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Bioorganic & Medicinal Chemistry Letters 15 (2005) 1793–1797
Bioorganic & Medicinal Chemistry Letters

Design, synthesis, biological evaluation and molecular docking of curcumin analogues as antioxidant, cyclooxygenase inhibitory and anti-inflammatory agents

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Received 22 December 2004; revised 14 February 2005; accepted 15 February 2005

Abstract—Curcuminoids were isolated from *Curcuma longa* and their pyrazole and isoxazole analogues were synthesized and evaluated for antioxidant, COX-1/COX-2 inhibitory and anti-inflammatory activities. The designed analogues significantly enhance COX-2/COX-1 selectivity and possess significant anti-inflammatory activity in carrageenan induced rat paw edema assay. Pyrazole, isoxazole analogues of curcumin (**4** and **7**) exhibited higher antioxidant activity than trolox. Molecular docking study revealed the binding orientations of curcumin analogues in the active sites of COX and thereby helps to design novel potent inhibitors.
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Nonsteroidal anti-inflammatory drugs (NSAIDs) are of huge therapeutic benefit in the treatment of rheumatoid arthritis and various types of inflammatory conditions. The target for these drugs is cyclooxygenase (COX), a rate limiting enzyme involved in the conversion of arachidonic acid into inflammatory prostaglandins. The two isozymes of COX involved in prostaglandin biosynthesis are COX-1 and COX-2. COX-1 is known as a housekeeping enzyme and constitutively expressed in all tissues, while COX-2 is constitutively expressed only in kidney, brain and ovaries. COX-2 is increasingly expressed during inflammatory conditions by pro-inflammatory molecules such as IL-1, TNF-α, LPS and agents such as carrageenan.[1–4]

As a part of our continuing program to discover COX-1 and COX-2 inhibitory compounds from Indian medicinal plants,[5–7] the rhizome of *Curcuma longa* was studied. The dichloromethane extract of *C. longa* exhibited significant COX-1 inhibitory activity in COX catalyzed prostaglandin biosynthesis assay in vitro. The CH_2Cl_2 extract was chemoprofiled and found to contain curcuminoids. These results prompted us to investigate structure activity relationship (SAR) studies on curcumin analogues.

Curcumin, 1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, is found as a major pigment in the Indian spice turmeric (*C. longa*, Zingiberaceae). The rhizome of the *C. longa* has been used in indigenous medicine for the treatment of inflammatory disorders and its medicinal activity has been known since ancient times. Curcumin is reported to have anti-inflammatory, antioxidant and anticancer properties.[8,9] From the literature it was found that curcumin was investigated for COX inhibitory activity using bovine seminal vesicles, microsomes and cytosol from homogenates of mouse epidermis showed IC_{50} value of 2 μM,[10] 52 μM[11] and 5–10 μM,[12] respectively. Moreover, one pyrazole analogue of curcumin was synthesized and investigated for lipoxygenase inhibitory activity[13] and the three pyrazole analogue of curcuminoids were synthesized and investigated for endothelial cell proliferation and cytotoxic activity.[14,15]

Keywords: Curcuminoids; Pyrazole analogues; Isoxazole analogues; COX-1; COX-2; Anti-inflammatory activity; Antioxidant activity.
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doi:10.1016/j.bmcl.2005.02.039

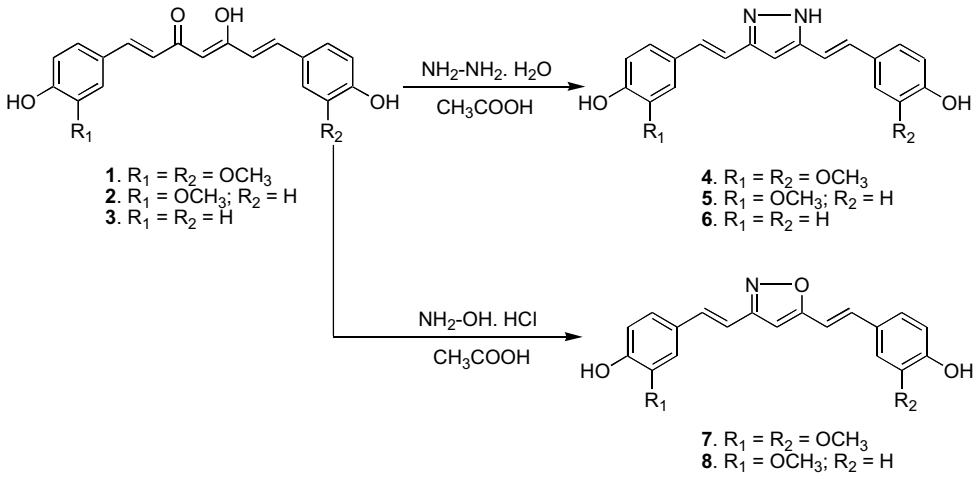
The curcuminoids such as curcumin (**1**), demethoxy curcumin (**2**) and bisdemethoxycurcumin (**3**) were isolated from the dichloromethane extract by silica gel column chromatography (CH_2Cl_2–MeOH).[16]

The pyrazole analogues were prepared (Scheme 1) by treating the CH_2Cl_2 extract (1 g) with hydrazine hydrate (5 mL) in acetic acid (50 mL). The reaction mixture was stirred at room temperature for 7 h. It was washed with water, extracted with CH_2Cl_2 and evaporated to obtain the crude residue. It was purified by column chromatography on silica gel to give pyrazole derivatives **4** (600 mg), **5** (95 mg) and **6** (90 mg).

The isoxazole analogues **7** and **8** were prepared by treating the CH_2Cl_2 extract with hydroxylamine hydrochloride in acetic acid at 85 °C for 6 h (Scheme 1). The reaction mixture was evaporated to dryness, washed with water and the residue was purified by column chromatography to give **7** (450 mg) and **8** (50 mg). Compound **7** was previously synthesized from vanillin and 3,5-dimethylisoxazole-MeI and not investigated for any biological activity so far.[17]

It has been found that in inflammatory disorders, excessive free radical generation takes place and several NSAIDs and phenolic compounds with anti-inflammatory activity are reported to act as radical scavengers.[18] The *o*-methoxyphenolic moiety has been found to be an essential structural feature for antioxidant property of curcuminoids.[19] Accordingly, antioxidant property of curcuminoids, pyrazole and isoxazole analogues was evaluated using DPPH radical scavenging in vitro assay.[20]

The radical scavenging activity results are shown in Table 1. All the compounds demonstrated significant antioxidant effect. Among them the pyrazole analogue



Scheme 1.

Table 1. Anti-oxidant, COX-1, COX-2 inhibitory and anti-inflammatory activities of curcumin analogues

Compound	Anti-oxidant activity[a]				% Inhibition (100 μM)[a]		Anti-inflammatory activity[b]
	IC_{50} (μM)	ARP ($1/ED_{50}$)	Stoichiometric value[c]	H atoms per molecule[d]	COX-1	COX-2	% Inhibition at 75 mg/kg
1	11.06	5.4	0.37	2.7	80.5 ± 2.1	35.0 ± 3.6	61.4 ± 2.7
2	21.36	2.8	0.71	1.4	67.8 ± 2.8	31.6 ± 0.4	—
3	36.37	1.7	1.21	0.8	41.7 ± 2.9	33.2 ± 0.8	—
4	9.70	6.2	0.32	3.1	87.0 ± 2.6	61.0 ± 2.0	68.8 ± 2.9
5	15.45	3.9	0.52	1.9	73.9 ± 3.0	43.8 ± 2.9	—
6	32.41	1.9	1.08	0.9	44.4 ± 1.5	35.8 ± 2.5	—
7	10.71	5.6	0.36	2.8	80.8 ± 3.6	58.1 ± 2.1	60.3 ± 2.9
8[g]	18.96	3.1	0.63	1.6	47.4 ± 3.7	35.0 ± 4.3	—
Trolox	13.1	4.5	0.44	2.2	—	—	—
Celecoxib	—	—	—	—	77.6 ± 0.6	2.05[e]	71.9 ± 2.2[f]
Ibuprofen	—	—	—	—	14.4[e]	265.5[e]	54.5 ± 1.4[h]

[a] $n = 3$–4.
[b] $n = 6$ animals/group.
[c] Theoretical concentration of antioxidant to reduce 100% of the DPPH.
[d] Moles reduced DPPH per mole antioxidant.
[e] IC_{50} value.
[f] 50 mg/kg.
[g] New compound.[22,23]
[h] 100 mg/kg.

(**4**) was found to be more potent than curcumin (**1**). But the isoxazole analogue **7** was equipotent to curcumin.

Compound **4** exhibited reduction of more than three molecules of DPPH per molecule of **4**, whereas curcumin reduces less than three molecules of DPPH/molecule. *O*-Methoxyphenolic compounds such as curcumin (**1**), pyrazole analogue of curcumin (**4**) and isoxazole analogue of curcumin (**7**) exhibited potent antioxidant activity in DPPH radical scavenging assay. It is well known that the *ortho*-methoxy substitution enhanced the stability of the phenoxy radical.[19] Thus the compounds **1**, **4** and **7** showed higher antioxidant activity than the reference compound trolox, and the other curcumin analogues, possessing two methoxy groups. Among these three compounds, compound **4** demonstrated better scavenging activity than the other two compounds; the reason may be the presence of pyrazole NH in **4**.

COX-1/COX-2 inhibitory activity was evaluated employing the COX catalyzed prostaglandin biosynthesis assay in vitro.[5–7,21] All the compounds were investigated for COX-1 and COX-2 inhibitory activity at 100 μM using COX catalyzed prostaglandin biosynthesis assay (Table 1). The structure activity relationship studies (SAR) revealed that curcumin to its pyrazole derivative (**4**) increased the COX-1 activity slightly (80.5–87.0% inhibition), whereas the COX-2 inhibitory activity increased twofold (35–61.0% inhibition). Thus pyrazole analogue (**4**) showed significant enhancement in the selectivity towards COX-2 enzyme (COX-2/COX-1 = 0.70) compared to curcumin (COX-2/COX-1 = 0.43). Compound **5** exhibited higher COX-2 inhibitory activity (43.8% ± 2.9%) than curcumin (35.0% ± 3.6%). Both isoxazole analogue **7** and **1** were equipotent towards COX-1 enzyme. For COX-2 enzyme isoxazole analogue **7** showed significantly increased COX-2 inhibitory activity as well as COX-2/COX-1 (0.72) ratio.

Both pyrazole **4** and isoxazole **7** analogues demonstrated better COX-2 inhibitory activity in comparison with curcumin. Since compounds **1**, **4** and **7** exhibited good COX inhibitory and antioxidant activities, they were investigated for in vivo anti-inflammatory activity using carrageenan induced rat paw edema assay at 75 mg/kg (Table 1). Among these three compounds pyrazole analogue of curcumin, **4** exhibited the highest activity (68.8% inhibition).

The level of COX-1/COX-2 inhibitory and anti-inflammatory activities of compounds **1**, **4** and **7**, prompted us to perform molecular docking studies to understand the ligand–protein interactions and COX-1/COX-2 selectivity in detail. All the calculations were performed using SYBYL6.9[24] software installed on SGI octane 2 workstation. The crystal structures of COX-1 and COX-2 enzymes complexed with indomethacin [1PGG·pdb, 4COX·pdb][25] were used for the docking. The active site of the enzyme was defined to include residues within a 6.5 Å radius to any of the inhibitor atoms.

The FlexX program[5,26] is an automated docking program, was used to dock compounds **1**, **4** and **7** on the active sites of both COX-1 and COX-2 enzymes. For each compound the most stable docking model was selected according to the best scored conformation predicted by the FlexX scoring function. The complexes were energy-minimized with a MMFF94[27] force field till the gradient convergence 0.05 kcal/mol was reached. The distance dependent dielectric function ($\varepsilon = 4r$) was used.

The three compounds could dock into the active site of COX-1 successfully. The binding energies of −36.45, −37.07 and −31.61 kcal/mol were obtained for **1**, **4** and **7**, respectively. The lower interaction energy observed for **4** rationalizes the tighter binding of pyrazole analogue (Fig. 2) into the COX-1 active site than that of the other two compounds. The tight binding can be explained in terms of extra hydrogen bonding with pyrazole NH and Tyr 355. All the three compounds were involved in the hydrogen bonding with a residue Ser 530. The hydrogen bonding distance between one of the methoxy group of curcumin with OH of Ser 530 was found to be 3.703 Å (O···O) 2.791 Å (O···H). One of the phenyl ring of curcumin was surrounded by active site amino acid residues Tyr 385, Leu 384, Phe 518, Met 522 and Ser 530. The heptanoid part was surrounded by residues Ile 523, Ala 526, Gly 526, Glu 524, Ser 353, Leu 359, Val 349 and Leu 352. The second phenyl ring (Fig. 1) was surrounded by Tyr 355, His 90, Leu 357 and Arg 120 and Glu 524. A similar trend was observed for **4** and **7** complexes.

The hydrogen bonding distance between Ser 530 and methoxy group of **4** was found to be 3.022 Å (O···O) 2.132 Å (O···H). Another hydrogen bonding between OH of the second phenyl ring and Arg 83 (3.319 Å, O···N, 2.406 Å, O···H–N) was observed. NH of the pyrazole was involved in hydrogen bonding interaction with Tyr 355 (3.373 Å, N···O, 2.564 Å, O···H) (Fig. 2). The isoxazole analogue **7** orients in a similar fashion to that of **1** and **4**. However, only one hydrogen bond was observed between the methoxy group and OH of Ser 530 (3.124 Å, O–O, 2.295 Å (O···H) (Fig. 3).

FlexX could dock only curcumin to the active site of COX-2. However, **4** and **7** were not docked into the active site by this method. Therefore using

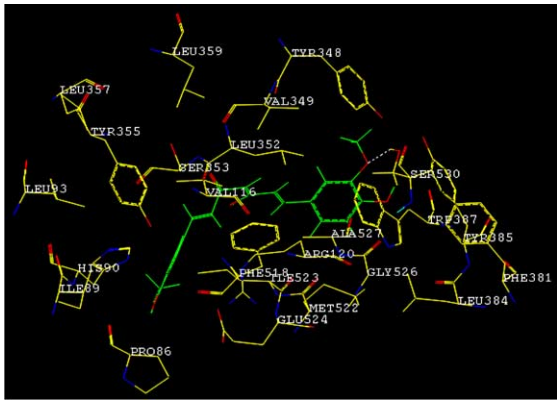


Figure 1. Binding of curcumin into the active site of COX-1.

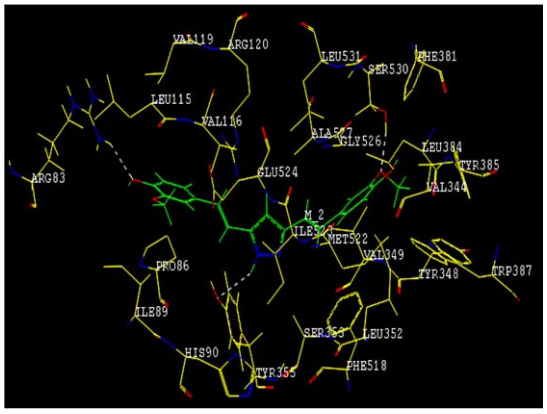


Figure 2. Binding of **4** into the active site of COX-1.

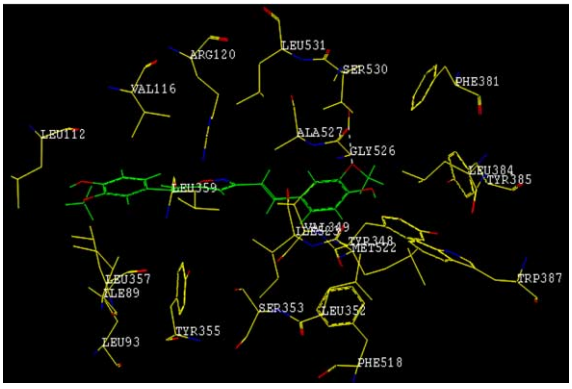


Figure 3. Binding of **7** into the active site of COX-1.

curcumin-COX-2 complex, compounds **4** and **7** were modeled in the active site of COX-2 enzyme. The carbonyl oxygen of curcumin was forming a hydrogen bonding interaction with Arg120 (3.562 Å, O···N, 2.78 Å, O···H). Whereas, compounds **4** and **7** did not produce any hydrogen bonding interactions with COX-2 enzyme. However, favourable van der Waals interactions between styryl carbon atoms and the hydrophobic residues such as Val 523, Val 116, between methoxy groups of **4** (Fig. 4) and **7** and Ala 516 and between

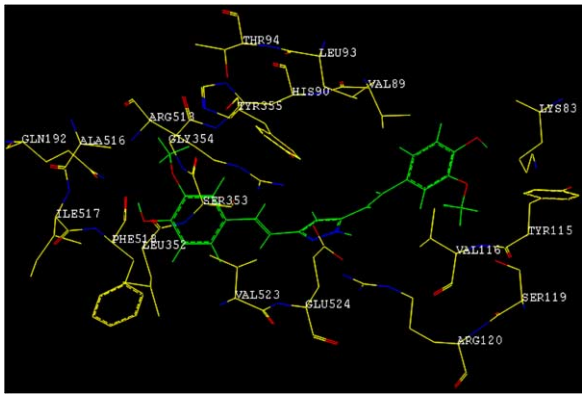


Figure 4. Binding of **4** into the active site of COX-2.

pyrazole/isoxazole rings and Tyr 355 contributed to stabilize the ligand–enzyme complexes. Molecular docking studies further supported the strong inhibitory activity of **4** compared to **1** and **7**.

In conclusion, eight curcumin analogues including one new compound were investigated for antioxidant and COX-1/COX-2 inhibitory activities. SAR studies revealed that replacement of the β-diketo fragment of curcumin by a pyrazole ring significantly enhances COX-2/COX-1 selectivity. The mixed functioning antioxidant and COX inhibitory properties may provide superior anti-inflammatory activity.[28] Thus, curcumin analogues were investigated for antioxidant activity and found to be good radical scavengers. Molecular docking studies further helps in understanding the various interactions between the ligands and enzyme active sites in detail and thereby helps to design novel potent inhibitors.

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