strongly indicate that the most probable biologically active conformation for the D-1 selective dopamine receptor agonist 1, as well as for the antagonist 8, is a chair conformation (or a conformation structurally close to it) with an equatorial phenyl ring. In both cases it is concluded that the orientation of the phenyl ring in the receptor-bound molecule does not deviate in terms of dihedral angles by more than about 30° from the preferred orientation. The *N*-methyl group in compound 8 most probably has an equatorial position in the active conformation.

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Synthesis and Biological Evaluation of 4-Fluoro-, 7-Fluoro-, and 4,7-Difluoro-5,6-dihydroxytryptamines

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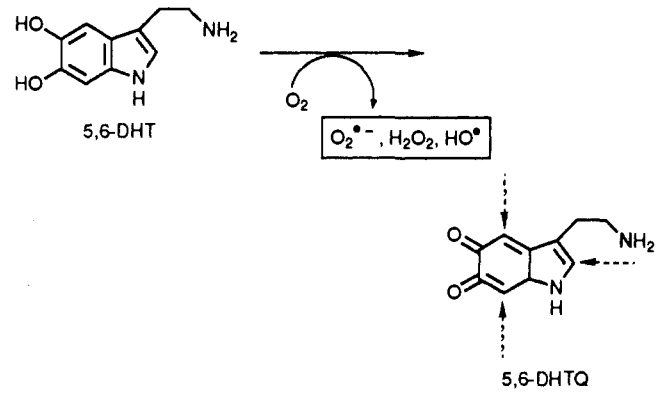
The 5,6-dihydroxytryptamine (5,6-DHT) derivatives 4-fluoro- and 7-fluoro-5,6-DHTs (**26a,b**) and 4,7-difluoro-5,6-DHT (**26c**) were synthesized from 3-fluoroanisole (1) and 1,4-difluoro-2,3-dimethoxybenzene (**13**), respectively. Efficient methods were developed for the conversion of 1 to 4-fluoro- and 7-fluoro-5,6-bis(benzyloxy)indoles (**12a,b**, respectively) and 13 to 4,7-difluoro-5,6-[(diphenylmethylene)dioxy]indole (**19**) via reductive cyclization of 2-nitro-β-(dialkylamino)styrenes prepared in situ from 2-nitrotoluenes. Indoles **12a,b** and **19** were then converted to **26a–c** via the corresponding indole-3-acetonitriles. The fluorine-substituted 5,6-DHTs displayed increased phenol acidities, determined spectrophotometrically, and decreased inherent potential to undergo oxidation as determined by cyclic voltammetry. Fluorine substitution did not have a significant adverse effect on the cytotoxic potential as judged from the IC_{50} values of 117, 125, 135, and 92 μM for **26a,c** and 5,6-DHT, respectively, for the inhibition of incorporation of [^{3}H]thymidine into the DNA of neuroblastoma clone N-2a cells in culture. Surprisingly, **26a–c** exhibited 32-, 23-, and 13-fold higher affinities, respectively, compared to 5,6-DHT for the serotonergic uptake system of N-2a cells as measured by the ability of **26a–c** and 5,6-DHT to antagonize the uptake of [^{3}H]5-HT into the N-2a cells. These desirable chemical and biological properties of **26a–c** should make them useful tools for the study of the molecular mechanism of neurodegenerative action of 5,6-DHT.

5,6-Dihydroxytryptamine (5,6-DHT, Chart I) is a general pharmacological tool used to produce selective destruction of 5-hydroxytryptamine (5-HT) containing nerve terminals.[1,2] It is selective due to its high-affinity, active uptake by the serotonergic membrane pumps. The neurodegeneration is believed to be initiated by the alkylation and free-radical-induced damage of essential neuronal constituents by the electrophilic quinones and the reduced O_2 species such as H_2O_2, $O_2^{\bullet-}$ and HO•, respectively, produced by the intraneuronal autoxidation of 5,6-DHT.[2-4] Because of the complexity of the autoxidation reaction, it has not yet been possible to characterize the DHT-derived product(s), postulated to be 5,6-DHTQ (Chart I). With radiolabeled 5,6-DHT, it has been shown that the DHT-derived autoxidation products undergo extensive covalent binding with protein nucleophiles both in vitro[4] and in vivo.[5] However, the nature of this protein–quinone interaction, including the relative importance of the postulated electrophilic sites of 5,6-DHTQ toward alkylation, remains to be determined. It was thought that if the unsubstituted positions in the 5,6-DHT ring were independently or simultaneously blocked, it might be possible to determine the relative importance not only of the putative electrophilic sites but also of the alkylation and free-radical-induced damage in neurodegeneration. Previously, we designed and synthesized 4-methyl-, 7-methyl-, and 4,7-dimethyl-5,7-DHTs for this purpose.[6,7] The methyl-substituted analogues suffered from two drawbacks. First, they underwent much more rapid autoxidation than 5,6-DHT, making them difficult to handle during in vivo studies. In addition, their affinity of uptake by the serotonergic membrane pumps was significantly

Chart I[a]



[a] The broken arrows point to putative sites of alkylation reactions.

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‡ Present address: Drug Metabolism Department, Glaxo, Inc., Five Moore Drive, Research Triangle Park, NC 27709.

(1) Baumgarten, H. G.; Bjorklund, A.; Lachenmayer, L.; Nobin, A.; Stenevi, U. *Acta Physiol. Scand. Suppl.* **1971**, *373*, 1. Baumgarten, H. G.; Jenner, S.; Klemm, H. P. *J. Physiol. (Paris)* **1981**, *77*, 309. Jonsson, G. In *Handbook of Chemical Neuroanatomy: Methods in Chemical Neuroanatomy*: Bjorklund, A., Hökfelt, T., Eds.; Elsevier: Amsterdam, 1983; Vol. 1, Chapter XII. Schallert, T.; Wilcox, R. E. In *Neuromethods: Vol. 1, General Neurochemical Techniques*; Boulton, A. A., Baker, G. B., Eds.; Humana Press: Clifton, NJ, 1985; pp 343–387.

(2) Baumgarten, H. G.; Klemm, H. P.; Sievers, J.; Schlossberger, H. G. *Brain Res. Bull.* **1982**, *9*, 131. Baumgarten, H. G.; Jenner, S.; Bjorklund, A.; Klemm, H. P.; Schlossberger, H. G. In *Biology of Serotonergic Transmission*: Osborne, N. N., Ed.; Wiley: New York, 1982, Chapter 10.

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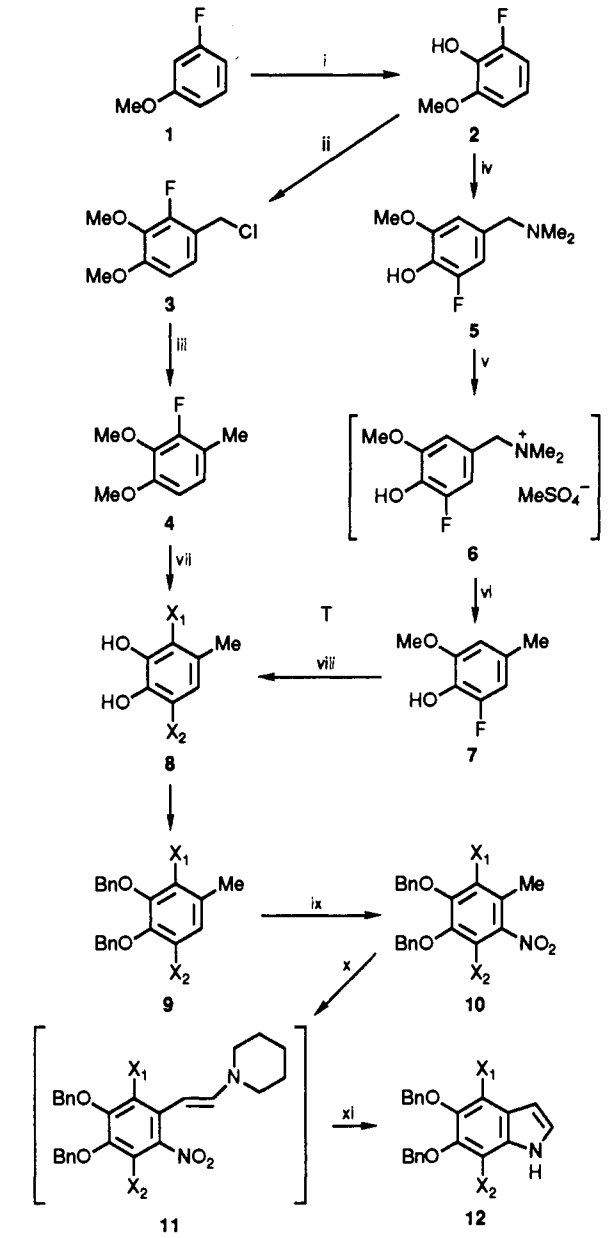
reduced, possibly due to the steric bulk of the methyl substituent.

It was thought that the fluorine-substituted analogues, 4-fluoro-, 7-fluoro-, and 4,7-difluoro-5,6-DHTs, would be superior to the corresponding methyl-substituted analogues as probes for the evaluation of the molecular mechanism of action of 5,6-DHT for the following reasons.[8,9] (1) The small size of the fluoro substituent would introduce minimum bulk in the resulting analogues, thereby minimizing loss of affinity of uptake due to bulk. (2) The electron-withdrawing effect of fluorine would make the fluorine-substituted analogues more stable toward autoxidation. (3) The electron-withdrawing effect of the fluoro substituent would render the fluorine-substituted quinoid products more electrophilic and hence more reactive toward nucleophiles when unsubstituted electrophilic sites are still available. In this paper we report on the efficient syntheses of these fluorine-substituted 5,6-DHTs, on the electronic effects of fluorine substitution, and on the evaluation of neurotoxic potency and uptake affinity of these analogues in neuroblastoma clone N-2a cells in culture.

Chemistry

The synthetic approach to **26a–c** was to transform appropriately substituted 2-nitrotoluenes to the corresponding indoles via 2-nitro-2′-piperidinostyrenes, followed by introduction of the aminoethyl side chain. Our previous studies on the synthesis of autoxidizable dihydroxytryptamines suggested a need for protecting the phenolic hydroxyl groups with groups (e.g., benzyl) that could be removed at the final step by hydrogenolysis under neutral conditions.[6,10] Thus, the indoles **12a,b** and **12c** ($X_1 = X_2 = F$) were required as precursors. For the synthesis of **12a** (Scheme I), fluoroanisole **1** was converted to the benzyl chloride **3** in three steps as described by Ladd and Weinstock.[11] Catalytic hydrogenation of **3** gave toluene **4**, which, upon O-demethylation and subsequent O-benzylation, gave benzyl ether **9a**. Nitration of **9a** to **10a** was done with HNO_2 in HOAc. Reaction of nitrotoluene **10a** with tripiperidinomethane (TPM), followed by reductive cyclization of the intermediate nitro enamine **11a** with Fe/HOAc in toluene in the presence of silica gel,[12] gave indole **12a**. For the synthesis of indole **12b** also, fluoroanisole **1** served as the starting material (Scheme I), which was converted to Mannich base **5** via phenol **2**. N-Methylation of **5** and subsequent reduction with NaBH_3CN in HMPA gave toluol **7**. O-Demethylation of **7** gave catechol **8b**, which was converted to indole **12b** following the same sequence of reactions established for the synthesis of **12a** from catechol **8a** (Scheme I). Attempted synthesis of 5,6-bis(benzyloxy)-4,7-difluoroindole (**12**, $X_1 = X_2 = F$), from toluene **9** ($X_1 = X_2 = F$), which was synthesized from 1,4-difluorobenzene, was not successful. Thus, in contrast to the successful nitration of **9a,b** to **10a,b** in high yields, toluene **9** ($X_1 = X_2 = F$) could not be satisfactorily nitrated to nitrotoluene **10** ($X_1 = X_2 = F$) under a variety of conditions and reagents tried. Side reactions initiated by the O-debenzylation during nitration may have been one of the major reasons for this failure. To circumvent these problems, difluorodimethoxytoluene **14**, which was synthesized from **13** via its aryllithium intermediate, was used as a substrate for nitration. Nitration of **14** with fuming HNO_3 in Ac_2O in the presence of H_2SO_4

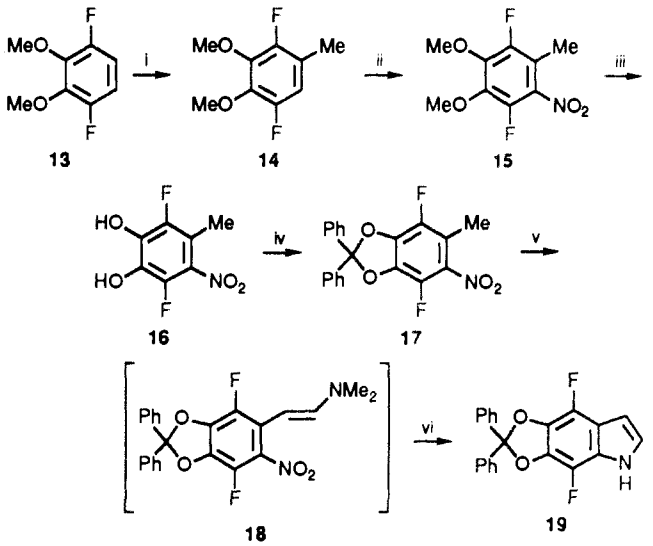
Scheme I[a]



[a] Series a: $X_1 = F$, $X_2 = H$. Series b: $X_1 = H$, $X_2 = F$. Reagents: (ia) BuLi/THF, (b) $B(OMe)_3$; (c) $HOAc/H_2O_2$; (ii) reference 11; (iii) $Pd/C/Et_3N/EtOAc$; (iv) $CH_2O/Me_2NH/EtOH$; (v) Me_2SO_4/THF; (vi) $NaBH_3CN/HMPA$; (vii) BBr_3/CH_2Cl_2; (viii) $PhCH_2Cl/K_2CO_3/NaI/Me_2CO$; (ix) $HNO_3/HOAc$; (x) tripiperidinomethane; (xi) Fe/HOAc/silica gel/PhMe.

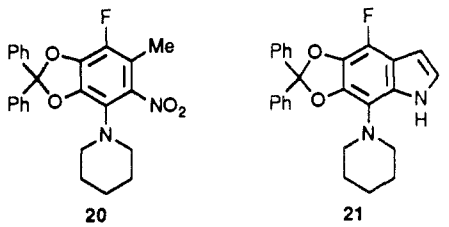
(3) Cohen, G.; Heikkila, R. E. *Ann. N.Y. Acad. Sci.* **1978**, *305*, 74.
(4) Creveling, C. R.; Rotman, A. *Ann. N.Y. Acad. Sci.* **1978**, *305*, 57.
(5) Baumgarten, H. G.; Klemm, H. P.; Lachenmayer, L.; Bjorklund, A.; Lovenberg, W.; Schlossberger, H. G. *Ann. N.Y. Acad. Sci.* **1978**, *305*, 3.
(6) Sinhababu, A. K.; Ghosh, A. K.; Borchardt, R. T. *J. Med. Chem.* **1985**, *28*, 1273.
(7) It was thought that the involvement of position 2 of 5,6-DHT in electrophilic reactions following autoxidation could be determined by observing the fate of a tritium label on C-2 of the4,7-disubstituted derivative of 5,6-DHT.
(8) Kirk, K. L.; Creveling, C. R. *Med. Res. Rev.* **1984**, *4*, 189.
(9) Goldman, P. *Science* **1969**, *164*, 1123. Sharpe, A. G. In *Carbon–fluorine Compounds: Chemistry, Biochemistry and Biological Activities*; A Ciba Foundation Symposium; Elsevier: Amsterdam, 1972; pp 33–54. Schlosser, M. *Tetrahedron* **1978**, *34*, 3. Ulrich, H.; Richter, R. In *Houben-Weyl-Miller: Methoden der Organischen Chemie*; Thieme: Stuttgart, 1977; Vol. VII/3a, Part I, p 726.
(10) Sinhababu, A. K.; Borchardt, R. T. *J. Am. Chem. Soc.* **1985**, *107*, 7618.
(11) Ladd, D. L.; Weinstock, J. *J. Org. Chem.* **1981**, *46*, 203.
(12) Kawase, M.; Sinhababu, A. K.; Borchardt, R. T. *J. Heterocycl. Chem.* **1987**, *24*, 1499.

Scheme II[a]



[a] Reagents: (ia) BuLi/THF, (b) Me_2SO_4; (ii) $HNO_3/Ac_2O/H_2SO_4$; (iii) BBr_3/CH_2Cl_2; (iv) Ph_2CCl_2; (v) $Me_2NCH(OMe)_2/Et_3N/PhMe$; (vi) Fe/HOAc/Florisil/PhMe.

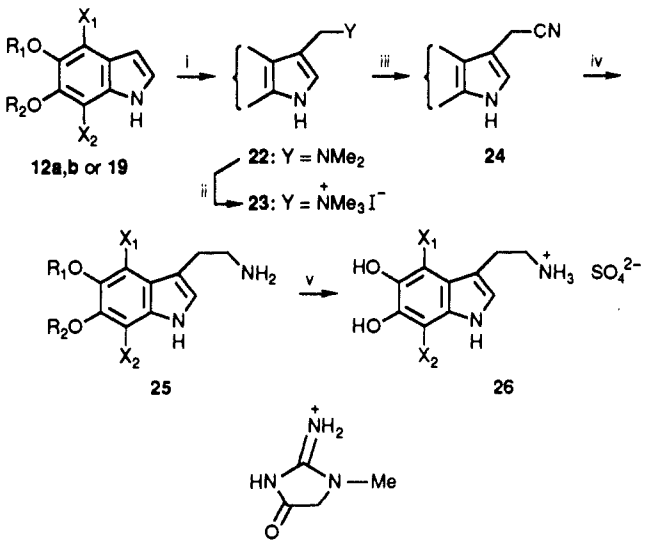
gave **15** in 71% yield. O-Demethylation of **15** gave catechol **16** in 98% yield, but this catechol could not be O-benzylated under a variety of conditions requiring the presence of a base. However, protection of the catechol moiety with another hydrogenolyzable group, namely, the (diphenylmethylene)dioxy group, by reaction of **16** with Ph_2CCl_2, was successful and gave **17** in 79% yield. Attempts to synthesize indole **19** from nitrotoluene **17** by the condensation of **17** with TPM and subsequent reductive cyclization following the method for the synthesis of **12** produced a mixture of products. Only two of these products could be isolated, and these were characterized as piperidinotoluene **20** and piperidinoindole **21**. It is apparent that the fluorine ortho to the nitro group of the starting nitrotoluene **17** was substituted by the piperidino group at the condensation step and **20** may have been an intermediate in the formation of **21**.



The most satisfactory method for the synthesis of indole **19** from nitrotoluene **17** was found to be the generation of nitro enamine **18** with $Me_2NCH(OMe)_2/Et_3N$ as the reagent and subsequent reductive cyclization with Fe/HOAc in the presence of Florisil. The indole was produced in 53% yield. This was surprising since only a 23% yield of indole **19** had been realized with the usual silica gel as the supporting agent for the cyclization step.

For the introduction of the aminoethyl side chain on C-3 of indoles **12a,b** and **19**, the first step involved reaction with CH_2O/Me_2NH to give gramine **22a-c**, respectively. Quaternization of gramine **22** and subsequent reaction with KCN gave nitrile **24**. Reduction [6,10,13] of nitrile **24** to tryptamine **25** could be effected satisfactorily only in relatively dilute solution of 1:1 Et_2O-PhH with a large excess of $LiAlH_4$. The hydrogen sulfate salts of **25a-c** were then subjected to catalytic hydrogenolysis, followed by treatment with creatinine to furnish the target dihydroxytryptamines **26a-c**, respectively. The target compounds as well as the synthetic intermediates were characterized by obtaining satisfactory spectral (^1H NMR, IR, MS) data and elemental analyses.

Scheme III[a]



[a] a: $X_1 = F$, $R_1 = R_2 = PhCH_2$, $X_2 = H$. b: $X_1 = H$, $R_1 = R_2 = PhCH_2$, $X_2 = F$. c: $X_1 = X_2 = F$, $R_1 + R_2 = CPh_2$. Reagents: (i) $CH_2O/Me_2NH/EtOH/HOAc$; (ii) MeI/EtOH/EtOAc; (iii) KCN/DMF/H_2O; (iv) $LiAlH_4/Et_2O/PhH$; (v) $H_2SO_4/Pd/C/H_2/EtOH$, (b) creatinine.

Table I. Ultraviolet Spectral Data[a]

compound (solvent)[b]	λ_{max}, nm	ϵ	compound (solvent)[b]	λ_{max}, nm	ϵ
5,6-DHT (A)	299	7650	**26b** (A)	290[c]	5060
5,6-DHT (B)	299	7790	**26b** (B)	292[c]	4540
5,6-DHT (C)	316	9390	**26b** (C)	305	7350
26a (A)	288[c]	5120	**26c** (A)	292[c,d]	2020
26a (B)	292[c]	4940	**26c** (B)	292[c]	2460
26a (C)	305	7880	**26c** (C)	300	7350

[a] All spectra were recorded in deoxygenated solvents. [b] Solvents: A, HCl buffer at pH 2.0; B, phosphate buffer at pH 7.4; C, 0.1 N NaOH, pH 12.6. [c] Shoulder. [d] The major absorption band occurred at 257 nm (ϵ 6220); it disappeared above pH 6.8.

Electronic Effects of Fluorine Substitution

The ionization constants and the redox potentials of 5,6-DHT and **26a-c** were determined to gain some insight into the electronic effects of fluorine substitution in 5,6-DHT. The ionization constants were determined spectrophotometrically.[14] The absorption maxima of 5,6-DHT and **26a-c** at three representative pH values are given in Table I. The electron-withdrawing effect of fluorine is reflected in the increased phenol acidity of **26a,b** with pK_a's of 8.58 and 8.04, respectively, compared to 9.28 for 5,6-DHT. This lowering of pK_a values upon fluorine substitution is in line with those observed with fluorine-substituted 5-HTs[15] and catecholamines.[7] The spectrophotometric technique could not be applied to determine the pK_a of **26c** accurately. The UV spectra of **26c** were pH-dependent but the relationship was complex probably due to partial autoxidation of **26c** during measurement of the UV spectra.

(13) Schlossberger, H. G.; Kuch, H. *Chem. Ber.* **1960**, *93*, 1318; *Liebigs Ann. Chem.* **1963**, *662*, 132.

(14) Albert, A.; Sergeant, E. P. *The Determination of Ionization Constants*, 3rd ed.; Chapman: London, 1984.

(15) Kirk, K. L. *J. Heterocycl. Chem.* **1976**, *13*, 1253.

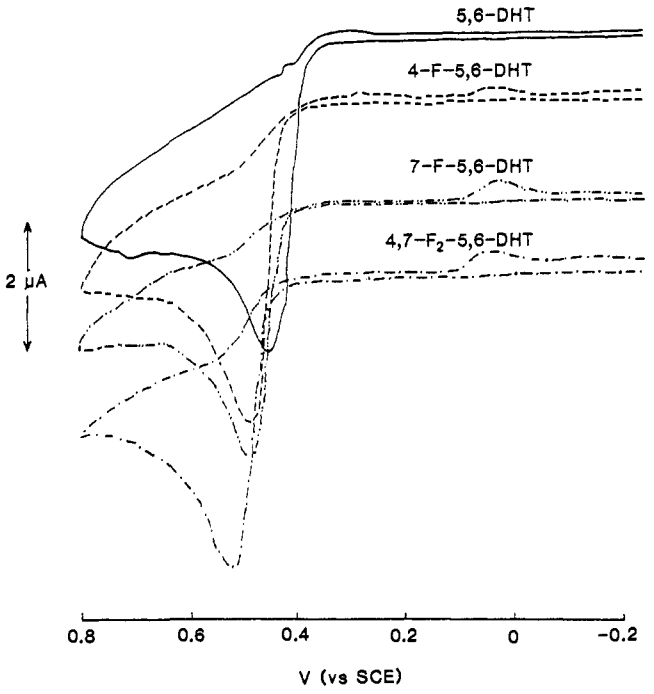


Figure 1. Cyclic voltammograms of the 5,6-DHTs ~0.5 mM in 1 M H_2SO_4 at a scan rate of 50 mV/s.

The redox potentials of 5,6-DHT and its fluorine-substituted analogues were determined by cyclic voltammetry with a carbon paste working electrode.[16] Even in 1 M H_2SO_4, none of the test compounds displayed any reversible redox behavior (Figure 1). The irreversible oxidation potentials (or, more accurately, the peak potentials) vs saturated calomel electrode observed in 1 M H_2SO_4 were (in mV) 452 for 5,6-DHT, 484 for 4-F-5,6-DHT, 483 for 7-F-5,6-DHT, and 523 for 4,7-F_2-5,6-DHT. These results suggest that the introduction of fluorine in 5,6-DHT leads to derivatives with slightly decreased ease of oxidation. This is expected due to the decreased electron density of the fluorine-substituted indole rings[9] and is consistent with the observed increase in phenol acidities. Similar decreases in the ease of electrochemical oxidation have been observed with 2- and 5-fluorodopamines (2- and 5-F-DAs) compared to dopamine (DA)[17] at pH 7.4. However, these results with 2- and 5-F-DAs as well as **26a–c** stand in contrast to those reported[17] with 6-F-DA, 2-, 5-, and 6-fluoronorepinephrines (2-, 5-, and 6-F-NEs), and 2-, 5-, and 6-fluoro-3,4-dihydroxyphenylalanines (2-, 5-, and 6-F-DOPAs), all of which displayed increased ease of oxidation compared to DA, NE, and DOPA, respectively, at pH 7.4. This increased ease of electrochemical oxidation has also been observed with 2-, 5-, and 6-F-NEs at pH 3.2.[17] Although it is not clear what accounts for the above differences in the observed effects of fluorine substitution on the ease of electrochemical oxidation of various catechol derivatives, pH of the electrolyte may have played a role.

Biology

The neurotoxic effects and the uptake affinities of 5,6-DHT and its fluorine-substituted analogues were evaluated in neuroblastoma clone N-2a cells in culture. These clonal cells have characteristics of serotonergic neurons, making them a simple in vitro model system for studying the mechanism of action of a serotonergic neurotoxin such as 5,6-DHT. The N-2a cells, differentiated by serum deprivation, have been shown to take up [^{3}H]5-HT from external medium with high affinity. This uptake is saturable, temperature-dependent, and partially sodium-dependent and follows Michaelis–Menten kinetics.[18]

Table II. Inhibition of [^{3}H]Thymidine Incorporation into the DNA of Differentiated N-2a Cells by the 5,6-DHTs[a]

drug	IC_{50}, μM
5,6-DHT	92 ± 21
4-F-5,6-DHT (**26a**)	117 ± 19
7-F-5,6-DHT (**26b**)	125 ± 32
4,7-F_2-5,6-DHT (**26c**)	135 ± 29

[a] The results are expressed as mean ± SD of at least three independent determinations in duplicate. Details of procedure are provided in the Experimental Section.

Table III. Effect of 5,6-DHTs on the Uptake of [^{3}H]5-HT into Differentiated N-2a Cells[a]

drug	IC_{50}, μM
5,6-DHT	17.8 ± 6
4-F-5,6-DHT (**26a**)	0.55 ± 0.21
7-F-5,6-DHT (**26b**)	0.76 ± 0.23
4,7-F_2-5,6-DHT (**26c**)	1.36 ± 0.35

[a] The results are expressed as mean ± SD of at least three independent determinations in duplicate. Details of procedure are given in the Experimental Section.

The cytotoxicities of 5,6-DHT and **26a–c** were evaluated by measuring the inhibition of incorporation of [^{3}H]thymidine into the DNA of N-2a cells as a function of neurotoxin concentrations ranging from 50 to 1000 μM. The plots of percent inhibition of [^{3}H]thymidine incorporation vs neurotoxin concentration were near linear up to 200 μM, at which concentration greater than 95% of the N-2a cells lost the ability to incorporate [^{3}H]thymidine. The IC_{50} values for 5,6-DHT and **26a–c**, calculated from such plots (not shown), were not significantly different from each other and ranged from 92 μM for 5,6-DHT to 135 μM for the difluoro derivative **26c** (Table II).

The uptake affinities of 5,6-DHT and **26a–c** were determined by measuring the inhibition of [^{3}H]5-HT uptake by the N-2a cells in suspension, in the presence of the respective neurotoxins either at 0–4 °C (energy-independent transport) or at 37 °C (total transport). The potencies of 5,6-DHT and **26a–c** in inhibiting active uptake of [^{3}H]5-HT, expressed as IC_{50} values, are shown in Table III. Each of the fluorine-substituted analogues proved to possess at least 1 order of magnitude higher affinity for the serotonergic uptake system of N-2a cells than the parent neurotoxin, 5,6-DHT.

Discussion

The objective of this study was to design and synthesize analogues of 5,6-DHT that would be similar to 5,6-DHT in terms of cytotoxic effects, uptake affinities, and redox potentials but would lead to quinoidal products with inherently different reactivities toward nucleophiles. The results of this study demonstrate that substitution with fluorine leads to analogues with similar cytotoxic effects and slightly higher redox potentials but with significantly increased affinity for the serotonergic uptake system of N-2a cells. This increase in uptake affinity was 32-, 23-, and 13-fold higher for 4-fluoro-, 7-fluoro-, and 4,7-difluoro-5,6-DHT, respectively, compared to 5,6-DHT. These results with fluorine-substituted analogues are in sharp contrast to those obtained with 4-methyl-, 7-methyl-,

(16) Adams, R. N. *Electrochemistry at Solid Electrodes*; Dekker: New York, 1969.
(17) Rice, M. E.; Moghaddam, B.; Creveling, C. R.; Kirk, K. L. *Anal. Chem.* **1987**, *59*, 1534.
(18) Yoffe, J. R.; Borchardt, R. T. *Mol. Pharmacol.* **1982**, *21*, 362.

and 4,7-dimethyl-5,6-DHT reported previously.[6] Thus, the two monomethylated analogues exhibited cytotoxic effects similar to those of 5,6-DHT, but the dimethyl analogue was at least 50-fold more cytotoxic. In addition, the methylated analogues exhibited lower redox potentials and 3–9-fold lower uptake affinities compared to 5,6-DHT.[6] It is likely that the small size of fluorine contributes to the retention of uptake affinities. However, it is not clear what factors led to the significant increases in uptake affinity. Recently, it has been shown that 4,6-difluoro-5-HT (phenolic pK_a = 7.97), which is substantially more acidic than 5-HT (phenolic pK_a = 10.73), is transported by the platelet 5-HT transporter with a rate identical with those for 5-HT both at pH 6.7, where the hydroxy group of 4,6-difluoro-5-HT is predominantly un-ionized, and at pH 9, where it is largely ionized.[19] These results suggest that the affinity of uptake of fluoro-5,6-DHTs by the 5-HT transporter should at least not be adversely affected by the increased phenol acidities of the analogues. In contrast to the difluoro-5-HT, the fluoro-5,6-DHTs contain two hydroxy groups, one of which will remain virtually completely un-ionized while the other will be ionized similar to that in the difluoro-5-HT, at physiological pH. It is possible that the presence of both an un-ionized hydroxyl group and a readily ionizable one in each of the fluoro-5-DHTs led to the observed increase in uptake affinity compared to 5,6-DHT.

The similarity of the cytotoxic potentials of all of the fluorine-substituted analogues with that of 5,6-DHT is somewhat surprising. It is possible that alkylation at either the 4- or 7-position or both is not essential for cytotoxic effects. Alkylation at position 2 and the damaging effects of the reduced O_2 species produced upon autoxidation may be sufficient to account for the observed cytotoxicity. There also exists the possibility that the quinones, derived from **26a–c** upon oxidation, may undergo alkylation at the sites occupied by fluorine with subsequent loss of F^-. It has been reported[17] that *o*-quinones derived from 6-F-DA, 6-F-NE, and 6-F-DOPA by bulk electrochemical oxidation all undergo intramolecular cyclization with 47, 47, and 60% loss of F^-. It should be noted that in all of these examples the fluorine was meta to the quinone carbonyl and not ortho as in fluoro-5,6-DHTs. Indeed, the extent of loss of F^- from the corresponding *o*-quinones (generated again by bulk electrochemical oxidation)[17] of 2- and 5-fluoro derivatives of DA, NE, and DOPA ranged from 6 to 9%. Finally, on the basis of the redox data, the fluoroquinones derived from **26a–c** are expected to be somewhat more electrophilic than 5,6-DHTQ, and consequently the chances of nucleophilic attack on the ring junctions (positions 3a and 7a) may be increased. The ring junctions of 5,6-DHTQ are generally not considered as potential sites of nucleophilic attack, although there is no evidence for or against it. However, results reported with the quinones derived from 5,6-dihydroxyindole derivatives would suggest that the ring junctions also may be involved in alkylation reactions.[20]

Experimental Section

Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 700 spectrophotometer. Ultraviolet spectra were recorded on a Shimadzu UV-260. The ^1H NMR spectra were recorded on Varian T-60, FT-80A, or XL-300 spectrometers with Me_4Si as internal standard. For compounds whose spectra were recorded in D_2O, chemical shifts were measured with *p*-dioxane (δ 3.56) as internal standard. The mass spectra were obtained on a Varian MAT CH-5. Combustion analyses were performed by Desert Analytics, Inc., Tucson, AZ, and by the Department of Medicinal Chemistry, University of Kansas. Column chromatography was performed on Merck silica gel 60 (70–230 mesh).

1-Fluoro-2-hydroxy-3-methoxybenzene (2). In a modification of the literature procedure,[11] a solution of 2.5 M *n*-BuLi in hexane (114 mL, 285 mmol) was added dropwise, over 1 h, to a stirred solution of **1** (37.8 g, 300 mmol) in dry THF (300 mL) under an Ar atmosphere below −65 °C. After 2 h at −78 °C, a solution of $B(OMe)_3$ (35 mL, 305 mmol) in dry THF (60 mL) was added over 1 h and stirring was continued for 0.5 h at −78 °C. To the solution, after it was allowed to warm to 0 °C, was added HOAc (26 mL, 450 mmol) all at once, followed by 30% H_2O_2 (34 mL, 330 mmol) dropwise at 0 °C. The mixture was stirred at 25 °C overnight, diluted with H_2O (300 mL) and then extracted with Et_2O (2 × 300 mL). The combined Et_2O extracts were washed successively with H_2O (200 mL), 10% ferrous ammonium sulfate (2 × 100 mL), and H_2O (200 mL) and then extracted with 10% NaOH (1 × 120 mL and then 1 × 50 mL). The combined NaOH extracts were acidified with concentrated HCl with cooling, and the product was extracted with CH_2Cl_2 (2 × 200 mL). The combined CH_2Cl_2 extracts were washed with H_2O (150 mL) and dried (Na_2SO_4), and the solvent was evaporated. The residue was distilled to give 35.4 g (83%) of **2**: bp 73–77 °C (0.2 mm) [lit.[11] bp 129.5–131 °C (36 mm)].

3,4-Dimethoxy-2-fluoro-1-methylbenzene (4). A solution of **3**[11] (4.79 g, 23.4 mmol) and Et_3N (3.3 mL, 23.4 mmol) in EtOAc (35 mL) containing 10% Pd/C (200 mg) was shaken in a Parr apparatus under an H_2 atmosphere at 40 psi for 5 h at 25 °C. The catalyst was removed by filtration and the filtrate, after dilution with Et_2O (200 mL), was washed successively with H_2O (80 mL), 3% $NaHCO_3$ (50 mL), H_2O (80 mL), 3% HCl (50 mL), and saturated NaCl (80 mL). The solution was dried (Na_2SO_4) and the solvent was evaporated to give 3.76 g (94%) of **4**. An analytical sample was prepared by vacuum distillation, giving a colorless oil: bp 109–113 °C (18 mm); ^1H NMR ($CDCl_3$) δ 2.18 (d, 3 H, J = 2.4 Hz, Me), 3.83 (s, 3 H, OMe), 3.90 (s, 3 H, OMe), 6.43–6.93 (m, 2 H); MS, *m/e* 170 (M^+); HR MS *m/e* calcd for $C_9H_{11}FO_2$, 170.0742, found 170.0744.

5-[(Dimethylamino)methyl]-1-fluoro-2-hydroxy-3-methoxybenzene (5). A solution of **2** (71 g, 500 mmol), 37% aqueous CH_2O (60 mL, 750 mmol), and 40% aqueous Me_2NH (90 mL, 750 mmol) in EtOH (300 mL) was stirred at 25 °C for 42 h and then diluted with H_2O (300 mL). The mixture was refrigerated overnight. The precipitated solid was collected by filtration and washed thoroughly with ice-cold EtOH. Recrystallization from benzene gave 86.7 g (87%) of **5** as a colorless solid: mp 142–143 °C (lit.[21] mp 140–142 °C); ^1H NMR ($CDCl_3$) δ 2.26 (s, 6 H, NMe_2), 3.37 (s, 2 H, CH_2), 3.73 (s, 3 H, OMe), 6.43–6.73 (m, 2 H), 8.10 (br, s, 1 H, OH); MS *m/e* 199 (M^+). Anal. ($C_{10}H_{14}FNO_2$) C, H, N.

1-Fluoro-2-hydroxy-3-methoxy-5-methylbenzene (7). To a stirred solution of the Mannich base **5** (10 g, 50 mmol) in dry THF (150 mL) was added Me_2SO_4 (9.5 mL, 100 mmol). The mixture was stirred at 25 °C for 3 h and then a solution of $NaBH_3CN$ (6.32 g, 100 mmol) in $(Me_2N)_3PO$ (50 mL) was added gradually. The mixture was refluxed for 6 h by which time 80 mL of the THF was distilled off to ensure dissolution of methiodide **6**. The cooled reaction mixture was diluted with H_2O (100 mL) and then extracted with Et_2O (2 × 200 mL). The combined Et_2O extracts were washed successively with saturated NaCl (4 × 150 mL), 2 N HCl (10 mL), 8% $NaHCO_3$ (100 mL), and saturated NaCl (150 mL). After drying (Na_2SO_4), the solvent was removed by evaporation. Distillation of the residue gave 6.39 g (82%) of **7** as a light yellow liquid: bp 85–88 °C (0.35 mm); ^1H NMR ($CDCl_3$) δ 2.22 (s, 3 H, Me), 3.79 (s, 3 H, OMe), 5.22 (s, 1 H, OH), 6.26–6.56 (m, 2 H); IR (Nujol) 3580 cm^{-1}; MS *m/e* 156 (M^+); HR MS *m/e* calcd for $C_8H_9FO_2$ 156.0586, found 156.0596.

3,4-Bis(benzoyloxy)-2-fluoro-1-methylbenzene (9a). A solution of BBr_3 (34 mL, 360 mmol) in CH_2Cl_2 (50 mL) was added

(19) Rudnick, G.; Kirk, K. L.; Fishkes, H.; Schuldiner, S. *J. Biol. Chem.* **1989**, *264*, 14865.

(20) Borchardt, R. T.; Bhatia, P. *J. Med. Chem.* **1982**, *25*, 263.

(21) Clark, M. T.; Miller, D. D. *J. Org. Chem.* **1986**, *51*, 4072.

dropwise to a stirred solution of 4 (51 g, 300 mmol) in CH_2Cl_2 (300 mL) at −78 °C under an Ar atmosphere. The reaction mixture was stirred at −78 °C for 2 h and then at 25 °C for 12 h. MeOH (50 mL) was added dropwise with ice-cooling and the mixture was stirred vigorously. The mixture was diluted with 1 N HCl (200 mL) and then extracted with EtOAc (2 × 300 mL). The combined EtOAc extracts were washed with saturated NaCl (2 × 200 mL) and dried (Na_2SO_4). Evapoaration of sovent gave 42 g (99%) of **8a** as a gum which was utilized in the next step without further purification: ^{1}H NMR ($CDCl_3$) δ 2.18 (d, 3 H, J = 2.4 Hz, Me), 5.70 (br s, 2 H, OH), 6.35–6.73 (m, 2 H).

A mixture of **8a** (42 g, 300 mmol), $PhCH_2Cl$ (86 mL, 750 mmol), powdered K_2CO_3 (124 g, 900 mmol), NaI (4.5 g, 30 mmol), and acetone (600 mL) was stirred and heated under reflux for 12 h. The cooled reaction mixture was filtered and the filtrate was evaporated in vacuo. To the residue was added H_2O (300 mL) and the resulting mixture was extracted with CH_2Cl_2 (2 × 300 mL). The combined CH_2Cl_2 extracts were dried (Na_2SO_4) and evaporated, and the residue was distilled to give 72.5 g (75%) of **9a** as a light yellow liquid: bp 199–201 °C (0.2 mm); ^{1}H NMR ($CDCl_3$) δ 2.17 (d, 3 H, J = 2.4 Hz, Me), 5.03 (s, 2 H, CH_2), 5.05 (s, 2 H, CH_2), 6.40–6.76 (m, 2 H), 7.13–7.50 (m, 10 H, Ph); MS m/e 322 (M^+); HR MS m/e calcd for $C_{21}H_{19}FO_2$ 322.1368, found 322.1371.

1,2-Bis(benzyloxy)-3-fluoro-5-methylbenzene (9b). Catechol **8b** was obtained in 98% yield from **7** following the procedure for the synthesis of **8a** from **4** and was utilized in the next step without further purification: ^{1}H NMR ($CDCl_3$) δ 2.20 (s, 3 H, Me), 5.00–5.63 (br, 2 H, OH), 6.18–6.52 (m, 2 H).

Benzylation of **8b** (as described above for **8a**) gave **9b** in 70% yield: mp 66–67 °C (hexane); ^{1}H NMR ($CDCl_3$) δ 2.22 (s, 3 H, Me), 4.92 (s, 4 H, CH_2), 6.17 (m, 2 H), 6.98–7.37 (m, 10 H, Ph); MS m/e 322 (M^+). Anal. ($C_{21}H_{19}FO_2$) C, H.

1,2-Bis(benzyloxy)-3-fluoro-4-methyl-5-nitrobenzene (10a). To a stirred solution of **9a** (32.2 g, 100 mmol) in HOAc (300 mL) was added 90% fuming HNO_3 (42 mL, 1 mol) dropwise, while the temperature was kept below 25 °C. The mixture was stirred at 25 °C for 0.5 h and then poured into crushed ice (600 g). The solid was collected by filtration, washed thoroughly with H_2O, and dried over P_2O_5. Recrystallization from EtOH gave 28.6 g (78%) of **10a** as a colorless solid: mp 88–89 °C; ^{1}H NMR ($CDCl_3$) δ 2.43 (d, 3 H, J = 2.4 Hz, Me), 5.13 (s, 2 H, CH_2), 5.22 (s, 2 H, CH_2), 5.22 (s, 2 H, CH_2), 7.23–7.60 (m, 11 H); MS m/e 367 (M^+). Anal. ($C_{21}H_{18}FNO_4$) C, H, N.

1,2-Bis(benzyloxy)-3-fluoro-5-methyl-4-nitrobenzene (10b). Nitration of **9b** (as described above for **9a**) gave **10b** in 77% yield: mp 129–131 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 2.32 (s, 3 H, Me), 5.03 (s, 2 H, CH_2), 5.07 (s, 2 H, CH_2), 6.40–6.61 (br, 1 H, H-6), 7.15–7.43 (m, 10 H, Ph); MS m/e 367 (M^+). Anal. ($C_{21}H_{18}FNO_4$) C, H, N.

5,6-Bis(benzyloxy)-4-fluoroindole (12a). A mixture of **10a** (734 mg, 2 mmol) and tripiperidinomethane (801 mg, 3 mmol) was heated to 120 °C and stirred under a water aspirator vacuum (to remove piperidine) for 3 h. The crude 2-nitro-β-piperidinostyrene **11a** was dissolved in a solution of PhMe–HOAc (5:3, 6 mL) and added to an efficiently stirred mixture of Fe (reduced, N.F. IX electrolytic, Mallinckrodt, 2 g, 35 mmol), silica gel (60–200 mesh, Mallinckrodt, 5 g) in PhMe–HOAc (5:3, 26 mL) at 25 °C. The mixture was refluxed under an Ar atmosphere for 1 h and then cooled to 25 °C, diluted with CH_2Cl_2 (50 mL), and filtered. The filter cake was washed thoroughly with CH_2Cl_2 (5 × 50 mL). The combined filtrates were washed successively with 10% sodium metabisulfite (50 mL), 10% Na_2CO_3 (3 × 80 mL) (until the aqueous layer was basic), and saturated NaCl (100 mL). After drying (Na_2SO_4), the solvent was evaporated in vacuo. The residue was chromatographed on a column of silica gel using CH_2Cl_2–hexane (2:1) as eluent to give 422.7 mg (61%) of **12a**. An analytical sample was prepared by recrystallization from PhH–hexane: mp 115–116 °C; ^{1}H NMR ($CDCl_3$) δ 5.09 (s, 4 H, OCH_2), 6.53 (t, 1 H, J = 2.4 Hz, H-3), 6.70 (s, 1 H, H-7), 7.01 (t, 1 H, J = 2.4 Hz, H-2), 7.28–7.49 (m, 10 H, Ph), 8.02 (br s, 1 H, NH); IR (Nujol) 3405 cm^{-1}; MS m/e 347 (M^+). Anal. ($C_{22}H_{18}FNO_2$) C, H, N.

5,6-Bis(benzyloxy)-7-fluoroindole (12b) was obtained in 53% yield from **10b** by the procedure for **12a**: mp 91–93 °C (PhH–hexane); ^{1}H NMR ($CDCl_3$) δ 5.13 (s, 4 H, OCH_2), 6.41–6.49 (m, 1 H, H-3), 6.96 (d, 1 H, J = 0.8 Hz, H-4), 7.13 (t, 1 H, J = 2.4 Hz, H-2), 7.28–7.47 (m, 10 H, Ph), 8.11 (br s, 1 H, NH); IR (Nujol) 3370, 3470 cm^{-1}; MS m/e 347 (M^+). Anal. ($C_{22}H_{18}FNO_2$) C, H, N.

1,4-Difluoro-2,3-dimethoxy-5-methylbenzene (14). To a solution of **13**[11] (34.8 g, 200 mmol) in dry THF (200 mL) at −78 °C was added under an Ar atmosphere 2.5 M n-BuLi in hexane (76 mL, 190 mmol) over 40 min. The mixture was stirred for 1 h at −78 °C and a solution of Me_2SO_4 (37.8 mL, 400 mol) in dry THF (40 mL) was added over 2 h at −78 °C. After being stirred at 25 °C for 12 h, the mixture was diluted with H_2O (150 mL) and extracted with Et_2O (2 × 250 mL). The combined Et_2O extracts were washed with saturated NaCl (200 mL) and concentrated in vacuo to ~125 mL. Aqueous NH_3 (10% 50 mL) was added and the mixture was stirred for 2 h at 25 °C. The mixture was then diluted with H_2O (200 mL) and extracted with Et_2O (2 × 200 mL). The combined Et_2O extracts were washed with saturated NaCl (3 × 200 mL) and dried (Na_2SO_4). Evaporation of the solvent and distillation of the residue afforded 28.6 g (76%) of **14** as a light yellow liquid: bp 110–112 °C (35 mm); ^{1}H NMR ($CDCl_3$) δ 2.20 (d, 3 H, J = 2.4 Hz, Me), 3.93 (m, 6 H, OMe), 6.40–6.78 (m, 1 H); MS m/e 188 (M^+); HR MS m/e calcd for $C_9H_{10}F_2O_2$ 188.0648, found 188.0653.

1,4-Difluoro-2,3-dimethoxy-5-methyl-6-nitrobenzene (15). To a stirred solution of 90% fuming HNO_3 (1.9 mL, 45 mmol) in Ac_2O (15 mL) (freshly distilled from P_2O_5) containing one drop of H_2SO_4 at −10 °C under an Ar atmosphere was added a solution of **14** (5.64 g, 30 mmol) in Ac_2O (5 mL) over 10 min. The mixture was stirred at −10 °C for 15 min and then poured into crushed ice (100 g). The resulting mixture was stirred at 25 °C for 30 min and extracted with EtOAc (2 × 80 mL). The combined extracts were washed successively with 8% $NaHCO_3$ (2 × 50 mL) and saturated NaCl (100 mL). After drying (Na_2SO_4), the solvent was evaporated in vacuo. The residue was chromatographed on a column of silica gel using EtOAc–hexane (1:20) as eluent to give 5.0 g (71%) of **15**. An analytical sample was prepared by Kugelrohr distillaton: bp 90 °C (bath temperature) (0.6 mm); mp 38–40 °C; ^{1}H NMR ($CDCl_3$) δ 2.25 (d, 3 H, J = 2.4 Hz, Me), 4.00 (m, 6 H, OMe); MS m/e 233 (M^+). Anal. ($C_9H_9F_2NO_4$) C, H, N.

1,4-Difluoro-2,3-[(diphenylmethylene)dioxy]-5-methyl-6-nitrobenzene (17). Catechol **16**, obtained in 98% yield by the demethylation of **13** (as described above for the synthesis of **8a**), was utilized in the next step without further purification: ^{1}H NMR ($CDCl_3$) δ 2.28 (d, 3 H, J = 2.4 Hz, Me), 5.53–6.18 (br, 2 H, OH).

A mixture of the crude catechol **16** (11.15 g, 54 mmol) and Ph_2CCl_2 (11.5 mL, 59 mmol) was maintained at 160 °C for 6 min. The cooled reaction mixture was extracted with EtOAc (3 × 80 mL). The combined extracts were washed successively with 8% $NaHCO_3$ (80 mL) and saturated NaCl (80 mL) and dried (Na_2SO_4). Evaporation of the solvent and subsequent recrystallization of the residue gave 15.9 g (79%) of **17**: mp 129–130 °C (Et_2O); ^{1}H NMR ($CDCl_3$) δ 2.23 (d, 3 H, J = 2.4 Hz, Me), 7.19–7.68 (m, 10 H, Ph); MS m/e 369 (M^+). Anal. ($C_{20}H_{13}F_2NO_4$) C, H, N.

4,7-Difluoro-5,6-[(diphenylmethylene)dioxy]indole (19). A solution of **17** (738 mg, 2 mmol), $Me_2NCH(OMe)_2$ (0.64 mL, 4.8 mmol), and Et_3N (0.33 mL, 2.4 mmol) in dry DMF (3 mL) was stirred under an Ar atmosphere at 120–130 °C for 15 h. The dark red solution of the enamine **18** was then evaporated at 80 °C (5 mm). The residue was dissolved in a solution of PhMe–HOAc (5:3, 6 mL) and added to an efficiently stirred mixture of Fe (2 g) and Florisil (100–200 mesh, Aldrich, 5 g) in PhMe–HOAc (5:3, 26 mL) at 25 °C. The mixture was then refluxed under an Ar atmosphere for 1.5 h. Indole **19** was isolated in 53% yield as described for **12a**. An analytical sample was prepared by recrystallization from PhH–hexane: mp 176–178 °C; ^{1}H NMR ($CDCl_3$) δ 6.53 (m, 1 H, H-3), 7.03 (t, 1 H, J = 2.4 Hz, H-2), 7.27–7.45 (m, 6 H), 7.54–7.71 (m, 4 H), 8.17 (br s, 1 H, NH); IR (Nujol) 3500 cm^{-1}; MS m/e 349 (M^+). Anal. ($C_{21}H_{13}F_2NO_2$) C, H, N.

1,2-[(Diphenylmethylene)dioxy]-3-fluoro-4-methyl-5-nitro-6-piperidinobenzene (20) and 5,6-[(Diphenylmethylene)dioxy]-4-fluoro-7-piperidinoindole (21). Nitrotoluene **17** (738 mg, 2 mmol) was subjected to the same reaction conditions which were used for the synthesis of **12a** from **10a**. The crude product was chromatographed on a column of silica gel with CH_2Cl_2–hexane (1:2) as the eluent to give **20** (159 mg, 18.3%) as

a gum: ^{1}H NMR ($CDCl_3$) δ 1.33–1.83 (m, 6 H, CH_2), 2.09 (d, 3 H, J = 2.2 Hz, Me), 2.85–3.23 (m, 4 H, CH_2), 7.18–7.77 (m, 10 H, Ph); MS m/e 434 (M^+); HR MS m/e calcd for $C_{25}H_{23}FN_2O_4$ 434.1640, found 434.1647.

The second compound eluted with CH_2Cl_2–hexane (2:1) was **21** (174 mg, 21%). It was further purified by recrystallization from PhH–hexane: mp 168–171 °C; ^{1}H NMR ($CDCl_3$) δ 1.49–1.89 (m, 6 H, CH_2), 2.96–3.28 (m, 4 H, CH_2), 6.43 (t, 1 H, J = 2.6 Hz, H-3), 6.90 (t, 1 H, J = 2.6 Hz, H-2 7.10–7.80 (m, 10 H, Ph), 8.40 (br s, 1 H, NH); MS m/e 414 (M^+); HR MS m/e calcd for $C_{26}H_{23}FN_2O_2$ 414.1742, found 414.1739.

5,6-Bis(benzyloxy)-3-(cyanomethyl)-4-fluoroindole (24a). To a stirred solution of 37% aqueous CH_2O (400 mg, 5 mmol) and 40% Me_2NH (600 mg, 5 mmol) in HOAc–EtOH (1:1, 16 mL) at 0–5 °C was added a solution of **12a** (794 mg, 2 mmol) in HOAc–EtOH (1:1, 5 mL). After being stirred at 0–5 °C for 2 h and then at 25 °C for 12 h, the mixture was diluted with H_2O (100 mL) and made strongly basic (pH > 10) with 4 N NaOH while cooling in an ice bath. The mixture was extracted with CH_2Cl_2 (3 × 20 mL). The combined extracts were washed with saturated NaCl (30 mL) and dried (K_2CO_3). The solvent was evaporated in vacuo to give the gramine **22a** as gum which was used in the next step without purification.

To a stirred solution of MeI (14.5 g, 100 mmol) in EtOH (20 mL) at 0–5 °C, protected from moisture, was added a solution of the crude gramine **22a** (808 mg, 2 mmol) in EtOAc (20 mL) dropwise. The mixture was refrigerated for 24 h, and then the volatiles were evaporated in vacuo at 30 °C to give the methiodide **23a** as a gum that was used in the next step without further purification.

To a stirred solution of crude **23a** in DMF (10 mL) at 75 °C was immediately added a solution of KCN (520 mg, 8 mmol) in H_2O (6 mL). The mixture was then heated with stirring at 75 °C for 1.5 h, cooled to 25 °C, and diluted with H_2O (150 mL). The mixture was kept at 0 °C for 1 h and then the supernatant was discarded. The residue was washed with H_2O (2 × 30 mL) and dissolved in CH_2Cl_2 (100 mL) and the CH_2Cl_2 solution was washed with saturated NaCl (50 mL). After drying (Na_2SO_4), the solvent was evaporated in vacuo. The residue was chromatographed on a column of silica gel using CH_2Cl_2 as eluent to give 548 mg (71%) of **24a**. An analytical sample was prepared by recrystallization from PhH: mp 139–141 °C; ^{1}H NMR ($CDCl_3$) δ 3.93 (s, 2 H, CH_2), 5.10 (s, 2 H, CH_2), 5.13 (s, 2 H, CH_2), 6.70 (s, 1 H, H-7), 7.08 (s, 1 H, H-2), 7.31–7.42 (m, 10 H, Ph), 7.99 (br s, 1 H, NH); IR (Nujol) 2250, 3450 cm^{-1}; MS m/e 386 (M^+). Anal. ($C_{24}H_{19}FN_2O_2$) C, H, N.

5,6-Bis(benzyloxy)-3-(cyanomethyl)-7-fluoroindole (24b) was obtained in 82% yield from **21b** by the procedure for **24a**: mp 130–132 °C (PhH); ^{1}H NMR ($CDCl_3$) δ 3.73 (d, 2 H, J = 0.8 Hz, CH_2), 5.13 (s, 2 H, OCH_2), 5.15 (s, 2 H, CH_2), 6.88 (d, 1 H, J = 1.6 Hz, H-4), 7.14 (dd, 1 H, J = 0.8 Hz, H-2), 7.27–7.50 (m, 10 H, Ph), 8.14 (br s, 1 H, NH); IR (Nujol) 2250, 3360 cm^{-1}; MS m/e 386 (M^+). Anal. ($C_{24}H_{19}FN_2O_2$) C, H, N.

3-(Cyanomethyl)-4,7-difluoro-5,6-[(diphenylmethylene)-dioxy]indole (24c) was obtained in 78% yield from **21c** by using a procedure similar to that for **24a**, except that 37% aqueous CH_2O and 40% aqueous Me_2NH were used in 5 molar excess and the reaction time was 48 h for the synthesis of gramine **22c**: mp 193–195 °C (PhH); ^{1}H NMR ($CDCl_3$) δ 3.87 (d, 2 H, J = 0.8 Hz, CH_2), 7.07 (m, 1 H, H-2), 7.29–7.53 (m, 6 H), 7.55–7.70 (m, 4 H), 8.15 (br s, 1 H, NH); IR (Nujol) 2260, 3360, 3430 cm^{-1}; MS m/e 388 (M^+). Anal. ($C_{23}H_{14}F_2N_2O_2$) C, H, N.

Dihydroxytryptamine Creatinine Sulfates (26a–c). General Procedure.[6,10,13] To a stirred suspension of $LiAlH_4$ (570 mg, 15 mmol) in dry Et_2O (30 mL) under an Ar atmosphere was gradually added a suspension of nitrile **24a**, **24b**, or **24c** (1 mmol) in dry PhH (30 mL) and the mixture was refluxed for 5 h. The mixture was cooled in an ice bath and excess $LiAlH_4$ was decomposed by carefully adding H_2O. The organic solution was collected by filtration and was washed with H_2O (2 × 50 mL), dried (K_2CO_3), and evaporated in vacuo to give the tryptamines **25a,b,c** in greater than 90% yield in each case. These tryptamines (pure by ^{1}H NMR) were utilized in the next step without further purification.

25a: ^{1}H NMR ($CDCl_3$) δ 1.41 (br s, 2 H, NH_2), 2.93–3.00 (m, 4 H, CH_2CH_2), 5.11 (s, 2 H, OCH_2), 5.15 (s, 2 H, OCH_2), 6.70 (s, 1 H, H-7), 6.87 (s, 1 H, H-2), 7.33–7.51 (m, 10 H, Ph), 7.88 (s, 1 H, NH).

25b: ^{1}H NMR ($CDCl_3$) δ 2.04 (br s, 2 H, NH_2), 2.76–3.06 (m, 4 H, CH_2CH_2), 5.11 (s, 2 H, OCH_2), 5.13 (s, 2 H, OCH_2), 6.90 (d, 1 H, J = 1.6 Hz, H-4), 6.98 (s, 1 H, H-2), 7.29–7.47 (m, 10 H, Ph), 8.05 (br s, 1 H, NH).

25c: mp, softening and color change at 198 °C, 236 °C dec; ^{1}H NMR ($CDCl_3$) δ 1.36 (s, 2 H, NH_2), 2.92 (m, 4 H, CH_2CH_2), 6.84 (s, 1 H, H-2), 7.28–7.45 (m, 6 H), 7.52–7.71 (m, 4 H), 8.09 (br s, 1 H, NH).

To a solution of **25a**, **25b**, or **25c** (1 mmol) in deoxygenated 95% EtOH (50 mL) were added 1 M H_2SO_4 (0.98 mL, 0.98 mmol) and 10% Pd/C (200 mg). The mixture was shaken in a Parr shaker at 40 psi of H_2 for 5 h at 25 °C. (All the operations described below were conducted, as far as practicable, in a positive Ar atmosphere.) The mixture was then filtered under gravity, and a solution of creatinine (109 mg, 0.96 mmol) in deoxygenated H_2O (1 mL) was added to the filtrate. The resulting cloudy mixture was evaporated in vacuo at <45 °C to dryness. The residue was washed with deoxygenated acetone and then dissolved in deoxygenated H_2O and filtered. To the filtrate was added deoxygenated acetone until precipitation of some solid had begun, and the mixture was then stored at −20 °C overnight. White solid was obtained in each case and was collected by filtration and dried under vacuum.

5,6-Dihydroxy-4-fluorotryptamine creatinine sulfate (26a): yield 73%; mp (color change at 198 °C) 216 °C dec; ^{1}H NMR (D_2O) δ 2.93 (s, 3 H, Me), 2.94 (m, 2 H, CH_2), 3.12 (m, 2 H, CH_2), 4.05 (s, 2 H, CH_2 of creatinine), 6.61 (s, 1 H, H-2), 6.88 (s, 1 H, H-7). Anal. ($C_{14}H_{20}FN_5O_7S\cdot 2H_2O$) C, H, N.

5,6-Dihydroxy-7-fluorotryptamine creatinine sulfate (26b): yield 79%; mp (color change at 183 °C) 213 °C dec; ^{1}H NMR (D_2O) δ 2.88 (m, 2 H, CH_2), 2.93 (s, 3 H, Me), 3.11 (m, 2 H, CH_2), 4.05 (s, 2 H, CH_2 of creatinine), 6.71 (s, 1 H, H-2), 7.00 (s, 1 H, H-4). Anal. ($C_{14}H_{20}FN_5O_7S\cdot 2H_2O$) C, H, N.

4,7-Difluoro-5,6-dihydroxytryptamine creatinine sulfate (26c): yield 79%; mp (color change at 188 °C) 196 °C dec; ^{1}H NMR (D_2O) δ 2.94 (m, 2 H, CH_2), 2.96 (s, 3 H, Me), 3.12 (m, 2 H, CH_2), 4.10 (s, 2 H, CH_2 of creatinine), 6.93 (s, 1 H, H-2). Anal. ($C_{14}H_{19}F_2N_5O_7S\cdot 2H_2O$) C, H, N.

Determination of Phenolic Ionization Constants of 5,6-DHT and 26a,b. The stock solution of each of the test compounds was made in deoxygenated H_2O just prior to the recording of the pH-dependent UV absorption spectra. An aliquot (0.05 mL) of the stock solution was diluted into 3 mL of the deoxygenated buffer which ranged in pH from 1.42 to 12.64. The analytical wavelength in each case was 310 nm. The search for an approximate value of pK_a and the exact determination of pK_a for each test compound were carried out as prescribed by Albert and Sergeant.[14] No attempts were made to account for possible effects of side-chain amine protonation/deprotonation. The absorption maxima of 5,6-DHT and **26a–c** at three representative pH values are given in Table I.

Cyclic Voltammetry. The oxidation potentials of 5,6-DHT and **26a–c** were measured by cyclic voltammetry. The electrochemical cell had the standard three-electrode configuration with a carbon paste working electrode, a saturated calomel reference electrode, and a platinum foil auxiliary electrode.[16] The carbon paste was prepared by mixing ultracarbon (Ultra F purity) and hexadecane in a ratio of 2:1 by weight. Each voltammogram was generated by using a freshly prepared electrode surface which was ~1.5 mm^2. The solvent/electrolyte was 1 M H_2SO_4 which was freed of dissolved O_2 by purging with Ar for at least 1 h. The cyclic voltammograms were recorded while the test solution was maintained quiet in an Ar atmosphere with an IBM EC 225 voltammetric analyzer.

Biology. Materials. Buffer A was an isotonic Dulbecco's phosphate-buffered saline containing 129 mM NaCl, 2.5 mM KCl, 7.4 mM $Na_2HPO_4\cdot 7H_2O$, and 1.3 mM KH_2PO_4. Buffer B (DMEM) was prepared fresh by supplementing buffer A with 0.63 mM $CaCl_2\cdot 2H_2O$, 0.74 mM $MgSO_4$, 5.3 mM glucose, and 0.1 mM ascorbic acid. [1,2-^{3}H]5-HT creatinine sulfate (23.4–26.7 Ci/mmol) was purchased from New England Nuclear Corp. (Boston, MA) and diluted with buffer B to give a final concentration of 0.1 μM in the incubation mixture. [*methyl*-^{3}H]Thymidine (20 Ci/mmol) was from Research Products International Corp. (Elk Grove

Village, IL). 5,6-DHT creatinine sulfate was from Sigma Chemical Co. (St. Louis, MO). Fetal calf serum (FCS) was from Hazelton Research Products, Denver, PA. GF/C glass-fiber filters were from Millipore Corp. (Bedford, MA).

Cell Cultures, Incubation Conditions, and Procedures. Starter cultures of neuroblastoma cells, clone N-2a, were kindly provided by Dr. X. O. Breakfield (Yale University School of Medicine). Conditions for growing and differentiating cells were similar to those described earlier[18] except that the medium used was DMEM supplemented with 8% FCS when first plated. All experiments to determine the cytotoxicity and inhibition of [^{3}H]5-HT uptake were conducted on cell suspensions rather than monolayer cultures. All experiments were done with differentiated N-2a cells that were suspended in buffer B by trituration, washed three times with use of a Dynac centrifuge, and resuspended in the same buffer. Cell viability, as determined by trypan blue exclusion, was found to be more than 85%. An aliquot of 10^6 cells per tube, counted by using a hemocytometer, was used for all experiments.

Cytotoxicity of 5,6-DHTs. Cytotoxicity was determined by measuring the inhibition of [^{3}H]thymidine incorporation into the DNA of the N-2a cells. The cells (10^6) were incubated at 37 °C in an incubation mixture of 1 mL of DMEM containing 50, 100, 250, 500, or 1000 μM concentrations of the DHTs. Solutions containing the neurotoxins were made in DMEM immediately before addition to the incubation mixture. The cells were separated by centrifugation and washed with buffer A. Finally, the cell pellets were suspended in 0.5 mL of DMEM containing 50 nM [^{3}H]thymidine (0.5 μCi) and incubated at 37 °C for an additional 90 min. The incubation was terminated by adding 2.5 mL of cold 10% trichloroacetic acid (TCA) and the mixture was kept for 12 h at 0-4 °C. The TCA-precipitated fraction was collected by filtration on GF/C glass fiber filters in a Millipore manifold previously wetted with TCA and washed three times each with 2.5 mL of 95% EtOH. The filter papers containing the precipitates were then carefully transferred to the scintillation vials, dried in an oven at 60-65 °C for 15 min and then digested with 0.5 mL of NCS tissue solubilizer (Amersham) for 30 min. To each sample was added 10 mL of complete counting cocktail, Biosafe II (Research Products International Corp.), and the samples were counted, after overnight storage in the dark, on a Beckman 5801 scintillation counter. Cytotoxicity was expressed (mean ± SD of at least three sets of duplicates) as plots of percent inhibition of [^{3}H]thymidine incorporation with respect to the cells not incubated with the neurotoxins. From these plots (not shown), concentrations required to give 50% inhibition of [^{3}H]thymidine incorporation (IC_{50} values) were calculated by using regression analysis (Table II).

Uptake Affinities. Inhibition of [^{3}H]5-HT uptake was determined by coincubation of cell suspension (10^6) and the respective neurotoxins either at 0-4 °C or at 37 °C for 2 min in an incubation mixture of 1 mL of buffer B containing 0.1 μM [^{3}H]5-HT (4 μCi). Neurotoxin solutions were made in buffer B immediately before addition to the incubation mixture to give a final concentration of 1-100 μM. The uptake experiment was stopped by the addition of 2.5 mL of cold buffer A followed by immediate filtration through GF/C glass fiber filters in a Millipore manifold and washing (5 × 2.5 mL) with the same cold buffer. The filter papers containing the cells were then carefully transferred to the scintillation vials, and the radioactivity was counted as described above. The active uptake is expressed (mean ± SD of at least three sets of duplicates) as the total accumulation of radioactivity at 37 °C minus the accumulation at 0-4 °C (passive diffusion). The degree of potency of the neurotoxins in inhibiting the [^{3}H]5-HT uptake was determined by comparing the concentrations required to give 50% inhibition of active uptake (IC_{50} values) measured at 37 °C. These were calculated by plotting the percentage inhibition of active uptake vs concentration and by using regression analysis (Table III).

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Synthesis, Phencyclidine-like Pharmacology, and Antiischemic Potential of Meta-Substituted 1-(1-Phenylcyclohexyl)-1,2,3,6-tetrahydropyridines

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A series of 1-[1-arylcyclohexyl]-1,2,3,6-tetrahydropyridines were prepared by the reaction between 1-(1-cyanocyclohexyl)-1,2,3,6-tetrahydropyridine (1) and an appropriately substituted Grignard reagent. The resulting compounds were tested for their phencyclidine binding site affinities. Selected compounds were then tested for their ability to produce ketamine appropriate responding in monkeys and/or to show neuroprotective effects in a baby rat hypoxia/ischemia model. While it was found that binding site affinity correlated well with discriminative stimulus effects, it was found to be a poor indicator of neuroprotective efficacy within this series.

Recent work by a number of investigators[1-6,11,12] has indicated that compounds with affinity phencyclidine (PCP) binding sites in the central nervous system can exhibit neuroprotective effects in animal models of ischemia. For example, the tricyclic MK-801 has high affinity for PCP binding sites[7] and has been shown to be effective in various animal models of neuroprotection.[8-11] PCP, with a much lower affinity for these sites, exhibits anti-ischemic neuroprotective effects only at much higher

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(1) Mc Donald, J. W.; Silverstein, F. S.; Cardona, D.; Uckele, J.; Chen, R.; Johnston, M. V. In *Sigma and Phencyclidine-Like Compounds as Molecular Probes in Biology*; Domino, E. F., Kamenka, J. M., Eds.; NPP Books: Ann Arbor, MI, 1988; pp 697-708.
(2) Goldberg, M. P.; Visekus, V.; Choi, D. W. In *Sigma and Phencyclidine-Like Compounds as Molecular Probes in Biology*; Domino, E. F., Kamenka, J. M., Eds.; NPP Books: Ann Arbor, MI, 1988; pp 709-716.

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