Notes

6,7-Dichloro-1-(3,4,5-trimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline. A Structurally Novel β-Adrenergic Receptor Blocking Agent

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Replacement of the catecholic hydroxyl groups of the β-adrenergic receptor agonist 6,7-dihydroxy-1-(3,4,5-trimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline (trimetoquinol) with chloro substituents results in a compound with marked β-adrenoceptor antagonist properties. This, therefore, parallels the similar transformation of the β-adrenoreceptor agonist isoproterenol into the antagonist dichloroisoproterenol. In a test for inhibition of isoproterenol-induced enhancement of the rate of contraction of spontaneously beating guinea pig atrial pairs the resultant 6,7-dichloro-1-(3,4,5-trimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline (**6b**) had a K_B value of $(6.7 \pm 2.3) \times 10^{-8}$ M. Although this is nearly 2 orders of magnitude less potent than propranolol ($K_B = 6.2 \times 10^{-10}$ M in this test), this compound represents the prototype of a new class of β-adrenergic receptor blockers, and unlike dichloroisoproterenol it is not a partial agonist. It has physicochemical properties, e.g., pK_a and distribution and partition coefficients, that differ from the prototypic β-blockers. These altered properties might impart advantageous tissue distribution and altered pharmacological properties to the new molecule. This new β-adrenoreceptor antagonist is suggested to merit further study.

The classification of adrenergic receptors into two distinct populations, namely the α- and β-subtypes[1] has provided the basis for the development of several classes of therapeutic agents having a substantial impact on the understanding and treatment of a broad range of disease states. Of the classes of therapeutic agents that modulate adrenergic receptors perhaps the one so far established to have the greatest therapeutic benefit is a group that blocks β-adrenergic receptors.[2] Initially introduced as antianginal agents,[3] these drugs have subsequently been found beneficial in the therapy of a number of cardiovascular disease states. Clearly, they are most widely used in hypertensive therapy[4,5] although the mode by which these drugs produce this well-recognized effect remains incompletely understood.[6,7]

The β-adrenergic receptors have been further subdivided into β_1 and β_2 subpopulations.[8-11] In general, activation of β_1 receptors results in increased force and rate of heart contraction, dilation of coronary blood vessels,[12,13] relaxation of smooth muscle in the alimentary tract, and lipolysis. Stimulation of β_2 receptors relaxes smooth muscle in the bronchi, uterus, and vasculature, decreases tension in some skeletal muscle, and promotes glycolysis and glycogenolysis. It follows that a serious problem in the use of agents that block these effects may be impairment of pulmonary function. This is likely a consequence of blockade of β_2-adrenoreceptors in the lungs. For this reason it was originally thought that selective β_1-adrenergic blocking agents would be most beneficial as they would lack the concomitant induction of bronchospasm. Clinical experience, however, has not confirmed this thinking. In fact, propranolol, the β-blocking drug that finds widest application in antihypertensive therapy, is a mixed β_1,β_2-adrenoreceptor antagonist. Experiments, in both humans and animals, indicate the three major differences between clinically used β-blockers are membrane-stabilizing activity, β_1 vs. β_2 subtype selectivity, and partial agonism (i.e., agents with lower intrinsic activities than those of the natural adrenergic agonists). The clinician must decide the importance of these properties in selecting a β-blocker for the treatment of individual patients.[14] Thus, it seems reasonable that new chemically different β-adrenergic receptor blocking agents with potentially different pharmacological, and particularly physicochemical features, might offer therapeutic advantages perhaps as a consequence of altered tissue distribution.

The most common chemical classes of β-adrenergic receptor antagonists so far described fall into two, or possibly three, chemical types. These are the arylethanolamines, e.g., dichloroisoproterenol (DCI, **1**)[15] and pronethalol (**2**),[16,17] aryloxypropanolamines, e.g., propranolol (**3**)[18] and

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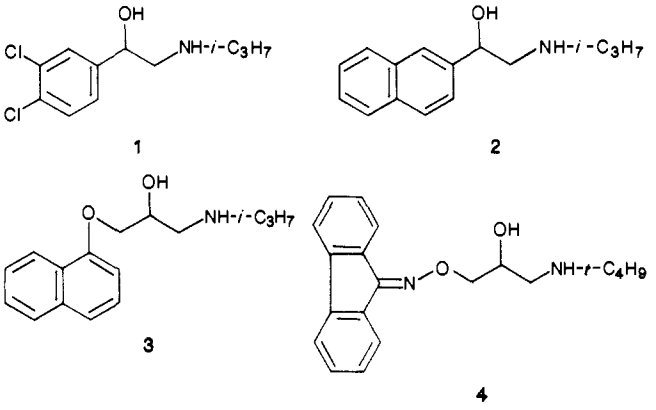
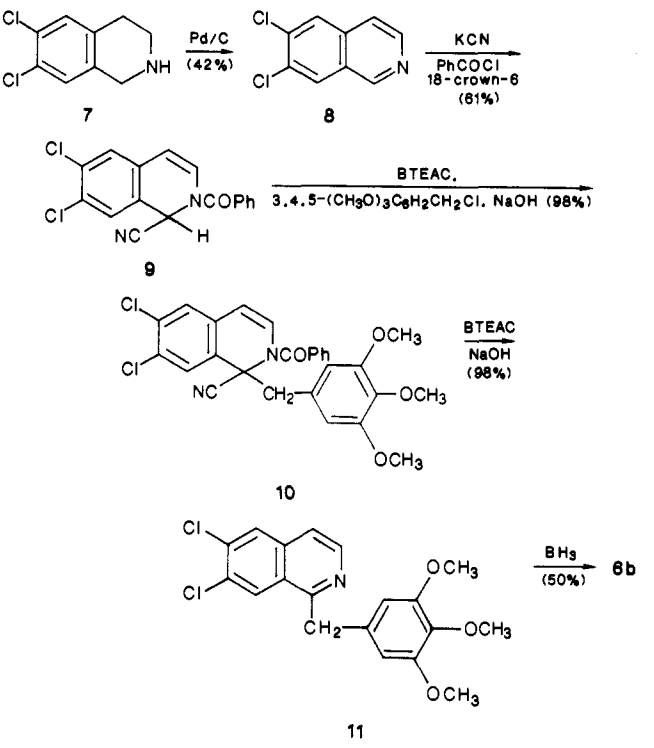
related compounds,[2,19,21] as well as the somewhat more chemically diverse 9-[[3-(*tert*-butylamino)-2-hydroxypropyl]oximino]fluorene (4) and similar oximino derivatives.[21]



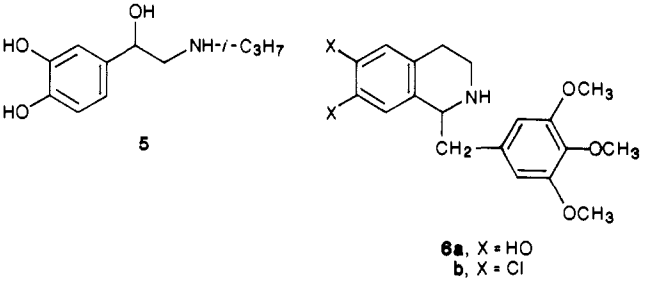
The derivation of these classes of β-adrenoreceptor blocking agents from the prototype of β-adrenergic agonists, i.e., isoproterenol (**5**) and related aryloxypropanolamines,[22] seems chemically straightforward, although the significance of the additional OCH_2 in **3** and the $=NOCH_2$ bridge in 4 is not entirely clear.[23-30] As in other types of receptor modulating drugs, however, structural modification of an agonist molecule provides a rational course toward the derivation of antagonists.

The prototype of another class of β-adrenoreceptor agonists is the tetrahydroisoquinoline trimetoquinol (**6a**) whose chemical relationship to the classical agonists is not readily apparent.[22,31] A critical difference between this compound and other β-adrenergic receptor agonists is the absence of an alcoholic hydroxyl group, a factor that might markedly change the physicochemical properties of the molecule. For this reason, we prepared and studied the β-adrenergic activity of 6,7-dichloro-1-(3,4,5-trimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline (**6b**), a modification of **6a** which might convert the agonist into an antagonist. In this report are described the preparation and results of pharmacological study of this structural analogue of **6a**

Scheme I



patterned after the transformation of the β-agonist isoproterenol (**5**) to the antagonist DCI (**1**).



Chemistry. The preparation of the target compound **6b** for this study was carried out as illustrated in Scheme I.

The requisite starting material 6,7-dichloro-1,2,3,4-tetrahydroisoquinoline (**7**) was obtained according to literature directions.[32] Dehydrogenation was effected with palladium on carbon in refluxing *p*-cymene to give the isoquinoline **8**, which was subjected to a Reissert reaction,[33] under phase-transfer conditions,[34] to obtain **9**. Treatment of **9** with 3,4,5-trimethoxybenzyl chloride utilizing a similar phase-transfer procedure gave **10**, which was converted to the 1-benzylated isoquinoline **11**. Borane reduction[35] of the isoquinoline nucleus provided the target compound **6b**.

Results and Discussion

6,7-Dichloro-1-(3,4,5-trimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline (**6b**) was studied for its ability to inhibit the isoproterenol-induced enhancement of the rate of contraction of isolated guinea pig atrial pairs as described

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Table I. Physicochemical Properties of Some β-Adrenoreceptor Antagonists

compd	log D^a	log P^a	apparent pK_a^a
1	+1.26	+2.55	8.7
3	+1.22	+2.75	8.9
6	+1.79	+2.23	7.6

[a] Determined as described in the Experimental Section.

in the Experimental Section. In this test, which measures predominantly β_1-adrenoreceptor blockade, **6b** behaved as a competitive β-receptor antagonist; it had a K_B value of $(6.7 \pm 2.3) \times 10^{-8}$ M ($N = 4$). Unlike dichloroisoproterenol it was not a partial agonist; it showed no agonist activity at concentrations as high as 10 μM, and it shifted the dose–response curve to isoproterenol to the right in a parallel fashion. Although it was about 2 orders of magnitude less potent than propranolol, which had a K_B value of 6.2×10^{-10} M in this test, and although its β_1,β_2-antagonist selectivity was not determined, **6b** is of particular interest because it represents the first member of a new class of β-adrenergic receptor antagonists.

This observation is particularly striking in light of the structure–activity relationship (SAR) differences between the tetrahydroisoquinolines and other types of β-adrenoreceptor agonists. For example, introduction of a hydroxyl-imitating group[22] into the 6- or 7-position of **6a** results in a loss of activity,[36] whereas replacement of the hydroxyl in the 3-position of catecholic β-agonists with hydroxyl-simulating moieties results in retention, or even enhancement, of activity.[22] Similarly, as noted earlier, the benzylic hydroxyl seems unimportant for the β-agonist activity of tetrahydroisoquinolines, and the absolute configuration *S* at position 1 is essential.[37] Apparently, however, trimetoquinol (**6a**) and arylethanolamine β-agonists act at β_1 and β_2 receptors in a similar fashion. Thus, **2** shifts the dose–response curve of **6a** to the right.[38] Similarly, in the current study the dose–response curve of **6a** (EC_{50} = 31 nM, intrinsic activity compared to **5** = 0.8, $N = 4$) was shifted to the right by the aryloxypropanolamine **3** (K_B vs. **6a** = 5.6 nM, $N = 2$). Therefore, it appears that the tetrahydroisoquinoline agonists and antagonists bind to the same β_1, and perhaps β_2, receptors in a similar fashion, although the marked difference in SAR between the two series of agonists clouds this conclusion. Possibly, they are interacting with the receptors in a different manner as has been proposed for the binding of various classes of analgetics with a common opioid receptor.[39] It is, therefore, particularly significant that similar modification of **5** and **6a**, i.e., replacement of the catecholic hydroxyls with Cl to give **1** and **6b**, respectively, results in transformation of an agonist into an antagonist. An extrapolation of this observation suggests that **6b**, like **1**, may be the prototype of a new, and possibly therapeutically beneficial, series of β-adrenoreceptor antagonists. Clearly, **6b** would be anticipated to have physicochemical properties markedly different from those of other prototypic β-adrenoreceptor antagonists, such as **1** and **3**. To enable comparison of some of these physicochemical parameters, distribution (log *D*) and partition (log *P*) coefficients between *n*-octanol and pH 7.4 phosphate buffer and apparent pK_as were determined for **1**, **3**, and **6b** (Table I). The experimentally derived values are quite similar for **1** and **3**, whereas those for **6b** differ significantly. Most strikingly, the pK_a for **6b** is more than one log unit lower than those for **1** and **3**, and the distribution coefficient values indicate much greater lipophilicity for **6b**. In summary, these data suggest that the physicochemical properties of **6b**, which differ markedly from other β-blockers, may result in altered tissue distribution with a concomitant variation in pharmacological activity. Further SAR study of tetrahydroisoquinoline β-blockers with emphasis toward derivation of agents with even greater differences in physicochemical properties from other drugs of this type therefore seems merited.

Experimental Section

Melting points were determined in open capillary tubes on a Thomas-Hoover Uni-Melt apparatus and were not corrected. Elemental analyses were determined by the Department of Analytical, Physical, and Structural Chemistry of Smith Kline & French Laboratories. Where analyses are reported by symbols of elements, results were within 0.4% of the calculated value. Although not presented, IR spectra (Perkin-Elmer 727 spectrophotometer), NMR spectra (Perkin-Elmer R-24 or Bruker WM-360 spectrometers using an internal standard of Me_4Si), and mass spectra (Perkin-Elmer RMV-6E spectrometer) were determined for all compounds described in this section and were evaluated as consistent with the assigned structures. All compounds were a single spot on TLC.

Chemistry. 6,7-Dichloroisoquinoline (8) Hydrochloride. A mixture of 6,7-dichloro-1,2,3,4-tetrahydroisoquinoline (**7**)[32] (6.2 g, 30 mmol), 0.6 g of 10% Pd/C, and 100 mL of *p*-cymene was stirred and refluxed under N_2 for 16 h. The mixture was filtered, and the filtrate was extracted with 2.5 N HCl. After the aqueous layer was extracted with Et_2O, it was made alkaline with 10 N NaOH and the mixture was extracted with EtOAc. The EtOAc extracts were washed with H_2O, dried ($MgSO_4$), and concentrated. The residue was dissolved in EtOH, the solution adjusted to pH 2 with HCl, and Et_2O added to give a crystalline solid (3.7 g, 42%) after recrystallization from EtOH: mp 227–228 °C. Anal. ($C_9H_6Cl_3N$) C, N; H: calcd, 2.58; found, 3.17.

2-Benzoyl-6,7-dichloro-1-cyano-1,2-dihydroisoquinoline (9). To a solution of **8** (3.2 g, 13.6 mmol) in 12 mL of CH_2Cl_2 was added 18-crown-6 (0.22 g, 0.43 mmol) followed by a solution of KCN (1.67 g, 26 mmol) in 12 mL of H_2O. The mixture was stirred vigorously under N_2 for 30 min, and then a solution of 2.4 g (17.2 mmol) of benzoyl chloride in 10 mL of CH_2Cl_2 was added dropwise. After the reaction mixture was stirred at 25 °C for 3 h, the layers were separated (additional CH_2Cl_2 and H_2O required). The organic layer was washed with H_2O, 5% aqueous $NaHCO_3$, H_2O, and brine, and then it was separated, dried ($MgSO_4$), and concentrated. The resulting residue was triturated with CH_3OH to give 1.7 g (61%) of crystals, mp 194–196 °C, after recrystallization from CH_3OH–EtOAc. Anal. ($C_{17}H_{10}Cl_2N_2O$) C, H, N.

2-Benzoyl-6,7-dichloro-1-cyano-1-(3,4,5-trimethoxybenzyl)-1,2-dihydroisoquinoline (10). A solution of **9** (1.7 g, 5.2 mmol), 3,4,5-trimethoxybenzyl chloride (1.2 g, 5.4 mmol) and benzyl triethylammonium chloride (BTEAC, 0.12 g, 0.52 mmol) in 25 mL of toluene was flushed with N_2. A 50% aqueous solution of NaOH (7.2 mL) was added in one portion, and the mixture was stirred under N_2 for 2 h at 25 °C. After addition of H_2O (25 mL) and EtOAc (25 mL), the mixture was filtered to remove 0.7 g of crystalline product. The organic phase was evaporated, and the residue was dissolved in CH_2Cl_2. After the organic solution was washed with H_2O and dried ($MgSO_4$), it was concentrated to give an additional 1.6 g of crystalline product: total yield, 2.3 g (98%); mp 193–195 °C after recrystallization from CH_3CN. Anal. ($C_{27}H_{22}Cl_2N_2O_4$) C, H, Cl, N.

6,7-Dichloro-1-(3,4,5-trimethoxybenzyl)isoquinoline (11) Hydrochloride. To a solution of **10** (2.3 g, 4.5 mmol) and BTEAC (0.1 g, 0.42 mmol) in 40 mL of toluene, flushed with N_2, was added 19 mL of a 50% aqueous solution of NaOH. After the reaction mixture was heated under reflux for 1.5 h, it was cooled and H_2O and EtOAc were added. The organic layer was separated and washed with H_2O and brine. It was then dried ($MgSO_4$) and concentrated. A solution of the residue in CH_3OH–Et_2O was

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acidified with HCl to give 1.8 g (98%) of pale-yellow crystals, mp 211-212 °C, after recrystallization from CH_3OH-EtOH. Anal. ($C_{19}H_{17}Cl_2NO_3$·HCl) C, H, Cl, N.

6,7-Dichloro-1-(3,4,5-trimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline (6b) Fumarate. A suspension of **11**·HCl (1.6 g, 3.9 mmol) in H_2O was converted to the base (10 N NaOH, CH_2Cl_2, H_2O wash, $MgSO_4$), which was dissolved in 5 mL of THF and treated with 18 mL of 0.97 M BH_3 in THF. After the solution was stirred at reflux under N_2 for 6 h, an additional 5 mL of the BH_3 solution was added, and refluxing and stirring were continued for an additional 1.5 h under N_2. The solution was cooled to 10 °C; CH_3OH was added dropwise with *caution*, and then the solution was concentrated. To the residue was added 20 mL of 2 N HCl, and the solution was refluxed for 30 min. After the solution was cooled to 20 °C it was made alkaline with 10 N NaOH, and the mixture was extracted with EtOAc. The EtOAc layer was washed with H_2O, dried ($MgSO_4$), and concentrated. The residue was dissolved in CH_3OH, and excess fumaric acid in CH_3OH was added. Et_2O was added to precipitate a solid (0.6 g, 50%), mp 163 °C, after recrystallization from EtOH. Anal. ($C_{19}H_{21}Cl_2NO_3$·$C_4H_4O_4$·0.25H_2O) C, H, N.

Determination of Distribution Coefficients. The compounds were partitioned by shaking between *n*-octanol and pH 7.4 phosphate buffer (prepared from 3.7 g of Na_2HPO_4 and 1.6 g of KH_2PO_4 in sufficient H_2O to make 9 L, adjusting the pH to 7.4 by addition of 0.02 M KH_2PO_4 or 0.01 M Na_2HPO_4 as required) until equilibrium was attained. The phases were allowed to separate and were clarified by centrifugation. Aliquots from each phase were then assayed by UV detection. The compounds were partitioned at four different concentrations, and the ratios (D = (milligrams of compound/milliliters of *n*-octanol)/(milligrams of compound/milliliters of pH 7.4 phosphate buffer)) were plotted vs. concentration. The value of D (or log D) was determined at infinite dilution by linear regression.

pK_a determinations were obtained by titration of the compounds in 1:2 CH_3OH-H_2O (to overcome solubility constraints).

True partition coefficient, P, was calculated from the distribution coefficient D as follows: $P = D/(1 - \alpha)$, where $\alpha = 1/[1 + \text{antilog}(\text{pH} - \text{p}K_a)]$.

Pharmacology. Inhibition of Isoproterenol-Induced Enhancement of Rate of Contraction of Spontaneously Beating Guinea Pig Atrial Pairs. Adult, male Hartley strain guinea pigs weighing 500-800 g were anesthetized with sodium pentobarbital (100 mg/kg, ip). The whole heart was quickly removed and immersed in 10 °C Krebs solution of the following concentration: NaCl (119 mM), $CaCl_2$ (2.5 mM), KH_2PO_4 (1.2 mM), KCl, 4.7 mM), $NaHCO_3^-$ (25 mM), glucose (11.1 mM), ascorbic acid (5 μM), and disodium EDTA (30 μM). The atrial pair was dissected free of the rest of the heart tissue and fat and was suspended via surgical thread secured to the apex of each atria in 50-mL tissue baths containing Krebs solution maintained at 37 °C and gassed continuously with a mixture of 95:5 O_2-CO_2. Cocaine (6 μM) was present throughout all experiments. Atrial contractions were recorded with a Grass FT 03D isometric force displacement transducer on a Beckman R-511 dynograph; atrial rate was recorded on the same instrument from cardiotachometers triggered by the atrial contractions.

The atrial pair was allowed to equilibrate for approximately 30 min prior to administration of test compounds. During this equilibration period the bathing solution was exchanged three or four times. A concentration-response curve to isoproterenol was then determined, increasing isoproterenol concentrations when the rate response to the previous dose had stabilized. After this determination, any β-agonist activity of a test compound was measured via a concentration-response curve. To determine β-antagonist activity, a concentration of test compound that produced no β response (3 μM for **6b** and propanolol, which lacked agonist activity at concentrations as high as 100 μM) was allowed to remain in the tissue bath while the curve for isoproterenol was repeated. The receptor dissociation constant (K_B) as a β-adrenergic antagonist was determined by using the following formula: K_B = antagonist concentration/(dose ratio - 1), where the dose ratio is the EC_{50} (the concentration increasing the rate of contraction to 50% of maximal for compound being tested) for isoproterenol in the presence of antagonist divided by the EC_{50} for isoproterenol. The reported K_B values are the average of 4-8 experiments.

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Registry No. **6b**·$C_4H_4O_4$, 103959-65-5; **7**, 75416-52-3; **8**·HCl, 73075-60-2; **9**, 103959-61-1; **10**, 103959-62-2; **11**·HCl, 103959-63-3; 3,4,5-trimethoxybenzyl chloride, 3840-30-0.

Synthesis and Biological Evaluation of Irreversible Inhibitors of Aldose Reductase

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5-Isothiocyanatoalrestatin (**1b**) and 5-azidoalrestatin (**1c**) were prepared synthetically and examined as potential affinity and photoaffinity inhibitors of rat lens aldose reductase. Both compound **1b** and **1c** under appropriate conditions at 10^{-4} M produced a 70% irreversible inactivation of aldose reductase within 1 min. The enzyme could, in part, be protected by preincubation with sorbinil **2**, a known potent inhibitor of aldose reductase.

The enzyme aldose reductase uses NADPH as a cofactor to reduce glucose to sorbitol. This reaction accounts for the first step of the polyol pathway, which subsequently leads to the formation of fructose. Although the polyol pathway has no known function in man, the enzyme aldose reductase is important because intracellular sorbitol accumulation occurs in the presence of excess glucose in various tissues and is linked to many of the disabling complications of diabetes including cataracts, retinopathy, corneal wound healing, and neuropathy.[1-3] These complications are believed to result in part from sorbitol accumulation, which leads to osmotic swelling and disturbances in cell function.[1] For this reason, inhibitors of aldose reductase are clinically desirable for reduction of sorbitol accumulation and possible alleviation of these diabetic complications.

Many structurally diverse compounds have been shown to inhibit aldose reductase. Among the more potent are

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