mothers who attempted suicide by taking large doses of alprazolam during pregnancy. *Toxicology and Industrial Health, 24*(1–2), 53–60. https://doi.org/10.1177/0748233708089017.

Grandison, L. (1982). Suppression of prolactin secretion by benzodiazepines in vivo. *Neuroendocrinology, 34*(5), 369–373.

Greenblatt, D. J., Allen, M. D., Locniskar, A., Harmatz, J. S., & Shader, R. I. (1979). Lorazepam kinetics in the elderly. *Clinical Pharmacology and Therapeutics, 26*(1), 103–113.

Greenblatt, D. J., Allen, M. D., MacLaughlin, D. S., Harmatz, J. S., & Shader, R. I. (1978). Diazepam absorption: effect of antacids and food. *Clinical Pharmacology and Therapeutics, 24*(5), 600–609.

Greenblatt, D. J., Divoll, M., Harmatz, J. S., MacLaughlin, D. S., & Shader, R. I. (1981). Kinetics and clinical effects of flurazepam in young and elderly noninsomniacs. *Clinical Pharmacology and Therapeutics, 30*(4), 475–486.

Greenblatt, D. J., Divoll, M., Harmatz, J. S., & Shader, R. I. (1982). Pharmacokinetic comparison of sublingual lorazepam with intravenous, intramuscular, and oral lorazepam. *Journal of Pharmaceutical Sciences, 71*(2), 248–252.

Greenblatt, D. J., Shader, R. I., Franke, K., & Harmatz, J. S. (1978). Pharmacokinetics of chlordiazepoxide and metabolites following single and multiple oral doses. *International Journal of Clinical Pharmacology and Biopharmacy, 16*(10), 486–493.

Greenblatt, D. J., Shader, R. I., & Koch-Weser, J. (1975a). Flurazepam hydrochloride. *Clinical Pharmacology and Therapeutics, 17*(1), 1–14.

Greenblatt, D. J., Shader, R. I., & Koch-Weser, J. (1975b). Flurazepam hydrochloride, a benzodiazepine hypnotic. *Annals of Internal Medicine, 83*(2), 237–241.

Greenblatt, D. J., Shader, R. I., MacLeod, S. M., & Sellers, E. M. (1978). Clinical pharmacokinetics of chlordiazepoxide. *Clinical Pharmacokinetics, 3*(5), 381–394.

Greenblatt, D. J., Shader, R. I., MacLeod, S. M., Sellers, E. M., Franke, K., & Giles, H. G. (1978). Absorption of oral and intramuscular chlordiazepoxide. *European Journal of Clinical Pharmacology, 13*(4), 267–274.

Greene, D. S., Salazar, D. E., Dockens, R. C., Kroboth, P., & Barbhaiya, R. H. (1995). Coadministration of nefazodone and benzodiazepines: III. A pharmacokinetic interaction study with alprazolam. *Journal of Clinical Psychopharmacology, 15*(6), 399–408.

Griffin, C. E., Kaye, A. M., Bueno, F. R., & Kaye, A. D. (2013). Benzodiazepine pharmacology and central nervous system–mediated effects. *The Ochsner Journal, 13*(2), 214–223.

Gupta, S. K., Ellinwood, E. H., Nikaido, A. M., & Heatherly, D. G. (1990). Simultaneous modeling of the pharmacokinetic and pharmacodynamic properties of benzodiazepines. I: Lorazepam. *Journal of Pharmacokinetics and Biopharmaceutics, 18*(2), 89–102.

Gurvich, T., & Cunningham, J. A. (2000). Appropriate use of psychotropic drugs in nursing homes. *American Family Physician, 61*(5), 1437–1446.

Gustavson, L. E., & Carrigan, P. J. (1990). The clinical pharmacokinetics of single doses of estazolam. *American Journal of Medicine, 88*(3A), 2S–5S.

Haeusler, M. C., Hoellwarth, M. E., & Holzer, P. (1995). Paralytic ileus in a fetus-neonate after maternal intake of benzodiazepine. *Prenatal Diagnosis, 15*(12), 1165–1167.

Harris, T. H. (1960). Methaminodiazepoxide. *Journal of the American Medical Association, 172*, 1162–1163.

Hilbert, J. M., Chung, M., Maier, G., Gural, R., Symchowicz, S., & Zampaglione, N. (1984). Effect of sleep on quazepam kinetics. *Clinical Pharmacology and Therapeutics, 36*(1), 99–104.

Hillestad, L., Hansen, T., & Melsom, H. (1974). Diazepam metabolism in normal man. II. Serum concentration and clinical effect after oral administration and cumulation. *Clinical Pharmacology and Therapeutics, 16*(3), 485–489.

Hillestad, L., Hansen, T., Melsom, H., & Drivenes, A. (1974). Diazepam metabolism in normal man. I. Serum concentrations and clinical effects after intravenous, intramuscular, and oral administration. *Clinical Pharmacology and Therapeutics, 16*(3), 479–484.

Hirvasniemi, A., Herrala, P., & Leisti, J. (1995). Northern epilepsy syndrome: clinical course and the effect of medication on seizures. *Epilepsia, 36*(8), 792–797.

Hung, O. R., Dyck, J. B., Varvel, J., Shafer, S. L., & Stanski, D. R. (1996). Comparative absorption kinetics of intramuscular midazolam and diazepam. *Canadian Journal of Anaesthesia = Journal Canadien d'anesthesie, 43*(5), 450–455.

Iorio, L. C., Barnett, A., & Billard, W. (1984). Selective affinity of 1-N-trifluoroethyl benzodiazepines for cerebellar type 1 receptor sites. *Life Sciences, 35*(1), 105–113.

Iqbal, M. M., Sobhan, T., & Ryals, T. (2002). Effects of commonly used benzodiazepines on the fetus, the neonate, and the nursing infant. *Psychiatric Services, 53*(1), 39–49.

Isjanovski, V., & Isjanovski, I. (2019). Comparison of the use of hypnotic in psychiatric patients with insomnia at the mental health centre prolet in skopje. *Open Access Macedonian Journal of Medical Sciences, 7*(17), 2786–2790. https://doi.org/10.3889/oamjms.2019.711.

Jonas, J. M., & Cohon, M. S. (1993). A comparison of the safety and efficacy of alprazolam versus other agents in the treatment of anxiety, panic, and depression: a review of the literature. *Journal of Clinical Psychiatry, 54*(Suppl 25-45), 46–48.

Kales, A. (1990). Quazepam: Hypnotic efficacy and side effects. *Pharmacotherapy, 10*(1), 1–14.

Kales, A., Allen, C., Scharf, M. B., & Kales, J. D. (1970). Hypnotic drugs and their effectiveness. All-night EEG studies of insomniac subjects. *Archives of General Psychiatry, 23*(3), 226–232.

Kales, A., Bixler, E. O., Soldatos, C. R., Vela-Bueno, A., Jacoby, J. A., & Kales, J. D. (1986a). Quazepam and temazepam: effects of short- and intermediate-term use and withdrawal. *Clinical Pharmacology and Therapeutics, 39*(3), 345–352.

Kales, J., Kales, A., Bixler, E. O., & Slye, E. S. (1971). Effects of placebo and flurazepam on sleep patterns in insomniac subjects. *Clinical Pharmacology and Therapeutics, 12*(4), 691–697.

Kiejna, A., Kantorska-Janiec, M., & Małyszczak, K. (1997). The use of chlorazepate dipotassium (Tranxene) in the states of restlessness and agitation. *Psychiatria Polska, 31*(6), 753–760.

Klosterskov Jensen, P., Abild, K., & Nøhr Poulsen, M. (1983). Serum concentration of clonazepam after rectal administration. *Acta Neurologica Scandinavica, 68*(6), 417–420.

Kripke, D. F. (2016). Hypnotic drug risks of mortality, infection, depression, and cancer: but lack of benefit. *F1000Research, 5*, 918. https://doi.org/10.12688/f1000research.8729.3

Kronenfeld, N., Berlin, M., Shaniv, D., & Berkovitch, M. (2017). Use of pmsychotropic medications in breastfeeding women. *Birth Defects Research, 109*(12), 957–997. https://doi.org/10.1002/bdr2.1077.

Kupietzky, A., & Houpt, M. I. (1993). Midazolam: A review of its use for conscious sedation of children. *Pediatric Dentistry, 15*(4), 237–241.

Kurowski, M., & Reim, H. G. (1986). Metabolic interaction between cimetidine, ranitidine and amitriptyline/chlordiazepoxide. *Zeitschrift Fur Gastroenterologie, 24*(10), 627–634.

Lader, M. H., Curry, S., & Baker, W. J. (1980). Physiological and psychological effects of clorazepate in man. *British Journal of Clinical Pharmacology, 9*(1), 83–90.

Lamphere, J., Roehrs, T., Zorick, F., Koshorek, G., & Roth, T. (1986). Chronic hypnotic efficacy of estazolam. *Drugs Under Experimental and Clinical Research, 12*(8), 687–691.

Lamy, P. P., & Kitler, M. E. (1971). Drugs and the geriatric patient. *Journal of the American Geriatrics Society, 19*(1), 23–33.

Lebedevs, T. H., Wojnar-Horton, R. E., Yapp, P., Roberts, M. J., Dusci, L. J., Hackett, L. P., & Ilett, K. F. (1992). Excretion of temazepam in breast milk. *British Journal of Clinical Pharmacology, 33*(2), 204–206.

Lelong-Boulouard, V., Quentin, T., Moreaux, F., Debruyne, D., Boulouard, M., & Coquerel, A. (2006). Interactions of buprenorphine and dipotassium clorazepate on anxiety and memory functions in the mouse. *Drug and Alcohol Dependence, 85*(2), 103–113.

Leung, S. Y. (2011). Benzodiazepines, opioids and driving: an overview of the experimental research. *Drug and Alcohol Review, 30*(3), 281–286. https://doi.org/10.1111/j.1465-3362.2011.00311.x.

Löbenberg, R., & Amidon, G. L. (2000). Modern bioavailability, bioequivalence and biopharmaceutics classification system. New scientific approaches to international regulatory standards. *European Journal of Pharmaceutics and Biopharmaceutics, 50*(1), 3–12.

Loftsson, T., Gudmundsdóttir, H., Sigurjónsdóttir, J. F., Sigurdsson, H. H., Sigfússon, S. D., Másson, M., & Stefánsson, E. (2001). Cyclodextrin solubilization of benzodiazepines: formulation of midazolam nasal spray. *International Journal of Pharmaceutics, 212*(1), 29–40.

López-Muñoz, F., Alamo, C., & García-García, P. (2011). The discovery of chlordiazepoxide and the clinical introduction of benzodiazepines: half a century of anxiolytic drugs. *Journal of Anxiety Disorders, 25*(4), 554–562. https://doi.org/10.1016/j.janxdis.2011.01.002.

Ma, J., Wang, S., Zhang, M., Zhang, Q., Zhou, Y., Lin, C., … Wang, X. (2015). Simultaneous determination of bupropion, metroprolol, midazolam, phenacetin, omeprazole and tolbutamide in rat plasma by UPLC-MS/MS and its application to cytochrome P450 activity study in rats. *Biomedical Chromatography: BMC, 29*(8), 1203–1212. https://doi.org/10.1002/bmc.3409.

MacKinnon, G. L., & Parker, W. A. (1982). Benzodiazepine withdrawal syndrome: A literature review and evaluation. *American Journal of Drug and Alcohol Abuse, 9*(1), 19–33.

Malinovsky, J. M., Lejus, C., Servin, F., Lepage, J. Y., Le Normand, Y., Testa, S., Cozian, A., & Pinaud, M. (1993). Plasma concentrations of midazolam after i.v., nasal or rectal administration in children. *British Journal of Anaesthesia, 70*(6), 617–620.

Mandelli, M., Tognoni, G., & Garattini, S. (1978). Clinical pharmacokinetics of diazepam. *Clinical Pharmacokinetics, 3*(1), 72–91.

Mariotti, M., & Ongini, E. (1983). Differential effects of benzodiazepines on EEG activity and hypnogenic mechanisms of the brain stem in cats. *Archives Internationales De Pharmacodynamie Et De Therapie, 264*(2), 203–219.

McElhatton, P. R. (1994). The effects of benzodiazepine use during pregnancy and lactation. *Reproductive Toxicology, 8*(6), 461–475.

McLean, M. J., & Macdonald, R. L. (1988). Benzodiazepines, but not beta carbolines, limit high frequency repetitive firing of action potentials of spinal cord neurons in cell culture. *Journal of Pharmacology and Experimental Therapeutics, 244*(2), 789–795.

Micallef, J., Frauger, E., & Lapeyre-Mestre, M. (2016). Misuse of benzodiazepines in France. In V. R. Preedy (Ed.), *Neuropathology of Drug Addictions and Substance Misuse* (pp. 1101–1111). Amsterdam, Netherlands: Elsevier Vol. 2. https://doi.org/10.1016/B978-0-12-800212-4.00102-3.

Milgrom, P., Beirne, O. R., Fiset, L., Weinstein, P., Tay, K. M., & Martin, M. (1993). The safety and efficacy of outpatient midazolam intravenous sedation for oral surgery with and without fentanyl. *Anesthesia Progress, 40*(3), 57–62.

Miller, J. A., & Richter, J. A. (1985). Effects of anticonvulsants in vivo on high affinity choline uptake in vitro in mouse hippocampal synaptosomes. *British Journal of Pharmacology, 84*(1), 19–25.

Miller, L. G., Greenblatt, D. J., Abernethy, D. R., Friedman, H., Luu, M. D., Paul, S. M., & Shader, R. I. (1988). Kinetics, brain uptake, and receptor binding characteristics of flurazepam and its metabolites. *Psychopharmacology, 94*(3), 386–391.

Mitler, M. M. (1981). Evaluation of temazepam as a hypnotic. *Pharmacotherapy, 1*(1), 3–11. https://doi.org/10.1002/j.1875-9114.1981.tb03546.x.

Munakata, M., & Tsuchiya, S. (2008). Residual effect of a 7-amino metabolite of clonazepam on GABA$_A$ receptor function in the nucleus reticularis thalami of the rat. *Epilepsia, 49*(10), 1803–1808. https://doi.org/10.1111/j.1528-1167.2008.01623.x.

Murphy, P., Hindmarch, I., & Hyland, C. M. (1982). Aspects of short-term use of two benzodiazepine hypnotics in the elderly. *Age and Ageing, 11*(4), 222–228.

Nordt, S. P., & Clark, R. F. (1997). Midazolam: A review of therapeutic uses and toxicity. *Journal of Emergency Medicine, 15*(3), 357–365.

Oishi, R., Nishibori, M., Itoh, Y., & Saeki, K. (1986). Diazepam-induced decrease in histamine turnover in mouse brain. *European Journal of Pharmacology, 124*(3), 337–342.

Ongini, E., Mariotti, M., & Mancia, M. (1982). Effects of a new benzodiazepine hypnotic (quazepam–SCH 16134) on EEG synchronization and sleep-inducing mechanisms in cats. *Neuropharmacology, 21*(5), 405–412.

Oo, C. Y., Kuhn, R. J., Desai, N., Wright, C. E., & McNamara, P. J. (1995). Pharmacokinetics in lactating women: prediction of alprazolam transfer into milk. *British Journal of Clinical Pharmacology, 40*(3), 231–236.

Ozdemir, M., Aktan, Y., Boydag, B. S., Cingi, M. I., & Musmul, A. (1998). Interaction between grapefruit juice and diazepam in humans. *European Journal of Drug Metabolism and Pharmacokinetics, 23*(1), 55–59.

Pakes, G. E., Brogden, R. N., Heel, R. C., Speight, T. M., & Avery, G. S. (1981). Triazolam: A review of its pharmacological properties and therapeutic efficacy in patients with insomnia. *Drugs, 22*(2), 81–110.

Phillips, J. P., Antal, E. J., & Smith, R. B. (1986). A pharmacokinetic drug interaction between erythromycin and triazolam. *Journal of Clinical Psychopharmacology, 6*(5), 297–299.

Piccione, P., Zorick, F., Lutz, T., Grissom, T., Kramer, M., & Roth, T. (1980). The efficacy of triazolam and chloral hydrate in geriatric insomniacs. *The Journal of International Medical Research, 8*(5), 361–367.

Pierce, M. W., Shu, V. S., & Groves, L. J. (1990). Safety of estazolam. The United States clinical experience. *American Journal of Medicine, 88*(3A), 12S–17S.

Post, G. L., Patrick, R. O., Crowder, J. E., Houston, J., Ferguson, J. M., Bielski, R. J., … Pierce, M. W. (1991). Estazolam treatment of insomnia in generalized anxiety disorder: A placebo-controlled study. *Journal of Clinical Psychopharmacology, 11*(4), 249–253.

Ramanujam, R., L, P, G, S., & Thimmaiah, R. S (2015). A comparative study of the clinical efficacy and safety of lorazepam and chlordiazepoxide in alcohol dependence syndrome. *Journal of Clinical and Diagnostic Research: JCDR, 9*(3), FC10–FC13. https://doi.org/10.7860/JCDR/2015/11887.5678.

Randall, L. O., Schallek, W., Scheckel, C. L., Stefko, P. L., Banziger, R. F., Pool, W., & Moe, R. A. (1969). Pharmacological studies on flurazepam hydrochloride (RO 5-6901), a new psychotropic agent of the benzodiazepine class. *Archives Internationales De Pharmacodynamie Et De Therapie, 178*(1), 216–241.

Rey, E., d'Athis, P., Giraux, P., de Lauture, D., Turquais, J. M., Chavinie, J., & Olive, G. (1979). Pharmacokinetics of clorazepate in pregnant and non-pregnant women. *European Journal of Clinical Pharmacology, 15*(3), 175–180.

Riker, R. R., & Fraser, G. L. (2005). Adverse events associated with sedatives, analgesics, and other drugs that provide patient comfort in the intensive care unit. *Pharmacotherapy, 25*(5), 8S–18S.

Riss, J., Cloyd, J., Gates, J., & Collins, S. (2008). Benzodiazepines in epilepsy: Pharmacology and pharmacokinetics. *Acta Neurologica Scandinavica, 118*(2), 69–86. https://doi.org/10.1111/j.1600-0404.2008.01004.x.

Rodgers, R. J., Cutler, M. G., & Jackson, J. E. (1997). Behavioural effects in mice of subchronic chlordiazepoxide, maprotiline and fluvoxamine. II. The elevated plus-maze. *Pharmacology, Biochemistry, and Behavior, 57*(1–2), 127–136.

Roehrs, T., Zorick, F., Lord, N., Koshorek, G. L., & Roth, T. (1983). Dose-related effects of estazolam on sleep of patients with insomnia. *Journal of Clinical Psychopharmacology, 3*(3), 152–156.

Roth, T., Roehrs, T. A., & Zorick, F. J. (1983). Pharmacology and hypnotic efficacy of triazolam. *Pharmacotherapy, 3*(3), 137–148.

Roth, T., Tietz, E. I., Kramer, M., & Kaffeman, M. (1979). The effect of a single dose of quazepam (Sch-16134) on the sleep of chronic insomniacs. *The Journal of International Medical Research, 7*(6), 583–587.

Rudolph, U., Crestani, F., Benke, D., Brünig, I., Benson, J. A., Fritschy, J. M., Möhler, H., … Möhler, H. (1999). Benzodiazepine actions mediated by specific γ-aminobutyric acid$_A$ receptor subtypes. *Nature, 401*(6755), 796–800.

Rylance, G. W., Poulton, J., Cherry, R. C., & Cullen, R. E. (1986). Plasma concentrations of clonazepam after single rectal administration. *Archives of Disease in Childhood, 61*(2), 186–188.

Saavedra, I. N., Aguilera, L. I., Faure, E., & Galdames, D. G. (1985). Phenytoin/clonazepam interaction. *Therapeutic Drug Monitoring, 7*(4), 481–484.

Sachdeva, A., Choudhary, M., & Chandra, M. (2015). Alcohol withdrawal syndrome: benzodiazepines and beyond. *Journal of Clinical and Diagnostic Research, 9*(9), VE01–VE07. https://doi.org/10.7860/JCDR/2015/13407.6538.

Salonen, M., Aantaa, E., Aaltonen, L., Hovi-Viander, M., & Kanto, J. (1986). A comparison of the soft gelatin capsule and the tablet form of temazepam. *Acta Pharmacologica Et Toxicologica, 58*(1), 49–54.

Santo, L., Rui, P., & Ashman, J. J. (2020). Physician office visits at which benzodiazepines were prescribed: findings from 2014–2016 National Ambulatory Medical Care Survey. *National Health Statistics Reports, 137*(January 17). https://www.cdc.gov/nchs/data/nhsr/nhsr137-508.pdf.

Schwartz, M. A., Postma, E., & Gaut, Z. (1971). Biological half-life of chlordiazepoxide and its metabolite, demoxepam, in man. *Journal of Pharmaceutical Sciences, 60*(10), 1500–1503.

Schwarz, H. J. (1979). Pharmacokinetics and metabolism of temazepam in man and several animal species. *British Journal of Clinical Pharmacology, 8*(1), 23S–29S.

Schweizer, E., Rickels, K., Weiss, S., & Zavodnick, S. (1993). Maintenance drug treatment of panic disorder. I. Results of a prospective, placebo-controlled comparison of alprazolam and imipramine. *Archives of General Psychiatry, 50*(1), 51–60.

Seigler, R. S. (1990). The administration of rectal diazepam for acute management of seizures. *Journal of Emergency Medicine, 8*(2), 155–159.

Shader, R. I., Greenblatt, D. J., Harmatz, J. S., Franke, K., & Koch-Weser, J. (1977). Absorption and disposition of chlordiazepoxide in young and elderly male volunteers. *Journal of Clinical Pharmacology, 17*(11–12), 709–718.

Shyken, J. M., Babbar, S., Babbar, S., & Forinash, A. (2019). Benzodiazepines in pregnancy. *Clinical Obstetrics and Gynecology, 62*(1), 156–167. https://doi.org/10.1097/GRF. 0000000000000417.

Siepmann, M., Heine, B., Kluge, A., Ziemssen, T., Mück–Weymann, M., & Kirch, W. (2007). The effects of lorazepam on skin conductance responses to aversive stimuli in healthy subjects. *Clinical Autonomic Research, 17*(3), 160–164.

Soyka, M. (2017). Treatment of benzodiazepine dependence. *New England Journal of Medicine, 376*(12), 1147–1157. https://doi.org/10.1056/NEJMra1611832.

Starcevic, V. J. A. (2012). Benzodiazepines for anxiety disorders: maximising the benefits and minimising the risks. *Advances in Psychiatric Treatment, 18*(4), 250–258. https://doi.org/ 10.1192/apt.bp.110.008631.

Sternbach, L. H. (1972). The discovery of librium. *Agents and Actions, 2*(4), 193–196.

Sternbach, L. H. (1978). The benzodiazepine story. Progress in drug research. *Fortschritte Der Arzneimittelforschung. Progres Des Recherches Pharmaceutiques,, 22,* 229–266.

Sternbach, L., Koechlin, B., & Reeder, E. (1962). Quinazolines and 1,4-benzodiazepines (VIII). 1 The photoisomerization of 7-chloro-2-methylamino-5-phenyl-3*H*-1,4-benzodiazepine 4-oxide 2. *Journal of Organic Chemistry, 27*(12), 4671–4672. https://doi. org/10.1021/jo01059a523.

Taft, W. C., & DeLorenzo, R. J. (1984). Micromolar-affinity benzodiazepine receptors regulate voltage-sensitive calcium channels in nerve terminal preparations. *Proceedings of the National Academy of Sciences of the United States of America, 81*(10), 3118–3122.

Takzare, N., Hosseini, M.-J., Bakhtiarian, A., Takzare, A., & Habibi, P. (2008). The teratogenic effects of flurazepam intake during organogenesis of the rat fetus. *Toxicology Mechanisms and Methods, 18*(9), 711–716. https://doi.org/10.1080/15376510801897739.

Terao, T., & Tani, Y. (1988). Two cases of psychotic state following normal-dose benzodiazepine withdrawal. *Journal of UOEH, 10*(3), 337–340.

The US Food and Drug Administration. (2019). Ativan injection (lorazepam). Accessed 5 May 2022. https://www.fda.gov/media/130820/download.

The US Food and Drug Administration. (2005). Librium (chlordiazepoxide hydrochloride) label. Accessed 5 May 2022. https://www.accessdata.fda.gov/drugsatfda_docs/ label/2016/012249s049lbl.pdf.

The US Food and Drug Administration. (2007). Dalmane (flurazepam hydrochloride) capsules for relief of insomnia. Accessed 5 May 2022. https://www.accessdata.fda.gov/ drugsatfda_docs/label/2007/016721s076lbl.pdf.

The US Food and Drug Administration. (2013). Klonopin (clonazepam) tablets. Accessed 5 May 2022. https://www.accessdata.fda.gov/drugsatfda_docs/label/2013/017533s053, 020813s009lbl.pdf.

The US Food and Drug Administration. (2016a). Doral (quazepam) label. Accessed 5 May 2022. https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/018708s023lbl. pdf.

The US Food and Drug Administration. (2016b). Restoril (temazepam) label. Accessed 5 May 2022. https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/018163s064lbl.pdf.

The US Food and Drug Administration. (2016c). Valium (diazepam) label. Accessed 5 May 2022. https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/013263s094lbl.pdf.

The US Food and Drug Administration. (2016d). Xanax label. Accessed 5 May 2022. https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/018276s052lbl.pdf.

Tokola, R. A., & Neuvonen, P. J. (1983). Pharmacokinetics of antiepileptic drugs. *Acta Neurologica Scandinavica, 68*(s97), 17–27.

Tóth, K., Csukly, G., Sirok, D., Belic, A., Kiss, Á., Háfra, E., … Monostory, K. (2016). Optimization of clonazepam therapy adjusted to patient's CYP3A status and NAT2 genotype. *The International Journal of Neuropsychopharmacology, 19*(12), 1–9. https://doi.org/10.1093/ijnp/pyw083.

Tranxene (clorazepate dipotassium) tablets label (2010). *The US Food and Drug Administration.* https://www.accessdata.fda.gov/drugsatfda_docs/label/2010/017105s076lbl.pdf. (Accessed 5 May 2022).

Tseng, T.-C., & Wang, S. C. (1971). Locus of central depressant action of some benzodiazepine analogues. *Experimental Biology and Medicine, 137*(2), 526–531. https://doi.org/10.3181/00379727-137-35613.

van Strien, A. M., Koek, H. L., van Marum, R. J., & Emmelot-Vonk, M. H. (2013). Psychotropic medications, including short acting benzodiazepines, strongly increase the frequency of falls in elderly. *Maturitas, 74*(4), 357–362. https://doi.org/10.1016/j.maturitas.2013.01.004.

Verster, J. C., & Volkerts, E. R. (2004). Clinical pharmacology, clinical efficacy, and behavioral toxicity of alprazolam: A review of the literature. *CNS Drug Reviews, 10*(1), 45–76.

Vogel, G. W., & Morris, D. (1992). The effects of estazolam on sleep, performance, and memory: A long-term sleep laboratory study of elderly insomniacs. *Journal of Clinical Pharmacology, 32*(7), 647–651.

von Moltke, L. L., Greenblatt, D. J., Court, M. H., Duan, S. X., Harmatz, J. S., & Shader, R. I. (1995). Inhibition of alprazolam and desipramine hydroxylation in vitro by paroxetine and fluvoxamine: comparison with other selective serotonin reuptake inhibitor antidepressants. *Journal of Clinical Psychopharmacology, 15*(2), 125–131.

von Moltke, L. L., Greenblatt, D. J., Harmatz, J. S., & Shader, R. I. (1993). Alprazolam metabolism in vitro: studies of human, monkey, mouse, and rat liver microsomes. *Pharmacology, 47*(4), 268–276.

Wang, R. I., & Stockdale, S. L. J. (1973). The hypnotic efficacy of triazolam. *Journal of International Medical Research, 1*(7), 600–607. https://doi.org/10.1177/030006057300100702.

Wang, S.-M., Kim, J.-B., Sakong, J. K., Suh, H.-S., Oh, K. S., Woo, J.-M., … Lee, K.-U. (2016). The efficacy and safety of clonazepam in patients with anxiety disorder taking newer antidepressants: a multicenter naturalistic study. *Clinical Psychopharmacology and Neuroscience, 14*(2), 177–183. https://doi.org/10.9758/cpn.2016.14.2.177.

Welt, T., Engelmann, M., Renner, U., Erhardt, A., Müller, M. B., Landgraf, R., … Keck, M. E. (2006). Temazepam triggers the release of vasopressin into the rat hypothalamic paraventricular nucleus: Novel insight into benzodiazepine action on hypothalamic-pituitary-adrenocortical system activity during stress. *Neuropsychopharmacology, 31*(12), 2573–2579.

Wermeling, D. P., Record, K. A., Archer, S. M., & Rudy, A. C. (2009). A pharmacokinetic and pharmacodynamic study, in healthy volunteers, of a rapidly absorbed intranasal midazolam formulation. *Epilepsy Research, 83*(2–3), 124–132. https://doi.org/10.1016/j.eplepsyres.2008.10.005.

Wick, J. Y. (2013). The history of benzodiazepines. *The Consultant Pharmacist, American Society of Consultant Pharmacists, 28*(9), 538–548. https://doi.org/10.4140/TCP.n.2013.538.

Willow, M., Kuenzel, E. A., & Catterall, W. A. (1984). Inhibition of voltage-sensitive sodium channels in neuroblastoma cells and synaptosomes by the anticonvulsant drugs diphenyl-hydantoin and carbamazepine. *Molecular Pharmacology, 25*(2), 228–234.

Witt, D. M., Ellsworth, A. J., & Leversee, J. H. (1993). Amiodarone-clonazepam interaction. *Annals of Pharmacotherapy, 27*(12), 1463–1464.

Wright, S. W., Chudnofsky, C. R., Dronen, S. C., Wright, M. B., & Borron, S. W. (1990). Midazolam use in the emergency department. *American Journal of Emergency Medicine, 8*(2), 97–100.

Yasui, N., Otani, K., Kaneko, S., Ohkubo, T., Osanai, T., Sugawara, K., … Ishizaki, T. (1996). A kinetic and dynamic study of oral alprazolam with and without erythromycin in humans: In vivo evidence for the involvement of CYP3A4 in alprazolam metabolism. *Clinical Pharmacology and Therapeutics, 59*(5), 514–519.

Yuan, R., Flockhart, D. A., & Balian, J. D. (1999). Pharmacokinetic and pharmacodynamic consequences of metabolism-based drug interactions with alprazolam, midazolam, and triazolam. *Journal of Clinical Pharmacology, 39*(11), 1109–1125.

Zampaglione, N., Hilbert, J. M., Ning, J., Chung, M., Gural, R., & Symchowicz, S. (1985). Disposition and metabolic fate of ^{14}C-quazepam in man. *Drug Metabolism and Disposition, 13*(1), 25–29.

Zhang, S. J., & Jackson, M. B. (1993). GABA-activated chloride channels in secretory nerve endings. *Science, 259*(5094), 531–534.

Non-Print Items

Abstract

This chapter explains the present pharmacological knowledge of twelve FDA–approved 1,4–benzodiazepines including general mechanisms of action, metabolic pathways, pharmacokinetic profiles, potential adverse effects, and drug-drug interactions. Furthermore, recommended dosages of these drugs for the management of anxiety, insomnia, seizures, and alcohol withdrawal are also summarized.

Structural features of 1,5-benzodiazepines and 1,5-benzothiazepines

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Chapter outline

6.1 Introduction

1,5–Benzodiazepines (**6.1.1–6.1.3**) and 1,5–benzothiazepines (**6.1.4**) are two structural isomers of benzodiazepines and benzothiazepines with the heteroatoms at positions 1 and 5 (Fig. 6.1). Considering the IUPAC nomenclature, these heterocycles are regarded as the 2,3–benzo fused analogs of 1,4–diazepines and 1,4–thiazepines, respectively (El-Bayouki, 2013; Lloyd & Cleghorn, 1974), which is why an another way of the numbering proceeds in the opposite direction of the diazepine or thiazepine ring. Thus, positions 1, 2, 3, 4, and 5 of the 1,5-benzodiazepines are attributed to positions 1, 7, 6, 5, and 4, respectively (Lloyd & Cleghorn, 1974). For example, 1,5–benzodiazepine **6.1.1** may also be named benzo[*b*] diazepine (Fig. 6.1). The saturation position in the diazepine or thiazepine ring should also be expressed as a prefix to the name of the compounds (Fig. 6.1), where the odd hydrogen should be given the lowest possible number. 1,5-Benzodiazepines usually exist in the bis–imino form **6.1.1** rather than in the isomeric conjugated forms **6.1.2** and **6.1.3** because the former enjoys excellent extra

6.1.1

benzo[b][1,4]diazepine
(1,5-benzodiazepine)

6.1.2

1H-benzo[b][1,4]diazepine

6.1.3

1H-benzo[b][1,4]diazepine

6.1.4

benzo[b][1,4]thiazepine
(1,5-benzothiazepine)

6.1.5

2,3-dihydro-benzo[b][1,4]thiazepine
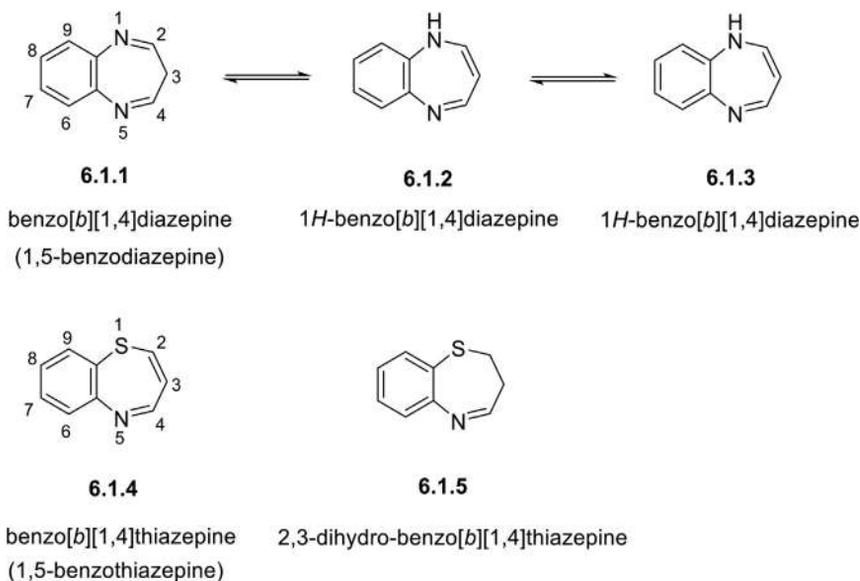
Figure 6.1 The general structures of 1,5-benzodiazepine and 1,5-benzothiazepine.

stabilization *via* the conjugation of the imine groups with the benzene ring (Lloyd & Cleghorn, 1974). In contrast, the cyclic conjugation in **6.1.2** and **6.1.3** does not meet Hückel's rule due to the interaction of 8π electrons around the diazepine ring in **6.1.2** or 12π electrons around the periphery of **6.1.3**. These interactions destabilize the structures. Spectral analyses (IR and ^{1}H NMR) of several 2,4–dialkyl– and 2,4–diaryl–1,5–benzodiazepines displayed no peaks for NH groups, but showed the presence of a methylene group (^{1}H NMR signal ≈ 2.5 ppm) and C=N bond (IR absorption peak ≈ 1600 cm^{-1}) (Barltrop, Richards, Russell, & Ryback, 1959; Barry, Finar, & Mooney, 1965; Lloyd & Cleghorn, 1974). These findings clearly confirm the dominant presence of the bis–imino form **6.1.1** as the major structural isomer of 1,5–benzodiazepines. Interestingly, conducting the ^{1}H NMR experiment at room temperature gives the methylene signal as a singlet peak, while this methylene signal appears as a doublet of doublets peaks at lower temperatures, indicating that the 1,5–benzodiazepine molecules exist as a boat conformation in solution, and rapidly invert at room temperature (See Section 6.4) (Lloyd & Mcnab, 1998).

This chapter discusses through the available data on aspects of structural and chemical properties of 1,5–benzodiazepines and 1,5–benzothiazepines. Most of these studies have been focused on the tautomerism, conformational isomerism, and chemical reactivity of these pharmaceutically critical fused heterocycles.
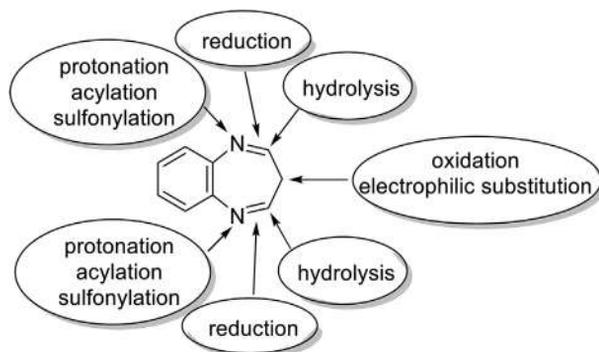
Figure 6.2 The general reactive sites of 1,5-benzodiazepines.

6.2 Chemical properties of 1,5-benzodiazepines

1,5–Benzodiazepines are less stable, more susceptible to hydrolysis, and have significantly weaker basicity than their dihydro counterparts (Lloyd & Cleghorn, 1974). The low basicity of these skeletons can be attributed to their tautomeric change to the less favored conjugated forms **6.2.1** and **6.2.2** (Lloyd & Cleghorn, 1974). Introducing a phenyl group to position 2 or 4 may further diminish the basicity of the benzodiazepine. Due to their relative instability in aqueous solution, there is a considerable paucity of reliable electrochemical studies on 1,5-benzodiazepines than their 1,4–benzodiazepines equivalents.

1,5–Benzodiazepines have a strong tendency to participate in various organic transformations at different reactive sites (Fig. 6.2). Oxidation usually occurs at position 3 by using oxidizing agents such as peroxy acids to generate 3-oxo–1,5-benzodiazepines (Lloyd & Cleghorn, 1974; Masakatsu, Akira, & Teijiro, 1970). The methylene group at position 3 is also susceptible to electrophilic substitutions under basic conditions because of the adjacent imine groups. The N1 and N5 centers can smoothly undergo acylation and sulfonylation reactions using suitable protecting groups, *e.g.*, benzoyl chloride and *para*-toluenesulfonyl chloride, in the presence of a base (Barltrop et al., 1959; Ichii, 1962; Paterson & Proctor, 1965). Reduction of 2,4–disubstituted–1,5-benzodiazepines readily occurs *via* treating with hydrogen gas over a palladium catalyst, affording *cis* and *trans* isomers of the corresponding tetrahydrobenzodiazepines.

Protonation of 1,5-benzodiazepines affords the formation of mono-cations **6.2.2** and **6.2.3**, and dication **6.2.5** (Fig. 6.3). Upon the protonation,^{1}H NMR methylene signal at 7.3 ppm in **6.2.1** disappears (where $R^1 = R^2 = $ Me and $R^3 = $ H), and it is replaced by a methane
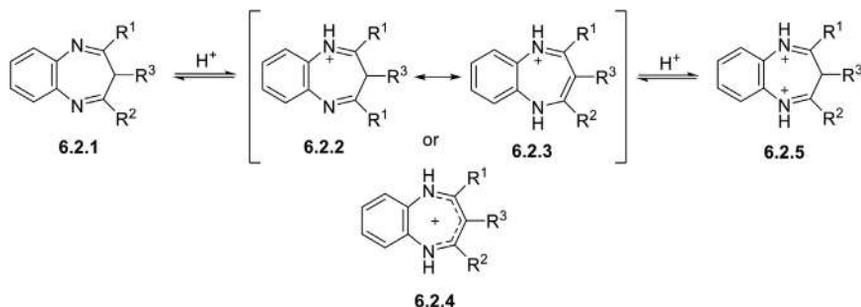
Figure 6.3 Protonation of 1,5-benzodiazepines.

signal at around 2.5 ppm, indicating the formation of **6.2.2** and **6.2.3** (Barry et al., 1965; Paterson & Proctor, 1965). Subsequently, the reappearance of the methylene signal confirms the generation of the dication **6.2.5** from the monocations. While the monocations **6.2.2** and **6.2.3** are dark blue or violet because of the formation of a conjugated system, the diacid salts **6.2.5** are colorless compounds due to the interference in conjugation by the presence of two NH$^+$ groups (Archer & Sternbach, 1968; Solomko & Kost, 1975). UV-vis analyses of 2,4-dialkyl-1,5-benzodiazpeines also display absorption bands at approximately 260 nm, which is significantly intensified to around 500 nm for their corresponding monocations because of their intense colors, conjugated systems, n–π^* transitions, and charge-transfer (Barltrop et al., 1959). Douglas Lloyd established the principle of quasi-aromaticity in 1971 to define the properties of diazepinium cations (Lloyd & Marshall, 1971). A quasi-aromatic structure is an acyclic conjugated π-electron system that exhibits a significant mesomeric effect and resonance stability, and typical properties of aromatic compounds. According to this concept, 1,5-benzodiazepinium salts (**6.2.2**-**6.2.5**) are considered quasi-aromatic scaffolds because they show similar chemical properties to typical aromatic compounds, *e.g.*, participating in electrophilic substitution reactions on the diazepine ring, without having a cyclic conjugated π-electron system (Lloyd & Mcnab, 1998). This is probably due to the delocalized vinamidinium system of the cations with electron–rich nature, containing six electrons contributed over the five atoms (Krygowski, Bankiewicz, Czarnocki, & Palusiak, 2015). In contrast, Alkorta *et al.* demonstrated that 1,5-benzodiazepinium cations are strongly *anti*-aromatic in the case of the diazepine ring, confirmed by Nuclear Independent Chemical Shifts (NICS) and ^{1}H NMR analyses (Claramunt, Alkorta, & Elguero, 2013). The NICS calculation of **6.2.4** showing NICS (1) = 41.1 ppm has indicated that the
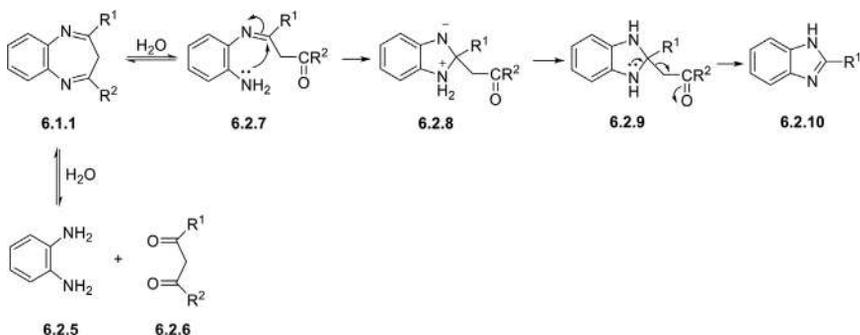
Figure 6.4 Hydrolysis of 1,5-benzodiazepines.

monocations **6.2.2** and **6.2.3** are *anti*-aromatic, which is in agreement with NMR data where the observed peak of the methylene group ($R^3 = H$) at around 4 ppm clearly belongs to an *anti*-aromatic compound. However, the harmonic oscillator model of aromaticity (HOMA) study has identified the 1,4–diazepine ring in **6.2.4** as a moderate aromatic with the HOMA value of approximately 0.73 (for benzene, HOMA = 1). Therefore, more experimental and computational strategies need to be designed to respond to these opposite conclusions. In this regard, some recently computational strategies for complex molecules might be of note (Assadi & Sahajwalla, 2014a, 2014b; Ramin, Assadi, & Sahajwalla, 2014). Although forming the stabilized cations of 1,5-benzodiazepines is advantageous, the possible interaction of the 8π electrons of the diazepine ring with the 6π–electron aromatic system of the benzene ring is not desirable. For minimizing such $4n$ circuit of π–electrons, the bonds linking the nitrogen atoms to the benzene ring of both benzodiazepinium cations **6.2.2** and **6.2.3** are abnormally longer (~1.42 Å) with lower bond orders compared with the other bonds (~1.38 Å) in the benzene ring (Lloyd & Mcnab, 1998), keeping two π–systems isolated.

1,5-Benzodiazepines and their benzodiazepinium salts are readily hydrolyzable in aqueous solution upon either heating or keeping at room temperature, undergoing a ring contraction process to form the corresponding benzimidazoles (Lloyd & Cleghorn, 1974). This phenomenon may proceed through either hydrolysis to *ortho*-phenylenediamine **6.2.5** and the diketone **6.2.6** or starting with ring-opening of the diazepine ring *via* nucleophilic attack of a water molecule to the position 2 or 4 to generate the phenylimine moiety (**6.2.7**), followed by a subsequent ring closure to give the benzimidazole derivative **6.2.10** (Fig. 6.4). 2,4-Disubstituted 1,5-benzodiazepines are
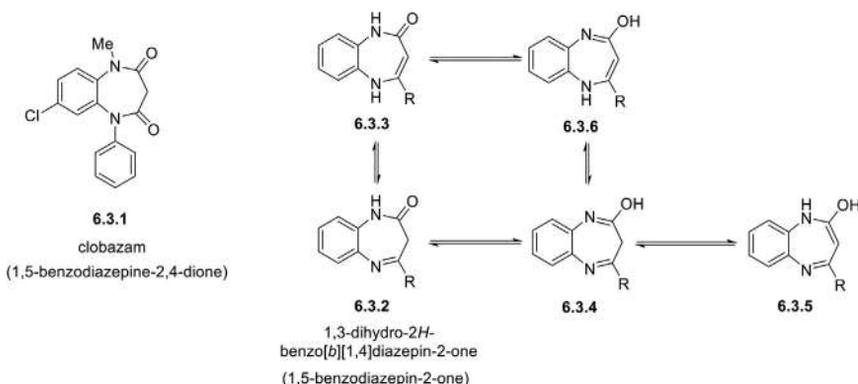
Figure 6.5 Clobazam and general tautomerization process of 1,5-benzodiazepin-2-ones.

less susceptible to be hydrolyzed than 2,3–di–substituted counterparts due to the steric hindrance caused by the substituents in the former. Furthermore, the presence of electron–withdrawing groups such as nitro and carboxyl at position 7 accelerates the hydrolysis rate of 1,5–benzodiazepines in basic media, generating the corresponding *ortho*-phenylenediamines (Archer & Sternbach, 1968; Lloyd & Cleghorn, 1974).

6.3 Chemical properties of 1,5-benzodiazepinones

Following the introduction of 1,5–benzodiazepine-2,4–dione clobazam **6.3.1** as the first anxiolytic drug from the 1,5–benzodiazepine family in 1969 (See Chapter 9), considerable efforts have been made on the development of new 1,5-benzodiazepinones. 1,5-Benzodiazepin-2-ones and 1,5-benzodiazepine-2,4-diones are the most common members of these fused heterocycles (Fig. 6.5). 1,5-Benzodiazepin–2-ones usually exist as two stable tautomers 3*H*-2-oxo **6.3.2** and the 1,5-di*H*-2-oxo **6.3.3** (Claramunt et al., 2013). The tautomerization process involves proton migration that is highly solvent–dependent. Density functional theory (DFT) calculations of 4-methyl–1,3–dihydro-2*H*-1,5-benzodiazepin-2-one (**6.3.2**, R = Me) have indicated that the keto forms (**6.3.2** and **6.3.3**) are more stable than the enol tautomers in the gas phase (no solvent), due to the occurrence of stronger intramolecular hydrogen bonds in the keto species (Okovytyy et al., 2010). Moreover, the tautomer preference is influenced by using protic solvents such as ethanol. Compared with the gas phase, intermolecular hydrogen bonds between ethanol and the keto/enol forms energetically facilitate
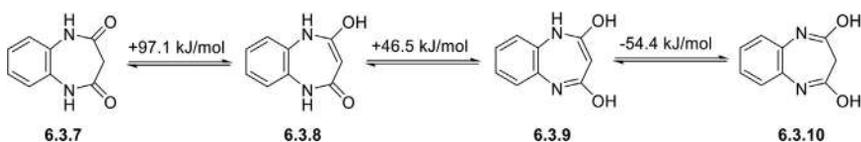
Figure 6.6 General tautomerization process of 1,5-benzodiazepine-2,4-diones.

the proton transfer and subsequently reduce the free energy barriers for tautomerization. Due to the largest dipole moment of enol form **6.3.5** ($\mu = 6.77$ D, R = Me), this tautomer is more stable than the other moieties in ethanol because of its higher interaction with ethanol, shifting the tautomeric equilibrium towards the formation of **6.3.5** (Okovytyy et al., 2010).

1,5–Benzodiazepine–2,4–diones are experimentally observed as only one tautomer (**6.3.7**) due to the significant difference in energy between the tautomers (Fig. 6.6) (Claramunt et al., 2013).

It is well–established that almost all 1,4–benzodiazepine–based anticonvulsants have at least one imine bond in the diazepine ring, which substantially contributes to their biological performances (Sternbach, Randall, Banziger, & Lehr, 1968). However, structure–activity relationship (SAR) studies have shown that the existence of an imine unit in the 1,5–benzodiazepines is not essential for their anxiolytic activity. Instead, as in 1,5–benzodiazepinones, the carboxamide function plays a key role in their psychotropic properties (Kuch, 1979). The imine group (ketimine) in 1,5–benzodiazepines is more reactive than the carboxamide in 1,5–benzodiazepinones, leading to making the former more susceptible to various chemical transformations than the latter. For example, while the hydrolysis of a ketimine to ketone and amine in 1,5–benzodiazpines is easily attained under mildly acidic conditions, the corresponding hydrolytic cleavage of a carboxamide in 1,5–benzodiazepinone counterparts generally requires strong acidic conditions at high temperatures (Kuch, 1979; Nicolaus, Bellasio, Pagani, Mariani, & Testa, 1965). This is the main reason that ring–opened metabolites of 1,5–benzodiazpines can be detected in the body after consumption, but not observed for 1,5–benzodiazepinones such as clobazam.

Due to carbonyl moiety in the structure of 1,5–benzodiazepin–2–ones and 1,5–benzodiazepine–2,4–diones, these fused heterocycles contain different reactive sites to participate in various organic transformations. The adjacent carbonyl functions activate the methylene group. Accordingly, the acidic hydrogen in position 3 is readily abstracted by a base and the resulting carbanion can participate in different nucleophilic addition
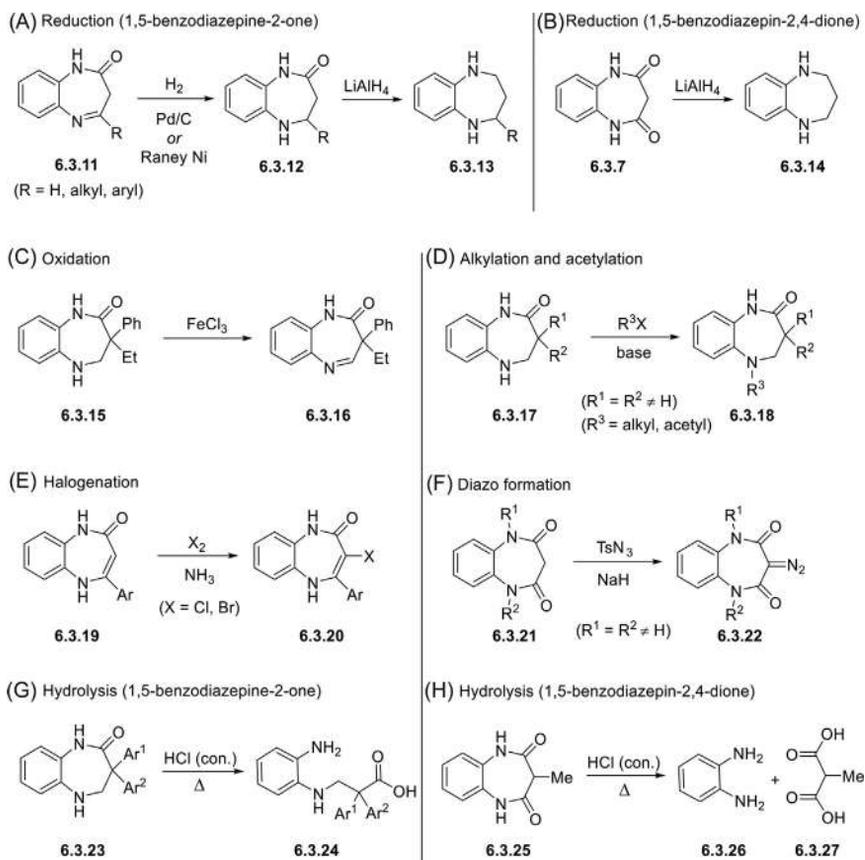
Figure 6.7 Examples of chemical reactivity of 1,5-benzodiazepin-2-ones and 1,5-benzodiazepin-2,4-diones.

reactions. The imine group of 1,5–benzodiazepin–2–ones **6.3.11** can undergo hydrogenation reaction using hydrogen gas catalyzed by either Pd/C or Raney nickel catalyst to afford the corresponding tetrahydro analogue **6.3.12** (Fig. 6.7A). Further reduction of **6.3.12** with lithium aluminum hydride (LiAlH₄) gives the 2,3,4,5-tetrahydro-l*H*-1,5-benzodiazepine **6.3.13** (Fig. 6.7A) (Davoll, 1960). 1,5–Benzodiazepin–2,4–dione **6.3.7** is also susceptible to get reduced by LiAlH₄, generating the corresponding 2,3,4,5-tetrahydro-1*H*-benzodiazepine **6.3.14** (Fig. 6.7B). It has been also shown that the tetrahydrobenzodiazepin–2–one **6.3.15** can be oxidized with ferric chloride to form the 1,3–dihydrobenzodiazepin–2–one **6.3.16** (Fig. 6.7C) (Nicolaus et al., 1965). Treatment of tetrahydrobenzodiazepin–2–ones **6.3.17** with either alkyl or acetyl halides in the presence of a base produces
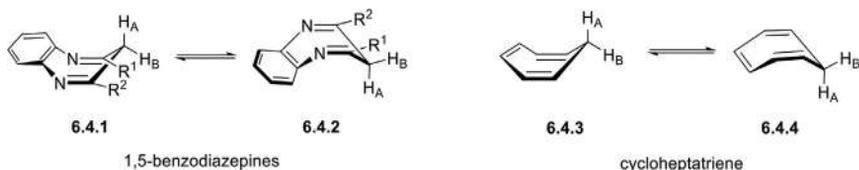
Figure 6.8 General boat-shaped conformations of 1,5-benzodiazepines and cycloheptatriene.

the 5–alkylated or 5–acetylated derivatives **6.3.18**, respectively (Fig. 6.7D) (Nicolaus et al., 1965). Dihydrobenzodiazepine–2–ones **6.3.19** can be easily halogenated using halogenating reagents such as Br_2 to generate 3-bromo analog **6.3.20** (Fig. 6.7E) (Barchet & Merz, 1964). Upon the reaction of 1,5-benzodiazepine–2,4–diones **6.3.21** tosyl azide in the presence of sodium hydride as a base, the corresponding diazo derivatives **6.3.22** are obtained, which can be further manipulated in various nucleophilic addition reactions (Fig. 6.7F) (Solomko & Kost, 1975). As mentioned earlier, the hydrolysis ring-opening reaction of 1,5-benzodiazepinones requires harsh acidic reaction conditions. For example, treatment of 1,5-benzodiazepin–2–one **6.3.23** and 1,5-benzodiazepine-2,4-dione **6.3.25** with hot concentrated hydrochloric acid for two days gives the amino acid **6.3.24** and *ortho*-phenylenediamine **6.3.26**, respectively (Fig. 6.7G and H) (Solomko & Kost, 1975).

6.4 Conformational studies of 1,5-benzodiazepines and 1,5-benzodiazepinones

1,5-Benzodiazepines are structurally not planar where the diazepine ring folds out of the plane from position 3 (**6.4.1** and **6.4.2**, Fig. 6.8). This boat-shaped structure of the seven–membered ring, which is similar to cycloheptatriene (**6.4.3** and **6.4.4**), can invert and minimize the steric hindrance of vicinal crowding (Eliel & Wilen, 1994). The interconversion barrier ($\Delta G^{\#}$) between the conformers highly depends upon the size of the substituents at positions 2 and 4. For example, 2,4-diphenyl substituted 1,5-benzodiazepine (**6.4.1**, $R^1 = R^2 = $ Ph) shows higher energy barrier ($\Delta G^{\#} = 12.6$ kcal/mol) compared to 2,4-dimethyl substituted derivative (**6.4.1**, $R^1 = R^2 = $ Me, $\Delta G^{\#} = 11.7$ kcal/mol), due to the greater steric requirements for the inversion (Mannschreck, Rissmann, Vogtle, & Wild, 1967).
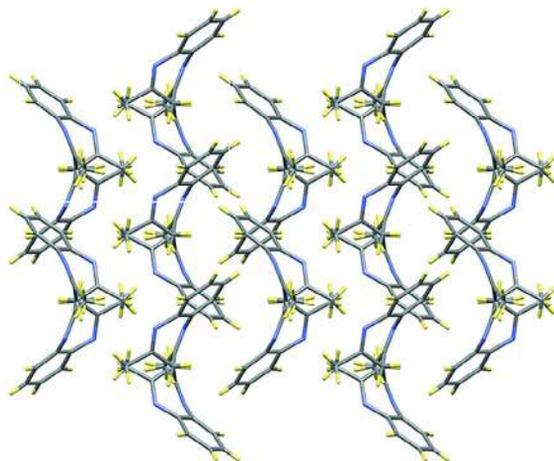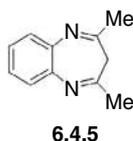
Figure 6.9 The crystal stacking of 2,4-dimethyl-3H-benzo[*b*][1,4]diazepine. Reproduced with permission of the International Union of Crystallography (Nieto et al., 2017).

In crystal forms, 1,5-benzodiazepines are basically stacked opposite each other to reduce electronic repulsion. The boat-shaped conformation of these compounds, the absence of hydrogen atoms, and consequently no hydrogen bonding between the molecules avoid forming a supramolecular framework in 1,5-benzodiazepines (Nieto, Claramunt, Torralba, Torres, & Elguero, 2017). As an example, Claramunt and coworkers analyzed the crystal structure of 2,4-dimethyl–3*H*–benzo[*b*][1,4]diazepine **6.4.5** as a model compound of 1,5-benzodiazepines (Fig. 6.9). The molecules have long intermolecular distances with no contact with each other, which are aligned oppositely. The crystal structure is the monoclinic space group $P2_1/c$ with one molecule in the asymmetric unit. C2, C3, C4 atoms of the diazepine ring together with the methyl substituents deviate from the benzene ring plane with a dihedral angle of 87.8 °, representing a boat-shaped conformation for the diazepine ring (Nieto et al., 2017).

1,5-Benzodiazepinones are also structurally not planar where the diazepine ring adopts a boat-shaped conformation. The interconversion barrier ($\Delta G^{\#}$) between the conformers depends on the substituents on the N1 atom. For example, both experimental and computational analyses of 4-substituted–6,7,8,9-tetrafluoro–1,5-benzodiazepine–2-ones (**6.4.6**, Fig. 6.10) have revealed that the energy barrier is much lower for $R^1 = H$ ($\Delta G^{\#} \approx$ 48 kJ/mol) compared with $R^1 = Me$ ($\Delta G^{\#} \approx$ 70 kJ/mol). Accordingly, the NMR experiments to distinguish the tautomers should be carried out

6.4.6

6.4.7

6.4.8

R^1 = H, Me
R^2 = C$_6$H$_5$, Me
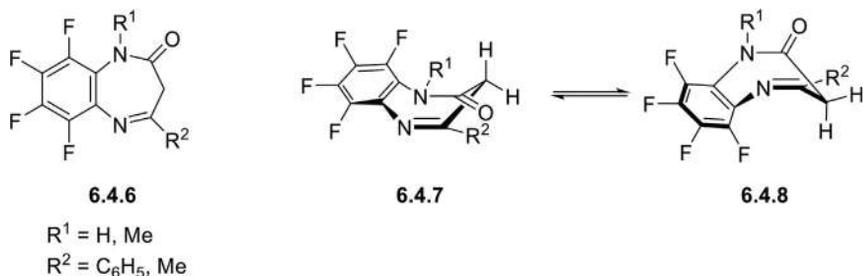
Figure 6.10 General boat-shaped conformations of 4-substituted-6,7,8,9-tetrafluoro-1,5-benzodiazepine-2-ones.

at low temperatures and high temperatures for the former and the latter, respectively (Martín et al., 2016). In solid-state, two **6.4.6** molecules (where R^1 = H and R^2 = Ph) are connected through the hydrogen bonding of the amide nitrogen and carbonyl oxygen atoms to create a dimer. These dimers are stacked by π-π interactions of the tetrafluoro substituted benzene ring of one subunit with the iminophenyl unit of an adjacent subunit, forming a chain along the *b*-axis respectively (Martín et al., 2016). Each of these overlapped dimeric systems attaches to four nearby moieties of different chains that lead to an extended 2D framework similar to the *bc* crystal plane.

6.5 Chemical properties of 1,5-benzothiazepines

Although many research studies have been focused on the synthesis of 1,5–benzothiazepines (Dehaen & Ngo, 2008; El–Bayouki, 2013), much less attention has been devoted to their chemical properties and conformations. These fused heterocycles can participate in various chemical transformations such as cycloaddition, ring contraction, and reduction reactions (Fig. 6.11). The imine bond of 1,5-benzothiazepine **6.5.1** can undergo the Staudinger [2 + 2] cycloaddition with ketene **6.5.4**, which is generated *in situ* from acyl chloride **6.5.2**, to form the fused β–lactam-fused 1,5–benzothiazepine **6.5.3** (Fig. 6.11A) (Liu, Zhang, Liu, & Xu, 2008). The 1,3–dipolar cycload-dition reaction of benzonitrile oxide **6.5.6** to the imine unit of the 1,5–benzothiazepines **6.5.5** gives the corresponding fused 1,2,4-oxadiazoline **6.5.7** (Fig. 6.11B) (Dong, Liu, Xu, & Yuan, 2011). Acetylation of 1,5–benzothiazepine **6.5.8** in the presence of pyridine leads to a ring contraction, producing 2,2–disubstituted–3–acetylated benzothiazoline **6.5.9** (Fig. 6.11C) (Levai & Jeko, 2003). Reduction of the imine bond of 1,5-benzothiazepines can occur with either boron–based reducing agents, *e.g.*, sodium borohydride
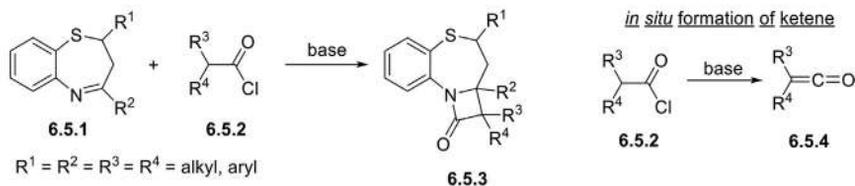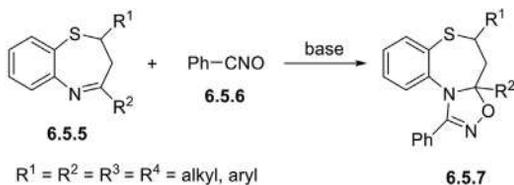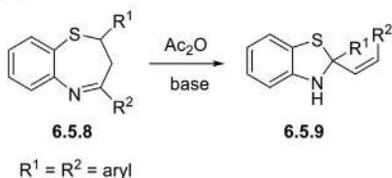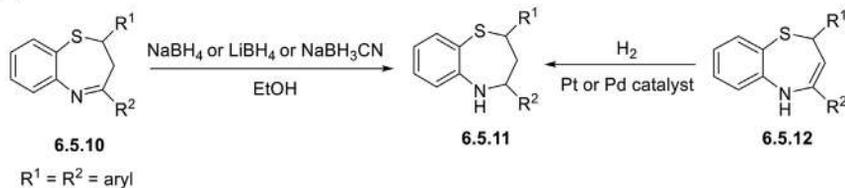
Figure 6.11 Examples of chemical reactivity of 1,5-benzothiazepines.

(NaBH$_4$), lithium borohydride (LiBH$_4$), and sodium cyanoborohydride (NaBH$_3$CN) or catalytic hydrogenation using H$_2$ gas (Fig. 6.11D) (Dehaen & Ngo, 2008; Rao & Reddy, 2006; Storck, Aubertin, & Grierson, 2005).

It is proven that 2,4-disubstituted-2,3-dihydro-1,5-benzothiazepines are structurally not planar where the thiazepine ring adopts a boat-like conformation (Lu, Jin, & Xing, 1988). The dihedral angle between the benzene ring and the seven-membered ring is calculated to be about 50°. According to the Wiberg bond order calculated by the MNDO program for **6.5.13** with R^2 = Ph, the bond order of N=C is found to be about

6.5.13   **6.5.14**   **6.5.15**

($R^1 = R^2 = H$, alkyl, aryl)
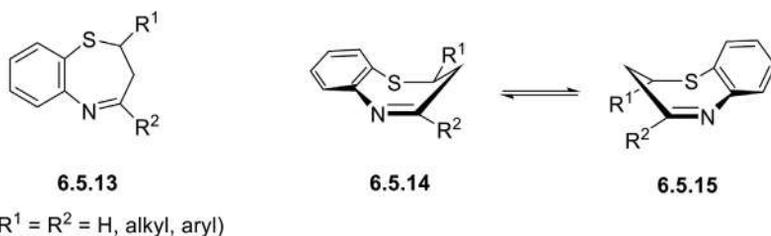
Figure 6.12 General boat-shaped conformations of 2,4-disubstituted-2,3-dihydro-1,5-benzothiazepines.

1.84, which is less than a typical isolated double bond. The C11–N5 and C4–Ph bonds also show the values of 1.04 and 0.98, respectively, which are larger than those of typical single bonds. These observations clearly suggest that the aromatic units and the nitrogen atom in Ar–N=C–Ar are conjugated, and the delocalization effect in Ar–N–C is greater than N=C–Ar (Lu et al., 1988). There are two boat conformations (**6.5.14** and **6.5.15**) for these skeletons based on the orientation of R^1 (Fig. 6.12), in which the energy difference between the conformers is approximately 2.0 kcal/mol. Therefore, both **6.5.14** and **6.5.15** are almost equally stable and exist in the crystal framework. Although the energy difference is slight, conformer **6.5.15** has a little higher energy than **6.5.14**. Note that the interaction of R^1 with other adjacent groups in the structure is not the main reason for the increased energy of conformer **6.5.15**. For example, the distance between R^1 and R^2 is much greater than the amounts of the van der Waals radii, which makes their van der Waals interactions negligible to less than 0.1 kcal/mol (Lu et al., 1988). Instead, it has been assumed that bending and torsion can be the key factors to impact the steric energies. The reason lies in the fact that some structural changes within the compound have occurred when R^1 moves from upward (**6.5.14**) to downward (**6.5.15**) (Lu et al., 1988). A direct relationship exists between the conformations of 2,3-dihydro-1,5-benzothiazepines and their crystal lattices, as conformers **6.5.14** and **6.5.15** typically crystalize in monoclinic ($P2_1/a$ and $P2_1/c$) and triclinic (P-1 and Ci^1-$P\bar{1}$) lattices, respectively.

2,4-Disubstituted-2,3,4,5-tetrahydro-1,5-benzothiazepines (**6.5.16**) usually exist in two twist-boat and chair conformations with an energy difference of about 2.0 kcal/mol (Fig. 6.13). The stability of the conformers highly depends on the heteroatoms and the substituents at positions 2 and 4 in the thiazepine ring (Lu et al., 1988). For example, the twisted boat form of the unsubstituted 2,3,4,5-tetrahydro-1,5-benzothiazepine (**6.5.16**,
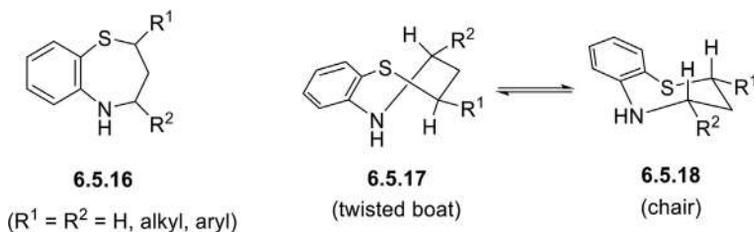
Figure 6.13 General conformations of 2,4-disubstituted-2,3,4,5-tetrahydro-1,5-benzothiazepines.

$R^1 = R^2 = H$) displays lower calculated energy (18.1 kcal/mol) than the 4-methyl-2-phenyl-2,3,4,5-tetrahydro-1,5-benzothiazepine (27.1 kcal/mol). Basically, the twist-boat conformation of all types of tetrahydro-1,5-benzothiazepines is more stable than their chair conformation. The bond angle of C11–N5–C4 is observed to be 120° which allows the nitrogen orbitals to act as the sp^2 orbitals, facilitating the conjugation of p-orbital with the benzene ring.

6.6 Conclusion

This chapter briefly reviewed the knowledge of the structural features of 1,5-benzodiazepines and 1,5-benzothiazepines. The knowledge includes tautomerization, conformational isomerism, crystal structure, and potential reactive sites, and the influencing parameters on these properties, which are important for designing new synthetic reactions as well as understanding the behavior of these versatile fused heterocycles in pharmacological systems. Despite the rich chemistry of benzodiazepine- and benzothiazepine-based drug molecules over the past 50 years, only a few examples have been reported to determine the structural features of 1,5-benzodiazepines and 1,5-benzothiazepines. Therefore, there is an imperative need for more research endeavors to explore the chemical and structural potentials of these molecules in the future.

References

Archer, G. A., & Sternbach, L. H. (1968). Chemistry of benzodiazepines. *Chemical Reviews, 68*(6), 747–784. https://doi.org/10.1021/cr60256a004.

Assadi, M. H. N., & Sahajwalla, V. (2014a). Polymers' surface interactions with molten iron: A theoretical study. *Chemical Physics, 443*, 107–111. https://doi.org/10.1016/j.chemphys.2014.09.007.

Assadi, M. H. N., & Sahajwalla, V. (2014b). Recycling end–of–life polycarbonate in steelmaking: *Ab* initio study of carbon dissolution in molten iron. *Industrial & Engineering Chemistry Research, 53*(10), 3861–3864. https://doi.org/10.1021/ie4031105.

Barchet, V. R., & Merz, K. W. (1964). Über kondensationen von nicotinoylessigester mit aromatischen diaminen. *Tetrahedron Letters, 5*(33), 2239–2244. https://doi.org/10.1016/S0040–4039(00)71694–7.

Barltrop, J. A., Richards, C. G., Russell, D. M., & Ryback, G. (1959). Seven–membered heterocyclic compounds. Part I. 1: 5-Benzodiazepines and derivatives of 3: 6-diaza-4: 5-benzotropone. *Journal of the Chemical Society*, 1132–1142. https://doi.org/10.1039/JR9590001132.

Barry, W. J., Finar, I. L., & Mooney, E. F. (1965). A comparison of the structures of some dianils with those of analogous diazepines. *Spectrochimica Acta, 21*(6), 1095–1099. https://doi.org/10.1016/0371–1951(65)80189–8.

Claramunt, R. M., Alkorta, I., & Elguero, J. (2013). A theoretical study of the conformation and dynamic properties of 1,5-benzodiazepines and their derivatives. *Computational and Theoretical Chemistry, 1019*, 108–115. https://doi.org/10.1016/j.comptc.2013.07.002.

Davoll, J. (1960). The reaction of *o*-phenylenediamine with α,β-unsaturated acids and with β-keto-esters. *Journal of the Chemical Society*, 308–314. https://doi.org/10.1039/JR9600000308.

Dehaen, W., & Ngo, T. H. (2008). 1,4-Oxazepines and 1,4-thiazepines. In A. R. Katritzky, C. Ramsden, E. Scriven, & R. Taylor (Eds.), *Comprehensive Heterocyclic Chemistry III*: vol. 13. Amsterdam: Elsevier. https://doi.org/10.1016/B978–008044992–0.01209–8.

Dong, Z.-Q., Liu, F.-M., Xu, F., & Yuan, Z.-L. (2011). Synthesis of 1,5-benzothiazepine derivatives bearing 2-phenoxy-quinoline moiety *via* 1,3-diplolar cycloaddition reaction. *Molecular Diversity, 15*(4), 963–970. https://doi.org/10.1007/s11030–011–9328–z.

El-Bayouki, K. A. M. (2013). Benzo[1,5]thiazepine: Synthesis, reactions, spectroscopy, and applications. *Organic Chemistry International,* Article ID 210474, 71 pages. https://doi.org/10.1155/2013/210474.

Eliel, E. L., & Wilen, S. H. (1994). Stereochemistry of Organic Compounds. Hoboken, New Jersey: John Wiley & Sons.

Ichii, T. (1962). Synthesis of phenothiazine derivatives. IV. *Journal of the Pharmaceutical Society of Japan, 82*(7), 992–998. https://doi.org/10.1248/yakushi1947.82.7_992.

Krygowski, T. M., Bankiewicz, B., Czarnocki, Z., & Palusiak, M. (2015). Quasi-aromaticity–what does it mean? *Tetrahedron, 71*(30), 4895–4908. https://doi.org/10.1016/j.tet.2015.05.074.

Kuch, H. (1979). Clobazam: Chemical aspects of the 1,4 and 1,5-benzodiazepines. *British Journal of Clinical Pharmacology, 7*(Suppl 1), 17S–21S.

Levai, A., & Jeko, J. (2003). Chemoselective oxidation of 3-acetyl-2,3-dihydrobenzothiazoles by dimethyldioxirane. *Arkivoc*, 19–27. https://doi.org/10.3998/ark.5550190.0004.503.

Liu, L., Zhang, L., Liu, G., & Xu, J. (2008). Synthesis of spiro-fused polycyclic β-lactam derivatives. *Arkivoc*, 318–326. https://doi.org/10.3998/ark.5550190.0009.g30.

Lloyd, D., & Cleghorn, H. P. (1974). Advances in Heterocyclic Chemistry: vol. 17. Amsterdam: Elsevier.

Lloyd, D., & Marshall, D. R. (1971). Aromaticity, pseudo-aromaticity, anti-aromaticity. In E. D. Bergmann, & B. Pullman (Eds.), *Proceedings of an International Symposium* (1st ed.). Cambridge, Massachusetts: Academic Press.

Lloyd, D., & Mcnab, H. (1998). 1,5-Benzodiazepines and 1,5-benzodiazepinium salts. *Advances in heterocyclic chemistry, 71*, 1–56. https://doi. org/10. 1016/S0065-2725(08)60830-2.

Lu, Y. C., Jin, S., & Xing, Q. Y. (1988). Theoretical conformational analysis of 1,5-benzodiazepines and benzothiazepines. *Journal of Molecular Structure: THEOCHEM, 167*(3–4), 253–267. https://doi.org/10.1016/0166–1280(88)80230–6.

Mannschreck, A., Rissmann, G., Vogtle, F., & Wild, D. (1967). Protonenresonanz-untersuchungen an siebengliedrigen ringverbindungen, I. Intramolekulare beweglichkeit

von azepinen und diazepinen. *Chemistche Berichte, 100*(1), 335–346. https://doi.org/10.1002/cber.19671000138.

Martín, O., Pérez-Torralba, M., García, M. Á., Claramunt, R. M., Torralba, M. C., Torres, M. R., et al. (2016). Static and dynamic properties of fluorinated 4-aryl-1,5-benzodiazepinones. *Chemistry Select, 1*(4), 861–870. https://doi.org/10.1002/slct.201600167.

Masakatsu, M., Akira, L., & Teijiro, Y. (1970). Oxidation of 2,4-diphenyl-l,5-benzodiazepine with peracetic acid. *Bulletin of the Chemical Society of Japan, 43*(1), 281–282. https://doi.org/10.1246/bcsj.43.281.

Nicolaus, B. J. R., Bellasio, E., Pagani, G., Mariani, L., & Testa, E. (1965). Über eine neu allgemeine Synthese von 3,3-disubstituierten 2,3,4,5-Tetrahydro-1*H*-(benzo[*b*]1,4-diazepin)-2-onen durch Ringerweiterung 3,3-disubstituierter 1-(*o*-Aminophenyl)-azetidin-2-one. *Helvetica Chimica Acta, 48*(8), 1867–1885. https://doi.org/10.1002/hlca.19650480806.

Nieto, C. I., Claramunt, R. M., Torralba, M. C., Torres, M. R., & Elguero, J. (2017). Crystal structure of (1*Z*,4*Z*)-2,4-dimethyl-3*H*-benzo[*b*][1,4]diazepine. *Acta Crystallographica Section E: Crystallographic Communications, 73*(5), 647–650. https://doi.org/10.1107/S2056989017004972.

Okovytyy, S. I., Sviatenko, L. K., Gaponov, A. A., Kasyan, L. I., Tarabara, I. N., & Leszczynski, J. (2010). DFT study on tautomerism of dihydro-2*H*-1,5-benzodiazepin-2-ones and dihydro-2*H*-1,5-benzodiazepine-2-thiones. *European Journal of Organic Chemistry*, 280–291. https://doi.org/10.1002/ejoc.200900689.

Paterson, W., & Proctor, G. R. (1965). The removal of toluene-*p*-sulphonyl groups from sulphonamides. Part I. Synthesis of schiff bases. *Journal of the Chemical Society*, 485–489. https://doi.org/10.1039/JR9650000485.

Ramin, L., Assadi, M. H. N., & Sahajwalla, V. (2014). High-density polyethylene degradation into low molecular weight gases at 1823K: An atomistic simulation. *Journal of Analytical and Applied Pyrolysis, 110*, 318–321. https://doi.org/10.1016/j.jaap.2014.09.022.

Rao, V. R., & Reddy, M. M. (2006). A facile one pot synthesis of 2-aryl-4-[2*H*-2-oxo-[1]benzopyran-3-yl] 2,3-dihydro and 2,5-dihydro-1,5-benzothiazepines. *Phosphorus, Sulfur and Silicon and the Related Elements, 181*(2), 461–471. https://doi.org/10.1080/104265091001326.

Solomko, Z. F., & Kost, A. N. (1975). 1,5-Benzodiazepines (review). *Chemistry of Heterocyclic Compounds, 11*, 1231–1248. https://doi.org/10.1007/BF00474444.

Sternbach, L. H., Randall, L. O., Banziger, R., & Lehr, H. (1968). Structure-activity relationships in the 1,4-benzodiazepine series. In A. Berger, & M. Decker (Eds.), *Drugs Affecting The Central Nervous System* (vol. 2, pp. 237–264). New York: Marcel Dekker.

Storck, P., Aubertin, A.-M., & Grierson, S. S. (2005). Tosylation/mesylation of 4-hydroxy-3-nitro-2-pyridinones as an activation step in the construction of dihydropyrido[3,4-*b*] benzo[*f*][1,4]thiazepin-1-one based anti-HIV agents. *Tetrahedron Letters, 46*(16), 2919–2922. https://doi.org/10.1016/j.tetlet.2005.02.128.

Non-Print Items

Abstract

In this chapter, the structural and chemical properties of 1,5-benzodiazepines and 1,5-benzothiazepines through available literature are discussed. Most of these studies have been focused on the tautomerism, conformational isomerism, and chemical reactivity of these pharmaceutically important fused heterocycles.

Synthesis of 1,5-benzodiazepines and 1,5-benzothiazepines

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Chapter outline

7.1 Introduction

Since 1,5–benzodiazepines and 1,5-benzothiazepines have exhibited a wide range of biological activity profiles in the pharmaceutical industry, the search for potentially useful newer derivatives is continuously pursued in organic synthesis. The most common synthetic strategies of these privileged scaffolds involve the condensation reactions of *ortho*-phenylenediamines or 2-aminothiophenols (**7.1.1**) with various carbonyl compounds in presence of an acid or a base (Fig. 7.1) (Ried & Torinus, 1959; Stahlhofen & Ried, 1957). Cycloaddition [4 + 3] reactions of **7.1.1** with, *α,β*-unsaturated carbonyl compounds (**7.1.2**), ketones (**7.1.4**), or 1,3-dicarbonyl compounds (**7.1.6**) provide access to disubstituted (**7.1.3**), tetrasubstituted (**7.1.5**), and disubstituted (**7.1.7**) 1,5-benzodia(thia)zepine derivatives, respectively. Cycloaddition [4 + 2 + 1] reaction of **7.1.1** and alkynoates (**7.1.8**) can afford the corresponding disubstituted–1,5-benzodia(thia)zepine (**7.1.9**) containing a
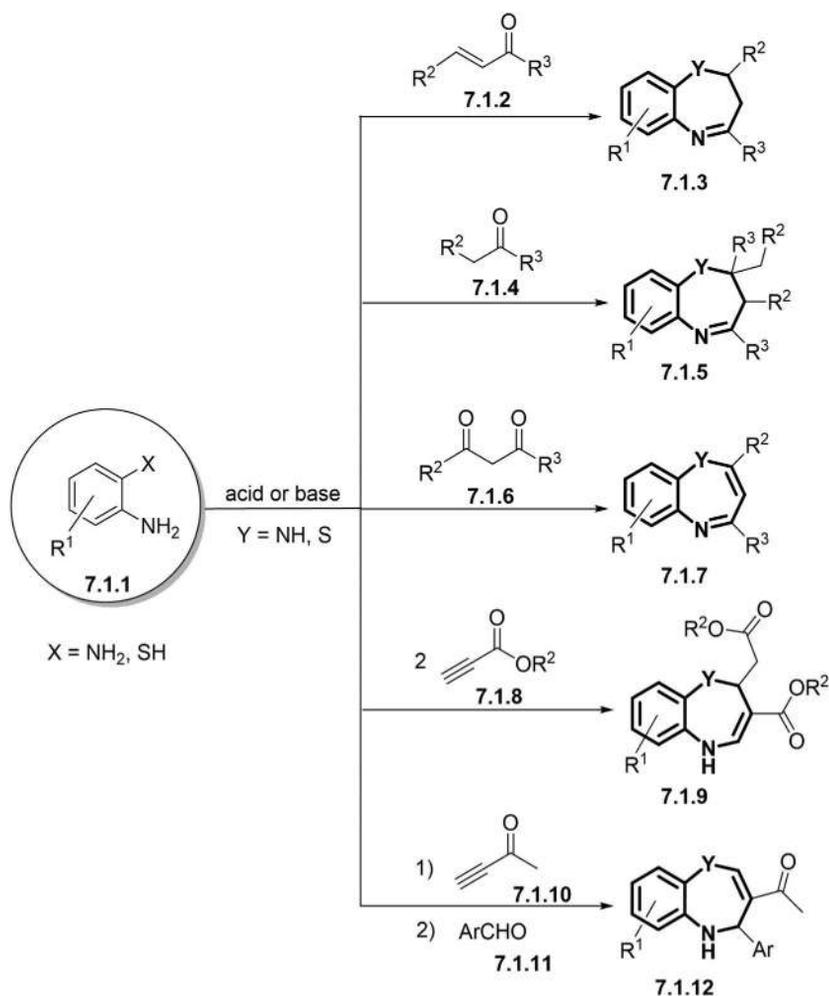
Figure 7.1 General synthetic approaches of 1,5-benzodiazepines and 1,5-benzothiazepines.

C=C bond in the heterocyclic structure for further manipulations. Michael addition of the substrate **7.1.1** to the propargylic ketone (**7.1.10**), followed by nucleophilic addition/cyclization with the aromatic aldehyde (**7.1.11**), also generates the disubstituted-1,5-benzodia(thia)zepine (**7.1.12**). These conventional protocols, however, suffer from various drawbacks including expensive reagents, low yields, harsh reaction conditions, tedious work-up procedures, and the formation of by-products. Thus, there is always a need to develop a simpler and sustainable approach to efficiently obtain

these versatile pharmacophores in an environmentally benign manner. This chapter does not provide an exhaustive literature list but instead aims to provide an overview of the most practical and attractive protocols that demonstrate the synthesis of 1,5-benzodiazepines and 1,5-benzothiazepines deploying various readily available starting materials and catalysts under mild reaction conditions.
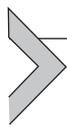
7.2 Synthesis of 1,5-benzodiazepines

Remarkable progress has been witnessed in the preparation of benzo-diazepines over the past decade due to their diverse spectrum of biological activities. In addition to the traditional methods, a large number of newer synthetic approaches have emerged to afford novel derivatives of these therapeutic candidates. In this section, recent advances in facile and useful procedures for the synthesis of 1,5-benzodiazepines *via* both, synthetic and catalytic approaches, are discussed.

7.2.1 Synthetic methods for 1,5-benzodiazepines

A simple procedure for the synthesis of several 4–naphtho[2,1–*b*]furan–2–yl–2(phenyl)–2,5–dihydro–1*H*–1,5–benzodiazepines (**7.2.7**) has been developed *via* the reaction of 2–acetylnaphtho[2,1–*b*]furan (**7.2.3**), benzaldehydes (**7.2.4**), and *ortho*–phenylenediamine (**7.2.6**) (Fig. 7.2) (Kumaraswamy et al., 2013). The key substrate (**7.2.3**) is initially prepared *via* the condensation reaction of 2–hydroxy–1–naphthaldehyde (**7.2.1**) and 1–chloropropan–2–one (**7.2.2**) in the presence of potassium carbonate, and the desired products (**7.2.7**) can be obtained upon recrystallization in aqueous DMF in good yields.

Five-membered ring heterocycles which are trisubstituted and appended to 1,5-benzodiazepines have been shown to significantly improve biological activities (Ghogare et al., 2010). A large number of thiophene- and thiazole-containing 1,5-benzodiazepines (**7.2.13, 7.2.14**) have been synthesized from readily available substrates under mild reaction conditions (Fig. 7.3) (Wang, Li, & An, 2015). *Ortho*–phenylenediamines (**7.2.8**) first undergo a nucleophilic addition reaction with ethyl acetoacetate (**7.2.9**) to give *N*–*ortho*–aminoaryl–β–enamino esters (**7.2.10**) at room temperature. This is followed by the reaction of either 2–thiophenecarboxaldehyde (**7.2.11**) or 2–thiazolecarboxaldehyde (**7.2.12**) with the adduct (**7.2.10**) in ethanol at 0 °C to produce the corresponding 1,5-benzodiazepine ester analogs (**7.2.13**) and
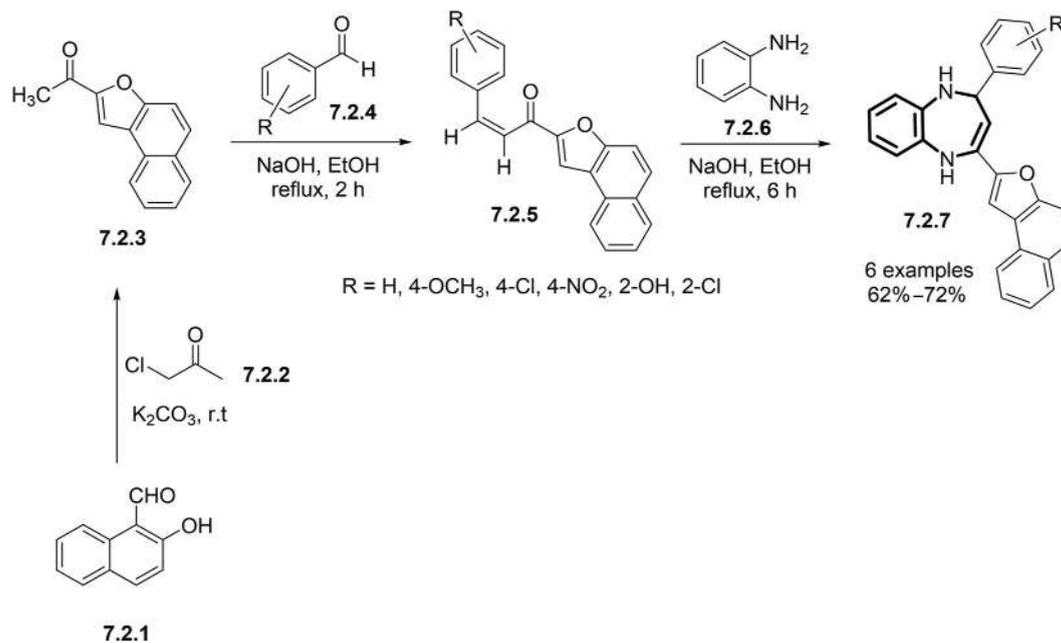
Figure 7.2 Synthesis of 4-naphtho [2,1-*b*] furan-2-yl-2(phenyl)-2,5-dihydro-1*H*-1,5-benzodiazepines.
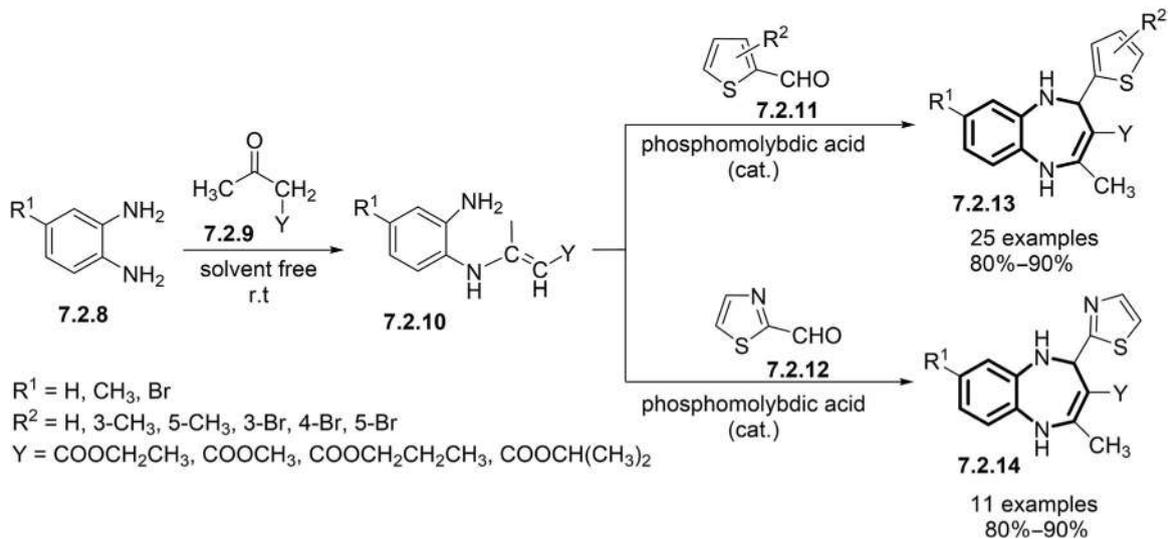
Figure 7.3 Synthesis of 2,3,4-trisubstituted 1,5-benzodiazepine derivatives.
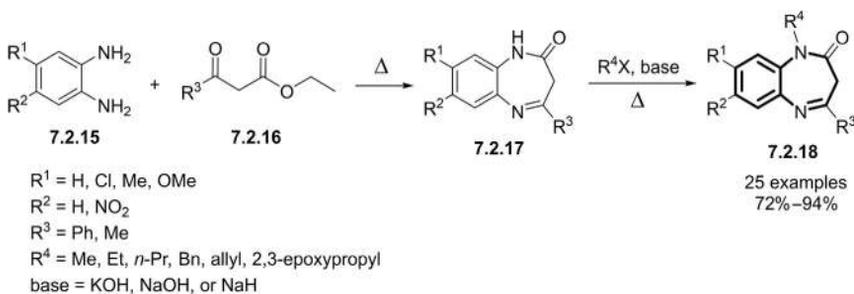
Figure 7.4 Synthesis of *N*-alkyl/allyl-1,5-benzodiazepine-2-ones.

(**7.2.14**), respectively, *via* recrystallization in dry ethanol. It is well understood that the presence of a free ester group in benzodiazepine skeletons can increase the pharmacological activity due to the higher hydrophobicity (Schütz, 1982), which highlights the significance of this reported methodology.

Benzodiazepin-2-ones are known as privileged structures in drug development due to their wide range of biological activities such as antisecretory, anticonvulsant, enzyme inhibitory, and antiarrhythmic properties (Spencer, Rathnam, & Chowdhry, 2010). It is also well understood that increasing hydrophobicity in drug molecules can enhance their cell permeation efficacy with possibly decreased cytotoxicity (Sakagami, Masuda, Kawano, & Futaki, 2018). As many FDA-approved 1,5-benzodiazepine-based drugs comprise an *N*-alkyl/allyl substituent, several *N*-alkyl-1,5-benzodiazepine-2-ones (**7.2.18**) through a facile two-step synthetic method have been synthesized (Fig. 7.4) (Gaponov et al., 2016). A boiling mixture of *ortho*-phenylenediamines (**7.2.15**) and acetoacetic esters (**7.2.16**) is stirred for 2 h, produced the corresponding 1,5-benzodiazepine-2-ones (**7.2.17**). *N*-alkylation reaction of the synthesized (**7.2.17**) is carried out using various alkyl/allyl halides in presence of a base. The target products (**7.2.18**) are obtained in high purities after flash chromatography on silica or recrystallization in hexane with high yields of 72%–94%.

N-Benzoyl substituted benzodiazepin-2-ones have been found to be potent inhibitors of HIV-1 reverse transcriptase (RT) in medicinal studies (Nichols et al., 2009). In this respect, Sankaranarayanan group described the preparation of a library of 5-benzoyl-4-methyl-1,3,4,5-tetrahydro-2*H*-1,5-benzodiazepin-2-one compounds (**7.2.22**) *via* a simple two-step synthetic pathway comprising the condensation of *ortho*-phenylenediamine (**7.2.6**) with crotonic acid (**7.2.19**), followed by *N*-acylation reaction using acid chlorides (**7.2.21**) and triethylamine as a base (Fig. 7.5) (Chander et al., 2017).
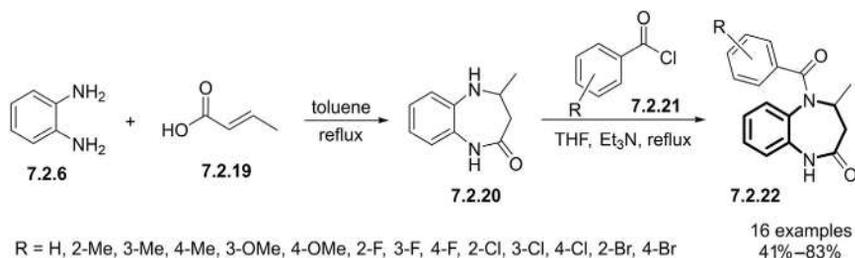
Figure 7.5 Synthesis of 5-benzoyl-4-methyl-1,3,4,5-tetrahydro-2*H*-1,5-benzodiazepin-2-ones.
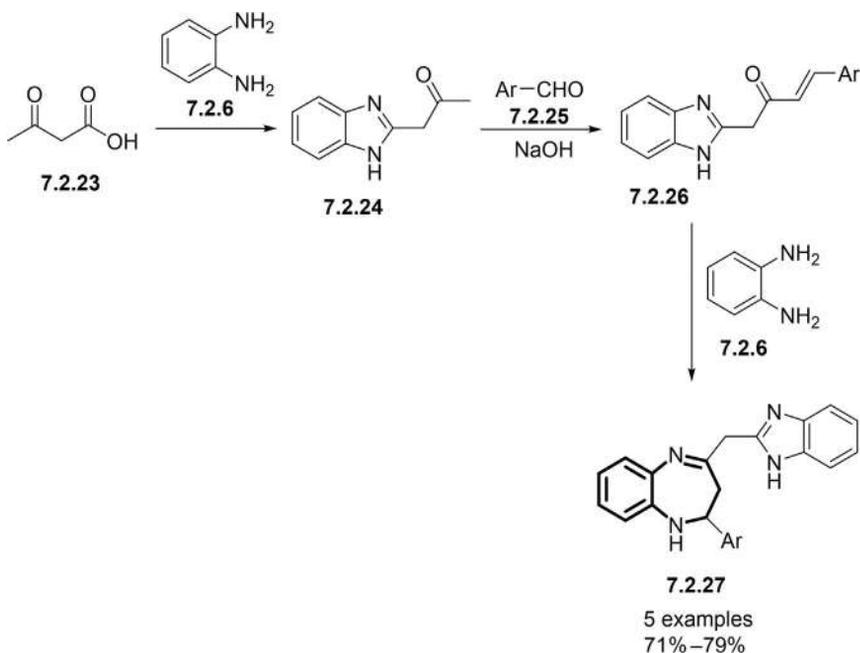
Figure 7.6 Synthesis of benzimidazole-substituted 1,5-benzodiazepines.

A multistep synthetic pathway for a range of benzimidazole–substituted 1,5–benzodiazepine derivatives (**7.2.27**) has been developed (Fig. 7.6) (Sharma, Tilak, Thakur, Gangwar, & Sutar, 2017). The reaction of *ortho*–phenylenediamines (**7.2.6**) with acetoacetic acid (**7.2.23**) initially yields the benzimidazole (**7.2.24**), which subsequently undergoes Claisen–Schmidt condensation with various aromatic aldehydes (**7.2.25**) to produce the corresponding chalcones (**7.2.26**). The desired 1,5–benzodiazepines (**7.2.27**) are finally obtained *via* the Michael addition reaction of *ortho*–phenylenediamines (**7.2.6**) and (**7.2.26**), purified by recrystallization in hot ethanol.
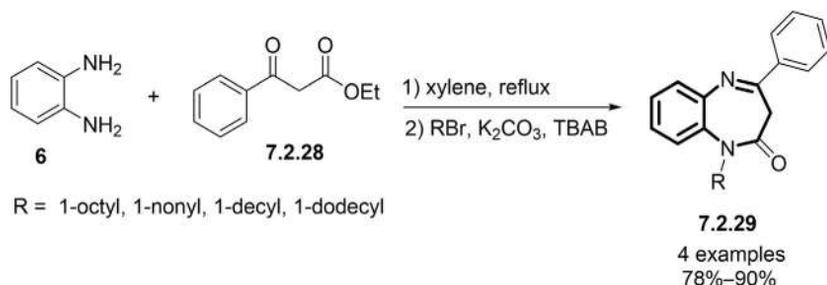
Figure 7.7 Synthesis of 4-phenyl-1,5-benzodiazepin-2-ones with long carbon chains.

The Ongone group has demonstrated the preparation of several 4–phenyl–1,5–benzodiazepin–2–ones (**7.2.29**) with long hydrophobic carbon chains (Fig. 7.7) (Ongone et al., 2019). This synthetic methodology initiated by the reaction of *ortho*–phenylenediamine (**7.2.6**) with ethyl benzoylacetate (**7.2.28**) in xylene under reflux conditions, is followed by *N*-alkylation of the diazepine ring with several alkyl bromides in presence of potassium carbonate and tetrabutylammonium bromide (TBAB) to generate the title compounds (**7.2.29**) in high yields (78%–90%) after flash chromatography on silica gel.

Benzodiazepines linked to a pyridazinone nucleus have exhibited significant pharmacological activities in drug development such as anticonvulsant, analgesic, cardiotonic, and antidiabetic (Varvounis, 2016). A simple and efficient synthetic protocol for several pyridazinone-containing 1,5–benzodiazepines has been elaborated (Tupare & Pawar, 2017). Various chalcone intermediates (**7.2.32**) are initially synthesized using a base-catalyzed condensation reaction of pyridazinone acetophenone (**7.2.30**) with a range of aromatic aldehydes (**7.2.31**), which are then subjected to microwave (MW) irradiation with *ortho*-phenylenediamine (**7.2.6**) in piperidine as a solvent to afford target 1,5-benzodiazepine compounds (**7.2.33**) (Fig. 7.8).

Click reaction is one of the most powerful synthetic tactics for building and linking the diverse classes of heterocyclic systems and converting them into a single molecule (Doustkhah et al., 2018). The incorporation of heterocyclic systems into the 1,5-benzodiazepine core structures could extend their spectrum of efficiency with new biological properties, due to their ability to interact with different biological targets. Synthesis of several 2-(3,5-disubstituted isoxazolyl)-1,5-benzodiazepines and 2,4-bis-(3,5-disubstituted isoxazolyl)-1,5-benzodiazepine analogs *via* the click chemistry approach has been reported (Abdallah, Daami-Remadi, Znati, Jannet, & Gharbi, 2017). The title 1,5-benzodiazepine products (**7.2.42**) and (**7.2.43**) are synthesized through three steps starting from the readily accessible

R = H, OH, Cl, NO$_2$, OCH$_3$
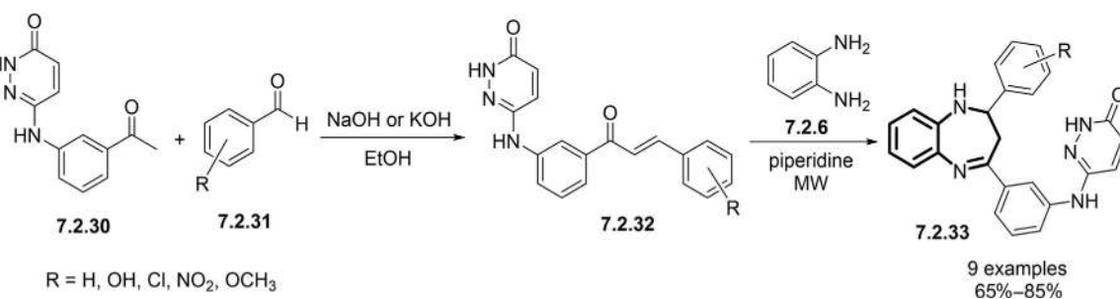
Synthesis of pyridazinone-containing 1,5-benzodiazepines in piperidine as solvent
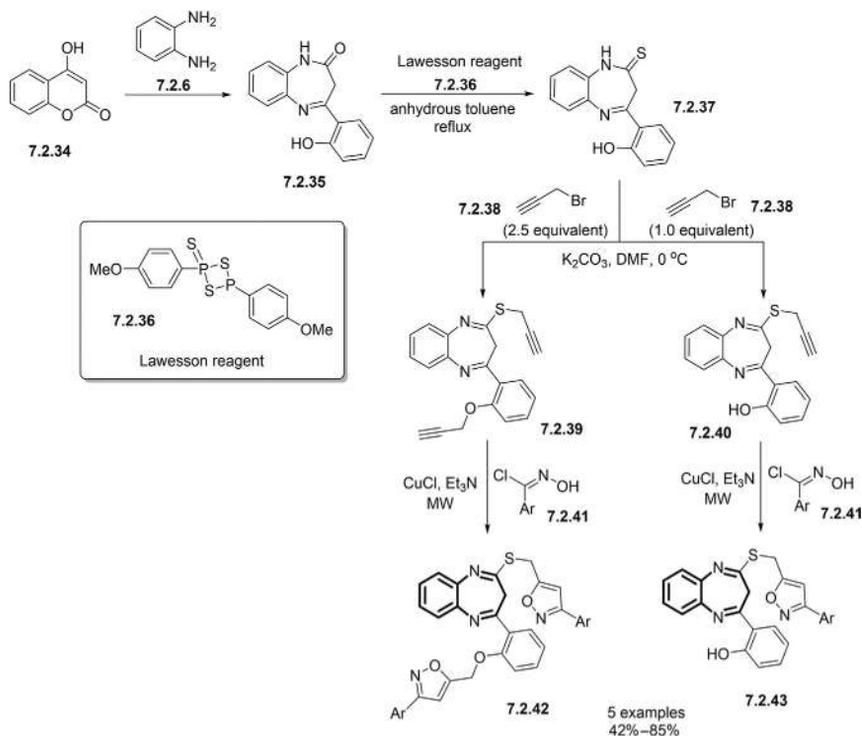
Figure 7.9 Synthesis of pyridazinone-containing 1,5-benzodiazepines in piperidine as solvent.

4-(2-hydroxyphenyl)-1,5-benzodiazepine-2-one (**7.2.35**) (Fig. 7.9). The intermediate 4-(2-hydroxyphenyl)-3*H*-1,5-benzodiazepine-2-thione (**7.2.37**) is first obtained by a thiation reaction of the precursor (**7.2.35**) using Lawesson's reagent (**7.2.36**). The treatment of the thioamide (**7.2.37**) with different equimolar ratios of propargyl bromide (**7.2.38**) then affords the *S,O*-dipropargylated 1,5-benzodiazepines (**7.2.39**) and the *S*-propargylated 1,5-benzodiazepines (**7.2.40**) and under mild reaction conditions. Subsequently, the synthesized propargylated compounds are reacted with various arylhydroxamoyl chlorides (**7.2.41**) in the presence of CuCl$_2$ and Et$_3$N under MW irradiation to generate the desired products (**7.2.42, 7.2.43**) in good yields.

The Dwivedi group has developed the synthesis of six different pyrimidine- and quinazoline-incorporated derivatives of 1,5-benzodiazepine (Misra et al., 2020). The required intermediates are synthesized using simple synthetic pathways from readily available precursors (Figs 7.10 and 7.11). Initially, the 1,5-benzodiazepines (**7.2.46, 7.2.49, 7.2.55, 7.2.60**) are prepared *via* the cyclo-condensation reaction of
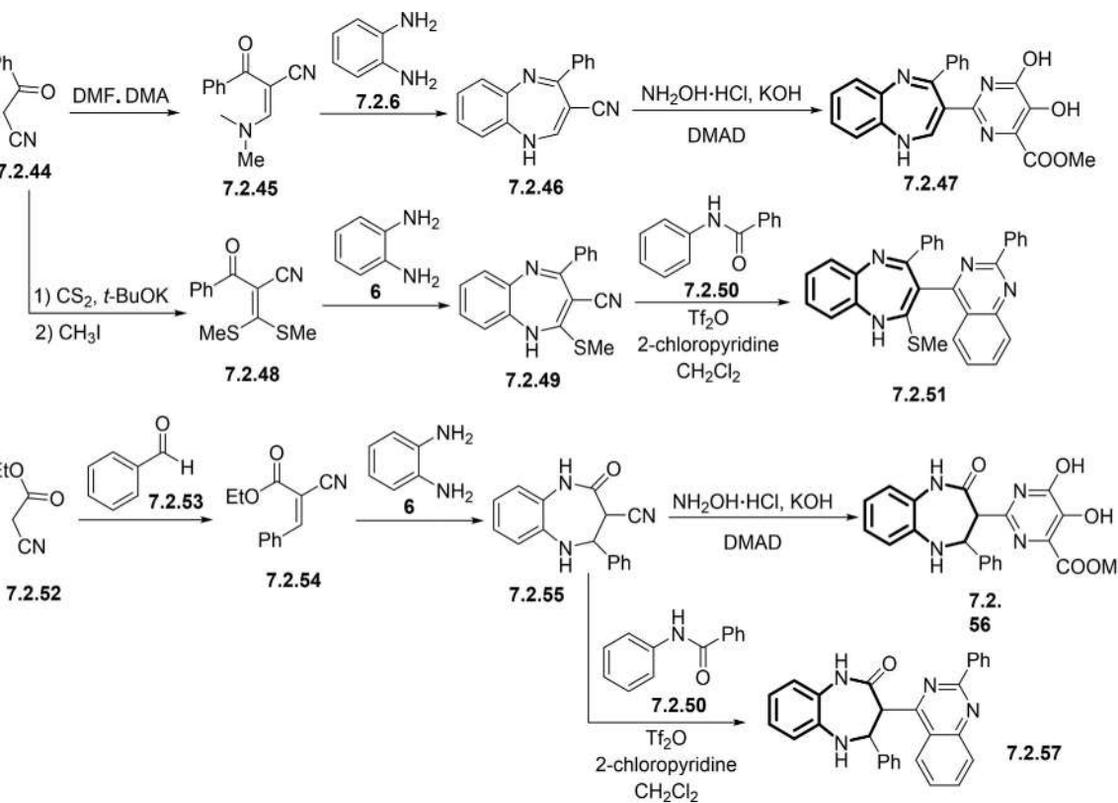
Synthesis of highly functionalized benzopyrimidine-containing 1,5-benzodiazepines.
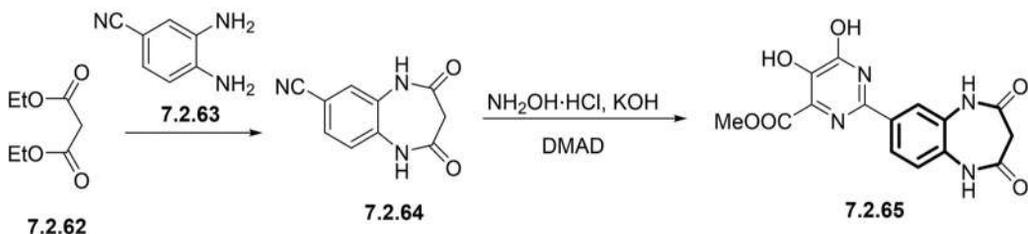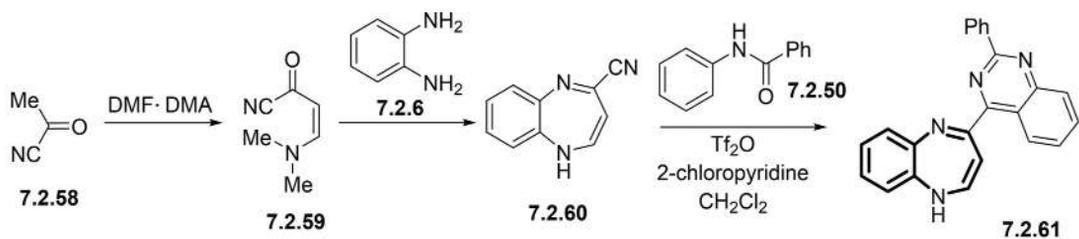
Synthesis of benzopyrimidine-containing 1,5-benzodiazepines.

ortho-phenylenediamine (**7.2.6**) with the nitriles (**7.2.45, 7.2.48, 7.2.54, 7.2.59**). The cyclo-condensation reaction of diethyl malonate (**7.2.62**) with 3,4-diaminobenzonitrile (**7.2.63**) also provides the analog (**7.2.64**). The desired products (**7.2.47, 7.2.51, 7.2.56, 7.2.57, 7.2.61, 7.2.65**) are finally obtained *via* two different pathways including the one-pot domino reaction **7.2.46, 7.2.55**, and **7.2.64** with dimethyl acetylene dicarboxylate (DMAD) in presence of 1,4-diazabicyclo[2.2.2]octane (DABCO) catalyst for the pyrimidine derivatives (**7.2.47, 7.2.56, 7.2.65**), and the reaction of **7.2.49, 7.2.55** and **7.2.60** with benzanilide **7.2.50** in presence of Tf$_2$O and 2-chloropyridine for the quinazoline analogs (**7.2.51, 7.2.57, 7.2.61**) (Figs. 7.10 and 7.11).

Triazole-bearing 1,5-benzodiazepine derivatives are recognized as superior anticonvulsant agents with excellent efficacy and low toxicity (Song & Deng, 2018). A facile synthesis of three new 1,2,4-triazolo-fused 1,5-benzodiazepine, 1,5-benzoxazepine, and 1,5-benzothiazepine has been disclosed (Sharma, Kishore, & Singh, 2018), wherein the required intermediates are prepared using multiple synthetic strategies from easily available starting materials (Fig. 7.12). In the case of 1,2,4-triazolo-fused 1,5-benzodiazepine (**7.2.73**, Z = NH), the synthetic protocol starts with the nitration of 4-chloroacetophenenone (**7.2.66**) using H$_2$SO$_4$/HNO$_3$ to obtain 4-chloro-3-nitroacetophenenone (**7.2.67**), which then undergoes Ullmann condensation reaction with 2-aminobenzoic acid (**7.2.68**) to form the corresponding product (**7.2.69**) (Z = NH). The reduction of the nitro group of (**7.2.69**) by iron powder and HCl in aqueous ethanol then affords the amino analog (**7.2.70**) (Z = NH). The obtained compound (**7.2.70**) is then treated with *N,N′*-dicyclohexylcarbodiimide (DCC) in THF to give the benzodiazepinone **70** (Z = NH). Upon treatment of **70** with Lawesson's reagent, the thioamide derivative (**7.2.71**) (Z = NH) is generated, which is then converted to the corresponding acetyl hydrazine followed by cyclization in acetic acid to secure the desired 1,2,4-triazolo-fused 1,5-benzodiazepine (**7.2.73**) (Z = NH) in 70% yield. The other analogs of **7.2.73** (Z = S and O) can also be synthesized through the same synthetic routes in good yields.

Styryl-substituted benzodiazepines are known for their substantial anti-inflammatory, antimicrobial, and antimalarial activities (Nowakowska, 2007). A simple synthetic protocol for various styryl-containing 1,5-benzodiazepines under mild reaction conditions has been demonstrated (Ishwar Bhat & Kumar, 2016). A series of 2,4-pentadien-1-one (**7.2.76**) are first prepared *via* the NaOH-mediated condensation
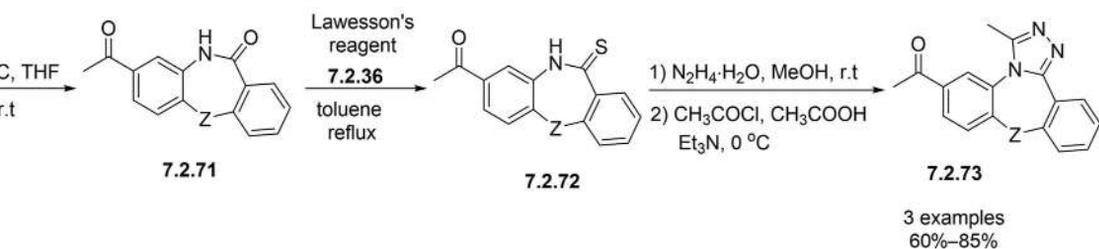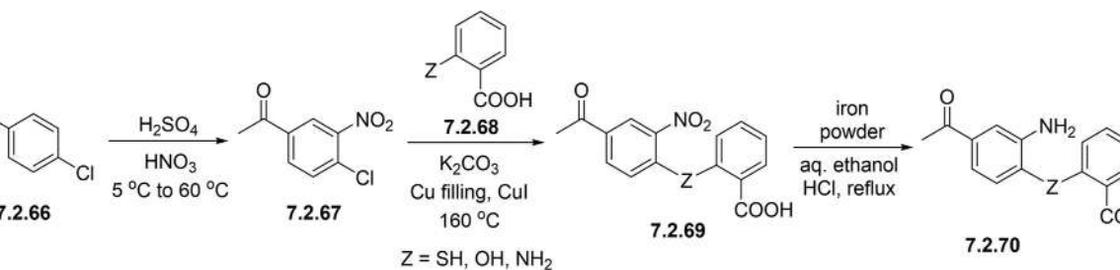
Synthesis of 1,2,4-triazolo-fused 1,5-benzodiazepines, 1,5-benzoxazepine, and 1,5-benzothiazepine.

Reagents and conditions (as shown in scheme):

7.2.66 → **7.2.67**: H_2SO_4, HNO_3, 5 °C to 60 °C

7.2.67 → **7.2.69**: **7.2.68**, K_2CO_3, Cu filling, CuI, 160 °C; Z = SH, OH, NH_2

7.2.69 → **7.2.70**: iron powder, aq. ethanol, HCl, reflux

7.2.70 → **7.2.71**: C, THF, r.t

7.2.71 → **7.2.72**: Lawesson's reagent **7.2.36**, toluene reflux

7.2.72 → **7.2.73**: 1) $N_2H_4 \cdot H_2O$, MeOH, r.t; 2) CH_3COCl, CH_3COOH, Et_3N, 0 °C

3 examples
60%–85%
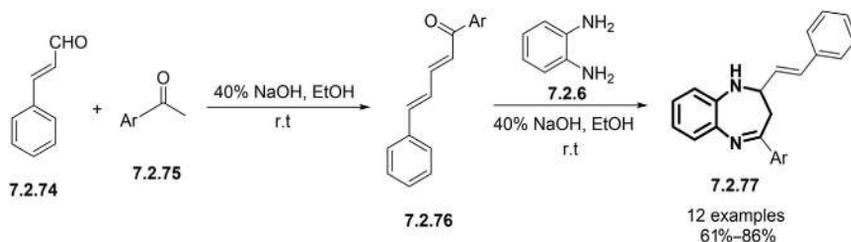
Figure 7.13 Synthesis of styryl-containing 1,5-benzodiazepines.

reaction of cinnamaldehyde (**7.2.74**) with various acetophenones (**7.2.75**) in ethanol at room temperature (Fig. 7.13). The Michael addition of *ortho*-phenylenediamine (**7.2.6**) to the α,β–unsaturated compound (**7.2.76**) followed by the cyclization reaction in the presence of NaOH provides the desired 1,5-benzodiazepine derivatives (**7.2.77**), recrystallized in ethanol, in good to high yields.

The introduction of a pyrimidine ring to the diazepine core of the 1,5-benzodiazepines results in a substantial increase of their pharmacological activity in drug-development applications (Chimirri et al., 1993). A facile enaminone–based approach for the preparation of several new tricyclic pyrimidine-fused 1,5-benzodiazepines has been revealed (Qomi & Habibi, 2017). Initially, the HCl–mediated condensation reaction of 1,3-propanedioic acid (**7.2.78**) with *ortho*-phenylenediamine (**7.2.79**) generates the corresponding 1*H*-1,5-benzodiazepine-2,4(3*H*,5*H*)–dione (**7.2.80**) (Fig. 7.14). This is followed by the formation of the C=C−NMe$_2$ next to the carbonyl groups through the reaction of (**7.2.80**) and *N,N*-dimethylformamide dimethylacetal (**7.2.81**) under solvent-free reaction conditions or in toluene/*p*-xylene. These key β–enaminonamide intermediates (**7.2.82**) are then reacted with thiourea or guanidine analogs (**7.2.83**) to deliver the desired tricyclic 1,5-diazepin-5-ones (**7.2.84**) in good to high yields.

A wide range of highly functionalized 3-acyl-1,5-benzodiazepine derivatives containing a carbonyl group at the 2-position has been synthesized through a catalyst-free domino sequence (Sun, Bei, & Wang, 2019), by one-pot reacting readily available starting materials of *N,N*-dimethylformamide dimethyl acetal (**7.2.87**), aromatic ketones (**7.2.85**), *ortho*–phenylenediamine (**7.2.86**), and aldehydes (**7.2.87**) in ethanol under reflux reaction conditions. Four new bonds including one C=C, one C–C, and two C–N are formed, providing the corresponding 1,5-benzodiazepines (**7.2.88**) in good to high yields (Fig. 7.15A). This protocol can offer several significant bene-
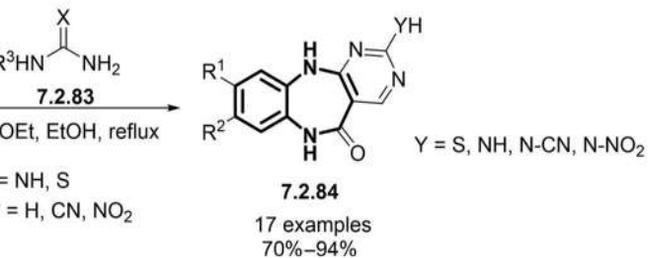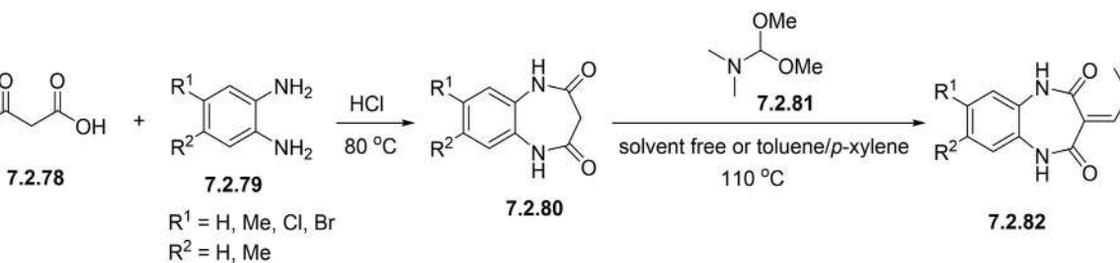
Synthesis of tricyclic pyrimidine-fused 1,5-benzodiazepines *via* an enaminone-based approach.
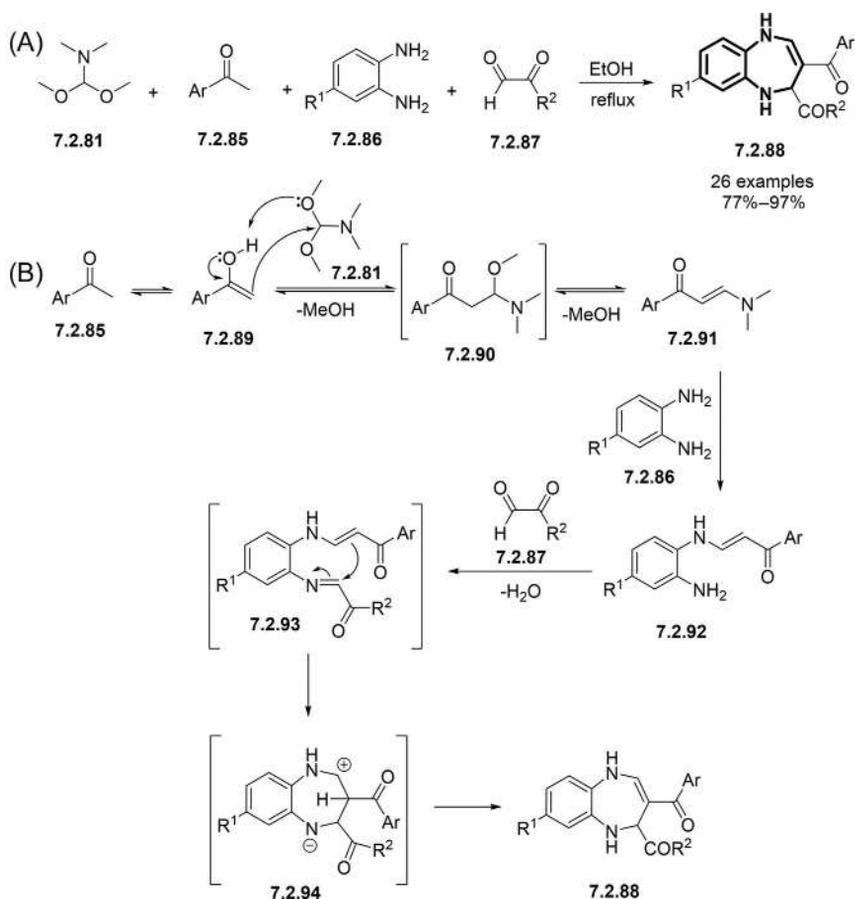
Figure 7.15 (A) One-pot synthesis of 3-acyl-1,5-benzodiazepines *via* a catalyst-free domino sequence. (B) Proposed mechanism for the formation of 1,5-benzodiazepines.

fits such as inexpensive substrates, greener one–pot operation, high yields, and excellent functional group tolerance. Mechanistically, two successive nucleophilic substitution reactions occur between *N*,*N*-dimethylformamide dimethyl acetal (**7.2.81**), and enolized aromatic ketone (**7.2.89**) to form the intermediate (**7.2.91**) (Fig. 7.15B). The nucleophilic attack of the *ortho*–phenylenediamine (**7.2.86**) on (**7.2.91**) produces the enamine (**7.2.92**) with high selectivity, which then undergoes a nucleophilic addition–dehydration reaction with the aldehyde (**7.2.87**) to fabricate the corresponding compound (**7.2.93**). The intramolecular cyclization of **7.2.93** followed by a proton transfer finally provides the desired 3–acyl-1,5–benzodiazepine product.

Spirooxindole-fused benzodiazepines have been distinguished as potent antiviral therapeutics with high selectivity and low toxicity among pharmacologically relevant drugs. A facile multicomponent synthesis of a series of isatin-fused benzodiazepines has been developed (Maury et al., 2021). Ultrasound-assisted one-pot three-component reactions of isatin (**7.2.95**), *ortho*-phenylenediamine (**7.2.96**), and 1,3-diketone (**7.2.97**) provide the corresponding benzodiazepines (**7.2.98**) in water at 80 °C (Fig. 7.16A). The reaction is simply initiated by the condensation reaction of *ortho*-phenylenediamine (**7.2.96**) and 1,3-diketone (**7.2.97**) to form the intermediate (**7.2.99**), which then attacks the carbonyl group of isatin (**7.2.95**) *via* its free amine and subsequently gives the final product after intramolecular cyclization. A gram–scale model reaction is conducted which affords the product (**7.2.102**) upon recrystallization in ethanol with a high yield of 88% (Fig. 7.16B).

Alizadeh and Bagherinejad reported the synthesis of several tetracyclic pyrido-fused dibenzodiazepines (**7.2.107**) *via* one-pot three-component reaction of dicyanochromones (**7.2.105**), *ortho*-phenylenediamine (**7.2.106**), and dimedone (**7.2.101**) in ethanol under reflux conditions (Fig. 7.17) (Alizadeh & Bagherinejad, 2020). Dicyanochromones (**7.2.105**) are first prepared *in situ* by the Knoevenagel condensation of 3-formylchromone (**7.2.103**) with malononitrile (**7.2.104**). The salient feature of this methodology is the catalyst-free cascade reaction conditions to afford the highly fused dibenzodiazepine analogs, where the Knoevenagel adduct (**7.2.105**) serves as a soft electrophile to initiate the cascade sequence. It has been shown that the desired products can be obtained under reflux conditions within 8 h, however, the reactions take 3 days to complete at room temperature.

7.2.2 Catalytic methods for synthesis of 1,5-benzodiazepines

The ever-increasing need for environmentally benign protocols with minimum chemical pollutions has unceasingly encouraged scientists to develop efficient single-step catalytic methods for the synthesis of pharmaceutically important scaffolds. A sustainable synthetic strategy for a library of 4-substituted-1,5-benzodiazepines has been reported by the Chaturbhuj group (Indalkar, Patil, & Chaturbhuj, 2017). In this methodology, sulfated polyborate-catalyzed one-pot three-component reaction of *ortho*-phenylenediamines (**7.2.108**), dimedone (**7.2.101**), and various aldehydes (**7.2.109**) afford the desired 1,5-benzodiazepines (**7.2.110**) under solvent-free reaction conditions. The polyborate catalyst can be practically prepared *via* dehydrative polymerization of boric acid followed by sulfonation with
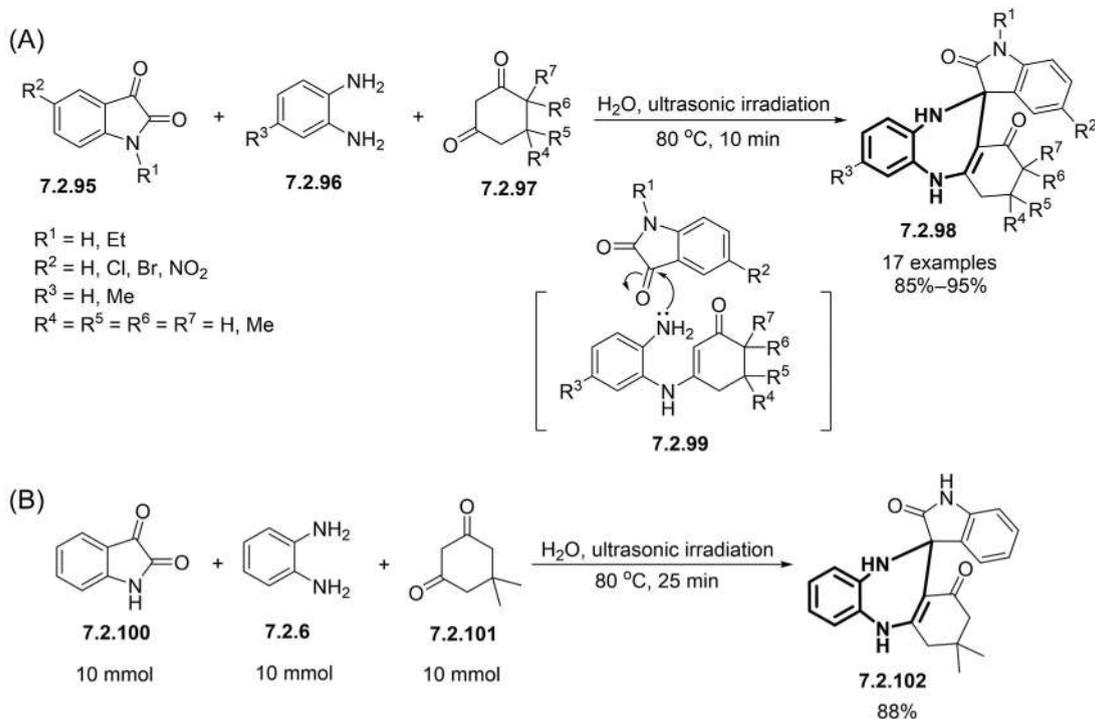
Figure 7.16 (A) Synthesis of isatin-fused benzodiazepines under ultrasonic irradiation. (B) The industrial applicability test through the gram scale reaction.
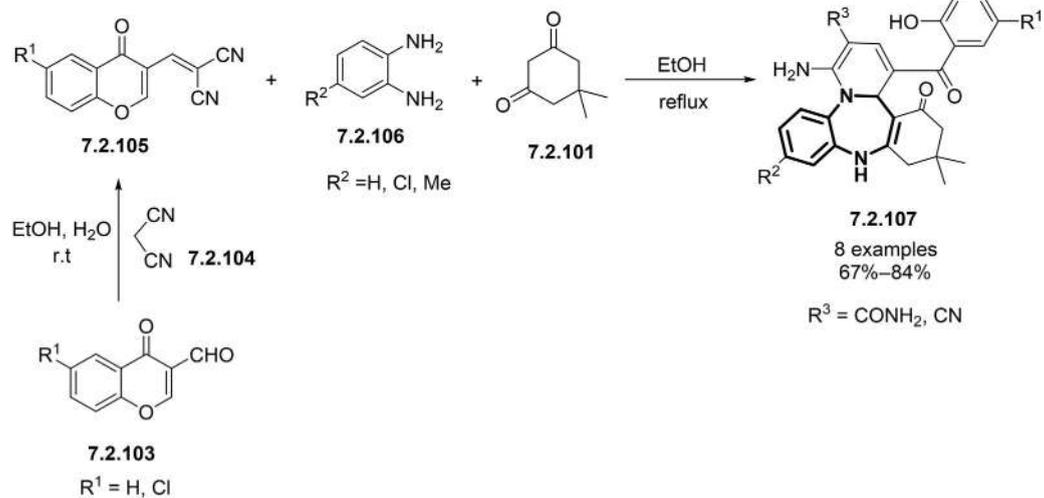
**Figure 7.17** Synthesis of tetracyclic pyrido-fused dibenzodiazepines in ethanol under reflux conditions.
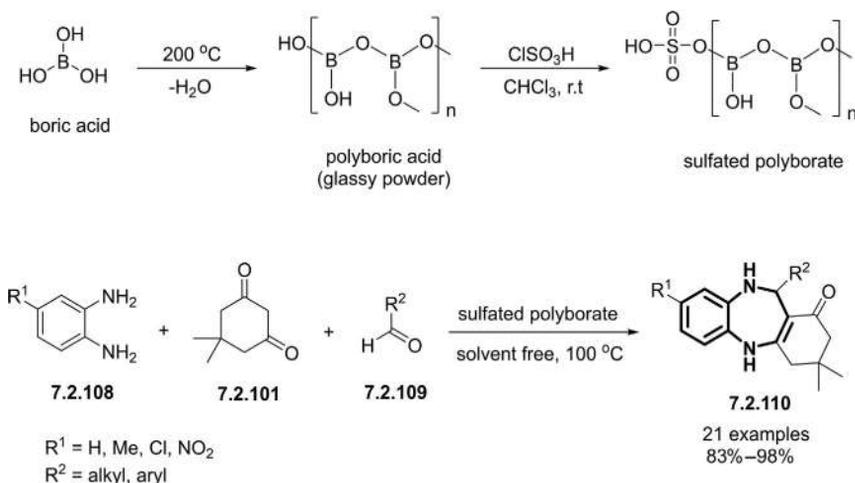
Figure 7.18 One-pot three-component synthesis of 4-substituted-1,5-benzodiazepines catalyzed by sulfated polyborate (10 wt%).

chlorosulfonic acid at room temperature (Fig. 7.18). The sulfated polyborate is known to have both Lewis acid and Brønsted acid sites due to the presence of electron–deficient boron and the sulfonic acid group, which exhibits outstanding catalytic activity in the preparation of 1,5-benzodiazepine analogs (**7.2.110**). The catalyst displays no significant loss of catalytic activity after four consecutive reaction cycles. The eco–friendly reaction conditions, inexpensive catalysts, excellent product yields, and the short reaction time (10-15 min) are the main advantages of this strategy.

Graphite-derived materials are a class of 2D carbon nanomaterials that have received considerable attention in synthetic methodology as eco–friendly metal-free catalysts endowed with high surface area, biocompatibility, commercial availability, and good reusability (Doustkhah, Mohtasham, Habeeb, & Rostamnia, 2019). Pal *et al.* used graphite oxide as a heterogeneous catalyst for synthesizing a series of 2,4-disubstituted 1,5-benzodiazepines (**7.2.113**) under mild reaction conditions (Jamatia, Gupta, Dam, Saha, & Pal, 2017). Graphite oxide can be easily obtained by oxidation of graphite powder using concentrated sulfuric acid and potassium permanganate ($KMnO_4$) (Fig. 7.19A). The acidic nature of the graphite oxide has a key role in the catalytic condensation reaction of diamines (**7.2.111**) and ketones (**7.2.112**) toward the production of the corresponding 1,5-benzodiazepines (Fig. 7.19B). The desired products can be realized in good to high yields under two different sets of reaction conditions: the solvent-free conditions at 80 °C or in ethanol at room temperature. Mechanistically, the graphite
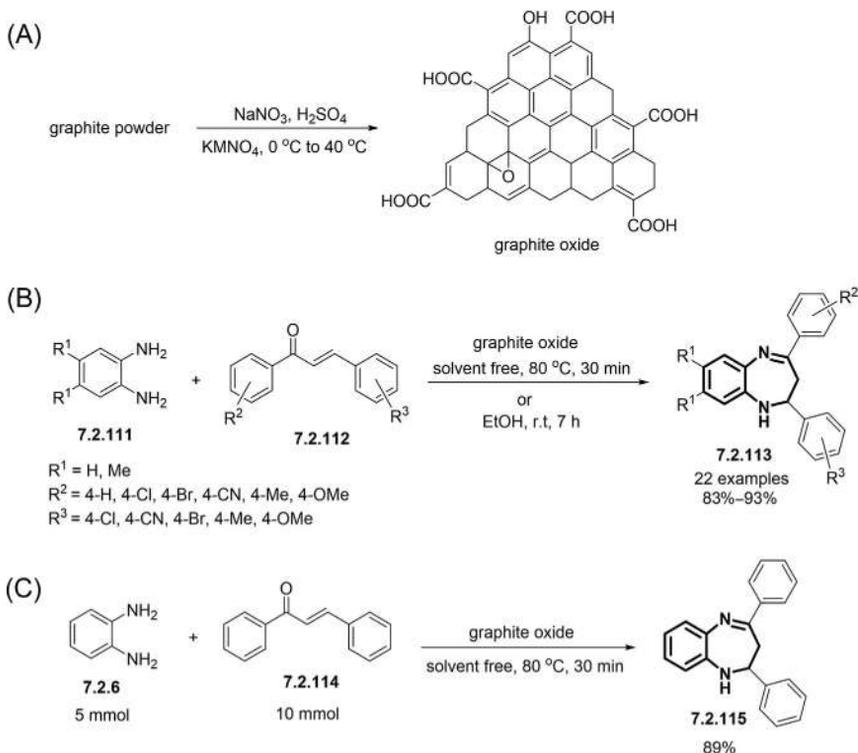
Figure 7.19 (A) Preparation of graphite oxide. (B) Synthesis of 2,4-disubstituted 1,5-benzodiazepines catalyzed by graphite oxide. (C) The industrial applicability test through the gram scale reaction.

oxide can increase the electrophilicity of the carbonyl group in chalcones *via* H-bonding, thus facilitating the diamine nucleophilic attack. In terms of industrial applicability, this methodology was tested in a gram scale reaction where a high yield of 89% was obtained for the compound (**7.2.115**) under solvent-free conditions (Fig. 7.19C).

ZnS nanoparticles are promising catalyst candidates due to their interesting properties such as good Lewis acidity, water insolubility, non-toxicity, that entail inexpensive preparative method (Fang et al., 2011). An efficient three-component synthetic platform for a range of 4-substituted 1,5-benzodiazepine analogs catalyzed by ZnS nanoparticles has been developed under grinding reaction conditions (Naeimi & Foroughi, 2016). The metal nanoparticles are easily prepared *via* an MW-assisted reaction of $Zn(OAc)_2 \cdot 2H_2O$ and thioacetamide at 110 °C (Fig. 7.20A). The Lewis acidity of the ZnS nanoparticles can facilitate the multicomponent reaction
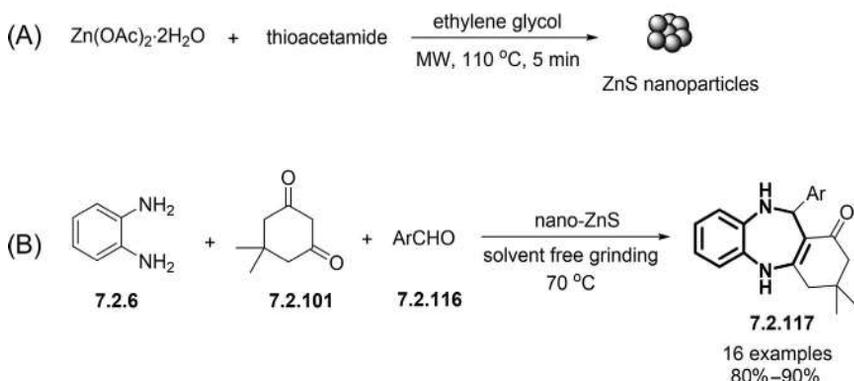
Figure 7.20 (A) Preparation of ZnS nanoparticles. (B) Synthesis of 4-disubstituted 1,5-benzodiazepines catalyzed by ZnS nanoparticles under solvent-free conditions.

via an enhancement in the electrophilicity of the carbonyl groups of the substrates. A wide range of aromatic aldehydes (**7.2.116**) are then reacted with dimedone (**7.2.101**) and *ortho*-phenylenediamine (**7.2.6**) in the presence of ZnS nanoparticles under solvent-free conditions to afford the corresponding 3,3–dimethyl–2,3,4,5,10,11–hexahydro–11-[aryl]–1*H*–dibenzo[*b,e*][1,4] diazepine–1–ones (**7.2.117**) in high yields (Fig. 7.20B). The 1,5-benzodiazepine products are solvent-free purified *via* two steps comprising crystallization from water and methanol (5:6) followed by recrystallization in ethanol.

Cerium(III) chloride heptahydrate (CeCl$_3$·7H$_2$O) is one of the most common, inexpensive, and green Lewis acids widely deployed in organic transformations (Bartoli, Marcantoni, Marcolini, & Sambri, 2010). The Wang group has reported a sustainable synthesis of a library of 2,3-substituted 1,5-benzodiazepines catalyzed by CeCl$_3$·7H$_2$O (10 mol%) as Lewis acid at room temperature (Yin & Wang, 2016). Several 2-aryl–3-acetyl–2,4-dihydro–1*H*–5*H*–1,5-benzodiazepine derivatives (**7.2.120**) are synthesized *via* the one-pot three–component reaction of *ortho*–phenylenediamine (**7.2.6**), 3-butyn–2-one (**7.2.118**), and aromatic aldehyde (**7.2.119**) in good to high yields (Fig. 7.21A). By using the asymmetric *ortho*–phenylenediamine (**7.2.121**), the chemo-/regio–selectivity of the reaction decreases significantly, leading to the formation of two inseparable isomers (**7.2.122**) and (**7.2.123**) (Fig. 7.21B). As a result, a stepwise protocol for the synthesis of 1,5–benzodiazepines is performed to increase the chemical selectivity, where the intermediate **7.2.124** is initially prepared by the reaction of *ortho*–phenylenediamine (**7.2.121**) and 3-butyn–2-one (**7.2.118**) (Fig. 7.21C).
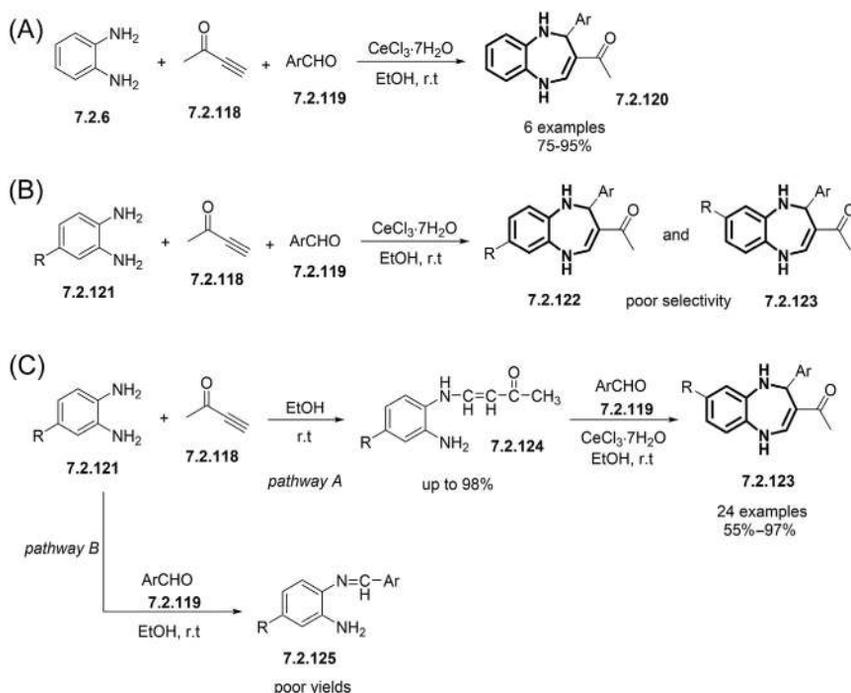
Figure 7.21 (A) One-pot synthesis of 2,3-substituted 1,5-benzodiazepines catalyzed by $CeCl_3 \cdot 7H_2O$ (10 mol%). (B) One-pot formation of two 2,3-substituted 1,5-benzodiazepine isomers using asymmetric ortho-phenylenediamines. (C) Stepwise synthesis of 2,3-substituted 1,5-benzodiazepines catalyzed by $CeCl_3 \cdot 7H_2O$.

Then, the $CeCl_3$–catalyzed condensation reaction of **7.2.124** with various aromatic aldehydes (**7.2.119**) at room temperature provides the desired 1,5–benzodiazepines (**7.2.123**) in good to high yields. Pathway B does not efficiently deliver the corresponding imines (**7.2.125**), probably due to steric reasons and the lower reactivity of aromatic aldehydes compared to the 3-butyn–2–one. Inexpensive substrates, mild reaction conditions, high yields, shorter reaction time (3-7 h), and eco–friendliness are the main advantages of this methodology.

N–Bromosuccinimide (NBS) is an inexpensive nontoxic reagent that has been used as a versatile organocatalyst in organic synthesis. Compared to transition metal catalysts, NBS is especially attractive for the synthesis of pharmaceutically relevant compounds constrained by metal contamination limits, thus avoiding the use of transition metals. The Yao group has developed a simple and mild synthetic protocol for a library of 1,5–benzodiazepine derivatives (**7.2.128**) using NBS as a catalyst (Kuo, More, & Yao, 2006).
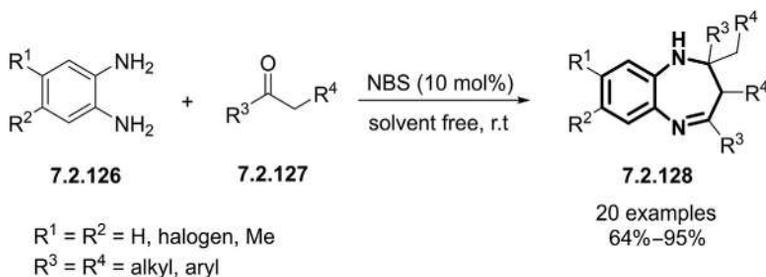
Figure 7.22 Synthesis of 1,5-benzodiazepine derivatives in the presence of 10 mol% NBS at room temperature.

The condensation reactions of various *ortho*-phenylenediamines (**7.2.126**) with a large number of ketones (**7.2.127**) in presence of 10 mol% NBS provide the corresponding 1,5-benzodiazepines under solvent-free conditions (Fig. 7.22). NBS plays a key catalytic role in the reaction mechanism through the activation of the amine group of *ortho*-phenylenediamine (**7.2.126**) in order to initiate the attack on the carbonyl group of the ketone. Upon using asymmetric *ortho*-phenylenediamines, a mixture of two inseparable regioisomers of 1,5-benzodiazepines is observed with low to good selectivity (1:1 to 1:2.3).

HY zeolite is a nontoxic microporous solid material that has been extensively used as an inexpensive powerful acidic catalyst in organic transformations, due to its high surface area, high stability, and well-defined pore size distributions (Lutz, 2014). Direct access to a series of densely functionalized 1,5-benzodiazepines catalyzed by HY zeolite has been reported under solvent-free reaction conditions (Jeganathan & Pitchumani, 2014). HY zeolite can be easily formed by the thermal deammonification of commercially available NH$_4$Y zeolite at 450 °C for 6 h. The reaction between various substituted *ortho*-phenylenediamines (**7.2.129**) and ketones (**7.2.130**) gives the desired 1,5-benzodiazepines (**7.2.131**) in good to high yields (Fig. 7.23). Notably, there is a connection between the size of the ketones and the reaction time, as the bulkier substrates require a longer reaction time. The catalyst can be recovered by simple filtration and be reused up to 6 experimental cycles with only a slight reduction (7%) in the catalytic activity.

A water-mediated mild methodology has been demonstrated to construct a vast array of 2,4–disubstituted 1,5-benzodiazepine derivatives (**7.2.134**) (Tamuli & Bordoloi, 2020). Several *ortho*-phenylenediamines (**7.2.132**) are reacted with a large number of aromatic and aliphatic ketones (**7.2.133**) in presence of biodegradable itaconic acid (20 mol%) as an acidic
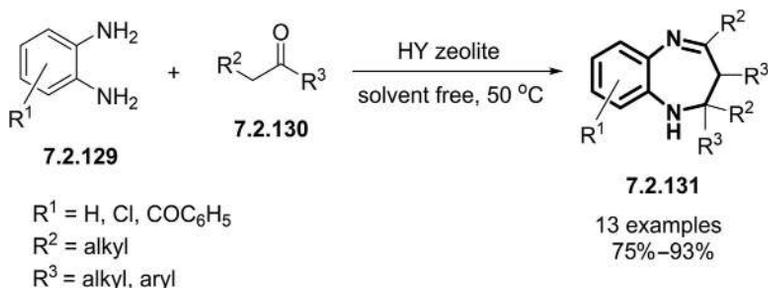
Figure 7.23 Synthesis of densely functionalized 1,5-benzodiazepines catalyzed by HY zeolite under solvent-free conditions.
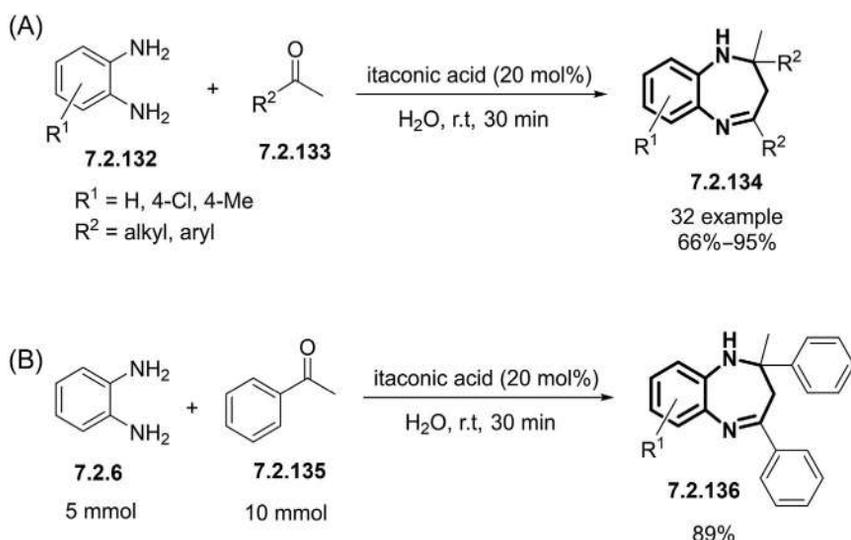

Figure 7.24 Synthesis of 2,4-disubstituted 1,5-benzodiazepine derivatives in the presence of itaconic acid (20 mol%) at room temperature.

promoter at room temperature (Fig. 7.24A). Carboxylic acid groups of ita‑ conic acid serve as mild activators to interact with the carbonyl groups of the ketones. This strategy displays excellent functional group tolerance affording the corresponding products in good to excellent yields. A gram scale model reaction is likewise conducted using *ortho*–phenylenediamine (**7.2.6**) and acetophenone (**7.2.135**) under the optimized reaction conditions, where the 1,5–benzodiazepine (**7.2.136**) is obtained in a high yield of 89% after a simple filtration and without purification by the column chromatography (Fig. 7.24B). Since the itaconic acid is miscible in water, the catalyst can be easily recovered and reused five times without significant loss of catalytic activity.

Fe$_3$O$_4$ nanoparticles are promising catalytic candidates widely employed as a Lewis acid, facilitating the multicomponent reaction through activating the carbonyl groups of the substrates (Sharma et al., 2016; Zamani & Izadi, 2013). An efficient strategy for the preparation of a number of benzo[*b*][1,5]diazepines (**7.2.140**) catalyzed by Fe$_3$O$_4$ nanoparticles (10 mol%) has been developed (Ghasemzadeh & Ghasemi-Seresht, 2015). Fe$_3$O$_4$ nanoparticles are easily prepared from FeSO$_4$·7H$_2$O and poly(ethylene glycol) in an aqueous ammonia solution (Fig. 7.25A). One-pot three-component reaction of *ortho*-phenylenediamines (**7.2.137**), isocyanides (**7.2.138**), and Meldrum's acid (**7.2.139**) provides the corresponding tetrahydro-2,4-dioxo-1*H*-benzo[*b*][1,5]diazepine-3-yl-2-methylpropanamides (**7.2.140**) in excellent yields under mild reaction conditions (Fig. 7.25B). This protocol offers several advantages including easily recoverable magnetic catalyst, inexpensive starting materials, excellent yields, and mild reaction conditions.

A number of 3,4-disubstituted-1,5-benzodiazepine analogs have been synthesized *via* ultrasonic-assisted [4 + 2 + 1] cycloaddition reactions of *ortho*-phenylenediamines (**7.2.41**) and ethyl propiolates (**7.2.42**) in presence of 10 mol% Ga(OTf)$_3$ catalyst under solvent-free conditions (Fig. 7.26A) (Jiang, Cai, Zou, & Zhang, 2010). The reaction of asymmetric *ortho*-phenylenediamines results in the formation of the corresponding 1,5-benzodiazepine as a mixture of two inseparable regioisomers (∼1:1 ratio). Mechanical interaction of Ga(OTf)$_3$ and ethyl propiolates (**7.2.42**) generates the complex (**7.2.44**). Michael addition of the amino groups of **7.2.41** to the Ga-propiolate complexes forms intermediate **7.2.45** followed by the cyclization reaction to finally deliver the product (Fig. 7.26B). A major merit of this methodology is the construction of highly functionalized benzodiazepine structures with two free NH groups, two carbonyl groups, and one C=C bond, which can be further elaborated to generate complex molecules.

7.3 Synthesis of 1,5-benzothiazepines

Since the approval of the first molecule of the 1,5-benzothiazepine family, diltiazem, as a cardiovascular drug in 1982, many research endeavors have been devoted to the synthesis of novel 1,5-benzothiazepine derivatives in both, the industry and academia (El-Bayouki, 2013). In this section, recent progress on the development of facile and practical procedures for
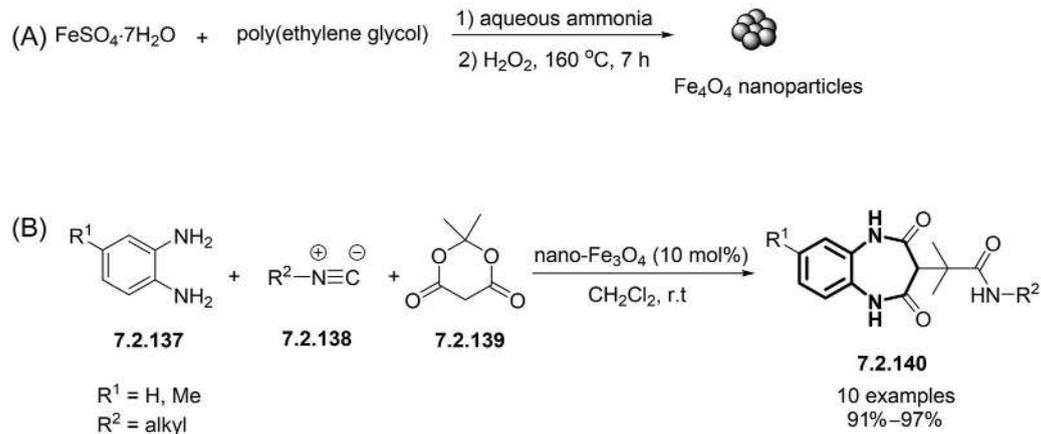
Figure 7.25 (A) Preparation of Fe$_3$O$_4$ nanoparticles. (B) Synthesis of benzo[b][1,5]diazepine derivatives in the presence of 10 mol% Fe$_3$O$_4$ nanoparticles at room temperature.
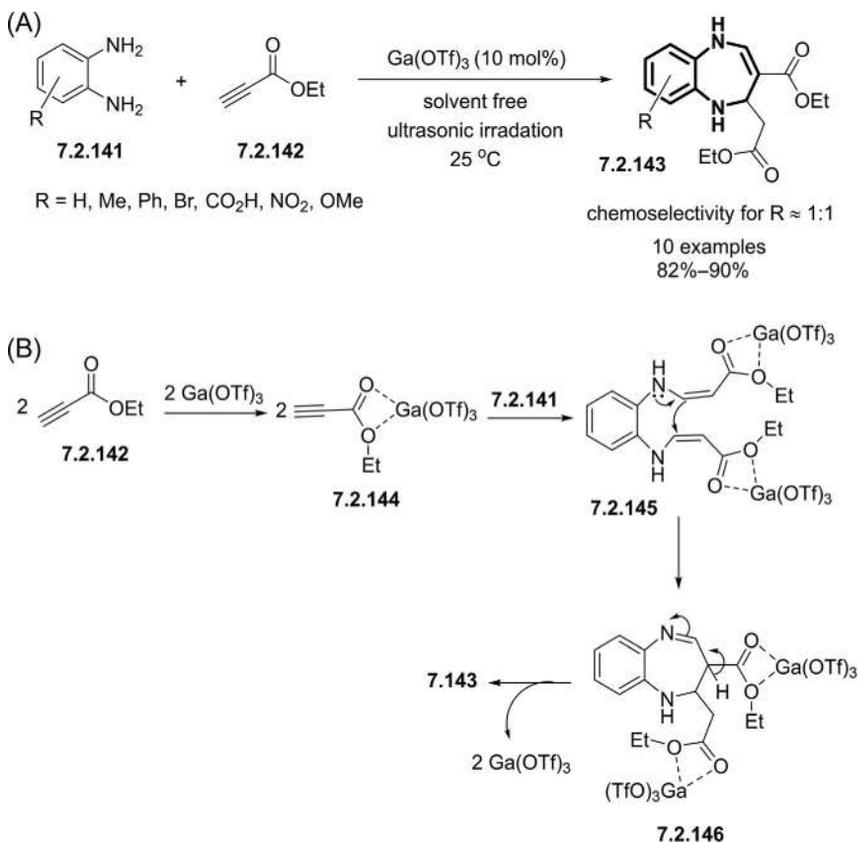
Figure 7.26 (A) Synthesis of novel 3,4-disubstituted-1,5-benzodiazepines catalyzed by Ga(OTf)$_3$. (B) Proposed mechanism for the formation of 1,5-benzodiazepines.

the assembly of 1,5–benzothiazepines *via* catalytic approaches including the enantioselective synthesis, are discussed.

7.3.1 Synthetic methods for 1,5-benzothiazepines

A straightforward strategy for the one–pot synthesis of two classes of 2,3–dihydro– and 2,5–dihydro–1,5–benzothiazepines (**7.3.5, 7.3.6**) has been described *via* a condensation reaction of 3-acetyl coumarins (**7.3.1**), benzaldehydes (**7.3.2**), and 2-aminothiophenol (**7.3.4**) in piperidine under solvent–free reaction conditions (Fig. 7.27) (Rao & Reddy, 2006). Upon using a weak acid in ethanol (*i.e.*, EtOH/CH$_3$COOH), the 2-aryl-4-(2*H*-2-oxo-[1]-benzopyran-3-yl]-2,3-dihydro-1,5-benzothiazepines (**7.3.5**) are
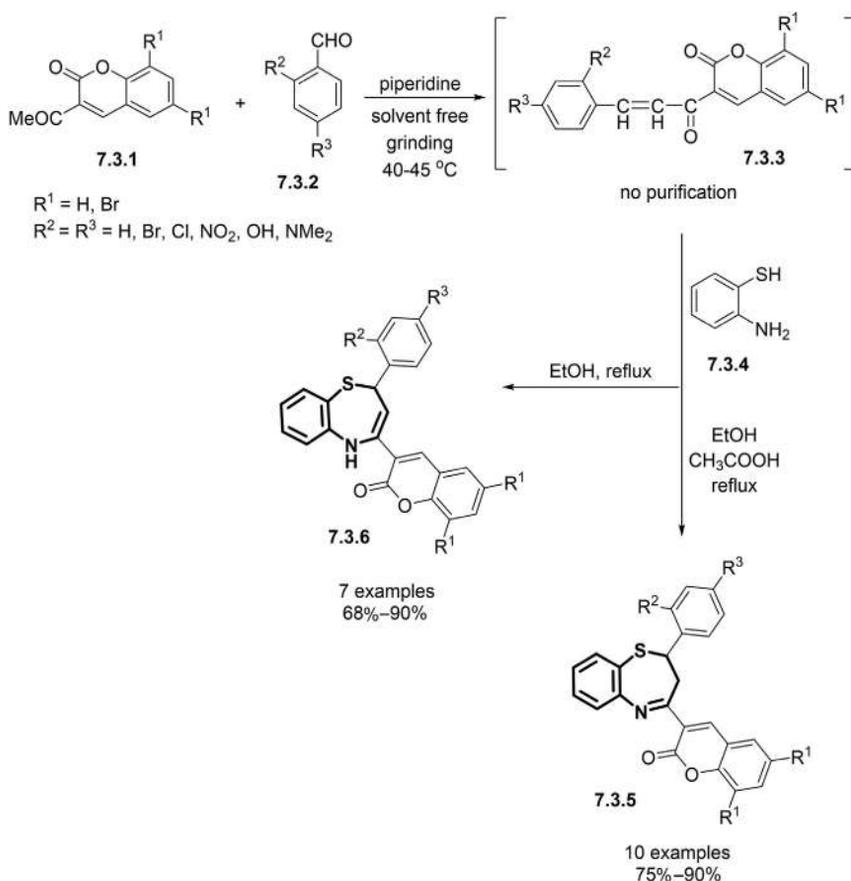
Figure 7.27 One-pot synthesis of 2,3-dihydro- and 2,5-dihydro-1,5-benzothiazepine derivatives in piperidine under solvent-free conditions.

obtained in good to high yields. However, refluxing the intermediate **7.3.3** and 2-aminothiophenol (**7.3.4**) in ethanol/piperidine provides the corresponding 2-aryl-4-[2*H*-2-oxo-[1]-benzopyran-2-one-3-yl]-2,5-dihydro-1,5-benzothiazepine analogs (**7.3.6**) with the high yields of 68%–90%.

It is well known that the presence of an acetyl group at the C3 position of 1,5-benzothiazepines considerably improves their antimicrobial and antifungal activities (Bariwal et al., 2008; Kaur, Singh, & Singh, 2016). Two classes of new highly functionalized 3-acetyl-1,5-benzothiazepines analogs are efficiently synthesized using readily available starting materials (L. Wang et al., 2009). 2,5-Dihydro-4-methyl-2-aryl-3-acetyl-1,5-benzothiazepines (**7.3.12**) are acquired *via* three simple steps starting from a Knoevenagel

condensation reaction of various substituted benzaldehydes (**7.3.7**) with 2,4–pentandione (**7.3.8**) catalyzed by piperidine (Fig. 7.28A). The ensuing α,β-unsaturated compound (**7.3.9**) then undergoes a Michael addition reaction with 2-aminobenzenethiols (**7.3.10**) to form the corresponding pentandione derivatives (**7.3.11**) at room temperature. An intramolecular cyclization reaction of **7.3.11** followed by dehydration in acidic conditions finally affords the desired 1,5-benzothiazepines (**7.3.12**) in good overall yields, purified by recrystallization in methanol. 2,5–Dihydro–2–aryl–3–ethoxycarbonyl–4–methyl–1,5–benzothiazepines (**7.3.16**) can be prepared through the same synthetic procedure as (**7.3.12**), except by replacing 2,4–pentandione (**7.3.8**) with ethyl acetoacetate (**7.3.13**) (Fig. 7.28B). The products are purified by recrystallization in methanol in the overall yields of 20%–33%.

The introduction of a heterocyclic ring fused to the thiazepine ring in 1,5-benzothiazepine can significantly improve their pharmacological activity (Ambrogi et al., 1995). Considering superior biological activities of quinoline and azole derivatives as drugs, Liu's group has developed a facile synthetic protocol for two new types of 1,5-benzothiazepines bearing 1,2,4-triazole, or 1,2,4-oxadiazoline units (Dong, Liu, Xu, & Yuan, 2011). Primarily, the reaction of 2-chloro-3-quinolinecarbaldehyde (**7.3.17**) with phenol (**7.3.18**) at 90 °C provides the compound (**7.3.19**) (Fig. 7.29A). This is followed by the Claisen–Schmidt condensation reaction between **7.3.19** and aromatic ketones (**7.3.20**), yielding α,β-unsaturated ketones (**7.3.21**). 1,5-Benzothiazepines (**7.3.22**) are then synthesized *via* the condensation reaction of **7.3.21** with 2-aminothiophenol (**7.3.4**) in acidic ethanol under reflux conditions. The desired 1,5–benzothiazepine analogs (**7.3.24**) and (**7.3.26**) are finally obtained *via* 1,3-dipolar cycloaddition reactions of **7.3.22** with benzohydroximinoyl chlorides (**7.3.23**) and hydrazonoyl chlorides (**7.3.25**), respectively, at room temperature. A possible mechanism for the intramolecular 1,3-dipolar cycloaddition is illustrated in Fig. 7.29B. The preferable orientation of the thiazepine ring would be a boat-like conformation, where a cyclic transition state (**7.3.27**) is formed *via* the interaction of the *in situ* generated nitrile oxide and C=N bond in the thiazepine ring followed by C–N and C–O bonds formation, resulting in 1,2,4-oxadiazole ring.

Fluorinated solvents are attractive reaction mediums deployed in organic transformations because of their unique properties. These solvents provide neutral and mild conditions for those reactions that are usually performed at elevated temperatures catalyzed by strong Brønsted or Lewis acids. For
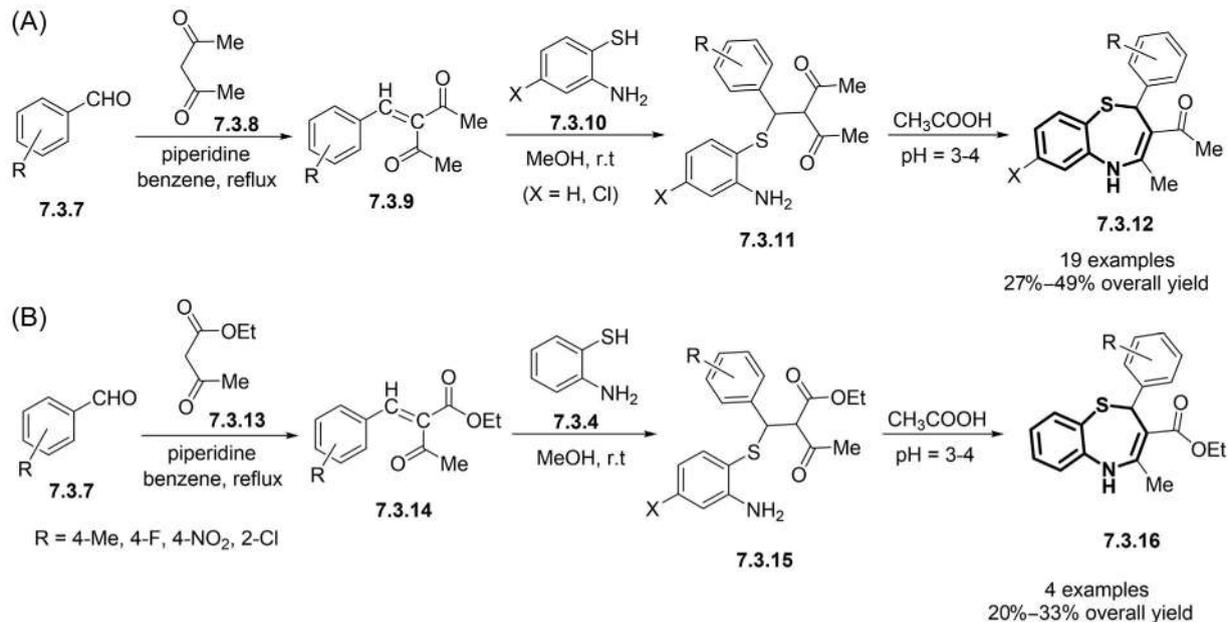
Figure 7.28 Synthesis of two classes of highly functionalized 1,5-benzothiazepine derivatives.
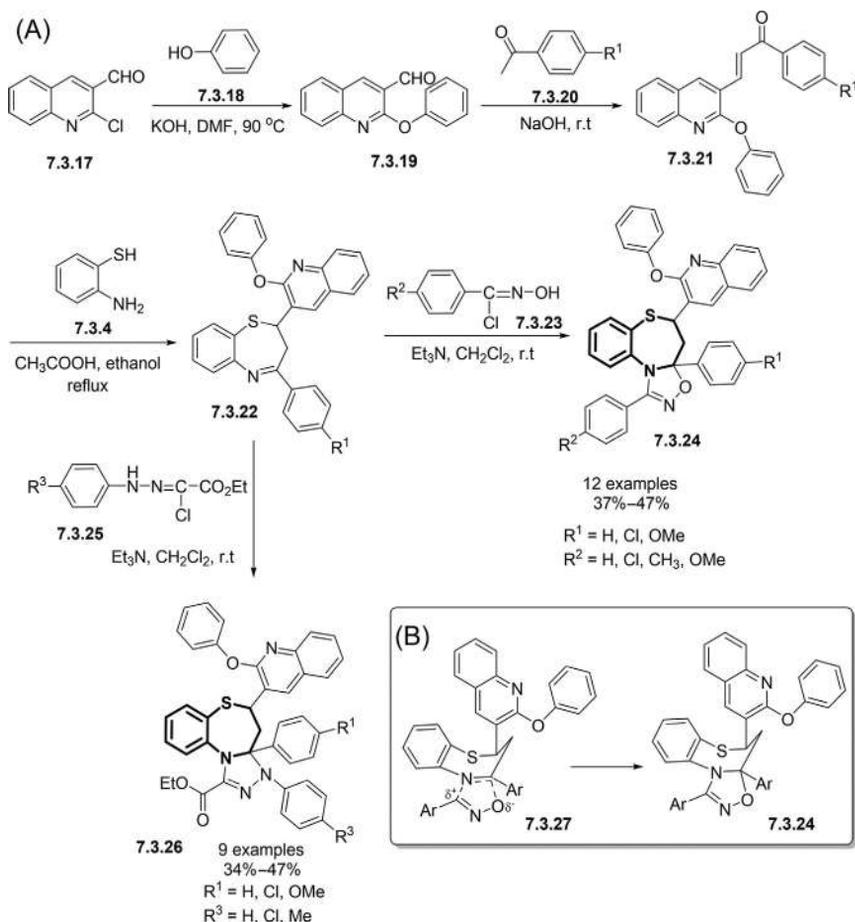
Figure 7.29 (A) Synthesis of new types of 1,5-benzothiazepines bearing 1,2,4-triazole or 1,2,4-oxadiazoline units. (B) Proposed intramolecular 1,3-diploar cycloaddition mechanism of the 1,2,4-oxadiazole ring.

example, hexafluoro–2-propanol (HFIP) offers an efficient medium displaying a weak acidic feature through the strong H-bond donor ability, low nucleophilicity, and strong ionization power (Vuluga et al., 2011). Gaggero's group has demonstrated the use of HFIP as an acidic medium for the synthesizing a number of 2,3-dihydro-1,5-benzothiazepine derivatives under mild reaction conditions (Albanese, Gaggero, & Fei, 2017). A Michael addition reaction of 2-aminothiophenol (**7.3.4**) to *α*,*β*–unsaturated ketones (**7.3.28**) in HFIP gives the intermediate (**7.3.33**), which subsequently undergoes *in situ* cyclization to yield 1,5–benzothiazepine products (**7.3.29**) (Fig. 7.30). In this study, HFIP facilitates the first step of the domino sequence *via* a dual
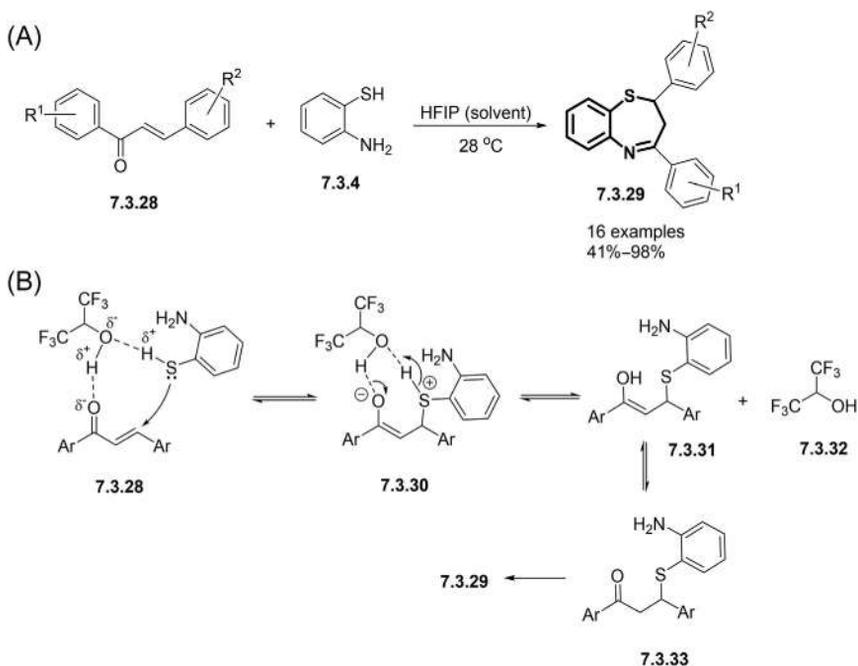
Figure 7.30 (A) Synthesis of 2,3-dihydro-1,5-benzothiazepines in hexafluoro-2-propanol (HFIP) as an acidic medium under mild reaction conditions. (B) HFIP's role in the first step of the domino reaction.

activation of both ketones **7.3.28** and 2–aminothiophenol (**7.3.4**), which can be easily recycled through distillation from the reaction mixture, making this protocol attractive for the large-scale synthesis 1,5-benzothiazepines.

A simple synthesis of a library of pyridine-containing 1,5–benzothiazepine analogs (**7.3.39**) has been documented (Li et al., 2017), in which the 1,3-dipolar cycloaddition reaction of various chalcones (**7.3.36**) and 2-aminothiophenol (**7.3.4**) followed by functionalization with 2-chloro–5-chloromethyl-pyridine (**7.3.38**) provide the desired products in moderate yields (Fig. 7.31). The α,β-unsaturated ketones (**7.3.36**) are initially prepared *via* the condensation reaction of hydroxyacetophenone (**7.3.34**) with several aromatic aldehydes (**7.3.35**), which then undergo a condensation reaction with 2-aminothiophenol (**7.3.4**) to deliver the intermediate **7.3.37** under reflux conditions. Finally, a mixture of **7.3.37** and 2-chloro–5-chloromethyl-pyridine (**7.3.38**) is refluxed in presence of K_2CO_3/KI as catalysts to yield the pyridine–containing 1,5-benzothiazepines (**7.3.39**).
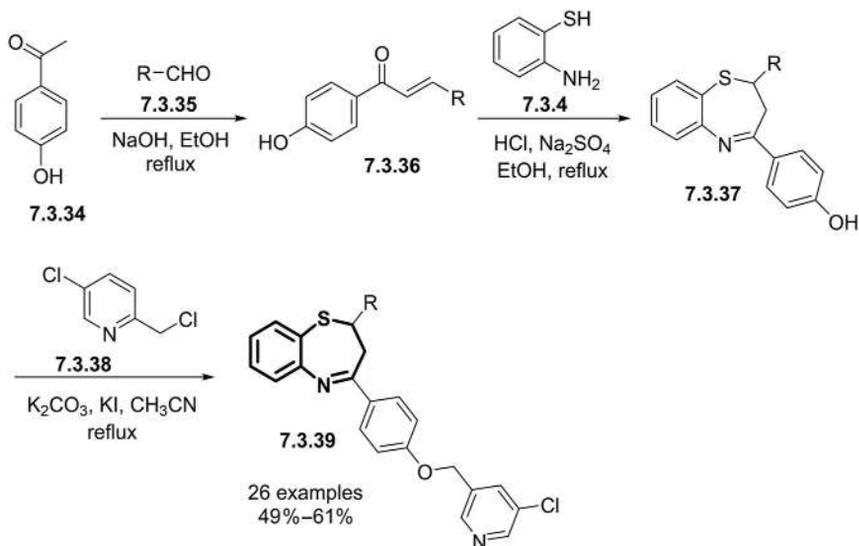
Figure 7.31 Three-step synthesis of pyridine-containing 1,5-benzothiazepines.

Shimotori *et al.* reported an efficient synthesis of several dibenzothiazepines bearing lactam, amidine, and imine units starting from commercially available 2–aminophenyl disulfides (**7.3.40**) (Shimotori et al., 2018). Primarily, a one-pot *S*-arylation reaction occurs *via* an S—S bond cleavage of the disulfides using L-cysteine in aqueous ammonia solution followed by an S_NAr reaction using various disubstituted nitrobenzenes (**7.3.41, 7.3.44, 7.3.47**) to quantitatively provide the corresponding diaryl sulfides (**7.3.42, 7.3.45, 7.3.48**) (Fig. 7.32). The presence of a nitro group as a strong electron–withdrawing group is necessary for the S_NAr reaction to reduce the electron density and increase the anion stabilization of the benzene ring. Dibenzothiazepine derivatives containing lactam (**7.3.43**) and amidine (**7.3.46**) units are synthesized *via* intramolecular cyclization of the diaryl sulfides **7.3.42** and **7.3.45** using *p*-TsOH·H$_2$O and HCl, respectively. The spontaneous intramolecular cyclization of the intermediate **7.3.48** generated from one–pot *S*-arylation of 2-bromo-5-nitrobenzaldehyde (**7.3.47**) delivers the dibenzothiazepines **7.3.49**.

7.3.2 Catalytic methods for synthesis of 1,5-benzothiazepines

Lanthanum (La)–exchanged Y-type zeolites can be easily prepared by ion exchange of naturally abundant NaY zeolites. The La^{3+} cations may provide strong Brønsted acid centers by water hydrolysis, nominating them as powerful nontoxic catalysts in acid–catalyzed organic transformations
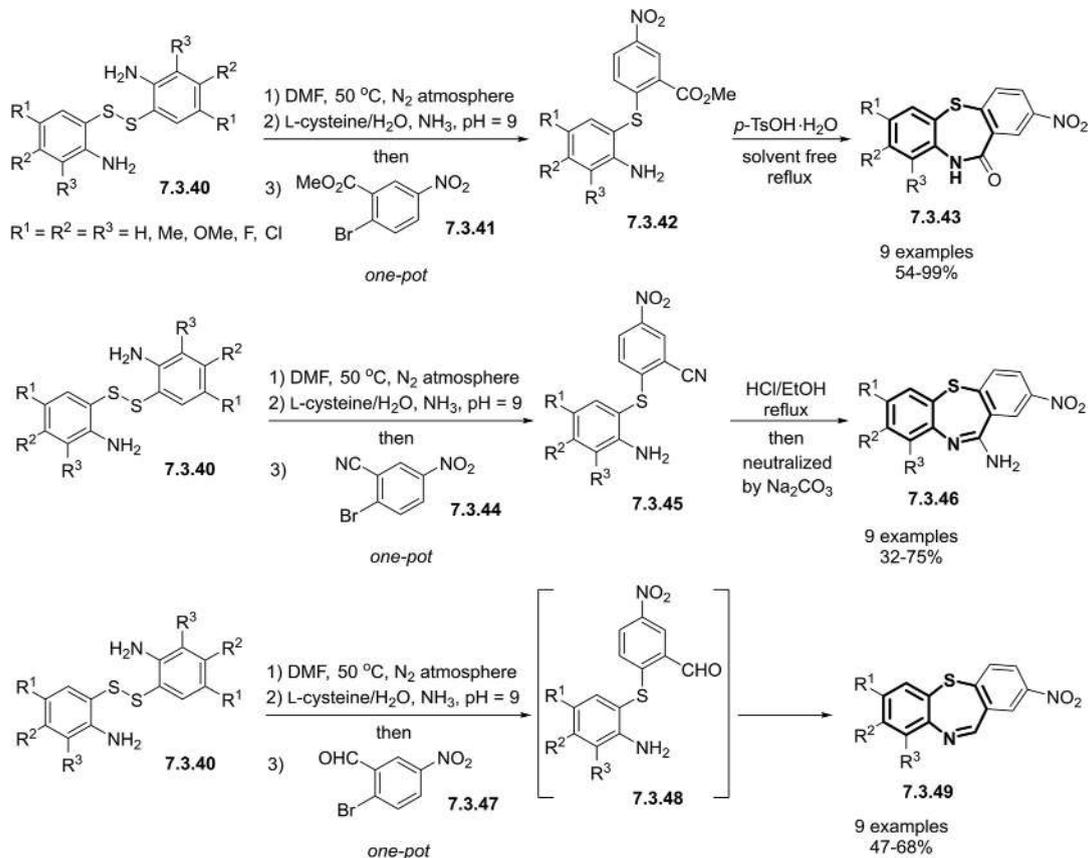
Figure 7.32 Synthesis of dibenzothiazepines bearing lactam, amidine, and imine units started from 2-aminophenyl disulfides.
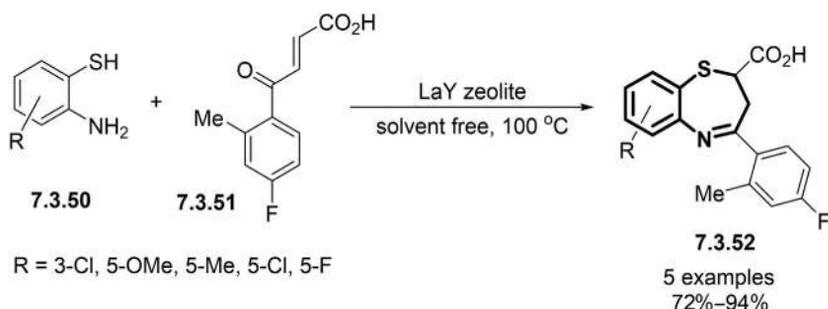
Figure 7.33 Synthesis of 2,3-dihydro-2,3-dihydro-1,5-benzothiazepine derivatives catalyzed by LaY zeolite.

(Sievers, Liebert, Stratmann, Olindo, & Lercher, 2008). A sustainable synthetic approach for a series of 2,3-dihydro-1,5-benzothiazepines (**7.3.52**) catalyzed by LaY zeolite has been disclosed (Arya & Dandia, 2008). The La-exchanged Y type zeolite is prepared by the treatment of zeolite NaY with an aqueous solution of lanthanum chloride at 95 °C for 8 h followed by drying at 120 °C for 4 h. Various 2-aminobenzenethiols (**7.3.50**) can then smoothly undergo the condensation reaction with 3-(4-fluoro-2-methyl benzoyl)-2-propenoic acid (**7.3.51**) in the presence of LaY zeolite under solvent-free conditions to give the corresponding 1,5-benzothiazepine derivatives (**7.3.52**) in high yields (Fig. 7.33).

Catalysis by nanocrystalline metal oxides has emerged as a green and economical method for the production of numerous pharmaceutically relevant compounds, mainly due to their viable preparation method and strong acidity (Fathi et al., 2018; Zamani, Hosseini, & Kianpour, 2013). The Hekmatshoar group reported a green strategy for the preparation of several 2,4-disubstituted 1,5-benzothiazepines (**7.3.54**) in presence of Al_2O_3 nanoparticles as a catalyst (Hekmatshoar, Sadjadi, Shiri, Heravi, & Beheshtiha, 2009). Nanocrystalline aluminum oxide is simply synthesized by a common precipitation method using aqueous ammonia and $Al(NO_3)_3$ (Fig. 7.34A). Refluxing an aqueous mixture of 1,3-diphenylpropenone (**7.3.53**) and 2-aminothiophenol (**7.3.4**) in presence of nano-Al_2O_3 provides the corresponding 1,5-benzothiazepines (**7.3.54**) in good to high yields (Fig. 7.34B). As the benzothiazepines are formed as sole products, it has been suggested that the reaction is initiated by 1,4-Michael addition of the thiol group on the C=C bond followed by the condensation reaction of the amine on the carbonyl group. By comparison of the catalytic performance of the nano-Al_2O_3 with commercial bulk Al_2O_3, the nanocatalyst exhibits superior
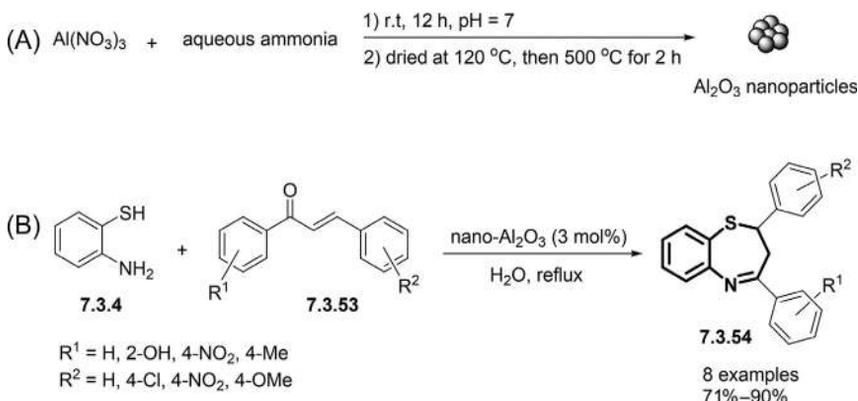
Figure 7.34 Synthesis of 2,4-disubstituted 1,5-benzothiazepines catalyzed by nano-Al$_2$O$_3$ in water.

activity which can be ascribed to its larger surface area and the larger surface concentration of the active sites.

Montmorillonite is a natural layered clay extensively investigated in heterogeneous catalysis due to its unique 2D structure and acidic nature, low cost, and eco–friendliness. Microwave-assisted rapid access to several new fused pyrazolo-[1,5]benzothiazepine derivatives using montmorillonite K10 as a catalyst has been demonstrated (Dandia, Singh, Singh, Laxkar, & Sivpuri, 2010). A mixture of 3-methyl–1-phenyl–2-pyrazolin-5-one (**7.3.55**) and benzaldehydes (**7.3.56**) reacts in presence of montmorillonite K10 under MW irradiation and solvent-free conditions led to *in situ* production of intermediate (**7.3.57**) (Fig. 7.35), which then undergoes the condensation reaction with 2-aminothiophenols (**7.3.58**) to provide the final pyrazolo[4,3-*c*][1,5]benzothiazepine products (**7.3.60**). This methodology offers a simple operation through inexpensive catalyst and starting materials while the synthesis conditions are environmentally benign.

Cerium (IV) ammonium nitrate (CAN) is relatively less toxic, air-stable, easy to handle, and an inexpensive reagent that can be employed as a high reactive Lewis acid and oxidant in organic synthesis. Gill and co–workers reported the synthesis of a series of 2,4–disubstituted 1,5-benzothiazepine analogs in presence of the catalytic amount of CAN as Lewis acid under ultrasonic irradiation (Chate, Joshi, Mandhane, & Gill, 2011). Initially, the Claisen-Schmidt condensation reaction of acetophenones (**7.3.61**) and benzaldehydes (**7.3.62**) using KOH/EtOH at room temperature provides the corresponding chalcones (**7.3.63**) (Fig. 7.36). These α,β-unsaturated carbonyl compounds then react with 2-aminothiophenol (**7.3.4**) in ethanol
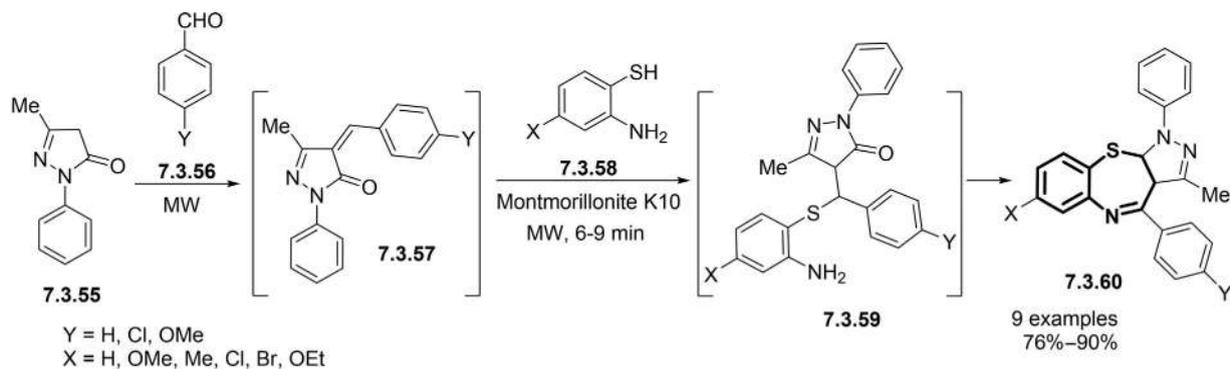
**Figure 7.35** Microwave-assisted rapid synthesis of pyrazolo [4,3-*c*][1,5]benzothiazepines catalyzed by montmorillonite K10.

Farzad Zamani, Esmail Doustkhah, Fatemeh Ahmadi and Rajender S. Varma
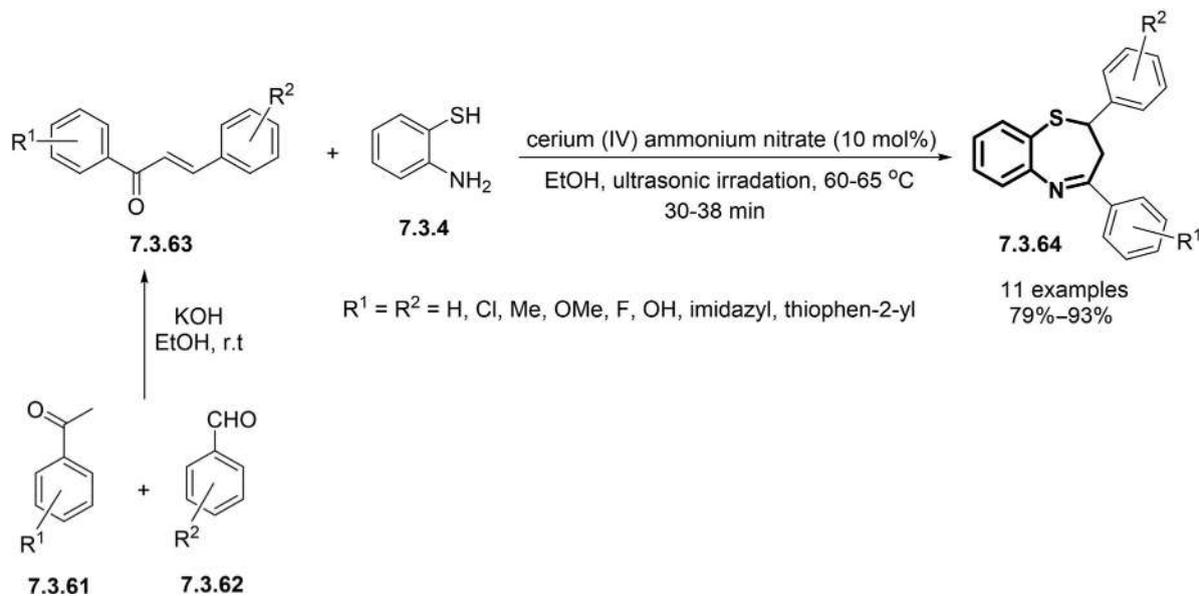
Figure 7.36 Synthesis of 2,4-disubstituted 1,5-benzothiazepines catalyzed by cerium (IV) ammonium nitrate under ultrasonic irradiation.

under ultrasonication at 60–65 °C to afford the final 1,5–benzothiazepine products (**7.3.64**) in good to high yields.

A sustainable synthetic protocol for a number of 2,3–dihydro–1,5–benzothiazepine analogs has been demonstrated (Farghaly & Hassaneen, 2017). The acidic form of zeolite ferrierite (H–FER zeolite) is employed as an inexpensive acidic catalyst in the condensation reaction of several 1,3–diaryl–2–propenones (**7.3.65**) with 2–aminothiophenol (**7.3.4**) under solvent–free conditions, providing the corresponding 1,5–benzothiazepines (**7.3.66**) in high yields (Fig. 7.37). These heterocyclic scaffolds further react with various hydrazonoyl chlorides (**7.3.67**) *via* 1,3–dipolar cycload–dition using a mixture of Na_2CO_3/THAC as a catalyst in water at room temperature to afford several new 1,2,4–triazolo[3,4–*d*][1,5]benzothiazepine derivatives (**7.3.68**). Green and mild reaction conditions, simple operation, cheap substrates and catalysts, and high yields make this protocol attractive for industrial–scale operation.

7.3.3 Enantioselective synthesis of 1,5-benzothiazepines

Despite a wide variety of strategies demonstrated for the racemic synthe–sis of 1,5–benzothiazepines, enantioselective protocols for the synthesis of optically active 1,5–benzothiazepines are receiving ever–increasing attention in procedural studies (Asano & Matsubara, 2018). The enantioselective catalysis is highly attractive for both academic and pharmaceutical purposes due to several advantages such as atom– and step economy, and diversity–oriented synthesis. In 2015, Matsubara and co–workers reported the first example of a highly enantioselective synthesis of various 2–substituted 1,5–benzothiazepines using the chiral isothiourea catalyst (**7.3.72**) (Fukata, Asano, & Matsubara, 2015). The organocatalyst (**7.3.72**) is synthesized in two synthetic steps starting from commercially available (*R*)-(+)-phenylglycinol (**7.3.69**) (Fig. 7.38A). A solution of the amino alcohol **7.3.69** in *i*–Pr₂EtN is treated with 2-chlorobenzothiazole (**7.3.70**) at 135 °C for 24 h. After purifi–cation of the corresponding product (**7.3.71**) by column chromatography, it is reacted with methanesulfonyl chloride in the presence of Et₃N to finally attain the chiral benzotetramisole (**7.3.72**) in a good yield. The [4 + 3] cycloaddition reaction of (*E*)-α,β–unsaturated anhydride (**7.3.73**) and 2-aminothiophenols (**7.3.74**) with 5 mol% of benzotetramisole organocat–alyst (**7.3.72**) delivers the target optically active (*R*)-1,5–benzothiazepines (**7.3.75**) in high yields and enantioselectivities (Fig. 7.38B). Interestingly, mechanistic studies have shown that *Z*–form of (**7.3.73**) also affords the corresponding (*R*)-1,5–benzothiazepines (**7.3.75**), indicating that the *Z*

Y = H, 4-Me, 4-F, 2,3,4-(OMe)$_3$
X = H, F, Br
R^1 = CO$_2$Et, C$_6$H$_5$
R^2 = C$_6$H$_5$, 4-ClC$_6$H$_4$, 4-NO$_2$C$_6$H$_4$
THAC = tetrahexylammonium chloride
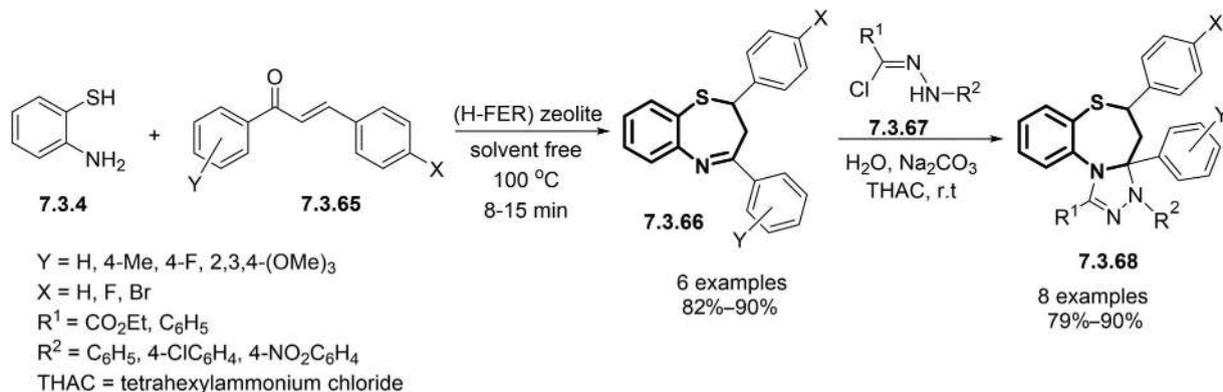
Figure 7.37 Synthesis of 2,3-dihydro-1,5-benzothiazepines and 1,2,4-triazolo[3,4-*d*][1,5]benzothiazepines catalyzed by H-FER zeolite under mild reaction conditions.
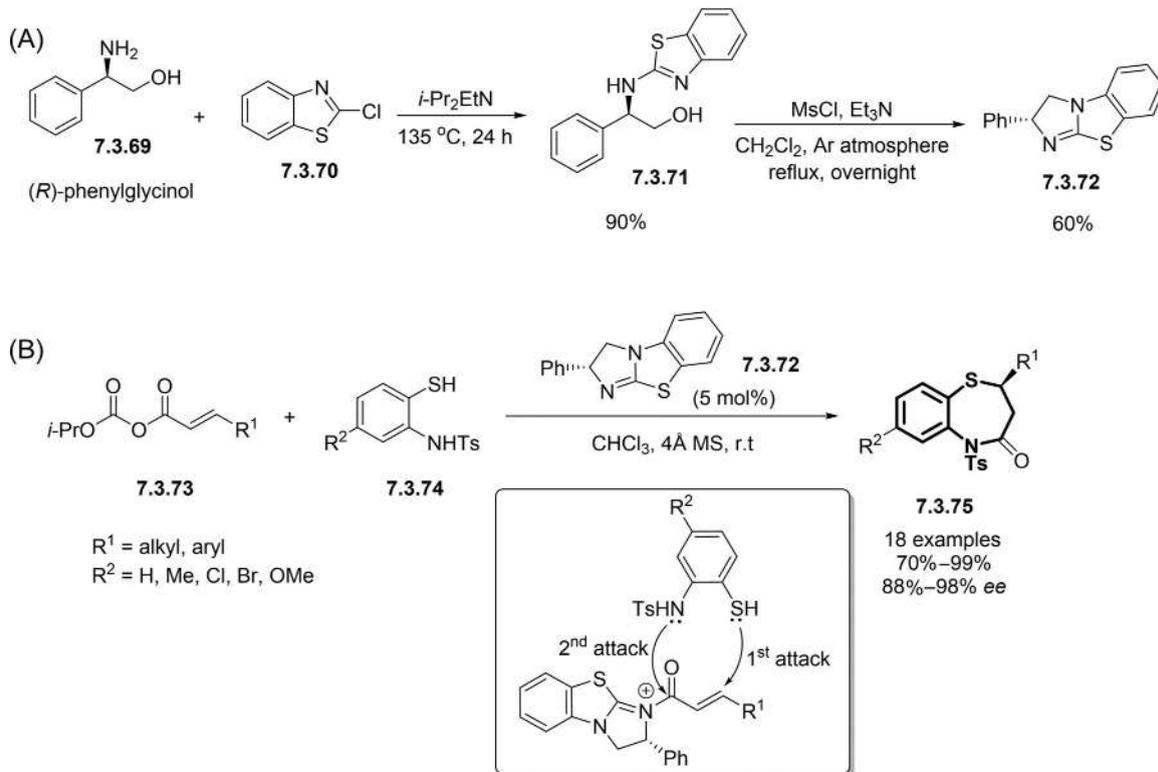
Figure 7.38 (A) Preparation of the chiral benzotetramisole organocatalyst. (B) Synthesis of chiral (*R*)-1,5-benzothiazepines catalyzed by the chiral benzotetramisole at room temperature.
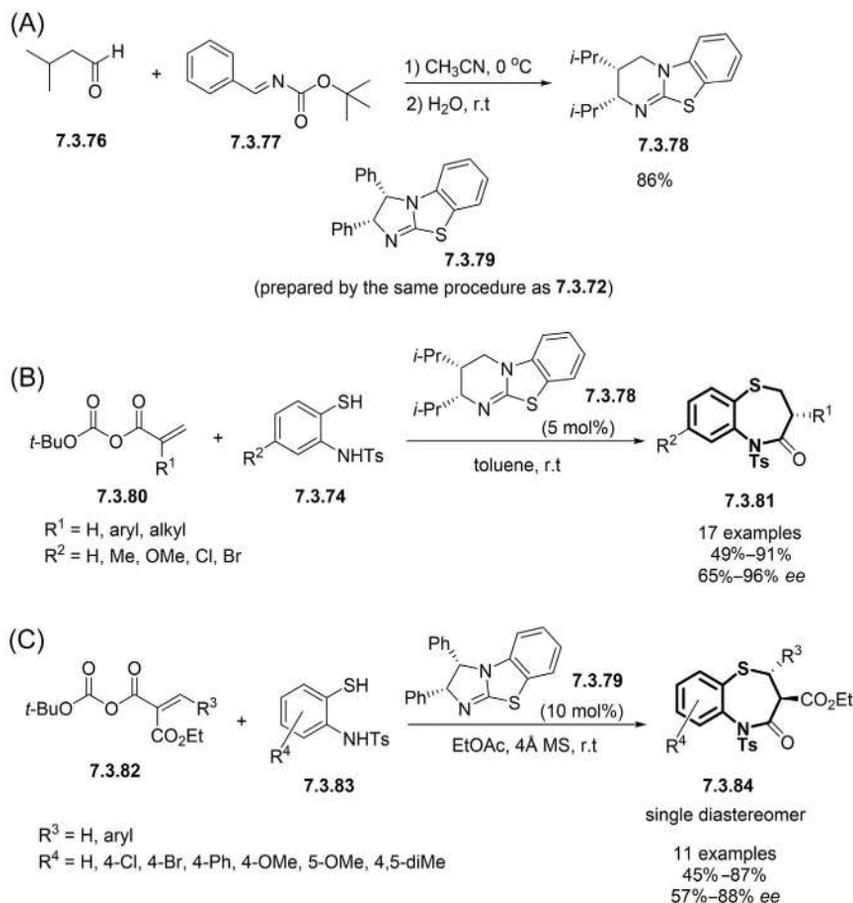
Figure 7.39 (A) Preparation of the chiral isothiourea organocatalyst. (B,C) Syntheses of two classes of chiral 1,5-benzothiazepines catalyzed by the chiral isothiourea organocatalyst at room temperature.

isomer can isomerize to the (E)-form in this protocol that leads to the same product.

In another method developed by the Matsubara group, two classes of chiral 1,5-benzothiazepines have been efficiently prepared *via* an isothiourea-catalyzed enantioselective reaction of several α,β-unsaturated carboxylic acid anhydrides with 2-aminothiophenols (Fukata, Yao, Miyaji, Asano, & Matsubara, 2017). The chiral isothiourea organocatalysts (**7.3.78**) and (**7.3.79**) are prepared from readily available substrates under mild reaction conditions (Fig. 7.39A). The asymmetric cycloaddition reactions of the carboxylic acid anhydride derivatives (**7.3.80** and **7.3.82**) and
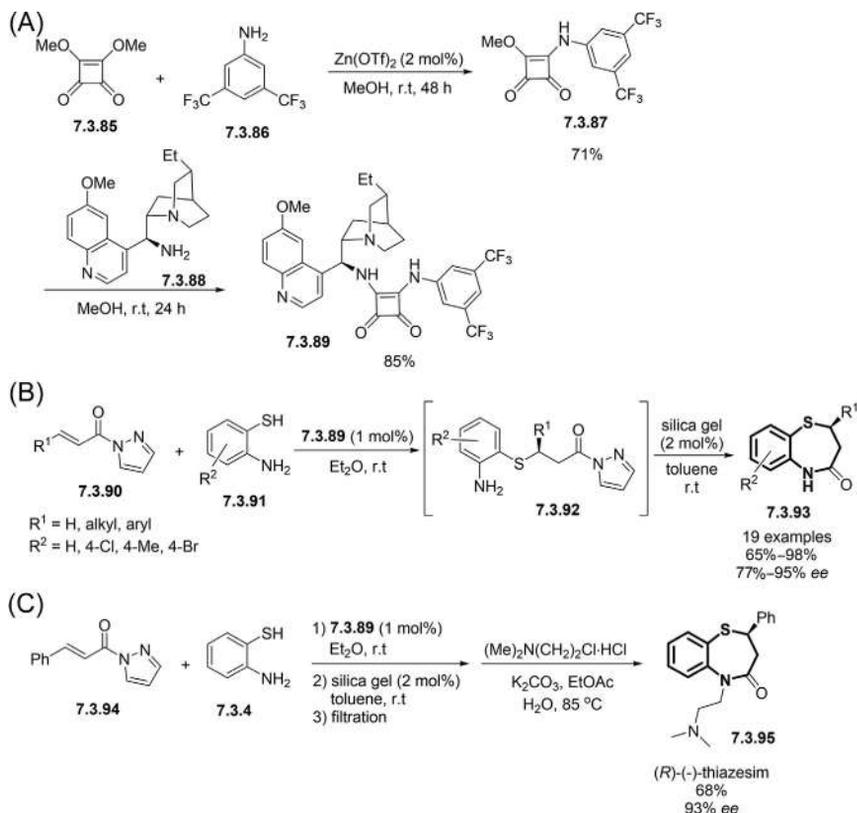
Figure 7.40 Synthesis of a library of 2,3-dihydro-1,5-benzothiazepinones using a hydroquinine-derived squaramide as chiral organocatalyst at room temperature.

2–aminothiophenols (**7.3.74** and **7.3.83**) are conducted in the presence of 5 mol% **7.3.78** and **7.3.79**, respectively, at room temperature, providing the corresponding 1,5-benzothiazepines **7.3.81** and **7.3.84** in high yields and enantioselectivities (Fig. 7.39B and C). A major benefit of this methodology is the delivery of a library of potential pharmaceutically active chiral 1,5-benzothiazepinones for various biological targets in drug–development applications.

Lattanzi's group has developed a one–pot enantioselective synthesis of a library of 2,3-dihydro-1,5-benzothiazepinones using a hydroquinine-derived squaramide as a chiral organocatalyst at room temperature (Meninno, Volpe, & Lattanzi, 2017). The catalyst **7.3.89** can be easily prepared in two steps starting from the commercially available 3,4-dimethoxycyclobut-3-ene-1,2-dione (**7.3.85**) and 3,5-bis(trifluoromethyl) aniline (**7.3.86**) (Fig. 7.40A). Several *trans* α,β–unsaturated *N*–acylpyrazoles

(**7.3.90**) are treated with 2–aminothiophenols (**7.3.91**) in presence of 1 mol% organocatalyst (**7.3.89**) to regioselectively generate the intermediate (**7.3.92**) (Fig. 7.40B), which then undergo a silica gel–catalyzed lactamization reaction, providing the final products (**7.3.93**) in high yields and enantioselectivity. Their synthetic protocol is applied in the synthesis of the antidepressant drug (*R*)–(–)–thiazesim (**7.3.95**) (Fig. 7.40C). After conducting the model reaction of **7.3.94** with **7.3.4**, the crude mixture is directly subjected to the alkylation process to afford the drug **7.3.95** in an overall yield of 68% with excellent enantioselectivity (93% *ee*).

7.4 Future directions

In view of their pharmaceutical importance, the future of 1,5-benzodiazepine chemistry will remain under focus sake of developing more efficient and sustainable synthetic protocols to construct new scaffolds for pharmaceutical purposes and drug discovery.

References

Abdallah, W., Daami-Remadi, M., Znati, M., Jannet, H. B., & Gharbi, R. (2017). Design and synthesis of (3,5-disubstituted isoxazole)-linked [1,5]-benzodiazepine conjugates: Evaluation of their antimicrobial and anti-tyrosinase activities. *Journal of Chemical Research, 41*(1), 12–17. https://doi.org/10.3184/174751917×14815427219121.

Albanese, D. C. M., Gaggero, N., & Fei, M. (2017). A practical synthesis of 2,3-dihydro-1,5-benzothiazepines. *Green Chemistry, 19*(23), 5703–5707. https://doi.org/10.1039/c7gc02097j.

Alizadeh, A., & Bagherinejad, A. (2020). Synthesis of tetracyclic pyrido-fused dibenzodiazepines *via* a catalyst-free cascade reaction. *Molecular Diversity*. https://doi.org/10.1007/s11030-020-10114-1.

Ambrogi, V., Grandolini, G., Perioli, L., Giusti, L., Lucacchini, A., & Martini, C. (1995). Studies on annulated 1,4-benzothiazines and 1,5-benzothiazepines. IX. Imidazo [2,1-*d*][1,5] benzothiazepines: Synthesis and *in vitro* benzodiazepine receptor affinity. *European Journal of Medicinal Chemistry, 30*(5), 429–437. https://doi.org/10.1016/0223-5234(96)88253-5.

Arya, K., & Dandia, A. (2008). The expedient synthesis of 1,5-benzothiazepines as a family of cytotoxic drugs. *Bioorganic and Medicinal Chemistry Letters, 18*(1), 114–119. https://doi.org/10.1016/j.bmcl.2007.11.002.

Asano, K., & Matsubara, S. (2018). Catalytic approaches to optically active 1,5-benzothiazepines. *ACS Catalysis, 8*(7), 6273–6282. https://doi.org/10.1021/acscatal.8b00908.

Bariwal, J. B., Upadhyay, K. D., Manvar, A. T., Trivedi, J. C., Singh, J. S., Jain, K. S., et al. (2008). 1,5-Benzothiazepine, a versatile pharmacophore: A review. *European Journal of Medicinal Chemistry, 43*(11), 2279–2290. https://doi.org/10.1016/j.ejmech.2008.05.035.

Bartoli, G., Marcantoni, E., Marcolini, M., & Sambri, L. (2010). Applications of $CeCl_3$ as an environmental friendly promoter in organic chemistry. *Chemical Reviews, 110*(10), 6104–6143. https://doi.org/10.1021/cr100084g.

Chander, S., Tang, C. R., Al-Maqtari, H. M., Jamalis, J., Penta, A., Hadda, T. B., et al. (2017). Synthesis and study of anti-HIV-1 RT activity of 5-benzoyl-4-methyl-1,3,4,5-tetrahydro-2*H*-1,5-benzodiazepin-2-one derivatives. *Bioorganic Chemistry, 72*, 74–79. https://doi.org/10.1016/j.bioorg.2017.03.013.

Chate, A. V., Joshi, R. S., Mandhane, P. G., & Gill, C. H. (2011). An improved procedure for the synthesis of 1,5-benzothiazepines using ceric ammonium nitrate (CAN). *Journal of the Korean Chemical Society, 55*(5), 776–780. https://doi.org/10.5012/jkcs.2011.55.5.776.

Chimirri, A., Gitto, R., Grasso, S., Monforte, A. M., Romeo, G., & Zappalà, M. (1993). Annelated 1,5-benzodiazepines. Part II. Six membered rings. *Heterocycles, 36*(4), 865–890. https://doi.org/10.3987/REV-92-442.

Dandia, A., Singh, R., Singh, D., Laxkar, A., & Sivpuri, A. (2010). Regioselective synthesis of diltiazem analogue pyrazolo[4,3-*c*][1,5] benzothiazepines and antifungal activity. *Phosphorus, Sulfur and Silicon and the Related Elements, 185*(12), 2472–2479. https://doi.org/10.1080/10426501003713064.

Dong, Z. Q., Liu, F. M., Xu, F., & Yuan, Z. L. (2011). Synthesis of 1,5-benzothiazepine derivatives bearing 2-phenoxy-quinoline moiety *via* 1,3-diplolar cycloaddition reaction. *Molecular Diversity, 15*(4), 963–970. https://doi.org/10.1007/s11030-011-9328-z.

Doustkhah, E., Heidarizadeh, M., Rostamnia, S., Hassankhani, A., Kazemi, B., & Liu, X. (2018). Copper immobilization on carboxylic acid-rich Fe_3O_4–Pectin: Cu^{2+}@Fe_3O_4–Pectin a superparamagnetic nanobiopolymer source for click reaction. *Materials Letters, 216*, 139–143. https://doi.org/10.1016/j.matlet.2018.01.014.

Doustkhah, F., Mohtasham, H., Habeeb, J., & Rostamnia, S. (2019). Exfoliated graphene-based 2D materials: Synthesis and catalytic behaviors. *Handbook of graphene set: Growth, synthesis and functionalization* (pp. 529–558). Hoboken, U.S.: Wiley.

El-Bayouki, K. A. M. (2013). Benzo[1,5]thiazepine: Synthesis, reactions, spectroscopy, and applications. *Organic Chemistry International*. https://doi.org/10.1155/2013/210474.

Fang, X., Zhai, T., Gautam, U. K., Li, L., Wu, L., Bando, Y., et al. (2011). ZnS nanostructures: From synthesis to applications. *Progress in Materials Science, 56*(2), 175–287. https://doi.org/10.1016/j.pmatsci.2010.10.001.

Farghaly, T. A., & Hassaneen, H. M. E. (2017). H-Ferrierite zeolite as an effective and reusable heterogeneous catalyst for synthesis of 1,5-benzothiazepine under solvent free condition and 1,3-dipolar cycloaddition in water. *Arabian Journal of Chemistry, 10*, S3255–S3262. https://doi.org/10.1016/j.arabjc.2013.12.024.

Fathi, Z., Doustkhah, E., Rostamnia, S., Darvishi, F., Ghodsi, A., & Ide, Y. (2018). Interaction of Yarrowia lipolytica lipase with dithiocarbamate modified magnetic carbon Fe_3O_4@C–NHCS$_2$H core-shell nanoparticles. *International Journal of Biological Macromolecules, 117*, 218–224. https://doi.org/10.1016/j.ijbiomac.2018.05.156.

Fukata, Y., Asano, K., & Matsubara, S. (2015). Facile net cycloaddition approach to optically active 1,5-benzothiazepines. *Journal of the American Chemical Society, 137*(16), 5320–5323. https://doi.org/10.1021/jacs.5b02537.

Fukata, Y., Yao, K., Miyaji, R., Asano, K., & Matsubara, S. (2017). Asymmetric net cycloaddition for access to diverse substituted 1,5-benzothiazepines. *Journal of Organic Chemistry, 82*(23), 12655–12668. https://doi.org/10.1021/acs.joc.7b02451.

Gaponov, A. A., Zlenko, E. T., Shishkina, S. V., Shishkin, O. V., Antypenko, O. M., Tretiakov, S. V., et al. (2016). Synthesis, spectroscopic characterization, X-ray structure, and *in vivo* neurotropic activity of new 1,5-benzodiazepin-2-ones. *Medicinal Chemistry Research, 25*(9), 1768–1780. https://doi.org/10.1007/s00044-016-1605-z.

Ghasemzadeh, M. A., & Ghasemi-Seresht, N. (2015). Facile and efficient synthesis of benzo[*b*][1,5]diazepines by three-component coupling of aromatic diamines, Meldrum's acid, and isocyanides catalyzed by Fe_3O_4 nanoparticles. *Research on Chemical Intermediates, 41*(11), 8625–8636. https://doi.org/10.1007/s11164-014-1915-z.

Ghogare, J. G., Bhandari, S. V., Bothara, K. G., Madgulkar, A. R., Parashar, G. A., Sonawane, B. G., et al. (2010). Design, synthesis and pharmacological screening of potential anticonvulsant agents using hybrid approach. *European Journal of Medicinal Chemistry, 45*(3), 857–863. https://doi.org/10.1016/j.ejmech.2009.09.014.

Hekmatshoar, R., Sadjadi, S., Shiri, S., Heravi, M. M., & Beheshtiha, Y. S. (2009). Green protocol for synthesis of 1,5-benzodiazepines and 1,5-benzothiazepines in the presence of nanocrystalline aluminum oxide. *Synthetic Communications, 39*(14), 2549–2559. https://doi.org/10.1080/00397910802657925.

Indalkar, K. S., Patil, M. S., & Chaturbhuj, G. U. (2017). An efficient, environmentally benign, and solvent-free protocol for the synthesis of 4-substituted 1,5-benzodiazepines catalyzed by reusable sulfated polyborate. *Tetrahedron Letters, 58*(48), 4496–4502. https://doi.org/10.1016/j.tetlet.2017.10.030.

Ishwar Bhat, K., & Kumar, A. (2016). Synthesis and anti-inflammatory activity of some novel 1,5-benzodiazepine derivatives. *Asian Journal of Pharmaceutical and Clinical Research, 9*(4), 63–66. http://innovareacademics.in/journals/index.php/ajpcr/article/download/9012/5793.

Jamatia, R., Gupta, A., Dam, B., Saha, M., & Pal, A. K. (2017). Graphite oxide: A metal free highly efficient carbocatalyst for the synthesis of 1,5-benzodiazepines under room temperature and solvent free heating conditions. *Green Chemistry, 19*(6), 1576–1585. https://doi.org/10.1039/c6gc03110b.

Jeganathan, M., & Pitchumani, K. (2014). Solvent-free syntheses of 1,5-benzodiazepines using HY zeolite as a green solid acid catalyst. *ACS Sustainable Chemistry and Engineering, 2*(5), 1169–1176. https://doi.org/10.1021/sc400560v.

Jiang, Y. J., Cai, J. J., Zou, J. P., & Zhang, W. (2010). Gallium(III) triflate-catalyzed [4 + 2 + 1] cycloadditions for the synthesis of novel 3,4-disubstituted-1,5-benzodiazepines. *Tetrahedron Letters, 51*(3), 471–474. https://doi.org/10.1016/j.tetlet.2009.11.049.

Kaur, R., Singh, K., & Singh, R. (2016). 1,5-benzothiazepine: Bioactivity and targets. *Chemical Biology Letters, 3*(1), 18–31. http://pubs.iscience.in/journal/index.php/cbl/article/download/453/286.

Kumaraswamy, M. N., Vaidya, V. P., Chandrashekhar, C., Mathias, D. A. P., Shivakumar, H., & Mahadevan, K. M. (2013). Synthesis of novel 2,5-dihydro-1*H*-1,5-benzodiazepines encompassing naphto [2,1-*b*] furan and evaluation of their pharmacological activities, Inter. *Journal of Pharmaceutical and Biological Sciences, 3*, 281–287.

Kuo, C. W., More, S. V., & Yao, C. F. (2006). NBS as an efficient catalyst for the synthesis of 1,5-benzodiazepine derivatives under mild conditions. *Tetrahedron Letters, 47*(48), 8523–8528. https://doi.org/10.1016/j.tetlet.2006.09.128.

Li, T., Zhang, J., Pan, J., Wu, Z., Hu, D., & Song, B. (2017). Design, synthesis, and antiviral activities of 1,5-benzothiazepine derivatives containing pyridine moiety. *European Journal of Medicinal Chemistry, 125*, 657–662. https://doi.org/10.1016/j.ejmech.2016.09.069.

Lutz, W. (2014). Zeolite Y: Synthesis, modification, and properties—A case revisited. *Advances in Materials Science and Engineering, Article ID 724248*, 20 pages. https://doi.org/10.1155/2014/724248.

Maury, S. K., Kumar, D., Kamal, A., Singh, H. K., Kumari, S., & Singh, S. (2021). A facile and efficient multicomponent ultrasound-assisted "on water" synthesis of benzodiazepine ring. *Molecular Diversity, 25*, 131–142. https://doi.org/10.1007/s11030-019-10031-y.

Meninno, S., Volpe, C., & Lattanzi, A. (2017). Catalytic enantioselective synthesis of protecting-group-free 1,5-benzothiazepines. *Chemistry (Weinheim An Der Bergstrasse, Germany), 23*(19), 4547–4550. https://doi.org/10.1002/chem.201700837.

Misra, A., Kishore, D., Verma, P., Dubey, S., Chander, S., Gupta, N., et al. (2020). Synthesis, biological evaluation and molecular docking of pyrimidine and quinazoline derivatives of 1,5-benzodiazepine as potential anticancer agents. *Journal of King Saud University - Science, 32*(2), 1486–1495. https://doi.org/10.1016/j.jksus.2019.12.002.

Naeimi, H., & Foroughi, H. (2016). Facile three-component preparation of benzodiazepine derivatives catalyzed by zinc sulfide nanoparticles via grinding method. *Research on Chemical Intermediates, 42*(5), 3999–4020. https://doi.org/10.1007/s11164-015-2254-4.

Nichols, S. E., Domaoal, R. A., Thakur, V. V., Tirado-Rives, J., Anderson, K. S., & Jorgensen, W. L. (2009). Discovery of wild-type and Y181C mutant non-nucleoside HIV-1 reverse transcriptase inhibitors using virtual screening with multiple protein structures. *Journal of Chemical Information and Modeling, 49*(5), 1272–1279. https://doi.org/10.1021/ci900068k.

Nowakowska, Z. (2007). A review of anti-infective and anti-inflammatory chalcones. *European Journal of Medicinal Chemistry, 42*(2), 125–137. https://doi.org/10.1016/j.ejmech.2006.09.019.

Ongone, T. N., Achour, R., El Ghoul, M., El Ouasif, L., El Jemli, M., Chemlal, L., et al. (2019). Analgesic and antioxidant activities of 4-phenyl-1,5-benzodiazepin-2-one and its long carbon chains derivatives. *Journal of Chemistry, 2019.* https://doi.org/10.1155/2019/9043570.

Qomi, H. R., & Habibi, A. (2017). Synthesis of a novel functionalized tricyclic pyrimidine-fused 1,5-benzodiazepine library. *Tetrahedron, 73*(21), 2991–3001. https://doi.org/10.1016/j.tet.2017.03.079.

Rao, V. R., & Reddy, M. M. M. (2006). A facile one pot synthesis of 2-aryl-4-[2*H*-2-oxo-[1]benzopyran-3-Yl] 2,3-dihydro and 2,5-dihydro-1,5-benzothiazepines. *Phosphorus, Sulfur, and Silicon and the Related Elements, 181*, 461–471. https://doi.org/10.1080/104265091001326.

Ried, W., & Torinus, E. (1959). Heterocyclic seven-ring systems. X: Syntheses of condensed 5,7-, and 8-membered heterocycles with 2 nitrogen atoms. *Chemische Berichte, 92*, 2902–2916.

Sakagami, K., Masuda, T., Kawano, K., & Futaki, S. (2018). Importance of net hydrophobicity in the cellular uptake of all-hydrocarbon stapled peptides. *Molecular Pharmaceutics, 15*(3), 1332–1340. https://doi.org/10.1021/acs.molpharmaceut.7b01130.

Schütz, H. (1982). *Benzodiazepines: A handbook. Basic Data, Analytical Methods, Pharmacokinetics and Comprehensive Literature* (pp. 1–439). Switzerland: Springer.

Sharma, A., Kishore, D., & Singh, B. (2018). An expedient method for the synthesis of 1,2,4-triazolo-fused 1,5-benzodiazepine, 1,5-benzoxazepine, and 1,5-benzothiazepine scaffolds: A novel seven-membered ring system of biological interest. *Journal of Heterocyclic Chemistry, 55*(3), 586–592. https://doi.org/10.1002/jhet.3060.

Sharma, R. K., Dutta, S., Sharma, S., Zboril, R., Varma, R. S., & Gawande, M. B. (2016). Fe_3O_4 (iron oxide)-supported nanocatalysts: Synthesis, characterization and applications in coupling reactions. *Green Chemistry, 18*(11), 3184–3209. https://doi.org/10.1039/c6gc00864j.

Sharma, R., Tilak, A., Thakur, R. N., Gangwar, S. S., & Sutar, R. C. (2017). Synthesis and pharmacological evaluation of substituted 1,5-benzodiazepine derivatives for its antidepressant activity in experimental animals. *World Journal of Pharmaceutical Research, 6*, 925–931.

Shimotori, Y., Hoshi, M., Murata, M., Ogawa, N., Miyakoshi, T., & Kanamoto, T. (2018). Synthesis of dibenzothiazepine analogues by one-pot *S*-arylation and intramolecular cyclization of diaryl sulfides and evaluation of antibacterial properties. *Heterocyclic Communications, 24*(4), 219–230. https://doi.org/10.1515/hc-2018-0099.

Sievers, C., Liebert, J. S., Stratmann, M. M., Olindo, R., & Lercher, J. A. (2008). Comparison of zeolites LaX and LaY as catalysts for isobutane/2-butene alkylation. *Applied Catalysis A: General, 336*(1–2), 89–100. https://doi.org/10.1016/j.apcata.2007.09.039.

Song, M. X., & Deng, X. Q. (2018). Recent developments on triazole nucleus in anticonvulsant compounds: A review. *Journal of Enzyme Inhibition and Medicinal Chemistry, 33*(1), 453–478. https://doi.org/10.1080/14756366.2017.1423068.

Spencer, J., Rathnam, R. P., & Chowdhry, B. Z. (2010). 1,4-benzodiazepin-2-ones in medicinal chemistry. *Future Medicinal Chemistry, 2*(9), 1441–1449. https://doi.org/10.4155/fmc.10.226.

Stahlhofen, P., & Ried, W. (1957). Heterocyclic seven-membered ring systems. V: Reaction of *o*-phenylenediamine with α,β-unsaturated carbonyl compounds. *Chemische Berichte, 90*, 815–824.

Sun, Y. W., Bei, Y. M., & Wang, L. Z. (2019). A catalyst-free four-component domino reaction for the synthesis of functionalized 3-acyl-1,5-benzodiazepines. *Organic and Biomolecular Chemistry, 17*(4), 930–938. https://doi.org/10.1039/c8ob02903b.

Tamuli, K. J., & Bordoloi, M. (2020). Recyclable itaconic acid with water as green catalytic system: Synthesis of substituted 1,5-benzodiazepine derivatives at room temperature. *ChemistrySelect, 5*(4), 1353–1358. https://doi.org/10.1002/slct.201904408.

Tupare, S. D., & Pawar, R. P. (2017). Highly efficient synthesis and antibacterial of 1,5-benzodiazepines under microwave irradiation. *International Journal of Applied Chemistry, 13*, 369–376.

Varvounis, G. (2016). An update on the synthesis of pyrrolo[1,4]benzodiazepines. *Molecules (Basel, Switzerland), 21*(2). https://doi.org/10.3390/molecules21020154.

Vuluga, D., Legros, J., Crousse, B., Slawin, A. M. Z., Laurence, C., Nicolet, P., et al. (2011). Influence of the structure of polyfluorinated alcohols on Brønsted acidity/hydrogen-bond donor ability and consequences on the promoter effect. *Journal of Organic Chemistry, 76*(4), 1126–1133. https://doi.org/10.1021/jo1023816.

Wang, L. Z., Li, X. Q., & An, Y. S. (2015). 1,5-Benzodiazepine derivatives as potential antimicrobial agents: Design, synthesis, biological evaluation, and structure-activity relationships. *Organic and Biomolecular Chemistry, 13*(19), 5497–5509. https://doi.org/10.1039/c5ob00655d.

Wang, L., Zhang, P., Zhang, X., Zhang, Y., Li, Y., & Wang, Y. (2009). Synthesis and biological evaluation of a novel series of 1,5-benzothiazepine derivatives as potential antimicrobial agents. *European Journal of Medicinal Chemistry, 44*(7), 2815–2821. https://doi.org/10.1016/j.ejmech.2008.12.021.

Yin, L., & Wang, L. (2016). Chemo-/regio-selective synthesis of 2-aryl-3-acetyl-2,4-dihydro-1*H*-5*H*-1,5-benzodiazepines using Lewis acid, $CeCl_3 \cdot 7H_2O$. *Tetrahedron Letters, 57*(52), 5935–5940. https://doi.org/10.1016/j.tetlet.2016.11.089.

Zamani, F., Hosseini, S. M., & Kianpour, S. (2013). Synthesis and characterization of sulfonated-mercaptopropanoic acid coated Fe_3O_4 nanoparticles as a novel acid magnetic catalyst for Biginelli reaction. *Solid State Sciences, 26*, 139–143. https://doi.org/10.1016/j.solidstatesciences.2013.10.007.

Zamani, F., & Izadi, E. (2013). Synthesis and characterization of sulfonated-phenylacetic acid coated Fe_3O_4 nanoparticles as a novel acid magnetic catalyst for Biginelli reaction. *Catalysis Communications, 42*, 104–108. https://doi.org/10.1016/j.catcom.2013.08.006.

Non-Print Items

Abstract

This chapter aims to provide an overview of the most practical and attractive protocols that demonstrate the syntheses of 1,5-benzodiazepines and 1,5-benzothiazepines using various readily available starting materials and catalysts under mild reaction conditions.

Keywords

1,5-Benzodiazepines; 1,5-Benzothiazepines; Synthetic methods; Organic reaction; Chemical substance; Chemical process

Biological behavior of 1,5-benzodiazepines and 1,5-benzothiazepines

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Chapter outline

8.1 Introduction

Heterocyclic compounds are abundant in nature and are considered a vital core in life processes and sustainability. Articles and experiments have been widely utilized to imitate and understand the biological behaviors of these bioactive compounds using *in silico, in vitro, in vivo* studies, and finally, to apply them in clinical trials to find some practical solutions to current human-being diseases or biologically associated issues. Most of these molecules possessing N– and S–containing heterocyclic nucleus usually incorporate with other organic skeletons to create more potent and selective biologically active fused heterocycles with potential therapeutic activities. As such, 1,5-benzodiazepines and 1,5-benzothiazepines are among the most important fused heterocyclic scaffolds displaying a diverse range of biological behaviors with tunable structure, which have been broadly used as sedatives, hypnotics, disinfectants, and pre-anesthetics in clinical treatments (Aastha, Navneet, Anshu, Pratima, & Dharma, 2013; Archer & Sternbach, 1968). Small changes in their structure can alter their biological activities and new applications of these compounds are constantly emerging. Apart from various anxiolytic effects, several 1,5-benzodiazepines and 1,5-benzothiazepines have been approved for therapeutic uses as antihypertensive, antibacterial, and antifungal agents (Bariwal et al., 2008; Henke et al., 1996; Nagao, Narita, Sato, Nakajima, & Kiyomoto, 1982; Ranran, Yang, Yanqing, & Ping, 2020; Wang et al., 2009). A broad range of studies has been directed to explain the action mechanisms of 1,5-benzodiazepines and 1,5-benzothiazepines in the body and to determine how their side effects appear. In this regard, it is generally believed that these fused compounds are highly active in regulating several cell receptors to exert their biological features (Verma & Snyder, 1989). This chapter will elucidate an inclusive compilation of biological activities of 1,5-benzodiazepines and 1,5-benzothiazepines.

8.2 Interaction of 1,5-benzodiazepines and 1,5-benzothiazepines with cell receptors

Cell receptors located within the plasma membrane differ in morphology and structure, which often provide a unique binding pocket to interact with a certain type of molecule. Binding bioactive compounds to the receptors can then effectively modify the receptors' performance, leading to significant changes in cell behavior. In this section, the ever-discovered
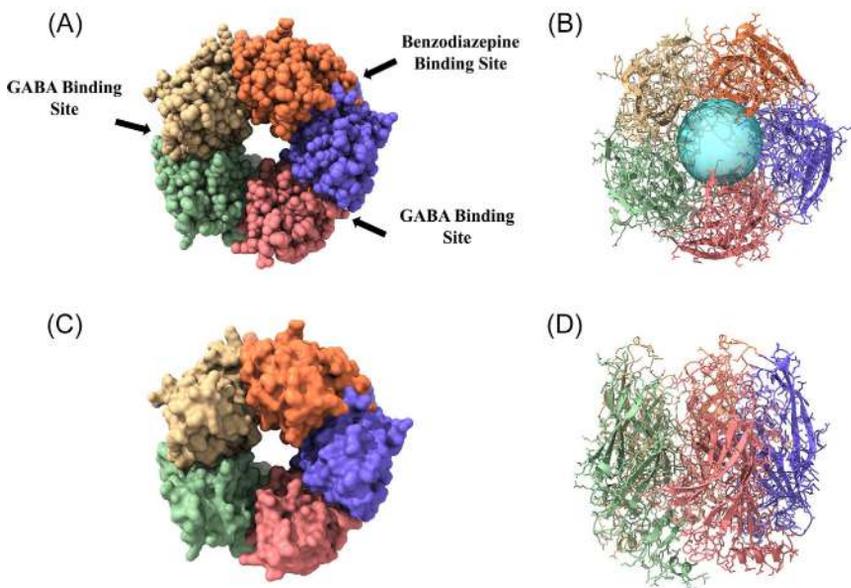
Figure 8.1 Structure of a GABA$_A$ receptor in the electronic chart display (ECD) map (PDB: 6DW1). (A, C) Top views of a GABA$_A$ receptor in the space-filling molecular model. (B, D) Ribbon representations of the benzodiazepine receptor from top and side views, respectively. The blue area indicates the chloride channel.

interactions of 1,5–benzodiazepines and 1,5–benzothiazepines with various cell receptors are discussed.

8.2.1 GABA$_A$ receptors

The GABA$_A$ receptor, widely located in the CNS, is one of the main cellular receptors that have a strong interaction with 1,5-benzodiazepines (Nicholson et al., 2018) (See Chapter 4 for a detailed discussion about these receptors). GABA$_A$ receptors consist of three main subunits (*i.e.*, $\alpha 1$, $\beta 2$, and $\gamma 2$) and one central chloride ion channel (Fig. 8.1). Binding extracellular biomolecules to the subunits *via* specific binding domains leads to opening the chloride channel and enhancing the intracellular concentration of chloride ions, which results in neuronal hyperpolarization. There are two binding sites for the agonist GABA located at the α/β subunit interfaces and one modulatory site for benzodiazepines at the α/γ interface (Fig. 8.1).

1,5–Benzodiazepines and 1,5-benzothiazepines interact with GABA$_A$ receptors at the corresponding binding domains, also known as benzodi–azepine binding sites or benzodiazepine receptors, and subsequently increase
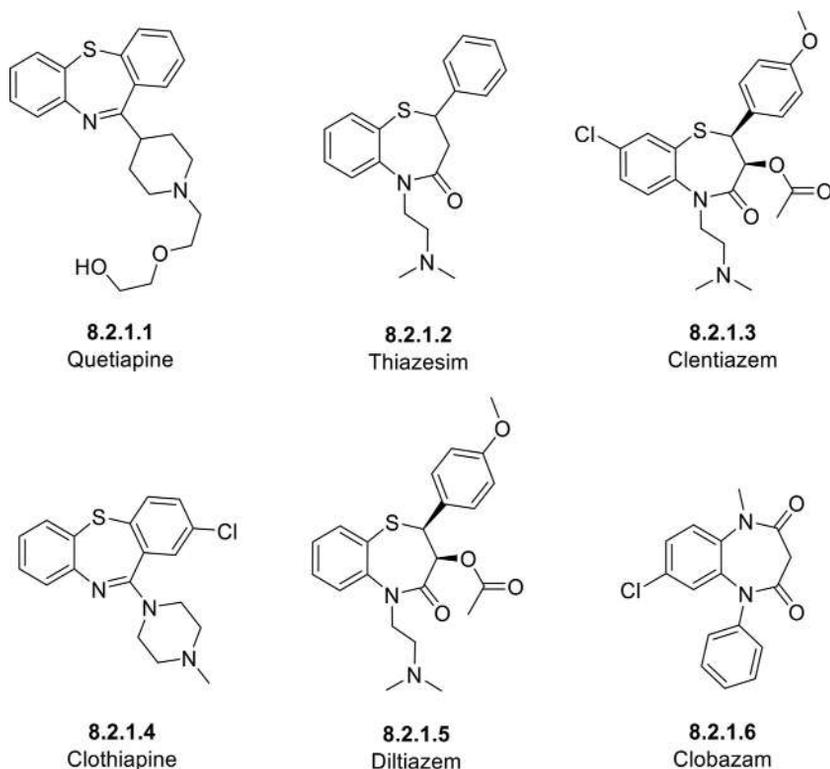
Figure 8.2 Several examples of known 1,5-benzodiazepine and 1,5-benzothiazepines as GABA$_A$ receptor agonists.

GABA–induced chloride ion influx (Sigel & Ernst, 2018). While 1,5-benzodiazepines and 1,5-benzothiazepines basically act as benzodiazepine-receptor agonists to facilitate the entry of chloride ions into the cells, GABA neurotransmitter binds to the GABA$_A$ receptors inhibiting the chloride ion influx (Nikmaram et al., 2017). Fig. 8.2 represents several examples of 1,5-benzodiazepines and 1,5-benzothiazepines (**8.2.1.1–8.2.1.6**) that behave as GABA$_A$ receptor agonists to cause neuronal hyperpolarization and consequently induce calming the CNS.

8.2.2　Peripheral benzodiazepine receptors (PBRs)

Peripheral benzodiazepine receptors (PBRs) (Fig. 8.3), also known as mitochondrial benzodiazepine receptors (MBRs), are another recognition binding site for benzodiazepines found in steroidogenic cells such as ovarian cells. Although the MBRs were initially recognized as the peripheral receptors,
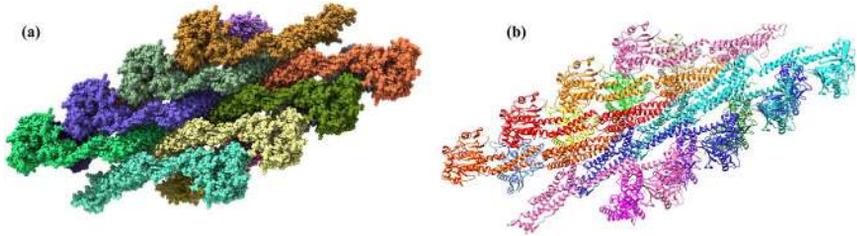
Figure 8.3 3D representation of peripheral benzodiazepine receptors (PDB: 5WP9). (A) Space-filling model. (B) Ribbon representation.
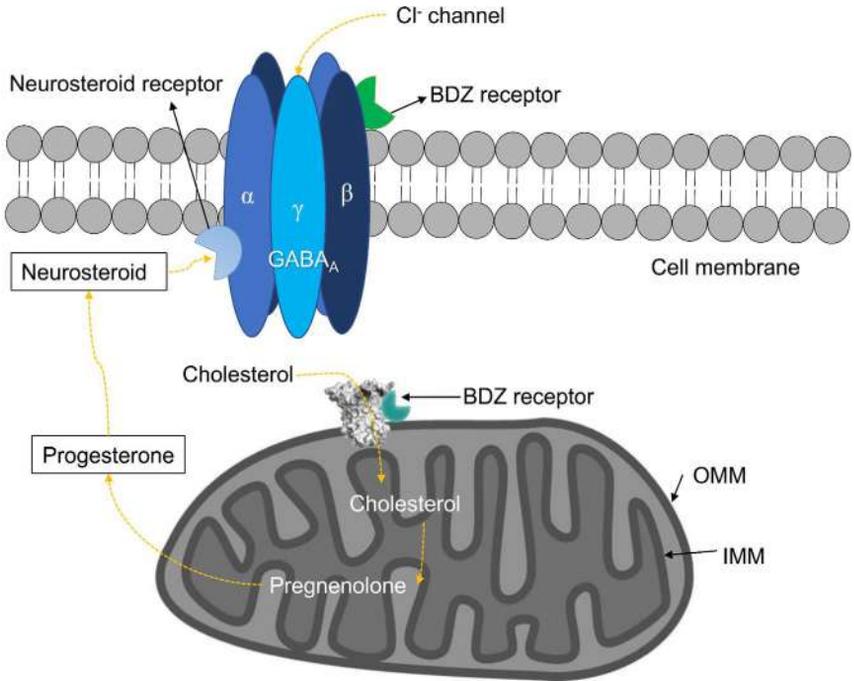

Figure 8.4 The mechanism of GABA$_A$ activation through endogenous steroid synthesis in mitochondria. BDZ, benzodiazepine; IMM, inner mitochondrial membrane; OMM, outer mitochondrial membrane.

it was later found that they also exist in the CNS astrocytes, regulating the axon guidance, synaptic performance, and blood flow (Beurdeley-Thomas, Miccoli, Oudard, Dutrillaux, & Poupon, 2000).

MBRs are located on the outer surface of an 18 kDa translocator protein (TSPO), which is found in the outer membrane of the mitochondria (Fig. 8.4). The endogenous MBRs can be activated by the entry of

cholesterol into the inner mitochondrial membrane (IMM), where the cholesterol biotransforms into the pregnenolone and progesterone and finally becomes neurosteroids inside the mitochondria. These neurosteroids then do their final task as they bind to the GABA$_A$ receptors *via* specific neurosteroid binding domains (Fig. 8.4), activating the chloride channel and inducing the cellular chloride ion concentration within the cell. The main endogenous neurosteroids include allopregnanolone, tetrahydroxy-desoxycorticosterone (THDOC), pregnenolone sulfate (PS, PREGS), and epipregnanolone (Chen et al., 2019). Therefore, the neurosteroids can be regarded as positive allosteric modulators (PAMs) of GABA$_A$ receptors, which potentiate the effects of GABA at nanomolar concentrations and directly activate currents at micromolar concentrations (Chen et al., 2019).

Diazepam is an example of an FDA-approved 1,4-benzodiazepine that exhibits a high affinity for the mitochondrial benzodiazepine receptors. This molecule stimulates endogenous steroid synthesis upon the activation of MBRs and then regulates the GABA$_A$ receptors' functions (Papadopoulos, 1993). In addition, several structure-activity relationships (SAR) and molecular modeling studies have shown that pyrolo-fused 1,5-benzothiazepine derivatives (**8.2.2.1–8.2.2.9**) show a high affinity for the MBRs (Fig. 8.5) (Campiani et al., 1996; Fiorini et al., 1994).

8.2.3 Muscarinic acetylcholine receptors

The muscarinic acetylcholine receptors (mAChRs) are a G protein-coupled receptor subfamily that is abundantly expressed in the central and peripheral nervous systems. These receptors consist of five different subtypes of M1–M5, encoded by the genes CHRM1 to CHRM5 (Eglen, 2006). Three of these receptor subtypes (M1, M3, and M5) couple to G$_q$/G$_{11}$ proteins, and the remaining M2 and M4 subtypes primarily signal through the G$_i$/G$_o$ proteins. The crystal structures of M1, M3, M5 muscarinic acetylcholine receptors are depicted in Fig. 8.6. The mAChRs play an important role in many CNS functions such as human physiology, controlling heart rate, glandular secretion, smooth muscle contraction (Eglen, 2006). These receptors are also promising therapeutic targets for the treatment of some psychiatric diseases such as schizophrenia and Alzheimer's disease (Jones, Byun, & Bubser, 2012). The acetylcholine activates the receptors *via* inducing a parasympathetic reaction in the corresponding organs and tissues, which are innervated by both postganglionic sympathetic and parasympathetic nerves innervating eccrine sweat glands.
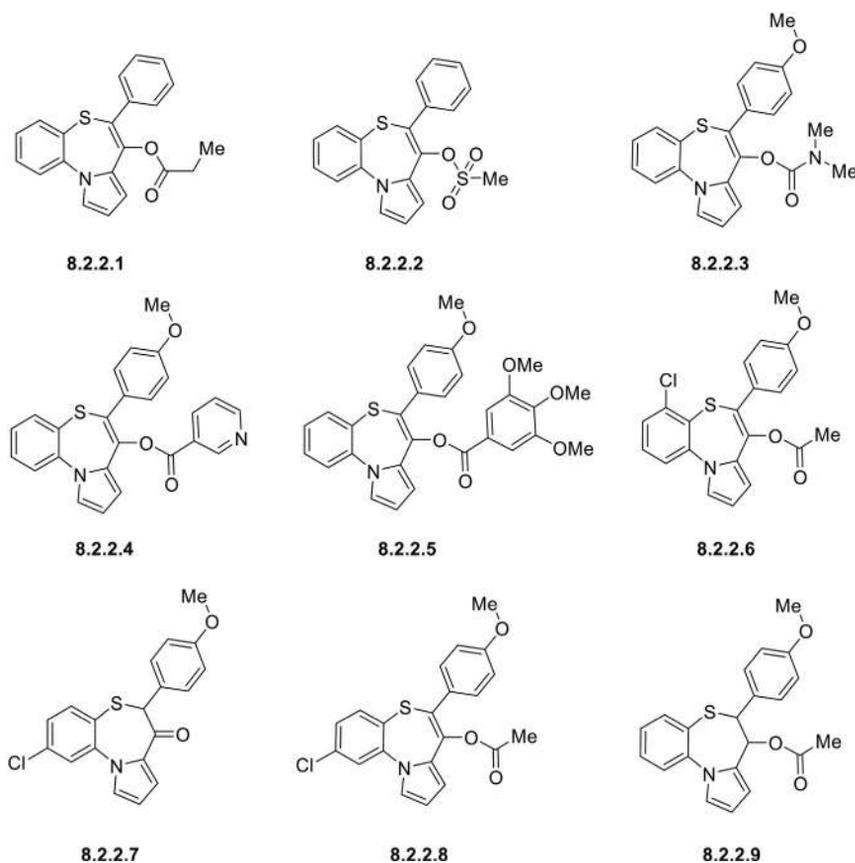
Figure 8.5 Several examples of pyrolo-fused 1,5-benzothiazepine derivatives with a high affinity for MBRs.

Olanzapine (**8.2.3.1**) and clozapine (**8.2.3.2**) are two examples of 1,5–benzodiazepine–derived antipsychotic drugs used for schizophrenia and bipolar disorders (Fig. 8.7). These medications manifest a muscarinic receptor antagonist profile through binding to mAChRs, preferentially the M2 subtypes, and subsequently enhancing acetylcholine release *via* interrupting muscarinic autoreceptor–induced autoinhibition (Svoboda, Popelikova, & Stuchlik, 2017; Tzavara, Bymaster, & Nomikos, 2006).

8.2.4 5-Hydroxytryptamine receptors (5-HT)

5-Hydroxytryptamine (5-HT), also known as serotonin, is an important neurotransmitter that exerts its diverse physiological effects through binding to various G protein–coupled 5-hydroxytryptamine receptors (serotonin

M5 Muscarinic acetylcholine receptor

M1 Muscarinic acetylcholine receptor

Muscarinic acetylcholine receptor 1-toxin 7 complex

M3 Muscarinic acetylcholine receptor
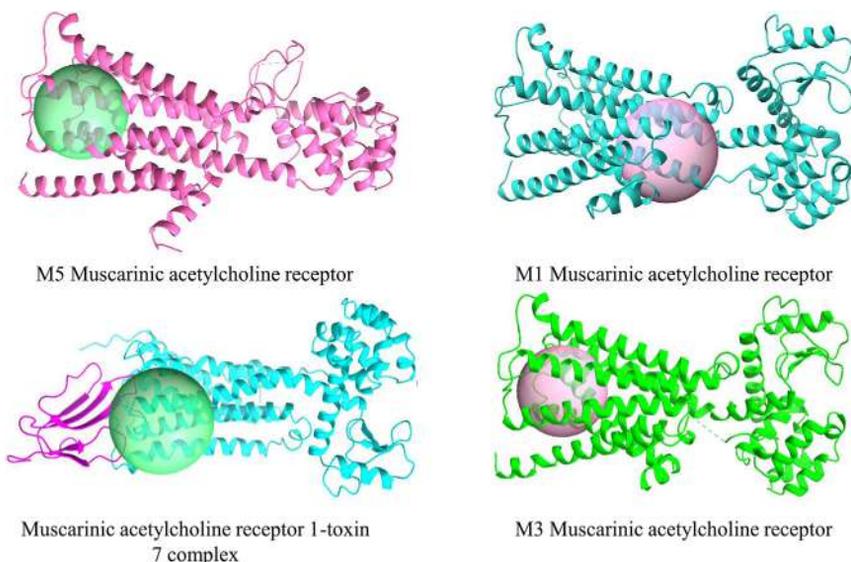
Figure 8.6 The crystal structures of the human M1 muscarinic acetylcholine (PDB: 5CXV), M3 muscarinic acetylcholine (PDB: 4DAJ), M5 muscarinic acetylcholine receptor (PDB: 6OL9), and complex of muscarinic acetylcholine receptor 1-muscarinic toxin 7 (PDB: 6WJC). Muscarinic toxin 7 is a biologically active M1 AChR antagonist. The pink and green spheres represent the active sites.

Olanzapine
8.2.3.1
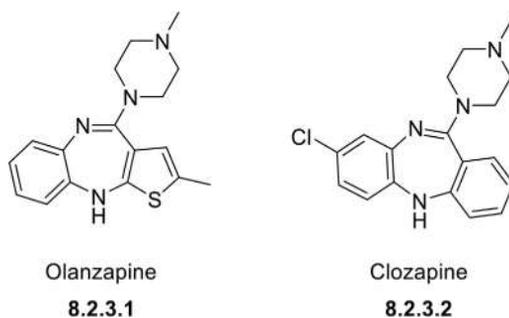
Clozapine
8.2.3.2

Figure 8.7 The chemical structures of olanzapine and clozapine.

receptors), classified into seven distinctive subtypes, 5-HT1 to 5-HT7, according to their structural diversity and mode of action (Barnes & Sharp, 1999). The 5-HT receptors are key elements in different human diseases such as depression, schizophrenia, migraine, obesity, and cardiovascular disorders. Fig. 8.8 represents the crystal structure of the chimeric protein of 5-hydroxytryptamine 1B in complex with dihydroergotamine (an alkaloid-based migraine drug). The 5-HT receptors are also responsible for modulating the release of many neurotransmitters (*e.g.*, dopamine, glutamine,
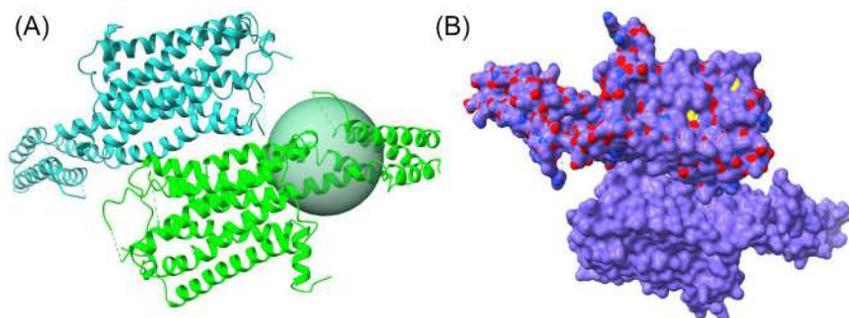
Figure 8.8 (A) Crystal structure of the chimeric protein of 5-hydroxytryptamine 1B (ribbon representation) in complex with dihydroergotamine (an alkaloid-based migraine drug) (PDB: 4IAQ). The green sphere shows the active site. (B) The space-filling model of 5-hydroxytryptamine 1B.

epinephrine/norepinephrine, and GABA) and hormones (*e.g.*, oxytocin, vasopressin, prolactin, cortisol, and corticotropin) in the CNS (Barnes & Sharp, 1999). For example, the serotonin system effectively inhibits dopaminergic function in both the midbrain and forebrain. Several tract-tracing and immunohistochemical experiments have demonstrated that serotonergic neurons arising in the dorsal raphe nucleus (DRN) project repeatedly through the medial forebrain bundle (MFB) to the striatum and cortex (Kapur & Remington, 1996). Stimulation of these raphe-striatal neurons causes inhibition of striatal neuronal firing, which is likely due to a reduction in synaptic dopamine (Kapur & Remington, 1996). This phenomenon is possibly triggered by the 5-HT2 receptors as a result of a diminished release or a declined dopamine synthesis in the nerve terminals (Kapur & Remington, 1996).

Prolonged drug administration of schizophrenia with conventional antipsychotics displays treatment resistance and several adverse effects such as extrapyramidal symptoms. Olanzapine (**8.2.3.1**) and clozapine (**8.2.3.2**) are recognized as the typical antipsychotics to address these limitations (Campbell, Young, Bateman, Smith, & Thomas, 1999). Serotonin receptor antagonists inhibit the serotonin system, leading to the release of the dopamine system from its restriction. This dopamine system disinhibition in the striatum may then reduce neuroleptic-stimulated extrapyramidal symptoms.

8.2.5 Bradykinin receptors

Bradykinin (BK) is a vasoactive peptide (Arg–Pro–Pro–Gly–Phe–Ser–Pro–Phe–Arg) that plays a key role in the physiological response to inflammation,
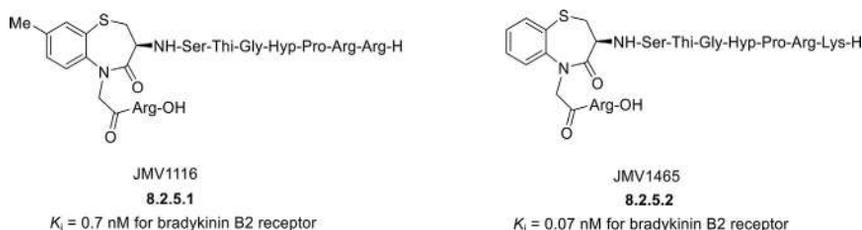
Figure 8.9 Two examples of highly potent and selective 1,5-benzothiazepine-derived B2R agonists.

trauma, and damage (Hess, Borkowski, Young, Strader, & Ransom, 1992; Menke et al., 1994). The release of endogenous bradykinin from kininogens followed by an interaction with the bradykinin B1 and B2 receptors (B1R and B2R) generates several biological effects (Hall, 1997). B2R is expressed in healthy tissues throughout the body and is responsible for smooth muscle contraction, vasodilation, nociceptor activation, and osmoregulation (Lau, Rousseau, Kwon, Bénard, & Lin, 2020). On contrary, B1R is absent in normal tissues and is extensively expressed in the case of tissue trauma or inflammation (Lau et al., 2020). B1R and B2R have been demonstrated to be promising therapeutic targets as they are associated with various human diseases such as vasculopathy, neuropathy, obesity, diabetes, and cancer (Howl & Payne, 2003; Marceau & Regoli, 2004). One of the most potent and selective B2R antagonists reported so far is a decapeptide HOE 140 (H–Arg–Arg–Pro–Hyp–Gly–Thi–Ser–Tic–Oic–Arg–OH), displaying an excellent affinity for the human cloned B2R with the K_i value of 0.65 nM, which may be related to its tendency to adopt C–terminal β–turn conformations (Kyle et al., 1993; Lembeck et al., 1991). However, this active ligand is prone to hydrolysis by angiotensin–converting enzyme (ACE) at positions 7 and 8 (Amblard et al., 1999). In this regard, the substitution of the ACE cleave site (Tic-Oic unit) of HOE 140 by a (3S)-[amino]-5-(carbonylmethyl)-2,3-dihydro-1,5-benzothiazepine-4(5H)–one moiety leads to the formation of a full potent and selective bradykinin B2 receptor agonist (JMV1116, **8.2.5.1**), presenting a high affinity for the human B2R (K_i = 0.7 nM) with much better stability against enzymatic (ACE) degradation (Fig. 8.9) (Amblard et al., 1999). It is believed that the interaction of 1,5-benzothiazepine with B2R can be one of the main reasons for the agonist's high potency. Replacement of the arginine unit with a lysine residue produces a 10-fold more powerful compound (JMV1465, **8.2.5.2**) exhibiting excellent agonist activity on B2R in the human umbilical vein (Amblard et al., 1999).

8.2.6 Vasopressin V2 receptor (V2R)

Arginine vasopressin (AVP), also known as the antidiuretic hormone, is the main physiological modulator of extracellular fluid volume (Verbalis, 2002). Although the primary function of AVP is the regulation of renal free water excretion, this hormone is also a vasoconstrictor and pressor agent, where the name "vasopressin" is originated. AVP acts on the collecting duct system in the kidney *via* specific AVP receptors, for example, V1aR, V1bR, and V2R subtypes, to improve water permeability of the renal tubules, resulting in reduced urine formation. This phenomenon subsequently affects blood volume, body fluid osmolality, cardiac output, arterial pressure, and myocardial contractile function (Finley, Konstam, & Udelson, 2008; Juul, Bichet, Nielsen, & Nørgaard, 2014). Vasopressin V2 receptor (V2R) is a G–protein coupled receptor mainly expressed in the distal convoluted tubule's cell membrane (Robben, Knoers, & Deen, 2004). The key function of this receptor is to modulate the homeostasis of body water by controlling the level of reabsorbed water from urine via Aquaporin-2 (AQP2) water channels (Robben et al., 2004). On binding AVP to the V2R receptors, adenylate cyclase is activated by the stimulatory G protein. This is followed by increased intracellular cAMP, induced AQP2 phosphorylation by protein kinase A (PKA), and subsequently redistribution of the protein from intracellular vesicles to the apical membrane, which finally results in urine concentration (Robben et al., 2004).

The use of antagonists for inhibiting the activity of V2Rs is a promising technique in polycystic kidney diseases (PKDs), hyponatremia, and heart failure (Gattone, Wang, Harris, & Torres, 2003; Palm, Reimann, & Gross, 2001). 1,5-Benzothiazepine derivatives display great inhibitory activity against vasopressin V_2 receptors. For example, the carboxymethyl–substituted 1,5-benzothiazepine analog **8.2.6.1** has shown approximately 140-fold higher selectivity for the V2R over the V1aR with the IC_{50} values of 0.008 μM and 1.14 μM, respectively (Fig. 8.10) (Urbanski et al., 2003). This antagonist is also orally active *in vivo*, exhibiting good aquaretic activity at a low dose of 3 mg/kg.

8.2.7 Cholecystokinin receptors

Cholecystokinin (CCK) is a regulatory neuropeptide in the body's nervous system, responsible for a wide range of biological events including modulation of anxiety, satiety, and analgesia in the CNS, as well as gastric emptying and motility in the gastrointestinal (GI) tract (Wank, 1995). Endogenously
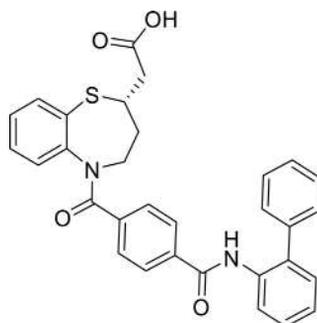
8.2.6.1

IC$_{50}$ = 0.008 μM for V2R

Figure 8.10 2,5-Disubstituted 3,4-dihydro-2*H*-benzo[*b*][1,4]thiazepines as a potent and selective V2R antagonist.
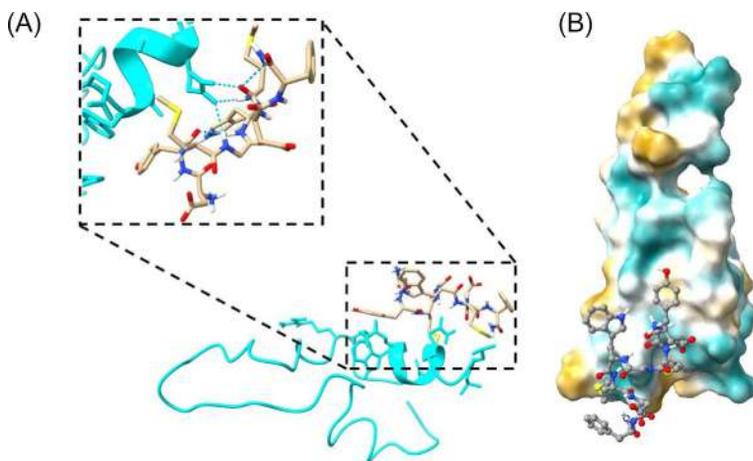

Figure 8.11 The molecular complex of CCK-8 (The CNS form of CCK) is bound to the *N*-terminus of the CCK$_1$ receptor (PDB: 1D6G). (A) Ribbon representation. (B) Electrostatic model.

released CCK also controls the normal exocrine activity of the pancreas and the brain's dopaminergic circuits (Hökfelt et al., 1980). CCK's activities are modulated by two subtypes of G protein–coupled receptors known as CCK$_1$ and CCK$_2$, in which CCK binding occurs at either the extracellular surface or the helical bundle of the receptors (Fig. 8.11).

Modulating the CCK receptors' functions using small molecules is a method for the treatment of acute and chronic pancreatitis, appetite
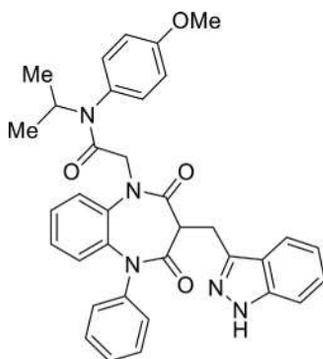
GW 5823

8.2.7.1

pIC_{50} = 7.64 for CCK$_1$ receptor

Figure 8.12 An example of a selective 1,5-benzodiazepine-derived CCK$_1$ agonist.

alterations, and gastrointestinal disorders such as dyspepsia and irritable bowel syndrome (Herranz, 2003; Peter, D'Amato, & Beglinger, 2006). Non–peptide CCK agonists or antagonists are particularly attractive due to the possibility of structural optimization and substitution pattern design. Henke *et al.* have developed a selective non–peptide 1,5–benzodiazepine–derived CCK$_1$ agonist (GW5823, **8.2.7.1**) that demonstrates the inhibitory effect on food intake in the rat feeding model, with pIC_{50} = 7.64 for CCK$_1$ receptors (Fig. 8.12) (Henke et al., 1997). However, the rat pharmacokinetic profile of GW5823 (**8.2.7.1**) indicates poor oral bioavailability (8%) and relatively high total body clearance (Cl_{tot} = 38 mL/min/kg), which may be due to delayed gastric emptying and rapid metabolism by the liver (Henke et al., 1997).

8.3 Interaction of 1,5-benzodiazepines and 1,5-benzothiazepines with enzymes

We know enzymes act as a biocatalyst in a wide range of cellular processes in the body, where the organs, tissues, and cells require energy, protein, proliferation, and other factors to keep the body's survival system running. However, the cells must regulate the activity of enzymes to control the corresponding biological events and to prevent the occurrence of cells' malfunctions. Accordingly, enzyme inhibitors/activators comprise more

8.3.1.1
39% apoptosis for HL-60 cells at 10 M
41% apoptosis for K562 cells at 10 M

8.3.1.2
55% apoptosis for HL-60 cells at 10 M
27% apoptosis for K562 cells at 10 M
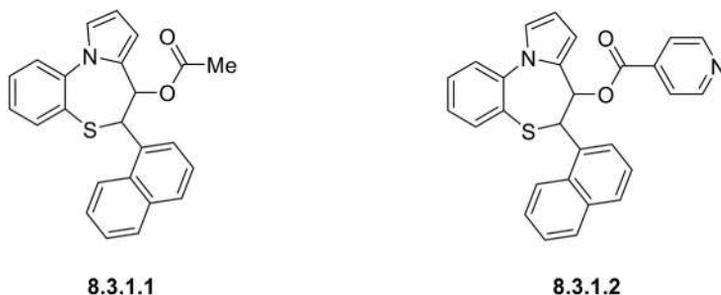
Figure 8.13 Two examples of 1,5-benzothiazepine-derived JNK activators in cancer cells.

than half of all marketed drugs in clinical treatments (Holdgate, Meek, & Grimley, 2018). The study of these agents provides useful information on the mechanisms of enzymatic action and helps to recognize metabolic pathways. In the following sections, we will discuss a series of enzymes that can be inhibited/activated by 1,5–benzodiazepines and/or 1,5–benzothiazepines.

8.3.1 Mitogen-activated protein kinases (MAPKs)

The regulation of apoptosis or programmed cell death is an essential task for maintaining tissue homeostasis, which is mainly modulated *via* many signaling pathways controlled by kinases (Grilo & Mantalaris, 2019). Developmental cellular events and environmental factors induce these evolutionarily conserved kinases that convey signals of regulating proliferation, survival, or cell death (Wada & Penninger, 2004). One of the main family of kinases is the mitogen–activated protein kinases (MAPK), which are accountable for transmitting signals from the cell membrane to the nucleus in response to growth and/or stress stimuli (Morrison, 2012; Wada & Penninger, 2004). The MAPKs consist of three subtypes in mammalian cells including *c*–Jun *N*–terminal kinase (JNK), p38–MAPK, and the extracellular signal–regulated kinase (ERK) (Morrison, 2012). Each subtype participates in different intracellular signaling pathways. For example, JNK controls cell growth, differentiation, transformation, and apoptosis. Two 1,5–pyrrolobenzothiazepine derivatives have shown to be potent apoptotic agents that trigger apoptosis in a couple of cancer cells, HL–60 and K562, through inducing JNK activation (Fig. 8.13) (Mc Gee et al., 2005). Although the detailed action mechanism of these molecules is not clear, the higher activity of **8.3.1.2** against HL–60 cells (55% apoptosis) compared to **8.3.1.1** (39% apoptosis) is hypothesized
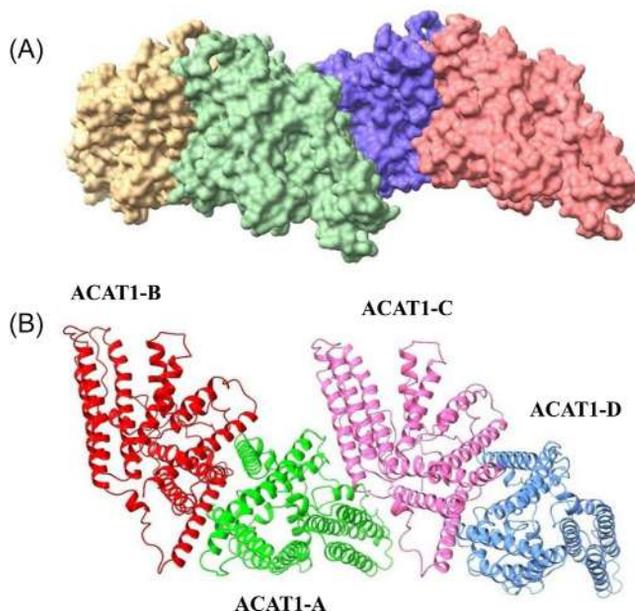
Figure 8.14 (A) Electrostatic and (B) Ribbon representation of the tetrameric structure of ACAT1 (PDB: 6P2P). ACAT1 exists as a dimer of dimers. The two protomers that regulate the dimers' interfaces are colored in green and pink.

to be due to the presence of a planar aromatic substituent (4–pyridyl) in the former, leading to a better accommodation of the ligand in the binding pockets of the kinase in the cells (Mc Gee et al., 2005).

8.3.2 Acyl-coenzyme A: cholesterol acyltransferase (ACAT)

Acyl–coenzyme A: cholesterol acyltransferase (ACAT), also known as sterol *O*–acyltransferase, is an essential enzyme involved in intracellular cholesterol homeostasis as well as systemic sterol metabolism (Tabas, Rosoff, & Boykow, 1988). This enzyme, comprising of two isomeric forms, ACAT-1 and ACAT-2, has been well–demonstrated to catalyze the esterification of both lipoprotein–derived cholesterol and cellular cholesterol in fibroblasts. This phenomenon makes the cholesterol less soluble in body fat, facilitating the storage of cholesterol (Chang, Li, Chang, & Urano, 2009). Both electrostatic and ribbon representations of the tetrameric structure of ACAT1 are represented in Fig. 8.14. Accordingly, inhibiting ACAT plays a significant role in preventing both high cholesterol absorption from the intestinal wall and the formation of suppressing foam cells. A large number of research efforts have been devoted to developing effective ACAT inhibitors to block
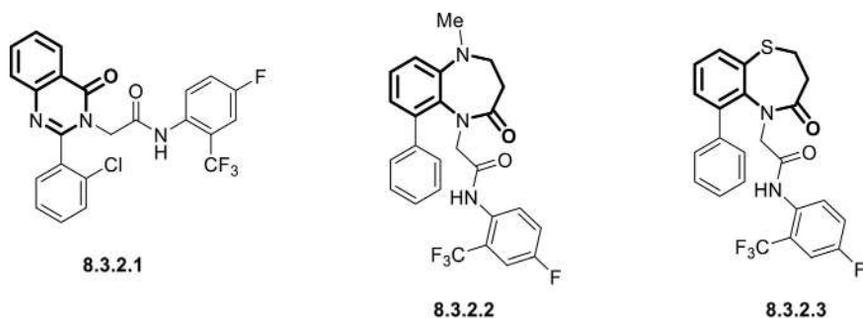
Figure 8.15 (A) Chemical structures and biological activity of the lead compound, 1,5-benzothiazepine, and 1,5-benzodiazepine derivatives developed as ACAT inhibitors.

cholesterol esterification and thereby preventing hypercholesterolemia and atherosclerosis in humans (Giovannoni, Piaz, Vergelli, & Barlocco, 2003). However, many of the reported inhibitors have given unsatisfactory results in terms of selectivity and efficacy in clinical trials together with observed adrenal toxicity (Giovannoni et al., 2003; López–Farré, Sacristán, Zamorano-León, San-Martín, & Macaya, 2008). Natsugari *et al.* discovered a couple of 1,5-benzodiazepine– and 1,5-benzothiazepine-derived ACAT inhibitors (**8.3.2.2** and **8.3.2.3**), which were inspired by the lead compound **8.3.2.1** (Fig. 8.15) (Tabata et al., 2012). They found that the presence of a highly hydrophilic group of *N*-(4–fluoro-2-(trifluoromethyl)phenyl)–acetamide, same as the lead compound (**8.3.2.1**), is necessary to induce the inhibitory activity. Both developed compounds **8.3.2.2** and **8.3.2.3** exhibit high potency against ACAT in rabbit liver microsomes with the IC_{50} values of 3.5 μM and 0.43 μM, respectively.

8.3.3 Reverse transcriptase (RT) and integrase (IN)

When a virus enters a host cell, the viral replication is initiated *via* the reverse transcriptase (RT)-biocatalytic production of a viral DNA copy from the virus genomic RNA. This double-stranded DNA then enters the nucleus of the host cell, where integrase (IN) catalyzes the covalent insertion of the DNA into the chromosomes of the infected cells (Delelis, Carayon, Saïb, Deprez, & Mouscadet, 2008). The resulting provirus acts as a template to replicate the viral genome that eventually leads to the formation of new
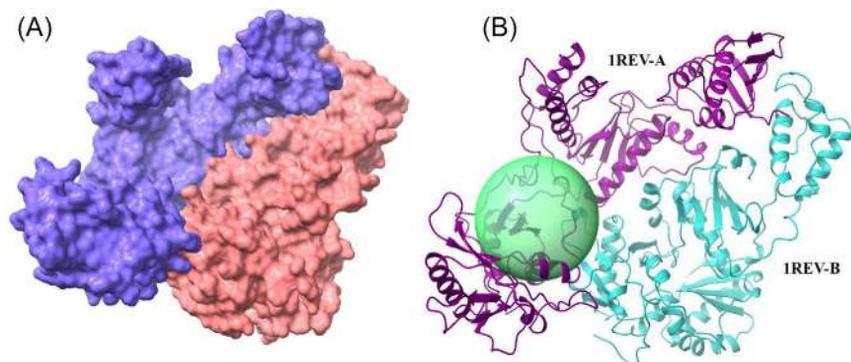
Figure 8.16 (A) Electrostatic and (B) ribbon representation of HIV-1 reverse transcriptase (PDB: 1REV). The active region is displayed by the green sphere.
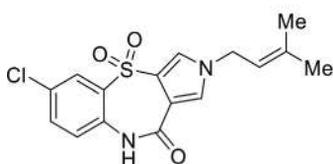
8.3.3.1

$EC_{50} = 0.3\ \mu M$
$CC_{50} = 68\ \mu M$
SI (selevtivity index) = 226
(HIV-1 infected MT-4 cells)
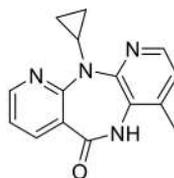
Nevirapine (8.3.3.2)

$EC_{50} = 0.25\ \mu M$
$CC_{50} = >200\ \mu M$
SI (selevtivity index) = >800
(HIV-1 infected MT-4 cells)

Figure 8.17 Nevirapine (FDA-approved anti-HIV-1 drug) and a 1,5-benzothiazepine-derived compound with high inhibitory activity against HIV-1 reverse transcriptase (HIV-1 RT) in infected MT-4 cells.

viruses and so forth (Delelis et al., 2008). HIV-1 (human immunodeficiency virus 1), as one of the most fatal viruses, can easily penetrate the cells in the human immune system, replicate HIV-1 viral DNA copy and RNA through HIV-1 reverse transcriptase and integrase, and consequently weaken the body's immune system against many infections and diseases which eventually may lead to death. Fig. 8.16 represents the ribbon representation and the active site of HIV-1 reverse transcriptase.

A pyrrol–containing 1,5-benzothiazepine derivative **8.3.3.1** has been shown to be highly potent against HIV-1–induced cytopathicity in MT-4 cells, targeting the HIV-1 reverse transcriptase in enzyme assays (Fig. 8.17) (Di Santo & Costi, 2005). This anti–HIV-1 agent displays good inhibitory activity and selectivity with $EC_{50} = 0.3\ \mu M$ and

$CC_{50} = 68$ μM compared with nevirapine (**8.3.3.2**), an FDA–approved anti–HIV drug. However, the high cytotoxicity of the 1,5–benzothiazepine compound limits its selectivity index (SI = 226) when compared with the SI of the reference drug (SI > 800) (Di Santo & Costi, 2005).

8.3.4 Nonstructural protein 5B (NS5B)

Nonstructural protein 5B (NS5B) is an RNA–dependent RNA polymerase (RdRp) in the hepatitis C virus (HCV). This enzyme is responsible for the copy of the RNA viral genome in the virus replication process, and it contains the active site Gly-Asp-Asp sequence to bind with magnesium ions (cofactor) for the enzymatic activities (Patil, Gupta, Samanta, & Masand, 2011). HCV NS5B starts RNA synthesis by a de novo process through binding to the 3′–terminus of the viral RNA genome to generate a template/enzyme complex (Shim, Larson, Wu, & Hong, 2002). The complex is subsequently joined by two incoming nucleotides that are complementary to the 3′–terminal and penultimate bases, forming Watson-Crick base pairs with the template. The 3′–hydroxyl group of the initiating nucleotide launches a nucleophilic attack onto the α–phosphate of the nucleotide to produce the first phosphodiester bond and subsequently a dinucleotide initiation product (Shim et al., 2002). A series of 1,5–benzodiazepine-derived compounds (**8.3.4.1**) has been shown to prevent the formation of the first phosphodiester bond by blocking the RNA–dependent RNA polymerase activity of NS5B (Nyanguile et al., 2008). The analog (*S*)-**8.3.4.1a** displays the best inhibitory efficacy and selectivity against the HCV replication in the affected Huh7-CMV-Luc cell line with the K_i and EC_{50} values of 1.7 μM and 1.9 μM, respectively (Fig. 8.18). To investigate whether the enantiomer–specific inhibitory activity observed in the cellular assay is due to the suppression of NS5B function in the de novo mechanism, both (*S*) and (*R*) enantiomers of **8.3.4.1a** small molecules have been examined in a primer–independent assay using a transcript related to the 3′ untranslated area of the HCV genome. The results indicate that only (*S*)-**8.3.4.1a** can inhibit the NS5B polymerase function in the de novo assay. Molecular modeling studies of the binding interaction between the lead compound **8.3.4.1a** and NS5B show that the benzodiazepine interacts with the enzyme mostly *via* hydrophobic and aromatic binding contacts. The terminal benzyl group is intensely bound in the binding pockets of the enzyme by Pro197, Arg200, Leu384, Met414, Tyr415, and Tyr448 (Shim et al., 2002). Only one intermolecular hydrogen bond is observed between the exocyclic carbonyl of **8.3.4.1a** and Tyr448 of the enzyme.

8.3.4.1

R^1 = Ac, H; R^2 = Aryl
R^3 = Me, 4-Ph-t-Bu
R^4 = Me, H; R^5 = H, OH

(S)-8.3.4.1a
K_i = 1.7 µM for NS5B
EC_{50} = 1.9 µM for Huh7-CMV-Luc cells
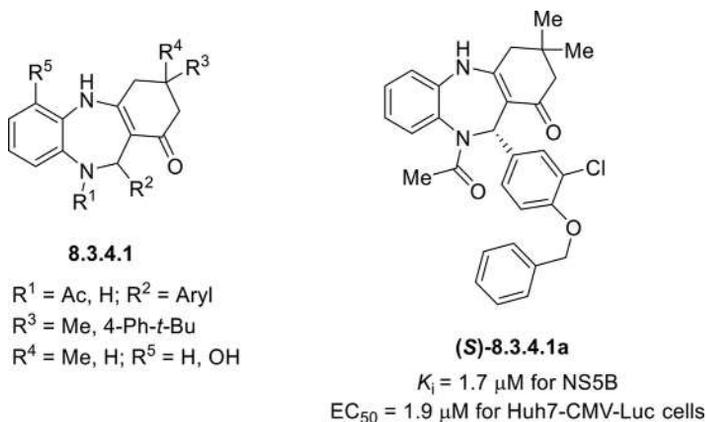
Figure 8.18 A series of 1,5-benzodiazepine-derived compounds as HCV NS5B polymerase inhibitors.

8.3.5 Angiotensin-converting enzyme (ACE)

Angiotensin–converting enzyme (ACE) is a dipeptidyl carboxypeptidase containing Zn^{2+} that transforms angiotensin I (A–I) into the powerful vaso-constrictor angiotensin II (A–II) (Bernstein, Martin, Edwards, & Bernstein, 1989). Angiotensin II is responsible for increasing total peripheral resistance by narrowing capillary arteries through activating the AT1 receptor, which is located on the vascular smooth muscle cells. Angiotensin II also facilitates peripheral noradrenergic neurotransmission by enhancing neuroprotein re-lease from sympathetic nerve terminals *via* inhibiting neuroprotein reuptake to the nerve terminal and increasing the vascular response to neuroprotein (Bernstein et al., 1989). The renin–angiotensin system plays a remarkable role in regulating arterial blood pressure in both the short and long term. In this respect, the suppression of ACE has been the topic of significant clinical research in the hope of lowering blood pressure in people with high renin levels (He, Liu, & Ma, 2013). Fig. 8.19 illustrates both ribbon and 3D space-filling models of the crystal structure of the human angiotensin–converting enzyme (ACE).

CV-5975 (**8.3.5.1**), (*R*)-3-[(*S*)-1-carboxy-5-(4-piperidyl)pentyl]amino-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepine-5-acetic acid, is the most well-known example of 1,5-benzothiazepine-derived ACE inhibitor (Fig. 8.20) (Inada, Itoh, Kamiya, Sugihara, & Nishikawa, 1988). Intravenous (i.v.) administration of rats with 0.3 mg/kg of CV-5975 displays high inhibitory potency against the ACE-induced conversion of angiotensin I (96% inhibition) with a high duration of activity (108 min). Furthermore,
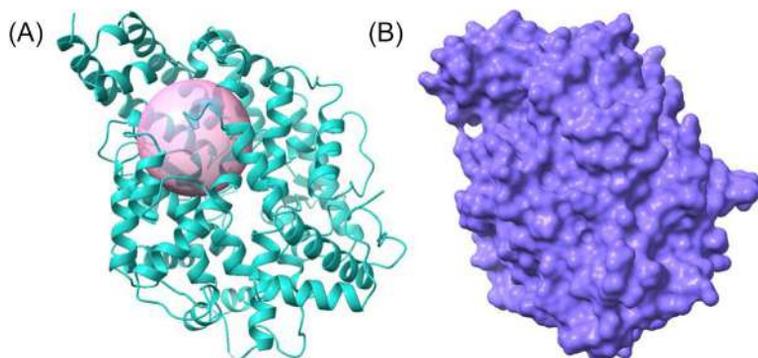
Figure 8.19 Crystal structure of the human angiotensin-converting enzyme (native): (A) The ribbon representation (PDB: 1O8A) and (B) 3D space-filling model. The pink sphere is shown as the enzyme active site.
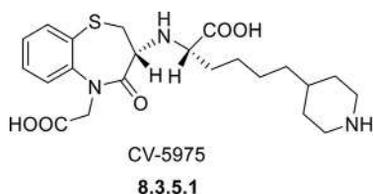
1) 96% inhibition (i.v.) of ACE-induced conversion of angiotensin I to angiotensin II in rats

2) IC_{50} = 0.0031 µM for ACE in rabbit lung

CV-5975

8.3.5.1

1) 75% inhibition (i.v.) of ACE-induced conversion of angiotensin I to angiotensin II in rats

2) IC_{50} = 0.00295 µM for ACE in rats

8.3.5.2

Figure 8.20 Two examples of chiral 1,5-benzothiazepines as angiotensin-converting enzyme (ACE) inhibitors.

the benzothiazepine derivative exhibits a strong ACE repressing effect on rabbit lung with IC_{50} = 0.0031 µM (i.v. = 0.3 mg/kg). *In vitro* analysis of the chiral 1,5–benzothiazepine **8.3.5.2** also shows a high ability to inhibit ACE in rats having an IC_{50} of 0.00295 µM, together with high efficacy against angiotensin I vasopressor responses in normotensive rats upon either oral (0.05 mg/kg, 75% inhibition) or i.v. (1.0 mg/kg, 39% inhibition) administration (Slade, Stanton, Ben–David, & Mazzenga, 1985).

8.3.6 Acetylcholinesterase (AChE)

Acetylcholinesterase (AChE), known as a cholinergic enzyme, is mainly located at postsynaptic neuromuscular junctions, especially in muscles
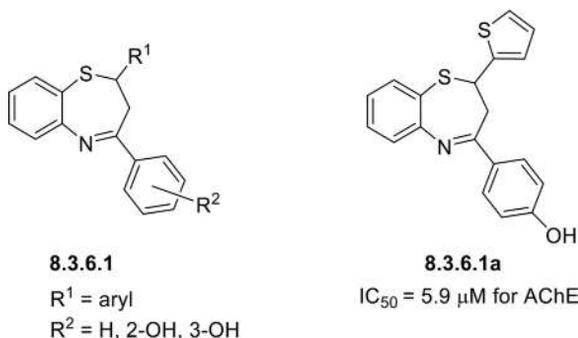
Figure 8.21 Two examples of 1,5-benzothiazepines as acetylcholinesterase (AChE) inhibitors.

and nerves. Acetylcholine (ACh), a neurotransmitter responsible for the conduction of electrical impulses among nerve cells, is easily hydrolyzed by AChE into acetic acid and choline, leading to the termination of the neural transmission and signaling between synapses (Anand & Singh, 2013). The inhibition of AChE can occur by some organic compounds that bear functional groups including the carbamate, quaternary or tertiary ammonium groups, and more importantly, can play as therapeutic agents in the diagnostic and/or treatment of several human diseases, such as myasthenia gravis, Alzheimer, dementia, post-operative ileus, and bladder distention (Anand & Singh, 2013). In simple words, the existence of acetylcholine in the neural system is vital for smooth muscle contraction, memorizing, and thinking, in which the AChE inhibitors can contribute to the preservation of ACh in the neural system.

A number of 2,3-dihydro-1,5-benzothiazepines have been demonstrated as active inhibitors against AChE (Ansari et al., 2012). The docking studies of these compounds with the AChE enzyme have revealed that both $\pi-\pi$ interactions and hydrogen bonding consist of the main nature of their binding contacts, the key contributing role of Trp84 in the ligand pocket. The lead compound **8.3.6.1a** is found to be the most active analog of the 1,5-benzothiazepines with an IC_{50} value of 5.9 μM against AChE *in silico* (Fig. 8.21). This high potency can be attributed to both strong hydrogen bonding between 3′-hydroxyl group of **8.3.6.1a** and the carbonyl oxygen of Glu199 of AChE as well as the $\pi-\pi$ interactions between the aromatic rings of the ligand with Trp84 and Phe330 of the enzyme (Ansari et al., 2012).
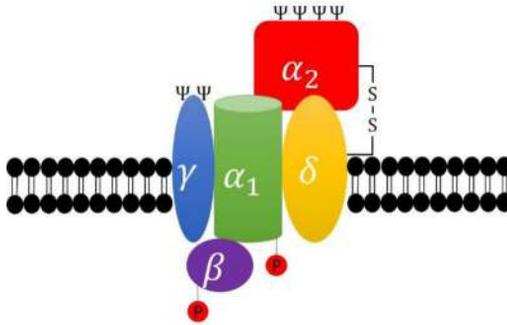
Figure 8.22   Structure of L-type Ca^{2+} channel.

8.4   Interaction of 1,5-benzodiazepines and 1,5-benzothiazepines with ion channels

8.4.1   Voltage-gated Ca^{2+} channels

Ca^{2+} is a versatile messenger of intracellular electrical signaling that can initiate many different cellular events. Voltage-gated calcium (Ca^{2+}) channels are the essential modulators of the depolarization–induced release of neurotransmitters through regulating calcium ions entry into neurons (Pelzer, Pelzer, & McDonald, 1990). There are four types of Ca^{2+} channels on the cells in vertebrates including L-type (located in cardiac and muscle smooth cells), T-type (located in pacemakers' cells), P-type (neuromuscular junctions), and N-type channels (located in neural cells) (Pelzer et al., 1990). The L-type channel is comprised of five protein subunits including $\alpha 1$, $\alpha 2$, β, γ, and δ (Fig. 8.22). The $\alpha 1$ subunit is the major body of the calcium channel and any change in the conformational form of this subunit may play a critical role in the channel function and membrane depolarization (Simms & Zamponi, 2014).

1,5–Benzodiazepines have been shown to interact with the L–type Ca^{2+} channel as antagonist ligands, regulating the channel's activity. The antagonistic activity of 1,5-benzothiazepines on calcium channels is a major reason that they can be considered as cardiovascular modulators for the treatment of cardiovascular diseases such as hypertension and arrhythmias (Nagao, Sato, Iwasawa, Takada, & Ishida, 1972; Nagao, Sato, Nakajima, & Kiyomoto, 1973). Since calcium ion is essential for muscle contraction, cell stimulation, and cell membrane depolarization, the right functioning of the calcium channels on
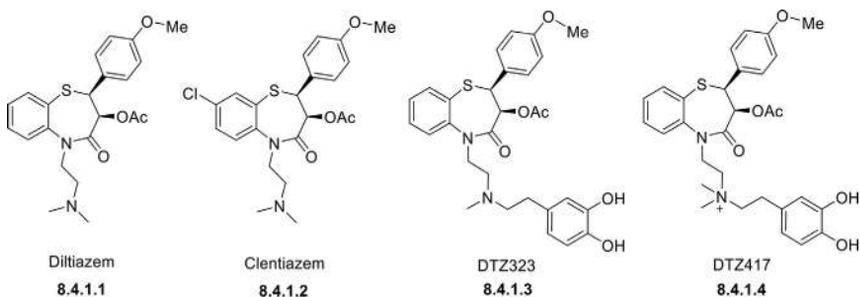
Compound	K_i (μM)	IC$_{50}$ (μM)[a]
Diltiazem	0.31	0.631
Clentiazem	0.061	0.00677[b]
DTZ323	0.0066	0.0257
DTZ417	0.041	1.2

[a]Measured using guinea pig ventricular myocytes.
[b]Measured using depolarized human coronary artery cells.

Figure 8.23 The chemical structures of diltiazem, clentiazem, DTZ232, and DTZ417 as efficient voltage-gated calcium channel blocking agents.

the cell entries is a crucial matter to keep the cardiovascular system devoid of any dysfunction or disease. The first discovery of a 1,5-benzothiazepine, diltiazem (CRD–401), as a calcium channel antagonist in 1971 was a great breakthrough in the treatment of heart diseases (Sato, Nagao, Yamaguchi, Nakajima, & Kiyomoto, 1971). Later, other 1,5-benzothiazepines, as well as 1,5–benzodiazepines, were discovered as effective blocking agents of the L–type calcium channels (Atwal, Bergey, Hedberg, & Moreland, 1987; Kendall & Okopski, 1986; Narita et al., 1988; Narita et al., 1990). Considering the action mechanism of 1,5-benzothiazepines, these compounds and calcium ions act in a competitive way to interact with calcium channels. Accordingly, the intracellular calcium concentration decrease leads to a reduction in the contraction of smooth muscle cells in the blood vessels. Consequently, an increase in the diameter of the blood vessels and a decrease in blood pressure occur, which can be a promising therapeutic strategy for the treatment of hypertensive heart diseases (Williams, 1990). The first clinically used 1,5–benzothiazepines for treating cardiovascular diseases are diltiazem (**8.4.1.1**) and clentiazem (**8.4.1.2**) (Fig. 8.23) (Kawakita et al., 1991; Pool et al., 1986). These molecules display high suppressing activity against L–type Ca^{2+} channel current with the K_i values of 0.31 μM and 0.061 μM, respectively. Apart from the calcium channel blocking effect, diltiazem (**8.4.1.1**) and

clentiazem (**8.4.1.2**) exhibit both vasorelaxant and negative inotropic actions, similar to other non-benzothiazepine Ca^{2+} antagonists such as nifedipine (Narita, Zera, & Ginsburg, 1990). Further modification of diltiazem (**8.4.1.1**) has resulted in the discovery of DTZ323 (**8.4.1.3**) as the most potent and selective L-type calcium channel antagonist ($K_i = 0.0066$ μM) among 1,5-benzothiazepines, displaying 48 times more potency than diltiazem and nine times more active than clentiazem (Hagiwara, Adachi-Akahane, & Nagao, 1997; Kurokawa, Adachi-Akahane, & Nagao, 1997). *In vivo* studies on Hartley guinea pigs have shown that DTZ323 blocks the L-type Ca^{2+} channels' currents more selectively than both T-type Ca^{2+} and Na^+ channels' currents, working more selectively on partially depolarized ischemic regions in the heart (Kurokawa et al., 1997). DTZ417 (**8.4.1.4**), a quaternary ammonium analog of DTZ323, is also a good inhibitor against L-type Ca^{2+} channel currents with a K_i value of 0.041 μM (Fig. 8.23).

8.4.2 Voltage-gated K$^+$ channels (Kv)

Potassium (K^+) channels, encoded by about 80 genes, are the most diverse and widely distributed class of ion channels in mammals. These channels control K^+ flux and are responsible for cell survival and growth, as well as the generation of electric potentials and currents across excitable cells such as neurons, cardiomyocytes, and muscles (Choe, 2002; Coetzee et al., 1999). The voltage-gated potassium (K^+) channel is the most abundant subtype of the K^+ channel with broad a distribution in the nervous system and cardiac tissues. Such a channel consists of four pore-forming α-subunits (Fig. 8.24a,b), where each subunit is composed of six transmembrane segments (S1-S6). While the first S1 to S4 transmembrane fragments constitute a voltage sensor, the last two S5 and S6 transmembrane units form a pore loop as the pore domain (S5-P-S6) (Fig. 8.24c) (González et al., 2012). There are 12 subfamilies of Kv channels, named Kv1 to Kv12, where each of them presents multiple subtypes (*i.e.*, Kv3.1, Kv3.2, Kv3.3, and Kv3.4). The voltage-gated potassium (K^+) channel is activated by fluctuations in transmembrane voltage or by binding small molecules to the membrane. Therefore, these channels offer enormous potential for the development of novel medications to treat cancer, autoimmune illnesses, as well as metabolic, neurological, and cardiovascular disorders (Wulff, Castle, & Pardo, 2009).

1,5-Benzothiazepine-derived L-type Ca^{2+} channel blockers have been shown to block voltage-gated potassium (K^+) channels. For example, diltiazem (**8.4.1.1**) at the concentration of 100 μM moderately suppresses
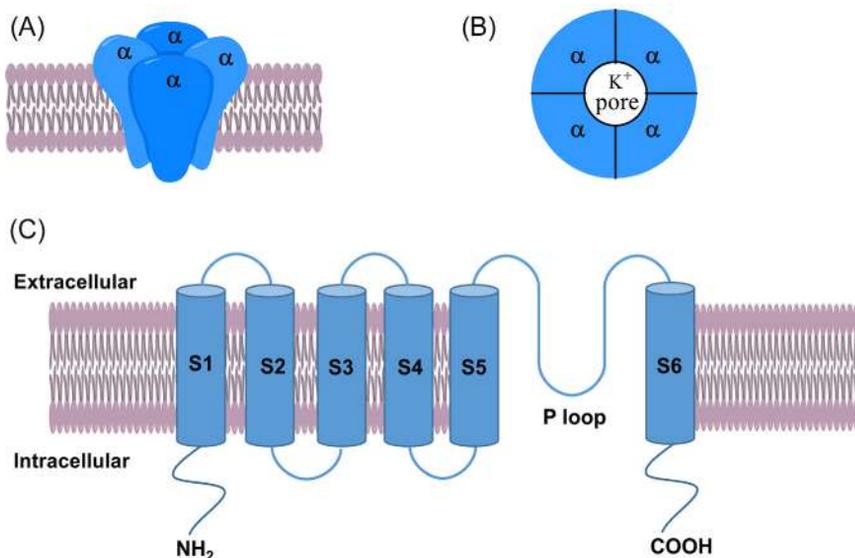
Figure 8.24 Schematic representation of the voltage-gated K^+ channel. (A, B) The α-subunits composition of the voltage-gated K^+ channel from side and up views, respectively. (C) Transmembrane segment compositions of an α-subunit.

Kv1.5 currents in mouse erythroleukemia (MEL) cells and *Xenopus* oocytes (Grissmer et al., 1994; Rolf et al., 2000). However, the detailed action mechanism of diltiazem has not been studied yet. Caballero *et al.* also investigated the effects of diltiazem (**8.4.1.1**) on the human Kv1.5 (hKv1.5) and Kv4.3 channels, which are responsible for the cardiac ultrarapid delayed rectifier (I_{Kur}) and the 4–aminopyridine sensitive transient outward (I_{to}) K^+ currents, respectively (Caballero et al., 2004). The results of concentration–dependent effects of diltiazem on the K^+ channels have demonstrated that the molecule, at low concentrations of up to 0.1 μM, reduces hKv1.5 and Kv4.3 currents by binding to both the open and inactivated state of the K^+ channels. It is hypothesized that one diltiazem molecule may reach the opened pores to prevent the K^+ flow. However, high concentrations of bulky diltiazem next to the binding site result in steric hindrance interactions, which may decrease the compound's blocking efficacy (Caballero et al., 2004). Furthermore, diltiazem alters the voltage dependency of hKv1.5 and Kv4.3 channels' inactivation, in which the channel blockade significantly increases with the occurrence of channel inactivation. These results also suggest that diltiazem also attaches to the inactivated state of the channels (Caballero et al., 2004).

8.5 Interaction of 1,5-benzodiazepines and 1,5-benzothiazepines with platelets

Platelets are the smallest human blood cells that play pivotal roles not only in hemostasis and thrombosis processes, but also in wound healing, inflammation, and tumor metastasis (Jurk & Kehrel, 2005). They are discharged into the blood as anucleated fragments from megakaryocytes in the bone marrow. Following a vessel wall injury, platelets are recruited from the circulatory system to the exposed subendothelial matrix, generating a hemostatic plug to seal the leak. Platelet immobilization at vascular injury sites is a complex process that requires the cooperation of various platelet ligands and receptors (Ramasamy, 2004). Atherothrombosis is a degenerative blood disease identified by the aggregation of lipids, minerals, and fibrous material inside the blood vessel. Platelet dysfunction is one of the main reasons for the occurrence of atherothrombosis in the arteries. As a result, platelet–inhibiting drugs are an important part of the main and secondary treatment of atherosclerosis and atherothrombosis (Bhatt, 2009).

Several 1,5-benzodiazepines and 1,5-benzothiazepines have been developed as potent antiplatelet agents. For example, diltiazem (**8.4.1.1**) displays high inhibitory effects on platelet aggregation *via* regulating Ca^{2+} blood concentration (Mehta, Mehta, Ostrowski, & Brigmon, 1983). Ca^{2+} flow across the platelet membranes stimulates the platelet aggregation induced by adenosine diphosphate (ADP, a platelet agonist). Diltiazem (**8.4.1.1**) at a low concentration of 11 μM prevents this platelet activation process by blocking Ca^{2+} channels and subsequently reducing Ca^{2+} flux in the platelet membranes. 4′,8-Dimethyl analog of diltiazem, known as TA-993 (**8.5.1**), and its metabolites have also been demonstrated to suppress platelet aggregation in human platelets *in vitro* (Odawara et al., 1996). MB3 (**8.5.2**) is the most potent metabolite of TA-993 (**8.5.1**) with an IC_{50} value of 0.3 μM (Fig. 8.25), which may be attributed to the high plasma level of MB3 after administration. TA-993 not only prevents both primary and secondary phases of platelet aggregation induced by ADP but also displays a disaggregating effect on human platelet aggregation (Odawara et al., 1996). While oral administration of TA-993 (**8.5.1**) to dogs inhibits platelet aggregation *ex vivo* in a dose-dependent manner (0.3 to 10 mg/kg), rats are significantly protected (65% survival) against both collagen- and epinephrine–induced thromboembolic death using 10 mg/kg *in vivo*. The Ca^{2+}-antagonistic activity of TA-993 (**8.5.1**) on Ca^{2+} channels is approximately 1/10 less than diltiazem in depolarized canine basilar arteries, indicating that its mechanism of antiplatelet action must be different from that of diltiazem (**8.4.1.1**).
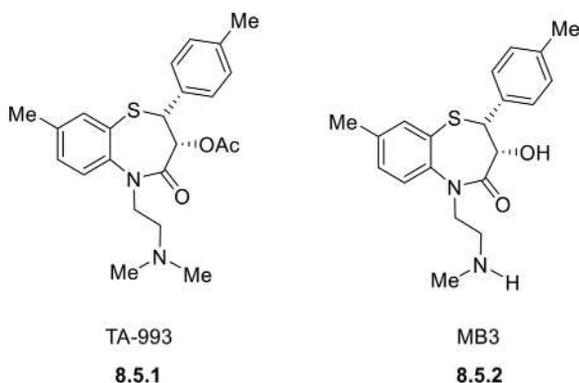
Compound	IC$_{50}$^{a} (μM)		
	Human	Dogs	Rats
TA-993	113.3	29.1	49.3
MB3	0.3	0.3	0.4

^{a}Measured against collagen-induced platelet aggregation

Figure 8.25 The chemical structures of TA-993 and its metabolite (MB3) as potent antiplatelet agents.

8.6 Conclusion

1,5-Benzodiazepines and 1,5-benzothiazepines are privileged scaffolds in a wide range of medications, such as analgesic, hypnotic, anticonvulsant, and cardiovascular drugs. Appropriate adjustment of binding selectivity through structural modifications is a fundamental objective in the discovery and optimization of these skeletons in therapeutic applications. 1,5-Benzodiazepines and 1,5-benzothiazepines work in a complex environment with numerous potential interaction partners. Various receptors, enzymes, and proteins have been shown to selectively interact with these heterocyclic compounds with different binding affinities. On many occasions, unexpected interactions may develop undesired adverse effects. This chapter demonstrated that the majority of the 1,5-benzodiazepine- and 1,5-benzothiazepine-derived molecules serve as antagonists *via* binding to the target biomolecules and regulating their biological activities through some molecular mechanisms yet to be precisely identified. Therefore, the management of patients who are receiving these medications requires a detailed understanding of both the action mechanisms of the drug and the relevant interactions with the biomolecules. Through continued expansion of experimental perceptions related to ligand-biomolecule interactions

and cellular events in biological systems, a greater definition of biological behaviors of 1,5-benzodiazepines and 1,5-benzothiazepines may become clearer as the prospective of these compounds.

References

Aastha, P., Navneet, K., Anshu, A., Pratima, S., & Dharma, K. (2013). 1,5-Benzodiazepines: Overview of properties and synthetic aspects. *Research Journal of Chemical Sciences, 3*(7), 90–103.

Amblard, M., Daffix, I., Bedos, P., Bergé, G., Pruneau, D., Paquet, J. L., et al. (1999). Design and synthesis of potent bradykinin agonists containing a benzothiazepine moiety. *Journal of Medicinal Chemistry, 42*(20), 4185–4192.

Amblard, M., Daffix, I., Bergé, G., Calmès, M., Dodey, P., Pruneau, D., et al. (1999). Synthesis and characterization of bradykinin B(2) receptor agonists containing constrained dipeptide mimics. *Journal of Medicinal Chemistry, 42*(20), 4193–4201.

Anand, P., & Singh, B. (2013). A review on cholinesterase inhibitors for Alzheimer's disease. *Archives of Pharmacal Research, 36*(4), 375–399. https://doi.org/10.1007/s12272-013-0036-3.

Ansari, F. L., Kalsoom, S., Ali, Z., & Jabeen, F. (2012). *In silico* studies on 2,3-dihydro-1,5-benzothiazepines as cholinesterase inhibitors. *Medicinal Chemistry Research, 21*, 2329–2339. https://doi.org/10.1007/s00044-011-9754-6.

Archer, G. A., & Sternbach, L. H. (1968). Chemistry of benzodiazepines. *Chemical Reviews, 68*(6), 747–784. https://doi.org/10.1021/cr60256a004.

Atwal, K. S., Bergey, J. L., Hedberg, A., & Moreland, S. (1987). Synthesis and biological activity of novel calcium channel blockers: 2,5-Dihydro-4-methyl-2-phenyl-1,5-benzothiazepine-3-carboxylic acid esters and 2,5-dihydro-4-methyl-2-phenyl-1,5-benzodiazepine-3-carboxylic acid esters. *Journal of Medicinal Chemistry, 30*(4), 635–640.

Bariwal, J. B., Upadhyay, K. D., Manvar, A. T., Trivedi, J. C., Singh, J. S., Jain, K. S., et al. (2008). 1,5-Benzothiazepine, a versatile pharmacophore: A review. *European Journal of Medicinal Chemistry, 43*(11), 2279–2290. https://doi.org/10.1016/j.ejmech.2008.05.035.

Barnes, N. M., & Sharp, T. (1999). A review of central 5-HT receptors and their function. *Neuropharmacology, 38*(8), 1083–1152.

Bernstein, K. E., Martin, B. M., Edwards, A. S., & Bernstein, E. A. (1989). Mouse angiotensin-converting enzyme is a protein composed of two homologous domains. *The Journal of Biological Chemistry, 264*(20), 11945–11951.

Beurdeley-Thomas, A., Miccoli, L., Oudard, S., Dutrillaux, B., & Poupon, M. F. (2000). The peripheral benzodiazepine receptors: A review. *Journal of Neuro-Oncology, 46*(1), 45–56.

Bhatt, D. L. (2009). Role of antiplatelet therapy across the spectrum of patients with coronary artery disease. *The American Journal of Cardiology, 103*(3), 11A–19A. https://doi.org/10.1016/j.amjcard.2008.11.018.

Caballero, R., Gómez, R., Núñez, L., Moreno, I., Tamargo, J., & Delpón, E. (2004). Diltiazem inhibits hKv1.5 and Kv4.3 currents at therapeutic concentrations. *Cardiovascular Research, 64*(3), 457–466.

Campbell, M., Young, P. I., Bateman, D. N., Smith, J. M., & Thomas, S. H. (1999). The use of atypical antipsychotics in the management of schizophrenia. *British Journal of Clinical Pharmacology, 47*(1), 13–22.

Campiani, G., Fiorini, I., De Filippis, M. P., Ciani, S. M., Garofalo, A., Nacci, V., et al. (1996). Cardiovascular characterization of pyrrolo[2,1-d][1,5]benzothiazepine derivatives binding selectively to the peripheral-type benzodiazepine receptor (PBR): From dual PBR affinity and calcium antagonist activity to novel and selective calcium entry blockers. *Journal of Medicinal Chemistry, 39*(15), 2922–2938.

Chang, T.-Y., Li, B.-L., Chang, C. C. Y., & Urano, Y. (2009). Acyl-coenzyme A: cholesterol acyltransferases. *American Journal of Physiology. Endocrinology and Metabolism, 297*(1), E1–E9. https://doi.org/10.1152/ajpendo.90926.2008.

Chen, Z.-W., Bracamontes, J. R., Budelier, M. M., Germann, A. L., Shin, D. J., Kathiresan, K., et al. (2019). Multiple functional neurosteroid binding sites on GABA$_A$ receptors. *PLoS Biology, 17*(3), e3000157. https://doi.org/10.1371/journal.pbio.3000157.

Choe, S. (2002). Potassium channel structures. *Nature Reviews. Neuroscience, 3*(2), 115–121.

Coetzee, W. A., Amarillo, Y., Chiu, J., Chow, A., Lau, D., McCormack, T., et al. (1999). Molecular diversity of K$^+$ channels. *Annals of the New York Academy of Sciences, 868*, 233–285.

Delelis, O., Carayon, K., Saïb, A., Deprez, E., & Mouscadet, J.-F. (2008). Integrase and integration: Biochemical activities of HIV-1 integrase. *Retrovirology, 5*, 114. https://doi.org/10.1186/1742-4690-5-114.

Di Santo, R., & Costi, R. (2005). 2*H*-Pyrrolo[3,4–*b*] [1,5]benzothiazepine derivatives as potential inhibitors of HIV-1 reverse transcriptase. *Farmaco (Societa Chimica Italiana: 1989), 60*(5), 385–392.

Eglen, R. M. (2006). Muscarinic receptor subtypes in neuronal and non-neuronal cholinergic function. *Autonomic & Autacoid Pharmacology, 26*(3), 219–233.

Finley, J. J., Konstam, M. A., & Udelson, J. E. (2008). Arginine vasopressin antagonists for the treatment of heart failure and hyponatremia. *Circulation, 118*(4), 410–421. https://doi.org/10.1161/CIRCULATIONAHA.108.765289.

Fiorini, I., Nacci, V., Ciani, S. M., Garofalo, A., Campiani, G., Savini, L., et al. (1994). Novel ligands specific for mitochondrial benzodiazepine receptors: 6-arylpyrrolo[2,1–*d*][1,5]benzothiazepine derivatives. Synthesis, structure-activity relationships, and molecular modeling studies. *Journal of Medicinal Chemistry, 37*(10), 1427–1438.

Gattone, V. H., Wang, X., Harris, P. C., & Torres, V. E. (2003). Inhibition of renal cystic disease development and progression by a vasopressin V2 receptor antagonist. *Nature Medicine, 9*(10), 1323–1326.

Giovannoni, M. P., Piaz, V. D., Vergelli, C., & Barlocco, D. (2003). Selective ACAT inhibitors as promising antihyperlipidemic, antiathero-sclerotic and anti-Alzheimer drugs. *Mini Reviews in Medicinal Chemistry, 3*(6), 576–584.

González, C., Baez-Nieto, D., Valencia, I., Oyarzún, I., Rojas, P., Naranjo, D., et al. (2012). K$^+$ channels: Function-structural overview. *Comprehensive Physiology, 2*(3), 2087–2149. https://doi.org/10.1002/cphy.c110047.

Grilo, A. L., & Mantalaris, A. (2019). Apoptosis: A mammalian cell bioprocessing perspective. *Biotechnology Advances, 37*(3), 459–475. https://doi.org/10.1016/j.biotechadv.2019.02.012.

Grissmer, S., Nguyen, A. N., Aiyar, J., Hanson, D. C., Mather, R. J., Gutman, G. A., et al. (1994). Pharmacological characterization of five cloned voltage-gated K$^+$ channels, types Kv1.1, 1.2, 1.3, 1.5, and 3.1, stably expressed in mammalian cell lines. *Molecular Pharmacology, 45*(6), 1227–1234.

Hagiwara, M., Adachi-Akahane, S., & Nagao, T. (1997). High-affinity binding of DTZ323, a novel derivative of diltiazem, to rabbit skeletal muscle L-type Ca^{++} channels. *The Journal of Pharmacology and Experimental Therapeutics, 281*(1), 173–179.

Hall, J. M. (1997). Bradykinin receptors. *General Pharmacology, 28*(1), 1–6.

He, H.-L., Liu, D., & Ma, C.-B. (2013). Review on the angiotensin-I-converting enzyme (ACE) inhibitor peptides from marine proteins. *Applied Biochemistry and Biotechnology, 169*(3), 738–749. https://doi.org/10.1007/s12010-012-0024-y.

Henke, B. R., Aquino, C. J., Birkemo, L. S., Croom, D. K., Dougherty, R. W., Ervin, G. N., et al. (1997). Optimization of 3-(1*H*-indazol-3-ylmethyl)-1,5-benzodiazepines as potent, orally active CCK-A agonists. *Journal of Medicinal Chemistry, 40*(17), 2706–2725.

Henke, B. R., Willson, T. M., Sugg, E. E., Croom, D. K., Dougherty, R. W., Queen, K. L., et al. (1996). 3-(1*H*-indazol-3-ylmethyl)-1,5-benzodiazepines: CCK-A agonists that demonstrate oral activity as satiety agents. *Journal of Medicinal Chemistry, 39*(14), 2655–2658.

Herranz, R. (2003). Cholecystokinin antagonists: Pharmacological and therapeutic potential. *Medicinal Research Reviews, 23*(5), 559–605.

Hess, J. F., Borkowski, J. A., Young, G. S., Strader, C. D., & Ransom, R. W. (1992). Cloning and pharmacological characterization of a human bradykinin (BK–2) receptor. *Biochemical and Biophysical Research Communications, 184*(1), 260–268.

Hökfelt, T., Rehfeld, J. F., Skirboll, L., Ivemark, B., Goldstein, M., & Markey, K. (1980). Evidence for coexistence of dopamine and CCK in meso-limbic neurones. *Nature, 285*(5765), 476–478.

Holdgate, G. A., Meek, T. D., & Grimley, R. L. (2018). Mechanistic enzymology in drug discovery: A fresh perspective. *Nature Reviews Drug Discovery, 17*(2), 115–132. https://doi.org/10.1038/nrd.2017.219.

Howl, J., & Payne, S. J. (2003). Bradykinin receptors as a therapeutic target. *Expert Opinion on Therapeutic Targets, 7*(2), 277–285.

Inada, Y., Itoh, K., Kamiya, K., Sugihara, H., & Nishikawa, K. (1988). (*R*)-3-[(*S*)-1-carboxy-5-(4-piperidyl)pentyl]amino-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepine-5-acetic acid (CV-5975): A new potent and long-lasting inhibitor of angiotensin converting enzyme. *Japanese Journal of Pharmacology, 47*(2), 135–141.

Jones, C. K., Byun, N., & Bubser, M. (2012). Muscarinic and nicotinic acetylcholine receptor agonists and allosteric modulators for the treatment of schizophrenia. *Neuropsychopharmacology, 37*(1), 16–42. https://doi.org/10.1038/npp.2011.199.

Jurk, K., & Kehrel, B. E. (2005). Platelets: Physiology and biochemistry. *Seminars in Thrombosis and Hemostasis, 31*(4), 381–392.

Juul, K. V., Bichet, D. G., Nielsen, S., & Nørgaard, J. P. (2014). The physiological and pathophysiological functions of renal and extrarenal vasopressin V2 receptors. *American Journal of Physiology. Renal Physiology, 306*(9), F931–F940. https://doi.org/10.1152/ajprenal.00604.2013.

Kapur, S., & Remington, G. (1996). Serotonin-dopamine interaction and its relevance to schizophrenia. *The American Journal of Psychiatry, 153*(4), 466–476.

Kawakita, S., Kinoshita, M., Ishikawa, H., Kagoshima, T., Katori, R., Ishikawa, K., et al. (1991). Efficacy and safety of clentiazem in patients with essential hypertension: Results of an early pilot test. *Clinical Cardiology, 14*(1), 53–60.

Kendall, M. J., & Okopski, J. V. (1986). Calcium antagonism—with special reference to diltiazem. *Journal of Clinical and Hospital Pharmacy, 11*(3), 159–174.

Kurokawa, J., Adachi-Akahane, S., & Nagao, T. (1997). Effects of a novel, potent benzothiazepine Ca^{2+} channel antagonist, DTZ323, on guinea-pig ventricular myocytes. *European Journal of Pharmacology, 325*(2–3), 229–236.

Kyle, D. J., Blake, P. R., Smithwick, D., Green, L. M., Martin, J. A., Sinsko, J. A., et al. (1993). NMR and computational evidence that high-affinity bradykinin receptor antagonists adopt C-terminal beta-turns. *Journal of Medicinal Chemistry, 36*(10), 1450–1460.

Lau, J., Rousseau, J., Kwon, D., Bénard, F., & Lin, K.-S. (2020). A systematic review of molecular imaging agents targeting bradykinin B1 and B2 receptors. *Pharmaceuticals (Basel, Switzerland), 13*(8), 199–219. https://doi.org/10.3390/ph13080199.

Lembeck, F., Griesbacher, T., Eckhardt, M., Henke, S., Breipohl, G., & Knolle, J. (1991). New, long-acting, potent bradykinin antagonists. *British Journal of Pharmacology, 102*(2), 297–304.

López-Farré, A. J., Sacristán, D., Zamorano-León, J. J., San-Martín, N., & Macaya, C. (2008). Inhibition of acyl-CoA cholesterol acyltransferase by F12511 (Eflucimibe): Could it be a new antiatherosclerotic therapeutic? *Cardiovascular Therapeutics, 26*(1), 65–74. https://doi.org/10.1111/j.1527-3466.2007.00030.x.

Marceau, F., & Regoli, D. (2004). Bradykinin receptor ligands: Therapeutic perspectives. *Nature Reviews Drug Discovery, 3*(10), 845–852.

Mc Gee, M. M., Gemma, S., Butini, S., Ramunno, A., Zisterer, D. M., Fattorusso, C., et al. (2005). Pyrrolo[1,5]benzoxa(thia)zepines as a new class of potent apoptotic agents. Biological studies and identification of an intracellular location of their drug target. *Journal of Medicinal Chemistry, 48*(13), 4367–4377.

Mehta, P., Mehta, J., Ostrowski, N., & Brigmon, L. (1983). Inhibitory effects of diltiazem on platelet activation caused by ionophore A23187 plus ADP or epinephrine in subthreshold concentrations. *The Journal of Laboratory and Clinical Medicine, 102*(3), 332–339.

Menke, J. G., Borkowski, J. A., Bierilo, K. K., MacNeil, T., Derrick, A. W., Schneck, K. A., et al. (1994). Expression cloning of a human B1 bradykinin receptor. *The Journal of Biological Chemistry, 269*(34), 21583–21586.

Morrison, D. K. (2012). MAP kinase pathways. *Cold Spring Harbor Perspectives in Biology, 4*(11), a011254. https://doi.org/10.1101/cshperspect.a011254.

Nagao, T., Narita, H., Sato, M., Nakajima, H., & Kiyomoto, A. (1982). Development of diltiazem, a calcium antagonist: Coronary vasodilating and antihypertensive actions. *Clinical and Experimental Hypertension. Part A, Theory and Practice, 4*(1–2), 285–296.

Nagao, T., Sato, M., Iwasawa, Y., Takada, T., & Ishida, R. (1972). Studies on a new 1,5-benzothiazepine derivative (CRD-401). 3. Effects of optical isomers of CRD-401 on smooth muscle and other pharmacological properties. *Japanese Journal of Pharmacology, 22*(4), 467–478.

Nagao, T., Sato, M., Nakajima, H., & Kiyomoto, A. (1973). Studies on a new 1,5-benzothiazepine derivative (CRD-401). IV. Coronary vasodilating effect and structure-activity relationship. *Chemical & Pharmaceutical Bulletin, 21*(1), 92–97.

Narita, H., Gaino, M., Suzuki, T., Kurosawa, H., Inoue, H., & Nagao, T. (1990). Synthesis and pharmacological properties of azido derivatives of 1,5-benzothiazepine Ca antagonist. *Chemical & Pharmaceutical Bulletin, 38*(2), 407–410.

Narita, H., Murata, S., Yabana, H., Kikkawa, K., Sugawara, Y., Akimoto, Y., et al. (1988). Long-lasting hypotensive and antihypertensive effects of a new 1,5-benzothiazepine calcium antagonist in hypertensive rats and renal hypertensive dogs. *Arzneimittel-Forschung, 38*(4), 515–520.

Narita, H., Zera, P. H., & Ginsburg, R. (1990). Mechanism of action of clentiazem on human coronary artery and myocardium. *Cardiovascular Drugs and Therapy, 4*(4), 1097–1104.

Nicholson, M. W., Sweeney, A., Pekle, E., Alam, S., Ali, A. B., Duchen, M., et al. (2018). Diazepam–induced loss of inhibitory synapses mediated by PLCδ/ Ca^{2+}/calcineurin signalling downstream of GABA$_A$ receptors. *Molecular Psychiatry, 23*(9), 1851–1867. https://doi.org/10.1038/s41380-018-0100-y.

Nikmaram, N., Dar, B. N., Roohinejad, S., Koubaa, M., Barba, F. J., Greiner, R., et al. (2017). Recent advances in γ-aminobutyric acid (GABA) properties in pulses: An overview. *Journal of the Science of Food and Agriculture, 9*7(9), 2681–2689. https://doi.org/10.1002/jsfa.8283.

Nyanguile, O., Pauwels, F., Van den Broeck, W., Boutton, C. W., Quirynen, L., Ivens, T., et al. (2008). 1,5-benzodiazepines, a novel class of hepatitis C virus polymerase nonnucleoside inhibitors. *Antimicrobial Agents and Chemotherapy, 52*(12), 4420–4431. https://doi.org/10.1128/AAC.00669-08.

Odawara, A., Kikkawa, K., Katoh, M., Toryu, H., Shimazaki, T., & Sasaki, Y. (1996). Inhibitory effects of TA-993, a new 1,5-benzothiazepine derivative, on platelet aggregation. *Circulation Research, 78*(4), 643–649.

Palm, C., Reimann, D., & Gross, P. (2001). The role of V2 vasopressin antagonists in hyponatremia. *Cardiovascular Research, 51*(3), 403–408.

Papadopoulos, V. (1993). Peripheral-type benzodiazepine/diazepam binding inhibitor receptor: Biological role in steroidogenic cell function. *Endocrine Reviews, 14*(2), 222–240.

Patil, V. M., Gupta, S. P., Samanta, S., & Masand, N. (2011). Current perspective of HCV NS5B inhibitors: A review. *Current Medicinal Chemistry, 18*(36), 5564–5597.

Pelzer, D., Pelzer, S., & McDonald, T. F.   (1990). Properties and regulation of calcium channels in muscle cells. *Reviews of Physiology, Biochemistry and Pharmacology, 114*, 107–207. https://doi.org/10.1007/BFb0031019.

Peter, S. A. S., D'Amato, M., & Beglinger, C. (2006). CCK1 antagonists: Are they ready for clinical use? *Digestive Diseases (Basel, Switzerland), 24*(1–2), 70–82.

Pool, P. E., Massie, B. M., Venkataraman, K., Hirsch, A. T., Samant, D. R., Seagren, S. C., et al. (1986). Diltiazem as monotherapy for systemic hypertension: A multicenter, randomized, placebo-controlled trial. *The American Journal of Cardiology, 57*(4), 212–217.

Ramasamy, I. (2004). Inherited bleeding disorders: Disorders of platelet adhesion and aggregation. *Critical Reviews in Oncology/Hematology, 49*(1), 1–35.

Ranran, W. Y., Yang, W., Yanqing, B., & Ping, Z. (2020). Synthesis and antifungal activity of 1,5-benzothiazepines containing 1,2,3-triazole. *Chinese Journal of Organic Chemistry, 40*(2), 398–407. https://doi.org/10.6023/cjoc201907056.

Robben, J. H., Knoers, N. V. A. M., & Deen, P. M. T. (2004). Regulation of the vasopressin V2 receptor by vasopressin in polarized renal collecting duct cells. *Molecular Biology of the Cell, 15*(12), 5693–5699.

Rolf, S., Haverkamp, W., Borggrefe, M., Musshoff, U., Eckardt, L., Mergenthaler, J., et al. (2000). Effects of antiarrhythmic drugs on cloned cardiac voltage-gated potassium channels expressed in *Xenopus* oocytes. *Naunyn-Schmiedeberg's Archives of Pharmacology, 362*(1), 22–31.

Sato, M., Nagao, T., Yamaguchi, I., Nakajima, H., & Kiyomoto, A. (1971). Pharmacological studies on a new l,5-benzothiazepine derivative (CRD-401). *Arzneimittel-Forschung, 21*(9), 1338–1343.

Shim, J. H., Larson, G., Wu, J. Z., & Hong, Z. (2002). Selection of 3'-template bases and initiating nucleotides by hepatitis C virus NS5B RNA-dependent RNA polymerase. *Journal of Virology, 76*(14), 7030–7039.

Sigel, E., & Ernst, M. (2018). Receptors. *Trends in Pharmacological Sciences, 39*(7), 659–671. https://doi.org/10.1016/j.tips.2018.03.006.

Simms, B. A., & Zamponi, G. W. (2014). Neuronal voltage-gated calcium channels: Structure, function, and dysfunction. *Neuron, 82*(1), 24–45. https://doi.org/10.1016/j.neuron.2014.03.016.

Slade, J., Stanton, J. L., Ben-David, D., & Mazzenga, G. C. (1985). Angiotensin converting enzyme inhibitors: 1,5-Benzothiazepine derivatives. *Journal of Medicinal Chemistry, 28*(10), 1517–1521.

Svoboda, J., Popelikova, A., & Stuchlik, A. (2017). Drugs interfering with muscarinic acetylcholine receptors and their effects on place navigation. *Frontiers in Psychiatry, 8*, 215. https://doi.org/10.3389/fpsyt.2017.00215.

Tabas, I., Rosoff, W. J., & Boykow, G. C. (1988). Acyl coenzyme A: cholesterol acyl transferase in macrophages utilizes a cellular pool of cholesterol oxidase-accessible cholesterol as substrate. *The Journal of Biological Chemistry, 263*(3), 1266–1272.

Tabata, H., Wada, N., Takada, Y., Nakagomi, J., Miike, T., Shirahase, H., et al. (2012). Active conformation of seven-membered-ring benzolactams as new ACAT inhibitors: Latent chirality at N5 in the 1,5-benzodiazepin-2-one nucleus. *Chemistry (Weinheim an Der Bergstrasse, Germany), 18*(6), 1572–1576. https://doi.org/10.1002/chem.201103264.

Tzavara, E. T., Bymaster, F. P., & Nomikos, G. G. (2006). The procholinergic effects of the atypical antipsychotic olanzapine are independent of muscarinic autoreceptor inhibition. *Molecular Psychiatry, 11*(7), 619–621.

Urbanski, M. J., Chen, R. H., Demarest, K. T., Gunnet, J., Look, R., Ericson, E., et al. (2003). 2,5-disubstituted 3,4-dihydro-2*H*-benzo[*b*][1,4]thiazepines as potent and selective V2 arginine vasopressin receptor antagonists. *Bioorganic & Medicinal Chemistry Letters, 13*(22), 4031–4034.

Verbalis, J. G. (2002). Vasopressin V2 receptor antagonists. *Journal of Molecular Endocrinology, 29*(1), 1–9.

Verma, A., & Snyder, S. H. (1989). Peripheral type benzodiazepine receptors. *Annual Review of Pharmacology and Toxicology, 29*, 307–322.

Wada, T., & Penninger, J. M. (2004). Mitogen-activated protein kinases in apoptosis regulation. *Oncogene, 23*(16), 2838–2849.

Wang, L., Zhang, P., Zhang, X., Zhang, Y., Li, Y., & Wang, Y. (2009). Synthesis and biological evaluation of a novel series of 1,5-benzothiazepine derivatives as potential antimicrobial agents. *European Journal of Medicinal Chemistry, 44*(7), 2815–2821. https://doi.org/10.1016/j.ejmech.2008.12.021.

Wank, S. A. (1995). Cholecystokinin receptors. *The American Journal of Physiology, 269*(5–1), G628–G646.

Williams, R. B. (1990). Do benzodiazepines have a role in the prevention or treatment of coronary heart disease and other major medical disorders? *Journal of Psychiatric Research, 24*(2), 51–56.

Wulff, H., Castle, N. A., & Pardo, L. A. (2009). Voltage-gated potassium channels as therapeutic targets. *Nature Reviews Drug Discovery, 8*(12), 982–1001. https://doi.org/10.1038/nrd2983.

Non-Print Items

Abstract

This chapter discusses the biological behavior of 1,5–benzodiazepines and 1,5–benzothiazepines through their molecular effects on various cellular receptors, enzymes, ion channels, and platelets.

Pharmaceutical applications of 1,5-benzodiazepines

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Chapter outline

9.1 Introduction

Epilepsy is one of the most common brain disorders affecting people of all ages with a growing global incidence of at least 1% (GBD 2016 Epilepsy Collaborators, 2019; Thurman et al., 2011). This chronic disease is generally characterized by epileptic seizures, which are recurrent bursts of neural electrical activity in the brain owing to hypersynchronous discharges of a large number of cortical neurons (Staley, 2015). The majority of people with epilepsy also suffer from other comorbid conditions such as depression and anxiety (Kwon & Park, 2014). Furthermore, several syndromes manifesting in adults are influenced by epileptic conditions arising during childhood, such as Lennox–Gastaut syndrome (LGS), infantile spasms (IS, or West syndrome), progressive myoclonic epilepsy (PME), and Dravet syndrome (Bureau et al., 2019). Sufferers of these early–childhood onset syndromes often experience loss of skills, cognitive impairment, cerebellum damage, intellectual disabilities, and even sudden death (Arzimanoglou et al., 2009; Guerrini, 2006; Orsini et al., 2019; Shields, 2006). The repetition of epileptic seizures and their subsequent severe physical and psychological effects have

marked epilepsy as a destructive neurological disease for many decades. It should also be noted that, due to a profound cross–over in symptoms, other neurological conditions such as functional neurological disorders (FND, which can involve non–epileptic seizures) are often misdiagnosed as epilepsy. Further research is therefore crucial in ensuring accurate diagnoses amongst related conditions which are comparable in their infancy in terms of understanding and treatment (Cock & Edwards, 2018).

Most of the common anti–epileptic drugs (AEDs), e.g., carbamazepine, lamotrigine, levetiracetam, sodium valproate, and topiramate, control epileptic seizures by inhibiting voltage-gated sodium, potassium, and T-type calcium channels in the affected cortical neurons (Sankaraneni & Lachhwani, 2015; Staley, 2015). However, seizures still persist in around 30% of patients using these medications (Kwan & Brodie, 2000; Mattson et al., 1985). In this regard, a continuing demand for more efficient treatments exists. There are several factors to be considered in identifying a suitable anti–epileptic drug, including toxicity, tolerability, pharmacokinetics, and contraindication with other drugs (Glauser et al., 2006). Clobazam, a 1,5–benzodiazepine, has garnered attention over a considerable time as adjunctive therapy for epilepsy and anxiety. This chapter details the properties of clobazam as an anti–epileptic drug, including its pharmacokinetics, clinical dosing, mechanism of action, and adverse effects.

9.2 Clobazam

9.2.1 Background

Clobazam is the only member of 1,5–benzodiazepine family to be introduced as an anxiolytic agent (Australia, 1970) or antiepileptic (France in, 1974) (de Leon, Spina, & Diaz, 2013; Gauthier & Mattson, 2015; Hanks, 1979). This bicyclic fused heterocycle with the chemical structure of 7-chloro-1-methyl-5-phenyl-1-H-1,5-benzodiazepine-2,4-[3H,5H]-dione ($C_{16}H_{13}N_2O_2Cl$) exists as a white crystalline powder that is soluble in both water and dichloromethane. The primary intent of the synthesis of clobazam was to provide greater anxiolytic efficacy with fewer destructive side effects compared to traditional benzodiazepines, especially 1,4-benzodiazepines such as diazepam (*Valium*). During clinical trials, clobazam exhibited outstanding efficacy with mild adverse effects in the treatment of anxiety and epilepsy (Brogden, Heel, Speight, & Avery, 1980; Gastaut & Low, 1979; Ng & Collins, 2007; Robertson, 1995;

Wieck, Blaha, & Heerklotz, 1979). The 1,5-benzodiazepine derivative also proved to have many other therapeutic advantages including small residual hypnotic impact, no lethargic post-treatment consequence, high effectiveness against several epilepsies, no increased duration of sleep, and high efficacy against LGS (Brogden et al., 1980; Hancock & Cross, 2013; Robertson, 1995; Wheless & Phelps, 2013). Having established its clinical benefits and safety over 40 years of continuous clinical use around the world, clobazam was finally approved by the FDA as a third-generation antiseizure drug for the adjunctive treatment of LGS in children aged two years or older under the brand name *Onfi* (Lundbeck, Deerfield, IL, USA) in 2011 (Deerfield, 2016; Giarratano, Standley, & Benbadis, 2012).

LGS is a devastating epileptic encephalopathy with primary onset in children between 3-5 years old, often persisting into adulthood (Arzimanoglou & Resnick, 2011; Asadi-Pooya, 2018; Faulkner, 2015; Gastraut et al., 1966). This neurological disorder is generally characterized by several generalized concurrent seizures, cognitive impairment, and electroencephalographic (EEG) abnormalities such as slow spike-and-wave discharges. The incidence of LGS is approximately 1–2% of all patients with epilepsy, with an accompanying mortality rate of 3–7% (Asadi-Pooya, 2018). LGS seizures are typically drug-resistant and corresponding medical treatment usually requires the administration of multiple AEDs.

9.2.2 Pharmacodynamics

Since recent progress in the structural understanding of $GABA_A$ receptors (which interact with γ-aminobutyric acid, GABA), the mechanism of action of benzodiazepine-based drugs is better understood. $GABA_A$ receptors are the main inhibitory neurotransmitter receptors in the central nervous system (CNS) gathered around the anion-conducting channels in either synapses or extrasynaptic membranes. These receptors mainly include three subunits of alpha, beta, and gamma with different stoichiometries such as $\alpha1\beta\gamma2$, $\alpha1\beta2\gamma2$, $\alpha2\beta\gamma2$, and $\alpha3\beta\gamma2$ (Has & Chebib, 2018; Sankar, 2012; Sigel & Steinmann, 2012).

Benzodiazepines typically bind to allosteric sites located at the α- and γ-subunit interface of the $GABA_A$ receptor, increasing the influx of chloride ions through the neuronal cell channels and consequently induce a state of calm and relaxation (Campo-Soria, Chang, & Weiss, 2006; Elgarf et al., 2018). The subunits of $GABA_A$ receptors have been implicated as key factors for defining the functional properties of the overall receptors. For example, the $\alpha1$ and $\alpha2$ subunits are responsible for sedation and anxiolytic effects in

the CNS, respectively. Clobazam and its metabolites display more selective binding affinity to the $\alpha 2$ and $\gamma 2$ interface of the GABA$_A$ receptor compared to other benzodiazepines, rationalizing its reduced sedative effects and improved anticonvulsant activity. The anti-epileptic effects of clobazam are associated with its allosteric activation of GABA$_A$ receptors, up-regulation of the GABA transporter type 3 (GAT3), and enhancement of GABA transporter type 1 (GAT1) in the hippocampus. These GABA transporters generally control the regulation of GABA concentration during a synaptic process in the extracellular space, rendering them an attractive target for anticonvulsant agents (Campo-Soria et al., 2006; Sankar, 2012).

9.2.3 Metabolism

Clobazam is converted to several metabolites by cytochrome P450 (CYP) enzymes in the human liver (Giraud et al., 2004; Volz et al., 1979). Approximately two–thirds of clobazam molecules undergo *N*-demethylation by the enzyme CYP3A4 to form the highly active (anti-epileptic) metabolite, norclobazam (*N*-desmethylclobazam) (Fig. 9.1) (Sennoune et al., 1992; Volz et al., 1979). Elimination of both clobazam and norclobazam occurs via hydroxylation at the *para* position of the *N*-phenyl substituent of the diazepine ring and is catalyzed by CYP2C18 and CYP2C19 enzymes, leaving the inactive metabolites 4′-hydroxyclobazam and 4′-hydroxy-*N*-desmethylclobazam, which are subsequently excreted in urine (Fig. 9.1) (Tolbert & Larsen, 2019; Volz et al., 1979).

9.2.4 Pharmacokinetics and dosage

Clobazam is commercially available in either tablet (5, 10, and 20 mg) or oral solutions (2–2.5 mg/mL) and is quickly absorbed from the gut with a $T_{\max}$ (time to maximum plasma concentration) of 30 min to 3 h (Tedeschi, Riva, & Baruzzi, 1981). It is considered a long–acting benzodiazepine with no effect on psychomotor performance and no incidence of hangover or sedation (Aucamp, 1982). The half-life ($t_{1/2}$) of clobazam is shortened in patients suffering from seizures, where $t_{1/2}$ is roughly 24 h for healthy adults compared to 12 h for those with epilepsy. Following the entry into the blood, the serum concentration of clobazam usually requires between 5 and 9 days to reach a steady-state level in young adults, and this can be extended to 13 days for the elderly. The metabolite, norclobazam, has a longer $t_{1/2}$ of 36–46 h (Sennoune et al., 1992; Volz et al., 1979). Clobazam is considered a protein–bound AED: both clobazam and norclobazam bind to serum proteins in a highly efficient
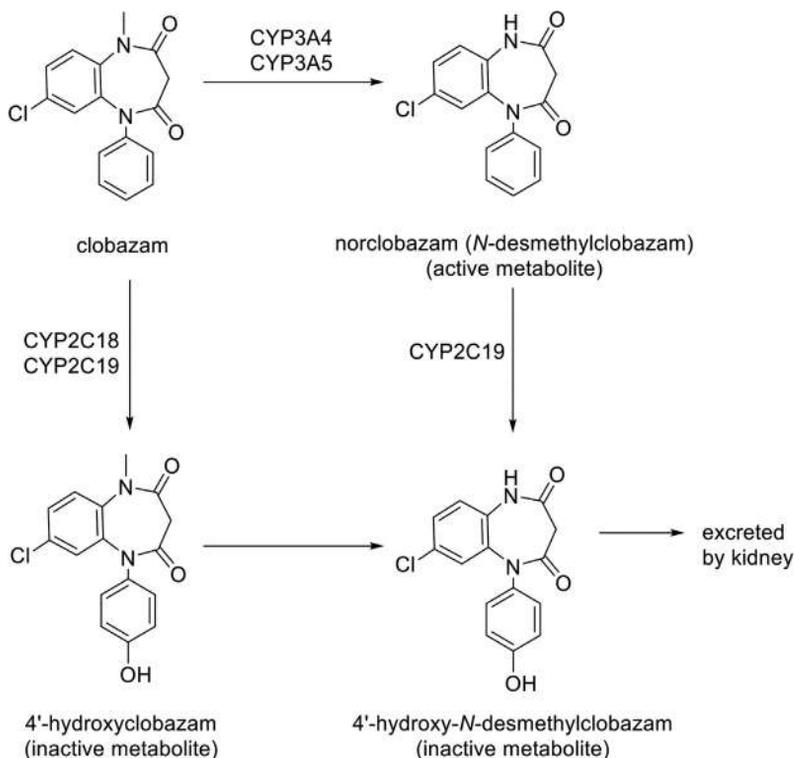
Figure 9.1 Metabolic pathway of clobazam in the human body.

manner, with 90% and 89% of molecules binding to plasma albumin and α1-acid glycoprotein, respectively (Patsalos et al., 2017). Only the remaining unbound molecules are free to pass across the blood–brain barrier and carry out their anti–epileptic effect in the brain. The significant advantage of protein-bound AEDs over other anti-epileptics is the enhanced ability to monitor their blood levels while clinically managing patients. While employing the therapeutic drug monitoring (TDM) technique, the amounts of bound and free drug molecules are measurable, allowing the minimization of dose-dependent adverse effects and toxicity of protein-bound AEDs (Patsalos et al., 2017). For example, in patients administered with clinical dosages of clobazam, respective serum levels have been determined to be 30–300 ng/mL (0.1–1.0 µmol/L) and 300–3000 ng/mL (1–10 µmol/L) for the parent drug and its norclobazam metabolite (Rupp et al., 1979). The pharmacokinetics of clobazam may be influenced by several parameters such as age, sex, drug interactions, and drug dosage. The absorption rate of clobazam is generally not influenced by the age or sex of patients. However,

its volume of distribution (V_D) is highly dependent on these two parameters and surges with age for both genders and is also significantly higher in women than in men (Greenblatt et al., 1981). It is well-documented that taking clobazam with food reduces its peak plasma level (C_{max}), indicating a reduction in the rate of absorption (Cenraud et al., 1983; Divoll et al., 1982). Furthermore, the area under the curve (AUC) is not influenced by the time of clobazam administration, which suggests no effect of food on the extent of absorption (Cenraud et al., 1983; Divoll et al., 1982).

Dosage adjustment of clobazam for both anxiety and epilepsy is mainly based on body weight, where people under 30 kg are initially given a dose of 5 mg once a day and increased to 20 mg per day in divided doses at weekly intervals. Patients over 30 kg are administered with 10 mg per day in divided doses, with a slow increase to 20 mg per day after 1 week, with a maximum of 40 mg per day after 2 weeks (Faulkner, 2015). A gradual increase in dosage every 5 days is recommended for children and infants until seizures are managed, with a maximum dosage of 1 mg/kg daily. In the case of elderly patients, while moderate impairment of CYP enzymes has no obvious effects on the pharmacokinetics of clobazam, it is advised that lower initial dosages are prescribed and that increments in dosage are more gradual (de Leon et al., 2013).

9.2.5 Adverse effects

The side effects of clobazam are comparatively mild with respect to other AEDs. A comprehensive toxicological examination of clobazam and its metabolites conducted for more than 3000 individuals with epilepsy demonstrated that the most frequent adverse effects included mild dizziness, sedation, and ataxia (Ng & Collins, 2007). Furthermore, the surveyance of over 1.1 million patients treated with clobazam between 1994 and 2004 revealed that only five individuals experienced severe side effects, such as status epilepticus (long-lasting seizures), hepatic failure, or death (Gauthier & Mattson, 2015; Ng & Collins, 2007). There is also strong evidence that clobazam causes fewer cognitive dysfunctions than 1,4-benzodiazepines. For example, healthy adults taking 10–20 mg doses of clobazam per day have been shown to exhibit fewer psychomotor side effects than those who took 0.5–1 mg daily (Gauthier & Mattson, 2015; Wildin, Pleuvry, Mawer, Onon, & Millington, 1990). Despite the safety of clobazam, there is still a risk of severe allergic reactions of the skin, often in combination with other anti-epileptic agents. A few case studies have demonstrated that clobazam coadministered with other AEDs, for example, gabapentin, clonazepam, lamotrigine, and

valproic acid, may cause Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) (Dang, Beets-Shay, & Kahn, 2015; Ertam, Sezgin, & Unal, 2009; Noguchi, Takaoka, Hayashi, Tachi, & Teramachi, 2020; Redondo et al., 1996; Yapici et al., 2014). SJS and TEN are cutaneous disorders identified by a significant loss of the epidermal skin layer, frequently leading to death.

As with other benzodiazepines, abrupt discontinuation of clobazam can lead to withdrawal symptoms such as seizures, irritability, behavioral disorders, and insomnia. In a summary report of 207 individuals with LGS, withdrawal symptoms were assessed after either abrupt or gradual discontinuation of clobazam (Tolbert, Harris, Bekersky, Lee, & Isojarvi, 2014). Two groups of people with short-term (≤15 weeks) or long-term (≤5 years) clobazam use were administered with different dosages including 20 and 40 mg/day (therapeutic) and 120 and 160 mg/day (supratherapeutic). Approximately 50% of patients experienced mild to moderate withdrawal symptoms when clobazam was discontinued instantly. In contrast, 87 participants given gradual discontinuation of clobazam over 2–3 weeks showed no withdrawal symptoms. These results suggest that discontinuation of clobazam in patients with epilepsy should be gradual, with careful monitoring for withdrawal symptoms.

9.2.6 Drug-drug interactions

Considering epileptic disorders bring on various forms of uncontrollable seizures, efficient treatment requires a combination of medications. It is, therefore, crucial to identify and characterize potential clobazam drug-drug interactions in patients with poly-therapeutic prescriptions. As previously mentioned, CYP enzymes such as CYP3A4 and CYP2C19 are the key catalysts for clobazam metabolism and the generation of its highly active norclobazam metabolite, and other compounds that interact with these same enzymes can therefore interfere with these processes. Many studies have investigated drug-drug interactions between clobazam and various types of medications. For example, etravirine-based antiretroviral drugs co-administered with clobazam can act as both CYP3A4 inducers and CYP2C19 inhibitors, increasing plasma concentrations of norclobazam (Naccarato, Yoong, Kovacs, & Gough, 2012). Therefore, it is recommended to closely monitor patients taking these two types of drugs together, to minimize toxicity. Aside from this, it is well-documented that there are no clinically obvious drug–drug interactions between clobazam and other AEDs metabolized by CYP enzymes (Tolbert, Bekersky, Chu, & Ette, 2016;

Walzer, Bekersky, Blum, & Tolbert, 2012), allowing its suitable and safe prescription as an adjunctive agent in polytherapy treatments for several epileptic disorders such as LGS.

References

Arzimanoglou, A., French, J., Blume, W. T., Cross, J. H., Ernst, J.-P., Feucht, M., et al. (2009). Lennox-Gastaut syndrome: A consensus approach on diagnosis, assessment, management, and trial methodology. *The Lancet Neurology, 8*(1), 82–93. https://doi.org/10.1016/S1474-4422(08)70292-8.

Arzimanoglou, A., & Resnick, T. (2011). All children who experience epileptic falls do not necessarily have Lennox-Gastaut syndrome… but many do. *Epileptic Disorders: International Epilepsy Journal with Videotape, 13*(1), S3–S13. https://doi.org/10.1684/epd.2011.0422.

Asadi-Pooya, A. A. (2018). Lennox-Gastaut syndrome: A comprehensive review. *Neurological Sciences, 39*(3), 403–414. https://doi.org/10.1007/s10072-017-3188-y.

Aucamp, A. K. (1982). Aspects of the pharmacokinetics and pharmacodynamics of benzodiazepines with particular reference to clobazam. *Drug Development Research, 2*(S1), 117–126. https://doi.org/10.1002/ddr.430010716.

Brogden, R. N., Heel, R. C., Speight, T. M., & Avery, G. S. (1980). Clobazam: A review of its pharmacological properties and therapeutic use in anxiety. *Drugs, 20*(3), 161–178.

Bureau, M., Genton, P., Delgado-Escueta, A., Dravet, C., Guerrini, R., Tassinari, C.A., et al. (2019). Epileptic syndromes in infancy, childhood, and adolescence (6th ed.). John Libbey Eurotex.

Campo-Soria, C., Chang, Y., & Weiss, D. S. (2006). Mechanism of action of benzodiazepines on GABA$_A$ receptors. *British Journal of Pharmacology, 148*(7), 984–990.

Cenraud, B., Guyot, M., Levy, R. H., Brachet-Liermain, A., Morselli, P. L., Moreland, T. A., et al. (1983). No effect of food intake on clobazam absorption. *British Journal of Clinical Pharmacology, 16*(6), 728–730.

Cock, H. R., & Edwards, M. J. (2018). Functional neurological disorders: Acute presentations and management. *Clinical Medicine (London, England), 18*(5), 414–417. https://doi.org/10.7861/clinmedicine.18-5-414.

Dang, C. D., Beets-Shay, L., & Kahn, E. C. (2015). Toxic epidermal necrolysis triggered by clobazam: A case report in a 13-year-old girl. *Pediatric Dermatology, 32*(3), e102–e103. https://doi.org/10.1111/pde.12527.

de Leon, J., Spina, E., & Diaz, F. J. (2013). Clobazam therapeutic drug monitoring: A comprehensive review of the literature with proposals to improve future studies. *Therapeutic Drug Monitoring, 35*(1), 30–47. https://doi.org/10.1097/FTD.0b013e31827ada88.

Deerfield, L. I. (2016). Onfi (clobazam). Full prescribing information. *US Food and Drug Administration (FDA).* https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/202067s004lbl.pdf.

Divoll, M., Greenblatt, D. J., Ciraulo, D. A., Puri, S. K., Ho, I., & Shader, R. I. (1982). Clobazam kinetics: Intrasubject variability and effect of food on adsorption. *Journal of Clinical Pharmacology, 22*(1), 69–73.

Elgarf, A. A., Siebert, D. C. B., Steudle, F., Draxler, A., Li, G., Huang, S., et al. (2018). Different benzodiazepines bind with distinct binding modes to GABA$_A$. *ACS Chemical Biology, 13*(8), 2033–2039. https://doi.org/10.1021/acschembio.8b00144.

Ertam, I., Sezgin, A. O., & Unal, I. (2009). A case with Stevens Johnson syndrome triggered by combination of clobazam, lamotrigine, and valproic acid treatment. *International Journal of Dermatology, 48*(1), 98–99. https://doi.org/10.1111/j.1365-4632.2009.03865.x.

Faulkner, M. A. (2015). Comprehensive overview: Efficacy, tolerability, and cost-effectiveness of clobazam in Lennox-Gastaut syndrome. *Therapeutics and Clinical Risk Management, 11*, 905–914. https://doi.org/10.2147/TCRM.S55930.

Gastaut, H., & Low, M. D. (1979). Antiepileptic properties of clobazam, a 1-5 benzodiazepine, in man. *Epilepsia, 20*(4), 437–446.

Gastraut, H., Roger, J., Soulayrol, R., Tassinari, C. A., Régis, H., Dravet, C., et al. (1966). Childhood epileptic encephalopathy with diffuse slow spike-waves (otherwise known as "petit mal variant") or Lennox syndrome. *Epilepsia, 7*(2), 139–179.

Gauthier, A. C., & Mattson, R. H. (2015). Clobazam: A safe, efficacious, and newly rediscovered therapeutic for epilepsy. *CNS Neuroscience & Therapeutics, 21*(7), 543–548. https://doi.org/10.1111/cns.12399.

GBD 2016 Epilepsy Collaborators. (2019). Global, regional, and national burden of epilepsy, 1990-2016: A systematic analysis for the Global Burden of Disease Study 2016. *The Lancet Neurology, 18*(4), 357–375. https://doi.org/10.1016/S1474-4422(18)30454-X.

Giarratano, M., Standley, K., & Benbadis, S. R. (2012). Clobazam for treatment of epilepsy. *Expert Opinion on Pharmacotherapy, 13*(2), 227–233. https://doi.org/10.1517/14656566.2012.647686.

Giraud, C., Tran, A., Rey, E., Vincent, J., Tréluyer, J.-M., & Pons, G. (2004). In vitro characterization of clobazam metabolism by recombinant cytochrome P450 enzymes: Importance of CYP2C19. *Drug Metabolism and Disposition: The Biological Fate of Chemicals, 32*(11), 1279–1286.

Glauser, T., Ben-Menachem, E., Bourgeois, B., Cnaan, A., Chadwick, D., Guerreiro, C., et al. (2006). ILAE treatment guidelines: Evidence-based analysis of antiepileptic drug efficacy and effectiveness as initial monotherapy for epileptic seizures and syndromes. *Epilepsia, 47*(7), 1094–1120.

Greenblatt, D. J., Divoll, M., Puri, S. K., Ho, I., Zinny, M. A., & Shader, R. I. (1981). Clobazam kinetics in the elderly. *British Journal of Clinical Pharmacology, 12*(5), 631–636.

Guerrini, R. (2006). Epilepsy in children. *Lancet (London, England), 367*(9509), 499–524.

Hancock, E. C., & Cross, J. H. (2013). Treatment of Lennox–Gastaut syndrome. *Cochrane Database Systematic Reviews*. https://doi.org/10.1002/14651858.CD003277.pub3.

Hanks, G. W. (1979). Clobazam: Pharmacological and therapeutic profile. *British Journal of Clinical Pharmacology, 7*(1), 151S–155S.

Has, A. T. C., & Chebib, M. (2018). receptors: Various stoichiometrics of subunit arrangement in $\alpha 1\beta 3$ and $\alpha 1\beta 3\varepsilon$ receptors. *Current Pharmaceutical Design, 24*(17), 1839–1844. https://doi.org/10.2174/1381612824666180515123921.

Kwan, P., & Brodie, M. J. (2000). Early identification of refractory epilepsy. *The New England Journal of Medicine, 342*(5), 314–319.

Kwon, O.-Y., & Park, S.-P. (2014). Depression and anxiety in people with epilepsy. *Journal of Clinical Neurology (Seoul, Korea), 10*(3), 175–188. https://doi.org/10.3988/jcn.2014.10.3.175.

Mattson, R. H., Cramer, J. A., Collins, J. F., Smith, D. B., Delgado-Escueta, A. V., Browne, T. R., et al. (1985). Comparison of carbamazepine, phenobarbital, phenytoin, and primidone in partial and secondarily generalized tonic-clonic seizures. *The New England Journal of Medicine, 313*(3), 145–151.

Naccarato, M., Yoong, D., Kovacs, C., & Gough, K. (2012). A case of a potential drug interaction between clobazam and etravirine-based antiretroviral therapy. *Antiviral Therapy, 17*(3), 589–592. https://doi.org/10.3851/IMP1953.

Ng, Y., & Collins, S. D. (2007). Clobazam. *Neurotherapeutics, 4*(1), 138–144.

Noguchi, Y., Takaoka, M., Hayashi, T., Tachi, T., & Teramachi, H. (2020). Antiepileptic combination therapy with Stevens-Johnson syndrome and toxic epidermal necrolysis: Analysis of a Japanese pharmacovigilance database. *Epilepsia, 61*(9), 1979–1989. https://doi.org/10.1111/epi.16626.

Orsini, A., Valetto, A., Bertini, V., Esposito, M., Carli, N., Minassian, B. A., et al. (2019). The best evidence for progressive myoclonic epilepsy: A pathway to precision therapy. *Seizure: The Journal of the British Epilepsy Association, 71*, 247–257. https://doi.org/10.1016/j.seizure.2019.08.012.

Patsalos, P. N., Zugman, M., Lake, C., James, A., Ratnaraj, N., & Sander, J. W. (2017). Serum protein binding of 25 antiepileptic drugs in a routine clinical setting: A comparison of free non-protein-bound concentrations. *Epilepsia, 58*(7), 1234–1243. https://doi.org/10.1111/epi.13802.

Redondo, P., Vicente, J., España, A., Subira, M. L., De Felipe, I., & Quintanilla, E. (1996). Photo-induced toxic epidermal necrolysis caused by clobazam. *The British Journal of Dermatology, 135*(6), 999–1002.

Robertson, M. (1995). The place of clobazam in the treatment of epilepsy: An update. *Human Psychopharmacology: Clinical and Experimental, 10*(1), S43–S63. https://doi.org/10.1002/hup.470100705.

Rupp, W., Badian, M., Christ, O., Hajdú, P., Kulkarni, R. D., Taeuber, K., et al. (1979). Pharmacokinetics of single and multiple doses of clobazam in humans. *British Journal of Clinical Pharmacology, 7*(1), 51S–57S.

Sankar, R. (2012). GABA$_A$ receptor physiology and its relationship to the mechanism of action of the 1,5-benzodiazepine clobazam. *CNS Drugs, 26*(3), 229–244. https://doi.org/10.2165/11599020-000000000-00000.

Sankaraneni, R., & Lachhwani, D. (2015). Antiepileptic drugs–a review. *Pediatric Annals, 44*(2), e36–e42. https://doi.org/10.3928/00904481-20150203-10.

Sennoune, S., Mesdjian, E., Bonneton, J., Genton, P., Dravet, C., & Roger, J. (1992). Interactions between clobazam and standard antiepileptic drugs in patients with epilepsy. *Therapeutic Drug Monitoring, 14*(4), 269–274.

Shields, W. D. (2006). Infantile spasms: Little seizures, big consequences. *Epilepsy Currents, 6*(3), 63–69.

Sigel, E., & Steinmann, M. E. (2012). Structure, function, and modulation of GABA$_A$ receptors. *The Journal of Biological Chemistry, 287*(48), 40224–40231. https://doi.org/10.1074/jbc.R112.386664.

Staley, K. (2015). Molecular mechanisms of epilepsy. *Nature Neuroscience, 18*(3), 367–372. https://doi.org/10.1038/nn.3947.

Tedeschi, G., Riva, R., & Baruzzi, A. (1981). Clobazam plasma concentrations: Pharmacokinetic study in healthy volunteers and data in epileptic patients. *British Journal of Clinical Pharmacology, 11*(6), 619–622.

Thurman, D. J., Beghi, E., Begley, C. E., Berg, A. T., Buchhalter, J. R., Ding, D., et al. (2011). Standards for epidemiologic studies and surveillance of epilepsy. *Epilepsia, 52*, 2–26. https://doi.org/10.1111/j.1528-1167.2011.03121.x.

Tolbert, D., Bekersky, I., Chu, H.–M., & Ette, E. I. (2016). Drug–metabolism mechanism: Knowledge-based population pharmacokinetic approach for characterizing clobazam drug-drug interactions. *Journal of Clinical Pharmacology, 56*(3), 365–374. https://doi.org/10.1002/jcph.603.

Tolbert, D., Harris, S. I., Bekersky, I., Lee, D., & Isojarvi, J. (2014). Withdrawal-related adverse events from clinical trials of clobazam in Lennox-Gastaut syndrome. *Epilepsy & Behavior, 37*, 11–15. https://doi.org/10.1016/j.yebeh.2014.05.016.

Tolbert, D., & Larsen, F. (2019). A comprehensive overview of the clinical pharmacokinetics of clobazam. *Journal of Clinical Pharmacology, 59*(1), 7–19. https://doi.org/10.1002/jcph.1313.

Volz, M., Christ, O., Kellner, H. M., Kuch, H., Fehlhaber, H. W., Gantz, D., et al. (1979). Kinetics and metabolism of clobazam in animals and man. *British Journal of Clinical Pharmacology, 7*(1), 41S–50S.

Walzer, M., Bekersky, I., Blum, R. A., & Tolbert, D. (2012). Pharmacokinetic drug interactions between clobazam and drugs metabolized by cytochrome P450 isoenzymes. *Pharmacotherapy, 32*(4), 340–353. https://doi.org/10.1002/j.1875-9114.2012.01028.x.

Wheless, J. W., & Phelps, S. J. (2013). Clobazam: A newly approved but well-established drug for the treatment of intractable epilepsy syndromes. *Journal of Child Neurology, 28*(2), 219–229. https://doi.org/10.1177/0883073812463609.

Wieck, H. H., Blaha, L., & Heerklotz, B. (1979). A clinical view of clobazam. *British Journal of Clinical Pharmacology, 7*(1), 109S–112S.

Wildin, J. D., Pleuvry, B. J., Mawer, G. E., Onon, T., & Millington, L. (1990). Respiratory and sedative effects of clobazam and clonazepam in volunteers. *British Journal of Clinical Pharmacology, 29*(2), 169–177.

Yapici, A. K., Fidanci, M. K., Kilic, S., Balamtekin, N., Mutluay Arslan, M., Yavuz, S. T., et al. (2014). Stevens-Johnson Syndrome triggered by a combination of clobazam, lamotrigine and valproic acid in a 7-year-old child. *Annals of Burns and Fire Disasters, 27*(3), 121–125.

Non-Print Items

Abstract

This chapter explains the present pharmacological knowledge of a FDA-approved 1,5-benzodiazepine, clobazam, including general mechanism of action, metabolic pathway, pharmacokinetic profile, recommended dosages, potential side effects, and drug-drug interactions.

CHAPTER TEN

Pharmaceutical applications of 1,5-benzothiazepines

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Chapter outline

295

10.1 Introduction

1,5-Benzothiazepines are extensively well-known through their numerous drug developments for various disorder treatments, cardio-therapeutic, and psycho–therapeutic scaffolds (Kaur, Singh, & Singh, 2016; Devi, Singh, & Monga, 2020). The majority of 1,5-benzothiazepine derivatives possess a wide spectrum of pharmacological activities by their biological impact on the brain, cancer cells, cardiac system, liver, kidney, blood, and microbes. Thanks to their biological impacts, these compounds play a pivotal role as therapeutic agents such as anticancer, anticonvulsant, hypolipidaemic, antiarrhythmic, antimicrobial, antifungal, anti-HIV, antitubercular, diuretic activities, antiviral, sedative, CNS depression antagonist, bradykinin agonists, antiplatelet aggregation, vasodilators, anticholinesterase, histone deacetylase 6 (HDAC6), and angiotensin-converting enzyme (ACE) inhibitor (Kaur et al., 2016; Bariwal et al., 2008). In this chapter, the pharmaceutical applications of the most common commercially available 1,5-benzothiazepine-drugs including diltiazem (**10.1.1**), clentiazem (**10.1.2**), thiazesim (**10.1.3**), quetiapine (**10.1.4**), and clotiapine (**10.1.5**) are discussed (Fig. 10.1).

10.2 Diltiazem

10.2.1 Introduction

Diltiazem (DTZ) (**10.1.1**, as a generic name) under the brand names of *Cardizem*, *Diltia*, *Dilzem*, *Diltzac*, *Taztia*, or *Tiazac*, and the IUPAC name of [(2*S*,3*S*)-5-[2-(dimethylamino)ethyl]-2-(4-methoxyphenyl)-4-oxo-2,3-dihydro-1,5-benzothiazepin-3-yl] acetate, is a nondihydropyridine calcium channel blocking agent for the treatment of cardiac rhythm abnormalities, which was first synthesized and developed in 1971. This molecule possesses two chiral centers and four different diastereomers, in which the (+) *cis* isomer has revealed the highest pharmaceutical activity in the treatment of angina pectoris, hypertension, certain heart-rhythm disorders, coronary vasodilating action, and supraventricular tachyarrhythmias (Britt, 1985; Markham & Brogden, 1993; Chaffman & Brogden, 1985).

10.2.2 Pharmacodynamics

Diltiazem has the potential to alleviate arteries contractions, which generally occur at high potassium (K^+) concentrations or norepinephrine (NE), via
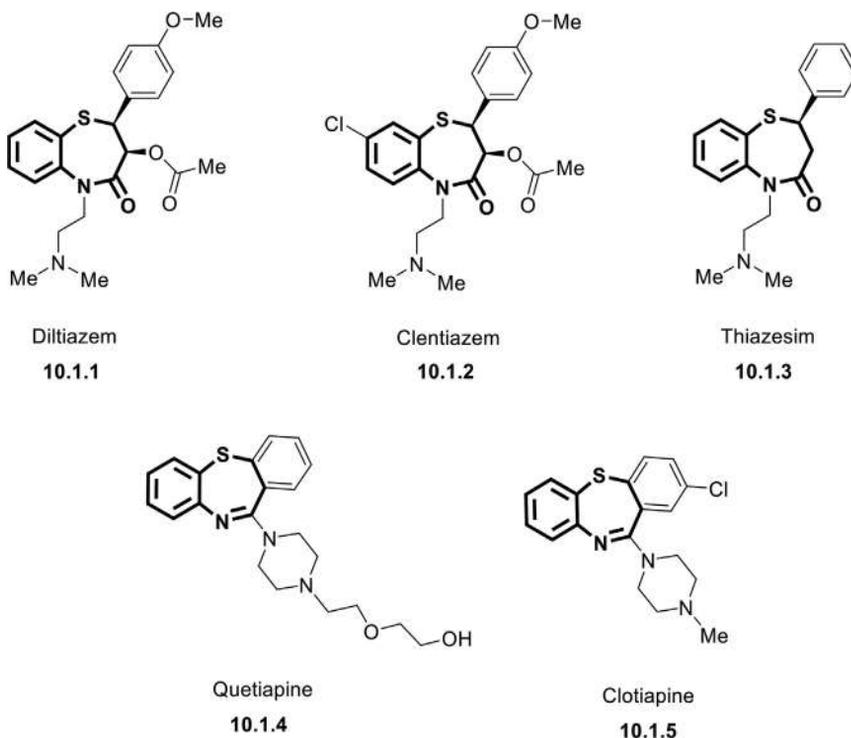
Figure 10.1 The most common 1,5-benzothiazepine-based commercially available drugs.

the reduction of intracellular Ca^{2+} influx reduction (Kikkawa, Murata, & Nagao, 1988; Nagao, Murata, & Sato, 1975). Since NE contractions are much more resistant to Ca^{2+} elimination than K^+ contractions, this inhibitory effect becomes more effective when the high K^+ concentration is the main cause of vasoconstriction rather than NE. There are two parts of Ca^{2+} extracellularly bound to a "weak link", which is triggered by K^+, and a "closely related" sensitive to NE. It is suggested that the release of intracellular Ca^{2+} may also be involved in agonist–induced contractions (Cauvin, Loutzenhiser, & Van Breemen, 1983; Cauvin, Saida, & van Breemen, 1982). The antihypertensive effect of DTZ has been clarified in various animal models such as hypertensive rats (SHR), as well as renal hypertensive and deoxycorticosterone acetate (DOCA)/saline rats. Diltiazem exerts its antihypertensive effect primarily through relaxation of vascular smooth muscle and a decrease in peripheral vascular resistance (Nagao et al., 1982). This medication significantly diminishes both fatal and non–fatal risks in cardiovascular events via vascular muscle relief and blood pressure reduction.

Diltiazem basically serves as a dose–dependent deterrence against the calcium flow into the heart, leading to longer intranodal (A-H interval) conduction time, effective and functional refractory periods of the atrioventricular node, and Wenckebach cycle lengths enhancement (Nagao et al., 1982). DTZ can also enhance collateral flow by simulating unstable angina pectoris to collateral–dependent myocardium (Nagao et al., 1975; Franklin, Millard, & Nagao, 1980). The negative inotropic effect of DTZ has been demonstrated *in vitro*, where this effect is found to be more impressive in the ischemic muscles. These observations highlight the DTZ benefits as an antianginal drug, which is likely due to lowering the myocardial oxygen demand, as favorable hemodynamic factors, and through decrement of transmembrane Ca^{2+} fluxes in ischemia and reperfusion (Bush et al., 1982; Szekeres, Udvary, & Végh, 1985). The protective effect of DTZ on the damage induced by cardiac ischemia-reperfusion or hypoxia/reoxygenation has been investigated in rat models (Ichihara & Abiko, 1983; Takeo et al., 1988; Tanonaka et al., 1999). Despite the inconsistency in the proposed molecular mechanisms, it is generally believed that this effect may be attributed to drug-induced attenuation of Ca^{2+} overload (Nishida et al., 1999) or the energy-sparing effect created by the negative inotropic effect (Sakamoto et al., 2000). Although L-*cis*-DTZ is at least 30 times weaker in blocking Ca^{2+} channels compared with D-*cis*-DTZ, the L isomer has still a marked cardioprotective efficacy, indicating that the DTZ cardioprotection ability is not only associated with Ca^{2+} channel blockade (Nasa, Ichihara, & Abiko, 1990). Both *in vivo* and *in vitro* experiments on rats have shown that DTZ can target *Egr-1* as one of the main elements in the myocardial ischemia/reperfusion injury and can act as a cardioprotective agent through downregulating *Egr-1* expression (Huang et al., 2009). Apart from the cardioprotective activity, DTZ appears to be an effective antrum and detrusor muscle relaxant through regulating intracellular Ca^{2+} fluctuations in the antrum, increasing bladder capacity, reducing both bladder and detrusor pressure (Ishikawa et al., 1985; Faustini et al., 1989).

Calcium concentration in neuronal cells is considered to play a vital role in the mechanism of anxiety, although the detailed mechanism has not been fully understood (Balon & Ramesh, 1996). Accordingly, calcium channel blockers such as diltiazem may be promising candidates in the treatment of anxiety disorders. However, no successful preclinical and clinical studies are available regarding the management of mental illnesses by using diltiazem. Nonetheless, several clinical investigations have been demonstrated that

diltiazem may reduce the symptoms of tardive dyskinesia, a potentially disfiguring facial movement disorder caused by the use of neuroleptic drugs (Soares & McGrath, 2000; Ross et al., 1987).

It is noteworthy to mention that DTZ may have several demerit effects on learning and cognition (Maxwell, Hogan, & Ebly, 1999). This medication has been shown to reduce nerve conduction along with local anesthetic effects (Leszczynska & Kau, 1992). Researchers have also identified that hampered lymphocyte function and cytokine release from mononuclear cells caused by DTZ can prove its immunosuppressive effects (Chitwood & Heim-Duthoy, 1993; D'Ambrosio et al., 1998). Furthermore, DTZ can enhance antibiotic resistance in cultures of *Pseudomonas aeruginousa in vitro* (Elkhatib, Haynes, & Noreddin, 2008).

10.2.3 Metabolism

Diltiazem is extensively metabolized by the liver through deacetylation, *N*-demethylation, *O*-demethylation, *N*-oxidation, and oxidative deamination processes, where the *N*-demethylation and deacetylation are the main metabolic routes. The major pharmacologically active metabolites identified in the human plasma include M_1 (**10.2.1**), M_4 (**10.2.4**), and M_5 (**10.2.5**) (Fig. 10.2). The deacetylation reaction of diltiazem is mediated by esterases, while *N*- and *O*-demethylation are catalyzed by CYP3A4 and CYP2D6 enzymes, respectively. The deacetyl DTZ (**10.2.1**) is converted to either the *N*-oxide species (**10.2.2**) or the *O*-demethylated analog (**10.2.3**). The *N*-demethylation of diltiazem provided the corresponding metabolite M_4 (**10.2.4**), which then undergoes a two-step deacetylation/*O*-demethylation sequence to give the inactive metabolite M_6 (**10.2.6**). The half-life elimination values of these metabolites are longer than that of diltiazem, and they are finally exerted through the urine (Maxwell et al., 1999; Yeung et al., 1993).

10.2.4 Pharmacokinetics and dosage

Diltiazem is absorbed in the digestive system in which approximately 30% of the drug is bound to albumin (Chaffman & Brogden, 1985; Cataldi, 2015). Following the oral administration of DTZ, the plasma concentration reaches the maximum of 50–200 ng/ml within 11–18 h (Table 10.1). In either single or multiple drug administration, the plasma elimination half-life of DTZ is almost 3.5 h. It is reported that the absolute bioavailability in oral administration is about 40% based on hepatic first-pass metabolism,
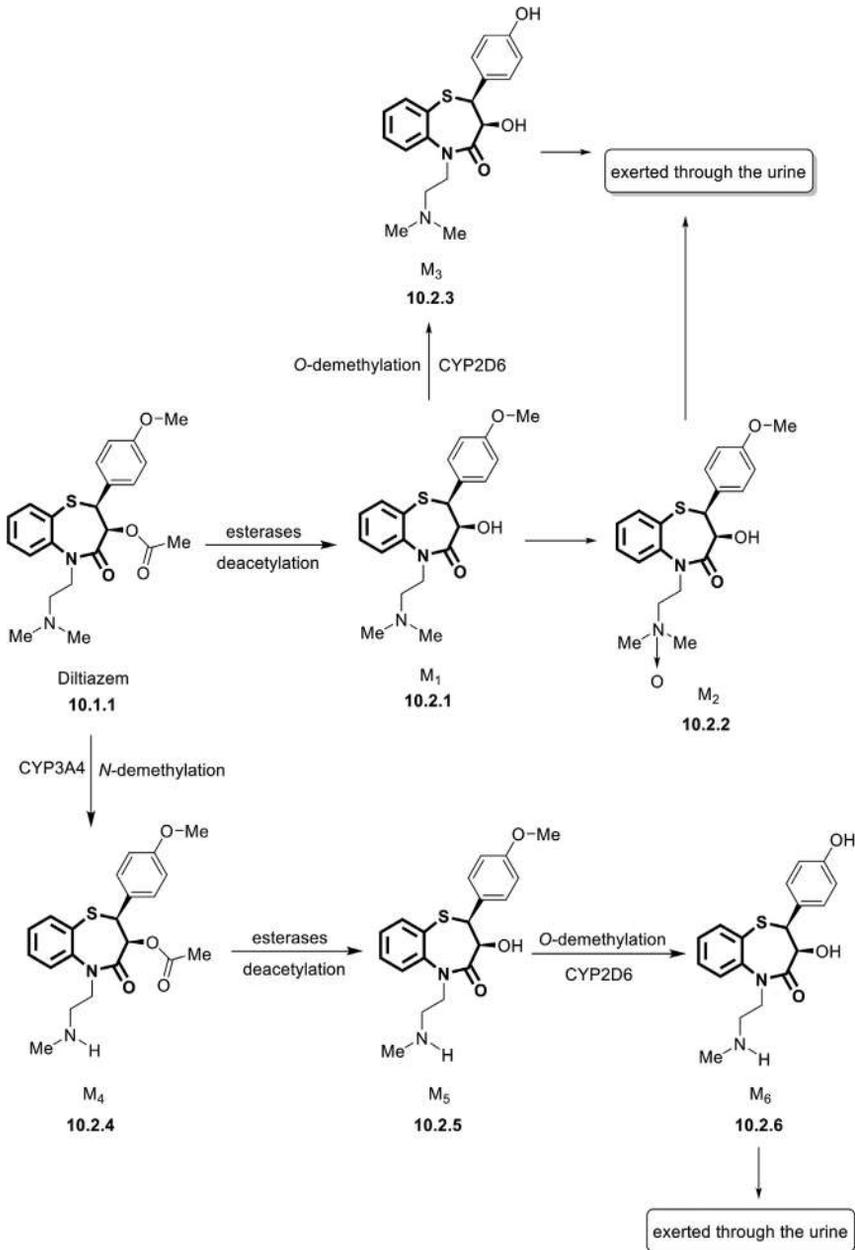
Figure 10.2 The metabolic pathways of diltiazem in humans.

Table 10.1 Pharmacokinetic parameters of diltiazem after oral administration.

Pharmacokinetic parameters	Values
Plasma albumin binding	30%
Maximum plasma concentration (C_{max})	50–200 ng/ml
Time to peak drug (T_{max})	11–18 h
Duration of action	3–4 h
Volume of distribution (V_d)	305 L/kg
Elimination half-life ($t_{1/2}$)	3.0–4.5 h
Bioavailability	∼40%

which 2% to 4% of the unchanged drug appears in the urine, and ranging in 24–74% considering high interindividual variation in the first pass effect (Chaffman & Brogden, 1985; Cataldi, 2015). In hepatic impairment patients, the bioavailability may tend to increase. The recommended DTZ dosage forstable angina or angina is approximately 120-180 mg per day, which can be split to 3 or 4 times daily based on the type of drug tablet or capsule (Chaffman & Brogden, 1985; Cataldi, 2015). The maximum recommended daily dose of this drug varies in different forms of the drug, which are 540, 480, and 360 mg/day for release capsules, coated capsules, and release tablets, respectively. For hypertension, the prescribed DTZ dosages are either 120–240 mg/day as extended–release capsules or 180–240 mg/day as extended–release tablets. The maximum daily dose for this treatment is up to 540 mg/day (Table 10.2) (Chaffman & Brogden, 1985; Cataldi, 2015).

10.2.5 Adverse effects

In patients treated with DTZ, some cardiovascular, dermatological, renal, lower tract urinary symptoms, hepatotoxicity, gingival overgrowth, neurological, and psychiatric side effects have been reported (Cataldi, 2015). In the case of DTZ effects on the cardiovascular system, it may cause adverse reactions such as bradyarrhythmias (first–, second–, or third-degree AV block), bradycardia, bundle branch block, hypotension with flushing, palpitations, and syncope. In addition, some rare cutaneous reactions including cutaneous vasculitis, exfoliative dermatitis, and erythematous rash have been observed (Cataldi, 2015). Besides, parkinsonism akathisia, myoclonus, and major depression are also some of the adverse events occurring in patients receiving DTZ. There are no well-controlled studies of DTZ administration in pregnant women, nevertheless, it is highly recommended to use DTZ only if the possible benefits outweigh the risks to the fetus (Cataldi, 2015).

Table 10.2 Administration profile of DTZ oral dosage in adults.

Treatment	Dosage schedule (daily)	Maintenance dose	Maximum dose (daily)
Angina Pectoris Prophylaxis	1) Extended-release capsule: 120–180 mg	Increment every 7–14 days as needed	540 mg
	2) Extended-release coated capsule: 120–180 mg	Increment every 7–14 days as needed	480 mg
	3) Extended-release tablet: 180 mg	Increment every 7–14 days as needed	360 mg
	4) Immediate-release tablet: 120 mg in 4 divided doses	180–360 mg/day in 3–4 divided doses	–
Hypertension	1) Extended-release capsule: 120–240 mg	120–540 mg/day	540 mg
	2) Extended-release coated capsule: 180–240 mg	240–360 mg/day	480 mg
	3) Extended-release tablet: 180–240 mg	–	540 mg

10.2.6 Interactions

Diltiazem has been demonstrated to act as the CYP3A4 enzyme inhibitor, which is why it may cause drug-drug interactions via interrupting the metabolic pathways of other medications. Thus, for patients with renal and/or hepatic disorders, dosage adjustment is required while DTZ is concomitantly administered to achieve optimal blood therapy levels. Generally, DTZ is subjected to various pharmacological drug interactions as follow: (1) calcium channel blockers (CCBs) (amiodarone, lithium, calcium supplements), (2) antihypertensive medications (sometimes desirable), (3) negative inotropic agents (beta-blockers), (4) drugs with anti-hypertensive side effects (anesthetics, antipsychotics, alprostadil, aldesleukin, baclofen), (5) barriers to absorption (bile sequestrants), and (6) drugs that increase blood pressure (corticosteroids, estrogens) (Ohno, Hisaka, & Suzuki, 2007; Edoute et al., 2000; Narimatsu & Taira, 1976; Christensen et al., 2002; Watson & Little, 1994). Furthermore, in coadministering DTZ with digitalis glycosides and/or a 3-adrenoceptor blocking agent, necessary precautions should be taken because of drug-drug interactions or additive depressant effects on the atrioventricular node (Markham & Brogden, 1993; Chaffman & Brogden, 1985). In the case of food interaction, alcohol–containing beverages may also

increase the effects of DTZ as they can lower blood pressure and result in dizziness, fainting, lightheadedness, or a rapid heartbeat. These effects may be created particularly after medicine initiating or taking some booster dose of DTZ (Watson & Little, 1994; Sica, 2006).
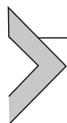
10.3 Clentiazem

10.3.1 Introduction

Clentiazem (as a generic name) with the IUPAC name of [(2*S*, 3*S*)-8-chloro-5-[2-(dimethylamino)ethyl]-2-(4-methoxyphenyl)-4-oxo-2,3-dihydro-1,5-benzothiazepin-3-yl] acetate was initially introduced by Tanabe Seiyaku a Japanese pharmaceutical company as a 1,5-benzothiazepine-based calcium channel antagonist (**10.1.2**) (Mecca & Love, 1992).

10.3.2 Pharmacodynamics

Clentiazem (CLZ) is categorized as an antiarrhythmic, antihypertensive, and ischaemic heart disorder therapeutic agent via blocking the calcium channels. This compound is five times more lipophilic than DTZ, as a result, the lipophilicity causes a longer half-life (10-13 h) compared with DTZ. Clentiazem reveals more vigorous and persistent hypotensive performance in various hypertension cases and vasorelaxant action in isolated canine, monkey arteries, and human myocardium (Narita, Zera, & Ginsburg, 1990). CLZ exhibits its antihypertensive effects by reducing peripheral arterial resistance (Bariwal et al., 2008). This medication selectively regulates the K^+ contraction in the coronary artery rather than in the mesentery. Therefore, it can be considered that CLZ is effective in angina treatment or cerebral vascular disorders (Mecca & Love, 1992). Due to decreasing heart rate, CLZ (40, 80, and 120 mg/day) reduces rate-pressure product at submaximal exercise (Waters & Garceau, 1993). Clentiazem also suppresses the proliferation of vascular smooth muscle cells (VSMCs) by blocking DNA synthesis through the activation of protein kinase C (PKC) (Kataoka et al., 1997). Furthermore, CLZ has been shown to reduce metabolic and functional abnormalities in an ischemia rat model, mainly due to the relative preservation of cerebral blood flow after carotid closure (Kikkawa et al., 1994). Calcium entry into smooth muscle, endothelial cells, and sympathetic nerve terminals is linked to vascular injury in chronic cerebral vasospasm. In an *in vivo* study on the rabbit basilar artery, CLZ has been found to significantly reduce

Table 10.3 Pharmacokinetic parameters of clentiazem after a single intravenous dose (20 mg) and an oral dose (80 mg).

Pharmacokinetic parameters	Intravenous dose (20 mg)	Oral dose (80 mg)
Maximum plasma concentration (C_{max})	6.37 ng/mL	37 ng/mL
$AUC_{(0\to\infty)}$	265 ng.h/ml	458.2 ng.h/ml
Time to peak drug (T_{max})	ND[a]	3.66 h
Duration of action	3.7 h	4–12 h
Volume of distribution (V_d)	756.1 L/kg	ND[a]
Elimination half-life ($t_{1/2}$)	10.6 h	13.7–15.5 h
Bioavailability	100%	∼45%
Clearance	63.6 L/h	ND[a]

[a] ND = not determined.

some key changes, e.g., wall force and wall stiffness, in the basilar artery caused by the subarachnoid hemorrhage. Therefore, it is suggested that CLZ can be a promising candidate for the management of chronic cerebral vasospasm (Vorkapic, Bevan, & Bevan, 1991). CLZ also protects the brain and significantly decreases infarct volume and arterial wall damage in rabbit middle cerebral artery occlusion (Kaminow & Bevan, 1991). In another study on rats, this calcium antagonist has reduced epinephrine-induced fibrosis and heart damage, probably through acting on the adrenergic system (Deisher et al., 1993).

10.3.3 Metabolism

The metabolic pathways of CLZ are similar to DTZ including deacetylation, *N*-demethylation (via cytochrome P-450), *O*-demethylation, deamination, aromatic hydroxylation, and conjugation processes. Seven basic metabolites (MB1-7, **10.3.1–10.3.7**) and four acidic metabolites (MA1-4, **10.3.8–10.3.11**) of CLZ were found in humans which are also shown in Fig. 10.3. Although all the basic metabolites (**10.3.1–10.3.7**) have revealed stronger inhibitory effects ($IC_{50} = 8$–22 pg/mL) on collagen–induced platelet aggregation in human platelets than clentiazem itself ($IC_{50} = 53$ pg/mL), the acidic metabolites (**10.3.8–10.3.11**) have shown no inhibitory effects (Odawara et al., 1994; Inoue et al., 1994).

10.3.4 Pharmacokinetics and dosage

Pharmacokinetic parameters of clentiazem after oral and intravenous administrations are summarized in Table 10.3. The maximum plasma concentration reaches 6.37 ng/ml after a single intravenous dose (20 mg) and 37 ng/mL after an oral dose (80 mg). Duration of actions is 3.7 h and 4–12 h for the
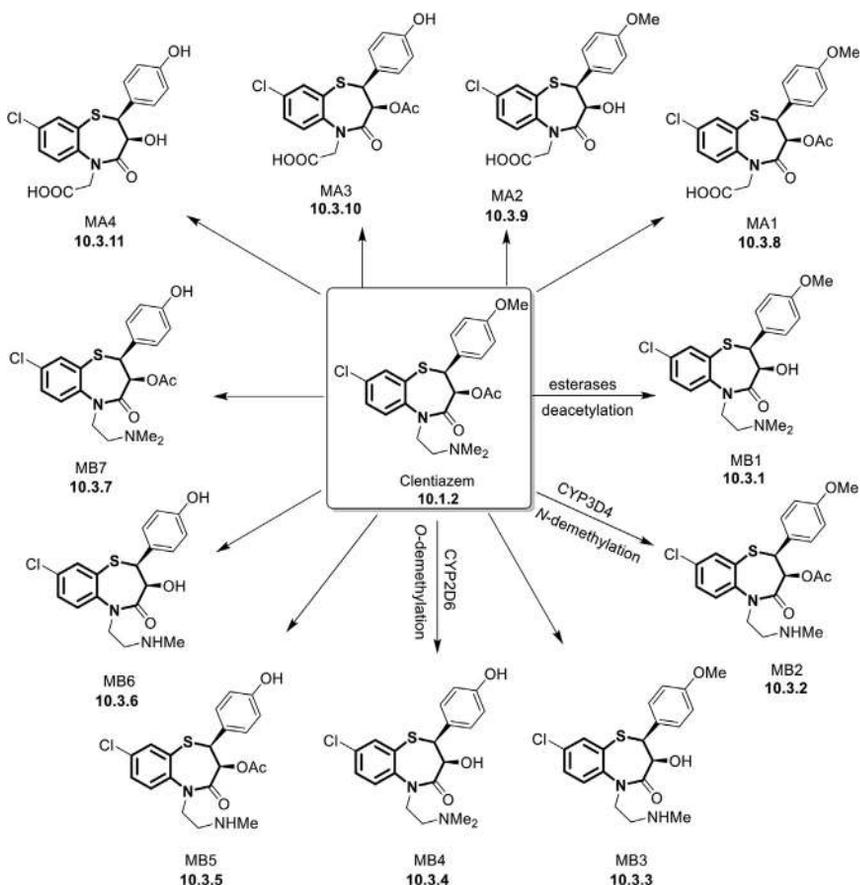
Figure 10.3 The metabolic pathways of clentiazem.

Table 10.4 Administration profile of clentiazem.

Treatment	Recommended dose
Stable angina	Twice daily in doses of 80–120 mg
Essential hypertension	30 mg/day

intravenous and oral routes, respectively. The plasma elimination half-life of CLZ is almost 10.6 h and 13.7–15.5 h for a single intravenous and oral dose, respectively. It is also known that the absolute bioavailability of CLZ after oral administration is approximately 45%. The CLZ treatment for stable angina is recommended twice daily in doses of 80 or 120 mg (Table 10.4). Furthermore, CLZ tablets are typically prescribed for patients with essential hypertension with a dose of 30 mg once daily for 2 weeks (Inoue et al., 1991; Suzuki, Mori, & Kusano, 1992).

10.3.5 Adverse effects

The most frequent adverse effects caused by CLZ are asthenia, headache, first-degree atrioventricular block, dizziness, nausea, cough, tired feeling, and stuffy nose. The obtained data indicate that administering CLZ twice a day in doses of 80 or 120 mg/day has confident mono-therapeutic results particularly in the case of stable angina (Waters & Garceau, 1993).

10.3.6 Interactions

Clentiazem is believed to act similarly to diltiazem in drug–drug and/or food interactions. Despite the promising preclinical and clinical results of CLZ, only a few research studies have been reported regarding the CLZ interaction with other drugs. Clentiazem diminishes the left atrial and ventricular papillary muscle's contractile response to isoproterenol, while this drug coadministered with epinephrine amplifies the left atrial's ventricular papillary response to isoproterenol (Deisher et al., 1993; Odawara et al., 1994). Coadministration of CLZ with aspirin or ticlopidine also significantly enhances the inhibitory effect of CLZ on collagen-induced thromboxane B2 produced by platelets in rats (Odawara et al., 1994). Furthermore, platelet accumulation is suppressed in the rats simultaneously treated with CLZ and aspirin. These results suggest that synergistic effects of aspirin or ticlopidine with CLZ can be influential in preventing and/or healing thrombotic disorders (Odawara et al., 1994).
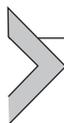
10.4 Thiazesim

10.4.1 Introduction

Thiazesim, also named as tiazesim, under the brand name of *Altinil* with the IUPAC name of 5-[2-(dimethylamino)ethyl]-2,3-dihydro-2-phenyl-1,5-benzothiazepin-4(5*H*)-one is a tricyclic antidepressant (TCA) and cardiovascular drug, which was initially developed by Squibb Corporation (currently Bristol–Myers Squibb) in 1966 (Ganellin & Triggle, 1996). However, this medication was soon later discontinued and became unavailable in the market due to its low efficiency and severe negative effects on emotion and learning. In this regard, our current knowledge about the mechanism of action, pharmacokinetics, and adverse effects of thiazesim is quite limited.

10.4.2 Pharmacodynamics

Horovitz and co-workers have demonstrated that thiazesim selectively interacts with the basolateral amygdala in the mouse-killing (muricide) test through unknown mechanisms (Horovitz et al., 1966), indicating that this medication may be considered as an influential drug in psychotic and emotional states. They hypothesized that the amygdala may be a common site of action for thiazesim. In another study reported by Watzman *et al.*, the effects of thiazesim on both unlearned behaviors, e.g., spontaneous motor activity, drinking, eating, mouse-killing, and forced motor activity, and learned behavior were investigated (Geyer, Watzman, & Buckley, 1970). The results show that thiazesim inhibits all unlearned and learned behaviors at high doses (20-44 mg/kg, intraperitoneal injection).

10.4.3 Metabolism

Our current knowledge of metabolic pathways of thiazesim is extremely limited. In a single *in vivo* study, Dreyfuss *et al.* have demonstrated three possible metabolites of thiazesim (**10.4.1–10.4.3**), which were observed in rats' urine and feces (Dreyfuss, Cohen, & Hess, 1968). Metabolites 1 and 2 (**10.4.1 and 10.4.2**) including hydroxyl groups on their 2-phenyl rings are formed from the hydroxylation of thiazesim (**10.1.3**) mediated by NADPH and oxygen in the liver (Fig. 10.4). Metabolite 3 (**10.4.3**) is derived from metabolite 1 (**10.4.1**) that is subsequently transformed to the glucuronide and sulfate analogs and are finally exerted in the urine.

10.5 Quetiapine

10.5.1 Introduction

Quetiapine (as a generic name) (**10.1.4**) under the brand name of *Seroquel* with the IUPAC name of 2-(2-(4-(dibenzo[*b*,*f*][1,4]thiazepin-11-yl)piperazin-1-yl)ethoxy)ethan-1-ol is a second-generation antipsychotic (SGA), which was first developed by AstraZeneca in 1992 and was then approved by the U.S. Food and Drug Administration (FDA) in 1997 for the short term treatment of schizophrenia and bipolar disorder (BD) (Kasper & Müller-Spahn, 2000; Srinivas et al., 2020). Quetiapine has also been demonstrated to be highly efficient in several non–FDA approved indications including generalized anxiety disorder, psychosis in patients suffering from Parkinson's disease, insomnia, chronic post-traumatic stress disorder (PTSD),

adjunctive treatment for obsessive-compulsive disorder (OCD), borderline personality disorder, decreasing aggression with psychiatric illness, major depressive disorder, and agitation (Juri et al., 2005; Villarreal et al., 2016; Shafti & Kaviani, 2019; Perrella et al., 2007; Walker, Thomas, & Allen, 2003; Ignácio et al., 2018; Gareri et al., 2015), via maintaining the balance of neurotransmitters in the brain. Oral administration of quetiapine may also reduce hallucinations, improving concentration, adjusting body energy level and sleep, increasing appetite, and decreasing severe mood swings (Ignácio et al., 2018; Fernandez et al., 2009).

10.5.2 Pharmacodynamics

Quetiapine has moderate antagonist activity against the brain serotonin 2A receptor (HTR_{2A}), against $\alpha 1$–adrenergic, muscarinic, and histaminergic (HTH_1) receptors. This drug also displays no affinity for the norepinephrine transporter (NET) and a minor affinity for serotonin 1A (HTR_{1A}), dopamine D_1 (DRD_1), dopamine D_2 (DRD_2), serotonin 2C (HTR_{2C}), and α_2–adrenergic receptors (Jensen et al., 2008; Schotte et al., 1996; Spanarello et al., 2005), although its detailed mechanism of action is still not fully understood. In bipolar and major depression, the action of quetiapine may be related to the binding of quetiapine or its metabolite to the norepinephrine transporter. Seroquel's antagonism of histamine H_1 and adrenergic α_1 receptors can explain somnolence and orthostatic hypotension respectively. Quetiapine also reveals low or no affinity for muscarinic receptors, while norquetiapine shows mild to high affinity for numerous muscarinic receptor subtypes leading to anticholinergic (muscarinic) effects. The antidepressant behavior of this compound is mainly originated from the drug interaction with HTR_{2A} and DRD_2 (Jensen et al., 2008).

10.5.3 Metabolism

Quetiapine is extensively metabolized by CYP3A4 and CYP3A5 enzymes in the liver through two main sulfoxidation and oxidation pathways, generating several metabolites such as quetiapine sulfoxide, *N*-desalkylquetiapine (**10.5.1**), *O*-desalkylquetiapine (**10.5.2**), and 7-hydroxyquetiapine (**10.5.3**) (Fig. 10.5). 7-Hydroxy-*N*-desalkylquetiapine and *N*-desalkylquetiapine are the pharmacologically active quetiapine metabolites, which are responsible for the biological behavior of the drug (Bakken et al., 2012; DeVane & Nemeroff, 2001). After hepatic metabolism, quetiapine and its metabolites
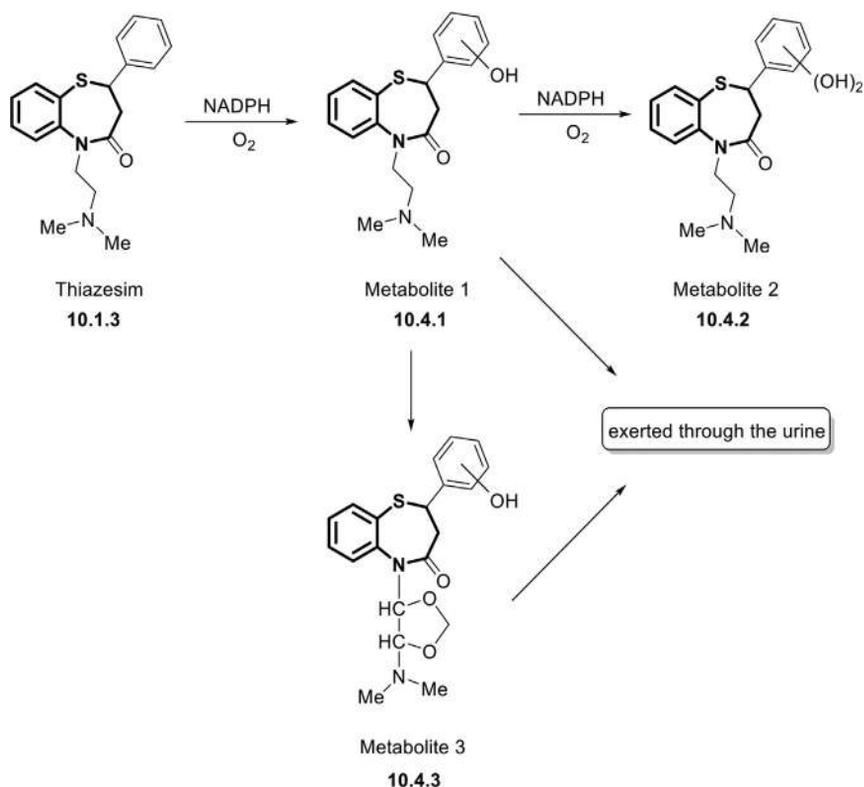
Figure 10.4 The metabolic pathways of thiazesim.

are mainly excreted by the kidneys (73%) and in feces (20%), and 1% of the drug is excreted in the unmetabolized form.

10.5.4 Pharmacokinetics and dosage

Quetiapine is rapidly absorbed in the gastrointestinal tract (GI) after oral administration, reaching the maximum plasma concentration in 1–2 h. In the human body, food has the least effect on the absorption of quetiapine as 83% of this drug can approximately bind to serum proteins (DeVane & Nemeroff, 2001). The drug elimination half-life and duration of action are 4-8 h and 7 h, respectively, depending on the drug dosage Table 10.5. The initial, recommended, and maximum doses of quetiapine according to various treatments are summarized in Table 10.6. In most prescriptions, the recommended dose is 400–800 mg/day, and the maximum dose is 300–800 mg/day. In the treatment of acute schizophrenia, the initial dose on the first day is 300 mg, which can be increased up to the maximum
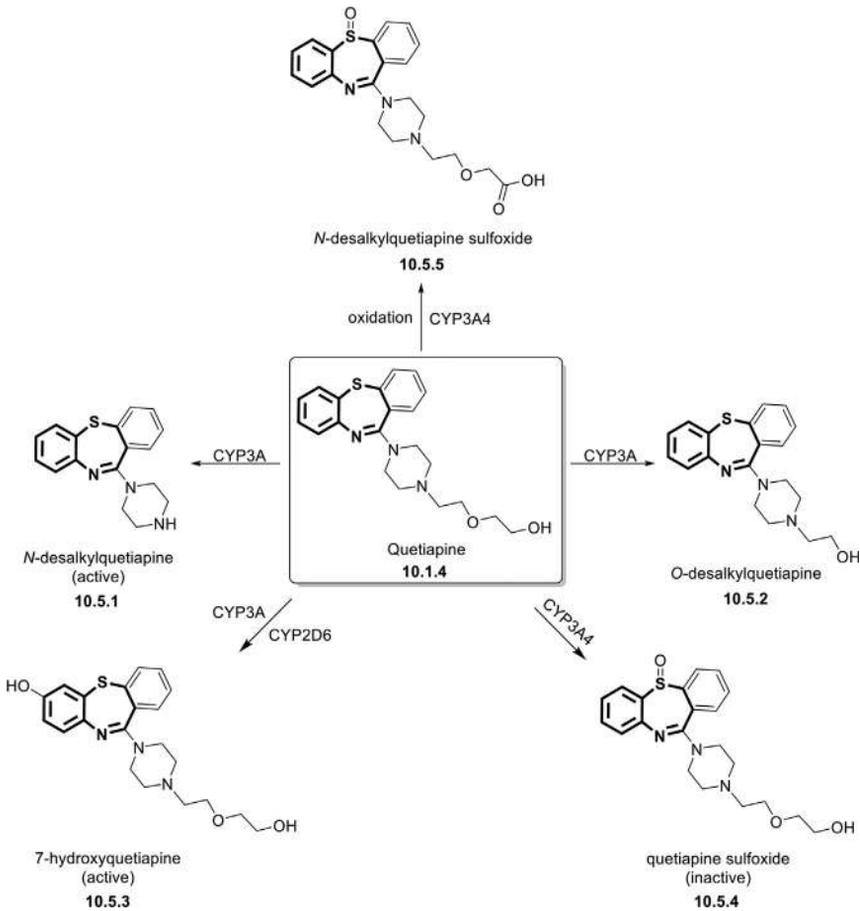
Figure 10.5   The metabolic pathways of quetiapine.

dose of 800 mg per day. For bipolar mania, dosing instructions say that 300 mg should be given on day 1, 600 mg on day 2, and up to a maximum of 800 mg on day 3. In the treatment of BD–associated depressive episodes, day 1 starts with 50 mg of quetiapine, increasing to 100 mg on day 2, and subsequently reaches 300 mg on day 4. The maximum dose for this treatment is 300 mg daily. For bipolar I maintenance, as an adjunct to lithium or divalproex, the recommended dose is 400–800 mg per day. When quetiapine is used for the treatment of major depressive disorders together with other antidepressants, the starting dose should be 50 mg on days 1 and 2, which can be increased to 150 mg on day 3. The recommended daily dosage of quetiapine as the adjunctive therapy of major depressive disorders is

Table 10.5 Pharmacokinetic parameters for oral administration of 10–25 mg quetiapine.

Pharmacokinetic parameters	Values
Plasma proteins binding	83%
Maximum plasma concentration (C_{max})	53–117 μg/mL
Time to peak drug (T_{max})	1.5–6 h
Steady-state concentrations	48 h
Duration of action	4–8 h
Volume of distribution (V_d)	~ 10 L/kg
Elimination half-life ($t_{1/2}$)	$\sim$7 h

150–300 mg/day (Muneer, 2015). Based on the reported data under multiple-dose conditions of quetiapine, it has been revealed that the pharmacokinetic parameters of this medication are time- and dose-independent, as well as gender-free (DeVane & Nemeroff, 2001; Muneer, 2015).

10.5.5 Adverse effects

Quetiapine may have a life-threatening risk for older patients suffering from dementia-related psychosis. In addition, the quetiapine prescription is forbidden for individuals younger than 10 years old (Muneer, 2015; Cabaleiro et al., 2015). General adverse effects of quetiapine include neurological (mood or behavior changes, headache, tiredness, dizziness, and somnolence), gastrointestinal (constipation, diarrhea, nausea, vomiting, dry mouth, and abdominal pain), psychiatric (nightmare and anhedonia), and cardiovascular disorders (hypotension, syncope, and prolongation of QTc) (Cabaleiro et al., 2015).

10.5.6 Interactions

Either long-term administration or high doses of quetiapine can result in a serious irreversible movement disorder. In the case of diabetic patients, it is recommended to check blood sugar levels regularly as it may cause high blood sugar (hyperglycemia). Monitoring weight and blood sugar levels during pregnancy are required since the adverse effects of the drug on the fetus are negligible (Cabaleiro et al., 2015). Some medications such as quinidine, sotalol, moxifloxacin, and thioridazine can change the heart rate when co-administered with quetiapine (Muneer, 2015; Guo et al., 2012). Quetiapine can also interrupt the biological efficiency of several drugs including Parkinson's medications, for example, levodopa, ropinirole, pergolide, bromocriptine, anticonvulsant medicines for epilepsy, and antidiabetics (DeVane & Nemeroff, 2001; Guo et al., 2012). While using quetiapine,

Table 10.6 Administration profile of quetiapine.

Treatment	Initial dose and titration (daily)	Recommended dose (daily)	Maximum dose (daily)
Schizophrenia-adults	300 mg	400–800 mg	800 mg
Schizophrenia-adolescents (13–17 years)	Day 1: 50 mg; Day 2: 100 mg; Day 3: 200 mg; Day 4: 300 mg; Day 5: 400 mg	400–800 mg	800 mg
Acute schizophrenia	200 mg	300 mg	800 mg
Schizophrenia maintenance	–	400–800 mg	800 mg
Bipolar disorder manic, mixed acute monotherapy, or adjunct to lithium and divalproex	Day 1: 300 mg; Day 2: 600 mg; Day 3: 800 mg	400–800 mg	800 mg
Depressive episodes associated with BD	Day 1: 50 mg; Day 2: 100 mg; Day 3: 200 mg; Day 4: 300 mg	300 mg	300 mg
Bipolar I maintenance-adjunct to lithium and divalproex	–	400–800 mg	800 mg
Major depressive disorder	Day 1: 50 mg; Day 2: 50 mg; Day 3: 150 mg	150–300 mg (adjunct to antidepressants)	300 mg
Hepatic impairment	25 mg	Depending on the patient's clinical response and tolerability	–

drinking alcohol should be avoided due to undesirable side effects (Cabaleiro et al., 2015; Guo et al., 2012).
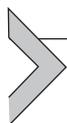
10.6 Clotiapine

10.6.1 Introduction

Clotiapine (as a generic name) (**10.1.5**) with the IUPAC name of 8-chloro-6-(4-methylpiperazin-1-yl)benzo[*b*][1,4]benzothiazepine is a neuroleptic dibenzothiazepine prescribed for atypical antipsychotic treatment. Although more than 45 years have passed since the discovery of clotiapine (Carpenter, Berk, & Rathbone, 2004; Lokshin et al., 1998), there is still insufficient information on the clotiapine therapeutic and toxic profile. This drug was initially marketed under the brand name *Entumine* in several countries in 1970. Clotiapine's biological behavior is similar to other antipsychotic medications, such as phenothiazines and clozapine (Lokshin et al., 1998; Geller et al., 2005). This medication can be a promising agent for the management of alcoholism and drug addiction. For example, it has been demonstrated that the oral administration of clotiapine is one of the most efficient strategies in opioid withdrawal using 40 to 100 mg/day co–administered with diazepam (10 to 20 mg i.m. per day) (Lokshin et al., 1998; Geller et al., 2005). Despite limited randomized controlled trials, the efficacy of this drug in the treatment and management of sleep disorder, drug withdrawal symptoms, inner uneasiness, hyperactivity, panic, states of depersonalization, agitation of endogenous or exogenous (drugs, alcohol) cause, bipolar disorder (particularly mania), acute or exacerbations of chronic schizophrenia, chronic schizophrenia, and other forms of acute psychotic illnesses have been reported (Carpenter et al., 2004; Geller et al., 2005).

10.6.2 Pharmacodynamics

Clotiapine can selectively block $5HT_3$ and a subset of $GABA_A$ receptors, providing sedative and anxiolytic effects (Carpenter et al., 2004; Lyseng–Williamson, 2015). It is also known that clotiapine has a high affinity for $5\text{-}HT_6$, $5\text{-}HT_7$, D_1, and D_4 receptors, but has poor affinity for the dopamine D_2 receptor (Carpenter et al., 2004; Lyseng–Williamson, 2015). This drug with a broad range of effects on various receptors may have superior clinical privileges compared with other common antipsychotic drugs in the treatment of schizophrenia (Lyseng–Williamson, 2015).
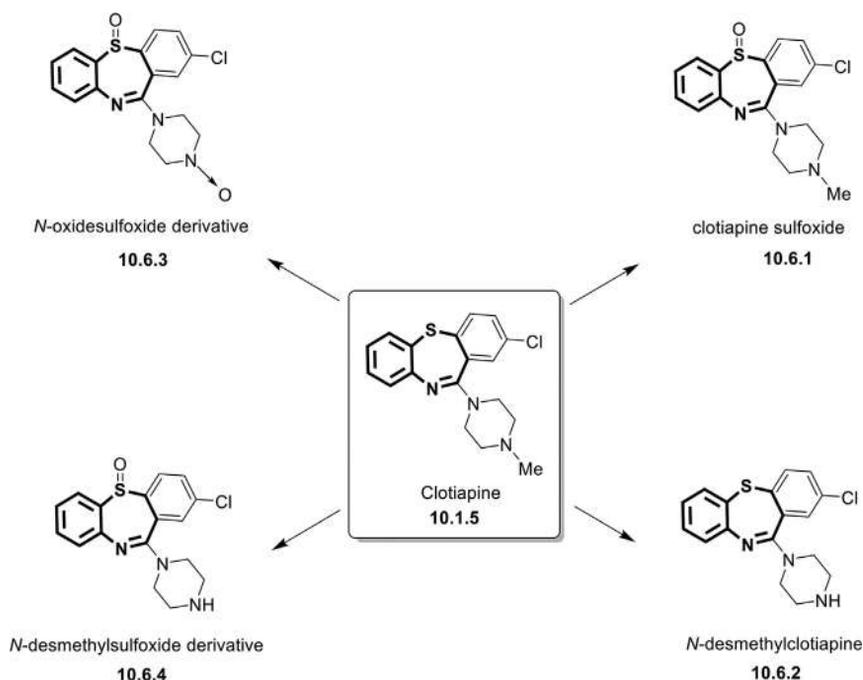
Figure 10.6 The metabolic pathways of clotiapine.

10.6.3 Metabolism

Clotiapine is extensively metabolized in the liver to several metabolites including clotiapine sulfoxide (**10.6.1**), N–desmethylclotiapine (**10.6.2**), N–oxidesulfoxide derivatives (**10.6.3**), and N–desmethylsulfoxide (**10.6.4**), which are finally excreted in the urine (Fig. 10.6) (Michaelis, 1969; Gauch & Lehner, 1969). However, the detailed mechanism of the metabolic pathways still remains to be identified.

10.6.4 Pharmacokinetics and dosage

There is very little information on the pharmacokinetics of clotiapine, as well as its clinical symptoms and toxicokinetics of overdose. The mean elimination half-life of clotiapine after single intravenous (i.v.) (15 mg) and oral (40 mg) administrations are approximately 4 h and 7 h, respectively (Sporkert et al., 2007). Clotiapine administration varies in different countries (Table 10.7). For example, while the initial dose of clotiapine for the treatment of acute psychosis is 120-200 mg/day in Spain, Switzerland, Belgium, Israel, Taiwan, and Argentina, 120–160 mg/day is prescribed for the same therapy

Table 10.7 Administration profile of clotiapine.

Treatment	Initial dose (daily)	Recommended dose (daily)	Maximum dose (daily)
Acute psychosis (anxiety, mania, schizophrenia)	1) 120–200 mg in Spain, Switzerland, Belgium, Israel, Taiwan, Argentina; 2) 120–160 mg in South Africa	20–160 mg	360 mg

in South Africa. The recommended dose for patients suffering from anxiety, mania, and schizophrenia is generally 20–160 mg daily, with a maximum dosage of 360 mg per day (Lyseng–Williamson, 2015).

10.6.5 Adverse effects

There are no life-threatening adverse effects reported for clotiapine. However, precautions should be taken to reduce the risk of negative effects in older people. The most common side effects of clotiapine can be categorized into three types as follows.

(1) Anticholinergic symptoms: Constipation, dry mouth, and blurred vision, which are temporary and may emerge at treatment initiation (Lyseng–Williamson, 2015).

(2) Dopaminergic and CNS–related symptoms: Extrapyramidal symptoms (EPS), confusion, dystonia, parkinsonism, sedation, tardive dyskinesia, and akathisia. Note that the occurrence and intensity of these events depend on the drug dosage, which can be generally controlled via the administration of antiparkinson medications, treatment discontinuation, and/or dose reduction (Lyseng–Williamson, 2015).

(3) Orthostatic hypotension risk: This symptom is scarcely observed in elderly patients who simultaneously receive antihypertensive medication and/or suffer from cardiovascular diseases (Lyseng–Williamson, 2015).

10.6.6 Interactions

Although clinical evidence for the potential interactions of clotiapine with other drugs is quite limited, the tolerability and safety profiles of clotiapine are generally assumed to be similar to other antipsychotics (Lyseng–Williamson, 2015). In this case, clotiapine displays minor to moderate drug–drug interactions with alcohol, levodopa, antiepileptics, antihypertensives, CNS active agents, and lithium (Lyseng–Williamson, 2015).

References

Bakken, G. V., Molden, E., Knutsen, K., Lunder, N., & Hermann, M. (2012). Metabolism of the active metabolite of quetiapine, *N*-desalkylquetiapine *in vitro*. *Drug Metab. Dispos., 40*(9), 1778–1784. https://doi.org/10.1124/dmd.112.045237.

Balon, R., & Ramesh, C. (1996). Calcium channel blockers for anxiety disorders? *Ann. Clin. Psychiatry, 8*(4), 215–220.

Bariwal, J. B., Upadhyay, K. D., Manvar, A. T., Trivedi, J. C., Singh, J. S., Jain, K. S., et al. (2008). 1,5-Benzothiazepine, a versatile pharmacophore: A review. *Eur. J. Med. Chem., 43*(11), 2279–2290. https://doi.org/10.1016/j.ejmech.2008.05.035.

Britt, B. A. (1985). Diltiazem. *Can. Anaesth. Soc. J., 32*(1), 30–44.

Bush, L. R., Romson, J. L., Ash, J. L., & Lucchesi, B. R. (1982). Effect of diltiazem on extent of ultimate myocardial injury resulting from temporary coronary artery occlusion in dogs. *J. Cardiovasc. Pharmacol., 4*(2), 285–296.

Cabaleiro, T., López-Rodríguez, R., Román, M., Ochoa, D., Novalbos, J., Borobia, A., et al. (2015). Pharmacogenetics of quetiapine in healthy volunteers: Association with pharmacokinetics, pharmacodynamics, and adverse effects. *Int. Clin. Psychopharmacol., 30*(2), 82–88. https://doi.org/10.1097/YIC.0000000000000047.

Carpenter, S., Berk, M., & Rathbone, J. (2004). Clotiapine for acute psychotic illnesses. *Cochrane Database Syst. Rev., 18*(4). CD002304. https://doi.org/10.1002/14651858.CD002304.pub2.

Cataldi, M. (2015). Diltiazem. *Reference Module in Biomedical Sciences*. https://doi.org/10.1016/B978-0-12-801238-3.07804-1.

Cauvin, C., Loutzenhiser, R., & Van Breemen, C. (1983). Mechanisms of calcium antagonist-induced vasodilation. *Annu. Rev. Pharmacol. Toxicol., 23*, 373–396.

Cauvin, C., Saida, K., & van Breemen, C. (1982). Effects of Ca antagonists on Ca fluxes in resistance vessels. *J. Cardiovasc. Pharmacol., 4*(3), S287–S290.

Chaffman, M., & Brogden, R. N. (1985). Diltiazem. A review of its pharmacological properties and therapeutic efficacy. *Drugs, 29*(5), 387–454.

Chitwood, K. K., & Heim-Duthoy, K. L. (1993). Immunosuppressive properties of calcium channel blockers. *Pharmacotherapy, 13*(5), 447–454.

Christensen, H., Asberg, A., Holmboe, A.-B., & Berg, K. J. (2002). Coadministration of grapefruit juice increases systemic exposure of diltiazem in healthy volunteers. *Eur. J. Clin. Pharmacol., 58*(8), 515–520.

D'Ambrosio, A., Giacomini, E., Camponeschi, B., & Quintieri, F. (1998). Diltiazem modulates monokine production in human mixed lymphocyte culture. *Transplantation, 65*(10), 1411–1413.

Deisher, T. A., Narita, H., Zera, P., Ginsburg, R., Bristow, M. R., Billingham, M. E., et al. (1993). Protective effect of clentiazem against epinephrine-induced cardiac injury in rats. *J. Pharmacol. Exp. Ther., 266*(1), 262–269.

DeVane, C. L., & Nemeroff, C. B. (2001). Clinical pharmacokinetics of quetiapine: An atypical antipsychotic. *Clin. Pharmacokinet., 40*(7), 509–522.

Devi, V., Singh, G., & Monga, V. (2020). Recent advances in the synthetic chemistry of 1,5-benzothiazepines: A minireview. *J. Heterocycl. Chem., 57*(9), 3255–3270. https://doi.org/10.1002/jhet.4062.

Dreyfuss, J., Cohen, A. I., & Hess, S. M. (1968). Metabolism of thiazesim, 5-(2-dimethylaminoethyl)-2,3-dihydro-2-phenyl-1,5-benzothiazepin-4(5*H*)-one, in the rat *in vivo* and *in vitro*. *J. Pharm. Sci., 57*(9), 1505–1511.

Edoute, Y., Nagachandran, P., Svirski, B., & Ben-Ami, H. (2000). Cardiovascular adverse drug reaction associated with combined beta-adrenergic and calcium entry-blocking agents. *J. Cardiovasc. Pharmacol., 35*(4), 556–559.

Elkhatib, W. F., Haynes, V. L., & Noreddin, A. M. (2008). Unexpected induction of resistant Pseudomonas aeruginosa biofilm to fluoroquinolones by diltiazem: A new

perspective of microbiological drug–drug interaction. *J. Infect. Public Health, 1*(2), 105–112. https://doi.org/10.1016/j.jiph.2008.10.004.

Faustini, S., Salvini, A., Pizzi, P., Conti, M., Magistretti, M. J., & Vescovini, R. (1989). Experimental study on the action of diltiazem on detrusor muscle and clinical evaluation in patients with detrusor hyperactivity. *Arzneimittelforschung, 39*(8), 899–903.

Fernandez, H. H., Okun, M. S., Rodriguez, R. L., Malaty, I. A., Romrell, J., Sun, A., et al. (2009). Quetiapine improves visual hallucinations in Parkinson disease but not through normalization of sleep architecture: Results from a double-blind clinical-polysomnography study. *Int. J. Neurosci., 119*(12), 2196–2205. https://doi.org/10.3109/00207450903222758.

Franklin, D., Millard, R. W., & Nagao, T. (1980). Responses of coronary collateral flow and dependent myocardial mechanical function to the calcium antagonist, diltiazem. *Chest, 78*(1), 200–204.

Ganellin, C., & Triggle, D. J. (1996). *Dictionary of pharmacological agents*: 2. United States, Boca Raton, Florida: CRC Press.

Gareri, P., Gallelli, L., Pirritano, D., Rende, P., Palleria, C., Russo, E., et al. (2015). The role of quetiapine in the treatment of Alzheimer's disease. *J. Gerontol. Geriatr. Res., 4*(1), 197. https://doi.org/10.4172/2167-7182.1000197.

Gauch, R., & Lehner, H. (1969). The metabolism of 2-chloro-11-(4-methyl-1-piperazinyl) dibenzo-(*b,f*)(1,4)-thiazepine (clotiapine). 3. The metabolism and excretion in the human. *Farmaco. Prat., 24*(2), 100–109.

Geller, V., Gorzaltsan, I., Shleifer, T., Belmaker, R. H., & Bersudsky, Y. (2005). Clotiapine compared with chlorpromazine in chronic schizophrenia. *Schizophr. Res., 80*(2–3), 343–347.

Geyer, H. M., Watzman, N., & Buckley, J. P. (1970). Effects of a tranquilizer and two antidepressants on learned and unlearned behaviors. *J. Pharm. Sci., 59*(7), 964–968.

Guo, J. J., Wu, J., Kelton, C. M. L., Jing, Y., Fan, H., Keck, P. E., et al. (2012). Exposure to potentially dangerous drug–drug interactions involving antipsychotics. *Psychiat. Serv., 63*(11), 1080–1088. https://doi.org/10.1176/appi.ps.201100443.

Horovitz, Z. P., Piala, J. J., High, J. P., Burke, J. C., & Leaf, R. C. (1966). Effects of drugs on the mouse-killing (muricide) test and its relationship to amygdaloid function. *Int. J. Neuropharmacol., 5*(6), 405–411.

Huang, Z., Li, H., Guo, F., Jia, Q., Zhang, Y., Liu, X., et al. (2009). Egr-1, the potential target of calcium channel blockers in cardioprotection with ischemia/reperfusion injury in rats. *Cell. Physiol. Biochem., 24*(1–2), 17–24. https://doi.org/10.1159/000227809.

Ichihara, K., & Abiko, Y. (1983). Effects of diltiazem and propranolol on irreversibility of ischemic cardiac function and metabolism in the isolated perfused rat heart. *J. Cardiovasc. Pharmacol., 5*(5), 745–751.

Ignácio, Z. M., Calixto, A. V., da Silva, R. H., Quevedo, J., & Réus, G. Z. (2018). The use of quetiapine in the treatment of major depressive disorder: Evidence from clinical and experimental studies. *Neurosci. Biobehav. Rev., 86*, 36–50. https://doi.org/10.1016/j.neubiorev.2017.12.012.

Inoue, H., Konda, M., Hashiyama, T., Otsuka, H., Takahashi, K., Gaino, M., et al. (1991). Synthesis of halogen-substituted 1,5-benzothiazepine derivatives and their vasodilating and hypotensive activities. *J. Med. Chem., 34*(2), 675–687.

Inoue, H., Nakamura, S., Otsuka, H., Gaino, M., Harada, T., Matsuki, K., et al. (1994). Synthesis of the metabolites of clentiazem. *Chem. Pharm. Bull., 42*(1), 167–172.

Ishikawa, S., Komori, K., Nagao, T., & Suzuki, H. (1985). Effects of diltiazem on electrical responses evoked spontaneously or by electrical stimulation in the antrum smooth muscle cells of the guinea-pig stomach. *Br. J. Pharmacol., 86*(4), 789–797.

Jensen, N. H., Rodriguiz, R. M., Caron, M. G., Wetsel, W. C., Rothman, R. B., & Roth, B. L. (2008). *N*-desalkylquetiapine, a potent norepinephrine reuptake inhibitor

and partial 5-HT1A agonist, as a putative mediator of quetiapine's antidepressant activity. *Neuropsychopharmacology, 33*(10), 2303–2312.

Juri, C., Chaná, P., Tapia, J., Kunstmann, C., & Parrao, T. (2005). Quetiapine for insomnia in Parkinson disease: Results from an open-label trial. *Clin. Neuropharmacol., 28*(4), 185–187.

Kaminow, L., & Bevan, J. (1991). Clentiazem reduces infarct size in rabbit middle cerebral artery occlusion. *Stroke, 22*(2), 242–246.

Kasper, S., & Müller-Spahn, F. (2000). Review of quetiapine and its clinical applications in schizophrenia. *Expert Opin. Pharmacother., 1*(4), 783–801.

Kataoka, S., Alam, R., Dash, P. K., & Yatsu, F. M. (1997). Inhibition of PDGF-mediated proliferation of vascular smooth muscle cells by calcium antagonists. *Stroke, 28*(2), 364–369.

Kaur, R., Singh, R., & Singh, K. (2016). 1,5-Benzothiazepine: Bioactivity and targets. *Chem. Biol. Lett., 3*(1), 18–31.

Kikkawa, K., Murata, S., & Nagao, T. (1988). Calcium antagonistic and spasmolytic activities of a new 1,5-benzothiazepine derivative in isolated canine and monkey arteries. *Arzneimittelforschung, 38*(4), 526–531.

Kikkawa, K., Yamauchi, R., Suzuki, T., Banno, K., Murata, S., Tetsuka, T., et al. (1994). Effects of clentiazem on cerebral ischemia induced by carotid artery occlusion in stroke-prone spontaneously hypertensive rats. *Stroke, 25*(2), 474–480.

Leszczynska, K., & Kau, S. T. (1992). A sciatic nerve blockade method to differentiate drug-induced local anesthesia from neuromuscular blockade in mice. *J. Pharmacol. Toxicol. Methods, 27*(2), 85–93.

Lokshin, P., Kotler, M., Kutzuk, D., & Belmaker, R. H. (1998). Clotiapine: An old neuroleptic with possible clozapine-like properties. *Prog. Neuropsychopharmacol. Biol. Psychiatry, 22*(8), 1289–1293. https://doi.org/10.1016/S0278-5846(98)00083-9.

Lyseng-Williamson, K. A. (2015). Clotiapine in schizophrenia: a guide to its use. *Drugs Ther. Perspect., 31*, 365–371. https://doi.org/10.1007/s40267-015-0248-3.

Markham, A., & Brogden, R. N. (1993). Diltiazem. A review of its pharmacology and therapeutic use in older patients. *Drugs Aging, 3*(4), 363–390.

Maxwell, C. J., Hogan, D. B., & Ebly, E. M. (1999). Calcium-channel blockers and cognitive function in elderly people: Results from the Canadian Study of Health and Aging. *Can. Med. Assoc. J., 161*(5), 501–506.

Mecca, T. E., & Love, S. D. (1992). Comparative cardiovascular actions of clentiazem, diltiazem, verapamil, nifedipine, and nimodipine in isolated rabbit tissues. *J. Cardiovasc. Pharmacol., 20*(4), 678–682.

Michaelis, W. (1969). The metabolism of 2-chloro-11-(4-methyl-1-piperazinyl) dibenzo-(*b,f*)(1,4)-thiazepine (clotiapine). I. Resorption, distribution in the body and excretion in rodents. *Farmaco Prat., 24*(2), 84–91.

Muneer, A. (2015). Pharmacotherapy of bipolar disorder with quetiapine: A recent literature review and an update. *Clin. Psychopharmacol. Neurosci., 13*(1), 25–35. https://doi.org/10.9758/cpn.2015.13.1.25.

Nagao, T., Murata, S., & Sato, M. (1975). Effects of diltiazem (CRD-401) on developed coronary collaterals in the dog. *Jpn. J. Pharmacol., 25*(3), 281–288.

Nagao, T., Narita, H., Sato, M., Nakajima, H., & Kiyomoto, A. (1982). Development of diltiazem, a calcium antagonist: Coronary vasodilating and antihypertensive actions. *Clin. Exp. Hypertens. A, 4*(1–2), 285–296.

Narimatsu, A., & Taira, N. (1976). Effects of atrio-ventricular conduction of calcium-antagonistic coronary vasodilators, local anaesthetics and quinidine injected into the posterior and the anterior septal artery of the atrio-ventricular node preparation of the dog. *Naunyn-Schmiedeb. Arch. Pharmacol., 294*(2), 169–177.

Narita, H., Zera, P. H., & Ginsburg, R. (1990). Mechanism of action of clentiazem on human coronary artery and myocardium. *Cardiovasc. Drugs Ther., 4*(4), 1097–1104.

Nasa, Y., Ichihara, K., & Abiko, Y. (1990). Both d–*cis*– and l–*cis*–diltiazem have anti-ischemic action in the isolated, perfused working rat heart. *J. Pharmacol. Exp. Ther., 255*(2), 680–689.

Nishida, M., Urushidani, T., Sakamoto, K., & Nagao, T. (1999). L–*cis* diltiazem attenuates intracellular $Ca^{(2+)}$ overload by metabolic inhibition in guinea pig myocytes. *Eur. J. Pharmacol., 385*(2–3), 225–230.

Odawara, A., Katoh, M., Karasawa, T., Tamura, K., & Sasaki, Y. (1994). Inhibitory effect of clentiazem (TA–3090) on platelet aggregation–alone and in combination with aspirin or ticlopidine. *Thromb. Res., 75*(2), 109–119.

Ohno, Y., Hisaka, A., & Suzuki, H. (2007). General framework for the quantitative prediction of CYP3A4–mediated oral drug interactions based on the AUC increase by coadministration of standard drugs. *Clin. Pharmacokinet., 46*(8), 681–696.

Perrella, C., Carrus, D., Costa, E., & Schifano, F. (2007). Quetiapine for the treatment of borderline personality disorder; an open–label study. *Prog. Neuropsychopharmacol. Biol. Psychiatry, 31*(1), 158–163.

Ross, J. L., Mackenzie, T. B., Hanson, D. R., & Charles, C. R. (1987). Diltiazem for tardive dyskinesia. *Lancet (London, England), 329*(8527), 268.

Sakamoto, K., Ishikawa, M., Koga, K., Urushidani, T., & Nagao, T. (2000). Energy preserving effect of l–*cis* diltiazem in isolated ischemic and reperfused guinea pig hearts: A ^{31}P–NMR study. *Jpn. J. Pharmacol., 83*(3), 225–232.

Schotte, A., Janssen, P. F., Gommeren, W., Luyten, W. H., Van Gompel, P., Lesage, A. S., et al. (1996). Risperidone compared with new and reference antipsychotic drugs: *In vitro* and in vivo receptor binding. *Psychopharmacology, 124*(1–2), 57–73.

Shafti, S. S., & Kaviani, H. (2019). Adjunctive quetiapine may help fluvoxamine-resistant obsessive-compulsive disorder among female in-patients: A randomized-controlled study. *Psychiatr. Clin. Psychopharmacol., 29*(2), 171–177. https://doi.org/10.1080/24750573.2019.1597584.

Sica, D. A. (2006). Interaction of grapefruit juice and calcium channel blockers. *Am. J. Hypertens., 19*(7), 768–773.

Soares, K. V., & McGrath, J. J. (2000). Diltiazem, nifedipine, nimodipine or verapamil for neuroleptic–induced tardive dyskinesia. *Cochrane Database Syst. Rev, 2*, CD000206.

Spanarello, S., Beoni, A. M., Mina, G., Amantini, K., & Colotto, A. (2005). Analysis of differential clinical profiles of different antipsychotic molecules in the first psychotic episode: A retrospective study. *Encephale, 31*(6–1), 692–697.

Sporkert, F., Augsburger, M., Giroud, C., Brossard, C., Eap, C. B., & Mangin, P. (2007). Determination and distribution of clotiapine (Entumine) in human plasma, post-mortem blood and tissue samples from clotiapine-treated patients and from autopsy cases. *Forensic Sci. Int., 170*(2–3), 193–199.

Srinivas, S., Parvataneni, T., Makani, R., & Patel, R. S. (2020). Efficacy and safety of quetiapine for pediatric bipolar depression: A systematic review of randomized clinical trials. *Cureus, 12*(6), e8407. https://doi.org/10.7759/cureus.8407.

Suzuki, S., Mori, M., & Kusano, S. (1992). Analysis of the daily variation in blood pressure and pharmacokinetics after single or repeated administration of clentiazem to patients with essential hypertension. *Arzneimittelforschung, 42*(4), 533–539.

Szekeres, L., Udvary, E., & Végh, A. (1985). Importance of myocardial blood flow changes in the protective action of diltiazem in a new model of myocardial ischaemia. *Br. J. Pharmacol., 86*(2), 341–350.

Takeo, S., Tanonaka, K., Tazuma, Y., Fukao, N., Yoshikawa, C., Fukumoto, T., et al. (1988). Diltiazem and verapamil reduce the loss of adenine nucleotide metabolites from hypoxic hearts. *J. Mol. Cell. Cardiol., 20*(5), 443–456.

Tanonaka, K., Kajiwara, H., Kameda, H., Takasaki, A., & Takeo, S. (1999). Relationship between myocardial cation content and injury in reperfused rat hearts treated with cation channel blockers. *Eur. J. Pharmacol., 372*(1), 37–48.

Villarreal, G., Hamner, M. B., Cañive, J. M., Robert, S., Calais, L. A., Durklaski, V., et al. (2016). Efficacy of quetiapine monotherapy in posttraumatic stress disorder: A randomized, placebo-controlled trial. *Am. J. Psychiatry, 173*(12), 1205–1212.

Vorkapic, P., Bevan, J. A., & Bevan, R. D. (1991). Clentiazem protects against chronic cerebral vasospasm in rabbit basilar artery. *Stroke, 22*(11), 1409–1413.

Walker, C., Thomas, J., & Allen, T. S. (2003). Treating impulsivity, irritability, and aggression of antisocial personality disorder with quetiapine. *Int. J. Offender Ther. Comp. Criminol., 47*(5), 556–567.

Waters, D., & Garceau, D. (1993). A dose–response study of clentiazem, a chloro-derivative of diltiazem, in patients with stable angina. CAMCAT Study Group. *J. Am. Coll. Cardiol., 21*(4), 964–970.

Watson, W. P., & Little, H. J. (1994). Interactions between diltiazem and ethanol: Differences from those seen with dihydropyridine calcium channel antagonists. *Psychopharmacology, 114*(2), 329–336.

Yeung, P. K., Prescott, C., Haddad, C., Montague, T. J., McGregor, C., Quilliam, M. A., et al. (1993). Pharmacokinetics and metabolism of diltiazem in healthy males and females following a single oral dose. *Eur. J. Drug Metab. Pharmacokinet., 18*(2), 199–206.

Non-Print Items

Abstract

The current pharmacological knowledge of five 1,5-benzothiazepine drugs, including general modes of action, metabolic routes, pharmacokinetic profiles, recommended dosages, adverse effects, and drug–drug interactions, is explained in this chapter.

Keywords

Diltiazem; Clentiazem; Quetiapine; Clotiapine; Pharmaceutical applications

Index

Page numbers followed by "*f*" and "*t*" indicate, figures and tables respectively.