Articles

Ultra-Short-Acting β-Adrenergic Receptor Blocking Agents. 1. (Aryloxy)propanolamines Containing Esters in the Nitrogen Substituent

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In an attempt to produce short-acting β-adrenergic receptor blocking agents, we prepared several (aryloxy)-propanolamines with ester functions incorporated into the nitrogen substituent. Many of these compounds exhibited a short duration of blocking activity after their continuous intravenous infusion for 40 min. However, their durations were found to increase considerably upon longer intravenous infusion.

The use of β-adrenergic receptor blocking agents to decrease myocardial oxygen need and to control dysrhythmia after acute myocardial infarction has been limited because of the potential for producing long-lasting cardiac depression.[1] Recently, it has been suggested that a β-adrenergic blocking agent having an ultrashort duration of action could remedy this limitation.[2] Such a compound (1) could be administered by intravenous infusion to rapidly obtain desired levels of β-adrenergic blockade; (2) would allow rapid adjustment of blockade as required to match a changing adrenergic state accompanying the dynamic character of an infarct in the acute phase; and (3) would reduce the risk of producing long-lasting cardiac depression, since the effects of the drug would rapidly dissipate upon termination of the infusion. This paper describes the preparation and structure–activity relationships for several β-adrenergic receptor blocking agents designed to have short durations of action.

Our approach was to incorporate into the typical β-blocker structural framework functionalities susceptible to rapid-phase one metabolic processes whereby biotransformation would be expected to inactivate the parent compound. Exploitation of ester groups for this purpose was chosen since the ability of the ubiquitous esterases to quickly terminate the action of a variety of ester-containing drug substances has been established.[3] Incorporation of ester moieties into the amine alkyl substituent of several (aryloxy)propanolamine structures produced the compounds listed in Table I. These compounds resemble known β-blockers having amide groups in this region of the molecule.[4] Because of the electronic similarities between ester and amide groups, it was felt that reasonable β-blocking potency could be expected from these esters. Furthermore, hydrolysis of these esters produces compounds (e.g., **5**) having a carboxylate anion at physiological pH. It was suspected that these negatively charged species would be unacceptable when presented to a region of the cardiac β receptor accustomed to recognizing either a protonated ammonium system or an unprotonated amine bearing a relatively lipophilic alkyl substituent. Therefore, it was felt that these metabolities would be inactive or only very weakly active as β-adrenergic blocking agents. In order to maximize the attenuation of blocking potency by the carboxylate anion, it seemed most desirable to place the ester moieties close to the (aryloxy)propanolamine β-blocking pharmacophore. However, consideration of the importance of the amine function in establishing receptor binding during β blockade led to the suspicion that the ester carboxy group might jeopardize an amine–receptor relationship requisite for good β-blocking activity. Therefore, several ester-containing target compounds were prepared in which the ester functions were removed from their close proximity to the pharmacophoric amino group by insertion of methylene units.

Chemistry. Appropriate phenols were condensed with excess epichlorohydrin employing potassium carbonate in acetone.[5] This approach minimizes second additions to the epoxide[6] and promotes ring closure to the epoxide when nucleophilic attack occurs at the epoxide rather than at the halide-containing carbon.[7] The epoxides were then opened by the various methods depicted in Scheme I.

Compound **2** was prepared directly by opening the epoxide with ethyl glycinate free amine (method A). However, obtaining the free amine substrates, in general, proved to be an inefficient process,[8] and these reagents were more conveniently handled as their hydrochloride salts with triethylamine in the reaction media.[9] In this fashion, method A was also employed to directly prepare compounds **10** and **12**, where $n = 1$ and 2. However, in an attempt to prepare **4**, where $n = 3$, cyclization to the γ-lactam, **6**, occurred simultaneously.

In method B,[10] epoxides were first reacted with succinimide and, after acid hydrolysis of the intermediate suc-

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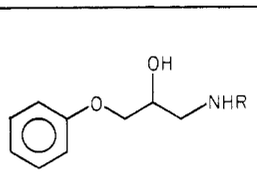
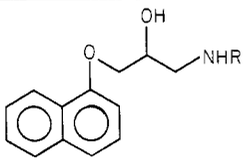
Table I. Structures and Pharmacological Data for Test Compounds



1-8



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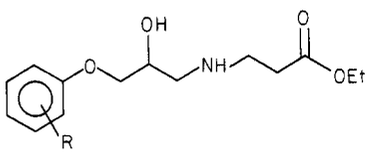
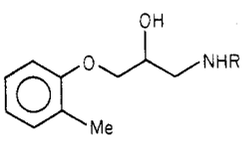
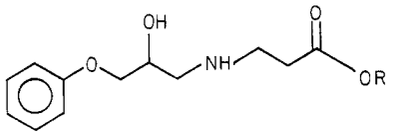


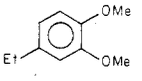
15-23



24-35

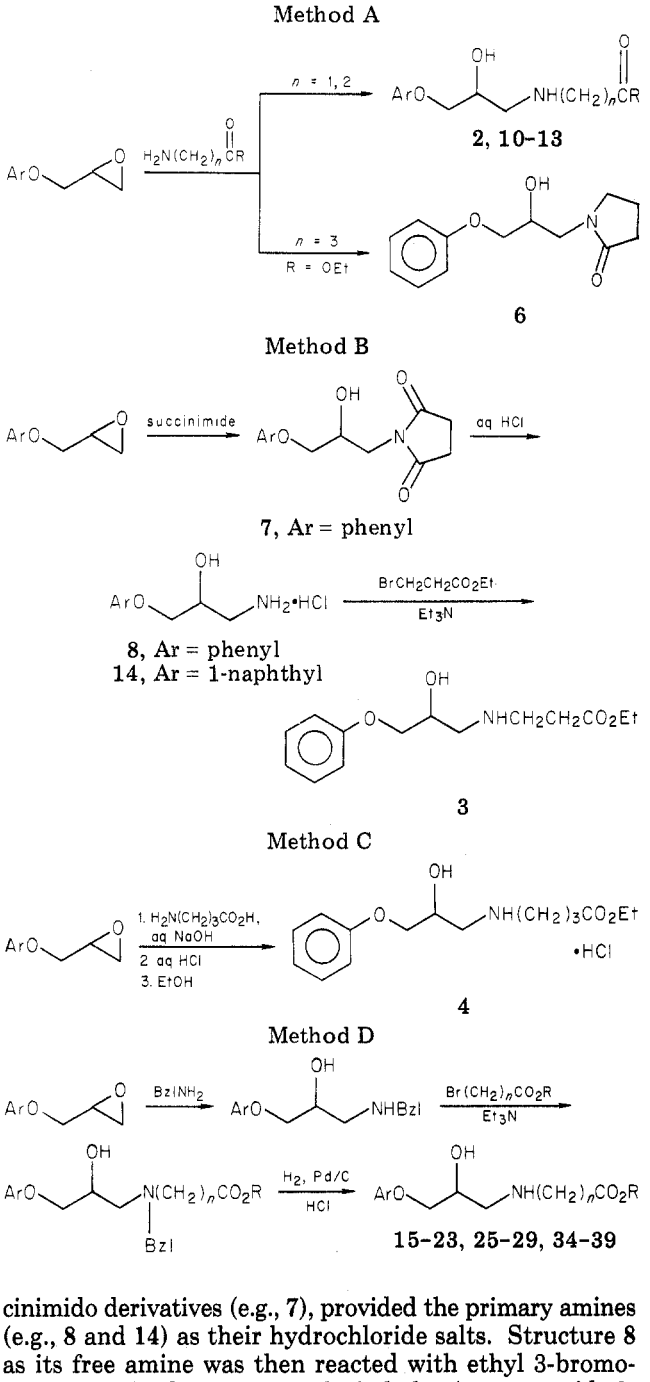


36-39

no.	R	in vitro data:[a] pA_2 atria	trachea	cardioselectivity[b]	in vivo data:[c] duration[d] 40 min	3 h
1	$CH_2(CH_3)_2$	8.0	7.4	5		
2	CH_2CO_2Et	5.7	5.6			
3	$(CH_2)_2CO_2Et$	7.3	5.9	25	29 ± 13	
4	$(CH_2)_3CO_2Et$	6.6	6.0	5	17 ± 4	
5	$CH_2COO^-K^+$	inact[e]	inact[e]			
6[f]		inact[e]	inact[e]			
7[f]		inact[e]				
8[g]	H	6.9	5.9	10		
9[h]	$CH_2(CH_3)_2$	8.7	8.9		46 ± 8	>60
10	CH_2CO_2Et	6.2	6.2		53 ± 11	
11	CH_2CONH_2	6.2	6.2			
12	$(CH_2)_2CO_2Et$	7.4	7.3		17 ± 3	
13	$(CH_2)_2CO_2Me$	7.1	7.0			
14[g]	H	6.5	6.3			
15	*o*-Cl	7.4	6.6	6		>60
16	*m*-Cl	7.4	6.7	5		>60
17	*p*-Cl	6.4	5.6	7		
18	*p*-Cl[i]	inact[e]				
19	*o*-CH_3	7.5	7.4		14 ± 2	
20	*m*-CH_3	6.7	6.5			
21	*m*-OCH_3	6.4	6.7			
22	*p*-OCH_3	5.8	4.9	8		
23	*o,o*-$(CH_3)_2$	6.1	6.0			
24	$CH_2(CH_3)_2$	8.6	8.2			
25	CH_2CO_2Et	5.9	6.2			
26	CH_2CO_2Me	5.5	5.7			
27	$(CH_2)_2CO_2Me$	7.5	7.0			
28	$(CH_2)_3CO_2Et$	7.4	6.7	5	15 ± 2	>60
29	$(CH_2)_4CO_2Et$	7.8	7.4		20 ± 4	
30	$C(CH_3)_2CH_2CO_2Et$	8.5	8.1			>60
31	$CH(CO_2Et)_2$	5.9				
32	Ph-CO_2Et-*p*	5.1				
33	Ph-CO_2Et-*m*	5.2				
34	CH_2-Ph-CO_2Et-*p*	6.6	5.9	5		>60
35	CH_2-Ph-CO_2Me-*p*	6.9	6.7			
36		7.0	6.6			
37	Ph	6.4				>60
38	Ph-F-*p*	5.7				
39	CH_2CF_3	5.7				

[a] Number of experiments is equal to or greater than two for each compound. Tabulated pA_2 data are mean values. The range for each value is equal to or less than ±0.2 unit. [b] Antilog [pA_2 (atria) − pA_2 (trachea)]. [c] Number of experiments is equal to or greater than three for each compound. The tabulated data are mean values ± SEM. [d] Time in minutes for 80% recovery from 50% blockade levels after 40 min and/or 3 h infusions of drug. [e] At 10^{-5} M. [f] These tertiary nitrogen cyclic amides do not contain a hydrogen on the nitrogen atom as suggested by the general structure. [g] An early report [N. C. Moran and M. E. Perkins, *J. Pharmacol. Exp. Ther.*, 124, 223 (1958)] that certain primary amine β blockers possessed short durations of action prompted the evaluation of these primary amine synthetic intermediates. They were tested during model development and found to have long durations of action before the 40-min and 3-h infusion models were adopted. [h] Propranolol. [i] Acid metabolite.

Scheme I



cinimido derivatives (e.g., 7), provided the primary amines (e.g., 8 and 14) as their hydrochloride salts. Structure 8 as its free amine was then reacted with ethyl 3-bromopropionate in the presence of triethylamine to provide 3.

In method C, the epoxide was first reacted with γ-aminobutyric acid under basic conditions.[11] An attempt to isolate a purified acid intermediate by this route also resulted in spontaneous cyclization to the γ-lactam 6 when the product was subjected to standard partitioning procedures. The resulting crude amino acid was, therefore, immediately esterified with ethanol under acidic conditions to prevent cyclization to the γ-lactam. Compound 4 was obtained in this fashion.

In a final approach, method D, epoxides were first opened with benzylamine, and the resulting secondary amines were alkylated with an appropriate ester-containing alkyl halide.[12] Subsequent hydrogenolyses of the tertiary amine benzyl groups were facile and were performed under acidic conditions to help stabilize the various ester moieties. Compounds 15–17, 20–23, 25–29, and 34–39 were prepared by this method.

As examples of the anticipated metabolic products, ester 2 was hydrolyzed by aqueous base to its corresponding acid 5, and acid 18 was prepared directly by method C. In addition, the non-ester-containing analogues, 1 and 24, and an N-external amide, 11, were prepared as standards for comparative purposes.

Biological Results and Discussion

In Vitro β-Blocking Potency Studies. Respective β_1- and β_2-blocking potencies were determined in guinea pig right atria and trachea preparations. As indicated in Table I, for the first two series of compounds, 2–7 and 10–13, highest β-blocking potency was observed when the ester carbonyl group was located β to the nitrogen atom as in 3, 12, and 13. This could reflect either an undesirable interaction when the carboxy group is α to the nitrogen atom (e.g., 2 and 10) or it could reflect decreased stability of the α-amino esters during in vitro incubation.[13] However, since low potency was also obtained for the more stable α-amide, 11, it seems unlikely that increased instability alone can explain the lower potency observed for the α-arranged esters. Because the esters extended from the nitrogen atom by three methylene units, e.g., 4, had been found to undergo ready cyclization at neutral or basic pH during synthesis, the γ-lactam 6 and a structurally related succinimido intermediate 7 were also tested for potential β-blocking activity. As expected,[14] these compounds were found to be inactive, and the cyclization process, therefore, represents an alternate, spontaneous pathway at physiological pH for the destruction of esters of this type. The inactivity observed for the acid 5 suggested that hydrolysis of these N-external esters could be relied upon to terminate their β-blocking activity.

Although the β-blocking potency observed for 3 was regarded as acceptable, series 2–7 and the standard 1 also exhibited undesirable partial agonist activity. Intrinsic activity has been observed previously for other aryl-unsubstituted phenoxypropanolamine systems.[4,15] Similarly, while 12 possessed reasonable potency, the naphthyl compounds 10–13 were found to have low aqueous solubilities, which detracted from their overall profile. These findings led to the development of series 15–23 where the phenyl ring was substituted to remove intrinsic activity. All of these compounds had acceptable aqueous solubilities and were found to lack intrinsic activity. The overall trend observed was that blocking potency was higher for the mono-ortho-substituted (15 and 19) than for the para-substituted systems (17 and 22), in accord with previous structure–activity relationships.[16,17] Di-o-aryl substitution, e.g., 23, resulted in low potency. Inactivity was obtained with an acid analogue (18) from this series, again suggesting that if enzymatic ester hydrolysis occurs in vivo there should be a termination of the blocking action of the parent compound.

(11) D. O. Rauls and J. K. Baker, *J. Med. Chem.*, 22, 81 (1979).
(12) P. W. Erhardt, *Synth. Commun.*, in press.
(13) Preliminary results from reverse-phase HPLC studies of various structural representatives of the nitrogen derived esters suggest that significant spontaneous hydrolysis does not occur at pH 7.4 in aqueous media within 1 h. However, the α-amino esters do appear to be more labile under these conditions. Detailed results will be published elsewhere.
(14) R. P. Ahlquist, *Prog. Drug Res.*, 20, 27 (1976).
(15) J. Augustein, D. A. Cox, A. L. Ham, P. R. Leeming, and M. Snarey, *J. Med. Chem.*, 16, 1245 (1973).
(16) A. M. Barrett, *Drug Des.*, 3 (1972).
(17) R. Clarkson in "Antihypertensive Agents", E. J. Englehardt, Ed.; American Chemical Society: Washington, DC, 1976.

The most potent compound from this series, **19**, was derivatized to determine the optimum chain length for attachment of an ester. The results obtained for this series, **25–29**, again show that highest potency occurs when at least two carbons separate the ester from the amine. The effect of chain branching is revealed by compounds **30** and **31**. While the excellent potency exhibited by **30** is appropriate for such branching,[18] the low potency obtained for **31** is not characteristic and probably reflects the fact that in this structure two esters are α to the amine. In compounds **32–35**, an aromatic system is employed to separate the amine and ester functions. For **32** it was hoped that its structural resemblence to the *p*-aminobenzoate portion of procaine would enhance its likelihood for rapid ester hydrolysis. In this regard, the low potencies exhibited by **32** and **33** were disappointing, although not totally unexpected, since the electronic influence of the aryl ring attached directly to the pharmacophoric amino group should be significant. This is supported by the increased potencies for **34** and **35** where a methylene is present to insulate the amine from the electronic effects of the aryl ring. In the final series of compounds, **36–39**, the ester alcohol adduct was modified. In **36** the desired enhancement in β_1 selectivity due to the 3,4-dimethoxyphenethyl group[19] was not obtained, perhaps because this function is too far from the amine when the propionic ester is also present. In compounds **37–39**, "good leaving groups" were employed as the ester alcohol adducts. Although attractive in that spontaneous hydrolysis could be expected at pH 7.4, their low potency and extremely labile nature suggested that this was not a practical approach.

In Vivo Duration Studies. The duration of β-blocking activity was determined in vivo for compounds that had β_1 pA_2 values $\gtrsim$7.5 and for structures that were regarded as representative of a specific series. Initially, a 40-min infusion of the test compound was employed to achieve a quasi-steady-state plasma concentration. Compounds showing a short duration, ~15 min, in this preliminary screen were then studied further in a 3-h infusion model. This model minimized the possibility that any observed reductions in β-blocking duration resulted from redistribution of the active substances rather than from their metabolism and/or elimination. Table I lists the results of the duration studies. In general, relative potencies for the compounds studied in vivo paralleled the relationships obtained in the in vitro studies. Several compounds, **4**, **12**, **19**, **28**, and **29**, were found to have durations of action in the 15- to 20-min range after their infusion for 40 min. This represents a significant reduction in duration when compared to the standard propranolol, **9**, which required ~45 min to allow 80% recovery from its 50% blocking dose. Although compounds **4** and **12** possessed durations in the desired range, their intrinsic activity and low aqueous solubility, respectively, eliminated them from further consideration and study. In contrast, compounds **19** and **28** appeared to have very desirable overall profiles. These two compounds and several other representatives of different structural approaches, **15**, **16**, **30**, **34**, and **37**, were studied in the 3-h infusion model. All of these compounds, including **37**, which was designed to be labile at pH 7.4, were found to have long durations of action, >60 min, after their infusion for 3 h.

In summary, the in vitro studies confirm that it is possible to design (aryloxy)propanolamines that are active as β-adrenergic receptor blocking agents when they contain esters incorporated into the nitrogen substituent and are essentially inactive when these esters are hydrolyzed to their corresponding carboxylic acids. Furthermore, when studied in vivo, after 40-min intravenous infusions several of these compounds have shortened durations of action compared to propranolol. However, after 3-h infusions, their durations increased considerably. Therefore, the definition of specific structural criteria that will allow continued rapid metabolic hydrolysis after prolonged drug infusion requires additional exploration. In this regard, the following paper[25] in this issue describes studies where esters have been placed on the aryl function of the typical β blocker pharmacophore as an alternate locale for incorporation of the desired enzymatic lability.

Experimental Section

Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. IR spectra were obtained with a Perkin-Elmer 283 spectrophotometer either as thin films or as KBr disks. NMR spectra were recorded on a Varian Associates T-60A spectrometer. Thin-layer chromatography was performed on Analtech 250 silica gel GF plates, and visualization was effected by fluorescence quenching while under 254-nm UV lamp irradiation. Column chromatography was performed on a Waters Associates Prep-500 system at a flow rate of 250 mL/min. Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, TN.

Condensation of Phenols with Epichlorohydrin.[5] **1-(2,3-Epoxypropoxy)benzene (40).** A mixture of 9.4 g (0.1 mol) of phenol, 28 g (0.2 mol) of potassium carbonate, and 30 mL (0.4 mol) of epichlorohydrin in 250 mL of acetone was heated to reflux for 12 h. The reaction medium was then filtered and evaporated, leaving an oil, which was taken up in toluene and successively washed with 100 mL of water, 2 × 100 mL of 1.0 N sodium hydroxide, and 2 × 100 mL of water. The toluene phase was dried with magnesium sulfate and evaporated to provide a clear oil, which was chromatographed on a silica gel column employing hexane/ethyl acetate (9:1) as the mobile phase. Collection of the major peak and evaporation of solvent provided 9 g (60%) of a clear oil: bp 116 °C (5 mm) [lit.[20] bp 115–116 °C (3–4 mm)]. Chromatographic and spectral data were identical with a commercial sample. Purification of intermediate 1-(2,3-epoxypropoxy)-2-methylbenzene was effected by vacuum distillation rather than by column chromatography: bp 90–94 °C (0.2 mm).

Reaction of Epoxides with Ester-Containing Alkylamines. Method A. Ethyl *N*-(2-Hydroxy-3-phenoxypropyl)glycinate Oxalate (2). A solution of 20 g (0.14 mol) of ethyl glycinate hydrochloride and 40 g of K_2CO_3 in 100 mL of water was extracted with ether (5 × 100 mL). The ethereal phase was then dried with Na_2SO_4 and evaporated under reduced pressure at a temperature not exceeding 40 °C to provide 10.5 g (71%) of ethyl glycinate free amine: NMR ($CDCl_3$) δ 4.1 (q, J = 7 Hz, 2, OCH_2), 3.3 (s, 2, NCH_2CO_2), 1.2 (t, J = 7 Hz, 3, CH_2CH_3). The free amine (0.10 mole) was used immediately by reacting it with 4.0 g (0.03 mol) of epoxide **40** in refluxing ethanol (50 mL). After 4 h, the reaction medium was evaporated under reduced pressure, and the resulting oil was taken up in 50 mL of toluene and washed with 4 × 50 mL of water. The organic phase was dried with $MgSO_4$ and evaporated to a yellow oil. An analytical sample of the free amine was obtained by crystallization from ethyl acetate, mp 49–50 °C. Anal. ($C_{13}H_{19}NO_4$) C, H, N. The major portion of this oil was converted to its oxalate salt and crystalled from ethanol–ether to provide

(18) A. F. Crowther, D. J. Gilman, B. J. McLoughlin, L. H. Smith, R. W. Turner, and T. M. Wood, *J. Med. Chem.*, **12**, 638 (1969).
(19) M. L. Hoefle, S. G. Hastings, R. F. Meyer, R. M. Corey, A. Holmes, and C. D. Stratton, *J. Med. Chem.*, **18**, 148 (1975).
(20) D. W. Stephens, *Chem. Ind. (London)*, **51**, 375 (1932).
(21) A. F. Crowther and L. H. Smith, *J. Med. Chem.*, **11**, 1009 (1968).
(22) H. R. Ing and W. E. Ormedrod, *J. Pharm. Pharmacol.*, **4**, 21 (1952).
(23) L. Fieser and M. Fieser, "Reagents for Organic Synthesis", Vol. 1, Wiley, New York, 1967, p 705.
(24) R. F. Furchgott, *Ann. N.Y. Acad. Sci.*, **139**, 553 (1967).
(25) P. W. Erhardt, C. M. Woo, W. G. Anderson, and R. J. Gorczynski, *J. Med. Chem.*, following paper in this issue.

0.8 g (8%) of **2**: mp 144–145 °C; IR (KBr) 1750 (ester carbonyl) cm^{-1}; NMR (D_2O) δ 7.2 (m, 5, Ar H), 1.3 (t, J = 7 Hz, 3, CH_2CH_3). Anal. ($C_{15}H_{21}NO_8$) C, H, N.

Method A with Triethylamine. Ethyl *N*-[2-Hydroxy-3-(1-naphthoxy)propyl]glycinate Oxalate Hemihydrate (10). A mixture of 4.0 g (0.02 mol) of 1-(2,3-epoxypropoxy)naphthylene, 5.6 g (0.04 mol) of ethyl glycinate hydrochloride, and 5.5 mL (0.04 mol) of triethylamine in 50 mL of ethanol was heated to reflux for 2 h. The reaction medium was then evaporated under reduced pressure, and the resulting oil was taken up in 50 mL of toluene and washed with 2 × 50 mL of water. The organic phase was dried over $MgSO_4$ and evaporated under reduced pressure. The resulting oil was crystallized as its oxalate salt from water and provided 1.1 g (14%) of **10**: mp 161–162 °C; NMR (Me_2SO-d_6) δ 7.6 (m, 7, Ar H), 1.1 (t, J = 7 Hz, 3, CH_2CH_3). Anal. ($C_{19}H_{23}NO_8 \cdot 0.5H_2O$) C, H, N. An anhydrous analytical sample was also obtained by crystallization from acetone, mp 169–170 °C. Anal. ($C_{19}H_{23}NO_8$) C, H, N.

Method A. Lactam Formation. Tetrahydro-*N*-(2-hydroxy-3-phenoxypropyl)-2-oxopyrrole (6). A mixture of 2.3 g (0.02 mol) of ethyl 4-aminobutyrate and 2.4 mL (0.02 mol) of epoxide **40** in 50 mL of ethanol was heated to reflux for 6 h. The reaction medium was then evaporated under reduced pressure, and the resulting oil was treated with ethyl acetate to provide 1.9 g (40%) of crystalline rosettes: mp 88–90 °C; NMR ($CDCl_3$) δ 7.0 (m, 5, Ar H), 4.0 [m, 4, OCH_2 and CH(OH)], 3.4 (m, 4, CH_2CH_2), 2.2 (m, 4, ring CH_2CH_2). Anal. ($C_{13}H_{17}NO_3$) C, H, N.

Method B. The Sequence Leading to Ethyl 3-[(2-Hydroxy-3-phenoxypropyl)amino]propionate Oxalate (3) Is Representative. 1-Succinimido-3-phenoxy-2-propanol (7). A mixture of 15 g (0.10 mol) of epoxide **40** and 9.9 g (0.10 mol) of succinimide in 100 mL of ethanol and 10 drops of pyridine was heated to reflux for 4 h.[21] The reaction medium was then allowed to stand at room temperature for 24 h and provided 20 g of crude crystalline product, which was recrystallized from 700 mL of ethyl acetate/hexane (6:1) to finally yield 18 g (72%) of **7**: mp 130 °C (lit.[10] mp 130 °C). Anal. ($C_{13}H_{15}NO_4$) C, N, N.

1-Amino-3-phenoxy-2-propanol Hydrochloride (8). A solution of 16 g (0.06 mol) of **7** in 100 mL of concentrated HCl and 100 mL of ethanol was heated to reflux for 6 h. The reaction medium was then evaporated to a white residue. This residue was taken up in 25 mL of water and washed with 3 × 50 mL of ether. The aqueous phase was again evaporated, and the resulting amorphous solid was crystallized from 20 mL of ethanol to provide 8.3 g (69%) of white crystals: mp 226–228 °C with prior softening at ~140 °C (lit.[22] mp 135–136 °C converts to an opaque residue which clears at 228 °C). Anal. ($C_9H_{14}NO_2Cl$) C, H, N.

Ethyl 3-[(2-Hydroxy-3-phenoxypropyl)amino]propionate Oxalate (3). A mixture of 3.4 g (0.02 mol) of **8**, 2.6 mL (0.02 mol) of ethyl 3-bromopropionate, and 2.8 mL (0.02 mol) of triethylamine in 20 mL of ethanol was heated to reflux for 24 h. The reaction medium was then filtered and evaporated under reduced pressure. The resulting residue was taken up in 25 mL of water and 50 mL of ether. The ether phase was washed a second time with 25 mL of water and then dried over $MgSO_4$ and evaporated. The resulting oil was converted to a soft, solid-free amine by crystallization from ethyl acetate. An oxalate salt was then produced by combining equivalent amounts of the amine and oxalic acid dihydrate, employing ethanol as solvent. The resulting crystals were recrystallized from acetone to finally provide 1.2 g (17%) of **3**: mp 137–138 °C; NMR (CD_3OD) δ 7.0 (m, 5, Ar H), 1.1 (t, J = 7 Hz, 3, CH_2CH_3). Anal. ($C_{16}H_{23}NO_8$) C, H, N.

Method C. Ethyl 3-[(2-Hydroxy-3-phenoxypropyl)amino]butyrate Hydrochloride (4). A mixture of 10 g (0.10 mol) of 4-aminobutyric acid, 6.6 mL of **40** (0.05 mol), and 4.0 g (0.10 mol) of NaOH in 160 mL of aqueous dioxane (1:3) was heated to reflux for 4 h. After the mixture was cooled, 100 mL of water was added, and the aqueous medium was washed with 400 mL of ether. The aqueous phase was acidified to pH 1 by adding concentrated HCl and then evaporated to a semisolid, which was extracted with ethyl acetate. This process removed 5.3 g (96%) of NaCl side product. Evaporation of ethyl acetate provided the crude amino acid product as an oil, which was immediately esterified by dissolving it in 500 mL of ethanol, which was then heated to reflux for 96 h in a Soxhlet extractor charged with 250 g of activated 3A molecular sieves.[23] Concentration of the ethanol and treatment with ether provided a crystalline material, which was subsequently recrystallized from ethyl acetate to provide 4.1 g (25%) of **4**: mp 109–110 °C; NMR (CD_3OD) δ 7.1 (m, 5, Ar H), 1.1 (t, J = 7 Hz, 3, CH_2CH_3). Anal. ($C_{15}H_{24}NO_4Cl$) C, H, N.

Method D. The Preparation of Ethyl 3-[[2-Hydroxy-3-(2-methylphenoxy)propyl]amino]propionate Hydrochloride (19) Is Representative. 1-(Benzylamino)-3-(2-methylphenoxy)-2-propanol Hydrochloride (41). A solution of 16.4 g (0.1 mol) of 1-(2,3-epoxypropoxy)-2-methylbenzene and 10.7 g (0.1 mol) of benzylamine in 90 mL of ethanol was heated to reflux for 4 h. The reaction medium was then concentrated under reduced pressure, leaving an oil. The oil was taken up in ether and the solution was filtered and treated with HCl gas to provide 13.8 g (45%) of crystalline product: mp 140–141 °C; NMR (Me_2SO) δ 7.4 (m, 5, Bnz-Ar), 6.9 (m, 4, Ar), 4.2 [m, 3, CH_2Ar and $CH(OH)$], 3.9 (d, 2, OCH_2), 3.1 (m, 2, CH_2N), 2.1 (s, 3, Ar CH_3). Anal. ($C_{17}H_{22}NO_2Cl$) C, H, N.

Ethyl 3-[[2-Hydroxy-3-(2-methylphenoxy)propyl]amino]propionate Hydrochloride (19). A solution of 6.3 g (0.023 mol) of free amine **41**, 4.2 g (0.023 mol) of ethyl 3-bromopropionate, and 2.3 g (0.023 mol) of triethylamine in 50 mL of ethanol was heated to reflux for 24 h. The reaction medium was then evaporated under reduced pressure. The resulting oil was taken up in aqueous ether (1:1), and the ethereal phase was separated, dried over magnesium sulfate, and evaporated to an oil. The NMR spectrum of this oil was appropriate for the desired tertiary amine intermediate. Without additional purification, 3.8 g (0.01 mol) of the oil was dissolved in 25 mL of ethanol and treated with HCl gas. A 400-mg catalytic quantity of 10% Pd/C was added to the acidified solution, which was then hydrogenated at 40 psi for 30 min. The reaction medium was then filtered and concentrated under reduced pressure. The resulting oil was taken up in chloroform and treated with ether to provide 1.3 g (44%) of crystalline product: mp 82–83 °C; NMR ($CDCl_3$) δ 6.8 (m, 4, Ar), 4.6 [m, 1, $CH(OH)$], 4.0 (m, 4, 2 OCH_2), 3.3 (m, 6, $CH_2NCH_2CH_2$), 2.2 (s, 3, Ar CH_3), 1.2 (t, J = 7 Hz, 3, CH_2CH_3). Anal. ($C_{15}H_{24}NO_4Cl$) C, H, N.

Ester Hydrolysis. Potassium *N*-(2-Hydroxy-3-phenoxypropyl)glycinate Hydrate (5). A solution of 0.67 g (0.003 mol) of free amine (**2**) in 50 mL of ethanol containing 0.34 g (0.005 mol) of 86% KOH in 10 mL of water was heated to reflux for 24 h. The reaction medium was then concentrated, while crystallization occurred slowly at room temperature to provide 0.4 g (48%) of **5**: mp 189–190 °C; NMR (D_2O) δ 7.0 (m, 5, Ar H), 4.0 [m, 3, OCH_2 and $CH(OH)$], 3.2 (s, 2, NCH_2CO), 2.7 (d, J = 5 Hz, 2, CH_2N); IR (KBr) 1580 (carboxylate anion) cm^{-1}. Anal. ($C_{11}H_{14}NO_4K \cdot H_2O$) C, H, N.

Compounds 1, 11–18, and 20–39. The remaining compounds were prepared according to the described methods employing the appropriate epoxide and amine reagents. Details are provided in Table II.

In Vitro Studies. β-Blockade was assessed in vitro with guinea pig right atria and tracheal strips mounted in tissue baths containing oxygenated (95% O_2–5% CO_2) Krebs physiological salt solution at 37 °C. Each tissue was suspended between a fixed glass rod and a Statham Universal Transducer connected to a Beckman recorder. Atria were allowed to beat spontaneously under a loading tension of approximately 0.5 g. Intrinsic depressant or stimulant activity was determined for each compound by progressively increasing concentrations in the tissue baths at 60-min intervals. Tissues were not washed between increments. A concentration showing little or no cardiodepressant or cardiostimulant activity was chosen for blockade experiments. Changes in rate in response to isoproterenol were measured in the absence and presence of test compounds. Spiral strips of trachea were suspended under 5-g resting tension and incubated with tropolone (3×10^{-5} M, 35 min), phentolamine (1.2×10^{-5} M, 25 min), and cocaine (9.8×10^{-6} M, 25 min). Active tension was generated by addition of carbachol (3.0×10^{-7} M, 15 min), and decreases in tension in response to isoproterenol were quantitated. Cumulative concentration–response curves were produced with isoproterenol both before and after 60-min incubation of test compounds[13] with atria and trachea. The blocking potency of test compounds was estimated by computing K_B values by the method of Furchgott.[24] Computation of the atria/trachea potency ratio with K_B values obtained from each tissue permitted

Table II. Experimental Data for Test Compounds[a]

no.	formula	method	salt form	cryst solvents	yield, %	mp, °C
1	$C_{12}H_{20}NO_2Cl$	A	HCl	MeOH/Et_2O	75	112-114[b]
11	$C_{15}H_{19}N_2O_3Cl$	A	HCl	H_2O/EtOH	24	175-176
12	$C_{18}H_{24}NO_4Cl$	A	HCl	acetone/EtOAc	10	123-124
13	$C_{19}H_{23}NO_8$	C	oxalate	MeOH/Et_2O	15	180
14	$C_{13}H_{16}NO_2Cl$	B	HCl	EtOH/Et_2O	53	204-206[c]
15	$C_{14}H_{20}NOCl \cdot 0.7C_2H_2O_4$	D	oxalate	EtOH	15	134-135
16	$C_{14}H_{21}NO_4Cl_2$	D	HCl	EtOAc/Et_2O	22	94-95
17	$C_{14}H_{21}NO_4Cl_2$	D	HCl	EtOH/Et_2O	41	119-120
18	$C_{12}H_{17}NO_4Cl_2$	C	HCl	acetone	15	124-125
20	$C_{17}H_{25}NO_8$	D	oxalate	EtOH	18	127-128
21	$C_{15}H_{23}NO_5 \cdot 0.7C_2H_2O_4$	D	oxalate	EtOH	22	129-132
22	$C_{15}H_{24}NO_5Cl$	D	HCl	EtOH/Et_2O	30	117-119
23	$C_{16}H_{25}NO_4 \cdot 0.5C_2H_2O_4$	D	oxalate	EtOH/EtOAc	20	148-149
24	$C_{13}H_{21}NO_2Cl$	A	HCl	EtOH/Et_2O	84	133-135
25	$C_{16}H_{23}NO_8 \cdot 0.5H_2O$	D	oxalate	EtOH	25	137-138
26	$C_{13}H_{20}NO_4Cl$	D	HCl	MeOH/EtOAc	25	128-129
27	$C_{16}H_{23}NO_8$	D	oxalate	MeOH	45	152-153
28	$C_{16}H_{26}NO_4Cl$	D	HCl	EtOAc/Et_2O	17	77-78
29	$C_{17}H_{28}NO_4Cl$	D	HCl	CH_2Cl_2/Et_2O	46	102-103
30	$C_{17}H_{27}NO_4 \cdot 0.5C_2H_2O_4 \cdot H_2O$	A	oxalate	acetone/Et_2O	15	127-128
31	$C_{17}H_{26}NO_6Cl$	A	HCl	EtOH/EtOAc	26	105-106
32	$C_{19}H_{23}NO_4$	A		EtOAc/hexane	70	88-89
33	$C_{21}H_{25}NO_8$	A	oxalate	EtOH/Et_2O	35	89-92
34	$C_{20}H_{26}NO_4Cl$	D	HCl	EtOH/Et_2O	42	144-145
35	$C_{19}H_{24}NO_4Cl$	D	HCl	MeOH/Et_2O	40	179-180
36	$C_{23}H_{31}NO_6 \cdot 0.5C_2H_2O_4 \cdot H_2O$	D[d]	oxalate	CH_2Cl_2/EtOAc	23	125-129
37	$C_{19}H_{24}NO_4Cl$	D[d]	HCl	EtOAc	16	148-149
38	$C_{19}H_{23}NO_4FCl$	D[d]	HCl	CH_2Cl_2/EtOAc	14	142-144
39	$C_{14}H_{20}NO_4F_3 \cdot 0.5C_2H_2O_4$	D[d]	oxalate	CH_2Cl_2/EtOAc	15	133-134

[a] C, H, and N elemental analyses data agree within ±0.40% of the theoretical values for all compounds, and the NMR spectra were appropriate for all compounds. [b] Literature [J. Zaagsma and W. T. Nauta, *J. Med. Chem.*, 17, 507 (1979)] mp 110-112 °C. [c] Literature [J. Zaagsma and W. T. Nauta, *J. Med. Chem.*, 17, 507 (1979)] mp 200 °C dec. [d] Because of the labile nature of the ester alcohol adduct for these compounds, it was necessary to remove all alcohol-containing solvents from the experimentals described in method D. Methylene dichloride was found to be a satisfactory substitute in all cases.

assessment of cardioselectivity of test compounds. Test drugs were dissolved in distilled water and added to the bath (30 mL) in a volume of 10 or 100 μL. Compounds isolated as their free amines were acidified with concentrated HCl to pH ~3–4 during their dissolution.

In Vivo Studies. The duration of β-blockade was determined in vivo with barbiturate-anesthetized dogs instrumented for measurement of heart rate with a Beckman cardiotachometer triggered electronically by a phasic aortic blood-pressure signal. Both vagus nerves were severed in the cervical region, and the animals were mechanically ventilated. Two experimental designs were used. The first employed a 40-min infusion of test compound and the second used a 3-h infusion of test compound. In the 40-min model, isoproterenol was infused into a foreleg vein at the rate of 0.5 (μg/kg)/min to induce a β-receptor-mediated tachycardia. Various doses of the test compound were then infused into a femoral vein over a period of 40 min. This infusion was then terminated, and recovery from blockade was quantitated. The percent inhibition of the heart rate response to isoproterenol after 40 min of infusion of the test compound was computed, along with the total cumulative dose received over the 40-min period. The time period required for 80% recovery of heart rate for each dose of test drug was also measured to quantitate duration of action. To facilitate comparison of data between animals, we normalized the data for potency and duration of action to a level of 50% inhibition of the isoproterenol response via least-squares regression of data from each animal. Blockers were dissolved in 0.9% NaCl and infused at a rate of 0.04 (μL/kg)/min or less. In the 3-h infusion model, bolus doses of isoproterenol (0.5 μg/kg) were used to assess the degree of β blockade and recovery from β blockade after termination of the blocker infusion. The doses were spaced at 10-min intervals and were given before, during, and following the infusion of test compounds. Doses were infused at a rate of 0.05 (mL/kg)/min or less and were adjusted during the first hour of infusion to produce 50% blockade of the standard isoproterenol challenges. This obviated the need for normalization of the data obtained from this experimental design.

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