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Synthesis and evaluation of novel fluorinated sulotroban-related sulfonamide derivatives as thromboxane A_2 receptor antagonists*

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Summary — A series of sulotroban-related arylsulfonamide derivatives possessing a fluorinated phenoxyacetic acid moiety was synthesized and tested for TXA_2 antagonizing ability on U-46619-induced platelet aggregation of rabbit platelet-rich plasma. Introduction of one or more fluorine atoms to the phenoxyacetic acid moiety increased this activity. The most potent compound among these compounds was **10c**, which was 40-fold more potent (IC_{50} 3.4 x 10^{-7} M) than sulotroban. **10c** exhibited high activity (ID_{50} 0.14 mg/kg) against a U-46619-induced acute thrombocytopenia model in mice when orally administrated. These findings and those of radioligand binding assays with various ligands showed **10c** to be a potent and selective systemic TXA_2 receptor antagonist.

tromboxane A_2 / TXA_2 / receptor antagonist / sulotroban

Introduction

Thromboxane A_2 (TXA_2), one of the most potent inducers of platelet aggregation, vasoconstriction and bronchoconstriction known [3–6], is now believed to play an important role in the pathogenesis of asthma and various circulatory disorders including myocardial infarction, unstable angina and stroke [7–9]. A number of studies have therefore been performed in an attempt to inhibit the production of TXA_2 for treatment of these diseases. One of the most promising approaches tested was examination of TXA_2 synthase inhibitors, which selectively inhibit the production of TXA_2 from arachidonic acid and did not affect the production of the other prostaglandins. While a number of TXA_2 synthase inhibitors have been studied, each has been found to be less effective than anticipated [10]. One of the reasons suggested for these findings is the accumulation of the endoperoxide intermediate PGH_2, itself a potent TXA_2 agonist [11]. TXA_2 receptor antagonists are now expected to overcome this disadvantage of TXA_2 synthase inhibitors, since they are not expected to induce the accumulation of PGH_2, and since they directly block the binding of both TXA_2 and PGH_2 to the TXA_2 receptor [11]. A number of clinical studies of TXA_2 receptors are therefore now underway [12].

The amino-acid sequence of the human TXA_2 receptor was recently determined [13], and construction of a TXA_2 receptor model and analyses of TXA_2 receptor–ligand interactions have been performed [14]. Despite these efforts, the conformation of TXA_2 bound to its receptor is still unknown. On the other hand, hairpin conformations like those of prostaglandins have been considered suitable for the 'active' conformation of TXA_2 [15]. This hypothesis has been proposed on the basis of the observation that the prostaglandin endoperoxide PGH_2 and stable analogs of it including U-46619 also stimulate the TXA_2 receptor [16]. Therefore, investigations of TXA_2 receptor antagonists were begun with studies of mimics of PGH_2 or TXA_2 and were classified as prostanoid-related derivatives. Prostanoid-related derivatives such as vapiprost (GR32191) [17] and S-145 [18] possess a rigid ring system and fit this hairpin-like conformation model. However, compounds classified as non-prostanoid derivatives such as sulotroban (BM-13,177) [19, 20] generally lack the rigid ring system. Since the flexible non-prostanoid antagonists are generally less potent

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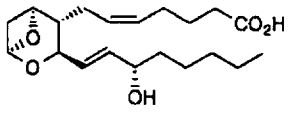
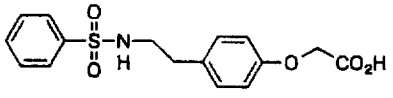
than rigid prostanoid-related antagonists, the relationship between the tendency to form the hairpin-like conformation and the activity of these compounds has been studied [21]. Takasuka *et al* studied the interaction of the acidic proton of the sulfonamide residue and the π-electron of the phenoxyacetic acid moiety of some TXA_2 receptor antagonists in apolar solvents, and reported that this intramolecular interaction participated in the formation of a hairpin-like conformation in such compounds [22].

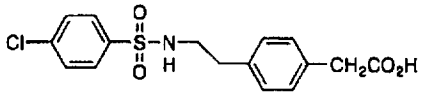
We have recently examined the synthesis and platelet aggregation inhibitory activities of a series of compounds with an –N–C–C–S– unit within the molecule such as 4-[2-(phenylsulfonylamino)ethylthio]phenoxyacetic acid **1** [23, 24]. A preliminary structure–activity relationship study of these compounds suggested that the TXA_2 antagonistic activities of these compounds



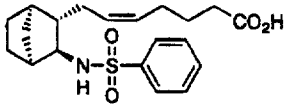
TXA_2



Sulotroban



Daltroban



S145

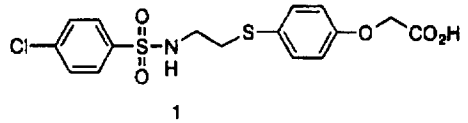


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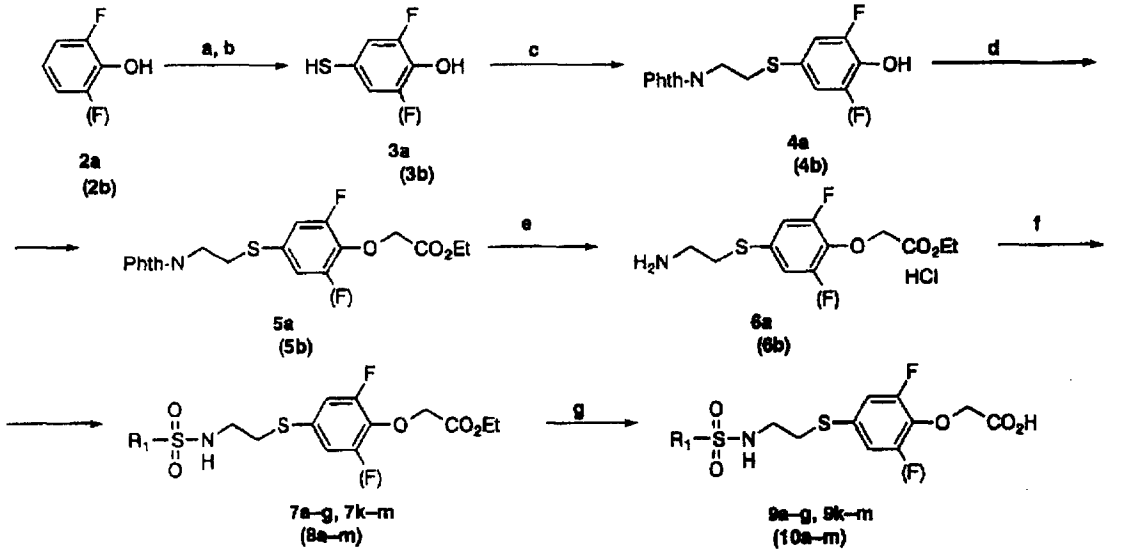
Chart 1.

are strongly influenced by the substituent on the phenyl ring attached to the carboxy residue.

In the present study, we examined the effects of the introduction of a fluorine atom into the phenoxy ring of **1**, which was expected to increase the intramolecular interaction described above. The synthesis of related compounds was also performed for further study of structure–activity relationships.

Chemistry

2-Fluoro- and 2,6-difluorophenoxyacetic acid derivatives **9a–g**, **9k–m** and **10a–m** were synthesized using the method shown in scheme 1. Introduction of a mercapto residue into the 4-positions of 2-fluorophenol (**2a**) and 2,6-difluorophenol (**2b**) were accomplished by treatment with $ClSO_3H$ followed by reduction of the resulting sulfonyl chlorides with Zn in 25% H_2SO_4. The relatively unstable thiophenol derivatives **3a,b** were alkylated stepwise with *N*-(2-bromoethyl)-phthalimide and ethyl bromoacetate to yield **5a,b**. The amino esters **6a,b** were obtained by treatment of **5a,b** with hydrazine, and isolated as crystalline hydrochlorides. Compounds **6a** and **6b** were treated with a variety of sulfonyl chlorides to obtain the corresponding sulfonamide derivatives (**7a–g**, **7k–m** and **8a–m**) in good yields, which were then hydrolyzed to obtain the test compounds (**9a–g**, **9k–m** and **10a–m**). 2-Fluoro-4-mercaptophenol (**3a**) was directly alkylated with *N*-(4-chlorophenylsulfonyl)-2-bromoethylamine (**11**) to yield the phenol derivative **12**. (Compound **11** was prepared by the treatment of a mixture of 4-chlophenylsulfonylchloride and 2-bromoethylamine hydrobromide in CH_2Cl_2 with one equivalent of Et_3N.) Bromination of **12** followed by alkylation of the phenol residue with methyl bromoacetate and hydrolysis of the ester group yielded the 2-bromo-6-fluorophenoxyacetic acid derivative **14** (scheme 2). Alkylation of 2,6-difluorophenol derivative **15**, prepared from **3b**, with corresponding haloalkyl esters followed by hydrolysis of the ester group yielded the other phenoxyalkanoic acid derivatives **16–18**. Reduction of chlorosulfonyl derivative **20** with $SnCl_2 \cdot 2H_2O$ in HCl/MeOH gave thiophenol derivative **21** in good yield, whereas reduction with Zn/H_2SO_4 or Sn/HCl/MeOH resulted in poor yield and product purity. The methyl ester **22** of **10c** was prepared by alkylation of **21** with halide **11**, and the sulfonamide moiety of **22** was alkylated with various halides followed by hydrolysis of the ester group to obtain **23a–d,f** (scheme 3). Compound **23e** was prepared by introduction of a 1-tosylimidazol-4-ylmethyl group into **22** using Mitsunobu's method [25], followed by deprotection with HOBt [26] and hydrolysis of the ester group. Carboxamide derivatives **24** and **25** and hydroxamic acid derivative **26** were prepared by treat-

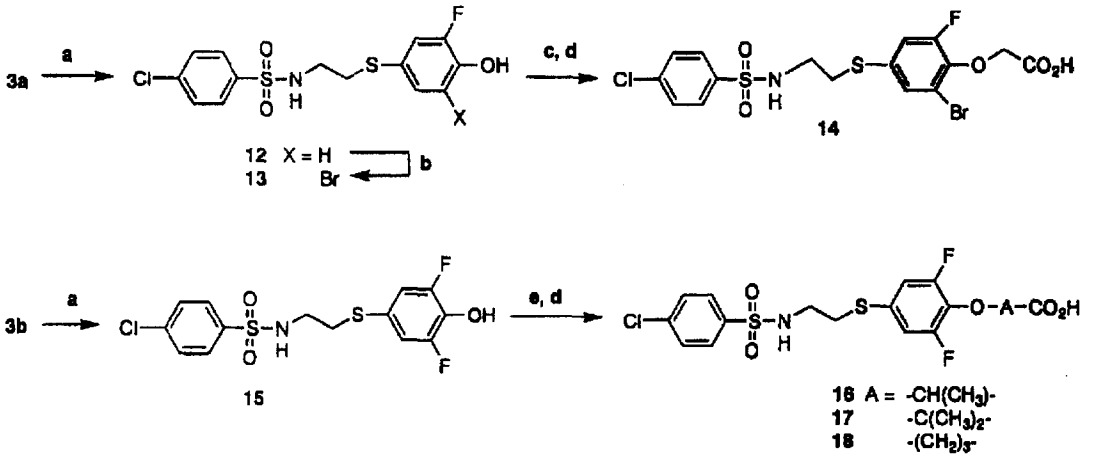


Scheme 1. (a) $ClSO_3H$; (b) Zn/25% H_2SO_4; (c) *N*-(2-bromoethyl)phthalimide/K_2CO_3/acetone/rt 16 h; (d) $BrCH_2CO_2Et/K_2CO_3$/DMF, rt 16 h; (e) $H_2NNH_2 \cdot H_2O/CH_2Cl_2$/MeOH rt 16 h, then 4 N HCl/EtOAc; (f) $R_1SO_2Cl/Et_3N/CH_2Cl_2$ rt; (g) NaOH aq, rt.

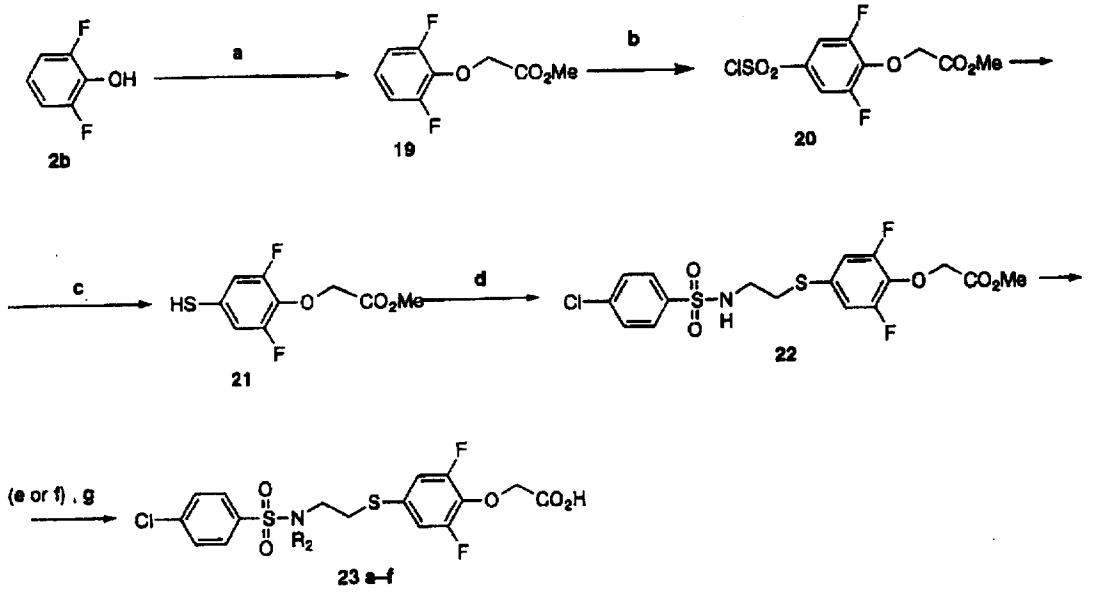
ment of **22** with corresponding amines in MeOH at room temperature (scheme 4). The 2-hydroxyethoxy derivative **27** was prepared by the reduction of the ethylester **8c** with $LiAlH_4$ in THF. Oxidation of **22** with *m*-chloroperbenzoic acid yielded an easily separable mixture of **28** and **29**, each of which was then hydrolyzed to obtain the sulfoxide derivative **30** and the sulfone derivative **31** (scheme 4).

Results and discussion

The inhibitory activity of the compounds against U-46619-induced (5 x 10^{-6} M) platelet aggregation of rabbit platelet-rich plasma (PRP) was measured using the method of Born [27], and the IC_{50} values are shown in table I–III. Sulotroban, daltroban and S-145 were also tested as reference compounds.

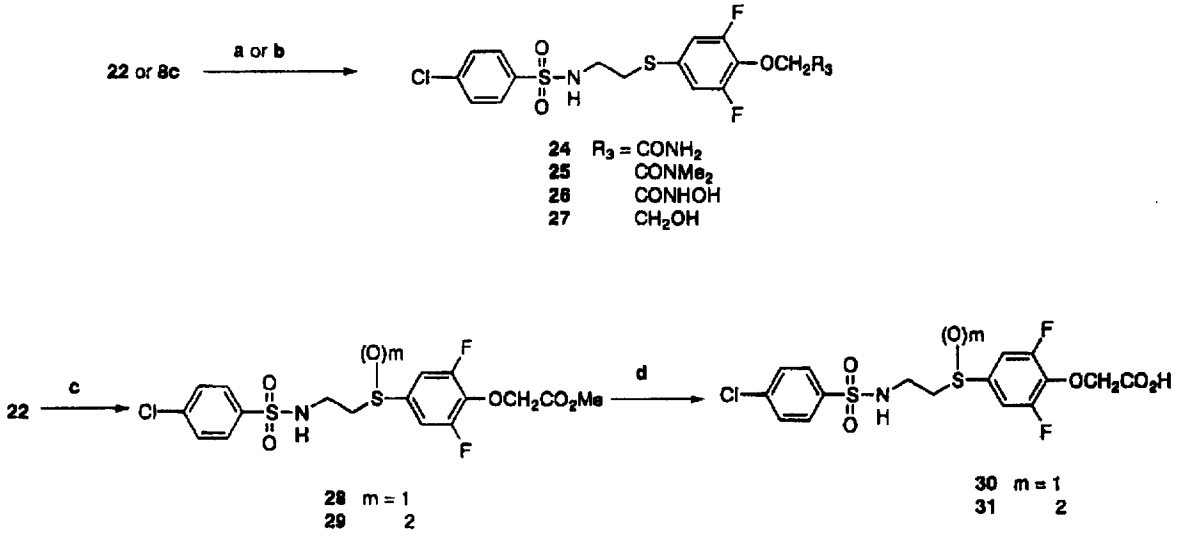


Scheme 2. (a) *N*-(2-Bromoethyl)-4-chlorophenylsulfonamide **11**/K_2CO_3/DMF rt; (b) Br_2/MeOH rt; (c) $BrCH_2CO_2Me/K_2CO_3$/acetone rt; (d) NaOH aq; (e) Hal-A-CO_2Me/K_2CO_3/DMF rt.



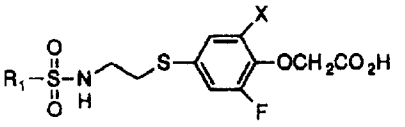
Scheme 3. (a) $BrCH_2CO_2Me/K_2CO_3$/acetone; (b) $ClSO_3H/CH_2Cl_2$ reflux; (c) $SnCl_2{\cdot}2H_2O$/HCl/MeOH reflux; (d) **11**/K_2CO_3/acetone; (e) R_2-Hal/K_2CO_3/DMF rt; (f) 1-tosyl-3-(hydroxymethyl)imidazole/$EtO_2CN{=}NCO_2Et/Ph_3P$/THF then HOBt/MeOH/THF; (g) NaOH aq rt.

The introduction of a fluorine atom into the phenoxy moiety of compound **1** (IC_{50} 6.10 x 10^{-6} M) increased activity, and the corresponding 2-fluorophenoxy derivative **9c** was 1.5 times more potent (IC_{50} 3.84 x 10^{-6} M) than **1**. Among the 2-fluorophenoxy acetic acid derivatives **9a–g** and **9k–m**, those compounds with a substituent on the 4-position of the phenylsulfonyl group such as a halogen atom **9b–d**, a methyl group **9e**, a methoxy group **9f** or a nitro group **9g** showed over four times higher activity than



Scheme 4. (a) NH_3, Me_2NH or NH_2OH/MeOH rt; (b) $LiAlH_4$/THF, rt; (c) *m*-CPBA/CH_2Cl_2, rt; (d) NaOH aq.

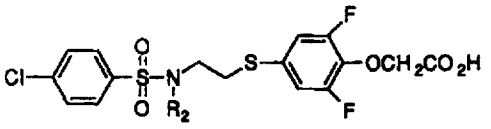
Table I. Structures and pharmacological activities *in vitro*.



Compd	R_1	X	mp, °C	formula[a]	Inhibition of platelet aggregation IC_{50}[b] μM
9a	Ph	H	122–126	$C_{16}H_{16}NO_5FS_2$	71.28±45.11
9b	4-F-Ph	H	126–128	$C_{16}H_{15}NO_5F_2S_2$	5.19±1.66
9c	4-Cl-Ph	H	111–113.5	$C_{16}H_{15}NO_5ClFS_2$	3.84±1.28
9d	4-Br-Ph	H	118–120.5	$C_{16}H_{15}NO_5BrFS_2$	2.44±0.69
9e	4-Me-Ph	H	120.5–122.5	$C_{17}H_{18}NO_5FS_2 \cdot 1/2H_2O$	9.13±2.26
9f	4-MeO-Ph	H	144–146	$C_{17}H_{18}NO_6FS_2$	8.30±1.02
9g	4-NO_2-Ph	H	140–142	$C_{16}H_{15}N_2O_7FS_2$	9.75±2.14
9k	CH_3	H	89–91.5	$C_{11}H_{14}NO_5FS_2 \cdot 1/2H_2O$	>100[c]
9l	$CH_3(CH_2)_7$	H	107.5–109.5	$C_{18}H_{28}NO_5FS_2$	>100[c]
9m	$CH_3(CH_2)_{15}$	H	120.5–123.5	$C_{26}H_{44}NO_5FS_2$	>100[c]
10a	Ph	F	91–93	$C_{16}H_{15}NO_5F_2S_2$	1.97±0.43
10b	4-F-Ph	F	85–88	$C_{16}H_{14}NO_5F_3S_2$	0.62±0.06
10c	4-Cl-Ph	F	110–111	$C_{16}H_{14}NO_5ClF_2S_2$	0.34±0.07
10d	4-Br-Ph	F	113.5–116.5	$C_{16}H_{14}NO_5BrF_2S_2$	0.33±0.02
10e	4-Me-Ph	F	93–95	$C_{17}H_{17}NO_5F_2S_2$	0.68±0.37
10f	4-MeO-Ph	F	76–78	$C_{17}H_{17}NO_6F_2S_2$	0.73±0.04
10g	4-NO_2-Ph	F	115.5–119	$C_{16}H_{14}N_2O_7F_2S_2$	0.79±0.06
10h	3-Cl-Ph	F	88–89	$C_{16}H_{14}NO_5ClF_2S_2$	11.60±1.61
10i	3,4-diCl-Ph	F	105–106	$C_{16}H_{13}NO_5Cl_2F_2S_2$	2.17±0.73
10j	4-Cl-3-NO_2-Ph	F	113–115	$C_{16}H_{13}N_2O_7ClF_2S_2$	36.85±1.74
10k	CH_3	F	118–121.5	$C_{11}H_{13}NO_5F_2S_2$	>100[c]
10l	$CH_3(CH_2)_7$	F	98–101.5	$C_{18}H_{27}NO_5F_2S_2 \cdot 1/2H_2O$	>100[c]
10m	$CH_3(CH_2)_{15}$	F	119–121	$C_{26}H_{43}NO_5F_2S_2$	>100[c]
14	4-Cl-Ph	Br	102.5–103.5	$C_{16}H_{14}NO_5BrClFS_2$	0.79±0.37
1	–	–			6.10±3.72
sulotroban					13.51±3.12
daltroban					0.36 ± 0.05
S-145					0.18±0.03

[a]C, H, N, Br, Cl, F, S analyses were within ±0.4% of the calculated values. All the compounds had ^1H NMR, IR and MS data consistent with their structure. [b]Concentration needed to inhibit U-46619-induced (5 μM) platelet aggregation of rabbit PRP by 50%. IC_{50} values represent means ± SEM and were calculated by regression analysis from the four dose groups of four different predictions. [c]$n = 2$.

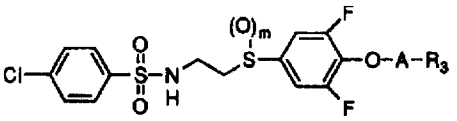
Table II. Structures and pharmacological activities *in vitro*.



Compd	R_2	mp,°C	formula[a]	Inhibition of platelet aggregation IC_{50}[b] μM
23a	CH_3	81.5–83	$C_{17}H_{16}NO_5ClF_2S_2$	70.92±13.46
23b	$CH_3(CH_2)_4$	93–94	$C_{22}H_{26}NO_5ClF_2S_2$	>100[c]
23c	$PhCH_2$	99–100	$C_{23}H_{20}NO_5ClF_2S_2$	>100[c]
23d	2-Py-CH_2 [d]	119.5–121	$C_{22}H_{19}N_2O_5ClF_2S_2$	>100[c]
23e	4-Im-CH_2 [e]	99–102	$C_{20}H_{18}N_3O_5ClF_2S_2$	>100[c]
23f	CH_2CO_2H	192–192.5	$C_{18}H_{16}NO_7ClF_2S_2$	>100[c]

[a]C, H, N, Br, Cl, F, S analyses were within ±0.4% of the calculated values. All compounds had ^1H NMR, IR, and MS data consistent with their structure. [b]Concentration needed to inhibit U-46619-induced (5 μM) platelet aggregation of rabbit PRP by 50%. IC_{50} values represent means ± SEM and were calculated by regression analysis from the four dose groups of four different predictions. [c]n = 2. [d]Pyridin-2-ylmethyl. [e]Imidazol-4-ylmethyl.

Table III. Structures and pharmacological activities *in vitro*.



Compd	m	A	R_3	mp, °C	formula[a]	Inhibition of platelet aggregation IC_{50}[b] μM
15	0	H	–	77.5–79	$C_{14}H_{12}NO_3ClF_2S_2$	22.03±3.33
16	0	$CH(CH_3)$	CO_2Na	102–108	$C_{17}H_{15}NO_5ClF_2NaS_2 \cdot H_2O$	5.54±0.46
17	0	$C(CH_3)_2$	CO_2Na	136–142	$C_{18}H_{17}NO_5ClF_2NaS_2$	>100 [c]
18	0	$(CH_2)_3$	CO_2H	71–73	$C_{18}H_{18}NO_5ClF_2S_2$	19.57±0.96
24	0	CH_2	$CONH_2$	158.5–160	$C_{16}H_{15}N_2O_4ClF_2S_2$	28.65±8.52
25	0	CH_2	$CONMe_2$	136.5–137.5	$C_{18}H_{19}N_2O_4ClF_2S_2$	57.37±2.41
26	0	CH_2	CONHOH	132–133.5	$C_{16}H_{15}N_2O_5ClF_2S_2$	1.73±0.22
27	0	CH_2	CH_2OH	43.5–45	$C_{16}H_{16}NO_4ClF_2S_2$	>100 [c]
30	1	CH_2	CO_2H	154.5–155.5	$C_{16}H_{14}NO_6ClF_2S_2$	18.94±1.16
31	2	CH_2	CO_2H	163–163.5	$C_{16}H_{14}NO_7ClF_2S_2$	1.84±0.17

[a]C, H, N, Cl, F, S analyses were within ±0.4% of the calculated values. All compounds had ^1H NMR, IR and MS data consistent with their structure. [b]Concentration needed to inhibit U-46619-induced (5 μM) platelet aggregation of rabbit PRP by 50%. IC_{50} values represent means ± SEM and were calculated by regression analysis from the four dose groups of four different predictions. [c]n = 2.

the non-substituted derivative **9a**, as reported in a previous study [24]. In contrast to the findings for S-145 derivatives reported by Narisada *et al* [28], alkylsulfonyl derivatives (**9k–m**) were much less active than arylsulfonyl derivatives, and were almost inactive at a concentration of 1×10^{-4} M (table I). This difference in activity was probably due to the inability of the intramolecular hydrogen bonding of the alkylsulfonamido moiety of these compounds. Moreover such an interaction is not essential for rigid S-145 derivatives. Since introduction of a fluorine atom at the 2-position of the π-acid moiety gave good results, a second fluorine atom was introduced to the phenoxy acetic acid moiety as a next step.

The introduction of the second fluorine atom at the 6-position of the phenoxyacetic acid moiety of **9a–g** improved the activities by over five-fold. The resulting 2,6-difluorophenoxyacetic acid derivatives **10b–g** had sub-micromolar IC_{50} values. Introduction of a substituent at the 4-position of the phenylsulfonyl moiety (**10b–g**) of **10a** also increased activity as was also the case for **9b–g**. Conformational searches of the potassium salt of **10c** and the corresponding non-fluorinated derivative **1** in aqueous solution was performed by ^1H-^1H nuclear Overhauser effect spectroscopy experiments and molecular dynamics calculations. The intramolecular hydrogen bond of the sulotroban-related derivative **1** was not observed in aqueous solution whereas that of sulotroban was observed in apolar solvents [22]. In contrast to the result of compound **1**, intramolecular hydrogen bond of difluorinated derivative **10c** was observed even in aqueous solution (Hirono *et al*, manuscript in preparation).

These findings suggest that introduction of two fluorine atoms into the phenoxy ring of **1** increases the intramolecular hydrogen-bonding interaction, and stabilized the hairpin-like conformations to enhance the TXA_2 receptor antagonizing abilities.

Further structural modification of 4-chlorophenylsulfonyl derivative **10c**, which is one of the most potent compounds (IC_{50} 3.4×10^{-7} M) of the 2,6-difluorophenoxyacetic acid derivatives, was attempted as the next step of the investigation.

Modification of the arylsulfonamide moiety was attempted first. The 3-chlorophenylsulfonyl derivative **10h** (IC_{50} 1.16×10^{-5} M) was about 40-fold less active than **10c**, and even less active than the non-substituted derivative **10a**. Introduction of a second substituent, such as a chlorine atom (**10i**) or nitro group (**10j**) at the 3-position of **10c**, also lowered activity. These unexpected findings suggested that the arylsulfonyl moiety is located in the sterically limited region of the TXA_2 receptor protein. Introduction of a bromine atom at the 6-position of the phenoxyacetic acid moiety of **9c** also increased the activity (**14**: IC_{50} 7.9×10^{-7} M), but was less effective than introduction of a second fluorine atom. In addition, the alkylsulfonyl derivatives **10k–m** were almost inactive, as was the case for **9k–m**. *N*-Methyl (**23a**) and *N*-hexyl (**23b**) sulfonamide derivatives exhibited less than 1% of the activity of the non-substituted derivative **10c**, and *N*-benzyl (**23c**), *N*-carboxymethyl (**23f**), *N*-(4-imidazolylmethyl) (**23e**) and *N*-(3-pyridylmethyl) (**23d**) derivatives were almost inactive at the concentration of 1×10^{-4} M. Introduction of a substituent on the nitrogen atom of the sulfonamide moiety probably decreased the intramolecular interaction of these compounds, which was required to maintain the preferable hairpin-like conformation (table II) [22].

Structural modification of the acidic moiety of compound **10c** was then attempted. The hydroxamic acid derivative **26** showed relatively high activity (IC_{50} 1.73×10^{-6} M), but non-acidic derivatives such as carboxamides **24** and **25** and the 2-hydroxyethoxy derivative **27** were much less active, and introduction of a methyl (**16**) or two methyl groups (**17**) into the α-position of the phenoxy acetic acid moiety of **10c** and extension of the methylene group (**18**) reduced activity (table III). These findings indicate that the acidic moiety on the other end of the molecule from arylsulfonyl moiety is also required for activity, and suggested that the acidic moiety interacts with a basic amino-acid residue in another pocket region of the TXA_2 receptor protein. In contrast to findings for the two ends of the molecule, there appeared to be a better tolerance for change near the 4-position of the phenoxyacetic acid moiety (table III), since the relatively bulky sulfone derivative **31** (IC_{50} 1.84×10^{-6} M) was 10-fold more potent than the less bulky sulfoxide derivative **30** (IC_{50} 1.89×10^{-5} M).

Study of these structural modifications showed that **10c** was the optimal compound *in vitro*. The IC_{50} value of **10c** against U-46619-induced rabbit platelet aggregation was 3.4×10^{-7} M; **10c** was thus over 40-fold more potent than the representative non-prostanoid TXA_2 antagonist sulotroban (IC_{50} 1.35×10^{-5} M) and comparable in potency to prostanoid TXA_2 antagonists such as S-145 (IC_{50} 1.8×10^{-7} M). (Inhibitory tests of ON-579 on rabbit platelet aggregation induced by other aggregents were also performed: aggregant (concentration) IC_{50} value: collagen (5 μg/ml) 5.3×10^{-6} M; arachidonic acid (0.5 mM) 1.4×10^{-6} M; ADP (5 μM) > 1×10^{-4} M; PAF (0.03 μM) > 1×10^{-4} M.) Compound **10c** also showed potent inhibitory activity on human platelet aggregation induced by U-46619 (1 μM) and collagen (2 μg/ml) with IC_{50} values of 6.7×10^{-7} M and 2.37×10^{-6} M. Compound **10c** inhibited U-46619-induced rat aorta constriction with a pA_2 [29] of 8.5, and the slope of the Schild's plot was 1.1 (fig 1). Compound **10c** also inhibited specific binding of [^3H]SQ29,548 [30] to gel-filtered guinea-pig platelets with an IC_{50} of 6.61×10^{-8} M (table IV),

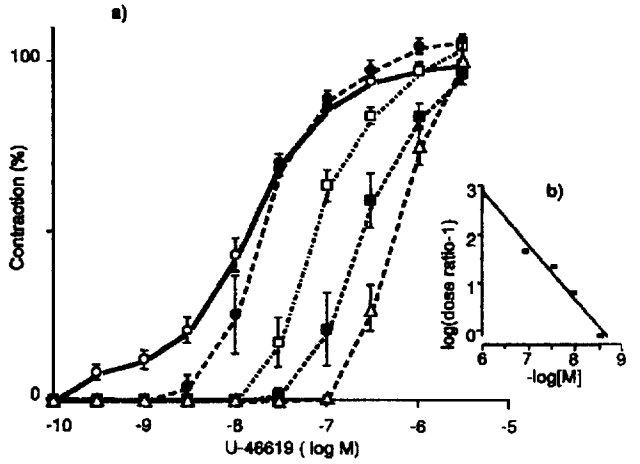


Fig 1. a) Comparative concentration–response curves of U-46619-induced contractions of isolated rat aorta in the presence of varying concentrations of **10c** (ON-579); ○: 0 nM, ●: 3 nM, □: 12 nM, ■: 30 nM, Δ: 120 nM. Values represent the mean values ± SE (n = 3–16). b) Schild plot of the antagonism by **10c**. The calculated pA_2 value was 8.6 with a slope value of –1.1 and r = 0.97. The slope value was not significantly different from –1.0.

but did not affect the binding of a variety of other chemical mediators to their receptors (data not shown). These findings indicate that **10c** is a potent and selective competitive TXA_2 receptor antagonist.

The platelet aggregation inhibitory ability of **9c**, **10a**, **10c** and **10e** were further evaluated using a U-46619-induced acute thrombocytopenia model [31–33] in mice *in vivo* (table V). Difluorophenoxy derivatives **10a**, **10c** and **10e** inhibited platelet aggregation *in vivo* at sub-milligram/kilogram dosages. Of these compounds **10c** exhibited the strongest activity with an ID_{50} of 0.14 mg/kg, and was 10-fold more potent than sulotroban (1.43 mg/kg). The durations of activity of **10c** and sulotroban were determined upon oral administration of about double the dose of the ID_{50} values. Oral administration of 0.3 mg/kg **10c** and 3.0 mg/kg of sulotroban almost completely inhibited U-46619-induced platelet aggregation *in vivo* during the 1 h period following administration. However, significant inhibition by sulotroban had disappeared by 2 h after administration, whereas **10c** continued to almost completely inhibit aggregation at that time. In addition, a higher dosage (3.0 mg/kg) of **10c** completely inhibited platelet aggregation for over 4 h (fig 2).

Table IV. Displacement of the specific binding of [^{3}H]-SQ29548 to guinea-pig gel-filtered platelets.

Compound	*Guinea-pig platelet binding assay IC_{50} (M)*
ON-579 (**10c**)	$(6.61 \pm 0.23) \times 10^{-8}$ (n = 3)
Sulotroban	6.47×10^{-6} (n = 1)

Aliquots of guinea-pig platelet suspension were incubated with 5 nM [^{3}H]SQ29548 plus various concentrations of test compounds for 60 min at 30°C. Specific binding was defined as the difference between the binding in the presence and absence of 10^{-5} M U-46619 and the IC_{50} values representing means ± SEM were calculated.

Table V. Inhibition of U-46619-induced acute thrombocytopenia in mice.

Compound	*ID_{50}[a] (mg/kg)*
9c	3.19 (0.35–18.62)
10a	0.40 (0.20–0.82)
ON-579 (**10c**)	0.15 (0.058–0.40)
10e	6.69 (1.91–23.55)
Sulotroban	1.45 (0.41–5.09)

Each compound was administered orally 60 min prior to injection of U-46619 (25 μg/kg). At 30 s after injection of U-46619, 20 μl of the blood was collected and the platelet counts were immediately measured. [a]ID_{50} value with 95% confidence limits (n = 7–11).

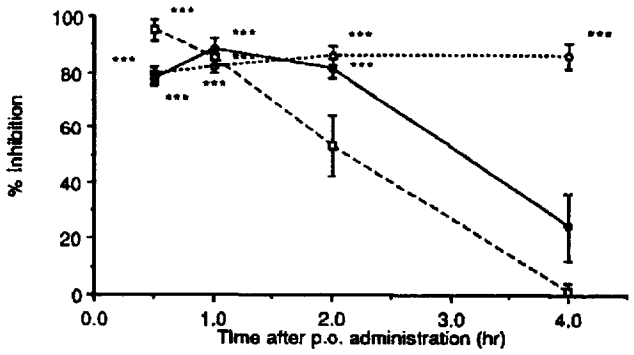


Fig 2. Inhibitory effects of **10c** (ON-579) and sulotroban against U-46619-induced acute thrombocytopenia in mice. Each compound was administered orally 0.5, 1, 2 or 4 h prior injection of U-46619 (25 μg/kg). At 30 s after injection of U-46619, 20 μl blood was collected and the platelet counts were immediately measured. The inhibition value of the platelet count decrease was calculated by comparison with that of vehicle-treated group. ***P < 0.001 vs control (0 h); n = 10. ○: ON-579 3 mg/kg; ●: ON-579 0.3 mg/kg; □: BM-13177 3 mg/kg.

In conclusion, introduction of two fluorine atoms to the phenoxy acetic acid moiety of sulotroban-related compound **1** markedly increased TXA_2 antagonist activity, and structure–activity relationship studies of these compounds demonstrated that **10c** is a potent and selective systemic TXA_2 receptor antagonist. (Researchers of Tanabe Seiyaku have also reported the increased *in vivo* potency of mono and difluoro derivatives of sulotroban in a patent [34] but the potency against TXA_2 receptor antagonizing ability of these compounds was unclear due to the lack of *in vitro* data.) Due to its attractive pharmacological and toxicological (data not shown) profiles, **10c**, which was named ON-579, has been selected for further evaluation.

Experimental protocols

Chemistry

Melting points were determined with a Mettler FP-60 melting point apparatus and are uncorrected. Infrared (IR) spectra were obtained with 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) using tetramethylsilane as an internal standard. Mass spectra (MS) were obtained with a Jeol JMS-SX102 spectrometer. Elemental analyses were within ±0.4% of the theoretical values. Organic solutions prepared 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, Kieselgel 60F-254).

N-[2-(3,5-Difluoro-4-hydroxyphenylthio)ethyl]phthalimide 4b
To a solution of 2,6-difluorophenol **2b** (25 g, 192 mmol) in CH_2Cl_2 (133 ml) was added dropwise chlorosulfonic acid (77 ml, 1.15 mol). The reaction mixture was heated under reflux for 2 h and then poured into crushed ice. The organic layer was separated and filtered through celite. The filtrate was dried and evaporated *in vacuo* to give 22.3 g (50.7%) of crude 4-chlorosulfonyl-2,6-difluorophenol as an orange oil; IR (neat) 3431 br, 1609, 1520, 1446, 1382, 1321, 1173, 1024 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.29 (s, 1 H), 7.68 (d, J = 8 Hz, 2H); MS (El) *m/z* 228 (M^+).

A mixture of crude sulfonylchloride obtained above, zinc dust (32 g, 0.48 mol) and 25% H_2SO_4 (341 ml) was heated under reflux for 4 h. The reaction mixture was poured into water and extracted with toluene. The organic layer was washed with brine, dried and evaporated *in vacuo* to give 11 g (69.5%) of 2,6-difluoro-4-mercaptophenol (**3b**) as a colorless oil that was used immediately without further purification.

A mixture of **3b** (11 g, 43.5 mmol), K_2CO_3 (11.7 g, 85.7 mmol), *N*-(2-bromoethyl)phthalimide (4.5 g, 27.8 mmol) and DMF (41 ml) was stirred for 16 h at room temperature under argon atmosphere. The reaction mixture was poured into 3% HCl and extracted with EtOAc. The organic layer was washed twice with brine, dried and evaporated *in vacuo*. The residue was triturated with CH_2Cl_2 to give 6.3 g (67%) of **4b** as a yellow powder: mp 153–154°C; IR (KBr) 3407, 1770, 1697, 1511, 1401, 1011, 719 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 3.21 (t, J = 7 Hz, 2H), 3.77 (t, J = 7 Hz, 2H), 7.08 (d, J = 8 Hz, 2H), 7.83 (s, 4H), 10.18 (s, 1H); MS (El) *m/z* 335 (M^+).

Ethyl 2,6-difluoro-4-[2-(phthalimid-2-yl)ethylthio]phenoxyacetate 5b
To a mixture of **4b** (15.0 g, 44.7 mmol), K_2CO_3 (9.1 g, 66 mmol) and DMF (80 ml) was added dropwise ethyl 2-bromoacetate (7.5 g, 45 mmol) at room temperature. The reaction mixture was stirred for 16 h at room temperature and then poured into 3% HCl and extracted with EtOAc. The organic layer was washed twice with brine, dried and evaporated *in vacuo*. The residue was triturated with CH_2Cl_2/hexane to give 17.0 g (90.2%) of **5b** as colorless prisms: mp 90.5–92°C; IR (KBr) 1764, 1737, 1715, 1504, 1400, 1291, 1031, 718 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.29 (t, J = 7 Hz, 3H), 3.19 (t, J = 7 Hz, 2H), 3.93 (t, J = 7 Hz, 2H), 4.26 (q, J = 7 Hz, 2H), 4.68 (s, 2H), 6.99 (d, J = 8 Hz, 2H), 7.72 (m, 2H), 7.82 (m, 2H); MS (El) *m/z*: 421 (M^+).

Ethyl 4-(2-aminoethylthio)-2,6-difluorophenoxyacetate hydrochloride 6b
To a solution of **5b** (6.0 g, 14.2 mmol) in EtOH/CH_2Cl_2 (100 ml/100 ml) was added hydrazine monohydrate (7.1 ml, 142 mmol). The reaction mixture was stirred for 16 h at room temperature and then resulting precipitate was removed by filtration. The filtrate was washed with water, and the aqueous layer was extracted three times with CH_2Cl_2. The organic layers were combined and washed with brine, dried and filtered. To the filtrate was added 4 N HCl/ EtOAc (7 ml, 28 mmol) and the mixture was evaporated *in vacuo* to give 2.9 g (62.1%) of **6b** as a colorless hygroscopic powder: mp 94–98°C; IR (KBr) 3436 br, 2990 br, 1760, 1505, 1313, 1205 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.20 (t, J = 7 Hz, 3H), 2.95 (br, 2H), 3.23 (t, J = 7 Hz, 2H), 4.15 (q, J = 7 Hz, 2H), 4.83 (s, 2H), 7.26 (d, J = 8Hz, 2H), 8.28 (br, 3H); MS (El) *m/z* 291 (M^+).

Ethyl 4-[2-(4-chlorophenylsulfonylamino)ethylthio]-2,6-difluorophenoxyacetate 8c
To a mixture of **6b** (1.0 g, 3.1 mmol), Et_3N (0.95 ml, 6.8 mmol) and CH_2Cl_2 (80 ml) was added a solution of 4-chlorophenylsulfonyl chloride (0.7 g, 3.3 mmol) in CH_2Cl_2 (3 ml) and the mixture was stirred for 30 min at room temperature. The reaction mixture was washed with 3% HCl, water, 5% $NaHCO_3$ and brine successively, dried and evaporated *in vacuo*. The residue was purified by flash chromatography using 2:3 EtOAc/ hexane to give 1.3 g (90.6%) of **8c** as a colorless powder: mp 70–71°C; IR (KBr) 3289, 1757, 1724, 1504, 1423, 1202, 1163 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.29 (t, J = 7 Hz, 3H), 2.98 (t, J = 6 Hz, 2H), 3.14 (m, 2H), 4.25 (q, J = 7 Hz, 2H), 4.73 (s, 2H), 4.88 (t, J = 5 Hz, 1H), 6.82 (d, J = 8 Hz, 2H), 7.49 (d, J = 8 Hz, 2H), 7.78 (d, J = 8 Hz, 2H); MS (El) *m/z* 465 (M^+).

4-[2-(4-Chlorophenylsulfonylamino)ethylthio]-2,6-difluorophenoxyacetic acid 10c, ON-579
To a solution of **8c** (1.4 g, 3.0 mmol) in EtOH (12 ml) was added 10% NaOH (3.0 ml, 7.5 mmol), and stirred for 1 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 triturated with MeOH aq to give 1.18 g (89.8%) of **10c** as colorless needles: mp 110–111°C; IR (KBr) 3301, 1722, 1502, 1319, 1223, 1152 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.99 (t, J = 7 Hz, 2H), 3.13 (m, 2H), 4.80 (s, 2H), 5.08 (t, J = 5 Hz, 1H), 5.75 (br, 1H), 6.83 (d, J = 8 Hz, 2H), 7.47 (d, J = 8 Hz, 2H), 7.76 (d, J = 8 Hz, 2H); MS (El) *m/z* 437 (M^+). Anal $C_{16}H_{14}NO_5ClF_2S_2$ (C, H, N, Cl, F, S).

4-[2-(4-Chlorophenylsulfonylamino)ethylthio]-2-fluorophenol 12
To a mixture of **3a** (6.4 g, 44.4 mmol), K_2CO_3 (11.7 g, 39 mmol) in acetone was added *N*-(2-bromoethyl)-4-chlorophenylsulfon-

amide (**11**) (11.7 g, 39 mmol), and the mixture was stirred for 3 h under an argon atmosphere. The resulting precipitate was removed by filtration and the filtrate was evaporated *in vacuo*. The residue was purified by flash chromatography using 20:1 CH_2Cl_2/EtOAc to give 11.3 g (80%) of **12** as colorless prisms: mp 83–84°C; IR (KBr) 3403, 3267, 1504, 1167 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.89 (t, J = 7 Hz, 2H), 3.08 (m, 2H), 4.92 (t, J = 5 Hz, 1 H), 5.22 (d, J = 3 Hz, 1H), 6.90 (t, J = 8 Hz, 1H), 6.95 (m, 1H), 7.02 (dd, J = 12, 2 Hz, 1H), 7.48 (d, J = 8 Hz, 2H), 7.75 (d, J = 8 Hz, 2H); MS (El) m/z 361 (M^+).

2-Bromo-4-[2-(4-chlorophenylsulfonylamino)ethylthio]-6-fluorophenol 13

To a solution of **12** (9.5 g, 26.3 mmol) in MeOH (100 ml) was added a solution of bromine (5.0 g, 31.6 mmol) in MeOH (10 ml). The reaction mixture was stirred for 50 min at room temperature and then quenched with 5% $Na_2S_2O_3$ solution and extracted with EtOAc. The organic layer was washed with 5% $NaHCO_3$, brine successively, dried and evaporated *in vacuo*. The residue was purified by flash chromatography using 1:2 EtOAc/hexane and triturated with ether/hexane to give 10.2 g (88%) of **13** as a colorless powder: mp 108–109.5°C; IR (KBr) 3329, 1572, 1476, 1305, 1222, 1159, 1089 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.93 (t, J = 7 Hz, 2H), 3.12 (m, 2H), 4.90 (t, J = 5 Hz, 1H), 5.56 (d, J = 2 Hz, 1H), 7.00 (dd, J = 12, 2 Hz, 1H), 7.22 (t, J = 2 Hz, 1H), 7.49 (d, J = 8 Hz, 2H), 7.78 (d, J = 8 Hz, 2H); MS (El) m/z 439 (M^+).

2-Bromo-4-[2-(4-chlorophenylsulfonylamino)ethylthio]-6-fluorophenoxyacetic acid 14

A mixture of **13** (4.4 g, 10 mmol), methyl bromoacetate (1.55 ml, 16 mmol), K_2CO_3 (3.9 g, 28.3 mmol) and acetone (40 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 50:1 CH_2Cl_2/ EtOAc to give 5.1 g (99%) of methyl 2-bromo-4-[2-(4-chlorophenylsulfonylamino)ethylthio]-6-fluorophenoxyacetate as colorless prisms: mp 78.5–79.5°C; IR (KBr) 3436, 3267, 1746, 1474, 1158 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.00 (t, J = 6 Hz, 2H), 3.12 (m, 2H), 3.80 (s, 3H), 4.73 (s, 2H), 4.95 (t, J = 5 Hz, 1H), 6.98 (dd, J = 12, 2 Hz, 1H), 7.25 (t, J = 2 Hz, 1H), 7.50 (d, J = 8 Hz, 2H), 7.78 (d, J = 8 Hz, 2H); MS (El) m/z 511 (M^+).

This methyl ester (1.2 g, 2.3 mmol) was hydrolyzed by the method described above to give 1.03 g (88%) of **14** as colorless prisms: mp 102–103.5°C; IR (KBr) 3436, 3290, 1720, 1483, 1157 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.90–3.10 (m, 4H), 4.70 (d, J = 2 Hz, 2H), 7.28 (dd, J = 12, 2 Hz, 1H), 7.36 (t, J = 2 Hz, 1H), 7.63 (d, J = 8 Hz, 2H), 7.78 (d, J = 8 Hz, 2H), 8.02 (t, J = 5 Hz, 1H), 13.05 (brs, 1H); MS (El) m/z 497 (M^+). Anal $C_{16}H_{14}NBrClO_5S_2$ (C, H, N, Br, Cl, F, S).

Methyl 4-[2-(4-chlorophenylsulfonylamino)ethylthio]-2,6-difluorophenoxyacetate 22

A mixture of **2b** (25 g, 0.19 mol), K_2CO_3 (39.5 g, 0.3 mol), methyl bromoacetate (17.8 ml, 0.19 mol) and acetone (200 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 distilled under reduced pressure to give 37.3 g (95.8%) of methyl 2,6-difluorophenoxyacetate **19** as a colorless oil: bp 72–75°C (0.3 mmHg); IR (neat) 2958, 1767, 1499 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.80 (s, 3H), 4.77 (s, 2H), 6.80–7.10 (m, 3H); MS (El) m/z 202 (M^+).

To a solution of **19** (35 g, 173 mmol) in CH_2Cl_2 (105 ml) was added dropwise chlorosulfonic acid (69 ml, 1.04 mol) at 35°C. The reaction mixture was heated under reflux for 1.5 h and then poured onto crushed ice. The organic layer was separated and washed three times with water, dried and evaporated *in vacuo* to give 43.8 g (84%) of methyl 2,6-difluoro-4-chlorosulfonylphenoxyacetate **20** as a faint yellow oil: IR (neat) 1762, 1505, 1439, 1385, 1216, 1175, 621, 577 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.81 (s, 3H), 4.97 (s, 2H), 7.63 (d, J = 8 Hz, 2H); MS (El) m/z 300 (M^+).

A mixture of **20** (37.6 g, 125 mmol), $SnCl_2{\cdot}2H_2O$ (98.8 g, 440 mmol), concentrated HCl (73 ml, 875 mmol) and MeOH (500 ml) was heated under reflux for 90 min. The reaction mixture was poured into crushed ice and extracted with toluene. The organic layer was washed 3 times with 12% HCl, brine, dried and evaporated *in vacuo* to give 25.48 g (87%) of methyl 2,6-difluoro-4-mercaptophenoxyacetate **21** as a yellow oil: ^1H NMR ($CDCl_3$) δ 3.50 (s, 1H), 3.80 (s, 3H), 4.70 (s, 2H), 6.85 (d, J = 8 Hz, 2H); MS (El) m/z 234 (M^+).

To a mixture of **21** (8.0 g, 34.1 mmol), K_2CO_3 (5.6 g, 40.6 mmol), and acetone (20 ml) was added dropwise a solution of **11** (10.5 g, 35 mmol) in acetone (30 ml). The reaction mixture was stirred for 16 h at room temperature, and then poured into 3% HCl and extracted with EtOAc. The organic layer was washed with water and brine successively, dried and evaporated *in vacuo*. The residue was recrystallized from 50% MeOH to give 12.7 g (82%) of **22** as colorless needles: mp 79.5–80.5°C; IR (KBr) 3264, 1747, 1510, 1217, 1157 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.99 (t, J = 7 Hz, 2H), 3.14 (m, 2H), 3.80 (s, 3H), 4.75 (s, 2H), 4.89 (t, J = 5 Hz, 1 H), 6.83 (d, J = 8 Hz, 2H), 7.49 (d, J = 8 Hz, 2H), 7.78 (d, J = 8 Hz, 2H); MS (El) m/z 451 (M^+).

4-[2-[N-(4-Chlorophenylsulfonyl)-N-methylamino]ethylthio]-2,6-difluorophenoxyacetic acid 23a

To a mixture of **22** (2.2 g, 5 mmol), K_2CO_3 (2.75 g, 20 mmol) and DMF (30 ml) was added MeI (1.7 g, 12 mmol), and stirred for 30 min at room temperature. The reaction mixture was poured into 3% HCl and extracted with EtOAc. The organic layer was washed with water, 5% $NaHCO_3$ and brine successively, dried and evaporated *in vacuo* to give 2.41 g (100%) of methyl 4-[2-[*N*-(4-chlorophenylsulfonyl)-*N*-methyl]aminoethylthio]-2,6-difluorophenoxyacetate as a yellow oil: IR (neat) 1762, 1504, 1207, 1163, 1093 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.81 (s, 3H), 3.08 (m, 2H), 3.20 (m, 2H), 3.79 (s, 3H), 4.74 (s, 2H), 6.90 (d, J = 8 Hz, 2H), 7.50 (d, J = 8 Hz, 2H), 7.68 (d, J = 8 Hz, 2H); MS (El) m/z 465 (M^+).

This methylester was hydrolyzed by the method described above to give **23a** as a colorless powder: mp 81.5–83°C; IR (KBr) 3436, 1745, 1505, 1323, 1093 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.82 (s, 3H), 3.09 (m, 2H), 3.20 (m, 2H), 4.80 (s, 2H), 6.94 (d, J = 8 Hz, 2H), 7.50 (d, J = 8 Hz, 2H), 7.72 (d, J = 8 Hz, 2H); MS (El) m/z 451 (M^+). Anal $C_{17}H_{16}NO_5ClF_2S_2$ (C, H, N, Cl, F, S).

4-[2-[N-(4-Chlorophenylsulfonyl)-N-(imidazol-4-ylmethyl)amino]ethylthio]-2,6-difluorophenoxyacetic acid 23e

To a mixture of **22** (1.36 g, 3.0 mmol), Ph_3P (0.79 g, 3.0 mmol) and 4-(hydroxymethyl)-1-(4-methylphenylsulfonyl)imidazole (0.76 g, 3.0 mmol) in THF (20 ml) was added dropwise diethyl azodicarboxylate (0.47 ml, 3.0 mmol) at 0°C under an argon atmosphere. The reaction mixture was stirred at room temperature for 16 h and then evaporated *in vacuo*. The residue was purified by flash chromatography using 1:1 hexane/EtOAc to give 1.81 g (87.9%) of methyl 4-[2-[*N*-(4-chlorophenylsulfonyl)-*N*-[1-(4-methylphenylsulfonyl)imidazol-4-ylmethyl]-

amino]ethylthio]-2,6-difluorophenoxyacetate as a colorless powder: mp 106.5–109°C; IR (KBr) 1768, 1698, 1506 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.46 (s, 3H), 3.00 (m, 2H), 3.34 (m, 2H), 3.80 (s, 3H), 4.30 (s, 2H), 4.75 (s, 2H), 6.84 (d, J = 8 Hz, 2H), 7.17 (s, 1 H), 7.25–7.42 (m, 4H), 7.60 (d, J = 8 Hz, 2H), 7.82 (d, J = 8 Hz, 2H), 7.83 (s, 1H); MS (EI) m/z 685 (M^+).

A mixture of the above product (1.03 g, 1.5 mmol), 1-hydroxybenzotriazole (HOBt) hydrate (0.46 g, 3.0 mmol) and MeOH/ THF (30 ml/ 5 ml) was stirred for 16 h at room temperature. The reaction mixture was poured into water and extracted with EtOAc. The organic layer was washed with 5% $NaHCO_3$, brine successively, dried and evaporated *in vacuo*. The residue was purified by flash chromatography using 1:1 EtOAc/hexane then 1:9 MeOH/$CHCl_3$ to give 0.57 g (86%) of methyl 4-[2-[*N*-(4-chlorophenylsulfonyl)-*N*-(imidazol-4-ylmethyl)amino]ethylthio]-2,6-difluorophenoxyacetate as a colorless oil: IR (neat) 3368, 3139, 1758, 1504 cm^{-1}; ^1H NMR (DMSO–d_6) δ 3.10–3.50 (m, 4H), 3.69 (s, 3H), 4.49 (s, 2H), 4.83 (s, 2H), 7.09 (d, J = 8 Hz, 2H), 7.50 (s, 1 H), 7.67 (d, J = 8 Hz, 2H), 7.82 (d, J = 8 Hz, 2H), 8.99 (s, 1H), 14.56 (brs, 1H); MS (FAB) m/z 532 (M+H).

This methylester was hydrolyzed by the method described above to give **23e** as a colorless powder: mp 99–102°C; IR (KBr) 3436, 2928, 1732, 1504 cm^{-1}; ^1H NMR (DMSO–d_6) δ 3.10 (m, 2H), 3.31 (m, 2H), 4.37 (s, 2H), 4.74 (s, 2H), 7.06 (s, 1 H), 7.08 (d, J = 8 Hz, 2H), 7.62 (d, J = 8 Hz, 2H), 7.77 (d, J = 8 Hz, 2H), 7.95 (s, 1 H); MS (FAB) m/z 518 (M+H). Anal $C_{20}H_{18}N_3O_5ClF_2S_2 \cdot 1/2\ H_2O$ (C, H, N, Cl, F, S).

4-[2-(4-Chlorophenylsulfonylamino)ethylthio]-2,6-difluorophenoxyacetamide 24

A mixture of **22** (1.0 g, 2.2 mmol) and 2 M NH_3/MeOH (50 ml, 100 mmol) was stirred for 16 h at room temperature. The resulting precipitate was collected to give 0.85 g (85%) of **24** as a colorless powder: mp 158.5–160°C; IR (KBr) 3476, 3118, 1694, 1504, 1335, 1161 cm^{-1}; ^1H NMR (DMSO–d_6) δ 2.9–3.1 (m, 4H), 4.49 (s, 2H), 7.10 (d, J = 8 Hz, 2H), 7.41 (br, 2H), 7.63 (d, J = 8 Hz, 2H), 7.78 (d, J = 8 Hz, 2H), 8.01 (br, 1H); MS (EI) m/z 436 (M^+). Anal $C_{16}H_{15}N_2O_4ClF_2S_2$ (C, H, N, Cl, F, S).

2-[4-[2-(4-Chlorophenylsulfonylamino)ethylthio]-2,6-difluorophenoxy]ethanol 27

To a solution of **8c** (466 mg, 1.0 mmol) in THF (30 ml) was added $LiAlH_4$ (88 mg, 2.3 mmol) at 0°C and the mixture was stirred for 1 h at room temperature. To the reaction mixture were added water (180 μl, 10 mmol), EtOAc (20 ml), Na_2SO_4 (1.0 g) successively, and the resulting precipitate was removed by filtration. The filtrate was evaporated *in vacuo* and the residue was purified by flash chromatography using 1:1 hexane/ EtOAc to give 110 mg (25%) of **27** as colorless needles: mp 43.5–45°C; IR (KBr) 3446, 3176, 1504, 1329, 1147, 1086 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.03 (br, 1 H), 2.98 (t, J = 7 Hz, 2H), 3.12 (m, 2H), 3.88 (t, J = 5 Hz, 2H), 4.21 (t, J = 5 Hz, 2H), 4.90 (t, J = 5 Hz, 1 H), 6.82 (d, J = 8 Hz, 2H), 7.28 (d, J = 8 Hz, 2H), 7.48 (d, J = 8 Hz, 2H); MS (EI) m/z 423 (M^+). Anal $C_{16}H_{16}NO_4ClF_2S_2$ (C, H, N, Cl, F, S).

Methyl 4-[2-(4-chlorophenylsulfonylamino)ethylsulfinyl]-2,6-difluorophenoxyacetate 28 and methyl 4-[2-(4-chlorophenylsulfonylamino)ethylsulfonyl]-2,6-difluorophenoxyacetate 29

To a solution of **22** (0.9 g, 2 mmol) in CH_2Cl_2 (32 ml) was added 70% *m*-chloroperbenzoic acid (0.5 g, 2 mmol) in portions at 0°C. The reaction mixture was stirred for 16 h at room temperature, and then washed with 3% $Na_2S_2O_3$, 5% $NaHCO_3$ and brine successively, dried and evaporated *in vacuo*. The residue was purified by flash chromatography using 3:7 EtOAc/CH_2Cl_2 to give 0.53 g (54.8%) of **28** and 0.2 g (20.7%) of **29**.

Compound 28. Colorless prisms: mp 140–141°C; IR (KBr) 3118, 1748, 1500, 1338, 1215, 1162, 1086, 1028 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.75 (m, 1H), 3.20 (m, 1H), 3.40 (m, 2H), 3.80 (s, 3H), 4.85 (s, 2H), 5.68 (t, J = 5 Hz, 1 H), 7.08 (d, J = 8 Hz, 2H), 7.51 (d, J = 8 Hz, 2H), 7.80 (d, J = 8 Hz, 2H); MS (EI) m/z 467 (M^+).

Compound 29. A colorless powder: mp 166–167°C; IR (KBr) 3292, 1758, 1505, 1338, 1297, 1221, 1166, 1136, 1086 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.28 (m, 2H), 3.43 (m, 2H), 3.80 (s, 3H), 4.92 (s, 2H), 5.35 (t, J = 5 Hz, 1H), 7.39 (d, J = 8 Hz, 2H), 7.53 (d, J = 8 Hz, 2H), 7.80 (d, J = 8 Hz, 2H); MS (EI) m/z 483 (M^+).

Pharmacology

Platelet aggregation test in vitro

Citrated blood (1 volume of 3.2% sodium citrate: 9 volumes of blood) was collected from the carotid artery of male New-Zealand white rabbits and centrifuged at 150 *g* at room temperature for 15 min to yield platelet-rich plasma (PRP) as a supernatant. The remaining blood was centrifuged at 1500 *g* for 10 min to yield 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 [27] with an aggregometer (PA-3210, Kyoto Daiichi Kagaku or PAM-8C, Mebanix). The compound to be tested was dissolved in DMSO, and 1 μl of the solution was added to 275 μl PRP and incubated at 37°C for 3 min with stirring at 1000 rpm, and then 25 μl of the U-46619 solution (final concentration: 5 μM) was added. The mixture was measured for 5 min with an aggregometer in order to obtain the maximum aggregation rate. The IC_{50} value (mean ± SEM) was calculated by regression analysis from the four dose groups with four different predictions.

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 filled with Krebs-Henseleit solution containing indomethacin (1 x 10^{-5} M). The organ baths were kept at 37°C and bubbled with 95 % O_2 and 5% CO_2. A resting tension of 1.0 g was applied to each strip. After equilibration for 1 h, the tensions developed by the strips were recorded on a polygraph (Nihon-Koden) through an isometric transducer (Nihon-Koden). The test compounds (3 x 10^{-9} to 1.2 x 10^{-7} M for **10c** and 1 x 10^{-4} M for sulotroban) were added 5 min before the addition of agonist (U-46619), and agonist–response curves were obtained using cumulative concentration methods (1 x 10^{-10} to 3 x 10^{-6} M).

ED_{50} values were obtained by regression analysis and used to calculate dissociation constants (pA_2 values) by the method of Schild [29].

TXA_2/PGH_2 receptor binding assay with guinea-pig platelets

Blood from male Hartrey guinea-pigs weighing 400–600 g was collected into syringes containing 0.2 volumes of acid-citrate-indomethacin-dextrose solution (trisodium citrate (85 mM), citric acid (70 mM), indomethacin (10 μM) and glucose (110 mM)). PRP was obtained by centrifugation at 160 *g* for 15 min. The PRP was recentrifuged at 1200 *g* for 15 min, and

resuspended in 2.0 ml of HEPES-Tyrode buffer (NaCl (130 mM), KCl (2.6 mM), Na_2HCO_3 (12 mM), HEPES (5 mM), glucose (5.5 mM), pH 7.4). Platelets were separated from plasma proteins by gel filtration through a Sepharose 2B column (60 ml). They were suspended in the HEPES-Tyrode buffer containing 1 mM $CaCl_2$ and 5 mM $MgCl_2$ to a final concentration of 3×10^8 cells /ml. Aliquots of the platelet suspension (0.5 ml) were incubated with 5 nM [^{3}H]SQ29548 plus various concentrations of test compounds for 60 min at 30°C. Specific binding was defined as the difference between the binding in the presence and absence of 10^{-5} M U-46619. After incubation, ice-cold HEPES-Tyrode buffer (1.25 ml) was added to each tube, and the reaction mixture was immediately centrifuged at 14 000 rpm for 2 min. The pellet was washed, recentrifuged and resuspended in HEPES-Tyrode buffer. Aquazol 2 scintillator (8 ml) was added to the suspension and radioactivity was measured in a liquid scintillation spectrometer (LSC 3500, Aloka).

Platelet aggregation in mice in vivo

Male ICR mice weighing about 25 g were used (7–11 animals for each group). U-46619 (25 μg/kg) was injected through the tail vein. At 30 s after the injection of U-46619, 20 μl blood was collected from the femoral artery, and platelet number was immediately measured with a microcell counter (CC-180A, Syntex). The compound to be tested was dissolved in 5% gum arabic solution, and administrated orally to the test animals 60 min before the injection of U-46619.

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