New, centrally acting dopaminergic agents with an improved oral bioavailability: synthesis and pharmacological evaluation

RIJKSUNIVERSITEIT GRONINGEN

New, centrally acting dopaminergic agents with an improved oral bioavailability: synthesis and pharmacological evaluation

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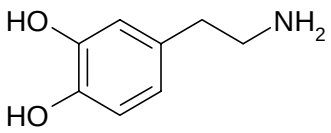
Chapter 1

Introduction

1.1 Dopamine, a neurotransmitter in the central nervous system

Neurotransmitters serve to transmit signals between neurons, which are separated by a synaptic cleft. One of the neurotransmitters is dopamine (DA), or β-(3,4-dihydroxyphenyl)ethylamine (**1**). Until the mid-1950s dopamine was exclusively considered to be an intermediate in the biosynthesis of the catecholamines noradrenaline and adrenaline. Significant tissue levels of dopamine were first demonstrated in peripheral organs of ruminant species.[1] A short time later it was found that dopamine was also present in the brain in about equal concentrations to those of noradrenaline.[2]



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When a stimulus depolarises the transmembrane potential in a spiking axon above the threshold level, an all-or-none action potential in a spiking axon is activated. The action potential propagates unattenuated to the nerve terminal where ion fluxes activate a mobilisation process leading to transmitter secretion.[3] The neurotransmitter binds reversibly to receptor proteins embedded in the membrane of a neuron, which triggers a certain effect. There are two types of receptors known, presynaptic receptors or autoreceptors which are present on the neurotransmitter 'releasing neurons', and postsynaptic or heteroreceptors, which are present on the neurotransmitter 'receiving neuron'. The former are supposed to perform a feed-back function, and slow down the release of neurotransmitter from these neurons when they are stimulated.[4]

Dopamine receptors are G-protein coupled receptors. After a receptor has been activated, this G-protein may activate or inhibit the second messenger system causing certain biochemical reactions to occur. In contrast to the ionotropic receptors that are linked to an ion-channel and respond very fast to activation by a neurotransmitter (millisecond processes), G-protein coupled receptors mediate slower responses (seconds to minutes) and in general have a modulatory function on other signal transduction processes.[4]

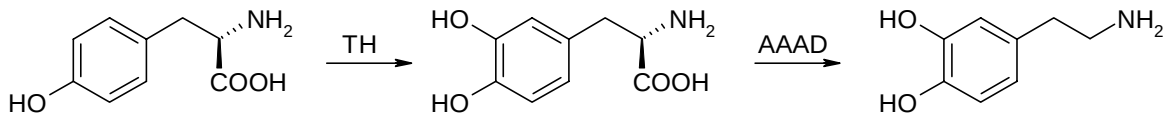
Although dopamine also has important peripheral functions, e.g. within the regulation of cardiovascular homeostasis, this thesis will deal with the effects of ligands on dopamine receptors in the central nervous system (CNS). Dopamine is the predominant catecholamine neurotransmitter in the mammalian brain and is involved in the regulation of a number of physiological activities, i.e. movement, emotion and hormone secretion. These activities are associated with three principal dopaminergic pathways: (1) The nigrostriatal pathway controls movements; partial degeneration of this system contributes to the pathogenesis of Parkinson's disease. (2) The mesocorticolimbic pathway is involved in emotion; imbalance in this pathway

is thought to contribute to the aetiology of schizophrenia. (3) The tuberoinfundibular pathway regulates e.g. prolactin secretion from the pituitary and influences lactation and fertility.[5]

1.2 General aspects of dopamine neurotransmission in the central nervous system

1.2.1 Biosynthesis of dopamine

The synthesis of dopamine originates from the precursor the amino acid L-tyrosine, which must be transported across the blood-brain barrier into the dopaminergic neuron. The rate limiting step in the synthesis is the conversion of L-tyrosine to L-dihydroxyphenylalanine (L-DOPA) by the enzyme tyrosine hydroxylase (TH). L-DOPA is subsequently converted to dopamine by aromatic L-amino acid decarboxylase. The latter enzyme turns over so rapidly that L-DOPA levels in the brain are negligible under normal conditions.[1]



2 **3** **1**

Chart 1.1 Biosynthesis of dopamine. Dopamine (**1**); L-Tyrosine (**2**); L-3,4-dihydroxyphenylalanine (**3**, L-DOPA); TH, tyrosine hydroxylase; AAAD, aromatic L-amino acid decarboxylase.

Under normal conditions it is not feasible to augment dopamine synthesis significantly by increasing brain levels of L-tyrosine, since the levels in the brain are already above the K_m for tyrosine hydroxylase. The activity of tyrosine hydroxylase is subject to four major regulatory influences: (1) Dopamine functions as end-product inhibitor of TH by competing with a tetrahydrobiopterin (BH-4) cofactor for a binding site on the enzyme. (2) The availability of BH-4 may also play a role in regulating TH activity. (3) Presynaptic dopamine receptors also modulate the rate of tyrosine hydroxylation. These receptors are activated by dopamine released from the nerve terminal, resulting in feedback inhibition of dopamine synthesis. (4) Dopamine synthesis also depends on the rate of impulse flow in the nigrostriatal pathway. On the other hand it is possible to enhance dramatically the formation of dopamine by increasing the levels of L-DOPA because of the high activity of aromatic L-amino acid decarboxylase and the low endogenous levels of L-DOPA normally present in the brain.[1]

1.2.2 Dopamine re-uptake and metabolism

Once the dopamine that is released into the synaptic cleft has exerted its action on the various dopamine receptors, these actions have to be terminated to prevent continuous

stimulation of these receptors. This inactivation is brought about by re-uptake mechanisms and by metabolism of dopamine.

Dopamine nerve terminals possess high-affinity dopamine uptake sites that are important in termination transmitter action and in maintaining transmitter homeostasis. Uptake is accomplished by a membrane carrier that is capable of transporting dopamine in either direction, depending on the existing concentration gradient. The dopamine transporter recycles extracellular dopamine by actively pumping it back into the nerve terminal. About 70-80 % of the dopamine, which is present in the synaptic cleft, is inactivated by this process. Certain drugs, such as cocaine, are able to block the action of the dopamine transporter, thereby sustaining the presence of dopamine in the synaptic cleft and its action on dopamine receptors.

After re-uptake by the nerve terminal a part of the released dopamine is converted to dihydroxyphenylacetic acid (DOPAC, **5**) by intraneuronal monoamine oxidase (MAO) and aldehyde dehydrogenase (AD). Released dopamine is also converted to homovanillic acid (HVA, **6**), probably at an extraneuronal site through the sequential action of catechol-O-methyltransferase (COMT) and MAO. In rat brain, DOPAC is the major metabolite and considerable amounts of DOPAC and HVA are present in sulfate-conjugated as well as free forms.[1]

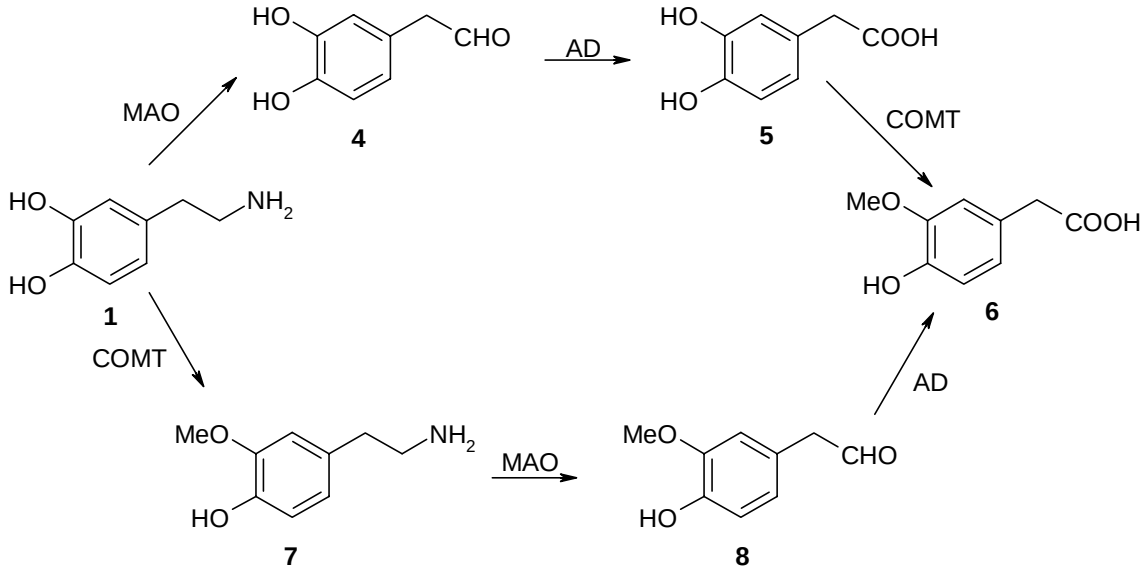


Chart 1.2 Neuronal metabolism of dopamine. Dopamine (**1**); 3,4-dihydroxyphenyl acetaldehyde (**4**); 3,4-dihydroxyphenylacetic acid (DOPAC, **5**); homovanillic acid (HVA, **6**); 3-methoxytyramine (3-MT, **7**); 3-methoxy-4-hydroxyphenyl acetaldehyde (**8**); MAO, monoamine oxidase; AD, aldehyde dehydrogenase; COMT, catechol-O-methyltransferase.

1.3 Dopamine receptors

1.3.1 General structural features of G-protein-coupled receptors

Upon release from dopaminergic neurons, dopamine exerts its action by interacting with specific dopamine receptors. With molecular biological techniques five subtypes of dopamine receptors have been identified so far. These receptors have been characterised anatomically, and to a certain extent biochemically and pharmacologically. All currently identified dopamine receptor subtypes belong to the superfamily of G-protein-coupled receptors (GPCRs).

A wide variety of membrane receptors for hormones and neurotransmitters are coupled to guanine-nucleotide-binding regulatory 'G' proteins, which upon activation by receptors, stimulate or inhibit various effectors such as enzymes or ion channels. Among the family of receptors coupled to G-proteins are those for catecholamines, acetylcholine and related muscarinic ligands, tachykinins, and for the pituitary glycoprotein hormones and many more.[6] The exact molecular structures of GPCRs are unknown, since attempts to crystallise these proteins have failed thus far. Nevertheless, biophysical, biochemical and molecular biological studies on various GPCRs suggest that these receptors have many structural features in common. The common features are: (1) a cell surface receptor; (2) an effector, such as an ion channel or the enzyme adenylyl cyclase; and (3) a G-protein, that is coupled to both the receptor and its effector.[7] All these receptors are made up of a single chain containing amino acid residues of which the number of residues differ for each receptor subtype. The amino-terminus, which has no signal sequence, may contain sites for N-linked glycosylation, which is the case for the dopamine receptors; the carboxy-terminus has typical sites for phosphorylation by protein kinase A and other kinases. Seven stretches of 22-28 hydrophobic conserved residues, separated by hydrophilic segments, are found in each of the members of the family of proteins, as has been seen earlier in the well-characterised rhodopsins, which are also coupled to a GTP binding protein. This similarity has led to the suggestion that these receptors share with bacteriorhodopsin its peculiar membrane topology by which the seven conserved hydrophobic segments form transmembrane domains, possibly constituting α-helices, although other configurations are also imaginable. The N-terminal region, by virtue of its glycosylation, is extracellular and the carboxy-terminal domain is intracellular. The various portions between the seven hydrophobic stretches are either extra- or intracellular. The third intracellular and the carboxy-terminal segments display an extensive variability in length and sequence, which has led to the hypothesis that these parts of the 7 transmembrane G-protein coupled receptors are responsible for the selective interaction with the various regulatory G-proteins.[6] All GPCRs cloned so far have been shown to possess a substantial degree of homology in their amino acid sequences, especially in the transmembrane regions.

The binding site for the endogenous ligand (the 'active site') is believed to be situated within the core formed by the seven transmembrane domains.[7] Binding of the endogenous

ligand to the active site presumably induces conformational changes in the receptor molecule, which trigger via the G-protein an intracellular response, e.g. the activation of a second messenger system. In this way, the 'information' carried by the ligand is transduced over the plasma membrane into the cell (for reviews and references on GPCRs, see refs. 6-8).

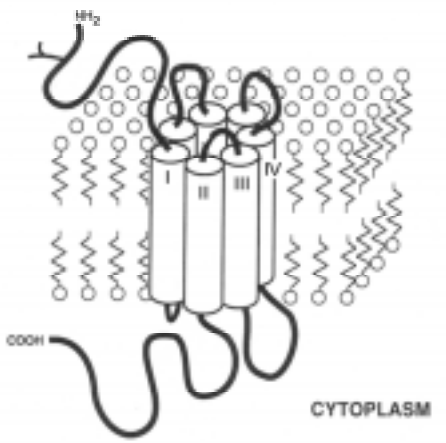


Figure 1.1 Schematic model for the insertion of G-protein-coupled receptors in the plasma membrane. The seven transmembrane domains are shown as cylinders spanning the lipid bilayer. The intra- and extracellular loops are represented by black ribbons. The ligand binding site is formed by the interaction of several transmembrane domains. Coupling to transducing and desensitisation systems involves the cytoplasmic loops. Glycosylation (represented with a Y) of the N-terminus is required for proper insertion of the receptors in the membrane but not for ligand binding. Figure adapted from ref. 6.

1.3.2 Dopamine receptor classification

The application of biochemical, pharmacological and physiological techniques to the study of dopamine receptors showed clearly that there were multiple receptors for dopamine, and in 1978 it was proposed that there were two subtypes of the dopamine receptor (D_1 and D_2).[9] The application of molecular biological techniques in the late 1980s showed that there were at least five dopamine receptor subtypes (D_1, D_2, D_3, D_4 and D_5).[10-18]

On the basis of structural, pharmacological, functional and distributional similarities, all dopamine receptor subtypes fall into one of the two initially recognised receptor categories, here designated dopamine D_1- or dopamine D_2-like receptors. Dopamine D_5 receptors share extensive similarities with dopamine D_1 receptors, while dopamine D_3 and D_4 receptors more closely conform to the features of dopamine D_2 receptors. The properties of the two subfamilies closely resemble those of the dopamine D_1 and D_2 receptor subtypes as originally defined by Kebabian and Calne.[9] The most important characteristics of the cloned human dopamine receptor subtypes are summarised in Table 1.1.

Table 1.1 Summary of the characteristics of cloned human dopamine receptor subtypes.

	D_1-like		D_2-like		
	D_1	D_5	D_2	D_3	D_4
Gene					
Chromosome localisation	5q35.1[19]	4p16.3[19]	11q22-23[19]	3q13.3[19]	11p15.5[19]
Introns	no[12-14]	no[10,18]	yes[15]	yes[17]	yes[16]
Expression	–	–	D_{2A}/D_{2B}	–	$D_{4.2}$-$D_{4.10}$
Protein					
Amino acids	446[12-14]	477[10,18,20]	443/414[15]	400[17]	387[16]
3rd Cytoplasmatic loop	short	short	long	long	long
C-terminus	long	long	short	short	short
Sequence homology[a] D_1	100	82	47	45	42
D_5		100	44	40	45
D_2			100	77	51
D_3				100	40
D_4					100
Localisation[b, 21]	Cput, NAc, ICj, OT	Hipp, Hyp	Cput, NAc, ICj, OT, Pit, SN, VTA	NAc, ICj, Sept, Thal, Hyp, Cer	FC, OT, Amyg, Mes, MO
Pharmacology					
Dopamine affinity (nM)[c]	2300	230	2000	30	450
Agonist	SKF 38393	SKF 38393	N-0923	PD 128907	PD 168077
Antagonist	SCH 23390	SCH 23390	Raclopride	S 14297	L-745,870
Biochemistry[d]					
G-protein coupled	yes	yes	yes	yes/?	yes
cAMP	+	+	–	n.e./–	n.e./–
IP_3	+	?	+	n.e.	n.e.
Ca^{2+}	+	?	–	–	–
Arachidonic acid	?	?	+	n.e./–	+
Dopamine release	?	?	–	n.e./–	n.e.
Mitogenesis	?	?	+	n.e./+	?
Acidification	?	?	+	+	+

Footnotes: [a] Sequence homology in transmembrane domains, expressed as percentages. [b] Based on rat brain mRNA distribution data, only the areas with a high density are mentioned. Abbreviations: Cput,

caudate putamen; NAc, nucleus accumbens; ICj, islands of Calleja; OT, olfactory tubercle; Hipp, hippocampus; Hyp, hypothalamus; Thal, thalamus; Pit, pituitary; SN, substantia nigra; VTA, ventral tegmental area; Sept, septum; Cer, cerebellum; FC, frontal cortex; Amyg, amygdala; Mes, mesencephalon; MO, medulla oblongata. [c] Values in the presence of Gpp(NH)p (taken from ref. 22). [d] Abbreviations: cAMP, cyclic adenosine monophosphate; IP_3, inositol triphosphate; +, increase; –, decrease; n.e., no effect; ?, unknown; n.e./– and n.e./+, in the literature there is a controversy about the effect (taken from ref. 23).

In addition to elucidating the molecular features of GPCRs, molecular cloning techniques have also allowed for the expression of GPCRs in cells that normally do not express such receptors. Thus, mammalian cell lines can be transiently or permanently transfected with cDNAs encoding the different dopamine receptor subtypes. Because these cells usually express a single receptor subtype in high density, they are very suitable for determining receptor binding affinities of drug candidates. Since most newly synthesised target compounds presented in the subsequent chapters of this thesis have been evaluated for their ability to bind to cloned human dopamine D_2 and D_3 receptors, the characteristics of these two receptor subtypes will be described in more detail in the next sections. For reviews and references on the other dopamine receptor subtypes, see refs. 21,24-26.

1.3.3 Dopamine D_2 receptors

Based on the presumed structural homology between different GPCRs a rat genomic library was screened by Bunzow et al.[15] They used the DNA sequence encoding the hamster β_2-adrenergic receptor as a hybridisation probe. Ultimately a cDNA was isolated which coded for a 415 amino acid protein, which possessed a relative molecular mass similar to that for the deglycosylated form of the dopamine D_2 receptor as determined by SDS-PAGE. Another conformation that this cloned receptor was the dopamine D_2 receptor was the fact that the mRNA distribution parallels that of the dopamine D_2 receptor. Several structural features of the protein deduced from this cDNA demonstrated that it belongs to the family of GPCRs. First, a hydrophobicity plot of the protein sequence shows the existence of seven stretches of hydrophobic amino acids, which could represent seven transmembrane domains. Second, the primary amino acid sequence shows a high degree of similarity with other GPCRs. Third, the protein has several structural characteristics common to the other members of the family of GPCRs. There are three consensus sequences for N-linked glycosylation in the N-terminus with no signal sequence. Aspartate 80 found in transmembrane domain II is conserved in all known GPCRs. In transmembrane domain III Asp114 corresponds to an Asp residue found in receptors that bind cationic amines. Phosphorylation has been proposed as a means of regulating receptor function. A potential site for phosphorylation by protein kinase A exists at Ser228 in the third cytoplasmic loop. The cloned protein contains a large cytoplasmic loop between transmembrane

domains V and VI with a short C terminus. This structural organisation is similar to other receptors, which are coupled to G_i.[15]

In situ hybridisation revealed the distribution of dopamine D_2 receptor mRNA in the rat brain. High abundance was found in regions which are classically associated with dopaminergic neurotransmission, including the caudate putamen, nucleus accumbens, olfactory tubercle, pituitary, substantia nigra pars compacta and ventral tegmental area.[27,28]

When the receptors were expressed in mouse fibroblast cells it was possible to determine the pharmacological features of the receptor. It turned out that the expressed receptors possessed features typical of the native striatal dopamine D_2 receptor. When all of the drug K_i values were compared, their rank order of potency (spiperone > (+)-butaclamol > haloperidol > sulpiride >> (–)-butaclamol) agrees closely with the published values for the dopamine D_2 receptor.[29]

Expression of the receptor in other cell lines has revealed that the receptor interacts productively with a G-protein, probably G_i to inhibit adenylyl cyclase activity,[30] and also appeared to inhibit prolactin secretion.[29] Taken together, these findings strongly suggest that the cloned protein indeed corresponded to the classical dopamine D_2 receptor (for reviews and references see ref. 29).

After cloning of the rat dopamine D_2 receptor by Bunzow et al.[15] the next step was the cloning of the human dopamine D_2 receptor.[11,31] The human and rat dopamine D_2 receptors are encoded by highly related genes, and are pharmacologically highly related.[11] The two receptors differ in only 18 amino acid substitutions and by 1 amino acid in length, the human form lacking an isoleucine in the third cytoplasmic loop.[11] In order to determine which amino acids were relevant in binding and effector-coupling and to test these models, dopamine D_2 receptor mutants were generated. Initially, the highly conserved Asp 80 was recognised as serving a central role in normal receptor coupling to adenylyl cyclase.[21,32] Ser 193, in particular, seems to play a critical role in binding dopamine[21,33] and Asp 114 was a prerequisite for agonist as well as antagonist binding.[21]

A number of different studies have revealed that the dopamine D_2 receptor exists in alternate splice forms in rats[31,34-38] and humans.[31,39] The two forms of the dopamine D_2 receptor exist both in human and rat, and are generated by alternative splicing. Specifically, these forms differ by a 29 residue peptide sequence located in the predicted intracellular domain between transmembrane domain V and VI (cytoplasmic loop 3). The presence of this peptide characterises the more abundant, larger form (D_{2L} receptor); its absence defines the rarer, shorter form (D_{2S} receptor). The fact that the difference between the two isoforms lies in the third cytoplasmic loop may be important for G-protein coupling. This suggests that alternative splicing is used to fine-tune receptor interaction with G_i and G_o proteins. In fact, in preliminary expression studies, the D_{2S} receptor inhibited adenylyl cyclase activity to a greater extent than did the D_{2L} receptor form, corresponding with the notion that the shorter form more effectively couples to a G_i protein.[31] Several groups tried to identify properties that may differentiate the two dopamine D_2 receptor variants by studying the mRNA expression of the two receptor

isoforms in brain and pituitary, and as expressed in different cell lines.[35-37,39-41] Both splice variants have been detected in human anterior pituitary[31,42] and in a variety of brain regions.[42] In general, the mRNA distribution of both splice variants agrees with the distribution pattern of total dopamine D_2 mRNA. However, the D_{2L}/D_{2S} mRNA ratio varies in different brain regions and the short isoform is the least abundant of the two. Receptor binding experiments show that for D_{2S} and D_{2L} the K_i values for the high and low affinity agonist binding are rather similar in three cell lines tested, it is the proportions of the sites that differ. However, some compounds seem to have a higher affinity for the dopamine D_{2S} receptor.[43,44]

Presumably via coupling to different G-proteins, dopamine D_2 receptors in various cell lines revealed that they utilise different signal transduction systems (for reviews see refs. 21, 25). Inhibition of adenylyl cyclase has been detected in all cellular environments,[30,45-51] but cell-specific signalling pathways may be present as well. Beside inhibition of intracellular cAMP production, stimulation of dopamine D_2 receptors may result in: (1) enhancement of phosphatidylinositol (PI) hydrolysis by activation of the enzyme phospholipase C;[49,51] (2) increase[48,49,51] or decrease[51] in the intracellular Ca^{2+} concentration; (3) opening of K^+ channels;[51] and (4) extracellular release of arachidonic acid.[48,52]

1.3.4 Dopamine D_3 receptors

In 1990 Sokoloff et al.[17] cloned the cDNA encoding a novel dopamine D_2-like receptor, which was designated as dopamine D_3 receptor. The human dopamine D_3 receptor consisted of 400 amino acid residues and displayed a homology of 46 % with the human dopamine D_2 receptor and a homology of 78 % if only the presumed transmembrane domains are considered.[53] In addition, the subtypes have more structural features in common, such as the presence of introns in the coding sequence, a long third intracellular loop, a short carboxylic acid terminal segment and several glycosylation sites. The existence of introns may give rise to the expression of splice variants encoded by the same gene. Various truncated forms of the dopamine D_3 receptor mRNA, generated by alternative splicing, have been detected in rat and human brain which do not correspond to a functional receptor.[19,54]

The anatomical distribution of the dopamine D_3 receptor mRNA partially overlaps but markedly differs from that of dopamine D_2 receptor mRNA. The dopamine D_3 receptor is mainly expressed in discrete brain areas belonging to or related to the limbic system, whereas dopamine D_1 and D_2 receptors are widely expressed in all major dopaminoceptive areas.[17] Both dopamine D_2 and D_3 receptors are expressed by dopamine neurons belonging to the A9 and A10 cell groups and it has been suggested that both act as autoreceptors. Such a role for the dopamine D_3 receptor is consistent with its high apparent affinity for dopamine. The pharmacological profile of the dopamine D_3 receptor is comparable to that of the dopamine D_2 receptor, and supports a possible role as an autoreceptor. Thus, all dopamine D_2 receptor agonists and antagonists bind with good affinities to dopamine D_3 receptors as well, but some

compounds, previously designated as putative dopamine D_2 autoreceptor agonists (e.g. 7-OH-DPAT) or antagonists (e.g. (+)-AJ 76 and (+)-UH 232), show preference for the dopamine D_3 receptor. Given this pharmacological profile it would favour a role for the dopamine D_3 receptor as an autoreceptor. If so, it could play a role in regulating impulse flow, as well as neurotransmitter synthesis and release. However, the function of the dopamine D_3 receptor is still subject of debate. Several studies have given some insight into this debate, using the dopamine D_3 receptor preferring ligands R-(+)-7-OH-DPAT and PD128907 (agonists) or U-99194A (antagonist). Some studies indicate that the dopamine D_3 receptor is an autoreceptor involved in the presynaptic regulation of dopamine release.[55-62] These data, however, could not be confirmed by dopamine D_2 and D_3 knock-out mice. Experiments with dopamine D_2 receptor-deficient mice strongly suggest that only D_2 but not D_3 receptors are involved in the autoreceptor-mediated inhibition of the evoked release of [^{3}H]-dopamine.[63] These data are confirmed by experiments with D_3-receptor knock-out mice.[64]

Other studies indicate that the dopamine D_3 receptor is located postsynaptically and is involved in locomotor activity and behaviour. Experiments with dopamine D_3 receptor agonists[65-72] and antagonists[73-76] show that these receptors have inhibitory effects on locomotor activity and behaviour. In the literature it is also described that administration of dopamine D_3 receptor antisense[67] and dopamine D_3 mutant mice[77] give hyperactivity in the animals. Svensson et al.[72,78] showed that the decrease in locomotor activity after agonist administration is not likely to be dependent upon effects on dopamine release or synthesis.

Using [^{3}H]-7-OH-DPAT and [^{3}H]-PD 128907 it was possible to determine the localisation of the dopamine D_3 receptor in the brain with autoradiographic techniques.[79,80] The abundance of the dopamine D_3 receptor mRNA seems to be several orders of magnitude lower than that of dopamine D_2 receptor mRNA.[81] Furthermore, the dopamine D_3 receptor mRNA is mainly expressed in discrete brain areas belonging or related to the limbic system,[82] whereas dopamine D_2 receptor mRNA is widely expressed in all major dopaminoceptive areas.[17] Northern blot[81] and *in situ* hybridisation[17] analyses showed that dopamine D_3 receptor mRNA is widely expressed in the olfactory tubercle-islands of Calleja complex, antero-medial part of the nucleus accumbens, the bed nucleus of the stria terminalis, amygdaloid, septal, medial mammillary or anterior thalamic nuclei and hippocampal formation. Since the dopamine D_3 receptors are mainly expressed in these 'limbic' areas of the brain it is suggested that these receptors mediate cognitive, emotional, neuro-endocrine and autonomic functions.[81] Hence, these receptors are major targets for antipsychotic drug action and anti-addictive drug therapy.

Whereas the signal transduction pathways of the dopamine D_2 receptor have been unravelled to a large extent, the biochemistry of the dopamine D_3 receptor is much less clear. Initial studies failed to demonstrate any coupling to G-proteins. Binding of agonists to dopamine D_3 receptors, expressed in various cell types, was not or only weakly affected by guanine nucleotides, and no second messenger generation was observed.[17,22,50,83-85] Such a lack of response may, however, be due to the absence of a suitable G-protein or to an inappropriate

expression of the dopamine D_3 receptor in the recipient cells.[17] Later studies have revealed functional coupling of dopamine D_3 receptors to different transduction mechanisms in various cell lines. These mechanisms are: (1) inhibition of cAMP production,[86] (2) aggregation of melanophore pigment,[87] (3) acidification of the extracellular environment,[88] (4) inhibition of Ca^{2+} currents,[89] and (5) mitogen-activated protein kinase.[90] It is interesting that the dopamine D_3 receptors mimic the signalling output of dopamine D_2 receptors in the same cell, but with a lower efficacy, implying a less efficient coupling of dopamine D_3 receptors. The different efficacy of dopamine D_2 and D_3 receptor activation may be an important means for varying the information resulting from dopamine neurotransmission.[86]

1.4 Structure-activity relationships of dopamine receptor agonists

Through the years a number of different structure-activity relationships (SARs) and receptor models have been developed (for reviews see refs. 91, 92). Therefore this introduction will only give a short overview of the general aspects of dopamine receptor agonist SARs.

The growing interest in unravelling the mechanism of action of dopamine and related compounds at the molecular level started with the considerations of the conformation(s) of the dopamine molecule when bound to the receptor site. Dopamine itself is a conformationally flexible molecule that can adopt specific conformation(s) needed to achieve appropriate three dimensional interaction with various groups located at or near the recognition (receptor) site(s).

When the conformation of the ethylamine side-chain with respect to the aromatic nucleus is considered as the starting-point, the dopamine molecule may exist either in a trans (extended) conformation, or in a cis (gauche) conformation. Single crystal x-ray analysis of dopamine hydrochloride indicates that the ethylamine chain is in a nearly completely extended conformation which resides on a plane almost perpendicular to the plane of the catechol ring.[93]

Cannon[94,95] defined α- and β-conformers of dopamine, in which the catechol ring is coplanar with the ethylamine side-chain (chart 1.3). In the α-conformer the meta-OH group is on the edge of the ring closer to the ethylamine chain, whereas in the β-conformer the meta-OH is on the edge of the ring away from the side-chain. Concluding from several studies with dopamine analogues, which simulate the gauche conformation leading to inactive compounds, dopamine is assumed to interact with the receptor in the extended conformation.

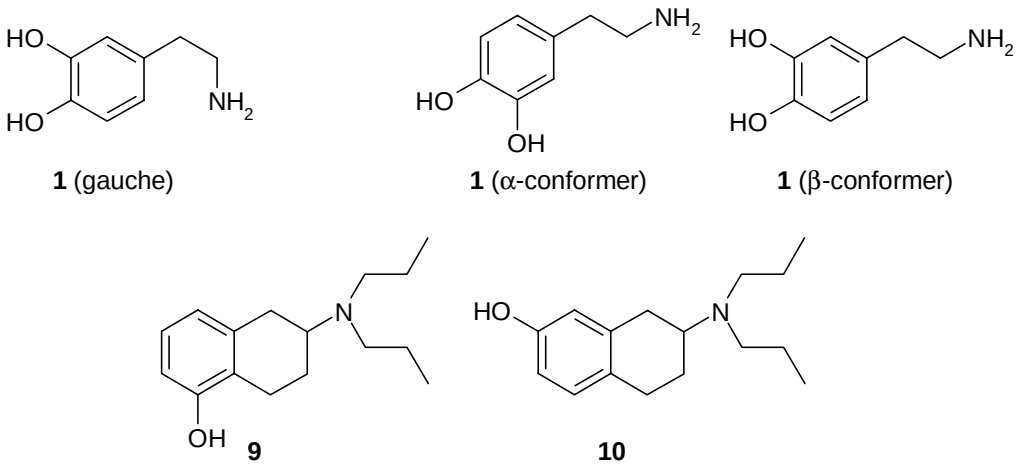


Chart 1.3 Gauche conformation of dopamine (**1**) and the two rotamers of the extended conformation of dopamine (**1** α-conformer and **1** β-conformer), and the corresponding 2-aminotetralins S-(–)-5-OH-DPAT (**9**) and R-(+)-7-OH-DPAT (**10**).

Studies with hydroxylated 2-aminotetralin analogues (among others using compounds **9** and **10**) and phenylethylamine analogues revealed that the first possessed a higher potency.[96,97] Apparently, the near co-planar arrangement is required for higher dopamine receptor agonist activity.

There are two main routes along which the development of dopamine agonists has initially proceeded, namely rigidification of the dopamine molecule (**1**), and dissection of one of the first known potent dopamine agonists, apomorphine (**11**).

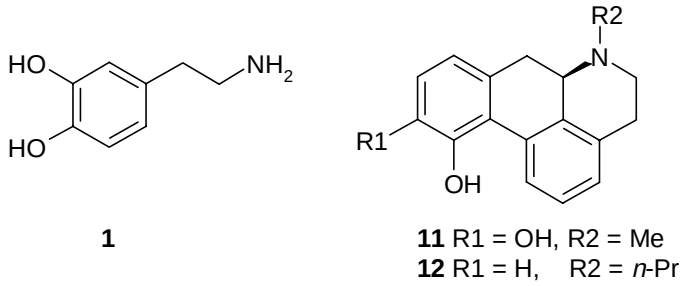


Chart 1.4 Chemical structures of dopamine (**1**), apomorphine (**11**) and 11-hydroxy-N-*n*-propylnoraporphine (**12**).

The rigidification of the dopamine molecule led to a number of dopamine receptor agonists in which the dopamine molecule, with its phenylethylamine moiety, is readily recognisable. In these dopamine agonists the amine moiety is either a part of a cyclic system, e.g. benzo[*f*]quinolines, naphthoxazines, and benzopyranoxazines, or an exocyclic amine, e.g. 2-aminotetralins and 2-aminoindans (Chart 1.5).

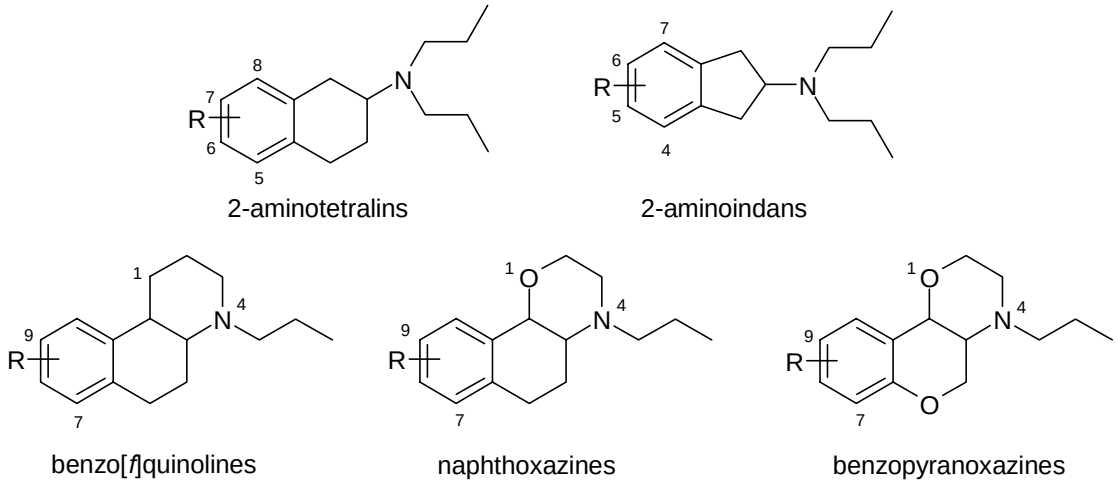
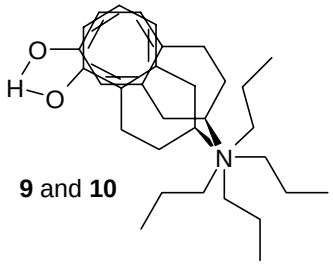


Chart 1.5 Some approaches for the rigidification of the dopamine ethylamine side chain. R-groups are usually -H or -OH.

SAR studies with these different classes of dopamine receptor agonists have led to the identification of a pharmacophore for activation of dopamine D_2 (-like) receptors consisting of the meta-hydroxyphenylethylamine structure. 2-Aminotetralins, hydroxylated at the 5- or 7-posistion, are potent dopamine receptor agonists, but their 6- or 8-hydroxylated analogues are less potent.[98] 8-Hydroxy-(N,N-di-*n*-propylamino)tetralin (8-OH-DPAT) is a potent serotonergic agent. Also dihydroxylated compounds like 5,6-di-OH-DPAT, which possess the catechol moiety also present in dopamine, are potent dopaminergic agents.

McDermed et al.[99,100] found that the interaction of dopamine receptor agonists with dopamine receptors is highly stereoselective. They observed that for compounds which are α-conformers the S-enantiomer is the most active (as is the case in S-(–)-5-OH-DPAT, **9**), whereas for β-conformers the R-enantiomer is the most active (as is the case in R-(+)-7-OH-DPAT, **10**). Their assumption then was that in order to achieve the proper orientation of the amino moiety and meta-OH groups, relative to the ethylamine side-chain, with respect to their presumed binding groups on the dopamine receptor surface, the β-conformer must be rotated with respect to the α-conformer (Chart 1.6). As a result of such a rotation the amine groups are pointing in the same direction.

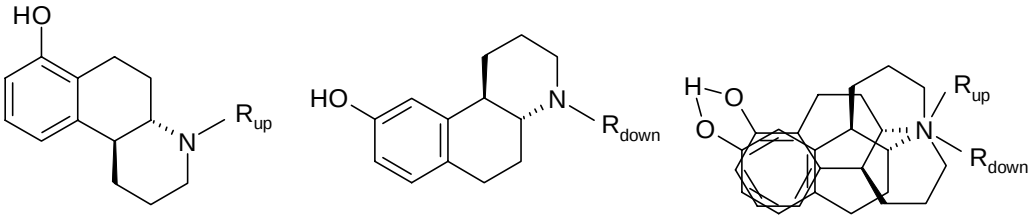


9 and **10**

Chart 1.6 Superimposition of S-(–)-5-OH-DPAT (**9**) with R-(+)-7-OH-DPAT (**10**).

McDermed and colleagues have also included a region in the receptor preventing the interaction with compounds having a steric bulk in this region.

In an extension of the McDermed model Wikström and colleagues,[101,102] applied to the dopaminergic monohydroxylated 3-phenylpiperidines and the octahydrobenzo[*f*]quinolines, have found that there is a relationship between the absolute configuration, the ring position of the hydroxyl group and the size of the N-alkyl substituent. In their model there are now two different directions possible for the N-alkyl substituents, namely 'upwards' and 'downwards' (Chart 1.7). An assumption they made is that, due to the different directions in which the N-alkyl substituents point, the space available for the N-substituent of compound **14** might be more restricted than for the corresponding isomer **13**. And indeed, they have found that the N-substituent 'downwards' is sterically restricted to maximally an *n*-propyl group, whereas the 'upwards' direction has less restricted demands.



13 **14**

Chart 1.7 Wikström's modification of McDermed's model. The structures of 7- (**13**) and 9-hydroxyoctahydrobenzo[*f*]quinolines (**14**) superimposed.

The distance from the amine-nitrogen to the hydroxyl-oxygen (meta or para) has been studied in several classes of dopamine agonists. The distances which were found vary considerably between these studies, which made clear that an optimal distance is hard to define. The nitrogen atom to meta-hydroxyl distance varied from 5.5 to 7.3 Å, the nitrogen to para-hydroxyl varied from 7.0-7.8 Å.[91] Therefore, it can be concluded that any distance between the nitrogen and the meta-hydroxyl that is less than that of dopamine in its fully extended form may be acceptable to a minimum of 5.5 Å.

Although some studies suggest the opposite,[103] it is generally believed that the nitrogen atom of a dopamine agonist interacts with the receptor in the protonated (ammonium) form. This idea is supported by studies with permanently charged dopamine analogues.[104] Furthermore, at physiological pH, dopamine exists mainly in the protonated form.[105]

In general, increasing the lipophilicity brings about increased potency for dopamine agonists. However, this is only true for compounds within the same series. When comparing compounds from different structural classes, other factors like conformation and stereochemistry are of primary importance for dopamine agonist activity. The oft-quoted rule[106] that an octanol-water partition coefficient, $\log P_{oct}$, of approximately 2 log units is optimum for ready entry into the brain is derived from studies on biological activity and not from rates of permeation nor from equilibria. Gratton et al.,[107] however, found that even if there is a parabolic relationship between the logarithm of a permeability surface area (logPS) and $\log P_{oct}$, this relationship is not good enough to use predictively.

The development of SARs for dopamine agonists was accompanied by the development of dopamine receptor models with computer programs. Basically, two kinds of dopamine receptor models emerged: indirect models and direct models. Indirect models take a series of (analogous) agents (agonists or antagonists) with certain receptor-binding characteristics as a starting point, and describe the receptor binding site as a collection of 'areas' with certain properties.[102,108-112] Later, the amino acid sequence and structure of the various dopamine receptor proteins became known with molecular biological techniques, which enabled the development of direct models.[113-115] These models take the receptor protein as a starting point, and describe the receptor binding site in terms of amino acids with different physical and sterical properties. Despite the development of these models for the different dopamine D_2-like receptors, it is still difficult to design selective ligands, because there is a high similarity in the transmembrane segments of the dopamine D_2 like receptors and the crystal structure of the receptor is not known yet. Since the models developed are all based on known compounds, their predictability declines when new, not included, compounds are tested in this model.

1.5 Pathogenesis of the dopaminergic system

Both movement and psychotic disorders in humans have been associated with disturbances in the functioning of the dopaminergic system. Examples of the motor disturbances are Parkinson's disease, Huntington's disease and Gilles de la Tourette's syndrome. These movement diseases can be categorised into either hypo- or hyperkinetic disorders. In this paragraph Parkinson's disease and schizophrenia with their current treatment will be discussed. Also drug abuse and its possible treatment will be discussed, since selective dopamine D_3 receptor agonists may be active as anti-addictive drugs.[116]

1.5.1 Parkinson's disease

1.5.1.1 Pathology of Parkinson's disease

Parkinson's disease is a progressive neurodegenerative disorder of the basal ganglia, which most often becomes apparent after the age of 55. It is a prototypic hypokinetic disorder, with tremor, rigidity, bradykinesia, and akinesia as the most prominent features.[117] Depression and a general slowing down of intellectual processes also occur, but are less well-defined. The neurological and psychiatric symptoms usually worsen with time (for review: ref. 118). The neuropathology of Parkinson's disease reveals a striking loss of the dopaminergic neurons of the nigrostriatal pathway terminating preferentially in the caudate nucleus and putamen.[119,120] Although the nigrostriatal system has a large reserve (only after a decline of approx. 70-80% of these cells, Parkinson's disease symptoms arise), progressive loss of these systems ultimately leads to Parkinson's disease, with its well-known movement disorders ('shaking palsy'). In contrast with some other neurodegenerative diseases (e.g. Huntington's disease), for Parkinson's disease no genetic component has been discovered yet. Usually the disease is not diagnosed before the first symptoms appear.

The control of normal motor behaviour is under influence of the basal ganglia through a so-called reinforcing basal ganglia-thalamocortical 'motor circuit'. Based on current data from a variety of experimental fields, a functional model of this 'motor circuit' has been proposed, as depicted, greatly simplified, in Figure 1.2.

First, there is a pathway called the 'direct' pathway consisting of neurones containing GABA and substance P. Activation of this pathway tends to disinhibit the thalamic stage of the circuit. The second pathway is the 'indirect' pathway, which passes first to the external segment of the globus pallidus (GPe) via striatal projection neurones that contain both GABA and enkephalin, then from GPe to the subthalamic nucleus (STN) via a purely GABAergic pathway, and finally to the output nuclei via an excitatory, probably glutaminergic, projection from the STN.[121] It should be apparent that dopamine D_1 receptors are primarily present on the medium spiny neurones, forming the 'direct' pathway, whereas dopamine D_2 receptors are primarily present on the medium spiny neurones at the start of the 'indirect' pathway.[122]

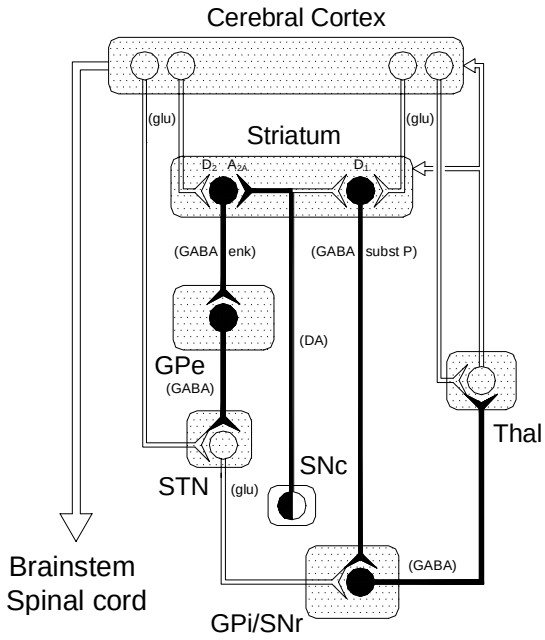


Figure 1.2 Schematic diagram of the circuitry and neurotransmitters of the basal ganglia-thalamocortical circuitry, indicating the parallel 'direct' and 'indirect' pathways from the striatum to the basal ganglia output nuclei in a normal situation. Inhibitory neurons are shown as filled symbols, excitatory neurons as open symbols. Abbreviations: DA, dopamine; D_1, dopamine D_1 receptor; D_2, dopamine D_2 receptor; A_{2A}, adenosine A_{2A} receptor; enk, enkephalin; GABA, γ-aminobutyric acid; GPe, external segment of globus pallidus; GPi, internal segment of globus pallidus; glu, glutamate; PPN, pedunculopontine nucleus; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; subst P, substance P; STN, subthalamic nucleus; Thal, thalamus. Adapted from references 121-123.

Considerable evidence indicates that shifts in the balance between the activity in the 'direct' and 'indirect' striatal output pathways and the resulting alterations in the output of the SNr and the GPi may account for the hypo- and hyperkinetic features of basal ganglia movement disorders. In summary, excessive inhibition of GPe within the indirect pathway leads to disinhibition of the STN, which in turn provides excessive excitatory drive to basal ganglia output nuclei (GPi/SNr), thus leading to excessive thalamic inhibition. This is reinforced by reduced inhibitory input to GPi/SNr through the direct pathway. Overall these effects are postulated to results in a reduction in the usual reinforcing influence of the motor circuit upon cortically initiated movements.[123] (For more reviews and references see ref. 117, 124-126).

Based on the altered neuronal activity in the 'motor' circuit in Parkinson's disease, it seems that parkinsonism symptoms can be treated by restoring the right balance between the activity in the 'direct' and 'indirect' pathways.

Although the exact cause of the progressive degeneration of nigrostriatal dopamine neurons in Parkinson's disease is still unknown, several mechanisms were proposed to explain the cell damaging processes at the molecular level that ultimately cause Parkinson's disease.[127] These mechanisms, which are probably related by interacting mechanisms, include: (1) excitotoxic mechanism,[128] (2) mitochondrial toxins,[129] and (3) oxidative stress.[130]

There is considerable body of (indirect) evidence which makes oxidative stress one of the best accepted hypothesis for explaining the cause of Parkinson's disease. For example, the Fe(II)/Fe(III) ratio in the substantia nigra is shifted from 2:1 in the normal brain to 1:2 in Parkinsonian brain.[131,132] In the Parkinsonian brain several enzymes which constitute the antioxidative defence mechanisms (glutathione peroxidase, catalase) have a decreased activity, while the activity of superoxide dismutase is increased, relative to the normal brain.[133] Furthermore, specific products of radical damage, such as lipid hydroperoxides, were detected at a 10-fold increased level in the Parkinsonian brain.[134]

1.5.1.2 Current treatment of Parkinson's disease

As Parkinson's disease is associated with a loss of dopamine, it is commonly treated with drugs, which replace or supply dopamine. Since dopamine itself cannot pass the blood-brain barrier, the most commonly used therapy is levodopa (L-DOPA), a precursor of dopamine. A complication of long-term treatment with L-DOPA, however, is the development of rapid fluctuations in clinical state where the patient switches suddenly between mobility and immobility; this phenomenon is known as the 'on-off effect'.[135,136] This effect might be caused by the loss of feed-back mechanisms.

An alternative approach to the treatment with L-DOPA is the use of drugs that mimic the action of dopamine. Treatment with dopamine-agonists, such as bromocriptine (**15**),[137] pergolide,[138,139] and lisuride,[138,140] has some advantages over treatment with L-DOPA. Dopamine agonists are effective in patients in the advanced stages of Parkinson's disease, unlike L-DOPA, because their action at postsynaptic dopamine receptors is unaffected by the lack of dopamine producing nerve cells.

Furthermore, there is an increasing interest in the direct effect of dopamine agonists to be potential neuro-protective agents. A number of studies in biologic and non-biologic systems have shown that dopamine agonists have antioxidant effects and can trap a variety of radical species.[141-143] Theoretically, such a protective effect might result from (a) a decrease in L-DOPA application, as L-DOPA itself may cause oxidative stress,[144] (b) stimulation of dopamine autoreceptors resulting in decreased dopamine synthesis, release, and turnover, as dopamine metabolism leads to reactive oxygen species,[145] and (c) direct anti-oxidant effects.[141-143]

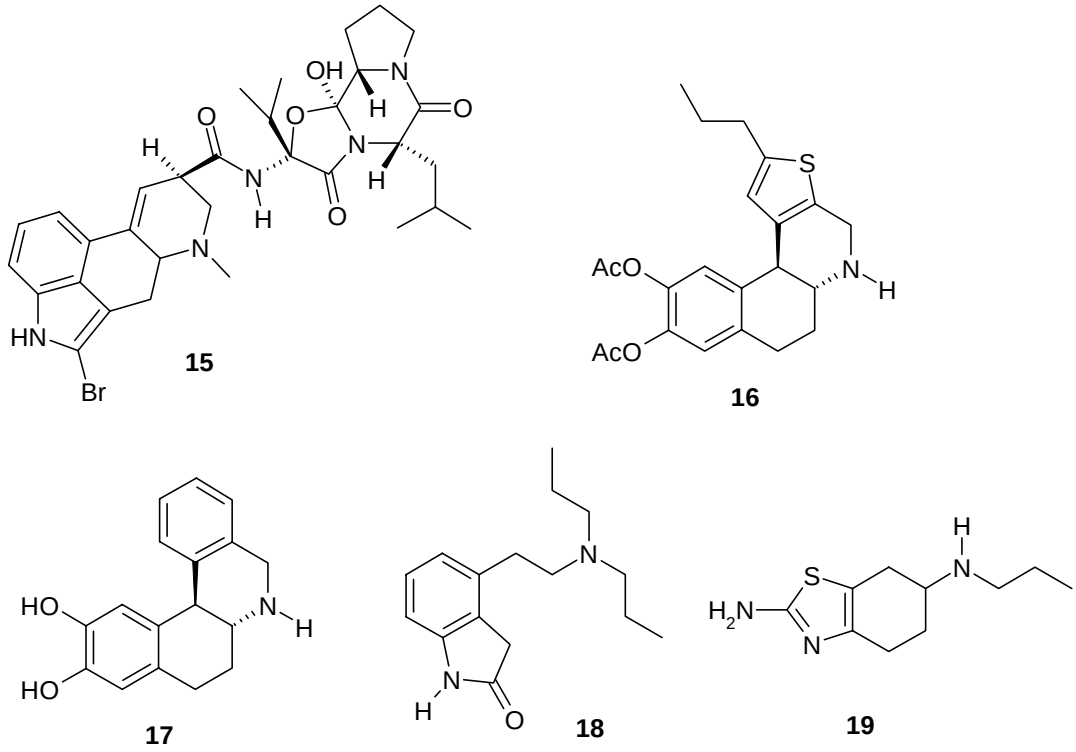


Chart 1.8 Chemical structures of bromocriptine (**15**), ABT-431 (**16**), dihydrexidine (**17**), ropinirole (**18**), and pramipexole (**19**).

Drug therapies or therapies which are in clinical development,[146-149] nowadays, include treatment with (prodrugs of) dopamine receptor agonists that are either selective for the dopamine D_1 receptor, such as ABT-431[150] (**16**) and dihydrexidine[151,152] (**17**), or are selective for the dopamine D_2/D_3 receptor subtypes, such as ropinirole[153] (**18**) and pramipexole[154,155] (**19**). An ideal antiparkinsonian treatment may require stimulation of both D_1 and D_2 dopamine receptors.[156] Literature shows that antiparkinsonian effects can be exerted either by a dopamine D_1 receptor agonist alone or by a dopamine D_2 receptor agonist alone, whereas hyperactivity and aggressiveness manifested by dopamine receptor agonists require co-activation of the D_1 and D_2 receptors. The antiparkinsonian effect can be dissociated from the adverse effect by therapeutic strategy. It is implied that imbalances in activation of the D_1 and D_2 receptors may provide a favourable approach for long-term treatment of parkinsonian patients with dopamine drugs.[157,158] Potent dopamine D_1 receptor agonists with an intermediate half-life may prove to be better adjuncts in the treatment of Parkinson's disease, because stimulation of the dopamine D_1 receptor may provide a better integration of neural inputs to the internal segment of the globus pallidus (referred to as the basal ganglia output) compared with L-DOPA and selective dopamine D_2 receptor agonist.[159,160] A drug with affinity for both the dopamine D_1 and D_2 receptors is apomorphine, which can be a good tool in restoring the imbalances in activation of the D_1 and D_2 receptors in Parkinson's disease.

Dopamine receptor agonists used in the therapy against Parkinson's disease often possess phenolic or catecholic moieties, which lead to low oral bioavailabilities for these compounds since they undergo considerable metabolic degradation in the liver.[161] Because of the low oral bioavailability of hydroxylated compounds, there has been a lot of interest in the development of prodrugs of such compounds, thereby circumventing the metabolic degradation.

Other approaches, some of which are still in clinical development, include restoration of the acetylcholine-dopamine balance in the basal ganglia, neuronal nicotinic receptor agonists, neurotrophic immunophilins, dopamine transport inhibitors, COMT-inhibitors, and adenosine A_{2A} receptor antagonists.[162,163] Also surgical therapies are used or under development, including stereotactic thalamotomy, continuous electric thalamus stimulation,[164] posteroventral pallidotomy and transplantation of embryonal substantia nigra cells.

1.5.2 Schizophrenia

1.5.2.1 Pathology of schizophrenia

Schizophrenia is a psychotic disorder of unknown aetiology in which patients suffer from a cluster of symptoms which may include both positive (delusions, hallucinations, disordered thoughts, and disorganised speech) and negative (flat effect, anhedonia, social withdrawal, emotional detachment, cognitive deficits, and poverty of speech) symptoms. This disease, which is relatively common (lifetime prevalence rate ~1 %), usually strikes its victims during adolescence or early adulthood. Since occupational and social function is severely affected, often leading to institutionalisation, the cost to society is very high.[165] Unfortunately, it is very difficult to diagnose schizophrenia and many different diagnostic systems have so far been developed. Moreover, there are strong indications that schizophrenia is not a homogeneous disorder, but rather consists of subgroups.[166]

Although there has been extensive research during several decades, still no definite explanation for the development of schizophrenia in man can be given. There is still a controversy between the very diverse theories to explain the development of the disease. It is generally accepted that genetic predisposition plays a significant etiological role in schizophrenia.[167] Although there has been a lot of research for finding genes for schizophrenia this search has not been successful yet. Crocq et al.[168] found an association between schizophrenia and homozygosity at the dopamine D_3 receptor gene. However, Yang et al.[169] found that this association was not present in schizophrenics. These two studies show that more research is necessary to find genes. Other etiological factors in schizophrenia are infection and autoimmunity,[170] and obstetric complications.[171] Also theories involving neurochemical alterations are related to the pathogenesis of schizophrenia. From this point of view the dopamine hypothesis has provided a framework for understanding the disease and proposing approaches to the treatment of schizophrenia. This hypothesis suggests that schizophrenia

results from increased dopaminergic neurotransmission and that treatments which decrease dopaminergic function will alleviate psychotic symptoms.[165] This dopamine hypothesis prevails despite much criticism and qualification. In addition to dopamine, there has been increased interest in other neurotransmitters that are hypothesised to play a role in schizophrenic pathophysiology through their interaction with dopamine or in their own right. The principal candidates in recent years have been serotonin, noradrenaline, glutamate and the neuropeptides cholecystokinin and neurotensin.[172]

Another line of research is focused on the neurobiological development of the human brain in relation to schizophrenia. It has been hypothesised that schizophrenia is due to abnormal neurodevelopment, which results in a static encephalopathy that usually becomes manifest in adolescence. This abnormal development leads to morphological deviations such as enlarged or reduced brain structures, a disrupted communication between different brain structures, alterations in neuron density or decreased neuron size, and abnormal neuronal migration or differentiation.[173]

1.5.2.2 Current treatment of schizophrenia

The first neuroleptic drug to be developed was chlorpromazine (**20**) in 1952, which belongs to the group of the phenothiazines. Reserpine (**21**) was the second drug, which was introduced into the psychiatric practice. Although it is a potent anti-psychotic drug, its side effects are so strong that it is hardly used as an anti-psychotic drug anymore. Another line of research was the development of butyrophenones. An important representative of this series of compounds is haloperidol (**22**).[166]

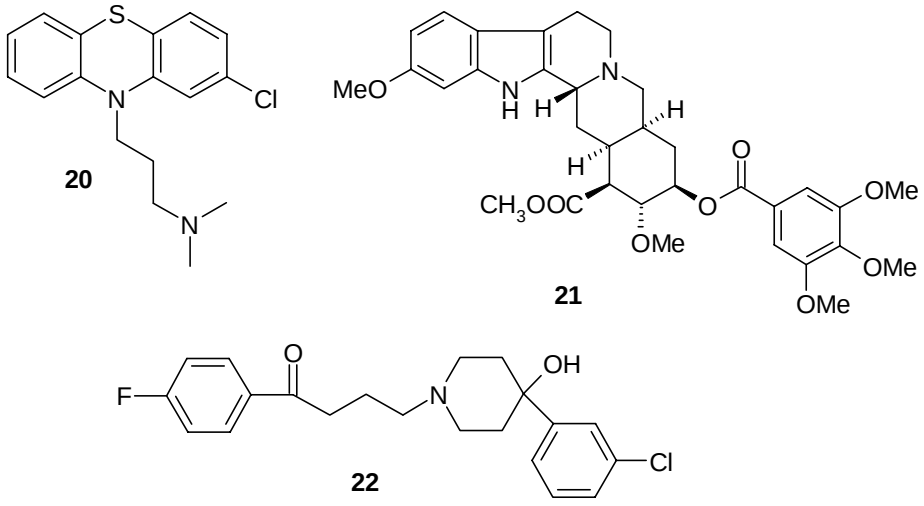


Chart 1.9 Chemical structures of chlorpromazine (**20**), reserpine (**21**), haloperidol (**22**).

Since the discovery of the first antipsychotic agents researchers have tried to classify the different drugs. First the drugs were classified according to the therapeutic profile of individual drugs, later the side effects were used to classify the anti-psychotic drugs. Nowadays, anti-psychotic drugs are divided into two groups. The typical (first generation) anti-psychotics include a variety of phenothiazine and butyrophenone type compounds (with chlorpromazine (**20**) and haloperidol (**22**), respectively, as well known examples), and have in common their (potent) antagonism at the dopamine D_2 receptor. These anti-psychotics are especially active against the positive symptoms of schizophrenia and usually show serious extrapyramidal side-effects (EPS) and elevation of serum prolactin levels. An atypical anti-psychotic is defined as a drug that has anti-psychotic efficacy, with minimal EPS and elevation of serum prolactin levels, and that does not cause tardive dyskinesia. An additional effect of the atypical anti-psychotics is its efficacy against negative symptoms (for review see ref. 166).

The prototypical atypical anti-psychotic drug, nowadays, is clozapine (**23**). The drug has affinity for a variety of receptor subtypes; i.e. dopamine, serotonin, histamine, muscarine and noradrenaline receptors.[174] Because of the variety of binding affinities of clozapine (**23**), new drugs were tried to be developed with a selectivity for one of the above-mentioned receptors. So, amongst others, selective dopamine and 5-HT_{2A} receptor antagonists were developed, but none of these drugs has reached the market yet.

Clozapine (**23**) shows that the atypicallity of a neuroleptic drug is explained by its affinity for more than one receptor subtype, and that no single hypothesis effectively explains the atypical character. Currently on the market are the following atypical anti-psychotics: clozapine (**23**), risperidone (**24**, in a low dose atypical properties), olanzapine (**25**) and seroquel (**26**).

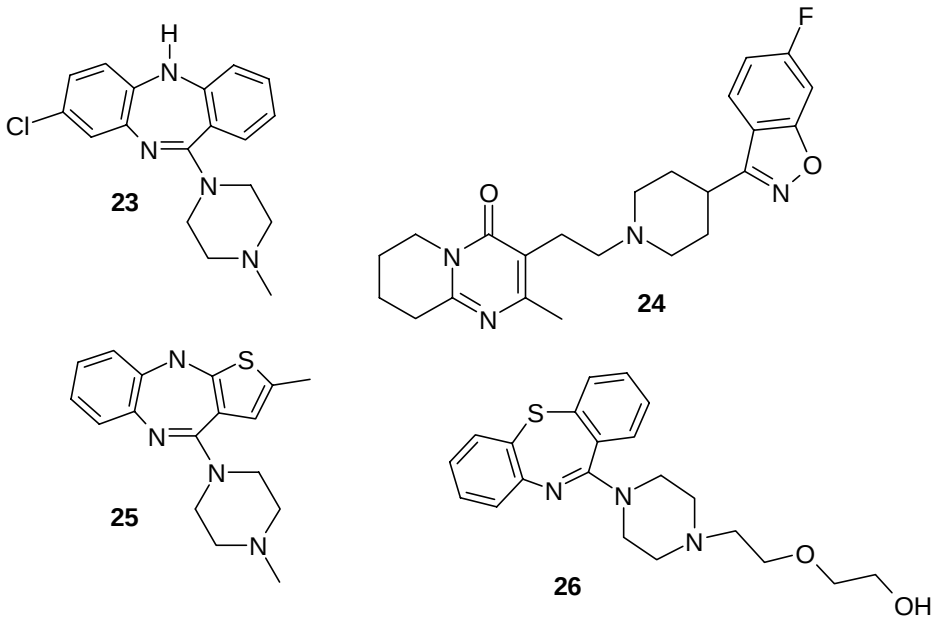


Chart 1.10 Chemical structures of clozapine (**23**), risperidone (**24**), olanzapine (**25**), seroquel (**26**).

1.5.3 Drug abuse

Cocaine and amphetamine are powerful psychostimulants and widespread abuse of these drugs creates a major problem in society. Two characteristics of drug addiction are the compulsive nature of substance use and the high propensity for relapse. Relapse is, in fact, so prevalent that attempts to stop using drugs often lead to repeated cycles of detoxification followed by a return to compulsive substance use.[175] The craving for drugs in an addict is driven not only by the pain and discomfort of withdrawal, but also by the expected rewarding effect of the drug. Craving can even be elicited by stimuli previously associated with taking the drug. Over the past several years, researchers have begun to work out the neurochemical substrates of such conditioned responses. Several studies indicate that the brain neurotransmitter dopamine plays a pivotal role in the 'reinforcing' effects of many drugs of abuse, including cocaine. Cocaine causes large increases in the amount of dopamine available to stimulate dopamine receptors. These increased levels are probably responsible for the 'reinforcing' effects, meaning that an addict is more likely to seek cocaine in the future.

The dopamine D_3 receptor is mainly expressed in the limbic regions of the brain, including the nucleus accumbens, olfactory tubercle, the ventral pallidum and the amygdala.[19,176] The nucleus accumbens has been implicated in motivated behaviours such as drug self-administration and conditioned-cue preference (CCP).[177] The limbic region-specific expression of the dopamine D_3 receptor has led to the speculation that this receptor may be involved in responses to psychostimulants. A number of different studies have shown that cocaine self-administration is attenuated by the co-administration of moderately selective dopamine D_3 receptor agonists, suggesting the potential importance of dopamine D_3 receptors for this behaviour.[116,178,179] Using a genetic approach Xu[177] found that dopamine D_3 receptor mutant mice exhibit increased sensitivity to amphetamine in the CCP paradigm. This finding suggests that the dopamine D_3 receptor is involved in behavioural responses to the rewarding effect of amphetamine and that dopamine D_3 receptor-associated mechanisms can either attenuate the positive effect or disrupt the conditioning process whereby the neutral cues in the conditioning environment become associated with this effect.[177] Furthermore, Staley and Mash[180] found in cocaine overdose victims, as compared with age-matched and drug-free control subjects, that the number of [^{3}H]-(+)-7-OH-DPAT binding sites was elevated. This demonstrates that adaptive changes in the dopamine D_3 receptor in the reward circuitry of the brain are associated with chronic cocaine abuse. These results suggest that the dopamine D_3 receptor may be a useful target for drug development. No medications have reached the clinical stage or the market yet.

1.6 Oral bioavailability of dopamine receptor agonists

Many dopamine receptor agonists, both of natural and synthetic origin, contain the catecholethylamine structure of dopamine embedded within them. The clinical utility of catechol and phenol-containing dopamine receptor agonists has been limited by their relatively low selectivity for presynaptic versus postsynaptic dopamine receptors, their low oral bioavailability and, consequently, their short duration of action. The catechol and phenol rings provide optimal sites for metabolism as well as conjugation and excretion.[181] To circumvent the problem of the low oral bioavailability of dopamine receptor ligands, synthesis of bioisosteres or prodrugs of the phenolic compounds could be useful.

1.6.1 Bioisosteric replacement

Throughout its development, there has been a number of different definitions proposed for bioisosterism. Friedman defined bioisosterism as the relationship of compounds, which fit the broadest definition of isosteres and have the same type of biological activity. Erlenmeyer was more restricted in his definition and he postulated that isosteres are atoms, ions or molecules in which the peripheral layers of electrons can be considered to be identical. Hansch tried to be less vague and defined bioisosterism as compounds causing identical biochemical or pharmacological response in a standard test system. The system might be an enzyme, membrane, mouse or man. Finally, Burger suggested an expanded statement which takes into account biochemical views of biological activity. Bioisosteres are compounds or groups that possess near-equal molecular shapes and volumes, approximately the same distribution of electrons, and which exhibit similar physical properties such as hydrophobicity. Bioisosteric compounds affect the same biochemically associated systems as agonists or antagonists and thereby produce biological properties that are related to each other. For a review on these definitions see ref. 182.

A number of different bioisosteric replacements can be found in literature, but here only the ones important for the experiments described in this thesis are given. (for review see ref. 182). A -CH=CH- group in a benzene moiety is replaced by divalent sulphur (-S-) giving a thiophene. The most popular bioisosteric substituents for the phenolic hydroxyl are methanesulfamide (CH_3SO_2NH-), hydroxymethyl ($HOCH_2$-) or hydroxyisopropyl ($HOC(CH_3)_2$), various amide groups (-NHCHO, -$NHCOCH_3$, -$NHCOC_6H_5$), methanesulfamidomethyl ($CH_3SO_2NHCH_2$-), dimethylaminosulfonamide ($(CH_3)_2NSO_2NH$-), and other with an ionizable proton next to, or near an aromatic ring. These groups should be not much larger than hydroxyl or they should have the same approximate acidity range, form hydrogen bonds and, on occasion, redox systems, in conjugation with a para-OH.

The structure of a catechol itself can be replaced by analogous heterocycles in various derivatives. All these compounds share the ability to chelate metal atoms and to form hydrogen-bonded second rings, the benzimidazole imitates this by way of a covalent ring structure.[182] A successful bioisosteric replacement of a phenol or a catechol moiety is the 2-aminothiazolyl moiety. Active dopaminergic compounds which possess such a moiety are B-HT 920 (**32**)[183] and pramipexole (**19**).[146,154,155]

One apparent shortcoming of bioisosterism was that what seemed to be a solid rule in one series of structurally related compounds failed to work out in a series with different overall structural characteristics. Experiments to rationally design a drug structure, may have disturbed one of several parameters that had proved to be necessary for inducing a given biological activity. Such parameters include size and bond angles, ability to form hydrogen bonds, pKa, chemical reactivity, hydrophilicity, lipophilicity, and also the metabolic fate of the compounds to be prepared. One will rarely seek to obtain a drug with completely identical properties but almost always try to improve efficacy and specificity and decrease side effects and/or toxicity. [182]

1.6.2 Pro-drugs of dopamine receptor agonists.

A prodrug is a pharmacologically inactive derivative of a parent drug molecule that requires spontaneous or enzymatic transformation within the body in order to release the active drug, and that has improved delivery properties over the parent drug molecule. A molecule with optimal structural configuration and physico-chemical properties for eliciting the desired therapeutic response at its target site does not necessarily possess the best molecular form and properties for its delivery to its point of ultimate action. Prodrugs are designed to overcome pharmaceutically and/or pharmacokinetically based problems associated with the parent drug molecule that would otherwise limit the clinical usefulness of the drug. In the design of a prodrug several criteria should be considered: (1) what functional groups on the parent drug molecule are sensitive to chemical derivatisation, (2) what mechanisms and systems are available in the organism for the required bioactivation of the prodrug, (3) synthesis and purification of the prodrug should be relatively simple, (4) the parent drug molecule must be regenerated from the prodrug *in vivo*, (5) toxicity of the transport group of the prodrug as well as of the prodrug per se must be considered.

Application of prodrugs has involved (1) enhancement of bioavailability and passage through various biological barriers, (2) increased duration of pharmacological effects, (3) increased site-specificity, (4) decreased toxicity and adverse reactions, (5) improvement of organoleptic properties and (6) improvement of stability and solubility properties.[184]

Since a lot of the dopamine receptor agonists possess catechol or phenol moieties, the most frequently investigated prodrugs are esters and carbamates of these hydroxylated compounds.[96,185-187]

1.7 Functional models used to study dopamine receptor ligands

There are a large number of generally accepted *in vitro* and *in vivo* test models available for the evaluation of centrally acting dopamine receptor agonists and antagonists. These models give information about the effect on pre- and postsynaptic dopamine receptors. The models for postsynaptic receptors can also be used to determine the involvement of different receptor subtypes on the effect. In this part only the *in vivo* models used in this thesis are discussed briefly.

1.7.1 Brain microdialysis

On-line brain microdialysis is an *in vivo* sampling technique in freely moving animals.[188] The technique is based on the dialysis principle in which a membrane, permeable to water and small molecules, separates two fluid compartments. The tubular membrane is constantly perfused with the extracellular fluid by diffusion in both directions. A microdialysis probe typically consists of a cylindrical dialysis membrane that is connected with in- and outlet tubes. The length of the membrane is adjusted to the brain region studied. Neurotransmitters and their metabolites in the dialysates can be determined by the use of HPLC techniques. The extracellular level of a given neurotransmitter that is determined by microdialysis is supposed to reflect the overflow of neurotransmitter. Using the microdialysis technique the dopamine D_2 receptor agonistic properties of the compounds can be monitored, as the release of dopamine is under the control of dopamine D_2 autoreceptors.[189] Dopamine agonists decrease and antagonists increase dopamine levels as measured by microdialysis.

1.7.2 Locomotor activity in reserpinised rats

The postsynaptic agonistic effects of dopaminergic agents can be determined in rats by measuring the effect on locomotor activity. These experiments are performed in rats which had been treated 18 h beforehand with reserpine. Reserpine reduces the monoamine levels by inhibiting the transport system of the storage granule membrane and thereby the nerve endings lose their ability to concentrate and store the amine.[190] After 18 h there is no endogenous dopamine present and the locomotor activity measured after administration of a dopamine receptor agonist is solely caused by the agonist.

1.7.3 Behavioural characteristics in reserpinised rats

During the locomotor activity experiments also the behavioural characteristics were observed. With these observations an indication can be given about which receptors are important for the behaviour. The dopaminergic stereotyped behaviour can be divided into two groups, namely behaviour caused by dopamine D_1-receptors (rearing) and dopamine D_2 receptors (licking and sniffing).[191] Indications for the serotonergic behavioural syndrome are flat body posture and lower lip retraction which is caused by 5-HT_{1A} receptor agonists.[192]

1.8 Scope of the thesis

The Department of Medicinal Chemistry at the University of Groningen has a long tradition in the design, synthesis, and pharmacological evaluation of drugs acting at the central nervous system. During the last 15 years research has been focused in particular on 2-aminotetralin-derived and structurally related compounds, such as octahydrobenzo[*f*]quinolines, hexahydronaphthoxazines and tetrahydrobenzopyranoxazines, with activity at dopamine, serotonin or melatonin receptors (e.g. see refs. 193-199). Examples of dopamine receptor ligands that have been developed within the department are the dopamine D_3 receptor preferring agonists (R)-7-OH-DPAT and PD 128907,[200] and the dopamine D_2 receptor preferring agonist N-0923,[201] which is currently undergoing clinical trials for the treatment of Parkinson's disease.

All the above mentioned compounds have the same problem, namely, they undergo considerable deactivation by means of glucuronidation in the liver, because of their phenol moiety. This thesis deals with the synthesis and pharmacological evaluation of dopamine receptor ligands, which were designed to possess a higher oral bioavailability than the currently known dopamine receptor agonists. The main goal was to bioisosterically replace the phenol moiety of 2-aminotetralins and hexahydronaphthoxazines with a thiophene moiety. Apomorphine and three of its analogues were tested to determine their relative oral bioavailabilities in a search for good targets for treatment of Parkinson's disease. Also a novel prodrug of hydroxylated 2-aminotetralins has been tested to find compounds with a better oral bioavailability, as compared to hydroxylated 2-aminotetralins.

Chapter 2

Further characterisation of structural requirements for ligands at the dopamine D_2 and D_3 receptor: studies with thienylethylamine as a possible pharmacophore*

Abstract

This chapter describes the synthesis and *in vitro* pharmacology of a novel series of dopamine receptor ligands, in which the classical phenylethylamine pharmacophore is replaced by a thienylethylamine moiety. In general, the novel compounds showed moderate affinity for the dopamine D_2 and D_3 receptors. The results showed that a thienylethylamine moiety can act as a dopamine pharmacophore on these receptors. When the thienylethylamine moiety is fixed in a rigid system the affinity for the dopamine receptor is increased, however, in the tricyclic hexahydrothianaphthoxazine structure, the affinity for the dopamine receptors is diminished.

* This chapter is based on: Rodenhuis, N.; Vermeulen, E.S.; Wikström, H.V.; Pugsley, T.A.; Wise, L.D.; Dijkstra, D. (2000) Further characterization of structural requirements for ligands at the dopamine D_2 and D_3 receptor: studies with thienylethylamines as a possible pharmacophore. *J. Med. Chem*. **submitted**.

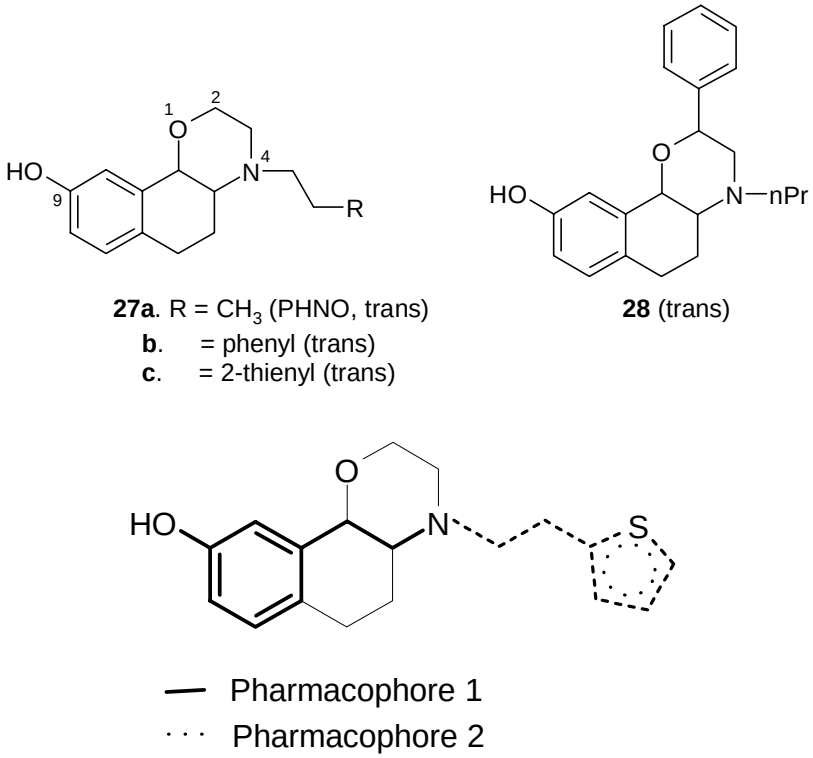
2.1 Introduction

Dopamine receptor agonists have attracted considerable attention due to their therapeutic potential against several neurological, endocrinological and cardiovascular diseases and drug abuse.[116,202-204] Dopamine receptor agonist activities can be found in several classes of compounds including 2-phenylethylamines, aporphines, aminotetralins, naphthoxazines, and ergoline derivatives.

With the application of molecular biological techniques to express receptors in cloned cells at least six different subtypes of dopamine receptors have been identified.[21,25] Their characterisation revealed two categories according to sequence homology, gene construction and second messenger system: D_1 and D_5, which are called "D_1-like receptors" and D_2 (D_{2L} and D_{2S}), D_3, D_4, which are called "D_2- like receptors". The mechanism by which dopamine-binding at the receptor induces G-protein activity is unknown but most likely involves a cascade of intermolecular reactions. In particular, charged and conserved amino acid residues found in transmembrane domains should participate in the dopamine recognition. Molecular models and site directed mutagenesis confirm that an Asp in transmembrane domain 3 and two Ser in transmembrane domain 5 are important for the interaction with the amine and the hydroxyl groups of dopamine, respectively.[21]

Dopamine, and most of the known dopamine receptor agonists, binds with higher affinity to the dopamine D_3 than to the dopamine D_2 receptor. Due to the close homology between the dopamine D_2 and D_3 receptors, especially in the transmembrane domains (~80%), it is difficult to predict dopamine D_2 versus dopamine D_3 receptor selectivity based on receptor models. Malmberg et al.[115] suggested that the observed dopamine D_3 receptor selectivity may not be due to a single specific interaction but rather to a small difference in conformation between the dopamine D_3 and D_2 receptors.

McDermed et al.[99] elegantly rationalised the heterochirality of the potent dopamine receptor agonists by suggesting a model in which different faces of the compound interact with a putative three-point pharmacophore. An attractive feature of this model is that it allows superposition of the nitrogen atoms, the nitrogen lone pairs, the oxygen atoms, and the aromatic rings, the pharmacophoric elements of several dopamine receptor agonists. In addition, the presence of two lipophilic sites which bind the N-alkyl groups have been postulated.[98] Wikström et al.[101] have shown with a series of octahydrobenzo[*f*]quinolines, using a pharmacological *in vivo* model measuring dopamine D_2 activity, that one of the N-alkyl binding sites can only tolerate N-substituents equal to an *n*-propyl. Seiler and Markstein[205] conclude that this space-limited accessory binding site, which they call "small N-alkyl binding site", exists in both main groups of dopamine receptors.



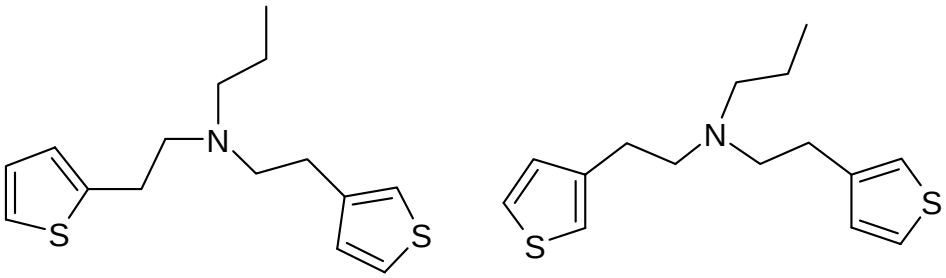
27a. R = CH_3 (PHNO, trans)
b. = phenyl (trans)
c. = 2-thienyl (trans)

28 (trans)

Chart 2.1 Chemical structures of *trans*-9-hydroxy-N-*n*-propyl-2,3,4a,5,6,10b-hexahydro-*4H*-naphth[1,2*b*][1,4]oxazine (PHNO, **27a**), *trans*-9-hydroxy-4-(2-phenylethyl)-2,3,4a,5,6,10b-hexahydro-*4H*-naphth[1,2*b*][1,4]oxazine (**27b**), *trans*-9-hydroxy-4-(2-thienylethyl)-2,3,4a,5,6,10b-hexahydro-*4H*-naphth[1,2*b*][1,4]oxazine (**27c**), *trans*-9-hydroxy-2-N-*n*-propyl-2,3,4a,5,6,10b-hexahydro-*4H*-naphth[1,2*b*][1,4]oxazine (**28**).

For a more extensive exploitation of this theory, we synthesised some derivatives of the potent dopamine D_2/D_3 agonist PHNO **27a**.[206,207] According to the Wikström/Seiler modification of McDermed's model N-substituents larger than *n*-propyl should give compounds inactive at the dopamine D_2/D_3 receptors, while the steric requirements for an R group on the 2-position should be less critical.

The *in vitro* pharmacology data of the naphthoxazines (Table 2.1) confirmed that an N-substituent should not be larger than an *n*-propyl, and that there is more structural freedom for a 2-substituent. However, a thienylethyl substituent on the nitrogen (**1c**) gave a compound with a significantly higher affinity for the dopamine D_3 receptor than the phenylethyl analogue **27b**. We hypothesised that the dopamine receptor pharmacophore of compound **27c** is the thienylethylamine moiety (pharmacophore **2**, chart 2.1) and not the 3-OH-phenylethylamine moiety (pharmacophore **1**, chart 2.1). To test this hypothesis the thienylethylamines **29** and **30** were synthesised and tested *in vitro*.



29 **30**

Chart 2.2 Chemical structures of N-*n*-propyl-(3-thiophen-2-yl-ethyl)-thiophen-3-ylethyl-amine (**29**), N-*n*-propyl-(2-thiophen-2-yl-ethyl)-thiophen-3-ylethyl-amine (**30**).

The *in vitro* pharmacology of compounds **29** and **30** (Table 2.1) showed that the thienylethylamine moiety could behave as a pharmacophore at the dopamine receptor.

It has been known for a long time that the 2-aminotetralin (2-amino-1,2,3,4-tetrahydronaphthalene) structure is pharmacologically important. Initially, 2-aminotetralins were characterised by their sympathomimetic action, causing mydriasis, contraction of the uterus, changes in blood pressure and respiration, and increased intestinal motility in test animals.[202-204] The 2-aminotetralin system has proved to be a valuable structural base for dopamine receptor, serotonin receptor and adrenoceptor ligands, as well as for compounds that interact with melatonin receptors.[208,209]

Some of these compounds have been studied by several research groups to elucidate their structure activity relationship for dopamine receptors.[98,100,101,201,205,210-212] Initially, these studies identified S-(–)-5-hydroxy-2-(N,N-di-*n*-propylamino)tetralin (S-(–)-5-OH-DPAT, S-(–)-**9**) as the most potent monohydroxy 2-aminotetralin.[98,100,205] Later S-(–)-5-hydroxy-2-(N-*n*-propyl-N-2-thienylethylamino)tetralin (S-(–)-N-0437, S-(–)-**31**) was found to be an even more potent dopamine receptor agonist.[201] Moreover, R-(+)-7-hydroxy-2-(N,N-di-*n*-propylamino)tetralin (R-(+)-7-OH-DPAT, R-(+)-**10**) was later shown to have preference for the dopamine D_3 receptor subtype.[79,213]

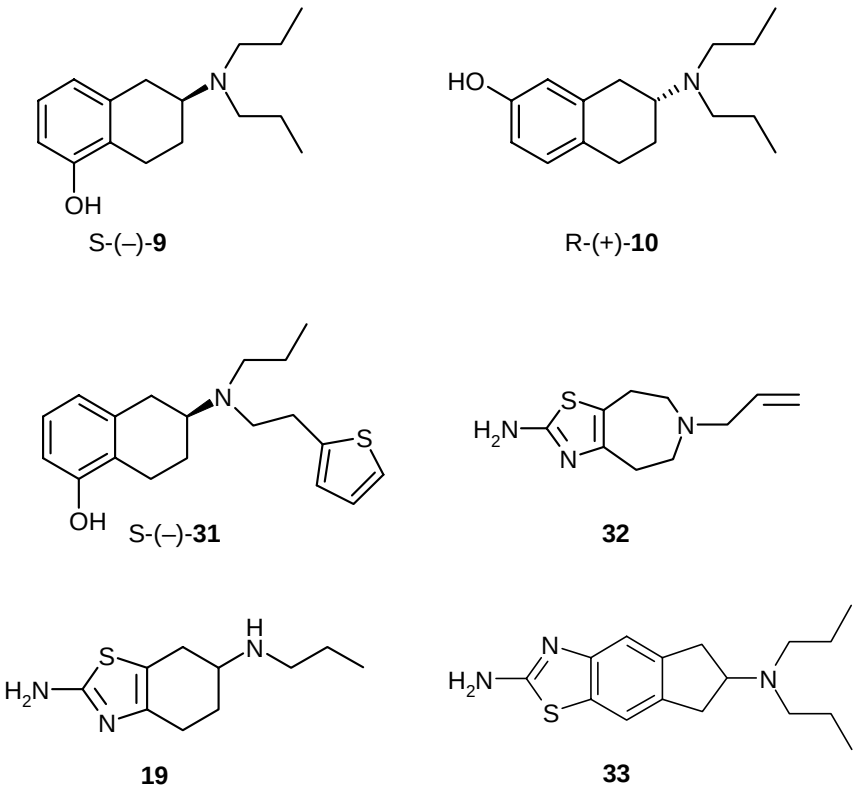


Chart 2.3 Chemical structures of S-(–)-5-hydroxy-2-(N,N-di-*n*-propylamino)tetralin (S-(–)-5-OH-DPAT, S-(–)-**9**), R-(+)-7-hydroxy-2-(N,N-di-*n*-propylamino)tetralin (R-(+)-7-OH-DPAT, R-(+)-**10**), S-(–)-5-hydroxy-2-(N-*n*-propyl-N-2-thienylethylamino)tetralin (S-(–)-N-0437, S-(–)-**31**), 5,6,7,8-tetrahydro-6-(2-propenyl)-4*H*-thiazolo[4,5-*d*]azepin-2-amine (BHT920, **32**), pramipexole (**19**) and 6-amino-2-(N,N-di-*n*-propylamino)-thiazolo[4,5-*f*]indan (GMC1111, **33**).

Their low oral bioavailability and their short duration of action have limited the clinical utility of catechol and phenol-containing drugs. The catechol and phenol rings provide optimal sites for glucuronidation. Thus, for many years, emphasis has been focused on the identification of bioisosteric replacements for catechols and phenols. The idea that neither catecholic nor phenolic hydroxyl groups are an absolute requirement for potent dopamine receptor activity was presented by Andén et al.,[183] who showed that the aminothiazolazepine derivative BHT920 (**32**) is a dopamine autoreceptor agonist, as well as an α_2-adrenoceptor agonist. Pramipexole (**19**), a benzothiazole analogue of the 2-aminotetralins, was found to be a potent dopamine receptor agonist with both dopamine D_2 and D_3 receptor stimulating properties. It is presently on the market for the treatment of Parkinson's disease.[146,154,155] Another example of an analogue with an aminothiazole moiety is GMC1111 (**33**), which possesses affinity for the dopamine D_2 and D_3 receptor, and also inhibits lipid peroxidation.[214]

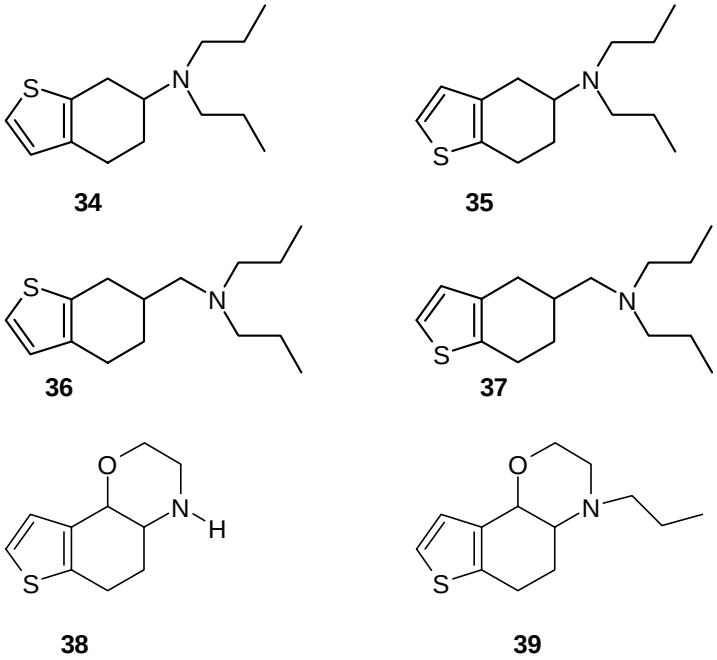
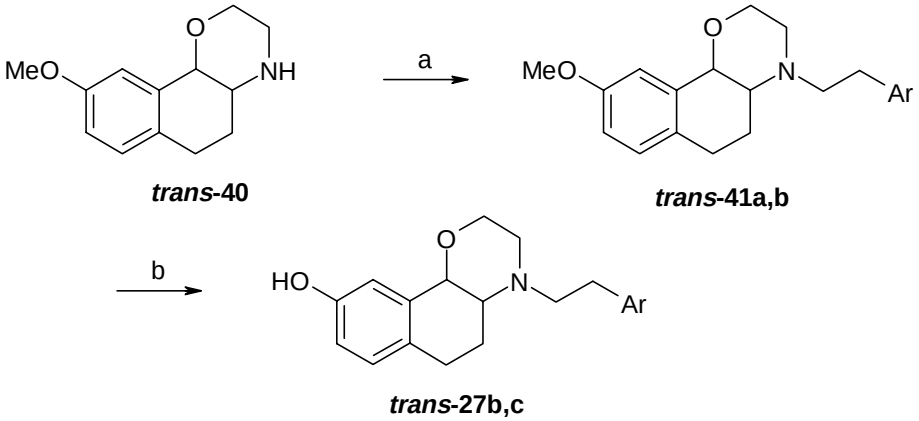


Chart 2.4 Chemical structures of 6-(N,N-di-*n*-propyl)amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene (**34**), 5-(N,N-di-*n*-propyl)amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene (**35**), 6- (N,N-di-*n*-propyl)aminomethyl-4,5,6,7-tetrahydro-benzo[*b*]thiophene (**36**), 5- (N,N-di-*n*-propyl)aminomethyl-4,5,6,7-tetrahydro-benzo[*b*]thiophene (**37**), *trans*-2,3,4a,5,6,9b-hexahydro-4*H*-thianaphth[4,5*e*][1,4]oxazine (**38**), *trans*-N-*n*-propyl-2,3,4a,5,6,9b-hexahydro-4*H*-thianaphth[4,5*e*][1,4]oxazine (**39**).

Since it was found that the thienylethylamine moiety might act as a dopamine receptor pharmacophore, we tested whether a thiophene moiety may act as a bioisostere for a phenol in 2-aminotetralins and hexahydronaphthoxazines. Therefore, thiophene analogues of the 2-aminotetralins and hexahydronaphthoxazines, **34-39** were synthesised. All the compounds synthesised were tested *in vitro* for their affinity at dopamine D_{2L} and D_3 receptors. The derivatives with interesting properties were further investigated for their *in vivo* dopamine receptor activity and bioavailability using the microdialysis technique in freely moving rats.[215]

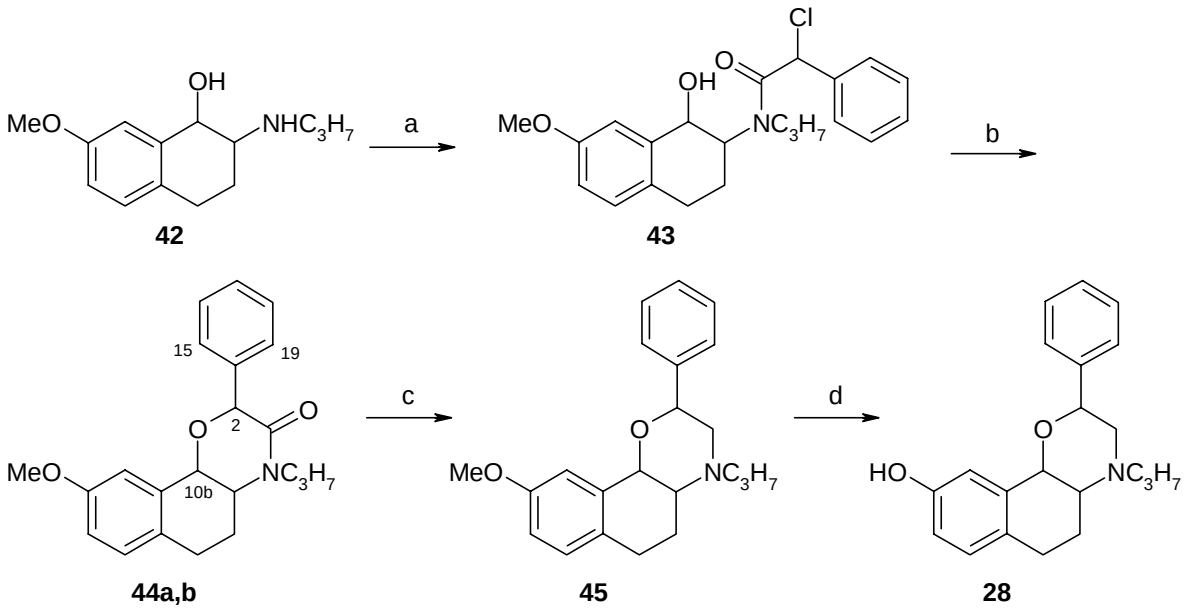
2.2 Chemistry

The *trans* N-arylalkyl substituted hexahydronaphthoxazines **27b** and **c** were synthesised from *trans*-9-methoxy secondary amine **40** via N-alkylation with the appropriate arylethyl halide or by reductive alkylation. The *trans*-9-methoxy secondary amine **40** was prepared by using known methods.[206,216,217] The phenols were achieved through ether cleavage with BBr_3 under N_2 (Scheme 2.1).



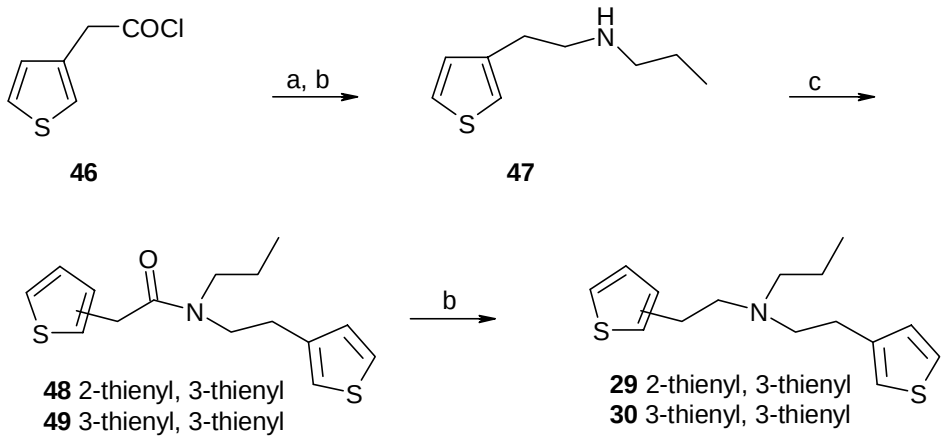
Scheme 2.1 Reagents: (a) $C_6H_5CH_2CH_2Br$, K_2CO_3, DMF or 2-thienyl acetic acid, $(CH_3)_3N.BH_3$, xylene; (b) BBr_3, CH_2Cl_2.

The synthesis of 2-phenyl-N-*n*-propylnaphthoxazine **28** is outlined in Scheme 2.2. The racemic *trans*-aminoalcohol **42** was acylated with 2-chloro-2-phenylacetyl chloride to afford a mixture of diastereomeric *trans*-amidoalcohols **43**. Cyclisation of the chloroacetamide **43** was achieved with NaOH in isopropanol, affording the mixture of *trans*-lactams **44a, b** with the 2-phenyl ring in equatorial or in axial position. The 2-(axial)phenyl *trans*-lactam and the 2-(equatorial)phenyl-*trans*-lactam could be separated by column chromatography. Theoretically the morpholine-ring can exist in two conformations namely a pseudo chair or boat conformation. The phenyl-ring can be adjusted in a pseudo-axial and pseudo-equatorial position. The morpholine ring exists probably most of the time in a chair conformation since this is energetically the most favourable conformation, but it is possible that the conformation changes to the boat-conformation. When the morpholine ring exists in a chair conformation with the phenyl-ring in the axial position there is an interaction in space between the protons on C10b and C15/C19. The other epimer with the phenyl-ring in the equatorial position shows an interaction in space between the protons on C10b and C2. The NOESY-experiments showed that the fast eluting compound has an interaction between the protons on C10b and C15/C19, so this is the compound with the phenyl in the axial position. The last eluting compound shows an interaction between the protons on C10b and C2, indicating that the compound has the phenyl-ring in the equatorial position. The 2-equatorial-phenyl isomer was used for the next reaction. After reduction of the amide with $BH_3.Me_2S$ complex, the final step was demethylation, which was achieved by applying BBr_3, giving the final product **28**.



Scheme 2.2 Reagents: (a) PhCHClCOCl, NaOH, CH_2Cl_2; (b) NaOH, i-PrOH; (c) $BH_3.Me_2S$, THF; (d) BBr_3, CH_2Cl_2.

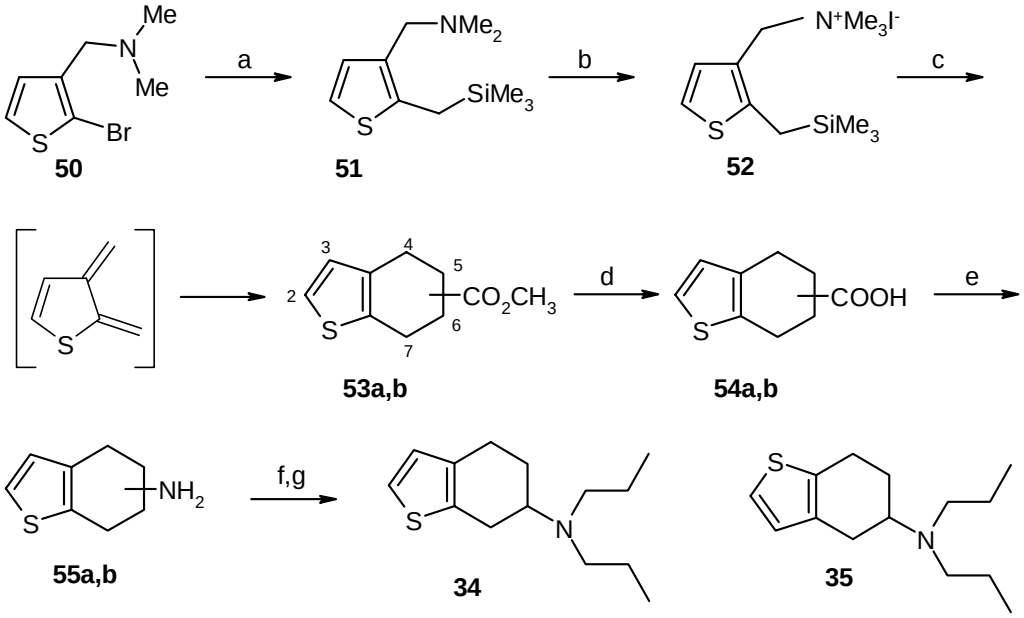
The thienylethylamines **29** and **30** were synthesised according to Scheme 2.3. The secondary amine **47** was acylated with 2- or 3-thienylacetylchloride. As a result of the rotation around a binding with a partial double bond character the aliphatic protons and the aliphatic carbon atoms of **48** and **49** were according to the ^{1}H- and ^{13}C-NMR spectra chemically non-equivalent. The resulting amides were reduced with $BH_3.Me_2S$ complex.



Scheme 2.3 Reagents: (a) $C_3H_7NH_2$, CH_2Cl_2; (b) $BH_3.Me_2S$, THF; (c) 2- or 3-thienylacetyl chloride, CH_2Cl_2, Et_3N.

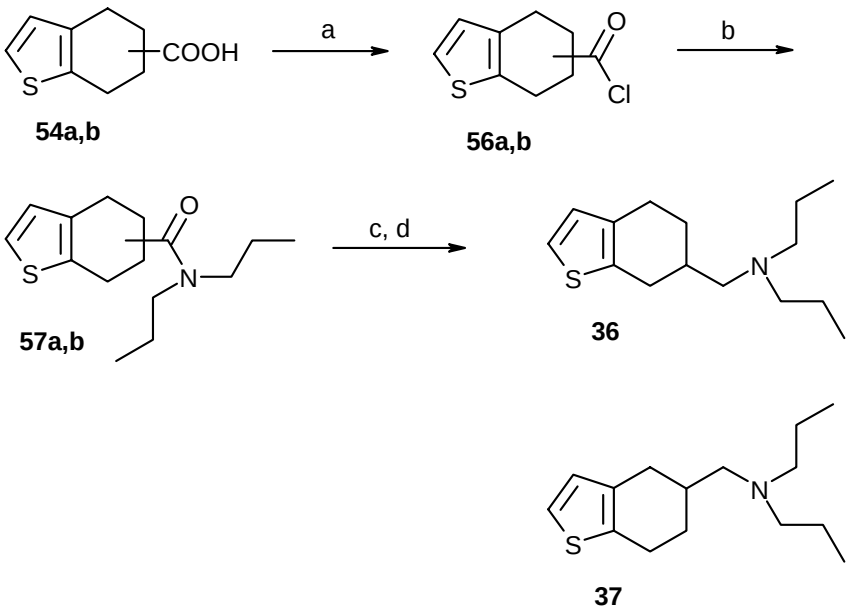
The synthesis of the tetrahydrobenzo[*b*]thiophenes is outlined in Scheme 2.4. A Grignard-reaction of **50** with $ClMgCH_2SiMe_3$ followed by quaternisation with methyl iodide gave the 3-(trimethylammoniummethyl)-2-(trimethylsilylmethyl)thiophene **52**, which is the precursor of

2,3-dimethylene-2,3-dihydrothiophene.[218] Treatment of **52** with *n*-tetrabutylammonium fluoride (TBAF) leads to the formation of 2,3-dimethylene-2,3-dihydrothiophene, which is an unstable intermediate. This intermediate was captured in a Diels-Alder [4 + 2] cycloaddition reaction with methyl acrylate as the dienophile. We did not succeed in the separation of the mixture of regioisomers obtained. Hydrolysis of this mixture of esters gave the carboxylic acids **54a, b** in good yield. A Curtius rearrangement gave the mixture of amines **55a, b**.[218] Only after conversion into the tertiary amines **34** and **35** was it possible to separate the mixture of regioisomers on a SiO_2 column.



Scheme 2.4 Reagents: (a) $ClMgCH_2SiMe_3$, $Ni(PPh_3)_2Cl_2$, Et_2O; (b) CH_3I, CH_3CN; (c) $CH_2{=}CHCO_2CH_3$, TBAF, CH_3CN; (d) NaOH; (e) 1) DPPA, Et_3N, dioxane; 2) HCl, dioxane, 120 °C; (f) C_3H_7I, K_2CO_3, DMF; (g) SiO_2 column chromatography, EtOAc:Hexane = 1:9.

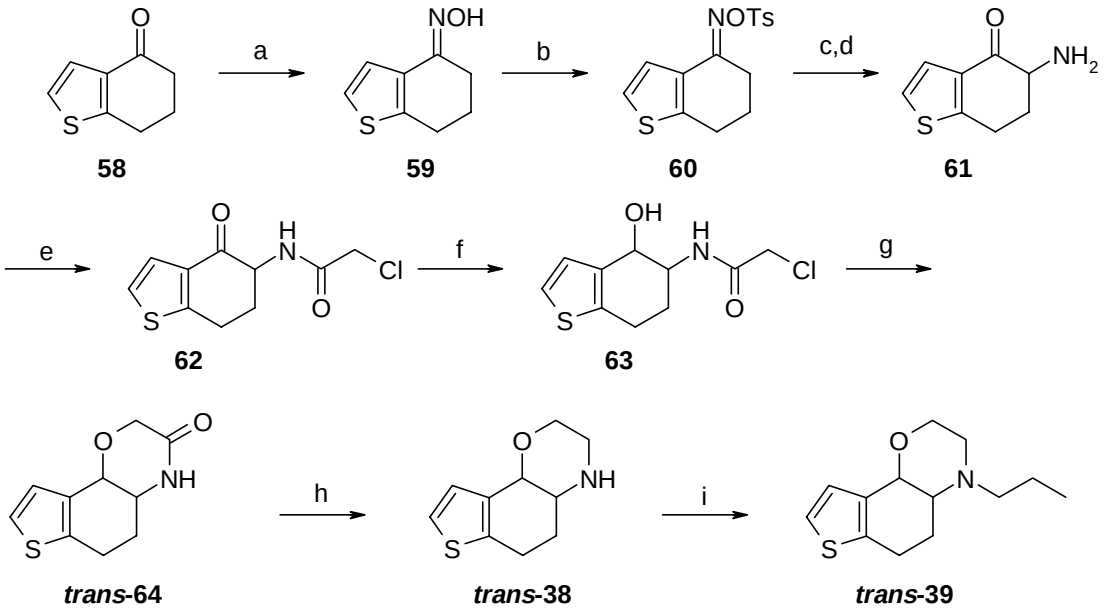
The aminomethyltetrahydrobenzo[*b*]thiophenes (**36** and **37**) were synthesised from the corresponding carboxylic acids (**54a** and **b**) in three steps by standard chemistry (Scheme 2.5). The two isomers could be separated on a SiO_2 column after conversion to the tertiary amines.



Scheme 2.5 Reagents: (a) $(COCl)_2$, CH_2Cl_2; (b) $(C_3H_7)_2NH$, Et_3N, CH_2Cl_2; (c) $LiALH_4$, THF; (d) SiO_2 column chromatography, CH_2Cl_2: MeOH = 20:1.

The hexahydrothianaphthoxazines **38** and **39** were synthesised from the commercially available ketone **58**, which was readily converted into the tosyloxime **60** in two steps. Neber rearrangement of **60** with potassium tert-butoxide afforded the desired amino-ketone **61**. The amino ketone **61** was readily acylated with chloroacetyl chloride. Reduction of the keto-chloroacetamide **62** with sodium borohydride gave only the trans isomer. The proton on C1 gave a doublet at δ 4.6 ppm with a coupling constant of 7.2 Hz indicating a di-axial coupling. The cis-compound would have a couplings constant of about 3.5 Hz.

The cyclization of the alcohol-chloroacetamide **63** by means of 50 % aqueous NaOH-solution in isopropanol at room temperature gave satisfactory yields of the lactam **64**, which was reduced with $LiAlH_4$ to the oxazine **38**. The reduction took place in a low yield. Alkylation of the amine **38** with propyl iodide in DMF afforded the tertiary amine **39** in a good yield.



Scheme 2.6 Reagents: (a) $NH_2OH.HCl$; (b) TsCl; (c) t-BuOK/ETOH; (d) HCl/H_2O; (e) $ClCH_2COCl$, NaOH, CH_2Cl_2; (f) $NaBH_4$; (g) NaOH; (h) $LiAlH_4$; (i) C_3H_7I, DMF, K_2CO_3.

2.3 Results and Discussion

The structural requirements for the N-substituents of dopaminergic 7- and 9-hydroxylated octahydrobenzo[*f*]quinolines (OHB[*f*]Q) and related compounds have been described previously.[101] On the basis of *in vivo* biochemical and behavioural data it was demonstrated that the nitrogen should be substituted with maximal an *n*-propyl group for optimal dopamine receptor activity. Larger N-substituents, (e.g. *n*-butyl) for the 9-hydroxy-OHB[*f*]Qs gave a dramatic reduction in potency of these compounds. The K_i values shown in Table 2.1 for the N-substituted 9-hydroxy-hexahydronaphthoxazines (9-OH-HNO) **27b** and **28** are in full agreement with the models of McDermed and Wikström.[98,101] According to these models there is space available for a 2-substituent. Interestingly, however, the dopamine D_3 receptor affinity of **27c** (K_i = 83 nM) showed that this compound does not fit these receptor models. This has led us to hypothesise that it is the thienylethylamine moiety of **27c**, which confers dopamine D_3 receptor properties to this compound. In the binding model for the dopamine receptors described by Miller et al., the protonated nitrogen of the ligand interacts with the receptor.[219] With the development of molecular biological techniques and the cloning of the dopamine receptors, the amino acid sequence of the different receptors were determined which lead to the assumption that the protonated nitrogen of ligands interacts with Asp 114 (D_2) or Asp 110 (D_3) in transmembrane domain 3 through a reinforced ionic bond (for review see ref. 21). In the hydroxylated 2-aminotetralins and OHB[*f*]Qs an additional hydrogen bond is formed from the phenolic hydrogen of the ligands to the Ser 193 (D_2) or Ser 192 (D_3) in transmembrane domain 5.[115] If a thiophene ring utilises the same interaction points as the phenol, it may be speculated

that the sulfur atom in the thienylethyl substituent may form a hydrogen bond with the hydroxyl moiety of a Ser residue. Sulfur can only act as a hydrogen bond acceptor and consequently this weaker interaction may provide an explanation for the lower affinity for the dopamine D_3 receptor of compound **27c**, as compared to compound **27a**.[220-222] Two alternative explanations for the diminished affinity are I) the non-optimal distance between the hydrogen bond forming moieties on the aromatic site and the nitrogen, II) alternative interaction points in the receptor for these essential atoms in a dopamine receptor.

Table 2.1 Receptor binding data of various dopamine receptor ligands.

Compound	Ki (nM)[a]	
	D_{2L} [^{3}H]N-0437	D_3 [^{3}H]Spiperone
(+)-PHNO ((+)-27a)	6.24	0.21
(±)-27b	>3676	1566
(±)-27c	3676	83
(±)-28	375	12
29	1080	117
30	439	108
34	27	28
35	20	40
36	3107[b]	60
37	2037	247
38	>4780[b]	3000
39	630[b]	240
(–)-5-OH-DPAT (9)[223]	14	0.54
(+)-7-OH-DPAT (10)[223]	34	0.57
(±)-N-0437 (31)[223]	0.06	4.0

Footnotes: [a] K_i values are means of three separate experiments; the results of which did not vary more than 25%. [b] [^{3}H]NPA was used as radiolabeled ligand.

To prove the assumption whether the thienylethylamine moiety can act as a pharmacophore, we synthesised compounds **29** and **30**, possessing only the thienylethylamine moieties. These compounds possess low to moderate affinity for the dopamine D_3 receptor, but confirmed our assumption that a thienylethylamine moiety can act as a non-optimal pharmacophore at the dopamine D_3 receptor.

It is known from the literature that hydroxylated 2-aminotetralins and hexahydronaphthoxazines are potent dopaminergic agonists, but their oral bioavailability is very low due to glucuronidation in the gut and the liver.[161]

The thiophene analogues of the 2-aminotetralins (compounds **34** and **35**) have considerable affinity for the dopamine D_2 and D_3 receptors, but significantly lower than e.g. 5-OH-DPAT. As stated earlier, one reason could be the less tight H-bonding of the sulfur atom, as compared to a hydroxyl moiety.[220-222] Calculating the distances between the sulfur and the nitrogen in compounds **34** and **35** in a minimised conformation using the computer program MacroModel shows that these distances are 5.4 Å and 6.0 Å, respectively. The distance between the nitrogen atom and the hydroxyl group in potent dopamine receptor agents should be between 5.5 and 7.4 Å.[91,108] The distance between the sulfur and the nitrogen atom in the dipropylaminotetrahydrobenzo[*b*]thiophenes is comparable with the distance between the oxygen atom of the hydroxyl group and the nitrogen atom in 4-hydroxy-2-aminoindans.[108] It is known that 2-aminoindans are less potent dopamine receptor ligands than the corresponding 2-aminotetralins.[97]

The results of compounds **34** and **35** confirmed the hypothesis that a thienylethylamine may act as a pharmacophore, moreover, the semi-rigid system increased the affinity for the dopamine receptor. This is in line with the higher potency of the hydroxylated 2-aminotetralin analogues compared to phenylethylamines.[97,224] Apparently, the near coplanar arrangement is required for higher dopamine agonist activity. Since a thienylethylamine moiety may act as a pharmacophore, it is stated that a thiophene may be a bioisostere for a phenol moiety. Bioisosteres are groups of molecules which have chemical and physical similarities producing broadly similar biological effects.[225] The substitution of –CH=CH– by –S– in aromatic rings has been one of the most successful applications of classical isosterism[182] (an example of such a replacement is found with the atypical antipsychotic olanzapine).

Using microdialysis experiments the relative oral bioavailabilities of the compounds **34**, **35** and 5-OH-DPAT could be calculated.[215] The relative oral bioavailabilities were calculated by comparing the Areas Under the Curve (AUCs) after oral and subcutaneous administration. When there was no significant difference between the AUCs the subcutaneous dose was divided by the oral dose and multiplied by 100 to give the relative oral bioavailability. These data show that, although the affinities of the benzo[*b*]thiophenes (**34** and **35**) for the dopamine receptors are lower as compared to 5-OH-DPAT, the relative oral bioavailability is higher. Therefore, the benzo[*b*]thiophenes are interesting compounds for further research.

Table 2.2 Summary of the microdialysis results of compounds **34**, **35** and 5-OH-DPAT. Results are given as Areas Under the Curve (AUCs). Adapted from reference 215.

	subcutaneous administration		oral administration		relative oral bioavailability (%)
Compound	dose (µmol/kg)	AUC	dose (µmol/kg)	AUC	
34	0.1	2650 ± 1000[a]	1	2730 ± 390[a]	10
	1	6000 ± 500	10	3700 ± 950	10
	10	12446 ± 335			
35	1	3150 ± 400	10	4100 ± 750	10
	10	6700 ± 800	10	4100 ± 750	100
	10	6700 ± 800	30	7000 ± 450	30
	30	9400 ± 960	30	7000 ± 450	100
5-OH-DPAT (**9**)	0.1	9700 ± 500	10	11500 ± 300	1

Footnotes: [a] Experiment lasted 150 min. All other experiments lasted 165 min. All the AUCs of s.c. and p.o. doses of each compound were compared, but only the doses that were not significantly different were put in line in the table.

Compounds **36** and **37** were synthesised to enlarge the distance between the nitrogen and sulfur atom. Compound **36** turned out to have no affinity for the dopamine D_2 receptor and a moderate affinity for the dopamine D_3 receptor. The distance between the sulfur atom and the nitrogen atom in an extended minimised conformation using the computer program MacroModel of compounds **12** and **13** is between 5.7 and 6.7 Å depending on the conformation of the methylene amino group.

Although the distances between the sulfur atom and the nitrogen atom in the hexahydrothianaphthoxazines (**38** and **39**) are comparable with those in 5-(N,N-di-*n*-propyl)amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene (**35**), the introduction of a morpholine ring gave a dramatic decrease in the dopamine D_2 and D_3 receptor affinity.

In conclusion, bioisosteric replacement of a phenol by a thiophene moiety gave dopamine receptor agonists with a lower affinity than the corresponding 2-aminotetralins. This loss in affinity is, however, partly compensated by a relative higher oral bioavailability of the tetrahydrobenzo[*b*]thiophenes **34** and **35**.

2.4 Experimental Section

2.4.1 Chemistry

Melting points were determined in open glass capillaries on an Electrothermal digital melting-point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded at 200 MHz and 50.3 MHz, respectively, on a Varian Gemini 200 spectrometer. The splitting patterns are designated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and dd (double doublet). Chemical shifts are given in δ units (ppm) and are relative to the solvent. Coupling constants are given in Hertz (Hz). The spectra recorded were consistent with the proposed structures. IR spectra were obtained on an ATI-Mattson spectrometer. Elemental analyses were performed by the Analytical Chemistry Section at Parke Davis (Ann Arbor, MI) or by the Microanalytical Department of the University of Groningen and were within ± 0.4 % of the theoretical values, except where noted.

All chemicals used were commercially available (Aldrich or Acros) and were used without further purification.

***trans*-9-Methoxy-4-(2-phenylethyl)-2,3,4a,5,6,10b-hexahydro-*4H*-naphth[1,2*b*][1,4]oxazine (41a).** A solution of *trans*-9-methoxy-2,3,4a,5,6,10b-hexahydro-*4H*-naphth[1,2*b*][1,4]oxazine (**40**) (0.42 g, 1.91 mmol), K_2CO_3 (1.5 g, 10.9 mmol), 2-bromo-phenylethyl (0.39 g, 2.10 mol) in 15 mL of DMF was stirred for 15 h at 60 °C under an atmosphere of nitrogen. The reaction mixture was allowed to cool to RT and poured into water and extracted 3 times with 30 mL of diethyl ether. The combined organic phases were extracted several times with brine, dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure. The residue was further purified by flash chromatography using a mixture of CH_2Cl_2 and MeOH (25/1) as the eluent. After evaporation of the solvent the yield was 0.39 g (63 %) **41a**, which was converted to the HCl-salt: mp 239-241 °C; ^{1}H NMR (CD_3OD) δ 1.9-2.1 (m, 1H), 2.5-2.7 (m, 1H), 2.9-3.0 (m, 2H), 3.1-3.3 (m, 2H), 3.4-3.6 (m, 3H), 3.7-3.9 (m, 2H), 3.8 (s, 3H), 4.2-4.4 (m, 2H), 4.8 (d, 1H, J = 9.3 Hz), 6.9-7.0 (m, 1H), 7.0-7.2 (m, 2H), 7.3-7.5 (m, 5H); ^{13}C NMR (CD_3OD) δ 20.3, 24.7, 27.7, 49.7, 51.9, 53.3, 62.2, 62.7, 74.8, 108.4, 113.0, 124.7, 125.7, 127.4, 127.8, 133.4, 135.2, 156.9; Anal ($C_{21}H_{25}NO_2$.HCl) C, H, N.

***trans*-9-Hydroxy-4-(2-phenylethyl)-2,3,4a,5,6,10b-hexahydro-*4H*-naphth[1,2*b*][1,4]oxazine (27b).** A 1 M solution of BBr_3 in CH_2Cl_2 was added to a cooled solution (-30 °C) of **41a**.HCl (0.22 g, 0.61 mmol) in 30 mL of dichloromethane, under an atmosphere of nitrogen. The reaction was initially stirred for 1 h at this temperature, which was then allowed to rise to RT after which the reaction was stirred a further 3 h. The reaction mixture was then poured into water, made alkaline by the addition of a solution of $NaHCO_3$. The separated organic layer was washed with brine, dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure. Conversion to the HCl-salt and recrystallization from acetonitril yielded 0.12 g (51 %) **27b**: mp of the free base 173-176 °C; ^{1}H NMR ($CDCl_3$) δ 1.6-

1.8 (m, 1H), 2.2-2.4 (m, 2H), 2.6-2.9(m, 6H), 3.0-3.2 (m, 2H), 3.9-4.1 (m, 2H), 4.4 (d, 1H, J = 9.7 Hz), 6.6-6.8 (m, 1H), 6.9-7.0 (m, 2H), 7.2-7.4 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 22.5, 25.6, 30.3, 50.7, 53.4, 60.9, 65.4, 77.1, 110.4, 113.2, 124.7, 125.2, 127.0, 127.2, 127.8, 152.8; Anal ($C_{20}H_{23}NO_2$.2HCl) C, H, N.

***trans*-9-Methoxy-4-(2-thienylethyl)-2,3,4a,5,6,10b-hexahydro-*4H*-naphth[1,2*b*][1,4]oxazine (41b).** To a solution of *trans*-9-methoxy-2,3,4a,5,6,10b-hexahydro-*4H*-naphth[1,2*b*][1,4]oxazine (**40**) (0.5 g, 2.3 mmol) and trimethylamine borane complex (0.34 g, 4.6 mmol) in 30 mL of xylene was added 2-thienyl acetic acid (0.65 g, 4.5 mmol). The mixture was heated under N_2 and refluxed for 15 h. The mixture was poured into water. The organic layer was separated and the aqueous layer was extracted with diethyl ether (2 x 25 mL). The combined organic layers were washed with $NaHCO_3$-solution and brine, dried over $MgSO_4$. Evaporation of the solvents yielded an oil which was converted to the HCl-salt and recrystallized from ethanol; yield 0.46 g (62.3%): mp 195.5-197 °C; ^{1}H NMR (CD_3OD) δ 2.5-2.8 (m, 1H), 3.1-3.3 (m, 1H), 3.5-3.7 (m, 2H), 4.0-4.2 (m, 5H), 4.4-4.6 (m, 2H), 4.4 (s, 3H), 4.8-5.1 (m, 2H), 5.5 (d, 1H, J = 9.5 Hz), 7.5-7.6 (m, 1H), 7.7-7.9 (m, 2H), 8.1 (d, 1H, J = 3.7 Hz); ^{13}C NMR (CD_3OD) δ 20.9, 22.7, 25.4, 52.4, 53.8. 63.1, 63.3, 75.4, 109.1, 113.6, 124.0, 125.3, 125.6, 126.4, 128.4, 133.9, 137.3, 157.6; Anal ($C_{19}H_{23}NO_2S$.HCl) C, H, N.

***trans*-9-Hydroxy-4-(2-thienylethyl)-2,3,4a,5,6,10b-hexahydro-*4H*-naphth[1,2*b*][1,4]oxazine (27c).** Compound **27c** was prepared from the methoxy compound **41b** by essential the same procedure as described for the preparation of **27b**. The yield was 65 %. An analytical sample was recrystallized from ethanol-diethyl ether to provide white crystals: mp free base 173-175 °C, HCl-salt: 231-234 °C; ^{1}H NMR (CD_3OD) δ 1.8-2.0 (m, 1H), 2.5-2.6 (m, 1H), 2.8-3.0 (m, 2H), 3.3-3.5 (m, 5H), 3.7-3.8 (m, 2H), 4.1-4.4 (m, 2H), 4.7 (d, 1H, J = 9.8 Hz), 6.6-6.7 (m, 1H), 6.9-7.1 (m, 4H), 7.3 (d, 1H, J = 4.9 Hz); ^{13}C NMR ($CDCl_3$) δ 20.3, 22.0, 24.6, 51.9, 54.0, 62.4, 62.8, 74.9, 109.9, 113.8, 122.9, 123.1, 124.7, 125.5, 127.3, 132.7, 136.1, 154.3; Anal ($C_{18}H_{21}NO_2S$.HCl.¼H_2O) C, H, N.

***trans*-9-Methoxy-2-phenyl-4-N-*n*-propyl-2,3,4a,5,6-tetrahydro-4*H*-naphth[1,2*b*][1,4]oxazin-3-one (44a) and (44b).**To a solution of compound **42** (1.14 g, 4.8 mmol) in 70 mL of dichloromethane was added NaOH (1.0 g) dissolved in 10 mL of water. 2-Chloro-2-phenyl acetyl chloride (1.0 g, 5.3 mmol) dissolved in 10 mL of dichloromethane was slowly added. The reaction mixture was stirred at RT for 2 h. The mixture was then poured into 60 mL of water. The two layers were separated and the aqueous layer was extracted with dichloromethane. The combined organic extracts were washed with water and thereafter dried over Na_2SO_4. After filtration the solvent was removed under reduced pressure to yield 1.7 g (91 %) oil as a mixture of diastereomers of chloroacetamide **43** and partly cyclized product: ^{1}H NMR ($CDCl_3$) δ 0.9 (t, 3H, J = 7.3 Hz), 1.4-1.6 (m, 1H), 1.6-1.9 (m, 2H), 2.35-2.5 (m, 1H), 2.9-3.0 (m, 2H), 3.1-3.3 (m, 1H), 3.7-3.9 (m, 2H), 3.8(s, 3H), 4.8 (d, 1H, J = 9.0 Hz), 5.4 (s, 1H), 6.7-6.8 (m, 1H), 7.0-7.15 (m, 2H), 7.3-7.4 (m, 3H), 7.5-7.6 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 9.7,

19.7, 23.7, 25.5, 41.6, 53.9, 55.2, 75.2, 79.8, 108.3, 113.2, 124.6, 126.5, 126.7, 127.7, 134.0, 136.8, 156.9, 167.3. The compound was used without further purification.

To a solution of the chloroacetamide **43** (1.7 g, 4.8 mmol) in 200 mL of isopropanol a solution of 1.2 g NaOH in 2.4 mL H_2O was added dropwise at RT. After stirring for 5 h at RT the mixture was neutralised with 1 N HCl. The solvents were evaporated as much as possible and the resulting residue was slurried in 200 mL of water and extracted with 4 x 25 mL of dichloromethane. The organic layer was washed with water, dried over Na_2SO_4 and then reduced to dryness. The residual solid was purified by column chromatography on silica gel 60 using a mixture of ethyl acetate and hexane (1/4) as the eluent resulting in the separation of the two stereoisomers (**44a** and **44b**). Recrystallization from *iso*-propylacetate gave the lactams as white crystals. Fast eluting compound (**44a**, axial): yield: 590 mg (38 %): mp 151.5-152 °C; ^{1}HNMR ($CDCl_3$) δ 1.0 (t, 3H, J = 7.3 Hz), 1.5-1.9 (m, 3H), 2.3-2.5 (m,1H), 2.8-3.0 (m, 2H), 3.4-3.5 (m, 1H), 3.6-3.8 (m, 2H), 3.8 (s, 3H), 4.6 (d, 1H, J = 9.5 Hz), 5.6 (s, 1H), 6.8 (dd, 1H), 7.0 (d, 1H, J = 8.3 Hz), 7.1 (br s, 1H), 7.3-7.4 (m, 3H), 7.6 (d, 2H, J = 7.4 Hz); ^{13}C NMR ($CDCl_3$) δ 11.3, 21.3, 25.2, 26.7, 43.5, 55.2, 57.2, 71.3, 78.2, 109.5, 113.8, 126.0, 127.3, 127.9, 128.4, 129.1, 135.8, 137.2, 158.1, 167.5; IR (NaCl) 1651 cm^{-1} (CO); MS (EIPI) m/e 351 (M^+).

Last eluting compound (**44b**, equatorial): yield: 840 mg (55 %): mp 112.5-113.5 °C ^{1}HNMR ($CDCl_3$) δ 0.9 (t, 3H, J = 7.4 Hz), 1.4-1.6 (m, 1H), 1.6-1.9 (m, 2H), 2.4-2.5 (m,1H), 2.9-3.0 (m, 2H), 3.1-3.3 (m, 1H), 3.8 (s, 3H), 3.8-4.0 (m, 2H), 4.80 (d, 1H, J = 9.3 Hz,), 5.4 (s, 1H), 6.9 (m, 1H), 7.0-7.2 (m, 2H), 7.5-7.6 (m,3H), 7.7-7.8 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 11, 21, 25, 27, 43, 55, 57, 77, 81, 110, 115, 126, 127.9, 128, 130, 135.5, 138, 158, 169; IR (NaCl) 1640 cm^{-1} (CO); MS (EIPI) m/e 351 (M^+). Anal ($C_{22}H_{25}NO_3$) C, H, N. The equatorial product is used for the next step.

***trans*-9-Methoxy-2-phenyl-4-N-*n*-propyl-2,3,4a,5,6,10b-hexahydro-*4H*-naphth[1,2*b*][1,4]oxazine (45).**

To a solution of amide **44b** (350 mg, 1.0 mmol) in anhydrous THF (25 mL) was added $LiAlH_4$ (200 mg). The mixture was refluxed for 3 h and then was added successively water (0.2 mL), 4 N NaOH (0.2 mL) and water (0.6 mL). This mixture was refluxed for another 15 min. The solid was filtered off and the filtrate dried over Na_2SO_4 and concentrated to yield 316 mg (94 %) oil. The amine was converted to the HCl-salt. mp 209-210 °C; ^{1}H NMR ($CDCl_3$) δ 0.9 (t, 3H, J = 7.3 Hz), 1.5-1.7 (m, 3H), 2.3-2.4 (m, 4H), 2.8-2.9 (m, 3H), 3.1 (dd, 1H, J = 11.7 Hz), 3.8 (s, 3H), 4.6 (d, 1H, J = 9.03 Hz), 4.9 (dd, 1H, J = 10.5 Hz), 6.7-6.8 (m, 1H), 7.0 (d, 1H, J = 8.3 Hz), 7.2 (m, 1H), 7.3-7.5 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 10.5, 17.0, 22.8, 25.8, 53.6, 53.9, 58.1, 60.3, 76.7, 77.5, 108.6, 112.2, 124.6, 125.6, 126.1, 126.8, 127.5, 136.0, 139.2, 156.36; Anal ($C_{22}H_{27}NO_2$.HCl) C, H, N.

***trans*-9-Hydroxy-2-phenyl-4-N-*n*-propyl-2,3,4a,5,6,10b-hexahydro-*4H*-naphth[1,2*b*][1,4]oxazine (28).** The phenol **28** was prepared from the methoxy compound **45** by essential the same procedure as described for the preparation of **27b** from **41a**. The yield was 60 %. An analytical sample was recrystallized from acetonitril to provide white crystals: mp 202-

204 °C; ^{1}H NMR (CD_3OD) δ 0.9 (t, 3H, J = 7.1 Hz), 1.6-1.9 (m, 3H), 2.3-2.4 (m, 1H), 2.7-2.9 (m, 2H), 3.0-3.2 (m, 1H), 3.3-3.5 (m, 3H), 4.2-4.3(d, 1H, J = 12.9 Hz), 4.7 (d, 1H, J = 9.5 Hz), 5.5 (br s, 1H), 6.9 (d, 1H, J = 8.5 Hz), 6.6-6.7 (m, 1H), 7.1 (s, 1H), 7.3-7.6 (m, 5H); ^{13}C NMR (CD_3OD) δ 9.8, 15.1, 20.4, 25.0, 49.5, 52.6, 61.9, 68.9, 69.6, 110.6, 114.4, 123.7, 124.9, 127.0, 128.0, 128.2, 133.3, 135.8, 154.7; Anal ($C_{21}H_{25}NO_2$.HCl.½H_2O) C, H, N.

N-*n*-Propyl-3-thiophen-2-yl-acetamide. To a solution of *n*-propylamine (5.9 g, 100 mmol) in dichloromethane (50 mL) and 2N NaOH (10 mL) was added dropwise 3-thienylacetyl chloride **46** (2.8 g, 17.4 mmol) dissolved in dichloromethane (10 mL). The reaction mixture was stirred for 2h at RT. The two layers were separated and the aqueous layer was extracted with dichloromethane (20 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to yield 2.3 g (72 %) oil which solidified upon standing which was recrystallized from ethyl acetate-hexane: ^{1}H NMR ($CDCl_3$) δ 0.8 (t, 3H, J = 7.3 Hz), 1.4-1.5 (m, 2H), 3.16-3.22 (m, 2H), 3.6 (s, 2H), 5.6 (br s, 1H), 7.0-7.1 (m, 1H), 7.1-7.2 (m, 1H), 7.3-7.4 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 9.7, 21.2, 36.6, 39.8, 122.2, 125.2, 127.0, 133.5, 169; IR (NaCl) cm^{-1} 1641 (C=O, amide). Anal ($C_9H_{13}NOS$) C, H, N.

N-*n*-Propyl-(3-thiophen-2-yl-ethyl)-amine (47). N-*n*-Propyl-3-thiophen-2-yl-acetamide (1.0 g, 5.5 mmol) was dissolved in anhydrous THF (25 mL) and 2 M BH_3.Me_2S (5.5 mL, 10.9 mmol) in anhydrous THF (20 mL) was slowly added at RT. The mixture was stirred at RT for 30 min and subsequently refluxed for 1 h. The mixture was allowed to cool to RT and successively MeOH (3.5 mL), H_2O (3.5 mL) and 4 N HCl (3.5 mL) was added and the mixture was stirred for another 30 min at RT. The solvent was evaporated and the residue dissolved in H_2O, washed with diethyl ether and the aqueous layer was made alkaline with $NaHCO_3$ and extracted with diethyl ether. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and the solvent was evaporated to yield 0.75 g (67 %) yellow oil: ^{1}H NMR ($CDCl_3$) δ 0.9 (t, 3H, J = 7.3 Hz), 1.5 (q, 2H, J = 7.3 Hz), 1.5-1.6 (m, 2H), 2.5 (t, 2H, J = 7.2 Hz), 2.8 (s, 4H), 6.9-7.0 (m, 2H), 7.2-7.3 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 10.2, 21.6, 29.2, 48.7, 50.2, 119.4, 124.0, 126.6, 138.8; Anal Calcd (Obsd) for $C_9H_{15}NS$.¾H_2O: C: 59.14 (59.60), H: 9.10 (8.68), N: 7.66 (7.30). The amine was converted to the HCl-salt and recrystallized from diethyl ether-isopropanol: mp 207-210 °C.

N-*n*-Propyl-(3-thiophen-2-yl-ethyl)-thiophen-2-yl-acetamide (48) To a solution of amine **47**.HCl (500 mg, 2.4 mmol) dissolved in dichloromethane (50 mL) and 10 % NaOH (10 mL) was added 2-thienylacetylchloride (2 mL). The mixture was stirred for 3 h at RT and poured into water. The organic layer was separated and the aqueous layer was extracted with dichloromethane. The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The oil was purified over a SiO_2-column with dichloromethane as eluent. Evaporation of the dichloromethane yielded 660 mg (93 %) oil: ^{1}H NMR ($CDCl_3$) δ 0.9 (t, 3H, J = 7.3 Hz), 1.4-1.7 (m, 2H), 2.8-2.9 (m, 2H), 3.1-3.2 (m, 1H), 3.3-3.4 (m, 1H), 3.5-3.7 (m, 4H), 6.8-7.0 (m, 4H), 7.2-7.3 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 9.7 (C10), 9.9 (C10), 19.3 (C9 or C12), 20.8 (C9 or C12), 26.7 (C9 or C12), 28.1 (C9 or C12), 33.3 (C6),

33.4 (C6), 46.0 (C8 or C11), 46.2 (C8 or C11), 47.6 (C8 or C11), 49.1 (C8 or C11), 119.8, 120.3, 123.2, 124.1, 124.5, 124.8, 125.2, 126.5, 126.7, 135.2, 136.5, 137.8, 168.2 (C7); Anal ($C_{15}H_{19}NOS_2$) C, H, N.

N-*n*-Propyl-(2-thiophen-2-yl-ethyl)-thiophen-3-ylethyl-amine (29). N-*n*-Propyl-(3-thiophen-2-yl-ethyl)-thiophen-2-yl-acetamide **48** (0.5 g, 1.70 mmol) was dissolved in anhydrous THF (40 mL) and 2 M $BH_3.Me_2S$ (3 mL) in anhydrous THF (10 mL) was slowly added at RT. The mixture was stirred at RT for 30 min and subsequently refluxed for 3h. The mixture was allowed to cool to RT and successively MeOH (3 mL), H_2O (3 mL) and 12 N HCl (3 mL) was added and the mixture was stirred for another 30 min at RT. The solvent was evaporated and the residue dissolved in H_2O, washed with diethyl ether and the aqueous layer was made alkaline with $NaHCO_3$ and extracted with diethyl ether. In both diethyl ether layers compound was present and therefore all the organic layers were combined. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and the solvent was evaporated to yield 0.33 g (69.3 %) light yellow solid: mp 141.5-142.5 °C; ^{1}H NMR ($CDCl_3$) δ 0.9 (t, 3H, J = 7.3 Hz), 1.5 (q, 2H, J = 7.3 Hz), 2.5-2.6 (m, 2H), 2.8-2.9 (m, 6H), 3.0-3.1 (m, 2H), 6.8 (m, 1H), 6.9-7.0 (m, 3H), 7.1-7.2 (m, 1H), 7.2-7.3 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 10.5, 19.0, 26.4, 26.6, 53.5, 54.4, 54.5, 119.1, 121.8, 123.1, 123.7, 125.1, 126.9, 139.4, 141.7. Anal ($C_{15}H_{21}NS_2$.ditoluoyl tartaric acid) C, H, N.

N-*n*-Propyl-(3-thiophen-2-yl-ethyl)-thiophen-3-yl-acetamide (49). N-*n*-Propyl-(3-thiophen-2-yl-ethyl)-amine **47** (1.0 g, 6.0 mmol) was dissolved in dichloromethane (50 mL) and 10 % NaOH-solution (10 mL) and 3-thienyl acetyl chloride (1.0 g, 6.2 mmol) in dichloromethane (20 mL) were added. The reaction mixture was stirred for 2 h at RT. The two layers were separated and the organic layer was washed with 3 N HCl-solution and water, dried over Na_2SO_4 and the solvent was evaporated to yield 1.2 g (85.7 %) yellow oil: ^{1}H NMR ($CDCl_3$) δ 0.9 (dt, 3H, J = 7.3 Hz), 1.4-1.7 (m, 2H), 2.7-2.9 (m, 2H), 3.0-3.2 (m, 1H), 3.3-3.4 (m, 1H), 3.5-3.7 (m, 4H), 6.8-7.0 (m, 4H), 7.2-7.3 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 9.7 (C10), 9.9 (C10), 19.3 (C9 or C12), 20.7 (C9 or C12), 26.7 (C9 or C12), 28.0 (C9 or C12), 34.3 (C6), 45.6 (C8 or C11), 46.0 (C8 or C11), 47.6 (C8 or C11), 48.9 (C8 or C11), 119.7, 120.2, 120.4, 120.5, 124.0, 124.3, 124.7, 126.5, 126.8, 127.2, 133.5 (C4 or C13), 133.6 (C4 or C13), 136.8 (C4 or C13), 137.8 (C4 or C13), 169.1 (C7); IR (NaCl) cm^{-1} 1642 (C=O, amide). Anal $C_{15}H_{19}NOS_2.½H_2O$ C, H, N.

N-*n*-Propyl-(3-thiophen-2-yl-ethyl)-thiophen-3-ylethyl-amine (30). This compound was synthesised in 67 % yield according to the method used for compound **29**: mp 117-119 °C; ^{1}H NMR ($CDCl_3$) δ 0.9 (t, 3H, J = 7.3 Hz), 1.5-1.8 (m, 2H), 2.8-3.0 (m, 4H), 3.0-3.2 (m, 4H) 6.9-7.1 (m, 4H), 7.2-7.3 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 10.1, 14.8, 23.0, 53.0, 58.5, 119.9, 124.6, 126.5, 136.7. Anal ($C_{15}H_{21}NS_2$.HCl.¼H_2O) C, H, N.

3-(Dimethylaminomethyl)-2-(trimethylsilylmethyl)thiophene (51). A solution of trimethylsilylmethylmagnesiumchloride, prepared from magnesium (8.9 g, 0.37 mol), a crystal iodine and chloromethyltrimethylsilane (40.9 g, 0.34 mol), in anhydrous diethyl ether (20 mL)

was added dropwise to a cooled solution of 2-bromo-3-(dimethylaminomethyl)-thiophene (**50**) (47 g, 0.21 mol), bis(triphenylphosphine)nickel(II)chloride (1.4 g, 2.6 mol-%) in anhydrous diethyl ether (500 mL). After refluxing for 20 h the mixture was cooled and slowly water (160 mL) and aqueous saturated NH_4Cl-solution (160 mL) were added. The two layers were separated and the aqueous layer was extracted with diethyl ether. The combined diethyl ether layers were washed with brine, dried over Na_2SO_4, filtered and the solvent was evaporated to yield 37 g (76 %) of an oil: ^{1}H NMR ($CDCl_3$) δ 0.02 (s, 9H), 2.16 (s, 6H), 2.2 (s, 2H), 3.2 (s,2H), 6.9 (s, 2H); ^{13}C NMR ($CDCl_3$) δ -1.7, 18.1, 45.0, 56.5, 119.3, 129.1, 132, 138 (compare ref. 218).

3-(Trimethylammonium)-2-(trimethylsilylmethyl)thiophene iodide (52). To a stirred solution of 3-(dimethylaminomethyl)-2-(trimethylsilylmethyl)thiophene (**51**) (3 g, 0.013 mol) in acetonitril (10 mL) was added iodomethane (1.3 mL, 0.021 mol). After refluxing for 1 h the mixture was cooled to RT and diethyl ether was added. The mixture was filtered to yield 3.45 g (71%) of a yellow solid compound: ^{1}H NMR (DMSO) δ 0.0 (s, 9H), 2.5 (s, 2H), 3.0 (s, 9H), 4.4 (s, 2H), 7.1 (d, J = 5.4 Hz, 1H), 7.3 (d, J = 5.4 Hz, 1H); ^{13}C NMR (DMSO) δ -1.4, 18.5, 51.7, 60.3, 122.3, 123.1, 130.8, 147.7 (compare ref. 218).

5- And 6-methylcarboxylate-4,5,6,7-tetrahydrobenzo[*b*]thiophene (53a, b). To a stirred solution of 2-(trimethylsilylmethyl)-3-(trimethylammonium)thiophene iodide (**52**) (10 g, 0.03 mol) and methylacrylate (136 mL) in acetonitril (270 mL) was added dropwise a solution of tetrabutylammoniumfluoride trihydrate (17.1 g, 0.05 mol) in acetonitril (540 mL) in 2 h. After the addition was complete the solution was concentrated and diethyl ether was added until no more precipitate was formed. The mixture was filtered and the solvent was evaporated. Bulb-to-bulb distillation at 100 °C (0.05 mm Hg) yielded 4.2 g (81 %) oil: ^{1}H NMR ($CDCl_3$) δ 1.8-2.0 (m, 1H), 2.15-2.3 (m, 1H), 2.6-3.1 (m, 5H), 3.7 (s, 3H), 6.7 (d, J = 5.1 Hz, 1H), 7.1 (d, J = 5.1 Hz, 1H); 5-isomer ^{13}C NMR ($CDCl_3$) δ 23.9, 26.1, 27.8, 39.6, 51.6, 122.3, 127.2, 133.3, 134.3, 175.3; 6-isomer ^{13}C NMR ($CDCl_3$) δ 24.4, 25.6, 27.1, 40.3, 51.6, 122.3, 127.1, 133.3, 134.3, 175.3; the ratio 6-isomer/5-isomer = 2:1, which was determined by ^{13}C NMR; IR (NaCl) cm^{-1} 1739 (C=O) (compare ref. 218).

4,5,6,7-Tetrahydrobenzo[*b*]thiophene-5 and 6-carboxylic acid (54a, b). A solution of 5- and 6-methylcarboxylate-4,5,6,7-tetrahydrobenzo[*b*]thiophene (**53a, b**) (4.29 g, 22 mmol) in an aqueous 16% NaOH-solution (35 mL) was refluxed for 45 min. After cooling to RT an aqueous 2 N HCl-solution was added until the pH was 1. A white solid was formed which dissolved in dichloromethane. The aqueous layer was saturated with NaCl and extracted with dichloromethane. The combined organic layers were dried over Na_2SO_4, filtered and the solvent was evaporated to yield 3.87 g (97 %) pale yellow solid: mp 82.5-84.5 °C; ^{1}H NMR ($CDCl_3$) δ 1.8-2.1 (m, 1H), 2.2-2.3 (m, 1H), 2.7-3.1 (m, 5H), 6.8 (d, J = 5.1 Hz, 1H), 7.1 (d, J = 5.1 Hz, 1H); 5-isomer ^{13}C NMR ($CDCl_3$) δ 23.7, 25.8, 27.5, 39.5, 122.3, 127.2, 133.0, 134.3, 181.5; 6-isomer ^{13}C NMR ($CDCl_3$) δ 24.3, 25.3, 26.8, 40.1, 122.3, 127.1, 133.0, 134.3, 181.2. ^{13}C NMR (compare ref. 218) determined a 2:1 ratio of the 6-isomer:5-isomer.

5-And 6-ammonium-4,5,6,7-tetrahydrobenzo[*b*]thiophene hydrochloride (55a, b). To a solution of mixture **54a, b** (4.26 g, 23 mmol) and triethylamine (3.62 g, 24 mmol) in dioxane (120 mL) was added diphenylphosphoryl azide (5.3 mL, 24 mmol) at 5°C. The reaction was stirred overnight at RT. Diethyl ether (300 mL) and water (300 mL) were added to the reaction mixture. After separation of the two layers the organic layer was washed with an 1% NaOH solution, dried over Na_2SO_4 and concentrated under reduced pressure yielding a red residue which was used without purification for the next step. To the residue was added aqueous 1N HCl (110 mL) and dioxane (110 mL) and heated for 2 h at 120 °C. The mixture was allowed to cool to RT and the mixture was basified (pH~10) with 4N NaOH and extracted with diethyl ether and dried over Na_2SO_4. Filtration and evaporation of the organic solvents gave a brown oil, which dissolved in diethyl ether. Slow addition of an ethereal HCl solution gave a light brown solid. The yield of the obtained mixture of regioisomers (**55a, b**) was 2.6 g (58%): mp 192.8- 194.1 ºC. ^{1}H NMR ($CDCl_3$) δ 1.5-1.7 (m, 1H), 1.9-2.0 (m, 1H), 2.4-3.0 (m, 4H), 3.1-3.3 (m, 1H), 6.7 (d, 1H, J=5.1 Hz), 7,0 (d, 1H, J = 5.1 Hz). 5-isomer ^{13}C NMR ($CDCl_3$) δ 21.9, 31.8, 34.0, 45.9, 121.0, 126.0, 132.1, 132.4; 6-isomer ^{13}C NMR ($CDCl_3$) δ 22.4, 31.1, 33.3, 46.4, 120.9, 125.7, 132.1, 132.9; IR (KBr) 3300 cm^{-1} and 1618 cm^{-1} (NH_2). ^{13}C NMR (compare ref. 218) determined a 2:1 ratio of the 6-isomer:5-isomer.

5-And 6-(N,N-di-*n*-propyl)amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene (34 and 35). To a stirred solution of 5- and 6-ammonium-4,5,6,7-tetrahydrobenzo[*b*]thiophene chloride (**55a, b**) (2.5 g, 0.013 mol) and K_2CO_3 (7.05 g, 0.05 mol) in DMF (370 mL) was added 1-iodopropane (9 mL, 0.092 mmol). After stirring for 24 h at 50 °C the solution was poured into water and extracted 5 times with diethyl ether. The combined diethyl ether layers were washed 6 times with brine, dried over Na_2SO_4, filtered and evaporated to yield 3 g (84%). Now it is possible to separate both isomers by column chromatography with as eluent ethyl acetate : Hexane (1: 9). After evaporation of the solvent the isomers were dissolved in anhydrous diethyl ether and 1 N HCl in diethyl ether was added to yield 1.3 g (36%) of the 6-isomer and 650 mg (18%) of the 5-isomer. 6-Isomer: mp 134-135 °C; ^{1}H NMR ($CDCl_3$) δ 1.0 (t, J = 7.3 Hz, 6H), 1.8 (q, J= 7.6 Hz, 4H), 2.0-2.2 (m, 1H), 2.3-2.4 (m, 1H), 2.7-3.0 (m, 2H), 3.0-3.4 (m, 6H), 3.7-3.9 (m, 1H), 6.8 (d, J = 5.1 Hz, 1H), 7.2 (d, J = 5.1 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 9.6, 18.0, 18.2, 23.5, 23.6, 24.7, 52.2, 52.7, 60.5, 123.4, 126.3; Anal ($C_{14}H_{23}NS.HCl$) C, H, N. 5-Isomer: mp 134-136 °C; ^{1}H NMR ($CDCl_3$) δ 1.0 (t, J 7.2 Hz, 6H), 1.8 (q, J = 7.3 Hz, 4H), 2.0-2.2 (m, 1H), 2.3-2.4 (m, 1H), 2.8-3.0 (m, 2H), 3.0-3.3 (m, 6H), 3.7-3.9 (m, 1H), 6.8 (d, J = 5.1 Hz, 1H), 7.2 (d, J = 5.1 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 9.6, 18.0, 18.3, 23.1, 24.1, 25.4, 52.2, 52.7, 60.2, 123.4, 126.7, 131, 133.5; Anal ($C_{14}H_{23}NS.HCl.¼H_2O$) C, H, N.

5- And 6- (N,N-di-*n*-propyl)aminomethyl-4,5,6,7-tetrahydro-benzo[*b*]thiophene (**36, 37**). To a cooled and stirred solution of 4,5,6,7-tetrahydrobenzo[*b*]thiophene-5 and 6-carboxylic acid (**54a, b**) (2.7 g, 0.015 mol) in anhydrous dichloromethane (40 mL) was added under nitrogen dropwise oxalyl chloride (5.9 mL, 8.9 g, 0.068 mol). The mixture was stirred overnight at RT and evaporated to yield 2.9 g (96%) oil of compound **56a, b**: ^{1}H NMR ($CDCl_3$) δ 1.9-2.2

(m, 1H), 2.3-2.5 (m, 1H), 2.7-3.3 (m, 5H), 6.8 (d, J = 5.0 Hz, 1H), 7.1 (d, J = 5.0 Hz, 1H); 5-isomer ^{13}C NMR ($CDCl_3$) δ 23.5, 26.3, 28.0, 51.6, 123.0, 127.0, 132.0, 134.3, 176.1; 6-isomer ^{13}C NMR ($CDCl_3$) δ 24.0, 25.8, 27.4, 52.0, 123.0, 127.0, 131.8, 134.1, 176.1. 13C NMR determined a 2:1 ratio of the 6-isomer:5-isomer. The reaction product was used for the next step without further purification and analysis.

To a stirred solution of 4,5,6,7-tetrahydrobenzo[*b*]thiophene-5 and 6-carbanoyl chloride (**56a, b**) (1.8 g, 8.8 mmol) in dichloromethane (100 mL) was added dropwise a mixture of di-*n*-propylamine (1.8 mL, 1.3 g, 0.013 mmol) and triethylamine (1.3 mL, 0.95 g, 0.010 mmol) in dichloromethane. After stirring for 3 h at RT the mixture was evaporated and the residue was dissolved in diethyl ether. The diethyl ether layer was extracted 4 times with 4N HCl, dried over Na_2SO_4 and evaporated. Purification with column chromatography with as eluent ethyl acetate: Hexane (1: 9) yielded 1.66 g (71%) oil of compound **57a, b**: ^{1}H NMR ($CDCl_3$) δ 0.9 (t, J = 7.4 Hz, 6H), 1.5-1.6 (m, 4H), 1.9-2.0 (m, 2H), 2.7-3.0 (m, 5H), 3.2-3.3 (m, 4H), 6.7 (d, J = 5.1 Hz, 1H), 7.0 (d, J = 5.1 Hz, 1H); 5-isomer ^{13}C NMR ($CDCl_3$) δ 10.9, 11.1, 20.7, 22.6, 24.4, 27.2, 28.8, 37.1, 47.4, 49.3, 122.1, 127.3, 134.1, 134.2, 174.6; 6-isomer ^{13}C NMR ($CDCl_3$) δ 10.9, 11.1, 20.7, 22.6, 24.9, 26.7, 28.1, 37.7, 47.4, 49.3, 122.0, 127.1, 134.1, 134.2, 174.6. ^{13}C NMR determined a 2:1 ratio of the 6-isomer:5-isomer. The reaction product was used for the next step without further purification and analysis.

A solution of 4,5,6,7-tetrahydrobenzo[*b*]thiophene-5 and 6-carboxylic amide (**57a, b**) (5 g, 19 mmol) in anhydrous THF (340 mL) was cooled to 0-5 °C and $LiAlH_4$ (3.6 g, 95 mmol) was added. The mixture was refluxed for 3 h and than cooled to RT and 3.6 mL water, 3.6 mL 4 N NaOH-solution and 10.8 mL water was added. The mixture was filtered, the precipitate washed with diethyl ether and the filtrate dried over Na_2SO_4. Evaporation of the solvent yielded 4.5 g (92 %) crude oil. Purification and separation over a column with as eluent CH_2Cl_2:MeOH= 20:1 yielded 3.7 g 6-isomer and 1 g 5-isomer: ^{1}H NMR ($CDCl_3$) δ 0.9 (t, J = 7.3 Hz, 6H), 1.4-1.5 (m, 6H), 1.9-2.1 (m, 2H), 2.3-2.5 (m, 6H), 2.7-3.0 (m, 3H), 6.8 (d, J = 5.1 Hz,1H), 7.1 (d, J = 5.1 Hz, 1H); 5-isomer: mp 118.5-120.5 °C; ^{13}C NMR ($CDCl_3$) δ 11.7, 20.2, 24.3, 28.3, 30.5, 32.9, 56.7, 60.2, 121.7, 127.5, 135.2, 135.3; Anal ($C_{15}H_{25}NS.¼H_2O$) C, H, N. 6-isomer: mp 79-81 °C; ^{13}C NMR ($CDCl_3$) δ 11.7, 20.2, 24.8, 27.6, 29.8, 33.8,56.7, 60.2, 121.6, 127.2, 135.2, 135.3. Anal ($C_{15}H_{25}NS.C_4H_4O_4$) C, H, N.

4-Oxim-4,5,6,7-tetrahydrothianaphthene (59).[226-228] 4-Keto-4,5,6,7-tetrahydrothia-naphthene (**58**) (10.0 g, 66.0 mmol) was dissolved in ethanol (120 mL) and water (12 mL). To this solution was added sodium acetate (11 g, 134 mmol) and hydroxylammoniumchloride (8.67 g, 125 mmol). This mixture was refluxed for 3 h and then cooled to RT. Cold water was added and the precipitate obtained was filtered, washed with water and dried: yield 13.06 g (118 %) not pure. Recrystallization of the white precipitate from ethanol[227] gave white crystals; mp 125-127 °C; IR (KBr) 3289 cm^{-1} (C=N); ^{1}H-NMR ($CDCl_3$) δ 2.0 (t, 2H, J = 6.3 Hz), 2.8-2.9 (m, 4H), 7.1 (d, 1H, J = 5.3 Hz), 7.3 (d, 1H, J = 5.4 Hz); ^{13}C-NMR ($CDCl_3$) δ22.2, 22.6, 24.8, 122.9, 123.1, 131, 143.7, 153.1; Anal (C_8H_9NOS) C, H, N.

4-Tosyloxim-4,5,6,7-tetrahydrothianaphthene (60).[227,228] A solution of 4-oxim-4,5,6,7-tetrahydrothianaphthene (**59**) (4.0 g, 23.9 mmol) in 25 mL pyridine was cooled to about 10°C in an ice-bath. p-Toluene sulfonyl chlorid (10.3 g, 53.9 mmol) was added slowly in small portions. This mixture was stirred for 2 h at about 10 °C and then 2 h at RT. Then the mixture was poured into ice water. The precipitate obtained was filtered, washed with water and dried. The yield was 7.84 g (100 %) not pure. Recrystallization of the white precipitate from ethyl acetate gave white crystals; mp 130-132 °C; IR (KBr) 1596 cm^{-1} (C=N); ^{1}H-NMR ($CDCl_3$) δ 1.9 (t, 2H, J = 6.2 Hz), 2.4 (s, 3H), 2.8-2.9 (m, 4H), 7.0 (d, 1H, J = 5.2 Hz), 7.2 (d, 1H, J = 5.3 Hz), 7.4 (d, 2H, J = 8.3 Hz), 7.9 (d, 2H, J = 8.8 Hz); ^{13}C-NMR ($CDCl_3$) δ 21.8, 22.3, 23.8, 24.6, 123.2, 123.4, 128.8, 128.9, 129.5, 133, 145, 148, 159; Anal ($C_{15}H_{15}NO_3S_2.½H_2O$) C, H, N.

4-Keto-5-amino-4,5,6,7-tetrahydrothianaphthene (61).[228,229] A solution of potassium tert-butoxide (5.7 g, 50.8 mmol), ethanol (43 mL) and toluene (107 mL) was cooled to 0-5 °C. To this solution was added 4-tosyloxim-4,5,6,7-tetrahydrothianaphthene (**60**) (10.0 g, 31.6 mmol). This mixture was stirred for 2 h at 0-5 °C and then stirred for 2 h at RT. The precipitate (potassium tosylate) obtained was filtered and washed with diethyl ether. To the filtrate was added 5 mL 37 % HCl. After stirring some time a precipitate arises of the ketamine.HCl **61**. The precipitate was filtered and washed with diethyl ether, the yield was 4.5 g (71 %) before recrystallization, after recrystallization of the precipitate from ethanol-diethyl ether yellow crystals were obtained; mp 197-199 °C; IR (KBr) 3430 cm^{-1} (NH), ~ 3000 cm^{-1} (NH), 1600 cm^{-1}, 1500 cm^{-1} (NH), 1676 cm^{-1} (C=O); ^{1}H-NMR (CD_3OD) δ 2.3-2.5 (m, 1H), 2.6-2.7 (m, 1H), 3.3-3.4 (m, 2H), 4.3-4.4 (dd, 1H, J = 13.7 Hz), 7.4 (s, 2H); ^{13}C-NMR (CD_3OD) δ 24.6, 30.2, 55.7, 124.9, 126.4, 135, 158, 188.

4-Keto-5-chloroacetamide-4,5,6,7-tetrahydrothianaphthene (62).[227] To a solution of 4-keto-5-amino-4,5,6,7-tetrahydrothianaphthene (**61**)(5.3 g, 21.1 mmol) in dichloromethane (290 mL) was added a solution of NaOH (7.1 g, 0.18 mol) in water (61 mL). To this stirred mixture was added chloroacetylchlorid (5.9 g, 4.2 mL, 51.9 mmol) and the mixture was stirred another 3 h. After the reaction was complete the organic layer was separated. To the aqueous layer was added water (145 mL) and 4 N HCl until the aqueous layer was neutral. The aqueous layer was extracted with dichloromethane (3 x 25 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and evaporated. Recrystallization from ethyl acetate-hexane yielded 6.0 g (95 %) brown crystals; mp 130-132 °C; IR (KBr) 3334 cm^{-1} (NH), 1681 cm^{-1} (C=O), 1639 cm^{-1} (C=O, amide); ^{1}H-NMR ($CDCL_3$) δ 1.9-2.1 (m, 1H), 2.8-2.9 (m, 1H), 3.15-3.25(m, 1H), 4.1 (s, 2H), 4.5-4.65 (m, 1H), 7.1 (d, 1H, J = 5.3 Hz), 7.3 (d, 1H, J = 5.4 Hz), 7.6 (m, 1H); ^{13}C-NMR (CD_3OD) δ 24.6, 31.3, 42.6, 55.9, 124.5, 124.7, 135.6, 155.7, 166.4, 189 Anal ($C_{10}H_{10}NO_2SCl$) C, H, N.

4-Hydroxy-5-chloroacetamide-4,5,6,7-tetrahydrothianaphthene (63). A solution of 4-keto-5-chloroacetamide-4,5,6,7-tetrahydrothianaphthene (**62**) (4.0 g, 16.4 mmol) in methanol (90 mL) under nitrogen was cooled to 5-8 °C in an ice-bath. While stirring the solution $NaBH_4$ (1.5 g, 39.6 mmol) was added in portions. The solution was stirred another h at 5-8 °C. To the

mixture was added 1 N HCl to remove excess of $NaBH_4$ and then the solvent was evaporated. Recrystallization of the crude product from ethyl acetate-hexane yielded 3.55 g (88 %) brown crystals: mp 148-150 °C; IR (KBr) ~ 3200 cm^{-1} (OH), ~ 3000 cm^{-1} (NH), 1646 cm^{-1} (C=O, amide); ^{1}H-NMR ($CDCl_3$) δ 1.85-2.05 (m, 1.5H), 2.15-2.3 (m, 1.5H), 2.8-3.0 (m, 2H), 4.1 (s, 2H), 4.2 (m, 1H), 4.6 (d, 1H, J = 7.17 Hz), 6.7 (br s, 1H), 7.1 (d, 1H, J = 5.0 Hz), 7.2 (d, 1H, J = 5.1 Hz); ^{13}C-NMR 23.3, 27.6, 42.9, 54.1, 69.8, 124.1, 127.4, 127.5, 128, 169; Anal ($C_{10}H_{12}NO_2SCl$) C, H, N.

***trans*-2,3,4a,5,6,9b-Hexahydro-4*H*-thianaphth[4,5*e*][1,4]oxazine-3-one (64).** To a solution of 4-hydroxy-5-chloroacetamide-4,5,6,7-tetrahydrothianaphthene (**63**) (5.2 g, 21.16 mmol) in isopropanol (275 mL) was added dropwise 50 % NaOH solution (3.6 mL) at RT. The solution was stirred for 15 h. After the reaction was complete the solvent was evaporated until almost dry. The suspension was diluted with water (180 mL), neutralised with 10 % HCl and extracted with dichloromethane. The combined organic layers were washed with brine, dried over Na_2SO_4 and evaporated. Recrystallization of the crude product from ethyl acetate-hexane yielded 2.0 g (45 %) brown crystals; mp 242-244 °C; IR (KBr) 3313 cm (NH), 1638 cm (C=O); ^{1}H-NMR ($CDCl_3$) δ 1.9-2.1 (m, 1H), 2.1-2.2 (m, 1H), 2.9-3.0 (m, 2H), 3.6-3.7 (m, 1H), 4.4 (d, 2H, J = 2.7 Hz), 4.47-4.53 (m, 1H), 7.0 (d, 1H, J = 5.2 Hz), 7.2 (d, 1H, J = 5.2 Hz), 7.9 (br s, 1H); ^{13}C-NMR 23.5, 27.6, 53.8, 68.3, 76.1, 124.2, 124.5, 133.9, 136.3, 170.2; Anal ($C_{10}H_{11}NO_2S$) C, H, N.

***trans*-2,3,4a,5,6,9b-Hexahydro-4*H*-thianaphth[4,5*e*][1,4]oxazine (38).** A solution of *trans*-2,3,4a,5,6,9b-hexahydro-4*H*-thianaphth[4,5*e*][1,4]oxazine-3-one (**64**) (1.3 g, 6.2 mmol) in anhydrous tetrahydrofuran (195 mL) was cooled to about 5 °C. To this solution was added $LiAlH_4$ (845 mg, 22.3 mmol). This mixture was refluxed for 2 h and then cooled to RT. Then successively water (0.9 mL), 4 N NaOH-solution (0.9 mL) and water (2.7 mL) were added to remove excess of $LiAlH_4$. The mixture was filtered, washed with diethyl ether and then the filtrate was dried over Na_2SO_4. Evaporation under reduced pressure of the solvent yielded an oil which was dissolved in anhydrous diethyl ether and diethyl ether saturated with gaseous HCl. Recrystallization from isopropanol-anhydrous diethyl ether yielded 245 mg (17%) white crystals: mp 264-266 °C; IR (KBr) 3213 cm^{-1} (NH); ^{1}H-NMR ($CDCl_3$) δ 1.2-1.3 (m, 1H), 2.1-2.3 (m, 1H), 2.4-2.6 (m, 1H), 2.9-3.1 (m, 2H), 3.2-3.3 (m, 1H), 3.3-3.5 (m, 2H), 3.7-3.8 (m, 1H), 3.9-4.0 (m, 1H), 4.8-4.9 (m, 1H), 7.0 (d, 1H, J = 5.4 Hz), 7.3 (d, 1H, 5.4 Hz); ^{13}C-NMR ($CDCl_3$) δ 23.9, 26.1, 45.2, 58.0, 64.8, 76.3, 125.1, 125.5, 134.5, 136.5; Anal ($C_{10}H_{13}NOS.HCl.¼H_2O$) C, H, N.

***trans*-N-*n*-Propyl-2,3,4a,5,6,9b-hexahydro-4*H*-thianaphth[4,5*e*][1,4]oxazine (39).** To a solution of *trans*-2,3,4a,5,6,9b-hexahydro-4*H*-thianaphth[4,5*e*][1,4]oxazine (**38**) (100 mg, 0.43 mmol) in DMF (12 mL) under nitrogen was added anhydrous K_2CO_3 (230 mg, 1.7 mmol) and 1-iodopropane (490 mg, 290 µL, 2.9 mmol). This mixture was stirred at 55 °C for 2½ h. From TLC (with CH_2Cl_2: MeOH = 10: 1 as eluent) it was clear that the reaction was not finished, so extra 1-iodopropane was added and the mixture was allowed to stand over the weekend at RT.

The mixture was poured into water (20 mL) and extracted with diethyl ether (5 x 20 mL). The combined extracts were washed with brine (6 x 20 mL), dried over Na_2SO_4 and evaporated. The resulting oil was dissolved in anhydrous diethyl ether and diethyl ether saturated with gaseous HCl was added to prepare the HCl-salt in a yield of 94.35 mg (80 %): mp 238-240 °C; ^{1}H-NMR ($CDCl_3$) δ 1.1 (t, 3H, J = 7.3 Hz), 1.7-2.1 (m, 3H), 2.6-2.8 (m, 1H), 3.0-3.2 (m, 3H), 3.3-3.7 (m, 4H), 4.1-4.2 (m, 2H), 4.8 (d, 1H, J = 9.1 Hz), 7.0 (d, 1H, J = 5.1 Hz), 7.2 (d, 1H, 5.2 Hz); ^{13}C-NMR ($CDCl_3$) δ 10.9, 17.6, 23.4, 24.1, 52.6, 55.4, 65.0, 65.6, 76.7, 125.3, 125.5, 135, 147; Anal ($C_{13}H_{19}NOS.HCl$) C, H, N.

2.4.2 Distance calculation

Conformational analyses were performed on a Silicon Graphics O_2 Workstation R5000 chipset, running IRIX 6.3. Conformational analyses were performed in MacroModel version 6.5,[230] using the Monte Carlo Multiple Minimum (MCMM) search protocol.[231] All ligands were considered in their protonated, positively charged forms. N-*n*-propyl groups were truncated to N-methyl groups during the conformational analyses in order to reduce the number of torsion angles. All minimizations were performed within the MM3* force field,[232-234] while simulating a distance-dependent GB/SA water continuum,[235] as implemented in MacroModel. Prior to submitting them to the MCMM protocol all ligands were minimised with default options. The starting conformations for the ligands were independently submitted to the MCMM protocol. To search conformational space 1000-5000 MC steps were performed on each starting conformation, dependent on the number of torsion angles. Starting conformations for each step were systematically generated using the SUMM option.[236] The number of torsion angles to be varied in each MC step was set between 2 and *n*-1, *n* being the total number of variable torsion angles. Ring closure bonds were defined in the 6-membered non-aromatic rings in order to allow torsion angles within these rings to be varied as well. Ring closure distances were limited to 0.5-2.0 Å. The randomly generated structures were minimised using the Truncated Newton Conjugate Gradient (TNCG) minimizer, allowing for 250 iterations per structure, until an initial gradient of 0.01 kcal/Å mol^{-1} was reached. Least squares superimposition of all non-hydrogen atoms was used to eliminate duplicate conformations. For non-chiral ligands, specifying the NANT options prevented rejection of mirror images. The minimum energy conformations thus obtained were submitted to a final minimisation, using the Full Matrix Newton Raphson (FMNR) minimiser, allowing for 1000 iterations per structure, until a final gradient of 0.002 kcal/Å mol^{-1} was reached. An energy cut-off of 12 kcal/mol was applied to the search results. (For ligands containing a 'chiral' protonated nitrogen atom, the search results of the independent analyses performed on the starting conformations with inverted nitrogen atoms were combined and subsequently filtered on energy ($\Delta E \leq 3.0$ kcal/mol) using the filter mode.) After the minimisation the distances were calculated.

2.4.3 Pharmacology

Cell lines expressing dopamine receptor isoforms. A cell line expressing the human dopamine D_{2L} was purchased from Dr. O. Civelli, Oregon Health Sciences University. The D_{2L} receptor cDNA was subcloned into the expression vector, pRc/CMV. The plasmids were transfected by electroporation into CHO K1 cells. A single stable transfectant, resistant to the antibiotic G418, was isolated and selected for use in the binding studies. The human dopamine D_3 receptor cDNA cloned in the pcDNAIneo plasmid was obtained from Dr. K. O'Malley and stably transfected into CHO K1 cells by a modified calcium phosphate precipitation technique[237] and transfectants were selected in G418, isolated and screened for expression of human D_3 receptors by radioligand binding as previously described.[50]

Cell culture and preparation of cell membranes. CHO K1 cells expressing either human dopamine D_{2L} and D_3 receptors were grown in 162 cm^2 culture flasks in F12 medium (Gibco Laboratories, Grand Island, N.Y., USA) supplemented with 10 % foetal bovine serum (FBS, Hyclone, Logan, UT) in an atmosphere of 5 % CO_2/ 95 % air at 37 ºC. Cells were grown until confluent after which growth medium was removed and replaced with 0.02 % EDTA in a phosphate-buffered saline solution (Sigma Chemical Co. St. Louis, MO, USA) and scraped from the flasks. The cells were centrifuged at about 1000 x g for 10 min at 4 ºC and than resuspended in TEM buffer (25 mM Tris-HCl, pH 7.4 at 37 ºC, 1 mM EDTA, and 6 mM $CaCl_2$) for D_{2L} and D_3 and homogenised with a Brinkman Polytron homogenizer at setting 5 for 10 sec. The membranes were pelleted by centrifugation at 20000 x g at 4 ºC for 20 min, then the pellets were resuspended in appropriate buffer at 1 ml/flask and stored at -70 ºC until used in the receptor binding assay.

Receptor binding assays: D_{2L} and D_3 dopamine receptors. A cell membrane preparation (400 µL) was incubated in triplicate with 50 µL [^{3}H]N-0437 (2nM for D_{2L}) or [^{3}H]spiperone (0.5 nM for D_3), 50 µL buffer, or competing drugs where appropriate to give a final volume of 0.5 mL. After 60 min incubation at 25 ºC, the incubations were terminated by rapid filtration through Whatmann GF/B glass fibre filters (soaked for 1 hr in 0.5 % polyethylenimine) on a Brandel MB-48R cell harvester, with 3 washes of 1 mL ice-cold buffer. Individual filter discs containing the bound ligand were placed in counting vials with 4 mL of scintillation fluid (Ready Gel, Beckman Instrument Inc., Fullerton, CA, USA) and then counted in a Beckman LS-6800 liquid scintillation counter at an efficiency of 45 %. Non-specific binding was defined in presence of 1 µM of haloperidol.

Data calculation. Saturation and competition binding data were analysed using the iterative non-linear least square curve-fitting Ligand program. In competition experiments, apparent K_i values were calculated from IC50 values by method of Cheng and Prusoff.[238] Experimental compounds were made up as stock solutions in dimethyl sulfoxide (DMSO). The final concentration of 0.1 % DMSO used in the incubation mixture had no effect on the specific binding. Each observation was carried out in triplicate. To allow these calculations, K_d values

were measured for the interaction of various ligands with the receptor. These were: [^{3}H]spiperone binding, human D_3, 0.15 ± 0.02 (n=3); [^{3}H]N-0437 binding, human D_{2L}, 2.24 ± 0.05, nM (n=3).

Chapter 3

Studies with a series of 2-substituted tetrahydrobenzo[*b*]thiophenes as dopamine receptor agents with selectivity for the dopamine D_3 receptor.

Abstract

This study describes the synthesis and *in vitro* pharmacology of a series of tetrahydrobenzo[*b*]thiophenes, which are substituted on the 2-position or which have a methylene bridge between the aliphatic ring and the nitrogen atom. 2-Substitution in 6-(N,N-di-*n*-propyl)amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene leads to compounds with a 50-100-fold selectivity and a moderate to high affinity for the dopamine D_3 receptor. For the 5-isomer the same introduction gives compounds with no affinity for the dopamine D_2 and D_3 receptor. This difference in affinity between the 5- and 6-isomer is not seen in the parent compounds. An explanation could be that for the 6-isomer the 2-substituted derivatives are structurally comparable with the dopamine D_3 receptor preferring agonist 7-OH-DPAT, while the 2-substituted 5-isomers are structurally more comparable with the low-affinity dopamine receptor ligand 6-OH-DPAT. Lengthening of the distance between the sulfur and the nitrogen atom also gives a dopamine D_3 receptor selective ligand. 2-Substitution of this compound leads to inactive compounds.

In a series of tetrahydrobenzo[*b*]thiophene analogues, we have identified novel selective dopamine D_3 receptor agents, one of which displays a 100-fold selectivity over dopamine D_2 receptors. These results provide information for the development of pharmacophoric models of the dopamine D_2 and D_3 receptor subtypes that can be used for the future development of selective agonists at these receptor subtypes.

3.1 Introduction

Hydroxylated 2-aminotetralins are potent dopamine receptor agonists. Examples of such are 5-hydroxy-2-(N,N-di-*n*-propylamino)tetralin (5-OH-DPAT, **9**)[98,100,205] and 7-hydroxy-2-(N,N-di-*n*-propylamino)tetralin (7-OH-DPAT, **10**).[79,213] These compounds have no clinical utility because of their low oral bioavailability and their short duration of action, due to glucuronidation in the liver and gut.[161] We have synthesised and tested 6-(N,N-di-*n*-propyl)amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene (**34**) and 5-(N,N-di-*n*-propyl)amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene (**35**), which are thiophene analogues of hydroxylated 2-aminotetralins. These tetrahydrobenzo[*b*]thiophenes turned out to possess a higher relative oral bioavailability than 5-OH-DPAT (chapter 4 and ref. 215). However, the affinity for the dopamine receptors is diminished, as compared to 5-OH-DPAT (chapter 2, ref. 239).

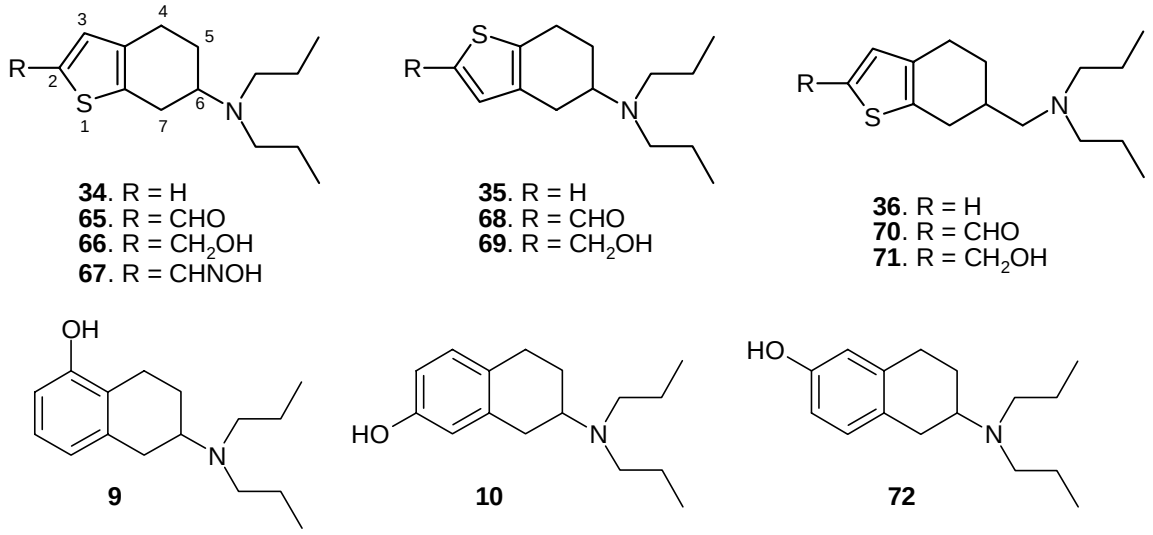


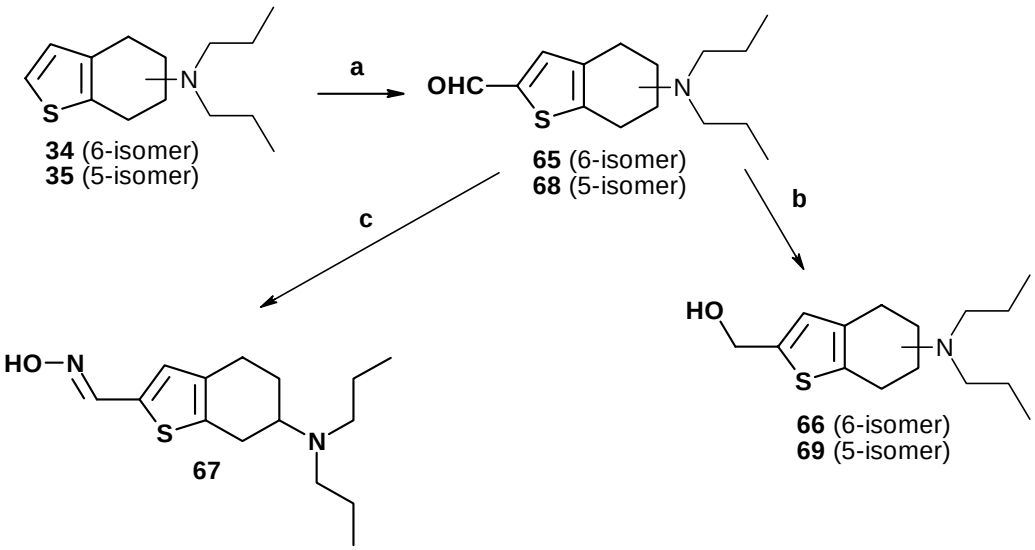
Chart 3.1 Chemical structures of 6-(N,N-di-*n*-propyl)amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene (**34**), 2-formyl-6-(N,N-di-*n*-propyl)amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene (**65**), 2-hydroxymethyl-6-(N,N-di-*n*-propyl)amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene (**66**), 2-carboxaldoxime-6-(N,N-di-*n*-propyl)amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene (**67**), 5-(N,N-di-*n*-propyl)amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene (**35**), 2-formyl-5-(N,N-di-*n*-propyl)amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene (**68**), 2-hydroxymethyl-5-(N,N-di-*n*-propyl)amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene (**69**), 6-(N,N-di-*n*-propyl)aminomethyl-4,5,6,7-tetrahydrobenzo[*b*]thiophene (**36**), 2-formyl-6-(N,N-di-*n*-propyl)aminomethyl-4,5,6,7-tetrahydrobenzo[*b*]thiophene (**70**), 2-hydroxymethyl-6-(N,N-di-*n*-propyl)aminomethyl-4,5,6,7-tetrahydrobenzo[*b*]thiophene (**71**), 5-hydroxy-2-(N,N-di-*n*-propylamino)tetralin (5-OH-DPAT, **9**), 7-hydroxy-2-(N,N-di-*n*-propylamino)tetralin (7-OH-DPAT, **10**) and 6-hydroxy-2-(N,N-di-*n*-propylamino)tetralin (6-OH-DPAT, **72**).

Some possible reasons for a diminished affinity for the dopamine receptors can be that: I) a sulfur atom is only a weak hydrogen bond acceptor and is not a hydrogen bond donor as is a hydroxyl moiety, II) there is a non-optimal distance between the hydrogen bond forming moieties on the aromatic ring and the nitrogen, III) there are alternative interaction points for the presumed essential atoms in a dopamine receptor.

In order to investigate the structure-activity relationships of tetrahydrobenzo[*b*]thiophenes, we synthesised a series of 2-substituted tetrahydrobenzo[*b*]thiophenes and analogues with a methylene moiety between the aliphatic ring and the nitrogen atom to enlarge the distance between the sulfur and the nitrogen atom.

3.2 Chemistry

The synthesis of 5- and 6-(N,N-di-*n*-propyl)amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene (**34** and **35**) has been described previously (Chapter 2). Using a Vilsmeier-Haack reaction, a formyl moiety was introduced on the 2-position of the thiophene ring.[240] An excess of N-methylformanilide was used which could be easily removed by column chromatography. Reduction of the formaldehydes **65** and **68** with $NaBH_4$ yielded the hydroxymethyl compounds **66** and **69**. From compound **65**, the aldoxime **67** was synthesised, using hydroxylamine.HCl in ethanol and 5N NaOH-solution.



Scheme 3.1 Reagents: (a) $POCl_3$, N-methylformanilide; (b) $NaBH_4$, EtOH; (c) $NH_4OH.HCl$, EtOH, 5N NaOH.

The NMR-data showed that reaction **c** resulted in two products, the *syn* and the *anti*-isomers. Chart 3.2 shows that only the *syn*-isomer possesses the possibility to form an internal hydrogen bond. Due to this intramolecular hydrogen bond are the protons of the thiophene ring

and the carbon atoms of the thiophene ring and the aldoxime moiety chemically non-equivalent for the *syn* and *anti*-isomer. The ^{13}C-NMR showed that the ratio between the two isomers was 1:2. On TLC there was a slight difference between the R_f-values, however, it was not possible to separate the isomers by column chromatography. The fast eluting isomer is probably the *syn*-isomer, since this isomer has less interaction with the column material due to its intramolecular hydrogen bond. The syn-anti mixture was used for pharmacological testing.

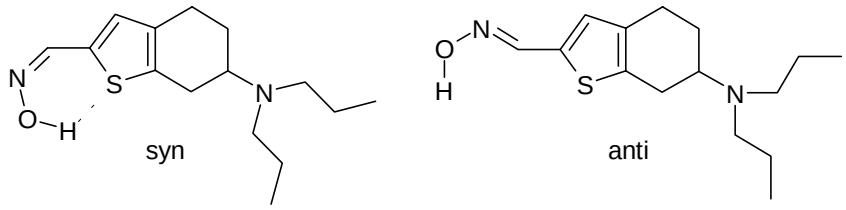
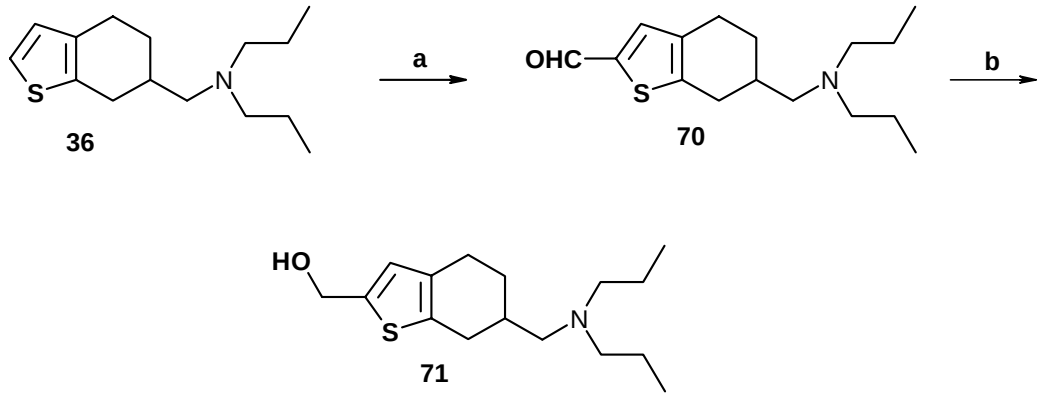


Chart 3.2 The *syn* and *anti*-isomers of compound **67**.

The synthesis of the 2-substituted 6-(N,N-di-*n*-propyl)aminomethyl-4,5,6,7-tetrahydrobenzo[*b*]thiophenes is outlined in Scheme 3.2. First the two enantiomers of 6-(N,N-di-*n*-propyl)aminomethyl-4,5,6,7-tetrahydrobenzo[*b*]thiophene (**36**) were separated. Since the affinity resides in the (+)-enantiomer, only the (+)-enantiomer was used for further reactions. The same reaction procedures were used as for the 2-substituted 5- and 6-(N,N-di-*n*-propyl)amino-4,5,6,7-tetrahydrobenzo[*b*]thiophenes.



Scheme 3.2 Reagents: (a) $POCl_3$, N-methylformanilide; (b) $NaBH_4$, EtOH.

3.3 Results and discussion

Tetrahydrobenzo[*b*]thiophenes **34** and **35** possess moderate affinity for the dopamine receptors. In order to potentially increase their affinity for the dopamine receptors, substituents were introduced on the 2-position of the tetrahydrobenzo[*b*]thiophenes. The extra interaction point and the fact that these substituents have better hydrogen bond forming capacities than a

sulfur atom may lead to compounds with a higher affinity for the dopamine receptors. For 6-(N,N-di-*n*-propyl)amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene (**34**) this has led to compounds (**65**, **66**, **67**) with moderate to high affinity for the dopamine D_3 receptor. However, the affinity for the dopamine D_2 receptor was dramatically decreased. Therefore, these compounds showed a high selectivity for the dopamine D_3 receptor. The introduction of 2-substituents in 5-(N,N-di-*n*-propyl)amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene (**35**) gave compounds with low to no affinity for the dopamine D_2 and D_3 receptors. Surprisingly, because the parent compounds **34** and **35** show a comparable affinity for the dopamine receptors. An explanation could be that compounds **65**, **66** and **67** are structurally comparable with the dopamine D_3-preferring agonist 7-OH-DPAT (**10**), while compounds **68** and **69** are structurally more comparable with the low affinity dopamine receptor ligand 6-OH-DPAT (**72**).

Table 3.1 Receptor binding data of various dopamine receptor ligands.

Compound	Ki (nM)[a]		Ratio D_{2L}/D_3
	D_{2L} [^{3}H]Spiperone	D_3 [^{3}H]Spiperone	
34	27[b]	28	1
65	>10000[c,d]	40	-
66	968[d]	9	108
67	>10000[c,d]	113	-
35	20[d]	40	0.5
68	100/11[e]	50/19[e]	-
69	100/-3[e]	50/6[e]	-
(±)-**36**	3107[d]	60	52
(+)-**36**	100/-7	50/43	
(–)-**36**	100/8[e]	50/17[e]	-
(+)-**70**	100/-9[e]	50/6[e]	-
(+)-**71**	100/18[e]	50/14[e]	-
7-OH-DPAT[223]	34[b]	0.57	60
PD128907[223]	42[b]	1.1	38
6-OH-DPAT[98,205]	1200[c,f]		

Footnotes: [a] K_i values are means of three separate experiments; the results of which did not vary more than 25%. [b] [^{3}H]N-0437 was used as radiolabeled ligand. [c] IC50 value instead of K_i. [d] [^{3}H]NPA. [e] First number gives the concentration of the tested compound in nM, second number gives the percentage of displacement of radioligand in %. [f] [^{3}H]dopamine, IC50 is determined in calf caudate nucleus homogenates.

The most potent and selective compound is derivative **66** with a hydroxymethyl moiety on the 2-position. Introduction of an extra interaction point on the 2-position of 6-(N,N-di-*n*-propyl)aminotetrahydrobenzo[*b*]thiophene has a negative effect on the affinity for the dopamine D_2 receptor and no or a positive effect on the affinity for the dopamine D_3 receptor.

Also the introduction of a methylene group between the six-membered ring and the nitrogen in 6-(N,N-di-*n*-propyl)amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene (**34**) gives a compound with a moderate affinity for the dopamine D_3 receptor and no affinity for the dopamine D_2 receptor. This affinity resides in the (+)-enantiomer of the compound. Introduction of substituents on the 2-position of 6-(N,N-di-*n*-propyl)aminomethyl-4,5,6,7-tetrahydrobenzo[*b*]thiophene (**36**) gives compounds (**70**, **71**) without affinity for the dopamine D_2 and D_3 receptors.

Literature data have shown that a phenol or catechol is not essential for binding at the dopamine receptors since a number of non-phenolic compounds possess affinity for the dopamine receptors. Examples of such compounds are pramipexole,[155] non-hydroxylated 2-aminotetralins,[241] conjugated enynes,[242] heterocyclic bioisosteres of 3-OH-N-phenylpiperazine,[243] and indolylcyclohexanes.[244] Recurrent phenomena in all these compounds are a conjugated system and a nitrogen which can be protonated to bind to the receptor.

The selectivity for the dopamine D_3 receptor is difficult to explain, since there is a high homology in the amino acid sequence in the transmembrane domains between the dopamine D_2 and D_3 receptors. Most receptor models developed deal with dopamine D_2-like receptors and make no difference between D_2 and D_3 receptors. Malmberg et al.[115] found that the putative interacting amino acids in the dopamine D_2 and D_3 receptors are not found on identical positions in the receptors.

Also the hypothesis of Hübner et al.[242] does not seem to be the most likely explanation, since there is only a marginal difference in amino acids between the dopamine D_2 and D_3 receptors. They hypothesised that the selectivity of their conjugated enynes could be explained from the fact that at, especially, the dopamine D_3 receptor the ability of the more lipophilic enyne system facilitates hydrophobic interactions.

Despite several reports on dopamine receptor ligands, until now no distinguishing models for the dopamine D_2 and D_3 receptors have been found. Therefore, more knowledge of pharmacophores, interacting points and receptor conformations are necessary to be able to develop distinguishing dopamine D_2 or D_3 receptor models, which can help to develop other selective agents.

In conclusion, in a series of tetrahydrobenzo[*b*]thiophene analogues, we have identified novel selective dopamine D_3 receptor agents, one of which displays a 100-fold selectivity over dopamine D_2 receptors.

3.4 Experimental Section

3.4.1 Chemistry

Melting points were determined in open glass capillaries on an Electrothermal digital melting-point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded at 200 MHz and 50.3 MHz, respectively, on a Varian Gemini 200 spectrometer. The splitting patterns are designated as follows: s (singlet), d (doublet), t (triplet), q (quartet) m, (multiplet). Chemical shifts are given in δ units (ppm) and are relative to the solvent. Coupling constants are given in Hertz (Hz). The spectra recorded were consistent with the proposed structures. IR spectra were obtained on a ATI-Mattson spectrometer. Electronic ionisation (EI) mass spectra were obtained on a Unicam 610-Automass 150 GC-MS system. Elemental analyses were performed by the Analytical Chemistry Section at Parke Davis (Ann Arbor, MI) or by the Microanalytical Department of the University of Groningen and were within ± 0.4 % of the theoretical values, except where noted.

All chemicals used were commercially available (Aldrich or Acros) and were used without further purification.

2-Formyl-6-(N,N-di-*n*-propyl)amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene (65). A mixture of phosphorous oxychloride (1.29 g, 8.4 mmol) and N-methylformanilide (1.14 g, 8.4 mmol) was stirred for 0.5 h at RT. Then 6-(N,N-di-*n*-propyl)amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene (**34**) (1.0 g, 4.2 mmol) was added and the mixture was stirred for 4 h at RT after which it was poured into water. The aqueous layer was extracted with dichloromethane and the combined organic layers were extracted with 4 N HCl. The acidic layer was basified and extracted with dichloromethane. The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The product was purified over a SiO_2 column using CH_2Cl_2: MeOH = 20:1 as the eluent yielding 1.1 g (99 %) of a yellow oil: ^{1}H NMR ($CDCl_3$) δ 0.9 (t, 6H, J = 7.3 Hz), 1.4 (q, 4H), 1.6-1.8 (m, 1H), 1.9-2.1 (m, 1H), 2.4-2.5 (m, 4H), 2.6-2.8 (m, 3H), 2.9-3.1 (m, 2H), 7.4 (s, 1H), 9.8 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 10.3, 20.7, 23.9, 24.1, 26.7, 51.1, 55.5, 135.3, 135.5, 139.4, 146.2, 181.2; IR (NaCl) cm^{-1} 1667 (C=O). A sample was converted to the HCl-salt for analysis, Anal Calcd (Obsd) for $C_{15}H_{23}NOS.HCl.1H_2O$: C: 56.32 (56.33), H: 8.19 (8.01), N: 4.38 (4.72).

2-Hydroxymethyl-6-(N,N-di-*n*-propyl)amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene (66). To a cooled solution of 2-formyl-6-(N,N-di-*n*-propyl)amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene (**65**) (200 mg, 0.75 mmol) in ethanol (5 mL) was added dropwise $NaBH_4$ (95 mg, 2.5 mmol) in ethanol (5 mL). After stirring for 30 minutes at RT the solvent was evaporated. The residue was dissolved in water and extracted with dichloromethane. The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting oil was dissolved in anhydrous ether and 1 N HCl in ether was added to yield 144 mg (63%): ^{1}H NMR ($CDCl_3$) δ 0.9 (t, 6H, J = 7.3 Hz), 1.5 (q, 4H, J = 7.3 Hz), 1.6-1.8 (m, 1H), 1.9-

2.1 (m, 1H), 2.4-2.5 (m, 4H), 2.6-2.9 (m, 4H), 3.0-3.1 (m, 1H), 4.7 (s, 2H), 6.6 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 10.4, 20.4, 24.1, 24.3, 25.6, 51.2, 56.3, 58.6, 124.3, 133.2, 139.7; Anal Calcd (Obsd) for $C_{15}H_{25}NOS.0.3\ H_2O$: C: 66.03 (65.80), H: 9.46 (9.31), N: 5.13 (5.15).

2-Carboxaldoxim-6-(N,N-di-*n*-propyl)amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene (67). To a solution of 2-formyl-6-(N,N-di-n-propyl)amino-4,5,6,7-tetrahydro-benzo[*b*]thiophene.HCl (**65**) (100 mg, 0.33 mmol) and hydroxylamine (80 mg, mol) in 5 mL ethanol was added 0.8 mL 5 N NaOH-solution. After stirring for 3 h at RT the solvent was evaporated, water was added and extracted with dichloromethane. The organic layer was dried over Na_2SO_4 and evaporated to yield 90 mg yellow oil (97 %). The compound consisted of a syn and anti-isomer which could not be separated. An analytical sample was converted to the HCl salt. ^{1}H NMR ($CDCl_3$) δ 0.9 (t, J = 7.2 Hz, 6H), 1.5 (q, J = 7.2 Hz, 4H), 1.6-1.8 (m, 1H), 2.0-2.2 (m, 1H), 2.4-2.6 (m, 4H), 2.6-3.2 (m, 5H), 6.8 (s, ½H), 7.0 (s, ½H), 7.5 (s, ½H), 8.1 (s, ½H); ^{13}C NMR ($CDCl_3$) δ 11.6, 21.4, 25.0, 25.4, 26.7, 52.5, 57.3, 128.6, 129.4, 131.3, 134.1, 135.0, 140.9, 144.5; IR (NaCl) cm^{-1} 1615 (C=N–OH); The ^{13}C-NMR showed that the ratio between the two isomers was 1:2. On TLC, with as eluent CH_2Cl_2:MeOH = 20:1, there was only a slight difference between the R_f-values. Anal Calcd (Obsd) for $C_{15}H_{24}N_2OS.HCl$: C: 56.85 (56.46), H: 7.95 (8.06), N: 8.84 (8.75).

2-Formyl-5-(N,N-di-*n*-propyl)amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene (68). This compound was synthesised in 53 % yield yellow oil according to the method used for compound **65**. ^{1}H NMR ($CDCl_3$) δ 0.9 (t, 6H, J = 7.3 Hz), 1.5 (q, 4H, J = 7.1 Hz), 1.6-1.8 (m, 1H), 2.1-2.2 (m, 1H), 2.4-2.6 (m, 4H), 2.6-2.7 (m, 1H), 2.7-3.0 (m, 2H), 3.0-3.2 (m, 2H), 7.3 (s, 1H), 9.8 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 10.3, 20, 24.1, 24.0, 24.5, 26.3, 51.1, 55.2, 136.0, 181.2; IR (NaCl) cm^{-1} 1669 (C=O); MS (EIPI) m/e 265 (M^+).

2-Hydroxymethyl-5-(N,N-di-*n*-propyl)amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene (69). This compound was synthesised according to the method used for compound **66**. Purification over a SiO_2 column using CH_2Cl_2:MeOH=9:1 yielded 146 mg yellow oil (87%). ^{1}H NMR ($CDCl_3$) δ 0.9 (t, 6H, J = 7.3 Hz), 1.5 (q, 4H, J = 7.5 Hz), 1.6-1.8 (m, 1H), 2.0-2.2 (m, 1H), 2.4-2.6 (m, 4H), 2.6-2.8 (m, 3H), 2.9-3.1 (m, 2H), 4.7 (s, 2H), 6.6 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 10.4, 20.3, 23.8, 24.6, 26.3, 51.2, 55.7, 58.6, 124.9; MS (EIPI) m/e 267 (M^+).

Resolution of 6-(N-di-n-propyl)aminomethyl-4,5,6,7-tetrahydrobenzo[*b*]thiophene (36).To a solution of 4 g (15.9 mmol) free amine **36** in 60 mL of isopropyl acetate was added dropwise a solution of 6.4 g (15.9 mmol) of (+)-di-p-toluoyl-D-tartaric acid in isopropyl acetate (60 mL). The precipitate was collected and three times recrystallised from ethanol/diethyl ether to give 3.8 g (36.5 %) of the diastereomeric salt (mp 173-174 °C); $[\alpha]_{589}^{21}$ = + 114.5°.

This salt was stirred with 1 N NaOH and 50 mL chloroform was added to the suspension. The layers were separated and the aqueous layer extracted with chloroform. The organic layers were washed with water, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting oil was converted to the HCl salt. The product was dried in vacuo to afford 1.4 g (30.6

%) of (+)-**12**.HCl as a white solid. An analytical sample was recrystallized from isopropanol-ether; mp 112-113 °C, $[\alpha]_{589}^{21}$ = + 62.4° (c 0.1, MeOH).

The filtrate of the salt formation and the filtrate of the first recrystallization were combined. After evaporation under vacuo and treatment of the residue with NaOH solution gave impure (–)-amine. This amine (2.0 g), 3.2 g (–)-di-p-toluoyl-L-tartaric acid and 400 mL of methanol was heated to solution. According to the procedure as described above, 1.3 g (28.4 %) of (–)-**13**.HCl was obtained with (mp 113-114 °C); $[\alpha]_{589}^{21}$ = - 62.5°.

(+)-2-Formyl-6-(N,N-di-*n*-propyl)aminomethyl-4,5,6,7-tetrahydrobenzo[*b*]thiophene (70). This compound was synthesised according to the method used for compound **65**. Purification over a SiO_2 column using CH_2Cl_2:MeOH=9:1 yielded 421 mg crude product. 156 mg was purified twice over a SiO_2 column with as eluent CH_2Cl_2:MeOH=20:1 yielding 32 mg (13 %) pure and 50 mg (21 %) not completely pure yellow oil. ^{1}H NMR ($CDCl_3$) δ 0.9 (t, 6H, J = 7.2 Hz), 1.3-1.6 (m, 5H), 1.9-2.1 (m, 2H), 2.2-2.5 (m, 7H), 2.6-2.8 (m, 1H), 2.8-2.9 (m, 1H), 2.9-3.1 (m, 1H), 7.3 (s, 1H), 9.8 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 10.4, 18.7, 23.2, 25.7, 29.3, 55.2, 58.3, 135.8, 181.3; IR (NaCl) cm^{-1} 1682 (C=O); MS (EIPI) m/e 279 (M^+).

(+)-2-Hydroxymethyl-6-(N,N-di-*n*-propyl)aminomethyl-4,5,6,7-tetrahydrobenzo[*b*]thiophene (71). This compound was synthesised according to the method used for compound **66** starting with 0.25 g crude 2-formyl-6-(N,N-di-*n*-propyl)aminomethyl-4,5,6,7-tetrahydrobenzo[*b*]thiophene (70). Purification over a SiO_2 column using CH_2Cl_2:MeOH=9:1 yielded 57 mg yellow oil. ^{1}H NMR ($CDCl_3$) δ 0.9 (t, 6H, J = 7.2 Hz), 1.4-1.7 (m, 5H), 1.9-2.1 (m, 3H), 2.3-2.7 (m, 6H), 2.7-3.0 (m, 3H), 4.7 (s, 2H), 6.6 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 10.2, 23.2, 26.2, 26.8, 28.6, 29.2, 54.8, 58.6, 64.3, 124.6; MS (EIPI) m/e 281 (M^+).

3.4.2 Pharmacology

For the compounds 6-(N,N-di-*n*-propyl)amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene (**34**), 2-formyl-6-(N,N-di-*n*-propyl)amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene (**65**), 2-hydroxymethyl-6-(N,N-di-*n*-propyl)amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene (**66**), 2-carboxaldoxim-6-(N,N-di-*n*-propyl)amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene (**67**), 5-(N,N-di-*n*-propyl)amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene (**35**) and (±)-6-(N,N-di-*n*-propyl)aminomethyl-4,5,6,7-tetrahydrobenzo[*b*]thiophene ((±)-**36**) the binding experiments were performed according to the method described in Chapter 2.

For the compounds 2-formyl-5-(N,N-di-*n*-propyl)amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene (**68**), 2-hydroxymethyl-5-(N,N-di-*n*-propyl)amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene (**69**), (+)-6-(N,N-di-*n*-propyl)aminomethyl-4,5,6,7-tetrahydro-benzo[*b*]thiophene ((+)-**36**), (–)-6-(N,N-di-*n*-propyl)aminomethyl-4,5,6,7-tetrahydrobenzo[*b*]thiophene ((–)-**36**), (+)-2-formyl-6-(N,N-di-*n*-propyl)aminomethyl-4,5,6,7-tetrahydrobenzo[*b*]thiophene (**70**) and

(+)-2-hydroxymethyl-6-(N,N-di-*n*-propyl)aminomethyl-4,5,6,7-tetrahydrobenzo[*b*]thiophene (**71**) the binding experiments were performed by Lundbeck, Denmark.

[^{3}H]Spiperone (D_2 binding) By this method the inhibition of drugs of the binding of [^{3}H]Spiperone (0.5 nM, K_d 0.20 nM) to dopamine D_2 receptors in membranes from rat corpus striatum is determined in vitro. Method and results are described by Hyttel and Larsen.[245]

[^{3}H]Spiperone (D_3 binding) By this method the inhibition by drugs of the binding of [^{3}H]Spiperone (0.3 nM, K_d 0.45 nM) to membranes of human cloned dopamine D_3 receptors expressed in CHO-cells is determined in vitro. Method modified from R.G. MacKenzie et al.[50]

CHO -cells expressing the human cloned D_3 dopamine receptor are harvested and the cell suspension centrifuged at 1000 rpm for 7 min at 4°C. The supernatant is frozen. At the day of experiment the cell pellet is thawed at room temperature and diluted in assay buffer (25 Mm TRIS-HCl pH 7.4 + 6.0 mM $MgCl_2$ + 1.0 mM EDTA) to the desired concentration.

50 µl Displacer (10 µM Haloperidol, test compound or assay buffer) and 230 µl buffer is added to a 96 well deep plate. Then 50 µl 0.3 nM [^{3}H]spiperone is added. The reaction is initiated by addition of 670 µL membrane suspension (test concentration 26 µg protein/670 µl). Packard GF/C unifilter (96 well) is pretreated with 0.1 % PEI-solution 10-15 min before filtration.

After 60 min. of incubation at 25°C the reaction is terminated by filtration at Tomtec unifilter. The filters are washed twice with ice cold assay buffer. The filters are dried for 1.5 hours at 50°C, 35 µl scintillation liquid is added and bound radioactivity is counted in Wallac Tri-Lux scintillation counters.

Chapter 4

Thiophene analogues of naphthoxazines and 2-aminotetralins: bioisosteres with improved relative oral bioavailability, as compared to 5-OH-DPAT[*]

Abstract

In the present study, a series of thiophene analogues of 2-aminotetralins and hexahydronaphthoxazines were studied *in vivo* for their ability to decrease striatal dopamine release, their effects on locomotor activity, and their behavioural characteristics in reserpinised rats, in order to investigate whether a thiophene moiety can act as a bioisostere for the phenol moiety.

In general, the new compounds showed lower *in vivo* activities than 5-hydroxy-2-(N,N,-di-*n*-propylamino)tetralin (5-OH-DPAT). However, the introduction of the thiophene moiety gave a significant improvement of the relative oral bioavailability, as compared to 5-OH-DPAT.

Our results suggest that the thiophene moiety can act as a bioisostere for a phenol group in hydroxylated 2-aminotetralins. For the hexahydrothianaphthoxazines it was not possible to discriminate between bioisosterism for a phenyl or a phenol moiety. The tetrahydrobenzo[*b*]thiophenes could be used as lead compounds for the development of novel dopamine receptor ligands with improved relative oral bioavailability.

[*] This chapter is based on: Rodenhuis, N.; Timmerman, W.; Wikström, H.V: Dijkstra, D. (2000) Thiophene analogs of naphthoxazines and 2-aminotetralins: bioisosteres with improved relative oral bioavailability, as compared to 5-OH-DPAT. *Eur. J. Pharmacol*. **394**, 255-263.

4.1 Introduction

The pharmacological importance of the 2-aminotetralin structure has been known for a long time.[202] Initially, aminotetralins were characterised by their sympathomimetic action, i.e. the induction of mydriasis, contraction of the uterus, changes in blood pressure and respiration, and increased intestinal motility in *in vivo* experiments.[202-204] During the late sixties central dopamine receptor activity of 2-aminotetralins was identified, which led to active synthesis programs around the world.

The 2-aminotetralin structure has proven to be a valuable structural base, not only for the development of dopamine receptor ligands, but also for the development of serotonin receptor and adrenoceptor ligands, as well as compounds that interact with melatonin receptors.[208,209] The position of the aromatic hydroxyl group appeared to determine the kind of activity of the 2-aminotetralins, namely, 5- and 7-hydroxy-2-(N,N-di-*n*-propylamino)tetralin (5- and 7-OH-DPAT) are potent dopamine receptor ligands while 8-hydroxy-2-(N,N-di-*n*-propylamino)tetralin (8-OH-DPAT) is a very potent and selective serotonin receptor ligand.

In a number of different *in vitro* and *in vivo* models it has been shown that 5-OH-DPAT (**9**) is a very potent dopamine receptor agonist,[98,205] which has affinity for both the dopamine D_2 and the dopamine D_3 receptors.[223] Another potent dopamine D_2/D_3 receptor agonist is 5-hydroxy-2-(N-*n*-propyl-N-2-(2-thienyl)ethylamino)tetralin (N-0437, **31**), which has reached the clinical stage as an anti-Parkinson agent. However, its use is limited to subcutaneous and intravenous administration because of its low oral bioavailability.[246] This accounts for all the hydroxylated 2-aminotetralins, since they undergo considerable inactivation by glucuronidation in the gut and the liver.[161] Therefore, for many years, the identification of bioisosteric catechol and phenol replacements has been emphasised. Neither the catecholic nor the phenolic hydroxyl groups appear to be an absolute requirement for potent dopamine receptor activity, as illustrated by the action of pramipexole (**19**), a benzothiazole analogue of the 2-aminotetralins, which is presently on the market as a therapeutic agent for Parkinson's disease.[146,154,155] Also, Andén and co-workers showed that the aminothiazolazepine derivative 5,6,7,8-tetrahydro-6-(2-propenyl)-4*H*-thiazolo[4,5-*d*]azepin-2-amine (BHT920, **32**) is a dopamine receptor agonist, with α-adrenoceptor properties.[183]

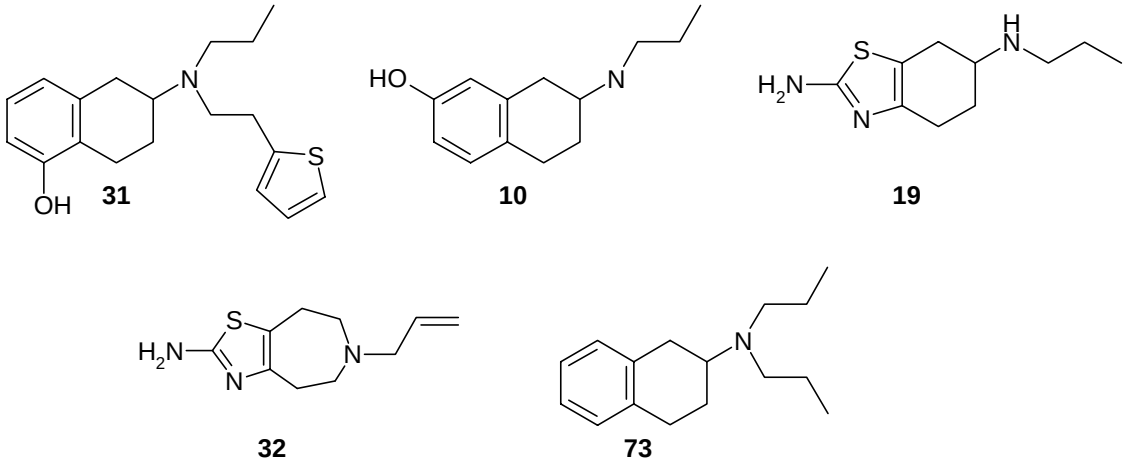


Chart 4.1 Chemical structures of 5-hydroxy-2-(N-*n*-propyl-N-2-thienyl)ethylamino)tetralin (N-0437, **31**), 7-hydroxy-(N,N-di-*n*-propylamino)tetralin (7-OH-DPAT, **10**), pramipexole (**19**), 5,6,7,8-tetrahydro-6-(2-propenyl)-4*H*-thiazolo[4,5-*d*]azepin-2-amine (BHT920, **32**), 2-(N,N-di-*n*-propylamino)tetralin (DPAT, **73**).

In an attempt to circumvent the problem of intensive first-pass metabolism, 6- and 5-(N,N-di-*n*-propylamino)tetrahydrobenzo[*b*]thiophenes (**34**, **35**) were synthesised. These two compounds possess moderate to high affinity for both the dopamine D_2 and D_3 receptor (Table 4.1). Tricyclic compounds like *trans*-9-hydroxy-4-(*n*-propyl)-2,3,4a,5,6,10b-hexahydro-4*H*-naphth[1,2*b*][1,4]oxazine (PHNO, **27a**) and hydroxylated octahydrobenzo[*f*]quinolines (**77** and **78**) also possess high affinity for the dopamine D_2 and D_3 receptor, but they display the same problem as hydroxylated 2-aminotetralins, they undergo considerable glucuronidation in the liver due to the phenol moiety.[161] Since these tricyclic compounds could be of interest, *trans*-2,3,4a,5,6,10b-hexahydro-4*H*-thianaphth[4,5*e*][1,4]oxazine (**38**) and *trans*-N-*n*-propyl-2,3,4a,5,6,10b-hexahydro-4*H*-thianaphth[4,5*e*][1,4]oxazine (**39**) were synthesised. Compounds **38** and **39** possessed negligible and low affinity, respectively for the dopamine D_2 and D_3 receptor (Table 4.1).

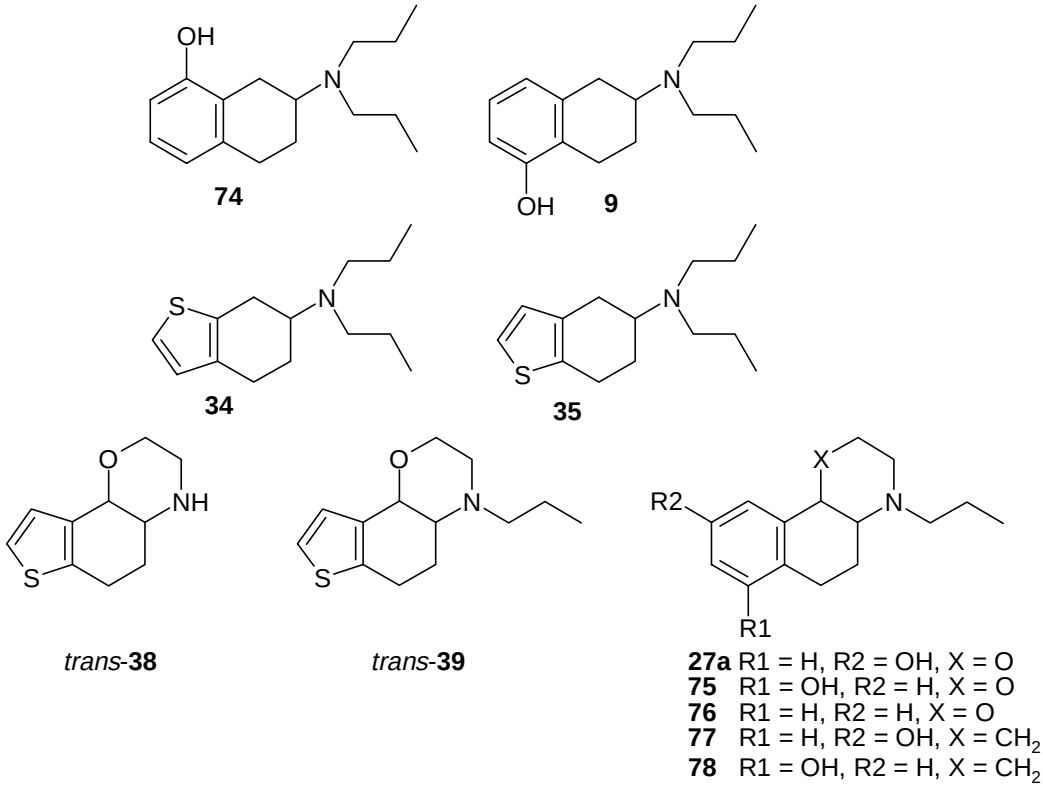


Chart 4.2 Chemical structures of 8-hydroxy-2-(N,N-di-*n*-propylamino)tetralin (8-OH-DPAT, **74**), 5-hydroxy-2-(N,N-di-*n*-propylamino)tetralin (5-OH-DPAT, **9**), 6-(N,N-di-*n*-propylamino)tetrahydrobenzo[*b*]thiophene (**34**), 5-(N,N-di-*n*-propylamino)tetrahydrobenzo[*b*]thiophene (**35**), *trans*-2,3,4a,5,6,10b-hexahydro-4*H*-thianaphth[4,5*e*][1,4]oxazine (**38**), *trans*-N-*n*-propyl-2,3,4a,5,6,10b-hexahydro-4*H*-thianaphth[4,5*e*][1,4]oxazine (**39**), *trans*-9-hydroxy-4-(*n*-propyl)-2,3,4a,5,6,10b-hexahydro-4*H*-naphth[1,2*b*][1,4]oxazine (**27a**), *trans*-7-hydroxy-4-*n*-propyl-2,3,4a,5,6,10b-hexahydro-4*H*-naphth[1,2*b*][1,4]oxazine (**75**), *trans*-4-*n*-propyl-2,3,4a,5,6,10b-hexahydro-4*H*-naphth[1,2*b*][1,4]oxazine (**76**), *trans*-9-hydroxy-4-*n*-propyl-1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]quinoline (**77**), *trans*-7-hydroxy-4-*n*-propyl-1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]quinoline (**78**).

Table 4.1. Binding affinities of some dopamine receptor compounds.

Compound	Reference	K_i (nM)		
		D_{2L} Agonist	D_3 [^{3}H]spiperone	5-HT_{1A} [^{3}H]8-OH-DPAT
N-0437 (**31**)	223	0.06[a]	4.0	–
7-OH-DPAT (**10**)	247	34[a]	1.4	–
pramipexole (**19**)	155	2.07[d]	0.49[d]	–
5-OH-DPAT (**9**)	248	6[a]	0.66	–
8-OH-DPAT (**74**)	249	3200[b]	250	0.9
R-DPAT (R-**73**)	241	32[c]	33	12
S-DPAT (S-**73**)	241	5.5[c]	35	38
34	239	27[b]	28	80
35	239	40[b]	20	–
38	239	>4780[b]	3003	–
39	239	631[b]	237	–
27a	206	*2.8*[e]		
75	206	*80*[e]		
76	206	*110*[e]		

Footnotes: [a] [^{3}H]N-0437; [b] [^{3}H]N-*n*-propylnorapomorphine; [c] [^{3}H]quinpirole; [d] [^{3}H]spiperone, high affinity binding; [e] IC_{50} (nM) in rat striatal membrane homogenates using [^{3}H]DP-5,6-ADTN.

To determine whether a thiophene moiety can act as a bioisostere for a phenol moiety compounds **34, 35, 38, 39** were tested for their effects on dopamine release using the microdialysis technique in freely moving rats. The effects of compounds **34** and **35** were compared with the effects of the prototypic dopamine receptor agonist 5-OH-DPAT. On the basis of structural similarities it was thought that compound **34** could be related to 8-OH-DPAT and compound **35** to 5-OH-DPAT. However, compound **34** also showed affinities for the dopamine D_2 and D_3 receptors and therefore this compound was also studied for its effects on dopamine release. Since all hydroxylated 2-aminotetralins possess a free phenolic hydroxyl group they are prone to conjugation reactions.[161] However, there is a difference to what extent the compound is glucuronidated depending on the position of the hydroxyl moiety. In this study the compounds are compared with 5-OH-DPAT which is the least glucuronidated of the isomeric dopaminergic monophenolic 2-aminotetralins.[161] The relative oral bioavailabilities of compounds **34** and **35** were determined. No such estimation was made for compounds **38** and **39** since these compounds displayed limited affinity for the dopamine D_2 and D_3 receptors. In addition, compounds **34** and **35** tested for their locomotor activity and dopamine and serotonin receptor behavioural characteristics in reserpinised rats.

4.2 Materials and methods

4.2.1 Animals

Male Wistar rats (from CDL, Groningen, The Netherlands) weighing 280-320 g were used for microdialysis experiments and rats weighing 180-220 g for the locomotor activity and behavioural characteristics experiments. The rats were housed in Plexiglas cages, eight animals in each cage, with free access to water and food. The cages were placed in a room with controlled environmental conditions (21 °C; humidity 60-65%; lights on at 8 a.m. and off at 8 p.m.). The animals were housed at least one week after arrival prior to surgery and use in the experiments. Animal procedures were conducted in accordance with guidelines published in the NIH Guide for the Care and Use of Laboratory Animals and all protocols were approved by the Groningen University Institutional Animal Care and Use Committee.

4.2.2 Drug treatment

The drugs were dissolved in saline and stored in a concentration of 100 µmol/ml for subcutaneous (s.c.) and 50 µmol/ml for per oral (p.o.) administration and diluted, if necessary, with saline before administration. A volume of 1 ml/kg was administered for s.c. administration and 2 ml/kg for p.o. administration. The drugs that were used were 6-(N,N-di-*n*-propylamino)tetrahydrobenzo[*b*]thiophene (**34**), 5-(N,N-di-*n*-propylamino)tetrahydrobenzo[*b*]thiophene (**35**), 5-hydroxy-2-(N,N-di-*n*-propylamino)tetralin (5-OH-DPAT, **9**), *trans*-2,3,4a,5,6,10b-hexahydro-4*H*-thianaphth[4,5*e*][1,4]oxazine (**38**) and *trans*-N-*n*-propyl-2,3,4a,5,6,10b-hexahydro-4*H*-thianaphth[4,5*e*][1,4]oxazine (**39**). All five drugs were synthesised at the Department of Medicinal Chemistry in Groningen.

4.2.3 Surgery and brain microdialysis

On-line brain microdialysis in freely moving animals has previously been described.[188] In brief, the rats were anaesthetised with midazolam (5 mg/kg s.c.), atropine nitrate (0.1 mg/kg s.c.), ketamine (50 mg/kg i.p.) and xylazine (8 mg/kg i.p.); 10% lidocaine was locally applied. The rats were then mounted into a stereotaxic frame (Kopf). The incisor bar was placed in position so that the skull was held horizontal. The skull was exposed and burr holes were drilled. A Y-shaped dialysis probe was used for the experiments, with an exposed tip length of 3 mm. The dialysis tube (ID: 0.22 mm; OD: 0.31 mm) was prepared from polyacrylonitrile/sodium methallyl sulfonate copolymer (AN 69, Hospal, Bologna, Italy). The microdialysis membrane was implanted in the striatum. The dura was removed with a sharp needle. Two anchor screws were positioned in different bone plates nearby. The following co-ordinates were used according to the atlas of Paxinos and Watson:[250] AP + 1.0, LM ± 3.0

relative to bregma, and VD − 6.0 below dura. Before insertion into the brain the dialysis probe was perfused successively with ultra pure water, methanol, ultra pure water and Ringer solution (1.2 mM Ca^{2+}). The dialysis probe was positioned in the burr hole under stereotaxic guidance. The probe was cemented in this position with dental cement. After the surgery, the rats received buprenorphine (0.1 mg/kg i.m.), an analgesic agent. The rats were housed solitary.

The experiments were performed in conscious rats 17-48 h after implantation of the cannula. The striatum was perfused with a Ringer solution (147 mmol/l NaCl, 4 mmol/l KCl, 1.2 mmol/l $CaCl_2$, 1.1 mmol/l $MgCl_2$) at 2 µl/min (CMA/102 microdialysis pump, Sweden).

Dopamine was quantitated by high-performance liquid chromatography (HPLC) with electrochemical detection with a detection limit of approximately 5 fmol/sample. An HPLC pump (LKB, Pharmacia) was used in conjunction with an electrochemical detector (Antec, Leiden) working at 625 mV versus an Ag/AgCl reference electrode. The analytical column was a Supelco Supelcosil LC-18 Column (3 µm particle size). The mobile phase consisted of a mixture of 4.1 g/l sodium acetate (Merck), 85 mg/l octane sulphonic acid (Aldrich), 50 mg/l EDTA (Merck), 1 mM tetramethylammonium chloride (ACROS), 8.5 % methanol (Labscan) and ultra pure water (pH=4.1 with glacial acetic acid).

After the experiments the rats were sacrificed and the brains were removed. After removal the brains were kept in 4% paraformaldehyde solution until they were sectioned to control the location of the dialysis probes.

4.2.4 Locomotor activity as monitored in automated cages and behavioural characteristics

Reserpine (10 mg/kg s.c.) was administered 18 h prior to the start of the experiments. On the day of the experiments the animals were placed alone in Plexiglas boxes during a period of 15 min for habituation. Subsequently, the test compounds were administered subcutaneously. The locomotor activity was registered during a period of 120 min using AUTOMEX II activity monitors (Columbus Instruments, Columbus, OH, USA).

During a period of 60 min the behaviour of the rats was scored manually every 5 minutes. The behaviour scored was repeated sniffing, repeated licking and rearing as dopamine receptor stereotyped behaviour and flat body posture and lower lip retraction as indications of the 5-hydroxytryptamine (5-HT, serotonin) behavioural syndrome. The behaviour was scored when it lasted for more than half the observation period. The effects of the compounds were compared to a saline-treated control group.

4.2.5 Statistics

Data of the microdialysis experiments were converted into percentage of the basal levels. The basal levels were determined from four consecutive samples (less than 20% variation), and set at 100%. During a period of 165 min after administration of the test compound the dopamine

release was measured. Microdialysis data were analysed using one-way Analysis of Variance (ANOVA) for repeated measurements, followed by Dunnett's Method post-hoc test. The relative oral bioavailabilities were determined by comparing the Area Under the Curves (AUCs) after p.o. and s.c. administration. When the AUCs were not significantly different, the relative oral bioavailability, as expressed in percent, was determined by dividing the s.c. dose by the p.o. dose and multiplying by 100. Statistical analysis of the AUCs was performed by a t-test. The data of the locomotor activity experiments were analysed using Two Way Repeated Measures ANOVA on One Factor Balanced Design, followed by Student-Newman-Keuls Method post-hoc test. In all cases a significance level of 0.05 was applied.

4.3 Results

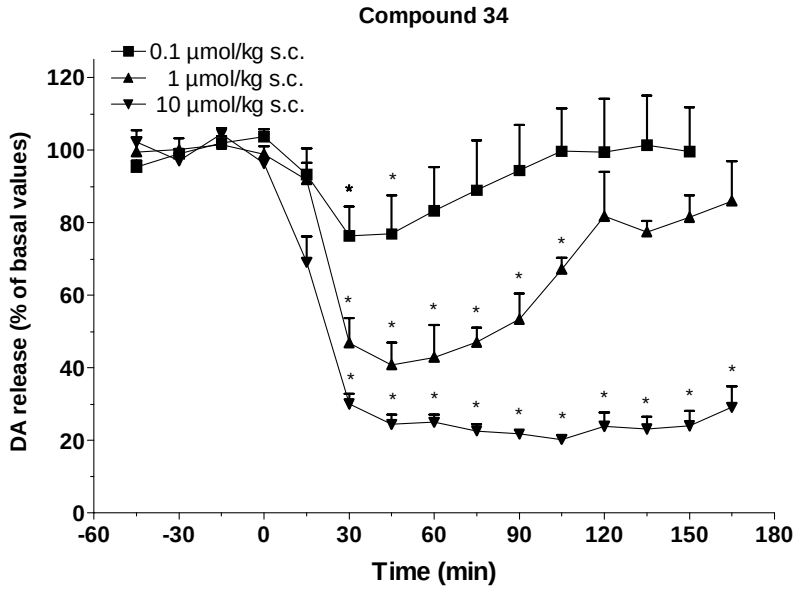
4.3.1 *In vivo* microdialysis

The basal dialysate concentrations in the striatum for the experiments were 11.1 ± 0.96 fmol/min (n = 62).

The results of the microdialysis experiments of the compounds **34**, **35**, 5-OH-DPAT, **38** and **39** are shown in Figure 4.1-4.4. S.c. administration of all compounds, except compound **38**, induced a dose-dependent and significant decrease in the release of dopamine in the striatum. Furthermore, compounds **34**, **35** and 5-OH-DPAT also induced a significant decrease in the release of dopamine in the striatum after p.o. administration. Effects of compounds **38** and **39** were not studied upon p.o. administration.

The significant decrease in dopamine release induced by s.c. administration of a dose of 0.1 µmol/kg of compound **34** lasted from t = 30 min to t = 45 min with a maximum decrease of 20 % of control values. For a dose of 1 µmol/kg this was from t = 30 min to t = 105 min with a maximum decrease of 60 % of control values and for a dose of 10 µmol/kg the significant decrease lasted from t = 30 min to t = 165 min with a maximum of 75 % of control values (Figure 4.1A). Figure 4.1B shows that the significant decrease after p.o. administration of compound **34** in a dose of 1 µmol/kg lasted from t = 15 min to t = 60 min with a maximum decrease of 25 % and in a dose of 10 µmol/kg from t = 30 min to t = 135 min with a maximum decrease of 40 % of control values.

A



B

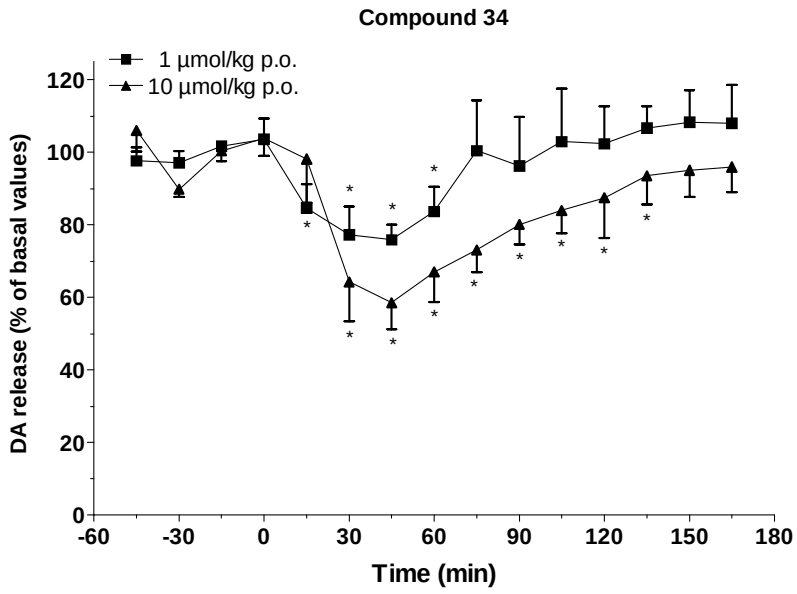
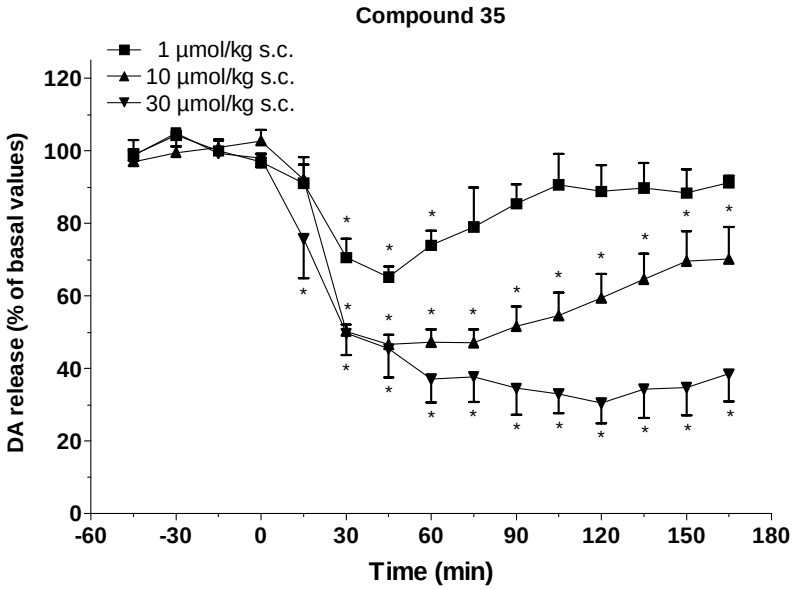


Figure 4.1 Effect of s.c. (A) and p.o. (B) administration of 6-(N,N-di-*n*-propylamino)tetrahydro-benzo[*b*]thiophene (**34**) on striatal dopamine release in freely moving rats. Data are presented as mean ± S.E.M. (n = 4). * P<0.05 (Dunnett's test).

Compound **35**, upon s.c. administration, induced a significant decrease in dopamine release of maximally 35 %, 55 %, and 65 % after doses of 1, 10 and 30 µmol/kg, respectively (Figure 4.2A). The decrease induced by a dose of 1 µmol/kg lasted only 30 min, while the doses of 10 and 30 µmol/kg both induced decreases in dopamine release that lasted until 165 min after administration. The significant effect of administration of a dose of 10 µmol/kg p.o. and a dose

of 30 µmol/kg p.o. of compound **35** lasted from t = 30 min to t = 90 min for both doses with a maximum decrease of 50 % and 60 % of control values, respectively (Figure 4.2B).

A



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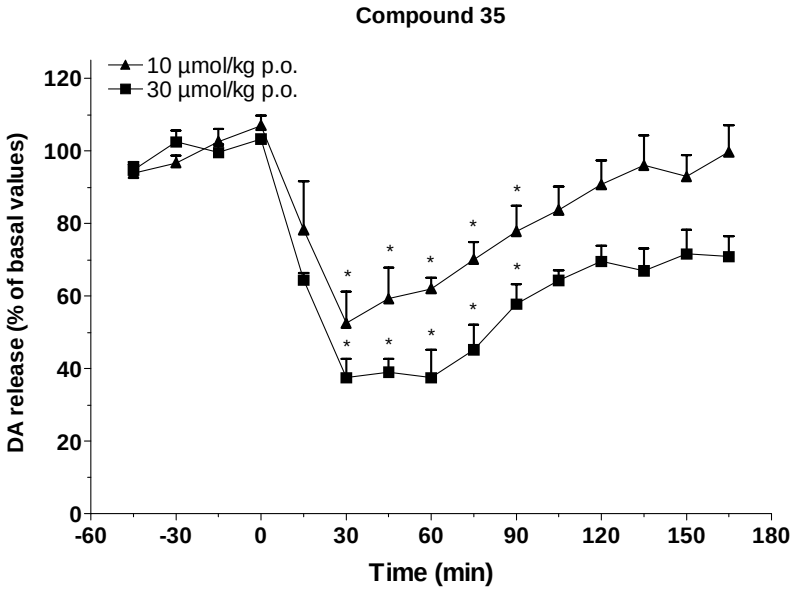


Figure 4.2 Effect of s.c. (A) and p.o. (B) administration of 5-(N,N-di-*n*-propylamino)tetrahydrobenzo[*b*]-thiophene (**35**) on striatal dopamine release in freely moving rats. Data are presented as mean ± S.E.M. (n = 4). * P<0.05 (Dunnett's test).

For comparison, administration of a s.c. dose of 0.1 µmol/kg and a p.o. dose of 10 µmol/kg of 5-OH-DPAT (**9**) induced very similar effects. Both treatments induced a significant decrease in dopamine release from 15 to 165 min with a maximum decrease in dopamine release of 70 %

and 75 % of control values for a s.c. dose of 0.1 µmol/kg and a p.o. dose of 10 µmol/kg, respectively (Figure 4.3).

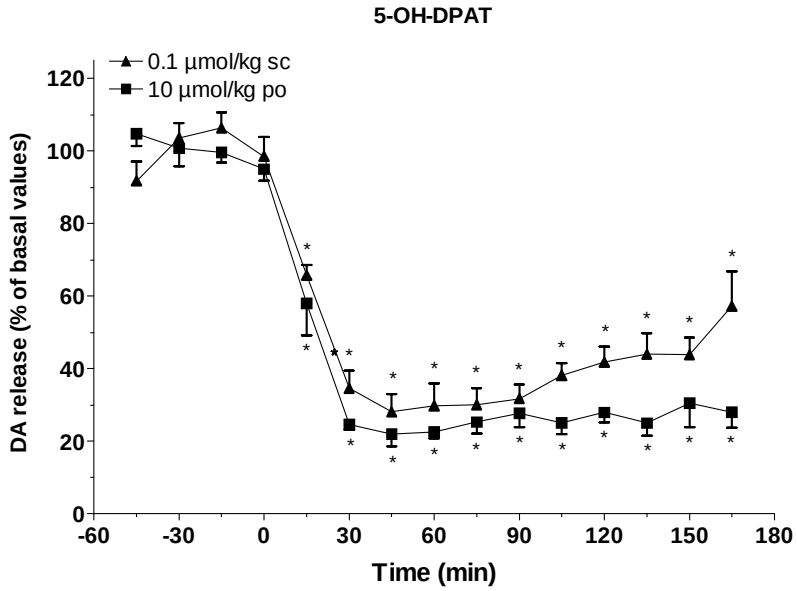


Figure 4.3 Effect of s.c. and p.o. administration of 5-OH-DPAT on striatal dopamine release in freely moving rats. Data are presented as mean ± S.E.M. (n = 4). * P<0.05 (Dunnett's test).

Figure 4.4 shows that *trans*-2,3,4a,5,6,10b-hexahydro-4*H*-thianaphth[4,5*e*][1,4]oxazine (**38**) had no significant effect on the release of dopamine in the striatum in a dose of 100 µmol/kg s.c.

Compound **39** in a s.c. dose of 1 µmol/kg had no effect on the release of dopamine, while s.c. administration of 10 µmol/kg induced a significant decrease in the release of dopamine in the striatum from t = 30 min to t = 60 min with a maximum decrease of 40 %. The significant effect of s.c. administration of 50 µmol/kg lasted from t = 15 min to t = 150 min with a maximum of 65 % (Figure 4.4).

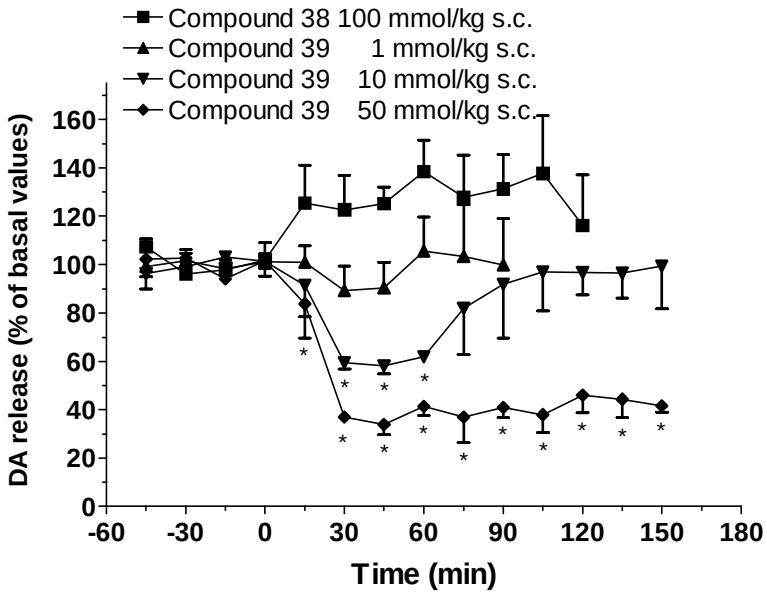


Figure 4.4 Effect of s.c. administration of *trans*-2,3,4a,5,6,10b-hexahydro-4*H*-thianaphth[4,5*e*][1,4]-oxazine (**38**) and *trans*-N-*n*-propyl-2,3,4a,5,6,10b-hexahydro-4*H*-thianaphth[4,5*e*][1,4]oxazine (**39**) on striatal dopamine release in freely moving rats. Data are presented as mean ± S.E.M. (n = 4). * P<0.05 (Dunnett's test).

The relative oral bioavailabilities, as determined by comparing the AUC after s.c. and p.o. administration, of 6-(N,N-di-*n*-propylamino)tetrahydrobenzo[*b*]thiophene (**34**), 5-(N,N-di-*n*-propylamino)tetrahydrobenzo[*b*]thiophene (**35**) and 5-OH-DPAT (**9**) were calculated from Figures 4.1-4.3, and are shown in Table 4.2. For compounds **34** and **35** the relative oral bioavailabilities were ≥ 10 %, while for the reference compound 5-OH-DPAT it was 1 %. In order to verify the fact that the decrease induced by a dose of 10 µmol/kg p.o. was not already induced by a lower dose, we have found that a dose of 1 µmol/kg p.o. of 5-OH-DPAT induced a decrease in the release of dopamine in the striatum of only 50-55 %. Furthermore, microdialysis experiments in our laboratory with the (–)-enantiomer of 5-OH-DPAT also showed that the relative oral bioavailability was about 1-3 % (Chapter 7).

Table 4.2 AUCs of the microdialysis experiments of 6-(N,N-di-*n*-propylamino)tetrahydrobenzo[*b*]thiophene (**34**), 5-(N,N-di-*n*-propylamino)tetrahydrobenzo[*b*]thiophene (**35**) and 5-OH-DPAT (**9**) after s.c. and p.o. administration.

	Subcutaneous administration		Oral administration		Relative oral bioavailability (%)
Compound	Dose (µmol/kg)	AUC	Dose (µmol/kg)	AUC	
34	0.1	2650 ± 1000[a]	1	2730 ± 390[a]	10
	1	6000 ± 500	10	3700 ± 950	10
	10	12446 ± 335			
35	1	3150 ± 400	10	4100 ± 750	10
	10	6700 ± 800	10	4100 ± 750	100
	10	6700 ± 800	30	7000 ± 450	30
	30	9400 ± 960	30	7000 ± 450	100
5-OH-DPAT (**9**)	0.1	9700 ± 500	10	11500 ± 300	1

Footnotes: [a] Experiment lasted 150 min. All other experiments lasted 165 min. All the AUCs of s.c. and p.o. doses of each compound were compared, but only the doses that were not significantly different were put in line in the table.

4.3.2 Locomotor activity in reserpinised rats

Both compounds **34** and **35** induced a significant increase in locomotor activity in reserpinised rats (Figure 4.5). The basal level of locomotor activity of reserpinised rats is maximally 48.5 ± 18.8 counts per 15 min (n = 4) as measured upon s.c. administration of saline. When comparing their maximum effect on locomotor activity as measured by the number of counts over 15 min, compound **34**, in a dose of 10 µmol/kg, induced an increase in locomotor activity to 600 counts/15 min, which returned to basal levels after 60 min. In a dose of 30 µmol/kg the maximum effect was 750 counts/15 min, which returned to basal levels after 90 min. The effects of compound **35** in the same doses were less pronounced than those for compound **34**. In a dose of 10 µmol/kg compound **35** induced an increase in locomotor activity to maximally 300 counts/15 min and in a dose of 30 µmol/kg the maximum effect was 650 counts/15 min The latter returned to basal levels after 105 min.

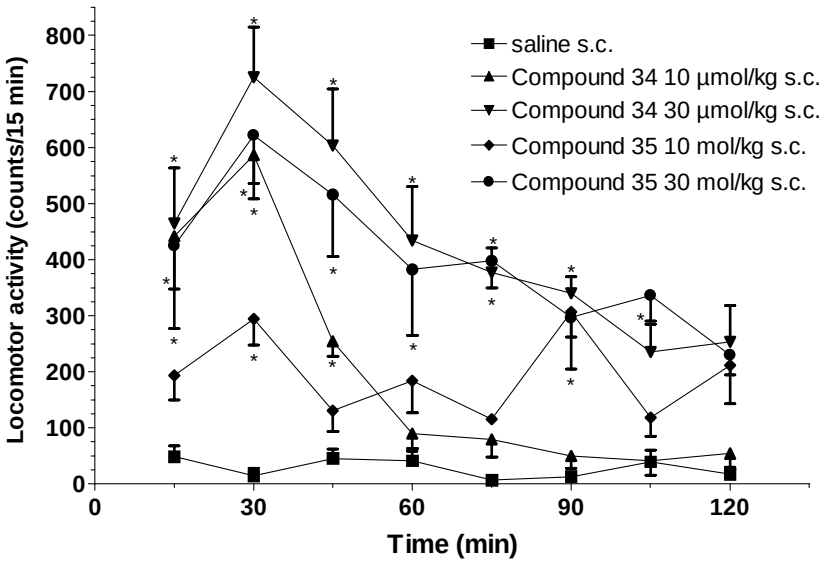


Figure 4.5 The effect of 6-(N,N-di-*n*-propylamino)tetrahydrobenzo[*b*]thiophene (**34**) and 5-(N,N-di-*n*-propylamino)tetrahydrobenzo[*b*]thiophene (**35**) on the locomotor activity of reserpinised rats. Data are presented as mean ± S.E.M. (n = 4). * P<0.05 (Student-Newman-Keuls test).

4.3.3 Behaviour in reserpinised rats

Table 4.3 shows that s.c. administration of compound **34** induced both dopamine receptor stereotyped behaviour (sniffing, licking and rearing) and a 5-HT behavioural syndrome (flat body posture and lower lip retraction) in the reserpinised rats. Administration of compound **35** induced dopamine receptor but no serotonin receptor behaviour.

Table 4.3 Behaviour after s.c. administration of the tetrahydrobenzo[*b*]thiophenes **34** and **35** represented as the number of animals which showed the behaviour of the total number of animals used in the experiment.

Compound	Dose µmol/kg s.c.	Sniffing	Licking	Rearing	Flat body posture	Lower lip retraction
34	10	4/4	0/4	0/4	2/4	2/4
34	30	4/4	2/4	3/4	4/4	4/4
35	10	4/4	4/4	0/4	0/4	0/4
35	30	4/4	4/4	4/4	0/4	0/4

4.4 Discussion

In the present study we investigated the effects of the bioisosteric replacement of a phenol moiety by a thiophene moiety. The effects of the compounds on dopamine release were determined using the microdialysis technique in freely moving rats. Systemic administration of compounds **34**, **35** and **39** but not **38** induced a decrease in the release of dopamine in the striatum, which results from the dopamine receptor agonistic properties of the compounds, as the release of dopamine is under the control of dopamine autoreceptors.[189] Compound **39** was less effective compared to compounds **34** and **35** in decreasing dopamine release in the striatum and compound **38** was without effect, which is in line with the differences of the four compounds in binding affinities found for the dopamine D_2 and D_3 receptor. All compounds were less potent than 5-OH-DPAT, again in agreement with the higher affinity at the dopamine D_2 and D_3 receptors of the latter compound. The role of the dopamine D_3 receptor as an autoreceptor is still under debate,[61,64] while it is generally accepted that the dopamine D_2 receptor functions as an autoreceptor.[63]

The affinity of compounds **34** and **35** for the dopamine D_2 and D_3 receptors is lower than that of 5-OH-DPAT, probably due to the fact that the sulfur atom in the thiophene ring is only a weak hydrogen bond acceptor unlike the hydroxyl moiety of a phenol, which is a strong hydrogen bond acceptor and donor. The fact that compound **39** was more effective compared to compound **38** is most likely due to a better fit into the receptor of the *n*-propyl substituent of compound **39** than the hydrogen of compound **38**.

For a compound to display dopamine receptor activity the distance between the nitrogen atom and the H-bond forming group is of importance. Previous studies indicated that the distance between the nitrogen and the hydroxyl moiety in dopamine receptor agents should be between 5.5 and 7.4 Å.[91,108] For 5-OH-DPAT and 8-OH-DPAT the distance between the nitrogen and the hydroxyl moiety in a minimised conformation using the computer program MacroModel is 6.6 and 5.2 Å, respectively (Chapter 2). For 5-OH-DPAT this has been formerly published by Malmberg et al.[115] This difference in distances might explain the difference in dopamine receptor activity of the two compounds, i.e. 5-OH-DPAT fits into the dopamine receptor, while the distance in 8-OH-DPAT seems to be too small. The distances between the sulfur and the nitrogen atom of compounds **34** and **35** in a minimised conformation are 5.4 and 6.0 Å, respectively (Chapter 2). This might explain why compound **34** displays dopamine receptor activity beside its serotonin receptor activity different than 8-OH-DPAT.

The relative oral bioavailability of compounds **34**, **35** and 5-OH-DPAT was determined by comparing the effects on the dopamine output after s.c. and p.o. administration, i.e. applying a pharmacodynamic method. Compounds **34** and **35** showed relative oral bioavailabilities of about 10 % and >10 %, respectively. The reference compound 5-OH-DPAT had a relative oral bioavailability of about 1 % (Table 4.2). Thus, the structural changes did influence the oral bioavailability in a positive manner. For hydroxylated 2-aminotetralins glucuronidation is the

main route of metabolism.[161] The thiophene ring is not a target for glucuronidation, which most likely explains the higher relative oral bioavailability of compounds **34** and **35**, as compared to 5-OH-DPAT.

The effects of compounds **34** and **35** on postsynaptic dopamine receptors were determined using a locomotor activity measure and looking at the behavioural characteristics after administration of the drugs. Compounds **34** and **35** induced a significant increase in locomotor activity in reserpinised rats, which again confirms that these compounds are dopamine receptor agonists. The behavioural scoring (Table 4.3) showed that compound **34** induced dopamine receptor stereotyped behaviour (sniffing, licking and rearing), as well as the 5-HT behavioural syndrome (flat body posture and lower lip retraction). Compound **35**, on the other hand, only induced dopamine receptor activity. Thus, the behavioural models confirm that both compounds are active at postsynaptic dopamine receptors. In the microdialysis experiments and in the locomotor activity experiments compound **34** was in low doses more potent than compound **35**. The behavioural scoring, however, does not show this difference in potency. It is speculated that this might have been caused by the fact that the serotonergic activity of compound **34** attenuated the dopamine receptor activity of this compound which was not the case for compound **35**.

Bioisosteres are groups or molecules, which have chemical and physical similarities producing broadly similar biological effects.[225] The substitution of -CH=CH- by -S- in aromatic rings has been one of the most successful applications of classical isosterism.[182] Since the dopamine D_2 and D_3 receptor binding affinity of compounds **34** and **35** are comparable to DPAT (**73**), it could be suggested that a thiophene moiety is just a bioisostere for a benzene moiety rather than for a phenol moiety. If this hypothesis were correct, the *in vivo* activity of compounds **34** and **35** should have been the same. However, compound **35** did not induce the 5-HT behavioural syndrome in reserpinised rats, whereas compound **34** and DPAT (**73**) both possess serotonin and dopamine receptor properties.[241] When the thiophene moiety is considered as a bioisostere for a phenol it is clear that there are similarities between compounds **34** and **35** and their alleged corresponding hydroxylated 2-aminotetralins, i.e. 8- and 5-OH-DPAT, but not all pharmacological aspects are identical. Due to differences in distances between the nitrogen atom and H-bond accepting or donating moieties of the different compounds, there is not a hydroxyl position in a hydroxylated 2-aminotetralin that exactly corresponds with the sulfur position in the thiophene analogues. This is, however, a general phenomenon of isosteric replacement; even though it represents a subtle structural change it might result in a modified profile, i.e. some properties of the parent molecule remain unaltered, others will be changed.

Compounds **38** and **39** were synthesised as possible bioisosteric analogues for PHNO (**27a**) or one of its analogues. After changing the structure of the hexahydronaphthoxazines (**27a**, **75**, **76**) to the hexahydrothianaphthoxazines (**38**, **39**) the position of the sulfur atom would suggest that the hexahydrothianaphthoxazines are bioisosteric analogues for *trans*-7-hydroxy-4-*n*-propyl-2,3,4a,5,6,10b-hexahydro-*4H*-naphth[1,2*b*][1,4]oxazine (**75**) which is not a potent dopamine

receptor ligand. However, small structural changes in the basic structure may have large influences on the dopamine receptor activity of compounds. For instance, in the series of the hydroxylated 2-aminotetralins the position of the hydroxyl moiety on the benzene ring determines the dopamine receptor activity of the compounds. For these compounds there is an order of dopamine receptor potency: 5-OH-DPAT > 7-OH-DPAT > 6-OH-DPAT > 8-OH-DPAT, the latter displaying negligible dopamine receptor affinity.[251] On the other hand, in the series of the hexahydronaphthoxazines (**27a**, **75**, **76**) only the 9-hydroxy analogue possesses potent dopamine receptor activity,[206] while in the series of the benzo[*f*]quinolines (**77** and **78**) both the 7- and 9-hydroxy isomers are potent dopamine receptor ligands.[101,102] Also this study shows that the structural changes of compounds **34** and **35** result in a higher dopamine receptor activity of compound **34** compared to compound **35**, which was unexpected based on the ranking of the monohydroxy 2-aminotetralins.

Still, the binding data indicate that indeed compounds **38** and **39** are ligands with low dopamine receptor affinity. Despite this low affinity for the dopamine D_2 and D_3 receptors the compounds were tested since it was not clear whether or not possible active metabolites could be formed *in vivo*. Sulfur atoms in molecules may be oxidised *in vivo* to sulfoxides, which may be active compounds. For instance, in the case of pergolide the sulfoxide metabolite retains its dopamine receptor activity.[252] The pharmacological data, however, show that the dopamine receptor activity of compounds **38** and **39** resembles the low dopamine receptor efficacy of compound **75** or its non-hydroxylated analogue **76**.[206] Given the small difference in binding affinities between compound **75** and its non-hydroxylated analogue **76**[206] and their comparable, low efficacy, it is not possible to determine whether a thiophene moiety is a bioisostere for a phenol or a phenyl moiety using the hexahydrothianaphthoxazines **38** and **39**.

Because of the diminished activity of compounds **34** and **35**, compared to 5-OH-DPAT, it is now an interesting challenge to develop new compounds based on the structure of tetrahydrobenzo[*b*]thiophenes, which possess the same, improved oral bioavailability as do our compounds **34** and **35**, but with a higher affinity and activity at the dopamine D_2 and D_3 receptor. These compounds will be of great interest for the development of new drugs in Parkinson's disease therapy.

In conclusion, we have shown that a thiophene moiety may qualitatively function as a bioisostere for a phenol moiety in hydroxylated 2-aminotetralins. For the hexahydrothianaphthoxazines it was not possible to discriminate between bioisosterism for a phenyl or a phenol moiety. The tetrahydrobenzo[*b*]thiophenes (**34** and **35**) possess higher relative oral bioavailabilities than 5-OH-DPAT.

Chapter 5

Dopamine D_2 activity of R-(–)-apomorphine and selected analogues: a microdialysis study*

Abstract

In the present study, R-(–)-apomorphine and three of its analogues were studied for their potency in decreasing the release of dopamine in the striatum after subcutaneous administration and for their oral bioavailability using the microdialysis technique in freely moving rats.

The analogues R-(–)-N-*n*-propylnorapomorphine and R-(–)-11-hydroxy-N-*n*-propylnoraporphine displayed a higher potency than R-(–)-apomorphine in decreasing the release of dopamine in the striatum. A high dose of R-(–)-11-hydroxyaporphine, a dopamine D_2 receptor partial agonist, had a small effect on the release of dopamine in the striatum. The catechols R-(–)-N-*n*-propylnorapomorphine and R-(–)-apomorphine displayed a comparable oral bioavailability (1%), while the monohydroxy analogue R-(–)-11-hydroxy-N-*n*-propylnoraporphine displayed a slightly higher oral bioavailability (3%).

In conclusion, R-(–)-N-*n*-propylnorapomorphine and R-(–)-11-hydroxy-N-*n*-propylnoraporphine did not show a substantial improvement in bioavailability. However, due to the clear difference in their efficacy in decreasing dopamine release, in spite of the similar agonist binding affinity for the dopamine D_2 receptor of the two analogues compared to R-(–)-apomorphine, they could be useful alternatives for apomorphine in the treatment of Parkinson's disease.

* This chapter is based on: Rodenhuis, N.; Dijkstra, D; Vermeulen, E.S.; Timmerman, W.; Wikström, H.V. (2000) Dopamine D_2 activity of R-(–)-apomorphine and selected analogs: a microdialysis study. *Eur. J. Pharmacol*. **387**, 39-45.

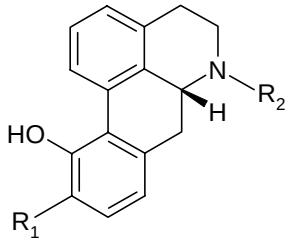
5.1 Introduction

Parkinson's disease is a progressive neurodegenerative disorder of the basal ganglia, which most often becomes apparent after the age of 55. It is a prototypic hypokinetic disorder, with akinesia, bradykinesia, rigidity and tremor as the most prominent features.[117] Depression and a general slowing of intellectual processes also occur, but are less well-defined. The neurological and psychiatric symptoms usually worsen with time (for review see ref. 118). The neuropathology of Parkinson's disease reveals a striking loss of the dopaminergic neurons of the nigrostriatal pathway.[119,120]

As Parkinson's disease is associated with a loss of dopamine, it is commonly treated with drugs which replace dopamine. Since dopamine itself cannot pass the blood-brain barrier, the most commonly used therapy is levodopa (L-DOPA), a precursor of dopamine. A complication of long-term treatment with L-DOPA, however, is the development of rapid fluctuations in clinical state where the patient switches suddenly between mobility and immobility; this phenomenon is known as the 'on-off' effect.[135,136]

An alternative approach to the treatment with L-DOPA is the use of drugs that mimic the action of dopamine. Treatment with dopamine receptor agonists has some advantages over treatment with L-DOPA. Dopamine receptor agonists are effective in patients in the advanced stages of Parkinson's disease unlike L-DOPA, because their action at postsynaptic receptors is unaffected by the lack of dopamine producing nerve cells. Furthermore, there is an increasing interest in the potential of dopamine receptor agonists to provide a neuroprotective effect. Theoretically, such a protective effect might result from (a) a decrease in L-DOPA application, as L-DOPA may cause oxidative stress,[144] (b) stimulation of dopamine autoreceptors resulting in decreased dopamine synthesis, release, and turnover, as dopamine metabolism leads to reactive oxygen species,[145] and (c) direct anti-oxidant effects.[141-143]

The dopamine D_1/D_2 receptor agonist R-(–)-apomorphine has proven to be very effective in Parkinson's disease. Subcutaneously administered R-(–)-apomorphine in combination with L-DOPA rapidly and consistently reverses the 'off' period motor deficits.[253-256] Beside its action as a dopamine D_1/D_2 receptor agonist, R-(–)-apomorphine can also act as a radical scavenger[257] and, therefore, may have neuroprotective properties. One of the major limitations of the clinical use of R-(–)-apomorphine, a catechol-aporphine, however, is its low oral activity.[258-260]



11. R_1=OH, R_2=CH_3 R-(–)-apomorphine
79. R_1=H, R_2=CH_3 R-(–)-11-hydroxy-aporphine
80. R_1=OH, R_2=*n*-C_3H_7 R-(–)-N-*n*-propylnorapomorphine
12. R_1=H, R_2=*n*-C_3H_7 R-(–)-11-hydroxy-N-*n*-propylnoraporphine

Chart 5.1 Chemical structures of R-(–)-apomorphine and selected analogues.

With respect to the low bioavailability of R-(–)-apomorphine we initiated a study of three analogues (**79**, **80**, **12**) of R-(–)-apomorphine (**11**). The selected analogues all possess affinity for the dopamine D_1 and D_2 receptors comparable to R-(–)-apomorphine. It was postulated that the monohydroxy compounds would have a higher oral bioavailability, as compared to the catechols, because they are likely to be less sensitive to metabolic degradation. Although a great deal has already been reported on the *in vitro* and *in vivo* pharmacology of apomorphine (**11**) and selected analogues (**79**, **80**, **12**), no study has been undertaken to examine this series of compounds with respect to their oral bioavailabilities *in vivo*. We have now examined these compounds with respect to their potencies and relative bioavailabilities, using the microdialysis technique in freely moving rats. By measuring dopamine release in the striatum, information on the degree of dopamine D_2 autoreceptor stimulation can be obtained. Dopamine D_1 receptor stimulation was not investigated in this study. Comparisons were made after subcutaneous (s.c.) and per oral (p.o.) administration in an attempt to estimate the importance of the first-pass effect for this series of apomorphine analogues.

5.2 Materials and Methods

5.2.1 Animals

Male Wistar rats (from CDL, Groningen, The Netherlands) weighing 280-320 g were used for microdialysis experiments. The rats were housed in plexiglas cages, eight animals in each cage, with free access to water and food. The cages were placed in a room with controlled environmental conditions (21 °C; humidity 60-65%; lights on at 8 a.m. and off at 8 p.m.). The animals were housed at least one week after arrival prior to surgery. Animal procedures were conducted in accordance with guidelines published in the NIH Guide for the Care and Use of Laboratory Animals and all protocols were approved by the Groningen University Institutional Animal Care and Use Committee.

5.2.2 Drug treatment

The drugs were dissolved in degassed ultra pure water with approximately 0.5 mg/ml ascorbic acid to prevent oxidation of the compounds and stocked in a concentration of 300 nmol/ml for subcutaneous administration and 10 µmol/2 ml for oral administration and diluted, if necessary, with degassed ultra pure water before administration. To dissolve R-(–)-11-hydroxy-aporphine a drop of glacial acetic acid was added. Drugs used were R-(–)-apomorphine.HCl (**11**), R-(–)-11-hydroxyaporphine (**79**), R-(–)-N-*n*-propylnorapomorphine.HCl (**80**) and R-(–)-11-hydroxy-N-*n*-propylnoraporphine.HBr (**12**). R-(–)-apomorphine.HCl was purchased from RBI, compounds **80** and **12** were provided by Prof. J.L. Neumeyer (Harvard Medical School, MA), R-(–)-11-hydroxy-aporphine (**79**) was synthesised in Groningen.

5.2.3 Surgery and brain microdialysis

On-line brain microdialysis in freely moving animals has previously been described.[188] In brief, the rats were anaesthetised with midazolam (5 mg/kg s.c.), atropine nitrate (0.1 mg/kg s.c.), ketamine (50 mg/kg i.p.) and xylazine (8 mg/kg i.p.); 10% lidocaine was locally applied. The rats were then mounted into a stereotaxic frame (Kopf). The incisor bar was placed in position so that the skull was held horizontal. The skull was exposed and burr holes were drilled. A Y-shaped cannula was used for the experiments, with an exposed tip length of 3 mm. The dialysis tube (ID: 0.22 mm; OD: 0.31 mm) was prepared from polyacrylonitrile/sodium methallyl sulfonate copolymer (AN 69, Hospal, Bologna, Italy). The microdialysis membrane was implanted in the striatum. The dura was removed with a sharp needle. Two anchor screws were positioned in different bone plates nearby. The following co-ordinates were used according to the atlas of Paxinos and Watson:[250] AP + 1.0, LM ± 3.0 relative to bregma, and VD − 6.0 below dura. Before insertion into the brain the dialysis probe was perfused successively with ultra pure water, methanol, ultra pure water and Ringer solution (1.2 mM Ca^{2+}). The dialysis probe was positioned in the burr hole under stereotaxic guidance. The probe was cemented in this position with phosphatine dental cement. After the surgery the rats received buprenorphine (0.1 mg/kg i.m.), an analgesic agent. The rats were housed solitary.

The experiments were performed in conscious rats 17-48 h after implantation of the cannula. The striatum was perfused with a Ringer solution (147 mmol/l NaCl, 4 mmol/l KCl, 1.2 mmol/l $CaCl_2$, 1.1 mmol/l $MgCl_2$) at 2 µl/min (CMA/102 microdialysis pump, Sweden). After the experiments, the rats were sacrificed and the brains were removed. After removal the brains were kept in 4% paraformaldehyde solution until they were sectioned to control the location of the dialysis probe.

Dopamine was quantitated by high-performance liquid chromatography (HPLC) with electrochemical detection with a detection limit of approximately 5 fmol/sample. An HPLC

pump (LKB, Pharmacia) was used in conjugation with an electrochemical detector (Antec, Leiden) working at 625 mV versus an Ag/AgCl reference electrode. The analytical column was a Supelco Supelcosil LC-18 Column (3 µm particle size). The mobile phase consisted of a mixture of 4.1 g/l Na-acetate (Merck), 85 mg/l octane sulphonic acid (Aldrich), 50 mg/l EDTA (Merck), 8.5 % methanol (Labscan) and ultra pure water (pH=4.1 with glacial acetic acid).

5.2.4 Data analysis

Data were converted into percentage of basal levels. The basal levels were determined from four consecutive samples (less than 20% variation), and set at 100%. During 180 min after administration of the compound the dopamine release was measured. This time course was chosen to be able to compare the effects and Areas Under the Curve (AUC) of the different compounds and routes of administration. The AUC was determined using GraphPad Prism for Windows (GraphPad Inc.). To determine the AUC the mean of the first 4 samples were taken as baseline and then the AUC was calculated from t=0 min to t=180 min. At t=180 min the program draws an imaginary vertical line and left from this line the AUC is calculated. The experiments were terminated after 180 minutes to be able to compare the AUCs. The relative oral bioavailabilities were determined by comparing the curves after p.o. and s.c. administration. When there was no significant difference between the effects on dopamine release the s.c. dose was divided by the p.o. dose and multiplied by 100 to give a percentage representing the relative oral bioavailability. Microdialysis data were compared using one-way analysis of variance (ANOVA) for repeated measurements, followed by Dunnett's Method post-hoc test. A significance level of 0.05 was applied. Statistical analysis of the AUCs was performed by a t-test. For comparison with R-(–)-apomorphine (**11**) 30 nmol/kg equal variance test failed and than Rank Sum Test followed by Mann-Whitney test was performed.

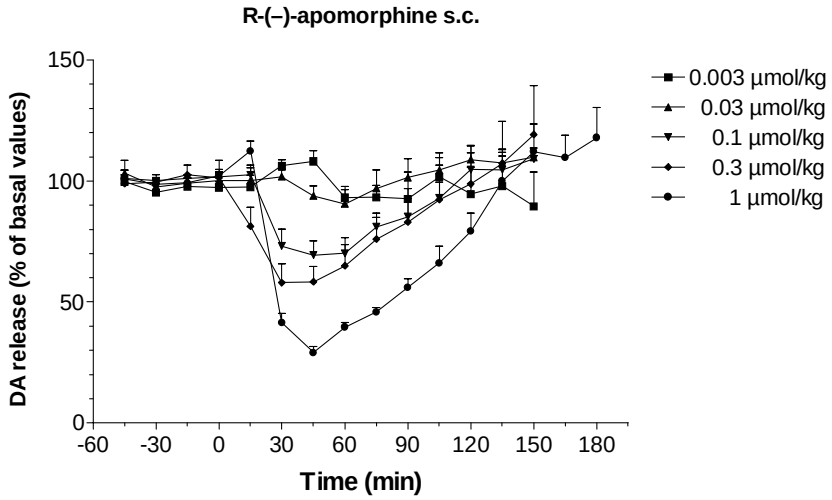
5.3 Results

The basal dialysate concentrations in the striatum for the experiments were 11.9 ± 0.7 (n = 79) fmol/min.

Figures 5.1A-D show that s.c. administration of R-(–)-apomorphine (**11**), R-(–)-N-*n*-propylnorapomorphine (**80**), and R-(–)-11-hydroxy-N-*n*-propylnoraporphine (**12**), but not R-(–)-11-hydroxyaporphine (**79**), induced a dose-dependent decrease in the release of dopamine in the striatum. R-(–)-apomorphine (**11**) induced a significant decrease in the release of dopamine in the striatum in a dose-range from 0.1 to 1 µmol/kg s.c. In a dose-range from 0.003 to 0.3 µmol/kg s.c., R-(–)-N-*n*-propylnorapomorphine (**80**) induced a significant decrease in the release of dopamine in the striatum. R-(–)-11-hydroxy-N-*n*-propylnoraporphine (**12**) induced a significant decrease in the release of dopamine in the striatum in a dose-range from 0.03 to 0.3 µmol/kg s.c. Although R-(–)-11-hydroxyaporphine (**79**) displays affinity for the dopamine D_2

receptor, it only induced a small significant decrease in the release of dopamine in the striatum in a dose of 1 µmol/kg s.c.

A



B

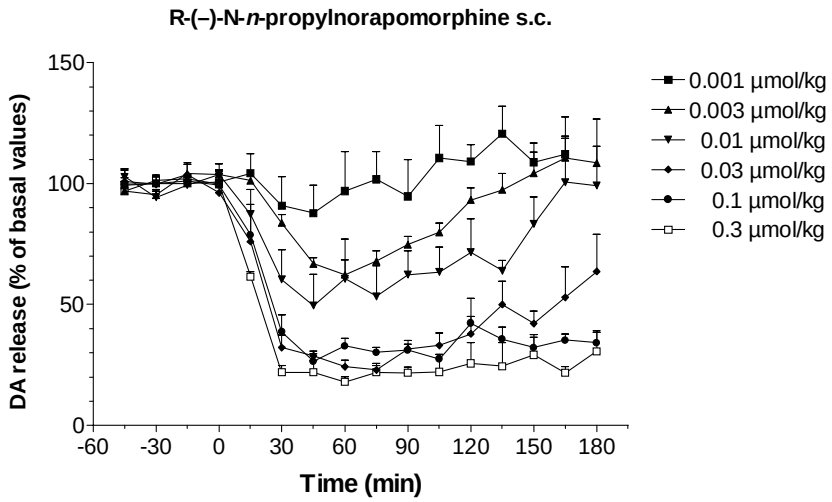
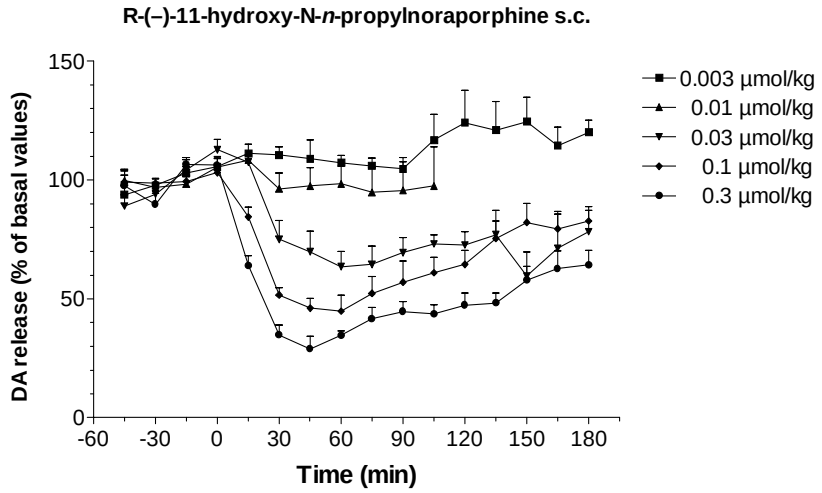


Figure 5.1 Effects on striatal dopamine release in freely moving rats after s.c. administration. The results are the mean ± S.E.M. of data obtained from 4 rats. (A) R-(–)-apomorphine (**11**); changes are significant ($p < 0.05$) from t = 30 min to t = 90 min for 0.1, and 0.3 µmol/kg s.c., and from t = 30 min to t = 120 min for 1 µmol/kg s.c. (B) R-(–)-N-*n*-propylnorapomorphine (**80**); changes are significant ($p < 0.05$) from t = 30 min to t = 105 min for 0.003 and 0.01 µmol/kg s.c., from t = 15 min to t = 180 min for 0.03, 0.1, and 0.3 µmol/kg s.c.

C



D

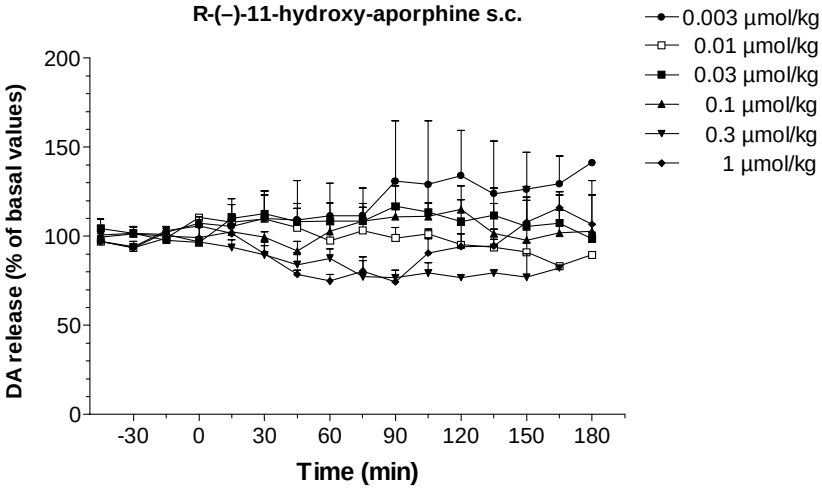


Figure 5.1 (continued) Effects on striatal dopamine release in freely moving rats after s.c. administration. The results are the mean ± S.E.M. of data obtained from 4 rats. (C) R-(–)-11-hydroxy-N-*n*-propylnoraporphine (**12**); changes are significant ($p < 0.05$) from t = 30 min to t = 180 min for 0.03 and 0.1 µmol/kg s.c., from t = 15 min to t = 180 min for 0.3 µmol/kg s.c. (D) R-(–)-11-hydroxyaporphine (**79**); changes are significant ($p < 0.05$) from t = 45 min to t = 75 min for 1 µmol/kg s.c.

The dose-response relationships of the test compounds are given in Figure 5.2. The response of the compounds is given as the AUC. To compare the AUCs, the experiments were stopped after 180 min. The rank order in the potency upon s.c. administration of the compounds is: R-(–)-N-*n*-propylnorapomorphine (**80**) > R-(–)-11-hydroxy-N-*n*-propylnoraporphine (**12**) > R-(–)-apomorphine (**11**).

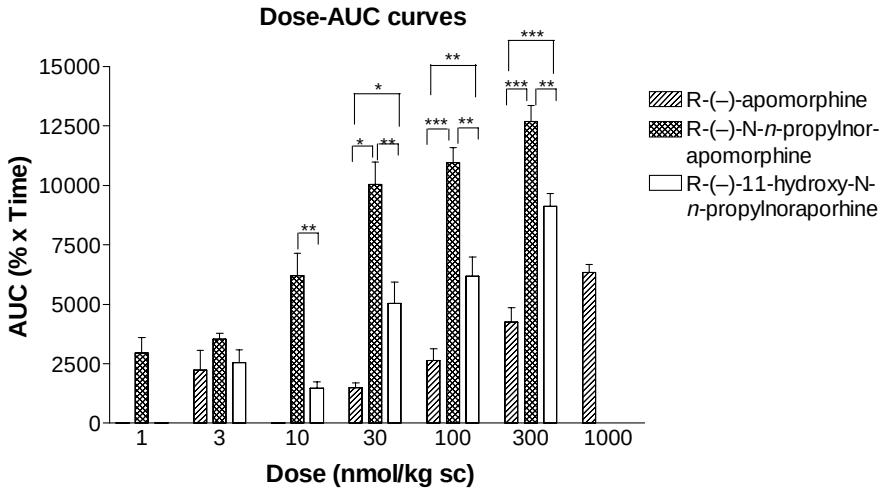


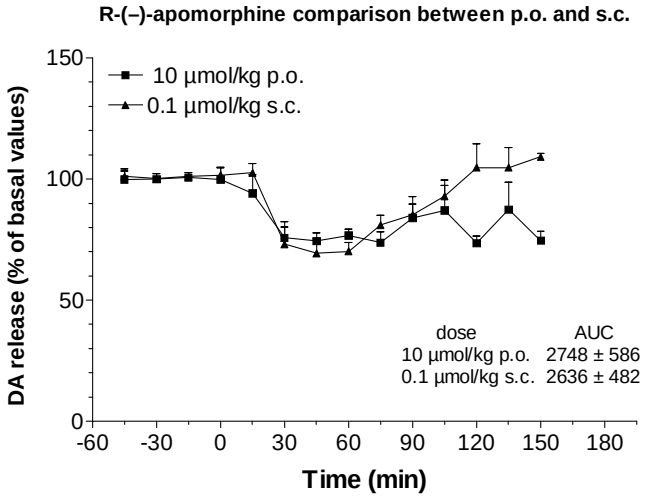
Figure 5.2 Comparison of the dose-AUC relationship of R-(-)-apomorphine (**11**), R-(-)-N-*n*-propylnorapomorphine (**80**) and R-(-)-11-OH-N-*n*-propylnoraporphine (**12**). Data represent mean values ± S.E.M. of 4 animals. Statistical analysis by t-test: * $p<0.05$, ** $p<0.01$, *** $p<0.001$. For comparison with R-(-)-apomorphine (**11**) 30 nmol/kg equal variance test failed and than Rank Sum Test followed by Mann-Whitney test was performed.

The relative oral bioavailabilities of R-(–)-apomorphine (**11**) and two of its analogues **80** and **12** can be found from Figures 5.3A-C. The relative oral bioavailability was determined by comparing the curves and the AUC after s.c. and p.o. administration. When the AUCs were not significantly different, the relative oral bioavailability was determined by dividing the s.c. dose by the p.o. dose and multiplying by 100. It was known that R-(–)-apomorphine (**11**) possessed a low oral bioavailability. It was expected that the oral bioavailability of the three compounds would be between 1 % and 10 %. Based on this assumption the oral doses were chosen. With this method, both the catechols R-(–)-apomorphine (**11**) and R-(–)-N-*n*-propylnorapomorphine (**80**) possess a relative oral bioavailability of about 1 %. The mono-hydroxy compound R-(–)-11-hydroxy-N-*n*-propylnoraporphine (**12**) possesses a relative oral bioavailability of about 3 %.

When the courses of the curves after s.c. administration of compounds **11**, **80**, and **12**, respectively, are compared it can be seen that the duration of action of the N-*n*-propyl analogues is longer than that of R-(–)-apomorphine.

Compound **79** does not induce an effect on the release of dopamine from the striatum (Figure 5.1D). The binding data together with literature data suggest that this compound is a partial dopamine D_2 receptor agonist and a dopamine D_1 receptor antagonist.[261] Therefore, the present pharmacodynamic method was not suitable to determine the relative oral bioavailability.

A



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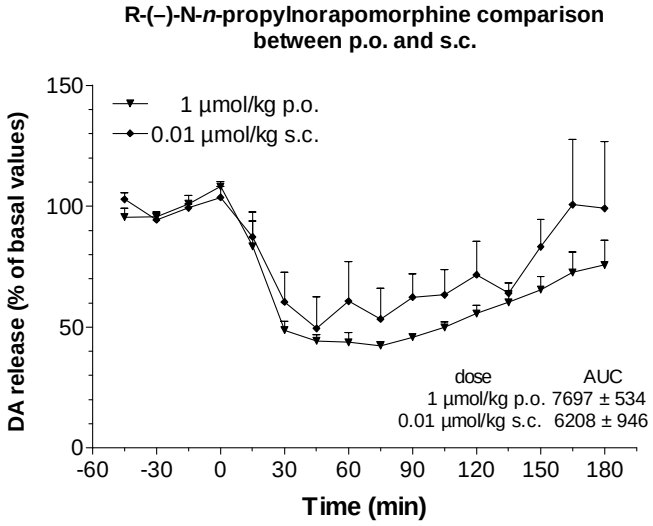


Figure 5.3 Effects on striatal dopamine release in freely moving rats after s.c. and p.o. administration. The results are the mean ± S.E.M. of data obtained from 4 rats.
(A) R-(–)-apomorphine (**11**); changes are significant ($p < 0.05$) from t = 30 min to t = 90 min for 0.1 µmol/kg s.c. and 10 µmol/kg p.o. (B) R-(–)-N-*n*-propylnorapomorphine (**80**); changes are significant ($p < 0.05$) from t = 30 min to t = 105 min for 0.01 µmol/kg s.c. and for t = 15 min to t = 180 min for and 1 µmol/kg p.o.

C

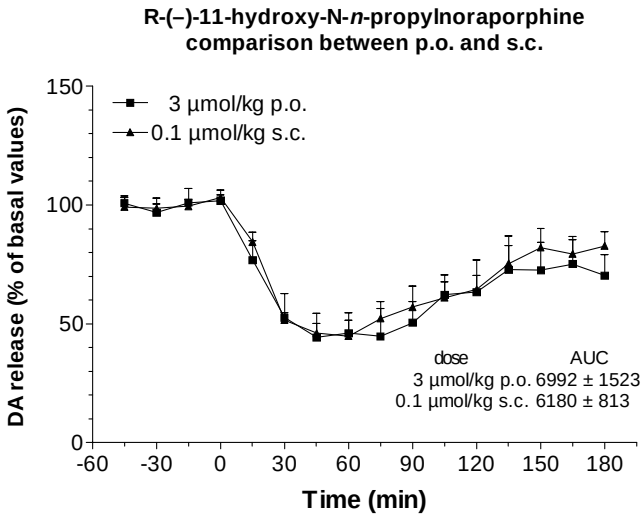


Figure 5.3 (continued) Effects on striatal dopamine release in freely moving rats after s.c. and p.o. administration. The results are the mean ± S.E.M. of data obtained from 4 rats. (C) R-(–)-11-hydroxy-N-*n*-noraporphine (**12**); changes are significant ($p < 0.05$) from t = 30 min to t = 180 min for 0.1 µmol/kg s.c., from t = 15 min to t = 180 min for 3 µmol/kg p.o.

5.4 Discussion

R-(–)-apomorphine (**11**) is a catechol and is known to have a low oral bioavailability.[262] However, the drug is very useful in the treatment of Parkinson's disease when L-DOPA treatment gives 'on-off' fluctuations.[253] An analogue which also displays dopamine D_1 and D_2 receptor agonistic properties, but possessing a higher oral bioavailability, would be beneficial as an alternative treatment in Parkinson's disease. The analogues tested (R-(–)-11-hydroxyaporphine (**79**), R-(–)-N-*n*-propylnorapomorphine (**80**) and R-(–)-11-hydroxy-N-*n*-propylnoraporphine (**12**)) possess affinity for the dopamine D_1 and D_2 receptors comparable to R-(–)-apomorphine (Table 5.1). In our experiments we monitored the dopamine D_2 receptor agonistic properties of the compounds, as the release of dopamine is under the control of dopamine D_2 autoreceptors.[189]

Figures 5.1A-C show that compounds **11**, **80**, and **12** act as dopamine D_2 receptor agonists, because they all induce a decrease in the release of dopamine in the striatum. R-(–)-11-hydroxyaporphine (**79**) (Figure 5.1D) induces, in a dose of 1 µmol/kg, a small significant decrease in the release of dopamine in the striatum. This lack of biochemical activity of compound **79** was not expected from a structure-activity point of view. However, Schaus et al.[261] already published that R-(–)-11-hydroxyaporphine (**79**) acts as a partial agonist at the dopamine D_2 receptor. This would explain our findings that this compound has a very weak effect on the dopamine D_2 autoreceptor.

Table 5.1 Affinities of R-(–)-apomorphine (**11**) and its selected analogues (**79**, **80**, **12**).

compound	K_i (nM)		
	[^{3}H]-SCH23390 (D_1)	[^{3}H]-spiperone (D_2-antagonist)	[^{3}H]-ADTN (D_2-agonist)
R-(–)-apomorphine (**11**)[a]	240	11.1	3.7
R-(–)-11-hydroxyaporphine (**79**)[b]	*107*[c]	*58*[c]	-
R-(–)-N-*n*-propylnorapomorphine (**80**)[a]	340	0.8	1.5
R(–)-11-hydroxy-N-*n*-propylnoraporphine (**12**)[d]	434	0.9	5.3

Footnotes: Affinity of R-(–)-apomorphine (**11**) and its selected analogues (**79**, **80**, **12**) as measured by their ability to displace *in vitro* [^{3}H]-SCH23390 (D_1-antagonist), [^{3}H]-spiperone (D_2-antagonist), and [^{3}H]-ADTN (D_2-agonist) from membrane preparations of rat brain corpus striatum tissue in order to measure the affinity for dopamine D_1 and D_2 receptors, respectively. [a] Values are taken from ref. 263; [b] values are taken from ref. 261; [c] *IC50 in nM*; [d] values are taken from ref. 264.

The dose-AUC relationships upon s.c. administration (Figure 5.2) clearly show that the N-*n*-propyl analogues **80** and **12** are more efficacious in the microdialysis experiments than the N-methyl analogue (R-(–)-apomorphine (**11**)). An explanation could be the presence of a propyl moiety on the nitrogen for R-(–)-N-*n*-propylnorapomorphine (**80**) because a propyl moiety has a better fit into the receptor than a methyl. This, however, should have been shown in differences in binding affinities, which are not observed. Although there is a clear distinction in efficiency in the microdialysis experiments between the three compounds, this difference cannot be explained by the affinity for the dopamine D_2 receptor (Table 5.1). The relevant dopamine D_2 receptor agonist binding is comparable for the three compounds.

Figures 5.3A and B show that both of the catechol-containing aporphines (R-(–)-apomorphine (**11**) and R-(–)-N-*n*-propylnorapomorphine (**80**)) possess a relative oral bioavailability of about 1%. The greater s.c. potency of R-(–)-N-*n*-propylnorapomorphine (**80**) over R-(–)-apomorphine (**11**) is not likely to be the result from different rates of metabolism in the periphery because the main route of metabolism for both compounds is glucuronidation of the catechol moiety.[265] The greater potency of the mono-hydroxy analogue R-(–)-11-hydroxy-N-*n*-propylnoraporphine (**12**) (Figure 5.3) could possibly be explained from differences in metabolism, R-(–)-11-hydroxy-N-*n*-propylnoraporphine (**12**) possesses a relative oral bioavailability of 3%. Although this represents an increase, it is still not an optimal oral bioavailability for a therapeutic agent. The improvement of the relative oral bioavailability of the mono-hydroxy analogue **12** may be explained from the fact that a mono-hydroxy analogue is less sensitive to metabolic degradation than a catechol moiety.[266] Beside glucuronidation, the catechol moiety is also sensitive to oxidative degradation, as well as to degradation by catechol-O-methyl transferase (COMT), which will predominantly result in 10-methoxy-11-

hydroxyaporphine, an inactive compound.[267,268] The catechol moiety is clearly not necessary for high affinity at the dopamine D_2 receptors. The presence of a free 11-hydroxy moiety is enough to confer dopamine D_2 receptor agonist-like activity, similar to that of 10,11-dihydroxyaporphines.[266]

An explanation could be differences in intrinsic efficacy and differences in the ability to pass the blood-brain barrier. For R-(–)-N-*n*-propylnorapomorphine (**80**) this difference in ability to pass the blood-brain barrier compared to R-(–)-apomorphine has previously been published.[265,269,270]

Beside comparable effects on the release of dopamine in the striatum, R-(–)-N-*n*-propylnorapomorphine (**80**) and R-(–)-11-hydroxy-N-*n*-propylnoraporphine (**12**) could also possess the same neuroprotective effects as R-(–)-apomorphine. This neuroprotective effect resides in the phenolic moiety, which can act as a radical scavenger.[141-143] Both compounds possess this moiety.

Based on our results, R-(–)-N-*n*-propylnorapomorphine (**80**) and R-(–)-11-hydroxy-N-*n*-propylnoraporphine (**12**) could be good targets for treatment of Parkinson's disease. Although their oral bioavailabilities are low, their greater efficacy results in the possibility of administering lower doses. R-(–)-N-*n*-propylnorapomorphine (**80**) has proven to be a useful adjunct in the long-term management of patients with unsatisfactory response to L-DOPA and produced a significant therapeutic benefit at doses much lower than the dose at which side-effects occur.[271]

In conclusion, this microdialysis study shows that R-(–)-N-*n*-propylnorapomorphine (**80**) and R-(–)-11-hydroxy-N-*n*-propylnoraporphine (**12**) are more potent than R-(–)-apomorphine (**11**) in inhibiting dopamine release in the striatum. This difference in potency on the presynaptic receptors resembles the difference in potency of these analogues on supersensitive postsynaptic receptors as described by Kelly et al.[269]

Chapter 6

Neuropharmacological evaluation of a new dopaminergic prodrug with anti-parkinsonian potential*

Abstract

In this study, a prodrug of hydroxylated 2-aminotetralins was tested against the prototypic dopamine receptor agonist S-(–)-5-OH-DPAT. The active S-enantiomer of the prodrug (DD9812) was able to decrease the release of dopamine in the striatum, but with a lower potency than S-(–)-5-OH-DPAT after s.c. administration. The R-enantiomer of the prodrug (DD9813) only had a limited effect on the release of dopamine in the striatum. The prodrug, however, showed an improved relative oral bioavailability, as compared to S-(–)-5-OH-DPAT.

* This chapter is based on: Rodenhuis, N.; Venhuis, B.J.; Timmerman, W.; Wikström, H.V.; Dijkstra, D; Meltzer, L.; Johnson, S.; Wise, L.D. (2000) Neuropharmacological evaluation of a new dopaminergic prodrug with anti-parkinsonian potential. **In preparation**.

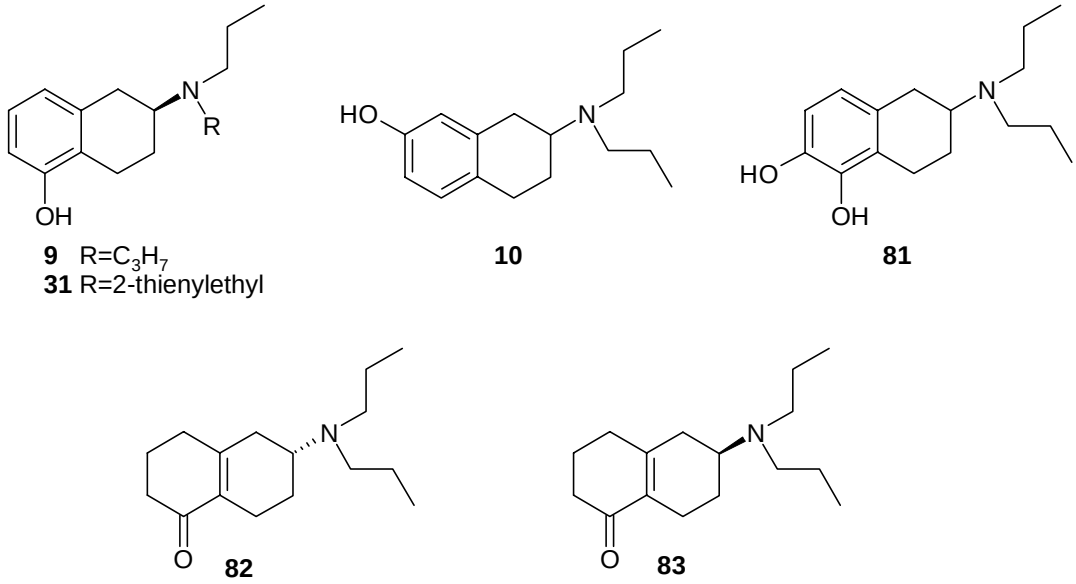
6.1 Introduction

Parkinson's disease is a neurodegenerative disease that is characterised by progressive damage of predominantly dopaminergic neurons in the substantia nigra. Damage to neurons in the substantia nigra causes a dopamine deficiency in the striatum, resulting in disturbed motor functioning. Only when approximately 70-80 % of these cells have degenerated, symptoms of Parkinson's disease arise.

Current drug-based therapies for Parkinson's disease are palliative therapies, i.e. they relieve the symptoms, but do not cure the disease. One of the current and expanding therapies is treatment with dopamine D_2/D_3 receptor agonists. There are a number of such therapeutics, e.g. bromocriptine, pergolide, apomorphine, ropinirole, and pramipexole.

A class of compounds with a high affinity for e.g. dopamine D_2 receptors are the hydroxylated 2-aminotetralins, e.g. 5-OH-DPAT (**9**), N-0437 (**31**), 7-OH-DPAT (**10**) and 5,6-di-OH-DPAT (**81**).[272,273] Preclinical data show that these compounds display limited activity upon oral administration. A major disadvantage of the hydroxylated 2-aminotetralins and other phenolic compounds is that they undergo considerable inactivation by glucuronidation in the gut and the liver.[161] One of the strategies to circumvent the problem of the low oral bioavailability of the hydroxylated 2-aminotetralins is to search for suitable prodrugs. Frequently investigated prodrugs of phenols are esters and carbamates.[96,185-187]

We have now synthesised 6-(N,N-di-*n*-propylamino-3,4,5,6,7,8-hexahydro-2*H*-naphthalen-1-one (PD148903), which is a dihydro analogue of 5-OH-DPAT and which may possibly act as a prodrug or a pharmacophore equivalent of 5-OH-DPAT. The individual enantiomers of racemic PD148903 were prepared in our laboratory (to be published). Johnson et al.[274] published that the *in vitro* biochemistry of PD148903 showed that the compound itself did not have any affinity for the dopamine D_2 receptor. However, *in vivo* experiments showed that it has potent dopamine receptor agonist-like activity. These two observations suggest that *in vivo* the prodrug is, at least partly, converted to hydroxylated derivatives.[274] Therefore, we have pharmacologically evaluated the S-(–)-enantiomer (DD9812, **83**) and the R-(+)-enantiomer (DD9813, **82**) of the potential prodrug PD148903 against the active enantiomer of the prototypic dopamine receptor agonist S-(–)-5-OH-DPAT. The exact mechanism of conversion and metabolism is still under investigation.



9 R=C_3H_7
31 R=2-thienylethyl

10 **81** **82** **83**

Chart 6.1 Chemical structures of S-(–)-5-hydroxy-2-(N,N-di-*n*-propylamino)tetralin (S-(–)-5-OH-DPAT, S-(–)-**9**), S-(–)-5-hydroxy-2-(N-*n*-propyl-N-2-thienylethylamino)tetralin (S-(–)-N-0437, S-(–)-**31**), R-(+)-7-hydroxy-2-(N,N-di-*n*-propylamino)tetralin (R-(+)-7-OH-DPAT, R-(+)-**10**), 5,6-dihydroxy-2-(N,N-di-*n*-propylamino)tetralin (5,6-di-OH-DPAT, **81**) and R- and S-6-(N,N-di-*n*-propylamino-3,4,5,6,7,8-hexahydro-2*H*-naphthalen-1-one (R-enantiomer, DD9813, **82**; S-enantiomer, DD9812, **83**).

6.2 Material and methods

6.2.1 Animals

Male Wistar rats (from CDL, Groningen, The Netherlands) weighing 280-320 g were used for microdialysis experiments. The rats were housed in Plexiglas cages, eight animals in each cage, with free access to water and food. The cages were placed in a room with controlled environmental conditions (21 °C; humidity 60-65%; lights on at 8 a.m. and off at 8 p.m.). The animals were housed at least one week after arrival prior to surgery and use in the experiments. Animal procedures were conducted in accordance with guidelines published in the NIH Guide for the Care and Use of Laboratory Animals and all protocols were approved by the Groningen University Institutional Animal Care and Use Committee.

6.2.2 Drug treatment

The drugs were dissolved in saline and stocked in a concentration of 1 mg/ml for subcutaneous (s.c.) and 1 mg/2 ml for oral (p.o.) administration and diluted, if necessary, with saline before administration. A volume of 1 ml/kg was administered per s.c. injection and 2

ml/kg per p.o. injection. Drugs used were DD9812, DD9813 and S-(–)-5-OH-DPAT and were synthesised at the Department of Medicinal Chemistry in Groningen. The amount of compound was recalculated to an amount of S-(–)-5-OH-DPAT, i.e. 1 mg/ml indicates and amount of compound equal to 1 mg/ml S-(–)-5-OH-DPAT.

6.2.3 Surgery and brain microdialysis

On-line brain microdialysis in freely moving animals has previously been described.[188] In brief, the rats were anaesthetised with midazolam (5 mg/kg s.c.), atropine nitrate (0.1 mg/kg s.c.), ketamine (50 mg/kg i.p.) and xylazine (8 mg/kg i.p.); 10% lidocaine was locally applied. The rats were then mounted into a stereotaxic frame (Kopf). The incisor bar was placed in position so that the skull was held horizontal. The skull was exposed and burr holes were drilled. A Y-shaped dialysis probe was used for the experiments, with an exposed tip length of 3 mm. The dialysis tube (ID: 0.22 mm; OD: 0.31 mm) was prepared from polyacrylonitrile/sodium methallyl sulfonate copolymer (AN 69, Hospal, Bologna, Italy). The microdialysis membrane was implanted in the striatum. The dura was removed with a sharp needle. Two anchor screws were positioned in different bone plates nearby. The following co-ordinates were used according to the atlas of Paxinos and Watson:[250] AP + 1.0, LM ± 3.0 relative to bregma, and VD − 6.0 below dura. Before insertion into the brain the dialysis probe was perfused successively with ultra pure water, methanol, ultra pure water and Ringer solution (1.2 mM Ca^{2+}). The dialysis probe was positioned in the burr hole under stereotaxic guidance. The probe was cemented in this position with dental cement. After the surgery, the rats received buprenorphine (0.1 mg/kg i.m.), an analgesic agent. The rats were housed solitary.

The experiments were performed in conscious rats 17-48 h after implantation of the cannula. The striatum was perfused with a Ringer solution (147 mmol/l NaCl, 4 mmol/l KCl, 1.2 mmol/l $CaCl_2$, 1.1 mmol/l $MgCl_2$) at 2 µl/min (CMA/102 microdialysis pump, Sweden).

Dopamine was quantitated by high-performance liquid chromatography (HPLC) with electrochemical detection with a detection limit of approximately 5 fmol/sample. An HPLC pump (LKB, Pharmacia) was used in conjunction with an electrochemical detector (Antec, Leiden) working at 625 mV versus an Ag/AgCl reference electrode. The analytical column was a Supelco Supelcosil LC-18 Column (3 µm particle size). The mobile phase consisted of a mixture of 4.1 g/l sodium acetate (Merck), 85 mg/l octane sulphonic acid (Aldrich), 50 mg/l EDTA (Merck), 1 mM tetramethylammonium chloride (ACROS), 8.5 % methanol (Labscan) and ultra pure water (pH=4.1 with glacial acetic acid).

After the experiments the rats were sacrificed and the brains were removed. After removal the brains were kept in 4% paraformaldehyde solution until they were sectioned to control the location of the dialysis probes.

6.2.4 Statistics

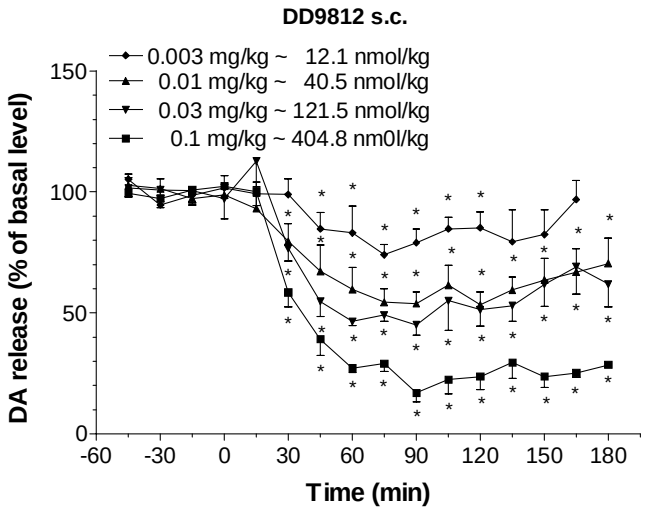
Data of the microdialysis experiments were converted into percentage of the basal levels. The basal levels were determined from four consecutive samples (less than 20% variation), and set at 100%. Dopamine release was measured for 180 min to be able to compare the curves and the Areas Under the Curve (AUCs). Microdialysis data were compared using one-way ANOVA for repeated measurements, followed by Dunnett's Method post-hoc test. The relative oral bioavailabilities were determined by comparing the AUCs after p.o. and s.c. administration. When there was no significant difference between the effects on dopamine release, the s.c. dose was divided by the p.o. dose and multiplied by 100 to give a percentage representing the relative oral bioavailability. For determining the AUC the mean of the first four samples (t = – 45 to 0 minutes) was taken as baseline and then the AUC was calculated. At the end of the curve, whether basal levels were reached again or not, the program draws an imaginary vertical line and left from this line the AUC is calculated. To be able to compare the AUCs the AUC was calculated using the same time course for the different doses. Statistical analysis of the AUC was performed by a t-test. In all cases a significance level of 0.05 was applied.

6.3 Results

The control dialysate concentrations in the striatum for the experiments were 12.0 ± 0.9 (n = 51) fmol/min.

The results of the microdialysis experiments of the compounds DD9812, DD9813 and S-(–)-5-OH-DPAT are shown in the figures 6.1-6.3. S.c. administration of DD9812 and S-(–)-5-OH-DPAT, but not DD9813 induced a dose-dependent, and significant, decrease in the release of dopamine in the striatum. Also after p.o. administration DD9812 and S-(–)-5-OH-DPAT induced a significant decrease in the release of dopamine in the striatum. The effect of DD9813 was not studied upon p.o. administration.

A



B

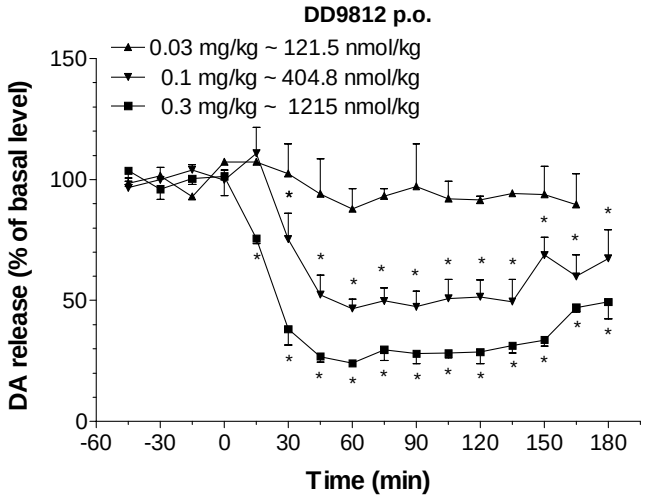


Figure 6.1 Effect of DD9812 on striatal dopamine release in freely moving rats after s.c. (A) and p.o. (B) administration. The results are the mean ± S.E.M. of data obtained from 4 rats (* $p<0.05$).

DD9812 induced a significant decrease in dopamine release by s.c. administration of a dose of 0.003 mg/kg, 0.01 mg/kg, 0.03 mg/kg and 0.1 mg/kg with a maximum decrease of 25 %, 45 %, 50 % and 80 % of control values, respectively (Figure 6.1A). Figure 6.1B shows that the maximum significant decrease after p.o. administration of DD9812 in a dose of 0.1 mg/kg and 0.3 mg/kg was 50 % and 70 % of control values, respectively. For both s.c. and p.o. administration the duration of action of the compound was longer with a higher dose.

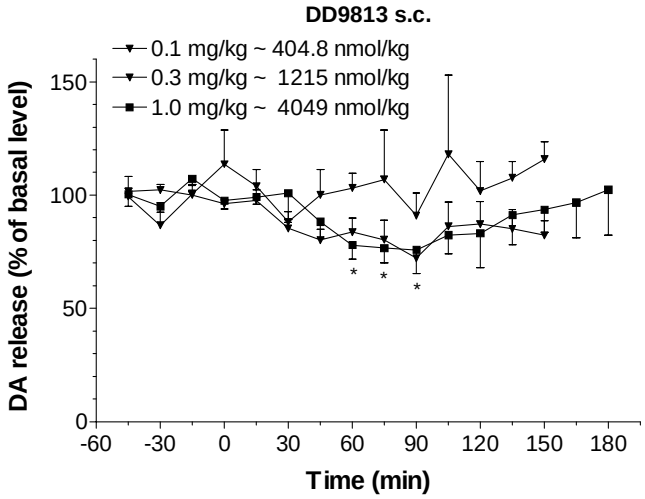
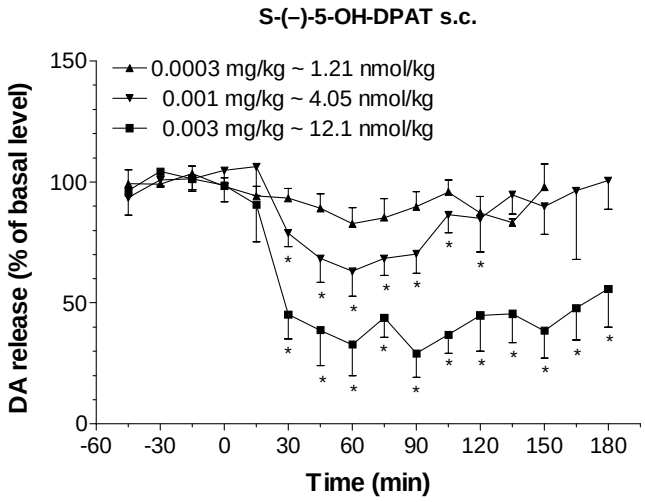


Figure 6.2 Effect of DD9813 on striatal dopamine release in freely moving rats after s.c. administration. The results are the mean ± S.E.M. of data obtained from 4 rats (* $p<0.05$).

Only in a high dose (1 mg/kg s.c.) DD9813 has a limited, significant effect on the release of dopamine in the striatum (Figure 6.2).

S-(–)-5-OH-DPAT, upon s.c. administration, induced a significant decrease in dopamine release of maximally 35 % and 70 % of control values after doses of 0.001, and 0.003 mg/kg , respectively (Figure 6.3A). The significant effect of administration of 0.1 mg/kg p.o. and 0.3 mg/kg p.o. of S-(–)-5-OH-DPAT lasted from t = 15 min to t = 180 min with a maximum decrease of 55 % and from t = 30 min to t = 180 min with a maximum decrease of 70 % of control values, respectively (Figure 6.3B).

A



B

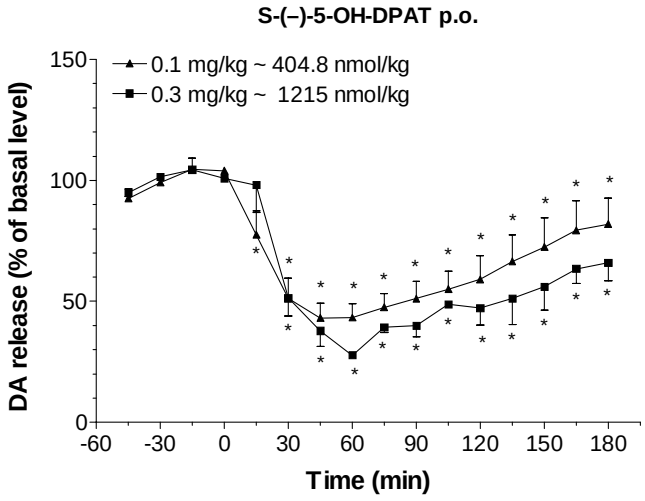


Figure 6.3 Effect of (–)-5-OH-DPAT on striatal dopamine release in freely moving rats after s.c. (A) and p.o. (B) administration. The results are the mean ± S.E.M. of data obtained from 4 rats (* $p<0.05$).

The relative oral bioavailabilities, as determined by comparing the AUCs after s.c. and p.o. administration, of DD9812 and S-(–)-5-OH-DPAT were calculated from data shown in Figures 6.1 and 6.3 and are presented in Table 6.1. The AUCs were calculated by taking the mean of the first four points of the basal level and than determining the area beneath this baseline until the end of the curve. To be able to compare the AUCs after different doses the same time courses of 180 min were used. When the AUCs were not significantly different, the relative oral bioavailability, as expressed in per cent, was determined by dividing the s.c. dose by the p.o. dose and multiplying by 100. For DD9812 the relative oral bioavailability was 10-100 %, while for the reference compound S-(–)-5-OH-DPAT it was 1-3 %.

Table 6.1 AUCs of the microdialysis experiments of DD9812 and S-(–)-5-OH-DPAT after p.o. and s.c. administration.

	Subcutaneous administration		Oral administration		Relative bioavailability
Compound	Dose (mg/kg)	AUC (± S.E.M.)	AUC (± S.E.M.)	Dose (mg/kg)	%
DD9812	0.003	2650 ± 800[a]			
	0.01	6240 ± 1200	7660 ± 960	0.1	10
	0.03	7430 ± 880	7660 ± 960	0.1	30
	0.1	9980 ± 1280	7660 ± 960	0.1	100
	0.1	9980 ± 1280	11260 ± 420	0.3	30
S-(–)-5-OH-DPAT	0.0003	1915 ± 420[b]			
	0.001	4060 ± 930	7190 ± 1270	0.1	1
	0.003	9750 ± 1630	7190 ± 1270	0.1	3
	0.003	9750 ± 1630	8455 ± 860	0.3	1

Footnotes: [a] Experiment lasted 165 min. [b] Experiment lasted 150 min. All other experiments lasted 180 min. All the AUCs of s.c. and p.o. doses of each compound were compared, but only the doses that were not significantly different were put in line in the table.

Statistical analysis of the AUCs of the microdialysis experiments of DD9812 and S-(–)-5-OH-DPAT after p.o. administration showed that there is no significant difference between the effects of the two compounds using the same dose.

6.4 Discussion

The enantiomers of the putative prodrug PD148903 were tested for their pharmacological effects on dopamine autoreceptors *in vivo*, by measuring dopamine release in the striatum using on-line microdialysis in freely moving rats. Stimulation of dopamine autoreceptors by dopamine D_2 receptor agonists cause a decrease in the release of dopamine.[189]

DD9812, which is the S-(–)-enantiomer of the prodrug, induces a dose-dependent decrease in the release of dopamine in the striatum after both s.c. and p.o. administration. This indicates that DD9812 acts as a dopamine receptor agonist *in vivo*. During the microdialysis experiments it was observed that in the highest doses administered (0.1 mg/kg s.c. and 0.3 mg/kg s.c.) DD9812 induced dopaminergic stereotyped behaviour, i.e. sniffing and rearing. DD9813, the R-(+)-enantiomer, induced only a very limited effect on the release of dopamine in the striatum, i.e. only in a high dose (1 mg/kg s.c.) there was a significant but limited decrease in the release

of dopamine. It is uncertain whether this decrease is caused by the R-(+)-enantiomer itself or by a contamination of the S-(–)-enantiomer. The enantiomeric purity of the (+)-enantiomer is >99.8 %. If the impurity of the S-(–)-enantiomer is 0.2 % this would correspond to 0.002 mg/kg of DD9812, which may be an active dose. Because of the limited activity of DD9813 we did not determine its relative oral bioavailability. S-(–)-5-OH-DPAT induced a dose-dependent decrease in the release of dopamine in the striatum after both s.c. and p.o. administration.

Compared to S-(–)-5-OH-DPAT the prodrug DD9812 displayed a lower potency in decreasing dopamine release in the striatum after s.c. administration. This difference in potency might be due to the time course of DD9812 uptake and/or bioactivation, which may influence the responses to the prodrug. On the other hand, the prodrug may also be sensitive to metabolism into inactive metabolites, before its conversion into the active metabolite. It is known from the metabolism of the N,N-dipropylated 2-aminotetralins that they are N-dealkylated.[275] For DD9812 this is also one of the possible routes of metabolism. The prodrug is compared with the very potent dopamine receptor agonist S-(–)-5-OH-DPAT, but this might not be correct since it has not been proven yet that bioactivation leads to this 2-aminotetralin. It is possible that DD9812 is converted into another active hydroxylated 2-aminotetralin. When the p.o. doses are compared the effects are similar, i.e. there are no significant differences in the AUCs for DD9812 and S-(–)-5-OH-DPAT at the same dose administered.

The relative oral bioavailabilities were calculated by measuring the pharmacological effect of the compounds. This is called the pharmacodynamic method of determining pharmacokinetic parameters. Only relative oral bioavailabilities can be determined in this manner. In order to be able to determine the absolute oral bioavailability pharmacokinetic experiments are necessary. DD9812 showed a relative oral bioavailability of 10-100 %. (–)-5-OH-DPAT possessed a low relative oral bioavailability of 1-3 %, which can be explained by the considerable inactivation via glucuronidation in the gut and the liver.[161]

Thus, DD9812 seems to be an active prodrug of a dopamine receptor agonist with a better relative oral bioavailability than the prototypic dopamine receptor agonist S-(–)-5-OH-DPAT. However, the prodrug has a lower potency than S-(–)-5-OH-DPAT in decreasing the release of dopamine in the striatum after s.c. administration. To find out whether DD9812 is an useful prodrug and an alternative for hydroxylated 2-aminotetralins, metabolism studies are in progress to elucidate the mechanism of bioactivation and to find the active species.

Chapter 7

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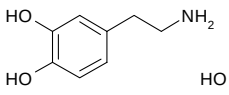
Appendix

Chemical structures of the compounds found in this thesis



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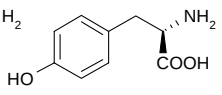

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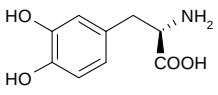

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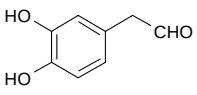

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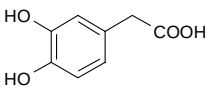

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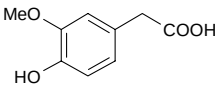

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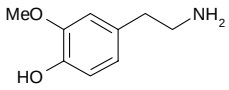

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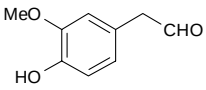

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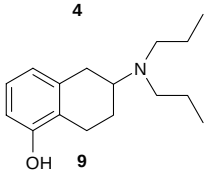

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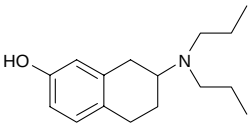

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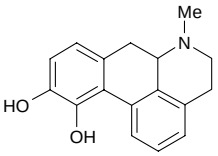

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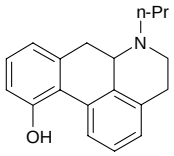

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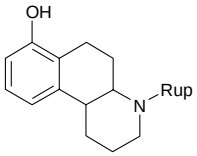

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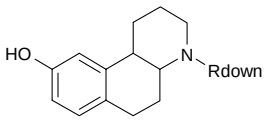

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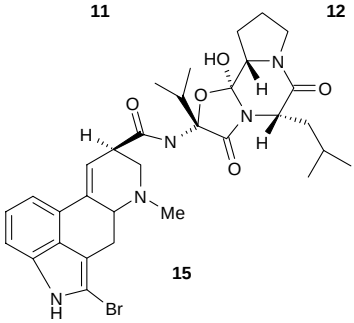

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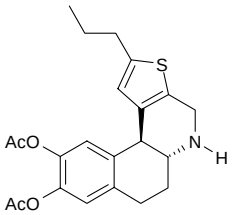

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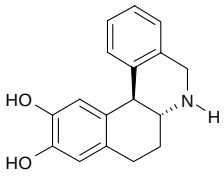

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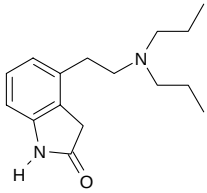

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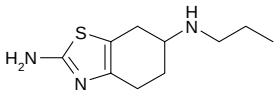

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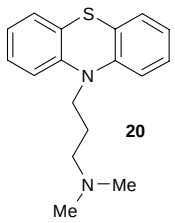

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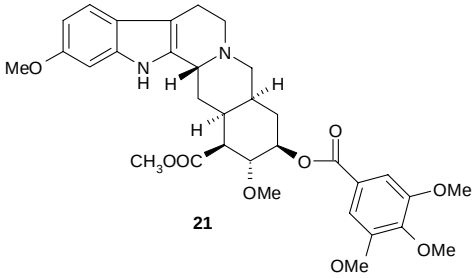

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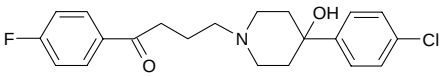

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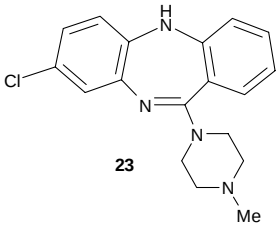

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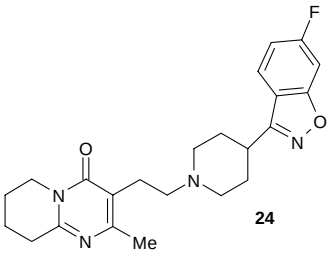

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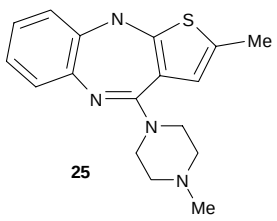

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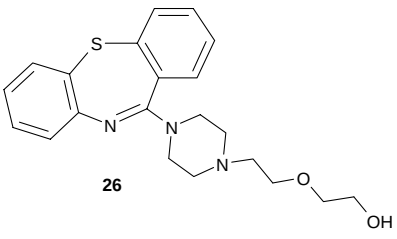

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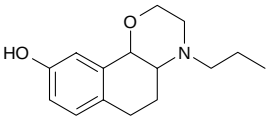

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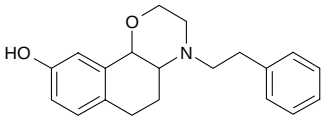

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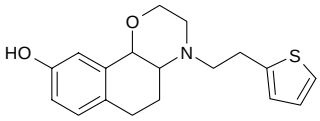

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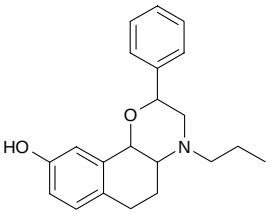

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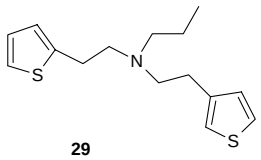

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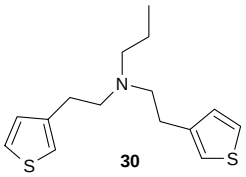

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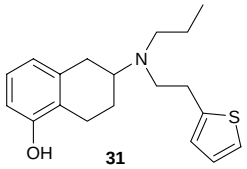

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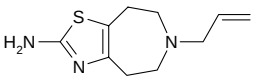

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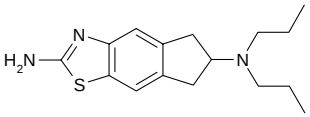

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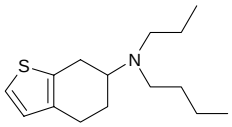

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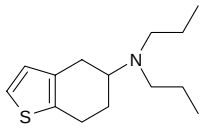

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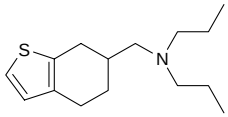

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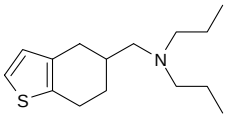

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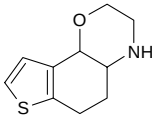

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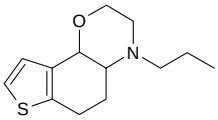

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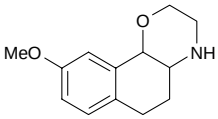

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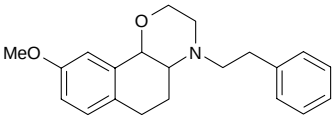

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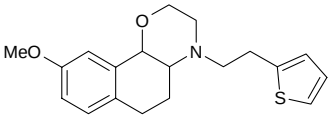

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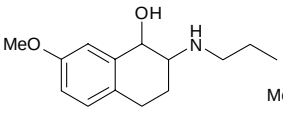

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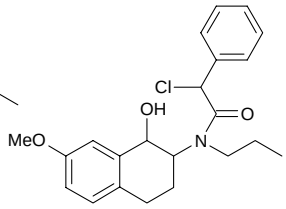

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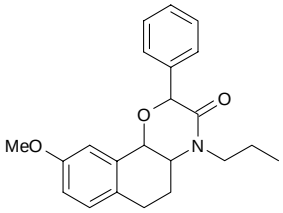

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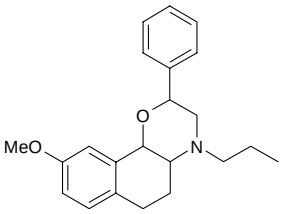

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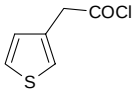

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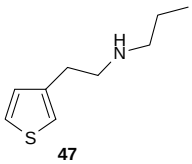

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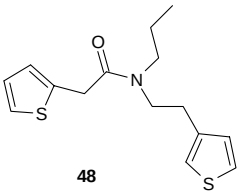

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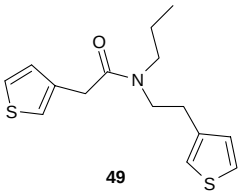

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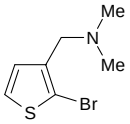

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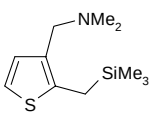

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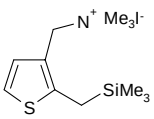

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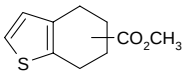

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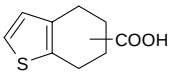

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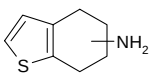

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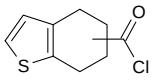
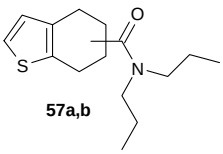


56a,b



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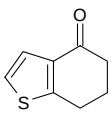

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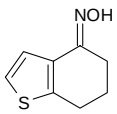

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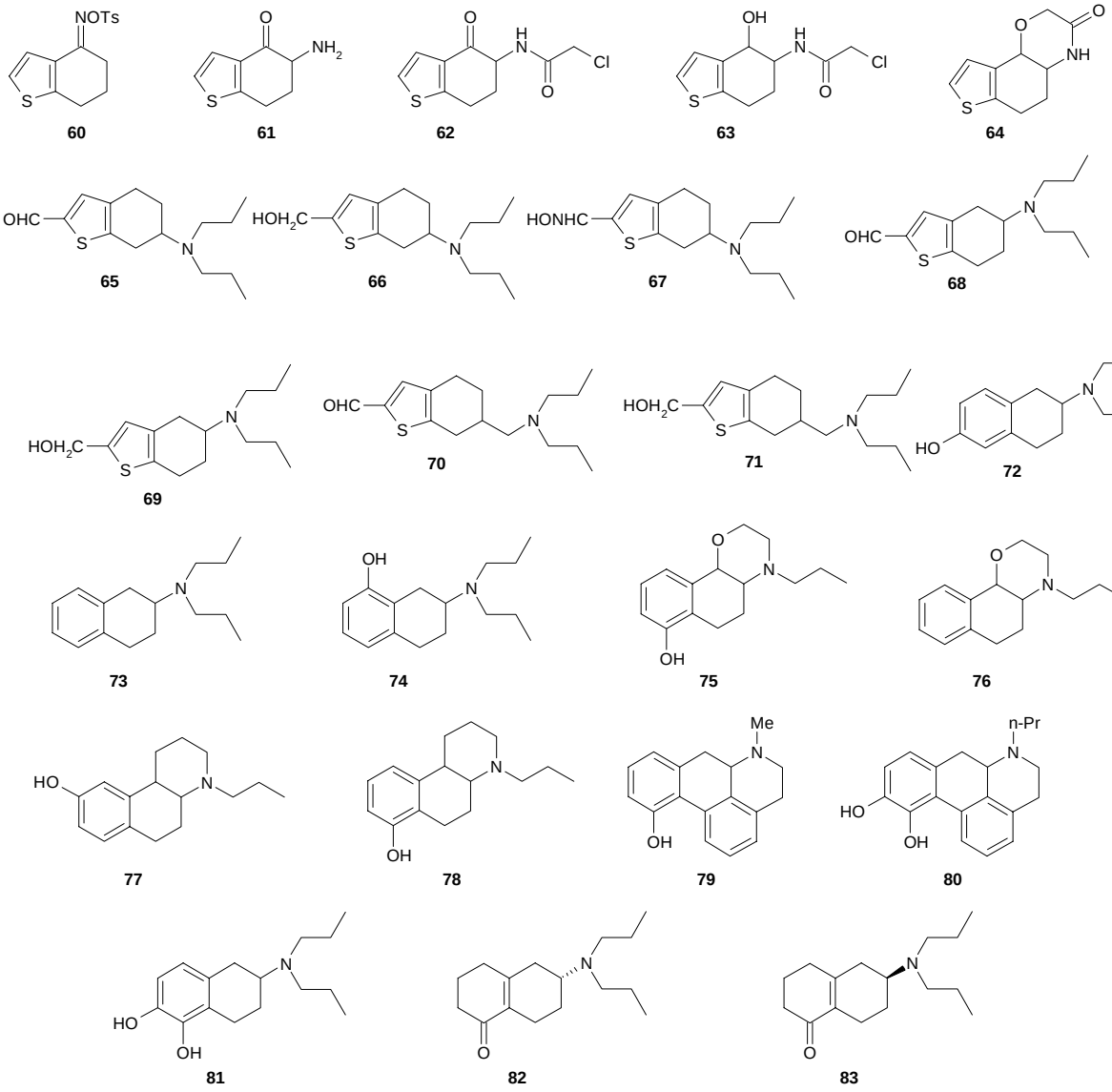
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Concluding remarks

Both newly synthesised and previously known compounds were tested for their affinity at cloned human dopamine D_2 and D_3 receptors. Compounds with an interesting binding profile were selected for *in vivo* testing. For a number of these compounds, their relative oral bioavailabilities were determined.

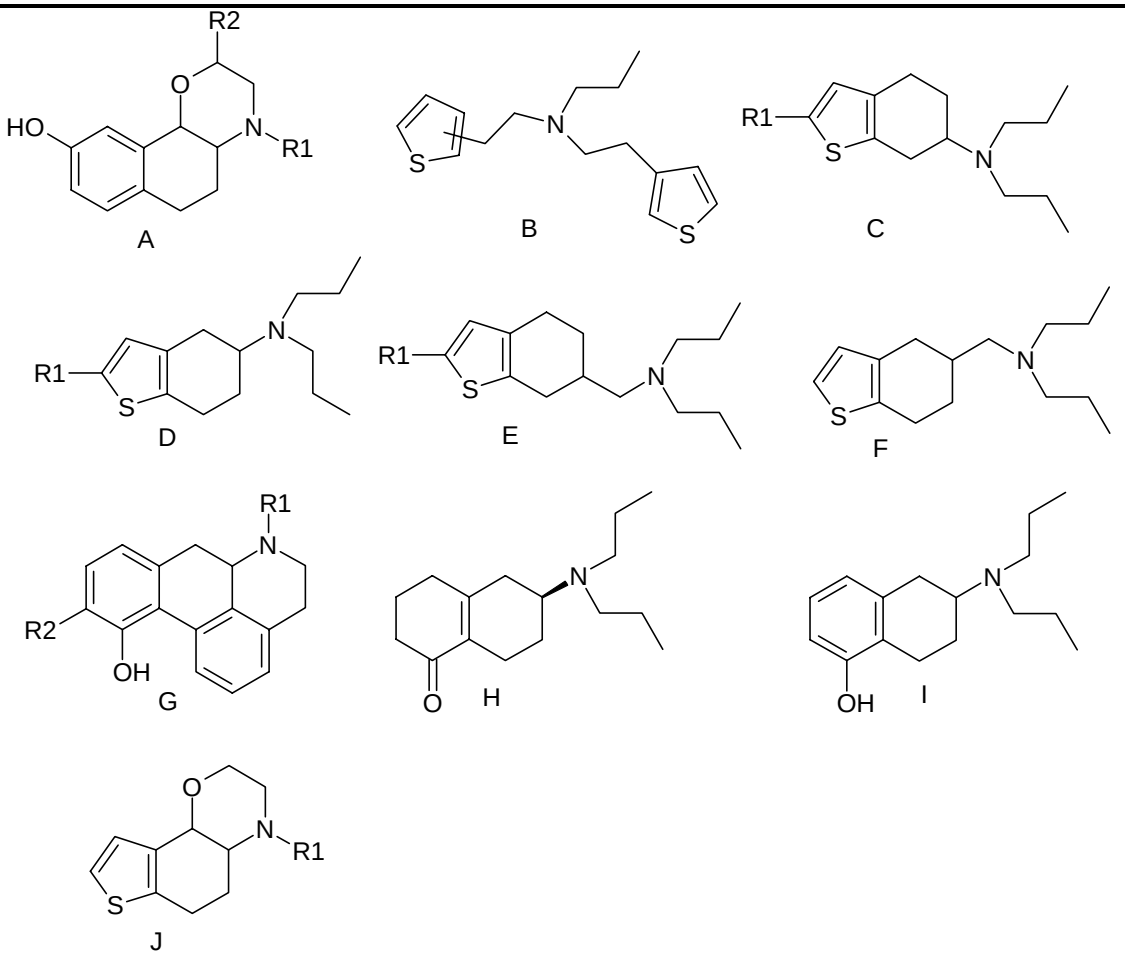
The binding affinities of the hexahydronaphthoxazines of type A showed that a large substituent on the nitrogen gives a compound which lacks affinity for the dopamine receptors (compound **27b**, Table 8.1). However, the compound with a thienylethyl moiety on the nitrogen possesses affinity for the dopamine D_3 receptor. This led us to hypothesise that a thienylethylamine moiety could act as a pharmacophore for the dopamine receptor. To test this hypothesis a series of thiophene containing compounds were synthesised (compounds **29**, **30**, **34** and **35**). These compounds possessed affinity for the dopamine receptors, but their affinities were lower than those of the corresponding phenolic analogues. This diminished affinity might be caused by I) the less tight H-bonding of the sulfur atom, as compared to a hydroxyl moiety; II) the non-optimal distance between the hydrogen bond forming moieties on the aromatic site and the nitrogen atom; III) by the fact that the essential atoms of the ligands have an interaction with alternative interaction points of the dopamine receptor. The results of compounds **29**, **30**, **34** and **35** confirmed the hypothesis that a thienylethylamine can act as a dopamine receptor pharmacophore. Although the distances between the sulfur atom and the nitrogen atom in the hexahydrothianaphthoxazines **38** and **39** are comparable with those in compound **35**, the introduction of a morpholine ring gave a dramatic decrease in the dopamine D_2 and D_3 receptor affinity.[239]

Since the tetrahydrobenzo[*b*]thiophenes **34** and **35** possess a diminished affinity for the dopamine receptors, as compared to hydroxylated 2-aminotetralins, a number of compounds were synthesised of which was expected that they would possess a higher affinity for the dopamine receptors. We have used two methods for such a strategy; i.e. I) increasing the distance between the sulfur and the nitrogen (compounds **36** and **37**); II) introduction of another and better H-bond forming moiety on the 2-position of the thiophene ring (compounds **65**, **66**, **67**, **68**, **69**, **70** and **71**). Introduction of substituents on the 2-position in 6-(N,N-di-*n*-propyl)amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene **34** lead to compounds with no affinity for the dopamine D_2 receptors and moderate to high affinity for the dopamine D_3 receptors. Therefore, they are very selective for the dopamine D_3 receptor. The same introduction in 5-(N,N-di-*n*-propyl)amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene **35** gives compounds with low to no affinity for the dopamine D_2 and D_3 receptors. Such a difference in affinity, which is not seen with the parent compounds **34** and **35**, might be explained from the fact that compounds **65**, **66**, and **67** are structurally comparable with the dopamine D_3 receptor preferring agonist

7-OH-DPAT, while compounds **68** and **69** are structurally more comparable with the less-active dopamine receptor ligand 6-OH-DPAT.[98,205]

Also the introduction of a methylene group between the aliphatic ring and the nitrogen atom in compound **34** gives a compound with selectivity and a moderate affinity for the dopamine D_3 receptor, which resides in the (+)-enantiomer. Substitution of the 2-position of compound **36** gives compounds without affinity for the dopamine D_2 and D_3 receptors.

Table 1 Binding affinities and relative oral bioavailabilities of dopamine receptor ligands used in this thesis.



Compound	Structural type	R1	R2	Ki (nM) D_2	Ki (nM) D_3	Relative oral bioavailability (%)
27a	A	*n*-propyl	H	6.24	0.21	NT
27b	A	phenylethyl	H	> 3676	1566	NT
27c	A	2-thienylethyl	H	3676	83	NT
28	A	*n*-propyl	phenyl	375	12	NT
29	B	2-thienyl		1080	117	NT

Table 1, continued

Compound	Structural type	R1	R2	Ki (nM) D_2	Ki (nM) D_3	Relative oral bioavailability (%)
30	B	3-thienyl		439	108	NT
34	C	H		27	28	10 %
65	C	CHO		>10000[a]	40	NT
66	C	CH_2OH		968	9	NT
67	C	CHNOH		>10000[a]	113	NT
35	D	H		20	40	≥ 10 %
68	D	CHO				NT
69	D	CH_2OH				NT
36	E	H		3107	60	NT
(+)-36	E	H		100/-7	50/43	NT
(–)-36	E	H		100/8	50/17	NT
70	E	CHO				NT
71	E	CH_2OH				NT
37	F			2037	247	NT
11	G	CH_3	OH	3.7		1 %
79	G	CH_3	H	58[a]		NT
80	G	*n*-propyl	OH	1.5		1 %
12	G	*n*-propyl	H	5.3		3 %
83	H					3-30 %
9	I			14	0.54	1-3 %
38	J	H		>4780	3000	NT
39	J	*n*-propyl		630	240	NT

Footnotes: [a] IC_{50}; NT: not tested.

Using microdialysis experiments the relative oral bioavailabilities of the compounds **34**, **35**, **11**, **80**, **12**, **83** and **9** could be calculated. These data show that a compound with a catechol or a phenol possesses a low relative oral bioavailability (compounds **11**, **80**, **12** and **9**). To circumvent such a low relative oral bioavailability a bioisostere of a phenol could be introduced or a prodrug approach could be applied. Compounds **34** and **35** are examples of a bioisosteric replacements and compound **83** is a prodrug of a catecholic or a phenolic 2-aminotetralin. Both types of compounds show an improved relative oral bioavailability, as compared to the hydroxylated 2-aminotetralins.[215,276]

In conclusion, the thienylethylamine moiety can act as a pharmacophore at the dopamine receptor and introduction of such a moiety yields compounds with an improved relative oral bioavailability, as compared to the hydroxylated 2-aminotetralins. The synthesised and tested thiophene analogues of 2-aminotetralins possess a diminished affinity for the dopamine

receptors, but this could be partly compensated by their higher relative oral bioavailability. Also the concept of a new kind of prodrug leads to a compound with a significantly improved relative oral bioavailability. Aporphines are still interesting and potent dopamine receptor agonists, however, they possess a low relative oral bioavailability.

Samenvatting

Eén van de methoden om in de hersenen een signaaloverdracht te bewerkstelligen is het gebruik van neurotransmitters. Eén van deze neurotransmitters is dopamine, waarvan tot midden jaren 50 werd gedacht dat het een tussenproduct was in de biosynthese van de neurotransmitters noradrenaline en adrenaline. Om een signaal te kunnen overdragen grijpt het dopamine aan op dopaminereceptoren. De hersenen bevatten drie belangrijke dopaminerge gebieden, namelijk: 1) het nigrostriatale systeem, dat lichaamshouding en beweging reguleert, 2) het mesolimbische systeem, dat betrokken is bij emotie, cognitie en geheugen, en 3) het tuberoinfundibulaire systeem, dat de prolactine-afgifte reguleert en lactatie en vruchtbaarheid beïnvloedt. Een aantal ziektebeelden, o.a. de ziekte van Parkinson en schizofrenie, worden veroorzaakt door een verstoorde signaaloverdracht in deze dopaminerge systemen. Ook is dopamine waarschijnlijk betrokken bij alcohol- en drugsverslaving.

Een algemene inleiding over dopaminereceptoren en dopamine als neurotransmitter wordt gegeven in **Hoofdstuk 1**. Dopaminereceptoren behoren tot de superfamilie van G-eiwit gekoppelde receptoren. Deze receptoren hebben een aantal gemeenschappelijke eigenschappen, te weten: 1) een membraangebonden receptor; 2) een effector, zoals een ionkanaal of een enzym; en 3) een G-eiwit dat gekoppeld is aan zowel de receptor als de effector (zie voor een illustratie figuur 1.1).

Tot eind jaren 80 waren twee typen dopaminereceptoren bekend, maar met behulp van moleculair-biologische technieken is dit aantal ondertussen uitgebreid tot minimaal vijf subtypen. Gebaseerd op structurele, farmacologische en functionele overeenkomsten worden de dopaminereceptoren verdeeld in twee families, namelijk de D_1-gelijkende receptoren bestaande uit D_1- en D_5-receptoren, en D_2-gelijkende receptoren bestaande uit D_2-, D_3- en D_4-receptoren. Omdat de in dit proefschrift beschreven verbindingen vooral affiniteit bezitten voor de dopamine D_2- en D_3-receptoren, wordt de locatie en functie van deze receptoren nader in dit hoofdstuk besproken. Dopamine D_2-receptoren worden gevonden in hersengebieden welke van oudsher worden geassocieerd met dopaminerge neurotransmissie, zoals het caudate putamen, nucleus accumbens, olfactory tubercle, pituitary, substantia nigra en ventral tegmental area. Dopamine D_2-receptoren kunnen gekoppeld zijn aan verschillende signaaltransductiesystemen. Dopamine D_3-receptoren worden voornamelijk gevonden in het limbisch gebied, dat te maken heeft met cognitieve, emotionele, neuro-endocriene en autonome functies. Dopamine D_3-receptoren zijn gekoppeld aan verschillende signaaltransductiesystemen; de functie van dopamine D_3-receptoren is nog niet geheel duidelijk.

Verder wordt in **Hoofdstuk 1** kort ingegaan op de structuur-activiteits relaties van dopaminereceptor-agonisten. De ontwikkeling van structuur-activiteits relaties gebeurt enerzijds via een conjunctieve benadering, uitgaande van dopamine en anderzijds via een disconjunctieve

benadering, uitgaande van de dopamine agonist apomorfine. De gemeenschappelijke farmacofoor, die via deze twee methoden gevonden wordt, bezit een fenylethylamine structuur.

Een aantal ziektebeelden wordt in verband gebracht met het niet goed functioneren van het dopaminerge systeem. Bij de ziekte van Parkinson is een groot deel van de dopaminerge neuronen van het nigrostriatale systeem verloren gegaan, zodat er een tekort aan de neurotransmitter dopamine is. Dit kan worden opgelost door een precursor van dopamine toe te dienen of een dopaminereceptor-agonist te geven. Het ontstaan van psychosen (o.a. bij schizofrenie) is niet helder, maar aangenomen wordt dat één van de oorzaken een verhoogde dopaminerge neurotransmissie is. Antipsychotica moeten dus in staat zijn om de dopaminerge functie te verlagen; dit kan met behulp van dopaminereceptor-antagonisten.

Vele verbindingen met een farmacologisch interessant profiel bezitten een fenol- of een catechol-functie. Het nadeel van dergelijke verbindingen is dat ze een lage biologische beschikbaarheid hebben. In dit proefschrift wordt een tweetal methoden besproken om de orale biologische beschikbaarheid te verbeteren, te weten: 1) bioisostere vervanging van het fenol/catechol-gedeelte; 2) synthese van prodrugs van fenolen of catecholen. Bioisosteren zijn verbindingen of functionele groepen welke bijna identieke moleculaire vormen en volumes hebben en vergelijkbare elektronverdeling en fysische eigenschappen bezitten. Prodrugs zijn inactieve verbindingen die in het lichaam worden omgezet in actieve verbindingen.

Tot slot worden in **Hoofdstuk 1** de technieken besproken die in dit proefschrift gebruikt zijn om de gesynthetiseerde verbindingen *in vivo* te testen. Deze technieken zijn microdialyse in de hersenen, waarbij de invloed van nieuwe verbindingen op de afgifte van dopamine wordt bekeken en locomotor-activiteit en gedragseigenschappen in met reserpine behandelde ratten. Reserpine verstoort de opslag van dopamine in de blaasjes. Na toediening van reserpine worden de blaasjes acuut geleegd en wordt het proefdier motorisch sterk geremd; het vertoonde gedrag wordt dan alleen veroorzaakt door de geteste verbinding.

In **Hoofdstuk 2** wordt de synthese van hexahydronaftoxazines, thienylethylamines, tetrahydrobenzo[*b*]thiofenen en hexahydrothianaftoxazines beschreven. Verder wordt in dit hoofdstuk de affiniteit van deze verbindingen voor dopamine D_2- en D_3-receptoren gegeven. De resultaten tonen aan dat een thienylethylamine-structuur als farmacofoor voor de dopaminereceptor kan dienen. Voor de tetrahydrobenzo[*b*]thiofenen, waar het thienylethylamine-gedeelte deel uitmaakt van een star systeem, wordt een verhoogde affiniteit voor de dopamine D_2- en D_3-receptor gevonden. Is het thienylethylamine-gedeelte echter gefixeerd in een tricyclisch systeem, zoals in de hexahydrothianaphthoxazine structuur, dan is de affiniteit voor de dopamine D_2- en D_3-receptoren verminderd.

Hoewel een aantal van de in hoofdstuk 2 beschreven verbindingen affiniteit bezit voor de dopamine D_2- en D_3-receptoren is deze affiniteit vergeleken met 5-OH-DPAT toch significant lager. Daarom wordt in **Hoofdstuk 3** de synthese van een aantal verbindingen beschreven met een substituent met betere waterstofbrugvormende eigenschappen, op de 2-postitie van de thiofeenring, met als doel verbindingen met een hogere affiniteit voor de dopamine D_2- en D_3-

receptoren te creëren. Uit de bindingsresultaten blijkt dat introductie van een substituent op de 2-positie van 6-(N,N-di-*n*-propyl)amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene verbindingen geeft die geen affiniteit voor de dopamine D_2-receptor bezitten, maar een verhoogde of gelijke affiniteit voor de dopamine D_3-receptor. Introductie van substituenten op de 2-positie van 5-(N,N-di-*n*-propyl)amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene geeft verbindingen zonder affiniteit voor de dopamine D_2- en D_3-receptoren. Het vergroten van de afstand tussen het zwavel- en het stikstofatoom door middel van een methyleen groep tussen de alifatische ring en het stikstofatoom geeft een verbinding die affiniteit voor de dopamine D_3-receptor bezit, maar niet meer voor de dopamine D_2-receptor. Na het splitsen van de enantiomeren bleek de (+)-enantiomeer de actieve verbinding te zijn. Introductie van substituenten op de 2-positie van deze verbinding geeft inactieve verbindingen. Omdat de dopamine D_2- en D_3-receptoren zoveel op elkaar lijken is het moeilijk om de gevonden verschillen in selectiviteit van de nieuwe verbindingen te verklaren. De 2-gesubstitueerde tetrahydrobenzo[*b*]thiofenen kunnen dienen als uitgangspunten voor de ontwikkeling van afzonderlijke dopamine D_2- en D_3-receptormodellen.

Daar uit de resultaten van hoofdstuk 2 blijkt dat een thienylethylamine kan dienen als farmacofoor voor de dopaminereceptor, wordt in **Hoofdstuk 4** van de tetrahydrobenzo[*b*]thiofenen het effect op de dopaminereceptoren en hun orale biologische beschikbaarheid bepaald. Met behulp van microdialyse en het reserpinemodel is gevonden dat de tetrahydrobenzo[*b*]thiofenen een lagere *in vivo* activiteit bezitten dan 5-OH-DPAT. De introductie van een thiofeen gedeelte geeft echter een significante verbetering in de relatieve orale biologische beschikbaarheid, vergeleken met 5-OH-DPAT. Uit de *in vivo* resultaten blijkt dat de tetrahydrobenzo[*b*]thiofenen als uitgangsstoffen kunnen dienen voor de ontwikkeling van nieuwe dopaminereceptor-liganden met een verbeterde orale biologische beschikbaarheid.

In **Hoofdstuk 5** worden de microdialyseresultaten besproken van R-(–)-apomorfine, R-(–)-11-hydroxyaporfine, R-(–)-N-*n*-propylapomorfine and R-(–)-11-hydroxy-N-*n*-propylaporfine. Met behulp van microdialyse is het effect op de dopamine-afgifte van deze verbindingen bepaald. Bovendien is door vergelijking van de resultaten na subcutane en orale toediening de relatieve orale biologische beschikbaarheid bepaald. De analogen R-(–)-N-*n*-propylapomorfine en R-(–)-11-hydroxy-N-*n*-propylaporfine blijken beter in staat te zijn om de dopamine-afgifte in het striatum te verlagen dan R-(–)-apomorfine. Alleen een hoge dosis R-(–)-11-hydroxyaporfine was in staat om de dopamine-afgifte in het striatum te verlagen. Uit de literatuur bleek echter dat deze verbinding een partiële dopaminereceptor-agonist was. Onze resultaten bevestigen deze vinding. Door het beperkte effect op de dopamine-afgifte was het niet mogelijk met behulp van de microdialyse techniek de orale biologische beschikbaarheid van R-(–)-11-hydroxyaporfine te bepalen. De catecholen R-(–)-apomorfine en R-(–)-N-*n*-propylapomorfine bezitten een vergelijkbare relatieve orale biologische beschikbaarheid (1 %), terwijl de monohydroxy-analoog R-(–)-11-hydroxy-N-*n*-propylaporfine een iets hogere relatieve orale biologische beschikbaarheid (3 %) bezit. R-(–)-N-*n*-propylapomorfine en R-(–)-11-hydroxy-N-*n*-propylaporfine vertonen dus geen substantiële verbetering in de orale biologische

beschikbaarheid. Echter, door het feit dat R-(–)-N-*n*-propylapomorfine en R-(–)-11-hydroxy-N-*n*-propylaporfine beter in staat zijn om de dopamine-afgifte te verlagen, kunnen het nuttige alternatieven zijn voor apomorfine tijdens de behandeling van de ziekte van Parkinson.

Hoofdstuk 7 behandelt de neurofarmacologische evaluatie van een nieuwe dopaminereceptor-prodrug. Zoals bekend is uit de literatuur bezit 5-OH-DPAT een hoge affiniteit voor dopaminereceptoren. Het nadeel van deze verbinding is dat het een lage orale biologische beschikbaarheid heeft. Een gebruikte methode om de orale biologische beschikbaarheid van fenolische verbindingen te verhogen is het synthetiseren van prodrugs, bijvoorbeeld esters of carbamaten. In dit hoofdstuk wordt het effect van een nieuw soort prodrug, een dihydro-analoog van 5-OH-DPAT, op de afgifte van dopamine in het striatum besproken. De beide enantiomeren van deze verbinding zijn getest in microdialyse-experimenten na subcutane en orale toediening en zijn vergeleken met S-(–)-5-OH-DPAT. De actieve S-enantiomeer van de prodrug (DD9812) is na subcutane toediening in staat om de afgifte van dopamine in het striatum significant te verlagen. De verbinding is enantioselectief, want de R-enantiomer (DD9813) heeft nauwelijks effect op de afgifte van dopamine in het striatum. De prodrug vertoont een verbeterde relatieve orale biologische beschikbaarheid, vergeleken met S-(–)-5-OH-DPAT.

In de **conclusies** wordt nog eens samengevat wat de bindingsaffiniteiten en orale biologische beschikbaarheden van de verschillende onderzochte verbindingen zijn. Dit onderzoek heeft een aantal verbindingen opgeleverd welke een veel betere orale biologische beschikbaarheid bezitten dan 5-OH-DPAT. Deze stoffen kunnen dienen als uitgangsstoffen voor de ontwikkeling van betere en selectievere liganden voor de dopamine D_2- en D_3-receptoren.

STELLINGEN

1 Thienylethylamine kan dienen als farmacofoor voor dopaminereceptoren (dit proefschrift).

2 Gezien het grote verschil in bindingsresultaten tussen de verschillende laboratoria verdient het aanbeveling om een afspraak te maken met één onderzoekslaboratorium, dat deze gedurende het gehele onderzoek de bindingsproeven zal uitvoeren.

3 Bindingsresultaten die met radioactief gelabeld spiperone zijn bepaald voor agonisten, zijn erg vertekend.

4 De door de industrie betaalde onderzoekers kunnen moeilijk onafhankelijke en objectieve resultaten publiceren. (n.a.v. Kantlijn in de Volkskrant).

5 Ondanks een bloeiende scheepvaartindustrie in de provincie Groningen blijkt het niet mogelijk om van de Eemshaven een goed draaiende zeehaven te maken.

6 Hoewel Nederland bekend staat als watersportland zijn grote bedrijven niet erg watersport-minded.

7 Toen Rinus Michels begin jaren zeventig zei: “Voetbal is oorlog.” kon hij niet weten dat dit 20 tot 30 jaar later het geval zou zijn tussen supportersgroepen.

8 Groningers vinden het vreemd dat ze subsidiepotjes voor Noord-Nederland moeten delen met Friesland en Drenthe.

9 De millenniumbrug in Londen is waarschijnlijk de enige millenniumbug.

10 Hoewel in alle media wordt gesproken over een tekort op de arbeidsmarkt, wordt (om)scholing of het zoeken van een baan door de arbeidsbureaus niet gestimuleerd.

11 Een promotieonderzoek is vier jaar hard werken om de “s” in je titel weer kwijt te raken.

Nienke Rodenhuis Groningen, 23 oktober 2000

Dankwoord

Na ruim vijf jaar is het dan zover. Op 23 oktober ga ik eindelijk promoveren. Naast al het werk dat ik zelf heb gedaan zijn er ook een aantal mensen geweest die in meer of mindere mate hebben bijgedragen aan het tot stand komen van dit proefschrift. Deze mensen wil ik op deze plaats bedanken.

Ten eerste wil ik mijn promotor Håkan Wikström bedanken, maar dan tegelijk ook mijn referent Durk Dijkstra. Samen hebben jullie me de kans gegeven om bij jullie vakgroep te promoveren en kon ik altijd bij jullie terecht met vragen en problemen. Ook wil ik jullie bedanken voor het nakijken van de manuscripten.

I would like to thank Prof. A. IJzerman, Prof. D. Nichols and Prof. T. Svensson for taking part in the evaluation committee for this thesis, for the time and effort you have put in reading the manuscript and for their useful suggestions.

Medicinal Chemistry is a multidisciplinary science and therefore you have to collaborate with other scientists. I would like to thank Tom Pugsley and Larry Wise from Parke Davis, Michelle Kagey, Chris Haber and Kjell Svensson from Pharmacia & Upjohn and Karina Søbye and Ejner Moltzen from Lundbeck for providing me with the binding data of all compounds.

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