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Review

Curcumin and curcuminoids in quest for medicinal status

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Curcumin, known for thousands of years as an Ayurvedic medicine, and popular as a spice in Asian cuisine, has undergone in recent times remarkable transformation into a drug candidate with prospective multipotent therapeutic applications. Characterized by high chemical reactivity, resulting from an extended conjugated double bond system prone to nucleophilic attack, curcumin has been shown to interact with a plethora of molecular targets, in numerous experimental observations based on spectral, physicochemical or biological principles. The collected preclinical pharmacological data support traditional claims concerning the medicinal potential of curcumin and its congeners but at the same time point to their suboptimal properties in the ADME (absorption, distribution, metabolism and excretion) area.

Key words: curcumin, curcumin derivatives and analogs, curcuminoids, diferuloylmethane, Michael acceptors, oleoresin, turmeric

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INTRODUCTION

Curcumin (**1**; Fig. 1) is a secondary metabolite, constituent of the Asian spice named turmeric, which has been known in Ayurvedic medicine for millennia as a remedy for numerous ailments, including inflammation, cancer and pathogenic invasions (Ammon & Wald, 1991; Kapoor, 1990). The principal pigment of turmeric was first isolated by Pelletier (Vogel & Pelletier, 1815), at the advent of great discoveries in natural products era, and almost a century later Polish chemists established its structure and subsequently confirmed it by synthesis (Miłobędzka *et al.,* 1910; Lampe & Miłobędzka, 1913). Today, unlike many other natural products within the ethno-medicinal tradition, curcumin still evokes great interest as a food additive, dietary supplement and prospective medicine. In the last decade about two hundred papers concerning curcumin were published annually, only in connection with pharmaceutical preparations involving its innovative nanotechnological formulations. At the same time, approximately hundred patents were filed each ear, globally. In 2011 the SciFinder gave over 12 000 hits for "curcumin". Among them were over 1 000 review articles, nearly 1 500 patents and 56 references to clinical trials. Obviously, this hype should have some rational explanation. A critical survey of the recent wave of scientific information on curcumin clearly suggests that the compound has made great promise, as a potent modulator of activity of many vital biomacromolecular targets involved in homeostasis of mammalian physiology (Aggarwal *et al.,* 2007; Aggarwal & Sung, 2009). However, it is generally agreed, that the advantageous molecular interactions of curcumin with functional proteins, known from model cellular experiments, are not reflected as clear health benefits at the systemic level. Hence, a new wave of interest, this time shifted to improvements in the active substance pharmacokinetics (PK) and delivery systems (Anand *et al.,* 2008; Aggarwal & Haricumar, 2009; Marathe *et al.,* 2011). The name curcumin is widely used in common language in a rather imprecise manner, often referring to the turmeric powder used as a cooking spice. In scientific literature the name refers only to the compound corresponding to formula **1** (of defined chemical purity), while a mixture of natural pigments represented by formulae **1–3** is collectively named curcuminoids. The latter name has been adopted to accommodate also synthetic analogs and derivatives of **1**, but in that case it should be noted that

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Abbreviations: ADME — absorption, distribution, metabolism and excretion — principal processes controlling pharmacokinetics (PK) and pharmacodynamic (PD) effects of drugs; Abbreviations used for molecular targets of curcumin: AP-1, activating protein-1; AR, androgenreceptor; Arh-R, aryl hydrocarbon receptor; ATPase, a class of enzymes that catalyze the decomposition of adenosine triphosphate; cAK, autophosphorylation-activated protein kinase; Bcl-2, B-cell lymphoma protein 2; Bcl-xL, anti-apoptotic proteine; CBP, CREB-binding protein; Ca^{2+}PK/CDPK, Ca^{2+}-dependent protein kinase; cPK, protamine kinase; CTGF, connective tissue growth factor; COX-2, cyclooxygenase-2; CXCR4 alpha-chemokine receptor; DFF40, DNA fragmentation factor, 40-kd subunit; DR-5, death receptor-5; EGF, epidermal growth factor; EGF-R, EGF-receptor; EGFRK, EGF receptor-kinase; Egr-1, early growth response gene-1; ELAM-1, endothelial leukocyte adhesion molecule-1; EPC-R, endothelial protein C-receptor; EpRE, electrophile response element; ER, estrogen receptor; ERK, extracellular receptor kinase; FAK, focal adhesion kinase; Fas-R, Fas receptor; FGF, fibroblast growth factor; FPTase, farnesyl protein transferase; Gcl, glutamatecysteine ligase; GST, gluthathione-*S*-transferase; H2-R, histamine (2)-receptor; HGF, hepatocytegrowth factor; HO, hemeoxygenase; HSP-70, heat shock protein 70; iNOS, inducible nitric oxide synthase; IAP, inhibitory apoptosis protein; IARK, interleukin-1 receptor-associated kinase; ICAM-1, intracellular adhesion molecule-1; InsP3-R, inositol 1,4,5-triphosphate receptor; IL, interleukin; IL-1 receptor-associated kinase; IL-8-R, interleukin-8-receptor; IR, integrin receptor; JAK, janus kinase; JNK, c-jun N-terminal kinase; LDL-R, low-density lipoprotein-receptor; LOX, lipoxygenase; MAPK, mitogen-activated protein kinase; MCP, monocyte chemoattractant protein; MDR, multidrug resistance; MIF, migration inhibition protein; MIP, macrophage inflammatory protein; MMP,matrix metalloproteinase; NAT, arylamine *N*-acetyltransferases; Nrf-2,nuclear factor erythroid 2-related factor; NF-κB, nuclear factor-kappaB; NGF, nerve growth factor; Notch-1, highly conserved cell signaling system; NQO-1, Nrf-2,nuclear factor erythroid 2-related factor; NAD(P)H dehydrogenase, quinone 1; ODC, Ornithine decarboxylase; STAT, signal transducers and activators of transcription; PDGF, platelet-derived growth factor; PhK, phosphorylase kinase; PKA, protein kinase A; PKB, protein kinase B; PKC, protein kinase C; pp60c-src, a nonreceptor protein tyrosine kinase c-Src, cellular src kinase; PPAR-γ, peroxisome preoliferator-activated receptor γ; PTK, protein tyrosine kinase; SHP-2, Src homology 2 domaincontaining tyrosine phosphatase 2; STAT, signal transducers and activators of transcription; TGF-1,transforming growth factor-1; TIMP, tissue inhibitor of metalloproteinase-3; TK, protein tyrosine kinase; TNF, tumor necrosis factor; uPA, urokinase-type plasminogen activator, VCAM-1, vascular cell adhesion molecule-1; VEGF, vascular endothelial growth factor.

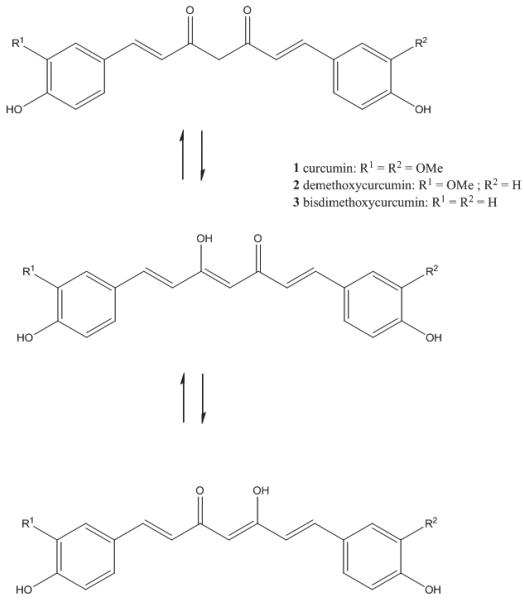

Figure 1. Principal curcuminoids of turmeric shown as ketone-enol equilibrium

curcuminoid characteristics require fully extended conjugation of the aromatic and the C_7 linker double bond system.

CHEMOTAXONOMY, AGRICULTURE AND BIOSYNTHESIS OF CURCUMINOIDS

Compounds depicted in Fig. 1, yellow-orange pigments accumulating in turmeric rhizomes, are known under the collective name of curcuminoids. Their presence seems to be a distinctive genetic and chemotaxonomical trait limited to the Asian perennial shrub *Curcuma*, (more than 100 *Curcuma* species are listed in botanical sources; Babu *et al.,* 2007) members of the ginger family (*Zingiberaceae*) of which *Curcuma longa* L. (turmeric) is the best known representative. The plant is also considered as a medicinal herb in the West, as evidenced by WHO and Commission E monographs (World Health Organization, 1999; Blumenthal, 2000). Turmeric is cultivated in many Asian countries but India is the most important producer by far (ca. 850 000 tons; 78% of the global market; productivity reaching 4.7 tons/ha) end exporter of the commodity, used as a spice and food colorant also in the USA and Europe. The Indian Institute of Spice Research in Calicut, Kerala, maintains a collection of ca. 600 cultivars, specified by regions and distinct locations of growth. Best of them are reported to contain 4–6% of curcuminoids based on dried root weight. The post-harvest process of turmeric powder production involves slicing the rhizomes, boiling in water, drying and powdering the residue, which is subsequently traded as such or used as the principal constituent of curry powder (containing also coriander, cardamom and cumin as well as minor admixtures of other spices). Turmeric powder is known to be photo-labile and require special conditions for storage. Another important product, which can be considered a fine chemical, is turmeric oleoresin, which is obtained by organic solvent extraction of cured curcuma rhizomes followed by evaporation. This material typically contains over 80% curcuminoids and constitutes convenient source of pure natural curcumin. (Babu, *et al.,* 2007; Li *et al.,* 2011). Both: the spice and its isolates, when provided by reliable sources, are presently subject to examination by modern analytical techniques and they are accompanied by detailed specifications using curcumin as the principal marker of quality.

The first biogenetic studies of *Curcuma* pigments were based on ^{14}C precursor feeding experiments and resulted in two pathways being proposed, in which feruloyl-CoA and malonyl-CoA were utilized in two different modes of condensations (Roughley, 1971; Roughley, 1973). In a recent study modern spectroscopic techniques were used in experiment focused on demethoxycurcumin (DMC, **2**) as the product. Using ^{13}C labeled phenylalanine **4** as basic precursor and ^{13}C NMR technique for spectral monitoring, allowed for effective tracing of non-symmetrical precursors, in which principally every carbon atom could be assigned and accounted for, on the way from phenylalanine derived cinnamic acids (**5, 8, 10** and their CoA derivatives **6**, **9**, **11**) to the final products — curcuminoids. Despite meticulous design of the experiment, there was no definite conclusion concerning the contribution of the alternative pathways. The authors postulated formation of a common intermediate — bis-deshydroxybisdesmethoxycurcumin **7** (Fig. 2; not found, allegedly because of rapid transformation into hydroxylated and methoxylated products), but did not exclude the participation of cumaroyl or feruloyl intermediates in the biogenetic process (Kita *et al.,* 2008).

PHYSICOCHEMICAL AND SPECTRAL PROPERTIES OF CURCUMIN

The chemical name of curcumin, **1** [CAS No. 458-37-7] is (1*E*, 6*E*) -1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione and it is often referred to as diferruloylmethane (in which case the central methylene carbon is numbered as C-1). Neither of these names reflects the fact that **1** exists, predominantly if not exclusively, as an equilibrium of equivalent enol forms, rather than tautomeric ketone — enol mixture. (Fig. 1) This concerns both: solutions (Payton *et al.,* 2007; Li *et al.,* 2009), and the solid state of **1** (Tønnesen *et al.,* 1982; Anand *et al.,* 2008; Parameswari *et al.,* 2011), thus the name 5-hydroxy-(1*E*, 4*Z*, 6*E*)-1,7-bis-(4-hydroxy-3-methoxyphenyl)-hepta-1,4,6-trien-3-one should be used more frequently (Parimita *et al.,* 2007).

Since curcumin is not only a popular spice, constituent of curry powder, but is also used as an ecological dye, it is known as Natural Yellow 3 and is assigned E100 number, when applied as a food coloring agent. The atomic composition $C_{21}H_{20}O_6$ corresponds to a molecular mass of 368.39 g/mol. The reference melting point 183°C is seldom recorded in practice for isolated samples, because of natural admixtures in commercial materials. For synthetic **1**, purified by crystallization from methanol, mp 187–188°C was reported (Buadonpri *et al.,* 2009). The pigment is practically insoluble in water and its solubility in most organic solvents (with the exception of acetone, acetic acid, acetonitrile and lower alcohols) is rather low. For biological experiments, stock solutions of **1** are prepared in DMSO, or less often in DMF. Curcumin is available commercially as an extract from *Curcuma* roots (where its content is estimated at 3–6%), and marketable yellow powders are usually assayed for sum of curcuminoid content (typically 77% of **1**, 17% of **2** and 6% of **3**). Such extracts can be easily analysed by HPLC (Jayaprakasha *et al.,* 2002; Sotanaphun *et al.,* 2007) and column chromatography or preparative TLC on sili-

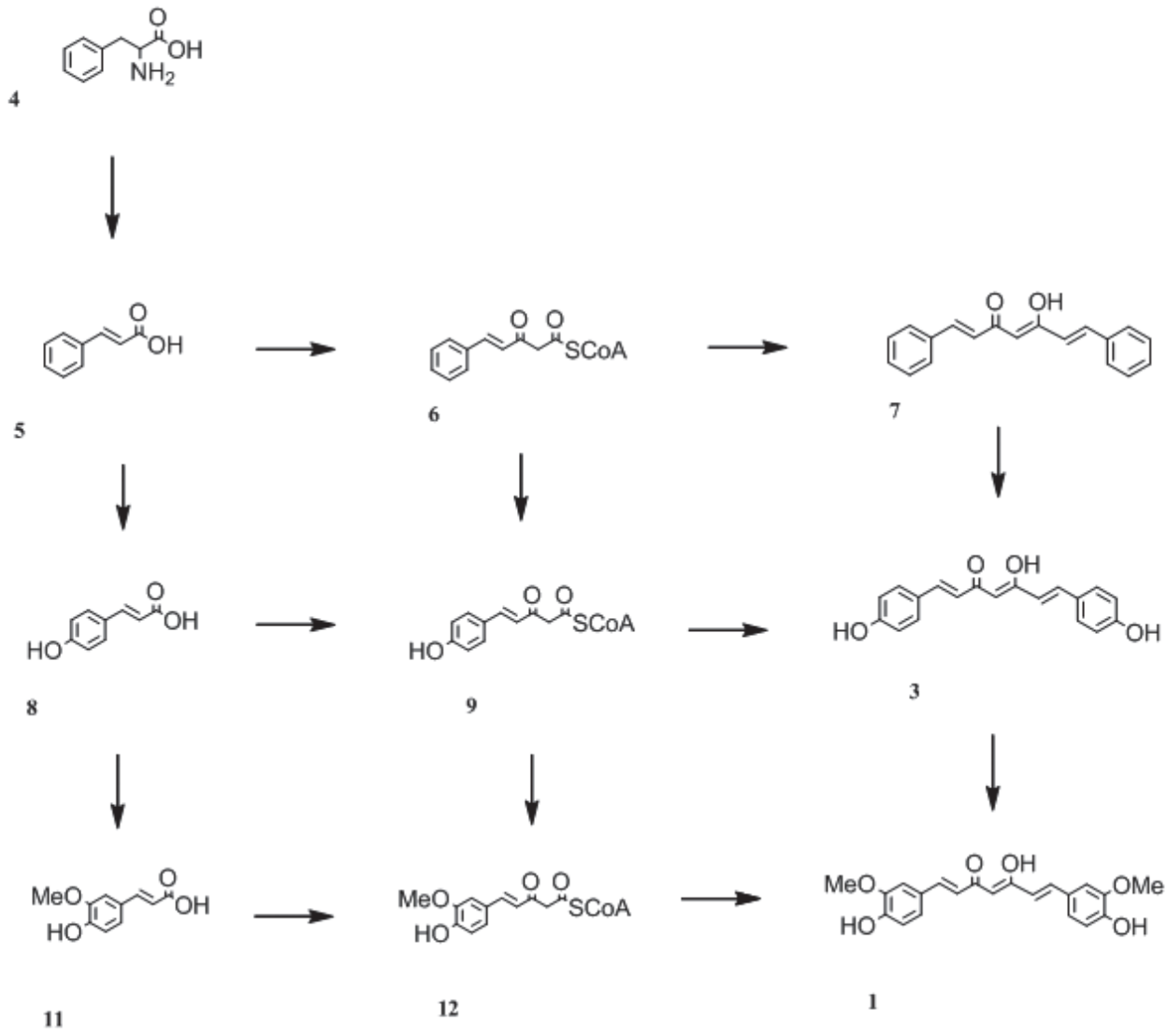

Figure 2. Biosynthetic pathways for curcuminoids. All paths are possible

cagel are advocated for isolation and purification of the main component (Peret-Almedia *et al.,* 2005; Pathania *et al.,* 2006).

The centrosymmetric crystal structure of **1** was first determined by X-ray diffraction (Tønnesen *et al.,* 1982) and it was demonstrated that the enol form is strongly stabilized by asymmetric intermolecular hydrogen bonds in the solid state. Recent measurements have revealed that **1** crystallizes in the orthorhombic space group Pca2 1 with two molecules in the asymmetric unit, each characterized by a strong hydrogen bond between the enol and ketone functions (Parameswari *et al.,* 2011). Density Functional Theory (DFT) was used to perform structural and conformational analysis of **1** and computations of essential frequencies in IR, Raman and vibrational spectra were performed (Kolev *et al.,* 2005). Considering four different rotation axes for the mono-enol structure of **1**, eight relatively stable conformers were proposed. In view of those results it was not surprising, that curcumin exhibits solid state polymorphism (Sanphuis *et al.,* 2011), with three crystalline forms revealed so far. Experimental UV/VIS spectra of **1** in solution show maxima at 420 nm (log ε = 4.77) and 265 nm (log ε = 4.18). The fluorescence emission band is at 511 nm for excitation wavelength of 433 nm for solutions in aprotic solvents. Photochemistry of **1** has been studied in some detail (Tønnesen *et al.,* 1982; Chignel *et al.,* 1994; Priyadarsini 2009), disclosing its rather limited photostability and tendency to generate singlet oxygen upon excitation. Calculations of HOMO and LUMO orbitals for curcumin have been plotted, in an attempt to justify experimental results which demonstrated its binding to beta-amyloid aggregates, *in vitro* and *in vivo* (Yang *et al.,* 2005; Balasubramanian 2006). There is no doubt that only deep analysis of the molecular properties of **1** in an idealized state, as well as in the form of a ligand, controlled by a force field of multifunctional biological macromolecule, can lead to proper understanding of its action in biological models and human physiology (Priyadarsini, 2009; Girija *et al.,* 2010). Curcumin is considered hydrophobic, based on predicted values of log P ranging from 2.56 to 3.29, which justifies such experimental observations as lipid membrane affinity, interactions with hydrophobic domains of proteins and ability to cross the blood-brain

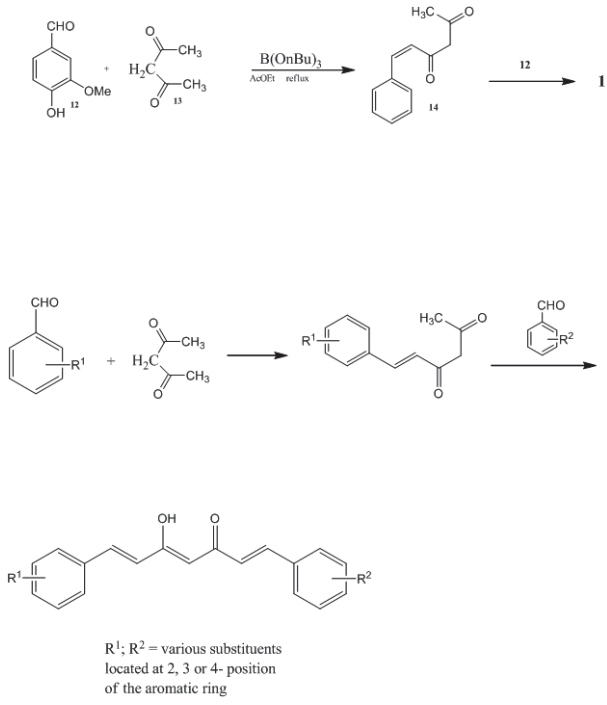

Figure 3. Stepwise synthesis of curcumin and non-symmetric curcuminoids

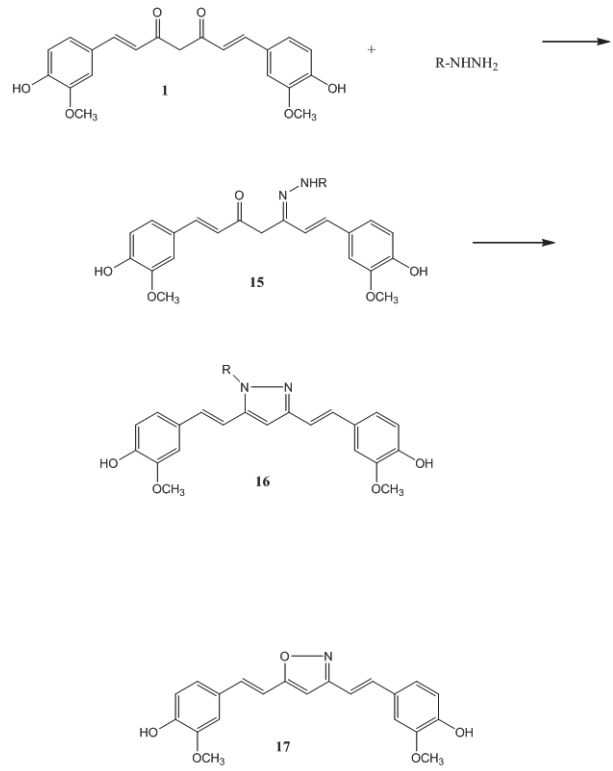

Figure 4a. Curcumine derived pyrazoles 16 and oxazole 17

barrier (Basnet & Skalko-Basnet, 2011; Sadpathy *et al.,* 2010). Owing to the mentioned spectral characteristics, curcumin is easy to detect and also to determine quantitatively. Various separation techniques have been adopted for analysis of curcuminoids in herbal sources and preparations derived from them: HP TLC, RP HPLC and CE, and UV/VIS spectroscopy is routinely used for detection (Peret-Almedia *et al.,* 2005; Jadhav *et al.,* 2007; Sotanaphun *et al.,* 2007; Wichidnithad *et al.*, 2009; Himesh *et al.,* 2011; Rohman, 2012). However, more efficient quantification of **1**–**3** can be achieved with application of fluorescence detection at λ_{ex} 426 nm and λ_{em} 539 nm, with the LOD of about 1 ng mL^{-1} for natural curcuminoids (Zhang *et al.*, 2009).

CHEMICAL REACTIVITY OF CURCUMIN

Curcumin was first obtained by synthesis in S. Kostanecki's laboratory in Berne (Lampe & Miłobędzka, 1913), where sequential acylation of diethyl malonate with cinnamoyl chloride was successfully employed as a synthetic concept. Simple retrosynthetic analysis suggests that **1** can also be made from acetyl acetone, by condensing it with an appropriate aromatic aldehyde, but in a direct reaction between these substrates the central methylene group of diketone is likely to react before any end methyl group (because of its higher acidity). It has been demonstrated, however, that formation of 1,3-dienolate complex with boric acid sufficiently protects the methylene group and allows for Knoevenagel type condensation, in which diaryl-heptenoates are easily formed. Importantly, the phenolic hydroxyls do not need protection in the process of assembling the molecular framework of curcumin. Symmetric analogs of **1** or **3** are easily obtainable in a base-catalyzed reaction of acetyl acetone (**13**, pentanone-2,4; typically applied as *in situ*-generated boro-

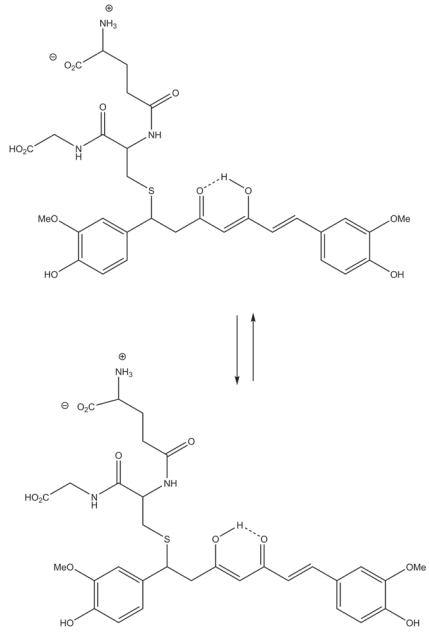

Figure 4b. Hypothetical Michael adduct of gluthathione and curcumin, pictured in form of tautomeric equilibrium

nate compex) with two equivalents of an aromatic aldehyde (Fig. 3; vanillin **12** is used in the case of curcumin). Preparation of curcuminoids with different substitution patterns in both aromatic rings involves a stepwise procedure, for which solid state synthesis technology is recommended (Anand *et al.,* 2008; Dubey *et al.,* 2008; Padhye *et al.,* 2010). Typical solvents for condensation range from aprotic (dimethylformamide, ethyl acetate) to acetic acid. As a basic catalyst *n*-butylamine, morpholine or diethanolamine have been used; while boron oxide or

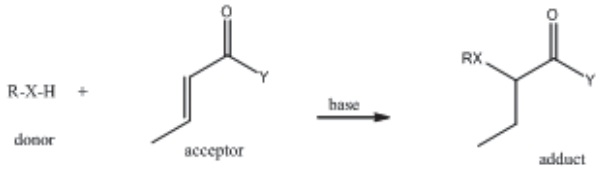

Figure 5. Schematic representation of Michael addition reaction. Nucleophiles (e.g. anions of OH, NH, SH and CH acids) are good donors. Unsaturated carbonyl compounds (e.g. conjugated aldehydes, ketaones and esters are good acceptors.

borate esters served as dienolic function protection. The reaction is usually carried out with reflux, but irradiation energy, ranging from sonic to microwave, has also been successfully employed for substrate activation (Nichols *et al.,* 2006; Buadonpri *et al.,* 2009).

The trivial name of curcumin — diferuloylmethane — is usefully descriptive when it comes to discussion of its chemical reactivity, because it underlines the formal symmetry, conjugated 1,3-unsaturated diketone characteristics, and typical phenolic features of the compound. Although the solubility of **1** in water is very low, both the food preparation conditions and the physiological parameters of human body, call for comments concerning the aqueous environment. Curcumin is a weak Brønsted acid, capable of three-step deprotonation as the pH of its solution increases. This process is accompanied by profound changes in light absorption in the visible region. The solubility of curcumin in water increases with basification and deep red coloration develops, but at the same time the chemical stability of the multiple hydroxyl anions decreases sharply and fragmentation of the conjugated system by carbon-carbon bond fission occurs. Ferulic acid and feruloylmethane are initial degradation products; the latter decomposes further into vanillin and acetone. Another prominent feature of curcumin reactivity in aqueous media is connected with typical reactivity of polyphenols, which features susceptibility to attack by free radicals, subsequently leading to various substitution and condensation reactions (Agnihotri *et al.*, 2011). The antioxidant properties of **1**, well documented in chemical laboratory experiments, is believed to correspond with physiological conditions, when scavenging of ROS and nitrogen radicals can protect important biological molecules (e.g., lipids) from peroxidation (Basnet & Skalko-Basnet, 2011). Details of the mechanisms involved in reaction of various phenols with free radicals have become a subject of separate studies, particulary in reference to phenolic hydrogen transfer within extended conjugated systems (Litwinienko & Ingold, 2007; Kowalewska *et al.,* 2010). In regard to other aspects of chemical reactivity, similarly to other natural phenolics, **1** can easily undergo acylation or alkylation. These possibilities have already been tested and corresponding derivatives of **1–3** applied in the syntheses of new chemical entities. (Dubey *et al.*, 2008; Wichitnithad *et al.,* 2011). Chemical glycosylation is a separate category of derivatization, because the facility of selective and efficient enzymatic glycosidation in plants or glucuronidation in animals can hardly be matched by synthetic laboratory procedures. Despite difficulties, desired glucosidic derivatives of curcuminoids have been obtained by using vanillin glycosides as substrates in acetylacetone condensation (Mohri *et al.,* 2003) and also in direct condensation of **1** with acylated glycosyl halides, under ultrasound-assisted PTC conditions (Parvathy *et al.,* 2008).

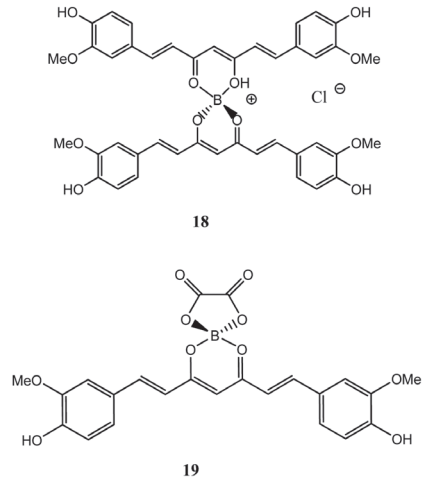

Figure 6. Rosocyanine 18 and rubrocurcumin 19

Curcumin is a typical acceptor in Michael addition — a property often mentioned in connection with the pleiotropic reactivity of **1** with proteins, deduced from the observed inhibition of their biological activity. Michael reaction, described at the end of XIX century, is illustrated by a simple schematic chemical equation (Fig 5), describing addition of an exemplary nucleophile to conjugated π-electron systems, typically an α,β-unsaturated ketone or ester (Bergmann *et al.,* 1959; Li, 2009). The principal feature of this reaction is reversibility, hence its biological importance. Since the most active nucleophiles in the biological environment are sulfhydryl (-SH) groups of peptides, the structure of a Michael adduct is further exemplified by a hypothetical conjugate of glutathione and **1** (Fig 4b).

However, some primary Michael addition products can be stabilized by subsequent transformations, like cyclization, rearrangement or dehydration, as demonstrated in innumerable syntheses of heterocyclic compounds. Many new derivatives of **1** have been synthesized based on this principle, as illustrated in Fig. 5. Typical nucleophiles used in these reactions were bifunctional, (e.g., hydrazines), which facilitated stabilization of intermediate Michael adducts, through secondary nucleophilic attack and ring closure (Anand *et al.*, 2008; Narlawar *et al.*, 2008). Since many of the synthetic heterocyclic derivatives of **1** exhibited interesting pharmacological properties, it has been observed, that feruloylacetone **14** could serve as a suitable model of minimal pharmacophore, capable of mimicking antioxidant activity as well as reactivity of curcumin towards nucleophiles. (This compound was obtained by condensation of equimolar amounts of vanillin and acetylacetone, in reaction catalyzed with boric acid and *n*-butylamine; Feng, 2011).

Curcumin is known to form strong molecular complexes with a variety of charged species, including bivalent metal cations. Tetraedric boron complexes (rosocyanine **18**; rubrocurcumin **19**; Fig. 6) are particularly well known, because of their application in analytical chemistry (Balaban, 2008). In connection with this, the metal ion complexing ability of curcumin has raised some concerns about possible sequestration of iron and a threat of anemia in some groups of its consumers.

MOLECULAR TARGET	DOWNREGULATION		UPREGULATION
ENZYMES	Telomerase	GICL	
	DNA Pol	COX-2	
	TMMP-3	MMP	
	Src-2	ATPase	
	GST	ATPase	
	FPT	GCL	
	ODC	AATF-1	
	NQO-1	Desalurasc	
	PhP D	5-LOX	
	INOS		
KINASES	FAK	PKA	JNK
	Pp60c-tk	AAK	
	IL-1R	AK MAPK	
	JAK	PKB	
	Ca^2PK	EGFR-K	
	ERK	PAK	
	PTK	PhK	
RECEPTORS	Fas R	IL-8 R	DR-5
	IR	CXCR4	
	EPCR	LDLR	
	H2R	ITR	
	EGFR	AHR	
	HER-2	AR	
	ER-α		
TRANSCRIPTION FACTORS	Notch-1	CREB-BP	Nrf-2
	ERG-1	NF-Xb	ERE
	WT-1	HIF-1	
	B-catenin	STAT-1	
	AP-1	STAT-3	
	PPAR-γ	STAT-5	
	STAT-4		
GROWTH FACTORS	CTGF	NGF	
	FGF	TGF-β1	
	EGF	TF	
	VEGH	PDGF	
	HGF		
INFLAMMATORY CYTOKINES	TNF-α	IL-5	
	MaIP	IL-6	
	MIP	IL-8	
	MCP	IL-12	
	IL-1	IL-18	
	IL-2		
OTHERS	MDRP	VCAM-1	DEF-40
	IAP-1	Hsp-70	p53
	ELAM-1	Bcl-2	
	Cyclin D1	ICAM-1	
	Bcl-Xl	uPA	

Figure 7. Principal molecular targets of curcumin (downregulated).
Arrows indicate molecular targets upregulated by curcumin. Full list of abbreviations follows the title page.

MOLECULAR PHARMACOLOGY

There is an extensive evidence indicating that curcumin interacts, directly or by modulating signaling pathways, with many molecular targets (Fig. 7). It should be pointed out that a wide array of high-tech methods have been applied in the process of accumulating knowledge about preclinical pharmacology of **1**, which is summarized in recent reviews. These include, apart from conventional biological activity tests, advanced spectroscopic techniques like NMR, fluorescence, Fourier transform infrared (FTIR) and circular dichroism (CD), surface plasmon resonance, competitive ligand binding, Forster type fluorescence resonance energy transfer (FRET), radiolabeling, site-directed mutagenesis, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), immunoprecipitation etc. (Gupta *et al.,* 2011 and references cited therein).

Curcumin is a powerful antioxidant – a property considered advantageous in a food constituent or dietary supplement, since the red-ox balance of living cells is constantly challenged by newly formed reactive oxygen species (ROS) and their nitrogen analogs (Fujisawa, 2004; Augustyniak, 2010). Organisms are equipped with protective enzymes (e.g., superoxide dismutase, catalase) and additionally a set of low molecular weight compounds, capable of neutralizing ROS in their hydrophilic (e.g., ascorbate, glutathione) as well as lipophilic version (e.g., tocopherols). Nevertheless, the intake of dietary antioxidants (plant phenolics, flavonoids, carotenoids) is considered very important for human well being. Scavenging of ROS by **1**, which is considered to be ten times more effective in this respect than alpha-tocopherol, leads to secondary free radicals which are considerably less harmful. It should not be ignored, however, that curcumin can also act as a photo-sensitizer of singlet oxygen and its congeners, thus exerting pro-oxidative action. A second important point in consideration of nonselective, systemic action of **1**, is its ability to up-regulate expression of some genes, in particular to enhance production of inducible enzymes. Studies along this line have confirmed curcumin influence on expression of various enzymes involved in biological red-ox processes (e.g., glutathione synthase GTS, cytochrome P 450 oxidases CYP-450, etc.). There is no doubt that the most important biological actions of **1** are those, connected with its highly selective interactions with certain proteins and leading to substantial inhibition of their activity. The assumption that the antioxidant activity of **1** is connected with anti-inflammatory effects is supported by findings on inhibition of lipooxygenase and cyclooxygenase (LOX and COX, the key enzymes responsible for transformation of arachidonic acid to prostaglandins) activities by natural and synthetic curcuminoids (Aggarwal & Harikumar, 2009; Shezad *et al.,* 2010). In fact, inhibition of lipid peroxidation by **1** has been observed in many experimental models. Additionally, curcumin inhibits the production of pro-inflammatory cytokines (e.g., interleukines: IL-1β and IL-8) as well as tumor necrosis factor alpha (TNF-α), xanthine oxidase, inducible nitrogen oxide synthase (iNOS) and monocyte inflammatory protein-1 (MIP-1). In contrast to a great majority of studies, in which the problem of the molecular mechanism of such inhibitions is virtually neglected, a report on inhibition of thioredoxin reductase (TrxR, overexpressed in cancer cells), describes irreversible modification of the enzyme by **1**, as a result of a Michael addition reaction between the reductase rich in sulfhydryl groups and the unsaturated conjugated ketone functionality of curcumin (Fang *et al.,* 2005). It seems crucial, however, that TrxR isoenzymes contain the very rare aminoacid selenocysteine, which should by virtue of high RSe-H nucleophilicity be a much stronger Michael donor than any sulfhydryl substrate. In fact, **1** is known to inhibit glutathione reductase only weakly at 50 μM, while affecting TrxR at much lower concentrations. Undoubtedly the topic of covalent modification of proteins by curcumin warrants more research, very much like its interactions with lipids of cellular and organellar membranes, which can lead to functional changes by modulating their fluidity and permeability. Generally, two lines of research on the interaction of **1** with proteins are actively pursued: *in silico* inhibition studies (Wilson *et al.,* 2006; Girija *et al.,* 2010; Kumar & Bore, 2011) and experimental affinity and binding measurements (Fang *et al.,* 2005; Mitra, 2007). It comes as no surprise that experiments, unlike molecular modeling, can reveal rather unexpected

aspects of curcumin biological activity. For example, investigation of complexation of **1** with soy lipooxygenase indicated formation, under X-ray irradiation, of a metastable adduct with the connectivity: Enz–Fe–O–O–R , in which R stands for a new, bicyclic derivative of curcumin with four newly formed chiral centers (Skrzypczak-Jankun *et al.*, 2003). This example indicates clearly that initial complexes of **1** with hydrophobic pockets of some proteins can relatively easily undergo transition from hydrogen bonding controlled structure to a new covalent bonding.

The list of established molecular targets of curcumin is exceptionally long. (Basile *et al.,* 2009; Shezed *et al.,* 2010; Ferrari *et al.,* 2011). Continuing with the enzyme category, a group of protein kinases should be mentioned: cAK, ERK, FAK, JAK, PKA, PKB, PKC, MAPK, TK etc. Other enzymes include: ATPases, DNA polymerases and telomerases. Perhaps even more important are other target categories: receptors, growth factors, transcription factors, inflammatory cytokines, cell adhesion molecules and gene expression regulators, with multiple examples, listed in numerous review articles (Padhye *et al.,* 2010; Shezad, 2010; Aggarwal & Haricumar, 2009; Anand *et al.*, 2008; Hatcher *et al.*, 2008). The effects of activity modulation of the mentioned targets by **1** can be arranged along known signaling pathways for which chain of molecular events are reasonably well established. For example, curcumin downregulates expression and activity of epidermal growth factor receptors (EGFR), effectively diminishing the effects of epidermal growth factors (EGFs), including those closely linked with cancer promotion (e.g., cancers of the breast, lung, kidney, prostate). Alternatively, **1** downregulates signaling through the JAK/STAT pathway, which leads to negative regulation of proinflamatory interleukines (IL-6, IL-2). In recent years many studies have focused on nuclear factor kappa B (NF-$\varkappa$B) as an agent which is constitutively overexpressed in key pathologies, from inflammation to cancer, controlling through various downstream effectors such important stages as tumor growth, invasion, angiogenesis and metastasis. It has been demonstrated that **1** inhibits NF-$\varkappa$B activation, not by chemical modification of the protein, but rather by neutralizing its activators or their products (Aggarwal *et al.,* 2007; Hatcher *et al.,* 2008; Lin *et al.,* 2008). A detailed discussion of the entire list of macromolecules targeted by **1**, is beyond the scope of this review. It will suffice to remark here, that pharmacological studies on curcumin (and curcuminoids) help to contest somewhat one-sided view, deeply rooted in theory and practice of the last century drug design, that the good drug lead should be selective towards one single target. Our present view, accepts multiple causes and mediators involved in development of such pathology as cancer, and is much more tolerant towards studies of the agents which exhibit pleiotropic biological activity. As has been stressed, an overall effect of pleiotropic action of **1** at the cellular level is that it acts as a chemosensitizer and radiosensitizer for tumors and as a chemoprotector and radioprotector for normal tissues (Jagetia, 2007; Goel & Aggarwal, 2010).

TARGET PATHOLOGIES

There is a natural tendency to translate basic scientific discoveries in biology, biochemistry and pharmacology into medicinal practice, but the way to such applications typical for contemporary new drug discovery is long, and costly, and the attrition rate of candidates is very high. Curcumin, which has an exceptionally long record of usage in traditional medicine practices, was considered a drug candidate by Western medicine relatively recently. Two papers which appeared in *Drugs of the Future* in a span of 23 years can serve as an illustration of a slow change in perception of the curcumin's therapeutic potential (Srimal, 1987; Shehzad & Lee, 2010). Remarkably, the first paper already suggested chemical synthesis of **1** as an efficient source of research material and stressed its lack of toxicity. Multifactorial anti-inflammatory action of curcumin, very low ulcerogenic index and a lack of antipyretic effects were underlined as arguments for potential application in treatment of rheumathoid arthritis, with some advantage over non-steroidal anti-inflammatory drugs. (Srimal, 1987) The more recent article in the same journal (Shehzad & Lee, 2010) presents an entirely different perspective, stressing the multitude of molecular targets for **1**, discussing the complexity of their cross-talk and indicating a wide span of its possible application in chemoprevention as well as therapy. Most of contemporary research tends to concentrate on the antitumor activity of **1** (Bar-Sela *et al.,* 2010; Basnet & Skalko-Basnet, 2011). Current knowledge of cancer biology offers a clear-cut distinction between the physiology of normal and tumor cells which are characterized by extensive proliferative signaling, replicate immortality, angiogenesis, and resistance to growth suppressors and apoptosis. Curcumin has been demonstrated to be an effective modulator of the activity of a majority of mediators responsible for tumor promotion and progression.

As we have pointed out, curcumin is a pigment, with sufficient affinity for various organic materials to be called (and used as) a dye. It is well known from the times of Paul Ehrlich and Hans Christian Gram, that organic dyes can exhibit differential affinity for various tissues, cells and even sub-cellular compartments, allowing their selective coloration, which facilitates identification of physiological (and pathological) preparates. It has been demonstrated that **1**, similarly to such synthetic dyes as Congo Red or Chrysamine G, shows selective affinity for pathological amyloid fibrils (plaques), a hallmark of Alzheimer's disease. This observation has led to the discovery that curcumin and synthetic curcuminoids have considerable potential as inhibitors of protein tau and amyloid beta aggregation (Yang *et al.,* 2005; Ringman *et al.,* 2005; Narlawar *et al.,* 2008). Other prospective medicinal applications of curcuminoids include arthritis, diabetes, cardiovascular disease, wound healing, and the list is growing with new experimental and clinical therapy papers published (Aggarwal & Sung, 2009; Shezad & Lee, 2010; Gupta *et al.,* 2011).

SYSTEMIC AND CLINICAL OBSERVATIONS

The United States Food and Drug Administration (FDA) has declared curcumin as "generally regarded as safe" (GRAS) and acceptable for food coloring and flavoring. The results from several pilot and phase I clinical trials in patients and volunteers confirm that low systemic bioavailability is achieved after oral dosing and it is related to rapid first-pass metabolism and some degree of intestinal pre-metabolism. Metabolite structures testify to the bio-oxidative nature of **1**: they result from partial or even total hydrogenation of the heptatrienone carbon chain (Herebian *et al.,* 2009). Recently, it has been found that curcumin-converting enzymes are of intesti-

nal microbial origin (Hassaninasab *et al.,* 2011). Products of conjugative metabolism are typical for phenolics and include glucuronates and sulfates (Ireson *et al.,* 2001; Ireson *et al.,* 2002).

The US National Toxicology Program (NTP) has evaluated the short-term and long-term toxicity of turmeric oleoresin (79–85% curcumin; NIH 1993) in F344/N rats and B6C3F1 mice. Animals were fed diets containing the turmeric extract at different concentrations with daily doses of 50, 250, 480, 1300 or 2600 mg/kg body weight for periods of 13 weeks or two years. In a 13-week study, no death was attributed to curcumin and the only toxicity noted was a relative increase in liver weight, stained fur, discolored feces, and hyperplasia of the mucosal epithelium in the cecum and colon of rats that received 2600 mg/kg. No sign of carcinogenic lesions was observed. In a 2-year study, the turmeric administration did not have any effect on the food consumption when compared to controls and no mortality was observed in male or female rats (NTP, 1993). A study in dogs similarly showed a lack of toxicity. An independent study of rats (1000 mg/kg/day) and monkeys (800 mg/kg/day) for 3 months failed to reveal any evidence of adverse effects on growth, behavioral, biochemical, or histopathological parameters (Kellof *et al*., 1996).

Animal studies also have shown that curcumin is rapidly metabolized, conjugated in the liver, and excreted in the feces, therefore having limited systemic bioavailability. A 40 mg/kg intravenous dose of curcumin given to rats resulted in complete plasma clearance at one hour postdose. An oral dose of 500 mg/kg given to rats resulted in a peak plasma concentration of only 1.8 ng/mL, with the major metabolites identified being curcumin sulfate and curcumin glucuronide (Ireson *et al*., 2001)

A phase I clinical trial involving 15 patients with colon cancer used oral doses of curcumin of 440–2200 mg daily and curcumin levels were analysed in the blood, urine, and feces for up to 29 days using high pressure liquid chromatography (HPLC). Though curcumin and its metabolites (curcumin glucuronide, curcumin sulfate, hexahydrocurcumin and hexahydrocurcuminol) were readily measured in feces, none were identifiable in the blood or urine (Sharma *et al*., 2001).

In another phase I clinical trial conducted on 25 patients with various precancerous lesions given oral doses of 4000, 6000, and 8000 mg curcumin daily for three months yielded serum curcumin concentrations of only 0.51±0.11, 0.63±0.06, and 1.77±1.87 μM, respectively, indicating that curcumin is poorly absorbed and may have limited systemic bioavailability. Peak blood levels of 1.77 μM were observed 2 hours after the 8000-mg dose with levels decreasing gradually after that. Blood levels in persons receiving 500–2000 mg doses were barely detectable. Serum levels peaked between one and two hours post-dose and declined rapidly. This study did not identify curcumin metabolites and urinary excretion of curcumin was undetectable. The low blood levels of curcumin seen in that study and the absence of curcumin seen with lower doses are consistent with extensive metabolism of curcumin in the intestinal wall and/or its poor absorption. The possibility that curcumin is rapidly metabolized to some active metabolites that were not studied would reconcile these findings with the observed biological activity of comparable doses in animal studies (Cheng *et al*., 2001).

Results of a phase I clinical trial published in 2004 on oral curcumin in patients with advanced colorectal cancer in which the US NCI criteria were used to assess potential toxicity, demonstrated that curcumin was well tolerated at all dose levels up to 3600 mg daily for up to four months. Adverse effects related to curcumin consumption reported by patients in those studies were mainly gastrointestinal (nausea and diarrhoea). Two abnormalities were detected in blood tests in the trial, but it is not clear whether those abnormal blood test results were related to the activity of the malignant disease in those patients or to curcumin toxicity (Sharma *et al*., 2004).

Although turmeric is often used to treat inflammatory skin conditions in traditional Asian medical systems, it is necessary to call attention to several reports of allergic dermatitis after contact with curcumin which have been published in the scientific literature (Liddle *et al*., 2006; Thompson *et al*., 2006). An allergic reaction to turmeric-related products was also described in one healthy volunteer enrolled in a phase I study testing the safety of turmeric oil and turmeric extract (Joshi *et al.,* 2003).

Despite the lack of systematic testing of the interaction between curcumin with other commonly used drugs, the US Department of Health and Human Services has recommended, based on published laboratory and animal studies, that co-administration of curcumin with nonsteroidal anti-inflammatory drugs (NSAIDs) or anticoagulant drugs (heparin, clopidogrel, aspirin) may result in an increased risk of bleeding. They have also suggested that interference may be found with other drugs that affect or are metabolized by the cytochrome P450 (CYP) enzyme system, resulting in the possibility of unintended drug levels in the blood (Strimpakos *et al*., 2008). In addition to this advice, it has been speculated that *Curcuma* extract (rather than curcumin) may potentially interfere with histamine 2-receptor antagonists (e.g., ranitidine) and proton-pump inhibitors (e.g., omeprazole) *via* inhibitory effects on histamine receptors (Kim *et al*, 2005). Based on animal studies, other scientists have proposed that curcumin may enhance the hypoglycaemic effect of anti-diabetic medication or the efficacy of anti-lipidemic drugs, *via* inhibition of the CYP enzyme system or by reducing the low-density lipoprotein fraction in the blood. (Fan *et al*, 2006).

ATTEMPTS TO MANAGE BIOAVAILABILITY AND PHARMACOKINETICS

Due to the poor systemic bioavailability after oral administration of curcumin, many research groups have focused on ways to improve its bioavailability. Like many other natural polyphenols, curcumin is poorly soluble in water. It is well documented that the main limitation of the use of curcumin-based formulations is its poor solubility and fast metabolism. Therefore, in order to increase its solubility, stability and pharmacological activities, study on chemically modified curcumin derivatives as well as improved formulations and delivery systems should be studied to achieve its optimum therapeutic effects. Various classical techniques based on physical parameters such as heat, pH, and complexations with metal ions, polymers or serum have been applied for improvement of curcumin solubility. It has been claimed that the solubility of curcumin can be increased by 12-fold by the use of heat without heat-mediated disintegration of curcumin (Kurien *et al*., 2009). Complexes of curcumin with metal ions (Zn^{2+}, Cu^{2+}, Mg^{2+} and Se^{2+}) were found to be readily soluble in water-glycerol (1:1; w/w) and quite stable towards light and heat (Zebib *et al*., 2010). Also, chemically modified 4-arylidene curcumin deriva-

tives were found to be more soluble and more potent anti-cancer targeted analogues (Qiu *et al.,*2010*)*. New information concerning solubility of curcumin complexed with serum albumin was provided by data showing that such complex reduced the toxic efect of amphotericin B by delaying the erythrocyte membrane damage (Kudva *et al.,* 2011). Water-soluble curcumin conjugates with two differently sized poly(ethyleneglycol) molecules exhibited enhanced cytotoxicity as compared to curcumin alone and demonstrated some potential in anti-cancer treatment (Safavy *et al.,* 2007). Chemically modified curcumin demonstrated increased activity against cancer (Weber *et al.,* 2006).

The bioavailability of curcumin is limited by its intestinal and hepatic glucuronidation. The roles of adjuvants, which can block the metabolism of curcumin, are of great interest. It has been demonstrated that piperine, an inhibitor of glucuronidation, could be administered concomitantly with curcumin to increase its bioavailability. When 20 mg of piperine was administered orally with 2 g of curcumin to volunteers, serum levels were significantly enhanced at 1 hour's time, increasing the total bioavailability by 20-fold. No toxicity was observed in the 10 subjects who participated in that study (Shoba et al., 1998). Various novel delivery systems have been proposed in recent years as means of improving the bioavailability of curcumin. The possible advantages attributed to the formulations are following: (1) they provide longer circulation; (2) they increase the cellular permeability, and (3) they slow down metabolic transformation processes.

Nanoparticles

Nanoparticles encapsulating curcumin have been prepared by the emulsion technique - thanks to this nano-delivery system it is possible to achive a 9-fold increase in curcumin oral bioavailability as compared to curcumin administrated with piperine (Shaikh *et al*., 2009). Optimized polylactic-co-glycolic acid (PLGA) nano-formulation of curcumin gave some 22-fold higher oral bioavailability in rats as compared to conventional curcumin (Tsai *et al*., 2011) Dextran sulfate-chitosan nanoparticles with curcumin showed preferential killing of cancer cells compared to normal cells (Anitha *et al*., 2011). Polymeric nanoparticle-encapsulated curcumin is readily dispersed in aqueous media and showed anti-cancer potential in preclinical *in vivo* models (Bhawana *et al*., 2011; Yallapu, 2012). Water-dispersible hybrid nanogels were also proposed for intracellular delivery of curcumin (Wu *et al*., 2011).

Micelles

Injectable curcumin-loaded poly(ethyleneoxide)-*b*-poly(ε-caprolactone) micelles for controlled delivery of curcumin confirmed that curcumin in this form retained its cytotoxicity in mouse melanoma (Ma *et al.,* 2008). Curcumin-loaded poly(D,L-lactide-co-glycolide)-β-poly(ethylene glycol)-β-poly (DL-lactide-co-glycolide; PLGA-PEG-PLGA) micelles showed improved area under the curve (AUC) and $t_{1/2}$ *in vivo*. Moreover, the micelles decreased curcumin uptake by liver and spleen, and at the same time, enhanced the distribution of curcumin in the lung and brain (Song *et al.,* 2011).

Phospholipid-based delivery systems

Several research groups have proposed curcumin-phospholipid complexes to improve curcumin delivery. Complexation of curcumin with phosphatidylcholine resulted in enhanced bioavailability, improved the pharmacokinetics and increased hepatoprotective activity as compared to plain mixtures of curcumin and phosphatidylcholine (Gupta *et al.,* 2011).

Phosphatidylcholine formulated curcumin showed increased bioavailability in rats. Curcumin-phospholipid complex administered orally resulted in higher serum concentrations of curcumin as compared to uncomplexed curcumin. Moreover, the complex maintained effective concentrations of curcumin over a longer period of time (Marczylo *et al*., 2007). Phospholipid vesicles and lipid nanospheres embedding curcumin improved intravenous delivery of curcumin to tissue macrophages, especially bone marrow and spleen macrophages. The pharmacokinetic profile of curcumin on solid lipid nanoparticles (SLN) in rats showed significant improvement as compared to dissolved curcumin (Kakkar *et al*., 2011).

Liposomes

Liposomes are a well established delivery system able to incorporate poorly soluble drugs and enable their administration in aqueous medium. Liposomal curcumin has a higher stability than free curcumin in phosphate buffered saline, human blood, and plasma. Novel liposomal delivery systems enhance stability, bioavailability and cellular uptake of curcumin. A liposomal delivery system for curcumin has been developed aiming at intravenous and oral administration. During anticancer research of the intravenous form antitumor and anti-angiogenesis effects have been found in pancreatic carcinoma, head and neck squamous cell carcinoma, prostatic adenocarcinoma and no demonstrable toxicity was detected. (Li *at al.,* 2007; Wang *et al*., 2008). Faster and better absorption was achieved after oral administration the liposomal delivery system of curcumin in rats as compared to non-liposomal curcumin. Those results indicated that liposomal encapsulation enhanced the gastrointestinal absorption of curcumin (Takahashi *et al.,* 2007).

A study on prepared curcumin in poly-(ε-caprolactone)-based implants confirmed the potential of polymeric implants to by-pass the oral route and provide sustained release of incorporated curcumin (Bansal *et al*., 2011).

A beta-cyclodextrin-curcumin complex has been shown to enhance curcumin delivery through higher uptake by cells (Murali *et al*., 2010).

CONCLUSIONS

It is generally believed that well over half of contemporary drugs are derived from (or inspired by) low molecular weight natural products, like secondary metabolites of microorganisms or higher plants (Kingston, 2011). At the same time an opinion prevails that natural products are not full-value drug candidates and are not worthy R&D investments, since they cannot be claimed as new chemical entities and therefore are not patentable. Many recent examples from the drug discovery and development area have proven this reasoning wrong. The case of curcumin clearly indicates that a massive academic effort to accumulate basic knowledge on constituents of a well established traditional medicine produces the snowball effect, inevitably evoking an avalanche of technical innovation aimed at commercial application. At the moment, only the dietary supplement sector (and its customers) seems to benefit from such development, but judging from the pace at which new synthetic curcumi-

noids and their delivery systems are being patented and disclosed, the emergence of a new medicinal product based on the natural constituents of turmeric is not unlikely in the foreseeable future.

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