Regular Article

Synthesis of Hydroxybenzofuranyl-pyrazolyl and Hydroxyphenyl-pyrazolyl Chalcones and Their Corresponding Pyrazoline Derivatives as COX Inhibitors, Anti-inflammatory and Gastroprotective Agents

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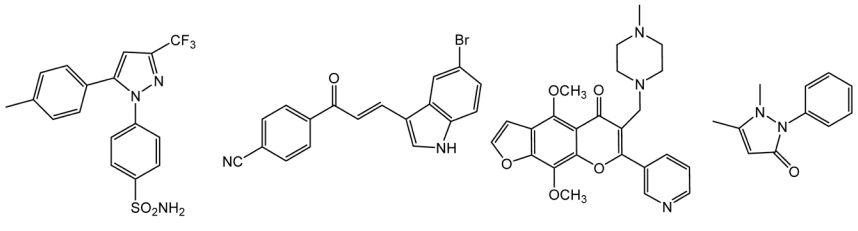
Five new series of hydroxybenzofuranyl-pyrazolyl chalcones 3a,b, hydroxyphenyl-pyrazolyl chalcones 6a–c and their corresponding pyrazolylpyrazolines 4a, d, 7a–c and 8a–f have been synthesized and evaluated for their *in vitro* cyclooxygenase (COX)-1 and COX-2 inhibitory activity. All the synthesized compounds exhibited dual COX-1 and COX-2 inhibitory activity with obvious selectivity against COX-2. The pyrazolylpyrazolines 4a–d and 8a–f bearing two vicinal aryl moieties in the pyrazoline nucleus showed more selectivity towards COX-2. Within these two series, derivatives 4c, d and 8d–f bearing the benzenesulfonamide group were the most selective. Compounds 4a–d and 8a–f were further subjected to *in vivo* anti-inflammatory screening, ulcerogenic liability and showed good anti-inflammatory activity with no ulcerogenic effect. In addition compounds 4c and 8d as examples showed prostaglandin (PG)E_2 inhibition % 44.23 and 51.4 respectively, tumor necrosis factor *α* (TNF*α*) inhibition % 33.48 and 41.41 respectively and gastroprotective effect in ethanol induced rodent gastric ulcer model. In addition, to explore the binding mode and selectivity of our compounds, 8d and celecoxib were docked into the active site of COX-1 and COX-2. It was found that compound 8d exhibited a binding pattern and interactions similar to that of celecoxib with COX-2 active site, while bitter manner of interaction than celecoxib to COX-1 active site.

Key words chalcone; pyrazolylpyrazoline; synthesis; anti-inflammatory activity; structure–activity relationship; molecular docking

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) comprise a major drug class due to their therapeutic use that ranges from the treatment of fever and mild pain up to severe chronic inflammatory disorder.[1–3] The clinical efficacy of most NSAIDs is due to their ability to inhibit cyclooxygenases (COXs) enzymes that responsible for catalyzing formation of prostanoids consisting of prostaglandins (mediators of inflammatory and anaphylactic reactions), thromboxanes (mediators of vasoconstrictions and stimulus for platelet aggregations) and the prostacyclin which is a vasodilator and antithrombotic agent.[4,5] COXs exist in 3 distinct forms COX-1, COX-2 and COX-3.[6] COX-1 is constitutive plays a physiological role and produced in most tissues and is important for protection of gastric mucosa, platelet aggregation and renal blood flow.[7] COX-2 is inducible in inflammation in response to pro inflammatory stimuli.[8,9] COX-3 is located in central nervous system which is selectively inhibited by acetaminophen and other antipyretic NSAIDs.[10] Gastrointestinal erosions and bleeding are the most common side effect of NSAIDs due to the high COX-1 *versus* COX-2 selectivity.[11,12] On the other hand, the altered balance between prostacyclin and thromboxane due to selective inhibition of COX-2 without inhibition COX-1 could promote a prothrombatic status and explain the observed increase in cardiovascular risk.[13,14] Consequently, the development of new anti-inflammatory drugs is still a strong clinical need, especially after the withdrawal of some selective COX-2 inhibitors as rofecoxib and valdecoxib and only celecoxib is the only coxib that is still approved by U.S. Food and Drug Administration (FDA).[15–17] Although celecoxib is the least COX-2 specific of all coxibs and shows a



Celecoxib **I** **II** **Phenazone**

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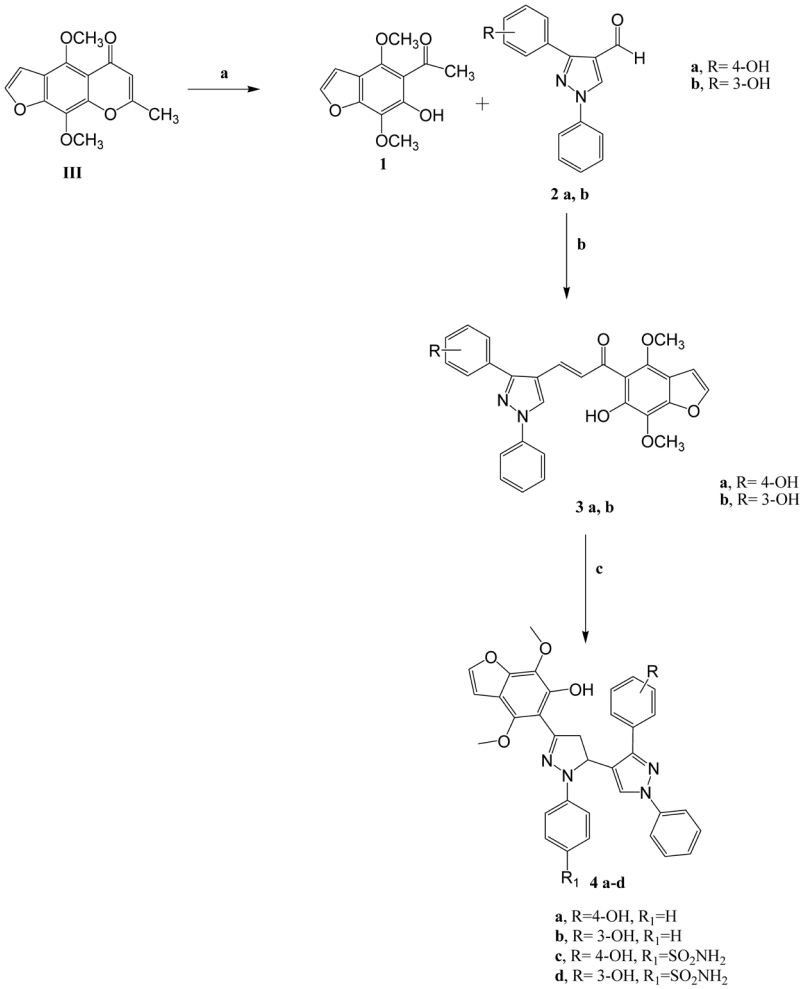


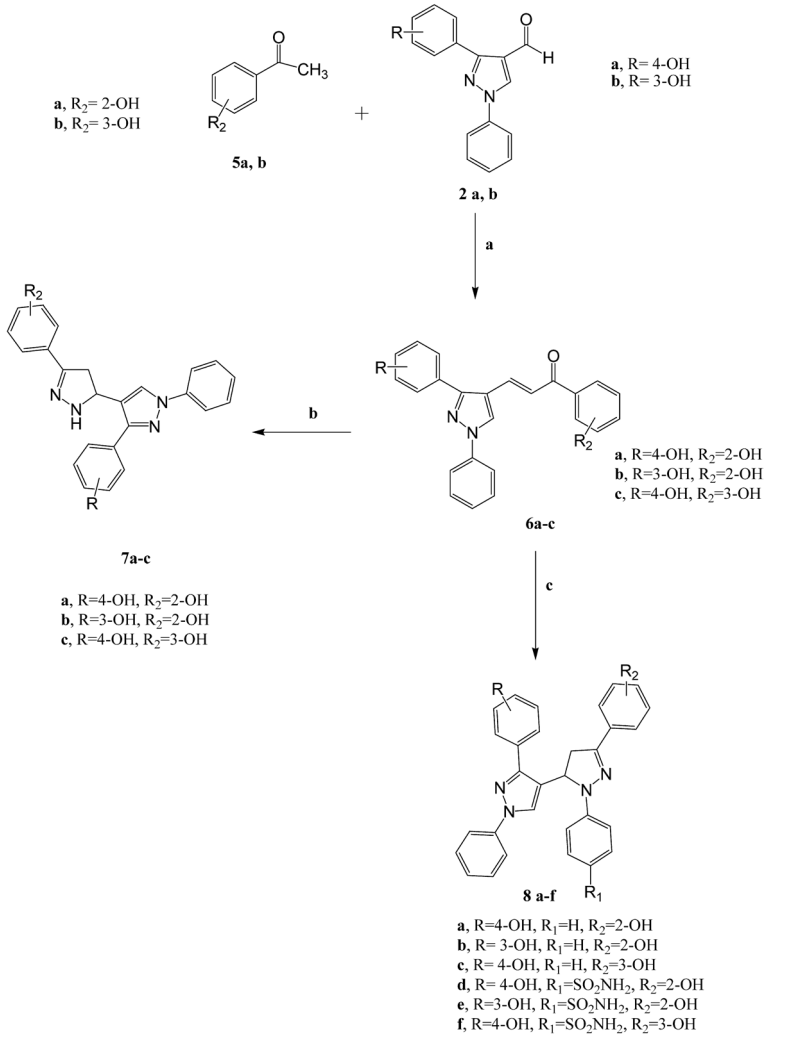
Solvents and Reagents: a: H_2O, 5%KOH; b: ethanol, NaOH; c: ethanol, phenylhydrazine or 4-sulfonamidephenylhydrazine.

Chart 1.

higher percentage of COX-1 inhibition than other coxibs.[18]

For millennia, medicinal plants have been a valuable source of therapeutic agents and still many of today's drugs are plant derived natural products or their derivatives which increase the interest of scientists towards the utility of natural compounds as substituted of synthetic drugs, chalcones also known as 1,3-diaryl-propenones either natural or synthetic have been reported to exhibit diverse biological activity including anti-inflammatory activity.[19–21] Some chalcones inhibit both the isoforms of COX and others being selectively inhibit COX-2.[22,23] The activity of chalcones was found to be dependent on the presence of hydroxy group in both the aryl moieties.[24] The hydroxy group is expected to increase COX inhibitory activity due its mild acidic character and reduction of superoxide radicals.[25,26] On the other hand, the pyrazole scaffold represents a common motif in many anti-inflammatory agents.[27] Among the highly marketed COX-2 inhibitors that comprise the pyrazole nucleus, celecoxib is used as anti-inflammatory and analgesic drug.[28] In addition the existence of benzofuran moiety in many naturally occurring molecules like khellin encourages the medicinal chemist to use it as a synthone in the search for new pharmacologically active molecules, benzofuran derivatives (bioisosteres of indole derivatives **I**)[29] exhibit dual COX-2 and 5-lipoxygenase (5-LOX) inhibitory activity.[30] Also benzofuran containing structures as the furoflavone **II** exhibits gastroprotective effect.[31–33]

Consequently the present investigation deals with the synthesis of hybrid hydroxybenzofuranyl-pyrazolyl chalcones **3a**, **b** and hydroxyphenyl-pyrazolyl chalcones **6a**–**c** to be tested as anti-inflammatory agents. Chalcones are known to be good starting materials for construction of various heterocyclic systems,[34] one of these systems is pyrazoline derivatives which are reported to exhibit marked anti-inflammatory activity with suppression of COX-2 enzyme and tumor necrosis factor α (TNFα) production.[35] The pyrazoline ring is present as a core in a variety of leading drugs as phenazone (antipyrine) and metamizole. Which possess anti-inflammatory, antipyretic and analgesic activity.[36] In addition several 1,3,5 trisubstituted pyrazoline derivatives have excellent activity as anti-inflammatory.[37–39] Accordingly, the synthesized chalcones **3a**, **b** and **6a**–**c** are used to construct the pyrazoline derivatives **4a**–**d**, **7a**–**c** and **8a**–**f** containing the three active anti-inflammatory motifs pyrazole, pyrazoline and hydroxybenzofuranyl or hydroxyphenyl which can enhance the total observed anti-inflammatory activity. In **4a**–**d** and **8a**–**f** the pyrazoline nucleus is disubstituted with vicinal aryl rings (substituted pyrazole and phenyl or benzene sulfonamide) to increase COX-2 selectivity through fitting in the second pocket inside COX-2 binding site,[40–43] while benzene sulfonamide moiety increases COX-2 selectivity targeting hydrophilic side pock-



Solvents and Reagents: a: ethanol, NaOH; b: ethanol, hydrazine hydrate; c: ethanol, phenylhydrazine or 4-sulfonamidephenylhydrazine.

Chart 2.

et.[44–46] All the synthesized compounds were tested *in vitro* for COX-1 and COX-2 inhibitory activity and the most active derivatives were screened for their *in vivo* anti-inflammatory and ulcerogenic activities. Furthermore, the ability of compounds **4c** and **8d** to inhibit prostaglandin (PG)E_2 and TNFα and their gastro protective effect were also carried out.

Results and Discussion

Chemistry The chalcones **3a**, **b** and **6a**–**c** have been synthesized by classical Claisen–Schmidt condensation[47–49] between the 1-(6-hydroxy-4,7-dimethoxy benzofuran-5-yl) ethanone **1** obtained by the alkaline hydrolysis of the natural furochromone **III** according to the reported method[50] or 2-hydroxy or 3-hydroxy acetophenones **5a**, **b** with pyrazole aldehydes **2a**, **b** (synthesized according to the reported method)[51–54] (Charts 1 and 2) . ^{1}H-NMR spectra of compounds **3a**, **b** and **6a**–**c** revealed beside other peaks two new doublets assigned to the two olefinic protons of α and β unsaturated ketones, while ^{13}C-NMR (attached proton test (APT)) showed beside other peaks the presence of peak corresponding to (C=O) at 189.22–194.34 ppm confirming chalcones formation. For example ^{1}H-NMR spectra of compound **6c** showed the presence of two doublet at 7.68 and 7.77 ppm corresponding to (–CO–CH=CH) J = 15.48, 15.44 Hz indicating the trans configuration, while ^{13}C-NMR (APT) for this compound showed the presence of peak at 189.22 ppm corresponding to (C=O). The pyrazolylpyrazolines derivatives **4a**–**d** and **8a**–**f** were obtained by reacting chalcones **3a**, **b** and **6a**–**c** with phenylhydrazine or 4-sulfonamidephenylhydrazine while **7a**–**c** were obtained by reacting chalcones **6a**–**c** with hydrazine hydrate (Charts 1 and 2). The structures of pyrazolylpyrazoline derivatives were confirmed on the basis of spectral analyses. For example ^{1}H-NMR spectra of compounds **4d** revealed beside other peaks three doublet of doublet at 3.53 ppm J = 6.12, 18.12 Hz, 4.25 ppm J = 12.08, 18.28 Hz and 5.64 ppm J = 6.08, 11.72 Hz corresponding to two protons of C_4 pyrazoline and one proton of C_5 pyrazoline respectively and the disappearance of the two doublet corresponding to (–CO–CH=CH), also show singlet peak at 7.05 ppm corresponding to the protons of SO_2NH_2 group (D_2O-exchangeable), while ^{13}C-NMR (APT) revealed beside other peaks the disappearance of the peak corresponding to (C=O) at 194.09 ppm and the appearance of two aliphatic carbon peak at 46.94 and 54.15 ppm corresponding to C_4 and C_5 of pyrazoline respectively.

Biological Screening

In Vitro Cyclooxygenase Enzyme Inhibition Assay

All the synthesized compounds have been screened for

their inhibitory activity of COX-1 and COX-2 isozymes using an ovine-COX-1/COX-2 assay kit (catalog No. 560131, Cayman Chemical, Ann Arbor, MI, U.S.A.).[55] The results were summarized in Table 1. It was observed that all the synthesized compounds exhibited dual COX-1 and COX-2 inhibitory activity with obvious selectivity toward COX-2. The IC_{50} against COX-1 ranged from 6.53–12.65 μM while against COX-2 ranged from 0.050–0.330 μM. Concerning the chalcones **3a** and **3b** bearing hydroxybenzofuranyl moiety as ring A showed selectivity indices (S.I.) of 90.45 and 73.75, respectively. Replacement the hydroxybenzofuranyl moiety in **3a** and **3b** with 2 or 3-hydroxyphenyl substituent to give chalcones **6a**–**c** decreased the S.I. to 64.69, 24.07 and 31.87, respectively. Conversion of **3a** and **3b** to the corresponding pyrazolylpyrazolines **4a**–**d** decreased the inhibitory activity toward COX-1 with marked increase toward COX-2 and expected increase in S.I. (S.I. 190.81, 185.74, 224.26 and 253.00, respectively). It was clear that the two derivatives **4c** and **4d** bearing benzene sulfonamide substituent were the most selective derivatives in this series (S.I. 224.26 and 253.00, respectively). However, construction of pyrazolylpyrazolines **7a**–**c** lacking the vicinal diaryl moiety in the pyrazoline nucleus did not markedly affect COX-1, COX-2 and S.I. (S.I. 61.92, 19.78 and 29.74, respectively) in comparison with the corresponding chalcones **6a**–**c** (S.I. 64.69, 24.07 and 31.87, respectively). On the other hand, the pyrazolylpyrazolines **8a**–**f** bearing two vicinal aryl moieties in the pyrazoline nucleus showed pronounced decrease in the inhibitory activity of COX-1, increase in the inhibitory activity of COX-2 and consequently increase in S.I (S.I. 98.70, 115.89, 135.36, 189.21, 218.39 and 223.64, respectively). It was noticed again that activity against COX-2 and S.I. were governed to a greater extent by the presence of benzene sulfonamide group, as derivatives **8d**–**f** showed the lowest COX-2 IC_{50} and the highest S.I. in this series (S.I. 189.21, 218.39 and 223.64 respectively). Finally according to S.I. the most selective compounds were **4d** (S.I. 253.00), **4c** (S.I. 224.26), **8f** (S.I. 223.64) and **8e** (S.I. 218.39).

Table 1. *In Vitro* COX-1 and COX-2 Inhibitory Activity and Selectivity Indices (S.I.) of the Tested Compounds **3a**, **b**, **4a**–**d**, **6a**–**c**, **7a**–**c** and **8a**–**f** and the Reference Drugs Celecoxib, Rofecoxib and Indomethacin

Compound	IC_{50} (μM)[a]		S.I.[b]
	COX-1	COX-2	
3a	9.95 ± .03*	0.110 ± .001*	90.45
3b	8.85 ± .04*	0.120 ± .002*	73.75
4a	11.83 ± .03*	0.062 ± .001*	190.81
4b	11.33 ± .03*	0.061 ± .002*	185.74
4c	12.11 ± .03*	0.054 ± .001*	224.26
4d	12.65 ± .02*	0.050 ± .001*	253
6a	8.41 ± .04*	0.130 ± .002*	64.69
6b	6.98 ± .02*	0.290 ± .001*	24.07
6c	7.65 ± .03*	0.240 ± .001*	31.875
7a	8.05 ± .03*	0.130 ± .001*	61.92
7b	6.53 ± .04*	0.330 ± .002*	19.78
7c	6.84 ± .02*	0.230 ± .001*	29.74
8a	9.87 ± .02*	0.100 ± .001*	98.7
8b	10.43 ± .04*	0.090 ± .002*	115.89
8c	10.83 ± .04*	0.080 ± .002*	135.357
8d	11.92 ± .03*	0.063 ± .001*	189.21
8e	12.23 ± .02*	0.056 ± .001*	218.39
8f	12.30 ± .01*	0.055 ± .001*	223.64
Celecoxib	14.7 ± .02	0.045 ± .001	326.67
Rofecoxib	14.5 ± .01*	0.025 ± .002*	580
Indomethacin	.1 ± .03*	0.080 ± .001*	1.25

a) The concentration of test compound produce 50% inhibition of COX-1, COX-2 enzyme. The result is the mean of three values obtained by assay of enzyme kits obtained from (Cayman Chemical Inc.). *b*) The *in vitro* COX-2 selectivity index (COX-1/COX-2). *: Statistical significance as compared to the celecoxib at $p < 0.05$.

In Vivo Anti-inflammatory Activity

Motivated by the good *in vitro* enzyme inhibitory activity demonstrated by compounds **4a**–**d** and **8a**–**f**, these derivatives have been evaluated for their *in vivo* anti-inflammatory activity using carrageenan-induced paw edema in rats model (50 mg/kg interperitoneal dose).[56] The results were presented in Table 2 and Fig. 1. After 4 h interval compounds **4a**–**d** bearing hydroxy-benzofuranyl substituent exhibited % inhibition of edema ranged from 63.18–75.00% while that of compounds **8a**–**f** bearing hydroxy-phenyl moiety ranged from 65.00–78.64% (celecoxib as a reference drug showed % inhibition 82.73). Within the series **4a**–**d** the most active derivatives

Table 2. *In Vivo* Anti-inflammatory Activity of the Target Compounds **4a**–**d** and **8a**–**f** and the Reference Drug Celcoxib

	Volume of edema and percentage of edema inhibition							
	1st h		2nd h		3rd h		4th h	
	Edema volume	% of inhibition	Edema volume	% of inhibition	Edema volume	% of inhibition	Edema volume	% of inhibition
Carrageenan	97 ± 9	—	201 ± 8.7	—	216 ± 6.8	—	220 ± 6	—
Celecoxib	46 ± 4*	52.58	60 ± 6*	70.15	49 ± 4.7*	77.31	38 ± 3.4*	82.73
4a	65 ± 6.7*	32.99	96 ± 3.9*	52.24	90 ± 3.6*■	58.33	81 ± 6.5*■	63.18
4b	73 ± 6.3■	24.74	88 ± 8.6*	56.22	78 ± 6*■	63.89	75 ± 7*■	65.91
4c	59 ± 5*	39.17	67 ± 5.6*	66.67	63 ± 5.8*	70.83	55 ± 4.9*	75
4d	63 ± 1.5*	35.05	89 ± 3.2*	55.72	76 ± 5*■	64.81	66 ± 2.9*■	70
8a	54 ± 5.6*	44.33	58 ± 3.7*	71.14	52.8 ± 4.8*	75.56	47 ± 4.4*	78.64
8b	49 ± 2*	49.48	73 ± 3.7*	63.68	69 ± 3*	68.06	56 ± 3*	74.55
8c	82 ± 6.5■	15.46	96.5 ± 9.9*	51.99	93 ± 3*■	56.94	77 ± 2.5*■	65
8d	47 ± 4.5*	51.55	74.8 ± 7*	62.79	61 ± 4.4*	71.76	47 ± 3.7*	78.64
8e	48 ± 3*	50.52	75.9 ± 5*	62.24	63 ± 4*	70.83	49 ± 3.5*	77.73
8f	68 ± 4*	29.89	89 ± 6.7*	55.72	66 ± 4*	69.44	60 ± 3*■	72.73

Values represent means ± S.E.M. of sex animals for each group. *: Statistical significance as compared to the control at $p < 0.05$. ■: Statistical significance as compared to the reference treated group at $p < 0.05$.

were **4c** and **4d** bearing sulfonamide group (% inhibition 75.00 and 70.00 respectively), while in series **8a**–**f** the most active members were **8a** and **8d** (with equal % inhibition 78.64). It was also noticed that the 2-hydroxyphenyl substituent attached to the 3-position of pyrazoline ring gave better activity than the 3-hydroxyphenyl substituent (compounds **8a** *versus* **8c** and **8d** *versus* **8f**). While concerning substitution in the pyrazole nucleus the 4-hydroxyphenyl substituent gave slightly better activity than that with 3-hydroxyphenyl congener (compounds **8a** *versus* **8b** and **8d** *versus* **8e**).

Ulcerogenic Liability

The ulcerogenic liability of **4a**–**d** and **8a**–**f** with reference to celecoxib and diclofenac sodium (in an oral dose 50 mg/kg) was evaluated.[57] The results revealed that all the tested compounds and celecoxib exhibited no ulcerogenic effect (ulcer index = 0), while that of diclofenac sodium caused marked ulcerogenic effect (ulcer index = 20.25).

Compound **4c** as an example of the series containing hydroxybenzofuranyl moiety and compound **8d** as an example of the series bearing hydroxyphenyl moiety were chosen for further investigation as PGE_2, TNFα inhibitors and gastro protective activity.

Evaluation of PGE_2 Inhibition Activity

PGE_2 is a potent inflammatory mediator that is generated by COX-2 conversion of arachidonic acid.[58] Inhibition of PGE_2 production may relieve inflammatory symptoms such as fever, arthritis and inflammatory pain.[59] Therefore, the % inhibition of PGE_2 by compounds **4c** and **8d** was measured. The results were presented in Table 3 and Fig. 2. **4c** and **8d** elicited % inhibition of PGE_2 44.23 and 51.4 respectively compared to celecoxib 72.54%.

Evaluation of TNFα Inhibition Activity

TNFα is an inflammatory cytokine produced by white blood cells (macrophages/monocytes) during acute inflammation and is responsible for a diverse range of signaling events within cell leading to necrosis or apoptosis.[60,61] TNFα

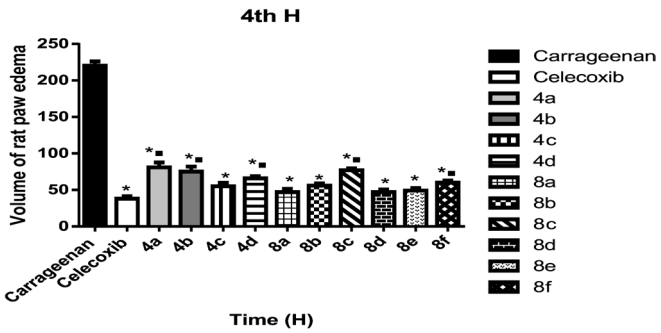


Fig. 1. Effect of Carrageenan, Celecoxib and Listed Compounds **4a**–**d** and **8a**–**f** on the Volume of Paw Edema after 4 h Interval

Columns represent means ± standard error of the mean (S.E.M.) of sex animals for each group. *: Statistical significance as compared to the control at $p < 0.05$. ■: Statistical significance as compared to the reference treated group at $p < 0.05$.

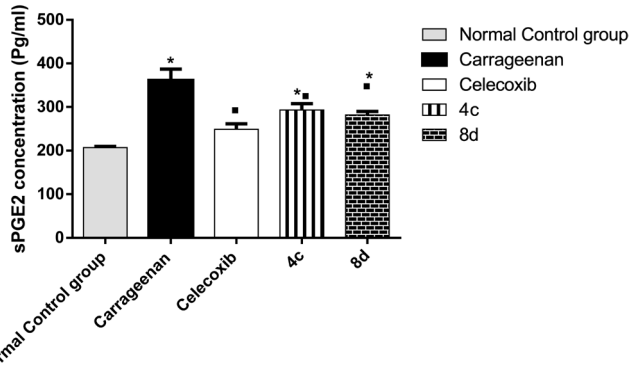


Fig. 2. Serum Concentration of PGE_2

Statistical analysis was carried out by one-way ANOVA followed by Tukey *post hoc* test. Values represent means ± S.E.M. of five blood sample for each group. *: Statistical significance as compared to the Normal control. ■: Statistical significance as compared to carrageenan treated group (Positive control).

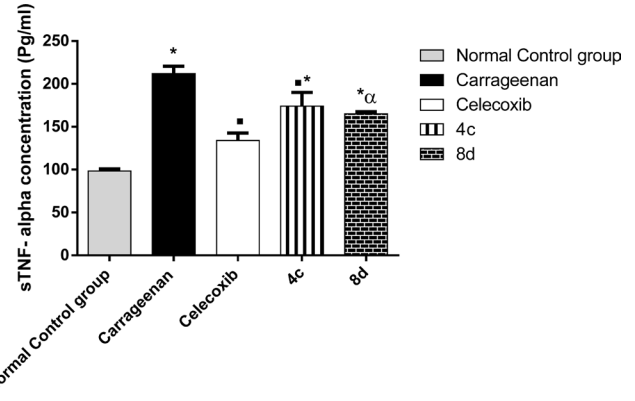


Fig. 3. Serum Concentration of TNFα

Statistical analysis was carried out by one-way ANOVA followed by Tukey *post hoc* test. Values represent means ± S.E.M. of five blood sample for each group. *: Statistical significance as compared to the Normal control. ■: Statistical significance as compared to carrageenan treated group (Positive control). α: Statistical significance as compared to celecoxib as standard anti-inflammatory agent.

Table 3. Concentration and Percentage Inhibition of PGE_2 and TNFα in Rat Serum

	Concentration and percentage inhibition of PGE_2 and TNFα			
	PGE_2		TNFα	
	CONC pg/mL	% inhibition	CONC pg/mL	% inhibition
4c	293 ± 14.5*■	44.23	174 ± 16*$^{\alpha}$	33.48
8d	282 ± 7.8*■	51.40	165 ± 2.7*■	41.41
Celecoxib	249.6 ± 12.5■	72.54	133.9 ± 8.9■	68.81
Normal control	207.5 ± 2.4	—	98.5 ± 2.4	—
Carrageenan	361 ± 26*	—	212 ± 8.5*	—

Statistical analysis was carried out by one-way ANOVA followed by Tukey *post hoc* test. Values represent means ± S.E.M. of five blood sample for each group. *: Statistical significance as compared to the Normal control. ■: Statistical significance as compared to carrageenan treated group (Positive control). α: Statistical significance as compared to celecoxib as standard anti-inflammatory agent.

may be involved in inflammation-associated carcinogenesis.[62] Compounds **4c** and **8d** were further tested for their ability to inhibit TNFα and the results were presented in Table 3 and Fig. 3. The two compounds showed good inhibition of TNFα production (33.48 and 41.41%, respectively compared to celecoxib 68.81%).

Gastro Protective Effect

Gastro protection is defined as the ability of certain drugs to counteract gastric mucosal damage through mechanisms in related to inhibition of acid secretion.[63] Many phenolic compounds have been reported to exhibit a good level of gastro protective effect.[64] Compounds **4c** and **8d** were tested as gastro protective agents in ethanol-induced rodent gastric ulcer model in comparison with famotidine (50 mg/kg) and a control group (ethanol only).[65–67] No ulcers were detected on using **4c**, **8d** and famotidine while control group (ethanol only) showed ulcer index 4.85.

Molecular Docking The structures of the two isozymes COX-1 and COX-2 differ in the volume of the active site, where the active site of COX-2 possesses an additional binding pocket, which is thought to be responsible for the selectivity of selective COX-2 inhibitors which are utilize this additional pocket in binding.[68] In order to elucidate the mechanism of selectivity shown by compound **8d**, our compound and ce-

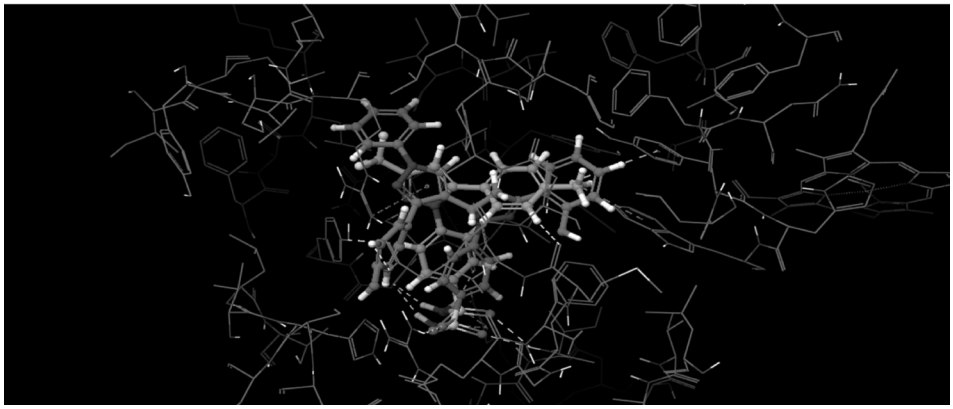


Fig. 4. Orientation of Compound **8d** and Celecoxib in Binding Pocket of COX-2 Enzyme

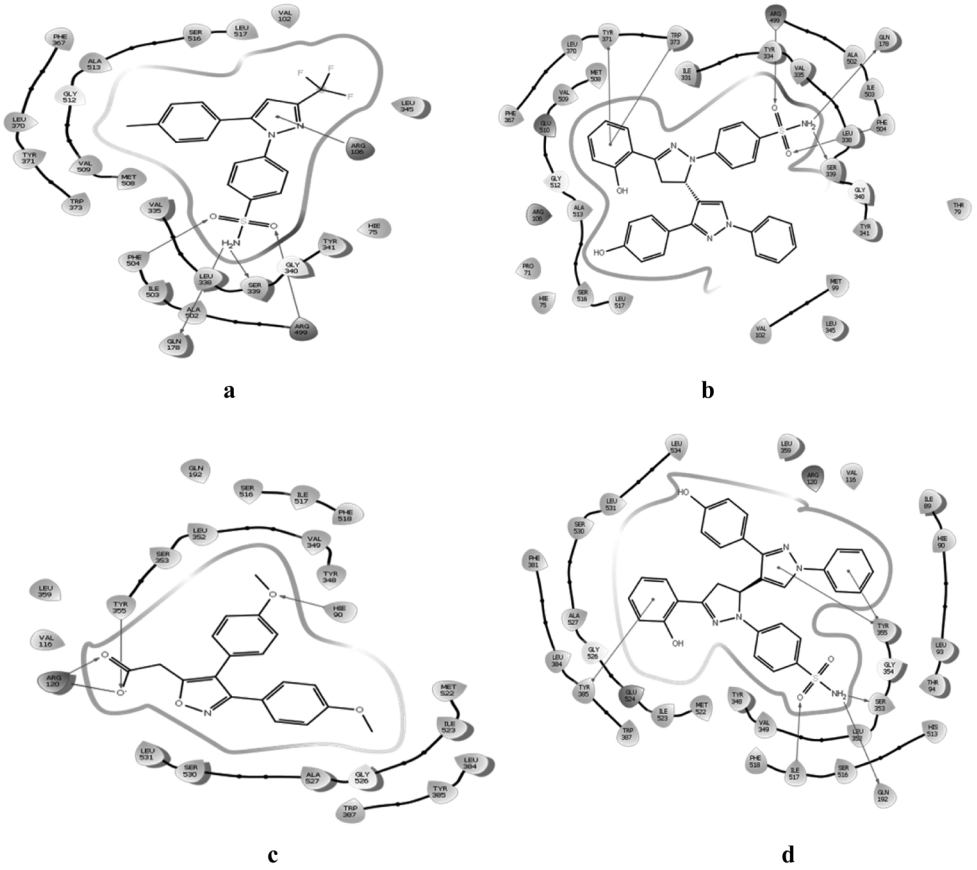


Fig. 5. a, 2D Interactions of the Celecoxib in the Active Site of COX-2 Enzyme; b, 2D Interactions of **8d** in the Active Site of COX-2 Enzymes; c, 2D Interactions of Mofezolac (Co-crystalized Ligand) in the Active Site of COX-1 Enzyme; d, 2D Interactions of **8d** in the Active Site of COX-1 Enzyme

lecoxib were docked into the active site of both COX-1 (pdb code: 5WBE)[69] and COX-2 (pdb code: 3LN1).[70] The computational findings supported those of the biology, where the compound **8d** and celecoxib were found to exhibit a binding pattern and interactions similar to each other in COX-2 active site where *N*-phenyl pyrazoline (**8d**) and *N*-phenyl pyrazole (celecoxib) fit into the additional binding pocket of COX-2 while the sulphonamide moiety of both compounds interact by hydrogen bonds (acceptor or donor) with the same amino acids (Phe 504, Gln 178, Ser 339 and Arg 499) with docking score −7.247 and −12.534, respectively (rmsd = 0.094883), while **8d** bind to the active site of the COX-1 enzyme with docking score −2.816 (rmsd = 0.086), celecoxib fail to comply our constrain (Figs. 4, 5).

Conclusion

New hydroxybenzofuranyl-pyrazolyl chalcones **3a**, **b**, hydroxyphenyl-pyrazolyl chalcones **6a**–**c** and the corresponding pyrazolylpyrazolines **4a**–**d**, **7a**–**c** and **8a**–**f** were synthesized and exhibited dual COX-1 and COX-2 inhibitory activity with obvious selectivity towards COX-2. Compounds **4a**–**d** and **8a**–**f** bearing two vicinal aryl moieties in the pyrazoline nucleus showed the highest selectivity. They also showed good *in vivo* anti-inflammatory activity and were non ulcerogenic. Compounds **4c**, **8a**, **8b**, **8d** and **8e** showed no significance difference from celecoxib in their *in vivo* anti-inflammatory activity. The pyrazolylpyrazoline **4c** bearing hydroxybenzofuranyl moiety and **8d** bearing hydroxyphenyl moiety showed reduction in PGE_2 and TNFα in serum samples. Moreover this two compounds **4c** and **8d** exhibited gastroprotective activity in ethanol induced ulcer model. The docking study of **8d** and celecoxib showed similar manner of interaction with COX-2 active site, while bitter manner of interaction than celecoxib to COX-1 active site. These two derivatives **4c** and **8d** with obvious selectivity against COX-2 and still maintain some degree of COX-1 inhibition may have lower cardiovascular side effects than those with exclusive inhibition of COX-2.

Experimental

Chemistry Melting points were determined on Electro thermal Stuart 5MP3 digital melting point apparatus and were uncorrected. NMR spectra (in dimethyl sulfoxide (DMSO)-d_6) were recorded on Bruker AVANCE III 400 MHz FT-NMR spectrometer (Bruker, Flawil, Switzerland, δ ppm) using trimethylsilyl (TMS) as internal Standard. ^{1}H-NMR spectra were run at 400 MHz and ^{13}C-NMR spectra were run at 100 MHz. Reactions were monitored by TLC using Macherey–Nagel AlugramSil G/UV_{254} silica gel plates and hexane–ethanol (4 : 1) as the eluting system. The spots were visualized using VilberLourmet ultraviolet lamp at ג = 254 and 266 nm.

General Procedure for the Synthesis of Hydroxybenzofuranyl-pyrazolyl Chalcones **3a**, **b** and Hydroxyphenyl-pyrazolyl Chalcones **6a**–**c**

To a mixture of khellinone **1** or acetophenone derivatives **5a**, **b** (2 mmol) in sodium hydroxide solution (10 mL 30% w/v) and ethanol (20 mL), a solution of the appropriate pyrazole aldehyde **2a**, **b** (2 mmol) in ethanol (60 mL) was added, the resulting red solution was allowed to stand for 48 h at room temperature. The mixture was diluted with water to 200 mL and neutralized with dilute acetic acid. The solid was filtered off, washed with water, dried and crystallized from ethanol.

(*E*)-1-(6-Hydroxy-4,7-dimethoxybenzofuran-5-yl)-3-(3-(4-hydroxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)prop-2-en-1-one **3a**

Yield 45%, mp 95–97°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 3.92 (s, 6H, 2 OCH_3), 6.82 (d, 2H, J = 8.44 Hz, Ar), 7.02 (d, 1H, J = 15.92 Hz, CH=CH), 7.15 (d, 1H, J = 2.04 Hz, CH furan), 7.28 (d, 1H, J = 15.88 Hz, CH=CH), 7.35–7.39 (m, 3H, J = 8.56 Hz, Ar), 7.55 (t, 2H, J = 7.88 Hz, Ar), 7.92 (d, 3H, J = 8.92 Hz, CH furan + 2Ar), 9.20 (s, 1H, C_5-H pyrazole), 9.75 (s, 1H, OH, D_2O-exchangeable), 9.87 (s, 1H, OH, D_2O-exchangeable). ^{13}C-NMR/APT (100 MHz, DMSO-d_6) δ: 61.09 (OCH_3), 61.31 (OCH_3), 105.91 (CH), 112.01 (C_q), 116.02 (2CH), 116.15 (C_q), 117.28 (C_q), 119.08 (2CH), 122.95 (C_q), 127.42 (CH), 128.12 (CH), 128.97 (C_q), 129.75 (CH), 130.03 (2CH), 130.09 (2CH), 136.64 (CH), 139.44 (C_q), 144.86 (CH), 145.57 (C_q), 146.23 (C_q), 149.17 (C_q), 153.20 (C_q), 158.46 (C_q), 194.34 (C=O).

(*E*)-1-(6-Hydroxy-4,7-dimethoxybenzofuran-5-yl)-3-(3-(3-hydroxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)prop-2-en-1-one **3b**

Yield 32%, mp 100°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 3.93 (d, 6H, 2OCH_3), 6.83 (d, 1H, J = 7.68 Hz, Ar), 6.93 (d, 1H, J = 7.64 Hz, Ar), 7.03–7.15 (m, 4H, 2Ar + CH=CH + CH furan), 7.23 (t, 1H, J = 8.05 Hz, Ar), 7.34–7.41 (m, 2H, J = 16.60 Hz, CH=CH + Ar), 7.56 (t, 2H, J = 7.75 Hz, Ar), 7.92 (t, 2H, J = 8.08 Hz, Ar + CH furan), 9.24 (s, 1H, C_5-H pyrazole), 9.65 (s, 1H, OH, D_2O-exchangeable), 9.96 (s, 1H, OH, D_2O-exchangeable). ^{13}C-NMR/APT (100 MHz, DMSO-d_6) δ: 61.11 (OCH_3), 61.27 (OCH_3), 105.99 (CH), 112.00 (C_q), 115.42 (CH), 116.09 (C_q), 116.21 (CH), 117.66 (C_q), 119.17 (2CH), 119.51 (CH), 127.57 (CH), 128.33 (CH), 128.96 (C_q), 129.15 (CH), 130.13 (2CH), 130.17 (CH), 133.51 (C_q), 135.71 (CH), 139.42 (C_q), 144.89 (CH), 145.82 (C_q), 146.45 (C_q), 149.32 (C_q), 153.05 (C_q), 158.14 (C_q), 194.09 (C=O).

(*E*)-1-(2-Hydroxyphenyl)-3-(3-(4-hydroxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)prop-2-en-1-one **6a**

Yield 50%, mp 115°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 6.95 (d, 2H, J = 8.52 Hz, Ar), 7.00–7.06 (m, 2H, Ar), 7.41 (t, 1H, J = 7.36 Hz, Ar), 7.50 (d, 2H, J = 8.48 Hz, Ar), 7.56–7.61 (m, 3H, Ar), 7.80 (d, 1H, J = 15.32 Hz, CH=CH), 7.93–7.96 (m, 3H, J = 15.12 Hz, CH=CH + 2Ar), 8.13 (d, 1H, J = 7.32 Hz, Ar), 9.43 (s, 1H, C_5-H pyrazole), 9.82 (s, 1H, OH, D_2O-exchangeable), 12.68 (s, 1H, OH, D_2O-exchangeable). ^{13}C-NMR/APT (100 MHz, DMSO-d_6) δ: 116.19 (2CH), 117.90 (C_q), 118.35 (CH), 119.10 (2CH), 119.52 (CH), 120.57 (CH), 120.89 (C_q), 122.97 (C_q), 127.58 (CH), 129.45 (CH), 130.17 (2CH), 130.30 (2CH), 130.62 (CH), 136.32 (CH), 136.71 (CH), 139.42 (C_q), 153.97 (C_q), 158.63 (C_q), 162.55 (C_q), 193.73 (C=O).

(*E*)-1-(2-Hydroxyphenyl)-3-(3-(3-hydroxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)prop-2-en-1-one **6b**

Yield 40%, mp 125–128°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 6.92 (d, 1H, J = 7.79 Hz, Ar), 7.01–7.10 (m, 4H, Ar), 7.37 (t, 1H, J = 8.08 Hz, Ar), 7.43 (t, 1H, J = 7.39 Hz, Ar), 7.57–7.63 (m, 3H, Ar), 7.84 (d, 1H, J = 15.24 Hz, CH=CH), 7.94–8.00 (m, 3H, J = 15.64, 8.84 Hz, CH=CH + 2Ar), 8.15 (d, 1H, J = 7.37 Hz, Ar), 9.47 (s, 1H, C_5-H pyrazole), 9.73 (s, 1H, OH, D_2O-exchangeable), 12.65 (s, 1H, OH, D_2O-exchangeable). ^{13}C-NMR/APT (100 MHz, DMSO-d_6) δ: 115.64 (CH), 116.35 (CH), 118.13 (C_q), 118.37 (CH), 119.20 (2CH), 119.57 (CH), 119.71 (CH), 120.85 (C_q), 120.95 (CH), 127.77 (CH), 129.61 (CH), 130.22 (2CH), 130.48 (CH), 130.67 (CH), 133.41 (C_q), 135.92 (CH), 136.82 (CH), 139.36 (C_q), 153.74 (C_q), 158.13

(C_q), 162.56 (C_q), 193.72 (C=O).

(*E*)-1-(3-Hydroxyphenyl)-3-(3-(4-hydroxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)prop-2-en-1-one **6c**

Yield 35%, mp 137–140°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 6.96 (d, 2H, J = 8.52 Hz, Ar), 7.07 (dd, 1H, J = 1.84, 8.00 Hz, Ar), 7.39 (t, 2H, J = 7.85 Hz, Ar), 7.44 (t, 1H, J = 2.03 Hz, Ar), 7.50 (d, 2H, J = 8.52 Hz, Ar), 7.54–7.60 (m, 3H, Ar), 7.68 (d, 1H, J = 15.48 Hz, CH=CH), 7.77 (d, 1H, J = 15.44 Hz, CH=CH), 7.94 (d, 2H, J = 7.84 Hz, Ar), 9.39 (s, 1H, C_5-H pyrazole), 9.83 (d, 2H, 2OH, D_2O-exchangeable). ^{13}C-NMR/APT (100 MHz, DMSO-d_6) δ: 115.02 (CH), 116.15 (2CH), 117.96 (C_q), 119.02 (2CH), 119.66 (CH), 120.57 (CH), 121.60 (CH), 123.13 (C_q), 127.44 (CH), 129.03 (CH), 130.15 (2CH), 130.27 (3CH), 135.01 (CH), 139.50 (C_q), 139.69 (C_q), 153.72 (C_q), 158.22 (C_q), 158.53 (C_q), 189.22 (C=O).

General Method for the Synthesis of Pyrazolylpyrazoline Derivatives **4a**–**d** and **8a**–**f**

To a hot solution of the appropriate chalcone **3a**, **b** or **6a**–**c** (2 mmol) in ethanol (100 mL) phenylhydrazine hydrochloride or 4-sulfonamidephenylhydrazine hydrochloride (10 mmol) were added. The reaction mixture was refluxed for 16–48 h (TLC). The precipitated products were filtered (if no precipitate occurred concentrate first then the solutions were left to crystalize). The products were washed with ethanol and crystallized from ethanol to afford the pyrazolines **4a**–**d** and **8a**–**f** in moderate to good yields.

General Method for the Synthesis of Pyrazolylpyrazoline Derivatives **7a**–**c**

To a solution of the appropriate chalcone **6a**–**c** (2 mmol) in ethanol (100 mL) hydrazine hydrate (10 mmol) were added. The reaction mixture was refluxed for 16–48 h (TLC). Concentrate then the solutions were poured into ice-water (10 mL) to precipitate (in case of compounds **7a** and **7b** a few drops of dil HCl is added to precipitate the compounds). The formed precipitates were filtered and washed with water and crystallized from ethanol to afford the pyrazolines **7a**–**c**.

5-(4,5-Dihydro-5-(3-(4-hydroxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)-1-phenyl-1*H*-pyrazol-3-yl)-4,7-dimethoxybenzofuran-6-ol **4a**

Yield 60%, mp 238–240°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 3.50 (dd, 1H, J = 8.04, 18.00 Hz, C_4-H pyrazoline), 3.94 (d, 6H, 2OCH_3), 4.23 (m, 1H, J = 11.88, 18.28 Hz, C_4-H pyrazoline), 5.45 (dd, 1H, J = 8.00, 11.84 Hz, C_5-H pyrazoline), 6.80 (t, 1H, J = 7.29 Hz, Ar), 6.89–6.92 (m, 4H, Ar), 7.13 (d, 1H, J = 2.28 Hz, CH furan), 7.20 (t, 2H, J = 7.93 Hz, Ar), 7.27 (t, 1H, J = 7.37 Hz, Ar), 7.46 (t, 2H, J = 7.95 Hz, Ar), 7.59 (d, 2H, J = 8.52 Hz, Ar), 7.84 (d, 2H, J = 7.80 Hz, Ar), 7.88 (d, 1H, J = 2.28 Hz, CH furan), 8.46 (s, 1H, C_5-H pyrazole), 9.68 (s, 1H, OH, D_2O-exchangeable), 11.60 (s, 1H, OH, D_2O-exchangeable). ^{13}C-NMR/APT (100 MHz, DMSO-d_6) δ: 46.80 (CH_2), 55.15 (CH), 60.94 (OCH_3), 61.36 (OCH_3), 105.80 (CH), 106.88 (C_q), 112.37 (C_q), 113.72 (2CH), 116.00 (2CH), 118.46 (2CH), 120.10 (CH), 122.48 (C_q), 123.95 (C_q), 126.60 (CH), 127.63 (CH), 129.13 (C_q), 129.57 (2CH), 129.83 (2CH), 129.94 (2CH), 139.73 (C_q), 144.59 (C_q), 144.79 (CH), 147.58 (C_q), 148.04 (C_q), 149.15 (C_q), 150.36 (C_q), 150.79 (C_q), 158.04 (C_q).

5-(4,5-Dihydro-5-(3-(3-hydroxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)-1-phenyl-1*H*-pyrazol-3-yl)-4,7-dimethoxybenzofuran-6-ol **4b**

Yield 40%, mp 125–127°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 3.52 (dd, 1H, J = 7.96, 18.00 Hz, C_4-H pyrazoline), 3.94 (d, 6H, 2OCH_3), 4.24 (m, 1H, J = 11.84, 18.24 Hz, C_4-H pyrazoline), 5.46 (dd, 1H, J = 8.00, 11.88 Hz, C_5-H pyrazoline,), 6.79–6.86 (m, 2H, Ar), 6.92 (d, 2H, J = 8.12 Hz, Ar), 7.13 (d, 1H, J = 2.32 Hz, CH furan), 7.19–7.34 (m, 6H, Ar), 7.47 (t, 2H, J = 7.83 Hz, Ar), 7.85 (d, 2H, J = 7.91 Hz, Ar), 7.88 (d, 1H, J = 2.24 Hz, CH furan), 8.48 (s, 1H, C_5-H pyrazole), 9.62 (s, 1H, OH, D_2O-exchangeable), 11.58 (s, 1H, OH, D_2O-exchangeable). ^{13}C-NMR/APT (100 MHz, DMSO-d_6) δ: 46.89 (CH_2), 55.15 (CH), 60.95 (OCH_3), 61.38 (OCH_3), 105.80 (CH), 106.89 (C_q), 112.37 (C_q), 113.73 (2CH), 115.19 (CH), 115.83 (CH), 118.59 (2CH), 119.24 (CH), 120.16 (CH), 122.97 (C_q), 126.85 (CH), 127.77 (CH), 129.12 (C_q), 129.59 (2CH), 129.99 (2CH), 130.32 (CH), 134.28 (C_q), 139.63 (C_q), 144.57 (C_q), 144.81 (CH), 147.58 (C_q), 148.02 (C_q), 149.14 (C_q), 150.26 (C_q), 150.54 (C_q), 158.00 (C_q).

5-(1-(4-Sulfonamidephenyl)-4,5-dihydro-5-(3-(4-hydroxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)-1*H*-pyrazol-3-yl)-4,7-dimethoxybenzofuran-6-ol **4c**

Yield 60%, mp 220–222°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 3.51 (dd, 1H, J = 6.20, 18.32 Hz, C_4-H pyrazoline), 3.94 (d, 6H, 2OCH_3), 4.22 (dd, 1H, J = 11.76, 18.08 Hz, C_4-H pyrazoline), 5.62 (dd, 1H, J = 6.24, 11.88 Hz, C_5-H pyrazoline), 6.91 (d, 2H, J = 8.37 Hz, Ar), 6.95 (d, 2H, J = 8.75 Hz, Ar), 7.04 (s, 2H, SO_2NH_2, D_2O-exchangeable), 7.14 (d, 1H, J = 2.28 Hz, CH furan), 7.28 (t, 1H, J = 7.41 Hz, Ar), 7.46 (t, 2H, J = 7.80 Hz, Ar), 7.57 (d, 2H, J = 8.35 Hz, Ar), 7.61 (d, 2H, J = 8.73 Hz, Ar) 7.82 (d, 2H, J = 8.05 Hz, Ar), 7.90 (d, 1H, J = 2.21 Hz, CH furan), 8.41 (s, 1H, C_5-H pyrazole), 9.70 (s, 1H, OH, D_2O-exchangeable), 11.15 (s, 1H, OH, D_2O-exchangeable). ^{13}C-NMR/APT (100 MHz, DMSO-d_6) δ: 46.83 (CH_2), 54.12 (CH), 61.03 (OCH_3), 61.42 (OCH_3), 105.83 (CH), 106.97 (C_q), 112.45 (C_q), 112.53 (2CH), 116.01 (2CH), 118.52 (2CH), 121.86 (C_q), 123.79 (C_q), 126.69 (CH), 127.53 (CH), 127.71 (2CH), 129.15 (C_q), 129.87 (2CH), 129.96 (2CH), 134.11 (C_q), 139.68 (C_q), 144.91 (CH), 145.97 (C_q), 147.76 (C_q), 147.79 (C_q), 149.32 (C_q), 150.87 (C_q), 151.70 (C_q), 158.10 (C_q).

5-(1-(4-Sulfonamidephenyl)-4,5-dihydro-5-(3-(3-hydroxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)-1*H*-pyrazol-3-yl)-4,7-dimethoxybenzofuran-6-ol **4d**

Yield 40%, mp 130°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 3.53 (dd, 1H, J = 6.12, 18.12 Hz, C_4-H pyrazoline), 3.95 (s, 6H, 2OCH_3), 4.25 (dd, 1H, J = 12.08, 18.28 Hz, C_4-H pyrazoline), 5.64 (dd, 1H, J = 6.08, 11.72 Hz, C_5-H pyrazoline), 6.87 (d, 1H, J = 8.12 Hz, Ar), 6.97 (d, 2H, J = 8.60 Hz, Ar), 7.05 (s, 2H, SO_2NH_2, D_2O-exchangeable), 7.14 (s, 1H, CH furan), 7.19 (d, 2H, J = 8.08 Hz, Ar), 7.28–7.36 (m, 2H, Ar), 7.47 (t, 2H, J = 7.78 Hz, Hz, Ar), 7.63 (d, 2H, J = 8.44 Hz, Ar), 7.84 (d, 2H, J = 8.00 Hz, Ar), 7.90 (s, 1H, CH furan), 8.44 (s, 1H, C_5-H pyrazole), 9.64 (s, 1H, OH, D_2O-exchangeable), 11.16 (s, 1H, OH, D_2O-exchangeable). ^{13}C-NMR/APT (100 MHz, DMSO-d_6) δ: 46.94 (CH_2), 54.15 (CH), 61.03 (OCH_3), 61.42 (OCH_3), 105.84 (CH), 106.97 (C_q), 112.45 (C_q), 112.55 (2CH), 115,25 (CH), 115.89 (CH), 118.65 (2CH), 119.28 (CH), 122.32 (C_q), 126.90 (CH), 127.69 (CH), 127.72 (2CH), 129.15 (C_q), 130.00 (2CH), 130.32 (CH), 134.17 (C_q), 134.19 (C_q), 139.61 (C_q), 144.91 (CH), 145.97 (C_q), 147.77 (C_q), 147.80 (C_q), 149.33 (C_q), 150.61 (C_q), 151.60 (C_q), 158.06 (C_q).

2-(4,5-Dihydro-5-(3-(4-hydroxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)-1*H*-pyrazol-3-yl)phenol **7a**

Yield 45%, mp 125–128°C, ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 3.13 (dd, 1H, J = 10.48, 16.32 Hz, C_4-H pyrazoline),

3.67 (dd, 1H, J = 10.40, 16.28 Hz, C_4-H pyrazoline), 4.95 (t, 1H, J = 10.80 Hz, C_5-H pyrazoline), 6.77–6.94 (m, 5H, Ar), 7.13–7.31 (m, 2H, Ar), 7.50–7.60 (m, 4H, Ar), 7.79–7.93 (m, 2H, Ar), 8.58 (s, 1H, C_5-H pyrazole), 9.63 (s, 1H, OH, D_2O-exchangeable), 11.20 (s, 1H, OH, D_2O-exchangeable), 13.41 (s, 1H, NH, D_2O-exchangeable). ^{13}C-NMR/APT (100 MHz, DMSO-d_6) δ: 40.83 (CH_2), 54.20 (CH), 115.90 (2CH), 116.20 (CH), 117.24 (C_q), 118.49 (2CH), 119.60 (CH), 119.77 (CH), 122.43 (C_q), 124.11 (C_q), 128.40 (CH), 129.40 (CH), 129.76 (2CH), 129.99 (2CH), 130.12 (CH), 139.97 (C_q), 151.19 (C_q), 153.71 (C_q), 157.72 (C_q), 157.88 (C_q).

2-(4,5-Dihydro-5-(3-(3-hydroxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)-1*H*-pyrazol-3-yl)phenol **7b**

Yield 30%, mp 115°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 3.17 (m, 1H, J = 10.76, 16.68 Hz, C_4-H pyrazoline), 3.68 (m, 1H, J = 10.90, 16.08 Hz, C_4-H pyrazoline), 5.00 (t, 1H, J = 10.48 Hz, C_5-H pyrazoline), 6.79–6.92 (m, 3H, Ar), 7.16–7.32 (m, 6H, Ar), 7.52–7.95 (m, 4H, Ar), 8.61 (s, 1H, C_5-H pyrazole), 9.54 (s, 1H, OH, D_2O-exchangeable), 11.20 (s, 1H, OH, D_2O-exchangeable), 13.42 (s, 1H, NH, D_2O-exchangeable). ^{13}C-NMR/APT (100 MHz, DMSO-d_6) δ: 40.98 (CH_2), 54.21 (CH), 115.12 (CH), 116.22 (CH), 117.21 (C_q), 118.63 (CH), 118.82 (2CH), 119.19 (CH), 119.62 (CH), 119.77 (CH), 123.00 (C_q), 128.39 (CH), 130.03 (2CH), 130.17 (2CH), 130.32 (CH), 134.96 (C_q), 139.89 (C_q), 150.93 (C_q), 153.66 (C_q), 157.94 (C_q), 158.76 (C_q).

3-(4,5-Dihydro-5-(3-(4-hydroxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)-1*H*-pyrazol-3-yl)phenol **7c**

Yield 55%, mp 101–103°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 2.92 (dd, 1H, J = 11.20, 16.48 Hz, C_4-H pyrazoline), 3.43 (m, 1H, J = 11.44, 16.68 Hz, C_4-H pyrazoline), 4.94 (t, 1H, J = 11.13 Hz, C_5-H pyrazoline), 6.74 (d, 1H, J = 8.24 Hz, Ar), 6.88 (d, 2H, J = 8.08 Hz, Ar), 7.04–7.08 (m, 2H, J = 8.16, 7.52 Hz, Ar), 7.18 (t, 1H, J = 7.86 Hz, Ar), 7.29 (t, 1H, J = 7.73 Hz, Ar), 7.48–7.51 (m, 3H, 2Ar + NH D_2O-exchangeable), 7.59 (d, 2H, J = 8.04 Hz, Ar), 7.88 (d, 2H, J = 8.20 Hz, Ar), 8.52 (s, 1H, C_5-H pyrazole), 9.46 (s, 1H, OH, D_2O-exchangeable), 9.62 (s, 1H, OH, D_2O-exchangeable). ^{13}C-NMR/APT (100 MHz, DMSO-d_6) δ: 40.11 (CH_2), 55.78 (CH), 112.47 (CH), 115.25 (CH), 115.87 (2CH), 118.44 (2CH), 119.37 (CH), 123.07 (C_q), 124.27 (C_q), 126.43 (CH), 129.74 (2CH), 129.98 (2CH), 130.13 (CH), 131.12 (CH), 134.95 (C_q), 140.01 (C_q), 149.98 (C_q), 151.06 (C_q), 157.77 (C_q), 157.83 (C_q),

2-(4,5-Dihydro-5-(3-(4-hydroxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)-1-phenyl-1*H*-pyrazol-3-yl)phenol **8a**

Yield 60%, mp 250°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 3.42 (dd, 1H, J = 7.84, 17.44 Hz, C_4-H pyrazoline), 4.19 (dd, 1H, J = 12.08, 17.56 Hz, C_4-H pyrazoline), 5.47 (dd, 1H, J = 7.80, 11.96 Hz, C_5-H pyrazoline), 6.79 (t, 1H, J = 7.32 Hz, Ar), 6.90–6.96 (m, 5H, Ar), 7.00 (d, 1H, J = 8.20 Hz, Ar), 7.20 (t, 2H, J = 7.73 Hz, Ar), 7.24–7.32 (m, 2H, Ar), 7.44 (t, 3H, J = 7.37 Hz, Ar), 7.61 (d, 2H, J = 8.16 Hz, Ar), 7.83 (d, 2H, J = 8.04 Hz, Ar), 8.40 (s, 1H, C_5-H pyrazole), 9.69 (s, 1H, OH, D_2O-exchangeable), 10.62 (s, 1H, OH, D_2O-exchangeable). ^{13}C-NMR/APT (100 MHz, DMSO-d_6) δ: 43.71 (CH_2), 55.57 (CH), 113.75 (2CH), 116.02 (2CH), 116.50 (CH), 117.05 (C_q), 118.45 (2CH), 119.98 (CH), 120.08 (CH), 122.36 (C_q), 123.86 (C_q), 126.59 (CH), 127.49 (CH), 128.61 (CH), 129.55 (2CH), 129.76 (2CH), 129.92 (2CH), 130.90 (CH), 139.72 (C_q), 144.50 (C_q), 150.52 (C_q), 150.99 (C_q), 156.77 (C_q), 158.06 (C_q).

2-(4,5-Dihydro-5-(3-(3-hydroxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)-1-phenyl-1*H*-pyrazol-3-yl)phenol **8b**

Yield 50%, mp 138–140°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 3.44 (dd, 1H, J = 7.76, 17.40 Hz, C_4-H pyrazoline), 4.19 (dd, 1H, J = 12.12, 17.56 Hz, C_4-H pyrazoline), 5.49 (dd, 1H, J = 7.76, 12.00 Hz, C_5-H pyrazoline), 6.80 (t, 1H, J = 7.25 Hz, Ar), 6.86 (d, 1H, J = 7.73 Hz, Ar), 6.91–6.96 (m, 3H, Ar), 7.00 (d, 1H, J = 8.19 Hz, Ar), 7.18–7.23 (m, 4H, Ar), 7.26–7.35 (m, 3H, Ar), 7.46 (t, 3H, J = 7.41 Hz, Ar), 7.84 (d, 2H, J = 8.02 Hz, Ar), 8.42 (s, 1H, C_5-H pyrazole), 9.62 (s, 1H, OH, D_2O-exchangeable), 10.61 (s, 1H, OH, D_2O-exchangeable). ^{13}C-NMR/APT (100 MHz, DMSO-d_6) δ: 43.80 (CH_2), 55.55 (CH), 113.74 (2CH), 115.13 (CH), 115.86 (CH), 116.51 (CH), 117.05 (C_q), 118.58 (2CH), 119.18 (CH), 119.99 (CH), 120.12 (CH), 122.87 (C_q), 126.83 (CH), 127.65 (CH), 128.60 (CH), 129.56 (2CH), 129.96 (2CH), 130 .34 (CH), 130.92 (CH), 134.20 (C_q), 139.63 (C_q), 144.48 (C_q), 150.28 (C_q), 150.89 (C_q), 156.77 (C_q), 158.04 (C_q).

3-(4,5-Dihydro-5-(3-(4-hydroxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)-1-phenyl-1*H*-pyrazol-3-yl)phenol **8c**

Yield 45%, mp 135–137°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 3.21 (dd, 1H, J = 7.84, 17.20 Hz, C_4-H pyrazoline), 4.01 (dd, 1H, J = 12.24, 17.36 Hz, C_4-H pyrazoline), 5.45 (dd, 1H, J = 7.96, 12.08 Hz, C_5-H pyrazoline), 6.73 (t, 1H, J = 7.26, 7.26 Hz, Ar), 6.79 (d, 1H, J = 7.58 Hz, Ar), 6.91 (d, 2H, J = 8.18 Hz, Ar), 6.97 (d, 2H, J = 8.08 Hz, Ar), 7.15 (t, 3H, J = 7.99 Hz, Ar), 7.21–7.28 (m, 3H, Ar), 7.44 (t, 2H, J = 7.74 Hz, Ar), 7.62 (d, 2H, J = 8.20 Hz, Ar), 7.82 (d, 2H, J = 8.06 Hz, Ar), 8.29 (s, 1H, C_5-H pyrazole), 9.54 (s, 1H, OH, D_2O-exchangeable), 9.69 (s, 1H, OH, D_2O-exchangeable). ^{13}C-NMR/APT (100 MHz, DMSO-d_6) δ: 43.01 (CH_2), 56.55 (CH), 112.60 (CH), 113.75 (2CH), 116.03 (2CH), 116.51 (CH), 117.41 (CH), 118.44 (2CH), 119.42 (CH), 122.81 (C_q), 123.94 (C_q), 126.56 (CH), 127.21 (CH), 129.31 (2CH), 129.74 (2CH), 129.93 (2CH), 130.14 (CH), 134.02 (C_q), 139.71 (C_q), 145.20 (C_q), 148.24 (C_q), 150.42 (C_q), 157.87 (C_q), 158.01 (C_q).

2-(1-(4-Sulfonamidephenyl)-4,5-dihydro-5-(3-(4-hydroxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)-1*H*-pyrazol-3-yl)phenol **8d**

Yield 35%, mp 160°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ : 3.49 (dd, 1H, J = 6.36, 17.72 Hz, C_4-H pyrazoline), 4.21 (dd, 1H, J = 12.12, 17.80 Hz, C_4-H pyrazoline), 5.64 (dd, 1H, J = 6.28, 11.92 Hz, C_5-H pyrazoline), 6.91–7.03 (m, 8H, 6Ar + SO_2NH_2 D_2O-exchangeable), 7.26 (t, 1H, J = 7.36 Hz, Ar), 7.32 (t, 1H, J = 7.75 Hz, Ar), 7.44 (t, 2H, J = 7.89 Hz, Ar), 7.52 (d, 1H, J = 7.65 Hz, Ar), 7.59–7.63 (m, 4H, Ar), 7.82 (d, 2H, J = 7.90 Hz, Ar), 8.36 (s, 1H, C_5-H pyrazole), 9.71 (s, 1H, OH, D_2O-exchangeable), 10.37 (s, 1H, OH, D_2O-exchangeable). ^{13}C-NMR/APT (100 MHz, DMSO-d_6) δ: 44.04 (CH_2), 54.65 (CH), 112.64 (2CH), 116.04 (2CH), 116.68 (CH), 117.04 (C_q), 118.51 (2CH), 120.05 (CH), 121.72 (C_q), 123.73 (C_q), 126.67 (CH), 127.42 (CH), 127.66 (2CH), 128.94 (CH), 129.81 (2CH), 129.92 (2CH), 131.37 (CH), 134.11 (C_q), 139.66 (C_q), 145.94 (C_q), 150.63 (C_q), 152.40 (C_q), 156.77 (C_q), 158.11 (C_q).

2-(1-(4-Sulfonamidephenyl)-4,5-dihydro-5-(3-(3-hydroxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)-1*H*-pyrazol-3-yl)phenol **8e**

Yield 55%, mp 168–170°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 3.51 (dd, 1H, J = 6.28, 17.76 Hz, C_4-H pyrazoline), 4.22 (dd, 1H, J = 12.08, 17.80 Hz, C_4-H pyrazoline), 5.65 (dd, 1H,

$J = 6.20$, 11.96 Hz, C_5-H pyrazoline), 6.87 (d, 1H, $J = 7.32$ Hz, Ar), 6.93–7.04 (m, 6H, 4Ar + SO_2NH_2 D_2O-exchangeable), 7.21 (d, 2H, $J = 7.88$ Hz, Ar), 7.26–7.36 (m, 3H, Ar), 7.45 (t, 2H, $J = 7.85$ Hz, Ar), 7.53 (d, 1H, $J = 7.28$ Hz, Ar), 7.62 (d, 2H, $J = 8.56$ Hz, Ar), 7.83 (d, 2H, $J = 8.00$ Hz, Ar), 8.39 (s, 1H, C_5-H pyrazole), 9.64 (s, 1H, OH, D_2O-exchangeable), 10.37 (s, 1H, OH, D_2O-exchangeable). ^{13}C-NMR/APT (100 MHz, DMSO-d_6) δ: 44.13 (CH_2), 54.63 (CH), 112.65 (2CH), 115.18 (CH), 115.94 (CH), 116.68 (CH), 117.03 (C_q), 118.64 (2CH), 119.24 (CH), 120.06 (CH), 122.20 (C_q), 126.91 (CH), 127.60 (CH), 127.68 (2CH), 128.94 (CH), 129.97 (2CH), 130.36 (CH), 131.39 (CH), 134.10 (C_q), 134.13 (C_q), 139.57 (C_q), 145.94 (C_q), 150.39 (C_q), 152.31 (C_q), 156.78 (C_q), 158.05 (C_q).

3-(1-(4-Sulfonamidephenyl)-4,5-dihydro-5-(3-(4-hydroxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)-1*H*-pyrazol-3-yl)phenol **8f**

Yield 30%, mp 170°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 3.30 (dd, 1H, $J = 6.72$, 17.60 Hz, C_4-H pyrazoline), 4.06 (dd, 1H, $J = 12.28$, 17.60 Hz, C_4-H pyrazoline), 5.62 (dd, 1H, $J = 6.40$, 11.92 Hz, C_5-H pyrazoline), 6.83 (d, 1H, $J = 7.29$ Hz, Ar), 6.93 (d, 2H, $J = 8.11$ Hz, Ar), 7.00–7.03 (m, 4H, 2Ar + SO_2NH_2 D_2O-exchangeable), 7.19–7.28 (m, 4H, Ar), 7.44 (t, 2H, $J = 7.80$ Hz, Ar), 7.60 (t, 4H, $J = 8.9$ Hz, Ar), 7.82 (d, 2H, $J = 8.02$ Hz, Ar), 8.27 (s, 1H, C_5-H pyrazole), 9.60 (s, 1H, OH, D_2O-exchangeable), 9.72 (s, 1H, OH, D_2O-exchangeable). ^{13}C-NMR/APT (100 MHz, DMSO-d_6) δ: 43.05 (CH_2), 55.64 (CH), 112.61 (CH), 112.91 (CH), 115.87 (CH), 116.07 (2CH), 117.04 (CH), 117.75 (CH), 118.52 (2CH), 122.04 (C_q), 123.80 (C_q), 126.67 (CH), 127.55 (2CH), 129.83 (2CH), 129.93 (2CH), 130.17 (CH), 130.22 (CH), 133.56 (C_q), 133.58 (C_q), 139.66 (C_q), 146.63 (C_q), 150.37 (C_q), 150.61 (C_q), 157.90 (C_q), 158.09 (C_q).

Biological Assays

Cyclooxygenase Inhibition Assays

The ability of compounds **3a**–**b**, **6a**–**c**, **4a**–**d**, **7a**–**c** and **8a**–**f** listed in Table 1 to inhibit COX-1 and COX-2 (IC_{50} value, μM) was determined using an enzyme immunoassay (EIA) kit (catalog No. 560131, Cayman Chemical, Ann Arbor, MI, U.S.A.) according to the previously reported method.[55]

In Vivo Anti-inflammatory Assay

The compounds **4a**–**d** and **8a**–**f** and the reference drug celecoxib were evaluated using the *in vivo* carrageenan-induced rat foot paw edema model and the measurement of paw volume was done after 1, 2, 3 and 4 h of carrageenan injection as the reported procedure.[56] Briefly, The left paw was measured once before (normal baseline) and then after carrageenan injection at 1, 2, 3, and 4 h intervals. Animals were divided to thirteen groups six in each one. The first group represented the normal control group (no carrageenan, no drug), the second represented the carrageenan group, the third was given Celecoxib (50 mg/kg IP) as reference drug and the remaining groups were treated with the tested compounds (50 mg/kg IP) one hour before carrageenan (Sigma, U.S.A.) injection (1% w/v, 0.1 mL/paw). Paw volume was measured by using a water displacement plethysmometer (UGO BASILE 21025 COMERIO, ITALY). The percent change in paw volume compared to base line measurement was taken as the criteria of comparison and was calculated as follows;

The percentage increase in paw volume was calculated using :

$$\%\text{Edema} = (\text{volume of test} / \text{baseline volume}) \times 100 - 100.$$

The percentage (%) inhibition was calculated using:

$$\text{Percent inhibition} = (1 - D / C) \times 100$$

Where, D-represents the percentage difference in increased paw volume after the administration of test drugs to the rats. C-represents the percentage difference of increased volume in the control group.

Ulcerogenic Liability

Ulcerogenic liability of ten compounds **4a**–**d** and **8a**–**f** in comparison with celecoxib and diclofenac sodium was evaluated using 50 mg/kg oral dose according to the reported procedure.[57]

Evaluation of PGE_2 Inhibition in Rat Serum Samples

Serum samples were collected 4 h after carrageenan injection and PGE_2 was measured by Rat PGE_2 (Prostaglandin E_2) enzyme-linked immunosorbent assay (ELISA) Kit (Elabscience, Catalog No: E-EL-R0107), and the results were expressed as pg/mL.

Evaluation of TNFα Inhibition in Rat Serum Samples

TNFα was assessed using Rat TNFα ELISA Kit (CUSABIO, Catalog Number. CSB-E11997r) and the results were expressed as pg/mL.

Ethanol-Induced Rodent Gastric Ulcer Model

Compounds **4c** and **8d** in comparison with famotidine were evaluated using 50 mg/kg oral dose. Animals were divided into four groups (six rats each). One group received saline as control; the second group received famotidine (50 mg/kg *per os* (*p.o.*)) and the remaining groups received the tested compounds **4c** and **8d** (50 mg/kg *p.o.*). One hour later, gastric lesion was induced in rats by intragastric administration of 1 mL ethanol (99% (v/v)) to rats that had been fasted for 18 h with access to water. Rats were sacrificed 1 h after ethanol administration by cervical dislocation after being lightly anesthetized with ether. Stomach of experimental rats was excised, washed with saline and ulcer index was measured.[66,67]

Molecular Docking In order to further elucidate the mechanism of binding and selectivity of the synthesized compounds a docking experiment was carried out. Compound showing the highest *in vivo* activity **8d** and celecoxib were docked in the active site of both COX-1 and COX-2 enzymes using Maestro 11.4 (Schrödinger Release 2017-4: Maestro; Schrödinger, LLC: New York, NY, U.S.A., 2017). The compound **8d**, celecoxib, crystal structures of COX-1 (pdb code: 5WBE)[69] and COX-2 (pdb code: 3LN1)[70] were prepared for docking using Maestro tools (Ligprep and protein preparation wizard). A grid box centered on the native ligand was used to define the binding pocket of the protein. Depending on the co-crystalized ligand, bond constrain have been used where we pick Gln 178, Arg 499 and Phe 504 for COX-2 and Arg 120, Tyr 355 and Hie 90 for COX-1 (at least one of these amino acids should participate in bond interaction during docking). Extra precision (XP) setting have been used during docking.

Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.

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