Design and synthesis of novel 2-phenyl-5-(1,3-diphenyl-1H-pyrazol-4-yl)-1,3,4-oxadiazoles as selective COX-2 inhibitors with potent anti-inflammatory activity.

ARTICLE *in* EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY · JANUARY 2014

Impact Factor: 3.45

8 AUTHORS, INCLUDING:

Varun Bhardwaj

Jaypee University of Information Technology

28 PUBLICATIONS **167** CITATIONS

SEE PROFILE

Alex Joseph

Manipal University

33 PUBLICATIONS **143** CITATIONS

SEE PROFILE

European Journal of Medicinal Chemistry 80 (2014) 167–174

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Short communication

Design and synthesis of novel 2-phenyl-5-(1,3-diphenyl-1*H*-pyrazol-4-yl)-1,3,4-oxadiazoles as selective COX-2 inhibitors with potent anti-inflammatory activity

Sumit Bansal [a,*,1], Manju Bala [b,1], Sharad Kumar Suthar [c,**,1], Shivani Choudhary [d], Shoumyo Bhattacharya [a], Varun Bhardwaj [e], Sumit Singla [f], Alex Joseph [c]

[a] School of Pharmaceutical Sciences, Shoolini University, Solan, Himachal Pradesh, India
[b] School of Pharmaceutical Sciences, Jaipur National University, Jaipur 302017, India
[c] Department of Pharmaceutical Chemistry, Manipal College of Pharmaceutical Sciences, Manipal 576104, India
[d] Doon Valley Institute of Pharmacy and Medicine, Karnal, Haryana 132001, India
[e] Department of Pharmaceutical Chemistry, ASBASJSM College of Pharmacy, Bela, Ropar, Punjab 140111, India
[f] Department of Pharmaceutical Chemistry, Rajendra Institute of Technology & Sciences, Sirsa, Haryana, India

ARTICLE INFO

Article history:
Received 19 October 2013
Received in revised form
9 April 2014
Accepted 14 April 2014
Available online 15 April 2014

Keywords:
Pyrazole
1,3,4-Oxadiazole
COX-2
Anti-inflammatory
Analgesic
Molecular docking analysis

ABSTRACT

A novel series of 2-phenyl-5-(1,3-diphenyl-1*H*-pyrazol-4-yl)-1,3,4-oxadiazoles were designed and synthesized for selective COX-2 inhibition with potent anti-inflammatory activity. Among the compounds tested, **9g** (2-(3-(4-nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-5-phenyl-1,3,4-oxadiazole) was found to be the most potent inhibitor of COX-2 with IC_{50} of 0.31 μM showing promising degree of anti-inflammatory activity in the carrageenan-induced rat paw edema model with ED_{50} of 74.3 mg/kg. The lead compound **9g** further showed suppression of acetic acid-induced writhes comparable to that of aspirin and gastro-sparing profile superior to the aspirin. Molecular docking analysis displayed higher binding affinity of ligands towards COX-2 than COX-1.

1. Introduction

The cyclooxygenase (COX) enzymes are chiefly responsible for the production of prostaglandins, a well-known mediator of inflammation, pain, and swelling [1]. COX enzymes exist in three isoforms: COX-1, COX-2, and COX-3 [2]. The COX-1 is a constitutive enzyme found in most of the cells and has important role in the protection of gastric mucosa, platelet aggregation, and renal blood flow. The COX-2 is an inducible isozyme; significantly expressed during inflammation, pain, and oncogenesis [3], while COX-3, a splice variant of COX-1, is considered as another target for anti-inflammatory drugs [2,4]. These findings have led to hypothesis that selective COX-2 inhibitors might provide good anti-inflammatory agents with improved therapeutic potency and reduced side effects associated with the use of conventional nonsteroidal anti-inflammatory drugs (NSAIDs). Many selective COX-2 inhibitors such as celecoxib [5], rofecoxib [6], valdecoxib [7], and etoricoxib [8] are marketed as new generation NSAIDs. However, because of several cardiovascular adverse effects associated with coxibs, they have been voluntarily withdrawn from the market [9,10]. Thus, novel COX-2 selective inhibitors having anti-inflammatory and analgesic activities with an improved safety profile is the need of the hour.

Many pyrazole derivatives are known to possess a wide range of bioactivities such as anti-inflammatory [5], analgesic [11], antibacterial [12], antifungal [13], and anticancer [14,15]. The recent success of celecoxib as selective COX-2 inhibitor has grown significant attention towards pyrazole nucleus in the designing of newer and safer anti-inflammatory agents [5]. Besides, a great variety of similar kinds of compounds having potent anti-inflammatory

* Corresponding author. Present address: Dept. of Pharmacy, Jaypee University of Information Technology, Waknaghat, Solan.
** Corresponding author. Dept. of Pharmaceutical Chemistry, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal 576104, India.
E-mail addresses: bansalsumit50@gmail.com (S. Bansal), sharadpune_2009@yahoo.com (S.K. Suthar).
[1] Authors hold equal contribution.

http://dx.doi.org/10.1016/j.ejmech.2014.04.045
0223-5234/

activity have been synthesized [16–18]. Pyrazoles bearing 1,3-disubstituted aryl moieties are reported as effective pharmacophore for selective COX-2 inhibition [19].

Substituted 1,3,4-oxadiazoles are one of the most important heterocyclic compounds, which have gained attention because of their remarkable biological and pharmacological properties, such as anti-inflammatory [20], anticancer [21], antitubercular [22], antibacterial [23], antiviral [24], antifungal [25], and insecticidal activities [26]. They have also attracted interest in medicinal chemistry as bioisosteres for carboxylic acids, esters, and carboxamides [27]. A number of molecules based upon this monocyclic heterocyclic template have been investigated for their anti-inflammatory activity [28,29]. Recently, Dekhane et al. [30] reported synthesis, COX-2 inhibitory potential, and anti-inflammatory activity of pyrazole substituted 1,3,4-oxadiazoles and 1,2,4-oxadiazoles. Encouraged by these observations and our ongoing efforts towards developing promising biologically active agents using a hybrid pharmacophore approach [31–33], it was considered worthwhile to design and synthesize novel hybrid compounds by integrating two five membered heterocyclic rings (pyrazole and oxadiazole) into a single molecule to investigate their COX inhibitory potential with promising anti-inflammatory activity.

2. Result and discussion

2.1. Chemistry

The synthetic route of title compounds **9a**–**n** and **10a**–**g** is shown in Scheme 2. The title compounds were synthesized by oxidative cyclization of various pyrazolylaldehyde N-acylhydrazones **7** and **8** with iodobenzenediacetate (IBD) in dichloromethane by stirring at room temperature for 20–25 min [34]. The intermediates **7a**–**n** and **8a**–**g** were synthesized by refluxing ethanolic solutions of formylpyrazoles **6** with acid hydrazides **2**–**3** and isoniazid in the presence of few drops of acetic acid for 4 h, respectively. Formylpyrazoles **6** were prepared by previous reported method of Kira et al. [35]. Acid hydrazides were synthesized according to Scheme 1.

Progresses of the reactions were monitored by TLC using n-hexane: ethylacetate (4:6) as solvent system. The synthesized compounds **9a**–**n** and **10a**–**g** were purified by column chromatography using silica gel 100–200 mesh to afford pale white or yellow products. A total of 21 compounds were synthesized with yield in the range of 51–84%.

2.2. Biological evaluation

2.2.1. In vitro cyclooxygenase (COX) inhibition assay

Newly synthesized 21 compounds and reference drugs celecoxib and diclofenac were evaluated for their inhibitory activities against COX-1 and COX-2 enzymes using an enzyme immunoassay (EIA) kit. The activities of the tested compounds were expressed as IC_{50} (concentration exhibiting 50% enzyme inhibition). The selectivity index (SI values) was calculated by IC_{50} (COX-1)/IC_{50}(COX-2). The IC_{50} values of celecoxib against COX-1 and COX-2 were found to be >100 and 0.28 μM, respectively, indicating that celecoxib is a selective COX-2 inhibitor (SI = 357), while diclofenac showed non-selectivity towards COX-1 and COX-2. The results showed that compounds **9a**, **9d**, **9g**, **9j**, **9k**, **9n**, **10c**, **10d**, and **10g** displayed potent COX-2 inhibition with IC_{50} in a range of 0.31–8.7 μM when compared to the inhibition of COX-1 with IC_{50} > 100 μM, as listed in Table 1. Among these compounds, **9g** was found to be the most potent COX-2 inhibitor with lowest IC_{50} i.e. 0.31 μM. The COX-2 inhibition depends on the electronic nature of substituents on phenyl ring of pyrazole nucleus. It was observed that compounds possessing electron withdrawing nitro group (**9g**, **9n**, and **10g**) demonstrated augmented COX-2 inhibitory activity, whereas introduction of electron donating methyl or methoxy group decreased the COX-2 activity and selectivity. Halogen substitutions also played a significant role in COX-2 inhibition. Chloro substitution was found more favorable towards COX-2 inhibition over fluoro substitution. However, bromo substitution resulted into complete loss of COX-2 selectivity. Moreover, 1,3,4-oxadiazoles bearing phenyl ring substitution at C-2 position showed higher inhibitory potency towards COX-2 compared to that of pyridyl ring substitution, while 4-chlorophenyl substitution yielded least potent inhibitors.

2.2.2. In vivo anti-inflammatory activity

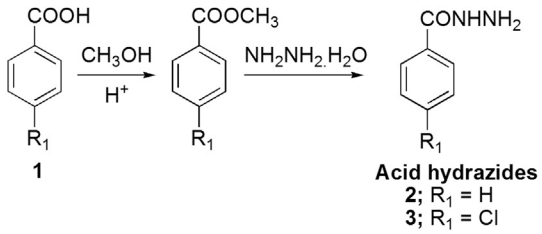
Ten compounds, namely **9a**, **9c**, **9d**, **9g**, **9j**, **9k**, **9n**, **10c**, **10d**, and **10g** were selected for *in vivo* anti-inflammatory activity based on *in vitro* results. These named compounds were evaluated using well-known rat carrageenan-induced foot paw edema model. Celecoxib and diclofenac were used as the reference drugs. The ED_{50} values of test and reference compounds were calculated after 2 h of treatment with Carrageenan, are represented in Table 1. All of the ED_{50} values were determined in three doses of 50, 100 and 200 mg/kg body wt. of the test compounds and 25, 50 and 100 mg/kg body wt. of the reference drugs diclofenac and celecoxib. Among the compounds tested, **9a**, **9g**, **10c**, and **10g** showed promising degree of anti-inflammatory activity with ED_{50} in the range of 72.6–125.4 mg/kg. ED_{50s} of the reference drugs celecoxib and diclofenac were found to be 81.7 and 110.4 mg/kg, respectively.

2.2.3. In vivo analgesic activity

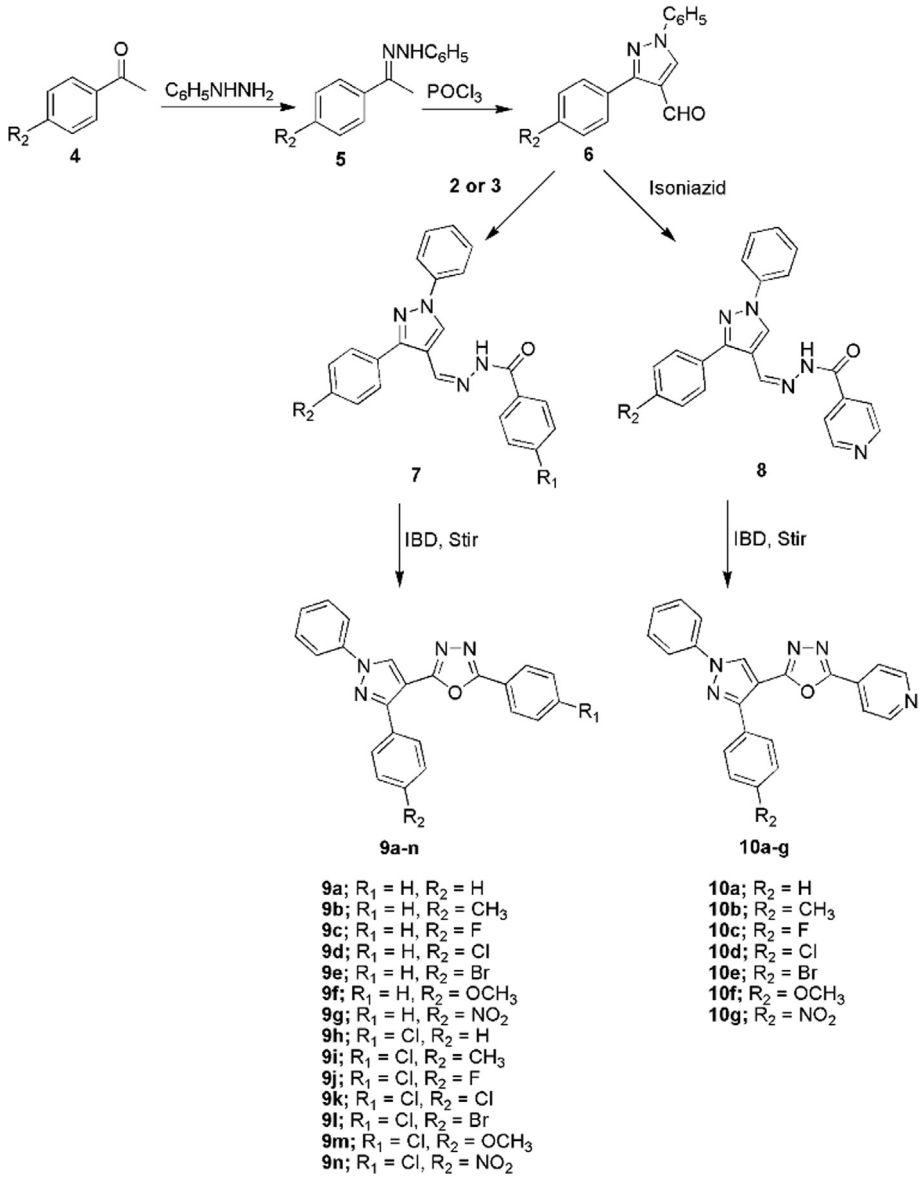
Analgesic effect of the lead compound **9g** was evaluated by employing the acetic acid-induced writhing test in mice, a visceral inflammatory analgesic test. Mice treated with **9g** exhibited 67.29% suppression of writhes compared with the control group, whereas the reference drug aspirin produced 72.23% suppression of writhes. Therefore, it can be stated that the lead compound **9g** possesses analgesic potential same as that of the clinically used drug aspirin and holds a great promise for the further development as an NSAID.

2.2.4. Gastric ulceration study

Gastric ulcers developed on the administration of the lead compound **9g** and aspirin were measured individually in terms of their length. Gastric ulceration study indicated extraordinary gastro-sparing effects of the lead compound **9g** with ulcer-index of 22.8 ± 3.2 only. Contrastingly in the same experimental conditions, clinically used drug aspirin demonstrated ulcer-index of 81 ± 8.9, which was >3.5-fold more than that produced by **9g**. These gastroprotective effects of **9g** provide evidence that it can be developed as a potential gastro-sparing NSAID.



Scheme 1. Synthesis of acid hydrazides (**2**–**3**).



Scheme 2. Synthesis of 2-Phenyl-5-(1,3-diphenyl-1*H*-pyrazol-4-yl)-1,3,4-oxadiazoles (**9a**–**n**, **10a**–**g**).

2.3. Molecular docking analysis

All the designed compounds were docked into the active sites of COX-1 and COX-2. Results of molecular docking analysis indicate that all the designed and subsequently synthesized compounds were more selective towards COX-2 than COX-1. Estimated free energy of binding of docked compounds towards COX-2 was found to be between −10.15 and −8.97 kcal/mol, whereas compounds docked to COX-1 showed estimated free energy of binding between −5.96 and −4.20 kcal/mol. The estimated free energy of binding of docked ligands signifies that synthesized compounds exhibited preferential selectivity towards COX-2 instead of COX-1. The most potent compound **9g** revealed by *in vitro* study showed minimum estimated free energy of binding among the docked compounds and it exhibited an estimated free energy of binding of −10.15 kcal/mol towards COX-2, whereas the estimated free energy of binding of clinically used drug diclofenac was found to be −9.23 kcal/mol towards the same target. This energy difference between **9g** and diclofenac indicates higher binding affinity of **9g** towards COX-2 than diclofenac.

Analysis of docked complex of **9g** and COX-2 proved that the nitro group of **9g** plays a crucial role in COX-2 inhibition. Moreover, oxygen of nitro group forms hydrogen bonding with amino group hydrogen of Arg-120 with a bond distance of 2.4 Å (O═N–O…H–N, 2.4 Å) (Fig. 1). Nitro group substituted phenyl ring of **9g** was placed in the hydrophobic pocket created by Val-116, Leu-117, Val-349, Leu-359, and Leu-531 residues of COX-2 (Fig. 2). Furthermore, phenyl ring placed on pyrazole moiety displayed hydrophobic and van der Waal interactions with Ile-517 and Phe-518 residues of the COX-2, while phenyl ring of oxadiazole moiety showed hydrophobic interaction with Val-523 residue of the target enzyme (Fig. 2). Stereoview of docked complex of **9g** into the active site of COX-1 indicates that **9g** showed hydrogen, hydrophobic, and van der Waal interactions with various residues of COX-1 *viz*. Leu-93, Val-349, Leu-352, Ser-353, Tyr-355, Phe-356, Leu-357, and Pro-514

Table 1
Data of *In vitro* COX-1/COX-2 enzyme inhibition assay and *in vivo* anti-inflammatory activity (ED_{50} mg/kg) of the synthesized compounds.

Compound	IC_{50} (μM)[a]		Selectiviy index[b]	ED_{50}[c] (mg/kg)
	COX-1	COX-2		
9a	>100	1.5	>67	125.4
9b	>100	25	>4	ND
9c	>100	6.5	>15.4	175.3
9d	>100	4.7	>21	170.7
9e	>100	>100	>1	ND
9f	>100	>100	>1	N
9g	>100	0.31	>222	74.3
9h	>100	38	>2.6	ND
9i	>100	58	>1.7	ND
9j	>100	5.5	>18.2	182.5
9k	>100	8.7	>11.5	198.7
9l	>100	50	>2	ND
9m	>100	67	>1.5	ND
9n	>100	1.2	>83.3	167.4
10a	>100	11.1	>9	ND
10b	>100	53	>1.9	ND
10c	>100	2.3	>43	122.8
10d	>100	4.4	>23	167.4
10e	>100	39	>2.6	ND
10f	>100	42	>2.4	ND
10g	>100	0.5	>200	72.6
Celecoxib	>100	0.28	>357	81.7
Diclofenac	0.18	2.7	0.06	110.4

[a] Deviation from the mean is <10% of the mean value.
[b] Selectivity index = (COX-1 IC_{50}/COX-2 IC_{50}).
[c] ED_{50} was the effective dose calculated after 2 h.

(Figs. 3 and 4) with estimated free energy of binding of −5.96 kcal/mol towards COX-1.

3. Conclusions

In conclusion, a novel series of 1,3,4-oxadiazole derivatives containing diarylpyrazole moiety were synthesized employing hybrid pharmacophore approach. Results of *in vitro* COX assay indicated that all the compounds showed considerable COX-2 selectivity over COX-1. Moreover, substitution of electron withdrawing nitro group (**9g**, **9n**, and **10 g**) was found to be more favorable for COX-2 inhibition, while compounds possessing electron releasing groups showed diminished COX-2 activity. Among the compounds tested, **9g** was found to be the most potent inhibitor of COX-2 with the highest selectivity. The *in vivo* carrageenan-induced rat paw edema and mice acetic acid-induced writhing assays further established the anti-inflammatory potential of **9g**. Additionally, the lead compound **9g** showed gastro-sparing profile superior to the aspirin. Molecular docking analysis exhibited higher binding affinity of **9g** and other ligands towards the COX-2 than COX-1, while **9g** displaying the lowest estimated free energy of binding for COX-2, among all the docked compounds. Taken together, synthesis of dual pharmacophores i.e. 1,3,4-oxadiazole and diarylpyrazole bearing hybrid molecules exhibited anti-inflammatory and analgesic effects comparable to those of clinically used drugs with better safety profile and further research is warranted for the optimization of current pharmacophore.

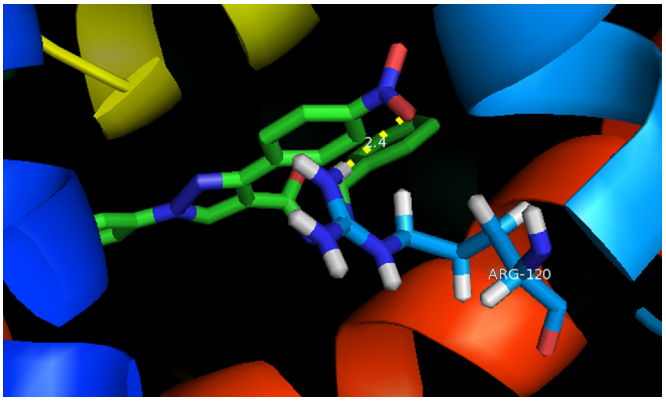


Fig. 1. Docking of **9g** into the active site of COX-2. The amino acid Arg-120 involved in hydrogen bond interaction with **9g**, is highlighted.

4. Experimental section

4.1. General experimental methods

All the chemicals were purchased from Merck, Sigma–Aldrich, Himedia, and Spectrochem, India of analytical or reagent grade and were used without further purification. Merck silica gel 60 F_{254} thin layer pre-coated plates were used for monitoring the chemical reactions. Melting points of the synthesized compounds were taken by one end open capillary tubes melting point apparatus and are uncorrected. Infrared spectra were recorded on a Shimadzu FTIR-8310 spectrophotometer (KBr). ^{1}H NMR spectra were recorded on a Bruker 400 MHz spectrophotometer and chemical shifts are reported in parts per million (δ) downfield from an internal standard tetramethylsilane. Mass spectra were obtained using electrospray ionization (ESI) technique by Agilent 1100 series LC-MS instrument. Elemental analyses were performed on Leco CHNS-932 (Leco, St. Joseph, MI, USA).

4.2. Synthesis of acid hydrazides (**2**–**3**)

A solution of substituted benzoic acid **1** (0.083 mol) in 20 ml methanol containing 2–3 ml of sulfuric acid was refluxed for 12 h (Scheme 1). The mixture was then allowed to cool at room temperature. A saturated solution of sodium bicarbonate was added to neutralize the mixture. Prepared ester was extracted by using dichloromethane (DCM). The DCM layer was dried using anhydrous sodium sulfate. The solvent was removed to dryness to afford ester which was used without further purification. To a solution of acid methyl ester in 15 ml of ethanol, 5 ml hydrazine hydrate was added and the resulting solution was refluxed for 16 h. Excess ethanol was distilled off and the concentrated solution was cooled to obtain acid hydrazide which was used without further purification.

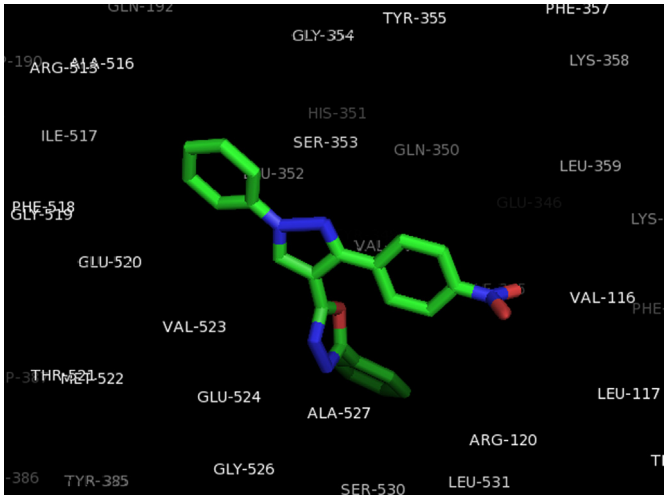


Fig. 2. Stereoview of docked complex of **9g** within the active site of COX-2. The amino acid residues of COX-2 involved in hydrogen, hydrophobic and van der Waal interactions with **9g**, are highlighted.

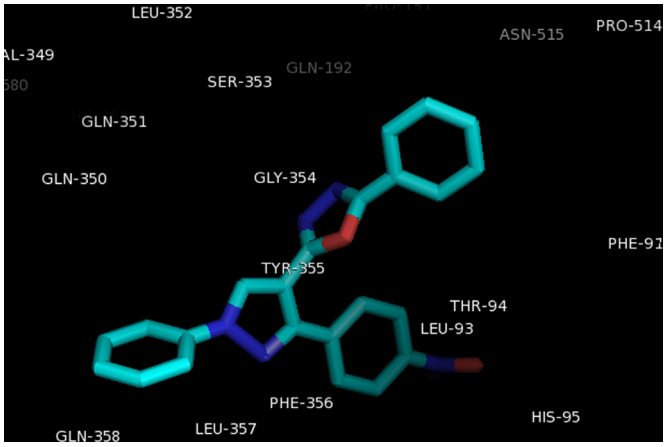


Fig. 3. Stereoview of docked complex of **9g** into the active site of COX-1. The amino acids of COX-1 involved in hydrogen, hydrophobic and van der Waal interactions with **9g**, are highlighted.

4.3. Synthesis of acetophenone phenylhydrazones (**5**)

A mixture of appropriate ketone **4** (0.01 mol) and phenylhydrazine (0.01 mol) in ethanol (10 ml) was refluxed in the presence of few drops of glacial acetic acid for 1 h. The progress of reaction was monitored by TLC using n-hexane:ethyl acetate (9.5:0.5) as a mobile phase. The mixture was cooled and the solid product obtained was filtered, washed with water (3–4 times) and recrystallized from ethanol.

4.4. Synthesis of 3-aryl-1-phenyl-1H-pyrazole-4-carbaldehyde (**6**)

To an ice cold dimethylformamide (10 ml), $POCl_3$ (0.03 mol) was added dropwise with continuous stirring over a period of 30 min. Stirring was continued for further 60 min, keeping the reaction temp at 0 °C. Acetophenone phenylhydrazone derivative **5** (0.01 mol) was then added, the reaction mixture was allowed to attain room temperature and stirred for further 4 h. The mixture was then refluxed for 2 h, allowed to cool and poured onto ice. A saturated solution of sodium bicarbonate was added to neutralize the mixture. The solid product obtained was filtered, washed with water followed by hexane (2–3 times) and recrystallized from methanol. The completion of reaction was monitored by TLC using n-hexane:ethyl acetate (9:1) as a mobile phase.

4.5. Synthesis of 3-aryl-1-phenyl-1H-pyrazole-4-carbaldehyde-acylhydrazones (**7a–n**, **8a–g**)

To an ethanolic solution of acid hydrazide or isoniazid (0.01 mol), aldehyde (0.01 mol) was added and the solution was refluxed in the presence of few drops of acetic acid for 4 h. The progress of reaction was monitored by TLC using n-hexane:ethyl acetate (4:6) as a mobile phase. After completion of reaction, solvent was evaporated under vacuum to half of its volume, cooled to room temperature and poured onto ice cold water. The precipitated product thus obtained was filtered, washed with water, dried, and recrystallized from ethanol.

4.6. Synthesis of 2,5-disubstituted 1,3,4-oxadiazoles (**9a–n**, **10a–g**)

An appropriate acylhydrazone **4** (0.01 mol) was dissolved in DCM (20 ml) and stirred. To this solution, IBD (0.11 mol) was added in four to five potions during 5 min and the mixture was stirred for 20–25 min at room temperature (Scheme 2). The solvent was evaporated in rotary evaporator and the residual mass was triturated with petroleum ether to give a solid product which was further purified by column chromatography using solvent system n-hexane:ethyl acetate (5:5).

4.6.1. 2-(1,3-Diphenyl-1H-pyrazol-4-yl)-5-phenyl-1,3,4-oxadiazole (**9a**)

Yield 62%, mp 118–120 °C. Anal. calcd. for $C_{23}H_{16}N_4O$ (364.40): %C, 75.81; H, 4.43; N, 15.38. Found: %C, 75.86; H, 4.40; N, 15.41. IR (KBr, cm^{-1}): 1616.5 (C=N), 1537.4 (C=C), 1212.6 (C–O–C). ^1H NMR (DMSO-d_6, 400 MHz, δ ppm): 8.41 (s, 1H, =CH– of pyrazole ring), 7.82–8.02 (m, 4H, Ar-H), 7.36–7.53 (m, 11H, Ar-H). ESI-MS (*m*/*z*): 364 (M^+).

4.6.2. 2-Phenyl-5-(1-phenyl-3-p-tolyl-1H-pyrazol-4-yl)-1,3,4-oxadiazole (**9b**)

Yield 69%, mp 196–198 °C. Anal. calcd. for $C_{24}H_{18}N_4O$ (378.43): %C, 76.17; H, 4.79; N, 14.81. Found: %C, 76.15; H, 4.82; N, 14.85. IR (KBr, cm^{-1}): 1618.2 (C=N), 1537.1 (C=C), 1209.5 (C–O–C). ^1H NMR (DMSO-d_6, 400 MHz, δ ppm): 8.44 (s, 1H, =CH– of pyrazole ring), 7.96–8.06 (m, 2H, Ar-H), 7.40–7.55 (m, 10H, Ar-H), 7.12–7.14 (m, 2H, Ar-H), 2.36 (s, 1H, CH_3). ESI-MS (*m*/*z*): 378 (M^+).

4.6.3. 2-(3-(4-Fluorophenyl)-1-phenyl-1H-pyrazol-4-yl)-5-phenyl-1,3,4-oxadiazole(**9c**)

Yield 71%, mp 132–134 °C. Anal. calcd. for $C_{23}H_{15}FN_4O$ (382.39): %C, 72.24; H, 3.95; N, 14.65. Found: %C, 72.28; H, 3.97; N, 14.62. IR (KBr, cm^{-1}): 1621.7 (C=N), 1538.8 (C=C), 1219.9 (C–O–C). ^1H NMR (DMSO-d_6, 400 MHz, δ ppm): 8.52 (s, 1H, =CH– of pyrazole ring), 7.84–7.96 (m, 4H, Ar-H), 7.40–7.55 (m, 8H, Ar-H), 7.06–7.10 (m, 2H, Ar-H). ESI-MS (*m*/*z*): 382 (M^+).

4.6.4. 2-(3-(4-Chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)-5-phenyl-1,3,4-oxadiazole (**9d**)

Yield 54%, mp 142–144 °C. Anal. calcd. for $C_{23}H_{15}ClN_4O$ (398.84): %C, 69.26; H, 3.79; N, 14.05. Found: %C, 69.23; H, 3.77; N, 14.10. IR (KBr, cm^{-1}): 1608.3 (C=N), 1555.8 (C=C), 1221.5 (C–O–C), 690.1 (C–Cl). ^1H NMR (DMSO-d_6, 400 MHz, δ ppm): 8.46 (s, 1H, =CH– of pyrazole ring), 7.83–7.95 (m, 4H, Ar-H), 7.43–7.63 (m, 10H, Ar-H). ESI-MS (*m*/*z*): 399 (M^+).

4.6.5. 2-(3-(4-Bromophenyl)-1-phenyl-1H-pyrazol-4-yl)-5-phenyl-1,3,4-oxadiazole (**9e**)

Yield 61%, mp 174–175 °C. Anal. calcd. for $C_{23}H_{15}BrN_4O$ (443.30): %C, 62.32; H, 3.41; N, 12.64. Found: %C, 62.35; H, 3.40; N, 12.67. IR (KBr, cm^{-1}): 1616.3 (C=N), 1556.7 (C=C), 1202.8 (C–O–C). ^1H NMR (DMSO-d_6, 400 MHz, δ ppm): 8.61 (s, 1H, =CH– of pyrazole ring), 7.77–7.89 (m, 4H, Ar-H), 7.39–7.59 (m, 10H, Ar-H). ESI-MS (*m*/*z*): 443 (M^+).

4.6.6. 2-(3-(4-Methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-5-phenyl-1,3,4-oxadiazole(**9f**)

Yield 64%, mp 143–145 °C. Anal. calcd. for $C_{24}H_{18}N_4O_2$ (394.43): %C, 73.08; H, 4.60; N, 14.20. Found: %C, 73.12; H, 4.63; N, 14.25. IR (KBr, cm^{-1}): 1614.5 (C=N), 1535.5 (C=C), 1212.7 (C–O–C). ^1H NMR (DMSO-d_6, 400 MHz, δ ppm): 8.49 (s, 1H, =CH– of pyrazole ring), 7.95–7.98 (m, 2H, Ar-H), 7.45–7.62 (m, 10H, Ar-H), 6.86–6.88 (m, 2H, Ar-H), 3.76 (s, 1H, OCH_3). ESI-MS (*m*/*z*): 394 (M^+).

4.6.7. 2-(3-(4-Nitrophenyl)-1-phenyl-1H-pyrazol-4-yl)-5-phenyl-1,3,4-oxadiazole (**9g**)

Yield 83%, mp 105–106 °C. Anal. calcd. for $C_{23}H_{15}N_5O_3$ (409.4): %C, 67.48; H, 3.69; N, 17.11. Found: %C, 67.52; H, 3.71; N, 17.08. IR (KBr, cm^{-1}): 1616.6 (C=N), 1595.5 (C=C), 1341.7 (N=O), 1211.4 (C–O–C). ^1H NMR (DMSO-d_6, 400 MHz, δ ppm): 8.39 (s, 1H, =CH– of

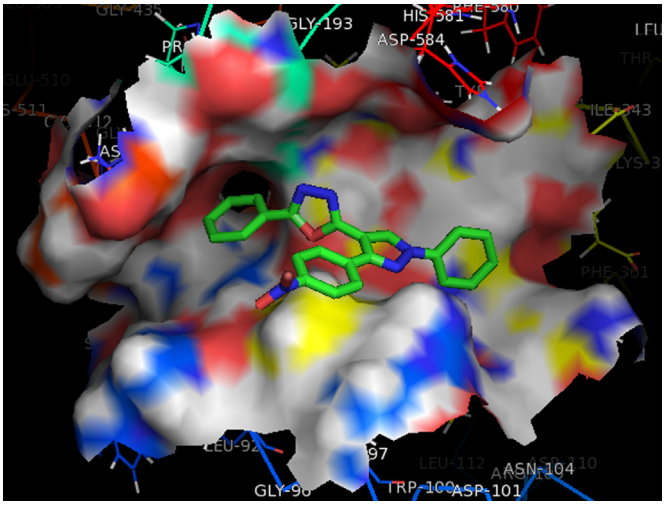


Fig. 4. Binding orientation of **9g** within the active site of COX-1.

pyrazole ring), 8.27–8.29 (m, 2H, Ar-H), 7.82–7.94 (m, 4H, Ar-H), 7.44–7.63 (m, 8H, Ar-H). ESI-MS (*m*/*z*): 409 (M^+).

*4.6.8. 2-(4-Chlorophenyl)-5-(1,3-diphenyl-1H-pyrazol-4-yl)-1,3,4-oxadiazole (**9h**)*

Yield 55%, mp 122–123 °C. Anal. calcd. for $C_{23}H_{15}ClN_4O$ (398.84): %C, 69.26; H, 3.79; N, 14.05. Found: %C, 69.30; H, 3.81; N, 14.10. IR (KBr, cm^{-1}): 1609.5 (C=N), 1595.1 (C=C), 1221.7 (C–O–C), 694.6 (C–Cl). ^1H NMR (DMSO-d_6, 400 MHz, δ ppm): 8.43 (s, 1H, =CH– of pyrazole ring), 7.85–7.98 (m, 4H, Ar-H), 7.32–7.59 (m, 10H, Ar-H). ESI-MS (*m*/*z*): 399 (M^+).

*4.6.9. 2-(4-Chlorophenyl)-5-(1-phenyl-3-p-tolyl-1H-pyrazol-4-yl)-1,3,4-oxadiazole (**9i**)*

Yield 65%, mp 120–122 °C. Anal. calcd. For $C_{24}H_{17}ClN_4O$ (412.87): %C, 69.84; H, 4.15; N, 13.57. Found: %C, 69.79; H, 4.16; N, 13.59. IR (KBr, cm^{-1}): 1612.6 (C=N), 1514.0 (C=C), 1215.9 (C–O–C), 694.5 (C–Cl). ^1H NMR (DMSO-d_6, 400 MHz, δ ppm): 8.45 (s, 1H, =CH– of pyrazole ring), 7.94–7.97 (m, 2H, Ar-H), 7.32–7.60 (m, 9H, Ar-H), 7.16–7.18 (m, 2H, Ar-H), 2.35 (s, 1H, CH_3). ESI-MS (*m*/*z*): 413 (M^+).

*4.6.10. 2-(4-Chlorophenyl)-5-(3-(4-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl)-1,3,4-oxadiazole (**9j**)*

Yield 69%, mp 105–106 °C. Anal. calcd. for $C_{23}H_{14}ClFN_4O$ (416.83): %C, 66.27; H, 3.39; N, 13.44. Found: %C, 66.24; H, 3.40; N, 13.48. IR (KBr, cm^{-1}): 1603.2 (C=N), 1594.0 (C=C), 1221.1 (C–O–C), 1002.7 (C–F), 679.6 (C–Cl). ^1H NMR (DMSO-d_6, 400 MHz, δ ppm): 8.52 (s, 1H, =CH– of pyrazole ring), 7.78–7.92 (m, 4H, Ar-H), 7.42–7.53 (m, 7H, Ar-H), 7.12–7.14 (m, 2H, Ar-H). ESI-MS (*m*/*z*): 417 (M^+).

*4.6.11. 2-(4-Chlorophenyl)-5-(3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)-1,3,4-oxadiazole(**9k**)*

Yield 80%, mp 168–170 °C. Anal. calcd. for $C_{23}H_{14}Cl_2N_4O$ (433.29): %C, 63.76; H, 3.26; N, 12.93. Found: %C, 63.78; H, 3.29; N, 12.96. IR (KBr, cm^{-1}): 1617.4 (C=N), 1598.0 (C=C), 1212.6 (C–O–C), 686.3 (C–Cl). ^1H NMR (DMSO-d_6, 400 MHz, δ ppm): 8.48 (s, 1H, =CH– of pyrazole ring), 7.87–7.98 (m, 4H, Ar-H), 7.38–7.58 (m, 9H, Ar-H). ESI-MS (*m*/*z*): 433 (M^+).

*4.6.12. 2-(3-(4-Bromophenyl)-1-phenyl-1H-pyrazol-4-yl)-5-(4-chlorophenyl)-1,3,4-oxadiazole (**9l**)*

Yield 51%, mp 174–175 °C. Anal. calcd. for $C_{23}H_{14}BrClN_4O$ (477.74): %C, 57.82; H, 2.95; N, 11.73. Found: %C, 57.79; H, 2.94; N, 11.79. IR (KBr, cm^{-1}): 1616.6 (C=N), 1504.0 (C=C), 1210.9 (C–O–C), 690.4 (C–Cl), 504.6 (C–Br). ^1H NMR (DMSO-d_6, 400 MHz, δ ppm): 8.65 (s, 1H, =CH– of pyrazole ring), 7.82–7.92 (m, 4H, Ar-H), 7.43–7.62 (m, 9H, Ar-H). ESI-MS (*m*/*z*): 478 (M^+).

*4.6.13. 2-(4-Chlorophenyl)-5-(3-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-1,3,4-oxadiazole (**9m**)*

Yield 59%, mp 100–101 °C. Anal. calcd. for $C_{24}H_{17}ClN_4O_2$ (428.87): %C, 67.21; H, 4.00; N, 13.06. Found: %C, 67.23; H, 4.03; N, 13.03. IR (KBr, cm^{-1}): 1608.8 (C=N), 1597.9 (C=C), 1245.7 (C–O–C), 684.8 (C–Cl). ^1H NMR (DMSO-d_6, 400 MHz, δ ppm): 8.45 (s, 1H, =CH– of pyrazole ring), 7.99–8.05 (m, 2H, Ar-H), 7.49–7.58 (m, 9H, Ar-H), 6.79–6.83 (m, 2H, Ar-H), 3.77 (s, 1H, OCH_3). ESI-MS (*m*/*z*): 429 (M^+).

*4.6.14. 2-(4-Chlorophenyl)-5-(3-(4-nitrophenyl)-1-phenyl-1H-pyrazol-4-yl)-1,3,4-oxadiazole (**9n**)*

Yield 72%, mp 156–158 °C. Anal. calcd. for $C_{23}H_{14}ClN_5O_3$ (443.84): %C, 62.24; H, 3.18; N, 15.78. Found: %C, 62.21; H, 3.17; N, 15.83. IR (KBr, cm^{-1}): 1624.6 (C=N), 1599.5 (C=C), 1343.7 (N=O), 1225.2 (C–O–C), 684.4 (C–Cl). ^1H NMR (DMSO-d_6, 400 MHz, δ ppm): 8.42 (s, 1H, =CH– of pyrazole ring), 8.22–8.24 (m, 2H, Ar-H), 7.79–7.89 (m, 4H, Ar-H), 7.39–7.58 (m, 7H, Ar-H). ESI-MS (*m*/*z*): 444 (M^+).

*4.6.15. 4-(5-(1,3-Diphenyl-1H-pyrazol-4-yl)-1,3,4-oxadiazol-2-yl) pyridine (**10a**)*

Yield 52%, mp 118–120 °C. Anal. calcd. for $C_{22}H_{15}N_5O$ (365.39): %C, 72.32; H, 4.14; N, 19.17. Found: %C, 72.29; H, 4.13; N, 19.15. IR (KBr, cm^{-1}): 1609.5 (C=N), 1585.5 (C=C), 1212.8 (C–O–C). ^1H NMR (DMSO-d_6, 400 MHz, δ ppm): 8.75–8.76 (m, 2H, Py-H), 8.45 (s, 1H, =CH– of pyrazole ring), 7.84–7.99 (m, 4H, Ar-H), 7.35–7.58 (m, 8H, Ar-H). ESI-MS (*m*/*z*): 365 (M^+).

*4.6.16. 4-(5-(1-Phenyl-3-p-tolyl-1H-pyrazol-4-yl)-1,3,4-oxadiazol-2-yl)pyridine (**10b**)*

Yield 60%, mp 108–110 °C. Anal. calcd. for $C_{23}H_{17}N_5O$ (379.41): %C, 72.81; H, 4.52; N, 18.46. Found: %C, 72.79; H, 4.54; N, 18.50. IR (KBr, cm^{-1}): 1605.6 (C=N), 1570.4 (C=C), 1224.7 (C–O–C). ^1H NMR (DMSO-d_6, 400 MHz, δ ppm): 8.80–8.81 (m, 2H, Py-H), 8.49 (s, 1H, =CH– of pyrazole ring), 7.89–7.95 (m, 2H, Ar-H), 7.37–7.54 (m, 7H, Ar-H), 7.16–7.18 (m, 2H, Ar-H), 2.36 (s, 1H, CH_3). ESI-MS (*m*/*z*): 379 (M^+).

*4.6.17. 4-(5-(3-(4-Fluorophenyl)-1-phenyl-1H-pyrazol-4-yl)-1,3,4-oxadiazol-2-yl)pyridine (**10c**)*

Yield 74%, mp 116–118 °C. Anal. calcd. for $C_{22}H_{14}FN_5O$ (383.38): %C, 68.92; H, 3.68; N, 18.27. Found: %C, 68.97; H, 3.65; N, 18.30. IR (KBr, cm^{-1}): 1609.3 (C=N), 1592.2 (C=C), 1229.9 (C–O–C), 1066.3 (C–F). ^1H NMR (DMSO-d_6, 400 MHz, δ ppm): 8.77–8.79 (m, 2H, Py-H), 8.49 (s, 1H, =CH– of pyrazole ring), 7.88–7.92 (m, 2H, Ar-H), 7.37–7.55 (m, 7H, Ar-H), 7.18–7.20 (m, 2H, Ar-H). ESI-MS (*m*/*z*): 383 (M^+).

*4.6.18. 4-(5-(3-(4-Chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)-1,3,4-oxadiazol-2-yl)pyridine (**10d**)*

Yield 59%, mp 192–194 °C. Anal. calcd. for $C_{22}H_{14}ClN_5O$ (399.83): %C, 66.09; H, 3.53; N, 17.52. Found: %C, 66.06; H, 3.54; N, 17.58. IR (KBr, cm^{-1}): 1600.2 (C=N), 1593.7 (C=C), 1245.1 (C–O–C), 684.4 (C–Cl). ^1H NMR (DMSO-d_6, 400 MHz, δ ppm): 8.83–8.85 (m,

2H, Py-H), 8.43 (s, 1H, =CH– of pyrazole ring), 7.84–7.96 (m, 4H, Ar-H), 7.41–7.60 (m, 7H, Ar-H). ESI-MS (*m*/*z*): 400 (M^+).

4.6.19. 4-(5-(3-(4-Bromophenyl)-1-phenyl-1H-pyrazol-4-yl)-1,3,4-oxadiazol-2-yl)pyridine (**10e**)

Yield 84%, mp 174–175 °C. Anal. calcd. for $C_{22}H_{14}BrN_5O$ (444.28): %C, 59.47; H, 3.18; N, 15.76. Found: %C, 59.53; H, 3.22; N, 15.82. IR (KBr, cm^{-1}): 1620.9 (C=N), 1573.5 (C=C), 1215.9 (C–O–C), 750.7 (C–Br). ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 8.83–8.85 (m, 2H, Py-H), 8.63 (s, 1H, =CH– of pyrazole ring), 7.75–7.83 (m, 4H, Ar-H),7.41–7.62 (m, 7H, Ar-H). ESI-MS (*m*/*z*): 444 (M^+).

4.6.20. 4-(5-(3-(4-Methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-1,3,4-oxadiazol-2-yl)-pyridine (**10f**)

Yield 60%, mp 140–142 °C. Anal. calcd. for $C_{23}H_{17}N_5O_2$ (395.41): %C, 69.86; H, 4.33; N, 17.71. Found: %C, 69.85; H, 4.31; N, 17.70. IR (KBr, cm^{-1}): 1611.7 (C=N), 1598.5 (C=C), 1249.1 (C–O–C). ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 8.80–8.81 (m, 2H, Py-H), 8.40 (s, 1H, =CH– of pyrazole ring), 7.93–7.96 (m, 2H, Ar-H), 7.49–7.61 (m, 7H, Ar-H), 6.82–6.84 (m, 2H, Ar-H), 3.82 (s, 1H, OCH_3). ESI-MS (*m*/*z*): 395 (M^+).

4.6.21. 4-(5-(3-(4-Nitrophenyl)-1-phenyl-1H-pyrazol-4-yl)-1,3,4-oxadiazol-2-yl)pyridine (**10g**)

Yield 70%, mp 240–241 °C. Anal. calcd. for $C_{22}H_{14}N_6O_3$ (410.38): %C, 64.39; H, 3.44; N, 20.48. Found: %C, 64.44; H, 3.49; N, 20.52. IR (KBr, cm^{-1}): 1618.5 (C=N), 1575.9 (C=C), 1345.7 (N=O), 1207.6 (C–O–C). ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 8.78–8.80 (m, 2H, Py-H), 8.45 (s, 1H, =CH– of pyrazole ring), 8.25–8.27 (m, 2H, Ar-H), 7.77–7.84 (m, 4H, Ar-H), 7.44–7.56 (m, 5H, Ar-H). ESI-MS (*m*/*z*): 410 (M^+).

4.7. Biological screening

4.7.1. In vitro COX inhibition assay [16]

COX-1 and COX-2 inhibition activities of tested compounds were determined by using a COX fluorescent inhibitor Screening assay kit consisting of ovine COX-1 and human recombinant COX-2 enzymes. A minimum volume of DMSO was used to prepare stock solutions of tested compounds. A 10 μL of test solutions (0.01, 0.1, 1, 10, 50, and 100 μM) were added to 960 μL supplied buffer solution (0.1 M Tris–HCl pH 8.0 containing 5 mM EDTA and 2 mM phenol) with either COX-1 or COX-2 (10 μL) enzyme in the presence of 10 μL heme and 10 μL fluorometric substrate ADHP (10-acetyl-3,7-dihydroxyphenoxazine). These solutions were kept at 37 °C for 5 min, 10 μL of arachidonic acid solution (100 μM) was added and the COX reaction was stopped by the addition of 50 μL of 1 M HCl after 2 min. Fluorescence of resorufin, produced by the reaction between PGG2 and ADHP, were measured with an excitation wavelength of 535 nm and an emission wavelength of 590 nm. The intensity of this fluorescence is proportional to the amount of resorufin, which is proportional to the amount of PGG2 present in the well during the incubation. Percentage inhibition was calculated by comparison with control value (no inhibitor). The IC_{50} values of tested compounds against COX-1 and COX-2 (μM) were calculated from the concentration inhibition response curve (triplicate determinations).

4.7.2. Animals

The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) and complied with the NIH guidelines on handling of experimental animals. The animals were kept at room temperature (27 ± 3 °C) with 14:10 h light and dark cycle and at controlled humidity conditions. They were housed under standard conditions and received autoclaved standard food pellets and water *ad libitum* during the maintenance but were fasted with only access to water at least 14–24 h prior to perform experiments.

4.7.3. Toxicological study (OECD 425 guidelines)

An acute toxicity study was done and safe dose was calculated as per OECD test guideline 425 [36].

4.7.4. Carrageenan-induced paw edema model

The anti-inflammatory activity was evaluated using *in vivo* rat carrageenan-induced paw edema method as described previously by Winter et al. [37]. The animals (Charles Foster male albino rats, weighing 150–200 g) were divided into three groups ($n = 6$) *viz.* test, standard, and control. The test compounds and standard drug diclofenac were suspended in 0.5% methyl cellulose and were given orally to each group of animals. After 1 h, 0.2 mL of 1% carrageenan in 0.9% NaCl was injected subcutaneously into the plantar surface of the right hind paw of each rat. The paw volume was recorded using a plethysmometer (UGO Basile 7140, model-7141, Biological research apparatus, Italy) immediately after the carrageenan injection and again 2 h later. The mean increase in paw volume was compared with that of control group (six rats received only carrageenan, no test compounds) and percent inhibition values were calculated using the formula: anti-inflammatory activity (%) = 1 − Dt/Dc × 100, where Dt represents the difference in paw volume before and after drug was administered to the rats, while Dc is the difference in paw volume in the control group. The ED_{50} obtained after 2 h of treatment with carrageenan are depicted in Table 1. All of the ED_{50} values were determined at three doses of 50, 100, and 200 mg/kg body wt. for the test compounds and 25, 50, and 100 mg/kg body wt. for the reference drugs diclofenac and celecoxib.

4.7.5. Acetic acid-induced writhing assay

The most active compound (**9g**) emerged in the anti-inflammatory study was further tested for its analgesic potential by employing acetic acid-induced writhing assay. The separate groups (each group consisting of 6 mice) were administered with saline, lead compound **9g** (200 mg/kg, p.o.), and reference drug aspirin (100 mg/kg, p.o.). After 60 min, an i.p. injection of 0.6% acetic acid was given to the mice of all the groups. The total number of writhes made by each mouse was counted between 5 and 10 min after acetic acid administration and the analgesic effect was calculated as the percentage inhibition of writhes as compared with the control group [30,38].

4.7.6. Ulcerogenic assay

The lead compound **9g** along with reference drug aspirin were examined for gastric ulcerogenic potential. Six rats in each group, weighing 180–200 g and fasted for 24 h were administered the lead compound **9g** and aspirin (each 200 mg/kg) by intragastric route. Three hours after, rats were sacrificed, their stomachs were removed, opened along the lesser curvature, and examined for the presence of gastric ulcers. The glandular mucosa was explored and each hemorrhagic lesion was measured individually along its maximum length (<1 mm: rank = 1; 1–2 mm: rank = 2; >2 mm: ranking according to their utmost length). The lengths of all the lesions were summed up to provide an overall total, defined as the ulcer-index. The results obtained are expressed as mean ± SEM [39].

4.8. Molecular docking study

Crystal structures of COX-1 and COX-2 were downloaded from Protein Data Bank (PDB ID: 1Q4G and 1CX2, respectively). The 3D structures of ligands were prepared in CS Chem Draw Ultra 8.0.

Molecular docking analysis was performed using AutoDock tools 1.5.4. Polar hydrogens were added to the protein and Gasteiger charges were assigned. Non-polar hydrogens were merged and partial charges added to their parent carbon atoms. The search space was defined as a grid box with dimensions 40 Å × 40 Å × 40 Å and center $x = 22$, $y = 35$, $z = 210$ for 1Q4G and dimensions 40 Å × 40 Å × 40 Å and center $x = 25$, $y = 23$, $z = 19$ for 1CX2. The Lamarckian genetic algorithm (LGA) was applied to search conformers that possessed lowest binding energy. Results of molecular docking analysis were obtained as estimated free energy of binding in kcal/mol (docking score).

Acknowledgments

The authors thank the SAIF labs of Panjab University, Chandigarh, India for carrying out spectral and CHN analyses.

References

[1] E. Palaska, G. Şahin, P. Kelicen, N.T. Durlu, G. Altinok, Il Farmaco 57 (2002) 101–107.
[2] N.V. Chandrasekharan, H. Dai, K.L. Roos, N.K. Evanson, J. Tomsik, T.S. Elton, D.L. Simmons, Proceedings of the National Academy of Sciences of the United States of America 99 (2002) 13926–13931.
[3] H.R. Herschman, Biochimica et Biophysica Acta 1299 (1996) 125–140.
[4] L. Parente, M. Perretti, Biochemical Pharmacology 65 (2003) 153–159.
[5] T.D. Penning, J.J. Talley, S.R. Bertenshaw, J.S. Carter, P.W. Collins, S. Docter, M.J. Graneto, L.F. Lee, J.W. Malecha, J.M. Miyashiro, R.S. Rogers, D.J. Rogier, S.S. Yu, G.D. Anderson, E.G. Burton, J.N. Cogburn, S.A. Gregory, C.M. Koboldt, W.E. Perkins, K. Seibert, A.W. Veenhuizen, Y.Y. Zhang, P.C. Isakson, Journal of Medicinal Chemistry 40 (1997) 1347–1365.
[6] P. Prasit, Z. Wang, C. Brideau, C.C. Chan, S. Charleson, W. Cromlish, D. Ethier, J.F. Evans, A.W. Ford-Hutchinson, J.Y. Gauthier, Bioorganic & Medicinal Chemistry Letters 9 (1999) 1773–1778.
[7] J.J. Talley, D.L. Brown, J.S. Carter, M.J. Graneto, C.M. Koboldt, J.L. Masferrer, W.E. Perkins, R.S. Rogers, A.F. Shaffer, Y.Y. Zhang, B.S. Zweifel, K. Seibert, Journal of Medicinal Chemistry 43 (2000) 775–777.
[8] D. Riendeau, M.D. Percival, C. Brideau, S. Charleson, D. Dube, D. Ethier, J.P. Falgueyret, R.W. Friesen, R. Gordon, G. Greig, J. Guay, J. Mancini, M. Ouellet, E. Wong, L. Xu, S. Boyce, D. Visco, Y. Girard, P. Prasit, R. Zamboni, I.W. Rodger, M. Gresser, A.W. Ford-Hutchinson, R.N. Young, C.C. Chan, The Journal of Pharmacology and Experimental Therapeutics 296 (2001) 558–566.
[9] J.M. Dogne, C.T. Supuran, D. Pratico, Journal of Medicinal Chemistry 48 (2005) 2251–2257.
[10] P. McGettigan, D. Henry, Jama: the Journal of the American Medical Association 296 (2006) 1633–1644.
[11] G. Menozzi, L. Mosti, P. Fossa, F. Mattioli, M. Ghia, Journal of Heterocyclic Chemistry 34 (1997) 963–968.
[12] A. Tanitame, Y. Oyamada, K. Ofuji, M. Fujimoto, N. Iwai, Y. Hiyama, K. Suzuki, H. Ito, M. Wachi, J. Yamagishi, Journal of Medicinal Chemistry 47 (2004) 3693–3696.
[13] R. Sridhar, P.T. Perumal, S. Etti, G. Shanmugam, M.N. Ponnuswamy, V.R. Prabavathy, N. Mathivanan, Bioorganic & Medicinal Chemistry Letters 14 (2004) 6035–6040.
[14] M.I. El-Gamal, Y.S. Park, D.Y. Chi, K.H. Yoo, C.H. Oh, European Journal of Medicinal Chemistry 65 (2013) 315–322.
[15] M.I. El-Gamal, H.S. Choi, K.H. Yoo, D. Baek, C.H. Oh, Chemical Biology & Drug Design 82 (2013) 336–347.
[16] S.H. Hwang, K.M. Wagner, C. Morisseau, J.Y. Liu, H. Dong, A.T. Wecksler, B.D. Hammock, Journal of Medicinal Chemistry 54 (2011) 3037–3050.
[17] M.A. El-Sayed, N.I. Abdel-Aziz, A.A. Abdel-Aziz, A.S. El-Azab, Y.A. Asiri, K.E. El-Tahir, Bioorganic & Medicinal Chemistry 19 (2011) 3416–3424.
[18] M. Amir, H. Kumar, S.A. Khan, Bioorganic & Medicinal Chemistry Letters 18 (2008) 918–922.
[19] Z. Sui, J. Guan, M.P. Ferro, K. McCoy, M.P. Wachter, W.V. Murray, M. Singer, M. Steber, D.M. Ritchie, D.C. Argentieri, Bioorganic & Medicinal Chemistry Letters 10 (2000) 601–604.
[20] B. Jayashankar, K.M. Lokanathrai, N. Baskaran, H.S. Sathish, European Journal of Medicinal Chemistry 44 (2009) 3898–3902.
[21] S. Bondock, S. Adel, H.A. Etman, F.A. Badria, European Journal of Medicinal Chemistry 48 (2012) 192–199.
[22] S.G. Kucukguzel, E.E. Oruc, S. Rollas, F. Sahin, A. Ozbek, European Journal of Medicinal Chemistry 37 (2002) 197–206.
[23] N.N. Farshori, M.R. Banday, A. Ahmad, A.U. Khan, A. Rauf, Bioorganic & Medicinal Chemistry Letters 20 (2010) 1933–1938.
[24] A.A. El-Emam, O.A. Al-Deeb, M. Al-Omar, J. Lehmann, Bioorganic & Medicinal Chemistry 12 (2004) 5107–5113.
[25] Z.N. Cui, Y.X. Shi, L. Zhang, Y. Ling, B.J. Li, Y. Nishida, X.L. Yang, Journal of Agricultural and Food Chemistry 60 (2012) 11649–11656.
[26] Z.N. Cui, L. Yang, X.C. Li, Z. Wang, X.L. Yang, Chinese Journal of Organic Chemistry 26 (2006) 1647–1656.
[27] J. Boström, A. Hogner, A. Llinàs, E. Wellner, A.T. Plowright, Journal of Medicinal Chemistry 55 (2012) 1817–1830.
[28] K. Manjunatha, B. Poojary, P.L. Lobo, J. Fernandes, N.S. Kumari, European Journal of Medicinal Chemistry 45 (2010) 5225–5233.
[29] M. Akhter, A. Husain, B. Azad, M. Ajmal, European Journal of Medicinal Chemistry 44 (2009) 2372–2378.
[30] D.V. Dekhane, S.S. Pawar, S. Gupta, M.S. Shingare, C.R. Patil, S.N. Thore, Bioorganic & Medicinal Chemistry Letters 21 (2011) 6527–6532.
[31] S.K. Suthar, V. Jaiswal, S. Lohan, S. Bansal, A. Chaudhary, A. Tiwari, A.T. Alex, A. Joseph, European Journal of Medicinal Chemistry 63 (2013) 589–602.
[32] S.K. Suthar, S. Bansal, S. Lohan, V. Modak, A. Chaudhary, A. Tiwari, European Journal of Medicinal Chemistry 66 (2013) 372–379.
[33] A. Joseph, C.S. Shah, S.K. Suthar, A.T. Alex, N. Maliyakkal, S. Moorkoth, J.E. Mathew, Acta Pharmaceutica: a Quarterly Journal of Croatian Pharmaceutical Society and Slovenian Pharmaceutical Society, Dealing With All Branches of Pharmacy and Allied Sciences 63 (2013) 397–408.
[34] R.Y. Yang, L.X. Dai, The Journal of Organic Chemistry 58 (1993) 3381–3383.
[35] M.A. Kira, M.O. Abdel-Rahman, K.Z. Gadalla, Tetrahedron Letters 10 (1969) 109–110.
[36] OECD Guidelines for the Testing of Chemicals: 425 Acute Oral Toxicity–Up-and-Down-Procedure (UDP), OECD, 2008, pp. 1–27.
[37] C.A. Winter, E.A. Risley, G.W. Nuss, Proceedings of the Society for Experimental Biology and Medicine 111 (1962) 544–547.
[38] G. Daidone, B. Maggio, D. Raffa, S. Plescia, M.L. Bajardi, A. Caruso, V.M.C. Cutuli, M. Amico-Roxas, European Journal of Medicinal Chemistry 29 (1994) 707–711.
[39] L. Lazzarato, C. Cena, B. Rolando, E. Marini, M.L. Lolli, S. Guglielmo, E. Guaita, G. Morini, G. Coruzzi, R. Fruttero, A. Gasco, Bioorganic & Medicinal Chemistry 19 (2011) 5852–5860.