Synthesis and Evaluation of Novel Thiazolidine Derivatives as Thromboxane A_2 Receptor Antagonists[1)]

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A series of 3-benzoyl or 3-phenylsulfonyl-2-substituted thiazolidine derivatives were synthesized, and evaluated for their thromboxane A_2 (TXA_2) receptor-antagonizing effect on (15*S*)-15-hydroxy-11α,9α-(epoxymethano)prosta-5(*Z*),13(*E*)-dienoic acid (U-46619)-induced aggregation of rabbit platelet-rich plasma (PRP). A simple 2-aryl-thiazolidine derivative, 3-benzoyl-2-(4-hydroxy-3-methoxyphenyl)thiazolidine (5a), showed mild TXA_2 receptor antagonist activity. Modification of 5a led to 2-chloro-4-[3-(4-chlorophenylsulfonyl)thiazolidin-2-ylmethyl]phenoxy-acetic acid (29d), which showed 10 times more potent TXA_2 receptor antagonist activity than 5a.

Keywords thromboxane A_2, TXA_2; receptor, antagonist; thiazolidine; structure–activity relationship

During a pharmacological investigation of marine products, Satake *et al.* reported that D-cysteinolic acid (**1**) isolated from sardine exhibits mild platelet aggregation-inhibitory activity, and 2-(4-hydroxy-3-methoxyphenyl)-thiazolidine (**2**), which is a synthetic analogue of this natural product, shows elevated activity (Chart 1).[2–4)]

In the course of the modification of **2**, we have found that the 3-benzoyl derivative (**5a**) has mild thromboxane A_2 (TXA_2) receptor antagonist activity. Compound **5a** selectively inhibited the U-46619[5)]-induced rabbit platelet-rich plasma (PRP) aggregation, while **2** showed no activity against U-46619-induced aggregation even at elevated concentration (1×10^{-3} M). Although some thiazolidine derivatives were recently reported as platelet activating factor (PAF) inhibitors,[6)] and a number of TXA_2 receptor antagonists such as sulotroban were found,[7)] **5a** is a first thiazolidine derivative to be reported as a TXA_2 receptor antagonist. Since TXA_2 is one of the most potent inducers of platelet aggregation, vasoconstriction and bronchoconstriction,[8)] it has been postulated that TXA_2 plays an important role in the pathogenesis of various circulatory disorders and asthma,[9)] and TXA_2 receptor antagonists are therefore expected to be effective for the treatment of these diseases. So, we have examined the modification of **5a** with the aim of enhancing the potency in order to obtain compounds with potential for the treatment of these diseases.

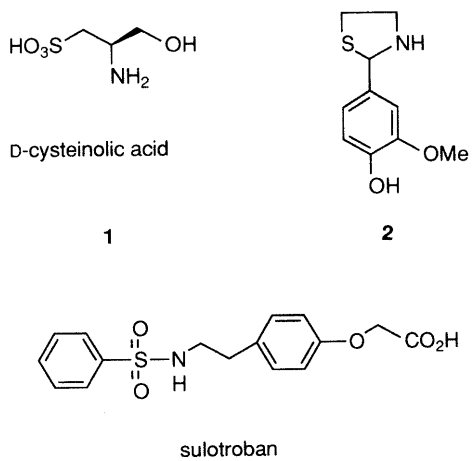


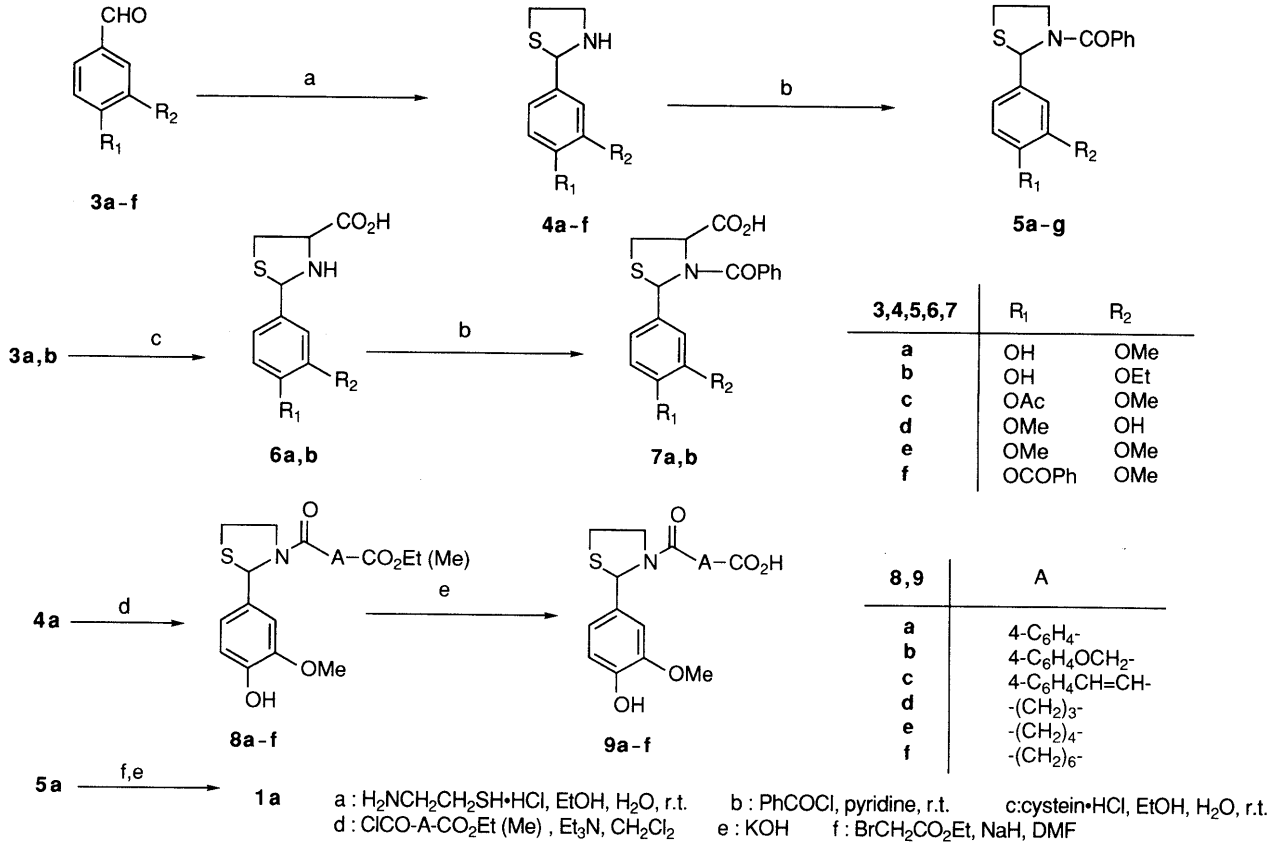
Chart 1

Chemistry

Compound **5a** was synthesized by the selective acylation of the nitrogen atom of the thiazolidine ring of **2**[3)] by treatment with one equivalent of benzoyl chloride in pyridine. The compounds which have modified substituents on the phenyl ring at the 2-position of the thiazolidine ring were synthesized from the corresponding 2-arylthiazolidines (**4a—f**) in the same way (Chart 2).

Introduction of a carboxy group at various positions of **5a** was examined (Chart 2) since a carboxy group exists in most synthetic TXA_2 receptor antagonists or agonists so far reported[7)] and in TXA_2 itself,[10)] and is postulated to be essential for the activity. Reaction of cysteine hydrochloride with arylaldehydes (**3a, b**) led to **6a, b**, which were directly acylated with benzoyl chloride without any protection to obtain the 4-carboxy derivatives (**7a, b**). Acylation of **4a** with various acyl chlorides possessing an ester group followed by hydrolysis of the ester group gave a series of compounds possessing a carboxy group at the 3-position substituent, and treatment of **5a** with ethyl bromoacetate followed by hydrolysis gave a phenoxy-acetate derivative (**10**).

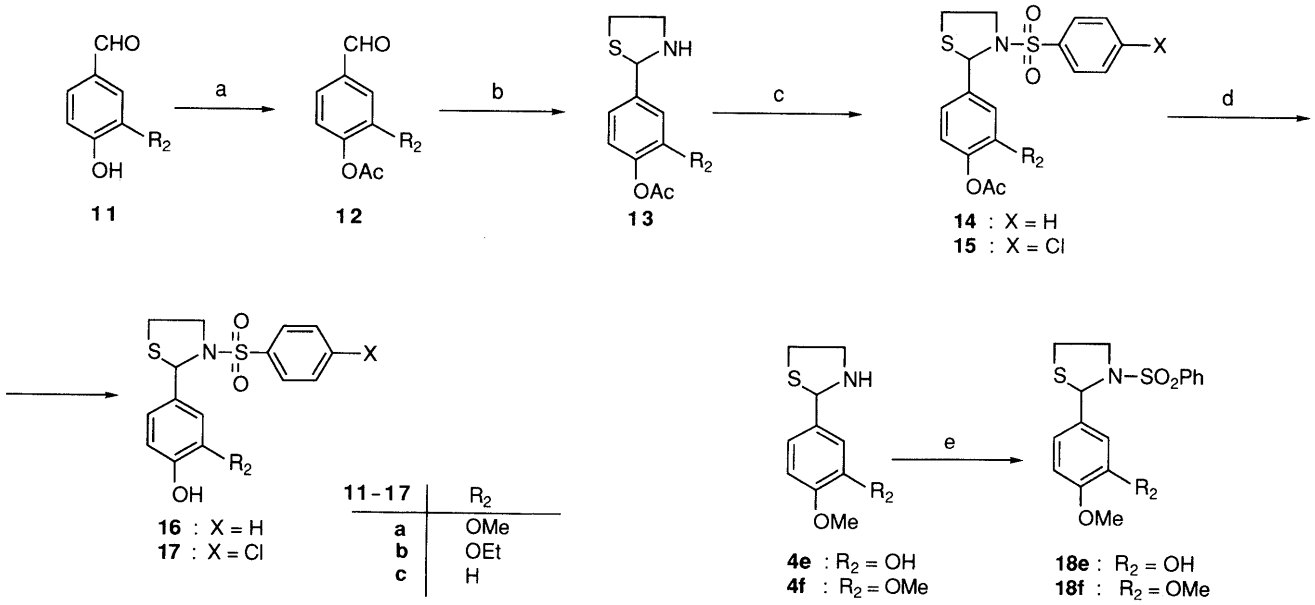
Next, introduction of an arylsulfonyl group at the 3-position of the thiazolidine ring of **2** was examined (Chart 3), because the arylsulfonylamino moiety is a characteristic moiety of the representative non-prostanoid TXA_2 receptor antagonist sulotroban[11)] and was reported as an "effective residue" in some other TXA_2 receptor antagonists.[12)] Treatment of **13** with $PhSO_2Cl$ and Et_3N in CH_2Cl_2 resulted in decomposition of the product to give the aldehyde **12**. Since the arylsulfonamide derivatives (**14** and **15**) were found to be unstable even to a weak acid such as $Et_3N \cdot HCl$, the sulfonamide derivatives (**14**, **15**) were synthesized by the use of $PhSO_2Cl$ and K_2CO_3 in



3,4,5,6,7	R_1	R_2
a	OH	OMe
b	OH	OEt
c	OAc	OMe
d	OMe	OH
e	OMe	OMe
f	OCOPh	OMe

8,9	A
a	4-C_6H_4-
b	4-$C_6H_4OCH_2$-
c	4-C_6H_4CH=CH-
d	-$(CH_2)_3$-
e	-$(CH_2)_4$-
f	-$(CH_2)_6$-

Chart 2

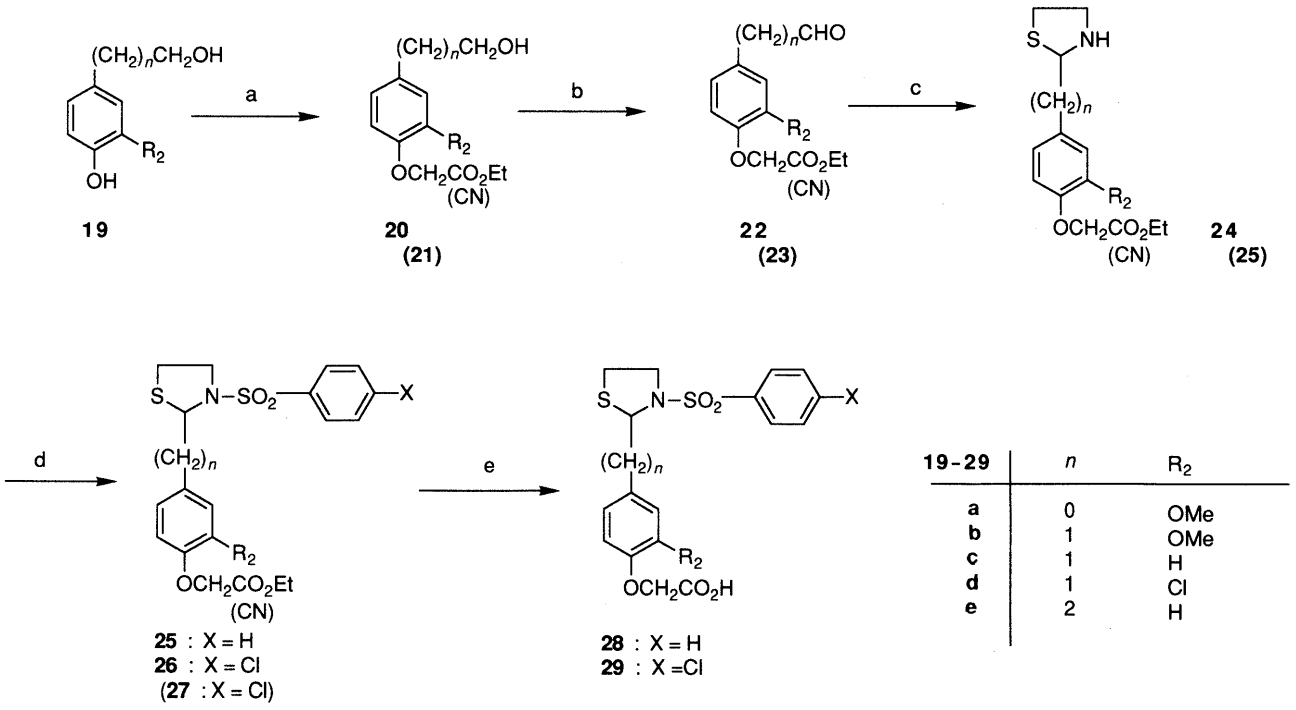


11-17	R_2
a	OMe
b	OEt
c	H

Chart 3

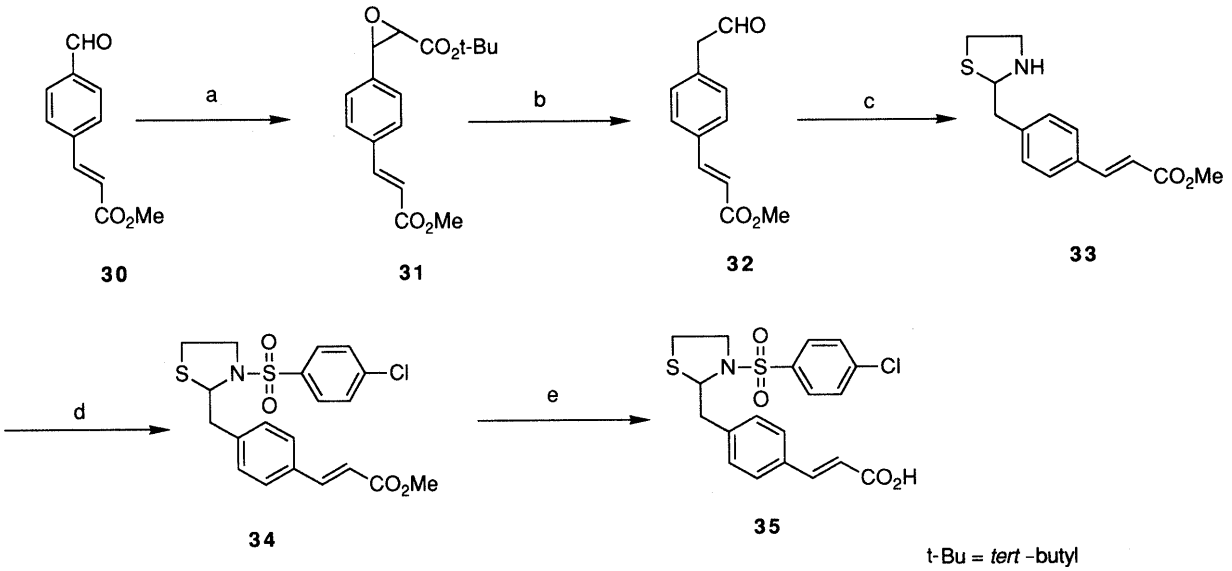
acetone. The phenol derivatives (**16**, **17**) were obtained by hydrolysis of the acetates (**14**, **15**) under alkaline conditions followed by careful neutralization with carbonate buffer.

Introduction of a carboxymethyl moiety at the phenolic oxygen atom of **16a** gave **28a** (Chart 4), and the compounds which have an alkylene group between the thiazolidine



a : $BrCH_2CO_2Et$ or $ClCH_2CN$, NaI, K_2CO_3, acetone, reflux b : CrO_3, pyridine or PCC, CH_2Cl_2 or DMSO, $(COCl)_2$, Et_3N, CH_2Cl_2
c : $H_2NCH_2CH_2SH \cdot HCl$, EtOH, H_2O d : 4-X-$C_6H_4SO_2Cl$, K_2CO_3, acetone e : NaOH aq.

Chart 4



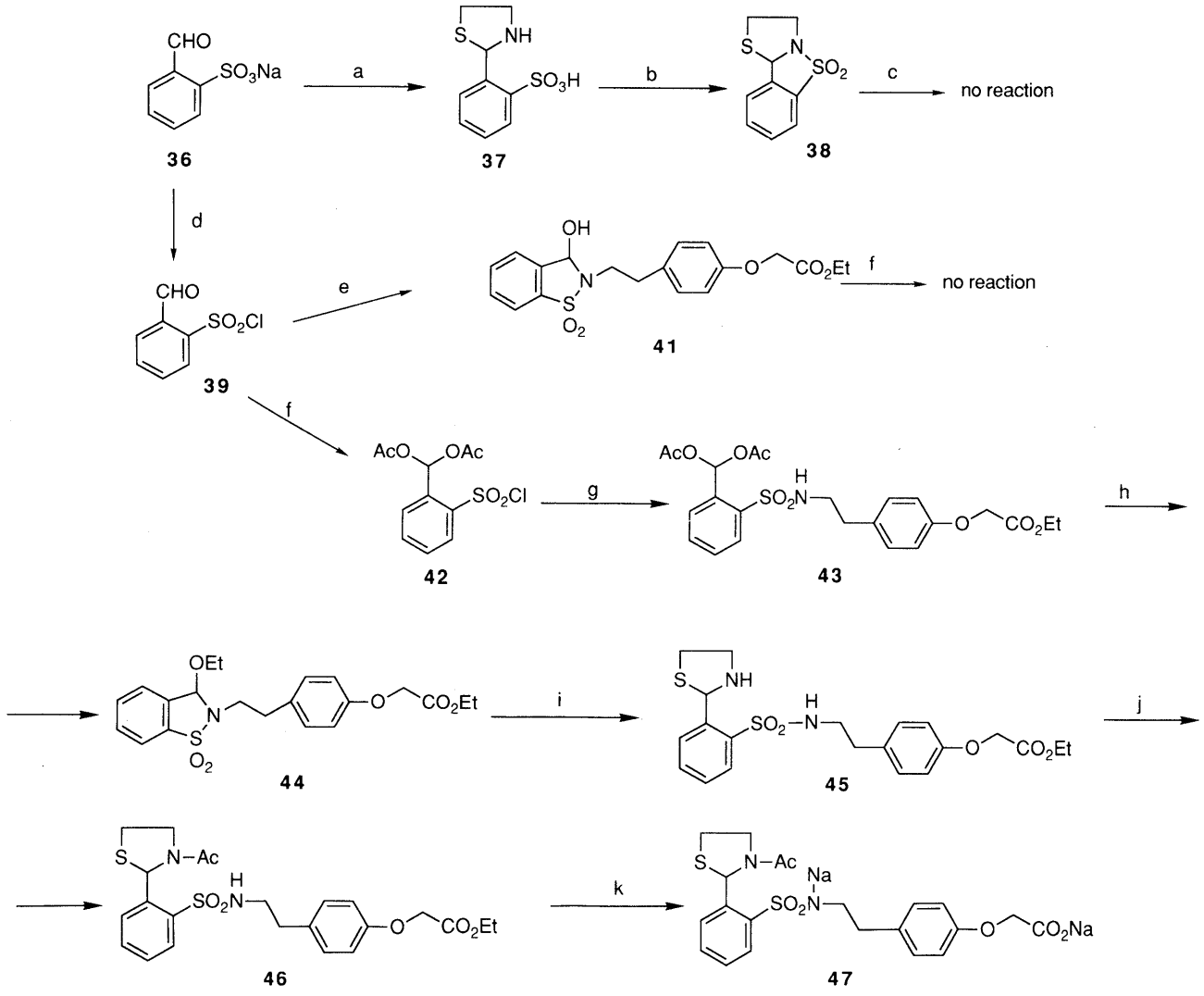
t-Bu = *tert*-butyl

a : $ClCH_2CO_2t$-Bu, t-BuOK, t-BuOH, THF b : heat c : $H_2NCH_2CH_2SH \cdot HCl$, MeOH, H_2O d : 4-Cl-$C_6H_4SO_2Cl$, K_2CO_3, acetone
e : NaOH aq.

Chart 5

ring and the phenyl ring (**28b, c** and **29c—e**) were synthesized from the corresponding aldehydes. The aldehydes (**22b—d** and **23**) were synthesized from phenethyl alcohol derivatives (**20b—d**) by oxidation with CrO_3/pyridine[13] or pyridium chlorochromate (PCC)[14] and from the phenylpropanol derivative (**21e**) by Swern's oxidation.[15] The aldehyde (**32**) was prepared by Darzens' reaction of **30**[16] with *tert*-butyl chloroacetate followed by the thermal rearrangement of the resulting oxirane[17] (Chart 5).

Finally, introduction of a thiazolidine ring at the phenylsulfonyl moiety of sulotroban was examined (Chart 6). 2-(2-Sulfophenyl)thiazolidine (**37**) was easily prepared from **36** and cysteamine hydrochloride. The protection of the amino residue of **37** with Ac_2O did not proceed, and the treatment of **37** with AcCl resulted in the formation



a : $H_2NCH_2CH_2SH \cdot HCl$, H_2O b : AcCl, 10% NaOH c : ethyl 4-(2-aminoethyl)phenoxyacetate·HCl (**40**), Et_3N, $CHCl_3$ d : $SOCl_2$, DMF e : **40**, Et_3N, CH_2Cl_2 f : Ac_2O, H_2SO_4 g : **40**, Et_3N, CH_2Cl_2 h : EtONa, EtOH i : $H_2NCH_2CH_2SH \cdot HCl \cdot EtOH$ j : AcCl, pyridine, CH_2Cl_2 k : 10%NaOH, EtOH

Chart 6

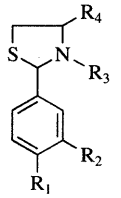
of the intramolecular sulfonamide (**38**), which was unreactive with the amino ester (**40**).[18] Chlorination of **36** with $SOCl_2$ in the presence of a catalytic amount of *N,N*-dimethylformamide (DMF) gave the relatively unstable sulfonyl chloride (**39**)[19] which was treated with **40** to give the sulfonamide derivative (**43**). Since this hemiacetal derivative failed to react with cysteamine hydrochloride, protection of the formyl group was examined. Compound **39** was treated with acetic anhydride in the presence of a catalytic amount of H_2SO_4 to give the *gem*-diacetoxy derivative (**42**), which was treated with **40** to afford **43** in excellent yield. Although the direct reaction of **43** with **40** did not proceed, the ketal derivative (**44**) reacted with **40** to give the desired thiazolidine derivative (**45**) in fair yield. Compound **44** was produced by the treatment of **43** with NaOEt in EtOH (treatment of **43** with aqueous alkali or acid gave **41**). Since the benzoylation of **45** with benzoyl chloride did not proceed, the amino group was acylated with acetyl chloride in pyridine followed by the hydrolysis of the ester function to give **47**. In addition, the NMR spectra of **46** and **47** showed duplicated signals due to inhibition of the free rotation of the thiazolidine ring, and these peaks coalesced to single broad peaks at elevated temperature (90 °C).

Pharmacological Results and Discussion

The compounds synthesized above were evaluated for inhibitory activity towards U-46619-induced rabbit platelet aggregation as described by Born,[20] as well as inhibitory activity against ADP-induced platelet aggregation.

Although the 2-arylthiazolidine derivative **2** was inactive against U-46619-induced aggregation even at an elevated concentration (1×10^{-3} M), the *N*-benzoyl derivative (**5a**) showed mild inhibitory activity against U-46619-induced aggregation with an IC_{50} value of 610 μM. Since **5a** was inactive against ADP- or PAF-induced aggregation at 1×10^{-3} M, and inhibited the specific binding of

TABLE I. Structure and Pharmacological Results of 3-Acylthiazolidine Derivatives



	R_1	R_2	R_3	R_4	mp	Formula	Analysis (%) Calcd (Found) C	H	N	Inhibition of platelet Agg. IC_{50} (10^{-5} M)[a] U46619	ADP
2b[b]	OH	OMe	H	H						>100	43
5a	OH	OMe	PhCO	H	137.5—138.5	$C_{17}H_{17}NO_3S$	64.74 (64.64)	5.43 (5.27)	4.44 (4.36)	61	>100
5b	OH	OEt	PhCO	H	159—161	$C_{18}H_{19}NO_3S$	65.63 (65.15)	5.81 (5.59)	4.25 (4.21)	>100	>100
5c	OAc	OMe	PhCO	H	96—98	$C_{19}H_{19}NO_4S$	63.85 (63.66)	5.36 (5.11)	3.92 (3.80)	47	>100
5d	OMe	OH	PhCO	H	136—137	$C_{17}H_{17}NO_3S$	64.74 (64.32)	5.43 (5.23)	4.44 (4.38)	>100	>100
5e	OMe	OMe	PhCO	H	Oil	$C_{18}H_{19}NO_3S$	329.1086[c] (329.1092)			>100	>100
5f	OCOPh	OMe	PhCO	H	104—106	$C_{24}H_{21}NO_4S$	68.72 (69.10)	5.05 (4.94)	3.34 (3.10)	>100	>100
7a	OH	OMe	PhCO	CO_2H	Oil	$C_{18}H_{17}NO_5S$	359.0828[c] (359.0828)			>100	>100
9a	OH	OMe	4-(HO_2C)PhCO	H	106—107	$C_{18}H_{17}NO_5S \cdot H_2O$	57.28 (57.55)	5.07 (4.89)	3.71 (3.73)	>100	>100
9b	OH	OMe	4-(HO_2CCH_2O)PhCO	H	Oil	$C_{19}H_{19}NO_6S$	389.0828[c] (389.0936)			>100	>100
9c	OH	OMe	4-($HO_2CCH=CH$)PhCO	H	191—194	$C_{20}H_{19}NO_5S \cdot 1/2H_2O$	60.90 (60.37)	5.11 (4.92)	3.55 (3.44)	>100	>100
9d	OH	OMe	$HO_2C(CH_2)_3CO$	H	57—59	$C_{15}H_{19}NO_5S$	55.37 (55.33)	5.89 (5.85)	4.30 (4.27)	78	100
9e	OH	OMe	$HO_2C(CH_2)_4CO$	H	118—120	$C_{16}H_{21}NO_5S$	56.62 (56.84)	6.24 (6.32)	4.13 (4.15)	55	100
9f	OH	OMe	$HO_2C(CH_2)_6CO$	H	89—93	$C_{18}H_{25}NO_5S$	58.83 (58.34)	6.86 (7.06)	3.81 (3.43)	>100	>100
10	OCH_2CO_2H	OMe	PhCO	H	Oil	$C_{19}H_{19}NO_5S \cdot 3/2H_2O$	56.98 (57.36)	5.54 (5.26)	3.50 (3.47)	>100	>100
Sulotroban										1.3	>100

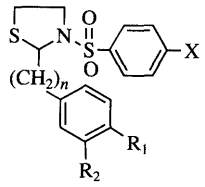
a) Concentration needed to inhibit U46619 (5 μM)- or ADP (5 μM)-induced platelet aggregation of rabbit PRP by 50% ($n=3$—4). b) Reference 3. c) Hi-MS.

[^{3}H]SQ29,548[21] to guinea pig platelets ($IC_{50}=336.3\ \mu M$), **5a** was regarded as a TXA_2 receptor antagonist.

Modification of the phenyl ring of **5a**, such as introduction of a methyl group (**5e**) or benzoyl group (**5f**) on the phenolic oxygen atom, or replacement of the methoxy group by an ethoxy group (**5b**), resulted in loss of the activity. Since the 4-hydroxy-3-methoxyphenyl moiety seemed to be essential for the activity, the substituents on the phenyl ring of **5a** were fixed and the introduction of a carboxy group at various positions of **5a** was examined. Although introduction of a carboxy group at an appropriate site of **5a** was expected to enhance the affinity to the TXA_2 receptor, introduction of a carboxy group at the 4-position of the thiazolidine ring (**7a**), and in the benzoyl moiety (**9a—c**) resulted in loss of the activity. In addition, introduction of a carboxymethyl group (**10**) on the phenolic oxygen atom also resulted in loss of the activity. Introduction of an ω-carboxy-alkanoyl moiety (**9e**) at the nitrogen atom of **2** instead of the benzoyl group somewhat improved the activity, but resulted in loss of the selective inhibitory activity against U-46619-induced platelet aggregation.

Since the introduction of a carboxy moiety into **5a** failed to enhance the activity, introduction of an arylsulfonyl moiety which is the other characteristic moiety of sulotroban, into **5a** was examined. Simple replacement of the benzoyl group of **5a**, or **10** by an arylsulfonyl group (**16a**, or **28a**) lowered the activity, but insertion of a methylene group (**28b**) between the thiazolidine ring and the phenyl ring of **28a** improved the activity. Compound **28b**, which has a common partial structure with sulotroban and corresponds to a bridged derivative of sulotroban, showed 3 times more potent activity than **5a**. The displacement of the methoxy group (**28c**) of **28b** did not affect the activity, but replacement of the methoxy group of **28b** by a chlorine atom enhanced the activity to give the most potent compound **29d** ($IC_{50}=60\ \mu M$) in this series. Compound **29d** showed essentially the same affinity ($IC_{50}=23.1\ \mu M$) to the TXA_2 receptor as sulotroban ($IC_{50}=25.1\ \mu M$) in a radioligand binding assay with gel-filtered guinea pig platelets. Finally, simple introduction of a thiazolidine ring into the structure of sulotroban

TABLE II. Structure and Pharmacological Results of 3-Arylsulfonylthiazolidine Derivatives



	R_1	R_2	n	X	mp	Formula	Analysis (%) Calcd (Found) C	H	N	Inhibition of platelet Agg.[a] IC_{50} (10^{-5} M) U46619	ADP
14a	OAc	OMe	0	H	151—152	$C_{18}H_{19}NO_5S_2$	54.95 (55.01	4.87 4.93	3.56 3.59)	>100	>100
15a	OAc	OMe	0	Cl	134.5—135.5	$C_{18}H_{18}NO_5ClS$	50.52 (50.43	4.24 4.22	3.27 3.24)	>100	>100
16a	OH	OMe	0	H	93—94.5	$C_{16}H_{17}NO_4S_2$	54.68 (54.63	4.83 4.86	3.99 3.89)	100	>100
17a	OH	OMe	0	Cl	135.5—136.5	$C_{16}H_{16}NO_4ClS$	49.80 (49.52	4.18 4.24	3.63 3.67)	>100	>100
16b	OH	OEt	0	H	Oil	$C_{17}H_{19}NO_4S_2$	365.0756[b] (365.0755)			44	100
16c	OH	H	0	H	154—155.5	$C_{15}H_{15}NO_3S_2$	56.05 (55.79	4.70 4.49	4.36 4.25)	31	80
18e	OMe	OH	0	H	127—129.5	$C_{16}H_{17}NO_4S_2$	54.68 (54.89	4.88 4.68	3.99 3.51)	69	100
18f	OMe	OMe	0	H	Oil	$C_{17}H_{19}NO_4S_2$	365.0756 (365.0743)			>100	>100
28a	OCH_2CO_2Na	OMe	0	H	171—176	$C_{16}H_{18}NO_6NaS_2 \cdot 1/2H_2O$	49.10 (49.01	4.35 4.19	3.18 3.09)	>100	>100
28b	OCH_2CO_2Na	OMe	1	H	130—132	$C_{19}H_{20}NO_6NaS \cdot H_2O$	49.23 (49.20	4.78 4.57	3.02 2.98)	22	>100
28c	OCH_2CO_2Na	H	1	H	142—148	$C_{18}H_{18}NO_5NaS_2$	52.04 (52.11	4.37 4.49	3.37 3.26)	100	>100
29c	OCH_2CO_2Na	H	1	Cl	166—168	$C_{18}H_{17}NO_5ClNaS_2$	48.05 (48.56	3.81 4.18	3.11 2.98)	53	>100
29d	OCH_2CO_2H	Cl	1	Cl	163.5—165	$C_{18}H_{17}NO_5Cl_2S_2$	46.76 (46.58	3.71 3.58	3.03 2.97)	6	>100
29e	OCH_2CO_2H	H	2	Cl	135.5—136.5	$C_{19}H_{20}NO_5ClS_2$	51.64 (51.57	4.56 4.62	3.17 3.08)	30	>100
35	$CH=CHCO_2H$	H	1	Cl	172.5—173.5	$C_{19}H_{18}NO_4ClS_2$	53.83 (54.10	4.28 4.36	3.30 3.07)	>100	>100
27	OCH_2CN	H	1	Cl	98—99	$C_{18}H_{17}N_2O_3ClS_2$	52.87 (52.82	4.19 4.10	6.85 6.85)	>100	>100
48					260—265	$C_{21}H_{22}N_2O_6Na_2S_2 \cdot C_2H_6O \cdot H_2O$	48.24 (47.75	5.28 4.85	4.89 4.69)	>100	>100
Sulotroban										1.3	>100

a) See footnote of Table I. *b*) Hi-MS.

(**47**), and syntheses of the cinnamic acid derivative (**35**) and phenoxyacetonitrile derivative (**27**) were examined. Since all of these compounds were inactive against the U-46619-induced platelet aggregation at 1×10^{-3} M, the existence of a flexible carboxy group was concluded to be essential for the activity. By the systematic modification of **5a** as described above, it was found that the appropriate introduction of the characteristic structural features of sulotroban to **5a** was required for the improvement of the activity.

In conclusion, we have found a unique non-prostanoid TXA_2 receptor antagonist (**5a**), and the modification of this molecule led to compound **29d** which has an affinity for the TXA_2 receptor as high as that of the representative non-prostanoid TXA_2 receptor antagonist sulotroban.

Experimental

Melting points were determined on a Mettler FP-60 melting point apparatus without correction. 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) value using tetramethylsilane as an internal standard. Mass spectra (MS) were taken on a JEOL JMS-SX102 spectrometer. Microanalytical data were obtained by using a Carlo Elba 1106R or a Perkin-Elmer 240C elemental analyzer. Organic solutions during work-up were dried over 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).

3-Benzoyl-2-(4-hydroxy-3-methoxyphenyl)thiazolidine (5a) Benzoyl chloride (6.7 g, 47.4 mmol) was added dropwise to a solution of 2-(4-hydroxy-3-methoxyphenyl)thiazolidine (**4a**) (10.0 g, 47.4 mmol) in pyridine (200 ml) at 0 °C. The reaction mixture was stirred for 15 h at room temperature and then evaporated *in vacuo*. The residue was dissolved in CH_2Cl_2 and washed with 3% HCl and water successively, dried and evaporated *in vacuo*. The residue was purified by flash chromatography using benzene–EtOAc (8 : 1) to give **5a** (7.9 g, 53%) as a colorless powder. mp 137.5—138.5 °C. IR (KBr): 3246, 1618, 1518, 1412, 1279, 794, 698 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.08 (1H, br s), 3.10

(2H, br t, J=8 Hz), 3.80 (2H, m), 3.85 (3H, s), 5.61 (1H, br s), 6.8—7.1 (3H, br m), 7.3—7.55 (5H, br m). MS (EI) m/z: 315 (M^+). *Anal.* Calcd for $C_{17}H_{17}NO_3S$: C, 64.74; H, 5.43; N, 4.44. Found: C, 64.64; H, 5.27; N, 4.36.

2-(4-Acetoxy-3-methoxyphenyl)-3-(4-chlorophenylsulfonyl)thiazolidine (15a) Acetic anhydride (9 ml, 100 mmol) was added to a solution of vanillin (**11a**) (5 g, 32.9 mmol) and pyridine (2.7 ml, 32.9 mmol) in CH_2Cl_2 (100 ml). The reaction mixture was stirred for 16 h at room temperature, washed with 3% HCl, water and brine successively, dried and evaporated *in vacuo*. The residue was crystallized from ether to give **12a** (5.9 g, 92.4%) as colorless prisms. mp 75—76 °C.

A mixture of **12a** (5.9 g, 30.4 mmol), cysteamine hydrochloride (3.7 g, 32.6 mmol) and EtOH (100 ml) was heated under reflux for 2 h and then evaporated *in vacuo*. The residue was dissolved in water and washed with ether. The aqueous layer was neutralized with $NaHCO_3$ and extracted with EtOAc. The EtOAc layer was washed with water and brine successively, dried and evaporated *in vacuo*. The residue was crystallized from ether to give 2-(4-acetoxy-3-methoxyphenyl)thiazolidine (**13a**) (6.0 g, 78%) as colorless prisms. mp 87.5—89 °C. IR (KBr): 3500, 1758 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.95 (1H, br s), 2.30 (3H, s), 3.10 (3H, m), 3.58 (1H, m), 3.83 (3H, s), 5.56 (1H, s), 7.00 (1H, d, J=8 Hz), 7.12 (1H, dd, J=8, 2 Hz), 7.14 (1H, d, J=2 Hz). MS (EI) m/z: 253 (M^+).

4-Chlorophenylsulfonyl chloride (0.85 g, 4.0 mmol) was added to a mixture of **13a** (1.02 g, 4.0 mmol), K_2CO_3 (1.7 g, 12 mmol) and acetone (30 ml) and stirred for 4 h at room temperature. The reaction mixture was poured into water and extracted with EtOAc. The organic layer was washed with water and brine successively, dried and evaporated *in vacuo*. The residue was purified by flash chromatography using hexane–EtOAc (3 : 1) to give **15a** (1.05 g, 60.9%) as colorless prisms. mp 134.5—135.5°C. IR (KBr): 1768, 1349 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.30 (3H, s), 2.70 (1H, m), 2.97 (1H, m), 3.52 (1H, m), 3.80 (3H, s), 4.05 (1H, m), 6.19 (1H, s), 6.97 (1H, m), 7.03 (2H, m), 7.50 (2H, m), 7.74 (2H, m). MS (EI) m/z: 427 (M^+). *Anal.* Calcd for $C_{18}H_{18}NO_5S_2Cl$: C, 50.52; H, 4.24; N, 3.27. Found: C, 50.43; H, 4.22; N, 3.24.

3-(4-Chlorophenylsulfonyl)-2-(4-hydroxy-3-methoxyphenyl)thiazolidine (17a) A 10% NaOH solution (1.5 ml, 6 mmol) was added dropwise to a mixture of **15a** (0.71 g, 1.66 mmol) and EtOH (50 ml) and stirred for 30 min at room temperature. The reaction mixture was neutralized carefully by adding 3% HCl in the presence of $NaHCO_3$ (1.0 g, 12 mmol), and then extracted with EtOAc. The organic layer was washed with water and brine successively, dried and evaporated *in vacuo*. The residue was triturated with EtOAc–hexane to give **17a** (0.52 g, 81.2%) as colorless prisms. mp 135.5—136.5 °C. IR (KBr): 3486, 1350, 1163 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.70 (1H, m), 2.98 (1H, m), 3.08 (1H, m), 3.85 (3H, s), 4.05 (1H, m), 5.62 (1H, s), 6.13 (1H, s), 6.84 (1H, d, J=8 Hz), 6.92 (1H, d, J=2 Hz), 6.94 (1H, dd, J=8, 2 Hz), 7.47 (2H, m), 7.73 (2H, m). MS (EI) m/z: 385 (M^+). *Anal.* Calcd for $C_{16}H_{16}NO_4ClS_2$: C, 49.80; H, 4.18; N, 3.63. Found: C, 49.52; H, 4.24; N, 3.67.

Ethyl 2-Chloro-4-(2-hydroxyethyl)phenoxyacetate (20d) A 1 N BH_3/tetrahydrofuran (THF) solution (100 ml, 100 mmol) was added dropwise to a solution of 3-chloro-4-hydroxyphenylacetic acid (12.5 g, 67 mmol) in THF (50 ml) at 3—5 °C under an argon atmosphere. The reaction mixture was stirred for 1 h at room temperature, then water (10 ml) was added and the whole was evaporated *in vacuo*. To the residue, 10% NaOH (30 ml), water (50 ml) and 3% HCl (9 ml) were added successively and the mixture was extracted three times with EtOAc. The organic layer was combined, washed with 5% $NaHCO_3$ and brine successively, dried and evaporated *in vacuo*. The residue was triturated with ether–hexane to give 3-chloro-4-hydroxyphenethyl alcohol (**19d**) (11.46 g, 99.1%). mp 64.5—65.5 °C. IR (KBr): 3488, 3269, 1600 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.60 (1H, br s), 2.78 (2H, t, J=7 Hz), 3.81 (2H, t, J=7 Hz), 5.67 (1H, br s), 6.93 (1H, d, J=8 Hz), 7.02 (1H, dd, J=8, 2 Hz), 7.18 (1H, d, J=2 Hz). MS (EI) m/z: 172 (M^+).

A mixture of **19d** (2.6 g, 15 mmol), ethyl 2-bromoacetate (1.66 ml, 15 mmol), K_2CO_3 (6.2 g, 45 mmol), NaI (2.25 g, 15 mmol) and acetone (75 ml) was heated under reflux for 10 h. The reaction mixture was filtered to remove the insolubles and the filtrate was evaporated *in vacuo*. The residue was purified by flash chromatography using hexane–EtOAc (2 : 1) to give **20d** (3.67 g, 94.9%) as a colorless oil. IR (neat): 3391, 1757, 1607, 1500 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.30 (2H, t, J=7 Hz), 1.55 (1H, br s), 2.79 (2H, t, J=7 Hz), 3.80 (2H, br s), 4.27 (2H, q, J=7 Hz), 4.68 (2H, s), 6.80 (1H, d, J=8 Hz), 7.04 (1H, dd, J=8 Hz), 7.25 (1H, d, J=2 Hz). MS (EI) m/z: 258 (M^+).

Ethyl 2-Chloro-4-formylmethylphenoxyacetate (22d) CrO_3 (8.58 g, 85.8 mmol) was added in portions to a mixture of pyridine (13.85 ml, 171.5 mmol) and CH_2Cl_2 (214 ml) at 0 °C and the mixture was stirred for 15 min at room temperature. A solution of **21d** (3.67 g, 14.3 mmol) in CH_2Cl_2 (17 ml) was added in one portion, and the mixture was stirred for 15 min. The resulting tar was removed by decantation and the solution was washed with 5% $NaHCO_3$, 3% HCl and brine successively, dried and evaporated *in vacuo*. The residue was purified by flash chromatography using hexane–EtOAc (2 : 1) to give **22d** (1.21 g, 33%) as a yellow oil. IR (neat): 1757, 1501 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.30 (3H, t, J=7 Hz), 3.62 (2H, d, J=2 Hz), 4.26 (2H, q, J=7 Hz), 4.70 (2H, s), 6.8—7.3 (3H, m), 9.72 (1H, t, J=2 Hz). MS (EI) m/z: 256 (M^+).

2-(4-Ethoxycarbonylmethoxy-3-chlorobenzyl)thiazolidine (24d) A mixture of **22d** (3.9 g, 15.2 mmol), cysteamine hydrochloride (1.72 g, 15.2 mmol), H_2O (20 ml) and EtOH (40 ml) was heated under reflux for 1 h in an argon atmosphere. The reaction mixture was poured into 5% $NaHCO_3$ 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 0 : 1) to give **24d** (4.03 g 84%) as a colorless oil. IR (neat): 3302, 1757, 1500 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.30 (3H, t, J=7 Hz), 1.73 (1H, br s), 2.8—3.15 (5H, m), 3.48 (1H, m), 4.27 (2H, q, J=7 Hz), 4.68 (1H, t, J=6 Hz), 4.69 (2H, s), 6.78 (1H, d, J=8 Hz), 7.10 (1H, dd, J=8, 2 Hz), 7.42 (1H, d, J=2 Hz). MS (EI) m/z: 315 (M^+).

3-(4-Chlorophenylsulfonyl)-2-(4-ethoxycarbonylmethoxy-3-chlorobenzyl)thiazolidine (26d) A mixture of **24d** (3.0 g, 9.5 mmol), 4-chlorophenylsulfonyl chloride (2.0 g, 9.5 mmol), K_2CO_3 (3.9 g, 28.5 mmol) and acetone (100 ml) was stirred for 3.5 h at room temperature. The reaction mixture was filtered and the filtrate was evaporated *in vacuo*. The residue was purified by flash chromatography using hexane–EtOAc (2 : 1) and triturated with ether to give **26d** (3.41 g, 73%) as colorless needles. mp 84.5—85.5 °C. IR (KBr): 1757, 1735, 1500 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.30 (3H, t, J=7 Hz), 2.50 (1H, m), 2.72 (1H, m), 2.94 (1H, dd, J=14, 7 Hz), 3.05 (1H, dd, J=14, 5 Hz), 3.43 (1H, m), 3.85 (1H, m), 4.27 (2H, q, J=7 Hz), 4.70 (2H, s), 5.21 (1H, dd, J=7, 5 Hz), 6.77 (1H, d, J=8 Hz), 7.10 (1H, dd, J=8, 2 Hz), 7.27 (1H, d, J=2 Hz), 7.50 (2H, m), 7.72 (2H, m). MS (EI) m/z: 489 (M^+).

3-(4-Chlorophenylsulfonyl)-2-(4-carboxymethoxy-3-chlorobenzyl)thiazolidine (29d) A mixture of **26d** (1.82 g, 3.7 mmol), 10% NaOH (2 ml, 5 mmol) and EtOH (20 ml) was 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, evaporated *in vacuo* and triturated with EtOAc to give **29d** (1.75 g, 100%) as a colorless powder. mp 163.5—165 °C. IR (KBr): 3436, 1744, 1498 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.40 (1H, m), 2.87 (1H, m), 2.85—3.1 (2H, m), 3.52 (1H, m), 3.95 (1H, m), 4.80 (2H, s), 5.35 (1H, t, J=6 Hz), 6.95 (1H, d, J=8 Hz), 7.15 (1H, dd, J=8, 2 Hz), 7.30 (1H, d, J=2 Hz), 7.63 (2H, m), 7.80 (2H, m), 13.10 (1H, s). MS (EI) m/z: 462 (M^+). *Anal.* Calcd for $C_{18}H_{17}NO_5Cl_2S_2$: C, 46.76; H, 3.71; N, 3.03. Found: C, 46.58; H, 3.58; N, 2.97.

1-*tert*-Butoxycarbonyl-2-[4-(2-methoxycarbonyl-(*E*)-ethenyl)]-(*cis* and *trans*)oxirane (31) A solution of *tert*-BuOK (3.37 g, 30 mmol) in *tert*-BuOH (50 ml) was added dropwise to a solution of methyl 4-formylcinnamate (**30**) (5.71 g, 30 mmol) and *tert*-butyl 2-chloroacetate (4.52 g, 30 mmol) in THF (40 ml) at room temperature over a period of 2 h. The reaction mixture was stirred for 1 h and evaporated *in vacuo*. The residue was dissolved in CH_2Cl_2 and the insolubles were removed by filtration. The filtrate was evaporated *in vacuo* and the residue was purified by flash chromatography using hexane–CH_2Cl_2 (2 : 1) to give **31** (1 : 1 mixture) (7.81 g, 85.5%) as a colorless oil. IR (neat): 1746, 1722, 1638 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.20 and 1.51 (9H, s), 3.40 (0.5H, d, J=1 Hz), 3.72 (0.5H, d, J=4 Hz), 4.03 (0.5H, d, J=1 Hz), 4.22 (0.5H, d, J=4 Hz), 6.43 (1H, d, J=17 Hz), 7.25—7.55 (4H, m), 7.66 (1H, d, J=17 Hz). MS (EI) m/z: 304 (M^+).

Methyl 4-(Thiazolidin-2-ylmethyl)cinnamate (33) Compound **31** (3.46 g, 11.4 mmol) was heated at 220 °C for 7 min to give a mixture of unreacted **31** and methyl 4-formylmethylcinnamate (**32**) as an orange oil. **32**: IR ($CHCl_3$): 1713 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 3.73 (2H, d, J=2 Hz), 3.80 (3H, s), 6.42 (1H, d, J=17 Hz), 7.24 (2H, m), 7.50 (2H, m), 7.68 (1H, d, J=17 Hz), 9.79 (1H, d, J=2 Hz). MS (EI) m/z: 204 (M^+).

A mixture of crude **32** (2.3 g, 11.4 mmol), cysteamine hydrochloride (1.29 g, 11.4 mmol), MeOH (30 ml) and water (2 ml) was stirred for 16 h at room temperature under an argon atmosphere and then evaporated *in vacuo*. The residue was dissolved in 3% HCl and washed with EtOAc. Unreacted **31** was recovered (1.95 g, 56.3%) from the organic layer. The

aqueous layer was neutralized with $NaHCO_3$ and extracted with EtOAc. The EtOAc layer was washed with water and brine successively, dried and evaporated *in vacuo* to give **33** (0.76 g, 25.4%) as a colorless oil. IR (neat): 3297, 1714, 1635 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.86 (1H, br s), 2.85—3.30 (5H, m), 3.50 (1H, m), 3.80 (3H, s), 4.72 (1H, t, J=6 Hz), 6.40 (1H, d, J=17 Hz), 7.28 (2H, m), 7.50 (2H, m), 7.65 (1H, d, J=17 Hz). MS (CI) m/z: 264 (M+H).

Methyl 4-[3-(4-Chlorophenylsulfonyl)thiazolidin-2-ylmethyl)cinnamate (34) A mixture of **33** (0.75 g, 2.85 mmol), 4-chlorophenylsulfonyl chloride (0.6 g, 2.85 mmol), K_2CO_3 (0.8 g, 5.79 mmol) and acetone (20 ml) was stirred for 16 h at room temperature. The reaction mixture was filtered and the filtrate was evaporated *in vacuo*. The residue was purified by flash chromatography using hexane–EtOAc (2:1) to give **34** (0.82 g, 65.7%) as a colorless oil. IR (neat): 1708 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.50 (1H, m), 2.75 (1H, m), 3.05 (1H, dd, J=13, 8 Hz), 3.20 (1H, dd, J=13, 6 Hz), 3.80 (3H, s), 5.25 (1H, dd, J=8, 6 Hz), 6.42 (1H, d, J=17 Hz), 7.28 (2H, m), 7.45 (4H, m), 7.68 (1H, d, J=17 Hz), 7.70 (2H, m). MS (EI) m/z: 437 (M^+).

4-[3-(4-Chlorophenylsulfonyl)thiazolidin-2-ylmethyl)cinnamic Acid (35) A mixture of **34** (0.8 g 1.82 mmol), 10% NaOH (2 ml, 5 mmol) and EtOH (50 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* to give **35** (0.8 g, 100%) as a colorless powder. mp: 172—173.5 °C. IR (KBr): 3400, 1684, 1626 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.40 (1H, m), 2.88 (1H, m), 2.99 (1H, dd, J=13, 8 Hz), 3.10 (1H, dd, J=13, 8 Hz), 3.33 (1H, br s), 3.55 (1H, m), 3.95 (1H, m), 5.37 (1H, t, J=8 Hz), 6.50 (1H, d, J=17 Hz), 7.29 (2H, m), 7.60 (1H, d, J=17 Hz), 7.50—7.70 (4H, m), 7.82 (2H, m). MS (EI) m/z: 423 (M^+). *Anal.* Calcd for $C_{19}H_{18}NO_4S_2Cl$: C, 53.83; H, 4.28; N, 3.30. Found: C, 54.10; H, 4.36; N, 3.07.

2-(2-Sulfophenyl)thiazolidine (37) A mixture of sodium 2-formylphenylsulfonate (**36**) (10.4 g, 50 mmol), cysteamine hydrochloride (5.65 g, 50 mmol) and water (100 ml) was allowed to stand for 16 h in refrigerator. The resulting crystals were collected to give **36** (8.2 g, 68%) as colorless prisms. mp 243—246 °C. IR (KBr): 3452, 2970 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 3.23 (1H,m), 3.45 (2H, m), 3.58 (1H, m), 6.89 (1H, s), 7.50 (2H, m), 7.85 (1H, m), 7.92 (1H, m), 9.73 (2H, m). MS (FAB) m/z: 246 (M+H).

4,4-Dioxo-2,3,8′-trihydrothiazolo[3,2-*b*]benzo-1,2-isothiazole (38) Acetyl chloride (100 mg, 1.27 mmol) was added to a solution of **37** (245 mg, 1.0 mmol) in 10% NaOH (0.88 ml, 2.2 mmol) at 0 °C and the mixture was stirred for 30 min. The resulting crystals were collected to give **37** (130 mg, 57%) as colorless prisms. mp 247.5—249.5 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.10 (1H, m), 3.25 (1H, m), 3.45 (1H, m), 4.40 (1H, m), 6.03 (1H, s), 7.45—7.80 (4H, m). MS (FAB) m/z: 242 (M+H).

2-Formylphenylsulfonyl Chloride (39) A mixture of **36** (10 g, 48 mmol) and $SOCl_2$ (40 ml, 540 mmol) was treated with DMF (4.0 ml, 53 mmol) (caution: exothermic and HCl gas evolved vigorously). The reaction mixture was heated for 3 min at 100 °C, then extracted three times with hexane. The hexane layers were combined, washed with water, dried and evaporated *in vacuo* to give **39** (3.53 g, 35.9%) as a colorless oil. IR (neat): 1704 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 7.85 (2H, m), 8.18 (2H, m), 10.91 (1H, s). MS (CI) m/z: 205 (M+H).

***S,S*-Dioxo-2,3-dihydro-2-[2-(4-ethoxycarbonylmethoxy)phenyl]-3-hydroxybenzoisothiazole (41).** To a mixture of ethyl 4-(2-aminoethyl)phenoxyacetate hydrochloride (**40**) (3.5 g, 17.1 mmol) and CH_2Cl_2 (80 ml) was added Et_3N (5.23 ml, 37.6 mmol), followed by a solution of **39** (4.44 g, 17.1 mmol) in CH_2Cl_2 (20 ml) at 0 °C. The reaction mixture was stirred for 1 h at room temperature and then washed with water, 5% $NaHCO_3$ and brine successively, dried and evaporated *in vacuo*. The residue was purified by flash chromatography using $CHCl_3$–MeOH (1:0 to 50:1) to give **41** (6.4 g, 95.6%) as a colorless oil. IR (neat): 3436, 1756 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.27 (3H, t, J=7 Hz), 2.9—3.2 (2H, m), 3.02 (2H, t, J=7 Hz), 3.35 (1H, d, J=12 Hz), 4.24 (2H, q, J=7 Hz), 4.58 (2H, s), 5.57 (1H, d, J=12 Hz), 6.82 (2H, d, J=9 Hz), 7.18 (2H, d, J=9 Hz), 7.5—7.75 (4H, m). MS (CI) m/z: 392 (M+H).

2-(Bis-acetoxymethyl)phenylsulfonyl Chloride (42) Concentrated H_2SO_4 (3 drops) was added to a solution of **39** (4.5 g, 22 mmol) in acetic anhydride (5 ml, 53 mmol), and the mixture was stirred for 30 min. The reaction mixture was purified by flash chromatography using hexane–EtOAc (5:2) to give **42** (5.6 g, 83%) as colorless prisms. mp 71—72 °C. IR (KBr): 1763 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.15 (6H, s), 7.68 (1H, m), 7.81 (1H, m), 7.86 (1H, m), 8.19 (1H, m), 8.39 (1H, s). MS (CI) m/z: 247 (M−OAc).

Ethyl 4-[2-(2-Bis-acetoxymethylphenylsulfonylamino)ethyl]phenoxyacetate (43) A solution of **42** (3.96 g, 38.7 mmol) in CH_2Cl_2 (10 ml) was added dropwise to a mixture of **40** (3.35 g, 12.9 mmol), Et_3N (5.4 ml, 38.7 mmol) and CH_2Cl_2 (100 ml) and the mixture was stirred for 30 min at room temperature. The reaction mixture was washed with 3% HCl, water and brine successively, dried and evaporated *in vacuo* to give **43** (6.03 g, 94.6%) as a colorless oil. IR (neat): 3286, 1757 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.30 (3H, t, J=7 Hz), 2.11 (6H, s), 2.72 (2H, t, J=7 Hz), 3.07 (2H, m), 4.27 (2H, q, J=7 Hz), 4.59 (2H, s), 5.62 (1H, br d, J=5 Hz), 6.80 (2H, m), 7.05 (2H, m), 7.54 (1H, m), 7.66 (1H, m), 7.80 (1H, m), 7.98 (1H, m), 8.31 (1H, s). MS (EI) m/z: 493 (M^+).

***S,S*-Dioxo-2,3-dihydro-3-ethoxy-2-[2-(4-ethoxycarbonylmethoxy)-phenyl]benzoisothiazole (44)** A solution of EtONa (1.66 g, 12.2 mmol) in EtOH (20 ml) was added to a solution of **43** (6.03 g, 12.2 mmol) in absolute EtOH (100 ml), and the mixture was stirred for 10 h at room temperature. The reaction mixture was evaporated *in vacuo* and the CH_2Cl_2-insolubles were removed to give **44** (3.83 g, 74.7%) as a colorless oil. IR (neat): 1757 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.06 (3H, t, J=7 Hz), 1.25 (3H, t, J=7 Hz), 2.92 (1H, m), 3.07 (2H, t, J=7 Hz), 3.10 (2H, m), 3.29 (1H, m), 4.25 (2H, q, J=7 Hz), 4.60 (2H, s), 5.71 (1H, s), 6.85 (2H, m), 7.22 (2H, m), 7.52 (1H, m), 7.64 (2H, m), 7.72 (2H, m). MS (CI) m/z: 420 (M+H).

Ethyl 4-{2-[2-(Thiazolidin-2-yl)phenylsulfonylamino]ethyl}phenoxyacetate (45) A mixture of **44** (3.8 g, 9.7 mmol), cysteamine hydrochloride (1.1 g, 9.7 mmol) and EtOH (50 ml) was stirred for 3 days at room temperature under an argon atmosphere. The reaction mixture was evaporated *in vacuo* and the residue was purified by flash chromatography using hexane–EtOAc (2:1) to give **45** (3.35 g, 82.4%) as a colorless oil. IR (neat): 3285, 1756, 1330 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.30 (3H, t, J=7 Hz), 2.6—3.1 (7H, m), 3.30 (1H, m), 4.27 (2H, q, J=7 Hz), 4.58 (2H, s), 6.45 (1H, s), 6.75 (2H, m), 6.90 (2H, m), 7.4—7.7 (3H, m), 8.08 (1H, m). MS (CI) m/z: 451 (M+H).

Ethyl 4-{2-[2-(3-Acetylthiazolidin-2-yl)phenylsulfonylamino]ethyl}-phenoxyacetate (46) A mixture of **45** (3.3 g, 7.32 mmol) and pyridine (0.6 g, 7.64 mmol) in CH_2Cl_2 (100 ml) was treated dropwise with AcCl (0.6 g, 7.64 mmol) at 0 °C. The reaction mixture was stirred for 1 h at room temperature and then washed with water, dried and evaporated *in vacuo*. The residue was purified by flash chromatography using $CHCl_3$–MeOH (50:1) to give **46** (1.54 g, 42.7%) as a colorless oil. IR (neat): 1756, 1646, 1512, 1407 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.29 and 1.30 (3H, t, J=7 Hz), 1.90 and 2.14 (3H, s), 2.80 (2H, t, J=7 Hz), 2.7—3.4 (4H, m), 4.12 (2H, m), 4.59 and 4.60 (2H, s), 4.24 and 4.25 (2H, q, J=7 Hz), 4.79 and 5.95 (1H, m), 6.80 (2H, m), 7.05 (2H, m), 6.91 and 6.97 (1H, s), 7.2—7.7 (3H, m), 7.92 (1H, m). MS (CI) m/z: 493 (M+H).

Disodium 4-{2-[2-(3-Acetylthiazolidin-2-yl)phenylsulfonylamino]-ethyl}phenoxyacetate (47) A mixture of **46** (1.45 g, 2.94 mmol), 4% NaOH (4.4 ml, 4.4 mmol) and EtOH–CH_2Cl_2 (20 ml/5 ml) was stirred for 1 h at room temperature. The reaction mixture was evaporated *in vacuo* at 40 °C and then dried over P_2O_5 under vacuum. The residue was dissolved in $CHCl_3$ and the solution was filtered through Celite. The filtrate was evaporated *in vacuo* and the residue was washed with EtOH to give **47**·EtOH·H_2O (1.17 g, 69.4%) as a colorless powder. IR (KBr): 3436, 1616, 1339 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.05 (3H, t, J=7 Hz), 1.75 and 2.10 (3H, s), 2.08 (2H, t, J=7 Hz), 3.00 (4H, m), 3.80 (1H, m), 3.42 (2H, q, J=7 Hz), 4.03 (2H, s), 4.14 (1H, m), 6.78 (2H, m), 6.90 (2H, m), 7.09 and 7.12 (1H, s), 7.2—7.4 (2H, m), 7.6—7.8 (2H, m). MS (FAB) m/z: 509 (M+H). *Anal.* Calcd for $C_{21}H_{22}N_2O_6Na_2S_2 \cdot C_2H_6O_2 \cdot H_2O$: C, 48.24; H, 5.28; N, 4.89. Found: C, 47.75; H, 4.85; N, 4.69.

Platelet Aggregation Test *in Vitro* Citrated blood (one 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 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 × $10^4/\mu l$ by dilution of PRP with PPP.

Platelet aggregation was measured by the turbidometric method of Born,[20] with an aggregometer (PA-3210, Kyoto Daiichi Kagaku and PAM-8C, Mebanix). The compound to be tested was dissolved in DMSO. Then 1 μl of the solution was added to 275 μl of PRP. The mixture was incubated 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. The mixture was examined for 5 min in an aggregometer to obtain the maximum aggregation rate. The IC_{50} value was calculated from the maximum

decrease in absorbency of the test compound-treated PRP ($n=3$—4) as compared with the vehicle-treated PRP.

TXA_2/PGH_2 Receptor Binding Assay with Guinea Pig Platelets Blood from male Hartley guinea-pigs weighing 400—500 g was collected into a syringe containing 0.2 volume of acid–citrate–indomethacin–dextrose [trisodium citrate (85 mM), citric acid (70 mM), indomethacin (10 μM) and glucose (110 mM)]. PRP was obtained by centrifugation at $160 \times g$ for 15 min. The PRP was recentrifuged at $1200 \times g$ 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 column of Sepharose 2B (60 ml) and 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 ($n=3$). After the incubation, ice-cold HEPES-Tyrode buffer (1.25 ml) was added to each tube and the reaction mixture was immediately centrifuged at 14000 rpm for 2 min. The pellet was washed, recentrifuged and resuspended in HEPES-Tyrode buffer. Aquasol 2 scintillator (8 ml) was added to the suspension and radioactivity was measured in a liquid scintillation spectrometer (LSC 3500, Aloka).

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References and Notes

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