Effect of Fluorine Substitution on the Adrenergic Properties of 3-(*tert*-Butylamino)-1-(3,4-dihydroxyphenoxy)-2-propanol

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The 2- and 6-fluoro derivatives of the potent β-adrenergic agonist 3-(*tert*-butylamino)-1-(3,4-dihydroxyphenoxy)-2-propanol were prepared and their adrenergic properties examined. The order of potency was as follows: β-adrenergic activity (simulation of cyclic AMP formation in C6 glioma cells), 2-F = parent ≫ 6-F; β_1-activity (rate of contraction, guinea pig atria), parent > 2-F ≫ 6-F; β_2-activity (relaxation of guinea pig tracheal strip), 2-F > parent ≫ 6-F. The affinity of the 2-fluoro analogue for β_1-adrenergic receptors (inhibition of the specific binding of [^{3}H]dihydroalprenolol, rat cerebral cortical membranes) was 2 times greater, while the 6-fluoro analogue was 1450 times less than the parent. These results suggest that the aromatic rings of phenoxypropanolamine adrenergic agonists and phenylethanolamine adrenergic agonists bind in similar fashion to the adrenergic receptor, and that if interactions between fluorine and the side-chain hydroxyl group are critical in defining β-adrenergic selectivity, the interactions are similar in both phenoxypropanolamines and phenylethanolamines.

The presence of fluorine on the aromatic ring of norepinephrine (NE) and other phenylethanolamine adrenergic agonists has a striking effect on their adrenergic activities, depending on the site of the fluorine substituent. This phenomenon was demonstrated initially by the discovery that 2-FNE is a selective β-adrenergic agonist and 6-FNE a selective α-adrenergic agonist, while 5-FNE has adrenergic potencies at both receptor types similar to NE.[1,2] Similar fluorine-induced changes in adrenergic potencies were observed subsequently for the potent β-selective agonist isoproterenol[3] and the α-selective agonist phenylephrine[4] and epinephrine.[5] Mechanisms advanced to rationalize fluorine-induced adrenergic selectivities include proposals that invoke an interaction of fluorine with the ethanolamine side chain and proposals that consider effects of fluorine substitution on electronic properties of the aromatic ring.[6] The apparent lack of adrenergic selectivities of ring-fluorinated dopamine led us initially to suspect an important role of the benzylic hydroxyl group in defining fluorine-induced selectivities in phenylethanolamines. Our initial proposal[1] that hydrogen bonding of this group with an ortho-situated fluorine could favor conformations favorable to binding to α- or β-adrenergic receptors was difficult to support on theoretical or experimental bases. As an alternative mechanism based on conformational effects, electrostatic repulsion between the negative charges of the C–F and C–OH dipoles was suggested as a mechanism by which fluorine could destabilize specific rotamers of phenylethanolamines, and thus introduce selectivites toward receptor subtypes.[5,7] Mechanisms involving electronic perturbations of the aromatic ring caused by fluorine substitution included our suggestion that a decrease in electronic charge on the carbon to which fluorine is bound might inhibit a bonding interaction between a positive charge on the receptor and the electron-rich catechol ring.[3] On the basis of theoretical calculations, Kocjan et al. made the alternative proposal that the negative end of the C–F dipole could be repulsed by a negative charge on the receptor binding site.[8] In each of the latter two formulations, selectivity would result from different locations of charged species on α- and β-adrenergic receptors with respect to the carbon bearing the fluorine substituent.

The speculative nature of all explanations for fluorine-induced adrenergic selectivities is apparent. We now report further experimental evidence pertinent to the question of binding and activity of fluorinated adrenergic agonists. The 2- and 6-fluoro derivatives (**1a,b**) of 3-(*tert*-butylamino)-1-(3,4-dihydroxyphenoxy)-2-propanol (**1c**), a potent β-adrenergic agonist,[9] have been prepared and effects of fluorine on β-adrenergic activity assessed. While interaction of the side-chain hydroxyl group with a 2- or 6-fluoro substituent is still possible, the spatial relationships between these substituents differ substantially in the two series. However, either electrostatic repulsion or hydrogen bonding will be possible if the hydroxyl group in the phenoxypropanolamine must assume the same position relative to the aryl ring as in phenylethanolamines. The present results indicate that fluoro substituents have the same effect on adrenergic activity in phenoxypropanolamines as in phenylethanolamines.

Results

Chemistry. 3-(*tert*-Butylamino)-1-(3,4-dihydroxyphenoxy)-2-propanol (**1c**) was prepared from 3,4-bis(benzyloxy)phenol according the procedure described by Kaiser et al.[9] To apply this procedure to the preparation of the 2-fluoro- (**1a**) and 6-fluoro analogue (**1b**), the corresponding 3,4-bis(benzyloxy)-2-fluoro- and 4,5-bis(benzyloxy)-2-fluorophenols (**2a,b**) were prepared by *m*-chloroperoxybenzoic acid oxidation of the previously reported benzaldehydes.[1,3] Alkylation of **2a** and **2b** with epichlorohydrin and aminolysis of the intermediate epoxide **3a** and **3b** with

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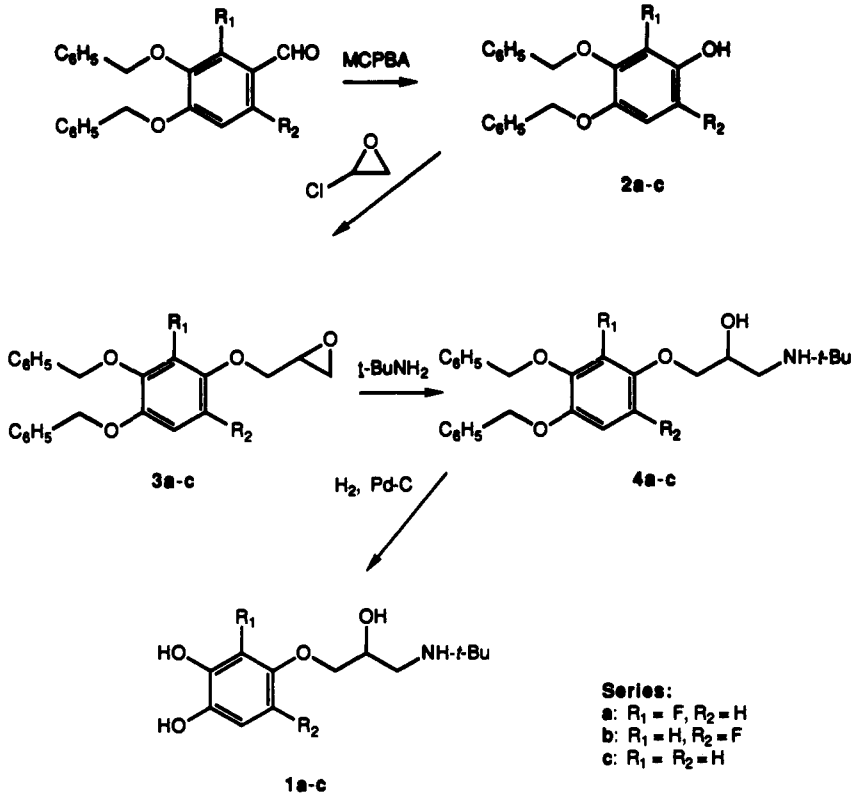
Scheme I



tert-butylamine gave the benzyl-protected intermediates **4a** and **4b**, purified as the oxalate salts. Hydrogenolysis of the oxalate of **4a** over Pd/C yielded **1a**, readily isolated and purified as the neutral oxalate salt. Substantial decomposition occurred during a similar procedure with **4b**·oxalate. Hydrogenolysis of **4b**, derived from **4b**·oxalate, in the presence of HCl gave much cleaner results. The final product (**1b**) from either procedure was purified as the noncrystalline trifluoroacetate by HPLC and characterized by NMR and mass spectral analysis (Scheme I).

Biology. The affinities of the 2- and 6-fluoro analogues of **1c** for β_1-adrenergic receptors were determined through inhibition of the binding of [^{3}H]dihydroalprenolol to rat cerebral cortical membranes. The agonist activities of the fluoro analogues on adrenergic receptor mediated responses were assayed as follows: For β_1-adrenergic activity, stimulation of the rate of contraction of the isolated guinea pig atria was determined. For β_2-adrenergic activity, the relaxation of isolated guinea pig tracheal strips was determined. For β-adrenergic activity, the stimulation of cyclic AMP accumulation in C6 glioma cells was determined. For details on these procedures see the Experimental Section.

The phenoxypropanolamine derivatives (**1a–c**) fully displaced [^{3}H]dihydroalprenolol from rat cerebral cortical membranes. As found for fluorinated phenylethanolamine β-adrenergic agonists, the affinity of the 2-fluoro derivative **1a** for β_1-adrenergic receptors is somewhat higher than the affinity of the parent compound, while the affinity of the 6-fluoro derivative **1b** is much lower. Thus, the order for β_1-adrenergic receptor binding of the phenoxypropanolamine derivatives was 2-F > parent ≫ 6-F (Table I). The order of potencies in the β-adrenergic receptor mediated stimulation of cyclic AMP accumulation in C6 glioma cells was 2-F = parent ≫ 6-F. The parent phenoxypropanolamine **1c** and the 2-fluoro derivative **1a** were partial agonists (60–80% efficacy) relative to isoproterenol. The order of potencies for the stimulation of β_1-adrenergic receptor mediated responses in the isolated guinea pig atria was parent > 2-F ≫ 6-F. Both **1a** and **1b** were partial agonists relative to isoproterenol, while the parent compound was a full agonist (Table I). The atrial responses were completely blocked by propanolol. The order of potencies for the β_2-adrenergic receptor mediated relaxation of guinea pig tracheal strips was 2-F > parent ≫ 6-F. The parent compound and the **1a** were full agonists, while **1b**, even at 10 μM, gave a relaxation only 15% of that of isoproterenol.

Table I. Adrenergic Properties of (±)-3-(*tert*-Butylamino)-1-(3,4-dihydroxyphenoxy)-2-propanols (**1a–c**)[a] and of (±)-Isoproterenol (Iso)

amine	affinity: K_i, μM β_1[b]	agonist potency: EC_{50}, nM β[c]	β_1[d]	β_2[e]
1c	0.12 ± 0.01 (3)	8.2[f]	4.7 ± 0.5 (5)	130 ± 16 (6)
1a	0.06 ± 0.01 (3)	9.2[f]	8.6 ± 1.7 (5)[g]	38 ± 7.2 (6)
1b	87 ± 6 (6)	≫10000	175 ± 27 (5)[h]	≫10000 (4)[i]
Iso	0.67[j]	7.0	6.0 ± 0.9 (5)	58 ± 12 (3)

[a] Data are expressed in terms of (±) racemate and have not been "corrected" to values that would have been obtained with the more active (−) enantiomer. Values in parentheses are the number of experiments. All compounds are full agonists unless otherwise noted. [b] K_i values ± SEM for the displacement of [^{3}H]dihydroalprenolol from rat cerebral cortical membranes. [c] EC_{50} values were from dose–response curves for the stimulation of accumulatioin of cyclic AMP in cultured C6 glioma cells. [d] EC_{50} values were from dose–response curves for the percent maximal increase in contraction rate of isolated guinea pig atria. [e] EC_{50} values were from dose–response curves for the percent maximal relaxation of isolated guinea pig trachea. [f] Partial agonist; 60–80% of isoproterenol response. [g] Partial agonist; 80% of isoproterenol response. [h] Partial agonist; 37% of isoproterenol response. [i] At 10 μM the response was not maximal, but was only 15% of the isoproterenol response. [j] Value from ref 3.

Discussion

Hydrogen bonding between the benzylic OH group and an aromatic fluorine substituent initially was proposed as

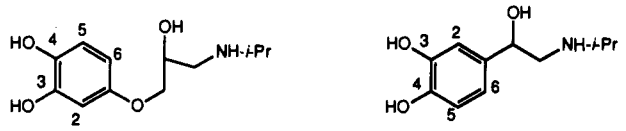


Figure 1. Proposed conformations of phenoxypropanolamine and phenylethanolamine adrenergic agonists that allow the side chain functional groups and the catechol rings in each class to occupy the same relative position on the receptor (ref 14).

a possible explanation for the fluorine-induced adrenergic selectivities of 2- and 6-FNE. An alternative mechanism that invoked effects of fluorine on side chain conformation involved a repulsive interaction between the benzylic OH group and the fluorine substituent. Fundamentally different mechanisms have been proposed that involve a direct effect of fluorine substitution on the electronic distributions of the aromatic ring, effects that would alter specific interactions of the aromatic ring with the receptor protein. Since any interaction of the fluorine substituent with the side-chain OH group in **1a** and **1b**, if present, should differ in energy substantially when compared to this interaction in 2- and 6-FNE (and related phenylethanolamines), the marked reduction in β-adrenergic activity of **1b** relative to **1a** and **1c**, and the significant increase in activity of **1a** relative to **1c**, appears to support a mechanism of fluorine-induced adrenergic selectivity that does not depend on such intramolecular interactions. Recent theoretical approaches to the elucidation of ligand–receptor interactions, in fact, stress the importance of conformational flexibility of ligands, a consequence of which flexibility is that an "active" conformation may not be that preferred in the crystal structure, solution, or in vacuo.[10,11] Recognition of this principle further places in doubt a mechanism of fluorine-induced adrenergic selectivities based on conformational biases based on intramolecular interactions of the fluorine substituent with the benzylic OH group.

These results also may have implications regarding the relative binding modes of the phenylethanolamines (type A adrenergic ligand) and (aryloxy)propanolamines (type B adrenergic ligand).[12] The 1-(aryloxy)-2-propanolamines can function as either agonists or antagonists, depending on the nature and pattern of substitution on the aromatic ring. β-Adrenergic antagonists in this group include the classic β-blockers propranolol, alprenolol, and pindolol, while certain of the phenoxypropanolamines having polar substituents meta and para to the ether function, for example **1c**, are potent β-adrenergic agonists. The common functional role and gauche relationship of the protonated amine and the β-hydroxyl group in receptor binding of both type of ligands has been demonstrated.[13] However, more controversial has been the relative positions of the aromatic rings of each class when bound to the receptor.

Comer[14] initially suggested that the potent β-adrenergic agonist activity of 3-(isopropylamino)-1-(3,4-dihydroxyphenoxy)-2-propanol (prenalterol) might be related to its ability to adopt the conformation shown in Figure 1. In this conformation the catechol ring and side-chain functional groups can be superimposed on the corresponding sites of a phenylethanolamine agonist in its favored extended conformation. Kaiser et al.[9] noted that this formulation transposes the relative positions of the aromatic substituents in the two series. Similar agonist properties exhibited by a series of substituted phenoxypropanolamines and the corresponding phenylethanolamine agonists suggested to these workers that the 3- and 4-positions of the aromatic ring in each series occupied the same relative positions when bound to the receptor. Citing this as evidence against the conformational relationship shown in Figure 1, they suggested a semirigid hydrogen-bonded conformation, shown in Figure 2, to explain the similar agonist properties in these series.[9] NMR evidence for such a conformation was obtained to support this proposal.[15] On the other hand, on the basis of NMR and IR spectroscopic data of a phenoxypropanolamine adrenergic antagonist (toliprolol) (**5**), Zaagsma[16] proposed an alternative seven-membered structure not involving ether oxygen participation in hydrogen bonding (Figure 3). Based on molecular orbital calculations, Macchia et al.[12] suggested that a completely unfolded conformation of (aryloxy)propanolamine adrenergic agonists such as **1c** is preferred and that similar "chemical effects" related to binding in the aromatic region are mediated by different chemical groups.

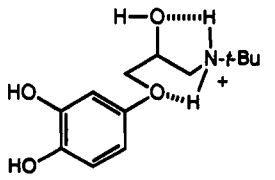


Figure 2. Proposed conformation of a phenoxypropanolamine adrenergic agonist that permits the 3- and 4-hydroxyl groups of the aromatic ring to occupy the same relative positions on the receptor that would be occupied by a (3,4-dihydroxyphenyl)-ethanolamine agonist (ref 9).

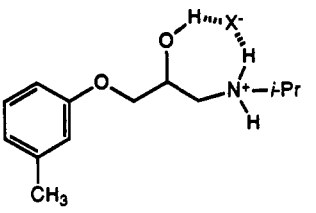


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Figure 3. Alternative hydrogen-bonded conformation proposed for the (aryloxy)propanolamine adrenergic antagonist, toliprolol (ref 16).

As part of an extensive investigation of quantitative relationships between structure and pharmacological activity of β-adrenergic agonists, Donne-Op den Kelder et al.[11] have addressed the important question of binding modes of type A (phenylethanolamine) and B (phenoxypropanolamine) ligands to adrenergic receptors. Modeling of binding to β_2-adrenergic receptors led to the proposal for one mode of binding for type A ligands to the receptor. Type B ligands with no para substituent could bind in two modes—one energetically less favorable due to "folding around the OCH_2 bridge" and the other corresponding to a favored, extended conformation (Figure 4). In the less-favored binding mode of class B ligands, spatial correspondence pertains with class A ligands with respect to

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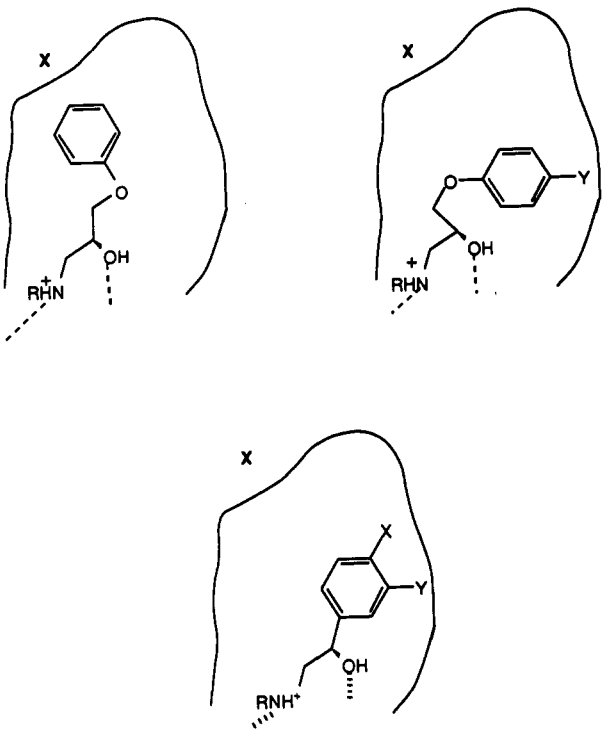

Figure 4. Different binding modes proposed for type B ((aryloxy)propanolamine) adrenergic agonists (above). In the less-favored binding mode (above right), spatial correspondence pertains with class A ligands (below) with respect to the aromatic moiety, the alcohol group, and the cationic group (ref 11).

the aromatic moiety, the alcohol group, and the cationic group. Their model further predicted that type B ligands substituted in the para position, because of an unfavorable interaction with a "filled site point" on the receptor ("X" in Figure 4) are forced in binding to adopt the less favorable conformation that closely overlaps the binding mode favored by type A ligands (agonists and antagonists). Binding in the latter mode to a functional high affinity state of the receptor and consequent receptor activation is favored by the presence of phenolic hydroxyl groups in both type A or B ligands (Figure 5).

As noted above, the demonstration that fluorine substitution has an effect on the adrenergic activity of **1c** comparable to that seen in the phenylethanolamine series is strong evidence against an effect mediated through conformational changes in the side chain. If, instead, an intermolecular interaction of the C–F bond with the adrenergic receptor binding site affects binding, this interaction should be an effective probe to study the characteristics of the aromatic binding site in adrenergic receptors. Thus, our results suggest that, for type B adrenergic agonists, the 2- and 6-positions of the aromatic ring occupy the same position as the 2- and 6-position of type A adrenergic agonists, a result of considerable significance in view of the above discussion of binding modes proposed for type A and B ligands. For example, it appears that if the binding mode shown in Figure 5 is adopted, the aromatic ether C–O bond should rotate such that the catechol OH groups will interact with their receptor binding sites. Such a rotation in **1a** or **1b** transposes the fluorine substituent such that an intermolecular interaction of the fluorine substituent of **1a** would correspond to a (3,4-dihydroxy-5-fluorophenyl)ethanolamine, while the fluorine substituent of 1b corresponds to the site of attachment of the side chain of a (3,4-dihydroxyphenyl)ethanolamine. Such a conclusion would be an extension of the interpretation advanced by Kaiser et al.[9] in their analysis of results

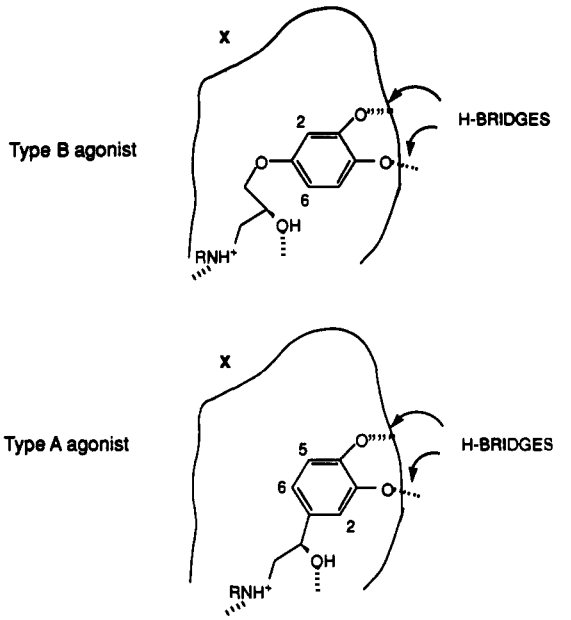

Figure 5. Relative ring positions of type A adrenergic agonists and type B adrenergic agonists after folding around the OCH_2 bridge of the latter.

obtained with 3- and 4-substituted type A and B adrenergic agonists. Thus, the comparable effects of fluorine substituted at the 2- and 6-position in each series of adrenergic agonists suggest that the phenylethanolamines and phenoxypropanolamines do not bind as suggested in Figure 5 where the 2- and 6-positions do not correspond, but, instead bind so that the aromatic rings do correspond. However, we cannot rule out the possibility that, even if phenylethanolamines and phenoxypropanolamines do bind differently to the receptor, a 6-fluoro substituent could still exert an unfavorable affect on binding to β-adrenergic receptors. The effects of a 2-fluoro substituent on β-adrenergic activity are much less pronounced than the effects of a 6-fluoro substituent. Depending on the receptor subtype and system being investigated, 2-fluoro analogues have been found to be equal, somewhat less, or somewhat more potent than their parents. Thus, any lack of correspondence of the site of the 2-fluoro substituent with respect to the proposed binding modes (Figure 5) in the two series of β-adrenergic agonists may not be that relevant to potency since more subtle factors appear to be involved.

We stress that this analysis, based on the results of adrenergic activity in one type B series, is quite speculative at this time. Clearly more data will be required to place any mechanistic proposals on a more secure basis. Research is in progress to that end.

Experimental Section

Purity and identity of products were monitored by thin-layer chromatography (silica gel GF plates) and chemical ionization mass spectrometry. All products gave mass spectra consistent with the assigned structures. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN.

1-(3,4-Dihydroxyphenoxy)-3-(*tert*-butylamino)-2-propanol·HCl (1c) was prepared according to the procedure of Kaiser et al.,[9] mp 171–174 °C (lit.[9] mp 175–177 °C).

3,4-Bis(benzyloxy)-2-fluorophenol (2a). A solution of 1.899 g (5.64 mmol) of 3,4-bis(benzyloxy)-2-fluorobenzaldehyde[3] and 1.580 g of *m*-chloroperbenzoic acid (9.15 mmol) in 25 mL of methylene chloride was heated at reflux for 25 h, during which time a white solid precipitated from the solution. The methylene chloride was removed by rotary evaporation, and the residue was dissolved in ethyl acetate. The ethyl acetate solution was washed three times with 10% sodium bicarbonate and once with a sat-

urated aqueous solution of sodium chloride and dried over sodium sulfate. Removal of the solvent by rotary evaporation gave an oil, which solidified. The oil was dissolved in 16 mL of methanol and 3.5 mL of 10% potassium hydroxide and stirred for 1 h. The solution then was cooled, acidified with 10% hydrochloric acid, and extracted three times with ethyl acetate. Evaporation of the solvent gave 1.337 g (73%) of **2a** as a reddish solid. The solid was recrystallized from ethyl acetate/petroleum ether to give colorless crystals: mp 61–62 °C; NMR ($CDCl_3$) δ 4.74 (1 H, s, O*H*), 5.03 (2 H, s, $C_6H_5CH_2$), 5.11 (2 H, s, $C_6H_5CH_2$), 6.51–6.64 (2 H, [AB portion of ABX multiplet] 5,6-Ar*H*), 7.29–7.42 (m, 10 H, $C_6H_5CH_2$). Anal. ($C_{20}H_{17}FO_3$) C, H.

4,5-Bis(benzyloxy)-2-fluorophenol (2b). By using the procedure described for the synthesis of **2a** (50 h reflux in 15 mL of methanol containing 950 mg of *m*-chloroperbenzoic acid), 1.217 g (3.62 mmol) of 4,5-bis(benzyloxy)-2-fluorobenzaldehyde[1,2] gave 0.622 g of **2b** (53%) (mp 90–91 °C after recrystallization from ethyl acetate/petroleum ether): NMR ($CDCl_3$) δ 4.70 (1 H, s [broad], O*H*), 5.04 (2 H, s, $C_6H_5CH_2$), 5.07 (2 H, s, $C_6H_5CH_2$), 6.61 (1 H, d, J = 11.9 Hz, 3 Ar*H*), 6.61 (1 H, J = 5.2, 6 Ar*H*), 7.26–7.42 (m, 10 H, $C_6H_5CH_2$). Anal. ($C_{20}H_{17}FO_3$) C, H.

3-[3,4-Bis(benzyloxy)-2-fluorophenoxy]-1,2-epoxypropane (3a). Following the procedure of Kaiser et al.,[9] a solution of 374 mg (1.15 mmol) of **2a** in 6 mL of water and 1 mL of ethanol was treated with 90 mg of potassium hydroxide and 0.3 mL (3.83 mmol) of epichlorohydrin. The solution was stirred at ambient room temperature for 21 h and then concentrated by rotary evaporation. The residue was suspended in water, and the mixture extracted with ether. The ether was dried (Na_2SO_4) and evaporated to give an oil (280 mg, 64%). This was used without further purification in the next step.

3-[4,5-Bis(benzyloxy)-2-fluorophenoxy]-1,2-epoxypropane (3b) was prepared in 67% crude yield from **2b** by following the same procedure. This material could not be crystallized and was used in the next step without further purification.

3-[3,4-Bis(benzyloxy)-2-fluorophenoxy]-3-(*tert*-butylamino)-2-propanol (4a). A solution of 280 mg of **3a** (0.736 mmol) and 2 mL of *tert*-butylamine in 5 mL of methanol was refluxed for 6 h. After rotary evaporation of the mixture, toluene was added, and the solution again was evaporated. After an additional toluene evaporation to remove traces of *tert*-butylamine, the residue was dissolved in methanol and 96 mg of oxalic acid dihydrate was added. After evaporation, the residue was recrystallized from methanol/ether to give 245 mg (61%) of **4a**·oxalate: mp 118–120 °C; NMR (CD_3OD) δ 1.38 (9 H, $(CH_3)_3C$), 3.04–3.12 (2 H, m, $NCH_2CH(OH)$), 3.97–4.04 (2 H, m, $OCH_2CH(OH)$, 4.15 (1 H, m C*H*(OH)), 5.07 (4 H, s, $C_6H_5CH_2$), 6.77–6.79 (2 H, m [AB portion of ABX multiplet]), 5,6-Ar*H*), 7.27–7.46 (10 H, m, $C_6H_5CH_2O$). Anal. ($C_{29}H_{34}NFO_8$) C, H, N.

3-[4,5-Bis(benzyloxy)-2-fluorophenoxy]-3-(*tert*-butylamino)-2-propanol (4b) was prepared in similar fashion by heating a solution of 532 mg (1.40 mmol) of **3b** and 3.5 mL of *tert*-butylamine in 9 mL of methanol for 6 h. Isolation as described for **4a** gave 550 mg of **4b·oxalate** (72%): mp 150–152 °C after recrystallization from methanol; NMR ($CDCl_3$) δ 1.32 (9 H, $(CH_3)_3C$), 2.95–3.05 (1 H, m, $NCHHCH(OH)$), 3.15–3.24 (1 H, m, $NCHHCH(OH)$), 3.88–4.02 (2 H, m, $OCH_2CH(OH)$, 4.30 (1 H, m, C*H*(OH)), 5.02 (s, $C_6H_5CH_2$), 5.04 (s, $C_6H_5CH_2$), 6.69 (1 H, d, J = 8.1 Hz, 6-Ar*H*), 6.70 (1 H, d, J = 12.0 Hz, 3-Ar*H*), 7.18–7.42 (10 H, m, $C_6H_6CH_2O$). Anal. ($C_{29}H_{34}NFO_8$) C, H, N.

3-(3,4-Dihydroxy-2-fluorophenoxy)-3-(*tert*-butylamino)-2-propanol (1a). A 100-mg sample (0.184 mmol) of **4a**·oxalate in 35 mL of methanol was hydrogenolyzed over 35 mg of 10% Pd/C at 40 psi for 6 h. The catalyst was removed by filtration, the solvent was evaporated, and the residue was recrystallized from methanol/ether to give 42 mg of **1a**·$^1/_2$oxalate (72%): mp 205–209 °C; NMR (CD_3OD) δ 1.36 (9 H, $(CH_3)_3C$), 2.98–3.10 (1 H, m, $NCHHCH(OH)$), 3.20–3.40 (1 H, m, $NCHHCH(OH)$), 3.90–4.15 (2 H, m, $OCH_2CH(OH)$, 4.12–4.22 (1 H, m, C*H*(OH)), 6.35–6.55 (2 H, m [AB portion of ABX multiplet] 5,6-Ar*H*). Anal. ($C_{14}H_{21}FNO_6$) C, H, N.

3-(4,5-Dihydroxy-2-fluorophenoxy)-3-(*tert*-butylamino)-2-propanol (1b). (a) Hydrogenolysis of a 100-mg sample of **4b**·oxalate (0.183 mmol) as above gave 60 mg of a product that could not be recrystallized by the usual manipulations. HPLC and TLC indicated the presence of significant amounts of polar and nonpolar impurities (<50% by HPLC). The compound was purified by semipreparative RP-HPLC (Beckman C_{18} column, 5 μm, 1 × 25 cm) by using a gradient of 0% A to 50% **B** over 30 min (solvent A = H_2O, 0.05% trifluoroacetic acid; solvent B = 70% acetonitrile, 0.05% trifluoroacetic acid). Under these conditions, the product had a retention time of 17.35 min. By this method, 20 mg of crude reduction product as the oxalate gave 11.6 mg of **1b**·trifluoroacetate as a colorless noncrystalline solid, homogeneous according to HPLC: EI mass spectrum, m/e 273 (calculated for $C_{13}H_{20}FNO_4$; m/e = 273); NMR (D_2O) δ 1.38 (9 H, $(CH_3)_3C$), 3.14–3.21 (1 H, m, $NCHHCH(OH)$), 3.28–3.34 (1 H, m, $NCHHCH(OH)$), 4.03–4.14 (2 H, m, $OCH_2CH(OH)$, 4.21–4.25 (1 H, m, C*H*(OH)), 6.72 (1 H, d, J = 8.4 Hz, 6-Ar*H*), 6.78 (1 H, d, J = 12.2 Hz, 3-Ar*H*). The ^{19}F-NMR spectrum (D_2O) gave a multiplet 64 ppm downfield from the trifluoroacetic acid peak.

(b) A 200-mg sample of **4b**·oxalate was suspended in 50 mL of ethyl acetate. The oxalic acid was removed by extraction five times (thorough shaking) with 25 mL of 10% Na_2CO_3. The ethyl acetate layer was washed two times with 25 mL of water, dried over Na_2SO_4, and evaporated. The residue was dissolved in 50 mL of methanol containing 0.1 mL of concentrated HCl. After hydrogenolysis at 40 psi over 60 mg of 10% Pd/C for 12 h, removal of catalyst and solvent gave 112 mg of crude of **1b**·HCl, estimated by HPLC to be at least 67% pure. The sample was free of nonpolar impurities. The material was purified as above by preparative HPLC.

Determination of [^{3}H]Dihydroalprenolol ([^{3}H]DHA) Binding to Rat Cerebral Cortical Membranes. Measurement of the specific binding of [^{3}H]dihydroalprenolol (SA = 48.1 Ci/mmol, New England Nuclear, Boston, MA) to β_1-adrenergic receptors in rat cerebral cortical membranes was carried out essentially as described.[17] [^{3}H]DHA was incubated in 50 mM Tris-HCl buffer, pH 8.0, for 20 min at 25 °C in a total volume of 1 mL. The reaction mixture was filtered through Whatman glass filters (EF/3) and washed three times with ice-cold buffer. Specific binding, defined as that blocked by 10 μM (±)-alprenolol, was 75–80% of total binding. Inhibition curves were performed with 1 nM [^{3}H]DHA.

Determination of Agonist Response in the Isolated Guinea Pig Atrium. Atria were obtained from male Hartley guinea pigs (255–360 g) and individually bathed in 20-mL organ chambers containing Tyrode's solution made of the following components (mM): NaCl 137, KCl 2.68, $CaCl_2$ 1.8, $MgCl_2$ 0.49, NaH_2PO_4 0.36, dextrose 5.55, and $NaHCO_3$ 11.9. The buffer was maintained at 37 °C and aerated with 95% O_2/5% CO_2 and had a pH of 7.15–7.2. Tissues were equilibrated for 40–90 min with four washes with fresh buffer. Diastolic base line tension was adjusted to 1.0 ± 0.1 g. Atrial contraction rate was monitored with a Gould force transducer and a Gould RS3600 physiograph. The test compounds were added to the bath in a cumulative fashion at 3-min intervals. The maximum contraction rate was determined by adding (±)-isoproterenol (10 μM) at the end of each experiment. Values are expressed as percent maximal increase in rate (PMI). The dose response for each test compound was repeated five times. EC_{50} values were determined by nonlinear regression analysis and the values reported as the mean ± standard error of the mean (SEM) for five separate experiments.

Tracheal Smooth Muscle Relaxation. Male Hartley guinea pigs (400–500 g) were sacrificed by stunning and exsanguination. Tracheal spiral rings were attached to a Gould force transducer and placed in an organ chamber (20 mL) containing Tyrode's buffer (see above). The buffer was aerated with 95% O_2/5% CO_2 and the temperature maintained at 37 °C. Tension was set at 2 g and readjusted to 2 g after 15 min. The preparation was allowed to equilibrate for 60–80 min with 3–4 washes with fresh buffer. Isometric tension was monitored on a Gould RS3600 physiograph. Tracheal contraction was induced by the addition of carbamylcholine (0.3 μM). The preparation was allowed to equilibrate for 30–40 min and then the text compound was added in a cumulative fashion at 5-min intervals. Maximal relaxation was determined by the addition of (±)-isoproterenol (1.0 μM) at the end of each experiment. EC_{50} values for relaxation were

(17) Bylund, B. D.; Snyder, S. H. *Mol. Pharmacol.* **1976**, *12*, 568–580.

determined by nonlinear regression analysis and expressed as the mean ± standard error of the mean (SEM) for 5–6 separate experiments.

Determination of Cyclic AMP Accumulation in Glioma Cells. The C6 glioma cells were cultured as previously described.[4] One day prior to the experiment the cells were subcultured in multidish trays, incubated overnight at 37 °C, the media removed and the cells washed twice with buffer made with the following components (mM): NaCl 118, KCl 4.7, $CaCl_2$ 3, $MgSO_4$ 1.2, KH_2PO_4, EDTA 0.5, glucose 10, and HEPES 20, pH 7.4. Fresh buffer (1 mL) containing the phosphodiesterase inhibitor rolipram (30 μM) was added to each well. Following a 10-min preincubation the test compounds were added and the incubation continued for an additional 10 min. Incubations were stopped by the removal of buffer and the addition of 0.1 N HCl (1.0 mL) to each well. After 30 min, the media was neutralized by the addition of 0.1 N NaOH, and cyclic AMP levels determined with a commercial kit (Amersham, Arlington Heights, IL). Results were expressed as picomole of cyclic AMP formed per 10 min. EC_{50} values were determined graphically.

Synthesis and Biological Evaluation of a Series of Substituted *N*-Alkoxyimides and -amides as Potential Atypical Antipsychotic Agents[1]

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In a continuing program to discover antipsychotic agents with a reduced propensity toward extrapyramidal side-effects, a series of *N*-alkoxyimides and -amides was prepared. Evaluation of these compounds in vitro revealed affinities for D_2, $5HT_2$ and $5HT_{1A}$ receptors. Several members of the series displayed a profile indicative of potential antipsychotic activity in preclinical assays. The most potent compound in these assays, **7**, also displayed possible effectiveness for the negative symptoms of schizophrenia. The synthesis of these compounds and details of their structure–activity relationships are described.

The inhibition of postsynaptic dopaminergic neurotransmission is traditionally assumed to be the mode of action of clinically available antipsychotic agents.[2] While treatment with these agents can be effective, it is often accompanied by the development of extrapyramidal side-effects (EPS),[3] and chronic treatment may result in tardive dyskinesia.[4]

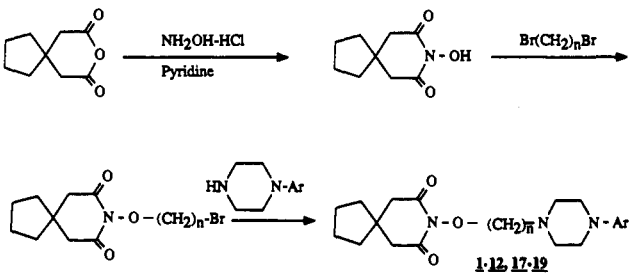
In research toward the development of a more selective therapeutic agent for schizophrenia, one emerging strategy is that the dopaminergic system can be more sensitively modulated through pharmacological manipulation of the serotonergic system. Consistent with this theory, clinical evidence exists for the involvement of the serotonergic receptor system in the pathology of schizophrenia. For example, in addition to its dopaminergic and α-adrenergic antagonist properties the atypical neuroleptic clozapine displays serotonergic antagonism at the $5\text{-}HT_2$ receptor site.[5] Another putative atypical neuroleptic, risperidone, also possesses both D_2 and $5\text{-}HT_2$ antagonist properties.[6] In addition to their reduced propensity to produce EPS, both compounds have been reported to improve type II (negative syndrome) schizophrenia, characterized by apathy and social withdrawal. Classical neuroleptics such as haloperidol are generally less effective against negative symptoms.[7]

We have prepared a series of compounds which would incorporate a $5HT_2$ antagonist component of action with dopamine D_2 antagonist activity and submitted them to a battery of tests predictive of antipsychotic activity. Several members of this series displayed a profile of activity indicative of potential antipsychotic efficacy with a reduced propensity for EPS liability. The standard neuroleptic agents haloperidol, clozapine, tiospirone and risperidone were assayed for comparison.

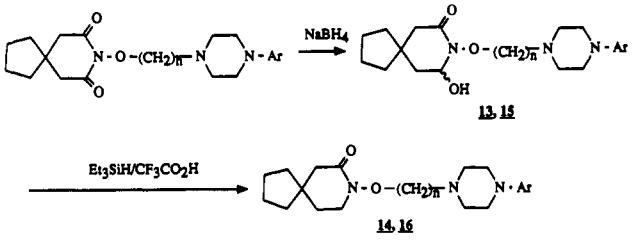
Chemistry

The target compounds reported here were synthesized according to the routes outlined below. The azaspiro[4.5]decane-7,9-dione derivatives were prepared via treatment of 8-oxaspiro[4.5]decane-7,9-dione with hydroxylamine hydrochloride and pyridine to provide 8-hydroxy-8-azaspiro[4.5]decane-7,9-dione. This compound

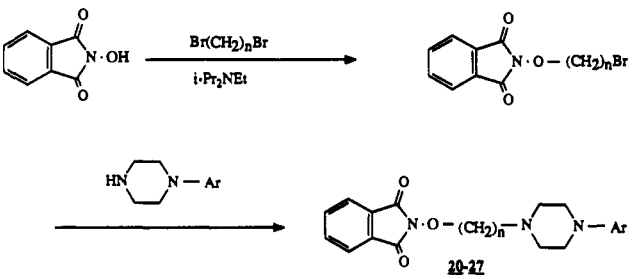
Scheme I



Scheme II



Scheme III



(1) Presented in part in poster form at the 197th American Chemical Society Meeting, Dallas, Tx, April 1989; Abstract MEDI 37.
(2) Carlsson, A. *Am. J. Psychiatry* **1978**, *135*, 164.
(3) Hollister, L. E. *Drug Dev. Res.* **1986**, *9*, 9.
(4) Tarsy, D.; Baldessarini, R. J. *Ann. Rev. Med.* **1984**, 35.

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