Synthesis and Dopamine Receptor Affinities of 2-(4-Fluoro-3-hydroxyphenyl)ethylamine and N-Substituted Derivatives

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The synthesis of 2-(4-fluoro-3-hydroxyphenyl)ethylamine (26) and of some N,N-dialkyl derivatives (27–30) starting from 4-fluoro-3-hydroxytoluene and their in vitro binding affinities for dopamine (DA) receptor are reported. The amine 26 can be regarded as a molecular modification of DA in which the para hydroxyl group is replaced by fluorine. The new compounds 26–30 were evaluated for their affinity at D-1 and D-2 DA receptor subtypes by displacement of [³H]SCH 23390 (D-1 selective) and [³H]spiperone (D-2 selective). The amine 26 had about 2-fold less affinity for D-1 and D-2 binding sites than DA. The substitution of the amino group with ethyl, n-propyl, and 2-phenylethyl groups decreased the affinity for D-1 binding sites but greatly enhanced the effectiveness on D-2 binding sites. The N-ethyl- (28) and N-n-propyl-N-(2-phenylethyl)-2-(4-fluoro-3-hydroxyphenyl)ethylamine (30) were the most potent members of the series with high selectivity for D-2 binding sites. A similar effect was observed with isomeric N-n-propyl-N-(2-phenylethyl)-2-(3-fluoro-4-hydroxyphenyl)ethylamine (31) which was approximately 65 times more selective for D-2 sites vs D-1 sites. The introduction of a 2-phenylethyl group on the nitrogen atom induce the highest effect, perhaps as a consequence of an increased liposolubility or of binding to a complementary lipophilic site on the receptor.

The potential clinical usefulness of centrally acting dopamine (DA) receptor agonists has stimulated intense research on new dopaminergic agents. DA is not suitable for therapeutic use due to the lack of selectivity on various subtypes of DA receptors as well as on norepinephrine receptors. Furthermore, DA does not cross the blood–brain barrier because of its polar nature, and it is a good substrate for catechol-O-methyltransferase (COMT) and monoamineoxidase (MAO). Structural modifications of the DA molecule to obtain novel chemical entities with agonist activity have concerned mainly the ethylamine chain and the amino group and only seldomly have involved the catechol moiety.[1-10]

Among the classes of compounds bearing the DA molecule embedded in a cyclic structure the 2-aminotetralins are widely studied.[1,2] Some halogenated mono- and dihydroxytetralins were recently prepared and evaluated for dopaminergic properties on D-1 and D-2 receptors.[11] Among these compounds the racemic 2-amino-6-fluoro-7-hydroxytetralin (1) was about 4-fold more potent than DA for D-2 binding sites, whereas it showed half the effectiveness of DA for D-1 binding sites.

By observing the molecular structure of tetralin 1 it may be supposed that the pharmacophore rigidly held in this compound is the moiety of the 2-(4-fluoro-3-hydroxyphenyl)ethylamine (26) which can be also regarded as a molecular modification of DA where the hydroxyl group at position 4 is replaced by a fluorine atom.
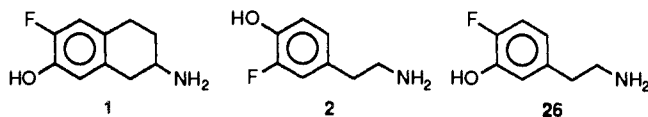
In a previous work[12] we described the synthesis of 2-(3-fluoro-4-hydroxyphenyl)ethylamine (2), and in vitro binding studies performed on the N,N-diethyl and N,N-di-n-propyl derivatives of 2 showed that these compounds have some degree of selectivity for the D-2 DA receptor.

In the present study we have synthesized the 2-(4-fluoro-3-hydroxyphenyl)ethylamine (26) and its N,N-dialkyl derivatives (27–30) (Table I), and we have evaluated

Scheme I[a]
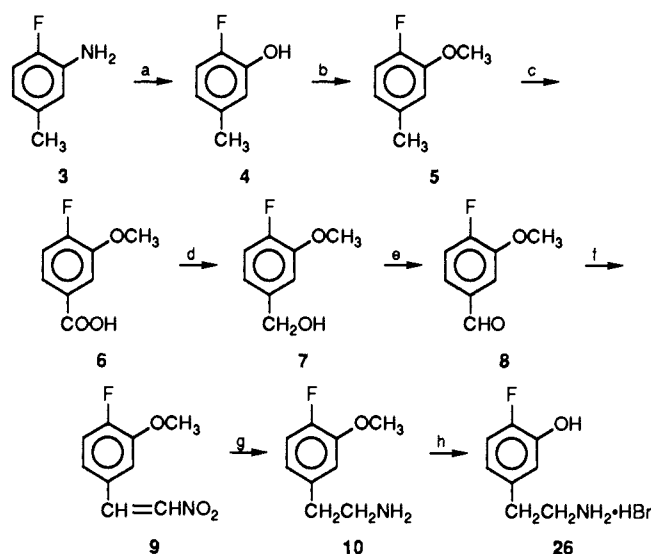
[a] Reagents: (a) H₂SO₄, NaNO₂, Δ, CuSO₄; (b) (CH₃)₂SO₄, K₂CO₃, acetone; (c) KMnO₄, pyridine, H₂O; (d) LiAlH₄; (e) pyrHCrO₃Cl; (f) CH₃NO₂; (g) LiAlH₄; (h) HBr.

the affinity of the new compounds for D-1 and D-2 subtypes of the DA receptor by using binding studies in order

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Table I. Inhibition of [^{3}H]SCH 23390 and [^{3}H]Spiperone Binding to Rat Striatal Membranes

drug	X	X$_1$	R	R$_1$	IC$_{50}$,^{a} μM [^{3}H]SCH 23390	IC$_{50}$,^{a} μM [^{3}H]spiperone	D-2/D-1 selectivity index^b
26	F	OH	H	H	6.15 ± 0.37	4.64 ± 0.12	1.3
27	F	OH	CH$_2$CH$_3$	n-C$_3$H$_7$	11.1 ± 0.46	1.97 ± 0.91	5.6
28	F	OH	CH$_2$CH$_3$	CH$_2$CH$_2$Ph	8.0 ± 0.93	0.23 ± 0.11	34.8
29	F	OH	n-C$_3$H$_7$	n-C$_3$H$_7$	11.4 ± 1.1	0.95 ± 0.31	12
30	F	OH	n-C$_3$H$_7$	CH$_2$CH$_2$Ph	10.9 ± 2.01	0.25 ± 0.05	43.6
31	OH	F	n-C$_3$H$_7$	CH$_2$CH$_2$Ph	38.0 ± 3.3	0.58 ± 0.15	65.5
dopamine					3.0 ± 0.45	2.2 ± 0.12	1.3
(R)-(–)-apomorphine					3.0 ± 0.27	0.07 ± 0.01	42.8

^{a}All values expressed as a mean IC$_{50}$ (concentration of agonist producing half-maximal inhibition of the binding) values ± SEM. ^{b}The index was obtained by division of the IC$_{50}$ for the D-1 receptor ([^{3}H]SCH 23390) by that for the D-2 receptor ([^{3}H]spiperone).

to assess whether one of the DA hydroxyl groups could be replaced by a fluorine atom. Compound **26** is not affected by COMT and retains the hydroxyl group in the position meta to the ethylamine side chain which is apparently sufficient for the activation of the DA D-2 receptor.[13] The replacement of a hydroxyl group of DA by a fluorine atom increases the liposolubility, and the strong electronegative influence of fluorine could alter both the physicochemical properties of the phenolic group and the affinity for DA receptors.

In the synthesis of dialkylderivatives of **26**, the amino group was substituted with ethyl, *n*-propyl, and phenylethyl groups. The isomeric *N-n*-propyl-*N*-(2-phenylethyl)-2-(3-fluoro-4-hydroxyphenyl)ethylamine (**31**) was also synthesized and tested in order to evaluate the contribution of the amino group substitution relative to the position of the fluorine atom in the phenolic ring.

Chemistry

The synthetic approach to 2-(4-fluoro-3-hydroxyphenyl)ethylamine (**26**), outlined in Scheme I, was based on the 4-fluoro-3-methoxybenzaldehyde (**8**) as an intermediate which can be prepared from 4-fluoro-3-hydroxybenzaldehyde. However, the synthetic method reported in the literature for this compound is unsatisfactory, due to the low yield (5%) of the process.[14]

To need to prepare large quantities of **8** prompted us to develop a new synthetic method. The alternative synthesis of compound **8** was accomplished by starting with the commercially available 3-amino-4-fluorotoluene (**3**), which was diazotized, and then the diazonium salt was hydrolyzed at 165 °C to form 4-fluoro-3-hydroxytoluene (**4**). Methylation of **4** with dimethyl sulfate provided the 4-fluoro-3-methoxytoluene (**5**) in 80% yield. The previous synthetic method to obtain **5** using a modified Schiemann reaction[15] from 4-amino-3-methoxytoluene had a yield of 45%. Oxidation of the methyl group with potassium permanganate, followed by reduction of 4-fluoro-3-methoxybenzoic acid (**6**) with lithium aluminum hydride gave

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Chart I. Derivatives of 2-(4-Fluoro-3-methoxyphenyl)ethylamine

	R	R$_1$		R	R$_1$
11	H	COCH$_3$	17	H	CH$_2$CH$_3$
12	H	COCH$_2$CH$_3$	18	H	n-C$_3$H$_7$
13	CH$_2$CH$_3$	COCH$_2$Ph	19	CH$_2$CH$_3$	n-C$_3$H$_7$
14	n-C$_3$H$_7$	COCH$_3$	20	CH$_2$CH$_3$	CH$_2$CH$_2$Ph
15	n-C$_3$H$_7$	COCH$_2$CH$_3$	21	n-C$_3$H$_7$	n-C$_3$H$_7$
16	n-C$_3$H$_7$	COCH$_2$Ph	22	n-C$_3$H$_7$	CH$_2$CH$_2$Ph

Chart II. Derivatives of 2-(3-Fluoro-4-methoxyphenyl)ethylamine and 2-(3-Fluoro-4-hydroxyphenyl)ethylamine

	R	R$_1$	R$_3$
23	H	COCH$_2$CH$_3$	CH$_3$
24	COCH$_2$Ph	n-C$_3$H$_7$	CH$_3$
25	CH$_2$CH$_2$Ph	n-C$_3$H$_7$	CH$_3$
31	CH$_2$CH$_2$Ph	n-C$_3$H$_7$	H

the 4-fluoro-3-methoxybenzyl alcohol (**7**). When pyridinium chlorochromate was used, **7** was oxidized to 4-fluoro-3-methoxybenzaldehyde (**8**) with an overall yield of 27%.

The aldehyde **8** was condensed with nitromethane, and the resulting nitrostyrene **9** was treated with lithium aluminum hydride to obtain the 2-(4-fluoro-3-methoxyphenyl)ethylamine (**10**). The reaction of **10** with acetic or propionic anhydride gave the *N*-acetyl (**11**) and *N*-propionyl (**12**) derivatives, respectively, which were then reduced by lithium aluminum hydride to N-ethyl- (**17**) and *N-n*-propyl-2-(4-fluoro-3-methoxyphenyl)ethylamine (**18**), respectively (Chart I). By reaction with acetyl, propionyl, or phenylacetyl chloride the latter compounds were converted into the acyl derivatives **13–16**. By reduction of the resulting compounds with lithium aluminum hydride the alkyl derivatives **19–22** were obtained. The ether cleavage of methoxy group was carried out with 48% hydrobromic acid and the hydrobromides **26–30** (Table I) were prepared.

The isomeric *N-n*-propyl-*N*-(2-phenylethyl)-2-(3-fluoro-4-hydroxyphenyl)ethylamine (**31**) was synthesized by using the 2-(3-fluoro-4-methoxyphenyl)ethylamine as starting material and following the procedures previously described[12] (Chart II).

Results and Discussion

The 2-(4-fluoro-3-hydroxyphenyl)ethylamine (**26**) and its *N,N*-dialkyl derivatives (**27-30**) were tested for their affinity for D-1 binding sites, labeled with [^{3}H]SCH 23390, and D-2 binding sites, labeled with [^{3}H]spiperone, in homogenates of rat striatum. The results are shown in Table I. Both DA and (*R*)-(-)-apomorphine were chosen as reference substances. The unsubstituted amine **26** shows about 2-fold lower affinity than DA for both [^{3}H]-SCH 23390 and [^{3}H]spiperone binding sites. However, the derivatives **27-30** when compared to DA and **26** have lower affinity for D-1 receptors and higher affinity for D-2 receptors. The *N*-ethyl- and *N-n*-propyl-*N*-(2-phenylethyl) derivatives **28** and **30** were the most potent and selective members of the series (about 10-fold more effective than DA).

The isomeric 2-(3-fluoro-4-hydroxyphenyl) derivative **31** was 13-fold less effective than DA for the D-1 receptor, but it was about 4-fold more effective than DA for the D-2 receptor. Thus, **31** is approximately 65 times more selective for D-2 vs D-1 sites. Compounds **28** and **30** were also selective but less than **31** (34.8 vs 43.6 and 65.5, respectively). Furthermore, compound **31** is more selective than (*R*)-(-)-apomorphine (65.5 vs 42.8, respectively) but less active.

We have previously[12] shown that **2** has a very low ability in displacing [^{3}H]SCH 23390 (IC$_{50}$ = 150 μM) and [^{3}H]-spiperone (IC$_{50}$ = 165 μM) binding, suggesting that the replacement of the hydroxyl group in the meta position of DA with a fluorine atom decreases the affinity for both D-1 and D-2 DA receptors. On the other hand when the para hydroxyl group of DA, instead of the meta one, is replaced by fluorine atom the new compound **26** retains affinity for both D-1 and D-2 receptors even if it is still not able to discriminate between the two subtypes of DA receptors. According to previous results from our laboratory[12] the substitution of the amino group greatly affects the selectivity for D-2 receptors. The introduction of a 2-phenylethyl group on the nitrogen atom results in the highest increase in binding affinity and selectivity towards D-2 receptors also in compound **2**, which itself has a very low affinity for DA receptors, probably by an increased liposolubility[16] or by binding to a complementary lipophilic site of the receptor which can recognize the lipophilic 2-phenylethyl moiety, as proposed by Olson et al.[17]

Experimental Section

Melting points were determined on a Buchi 510 apparatus and are uncorrected. Mycroanalyses were performed on a 1106 Carlo Erba C H and N Analyzer, and the results were within ±0.4% of the calculated values. Proton magnetic resonance (NMR) spectra were recorded on a Varian VXR 300 MHz spectrometer with CDCl$_3$ as solvent and are reported in parts per million (δ) downfield from the internal standard tetramethylsilane (Me$_4$Si). All NMR spectra were consistent with the structures assigned. The IR spectra were run on a Perkin-Elmer Model 297 spectrophotometer as Nujol mulls or liquid films. The identity of all new compounds was confirmed by both elemental analysis and NMR data; homogeneity was confirmed by TLC. Solutions were routinely dried over anhydrous sodium sulfate prior to evaporation. Chromatographic purifications were accomplished on Merck-60 silica gel columns 70-230 mesh ASTM with a reported solvent.

4-Fluoro-3-hydroxytoluene (4). 3-Amino-4-fluorotoluene (**3**) (15.64 g, 125 mmol) was added dropwise to a solution of 6 M sulfuric acid (104 mL, 625 mmol). The solution was cooled to

-5 °C, and NaNO$_2$ (9.25 g, 130 mmol) in water (15 mL) was added dropwise with stirring, while the temperature was maintained between -5 and 5 °C. The reaction was allowed to warm to room temperature, and the excess nitrite was decomposed with few crystals of urea. In a 1-L three-necked flask, equipped with a thermometer, a dropping funnel, and a steam distillation apparatus, CuSO$_4$·5H$_2$O (56.25 g, 125 mmol), H$_2$O (125 mL), and 96% H$_2$SO$_4$ (156 mL) were placed. The mixture was heated to 165 °C, the solution of diazonium salt was added dropwise, with stirring, and a slow current of steam was passed through the system to remove the phenol. The distillate was extracted with ether. After drying, the solvent was removed in vacuo, and the residue was distilled: bp 72 °C (28 mm) [lit.[15] bp 86-93 °C (30 mm)]; yield 80%; NMR δ 6.95 (dd, 1 H, H-5, J_{HF} = 11.4 Hz, J_{HHo} = 8.2 Hz), 6.82 (dd, 1 H, H-2, J_{HF} = 8.2 Hz, J_{HHm} = 2 Hz), 6.65 (ddd, 1 H, J_{HHo} = 8.2 Hz, J_{HF} = 4.3 Hz, J_{HHm} = 2 Hz), 5.30 (bs, 1 H, OH), 2.26 (s, 3 H, CH$_3$). Anal. (C$_7$H$_7$FO) C, H, N.

4-Fluoro-3-methoxytoluene (5). A suspension of 4-fluoro-3-hydroxytoluene (**4**) (18.05 g, 143 mmol), K$_2$CO$_3$ (20 g, 144 mmol), and (CH$_3$)$_2$SO$_4$ (14.3 mL, 150 mmol) in acetone (250 mL) was refluxed for 5 h. After cooling, the reaction mixture was filtered and evaporated in vacuo. The residue was diluted with water, and the product was extracted with ethyl acetate. The extracts were washed with 1 N NH$_4$OH and 1 N NaOH and dried, and the solvent was removed in vacuo. The residue was distilled: bp 92 °C (40 mm) [lit.[15] bp 93-5 °C, yield 45%]; yield 84%; NMR δ 6.95 (dd, 1 H, H-5, J_{HF} = 11.4 Hz, J_{HHo} = 8.2 Hz), 6.78 (dd, 1 H, H-2, J_{HF} = 8.2 Hz, J_{HHm} = 2 Hz), 6.68 (ddd, 1 H, H-6, J_{HHo} = 8.2 Hz, J_{HF} = 4.3 Hz, J_{HHm} = 2 Hz), 3.87 (s, 3 H, OCH$_3$), 2.32 (s, 3 H, CH$_3$). Anal. (C$_8$H$_9$FO) C, H, N.

4-Fluoro-3-methoxybenzoic Acid (6). Potassium permanganate (35 g, 221 mmol) was added in small portions to a vigorously stirred mixture of **5** (10 g, 71 mmol), pyridine (36 mL), and water (107 mL) at 50 °C. After stirring for 3 h at the same temperature and 3 h at room temperature, the mixture was filtered. MnO$_2$ was suspended in hot water and again filtered off. The pyridine was removed from filtrates, as an azeotrope with water, by distillation under reduced pressure. The resulting aqueous solution was extracted with Et$_2$O and acidified with 2 N H$_2$SO$_4$. The precipitate was filtered off and recrystallized from EtOH/H$_2$O (8/2): mp 207 °C [lit.[18] mp 189-90 °C]; yield 70%; IR 1680 (C=O) cm^{-1}; NMR (acetone-*d$_6$*) δ 7.72 (dd, 1 H, H-2, J_{HHm} = 2 Hz, J_{HF} = 8.3 Hz), 7.67 (ddd, 1 H, H-6, J_{HHo} = 8.4 Hz, J_{HHm} = 2 Hz, J_{HF} = 4.4 Hz), 7.26 (dd, 1 H, H-5, J_{HF} = 11.1 Hz, J_{HHo} = 8.4 Hz), 5.58 (s, 1 H, OH), 3.95 (s, 3 H, OCH$_3$). Anal. (C$_8$H$_7$FO$_3$) C, H, N.

4-Fluoro-3-methoxybenzyl Alcohol (7). To a magnetically stirred suspension of **6** (2 g, 11.7 mmol) in anhydrous Et$_2$O (50 mL) in an ice bath was added portionwise LiAlH$_4$ (0.5 g, 13 mmol). The ice bath was removed, and the mixture was heated for 5 min, stirred at room temperature for 3 h, and the excess LiAlH$_4$ was quenched by successive dropwise additions of 0.5 mL of H$_2$O, 0.5 mL 15% NaOH, and 1.5 mL H$_2$O. The solution was filtered, and the filtrate was dried and concentrated under reduced pressure. The residue was recrystallized from petroleum ether: mp 48-9 °C, bp 132 °C (22 mm); yield 95%; NMR δ 7.02 (dd, 1 H, H-5, J_{HF} = 11.2 Hz, J_{HHo} = 8.2 Hz), 6.98 (dd, 1 H, H-2, J_{HF} = 8.2 Hz, J_{HHm} = 2 Hz), 6.83 (ddd, 1 H, H-6, J_{HHo} = 8.2 Hz, J_{HF} = 4.3 Hz, J_{HHm} = 2 Hz), 4.62 (s, 2 H, OCH$_2$), 3.88 (s, 3 H, OCH$_3$), 2.02 (bs, 1 H, OH). Anal. (C$_8$H$_9$FO$_2$) C, H, N.

4-Fluoro-3-methoxybenzaldehyde (8). To the magnetically stirred suspension of pyridinium chlorochromate (3.07 g, 142 mmol) in anhydrous CH$_2$Cl$_2$ (15 mL) was added a solution of **7** (1.45 g, 9.3 mmol) in anhydrous CH$_2$Cl$_2$ (20 mL). After 2 h Et$_2$O (20 mL) was added, and the reaction mixture was allowed to stand in a refrigerator overnight. The suspension was filtered on silica gel, and the solvent was evaporated under reduced pressure. The oily residue was recrystallized from EtOH/H$_2$O (7/3): mp 72 °C; yield 95%; IR 1700 (C=O) cm^{-1}; NMR δ 9.92 (s, 1 H, CHO), 7.51 (dd, 1 H, H-2, J_{HHm} = 2 Hz, J_{HF} = 8.3 Hz), 7.45 (ddd, 1 H, H-6, J_{HHo} = 8.3 Hz, J_{HF} = 4.4 Hz, J_{HHm} = 2 Hz), 7.23 (dd, 1 H, H-5,

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J_{HF} = 10.5 Hz, J_{HHo} = 8.3 Hz), 3.96 (s, 3 H, OCH$_3$). Anal. (C$_8$H$_7$FO$_2$) C, H, N.

2-(4-Fluoro-3-methoxyphenyl)nitroethylene (9). A mixture of Na$_2$CO$_3$ (2 g, 18.8 mmol) and methylamine hydrochloride (2 g, 29.6 mmol) in EtOH (20 mL) was stirred at room temperature for 15 min and filtered into a solution of 8 (9.4 g, 61 mmol) in EtOH (25 mL). Nitromethane (5.26 mL, 94 mmol) was added, and the mixture was stoppered and left in the dark at room temperature for 3 days. The yellow crystalline product was filtered and washed with EtOH: mp 147–9 °C; yield 84%; NMR (acetone-d_6) δ 8.08 (d, 1 H, CHNO$_2$, J = 13.7 Hz), 8.00 (d, 1 H, ArCH, J = 13.7 Hz), 7.68 (dd, 1 H, H-2, J_{HHm} 2 Hz, J_{HF} = 8.2 Hz), 7.43 (ddd, 1 H, H-6, J_{HHo} = 8.3 Hz, J_{HHm} = 2 Hz, J_{HF} = 4.4 Hz), 7.27 (dd, 1 H, H-5, J_{HF} = 11.4 Hz, J_{HHo} = 8.3 Hz), 3.98 (s, 3 H, OCH$_3$). Anal. (C$_9$H$_8$FNO$_3$) C, H, N.

2-(4-Fluoro-3-methoxyphenyl)ethylamine (10). A solution of 9 (5 g, 25.2 mmol) in anhydrous THF (50 mL) was added to a stirred solution of LiAlH$_4$ (3.36 g, 80 mmol) in anhydrous THF. The mixture was stirred at room temperature for 12 h. The excess LiAlH$_4$ was quenched by successive dropwise additions of H$_2$O (3.4 mL), 15% NaOH (3.4 mL), and H$_2$O (10 mL). After filtration the solution was dried and evaporated to an oil: bp 90 °C (1.5 mm); yield 54%; NMR δ 6.94 (dd, 1 H, H-5, J_{HF} = 11.2 Hz, J_{HHo} = 8.2 Hz), 6.76 (dd, 1 H, H-2, J_{HHm} = 2 Hz, J_{HF} = 8.2 Hz), 6.67 (ddd, 1 H, H-6, J_{HHo} = 8.2 Hz, J_{HHm} = 2 Hz, J_{HF} = 4.4 Hz), 3.82 (s, 3 H, OCH$_3$), 2.91 (t, 2 H, ArCH$_2$, J = 6.7 Hz), 2.66 (t, 2 H, CH$_2$N, J = 6.7 Hz), 1.45 (bs, 2 H, NH$_2$). Anal. (C$_9$H$_{12}$FNO) C, H, N.

General Procedure for Acylation. To a stirred solution of the appropriate amine (10 mmol) and triethylamine (10 mmol) in anhydrous Et$_2$O (15 mL) in a ice bath was added dropwise a solution of acyl chloride (10 mmol) in anhydrous Et$_2$O (10 mL). After ice bath removal, the mixture was stirred at room temperature for 1 h. The solution was washed with H$_2$O (5 mL) and 2 N HCl (5 mL). The organic layer was dried. Concentration in vacuo gave an oil. The compounds 13–16 and 24 are uncrystallizable.

N-Acetyl-2-(4-fluoro-3-methoxyphenyl)ethylamine (11). Compound 11 was prepared from 10 and acetyl chloride. The oil was recrystallized from benzene/petroleum ether: yield 88%; mp 72–4 °C; IR (neat) 3260 (NH), 1640 (C=O) cm^{-1}; NMR δ 7.01 (dd, 1 H, H-5), 6.82 (dd, 1 H, H-2), 6.72 (m, 1 H, H-6), 5.65 (bs, 1 H, NH), 3.88 (s, 3 H, OCH$_3$), 3.48 (q, 2 H, NCH$_2$), 2.78 (t, 2 H, ArCH$_2$), 1.94 (s, 3 H, CH$_3$). Anal. (C$_{11}$H$_{14}$FNO$_2$) C, H, N.

N-Propionyl-2-(4-fluoro-3-methoxyphenyl)ethylamine (12). Compound 12 was prepared from 10 and propionyl chloride. The oil was recrystallized from ethyl acetate/*n*-hexane: yield 82%; mp 52–3 °C; IR (neat) 3305 (NH), 1635 (C=O) cm^{-1}; NMR δ 6.98 (dd, 1 H, H-5), 6.82 (dd, 1 H, H-2), 6.70 (m, 1 H, H-6), 5.65 (bs, 1 H, NH), 3.87 (s, 3 H, OCH$_3$), 3.45 (q, 2 H, NCH$_2$), 2.77 (t, 2 H, ArCH$_2$), 2.13 (q, 2 H, COCH$_2$), 1.09 (t, 3 H, CH$_3$). Anal. (C$_{12}$H$_{16}$FNO$_2$) C, H, N.

N-Ethyl-N-(phenylacetyl)-2-(4-fluoro-3-methoxyphenyl)ethylamine (13). Compound 13 was prepared from 17 and phenylacetyl chloride. The oily residue was purified by column chromatography on silica gel with cyclohexane/ethyl acetate (1/1) as eluent: yield 70%; IR (neat) 1640 (C=O) cm^{-1}. Anal. (C$_{19}$H$_{22}$FNO$_2$) C, H, N.

N-*n*-Propyl-N-acetyl-2-(4-fluoro-3-methoxyphenyl)-ethylamine (14). Compound 14 was prepared from 18 and acetyl chloride. The oily residue was purified by column chromatography on silica gel with cyclohexane/ethyl acetate (1/1) as eluent: yield 70%; IR (neat) 1640 (C=O) cm^{-1}. Anal. (C$_{14}$H$_{20}$FNO$_2$) C, H, N.

N-*n*-Propyl-N-propionyl-2-(4-fluoro-3-methoxyphenyl)-ethylamine (15). Compound 15 was prepared from 18 and propionyl chloride: yield 93%; IR (neat) 1640 (C=O) cm^{-1}. Anal. (C$_{15}$H$_{22}$FNO$_2$) C, H, N.

N-*n*-Propyl-N-(phenylacetyl)-2-(4-fluoro-3-methoxyphenyl)ethylamine (16). Compound 16 was prepared from 18 and phenylacetyl chloride: yield 82%; IR (neat) 1640 (C=O) cm^{-1}. Anal. (C$_{20}$H$_{24}$FNO$_2$) C, H, N.

N-Propionyl-2-(3-fluoro-4-methoxyphenyl)ethylamine (23). Compound 23 was prepared from 2-(3-fluoro-4-methoxyphenyl)ethylamine[12] and propionyl chloride. The oil was recrystallized from ethyl acetate/cyclohexane: yield 88%; mp 101–3 °C; IR (neat) 3280 (NH), 1645 (C=O) cm^{-1}; NMR δ 6.92 (d, 1

H, H-2), 6.86 (m, 2 H, H-5,6), 5.62 (bs, 1 H, NH), 3.87 (s, 3 H, OCH$_3$), 3.46 (q, 2 H, NCH$_2$), 2.74 (t, 2 H, ArCH$_2$), 2.18 (q, 2 H, COCH$_2$), 1.12 (t, 3 H, CH$_3$). Anal. (C$_{12}$H$_{16}$FNO$_2$) C, H, N.

N-*n*-Propyl-N-(phenylacetyl)-2-(3-fluoro-4-methoxyphenyl)ethylamine (24). Compound 24 was prepared by reduction of 23 with LiAlH$_4$ in a similar method to that reported for the preparation of amines. Then, the crude amine was treated with phenylacetyl chloride by the same procedure used for acylation. The oily residue was purified by column chromatography on silica gel with cyclohexane/ethyl acetate (1/1) as eluent: yield 83%; IR (neat) 1640 (C=O) cm^{-1}. Anal. (C$_{20}$H$_{24}$FNO$_2$) C, H, N.

General Procedure for the Preparation of Amines. A solution of the amide (30 mmol) in anhydrous Et$_2$O (25 mL) was added dropwise under N$_2$ over a 20-min period to a stirred suspension of LiAlH$_4$ (60 mmol) in anhydrous Et$_2$O (25 mL). The mixture was stirred at room temperature for 4 h. The excess LiAlH$_4$ was quenched by successive dropwise additions of H$_2$O (2.3 mL), 15% NaOH (2.3 mL), and H$_2$O (7 mL). After filtration, the solution was dried and evaporated in vacuo. The remaining oily residue was used without further purification.

N-Ethyl-2-(4-fluoro-3-methoxyphenyl)ethylamine (17). Compound 17 was prepared from 11: yield 85%; NMR δ 6.99 (dd, 1 H, H-5), 6.80 (dd, 1 H, H-2), 6.72 (m, 1 H, H-6), 3.88 (s, 3 H, OCH$_3$), 2.85 (m, 2 H, ArCH$_2$), 2.66 (q, 2 H, NCH$_2$), 1.68 (bs, 1 H, NH), 1.08 (t, 3 H, CH$_3$). Anal. (C$_{11}$H$_{16}$FNO) C, H, N.

N-*n*-Propyl-2-(4-fluoro-3-methoxyphenyl)ethylamine (18). Compound 18 was prepared from 12: yield 87%; NMR δ 6.98 (dd, 1 H, H-5), 6.80 (dd, 1 H, H-2), 6.72 (m, 1 H, H-6), 3.88 (s, 3 H, OCH$_3$), 2.85 (m, 2 H, ArCH$_2$), 2.76 (m, 2 H, ArCCH$_2$), 2.58 (t, 2 H, NCH$_2$), 1.55 (bs, 1 H, NH), 1.48 (m, 2 H, CCH$_2$C), 0.88 (t, 3 H, CH$_3$). Anal. (C$_{12}$H$_{18}$FNO) C, H, N.

N-Ethyl-N-*n*-propyl-2-(4-fluoro-3-methoxyphenyl)-ethylamine (19). Compound 19 was prepared from 14: yield 79%; NMR δ 6.98 (dd, 1 H, H-5), 6.80 (dd, 1 H, H-2), 6.68 (m, 1 H, H-6), 3.88 (s, 3 H, OCH$_3$), 2.70 (m, 4 H, ArCH$_2$CH$_2$), 2.60 (q, 2 H, NCH$_2$), 2.46 (t, 2 H, NCH$_2$), 1.48 (m, 2 H, CCH$_2$C), 1.06 and 0.88 (two t, 6 H, 2 CH$_3$). Anal. (C$_{14}$H$_{22}$FNO) C, H, N.

N-Ethyl-N-(2-phenylethyl)-2-(4-fluoro-3-methoxyphenyl)ethylamine (20). Compound 20 was prepared from 13: yield 96%; NMR δ 7.28 and 7.20 (two m, 5 H, Harom), 6.98 (dd, 1 H, H-5), 6.78 (dd, 1 H, H-2), 6.68 (m, 1 H, H-6), 3.88 (s, 3 H, OCH$_3$), 2.78 (m, 10 H, 5 CH$_2$), 2.68 (q, 2 H, NCH$_2$), 1.10 (t, 3 H, CH$_3$). Anal. (C$_{19}$H$_{24}$FNO) C, H, N.

N,N-Di-*n*-propyl-2-(4-fluoro-3-methoxyphenyl)ethylamine (21). Compound 21 was prepared from 15: yield 92%; NMR δ 6.98 (dd, 1 H, H-5), 6.71 (dd, 1 H, H-2), 6.78 (m, 1 H, H-6), 3.88 (s, 3 H, OCH$_3$), 2.68 (m, 4 H, ArCH$_2$CH$_2$), 2.45 (m, 4 H, 2 NCH$_2$), 1.48 (m, 4 H, 2 CCH$_2$C), 0.90 (t, 6 H, 2 CH$_3$). Anal. (C$_{15}$H$_{24}$FNO) C, H, N.

N-*n*-Propyl-N-(2-phenylethyl)-2-(4-fluoro-3-methoxyphenyl)ethylamine (22). Compound 22 was prepared from 16: yield 92%; NMR δ 7.28 and 7.20 (two m, 5 H, Harom), 6.98 (dd, 1 H, H-5), 6.78 (dd, 1 H, H-2), 6.68 (m, 1 H, H-6), 3.88 (s, 3 H, OCH$_3$), 2.75 (m, 8 H, 4 CH$_2$), 3.55 (t, 2 H, NCH$_2$), 1.52 (m, 2 H, CCH$_2$C), 0.90 (t, 3 H, CH$_3$). Anal. (C$_{20}$H$_{26}$FNO) C, H, N.

N-*n*-Propyl-N-(2-phenylethyl)-2-(3-fluoro-4-methoxyphenyl)ethylamine (25). Compound 25 was prepared from 24: yield 94%; NMR δ 7.30 and 7.28 (two m, 5 H, Harom), 6.92 (dd, 1 H, H-2), 6.88 (m, 2 H, H-5,6), 3.88 (s, 3 H, OCH$_3$), 2.75 (m, 8 H, 4 CH$_2$), 2.55 (m, 2 H, NCH$_2$), 1.52 (m, 2 H, CCH$_2$C), 0.91 (t, 3 H, CH$_3$). Anal. (C$_{20}$H$_{26}$FNO) C, H, N.

General Procedure for Demethylation. A stirred solution of the appropriate methoxylated amine (10 mmol) and freshly distilled 48% HBr (30 mL) was heated, under N$_2$, at 125–30 °C for 3 h. The solution was evaporated in vacuo, and the residue was dissolved in absolute ethanol and evaporated in vacuo. This procedure was repeated twice. The residue was dissolved in 2-propanol (10 mL), and the hydrobromide was precipitated by addition of anhydrous Et$_2$O. Compounds 27 and 28 are uncrystallizable.

2-(4-Fluoro-3-hydroxyphenyl)ethylamine Hydrobromide (26). Compound 26 was prepared from 10: yield 63%; mp 211–13 °C; NMR (DMSO-d_6) δ 9.82 (s, 1 H, OH), 8.78 (bs, 3 H, NH$_3^+$), 7.08 (dd, 1 H, H-5), 6.84 (dd, 1 H, H-2), 6.67 (m, 1 H, H-6), 2.98 (m, 2 H, NCH$_2$), 2.77 (t, 2 H, ArCH$_2$). Anal. (C$_8$H$_{11}$BrFNO) C, H, N.

N-Ethyl-N-n-propyl-2-(4-fluoro-3-hydroxyphenyl)-ethylamine Hydrobromide (27). Compound 27 was prepared from 19: yield 92%; uncrystallizable; NMR (DMSO-d_6) δ 9.52 (bs, 1 H, OH), 7.08 (dd, 1 H, H-5), 6.88 (dd, 1 H, H-2), 6.72 (m, 1 H, H-6), 4.0 (bs, 1 H, NH$^+$), 3.20, 3.08, and 2.92 (three m, 8 H, 4 CH$_2$), 1.67 (m, 2 H, CCH$_2$C), 1.25 (t, 3 H, NCCH$_3$), 0.90 (t, 3 H, CH$_3$). Anal. (C$_{13}$H$_{21}$BrFNO) C, H, N.

N-Ethyl-N-(2-phenylethyl)-2-(4-fluoro-3-hydroxy-phenyl)ethylamine Hydrobromide (28). Compound 28 was prepared from 20: yield 89%; uncrystallizable; NMR (DMSO-d_6) δ 9.90 (bs, 1 H, OH), 7.32 and 7.25 (two m, 5 H, Harom), 7.05 (dd, 1 H, H-5), 6.92 (dd, 1 H, H-2), 6.73 (m, 1 H, H-6), 4.42 (bs, 1 H, NH$^+$), 3.32 (m, 6 H, 3 NCH$_2$), 3.05 and 2.96 (two t, 4 H, ArCH$_2$), 1.28 (t, 3 H, CH$_3$). Anal. (C$_{18}$H$_{23}$BrFNO) C, H, N.

N,N-Di-n-propyl-2-(4-fluoro-3-hydroxyphenyl)ethylamine Hydrobromide (29). Compound 29 was prepared from 21: yield 75%; mp 123-4 °C; NMR (DMSO-d_6) δ 9.85 (s, 1 H, OH), 9.22 (bs, 1 H, NH$^+$), 7.10 (dd, 1 H, H-5), 6.88 (dd, 1 H, H-2), 6.72 (m, 1 H, H-6), 3.22 and 3.05 (two m, 6 H, 3 NCH$_2$), 2.90 (m, 2 H, ArCH$_2$), 1.50 (m, 4 H, 2 CCH$_2$C), 0.90 (t, 6 H, 2 CH$_3$). Anal. (C$_{14}$H$_{23}$BrFNO) C, H, N.

N-n-Propyl-N-(2-phenylethyl)-2-(4-fluoro-3-hydroxy-phenyl)ethylamine Hydrobromide (30). Compound 30 was prepared from 22: yield 83%; mp 138-40 °C; NMR (DMSO-d_6) δ 9.85 (s, 1 H, OH), 9.52 (bs, 1 H, NH$^+$), 7.28 (m, 5 H, Harom), 7.10 (dd, 1 H, H-5), 6.90 (m, 1 H, H-2), 6.72 (m, 1 H, H-6), 3.52, 3.31, and 3.26 (three m, 6 H, 3 NCH$_2$), 2.99 and 2.90 (two t, 4 H, 2 ArCH$_2$), 1.70 (m, 2 H, 2 CCH$_2$C), 0.90 (t, 3 H, CH$_3$). Anal. (C$_{19}$H$_{25}$BrFNO) C, H, N.

N-n-Propyl-N-(2-phenylethyl)-2-(3-fluoro-4-hydroxy-phenyl)ethylamine Hydrobromide (31). Compound 31 was prepared from 25: yield 86%; mp 154-55 °C; NMR (DMSO-d_6) δ 9.78 (s, 1 H, OH), 9.65 (bs, 1 H, NH$^+$), 7.30 (m, 5 H, Harom), 7.16 (dd, 1 H, H-2), 6.92 (m, 2 H, H-5,6), 3.45 and 3.17 (two m, 6 H, 3 NCH$_2$), 3.05 and 2.95 (two t, 4 H, 2 ArCH$_2$), 1.72 (m, 2 H, CCH$_2$C), 0.90 (t, 3 H, CH$_3$). Anal. (C$_{19}$H$_{25}$BrFNO) C, H, N.

Pharmacology. Binding Studies. Adult Sprague-Dawley rats were obtained from Charles River (Calco, Italy). [^{3}H]SCH 23390 (specific activity 77.7 Ci/mmol) and [^{3}H]spiperone (specific activity 24 Ci/mmol) were purchased from New England Nuclear. Unlabeled SCH 23390 was a generous gift of Dr. Ongini (Essex, Italy). The following substances were obtained commercially: apomorphine hydrochloride, dopamine hydrochloride (Sigma Chemical Co., St. Louis, MO).

Radioreceptor binding studies were performed by using rat striatal membrane preparations. The tissue was homogenized in 100 volumes of Tris-HCl buffer (pH 7.4). The homogenate was centrifuged at 20.000g for 10 min. The resultant pellet was rehomogenized in buffer and centrifuged again. The final pellet was resuspended in 50 mM Tris-HCl containing 120 mM NaCl, 5 mM KCl, 2 mM CaCl$_2$, and 1 mM MgCl$_2$ to a final concentration of 3.5 mg wet weight/mL. Aliquots of 400 μL of membrane suspension were added to ice cold tubes containing 50 μL of [^{3}H]spiperone (final concentration 0.1 nM) or [^{3}H]SCH 23390 (final concentration 1 nM), 50 μL of displacer (6-7 different concentrations) in a final volume of 0.5 mL. Specific binding was determined in the presence of (+)-butaclamol (1 μM) for [^{3}H]-spiperone, and unlabeled SCH 23390 (10 μM) for [^{3}H]SCH 23390 binding. The experiments were performed in triplicate, and replicated three times on different days. Displacement analysis and IC$_{50}$ determinations were carried out by using a computerized log-probit plot program. The new compounds were tested as hydrobromides.

Synthesis of Substituted 7,12-Dihydropyrido[3,2-b:5,4-b']diindoles: Rigid Planar Benzodiazepine Receptor Ligands with Inverse Agonist/Antagonist Properties

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A series of 1-, 2-, 3-, 4-, 5-, 6-, 7-, 10-, and 12-substituted pyridodiindoles were synthesized and screened in vitro against [^{3}H]diazepam for activity at the benzodiazepine receptor (BzR). In vitro, the 2-substituted pyridodiindoles were found to be the most potent (IC$_{50}$ < 10 nM) of this new class of BzR ligands. In vivo, 2-methoxypyridodiindole 19a (IC$_{50}$ = 8 nM) was found to be the most potent partial inverse agonist (proconvulsant) of the series. The parent compound 2 (IC$_{50}$ = 4 nM) was only slightly less potent. In addition, 2-hydroxypyridodiindole 21a (IC$_{50}$ = 6 nM) was found to exhibit potent proconvulsant activity when administered as a prodrug derivative, pivaloyl ester 22. 2-Chloropyridodiindole 16a (IC$_{50}$ = 10 nM) was devoid of proconvulsant activity; however, 16a was found to be the most potent antagonist of the anticonvulsant effects of diazepam in this class of BzR ligands. From the in vivo data available, substitution on ring E of 2 with electron-withdrawing groups results in antagonists at BzR, while replacement of hydrogen at C-2 with electron-releasing groups provides enhanced inverse agonist activity. The pyridodiindoles were used as "templates" for the formulation of a model of the inverse agonist/antagonist active site of the BzR. The proposed model consists of a hydrogen bond acceptor site (A^1) and a hydrogen bond donor site (D^2) disposed 6.0-8.5 Å from each other on the receptor protein. The hydrogen-bonding sites are believed to be located at the base of a narrow cleft. A large lipophilic pocket at the mouth of the narrow cleft serves to direct molecules into the binding site, while the presence of a small lipophilic pocket permits substitution only at position 2 of the pyridodiindole nucleus for maximum binding potency.

Since the discovery of benzodiazepine receptors (BzR) in 1977[1,2] more than a half-dozen structurally unique classes of ligands have been identified.[3-13] These ligands exhibit actions along a pharmacological continuum ranging from complete mimicry of the 1,4-benzodiazepines (full

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