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5-Chloro-2-(2'-((dimethylamino)methyl)-4'-iodophenyl thio)benzenamine: A New Serotonin Transporter Ligand

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Abstract

Two novel ligands with a 4'-substitution on the phenyl ring B of biphenylthiol: 5-chloro-2-(2'-((dimethylamino)methyl)-4'-iodophenylthio)benzenamine, **7**, and 2-(2'-((dimethylamino)methyl)-4'-methoxyphenylthio)-5-iodobenzenamine, **8**, were prepared and tested as potential serotonin transporter (SERT) imaging agents. The new ligands displayed extremely high binding affinities to SERT ($K_i = 0.22 \pm 0.09$ and 0.11 ± 0.04 nM, respectively), with very low binding affinities to dopamine and norepinephrine transporters ($K_i > 1,000$ nM). The corresponding [¹²⁵I]**7** and [¹²⁵I]**8** were successfully prepared from the tri-n-butyltin derivatives. They showed good brain uptakes and prolonged retention after *iv* injection in rats (brain uptake was 1.77 and 0.98 %dose/g for [¹²⁵I]**7** and 0.92 and 0.29 %dose/g for [¹²⁵I]**8** at 2 and 120 minutes, respectively). Significantly, [¹²⁵I]**7** showed excellent uptake and prolonged retention in the hypothalamus, where the SERT concentration is the highest. The hypothalamus/cerebellum ratios (target to background ratios) were 4.24, 7.10, 8.24 and 12.6 at 2, 4, 6 and 12 hours, respectively. The hypothalamus/cerebellum ratios for [¹²⁵I]**8** were 3.97, 5.57 and 5.06 at 1, 2 and 4 hours, respectively. Adding the 4'-iodo- group to the phenyl ring B of **7** appeared to reduce the rate of clearance from the brain, and the kinetics favored uptake and retention in the hypothalamus. The localization of [¹²⁵I]**7** in the hypothalamus region in the brain of rats could be blocked by pretreatment with (+)McN5652, escitalopram and ADAM (**2**), all selective serotonin transporter ligands (at 2 mg/Kg dose, *iv*, 5 min pretreatment). *Ex vivo* autoradiograms of rat brain sections (at 4 hr after *iv* injection of [¹²⁵I]**7**) showed intense labeling in regions of the brain known to have high SERT density. The excellent selective uptake and retention in the hypothalamus region suggests that [¹²⁵I]**7** is a potential lead compound for developing new imaging agents targeting SERT binding sites with single photon emission computed tomography (SPECT).

Introduction

The serotonin transporter (SERT) is a transmembrane protein located in the synapse which is responsible for removing serotonin from the synaptic cleft. The associations between serotonergic function and depression have been well documented [4–6]. It is commonly held that patients with major depression experience changes in the serotonergic neuronal transmission function in the brain [1–3]. A series of newer antidepressants preferentially increase 5-HT (serotonin) transmission by inhibiting serotonin reuptake. Selective serotonin reuptake inhibitors (a.k.a. SSRIs, i.e. citalopram, fluoxetine, fluvoxamine, paroxetine and sertraline) preferably inhibit serotonin uptake compared with dopamine or norepinephrine uptakes. SSRIs have revolutionized the management of depression for millions of patients.

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They are also widely prescribed for treating various other mental disorders such as obsessive compulsive and social phobic disorders [7,8].

Recently, *in vivo* imaging of SERT binding sites in the brain by position emission tomography (PET) has been significantly improved. Encouraging results from human studies using the leading PET imaging agent, [¹¹C]DASB (**3**) (Table 1) have demonstrated the potential of studying drug occupancy in living human brains [9–14]. Progress has also been made in imaging SERT binding sites in the brain by single photon emission computed tomography (SPECT) [15–17]. SPECT imaging studies using [¹²³I]ADAM (**2**) were performed to investigate SERT occupancies after a single dose of escitalopram or citalopram. A test-retest study showed that the drug occupancy in the brain could be correlated with the level of antidepressant dose with good reproducibility [13,15,16,18–20].

Up to this point, the development of SPECT or PET imaging agents targeting SERT binding sites has relied on four different core structures, 5-iodo-nitroquipazine [21–23], tropanes [24–26], (+)-McN5652 [27–31] and biphenylthiols [32–40]. Biphenylthiol derivatives, [¹²³I]IDAM (**1**) [41] and [¹²³I]ADAM (**2**) [39,42], have been evaluated as potential agents for SPECT because there are several unique benefits associated with using this core structure (Table 1). Biphenylthiol contains no optical center and the synthesis is relatively simple. Most importantly, this group of compounds is highly selective, showing a high binding affinity to SERT (K_i in nM or sub nM) and relatively low binding affinity to the other monoamine transporters (dopamine transporter, DAT and norepinephrine transporter, NET) and other CNS receptors or binding sites. The biphenylthiol core structure is neutral and relatively small, properties essential for penetrating the intact blood-brain barrier and delivering imaging agents to SERT binding sites in the brain. These distinctive features have attracted many researchers to work on this series of compounds [32–37,43].

Most of the biphenylthiols reported are derivatives containing substitutions on the phenyl ring A [32,34]. Our laboratory has previously reported IDAM (**1**) and ADAM (**2**). In the ADAM (**2**) series of compounds, we have recently prepared two derivatives, the O-bridged, OADAM (**3**) and C-bridged, **4**, and measured the kinetics of the brain uptake of the ¹²⁵I labeled compounds in rats [44] (Table 1). The specific hypothalamus uptake of C-bridged, [¹²⁵I]**4**, was inferior to that of OADAM (**3**) and ADAM (**2**). It was found that OADAM (**3**) consistently displayed a higher uptake in the hypothalamus (between 60–240 minutes post iv injection) than that of ADAM (**2**). The specific uptake of OADAM (**3**) in the hypothalamus exhibited the highest target to non-target ratio (hypothalamus/cerebellum, HY/CB, was 5.3 at 240 minutes post iv injection). But, one major drawback of OADAM (**3**) was that it appeared to show less selectivity. It had a higher binding affinity for norepinephrine transporter (NET) ($K_i = 20 \pm 2$ nM) (Table 1), making it the least selective SERT ligand among the biphenylthiols reported so far.

In order to further explore the potential of compounds with a biphenylthiol core, we developed several derivatives, **7** and **8**, with 4'-substitutions on the phenyl ring B. To our surprise, these 4'-substituted derivatives showed a remarkable *in vivo* biodistribution. In particular, the 4'-iodo-biphenylthiol derivative, **7**, displayed extraordinary uptake and retention in the hypothalamus, a target region for SERT. In this paper, we report the first examples of iodinated biphenylthiol derivatives with a 4'-substitution on the phenyl ring B. When labeled with ¹²³I ($T_{1/2} = 13$ hr and 159 KeV), they were promising agents for imaging SERT with SPECT.

Chemistry

The biphenylthiol derivatives described in this paper were prepared as outlined in Scheme 1. Commercially available 2-amino-5-bromobenzoic acid (**9**) and 2-amino-5-methoxybenzoic acid (**10**) were converted to corresponding thiols **11** and **12**, which were coupled with either

2,5-dichloronitrobenzene or 2,5-dibromonitrobenzene. The resulting carboxylic acids **13–15** were treated with thionyl chloride and reacted with dimethylamine to yield amides **16–18**. Reductions of nitro and amide groups simultaneously by excess borane-THF provided compounds **19, 20** and **21**. The bromide groups of **19** and **20** were converted to the corresponding tri-*n*-butyltin derivatives. The compounds **19** and **20** were used as radioiodination precursors. Non-radioactive iodinated compounds **7** and **8** were also prepared from **19** and **20** respectively by treatments with iodine in chloroform.

Similar to that reported for this series of ligands [41,42,44], the preparation of [¹²⁵I]**7** and [¹²⁵I]**8** was successfully performed by using an oxidative iodination of the corresponding tri-*n*-butyltin derivatives (Scheme 2). The radiolabeling yields for [¹²⁵I]**7** and [¹²⁵I]**8** were 87% and 70%, respectively and the radiochemical purities were 99% and 99%, respectively.

Biological studies and discussion

We used an *in vitro* binding assay to determine the inhibition constants (K_i , nM) with membrane preparations of three different groups of LLC-PK₁ cells, each expressing one specific monoamine transporter, either SERT, DAT or NET [45]. As expected, the binding affinities of **7** and **8** to SERT were excellent ($K_i = 0.22 \pm 0.07$ and 0.11 ± 0.04 nM, respectively), while the affinities to DAT and NET were very low ($K_i > 1,000$ nM). The results are consistent with the binding profiles reported for other biphenylthiol derivatives [35–37,39,40,42]. Other 4'-substituted derivatives of this series, **19, 20, 21** and **25** similarly showed sub-nM binding affinities and excellent selectivity (low binding affinities to other monoamine transporters) (Table 1).

The most surprising and unexpected observation for this series was their relatively good brain uptake and the prolonged retention of [¹²⁵I]**7** and [¹²⁵I]**8**. Biodistribution studies of [¹²⁵I]**7** and [¹²⁵I]**8** in normal rats (Table 2 and 3) showed that they penetrated the intact blood-brain barrier with initial brain uptakes of 1.77 and 0.92 %dose/organ at 2 min post *iv* injection, respectively. The brain uptake values showed a low, but steady, decline for both compounds. [¹²⁵I]**7** showed a slower decline, and at 2 and 4 hours there was 0.98 and 0.75 %dose/organ remaining in the brain. The brain uptake stayed relatively high at 6 hours (0.57 %dose/organ) (Table 2 and Fig. 1). In a similar biodistribution study, the brain uptake of [¹²⁵I]**8** declined faster and at 2 hours only 0.29 %dose/organ remained in the brain. There was a slightly slower washout rate from the lungs for [¹²⁵I]**7**. We also observed a prolonged retention of [¹²⁵I]**8** in skin tissue. Since the other major organs (lungs, liver, kidneys and muscle) showed comparable uptake and washout, it is not apparent that the unique brain uptake and retention simply reflect the equilibrium of distribution in the major organs (Table 2 and 3). The partition coefficients were comparable (2,061 and 2,206 for [¹²⁵I]**7** and [¹²⁵I]**8**, respectively). Therefore, it may not be a significant factor for consideration. It is likely that the unique brain kinetics of [¹²⁵I]**7** may be related to adding the 4'-substitution at the phenyl ring B. We decided to focus on [¹²⁵I]**7** for further characterization because **7** exhibited a slower decline in brain uptake and a higher retention than **8**. We compared biodistribution data of [¹²⁵I]**7** with that of two other previously reported leading SERT imaging agents, ADAM (**2**) and OADAM (**3**) [42,44].

It is apparent that [¹²⁵I]**7** showed a prolonged retention in the hypothalamus. The hypothalamus retention at 4 hours (0.75 %dose/organ) was higher than that of ADAM, **4** or OADAM, **3** [42,44] (Fig. 1A). Regional brain distribution of [¹²⁵I]**7** showed the highest uptake in the hypothalamus, where SERT binding sites are densely packed. The hypothalamus to cerebellum ratios (HY/CB) displayed a steady increase between 2 and 720 minutes, and the ratios were very high between 2 and 24 hours (HY/CB ratio = 4.24, 7.10, 8.24, 12.56 and 8.67 for 2, 4, 6, 12 and 24 hr, respectively, see Table 2 and Fig. 1B). The absolute values of HY/CB ratios for [¹²⁵I]**7** were higher than those of [¹²⁵I]ADAM (**2**) [42] and OADAM (**3**) [44] (HY/CB ratios

peaked between 5–6 in 4 hours) (Fig. 1B). It is important to note that the absolute peak hypothalamus uptake value for [125 I]7 was higher than that for OADAM (3) (0.66 and 0.32 % dose/g at 4 hr, for [125 I]7 and OADAM (3), respectively) (Table 2 and Fig. 1) [44]. The high brain uptake and HY/CB ratio may be an advantage, because it translates to a higher specific binding for SPECT imaging. HY/CB ratios for [125 I]8 were comparable to those of [125 I]ADAM(2) [42], but the hypothalamus uptake and retention were lower than those of OADAM (3) and [125 I]7 [44]. These critical differences in brain uptake and hypothalamus retention are clearly associated with the substitutions on the biphenylthiols and by the particular positions from which substituents are attached. The biodistribution studies of [125 I]7 in rat brains demonstrated the unique effects of the 4'-iodo substitution on the hypothalamus uptake and retention. We believe that this is the first example of an iodinated SPECT agent based on a biphenylthiol core structure with a 4'-iodo substitution. The improved hypothalamus uptake may be useful. However, the uptake kinetics, peaking at 6–12 hours may be too slow for SPECT imaging. It may be worth testing an alternative substitution group, other than the chlorine atom at the 5-position of ring A, while retaining the 4'-iodo group on the phenyl ring B. We are currently investigating additional derivatives to further explore this novel series of compounds.

In a separate effort focused on developing 18 F labeled SERT imaging agents for PET, we prepared 4'-(2-fluoroethoxy)- derivative, 5, (see Table 1). It also showed excellent uptake and retention in the hypothalamus region of the rat brain (Kung et al., un-published data). The results of *in vivo* biodistribution in rats for [18 F]5 also displayed the same unique kinetics in the hypothalamus as those observed for [125 I]7. It is likely that the 4'-substitution with the 2-fluoroethoxy- group led to an excellent *in vitro* binding affinity and a high uptake and retention in the hypothalamus, properties that would be useful in a SERT imaging agent for PET.

To demonstrate that the uptake in the hypothalamus region of the brain for [125 I]7 was specifically linked to SERT binding, we performed a blocking study. The results (Fig. 2) clearly indicated that the binding of [125 I]7 in the hypothalamus is due to specific SERT binding. Only the selective SERT ligands, (+)McN5652, escitalopram and ADAM (2), were able to block uptake in the hypothalamus region. Other drugs, nioxetine-NET ligand; GBR-12909-DAT ligand, showed no inhibition. (+)McN5652 and escitalopram were able to block more efficiently than ADAM (2). It is likely that binding of the “cold” ADAM (2) (a dose of 2 mg/kg, injected at 5 min prior to [125 I]7 injection) may be slower than the delivery of the tracer. The timing of the peak uptake and total retention of ADAM (2) may not be fast enough to compete with the binding of [125 I]7 to SERT binding sites in the hypothalamus, resulting in less than complete blockage (Fig. 2).

Ex vivo autoradiography was performed 4 hours after an iv injection of [125 I]7 in rat brains. The brain sections showed intense labeling in several regions [46], i.e. the lateral hypothalamic area (LH), dorsomedial hypothalamic nucleus (DM), interpeduncular nucleus (IP), thalamus nuclei (ThN), dorsal raphe (DR), medial raphe (MR), superior colliculus (SC), locus coeruleus (LC), substantia nigra (SN), globus pallidus (GP), all areas known to have high densities of SERT sites (Fig. 3) [47]. Lower, but detectable, labeling was also found in the frontal cortex, caudate putamen, ventral pallidum and hippocampus, areas containing a lower number of SERT sites. The regional distribution observed with [125 I]7 is consistent with those reported for other SERT ligands [48,49]. The distribution is completely in agreement with those of [125 I]ADAM (2) [42] and [125 I]ADAM (1) [41]. But, due to its higher selectivity, (note the contrast of the autoradiography at 4 hr post *iv* injection) [125 I]7 is likely to be superior to any other previously reported agent for mapping SERT binding sites in the brain.

In conclusion, a novel biphenylthiol derivative, [125 I]7, was prepared and tested as a potential lead compound for developing new SPECT imaging agents targeting SERT binding sites. This new ligand has a 4'-iodo- substitution group on the phenyl ring B, which preserves the binding

affinity and selectivity. Yet, the 4'-iodo-biphenylthiol derivative dramatically changed the kinetics of *in vivo* biodistribution resulting in improved hypothalamus uptake and retention, a property desirable for a SPECT imaging agent. This novel ligand, or analogs with similar substitutions, may be potential lead candidates for studying serotonin transporters with *in vivo* SPECT imaging.

Experimental

¹H and NMR spectra were performed on a Bruker DPX 200 spectrometer using tetramethylsilane as an internal standard. High resolution mass spectrometries were measured at McMaster Regional Centre for Mass Spectrometry. Microwave reactions were performed on a Biotage InitiatorTM microwave reactor. Tetrahydrofuran (THF) was distilled immediately before use from sodium benzophenone ketyl. All other chemicals were purchased from Aldrich Chemical Co. and used without further purification. Two HPLC systems were used to determine the purities of target compounds. Two HPLC measurements were performed using an Agilent 1100 Series with an isocratic pump and a UV detector. Measurements were performed on a Phenomenex Gemini analytical C-18 column (250 × 4.6 mm, 5 micron) with acetonitrile/ammonium formate buffer (10 mM) 80/20, v/v flow rate 1.0 mL/min at 254 nm (System A), and on a Hamilton PRP-1 analytical column (250 × 4.1, 10 micron) with acetonitrile/dimethyl glutarate buffer (5 mM pH 7) 90/10, v/v flow rate 1.0 mL/min at 254 nm (System B).

Male CD1 rats weighing 200–225 g were used in all the studies. No carrier added [¹²⁵I]NaI (0.1N NaOH solution) was purchased from NEN Research Products (PerkinElmer Life Sciences, Boston, MA). IDAM and ADAM, as well as the respective corresponding tri-n-butyltin precursors needed for the radioiodination of IDAM and ADAM, were prepared in our laboratory [38,42]. (+)McN5652 was kindly provided by Research Biochemicals Int. (Natick, MA) through a drug synthesis program supported by the National Institute of Mental Health. The radioiodinated nioxetine was prepared in our laboratory as described previously [50]. All other chemicals used were of reagent grade.

5'-Bromo-2'-(4-chloro-2-nitrophenylthio)benzoic acid (13)

To a solution of 2-amino-5-bromobenzoic acid (**9**) (3.57 g, 16.5 mmol) in 1.6 mL of 50% sodium hydroxide, water (22 mL) and NaNO₂ (1.14 g, 16.5 mmol) were slowly added. The resulting mixture was poured into a mixture of concentrated HCl (5 mL) and 7 g of ice with external cooling with salt/ice. The mixture was stirred at 0°C for 1 h and neutralized by an addition of potassium acetate (~1 g). The mixture was slowly added with vigorous stirring to a solution of *O*-ethylxanthic acid, potassium salt (7.7 g, 48 mmol) in 28 mL water at 80 °C. The temperature was maintained at 80 °C during the addition and stirring was continued until the evolution of N₂ gas had subsided. After cooling down to room temperature by external cooling in an ice bath, the mixture was acidified by the addition of concentrated HCl to pH 3 and extracted by dichloromethane (20 mL × 2) under argon atmosphere. The organic layer was dried with sodium sulfate under argon and the solvent was removed *in vacuo*. The crude product, 2-mercapto-5-bromobenzoic acid (**11**), was quickly dissolved in 15 mL of hot ethanol and added to a mixture of 2,5-dichloronitrobenzene (3.2 g, 16.7 mmol) and sodium ethoxide (0.76 g sodium in 33 mL ethanol). The resulting solution was heated to reflux for 2 hours. After cooling down, the solvent was removed *in vacuo* and water was added to the residue. The solution was acidified by an addition of concentrated HCl and extracted with ethyl acetate. Combined organic layers were washed with water and dried with sodium sulfate. The solvent was removed *in vacuo*. The residue was purified by silica gel column (ethyl acetate/methanol 10%) to provide an oily yellow solid. (2.6 g, 41 %): ¹H NMR (CDCl₃): δ 8.14 (d, *J* = 2.0 Hz, 1H), 8.12 (d, *J* = 2.0 Hz, 1H), 7.69 (dd, *J* = 8.4 Hz, 2.0 Hz, 1H), 7.46 (dd, *J* = 8.4 Hz, 2.0 Hz, 1H), 7.24 (d, *J* = 8.4 Hz, 1H), 7.12 (d, *J* = 8.4 Hz, 1H).

2'-(4-Bromo-2-nitrophenylthio)-5'-methoxybenzoic acid (14)

The same procedure described above for compound **13** was followed starting with 2-amino-5-methoxybenzoic acid (**10**) (2.5 g, 15 mmol). The crude intermediate, 2-mercapto-5-methoxybenzoic acid (**12**), was coupled with 2,5-dibromonitrobenzene (4.64 g, 16.5 mmol). Yellow solid. (3.2 g, 56 %): mp-80–82°C; ¹H NMR (CDCl₃): δ 13.4 (s, 1H), 8.39 (s, 1H), 7.81 (d, *J* = 8.8 Hz, 1H), 7.59 (d, *J* = 8.6 Hz, 1H), 7.44 (d, *J* = 2 Hz, 1H), 7.26 (dd, *J* = 8.6 Hz, *J* = 2 Hz, 1H), 6.86 (d, *J* = 8.8 Hz, 1H), 3.91 (s, 3H).

5'-Bromo-2'-(4-bromo-2-nitrophenylthio)benzoic acid (15)

5-Bromo-2-mercaptobenzoic acid (**11**), prepared as described above from 2-amino-5-bromobenzoic acid (3.57 g, 16.5 mmol) and was coupled with 2,5-dibromonitrobenzene (4.67 g, 16.7 mmol) to provide title compound, a yellow oil (2.9 g, 41%). ¹H NMR (CDCl₃/CD₃OD 10%): 8.23 (d, *J* = 2.1 Hz, 1H), 8.08 (d, *J* = 2.1 Hz, 1H), δ7.45–7.67 (m, 2H), 6.96 (d, *J* = 8.6 Hz, 1H), 7.21 (d, *J* = 8.4 Hz, 1H).

5'-Bromo-2'-(4-chloro-2-nitrophenylthio)-*N,N*-dimethylbenzamide (16)

A solution of **13** (0.8 g, 2.06 mmol) and thionyl chloride (1.5 mL) in chloroform (30 mL) was heated to reflux for 2 hours. The solvent and excess thionyl chloride were removed *in vacuo*. The residue was dissolved in chloroform (20 mL) and added to the 2M solution of dimethylamine in tetrahydrofuran (10 mL) at 0 °C. The mixture was stirred at room temperature for 3 hours. The solvent was removed and the residue was dissolved in ethyl acetate and washed with water and brine. The organic layer was passed through a short pad of silica gel and the solvent was removed *in vacuo*. The residue was spectroscopically pure without further purifications. Yellow oil (0.9 g, 69%); ¹H NMR (CDCl₃): δ 8.17 (d, *J* = 2.2 Hz, 1H), 7.56–7.77 (m, 2H), 7.43 (d, *J* = 8.2 Hz, 1H), 7.34 (dd, *J* = 8.6 Hz, 2.1 Hz, 1H), 6.88 (d, *J* = 8.6 Hz, 1H), 3.02 (s, 3H), 2.87 (s, 3H).

2'-(4-Bromo-2-nitrophenylthio)-5'-methoxy-*N,N*-dimethylbenzamide (17)

The procedure described above to prepare **16** was applied to **14** (2 g, 5.2 mmol) provided a title compound, a yellow oil (1.5 g, 68%); ¹H NMR (CDCl₃): δ 8.31 (d, *J* = 2.2 Hz, 1H), 7.39–7.50 (m, 2H), 6.92–7.02 (m, 2H), 6.80 (d, *J* = 8.8 Hz, 1H), 3.87 (s, 3H), 3.02 (s, 3H), 2.85 (s, 3H).

5'-Bromo-2'-(4-bromo-2-nitrophenylthio)-*N,N*-dimethylbenzamide (18)

The procedure described above to prepare **16** was applied to **15** (1 g, 2.31 mmol) provided a title compound, also a yellow oil (0.8 g, 75%); ¹H NMR (CDCl₃): δ 8.32 (d, *J* = 2.1 Hz, 1H), 7.56–7.63 (m, 2H), 7.40–7.50 (m, 2H), 6.81 (d, *J* = 8.6 Hz, 1H), 3.02 (s, 3H), 2.87 (s, 3H).

2-(4'-Bromo-2'-((dimethylamino)methyl)phenylthio)-5-chlorobenzenamine (19)

To a solution of **16** (200 mg, 0.48 mmol) in THF at 0°C, 1.0 M BH₃-THF (5 mL) was added. The mixture was heated to reflux for 24 hours. After cooling down, 0.5 mL concentrated HCl was cautiously added and the solvent was removed *in vacuo*. Water (5 mL) was added to the residue and the mixture was heated to reflux for 30 min. The mixture was adjusted to pH 10 with 1N NaOH and then extracted with ethyl acetate (5 mL × 2). The organic layer was dried (Na₂SO₄) and purified by silica gel column (ethyl acetate). Pale yellow oil (150 mg, 83 %); ¹H NMR (CDCl₃): δ 7.34–7.39 (m, 2H), 7.19 (dd, *J* = 8.4 Hz, 2.1 Hz, 1H), 6.66–6.76 (m, 3H), 4.63 (br s, 2H), 3.50 (s, 2H), 2.30 (s, 6H); HRMS calcd for C₁₅H₁₆BrClN₂S [MH⁺] 370.9984 obsd 370.9971; HPLC: 99% t_R = 11.7 min (System A), 97 % t_R = 6.58 min, (System B).

5-Bromo-2'-((dimethylamino)methyl)-4' methoxyphenylthio)benzenamine (20)

The same procedure described above to prepare **19** was applied to **17** (200 mg, 0.48 mmol) to provide the title compound, a pale yellow oil (89 mg, 50 %); ^1H NMR ($\text{CDCl}_3/\text{CD}_3\text{OD}$ 10%): δ 7.19–7.25 (m, 2H), 6.82–6.91 (m, 3H), 6.75 (dd, J = 8.2 Hz, 2.0 Hz, 1H), 4.41 (s, 2H), 3.80 (s, 3H), 2.90 (s, 6H); HRMS calcd for $\text{C}_{16}\text{H}_{19}\text{BrN}_2\text{OS}$ [M^+] 366.0401 obsd 366.0403; HPLC: 96% t_R = 5.9 min (System A), 96% t_R = 4.15 min, (System B).

5-Bromo-2-(4'-bromo-2'-((dimethylamino)methyl)phenylthio)benzenamine (21)

The same procedure described above to prepare **19** was applied to **18** (200 mg, 0.43 mmol) to provide the title compound, a pale yellow oil (120 mg, 66%); ^1H NMR (CDCl_3): δ 7.38 (d, J = 2.2 Hz, 1H), 7.29 (d, J = 8.2 Hz, 1H), 7.19 (dd, J = 8.4 Hz, 2.2 Hz, 1H), 6.82–6.87 (m, 2H), 6.74 (d, J = 8.4 Hz, 1H), 4.62 (br s, 2H), 3.50 (s, 2H), 2.29 (s, 6H); HRMS calcd for $\text{C}_{15}\text{H}_{16}\text{Br}_2\text{N}_2\text{S}$ [M^+] 415.9380 obsd 415.9387; HPLC: 98% t_R = 12.8 min (System A), 98% t_R = 7.96 min, (System B).

5-Chloro-2-(2'-((dimethylamino)methyl)-4' (tributylstannyl)phenylthio)benzenamine (22)

A mixture of **19** (48 mg, 0.129 mmol) and dibutyltin (400 mg) and tetrakis(triphenylphosphine)palladium(0) (25 mg) in triethyl amine (1 mL) was heated at 120° C in a sealed tube overnight. The crude mixture was purified by silica gel preparatory plate (ethyl acetate/dichloromethane 1/1) (35 mg, 47%) ^1H NMR (CDCl_3): δ 7.41 (d, J = 8.0 Hz, 1H), 7.18 (m, 1H), 6.80–6.86 (m, 2H), 6.64–6.76 (m, 2H), 4.75 (br s, 2H), 3.55 (s, 2H), 2.29 (s, 6H), 0.82–1.50 (m, 27H).

2-(2'-((Dimethylamino)methyl)-4'-methoxyphenylthio)-5-(tributylstannyl)benzenamine (23)

The procedure above was applied to compound **20** (44 mg, 0.12 mmol) to yield the title compound (40 mg, 58%); ^1H NMR (CDCl_3): δ 7.23 (m, 1H), 6.89–7.02 (m, 2H), 6.66–6.98 (m, 3H), 3.77 (s, 3H), 3.64 (s, 2H), 2.25 (s, 6H), 0.88–1.57 (m, 27H).

5-Chloro-2-(2'-((dimethylamino)methyl)-4' iodophenylthio)benzenamine (7)

To a solution of **22** (10 mg, 0.0172 mmol) in chloroform, 1N iodine in chloroform was added dropwise until the red color of the iodine persisted. The mixture was stirred 10 more minutes at room temperature and washed with 1N solution of sodium thiosulfate and purified by prep plate. Colorless oil (4.6 mg, 64%); ^1H NMR (CDCl_3): δ 7.56 (d, J = 2.0 Hz, 1H), 7.34–7.40 (m, 2H), 6.66–6.71 (m, 2H), 6.58 (d, J = 8.2 Hz, 1H), 4.62 (br s, 2H), 3.48 (s, 2H), 2.29 (s, 6H); $\text{C}_{15}\text{H}_{16}\text{ClIN}_2\text{S}$ [M^+] 417.9768 obsd 417.9753; HPLC: 96%, t_R = 12.9 min (System A), 98% t_R = 8.06 min, (System B).

2-(2'-((Dimethylamino)methyl)-4'-methoxyphenylthio)-5-iodobenzenamine (8)

The above procedure was applied to compound **23** (10 mg, 0.0173 mmol) and yielded the title compound (6 mg, 83 %); ^1H NMR (CDCl_3): δ 6.88–7.08 (m, 4H), 6.88 (d, J = 2.8 Hz, 1H), 6.70 (dd, J = 8.4 Hz, 2.8 Hz, 1H), 4.67 (br s, 2H), 3.77 (s, 3H), 3.58 (s, 2H), 2.31 (s, 6H); $\text{C}_{16}\text{H}_{19}\text{IN}_2\text{OS}$ [M^+] 414.0263 obsd 414.0208; HPLC: 96% t_R = 6.5 min (System A), 97% t_R = 5.3 min, (System B).

2'-(4-Bromo-2-nitrophenylthio)-5'-hydroxy-*N,N*-dimethylbenzamide (24)

A solution of **17** (320 mg, 0.78 mmol) in dichloromethane (3 mL) was added to a 1.0 M solution of BBr_3 in dichloromethane (1.1 mL). The mixture was irradiated with microwave at 100°C for 20 minutes and washed with water (2 mL). The organic layer was dried (Na_2SO_4) and purified by silica gel column (ethyl acetate/dichloromethane 1/4) to yield a yellow oil (300 mg,

97 %). ^1H NMR (CDCl_3): δ 8.92 (br s, 1H), 8.34 (d, $J = 2.1$ Hz, 1H), 7.53 (dd, $J = 8.8$ Hz, 2.1 Hz, 1H), 7.37 (d, $J = 8.2$ Hz, 1H), 6.70–6.90 (m, 3H), 3.08 (s, 3H), 2.92 (s, 3H).

4'-(2-Amino-4-bromophenylthio)-3'-((dimethylamino)methyl)phenol (25)

To a solution of **24** (20 mg, 0.05 mmol) in THF at 0°C , 1.0 M BH_3 -THF (3 mL) was added. The mixture was heated to reflux for 24 hours. After cooling down, 0.5 mL concentrated HCl was cautiously added and the solvent was removed *in vacuo*. Water (5 mL) was added to the residue and the mixture was heated to reflux for 30 minutes. The mixture was adjusted to pH 10 with 1N NaOH and extracted with ethyl acetate (5 mL $\times$ 2). The organic layer was dried (Na_2SO_4) and purified by silica gel column (ethyl acetate). Pale yellow oil (10 mg, 56%); ^1H NMR (CDCl_3): δ 7.19 (d, $J = 8.0$ Hz, 1H), 6.89 (d, $J = 8.4$ Hz, 1H), 6.75–6.83 (m, 3H), 6.60 (dd, $J = 8.4$ Hz, 2.8 Hz, 1H), 3.52 (s, 2H), 2.29 (s, 6H); HRMS calcd for $\text{C}_{15}\text{H}_{17}\text{BrN}_2\text{OS}$ [M^+] 352.0245 obsd 352.0225; HPLC: 96% t_R 3.7 min (System A), 97 % $t_R = 3.6$ min (1 mL/min) (System B).

In vitro binding assays

Membrane homogenates from LLC-PK1 cells expressing three separate monoamine transporters (DAT, NET, and SERT) [45] were used for the binding assays. Competitive binding assays were performed in a final volume of 0.25 mL. Aliquots of membrane suspensions (100 μL corresponding to 30–40 μg protein) were mixed with 50 μM Tris-HCl, pH 7.4, 120 mM NaCl and 0.1% bovine serum albumin, 0.4 nM of either [^{125}I]IPT [51], 0.2 nM [^{125}I]IDAM [41] or 0.1 nM [^{125}I]2-iodo-nisoxetine [50] and 8–10 concentrations (10^{-10} to 10^{-5}M) of competing drugs. Nonspecific binding was defined by using 1 μM of GBR12909 for DAT, 5 μM nisoxetine for NET and 1 μM IDAM for SERT assays. Incubation was carried out for 1 hour at room temperature and the bound ligand was collected on glass fiber filters presoaked with 1% polyethylenimine (Sigma, St. Louis, MO) and counted in a gamma counter (Packard 5000, Downers Grove, IL). The results of competition experiments were subjected to nonlinear regression analysis using Graphpad Prism 4 software (Graphpad Software, San Diego, CA).

Biodistribution in rats

Three rats per group were used for each biodistribution study. While under isoflurane anesthesia, 0.2 mL of a saline solution containing 10–100 mCi of radioactive tracer was injected into the femoral vein. The rats were sacrificed at the time indicated by cardiac excision while under anesthesia. Organs of interest were removed and weighed, and the radioactivity was counted. The percent dose per organ was calculated by comparing the tissue counts to counts of 1% of the initial dose (aliquots of the injected material diluted 100 times) measured at the same time. Regional brain distribution was measured after an *iv* injection of the radioactive tracer. Samples from different brain regions [cortex (CX), striatum (ST), hippocampus (HP), cerebellum (CB) and hypothalamus (HY)] 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, as described above. The ratio of specific binding in each region was obtained by dividing the percentage dose/g of each region by that of the cerebellum [region/CB]. The cerebellum was used as a background region because it contains very low concentration of SERT.

In vivo competitive binding of various compounds in the regional uptake of [^{125}I]**7** was investigated by pretreating animals with various competing drugs (2 mg/kg, each, injected *iv* 5 min prior to injection of [^{125}I]**7**). The competing drugs included: (+)McN5652, Escitalopram and ADAM (**2**), which are serotonin transporter (SERT) ligands, nisoxetine, a selective norepinephrine transporter (NET) ligand and GBR-12909, a dopamine transporter (DAT)

ligand. Regional brain distributions were then determined at 4 hr after injection of [¹²⁵I]**7**, as described above. The reduction of regional specific binding in the drug-pretreated rats was compared to binding in control animals with saline pretreatment.

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Figure 1.

Comparison of total hypothalamus uptakes (A) and hypothalamus/cerebellum (HY/CB) ratios (B) between ADAM (2) [42], OADAM (3) [44] and $[^{125}\text{I}]7$ in rats. The kinetics of the brain uptake and retention for $[^{125}\text{I}]7$ was slower than that of ADAM (2) or OADAM (3). The hypothalamus/cerebellum ratios of ADAM (2) and OADAM (3) reached the highest value at 4 hr post *iv* injection (HY/CB = 5), while $[^{125}\text{I}]7$ peaked at 12 hr after the tracer injection (HY/CB = 12.6).

Figure 2.

Competitive studies of [125 I]7 in rat brains *in vivo*. Rats were pretreated with drugs with a dose of 2 mg/kg, iv. 5 min prior to the tracer administration. At 4 hr after the tracer injection, hypothalamus/cerebellum (HY/CB) ratios were compared between saline-pretreated (control) and drug-pretreated rats. The values are presented as the average $\pm$ SD of three rats in each point. (+)McN5652, Escitalopram and ADAM (**2**) are serotonin transporter (SERT) ligands. Nisoxetine is a norepinephrine transporter (NET) ligand and GBR-12909 is a dopamine transporter (DAT) ligand.

Figure 3.

Ex vivo autoradiographic localization of $[^{125}\text{I}]\mathbf{7}$ binding sites in rats 4 hours post-injection in a normal rat – High levels of radioactivity were observed in areas containing high densities of serotonin transporter sites. Rat brain sections showed intense labeling in several regions [46], i.e. lateral hypothalamic area (LH), dorsomedial hypothalamic nucleus (DM), interpeduncular nucleus (IP), thalamus nuclei (ThN), dorsal raphe (DR), medial raphe (MR), superior colliculus (SC), locus coeruleus (LC), substantia nigra (SN), globus pallidus (GP), areas known to have high densities of SERT sites [47]. Lower, but detectable, labeling was also found in the frontal cortex, caudate putamen, ventral pallidum and hippocampus, areas containing a significantly lower number of SERT sites. The coronal sections correspond to the stereotaxic atlas [46].

^a Reaction conditions: (a) 1) NaNO_2 , 2) KS_2COEt , 3) HCl ; (b) EtONa ; (c) 1) SOCl_2 , 2) Me_2NH ; (d) 1) $\text{BH}_3\text{-THF}$, 2) SnCl_2 ; (e) $(\text{SnBu}_3)_2$, $(\text{PPh}_3)_4\text{Pd}(0)$; (f) I_2 .

^a Reaction conditions: (g) BBr_3 ; (h) $\text{BH}_3\text{-THF}$.

^a Reaction conditions: (i) $\text{Na}^+/\text{H}_2\text{O}_2$, HCl

Scheme 1.

Synthesis of SERT ligands 7, 8, 19–21, 25^a

Table 1*In vitro* inhibition constants (K_i , nM) of SERT ligands

Compd	X	R ₁	R ₂	R ₃	K_i (nM)		
					SERT	NET	DAT
1 IDAM ^a	S	CH ₂ OH	Cl	H	0.1 ± 0.01	234 ± 26	>1000
2 ADAM ^b	S	NH ₂	I	H	0.01 ± 0.00	699 ± 80	840 ± 100
3 OADAM ^c	O	NH ₂	I	H	0.12 ± 0.02	20 ± 2	>1,000
4 ^c	CH ₂	NH ₂	I	H	48.6 ± 4.10	17 ± 4	>1,000
5 ^d	S	NH ₂	Br	O(CH ₂) ₂ F	0.09 ± 0.07	>1,000	>1,000
6 DASB ^e	S	NH ₂	CN	H	1.1 ± 0.04	>1,000	>1,000
7	S	NH ₂	Cl	I	0.22 ± 0.09	>1,000	>1,000
8	S	NH ₂	I	OMe	0.11 ± 0.04	>1,000	>1,000
19	S	NH ₂	Cl	Br	0.12 ± 0.09	>1,000	>1,000
21	S	NH ₂	Br	Br	0.09 ± 0.01	>1,000	>1,000
20	S	NH ₂	Br	OMe	0.03 ± 0.01	>1,000	>1,000
25	S	NH ₂	Br	OH	0.14 ± 0.06	>1,000	>1,000

^a[41].^b[42].^c[44];^dKung et al., unpublished data,^evalues reported previously by Wilson et al., [52].

Table 2
Organ distribution (% dose/organ, n = 3) and regional brain uptake (% dose/g) of [¹²⁵I]7 in rats (iv injection)

Organ	2 min	30 min	60 min	2 hr	4 hr	6 hr	12 hr	24 hr
Blood	3.45 ± 0.47	3.32 ± 0.13	4.06 ± 0.31	3.67 ± 0.28	2.43 ± 0.27	2.25 ± 0.59	2.34 ± 0.20	1.09 ± 0.11
Heart	1.71 ± 0.20	0.18 ± 0.05	0.11 ± 0.02	0.09 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.02 ± 0.00
Muscle	9.90 ± 1.41	11.7 ± 2.47	7.30 ± 0.28	4.93 ± 0.25	3.75 ± 0.13	3.02 ± 0.26	2.09 ± 0.38	1.24 ± 0.36
Lung	7.08 ± 1.21	2.34 ± 0.19	1.66 ± 0.17	0.79 ± 0.05	0.45 ± 0.03	0.34 ± 0.06	0.16 ± 0.03	0.06 ± 0.01
Kidney	5.28 ± 0.48	1.33 ± 0.04	1.03 ± 0.07	0.73 ± 0.03	0.68 ± 0.09	0.53 ± 0.15	0.40 ± 0.07	0.23 ± 0.05
Spleen	1.10 ± 0.30	0.47 ± 0.12	0.40 ± 0.06	0.21 ± 0.04	0.12 ± 0.02	0.09 ± 0.02	0.03 ± 0.00	0.02 ± 0.00
Liver	22.7 ± 5.03	5.82 ± 0.51	4.67 ± 0.35	3.55 ± 0.24	2.85 ± 0.21	2.74 ± 0.43	2.13 ± 0.11	1.30 ± 0.13
Skin	5.14 ± 0.71	5.28 ± 0.74	5.97 ± 0.44	4.49 ± 0.61	4.66 ± 0.16	4.65 ± 1.41	2.18 ± 0.50	1.16 ± 0.24
Brain	1.77 ± 0.37	1.17 ± 0.09	1.21 ± 0.12	0.98 ± 0.10	0.75 ± 0.05	0.57 ± 0.11	0.26 ± 0.04	0.05 ± 0.01
Thyroid	0.07 ± 0.01	0.04 ± 0.01	0.03 ± 0.00	0.04 ± 0.01	0.08 ± 0.01	0.14 ± 0.02	0.23 ± 0.05	0.15 ± 0.02

Regional brain distribution (% dose/g)								
Region	2 min	30 min	60 min	2 hr	4 hr	6 hr	12 hr	24 hr
Cerebellum	0.95 ± 0.22	0.38 ± 0.04	0.27 ± 0.04	0.17 ± 0.01	0.09 ± 0.01	0.06 ± 0.01	0.02 ± 0.01	0.01 ± 0.00
Striatum	0.81 ± 0.14	0.69 ± 0.04	0.68 ± 0.05	0.63 ± 0.02	0.51 ± 0.02	0.38 ± 0.12	0.16 ± 0.02	0.04 ± 0.01
Hippocampus	0.76 ± 0.17	0.56 ± 0.04	0.58 ± 0.05	0.50 ± 0.06	0.39 ± 0.07	0.28 ± 0.06	0.12 ± 0.03	0.02 ± 0.00
Cortex	1.16 ± 0.25	0.68 ± 0.03	0.68 ± 0.07	0.54 ± 0.04	0.40 ± 0.08	0.25 ± 0.07	0.08 ± 0.03	0.02 ± 0.00
Hypothalamus	0.85 ± 0.17	0.73 ± 0.05	0.77 ± 0.02	0.73 ± 0.04	0.66 ± 0.06	0.48 ± 0.08	0.26 ± 0.05	0.06 ± 0.01

Ratio to Cerebellum								
Region	2 min	30 min	60 min	2 hr	4 hr	6 hr	12 hr	24 hr
Striatum	0.85 ± 0.10	1.81 ± 0.11	2.52 ± 0.19	3.64 ± 0.15	5.51 ± 0.22	6.24 ± 0.97	7.84 ± 2.18	5.36 ± 1.80
Hippocampus	0.80 ± 0.05	1.47 ± 0.04	2.17 ± 0.17	2.90 ± 0.26	4.15 ± 0.47	4.77 ± 0.14	5.61 ± 0.40	3.43 ± 0.62
Cortex	1.22 ± 0.09	1.79 ± 0.10	2.51 ± 0.13	3.10 ± 0.16	4.23 ± 0.65	4.27 ± 0.69	3.72 ± 0.62	2.68 ± 0.49
Hypothalamus	0.89 ± 0.03	1.91 ± 0.07	2.90 ± 0.40	4.24 ± 0.22	7.10 ± 0.47	8.24 ± 0.40	12.6 ± 1.11	8.67 ± 1.23

Table 3
Organ distribution (% dose/organ, n = 3) and regional brain uptake (% dose/g) of [¹²⁵I]8 in rats (iv injection)

Organ	2 min	30 min	60 min	120 min	240 min
Blood	11.22 ± 0.26	5.19 ± 0.63	4.48 ± 0.07	4.11 ± 0.24	3.76 ± 0.20
Heart	0.57 ± 0.05	0.14 ± 0.03	0.09 ± 0.01	0.10 ± 0.01	0.06 ± 0.01
Muscle	15.37 ± 1.42	9.23 ± 1.27	6.64 ± 0.14	6.03 ± 0.60	4.95 ± 0.67
Lung	3.90 ± 0.44	1.41 ± 0.20	0.63 ± 0.00	0.36 ± 0.07	0.22 ± 0.01
Kidney	2.79 ± 0.11	1.66 ± 0.23	1.32 ± 0.14	0.74 ± 0.10	0.45 ± 0.05
Spleen	0.64 ± 0.19	0.37 ± 0.11	0.19 ± 0.00	0.13 ± 0.03	0.08 ± 0.00
Liver	21.2 ± 1.80	5.70 ± 0.65	5.32 ± 1.10	4.81 ± 0.47	3.81 ± 0.30
Skin	8.44 ± 0.91	13.2 ± 0.17	11.76 ± 0.48	11.0 ± 1.54	11.5 ± 4.49
Brain	0.92 ± 0.12	0.74 ± 0.16	0.55 ± 0.01	0.29 ± 0.07	0.08 ± 0.01
Thyroid	0.07 ± 0.00	0.16 ± 0.04	0.44 ± 0.07	1.24 ± 0.47	3.75 ± 1.00

Regional brain distribution (% dose/g)				
Region	2 min	30 min	60 min	120 min
Cerebellum	0.48 ± 0.06	0.22 ± 0.06	0.11 ± 0.01	0.04 ± 0.01
Striatum	0.46 ± 0.05	0.43 ± 0.09	0.35 ± 0.01	0.19 ± 0.03
Hippocampus	0.41 ± 0.06	0.38 ± 0.08	0.30 ± 0.02	0.16 ± 0.04
Cortex	0.59 ± 0.08	0.45 ± 0.09	0.30 ± 0.02	0.13 ± 0.03
Hypothalamus	0.46 ± 0.05	0.50 ± 0.09	0.43 ± 0.04	0.24 ± 0.05

Ratio to Cerebellum				
Region	2 min	30 min	60 min	120 min
Cerebellum	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
Striatum	0.95 ± 0.03	2.01 ± 0.11	3.28 ± 0.31	4.39 ± 0.16
Hippocampus	0.86 ± 0.03	1.76 ± 0.07	2.77 ± 0.23	3.57 ± 0.16
Cortex	1.24 ± 0.02	2.11 ± 0.11	2.76 ± 0.05	2.87 ± 0.07
Hypothalamus	0.97 ± 0.01	2.32 ± 0.17	3.97 ± 0.08	5.57 ± 0.12