Iodinated Tomoxetine Derivatives as Selective Ligands for Serotonin and Norepinephrine Uptake Sites

Sumalee Chumpradit, Mei-Ping Kung,† Chitchanum Panyachotipun, Vichukorn Prapansiri, Catherine Foulon, Brian P. Brooks, Stephen A. Szabo, Shanaz Tejani-Butt,† Alan Frazer,† and Hank F. Kung*

Departments of Radiology and Psychiatry, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Received March 18, 1992

In order to develop selective radioactive ligands for the study of presynaptic monoamine uptake sites, iodinated derivatives of tomoxetine were synthesized and evaluated in radioligand binding assays. Iodotomoxetine derivatives showed high affinity for serotonin (5-HT) uptake sites using a rat cortical membrane preparation. Compound **1R**, (*R*)-(−)-*N*-methyl-3-(4-iodo-2-methylphenoxy)-3-phenylpropanamine, was the most potent and showed high stereoselectivity for 5-HT uptake sites (K_i, *R* isomer = 0.65 nM, *S* isomer = 13.9 nM). Changing the position of the methyl group or eliminating the methyl group at the phenoxy ring resulted in a loss of stereoselectivity. Substitution of the methyl group of tomoxetine with iodine gave the *R* and *S* isomers of *N*-methyl-3-(2-iodophenoxy)-3-phenylpropanamine **4R** and **4S**. These compounds displayed stereoselectivity for the norepinephrine (NE) (K_i values = 0.24 and 9.35 nM for *R* and *S* isomers, respectively). The in vitro binding data suggest that **1R** and **4R** are potential radioiodinated ligands for pharmacological studies of 5-HT and NE uptake sites, respectively.

Introduction

Abnormalities in monoamine neurotransmission have been implicated in the etiology of affective disorders. Increased noradrenergic or serotonergic transmission, caused by inhibition of norepinephrine (NE) or 5-hydroxytryptamine (5-HT) uptake, has been suggested as one of the major mechanisms of the action of antidepressive agents.[1,2] For example, the antidepressant properties of fluoxetine (Figure 1) have been attributed, at least in part, to its potent and selective inhibition of 5-HT uptake.[3,4] Fluoxetine represents the first selective 5-HT uptake inhibitor to be approved for clinical use in the U.S.

Radiolabeled ligands selective for specific monoamine uptake sites provide powerful tools with which to further evaluate the involvement of these neuronal systems in affective disorders. Radioligands which have been developed to study 5-HT uptake sites in brain tissue include [^{3}H]imipramine,[5] [^{3}H]paroxetine,[6,7] [^{3}H]citalopram,[8] and [^{3}H]-6-nitroquipazine.[9,10] A number of selective ligands for studying NE uptake sites have also been reported, namely, [^{3}H]desipramine,[11,12] [^{3}H]mazindo,[13,14] and [^{3}H]-nisoxetine.[15,16] However, [^{3}H]desipramine has been shown to bind to both high- and low-affinity sites and shows high nonspecific binding. [^{3}H]Mazindol is nonselective and

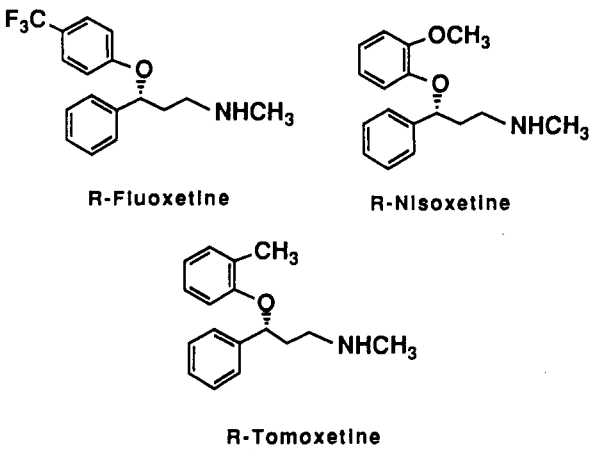


Figure 1. The structures of (*R*)-fluoxetine, (*R*)-nisoxetine and (*R*)-tomoxetine.

* Address for correspondence: Hank F. Kung, Ph.D., University of Pennsylvania, Department of Radiology, Rm 305, 3700 Market St., Philadelphia, PA 19104. Phone: (215) 662-3989. FAX: (215) 349-5035.
† Department of Psychiatry.

(1) Bøgesø, K.; Christensen, A.; Hyttel, J.; Liljefors, T. 3-Phenyl-1-indanamines. Potential antidepressant activity and potent inhibition of dopamine, norepinephrine, and serotonin uptake. *J. Med. Chem.* **1985**, *28*, 1817-1828.
(2) Ives, J.; Heym, J. Antidepressant agents. *Annu. Rep. Med. Chem.* **1989**, *24*, 21-29.
(3) Wong, D. T.; Horng, J. S.; Bymaster, K. P.; Hauser, K. L.; Molloy, B. B. A selective inhibitor of serotonin uptake: Lilly 11014, 3-(p-trifluoromethylphenoxy)-N-methyl-3-phenylpropylamine. *Life Sci.* **1974**, *15*, 471.
(4) Wong, D. T.; Bymaster, F. P.; Horng, J. S.; Molly, B. B. A new selective inhibitor for uptake of serotonin into synaptosomes of rat brain: 3-(p-trifluoromethylphenoxy)-N-methyl-3-phenylpropylamine. *J. Pharmacol. Exp. Ther.* **1975**, *193*, 804-811.
(5) Rehavi, M.; Paul, S. M.; Skolnick, A.; Goodwin, F. K. Demonstration of specific high affinity binding sites for [^{3}H]imipramine in human brain. *Life Sci.* **1980**, *26*, 2273-2279.
(6) Habert, E.; Graham, D.; Tahraoui, L.; Claustre, Y.; Langer, S. Characterization of [^{3}H]paroxetine binding to rat cortical membranes. *Eur. J. Pharmacol.* **1985**, *118*, 107-114.
(7) Scheffel, U.; Hartig, P. In vivo labeling of serotonin uptake sites with [^{3}H]paroxetine. *J. Neurochem.* **1989**, *52*, 1605-1612.
(8) D'Amato, R.; Largent, B.; Snowman, A.; Snyder, S. Selective Labeling of Serotonin Uptake Sites in Rat Brain by [^{3}H]Citalopram Contrasted to Labeling of Multiple Sites by [^{3}H]Imipramine. *J. Pharmacol. Exp. Ther.* **1987**, *242*, 364-371.
(9) Hashimoto, K.; Inoue, O.; Goromaru, T.; Yamasaki, T. Changes in in vivo binding of 3H-Ro 15-1788 in mouse brain by reserpine. *Int. J. Radiat. Appl. Instrum, Part B* **1988**, *15*, 637-644.
(10) Hashimoto, K.; Goromaru, T. J. In vitro labeling of 5-hydroxytryptamine uptake sites in mouse brain with [^{3}H]-6-nitroquipazine. *J. Pharmacol. Exp. Ther.* **1990**, *255*, 146-153.
(11) Lee, C. M.; Snyder, S. H. Norepinephrine neuronal uptake binding sites in rat brain membranes labeled with [^{3}H]desimipramine. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 5250-5254.
(12) Lee, C. M.; Javitch, J. A.; Snyder, S. H. Characterization of [^{3}H]-desimipramine binding associated with neuronal norepinephrine uptake sites in rat brain membranes. *J. Neurochem.* **1982**, *2*, 1515-1525.
(13) Javitch, J. A.; Blaustein, R. O.; Snyder, S. H. [^{3}H]Mazindol binding associated with neuronal dopamine and norepinephrine uptake sites. *Mol. Pharmacol.* **1984**, *26*, 35-44.
(14) Javitch, J. A.; Strittmatter, S. M.; Snyder, S. H. Differential visualization of dopamine and norepinephrine uptake sites in rat brain using [^{3}H]mazindol autoradiography. *J. Neurosci.* **1985**, *5*, 1513-1521.
(15) Tejani-Butt, S. M.; Brunswick, D. J.; Frazer, A. [^{3}H]Nisoxetine: A new radioligand for norepinephrine uptake sites in brain. *Eur. J. Pharmacol.* **1990**, *191*, 239-243.

0022-2623/92/1835-4492$03.00/0

also binds with high affinity to dopamine (DA) uptake sites. In contrast, nisoxetine was a potent and selective NE uptake inhibitor, blocking the uptake of NE approximately 1000 times more effectively than 5-HT and 400 times more effectively than dopamine.[17,18] Consequently, the high specificity of [^{3}H]nisoxetine for NE uptake sites makes it useful for in vitro radioligand binding and autoradiographic studies involving the NE uptake site.[16]

Recently, a close structural analog of nisoxetine, tomoxetine (Figure 1), was reported as a potent and selective inhibitor of NE uptake sites and is currently under clinical evaluation as a potential antidepressant agent.[18,19] In contrast to nisoxetine, which shows no stereoselectivity (K_i values = 3.8 and 8.4 nM for the *R* and *S* isomers, respectively), the *R* and *S* isomers of tomoxetine exhibit significant stereoselectivity for the NE uptake site. The K_i for inhibition of NE uptake is 1.9 nM for the *R*(−) isomer and 16.8 nM for the *S*(+) isomer.[18] (*R*)-Tomoxetine also shows specificity for NE uptake sites relative to other monoamine uptake sites. Thus, the inhibition of NE uptake by (*R*)-tomoxetine is 400 times greater than its inhibition of 5-HT uptake sites and ~800 times greater than for dopamine uptake sites. (*R*)-Tomoxetine has also been shown to have little or no affinity for other receptor sites in rat brain, including adrenergic, acetylcholine muscarinic, and histaminergic receptors.[18] This high selectivity may have clinical advantages, since the undesirable side effects of some antidepressants may be attributed to their affinity for other receptor sites.[20] Therefore, apart from the potential clinical utility, tomoxetine or derivatives, may provide more selective radioligands for in vitro and in vivo studies of the NE uptake site.

Positron emission tomography (PET) and single photon emission computed tomography (SPECT) make possible direct study and the quantitation of neurotransmitter system in the intact human brain. Several C-11 and F-18 ligands for in vivo studies of 5-HT uptake sites with PET were reported; these include [^{11}C]cyanoimipramine,[21] [^{11}C]-fluoxetine,[22] [^{11}C]setraline,[23] [*fluoropropyl*-^{18}F]paroxetine,[24] [^{11}C]citalopram,[25,26,22] McN5652,[27] *cis*-[^{11}C]-DPPI,[28] as well as [^{11}C]nisoxetine[29] for NE uptake sites. The high specific activity of the radioiodinated ligands (specific activity 2000 Ci/mmol for I-125) compared to tritiated ligands (specific activity ~85 Ci/mmol) allows the detection of low concentrations of receptor sites and offers advantages of quantitative autoradiography. In addition when labeled with I-123, the optimal physical characteristic (K_{ev} = 150, $t_{1/2}$ = 13.5 h) will provide direct information on the location and quantitation of the uptake sites in conjunction with SPECT.

We are interested in developing selective radioiodinated fluoxetine and tomoxetine derivatives as tools for in vitro (radioligand binding and autoradiographic studies) and potentially, in vivo (SPECT imaging techniques) mapping of 5-HT and NE uptake sites. Previous studies of iodofluoxetine, where the iodine was introduced at the para position of the phenyl ring, showed decreased affinity for 5-HT uptake sites.[30] This result is consistent with an earlier literature report which indicated that trifluoromethyl placed at the para position of the phenyl ring significantly reduces the affinity of the compound for 5-HT uptake sites (IC_{50}, 2.6×10^{-6} M vs 0.07×10^{-6} M).[4,17] Therefore, structural changes in the phenyl ring of the fluoxetine molecule have been shown to have detrimental effects on binding to the 5-HT uptake site. The goal of the present work was to further investigate the structure–activity relationships of derivatives with substitutions on the phenoxy ring. Consequently, derivatives possessing a variety of aromatic substitution patterns have been synthesized and evaluated as potential ligands for the 5-HT, NE, and DA uptake sites. The results of the study demonstrate that small changes in the substitution pattern of the phenoxy ring of these compounds can have profound effects on the selectivity of the ligands for 5-HT vs NE uptake sites.

Chemistry

Using Mitsunobu reaction conditions,[31,32] iodinated and brominated cresols or iodophenols were condensed with *R*-(+)- or *S*-(−)-3-chloro-1-phenyl-1-propanol in the presence of diethyl azodicarboxylate (DEAD) and triphenyl phosphine.[32] As expected, the reactions resulted in

(16) Tejani-Butt, S. M. [^{3}H]Nisoxetine: A radioligand for quantitation of norepinephrine uptake sites by autoradiography or by homogenate binding. *J. Pharmacol. Exp. Ther.* **1992**, *260*, 427–436.

(17) Wong, D. T.; Horng, J. S.; Bymaster, F. P. dl-N-Methyl-3-(2-methoxyphenoxy)-3-phenylpropylamine hydrochloride, Lilly 94939, a potent inhibitor for uptake of norepinephrine into rat brain synaptosomes and heart. *Life Sci.* **1975**, *17*, 755–760.

(18) Wong, D. T.; Threlkeld, P. G.; Best, K. L.; Bymaster, F. P. A new inhibitor of norepinephrine uptake devoid of affinity for receptors in rat brain. *J. Pharmacol. Exp. Ther.* **1982**, *222*, 61–65.

(19) Zerbe, R. L.; Rowe, H.; Enas, G. G.; Wong, D.; Farid, N.; Lemberger, L. Clinical pharmacology of tomoxetine, a potential antidepressant. *J. Pharmacol. Exp. Ther.* **1985**, *232*, 139.

(20) Richelson, E. Tricyclic antidepressants: Interaction with histamine and muscarinic acetylcholine receptors. In *Antidepressants: Neurochemical Behavioral and Clinical Perspectives*; Enna, S. J., Malick, J. B., Richelson, E., Eds.; New York: Raven Press, 1981.

(21) Hashimoto, K.; Hashimoto, K.; Inoue, O.; Suzuki, K.; Yamasaki, T.; Kojima, M. Synthesis and evaluation of ^{11}C-cyanoimipramine. *Nucl. Med. Biol.* **1987**, *14*, 587–592.

(22) Scheffel, U.; Dannals, R.; Suehiro, M.; Wilson, A.; Ravert, H.; Stathis, M.; Wagner, H., Jr.; Ricaurte, G. Evaluation of ^{11}C-citalopram and ^{11}C-fluoxetine as in vivo ligands for the serotonin uptake site (abstract). *J. Nucl. Med.* **1990**, *31*, 883.

(23) Lasne, M. C.; Pike, V. W.; Turton, D. R. The radiosynthesis of [N-methyl-^{11}C]-sertraline. *Int. J. Radiat. Appl. Instrum., Part A* **1989**, *40*, 147–151.

(24) Suehiro, M.; Wilson, A. A.; Scheffel, U.; Dannals, R. F.; Ravert, H. T.; Wagner, H. J. Radiosynthesis and evaluation of N-(3-^{18}F fluoropropyl)paroxetine as a radiotracer for in vivo labeling of serotonin uptake sites by PET. *Int. J. Radiat. Appl. Instrum., Part B.* **1991**, *18*, 791–796.

(25) Pascali, C.; Hume, S.; Turton, D.; Bateman, D.; Pike, V. Citalopram: Labelling with ^{11}C and evaluation in rat as a potential PET radioligand for 5-HT re-uptake sites [abstract]. *J. Labelled Compd. Radiopharm.* **1991**, *30*, 392–393.

(26) Ram, S.; Krishnan, K.; Bissette, G.; Knight, D.; Coleman, R. Synthesis of [^{11}C]citalopram and brain distribution studies in rats. *J. Labelled Compd. Radiopharm.* **1991**, *30*, 405–407.

(27) Suehiro, M.; Scheffel, U. Synthesis and Biological Evaluation of a PET Radiotracer for Studying Serotonin Uptake Sites (abstract). *J. Nucl. Med.* **1992**, *33*, 860.

(28) Suehiro, M.; Scheffel, U.; Dannals, R. F.; Wilson, A. A.; Ravert, H. T.; Wagner, H. N. J. Synthesis and Biodistribution of a New Radiotracer for *In Vivo* Labeling of Serotonin Uptake Sites by PET. *Nucl. Med. Biol.* **1992**, *19*, 549–553.

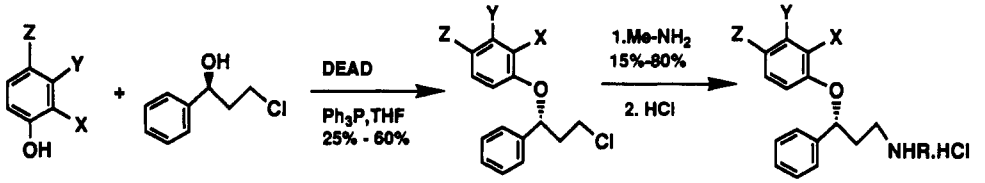
(29) Haka, M.; Kilbourn, M. Synthesis and regional distribution of [^{11}C]nisoxetine, a norepinephrine uptake inhibitor. *Nucl. Med. Biol.* **1989**, *16*, 771–774.

(30) Kung, M. P.; Chumpradit, S.; Panyachotipun, C.; Prapansiri, N.; Billings, J.; Kung, H. F. Radioiodinated analogs of fluoxetine: Potential agents for imaging serotonin reuptake sites with SPECT (abstract). *J. Nucl. Med.* **1991**, *32*, 964.

(31) Mitsunobu, O. The use of diethyl azodicarboxylate and triphenylphosphine in synthesis and transformation of natural products. *Synthesis* **1981**, 1–30.

(32) Srebnik, M.; Ramachandran, P. V.; Brown, H. C. Chiral synthesis via organoboranes. 18. Selective reductions. 43. Diisopinocamphenyl chloroboranes as an excellent chiral reducing reagent for the synthesis of halo alcohols of high enantiomeric purity. A higher enantioselective synthesis of both optical isomers of tomoxetine, fluoxetine, and nisoxetine. *J. Org. Chem.* **1990**, *55*, 5287–5291.

Scheme I. Synthesis of Iodotomoxetine, Demethylated Iodotomoxetine Derivatives* and Inhibition of Binding of Different Monoamine Uptake Sites in Rat Cortical and Striatal Membranes[a]



*Same scheme was used for the preparation of both R- and S- isomers.

					Ki(nM±SEM)		
Compound	X	Y	Z	R	[^{3}H]Citalopam (5-HT)	[^{3}H]Nisoxetine (NE)	[^{125}I]-β-CIT (DA)
1, R	CH_3	H	I	CH_3	0.65±0.04	69±27	845±93
S					13.9±1.0	1159±182	636±95
2, R	H	CH_3	I	CH_3	0.69±0.13	128±32	220±60
S					0.30±0.60	351±53	200±50
3, R	I	H	CH_3	CH_3	5.18±0.51	3.4±0.8	1100±300
S					5.73±1.14	154±22	410±61
4, R	I	H	H	CH_3	17.1±1.2	0.24±0.03	-----
S					13.1±1.7	9.35±1.85	-----
5, R	H	I	H	CH_3	14.4±3.0	1.4±0.17	486±82
S					4.0±0.5	1.8±0.24	208±41
6, R	H	H	I	CH_3	1.27±0.2	3.21±6.5	-----
S					0.80±0.2	245±25	-----
R-TMT	CH_3	H	H	CH_3	25.1±1.0	-----	-----

[a] Radioligand was incubated in the presence of the indicated compound in 7–11 concentrations of rat membrane preparation. Each value represents the mean ± SEM of two to three separate experiments performed in duplicate.

complete inversion of the chiral benzylic carbon to give the corresponding chloro compounds (Scheme I). Treatment of these chloro compounds with excess alkylamine in ethanol followed by conversion to the hydrochloride salt gave compounds 1–6 (Scheme I).

In order to confirm that no racemization occurred during the two-step reaction, and that substitution of the phenoxy ring did not change the absolute configuration of the compounds, the following steps were taken. The optical purities of 1–7 were measured with HPLC (chiral column). The HPLC method is a very sensitive method for measurement of optical purity (sensitivity ≤1%).[33] In all cases, the optical purity as measured by HPLC was >99%. To determine the absolute configuration, **7R** and **7S** were debrominated by lithiation, followed by addition of water to give (*R*)- and (*S*)-tomoxetine, respectively. Both samples showed the same retention time as the respective isomer with the same configuration (HPLC, chiral column), and the optical rotation values were consistent with previously reported values.[32]

Results and Discussion

Utilizing mild Mitsonobu reaction conditions, the *R* and *S* isomers of a series of tomoxetine and derivatives have been prepared and evaluated as ligands for DA, NE, and 5-HT uptake sites. The results of these studies are summarized in Scheme I. The affinity of the test compounds to inhibit DA uptake was evaluated using [^{125}I]-β-CIT, a high-affinity ligand for the uptake site. None of the compounds tested displayed high affinity for the DA uptake site.

The binding affinity of (*R*)-tomoxetine for NE uptake sites has been reported to be 400 times greater than its affinity for 5-HT uptake sites (K_i 1.9 nM for NE vs 750 nM for 5-HT).[18] In order to evaluate the affinity of the test compounds for the 5-HT uptake sites, in vitro binding studies were performed using [^{3}H]citalopam with a rat cortical membrane preparations (Scheme I). These studies indicate that the addition of an iodine atom to the phenoxy ring of tomoxetine can dramatically change the pharmacological profile of this ligand. For example, in sharp contrast to tomoxetine, the iodotomoxetine derivative **1R** displays substantially higher affinity for 5-HT uptake sites than for NE uptake sites. Additionally, compounds **1R**, **2R**, **2S**, **6R**, and **6S**, with an iodine atom at the 4-position of the phenoxy ring, displayed higher binding affinity toward 5-HT uptake sites than compounds with iodine at the 2- or 3-position. Similarly, radioligand binding studies with fluoxetine and related derivatives indicate that the 4-substituted trifluoromethyl derivative exhibited higher affinity for 5-HT uptake sites than the 2- or 3-substituted compounds.[30] These results and similar studies on fluoxetine[30] demonstrate that substitution at the 4-position of the phenoxy ring plays an important role in enhancing the binding of this series of compound to 5-HT uptake sites. Of the iodinated tomoxetine compounds studied here (1–3), **1R**, **2**, and **6** showed the highest affinity and selectivity for 5-HT uptake sites. The stereoselectivity of **1R** for 5-HT uptake sites is 20 times larger than **1S**; however, compounds **2** and **6** showed little stereoselectivity toward 5-HT reuptake site binding.

Binding studies were also performed using [^{3}H]nisoxetine to determine the ability of the test compounds to

(33) Chumpradit, S.; Kung, M. P.; Billings, J.; Kung, H. F. (±)-7-Chloro-8-hydroxy-1-(3-iodophenyl)-3-methyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (TISCH): A High Affinity and Selective Iodinated Ligand for CNS D-1 Dopamine Receptor. *J. Med. Chem.* **1991**, *34*, 877–883.

inhibit the specific binding of this radioligand to NE uptake sites. Compound **4R**, with iodine at the 2-position of the phenoxy ring, showed high affinity to NE uptake sites (K_i = 0.24 nM). While the work was in progress, Gackenheimer et al.[34] reported a similar binding affinity value for the same compound **4R**, using in vitro autoradiography technique. When the 2-position of phenoxy ring is substituted with a methoxy or methyl group (as in the case of nisoxetine or tomoxetine, respectively), both compounds also exhibited high affinity for NE binding sites (K_i, (*R*)-nisoxetine = 3.8 nM, (*R*)-tomoxetine = 1.9 nM).[18] These preliminary results suggest that the 2-position of the phenoxy ring may be important for binding to the NE uptake site. Changing the *N*-methyl to other *N*-alkyl groups of **4R** dramatically decreases the binding affinity to the 5-HT reuptake sites (data not shown).

In conclusion, the series of iodinated derivatives of tomoxetine and demethylated tomoxetine were synthesized and characterized. Among the iodinated derivatives examined, **1R** is the most potent and selective ligand for 5-HT uptake sites; **4R** displayed high affinity and high selectivity for NE uptake sites. Both compounds showed stereoselectivity, with the *R* isomer being more potent than the *S* isomer in each case. Compounds **1R** and **4R** are useful iodinated ligands for future pharmacological studies of 5-HT and NE uptake sites, respectively. In addition, both *R* and *S* isomers of compounds **2** and **6** also displayed high affinity to 5-HT reuptake sites. Results of radiolabeling and more extensive biological studies of **1R** published elsewhere[35] suggested that in vivo imaging of [^{123}I]**1R** in monkeys showed no selective 5-HT uptake site binding. Therefore, [^{123}I]**1R** may not be the agent of choice for in vivo imaging. A new iodinated ligand for the 5-HT reuptake site, 5-iodo-6-nitroquipazine, displayed high affinity (K_d = 0.02 nM) and excellent central nervous system (CNS) 5-HT binding of monkey brain by in vivo imaging.[36,37] This new compound may be a better candidate for in vivo imaging studies.

Experimental Section

General Methods. NMR were recorded on a Varian EM 360A, a Bruker WM-250 (250 MHz), or a Bruker AM 500 (500 MHz) spectrometer. The chemical shifts were reported in ppm downfield from an internal tetramethylsilane standard. Infrared spectra were obtained with a Mattson Polaris FT-IR spectrophotometer. Melting points were determined on a Meltemp apparatus and are reported uncorrected. HPLC was performed on Model Rabbit HP of Rainin Instrument Co., Inc. (Emeryville, CA) using a chiral column (chiralcel-OD, 4.1 × 250 mm) from Diacel Inc., Los Angeles, CA. Unless indicated, the mixture of hexane and ethanol (95:5, 0.5 mL/min) were mobile phases. Optical rotation of compounds was measured on a Perkin-Elmer 243B polarimeter. Mass spectra was performed on the mass spectrometer VG 70-70 HS with chemical ionization (CI), using methane or ammonia gas. Elemental analyses were performed by Atlantic Microlabs, Inc., of Norcross, GA and were within 0.4% of the theoretical values.

Materials. Tetrahydrofuran (THF) and diethyl ether were distilled from benzophenone ketyl under argon. Compound 2-iodophenol was purchased from Kodak (Rochester, NY); 3-iodophenyl, 4-iodophenol, 3-bromophenol, 4-iodo-2-methylphenol, (*R*)-(+)-3-chloro-1-phenylpropane, and (*S*)-(−)-3-chloro-1-phenylpropane were purchased from Aldrich (Milwaukee, WI) and used without further purification, except (*S*)-(−)-3-chloro-1-phenylpropane, which was recrystallized in hexane to improve the optical purity from $[\alpha]_D$ −25° (c = 1, $CHCl_3$) to −26° (c = 1, $CHCl_3$) to −26° (c = 1, $CHCl_3$). The rest of the bromo and iodo compounds were synthesized as reported previously.[38,39] [^{3}H]-Citalopram (specific activity 86.8 Ci/mmol, 98% pure) was purchased from Dupont NEN, Boston, MA. [^{3}H]Nisoxetine (specific activity 82.6 Ci/mmol, 98% pure) was purchased from Amersham, Arlington Heights, IL. The tributyltin precursor of [^{125}I]-β-CIT was prepared at Research Biochemicals Inc., Boston, MA. The specific activity of [^{125}I]-β-CIT was 500 Ci/mmol and 98% pure.[40]

(*R*)-(+)-1-Chloro-3-(4-iodo-2-methylphenoxy)-3-phenylpropane.[32] Triphenylphosphine (1.54 g, 5.88 mmol) and diethyl azodicarboxylate (0.93 mL, 1.03 g, 5.88 mmol) were added to a solution of (*S*)-(−)-3-chloro-1-phenylpropanol (1.00 g, 5.88 mmol) and 4-iodo-2-methylphenol (1.38 g, 5.88 mmol) in THF (15 mL). The mixture was stirred at room temperature overnight. THF was removed in a rotoevaporator. The residue was triturated with petroleum ether (3 × 15 mL). The combined petroleum ether fractions were concentrated, and the crude product was purified by column chromatography (silica gel, petroleum ether). The solvent was removed to give a thick pale yellow liquid: 1.60 g (75%) of the title compound; $[\alpha]_D$ + 14.02° (c = 7.7, $CHCl_3$); FT-IR (neat) ν 3080, 3020, 2976, 2915, 1585 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.20 (m, 7 H), 6.39 (d, J = 8.8 Hz, 1 H), 5.34 (dd, J = 4.4, 8.8 Hz, 1 H), 3.81–3.72 (m, 1 H), 3.63–3.55 (m, 1 H), 2.55–2.41 (m, 1 H), 2.31–2.16 (m, 1 H), 2.27 (s, 3 H). Anal. ($C_{17}H_{20}NOI$) C, H.

(*R*)-(−)-*N*-Methyl-3-(4-iodo-2-methylphenoxy)-3-phenylpropanamine Hydrochloride (1R).[32] The mixture of the above chloropropane (0.58 g, 1.50 mmol), 40% aqueous methylamine (4 mL), and ethanol (1.5 mL) in a sealed tube was heated at 130 °C for 3 h. The reaction mixture was cooled to room temperature and poured into water (5 mL). The mixture was extracted with dichloromethane (3 × 5 mL). The combined organic layers were dried over anhydrous sodium sulfate, filtered, and concentrated to give a yellowish oil. Flash-column chromatography of the crude product (silica gel, 5% MeOH/CH_2Cl_2) afforded 0.25 g (44%) of free base **1R**. HCl gas was bubbled through a solution of **1R** in a minimum amount of 1:1 ether/dichloromethane. The cloudy mixture was evaporated into dryness to give the title compound as a hydroscopic solid: $[\alpha]_D$ = −8.34° (c = 0.82, $CHCl_3$); FT-IR (free base, neat) ν 3400 (weak br, NH) 3100–3000 (Br, ArH), 3000–2600 (br, CH), 1480, 1240 cm^{-1}; ^{1}H NMR (free base, $CDCl_3$) δ 7.43–7.20 (m, 7 H), 6.38 (d, J = 5.4 Hz, 1 H), 5.23 (dd, J = 3.0, 5.4 Hz, 1 H), 2.78 (br t, 2 H), 2.45 (br s, 3 H), 2.28 (s, 3 H), 2.28–2.12 (m, 1 H), 2.10–2.00 (m, 1 H). Anal. ($C_{17}H_{21}NOICl$) C, H, N. Retention time = 7.35 min (HPLC, chiral column, hexanes/ethanol = 95/5, 0.5 mL/min).

Unless indicated, the following compounds were synthesized in the same manner as described above. The amination reaction was performed in a mini-Parr reactor in the same manner as it was done in the sealed tube.

(*S*)-(+)-*N*-Methyl-3-(4-iodo-2-methylphenoxy)-3-phenylpropanamine Hydrochloride (1S). After workup and purification, the yellowish oil of the base **1S** was obtained in 48% yield. The hydrochloride salt of **1S** was hygroscopic: $[\alpha]_D$ = +6.43° (c = 1.14, $CHCl_3$). The IR and NMR spectra of **1S** are the same as those of **1R**. Anal. calcd (CI high resolution mass

(34) Gackenheimer, S. L.; Littlefield, E. S.; Mais, D. E.; Robertson, D. W.; Gehlert, D. R. Autoradiographic localization of norepinephrine uptake sites in rat brain using [^{125}I]-iodotomoxetine (abstract). *Soc. Neurosci.* **1991**, 1553.

(35) Kung, M. P.; Chumpradit, S.; Billings, J.; Kung, H. F. 4-Iodotomoxetine: A novel ligand for serotonin uptake sites. *Life Sci.* **1992**, *51*, 95–106.

(36) Mathis, C.; Biegon, A.; Taylor, S.; Enas, J.; Hanrahan, S.; Jagust, W. [I-125]5-iodo-6-nitroqiupazine: Synthesis and evaluation of a potent and selective presynaptic serotonin ligand (abstract). *J. Nucl. Med.* **1992**, *32*, 890.

(37) Mathis, C. A.; Biegon, A.; Taylor, S. E.; Enas, J. D.; Hanrahan, S. M. [^{125}I]5-Iodo-6-nitro-2-piperazinylquinoline: a potent and selective ligand for the serotonin uptake complex. *Eur. J. Pharmacol.* **1991**, *210*, 103–104.

(38) Adam, R.; Marvel, C. S. p-Bromophenol. *Organic Syntheses*; Wiley: New York, **1941**; Vol. *1*, 128.

(39) Edgar, K. J.; Falling, S. N. An efficient and selective method for the preparation of iodophenols. *J. Org. Chem.* **1990**, *55*, 5287–5291.

(40) Neumeyer, J. L.; Wang, S. Y.; Milius, R. A.; Baldwin, R. M.; Zea, P. Y.; Hoffer, P. B.; Sybirska, E.; Tikriti, M.; Charney, D. S.; Malison, R. T. [^{123}I]-2-β-carbomethoxy-3β-(4-iodophenyl)tropane: high-affinity SPECT radiotracer of monoamine reuptake sites in brain. *J. Med. Chem.* **1991**, *34*, 3144–3146.

spectrum) for $C_{17}H_{21}NIO$ (M + H) 382.0668, found 382.0715. Retention time = 6.83 min (HPLC, chiral column, hexanes/ethanol = 95/5, 0.5 mL/min).

(*S*)-(-)-*N*-Methyl-3-(4-iodo-3-methylphenoxy)-3-phenylpropanamine Hydrochloride (2S). The free base was obtained in 58% yield: $[\alpha]_D$ = -20.4° (*c* = 7.6, MeOH); FT-IR (free base, neat) ν 3310 (br, s, NH) 3100–300 (Br, ArH), 2900–2750 (br CH), 1475, 1245 cm^{-1}; ^1H NMR (free base, $CHCl_3$) δ 7.42 (d, 1 H), 7.22–7.20 (m, 4 H), 7.15 (m, 1 H), 6.7 (m, 1 H), 6.30–6.28 (m, 1 H); 5.11–5.08 (dd, 1 H), 2.62 (m, 2 H), 3.32 (s, 3 H), 2.21 (s, 3 H), 2.05 (m, 1 H), 1.84 (m, 1 H), 1.30 (br, s, 1 H). Anal. ($C_{17}H_{21}$-NOICl) C, H, N. Retention time = 5.64 min (HPLC, chiral column, hexanes/ethanol = 95/5, 0.5 mL/min).

(*R*)-(+)-*N*-Methyl-3-(4-iodo-3-methylphenoxy)-3-phenylpropanamine Hydrochloride (2R). The free base of **2R** was obtained in 28%. Spectra are the same as **2S**. The salt is hygroscopic: $[\alpha]_D$ = +16.14° (*c* = 2.7, MeOH). Anal. calcd (CI high resolution mass spectrum) for $C_{17}H_{21}NIO$ (M + H) 382.0668, found 382.0674. Retention time = 7.32 min (HPLC, chiral column, hexanes/ethanol = 95/5, 0.5 mL/min).

(*R*)-(-)-*N*-Methyl-3-(2-iodo-4-methylphenoxy)-3-phenylpropanamine Hydrochloride (3R). The free base **3R** was obtained in 56% yield: $[\alpha]_D$ = -89.45° (*c* = 3.0, MeOH); FT-IR (free base, neat) ν 3340 (weak, br, NH) 3100–300 (Br, ArH), 2990–2700 (br, CH), 1475, 1250 cm^{-1}; ^1H NMR (free base, $CDCl_3$) δ 7.55 (d, 1 H), 7.30–7.27 (m, 4 H), 7.24 (m, 1 H), 6.84 (dd, 1 H), 6.45 (d, 1 H), 5.25 (dd, 1 H), 2.82–2.73 (m, 2 H), 2.41 (s, 3 H), 2.24–2.14 (m, 1 H), 2.15 (s, 3 H), 2.06–2.01 (m, 1 H), 1.45 (br s, 1 H). Anal. ($C_{17}H_{21}NOICl$) C, H, N. Retention time = 7.51 min (HPLC, chiral column, hexanes/ethanol = 95/5, 0.5 mL/min).

(*S*)-(+)-*N*-Methyl-3-(2-iodo-4-methylphenoxy)-3-phenylpropanamine Hydrochloride (3S). The hygroscopic salt **3S** was obtained in 48% yield. The spectra of the free base of **3S** are the same as those of the free base of **3R**: $[\alpha]_D$ = +66.69° (*c* = 3.4, MeOH). Anal. ($C_{17}H_{20}NOI \cdot {}_{1.25}H_2O$) C, H, N. Anal. calcd (CI high resolution mass spectrum) for $C_{17}H_{21}NOI$ (M + H) 382.0668, found 382.0635. Retention time = 6.45 min (HPLC, chiral column, hexanes/ethanol = 95/5, 0.5 mL/min).

(*R*)-(-)-*N*-Methyl-3-(2-iodophenoxy)-3-phenylpropanamine (4R). Compound **4R** was obtained in 19% yield: $[\alpha]_D$ = -113° (*c* = 2.5, MeOH); FT-IR (neat) ν 3400 (weak br, NH), 3100–3000 (br, ArH), 3000–2600 (br, CH), 1600, 1570, 1475, 1240 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.70 (m, 2 H), 7.30–7.20 (m, 5 H), 7.05 (m, 1 H), 6.60 (m, 1 H), 5.35 (dd, 1 H, *J* = 8.20, 4.5), 2.80 (m, 2 H), 2.45 (s, 3 H, NCH_3), 2.25 (tdd, 1 H, *J* = 13.7, 7.3), 2.10 (tdd, 1 H, *J* = 14.3, 7.2) 1.75 (s, 1 H, NH). Anal. ($C_{16}H_{19}NOICl \cdot {}_1/_3H_2O$) C, H, N. Retention time = 13.69 min (HPLC, chiral column, hexanes/ethanol = 95/5, 0.5 mL/min).

(*S*)-(+)-*N*-Methyl-3-(2-iodophenoxy)-3-phenylpropanamine (4S). Using the same procedure as **4R**, compound **4S** was obtained in 32% yield: $[\alpha]_D$ = +136° (*c* = 3, MeOH); FT-IR and ^1H NMR are the same as those for **4R**. Anal. ($C_{16}H_{19}NOICl$) C, H, N. Retention time = 7.18 min (HPLC, chiral column, hexanes/ethanol = 95/5, 0.5 mL/min).

(*S*)-(+)-*N*-Methyl-3-(3-iodophenoxy)-3-phenylpropanamine Hydrochloride (5S). The reaction gave free base **5S** (57%). **5S** was hygroscopic: $[\alpha]_D$ = +16.42° (*c* = 2.12, $CHCl_3$). FT-IR (free base, neat) ν 3450 (weak br, NH), 3090–3000 (br, ArH), 3000–2700 (br, CH), 1600, 1475, 1225 cm^{-1}; ^1H NMR (free base, $CDCl_3$) δ 7.37–7.19 (m, 7 H), 6.90–6.75 (m, 2 H), 5.22 (dd, *J* = 4.6, 8.5 Hz, 1 H), 2.78 (br t, 2 H), 2.45 (br s, 3 H), 2.27–2.12 (m, 1 H), 2.08–1.95 (m, 1 H). Anal. ($C_{16}H_{18}NIO$) C, H, N. Retention time = 5.49 min (HPLC, chiral column, hexanes/ethanol = 95/5, 0.5 mL/min).

(*R*)-(-)-*N*-Methyl-3-(3-iodophenoxy)-3-phenylpropanamine Hydrochloride (5R). Workup and purification afforded a pale yellow oil of free base **5R** (51%). Compound **5R** was hygroscopic: $[\alpha]_D$ = -15.39° (*c* = 1.37, $CHCl_3$). The spectra of **5R** are the same as for **5S**. Anal. ($C_{16}H_{19}NOICl \cdot {}_1/_3H_2O$) C, H, N. Retention time = 9.03 min (HPLC, chiral column, hexanes/ethanol = 95/5, 0.5 mL/min).

(*R*)-(+)-*N*-Methyl-3-(4-iodophenoxy)-3-phenylpropanamine (6R). The compound was obtained in 16% yield: $[\alpha]_D$ = +26° (*c* = 3, MeOH), FT-IR (neat) ν 3400 (weak br broad, NH), 3000–2600 (br, ArH, CH), 1575, 1480, 1225 cm^{-1}; ^1H NMR (free base, $CDCl_3$) δ 7.40 (dd, 2 H), 7.30–7.20 (m, 5 H), 6.60 (dd, 2 H), 5.20 (dd, 1 H), 2.70 (m, 2 H), 2.40 (s, 3 H), 2.15 (tdd, 1 H), 1.95 (tdd, 1 H), 1.50 (s, 1 H). Anal. ($C_{16}H_{18}NOICl$) C, H, N. Retention time = 7.54 min (HPLC, chiral column, hexanes/ethanol = 95/5, 0.5 mL/min).

(*S*)-(-)-*N*-Methyl-3-(4-iodophenoxy)-3-phenylpropanamine (6S). The compound was obtained in 61% yield: $[\alpha]_D$ = -11° (*c* = 3, MeOH); the NMR and IR spectra are the same as those for **6R**. Anal. ($C_{16}H_{18}NOICl$) C, H, N. Retention time = 7.44 min (HPLC, chiral column, hexanes/ethanol = 95/5, 0.5 mL/min).

(*R*)-(-)-*N*-Methyl-3-(4-bromo-2-methylphenoxy)-3-phenylpropanamine Hydrochloride (7R). The reaction gave 64% yield of **7R**: $[\alpha]_D$ = -12.5° (*c* = 3.3, MeOH); FT-IR (free base, neat) 3300 (br, NH) 3100–2580, 1490, 1250 cm^{-1}; ^1H NMR (free base, $CDCl_3$) δ 7.30–6.90 (m, 7 H); 6.36 (d, 1 H), 5.18–5.10 (m, 1 H), 2.90 (br, 3 H), 2.45 (s, 3 H), 2.15 (s, 3 H), 2.15–2.06 (m, 1 H), 2.05–1.95 (m, 1 H). Anal. ($C_{17}H_{21}NOBrCl$) C, H, N. Retention time = 7.17 min (HPLC, chiral column, hexanes/ethanol = 95/5, 0.5 mL/min).

(*S*)-(+)-*N*-Methyl-3-(4-bromo-2-methylphenoxy)-3-phenylpropanamine Hydrochloride (7S). The reaction gave 71% yield of **7S**: $[\alpha]_D$ = +12.3° (*c* = 3, MeOH). The FT-IR and NMR spectra are the same as those for **7R**. Anal. ($C_{17}H_{21}NOBrCl$) C, H, N. Retention time = 6.55 min (HPLC, chiral column, hexanes/ethanol = 95/5, 0.5 mL/min).

(*R*)-(-)-*N*-Methyl-3-(2-methylphenoxy)-3-phenylpropanamine Hydrochloride: (*R*)-(-)-Tomoxetine.[32] The product was obtained in 58.3% yield: $[\alpha]_D$ = -45.5° (*c* = 4.32, MeOH) (lit.[32] $[\alpha]_D$ = -43.1° (*c* = 5.9, MeOH)); FT-IR (free base, neat) ν 3310 (br, NH), 3100–300 (ArH), 2750–300 (CH), 1600, 1500, 1250 cm^{-1}; ^1H NMR (free base, $CDCl_3$) δ 7.29–7.22 (m, 4 H), 7.18–7.14 (m, 1 H), 7.04–7.03 (d, 1 H), 6.87 (t, 1 H), 6.69 (t, 1 H), 6.53 (d, 1 H), 5.19 (dd, 1 H), 2.73–2.69 (m, 2 H), 2.35 (s, 3 H, NCH_3), 2.24 (s, 3 H, CH_3), 2.17–2.09 (m, 1 H), 2.00–1.95 (m, 1 H), 1.64 (s, NH). Anal. ($C_{17}H_{22}ClNO$) C, H, N. Retention time = 8.44 min (HPLC, chiral column, hexanes/ethanol = 95/5, 0.5 mL/min).

(*S*)-(+)-*N*-Methyl-3-(2-methylphenoxy)-3-phenylpropanamine Hydrochloride: (*S*)-(-)-Tomoxetine. The product was obtained in 46.70% yield: $[\alpha]_D$ = +45.6° (*c* = 3.24, MeOH) (lit.[32] $[\alpha]_D$ = +42.9° (*c* = 6.0, MeOH)); FT-IR and ^1H NMR are the same as those for the *R* isomer. Anal. ($C_{17}H_{22}ClNO$) C, H, N. Retention time = 6.51 min (HPLC, chiral column, hexanes/ethanol = 95/5, 0.5 mL/min).

Determination of Configuration of 7R and 7S. To the solution of **7R** (300 mg, 0.89 mmol) in THF (20 mL) was added *n*-BuLi (1.24 mL, 1.60 mmol) at -78 °C. After 4 min stirring at -78 °C, the reaction mixture was quenched with water (0.5 mL). The mixture was allowed to warm to room temperature. THF was removed and the residue was dissolved in dichloromethane. The organic layer was separated, washed once with water, and dried over anhydrous sodium sulfate. After concentration of the solvent, it was purified by column chromatography (silica gel, CH_2Cl_2/MeOH/NH_4OH = 90/10/1) to obtain 184 mg (80%) of (*R*)-tomoxetine. The yield was less than expected due to a sticking problem. The spectra of the product are identical with those reported in the literature:[32] $[\alpha]_D$ = -43.3° (*c* = 4.5, MeOH) (lit.[32] $[\alpha]_D$ = -43.1° (*c* = 5.9, MeOH)).

The same procedure was applied to **7S** to yield (*S*)-tomoxetine (61%) with $[\alpha]_D$ = +42.6° (*c* = 3.84, MeOH) (lit.[32] $[\alpha]_D$ = +42.9° (*c* = 6, MeOH)).

HPLC profiles (chiral column) of the debrominated compound tomoxetine showed that the optical purity of both compounds was >99%. Coelution (hexane/ethanol = 95:5; 0.5 mL/min) of the debrominated compound with authentic tomoxetine gives a single peak with retention times of 6.72 and 8.66 min for (*S*)-tomoxetine and (*R*)-tomoxetine, respectively.

In Vitro Binding Studies. The rat cortical and striatal membranes were prepared as described previously.[35] The radioligands chosen for binding studies were [^{3}H]citalopram (selective ligand for 5-HT uptake sites), [^{3}H]nisoxetine (selective ligand for NE uptake sites),[15] and [^{125}I]-2β-carbomethoxy-3β-(4-iodophenyl)tropane ([^{125}I]-β-CIT), selective ligand for DA uptake sites.[40] The assay with [^{3}H]citalopram was performed according to the procedure reported by D'Amato.[8] Inhibition of [^{3}H]nisoxetine binding was carried out with freshly prepared rat

cortical membranes and the assay conditions were consistent with the published method.[15] Inhibition of [^{125}I]-β-CIT binding by various iodinated tomoxetine derivatives was examined with rat striatal membranes. Assay conditions were similar to those reported.[40]

After incubation of the specific radioligands with various concentrations of iodinated tomoxetine derivatives, samples in triplicate were separated by filtration through GF/B filters and rinsed with cold buffer. Radioactivity associated with the filters was either measured by liquid scintillation spectrophotometry (Ecolite, ICN, ~60% efficiency) in the cases of [^{3}H]citalopram and [^{3}H]nisoxetine or counted in a γ counter (Packard 5000) in the case of [^{125}I]-β-CIT at an efficiency of 70%. The data was analyzed using the iterative nonlinear least squares curve-fitting program LIGAND.[41]

Acknowledgment. The tributyltin precursor for radioiodination of β-CIT was prepared at Research Biochemicals Inc. and kindly supplied by Drs. J. L. Neumeyer, R. Innis, and R. Baldwin. Authors thank Catherine Cartwright and Karen Loomis for their editorial assistance and Dr. D. Canney for his useful discussion. This work is supported by grants awarded by USPH (NS-24538, MH-48125, and MH-48539) and Region Centre, France 91 298012.

(41) Munson, D.; Rodbard, D. LIGAND: a versatile computerized approach for characterization of ligand binding system. *Anal. Biochem.* 1980, *107*, 220–239.