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Chapter

ELUCIDATING THE STRUCTURE-ACTIVITY RELATIONSHIP OF CURCUMIN AND ITS BIOLOGICAL ACTIVITIES

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ABSTRACT

Curcumin is a major constituent of the turmeric plant *Curcuma longa*, a member of the Zingiberaceae family, which is cultivated in India, most parts of Southeast Asia, Asia and other parts of the world. Curcumin has been shown to afford a wide range of pharmacological activities encompassing antioxidative, anti-inflammatory, antibacterial, antifungal, antiviral, antiproliferative, proapoptotic and anti-atherosclerotic effects as well as medicinal benefits against neurodegenerative diseases, arthritis, allergy, inflammatory bowel disease, nephrotoxicity, AIDS, psoriasis, diabetes, multiple sclerosis, cardiovascular disease and lung fibrosis. Moreover, curcumin could suppress inflammatory cytokines as well as suppress various target proteins in cancer cell lines. Owing to its multi-faceted health benefits, curcumin has been used as health supplements as well as natural remedy while several clinical trials are under way to investigate its potential therapeutic usage. This chapter discusses the origins of curcumin's biological activities in light of its structure-activity relationship. The structure of curcumin is comprised of the central 1,6-heptadiene-3,5-dione bearing two terminal phenolic rings.

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Structural modification of this compound alters its biological activities either by affecting its selectivity, specificity or potency. Understanding of such structure-activity relationship may provide the impetus for further expanding its biological activity repertoire. Although it is an ambitious task to review the current state-of-the-art on the structure-activity relationship of curcumin, it should be mentioned that it is impossible for this chapter to provide a comprehensive account but rather a representative overview is given herein.

Keywords: curcumin; turmeric; structure-activity relationship; SAR; QSAR

LIST OF ABBREVIATIONS

ADMET, absorption, distribution, metabolism, excretion and toxicity;
AGEs, advanced glycation end-products;
AIDS, acquired immunodeficiency syndrome;
ALR2, aldose reductase 2;
AMPK, AMP-activated protein kinase;
ANN, artificial neural network;
AP-1, activator protein 1;
AR, androgen receptor;
BACE1, β-site amyloid precursor protein cleaving enzyme 1;
Bcl-2, B-cell lymphoma 2;
BDMC, bisdemethoxy curcumin;
CHIP, carboxyl terminus of Hsc70-interacting protein;
Cu, copper;
COX, cyclooxygenase;
DAC, diacetyl curcumin;
DMC, dimethoxycurcumin;
DT, decision tree;
DR, death receptors;
DNMT, DNA methyltransferase;
EC, European Commission;
ER, estrogen receptor;
ERK, extracellular signal-regulated kinase;
EGFR, epidermal growth factor receptor;
HER2/neu, human epidermal growth factor receptor 2/proto-oncogene Neu;
FN, fibronectin;
Fe, iron;
GLO1, glyoxalase 1;
GSK, glycogen synthase kinase;
g, gram;
h, hour;
HAT/p300, histone acetyltransferase/p300;
IκBα, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha
iNOS, inducible nitric oxide synthase;

IUPAC, International Union of Pure and Applied Chemistry;
JAK/STAT, Janus kinase/signal transducer and activator of transcription;
JECFA, Joint FAO/WHO Expert Committee on Food Additives;
kg, kilogram;
L, Liter;
LC-MS/MS, liquid chromatography-mass spectrometry;
LOX, Lipoxygenase;
mg, milligram;
MMPs, matrix metalloproteinases;
MLR, multiple linear regression;
MM-PBSA, molecular mechanic Poisson-Boltzmann surface area;
MD, molecular dynamics;
MAPK, mitogen-activated protein kinases;
MD-2, myeloid differentiation protein 2;
NF-κB, nuclear factor kappa B transcription factor;
NSAIDs, nonsteroidal anti-inflammatory drugs;
NO, nitric oxide;
nNOS, neuronal nitric oxide synthase;
NOS, nitric oxide synthase;
OH^-, hydroxide;
PLS, partial least squares;
PrP, prion protein;
PCA, principal component analysis;
PPAR-γ, peroxisome proliferator-activated receptor;
PI3K/Akt/mTOR, phosphoinositide3-kinase/protein kinase B/mammalian target of rapamycin;
PKC, protein kinase C;
QSAR, quantitative structure-activity relationship;
QSPR, quantitative structure-property relationship;
RF, random forest;
RNase, ribonuclease A;
ROS, reactive oxygen species;
SULTs, sulfotransferases;
SVM, support vector machine;
TNF, tumor necrosis factor;
TGF-β, transforming growth factor-beta;
TRAIL, TNF-related apoptosis-inducing ligand;
THC, tetrahydrocurcumin;
TRE, TPA responsive element;
TLR4, toll-like receptor 4;
PR, progesterone receptor;
UPS, ubiquitin-proteasome system;
UGTs, UDP-glucuronosyltransferases;
VDR, vitamin D receptor;
VEGF, vascular endothelial growth factor;
XO, xanthine oxidase;

μM, micromolar;
μg, microgram;
1,25D, 1α,25-dihydroxyvitamin D3;
3D-QSAR, three-dimensional quantitative structure-activity relationship.

INTRODUCTION

Historically, the turmeric plant *Curcuma longa*, belonging to the ginger family Zingiberaceae, has been used in India for more than 5,000 years [1] as it is a major consumer and exporter of turmeric accounting for an approximately 80% of its production. It can be cultivated in many parts of the country: Southern (Tamil Nadu and Andhra Pradesh), Central (Maharashtra) and Eastern (West Bengal) parts of the country [2]. Furthermore, turmeric plants are grown in other countries as well namely China, Thailand, Taiwan, South America and the Pacific islands. In 1280, Marco Polo who was enlightened by turmeric wrote in his journal as follows: "*Turmeric is a vegetable, contains all the properties of saffron and also has the color and smell, yet it is not actually saffron"* [1]. From its origin in India, by 700 BC turmeric reached the coast of China, then after 100 years reached East Africa, 500 years later to West Africa. During the 13th century, Arabs took the Turmeric plant by trade to the European continent [3]. In the 15th century, Vasco de Gama (a Portuguese sailor) also introduced spices to the West during his visit to India.

Turmeric has long been used by the Indian system of medicine or Ayurveda as remedies for curing a plethora of ailments such as cough, inflammation, rheumatism, diarrhea, indigestion, anemia, atherosclerosis, diabetes, edema, haemorrhoids, hepatitis, liver disorder, hysteria, skin disease, urinary disease, sinusitis, anorexia, psoriasis, wound and bruise healing [4]. As for its culinary utilizations, curcumin has been the essential spice for several different curry dishes as well as being used as food additives, coloring agent and flavor enhancer. Particularly, curcumin has been used throughout the world in various cuisines encompassing Indian, Thai, Chinese, Malaysian and Indonesian [5]. Aside from being used in culinary, curcumin has found textile usage as color dyes and in fact they are much better suited for human as synthetic dyes afford unwanted properties such as being carcinogenic, allergenic and non-biodegradable [6]. The use of curcumin as a color dye in cotton and wool fabrics not only give rise to strong yellow color but also possess antimicrobial activity [7, 8]. In regards to the former, Bhatti et al. reported that γ radiation causes an increase in the color strength and fastness of curcumin-dyed cotton fabric [9].

Skin acts as a major barrier that shields the body against harmful sunlight, helps to regulate temperature, offers protection against chemicals, ultraviolet rays and harmful microbes. Ever since ancient times, curcumin has been considered to be a popular cosmetic among Indian women as it has been used for skin nourishment [10]. Khiljee et al. [11] claimed that curcumin is prominently used in the treatment of eczema, which is the inflammation of the skin characterized by itchiness, redness and swollen skin. Addtionally, Saraf et al. [12] stressed that curcumin could be used to treat phototoxicity and photoallergic reactions arising from photosensitization as well as help the rejuvenating process of the skin as it has the ability to fight against free radicals. Furthermore, curcumin has also found prominent usage as home remedies. For instance, curcumin can be mixed with boiled milk to

cure various respiratory diseases. In addition, women in their postpartum period of Northern India ingest drinks made of turmeric paste, ginger roots, honey and boiled milk twice a day. Furthermore, curcumin paste can also be applied to cuts, eye infections, bites, abrasions, aces and various infections [13]. Not only that, this natural remedy can also be used to treat various dental diseases, indigestion, asthma and hallucination from the effects of hashish, the recreational drug from *Cannabis sativa* and *C. indica* plants that contain over 60 psychoactive compounds [5]. As will be discussed in a further section in more detail, curcumin has been shown to be linked to various beneficial activities such as antiinflammatory, antioxidant activities, chemopreventive, chemotherapeutic, radiosensitization, radioprotection, etc. [14].

The ability to understand the compositions that make up the yellow color turmeric could further be used in improving their properties. As such, the first attempt to elucidate the composition of turmeric was performed by Leach in 1904 [15] in which he roughly described that *"the chief ingredients of turmeric are starch, a slightly fluorescent, orange-yellow, volatile oil, a deep yellow coloring-matter (curcumin), soluble in alcohol, but insoluble in cold water, cellulose and a gum."* Subsequent studies in the late and early 2000 addressed this issue by investigating the chemical composition of turmeric [16-18]. Afterwards, Braga et al. [19] studied the effect of different solvent extraction methods on yield, composition and antioxidant activity of turmeric. Bansal et al. [20] characterized the different chemical composition of essential oils of the shoot organs, rhizomes and rhizoids in turmeric. Efforts have also been invested in studying the chemical compositions in fresh and dried forms of turmeric [21].

Notably, turmeric are enriched with the following compounds: curcumin, demethoxycurcumin (DMC), bisdemethoxycurcumin (BDMC), eugenol, dihydrocurcumin, azulene, borneol, D-camphene, caprylic acid, cineol, turmerone and zingiberine [22]. The former three are phenolic compounds that constitute what is known as curcuminoids (Figure 1) and gives rise to the characteristic yellow color of turmeric.

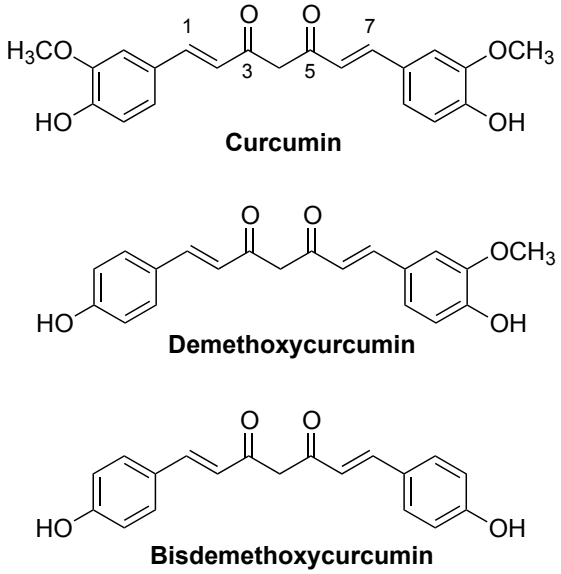

Figure 1. Chemical structure of curcuminoids: curcumin, demethoxycurcumin and bisdemethoxycurcumin.

I. CHEMISTRY OF CURCUMIN

Curcumin was first described by Trommsdorff [23] in 1808, isolated in impure form from the rhizomes of turmeric in 1818 by Vogel and Pelletier [24] and in the crystalline form in 1870 by Daube [25]. Its chemical structure was first described by Milobedeska and Lampe [26] in 1910, which was shortly followed by its synthesis by the same group [27] in 1913.

Curcumin has a molecular weight of 368.37 Da and is chemically known by its IUPAC name as 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione or simply as diferuloylmethane. It is a member of the diarylheptanoid class as well as being the first diarylheptanoid ever to be discovered. As the name implies, this class is comprised of two aromatic rings (i.e. aryl groups) linked by 1,6-heptadiene-3,5-dione moiety. Diarylheptanoid constitute a class of plant secondary metabolites comprising of over 409 compounds from more than 50 plants as compiled from the literature by Lv and She [28, 29]. These chemotypes are mainly distributed in the roots, rhizomes and bark of *Alpinia*, *Zingiber*, *Curcuma* and *Alnus* species.

Curcumin exists in multiple tautomeric states in which the diketone is stable in the enol state while being easily deprotonated to the keto state (Figure 2).

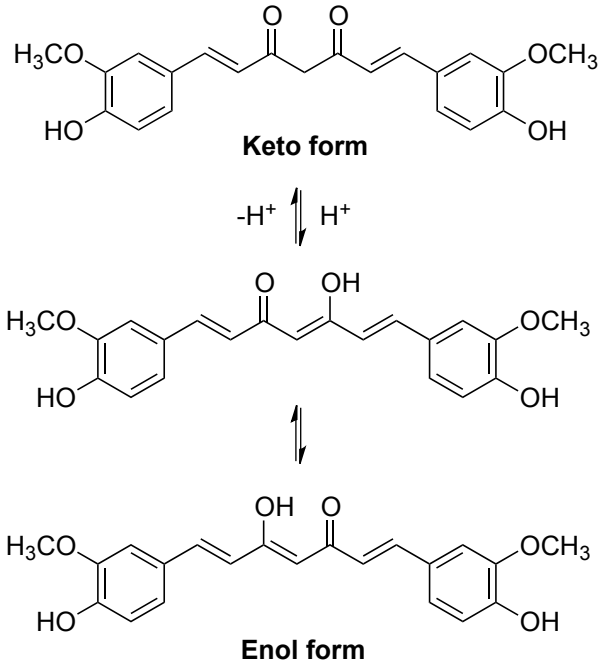

Figure 2. Chemical structures of keto and enol forms of curcumin.

As β-diketone facilitates the deprotonation of active methylene proton, this generates carbanion where electrons on the carbon atom can be delocalized to oxygen atoms of the keto groups. Under acidic to neutral pH ranges of 3-7 curcumin can exist in the enol form [30]. In strike contrast, under basic pH condition of 8 or more, the enolate form of curcumin is more prevalent. Furthermore, the α,β-unsaturated ketone of the curcumin is also known to be a good Michael acceptor that can readily undergo nucleophilic addition.

In regards to the solvent extraction of curcumin from turmeric, the JECFA monograph for curcumin (FNP 52 Add. 9, 2001) reported the following as appropriate solvents: acetone, methanol, ethanol, and isopropanol [31]. In addition, the European Commission Directive 95/45/EC reported the following solvents: acetone, supercritical carbon dioxide, ethyl acetate, dichloromethane, *n*-butanol, methanol, ethanol and hexane [32]. Curcumin is subsequently recovered by crystallization from the solvent extract where minor amounts of oils and resins may be inherently present in turmeric [33].

Historically, curcumin represents the first synthesis of natural diarylheptanoid as described by Lampe and co-workers [27, 34]. Owing to the simplicity of the retrosynthetic approach, curcumin can also be synthesized via the condensation of acetyl acetone (as *in situ* generated boronated complex) with two equivalent of aromatic aldehyde (vanillin) [35] as summarized in Figure 3.

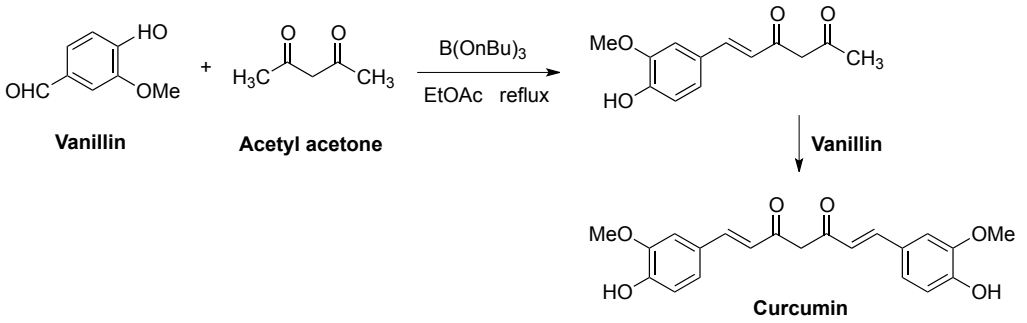

Figure 3. Synthesis of curcumin by condensation of acetyl acetone and vanillin.

1) Naturally Occurring Curcumin

Curcumin and its derivatives (i.e. bisdemethoxycurcumin and demethoxycurcumin) are collectively known as curcuminoids and are found in the rhizomes of turmeric, particularly *Curcuma longa* Linn. as well as in *Curcuma* spp. In addition to curcuminoids, *Curcuma* spp. also contains other substances such as turmerin (a water-soluble 40 amino acid peptide) [36] and essential oils. Comparatively, curcuminoids are the most bioactive components as it was found to afford a wide variety of pharmacological activities [37]. More specifically, curcumin has been shown to afford more potent biological activities than bisdemethoxycurcumin and demethoxycurcumin [38].

1.1. Chemical Stability

Curcumin is quite unstable at basic pH and it readily degrades within 30 min to form *trans*-6-(4′-hydroxy-3′-methoxyphenyl)-2,4-dioxo-5-hexanal, ferulic acid, feruloylmethane and vanillin [39]. However, at acidic pH, curcumin degrades much slower with less than 20% decomposing in 1 h [40]. Complex kinetics of pH-dependent degradation of curcumin in aqueous solution was first reported by Tonnesen and Karlsen [41]. Interestingly, the presence of fetal calf serum or human blood, the addition of antioxidants (i.e. ascorbic acid, N-acetylcysteine or glutathione) could had been shown to prevent its degradation in culture media or phosphate buffer with basic pH [40]. Furthermore, curcumin has been shown to be

rather photochemical sensitive and should therefore be protected from light [12]. A study by Suresh et al. [42] suggested that during domestic cooking the chemical structure of curcumin was degraded to ferulic acid, vanillin and vanillic acid. On the other hand, Dahmke [43] reported that cooking enhances the anticancer activity of curcumin by pyrolytic formation of so-called deketene curcumin (1,5-bis(4-hydroxy-3-methoxyphenyl)-1,4-pentadien-3-one). Taken together the thermal effects appear to alter the chemical structure of curcumin and in the former study by Suresh et al. the three metabolites that are formed have also been reported elsewhere in the literature [44, 45] to exhibit antioxidant activities irrespective of the pyrolytic formation as proposed in the latter study.

1.2. Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) Properties

1.2.1. Absorption

The uptake, distribution and excretion properties of curcumin was first reported in 1978 by Wahlstrom and Blennow [46] in an experiment performed on Sprague–Dawley rats in which they were administered 1g/kg of curcumin. Results indicated that there were insignificant amounts of curcumin in blood plasma and that it was poorly absorbed from the gut.

Ravindranath and Chandrasekhara [47] orally administered 400 mg of curcumin to rats and found that 60% of the dose could be absorbed. It was observed that no curcumin could be found in heart blood, trace amount of less than 5 μg/mL was found in the portal blood, small amounts of <20 μg/tissue could be measured in the liver and kidney while after 24 h of administration 38% of the dose could be found in the cecum and large intestine. Furthermore, it was also measured in urine upon applying a dose of 3.6 g/day. Absorption was found to be quite poor after ingestion in mice and rats with excretion of 38-75% of ingested dose as feces. However, absorption was found to improve when taken with food. On a different note, a clinical trial carried out in Taiwan indicated that the levels of serum curcumin maximally increased in one to two hours after oral dosage to 0.5, 0.6 and 1.8 μM at doses of 4, 6 and 8 g/day, respectively [48]. The poor bioavailability of curcumin can be attributed to its biotransformation in the intestine and liver to form curcumin glucuronides [49].

1.2.2. Distribution

The lipophilic nature of curcumin confers its ability to pass through cell membranes as well as cross the blood brain barrier to exert its intracellular effects. For example, this ability allows it to binds plaques in Alzheimer's disease as it affords relatively good inhibitory properties against Amyloid-β [50].

Upon oral administration, curcumin is distributed in various tissues encompassing blood, liver, kidney and intestine. Ravindrath and Chandrasekhara [47] administered a dose of 500 mg/kg of curcumin to rats and determined peak concentration in the intestine at 1 h while its peak concentrations were observed in blood, liver and kidney at 6 h. Curcumin could not be detected in the kidney after 24 h of administration, however it remains in the liver even 4 days after its administration.

1.2.3. Metabolism

Curcumin is known to undergo rapid metabolism in hepatic and intestinal systems [51]. Major metabolic pathways of curcumin includes reduction and conjugation [52] and some of the drug metabolizing enzymes involved in these processes include alcohol dehydrogenase, UDP-glucuronosyltransferases (UGTs) or sulfotransferases (SULTs). The metabolism of curcumin and other curcuminoids occurs via stepwise reduction of the olefinic heptanoid chain followed by conjugation of parent compounds and reduced metabolites with glucuronic acid and sulfate [51]. Particularly, orally administered curcumin is metabolized to O-conjugation products of OH group to form curcumin glucuronide and curcumin sulfate while those taken intraperitoneally undergo bioreduction to form dihydrocurcumin, tetrahydrocurcumin, hexahydrocurcumin and octahydrocurcumin (Figure 4) as demonstrated *in vivo* in rats and mice [53-55] as well as in suspensions of human and rat hepatocytes [54].

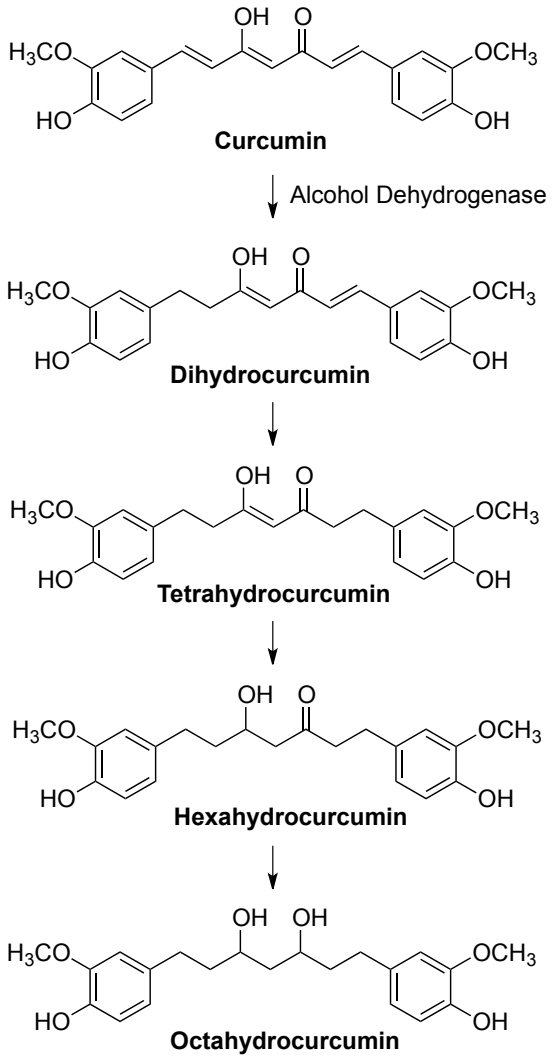

Figure 4. Chemical structures of reduced curcumin metabolites.

Tetrahydrocurcumin and hexahydrocurcumin were identified by mass spectrometry as the major metabolites, together with a small amount of dihydroferulic acid [51, 56]. Tetrahydrocurcumin has been demonstrated to prevent oxidative stress and inflammation, act against neurodegeneration as well as possess anticancer activity [56]. Such biological activities may account for the observed poor oral bioavailability of curcumin and yet potent effects *in vivo* [52]. Two other reductive metabolites, dihydrocurcumin and octahydrocurcumin, also exist but represent minor metabolites. Holder et al. [57] reported that the major biliary metabolites of curcumin in rats include glucuronide forms of tetrahydrocurcumin and hexahydrocurcumin while the minor biliary metabolite was dihydroferulic acid along with traces of ferulic acid.

Aside from the aforementioned major metabolic pathways, the following pathways can also occur *in vivo*: dehydroxylation, cyclization and methylation. In all, there are more than thirty metabolites of curcuminoids that have been previously identified in plasma, urine and bile from rats or humans via LC-MS/MS analysis and other methods [51]. Of particular note, curcumin was not metabolized by cytochrome P450 as there were no products of demethylation or hydroxylation that could be detected after incubating curcumin with rat liver microsomes [51].

Investigation by Ravindrath and Chandrasekhara [58] deduced that the major route of elimination of curcumin to be in the feces and that urinary excretion was found to be very low regardless of the dose. Administration of 80 mg of curcumin resulted in its excretion within 72 h while administration of 400 mg of curcumin resulted in its excretion 12 days after the dosage. In addition, they observed that the level of curcumin absorbed, which was 60-66%, remained the same irrespective of the dose that was administered. Pan et al. [53] observed that approximately 2.25 μg/mL of curcumin could be detected in plasma during the first 15 min and that it declined following its intraperitoneal administration of 0.1 g/kg of curcumin to mice.

1.2.4. Excretion

After an hour following administration, the levels of curcumin in the intestine, liver and kidney were found to be 177.0, 26.9, and 7.51 μg/g, respectively, while only traces (0.41 μg/g) could be detected in the brain at 1 h. In an earlier study by Wahlström and Blennow [46], 1 g/kg of curcumin was administered to rats and approximately 75% of the dose was excreted in the feces while a negligible amount could be detected in the urine. It was found that intravenous and intraperitoneal administration of curcumin in rodents led to large quantities of curcumin and metabolites (i.e. tetrahydrocurcumin and hexahydrocurcumin glucuronides) being trapped in the bile [57, 59]. Five hours after intravenous dosing, more than 50% of the dose was found to be excreted in the bile thereby suggesting that curcumin undergoes transformation via its absorption in the intestine and is possibly subjected to entero-hepatic recirculation [20]. Such hypothesis was originally proposed by Holder et al. [57] in their studies on the fate of curcumin in rats.

1.2.5. Toxicity

Studies on the toxicity of curcumin in both animal and human models have both revealed that the oral administration of curcumin was safe [60]. Particularly, toxicity studies conducted in rats, guinea pigs and monkeys found curcumin to be safe even at high doses [61]. In human, curcumin intake is considered safe as turmeric is commonly used as spice as well as

household remedy in India. The average 60 kg Indian consumes approximately 2-2.5 g of turmeric daily that is equivalent to 60-100 mg of curcumin [62]. However, owing to the rather low bioavailability of curcumin in plasma, high oral dose is necessary as to obtain desired pharmaceutical effect.

Several dose escalation studies also revealed no dose-limiting toxicity. An early phase I clinical trial of curcumin reported by Cheng et al. in patients with high-risk or premalignant lesions reveals no toxicity at the dose of 4g and 8 g [48]. In a subsequent study, healthy volunteers were given a dose of up to 12 g taken daily and yet no toxicity was found [63, 64]. However, minor side effects (i.e. headache, diarrhea and rash), which was not dose-related, have been reported [63]. Most patients with colorectal cancer could tolerate a dose of 3.6 g daily although with some reported cases of gastrointestinal adverse effects (i.e. diarrhea with National Cancer Institute grades 1 and 2) that may be related to the side effects of curcumin [65]. In phase II clinical studies, oral dose of 4 g and 8 g were tolerable in subjects with colorectal neoplasia [66] and patients with pancreatic cancer, respectively [67]. Taken together, curcumin were mostly found to be rather safe even at high dosage while a few studies reported mild side effects.

1.3. Drug Delivery of Curcumin

It was evident from previous studies that curcumin has poor absorption, biodistribution, metabolism and bioavailability. This had therefore sparked interests on devising new ways for overcoming these inherent problems by preparing delivery vectors for curcumin employing nanoparticles, liposomes, micelles and phospholipid complexes [68-70]. In addressing this problem, Bisht et al. reported the synthesis and characterization of nanoparticles. An *in vitro* intestinal absorption study by Suresh and Srinivasan [71] indicated that the absorption of curcumin increased from 47-56% when it was prepared in micelles. Similarly, pharmacokinetic studies in rats by Ma et al. observed that curcumin had a 60-fold higher biological half-life when prepared in a polymeric micellar formulation than curcumin solubilized in a mixture of dimethylacetamide, polyethylene glycol and dextrose.

2) Synthetic Analogs of Curcumin

The chemical structure of curcumin is important for its multiple biological activities and therefore great effort have been invested on improving such properties via the synthesis of different analogs by replacing or introducing different functional moieties at different positions of the structure [72]. In our effort to explore the chemical space of curcumin and its derivatives, we had compiled an exhaustive set of synthetic analogs of curcumin from the literature [73]. Briefly, in this forthcoming report we searched for all research article titles containing the keywords *curcumin* AND synthes** using the Scopus Database. The asterisk (*) symbol was used as a wild card as to also retrieve other deviants of the search query such as curcumin* would yield curcumin, curcumins, curcuminoid and curcuminoids. From the total of 221 documents, 64 research articles containing various synthetic analogs of curcumin were manually extracted. It is known that naturally occurring curcumin is comprised of three essential functional moieties: biaryl rings, β-diketone and diene chain (Figure 5). This is in concomitant with the fact that curcumin possessed rather poor solubility as mentioned in

previous sections and therefore several of these reports made efforts in not only improving the biological activities but also their pharmacokinetic properties.

Preliminarily, an analysis of the compiled literature revealed that 45 studies reported chemical substitution at the biaryl moiety, 6 studies reported chemical substitution at β-diketone moiety, 9 studies reported chemical substitution at both biaryl and diketone moieties while only one study reported chemical substitution at the heptene linker.

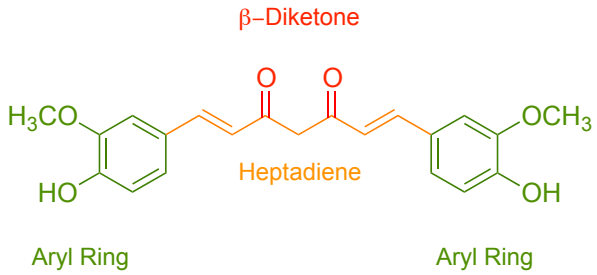

Figure 5. Chemical structure of curcumin and its essential functional moieties.

Firstly, since the majority of studies reported chemical substitution at the biaryl moiety, therefore this peripheral functional group is considered to be a crucial feature in eliciting multiple activities observed in curcumin. Priyadarsini et al. [74] showed that the essential part accounting for antioxidant activity of curcumin arise from the phenolic OH group. For this reason, different substituents have been introduced to the biaryl moiety as to understand their role towards antioxidant activity. Bayaomi et al. [75] synthesized different series of curcumin analogs as to evaluate their antioxidant activities and the results showed that the substitution of 4-methoxy with 4-hydroxy at the aryl group afforded high antioxidant activities. Furthermore, several evidences suggested that curcumin possessed potent property against Alzheimer's diseases as it could prevent the aggregation of amyloid-β proteins [76]. Moreover, Murray et al. performed fragment screening as to identify inhibitors of β-site amyloid precursor protein cleaving enzyme 1 (BACE1). As a result, they reported three different chemotypes as potential inhibitors. Of these chemotypes, one had an aliphatic hydroxyl group reminiscent of the characteristic feature of biaryl groups in curcumin [77]. Evidences suggested that the phenolic ring is a critical feature for inhibiting BACE1. As such, Konno et al. reported the synthesis of 37 curcumin derivatives containing substituents at the biaryl moiety and tested their inhibitory property against BACE1 [78].

Secondly, the observed poor bioavailability and absorption of curcumin arises from the β-diketone moiety because this functional group serves as a substrate for aldoketo reductases present in the liver [79]. Since this β-diketone moiety is responsible for its instability and degradation, a series of mono-carbonyl analogs have been synthesized as to improve the stability of curcumin [80]. In spite of the limitation caused by the β-diketone moiety it is still responsible for several pharmacological activities as substituted β-diketone have been shown to afford improved pharmacological properties. For instance, Simoni et al. synthesized a series of curcumin analogs substituted at the β-diketone site and these compounds were shown to be effective in inhibiting cancer cells with multiple drug resistance [81]. Moreover, Lal et al. demonstrated synergic anti-bacterial effects in their series of substituted sulfonamides at the β-diketone [82].

Finally, the aliphatic heptadienone chain serves as a linker between the biaryl moieties. Several studies reported substitution at biaryl and β-diketone moieties as they were deemed to be important structural features giving rise to multiple pharmacological properties whereas few studies report substitution at this aliphatic chain. Although such heptadienone is considered to be just a linker of the biaryl moieties, it has been shown to be required for potency against inflammation and parasites. Flynn et al. stressed that at least one or two olefinic bonds is needed in affording improved potency of antiinflammatory effect [83]. In addition, Changtam et al. reported that the olefinic function in curcumin was important as derivatives having olefinic linker were found to be more potent than their hydrogenated aliphatic counterpart as antiparasitic agents [84]. In addition, the length of the olefinic linker was shown to be associated with its protective activity against Alzheimer's disease. Particularly, Reinke and Gestwicki [85] reported that the length of the linker should be within 6-19 Å as to fit into the amyloid-β in prevention of its aggregation. Furthermore, Campos et al. [86] reported the synthesis of a series of curcumin analogs with olefins at various positions on the heptadienone chains as to understand their influence on the biological activities of curcumin.

II. BIOLOGICAL ACTIVITIES OF CURCUMIN

As noted previously, curcumin has had a long history of usage in the ancient Indian medicine of Ayurveda in which plant-derived natural products play a major part. In modern medicine, the first study on curcumin and human diseases was reported by Oppenheimer [87] in 1937. Thus far, there have been more than 4,000 reports on the wide spectrum of biological activities afforded by curcumin, which includes antioxidant, antibacterial, antifungal, antiviral, anti-inflammatory, antiproliferative, pro-apoptotic and anti-atherosclerotic effects. Particularly, curcumin has been shown to be effective against the following diseases: arthritis, allergy, inflammatory bowel disease, nephrotoxicity, acquired immunodeficiency syndrome (AIDS), psoriasis, diabetes, multiple sclerosis, cardiovascular disease, and lung fibrosis. In-depth information on the biological activities of curcumin has previously been reviewed [88, 89].

Such pleiotropic effects are highly dependent on its ability to interact and regulate multiple molecular targets. Curcumin is therefore a classical example of polypharmacology in which a small molecule has the capacity to interact with several molecular targets [90]. Polypharmacology takes a new stance on the traditional viewpoint of one drug-one target by illuminating the concept of one drug-multiple target. Explanation for such viewpoint may be attributed to experimental limitations that may have allowed the screening of the compound of interest against only a handful of potential target proteins but not systematically to a large panel of proteins as to derive a complete target profile. Great interests in the polypharmacology of drugs stems not only from the potential adverse effects that it may exert but also to the ability to repurpose or reposition such drugs for treating a new disease. Such polypharmacological effects can be attributed to target phylogeny in which the target protein shares similar binding site. Milletti and Vulpetti [91] investigated the polypharmacology of a set of 17 inhibitors against 189 kinases in which they constructed the inhibition profile by comparing their binding sites. Several tools are already available for analyzing such

similarities in the binding site of putative target proteins [92-96]. The fundamental basis of target phylogeny had prompted the development of chemogenomic [97, 98] and proteochemometric [99] approaches in unraveling the molecular interaction profiles of several compounds with several target proteins. A recent example is the development of a unified proteochemometric model for predicting the inhibition of 5 cytochrome P450 isoforms against a panel of 17,143 compounds [100]. Such computational methods are an integral part of current drug discovery effort in light of the inherent cross-pharmacology and drug promiscuity in drugs that may hinder its further progress.

Biological activities of curcumin are exhibited through direct binding to its targets as well as indirect modulation of targets via up-regulation or down-regulation [101]. The chemical structure of curcumin comprising two phenyl groups connected by a flexible linker allows its different conformations. Such flexible chemical structure confers the versatility in direct interaction with a wide range of targets in giving rise to its ability to modulate or interfere many biochemical pathways [102]. Molecular targets of curcumin among others include inflammatory molecules, enzymes, growth factors, transcription factors, kinases, receptors and metal ions. The following sections describe the different biological activities of curcumin in relation to their molecular targets.

1) Anti-Inflammatory Activity

Nuclear factor transcription factor (NF-κB) represents a key target for the anti-inflammatory activity of curcumin. NF-κB is an important transcription factor that is activated by various stimuli including free radicals, cytokines such as tumor necrosis factor (TNF), bacterial or viral antigens as well as radiation [102]. NF-κB is located in the cytoplasm in its inactive form and bound to IκBα. Upon activation, IκBα is phosphorylated by IκB kinase and free NF-κB is released and is subsequently translocated into nucleus as to activate the transcription of responsive genes [102].

Curcumin is a potent NF-κB blocker in which the inhibition can be triggered via many ways [102]. First, curcumin inhibits IκBα phosphorylation thereby preventing NF-κB translocation into the nucleus. This leads to the inhibition of transcription for several responsive genes [102] including genes coding for inflammatory mediators such as cyclooxygenase (COX). A study on the effects of curcumin on COX-2 expressing pancreatic cancer cell lines indicated that curcumin downregulates COX-2 expression suggesting its role as NF-κB inhibitors [103]. Secondly, curcumin is capable of direct binding with TNF-α and COX-2. In fact, findings from molecular docking studies suggested that Cys129 of TNF-α is the binding site for curcumin where hydrophobic, van der Waals forces and hydrogen bonding interactions are central for its binding [104]. As a result, the binding of TNF-α to its receptor is blocked thereby inactivating the NF-κB pathway. Curcumin can directly inhibit COX-2 via direct interaction with Val523, Val116, Ala516 and Tyr355 [105] and via hydrogen bonds with Ala562 thereby leading to the inhibition of PGE-2 production [106].

Myeloid differentiation protein 2 (MD-2) is a LPS-binding component of the endotoxin surface receptor complex MD-2/toll-like receptor 4 (TLR4), which is associated with the LPS signaling pathway [101]. Curcumin exhibits inhibitory effect against chronic inflammation as evoked by bacterial infection via MD-2 [107]. Curcumin is directly bound to the Cys133

residue inside the hydrophobic pocket of MD-2 via Michael addition reaction [107] thereby inhibiting LPS signaling.

Lipoxygenase (LOX) is an iron-containing enzyme that orchestrates crucial roles in the arachidonic acid pathway involving the biosynthesis of eicosanoids [101]. There are also some reports suggesting its role on the carcinogenesis and metastasis of many cancers [108]. Findings from Skrzypczak-Jankun et al. indicated that curcumin can inhibit LOX enzyme by blocking the enzyme's active site [109] in a non-competitive manner [110].

The ability of curcumin to inactivate the NF-*κ*B pathway and thereby modulate several downstream responsive genes involving inflammation and immune response is essentially related with several biological activities such as antipyretic, analgesic, anticancer as well as protective effects against cardiovascular diseases and diabetes. Likewise, it follows that curcumin is a natural compound affording a multitude of therapeutic potential for the treatment of several inflammatory-related diseases (i.e. cardiovascular diseases, diabetes mellitus, autoimmune diseases, allergy, asthma and cancers) [89].

2) Antioxidant Activity

Curcumin has also been reported to be a potent antioxidant with comparable levels of activity to those of vitamin C and vitamin E [111]. Molecular targets related to antioxidants includes NF-*κ*B [89], xanthine oxidase (XO) and metal ions [101]. Potent antioxidant property of curcumin gives rise to its therapeutic applications in the treatment of neurodegenerative diseases [112], cardiovascular diseases [113] as well as the prevention of diabetic complications [113].

XO is an enzyme that facilitates the conversion of hypoxanthine to xanthine and uric acid. This enzyme is capable of generating ROS and therefore plays central roles in the pathogenesis of many diseases [114]. Several *in vivo* studies demonstrated that curcumin is capable of inhibiting the XO activity as well as scavenging superoxides [89]. Molecular docking studies indicated that XO is a direct target of curcumin where the following residues serve as binding pocket: Phe914, Phe1009 and Thr1010 [115].

Owing to its potent antioxidant activity, curcumin is also used for the treatment of other oxidative-related diseases. Curcumin was reported to reduce oxidative stress and lipid peroxidation of LDL in atherosclerotic rabbits further indicating its protective effects against cardiovascular diseases [116]. Oxidative stress condition is a crucial factor facilitating diabetic complications. The protective effect of curcumin on diabetic rats was also reported to arise from its contribution to antioxidant defense [117].

3) Wound Healing Effect

Curcumin has been used to promote wound healing ever since ancient time [111]. The wound healing process starts with inflammation, granulation and tissue modeling involving several cell types, cytokines, extracellular matrix proteins as well as growth factors [111]. As such, curcumin was found to promote wound healing by acting at different levels of the healing process [111].

Transforming growth factor (TGF-β) plays a role in the wound healing process by which it activates the expression of fibronectin (FN) and collagen in fibroblasts thereby increasing granulation tissue formation [118]. An *in vivo* study in diabetic rats indicated that curcumin regulates TGF-β thereby resulting in an increase of fibronectin and collagen expression. This finding suggests that curcumin is capable of promoting granulation tissue formation, neovascularization and re-epithelialization of wounds in both diabetic rats and hydrocortisone-impaired wounds [119]. Therefore, the therapeutic potential of curcumin for treating diabetic-impaired wound healing was also documented [89].

As a potent NF-*κ*B blocker, curcumin has been reported to enhance muscle regeneration after trauma [120]. Moreover, curcumin has been demonstrated to promote cutaneous wound healing in normal and diabetic rats by modulating nitric oxide synthase (NOS) during wound healing by suppressing NF-*κ*B as well as increasing the expression of TGF-β [121]. Moreover, curcumin was shown to afford antiulcer activity in acute gastric ulcer rat model. The study indicated that curcumin prevented lipid peroxidation, depletion of glutathione and protein oxidation as well as promoted re-epithelialization of gastric lumen. The mechanism by which re-epithelialization occurs was suggested to involve the modulation of matrix metalloproteinases (MMPs) activities [122].

MMPs are zinc-dependent enzymes playing crucial roles in the degradation of various extracellular matrix proteins [101]. Functions of MMPs in physiological conditions are numerous and encompasses cell proliferation, migration, differentiation, angiogenesis, host defense and apoptosis as well as pathological conditions (i.e. inflammatory diseases, auto-immune diseases and vascular disorders) [101]. MMPs are direct target of curcumin [101] and a docking study of curcumin derivatives, THC and BDMC, revealed that interaction is formed via hydrogen bonding between the hydroxyl group of the phenyl ring with the carboxyl group of Pro421 while another hydrogen bond was shown to be formed between the oxygen atom of β-diketo moiety of the curcumin with the amine group of Arg424 [123].

In addition, the antioxidant effect of curcumin was also reported to enhance wound repair by reducing oxidative damages caused by hydrogen peroxides in human fibroblasts and keratinocytes [124].

4) Anticancer Activity

The ability of curcumin to treat various types of cancer (i.e. breast, prostate, kidney, colon, blood and liver cancers) has been reported in both culture cells and animal models [125]. Curcumin and its derivatives are versatile anticancer agents owing to their ability to modulate several biochemical pathways relating to cell proliferation and survival of cancers such as NF-*κ*B, activator protein 1 (AP-1)**,** B-cell lymphoma 2 (Bcl-2), Janus kinase/signal transducer and activator of transcription **(**JAK/STAT), phosphoinositide3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/Akt/mTOR), mitogen-activated protein kinases (MAPK), TNF-related apoptosis-inducing ligand (TRAIL) and steroid receptors [102]. As curcumin is a compound that can hit multiple intracellular targets without causing any side effects, this property renders it a promising candidate for potential usage in the eradication of cancer [126].

The inhibition of NF-*κ*B by curcumin affords anticancer activity through multiple ways such as through direct binding to TNF-α and COX-2 as reported in human pancreatic cancer

cell lines by Youns et al. [103] as well as via the indirect inhibition of NF-κB translocation by inhibiting IκBα phosphorylation [102] as reported in breast cancer cells by Vinod et al. [127].

AP-1 is a transcription factor that can be exploited as an anticancer target owing to its functions in differentiation, proliferation, apoptosis and oncogenic transformation [128]. AP-1 is activated by several stimuli such as bacterial or viral infections, growth factors, cytokines, stress signal and oncogenic factors [102]. Upon activation, AP-1 binds to TPA responsive element (TRE) that consequently activates the expression of many downstream genes involved with growth and angiogenesis of cancer cells (i.e. cyclin-D1, MMP and VEGF) [129]. The anticancer activity of curcumin occurring via the inhibition of AP-1 was reported in transformed keratinocytes [130], human leukemic cells [131], prostate cancer [132] and human fibrosarcoma cells [133]. Particularly, it was proposed that curcumin inhibits AP-1 by direct binding with the AP-1 DNA-binding site [134]. Evidences from spectroscopic study demonstrated that curcumin was capable of binding to DNA via the carbonyl oxygen atom of thymine in the minor groove, N7 of guanine and adenine from the major groove as well as phosphate moieties from the DNA backbone [135].

Bcl-2 is a cell survival protein in which expression is partly related with cancer resistance [136]. Curcumin directly bind and inactivate Bcl-2 thereby increasing the expression of pro-apoptotic protein, Bax, that subsequently leads to apoptosis via caspase cascades [137]. An effective antiproliferative activity of the curcumin derivative (DMC) occurs via Bcl-2-regulated pathways as reported by Luthra et al. [138]. Findings from molecular docking indicated that curcumin directly interacts with cavity 2 of Bcl-2 involving several amino acids comprising of Tyr108, Glu136, Gly141, Asn143, Trp144, Gly145, Arg146, His184, Trp188 and Tyr202 [138].

The JAK/STAT signaling pathway has been implicated in cancer progression and immune response [139]. Activated JAK induces the phosphorylation of tyrosine residues, which in turn generates phosphotyrosine sites on the receptor that subsequently serves as binding sites for other STATs. STATs themselves are also phosphorylated and also generate phosphotyrosine sites leading to STAT dimerization. The accumulation of STAT dimers in the nucleus results in the activation of many target genes related with cancer proliferation and migration [102]. Curcumin inhibits the JAK/STAT pathway by inhibiting STAT3 phosphorylation as observed in small lung cancer cells [140], prostate cancer cells [141] and glioblastomas [142].

PI3K/Akt/mTOR pathway is associated with proliferation of cancer cells [143]. Upon binding of ligand with its receptor, PI3K is activated and is recruited into the inner cell membrane. Activated PI3K then activates AKT thereby giving rise to mTOR phosphorylation that leads to the promotion of cell survival [144]. Curcumin inactivates this pathway by inhibiting mTOR phosphorylation thereby inducing apoptosis [102].

MAPK pathway is activated by a series of phosphorylation cycles as responses to several stimuli. The activation of the MAPK pathway leads to the induction of apoptosis. A study on colorectal carcinoma cells by Rodon et al. indicated that curcumin was capable of enhancing the MAPK pathway via activation of MAPK subgroup, ERK1/2 [144].

TRAIL is a protein that acts as a ligand for death receptors (DR)-4 and -5. Upon binding, caspase-8 is activated thereby resulting in activation of procaspase-3, -6 and -7 that subsequently leads to apoptosis [102]. Park et al. [145] demonstrated that curcumin could enhance TRAIL-induced apoptosis of breast cancer cells by modulating apoptosis-related proteins.

Steroid receptors comprising of estrogen receptor (ER), progesterone receptor (PR) and androgen receptor (AR) act as transcription factors for the activation of many downstream responsive genes. Abnormal expression of steroid receptors is known to be associated with the onset and progression of many types of hormone-dependent cancer, which implies the use of steroid receptor antagonist as anticancer agent [102]. Findings from Ohtsu et al. indicated that curcumin analogs provided anti-androgenic activity against human prostate cancer [146]. Moreover, results from structure-activity relationship study suggested that the conjugated and coplanarity β-diketone moiety, bis(3,4-dimethoxyphenyl) moieties, intramolecular symmetry of the molecules and strong hydrogen bond donor groups were crucial factors for origins of its anti-androgen property [146]. Vitamin D receptor (VDR) is a member of the nuclear and steroid receptor superfamily. 1α,25-Dihydroxyvitamin D3 (1,25D) is an active hormonal metabolite of vitamin D that serves as a ligand for vitamin-D receptor (VDR). The binding of VDR and its ligand regulates many vitamin-D responsive genes [147]. The role of 1,25D in chemoprevention is exerted via cell cycle regulation by arresting cells in the G1/G0 phase as well as upregulating tumor suppressor genes (i.e. p21) [148]. Curcumin was also found to be a ligand of VDR [147] and its chemoprotective effect arises from its ability to inhibit VDR and upregulate p21 as observed in human colon cancer [147], breast cancer and prostate cancer cells [149-151].

Aromatase is a member of the cytochrome P450 family that orchestrates essential role in the biosynthesis of estrogens, which is the main hormone responsible for the development of breast cancer [152]. In hormone-sensitive breast cancers, cyclooxygenase COX-2 raises the level of aromatase activity that subsequently leads to high levels of estrogen and likewise contributes to the development of breast cancer [153]. Therefore, suppression of the catalytic activity of aromatase represents one of the lucrative therapeutic targets for breast cancer. Curcumin decreases aromatase expression by inhibiting COX-2 activity. Kunnumakkara et al. [154] observed that curcumin can physically bind to COX-2 thereby downregulating its expression as shown both in culture cells and in animal models. In addition, Liang et al. [155] reported that letrozole, the third-generation inhibitor of aromatase, and curcumin could be combined as to create synergic inhibitory effects to carcinoma growth. Recently, we had explored the chemical space of all known aromatase inhibitors comprising of 973 compounds as to unravel insights into the origins of aromatase inhibitory activity [156]. This was performed by critically analyzing active and inactive aromatase inhibitors using statistical, histogram and fragment analysis as well as constructing classification models of the structure-activity relationship. Furthermore, we had also developed QSAR models to deduce the origins of aromatase inhibitory activity for a set of 54 letrozole analogs [157] and 63 flavonoids [158]. In the former study, 3 descriptors (i.e. number of rings, ALogP and HOMO-LUMO gap) were found to be pertinent in quantitating the aromatase inhibitory activity. As for the latter study, significant descriptors employed in modeling the flavonoids included the number of rotational bonds, number of rings, number of hydrogen bond donors, number of hydrogen bond acceptors and dipole moment.

Many enzymes acquiring abnormal function are closely related with cancers and were found to be a direct target of curcumin such as DNA methyltransferase, ribonuclease, proteasomes and lipoxygenase [101].

Proteasomes represents essential enzymes in the ubiquitin-proteasome system (UPS), which is a system that functions to maintain cellular homeostasis by regulating protein synthesis and degradation [159]. As the UPS pathway is associated with cell cycle regulation

and DNA damage repair while dysfunction of the system is known to lead to tumorigenesis [160].

Inhibition of the UPS pathway leads to apoptosis of cancer cells therefore making it a lucrative anticancer strategy. Recently, curcumin was reported to inhibit the proteasome activity in human colon cancer cells [161]. The proteasome inhibitory effect and antiproliferative activity of acetates and amino conjugates of curcumin against several cancer cell lines were investigated by Wan et al. [162]. Findings from molecular docking study indicated that curcumin could directly bind to the N-terminal threonine [161] from the β5 subunit of proteasome [161, 162].

DNA methyltransferase (DNMT) is an enzyme that functions in methylating promoter CpG from many tumor-suppressor genes thereby leading to their transcriptional silencing [101].

Particularly, promoter hypermethylation is associated with many solid and blood cancers [163]. Curcumin was found to exhibit DNMT inhibitory activity thereby suggesting its potential as a DNA hypomethylating agent. The mechanism of inhibition was found to occur via covalent blockage at the catalytic Cys1226 residue of DNMT [164].

Ribonuclease A (RNase A) is an enzyme that plays a crucial role in the junction of transcription and translation processes in which its hyperactivity gives rise to undesired RNA cleavage thereby inhibiting protein synthesis subsequently leading to cell death [101]. The direct binding of RNase and diacetylcurcumin (DAC), a curcumin analog, was reported via formation of hydrogen bonds between oxygen atoms in positions 3 and 5 of DAC and Tyr97, Gln11 and Lys7 of RNase [165]. This finding suggested that curcumin is a potential RNase inhibitor.

Lipoxygenases (LOXs) are enzymes in the arachidonic pathway that is responsible for eicosanoids production. Several isoforms of LOXs play essential roles in carcinogenesis whether as pro-tumorigenic or anti-tumorigenic factors [101]. LOX pathways have been known to be related with the spread and metastasis of many cancers via activation of many signaling pathways that modulate the expression of genes pertaining to cellular proliferation, survival, migration and extracellular matrix production [108]. Curcumin was found to act as a LOX inhibitor against soybean LOX L3 by blocking the active site [109] in a non-competitive manner [110].

ErbB2 (HER2/neu) is a transmembrane tyrosine kinase that plays an essential role in development and functions of normal cells as well as metastasis of many cancers. The overexpression of ErbB2 is closely related with increased chances of metastasis and resistance to anticancer drugs [166].

Hence, the suppression of ErbB2 expression is considered to be viable treatment strategies for ErbB2-overexpressed cancers [166]. Curcumin is capable of down-regulating ErbB2 by inducing its degradation [167].

Particularly, this occurs by increasing association of chaperone-dependent ubiquitin ligase, carboxyl terminus of Hsc70-interacting protein (CHIP), with ErbB2 leading to ubiquitination and degradation of ErbB2 [167]. Findings from molecular docking and site-directed mutagenesis studies suggested that curcumin attenuates the ErbB2 activity via binding at the kinase domain of ErbB2 while the Michael acceptor functionality of curcumin is essential for both covalent binding of curcumin with ErbB2 as well as curcumin-mediated ErbB2 depletion [167].

5) Antineurodegenerative Activity

Antineurodegenerative activity arises from its potent antioxidant activity as well as its ability to directly bind to redox-reactive metal ions and prion proteins [101]. Curcumin is capable of binding to redox-reactive transition metal ions such as copper (Cu) and iron (Fe) [101, 112]. Redox-reactive metal ions play crucial roles in neurodegenerative diseases (i.e. Alzheimer's disease) owing to their ability to generate reactive oxygen species (ROS) that consequently leads to oxidative damages of neurons. Moreover, metal ions can induce the formation and aggregation of abnormal proteins in the brain. Metal ions accumulated in abnormal protein plaque can also produce ROS thereby leading to neurotoxicity and neuronal damages [168]. Furthermore, curcumin-metal chelates (i.e. Cu and Mn) were found to exhibit potent antioxidant activity (i.e. superoxide dismutase mimic activity) [169]. In addition, findings from *in vitro* studies demonstrated that curcumin was capable of inhibiting plaque formation of abnormal protein in a mouse model [170, 171]. Moreover, curcumin reduces the expression of inducible nitric oxide synthase (iNOS) and neuronal nitric oxide synthase (nNOS) via the inhibition of NF-κB that in turn reduce oxidative stress and lipid peroxidation in the brain [172].

Prion protein (PrP) is the key etiologic protein of prion diseases (i.e. transmissible spongiform encephalopathies and others) [173]. Prion diseases occur when the native helical conformation of PrP is converted to the β-stranded conformation thereby giving rise to protein misfolding, aggregation, plaque formation and eventually neurotoxicity. Curcumin was shown to inhibit the conversion of PrP as well as the formation of protease-resistant PrP in neuroblastoma cell lines [174]. In addition, curcumin recognizes and selectively interacts with the converted form of PrP rather than the native form. However, the exact amino acid within the binding site has not yet been identified.

6) Antidiabetic Activity

Anti-diabetic effects of curcumin arise from its ability to interact with many targets such as NF-κB, TNF-α, VEGF [89] and aldose receptor [101] as well as arising from its potent antioxidant properties [89]. Curcumin has been found to exert hypoglycemic effect by means of modulating multiple targets. Human peroxisome proliferator-activated receptor-gamma (PPAR-γ) belongs to the superfamily of nuclear receptors, which plays significant roles in lipid and glucose metabolism and it is considered to be a molecular target for insulin-sensitizing thiazolidinedione drugs [175]. As curcumin is capable of binding to PPAR-γ, therefore it was assumed to improve insulin resistance and ameliorate type 2 diabetes mellitus [175]. NF-κB pathway and TNF-α are both influenced by PPAR-γ as well as being closely related with insulin resistance and high blood glucose in which activation of PPAR-γ may oppose the effects of TNF-α in adipocytes as well as modulate cytokine-mediated signaling pathway, which would enhance insulin action [176].

Owing to the potent antioxidant properties of curcumin and its ability to modulate many oxidative condition-related pathways, the effects of curcumin in the prevention of diabetic complications were documented.

Aldose reductase 2 (ALR2) is the rate-limiting enzyme in the polyol pathway and it belongs to the aldo-keto reductase superfamily [101]. ALR2 is responsible for the process of

reducing glucose to sorbitol using NADPH as a cofactor. Consequently, sorbitol is converted to fructose by means of sorbitol dehydrogenase [177]. Diabetes increases ALR2 activity that subsequently leads to the accumulation of intracellular sorbitol, which gives rise to many diabetes complications [178]. Many animal models suggested that the inhibition of ALR2 is effective for preventing diabetic complications (i.e. cataract, retinopathy, nephropathy and neuropathy) [179, 180]. Curcumin was reported to selectively inhibit the ALR2 activity in a non-competitive manner and was capable of reducing sorbitol accumulation as observed in human erythrocytes under high glucose condition [178]. In addition, molecular docking demonstrated that curcumin interacts at the ALR2 active site via the following residues: Tyr48, Lys21, Thr19, Gln183, Leu300 and Trp111 [178].

Oxidative stress along with hyperglycemia are crucial factors accelerating the accumulation of advanced glycation end-products (AGEs) and cross-linking collagen thereby subsequently leading to many diabetic complications such as impaired wound healing and cellular damages [89]. Curcumin was found to effectively prevent AGE-induced diabetic complication by inhibiting protein glycosylation, free radicals production and lipid peroxidation.

In addition, curcumin and its metabolite tetrahydrocurcumin (THC) have been shown to exhibit hypoglycemic effect in diabetic rats via modulation of key hepatic enzyme via reduction of lipid peroxidation and oxidative stress [181].

Cardiac hypertrophy is found in advanced cardiovascular diseases as well as diabetic patients. Furthermore, this condition involves remodeling of the left ventricle wall as a response to high blood pressure and volume overload [182]. Long-term hypertrophy may lead to the loss of cardiac plasticity and heart failure [183]. NF-AT is a transcription factor associated with metabolic and biochemical changes within cardiac myocytes subsequently leading to cardiac hypertrophy.

NF-AT is regulated by histone acetyltransferase (HAT/p300) while glucose-induced cardiac hypertrophy is mediated by p300 [184]. Therefore, inhibition of HAT is implied to prevent cardiac hypertrophy and diabetes-induced cardiac hypertrophy [113]. Marcu et al. demonstrated that curcumin could act as p300 inhibitor [185]. Particularly, curcumin was found to be covalently bound to p300 subsequently leading to proteasome-dependent degradation of p300 as well as inactivation of p300 activity [185].

Neo-vascularization is responsible for the pathogenesis of diabetic retinopathy and is induced by hyperglycemic conditions via the induction of vascular endothelial growth factor (VEGF) [89]. Owing to the ability of curcumin to inactivate upstream transcription factor (i.e. AP-1) it can therefore suppress VEGF expression that consequently prevents retinopathy. Furthermore, curcumin was reported to prevent diabetic nephropathy in an animal model [186]. Moreover, diabetes neuropathic pain is an important microvascular diabetic complication that is the most difficult type of pain to treat [89]. Curcumin is capable of modulating nitric oxide (NO) release while also inhibiting TNF-α in a dose-dependent manner thereby conferring it with anti-nociceptive activity implicating its therapeutic potential for the treatment of diabetic neuropathic pain [186].

Owing to its ability to bind to multiple protein targets and give rise to multifaceted activities, makes curcumin a promising bioactive compound that could potentially be utilized as therapeutic agent. In fact, at the time of writing this chapter there have been 92 reported clinical trials on a wide range of ailments. As previously mentioned, curcumin have been widely used in culinary preparation, marketed as dietary supplements, used in cosmetics, etc.

III. STRUCTURE-ACTIVITY RELATIONSHIP

Drug design constitutes of both receptor- and ligand-based approaches in which the former entails the use of protein structures while the latter makes use of only the ligand. From the perspective of receptor-based drug design, X-ray crystallographic structures of enzymes bound to its substrates or inhibitors provides a great deal of information on their interactions that could be used to aid the design of new or more specific inhibitors. The ease of crystallization of a protein-ligand complex is dependent upon several factors from both protein and ligand themselves, which may limit or make it difficult to obtain protein crystals. Alternatively, computational techniques pertaining to three-dimensional structure-based modeling (i.e. homology modeling and *ab initio* protein structure prediction) makes it possible to obtain predicted protein structures for those proteins whose crystal structures are unavailable thereby allowing the use of receptor-based approaches. Molecular docking and molecular dynamics (MD) simulation are complementary tools that are widely used to gain insights on the binding modalities of such protein-ligand complexes as well as shed light into their dynamicity. [187]. These techniques have been used for solving specific problems of drug design and it should be noted that each of these tools has its advantages and disadvantages. For example, although molecular docking can help in discerning the binding modalities of protein-ligand interactions, its limitation stems from potential pitfalls such as the scoring functions used, the handling of rigidity/flexibility of the studied protein-ligand entities and typical docking in vacuum may afford irrelevant answers. As for MD simulations, its strengths includes the ability to get a glimpse of protein dynamicity, its account of solvation and relatively accurate estimation of binding free energies while its disadvantages stems from its computationally expensive nature thereby limiting its usage to only a few privileged groups [188, 189].

As previously noted, ligand-based approaches primarily use information from the ligands alone mostly in the absence of protein structures. Commonly used methods in this paradigm include quantitative structure-activity relationship (QSAR) when modeling biological activities and quantitative structure-property relationship (QSPR) when handling the prediction of chemical properties. An in-depth treatment on the principles of QSAR/QSPR has been previously reviewed [190, 191]. Briefly, QSAR/QSPR essentially correlate the chemical structures from a congeneric series of compounds with their respective biological activity or chemical property. Chemical structures are defined by their molecular descriptors, which are essentially numerical description of physicochemical properties that uniquely describe its molecular features such as molecular weight, charge, number of hydrogen bond donors, hydrogen bond acceptors, number of rotational bonds, dipole moment, highest occupied molecular orbital, lowest unoccupied molecular orbital, etc. Multivariate analysis is then used to relate the **X** blocks of molecular descriptors with the **Y** block (i.e. the biological activity or chemical property of interest). Typical learning methods include the traditional multiple linear regression (MLR), artificial neural network (ANN), support vector machine (SVM), random forest (RF), decision tree (DT), partial least squares (PLS) and principal component analysis (PCA).

As previously mentioned, curcumin is capable of modulating a wide number of protein molecules either indirectly or directly by binding through covalent and non-covalent interactions. A number of biophysical tools have been performed to investigate direct

interactions of curcumin with target proteins. Most of these studies have utilized molecular modeling and docking as a computational tool to study their mode of binding. It has been shown that curcumin as well as its analogs directly interact with various kinds of proteins. These include inflammatory molecules, enzymes, carrier proteins and nucleic acids, for examples tumor necrosis factor-α, cyclooxygenase 1 and 2, histone acetylase, histone deacetylase, glyoxalase 1 (GLO1), proteasome, HIV-1 integrase, HIV-1 protease, DNA methyltransferase 1, matrix metalloproteinase, protein kinases, protein reductases [101] as well as cytochrome P450 2C9 [192], amyloid-β protein [193, 194], phospholipase A2 [195]. The binding constant of curcumin and its analogs to most of these proteins has been detected in the nM-μM range. Detailed intermolecular interactions between curcumins and such target molecules have been extensively reviewed by Gupta et al. [101].

The ability of curcumin to directly bind diverse proteins with high affinity can be accounted by its inherent chemical functionality. Structurally, curcumin has two hydrophobic phenyl rings connected by a flexible methylene bridge and molecular docking analyses have revealed that curcumin can exhibit several different conformations suitable for maximizing hydrophobic contacts with the protein to which it is bound. For example, the phenyl domains of curcumin can participate in π-π interactions with aromatic amino acid side chains. In addition to the hydrophobic nature of curcumin structure, the hydroxyl, methoxyl and the 1,3-dicarbonyl functional groups, which are located at the peripheral ends and in the center of the molecule, can participate in hydrogen bonding with their target macromolecules. These moieties provide a strong and directed electrostatic interaction as to increase favorable binding free energies. Furthermore, since curcumin undergoes the so-called keto-enol tautomerism as a result of the β-diketone moiety, it can therefore exert additional chemical functionality. Particularly, the predominant enol form allows the midsection of the molecule to act as both hydrogen bond donor and acceptor. Moreover, the enol form may also serve as a chelator for positively charged metals, which are often found in the active sites of target proteins.

The combination of hydrophobic π-π interactions, the extensive hydrogen bonding as well as metal chelation that covers such a large surface area of the molecule confer curcumin the capability to participate in interactions with several target proteins [101].

The most successful case in which ligand- and receptor-based approaches of rational drug design was employed is the identification of curcumin analogs as inhibitors against GLO1, which is a potential target for anti-tumor drug development [196, 197]. In these reports, molecular docking was performed on a series of curcumin analogs as to observe the possible binding poses for these compounds to the enzyme and they were shown to afford high inhibitory activity against human GLO1.

The docking studies showed that the enol form of curcumin analogs was coordinated to Zn^{2+} via oxygen atoms of the carbonyl group as well as forming strong hydrogen bonds with the positively-charged amino acid residues at the active site of the enzyme. MD simulations were carried out to confirm the reasonable binding model of the selected complex as explored by docking studies. Three-dimensional structure-activity relationship (3D-QSAR) analyses based on the docking results indicated good correlation and predictive power with the experimental K_i values. This information provided in-depth insights into the interaction mechanism between curcumin analogs and the active site of human GLO1, which could be used to further develop novel and highly selective inhibitors on the basis of existing rational receptor-ligand binding modes.

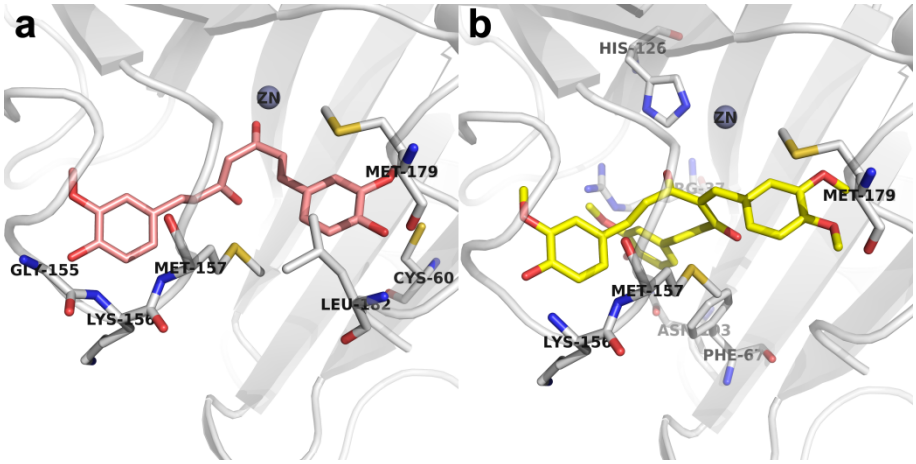

Figure 6. Docking poses of curcumin (a) and its analog ChEMBL 1669727 (b) inside the binding pocket of GLO1 as displayed in pink and yellow, respectively. The figure shown was created using PyMol software from docking results together with the best-ranked poses having the lowest energy as calculated by AutoDock 4.2.

The combined use of experimental and high-throughput virtual screening has been extensively applied for the identification of how curcumin and its analogs inhibits target proteins including, for example, DNA methyltransferase [198-201] and HIV-1 integrase [202-204] as well as protein kinases where the latter comprises GSK [205], AMPK [206], PKC [207] and EGFR [208, 209]. Ngo et al. [194] employed molecular docking followed by long time scale MD simulations in their investigations of how curcumin and two nonsteroidal anti-inflammatory drugs (NSAIDs) interacts with the binding site of $A\beta_{1-40}$ mature fibrils. Their results suggested that both curcumin and NSAIDs share the same binding pocket buried inside the fibrils. Binding free energies as obtained from the molecular mechanic Poisson-Boltzmann surface area (MM-PBSA) method indicated that curcumin afforded higher binding affinity than those of NSAIDs, which was found to be in good agreement with experimental finding [194]. This result implicates the role of curcumin in inhibiting amyloid-β aggregation, which is the hallmark of Alzheimer's disease.

In summary, although X-ray crystallography is a more accurate method for analyzing the interaction of a protein with bound ligands, it may not be practical to grow crystals for such protein-curcumin systems owing to inherent technicalities. In such cases, receptor-based computational modeling, which requires the availability of protein structures and when coupled to high-throughput molecular docking and subsequently analyzed by MD simulations, one can gain insights on such interactions of curcumins with their target proteins as to afford better understanding on the role of curcumins in modulating a wide range of biological activities.

CONCLUSION

Curcumin has been exploited by humankind for more than 5,000 years with a rich history of utilizations (i.e. food, cosmetics, textiles, household medicine, etc.). This had drawn great interest by the scientific community in gaining more understanding on the mechanistic basis

of its therapeutic action. It is inevitable to perform such task without the knowledge on its structure-activity relationship. This chapter addressed this point by reviewing our current understanding on the structural details of curcumin and its analogs with relation to its observed biological activities. Such endeavor is augmented through the use of molecular simulation that may further facilitate the rational design of novel analogs with robust biological activities as well as improved pharmacokinetic properties. As we had previously emphasized, there is simply too much to be learned and understood with respect to the therapeutic usage of curcumin. In light of the concept of polypharmacology, there is ample space to further explore in understanding the full spectrum of its possible pharmacological activities. It is the focus and ambition of this chapter to provide a glimpse of the structure-activity relationship of curcumin as well as encourage continual progress in realizing the full therapeutic utilization of curcumin.

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