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Analgesic agents without gastric damage: Design and synthesis of structurally simple benzenesulfonanilide-type cyclooxygenase-1-selective inhibitors

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Abstract—In order to create novel analgesic agents without gastric disturbance, structurally simple cyclooxygenase-1 (COX-1) inhibitors with a benzenesulfonanilide skeleton were designed and synthesized. As a result, compounds **11f** and **15a**, which possess a *p*-amino group on the benzenesulfonyl moiety and *p*-chloro group on the anilino moiety, showed COX-1-selective inhibition. Moreover compound **11f**, which is the most potent compound in this study showed more potent analgesic activity than that of aspirin at 30 mg/kg by po. The anti-inflammatory activity and gastric damage, however, were very weak or not detectably different from aspirin. Since the structure of our COX-1 inhibitors are very simple, they may be useful as lead compounds for superior COX-1 inhibitors as analgesic agents without gastric disturbance.

1. Introduction

Patients with cancer or rheumatism suffer from physical and mental pains due to anxiety produced by the disease. Therefore, it is very important to reduce these pains for the patients to achieve a better quality of life. One of the current methods to relieve their physical pains is the usage of non-steroidal anti-inflammatory drugs (NSAIDs), for example, aspirin, acetaminophene, ibuprofen and so on. However, these NSAIDs produce a serious problem, gastric disturbance.

In general, most NSAIDs inhibit cyclooxygenases (COXs), which catalyze the synthesis of prostaglanidins from arachidonic acid. They have three subtypes, COX-1, COX-2, and COX-3.[1–3] Until recently, the gastric disturbance was thought to be due to inhibition of COX-1 on the stomach mucous membrane by NSAIDs.[4] Furthermore, it was thought that the anti-inflammatory effect of NSAIDs arises from the inhibition of COX-2 in the inflamed region.[5] Consequently, COX-2-selective inhibitors drew much attention as candidates of anti-inflammatory agents with reduced side effects.[6] However, very recently, rofecoxib (Vioxx™),[7] a COX-2-selective inhibitor, was withdrawn from the market because of possible association with an increased incidence of cardiovascular events, such as heart attack and stroke.[8]

Meanwhile, NSAID-induced gastric damage was reported to be caused by the inhibition of both COX-1 and COX-2.[9] Moreover, it has been suggested that COX-1 may play an important role in pain processing and sensitization of the spinal cord and gracile nucleus after surgery.[10] Therefore, COX-1-selective inhibitors are anticipated to be candidates as novel analgesic agents with reduced gastroenteric disturbance. For example, mofezolac (**1**)[11] and FR122047

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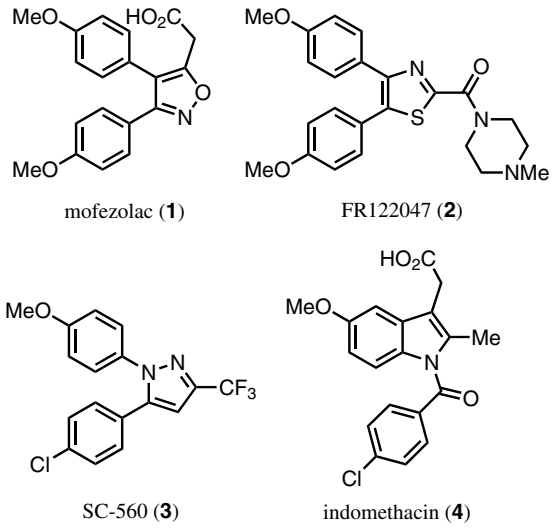


Figure 1. Chemical structures of representative COX-1 inhibitors **1**–**3** and a COX-1 relatively selective inhibitor **4**.

(**2**),[12] two representative COX-1-selective inhibitors, show analgesic activity and are recognized to reduce hyperplastic polyp. In addition, COX-1-selective inhibitors have started to garner attention as anti-tumor agents because of their anti-angiogenesis activity.[13] For example, low-dose aspirin, which inhibits mainly COX-1, is reported to decrease intestinal cancer incidence by 40–50% as compared with non-aspirin usage[14] and has also been applied in a clinical setting as an anti-thrombotic agent.[15] Thus, COX-1 is also thought to be a therapeutic target. However, very few well-known COX-1-selective inhibitors are available at this point. These factors motivated us to search for new COX-1-selective inhibitors as novel analgesic agents without gastric disturbance.

In Figure 1, representative COX-1 inhibitors are shown. These compounds possess the characteristic structure, which contains two *para*-substituted phenyl groups which oriented in a *cis*-conformation. The structural similarity of these COX-1 inhibitors led us to invent novel COX-1 inhibitors by modifying synthesized analogues with a similar chemical structure. Thus we chose benzenesulfonanilide (**5**), which contains two phenyl groups bridged by a SO_2-NH unit, as a simple model compound, because sulfonamide derivatives were reported to orient the substituents in an *s-cis* conformation[16] and to show various bioactivities.[17] To elucidate the conformation of the two phenyl groups of **5**, an X-ray crystallographic analysis was carried out (Tables 1 and 2). The molecular structure is shown in Figure 2. The torsional angle of C(1)–S(1)–N(1)–C(7) is 58.1(3)° indicating that two phenyl groups of **5** are in *s-cis* form. Based on the above ideas, we selected **5** as a novel COX-1-selective inhibitor framework and performed molecular design by the introduction of various substituents (e.g., carboxyl, nitro, amino and methanesulfonamide groups) onto each phenyl group of **5** (Fig. 3).

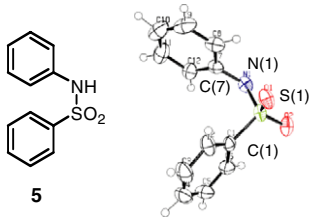


Figure 2. Chemical structure and ORTEP drawing of benzenesulfonanilide (**5**).

2. Chemistry

The benzenesulfonanilide skeleton was obtained by coupling benzenesulfonyl chlorides and anilines in pyridine. Most compounds except for carboxy derivatives **6** were obtained in at least 60% yield. N-Methylation of the benzenesulfonanilide moiety was performed with compound **7**, followed by reduction of the nitro group to afford **11**. For compounds bearing halogen atoms, reduction of the nitro group was carried out with tin and hydrogen chloride because halogen atoms except for fluorine departed under palladium on carbon reduction conditions. The structures of all the compounds were confirmed by spectroscopy (^{1}H NMR, IR, and mass spectrometry) and elemental analyses.

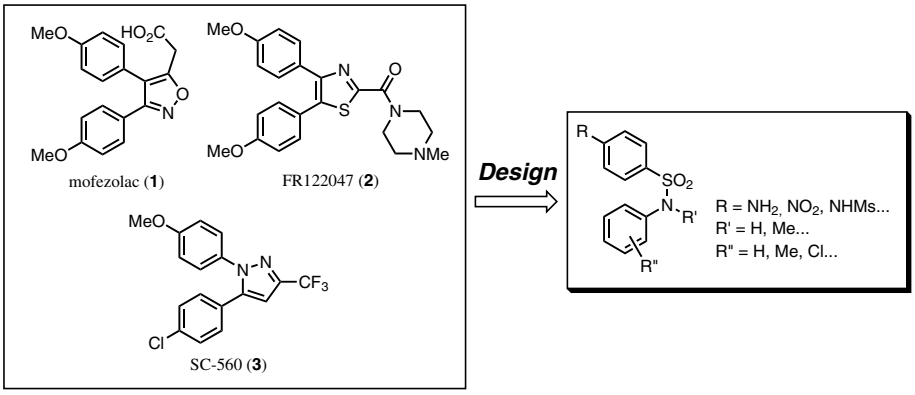


Figure 3. Molecular design strategy of COX-1-selective inhibitors with a benzenesulfonanilide skeleton.

Table 1. Crystallographic data for **5**

Formula	$C_{12}H_{11}NO_2S$
Crystal system	Tetragonal
Space group	P41212(#92)
a (Å)	8.7746(8)
c (Å)	30.037(2)
v (Å^3)	30312.6(3)
Z value	8
D (g/cm^{-1})	1.340
No. of obsd reflns	1627
No. of variables	179
*R*1;*wR*2	0.037, 0.087

Table 2. Selected bond distances (Å) and angles (°) for **5**

C(1)–S(1)	1.756(3)	S(1)–N(1)	1.618(3)
S(l)–O(l)	1.434(2)	S(1)–O(2)	1.424(2)
N(1)–C(7)	1.448(3)	N(1)–H(11)	1.12(4)
C(1)–S(1)–O(1)	106.3(1)	C(1)–S(1)–O(2)	108.5(1)
C(1)–S(1)–O(2)	108.5(1)	C(1)–S(1)–N(1)	108.9(1)
O(1)–S(1)–O(2)	119.4(1)	N(1)–S(1)–O(1)	107.8(1)
N(1)–S(1)–O(2)	105.7(1)	S(1)–N(1)–C(7)	121.7(2)
S(l)–N(l)–H(ll)	115(2)	C(7)–N(1)–H(11)	110(1)

3. Biology

The biological activity of the compounds was assessed with a colorimetric COX (ovine) inhibitory screening assay kit (Cayman Chemical; catalog No. 760111) according to the supplier's protocol. Each experiment was initially performed at 100 μM of the test compound (final concentration). IC_{50} values were calculated for only potent compounds discovered in this assay system. Lineweaver–Burk plot analysis was performed with the same assay kit described above at 100 μM (final concentration) of the compound and arachidonic acid was used as a substrate at a concentration of 25, 50, 100, 200, and 400 μM, respectively. Each experiment was performed at least twice and their mean value was calculated.

4. Molecular docking

The docking program used was AutoDock 3.05 developed by Morris et al.[18] The 3D coordinate structure of COX-1 (1PGF) was retrieved from the Brookhaven Protein Data Bank: URL*http://www.rcsb.org/pdb/ Welcome.do. and water molecules and ions were removed before the docking.

5. In vivo assay

5.1. Animals

Male mice and male rats, weighting 15–30 and 170–200 g, respectively, were acquired from the Charles River Co. Ltd. Only water was provided ad libitum during the 12 h before experimentation. The study was conducted according to internationally accepted principles of laboratory animal use.

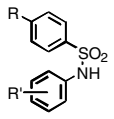
5.2. Writhing test[12]

Groups of mice (n = 9–11) were treated with the solutions of compound **11f**, aspirin at a dose of 30 mg/kg, and the solvent (1% ethanol and 0.5% CMC (carboxymethyl cellulose) in milliQ water) at a dose of 0.3 mL/10 g of animal via po. The muscular contraction was induced by an ip administration of 0.7% acetic acid at a dose 0.1 mL/10 g for 30 min after the treatment. The number of muscular contractions was counted starting at 10 min after injection for a period of 10 min. Data represent average of the total writhes observed.

5.3. Rat paw edema induced by carrageenan[19]

Groups of rats (n = 3–8) were treated with solutions of compound **11f**, aspirin (30 mg/kg, each), and indomethacin (**4**) (10 mg/kg). The solvent (1% ethanol and 0.5% CMC in milliQ water) at a dose of 1 mL/200 g of animal

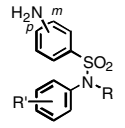
Table 3. COX-inhibitory activity of indomethacin (**4**) and compounds **6**–**9**



Compound	R′	% Inhibition of COX at 100 μM							
		6 (R = CO_2H)		**7** (R = NO_2)		**8** (R = NH_2)		**9** (R = NHMs)	
		COX-1	COX-2	COX-1	COX-2	COX-1	COX-2	COX-1	COX-2
4	—	90	90	90	90	90	90	90	90
a	H	11	0	0	0	0	0	0	0
b	2-Me	21	6	0	4	5	0	0	0
c	3-Me	0	19	3	0	8	2	3	0
d	4-Me	n.t.	n.t.	44	0	52	15	12	0
e	3,5-DiMe	n.t.	n.t.	0	0	13	28	0	0
f	4-OMe	3	0	0	0	n.t.	n.t.	8	13
g	4-CF_3	0	12	1	1	14	35	8	0
h	3,5-DiCF_3	25	8	19	19	30	49	0	0
i	4-Cl	n.t.	n.t.	6	0	70	50	11	4

n.t., not tested.

Table 4. COX-inhibitory activity of compounds **8** and **11**



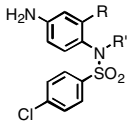
Compound	NH_2	R	R′	% Inhibition of COX at 100 μM	
				COX-1	COX-2
4	—	—	—	90	90
8i	*p*	H	4-CI	70	50
8j	*p*	H	3-CI	18	16
8k	*p*	H	2-CI	53	8
81	*p*	H	2,4-DiCI	6	12
8m	*p*	H	4-F	15	26
8n	*p*	H	4-Br	23	25
8o	*p*	H	4-I	18	50
11a	*p*	Me	H	48	16
11b	*p*	Me	4-Me	21	16
11c	*p*	Me	4-OMe	6	4
11d	*p*	Me	4-CF_3	9	6
11e	*p*	Me	4-F	0	5
11f	*p*	Me	4-CI	78	29
11g	*m*	Me	4-CI	39	4
11h	*p*	Et	4-CI	10	19
11i	*p*	Pr	4-CI	0	5
11j	*p*	Me	4-Br	21	8
11k	*p*	Me	4-I	18	45

was administered via po or ip. Paw edema was induced by 0.1 mL of 1% carrageenan in saline solution into the hind paw of a rat 1 h after the administration of the solution.[19] Edema was measured with a thickness gauge (Mitsutoyo Co. Ltd., Tokyo, No. 7331).

5.4. Acute gastric damage scoring[9]

An acute gastric damage experiment was performed according to Wallace et al.[9] The 0.5% CMC vehicle, indomethacin (10 mg/kg), compound **11f** (30 mg/kg), and aspirin (30 mg/kg) were orally administered to the rats ($n = 3$–5), respectively. Five hours later, the rats were anesthetized with diethyl ether. The stomach was taken out and viewed using a stereoscopic microscope. The stomach damage images were captured by hemorrhagic damage analysis using a CCD camera (Monicam2000, Motic China Group) and analyzed using Motic Images Plus 2.0S software. The damage scoring was performed by measuring the lengths of the ulcers in millimeters, and the sum of the damage values gave an overall gastric damage score for each rat.

Table 5. COX-inhibitory activity of indomethacin (**4**), compounds **13** and **15**

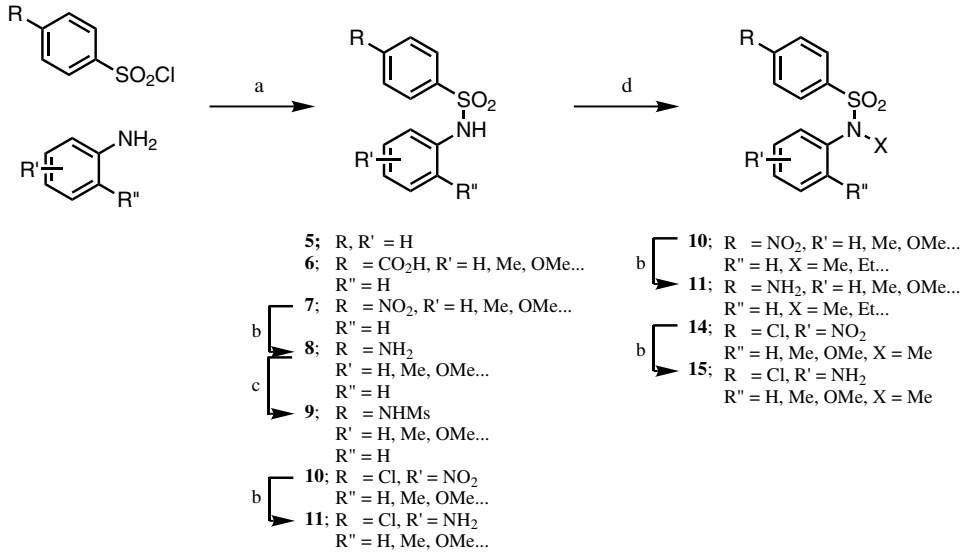


Compound	R	% Inhibition of COX at 100 μM			
		13 (R′ = H)		**15** (R′ = Me)	
		COX-1	COX-2	COX-1	COX-2
4	—	90	90	90	90
a	H	3	4	68	24
b	Me	7	28	77	5
c	OMe	1	22	73	44
d	CF_3	7	23	n.t.	n.t.

n.t., not tested.

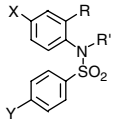
5.5. Statistical analysis

All data were expressed as means ± SEM. Comparisons among groups of data were performed using a one-way analysis of variance followed by the Dunnett's multiple comparison test. An associated probability (p value) of <5% was considered to be significant.



Scheme 1. Reagents and conditions: (a) pyridine, (b) H_2, Pd/C, EtOH or Sn, HCl, EtOH, (c) MsCl, pyridine, (d) MeI, NaH, DMF.

Table 6. COX-inhibitory activity of indomethacin (**4**), aspirin, and compounds **8i**, **11f**, and **15a–c**



Compound	X	Y	R	R^1	IC_{50} (μM)	
					COX-1	COX-2
Si	Cl	NH_2	H	H	12.0	>100
11f	Cl	NH_2	H	Me	3.2	>100
15a	NH2	Cl	H	Me	9.2	>100
15b	NH2	Cl	Me	Me	26.0	>100
15c	NH2	Cl	OMe	Me	20.0	>100
Aspirin					100	>100
4					0.03	7.7

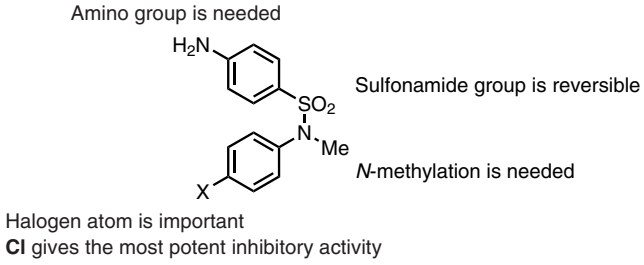


Figure 4. Important factors for benzenesulfonanilide-type COX-1 inhibitors.

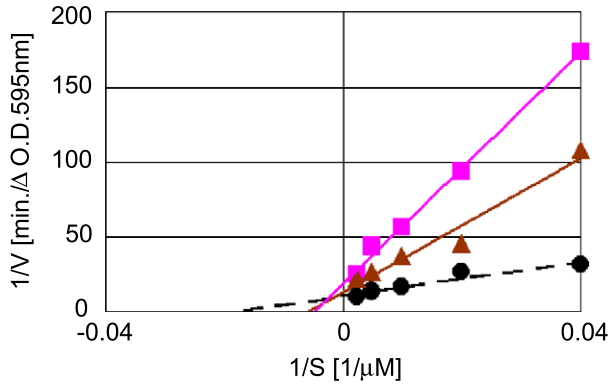


Figure 5. Lineweaver–Burk plot analysis for compounds **11f** and **15a**. DMSO (circle), **11f** (square), and **15a** (triangle), respectively. The compound concentration is 100 μM.

6. Results and discussion

The inhibitory activities toward individual COX isozymes are shown in Table 3. Most of the compounds showed no COX-inhibitory activity, but amino derivatives, for example, **8d** and **8i**, were shown to be active (Table 3). The chloro compound **8i** showed a potent COX-inhibitory activity. Since chloro compound **8i** showed COX-inhibitory activity, other

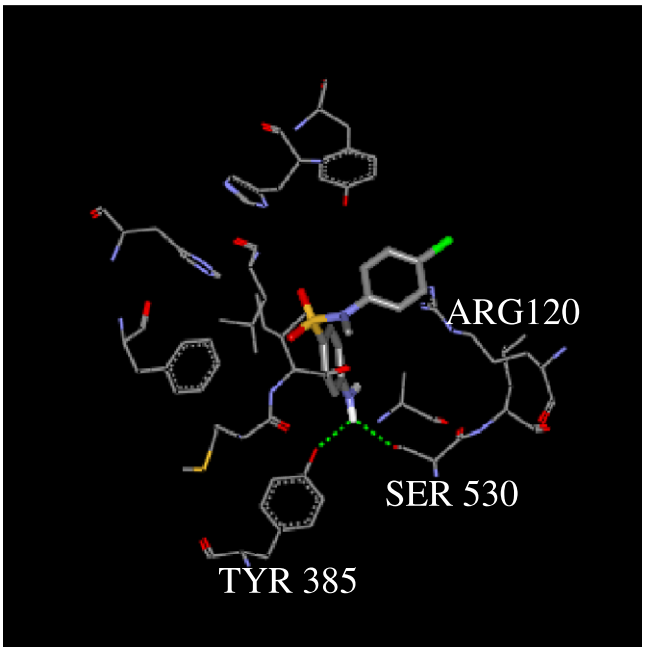


Figure 6. Potential binding mode of **11f** (colored by element, stick) in the COX-1 active site. 2H-bonds are indicated with green dotted lines.

halogen derivatives **8m**,**8n**, and **8o** were prepared and assessed. (Table 4). However, these compounds possessed little COX-inhibitory activity. Thus this COX-inhibitory activity was thought to be specific to chloro derivatives. Moreover, although chlorine regioisomers **8j** and **8k** were prepared, *p*-isomer **8i** was more potent than these chlorine regioisomers. Next, in order to increase the hydrophobicity of the compounds, N-alkylation of the sulfonanilide skeleton was performed (Table 4, compounds **11**). Compound **11f**, the N-methylated derivative of **8i**, showed the most potent and COX-1-selective inhibitory activity. In addition, compound **11a**, which has no substituted group on the *N*-aromatic ring, showed moderate COX-1-inhibitory activity. Other halogen derivatives **11e**, **11j** , and **11k** did not possess COX-inhibitory activity. Other N-alkylated compounds **11h** and **11i** also did not show COX-inhibitory activity. Additionally, *m*-amino derivative **11g** showed a weak COX-1-inhibitory activity. These results suggested that *p*-chlorine, *N*-methyl, and *p*-amino groups are important to exhibit a COX-inhibitory activity and a COX-1 sub-type selectivity Scheme 1.

Moreover, compounds with the sulfonamide group reversed in the structure and compounds bearing various substituents on the aniline moieties were also prepared (synthetic scheme not shown) (Table 5). As a result, the N-methylated compounds showed a potent COX-1-inhibitory activity. Then, the IC_{50} values were assessed as shown in Table 6. One of sulfonamide-reversed compounds **15a** possessed a COX-1-selective inhibitory activity as observed in compound **11f**. On the other hand, introduction of substituents to aniline moieties made COX-inhibitory activity slightly weaker.

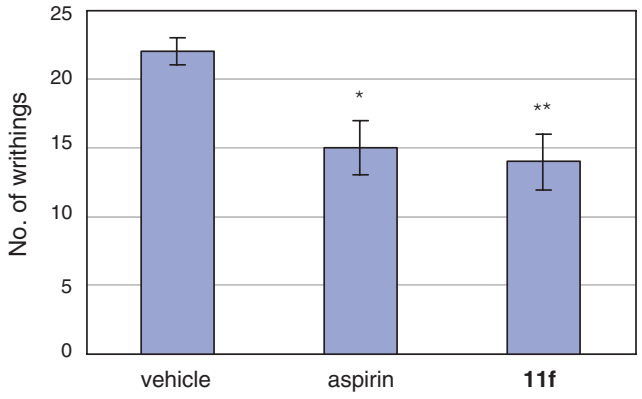


Figure 7. Analgesic effect on acetic acid-induced writhing response in mice. Data shown are the average of the total writhes observed (n = 9–11) ± SEM. *$p < 0.05$, **$p < 0.01$.

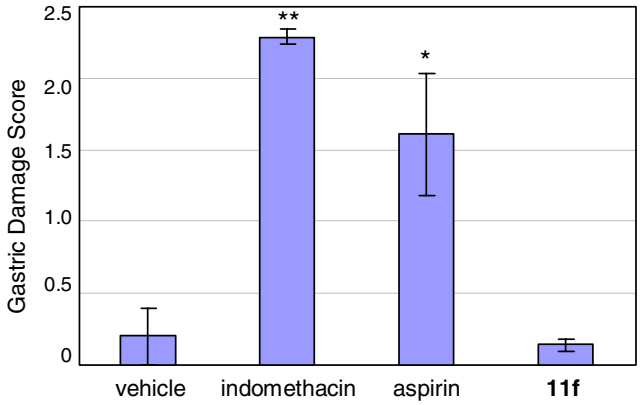


Figure 9. Gastric damaging effects of indomethacin (**4**) (10 mg/kg), aspirin (30 mg/kg) and **11f** (30 mg/kg). The gastric damage score was calculated by measuring the lengths, in millimeters, and summing the values for each rat. *$p < 0.05$, **$p < 0.01$. Data shown are means (n = 3–5/group) ± SEM.

Based on these results, the structure–activity relationship of benzenesulfonanilide COX-1-inhibitory activity is summarized in Figure 4. In order to produce COX-1-selective inhibitory activity, sulfonanilide derivatives need to possess a *p*-chlorine or *p*-amino group on each aromatic ring, whose sulfonamide moiety should be N-methylated. Though sulfonamide-type COX inhibitors have been reported, their inhibitory activities are less than those of our compounds.[20] The reason might come from lack of the SAR conditions discussed above.

In order to examine the inhibitory mode of these compounds to COX-1, Lineweaver–Burk plot analysis was performed. It is difficult to calculate the appropriate

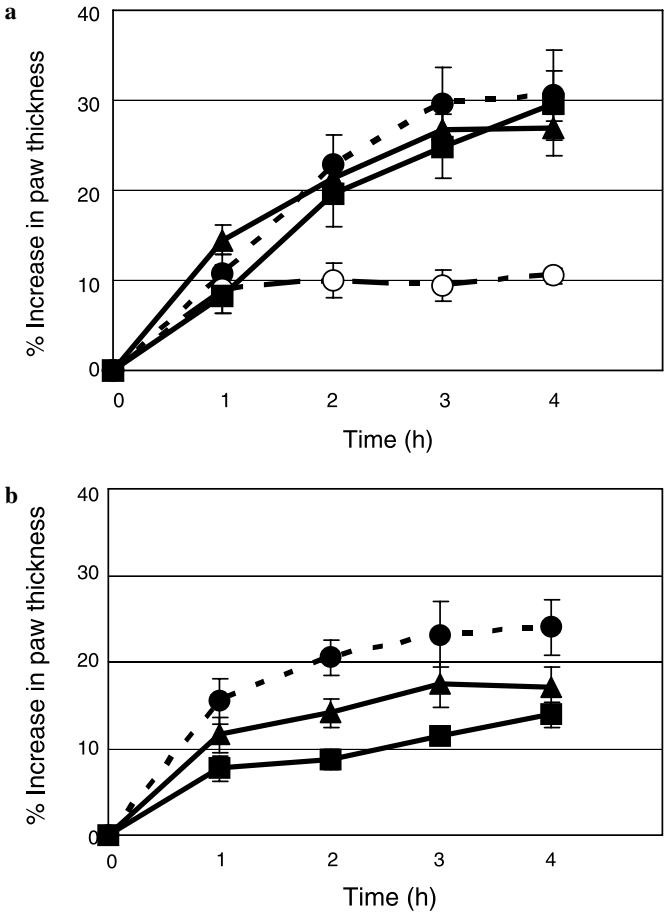


Figure 8. Effect of COX inhibitors on carrageenan-induced edema. (a) Administered via po, (b) administered via ip. Single doses of vehicle (circle), 30 mg/kg **11f** (triangle), 30 mg/kg aspirin (square) or 10 mg/kg indomethacin (**4**) (open circle) were administered by oral gavage 1 h before initiation of inflammation with carrageenan. Edema was measured 4 h after carrageenan injection. Data shown are means (n = 3–8) ± SEM. *$p < 0.05$, **$p < 0.01$.

amount of reacted arachidonic acid in this assay system. Therefore, the vertical axis ($1/V$) was represented with the amount of OD change at 595 nm used in this assay. As shown in Figure 5, the $1/V_{max}$ (y intercept) values of each inhibitor are as same as that of no inhibitor, suggesting that each inhibitor reduced the affinity of COX-1 for its substrate, arachidonic acid. These results suggest that the investigated compounds are competitive COX-1 inhibitors, which bind to the catalytic site of COX-1.

Furthermore, to understand the binding mode in COX-1, a docking study was performed. As shown in Figure 6, compound **11f** binds to the catalytic site of COX-1. In addition, the amino group of compound **11f** exhibited two hydrogen bonds with Tyr385 and Ser530, supporting the importance of the amino group on these benzenesulfonanilide COX-1 inhibitors.

Since mofezolac (**1**) and FR122047 (**2**) show the analgesic activity, the analgesic effect of our COX-1-selective inhibitor **11f** was assessed by the acetic acid-induced writhing test in mice (Fig. 7). As a result, moderate analgesic activity similar to that of aspirin was recognized.

Meanwhile, the anti-inflammatory effect of compound **11f** is shown in Figure 8. No anti-inflammatory effect was detected for compound **11f** in either po or iv as compared with that of indomethacin (**4**). Since mofezolac (**1**), FR122047 (**2**), and SC-560 (**3**) are reported not to possess a potent anti-inflammatory effect, this result is considered to be appropriate.

We also examined whether compound **11f** causes gastric ulcers or not. As shown in Figure 9, indomethacin (**4**) induced visible gastric ulcers when administered orally at 10 mg/kg and aspirin also induced small gastric ulcers when administered orally at 30 mg/kg. On the other hand, compound **11f** seldom produced them at a dose of 30 mg/kg. Mofezolac (**1**) and SC-560 (**3**) are reported not to produce macroscopically or histologically detectable gastric damage.[11,12,18] Therefore, as compared with reported data, this result also seems appropriate.

7. Conclusion

In conclusion, we have succeeded in the creation of novel analgesic agents without gastric damage by designing a structurally simple benzenesulfonanilide-type COX-1-selective inhibitor, compound **11f**. Since its structure is very simple and its synthesis is also very easy, this compound may be useful as a lead compound for superior COX-1 inhibitors. Moreover, compound **11f** did not induce gastric ulcer in the rats which were also not observed in the case of COX-1-selective inhibitors, mofezolac (**1**) and SC-560 (**3**). Recently, COX-1-selective inhibitors have also started to garner attention as anti-angiogenic agents,[13] therefore, compound **11f** may open a new field of novel anti-cancer drugs.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2006.10.029.

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