Structure–Activity Relationship Study of TXA₂ Receptor Antagonists. 4-[2-(4-Substituted Phenylsulfonylamino)ethylthio]phenoxyacetic Acids and Related Compounds¹⁾

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We have recently reported that 4-[2-(4-substituted phenylsulfonylamino)ethylthio]phenoxyacetic acids and related compounds showed potent thromboxane A₂ (TXA₂) receptor antagonist activity. To understand how substituents affect the biological activity, the quantitative structure–activity relationship (QSAR) was analyzed by using the Hansch–Fujita method for 36 compounds, including newly synthesized compounds. The positive coefficient for π_R and F_R in the results of the QSAR study suggested that a hydrophobic and σ electron-withdrawing substituent R at the *para*-position of the phenylsulfonyl moiety is required to improve the activity. Further, a substituent R which is long and moderately wide, was suggested to be preferable for the activity. The positive coefficients for $\pi_{X,Y,W-COOH}$ and $\Sigma Q_{(1)-(6)}$ may indicate that the introduction of a hydrophobic and electron-withdrawing group on the benzene ring of the phenoxy acetic acid moiety enhances the activity. The length of the W-COOH moiety may also be important. On the other hand, the effect of the presence of methylene ($n=1$) was not clear.

Key words TXA₂ receptor antagonist; structure–activity relationship; Hansch–Fujita method; [(phenylsulfonylamino)-ethylthio]phenoxyacetic acid

Thromboxane A₂ (TXA₂), an unstable metabolite of arachidonic acid, is one of the most potent inducers of platelet aggregation, vasoconstriction and bronchoconstriction,^{2–5)} and is believed to play an important role in the pathogenesis of asthma and various circulatory disorders, including myocardial infarction, unstable angina and stroke.^{6–8)} Therefore, TXA₂ receptor antagonists are expected to be effective for the treatment of these diseases, and a number of TXA₂ receptor antagonists have been clinically investigated.⁹⁾

Recently, we have found that the 2-substituted thiazolidine derivative (I) shown in Chart 1 exhibits a mild TXA₂ receptor antagonist activity.¹⁰⁾ In previous papers,^{11,12)} we have reported the synthesis of 4-[2-(substituted phenylsulfonylamino)ethylthio]phenoxyacetic

acid and related compounds (II), which can be regarded as thiazolidine ring-opened derivatives of compound I. Among the compounds II, **21** showed a pronounced TXA₂ receptor antagonist activity, and a preliminary structure–activity relationship study of these compounds led us to consider that the introduction of an electron-withdrawing substituent R at the *para*-position of the phenylsulfonyl moiety, the introduction of a fluorine atom(s) into the phenoxyacetic moiety, and the replacement of the oxygen atom of the phenoxyacetic acid moiety by a methylene group increase the activity. This consideration prompted us to synthesize some related compounds and to perform a quantitative structure–activity relationship (QSAR) analysis of these compounds. The QSAR studies were performed by using the Hansch–Fujita

Chart 1

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method¹³⁾ for 36 compounds, including the newly synthesized compounds.

Chemical Synthesis and Pharmacological Results

Seven compounds (**29**, **31–36**) were synthesized according to the method described in the previous paper¹²⁾ and evaluated for TXA₂ receptor antagonist activity. The general synthetic routes to compound **31–36** are shown in Chart 2. Chlorosulfonylation of ethyl phenylacetate (**III**) with ClSO₃H followed by reduction of the chlorosulfonyl moiety with tin powder in acidic MeOH gave methyl 4-mercaptophenylacetate (**V**). Relatively unstable **V** was immediately alkylated with *N*-(2-bromoethyl)phthalimide, and then treated with hydrazine to deprotect the primary amino group. The solution of the aminoester derivative **VII** was treated with substituted phenylsulfonyl chlorides followed by hydrolysis of the ester group to give compounds **31–36**.

The inhibitory activity of the newly synthesized compounds against U-46619¹⁴⁾ (5×10^{-6} M)-induced platelet aggregation of rabbit platelet-rich plasma (PRP) was measured by using the method of Born,¹⁵⁾ and the IC₅₀ values were determined. The IC₅₀ values are shown in Table 1.

Each of the newly synthesized compounds showed pronounced TXA₂ receptor antagonist activity compared with the positive control (sulotroban). The activities of compounds **33–36** were 14–44 fold higher than that of sulotroban. Compound **36** showed the most potent activity (IC₅₀ = 0.3×10^{-6} M) among the newly synthesized compounds, which was comparable to that of the most potent compound **21** in the previous paper.

QSAR of 4-[2-(4-Substituted Phenylsulfonylamino)ethylthio]phenoxyacetic Acids and Related Compounds The compounds used in the QSAR analysis are listed in Table 2 along with the structural descriptors and activity. In the parametrization of structural features for the Hansch study, we investigated physicochemical descriptors generally used in QSAR studies and indicator variables. The results obtained by regression analysis are summarized in

Table 3.

In Eqs. 1–3 (Table 2), π_R and $\pi_{X,Y,W-COOH}$ are hydrophobicity parameters, and F_R is the Swain–Lupton field constant for substituent R cited from the compilation by Hansch and Leo.¹⁶⁾ The value of $\Sigma Q_{(1)-(6)}$ is the total electronic charge on the B benzene ring. $Q_{\alpha C}$ is the electronic charge of the carbon atom adjacent to the carboxylate anion. These values were calculated using complete neglect of differential overlap (CNDO),¹⁷⁾ a semi-empirical molecular orbital method. The Sterimol parameters L_R , B_1 , and B_5 are for the length, minimum width, and maximum width of substituent R, and L_{W-COOH} is for the length of the W-COOH moiety. The squared cross-correlation matrix of the descriptors used in Eqs. 1–3 is shown in Table 3. There seems to be no significant cross-correlation between descriptors in these equations.

The positive coefficients for π_R and F_R suggest that the hydrophobicity and σ electron-withdrawing property of substituent R in benzene ring A are important, as shown in Eqs. 1–3. Further, a long length and moderate width for a substituent R seemed to be favorable to the activity.

For the B benzene moiety, the positive coefficients for $\pi_{X,Y,W-COOH}$ and $\Sigma Q_{(1)-(6)}$ may indicate that introduction of a hydrophobic electron-withdrawing group on the benzene ring enhances the activity. The length of the W-COOH moiety may also be important. On the other hand, the effect of the presence of methylene ($n=1$) was not clear.

The TXA₂ receptor belongs to the G protein-coupled receptor class. Its amino acid sequence was determined from its cDNA sequence.¹⁸⁾ However, the 3D structure has not yet been determined. A model of the receptor has recently been constructed by Yamamoto *et al.*¹⁹⁾ According to the model, the ligand-binding pocket includes Ser-201, Arg-295, and a large hydrophobic pocket between these two residues. Our QSAR results suggest that the W-COOH moiety interacts with Arg-295. If the sulfonamido group of the antagonists interacts with hydroxyl of Ser-201, the importance of hydrophobicity around benzene B and the length of W-COOH would be explicable. The

a, ClSO₃H. b, Sn /CHCl₃/ MeOH reflux. c, *N*-(2-bromoethyl)phthalimide /K₂CO₃/ DMF/ rt. d, H₂NNH₂·H₂O /CH₂Cl₂/ EtOH. e, 4-R-C₆H₄-SO₂Cl /Et₃N/ CH₂Cl₂. f, aqueous NaOH /MeOH.

Chart 2

Table 1. IC_{50} Values and Structural Parameters of Compounds

No.	R	W	X	Y	n	Inhibition of U-46619 induced platelet aggregation IC ₅₀ μM (rabbit PRP)	-log C ^{a)} (Obs.)	-log C ^{b)} (Calc.)	Substituent R						B moiety			
									L _R	B ₁	B ₅	π _R	F _R	L _{W-COOH}	π _{X+Y+W-COOH}	Q _{σC} ^{c)}	ΣQ ₍₁₎₋₍₆₎ ^{d)}	
1	H	OCH ₂	H	H	0	50.00	4.30	4.22	2.06	1.00	1.00	0.00	0.00	5.85	-0.87	-0.56	0.16	
2	OCH ₃	OCH ₂	H	H	0	40.10	4.40	4.68	3.98	1.35	9.42	-0.02	0.26	5.85	-0.87	-0.56	0.16	
3	F	OCH ₂	H	H	0	27.40	4.56	4.58	2.65	1.35	1.82	0.14	0.43	5.85	-0.87	-0.56	0.16	
4	CH ₃	OCH ₂	H	H	0	23.10	4.64	4.46	2.87	1.52	4.16	0.56	-0.04	5.85	-0.87	-0.56	0.16	
5	NO ₂	OCH ₂	H	H	0	16.30	4.79	4.69	3.44	1.70	5.95	-0.28	0.67	5.85	-0.87	-0.56	0.16	
6	Br	OCH ₂	H	H	0	5.20	5.28	5.25	3.82	1.95	3.80	0.86	0.44	5.85	-0.87	-0.56	0.16	
7	Cl	OCH ₂	H	H	0	4.50	5.35	5.08	3.52	1.80	3.24	0.71	0.41	5.85	-0.87	-0.56	0.16	
8	H	OCH ₂	H	F	0	37.00	4.43	4.91	2.06	1.00	1.00	0.00	0.00	5.85	-0.73	-0.56	0.30	
9	NO ₂	OCH ₂	H	F	0	8.50	5.07	5.38	3.44	1.70	5.95	-0.28	0.67	5.85	-0.73	-0.56	0.30	
10	CH ₃	OCH ₂	H	F	0	8.10	5.09	5.15	2.87	1.52	4.16	0.56	-0.04	5.85	-0.73	-0.56	0.30	
11	OCH ₃	OCH ₂	H	F	0	7.40	5.13	5.37	3.98	1.35	9.42	-0.02	0.26	5.85	-0.73	-0.56	0.30	
12	F	OCH ₂	H	F	0	4.50	5.35	5.27	2.65	1.35	1.82	0.14	0.43	5.85	-0.73	-0.56	0.30	
13	Br	OCH ₂	H	F	0	2.00	5.70	5.94	3.82	1.95	3.80	0.86	0.44	5.85	-0.73	-0.56	0.30	
14	H	OCH ₂	F	F	0	1.80	5.74	5.64	2.06	1.00	1.00	0.00	0.00	5.85	-0.59	-0.56	0.44	
15	Cl	OCH ₂	H	F	0	1.50	5.82	5.77	3.52	1.80	3.24	0.71	0.41	5.85	-0.73	-0.56	0.30	
16	NO ₂	OCH ₂	F	F	0	0.75	6.12	6.11	3.44	1.70	5.95	-0.28	0.67	5.85	-0.59	-0.56	0.44	
17	OCH ₃	OCH ₂	F	F	0	0.67	6.17	6.11	3.98	1.35	9.42	-0.02	0.26	5.85	-0.59	-0.56	0.44	
18	F	OCH ₂	F	F	0	0.58	6.24	6.00	2.65	1.35	1.82	0.14	0.43	5.85	-0.59	-0.56	0.44	
19	CH ₃	OCH ₂	F	F	0	0.54	6.27	5.89	2.87	1.52	4.16	0.56	-0.04	5.85	-0.59	-0.56	0.44	
20	Br	OCH ₂	F	F	0	0.33	6.48	6.67	3.82	1.95	3.80	0.86	0.44	5.85	-0.59	-0.56	0.44	
21	Cl	OCH ₂	F	F	0	0.30	6.52	6.51	3.52	1.80	3.24	0.71	0.41	5.85	-0.59	-0.56	0.44	
22	H	CH ₂ CH ₂	H	H	0	9.40	5.03	5.20	2.06	1.00	1.00	0.00	0.00	5.97	-0.29	-0.57	0.08	
23	CH ₃	CH ₂ CH ₂	H	H	0	5.80	5.24	5.44	2.87	1.52	4.16	0.56	-0.04	5.97	-0.29	-0.57	0.08	
24	F	CH ₂ CH ₂	H	H	0	2.40	5.62	5.56	2.65	1.35	1.82	0.14	0.43	5.97	-0.29	-0.57	0.08	
25	Br	CH ₂ CH ₂	H	H	0	1.70	5.77	6.23	3.82	1.95	3.80	0.86	0.44	5.97	-0.29	-0.57	0.08	
26	NO ₂	CH ₂ CH ₂	H	H	0	1.50	5.82	5.67	3.44	1.70	5.95	-0.28	0.67	5.97	-0.29	-0.57	0.08	
27	Cl	CH ₂ CH ₂	H	H	0	1.10	5.96	6.06	3.52	1.80	3.24	0.71	0.41	5.97	-0.29	-0.57	0.08	
28	H	OCH ₂	H	H	1	230.00	3.64	3.81	2.06	1.00	1.00	0.00	0.00	5.85	-0.87	-0.56	0.08	
29	Cl	OCH ₂	H	H	1	8.30	5.08	4.67	3.52	1.80	3.24	0.71	0.41	5.85	-0.87	-0.56	0.08	
30	Cl	CH ₂	H	H	0	1.50	5.82	6.16	3.52	1.80	3.24	0.71	0.41	4.74	-0.72	-0.57	0.09	
31	H	CH ₂	H	H	0	8.50	5.07	5.30	2.06	1.00	1.00	0.00	0.00	4.74	-0.72	-0.57	0.09	
32	CH ₃	CH ₂	H	H	0	3.10	5.51	5.54	2.87	1.52	4.16	0.56	-0.04	4.74	-0.72	-0.57	0.09	
33	NO ₂	CH ₂	H	H	0	0.93	6.03	5.77	3.44	1.70	5.95	-0.28	0.67	4.74	-0.72	-0.57	0.09	
34	OCH ₃	CH ₂	H	H	0	0.92	6.04	5.76	3.98	1.35	9.42	-0.02	0.26	4.74	-0.72	-0.57	0.09	
35	F	CH ₂	H	H	0	0.63	6.20	5.66	2.65	1.35	1.82	0.14	0.43	4.74	-0.72	-0.57	0.09	
36	Br	CH ₂	H	H	0	0.30	6.52	6.32	3.82	1.95	3.80	0.86	0.44	4.74	-0.72	-0.57	0.09	
Sulotroban						13.20												

a) $C = IC_{50}$. b) From Eq. 3. c, d) From charge obtained by the CNDO method.

Table 2. Results of Hansch-Fujita Analysis for 36 TXA_2 Receptor Antagonists (II)

No.	Regression equation	$n^a = 36$	R^b	S^c	R^d (pred.)
1	$-\log C = 1.06B_1 - 0.98L_{W-COOH} + 2.09\pi_{X+Y+W-COOH} + 3.04\Sigma Q_{(1)-(6)} + 10.12$ ($t^e = 7.38$) (8.89) (8.66) (8.65)		0.93	0.27	0.91
2	$-\log C = 0.61\pi_R + 1.14F_R - 9.33Q_{\sigma C} + 4.99\Sigma Q_{(1)-(6)} + 3.93$ (5.85) (6.55) (11.83) (12.30)		0.94	0.25	0.92
3	$-\log C = 0.78L_R - 0.12(B_5)^2 - 9.47Q_{\sigma C} + 5.00\Sigma Q_{(1)-(6)} + 2.48$ (7.63) (4.56) (11.52) (11.77)		0.94	0.25	0.92

a) Number of compounds. b) Correlation coefficient. c) Standard error of estimate. d) Correlation coefficient in prediction (leave-one-out). e) t statistics (95% confidence).

contribution of other structural parameters may become clearer when the 3D structure of TXA_2 receptor is determined.

Among these compounds, **21** showed the most potent and selective TXA_2 receptor antagonist activity *in vitro*, and is one of the most potent non-prostanoid TXA_2 antagonists. Compound **21** showed pronounced pharmacological activity in an *in vivo* study. To examine the

relation between the tendency for formation of hairpin-like conformation and the results of this QSAR study, conformational analysis of 4-[2-(substituted phenylsulfonamino)ethylthio]phenoxyacetic acids (II) is in progress.

Experimental

Melting points were determined on a Mettler FP-60 melting point apparatus and are uncorrected. Infrared (IR) spectra were taken on a

Table 3. Correlation Matrix for Colinearity between Variables Used

	L_R	B_1	$(B_5)^2$	π_R	F_R	L_{W-COOH}	$\pi_{X+Y+W-COOH}$	Q_{ac}	$\Sigma Q_{(1)-(6)}$
L_R	1								
B_1	0.777	1							
$(B_5)^2$	0.757	0.277	1						
π_R	0.312	0.602	-0.219	1					
F_R	0.581	0.627	0.275	-0.106	1				
L_{W-COOH}	-0.039	0.001	-0.062	-0.019	-0.007	1			
$\pi_{X+Y+W-COOH}$	-0.029	0.055	-0.064	0.030	0.038	0.231	1		
Q_{ac}	0.018	-0.037	0.041	-0.020	-0.026	0.589	-0.550	1	
$\Sigma Q_{(1)-(6)}$	0.066	0.102	0.102	-0.030	0.018	0.366	-0.042	0.669	1

Perkin Elmer 1760 spectrometer. Proton nuclear magnetic resonance spectra ($^1\text{H-NMR}$) were recorded on a Varian VXL-200 spectrometer. Chemical shifts are reported in ppm (δ value) with tetramethylsilane as an internal standard. Electron impact-mass spectra (EI-MS) were taken on a JEOL JMS-SX102 spectrometer. Microanalytical data were obtained by using a Carlo Erba 1106R or a Perkin-Elmer 240C elemental analyzer. Organic solutions used during work up were dried using anhydrous MgSO_4 . Flash chromatography was performed using Micro Sphere Gel D75-60A (Asahi Glass Co.). Thin layer chromatography was performed on silica gel pre-coated plates (Merck, Kiesegel 60F-254).

Ethyl 4-(Chlorosulfonyl)phenyl Acetate (IV) Chlorosulfonic acid (87 ml, 1.3 mol) was added dropwise to ethyl phenylacetate (III) (48 g, 0.29 mol) at 40°C . The reaction mixture was stirred for 30 min at room temperature, then poured carefully onto crushed ice and extracted with CH_2Cl_2 . The organic layer was washed with water and brine successively, dried and evaporated *in vacuo* to give 42 g (60%) of crude IV as a colorless oil. IR (neat): 2984, 1737, 1380, 1176, 577 cm^{-1} . $^1\text{H-NMR}$ (CDCl_3) δ : 1.25 (3H, t, $J=7\text{ Hz}$), 3.11 (2H, s), 4.20 (2H, q, $J=7\text{ Hz}$), 7.55 (2H, m), 8.00 (2H, m). EI-MS m/z : 262 (M^+).

Methyl 4-[2-(Phthalimido)ethylthio]phenyl Acetate (VI) Concentrated HCl (80 ml) was added dropwise to a mixture of IV (42 g, 0.16 mol), powdered tin (96 g, 0.775 mol) and MeOH (32 ml) and the mixture was heated under reflux for 3 h, then poured onto crushed ice and extracted with CH_2Cl_2 . The organic layer was washed successively with water and brine, dried and evaporated *in vacuo* to give methyl 4-mercaptophenylacetate (V) as a colorless oil, which was used immediately for the next reaction without further purification.

A solution of *N*-(2-bromoethyl)phthalimide (20.3 g, 79.9 mmol) in *N,N*-dimethylformamide (DMF) (100 ml) was added dropwise to a mixture of the crude product obtained above, K_2CO_3 (16.4 g, 118 mmol) and DMF (100 ml) under an argon atmosphere. The reaction mixture was stirred for 16 h at room temperature and then poured into 6% HCl (1200 ml) and extracted with EtOAc. The organic layer was washed with brine, dried and evaporated *in vacuo*. The residue was purified by flash chromatography using 1:1 CH_2Cl_2 /hexane to give 9.1 g (30%) of VI as colorless prisms: mp $90-92^\circ\text{C}$. IR (KBr): 1766, 1710, 1398, 715 cm^{-1} . $^1\text{H-NMR}$ (CDCl_3) δ : 3.21 (2H, t, $J=7\text{ Hz}$), 3.54 (2H, s), 3.69 (3H, s), 3.92 (2H, t, $J=7\text{ Hz}$), 7.10-7.45 (4H, m), 7.77 (4H, m). EI-MS m/z : 355 (M^+).

Methyl 4-[2-(Phenylsulfonylamino)ethylthio]phenylacetate (VIII) Hydrazine monohydrate (0.82 ml, 16.9 mmol) was added to a solution of VI (3.0 g, 8.44 mmol) in CH_2Cl_2 /MeOH (50 ml/50 ml) and the mixture was stirred for 16 h at room temperature. The resulting precipitate was removed by filtration and the filtrate was washed with water and brine successively, dried and filtered to give a solution of methyl 4-(2-aminoethylthio)phenylacetate (VII). Et_3N (1.4 ml, 10.1 mmol) was added to the above solution, followed by dropwise addition of a solution of phenylsulfonyl chloride (1.08 ml, 8.44 mmol) in CH_2Cl_2 (2 ml) at 0°C . The reaction mixture was stirred at room temperature for 3.5 h and then washed successively with water and brine, dried and evaporated *in vacuo*. The residue was purified by flash chromatography using 2:5 EtOAc/hexane to give 1.8 g (58.7%) of methyl 4-[2-(phenylsulfonylamino)ethylthio]phenylacetate as a colorless oil. IR (neat): 3284, 1732, 1328, 1158, 1094, 1016 cm^{-1} . $^1\text{H-NMR}$ (CDCl_3) δ : 2.96 (2H, t, $J=6\text{ Hz}$), 3.14 (2H, q, $J=6\text{ Hz}$), 3.59 (2H, s), 3.70 (3H, s), 4.97 (1H, br m), 7.18 (4H, m), 7.45-7.60 (3H, m), 7.82 (2H, m). EI-MS m/z : 365 (M^+).

Sodium 4-[2-(4-Phenylsulfonylamino)ethylthio]phenylacetate (31) A mixture of the methyl ester obtained above (1.75 g, 4.79 mmol), 10% NaOH solution (5 ml) and MeOH (50 ml) was stirred for 1 h at room

temperature. The reaction mixture was acidified with 3% HCl and then extracted with EtOAc. The organic layer was washed with brine, dried and evaporated *in vacuo* to give 1.6 g (95%) of 4-[2-(4-phenylsulfonylamino)ethylthio]phenylacetic acid as a colorless oil. IR (neat): 3286, 3000 (br), 1724, 1698, 1324, 1160, 1094 cm^{-1} . $^1\text{H-NMR}$ ($\text{DMSO}-d_6$) δ : 2.92 (4H, br m), 3.52 (2H, s), 7.20 (4H, s), 7.50-7.60 (3H, m), 7.75 (2H, m), 7.90 (1H, br t), 12.28 (1H, br s). EI-MS m/z : 351 (M^+).

The free acid obtained above was treated with NaOMe in MeOH to give 0.92 g (49%) of the sodium salt (31) as a colorless powder, mp $157.5-159.5^\circ\text{C}$. IR (KBr): 3500 (br), 1581, 1155 cm^{-1} . $^1\text{H-NMR}$ ($\text{DMSO}-d_6$) δ : 2.89 (4H, s), 3.22 (2H, s), 7.15 (4H, s), 7.52-7.65 (3H, m), 7.75 (2H, m), 8.10 (1H, br s). Anal. Calcd. for $\text{C}_{16}\text{H}_{16}\text{NaNO}_4\text{S}_2 \cdot \text{H}_2\text{O}$: C, 49.10; H, 4.63; N, 3.68. Found: C, 48.89; H, 4.34; N, 3.36.

The following compounds were synthesized by the same method as described above. **32**: mp $95-97^\circ\text{C}$. Anal. Calcd. for $\text{C}_{17}\text{H}_{19}\text{NO}_4\text{S}_2$: C, 55.87; H, 5.24; N, 3.83. Found: C, 55.49; H, 5.17; N, 3.79. **33**: mp $114.5-118^\circ\text{C}$. Anal. Calcd. for $\text{C}_{16}\text{H}_{16}\text{N}_2\text{O}_6\text{S}_2$: C, 48.47; H, 4.07; N, 7.07. Found: C, 48.20; H, 3.82; N, 7.10. **34**: mp $84.5-87.5^\circ\text{C}$. Anal. Calcd. for $\text{C}_{17}\text{H}_{19}\text{NO}_5\text{S}_2$: C, 53.53; H, 5.02; N, 3.67. Found: C, 53.75; H, 4.80; N, 3.53. **35**: mp $103-105.5^\circ\text{C}$. Anal. Calcd. for $\text{C}_{16}\text{H}_{16}\text{FNO}_4\text{S}_2$: C, 52.02; H, 4.37; N, 3.79. Found: C, 51.79; H, 4.11; N, 3.81. **36**: mp $126-129.5^\circ\text{C}$. Anal. Calcd. for $\text{C}_{16}\text{H}_{16}\text{BrNO}_4\text{S}_2$: C, 44.66; H, 3.75; N, 3.25. Found: C, 44.37; H, 3.48; N, 3.19.

Ethyl 4-[2-(4-Chlorophenylsulfonylamino)ethylthiomethyl]phenoxyacetate (37) Methanesulfonyl chloride (2.6 ml, 33.7 mmol) was added dropwise to a solution of ethyl 4-(hydroxymethyl)phenoxyacetate (7.08 g, 33.7 mmol) and triethylamine (5.2 ml, 37 mmol) in CH_2Cl_2 (100 ml) at room temperature. The reaction mixture was stirred for 16 h, washed successively with water, 5% NaHCO_3 and brine, then dried and evaporated *in vacuo*. The residue was purified by flash chromatography using EtOAc/hexane (1:4) to give 3.2 g (41.5%) of ethyl 4-(chloromethyl)phenoxyacetate as a colorless powder, mp $37-39^\circ\text{C}$. IR (KBr): 1757, 1612, 1514, 1201, 1179 cm^{-1} . $^1\text{H-NMR}$ (CDCl_3) δ : 1.30 (t, $J=7\text{ Hz}$, 3H), 4.26 (q, $J=7\text{ Hz}$, 2H), 4.56 (s, 2H), 4.60 (s, 2H), 6.88 (m, 2H), 7.30 (m, 2H). EI-MS m/z : 228 (M^+).

Tributylphosphine (3.2 ml, 13 mmol) was added dropwise to a mixture of bis[2-(4-chlorophenylsulfonylamino)ethyl]disulfide (5.21 g, 10.4 mmol) and 90% aqueous MeOH (100 ml) at room temperature under an argon atmosphere. The reaction mixture was stirred for 20 min and then evaporated *in vacuo* and dried over P_2O_5 under vacuum to give crude 2-(4-chlorophenylsulfonylamino)ethylmercaptan.

A mixture of the above crude mercaptan, ethyl 4-(chloromethyl)phenoxyacetate (4.75 g, 20.8 mmol), K_2CO_3 (5.7 g, 41.6 mmol) and DMF (100 ml) was stirred for 16 h at room temperature, then poured into 3% HCl and extracted with EtOAc. The organic layer was washed with brine, dried and evaporated *in vacuo*. The residue was purified by flash chromatography using hexane/EtOAc (2:1) to give 7.34 g (79.5%) of ethyl 4-[2-(4-chlorophenylsulfonylamino)ethylthiomethyl]phenoxyacetate as a colorless powder, mp $52.5-53.5^\circ\text{C}$. IR (KBr): 3272, 1756 cm^{-1} . $^1\text{H-NMR}$ (CDCl_3) δ : 1.30 (3H, t, $J=7\text{ Hz}$), 2.50 (2H, br s), 3.02 (2H, br s), 3.56 (2H, br s), 4.27 (2H, q, $J=7\text{ Hz}$), 4.61 (2H, s), 5.00 (1H, br t), 6.82 (2H, m), 7.15 (2H, m), 7.50 (2H, m), 7.85 (2H, m). EI-MS m/z : 443 (M^+).

4-[2-(4-Chlorophenylsulfonylamino)ethylthiomethyl]phenoxyacetic Acid (29) A mixture of the methyl ester (37) (1.1 g, 2.48 mmol), 10% NaOH (2.5 ml, 10 mmol) and MeOH (20 ml) was stirred for 2 h at room temperature and then poured into 3% HCl and extracted with EtOAc. The organic layer was washed with brine, dried and evaporated *in vacuo*. The residue was triturated with ether to give 0.9 g (87%) of **29** as colorless

needles, mp 123–124°C. IR (KBr): 3270, 1739 cm^{-1} . $^1\text{H-NMR}$ ($\text{DMSO-}d_6$) δ : 2.40 (2H, m), 2.90 (2H, m), 3.62 (2H, s), 4.65 (2H, s), 6.82 (2H, m), 7.15 (2H, m), 7.67 (2H, m), 7.77 (2H, m), 7.90 (1H, t, $J=5\text{ Hz}$), 12.98 (1H, brs). EI-MS m/z : 415 (M^+). *Anal.* Calcd for $\text{C}_{17}\text{H}_{18}\text{ClNO}_5\text{S}_2$: C, 49.09; H, 4.36; N, 3.37. Found: C, 49.07; H, 4.42; N, 3.22.

Platelet Aggregation Test *in Vitro* Citrated blood (one volume of 3.2% sodium citrate:9 volumes of blood from the carotid artery of male New Zealand white rabbits) was centrifuged at 150 g at room temperature for 15 min to give PRP as a supernatant. The remaining blood was centrifuged at $1500 \times g$ for 10 min to give platelet-poor plasma (PPP). The platelet count of PRP was adjusted to $50\text{--}60 \times 10^4/\mu\text{l}$ by dilution of PRP with PPP. The compound to be tested was dissolved in *N,N*-dimethyl sulfoxide (DMSO) and adjusted to the desired concentration with physiological saline solution. Then, $1\ \mu\text{l}$ of the solution was added to $275\ \mu\text{l}$ of PRP and incubated at 37°C for 3 min, and $25\ \mu\text{l}$ of U-46619 solution (final concentration, $5\ \mu\text{M}$) was added. The mixture was followed for 5 min with an aggregometer (Aggregometer PA-3210, Kyoto Daiichi Kagaku Co.) at 37°C under stirring at 1000 rpm to obtain the maximum aggregation rate. The IC_{50} value was calculated from the maximum decrease in absorbency of PRP in comparison with the vehicle-treated PRP.

References and Notes

- 1) A part of this work was presented at the 21st Symposium on Structure-Activity Relationships, Tokushima, November 1993.
- 2) Hamberg M., Sbensson J., Samuelsson B., *Proc. Natl. Acad. Sci. U.S.A.*, **72**, 2994 (1975).
- 3) Bhagwat S. S., Hamann P. R., Still W. C., Bunting S., Fitzpatrick F. A., *Nature* (London), **315**, 511 (1985).
- 4) Bhagwat S. S., Hamann P. R., Still W. C., *J. Am. Chem. Soc.*, **107**, 6372 (1985).
- 5) Arita H., Kano T., Hanasaki K., *Prog. Lipid Res.*, **28**, 273 (1989).
- 6) Coleman R. A., Sheldrich R. L. G., *Br. J. Pharmacol.*, **96**, 688 (1989).
- 7) Moncada S., Vane J. R., *Pharmacol. Rev.*, **30**, 293 (1979).
- 8) Majerus P. W., *J. Clin. Invest.*, **72**, 1521 (1983).
- 9) Hall S. E., *Med. Res. Rev.*, **11**, 503 (1991).
- 10) Sato M., Kawashima Y., Goto J., Yamane Y., Chiba Y., Jinno S., Satake M., Imanishi T., Iwata C., *Chem. Pharm. Bull.*, **42**, 521 (1994).
- 11) Sato M., Kawashima Y., Goto J., Yamane Y., Chiba Y., Jinno S., Satake M., Iwata C., *Eur. J. Med. Chem.*, **29**, 185 (1994).
- 12) Sato M., Kawashima Y., Goto J., Yamane Y., Chiba Y., Jinno S., Satake M., Iwata C., *Eur. J. Med. Chem.*, submitted.
- 13) Hansch C., Fujita T., *J. Am. Chem. Soc.*, **86**, 16216 (1964).
- 14) Malmsten C., *Life Sci.*, **18**, 169 (1976).
- 15) Born G. V. R., *Nature* (London), **194**, 927 (1962).
- 16) Hansch C., Leo A., "Substituent Constants for Correlation Analysis in Chemistry and Biology," John Wiley & Sons, New York, 1979.
- 17) Pople J. A., Santry D. P., Segal G. A., *J. Chem. Phys.*, **43**, 129 (1965).
- 18) Hirata M., Hayashi Y., Ushikubi F., Yokota Y., Kageyama R., Nakanishi S., Narumiya S., *Nature* (London), **349**, 617 (1991).
- 19) Yamamoto Y., Kamiya K., Terao S., *J. Med. Chem.*, **36**, 820 (1993).