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]

Acknowledgment. The authors thank members in Analytical Laboratory, Mitsubishi Kasei Corp., for elemental analyses.

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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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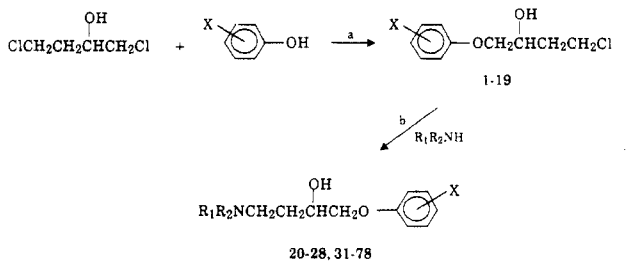
(1) Witte, E. C.; Stach, K.; Thiel, M.; Sponer, G.; Roesch, E. U.S. Patent 3 997 666, 1976; *Chem. Abstr.* **1975**, *83*, 179122e.

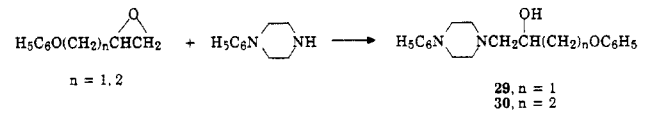
0022-2623/90/1833-1823$02.50/0

Scheme I[a]



[a] (a) H_2O, NaOH, 30 h; (b) C_4H_9OH, Na_2CO_3, KI, Δ, 18 h.

Scheme II



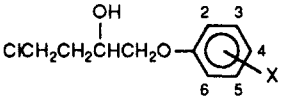
previously prepared a series of 1-(aryloxy)-4-amino-2-butanol derivatives for antihypertensive testing. It was anticipated that these compounds might have activity against allergic reactions. When the compounds were tested in our primary screening model, the passive foot anaphylaxis (PFA) assay in rats,[3] an IgE-mediated model useful in the detection of compounds possessing antiallergy activity, several showed good activity. A systematic structure–activity study was undertaken and the results are reported herein.

Chemistry. The general synthetic procedure for the preparation of all target compounds except **29** and **30** is depicted in Scheme I. A substituted phenol or 1-naphthol was reacted with 1,4-dichloro-2-butanol in the presence of aqueous sodium hydroxide to give the intermediate 1-(aryloxy)-4-chloro-2-butanol derivatives (**1–19**, Table I). A secondary amine was then used to displace the second chloro group to yield the desired target compounds **20–28** (Tables II and III) and **31–78** (Table IV). Scheme II displays the preparation of **29**[4] and **30** by the addition of 4-phenylpiperazine to the appropriate epoxide.

Results and Discussion. The data presented in Tables II and III indicate that there are very specific structural requirements for activity in this chemical series. Piperidine derivatives **20–22** and isoquinoline (**23**) were inactive in the PFA assay at a dose of 31.6 mg/kg. Of the two piperazine derivatives (**24**, **25**) shown in Table II, only the phenyl-substituted derivative (**25**) possessed good activity at 31.6 mg/kg. When the piperazine ring was opened to give **26**, all activity was lost at the dose tested.

Compounds related to **27** and **28** were claimed[5] to possess a wide range of pharmacological effects including antihistaminic activity. In the PFA assay, these compounds were only weakly active at 31.6 mg/kg (Table III). Although certain 2-propanol derivatives were reported[1] to inhibit allergic reactions, **29** possessed no antiallergy activity at 31.6 mg/kg. Surprisingly, 3-butanol isomer **30** also had no activity in the PFA. These relatively small structural variations in the carbon chain between the piperazinyl and aryloxy groups resulted in a large change in antiallergy activity in the PFA assay.

Table I. Substituted 1-(Aryloxy)-4-chloro-2-butanol Intermediates



no.	X	formula[a]	mp, °C (solv)[b]	% yield
1	H	$C_{10}H_{13}ClO_2$	52–54 (n)	72
2	4-F	$C_{10}H_{12}ClFO_2$	53–55 (no)	30
3	4-Cl	$C_{10}H_{12}Cl_2O_2$	62–64 (p)	72
4	4-Br	$C_{10}H_{12}BrClO_2$	75–78 (no)	37
5	2-OCH_3	$C_{11}H_{15}ClO_3$	48–50	86
6	4-OCH_3	$C_{11}H_{15}ClO_3$	65–68 (o)	42
7	4-CH_3	$C_{11}H_{15}ClO_2$	62–64 (no)	42
8	4-$C(CH_3)_3$	$C_{14}H_{21}ClO_2$	45–47 (n)	38
9	4-C_6H_5	$C_{16}H_{17}ClO_2$	123–124 (p)	65
10	3-CF_3	$C_{11}H_{12}ClF_3O_2$	50–52	34
11	4-CN	$C_{11}H_{12}ClNO_2$	78–80 (np)	13
12	4-$CONH_2$	$C_{11}H_{14}ClNO_3$	150–153 (q)	25
13	4-$COCH_3$	$C_{12}H_{15}ClO_3$	75–77 (oq)	22
14	4-NO_2	$C_{10}H_{12}ClNO_4$	60–63 (nr)	8
15	2,3- 	$C_{14}H_{15}ClO_2$	75–77 (no)	51
16	2-CH_3, 4-Cl	$C_{11}H_{14}Cl_2O_2$	oil	46
17	3-CH_3, 4-Cl	$C_{11}H_{14}Cl_2O_2$	87–89 (nr)	23
18	3,4-Cl_2	$C_{10}H_{11}Cl_3O_2$	82–84 (no)	35
19	3,5-$(CH_3)_2$	$C_{12}H_{17}ClO_2$	74–76 (r)	82

[a] All compounds were analyzed for C, H, and N, and results agreed to ±0.4% of the theoretical values. [b] n = petroleum ether (30–60 °C), o = ethyl ether, p = 2-propanol, q = ethanol, r = isopropyl ether. [c] 1-Naphthyl.

Since **25** was the only compound to show appreciable activity in the initial group of compounds tested, substituted derivatives of **25** were prepared (Table IV) in an attempt to increase potency. The substituent was first varied on the phenyl group attached to the piperazine ring (**31–44**). These compounds substituted in the para position with fluoro (**32**), methyl (**40**), and acetyl (**42**) showed a slight increase in potency relative to that of **25** at 10 mg/kg. When the substituent was varied on the phenoxy group (**45–62**), seven compounds were active at 10 mg/kg, and one compound (**46**) possessing a *p*-chloro group showed activity at 3.16 mg/kg. When substituents were varied on both aromatic rings of **25**, three compounds (**63**, **67**, **73**), each possessing a *p*-fluoro substituent on the aryloxy group, had some activity at 3.16 mg/kg. In general, compounds that contained a *p*-chloro or *p*-fluoro group on the aromatic ring attached to the piperazine ring and a *p*-fluoro group on the aryloxy ring possessed the greatest potency in the PFA assay.

Six of the most potent compounds in the PFA were compared with **25** in a classical model of immediate hypersensitivity, the guinea pig anaphylaxis (GPA) assay[3] (Table V). Of the six compounds tested, only **73** was less potent than **25** in the GPA. Compound **67** was the most potent derivative tested (PD_{50} = 3 mg/kg) in the GPA from this chemical series.

Since compounds related to **27** and **28** were reported[5] to have antihistaminic activity and the H_1-antihistamine diphenhydramine was active in the GPA assay (Table V), three compounds were compared with diphenhydramine for inhibition of tritiated mepyramine binding to H_1-histamine receptors isolated from guinea pig cerebral cortex. The two most potent compounds in the GPA assay from this series (**46** and **47**) had IC_{50}s of 1100 and 258 nM,

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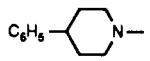
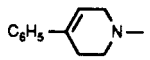
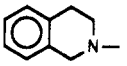
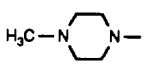
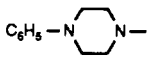
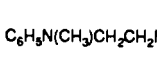
Table II. Oral Antiallergy Activity (1-h Pretreatment) in the Passive Foot Anaphylaxis (PFA) Assay of Various Amines

$RCH_2CH_2C(OH)HCH_2OC_6H_5$

no.	R	formula[a]	mp, °C (solv)[b]	% yield	PFA[c] (31.6 mg/kg)
20		$C_{15}H_{23}NO_2 \cdot C_2H_2O_4$[d]	142.5–143.5 (n)	65	−26[e]/−81
21		$C_{21}H_{27}NO_2$	80–82 (o)	54	−29[e]/−47
22		$C_{21}H_{25}NO_2$	102–103 (p)	60	−30[e]/−81
23		$C_{19}H_{23}NO_2$	85.5–86.5 (p)	43	+7[e]/−47
24		$C_{15}H_{24}N_2O_2 \cdot 2C_4H_4O_4$[f]	177–178 (nq)	58	−24[e]/−58
25		$C_{20}H_{26}N_2O_2$	93–94 (p)	68	−88/−75
26		$C_{20}H_{28}N_2O_2 \cdot C_2H_2O_4$[d]	118–120 (p)	59	−41[e]/−61

[a] All compounds were analyzed for C, H, and N, and results agreed to ±0.4% of theoretical values. [b] n = absolute ethanol, o = isopropyl ether, p = 2-propanol, q = water. [c] Percent change in volume of foot edema of test compound/percent change in volume of foot edema of positive control (aminophylline orally at 100 mg/kg). [d] Oxalate. [e] Not significantly different from negative control group at $p < 0.05$ as determined by the Dunnett's t test. [f] Maleate. [g] Reference 6.

Table III. Oral Antiallergy Activity (1-h Pretreatment) in the Passive Foot Anaphylaxis (PFA) Assay of Various Phenylpiperazine Derivatives

no.	Y	formula[a]	mp, °C (solv)[b]	% yield	PFA[c] (31.6 mg/kg)
27	$-(CH_2)_2-$[d]	$C_{19}H_{24}N_2O$	57–58 (n)	45	−40/−81
28	$-(CH_2)_4-$[d]	$C_{20}H_{26}N_2O \cdot C_4H_4O_4$[e]	151–153 (o)	65	−42/−81
29	$-CH_2CH(OH)CH_2-$[f]	$C_{19}H_{24}N_2O_2$	118–120 (o)	79	−23[g]/−81
25	$-CH_2CH_2CH(OH)CH_2-$	$C_{20}H_{26}N_2O_2$	93–94 (o)	68	−88/−75
30	$-CH_2CH(OH)CH_2CH_2-$	$C_{20}H_{26}N_2O_2$	104–105 (o)	55	−17[g]/−70

[a] All compounds were analyzed for C, H, and N, and results agreed to ±0.4% of the theoretical values. [b] n = petroleum ether (30–60 °C), o = 2-propanol. [c] Percent change in volume of foot edema of test compound/percent change in volume of foot edema of positive control (aminophylline orally at 100 mg/kg). [d] Reference 5. [e] Fumarate. [f] Reference 4. [g] Not significantly different from negative control group at $p < 0.05$ as determined by the Dunnett's t test.

respectively, while an inactive compound (**73**) had an IC_{50} = 930 nM compared with that of diphenhydramine (IC_{50} = 129 nM), a relatively weak H_1-antihistamine. From this data, the series of 2-butanol derivatives represented by **25** may possess weak H_1-antihistaminic activity and this component may contribute to this series' antiallergy activity, but it does not completely account for the level of activity observed for this series in the PFA and GPA assays.

Further work on this chemical series as antiallergy agents[8] has been suspended in favor of other series[3] which have shown greater therapeutic potential.

Experimental Section

Pharmacology. A. Primary Antiallergy Screen. A passive foot anaphylaxis model in rats was used as the primary test for antiallergy activity. Fed, male, Sprague–Dawley rats were injected in the right hind paw with 0.2 mL of rat and anti egg albumin serum at a dilution previously shown to produce significant edema upon antigen challenge. The animals were then fasted but allowed water ad libitum. The next day they were randomized into groups of six by means of tables generated by an IBM scrambler. Random-number tables were also used to determine the groups receiving the control, reference, and test compounds.

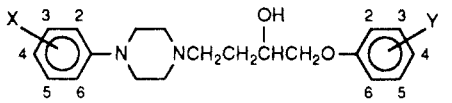
On the test day the right foot volume of each rat was determined plethysmographically; the hairline was used as the reference point. The volume of the foot was measured with a mercury-filled tube that was connected to a P23A Grass pressure transducer that in turn was connected to a Linear Cole-Parmer recorder (Model No. 255). The instrument was adjusted so that a pen deflection of 25 mm was equivalent to a 1-mL volume.

The reference, test, and control compounds were dissolved or suspended in 0.5% Tween 80 in distilled water. Sonification was used to facilitate solubilization or reduction in particle size. The animals were dosed orally (10 mL/kg) by gavage 1 h prior to the intravenous injection of the antigen: 2 mg of egg albumin in 0.2 mL of sterile saline. Thirty minutes later the foot volume of the right foot was measured again, and edema was determined by difference. Results were expressed as the average foot edema (mL) ± SD. A significant decrease ($p < 0.05$) in the edema of the treated group from that of the control group was considered as indicative of antiallergic activity. The results were acceptable only if the group receiving the reference compound showed a significant decrease in foot edema. The data were analyzed with the Dunnett's t test that compares several treated groups with a control group. Differences between groups were determined by the Studentized Range Test. Regression analysis was used to determine relative potency.

B. Secondary Antiallergy Screen. Secondary antiallergy testing of selected compounds was done in the guinea pig anaphylaxis (GPA) model. Guinea pigs were actively sensitized to egg albumin (EA, Sigma Chemical Co., St. Louis, MO) at least 20 days prior to aerosol challenge by injecting 0.5 mL of EA–

(8) Walsh, D. A.; Yanni, J. M. U.S. Patent 4 882 330, 1989; *Chem. Abstr.* 1988, *109*, 129056*w*.

Table IV. Oral Antiallergy Activity (1-h Pretreatment) in the Passive Foot Anaphylaxis (PFA) Assay of 1-(Aryloxy)-4-(4-arylpiperazinyl)-2-butanol Derivatives



no.	X	Y	formula[a]	mp, °C (solv)[b]	% yield	PFA (mg/kg)[c] 31.6	10	3.16
25	H	H	$C_{20}H_{26}N_2O_2$	93–94 (n)	68	−88/−75	−34[d]/−78	
31	2-N[e]	H	$C_{19}H_{25}N_3O_2 \cdot 2C_4H_4O_4$[f]	123–125 (n)	67	−70/−84	−39[d]/−78	
32	4-F	H	$C_{20}H_{25}FN_2O_2$	97–99 (n)	74	−88/−63	−53/−77	−18[d]/−77
33	2-Cl	H	$C_{20}H_{25}ClN_2O_2$	53–55 (o)	24	−27[d]/−65		
34	3-Cl	H	$C_{20}H_{25}ClN_2O_2$	76–78 (n)	69	−85/−78	+22[d]/−73	
35	4-Cl	H	$C_{20}H_{25}ClN_2O_2$	108–110 (n)	70	−65/−78	−43[d]/−77	
36	4-Br[g]	H	$C_{20}H_{25}BrN_2O_2$	125–126 (n)	73	−59/−83	−2[d]/−65	
37	2-OCH_3	H	$C_{21}H_{28}N_2O_3$	74–76 (p)	75	−79/−65	+22[d]/−73	
38	3-OCH_3	H	$C_{21}H_{28}N_2O_3$	58–60 (oq)	45	−69/−78	+3[d]/−73	
39	4-OCH_3	H	$C_{21}H_{28}N_2O_3$	95–97 (n)	72	−67/−63	−34[d]/−77	
40	4-CH_3	H	$C_{21}H_{28}N_2O_2$	90–92 (n)	62	−85/−82	−50/−80	−34/−79
41	3-CF_3	H	$C_{21}H_{25}F_3N_2O_2$	83–84 (n)	66	39[d]/−78		
42	4-$COCH_3$	H	$C_{22}H_{28}N_2O_3$	113.5–117 (n)	81	−80/−83	−36/−65	
43	3,4-Cl_2	H	$C_{20}H_{24}Cl_2N_2O_2$	120.5–121.5 (n)	63	−61/−63	−9[d]/−80	
44	3,4,5-$(OCH_3)_3$	H	$C_{23}H_{32}N_2O_5 \cdot 1.5C_2H_2O_4$[h]	154–156 (r)	58	−26[d]/−74		
45	H	4-F	$C_{20}H_{25}FN_2O_2$	71–73 (qs)	53	−73/−65	−43/−81	
46	H	4-Cl	$C_{20}H_{25}ClN_2O_2$	83–85 (n)	53	−83/−69	−53/−76	−50/−84
47	H	4-Br	$C_{20}H_{25}BrN_2O_2$	100–103 (rs)	35	−56/−65	−49/−81	−20[d]/−78
48	H	2-OCH_3	$C_{21}H_{28}N_2O_3$	101–102 (n)	64	−69/−69	−14[d]/−62	
49	H	4-OCH_3	$C_{21}H_{28}N_2O_3$	103–105 (n)	27	−54/−44	−10[d]/−44	
50	H	4-CH_3	$C_{21}H_{28}N_2O_2$	123–125 (qs)	63	−66/−66	−11[d]/−65	
51	H	4-$C(CH_3)_3$	$C_{24}H_{34}N_2O_2 \cdot HCl$	190–193 (ntu)	48	−57/−83	−39/−72	
52	H	4-C_6H_5	$C_{26}H_{30}N_2O_2$	101–106 (n)	62	−65/−69	−29[d]/−62	
53	H	3-CF_3	$C_{21}H_{25}F_3N_2O_2$	73–74 (v)	42	−49/−78	−26[d]/−62	
54	H	4-CN	$C_{21}H_{25}N_3O_2 \cdot 2HCl$	195–198 (ot)	35	−64/−73	−15[d]/−73	
55	H	4-$CONH_2$	$C_{21}H_{27}N_3O_3 \cdot HCl$	238–241 (tu)	40	−90/−83	−46/−83	−32[d]/−78
56	H	4-$COCH_3$	$C_{22}H_{28}N_2O_3 \cdot 2HCl$	203–208 (ru)	42		−25[d]/−93	
57	H	4-NO_2	$C_{20}H_{25}N_3O_4 \cdot 2HCl$	195–200 (tu)	5	−92/−81	−28[d]/−81	
58	H	2,3-[i]	$C_{24}H_{28}N_2O_2$	112–114 (n)	47	−35[d]/−69		
59	H	2-CH_3, 4-Cl	$C_{21}H_{27}ClN_2O_2 \cdot 2HCl$	186–188 (w)	52	−69/−78	−50/−62	+22[d]/−62
60	H	3-CH_3, 4-Cl	$C_{21}H_{27}ClN_2O_2$	102–103 (n)	64	−71/−69	−45/−76	−33[d]/−84
61	H	3,4-Cl_2	$C_{20}H_{24}Cl_2N_2O_2 \cdot 2HCl$	210–213 (ot)	37	−49/−44	−25[d]/−44	
62	H	3,5-$(CH_3)_2$	$C_{22}H_{30}N_2O_2$	88–90 (tu)	56	−48/−84	+14[d]/−44	
63	2-N[e]	4-F	$C_{19}H_{24}FN_3O_2 \cdot 2HCl$	>245 (tu)	72	−90/−79	−67/−72	−52/−79
64	2-N[e]	4-Cl	$C_{19}H_{24}ClN_3O_2 \cdot 2HCl$	>245 (nu)	62	−78/−78	−48/−68	−13[d]/−68
65	2-N[e]	4-$CONH_2$	$C_{20}H_{26}N_4O_3$	172–173.5 (r)	48	−73/−68	−38/−69	−13[d]/−73
66	2-N[e]	3-CH_3, 4-Cl	$C_{20}H_{26}ClN_3O_2 \cdot 2HCl$	>250 (otu)	65	−79/−81	−36/−88	
67	4-F	4-F	$C_{20}H_{24}F_2N_2O_2 \cdot HCl$	161–165 (otu)	56	−88/−83	−72/−83	−32/−93
68	4-F	4-Cl	$C_{20}H_{24}ClFN_2O_2$	90–92 (x)	37	−78/−69	−55/−63	−36[d]/−80
69	4-F	4-$CONH_2$	$C_{21}H_{26}FN_3O_3$	193–194 (uy)	57	−82/−69	−56/−69	−9[d]/−69
70	4-F	3-CH_3, 4-Cl	$C_{21}H_{26}ClFN_2O_2 \cdot 2HCl$	199–203 (otu)	36	−74/−67	−41[d]/−59	
71	4-Cl	4-F	$C_{20}H_{24}ClFN_2O_2 \cdot HCl \cdot H_2O$	183–186 (otu)	40	−87/−67	−73/−59	−8[d]/−67
72	4-Cl	4-Cl	$C_{20}H_{24}Cl_2N_2O_2 \cdot 2HCl$	195–200 (otu)	47	−54/−67	−5[d]/−59	
73	4-OCH_3	4-F	$C_{21}H_{27}FN_2O_3 \cdot HCl \cdot H_2O$	195–202 (otu)	48	−91/−83	−70/−88	−42/−78
74	4-OCH_3	4-Cl	$C_{21}H_{27}ClN_2O_3 \cdot 2HCl$	220–225 (tu)	42	−79/−81	−40/−81	
75	4-CH_3	4-F	$C_{21}H_{27}FN_2O_2 \cdot 2HCl$	200–205 (otu)	41	−77/−67	−41[d]/−59	
76	4-CH_3	4-Cl	$C_{21}H_{27}ClN_2O_2 \cdot HCl \cdot 1.5H_2O$	190–195 (oru)	31	−60/−74	−54/−72	−4[d]/−78
77	4-$COCH_3$	4-F	$C_{22}H_{27}FN_2O_3$	114–116 (n)	69	−87/−83	−38/−88	
78	4-$COCH_3$	4-Cl	$C_{22}H_{27}ClN_2O_3$	123–125 (n)	70	−75/−83	−61/−88	−38[d]/−78
diphenhydramine						−15[d]/−60		

[a] All compounds were analyzed for C, H, and N, and results agreed to ±0.4% of the theoretical values. [b] n = 2-propanol, o = ethyl ether, p = cyclohexane, q = petroleum ether (30–60 °C), r = ethanol, s = benzene, t = methanol, u = water, v = petroleum ether (60–110 °C), w = acetone, x = isopropyl ether, y = acetonitrile. [c] Percent change in volume of foot edema of test compound/percent change in volume of foot edema of positive control (aminophylline orally at 100 mg/kg). [d] Not significantly different from negative control group at $p < 0.05$ as determined by the Dunnett's t test. [e] 2-Pyridyl. [f] Maleate. [g] Reference 7. [h] Oxalate. [i] 1-Naphthyl.

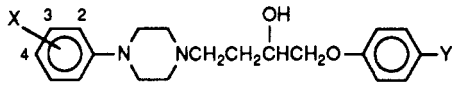
$Al(OH)_3$ conjugate (33 μg of EA/mL) intramuscularly in each hind leg.

On the test day, fasted, sensitized, Dunkin–Hartley guinea pigs were randonized by using random-number tables generated by an IBM scrambler into control ($N = 8$) and test ($N = 4$) groups. The control group was always labeled group I. The order in which compounds were administered to the other groups was also determined by random-number tables from the IBM scrambler.

The reference (theophylline at 100 mg/kg po), test, and control compounds were dissolved or suspended in 0.5% Tween 80 in distilled water, and the concentration was adjusted so that each animal received 10 mL/kg. Compounds were administered by gavage with syringes having rubber catheters attached to their tip.

At a specified time (1–24 h) following the administration of the test, reference, or control compound, each animal was placed in an aerosolization chamber. A 1% solution (w/v) of EA was aerosolized at a flow rate of 10 L of air/min into the chamber for a maximum of 5 min. An electronic timer was started when the aerosolization began. The anaphylactic response consisted of coughing, dyspnea, reeling, collapse, and death. Upon collapsing, the animals were removed from the chamber. The chamber was

Table V. Oral Activity (1-h Pretreatment) of Selected Compounds in the Guinea Pig Anaphylaxis (GPA) Model



no.	X	Y	PD_{50},[a] mg/kg (95% confidence limits)
25	H	H	39.98 (12.47–94.44)
46	H	Cl	6.69 (1.56–30.55)
63	2-N[b]	F	13.88 (2.62–20.36)
64	2-N[b]	Cl	10.17 (4.78–21.27)
67	4-F	F	3.06 (0.03–20.34)
71	4-Cl	F	7.85 (2.90–21.20)
73	4-OCH_3	F	>178
theophylline			75 (52–109)
diphenhydramine			23.3 (18.0–33.8)

[a] Dose that protects 50% of the guinea pigs from collapse. [b] 2-Pyridyl.

then flooded with air before the next animal was placed inside. Animals were considered protected if they did not collapse within 5 min of exposure to the aerosolized antigen. The number of animals that collapse in each group is recorded. PD_{50} for collapse is calculated by the method of Litchfield and Wilcoxon[9] for evaluation of dose–effect experiments.

Comparisons of PD_{50}s from different experimental trials and determinations of relative potency are determined by the Litchfield and Wilcoxon method. The following conditions were met before an experiment was acceptable. (1) The control group showed collapse in 7/8 or 8/8 animals. (2) The theophylline reference group showed protection in 3/4 or 4/4 animals. A compound was judged active if it showed protection in 3/4 or 4/4 animals.

The decision to accept a compound for further study was based on the Fisher's Exact 2 × 2 test. The χ^2 test was used to test similarity between control groups to allow pooling of data.

C. Tritiated Mepyramine Binding to H_1-Histamine Receptors in Guinea Pig Cerebral Cortex. (1) Preparation of Cerebral Cortical Membrane. The procedure was a modification of procedures reported by Wallace and Young[10] and Chang et al.[11] Dunkin–Hartley guinea pigs were killed by decapitation, and the cerebral cortex was quickly removed and weighed. The cerebral cortex was placed in 30 volumes of cold 50 mM Na–K phosphate (pH 7.5) buffer and then processed in a Polytron homogenizer for 30 s at a power setting of 6. The homogenate was centrifuged at 48000*g* for 10 min at 4 °C, and the resultant pellet was resuspended in 30 volumes of fresh buffer. The centrifugation and suspension procedure was repeated twice, and the final pellet was suspended in 30 mL of buffer/g of wet tissue.

(2) Procedure for Ligand-Binding Assay. Test and reference compounds were dissolved in buffer or the appropriate vehicles at a concentration of 1×10^{-3} M. The assay mixtures consisted of 100 μL of 15 nM tritiated mepyramine (1.5 nM final concentration); 100 μL of test, control, or reference compound; and 800 μL of membrane preparation for a total volume of 1.0 mL. The assay mixture was incubated for 20 min at 25 °C. The reaction was stopped when the assay mixture was washed (3 × 5 mL) with cold buffer and filtered through GF/B glass-filters. The filters were then transferred to vials; scintillation cocktail was added; and the radioactivity in each vial was determined by liquid-scintillation counting.

For the determination of an IC_{50} value, six concentrations (1×10^{-5}–1×10^{-10} M) of the various compounds were tested in triplicate. An IC_{50} value was calculated by means of regression analysis of the logits of the percent of control binding vs the log of the molar compound concentration.

General Procedures. Melting points were determined in open capillary tubes in a Thomas-Hoover melting point apparatus and are uncorrected; ^1H NMR spectra were obtained in $CDCl_3$ or Me_2SO-d_6 with Me_4Si as internal standard on a Varian A-60 or Varian EM-360L spectrometer; ^{13}C NMR spectra were obtained in the same solvents on a Varian FT-80A spectrometer; mass spectra were determined on a Varian MAT-44 mass spectrometer; IR spectra were run as KBr pellets on a Beckman IR8 or Perkin-Elmer 297 IR spectrophotometer. Spectral data for all reported compounds were consistent with assigned structures. Purifications were done by column chromatography on silica gel or Florisil and by high-pressure liquid chromatography with use of a Waters Prep LC-500A apparatus with a PrepPAK-500 silica cartridge. Analytical results for compounds followed by elemental symbols are within ±0.4% of the theoretical values and were determined on a Perkin-Elmer Model 240 CHN analyzer or on a Control Equipment Corp. 240-XHA CHN analyzer. All starting secondary amines could be obtained commercially or prepared by literature procedures, and 1,4-dichloro-2-butanol was purchased from Penick Chemical Co.

General Procedure for the Preparation of 1-(Aryloxy)-4-chloro-2-butanols (Table I). To a vigorously stirred solution of 22 g (0.55 mol) of NaOH in 400 mL of H_2O was added dropwise 71.5 g (0.50 mol) of freshly distilled 1,4-dichloro-2-butanol. The mixture was stirred at ambient temperature for 15 min and then treated dropwise over a 45-min period with a solution of 0.4 mol of an appropriate phenol or 1-naphthol, 18 g (0.45 mol) of NaOH, and 450 mL of H_2O. The mixture was stirred vigorously for 30 h and then chilled in an ice bath. The suspended solid was collected by filtration; the filter cake rinsed with several portions of H_2O, dried, and recrystallized to yield the desired 1-(aryloxy)-4-chloro-2-butanol derivative.

General Procedure for the Preparation of 4-Amino-1-(aryloxy)-2-butanols (20–28, 31–78). A stirred mixture of 0.03 mol of a secondary amine, 0.03 mol of the appropriately substituted 1-(aryloxy)-4-chloro-2-butanol (**1–19**), 15.9 g (0.15 mol) of anhydrous Na_2CO_3, and 0.4 g of KI in 250 mL of 1-butanol was heated at reflux for 18 h. The mixture was concentrated and the residue was partitioned between C_6H_6 and H_2O. The organic layer was washed with H_2O and brine, dried (Na_2SO_4), and concentrated. If the residue was a solid, it was purified by recrystallization. If the residue was a gum, it was converted to a crystalline salt and the salt was purified by recrystallization.

α-(2-Phenoxyethyl)-4-phenyl-1-piperazineethanol (30). A mixture of 2.4 g (0.015 mol) of 1,2-epoxy-4-phenoxybutane[12] was allowed to stand at ambient temperature over a weekend. The resulting solid was triturated with petroleum ether (30–60 °C), collected by filtration, and recrystallized from 2-propanol to yield 2.7 g (55%) of **30** as a white powder, mp 104–105 °C. Anal. ($C_{20}H_{26}N_2O_2$) C, H, N.

Acknowledgment. We thank Dr. Larry W. Jaques, Ashby F. Johnson, John B. Forehand, Joanne E. Anderson, and Malcolm D. Stone who determined the spectral and analytical data; Stephen K. Franzyshen who prepared **26**; Marianne H. Foxwell and Laura L. Whitman who provided technical assistance in conducting the pharmacological assays; Dr. Brian F. Kilpatrick and Robin D. Tabor who determined the H_1-histamine binding data; and Drs. William J. Welstead, Jr. and Mark C. Sleevi who kindly proofread the manuscript.

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