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Symmetrical and un-symmetrical curcumin analogues as selective COX-1 and COX-2 inhibitor

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ABSTRACT

Curcumin, a popular herbal medicine derived from turmeric, blocks the synthesis of prostaglandins by inhibiting Cyclooxygenase-1 and 2 (COX-1 and COX2). We have recently reported an efficient method of synthesizing curcumin and synthesised analogues. In the present study, we have investigated sixteen novel analogues of curcumin for their ability to inhibit COX-1 and COX-2. We report here that most of the curcumin analogues display selective inhibition of COX-2, whereas a few suppress COX-1 activity. Further, we examined the binding of these inhibitors by molecular docking and observed that the compound with pronounced selectivity for COX-2 displayed better binding to COX-2 compared to curcumin.

Introduction

The Biosynthesis of prostaglandins from the substrate arachidonic acid is catalyzed cyclooxygenase (COX) enzyme. Nonsteroidal anti-inflammatory drugs (NSAIDs), commonly used for fever, pain and inflammation, act by inhibiting COX catalyzed biosynthesis of inflammation mediators. However, prolonged use of classical NSAIDs is linked to side effects such as gastrointestinal bleeding and renal system damage. There are two isoforms of cyclooxygenase (COX) enzymes, COX-1 and COX-2. COX-1 is constitutively expressed in many tissues and is responsible for maintaining normal physiologic level of prostaglandins.[1] Although not considered generally as drug target, recent studies have implicated role of COX-1 in angiogenesis and shown to be overexpressed in ovarian cancer.[2–4] However, there are few COX-1-selective inhibitors available. Unlike COX-1, COX-2 is inducible and up-regulated by growth factors, tumor promoters, hormones, bacterial endotoxins, cytokines and shear stress.[5,6] Unlike COX-1, COX-2 is inducible and up-regulated by cytokines. COX-2 expression is also increased in certain colon tumors, pancreatic cancers and pre-malignant skin lesions.[7–9] Epidemiological studies have established that COX-2 is attractive target for preventing various types of cancer.[10] Therefore, we were interested in identifying a small molecule that selectively inhibits COX-2 enzyme. While COX-2 selective inhibitors rofecoxib and valdecoxib resulted improved anti-inflammatory properties and reduced gastrointestinal toxicity compared to classical NSAIDs, clinical trials of these inhibitors were discontinued due to severe cardiovascular side-effects.[11] Safety concerns of non-selective NSAIDs, failure of the COX-2-selective drugs and lack of any COX-1-specific drug have created enormous scope to develop alternative COX-1 and COX-2 inhibitors without any side-effects and safety concerns. Therefore, natural product-derived compounds as COX inhibitors, have gained significant recognition.

Curcumin is found in the rhizomes of the Indian spice turmeric (*Curcuma longa*). Several studies demonstrated anti-carcinogenic, anti-inflammatory, antioxidant, antiviral, and wound healing properties of curcumin.[12] One of the key targets of curcumin activity is the COX enzyme.[13] We have synthesized sixteen novel analogs of curcumin[14] and investigated whether these analogues might achieve selective COX-1 and COX-2 inhibition. Also, molecular docking studies were performed to investigate the ligand-protein interactions responsible for the biological activity.

Materials and Methods

COX inhibition assay- Inhibition of ovine COX-1 and human recombinant COX-2 was assessed using a COX fluorescent inhibitor screening assay kit from Cayman Chemical®. The assay monitors conversion of Prostaglandin-G2 to Prostaglandin-H2 by COX using a fluorescent dye and inhibition of COX activity is demonstrated by a decrease in fluorescence due to diminishing Prostaglandin-H2 formation. The selective inhibition of COX-1 and COX-2 by sixteen novel analogs of

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Table 1
COX-1 and COX-2 enzyme inhibitory activity of curcumin derivatives.

Sl. No.	Compound	IC_{50} COX-1 (μM)	IC_{50} COX-2 (μM)	Selectivity IndexIC_{50} COX-1/IC_{50} COX-2
1	Curcumin	**8.56**	**3.19**	2.68
2	**1h**	3.82	5	0.76
3	**1a**	n.d	5	
4	**1b**	7.13	n.d	
5	**1c**	7.79	n.d	
6	**1d**	2.81	5	0.56
7	**1e**	1.93	**0.04**	48.25
8	**1f**	8.02	4.73	1.69
9	**1g**	0.88	**0.01**	88
10	**1j**	0.63	4	0.16
11	**1i**	26.34	79.81	0.33
12	**1k**	0.61	10.84	0.056
13	**1l**	**0.13**	**0.01**	13
14	**1m**	1.88	2.71	0.69
15	**1n**	17.55	3.63	4.83
16	**1p**	0.41	n.d	
17	**1q**	0.43	**0.07**	6.14

curcumin was analyzed.[12] The compound **1r** is completely water insoluble and precipitated. So, the effect of **1r** on COX-1 and COX-2 activity could not be tested. All reactions were performed in a final volume of 200 μl in a black 96-well non-binding microplate (Cayman) according to the manufacturer's protocol. Briefly, to 150 μl assay buffer (Tris–HCl, 0.1 M, pH 8), 10 μl heme, 10 μl COX-1 or COX-2 and 10 μl curcumin analogs dissolved in DMSO were added and samples were incubated for 5 min at room temperature. The reaction was started by addition of 10 μl arachidonic acid in DMSO (final 100 μM) followed by immediate addition of 10ul of fluorometric substrate ADHP (10-acetyl-3,7-dihydroxyphenoxazine) that reacts with the Prostaglandin-G2 to form a fluorescent compound rusorufin. Fluorescence signals were measured after 2 min of incubation with an excitation wavelength of 540 nm and an emission wavelength of 595 nm using SpectraMax multimode reader. Compound SC-560 and DuP-697 (Cayman Chemical®) were used as positive control for COX-1 and COX-2, respectively. Data are expressed as relative fluorescence units (RFU) according to COX enzyme activity in % of DMSO control. IC_{50} values express the concentration exerting 50% inhibition of COX activity and were obtained after GraphPad®. Data are reported as means of 5 independent experiments performed in triplicates.

Docking of curcumin analogues with COX-2 using Autodock

Molecular docking analysis of COX-2 with curcumin analogues was performed using AutoDock tools 1.5.6 (ADT 4.2). The 3-dimensional structures of curcumin analogues were prepared using Maestro version 9.9 *(13)*. The coordinates for COX-1 (PDB entry code 6Y3C) and COX-2 (PDB entry code 1CX2) enzyme was obtained from the Protein Data Bank *(14)*. Water molecules were removed and polar hydrogens were added for the accurate calculation of partial charges. The grid box size was set at 64, 64 and 64 Å (x, y, and z) with center x = 25.0, y = 23.0 and z = 19.0 for the protein *(15)*. Docking simulations was performed using the Lamarckian genetic algorithm; populations of 150 individuals with a mutation rate of 0.02 were evolved for 10 generations. The program automatically grouped potential protein-ligand complex conformations into clusters based on their RMSD. The best docked complex for COX-2 with curumin analogues was selected on the basis of binding free energy value.

Results

All the curcumin analogs were evaluated for their ability to inhibit COX-1 and COX-2 and results obtained are presented in Table 1. We synthesized curcumin and determined IC_{50} against purified recombinant ovine COX-1 and human COX-2. Earlier, Hong et al *(12)* calculated IC_{50} of curcumin against COX-1 and COX-2 to be 50 μM and 100 μM, respectively, using crude cell extracts as the source of the enzymes. We found that curcumin inhibited purified recombinant ovine COX-1 and human COX-2 activity with IC_{50} of 8.56 μM and 3.19 μM, respectively. To our knowledge, no other report determined IC_{50} of curcumin using purified recombinant ovine COX-1 and human COX-2 and our results suggest that curcumin might be more potent inhibitor of COX-1 and COX-2 than presumed before.

Table 2
Molecular docking analysis of COX-2 enzyme

S. no	Curcumin analogues	Residues of COX-2 interacting with curcumin analogues	Binding energy (Kcal/mol)
1	**Curcumin**	SER353, GLN192, TYR355, HIS90, ALA527, ASP347	-5.52 Kcal/mol
2	**1e**	TYR355, ASP515, ASP347, SER 353, GLN192, GLN350	-8.21 Kcal/mol
3	**1g**	HIS90, GLN192, ASP34, ASN581, SER579, GLN350, TYR355	-8.37 Kcal/mol
4	**1l**	ARG513, ASP515, TYR355, SER353, VAL349, TYR385, ARG120, ASP375	-7.64 Kcal/mol
5	**1q**	SER530, ARG120, TYR355, SER353, LEU351, ALA527, GLN192, HIS90, TYR385	-8.50 Kcal/mol

Table 2 and Table 3.

Among the tested compounds, some compounds displayed selective inhibition of COX-1, whereas most were selective to COX-2. IC_{50} values of compounds tested for COX-1 ranged from 70 nM to 26.3 μM. 12 compounds (**1h, 1b, 1c, 1d, 1e, 1g, 1j, 1k, 1l, 1m, 1p** and **1q**) showed IC_{50} less than curcumin. Compound **1l** (IC_{50} = 130 nM) showed strong inhibition of COX-1. Analysis of inhibition of COX-2 by these compounds reveals that four compounds (**1e, 1g, 1l, 1m** and **1q**) showed IC_{50} less than curcumin for COX-2. Interestingly, all these compounds also displayed COX-1 inhibition stronger than curcumin. Out of these seven compounds, four compounds, namely, **1e** (IC_{50} = 40 nM), **1g** (IC_{50} = 10 nM), **1l** (IC_{50} = 10 nM) and **1q** (IC_{50} = 70 nM) showed very strong inhibition of COX-2. Interestingly, compound **1e (**SI= 48.25**)** and **1g (**SI=88**)** showed pronounced selectivity towards COX-2 and compound **1k** showed selectivity towards COX-1.

The docked pose of COX-2 enzyme with curcumin analogues is shown in Fig. 1. The protein-ligand complex generated after docking was monitored for the interactions in the active site (Table 2). One phenyl ring of the curcumin interacts with Tyr 385, Leu 384, Phe 518, Met 522 and Ser 530 residues while the second phenyl ring interacts with Tyr 355, His 90, Leu 357 and Arg 120 and Glu 524 residues *(16)*. The heptanoid portion of the compound **1g** forms hydrogen bonds with Asn581 and Gln192. This compound also interacts with His90, Asp34, Asn581, Ser579, Gln350 and Tyr355. Compound **1l** forms hydrogen bond His90 and also interacts with Arg120, Gln192, Val349, Leu352, Ser350, Ser533, Tyr385 and Leu531. The binding energies of **1e, 1g, 1l and 1q** are 8.21, -8.37, -7.64 and -8.50 Kcal/mol respectively. The higher binding affinity values of the analogues than curcumin explains the tighter binding in the COX-2 active site. Interestingly, it was found that 1p failed to inhibit the COX-2 activity while the IC_{50} values of **1p** with COX-1 was found to be 0.41μM. In order to understand the specificity of **1p** to selectively inhibit COX-1 molecular docking analysis was performed. It was found that the binding energy of **1p** with COX-1 and COX-2 were -7.60 and -1.50 Kcal/mol respectively. It was found that curcumin analogue **1p** bound outside the active site pocket of COX-2. While, **1p** interacted with catalytically active residues of COX-1 namely Gln 192, Ser 353, Ser 516, Ile 517, Phe 518 and Ile 523 (Figure 2).

Molecular parameters of curcumin analogues

Drug likeness of the compounds were evaluated by studying the Lipinski's rule of five. This rule considers the pharmacokinetic

Table 3
Molecular parameters of curcumin analogues

Compound	TPSA	n-rotb	nON	nOHNH	miLogp	MW	No. of violation
Rule of 5	—	—	≤10	≤5	≤5	≤500	≤1
1h	92.703	11	8	1	5.142	614.283	2
1a	37.294	5	2	1	6.578	414.115	1
1b	74.235	11	6	1	4.403	424.493	0
1c	37.299	5	2	1	5.94	434.127	1
1d	37.299	7	2	1	6.199	332.443	1
1e	37.299	7	2	1	7.16	428.531	1
1f	37.299	7	2	1	5.533	434.127	1
1g	55.767	7	4	1	6.347	436.507	1
1j	108.377	11	8	1	2.653	452.459	0
1i	55.767	7	4	1	8.436	809.763	2
1k	74.235	9	6	1	5.567	554.291	2
1l	94.463	9	7	2	4.075	491.334	0
1m	86.989	6	5	3	5.521	575.047	2
1n	74.235	9	6	1	5.744	525.395	2
1o	74.235	9	6	1	4.97	446.499	0
1p	65.001	8	5	1	5.22	416.473	1
1q	46.533	7	3	1	7.153	432.519	1
Curcumin	93.066	8	6	2	2.303	368.385	0

Where, **TPSA**, topological polar surface area; **n-rotb**, number of rotatable bonds; **nON**number of hydrogen bond acceptors; **nOHNH**, number of hydrogen bond donors; **miLogP**, logarithm of compound partition coefficient between n-octanol and water; **MW**, molecular weight.

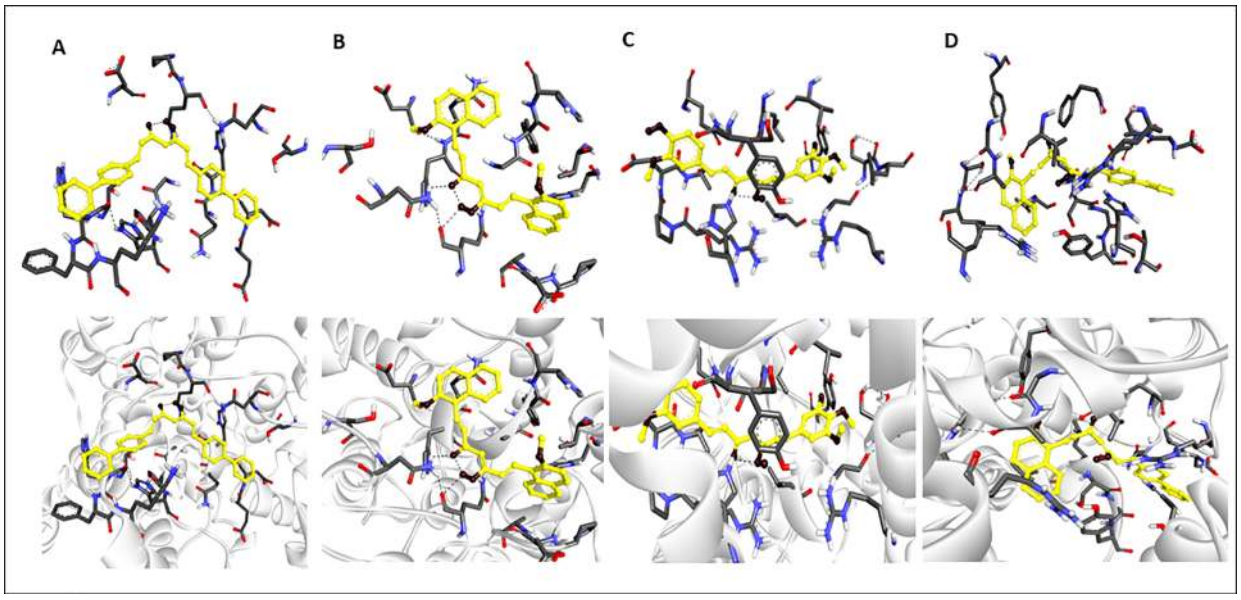

Fig. 1. Docking of curcumin analogues with COX-2 protein. COX-2 protein was docked with curcumin analogues A) **1e** B) **1g** C) **1l** D) **1q**. The interactions of curcumin analogues with the COX-2 active site residues were analyzed. COX-2 protein is represented in white ribbon and curcumin analogues are shown yellow ball and stick model.

parameters such as drug absorption, distribution, metabolism and excretion in the human body. The four criteria includes molecular mass less than 500 Da, high lipophilicity i.e LogP value less than 5, hydrogen bond donors less than 5 and hydrogen bond acceptors less than 10 *(17)*. Results showed that compounds **1b, 1j, 1l** and **1o** obey the rule of five (Table 3). Compounds **1a, 1c, 1e, 1f, 1g, 1p** and **1q** have logP values greater than 5, but since the number of violations is not more than one, therefore these compounds also satisfy the Lipinski's rule.

Credit Author Statement

MAH synthesized the compounds, MM and GS performed the experiments, RA and FAK conceived the idea, analyzed the result and wrote the manuscript.

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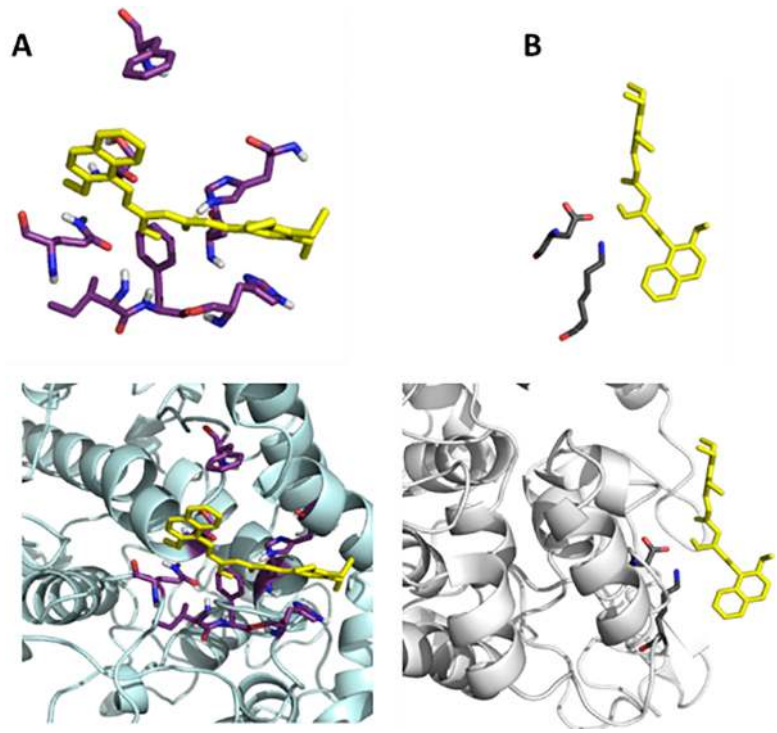

Fig. 2. Docking of curcumin analogue **1p** with COX-1 and COX-2 protein. (A) COX-1 and (B) COX-2 protein was docked with curcumin analogue **1p.** The interactions of **1p** with the COX-1 and COX-2 active site residues were analyzed. COX-1 and COX-2 protein is represented in cyan and white ribbon respectively. Curcumin analogue **1p** is represented as yellow ball and stick model.

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