Grant RD 262 (to University of North Carolina) and National Cancer Institute Research Grants CA 41314 and BRSG-RR 05404 (to Bowman Gray School of Medicine, Wake Forest University).

Registry No. 1, 124581-78-8; 2, 124581-79-9; 3, 124581-80-2; 4, 124581-94-8; 5, 126614-04-8; 5 (starting iodide), 126614-27-5; 6, 124581-82-4; 7, 126614-05-9; 8, 124581-83-5; 9, 124581-81-3; 10, 126614-06-0; 10 (starting bromide), 126614-21-9; 11, 126614-07-1; 11 (starting bromide), 126614-36-6; 12, 126614-08-2; 13, 126644-53-9; 13 (starting bromide), 126614-26-4; 14, 126614-09-3; 15, 126614-10-6; 15 (starting bromide), 126614-32-2; 16, 124581-92-6; 17, 126614-11-7; 17 (starting bromide), 126614-28-6; 18, 126614-12-8; 18 (starting bromide), 126614-23-1; 19, 126614-13-9; 19 (starting bromide), 126614-29-7; 20, 126614-14-0; 20 (starting bromide), 126614-33-3; 21, 126614-15-1; 21 (starting bromide), 126614-34-4; 22, 126614-16-2; 23, 126614-17-3; 23 (starting bromide), 126614-30-0; 24, 126614-18-4; 24 (starting bromide), 126614-35-5; 25, 126614-19-5; 25 (starting bromide), 126614-31-1; 26, 126614-20-8; (±)-$MeSO_3CH_2CH(OMe)CH_2OC_{16}H_{33}$, 126614-22-0; (±)-$HOCH_2CH(OMe)CH_2OC_{16}H_{33}$, 111188-59-1; (±)-$H_2C{=}CHCH_2CH_2OCH_2CH(OMe)CH_2OC_{16}H_{33}$, 126614-24-2; $H_{33}C_{16}OCH_2CH(OMe)CH_2O(CH_2)_4OCH_2CH(OMe)CH_2OC_{16}H_{33}$, 126614-25-3; $Br(CH_2)_4Br$, 110-52-1; $HO(CH_2)_3OC_{16}H_{33}$, 23377-40-4; CBr_4, 558-13-4; $MeNHCH_2CH_2OH$, 109-83-1; *N*-methyl-4-hydroxypiperidine, 106-52-5.

Syntheses and Platelet Aggregation Inhibitory and Antithrombotic Properties of [2-[(ω-Aminoalkoxy)phenyl]ethyl]benzenes

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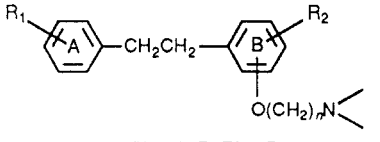
A series of [2-[(ω-aminoalkoxy)phenyl]ethyl]benzene derivatives were synthesized and evaluated for their ability to inhibit collagen-induced platelet aggregation in vitro and to protect experimental thrombosis in mice. The results showed that the compounds were in vitro inhibitors of collagen-induced platelet aggregation. Most of them were also effective in the mouse antithrombotic assay. The compounds were found to be potent antagonists to S_2 serotonergic receptor, and good correlation (r = 0.85) between their S_2 serotonergic receptor antagonism and their potency as platelet antiaggregatory drugs was observed. Among the compounds studied, mono[2-(dimethylamino)-1-[[2-[2-(3-methoxyphenyl)ethyl]phenoxy]methyl]ethyl] succinate hydrochloride (**12b**, MCI-9042) was selected for further pharmacological and toxicological evaluation.

The intravascular formation of platelet aggregates is an important pathogenic factor in the development of cardiovascular disease state such as atherosclerosis, myocardial infarction, transient ischemic attacks, and stroke.[1-4] Hence, one rational approach in the research for antithrombotic drugs is to search for inhibitors of platelet aggregation. Platelets are activated by exposed collagen on an injured vessel wall and subsequently aggregate to form a platelet plug and secrete intracellular granules containing serotonin, ADP, and some active substances. Since the first trigger of platelet aggregation in vivo is the activation by collagen of subendothelial tissues, we focused on an agent which would inhibit collagen-induced platelet aggregation.

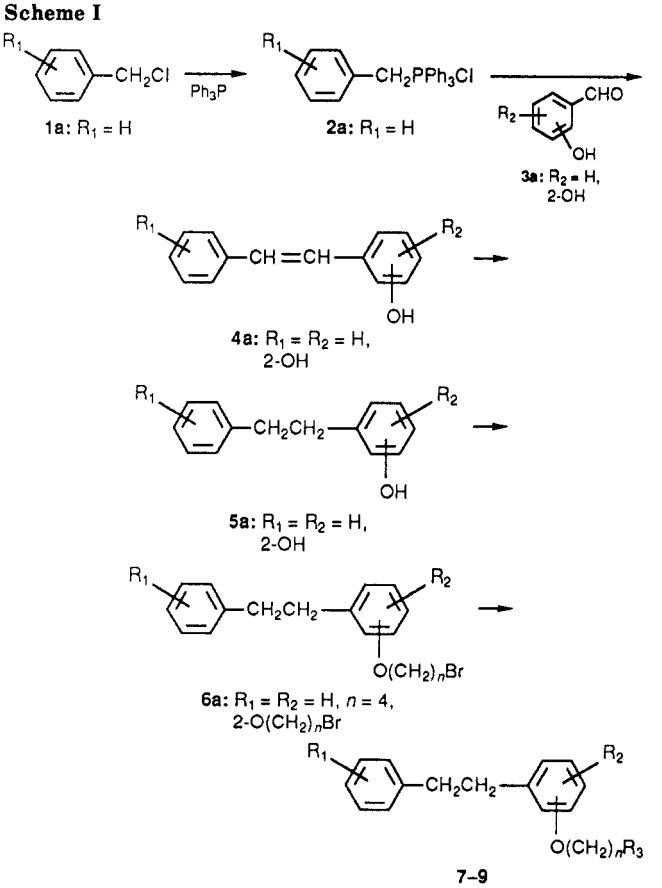
In our efforts to search for inhibitors of collagen-induced platelet aggregation, we have discovered a novel class of compounds, [2-(ω-aminoalkoxy)phenyl]ethyl]benzenes (I).



I (A; Ring A, B; Ring B)

In this paper are described the relationship between the structure of [2-[(ω-aminoalkoxy)phenyl]ethyl]benzenes and the inhibition of in vitro collagen-induced platelet aggregation and in vivo thrombus formation in mice. During the course of study with the compounds, we found that this series of compounds exhibited a potent S_2 serotonergic receptor antagonism, which highly correlated with an inhibitory effect on in vitro collagen-induced platelet aggregation.

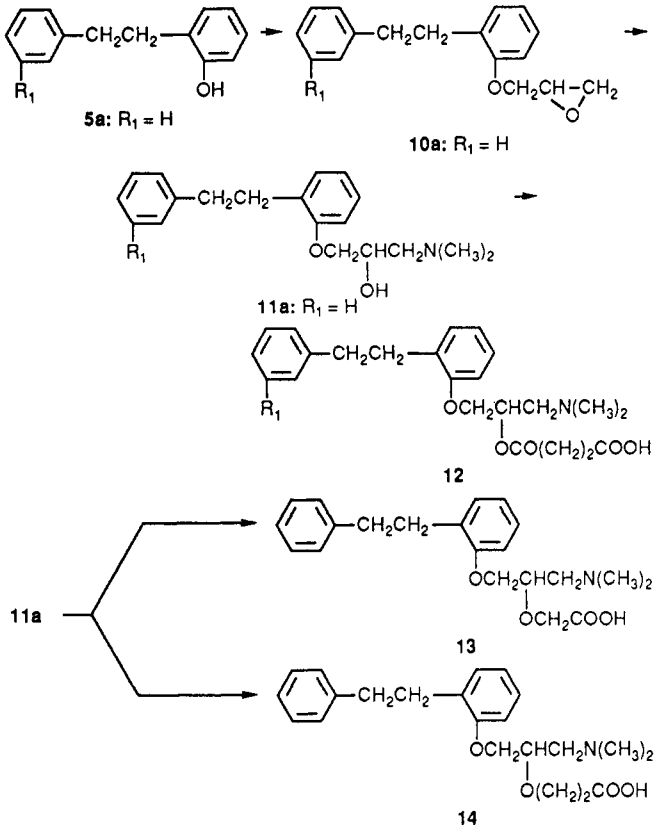
Scheme I



(1) Woolf, N. *Br. Med. Bull.* **1978**, *34*, 137.
(2) Mitchell, J. R. A. *Br. Med. Bull.* **1978**, *34*, 103.
(3) Bouchier-Hayes, D. *Ir. Med. J.* **1983**, *76*, 101.
(4) Koch-Weser, J. *N. Engl. J. Med.* **1978**, *298*, 1403.

0022-2623/90/1833-1818$02.50/0

Scheme II



Chemistry

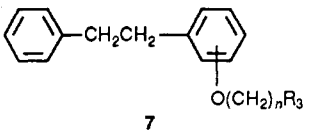
The [2-[(ω-aminoalkoxy)phenyl]ethyl]benzenes (I) were prepared by the sequence shown in Scheme I. Substituted benzyl chlorides **1** were converted to the corresponding phosphonium salts **2**.[5] Then, Wittig reactions were carried out with the substituted hydroxybenzaldehyde **3** to give stilbenes **4** as mixtures of cis and trans isomers,[6,7] which were hydrogenated to the corresponding diphenylethanes **5**. The [2-[(ω-bromoalkoxy)phenyl]ethyl]benzenes **6** were obtained by the reaction of the potassium salts of **5** with α,ω-dibromoalkane in *t*-BuOH, then aminated with appropriate amines to give the desired compounds **7–9**.

The preparation of the mono esters of succinic acid (**12**) is shown in Scheme II, route 1. Epoxy derivatives **10**, which were obtained from substituted 2-(2-phenylethyl)-phenols **5b** with epichlorohydrin in the presence of NaH in DMF by the usual manner, were treated with dimethylamine to give amino alcohols **11**. The mono esters (**12**) of succinic acid were prepared by the esterification of **11** with succinic anhydride.

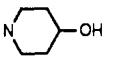
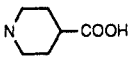
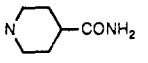
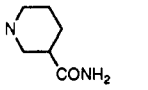
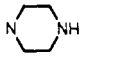
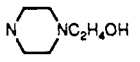
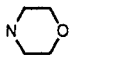
Compound **13** was prepared from **11a** by etherification with *tert*-butyl chloroacetate in the presence of *t*-BuOK in *t*-BuOH and hydrolysis of the ester group (Scheme II, route 2).

Compound **14** was obtained by Michael addition of **11a** to methyl acrylate in the presence of NaH and hydrolysis of the ester group (Scheme II, route 3). See Tables I–IV for the physical data of the compounds prepared.

Table I. [2-[2-(ω-Aminoalkoxy)phenyl]ethyl]benzene Derivatives and Their Inhibition of Platelet Aggregation



7

compd	R_3	*n*	mp, °C	formula[a]	in vitro[b] IC_{50}, μM
7a	$N(CH_3)_2$	2	172–3	$C_{18}H_{23}NO\cdot HCl$	0.67
7b	$N(CH_3)_2$	3	134–6	$C_{19}H_{25}NO\cdot HCl$	0.54
7c	$N(CH_3)_2$	4	113–8	$C_{20}H_{27}NO\cdot HCl$	0.70
7d	$N(CH_3)_2$	5	94–6	$C_{21}H_{29}NO\cdot HCl$	1.15
7e	$N(CH_3)_2$	6	powder	$C_{22}H_{31}NO\cdot HCl$	1.7
7f[c]	$NHCH_3$	4	106–11	$C_{19}H_{25}NO\cdot HCl$	4.5
7g	NH_2	4	111–2	$C_{18}H_{23}NO\cdot HCl$	>50
7h	$N(C_2H_5)_2$	4	122–9	$C_{22}H_{31}NO\cdot HCl$	0.70
7i		4	110–4	$C_{22}H_{29}NO\cdot HCl$	0.62
7j		4	118–24	$C_{23}H_{29}NO_3\cdot HCl$	18
7k		4	125–7	$C_{23}H_{31}NO\cdot HCl$	2.4
7l		4	132–4	$C_{23}H_{31}NO_2\cdot HCl$	0.89
7m		4	155–62	$C_{24}H_{31}NO_3\cdot HCl$	6.2
7n		4	106	$C_{24}H_{32}N_2O_2$	2.4
7o		4	114–8	$C_{24}H_{32}N_2O_2$	1.4
7p		4	134–40	$C_{22}H_{30}N_2O\cdot 2HCl$	2.0
7q		4	177–80	$C_{24}H_{34}N_2O_2\cdot 2HCl$	2.2
7r		4	125–30	$C_{22}H_{29}NO_2\cdot HCl$	19
7s	$N(CH_3)C_4H_9$	4	90–3	$C_{23}H_{33}NO\cdot HCl$	3.2
7t	$N(CH_3)C_6H_6$	4	70–8	$C_{25}H_{29}NO\cdot HCl$	>50
7u	$N(CH_3)CH_2C_6H_6$	4	70–5	$C_{26}H_{31}NO\cdot HCl$	>50
7v	$N(CH_3)C_2H_4OH$	4	powder	$C_{21}H_{29}NO_2\cdot HCl$	1.4
7w	$N(CH_3)CH_2COOH$	4	114–5	$C_{21}H_{27}NO_3$	29
7x	$N(CH_3)C_3H_6COOH$	4	84–5	$C_{23}H_{31}NO_3\cdot HCl$	13
aspirin					23

[a] Analysis for C, H, and N are within ±0.4% of the theoretical values. [b] Inhibitory activities of compounds on platelet aggregation in vitro. Values represent the concentration required for 50% inhibition of platelet aggregation. They are the average of all the IC_{50}s obtained and IC_{50} values in individual experiment varied less than a factor of 0.5–2 times the average value. [c] See ref 9.

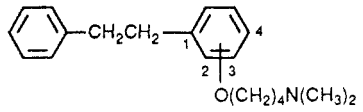
Biological Results and Discussion

The compounds in this study were primarily examined for the inhibition of collagen-induced platelet aggregation of rabbit platelet-rich plasma in vitro. Most of the compounds exhibiting a potent inhibition of platelet aggregation were further evaluated for their suppressive activities on platelet thrombus formation on injured endothelium by electrical stimulation in mice.

Among the compounds with various carbon chain length of the alkoxy portion of [2-[[ω-(dimethylamino)alkoxy]-phenyl]ethyl]benzenes **7a–e**, the compounds with a chain length of two to four carbon atoms (**7a–c**) exhibited a potent inhibition with IC_{50} of 0.54–0.70 μM, while the compounds with a chain length of five or six carbon atoms

(5) Friedrich, K.; Henning, H. G. *Chem. Ber.* **1959**, *92*, 2756.
(6) Kikumoto, R.; Tobe, A.; Fukami, H.; Ninomiya, K.; Egawa, M. *J. Med. Chem.* **1984**, *27*, 645.
(7) Kikumoto, R.; Tobe, A.; Fukami, H.; Egawa, M. *J. Med. Chem.* **1983**, *26*, 246.
(8) Blatt, A. H. *Organic Syntheses*; John Wiley and Sons Inc.: New York, 1967; Collective Volume 1, p 115.
(9) Kikumoto, R.; Tobe, A.; Tonomura, S. *J. Med. Chem.* **1981**, *24*, 145.

Table II. Positional Isomers of [2-[[4-(Dimethylamino)butoxy]phenyl]ethyl]benzenes and Their Inhibition of Platelet Aggregation in Vitro



8

compd	position	mp, °C	formula	in vitro IC_{50}, μM
7c	2			0.70
8a	3	128–9	$C_{20}H_{27}NO·HCl$	19
8b	4	169–71	$C_{20}H_{27}NO·HCl$	3.4

(**7d,c**) exhibited less potent inhibition. Subsequently, the effect of the N-substitution of the amino group was examined with the compound having a four carbon chain length alkoxy portion (**7f–x**). The necessity of disubstitution of the amino group was apparent from the reduction or lack of activity of N-monosubstituted amino derivative **7f** or N-unsubstituted amino derivative **7g**. Cyclic amino derivatives **7i,k,l,n–q** also showed potent activity. However, the substitution with a very bulky group such as aryl (**7t** and **7u**) or with the carboxylic acid containing groups (**7j**, **7m**, **7w** and **7x**) caused a decrease of the activity, suggesting some steric or ionic effects around this position. Among the N-substituted derivatives having a four carbon chain length alkoxy portion, dimethylamino (**7c**), diethylamino (**7h**), pyrrolidino (**7i**), and 4-hydroxypiperidino (**7l**) derivatives exhibited a potent inhibition with IC_{50} of less than 1 μM.

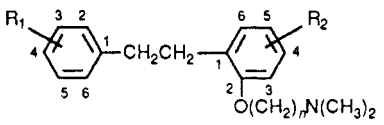
Since compound **7c** was a potent inhibitor as shown above, the effect of the substitution position of the 4-(dimethylamino)butoxy group on the benzene ring B was compared in Table II. The substitution at the position 3 (**8a**) or 4 (**8b**) gave a less potent inhibitor than substitution at position 2 (**7c**).

The effect of a substituent on benzene ring A or B of compound **7b** and **7c** was examined in Table III. The derivatives with a benzene ring substituted with a relatively small group such as methyl, methoxy, ethoxy, or halogen, exhibited a potent inhibition of in vitro platelet aggregation comparable to that of **7b** or **7c** regardless of the position of substitution examined. However, the in vivo antithrombotic activity was strikingly reduced in the compounds with substitution at position 4 (**9k–m**) and was considerably reduced in the compounds with substitution at position 2 (**9c** and **9e**). The discrepancy between in vitro and in vivo effectiveness was possibly caused by the change in pharmacokinetic properties such as absorption, distribution, and metabolism brought on by substitution at 4- or 2-position.

Subsequently the introduction of a substituent on the (dimethylamino)propoxy side chain of compound **7b** was examined mainly to test the effect of a carboxylic acid containing side chain on platelet aggregation inhibitory potency. As shown in Table IV, the compounds having a carboxylic acid containing side chain exhibited potent inhibition in both in vitro platelet aggregation and in vivo thrombus formation. In a series of compounds in Table IV, substitution at position 2 of benzene ring A resulted in the potentiation of in vitro inhibition (**11a** vs **11b** and **12a** vs **12b–d**). Interestingly, this introduction of side chain with carboxylic acid reduced acute toxicity in mice. The introduction of succinic acid to compound **11a** with (LD_{50} of 140 mg/kg ip) and **11b** (LD_{50} of 150 mg/kg ip) gave compounds 12a and 12b, with reduced acute toxicity (LD_{50} of 580 and 330 mg/kg ip, respectively). Thus, [2-[(ω-aminoalkoxy)phenyl]ethyl]benzene derivatives exhibited a potent inhibition on collagen-induced platelet aggregation, and their structural modification gave a group of compounds with potent in vivo effectiveness and low acute toxicity.

Most known inhibitors of collagen-induced platelet aggregation have been shown to be mediated by inhibition of cyclooxygenase or thromboxane A_2 synthetase or by a rise of cyclic-AMP levels in platelets with cyclic-AMP phosphodiesterase inhibition or adenylate cyclase stimulation. A series of compounds in this study neither in-

Table III. Benzene Ring Substituents of [[[(Dimethylamino)alkoxy]phenyl]ethyl]benzenes and in Vitro Platelet Aggregation Inhibitory Activities and in Vivo Antithrombotic Activities

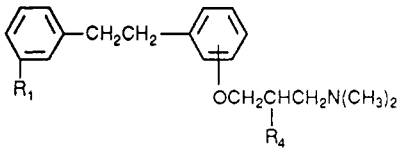


9

compd	R_1	R_2	n	mp, °C	formula	in vitro IC_{50}, μM	in vivo[a] PD_{50}, mg/kg po
7c	H	H	4			0.70	26
9a	2-CH_3	H	4	146–7	$C_{21}H_{29}NO·HCl$	1.2	
9b	2-OH	H	4	126–9	$C_{20}H_{27}NO_2·HCl$	0.96	
9c	2-OCH_3	H	4	150–4	$C_{21}H_{29}NO_2·HCl$	0.60	45
9d	2-OC_2H_5	H	4	184–8	$C_{22}H_{31}NO_2·HCl$	0.90	
9e	2-Cl	H	4	122–3	$C_{20}H_{26}ClNO·HCl$	0.50	50
9f	2-COOH	H	4	128–31	$C_{21}H_{27}NO_3·HCl$	1.5	
9g	3-OCH_3	H	4	101–2	$C_{21}H_{29}NO_2·HCl$	0.53	19
9h	3-Cl	H	4	93–4	$C_{20}H_{26}ClNO_2·HCl$	0.53	27
9i	3-F	H	4	90–1	$C_{20}H_{26}FNO·HCl$	0.48	27
9j	4-Cl	H	3	153–4	$C_{19}H_{24}ClNO·HCl$	0.54	>50
9k	4-F	H	4	137	$C_{20}H_{26}FNO·HCl$	0.76	>50
9l	4-$N(CH_3)_2$	H	3	164–7	$C_{21}H_{30}N_2O·2HCl$	0.31	130
9m	3,4-Cl	H	3	136–7	$C_{19}H_{23}Cl_2NO·HCl$	0.58	94
9n	H	3-OCH_3	4	117	$C_{21}H_{29}NO_2·HCl$	2.3	40
9o	H	5-Cl	3	122–4	$C_{19}H_{24}ClNO·HCl$	2.5	21
ticlopidin							120

[a] Antithrombotic activities in vivo. Values represent the dose required for 50% prolongation of occlusion time. They were derived from a experiment with five to 10 animals at each of at least three doses.

Table IV. Derivatives of [2-[2-[3-(Dimethylamino)-2-hydroxypropoxy]phenyl]ethyl]benzenes and Their Activities



compd	R_1	R_4	mp, °C	formula	in vitro IC_{50}, μM	in vivo PD_{50}, mg/kg po
7b	H	H			0.54	
11a	H	OH	101–3	$C_{19}H_{25}NO_2$·HCl	1.2	
11b	OCH_3	OH	120–1	$C_{20}H_{27}NO_3$·HCl	0.48	29
12a	H	$OCO(CH_2)_2COOH$	146–9	$C_{23}H_{29}NO_5$·HCl	2.2	27
12b	OCH_3	$OCO(CH_2)_2COOH$	139–142	$C_{24}H_{31}NO_6$·HCl	0.97	24
12c	F	$OCO(CH_2)_2COOH$	142–4	$C_{23}H_{28}FNO_5$·HCl	1.2	27
12d	Cl	$OCO(CH_2)_2COOH$	158–161	$C_{23}H_{28}ClNO_5$·HCl	0.8	27
13	H	OCH_2COOH	107–110	$C_{21}H_{27}NO_4$·HCl	3.6	24
14	H	OC_2H_4COOH	109–110	$C_{22}H_{29}NO_4$·HCl	3.3	25

Table V. 5-HT_2 Receptor Antagonism of [2-[(ω-Aminoalkoxy)phenyl]ethyl]benzenes on the Vasoconstriction of Rat Caudal Artery and the Receptor Binding of Rat Frontal Cortex Membrane

compd	vasoconstriction K_i, nM	receptor binding IC_{50}, nM
7a	6.33	96.2
7b	4.55	33.8
7c	9.78	115.4
7d	37.6	377.0
7e	74.9	152.4
7f	70.6	156.5
7s	67.6	974.6
7u	198.0	4479.0
8a	517.0	3328.3
8b	416.0	1976.0
9g	13.1	62.2
9l	23.3	53.6
9o	25.7	881.4
11a	13.9	54.8
11b	1.16	12.5
12b	17.8	51.3
12c	41.3	132.5

hibited thromboxane A_2 synthesis from arachidonic acid nor raised cyclic-AMP level in platelets (data not shown). However these compounds were found to exhibit a S_2 serotonergic receptor antagonism. In Table V are shown the effects of typical compounds of this study on the constriction of rat caudal artery by serotonin and the binding to S_2 serotonergic receptor of rat frontal cortex. The serotinin-induced constriction of rat caudal artery has been shown to be mediated by S_2 type serotonergic receptor on the arterial smooth muscle cells.[10,11] The potency of a S_2 serotonergic receptor antagonistic effect was found to be well correlated with that of an inhibition of collagen-induced platelet aggregation. Figure 1 shows that correlation between K_i in the serotonin-induced vasoconstriction assay or IC_{50} in the S_2 serotonergic receptor binding assay and IC_{50} in the collagen-induced platelet aggregation. In both the receptor binding assay and the vasoconstriction assay, good correlations were observed (r = 0.85 in both cases). These results suggest that serotonin participates in the mechanism of collagen-induced platelet aggregation. The existence of serotonergic receptors on platelet membrane has been demonstrated with radio-

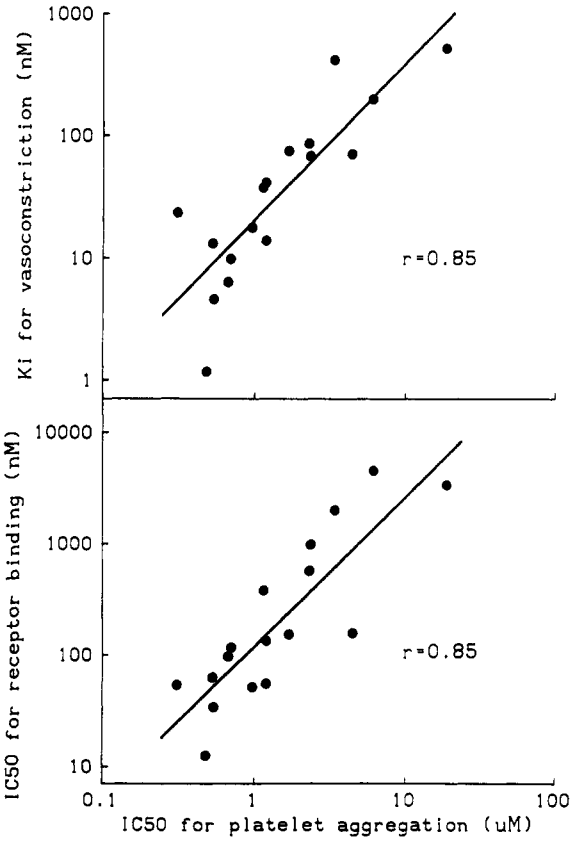


Figure 1. Correlation between the inhibitory activity of compounds on collagen-induced platelet aggregation and their potency to antagonize the serotonin-induced vasoconstriction of rat caudal artery (upper) or the [^{3}H]ketanserin binding to rat frontal cortex membrane (lower).

ligand-binding techniques, and the receptors on the platelet membrane display the same properties as the S_2 serotonergic receptor in the brain.[12,13] Serotonin is accumulated into platelets, stored, and later released from stimulated platelets. It induces platelet aggregation only with a weak potency but synergistically potentiates the aggregation induced by other agonist, e.g. collagen and epinephrine.[14,15] Thus, in collagen-induced platelet ag-

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gregation, serotonin behaves as a potent activator of platelets. Therefore, the inhibition of present compounds on collagen-induced platelet aggregation is most likely explained by their S_2 serotonergic receptor blocking properties. The antithrombotic effect of serotonin antagonists has been recently reported by some investigators[16-18] and these facts indicate that serotonin antagonists may be a new class of antiplatelet drug.

Among the compounds obtained in this study, mono-[2-(dimethylamino)-1-[[2-[-(3-methoxyphenyl)ethyl]-phenoxy]methyl]ethyl] succinate hydrochloride (**12b**) was selected for further pharmacological and toxicological evaluation.

Experimental Section

Chemical Methods. Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. All compounds were analyzed for C and H or C, H, and N, and analytical results were within ±0.4% of the theoretical values. Compounds were checked by IR spectra on a JASCO IR-A2. ^1H NMR spectra were taken on a JEOL PS-100 (Me_4Si as internal standard).

Synthesis of [2-[(ω-Aminoalkoxy)phenyl]ethyl]benzenes. The [2-[(ω-aminoalkoxy)phenyl]ethyl]benzenes were all prepared by the same method, and the procedure is exemplified by preparation of 2-[4-(dimethylamino)butoxy]-1-(2-phenylethyl)benzene (**7c**).

2-(2-Phenylethyl)phenol (5a). A solution of benzyl chloride (**1a**, 20.0 g, 0.16 mol) and Ph_3P (41.5 g, 0.17 mol) in CH_3CN (100 mL) was stirred for 5 h under reflux. The reaction mixture was concentrated to give a residue, which was crystallized from benzene to yield 58.3 g (95%) of phosphonium salt **2a**. The mixture of phosphonium salt (50.0 g, 0.13 mol), salicylaldehyde (**3a**, 15.7 g, 0.13 mol), DBU (20.5 g, 0.135 mol), and CH_3CN(200 mL) was stirred for 4 h under reflux. The resulting mixture was evaporated, and the organic material was extracted with benzene. Extracts were washed with H_2O, 1 N HCl, and H_2O successively, dried (Na_2SO_4), and evaporated to a syrup, which was chromatographed on silica gel (Wakogel C-200, 350 g) using benzene-*n*-hexane as eluant to remove triphenylphosphine oxide. The oily product obtained consisted of *cis*- and *trans*-stilbene (**4a**) and was hydrogenated in EtOH (200 mL) in the presence of 5% Pd–C(4 g) for 20 h. Then, the catalyst was filtered off, and the solution was evaporated to dryness. The residual material was crystallized from benzene–*n*-hexane to give 20.6 g (80.9%) of 2-(2-phenylethyl)-phenol (**5a**), mp 81.5 °C (lit.[19] 81 °C). Anal. ($C_{14}H_{13}O$) C, H.

2-[4-(Dimethylamino)butoxy]-1-(2-phenylethyl)benzene (7c). To a solution of KOH (3.1 g, 0.055 mol) in H_2O (3 mL) were added *t*-BuOH (60 mL), **5a** (10 g, 0.051 mol), and 1,4-dibromobutane (43.6 g, 0.20 mol), and the mixture was stirred for 2 h under reflux. The reaction mixture was evaporated and the organic material was extracted with benzene. Extracts were washed with H_2O and dried (Na_2SO_4). After evaporation of the solvent, the residual oil was distilled under reduced pressure to give 15.3 g (90.8%) of 2-(4-bromobutoxy)-1-(2-phenylethyl)benzene (**6a**, bp 180–5 °C/1 mmHg). A solution of **6a** (15 g, 0.045 mol) and 50% aqueous $(CH_3)_2NH$ (50 mL) in THF (50 mL) was stirred at room temperature for 5 h. The organic material was extracted with Et_2O, and the extracts were washed with brine, dried (Na_2SO_4), and evaporated to a syrup, which was dissolved in Et_2O. To the solution was added ethanolic HCl (20%, 9 g) to give crystals, which were recrystallized from CH_3COCH_3–Et_2O to obtain 12.0 g (79.9%) of **7c**, mp 113–8 °C. Anal. ($C_{20}H_{28}NOCl$) C, H, N.

[2-[2-[4-(4-Carboxypiperidino)butoxy]phenyl]ethyl]-benzene Hydrochloride (7m). To a solution of isonipecotic acid (1.9 g, 0.015 mol) and NaOH (1.2 g, 0.03 mol) in EtOH (50 mL) was added **6a** (5 g, 0.015 mol) dropwise under reflux. Reflux was continued for an additional 30 min and the reaction mixture was concentrated in vacuo to remove the solvent. To the residue was added water and then the mixture was adjusted to pH 2–3 with 2 N HCl. The product was extracted with $CHCl_3$, and the extract was washed with brine and dried (Na_2SO_4). The solvent was distilled off in vacuo to give a syrup, which was dissolved in acetone. To the solution was added 20% HCl in AcOEt to precipitate the crystals, which were recrystallized from acetone to give 4.5 g (77% yield) of **7m**, mp 155–62 °C. Anal. ($C_{24}H_{32}NO_3Cl$) C, H, N.

[2-[2-[4-(4-Carbamoylpiperidino)butoxy]phenyl]ethyl]-benzene (7n). A solution of **6a** (20 g, 0.06 mol), isonipectoamide (9.2 g, 0.072 mol), and Et_3N (16.6 mL) in THF (100 mL) and water (25 mL) was stirred at 70 °C for 10 h. The reaction mixture was concentrated, and 1 N NaOH was added to the residue. The resultant crystals were filtered, washed well with water, and then recrystallized from EtOH to give 16.2 g (71% yield) of **7n**, mp 106 °C. Anal. ($C_{24}H_{32}N_2O_2$) C, H, N.

[2-[2-[3-(Dimethylamino)-2-hydroxypropoxy]phenyl]-ethyl]benzene Hydrochloride (11a). To a solution of NaH (60% oil dispersion, 2.2 g, 0.055 mol) in DMF (30 mL) was added dropwise a solution of **5b** (10 g, 0.051 mol) in DMF (30 mL) under ice cooling. After the addition, epichlorohydrin (23.4 g, 0.25 mol) was added in one portion and the mixture was stirred for 4 h at room temperature. The resulting solution was evaporated off in vacuo to a residue, which was extracted with benzene. Extracts were washed with water, dried, and evaporated to an oil (**10a**). To a solution of **10a** in THF (40 mL) was added 50% aqueous $(CH_3)_2NH$ (53 mL) and the mixture was stirred for 5 h. The resulting solution was extracted with isopropyl ether, and the extracts were washed with water, dried, and evaporated to dryness. The residue was dissolved in isopropyl ether and 20% HCl in AcOEt was added to the solution to precipitate crystals, which were recrystallized from AcOEt to give 14.4 g (85% yield) of **11a**, mp 101–3 °C. Anal. ($C_{19}H_{26}NO_2Cl$) C, H, N.

Mono[2-(dimethylamino)-1-[[2-(2-phenylethyl)phenoxy]-methyl]ethyl] Succinate Hydrochloride (12a). The mixture of **11a** (5 g, 0.017 mol) and succinic anhydride (1.9 g, 0.019 mol) in acetone (25 mL) was refluxed for 1 h. To the resulting solution was added 20% HCl in AcOEt under cooling to precipitate the crystals, which were recrystallized from acetone to give 6.5 g (89.3% yield) of **12a**, mp 146–9 °C. Anal. ($C_{23}H_{30}NO_5Cl$) C, H, N.

[2-(Dimethylamino)-1-[[2-(2-phenylethyl)phenoxy]-methyl]ethoxy]acetic Acid Hydrochloride (13). To a solution of **11a** (8.1 g, 0.027 mol) in *t*-BuOH (60 mL) was added *t*-BuOK (6.2g, 0.0554 mol) and the mixture was stirred at 70 °C for 20 min. After cooling to room temperature, *tert*-butyl chloroacetate (4.9 g, 0.032 mol) was added dropwise under stirring. After completion of the dropwise addition, the reaction mixture was allowed to stir for 1 h, then *tert*-butyl chloroacetate (1.6 g, 0.011 mol) was further added dropwise, and the mixture was stirred for 2 h. Then, 2 N NaOH (40 mL) was added and the resulting mixture was stirred at 50 °C for 2 h. After the reaction was complete, the mixture was washed with ether. The ether layer was extracted twice with 0.5 N NaOH and then the extract was combined with the original aqueous layer. The solution was adjusted to pH 3 with concentrated HCl and the organic material was extracted with $CHCl_3$. Extracts were dried and evaporated to a residue, which was dissolved in acetone. Then 20% HCl in AcOEt was added to the solution under cooling to precipitate the crystals, which were recrystallized from acetone to obtain 6.6 g (62% yield) of **13**, mp 107–10 °C. Anal. ($C_{21}H_{28}NO_4Cl$) C, H, N.

3-[2-(Dimethylamino)-1-[[2-(2-phenylethyl)phenoxy]-methyl]ethoxy]propionic Acid Hydrochloride (14). To **11a** (10 g, 0.033 mol) was added NaH (60% oil dispersion, 0.1 g, 0.0025 mol) and the mixture was stirred for 15 min. Thereafter, methyl acrylate (14.4 g, 0.17 mol) was added in one portion and the mixture was stirred for 3 h at room temperature. After the reaction was complete, the product was extracted with ether. Extracts were washed with water and evaporated in vacuo to a residue, which was dissolved in MeOH (30 mL) and 2 N NaOH (33 mL) and stirred for 3 h at room temperature. The mixture was washed with ether and the aqueous layer was adjusted to pH

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3 with concentrated HCl and the organic material was extracted with $CHCl_3$. The extracts were dried and evaporated to a residue, which was dissolved in acetone. To the solution was added 20% HCl in AcOEt under cooling to precipitate the crystals, which were recrystallized from acetone to give 9.8 g (72% yield) of 14, mp 109–10 °C. Anal. ($C_{22}H_{30}NO_4Cl$) C, H, N.

Biological Methods. Inhibitory Activity to Collagen-Induced Platelet Aggregation in Vitro. Rabbit blood was obtained from the central artery of the ear lobe and was treated with 3.8% sodium citrate (1 part citrate to 9 parts of blood). Platelet-rich plasma (PRP) was prepared by centrifugation at 1200 rpm for 30 min. Platelet aggregation was measured at 37 °C by the turbidimetric method of Born and Cross[20] using an aggregometer (Sienco type DP-247E). A cuvette containing 200 μL of PRP, 10 μL of 0.1 M $CaCl_2$ in saline, and 25 μL of test compound solution in saline was placed in the aggregometer and allowed to incubate for 3 min. PRP was challenged with 15 μL of a collagen suspension and platelet aggregation was recorded continuously. A concentration of collagen was selected to produce a submaximal response for each PRP sample. The inhibitory activities of compounds were measured with various concentrations, and the concentration producing 50% inhibition (IC_{50}) was calculated by probit analysis.[21] The values in the tables represent average results from two or three experiments. IC_{50} values in individual experiments varied by less than a factor of 0.5–2 times the mean value.

Antithrombotic Activities in Vivo. The platelet thrombosis model induced by electrical stimulation in mice was performed by a modification of the technique described by Cowan and Monkhouse.[22] Male albino mice, weighing 20–25 g, were anesthetized with urethane (1.5 mg/kg sc). From a midline incision a section of the small intestine was placed over the viewing window of a mouse holder, and the mesenteric blood vessels were layed flat on the window for viewing under a microscope. The preparation was placed on the stage of the microscope and transilluminated from below. A glass capillary electrode filled with a 2 M KCl solution was manipulated into contact with a mesenteric artery with a diameter of about 100 μm. The solution in the glass capillary electrode was connected to a silver electrode via external circuit to a reference stainless steel electrode which was placed on the mesentery. A negative direct current of 500 μA via the glass capillary electrode was applied for 4 min, and the time, occlusion time, taken for the resultant thrombus on the electrically damaged site to occlude the blood flow for 80 s and above was recorded with a stop watch. In the control experiment, the occlusion time as 474.6 ± 9.96 s. The test compounds were suspended in 0.5% tragacanth and administered orally to mice that were fasted overnight. From 2 h after dosing, the measurement was started. PD_{50} values for each compound were estimated from graphical plots of occlusion time versus log dose of each compound and were defined as the dose required to produce 50% prolongation of the occlusion time in the control experiment. PD_{50} values in the tables were derived from an experiment with several animals (n = 5–10) at each of three or four dose levels. The standard deviation of occlusion time at each dose level was about ±10% of the mean value.

Vasoconstriction Assay. Spiral strips of rat caudal artery were freshly prepared and their constriction by serotonin was determined by the method of Van Neueten et al.[12] Assays were performed in duplicate at three different inhibitor concentrations. The concentration causing 50% inhibition of the original response (IC_{50}) was determined by probit analysis, and then the K_i value was calculated from the IC_{50} value according to the equation of Cheng and Prusoff.[23] Reported K_i values in the tables were the average of results at two concentrations and the standard error of K_i was less than ±30% of the mean value.

Serotonergic Receptor Binding Assay. Receptor binding assay for the determination of antagonist activities of the compounds was carried out according to the method of Leysen et al.[24] with [^{3}H]ketanserin as S_2 specific radioligand. All compounds were tested in at least duplicate at each of several different concentrations and IC_{50} values (concentration producing 50% inhibition of the specific binding of [^{3}H]ketanserin) were derived from graphical plots of the percentage inhibition of ligand binding versus the log concentration of the compounds.

Acute Toxicities. The compounds that were suspended in 0.5% tragacanth/saline were intrapenetoneally administered to male albino mice, and the mortality at 24 h later was measured. LD_{50} was estimated with the method of Litchfield and Wilcoxon.[25]

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The Synthesis and Antiallergy Activity of 1-(Aryloxy)-4-(4-arylpiperazinyl)-2-butanol Derivatives

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A series of 1-(aryloxy)-4-(4-arylpiperazinyl)-2-butanol derivatives were prepared and evaluated for antiallergy activity in the passive foot anaphylaxis (PFA) assay in rats. Twenty-seven derivatives had activity equal to or greater than the parent, α-(phenoxymethyl)-4-phenyl-1-piperazinepropanol. Six derivatives that possessed greater activity in the PFA than the parent compound were then tested in the guinea pig anaphylaxis (GPA) assay. Five of the derivatives were more potent than the parent (PD_{50} = 40 mg/kg) in the GPA with α-[(4-fluorophenoxy)methyl]-4-(4-fluorophenyl)-1-piperazinepropanol (PD_{50} = 3 mg/kg) having the greatest potency.

For several years a program to find a suitable clinical candidate for the treatment of allergic disorders has been underway in our laboratories. A report[1] that certain 1-(aryloxy)-3-amino-2-propanol derivatives inhibited dextran-induced anaphylactic reactions in rats piqued our interest since Chen and Lunsford[2] in our laboratories had

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