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Articles

Design, Synthesis, and Pharmacology of 3-Substituted Sodium Azulene-1-sulfonates and Related Compounds: Non-Prostanoid Thromboxane A₂ Receptor Antagonists

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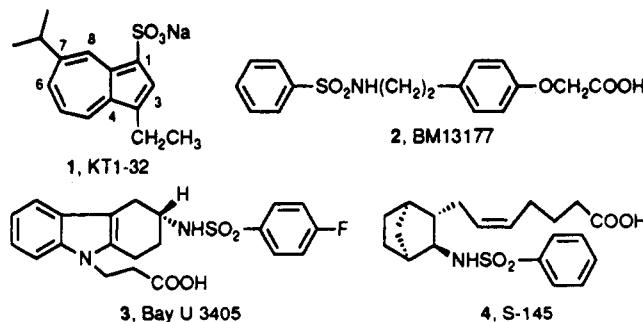
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A series of novel azulene-1 carboxylic acid derivatives 28–30, azulene-1 sulfonic acid sodium salts 41a–c, and related compounds were synthesized. These compounds were tested for TXA₂ receptor antagonistic activity. The inhibitory concentrations (IC₅₀) of these compounds for vascular contraction (TXA₂ τ receptor) and platelet aggregation (TXA₂ α receptor) induced by (15*S*)-15-hydroxy-11 α ,9 α -(epoxymethano)prosta-5(*Z*),13(*E*)-dienoic acid (U-46619) were obtained. Azulene-1-sulfonic acid sodium salts 41a–c were over 3 times more potent than azulene-1-carboxylic acids 28–30. The most potent compound, 41b, was 4 orders of magnitude more potent than a TXA₂ antagonist, BM13,177, in inhibiting vascular contraction (τ receptor) and had an IC₅₀ of 9.0×10^{-10} M. Compound 41b was also found to be a τ receptor selective antagonist (IC₅₀ of contraction/IC₅₀ of aggregation = 378) and to have no TXA₂ synthetase inhibitory activity at concentrations up to 10^{-4} M and no partial agonistic activity at concentrations up to 10^{-5} M in rabbit aorta (τ receptor) and up to 10^{-4} M in rabbit platelet-rich plasma (α receptor). In a radioligand binding assay using rabbit gel-filtered platelets, compound 41b had a high-affinity binding for the TXA₂ receptor. In an in vivo study, compound 41b inhibited U-46619-induced sudden death in mice at a dose of 0.3 mg/kg and its duration of action was over 8 h when administered orally at 3 mg/kg.

Introduction

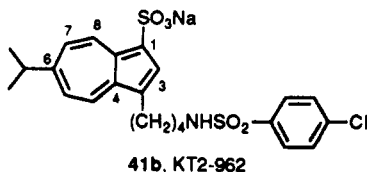
TXA₂ has been implicated in the etiology and pathology of many disorders such as coronary vasospasm,¹ myocardial ischemia,² asthma,³ and peptic ulcer.^{4,5} TXA₂ receptor antagonists may be more useful than TXA₂ synthetase inhibitors, because these compounds antagonize the effects of TXA₂ and PGH₂ and do not lead to accumulation of endoperoxide intermediates.⁶ TXA₂ receptor antagonists also do not inhibit the synthesis of vasodilating prostaglandins like prostacyclin.⁷ This implies that the synthetase inhibitors inhibit prostacyclin synthesis, which is incorrect. On the other hand, TXA₂ synthetase inhibitors are less effective in diseases in which TXA₂ may be produced.⁶

During the course of our screening for antiulceratives, we reported an azulene derivative, 3-ethyl-7-isopropylazulene-1-sulfonic acid sodium salt (KT-32, 1), as an antiulcerative agent.⁸ Recently, we have found that compound 1 has a weak TXA₂ antagonistic activity.⁹



Therefore, a derivative of compound 1 may prove to be a novel non-prostanoid TXA₂ antagonist.

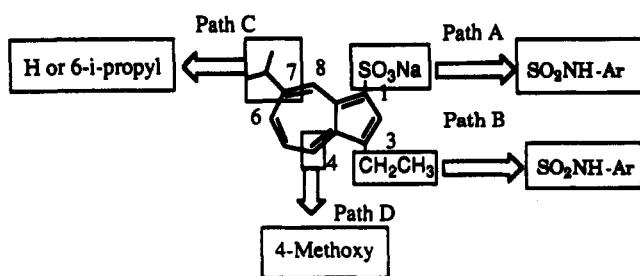
In a previous paper,¹⁰ we reported the synthesis and pharmacological activity of 3-substituted sodium azulene-1-sulfonate 41b. This compound was shown to be a selective thromboxane A₂ (TXA₂) τ -receptor¹¹ antagonist without partial agonistic activity.



In this paper, we describe the details of design, synthesis, and pharmacology of 3-substituted sodium azulene-1-sulfonate compounds which possess TXA₂ receptor antagonistic activities.

Chemistry

Modification of 1-Sodium Sulfonate (Path A) and the 3-Ethyl Group (Path B). TXA₂ receptor antagonists like BM13,177 (2),¹² S-145,¹³ and Bay U 3405 (3)¹⁴ have a sulfonamide group in the molecule and it seems that this group is necessary for the biological activity. Therefore, we introduced an arylsulfonamide group at the 1-position of compound 1 (Path A).

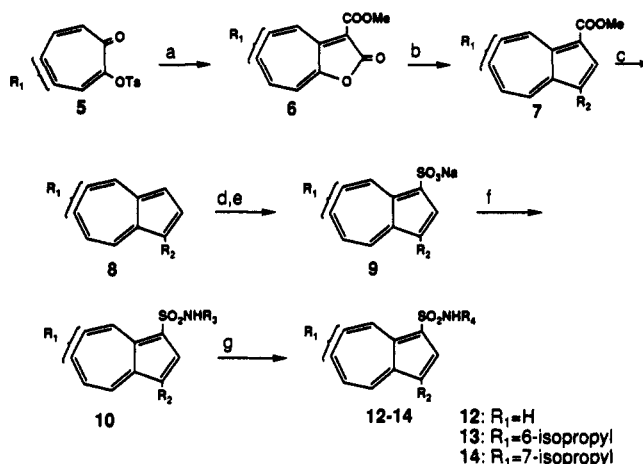


Modification of the 3-ethyl group with arylsulfonamide was accomplished by fixing the 1-position of azulene with a carboxylic acid, which was a precursor of azulene-1-sulfonic acid sodium salt as shown in Path B. In addition, the length of alkyl chain in (CH₂)_nNHSO₂-aryl at the 3-position of azulene was varied from *n* = 2 to 8 to optimize the biological activity. Furthermore, replacement of the carboxylic acid at the 1-position of azulene with CH₂COOH or CH=CHCOOH was performed to investigate whether these groups have the same activities as sodium sulfonate.

Effects of the 7-Isopropyl (Path C) and 4-Methoxy Group (Path D). To explore the effect of the 7-isopropyl group of compound 1 on TXA₂ receptor antagonistic activity, the activity of 7-isopropyl-substituted azulenes was compared with those of 6-isopropyl-substituted and unsubstituted azulenes (Path C). In the azulene chemistry, modification of the azulene ring was limited to introduction of an isopropyl group at the 6- or 7-position and a methoxy group at the 4-position (Path D).¹⁵ In order to compare the potencies of 7-unsubstituted, 7-isopropyl-, 6-isopropyl-, and 4-methoxyazulene analogues, the substituent group at the 1-position of azulene was fixed with phenoxycetic acid bearing a sulfonamide.

The synthesis of target compounds was started from the known tosyltropone 5 and Scheme I outlines the general synthesis of 6,7-unsubstituted, 6-isopropyl, or 7-isopropyl-3-alkylazulene-1-sulfonamides 12–14. We have recently reported the preparation of 7-unsubstituted and 7-isopropyl-3-alkylazulene-1-sulfonic acid sodium salt⁸ and 6-isopropyl-3-alkylazulene-1-sulfonic acid sodium salt.¹⁶ Tosyltropone 5 was reacted with dimethyl malonate in sodium methoxide to give 6- or 7-isopropyl-3-(methoxy-

Scheme I^a



R₁=H or isopropyl, R₂=alkyl, R₃=(CH₂)₂C₆H₄OCH₂COOMe
R₄=(CH₂)₂C₆H₄OCH₂COOH

^a Reagents: (a) dimethyl malonate, MeONa/MeOH, 0 °C; (b) R₂CH₂CHO, morpholine/EtOH, reflux; (c) 100% phosphoric acid, 95 °C; (d) SO₃-Py complex, benzene, reflux; (e) MeONa/MeOH; (f) SOCl₂/THF, 0 °C; R₃NH₂/HCl (11), Et₃N, THF/DMF, 0 °C; (g) 1 N KOH/EtOH.

carbonyl)-2H-cyclohepta[b]furan-2-one (6). Condensation of 6 with an in situ generated morpholino enamine of the appropriate aldehyde gave 1-(methoxycarbonyl)-azulene 7.¹⁷ Decarboxylation of 7 with 100% phosphoric acid at 95 °C yielded compound 8. Sulfonation of 8 with pyridine-sulfur trioxide complex in benzene, followed by treatment with sodium hydroxide, yielded the corresponding sulfonic acid sodium salt 9. Chlorination of 9 with thionyl chloride and subsequent amide formation with corresponding amine 11 in the usual manner yielded compound 10. Hydrolysis of 10 in 1 N KOH/EtOH gave 1-sulfonamides 12–14 (Table I).

The 4-methoxyazulene derivatives were also prepared according to the method previously reported⁸ (Scheme II). The reaction of 5 with diethyl malonate and sodium ethoxide, followed by methylation with diazomethane, yielded the corresponding 4-methoxy-3-(ethoxycarbonyl)-2H-cyclohepta[b]furan-2-one 15. Under more basic conditions using sodium ethoxide instead of sodium methoxide in this reaction, 4-hydroxy-3-(ethoxycarbonyl)-2H-cyclohepta[b]furan-2-one 15 was obtained as a major product, whereas 3-(methoxycarbonyl)-2H-cyclohepta[b]furan-2-one 6 was obtained by using sodium methoxide.¹⁸ Condensation of 15 with morpholino enamine as shown in Scheme II yielded 4-methoxy-1-(ethoxycarbonyl)azulene 16. Compounds 19 and 20 were prepared from 16 in 6 steps similar to those used for the conversion of 7 to 12 and 14 (Table I). The introduction of the sulfonylamino group on the side chain at the 3-position is shown in Scheme III. 6- or 7-isopropyl-3-(methoxycarbonyl)-2H-cyclohepta[b]furan-2-one 6 reacted with BnO(CH₂)_nCH₂CHO 21 and morpholine to give compound 22, and subsequent debenzoylation with AlCl₃ in anisole yielded compound 23. Mitsunobu reaction¹⁹ of 23 with 24, followed by treatment with hydrazine hydrate, resulted in the corresponding amino esters 26. Condensation of compound 26 with benzenesulfonyl chloride and hydrolysis with 10% NaOH-EtOH yielded compounds 28–30 (Table II). Alternatively, compound 26 was obtained by the reaction of compound 6 with CH(O)CH₂(CH₂)_n-N-phthalimide 24 followed by treatment with hydrazine hydrate.

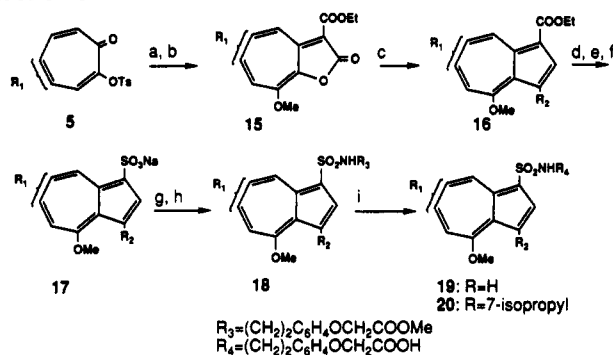
Table I. Unsubstituted and Substituted Azulene-1-sulfonamide 12–14 and 4-Methoxyazulene-1-sulfonamide 19–20

12: R₁ = H
13: R₁ = 6-isopropyl
14: R₁ = 7-isopropyl

19: R₁ = H
20: R₁ = 7-isopropyl

compd	R ₁	R ₂	mp, (°C) ^a	formula	ana. ^b	IC ₅₀ , ^c M
12a	H	CH ₃	oil	C ₂₁ H ₂₂ NO ₅ S	C, H, N	(1.9 ± 0.3) × 10 ⁻⁴
12b	H	C ₂ H ₅	147–148	C ₂₂ H ₂₃ NO ₅ S	C, H, N	(5.6 ± 0.1) × 10 ⁻⁵
12c	H	<i>n</i> -C ₃ H ₇	139–140	C ₂₆ H ₃₁ NO ₅ S	C, H, N	(7.7 ± 1.3) × 10 ⁻⁵
12d	H	<i>i</i> -C ₃ H ₇	oil	C ₂₃ H ₂₅ NO ₅ S	C, H, N	(1.6 ± 0.5) × 10 ⁻⁴
12e	H	<i>n</i> -C ₄ H ₉	oil	C ₂₃ H ₂₅ NO ₅ S	C, H, N	(5.5 ± 0.5) × 10 ⁻⁵
13a	6-isopropyl	CH ₃	58–59	C ₂₄ H ₂₇ NO ₅ S	C, H, N	(8.4 ± 0.4) × 10 ⁻⁵
13b	6-isopropyl	C ₂ H ₅	58–59	C ₂₅ H ₂₉ NO ₅ S	C, H, N	(6.5 ± 0.6) × 10 ⁻⁵
13c	6-isopropyl	<i>n</i> -C ₃ H ₇	59–60	C ₂₆ H ₃₁ NO ₅ S	C, H, N	(5.0 ± 0.2) × 10 ⁻⁵
13d	6-isopropyl	<i>i</i> -C ₃ H ₇	58–59	C ₂₆ H ₃₁ NO ₅ S	C, H, N	(2.5 ± 0.3) × 10 ⁻⁵
13e	6-isopropyl	<i>n</i> -C ₄ H ₉	61–62	C ₂₇ H ₃₃ NO ₅ S	C, H, N	(1.9 ± 0.2) × 10 ⁻⁵
14a	7-isopropyl	CH ₃	oil	C ₂₄ H ₂₇ NO ₅ S	C, H, N	(1.2 ± 0.1) × 10 ⁻⁴
14b	7-isopropyl	C ₂ H ₅	oil	C ₂₅ H ₂₉ NO ₅ S	C, H, N	(5.6 ± 0.5) × 10 ⁻⁵
14c	7-isopropyl	<i>n</i> -C ₃ H ₇	oil	C ₂₆ H ₃₁ NO ₅ S	C, H, N	(4.5 ± 0.5) × 10 ⁻⁵
14d	7-isopropyl	<i>n</i> -C ₄ H ₉	oil	C ₂₇ H ₃₃ NO ₅ S	C, H, N	(3.2 ± 0.2) × 10 ⁻⁵
19a	H	CH ₃	134–135	C ₂₂ H ₂₃ NO ₆ S	C, H, N	(1.9 ± 0.1) × 10 ⁻⁴
19b	H	C ₂ H ₅	132	C ₂₃ H ₂₅ NO ₆ S	C, H, N	(1.1 ± 0.1) × 10 ⁻⁴
20a	7-isopropyl	C ₂ H ₅	79–80	C ₂₆ H ₃₁ NO ₆ S	C, H, N	(1.2 ± 0.1) × 10 ⁻⁴

^a Melting points are uncorrected ^b C, H, N analyses were within ±0.4% of the calculated values. ^c Inhibition of U-46619 (3 × 10⁻⁸ M) induced contraction of rat aorta IC₅₀ values represent the mean ± SEM and were calculated by regression analysis from the three dose groups of four different preparations.

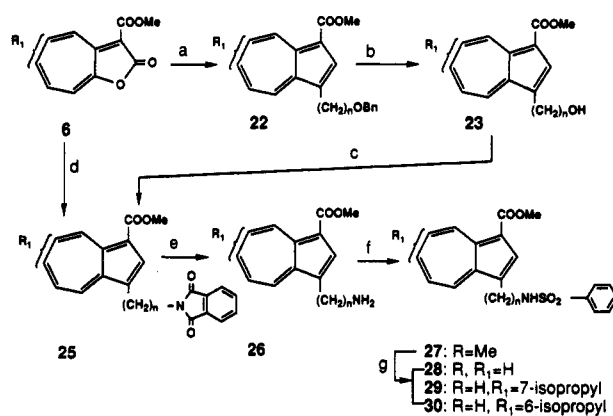
Scheme II^a

^a Reagents: (a) diethyl malonate, EtONa/EtOH, 0 °C; (b) CH₂N₂/THF; (c) R₂CH₂CHO, morpholine/EtOH, reflux; (d) 100% phosphoric acid, 95 °C; (e) SO₃-Py complex, benzene, reflux; (f) MeONa/MeOH; (g) SOCl₂/THF, 0 °C; (h) R₃NH₂·HCl (11), Et₃N, THF/DMF, 0 °C; (i) 1 N KOH/EtOH.

The homologation of the carboxylic acid at the 1-position is shown in Scheme IV. Demethoxycarbonylation of 27 with 100% phosphoric acid yielded 31, which upon homologation using Anderson's stepwise construction²⁰ yielded azulene-1-acetic acid 34. Compound 31 was formylated by Vielsmeier-Haack reaction,²¹ subjected to Horner-Emmons reaction²² with triethyl phosphonoacetate, followed by hydrolysis with 10% NaOH, and yielded α,β-unsaturated acid 37 (Table III). The compounds with a phenyl group or an olefin were also prepared 38–40.

Compound 38 was prepared as shown in Scheme V. The key aldehyde 47 was prepared in a four-step sequence from 43, prepared from reduction of 42. 43 was subjected to Wittig reaction to give olefin 44, followed by benzylation to give 45. Then, 45 was subjected to hydrogenation and oxidation to give 47. 47 was condensed with 6 in a same manner outlined in Scheme I to give target compound 38.

Preparation of *cis*- and *trans*-olefin compounds 39 and 40 is summarized in Scheme VI. The aldehyde intermediates 50 and 53 were obtained after PCC oxidation of

Scheme III^a

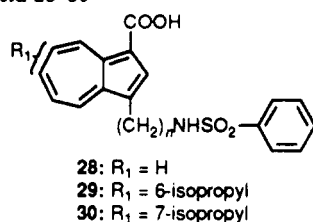
^a Reagents: (a) BnO(CH₂)_nCH₂CHO (21), morpholine/EtOH, reflux; (b) AlCl₃/anisole, 0 °C; (c) phthalimide, Ph₃P, EtOOCN=NCOOEt/THF, 0 °C; (d) CH(O)CH₂(CH₂)_nNpht (24), morpholine/EtOH; (e) NH₂NH₂·H₂O/EtOH; (f) ClSO₂Ph, NaHCO₃/acetone-H₂O; (g) 10% NaOH/MeOH.

reported *cis*-alcohol 49²³ and *trans*-alcohol 52.²⁴ 50 and 53 were reacted with 6 in an analogous manner in Scheme I to give 39 and 40.

Results and Discussion

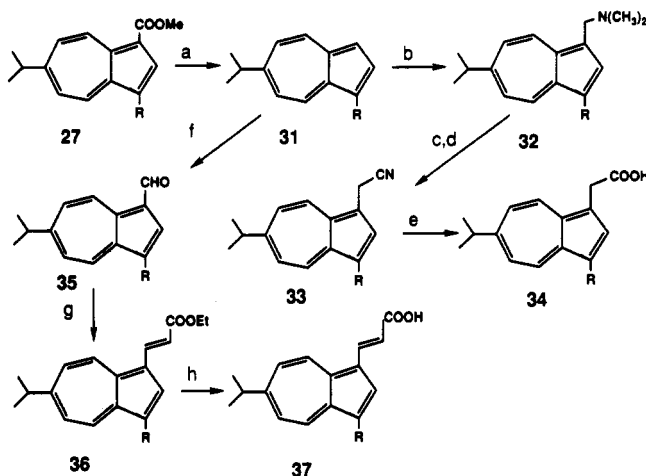
The compounds described herein were initially tested for their thromboxane receptor antagonistic activities. They were tested in isolated rat thoracic aorta precontracted by the TXA₂ mimetic (15*S*)-hydroxy-11α,9α-(epoxymethano)prosta-5(*Z*),13(*E*)-dienoic acid, U-46619²⁵ (3 × 10⁻⁹ M). The concentrations that caused 50% relaxation are shown in Tables I–III. The modification of the 1-sulfonic acid group of KT1-32 to a 1-sulfonamide (12–14) did not affect TXA₂ receptor antagonistic activity and there was no difference in the activity among the unsubstituted compounds 12a–e, the 6-substituted derivatives 13a–e, and the 7-substituted analogues 14a–d (Table I). At the same time, there appears to be no

Table II. 3-(Sulfonylamino)azulene-1-carboxylic Acid 28-30



compd	R_1	n	mp, ($^{\circ}C$) ^a	formula	anal. ^b	IC ₅₀ , ^c M
28a	H	2	193-195	C ₁₉ H ₁₇ NO ₄ S	C, H, N, S	$(2.1 \pm 0.3) \times 10^{-5}$
28b	H	3	187-188	C ₂₀ H ₁₉ NO ₄ S	C, H, N, S	$(4.8 \pm 0.7) \times 10^{-6}$
28c	H	4	168-169	C ₂₁ H ₂₁ NO ₄ S	C, H, N, S	$(5.7 \pm 0.6) \times 10^{-7}$
28d	H	5	170-171	C ₂₂ H ₂₃ NO ₄ S	C, H, N, S	$(8.4 \pm 3.1) \times 10^{-7}$
28e	H	6	168-169	C ₂₃ H ₂₅ NO ₄ S	C, H, N, S	$(1.4 \pm 0.1) \times 10^{-7}$
28f	H	7	143-144	C ₂₄ H ₂₇ NO ₄ S	C, H, N, S	$(1.8 \pm 0.1) \times 10^{-8}$
28g	H	8	182-184	C ₂₅ H ₂₉ NO ₄ S	C, H, N, S	$(3.8 \pm 0.4) \times 10^{-8}$
29a	6-isopropyl	2	165-166	C ₂₂ H ₂₃ NO ₄ S	C, H, N, S	$(6.0 \pm 0.1) \times 10^{-5}$
29b	6-isopropyl	3	120-122	C ₂₃ H ₂₅ NO ₄ S	C, H, N, S	$(4.8 \pm 0.1) \times 10^{-6}$
29c	6-isopropyl	4	167-168	C ₂₄ H ₂₇ NO ₄ S	C, H, N, S	$(7.0 \pm 0.6) \times 10^{-8}$
29d	6-isopropyl	5	75-76	C ₂₅ H ₂₉ NO ₄ S	C, H, N, S	$(1.2 \pm 0.2) \times 10^{-7}$
29e	6-isopropyl	6	117-118	C ₂₆ H ₃₁ NO ₄ S	C, H, N, S	$(7.0 \pm 0.5) \times 10^{-8}$
29f	6-isopropyl	7	119-120	C ₂₇ H ₃₃ NO ₄ S	C, H, N, S	$(1.2 \pm 0.2) \times 10^{-7}$
30a	7-isopropyl	2	166-167	C ₂₂ H ₂₃ NO ₄ S	C, H, N, S	$(1.5 \pm 0.1) \times 10^{-5}$
30b	7-isopropyl	3	78-80	C ₂₃ H ₂₅ NO ₄ S	C, H, N, S	$(7.4 \pm 0.1) \times 10^{-6}$
30c	7-isopropyl	4	142-143	C ₂₄ H ₂₇ NO ₄ S	C, H, N, S	$(3.1 \pm 0.2) \times 10^{-6}$
30d	7-isopropyl	5	146-147	C ₂₅ H ₂₉ NO ₄ S	C, H, N, S	$(2.6 \pm 0.1) \times 10^{-6}$
30e	7-isopropyl	6	85-88	C ₂₆ H ₃₁ NO ₄ S	C, H, N, S	$(9.2 \pm 0.8) \times 10^{-7}$
30f	7-isopropyl	7	124-125	C ₂₇ H ₃₃ NO ₄ S	C, H, N, S	$(9.3 \pm 1.2) \times 10^{-7}$

^a Melting points are uncorrected ^b C, H, N analyses were within $\pm 0.4\%$ of the calculated values. ^c Inhibition of U-46619 (3×10^{-8} M) induced contraction of rat aorta. IC₅₀ values represent the mean \pm SEM and were calculated by regression analysis from the three dose groups of four different preparations.

Scheme IV^a

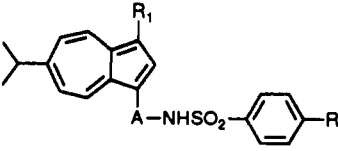
^a Reagents: (a) 100% phosphoric acid, 120 $^{\circ}C$; (b) $(Me)_2NCH_2N(Me)_2$, $(HCHO)_n$, AcOH; (c) MeI; (d) KCN; (e) 0.6 N KOH; (f) $POCl_3$, DMF; (g) $(EtO)_2P(O)CH_2COOEt$, NaH; (h) 10% NaOH; (i) $SO_3 \cdot Py$ complex; (j) MeONa/MeOH.

correlation between TXA₂ receptor antagonistic activity and the increase in alkyl chain length at the 3-position of these compounds. The introduction of a 4-methoxy group to the compounds with 1-sulfonamides (19) caused a loss in TXA₂ receptor antagonistic activity (IC₅₀) of about 1 order of magnitude from 10^{-5} to 10^{-4} M.

As shown in Table II, the introduction of an (sulfonylamino)alkyl group at the 3-position of compound 1 gave a stepwise increase in activity as the number of methylenes was increased. For example, with compounds 28a-g, the maximum activity was obtained when the alkyl chain was heptyl ($n = 7$, IC₅₀ = 1.8×10^{-8} M). In the 6-isopropyl-substituted compounds 29a-f, the maximum activity was obtained when the alkyl group was butyl ($n = 4$, 29c, IC₅₀ = 7.0×10^{-8} M). Further homologation of the alkyl chain

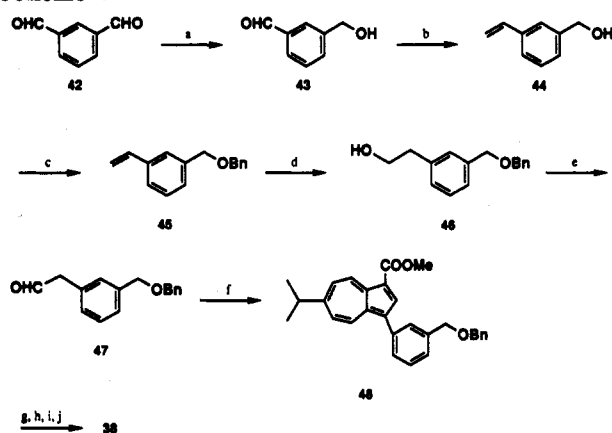
to heptyl ($n = 7$) did not increase the activity. The activities of 7-isopropyl-substituted compounds 30a-f were weaker than those of unsubstituted compounds 28a-g and 6-isopropyl-substituted compounds 29a-f, and a clear activity relationship with the lengthening of the alkyl chain was not demonstrated. Among the series of unsubstituted, 6-substituted, and 7-substituted compounds, 6-substituted compounds were selected for further optimization. The activities of 6-substituted compounds 29a-f correlated to alkyl-chain length better than those of unsubstituted or 7-isopropyl-substituted compounds. Among the 6-substituted compounds, the greatest activity was found in compound 29c as compared to the shorter and the longer chain compounds 29b and 29d (Table II). This suggests that there is an optimal chain length of carbon atoms for the maximum biological activity. Compound 29c was selected for further modification. As shown in Table III, unsaturation of the $(CH_2)_4$ side chain at the 3-position, to yield vinyl- or phenyl-containing compounds 38-40 failed to increase the potency, though cis-olefinic compound 39 retained the same potency as 29c. Addition of the electron-withdrawing and -donating substituents to the phenyl group of 29c (compounds 29g and 29h) also increased the potencies about 70 and 4 times compared to the potency of compound 29c. Replacement of the carboxylic acid at the 1-position of azulene (29c and 29g) with sulfonic acid sodium salt (41a, IC₅₀ = 1.7×10^{-8} M, and 41b, IC₅₀ = 9×10^{-10} M) increased the potency over 3 times compared to that of 29c. Replacement of the carboxylic group with sodium sulfonate seemed to be useful for providing TXA₂ antagonists. To our knowledge, this is the first example of the use of the sodium sulfonate group to prepare TXA₂ receptor antagonists. Lee et al.²⁶ reported that a neutral ion pair drug could permeate through hydrophobic membranes due to its lipophilicity, and 41a,b might be well-

Table III. Structure and Pharmacological Activity in Vitro



compd	R ₁	A	R	formula ^a	IC ₅₀ , ^b M
34	CH ₂ COOH	(CH ₂) ₄	H	C ₂₅ H ₂₉ NO ₄ S	(3.0 ± 0.7) × 10 ⁻⁷
37	<i>trans</i> -CH=CHCOOH	(CH ₂) ₄	H	C ₂₆ H ₂₉ NO ₄ S	(2.1 ± 0.64) × 10 ⁻⁶
38	COOH	C ₆ H ₄ - <i>m</i> -(CH ₂) ₂	H	C ₂₇ H ₂₅ NO ₄ S	(1.4 ± 0.1) × 10 ⁻⁷
39	COOH	<i>cis</i> -(CH ₂) ₂ CH=CH(CH ₂) ₂	H	C ₂₄ H ₂₅ NO ₄ S	(5.2 ± 0.02) × 10 ⁻⁸
40	COOH	<i>trans</i> -(CH ₂) ₂ CH=CH(CH ₂) ₂	H	C ₂₄ H ₂₅ NO ₄ S	(4.6 ± 0.3) × 10 ⁻⁷
29g	COOH	(CH ₂) ₄	<i>p</i> -Cl	C ₂₅ H ₂₈ ClNO ₄ S	(5.3 ± 0.1) × 10 ⁻⁹
29h	COOH	(CH ₂) ₄	<i>p</i> -OMe	C ₂₆ H ₃₁ NO ₅ S	(1.6 ± 0.03) × 10 ⁻⁷
29i	COOH	(CH ₂) ₄	<i>p</i> -F	C ₂₅ H ₂₈ FNO ₄ S	(1.1 ± 0.1) × 10 ⁻⁸
29j	COOH	(CH ₂) ₄	<i>m</i> -CF ₃	C ₂₆ H ₂₈ F ₃ NO ₄ S	(1.6 ± 0.03) × 10 ⁻⁶
41a	SO ₃ Na	(CH ₂) ₄	H	C ₂₃ H ₂₆ NNaO ₅ S	(1.7 ± 0.04) × 10 ⁻⁸
41b (KT2-962)	SO ₃ Na	(CH ₂) ₄	<i>p</i> -Cl	C ₂₃ H ₂₅ ClNNaO ₅ S	(9.0 ± 0.7) × 10 ⁻¹⁰
41c	SO ₃ Na	(CH ₂) ₄	<i>p</i> -OMe	C ₂₄ H ₂₇ NNaO ₆ S	(3.2 ± 0.8) × 10 ⁻⁹
BM13,177					(1.5 ± 0.1) × 10 ⁻⁶

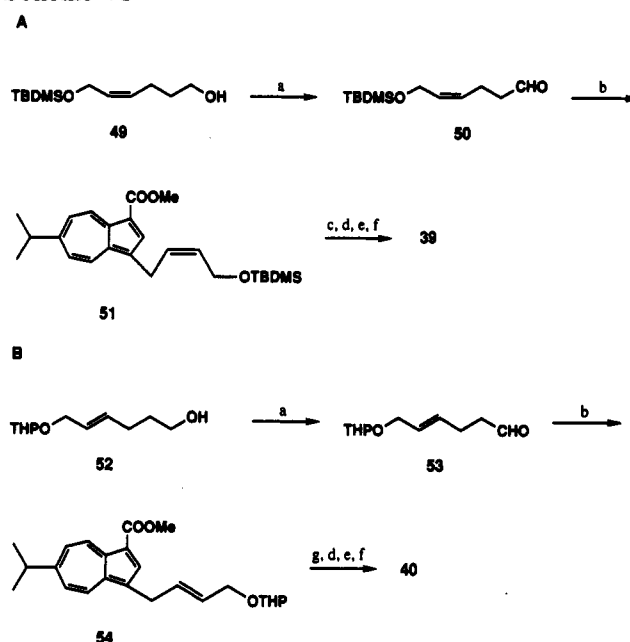
^a C, H, N analyses were with ±0.4% of the calculated values. ^b Inhibition of U-46619 (3 × 10⁻⁸ M) induced contraction of rat aorta. IC₅₀ values represent the mean ± SEM and were calculated by regression analysis from the three dose groups of four different preparations.

Scheme V^a

^a Reagents: (a) NaBH₄, THF, -45 °C, 67%; (b) methyltriphenylphosphonium bromide, *t*-BuOK, THF, rt, 91%; (c) benzyl bromide, NaH, DMF, rt, 94%; (d) (i) 9-BBN, THF, rt; (ii) 6 N NaOH, 31% H₂O₂, rt, 82%; (e) SO₃-Py, NEt₃, DMSO, rt, 91%; (f) 6, morpholine, EtOH, reflux, 39%; (g) AlCl₃, anisole, 0 °C, 87%; (h) PPh₃, CBr₄, CH₂Cl₂, 0 °C, 96%; (i) benzenesulfonamide, NaH, THF-HMPA, reflux, 34%; (j) 10% NaOH, MeOH, reflux, 45%.

absorbed from the gastrointestinal tract if this concept of ion formation is correct.

Extension of the carbon chain of the azulene nucleus 1-position (compounds 34 and 37) decreased the activities. Compound 41b was the most potent inhibitor of U-46619-induced contraction in rat aorta (IC₅₀ = 9.0 × 10⁻¹⁰ M), the pA₂²⁷ value of 41b being 9.78 (Schild analysis:²⁸ slope = 1.04, *n* = 4), and also had no inhibitory effect up to 10⁻⁵ M on contractile responses induced by norepinephrine, Ca²⁺, histamine, or serotonin. On the basis of these findings, 41b is a specific TXA₂ receptor antagonist. Needleman et al. first reported that platelet and vascular TXA₂ receptors might be different,²⁹ and mediation by the α receptor for aggregation and τ receptor for vasoconstriction has been postulated.¹¹ The selectivities of these compounds for τ (rabbit aorta) versus α [rabbit platelet-rich plasma (PRP)] receptor are shown in Table IV. In this comparison, 29g, 29h, 41b, and 41c are >667, >233, 378, and 660 times more potent against the τ receptor than the α receptor and are more selective for the τ receptor (higher indices) than BM13,177 in an assay. These findings are in agreement with the report that non-prostanoid TXA₂

Scheme VI^a

^a Reagents: (a) PCC, AcONa, CH₂Cl₂, 0 °C; (b) 6, morpholine, EtOH, reflux; (c) *n*-Bu₄NF, THF, rt; (d) PPh₃, CBr₄, CH₂Cl₂, 0 °C; (e) benzenesulfonamide, NaH, THF-HMPA, reflux; (f) 10% NaOH, MeOH, reflux; (g) AcOH, H₂O, THF, 45 °C.

antagonists have different structural requirements for α versus τ receptors.³⁰ A critical part in the development of TXA₂ receptor antagonists is whether these agents possess a partial agonistic activity,³¹ and efforts to diminish the partial agonistic activity of TXA₂ receptor antagonists have been described.³² As shown in Table V, section A, these compounds were evaluated as to whether they possess any partial agonistic activity in rabbit aorta (τ receptor) and in rabbit PRP (α receptor). These compounds had neither partial τ receptor agonistic activities at concentrations up to 10⁻⁵ M in rabbit aorta nor partial α receptor agonistic activities at concentrations up to 10⁻⁴ M in rabbit PRP, although other prostanoid TXA₂ receptor antagonists tend to be partial agonists.³³⁻³⁵

For further characterization of 41b,c, the TXA₂ synthetase inhibitor activities of these compounds were examined. 41b and 41c had no effect on TXA₂ formation in human platelets when evaluated at concentrations up

Table IV. Inhibition of U-46619-Induced Rabbit Aorta Contraction and Rabbit Platelet Aggregation

compd	R ₁	R	IC ₅₀ , ^a M		selectivity index: ^d vascular
			cont ^b	aggrn ^c	
29c	COH	H	(1.6 ± 0.1) × 10 ⁻⁶	>10 ⁻⁴	>63
29g	COOH	Cl	(1.5 ± 0.1) × 10 ⁻⁷	>10 ⁻⁴	>667
29h	COOH	OMe	(4.3 ± 0.5) × 10 ⁻⁷	>10 ⁻⁴	>233
41a	SO ₃ Na	H	(1.1 ± 0.1) × 10 ⁻⁶	>10 ⁻⁴	>91
41b (KT2-962)	SO ₃ Na	Cl	(2.3 ± 0.5) × 10 ⁻⁸	(8.7 ± 0.5) × 10 ⁻⁶	378
41c	SO ₃ Na	OMe	(9.7 ± 1.3) × 10 ⁻⁸	(6.4 ± 0.7) × 10 ⁻⁵	660
BM13,177			(1.3 ± 0.2) × 10 ⁻⁶	(7.1 ± 0.4) × 10 ⁻⁶	5

^a IC₅₀ values represent the mean ± SEM and were calculated by regression analysis from the three dose groups of four different preparations.

^b Contraction of rabbit aorta was induced by 3 × 10⁻⁸ M of U-46619. ^c Aggregation of rabbit platelet-rich plasma was induced by 2.5 × 10⁻⁶ M U-46619. ^d The selectivity index for the τ receptor was the difference between the IC₅₀ values in the aggregation and contraction assays.

Table V

A. Evaluation of Partial Agonistic Activities		
compd	rabbit aorta ^a	rabbit PRP ^b
29h	0	0
41a	0	0
41b (KT2-962)	0	0
41c	0	0

B. Effects of 41b and 41c on TXB ₂ Formation of Human Platelets	
compd	IC ₅₀ , ^c M
41b (KT2-962)	>10 ⁻⁴
41c	>10 ⁻⁴

C. Displacement of the Specific Binding of [³ H]SQ29548 ^b to Rabbit Gel-Filtered Platelets	
compd	IC ₅₀ , ^c M
41b (KT2-962)	1.4 × 10 ⁻⁸

^a Relative values (the value of 40 mM KCl is taken as 100). The concentration of each compound is 10⁻⁵ M. ^b Relative values (the value of 0.1 μ M U-46619 is taken as 100). The concentration of each compound is 10⁻⁴ M. ^c The concentration which caused 50% inhibition of TXB₂ formation. ^d SQ29,548 is a TXA₂/PGH₂ receptor antagonist; see ref 36. ^e IC₅₀ values were calculated as per ref 36 with slight modifications.

to 10⁻⁴ M (Table V, section B). In a radioligand binding assay with rabbit gel-filtered platelets,³⁶ compound 41b inhibited the binding of SQ 29,548³⁷ with a IC₅₀ value of 0.014 μ M and possessed high affinity for the TXA₂ receptor (Table V, section C).

In an in vivo experiment of U-46619-induced sudden death in mice, a minimum orally effective dose of 41b was 0.3 mg/kg and the duration of the effect was over 8 h at a dose of 3 mg/kg, whereas the effective duration of sulotroban was 4 h at a dose of 30 mg/kg (Figure 1). Since U-46619-induced mortality in mice may be due to a combination of vasoconstriction and thrombosis,³⁸ these results suggest that 41b prevents TXA₂-induced vasoconstriction and thrombosis.

Conclusion

In summary, we have shown the modification of 7-isopropyl-3-ethylazulene-1-sulfonic acid (compound 1) and the effects of a 7-isopropyl group and the introduction of a sulfonamide group at the 3-position of compound 1. 6-Isopropyl-substituted series having a sulfonamide group at the 3-position of the azulene ring showed more potent TXA₂ receptor antagonistic activities. After the optimi-

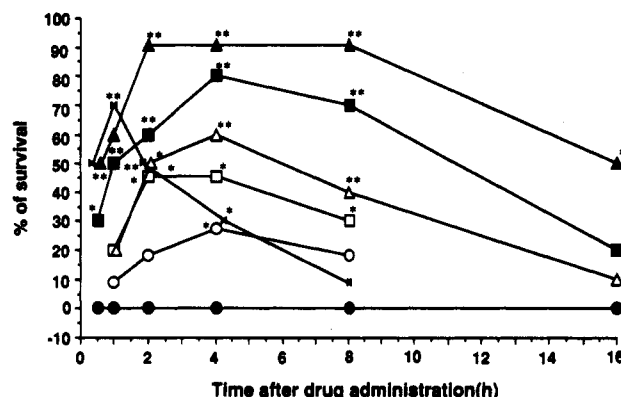


Figure 1. Protective effects of 41b (KT2-962) and BM13,177 against U46619-induced sudden death in mice; ●, control; ○, 0.3 mg/kg; □, 1 mg/kg; △, 3 mg/kg; ■, 10 mg/kg; ▲, 30 mg/kg; ×, 30 mg/kg BM13,177. Each drug orally administered prior to injection of U46619 (800 μ g/kg). At the indicated time after administration of drug, U46619 was injected intravenously and the incidence of death within 5 min was determined. Each point represents the survival percent in a group of 10–20 mice. **P* < 0.05 and ***P* < 0.01, compared with control group.

zation of the 6-substituted series, compound 41b was found to be the most potent of this series and also found to be a τ receptor selective TXA₂ antagonist with no partial agonistic and TXA₂ synthetase activities. In an in vivo study, compound 41b inhibited U-46619-induced sudden death and had a long duration of action. Due to its in vivo activity, compound 41b has been selected for clinical evaluation.

Experimental Section

Analysis. Melting points were recorded on a Yamato MP-21 apparatus and are uncorrected. ¹H-NMR spectra were recorded on a Hitachi R-90H (90-MHz) spectrometer. Chemical shifts were expressed in parts per million relative to internal tetramethylsilane (δ = 0) or chloroform (δ = 7.26). IR spectra were obtained on a Hitachi 270-30 spectrophotometer. Mass spectra were recorded with a Hitachi M-80B mass spectrometer. Thin-layer chromatography (TLC) was carried out on 0.25-mm precoated silica gel plates from E. Merck (60F-254) with UV light and/or 7% phosphomolybdic acid in ethanol-heat as visualizing agent. Column chromatography was conducted by using silica gel (Fuji Devision BW-200, 150–325 mesh).

Solvents. Ether and THF were distilled over sodium-benzophenone ketyl under an Ar atmosphere. CH₂Cl₂, toluene, triethylamine, pyridine, and DMSO were distilled over CaH₂ under an Ar atmosphere.

N-[2-[4-(carboxymethoxy)phenyl]ethyl]-3-methylazulene-1-sulfonamide (10a). A solution of 1.50 g (6.14 mmol) of 3-methylazulene-1-sulfonic acid sodium salt³⁹ in 20.0 mL of DMF at 0 °C was treated with 1.18 mL (18.4 mmol) of SOCl₂. The mixture was stirred for 30 min at 0 °C and then treated with a solution of 1.59 g (6.14 mmol) of ethyl 4-(2-aminoethyl)-phenoxyacetate hydrochloride in 20 mL of DMF and 20.7 mL (100.0 mmol) of Et₃N and stirred for 1 h at 0 °C. The mixture was poured into ice-water, extracted with ethyl acetate, washed with 10% aqueous HCl, saturated aqueous NaHCO₃, water, and brine, and dried over MgSO₄. Filtration and evaporation of the solvent under reduced pressure provided the crude sulfonamide, which was chromatographed on silica gel. Elution with EtOAc-*n*-hexane (1:1) gave 1.97 g (75.0% yield) of 10a as a violet oil: IR (neat) 3274, 2968, 2920, 1746, 1443, 1419, 1386, 1299, 1197, 1146, 1125 cm⁻¹; ¹H NMR (CDCl₃) δ = 1.31 (t, 3 H), 2.43 (s, 3 H), 2.75 (t, 2 H), 3.15 (q, 2 H), 4.27 (q, 2 H), 4.48 (bt, 1 H), 4.52 (s, 2 H), 7.27 (q, 4 H), 7.38 (t, 2 H), 7.74 (d, 1 H), 7.98 (s, 1 H), 8.37 (d, 1 H), 9.02 (d, 1 H); MS *m/z* 427 (M⁺).

N-[2-[4-(Carboxymethoxy)phenyl]ethyl]-3-methylazulene-1-sulfonamide (12a). To a solution of 1.90 g (4.44 mmol) of ester in 20 mL of MeOH was added 15.0 mL of 1 N aqueous KOH at 0 °C, and the mixture was stirred for 1 h. The mixture was adjusted to pH 7.0 with 10% aqueous HCl; the solvent was removed under reduced pressure. The pH was adjusted to pH 2-3 with 10% aqueous HCl, and the solution was extracted with EtOAc. The EtOAc extract was washed with water and brine and dried over MgSO₄. Filtration and evaporation of the solvent under reduced pressure provided the crude acid, which was chromatographed on silica gel. Elution with CHCl₃-MeOH (10:1) gave 1.73 g (97.4% yield) of 12a as violet crystals: mp 38-39 °C, IR (KBr) 3016, 2926, 1734, 1518, 1299, 1215, 1146 cm⁻¹; ¹H NMR (DMSO-*d*₆) δ = 2.50 (s, 3 H), 2.54 (t, 2 H), 3.06 (t, 2 H), 4.55 (s, 2 H), 6.55-6.94 (m, 4 H), 7.44 (q, 2 H), 7.80 (d, 1 H), 7.97 (s, 1 H), 8.46 (d, 1 H), 9.02 (d, 1 H); MS *m/z* 399 (M⁺).

Methyl 6-Isopropyl-3-(4-phthalimidobutyl)azulene-1-carboxylate (25c). To a stirred, ice-cold solution of 0.50 g (1.66 mmol) of methyl 6-isopropyl-3-(4-hydroxybutyl)azulene-1-carboxylate were added 0.54 g (3.67 mmol) of phthalimide and 0.963 g (3.67 mmol) of triphenylphosphine in 5.0 mL of THF, followed by 0.64 g (3.67 mmol) of diethyl azodicarboxylate. The mixture was allowed to warm to room temperature and stirred for 12 h. Removal of the solvent under reduced pressure followed by purification by chromatography on a silica gel column with ether-*n*-hexane (1:2) gave 0.68 g (96.8% yield) of 25c as a violet oil: IR (neat) 3004, 2950, 1767, 1704, 1578, 1446, 1398, 1368, 1218, 1050 cm⁻¹; ¹H NMR (CDCl₃) δ = 1.37 (d, 6 H), 1.66-1.91 (m, 4 H), 2.90-3.24 (t + m, 3 H), 3.74 (t, 2 H), 3.82 (s, 3 H), 7.20-7.90 (m, 6 H), 8.09 (s, 1 H), 8.32 (d, 1 H), 9.45 (d, 1 H); MS *m/z* 429 (M⁺).

Methyl 3-[4-[(Phenylsulfonyl)amino]butyl]-6-isopropylazulene-1-carboxylate (27c). To a solution of 0.68 g (1.59 mmol) of phthalimide in 5.0 mL of EtOH was added 0.20 g (3.98 mmol) of hydrazine hydrate, and mixture was refluxed for 2 h. The reaction mixture was cooled to room temperature, diluted with CHCl₃, and filtered. The filtrate was concentrated under reduced pressure to give an oily product which was dissolved in 10 mL of acetone. To this stirring solution at 0 °C were added a solution of 0.16 g (1.91 mmol) of NaHCO₃ in 5 mL of water and then dropwise a solution of 0.25 mL (1.91 mmol) of benzenesulfonyl chloride in 5.0 mL of acetone. The mixture was stirred at 0 °C for 30 min and the organic was removed under reduced pressure. The remaining aqueous solution was extracted with ethyl acetate. The ethyl acetate extract was washed with water and brine and dried over MgSO₄. Filtration and evaporation of the solvent under reduced pressure provided the crude sulfonamide, which was chromatographed on silica gel. Elution with ethyl acetate-*n*-hexane (1:4) gave 0.79 g (98.9% yield) of 27c as violet crystals: mp 79-80 °C; IR (KBr) 3320, 3010, 1677, 1449, 1422, 1326, 1215, 1158 cm⁻¹; ¹H NMR (CDCl₃) δ = 1.35 (d, 6 H), 1.20-1.93 (m, 4 H), 2.80-3.27 (t + m, 5 H), 3.90 (s, 3 H), 4.69 (bt, 1 H), 7.17-7.89 (m, 7 H), 8.00 (s, 1 H), 8.22 (d, 1 H), 9.43 (d, 1 H); MS *m/z* 440 (M⁺).

3-[4-[(Phenylsulfonyl)amino]butyl]-6-isopropylazulene-1-carboxylic Acid (29c). To a solution of 1.10 g (2.50 mmol) of 27c in 22.0 mL of MeOH was added 11.0 mL of 10% aqueous

NaOH, and the mixture was refluxed for 4 h. The mixture was cooled to room temperature, with removal of solvent under reduced pressure. The aqueous layer was washed with CHCl₃, and then the solution was adjusted to pH 2-3 with 10% aqueous HCl and extracted with ethyl acetate. The ethyl acetate was washed with water and brine and dried over MgSO₄. Filtration and evaporation of the solvent under reduced pressure provided the crude oil, which was chromatographed on silica gel. Elution with ethyl acetate-*n*-hexane (2:1) gave 0.89 g (83.6% yield) of 29c as violet crystals: mp 167-168 °C; IR (KBr) 3274, 2950, 1653, 1581, 1461, 1326, 1248, 1155 cm⁻¹; ¹H NMR (DMSO-*d*₆) δ = 1.35 (d, 6 H), 1.20-1.80 (m, 4 H), 2.62-3.40 (t + m, 5 H), 3.30 (bt, 1 H), 7.31-7.84 (m, 7 H), 7.98 (s, 1 H), 8.37 (d, 1 H), 9.36 (d, 1 H), 12.01 (bs, 1 H); MS *m/z* 381 (M⁺ - 44).

1-[4-[(Phenylsulfonyl)amino]butyl]-6-isopropylazulene (31). A mixture of 1.53 g (3.46 mmol) of 27 (R = (CH₂)₄-NHSO₂Ph, R₁ = 6-isopropyl) and 7.5 mL of anhydrous phosphoric acid was heated and stirred at 120 °C for 20 min. The reaction mixture was then poured into ice-water and extracted with ethyl acetate. The ethyl acetate extract was washed with water and dried over MgSO₄. Filtration and evaporation of the solvent under reduced pressure provided the crude product, which was chromatographed on silica gel. Elution with ethyl acetate-*n*-hexane (1:3) gave 1.23 g (93.1% yield) of 31 as violet crystals: mp 75-76 °C; IR (KBr) 3236, 2920, 1575, 1446, 1326, 1152, 1062 cm⁻¹; ¹H NMR (CDCl₃) δ = 1.33 (d, 6 H), 1.20-1.95 (m, 4 H), 2.76-3.20 (t + m, 5 H), 4.35 (bt, 1 H), 6.98 (d, 2 H), 7.11-7.90 (s + m, 7 H), 8.00 (d, 1 H), 8.17 (d, 1 H); MS *m/z* 381 (M⁺).

3-[4-[(Phenylsulfonyl)amino]butyl]-6-isopropylazulene-1-sulfonic Acid Sodium Salt (41b). A mixture of 0.50 g (1.31 mmol) of 31 and 0.42 g (2.62 mmol) of SO₃-pyridine complex in 10 mL of benzene was refluxed for 3 h. The solvent was removed under reduced pressure, and the residue was dissolved in 10 mL of MeOH. Next, 0.25 mL of 10% sodium methoxide was added at room temperature, and the mixture was stirred for 24 h. The solvent was removed under reduced pressure, and the residue was dissolved in water. The mixture was extracted with THF, washed with brine, and dried over Na₂SO₄. Filtration and evaporation of the solvent under reduced pressure provided the crude sodium salt, which was chromatographed on silica gel. Elution with CHCl₃-MeOH (10:1) gave 0.46 g (72.6% yield) of 41b as violet crystals: mp 207-208 °C; IR (KBr) 3268, 2944, 1578, 1446, 1401, 1323, 1155, 1092, 1038 cm⁻¹; ¹H NMR (CD₃OD) δ = 1.35 (d, 6 H), 1.20-1.79 (m, 4 H), 2.75-3.04 (t + m, 5 H), 7.20-7.31 (d, 2 H), 7.40-7.90 (s + m, 6 H), 8.30 (d, 1 H), 9.00 (d, 1 H).

1-(Cyanomethyl)-3-[4-[(Phenylsulfonyl)amino]butyl]-6-isopropylazulene (33). A mixture of 0.054 g (0.53 mmol) of *N,N,N',N'*-tetramethyldiaminomethane, 0.010 g (0.34 mmol) of paraformaldehyde, and 1.0 mL of acetic acid was heated to give a clear solution. This solution was cooled to room temperature and added dropwise with stirring to solution of 0.184 g (0.481 mmol) of 31 in 10 mL of CH₂Cl₂ at 0 °C. This mixture was allowed to stir for 1 h at 0 °C, placed in a refrigerator for 15 h, and then diluted with 20 mL of CH₂Cl₂, washed with water, and dried over MgSO₄. Filtration and evaporation of the solvent under reduced pressure provided the crude amine. This amine was dissolved in 10 mL of EtOH. To the stirring solution at room temperature were added 5.0 mL of methyl iodide and the mixture stirred for 2 h. Removal of solvent under reduced pressure gave the crude quaternary ammonium iodide. The quaternary ammonium iodide in 10 mL of EtOH was added to 0.094 g (1.44 mmol) of KCN. The mixture was heated under reflux for 1 h, diluted with 50 mL of water, and extracted with ether. The ether extract was washed with water and brine and dried over MgSO₄. Filtration and evaporation of the solvent under reduced pressure provided the crude cyanide, which was chromatographed on silica gel. Elution with ethyl acetate-*n*-hexane (1:2) gave 0.130 g (64.2% yield) of 33 as a violet oil: IR (neat) 3274, 2944, 2250, 1578, 1446, 1323, 1155, 1089 cm⁻¹; ¹H NMR (CDCl₃) δ = 1.42 (d, 6 H), 1.11-1.87 (m, 4 H), 2.70-3.20 (t + m, 5 H), 4.02 (s, 1 H), 4.91 (bt, 1 H), 7.02 (d, 2 H), 7.27-7.90 (d + m, 6 H), 8.00 (d, 1 H), 8.11 (d, 1 H); MS *m/z* 420 (M⁺).

3-[4-[(Phenylsulfonyl)amino]butyl]-6-isopropylazulene-1-acetic Acid (34). To a solution of 0.11 g (0.26 mmol) of 33 in 10 mL of EtOH was added 10 mL of 0.6 M aqueous KOH, and

the mixture was refluxed for 12 h. The mixture was cooled to room temperature and the solvent removed under reduced pressure. The aqueous layer was washed with CHCl_3 , adjusted to pH 2–3 with 10% aqueous HCl, and extracted with ethyl acetate. The ethyl acetate extract was washed with water and brine and dried over MgSO_4 . Filtration and evaporation of the solvent under reduced pressure provided the crude acid, which was chromatographed on silica gel. Elution with ethyl acetate gave 0.084 g (73.5% yield) of 34 as violet crystals: mp 51–52 °C; IR (KBr) 3268, 2944, 1704, 1578, 1446, 1323 cm^{-1} ; ^1H NMR (CDCl_3) δ = 1.31 (d, 6 H), 1.15–1.83 (m, 4 H), 2.77–3.15 (t + m, 5 H), 4.01 (s, 1 H), 4.30–4.60 (m, 2 H), 6.86–7.07 (dd, 2 H), 7.28–7.96 (s + m, 6 H), 7.97 (d, 1 H), 8.17 (d, 1 H); MS m/z 439 (M^+).

1-Formyl-3-[4-[(Phenylsulfonyl)amino]butyl]-6-isopropylazulene (35). DMF (4.0 mL) was treated with 0.18 mL (1.93 mmol) of POCl_3 at 0 °C under an Ar atmosphere. The resulting solution was stirred for 30 min and then treated with a solution of 0.490 g (1.28 mmol) of 31 in 1.8 mL of DMF and stirred for 30 min. The reaction mixture was poured into cold, 10% aqueous NaOH and extracted with ethyl acetate. The ethyl acetate extract was washed with water and brine and dried over MgSO_4 . Filtration and evaporation of the solvent under reduced pressure provided the crude aldehyde, which was chromatographed on silica gel. Elution with ethyl acetate–*n*-hexane (1:2) gave 0.48 g (91.4% yield) of 35 as a violet oil: IR (neat) 3268, 2950, 1629, 1578, 1446, 1368, 1326, 1156, 1092 cm^{-1} ; ^1H NMR (CDCl_3) δ = 1.36 (d, 6 H), 1.50–1.90 (m, 4 H), 2.73–3.30 (t + m, 5 H), 5.03 (bt, 1 H), 7.30–7.60 (m, 5 H), 7.76–8.00 (s + m, 3 H), 8.30 (d, 1 H), 9.35 (d, 1 H), 10.33 (s, 1 H); MS m/z 409 (M^+).

(E)-3-[4-[(Phenylsulfonyl)amino]butyl]-6-isopropylazulene-1-acrylic Acid Ethyl Ester (36). To a stirred suspension of 0.15 g (3.15 mmol) of a 60% NaH dispersion in 1.5 mL of THF cooled to 0 °C under an Ar atmosphere was added dropwise a solution of 0.79 g (3.51 mmol) of ethyl phosphonoacetate in 7.8 mL of THF. After the solution was stirred for 20 min, a solution of 0.48 g (1.17 mmol) of 35 in 4.8 mL of THF was added and the mixture was stirred for 2 h at room temperature. The reaction was then quenched with saturated aqueous NH_4Cl . The mixture was extracted with ethyl acetate, and the organic layer was washed with brine and dried over MgSO_4 . Filtration and evaporation of the solvent under reduced pressure provided the crude ester, which was chromatographed on silica gel. Elution with ethyl acetate–*n*-hexane (1:3) gave 0.54 g (96.1% yield) of 36 as a green oil: IR (neat) 3268, 2950, 1683, 1605, 1578, 1368, 1449, 1326, 1293 cm^{-1} ; ^1H NMR (CDCl_3) δ = 1.33 (d, 6 H), 1.34 (t, 3 H), 1.40–1.85 (m, 4 H), 2.70–3.13 (t + m, 5 H), 4.27 (q, 2 H), 5.05 (bt, 1 H), 6.36 (d, 2 H), 7.00–7.60 (m, 5 H), 7.60–7.90 (m, 3 H), 8.00 (s, 1 H), 8.14 (d, 1 H), 8.39 (d, 1 H); MS m/z 479 (M^+).

(E)-3-[4-[(Phenylsulfonyl)amino]butyl]-6-isopropylazulene-1-acrylic Acid (37). To a solution of 0.51 g (1.06 mmol) of 36 in 10 mL of MeOH was added 5.0 mL of 10% aqueous NaOH, and the mixture was stirred for 14 h at room temperature. After removal of the organic solvent under reduced pressure, the aqueous layer was washed with ethyl acetate. The solution was adjusted to pH 2–3 with 10% aqueous HCl and extracted with ethyl acetate. The ethyl acetate extract was washed with water and brine and dried over MgSO_4 . Filtration and evaporation of the solvent under reduced pressure provided the crude acid, which was chromatographed on silica gel. Elution with CHCl_3 –MeOH (10:1) gave 0.450 g (94.0% yield) of 37 as green crystals: mp 58–60 °C; IR (KBr) 3262, 2944, 1575, 1563, 1269, 1155 cm^{-1} ; ^1H NMR (CDCl_3) δ = 1.33 (d, 6 H), 1.45–1.90 (m, 4 H), 2.75–3.20 (t + m, 5 H), 4.98 (bs, 1 H), 6.37 (d, 2 H), 6.95–7.60 (m, 5 H), 7.65–7.95 (m, 3 H), 8.20 (d, 1 H), 8.20 (d, 1 H), 8.43 (d, 1 H), MS m/z 407 (M^+ – 44).

Methyl 3-[3-[(Benzyloxy)methyl]phenyl]-6-isopropylazulene-1-carboxylate. A mixture of 2.9 g (11.96 mmol) of [3-[(benzyloxy)methyl]phenyl]acetaldehyde, 1.1 mL (11.96 mmol) of morpholine, and 1.47 g (5.98 mmol) of 6 in 30 mL of EtOH was heated under reflux for 20 h. After the solvent was removed under reduced pressure, the residue was dissolved in ethyl acetate. The solution was washed with water and brine and dried over MgSO_4 . Filtration and evaporation of the solvent under reduced pressure provided crude azulene, which was chromatographed on silica gel. Elution with ethyl acetate–*n*-hexane (1:4) gave 1.18 g (46.5% yield) of azulene 7 (R_2 =

(PhCH_2)₂O) as a violet oil: IR (neat) 2950, 2848, 1689, 1578, 1446, 1419, 1227, 1200 cm^{-1} ; ^1H NMR (CDCl_3) δ = 1.36 (d, 6 H), 2.90–3.30 (m, 1 H), 3.95 (s, 3 H), 4.61 (s, 2 H), 7.15–7.65 (m, 11 H), 8.35 (s, 1 H), 8.56 (d, 1 H), 9.60 (d, 1 H); MS m/z 424 (M^+).

Methyl 3-[3-(Hydroxymethyl)phenyl]-6-isopropylazulene-1-carboxylate. To a solution of 1.18 g (2.78 mmol) of the azulene prepared above in 30 mL of anisole was added 0.74 g (5.56 mmol) of AlCl_3 at 0 °C and the mixture was stirred for 15 min. The mixture was poured into ice-water and extracted with ethyl acetate. The ethyl acetate extract was washed with water, saturated aqueous NaHCO_3 , and brine and dried over MgSO_4 . Filtration and evaporation of the solvent under reduced pressure provided the crude alcohol, which was chromatographed on silica gel. Elution with ethyl acetate–*n*-hexane (1:1) gave 0.81 g (87.1% yield) of alcohol as a violet oil: IR (neat) 3400, 2944, 2860, 1689, 1449, 1419, 1227, 1200 cm^{-1} ; ^1H NMR (CDCl_3) δ = 1.36 (d, 6 H), 2.90–3.30 (m, 1 H), 3.93 (s, 3 H), 4.76 (s, 2 H), 7.20–7.65 (m, 6 H), 8.33 (s, 1 H), 8.55 (d, 1 H), 9.57 (d, 1 H); MS m/z 334 (M^+).

Methyl 3-[3-(Bromomethyl)phenyl]-6-isopropylazulene-1-carboxylate. To a solution of 0.80 g (2.39 mmol) of the above alcohol in 10.0 mL of CH_2Cl_2 were added 0.75 g (2.87 mmol) of triphenylphosphine and 0.95 g (2.87 mmol) of carbon tetrabromide at –25 °C under an Ar atmosphere. The reaction mixture was stirred for 1 h at room temperature. The reaction was quenched with saturated aqueous NaHCO_3 and extracted with CHCl_3 . The CHCl_3 extract was washed with brine and dried over MgSO_4 . Filtration and evaporation of the solvent under reduced pressure provided the crude bromide, which was chromatographed on silica gel. Elution with ethyl acetate–*n*-hexane (1:4) gave 0.91 g (95.8% yield) of bromide as a violet oil: IR (neat) 2950, 1689, 1578, 1449, 1419, 1233, 1197 cm^{-1} ; ^1H NMR (CDCl_3) δ = 1.36 (d, 6 H), 2.90–3.30 (m, 1 H), 3.94 (s, 3 H), 4.57 (s, 2 H), 7.20–7.63 (m, 6 H), 8.33 (s, 1 H), 8.52 (d, 1 H), 9.60 (d, 1 H); MS m/z 398 (M^+).

Methyl 3-[3-[(Phenylsulfonyl)amino]phenyl]-6-isopropylazulene-1-carboxylate. To a stirred suspension of 0.15 g (3.40 mmol) of 60% sodium hydride dispersion in 5.0 mL of THF cooled to 0 °C under an Ar atmosphere was added dropwise a solution of 0.54 g (3.40 mmol) of benzenesulfonamide in 5.0 mL of THF. After the solution was stirred for 20 min, a solution of 0.89 g (2.26 mmol) of bromide in 5.0 mL of HMPA was added and the mixture was stirred for 1 h at room temperature. The reaction was then quenched with saturated aqueous NH_4Cl and extracted with EtOAc. The ethyl acetate extract was washed with water and brine and dried over MgSO_4 . Filtration and evaporation of the solvent under reduced pressure provided the crude sulfonamide, which was chromatographed on silica gel. Elution with ethyl acetate–*n*-hexane (1:3) gave 0.36 g (33.6% yield) of sulfonamide as a violet oil: IR (neat) 2968, 1692, 1578, 1449, 1422, 1374, 1239, 1200 cm^{-1} ; ^1H NMR (CDCl_3) δ = 1.32 (d, 6 H), 2.90–3.30 (m, 1 H), 3.93 (s, 3 H), 4.49 (s, 2 H), 7.05–7.60 (m, 9 H), 7.75–8.00 (m, 6 H), 8.11 (s, 1 H), 8.30 (d, 1 H), 9.52 (d, 1 H); MS m/z 473 (M^+).

3-[3-[[[(Phenylsulfonyl)amino]methyl]phenyl]-6-isopropylazulene-1-carboxylic Acid (38). To a solution of 0.26 g (0.55 mmol) of the ester prepared above in 10 mL of MeOH was added 5.0 mL of 10% aqueous NaOH and the mixture was refluxed for 10 h. After removal of the organic solvent under reduced pressure, the aqueous layer was washed with CHCl_3 . The solution was adjusted to pH 2–3 with 10% aqueous HCl and extracted with ethyl acetate. The ethyl acetate extract was washed with water and brine and dried over MgSO_4 . Filtration and evaporation of the solvent under reduced pressure provided the crude acid, which was chromatographed on silica gel. Elution with CHCl_3 –MeOH (50:1) gave 0.035 g (13.8% yield) of 38 as violet crystals: mp 95–97 °C; IR (KBr) 3010, 2950, 1650, 1578, 1485, 1236, 1155 cm^{-1} ; ^1H NMR (CDCl_3) δ = 1.36 (d, 6 H), 2.90–3.30 (m, 1 H), 4.25 (d, 2 H), 7.00–7.65 (m, 9 H), 7.70–8.00 (m, 2 H), 9.30 (s, 1 H), 9.48 (d, 1 H), 9.60 (d, 1 H); MS m/z 459 (M^+).

Biological Methods. Relaxing Effects on U-46619-Induced Contraction of Rat Aorta. The thoracic aortas from male rats (Wistar) were excised, cleaned, and cut into ring strips (2-mm width and 4-mm length). The tissues were placed in 10-mL organ baths containing a Krebs bicarbonate solution [(mM) NaCl (120.3), KCl (4.8), CaCl_2 (1.2), KH_2PO_4 (1.2), MgSO_4 (1.3), NaHCO_3 (24.2), dextrose (10.0)] that was kept at 37 °C while

being bubbled with 95% O₂ and 5% CO₂. A resting tension of 1.0 g was applied, and after equilibration for 1 h, the isomeric tension was recorded on a polygraph (Nihon Kohden) through a force-displacement transducer (Nihon Kohden). After the increase in isomeric tension due to addition of 3×10^{-8} M U-46619 became stable, cumulative concentrations of each compound were added to the bath. The concentration which caused a 50% relaxation of a U-46619-induced maximal change in tension was obtained by regression analysis of the concentration-relaxation curve.

Inhibitory Effects of U-46619-Induced Platelet Aggregation in Rabbit. Male rabbits (New Zealand) weighing 2.0–2.5 kg were anesthetized with ether; blood was withdrawn from the carotid artery through a cannulation tube with a syringe containing trisodium citrate (3.18%, $1/10$ volume). Platelet-rich plasma (PRP) was then prepared by centrifugation at 300g for 15 min. The supernatant was decanted, and the remaining pellet was centrifuged at 1000g for 20 min to produce platelet-poor plasma (PPP), which was used as a zero calibration. Platelet aggregation was measured with an aggregometer (Chrono-Log) by the method of Born.⁴⁰ PRP (500 μ L) placed in a cuvette was warmed at 37 °C for 2 min, and then a solution of the compound or vehicle was added. Exactly 2 min later, 2.5×10^{-6} M U-46619 was added to PRP. The change in light transmission was recorded, with the light transmissions for PRP and PPP being taken as 0% and 100%, respectively, and the maximum light transmission after addition of U-46619 as the maximum aggregation. The concentration which caused 50% inhibition of a U-46619-induced maximum aggregation was obtained by regression analysis of the concentration-inhibition curve.

Binding Assay with Rabbit Gel-Filtered Platelets. The experiments were done according to the method described by Hanasaki et al.⁴¹ with slight modifications. Blood from male New Zealand rabbits was collected into a syringe containing a 0.15 volume of acid citrate dextrose [(mM) trisodium citrate (85.0), citric acid (70.0), glucose (110.0)]. PRP was obtained by centrifugation at 300g for 15 min. The PRP was mixed with PGI₂ (0.5 μ g/mL) and apyrase (25 μ g/mL) and layered on 40% bovine serum albumin. Platelets were centrifuged at 1200g for 15 min and resuspended in 1.0 mL of resuspension buffer [(mM) NaCl (137), KCl (2.7), MgCl₂ (1.0), Na₂HPO₄ (3.8), HEPES (3.8), glucose (5.6), 0.035% bovine serum albumin, pH 7.35]. Platelets were separated from plasma proteins by gel filtration through a column of Sepharose 2B and suspended in the resuspension buffer to a final concentration of 3×10^8 cells/mL. Aliquots of the platelet suspension (0.46 mL) were incubated with 5 nM [³H]SQ29548 (0.075 μ Ci) plus 10^{-5} M BM13,505 or various concentrations of compound 41b (KT2-962) for 30 min at 25 °C. Specific binding is defined as the difference between the binding in the presence and absence of 10^{-5} M BM13505. After the incubation, ice-cold saline (3.0 mL) was added to each tube and the reaction mixture was immediately filtered through Whatman GF/C glass-filter disks, which were then washed four times with ice-cold saline. All filtration procedures were completed within 20 s. The radioactivity was measured in a liquid scintillation counter (Aloka, LSC-700).

Effects on Thromboxane B₂ Formation. Human blood was obtained from the antecubital vein of healthy volunteers. PRP was prepared by centrifugation at 200g for 20 min. The PRP was further centrifuged at 2000g for 10 min, and the resulting pellet was washed twice with 25 mM phosphate buffer. The washed pellets were resuspended in 50 mM phosphate buffer (pH 7.4) containing 1 mM EDTA, sonicated, and stored at -80 °C until use. Sonicated platelets were preincubated with test compounds in the presence of 1 mM GSH at 37 °C for 5 min, and then the mixture was incubated with [1-¹⁴C]arachidonic acid (0.1 μ Ci) at 37 °C for 5 min. Each reaction was terminated by the addition of 2.5 mL of cold ether/methanol (30:4, v/v) and the mixture was acidified with 200 mM citric acid. ¹⁴C-labeled eicosanoids were extracted and separated by thin-layer chromatography. The areas corresponding to each eicosanoid were removed, and the radioactivity was counted. Thromboxane formation was calculated from the amount of thromboxane B₂ generated from [¹⁴C]arachidonic acid. The IC₅₀ values were obtained by regression analysis of the concentration-inhibition curve.

Protective Effects against U-46619-Induced Sudden Death in Mice. This investigation was carried out according to the modified method of Myers et al.³⁸ using conscious male ddY mice. KT2-962 or BM13,177 (sulotroban) was orally administered in 0.5% methylcellulose solution at doses from 0.3 to 30 mg/kg. Control animals were given only 0.5% methylcellulose solution. At the indicated time after administration of each drug or vehicle, U-46619 (800 μ g/kg) was injected intravenously. The effect of each drug was evaluated by measuring the incidence of death within 5 min of injection. The point of death was determined by monitoring respiration. Results are expressed as percent survival. Each group was comprised of 10–20 mice.

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