Synthesis and Irreversible β-Adrenergic Blockade with a Bromoacetamido Derivative of Betaxolol

NOURDINE AMLAIKY*[x] AND GERARD LECLERC

Received September 19, 1983, from the *Department of Medicinal Chemistry, Institut de Pharmacologie, 67000 Strasbourg, France.* Accepted for publication July 10, 1985. Present address: *Departments of Medicine and Physiology, Box 3287, Duke University Medical Center, Durham, NC 27710.

Abstract □ 1-[4-(2-Cyclopropylmethoxyethyl)phenoxy]-3-[1-*p*-(bromoacetamidophenyl)-2-methyl-2-propylamine]-2-propranol (**8**), which is a derivative of the β_1-adrenergic agent betaxolol, was synthesized. Compound **8** showed less potent β-adrenergic blocking activity than betaxolol in an in vitro test with guinea pig tracheal muscle and left atrium but retained high β_1-selectivity. Irreversible β-adrenoceptor antagonism of **8** was assessed by the blockade of the isoproterenol response of the guinea pig atria and by ligand binding studies with rat cerebral cortex.

The properties of α- and β-adrenergic receptors have mainly been studied with reversible ligand but, to characterize and purify these receptors and to study their turnover, irreversible or long-lasting ligands are most desirable.[1-6] The present study describes the synthesis and characterization of a potent β_1-adrenergic ligand containing a bromoacetamido functional group. This compound, the bromoacetamidobenzyl derivative of betaxolol, was designed as an affinity label for the β_1-adrenergic receptor.

Experimental Section

2-Nitropropane and *p*-nitrobenzyl chloride were purchased from Aldrich Chemical Co., Bersse, Belgium. Isoprenaline was purchased from Fluka Chemical Co., Buchs, Switzerland. 4-(2-Cyclopropylmethoxyethylphenol was a gift from Synthelabo, Paris, France.

Melting points were obtained on a calibrated Kofler hot-stage apparatus and are uncorrected. Infrared spectra were measured in $CHCl_3$ with a Beckman IR 33 spectrophotometer. NMR spectra were recorded on a Perkin-Elmer R-24-B spectrometer with Me_4Si in a capillary as an external reference. Spectral data were consistent with the assigned structures. Mass spectra were recorded at 20 eV with an LKB 2091 spectrometer. Microanalyses were performed by the Service Central de Microanalyses du Centre National de la Recherche Scientifique, Strasbourg, France.

1-[4-(2-Cyclopropylmethoxyethyl)phenoxy]-2,3-epoxypropane (5)—Compound **5** was prepared as described previously.[7]

1-(*p*-Aminophenyl)-2-methyl-2-propylamine (2)—A solution of 2-methyl-2-nitro-1-(*p*-nitrophenyl)propane (**1**)[8] in 700 mL of EtOH containing 7.2 g of Pd/C was hydrogenated at room temperature and atmospheric pressure. After 15 L of H_2 had been absorbed, hydrogenation was continued for 8 h with heating at 35°C, which resulted in the absorption of an additional 7 L of H_2. The mixture was filtered, and the volume of the organic phase was reduced to about 200 mL. Concentrated HCl (50 mL) was added, and the dihydrochloride was removed by filtration to give 47.5 g (90%). The salt was converted to the free base by treatment with 2 M NaOH. The solution was extracted with EtOAc, washed with H_2O, and dried, and the solvent was removed under reduced pressure to give **2** as a yellow oil; mp 81–82°C [lit.[8] mp 84–85°C]; ^1H NMR ($CDCl_3$): δ 1.05 [s, 6, $C(CH_3)_2$], 2.5 (s, 2, CH_2), 6.5 (d, 2, J = 9 Hz, ArH), and 6.9 ppm (d, 2, J = 9 Hz, ArH).

1-(*p*-Azidophenyl)-2-methyl-2-propylamine (3)—Amine **2** (2 g, 12.1 mmol) was dissolved in 18 mL of 2 M HCl and 1.2 mL of H_2O. The mixture was ice-cooled, and $NaNO_2$ (0.88 g, 12.7 mmol) in 3 mL of H_2O was added in a dropwise manner while stirring. When the addition was complete, the reaction was allowed to proceed for 15 min, and NaN_3 (0.78 g, 12.1 mmol) in 3 mL of H_2O was then added in a dropwise manner. After another 15 min, the mixture was made alkaline with aqueous $NaHCO_3$ and extracted with EtOAc, and the solvent was then evaporated to give 2.2 g (96%) of **3** as yellow oil; IR ($CHCl_3$): 2130 cm^{-1} (N_3); ^1H NMR (Me_2SO-d_6): δ 1.05 [s, 6, $C(CH_3)_2$], 2.5 (s, 2, CH_2), and 6.9 ppm (d, 2, J = 9 Hz, ArH).

1-[4-(2-Cyclopropylmethoxyethyl)-phenoxy]-3-[1-*p*-(azidophenyl)-2-methyl-2-propylamine]-2-propanol (6)—A mixture of **5** (1 g, 4 mmol) and **3** (0.255 g, 1.3 mmol) was dissolved in 20 mL of EtOH. The mixture was refluxed in the dark for 6 h and the solvent was evaporated. The resulting material was purified by flash column chromatography with EtOAc:$NHEt_2$ (95:5) to give 0.36 g (60%) of an oil. IR ($CHCl_3$):2120 cm^{-1} (N_3); ^1H NMR ($CDCl_3$): δ 1.05 [s, 6, $C(CH_3)_2$], 2.55 (s, 2, CH_2), 2.7–3.85 (m, 18, aliphatic CH_2, ring CH), 7.0 (d, 2, J = 9 Hz, ArH), 7.3 (d, 4, ArH), and 7.7 ppm (d, 2, J = 9 Hz, ArH).

1-[4-(2-Cyclopropylmethoxyethyl)phenoxy]-3-[1-*p*-(aminophenyl)-2-methyl-2-propylamine]-2-propanol (7)—A solution of 1 g (2.28 mmol) of the azide **6** in 30 mL of EtOH was hydrogenated at room temperature and atmospheric pressure in the presence of 0.1 g of Pd/C. After 15 min, the catalyst was removed by filtration and the ethanolic solution was concentrated under reduced pressure to give 0.96 g (98%) of **7** as an oil. The dihydrochloride of **7** was prepared and recrystallized from isopropyl alcohol to yield a pale-yellow solid, mp 208–210°C; IR ($CHCl_3$): 3300 cm^{-1} (NH_2); ^1H NMR ($CDCl_3$): δ 1.1 [s, 6, $C(CH_3)_2$], 2.7 (s, 2, CH_2), 2.8–3.7 (m, 18, aliphatic CH_2, ring CH), 6.9 (d, 2, J = 9 Hz, ArH), 7.3 (d, 2, ArH), and 7.7 ppm (d, 2, J = 9 Hz, ArH); MS: m/z 397 ($M^+ - 15$).

Anal—Calc. for $C_{25}H_{38}Cl_2N_2O_3$: C, 61.85; H, 7.89; N, 5.76. Found: C, 61.68; H, 7.84, N, 5.72.

DL-1-[4-(2-Cyclopropylmethoxyethyl)phenoxy]-3-[1-*p*-(bromoacetamidophenyl)-2-methyl-2-propylamine]-2-propanol (the Bromoacetamidobenzyl Derivative of Betaxolol) (8)—Bromoacetyl bromide (0.106 mL, 1.2 mmol) in 3 mL of benzene was added in a dropwise manner to a solution of **7** (0.5 g, 1.2 mmol) in 20 mL of benzene. After 15 min, the hydrobromide of **8** was removed by filtration to give 0.71 g (97%) of colorless needles that were recrystallized from MeOH–H_2O to give 0.60 g (82%) of light tan-colored crystals, mp 219–220°C; IR (KBr): 1640 cm^{-1} (NHCO); ^1H NMR (Me_2SO-d_6): δ 1.1 [s, 6, $C(CH_3)_2$], 2.7 (s, 2, CH_2), 2.6–3.9 (m, 18, aliphatic CH_2, ring CH), 4.05 (s, 2, CH_2), 7.0 (d, 2, J = 9 Hz, ArH), 7.3 (d, 4, ArH), 7.7 (d, 2, J = 9 Hz, ArH), and 8.5 ppm (br s, 1, amide NH); MS: m/z 517, 519 ($M^+ - 15$).

Anal—Calc. for $C_{27}H_{38}Br_2N_2O_4$: C, 52.77; H, 6.23; N, 4.56. Found: C, 52.62; H, 6.22, N, 4.64.

Pharmacological Tests of β-Adrenergic Blocking Activity—An in vitro guinea pig model was used to determine the β- and β-adrenergic blocking activities. The antagonism of isoprenaline-induced positive chronotropism was measured on isolated spontaneously beating right atria according to the method of Horii et al.[9] The preparations were suspended in Krebs-Henseleit solution at 32°C and aerated with a mixture of 95% O_2 and 5% CO_2. Contractions were recorded isometrically. Diastolic tension was set to 0.5 g. Preincubation time with the antagonist **8** (10^{-7}, 3.10^{-7}, and 10^{-6} M) was 30 min before the next cumulative dose-response curve with isoprenaline. Ascorbic acid, 10^{-4} g/mL, was present during the elaboration of each curve.

β_2-Adrenergic blocking activity was assessed by the method described by Levy and Wilkenfield,[10] slightly modified. Two equal segments of trachea were placed in Krebs–Henseleit solution at 37°C and treated with 5% CO_2 in O_2. Contractions were recorded isotoni-

0022-3549/85/1000-1117$01.00/0

cally with 1 g preload. The bath fluid contained ascorbic acid (10^{-4} g/mL) and phentolamine (10^{-7} g/mL). Tracheal chain preparations were allowed to gain tone spontaneously. Preincubation time with the antagonists was 30 min.

β-Adrenergic blocking activity was expressed in terms of pA_2 values (logarithm of the reciprocal concentration of antagonist which necessitates doubling the concentration of agonist to keep the effect constant), as determined by the method of Arunlakshana and Schild.[11] A plot of log (x − 1) = f (colog A) gives straight lines of slope 1 for competitive antagonists.

Fraction of Free Receptors after Blockage—The isoprenaline dose-response curves obtained before and after the addition of 10^{-5} M of 8 on guinea pig right atria were used to calculate the fraction of free receptors remaining after the blockage by the method of Furchgott and Bursztyn.[12] The equation:

$$\frac{1}{[A]} = \frac{1-q}{q \cdot K_A} + \frac{1}{q[A']}$$

was applied to the results, where q is the fraction of active receptors remaining after treatment with 8. The concentrations of A and A' are the equimolar concentrations of isoprenaline before (A) and after (A') of 8, and K_A is the equilibrium activation constant for the agonist. Reciprocal values were plotted as 1/[A] against 1/[A'] and were used to calculate the regression line which gave a value for q of 1/slope.

Preparation of Rat Cerebral Cortex—Adult Wistar rats (150–250 g) were killed by decapitation. Shortly after decapitation, the cortex was dissected and homogenized at 0°C in 30 volumes of 20 mM Tris HCl (pH 7.4) with a polytron (Beckman) setting number 6 for 30 s. The homogenate was then centrifuged at 50,000 × *g* for 15 min. The supernatant was discarded and the pellet was resuspended in 30 volumes of the same buffer and then centrifuged as before. Pellets were then frozen and stored at −80°C.

[^{125}I]Iodocyanopindolol Binding Assay—A membrane preparation (5 mL) was incubated in 10 mM Tris HCl (pH 7.4) alone, with alprenolol, with betaxolol for the protective experiment, or with 8 for 30 min at 25°C. Membranes were then washed three times by centrifugation, resuspended in 5 mL of 10 mM Tris HCl–154 mM NaCl (pH 7.4) and assayed for [^{125}I]iodocyanopindolol binding by incubating 175 μL of membranes, 50 μL of ligand, and 25 μL of the competing drug for 1 h at 25°C. At the end of the incubation period, each suspension was filtered through Whatman GF/C glass fiber filters and washed three times with 5 mL of the incubation buffer. The radioactivity of the wet filters was determined in an Autogamma Packard counter at 75% counting efficiency. Specific [^{125}I]iodocyanopindolol binding to the β-adrenoreceptor was calculated as the difference between total binding in the absence of added competitor and the nonspecific binding determined in the presence of 10 μM (±)-propranolol. All assays were performed in triplicate. Protein concentration was measured by the method of Lowry et al.[13] with serum albumin as a standard.

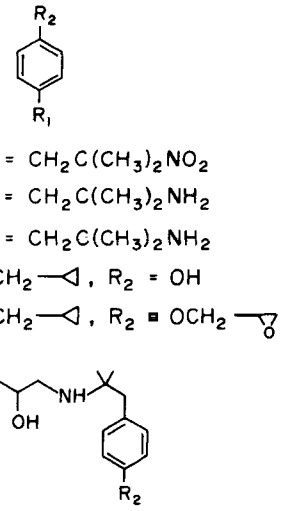
Results and Discussion

Chemistry—Scheme I illustrates the synthetic route to compound 8. The azido derivative 3 was obtained from the amine precursor 2. Compound 6 was obtained by opening the epoxide 5 with the azido derivative 3. The azido group of 6 was then reduced catalytically (Pd/C) to give 7, which was then treated with bromoacetyl bromide in benzene to give 8 as a pure hydrobromide salt. In conclusion, Scheme I allows not only the preparation of 8 with an overall yield of 48% but also the preparation of the azido derivative 6, which can be used as a photoaffinity label.

Adrenergic Activity—β-Adrenergic blocking activity was measured in vitro in guinea pigs as described previously.[9] The activity of the bromoacetyl derivative 8, expressed as pA_2, is shown in Table I. Betaxolol activity is also given for the sake of comparison.

Compound 8 has about one-one hundreth of the potency of betaxolol but remains highly cardioselective.

The long-lasting action (alkylating property) of compound 8 was studied on the guinea pig right atria. After 30 min of



Scheme I

preincubation with 8 at a concentration of 10^{-5} M, followed by six washings every 5 min for 30 min, the isoprenaline dose-response curve was shifted to the right and the maximal response decreased to 81% (Fig. 1). The fraction of β-adrenergic receptor population not occupied by 8 fell to 1%, according to the equation of Furchgott and Bursztyn.[12]

Irreversible Inhibition of [^{125}I]Iodocyanopindolol Binding—The specificity of the irreversible interaction of 8 with the β-adrenergic receptor is shown in Fig. 2. Exposure of rat cortical membranes to compound 8 for 30 min caused a 60–70% decrease in the amount of measurable binding sites after an extensive wash procedure. The presence of betaxolol (100 μM) in the incubation afforded partial protection of the sites against irreversible inactivation. As shown by the inclusion

Table I—Pharmacological Results

Compound	pA_2 ± SEM (n) Guinea Pig[a]		Selectivity, β_1/β_2[b]
	Atria	Trachea	
8	7.66 ± 0.40 (5)	5.28 ± 0.15 (4)	240
Betaxolol	9.49 ± 0.42 (8)	7.16 ± 0.21 (8)	214

[a] pA_2 with isoprenaline as agonist was determined according to Arunlakshana and Schild.[11] Although it applies to competitive antagonists, pA_2 was used for the alkylating **8** to facilitate its comparison with betaxolol. [b] Selectivity (β_1/β_2 is the antilog of (pA_2 on atria) − (pA_2 on trachea).

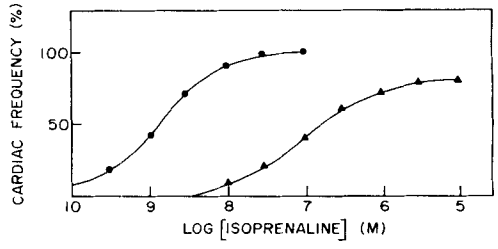


Figure 1—*Isoprenaline chronotropic effect on guinea pig right atria before (●) and after (▲) pretreatment with 8 (10^{-5} M) for 30 min. Preparations were washed every 5 min for 30 min before the isoprenaline challenge of the tissue.*

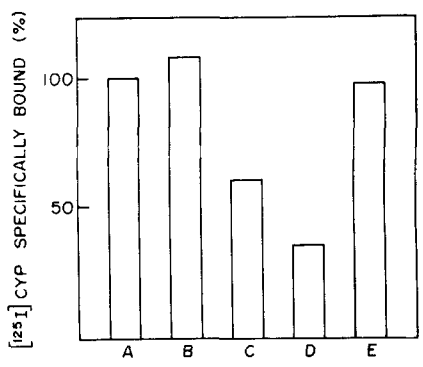


Figure 2—*Specificity of the interaction of* ***8*** *with the β-adrenergic receptor assessed by ligand binding. Membranes were incubated for 30 min at 25°C with various concentrations of drugs. Key:* (A) *control;* (B) *10 μ (±)-alprenolol;* (C) *betaxolol (100 μM) plus* ***8*** *(10 μM);* (D) ***8*** *(10 μM);* (E) ***8*** *(10 μM) plus cysteine (10 mM). Compound* ***8*** *(10^{-5} M) was then added, and the incubation was continued for 1 h at 25°C. Membranes were then washed three times by centrifugation and [^{125}I]iodocyanopindol ([^{125}I]CYP) binding assayed in triplicate. Incubation of a separate membrane with (±)-alprenolol at 10^{-5} M was incorporated in the experiment to serve as a control for the washing procedures. To assess the effect of quenching the alkylating function of compound* ***8*** *on its irreversible character, compound* ***8*** *(10 μM) was incubated with 10 mM cysteine for 30 min before addition to the membrane preparations. Results shown are the averages of three experiments.*

of (±)-alprenolol (10 μM) in the incubation mixture, the washing procedure was adequate to remove most reversibly bound ligand. This fact is also supported by the data obtained when the membrane preparation was incubated with compound 8 which had been prereacted with cysteine to quench its alkylating activity. As shown on the right of Fig. 2, the binding of [^{125}I]iodocyanopindolol virtually completely recuperated, indicating that the irreversible character of compound 8 appears to be due specifically to its alkylating properties.

References and Notes

1. Atlas, D.; Steer, M. L.; Levitzki, A. *Proc. Natl. Acad. Sci. USA* **1976**, *73*, 1921.
2. Leclerc, G.; Rouot, B.; Schwartz, J.; Velly, J.; Wermuth, C. G. *Br. J. Pharmacol.* **1980**, *71*, 5.
3. Pitha, J.; Zjawiony, J.; Nasrin, N.; Lefkowitz, R. J.; Caron, M. G. *Life Sci.* **1980**, *27*, 1791.
4. Baker, S. P.; Pitha, J. *J. Pharmacol. Exp. Ther.* **1982**, *220*, 247.
5. Wrenn, S. M.; Homcy, C. J. *Proc. Natl. Acad. Sci. USA* **1980**, *77*, 4449.
6. Rashidbaigi, A.; Ruoho, A. E. *J. Pharm. Sci.* **1982**, *71*, 305.
7. Leclerc, G.; Decker, N.; Schwartz, J. *Arzneim.-Forsch.* **1985**, in press.
8. Hass, H. B.; Berry, E. J.; Bender, M. L. *J. Am. Chem. Soc.* **1949**, *71*, 2290.
9. Horii, D.; Kanada, T.; Takeda, K.; Imai, S. *Arzneim.-Forsch.* **1974**, *24*, 1275.
10. Levy, B.; Wilkenfeld, B. E. *Eur. J. Pharmacol.* **1970**, *11*, 67.
11. Arunlakshana, O.; Schild, H. O. *Br. J. Pharmacol.* **1959**, *14*, 48.
12. Furchgott, R. F.; Bursztyn, P. *Ann. N.Y. Acad. Sci.* **1967**, *144*, 882.
13. Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. *J. Biol. Chem.* **1951**, *193*, 265.

Acknowledgments

The authors express their sincere thanks to Pr. J. Schwartz, Director of the Institut de Pharmacologie, to N. Decker and her associates for the biological evaluations, to Dr. J. D. Ehrhardt for the mass spectra, and to Dr. Marc G. Caron for helpful discussion and suggestions.