nM) alone or in the presence of 1 or 2 pM unlabeled TCDQ. Incubations were allowed to achieve equilibrium at 25 °C for 2 h, and bound and free hormone were separated by gel filtration as described above. Results are the mean value of two experimental determinations. Linear regression analysis gave correlation coefficients of >0.9 for all lines.

In sucrose gradient experiments, 100 μg of perfused rat liver nuclear extract protein, various concentrations of competitors in 10 μL of Me_2SO, and 0.6 nM [^{125}I]L-T_4, all in a final volume of 0.5 mL of buffer B, were incubated for 2 h at 25 °C. [^{125}I]L-T_4 bound to protein was separated from free ligand by gel filtration on Sephadex G-25 columns (bed volume of 2.0 mL, equilibrated in buffer B at 4 °C) as follows: a 0.4-mL aliquot of the incubation mixture cooled to 4 °C was applied to the column. Elution with 1.4 mL of buffer B gave the bound fraction (termed "labeled nuclear extract"). Free iodide was eluted with an additional 2.6 mL of buffer. Free hormone remains tightly bound to the gel and does not elute with volumes used. One milliliter of the labeled nuclear extract was layered onto a linear 10–40% (w/v) sucrose gradient made by allowing 0.6 mL each of 40, 30, 20, and 10% sucrose to diffuse at room temperature for 2 h. The gradients were spun for 17 h at 250000g in a SW 60_{Ti} rotor. Fractions (3 drops ~ 110 μL) were collected by puncturing the bottom of the tubes.

For Scatchard analysis, groups of five rats were administered TCDD at doses of 0, 12, 25, 37, or 50 μg/kg body weight. Each animal was given TCDD in corn oil (0.1 mL/kg body wt) by gavage and housed and maintained according to standard procedures. Control animals were dosed similarly with corn oil alone. The rats were sacrificed after 9 days, and the livers were frozen in liquid nitrogen and stored at −70 °C until use. The number of T_4 binding sites was estimated by Scatchard analysis. Rat livers were pooled for these analysis. Nuclear extracts (10 μg of nuclear protein) were incubated for 2 h at 25 °C with [^{125}I]T_4 (0.1–2.4 nM), and specific binding was determined as described above. The lines were determined by linear regression analysis and gave correlation coefficients >0.9. The intercept with the abscissa indicates the number of binding sites (nM/mg of protein), and the negative reciprocal of the slope provides an estimate of the equilibrium dissociation constant for the interaction of [^{125}I]T_4 with the binding sites. Values shown represent the mean ± SE of duplicate determinations. Similar results were obtained in independent experiments with rats dosed at 37 μg/kg and 25 and 50 μg/kg TCDD vs. controls. The Scatchard plot presented in Figure 1 is from control rats that were not treated with corn oil.

Calculations. A single binding site model was used to determine the $K_{A(T_4)}$ and $K_{A(PCB)}$ by nonlinear least squares for prealbumin binding.[52] For this purpose we used the SAS program DUD (*D*oesn't *U*se *D*erivatives) adapted for the VAX-11/780. A similar binding model was used to determine the $K_{A(PCB)}$ for nuclear receptor binding. In this case, since the total protein concentration is not known, it was necessary to fix the $K_{A(T_4)}$ at 0.142×10^{10} M^{-1} as determined independently (see Table II) by Scatchard analysis. The following equation for the fraction of the receptor bound (without and with PCB competitor, f_1 and f_2, respectively) was derived:[53]

$$\frac{f_2}{f_1} = \frac{[T_4]_2}{[T_4]_1} \frac{1 + [T_4]_1 K_{A(T_4)}}{1 + [T_4]_2 K_{A(T_4)} + [PCB] K_{A(PCB)}}$$

$K_{A(PCB)}$ was similarly calculated by nonlinear least-squares analysis.

The molecular modeling program MODEL 1.3 kindly provided to UNC—CH by W. Clark Still (Columbia University) was used to compute molecular surface areas and for molecular comparisons of the TCDD and TCDQ structures (Chart III). This program was modified in our laboratory to utilize a Tektronix 4107 color display terminal with graphics tablet input and the MM2p force field. In our hands,[54] MM2p has given energy-minimized structures for π-systems in excellent agreement with X-ray. MM2p was used to derive the starting geometry for TCDQ using standard parameters,[14] and the X-ray structure of TCDD was used.[9] The TCDD structure shown in Chart I (top) is from MM2p optimization and shows some deviation ($\phi_{min} \cong 142°$) from planarity. The method used to calculate their molecular polarizabilities has been previously reported.[40]

Acknowledgment. We thank Dr. P. Albro for helpful comments and suggestions and Dr. T. Darden for assistance with the molecular modeling work.

Registry No. L-T_4, 51-48-9; L-T_3, 6893-02-3; L-rT_3, 5817-39-0; DL-BpT_4, 100837-36-3; TIP, 609-23-4; BP, 92-52-4; 26TCB, 15968-05-5; 352DHB, 5335-24-0; 345HCB, 32774-16-6; 35TCB, 33284-52-5; 354DHB, 1137-59-3; TCDB-2, 100702-98-5; TCDB-1, 13049-13-3; TCDQ, 27728-29-6; TCDD, 1746-01-6.

(52) Pedersen, L. G.; Darden, T.; Oatley, S. J.; McKinney, J. D. *J. Med. Chem.* **1986**, *29*, 2451.
(53) Levitzki, A. *Receptor. A Quantitative Approach*; Benjamin-Cummings: Menlo Park, CA, 1984; p 24.
(54) Darden, T.; McKinney, J. D.; Gottschalk, K.; Maynard, A. T.; Pedersen, L. G. *J. Am. Chem. Soc.* **1986**, *108*, 207.
(55) Williams, D. A. *Biometrics* **1971**, *27*, 103.

5-Fluoro- and 8-Fluorotrimetoquinol: Selective β_2-Adrenoceptor Agonists

Michael T. Clark, Adeboye Adejare, Gamal Shams, Dennis R. Feller, and Duane D. Miller*

Divisions of Medicinal Chemistry and Pharmacognosy and of Pharmacology, College of Pharmacy, The Ohio State University, Columbus, Ohio 43210. *Received April 10, 1986*

The 5-fluoro and 8-fluoro analogues of trimetoquinol, TMQ, have been synthesized and evaluated for β_2- and β_1-adrenoceptor activity in guinea pig trachea and atria, respectively. The fluoro analogues of TMQ maintained potent β_2-adrenoceptor agonist activity but had reduced β_1-adrenoceptor agonist activity. The changes in β_1-activity of these compounds were correlated to differences in phenolic pK_a's. The β_1- and β_2-adrenoceptor actions of **2** and **3** were blocked in a competitive manner by propranolol. The enhanced β_2/β_1 selectivity for the analogues was found to be 8-fluoro analogue **3** > 5-fluoro analogue **2** > trimetoquinol (**1**).

Kirk et al.[1] have reported that fluorine substitution at the 2-position of norepinephrine produces a selective β-adrenergic agonist, while the 6-fluoro analogue of norepinephrine provides a selective α-adrenergic agonist. Several explanations have been presented for the selectivity conferred by the fluorine substitution on norepinephrine. One explanation suggests[2,3] that hydrogen-bond formation between the benzylic hydroxy group and the aromatic fluorine atom at the 2- or 6-position provides

(1) Kirk, K. L.; Cantacuzene, D.; Nimitkitpaisan, Y.; McCulloh, D.; Padgett, W. L.; Daly, J. W.; Creveling, C. R. *J. Med. Chem.* **1979**, *22*, 1493.
(2) Goldberg, L. I.; Kohli, J. D.; Cantacuzene, D.; Kirk, K. L.; Creveling, C. R. *J. Pharmacol. Exp. Ther.* **1980**, *213*, 509.
(3) Cantacuzene, D.; Kirk, K. L.; McCulloh, D. H.; Creveling, C. R. *Science (Washington, D.C.)* **1979**, *204*, 1217.

0022-2623/87/1830-0086$01.50/0

Table I. Comparison of Trimetoquinol (TMQ) and Fluorinated Analogues on β_2- and β_1-Adrenoceptors: Effect of Propranolol[a]

	trachea (β_2)[b]				atria (β_1)[b]				selectivity ratio[e]
compd	$pD_2 \pm$ SEM	IAR ± SEM[c]	$pK_B \pm$ SEM	potency ratio[d]	$pD_2 \pm$ SEM	IAR ± SEM[c]	$pK_B \pm$ SEM	potency ratio[d]	(β_2/β_1)
TMQ (1)	7.24 ± 0.02 (5)	0.95 ± 0.01		1.00	7.52 ± 0.21 (5)	1.00 ± 0.0		1.00	1.0
5-FTMQ (2)	7.26 ± 0.11 (5)	0.95 ± 0.01		1.05	6.95 ± 0.13 (6)	0.96 ± 0.04		0.27	3.88
5-FTMQ plus 3×10^{-8} M propranolol	6.38 ± 0.11 (4)	0.98 ± 0.01	8.69 ± 0.13		5.95 ± 0.04 (4)	1.00 ± 0.0	8.48 ± 0.04		
8-FTMQ (3)	7.15 ± 0.14 (5)	0.86 ± 0.02		0.81	6.53 ± 0.11 (5)	0.74 ± 0.05		0.102	7.94
8-FTMQ plus 3×10^{-8} M propranolol	5.38 ± 0.03 (4)	0.91 ± 0.04	8.83 ± 0.01		5.55 ± 0.10 (4)	0.66 ± 0.06	8.45 ± 0.11		

[a] Drug concentrations used varied from 10^{-9} to 3×10^{-5} M. Concentration of propranolol was 3×10^{-8} M. [b] Values are the mean ± SEM of N = 4–6 (numbers given in parentheses). [c] IAR = intrinsic activity ratio = ratio of maximal drug effect to maximal response of TMQ. [d] Potency ratio = EC_{50} (trimetoquinol)/EC_{50} (drug). [e] Selectivity ratio = potency ratio (β_2)/potency ratio (β_1) for each drug.

Table II. Ultraviolet Spectral and pK_a Data for TMQ, 5-FTMQ, and 8-FTMQ[a]

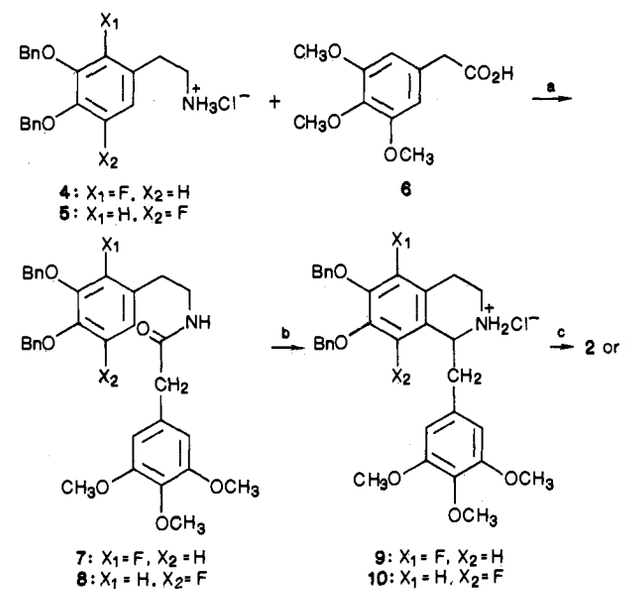
	λ_{max} (ϵ)		
compd	solvent A[b]	solvent B[c]	pK_a
TMQ	280 (12000)	298 (15000)	8.77 ± 0.15
5-FTMQ	275 (6700)	285 (10000)	8.11 ± 0.15
8-FTMQ	275 (7600)	287 (12000)	7.86 ± 0.15

[a] Spectra were measured on a Beckman DU-40 spectrophotometer. [b] Solvent A, 0.1 M HCl. [c] Solvent B, 0.1 M tris–(hydroxymethyl)aminomethane.

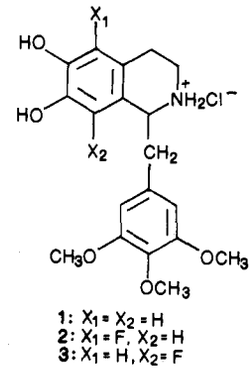
different conformations of the side chain relative to the aromatic ring and, thus, each drug shows a preference for binding to either the α- or β-adrenergic receptor. Recently, the specificity of 2- and 6-fluoronorepinephrine for β- and α-adrenergic receptors has been postulated to arise from a conformational bias induced by electrostatic repulsion between the aromatic fluorine at either the 2- or 6-position and the side-chain β-hydroxyl group.[4] This latter hypothesis was used in the design of some new selective α-adrenergic agonists. It is interesting to note that the corresponding 2-fluoro- and 6-fluorodopamine analogues do not show selectivity for adrenergic receptors.[2]

Trimetoquinol (1) has been reported[5,6] to be a potent β-adrenergic agonist. The S (−)-isomer of 1 is marketed in Japan as a bronchodilator for the treatment of asthma. We have employed a variety of structural modifications of 1 in an effort to obtain potent selective adrenergic stimulants.[7] Since no information was available as to the precise nature of the effect of fluorine substitution on 1, we felt that substitution of fluorine on the catechol segment of 1 would provide valuable insight as to the possibility of separating β_2- from β_1-adrenergic activity. We now report the syntheses of the 5-fluoro and 8-fluoro analogues of trimetoquinol, 2 (5-FTMQ) and 3 (8-FTMQ), and their biological actions on β_2- and β_1-adrenergic receptors (trachea and atria, respectively). Compounds related to 1 possessing selective and potent β_2-adrenergic agonist activity while having minimum β_1-adrenergic agonist activity should be of considerable therapeutic interest and value.

Scheme I[a]



[a] (a) $PhCH_3$, Δ; (b) (1) $POCl_3$, (2) $NaBH_4$, (3) HCl; (c) H_2, Pd/C.



Chemistry

Scheme I outlines the synthesis of 2 and 3. The pathway begins with the condensation of the appropriately substituted phenethylamine, 4 or 5, with trimethoxyphenylacetic acid (6) to afford the phenethylacetamide 7 or 8, respectively. The amides were cyclized under Bischler–Napieralski conditions[7] with use of $POCl_3$ in toluene to give the intermediate dihydroisoquinolines. Without isolation, the dihydroisoquinolines were reduced with $NaBH_4$ to give

(4) DeBernardis, J. F.; Kerkman, D. J.; Winn, M.; Bush, E. N.; Arendsen, D. L.; McClellan, W. J.; Kyncl, J. J.; Basha, F. Z. *J. Med. Chem.* **1985**, *28*, 1398.
(5) Yamato, E.; Hirakura, M.; Sugasawa, S. *Tetrahedron, Suppl.* **1966**, *8* (Part 1), 129.
(6) Iwasawa, Y.; Ohashi, M.; Yamamura, S.; Saito, S.; Kiyomoto, A. *Jpn. J. Pharmacol.* **1976**, *26*, 133.
(7) Sober, D. J.; Chang, J.; Fowble, J. W.; Mukhopadhyay, A.; Feller, D. R.; Miller D. D. *J. Med. Chem.* **1981**, *24*, 970 and references cited therein.

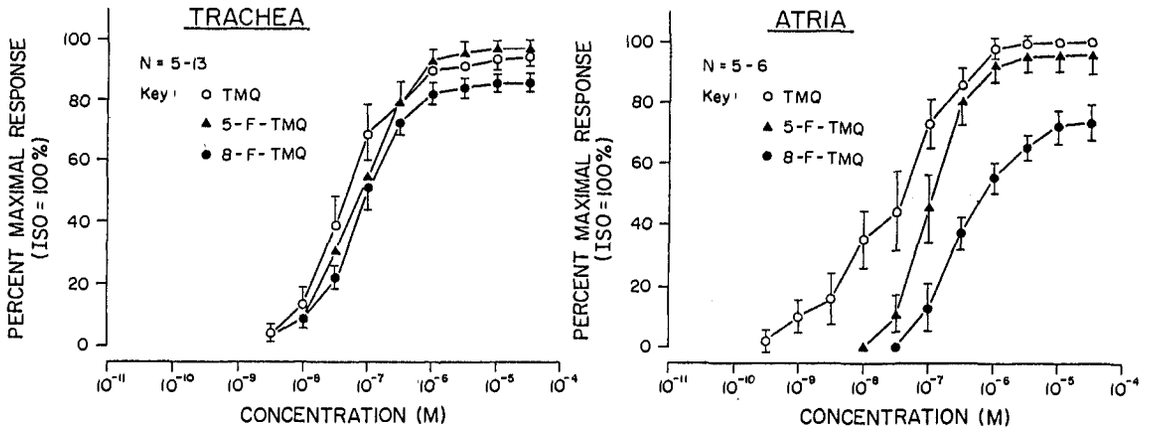
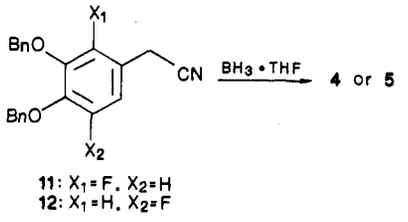


Figure 1. Tracheal relaxant (left panel) and chronotropic (right panel). Effects of trimetoquinol (TMQ, 1) and the 5-fluoro (2) and 8-fluoro (3) analogues. Key: 1, ○; 2, ▲; and 3, ●. Data are expressed as the mean response ± SEM (n = 5–13).

Scheme II

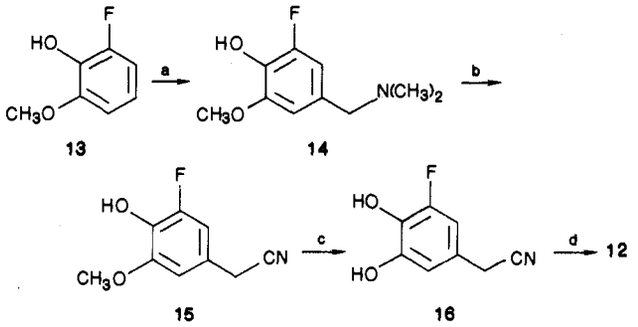


the tetrahydroisoquinoline **9** or **10**. The structure of **9** or **10** was assigned on the basis of ^{1}H NMR (Table II). The hydrochloride salt of **9** or **10** was prepared and subjected to hydrogenolysis, to give rise to the catechol **2** or **3**, respectively.

Of importance in the synthetic pathway was the availability of the previously unreported phenethylamines **4** and **5**. It was envisioned that **4** and **5** could be synthesized from benzyl cyanides **11** and **12** via reduction (Scheme II). The synthesis of nitrile **11** has been previously reported from our laboratory.[8]

The synthesis of **12** is outlined in Scheme III and utilized 2-fluoro-6-methoxyphenol (**13**).[9] It was anticipated that **13** could be converted into **14** if the phenol moiety was left unprotected. The Mannich reaction is known to occur on phenols, and the reaction usually gives both ortho and para products.[10] However, both ortho positions in **13** are substituted, so it was anticipated that only the para product would form. Under aminomethylation conditions with formaldehyde and *N,N*-dimethylamine, **13** was converted into *N,N*-dimethyl-4-hydroxy-3-methoxy-5-fluorobenzylamine (**14**). This was the sole product isolated from the reaction and provides a convenient method to substitute **13** in the para position. The benzylamine **14** was readily converted to benzyl nitrile **15** by treatment of **14** with methyl iodide and displacement with NaCN.[11] Next, a functional-group shuffle was carried out by the treatment of **15** with BBr_3 followed by dibenzylation of the catechol **16** to give **12**.[1,8] Initially, demethylation of **15** with BBr_3 gave a low yield of **16**. We determined that the low yield was due to the method of workup. Rather than the use of methanol to break down the excess BBr_3, we found that removal of the excess BBr_3 by evaporation yielded residue, which was dissolved in EtOAc and washed with brine to

Scheme III[a]



[a] (a) 37% CH_2O, 40% $(CH_3)_2NH$; (b) (1) CH_3I, (2) NaCN, (c) BBr_3; (d) $PhCH_2Cl$, K_2CO_3, KI.

give the catechol **16**. The catechol was dibenzylated with benzyl chloride to afford a good overall yield of **12**.

Either benzyl cyanide **11** or **12** was converted into phenethylamine **4** or **5** by reduction with diborane.[12] Thus, we have developed a convenient route to **4** or **5**, from a common synthetic intermediate, 2-fluoro-6-methoxyphenol (**13**).[9]

Biological Results and Discussion

The concentration-dependent effects of trimetoquinol (**1**) and the 5-fluoro (**2**) and 8-fluoro (**3**) analogues were evaluated with use of guinea pig right atria and tracheal strips as representative β_1- and β_2-adrenergic systems, respectively (Figure 1). Each compound was nearly equally active as an agonist on tracheal relaxation. In contrast, the rank order of stimulatory potency for these compounds in atria was **1** > **2** > **3**. Whereas **1** and **2** gave similar maximal effects in both of these β-adrenoceptor tissues, analogue **3** was found to be a partial stimulant in atria.

Additional experiments were undertaken in the presence of propranolol to determine whether the fluoro analogues produced their stimulatory effects by activation of β-adrenoceptors. The concentration–response curves of analogues **2** and **3** were shifted to the right in a parallel fashion (data not presented), and the experimentally calculated pK_B values of propranolol against each compound were nearly identical in atria and trachea (Table I). These concentration ratio shifts in the presence of propranolol are similar to that seen with trimetoquinol in these same β-adrenoceptor systems.[13]

(8) Adejare, A.; Miller, D. D. *J. Chem. Res., Synop.* **1986**, 14–15.
(9) Ladd, D. L.; Weinstock, J. *J. Org. Chem.* **1981**, *46*, 203.
(10) Mannich, C.; Krosche, W. *Arch. Pharm. (Weinheim, Ger.)* **1912**, *250*, 647.
(11) Simmons, J.; Borchardt, R. J. *J. Labelled Compd Radiopharm.* **1983**, *20*, 325.
(12) Kador, P. F.; Venkatraman, R.; Feller, D. R. Miller, D. D. *J. Med. Chem.* **1977**, *20*, 891.

Data on the potency ratio for the fluorine analogues relative to 1 are given for each tissue system (Table I) and show that the potencies of analogue 2 and 3 on β_1-adrenoceptors are reduced by about four- and eightfold, respectively. In contrast to 1, our results also show that each fluorinated analogue was more potent as an agonist in the β_2-adrenoceptor as compared to β_1-adrenoceptor tissue. Accordingly, the β_2/β_1 selectivity ratios of 2 and 3 were four- and eightfold greater, respectively, than that of the parent drug (1) and the rank order of β_2/β_1 selectivity was 3 > 2 > 1. It should also be noted that trimetoquinol (1) is more potent than isoproterenol as a β_2-adrenoceptor stimulant,[14] and others have classified 1 as a β_2-selective agonist.[13,15]

Our results clearly demonstrate that the substitution of a fluorine atom at either the 5- or 8-position of the tetrahydroisoquinoline nucleus does not produce any major change in the stimulatory activity of the parent drug (1) on β_2-adrenoceptors. However, a progressive reduction in the activation of β_1-adrenoceptors was seen with 5-fluoro and 8-fluoro substitution, respectively. Therefore, these stuides show that a reduced selectivity for β_1-adrenoceptors occurs by substitution of a fluorine atom for a hydrogen on the catechol ring system of 1.

Structural requirements for optimal β-adrenoceptor activity of trimetoquinol (1) include the catechol moiety, amino nitrogen, and 1-benzyl substituent.[14] Previous work[1-4] has suggested that the changes in α- and β-adrenoceptor activities of 2- or 6-fluorine-substituted norepinephrine were due to an interaction between the aromatic ring and the side chain containing the β-hydroxy group. From our studies, fluorine addition to 1 could not be expected to produce a dramatic effect on the conformation since the phenethylamine segment is contained within the tetrahydroisoquinoline nucleus. Further, an interaction of fluorine atoms with the 1-benzyl substituent is unlikely since the steric bulk of fluorine is comparable to that of hydrogen. On the basis of the presence of fluorine atoms in the catechol moiety, we suggest that the electronic effects attributable to the fluorine atom may alter the acidity of adjacent phenolic groups. In this regard, the ionization of phenols is increased by the presence of fluorine atoms placed in adjacent positions (Table II). The greatest effect on reduction of β_1-adrenoceptor activity was found with the 8-fluoro analogue 3. This may suggest that the relative ionization of the tetrahydroisoquinolines plays an important role in the interaction of these molecules with β-adrenoceptors, and in particular β_1-adrenoceptor tissues. In support of this hypothesis, we have determined that 5-FTMQ (2) is 4 times more acidic than 1 and is also about 4 times less active on β_1-adrenoceptors. In agreement with this observation, we have noted that 8-FTMQ (3) is 8 times more acidic than 1 and is 8 times less active on β_1-adrenoceptors. Taken together, the reduced potency on the β_1-adrenoceptor tissues appears related to the relative degree of ionization of the tetrahydroisoquinolines.

In summary, our results demonstrate that the 5-fluoro (2) and 8-fluoro (3) analogues of 1 maintain potency for stimulation of β_2-adrenoceptors but not of β_1-adrenoceptors. We conclude that these changes in β_2/β_1 selectivity are due to the electronic influence of fluorine and its effect on ionization of the phenolic groups and the binding of the catechol segment of 1 to β_1- and β_2-adrenoceptors.

Experimental Section

Melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. Infrared data were collected on a Beckman 4230 spectrophotometer. The ^{1}H NMR spectra were recorded on a Bruker HX-90E or an IBM 270 spectrometer with tetramethylsilane as the internal standard. The mass spectra were obtained at The Ohio State University Chemical Instrument Center, by use of a Kratos MS-30 mass spectrometer. Chemical analyses were determined by Galbraith Laboratories, Inc., Knoxville, TN, and all were found to be within ±0.4% of theoretical values. TLC was performed on silica gel 60 F precoated aluminum-backed plates from EM Reagents. Column chromatography was performed on silica gel 60, 70–230 mesh, from EM Reagents. Flash chromatography was performed on flash silica gel 60, 40–240 mesh, from EM Reagents. All reagents were dried prior to use.

***N,N*-Dimethyl-3-methoxy-4-hydroxy-5-fluorobenzylamine** (14). 2-Fluoro-6-methoxyphenol[9] (10 g, 76 mmol) was added to a solution of 40% aqueous dimethylamine (24 g) and 37% aqueous formaldehyde (9 mL) in absolute EtOH (20 mL). The mixture was heated at reflux for 2 h, cooled, and concentrated under reduced pressure to give a solid, which was crystallized from Et_2O to yield 13.5 g (95%) of 14 as colorless needles: mp 140–142 °C; IR (KBr) 3400 cm^{-1}; ^{1}H NMR δ 6.71–6.58 (m, 2 H, Ar H), 3.83 (s, 3 H, Ar OCH_3), 3.33 (s, 2 H, Ar CH_2N), 2.23 (s, 6 H, $N(CH_3)_2$). Anal. ($C_{10}H_{14}FNO_2$) C, H, N.

3-Methoxy-4-hydroxy-5-fluorobenzyl Cyanide (15). Iodomethane (12 mL) was added to a solution of *N,N*-dimethyl-3-methoxy-4-hydroxy-5-fluorobenzylamine (14, 5 g, 25 mmol) in $CHCl_3$ (100 mL). The mixture was stirred for 18 h at 25 °C. The precipitate that formed was collected to give 8.9 g of a white solid. Without further purification, the white solid was dissolved in Me_2SO (50 mL), and NaCN (2.25 g, 46 mmol) was added. The mixture was stirred for 7 h at 25 °C. The mixture was added to H_2O (25 mL) and acidified with 6 N HCl. The solution was extracted with EtOAc (3 × 50 mL), and the EtOAc extracts were washed with brine (2 × 150 mL) and H_2O (2 × 150 mL), dried with anhydrous $MgSO_4$, and concentrated under reduced pressure to give a solid. The solid was crystallized from EtOAc/hexanes to give 2.42 g (58%) of 15 as light yellow needles: mp 70–80 °C; IR (KBr) 3200, 2400 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.76–6.64 (m, 2 H, Ar H), 5.43 (br, 1 H, Ar OH), 3.92 (s, 3 H, Ar OCH_3), 3.66 (s, 2 H, Ar CH_2CN). Anal. ($C_9H_8FNO_2$) C, H, N.

3,4-Dihydroxy-5-fluorobenzyl Cyanide (16). Boron tribromide (1.03 mL, 11 mmol) was added dropwise to a cool (0 °C) solution of 3-methoxy-4-hydroxy-5-fluorobenzyl cyanide (15, 1 g, 5.5 mmol) in CH_2Cl_2 (10 mL). The mixture was warmed to 25 °C and stirred at that temperature for 18 h. The mixture was concentrated under reduced pressure to give a solid. The solid was dissolved in EtOAc (50 mL), washed with brine (3 × 50 mL) and H_2O (3 × 5 mL), dried ($MgSO_4$), and concentrated under reduced pressure to give a solid. The solid was crystallized from EtOAc/Et_2O to give 900 mg (90.1%) of 16 as a white solid: mp 100–101 °C; IR (KBr) 3400, 2400 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.60–6.646 (m, 2 H, Ar N), 3.72 (s, 2 H, Ar CH_2CN). Anal. ($C_8H_6FNO_2$) C, H, N.

3,4-Bis(benzyloxy)-5-fluorobenzyl Cyanide (12). Benzyl chloride (0.54 mL, 4.5 mmol) was added to a solution of 3,4-dihydroxy-5-fluorobenzyl cyanide (330 mg, 2 mmol), K_2CO_3 (620 mg, 4.5 mmol), and KI (50 mg) in acetone (10 mL). The mixture was heated at reflux for 4 h, cooled, and concentrated under reduced pressure. The residue was dissolved in H_2O (10 mL) and EtOAc (10 mL). The organic layer was washed with brine (2 × 10 mL) and H_2O (2 × 10 mL), dried with anhydrous $MgSO_4$, and concentrated under reduced pressure to an oil. The oil was purified by flash chromatography (20% EtOAc/hexane) to give an oil, which solidified on standing. The solid was crystallized from CH_2Cl_2/hexanes to give 555 mg (80%) of 12 as white needles: mp 120–121 °C; IR (KBr) 2400 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.47–7.25 (m, 10 H, Ar H), 6.76–6.61 (m, 2 H, Ar H), 5.11 (s, 4 H, Ar CH_2), 3.63 (s, 2 H, Ar CH_2CN). Anal. ($C_{22}H_{18}FNO_2$) C, H, N.

General Synthesis of Phenethylamines 4 and 5. To a cold solution (0 °C) of the benzyl cyanide 11 or 12 (2 g, 5.8 mmol) in

(13) Mukhopadhyay, A.; Sober, D. J.; Chang, J.; Slenn, R. T.; Amin, H. M.; Miller, D. D.; Feller, D. R. *Eur. J. Pharmacol.* **1982**, *77*, 209.
(14) Iwasawa, Y.; Kiyomoto, A. *Jpn. J. Pharmacol.* **1967**, *17*, 143.
(15) Farmer, J. B.; Kennedy, I.; Levy, G. P.; Marshall, R. J. *J. Pharm. Pharmacol.* **1970**, *22*, 61.

dry THF (30 mL) was added dropwise a 1 M BH_3·THF solution (100 mL, 100 mmol). The mixture was heated at reflux for 18 h and cooled to 0 °C, and MeOH (15 mL) was added cautiously. The mixture was concentrated under reduced pressure to an oil. The oil was dissolved in MeOH (15 mL) and reconcentrated under reduced pressure (this was repeated two more times) to give an oil. The oil was dissolved in Et_2O (30 mL), washed with 10% $NaHCO_3$ (3 × 30 mL) and H_2O (3 × 30 mL), dried with anhydrous $MgSO_4$, and concentrated under reduced pressure to give an oil. The oil was dissolved in MeOH (20 mL), and HCl gas was added to the solution. Diethyl ether was added until the solution became cloudy and a solid crystallized from the solution. The solid was collected to give **4** or **5** as the hydrochloride salt.

Compound **4**·HCl (85%): mp 111–113 °C; ^{1}H NMR ($CDCl_3$) δ 7.43–7.25 (m, 10 H, Ar H), 6.90–6.60 (m, 2 H, Ar H), 5.10 (s, 4 H, Ar CH_2O), 2.90–2.60 (m, 4 H, CH_2), 1.26 (s, 2 H, NH_2). Anal. ($C_{22}H_{23}ClFNO_2$) C, H, N.

Compound **5**·HCl (90%): mp 128–130 °C; ^{1}H NMR ($CDCl_3$) δ 7.44–7.25 (m, 10 H, Ar H), 6.53–6.39 (m, 2 H, Ar H), 5.05 (s, 2 H, Ar CH_2O), 5.03 (s, 2 H, Ar CH_2O), 3.2–2.9 (m, 4 H, CH_2). Anal. ($C_{22}H_{23}ClFNO_2 \cdot {}^1/_2H_2O$) C, H, N.

General Synthesis of Phenylacetamides 7 and 8. The hydrochloride salt of **4** or **5** (2.2 g, 5.6 mmol) was dissolved in CH_2Cl_2 (50 mL), washed with a 10% $NaHCO_3$ solution (3 × 5 mL) and H_2O (3 × 50 mL), dried with anhydrous $MgSO_4$, and concentrated under reduced pressure to give the free base as an oil. The oil was dissolved in toluene (50 mL), and 3,4,5-trimethoxyphenylacetic acid (1.29 g, 5.6 mmol) was added. The mixture was heated at reflux for 72 h with removal of H_2O via a Dean–Stark trap. The mixture was cooled and concentrated under reduced pressure to give a solid. The solid was dissolved in CH_2Cl_2 (50 mL), washed with H_2O (50 mL), 10% HCl (2 × 50 mL), H_2O (50 mL), 10% $NaHCO_3$ (2 × 50 mL), and H_2O (2 × 50 mL), dried with anhydrous $MgSO_4$, and concentrated under reduced pressure to give a solid. The solid was crystallized from EtOAc to give **7** or **8** as a white solid.

Compound **7** (75%): mp 109–111 °C; ^{1}H NMR ($CDCl_3$) δ 7.54–7.29 (m, 10 H, Ar H), 6.62–6.57 (m, 2 H, Ar H), 6.39 (s, 2 H, Ar H), 5.54–5.35 (br, 1 H, NH), 5.10–5.07 (s, 4 H, 2 × Ar CH_2O), 3.86 (s, 3 H, Ar OCH_3), 3.80 (s, 6 H, 2 × Ar OCH_3), 3.56–3.32 (m, 4 H, Ar CH_2C), 2.79–2.64 (t, 2 H, CH_2N). Anal. ($C_{33}H_{34}FNO_6$) C, H, N.

Compound **8** (75%): mp 94–95 °C; ^{1}H NMR ($CDCl_3$) δ 7.44–7.24 (m, 10 H, Ar H), 6.53–6.39 (m, 4 H, Ar H), 5.45 (br, 1 H, NH), 5.06 (s, 4 H, 2 × Ar CH_2), 3.83 (s, 9 H, Ar OCH_3), 3.55 (s, 2 H, $COCH_2$ Ar), 3.44 (m, 2 H, Ar CH_2C), 2.65 (t, 2 H, CH_2N). Anal. ($C_{33}H_{34}FNO_6$) C, H, N.

General Synthesis of Protected 1,2,3,4-Tetrahydroisoquinolines 9 and 10. Phosphorus oxychloride (0.83 mL, 9 mmol) was added to the phenylacetamide **7** or **8** (2 g, 3.6 mmol) in $PhCH_3$ (24 mL). The mixture was heated at 80 °C for 5 h under Ar atmosphere. The mixture was cooled and concentrated under reduced pressure to give an oil. The oil was dissolved in EtOH (100 mL) and cooled to 0 °C whereupon $NaBH_4$ (4.24 g, 11.2 mmol) was added. The mixture was stirred for 18 h at 25 °C and concentrated under reduced pressure to a solid. The solid was dissolved in H_2O (50 mL), and 10% NaOH (10 mL) was added. The mixture was extracted with Et_2O (2 × 50 mL), and the organic layer was washed with H_2O (2 × 5 mL), dried with anhydrous $MgSO_4$, and concentrated to an oil. The oil was purified by flash chromatography (5% MeOH/CH_2Cl_2 to give a clear oil that was converted to HCl salt **9** or **10**.

Compound **9** (25%): mp 125–127 °C; ^{1}H NMR ($CDCl_3$) δ 7.43–7.26 (m, 10 H, Ar H), 6.39 (s, 2 H, Ar H), 6.11 (d, 1 H, Ar H, J_{HF} = 1.3 Hz), 5.07 (s, 2 H, Ar CH_2), 4.79 (q, 2 H, Ar CH_2), 4.69 (m, 1 H, Ar CHNH), 3.81 (s, 3 H, Ar OCH_3), 3.75 (s, 6 H, Ar OCH_3), 3.52–2.98 (m, 6 H, CH_2). Anal. ($C_{33}H_{35}ClFNO_5$) C, H, N.

Compound **10** (65%): mp 179–181 °C; ^{1}H NMR ($CDCl_3$) δ 7.48–7.26 (m, 10 H, Ar H), 6.84 (d, 1 H, Ar H), 6.51 (s, 2 H, Ar H), 5.16 (s, 2 H, Ar CH_2), 5.02 (s, 2 H, Ar CH_2), 4.95 (m, 1 H, Ar CHNH), 3.78 (s, 6 H, Ar OCH_3), 3.72 (s, 3 H, Ar OCH_3), 3.56–3.05 (m, 6 H, CH_2). Anal. ($C_{33}H_{35}ClFNO_5 \cdot 3H_2O$) C, H, N.

General Synthesis of 1,2,3,4-Tetrahydroisoquinolines 2 and 3. The protected 1,2,3,4-tetrahydroisoquinoline **9** or **10** was dissolved in Et_2O, and HCl gas was added to give the hydrochloride salt of **9** or **10**. The hydrochloride salt was dissolved in EtOH and added to a suspension of 10% Pd/C in EtOH. The mixture was hydrogenated for 8 h at 45 psi at 25 °C. The mixture was filtered and concentrated under reduced pressure to give an oil, which solidified upon standing. The oil was crystallized from MeOH/Et_2O or MeOH/CH_2Cl_2 to give **2** or **3** as a white solid.

Compound **2** (65%): mp 165–169 °C; ^{1}H NMR (CD_3OD) δ 6.63 (s, 2 H, Ar H), 6.50 (d, 1 H, Ar H, J_{HF} = 0.9 Hz), 3.83 (s, 6 H, Ar OCH_3), 3.76 (s, 3 H, Ar OCH_3), 3.60–2.90 (m, 7 H, 3 × CH_2, CH). Anal. ($C_{19}H_{23}ClFNO_5 \cdot 2{}^1/_2H_2O$) C, H, N.

Compound **3** (65%): mp 219–221 °C; ^{1}H NMR (CD_3OD) δ 6.56 (s, 2 H, Ar H), 6.51 (d, 1 H, Ar H, J_{HF} = 0.8 Hz), 3.81 (s, 6 H, Ar OCH_3), 3.75 (s, 3 H, Ar OCH_3), 3.40–2.93 (m, 7 H, CH_2CH). Anal. ($C_{19}H_{23}ClFNO_5 \cdot {}^1/_2H_2O$) C, H, N.

Biological Studies. β-Adrenergic Studies. Male albino Hartley guinea pigs (400–600 g) were employed in all experiments. The isolation and procedures for testing of each compound (10^{-9} to 3×10^{-5} M) with isolated guinea pig atria and trachea were identical with those described by Sober et al.[7] Each drug concentration was added only after the effects of the previous concentration reached a maximum and remained constant. The final maximum concentration of the testing compound did not increase the effect. Responses for agonists were expressed as pD_2 (−log EC_{50}) values and were calculated directly from graphical plots of % maximal response vs. log molar drug concentration. Other experiments were done in the presence of propranolol (3×10^{-8} M), and the pK_B value of the antagonist was determined by using the equation pK_B = −log ([I]/CR-1), where CR = concentration ratio = EC_{50} of drug (presence of propranolol)/EC_{50} of drug (absence of propranolol) and [I] = molar concentration of propranolol, 3×10^{-8} M.

pK_a Determinations. Phenol acidities were determined spectrophotometrically, as described by Albert and Serjeant,[16] by measuring the absorption as a function of pH in tris(hydroxymethyl)aminomethane buffer. The spectral data of the neutral and ionized species are given in Table II. Because the catechols tend to oxidize in basic media, the buffers were degassed and flushed with argon prior to use.

Acknowledgment. We thank the National Institutes of Health for their support in the form of Grant HL-22533. G.S. is grateful for his financial support through Amideast.

Registry No. **2**, 104716-86-1; **2** (free base), 104716-88-3; **3**, 104716-87-2; **3** (free base), 104716-89-4; **4**, 103138-29-0; **4** (free base), 103138-28-9; **5**, 104716-78-1; **5** (free base), 104716-81-6; **7**, 104716-79-2; **8**, 104716-80-5; **9**, 104716-84-9; **9** (free base), 104716-82-7; **10**, 104716-85-0; **10** (free base), 104716-83-8; **12**, 104716-77-0; **13**, 73943-41-6; **14**, 103905-49-3; **15**, 104716-74-7; **16**, 104716-76-9; 3-methoxy-4-hydroxy-5-fluorobenzyl iodide, 104716-75-8; (3,4,5-trimethoxyphenyl)acetic acid, 951-82-6.

(16) Albert, A.; Serjeant, E. P. *The Determination of Ionization Constants*, 3rd ed.; Chapman and Hall: London, 1984.