Synthesis and Cyclooxygenase-2 Inhibiting Property of 1,5-Diarylpyrazoles with Substituted Benzenesulfonamide Moiety as Pharmacophore: Preparation of Sodium Salt for Injectable Formulation†

Manojit Pal,*,‡ Manjula Madan,‡ Srinivas Padakanti,‡ Vijaya R. Pattabiraman,‡ Srinivas Kalleda,‡ Akhila Vanguri,§ Ramesh Mullangi,§ N. V. S. Rao Mamidi,§ Seshagiri R. Casturi,§ Alpeshkumar Malde,‡ B. Gopalakrishnan,‡ and Koteswar R. Yeleswarapu*,‡

Discovery-Chemistry and Discovery-Biology, Dr Reddy's Laboratories Ltd., Bollaram Road, Miyapur, Hyderabad 500050, India

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A series of 1,5-diarylpyrazoles having a substituted benzenesulfonamide moiety as pharmacophore was synthesized and evaluated for cyclooxygenase (COX-1/COX-2) inhibitory activities. Through SAR and molecular modeling, it was found that fluorine substitution on the benzenesulfonamide moiety along with an electron-donating group at the 4-position of the 5-aryl ring yielded selectivity as well as potency for COX-2 inhibition in vitro. Among such compounds 3-fluoro-4-[5-(4-methoxyphenyl)-3-trifluoromethyl-1*H*-1-pyrazolyl]-1-benzenesulfonamide **3** displayed interesting pharmacokinetic properties along with antiinflammatory activity in vivo. Among the sodium salts tested in vivo, **10**, the propionyl analogue of **3**, showed excellent antiinflammatory activity and therefore represents a new lead structure for the development of injectable COX-2 specific inhibitors.

Introduction

The discovery that the key enzyme cyclooxygenase in the arachidonic acid metabolism exists in two isoforms, namely, the constitutive cyclooxygenase-1 (COX-1) and the inducible cyclooxygenase-2 (COX-2), has generated new avenues for drug design.[1] The isoforms differ in regulation and expression, depending on the tissue. COX-1 is constitutively expressed in the kidney and the gastrointestinal tract and is responsible for the biosynthesis of prostaglandins (PGs) involved in the cytoprotection of the gastrointestinal tract and platelet aggregation. Thus, interruption of COX-1 activity may lead to gastrointestinal toxicity such as ulceration, bleeding, and perforation.[2a] In contrast, COX-2 is inducible by proinflammatory molecules such as 1L-1, TNF-α, LPS, and carrageenan and plays a major role in the biosynthesis of PGs in inflammatory cells (monocytes and macrophages). COX-2, however, has also been reported to be produced in the normal kidney.[2b] The traditional NSAIDs inhibit both COX-1 and COX-2 and hence down regulate the PGs in almost all cells and tissues. This accounts for their antiinflammatory activity as well as side effects. However, selective inhibition of COX-2 over COX-1 is beneficial for the treatment of inflammatory diseases with reduced ulcerogenic side effects.

The sulfonyl-substituted diarylheterocycle is the most widely explored pharmacophore for the development of selective COX-2 inhibitors and is exemplified by 5-bromo-2-(4-fluorophenyl)-3-(4-methylsulfonylphenyl)-thiophene (**I**).[3] The pharmacophore for this series includes either a sulfonamide or a methanesulfone, which is at the 4-position of the phenyl ring. This phenyl moiety and a lipophilic moiety are attached to the adjacent positions of the central ring. The central ring may consist of a carbocycle, heterocycle, or even acyclic structure, which may adopt favorable geometry. In contrast to this, a similar type of structural generalization is not feasible for nonselective inhibitors of COX isoforms, e.g. aspirin, indomethacin.[1] After the discovery of the inducible enzyme COX-2 in 1991, the diarylheterocycle **I** and the methanesulfonanilide **II** (Figure 1) were first identified as nonulcerogenic antiinflammatory agents.[4,5] Subsequently, based on rational drug design, a wide range of diarylheterocycles have been identified and developed as selective COX-2 inhibitors. As a result, celecoxib[6a] and rofecoxib[7] (Figure 1) were the first to enter the market for the treatment of acute pain, rheumatoid arthritis, and osteoarthritis. They are also in clinical trials for the treatment of Alzheimer's disease and different types of cancer based on the role of COX-2 in the etiopathology.[8,9a]

Recent studies have shown that more than 70% of patients who undergo surgery experience moderate to extreme postoperative pain and it is desirable to control and inhibit the processes that cause pain and inflammation with nonnarcotic drugs. Injectable analgesics are favored because they have a rapid and reliable onset of effect without addiction. Ketorolac (Figure 2), the only injectable nonsteroidal antiinflammatory drug (NSAID) approved for use in the United States, is a potent inhibitor of both COX-1 and COX-2. Therefore, its use is associated with unwanted side effects such as gastrointestinal ulceration and bleeding.[10] To overcome these side effects parecoxib sodium[11a] (Figure 2) has been developed as a prodrug for the potent and selective COX-2 inhibitor valdecoxib for parenteral administration (parecoxib sodium has been marketed in Europe very recently). Like other COX-2 specific inhibitors, it

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* Corresponding author. Tel: 91-40-3045439. Fax: 91-40-3045438/3045007. E-mail: (M.P.) manojitpal@drreddys.com; (K.R.Y.) koteswarraoy@drreddys.com.

‡ Discovery-Chemistry.

§ Discovery-Biology.

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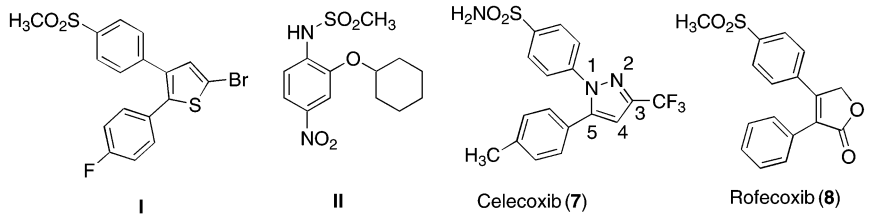


Figure 1. Some selective COX-2 Inhibitors.

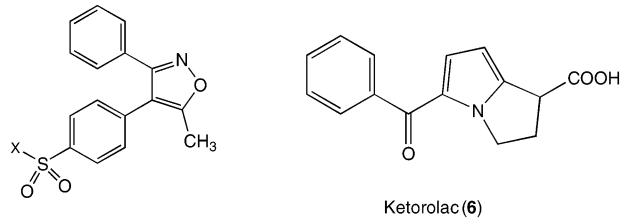


Figure 2. Injectable antiinflammatory agents.

is believed to reduce pain and inflammation by targeting the COX-2 enzyme, which is expressed at sites of injury, including surgical incisions. At therapeutic doses, parecoxib sodium does not affect COX-1. However, apart from parecoxib sodium, there is no other injectable COX-2-specific inhibitor known or reported to be in any phase of clinical investigation. Therefore, development of an alternative, new, injectable analgesic for better management of surgical pain with minimal side effects is desirable. These can also be used in the therapy of ocular inflammation.[9b]

Celecoxib as well as other coxibs such as rofecoxib, valdecoxib, and etoricoxib are considered as selective COX-2 inhibitors with fewer gastrointestinal side effects of traditional NSAIDs. However, a recent report has raised a cautionary flag regarding the use of COX-2 inhibitors in patients at risk for cardiovascular morbidity such as myocardial infarction.[12] Thus, despite their excellent antiinflammatory activities, there is still further scope and demand for the development of antiinflammatory agents with improved efficacy and safety compared to the existing COX-2 inhibitors. Celecoxib belongs to the 1,5-diarylpyrazole class of compounds, and a recent study revealed that this class of compounds could be useful as dual COX-2/5-LO (5-lipooxygenase) inhibitors for the effective management of inflammatory diseases.[6c] Therefore, in pursuance of our synthesis of various diaryl heterocycles,[13] we preferred 1,5-diarylpyrazoles over others for the development of novel class of COX-2 inhibitors.

The benzenesulfonamide group of celecoxib attached to the nitrogen atom of the pyrazole ring is thought to be responsible for its high potency and COX-2 selectivity in different models of inflammation. Extensive SAR study has been carried out on a large number of celecoxib analogues, including modification of aryl or pyrazole moiety.[6] However, systematic study of the attachment of appropriate substituents on the 1-benzenesulfonamide group[14] and its effect on COX activity has not yet been explored. We rationalized that introduction of an appropriate substituent at the 2 or 3 position of the 1-benzenesulfonamide group would reinforce the binding of this group with specificity to the COX-2 pocket. In the light of this hypothesis, a new class of 1,5-diarylpyrazole derivatives[14b] have been synthesized, some of which are in preclinical development. We now describe the synthesis and structure−activity relationship (SAR) studies of some 1,5-diarylpyrazole derivatives containing a substituted 1-benzenesulfonamide group as a pharmacophore and also explore the possibility of developing an injectable form for better management of pain. Our work involves identification of a potent COX-2 inhibitor followed by the preparation of corresponding prodrug.

Chemistry

The diarylpyrazoles listed in Table 1 were synthesized according to Schemes 1−3. The arylhydrazine hydrochloride **25**, a key intermediate for the synthesis of most of the analogues, was synthesized according to the procedure outlined in Scheme 1. Thus, chlorosulfonation of appropriately substituted flourobenzene **21** at low temperature followed by ammonolysis using aqueous ammonia in dioxane led to the formation of corresponding sulfonamide **23** in good yield. On treatment with anhydrous hydrazine in acetonitrile under refluxing condition, **23** provided **24**, which was then converted to the hydrochloride salt **25**.

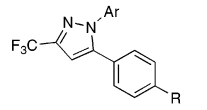
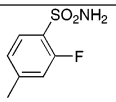
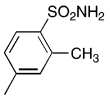
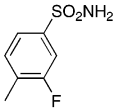
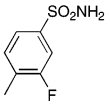
Synthesis[6a,15] of dicarbonyl derivatives **27** was carried out using (Scheme 2) Claisen condensation of an appropriate acetophenone **26** with either ethyltrifluoroacetate or ethyl difluoroacetate in the presence of sodium hydride as a base in dimethylformamide (DMF). Reaction of **27** with the hydrochloride salt **25** in ethanol under refluxing conditions led to the formation of the expected 1,5-diarylpyrazoles (**1**−**4**) as major products along with trace amounts of other regioisomers. The latter were removed by chromatography.

Syntheses of sodium salts **10**−**16** (Table 3) are outlined in Scheme 3. Sulfonamides **3** and **4** were acylated using the required acyl anhydride in the presence of triethylamine to give the corresponding acylated sulfonamides. These were treated with sodium bicarbonate in methanol to afford the required sodium salts. Other salts **17**−**20** were also prepared according to a similar procedure. 1,5-Diarylpyrazoles were characterized by the appearance of a peak at ∼6.8 and ∼5.7−4.9 ppm in the ^{1}H NMR spectra, which were assigned as due to the C-4 proton signal of the pyrazole ring and −NH of sulfonamide moiety, respectively. Salts were characterized by their high melting points (>240 °C) and the disappearance of the −NH signal of the acylated sulfonamide moiety (R′CONHSO$_{2-}$), which appeared at ∼8.50−12.50 ppm in the ^{1}H NMR spectra.

Biological Assays

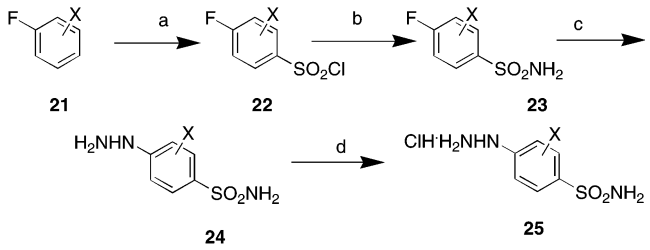
All the compounds synthesized were tested initially at 100 μM for selectivity and potency against human COX-2 (expressed in *sf9* insect cells using baculovirus)

Table 1. InVitro and in Vivo Data for 1,5-Diarylpyrazoles

Compd			InVitro % Inhibition @ 10 μM[a]		InVivo (30 mg/ kg)
			COX-1	COX-2	Rat paw edema[b]
	Ar	R			
1		OMe	0	34	52 ± 4
2		OMe	75	79	nd
3		OMe	13	75	26 ± 2
4		SMe	29	88	33 ± 5
7	Celecoxib		0	100, 98 (1)	49 ± 2
8	Rofecoxib		0	100	37 ± 7
9	Indomethacin		100	97	nd

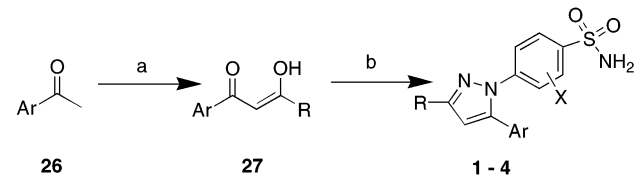
[a] Human COX-2 (expressed in *sf*9 insect cells using baculovirus) and COX-1 (ram seminal vesicles) enzyme. Figures in the brackets indicate concentration in μM. The result is the average of at least three determinations, and the deviation from the mean is <10% of the average value. [b] The carrageenan-induced rat paw edema assay was carried out using six animals (male Wistar rats)/group following oral dose of the test compound. [c] nd = not determined.

Scheme 1



(a) $ClSO_3H$, 0 °C, 4 h, 74% yield; (b) NH_3, dioxane, 0 °C, 6 h, 44−79% yield; (c) NH_2NH_2, CH_3CN, reflux, 6 h, 67−81% yield; (d) HCl, EtOH, quantitative yield.

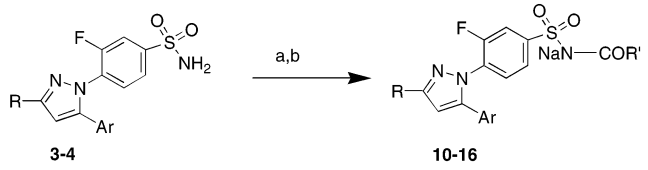
Scheme 2



(a) NaH, DMF, 0 °C to rt, RCO_2Et, 24 h, 12−97% yield; (b) **25**, EtOH, reflux, 44−64% yield.

and COX-1 (ram seminal vesicles) enzymes. On the basis of their in vitro efficacy, selected compounds were tested further at lower concentration such as 10 μM followed by 1 μM. IC_{50} values for COX-1 and COX-2 were determined[16] for selected/promising compounds. Celecoxib, rofecoxib, and indomethacin were used as reference compounds for the in vitro assay. Few compounds were selected for further investigation on the basis of their promising in vitro potencies and were tested in vivo for antiinflammatory activity at 30 mg/kg dose in the carrageenan-induced rat paw edema model.[17]

Scheme 3



(a) $(R'CO)_2O$, triethylamine, 24 h, rt, quantitative yield; (b) $NaHCO_3$, MeOH, 20 h, 25 °C, 60−96% yield.

Table 2. H-Bond Interactions of Molecules **1** and **3** with Residues in the Active Site of COX-2

compd	functional group	amino acid residue in the active site	distance X−Y (Å) for X−H···Y	angle X−H−Y (deg)
1	CF_3	Arg^{120} side chain	2.69	130.0 ± 0.3
	SO_2	NH of Phe^{518}	3.41	158.4 ± 0.1
	SO_2	Arg^{513} side chain	3.70	137.6 ± 0.1
	SO_2NH_2	Gln^{192} side chain	3.36	160.4 ± 0.1
	3-F	Arg^{513} side chain	3.01	124.0 ± 0.1
3	CF_3	Arg^{120} side chain	2.70	137.7 ± 0.1
	SO_2	NH of Phe^{518}	3.28	141.0 ± 0.2
	SO_2NH_2	Gln^{192} side chain	3.42	155.4 ± 0.2
	SO_2NH_2	CO of Ser^{353}	2.88	151.9 ± 0.1
	2-F	OH of Tyr^{355}	2.83	154.9 ± 0.1

Results and Discussion

Results of in vitro assays of the compounds synthesized are summarized in Table 1. The design of compound **1** was based on the assumption that attachment of fluorine at the C-2 position of benzenesulfonamide ring may enhance the interaction of this group with

Table 3. In Vitro Data for 1,5-Diarylpyrazoles: IC_{50} Values[a]

compd	IC_{50} (μM) COX-1	IC_{50} (μM) COX-2	volume[b] (Å^3)	log p[b]	selectivity index (COX-1/COX-2)
1	>170[c]	13.5 ± 0.13			>13
3	>30[c]	0.15 ± 0.01	261 ± 7.0	3.14 ± 0.92	>200
4	24.3 ± 0.1	0.034 ± 0.003	270 ± 7.0	3.81 ± 0.93	~714
celecoxib	15.33 ± 0.03	0.07 ± 0.005	252 ± 7.0	3.01 ± 0.86	~219
indomethacin	0.067 ± 0.001	7.8 ± 0.11	270 ± 7.0		~0.0085

[a] The result is the mean value of two determinations, and the deviation from the mean is <10% of the mean value. [b] Calculated using the ACDLabs program developed by Advanced Chemistry Development Inc. [c] Precipitation of compound observed beyond this concentration.

COX-2. We have chosen fluorine atom as a substituent because of its small size, lipophilic nature, and ability to form a strong H-bond. Thus, the basic skeleton of celecoxib has been modified in compound **1** with the introduction of a fluorine atom at the C-2 position of the benzenesulfonamide moiety along with the replacement of a methyl group by a methoxy group.

Compound **1** exhibited COX-2 selectivity with moderate potency in COX-2 inhibition (Tables 1 and 3). However, it showed good inhibition of edema in the in vivo rat paw model at a dose of 30 mg/kg. This result motivated us for further SAR studies in this series. Replacement of the methoxy substituent on the 5-aryl group of **1** with a halogen such as fluoro, chloro, or bromo led to either inactive or COX-1-selective compounds (data not shown). Thus, the methoxy group at the 4-position of the 5-aryl moiety appeared to be essential for the COX-2 selectivity and potency in this series. The fluorine substituent of the benzenesulfonamide group was found to be sensitive, and its replacement by other groups led to the alteration of selectivity. This is exemplified by compound **2**, wherein the replacement of fluorine by a methyl group resulted in loss of selectivity (Table 1). A similar effect of the methyl group was also observed in diaryl furanones.[14d] However, the reason for such observation is not clear at present. Change of the position of fluorine from C-2 (**1**) to C-3 (**3**) improved in vitro potency with a moderate change in selectivity. Although the replacement of the 4-methoxyphenyl moiety by other groups such as 4-XC_6H_4 (X = fluoro, chloro, or nitro) reduced the in vitro activity drastically (data not shown), a 4-methylsulfanylphenyl group (**4**) resulted in increase of both in vitro potency and selectivity (Table 1). These observations suggest that electron-donating groups at the 4-position of the 5-aryl moiety play a crucial role, and being good electron-donating groups, methoxy and methylsulfanyl substituents showed a similar effect on COX-2 selectivity.

The selectivity[18] of celecoxib has been shown to result from the 1-benzenesulfonamide moiety (Figure 3). This group binds in a COX-2 pocket, which is relatively polar and is less restricted compared to the COX-1 enzyme. This could also be the reason for the COX-2 selectivity of **1**, **3**, and **4**. Since the total volume[19] of the COX-2 primary binding site (394 Å^3) is about 25% larger than that of the COX-1 (316 Å^3), a fluorine-substituted benzene sulfonamide moiety should enhance its interaction with the COX-2 pocket significantly. Also it is evident from the in vitro data that fluorine at the C-3 position of the phenyl ring is more promising than at C-2. In search of a possible explanation for this observation we carefully analyzed the nature of interaction of SC-558 with the active site of COX-2 enzyme (Figure 3a).[18] The phenolic −OH of Tyr^{355} seems to have an important role in the hydrogen-bond network of the active site with the fluorine substituent of the benzenesulfonamide moiety, thereby enhancing its interaction with the COX-2 pocket. A fluorine at C-3 would be in close proximity to the Tyr^{355} side chain rather than when it is at C-2 for better electrostatic interactions. This is corroborated well by the results of the docking calculations for the molecules **1** and **3** in the COX-2 binding site. The calculated energy of interaction between COX-2 and **1** is −34.02 kcal/mol, while that of **3** is −36.06 kcal/mol, suggesting that molecule **3** interacts better. The salient hydrogen bond interactions (Figure 3b and 3c) and respective distances and angles (Table 2) clearly implicate the positional influence of fluorine substitution on the aryl sulfonamide moiety. The fluorine substituent at the C-3 position of the aryl sulfonamide in molecule **3** shows strong hydrogen-bonding interaction with −OH of the Tyr^{355} side chain. However, in molecule **1**, wherein the fluorine is at the C-2 position, it is interacting weakly with Arg^{513}. The additional interaction with Tyr^{355} may be responsible for the relatively higher potency of molecule **3** vis-à-vis molecule **1**. To gain further evidence in support of this hypothesis, the corresponding flouro analogue of celecoxib (i.e., **7a**, 3-fluoro-4-[5-(4-methylphenyl)-3-trifluoromethyl-1*H*-pyrazolyl]-1-benzenesulfonamide) was prepared and tested in vitro. The flouro analogue **7a** was found to be more potent {IC_{50} (COX-1) = 7.13; IC_{50} (COX-2) = 0.05} than celecoxib, confirming the favorable role of fluorine in binding with the COX-2 pocket.

On the basis of in vitro data, a few of these 1,5-diarylpyrazole derivatives were selected for further study. The IC_{50} values for compounds **3** and **4** are given in Table 3 and both of them were identified as potent and selective COX-2 inhibitors. They are either comparable or superior to celecoxib in terms of selectivity index (SI > 200 or 714 vs 219 for celecoxib). Interestingly, the computed molar volumes of **3** and **4** (Table 3) are comparable to those of celecoxib and indomethacin, which accounts for their ability to bind deeply at the active site of COX pockets. The antiinflammatory activity of these compounds was tested in the standard rat model of inflammation and compared with standard COX-2 inhibitors such as celecoxib or rofecoxib. However, their in vitro potencies did not translate into in vivo inhibitory efficacies. In carrageenan-induced rat paw edema assay, compounds **3** and **4** showed 26% and 33% inhibition, respectively, when compared to 52% inhibition of compound **1** when dosed orally at 30 mg/kg (Table 1). The reason for the good in vivo activity of **1** despite its inferior in vitro activity is not clear at the moment. Since none of these compounds exhibited improved antiinflammatory activity over celecoxib's 49%

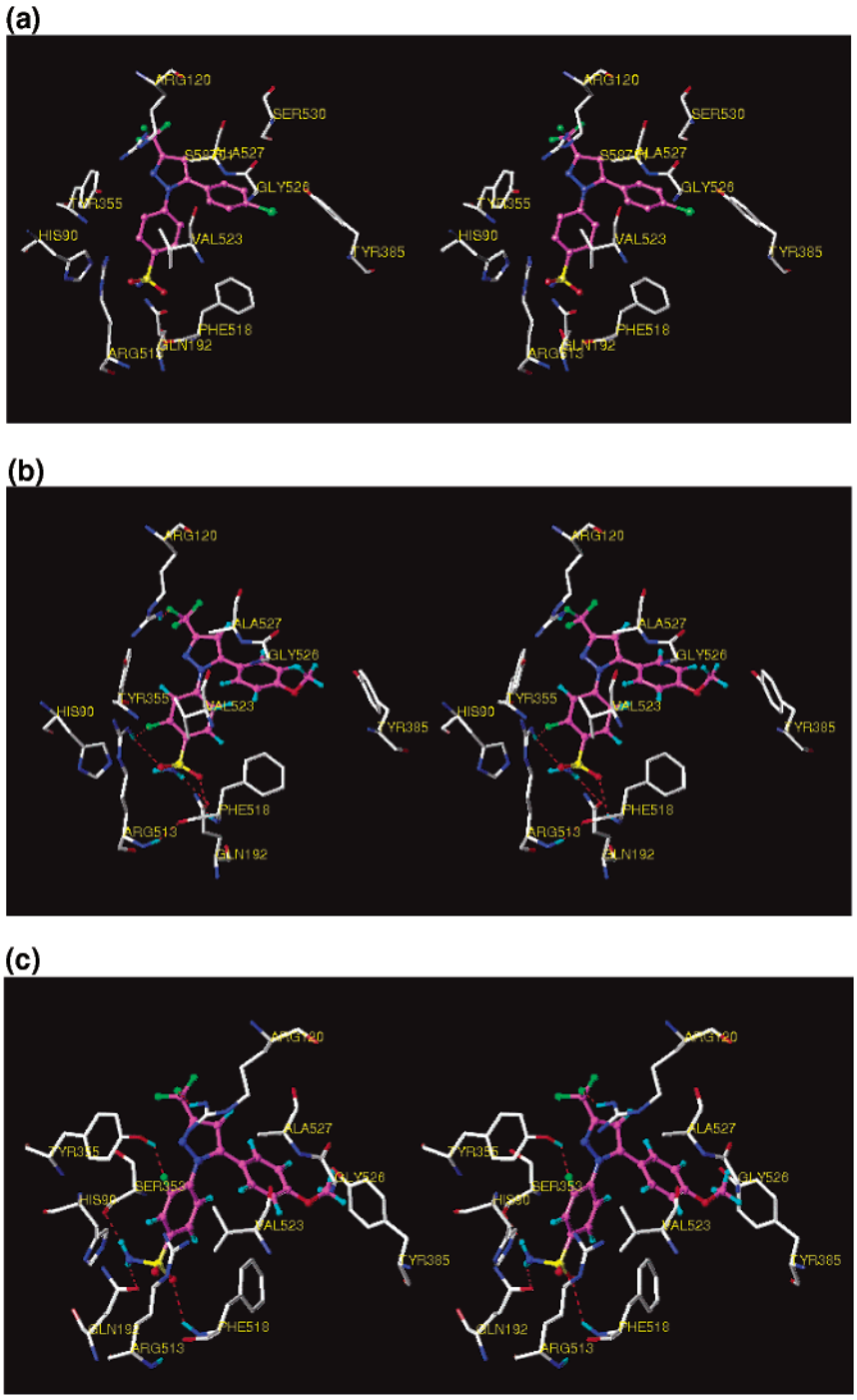
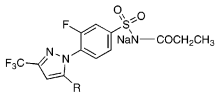
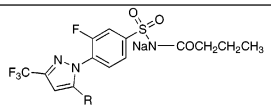
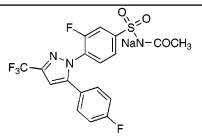
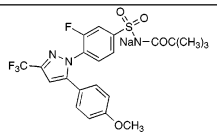
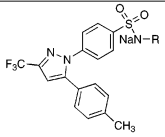


Figure 3. (a) Stereoview of SC558−COX-2 complex (6COX crystal structure). The ligand is shown in magenta. (b) Stereoview of the molecule **1**−COX-2 complex. The hydrogen-bonding interactions are shown as red broken lines. The ligand is shown in magenta. All protein hydrogens are removed for clarity. (c) Stereoview of the molecule **3**−COX-2 complex.

inhibition at 30 mg/kg po under similar conditions, it was felt that poor bioavailability could be one of the reasons for their lower in vivo potency. The calculated log *p* value for compound **3** showed greater similarity to celecoxib than **4** (Table 3) and was reflected by their in vivo pharmacokinetic study. Compound **3** displayed better pharmacokinetics than **4** and was comparable to celecoxib (see Table 6). However, the pharmacokinetic profile indicates that there could be absorption rate limitation for **3** because of its low water solubility, which could cause prolonged absorption and thereby delayed T_{max}. Since diarylheterocycles possess modest aqueous solubility, the easiest way to address this problem is to convert them into prodrugs that are comparatively more soluble in water.

Prodrugs[11b] are usually converted to the parent drug in plasma, which perhaps would allow them to pass through the cell membrane easily to reach the target for further action. The prodrug would also allow a better absorption of the drug through the gastrointestinal wall, and after being absorbed it would convert into the parent compound through hepatic metabolism. Nevertheless, we were also interested in the development of injectable formulation of a selective COX-2 inhibitor for parenteral administration. Therefore, we prepared water-soluble forms of compounds **3** and **4** and their deriva-

Table 4. In Vitro and in Vivo Data for 1,5-Diarylpyrazole Salts

Compd		InVitro % Inhibition @ 10 μM[a]		InVivo Rat paw edema[b] (30 mg/ kg)
		COX-1	COX-2	
10	4-Methoxyphenyl	18	38	78 ± 2
11	4-Methylsulfanylphenyl			37 ± 4
12	4-Fluorophenyl	0	32	38 ± 2
				
13	4-Methoxyphenyl	32	36	42 ± 5
14	4-Methylsulfanylphenyl	34	4	40 ± 3
15	4-Fluorophenyl	0	63	22 ± 5
16		26	71	44 ± 3
17		16	15	Not active
Celecoxib salts				
18	Butyryl	2	0	63 ± 2
19	Propionyl	0	0	30 ± 3
20	Acetyl	10	3	27 ± 3

[a] See footnote *a* of Table 1. [b] Carrageenan-induced rat paw edema assay was carried out using six animals (male Wistar rats)/group following oral dose of the test compound.

Table 5. In Vivo Data (Rat Paw Edema Assay[a])

entry	compd	ED_{30} (mg/kg)	ED_{50} (mg/kg)
1	**1**	1.00 ± 0.13	12.70 ± 1.43
2	**10**	nd	6.00 ± 0.21
3	celecoxib (**7**)	nd	7.90 ± 0.14
4	**18**	nd	6.69 ± 0.27
5	parecoxib sodium	nd	5.00[b]

[a] ED_{30} and ED_{50} values were determined using a minimum of four dose points, six animals (male Wistar rats)/group followed by oral administration. [b] Data from ref 11a. [c] nd = not determined.

tives, which showed substantial inhibition either in vitro or in vivo. A water-soluble form was accessible by N-acylation of the sulfonamide followed by preparation of its sodium salt, which would serve as possible prodrug for the parent compound. Thus, a variety of sodium salts **10**−**17** were prepared according to the procedure described above and evaluated in vivo. All these salts were found to be highly soluble in water and their in vitro and in vivo activities are summarized in Table 4. Prodrugs have been reported to be very weak inhibitors of both COX-1 and COX-2 earlier[11a] and were found to be the same in the present case, too. However, all the analogues exhibited good to excellent inhibition with respect to the various prodrugs of celecoxib (**18**−**20**, Table 4) when dosed orally at 30 mg/kg under similar conditions. Maximum inhibition was achieved with the propionyl analogue **10** (78%). Interestingly, the butyryl analogue (**18**) of celecoxib was found to be better than its propionyl (**19**) or acetyl (**20**) congeners. The differences in their in vivo drug-releasing properties for different prodrugs of celecoxib, i.e., **18**−**20**, as well as of compounds **3** and **4** and their derivatives, i.e., **10**−**17**, could be the reason for such observation. This, perhaps, provides an explanation for the lower activity of prodrug **11**, taking into account that, like **10**, it is the same type of derivative of the more in vitro potent **4**. The ED_{50} of compound **10** was found to be 6 mg/kg (Table 5), which is closer to that of celecoxib at 7.9 mg/kg (The ED_{50} of the butyryl analogue of celecoxib in carrgeenan-induced rat paw edema is 6.69 mg/kg.) and comparable with that of parecoxib sodium (5 mg/kg).[11a] The excellent antiinflammatory activity of **10** suggested that the salt might be protonated followed by the cleavage of acyl moiety in vivo to deliver the parent molecule at the target.[11] This was further supported by

Table 6. Oral Pharmacokinetics of 1,5-Diarylpyrazoles

parameters[a]	**3**	**4**	celecoxib	rofecoxib
AUC_{0-t} (μg h/mL)	46.45 ± 14.68	6.65 ± 1.33	23.16 ± 1.17	57.88 ± 4.53
C_{max} (μg/mL)	3.30 ± 0.98	3.21 ± 0.89	2.47 ± 0.63	5.61 ± 1.10
T_{max} (h)	7.50 ±3.32	1.26 ± 0.50	3.25 ± 1.26	3.00 ± 0.00

[a] Pharmacokinetic parameters were determined using four animals (male Wistar rats)/group following 100 mg/kg oral dose of the test compound.

the pharmacokinetic evaluation of **10** in male rats (100 mg/kg dose), where the plasma levels of **3** (after oral administration of **3** as such) and **10** were monitored. Appearance of **3** (HPLC retention time of **10**, **3**, and **3** released from **10** is 13.4, 9.0, and 9.2 min, respectively) at the first time point (0.5 h) in plasma suggested that the conversion of the prodrug into the parent compound occurred presystemically. Interestingly, this conversion of prodrug was found to be susceptible to steric influence, which has been reflected by the complete loss of in vivo activity in the case of pivolyl analogue **17** (Table 4, prodrug of **3**). The significant change in T_{max} observed for **3** (3.00 ± 1.41 h) released from **10** following oral administration at 100 mg/kg indicated rapid absorption of **3** in the present form compared to **3** as such (Table 6). Although the other pharmacokinetic parameters of **3** from **10** and **3** alone were comparable even at the lower dose (10 mg/kg), the higher efficacy of **10** observed in the inflammation model could be accounted for by a possible differential distribution of **3**, relatively higher exposure to **3**, and the inherent cyclooxygenase activity of **10**. Thus, the sodium salt **10** was identified as a water-soluble prodrug of **3** with good in vivo potency.

Conclusion

We have synthesized and described 1,5-diarylpyrazole derivatives having a substituted benzenesulfonamide moiety as potent inhibitors of COX-2 enzymes. Molecular modeling and SAR studies revealed that fluorine substitution on the phenyl sulfonamide moiety along with an electron-donating group at the 4-position of the 5-aryl ring determine the selectivity as well as potency for COX-2 inhibition. Compound **3** has been identified as the best molecule in this series and the water-soluble prodrug **10** has good activity in vivo. Compound **10** is the first 1,5-diarylpyrazole derivative reported that has potential to become an investigational COX-2 specific inhibitor for parenteral administration. The good COX-2 specificity of its parent compound **3** and the in vivo efficacy of prodrug **10** may provide better nonnarcotic management of acute to moderate pain compared to the nonselective ketorolac.

Experimental Section

Chemical Methods. All the solvents used were commercially available and distilled before use. Reactions were monitored by thin-layer chromatography (TLC) on silica gel plates (60 F254; Merck), visualizing with ultraviolet light or iodine spray. Flash chromatography was performed on silica gel (SRL 230−400 mesh) using distilled petroleum ether, ethyl acetate, dichloromethane, chloroform, and methanol. ^{1}H and ^{13}C NMR spectra were determined in $CDCl_3$, DMSO-d_6,or MeOH-d_4 solution on Varian Gemini 200 MHz spectrometers. Proton chemical shifts (δ) are relative to tetramethylsilane (TMS, $\delta = 0.00$) as internal standard and expressed in ppm. Spin multiplicities are given as s (singlet), d (doublet), t (triplet), and m (multiplet) as well as b (broad). Coupling constants (J) are given in hertz. Infrared spectra were recorded on a Perkin-Elmer 1650 FT-IR spectrometer. UV spectra were recorded on a Shimadzu UV 2100S UV-vis recording spectrophotometer. Melting points were determined using a Buchi melting point B-540 apparatus and are uncorrected. Thermal analysis data was generated with the help of a Shimadzu DSC-50 detector. MS spectra were obtained on a HP-5989A mass spectrometer. Purity was determined by HPLC (AGIL-AUTO) using the conditions specified in each case: column, mobile phase (range used), flow rate (ranges used), detection wavelength, retention times. Microanalyses were performed using a Perkin-Elmer 2400 C H N S/O analyzer. Elemental data are within ±0.4% of the theoretical values. All yields reported are unoptimized. Celecoxib was prepared according to the literature[6a] procedure. Rofecoxib was prepared according to the procedure described in WO 9500501. Acetophenones were either purchased or prepared according to the procedure described in the literature.

Preparation of Arylhydrazine Hydrochloride (25). Step a: Preparation of Arylsulfonyl Chloride (22). To a cold solution (0 °C) of chlorosulfonic acid (450 mmol) was added appropriately substituted fluorobenzene **21** (90 mmol) slowly at 0 °C. The mixture was then stirred for 4 h at 0 °C and allowed to stand for overnight at the same temperature. The reaction mixture was poured into crushed ice. The oily layer that separated was collected and washed with water to give the desired compound as a liquid.

Step b: Preparation of Arylsulfonamide (23). To a cold solution of **22** (67 mmol) in dioxane (30 mL) was added 25% aqueous solution of ammonia (140 mL) with vigorous stirring. The stirring continued for 6 h at 0 °C and separated solid was filtered and washed with water (2 × 50 mL) to give the title compound as a white solid.

3,4-Difluoro-1-benzenesulfonamide (23a). Yield 44%; mp 90−92 °C; ^{1}H NMR ($CDCl_3$) δ 7.82−7.74 (m, 1H), 7.39−7.32 (m, 2H), 5.05 (bs, D_2O exchangeable, 2H); IR (KBr, cm^{-1}) 3364, 3268, 1512; MS (CI, isobutane) 194 (M^{+1}, 100). Anal. ($C_6H_5F_2NO_2S$) C, H, N.

2,4-Difluoro-1-benzenesulfonamide (23b). Yield 53%; mp 157−158 °C; ^{1}H NMR (CD_3OD) δ 7.98−7.87 (m, 1H), 7.24−7.06 (m, 2H); IR (KBr, cm^{-1}) 3366, 3263; MS (CI, isobutane) 194 (M^{+1}, 100%). Anal. ($C_6H_5F_2NO_2S$) C, H, N.

4-Fluoro-2-methyl-1-benzenesulfonamide (23c). Yield 79%; mp 170−171°C; ^{1}H NMR (DMSO-d_6) δ 7.9 (m, 1H), 7.4 (s, 2H, D_2O exchangeable), 7.2 (m, 2H), 2.59 (s, 3H). Anal. ($C_7H_8FNO_2S$) C, H, N.

Step c: Preparation of 4-Hydrazino-1-arylsulfonamide (24). A solution of **23** (2.94 mmol) in acetonitrile (10 mL) was treated with anhydrous hydrazine (0.6 mL) and the mixture was refluxed for 6 h. The solvent was then removed under reduced pressure and the residue was treated with water (10 mL). The separated solid was filtered and washed with cold water (2 × 50 mL) to give the required product.

3-Fluoro-4-hydrazino-1-benzenesulfonamide (24a). Yield 81%; low-melting solid; ^{1}H NMR ($CDCl_3$) δ 7.76−7.64 (m, 2H), 7.38−7.27 (m, 1H); IR (KBr, cm^{-1}) 3340, 1512; MS (CI, isobutane) 206 (M + 1, 100). Anal. ($C_6H_8FN_3O_2S$) C, H, N.

2-Fluoro-4-hydrazino-1-benzenesulfonamide (24b). Yield 73%; low-melting solid; ^{1}H NMR (CD_3OD) δ 7.92−7.85 (m, 1H), 6.98−6.75 (m, 2H); IR (KBr, cm^{-1}) 3376, 3272, 1569; MS (CI, isobutane) 205 (30), 95 (100). Anal. ($C_6H_8FN_3O_2S$) C, H, N.

4-Hydrazino-2-methyl-1-benzenesulfonamide (24c). Yield 67%; low-melting soild; ^{1}H NMR (DMSO-d_6) δ 7.6 (m, 1H), 7.4 (m, 2H), 2.57 (s, 3H); IR (KBr, cm^{-1}) 3375, 3270, 1569; MS (CI, isobutane) 202 (M^+, 100). Anal. ($C_7H_{11}N_3O_2S$) C, H, N.

Step d: Preparation of Hydrazine Hydrochloride (25). To a solution of **24** (0.4 g) in ethanol (5 mL) was added 2-propanol (3 mL) saturated with dry hydrochloric acid at 25 °C and the mixture was stirred for 1.5 h at the same temperature. The solvent was removed under low pressure to yield the required compound in quantitative yield.

General Method for the Preparation of 1,5-Diarylpyrazoles.[6a,15] Step a: Preparation of 1,3-Diketones (27). General Procedure. To a solution of acetophenone (**26**, 2.7 mmol) in dimethylformamide was added a 60% oil suspension of sodium hydride (3.24 mmol) at 10 °C under nitrogen atmosphere and the mixture was stirred for 10 min. To this mixture was added fluorinated ethyl acetate (3.24 mmol) at the same temperature and stirring was continued for 12 h. This mixture was then poured into ice-cold hydrochloric acid solution (100 mL) and was stirred for 10 min. The separated solid was extracted with ethyl acetate (2 × 50 mL). The combined organic layer was collected, washed with water (2 × 50 mL), dried over anhydrous Na_2SO_4, and concentrated under vacuum. The residue isolated was purified by column chromatography using 5% ethyl acetate−petroleum ether to give required compound **27**.

Step b: A mixture of hydrazine hydrochloride **25** and 1,3-diketone **27** in ethanol was heated to reflux with vigorous stirring under nitrogen atmosphere for 12 h. Ethanol was removed under low vacuum and the isolated residue was purified by column chromatography using ethyl acetate−petroleum ether to give the expected product.

2-Fluoro-4-[5-(4-methoxyphenyl)-3-trifluoromethyl-1*H*-1-pyrazolyl]-1-benzenesulfonamide (1). The title compound was synthesized from 4′-methoxyacetophenone using the two-step procedure as described above to give a white powder: yield 44%; R_f 0.50 (30% ethyl acetate−petroleum ether); mp 124−125 °C; ^{1}H NMR ($CDCl_3$) δ 8.30−8.23 (m, 1H), 7.27−7.17 (m, 3H), 6.87−6.71 (m, 4H), 5.78 (s, D_2O exchangeable, 2H), 3.81 (s, 3H); IR (KBr, cm^{-1}) 3386 (b, s); UV (MeOH, nm) 255; MS (EI) 415 (M$^+$, 100); HPLC 98.7%, SYMMETRY SHIELD RP 18 (250 mm), water−acetonitrile (20:80), 1 mL/min, 255 nm, t_R 4.4 min. Anal. ($C_{17}H_{13}F_4N_3O_3S$) C, H, N.

2-Methyl-4-[5-(4-methoxyphenyl)-3-trifluoromethyl-1*H*-1-pyrazolyl]-1-benzenesulfonamide (2). The title compound was synthesized from 4′-methoxyacetophenone using the two-step procedure as described above to five a white powder: yield 62%; R_f 0.80 (30% ethyl acetate−petroleum ether); mp 138−140 °C; ^{1}H NMR (DMSO-d_6) δ 7.88−7.81 (m, 2H), 7.50−7.47 (m, 3H), 7.40−7.12 (m, 3H), 6.95 (d, J = 8.7 Hz, 2H), 3.75 (s, 3H), 2.57 (s, 3H); IR (KBr, cm^{-1}) 3374 (b, s), 2361; UV (MeOH, nm) 250; MS (CI, isobutane) 411 (M$^+$, 38), 323, 303 (100); HPLC 97.37%, SYMMETRY SHIELD RP 18 (150 mm), water−acetonitrile (50:50), 1 mL/min, 210 nm, t_R 13.2 min.

3-Fluoro-4-[5-(4-methoxyphenyl)-3-trifluoromethyl-1*H*-1-pyrazolyl]-1-benzenesulfonamide (3). The title compound was synthesized from 4′-methoxyacetophenone using the two-step procedure as described above to give white flakes: yield 64%; R_f 0.2 (30% ethyl acetate−petroleum ether); mp 116−120 °C; ^{1}H NMR ($CDCl_3$) δ 7.81−7.71 (m, 3H), 7.13 (d, J = 8.3 Hz, 2H), 6.86 (d, J = 8.3 Hz, 2H), 6.74 (s, 1H), 4.91 (s, D_2O exchangeable, 2H), 3.82 (s, 3H); IR (KBr, cm^{-1}) 3352, 3079, 1612, 1473; UV (MeOH, nm) 255; MS (CI, isobutane) 417 (M + 2, 25), 416 (M + 1, 100); HPLC 99.85%, SYMMETRY SHIELD RP 18 (250 mm), water−acetonitrile (50:50), 1 mL/min, 225 nm, t_R 20.3 min. Anal. ($C_{17}H_{13}O_3N_3SF_4$) C, H, N.

3-Fluoro-4-[5-(4-methylsulfanylphenyl)-3-trifluoromethyl-1*H*-1-pyrazolyl]-1-benzenesulfonamide (4). The title compound was synthesized from 4′-methylsulfanylacetophenone using the two-step procedure as described above to give a white solid: yield 61%; R_f 0.5 (30% ethyl acetate−petroleum ether); mp 156−159 °C; ^{1}H NMR ($CDCl_3$) δ 7.86−7.66 (m, 3H), 7.18 (d, J = 8.8 Hz, 2H), 7.10 (d, J = 8.8 Hz, 2H), 6.78 (s, 1H), 4.95 (s, D_2O exchangeable, 2H), 2.48 (s, 3H); IR (KBr, cm^{-1}) 3386, 3249, 1603, 1416; UV (MeOH, nm) 225; MS (CI, isobutane) 433 (M + 2, 33), 432 (M + 1, 100); HPLC 97.3%, HICHROM RPB, water−acetonitrile (30:70), 1 mL/min, 225 nm, t_R 7.7 min. Anal. ($C_{17}H_{13}O_2N_3S_2F_4$) C, H, N.

General Method for the Preparation of Sodium Salt of 1,5-Diarylpyrazoles. Step 1. To a solution of appropriate pyrazoles (**3**, **4**, or **7**, 0.62 mmol) in dichloromethane (12 mL) was added the required acyl anhydride (1.862 mmol) and triethylamine (1.862 mmol) and the mixture was stirred for 24 h at room temperature. After completion of the reaction, solvent was removed under vacuum and the residue was treated with water followed by extraction with dichloromethane (3 × 50 mL). Combined organic layer was dried over anhydrous Na_2SO_4 and concentrated under vacuum. The isolated residue was purified by column chromatography using ethyl acetate−petroleum ether to give required acylated compound.

Step 2. To a solution of acylated compound (0.57 mmol) obtained above in methanol (15 mL) was added $NaHCO_3$ (0.79 mmol) and the mixture was stirred at room temperature for 20 h. Methanol was removed under vacuum and the residue was treated with dry diethyl ether (3 × 10 mL). The powder was collected after filtration and dried under vacuum to give the required sodium salt of 1,5-diarylpyrazoles.

Sodium Salt of *N*1-Propionyl-3-fluoro-4-[5-(4-methoxyphenyl)-3-trifluoromethyl-1*H*-1-pyrazolyl]-1-benzenesulfonamide (10). White powder; yield 82%; R_f 0.1 (40% ethyl acetate−petroleum ether); mp 278−284 °C; ^{1}H NMR (DMSO-d_6) δ 7.65 (d, J = 7.8 Hz, 2H), 7.58 (s, 2H), 7.18 (d, J = 4.4 Hz, 2H), 6.93 (d, J = 8.8 Hz, 2H), 3.74 (s, 3H), 1.92 (q, J = 7.6 Hz, 2H), 0.88−0.81 (t, J = 7.3 Hz, 3H); IR (KBr, cm^{-1}) 1609; UV (MeOH, nm) 245; MS (CI, isobutane) 472 (M$^+$ − 21, 5), 337, 155 (100); HPLC 99.23%, SYMMETRY C^{18} (150 mm), water−acetonitrile (35:65), 1 mL/min, 245 nm, t_R 4.7 min. Anal. ($C_{20}H_{16}F_4N_3O_4SNa$) C, H, N.

Sodium Salt of *N*1-Propionyl-3-fluoro-4-[5-(4-methylsulfanylphenyl)-3-trifluoromethyl-1*H*-1-pyrazolyl]-1-benzenesulfonamide (11). White powder; yield 96%; R_f 0.1 (40% ethyl acetate−petroleum ether); mp 309−312 °C; ^{1}H NMR (DMSO-d_6) δ 7.65−7.60 (m, 3H), 7.25−7.23 (m, 5H), 2.5 (s, 3H), 2.0−1.85 (m, 2H), 0.83 (t, J = 7.3 Hz, 3H); IR (KBr, cm^{-1}) 3426, 2974, 2358, 1610; UV (MeOH, nm) 245; MS (CI, isobutane) 466 (10), 424 (100); HPLC 98%, SYMMETRY C^{18} (150 mm), 0.01 M potassium dihydrogen phosphate−acetonitrile (35:65), pH 3.5, 0.8 mL/min, 245 nm, t_R 8.4 min. Anal. ($C_{20}H_{16}F_4N_3O_3S_2$-Na) C, H, N.

Sodium Salt of *N*1-Propionyl-4-[5-(4-fluorophenyl)-3-trifluoromethyl-1*H*-1-pyrazolyl]-3-fluoro-1-benzenesulfonamide (12). White powder; yield 60%; R_f 0.1 (40% ethyl acetate−petroleum ether); mp 245−250 °C; ^{1}H NMR (DMSO-d_6) δ 7.67−7.58 (m, 3H), 7.35−7.24 (m, 5H), 1.98−1.82 (m, 2H), 0.92 (t, J = 7.3 Hz, 3H); IR (KBr, cm^{-1}) 3502, 1612; UV (MeOH, nm) 250; MS (CI, isobutane) 460 (M$^+$ − 21, 10), 404, 396, 250 (100); HPLC 99%, INERTSIL ODS 3V (4.6 × 250 mm), 0.01 M potassium dihydrogen phosphate−acetonitrile (30:70), pH 3.5, 1 mL/min, 250 nm, t_R 7.0 min. Anal. ($C_{19}H_{13}F_5N_3O_3SNa$) C, H, N.

Sodium Salt of *N*1-Butryl-3-fluoro-4-[5-(4-methoxyphenyl)-3-trifluoromethyl-1*H*-1-pyrazolyl]-1-benzenesulfonamide (13). White hygroscopic powder; yield 87%; R_f 0.1 (40% ethyl acetate−petroleum ether); ^{1}H NMR (DMSO-d_6) δ 8.00−7.72 (m, 3H), 7.11 (d, J = 8.8 Hz, 2H), 6.84 (d, J = 8.8 Hz, 2H), 6.74 (s, 1H), 3.81 (s, 3H), 2.24 (t, J = 7.3 Hz, 2H), 1.68−1.56 (m, 2H), 0.92 (t, J = 7.3 Hz, 3H); IR (KBr, cm^{-1}) 1610, 1469; UV (MeOH, nm) 250; MS (CI, isobutane) 486 (M$^+$ − 21, 100); HPLC 97.6%, INERTSIL ODS 3V (4.6 × 250 mm), 0.01M potassium dihydrogen phosphate−acetonitrile (30:70), pH 3.5, 1 mL/min, 250 nm, t_R 8.3 min. Anal. ($C_{21}H_{18}F_4N_3O_4SNa$) C, H, N.

Sodium Salt of *N*1-Butryl-3-fluoro-4-[5-(4-methylsulfanylphenyl)-3-trifluoromethyl-1*H*-1-pyrazolyl]-1-benzenesulfonamide (14). White powder; yield 96%; R_f 0.1 (40% ethyl acetate−petroleum ether); mp 300−304 °C; ^{1}H NMR (DMSO-d_6) δ 7.67−7.59 (m, 3H), 7.25−7.22 (m, 5H), 2.5 (s, 3H), 1.89 (t, J = 7.3 Hz, 2H), 1.49−1.38 (m, 2H), 0.83 (t, J = 7.3 Hz, 3H); IR (KBr, cm^{-1}) 3447, 2963, 1605, 1469; UV (MeOH,

nm.) 250; MS (CI, isobutane) 502 ($M^+ - 21$, 2), 438 (100); HPLC 98.3%, INERTSIL ODS 3V (250 mm), 0.01M potassium dihydrogen phosphate−acetonitrile (30:70), pH 3.5, 1 mL/min, 250 nm, t_R 10.1 min. Anal. ($C_{21}H_{18}F_4N_3O_3S_2Na$) C, H, N.

Sodium Salt of *N*1-Butryl-4-[5-(4-fluorophenyl)-3-trifluoromethyl-1*H*-1-pyrazolyl]-3-fluoro-1-benzenesulfonamide (15). White hygroscopic powder; yield 70%; R_f 0.1 (40% ethyl acetate−petroleum ether); ^{1}H NMR (DMSO-d_6) δ 8.02−7.71 (m, 3H), 7.22−6.99 (m, 4H), 6.79 (s, 1H), 2.24 (t, $J = 7.3$ Hz, 2H), 1.68−1.57 (m, 2H), 0.91 (t, $J = 7.3$ Hz, 3H); IR (KBr, cm^{-1}) 1604, 1470; UV (MeOH, nm.) 245; MS (CI, isobutane) 474 ($M^+ - 21$, 100); HPLC 98%, SYMMETRY C^{18} (4.6 × 150 mm), 0.01 M potassium dihydrogen phosphate−acetonitrile (40:60), pH 3.5, 1 mL/min, 245 nm, t_R 9.7 min. Anal. ($C_{20}H_{15}F_5N_3O_3SNa$) C, H, N.

Sodium Salt of *N*1-Acetyl-4-[5-(4-fluorophenyl)-3-trifluoromethyl-1*H*-1-pyrazolyl]-3-fluoro-1-benzenesulfonamide (16). White powder; yield 60%; R_f 0.1 (40% ethyl acetate−petroleum ether); mp 242−246 °C; ^{1}H NMR (DMSO-d_6) δ 7.99 (d, $J = 8.3$ Hz, 1H), 7.85−7.71 (m, 2H), 7.23−7.01 (m, 4H), 6.79 (s, 1H), 2.09 (s, 3H); HPLC 97.5%, INERTSIL ODS 3V (4.6 × 250 mm), 0.01 M potassium dihydrogen phosphate−acetonitrile (30:70), pH 3.5, 1 mL/min, 250 nm, t_R 7.0 min. Anal. ($C_{18}H_{11}F_5N_3O_3SNa$) C, H, N.

Sodium Salt of *N*1-Pivolyl-3-fluoro-4-[5-(4-methoxyphenyl)-3-trifluoromethyl-1*H*-1-pyrazolyl]-1-benzene-sulfonamide (17). White powder; yield 82%; R_f 0.1 (40% ethyl acetate−petroleum ether); mp >320 °C; ^{1}H NMR (DMSO-d_6) δ 7.64−7.54 (m, 3H), 7.23−7.18 (m, 3H), 6.92 (d, $J = 8.3$ Hz, 2H), 3.74 (s, 3H), 0.96 (s, 9H); HPLC 98%, HICHROM RPB (250 mm), 0.01 M potassium dihydrogenphosphate−acetonitrile (40:60), pH 3.5, 1 mL/min, 245 nm, t_R 18.0 min. Anal. ($C_{22}H_{20}F_4N_3O_4SNa$) C, H, N.

Biological Assays. In Vitro Biochemical Assays. Spectrophotometric Assay of COX-1 and COX-2. Microsomal fraction of ram seminal vesicles were used as a source of COX-1 enzyme,[20] and microsomes from *sf-9* cells infected with baculovirus containing human COX-2 c-DNA were used as a source of COX-2 enzyme.[21] 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 as per the procedure described by Copeland et al.,[22] with the following modifications. The assay mixture (1000 μL) contained 100 mM Tris pH 8.0, 3 mM EDTA, 15 μM hematin, 150 units of enzyme, and 8% DMSO. The mixture was preincubated at 25 °C for 15 min before initiation of enzymatic reaction in the presence of compound/vehicle. The reaction was initiated by the addition of 100 μM arachidonic acid and 120 μM TMPD. The enzyme activity was measured by estimation of the initial velocity of TMPD oxidation over the first 25 s of the reaction followed by tracking the increase in absorbance at 603 nM. The IC_{50} values were calculated using nonlinear regression analysis.

In Vivo Screening Methods. Carrageenan-Induced Rat Paw Edema. Male Wistar rats (120−140 g) were fasted for 16 h before the experiment. Compounds were suspended in 0.25% carboxymethylcellulose and administered orally in a volume of 10 mL/kg 2 h before carrageenan injection. Paw edema was induced in rats by intradermal injection of 50 μL of 1% λ-carrageenan in saline into the plantar surface of the right hind paw. Paw volume was measured before and 3 h after carrageenan injection by plethysmometer (Ugo-Basile, Italy). Paw edema was compared with the vehicle control group and percent inhibition was calculated in comparison to the vehicle group. ED_{30} and ED_{50} values were calculated using linear regression plot.

Molecular Modeling. The molecules **1** and **3** were sketched and minimized using the MMFF94 force field and charges in SYBYL 6.8.[23] A cocrystal structure of COX-2 with the selective ligand SC-558 (PDB: 6COX)[18,24] was used for docking. The molecules to be docked were aligned with respect to SC-558 in the active site of COX-2 using FIT ATOMS in SYBYL. The minimization of the ligand−protein complex was carried out in two steps: optimization of hydrogen atoms that were added followed by optimization of active site. The energy-minimized complexes were analyzed for ligand−receptor interactions in the active site.

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