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Original article

Synthesis and SAR/3D-QSAR studies on the COX-2 inhibitory activity of 1,5-diarylpyrazoles to validate the modified pharmacophore

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Abstract

Diverse analogs of 1,5-diarylpyrazoles having 3-hydroxymethyl-4-sulfamoyl (SO_2NH_2)/methyl sulfonyl (SO_2Me)-pheny group at N^1 were synthesized and evaluated for their in vitro cyclooxygenase (COX-1/COX-2) inhibitory activity. The SAR study mainly involved the variations at positions C-3, C-5 and N^1 of the pyrazole ring. Several small hydrophobic groups at/around position-4 of C-5 phenyl, viz. 3,4-dimethylphenyl analog **9**, 3-methyl-4-methylsulfanylphenyl analog **14** and 2,3-dihydrobenzo[b]thiophenyl analog **17**, exhibited impressive COX-2 inhibitory potency. In general, the sulfonamide analogues with a CHF_2 at C-3 were found to be more potent than those having a CF_3 group. The three dimensional quantitative structure activity relationship comprising comparative molecular field analysis (3D-QSAR-CoMFA) afforded the models with high predictivity which further validated the acceptance of hydroxymethyl (CH_2OH) group in the hydrophilic pocket of the COX-2 enzyme.

Keywords: COX-2 inhibitors; 1,5-Diarylpyrazoles; SAR; 3D-QSAR

1. Introduction

The cyclooxygenase (COX) exists in two isoforms [1–3]. The COX-1, a constitutive enzyme, primarily expressed in gastrointestinal (GI) tract, is responsible for the biosynthesis of prostaglandins (PGs) required for the cytoprotection and platelet aggregation [4,5]. Hence, any disturbance in its routine function for long time leads to gastrointestinal ulceration, bleeding and perforation [6,7]. In contrast, the COX-2, an inducible enzyme, produced during injury by the pro-inflammatory cytokines, viz. tumor necrosis factor-α (TNF-α), interleukines, mitogens and endotoxins, plays a major role in the biosynthesis of PGs required by inflammatory cells such as monocytes and macrophages to cause pain, inflammation and fever [8–10]. Thus, the selective inhibition of COX-2, sparing COX-1, emerged as the new way of treating inflammatory disorders with greater GI safety. This new concept was proved in clinic with the subsequent launch of two drugs, viz. celecoxib [11] and rofecoxib [12] for the treatment of rheumatoid and osteoarthritis. The continued effort in this area further introduced valdecoxib [13] and etoricoxib [14,15] as second generation therapy for the chronic inflammation (Fig. 1). Apart from inflammation, few COX-2 inhibitors are also being studied for treating other ailments like cancer [16,17] and Alzheimer's disease [18]. Though few adverse reports have also appeared about this treatment [19], by and large the patients on COX-2 inhibitors enjoy a greater benefit over the conventional NSAIDs therapy.

Unlike the diverse chemical classes of non-selective NSAIDs, the COX-2 inhibitors have two major structural motifs: the acidic methanesulfonamide ($MeSO_2NH$) containing diphenyl ethers exemplified by nimesulide [20,21] and sulfamoyl (SO_2NH_2)/methanesulfonyl (SO_2Me) containing vicinal diarylheterocycles represented by celecoxib, valdecoxib, rofecoxib and etoricoxib (Fig. 1). The latter class received more attention due to the known COX-2 enzyme-ligand co-crystal structure [22], and various carbocycles

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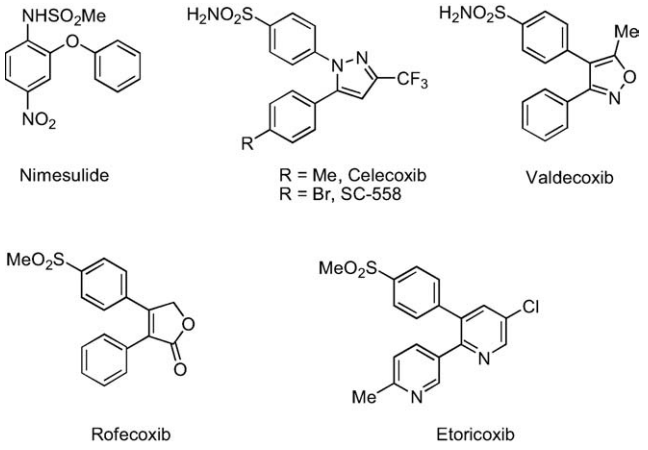


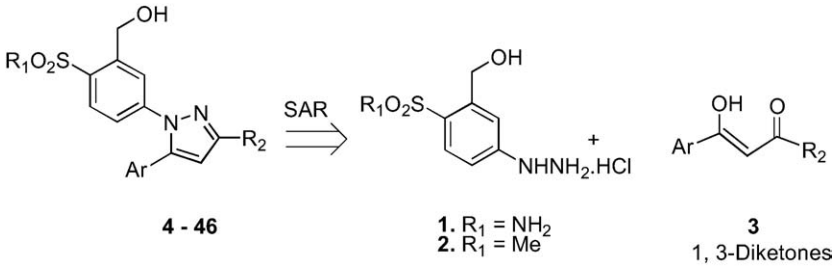
Fig. 1. COX-2 inhibitors.

[23,24] and heterocycles [25–27] were discovered on this basis. Though many COXIBs (COX-2 inhibitors) have been launched so far, there still remains a need to develop more effective drugs as an alternative to the steroidal and narcotic drugs used in severe pains.

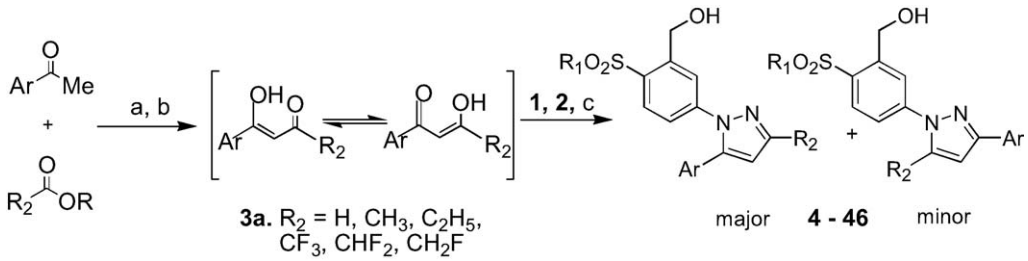
While introduction of small hydrophobic groups (F, Me) adjacent to sulfonamide in diarylheterocycles and their influence on binding with the hydrophilic region of COX-2 enzyme have yielded encouraging results [28,29], the effect of hydrophilic groups at this position has not yet been explored. Thus, we contemplated to introduce few such groups, viz. hydroxyalkyl and acylaminoalkyl adjacent to sulfonamide to study their binding as well as pharmacological impact on the COX-2 enzyme. Few compounds with the smallest hydroxymethyl (CH_2OH) group have already shown effective binding with the enzyme and impressive COX-2 selectivity [30,31]. With a view to optimize and validate the effect of this change, we synthesized its diverse analogues involving C-3, C-5 and N^1, and report herein the outcome of the SAR and 3D-QSAR-CoMFA [32] study on the series.

2. Chemistry

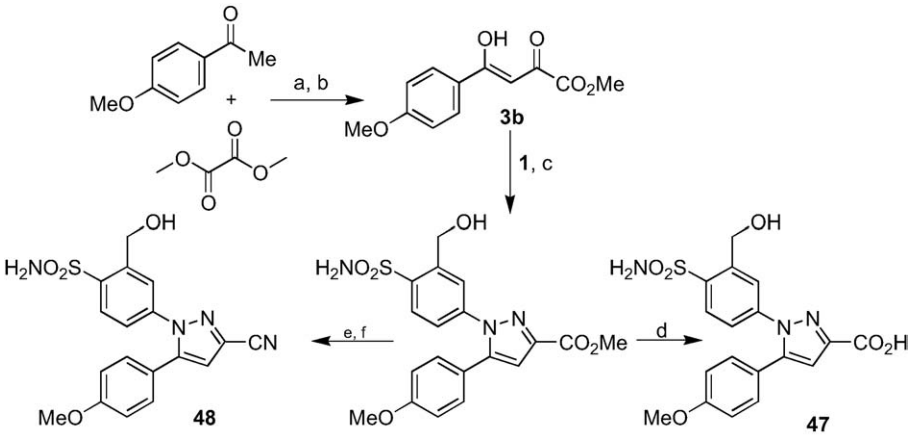
Disconnection for the synthesis of desired 1,5-diarylpyrazoles **4–46** for SAR study is depicted in Scheme 1. The basic requirement for the synthesis of these pyrazoles was the availability of corresponding phenyl hydrazine hydrochlorides **1–2**, the multi-step synthesis of which will be discussed elsewhere. Simple coupling of the latter with appropriate 1,3-diketones **3** in absolute ethanol under heating condition afforded the desired diarylpyrazoles **4–46** (Scheme 2) [29–31,33]. Though the regiomeric bias was normally in favor of 1,5-diarylpyrazoles, the minor undesired regio isomers were easily eliminated by triturating the product with a mixture of ethyl acetate–toluene after column chromatographic purification. The required 1,3-diketones **3** were synthesized by Claisen condensation using appropriate ethanones and ethyl/methyl ester of trifluoroacetic acid, difluoroacetic acid, fluoroacetic acid, acetic acid, formic acid, propionic acid and oxalic acid. This reaction was carried out under slightly modified condition involving sodium hydride in dry DMF at a temperature of –5 to 30 °C to afford 1,3-diketones in 95–98% yield. The commercially unavailable ethanones (substituted acetophenones) were prepared according to the standard literature procedures [11]. The sulfoxide (SOMe) analog **16** was prepared by the controlled oxidation of corresponding methylsulfanyl (SMe) analog **14** using m-CPBA at 0–5 °C [34]. The 3-carboxylic acid analog **47** was synthesized by the saponification of corresponding methyl ester of 1,5-diarylpyrazole (Scheme 3). The 3-cyano analog **48** was prepared by converting this ester to corresponding carboxamide by stirring with aqueous ammonia in a sealed flask at room temperature and dehydrating the latter with TFFA–pyridine [35]. All the compounds reported herein were characterized spectroscopically.



Scheme 1.



Scheme 2. (a) NaH/DMF, –5 → 30 °C, 4–5 h. (b) 2 N HCl. (c) Absolute ethanol, 50–60 °C, 12 h.



Scheme 3. (a) NaH/DMF, –5 → 30 °C, 4–5 h. (b) 2 N HCl. (c) Absolute ethanol, 50–60 °C, 12 h. (d) NaOH/MeOH, 5 → 35 °C, 3–4 h. (e) Aq. NH_3/MeOH, 20–25 °C, 15 h. (f) TFAA/Pyridine, dioxane, room temperature, 14 h.

3. Pharmacology

Initially, all the compounds were screened for their ability to inhibit COX-2 (recombinant human enzyme, expressed in sf-9 cells infected with baculovirus) and COX-1 (obtained from microsomal fraction of Ram Seminal Vesicles) at 100 μM. Based on the initial in vitro efficacy, the promising compounds were further tested at lower concentrations. The enzyme activity was measured by TMPD method and IC_{50}s were calculated using non-linear regression analysis of percent inhibitions [36,37]. The in vitro potent compounds were preliminary screened in the carrageenan induced rat paw edema model of inflammation [38]. Celecoxib and indomethacin were used as reference COX-2 selective and non-selective inhibitors respectively.

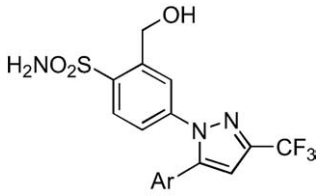
3.1. Results and discussion

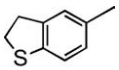
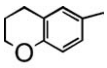
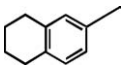
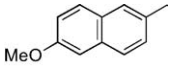
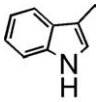
The analogs of the new series have broadly been divided into three classes based on the site of variation. Compound **25** was selected from the previous study for the ease of comparison.

3.1.1. Variation at position C-5

Since we already observed a trend of COX-2 selectivity due to monosubstituted phenyl group at this position [30], we opted here the diverse variations like disubstituted phenyls, heterocycles, fused carbocycles, fused heterocycles, saturated carbocycles, partially saturated fused carbocycles and partially saturated fused heterocycles for the study. The in vitro COX-1/COX-2 inhibitory activity of these analogs is depicted in Table 1. Among dihalogenated phenyls, 2,4-difluoro analog **4** (1.680 μM) was found to be more potent than the corresponding dichloro analog **6** (5.100 μM). While 3,4-difluoro analog **5** suffered a loss in activity (6.900 μM), the combination of both, 3-chloro-4-fluoro analog **7** drastically improved the COX-2 potency (0.570 μM). Lesser potency of compound **5** was not surprising because simple 4-fluoro analog also showed least potency among the mono halogenated analogs [30]. Combination of two methyl groups such as 3,4-dimethyl analog **9** enhanced the COX-2 potency further (0.411 μM) whereas 2,4-dimethyl analog **8** lost the activity drastically (8.500 μM). Generally 3,4-disubstituted analogs such as 3-methyl-4-fluoro analog **10** (0.830 μM), 3-chloro-4-methoxy analog **13** (1.300 μM), 3-methyl-4-methylsulfanyl **14** (0.139 μM) and 3-methoxy-4-methylsulfanyl **15** (1.390 μM) exhibited a far superior potency than the 2,4-disubstituted analogs **6**, **8** and **11**. This observation can be attributed to the bulkier groups which when near to the central heterocyclic ring (e.g. position-2 of the C-5 phenyl ring) may be causing distortion to the *cis*-stilbene geometry of the vicinal diaryl system required for COX-2 potency. Electronic factor seems to be less important at position-2, as fluorine even being highly electronegative shows better activity because of its small size (e.g. compound **4**) causing less geometrical distortion. On the other hand the electronic factor becomes very important at/around position-4 and fluorine being highly electronegative decreases the activity of compound **5**. Such could be the reason for the most potent compound **14** becoming inactive when converted to the corresponding sulfoxide analog **16**. However, suitable hydrophobic groups such as in 3,4-disubstituted compounds **7**, **9**, **10**, **13**–**15** being favorable, enhanced the potency dramatically. Observing the favorable effect of hydrophobic groups at positions-3 and 4, a similar hydrophobic environment was created by introducing partially saturated fused heterocycles and carbocycles such as 2,3-dihydrobezothiophenyl analog **17**, 6-chromanyl analog **18** and 1,2,3,4-tetrahydronaphthyl analog **19**. While **17** being prototype of the most potent compound **14**, remained the best (0.227 μM) among these, **18** and **19** also showed COX-2 activity even after being reasonably bulkier. But the cyclohexyl analog **20** completely turned out to be non-selective. The fused aromatics, 6-methoxynaphthyl analog **21** and 3-indolyl analog **22** also exhibited very good COX-2 activity but the simple heterocycles such as 3-thienyl **23** and 3-pyridyl **24** were found neither active nor selective. Possible reason for the poor activity of **23** and **24** could be the lack of effective access of these C-5 substitutions to the hydrophobic region of the COX-2 enzyme.

Table 1
In vitro data of C-5 substituted 1,5-diarylpyrazoles



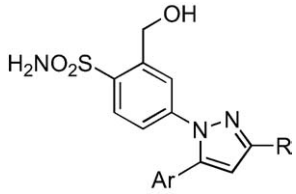
Compound	Ar	IC_{50} (μM) [a]	
		COX-1	COX-2
4	2,4-F_2-phenyl	>850.0	1.680
5	3,4-F_2-phenyl	500.0	6.900
6	2,4-Cl_2-phenyl	500.0	5.100
7	3-Cl,4-F-phenyl	393.0	0.570
8	2,4-Me_2-phenyl	500.0	8.500
9	3,4-Me_2-phenyl	108.0	0.411
10	3-Me,4-F-phenyl	199.0	0.830
11	2,4-OMe_2-phenyl	500.0	11.600
12	3-F,4-OMe-phenyl	500.0	4.450
13	3-Cl,4-OMe-phenyl	>500.0	1.300
14	3-Me,4-SMe-phenyl	130.0	0.139
15	3-OMe,4-SMe-phenyl	300.0	1.390
16	3-Me,4-SOMe-phenyl	9 [b]	3 [b]
17		64.0	0.227
18		100.0	1.000
19		100.0	2.100
20		100 [c,d]	100 [c,d]
21		>200.0	0.930
22		>200.0	1.546
23		0 [c]	52 [c]
24		26 [c]	2 [c]
Indomethacin	–	0.067	7.810
Celecoxib	–	10.750	0.076

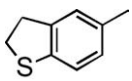
[a] Mean of three determinations.
[b] % Inhibition at 100 μM.
[c] % Inhibition at 10 μM.
[d] IC_{50} not determined. [>] Precipitation observed beyond this concentration (IC_{50}s may be much higher than the reported values).

3.1.2. Variation at position C-3

Since the newly introduced 3-hydroxymethyl-4-sulfamoylphenyl group at N^1 has generated encouraging SAR with respect to C-5 substitution, it became obvious to reassess the suitability of C-3 substitution. The effect of the systematic variation at this position is depicted in Table 2. Number of fluorine atoms were gradually removed from one of the most COX-2 selective compounds **25** containing a CF_3 group [30]. The CHF_2 analog **26** exhibited a reasonable improvement in potency (0.236 μM) whereas CH_2F analog **27** was found to be almost inactive. In contrast, the CH_3 analog **28**, again turned out to be selective inhibitor of COX-2 (0.660 μM). But, complete removal of this methyl group, e.g. compound **29** and its replacement with an ethyl group e.g. compound **30**, led to complete loss of activity. This was a unique observation where methyl group at C-3 was found to bring COX-2 selectivity. Compound **31** was synthesized to further confirm this observation and was found to have similar effect. The variation observed due to the above fluorinated methyl substituents at C-3 in compounds **25**–**27** can mainly be attributed to the number of fluorine atoms causing deviation in the net dipole moment and finally in the orientation of the molecules from the COX-2 active site. But, the activity of the C-3 methyl analogs **28** and **31** could only be explained on the basis of its suitable size for hydrophobic interaction with the COX-2 enzyme. The corresponding hydrogen and ethyl replacement of this methyl group was found to be inactive probably due to the ineffective hydrophobic interaction with

Table 2
In vitro data of C-3 and C-5 substituted 1,5-diarylpyrazoles



Compounds	Ar	R	IC_{50} (μM)[a]	
			COX-1	COX-2
25	4-OMe-phenyl	CF_3	63.0	0.365
26	4-OMe-phenyl	CHF_2	39.4	0.236
27	4-OMe-phenyl	CH_2F	82 [b]	58 [b]
28	4-OMe-phenyl	Me	67.0	0.660
29	4-OMe-phenyl	H	0 [b]	64 [b]
30	4-OMe-phenyl	Et	10 [b]	54 [b]
31	4-Cl-phenyl	Me	349.2	1.007
32	4-Me-phenyl	CHF_2	412.0	0.502
33	3-Me-phenyl	CHF_2	500.0	1.300
34	4-Et-phenyl	CHF_2	>500.0	1.000
35	3-Cl-phenyl	CHF_2	>500.0	2.200
36	4-SMe-phenyl	CHF_2	41.0	0.213
37	4-NHEt-phenyl	CHF_2	93.0	1.45
38	2,4-F_2-phenyl	CHF_2	156.0	1.000
39	3,4-Cl_2-phenyl	CHF_2	59.0	0.371
40	3,4-Me_2-phenyl	CHF_2	134.0	1.140
41	3-Me,4-F-phenyl	CHF_2	100.0	1.000
42	3-Me,4-SMe-phenyl	CHF_2	48 [b], 0 [c]	99 [b], 26 [c]
43		CHF_2	50 [b,d]	100 [b], 23[c]

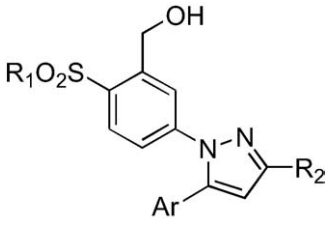
[a] Mean of three determinations.
[b] % Inhibition at 100 μM.
[c] % Inhibition at 10 μM.
[d] IC_{50} not determined. [>] Precipitation observed beyond this concentration (IC_{50}s may be much higher than the reported values).

the COX-2 pocket. Since we observed a significant improvement caused by CHF_2 over CF_3, more number of analogs were studied for this change. But this improvement was found to be limited to the smaller groups at/around position-4 of the C-5 phenyl ring, e.g. 4-methyl analog **32** (0.502 μM), 4-methylsulfanyl analog **36** (0.213 μM) and 3,4-dichloro analog **39** (0.371 μM) only exhibited better potency. Other CHF_2 analogs of potent C-5 substituted compounds selected from Table 1, such as 3,4-dimethyl analog **40** and 3-methyl-4-fluoro analog **41** suffered a reasonable loss in potency, while 3-methyl-4-methylsulfanyl analog **42** and 2,3-dihydrobezothiophenyl analog **43** even turned out to be inactive. The reason for this adverse observation may be the imbalance caused by deficiency of fluorine atoms in case of CHF_2 from the optimally balanced system due to hydrophobic groups at/around position-4 of C-5 phenyl ring in conjugation with CF_3. An entirely different variation at C-3 was brought about by introducing a carboxylic acid group e.g. in compound **47** and a cyano group in compound **48** which were, respectively, found to be inactive and COX-1 selective (Table 3).

3.1.3. Variation at N^1

Different polar and non-polar groups were studied adjacent to sulfonamide/methylsulfonyl during the search of second substitution in this benzene ring and a few best performing C-5 substitutions were selected here to conclude the SAR for sulfonamide vs. methylsulfonyl group [30]. The results are mentioned in Table 3. The compounds with methylsulfonyl (SO_2Me) group, such as 4-methoxyphenyl analog **44**, and 4-methylsulfanylphenyl analogs **45** and **46** exhibited lesser potency when compared to the corresponding sulfonamide (SO_2NH_2) analogs. The poor in vitro potency exhibited by methylsulfonyl group is a general observation, and is due to its comparatively lesser tendency to form hydrogen bond (donor) with the polar region of COX-2 enzyme.

Table 3
In vitro data of 4-methylsulfonyl and 4-sulfamoylphenyl containing 1,5-diarylpyrazoles



Compounds	R_1	Ar	R_2	IC_{50} (μM)[a]	
				COX-1	COX-2
44	SO_2Me	4-OMe-phenyl	CF_3	>300.0	3.200
45	SO_2Me	4-SMe-phenyl	CF_3	300.0	3.000
46	SO_2Me	4-SMe-phenyl	CHF_2	300.0	2.000
47	SO_2NH_2	4-OMe-phenyl	CO_2H	0 [b]	61 [b]
48	SO_2NH_2	4-OMe-phenyl	CN	96 [b]	0 [b]

[a] Mean of three determinations.
[b] % Inhibition at 100 μM. > Precipitation observed beyond this concentration (IC_{50}s may be much higher than the reported values).

3.2. 3D-QSAR-CoMFA study

Since the percentage inhibition could not be converted to IC_{50}/MIC values, the compounds having experimental IC_{50}s were only considered for CoMFA study. Out of 34 such compounds, 24 were incorporated in training set and the remaining 10 in the test set. These sets were formed based on the diversity in structure and enzyme activity. The statistical validity of the model was judged by the high values of q^2 (more than 0.5) and r^2 (more than 0.9) with low standard error of estimate (SEE). Additional descriptors such as ClogP, CMR and dipole moment were used as independent variables to obtain an improved 3D-QSAR model (Table 4) [39,40]. However, these descriptors led to the reduced values of r^2 and q^2. Among significant models, the one with highest q^2 value was utilized for further analysis. While value of the cross validated q^2 was found to be 0.571 with six components, the non-cross validated r^2 was found to be 0.986 (SEE = 0.071). The CoMFA contour plots for the steric and electrostatic fields are shown in Fig. 3a, b, respectively.

The actual and predicted pIC_{50} values of the training set are shown in Table 5. The graph of predicted versus actual activity for the training set revealed an excellent correlation between the two. The model was validated by predicting the activity of the test set compounds (Table 6). This model also afforded a significant value of r^2_{pred} as 0.591, and the predicted activity was highly consistent with the experimental data. A graph depicting the actual versus predicted activity of training and test set compounds is shown in Fig. 4. All the residual values for the prediction were less than 1 log order of activity. The CoMFA analysis provided contour plots of steric and electrostatic interactions (Fig. 3). The green contour near position-3 of C-5 phenyl ring indicated that a medium size substituent would enhance the activity, and it was found that the 3-methyl group of the most active compound **14** occupied the same contour. The 2,3-dihydrobenzothiophenyl ring of compound **17** also extended towards this green contour and this could be the reason for its high potency. The 3-fluoro group of compound **5** keeping away from this contour was found to be less potent. The yel-

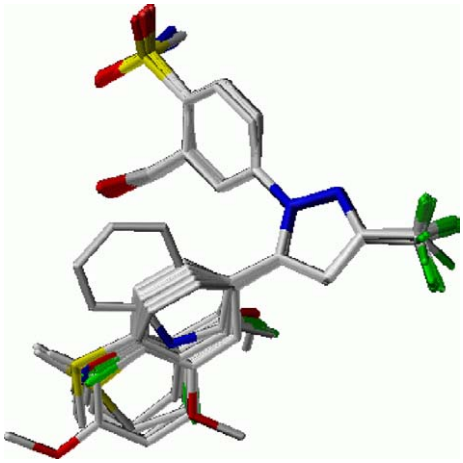
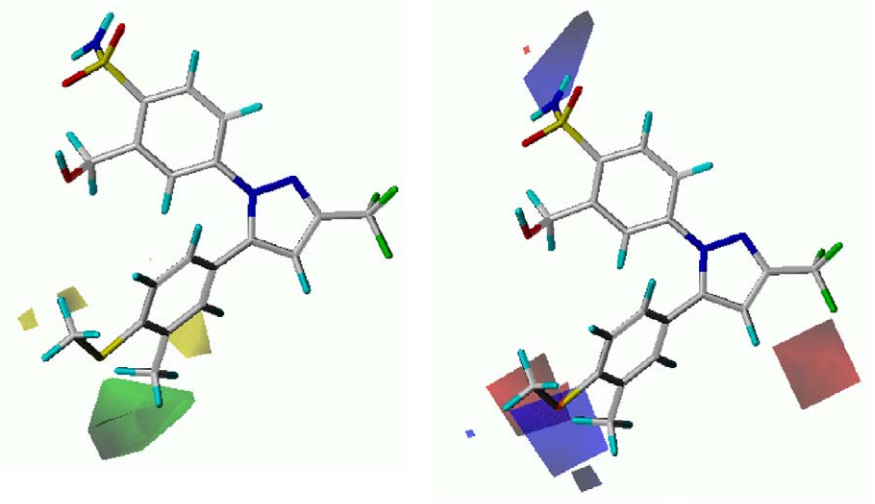


Fig. 2. Alignment of 1,5-diarylpyrazoles.

Table 4
PLS statistics of CoMFA studies

Fields	CoMFA	CoMFA with additional descriptors		
		ClogP	Dipole moment	CMR
r^{2a}	**0.986**	0.978	0.973	0.976
N[b]	6	6	6	6
q^{2c}	**0.571**	0.530	0.519	0.517
SEE[d]	0.071	0.089	0.098	0.093
F-value[e]	197.865	126.147	103.171	114.527
Field contribution[f]	0.524(S)	0.493 (S)	0.516 (S)	0.507 (S)
	0.476(E)	0.463 (E)	0.483 (E)	0.482 (E)
		0.046 (ClogP)	0.001 (DM)	0.011 (CMR)

[a] Non-cross validated correlation coefficient.
[b] Optimum number of components obtained form cross validated PLS analysis and used in final non-cross validated analysis.
[c] Cross validated correlation coefficient.
[d] SEE.
[e] *F*-test value.
[f] Field contributions: steric (S), electrostatic (E), and dipole moment (DM) fields from CoMFA.



a b

Fig. 3. CoMFA contour plots with compound **14** (a) steric: green-bulky groups favorable; yellow-bulky groups unfavorable (b) electrostatic: blue-electropositive groups favorable; red-electronegative groups favorable.

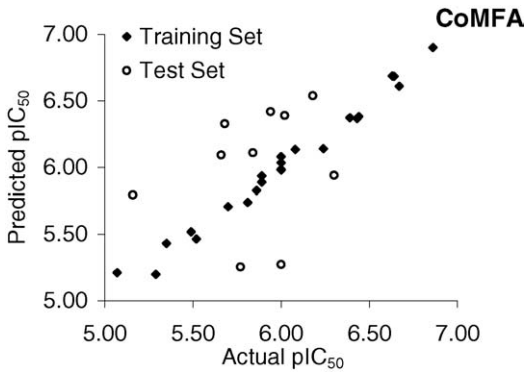


Fig. 4. Predicted values vs. experimental values for the training and test sets of CoMFA.

low contour accommodated position-2 of C-5 phenyl ring, thereby indicating a steric group to be less favorable, e.g. 2,4-dimethylphenyl compound **8** and 2,4-dimethoxy analog **11**. Blue contour near position-3 of phenyl ring indicated electropositive groups enhancing the activity, and that was probably the reason for compound **14** to be so potent. The presence of electronegative groups at this position, viz. 3-chloro and 3-fluoro could be the reason for the less potency of compounds **5**, **12**, **13** and **35**. Red contour seen near position-4 of C-5 phenyl ring indicated electronegative groups enhancing the activity profile e.g. in compounds **7**, **10** and **31** (Tables 1 and 2). The electropositive groups at this position in compounds **8** and **11** led to poor activity. Red contour seen near CF_3 group indicated electronegative groups favorable at this position. This could be the reason for most of the compounds having CF_3/CHF_2 group at this position to be potent inhibitors of COX-2. Blue contour surrounding NH_2 (SO_2NH_2) group indicates electropositive groups enhancing the activity. This could be the possible reason for sulfonamide (SO_2NH_2) containing compounds to be more potent than those having methylsulfonyl (SO_2Me) group.

Superposition of the CoMFA coefficient contour maps with the most active compound **14** in the active site of COX-2 is shown in Fig. 5. These studies established a meaningful cor-

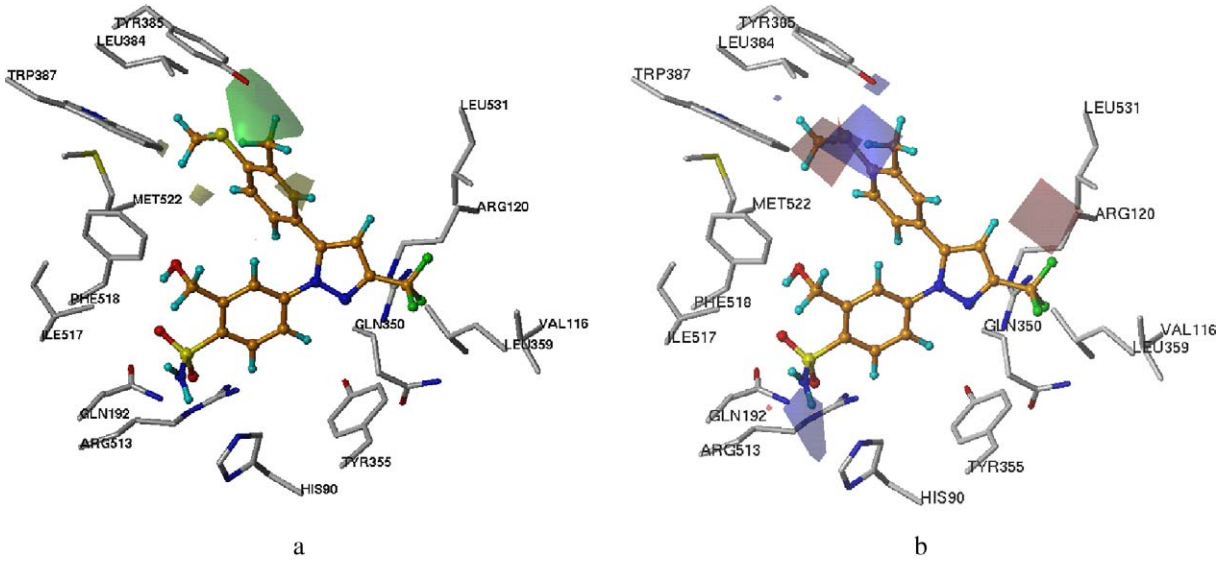


Fig. 5. Superimposition of (a) steric and (b) electrostatic CoMFA contour plots in the active site of COX-2. Most active compound **14** is shown in the background.

Table 5
Experimental pIC_{50}, corresponding predicted values and residuals of molecules used in training set for CoMFA

Compounds	Actual pIC_{50}	Predicted pIC_{50}	Residuals
6	5.29	5.20	0.09
7	6.24	6.14	0.10
8	5.07	5.21	–0.14
9	6.39	6.37	0.02
10	6.08	6.13	–0.05
11	4.94	4.92	0.02
12	5.35	5.43	–0.08
13	5.89	5.94	–0.05
14	6.86	6.90	–0.04
15	5.86	5.83	0.03
17	6.64	6.68	–0.04
18	6.00	5.98	0.02
22	5.81	5.74	0.07
25	6.44	6.38	0.06
26	6.63	6.69	–0.06
31	6.00	6.04	–0.04
33	5.89	5.89	0.00
34	6.00	6.08	–0.08
36	6.67	6.61	0.06
39	6.43	6.37	0.06
41	6.00	5.99	0.01
44	5.49	5.52	–0.03
45	5.52	5.46	0.06
46	5.70	5.71	–0.01

relation between receptor–ligand binding and the biological activity. Superposition of the steric fields on the binding site of COX-2 enzyme is shown in Fig. 5a. The 3-methyl group of C-5 phenyl ring in compound **14** nearing green contour could be mapped to the hydrophobic residues such as Phe-381, Leu-384 and Tyr-385 of the COX-2 pocket. Yellow contour seen near position-2 of the C-5 phenyl ring indicated that the groups with appropriate size were required to bind this narrow pocket. Superposition of the electrostatic fields

Table 6
Experimental pIC_{50}, corresponding predicted values and residuals of molecules used in test set

Compounds	Actual pIC_{50}	Predicted pIC_{50}	Residuals
4	5.77	5.29	0.48
5	5.16	5.73	–0.57
19	5.68	6.20	–0.52
21	6.03	6.23	–0.20
28	6.18	6.47	–0.29
32	6.30	5.74	0.56
35	5.66	5.95	–0.29
37	5.84	6.07	–0.23
38	6.00	5.29	0.71
40	5.94	6.30	–0.36

on the binding site of COX-2 enzyme is shown in Fig. 5b. The blue contour seen at position-3 of the C-5 phenyl ring indicated the electropositive groups causing increased interactions with residues Val-349, Leu-352 and Leu-384 whereas red contour near position-4 of this ring indicated electronegative groups showing favorable interactions with Tyr-385 and Trp-387. Red contour above the CF_3 group indicated electronegative substituent forming more favorable electrostatic interactions with guanidyl group of Arg-120. Blue contour above the amino group of sulfonamide indicated an electropositive group interacting better with hydrophilic region formed by His-90, Gln-192 and Ile-517.

3.3. In vivo results

The in vitro potent compounds were tested in the carrageenan induced rat paw edema model at 30 mg kg^{-1} where compounds **4**, **10**, **14**, **17, 18**, **21**, **26**, **28**, **31**, **32** and **36** showed 50–60% reduction in paw volume [38].

4. Conclusion

Herein, we presented the synthesis and a systematic SAR study on 1,5-diarylpyrazole class of COX-2 inhibitors with a modified pharmacophore. The results of the variations at C-3, C-5 and N^1 afforded many potent inhibitors of COX-2. Of many variations at the most sensitive C-5 phenyl ring, the small hydrophobic groups at position-3 and 4, e.g. 3-methyl-4-methylsulfanyl analog **14** and their saturated cyclic prototype 2,3-dihydrothiophenyl analog **17** exhibited excellent COX-2 inhibitory potency. Generally, the sulfonamide analogues with a CHF_2 group at C-3 were found to be better than those having CF_3. Thus, the biological findings coupled with a systematic 3D-QSAR-CoMFA study supported the acceptance of newly introduced hydroxymethyl (CH_2OH) group adjacent to sulfonamide in the hydrophilic region of the COX-2 enzyme.

5. Experimental section

5.1. Chemistry

Acetophenones and halo esters were either purchased from Lancaster/Aldrich Co. or prepared according to the standard procedures. While celecoxib was prepared according to the literature procedure [11], indomethacin was extracted from the capsules purchased from the medical store. The yields reported are unoptimized. Melting points were determined on Buchi melting point B-540 apparatus and are uncorrected. IR spectra were recorded on Perkin–Elmer FT-IR 1650 spectrometer. ^{1}H NMR and ^{13}C NMR experiments were, respectively, performed at 200 and 50 MHz on Varian Gemini 200 spectrometer and their chemical shifts are reported in δ units with respect to TMS as internal standard. Mass spectra were recorded on HP-5989A spectrometer. Elemental analyses were carried out for C, H, N using Perkin–Elmer 2400 series II CHN-O analyzer. All the analyses were performed by the Analytical Research Group of Discovery Research-Dr. Reddy's Laboratories Ltd. The purity of the final compounds were determined by HPLC using "System 1" which consisted column Hichrom RPB (250 mm), mobile phase 0.01 M KH_2PO_4/CH_3CN (50:50) and "System 2" which comprised column Intersil ODS 3V (250 mm), mobile phase H_2O/CH_3CN (50:50), both running at 1.0 ml min^{-1} with UV detection at respective λ_{max}.

5.2. General procedure for 1,3-diketones *3*. Representative preparation of 4,4,4-trifluoro-1-(4-methoxyphenyl)-1,3-butanedione *3a* (Ar = 4-OMe-Ph; $R_2 = CF_3$)

4-Methoxyacetophenone (5.00 g, 33.33 mmol) was dissolved in 25 ml of dry DMF under argon atmosphere and 60% NaH (1.66 g, 41.66 mmol) was added in three lots maintaining temperature between –5 and 0 °C. After stirring at this temperature for 0.5 h, ethyl trifluoroacetate (4.97 ml, 41.66 mmol) was injected and the reaction mixture was allowed to stir at ambient temperature for 4–5 h. The reaction mixture was poured into ice water, acidified with 2 N HCl and extracted with ethyl acetate. The combined organic layer was washed with water, dried and evaporated to get a residue which was stirred with petroleum ether to afford a low melting solid of the title compound (7.37 g, 90%). This solid was used in the next step without further purification [11]. IR (Neat) 3330, 1614, 1405 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 7.80 (d, J = 8.2 Hz, 2H), 7.35 (d, J = 8.0 Hz, 2H), 6.52 (s, 1H), 3.85 (s, 3H). MS (CI Method) 246 (M^+).

5.3. General procedure for 1,5-diarylpyrazoles. Representative preparation of 2-hydroxymethyl-4-[5-(4-methoxyphenyl)-3-trifluoromethyl-1H-1-pyrazolyl]-1-benzenesulfonamide *25*

4-Hydrazino-2-hydroxymethyl-1-benzenesulfonamide **1** (2.00 g, 9.21 mmol) was dissolved in MeOH (10 ml) under argon atmosphere and acidified to pH 1–2 using IPA-HCl. The reaction mixture was stirred at room temperature for 0.5 h and solvent was completely removed under high vacuum at 40–50 °C to afford a solid. The mixture of this solid and above prepared 4,4,4-trifluoro-1-(4-methoxyphenyl)-1,3-butanedione (2.04 g, 8.29 mmol) taken in absolute alcohol (30 ml), was heated at 50–60 °C for 10–12 h under argon atmosphere. The reaction mixture was cooled, poured over ice cold water and extracted with ethyl acetate. The combined organic layer after washing with brine and water, was dried over anhyd. Na_2SO_4. The residue obtained after solvent evaporation was purified by column chromatography using 30% ethyl acetate–petroleum ether, and the product so obtained was finally triturated with ethyl acetate–toluene to get the colorless solid of the title compound **25** (4.84 g, 79%). M.p. 188–189 °C. IR (Neat) 3311, 1609 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 7.97 (d, J = 8.6 Hz, 1H), 7.62 (d, J = 1.8 Hz, 1H), 7.25 (d, J = 8.0 Hz, 1H), 7.15 (d, J = 8.4 Hz, 2H), 6.88 (d, J = 8.6 Hz, 2H), 6.71 (s, 1H), 5.43 (bs, 2H), 5.01 (d, J = 3.8 Hz, 2H), 3.83 (s, 3H), 2.65 (bs, 1H). ^{13}C NMR (DMSO-d_6) δ 160.1, 145.1, 142.8, 142.0 (q, J = 35.5 Hz, 1C), 141.5, 139.9, 130.3 (2C), 128.1, 123.9, 123.3, 121.5 (q, J = 267.4 Hz, 1C), 120.6, 114.4 (2C), 105.9, 59.3, 55.3. MS (CI Method) 428 $(M + H)^+$, 409 (100%), 392, 346. HPLC (System 1) 98.35%. Anal. ($C_{18}H_{16}F_3N_3O_4S$) C, H, N.

5.3.1. Compound *4*

Yield 76%. M.p. 142–144 °C. IR (KBr) 3425, 3260, 1625 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 8.00 (d, J = 8.6 Hz, 1H), 7.62 (d, J = 1.8 Hz, 1H), 7.26–7.19 (m, 2H), 6.97–6.88 (m, 2H), 6.87 (s, 1H), 5.41 (bs, 2H), 5.04 (d, J = 4.2 Hz, 2H), 2.64 (bs, 1H). MS (CI Method) 434 $(M + H)^+$, 416, 398, 352. HPLC (System 1) 99.62%. Anal. ($C_{17}H_{12}F_5N_3O_3S$) C, H, N.

5.3.2. Compound *5*

Yield 67%. M.p. 120–122 °C. IR (KBr) 3423, 1606, 1486 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 8.05 (d, J = 8.6 Hz, 1H), 7.65

(s, 1H), 7.35–6.95 (m, 4H), 6.79 (s, 1H), 5.40 (bs, 2H), 5.08 (s, 2H), 2.60 (bs, 1H). MS (CI Method) 433 (M^+), 414, 352. HPLC (System 2) 97.32%. ($C_{17}H_{12}F_5N_3O_3S$) C, H, N.

5.3.3. Compound ***6***

Yield 71%. M.p. 126–128 °C. IR (KBr) 3371, 3228, 1600 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.94 (d, J = 8.4 Hz, 1H), 7.60 (d, J = 2.4 Hz, 1H), 7.46 (d, J = 1.8 Hz, 1H), 7.35 (d, J = 1.8 Hz, 1H), 7.23 (d, J = 7.2 Hz, 1H), 7.10 (dd, J = 6.2 & 2.2 Hz, 1H), 6.78 (s, 1H), 5.41 (bs, 2H), 5.00 (d, J = 3.2 Hz, 2H), 2.72 (bs, 1H). MS (CI Method) 466 (M^+), 447, 384. HPLC (System 1) 99.62%. Anal. ($C_{17}H_{12}Cl_2F_3N_3O_3S$) C, H, N.

5.3.4. Compound ***7***

Yield 80%. M.p. 148–150 °C. IR (KBr) 3458, 3237, 1604, 1473 cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.00 (d, J = 8.6 Hz, 1H), 7.63 (d, J = 1.8 Hz, 1H), 7.39 (d, J = 5.0 Hz, 1H), 7.26–7.01 (m, 3H), 6.78 (s, 1H), 5.02 (s, 2H). MS (CI Method) 450 (M^+), 414, 368, 340, 319. HPLC (System 2) 98.62%. Anal. ($C_{17}H_{12}ClF_4N_3O_3S$) C, H, N.

5.3.5. Compound ***8***

Yield 74%. M.p. 96–98 °C. IR (KBr) 3420, 1604, 1470 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.86 (d, J = 8.2 Hz, 1H), 7.59 (s, 1H), 7.20–7.00 (m, 4H), 6.65 (s, 1H), 5.45 (bs, 2H), 4.95 (s, 2H), 2.80 (bs, 1H), 2.35 (s, 3H), 1.95 (s, 3H). MS (CI Method) 425 (M^+), 407, 344. HPLC (System 1) 96.52%; (System 2) 97.10%. Anal. ($C_{19}H_{18}F_3N_3O_3S$) C, H, N.

5.3.6. Compound ***9***

Yield 58%. M.p. 132–134 °C. IR (KBr) 3522, 1605, 1472 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.95 (d, J = 8.4 Hz, 1H), 7.66 (s, 1H), 7.25 (d, J = 7.8 Hz, 1H), 7.16 (m, 2H), 6.86 (d, J = 7.4 Hz, 1H), 6.74 (s, 1H), 5.50 (bs, 2H), 5.03 (s, 2H), 2.80 (bs, 1H), 2.28 (s, 3H), 2.24 (s, 3H). ^{13}C NMR (DMSO-d_6) δ 145.4, 142.8, 142.2 (q, J = 37.5 Hz, 1C), 141.5, 140.0, 137.9, 137.1, 129.8 (2C), 128.0, 126.2, 125.8, 123.9, 123.3, 121.5 (q, J = 266.8 Hz, 1C), 106.1, 59.4, 19.3, 19.2. MS (CI Method) 425 (M^+), 407, 379, 327. HPLC (System 1) 97.55%; (System 2) 97.45%. Anal. ($C_{19}H_{18}F_3N_3O_3S$) C, N; H: calcd, 4.26; found, 4.68.

5.3.7. Compound ***10***

Yield 66%. IR (KBr) 3455, 3274, 1603, 1481 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.98 (d, J = 8.4 Hz, 1H), 7.63 (d, J = 1.6 Hz, 1H), 7.30–7.15 (m, 2H), 7.05–6.96 (m, 2H), 6.74 (s, 1H), 5.54 (bs, 2H), 5.02 (s, 2H), 3.05 (bs, 1H), 2.27 (s, 3H). MS (CI Method) 429 (M^+), 410, 394, 348. HPLC (System 1) 95.35%; HPLC (System 2) 96.12%. Anal. ($C_{18}H_{15}F_4N_3O_3S$) C, N; H: calcd, 3.52; found, 3.95.

5.3.8. Compound ***11***

Yield 68%. M.p. 125–127 °C. IR (KBr) 3343, 1602, 1484 cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.02 (d, J = 8.4 Hz, 1H), 7.65 (s, 1H), 7.30 (d, J = 1.8 Hz, 1H), 6.80 (d, J = 8.8 Hz, 1H), 6.75–6.72 (m, 2H), 6.71 (s, 1H), 5.45 (bs, 2H), 5.05 (s, 2H), 3.92 (s, 3H), 3.80 (s, 3H), 2.65 (bs, 1H). MS (CI Method) 457 (M^+) (440, 100%). HPLC (System 1) 97.97%. Anal ($C_{19}H_{18}F_3N_3O_5S$) C, H, N.

5.3.9. Compound ***12***

Yield 78%. M.p. 206–208 °C. IR (KBr) 3407, 3313, 1476, 1348 cm^{-1}. ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 7.98 (d, J = 8.3 Hz, 1H), 7.70 (s, 1H), 7.22–7.16 (m, 1H), 7.04–6.90 (m, 3H), 6.74 (s, 1H), 5.00 (d, J = 4.0 Hz, 2H), 3.92 (s, 3H). MS (EI Method) 445 (M^+), 426, 410, 364. HPLC (System 1) 98.25%. Anal. ($C_{18}H_{15}F_4N_3O_4S$) C, H, N.

5.3.10. Compound ***13***

Yield 69%. M.p. 211–212 °C. IR (KBr) 3429, 1605, 1473 cm^{-1}. ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 7.95 (d, J = 8.3 Hz, 1H), 7.79 (s, 1H), 7.34 (s, 1H), 7.16 (dd, J = 10.0 & 1.6 Hz, 1H), 7.08 (d, J = 8.6 Hz, 1H), 7.00 (s, 2H), 6.95 (d, J = 8.8 Hz, 1H), 6.77 (s, 1H), 5.28 (bs, 1H), 5.00 (d, J = 4.0 Hz, 2H), 3.92 (s, 3H). MS (EI Method) 461 (M^+), 441, 426, 380. HPLC (System 1) 99.16%; (System 2) 99.20%. Anal. ($C_{18}H_{15}Cl\ F_3N_3O_4S$) C, H; N: calcd, 9.10; found, 8.54.

5.3.11. Compound ***14***

Yield 59%. M.p. 115–117 °C. IR (KBr) 3422, 1604 cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.00 (d, J = 8.8 Hz, 1H), 7.60 (s, 1H), 7.30–7.20 (m, 1H), 7.10–7.04 (m, 2H), 6.94 (d, J = 7.0 Hz, 1H), 6.75 (s, 1H), 5.40 (bs, 2H), 5.00 (d, J = 4.4 Hz, 2H), 2.65 (bs, 1H), 2.47 (s, 3H), 2.28 (s, 3H). MS (CI Method) 457 (M^+), 443. HPLC (System 1) 98.28%. Anal. ($C_{19}H_{18}F_3N_3O_3S_2$) C, H, N.

5.3.12. Compound ***15***

Yield 48%. M.p. 168–170 °C. IR (KBr) 3467, 1602, 1472 cm^{-1}. ^1H NMR (CD_3OD) δ 7.90 (d, J = 8.4 Hz, 1H), 7.73 (s, 1H), 7.20 (dd, J = 6.4 & 2.0 Hz, 1H), 7.05 (dd, J = 6.4 & 2.0 Hz, 1H), 6.85–6.75 (m, 2H), 6.73 (s, 1H), 4.90 (s, 2H), 3.75 (s, 3H), 2.02 (s, 3H). MS (CI Method) 473 (M^+), 456, 392. HPLC (System 1) 98.01%. (System 2) 96.93%. Anal. ($C_{19}H_{18}F_3N_3O_4S_2$) C, H, N.

5.3.13. Compound ***17***

Yield 82%. M.p. 158–159 °C. IR (Nujol) 3319, 1598, 1461 cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.00 (d, J = 8.4 Hz, 1H), 7.67 (d, J = 2.2 Hz, 1H), 7.40–7.20 (m, 2H), 7.07 (s, 1H), 6.94 (d, J = 7.8 Hz, 1H), 6.72 (s, 1H), 5.45 (bs, 2H), 5.04 (d, J = 4.8 Hz, 2H), 3.43 (t, J = 7.4 Hz, 2H), 3.30 (t, J = 7.6 Hz, 2H), 2.75 (bs, 1H). MS (CI Method) 455 (M^+), 437, 355. HPLC (System 2) 98.25%. Anal. ($C_{19}H_{16}F_3N_3O_3S_2$) C, H, N.

5.3.14. Compound ***18***

Yield 64%. M.p. 92–94 °C. IR (KBr) 3386, 1603, 1475 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.98 (d, J = 8.6 Hz, 1H), 7.67 (d, J = 2.2 Hz, 1H), 7.26 (d, J = 8.2 Hz, 1H), 6.97 (s, 1H),

6.86 (d, J = 6.6 Hz, 1H), 6.74 (d, J = 6.8 Hz, 1H), 6.65 (s, 1H), 5.52 (bs, 2H), 5.04 (s, 2H), 4.25 (t, J = 5.0 Hz, 2H), 2.75 (t, J = 5.0 Hz, 2H), 2.06-1.97 (m, 2H). MS (EI Method) 453 (M^+), 435. HPLC (System 1) 98.96%. Anal. ($C_{20}H_{18}F_3N_3O_4S$) C, H, N.

5.3.15. Compound ***19***

Yield 73%. M.p. 122–125 °C. IR (KBr) 3488, 1602, 1468 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.96 (d, J = 8.6 Hz, 1H), 7.70 (d, J = 2.0 Hz, 1H), 7.24–7.19 (dd, J = 6.4 & 2.0 Hz, 1H), 7.04–7.00 (m, 2H), 6.81 (dd, J = 6.6 & 1.4 Hz, 1H), 6.71 (s, 1H), 5.44 (bs, 2H), 5.04 (s, 2H), 2.78–2.71 (m, 4H), 2.65 (bs, 1H), 1.80–1.75 (m, 4H). MS (CI Method) 451 (M^+), 433, 353. HPLC (System 1) 99.04%. Anal. ($C_{21}H_{20}F_3N_3O_3S$) C, H, N.

5.3.16. Compound ***20***

Yield 55%. M.p. 85–87 °C. IR (KBr) 3380, 3275, 1492, 1395 cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.15 (d, J = 8.5 Hz, 1H), 7.60 (s, 1H), 7.45 (d, J = 8.3 Hz, 1H), 6.49 (s, 1H), 5.06 (s, 2H), 2.82–2.63 (m, 1H), 1.87–1.74 (m, 6H), 1.47–1.22 (m, 4H). MS (EI Method) 403 (M^+), 384, 368, 322. HPLC (System 1) 98.93%. Anal. ($C_{17}H_{20}F_3N_3O_3S$) C, H, N.

5.3.17. Compound ***21***

Yield 82%. M.p. 207–208 °C. IR (KBr) 3427, 3329, 3252, 1630 cm^{-1}. ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 7.90 (d, J = 8.4 Hz, 1H), 7.77–7.71 (m, 2H), 7.69–7.67 (m, 2H), 7.18 (d, J = 6.4 Hz, 1H), 7.16–7.14 (m, 3H), 6.85 (s, 1H), 6.45 (s, 2H), 4.96 (s, 2H), 3.94 (s, 3H), 2.46 (bs, 1H). MS (CI Method) 477 (M^+), 396, 368. HPLC (System 2) 98.52%. Anal. ($C_{22}H_{18}F_3N_3O_4S$) C, H, N.

5.3.18. Compound ***22***

Yield 38%. M.p. 230–232 °C. IR (KBr) 3403, 3298, 1592, 1459 cm^{-1}. ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 10.63 (bs, 1H), 7.91 (d, J = 4.4 Hz, 1H), 7.78 (d, J = 1.4 Hz, 1H), 7.51–7.33 (m, 3H), 7.25–7.08 (m, 2H), 6.93 (d, J = 2.4 Hz, 1H), 6.86 (s, 1H), 6.62 (s, 2H), 4.94 (s, 2H), 2.68 (bs, 1H). MS (CI Method) 437 (M^+), 419. HPLC (System 1) 97.53%; (System 2) 96.82%. Anal. ($C_{19}H_{15}F_3N_4O_3S$) C, H, N.

5.3.19. Compound ***23***

Yield 80%. M.p. 167–168 °C. IR (KBr) 3320, 3246, 1604, 1488, 1348 cm^{-1}. ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 8.00 (d, J = 8.3 Hz, 1H), 7.74 (s, 1H), 7.39–7.27 (m, 3H), 6.92 (d, J = 4.9 Hz, 1H), 6.81 (s, 1H), 5.00 (d, J = 4.5 Hz, 2H). MS (CI Method) 403 (M^+), 384, 368, 322. HPLC (System 2) 98.50%. Anal. ($C_{15}H_{12}F_3N_3O_3S_2$) C, H, N.

5.3.20. Compound ***24***

Yield 47%. M.p. 155–156 °C. IR (KBr) 3380, 3212, 1585, 1455 cm^{-1}. ^1H NMR (DMSO-d_6) δ 9.30 (s, 1H), 8.85–8.78 (m, 2H), 8.00–7.95 (m, 4H), 7.35 (s, 1H), 6.00 (bt, 1H), 4.95 (d, J = 4.4 Hz, 2H). MS (CI Method) 399 (M + H, 100%)$^+$, 381. HPLC (System 2) 98.73%. Anal. ($C_{16}H_{13}F_3N_4O_3S$) C, H, N.

5.3.21. Compound ***26***

Yield 83%. M.p. 166–168 °C. IR (Nujol) 3300, 1608, 1460 cm^{-1}. ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 7.94 (dd, J = 6.0 & 2.6 Hz, 1H), 7.61 (s, 1H), 7.31 (s, 1H), 7.17 (d, J = 8.0 Hz, 2H), 6.90 (d, J = 7.0 Hz, 2H), 6.76 (t, J = 53.4 Hz, 1H), 6.67 (s, 1H), 6.28 (bs, 2H), 4.94 (s, 2H), 3.82 (s, 3H), 2.70 (bs, 1H). MS (EI Method) 409 (M^+), 389, 374. HPLC (System 1) 99.50%; (System 2) 99.71%. Anal. ($C_{18}H_{17}F_2N_3O_4S$) C, H; N: calcd, 10.26; found, 9.58.

5.3.22. Compound ***27***

Yield 33%. M.p. 148–150 °C. IR (KBr) 3302, 1611, 1511 cm^{-1}. ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 7.87 (d, J = 8.4 Hz, 1H), 7.60 (d, J = 2.0 Hz, 1H), 7.36 (s, 1H), 7.12 (d, J = 8.8 Hz, 2H), 6.84 (d, J = 8.8 Hz, 2H), 6.54 (s, 1H), 6.53 (bs, 2H), 5.40 (d, J = 48.2 Hz, 2H), 4.90 (s, 2H), 3.80 (s, 3H), 2.90 (bs, 1H). MS (CI Method) 391 (M^+), 356, 310. HPLC (System 1) 97.22%; HPLC (System 2) 96.20%. Anal. ($C_{18}H_{18}FN_3O_4S$) C, H, N.

5.3.23. Compound ***28***

Yield 75%. M.p. 177–179 °C. IR (KBr) 3320, 3196, 1610, 1578, 1511 cm^{-1}. ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 7.90 (d, J = 8.6 Hz, 1H), 7.62 (d, J = 1.8 Hz, 1H), 7.12 (d, J = 8.8 Hz, 2H), 7.10 (m, 1H), 6.85 (t, J = 8.8 Hz, 2H), 6.31 (s, 1H), 5.40 (bs, 2H), 4.96 (s, 2H), 3.82 (s, 3H), 2.64 (bs, 1H), 2.38 (s, 3H). MS (CI Method) 374 (M + H)$^+$, 356, 322, 295. HPLC (System 1) 97.86%; HPLC (System 2) 97.65%. Anal. ($C_{18}H_{19}N_3O_4S$) H; C: calcd, 57.90; found, 58.43; N: calcd, 11.25; found, 10.66.

5.3.24. Compound ***29***

Obtained by the general method using hydrazine hydrochloride **1** and 3-(4-methoxyphenyl)-3-oxopropanal in 35% yield. M.p. 166–167 °C. IR (KBr) 3320, 1608 cm^{-1}. ^1H NMR ($CDCl_3$ + CD_3OD) δ 7.90 (d, J = 8.4 Hz, 1H), 7.73 (d, J = 2.0 Hz, 1H), 7.64 (d, J = 2.0 Hz, 1H), 7.15 (d, J = 8.8 Hz, 2H), 7.06 (d, J = 2.2 Hz, 1H), 6.88 (d, J = 8.8 Hz, 2H), 6.49 (d, J = 2.0 Hz, 1H), 4.96 (s, 2H), 3.82 (s, 3H). MS (CI Method) 359 (M^+), 342, 324. HPLC (System 1) 98.47%; HPLC (System 2) 98.08%. Anal. ($C_{17}H_{17}N_3O_4S$) C, H, N.

5.3.25. Compound ***30***

Yield 48%. M.p. 168–170 °C. IR (KBr) 3481, 1606, 1511 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.75 (d, J = 8.8 Hz, 1H), 7.45 (d, J = 2.0 Hz, 1H), 7.06 (d, J = 8.8 Hz, 2H), 7.00–6.90 (dd, J = 8.4 & 2.4 Hz, 1H), 6.81 (d, J = 8.8 Hz, 2H), 6.27 (s, 1H), 5.88 (bs, 2H), 4.88 (s, 2H), 3.82 (s, 3H), 2.74 (q, J = 7.4 Hz, 2H), 2.65 (bs, 1H), 1.30 (t, J = 7.4 Hz, 3H). MS (CI Method) 387 (M^+), 372, 352. HPLC (System 1) 99.01%; HPLC (System 2) 99.19%. Anal. ($C_{19}H_{21}N_3O_4S$) H, N; C: calcd, 58.90; found, 59.41.

5.3.26. Compound ***31***

Yield 65%. M.p. 167–169 °C. IR (KBr) 3314, 3215, 1600, 1497 cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.76 (s, 1H), 7.72 (d,

J = 8.2 Hz, 1H), 7.47 (bs, 2H), 7.45 (d, *J* = 8.4 Hz, 2H), 7.23 (d, *J* = 8.2 Hz, 2H), 7.08 (d, *J* = 8.8 Hz, 1H), 6.51 (s, 1H), 5.46 (bt, *J* = 5.4 Hz, 1H), 4.84 (d, *J* = 5.0 Hz, 2H), 2.27 (s, 3H). MS (EI Method) 378 $(M + H)^+$, 360. HPLC (System 1) 97.21%; HPLC (System 2) 96.80%. Anal. ($C_{17}H_{16}ClN_3O_3S$) H; C: calcd, 54.04; found, 54.66; N: calcd, 11.12; found, 10.41.

5.3.27. Compound ***32***

Yield 71%. M.p. 138–139 °C. IR (KBr) 3333, 3211, 1607, 1492 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.95 (d, *J* = 8.3 Hz, 1H), 7.60 (d, *J* = 1.7 Hz, 1H), 7.26–7.09 (m, 5H), 6.77 (t, *J* = 54.8 Hz, 1H), 6.72 (s, 1H), 5.02 (d, *J* = 4.1 Hz, 2H), 2.38 (s, 3H). MS (CI Method) 393 (M^+), 375, 358, 312. HPLC (System 2) 98.21%. Anal. ($C_{18}H_{17}F_2N_3O_3S$) C, H, N.

5.3.28. Compound ***33***

Yield 58%. M.p. 134–136 °C. IR (KBr) 3315, 1601, 1579, 1486, 1415 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.96 (d, *J* = 8.5 Hz, 1H), 7.59 (d, *J* = 1.9 Hz, 1H), 7.26–7.12 (m, 4H), 6.96–6.93 (m, 2H), 6.77 (t, *J* = 54.8 Hz, 1H), 5.00 (s, 2H), 2.34 (s, 3H). MS (CI Method) 393 (M^+), 374, 358, 347, 312. HPLC (System 1) 98.50%. Anal. ($C_{18}H_{17}F_2N_3O_3S$) C, H, N.

5.3.29. Compound ***34***

Yield 65%. M.p. 133–135 °C. IR (KBr) 3273, 2479, 1831, 1636 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.97 (d, *J* = 8.3 Hz, 1H), 7.60 (s, 1H), 7.28–7.12 (m, 5H), 6.79 (t, *J* = 54.8 Hz, 1H), 6.73 (s, 1H), 5.01 (s, 2H), 2.75 (q, *J* = 7.4 Hz, 2H), 1.27 (t, *J* = 8.0 Hz, 3H). MS (CI Method) 407 (M^+), 389, 372, 354, 325. HPLC (System 1) 98.82%. Anal. ($C_{19}H_{19}F_2N_3O_3S$) C, H, N.

5.3.30. Compound ***35***

Yield 62%. M.p. 146–149 °C. IR (KBr) 3361, 1600, 1580, 1485, 1413 cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.00 (d, *J* = 8.3 Hz, 1H), 7.59 (d, *J* = 2.0 Hz, 1H), 7.42–7.19 (m, 4H), 7.06–7.02 (m, 1H), 6.79 (t, *J* = 54.9 Hz, 1H), 6.77 (s, 1H), 5.03 (s, 2H). MS (EI Method) 413(M^+), 394 (100%), 378, 332. HPLC (System 1) 99.21%. Anal. ($C_{17}H_{14}ClF_2N_3O_3S$) C, H, N.

5.3.31. Compound ***36***

Yield 67%. M.p. 166–167 °C. IR (KBr) 3306, 1600 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.98 (d, *J* = 8.4 Hz, 1H), 7.61 (d, *J* = 2.0 Hz, 1H), 7.25 (d, *J* = 5.4 Hz, 2H), 7.15 (d, *J* = 8.6 Hz, 2H), 7.12–7.05 (m, 1H), 6.77 (t, *J* = 54.8 Hz, 1H), 6.73 (s, 1H), 5.48 (bs, 2H), 5.03 (d, *J* = 3.8 Hz, 2H), 2.80 (bs, 1H), 2.51 (s, 3H). ^{13}C NMR (DMSO-d_6) δ 147.3 (t, *J* = 28.8 Hz, 1C), 144.3, 142.7, 141.7, 140.1, 139.5, 129.1 (2C), 128.1, 125.6 (2C), 125.0, 123.6, 123.0, 111.4 (t, *J* = 231.7 Hz, 1C), 105.6, 59.4, 14.2. MS (CI Method) 425 (M^+), 406, 390, 344. HPLC (System 2) 98.64%. Anal. ($C_{18}H_{17}F_2N_3O_3S_2$) C, H, N.

5.3.32. Compound ***37***

Yield 65%. M.p. 165–166 °C. IR (KBr) 3344, 3302, 1613, 1453 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.96 (d, *J* = 8.3 Hz, 1H), 7.60 (s, 1H), 7.26 (s, 1H), 7.00 (d, *J* = 8.4 Hz, 2H), 6.63 (t, *J* = 54.9 Hz, 1H), 6.63 (s, 1H), 6.53 (d, *J* = 8.2 Hz, 2H), 5.02 (s, 2H), 3.19 (q, *J* = 7.2 Hz, 2H), 1.25 (t, *J* = 7.4 Hz, 3H). MS (CI Method) 423 $(M + H)^+$, 389, 344, 242. HPLC (System 2) 98.31%. Anal. ($C_{19}H_{20}F_2N_4O_3S$) C, H, N.

5.3.33. Compound ***38***

Yield 56%. M.p. 155–157 °C. IR (KBr) 3481, 1599, 1563, 1487, 1457 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.94 (d, *J* = 8.4 Hz, 1H), 7.59 (d, *J* = 1.9 Hz, 1H), 7.30–7.17 (m, 2H), 7.06–6.87 (m, 2H), 6.79 (s, 1H), 6.79 (t, *J* = 54.6 Hz, 1H), 5.02 (d, *J* = 4.1 Hz, 2H). MS (CI Method) 415 (M^+), 396 (100%), 380, 334. HPLC (System 1) 98.90%. Anal. ($C_{17}H_{13}F_4N_3O_3S$) C, H, N.

5.3.34. Compound ***39***

Yield 68%. M.p. 160–162 °C. IR (KBr) 3441, 3210, 3094, 1601, 1575 cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.02 (d, *J* = 8.4 Hz, 1H), 7.61 (s, 1H), 7.45–7.41 (m, 2H), 7.30–7.19 (m, 2H), 7.00 (d, *J* = 6.6 Hz, 1H), 6.77 (t, *J* = 54.6 Hz, 1H), 5.04 (s, 2H). MS (CI Method) 448 (M^+), 429, 412, 366. HPLC (System 2) 98.72%. Anal. ($C_{17}H_{13}Cl_2F_2N_3O_3S$) C, H, N.

5.3.35. Compound ***40***

Yield 70%. M.p. 140–142 °C. IR (KBr) 3403, 1603 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.93 (d, *J* = 8.6 Hz, 1H), 7.60 (s, 1H), 7.26–7.10 (m, 2H), 7.05 (s, 1H), 6.85 (d, *J* = 7.4 Hz, 1H), 6.77 (s, 1H), 6.69 (t, *J* = 54.8 Hz, 1H), 5.55 (s, 2H), 5.00 (s, 2H), 2.28 (s, 3H), 2.23 (s, 3H), 1.92 (bs, 1H). MS (CI Method) 407 (M^+), 389, 361. HPLC (System 1) 96.41%; HPLC (System 2) 95.82%.

5.3.36. Compound ***41***

Yield 72%. M.p. 134–136 °C. IR (KBr) 3474, 3329, 1741, 1604, 1553 cm^{-1}. ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 7.94 (d, *J* = 8.4 Hz, 1H), 7.62 (s, 1H), 7.16 (d, *J* = 7.6 Hz, 2H), 6.97 (d, *J* = 7.2 Hz, 2H), 6.76 (t, *J* = 54.6 Hz, 1H), 6.69 (s, 1H), 4.95 (s, 2H), 2.26 (s, 3H). MS (CI Method) 411 (M^+), 391, 376, 357. HPLC (System 1) 98.33%. Anal. ($C_{18}H_{16}F_3N_3O_3S$) C, H, N.

5.3.37. Compound ***42***

Yield 58%. M.p. 170–172 °C. IR (KBr) 3332, 1603 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.96 (d, *J* = 8.6 Hz, 1H), 7.64 (d, *J* = 1.8 Hz, 1H), 7.27 (s, 1H), 7.23 (dd, *J* = 6.6 & 2.0 Hz, 1H), 7.08 (d, *J* = 8.6 Hz, 2H), 6.82 (t, *J* = 56.0 Hz, 1H), 6.66 (s, 1H), 5.50 (bs, 2H), 5.03 (s, 2H), 2.80 (bs, 1H), 2.49 (s, 3H), 2.30 (s, 3H). MS (CI Method) 439 (M^+), 419, 358. HPLC (System 1) 95.95%; HPLC (System 2) 96.20%.

5.3.38. Compound ***43***

Yield 55%. M.p. 160–162 °C. IR (KBr) 3313, 1602, 1452 cm^{-1}. ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 7.90 (d, *J* = 8.4 Hz, 1H), 7.67 (d, *J* = 1.6 Hz, 1H), 7.20 (d, *J* = 6.8 Hz, 2H), 7.16 (d, *J* = 5.8 Hz, 1H), 6.98 (d, *J* = 5.8 Hz, 1H), 6.85 (s, 2H), 6.55 (s, 1H), 6.50 (t, *J* = 54.8 Hz, 1H), 4.99 (s, 2H),

3.47 (t, J = 4.5 Hz, 2H), 3.22 (t, J = 4.8 Hz, 2H), 2.54 (bs, 1H). MS (CI Method) 437 (M^+), 419, 338. HPLC (System 1) 98.92%; HPLC (System 2) 98.62%. Anal. ($C_{19}H_{17}F_2N_3O_3S_2$) C, H, N.

5.3.39. Compound ***44***

Prepared using hydrazine hydrochloride **2** and the corresponding diketone. Yield 74%. M.p. 136–139 °C. IR (KBr) 3517, 1613, 1473 cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.25 (d, J = 1.4 Hz, 1H), 7.84 (dd, J = 8.2 & 2.0 Hz, 1H), 7.19 (d, J = 8.2 Hz, 1H), 7.06 (d, J = 8.4 Hz, 2H), 6.85 (d, J = 8.8 Hz, 2H), 6.81 (s, 1H), 4.60 (s, 2H), 3.88 (s, 3H), 3.17 (s, 3H), 1.95 (bs, 1H). ^{13}C NMR (DMSO-d_6) δ 160.0, 146.1, 142.3 (q, J = 37.5 Hz, 1C), 141.8, 141.0, 140.1, 129.8 (2C), 129.0, 126.5 (2C), 121.2 (q, J = 267.0 Hz, 1C), 120.0, 114.3 (2C), 104.4, 58.3, 55.2, 43.4. MS (CI Method) 426 (M^+), 409, 397. HPLC (System 1) 98.16%. Anal. ($C_{19}H_{17}F_3N_2O_4S$) C, H, N.

5.3.40. Compound ***45***

Yield 64%. M.p. 97–99 °C. IR (KBr) 3439, 1603 cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.27 (d, J = 1.80 Hz, 1H), 7.84 (dd, J = 7.4 & 1.8 Hz, 1H), 7.28–7.16 (m, 3H), 7.05 (d, J = 8.8 Hz, 2H), 6.85 (s, 1H), 4.60 (d, J = 7.0 Hz, 2H), 3.20 (bt, 1H), 3.11 (s, 3H), 2.54 (s, 3H). MS (CI Method) 442 (M^+), 425, 413. HPLC (System 1) 96.05%; HPLC (System 2) 95.20%. Anal. ($C_{19}H_{17}F_3N_2O_3S_2$) C, H; N: calcd, 6.33; found, 6.74.

5.3.41. Compound ***46***

Yield 58%. M.p. 86–88 °C. IR (KBr) 3434, 1602, 1451 cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.24 (d, J = 1.8 Hz, 1H), 7.80 (dd, J = 5.8 & 2.4 Hz, 1H), 7.20–7.10 (m, 3H), 7.04 (d, J = 8.4 Hz, 2H), 6.82 (s, 1H), 6.76 (t, J = 54.4 Hz, 1H), 4.61 (d, J = 6.4 Hz, 2H), 3.47 (bt, 1H), 3.11 (s, 3H), 2.49 (s, 3H). MS (CI Method) 424 (M^+), 407, 395. HPLC (System 1) 98.10%. HPLC (System 2) 97.71%. Anal. ($C_{19}H_{18}F_2N_2O_3S_2$) C, H, N.

5.3.42. 2-Hydroxymethyl-4-[5-(3-methyl-4-methylsulfinylphenyl)-3-trifluoromethyl-1H-1-pyrazolyl]-1-benzenesulfonamide ***16*** *[34]*

m-Chloroperbenzoic acid (202 mg, 1.74 mmol) was slowly added to a pre-cooled (0–5 °C) solution of compound **14** (400 mg, 0.87 mmol) in dichloromethane (10 ml) and stirred the reaction mixture at room temperature for 2 h. The reaction mixture was poured over ice water and extracted with dichloromethane. The combined organic layer was sequentially washed with 10% aqueous sodium bicarbonate and water. The solvent was evaporated after drying to get a viscous liquid which on further purification by column chromatography using 20% ethyl acetate–petroleum ether afforded a white solid of the title compound **16** (380 mg, 92%). M.p. 174–176 °C. IR (KBr) 3359, 1733, 1602, 1467 cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.86–7.75 (m, 3H), 7.42 (s, 1H), 7.31–7.19 (m, 3H), 4.91 (d, J = 3.8 Hz, 2H), 2.71 (s, 3H), 2.33 (s, 3H). MS (CI Method) 474 $(M + H)^+$, 457, 440, 392. HPLC (System 1) 98.57%. Anal. ($C_{19}H_{18}F_3N_3O_4S_2$) C, H, N.

5.3.43. 1-(3-Hydroxymethyl-4-sulfamoylphenyl)-5-(4-methoxyphenyl)-1H-3-pyrazolecarboxylic acid ***47***

Sodium hydroxide (57 mg, 1.43 mmol) was dissolved in methanol (5 ml) and methyl 1-(3-hydroxymethyl-4-sulfamoylphenyl)-5-(4-methoxyphenyl)-1*H*-3-pyrazolecarboxylate (500 mg, 1.19 mmol), prepared by the general method using hydrazine hydrochloride **1** and methyl 4-(4-methoxyphenyl)-2,4-dioxobutanoate **3b** (Scheme 3), was dumped at room temperature, and stirred for 5 h. The reaction mixture was poured in ice water, acidified with 2 N HCl and extracted with ethyl acetate. The combined organic layer was washed with water and evaporated to get a solid which on further trituration with ethyl acetate–petroleum ether afforded a white solid of the title compound **47** (330 mg, 68%). M.p. 250–252 °C. IR (KBr) 3333, 1700, 1608, 1465 cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.86–7.83 (m, 2H), 7.24–7.19 (m, 3H), 7.01–6.93 (m, 2H), 6.87 (s, 1H), 4.89 (s, 2H), 3.76 (s, 3H). MS (CI Method) 403 (M^+, 100%), 383, 322, 307. HPLC (System 2) 98.92%. Anal. ($C_{18}H_{17}N_3O_6S$) C, H, N.

5.3.44. 4-[3-Cyano-5-(4-methoxyphenyl)-1H-1-pyrazolyl]-2-hydroxymethyl-1-benzenesulfonamide ***48*** *[35]*

Methyl 1-(3-hydroxymethyl-4-sulfamoylphenyl)-5-(4-methoxyphenyl)-1*H*-3-pyrazolecarboxylate (500 mg, 1.19 mmol), prepared by the general method using hydrazine hydrochloride **1** and methyl 4-(4-methoxyphenyl)-2,4-dioxobutanoate **3b** (Scheme 3), was taken in aq. ammonia (25%, 5 ml) and stirred at ambient temperature in a closed flask for 15 h. The reaction mixture was poured in ice water, acidified with 2 N HCl and extracted with ethyl acetate. The combined organic layer was washed with water and evaporated to get a gummy mass which on trituration with dichloromethane afforded an expected off white solid of carboxamide (300 mg, 62%). The sample was used in the next step without further purification. IR (KBr) 3432, 2926, 1680, 1600, 1328 cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.85–7.80 (m, 2H), 7.78 (bs, 1H), 7.62 (bs, 2H), 7.42 (bs, 1H), 7.40–7.20 (m, 3H), 7.25–6.98 (m, 3H), 5.50 (bt, 1H), 4.89 (d, J = 4.2 Hz, 2H), 3.78 (s, 3H). MS (CI Method) 402 (M^+, 100%), 384, 305. The carboxamide (100 mg, 0.24 mmol) was dissolved in dry dioxane (5 ml), treated with pyridine (20 mg, 0.25 mmol) and cooled to 0–5 °C. Trifluoroacetic anhydride (100 mg, 0.47 mmol) was added at this temperature and reaction mixture was left for stirring overnight at room temperature. The reaction mixture was poured over ice water and extracted with ethyl acetate. The combined organic layer was washed with water and evaporated to get a gummy mass which was purified by column chromatography to get the title product **48** as viscous liquid (50 mg, 52%). IR (Neat) 3252, 2244, 1612, 1498 cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.00 (d, J = 8.2 Hz, 1H), 7.61 (s, 1H), 7.25–7.14 (m, 1H), 7.12 (d, J = 8.8 Hz, 2H), 6.90 (d, J = 8.6 Hz, 2H), 6.82 (s, 1H), 5.42 (bs, 2H), 5.03 (d, J = 4.2 Hz, 2H), 3.83 (s, 3H), 2.86 (bs, 1H). MS (CI Method) 384 (M^+), 349, 303. HPLC (System 1) 96.28%; HPLC (System 2) 97.20%. Anal. ($C_{18}H_{16}N_4O_4S$) C, H, N.

5.4. In vitro biochemical assays.
Spectrophotometric assay for COX-1 and COX-2 [36,37]

Microsomal fraction of ram seminal vesicles were used as a source of COX-1 enzyme, and microsomes from sf-9 cells infected with baculovirus containing human COX-2 c-DNA were used as a source of COX-2 enzyme. Enzyme activity was measured using a chromogenic assay based on oxidation of *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine (TMPD) during the reduction of PGG_2 to PGH_2. The assay mixture (1000 μl) contained Tris pH 8.0 (100 mM), EDTA (3 mM), hematin (15 μM), enzyme (150 units) and DMSO (8%). The mixture was pre-incubated at 25 °C for 15 min before initiation of enzymatic reaction in presence of compound/vehicle. The reaction was initiated by the addition of arachidonic acid (100 μM) and TMPD (120 μM). The enzyme activity was measured by the estimation of initial velocity of TMPD oxidation over the first 25 s of the reaction followed by tracking the increase in absorbance at 603 nM.

5.5. Computational studies

Three dimensional structure building and molecular modeling studies were performed using Sybyl program package, version 6.9 [41] on Silicon Graphics Octane2 workstations with the IRIX 6.5 operating system. All the molecular structures were sketched using the coordinates of SC-558 (6COX) [22] and their energies were minimized using MMFF94 force field with a gradient convergence of 0.05 kcal mol^{-1}. Further geometrical optimizations were performed using AM1 [42] method in order to obtain electrostatic potential charges. The negative logarithm of the molar IC_{50} values (pIC_{50}) was used for the study in order to achieve a normal distribution of activity values. The most active compound **14** was used as the template, and others were aligned to it by atom fit method (Fig. 2).

5.6. CoMFA

The steric and electrostatic potential fields for CoMFA were calculated at each lattice intersection of a regularly spaced grid of 2.0 Å. A sp^3 carbon atom with + 1.0 charge was selected as a probe for this calculation. Values of the steric and electrostatic energies were truncated at 30 kcal mol^{-1}. The partial least-square (PLS) [43] method was used for fitting the 3D structural features and their biological activities. It was cross validated using the leave one out method with a 2 kcal mol^{-1} column filter to check the consistency and predictivity. The optimum number of components used to derive the non-cross validated model was defined as the one leading to the considerably higher value of q^2 with the least standard error of prediction (SEP). Statistical validity of the model was assessed by the variance (r^2), SEE and the *F*-value. Models were further evaluated on the basis of their ability to predict activities of the compounds in the set. The predictive r^2 for the test set was calculated using the formula:

$$r^2_{\text{pred}} = \frac{SD - PRESS}{SD}$$

where SD is the sum of the squared deviations between the biological activities of the test set and mean activity of the training set molecules, PRESS is sum of squared deviation between predicted and actual activity values from each molecule in the test set.

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