2-(2-(Dimethylaminomethyl)phenoxy)-5-iodophenylamine: An Improved Serotonin Transporter Imaging Agent

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Imaging serotonin transporters (SERT) is an emerging research tool potentially useful to cast light on the mechanisms of drug action as well as to monitor the treatment of depressed patients. We have prepared two new derivatives of **3**, 2-(2-(dimethylaminomethyl)phenoxy)-5-iodophenylamine (**4**) and 2-(2-(dimethylaminomethyl)benzyl)-5-iodophenylamine (**5**) (K_i for SERT = 0.37 and 48.6 nM, respectively). Both [^{125}I]**4** and [^{125}I]**5** displayed excellent brain uptakes in rats, and they showed a highest uptake in hypothalamus (between 60 and 240 min), a region populated with the highest density of SERT. The specific uptake of [^{125}I]**4** in the hypothalamus resulted in a target to nontarget ratio ([hypothalamus-cerebellum]/cerebellum) of 4.3 at 2 h. Autoradiography of rat brain sections (ex vivo at 2 h) of [^{125}I]**4** showed an excellent regional distribution pattern consistent with known SERT localization. These data suggest that [^{123}I]**4** may be useful for imaging SERT binding sites in the brain by single photon emission computed tomography (SPECT).

Introduction

Alterations in serotonergic neuronal function in the central nervous system (CNS) are implicated in patients with major depression.[1−5] A series of newer antidepressants preferentially increase 5-HT (serotonin) transmission by inhibiting serotonin reuptake. Selective serotonin reuptake inhibitors (a.k.a. SSRIs) are those drugs, which preferably inhibit serotonin uptake compared with dopamine or norepinephrine uptakes. They have no or only slight effect on other uptake mechanisms, neurotransmitter receptors and enzymes, etc. The FDA has approved various SSRIs, such as citalopram, fluoxetine, fluvoxamine, paroxetine and sertraline, etc. These drugs have revolutionized the management of depression for millions of patients. They are now widely prescribed for treating various other mental disorders such as compulsive obsessive and social phobic disorders. By blocking the serotonin reuptake sites, the concentration of serotonin in the synaptic cleft is greatly increased. A substantial increase in serotonin signal is credited for apparent symptomatic improvements.[3] While the SSRI drugs have helped many patients, a significant segment of patients does not respond to the treatment. There is a compelling need to find a simple method to measure the drug occupancy (or the lack thereof) of the target sites in the brain of nonresponders. Noninvasive imaging methods are important for studying binding sites of psychoactive drugs and monitoring the effectiveness of such drug treatment in the living human brain.

Novel iodinated ligands, IDAM (**1**)[6,7] and ADAM (**3**)[8,9] (Table. 1), were previously developed in our laboratory. In vitro binding study showed that they displayed excellent binding affinities and selectivities to SERT sites.[7,9] Biodistribution of [^{125}I]IDAM (**1**) and [^{125}I]-ADAM (**3**) in the rat brain showed high initial uptakes with the specific binding peaked at 60 and 120 min postinjection, respectively. SPECT imaging using [^{123}I]-ADAM (**3**) in baboons demonstrated an excellent localization of regions in the midbrain area known to have a high concentration of SERT binding sites.[10] Many new derivatives of IDAM (**1**), DASB and ADAM (**3**) have been prepared and evaluated (Table 1).[11−16] Using the same LLC-PK$_1$ cells specifically expressing the individual transporter, ODAM (**2**) (Table. 1) displayed a good binding affinity for SERT (K_i = 0.12 nM) with much lower affinities for the other two transporters (K_i > 1000 nM and 20.0 nM for DAT and NET, respectively). However, biodistribution study of [^{125}I]ODAM (**2**) in rats displayed a much lower target to nontarget ratio as compared to the previous compound [^{125}I]IDAM (**1**) (0.86 vs 1.75 of [hypothalamus-cerebellum]/cerebellum ratio at 60 min post iv injection). This observation was confirmed by SPECT imaging studies in nonhuman primates with the brain images of [^{123}I]IDAM (**1**) and [^{123}I]ADAM (**3**) superior to that of [^{123}I]ODAM (**2**).[17] In addition, several O-bridged analogues have been reported, but their biological properties were not promising (unpublished results). Most of the biphenyl derivatives were highly potent in binding to SERT. However, it appears that the brain uptake and washout from the SERT target sites in vivo were highly variable depending on the substitution on the phenyl rings. It is not readily apparent, even with molecular modeling,[18] if the O-bridged and C-bridged derivatives of ADAM would have improved in vivo biological properties. To further explore our investigation, additional derivatives of this

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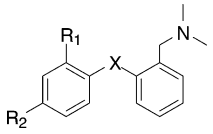
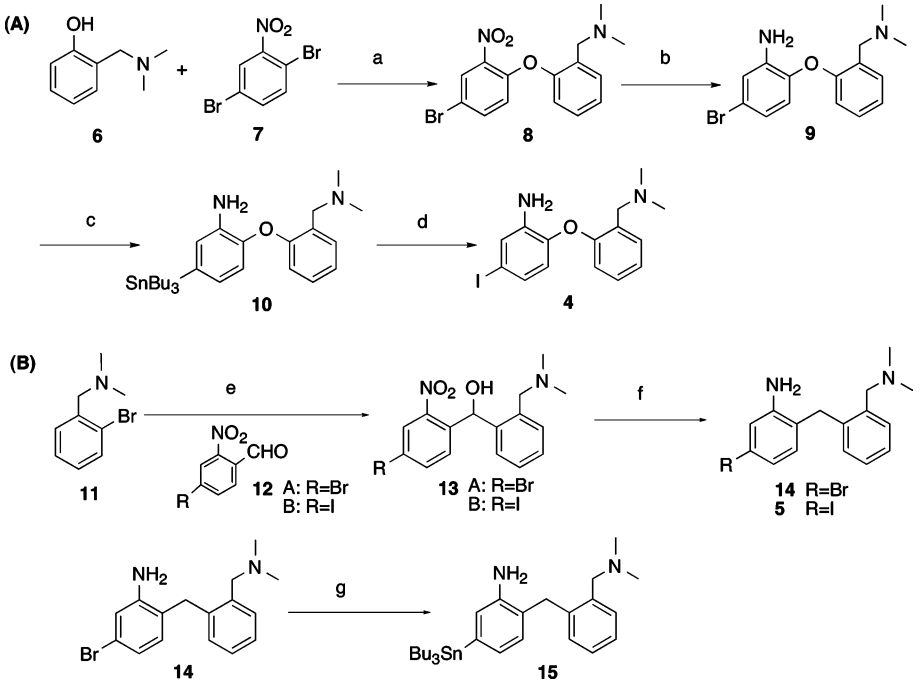
Table 1. Chemical Structures and in Vitro Binding Affinities of Serotonin Imaging Agents[a]



	X	R_1	R_2	K_i (nM) SERT[b]	DAT[b]	NET[b]	references
1. IDAM	S	CH_2OH	I	0.097 ± 0.013	>1000	234 ± 26	8
2. ODAM	O	CH_2OH	I	0.12 ± 0.02	>1000	20 ± 2	27
3. ADAM	S	NH_2	I	0.013 ± 0.003	840 ± 100	699 ± 80	8
4.	O	NH_2	I	0.37 ± 0.11	309 ± 65	286 ± 36	
5.	CH_2	NH_2	I	48.6 ± 4.10	>1000	17 ± 4	
DASB	S	NH_2	CN	1.1 ± 0.04	>1000	>1,000	12, 15, 16
(+)McN5652				0.009 ± 0.002	112 ± 23	11.3 ± 3.3	8, 23, 28
nisoxetine				203 ± 4	>1000	1.6 ± 0.14	29

[a] (+)McN5652 and nisoxetine were added to this list as specific ligands for SERT and NET, respectively. [b] SERT: serotonin transporters. DAT: dopamine transporters. NET: norepinephrine transporters.

Scheme 1[a]



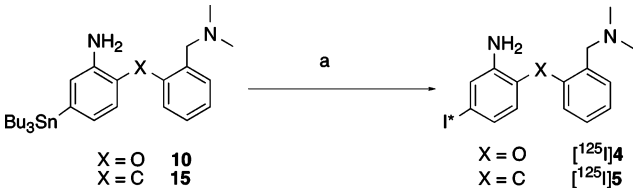
[a] Reagents: (a) KOH, DMSO heat; (b) $SnCl_2$, HCl; (c) $(SnBu_3)_2$, Pd(0); (d) I_2; (e) BuLi; (f) BH_3; (g) $(SnBu_3)_2$, Pd(0).

series of compounds with different bridge atoms between the phenyl groups were prepared and characterized. We report herein the synthesis, radiolabeling and biodistribution of 2-(2-(dimethylaminomethyl)phenoxy)-5-iodophenylamine, "O-bridge", **4**, and 2-(2-(dimethylaminomethyl)benzyl)-5-iodophenylamine, "C-bridge", **5**, as alternative candidates for SPECT imaging of SERT in vivo.

Results and Discussion

Synthesis of **4**, an O-bridged derivative, is outlined in Scheme 1A. 2-Dimethylaminomethylphenol (**6**) and 2,5-dibromonitrobenzene (**7**) were coupled to afford compound **8**. The nitro group of **8** was reduced to corresponding amine **9** by $SnCl_2$. Compound **9**, bromo derivative of target compound **4**, was converted to a tributyltin intermediate **10** by a palladium(0)-catalyzed coupling reaction with bis(tributyltin). The tin compound **10** was treated with iodine in chloroform to produce the desired compound, **4**.

Scheme 2. [a] Preparation of Radioiodinated Lignads



[a] Reagents: (a) Na*I, H_2O_2, HCl.

Synthesis of **5**, a C-bridged derivative, is outlined in Scheme 2B. 2-Bromo-*N*,*N*-dimethylbenzylamine (**11**) was lithiated by n-BuLi and reacted with either 4-bromo-2-nitrobenzaldehyde[19] (**12a**) or 4-iodo-2-nitrobenzaldehyde[20] (**12b**), leading to the carbon-bridged alcohols **13a** and **13b**. The alcohols were readily reduced by borane to give the bromo derivative **14** and the desired "cold" compound **5**. The bromo derivative **14** was converted to the tributyltin derivative **15**.

Table 2. Biodistribution in Rats after an iv Injection of [^{125}I]**4** (% dose/organ, avg of three rats ± SD)[a]

	2 min	30 min	60 min	120 min	240 min	6 h	11 h	24 h
				Organ Distribution				
blood	5.75 ± 0.54	4.56 ± 0.20	3.89 ± 0.28	3.15 ± 0.44	3.12 ± 0.32	3.29 ± 0.27	2.52 ± 0.47	0.70 ± 0.06
heart	1.51 ± 0.13	0.32 ± 0.04	0.20 ± 0.01	0.13 ± 0.01	0.08 ± 0.02	0.08 ± 0.00	0.06 ± 0.00	0.02 ± 0.00
muscle	25.9 ± 4.82	18.1 ± 0.55	12.9 ± 0.84	9.18 ± 0.65	6.27 ± 0.06	5.01 ± 0.42	4.01 ± 0.85	1.13 ± 0.13
lung	17.2 ± 2.32	3.56 ± 0.96	1.79 ± 0.06	0.88 ± 0.04	0.37 ± 0.03	0.25 ± 0.01	0.16 ± 0.00	0.04 ± 0.00
kidney	4.27 ± 0.90	2.67 ± 0.48	1.67 ± 0.21	1.24 ± 0.15	0.61 ± 0.09	0.55 ± 0.07	0.40 ± 0.10	0.17 ± 0.02
spleen	0.44 ± 0.08	0.84 ± 0.05	0.47 ± 0.02	0.27 ± 0.01	0.13 ± 0.02	0.11 ± 0.01	0.09 ± 0.01	0.05 ± 0.01
liver	5.71 ± 0.88	7.10 ± 0.66	5.79 ± 0.70	4.90 ± 0.38	3.52 ± 0.20	3.59 ± 0.23	2.64 ± 0.19	1.83 ± 0.07
skin	4.45 ± 0.52	11.3 ± 0.78	13.9 ± 1.00	12.5 ± 2.14	12.6 ± 0.48	15.5 ± 1.50	10.9 ± 4.62	4.02 ± 0.26
brain	1.77 ± 0.07	2.12 ± 0.15	1.74 ± 0.08	1.08 ± 0.06	0.33 ± 0.02	0.11 ± 0.01	0.02 ± 0.00	0.01 ± 0.00
thyroid	0.10 ± 0.02	0.15 ± 0.04	0.41 ± 0.11	0.96 ± 0.06	2.34 ± 1.16	2.68 ± 0.66	5.15 ± 1.49	4.61 ± 1.21
				Regional Brain Distribution (% dose/g)				
CB	0.79 ± 0.21	0.64 ± 0.09	0.41 ± 0.03	0.21 ± 0.01	0.06 ± 0.00	0.027 ± 0.002	0.011 ± 0.0013	0.0049 ± 0.0009
ST	0.77 ± 0.04	1.16 ± 0.10	1.02 ± 0.11	0.72 ± 0.01	0.24 ± 0.02	0.068 ± 0.005	0.012 ± 0.0000	0.0039 ± 0.0004
HP	0.86 ± 0.07	1.06 ± 0.12	0.94 ± 0.08	0.65 ± 0.04	0.20 ± 0.02	0.059 ± 0.005	0.012 ± 0.0021	0.0040 ± 0.0005
CX	1.16 ± 0.14	1.25 ± 0.13	0.95 ± 0.07	0.51 ± 0.04	0.12 ± 0.01	0.042 ± 0.002	0.010 ± 0.0010	0.0040 ± 0.0007
HY	0.96 ± 0.15	1.30 ± 0.06	1.18 ± 0.08	0.92 ± 0.06	0.32 ± 0.03	0.097 ± 0.015	0.016 ± 0.0025	0.0060 ± 0.0010
				Ratio of (Region-Cerebellum)/Cerebellum				
ST	0.01 ± 0.27	0.83 ± 0.23	1.51 ± 0.12	2.53 ± 0.17	3.11 ± 0.35	1.56 ± 0.41	0.14 ± 0.13	--
HP	0.13 ± 0.22	0.68 ± 0.22	1.30 ± 0.05	2.19 ± 0.07	2.47 ± 0.28	1.21 ± 0.11	0.09 ± 0.08	--
CX	0.56 ± 0.55	0.96 ± 0.14	1.35 ± 0.07	1.48 ± 0.13	1.12 ± 0.17	0.58 ± 0.09	0.00 ± 0.16	--
HY	0.24 ± 0.17	1.06 ± 0.22	1.92 ± 0.20	3.49 ± 0.09	4.49 ± 0.12	2.64 ± 0.54	0.44 ± 0.19	0.23 ± 0.15

[a] Cerebellum: CB; striatum: ST; hippocampus: HP; cortex: CX; hypothalamus: HY.

To prepare the radioiodinated ligands, compound **10** was treated with radioactive [^{123}I]/[^{125}I]sodium iodide under oxidative conditions (H_2O_2) to produce the labeled compound [^{123}I]/[^{125}I]**4** in excellent yields (>90%). The radioactive material [^{125}I]**4** is stable in a refrigerator for at least three weeks, and the corresponding [^{123}I]**4** was stable for 24 h after labeling. Tin compound **15** was treated with radioactive [^{125}I]sodium iodide in an oxidative condition (H_2O_2) to produce the labeled compound [^{125}I]**5** in excellent yields (>90%). The radioactive material [^{125}I]**5** is stable in a refrigerator for at least for three weeks.

Using an in vitro binding assay with membrane preparations containing the specific SERT expressed in LLC-PK$_1$ cells, **4** displayed good affinity to SERT sites, showing a K_i of 0.37 ± 0.11 nM, which is more than 500-fold selective for SERT over NET and DAT (K_i = 286 ± 36 and 309 ± 65 nM, for NET and DAT, respectively). Using the same cell line expressing SERT, **5** exhibited a moderate binding affinity for SERT (K_i = 48.6 ± 4.1 nM) and showing less selectivity between SERT and NET (K_i = 17 ± 4 nM for NET). Unexpectedly, a slight modification of the bridgehead atom between the two phenyl groups results in a dramatic shift in selective binding between SERT and NET. It is reasonable to assume that compound **5**, in addition to binding to SERT, likely have a significant NET binding component in vivo.

Partition coefficient of **4** was determined to be 418 between 1-octanol/buffer. The value is comparable to that of [^{125}I]ADAM (**3**) measured under the same condition (PC = 335), indicating both ligands have comparable lipophilicity. It is interesting to note that the partition coefficient determined for [^{125}I]**5** was 1730 between 1-octanol/buffer. Nevertheless, the higher partition coefficient did not lead to a higher brain uptake in rats (see discussion below).

Biodistribution of [^{125}I]**4** in the rats showed a high initial uptake in the brain (1.77%dose at 2 min after iv injection) (Table 2). The total brain uptake did not decrease in the initial 60 min and then dropped significantly after 120 min. The hypothalamus region, a SERT rich area of the brain, showed high uptake and retention ([hypothalamus-cerebellum]/cerebellum ratio was 1.92, 3.49, 4.49, and 2.64 at 60, 120, 240, and 360 min after injection, respectively) (Table 2). Comparable values ([hypothalamus-cerebellum]/cerebellum ratios) were observed for [^{125}I]ADAM (Figure 1). Biodistribution of [^{125}I]-**4** in other organs or tissues was similar to that reported previously for [^{125}I]ADAM (**3**).[8,9] Interestingly, the less potent and selective SERT ligand, [^{125}I]**5** exhibited a similar initial brain uptake (1.77% dose at 2 min postinjection) but with a fast kinetic washout as compared to [^{125}I]**4**. The highest specific binding in hypothalamus ([hypothalamus-cerebellum]/cerebellum) at 120 min postinjection was found to be 2.43. Likely, this specific signal could be due to a contamination of NET binding for [^{125}I]**5**. As expected, pretreatment of rats with nisoxetine (2 mg/kg) prior to [^{125}I]**5** injection did show a significant decrement for the labeling in several regions (data not shown). As such, [^{125}I]**5** will not be a selective imaging agent for SERT binding sites.

A surprising and unexpected observation for this series of compounds as SERT specific imaging agents was the high brain uptake of **4**, exhibited at 2 and 4 h after iv injection (1.08 and 0.33 vs 0.42 and 0.11%dose/brain for **4** vs ADAM (**3**)). This represents a two to 3-fold increase in the brain uptake. It is important to note that between 2 and 4 h after an iv injection is the time range important for in vivo imaging studies; therefore, the increase in uptake will significantly benefit the imaging study. Significantly, the main target area in the brain was the hypothalamus, where SERT density was the highest; **4** displayed more than 200% higher uptake in the hypothalamus between 2 and 4 h than that of ADAM (**3**) (0.92 and 0.32 vs 0.44 and 0.13%dose/g for **4** and ADAM (**3**), respectively). Compound **4** exhibits the highest hypothalamus uptake among all of the radioiodinated compounds in this series of SERT imaging agents (Figure 1A). However, the washout rate of [^{125}I]-**4** from cerebellum, a non-SERT-containing region, was relatively slow. Thus, the specific uptake ratio [(hypo-

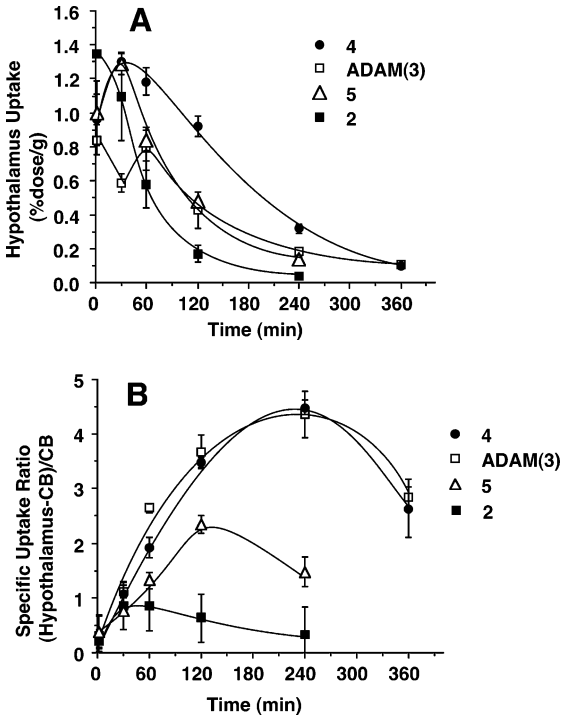


Figure 1. Comparison of uptake in hypothalamus (A) and specific uptake ratio (B) of [^{125}I]**2,** ADAM **(3), 4**, and **5** at different time points after an iv injection into rats. The specific uptake in hypothalamus (A) is expressed as % dose/g tissue, while the specific uptake ratio (B) is expressed as % dose/g ratio of (hypothalamus-cerebellum)/cerebellum. It is important to note that at 120 min iv postinjection, compound **4** displayed two times higher uptake in the targeted hypothalamus region as compared to that of ADAM (**3**) (Figure 1A)**.** The specific uptake ratios of ADAM (**3**) and **4** showed the same at all of the time points evaluated (Figure 1B), while compounds **2** and **5** exhibited lower specific uptake.

thalamus-cerebellum)/cerebellum] between 2 and 4 h showed a similar ratio for **4** and ADAM (**3**) (Figure 1B). The higher SERT uptake will likely to provide improved count rates for imaging studies; therefore, it is of great advantage to have this unanticipated finding. The O-bridged derivatives of ADAM may have improved brain uptakes; however, comparing the ODAM (**2**) vs **4** suggests that only **4** has a superior hypothalamus uptake and retention suitable for imaging study. However, this simple explanation may not be sufficient to account for the reasons why a minor change in chemical structure would have suggested a great disparity in SERT targeting effect. We do not know the exact mechanisms contributing to this phenomenon. Additional studies will be needed.

Ex vivo autoradiography of [^{125}I]**4** at 2 h after an iv injection into rats showed a distinctive distribution pattern in the brain, which reflected regional distribution of SERT binding sites. Intense labeling of olfactory tubercle (OT), lateral hypothalamic (LH) and thalamic nuclei (ThN), globus pallidus (GP), superior colliculus (SC), substantia nigra (SN), interpeduncular nucleus (IP), dorsal (DR) and median raphes (MnR) (Figure 2A), areas known to have high densities of SERT sites.[21,22] Lower but detectable labeling was also found in frontal cortex (Cx), caudate putamen (CP), ventral pallidum and hippocampus, areas containing a significantly lower amount of SERT sites. The regional distribution is consistent with those reported for other SERT ligands, [^{125}I]ADAM (**3**),[9] paroxetine,[21] (+)McN5652[23,24] and [^{125}I]5-iodo-6-nitroquipazine.[22] After a rat was pretreated with a dose of (+)McN5652 (2 mg/kg body weight) at 5 min prior to the iv injection of [^{125}I]**4**, the specific uptake in the regions of the brain was totally eliminated, suggesting that these two agents are competing for the same SERT binding sites (Figure 2B). The blocking study by ex vivo autoradiography lends support to the contention that this tracer binds to the SERT binding sites and the binding can be blocked by a chemical dose of a competing drug, (+)McN5652.

Blocking studies with selective SERT ligands, i.e., citalopram and (+)McN5652, completely eliminated the specific binding in the hypothalamus region, indicating the in vivo [^{125}I]**4** binding to SERT (Figure 3). Non-SERT agents, i.e., methylphenidate (DAT), nisoxetine (NET), raclopride (D2/D3) and ketanserin (5-HT_{2A}), did not show any significant effect on the specific uptake. The data suggest that this tracer is highly selective in binding to the SERT binding sites in vivo.

In summary, These radioiodinated imaging agents, including IDAM (**1**), ODAM (**2**), ADAM (**3**), **4** and **5**, are relatively small molecules, have no optical centers, and demonstrated high to moderate binding affinities in vitro and in vivo for SERT binding sites in the brain. A minor change in the chemical structure appears to have significant effects on the biodistribution, especially in the brain. Biological evaluations in rats demonstrated that **4** is a potentially useful candidate as a SERT imaging agent. Further characterization of this new ligand as a SERT imaging agent in human is planned for the near future.

Experimental Section

General. The ^{1}H and ^{13}C NMR spectra were performed on a Bruker 350 or 200 MHz spectrometer using tetramethylsilane as an internal standard. Elemental analyses were performed by Atlantic Microlab. Inc., GA. Mass spectrometry was performed at Department of Chemistry, University of Pennsylvania. Tetrahydrofuran (THF) was distilled immediately before use from sodium benzophenone kethyl. All other chemicals were purchased from Aldrich Chemical Co. and used without further purification.

[2-(4-Bromo-2-nitrophenoxy)benzyl]dimethylamine (8). To a stirred solution of potassium hydroxide (741 mg, 13.2 mmol) in water (18.2 mL) was added 2-dimethylaminomethylphenol (**6**) (2.0 g, 13.2 mol). The resulting solution was stirred at room temperature for 30 min and then concentrated to yield the phenoxide, which was used without further purification. DMSO (21 mL) was added to the phenoxide and then 2,5-dibromonitrobenzene (**7**) (5.1 g, 18.2 mmol) with stirring. The resulting mixture was heated to 120 °C for 8 h. The mixture was then cooled to room temperature and diluted with ethyl acetate and water. The aqueous phase was extracted three times with ethyl acetate, and the combined organic extracts were washed with brine, dried with magnesium sulfate, filtered, and concentrated to yield a dark orange oil. The product was isolated by column chromatography using silica gel and a solvent system of 40% ethly acetate: 60% hexane. The R_f value of the product **8**, in 40% ethyl acetate: 60% hexane was 0.2. The reaction yielded compound **8** (3.7 g, 80%): IR (cm^{-1}, neat) 3375, 2941, 2817, 2768, 1602, 1581, 1530, 1475, 1349, 1246; ^{1}H NMR (500 MHz $CDCl_3$) δ 8.07 (d, J = 2.3 Hz, 1H), 7.52−7.54 (m, 2H), 7.24−7.30 (m, 2H), 6.94 (d, J = 8.2 Hz, 1H), 6.75 (d, J = 8.2 Hz, 1H), 3.49 (s, 2H), 2.24 (s, 6H); HRMS (ES^+) calcd for $C_{15}H_{16}N_2O_3Br$ (MH^+) 351.0344, found 351.0344.

5-Bromo-2-(2-dimethylaminomethyl)phenoxy)phenylamine (9). To a suspension of **8** (760 mg, 2.17 mmol) in

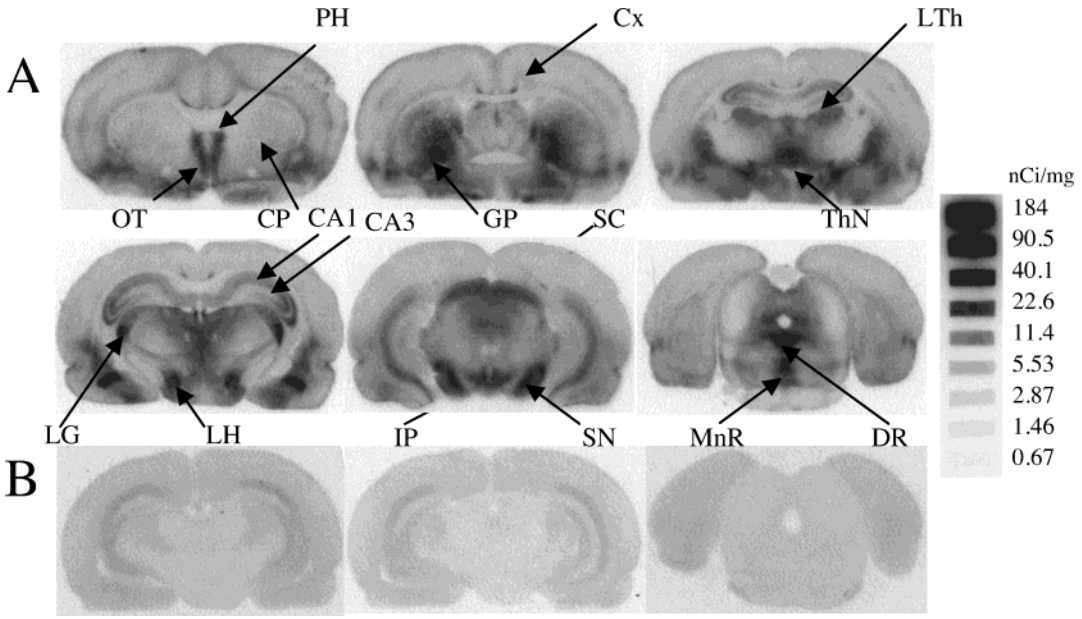


Figure 2. Ex vivo autoradiographic localization of [^{125}I]**4** binding sites in rats after 120 min postinjection. (A) Control rat: High levels of radioactivity were observed in areas containing high densities of serotonin transporter sites. The coronal sections corresponding to the stereotaxic atlas.[26] Abbreviations: CA1, CA3: CA1, CA3 Ammon's horn; CP: caudate putamen; Cx: cortex; DR: dorsal raphe nucleus; GP: globus pallidus; IP: interpedunclar nucleus; MnR: median raphe nucleus; LH: lateral hypothalamic area; LThN: laterodorsal thalamic nucleus; OT: olfactory tubercle; PH: periventricular hypothalamic nucleus; SC: superior colliculus; SN: substantia nigra; ThN: thalamus nucleus; VLG: ventricular lateral geniculate nucleus. (B) A rat pretreated with a dose of (+)McN5652 (2 mg/kg body weight) at 5 min prior to the iv injection of [^{125}I]**4**.

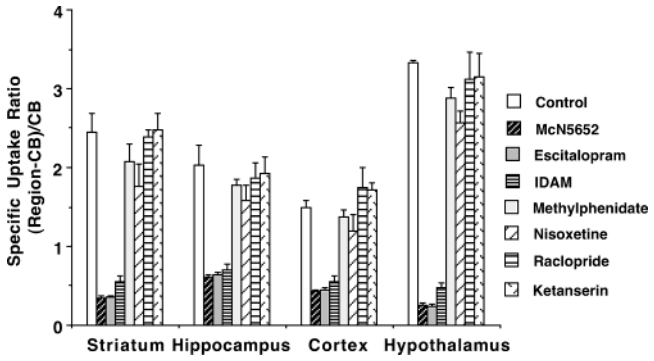


Figure 3. In vivo blockade of [^{125}I]**4** after pretreatment with various compounds on specific binding in rat brain regions. Rats were pretreated with various drugs with a dose of drug (2 mg/kg, iv at 5 min prior to tracer administration). Two hours after the tracer injection, specific binding in each brain region was compared between saline-pretreated (control) and drug-pretreated rats. The specific binding is expressed as the ratios of (region-cerebellum)/cerebellum, [(Region-CB)/CB], on a % dose/g basis. Values are presented as the average ± SD of three rats in each point. (+)McN5652, escitalopram and IDAM—serotonin transporter ligand; methylphenidate—dopamine transporter ligand; nisoxetine—norepinephrine transporter ligand; raclopride—dopamine D_2/D_3 receptor antagonist; ketanserin—5-HT_2 receptor antagonist.

concentrated HCl (8 mL) and methanol (16 mL) was added $SnCl_2$ (1.6 g) at 0 °C. The reaction mixture was stirred and warmed to room temperature. The reaction was stirred at room-temperature overnight. The acidic solution was diluted with water (20 mL) and extracted one time with ethyl acetate. The organic layer was discarded. The aqueous layer was basified to pH = 12 with 20% NaOH. The aqueous layer was then extracted three times with ethyl acetate. The combined organic extracts were then washed with brine, dried with magnesium sulfate, filtered, and concentrated to yield **9** (480 mg, 68%) as a brown solid. The R_f value of the product in 10% methanol:90% dichloromethane is 0.4: IR (cm^{-1}, neat) 3450, 3293, 3139, 2940, 2819, 2772, 2337, 1596, 1495, 1452, 1421, 1224, 1197; ^{1}H NMR (500 MHz $CDCl_3$) δ 7.26 (m, 1H), 7.17 (m, 1H), 7.02 (m, 1H), 6.78−6.82 (m, 4H), 4.74 (broad s, 2H), 3.53 (s, 2H), 2.26 (s, 6H); HRMS (ES^+) calcd for $C_{15}H_{17}N_2OBr$ (M^+) 320.0524, found 320.0523.

2-(2-(Dimethylaminomethyl)phenoxy)-5-(tributyltin)-phenylamine (10). Compound **9** (101.8 mg, 0.32 mmol) was placed in a sealed tube with dioxane (6.7 mL) and triethylamine (1.7 mL). The solution was stirred at room temperature, and bistribulyl tin (0.8 mL) was added followed by $Pd(PPh_3)_4$ (40 mg). The tube was sealed, and the reaction mixture was heated to 125 °C with stirring. The reaction was monitored by TLC and was complete as indicated by the absence of starting material in 8 h. The crude reaction mixture was cooled to room temperature, and solvent was removed in vacuo. The product was purified by silica gel preparative TLC twice using a solvent system of 10% methanol:90% dichloromethane. The R_f value of the product in that solvent system is 0.6. Compound **10** (68.7 mg, 43.4%) was isolated as a brown oil: IR (cm^{-1}, neat) 3308, 2954, 2923, 2851, 2356, 1582, 1486, 1453, 1227, 1197; ^{1}H NMR (500 MHz $CDCl_3$): δ 7.44 (d, J = 7.5 Hz, 1H), 7.25 (m, 1H), 7.06 (m, 1H), 6.75−6.87 (m, 4H), 3.85 (s, 2H), 2.48 (s, 6H), 0.87−1.53 (m, 27H); HRMS (ES^+) calcd for $C_{27}H_{45}N_2OSn$ (MH^+) 533.2554, found 533.2531.

2-(2-(Dimethylaminomethyl)phenoxy)-5-iodophenylamine, 4. To a solution of compound **10** (41.5 mg, 0.078 mmol) in $CHCl_3$ (5.9 mL) was added a solution of iodine in $CHCl_3$ (0.1 M) until the iodine color persisted. At this time the TLC showed no starting material. The reaction mixture was treated with KF in methanol (1.0 M, 0.2 mL) and stirred with sodium bisulfite (5%, 0.5 mL) for 5 min. The reaction mixture was diluted with water and chloroform. Two layers were separated, and the aqueous layer was discarded. The organic layer was washed with brine, dried with magnesium sulfate, filtered, and concentrated. The crude reaction was purified by silica gel preparative TLC to yield **4** (21.3 mg, 74%) as a brown solid. For analytical purpose, HCl salt was prepared as a white powder. Compound **4** has an R_f value of 0.5 in 10% methanol: 90% dichloromethane: IR (cm^{-1}, neat) 3317, 2952, 2921, 2851, 2358, 1592, 1492, 1461, 1413, 1225, 1196; ^{1}H NMR (500 MHz $CDCl_3$) δ 7.31 (d, J = 7.5 Hz, 1H), 7.20 (m, 1H), 7.02−7.08 (m, 2H), 6.96 (d, J = 8.0 Hz, 1H), 6.81 (d, J = 8.0 Hz, 1H), 6.61 (d,

J = 8.0 Hz, 1H), 4.65 (broad s, 2H), 3.60 (s, 2H), 2.32 (s, 6H); HRMS (ES^+) calcd for $C_{15}H_{18}N_2OI$ (MH^+) 369.0464, found 369.0462.

([^{125}I]/[^{123}I]2-(2-(Dimethylaminomethyl)phenoxy)-5-iodophenylamine, [^{125}I]/[^{123}I]4. The tin compound **10** (50 μg in 50 μL of ethanol), [I-125]/[I-123]sodium iodide, and 1 N HCl (100 μL) were placed in a sealed vial. To this mixture was added 100 μL of H_2O_2 (3% solution in water) via a syringe at room temperature. The iodination reaction was terminated after 10 min by an addition of saturated $NaHSO_3$ and the resulting solution was neutralized by adding a saturated $NaHCO_3$ solution. The mixture was purified by a C4 mini-column method (radiochemical yield > 90%). The purity was determined by HPLC (PRP-1 column, acetonitrile/5 mM 3,3-dimethylglutaric acid, pH 7.0: 90/10, flow rate = 1 mL/min; retention time = 9.3 min). Radiochemical purity was 99%.

(4-Bromo-2-nitrophenyl)(2-dimethylaminomethylphenyl)methanol (13a). A solution of 2-bromo-*N*,*N*-dimethylbenzylamine (**11**) (268 mg, 1.25 mmol) in THF (5 mL) was added to a solution of BuLi (1.7 mL, 1.6 M in THF, 2.7 mmol) in THF (5 mL) dropwise at RT. The mixture was stirred at RT for 10 min. The resulting lithium species was added to a solution of 4-bromo-2-nitrobenzaldehyde[19] (**12a**) (311 mg, 1.35 mmol) in THF (5 mL) dropwise at −78 °C. The mixture was stirred at RT overnight. Ice−water was added, and the mixture was extracted with CH_2Cl_2. The organic phase was dried under Na_2SO_4 and filtered, and the filtrate was concentrated to give crude product which was purified by PTLC (hexane/ethyl acetate (1/1)) to give 60 mg of product (12%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.28 (s, 6H), 3.11 (d, J = 12.5 Hz, 1H), 4.14 (d, J = 12.5 Hz, 1H), 6.56 (s, 1H), 6.58 (d, J = 8.8 Hz, 1H), 7.07−7.24 (m, 3H), 7.86 (d,d, J = 8.5, 2.2 Hz, 1H), 8.05 (d, J = 8.5 Hz, 1H), 8.16 (d, J = 2.0 Hz, 1H).

(2-Dimethylaminomethylphenyl)(4-iodo-2-nitrophenyl)-methanol (13b). The same procedure described above for preparation of **13a** was employed to prepare **13b**. Product **13b** was obtained (68 mg, 16%) from 4-iodo-2-nitrobenzaldehyde (**12b**) (277 mg, 1 mmol): ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.32 (s, 6H), 3.15 (d, J = 12.5 Hz, 1H), 4.31 (d, J = 12.5 Hz, 1H), 6.03 (s, 1H), 6.41 (d, J = 7.2 Hz, 1H), 7.09−7.29 (m, 3H), 8.02 (d, J = 8.5, Hz, 1H), 8.39 (d,d, J = 6.8, 2.3 Hz, 1H), 8.66 (d, J = 2.3 Hz, 1H).

5-Bromo-2-(2-(dimethylaminomethyl)benzyl)phenylamine (14). To a solution of nitro compound **13a** (67 mg, 0.18 mmol) in THF (5 mL) was added a solution of BH_3 (2 mL, 1 M in THF) dropwise at RT. The resulting mixture was stirred under reflux overnight. Water was added by caution to destroy the excess of BH_3. The solvent was removed on the Rotavapor. HCl solution (20 mL, 2% solution) was added, and the mixture was refluxed for 30 min NH_4OH (concentrated) was added after cooling to adjust the pH of the solution to basic. The mixture was extracted with mixed solvent (CH_2Cl_2/MeOH (95/5)). The organic phase was dried under Na_2SO_4 and filtered, and the filtrate was concentrated to give crude product which was purified by PTLC (hexane/ethyl acetate (1/1)) to give 34 mg of product (58%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.25(s, 6H), 3.47 (s, 2H), 3.90 (s, 2H), 6.71 (d, J = 2.0 Hz, 1H), 6.80 (d,d, J = 8.0, 2.0 Hz, 1H), 6.96 (d, J = 7.9 Hz, 1H), 7.03−7.08 (m, 1H), 7.12−7.20 (m, 3H).

2-(2-(Dimethylaminomethyl)benzyl)-5-iodophenylamine (5). The same procedure described above for preparation of **14** was employed to prepare **5**. Product **5** (13 mg, 21%) was obtained from **13b** (68 mg, 0.16 mmol). The HCl salt was prepared for analytical purposes: ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.23 (s, 6H), 3.34 (s, 2H), 4.00 (s, 2H), 6.54 (d,d, J = 8.2, 2.3 Hz, 1H), 6.62 (d, J = 8.2 Hz, 1H), 6.97 (m, 1H), 7.13−7.25 (m, 3H), 7.32−7.40 (m, 1H); HRMS (CI^+) calcd for $C_{16}H_{19}N_2I$ (M^+) 366.0593, found 366.0587.

2-(2-(Dimethylaminomethyl)benzyl)-5-(tributyltin)-phenylamine (15). The mixture of bromide **14** (14 mg, 0.04 mmol), bistributyltin (0.1 mL), $(PPh_3)_4Pd$ (10 mg) and triethylamine (0.1 mL) in dioxane (2 mL) was stirred at 90 °C overnight. Solvent was removed, and the residue was purified by PTLC (hexane/ethyl acetate (1/1)) to give 6 mg of product **15** (26%): ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.92 (t, J = 7.8 Hz, 9H), 1.26−2.03 (m, 18H), 2.26 (s, 6H), 3.49 (s, 2H), 3.96 (s, 2H), 6.69 (s, 1H), 6.78 (d, J = 7.1 Hz, 1H), 7.02 (d, J = 7.0 Hz, 1H), 7.05−7.20 (m, 4H).

2-(2-(Dimethylaminomethyl)benzyl)-5-[^{125}I]iodophenylamine ([^{125}I]5). The tin compound **17** (50 μg in 50 μL of ethanol), [I-125]/[I-123]sodium iodide, and 1 N HCl (100 μL) were placed in a sealed vial. To this mixture was added 100 μL of H_2O_2 (3% solution in water) via a syringe at room temperature. The iodination reaction was terminated after 10 min by an addition of saturated $NaHSO_3$. The resulting solution was neutralized by adding a saturated $NaHCO_3$ solution. The mixture was loaded on a C4 minicolumn. The cartridge was first washed with water. The desired product was washed off the cartridge by ethanol. Radiochemical yield >90%. Radiochemical purity after the purification was 99%.

Preparation of Membrane Homogenates. LLC-PK_1 cells from pig kidney permanently expressing DAT, NET and SERT, respectively (LLC-DAT, LLC-NET and LLC-SERT) were kindly provided by Dr. G. Rudnick at Yale University. These cells were grown to confluence as a monolayer on 15 cm Petri dish as reported previously.[25] Cells were then washed once with phosphate buffer saline (w/Ca^{2+} and Mg^{2+}) (Bio-Whittaker) and harvested with 10 mL of PBS by scraping. The cell suspensions were homogenized on ice for 20 strokes and centrifuged at 17 000 rpm for 20 min at 4 °C. The pellets were resuspended in PBS, frozen quickly in liquid N_2 and stored in a −70 °C freezer.

Binding Assays. Binding assays were performed in a final volume of 0.2 mL. Aliquots of membrane suspensions (100 μL, corresponding to 30−40 μg of protein) were mixed with 50 mM Tris-HCl, pH 7.4, 120 mM NaCl and 0.1% BSA (all from Sigma, St. Louis, MO), 0.4 nM [^{125}I]IPT or [^{125}I]IDAM, and 8−10 concentrations (10^{-10} to 10^{-5} M) of competing drugs. Nonspecific binding was defined with 10 μM CFT for [^{125}I]IPT assays and 1 μM IDAM for [^{125}I]IDAM assays. Incubation was carried out for 1 h at room temperature, and the bound ligand was collected on glass fiber filters (Schleicher & Schuell No. 25, Keene, NH) presoaked with 1% polyethylenimine (Sigma, St. Louis, MO) and counted in a gamma counter (Packard 5000). Results of competition experiments were subjected to nonlinear regression analysis using EBDA (Elsevier-BIOSOFT, Cambridge, UK).

Partition Coefficients. Partition coefficients were measured by mixing [^{125}I] compound with 3 g each of 1-octanol and buffer (pH 7.4, 0.1 M phosphate) in a test tube. The test tube was vortexed for 3 min at room temperature, then centrifuged for 5 min Two weighed samples (2 g each) from the 1-octanol and buffer layers were counted in a well counter. The partition coefficient was determined by calculating the ratio of cpm/g of 1-octanol to that of buffer. Samples from the 1-octanol layer were repartitioned until consistent partition coefficient values were obtained. The measurement was repeated three times.

Biodistribution in Rats. Three rats per group were used for each biodistribution study. While under ether anesthesia, 0.2 mL of a saline solution containing 10 μCi of radioactive tracer was injected into the femoral vein. The rats were sacrificed at the time indicated by cardiac excision while under ether anesthesia. Organs of interest were removed and weighed, and the radioactivity was counted. The percent dose per organ was calculated by a comparison of the tissue counts to counts of 1% of the initial dose (100 times diluted aliquots of the injected material) measured at the same time.

Regional brain distribution in rats was measured after an iv injection of the radioactive tracer. Samples from different brain regions: cortex (frontal plus occipital), striatum, hippocampus, cerebellum and hypothalamus were dissected, weighed and counted. The percentage dose/g of each sample was calculated by comparing sample counts with the counts of the diluted initial dose described above. The specific binding ratio in each region was obtained by dividing the %dose/gram values between region by that in the cerebellum ([region-

cerebellum/cerebellum]). The cerebellum region containing little or no serotonin transporters was used as the background region.

In vivo competitive binding of various compounds in the regional uptake of [^{125}I]**4** was investigated by pretreating animals with various competing drugs (2 mg/kg, iv at 5 min prior to injection of [^{125}I]**4**). The competing drugs included ketanserin, raclopride, methylphenidate, nisoxetine, (+)-McN5652, escitalopram and IDAM. Similar regional brain distributions were determined at 120 min after [^{125}I]**4** injection as described above. The reduction of regional specific binding in the drug-pretreated rats was compared to the control animals with saline pretreatment.

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Supporting Information Available: Elemental analyses data for **4** and **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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