Design, Docking, and Synthesis of Some New Pyrazoline and Pyranopyrazole Derivatives as Anti-inflammatory Agents

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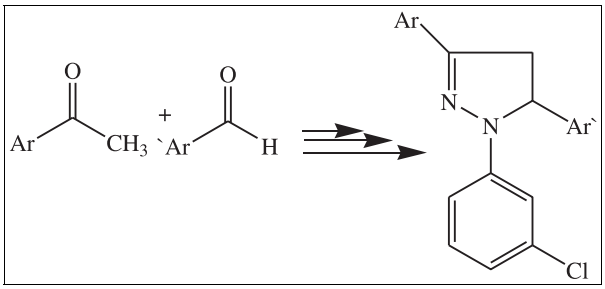

Design and synthesis of some novel pyrazoline and pyranopyrazole derivatives as potential anti-inflammatory agents are described. Most of the compounds were tested for their anti-inflammatory (*in vitro* and *in vivo*) and ulcerogenic activities. In all tested compounds, it was found that pyrazolines, **2a**, and pyrazolopyrano[2,3-*d*]pyrimidine **9** are the potent anti-inflammatory and selective cyclooxygenase-2 (COX-2) inhibitor. All compounds are mainly in the safe level. Docking study of **2a** and **9** revealed higher affinity for binding with the active site of COX-2 enzyme like SC-558, a selective COX-2 inhibitor.

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INTRODUCTION

Prostaglandins are endogenous substances involved in different processes of physiological nature and are potent mediators of inflammation. Prostaglandins are produced, together with other prostanoids, in the arachidonic acid metabolism, whose first step, consisting of the oxidative conversion of arachidonic acid into prostaglandin H_2, is catalyzed by cyclooxygenase (COX) [1]. This enzyme exists at least as two isoforms, one constitutive (COX-1) and the other inducible (COX-2) [2]. COX-1 is found in platelets, kidneys, and in the gastrointestinal (GI) tract and is believed to be responsible for the homeostatic maintenance of the kidneys and GI tract. The COX-2 enzyme is the inducible isoform that is produced by various cell types upon exposure to cytokines, mitogens, and endotoxins released during injury [3]. The COX-2 enzyme, after being overexpressed at the site of injury, is a catalyst for the production of the prostaglandins that result in inflammation and pain at the site. COX-1 is involved in the maintenance of the GI tract, so all nonsteroidal anti-inflammatory drugs that are inhibitors of both COX-2 and COX-1 have been found to cause side effects associated with GI ulcers [4]. Thus, it was thought that a more selective COX-2 inhibitor would have reduced GI side effects [3]. Several COX-2 selective inhibitors, including celecoxib (Celebrex) [5], valdecoxib (Bextra) [6], rofecoxib (Vioxx) [7], and etoricoxib (Arroxin) (Fig. 1), have shown excellent efficacy in humans with few side effects. Pyrazolines are well known for their pronounced anti-inflammatory activity. [8] Pyrano[2,3-*c*]pyrazole [9,10] had been reported to display good anti-inflammatory activity. The present investigation aims to achieve two goals; the first goal is design and synthesis of two novel series, trisubstituted-2-pyrazolines with different substituents at position 3 and 5, pyranopyrazoles and condensed pyranopyrazoles trying to obtain better and selective COX-2 inhibitors. The second goal is evaluation of their anti-inflammatory and ulcerogenic activities and use of docking technique to detect the compounds with high affinity to the active site of COX-2 enzyme; therefore, it might be of the best activity. Accordingly, docking of the designed compounds were performed into COX-2 complexed with its bound inhibitor, SC-558: 4-[5-(4-bromophenyl)-3-(trifluoromethyl)pyrazol-1-yl] benzenesulfonamide [11] (Fig. 1) using Molsoft ICM 3,4-8c program was performed to predict the affinity and orientation of these new series at the active site. The Internal Coordinate Mechanic (ICM) score values combined with hydrogen bonds formed with the surrounding amino acid residues are the prediction of the correct binding geometry (affinity and orientation) for each binder at the active site. This reflects the picture about the COX-2 inhibitory activity.

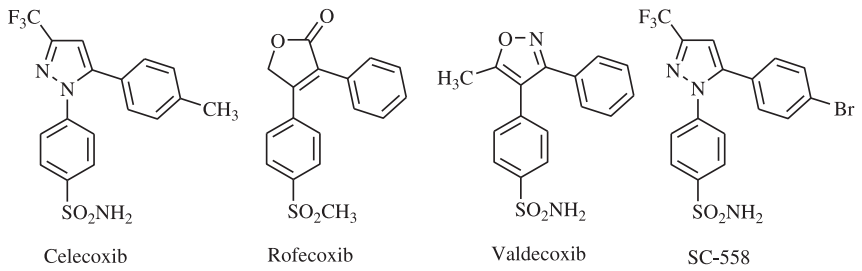


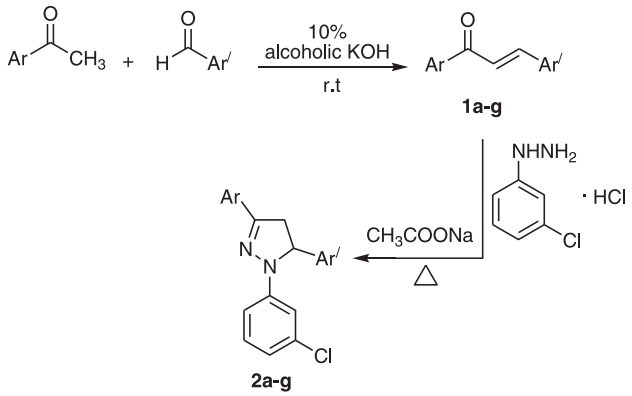
Figure 1. Structures of marketed COX-2 inhibitors and selective COX-2 inhibitor, SC-558.

RESULTS AND DISCUSSION

Chemistry. ***Synthesis of 1,3,5-trisubstituted pyrazoline derivatives 1a–g and 2a–g (Scheme 1).*** Claisen–Schmidt condensation reaction of methylarylketones with different aromatic or heterocyclic aldehydes in the presence of 10% alcoholic potassium hydroxide [12,13] gave the respective yellow-orange colored α,β-unsaturated ketones **1a–g**. Chalcones **1a–g** were condensed with *m*-chlorophenyl hydrazine hydrochloride in absolute ethanol in presence of catalytic amount of anhydrous sodium acetate [14] to give the respective pyrazolines **2a–g** (Scheme 1). The spectral and microanalytical data for compounds **1a–g** and **2a–g** were consistent with their chemical structures.

***Synthesis of pyrano[2,3-c]*pyrazole derivatives 4–10 (*Scheme 2*).** The one-pot reaction of 2-[2-(3-methyl-5-oxo-4,5-dihydro-*1H*-pyrazol-1-yl)-2-oxoethoxy]benzamide (**3**) [15] with different arylidene malononitriles/ piperidine afforded the bifunctional compounds 2-[2-(6-amino-4-(4-substituted-phenyl)-5-cyano-3-methylpyrano[2,3-*c*]pyrazol-1-(4*H*)-yl)-2-oxoethoxy]benzamides **4a–d**. Pyrano [2,3-*c*]pyrazole **4c** was employed as key intermediate for preparation of novel bicyclic and tricyclic derivatives. Treatment of **4c** with hydroxylamine hydrochloride/TEA [16] gave the 5-carboxamidoxime **5**. Also, **4c** was allowed to react with malononitrile in the presence of ethanolic piperidine [16] to give the pyrazolopyranopyridine **6**. On reaction of **4c** with triethylorthoformate, in the presence of acetic anhydride [17], the expected product **7** was not formed, and the product was identified as the corresponding 6-acetamido derivative **8**. Moreover, the reaction of **4c** with either formic acid [18] and/or formamide [17] furnished the pyranopyrazolopyrimidines **9** and **10**, respectively. Structures of target compounds, **4–10** were established on the basis of their elemental and spectral data.

Scheme 1. Synthesis of 1,3,5-trisubstituted pyrazoline derivatives **1a–g.** and **2a–g**.



Ar	Ar/
a 2-thienyl	$C_6H_3(OCH_3)$-2,5
b 2-thienyl	5-(benzo-[d][1,3]dioxol-5-yl)
c 3- indolyl	C_6H_4-Br-4
d 3-indolyl	C_6H_4-Cl-4
e C_6H_4-Cl-4	C_6H_4-Cl-4
f C_6H_4-(OCH_3)-4	C_6H_4-(OCH_3)-4
g 2-pyridyl	$C_6H_4N(CH_3)_2$-4

Molecular modeling and docking studies. All docking studies were performed using "Internal coordinate Mechanic (Molsoft ICM 3.4-8C)".

All compounds were evaluated through molecular modeling and docking techniques using program. ICM docking is probably the most accurate predictive tool of binding geometry today [19].

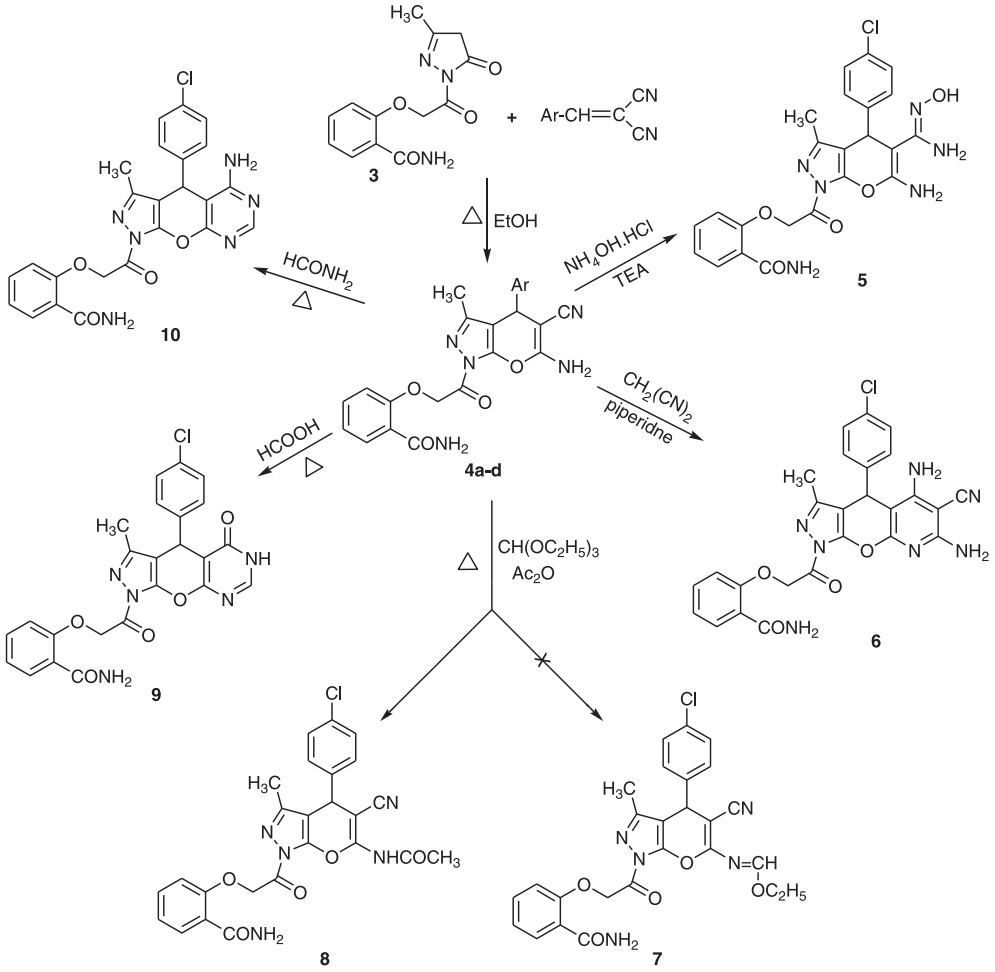
The aim of the flexible docking calculations is the prediction of correct binding geometry for each binder. The goal is to have an adequate 3D model of the receptor pocket we are planning to dock compounds to. Usually, dock the tested compounds to determine ICM scores.

To compare the binding affinity of the new pyrazolines and pyranopyrazoles, we docked compounds **2–10** into the empty binding site of the experimentally known crystal structure of COX-2 (prostaglandin synthase-2), complexed with a selective inhibitor, SC-558: 4-[5-(4-bromophenyl)-3-(trifluoromethyl)pyrazol-1-yl]benzene sulfonamide [1CX2] [11].

As shown in Table 1 and Fig. 2, SC-558 (original ligand) reveals ICM score of (−84.79) and forms three strong H bonds with the hydrophilic pocket of COX-2, one H bond with His 90, one H bond with Gln 192 in addition to the H bond with Arg 513.

1,3,5-Trisubstituted pyrazoline **2a** has a reasonable ICM score (−78.91) and forms one strong H bond (1.76 A) between oxygen of 5-OCH_3 group and His90. In addition, hydrophobic interactions between 3-thienyl and secondary pocket contains Ser 530, Tyr 385, His 386, and Phe 518.

Scheme 2. Synthesis of pyrano[2,3-*c*]pyrazole derivatives **4–10**.



Moreover, hydrophobic interaction between 5-Ph and Ser 353 was observed (Fig. 3), whereas other pyrazolines **2d**, **f**, and **g** bind to the important amino acids in the active site of COX-2, namely, Tyr 385, Trp 387, and Arg 120, respectively.

Pyrazolopyrano[2,3-*d*]pyrimidine **9** forms three strong H bonds with the hydrophilic pocket of COX-2 enzyme, one H bond between ketone-O attached to pyrazole with His 90 (2.27 A) and two H bonds between ketone-O and pyran-O with Arg 513 (1.23, 2.46 A). In addition; such interaction forces the 4-Cl-Ph moiety to adopt a specific orientation at the top of the channel with Ser 353, Ser 530, Tyr 385, His 386, Phe 518, and Trp 387 (Fig. 4). These interactions in compounds **2a** and **9** are responsible for the COX-2 selectivity and contributed to stabilize the ligand–enzyme complexes (Figs 3 and 4).

Pyranopyrazole **4d** has a reasonable ICM score of −85.22 and forms 10 H bonds with active sites of the enzyme: four H bonds with His 386, two H bonds with Trp387 and His388, and another two H bonds with Tyr 385. However, counterparts **4a–c** having lower energy (−101.61 to −102.06) bind to Trp 387 (Table 1).

Pharmacological activity. On the basis of the molecular modeling, compounds **2a**, **2d**, **2f**, **2g**, **4a–d**, **8**, and **9** were subjected for pharmacological screening.

Anti-inflammatory activity (*in vitro* and *in vivo*). All tested compounds and celecoxib, as a reference drug, were subjected to *in vitro* and *in vivo* anti-inflammatory studies using human whole blood assay [20] and carrageenan-induced rat paw edema (RPE) bioassay [21], respectively.

Most of the tested compounds at a dose level of 2.5 and 5 mg/kg showed significant anti-inflammatory activities with prostaglandin inhibition (Table 2). Some general features could be concluded from the pharmacological screening as follows.

In pyrazoline derivatives, the anti-inflammatory activity of the tested compounds using carrageenan-induced paw edema method produced significant reduction of paw size ranged from 88.16–92.27% as compared with that of celecoxib (at dose 2.5 mg/kg). Moreover, compound **2f**

Table 1

Docking of compounds on cyclooxygenase-2.

Compounds	ICM scores	No. of H bonds	Involved group of amino acid	Atom of ligand involved	Length of H bond (Å)
SC-558	−84.79	3	His 90 . . . He 2r	O-1	2.24
			Arg 513 . . . Hh 11	O-1	2.36
			Gln 192 . . . Hn	O-2	1.57
2a	−78.91	1	His 90 . . . He 2	O-1	1.76
2b	−82.22	1	Lys 493 . . . Hz 1	N-2	1.88
2c	−88.07	1	Cys 47 . . .. Hn	N-1	2.77
2d	−88.89	1	Tyr 385 . . . O	H-8	2.46
2e	−75.69	0	—	—	—
2f	−86.38	2	His 207 . . . He 2	N-1	2.75
			Trp 387 . . . Hn	O-3	2.22
2g	−80.07	1	Arg 120 . . . Hh 11	N-3	2.35
4a	−102.06	4	Asn 382 . . . Hd 21	N-5	2.31
			Trp 387 . . . Hn	O-3	2.77
			Asn 382 . . . Od 1	H-2	2.63
			Asn 382 . . . O	H-2	2.76
4b	−102.06	4	Asn 382 . . . Hd 21	N-5	2.31
			Trp 387 . . . Hn	O-3	2.77
			Asn 382 . . . Od 1	H-2	2.63
			Asn 382 . . . O	H-2	2.76
4c	−101.61	1	Trp 387 . . . Hn	O-3	2.66
4d	−85.22	10	Asn 382 . . . Hd 21	O-4	1.60
			His 386 . . . Hd 1	O-3	2.06
			His 386 . . . Hd 1	O-1	2.43
			His 386 . . . He 2	O-2	2.54
			His 386 . . . He 2	O-4	2.69
			Trp 387 . . . Hn	O-5	2.52
			His 388 . . . Hn	O-5	2.49
			Thr 212 . . . Hg 1	O-4	2.74
			Tyr 385 . . . O	H-2	1.09
			Tyr 385 . . . O	H-3	1.13
5	−110.40	2	Cys47 . . . Hn	N-1	2.38
			Cys 41 . . . O	H-7	1.84
6	−106.98	1	Asp 125 . . . Od 2	H-11	1.76
8	−98.48	3	His 207 . . . He 2	O-3	2.56
			His 207 . . . He 2	O-1	2.08
			His 207 . . . He 2	O-2	2.58
9	−90.17	3	His 90 . . . He 2	O-2	2.27
			Arg 513 . . . Hh 12	O-2	1.23
			Arg 513 . . . Hh 12	O-1	2.46
10	−104.21	2	Cys 47 . . . Hn	N-1	2.38
			Gly 135 . . . O	H-12	2.46

displayed potent anti-inflammatory activity. This may be due to the presence of electron-releasing groups at position 3 (4-methoxyphenyl) and position 5 (3,5-dimethoxyphenyl) of pyrazoline nucleus.

In pyranopyrazole derivatives, **4d** (RPEI% = 94.33), bearing 2,5-dimethoxyphenyl group at position 4, is the most potent anti-inflammatory than the other analogs **4a–c** (RPEI% = 70.15–78.18), having 4-Cl-/4-Br-phenyl group. Hence, it seems essential for activity to have an electron-releasing group at position 4 of pyranopyrazole nucleus.

In pyranopyrazole derivatives, it was found that pyranopyrazole **4d** showed good prostaglandin inhibitory activity (PGEI% = 73.17) compared with that of celecoxib (PGEI% = 92.88) at dose 2.5 mg/kg.

Ulcerogenic activity. All tested compounds and celecoxib as a reference drug were studied for ulcerogenic activity [22]. The animals tolerated the tested compounds quite well, and no mortalities have been recorded among them. The ulcer indices of all tested compounds are mainly in the safe level (UI = 2.11–4.26) (Table 3).

EXPERIMENTAL

Melting points were measured in open capillary tubes using Griffin apparatus (UK) and were uncorrected. The IR spectra were recorded using potassium bromide disk technique on Schimadzu 435 IR spectrophotometer (Tokyo, Japan) at Microanalytical Unit, National Research Centre. The proton nuclear magnetic resonance (^{1}H NMR) spectra were performed on Varian Gemini 300 MHz spectrophotometer (PoloAlto, Ca, USA) using

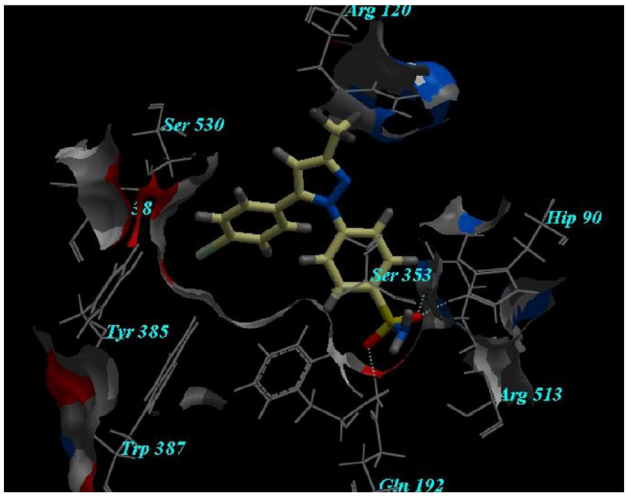


Figure 2. The proposed binding mode of ligand SC-558 inside the active site COX-2. Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.

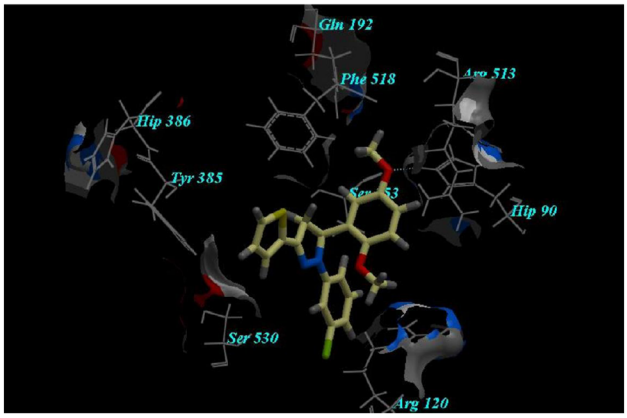


Figure 3. The proposed binding mode of **2a** inside the active site COX-2. Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.

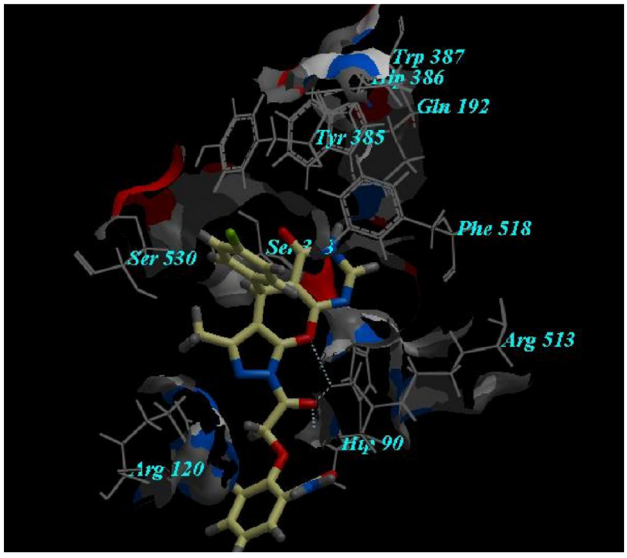


Figure 4. The proposed binding mode of **9** inside the active site COX-2. Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.

Table 2

Anti-inflammatory activities (*in vitro* and *in vivo*) of the tested compounds and reference standard at doses 2.5 and 5 mg/kg after 4 h.

Compound no.	*In vitro* %inhibition of plasma PGE		*In vivo* %inhibition of RPE	
	2.5 mg/kg	5 mg/kg	2.5 mg/kg	5 mg/kg
2a	70.18	89.12	90.44	98.56
2d	71.02	90.00	91.41	98.89
2f	71.15	90.08	92.27	99.00
2g	68.77	87.93	88.16	97.77
4a	61.23	80.50	77.12	88.88
4b	58.23	77.34	70.15	75.23
4c	63.22	81.98	78.18	91.19
4d	73.17	91.14	94.33	99.94
8	69.15	88.99	88.14	98.16
9	64.13	82.24	80.80	92.90
Celecoxib	92.88	97.55	96.44	99.93

tetramethylsilane (TMS) as internal standard. Chemical shift values (δ) are given using parts per million scale (ppm) at Microanalytical Unit, National Research Centre. Mass spectra were recorded on Hewlett Packard 5988 spectrometer (California, USA) at Microanalytical Unit, National Research Centre. Elemental microanalyses were carried out at Microanalytical unit, National Research Centre. All reactions were monitored by TLC using precoated aluminum sheet silica gel Merck 60F254 and were visualized by UV lamp. Chemical naming, calculation of molecular weight (MW), and microanalyses of new compounds were performed by ChemDraw program, whereas chemical naming of compounds **4–10** was performed by ChemSketch program. (E)-3-(benzo[*d*][1,3]dioxol-5-yl)-1-(thiophen-2-yl)prop-2-en-1-one (**1b**) [23], (E)-3-(4-bromophenyl)-1-(*1H*-indol-3-yl) prop-2-en-1-one (**1c**) [24], (E)-3-(4-chlorophenyl)-1-(*1H*-indol-3-yl)prop-2-en-1-one (**1d**) [25], (E)-1,3-bis(4-chlorophenyl) prop-2-en-1-one (**1e**) [26], (E)-3-(4-(dimethylamino)phenyl)-1-(pyridin-2-yl)prop-2-en-1-one (**1g**) [27], and 2-[2-(3-methyl-5-oxo-4,5-dihydro-*1H*-pyrazol-1-yl)-2-oxoethoxy]benzamide (**3**) [15] were prepared following the procedures reported in the literature.

General method for the synthesis of α,β-unsaturated ketones 1a–g. To a solution of the proper methyl aryl ketone, namely, 2-acetyl thiophene and/or *p*-methoxy acetophenone (0.01 mol) in ethanol (50 mL), the appropriate aromatic aldehydes, namely, 2,5- and 3,5-dimethoxy benzaldehyde (0.01 mol) were added in the presence of 10% alcoholic potassium hydroxide (5 mL). The reaction mixture was stirred for 10–12 h at room temperature and stand overnight at the same temperature. The solid obtained was filtered, washed with water, air dried, and crystallized from the ethanol.

(E)-3-(2,5-Dimethoxyphenyl)-1-thien-2-yl)prop-2-en-1-one (1a). Yield, 89%; mp 80–85°C; IR (KBr) ν_{max}/cm^{-1}: 3088 (CHAr), 1638 (C═O), 1573 (C═C). ^{1}H NMR (DMSO-d_6) (δ, ppm): 3.70 and 3.75 (2s, 6H, 2 OCH_3), 6.32–8.17 (m, 6H, Ar-H and thiophen protons), 7.71and 7.92 (dd, 2H, ethylenic protons). MS *m/z* (%): 274 (15.5, $M^{+\cdot}$), 243 (82), 137 (6), 111 (100), 107 (4), 83 (27), 77 (39). *Anal*. Calcd For $C_{15}H_{14}O_3S$ (%): C, 65.67; H, 5.14. Found: C, 65.8; H, 5.12.

Table 3

Ulcerogenic activity of the tested compounds and reference standard at dose 5 mg/kg.

Compound no.	Average no. of ulcers $X \pm SE$	Average no. of severity of ulcers $Y \pm SE$	% of incidence of ulcer divided by 10	Ulcer index (UI)
2a	2.10 ± 0.29	1.33 ± 0.17	5.60	4.10
2d	1.913 ± 0.26	1.23 ± 0.13	5.10	3.70
2f	1.414 ± 0.19	1.00 ± 0.06	3.90	2.90
2g	1.71 ± 0.22	1.17 ± 0.15	4.44	3.12
4a	1.10 ± 0.061	0.71 ± 0.021	2.90	2.11
4b	2.88 ± 0.34	2.01 ± 0.23	5.93	4.26
4c	2.56 ± 0.322	1.81 ± 0.22	5.91	4.22
4d	1.64 ± 0.21	1.10 ± 0.11	4.3	3.00
8	1.66 ± 0.20	1.12 ± 0.12	4.35	3.05
9	1.12 ± 0.073	0.81 ± 0.029	3.01	2.21
Celecoxib	1.88 ± 0.20	1.33 ± 0.18	4.05	3.00

(E)-3-(3,5-Dimethoxyphenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (1f). Yield, 87%; mp 100–104°C; IR (KBr) ν_{max}/cm^{-1} 3076 (CHAr), 1659 (C═O), 1599 (C═C). ^{1}H NMR (DMSO-d_6) (δ, ppm): 3.84 (s, 6H, 2 m.OCH_3), 3.87 (s, 3H, p.OCH_3), 6.58–8.17 (m, 7H, Ar-H), 7.64, 7.87 (dd, 2H, ethylenic protons). MS *m/z* (%):298 (100.0, $M^{+\cdot}$), 267 (60), 163 (8), 135 (75), 107 (16). *Anal*. Calcd For $C_{18}H_{18}O_4$ (%): C, 72.47; H, 6.08. Found: C, 72.18; H, 6.10.

1-(3-Chlorophenyl)-3,5-diaryl-2-pyrazoline derivatives 2a–g

General method. To a solution of the proper chalcones **1a–g** (0.001 mol) in absolute ethanol (15 mL), *m*-chlorophenyl hydrazine hydrochloride (0.001 mol, 0.18 g) and anhydrous sodium acetate (0.001 mol, 0.08 g) in absolute ethanol (15 mL) were added. The reaction mixture was refluxed for 6–8 h. After cooling, the solid obtained was filtered off, washed with ethanol, air dried, and crystallized from ethanol.

1-(3-Chlorophenyl)-4,5-dihydro-5-(2,5-dimethoxyphenyl)-3-(thiophen-2-yl)-1H-pyrazole (2a). Yield, 70%; mp 140–142°C; IR (KBr) ν_{max}/cm^{-1} 3072 (CHAr), 1659 (C═N), 1592 (C═C). ^{1}H NMR (DMSO-d_6) (δ, ppm): 3.13 (dd, 1H, CH_A), 3.61 and 3.87 (2s, 6H, 2 OCH_3), 3.93 (dd, 1H, CH_B), 5.60 (dd, 1H, CH_x), 6.56–7.61 (m, 10H, Ar-H and thiophene protons). MS *m/z* (%); 400 (17.3, $M^{+\cdot}+2$), 398 (100.0, $M^{+\cdot}$), 369 (4), 367 (9), 338 (0.1), 336 (0.3), 287 (0.2), 263 (7), 261 (21), 113 (2), 111 (6). *Anal*. Calcd For $C_{21}H_{19}ClN_2O_2S$ (%): C, 63.23; H, 4.80; N, 7.02. Found: C, 63.48; H, 4.82; N, 7.04.

5-(Benzo[d][1,3]dioxol-5-yl)-1-(3-chlorophenyl)-4,5-dihydro-3-(thiophen-2-yl)-1H-pyrazole (2b). Yield, 87%; mp 100–102°C; IR (KBr) ν_{max}/cm^{-1}: 3030 (CHAr), 1667 (C═N), 1593 (C═C). ^{1}H NMR (DMSO-d_6) (δ, ppm): 3.19 (dd, 1H, CH_A), 3.93 (dd, 1H, CH_B), 5.44 (dd, 1H, CH_x), 5.97 (s, 2H, CH_2 of piperonal), 6.71–7.63 (m, 10H, Ar-H and thiophene protons). MS *m/z* (%); 384 (42.7, $M^{+\cdot}+2$), 382 (83.5, $M^{+\cdot}$), 271 (3.1), 263 (22.0), 261 (35.1), 113 (21.2), 111 (79.4), 90 (100.0). *Anal*. Calcd For $C_{20}H_{15}ClN_2O_2S$ (%): C, 62.74; H, 3.95; N, 7.32. Found: C, 62.99; H, 3.93; N, 7.29.

3-(5-(4-Bromophenyl)-1-(3-chlorophenyl)-4,5-dihydro-1H-pyrazole-3-yl)-1H-indole (2c). Yield, 67%; mp 137–139°C; IR (KBr) ν_{max}/cm^{-1} 3407 (NH), 3060 (CHAr), 1659 (C═N), 1591 (C═C). ^{1}H NMR (DMSO-d_6) (δ, ppm): 3.15 (dd, 1H, CH_A), 3.95 (dd, 1H, CH_B), 5.40 (dd, 1H, CH_x), 6.67–8.25 (m, 13H, Ar-H and indole protons), 11.40 (s, 1H, NH exchangeable with D_2O). MS *m/z* (%); 455 (0.3, $M^{+\cdot}+6$), 453 (10.2, $M^{+\cdot}+4$), 451 (57.5, $M^{+\cdot}+2$), 449 (100.0, $M^{+\cdot}$), 372 (0.5), 370 (2.1), 296 (2.7), 294 (9.5), 116 (1.7), 113 (2.6), 111 (7.4). *Anal*. Calcd For $C_{23}H_{17}BrClN_3$ (%): C, 61.28; H, 3.80; N, 9.32. Found: C, 61.53; H, 3.78; N, 9.35.

3-(1-(3-Chlorophenyl)-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-1H-indole (2d). Yield, 68%; mp 160–162°C; IR (KBr) ν_{max}/cm^{-1} 3437 (NH), 3068 (CHAr), 1652 (C═N), 1588.8 (C═C). ^{1}H NMR (DMSO-d_6) (δ, ppm): 3.15 (dd, 1H, CH_A), 3.94 (dd, 1H, CH_B), 5.45 (dd, 1H, CH_x), 6.65–8.30 (m, 13H, Ar-H and indole protons), 11.55 (s, 1H, NH exchangeable with D_2O). MS *m/z* (%); 409 (12.1, $M^{+\cdot}+4$), 407 (71.5, $M^{+\cdot}+2$), 405 (100.0, $M^{+\cdot}$), 372 (0.5), 370 (1.3), 373 (0.2), 371 (0.7), 336 (0.4), 296 (5.0), 294 (15.1), 183 (1.3), 116 (3.4). *Anal*. Calcd For $C_{23}H_{17}Cl_2N_3$ (%): C, 67.99; H, 4.22; N, 10.34. Found: C, 68.26; H, 4.23; N, 10.31.

1-(3-Chlorophenyl)-3,5-bis(4-chlorophenyl)-4,5-dihydro-1H-pyrazole (2e). Yield, 73%; mp 120–122°C; IR (KBr) ν_{max}/cm^{-1}: 3076 (CHAr), 1682 (C═N), 1592 (C═C). ^{1}H NMR (DMSO-d_6) (δ, ppm): 3.14 (dd, 1H, CH_A), 3.90 (dd, 1H, CH_B), 5.57 (dd, 1H, CH_x), 6.75–7.95 (m, 12H, Ar-H). MS *m/z* (%); 404 (11.7, $M^{+\cdot}+4$), 402 (41.9, $M^{+\cdot}+2$), 400 (72.6, $M^{+\cdot}$), 293 (2.4), 291 (11.5), 289 (17.6), 113 (33.8), 111 (90.2), 75 (100.0). *Anal*. Calcd For $C_{21}H_{15}Cl_3N_2$ (%): C, 62.79; H, 3.76; N, 6.97. Found: C, 62.54; H, 3.77; N, 6.99.

1-(3-Chlorophenyl)-4,5-dihydro-5-(3,5-dimethoxyphenyl)-3-(4-methoxyphenyl)-1H-pyrazole (2f). Yield, 69%; mp 105–107°C; IR (KBr) ν_{max}/cm^{-1} 3045 (CHAr), 1682 (C═N), 1592 (C═C). ^{1}H NMR (DMSO-d_6) (δ, ppm): 3.10 (dd, 1H, CH_A), 3.65 (s, 6H, 2 m.OCH_3), 3.76 (s, 3H, p.OCH_3), 3.90 (dd, 1H, CH_B), 5.35 (dd, 1H, CH_x), 6.40–7.70 (m, 11H, Ar-H). MS *m/z* (%); 424 (37.6, $M^{+\cdot}+2$), 422 (100.0, $M^{+\cdot}$), 287 (23.6), 285 (65.7), 137 (3.3), 113 (14.5), 111 (43.3), 107 (6.6). *Anal*. Calcd For $C_{24}H_{23}ClN_2O_3$ (%): C, 68.16; H, 5.48; N, 6.62. Found: C, 68.43; H, 5.45; N, 6.64.

4-(1-(3-Chlorophenyl)-4,5-dihydro-3-pyrid-2-yl)-1H-pyrazol-5-yl)-N,N-dimethylbenzenamine (2g). Yield, 84%; mp 152–154°C; IR (KBr) ν_{max}/cm^{-1} 3076 (CHAr), 1679 (C═N), 1594 (C═C). ^{1}H NMR (DMSO-d_6) (δ, ppm) 2.85 (s, 6H, $N(CH_3)_2$), 3.15 (dd, 1H, CH_A), 3.90 (dd, 1H, CH_B), 5.46 (dd, 1H, CH_x), 6.65–8.57 (m, 12H, Ar-H and pyridine protons). MS *m/z* (%); 378 (36.2, $M^{+\cdot}+2$), 376 (90.6, $M^{+\cdot}$), 258 (30.1), 256 (79.7), 259 (10.3), 257 (33.0), 145 (10.0), 146 (100.0), 113 (16.1), 111 (57.4). *Anal*. Calcd For $C_{22}H_{21}ClN_4$ (%): C, 70.11; H, 5.62; N, 14.87. Found: C, 69.83; H, 5.64; N, 14.91.

2-[2-(6-Amino-4-(substitutedphenyl)-5-cyano-3-methylpyrano[2,3-c]pyrazol-1-(4H)-yl)-2-oxoethoxy]benzamides (4a–d)

General method. To a mixture of compound **3** (0.01 mol, 2.75 g) in absolute ethanol (25 mL), containing piperidine (0.3 mL), the proper arylidine malononitrile (0.01 mol) was added. The reaction mixture was refluxed for 6 h and then concentrated, cooled, and the solid formed was filtered off, washed with cold diethyl ether and then recrystallized from dimethyl formamide.

2-[2-(6-Amino-4-(2-bromophenyl))-5-cyano-3-methylpyrano[2,3-c]pyrazol-1(4H)-yl)-2-oxoethoxy]benzamide (4a). Yield, 55%; mp 230–233°C; IR (KBr) ν_{max}/cm^{-1} 3390, 3308 (2 NH_2), 2190 (C≡N), 1739 (CO-CH_2), 1654 (CO-NH_2), 1611 (C=C). ^{1}H NMR (DMSO-d_6) (δ, ppm): 2.94 (s, 3H, CH_3), 5.05 (s, 1H, CH-pyran), 5.69 (s, 2H, OCH_2), 6.95 (s, 2H, NH_2 exchangeable with D_2O), 7.14–7.93 (m, 8H, Ar-H), 12.14 (s, 2H, NH_2 exchangeable with D_2O). *Anal.* Calcd For $C_{23}H_{18}BrN_5O_4$ (%): C, 54.34; H, 3.57; N, 13.78. Found: C, 54.45; H, 3.58; N, 13.75.

2-[2-(6-Amino-4-(4-bromophenyl)-5-cyano-3-methylpyrano[2,3-c]pyrazol-1(4H)-yl)-2-oxoethoxy]benzamide (4b). Yield, 51%; mp 240–243°C; IR (KBr) ν_{max}/cm^{-1} 3416, 3292 (2 NH_2), 2237 (C≡N), 1731 (CO-CH_2), 1673 (CO-NH_2), 1597 (C=C). MS *m/z* (%); 509 (2.7, $M^{+\cdot}+2$), 414 (6.7), 296 (2.3), 219 (5.1), 203 (4.7), 121 (11.2), 120 (8.7), 81 (4.9), 79 (5.0), 80 (13.9), 62 (100.0). *Anal.* Calcd For $C_{23}H_{18}BrN_5O_4$ (%): C, 54.34; H, 3.57; N, 13.78. Found: C, 54.18; H, 3.58; N, 13.82.

2-[2-(6-Amino-4-(4-chlorophenyl)-5-cyano-3-methylpyrano[2,3-c]pyrazol-1(4H)-yl)-2-oxoethoxy]benzamide (4c). Yield, 62%; mp 226–228°C; IR (KBr) ν_{max}/cm^{-1} 3416, 3378 (2 NH_2), 2237 (C≡N), 1733 (CO-CH_2), 1674 (CO-NH_2), 1597 (C=C). ^{1}H NMR (DMSO-d_6) (δ, ppm): 2.84 (s, 3H, CH_3), 4.03 (s, 2H, NH_2 exchangeable with D_2O), 4.24 (s, 1H, CH-pyran), 5.63 (s, 2H, OCH_2), 7.05–7.63 (m, 8H, Ar-H), 7.86 (s, 2H, NH_2 exchangeable with D_2O). MS *m/z* (%); 465 (0.1, $M^{+\cdot}+2$), 463 (0.3, $M^{+\cdot}$), 353 (7.9), 352 (0.6), 175 (0.9), 121 (3.7), 96 (0.5), 80 (5.9), 62 (100.0). *Anal.* Calcd For $C_{23}H_{18}ClN_5O_4$ (%): C, 59.55; H, 3.91; N, 15.10. Found: C, 59.78; H, 3.92; N, 15.14.

2-[2-(6-Amino-5-cyano-4-(2,5-dimethoxyphenyl)-3-methylpyrano[2,3-c]pyrazol-1(4H)-yl)-2-oxoethoxy]benzamide (4d). Yield, 57%; mp 190–192°C; IR (KBr) ν_{max}/cm^{-1} 3433, 3325 (2 NH_2), 2210 (C≡N), 1739 (CO-CH_2), 1641 (CO-NH_2), 1591 (C=C). ^{1}H NMR (DMSO-d_6) (δ, ppm): 3.30 (s, 3H, CH_3), 3.68, 3.72 (2s, 6H, two OCH_3), 5.02 (s, 3H, CH-pyran, OCH_2), 6.86–7.93 (m, 7H, Ar-H), 7.52 (s, 2H, NH_2 exchangeable with D_2O), 8.78 (s, 2H, NH_2 exchangeable with D_2O). MS *m/z* (%): 489 (0.7, $M^{+\cdot}$), 354 (1.3), 353 (2.9), 352 (1.3), 137 (2.7), 125 (100.0), 124 (1.5). *Anal.* Calcd For $C_{25}H_{23}N_5O_6$ (%): C, 61.34; H, 4.74; N, 14.13. Found: C, 61.16; H, 4.75; N, 14.28.

2-[2-[6-Amino-4-(4-chlorophenyl)-5-(hydroxycarbamimidoyl)-3-methylpyrano-[2,3-c]pyrazol-1(4H)-yl)-2-oxoethoxy]benzamide (5). A mixture of compound **4c** (0.01 mol, 4.6 g) and hydroxylamine hydrochloride (0.01 mol, 0.6 g) in absolute ethanol (30 mL) containing few drops triethylamine was warmed until a clear solution was obtained and then left overnight; the solid formed was filtered off, dried, and crystallized from ethanol. Yield, 69%; mp 200–203°C; IR (KBr) ν_{max}/cm^{-1} 3412 (OH), 3322, 3184 (two NH_2), 1731 (CO-CH_2), 1650 (CO-NH_2), 1586 (C=C). ^{1}H NMR (DMSO-d_6) (δ, ppm): 2.82 (s, 3H, CH_3), 5.01 (s, 1H, CH-pyran), 5.62 (s, 2H, OCH_2), 7.07–8.24 (m, 8H, Ar-H), 8.31, 10.03, 10.23 (3s, 6H, 3 NH_2 exchangeable with D_2O), 11.00 (s, 1H, OH exchangeable with D_2O). MS *m/z* (%); 498 (0.04, $M^{+\cdot}+2$), 496 (0.1, $M^{+\cdot}$), 385 (1.0), 209 (1.1), 194 (2.1), 193 (2.0), 178 (2.4), 136 (100.0). *Anal.* Calcd For $C_{23}H_{21}ClN_6O_5$ (%): C, 55.59; H, 4.26; N, 16.91. Found: C, 55.70; H, 4.27; N, 16.84.

2-[2-(5,7-Diamino-4-(4-chlorophenyl)-6-cyano-3-methylpyrazolo[4′,3′:5,6]pyrano[2,3-b]pyridin-1(4H)-yl)-2-oxoethoxy]benzamide (6). To a solution of compound **4c** (0.01 mol, 4.6 g) in absolute ethanol (20 mL), malononitrile (0.01 mol, 0.66 g) and piperidine (0.3 mL) were added. The reaction mixture was refluxed for 3 h, concentrated, and cooled. The solid formed was filtered off, dried, and crystallized from ethanol. Yield, 73%; mp > 300°C; IR (KBr) ν_{max}/cm^{-1} 3464, 3355 and 3160 (3 NH_2), 2212 (C≡N), 1723 (CO-CH_2), 1648 (CO-NH_2), 1590 (C=C). ^{1}H NMR (DMSO-d_6) (δ, ppm): 2.83 (s, 2H, NH_2 exchangeable with D_2O), 2.85 (s, 3H, CH_3), 5.51 (s, 3H, CH-pyran and CH_2O), 5.59 (s, 2H, NH_2 exchangeable with D_2O), 7.11–7.77 (m, 8H, Ar-H), 8.01 (s, 2H, NH_2 exchangeable with D_2O). *Anal.* Calcd For $C_{26}H_{20}ClN_7O_4$ (%): C, 58.93; H, 3.80; N, 18.50. Found: C, 59.11; H, 3.79; N, 18.57.

2-[2-(6-Acetamido-4-(4-chlorophenyl)-5-cyano-3-methylpyrano[2,3-c]pyrazol-1(4H)-yl)-2-oxoethoxy]benzamide (8). A mixture of **4c** (0.007 mol, 3.2 g), triethylorthoformate (15 mL), and acetic anhydride (5 mL) was heated under reflux for 4 h. After cooling, the solid formed was filtered off and washed with water and crystallized from ethanol. Yield, 54%; mp 155–158°C; IR (KBr) ν_{max}/cm^{-1} 3418, 3334 (NH_2 and NH), 2246 (C≡N), 1729 (CO-CH_2), 1674 ($COCH_3$), 1663 (CO-NH_2), 1596 (C=C). ^{1}H NMR (DMSO-d_6) (δ, ppm): 2.29 (s, 3H, $COCH_3$), 2.88 (s, 3H, CH_3), 5.37 (s, 1H, CH-pyran), 5.71 (s, 2H, OCH_2), 6.97–8.13 (m, 8H, Ar-H), 8.49 (s, 2H, NH_2 exchangeable with D_2O), 9.20 (s, 1H, NH exchangeable with D_2O). *Anal.* Calcd For $C_{25}H_{20}ClN_5O_5$ (%): C, 59.35; H, 3.98; N, 13.84. Found: C, 59.46; H, 3.96; N, 13.88.

2-[2-(4-(4-Chlorophenyl)-3-methyl-5-oxo-5,6-dihydropyrazolo-[4′,3′:5,6]pyrano-[2,3-d]pyrimidin-1(4H)-yl)-2-oxoethoxy]benzamide (9). A solution of compound **4c** (0.005 mol, 2.3 g), in formic acid (10 mL), was refluxed for 4 h. The solid formed was filtered off, dried, and crystallized from ethanol. Yield, 55%; mp 207–210°C; IR (KBr) ν_{max}/cm^{-1} 3394, 3206 (NH_2, NH), 1713 (CO-CH_2), 1650 (CO-NH_2), 1603 (C=C). ^{1}H NMR (DMSO-d_6) (δ, ppm): 2.88 (s, 3H, CH_3), 4.75 (s, 1H, CH-pyran), 5.65 (s, 2H, OCH_2), 7.66 (s, 1H, CH-pyrimidine), 7.06–7.92 (m, 8H, Ar-H), 8.10 (s, 2H, NH_2 exchangeable with D_2O), 13.40 (br.s, 1H, NH exchangeable with D_2O). *Anal.* Calcd For $C_{24}H_{18}ClN_5O_5$ (%): C, 58.60; H, 3.69; N, 14.24. Found: C, 58.83; H, 3.70; N, 14.18.

2-[2-(5-Amino-4-(4-chlorophenyl)-3-methylpyrazolo[4′,3′:5,6]pyrano[2,3-d] pyrimidin-1(4H)-yl)-2-oxoethoxy]benzamide (10). A solution of **4c** (0.005 mol, 2.3 g) in formamide (20 mL) was refluxed for 6 h. The reaction mixture was cooled, diluted with water, and the resulting precipitate was collected by filtration and crystallized from ethanol. Yield, 57%; mp 245–247°C; IR (KBr) ν_{max}/cm^{-1} 3393, 3318 (2 NH_2), 1728 (CO-CH_2), 1646 (CO-NH_2), 1602 (C=C). ^{1}H NMR (DMSO-d_6) (δ, ppm): 2.87 (s, 3H, CH_3), 5.02 (s, 1H, CH-pyrane), 5.63 (s, 2H, OCH_2), 7.34–8.09 (m, 9H, Ar-H and CH-pyrimidine), 9.92, 10.58 (2s, 4H, 2 NH_2 exchangeable with D_2O). *Anal.* Calcd For $C_{24}H_{19}ClN_6O_4$ (%): C, 58.72; H, 3.90; N, 17.12. Found: C, 58.49; H, 3.91; N, 17.19.

Molecular modeling and docking studies. 1CX2 and 4COX [19] were downloaded through the Protein Data Bank PDB/ RCSB site and saved as *.pdb file. A set of synthesized pyrazoline and pyranopyrazole derivatives to inhibit COX-2 were compiled earlier. Molecular modeling of the target compounds was built using ChemDraw Ultra version 9.0.3 and minimized their energy through Chem3D Ultra version 9.0.3/MOPAC, Jop

Type: Minimum RMS Gradient of 0.010 kcal/mol and RMS distance of 0.1 Å and saved as MDL MolFile (*.mol). Docking procedure was followed using the standard protocol implemented in Molsoft ICM 3.4-8C program (Table 1).

PHARMACOLOGY

Anti-inflammatory activity. ***Human whole blood assay.*** Heparinized blood samples were collected from the treated rats ($n = 8$), and then, plasma were separated by centrifugation of these samples at 12000 g for 2 min at 4°C and immediately stored frozen at 20°C until use, where PG was estimated by kits of immuno assay. The design's correlate-EIA prostaglandin E_2 (PGE_2) kit is a competitive immuno assay for the quantitative determination of PGE_2 in biological fluids. The kit uses a monoclonal antibody to PGE_2 to bind, in a competitive manner, the PGE_2 in the sample. After a simultaneous incubation at room temperature, the excess reagents were washed away, and the substrate was added. After a short incubation time, the enzyme reaction was stopped, and the yellow color generated was read on a microplate reader (DYNATCH, MR 5000) at 405 nm. The intensity of the bound yellow color is inversely proportional to the concentration of PGE_2 in either standard or samples (Table 2).

Carrageenan-induced rat paw edema bioassay. The inhibitory activity of the studied compounds on carrageenan-induced rat's paw edema was carried out according to the method of Winter *et al*. Groups of adult male albino rats (150–180 g), each of eight animals were orally dosed with the test compounds at a dose level of 2.5 and 5 mg/kg 1 h before carrageenan challenge. Foot paw edema was induced by subplantar (injection was performed subcutaneously on the foot paw) injection of 0.05 mL of 1% suspension of carrageenan in saline into the plantar tissue of one hind paw. An equal volume of saline was injected to the other hand paw and served as control. Four hours after drug administration, the animals were decapitated; blood was collected, and the paws were rapidly excised. The average weight of edema was estimated for the treated as well as the control group, and the percentage inhibition of weight of edema was also evaluated. Celecoxib (2.5 and 5 mg/kg) was employed as the standard reference (Table 2).

Ulcerogenic activity. Groups of 10 male Wistar rats with weight between 150–175 g are used. They are starved 48 h prior to drug administration. The test compounds are administered orally in 10 mL/kg as aqueous suspension. Doses are chosen that are highly active in the activity (5 mg/kg) and used. The animals are sacrificed after 7 h. Stomachs are removed and placed on saline soaked filter paper until inspection. A longitudinal incision along the greater curvature is made with fine scissor. The stomach is inverted over the index finger and the presence or the absence of gastric irritation is determined. The presence of a single or multiple lesions (erosion, ulcer, or perforation) is considered to be positive. The number of ulcers and the occurrence of hyperemia are noted to determine the ulcer index (Table 3).

CONCLUSION

From the previous results, it seems that ICM score of the original ligand is lower than the ICM score of pyranopyrazolopyrimidine **9**, which means that it has higher affinity for binding with the active site of COX-2 enzyme. In addition, the original ligand, SC-558, forms three H bonds with Arg 513, Gln 192, and His 90, whereas pyrazoline **2a** binds with His 90 and pyranopyrazolopyrimidine **9** binds with three H bonds: one with His 90 and two with Arg 513 in the active site of COX-2 enzyme. Therefore, these two compounds are promising as selective COX-2 inhibitors.

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