Synthesis and in Vitro Activity of Various Derivatives of a Novel Thromboxane Receptor Antagonist, (±)-(5*Z*)-7-[3-*endo*-[(Phenylsulfonyl)amino]bicyclo[2.2.1]hept-2-*exo*-yl]heptenoic Acid[1,2]

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Several sulfonyl derivatives (**13a–t**) of (±)-(5*Z*)-7-(3-*endo*-aminobicyclo[2.2.1]hept-2-*exo*-yl)heptenoic acid (VI) were synthesized via its methyl ester **10**. Sulfonylation of **10** with **11a–t** followed by saponification yielded **13a–t**. Inhibitory concentrations (IC_{50}) of the corresponding sodium salts **14a–t** for platelet aggregation were measured with rat washed platelets (WP) and rabbit platelet-rich plasma (PRP). IC_{50} values of some derivatives for contraction of the rat aorta were also measured. The IC_{50} values for rat WP increased from 2.9 to 26 nM in the order of **14a**, **14c**, **14d**, and **14b** for derivatives with an arylsulfonyl residue, depending on the number of intervening methylene groups. Methyl derivative **14e** exhibited a higher IC_{50} value than *n*-hexyl derivative **14f**. Substitution with a *p*-methyl, *p*-fluoro-, or *p*-chloro group in **14a** retained or slightly reduced its IC_{50} value, while a *p*-*n*-pentyl or *p*-oxycarbonyl group augmented it significantly. The representative **14a** suppressed (15*S*)-15-hydroxy-11,9-(epoxymethano)prosta-5(*Z*),13(*E*)-dienoic acid (U-46619) induced aggregation of human WP with an IC_{50} value of 7.7 nM, which corresponds well to the IC_{50} value of 3 nM obtained for each displacement by **14a** of [^{3}H]-U-46619 or (5*Z*,15ξ)-9α,11α-(dimethylmethano)-15-hydroxy-16-(3-[^{125}I]iodo-4-hydroxyphenyl)-17,18,19,20-tetranor-13-aza-11a-carbathrombo-5-enoic acid ([^{125}I]-PTA-OH) bound to human WP. Synthesis of thromboxane A_2 (TxA_2) in human WP stimulated by thrombin was not inhibited by **14a** at a concentration up to 10 μM. From these observations, the corresponding acid **13a** (S-145) was concluded to be a potent TxA_2 receptor antagonist.

Thromboxane A_2 (TxA_2, I), which displays potent platelet aggregation, vasoconstriction, and bronchoconstriction activities, has been considered to be a mediator causing problems in circulatory disorders and asthmatic conditions. Thus, inhibitors of TxA_2 biosynthesis and TxA_2 receptor antagonists can be effective therapeutic agents for these diseases.[3] However, the biosynthesis inhibitors have recently been recognized to be less efficacious than expected, probably because prostaglandin H_2 (PGH_2, II) and TxA_2 are considered[4] to share a common receptor, and thus, the PGH_2 that accumulates with inhibition of the TxA_2 biosynthesis may act as an agonist of TxA_2. Several drugs with prostanoid,[5–14] e.g., SQ-29548 (III) or ONO-3708 (IV), and non-prostanoid[15,16] structures, e.g., BM-13177 (V), which possess the characteristics of TxA_2 receptor antag-

(1) British Patent 2184118A, Nov. 18, 1985. During the revision of the manuscript, a relevant synthesis of optically active **13g** was reported.[2]

(2) Furuta, K.; Hayashi, S.; Miwa, Y.; Yamamoto, H. *Tetrahedron Lett.* **1987**, *28*, 5841.

(3) See recent reviews: (a) Brezinski, M. E.; Yanagisawa, A.; Lefer, A. M. *J. Cardiovasc. Pharmacol.* **1987**, *9*, 65. (b) Ogletree, M. L. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1987**, *46*, 133. (c) Lefer, A. M.; Darius, H. *Ibid.* **1987**, *46*, 144. (d) Halushka, P. V.; Mais, D. E.; Saussy, D. L., Jr. *Ibid.* **1987**, *46*, 149.

(4) (a) Charo, I. F.; Feinman, R.; Detwiler, T.; Smith, J. B.; Ingerman, C. M.; Silver, M. J. *Nature (London)* **1977**, *269*, 66. (b) Coleman, R. A.; Humphrey, P. P. A.; Kennedy, I.; Levy, G. P.; Lumley, P. *Br. J. Pharmacol.* **1981**, *73*, 773.

(5) A review: Lefer, A. M. *Drugs Today* **1985**, *21*, 283–291.

(6) (a) Nicolaou, K. C.; Magolda, R. L.; Smith, J. B.; Aharony, D.; Smith, E. F.; Lefer, A. M. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 2566. (b) Burke, S. E.; Lefer, A. M.; Nicolaou, K. C.; Smith, G. M.; Smith, J. B. *Br. J. Pharmacol.* **1983**, *78*, 287.

(7) Le Breton, G. C.; Venton, D. L.; Enke, S. E.; Halushka, P. V. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 4097.

(8) (a) Sprague, P. W.; Heikes, J. E.; Gougoutas, J. Z.; Malley, M. F.; Harris, D. N.; Greenberg, R. *J. Med. Chem.* **1985**, *28*, 1580. (b) Hall, S. E.; Han, W.-C.; Haslanger, M. F.; Harris, D. N.; Ogletree, M. L. *Ibid.* **1986**, *29*, 2335. (c) Friedhoff, L. T.; Manning, J.; Funke, P. T.; Ivashkiv, E.; Tu, J.; Cooper, W.; Willard, D. A. *Clin. Pharmacol. Ther.* **1986**, *40*, 634.

(9) (a) Jones, R. L.; Peesapati, V.; Wilson, N. H. *Br. J. Pharmacol.* **1982**, *76*, 423. (b) Armstrong, R. A.; Jones, R. L.; Peesapati, V.; Will, S. G.; Wilson, N. H. *Ibid.* **1985**, *84*, 595.

(10) (a) Brittain, R. T.; Boutal, L.; Carter, M. C.; Coleman, R. A.; Collington, E. W.; Geisow, H. P.; Hallett, P.; Hornby, E. J.; Humphrey, P. P. A.; Jack, D.; Kennedy, I.; Lumley, P.; McCabe, P. J.; Skidmore, I. F.; Thomas, M.; Wallis, C. J. *Circulation* **1985**, *72*, 1208. (b) Dodd, N. J.; Humphrey, P. P. A.; Lumley, P.; Weston, M. J. *Br. Pharmacol.* **1985**, *85* (Suppl.), 285P. (c) Coker, S. J.; Parratt, J. R. *Ibid.* **1985**, *86*, 259.

(11) Brown, G. R.; Foubister, A. J. *J. Pharm. Pharmacol.* **1986**, *38*, 706.

(12) (a) Burch, R. M.; Mais, D. E.; Saussy, D. L., Jr.; Halushka, P. V. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82* 7434. (b) Mais, D. E.; Dunlap, C.; Hamanaka, N.; Halushka, P. V. *Eur. J. Pharmacol.* **1985**, *111*, 125. (c) Mais, D. E.; Saussy, D. L., Jr.; Chaikhouni, A.; Kochel, P. J.; Knapp, D. R.; Hamanaka, N.; Halushka, P. V. *J. Pharmacol. Exp. Ther.* **1985**, *233*, 418. (d) Mais, D.; Knapp, D.; Halushka, P.; Ballard, K.; Hamanaka, N. *Tetrahedron Lett.* **1984**, *25*, 4207.

(13) (a) Ogletree, M. L.; Harris, D. N.; Greenberg, R.; Haslanger, M. F.; Nakane, M. *J. Pharmacol. Exp. Ther.* **1985**, *234*, 435. (b) Stahl, G. L.; Darius, H.; Lefer, A. M. *Life Sci.* **1986**, *38*, 2037. (c) Lefer, A. M.; Darius, H. *Drugs Future* **1987**, *12*, 367.

(14) (a) Yui, Y.; Sakaguchi, K.; Susawa, T.; Hattori, R.; Takatsu, Y.; Yui, N.; Kawai, C. *Cardiovasc. Res.* **1987**, *21*, 119. (b) Toda, N.; Nakajima, M.; Okumura, T.; Miyazaki, M. *J. Cardiovasc. Pharmacol.* **1986**, *8*, 818. (c) Fujioka, M.; Nagao, T.; Kuriyama, H. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1986**, *334*, 468.

(15) (a) Patscheke, H.; Stegmeier, K. *Thrombosis Res.* **1984**, *33*, 277. (b) Stegmeier, K.; Pill, J.; Müller-Beckmann, B.; Schmidt, F. H.; Witte, E.-C.; Wolff, H.-P.; Patscheke, H. *Ibid.* **1984**, *35*, 379. (c) Seeger, W.; Ernst, C.; Waimrath, D.; Neuhof, H.; Roka, L. *Ibid.* **1985**, *40*, 793. (d) Brezinski, M. E.; Lefer, D. J.; Bowker, B.; Lefer, A. M. *Prostaglandins* **1987**, *33*, 75.

(16) (a) Chan, C. C.; Nathaniel, D. J.; Yusko, P. J.; Hall, R. A.; Ford-Hutchinson, A. W. *J. Pharmacol. Exp. Ther.* **1984**, *229*, 276. (b) Carrier, R.; Cragoe, E. J.; Ethier, D.; Ford-Hutchinson, A. W.; Girard, Y.; Hall, R. A.; Hamel, P.; Rokach, J.; Share, N. N.; Stone, A. A.; Yusko, P. *Br. J. Pharmacol.* **1984**, *82*, 389.

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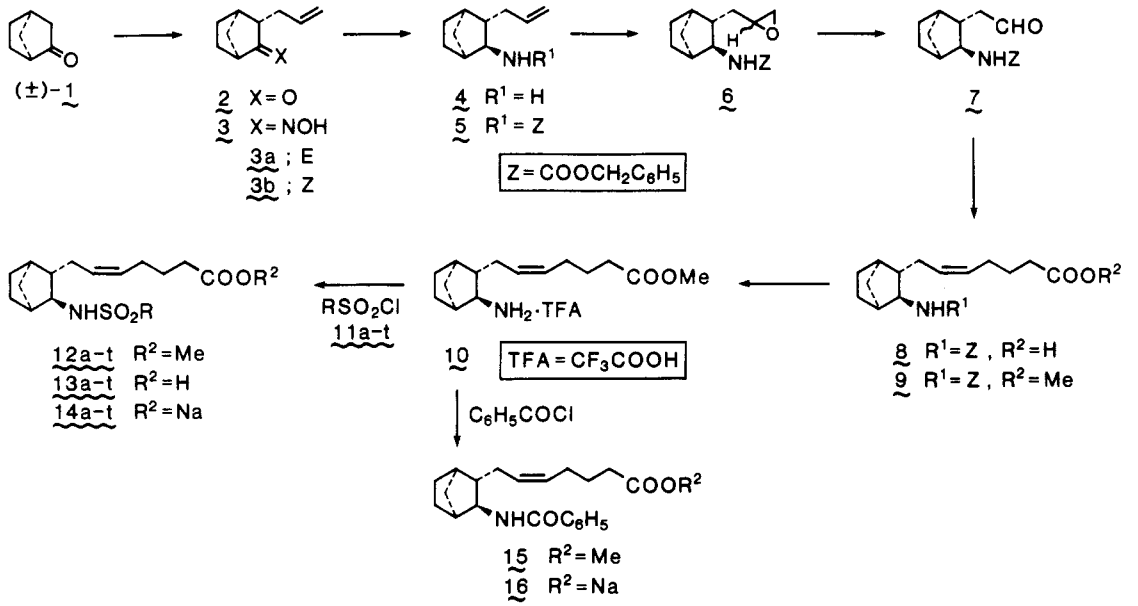
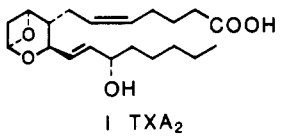
Scheme I



onists have been reported to be promising in preclinical evaluations.[17-20]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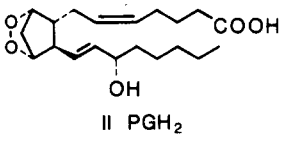
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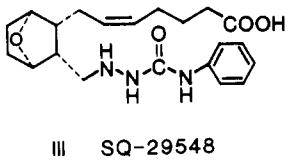
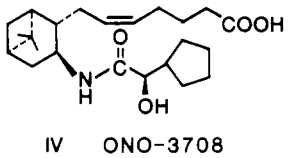
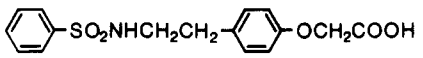
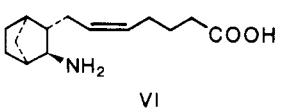
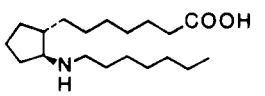

III SQ-29548


IV ONO-3708


V BM-13177

Chemical studies in our laboratories have been focused on the design and synthesis of novel sulfonyl derivatives **13a–t** of (±)-(5*Z*)-(3-*endo*-aminobicyclo[2.2.1]hept-2-*exo*-yl)heptenoic acid (VI), the structure of which was chosen as a rigid ring analogue of 13-azaprostanoic acid (13-APA, VII),[17] a TxA_2 antagonist. The phenylsulfonyl derivative **13a** of VI, designed on the basis of the latent similarity to the non-prostanoid antagonist BM-13177 (V),[15] was found to possess powerful binding affinity to the TxA_2 receptor of human platelets and very potent anti-TxA_2 activity. Structure–activity relationships among its derivatives were also investigated.


VI

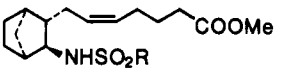

VII 13-APA

Chemistry

The common intermediate **10** was prepared from (±)-norcamphor (1) as depicted in Scheme I. Alkylation of the lithium enolate of 1 with allyl bromide at −15 °C in THF gave exclusively the 2-*exo*-allyl compound **2** in a 76% yield.[21] Treatment of **2** with hydroxylamine afforded a 2:1 mixture of *E* and *Z* oximes **3a** and **3b**. Subsequent reduction of the mixture of oximes with 4.0 equiv of $LiAlH_4$ for 1.5 h under reflux in THF to give **4** proceeded stereoselectively. The stereochemistry of the amino group in **4** was confirmed after its conversion into the *N*-benzyloxycarbonyl derivative **5** in a 25.6% yield from **2**. The ^1H NMR spectrum of **5** showed a signal at δ 3.53 (dt, J = 7, 4 Hz, 1 H) assignable to the CHN group, of which the coupling constants are typical for the 3-proton of the 2,3-disubstituted bicyclo[2.2.1]heptane system when a 3-heteroatom side chain is oriented in the endo-trans direction.[22] Epoxidation of **5** with *m*-chloroperbenzoic acid in methylene chloride at 20 °C gave a diastereomeric mixture of epoxides **6**, and subsequent treatment with HIO_4 in 50% aqueous dioxane at 10 °C yielded the aldehyde **7**. Wittig reaction of **7** with 3.0 equiv of ylide, prepared from (4-carboxybutyl)triphenylphosphonium bromide and dimsylsodium in DMSO at 20 °C, produced the desired 5(*Z*)-heptenoic acid **8** as the major product. Esterification of the product with diazomethane as usual in ether gave the methyl ester **9** in a 48% yield from **5**. Deprotection of the benzyloxycarbonyl group in **9** was attained by heating with a large excess of trifluoroacetic acid in anisole for 4 h at 45 °C, giving the primary amine as trifluoroacetate **10**. Sulfonylation of **10** with various sulfonyl chlorides **11a–t** and triethylamine in methylene chloride afforded the corresponding sulfonylamino esters **12a–t** in satisfactory yields. Finally, hydrolysis of these esters with KOH in aqueous methanol afforded the desired acids **13a–t**. The acids were frequently contaminated with organic solvents. Thus, for the analysis as well as the biological assay in vitro, these acids were transformed into their sodium salts **14a–t** by neutralizing the acids with

(17) Fitzgerald, D. J.; Doran, J.; Jackson, E.; Fitzgerald, G. A. *J. Clin. Invest.* **1986**, *77*, 496.
(18) Schrör, K.; Smith, E. F., III; Bickerton, M.; Bryan Smith, J.; Nicolaou, K. C.; Magolda, R.; Lefer, A. M. *Am. J. Physiol.* **1980**, *238*, H87.
(19) (a) Hock, C. E.; Brezinski, M. E.; Lefer, A. M. *Eur. J. Pharmacol.* **1986**, *122*, 213. (b) Darius, H.; Bryan Smith, J.; Lefer, A. M. *J. Pharmacol. Exp. Ther.* **1985**, *235*, 274.
(20) (a) Schrör, K.; Thiemermann, C. *Br. J. Pharmacol.* **1986**, *87*, 631. (b) Brezinski, M. E.; Yanagisawa, A.; Darius, H.; Lefer, A. M. *Am. Heart J.* **1985**, *110*, 1161.
(21) Barraclough, P. *Tetrahedron Lett.* **1980**, *21*, 1897.
(22) Jackman, L. M.; Sternhell, S. *Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*, 2nd ed.; Pergamon: London, 1969; pp 83, 229, 286.

Table I

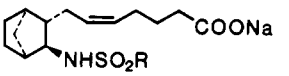


12a–t

compd 12	R	mp, °C	yield, %	formula	anal.
a	Ph	30–35	86	$C_{21}H_{29}NO_4S$	C, H, N, S
b	benzyl	oil	71	$C_{22}H_{31}NO_4S \cdot 0.1C_6H_6$[a]	C, H, S
c	2-phenethyl	oil	52	$C_{23}H_{33}NO_4S$	C, H, N, S
d	3-phenylpropyl	oil	32	$C_{24}H_{35}NO_4S$	C, H, N, S
e	Me	oil	74	$C_{16}H_{27}NO_4S \cdot 0.1C_6H_6$[a]	C, H, N
f	*n*-hexyl	oil	42	$C_{21}H_{37}NO_4S$	C, H, N, S
g	4-MePh	69–70	71	$C_{22}H_{31}NO_4S$	C, H, N, S
h	2-MePh	oil	48	$C_{22}H_{31}NO_4S \cdot 0.1C_6H_6$[a]	C, H, N, S
i	3-MePh	oil	30	$C_{22}H_{31}NO_4S \cdot 0.8H_2O$[b]	C, H, N, S
j	4-EtPh	69–72	78	$C_{23}H_{33}NO_4S$	C, H, N, S
k	4-*n*-pentyl-C_6H_4	85–87	34	$C_{26}H_{39}NO_4S$	C, H, N, S
l	naphthyl	oil	75	$C_{25}H_{31}NO_4S$	C, H, N, S
m	4-PhPh	116–117	32	$C_{27}H_{33}NO_4S$	C, H, N, S
n	4-OAcPh	84–86	39	$C_{23}H_{31}NO_6S$	C, H, N, S
o	4-MeOPh	oil	71	$C_{22}H_{31}NO_5S \cdot 0.1C_6H_6$[a]	C, H, N, S
p	4-NO_2Ph	oil	53	$C_{21}H_{28}N_2O_6S \cdot 0.2C_6H_6$[a]	C, H, N
q	4-FPh	oil	73	$C_{21}H_{28}FNO_4S$	C, H, N, S
r	4-ClPh	oil	72	$C_{21}H_{28}ClNO_4S \cdot 0.1C_6H_6$[a]	C, H, Cl, N, S
s	4-COOHPh	foam	23	$C_{22}H_{29}NO_6S \cdot 0.2C_6H_6$[a]	C, H, N
t	4-NMe_2Ph	94–98	33	$C_{23}H_{34}N_2O_4S$	C, H, N, S

[a] In order to remove solvents, oily compounds were flushed several times with benzene, giving benzene-containing products; in cases of **12e**, **12o**, **12p**, **12r**, and **12s**, contamination with benzene was confirmed by ^1H NMR spectra or by gas chromatography. [b] Hygroscopic.

Table II



14a–t, powder

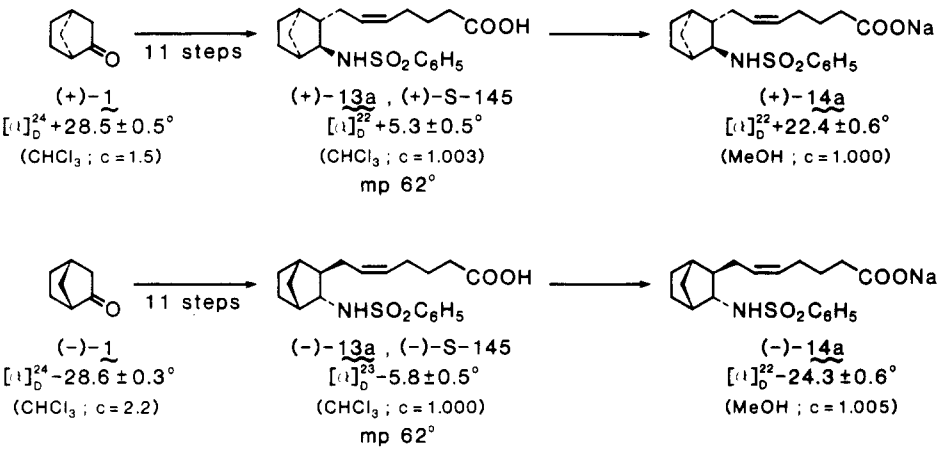
			IC_{50}			
compd 14	R	yield, %	rabbit PRP[a] (μM)	rat WP[b] (nM)	formula[e]	anal.
a	Ph	88	1.0[c]	2.9[d]	$C_{20}H_{26}NO_4NaS \cdot 0.1H_2O$	C, H, N, Na, S
b	benzyl	89	113	26	$C_{21}H_{28}NO_4NaS \cdot 0.4H_2O$	C, H, N, Na, S
c	2-phenethyl	97	8.2	3.7	$C_{22}H_{30}NO_4NaS \cdot 0.3H_2O$	C, H, N, Na, S
d	3-phenylpropyl	84	14	10.9	$C_{23}H_{32}NO_4NaS \cdot 0.6H_2O$	C, H, N
e	Me	93	640	370	$C_{15}H_{24}NO_4NaS \cdot 0.2H_2O$	C, H, N, Na, S
f	*n*-hexyl	84	13.3	19	$C_{20}H_{34}NO_4NaS \cdot 0.1H_2O$	C, H, N, Na
g	4-MePh	94	0.5	2.4	$C_{21}H_{28}NO_4NaS \cdot 0.5H_2O$	C, H, N, Na, S
h	2-MePh	94	6.2	4.3	$C_{21}H_{28}NO_4NaS \cdot 0.6H_2O$	C, H, N, Na, S
i	3-MePh	96	1.7	5.6	$C_{21}H_{28}NO_4NaS \cdot 1.3H_2O$	C, H, N, Na, S
j	4-EtPh	96	4.6	3.3	$C_{22}H_{30}NO_4NaS \cdot 0.2H_2O$	C, H, N, Na, S
k	4-*n*-pentyl-C_6H_4	83	800	14.5	$C_{25}H_{36}NO_4NaS \cdot 0.4H_2O$	C, H, N, Na, S
l	naphthyl	84	1.1	2.7	$C_{24}H_{28}NO_4NaS \cdot 0.4H_2O$	C, H, N, Na, S
m	4-PhPh	89	200	6.0	$C_{26}H_{30}NO_4NaS \cdot 0.6H_2O$	C, H, N, S
n	4-OHPh	87	0.5	4.0	$C_{20}H_{26}NO_5NaS \cdot 0.8H_2O$	C, H, N, Na, S
o	4-MeOPh	93	1.7	3.9	$C_{21}H_{28}NO_5NaS \cdot 0.2H_2O$	C, H, N, Na, S
p	4-NO_2Ph	89	1.4	8.9	$C_{20}H_{25}N_2O_6NaS$	C, H, N, S
q	4-FPh	79	0.6	2.9	$C_{20}H_{25}FNO_4NaS \cdot 0.5H_2O$	C, H, F, N, Na, S
r	4-ClPh	92	0.3	2.9	$C_{20}H_{25}ClNO_4NaS \cdot 0.5H_2O$	C, H, Cl, N, Na, S
s	4-COONaPh	25	800	>1000	$C_{21}H_{25}NO_6Na_2S \cdot 1.5H_2O$	C, H, N, S
t	4-NMe_2Ph	90	7.1	52	$C_{22}H_{31}N_2O_4NaS$	C, H, N, S
16			>800	450	$C_{21}H_{26}NO_3Na$	C, H, N
SQ-29548 (III)			9.0	3.0		
ONO-3708 (IV)			>800	3.7		
(+)-**14a**	Ph		0.6	1.8	$C_{20}H_{26}NO_4NaS \cdot 0.5H_2O$	C, H, N, Na, S
(−)-**14a**	Ph		7.4	13.4	$C_{20}H_{26}NO_4NaS \cdot 0.4H_2O$	C, H, N, Na, S

[a] Aggregation of platelet-rich plasma (PRP) was induced by 500 μM of arachidonic acid. [b] Aggregation of washed platelets (WP) was induced by 4 μg/mL of collagen. [c] The value varied from 0.9 to 1.2 μM for every measurement as the standard compound; thus, each IC_{50} measured by three experiments for the other compound was corrected for the value for **14a**. [d] The value varied from 1.5 to 4.3 nM on every measurement as the standard compound; thus, each IC_{50} measured by a single experiment for the other compound was corrected for the value for **14a**. [e] All compounds were freeze-dried, and the final products were amorphous and hygroscopic. In the cases of **14a** and **14i**, the water content was determined by the Karl–Fischer method.

sodium methoxide in methanol followed by freeze-drying the aqueous solution of the resulting sodium salts. The obtained sulfonyl derivatives **12a–t** and **14a–t** were characterized and the results are shown in Tables I and II, respectively.

In order to examine the effect of the absolute configuration of **14a** on the biological activity, (+)- and (−)-norcamphor, prepared by Jones' method,[23,24] were converted

Scheme II. Optically Pure (+)-*S*-145, (-)-*S*-145, and Their Sodium Salts



into both (+) and (-) enantiomers of **13a** and **14a**, respectively, in a way similar to that used for the synthesis of racemic congeners.[25] The optical properties of the products are shown in Scheme II.

For comparison of the biological activity, benzamide **16** was prepared from **10** as follows. Benzylation of **10** with benzoyl chloride and triethylamine in methylene chloride gave **15**. Hydrolysis of **15** with KOH to give the carboxylic acid, followed by neutralization with sodium methoxide, afforded **16** in 70% yield.

Biological Results and Discussion

Compounds **14a–t** were examined for their inhibitory activity against biological responses induced by TxA_2-related inducers: (i) aggregation of rabbit platelet-rich plasma (PRP) induced by arachidonic acid, (ii) aggregation of rat washed platelets (WP) induced by collagen, and (iii) contraction of rat aorta induced by (15*S*)-15-hydroxy-11,9-(epoxymethano)prosta-5(*Z*),13(*E*)-dienoic acid (U-46619, a TxA_2 agonist with a PGH_2 structure).[26] Difficulty in obtaining human platelets for frequent use with constant aggregatory property forced us to use readily available substitutes obtained from experimental animals.

The IC_{50} values of **14a–t** obtained for aggregations of rabbit PRP and rat WP are shown in Table II. The IC_{50} values for collagen-induced aggregation of rat WP are regarded as more important indices than those for arachidonic acid induced aggregation of rabbit PRP, because the former values obtained for a wide variety of compounds were reported to correlate fairly well with the corresponding inhibition constants for [^{3}H]-U-46619 binding to the TxA_2 receptor on human platelets,[27] while the IC_{50} value of (5*Z*,15ξ)-9α,11α-(dimethylmethano)-15-hydroxy-16-phenyl-17,18,19,20-tetranor-13-aza-11a-carbathrombo-5-enoic acid (ONO-11120)[12d] for aggregation of rabbit platelets was reported to be much greater than that for human platelets.[28] ONO-3708 (IV) possessing a pinane ring system in common with ONO-11120 also exhibited an extraordinarily large IC_{50} value for aggregation of rabbit PRP. For the compounds newly synthesized, the relative inhibitory activity obtained for item (i) agreed well with that obtained for item (ii). The large differences between the two kinds of IC_{50} values (μM vs nM) are considered to have arisen mostly from the probable binding of the test compounds to serum proteins and not from the species difference. Compound **14a** exhibited a much lower IC_{50} value than the reference compounds III and IV.[29] The very high IC_{50} value for the amide analogue **16** compared with that of **14a** indicates the importance of the sulfonylamino group in relation to its binding to the receptor. With respect to the stereochemistry of eight natural ω-side chain analogues of SQ-29548 (III), 2,3-*cis*- and -*trans*-α-heptenoic acids with an α-oriented epoxy bridge were reported to exhibit the highest level of inhibitory activity.[13] In agreement with this, (±)-**14a**, which exhibited one of the lowest IC_{50} values among the compounds obtained, contains the 2,3-*trans*-α-heptenoic acid with an α-oriented methylene bridge in the (+) enantiomer. In fact, the IC_{50} value for (+)-**14a** was almost half that for (±)-**14a** and several times lower than that for (-)-**14a**.

The effects of variation in the structure of sulfonyl residues on the IC_{50} values are prominent. *n*-Hexyl compound **14f**, which may possess comparable lipophilicity to that for the ω-side chain of the natural prostanoids, exhibited moderate inhibitory activity in rabbit, while the methyl compound **14e** gave a very high IC_{50} value. The IC_{50} values of the derivatives with phenylsulfonyl or a (phenylalkyl)sulfonyl residue for rat WP increases from 2.9 to 26 nM in the order of **14a**, **14c**, **14d**, and **14b** de-

Table III. Inhibitory Concentrations (IC_{50}) for Rat Aorta Contraction[a]

compd	IC_{50},[b] nM (CL $p < 0.05$)	compd	IC_{50},[b] nM (CL $p < 0.05$)
14a	1.4 (1.1–1.7)	**14j**	1.5 (1.0–2.0)
14b	111 (70–213)	**14l**	2.7 (1.8–4.0)
14c	3.4 (2.3–5.3)	**14m**	9.8 (7.8–12.5)
14d	10.9 (9.3–12.7)	**16**	3410 (2640–4460)
14e	1580 (1130–2520)	SQ-29548 (III)	14.5 (12.2–17.1)
14f	17.2 (12.8–22.9)	ONO-3708 (IV)	52.6 (42.2–65.3)
14g	0.7 (0.5–1.1)	(+)-**14a**	0.49 (0.37–0.69)
14h	3.7 (2.9–4.8)	(-)-**14a**	15.3 (11.5–22.1)
14i	3.2 (2.6–3.8)		

[a] Contraction of rat thoracic aorta induced by 30 nM U-46619. [b] IC_{50} values and their confidence limits are indicated.

(23) Irwin, A. J.; Jones, J. B. *J. Am. Chem. Soc.* **1976**, *98*, 8476.
(24) The (+) and (-) enantiomers of **1** (ee 99.3 and 98.8%, respectively) were prepared by Dr. T. Toyoda of Shionogi Research Laboratories and his contribution to this study is gratefully acknowledged.
(25) A crude sample of (+)-**14a** showed $[\alpha]^{24}_D$ + 14.3 ± 0.5° (MeOH, *c* 1.012).
(26) (a) Bundy, G. L. *Tetrahedron Lett.* **1975**, 1957. (b) Coleman, R. A.; Humphrey, P. P. A.; Kennedy, I.; Levy, G. P.; Lumley, P. *Br. J. Pharmacol.* **1981**, *73*, 773.
(27) Hanasaki, K.; Arita, H. *Thrombosis Res.*, in press.
(28) Narumiya S.; Okuma, M.; Ushikubi, F. *Br. J. Pharmacol.* **1986**, *88*, 323.
(29) Reference compounds III and sodium salt of IV were prepared by Drs. S. Hagishita and K. Seno of Shionogi Laboratories and their contribution to this study is gratefully acknowledged. Compound III was used as a solution dissolved in 0.5 M sodium bicarbonate solution.

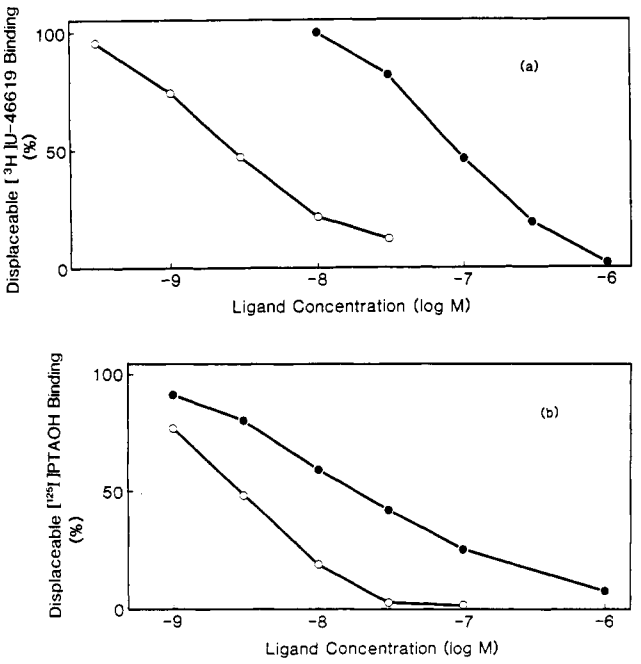


Figure 1. Displacement of the specific [^{3}H]-U-46619 or [^{125}I]-PTA-OH binding by various compounds. (a) Washed human platelets, 10^8 cells were incubated with 12 nM [^{3}H]-U-46619 (22.4 Ci/mmol; N.E.N., Boston) in the presence of various concentrations of **14a** (○) or U-46619 (●); and (b) with 0.2 nM [^{125}I]-PTA-OH (2000 Ci/mmol) in the presence of various concentrations of **14a** (○) and ONO-11120 (●). Incubation was carried out at 37 °C for 20 min. Mean values (n = 2) are shown.

pending on the number of intervening methylene groups, suggesting that the two relevant lipophilic binding sites may be present in the receptor. Introduction of the fused benzene ring (**14l**) did not change the IC_{50} value, while that of the *p*-phenyl group (**14m**) decreased the activity. With introduction of a *p*-methyl (**14g**), *p*-fluoro (**14q**), or *p*-chloro (**14r**) group, the activity was retained or slightly improved, while introduction of a *p*-hydroxy (**14n**), *p*-methoxy (**14o**), or *p*-nitro (**14p**) group had almost no effect. Substitution at the para, ortho, or meta position with a lower alkyl group (**14g–i**) other than the compounds described above did not significantly change the IC_{50} value of **14a**. Substitution with *p*-*n*-pentyl (**14k**) or *p*-oxycarbonyl (**14s**) significantly decreased the activity.

Table III shows IC_{50} values for the contraction induced by U-46619 in isolated rat thoracic aorta of compounds with fundamentally different structures in the sulfonyl side chains, i.e., **14a–j,l–m**, (+)-**14a**, and (−)-**14a**. Compound **14a** exhibited an IC_{50} value of 1.4 nM, which is lower than those for the reference compounds III and IV (14.5 and 52.6 nM, respectively).[29] The (+) enantiomer of **14a** also exhibited an IC_{50} value (0.49 nM) 30 times lower than that of (−)-**14a** (15.3 nM). The IC_{50} values for the U-46619-induced contraction in isolated rat thoracic aorta were not substantially different from the IC_{50} values for collagen-induced aggregation of rat WP.[30]

On the basis of the structure–activity relationship described above, compound **14a** was chosen as the candidate for further evaluation. Compound **14a** inhibited U-46619-and collagen-induced aggregation of human WP (IC_{50} = 7.7 and 4.5 nM, respectively). As shown in Figure 1, experiments of displacement with **14a** as well as U-46619 for [^{3}H]-U-46619 bound to human WP and that with **14a** as well as ONO-11120 for (5*Z*,15*ξ*)-9*α*,11*α*-(dimethylmethano)-15-hydroxy-16-(3-[^{125}I]iodo-4-hydroxyphenyl)-17,18,19,20-tetranor-13-aza-11a-carbathrombo-5-enoic acid ([^{125}I]-PTA-OH)[12d] bound to human WP revealed that **14a** strongly inhibits the labeled ligands from binding to the TxA_2 receptor in a similar manner to U-46619 and ONO-11120, respectively.[28,31,32] The IC_{50} value (about 3 nM for each experiment) corresponds well to those for the U-46619- and collagen-induced aggregation of human WP. Table IV indicates the effect of **14a** on the synthesis of metabolites of arachidonic acid in human platelets. Little influence of **14a** on the synthesis of TxA_2 and cyclooxygenase or lipoxygenase-derived products (HHT and HETE) in human WP, stimulated by thrombin (2 units/mL), was observed, while indomethacin (a cyclooxygenase inhibitor) and dazoxiben (a TxA_2 synthetase inhibitor) markedly inhibited the synthesis of TxA_2.

Table IV. Effect of **14a** on the Product Formation of [^{3}H]Arachidonic Acid Labeled Human Platelets Stimulated with Thrombin[a]

treatment	arachidonic acid	TxB_2	HHT + HETE
vehicle	14.0 ± 0.6	2.4 ± 0.1	24.0 ± 0.5
14a (1 μM)	14.3 ± 0.2	2.3 ± 0.3	24.0 ± 0.9
14a (10 μM)	14.2 ± 0.1	2.1 ● 0.1	23.2 ± 0.5
indomethacin (20 μM)	28.4 ± 0.2	0.34 ± 0.1	15.0 ± 0.0
dazoxiben (20 μM)	11.8 ± 2.1	0.74 ± 0.1	19.3 ± 4.5
control	1.2 ± 0.0	0.26 ± 0.0	1.5 ± 0.15

[a] The results are expressed as the percentage of total radioactivity recovered, where the remaining radioactivity was found in the phospholipid fractions at the origin of the chromatograms.

Conclusion

Several sulfonyl derivatives of VI were synthesized. Study of the structure–activity relationships revealed that the phenyl group attached directly to the sulfonylamino group is important in the binding to the TxA_2 receptor. Compound **14a** did not inhibit synthesis of TxA_2 in human WP stimulated by thrombin, while it strongly inhibited platelet aggregation, induced by arachidonic acid, U-46619, and collagen, and contraction of rat aorta induced by U-46619. Compound **14a** strongly inhibited labeled TxA_2 analogues from binding to human WP (IC_{50} = 3 nM). These findings imply that the corresponding free acid **13a** (S-145) is a potent TxA_2 receptor antagonist. The stereochemistry corresponding to (+)-**14a** was concluded to be important for the exhibition of potent inhibitory activity.

Experimental Section

Reactions using anhydrous solvents that had been dried over type 4A molecular sieves were carried out in a nitrogen atmosphere. Melting points were determined on a Yanagimoto apparatus and were not corrected. Infrared (IR) spectra were recorded on a JASCO A702 spectrometer. Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained on a Varian EM-390 spectrometer using deuteriochloroform unless otherwise stated with tetramethylsilane as an internal or external (D_2O) reference. When an oily product was contaminated with organic solvents, the sample was dissolved in CCl_4 and concentrated in vacuo. By

(30) A correlation similar to that obtained for IC_{50} values between the aggregation of rat WP and binding to rat WP[27] has recently been found for the IC_{50} values between rat aorta contraction and binding to rat aorta smooth muscle cells in culture. See: Hanasaki, K.; Nakano, K.; Kasai, H.; Arita, H.; Ohtani, K.; Doteuchi, M. *Biochem. Biophys. Res. Commun.* **1988**, *150*, 1170.

(31) Dr. S. Narumiya, Department of Pharmacology, School of Medicine, Kyoto University, kindly carried out the experiment and analyzed the data.

(32) Similarly, displacement experiments using rat WP have recently confirmed strong binding affinity of **14a** to rat WP. See ref 27.

repeating the procedures, peaks assignable to the organic solvents were removed. To dry the organic solution of the extraction, anhydrous magnesium sulfate was used. For column chromatography, silica gel (Merck silica gel 60) or Merck's Lobar column was used. Elemental analyses were within ±0.5% of the theoretical values.

2-*exo*-Allyl-3(*E*)- and -(*Z*)-hydroximinobicyclo[2.2.1]heptane (3a and 3b). A mixture of 6.0 g (40 mmol) of compound **2**, 5.56 g (80 mmol) of hydroxylamine hydrochloride, and 4.49 g (80 mmol) of powdered KOH in 60 mL of methanol was stirred for 30 min at 0 °C. The reaction mixture was diluted with ether, and the organic solution was washed with 0.1 N HCl, dried, and concentrated in vacuo, giving 6.44 g (97.4%) of the crude product, which was chromatographed on silica gel (*n*-hexane–ethyl acetate, 95:5). The nonpolar fraction gave 3.76 g (56.9%) of *E*-isomer **3a** as a colorless oil: IR ($CHCl_3$) 3590, 3285, 3140, 3080, 1680, 1640 cm^{-1}; ^1H NMR δ 1.03–1.90 (m, 7 H), 1.90–2.60 (m, 4 H), 3.50 (br s, 1 H), 4.86–5.25 (m, 2 H), 5.60–6.18 (m, 1 H). Anal. ($C_{10}H_{15}NO$) C, H, N. The polar fraction afforded 2.02 g (30.6%) of *Z*-isomer **3b** as a colorless oil: IR ($CHCl_3$) 3590, 3280, 3140, 3085, 1678, 1641 cm^{-1}; ^1H NMR δ 1.15–2.00 (m, 7 H), 2.15–3.15 (m, 5 H), 4.90–5.22 (m, 2 H), 5.60–6.12 (m, 1 H). Anal. ($C_{10}H_{15}NO$) C, H, N.

2-*exo*-Allyl-3-*endo*-(carbobenzoxyamino)bicyclo[2.2.1]heptane (5). To a solution of 4.5 g (27 mmol) of a mixture of oximes **3a** and **3b** in 50 mL of THF was added 1.0 g (27 mmol) of $LiAlH_4$ at 25 °C. After refluxing for 4 h, the mixture was decomposed with water as usual, extracted with ethyl acetate, dried, and concentrated in vacuo, giving 3.2 g (78.4%) of the crude amine **4** as a pale yellow oil, which was used for the further reaction without purification. To a solution of **4** (3.2 g) in 30 mL of methylene chloride were added 2.0 mL of pyridine and 3.6 mL of carbobenzoxy chloride at 0 °C. The resulting mixture was stirred for 30 min at the same temperature and partitioned between ethyl acetate and 0.1 N HCl. The organic layer was washed with water, dried, and concentrated in vacuo, giving a crude oil, which when chromatographed on silica gel (*n*-hexane–ethyl acetate, 98:2), gave 1.97 g (25.6% from **2**) of compound **5** as a colorless oil: IR ($CHCl_3$) 3430, 1715, 1505 cm^{-1}; ^1H NMR δ 0.74–1.89 (m, 7 H), 1.90–2.30 (m, 3 H), 2.43 (br s, 1 H), 3.53 (td, *J* = 4, 7 Hz, 1 H), 4.80 (br s, 1 H), 4.91 (m, 1 H), 5.09 (s, 2 H), 5.50–6.00 (m, 1 H), 7.36 (s, 5 H). Anal. ($C_{18}H_{23}NO_2$) C, H, N.

2-*exo*-(2α- and 2β-Epoxypropyl)-3-*endo*-(carbobenzoxyamino)bicyclo[2.2.1]heptane (6). To a solution of 6.8 g (23.8 mmol) of **5** in 150 mL of methylene chloride was added 10.3 g (23.8 mmol × 2) of *m*-chloroperbenzoic acid at 20 °C. After the mixture was stirred for 3 h, the resulting crystals were removed by filtration, and the filtrate was concentrated in vacuo. The residue was partitioned between ethyl acetate and 10% aqueous sodium thiosulfate. The organic solution was washed with 5% aqueous sodium bicarbonate and water, dried, and concentrated in vacuo. Chromatography of the residue on silica gel (*n*-hexane–ethyl acetate, 4:1) gave 7.17 g (100%) of **6** as a colorless oil. Although the oil was considered to be a mixture of diastereomers of the epoxides, it was analyzed without attempting to separate it into the two components: IR ($CHCl_3$) 3455, 1717, 1505, 1479, 1456 cm^{-1}; ^1H NMR δ 1.00–1.85 (m, 9 H), 2.05 (br s, 1 H), 2.40 (br s, 1 H), 2.43 (m, 1 H), 2.70 (m, 1 H), 2.89 (m, 1 H), 3.55 (m, 1 H), 4.90 (m, 1 H), 5.06 (s, 2 H), 7.32 (s, 5 H). Anal. ($C_{18}H_{23}NO_3$) C, H, N.

(5*Z*)-7-[3-*endo*-(Carbobenzoxyamino)bicyclo[2.2.1]hept-2-*exo*-yl]heptenoic Acid Methyl Ester (9). To a solution of 4.52 g (15 mmol) of compound **6** in 50 mL of dioxane was added a solution of 6.84 g (30 mmol) of HIO_4 in 15 mL of water at 25 °C, and the reaction mixture was stirred for 4 h at the same temperature and partitioned between ethyl acetate and water. The organic solution was washed with water, dried, and concentrated in vacuo, giving the crude aldehyde **7**, which was unstable and therefore used immediately for the next reaction. In order to prepare dimsylsodium in DMSO, 2.88 g (13.8 mmol × 5.4) of sodium hydride (60% in mineral oil) was added to 100 mL of DMSO at 25 °C and the mixture was stirred for 1.5 h at 70 °C. To the resulting dimsylsodium in DMSO was added 17.8 g (13.8 mmol × 3) of (4-carboxybutyl)triphenylphosphonium bromide at 18 °C, and the mixture was stirred for 20 min at 20 °C. To the red solution of the resulting ylide was added 3.79 g (13.8 mmol) of the above-described crude aldehyde **7** in 60 mL of DMSO at 15 °C. After the reaction mixture was stirred for 4 h at 25 °C, the solution was partitioned between ethyl acetate and 1 N HCl. The organic layer was washed with water, dried, and concentrated in vacuo. The residue was dissolved in 50 mL of ether and esterified by treatment with a solution of diazomethane in ether as usual. Concentration of the solution in vacuo, followed by chromatographic purification of the residue on silica gel (*n*-hexane–ethyl acetate, 9:1), afforded 2.8 g (48.4% from **6**) of **9** as a colorless oil: IR ($CHCl_3$) 3450, 1721, 1602, 1501, 1453, 1437 cm^{-1}; ^1H NMR δ 1.00–1.85 (m, 9 H), 1.85–2.30 (m, 5 H), 2.30 (t, *J* = 7 Hz, 2 H), 2.40 (br s, 1 H), 3.50 (td, *J* = 4, 7 Hz, 1 H), 3.63 (s, 3 H), 4.93 (d, *J* = 7 Hz, 1 H), 5.09 (s, 2 H), 5.36 (m, 2 H), 7.34 (s, 5 H). Anal. ($C_{23}H_{31}NO_4$) C, H, N.

(5*Z*)-7-(3-*endo*-Aminobicyclo[2.2.1]hept-2-*exo*-yl)heptenoic Acid Methyl Ester Trifluoroacetate (10). A mixture of compound **9** (771 mg, 2 mmol), trifluoroacetic acid (10 mL), and anisole (2 mL) was stirred for 3 h at 45 °C and concentrated in vacuo. Washing the residue with petroleum ether gave compound **10** (500 mg, 99.6%) as a light brown oil of sufficient purity for use in the following reactions: ^1H NMR δ 1.10–2.50 (m, 15 H), 2.32 (t, *J* = 7 Hz, 2 H), 3.08 (m, 1 H), 3.68 (s, 3 H), 5.40 (m, 2 H), 7.60 (br s, 2 H), 8.85 (br s, 1 H); IR ($CHCl_3$) 3100, 2560, 1779, 1725, 1675, 1522, 1436 cm^{-1}.

(5*Z*)-7-[3-*endo*-[(Phenylsulfonyl)amino]bicyclo[2.2.1]hept-2-*exo*-yl]heptenoic Acid Methyl Ester (12a). To a solution of compound **10** (204 mg, 0.557 mmol) in 3 mL of methylene chloride were added triethylamine (310 μL, 0.557 mmol × 4) and phenylsulfonyl chloride **11a** (107 μL, 0.557 mmol × 1.5) at 0 °C. The resulting mixture was stirred for 15 min at 23 °C and partitioned between ethyl acetate and 0.1 N HCl. The organic layer was washed with 5% aqueous sodium bicarbonate and water, dried, and concentrated in vacuo, giving a yellow oil, which when chromatographed on silica gel (*n*-hexane–ethyl acetate, 9:1) gave 188 mg (86.2%) of **12a** as a colorless oil: IR ($CHCl_3$) 3375, 1725, 1158, 1090 cm^{-1}; ^1H NMR δ 0.80–2.10 (m, 15 H), 2.17 (br s, 1 H), 2.26 (t, *J* = 7 Hz, 2 H), 3.02 (m, 1 H), 3.67 (s, 3 H), 5.20 (m, 2 H), 7.40–7.65 (m, 3 H), 7.83–8.03 (m, 2 H). Anal. ($C_{21}H_{29}NO_4S$) C, H, N, S.

Compound **12b–t** were also prepared with use of sulfonyl chlorides **11b–t**, respectively, in a manner similar to that described above.

(5*Z*)-7-[3-*endo*-[(Phenylsulfonyl)amino]bicyclo[2.2.1]hept-2-exo-yl]heptenoic Acid (13a). To a solution of **12a** (150 mg, 0.383 mmol) in 2 mL of methanol was added 1 N KOH (0.77 mL, 0.383 mmol × 2) at 23 °C. The reaction mixture was stirred for 6 h at the same temperature and partitioned between ether and water. The aqueous layer was washed with ether, acidified with 1 N HCl, and extracted with ethyl acetate. The organic solution was washed with water, dried, and concentrated in vacuo, giving crude crystals, which were recrystallized from a mixture of ether and *n*-hexane to obtain the target compound **13a** (126 mg, 87.5%) as colorless plates: mp 85–86 °C; IR ($CHCl_3$) 3380, 3550, 2500, 1710, 1160, 1090 cm^{-1}; ^1H NMR δ 0.85–2.30 (m, 15 H), 2.31 (t, *J* = 7 Hz, 2 H), 3.00 (td, *J* = 4, 7 Hz, 1 H), 5.20 (m, 2 H), 5.69 (d, *J* = 7 Hz, 1 H), 7.43–7.70 (m, 3 H), 7.83–8.10 (m, 2 H), 9.52 (br s, 1 H). Anal. ($C_{20}H_{27}NO_4S$) C, H, N, S. Compounds **13b–t** were also prepared from compounds **12b–t** in the same manner. These acids were analyzed after transformation into their sodium salts, because they were, except for **13s** (mp 190 °C), difficult to remove organic solvents.

(5*Z*)-7-[3-*endo*-[(Phenylsulfonyl)amino]bicyclo[2.2.1]hept-2-*exo*-yl]heptenoic Acid Sodium Salt (14a). To a solution of **13a** (2.727 g, 7.22 mmol) in 50 mL of methanol was added 6.23 mL (7.22 mmol) of a solution of sodium methoxide (1.16 mol in methanol) at 0 °C. The mixture was stirred for 10 min at the same temperature, concentrated in vacuo, and dissolved in 60 mL of water. Freeze-drying of the aqueous solution afforded **14a** (2.88 g, 100%) as a colorless powder: IR (KBr) 3390, 3270, 1560, 1445, 1408 cm^{-1}; ^1H NMR (D_2O) δ 1.40–2.65 (m, 17 H), 3.36 (m, 1 H), 5.53 (m, 2 H), 8.00–8.39 (m, 5 H). Anal. ($C_{20}H_{26}NO_4SNa \cdot 0.1H_2O$) C, H, N, S, Na. Sodium salts **14b–t** were also prepared from acids **13b–t**, respectively, in a way similar to that described here. Although the well-dried sodium salts were used for elemental analysis, they were fairly hygroscopic in general and contaminated with non-integer molecules of water.

(5Z)-7-[3-*endo*-(Benzoylamino)bicyclo[2.2.1]hept-2-*exo*-yl]heptenoic Acid Methyl Ester (15). To a solution of compound **10** (197 mg, 0.54 mmol) in 3 mL of methylene chloride were added benzoyl chloride (75 μL, 0.54 mmol × 1.5) and triethylamine (226 μL, 0.54 mmol × 3) at 0 °C. The mixture was stirred for 25 min at 0 °C, 1 N HCl was added, and the organic layer was washed with 5% aqueous sodium bicarbonate and water, then dried, and concentrated in vacuo. Chromatography of the residue on silica gel (*n*-hexane–ethyl acetate, 9:1) gave compound **15** (135 mg, 70.3%) as colorless plates: mp 58–60 °C; IR ($CHCl_3$) 3488, 1730, 1655, 1602, 1580, 1510, 1486 cm^{-1}; ^1H NMR δ 1.00–2.18 (m, 14 H), 2.30 (t, J = 7 Hz, 2 H), 2.59 (s, 1 H), 3.61 (s, 3 H), 3.90 (m, 1 H), 5.38 (m, 2 H), 6.45 (d, J = 7 Hz, 1 H), 7.35–7.85 (m, 5 H). Anal. ($C_{22}H_{29}NO_3$) C, H, N.

(5Z)-7-[3-*endo*-(Benzoylamino)bicyclo[2.2.1]hept-2-*exo*-yl]heptenoic Acid Sodium Salt (16). Hydrolysis of compound **15** followed by neutralization with sodium methoxide in methanol and freeze-drying in water afforded compound **16** as a colorless powder (100%): IR (KBr) 3430, 3350, 1630, 1600, 1577, 1560, 1534, 1490 cm^{-1}; ^1H NMR δ 1.20–2.26 (m, 16 H), 2.52 (m, 1 H), 3.79 (m, 1 H), 5.38 (m, 2 H), 7.38–7.84 (m, 5 H). Anal. ($C_{21}H_{26}NO_3Na$) C, H, N.

Biology. Inhibitory Effect on Rabbit PRP Aggregation. Preparation of Rabbit PRP. Mature male rabbits (NIBS-JW) weighing 2.2–2.6 kg were used. With the animal under sodium pentobarbital anesthesia (Somnopentyl, Pitman Moore, ca. 20 mg/kg, iv), blood was withdrawn from the carotid artery through a cannulation tube using a syringe containing sodium citrate (3.8%, 1/10 volume). The sample was left standing for 20 min at room temperature and then centrifuged at 210*g* for 10 min at 22 °C to obtain PRP. The remaining blood was centrifuged at 3000 rpm for 10 min to obtain platelet-poor plasma (PPP).

Measurement of Inhibition of Platelet Aggregation. Platelet aggregation was examined by the method of Born,[33] with an AUTO-RAM61 type aggregometer (Rika-Denki Co., Ltd., Tokyo) as reported previously.[34] A pair of samples of PRP (400 μL) placed in a cuvette were warmed at 37 °C for 1 min with stirring (1200 rpm), and then a saline solution of the test compound (50 μL) or saline was added. Exactly 2 min later, a solution of sodium arachidonate (50 μL) was added to each of the samples, and the changes in light transmission were recorded, with the light transmissions for PRP and PPP taken as 0% and 100%, respectively, and the maximum light transmissions after addition of sodium arachidonate as the maximum aggregations. The percent inhibition α was expressed as the difference between 1 and the ratio of the maximum aggregation with the test compound to that with the saline.

The IC_{50} value for each compound was obtained by regression analysis of the concentration–inhibition relationship among 12–16 points of α covering three concentrations and ranging from 20 to 80%, obtained by three experiments. The IC_{50} values obtained were calibrated for the IC_{50} value (standard: 1.0 μM) of **14a** obtained with the same PRP sample and are shown in Table II. The IC_{50} values for **14a** fluctuated [1.29 ± 0.42 (SD) μM, n = 21] for each sample of platelets, and thus the confidence limits of the IC_{50} values shown in Table II are believed to be of this level.

Inhibitory Effect on WP Aggregation. Preparation of WP. Blood was collected from rat or human volunteers into 0.15 volume of acid citrate dextrose (85 mM disodium citrate, 70 mM citric acid, and 110 mM glucose) containing 12 μg/mL PGE_1. PRP, obtained by centrifugation at 160*g* for 10 min, was layered on 40% bovine serum albumin. Platelets were sedimented at 1200*g* for 25 min and resuspended in 0.5 mL of resuspension buffer (137 mM NaCl, 2.7 mM KCl, 1.0 mM $MgSO_4$, 3.8 mM NaH_2PO_4, 3.8 mM Hepes, 5.6 mM glucose, and 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.

Measurement of Inhibition of Platelet Aggregation. (i) Rat WP (5×10^8 cells/mL) were preincubated with 1 mM $CaCl_2$ for 2 min at 37 °C in the presence of various concentrations of the test compounds dissolved in water, and then 4 μg/mL of collagen (Hormon-Chemie, München) was added. The aggregation was monitored with the aggregometer in terms of the decrease in light transmission. The IC_{50} values for each compound, calculated from the values of percent inhibition obtained by a single experiment and calibrated (standard: 2.9 nM) in a way similar to that described above, are shown in Table II. The IC_{50} values of **14a** fluctuated for every experiment [3.68 ± 1.29 (SD) nM, n = 49]. (ii) In a way similar to that described above, the IC_{50} value of **14a** for aggregation of human WP induced by collagen (2 μg/mL) was found to be 4.5 ± 0.9 nM (SE) (n = 4) and that by U-46619 (500 nM) as 7.7 ± 0.6 nM (n = 3).

Binding Studies. Studies were carried out in a way similar to that reported previously.[28] The results are shown in Figure 1.

Inhibitory Effect on Rat Thoracic Aorta Contraction. Male SD rats (Japan Clea Laboratory) weighing 200–250 g were used. From each animal killed by decapitation, the thoracic aorta was excised, cleaned, and cut into two spiral strips (3-mm width and 15-mm length).[35] The tissues were placed in 8-mL organ baths containing Krebs bicarbonate solution (NaCl, 118; $CaCl_2$, 2.5; KH_2PO_4, 1.2; KCl, 4.6; $MgSO_4$, 1.2; $NaHCO_3$, 24.8; dextrose, 10 mM; in the case of KCl contraction, NaCl, 69 mM), kept at 37 °C and bubbled with 95% O_2 and 5% CO_2, and then equilibrated for 1 h under a resting tension maintained at 1 g. Isometric tension was recorded on a polygraph (Nihon Kohden RM-6000) through a force displacement transducer (Nihon Kohden TB-612T). After contractile responses to 30 mM KCl became stable, the pair of the tissues was treated with the test compound dissolved in distilled water or vehicle and 30 min later with 30 nM of U-46619. The percent inhibition α was calculated as the difference between 1 and the ratio of the magnitude of contraction of the tissue treated with the test compound to that treated with the vehicle. The IC_{50} value and confidence limit ($p < 0.05$) for each compound were obtained by regression analysis of concentration–inhibition relationship among 12–16 points of α covering three to four different concentrations and ranging from 20 to 80%. The results are shown in Table III.

Assay for TxA_2 Synthetase Inhibition. Human PRP was centrifuged at 1200*g* for 15 min. Platelets were suspended in the resuspension buffer containing 1 μM PGE_1 at 4×10^8 cells/mL and incubated with [^{3}H]arachidonic acid (10 μCi/mL) for 1.5 h at room temperature. Labeled platelets (500 μL) were preincubated with **14a** as well as the reference compounds for 1 min at 37 °C, and 2 units/mL of thrombin was added. After 3 min, the reactions were terminated by adding 0.5 mL of $CHCl_3$–MeOH (1:2). ^{3}H-labeled eicosanoids were extracted and separated by thin-layer chromatography according to the method of Salmon et al.[36] The area corresponding to each eicosanoid was scraped off and the radioactivity was counted. The results are shown in Table IV.

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Registry No. (+)-1, 2630-41-3; (−)-1, 29583-35-5; **2**, 75040-20-9; **3a**, 115266-68-7; **3b**, 115266-69-8; **4**, 115266-70-1; **5**, 115266-71-2; **6** (isomer 1), 115266-72-3; **6** (isomer 2), 115268-40-1; **7**, 115266-73-4; **8**, 17814-85-6; **9**, 115266-74-5; **10**, 115266-76-7; **11a**, 98-09-9; **11b**, 1939-99-7; **11c**, 4025-71-2; **11d**, 63014-04-0; **11e**, 124-63-0; **11f**, 14532-24-2; **11g**, 98-59-9; **11h**, 133-59-5; **11i**, 1899-93-0; **11j**, 16712-69-9; **11k**, 73948-18-2; **11l**, 26588-36-3; **11m**, 1623-93-4; **11n**, 79119-26-9; **11o**, 98-68-0; **11p**, 98-74-8; **11q**, 349-88-2; **11r**, 98-60-2; **11s**, 10130-89-9; **11t**, 19715-49-2; **12a**, 115266-77-8; **12b**, 115266-78-9; **12c**, 115266-79-0; **12d**, 115206-10-5; **12e**, 115266-80-3; **12f**,

(33) Born, G. V. R. *Nature (London)* **1962**, *194*, 927.
(34) Uchida, K.; Nakamura, M.; Konishi, M.; Ishigami, T.; Komeno, T. *Jpn. J. Pharmacol.* **1987**, *43*, 9.
(35) Furchgott, R. F. *Methods in Medical Research*; 8; Bruner, H. D., Ed.; The Yearbook Publishers: Chicago, 1960; Vol. 8, p 177.
(36) Salmon, J. A.; Flower, R. J. *Methods in Enzymology*; Lands, W. E., Smith, W. L., Eds.; Academic: New York, 1982; Vol. 86, pp 447–493.

115266-81-4; **12g**, 115266-82-5; **12h**, 115266-83-6; **12i**, 115266-84-7; **12j**, 115266-85-8; **12k**, 115266-86-9; **12l**, 115227-01-5; **12m**, 115206-11-6; **12n**, 115206-12-7; **12o**, 115266-87-0; **12p**, 115266-88-1; **12q**, 115206-13-8; **12r**, 115266-89-2; **12s**, 115266-90-5; **12t**, 115266-91-6; (±)-**13a**, 115266-92-7; (+)-**13a**, 112966-96-8; (−)-**13a**, 115267-10-2; **13b**, 115266-93-8; **13c**, 115266-94-9; **13d**, 115206-14-9; **13e**, 115266-95-0; **13f**, 115266-96-1; **13g**, 115266-97-2; **13h**, 115266-98-3; **13i**, 115266-99-4; **13j**, 115267-00-0; **13k**, 115267-01-1; **13l**, 115227-02-6; **13m**, 115206-15-0; **13n**, 115206-16-1; **13o**, 115267-02-2; **13p**, 115267-03-3; **13q**, 115267-04-4; **13r**, 115303-09-8; **13s**, 115267-05-5; **13t**, 115267-06-6; (±)-**14a**, 115303-10-1; (+)-**14a**, 115267-11-3; (−)-**14a**, 115303-25-8; **14b**, 115303-11-2; **14c**, 115303-12-3; **14d**, 115267-07-7; **14e**, 115303-13-4; **14f**, 115303-14-5; **14g**, 115303-15-6; **14h**, 115303-16-7; **14i**, 115303-17-8; **14j**, 115303-18-9; **14k**, 115303-19-0; **14l**, 115268-41-2; **14m**, 115267-08-8; **14n**, 115267-09-9; **14o**, 115303-20-3; **14p**, 115303-21-4; **14q**, 115303-22-5; **14r**, 115361-70-1; **14s**, 115303-23-6; **14t**, 115303-24-7; **15**, 115206-17-2; **16**, 115206-18-3.

Synthesis of Novel 3-Substituted β-Carbolines as Benzodiazepine Receptor Ligands: Probing the Benzodiazepine Receptor Pharmacophore

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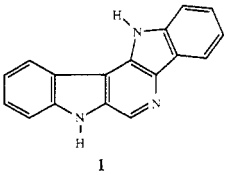
The 3-substituted β-carbolines **2–4** and **5–7** were prepared from 3-amino-β-carboline (**8**) in one step via diazotization, followed by reaction with the appropriate nucleophile in order to determine their binding affinity for benzodiazepine receptors (BzR). All three of the 3-alkoxy-β-carbolines **2** (IC_{50} = 124 nM), **3** (IC_{50} = 24 nM), and **4** (IC_{50} = 11 nM) have high affinities for BzR. The β-carbolines substituted with electron-withdrawing groups including **5** (Cl; IC_{50} = 45 nM), **6** (NO_2; IC_{50} = 125 nM), and **7** (N=C=S; IC_{50} = 8 nM) also had high affinities for BzR. The affinities of **5–8** clearly indicate that a carbonyl moiety at position 3 of a β-carboline is not required for high-affinity binding to BzR. These findings have led to the development of a model for the binding of ligands to an inverse agonist domain at BzR. This model is supported by the recent synthesis of 3-ethoxy-β-carboline (**3**), a potent, long-lived partial inverse agonist, and **7**, an irreversible BzR ligand.

The recent development of models for ligand binding to an inverse agonist site on the (BzR) in these[1a] and other laboratories[2a–d] has prompted the synthesis of a new class of 3-substitued β-carbolines to more rigorously test these models. The β-carbolines possess a broad spectrum of pharmacological actions (inverse agonist,[3–6] antagonist,[7] and agonist[8]) mediated via occupation of benzodiazepine receptors in the central nervous system. Previous studies on the structure–activity relationships (SAR) of β-carbolines had indicated that an ester moiety was required at position 3 in order for the compound to display high affinity for the BzR.[2a–d,4,9] Recently we reported that several β-carboline derivatives lacking a carbonyl group at position 3 possess moderate to high affinities for BzR. Examples of these include the 3,4-disubstituted β-carboline, 7,12-dihydropyrido[3,2-*b*:5,4-*b*']diindole (**1**) (IC_{50} = 4 nM),[10]



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6-(benzylamino)-β-carboline (**12**) (IC_{50} = 106 nM),[11] and the 3-substituted β-carbolines **2–7**, which comprise the subject of the present paper. Consideration of the SAR of the planar, rigid analogue **1**, coupled with other SAR studies,[12–17] has resulted in a model for the requirements of ligand interactions with the inverse agonist binding site on the BzR (Figure 1).[1a] The 3-substituted derivatives of β-carboline were synthesized from 3-amino-β-carboline (**8**) to more rigorously test this model.[18a,b]

Chemistry

The choice of 3-amino-β-carboline (**8**) as the starting material provided facile entry into a variety of 3-substituted β-carbolines, as depicted in Scheme I. Substitution of an electron-withdrawing group (CO_2CH_3,[13] CN,[2a] etc.) at position 3 of a β-carboline is thought to facilitate the interaction of the indole N(9)-H with a hydrogen bond donor[1b] (D_2) site on the receptor.[1a] Thus the chloro **5**, nitro **6**, and isothiocyanate **7** analogues were prepared; these groups exhibit the properties of electron withdrawal in varying degree. Replacement of the diazonium group at position 3 of **8** by a chloride anion has been carried out under classical diazotization conditions ($NaNO_2$, HCl). Moreover the 3-hydroxyl analogue **9** can be prepared by a simple modification. It is important to note that 3-hydroxy-β-carboline (**9**) exists in the pyridone form (Scheme I). This β-carboline was formed from diazotization of the amine **8** with sodium nitrite in 3 N sulfuric acid at 0 °C. The amine functionality on the β-carboline nucleus disposed α (C-3) to the pyridine nitrogen function does not undergo diazotization in the same manner as do other arylamines.[19] In the present case, formation of the diazonium ion does occur. However, because of an apparent lack of stability,[19] it reacts with nucleophiles present in high concentrations such as water or halide in the case of a mineral acid. Chichibabin reported that on treatment of 2-aminopyridine with sodium nitrite and dilute hydrochloric acid, 2-chloropyridine could be isolated in yields of approximately 50%.[20] Under analogous conditions, **8** was diazotized with sodium nitrite in 3 N HCl to furnish the 3-chloro-β-carboline (**5**) in yields of 50–55%, accompanied by the 3-hydroxyl analogue **9**.

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