Antidopaminergic Activities. Inhibition of [^{3}H]Spiperone Binding. The assays were performed in the rat striatal membranes using a previously described method.[14] Briefly, rat striata were homogenized in 100 volumes of ice-cold Tris-HCl buffer (50 mM, pH 7.7) and centrifuged (500*g*, 10 min, 0 °C). The supernatant was centrifuged at 50000*g* for 15 min. The pellet was suspended in 100 volumes of ice-cold Tris-HCl buffer (50 mM, pH 7.7) and recentrifuged (50000*g*, 15 min, 0 °C). The final pellet was resuspended in 150 volumes of ice-cold Tris-HCl buffer (50 mM, pH 7.1) containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 1.1 mM ascorbic acid, and 10 μM pargyline and incubated at 37 °C for 10 min. A portion of this membrane suspension (900 μM) was placed in a tube, and 50 μL of either test compound or vehicle solution was added, followed by 50 μL of [^{3}H]spiperone (40 Ci/mmol) at a final concentration of 0.2 nM. The tubes were incubated at 37 °C for 20 min and filtered through Whatman GF/B glass filters, which were then washed three times with 3 mL of the Tris-HCl buffer (50 mM, pH 7.7). (±)-Sulpiride (100 μM) was used for the determination of nonspecific binding. The radioactivity trapped on the filters was measured by liquid-scintillation spectrometry. The IC_{50} values were determined from concentration–inhibition curves.

Inhibition of Apomorphine-Induced Hyperactivity in Mice. Motor activity was measured with Varimex (Columbus Instruments) in groups of three male mice. Apomorphine hydrochloride (0.5 mg/kg) was injected subcutaneously 1 h after the oral or intraperitoneal administration of test compounds. The motor activity was measured for 20 min immediately after the apomorphine injection.

Supplementary Material Available: Tables of final atomic positional parameters, atomic thermal parameters, and bond distances and angles of compound **9** and the cinchonine salt of compound **21** (10 pages). Ordering information is given on any current masthead page.

(14) Creese, I.; Schneider, R.; Snyder, S. H. *Eur. J. Pharmacol.* **1977**, *46*, 377.

Dual Inhibitors of Thromboxane A_2 Synthase and 5-Lipoxygenase with Scavenging Activity of Active Oxygen Species. Synthesis of a Novel Series of (3-Pyridylmethyl)benzoquinone Derivatives

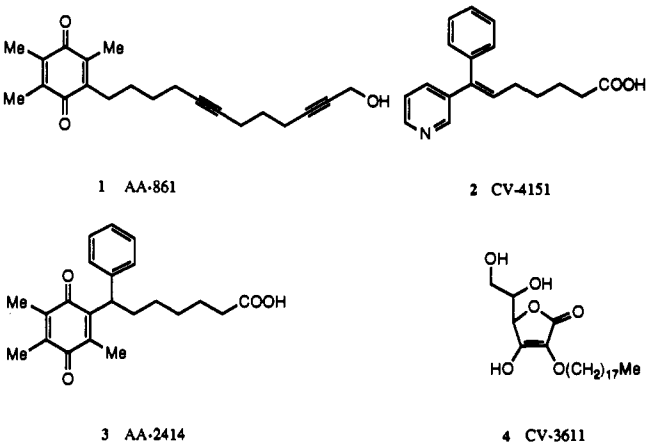
Shigenori Ohkawa,*,† Shinji Terao,† Zen-ichi Terashita,‡ Yumiko Shibouta,‡ and Kohei Nishikawa‡

Research and Development Division, Takeda Chemical Industries, Ltd., 17-85 Jusohonmachi 2-chome, Yodogawa-ku, Osaka 532, Japan. *Received March 5, 1990*

A novel series of (3-pyridylmethyl)benzoquinone derivatives was molecular designed and synthesized for the dual purpose of inhibiting thromboxane A_2 and leukotriene biosynthesis enzymes and scavenging active oxygen species (AOS). They were evaluated for inhibition of TXA_2 synthase, inhibition of 5-lipoxygenase, and for their scavenging activity of AOS using the thiobarbituric acid method. 2,3,5-Trimethyl-6-(3-pyridylmethyl)-1,4-benzoquinone (**24**, CV-6504) was the most promising derivative since it showed efficient AOS scavenging activity (inhibition of lipid peroxidation in rat brain homogenates: $IC_{50} = 1.8 \times 10^{-6}$ M) as well as potent, specific, and well-balanced inhibitory effects on both enzymes (inhibitory effect on TXA_2 synthase in human blood, $IC_{50} = 3.3 \times 10^{-7}$ M; inhibitory effect on 5-lipoxygenase in human blood, $IC_{50} = 3.6 \times 10^{-7}$ M). In adriamycin-induced proteinuria in a rat model, compound **24** at 10 mg/kg per day (po) suppressed proteinuria by more than 50%. The proteinuria, however, could not be reduced by single administration of an inhibitor specific for thromboxane A_2 synthase [(*E*)-7-phenyl-7-(3-pyridyl)-6-heptenoic acid (**2**, CV-4151)] or for 5-lipoxygenase [2-(12-hydroxy-5,10-dodecadiynyl)-3,5,6-trimethyl-1,4-benzoquinone (**1**, AA-861)]. The proteinuria was also not reduced by administration of an AOS scavenger, 2-*O*-octadecylascorbic acid (**4**, CV-3611). Triple function compounds such as compound **24** that specifically inhibit both enzymes as well as scavenge AOS possess a variety of pharmacologically beneficial effects.

Arachidonic acid liberated from phospholipid by various stimuli can be metabolized by the cyclooxygenase (CO) pathway to prostaglandins (PGs) and thromboxane A_2 (TXA_2) or by lipoxygenase (LO) pathways to hydroxyeicosatetraenoic acid (HETEs) and leukotrienes (LTs). These oxidative metabolites of arachidonic acid have been implicated as important mediators in a variety of diseases including stroke, myocardial infarction, inflammation, ulcerative colitis, and rheumatoid arthritis.[1] In addition, recent reports suggest that active oxygen species (AOS), including superoxide (O_2^-), hydrogen peroxide, hydroxyl radical, and ferryl radical, mediate cell damage in a variety of pathological conditions.[2] Intensive research in the perturbation of the arachidonate cascade system resulted in the discovery of many interesting agents, some of which have found therapeutic application. Most of these compounds are selective enzyme inhibitors and specific receptor antagonists. For example, we have developed a potent and selective 5-lipoxygenase inhibitor, 2-(12-hydroxy-5,10-dodecadiynyl)-3,5,6-trimethyl-1,4-benzoquinone (**1**, AA-861),[3] a potent, selective, long-acting thromboxane A_2 synthase inhibitor, (*E*)-7-phenyl-7-(3-pyridyl)-6-heptenoic acid (**2**, CV-4151),[4] a specific throm-

Chart I



† Chemistry Research Laboratories.
‡ Biology Research Laboratories.

(1) (a) Samuelsson, B. *Science*, **1983**, *220*, 568. (b) Ford-Hutchinson, A. W. *Fed. Proc.* **1985**, *44*, 25.

0022-2623/91/1834-0267$02.50/0

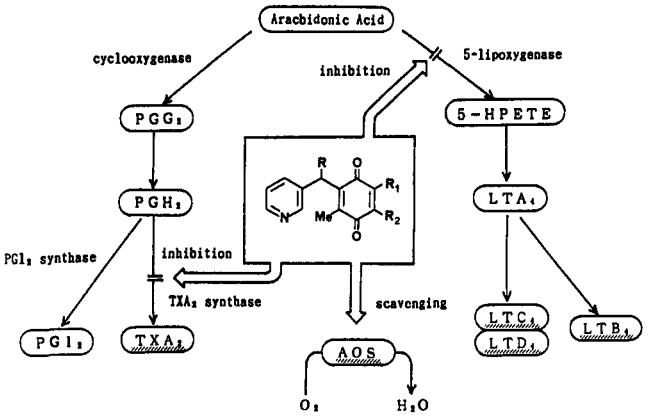


Figure 1. Activities of (3-pyridylmethyl)benzoquinone derivatives.

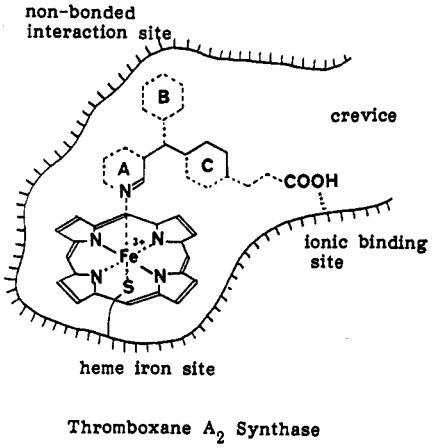


Figure 2. Conceptual model for the TXA_2 synthase-TXA_2 synthase inhibitor interactions.

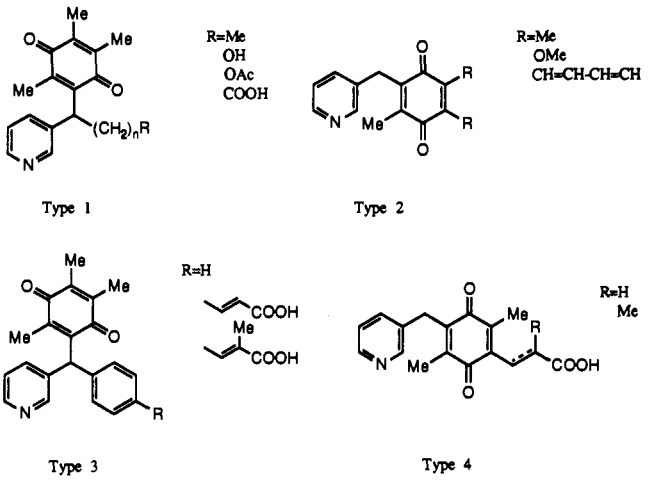
boxane A_2 receptor antagonist, 7-(3,5,6-trimethyl-1,4-benzoquinon-2-yl)-7-phenylheptanoic acid (3, AA-2414),[5] and a membrane-associated AOS scavenger, 2-*O*-octadecylascorbic acid (4, CV-3611).[6] These compounds are in clinical trials (Chart I).

These noxious mediators of the arachidonate cascade and AOS generating systems act together or sometimes synergistically in many disease states. Consequently dual-purpose compounds that inhibit two enzymes of thromboxane A_2 and leukotriene formation and which scavange AOS might be more effective than individual enzyme inhibitors, receptor antagonists, or specific AOS scavengers. Examples of these are TXA_2 synthase inhibitors, TXA_2 receptor antagonists, 5-lipoxygenase inhibitors, leukotriene receptor antagonists, and single AOS scavengers (Figure 1).

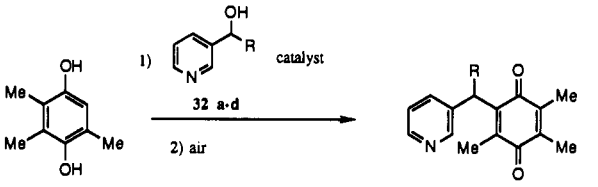
Herein, we report the synthesis and biological evaluation of (3-pyridylmethyl)benzoquinone derivatives. These compounds inhibit both TXA_2 synthase and 5-lipoxygenase and also scavenge AOS.

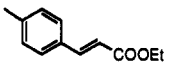
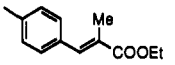
(2) (a) Fridvich, I. *Annu. Rev. Pharmacol. Toxicol.* **1983**, *23*, 239. (b) McCord, J. M. *N. Engl. J. Med.* **1985**, *312*, 159. (c) Halliwell, B.; Gutteridge, J. M. *Trends Biochem. Sci. (Pers. Ed.)* **1986**, *11*, 372. (d) Youngman, R. Y. *Ibid.* **1984**, *9*, 280.
(3) Terao, S.; Shiraishi, M.; Kato, K.; Ohkawa, S.; Ashida, Y.; Maki, Y. *J. Chem. Soc. Perkin Trans. 1* **1982**, 2909.
(4) Kato, K.; Ohkawa, S.; Terao, S.; Terashita, Z.; Nishikawa, K. *J. Med. Chem.* **1985**, *28*, 287.
(5) Shiraishi, M.; Kato, K.; Terao, S.; Ashida, Y.; Terashita, Z.; Kito, G. *J. Med. Chem.* **1989**, *32*, 2214.
(6) Kato, K.; Terao, S.; Shimamoto, N.; Hirata, M. *J. Med. Chem.* **1988**, *31*, 793.

Chart II



Scheme I



	R	catalyst	yield (%)
a	Me	CF_3SO_3H	61
b		H_2SO_4	73
c		H_2SO_4	73
d		H_2SO_4	83

Drug Design

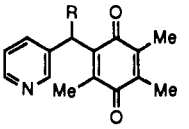
We reported previously that benzoquinone derivative 1 has a potent inhibiting activity on 5-lipoxygenase.[3] We also proposed a model for the interaction between PGH_2 and TXA_2 synthase in the molecular design of potent TXA_2 synthase inhibitors such as compound 2 (Figure 2).[4] In our continuous efforts of new drug evaluation, four novel types of (3-pyridylmethyl)-1,4-benzoquinones were molecular designed (Chart II). The *p*-quinone fragment imparts 5-lipoxygenase inhibiting activity and can also serve the function of hydrophobic fragment, which is desirable for TXA_2 synthase inhibitory activity. These compounds are also expected to have antioxidant activities because the redox nature of the *p*-quinone moiety may emerge in the environment of the living system.

Type I compounds have substituted alkyl chains at the methylene group between the pyridyl and *p*-quinonyl moieties. Type 2 compounds have three aromatic groups including a pyridine and *p*-quinone. Type 3 compouns are simple (pyridylmethyl)quinones. Type 4 compounds have a side chain on the quinone moiety.

Chemistry

The general synthetic pathways for preparation of compounds listed in Table I are shown in Schemes I–VI,

Table I. Physical Properties of (3-Pyridylmethyl)-1,4-benzoquinone Derivatives

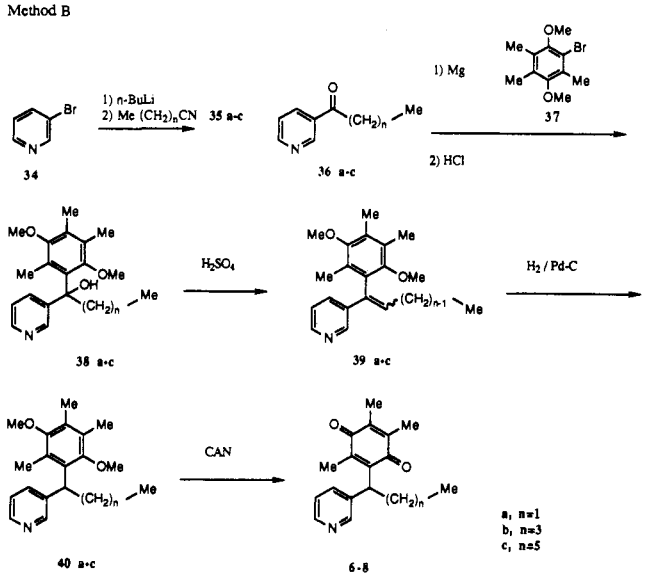


no.	R	synth method	% yield[a]	mp, °C	recrystn solvent	formula (fw)[b]	type[c]	log P
5	Me	A	61	oil		$C_{16}H_{17}NO_2$ (255.32)	1	2.85
6	CH_2Me	B	64	56–57	IPE[d]–hexane	$C_{17}H_{19}NO_2$ (269.35)	1	3.35
7	$(CH_2)_3Me$	B	62	oil		$C_{19}H_{23}NO_2$ (297.40)	1	4.35
8	$(CH_2)_5Me$	B	63	44–45	hexane	$C_{21}H_{27}NO_2$ (325.45)	1	5.35
9	$=CH(CH_2)_4Me$ (*Z*)	B	77	oil		$C_{21}H_{25}NO_2$ (323.44)	1	5.05
10	$=CH(CH_2)_4Me$ (*E*)	B	77	oil		$C_{21}H_{25}NO_2$ (323.44)	1	5.05
11	$(CH_2)_4OH$	C	46	104–105	IPE–EtOAc	$C_{19}H_{23}NO_3$ (313.40)	1	3.19
12	$(CH_2)_5OH$	C	68	oil		$C_{20}H_{25}NO_3$ (327.43)	1	3.69
13	$(CH_2)_4OAc$	C	93	76–77	IPE–EtOAc	$C_{21}H_{25}NO_4$ (355.44)	1	4.08
14	$(CH_2)_5OAc$	C	91	60–61	IPE–EtOAc	$C_{22}H_{27}NO_4$ (369.46)	1	4.58
15	$(CH_2)_3COOH$	C	54	82–84	EtOH	$C_{19}H_{21}NO_4$ (327.39)	1	0.35
16	$(CH_2)_4COOH$	C	58	68–69	EtOAc	$C_{20}H_{23}NO_4$ (341.41)	1	0.85
17	$(CH_2)_5COOH$	C	57	126–127	IPE–EtOAc	$C_{21}H_{25}NO_4$ (355.44)	1	1.35
18	$(CH_2)_6COOH$	C, D	75	113–114	EtOAc	$C_{22}H_{27}NO_4$ (369.46)	1	1.85
19	$=CH(CH_2)_4COOH$	C	60	oil		$C_{21}H_{23}NO_4$ (353.42)	1	1.05
20	Ph	A	72	oil		$C_{21}H_{19}NO_4$ (317.39)	2	4.48
21	PhCH=C(Me)COOH	A	85	199–201	EtOAc	$C_{25}H_{23}NO_4$ (401.47)	2	2.08
22	PhCH=CHCOOH	A	81	232–233	EtOAc	$C_{24}H_{21}NO_4$ (387.44)	2	1.78
23	$Ph(CH_2)_2COOH$	A	44	205–207	EtOAc	$C_{24}H_{23}NO_4$ (389.45)	2	2.08

[a] No attempt was made to optimize yields. Numbers represent the yield for the last step. [b] All compounds were analyzed for C, H, and N within ±0.4% of the calculated values. [c] See Chart II. [d] IPE = isopropyl ether.

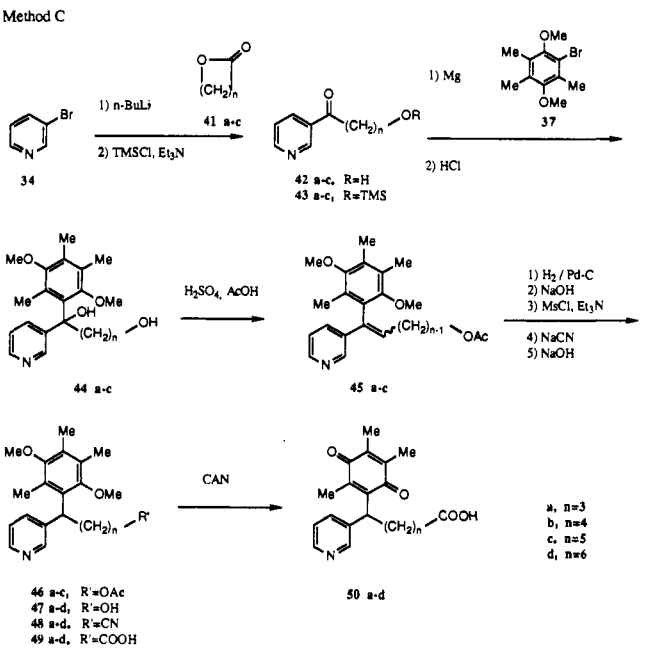
Scheme II

Method B



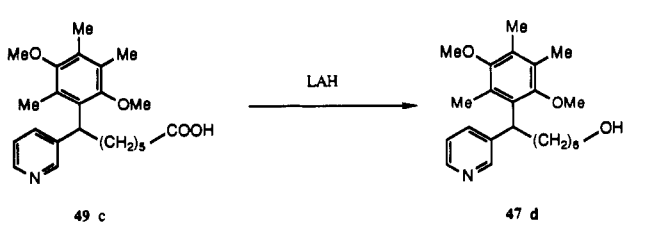
Scheme III

Method C



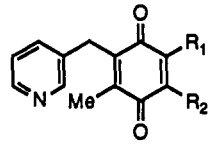
Scheme IV

Method D



methods A–F. Some type 1 and type 2 compounds were synthesized by applying Friedel–Crafts reactions of trimethylhydroquinone (**31**) with various pyridylmethanols (**32a–d**) in the presence of an acid catalyst (method A). Since solid Lewis acids like $AlCl_3$ formed insoluble complexes with pyridylmethanols, liquid acids like sulfuric acid and trifluoromethanesulfonic acid were used as catalysts. The obtained hydroquinone compounds were easily oxidized in CH_2Cl_2 solution via air contact to afford quinone compounds (**33a–d**). Hydrolysis of esters of **33c** and **33d** yielded free acids (**22**, **21**). Catalytic hydrogenation of **33c** on 5% Pd–C followed by air oxidation gave a propionic acid derivative (**23**). Since various attempts to couple trimethylhydroquinone (**31**) with pyridylmethanols (**32**) having alkyl groups of at least two carbons long were unsuccessful, most type 1 compounds were synthesized by the following basic condensation (method B). Reaction of 3-lithiopyridine, prepared from 3-bromopyridine (**34**) and *n*-butyllithium in ethyl ether, with various nitriles (**35a–c**) gave alkanoylpyridines **36a–c**. Coupling **36a–c**

Table II. Physical Properties of (3-Pyridylmethyl)-1,4-benzoquinone Derivatives

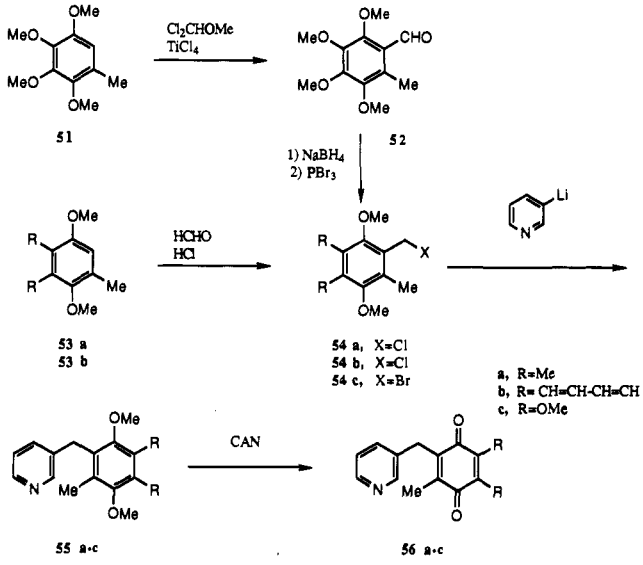


no.	R_1	R_2	synth method	% yield[a]	mp, °C	recrystn solvent	formula (fw)[b]	type[c]	log P
24	Me	Me	E	88	66–67	IPE–EtOAc	$C_{15}H_{15}NO_2$ (241.30)	3	2.55
25	-CH=CHCH=CH-		E	94	102–103	IPE–EtOAc	$C_{17}H_{13}NO_2$ (263.30)	3	2.77
26[d]	OMe	OMe	E	82	152–153	EtOAc–EtOH	$C_{15}H_{16}NO_4Cl$ (309.75)	3	0.61
27	Me	$(CH_2)_2COOH$	F	72	149–150	EtOAc–THF	$C_{17}H_{17}NO_4$ (299.33)	4	-0.65
28	Me	CH=CHCOOH	F	72	175–180	THF	$C_{17}H_{15}NO_4$ (297.32)	4	-0.95
29	Me	CH=C(Me)COOH	F	70	196–200	THF	$C_{18}H_{17}NO_4$ (311.34)	4	-0.65
30	Me	$(CH_2)_2COOEt$	F	81	oil		$C_{19}H_{21}NO_4$ (327.38)	4	3.28

[a–c] See footnotes of Table I. [d] This compound was prepared as the hydrochloride.

Scheme V

Method E



with Grignard reagent prepared from bromobenzene derivative **37** and magnesium gave tertiary alcohols **38a–c**. Acid-catalyzed dehydration of **38a–c** in acetic acid gave compounds **39a–c**, which were converted to compounds **40a–c** by hydrogenation using 5% Pd–C as catalyst. Oxidative demethylation of **40a–c** with cerium ammonium nitrate (CAN)[7] gave quinones **6–8** in good yield. Reaction of 3-lithiopyridine with various lactones (**41a–c**) gave ω-hydroxy pyridyl ketones **42a–c** in good yields (method C). Protection of the hydroxy group with a trimethylsilyl group followed by coupling with Grignard reagent and deprotection of the trimethylsilyl group gave tertiary alcohols **44a–c**. Dehydrations of tertiary alcohols were carried out under acidic conditions and led to the formation of compounds **45a–c**. Hydrogenation of the obtained acetates **45a–c** gave compounds **46a–c**, which were converted to alcohols **47a–c** by alkaline hydrolysis. The hydroxy group of **47a–c** was converted to a carboxy group in subsequent reactions: mesylation, cyanation, and base-catalyzed hydrolysis. Oxidative demethylation of carboxylic acids **49a–c** with CAN gave quinone compounds **50a–c**.

Reduction of **49c** with lithium aluminum hydride gave alcohol **47d**, which was converted to quinone (**18**) with CAN (method D). Compound **47d** was also converted to carboxylic acid derivative **50d** in a similar method as compound **50c**.

Protected (3-pyridylmethyl)quinones **55a–c** were prepared by coupling 3-lithiopyridine with (halomethyl)benzene derivatives **54a–c**, which were synthesized by chloromethylation of compound **53a,b** or formylation[8] of compound **51** followed by reduction and bromination (method E). Oxidative demethylation of compound **55a–c** was carried out with CAN.

Formylation of compound **58**, which was prepared from quinone (**57**) according to method E, gave aldehyde **59** in good yield (method F). A coupling reaction of **59** and triethyl phosphonoacetates **60** yielded α,β-unsaturated carboxylates **61** and **62**, which led to quinones **63–65** by acidic hydrolysis, catalytic hydrogenation, and oxidative demethylation with CAN.

Pharmacological Results and Discussion

In vitro and ex vivo inhibitory actions of synthesized compounds on TXA_2 synthase, in vitro inhibitory actions on 5-lipoxygenase, and in vitro inhibitory actions on lipid peroxidation are summarized in Tables III and IV.

A. Thromboxane A_2 Synthase Inhibition. Thromboxane A_2 synthase inhibition by each (3-pyridylmethyl)benzoquinone was evaluated by measuring the inhibition of TXA_2 production in horse platelet microsomes (in vitro)[9] and in rat serum (ex vivo). Type 1 compounds **17** and **18**, having carboxyl moieties on the terminus of alkyl chains, exhibited the most potent activities in vitro. Compound **15**, containing a shorter alkyl chain, tended to be less active. These results are in accord with this feature of TXA_2 synthase inhibition which was reported in our previous work.[4] Introduction of a double bond into the side chain that is conjugated with the pyridine ring either reduced inhibition (compare **8** with **9** and **10**) or did not show any effect on the activity (compare **17** and **19**). Potent activity was also exhibited by both the compounds possessing a short alkyl chain (**5**) and no side chain (**24**, type 3). Introduction of a phenyl ring into the side chains (type 2 compounds) reduced the activities (compare **18** and **23**). Type 4 compounds (**27–30**), which have carboxyl side chains on the quinone ring, exhibited rather potent activities. In the ex vivo test, type 1 compounds **5**, **7**, **14**, and **17–19**, type 3 compounds **24** and **25**, and type 4 compounds **27** and **28** showed potent activities. We also investigated the effects of compounds **17** and **24**

(7) Syper, L.; Kloc, K.; Mlochowski, J.; Szulc, Z. *Synthesis* **1979**, 521.

(8) Reiche, A.; Gross, H.; Höft, E. *Chem. Ber.* **1960**, *93*, 88.

(9) Needleman, P.; Minkes, M.; Raz, A. *Science* **1979**, *193*, 163.

Table III. Screening Results of (3-Pyridylmethyl)-1,4-benzoquinone Derivatives

no.	R	inhibn of TXB_2 production: in vitro, % (10^{-6} M)[a]	in vitro, IC_{50} × 10^{-7} M[b]	ex vivo:[c] 10 mg/kg	inhibn of LTB_4 production: IC_{50} × 10^{-8} M[d]	inhibn of lipid peroxidation: % (10^{-6} M)[e]	IC_{50} × 10^{-7} M[f]
5	Me	89	7.2 (2)	90	950 (2)	93	7.2 (4)
6	CH_2Me	76	8.3 (2)	75	66 (2)	83	8.4 (2)
7	$(CH_2)_3Me$	28	–[g]	97	83 (2)	83	8.4 (2)
8	$(CH_2)_5Me$	46	29 (2)	70	7.3 (2)	83	6.0 (2)
9	$=CH(CH_2)_4Me$ (*Z*)	7	–	nt[h]	26 (2)	80	6.2 (2)
10	$=CH(CH_2)_4Me$ (*E*)	6	–	nt	22 (2)	77	8.6 (2)
11	$(CH_2)_4OH$	44	11 (2)	40	10 (2)	67	9.2 (2)
12	$(CH_2)_5OH$	61	9.0 (2)	72	10 (2)	83	8.2 (2)
13	$(CH_2)_4OAc$	45	15 (2)	37	25 (2)	77	8.2 (2)
14	$(CH_2)_5OAc$	39	9.5 (2)	84	8.7 (3)	73	8.7 (2)
15	$(CH_2)_3COOH$	41	–	36	1000 (2)	37	–
16	$(CH_2)_4COOH$	90	0.89 (2)	64	6000 (2)	34	–
17	$(CH_2)_5COOH$	86	0.63 (4)	90	930 (2)	49	–
18	$(CH_2)_6COOH$	90	0.44 (2)	97	700 (2)	52	9.9 (2)
19	$=CH(CH_2)_4COOH$ (*E* + *Z*)	92	1.8 (2)	91	720 (2)	28	–
20	Ph	29	–	nt	760 (2)	73	8.6 (2)
21	PhCH=C(Me)COOH	68	5.2 (2)	19	10000 (2)	41	–
22	PhCH=CHCOOH	22	–	nt	8700 (2)	39	–
23	$Ph(CH_2)_2COOH$	25	–	nt	10000 (2)	48	–

[a] Percent inhibition of the amount of TXB_2 formed by incubating PGH_2 with horse platelet microsomes. Values are the mean of duplicate experiments. [b] The molar concentration of test compound required to reduce by 50% the amount of TXB_2 formed by incubating PGH_2 with horse platelet microsomes. The *n* values (in parentheses) are the number of experiments in which a dose–response curve was determined from two to six replicates per dose level. [c] Percent inhibition of the amount of serum TXB_2 24 h after oral administration of test compound to the rat. Values are the mean of duplicate experiments. [d] The molar concentration of test compound required to reduce by 50% the amount of LTB_4 formed by RBL-1 cells. The *n* values (in parentheses) are the number of experiments in which a dose–response curve was determined from two to six replicates per dose level. [e] Inhibition of lipid peroxidation in rat brain homogenates. Values are the mean of duplicate experiments. [f] The molar concentration of test compound required to reduce by 50% the amount of lipid peroxide formed in rat brain homogenates. The *n* values (in parentheses) are the number of experiments in which a dose–response curve was determined from two to six replicates per dose level. [g] Not determined. [h] Not tested.

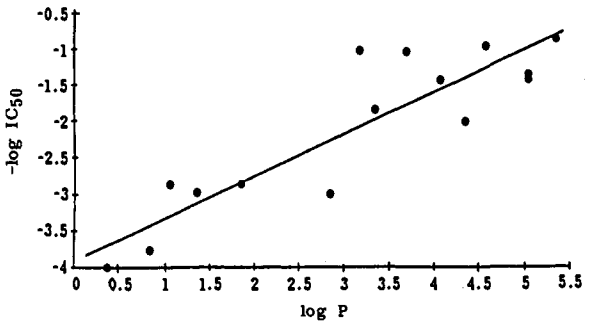
Table IV. Screening Results of (3-Pyridylmethyl)-1,4-benzoquinone Derivatives

no.	R_1	R_2	inhibn of TXB_2 production: in vitro, % (10^{-6} M)[a]	in vitro, IC_{50} × 10^{-7} M[b]	ex vivo,[c] 10 mg/kg	inhibn of LTB_4 production: IC_{50} × 10^{-8} M[d]	inhibn of lipid peroxidation: % (10^{-6} M)[a]	IC_{50} × 10^{-7} M[f]
24	Me	Me	60	4.0 (4)	86	6.2 (6)	80	6.8 (6)
25	–CH=CHCH=CH–		57	4.9 (2)	92	8.2 (4)	42	–[g]
26	OMe	OMe	40	–	74	520 (2)	39	–
27	Me	$(CH_2)_2COOH$	53	9.7 (2)	86	500 (2)	14	–
28	Me	CH=CHCOOH	79	5.0 (2)	90	880 (2)	14	–
29	Me	CH=C(Me)COOH	45	–	73	870 (2)	4	–
30	Me	CH=CHCOOEt	28	–	80	80 (2)	35	–
1 (AA-861)			nt[h]		nt	9.0 (6)	nt	
2 (CV-4151)			92	0.26 (4)	90	nt	nt	
4 (CV-3611)			nt		nt	nt	45	43 (6)

[a–h] See footnotes of Table III.

on the production of PGI_2 in rat serum (ex vivo) and found that its production was not reduced by these compounds (data not shown). This result suggests that these compounds have no cyclooxygenase inhibitory effects.

B. 5-Lipoxygenase Inhibition. 5-Lipoxygenase inhibition was evaluated by inhibition of LTB_4 production in RBL-1 cells.[10] Type 3 compounds **24** and **25**, which are potent TXA_2 synthase inhibitors as described above, were highly effective. Type 1 compounds **11**, **12**, **14** and **8**, having hydroxyl group, acetoxy group, or no substituent at the alkyl chain terminus, also exhibited significant activities. The substitution of methyl groups by methoxy groups on the quinone ring abolished this activity (compare **24** and **26**) and introduction of polar substituents reduced activities in common (compare **8** and **17**). There seemed to be a relationship between the log of the octanol/water partition coefficient (log *P*) and LTB_4 biosynthesis inhibition by these compounds. Thus, log *P* was estimated by calculation using log *P* of compound **24** (determined



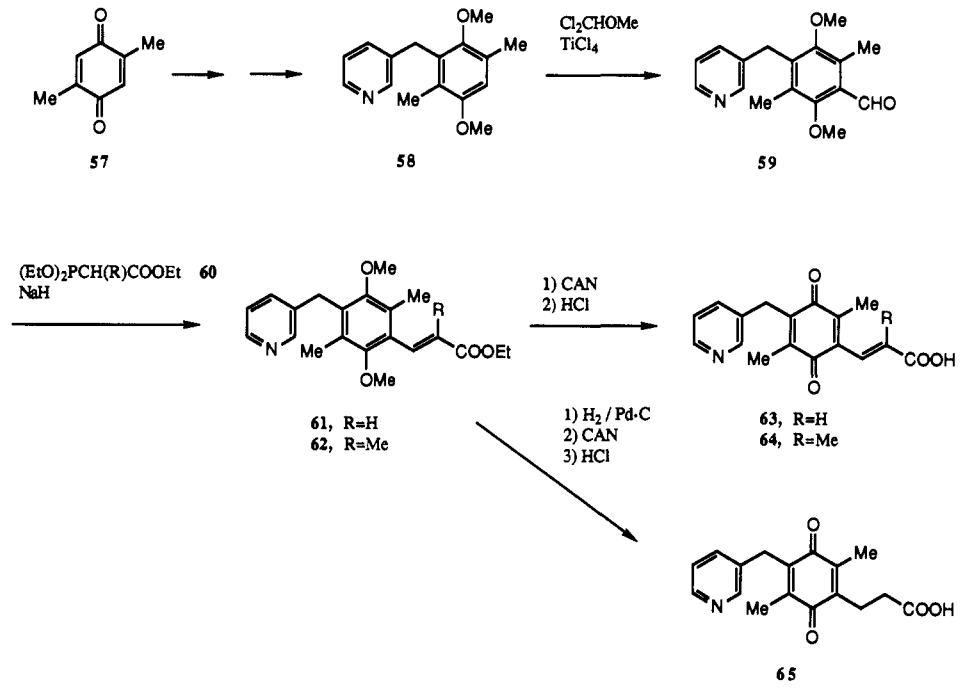
$\log IC_{50} = -0.565 \log P + 3.85$ $r = 0.88$

Figure 3. The linear dependence of LTB_4 biosynthesis inhibition (log IC_{50}) on log *P* (type 1 compounds).

(10) Amano, T.; Matsumoto, M.; Terao, S. *Vitamin (Tokyo)* **1985**, *59*, 211.

Scheme VI

Method F



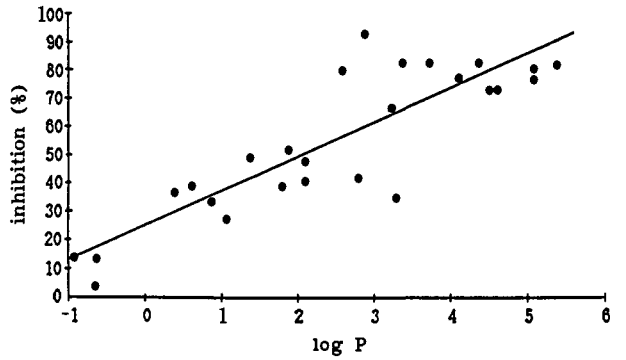


Figure 4. The linear dependence of lipid peroxidation inhibition (%, 10^{-6} M) on log *P*.

experimentally) and π values of each substituent[11] (log *P* values are listed in Tables I and II). This relationship is shown graphically in Figure 3 for compounds 5–19. There was a linear relationship between log IC_{50} for leukotriene B_4 biosynthesis inhibition and log *P*. Similar observations were reported by a Merck group.[12]

C. Lipid Peroxidation Inhibition. Lipid peroxidation inhibition was evaluated with rat brain homogenates.[13] Since these activities also correlated with log *P*, the relationship between structure and lipid peroxidation inhibition was similar to that of the 5-lipoxygenase inhibition activity (Figure 4). This correlation may be explained by the following mechanism. 5-Lipoxygenase exists in two states, an active state having ferric nonheme iron and a resting state having ferrous nonheme iron in a putative active center. This enzyme translocates from the cytosol to a membrane where its catalytic activity emerges.[14] The mechanism of inhibition of 5-lipoxygenase by antioxidants such as hydroquinones and phenols is thought to involve reduction of the ferric nonheme iron to the ferrous state.[15] Additionally the concentration of lipid hydroperoxides which are thought to activate the enzyme[16] is reduced. In other words, these two activities result from presence of an antioxidant (the quinone compound) in the lipophilic membrane.

D. Evaluation of (3-Pyridylmethyl)benzoquinones as Dual Inhibitors Having AOS Scavenging Activity. The above results show that within these series, type 3 compound **24** has the most potent and well-balanced inhibitory activity on TXA_2 synthase, 5-lipoxygenase, and lipid peroxidation. Although type 1 compounds **8**, **12**, and **14** showed potent and well-balanced activities, they were less active than compound **24**. The most promising compound (**24**) was tested for inhibition of TXA_2 and LTB_4 production in human whole blood in vitro. Compound **24**, which was used in the hydrochloride form for convenience, inhibited LTB_4 production in a dose-dependent manner. Its IC_{50} value was 3.3×10^{-7} M. Compound **24** also inhibited TXA_2 production in a dose-dependent manner and its IC_{50} value was 3.6×10^{-7} M, which was close to that of 5-LO inhibition. Furthermore, compound **24** did not inhibit PGI_2 synthase and tended to slightly increase the amount of PGI_2 in the ex vivo experiments (30% increase at 10^{-7} M).

It is concluded that compound **24** has potent and well-balanced inhibitory activity on TXA_2 synthase and on 5-lipoxygenase in human whole blood.

E. Effect of Compound 24 on Preventing Adriamycin-Induced Proteinuria in Rats. Its was suggested

(11) Tute, M. S. *Advances in Drug Research*; Academic press: New York and London, 1971; Vol. 6 p 1.
(12) Hammond, M. L.; Kopka, I. E.; Zambias, R. A.; Caldwell, C. G.; Boger, J.; Baker, F.; Bach, T.; Luell, S.; MacIntyre, D. E. *J. Med. Chem.* **1989**, *32*, 1006.
(13) Ohkawa, H.; Ohishi, N.; Yagi, K. *Anal. Biochem.* **1979**, *95*, 351.
(14) Samuelsson, B.; Dahlen, S.; Lindgren, J. A.; Rouzer, C. A.; Serhan, C. N. *Science* **1987**, *237*, 1171.
(15) R. W. Egan *Abstract from 21st National Medicinal Chemistry Symposium*, Minneapolis; American Chemical Society: Washington, DC, 1988; pp 145.
(16) Rouzer, C. A.; Samuelsson, B. *FEBS Lett.* **1986**, *204*, 293.

Table V. Protective Effect of Compound 24 on Adriamycin-Induced Proteinuria in Rats

group	no. of rats	total protein,[a] mg/100 g per 24 h	albumin,[a] mg/100 g per 24 h
vehicle	8	142 ± 20	93 ± 13
compound 24[b]	8	60 ± 7**	39 ± 5**
normal	5	5 ± 2	2 ± 0.7

[a] Values represent means ± SEM. Asterisks (**) distinguish statistically significant values using Dunnett's test ($P < 0.01$ vs vehicle). [b] Compound 24 (hydrochloride, 10 mg/kg per day) was administered orally for 4 weeks.

Table VI. Protective Effect of Compounds 1, 2, and 4 on Adriamycin-Induced Proteinuria in Rats

group	dose, mg/kg per day	no. of rats	total protein,[a] mg/100 g per 24 h	albumin,[a] mg/100 g per 24 h
vehicle		15	115 ± 20	63 ± 11
compound 1[b]	100	7	95 ± 14	49 ± 8
compound 2[b]	30	7	131 ± 21	75 ± 11
compound 4[b]	50	7	122 ± 23	70 ± 11

[a] Values represent means ± SEM. [b] All compounds were administered orally for 4 weeks.

that various chemical mediators, including TXA_2,[17] LTs,[18] and AOS,[19] are involved in the pathogenesis of renal glomerular diseases. It was considered that agents which inhibit more than one of these mediators simultaneously may be more effective than single inhibitors of individual mediators for treatment of renal diseases. Adriamycin (7.5 mg/kg iv) increased urinary protein excretion and also the level of malondialdehyde in plasma and urine.[20] Furthermore, the production of renal cortical TXA_2 and LTs was enhanced in these rats.[21] Oral continuous administration of compound 24 (10 mg/kg per day po) for 4 weeks significantly reduced urinary protein (total protein and albumin) by more than 50% (Table V). In this experiment the urine volume was not affected by administration of 24. In the same test system, a selective TXA_2 synthase inhibitor (2), selective 5-LO inhibitor (1), and AOS scavenger (4) could not reduce urinary protein (Table VI). Therefore, the multiple effects of compound 24 may be necessary for the activity.[22] Thus, compound 24 showed significant beneficial effects in adriamycin-induced nephrosis, which is a model for chronic progressive glomerular disease. Furthermore, compound 24 reduced proteinuria in puromycin aminonucleoside-induced nephrosis in rats. It also inhibited generation of renal TXA_2 and LT synthesis and the increase of plasma MDA levels.[23]

In conclusion, we have shown that compound 24 has an efficient AOS scavenging activity as well as potent, specific, and well-balanced inhibitory effects on both TXA_2 synthase and 5-LO. Compound 24 is a promising compound for the treatment of renal diseases and is now under clinical investigation for chronic glomerular nephritis.

Experimental Section

Melting points were obtained with a Yanaco micro melting apparatus and are uncorrected. ^1H NMR spectra were recorded on a Varian EM-390 spectrometer in $CDCl_3$ with tetramethylsilane as an internal standard. Where elemental analyses are given, results obtained were within ± 0.4% of the theoretical values. Solutions in organic solvents were dried over anhydrous $MgSO_4$. Column chromatography was carried out on silica gel (E. Merck, particle size 0.70–230 mesh). Yields were not maximized.

3,5,6-Trimethyl-2-[1-(3-pyridyl)ethyl]-1,4-benzoquinone (5). A mixture of 1-(3-pyridyl)ethanol (4.0 g, 32.5 mmol), 2,3,5-trimethylhydroquinone, and trifluoromethanesulfonic acid in dichloroethane (25 mL) was refluxed for 20 h under an argon atmosphere and then cooled. The mixture was poured into ice–water, washed with EtOAc, and neutralized with saturated aqueous $NaHCO_3$. The organic layer was separated and the aqueous layer was extracted with $CHCl_3$. The combined organic layer was washed with water, dried, oxidized with air, and evaporated. The residue was chromatographed on silica gel with isopropyl ether (IPE)–EtOAc (1:2) as eluent to give 1 (5.1 g, 61%). Other compounds listed in Scheme I were prepared by the same method from corresponding secondary alcohols obtained by the following method. 1-(3-Pyridyl)ethanol was prepared by $NaBH_4$ reduction of 3-acetylpyridine. 3-Pyridyl-2-thienylmethanol, ethyl 3-[4-(hydroxy-3-pyridylmethyl)phenyl]acrylate, and ethyl 3-[4-(hydroxy-3-pyridylmethyl)phenyl]-2-methylacrylate were prepared by the methods described previously.[24]

3-[4-[(3,5,6-Trimethyl-1,4-benzoquinon-2-yl)-3-pyridylmethyl]phenyl]-2-methylacrylic Acid (21). A solution of ethyl 3-[4-[(3,5,6-trimethyl-1,4-benzoquinon-2-yl)-3-pyridylmethyl]-phenyl]-2-methylacrylate (1.2 g, 2.8 mmol) in concentrated HCl (20 mL) was refluxed for 2 h and then cooled. The mixture was neutralized with saturated aqueous $NaHCO_3$ and the product was extracted with EtOAc. The extract was washed with water, dried, and evaporated. The residue was chromatographed on silica gel with EtOAc–EtOH (9:1) as eluent followed by recrystallization from EtOAc to give 21 (0.96 g, 86%). In a similar manner compound 22 was synthesized from corresponding esters.

3-[4-[(3,5,6-Trimethyl-1,4-benzoquinon-2-yl)-3-pyridylmethyl]phenyl]propionic Acid (23). A solution of 3-[4-[(3,5,6-trimethyl-1,4-benzoquinon-2-yl)-3-pyridylmethyl]-phenyl]acrylic acid (22, 0.70 g, 1.75 mmol) in acetic acid (6 mL) was hydrogenated on 5% Pd–C (0.2 g). The catalyst was removed by filtration and the filtrate was evaporated. The residue was dissolved in EtOAc and shaken with a solution of $FeCl_3$. The organic layer was separated, washed with aqueous $NaHCO_3$, dried, and evaporated. The residue was chromatographed on silica gel with EtOAc–EtOH (9:1) as eluent followed by recrystallization to give 23 (0.30 g, 44%).

4-(3-Pyridyl)-4-oxobutanol (42a). To a stirred solution of 3-bromopyridine (10 g, 63.3 mmol) in Et_2O (100 mL) was added *n*-butyllithium (1.6 M hexane solution, 40 mL, 64 mmol) dropwise at −78 °C. The mixture was stirred for 15 min. A solution of γ-butyrolactone (5.45 g, 63.3 mmol) in Et_2O (15 mL) was added to the resulting mixture and then stirred for another 1 h at ambient temperature. The mixture was diluted with brine and

(17) (a) Lianos, E. A.; Andres, G. A.; Dunn, M. J. *J. Clin. Invest.* **1983**, *72*, 1439. (b) Remuzzi, G.; Imberit, L.; Rossini, M.; Morelli, C.; Carminati, C.; Cattanso, G. M.; Bertani, T. *J. Clin. Invest.* **1985**, *75*, 94.

(18) (a) Lefkowith, J. B.; Morrison, A. R.; Schreiner, G. F. *J. Clin. Invest.* **1988**, *82*, 1655. (b) Failer, J.; Wiemeyer, A.; Marx, K. H.; Kuhn, K.; Koch, K. M.; Frolich, J. C. *Kidney Int.* **1989**, *36*, 46. (c) Lianos, E. A. *J. Clin. Invest.* **1988**, *82*, 427. (d) Rahman, M. A.; Nakazawa, M.; Emancipator, S. N.; Dunn, M. J. *J. Clin. Invest.* **1988**, *81*, 1945.

(19) (a) Rehan, A.; Johnson, K. J.; Wiggins, R. C.; Kunkel, R. G.; Ward, P. A. *Lab. Invest.* **1984**, *51*, 396. (b) Johnson, K. J.; Rehan, A.; Ward, P. A. *Abstract from Upjohn Symposium on the Oxygen Radical*, Bethesda; *Fed. Am. Soc. Exp. Biol.*; 1987; pp 115. (c) Laurent, B.; Ardaillou, R. *Am. J. Physiol.* **1986**, *251*, F765. (d) Thakur, V.; Walker, P. D.; Shah, S. V. *Kidney Int.* **1988**, *34*, 494.

(20) Okuda, S.; Oh, Y.; Tsuruda, H.; Onoyama, K.; Fujimi, S.; Fujishima, M. *Kidney Int.* **1986**, *29*, 502.

(21) Terashita, Z.; Imura, Y.; Shibouta, Y.; Ohkawa, S.; Nishikawa, K., *Abstract from XIth International Congress of Pharmacology*, Amsterdam, July 1–6, 1990.

(22) The results of a corresponding assay where the individual compounds are mixed in combination will be reported elsewhere.

(23) Shibouta, Y.; Terashita, Z.; Imura, Y.; Shino, A.; Nishikawa, K.; Fujiwara, Y. *Abstract from XIth International Congress of nephrology*, Tokyo, July 15–22, 1990.

(24) Tanouchi, T.; Kawamura, M.; Ohyama, I.; Kajiwara, I.; Iguchi, Y.; Okada, T.; Iizuka, K.; Nakazawa, M. *J. Med. Chem.* **1981**, *24*, 1149.

the product was extracted with *n*-BuOH. The extract was washed with brine and evaporated. The residue was chromatographed on silica gel with $CHCl_3$–MeOH (9:1) as eluent followed by recrystallization from EtOAc–IPE to give **42a** (8.0 g, 77%, mp 36–37 °C). 5-(3-Pyridyl)-5-oxopentanol (**42b**, 71%, oil) and 6-(3-pyridyl)-6-oxohexanol (**42c**, 57%, oil) were prepared by the same method from δ-valerolactone and ε-caprolactone, respectively.

1-(3-Pyridyl)-4-[(trimethylsilyl)oxy]butan-1-one (43a). To a stirred solution of **42a** (12.5 g, 69.8 mmol) and Et_3N (12.6 mL, 90.7 mmol) in DMF (100 mL) was added trimethylchlorosilane (9.1 g, 83.8 mmol) dropwise at 0 °C. After the addition was completed, the mixture was stirred for 30 min at ambient temperature and then poured into water. The product was extracted with EtOAc and the extract was washed with water, dried, and evaporated. The residue was distilled under reduced pressure to give **43a** (13.4 g, 76%, bp_1 140–143 °C). 1-(3-Pyridyl)-5-[(trimethylsilyl)oxy]pentan-1-one (**43b**, 72%, bp_1 134–138 °C) and 1-(3-pyridyl)-6-[(trimethylsilyl)oxy]hexan-1-one (**43c**, 82%, bp_1 140–143 °C) were prepared by the same method.

1-Acetoxy-4-(2,5-dimethoxy-3,4,6-trimethylphenyl)-4-(3-pyridyl)-3-butene (45a). A solution of 1-(3-pyridyl)-4-[(trimethylsilyl)oxy]butan-1-one (**43a**, 6.0 g, 23.9 mmol) in THF (10 mL) at 0 °C was added to a Grignard reagent prepared from 1-bromo-2,5-dimethoxy-3,4,6-trimethylbenzene (7.7 g, 29.8 mmol), magnesium (0.70 g), and THF (50 mL) with stirring. After the addition was completed, the mixture was stirred for 1 h at ambient temperature and then diluted with water. The product was extracted with EtOAc, and the extract was washed with water, dried, and evaporated. The residue was dissolved in EtOH (50 mL), and 2 N HCl (10 mL) was added. The mixture was stirred for 1 h at room temperature, diluted with water, and neutralized with saturated aqueous $NaHCO_3$. The product was extracted with EtOAc; the extract was washed with water, dried, and evaporated. The residue was dissolved in AcOH (80 mL), and concentrated H_2SO_4 (15 mL) was added. The mixture was stirred for 30 min at 60 °C, and then neutralized with $NaHCO_3$. The product was extracted with EtOAc. The extract was washed with water, dried, and evaporated. The residue was chromatographed on silica gel with $CHCl_3$–EtOAc (1:1) as eluent to give **45a** (4.09 g, 44%, oil). 1-Acetoxy-5-(2,5-dimethoxy-3,4,6-trimethylphenyl)-5-(3-pyridyl)-4-pentene (**45b**, 61%, oil) and 1-acetoxy-4-(2,5-dimethoxy-3,4,6-trimethylphenyl)-6-(3-pyridyl)-5-hexene (**45c**, 67%, oil) were prepared by the same method.

1-Acetoxy-4-(2,5-dimethoxy-3,4,6-trimethylphenyl)-4-(3-pyridyl)butane (46a). A solution of **45a** (1.0 g, 2.7 mmol) in AcOH (10 mL) was hydrogenated on 5% Pd–C (0.40 g) at 80 °C. After the theoretical amount of hydrogen was absorbed, the catalyst was removed by filtration. The filtrate was evaporated and the residue was dissolved in EtOAc. The solution was washed with saturated aqueous $NaHCO_3$, dried, and evaporated. The residue was chromatographed on silica gel (EtOAc) to give **46a** (0.75 g, 75%, oil). 1-Acetoxy-6-(2,5-dimethoxy-3,4,6-trimethylphenyl)-6-(3-pyridyl)hexane (**46b**, 83.1%, oil) and 1-acetoxy-5-(2,5-dimethoxy-3,4,6-trimethylphenyl)-5-(3-pyridyl)pentane (**46c**, 86%, oil) were prepared by the same method.

4-(2,5-Dimethoxy-3,4,6-trimethylphenyl)-4-(3-pyridyl)-1-butanol (47a). NaOH (1 N, 5 mL) was added to a solution of **46a** (0.70 g, 1.88 mmol) in MeOH (3 mL), and the mixture was stirred for 30 min at room temperature. The mixture was diluted with water and the product was extracted with EtOAc. The extract was washed with water, dried, and evaporated. The residue was purified with a short silica gel column (EtOAc) to give **47a** (92.0%, oil). 5-(2,5-Dimethoxy-3,4,6-trimethylphenyl)-5-(3-pyridyl)-1-pentanol (**47b**, 92.0%, mp 99–100 °C) and 6-(2,5-dimethoxy-3,4,6-trimethylphenyl)-6-(3-pyridyl)-1-hexanol (**47c**, 96%, mp 90–91 °C) were prepared by the same method.

4-Cyano-1-(2,5-dimethoxy-3,4,6-trimethylphenyl)-4-(3-pyridyl)butane (48a). To a stirred solution of **47a** (525 mg, 1.6 mmol) and Et_3N (0.33 mL) in dichloroethane (3.5 mL) was added methanesulfonyl chloride (0.15 mL, 1.94 mmol) at 0 °C with stirring. The mixture was stirred for 30 min at the same temperature and diluted with water. The organic layer was separated, the aqueous layer was extracted with dichloromethane, and the extract was combined with the organic layer. The organic layer was washed with water, dried, and evaporated. The residue was dissolved in DMSO (5 mL), and sodium cyanide (148 mg, 2.9 mmol) was added. The mixture was stirred for 2 h at 80 °C and then diluted with water. The product was extracted with EtOAc; the extract was washed with water, dried, and evaporated. The residue was chromatographed on silica gel (EtOAc) to give **48a** (445 mg, 83%, oil). 5-Cyano-1-(2,5-dimethoxy-3,4,6-trimethylphenyl)-1-(3-pyridyl)pentane (**48b**, 93%, oil), 6-cyano-1-(2,5-dimethoxy-3,4,6-trimethylphenyl)-1-(3-pyridyl)hexane (**48c**, 93%, oil), and 7-cyano-1-(2,5-dimethoxy-3,4,6-trimethylphenyl)-1-(3-pyridyl)heptane (**48d**, 94%, oil) were prepared by the same method.

5-(2,5-Dimethoxy-3,4,6-trimethylphenyl)-5-(3-pyridyl)-pentanoic Acid (49a). To a solution of **48a** (445 mg, 1.32 mmol) in MeOH (3 mL) was added 8 N NaOH (5 mL), and the mixture was refluxed for 3 h. The refluxed mixture was diluted with water and neutralized with 2 N HCl, and the product was extracted with EtOAc. The extract was washed with water, dried, and evaporated. The residue was chromatographed on silica gel ($CHCl_3$–MeOH, 9:1), followed by recrystallization from IPE–EtOAc, to give **49a** (400 mg, 85%, mp 82–84 °C). 6-(2,5-Dimethoxy-3,4,6-trimethylphenyl)-6-(3-pyridyl)hexanoic acid (**49b**, 85%, mp 183–184 °C), 7-(2,5-dimethoxy-3,4,6-trimethylphenyl)-7-(3-pyridyl)heptanoic acid (**49c**, 91%, oil), and 8-(2,5-dimethoxy-3,4,6-trimethylphenyl)-8-(3-pyridyl)octanoic acid (**49d**, 89%, oil) were prepared by the same method.

7-(2,5-Dimethoxy-3,4,6-trimethylphenyl)-7-(3-pyridyl)-heptanol (47d). To a stirred solution of **49c** (3.0 g, 7.8 mmol) in THF (40 mL) was added $LiAlH_4$ (450 mg, 11.9 mmol) portionwise at 0 °C. The mixture was stirred for 30 min at ambient temperature and diluted with water carefully. The product was extracted with EtOAc. The extract was washed with water, dried, and evaporated. The residue was chromatographed on silica gel (EtOAc) to give **47d** (2.3 g, 80%, oil).

3-Heptanoylpyridine (36a). To a stirred solution of 3-bromopyridine (10.0 g, 63.3 mmol) in Et_2O (100 mL) was added n-butyllithium (1.6 M hexane solution, 40 mL, 64 mmol) at −78 °C. The mixture was stirred for 15 min. To the mixture was added heptanenitrile (7.52 g, 67.7 mmol), and stirring was continued for another 1 h at ambient temperature. The mixture was poured into water and the organic layer was separated. The aqueous layer was extracted with EtOAc. The extract was combined with the organic layer. The combined extract was washed with water, dried, and evaporated. The residue was chromatographed on silica gel (IPE) to give **36a** (3.9 g, 32%, oil). 3-Propionylpyridine (**36b**, 30%, oil) and 3-pentanoylpyridine (**36c**, 44%, oil) were prepared by the same method from propionitrile and valeronitrile, respectively. The transformations of **32a–c** to **2-4** were performed by a method similar to that of **10a**.

2,5-Dimethoxy-3,4,6-trimethylbenzyl Chloride (54a). The mixture of 1,4-dimethoxy-2,3,5-trimethylbenzene (2.9 g, 11.1 mmol), formaline (2 mL), and concentrated HCl (8 mL) was stirred for 3 h at 80 °C. The product was extracted with IPE, and the extract was washed with water, dried, and evaporated. The residue was recrystallized from MeOH to give **54a** (2.1 g, 83%, mp 63–64 °C).

3-(2,5-Dimethoxy-3,4,5-trimethylbenzyl)pyridine (55c). To a stirred solution of 3-bromopyridine (1.0 g, 6.33 mmol) in Et_2O was added *n*-butyllithium (1.6 M hexane solution, 4.0 mL, 6.4 mmol) at −78 °C. After the addition was completed, the mixture was stirred for another 20 min at the same temperature. To the resulting mixture was added a solution of 2,5-dimethoxy-3,4,6-trimethylbenzyl chloride (**54c**, 1.45 g, 6.36 mmol) in Et_2O (5 mL). Stirring was continued for another 1 h at ambient temperature. The mixture was diluted with water and the product was extracted with EtOAc. The extract was washed with water, dried, and evaporated. The residue was chromatographed on silica gel (EtOAc) to give **55c** (1.3 g, 76%).

2,3,4,5-Tetramethoxy-6-methylbenzaldehyde (52). To a stirred solution of 1,2,3,4-tetramethoxy-5-methylbenzene (9.0 g, 42.5 mmol) in CH_2Cl_2 (60 mL) was added dichloromethyl methyl ether (14.4 g, 125 mmol) at 0 °C followed by addition of $TiCl_4$ (13.8 mL, 125 mmol). The resulting mixture was stirred for 4 h at ambient temperature and then poured into ice–water. After stirring vigorously for 10 min, the organic layer was separated. It was washed with water, dried, and evaporated. The residue was chromatographed on silica gel (IPE–hexane) to give **52** (10.0 g, 98%, oil).

2,3,4,5-Tetramethoxy-6-methylbenzyl Bromide (54c). To a solution of **52** (10 g, 41.7 mmol) in EtOH (150 mL) was added $NaBH_4$ (0.78 g, 20.6 mmol) at 0 °C. The mixture was stirred for 30 min at the same temperature. The mixture was diluted with brine and the product was extracted with EtOAc. The extract was washed with water, dried, and evaporated. The residue was dissolved in THF (60 mL). PBr_3 (4.4 g, 16.3 mmol) was then added at 0 °C. The resulting mixture was stirred for 30 min and diluted with water. The product was extracted with IPE, the extract was washed with saturated aqueous $NaHCO_3$, dried, and evaporated. The obtained crude product **54c** (8.7 g, 70%) was used in the next reaction without further purification.

3-(4-Formyl-2,5-dimethoxy-3,6-dimethylbenzyl)pyridine (59). 2,5-Dimethoxy-3,4,6-trimethylbenzene was transformed to 3-(2,5-dimethoxy-3,6-dimethylbenzyl)pyridine (**58**) by the same method as described in the synthesis of compound (**55c**). Compound **58** was formylated with dichoromethyl methyl ether and $TiCl_4$ to give compound **59** (94%).

Ethyl 3-[4-(3-Pyridylmethyl)-2,5-dimethoxy-3,6-dimethylphenyl]acrylate (61). To a solution of triethyl phosphonoacetate (**60**, 1.24 g, 5.53 mmol) in DMF (10 mL) was added NaH (60% dispersion in mineral oil, 244 mg, 6.08 mmol) at 0 °C. The mixture was stirred for 20 min at the same temperature. To the mixture was added a solution of **59** in DMF (5 mL). The mixture was stirred for 1 h at ambient temperature. The mixture was poured into water and the product was extracted with EtOAc. The extract was washed with water, dried, and evaporated. The residue was recrystallized from IPE to give **61** (1.6 g, 86%, mp 77–78 °C). Ethyl 3-[4-(3-pyridylmethyl)-2,5-dimethoxy-3,6-dimethylphenyl]-2-methylacrylate (**62**, 77%, oil) was prepared by the same method.

Oxidation of 1,4-Dimethoxybenzene Derivatives to Quinones. General Procedure. To a solution of 1,4-dimethoxybenzene derivative (3 mmol) in 50% aqueous acetonitrile (20 mL) was added a solution of cerium ammonium nitrate (8 mmol) in 50% aqueous acetonitrile (15 mL) at 0°C. The mixture was stirred for 1 h at the same temperature, then diluted with water, and neutrallized with saturated aqueous $NaHCO_3$. The product was extracted with EtOAc, and the extract was washed with water, dried, and evaporated. The residue was purified by silica gel chromatography and/or recrystallization to give the quinone derivative.

Determination of log P. log P of compound **24** (log P^*) was determined experimentally. A suspension of compound **24** (5.00 mg) in 0.1 M phosphate buffer (10 mL, pH 7.4, 8.0, and 9.0 in each experiment) was shaken for 2.5 h at room temperature. The insoluble material was removed by filtration and 2.5 mL of the filtrate was mixed with 1-octanol (2.5 mL) and shaken for 2.5 h at room temperature. The mixture was centrifuged (3000 rpm, 5 min) and the concentration of compound **24** in the aqueous layer before (C_a) and after (C_b) shaking with 1-octanol was determined by HPLC [column, YMC A-302; 0.01 M KH_2PO_4–MeCN (7:3); flow rate, 0.7 mL/min; detection, UV 254 nm]. The partition coefficient of compound **24** (P^*), which is expressed by $P^* = (C_a - C_b)/C_a$, did not changed with pH (7.4, 8.0, and 9.0). log P^* was determined to 2.55 ± 0.022 (SD, $n = 9$). log P values of other compounds were calculated by the expression (log P = log P^* + π) using π values described previously.[11]

Biological Methods. Inhibition of TXA_2 Synthase Activity. In Vitro Experiments. Horse platelet microsomes (HPM) were prepared and used as the enzyme source of thromboxane synthase according to the method of Needleman et al.[9] Enzyme inhibition by the prepared compounds was examined by measuring thromboxane B_2 (the stable metabolite of thromboxane A_2) produced by incubating PGH_2 with horse platelet microsomes. Duplicate samples of PGH_2 (30 mg/20 μL) in 50 mM Tris buffer (pH 7.50) were preincubated at room temperature for 5 min in the absence and presence of each of the synthesized compounds (final concentration of each compound was 10^{-6} M in dimethyl sulfoxide). Thromboxane synthase (HPM) (23 μg of protein/50 μL in Tris buffer) was then added and the incubation continued for 5 min at 4 °C. Each reaction was stopped by addition of Tris buffer, and each mixture was stored at −78 °C until TXB_2 determination by radioimmunoassay. Five microliters of the reaction mixture was used to assay TXB_2. Rabbit antiserum to TXB_2 was diluted 1:1000 with buffer I (0.1 M phosphate buffer at pH 7.50 containing 0.1% gelatin, 0.9% NaCl, and 0.01% NaN_3). Authentic TXB_2 (10–1000 pg) for the standard curve was dissolved in 50 μL of buffer I. [^{3}H]TXB_2 in buffer (10 000 cpm/100 μL) was added to the reaction mixture or to authentic TXB_2, and 50 μL of diluted rabbit antiserum was then added. The tubes were shaken for 15 s, incubated for 1 h at 25 °C, and stored for 16–20 h at 4 °C. To separate the antiserum-bound from free TXB_2, dextran-coated charcoal (0.1 mL of buffer I containing 2.5 mg of charcoal and 0.25 mg of dextran) was added to each tube and mixed for 15 s. The tubes were left standing on ice for 10 min and then centrifuged for 5 min at 3000 rpm at 4 °C. The radioactivity of each supernatant (180 μL) was measured in 4 mL of ACS II scintillator with a scintillation counter (LKB-1216, Rackbeta). The antiserum nonspecific binding tubes (containing no antiserum) were subtracted from counts of other tubes. The standard curve was plotted as a function of logit vs the amount of authentic TXB_2. The binding of each sample was determined from the standard curve.

Ex Vivo Experiments. Compounds at a dose of 10 mg/kg were given orally to 7–8-week-old male Sprague–Dawley rats. Control rats were given the vehicle (2 mL/kg of water containing small amounts of gum arabic) alone. Twenty-four hours later, blood (1 mL) was withdrawn from the abdominal aorta under anesthesia with sodium pentobarbital (50 mg/kg, intraperitoneally) and incubated for 90 min at 25 °C. Serum was then separated (15 000 rpm, 2 min). TXB_2 produced during incubation was determined by radioimmunoassay.

Inhibition of 5-Lipoxygenase Activity. Rat basophilic leukemia cells were suspended (10^7 cells) in 0.5 mL of mast-cell medium. To the suspension was subsequently added a solution consisting of 0.5 mL of mast-cell medium, 50 μg of arachidonic acid, and test compound at a final concentration of 10 μM, 1 μM, or 0.1 μM. The mixture was incubated at 37 °C for 20 min. Then, 4 mL of ethanol was added, and the mixture was well shaken and kept at room temperture for 10 min. The mixture was centriguted for 10 min (2000 rpm), and the supernatant was separated. The supernatant obtained was concentrated to dryness under reduced pressure. Then, 0.5 mL of 60% (v/v) aqueous methanol was added to the residue. One hundred microliters of this solution was subjected to high-performance liquid chromatography for quantitative analysis of 5-HETE (5-hydroxyeicosatetraenoic acid). The amount of 5-HETE was determined by its absorbance at 237 nm. The inhibitory effect (IE) of 5-HETE production is expressed by $(1 - b/a) \times 100$, wherein a is the peak height or the area of the peak corrected with the internal standard in the absence of the compound and b is the peak height or peak area corrected with the internal standard in the presence of the compound.

Inhibitory Action on Lipid Peroxide Production in Rat Brain Homogenates. Male Sprague–Dawley rat (12 weeks) brain tissue was extracted under anesthesia after depletion of blood. The extracted brain tissue was used as a 5% (v/v) homogenate in phosphate buffer (pH 7.4). After incubation of the homogenate for 1 h at 37 °C, the peroxide produced was determined by the thiobarbituric acid method according to the method of Ohkawa et al.[13] Before incubation, test compounds were added to the 5% (v/v) homogenate to a final concentration of 10^{-6} M. The inhibitory action on lipid peroxide production is expressed as percent inhibition compared with the amount of production in the vehicle (dimethyl sulfoxide) group.

Inhibition of TXA_2 and LTB_4 Production by Compound 24 (in Vitro). A mixture of human whole blood (500 μL, heparinized) and an aqueous solution of test compound (50 μL) was incubated at 37 °C for 5 min. The mixture was centrifuged (3000 rpm, 5 min) and the supernatant was extracted with petroleum ether and EtOAc. The amounts of LTB_4, TXB_2, and 6-keto-$PGF_{1\alpha}$ extracted with EtOAc were determined by radioimmunoassay independently.

Prevention of Adriamycin-Induced Proteinuria by Compound 24 in Rats. Male Sprague–Dawley rats (5-week-old) were used. Nephrotic syndrome was induced by the intravenous injection of adriamycin (7.5 mg/kg) through the tail vein according to the method of Remuzzi et al.[17b] One week after the injection of adriamycin, test compound or vehicle (gum arabic–water solution) in a volume of 10 mL/kg was administered orally for 4 weeks. After the final administration of test compound or vehicle, 24-h urine samples were collected. Urinary total protein and

albumin were measured by the A/G-B test (Wako, Japan) and albumin B-tests (Wako, Japan), respectively.

Acknowledgment. We wish to thank Dr. N. Shimamoto for the assay of lipid peroxidation inhibition, Dr. Y. Yoshimura for measuring log P of compound **24**, and Dr. M. Nishikawa, Dr. A. Imada, Dr. G. Goto, and Dr. Y. Inada for their encouragement and helpful discussions throughout this work. Thanks are also due to Dr. M. Motsenbocker for reviewing the manuscript.

Registry No. **1**, 80809-81-0; **2**, 89667-40-3; **4**, 98829-12-0; **5**, 117574-29-5; **6**, 117574-41-1; **7**, 117574-35-3; **8**, 117574-42-2; **9**, 117574-73-9; **10**, 117574-72-8; **11**, 117585-81-6; **12**, 117574-37-5; **13**, 117585-82-7; **14**, 117574-38-6; **15**, 117574-34-2; **16**, 117574-36-4; **17**, 117574-39-7; **18**, 117574-49-9; **19**, 117574-52-4; **20**, 117574-30-8; **21**, 117574-33-1; **22**, 117574-45-5; **23**, 117574-32-0; **24**, 117574-40-0; **25**, 117574-44-4; **26**, 129813-29-2; **27**, 129813-30-5; **28**, 129813-31-6; **29**, 129813-32-7; **30**, 129833-16-5; **31**, 700-13-0; **32a**, 4754-27-2; **32b**, 6270-47-9; **32c**, 129813-33-8; **32d**, 100181-52-0; **33c**, 129813-34-9; **33d**, 117574-31-9; **34**, 626-55-1; **36a**, 1570-48-5; **36b**, 1701-72-0; **36c**, 6294-61-7; **37**, 69821-05-2; **38a**, 129813-35-0; **38b**, 129813-36-1; **38c**, 129813-37-2; **39a**, 129813-38-3; **39b**, 129813-39-4; **39c**, 117585-86-1; **40a**, 129813-40-7; **40b**, 129813-41-8; **40c**, 117574-51-3; **41a**, 96-48-0; **41b**, 542-28-9; **41c**, 502-44-3; **42a**, 59578-62-0; **42b**, 117574-57-9; **42c**, 117574-58-0; **43a**, 129813-42-9; **43b**, 129813-43-0; **43c**, 129813-44-1; **44a**, 129813-45-2; **44b**, 129813-46-3; **44c**, 129833-17-6; **45a**, 117574-62-6; **45b**, 129813-47-4; **45c**, 129813-48-5; **46a**, 117574-63-7; **46b**, 117574-66-0; **46c**, 117574-65-9; **47a**, 117574-64-8; **47b**, 117574-67-1; **47c**, 117574-68-2; **47d**, 117574-69-3; **48a**, 117574-70-6; **48b**, 117574-71-7; **48c**, 117574-77-3; **48d**, 117574-78-4; **49a**, 117574-50-2; **49b**, 129813-49-6; **49c**, 117574-55-7; **49d**, 117574-56-8; **50a**, 117574-34-2; **50b**, 117574-36-4; **50c**, 117574-39-7; **50d**, 117574-49-9; **51**, 35896-58-3; **52**, 89048-25-9; **53a**, 4537-09-1; **53b**, 53772-19-3; **54a**, 54757-17-4; **54b**, 34824-73-2; **54c**, 89048-14-6; **55a**, 117574-76-2; **55b**, 129813-50-9; **55c**, 129813-51-0; **56a**, 117574-40-0; **56b**, 117574-44-4; **57**, 137-18-8; **58**, 129813-52-1; **59**, 129813-53-2; **60**, 867-13-0; **61**, 129813-54-3; **62**, 129813-55-4; ethyl 3-[4-(hydroxy-(3-pyridyl)methyl)phenyl]-2-methyl acrylate, 100181-52-0; trimethylchlorosilane, 75-77-4; propionitrile, 107-12-0; valeronitrile, 110-59-8; hetanenitrile, 629-08-3; 5-lipoxygenase, 80619-02-9; thromboxane A_2 synthase, 60832-04-4; thromboxane A_2, 57576-52-0; leucotriene B_4, 71160-24-2.

2,3-Dihydrobenzofuran Analogues of Hallucinogenic Phenethylamines

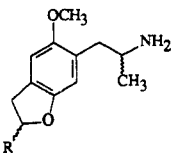
David E. Nichols,* Scott E. Snyder, Robert Oberlender, Michael P. Johnson, and Xuemei Huang

Departments of Medicinal Chemistry and Pharmacognosy, and Pharmacology and Toxicology, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907. *Received May 29, 1990*

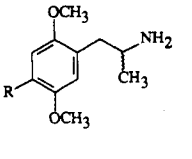
Two 2,3-dihydrobenzofuran analogues of hallucinogenic amphetamines were prepared and evaluated for activity in the two-lever drug-discrimination paradigm in rats trained to discriminate saline from LSD tartrate (0.08 mg/kg) and for the ability to displace [^{125}I]-(*R*)-DOI ([^{125}I]-(*R*)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane) from rat cortical homogenate 5-HT_2 receptors. The compounds, 1-(5-methoxy-2,3-dihydrobenzofuran-4-yl)-2-aminopropane (**6a**) and its 7-brominated analogue **6b**, possessed activity comparable to their conformationally flexible counterparts 1-(2,5-dimethoxyphenyl)-2-aminopropane (**3**) and its 4-bromo derivative DOB (**5**), respectively. The results suggest that the dihydrofuran ring in **6a** and **6b** models the active conformation of the 5-methoxy groups in **3** and **5**. Free energy of binding, derived from radioligand displacement K_A values, indicated that addition of the bromine in either series contributes 2.4–3.2 kcal/mol of binding energy. On the basis of surface area of the bromine atom, this value is 2–3 times higher than would be expected on the basis of hydrophobic binding. Thus, hydrophobicity of the para substituent alone cannot account for the dramatic enhancement of hallucinogenic activity. Although this substituent may play a minor role in orienting the conformation of the 5-methoxy group in derivatives such as **4** and **5**, there appears to be some other, as yet unknown, critical receptor interaction.

Recently, we reported the synthesis and LSD-like biological activity in rats of compounds **1** and **2**, which were considered to be analogues of the hallucinogenic phenethylamine derivative 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (**4**, DOM, STP).[1] Evaluation of **1** and **2** in the two-lever drug-discrimination paradigm, in rats trained to discriminate saline from LSD tartrate (0.08 mg/kg), revealed marked attenuation of potency in substituting for LSD when compared with the prototype **4**.

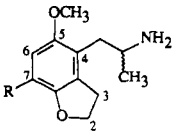
One explanation offered for this loss of activity was the possibility that the 5-methoxy group of **4** (and hence the unshared electron pairs of the methoxy oxygen) must adopt a particular conformation at the receptor, where the *O*-methyl is directed away from the 4-substituent. That is, the 4-methyl group of **4**, through a nonbonded interaction, forces the 5-methoxy to lie in an anti conformation. We have earlier reported the results of molecular mechanics calculations that illustrate this effect.[2] This was an attractive hypothesis, since it was known that in 2,5-dimethoxy-substituted phenethylamine derivatives such as **4**, virtually any substituent in the 4-position confers high activity on the molecule.[3] Substituents spanning a range of electronic and hydrophobic properties are effective, from



1: R = H
2: R = CH_3



3: R = H
4: R = CH_3
5: R = Br



6a: R = H
6b: R = Br

(1) Nichols, D. E.; Hoffman, A. J.; Oberlender, R. A.; Riggs, R. M. *J. Med. Chem.* **1986**, *29*, 302.
(2) Nichols, D. E. *VIIIth International Symposium on Medicinal Chemistry*; Dahlbom, R., Nilsson, J. L. G., Eds.; Swedish Pharmaceutical Press: Stockholm, 1985; Vol. 2, pp 103–115.
(3) Nichols, D. E.; Glennon, R. A. *Hallucinogens: Neurochemical, Behavioral, and Clinical Perspectives*; Raven Press: New York, 1984; pp 95–142.

0022-2623/91/1834-0276$02.50/0