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Synthesis and evaluation of novel sulfonamide derivatives as thromboxane A_2 receptor antagonists I

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Summary — A series of 4-[2-(arylsulfonylamino)ethylthio]phenoxyacetic acids and related compounds were synthesized. The compounds were tested for their thromboxane A_2 (TXA_2) receptor antagonizing effects on (15*S*)-15-hydroxy-11a,9a-(epoxymethano)prosta-5(*Z*),13(*E*)-dienoic acid (U-46619)-induced aggregation of rabbit platelet-rich plasma (PRP). Among the compounds synthesized, 3-{4-[2-(arylsulfonylamino)ethylthio]phenyl}propionic acids **26a–e,g** showed potent TXA_2 receptor antagonist activity. The most potent compound, 3-{4-[2-(4-chlorophenylsulfonylamino)ethylthio]phenyl}propionic acid **26c** was more than 10-fold more potent in TXA_2 receptor antagonizing activity (IC_{50} = 1.1 x 10^{-6} M) than sulotroban (BM-13177) on rabbit platelets. Compound **26c** was also more than 10-fold more potent in TXA_2-inhibitory activity than sulotroban on rat aorta smooth muscle (pA_2 7.7).

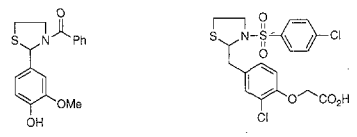
thromboxane A_2 / TXA_2 / receptor antagonist / sulfonamide derivative / sulotroban

Introduction

Thromboxane A_2 (TXA_2), an unstable metabolite of arachidonic acid, is one of the most potent inducers of platelet aggregation, vasoconstriction, and bronchoconstriction [1–4]. It has been postulated that TXA_2 plays an important role in the pathogenesis of various circulatory disorders and asthma [5–7].

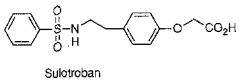
Recently, a number of TXA_2 synthase inhibitors have been studied for the treatment of these diseases and were determined to be less effective than expected [8]. One of the reasons postulated for these results is the accumulation of the endoperoxide intermediate PGH_2, which is itself a potent TXA_2 agonist [9]. TXA_2 receptor antagonists are expected to be more effective than TXA_2 synthase inhibitors for the treatment of these diseases, because they can avoid the accumulation of PGH_2 and inhibit the binding of both TXA_2 and PGH_2 on the receptor [9]. A number of TXA_2 receptor antagonists are now under clinical investigation [10], and they are classified into prostanoid-related compounds and non-prostanoid compounds such as sulotroban [11].

We have recently investigated the synthesis and platelet aggregation inhibitory activities of a series of compounds with an -N-C-C-S- unit within the molecule. During the course of these investigations, we have reported that some 2-substituted thiazolidine derivatives, such as **1** or **2**, have relatively low potency but selective TXA_2 receptor antagonist activity [12].



1

2



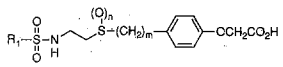
Sulotroban

This paper reports our investigation into introducing an -N-C-C-S- unit into the chemical structure of sulotroban, which corresponds to the opening of the thiazolidine ring of the compounds **1** or **2**. Structure–activity relationships between these derivatives were also investigated.

Chemistry

The compounds listed in tables I-II were synthesized by the method shown in schemes 1 and 2. The compounds **13**, **14**, and **15** were prepared by alkylation of *N*-phenylsulfonylcysteamine **9** with the benzyl halide **6** or aralkylmesylates **7** or **8**. Since the mercaptan **9** was very sensitive to air oxidation, it was prepared from the disulfide derivative by reduction with Bu_3P in aqueous MeOH/acetone at room temperature [13], and was used *in situ* after evaporation of the solvent. The esters **10**, **11**, and **12** were hydrolyzed to give compounds **13**, **14**, and **15** (scheme 1). Introduction of an -N-C-C-S- unit to the compounds **25a–n**, **26a–e,g–n**, and **27** was conducted by alkylation of arylthiols **16**, **17**, or **18** by protected 2-bromoethylamine (scheme 2). 4-Mercaptophenol **16** was successively alkylated by *N*-(2-bromoethyl)-phthalimide and ethylbromoacetate, and then the protective group of the primary amine residue was removed by hydrazine in an $EtOH/CH_2Cl_2$ mixture. The resulting aminoester **22** was used as a solution for the next step without isolation. After removal of the insoluble phthalhydrazide and excess hydrazine, **22** was treated with arylsulfonylchlorides followed by hydrolysis to give **25a–n** in moderate yields (scheme 2). The syntheses of **26a–e,g–n** and **27** were accomplished in a manner similar to that used for **25a–n**. Compound **20** was deprotected by hydrazine monohydrate without any solvent and the resulting amino acid **23** was purified by Toyo Soda Gel HP-20 using MeOH as eluent, and then treated with arylsulfonylchlorides. Methyl-4-mercaptophenylacetate **18** was prepared by chlorosulfonylation of ethylphenylacetate by $ClSO_3H$, followed by reduction with tin powder in acidic MeOH.

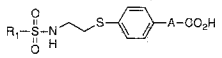
Table I. Structure and pharmacological activities *in vitro*.



Compd	R_1	*m*	*n*	*mp*	*Formula*[a]	*Platelet aggregation* IC_{50}[b] *(μM)*
13	Ph	1	0	105–107.5	$C_{17}H_{19}NO_5S_2 \cdot 1/2H_2O$	230
14[c]	Ph	2	0	> 300	$C_{18}H_{19}NO_5Na_2S_2 \cdot H_2O$	> 300[d]
15[c]	Ph	3	0	> 300	$C_{19}H_{21}NO_5Na_2S_2 \cdot H_2O$	> 300[d]
25a	Ph	0	0	156–159	$C_{16}H_{17}NO_5S_2$	50
25b	4-F-Ph	0	0	150.5–153.5	$C_{16}H_{16}NO_5FS_2$	27.4
25c	4-Cl-Ph	0	0	142.5–144	$C_{16}H_{16}NO_5ClS_2$	4.5
25d	4-Br-Ph	0	0	152–153.5	$C_{16}H_{16}NO_5BrS_2$	5.2
25e	4-Me-Ph	0	0	153–155	$C_{17}H_{19}NO_5S_2$	23.1
25f	4-MeO-Ph	0	0	128.5–131	$C_{17}H_{19}NO_6S_2$	40.1
25g	4-NO_2-Ph	0	0	160.5–162.5	$C_{16}H_{16}N_2O_7S_2$	16.3
25h[e]	4-AcNH-Ph	0	0	> 300	$C_{18}H_{18}N_2O_6K_2S_2 \cdot 3.5H_2O$	> 100[d]
25i	4-Cl-3-NO_2-Ph	0	0	91.5–95.8	$C_{16}H_{15}N_2O_7ClS_2$	> 100[d]
25j	2,4,5-Trichloro-Ph	0	0	125.5–127	$C_{16}H_{14}NO_5Cl_3S_2$	> 100[d]
25k	Pentafluoro-Ph	0	0	113.5–116.5	$C_{16}H_{12}NO_5F_5S_2$	> 100[d]
25l	2,4,6-Trimethyl-Ph	0	0	129.5–131.5	$C_{19}H_{23}NO_5S_2$	> 100[d]
25m	2-Naphthyl	0	0	74–79.5	$C_{20}H_{19}NO_5S_2 \cdot 1/2H_2O$	> 100[d]
25n	3-Pyridyl	0	0	163–165	$C_{15}H_{16}N_2O_5S_2$	> 100[d]
28	Ph	0	1	182.5–183.5	$C_{16}H_{17}NO_6S_2$	> 100[d]
29	Ph	0	2	156–158.5	$C_{16}H_{17}NO_7S_2$	100
Sulotroban						13.2

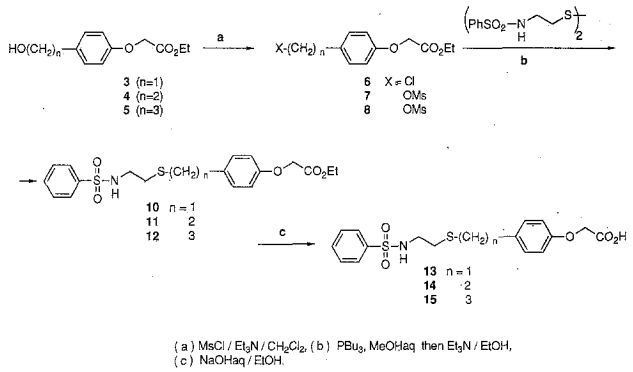
[a]C, H, N analyses were within ± 0.4% of the calculated values. All the compounds had ^{1}H-NMR, IR, and MS data that were consistent with their structures; [b]concentration needed to inhibit U-46619 (5 μM)-induced platelet aggregation in rabbit PRP by 50%. Values represented were means of 4 tests; [c]sodium salt; [d]*n* = 2; [e]potassium salt.

Table II. Structure and pharmacological activities *in vitro*.



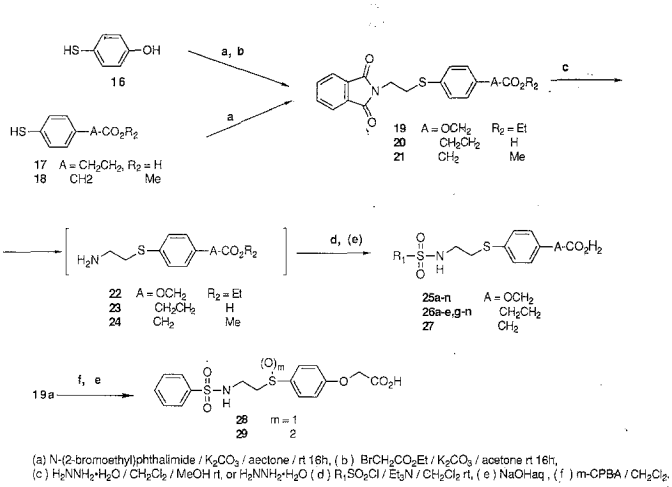
Compd	R_1	*A*	*mp*	*Formula*[a]	*Inhibition of platelet aggregation* IC_{50}[b] *(μM)*
26a	Ph	CH_2CH_2	146–148	$C_{17}H_{19}NO_4S_2$	9.4
26b	4-F-Ph	CH_2CH_2	135–136.5	$C_{17}H_{18}NO_4FS_2$	2.4
26c	4-Cl-Ph	CH_2CH_2	117–119	$C_{17}H_{18}NO_4ClS_2$	1.1
26d	4-Br-Ph	CH_2CH_2	130.5–131.5	$C_{17}H_{18}NO_4BrS_2$	1.7
26e	4-Me-Ph	CH_2CH_2	127.5–130.5	$C_{18}H_{21}NO_4S_2$	5.8
26g	4-NO_2-Ph	CH_2CH_2	154–156	$C_{17}H_{18}N_2O_6S_2$	1.5
26h	4-AcNH-Ph	CH_2CH_2	146.5–149	$C_{19}H_{22}N_2O_5S_2$	> 100[c]
26i	4-Cl-3-NO_2-Ph	CH_2CH_2	125–127.5	$C_{17}H_{17}N_2O_6ClS_2$	28.6
26j	2,4,5-Trichloro-Ph	CH_2CH_2	123.5–126.5	$C_{17}H_{16}NO_4Cl_3S_2$	47.6
26k	Pentafluoro-Ph	CH_2CH_2	131.5–133.5	$C_{17}H_{14}NO_4F_5S_2$	> 100[c]
26l	2,4,6-Trimethyl-Ph	CH_2CH_2	85–87	$C_{20}H_{25}NO_4S_2$	> 100[c]
26m	2-Naphthyl	CH_2CH_2	132–134.5	$C_{20}H_{19}NO_5S_2$	16.5
26n	3-Pyridyl	CH_2CH_2	146.5–150	$C_{16}H_{18}N_2O_4S_2$	> 100[c]
27	4-Cl-Ph	CH_2	129–131	$C_{16}H_{16}NO_4ClS_2$	1.5
sulotroban					13.2

[a]C, H, N analyses were within ± 0.4% of the calculated values. All the compounds had ^{1}H-NMR, IR, and MS data that were consistent with their structures; [b]concentration needed to inhibit U-46619 (5 μM)-induced platelet aggregation in rabbit PRP by 50%. Values represented were means of 4 tests; [c]n = 2.



(a) MsCl / Et_3N / CH_2Cl_2, (b) PBu_3, MeOHaq then Et_3N / EtOH, (c) NaOHaq / EtOH.

Scheme 1.



(a) N-(2-bromoethyl)phthalimide / K_2CO_3 / aectone / rt 16h, (b) $BrCH_2CO_2Et$ / K_2CO_3 / acetone rt 16h, (c) $H_2NNH_2{\cdot}H_2O$ / CH_2Cl_2 / MeOH rt, or $H_2NNH_2{\cdot}H_2O$ (d) R_1SO_2Cl / Et_3N / CH_2Cl_2 rt, (e) NaOHaq, (f) m-CPBA / CH_2Cl_2.

Scheme 2.

Results and discussion

The thromboxane A_2 (TXA_2) receptor antagonist activity of the compounds shown in tables I and II was tested on rabbit platelets. The inhibitory activity of the compounds against U-46619 [14] (5 x 10^{-6} M)-induced platelet aggregation of rabbit platelet-rich plasma (PRP) was measured by the method of Born [15], and the IC_{50} values were calculated. Sulotroban was also tested as a reference compound.

We first investigated the influence of the length of alkylene group between the sulfur atom and the phenyl ring. The IC_{50} values of compounds **14** and **15**,

which have an ethylene or trimethylene group, were over 300 μM. As the length of the alkylene group becomes shorter, the activity was enhanced, and compound **25a**, whose sulfur atom was directly attached to the phenyl ring, showed the best result (IC_{50} = 5 x 10^{-5} M) (table I). Next, we examined the introduction of a substituent on the *para* position of the phenylsulfonyl moiety of **25a**. Introduction of a variety of substituents, such as fluorine (**25b**), chlorine (**25c**) or bromine atoms (**25d**), or methyl (**25e**), methoxy (**25f**), or nitro groups (**25g**), into the *para*-position of the phenylsulfonyl moiety of **25a** was examined. Even though these substituents have different electron-donating or withdrawing abilities, all of the compounds showed a higher activity than **25a**, and the *para*-Cl derivative **25c** showed the best result (IC_{50} = 4.5 x 10^{-6} M) of the compounds, whereas the introduction of a polar residue, such as 4-acetamidophenyl (**25h**) or 3-pyridyl (**25n**) group resulted in a large decrease in activity (table I). Moreover, introduction of one or more second substituents to **25c**, such as 3-NO_2 (**25i**) or 2,6-dichloro (**25j**), decreased the activity, and the IC_{50} values of the compounds produced were over 20-fold larger than that of **25c**. Introduction of more bulky groups to the arylsulfonyl moiety, such as the poly-substituted phenyl (**25k**, **l**) or naphthyl (**25m**) groups, also decreased the activity. From these results, it was postulated that the arylsulfonyl moiety of the compounds of this series should be associated with the lipophilic pocket lesion of the thromboxane A_2 receptor, and the *para*-chloro- or *para*-bromo-phenylsulfonyl moieties may be sufficiently large for this binding site.

As the next step, the influence of the substituents on the other phenyl ring that was attached to the carboxy moiety was investigated. Oxidation of the sulfur atom of **25a** decreased the activity, and the more charged sulfoxide derivative **28** was much less active than sulfone derivative **29**. Substitution of the oxygen atom of **25a** by a methylene group resulted in a large enhancement of the activity. The phenylpropionic acid derivatives **26a–e,g** were over 4-fold more potent than corresponding phenoxyacetic acid derivatives. Since there is a little difference between the phenoxyacetic acid derivatives **25** and the phenylpropionic acid derivatives **26** in the length of the molecule, the electron density of the phenyl moiety seemed to have much influence on the activity. Of these compounds, the 4-chlorophenyl derivative **26c** showed the most potent activity on rabbit PRP (IC_{50} = 1.1 x 10^{-6} M), and **26c** also showed potent TXA_2 antagonist activity on rat aorta smooth muscle. The pA_2 value of **26c** on U-46619-induced rat aorta constriction was 7.7, which is about 15 times the potent activity of sulotroban (pA_2 6.5). Introduction of a poly-substituted phenyl or polar moiety to the arylsulfonyl moiety of the phenylpropionic acid derivatives **26h–n** also decreased the activity, as in the case of the phenoxyacetic acid derivatives **22h–n**.

Finally, the influence of the overall molecule length was investigated, because the length of the alkylene group between the sulfur atom and the phenyl ring had a strong influence on the activity. The phenylacetic acid derivative **27** corresponds to the replacement of the ethylene group of **26c** with a methylene group and is closely related to the structure of daltroban [10], which was reported to be much more potent than the phenoxyacetic acid derivative sulotroban. Compound **27** showed potent activity (IC_{50} = 1.5 x 10^{-6} M), but was less potent than **26c** (IC_{50} = 1.1 x 10^{-6} M) (table II). Thus, the phenylpropionic acid derivative was found to be more potent than the phenylacetic acid derivative in this series of compounds, which have longer full molecular length than all non-prostanoid TXA_2 receptor antagonists reported so far.

In conclusion, we have investigated the introduction of a sulfur atom into the molecular structure of sulotroban. Among the compounds synthesized, 3-{4-[2-(4-chlorophenylsulfonylamino)ethylthio]-phenyl}propionic acid **26c** was the optimal compound in this series, and showed over 10 times more potent TXA_2 receptor antagonist activity than sulotroban on rabbit platelets and rat aorta smooth muscle.

Experimental protocols

Chemistry

Melting points were determined by a Mettler FP-60 melting point apparatus and are uncorrected. Infrared (IR) spectra were taken on a Perkin–Elmer 1760 spectrometer. Proton nuclear magnetic resonance spectra (^{1}H-NMR) were recorded on a Varian VXL-200 spectrometer. Chemical shifts are reported in ppm (δ) values, based on tetramethylsilane as an internal standard. Mass spectra (MS) were taken on a JEOL JMS-SX102 spectrometer. Elemental analyses were within ± 0.4% of the theoretical values. Organic solutions during workup 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, Kieselgel 60F-254).

Ethyl 4-[2-(phenylsulfonylamino)ethylthiomethyl]phenoxyacetate ***10***

To a solution of ethyl 4-(hydroxymethyl)phenoxyacetate **3** (7.08 g, 33.7 mmol) and triethylamine (5.2 ml, 37 mmol) in CH_2Cl_2 (100 ml) was added methanesulfonylchloride (2.6 ml, 33.7 mmol) dropwise at room temperature. The reaction mixture was stirred for 16 h and was then washed successively with water, 5% $NaHCO_3$ and brine; it was 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%)

ethyl 4-(chloromethyl)phenoxyacetate **6** as a colorless powder: mp 37–39°C; IR (KBr) 1757, 1612, 1514, 1201, 1179 cm^{-1}; ^1H-NMR ($CDCl_3$) δ 1.30 (t, J = 7 Hz, 3H), 4.26 (q, J = 7 Hz, 2H), 4.56 (s, 2H), 4.60 (s, 2H), 6.88 (m, 2H), 7.30 (m, 2H); MS (EI) m/z 228 (M^+).

To a mixture of bis-[2-(phenylsulfonylamino)ethyl]disulfide (1.0 g, 2.3 mmol) and 90% MeOH aq/acetone (20 ml/10 ml) was added dropwise, tributylphosphine (0.72 ml, 2.9 mmol) at room temperature under argon. The reaction mixture was stirred for 20 min; it was then evaporated *in vacuo* and dried over P_2O_5 *in vacuo*, to give crude 2-(phenylsulfonylamino)-ethylmercaptan.

A mixture of the above crude mercaptan **9**, **6** (1.05 g, 4.6 mmol), Et_3N (1.4 ml, 10 mmol), and EtOH (20 ml) was stirred for 16 h at room temperature. The reaction mixture was 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 3:1, to give 1.04 g (55.2%) **10** as a colorless oil: IR (neat) 3285, 1756, 1511, 1327, 1206, 1162 cm^{-1}; ^1H-NMR δ 1.30 (t, J = 7 Hz, 3H), 2.49 (t, J = 6 Hz, 2H), 3.02 (brs, 2H), 3.51 (s, 2H), 4.25 (q, J =7 Hz, 2H), 4.59 (s, 2H), 4.98 (brs, 1H), 6.80 (m, 2H), 7.13 (m, 2H), 7.55 (m, 3H), 7.83 (m, 2H); MS (EI) m/z 409 (M^+).

4-[2-(Phenylsulfonylamino)ethylthiomethyl]phenoxyacetic acid ***13***

To a solution of **10** (0.54 g, 1.32 mmol) in EtOH (10 ml) was added 10% NaOH (2 ml, 5 mmol). The reaction mixture was stirred for 2 h at room temperature and was 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 0.39 g (77%) of **13** as a colorless powder: mp 105–107.5°C; IR (KBr) 3436, 3288, 1743, 1708, 1511, 1326, 1245, 1155, 1092 cm^{-1}; ^1H-NMR (acetone-d_6) δ 2.50 (dd, J = 7, 8 Hz, 2H), 3.8 (m, 2H), 3.65 (s, 2H), 4.70 (s, 2H), 6.60 (brt, 1H), 6.87 (m, 2H), 7.22 (m, 2H), 7.52–7.67 (m, 3H), 7.85 (m, 2H); MS (EI) m/z 387 (M^+). Anal $C_{17}H_{19}NO_5S$ (C, H, N).

Ethyl 4-[2-(phthalimid-2-yl)ethylthio]phenoxyacetate ***19***

To a mixture of 4-mercaptophenol **16** (3.78 g, 30 mmol), K_2CO_3 (8.2 g, 60 mmol) and DMF (40 ml) was added a solution of *N*-(2-bromoethyl)phthalimide **17** (7.62 g, 30 mmol) in DMF (50 ml), dropwise under an argon atmosphere. The reaction mixture was stirred for 16 h at room temperature; it was then poured into 3% HCl (200 ml) and extracted with EtOAc. The organic layer was washed twice with brine, dried and evaporated *in vacuo*; the residue was then triturated with EtOH, to give 7.84 g (87.3%) of *N*-[2-(4-hydroxyphenylthio)-ethyl]phthalimide as colorless prisms: mp 153–154°C; IR (KBr) 3406, 1769, 1698, 1515, 1400, 1084, 1011, 719 cm^{-1}; ^1H-NMR (DMSO–d_6) δ 3.21 (t, J = 6 Hz, 2H), 3.76 (t, J = 6 Hz, 2H), 7.08 (m, 2H), 7.82 (s, 4H), 10.20 (s, 1H); MS (EI) m/z 335 (M^+).

A solution of ethyl 2-bromoacetate (2.9 ml, 26.1 mmol) in DMF (5 ml) was added dropwise to a mixture of *N*-[2-(4-hydroxyphenylthio)ethyl]phthalimide, as obtained above (7.8 g, 26.1 mmol), K_2CO_3 (7.2 g, 52.2 mmol) and DMF (45 ml) at room temperature. The reaction mixture was stirred for 3 h at 60°C and then was poured into 3% HCl and extracted with EtOAc. The organic layer was washed twice with brine, dried, evaporated *in vacuo*, and the residue was triturated by hexane, to give 10.0 g (99.7%) of **19** as colorless prisms: mp 91–92°C; IR (KBr) 1750, 1713, 1204 cm^{-1}; ^1H-NMR ($CDCl_3$) δ 1.30 (t, J = 7 Hz, 3H), 3.13 (t, J = 7 Hz, 2H), 3.88 (t, J = 7 Hz, 2H), 4.28 (q, J = 7 Hz, 2H), 4.55 (s, 2H), 6.80 (m, 2H), 7.40 (m, 2H), 7.70 (m, 2H), 7.80 (m, 2H); MS (EI) m/z 385 (M^+).

4-[2-(Phenylsulfonylamino)ethylthio]phenoxyacetic acid ***25a***

To a solution of **19** (9.9 g, 25.7 mmol) in EtOH/CH_2Cl_2 (100 ml/100 ml) was added hydrazine monohydrate (2.5 ml, 51.4 mmol). The reaction mixture was stirred for 16 h at room temperature, and then the resulting precipitate was removed by filtration. The filtrate was washed with water, and the aqueous layer was extracted 3 times with CH_2Cl_2. The organic layers were combined, washed with brine, dried, and filtered, to give a solution of ethyl 4-(2-aminoethylthio)phenoxyacetate **22**.

To the above solution was added triethylamine (3.8 ml, 27 mmol), followed by dropwise addition of a solution of phenylsulfonylchloride (4.54 g, 25.7 mmol) in CH_2Cl_2 (10 ml) at 0°C, and the mixture was stirred for 30 min at room temperature. The reaction mixture was washed successively with water, 5% $NaHCO_3$ solution, and brine; it was then dried and evaporated *in vacuo*. The residue was purified by flash chromatography using EtOAc/hexane 2:3, to give 5.9 g (58.1%) ethyl 4-[2-(phenylsulfonylamino)ethylthio]phenoxy-acetate as a yellow oil: IR (neat) 3020, 1756, 1734, 1494 cm^{-1}; ^1H-NMR ($CDCl_3$) δ 1.29 (t, J = 7 Hz, 3H), 2.83 (t, J = 6 Hz, 2H), 3.04 (brm, 2H), 4.27 (q, J = 7 Hz, 2H), 4.60 (s, 2H), 5.10 (brs, 1H), 6.76 (m, 2H), 7.20 (m, 2H), 7.51 (m, 3H), 7.80 (m, 2H); MS (EI) m/z 395 (M^+).

This ester was hydrolyzed to give 1.76 g (94.7%) of **25a** as colorless prisms: mp 156–159°C; IR (KBr) 3436, 3262, 1735, 1708, 1494, 1241, 1156 cm^{-1}; ^1H-NMR (DMSO–d_6) δ 2.83 (brs, 4H), 4.68 (s, 2H), 6.86 (m, 2H), 7.25 (m, 2H), 7.5–7.7 (m, 3H), 7.73 (m, 2H), 7.82 (brt, 1 H), 13.0 (brs, 1H); MS (EI) m/z 367 (M^+). Anal $C_{16}H_{17}NO_5S_2$ (C, H, N).

4-[2-(4-Phenylsulfonylamino)ethylsulfinyl]phenoxyacetic acid ***28***

To a solution of the ethylester of **25a** (2.0 g, 5.1 mmol) in CH_2Cl_2 (50 ml) was added 70% *m*-CPBA (1.2 g, 5 mmol) in portions at 0°C. The reaction mixture was stirred for 1 h at room temperature; it was then washed successively with 3% $Na_2S_2O_3$, 5% $NaHCO_3$, and brine; it was then dried and evaporated *in vacuo*. The residue was purified by flash chromatography using EtOAc, to give 1.76 g (84.6%) of ethyl 4-[2-(4-phenylsulfonylamino)ethylsulfinyl]phenoxyacetate as a colorless powder: mp 116–118.5°C; IR (KBr) 3436, 3116, 1756, 1593, 1496, 1326, 1203, 1166, 1088, 1031 cm^{-1}; ^1H-NMR ($CDCl_3$) δ 1.30 (t, J = 7 Hz, 3H), 2.70 (m, 1H), 3.10 (m, 1H), 3.38 (t, J = 6 Hz), 4.29 (q, J = 7 Hz, 2H), 4.68 (s, 2H), 5.80 (br, 1H), 7.00 (m, 2H), 7.40 (m, 2H), 7.50–7.65 (m, 3H), 7.85 (m, 2H); MS (EI) m/z 411 (M^+).

The ethylester obtained was hydrolyzed by the method described above, to give **28** as a colorless powder: mp 182.5–183.5°C; IR (KBr) 3500 br, 3223, 1718, 1594, 1494, 1329, 1093, 991, 830, 690 cm^{-1}. ^1H-NMR (DMSO–d_6) δ 2.75–3.20 (m, 4H), 4.75 (s, 2H), 7.08 (m, 2H), 7.52 (m, 2H), 7.5–7.65 (m, 3H), 7.75 (m, 2H), 7.88 (brt, J = 5 Hz, 1H), 13.09 (brs, 1H); MS (EI) m/z 383 (M^+). Anal $C_{16}H_{17}NO_6S_2$ (C, H, N).

4-[2-(Phenylsulfonylamino)ethylsulfonyl]phenoxyacetic acid ***29***

The ethylester of **25a** was treated with 2 equivalents of *m*-CPBA and the resulting ester was hydrolyzed, to give **29** as a colorless powder: mp 156–158.5°C; IR (KBr) 3435, 3277, 1737, 1594, 1322, 1294, 1225, 1158, 1136 cm^{-1}; ^1H-NMR (DMSO–d_6) δ 2.95 (m, 2H), 3.40 (m, 2H), 4.82 (s, 2H), 7.15 (m, 2H), 7.55–7.75 (m, 8H), 13.18 (brs, 1H); MS (Cl) m/z 400 (M + H). Anal $C_{16}H_{17}NO_7S_2$ (C, H, N).

Pharmacology

Platelet aggregation test in vitro

Citrated blood (1 vol 3.2% sodium citrate/9 vol blood) was collected from the carotid arteries of male New-Zealand white rabbits and centrifuged at 150 *g* at room temperature for 15 min to give platelet-rich plasma (PRP) as a supernatant. The remaining blood was centrifuged at 1500 *g* for 10 min to give platelet-poor plasma (PPP). The platelet count of PRP was adjusted to 40–60 x 10^4/μl by dilution of PRP with PPP.

Platelet aggregation was measured by the turbidometric method of Born with an aggregometer (PA-3210, Kyoto Daiichi Kagaku and PAM-8C, Mebanix). The compound to be tested was dissolved in DMSO. A portion of 1 μl solution was added to 275 μl of PRP followed by incubation at 37°C for 3 min under stirring at 1000 rpm, and then 25 μl of U-46619 solution (final concentration: 5 μM) was added to the mixture. The mixture was measured for 5 min by the aggregometer to obtain the maximum aggregation rate. The IC_{50} value was calculated by the maximum decrease in absorbency of test-compound-treated PRP in comparison with the vehicle-treated PRP.

Inhibition of U-46619-induced constriction of rat aorta

Thoracic aortas from male rats (Wistar) were cut into spinal strips. The tissues were mounted isometrically in 10 ml organ baths containing a Krebs–Henseleit solution, containing indomethacin (1×10^{-5} M) which was kept at 37°C while being bubbled with 95% O_2 and 5% CO_2. A resting tension of 1.0 g was applied and, after equilibration for 1 h, the tension developed by the tissues was recorded on a polygraph (Nihon–Koden) through an isometric transducer (Nihon–Koden). The test compounds were added 5 min before the addition of agonist (U-46619) and agonist–response curves were obtained using cumulative concentration methods.

ED_{50} values were obtained by regression analysis and were used to calculate dissociation constants (pA_2 values) by the method of Schild [16].

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