New Celecoxib Derivatives as Anti-Inflammatory Agents

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A series of 1,5-diarylpyrazoles with a substituted benzenesulfonamide moiety was synthesized and evaluated for cyclooxygenase (COX-1/COX-2) inhibitory activities. Some compounds, for example, (±)-2-[4-(5-*p*-tolyl-3-trifluoromethyl-pyrazole-1-yl)-benzenesulfonylaminooxy]-propionic acid **16** and its disodium salt **21**, had a higher in vivo anti-inflammatory activity compared to celecoxib, despite having no in vitro COX-1 or COX-2 inhibitory activity. Their gastrointestinal side effect profile is essentially more favorable than that of celecoxib.

Introduction

The discovery that a key cyclooxygenase enzyme in arachidonic acid metabolism exists in two isoforms, namely, COX-1[a] and COX-2, the constitutive and inducible forms, has generated new avenues for drug design. COX-1 is present in the stomach, intestines, kidneys, and platelets, while COX-2 is expressed during inflammation[1–3] and is also constitutively expressed in brain, spinal cord,[4] kidneys, and pancreatic cellate cells (PCCs).[5] The traditional nonsteroid anti-inflammatory agents (NSAIDs) inhibit both cyclooxygenase enzymes and hence downregulate prostaglandin formation in almost all cells and tissues. This broad inhibitory profile accounts for their anti-inflammatory activity as well as their pronounced side effects. It was proposed that a selective inhibitor of COX-2 would be an attractive approach to the treatment of inflammatory conditions, without concomitant gastric and renal toxicity. Celecoxib **1**[6] and other coxibs, such as rofecoxib **2**,[7] etoricoxib **3**,[8] and valdecoxib **4**[9] (Figure 1), are selective COX-2 inhibitors with fewer gastrointestinal side effects to traditional NSAIDs. However, the long-term use of both traditional NSAIDs and coxibs have been reported to cause significant cardiovascular side effects.[10] Celecoxib has advantages in this respect, because it is not associated with an increased incidence of cardiovascular events compared with placebo and with nonselective NSAIDs.[11] There are, however, some characteristics of celecoxib that could be improved. For example, Celecoxib is not effective in all patients and has some gastrointestinal side effects. Following the successful introduction of celecoxib and rofecoxib, subsequent coxib research has focused on more selective COX-2 analogues. However, the COX-1/COX-2 selectivities of celecoxib and rofecoxib were different, and the VIGOR study[12] raised the question of its cardiovascular side effects. The possible reasons of this phenomenon are described in the study of D. Mukherjee.[13] Celecoxib has a lower COX1/COX2 selectivity, therefore, we focused our research work on its derivatives. Accordingly, our aim was to synthesize analogues by the method of Analogue Based Drug Discovery (ABDD)[14] to obtain more effective celecoxib derivatives with a better gastrointestinal side-effect profile.

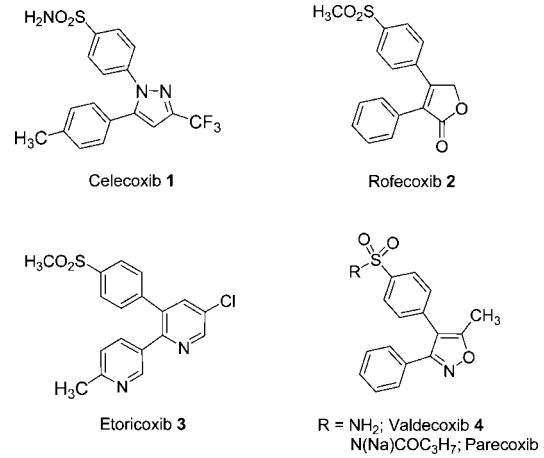

Figure 1. COX-2 inhibitors.

In our previous work, we prepared an *N*-hydroxy derivative of valdecoxib[15] (compound **6**) that showed marginal in vitro activity (Table 1), but nevertheless, was more effective than the parent compound in various animal models of acute and chronic inflammation.[16] Following a similar approach, we synthesized the *N*-hydroxy analogue of celecoxib (compound **12**; Figure 2), but it proved to be an unstable molecule. In contrast to **6**, which could be isolated as a stable monohydrate following the elimination of traces of free radicals by recrystallization from aqueous ethanol containing L-ascorbic acid, the same method was not successful with compound **12**. In this report, we discuss our attempt to prepare stable derivatives of **12**. One of its derivatives, **16**, and its disodium salt, **21**, were identified as potential analgesic and anti-inflammatory candidates for oral as well as intravenous administration.[17]

Synthesis. The key intermediate, **11**, required for the synthesis of compounds **12**−**22** was prepared according to the procedure outlined in Scheme 1. Claisen condensation of the commercially available *p*-methyl-acetophenone **7** and trifluoroacetic acid ethyl ester gave diketone **8** in good yield.[6] Compound **8** was then reacted with the commercially available *p*-hydrazino-benzenesulfonic acid **9** in refluxing ethanol to give sulfonic acid derivative **10** (yield over 80%). This reaction is a regioselective

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[a] Abbreviations: COX, cyclooxygenase; PCCs, pancreatic cellate cells; NSAIDs, nonsteroid anti-inflammatory drugs; ABDD, analogue-based drug discovery; VIGOR, Vioxx Gastrointestinal Outcome Research.

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Table 1. Effect of valdecoxib and **6** on human recombinant COX-2 and ovine COX-1 *in vitro*

cmpd	$IC_{50} \pm$ S.E.M., μM		
	human recombinant COX-2	ovine COX-1	COX-1/COX-2 selectivity
4, valdecoxib	0.04 ± 0.02	157.2	3930
6	1.1 ± 0.2	96.2 ± 10.2	88

transformation and the 1,5-diarylpyrazole could be generated almost exclusively by carrying out the condensation in the presence of the hydrochloride salt of the phenyl-hydrazine. The intermediate **10** was converted into its sulfonyl chloride **11** with phosphorus pentachloride in dry dichloromethane, and the product could be obtained in high purity after recrystallization from cyclohexane. The general method employed for the preparation of the aminooxy-carboxylic acid derivatives **12–18** and its sodium salts **19–22** is illustrated in Scheme 2. Reaction of **11** with hydroxylamine hydrochloride derivatives in a mixture of dioxane and water at room temperature provided the aminooxy derivatives **12–18**. Their respective disodium salts **20–22** were prepared in 80–88% yield using 2 equiv of aqueous NaOH solution in ethanol. From the acetic acid derivative **13**, only the monosodium salt **19** could be isolated in stable crystalline form using 1 equiv aqueous NaOH solution in ethanol. As the aminooxy-carboxylic acid moiety of **16** contains a stereogenic center, the prepared target compound **16** is a racemate. To investigate the in vivo activity of the pure enantiomers, the racemic compound was resolved by crystallization using optically active ephedrine in ethanol to furnish two optically pure compounds **23** and **24** (Scheme 3).

Biological Results and Discussion. As an initial screen for activity, **13–22** were evaluated against human recombinant COX-1 and COX-2 enzymes,[18] but despite exhibiting neither COX-1 nor COX-2 inhibition at 10 μM concentrations, they were evaluated for in vivo anti-inflammatory activity at a 10 mg/kg single dose using the carrageenan-induced paw edema method in rats. Table 2 shows the anti-inflammatory activities of **13–24** compared to celecoxib as a reference compound in

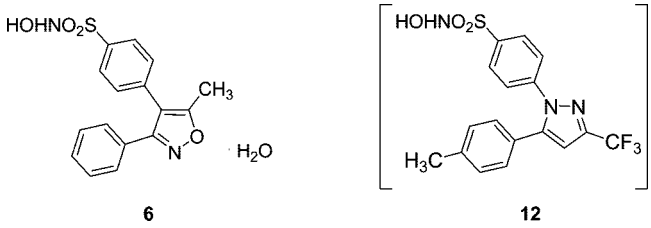
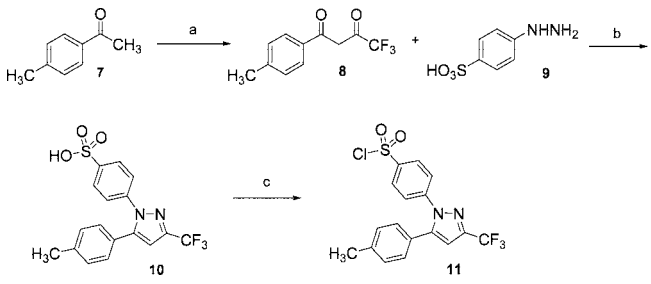


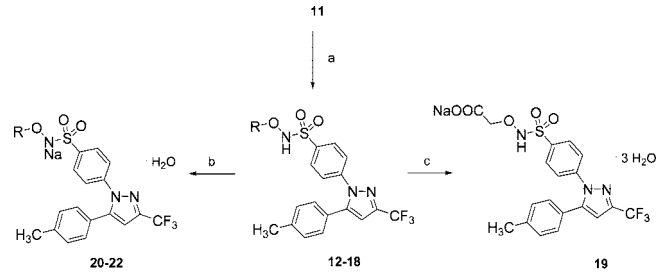
Figure 2. Structure of *N*-hydroxy-valdecoxib monohydrate and *N*-hydroxy-celecoxib.

Scheme 1. Synthesis of the Key Intermediate[a]



[a] Reagents and conditions: (a) CF_3COOEt, NaOMe/MeOH, reflux, 95%; (b) EtOH/HCl, reflux, 82%; (c) PCl_5, CH_2Cl_2, reflux, 74%.

Scheme 2. Synthesis of the Target Compounds[a]

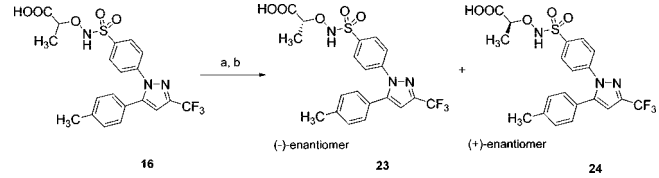


20 R = $-(CH_2)_2COONa$
21 R = $-CH(CH_3)COONa$
22 R = $-C(CH_3)_2COONa$

12 R = H
13 R = $-CH_2COOH$
14 R = $-CH_2COOEt$
15 R = $-(CH_2)_2COOH$
16 R = $-CH(CH_3)COOH$
17 R = $-CH(CH_3)COOEt$
18 R = $-C(CH_3)_2COOH$

[a] Reagents and conditions: (a) $RONH_2 \cdot HCl$, dioxan/H_2O, rt, 76−92%; (b) 2 equiv aq NaOH, ethanol, rt, 80−88%; (c) 1 equiv aq NaOH, ethanol, rt, 90%.

Scheme 3. Resolvation of **16**[a]



[a] Reagents and conditions: (a) active ephedrine, ethanol, rt, 60%; (b) 1 N HCl, 60%.

Table 2. Anti-Inflammatory Activity of Compounds at 10 mg/kg p.o. Dose in Carrageenan-Induced Edema Model in Rats (n = 6–12 Animals/Group)

cmpd	% inhibition 4 h post dose, mean ± SEM	% inhibition 6 h post dose, mean ± SEM	% inhibition 7 h post dose, mean ± SEM
1, celecoxib	24.2 ± 4.3	18.6 ± 2.3	12.3 ± 4.3
13	13.1 ± 7.1	21.3 ± 5.1	17.1 ± 5.4
14	13.3 ± 6.9	23.1 ± 7.2	20.6 ± 5.9
15	23.6 ± 4.3	12.8 ± 3.6	11.9 ± 3.9
16	41.0 ± 6.1**	31.1 ± 4.5*	17.9 ± 5.2
17	23.5 ± 4.2*	15.7 ± 3.1	7.3 ± 1.9
19	26.0 ± 5.5*	33.2 ± 4.1**	24.7 ± 3.5*
20	32.0 ± 3.5**	33.9 ± 3.7**	24.9 ± 3.2*
21	38.4 ± 3.9**	21.8 ± 7.0	16.2 ± 6.2
23	18.4 ± 4.2	15.3 ± 4.3	6.7 ± 3.1
24	0.9 ± 4.2	-9.3 ± 2.8	-6.3 ± 2.4

* $p < 0.05$. ** $p < 0.01$ vs control (5% Tween−80% phys. saline), Tukey's multiple comparison test.

this model. The aminooxy-acetic acid **13** and its ester derivative **14** showed a weak in vivo activity, while the propionic acid derivatives (**15** and **16**) and their disodium salts (**20** and **21**) exhibited impressive activity in this model. The best compound, **16**, was further tested at 3, 10, and 30 mg/kg in 6−12 animals per group to calculate the ED_{30}. These results are depicted in Figure 3. Celecoxib was effective at 4 h after treatment with an ED_{30} of 23 mg/kg (0.06 mmol/kg), but its activity practically disappeared after 6 h. Compound **16** was potent at 4 and 6 h after treatment with an ED_{30} of 5.7 mg/kg (0.012 mmol/kg) and ED_{30} of 8.4 mg/kg (0.018 mmol/kg), respectively. These results show that **16** significantly inhibited paw edema at both investigated time points and exhibited a 4-fold better anti-inflammatory potency (ED_{30}, 5.7 mg/kg, 0.012 mmol/kg) than the parent compound celecoxib (ED_{30}, 23 mg/kg, 0.06 mmol/kg) in this model. Surprisingly, we found that the pure enantiomers (**23** and **24**) of **16** were each less active than the

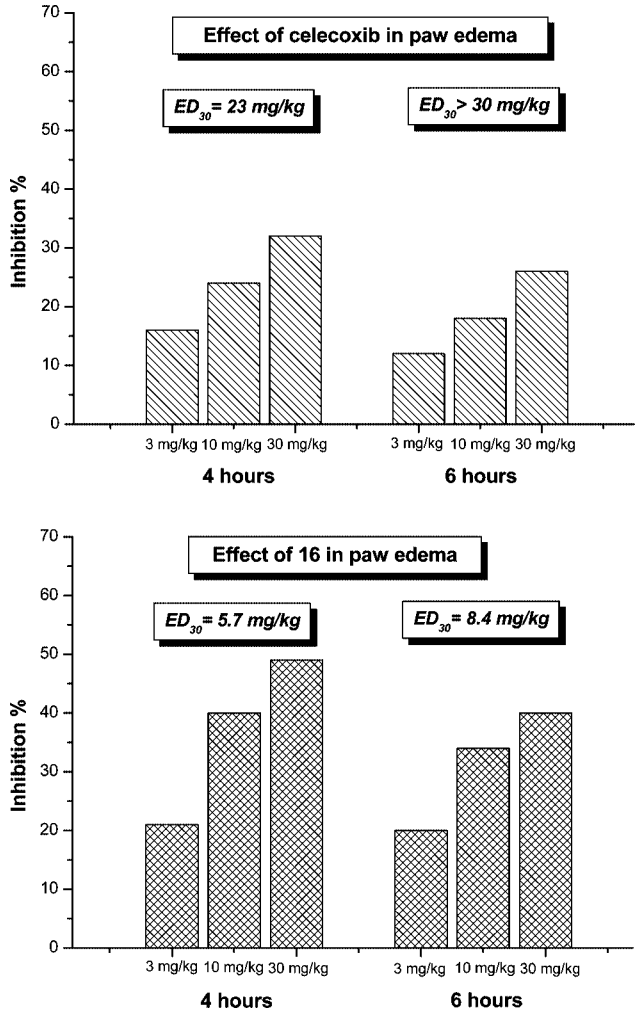


Figure 3. Anti-inflammatory activity of celecoxib **1** and **16** at 3–30 mg/kg p.o. dose range in carrageenan-induced edema model in rats (n = 6–12 animals/group).

Table 3. The Analgesic Activity of Celecoxib **1** and **16** in Carrageenan-Induced Mechanical Hyperalgesia Test in Rats

		reversal%			
cmpd	dose, mg/kg p.o.	30 min	60 min	120 min	180 min
		Acute Model			
1	10		0	0	0
	30		24	3	0
16	10		24	30	15
	30		89**	59*	35
		Chronic Model			
1	10	24	17	0	0
	30	54*	34	34	26
16	10	23	22	28	20
	30	42	54*	34	19

* $p < 0.05$. ** $p < 0.01$ vs control (5% Tween−80% phys. saline), Tukey's multiple comparison test.

racemate, which can be the result of a synergistic effect. The investigation with **16** was extended to the carrageenan-induced acute and chronic hyperalgesia model (Randall−Selitto model)[19,20] to monitor its capacity to reverse inflammatory hyperalgesia. The data are reported in Table 3 compared to those of the reference compound celecoxib. In both models (acute and chronic treatment), celecoxib at a 30 mg/kg p.o. dose showed a partial analgesic effect (24 and 54% reversal). In the acute model, **16** showed almost complete reversal (90%) at a 30 mg/kg p.o. dose and, thus, a significantly better anti-inflammatory/analgesic effect compared to celecoxib. In the chronic model, **16** showed 54% reversal, which is comparable with the analgesic effect of celecoxib. Additionally, **16** was investigated in different in vivo rat ulcer models to compare its gastrointestinal side effects relative to celecoxib. In the acid-independent ulcer model (ethanol induced gastric mucosal damage), acidified ethanol was used to induce deep hemorrhagic bends on the surface of gastric mucosa. The ulcer indexes were 60.8, 66.2, and 97.8. Celecoxib was ineffective in the dose range of 15–30 mg/kg, while at 60 mg/kg, it induced 55% inhibition ($p < 0.05$). Pretreatment with **16** resulted in 35, 43, and 75% inhibition of mucosal lesions at doses of 15, 30, and 60 mg/kg (Table 4). Thus, **16** was significantly more effective than celecoxib in its gastroprotective effects in this model. In the model of acetic acid induced gastric mucosal damage, treatment was started 5 days after the subserosal application of acetic acid. In the control (methylcellulose-treated) group, the ulcerated area was 3 mm^3. Indomethacin increased the ulcerated area by a factor of 10 times (39 mm^2). Celecoxib also increased the size of the ulcer (8.2 mm^2). In contrast, **16** reduced the ulcerated area (2.4 mm^2; Table 5). In conclusion, healing of the acetic acid induced ulcer, which is analogous to the human ulcer healing, was delayed significantly by celecoxib, while **16** did not influence it.

Table 4. Effect of Celecoxib **1** and **16** on Gastric Mucosal Damage Induced by Ethanol in Rats (n = 10)

cmpd	dose, mg/kg p.o.	ulcer index	inhibition%
methylcellulose		60.8 ± 4.1	
1	30	49.4 ± 4.5	19
16	30	35.0 ± 4.4*	43
methylcellulose		66.2 ± 1.7	
16	15	43.0 ± 4.4	35
	60	15.8 ± 2.8*	76
methylcellulose		97.8 ± 2.8	
1	15	80.6 ± 2.3	18
	60	44.4 ± 6.4*	55

* $p < 0.05$.

Table 5. Effect of Indomethacin and Celecoxib **1** and **16** on the Healing of Acetic Acid Induced Chronic Gastric Ulcer in Rats

cmpd	dose, mg/kg p.o.	change of bodyweight, g (+, increase; −, decrease)	area of ulcer (mm^2)
methylcellulose		+58 ± 3	3.0 ± 0,8
indomethacin	2.0	+2 ± 0.6	39.0 ± 5**
1	30	+32 ± 4	8.2 ± 2.0*
16	30	+43 ± 5	2.4 ± 0.7

* $p < 0.05$. ** $p < 0.01$.

Conclusion

In this report, we have described the design and synthesis of new celecoxib derivatives substituted at the benzenesulfonamide moiety. Although our intention was to prepare stable derivatives of **12**, it is not impossible that these derivatives act as prodrugs. The investigation of this issue is complicated by the unstable nature of **12**. Summarizing the biological results, it can be seen that the pharmacological efficacy of **16** in vivo is greater and of longer duration compared to celecoxib. In the carrageenan-induced edema test, the anti-inflammatory activity of **16** was significantly higher than that of celecoxib. In the inflammation-induced chronic hyperalgesia model, **16** showed significant analgesic effect and its duration of action was several hours longer than that of the reference compound. In comparing the gastrointestinal effects, **16** showed a significantly more favorable side effect profile than celecoxib. Thus, **16** and its water soluble

disodium salt **21** have been identified as potential analgesic and anti-inflammatory agents for both oral and parenteral administration.

Experimental Section

Chemistry. Research chemicals were either purchased from Aldrich Co. or Fluka and used without further purification in the reactions or were prepared according to procedures described in the literature. Reactions were monitored by thin-layer chromatography (TLC) on silica gel plates (60 F_{254}; Merck) visualizing with ultraviolet light or iodine. Column chromatography was performed on Silica Gel 60 (0.043–0.060 mm), Merck. The main reference compound, Celecoxib was prepared according to the literature procedure.[6] The yields of the products reported here are unoptimized. Melting points were determined with a Büchi 535 apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian INOVA-500 spectrometer, operating at 500 MHz. Chemical shifts (δ) are reported in parts per million downfield from tetramethylsilane. Mass spectra were scanned on a Finnigan MAT 95SQ spectrometer.

4,4,4-Trifluoro-1-(4-methyl-phenyl)-butane-1,3-dione (8). In a solution of 5.7 g (0.25 mol) of sodium in 80 mL of methanol, 22 mL (0.16 mol) of ethyl trifluoroacetate was added, and afterward over 30 min, 21 g (0.15 mol) of 4-methyl acetophenone **7** dissolved in 40 mL of methanol was added. The resulting solution was stirred for 10 h at 80 °C then evaporated to dryness. The sodium salt obtained was dissolved in 50 mL of water and acidified with 120 mL of 1 N HCl and extracted three times with 80 mL of ethyl acetate. The organic layer was dried over $MgSO_4$ and evaporated in vacuo to give 34.6 g of a brown solid, which was taken forward without further purification. Yield 95%.

4-(5-*p*-Methylphenyl-3-trifluoromethyl-pyrazole-1-yl)-benzenesulfonic Acid (10). A total of 42 g (0.22 mol) of **9** was added to a stirred solution of 51.4 g (0.223 mol) of **8** in 450 mL of ethanol and 74 mL (0.446 mol) of 6 N HCl. The mixture was heated to reflux and stirred for 8 h. After cooling to room temperature, the reaction mixture was concentrated in vacuo. The residue was taken up with ethyl acetate and washed with 100 mL of water and 100 mL of brine, dried over $MgSO_4$, filtered, and evaporated in vacuo to give an oil that was crystallized from 300 mL of diisopropylether to give 70.12 g of the pyrazole **10**. Yield 82%; mp 258 °C (decomposition).

4-(5-*p*-Methylphenyl-3-trifluoromethyl-pyrazole-1-yl)-benzenesulfonyl Chloride (11). To a suspension of 43 g (0.11 mol) of **10** in 250 mL of dichloromethane was added 34.2 g (0.168 mol) of phosphorus pentachloride in portions. To the resulting solution was added 10 mL of DMF. After stirring at reflux for 8 h, the mixture was concentrated in vacuo. To the residue was added water and twice extracted with ethyl acetate, dried over $MgSO_4$, and evaporated to dryness. The residue could be crystallized from cyclohexane to yield 34.2 g of product as a white solid. Yield 77%; mp 97–98 °C (cyclohexane).

***N*-Hydroxy-4-(5-*p*-methylphenyl-3-trifluoromethyl-pyrazole-1-yl)-benzenesulfonamide (12).** A total of 1.0 g (15 mmol) of hydroxylamine hydrochloride was suspended in 10 mL of dioxane and treated dropwise with a solution of 1.2 g (15 mmol) of sodium acetate in 5 mL of water. To this solution was added dropwise over 10 min a solution of 1.5 g (3.7 mmol) of **11** in 20 mL of CH_2Cl_2 and allowed to stir for 1 h, which was then concentrated in vacuo. To the residue was added water and extracted twice with ethyl acetate. The combined organic layers were washed three times with water, dried over $MgSO_4$, and evaporated in vacuo to give an oil, which could be crystallized from ethanol (75%) to give the pyrazole as a white solid. Yield 76%; mp 203 °C (ethanol/water); ^{1}H NMR (300 MHz, DMSO-d_6) 2.32 (s, 3H), 7.18–7.26 (m, 5H), 7.56–7.62 (m, 2H), 7.86–7.92 (m, 2H), 9.71 (d, 1H), 9.74 (d, 1H); HRMS calcd for $C_{17}H_{14}O_3N_3F_3S$, 397.07002; found, 397.07025.

[4-(5-*p*-Methylphenyl-3-trifluormethyl-pyrazole-1-yl)-benzenesulfonylaminooxy]-acetic Acid (13). Compound **13** was obtained from **11** and aminooxy-acetic acid according to the procedure described for **12** and was isolated as a white semisolid. Yield 88%; mp 224–225 °C (ethanol/water); ^{1}H NMR (300 MHz, DMSO-d_6) 2.32 (s, 3H), 4.44 (s, 2H), 7.19–7.21 (m, 1H), 7.19–7.24 (m, 4H), 7.58–7.64 (m, 2H), 7.91–7.97 (m, 2H), 10.98 (br, 1H), 12.99 (br, 1H); HRMS calcd for $C_{19}H_{16}O_5N_3F_3S$, 455.107559; found, 455.07573.

[4-(5-*p*-Methylphenyl-3-trifluormethyl-pyrazole-1-yl)-benzenesulfonylaminooxy]-acetic Acid Ethyl Ester (14). Compound **14** was obtained from **11** and aminooxy-acetic acid ethyl ester according to the procedure described for **12** and was isolated as a white semisolid. Yield 82%; mp 143–144 °C (ethanol); ^{1}H NMR (500 MHz, DMSO-d_6) 1.22 (t, 3H), 2.32 (s, 3H), 4.16 (q, 2H), 4.53 (s, 2H), 7.19–7.21 (m, 1H), 7.19–7.24 (m, 4H), 7.58–7.64 (m, 2H), 7.91–7.97 (m, 2H), 11.00 (s, 1H); HRMS calcd for $C_{21}H_{20}O_5N_3F_3S$, 483.10726; found, 483.10703.

3-[4-(5-*p*-Methylphenyl-3-trifluoromethyl-pyrazole-1-yl)-benzenesulfonylaminooxy]-propionic Acid (15). Compound **15** was obtained from **11** and aminooxy-propionic acid according to the procedure described for **12** and was isolated as a white semisolid. Yield 92%; mp 176–178 °C (ethanol/water); ^{1}H NMR (300 MHz, DMSO-d_6) 2.32 (s, 3H), 2.52 (t, 2H), 4.09 (t, 2H), 7.19–7.21 (m, 1H), 7.19–7.24 (m, 4H), 7.56–7.63 (m, 2H), 7.88–7.93 (m, 2H), 10.60 (br s, 1H), 12.30 (br s, 1H); HRMS calcd for $C_{20}H_{18}F_3N_3O_5S$, 469.09192; found, 469.0921.

(±)-2-[4-(5-*p*-Methylphenyl-3-trifluoromethyl-pyrazole-1-yl)-benzenesulfonylaminooxy]-propionic Acid (16). Compound **16** was obtained from **11** and 2-aminooxy-propionic acid according to the procedure described for **12** and was isolated as a white semisolid. Yield 85%; mp 187–189 °C (toluene); ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] 1.27 (d, 3H), 2.31 (s, 3H), 4.47 (q, 1H), 7.18 (s, 1H), 7.19–7.24 (m, 4H), 7.59– 7.64 (m, 2H), 7.92–7.96 (m, 2H), 10.80 (br s, 1H, NH), 12.94 (br s, 1H, COOH); HRMS calcd for $C_{20}H_{18}O_5N_3F_3S$, 469.09063; found, 469.09138.

(±)-2-[4-(5-*p*-Methylphenyl-3-trifluoromethyl-pyrazole-1-yl)-benzenesulfonylaminooxy]-propionic Acid Ethyl Ester (17). Compound **17** was obtained from **11** and 2-aminooxy-propionic acid ethyl ester according to the procedure described for **12** and was isolated as a white semisolid. Yield 82%; mp 137–138 °C (cyclohexane); ^{1}H NMR (500 MHz, $CDCl_3$) 1.29 (t, 3H), 1.38 (d, 3H), 2.39 (s, 3H), 4.21 (q, 2H), 4.63 (q, 1H), 6.74 (s, 1H), 7.10–7.13 (m, 2H), 7.17–7.21 (m, 2H), 7.49–7.54 (m, 2H), 7.88–7.92 (m, 2H); HRMS calcd for $C_{22}H_{22}O_5N_3F_3S$, 497.12366; found, 497.12268.

2-Methyl-2-[4-(5-p-methylphenyl-3-trifluoromethyl-pyrazole-1-yl)-benzenesulfonylaminooxy]-propionic Acid (18). Compound **18** was obtained from **11** and 2-aminooxy-2-methyl-propionic acid according to the procedure described for **12** and was isolated as a white semisolid. Yield 80%; mp: 94–96 °C (ethanol); ^{1}H NMR (400 MHz, DMSO-d_6) 1.36 (s, 6H), 2.32 (s, 3H), 7.18–7.25 (m, 5H), 7.57–7.63 (m, 2H), 7.90–7.96 (m, 2H), 10.26 (br s, 1H), 12.89 (br s, 1H); HRMS calcd for $C_{21}H_{20}F_3N_3O_5S$, 483.10737; found, 483.10694.

[4-(5-*p*-Methylphenyl-3-trifluoromethyl-pyrazole-1-yl)-benzenesulfonylaminooxy]-acetic Acid Sodium Salt Trihydrate (19). To a solution of 0.40 g (0.88 mmol) of **13** in 7 mL of ethanol was added 0.44 mL (0.88 mmol) of 2 N NaOH, and the mixture was stirred for 2 h at 0 °C. The precipitated product was collected by filtration to give a white solid. Yield 90%; mp > 300 °C; ^{1}H NMR (400 MHz, DMSO-d_6) 2.32 (s, 3H), 3.93 (s, 2H), 7.18–7.26 (m, 5H), 7.52–7.58 (m, 2H), 7.89–7.95 (m, 2H); $C_{19}H_{15}F_3N_3NaO_5S \cdot 3H_2O$ MW, 531.44.

3-[4-(5-*p*-Methylphenyl-3-trifluoromethyl-pyrazole-1-yl)-benzenesulfonylaminooxy]-propionic Acid Disodium Salt Monohydrate (20). To a solution of 1.4 g (3 mmol) of **15** in 20 mL of ethanol was added 1.9 mL (7.5 mmol) of 3.9 N NaOH, and the mixture was stirred for 2 h at 0 °C. The precipitated product was collected by filtration to give a white solid. Yield 87%; mp > 300 °C; ^{1}H NMR (400 MHz, DMSO-d_6) 1.98 (t, 2H), 2.30 (s, 3H), 3.51 (t, 2H), 7.12–7.14 (m, 1H), 7.15–7.23 (m, 4H), 7.24–7.30 (m, 2H), 7.66–7.71 (m, 2H); $C_{20}H_{18}F_3N_3Na_2O_6S$ MW, 531.41.

(±)2-[4-(5-*p*-Methylphenyl-3-trifluoromethyl-pyrazole-1-yl)-benzenesulfonylaminooxy]-propionic Acid Disodium Salt Mono-

hydrate (21). Compound **21** was obtained from **16** according to the procedure described for **20** and was isolated as a white solid. Yield 88%; mp > 300 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] 0.97 (d, 3H), 2.28 (s, 3H), 3.58 (q, 1H), 7.12 (s, 1H), 7.12–7.21 (m, 4H), 7.26–7.32 (m, 2H), 7.76–7.83 (m, 2H); $C_{20}H_{18}F_3N_3Na_2O_6S$ MW, 532.42.

2-Methyl-2-[4-(5-*p*-methylphenyl-3-trifluoromethyl-pyrazole-1-yl)-benzenesulfonylaminooxy]-propionic Acid Disodium Salt Monohydrate (22). Compound **22** was obtained from **18** according to the procedure described for **20** and was isolated as a white solid. Yield 80%; mp > 300 °C; ^{1}H NMR (300 MHz, DMSO-d_6) 0.90 (s, 6H), 2.29 (s, 3H), 7.10–7.17 (m, 5H), 7.26–7.32 (m, 2H), 7.68–7.73 (m, 2H); $C_{21}H_{20}F_3N_3Na_2O_6S$ MW, 545.42.

(−)-2-[4-(5-*p*-Methylphenyl-3-trifluoromethyl-pyrazol-1-yl)-benzenesulfonylaminooxy]-propionic Acid (23). To a stirred solution of 10 g (21 mmol) of **16** in 50 mL of 97% ethanol was added 1.76 g (10 mmol) of (−)-ephedrine. The mixture was stirred at room temperature for 1 day, and the resulting crystals were filtered off and washed with 97% ethanol to yield 3.38 g (25%) of product, calculated on the basis of the starting racemic mixture. The so-obtained crystals were recrystallized twice from 97% ethanol to yield 2.02 g (60%) of white crystalline material. The diastereomer salt was suspended in 30 mL of ethyl acetate, and 15 mL of 1 N hydrochloric acid was added. The organic layer was separated, dried over $MgSO_4$, filtered, and concentrated in vacuo. The obtained oil was crystallized from petroleum ether to yield 0.9 g (60%) of the title compound. Mp 158–160 °C; $[\alpha]^{20}_D = -505.3°$ (*c* 1, methanol).

(+)-2-[4-(5-*p*-Methylphenyl-3-trifluoromethyl-pyrazol-1-yl)-benzenesulfonylaminooxy]-propionic Acid (24). Compound **24** was obtained from **16** according to the procedure described for **23** using (+)-ephedrine as resolving agent and isopropanol as solvent. Yield 0.62 g, mp 161–163 °C; $[\alpha]^{20}_D = +457.6°$ (*c* 1, methanol).

Biological Methods. In Vitro Screening. Spectrophotometric Assay with Human Recombinant COX-2 and Ovine COX-1. Enzymatic activities of the human recombinant COX-2 and ovine COX-1 were measured using a spectrophotometric assay based on the oxidation of TMPD during the reduction of PGG2 to PGH2.

In Vivo Screening. Carrageenan-Induced Edema in Rats. Edema was induced by subcutaneous injection of 50 μL of 1% carrageenan (CARR) suspension in the subplantar region of the right hind paw of male Wistar rats (140–150 g). The injected carrageenan induced paw inflammation. The edema, that is, the difference between the pre- and post-treatment volume (in mL) of the injected hind paw was measured using a water displacement Plethysmometer (Ugo Basile, type 7150). The treated paw was immersed up to the tibio-tarsal articulation into the chamber, and the volume of displaced liquid was determined as the degree of the inflammation: Degree of inflammation (mL) = volume after the CARR treatment (mL) − volume before the CARR treatment (mL).

Carrageenan Induced Acute Hyperalgesia in Rats (Randall−Selitto Model). Edema (inflammation) was induced by subcutaneous injection of carrageenan (CARR) suspension in the subplantar region of the right hind paw of male Wistar rats, weighted 140–190 g (n = 8–12 animal/group). The nociceptive thresholds of the inflammed hind paw after painful mechanical stimuli were measured with an Analgesimeter (Ugo Basile, type 37215, Italy). The apparatus is suitable for the measuring of the extent and the latency of the pain reaction threshold of the sensitized paw after painful stimuli. The analgesics elevate the low pain reaction threshold of the inflammed paw and the degree of its antinociceptive effect is expressed in reversal%.

An increasing pressure was applied to the paw and the withdrawal threshold was determined as the first sign (squeaking and/or struggling) of pain response. The pressure threshold was shown in grams. The average value of the hind paw withdrawal obtained with untreated right paw was regarded as the basal hind paw withdrawal (average 80–110 g). After determining the baseline threshold, the animals received carrageenan injection that produced an intense inflammation associated with hyperalgesia. The mechanical threshold was determined at various times to establish the magnitude and duration of the hyperalgesia. The maximum reduction in threshold was measured 2–3 h after injection (the pain threshold of the inflammed paw is 20–30 g, which was decreased by 65–80% in comparing to the basic value).

Measurement of Gastrointestinal Side Effects. Animals. Experiments were performed in male Wistar rats weighing 140–160 g. They were housed in wire mesh bottom cages to prevent coprophagy. The rats were kept on a 12 h light−dark cycle and under condition of controlled temperature.

Gastric Mucosal Damage Induced by Acidified Ethanol. After 24 h food deprivation, the animals were given orally 0.5 mL of acidified ethanol (98% ethanol in 200 mmol/l HCl). One hour later, the animals were killed by overdose of ether and the stomachs were excised, opened along the greater curvature, rinsed with saline, and examined for lesions. Total number of mucosal lesions was assessed in a blinded manner by calculation of lesion index based on a 0–4 scoring system described previously.[21] The lesion index was calculated as the total number of lesions multiplied by the respective severity factor. The percentual inhibition of mucosal damage was calculated as follows 100 − [lesion index in treated group lesion index in control group] × 100.

Chronic Gastric Ulcer Induced by Acetic Acid−Study on Healing Process. The method of Okabe[22] was applied. Male Wistar rats (150–170 g) were used. Laparotomy by a midline, epigastric incision was made in rats under ether anesthesia. After exteriorizing the stomach, 0.05 mL/rat of 20% acetic acid was injected into the submucosal layer of the anterior wall of the glandular stomach. Care was taken not to puncture any of the superficial, large blood vessels. After injection, pressure (by hand) was applied over the inserted needle to prevent leakage of the acetic acid via the puncture wound and was maintained momentarily after needle withdrawal. The site of the injection was readily distinguished by the localized swelling and blanching of the serosa at the injection site. After surgery, the abdomen was closed and all rats were maintained conventionally on laboratory chow and water ad libitum. Animals were sacrificed 12 days later. Then the stomach was opened along the greater curvature and examined grossly for lesions. Because ulcers produced by the acetic acid are round or oval, the length and width were measured, and an ulcer index based upon the product of length and width was used. The test compounds were given from the fifth day after the operation for 7 days. A total of 24 hours after the last dose, the animals were sacrificed and the ulcer index was determined.

Supporting Information Available: Spectroscopic (^{1}H NMR and HRMS) data for all synthesized compounds, HPLC purity data for target compounds **12**−**24**, and analytical HPLC trace for compound **16**. This material is available free of charge via the Internet at http://pubs.acs.org.

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