

Novel Radiotracers for Imaging the Serotonin Transporter by Positron Emission Tomography: Synthesis, Radiosynthesis, and in Vitro and ex Vivo Evaluation of ^{11}C -Labeled 2-(Phenylthio)araalkylamines

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Received February 22, 2000

A series of four 2-(phenylthio)araalkylamines have been radiolabeled with ^{11}C and evaluated as potential radiotracers for imaging the serotonin transporter (SERT) by positron emission tomography (PET). All four candidates display high affinity for SERT and low affinity for the dopamine or norepinephrine transporters using in vitro binding assays. Biodistribution studies in rats demonstrated that tail-vein injection of the ^{11}C -labeled radiotracers resulted in high brain uptake of radioactivity with a preferential distribution in brain regions known to be rich in SERT such as hypothalamus and thalamus. The most promising candidate, **16**, had hypothalamus-to-cerebellum ratios of 9:1, 1 h postinjection, an indication of high specific to nonspecific binding. Ex vivo pharmacological studies demonstrated that uptake in SERT-rich brain regions was both saturable and selective for SERT. Two of the tested radiotracers, **15** and **16**, have highly favorable properties for imaging SERT and will be used in pilot human PET imaging studies.

Introduction

As the central site of action of many existing antidepressant drugs of the selective serotonin reuptake inhibitor (SSRI) type, the serotonin transporter (SERT) has been the focus of intense study for many years.^{1–4} In vivo imaging of SERT in living human brain, either by positron emission tomography (PET) or by single photon emission computed tomography (SPECT), has also been pursued vigorously by many groups but has met with limited success.⁵ Most of the candidate radiotracers, while possessing high affinity for SERT in vitro, have shown poor ex vivo binding characteristics.⁶ The most common defects have been poor signal-to-noise ratios and a lack of selectivity for SERT over other monoaminergic transporters: the dopamine transporter (DAT) and the norepinephrine transporter (NET). Inappropriate pharmacokinetics ex vivo, often due to very slow clearance of nonspecific binding, have been a common characteristic of failed SERT imaging ligands. Arguably the most useful SERT radiotracer developed to date for PET studies is (+)-[^{11}C]McNeil 5652.⁷ In human PET studies with this selective SERT ligand, modest midbrain-to-cerebellum ratios of 1.8:1 are obtained 115 min postinjection.⁸ Nevertheless, the modest signal, slow pharmacokinetics, and complicated modeling may limit the usefulness of this radiotracer.⁹

A few new candidates have appeared in the literature of late which may have the potential to overcome these limitations. In the phenyltropane class of compounds, [^{18}F]FEBRNT and [^{11}C]RTI-357 both display high selectivity for SERT in vitro over other monoaminergic transporters.^{10,11} Information on the ex vivo binding

data of the former is sparse however, while the latter displays moderate signal-to-noise ratios (1.2:1) in PET Cynomolgus monkey scans. A novel radioiodinated SERT radiotracer for SPECT imaging, [^{123}I]IDAM, has been described recently which was based on the 2-(phenylthio)araalkylamine structure, originally developed as nontricyclic antidepressants.^{12–14} IDAM was shown to have high specificity and selectivity for SERT in vitro and ex vivo in rat biodistribution studies,¹⁵ while SPECT studies on baboons using [^{123}I]IDAM demonstrated that a transient equilibrium was quickly reached with midbrain-to-cerebellum ratios of 1.8:1 obtained at 120 min postinjection.¹⁶

Recently we reported the radiosynthesis of a ^{11}C -labeled potential SERT ligand in the 2-(phenylthio)araalkylamines series (5-trifluoromethyl-2-(2-dimethylaminomethylphenylsulfanyl)phenylamine) and showed that it readily crossed the blood–brain barrier (BBB).¹⁷ Expanding upon this work, we report here the synthesis and radiosynthesis of three new, less lipophilic analogues of our prototype SERT ligand for PET studies. All four ^{11}C -labeled radiotracers [^{11}C]-**13**–**16** (Scheme 1) were evaluated by in vitro binding studies and ex vivo rat biodistribution experiments as potential ligands for imaging SERT by PET. In addition, ex vivo pharmacological and metabolic studies were also carried out on two of the more promising candidates.

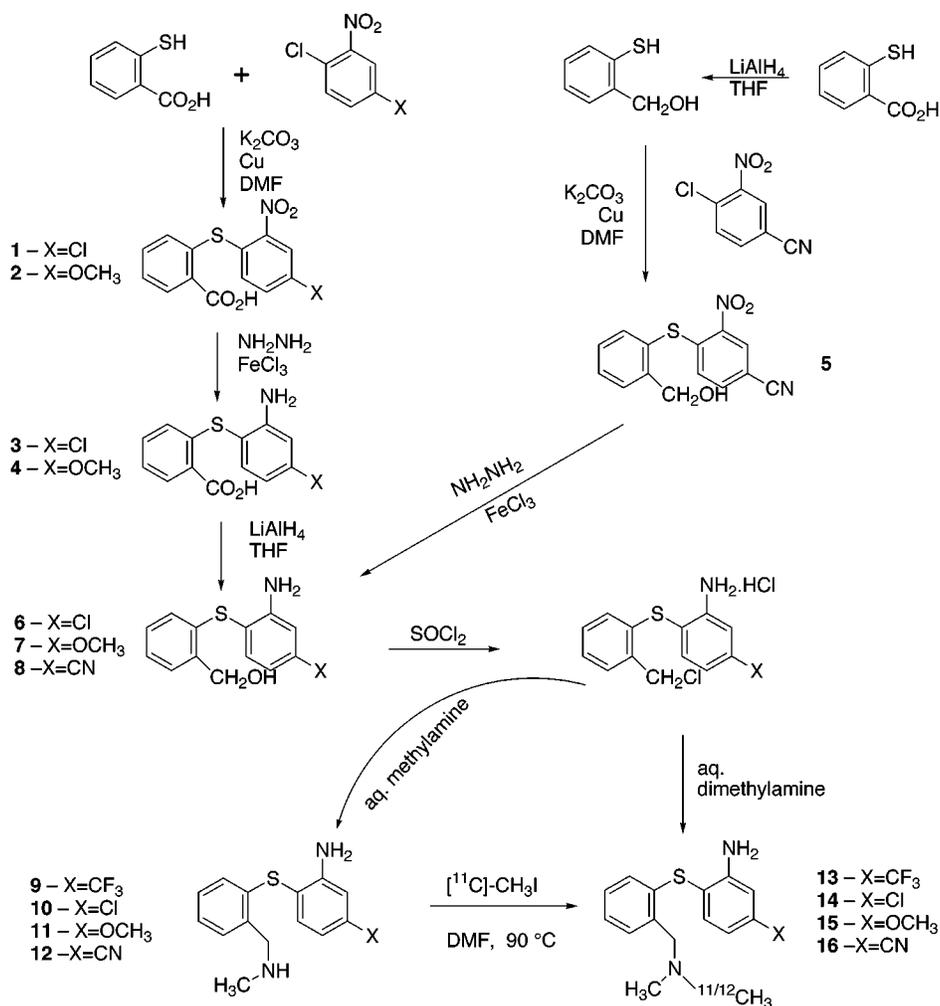
Experimental Section

Purification and analyses of radioactive mixtures were performed by HPLC with an in-line UV (254 nm) detector in series with a NaI crystal radioactivity detector (synthesis and QC) or a Berhhold LB507A radioactivity detector (metabolite analysis). Isolated radiochemical yields were determined with a dose-calibrator (Capintec CRC-712M). THF was freshly distilled under nitrogen from LiAlH_4 and DMF was distilled from BaO and stored over 4 Å molecular sieves. (+)-McNeil

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Scheme 1



5652 was obtained from the NIMH's Chemical Synthesis and Drug Supply Program. All other chemicals were obtained from commercial sources. Column chromatography used silica gel 60 (70–230 mesh). Elemental analyses were performed by Atlantic Microlab (Georgia). NMR spectra were run on a Varian Unity 500 at 500 MHz (¹H) or 127.5 MHz (¹³C) in CDCl₃ (unless otherwise stated) with tetramethylsilane or deuterated solvent as internal standard; *J* values are given in hertz (Hz). Radio-TLC of radioactive solutions were performed on a Berthold Tracemaster 20 linear analyzer. Melting points are uncorrected. All animal experiments were carried out under humane conditions, with approval from the Animal Care Committee at the CAMH, and in accordance with the guidelines set forth by the Canadian Council on Animal Care.

2-(4-Chloro-2-nitrophenylsulfanyl)benzoic Acid (1). A mixture of thiosalicylic acid (3.4 g, 22 mmol), 2,5-dichloronitrobenzene (4.5 g, 23.2 mmol), K₂CO₃ (7 g, 50.6 mmol) and copper powder (0.4 g, 6.3 mmol) in DMF (40 mL) was stirred at 65 °C for 36 h. After dilution with water (300 mL), the mixture was filtered through diatomaceous earth and the filtrate acidified with aqueous HCl (6 N) and cooled (4 °C). The yellow precipitate was filtered off and recrystallized from 50% aqueous ethanol to give 4.1 g (60%) of yellow crystals: mp 149–152 °C; ¹H NMR (DMSO-*d*₆) 8.27 (d, 1H, *J* = 2.4), 7.90 (dd, 1H, *J* = 1.7 and 7.8), 7.71 (dd, 1H, *J* = 1.5 and 8.5), 7.56 (dt, 1H, *J* = 1.7 and 7.6), 7.50 (dt, 1H, *J* = 1.2 and 7.8), 7.32 (dd, 1H, *J* = 1.2 and 7.8), 7.20 (d, 1H, *J* = 8.5). Anal. (C₁₃H₈ClNO₄S) C, H, N.

2-(4-Methoxy-2-nitrophenylsulfanyl)benzoic Acid (2). Using 4-bromo-3-nitroanisole and the procedure described for **1** above yielded 65% of **2** as yellow crystals after recrystallization from 95% aqueous ethanol: mp 216–222 °C; ¹H NMR

(DMSO-*d*₆) 7.91 (dd, 1H, *J* = 1.5 and 7.8), 7.67 (d, 1H, *J* = 2.9), 7.48 (d, 1H, *J* = 8.8), 7.44 (dt, 1H, *J* = 1.7 and 7.3), 7.28–7.33 (2H, m), 6.85 (dd, 1H, *J* = 1.0 and 8.0), 3.88 (s, 3H). Anal. (C₁₄H₁₁NO₅S) C, H, N.

2-(2-Amino-4-chlorophenylsulfanyl)benzoic Acid (3). A mixture of **1** (7 g, 22.6 mmol), ferric chloride (0.1 g, 0.6 mmol), and charcoal (0.5 g) in ethanol was stirred and treated with a solution of hydrazine hydrate (1.2 mL) in ethanol (4 mL). After heating to reflux for 26 h, the hot mixture was filtered through diatomaceous earth and washed with copious amounts of hot ethanol until filtrate ran colorless. Evaporation left a yellow solid which was dissolved in 10% aqueous NaOH (40 mL) and acidified with acetic acid. The pale yellow solid was collected and dried in vacuo (3.3 g, 45%). An analytical sample was recrystallized from 95% aqueous ethanol: mp 186–191 °C; ¹H NMR (DMSO-*d*₆) 7.92 (dd, 1H, *J* = 1.2 and 7.8), 7.35 (dt, 1H, *J* = 1.2 and 7.7), 7.28 (d, 1H, *J* = 8.3), 7.18 (dt, 1H, *J* = 1.2 and 7.8), 6.85 (d, 1H, *J* = 2.4), 6.60–6.63 (m, 2H), 3.0–3.3 (br s, 3H). Anal. (C₁₃H₁₀ClNO₂) C, H, N.

2-(2-Amino-4-methoxyphenylsulfanyl)benzoic Acid (4). Synthesized in a similar fashion from **2** in 43% yield to give light brown crystals from 95% aqueous ethanol: mp 194–202 °C; ¹H NMR (DMSO-*d*₆) 7.90 (dd, 1H, *J* = 1.5 and 7.8), 7.33 (dt, 1H, *J* = 1.5 and 7.8), 7.12–7.19 (m, 2H), 6.64 (d, 1H, *J* = 8.1), 6.39 (d, 1H, *J* = 2.7), 6.24 (dd, 1H, *J* = 2.7 and 8.1), 5.3 (br s, 1H), 3.72 (s, 3H), 3.2–3.5 (br s, 2H). Anal. (C₁₄H₁₃NO₃S) C, H, N.

4-(2-Hydroxymethylphenylsulfanyl)-3-nitrobenzotriole (5). A solution of thiosalicylic acid (4.04 g, 26.2 mmol) in THF (45 mL) was added dropwise to a solution of LiAlH₄ (1.85 g, 48.7 mmol) in THF (74 mL) at ambient temperature. After 4 h of stirring, the reaction was carefully quenched by the

addition of ethyl acetate (10 mL) and 10% aqueous sulfuric acid (10 mL). The organic layer was filtered through a plug of Na₂SO₄ under nitrogen and evaporated to dryness to leave a yellow oil (3.43 g). This was treated with K₂CO₃ (5.5 g, 40 mmol), 4-chloro-2-nitrobenzotrile (3.9 g, 21.3 mmol), copper powder (0.38 g, 6 mmol) and DMF (30 mL). The mixture was stirred under nitrogen for 1 h, diluted with water (100 mL), and extracted with ethyl acetate (3 × 60 mL). The combined extracts were washed with water (100 mL), dried (K₂CO₃), and filtered, and volatiles removed to leave a yellow foam (6.67 g, 89%). A portion (0.5 g) was purified by flash chromatography on silica gel with ethyl acetate followed by recrystallization from 80% aqueous ethanol to give an analytical sample of **5** as a yellow powder (0.35 g): mp 120–126 °C; ¹H NMR 8.54 (d, 1H, *J* = 1.8), 7.74 (dd, 1H, *J* = 1.3 and 7.7), 7.63 (dt, 1H, *J* = 1.3 and 7.6), 7.58 (dd, 1H, *J* = 1.2 and 7.7), 7.51 (dd, 1H, *J* = 1.8 and 8.5), 7.47 (dt, 1H, *J* = 1.5 and 7.7), 6.81 (d, 1H, *J* = 8.5), 4.77 (s, 2H), 1.94 (br s, 1H). Anal. (C₁₄H₁₀N₂O₃S) C, H, N.

[2-(2-Amino-4-chlorophenylsulfanyl)phenyl]methanol (6). A solution of **3** (1.67 g, 6 mmol) in THF (15 mL) was added dropwise to a solution of LiAlH₄ (0.5 g, 13.2 mmol) in THF (15 mL) and stirred for 1 h. The mixture was quenched with aqueous NaOH (2.1 mL, 4%) at 0 °C and filtered through diatomaceous earth, washing solids well with ether. Evaporation left a brown solid which was recrystallized from cyclohexane/toluene to give a light brown solid (1.24 g, 77%): mp 109–111 °C; hydrochloride salt mp 153–156 °C; ¹H NMR 7.40 (dd, 1H, *J* = 1.5 and 7.3), 7.31 (d, 1H, *J* = 8.3), 7.11–7.19 (m, 2H), 6.86 (dd, 1H, *J* = 1.3 and 7.7), 6.77 (d, 1H, *J* = 2.2), 6.71 (dd, 1H, *J* = 2.2 and 8.3), 4.83 (s, 2H), 4.35 (s, 2H), 2.06 (s, 1H). Anal. (C₁₃H₁₂ClNOS·HCl) C, H, N.

[2-(2-Amino-4-methoxyphenylsulfanyl)phenyl]methanol (7). Prepared in 61% yield from the carboxylic acid **4** by reduction with LiAlH₄ as described for **6** above. Column chromatography (silica, EtOAc/petroleum ether/Et₃N 45/50/5) gave a pale yellow oil which yielded a waxy solid upon drying under high vacuum: mp 92–93 °C; ¹H NMR 7.37 (dd, 1H, *J* = 2.7 and 6.3), 7.32 (d, 1H, *J* = 8.5), 7.07–7.13 (m, 2H), 6.80 (dd, 1H, *J* = 1.7 and 6.6), 6.35 (dd, 1H, *J* = 2.7 and 8.5), 6.31 (d, 1H, *J* = 2.7), 4.81 (s, 2H), 4.30 (br s, 2H), 3.77 (s, 3H), 2.7 (br s, 1H). Anal. (C₁₄H₁₅NO₂S) C, H, N.

3-Amino-4-(2-hydroxymethylphenylsulfanyl)benzotrile (8). A mixture of crude **5** (90% pure by HPLC, 12.1 g, 38 mmol), ferric chloride (0.19 g, 1.2 mmol), and charcoal (0.95 g) in ethanol (100 mL) was stirred at reflux and treated with a solution of hydrazine hydrate (2 mL) in ethanol (5 mL). After 3 days of heating, the hot mixture was filtered through diatomaceous earth and evaporated to dryness. The residue was partitioned between ethyl acetate (200 mL) and saturated aqueous NaHCO₃ (150 mL); the organic layer was washed with brine (100 mL), dried (K₂CO₃), filtered, and evaporated to give a red sticky solid (12.3 g). Purification by column chromatography on silica gel (ethyl acetate/petroleum ether/Et₃N 40/55/5) followed by recrystallization from 80% aqueous ethanol gave off-white needles (4.7 g 49%): mp 121–124 °C; ¹H NMR 7.47 (d of m, 1H, *J* = 7.5), 7.29 (dt, 1H, *J* = 1.3 and 7.5), 7.27 (d, 1H, *J* = 7.9), 7.21 (dt, 1H, *J* = 1.5 and 8.9), 7.04 (dd, 1H, *J* = 1.1 and 8.9), 6.96 (d, 1H, *J* = 1.7), 6.94 (dd, 1H, *J* = 1.7 and 7.9), 4.82 (s, 2H), 4.44 (br s, 2H), 2.17 (br s, 1H). Anal. (C₁₄H₁₂N₂O₃S) C, H, N.

5-Chloro-2-(2-methylaminomethylphenylsulfanyl)phenylamine (10). A solution of **6** (0.23 g, 0.87 mmol) in diethyl ether (1.5 mL) was treated with a solution of HCl in diethyl ether (5 mL, 1 N). The ether was evaporated; the solid stirred at ambient temperature with toluene (4 mL) and was treated with thionyl chloride (0.5 mL). After 2 h volatiles were removed, toluene (5 mL) was added, and the mixture evaporated to dryness. The crude benzyl chloride was stirred with toluene (5 mL) and treated with 40% aqueous methylamine (1 mL). After 24 h water (5 mL) was added, the organic layer separated, and the aqueous fraction extracted with toluene (5 mL). The combined organic extracts were dried (K₂CO₃),

filtered, and evaporated to dryness to give crude **10**. Purification by column chromatography on silica gel (ethyl acetate/petroleum ether/Et₃N 55/45/5) gave a light brown oil (0.09 g, 37%), which partially crystallized upon drying under high vacuum: ¹H NMR 7.34 (d, 1H, *J* = 8.3), 7.28 (dd, 1H, *J* = 1.7 and 7.3), 7.06–7.13 (m, 2H), 6.84 (dd, 1H, *J* = 1.7 and 7.3), 6.73 (d, 1H, *J* = 2.2), 6.69 (dd, 1H, *J* = 2.2 and 8.3), 4.51 (br s, 2H), 3.90 (s, 2H), 2.50 (s, 3H), 1.49 (br s, 1H); ¹³C NMR 149.7, 138.2, 137.7, 136.6, 135.3, 129.4, 127.9, 127.2, 125.8, 118.5, 114.8, 113.0, 54.1, 36.1; malonate salt mp 128–130 °C. Anal. (C₁₄H₁₅ClN₂S·C₃H₄O₄) C, H, N.

In a similar fashion with similar yields, using either 40% aqueous methylamine or 40% aqueous dimethylamine were prepared the following, either as free base, hydrochloride salt, or malonate salt:

5-Methoxy-2-(2-methylaminomethylphenylsulfanyl)phenylamine (11): ¹H NMR 7.34 (d, 1H, *J* = 8.3), 7.27 (dd, 1H, *J* = 2.6 and 6.7), 7.04–7.09 (m, 2H), 6.78 (dd, 1H, *J* = 2.2 and 6.5), 6.35 (dd, 1H, *J* = 2.7 and 8.5), 6.32 (d, 1H, *J* = 2.7), 3.91 (s, 2H), 3.78 (s, 3H), 2.50 (s, 3H); ¹³C NMR 162.4, 150.4, 138.9, 136.9, 136.8, 129.2, 127.7, 126.1, 125.1, 105.5, 105.2, 100.4, 55.2, 53.9, 36.0; hydrochloride salt mp 162–166 °C dec. Anal. (C₁₅H₁₈N₂OS) C, H, N.

3-Amino-4-(2-methylaminomethylphenylsulfanyl)benzotrile (12): ¹H NMR 7.36 (d, 1H, *J* = 8.3), 7.33 (dd, 1H, *J* = 1.6 and 7.4), 7.21 (dt, 1H, *J* = 1.3 and 7.4), 7.15 (dt, 1H, *J* = 1.6 and 7.6), 7.00 (dd, 1H, *J* = 1.3 and 7.8), 6.91–6.94 (m, 2H), 4.73 (br s, 2H), 3.89 (s, 2H), 2.47 (s, 3H), 1.75 (br s, 1H); ¹³C NMR 148.3, 139.6, 136.0, 133.6, 130.2, 130.1, 128.5, 127.4, 122.5, 121.3, 119.1, 117.8, 113.2, 54.4, 36.3. Anal. (C₁₄H₁₁NO₅S) C, H, N.

5-Chloro-2-(2-dimethylaminomethylphenylsulfanyl)phenylamine (14): ¹H NMR 7.38 (d, 1H, *J* = 7.8), 7.19–7.23 (m, 1H), 7.07–7.71 (m, 2H), 6.89–6.92 (m, 1H), 6.67–6.70 (m, 2H), 4.54 (br s, 2H), 3.55 (s, 2H), 2.29 (s, 6H); ¹³C NMR 149.9, 138.4, 137.2, 136.7, 136.5, 130.3, 128.2, 128.0, 125.6, 118.1, 114.7, 114.2, 62.5, 45.2; hydrochloride salt mp of 237–238 °C dec. Anal. (C₁₅H₁₇ClN₂S·HCl) C, H, N.

2-(2-Dimethylaminomethylphenylsulfanyl)-5-methoxyphenylamine (15): ¹H NMR 7.39 (d, 1H, *J* = 8.3), 7.22–7.25 (m, 1H), 7.05–7.09 (m, 2H), 6.84–6.87 (m, 1H), 6.33 (dd, 1H, *J* = 2.7 and 8.5), 6.29 (d, 1H, *J* = 2.7), 4.51 (br s, 2H), 3.78 (s, 3H), 3.57 (s, 2H), 2.31 (s, 6H); ¹³C NMR 162.4, 150.7, 139.2, 138.2, 136.5, 130.3, 128.0, 127.0, 125.1, 106.9, 105.0, 100.5, 62.5, 55.4, 45.5; malonate salt mp 102–105 °C dec. Anal. (C₁₅H₁₅N₂S·C₃H₄O₄) C, H, N.

3-Amino-4-(2-dimethylaminomethylphenylsulfanyl)benzotrile (16): ¹H NMR 7.47 (d, 1H, *J* = 7.8), 7.23 (d, 1H, *J* = 7.3), 7.11–7.18 (m, 2H), 6.99 (d, 1H, *J* = 8.5), 6.93 (dd, 1H, *J* = 1.5 and 7.8), 6.88 (d, 1H, *J* = 1.7), 4.97 (br s, 2H), 3.55 (s, 2H), 2.28 (s, 6H); ¹³C NMR 148.6, 138.3, 136.7, 135.0, 130.7, 130.1, 128.3, 126.6, 121.2, 120.6, 118.8, 117.5, 113.2, 62.6, 45.1; hydrochloride salt mp 212–218 °C dec. Anal. (C₁₅H₁₇N₃S·HCl·0.25H₂O) C, H, N.

Radiochemistry. 1. Radiosynthesis of [¹¹C]-16. [¹¹C]-Iodomethane, produced from ¹¹C₂O₂ as described previously,¹⁸ was swept by a flow of N₂ gas (10 mL/min) into a solution of **12** (1.0 mg) in DMF (200 μL) at –20 °C. When radioactivity had peaked the solution was heated to 90 °C for 4 min and quenched with HPLC buffer (0.5 mL). The mixture was purified by semipreparative HPLC; Phenomenex Prodigy C18 (250 × 10 mm, 35% CH₃CN:65% H₂O + 0.1 N NH₄HCO₂, 10 mL/min (*t*_R of **16** = 8 min, *t*_R of **12** = 3 min). The desired fraction was collected and evaporated to dryness at 50 °C and the residue taken up in 10 mL of sterile saline. The saline solution of [¹¹C]-**16** was passed through a sterile 0.22-μm filter into a sterile, pyrogen-free bottle containing aqueous sodium bicarbonate (1 mL, 8.4%). An aliquot (100 μL) of the formulated solution was used to establish the chemical and radiochemical purity and specific activity of the final solution by analytical HPLC; Phenomenex Prodigy C18 (250 × 4.5 mm, 40% CH₃CN:60% H₂O + 0.1 N NH₄HCO₂, 3 mL/min (*t*_R of **16** = 4 min). [¹¹C]-**14** and [¹¹C]-**15** were prepared in a similar manner with

minor adjustments to the HPLC conditions. [¹¹C]-**13** was prepared as described previously.¹⁷

Further evidence for the identity of the radiolabeled products was achieved by coinjection with authentic "cold" material using a further two different HPLC columns (Alltech C₈, Econosil; and Waters C₁₈, Novapak). In addition, radio-scanning TLC (silica gel, EtOAc:Et₃N 95:5 (v/v)) showed one radioactive peak with the same retention factor as authentic material.

2. log *P* Measurements. The determination of the partition coefficients of radioactive compounds between 1-octanol and 0.02 M phosphate buffer at pH 7.4 was performed by a previously described method.¹⁷

3. Animal Studies. Rats (male, Sprague–Dawley, 200–230 g) were kept on a 12-h light/12-h dark cycle and allowed food and water ad libitum. Rats received 3–30 MBq of high-specific activity radiotracer (350–450 ng) in 0.3 mL of buffered saline via the tail vein, vasodilated in a warm water bath, and sacrificed at various time points after injection. A previously described method was used to determine the regional brain uptake of radioactivity.¹⁹ For pharmacological blocking studies, rats received a tail-vein injection of a solution (1 mL/kg body wt, saline or 5% ethanol in saline) of the putative blocker (2 mg/kg) 17–20 min before administration of the radiotracer while controls received only saline before the radiotracer injection. Experiments were conducted on groups of 3 rats/time point.

4. Serotonergic Neurotoxin (PCA) study. Rats were injected ip with either *p*-chloroamphetamine (5 mg/kg/day) or saline (controls) on 2 consecutive days (*n* = 6 for both groups). Six days later, [¹¹C]-**16** was injected into each rat, the rats were killed 60 min postradiotracer administration, and regional brain uptake of the radiotracer was determined as above.

5. Metabolite Analysis in Rat Brain. A male rat (Sprague–Dawley, 250 g) was injected (tail vein) with [¹¹C]-**16** (350 MBq, 11 nmol, 0.5 mL) in saline and killed by decapitation 20 min later. Blood was collected from the trunk and the brain surgically removed and stored on ice. A control rat which received only saline was also killed after 20 min and its brain removed. To each excised brain was added 10 μL of a 4 mM solution of "cold" **16**, and 1 MBq of [¹¹C]-**16** in 10 μL of saline was added to the control brain only. Both brains were homogenized (Polytron, setting 7) in 5 mL of ice-cold 80% aqueous ethanol and centrifuged (17 000 rpm, 15 min). The supernatants and pellets were counted for radioactivity; the supernatants were then evaporated to near dryness and reconstituted in HPLC buffer (500 μL) for analysis using a Waters Novapak C18 column (300 × 7.6 mm, 40% CH₃CN; 60% H₂O + 0.1 N NH₄HCO₂, 5 mL/min). The procedure was repeated using [¹¹C]-**15**.

6. Binding Assays Using Cloned Human Monoamine Transporters (HSERT, HNET, and HDAT). Membranes, with a protein concentration of 10.3 mg/mL (HSERT), 16.9 mg/mL (HNET), or 6.2 mg/mL (HDAT) were obtained from Receptor Biology, Inc. (Beltsville, MD). All assays were performed in triplicate in a final volume of 0.8 mL containing each of the following: 0.2 mL drug, 0.2 mL of [³H]paroxetine (0.2 nM), [³H]nisoxetine (1.0 nM), or [³H]WIN 35,428 (1.0 nM) for HSERT, HNET, or HDAT, respectively, 0.2 mL of membrane resuspension (10.3, 16.9, or 6.2 μg protein for HSERT, HNET, or HDAT, respectively) and 0.2 mL buffer (50 mM TrisCl, pH 7.4, 150 mM NaCl, 5 mM KCl for HSERT and HNET; 50 mM TrisCl, pH 7.4, 100 mM NaCl for HDAT). After incubation at 37 °C for 40 min, binding was terminated by rapid vacuum filtration over Whatman GF/B filters (presoaked in 0.5% poly(ethylenimine)) and filters were washed four times with cold TrisCl. Nonspecific binding was determined by including separate samples of 1 μM duloxetine (HSERT), 10 μM desipramine (HNET), or 10 μM nomifensin (HDAT). Inhibition curves were analyzed by nonlinear least-squares curve fitting to obtain IC₅₀ values. The *K_i* values were calculated from IC₅₀ and the *K_d* values according to the method of Cheng and Prusoff.²⁰

Results

Chemistry. The synthesis and radiolabeling of **13**, which has the trifluoromethyl substituent in the 3-position, has been described in a previous paper.¹⁷ The synthesis of the new target ligands **14–16** and their normethyl derivatives **10–12**, which were required as precursors for radiolabeling with [¹¹C]iodomethane, is outlined in Scheme 1. Copper-catalyzed coupling of thiosalicylic acid with either 2,5-dichloronitrobenzene or 4-chloro-3-nitroanisole gave the aryl thioethers **1** and **2** in good yields. Ferrous chloride-catalyzed reduction of the nitro group using hydrazine provided **3** and **4** which was followed by reduction of the carboxylic acid group with lithium aluminum hydride to give benzyl alcohols **6** and **7**.

The presence of the reducible cyano group in the targeted **12** and **16** necessitated a different synthetic approach. Initially, a route involving the coupling of *o*-thiocresol with 4-chloro-3-nitrobenzotrile followed by benzylic bromination and reaction with either methylamine or dimethylamine was pursued.¹³ However the amination reactions resulted in a complex mixture of products from which only small quantities of the desired material could be isolated. The alternative route outlined in Scheme 1 was more successful. Copper-catalyzed coupling of 4-chloro-3-nitrobenzotrile with freshly prepared 2-thiobenzyl alcohol proceeded smoothly in DMF yielding **5** which was reduced to **8** with hydrazine. Treatment of the benzyl alcohols **6–8** with thionyl chloride in toluene gave crude benzyl chlorides as hydrochloride salts which were not isolated but which reacted with either methylamine or dimethylamine to yield the target amines **9–12** and **13–16**, respectively. The reaction of **6–8** with thionyl chloride produced much cleaner products when the hydrochloride salts were employed rather than the free bases.

Radiochemistry. The radiotracers [¹¹C]-**13–16** were all synthesized in a similar fashion as depicted in Scheme 1. Cyclotron-produced [¹¹C]iodomethane¹⁸ was trapped in solutions of the normethyl precursor **9–12** at –20 to –30 °C and heated to 90 °C for 2–5 min. Purifications of the resultant reaction mixtures were effected by reverse-phase HPLC followed by evaporation, filtration, and formulation in buffered saline to give isolated radiochemical yields of [¹¹C]-**13–16** of 25–55% (uncorrected, from [¹¹C]iodomethane). Final products were sterile, pyrogen-free, and radiochemically pure (>98%) by TLC and HPLC and contained less than 0.05 μg/mL of normethyl precursor. Synthesis times, including quality control, were 25–30 min from end-of-bombardment, and specific activities of 25–55 GBq/μmol at end-of-synthesis were obtained from bombardments of 10 μAh.

In Vitro Binding Assays. The affinities (*K_i*) of the test compounds **13–16** for cloned human monoamine transporters are shown in Table 1. All four are highly selective (>1000-fold) for SERT over NET and DAT. There was little species difference as binding studies using rat cortical and striatal membranes gave very similar results (data not shown).

Biodistribution in Rat Brain. Five minutes after tail-vein injection whole brain uptake for the four radiotracers, [¹¹C]-**13–16**, was 1.02, 1.27, 1.18, and 1.22% injected dose/g (%ID/g), respectively, demonstrat-

Table 1. Biological and Physicochemical Properties of 2-(Phenylthio)araalkylamines

compd	K_i (nM)			signal/noise ratio ^{a,b}	cerebellar clearance (min) ^c	log P^d
	HSERT	HNET	HDAT			
13	0.33 ± 0.01	1205 ± 1	2038 ± 101	2.5	32.3	3.774
14	0.27 ± 0.02	230 ± 9	115 ± 4	4.2	25.4	3.552
15	1.89 ± 0.06	1992 ± 43	2651 ± 193	6.3	18.8	2.825
16	1.10 ± 0.04	1350 ± 221	1423 ± 103	7.9	15.6	2.707

^a Rats were injected with a radiotracer, [¹¹C]-**13**–**16**, and brain regions dissected and counted. See text for details. ^b Hypothalamus-cerebellum/cerebellum ratio at 60 min postinjection. ^c Half-life of washout rate of radioactivity from the cerebellum. ^d Measured at pH 7.4.

Table 2. Biodistribution of Radioactivity in Rats (% ID/g) upon Tail-Vein Injection of ¹¹C-Labeled (Phenylthio)araalkylamines (mean ± SD, $n = 3$)

min	cerebellum	hypothalamus	thalamus	hippocampus	striatum	cortex	blood
[¹¹C]-13							
5	0.92 ± 0.07	1.02 ± 0.08	1.07 ± 0.03	0.92 ± 0.09	1.05 ± 0.07	1.09 ± 0.08	0.35 ± 0.05
15	0.70 ± 0.01	1.17 ± 0.07	1.06 ± 0.02	0.91 ± 0.03	1.02 ± 0.03	1.06 ± 0.04	0.21 ± 0.04
30	0.47 ± 0.04	1.23 ± 0.03	1.07 ± 0.05	0.88 ± 0.02	0.99 ± 0.02	0.98 ± 0.02	0.21 ± 0.02
60	0.28 ± 0.05	0.99 ± 0.11	0.89 ± 0.12	0.75 ± 0.12	0.72 ± 0.09	0.69 ± 0.12	0.12 ± 0.02
[¹¹C]-14							
5	1.03 ± 0.09	1.36 ± 0.10	1.43 ± 0.10	1.06 ± 0.10	1.36 ± 0.09	1.39 ± 0.11	0.39 ± 0.06
15	0.75 ± 0.06	1.48 ± 0.03	1.54 ± 0.11	1.12 ± 0.07	1.34 ± 0.12	1.33 ± 0.11	0.28 ± 0.04
30	0.47 ± 0.03	1.47 ± 0.09	1.65 ± 0.15	1.04 ± 0.09	1.17 ± 0.07	1.14 ± 0.07	0.14 ± 0.01
60	0.23 ± 0.00	1.19 ± 0.03	1.24 ± 0.10	0.74 ± 0.02	0.79 ± 0.06	0.73 ± 0.01	0.08 ± 0.02
[¹¹C]-15							
5	0.80 ± 0.06	1.22 ± 0.07	1.12 ± 0.04	1.09 ± 0.03	1.19 ± 0.06	1.37 ± 0.07	0.30 ± 0.02
15	0.52 ± 0.06	1.31 ± 0.15	1.13 ± 0.17	0.98 ± 0.09	1.07 ± 0.14	1.11 ± 0.15	0.17 ± 0.01
30	0.27 ± 0.02	1.08 ± 0.05	0.83 ± 0.08	0.74 ± 0.04	0.76 ± 0.08	0.70 ± 0.01	0.13 ± 0.00
45	0.20 ± 0.04	0.89 ± 0.08	0.71 ± 0.07	0.56 ± 0.05	0.58 ± 0.06	0.54 ± 0.07	0.10 ± 0.01
60	0.10 ± 0.01	0.63 ± 0.09	0.48 ± 0.09	0.38 ± 0.06	0.38 ± 0.05	0.36 ± 0.05	0.07 ± 0.01
[¹¹C]-16							
5	0.76 ± 0.04	1.25 ± 0.07	1.15 ± 0.00	1.13 ± 0.05	1.24 ± 0.03	1.46 ± 0.04	0.58 ± 0.03
15	0.54 ± 0.02	1.39 ± 0.17	1.21 ± 0.14	1.06 ± 0.14	1.24 ± 0.24	1.29 ± 0.20	0.31 ± 0.06
30	0.27 ± 0.06	1.02 ± 0.06	0.80 ± 0.09	0.72 ± 0.05	0.77 ± 0.09	0.74 ± 0.06	0.22 ± 0.08
45	0.13 ± 0.01	0.80 ± 0.06	0.64 ± 0.06	0.49 ± 0.03	0.55 ± 0.06	0.48 ± 0.04	0.10 ± 0.02
60	0.07 ± 0.00	0.62 ± 0.02	0.49 ± 0.12	0.35 ± 0.03	0.35 ± 0.05	0.32 ± 0.04	0.06 ± 0.01

ing facile crossing of the BBB in each case. An examination of the regional brain biodistribution (Table 2) showed that, for all four radiotracers, there was a slower washout of radioactivity from SERT-rich brain regions such as hypothalamus and thalamus than from cerebellum; a region which possesses a low density of SERT receptors in the rat.^{21,22} Regions of moderate SERT density such as striatum, hippocampus, and whole cortex had intermediate washout rates. Uptake of radioactivity (%ID/g) in the cerebellum was used as an index of nonspecific binding (NSB) and region minus cerebellum (%ID/g) as an index of specific binding (SB). SB-to-NSB ratios of 2.5, 4.2, 6.3, and 7.9 were obtained for [¹¹C]-**13**–**16**, respectively, at 60 min postinjection in the hypothalamus. Because of their higher ratios, [¹¹C]-**15** and [¹¹C]-**16** were chosen for further ex vivo studies.

Ex Vivo Pharmacology. To demonstrate ex vivo selectivity of binding of the radiotracers to SERT, a variety of pharmacologically active drugs (at 2 mg/kg) were administered iv to rats 17–20 min prior to administration of the radiotracers [¹¹C]-**15** and [¹¹C]-**16**. Administration of “cold” **15** or **16** resulted in almost complete reduction of SB, when compared to control rats receiving saline injections, showing that radiotracer binding was saturable (Figure 1). Similar reductions in SB were also observed upon preinjection of the selective SERT inhibitor (+)-McNeil 5652⁷ for both [¹¹C]-**15** and [¹¹C]-**16** (Figure 1). In contrast, no significant changes in SB were observed upon preinjection of either GBR 12909 (a selective DAT inhibitor) or desipramine (a selective NET inhibitor). Similarly, preinjection of WAY

100635 (5-HT_{1A}), ketanserin (5-HT_{2A/B}), raclopride (D₂/D₃), or haloperidol (D₂, sigma) had no significant effect on the SB of either of the radiotracers in any examined brain region (data not shown).

Metabolism. Ethanol extraction of whole brain homogenates from rats receiving an injection of either [¹¹C]-**15** or [¹¹C]-**16** recovered >93% of the radioactivity associated with the brain tissue. Upon concentration, the extracts were examined by reverse-phase HPLC for the presence of radioactive metabolites. Analysis of the brain extracts, for both radiotracers, showed a major peak (94–96% of total brain radioactivity) which co-eluted with the administered radiotracer and two small radioactive peaks (both 2–3% of total brain radioactivity) with earlier retention times. A comparison of retention times with standards suggested that in the case of [¹¹C]-**15**, the two metabolites corresponded to N- and O-demethylation products of **15**.

PCA Treatment. Systemic treatment of rats with the serotonergic neurotoxin *p*-chloroamphetamine ($n = 6$) or saline ($n = 6$) was followed 6 days later by administration of [¹¹C]-**16**. Significant reductions in the SB of [¹¹C]-**16** were found in all brain regions. Reductions ranged from 69% in the thalamus and hypothalamus to 82% in the cortex in treated rats as compared to controls (Figure 2).

Discussion

The radiosynthesis of the four radiotracers was achieved under very similar conditions by alkylation of

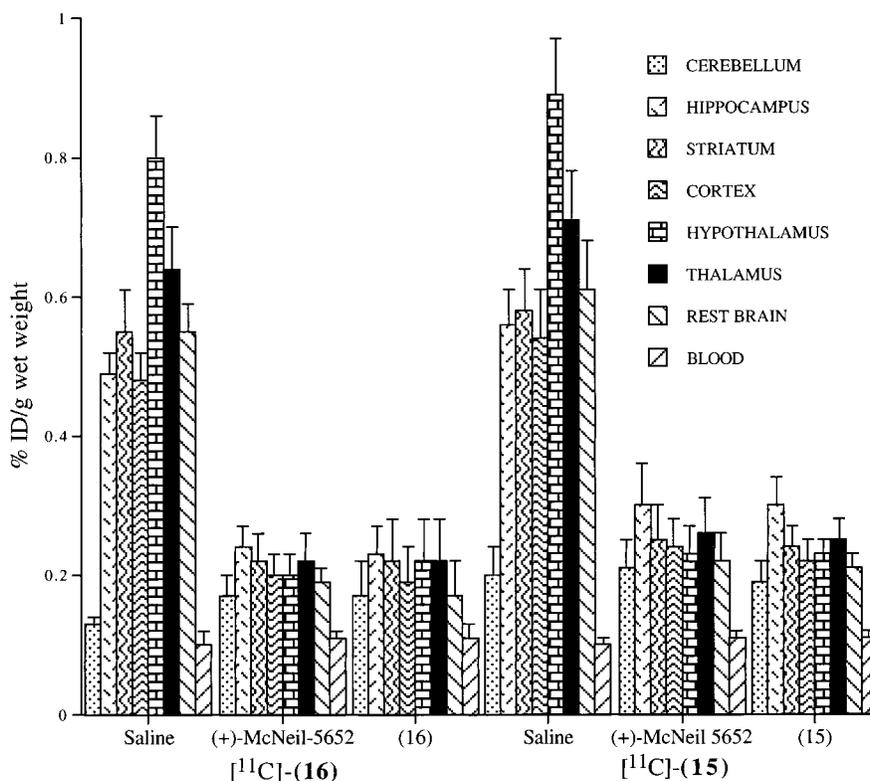


Figure 1. Effect of preinjection of saline (control), (+)-McNeil 5652, "cold" **15**, or **16** (all 2 mg/kg tail vein) on the regional brain distribution of [^{11}C]-**15** and [^{11}C]-**16** in rats. Drugs were injected 17–20 min prior to administration of the radiotracer and the rats killed 60 min later. Values are mean \pm SD ($n = 3$).

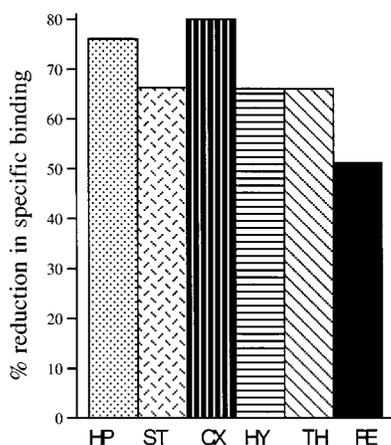


Figure 2. Reduction in ex vivo regional specific binding of [^{11}C]-**16** in rat brain upon systemic treatment with *p*-chloroamphetamine. HP, hippocampus; ST, striatum; CX, cortex; HY, hypothalamus; TH, thalamus; RE, rest of brain. See text for details.

the normethyl precursor, a secondary benzylamine, with [^{11}C]iodomethane in DMF to give the desired ^{11}C -labeled tertiary benzylamines. A possible side reaction in these syntheses is methylation of the aromatic amino group, a site that should be less nucleophilic than the targeted aliphatic amino group. This seems to be the case as only minor radioactive byproducts are observed. In the specific case of labeling **13**, reaction with [^{13}C]iodomethane under similar conditions, followed by HPLC purification, gave only [^{13}C]-**13** as determined by ^{13}C NMR.¹⁷ It should be noted that both [^{11}C]-**15** and [^{11}C]-**16** occasionally contained radiochemical impurities arising from radiolytic degradation during the evaporation of

the HPLC eluent. Reduction of evaporation temperature minimized this problem.

The first targeted radiotracer in this work, [^{11}C]-**13**, was identified in the patent literature as a potent selective inhibitor of serotonin reuptake,²³ a result that was confirmed in our binding assays (Table 1). The rat brain biodistribution results from [^{11}C]-**13** were encouraging, showing excellent brain penetration and selective uptake in SERT-rich regions such as hypothalamus, resulting in a signal-to-noise ratio of 2.5:1 after 60 min, a moderate improvement over [^{123}I]IDAM (1.75:1).¹⁵ However we thought that the clearance rate from the cerebellum ($t_{1/2}$ 34 min) was too slow for good pharmacokinetics in human PET studies with the short-lived radiotracer ^{11}C ($t_{1/2}$ 20.4 min). Since slow clearance of NSB is often attributed to high lipophilicity, other analogues were designed with more polar groups in the 3'-position to replace the lipophilic trifluoromethyl group.²⁴ The limited structure–affinity data available suggested that a variety of groups could be tolerated at this position.^{13,23} This simple approach of reducing lipophilicity was very successful in increasing the washout rate of the radiotracers from the cerebellum and, more significantly, resulting in a concomitant increase in SB to NSB ratios: e.g. 6.3 and 7.9 for **15** and **16**, respectively, in the hypothalamic region (Tables 1 and 2). Even though **15** and **16** have 5–10-fold lower affinity for SERT (Table 1) than **13** and **14**, the former pair of analogues displays significantly better ex vivo binding characteristics as potential SERT imaging agents than do the latter pair. This would suggest that, for these analogues, the reduction in lipophilicity with concomitant faster washout of nonspecific binding plays

just as significant a role in obtaining high signal contrast as does affinity for the receptor.

Upon the basis of their superior ex vivo pharmacokinetics, the two radiotracers [^{11}C]-**15** and [^{11}C]-**16** were selected for further ex vivo studies in rats. Both candidates showed saturability and specificity of binding for SERT binding sites as demonstrated by the ex vivo blocking studies with either "cold" ligand or (+)-McNeil 5652 (Figure 1). In these studies reduced regional brain uptake of radioactivity to cerebellar levels was found for all brain regions except in the hippocampus where reductions of 69% and 83% were observed for [^{11}C]-**15** and [^{11}C]-**16**, respectively (Figure 1). Nonsaturable uptake in hippocampal areas has previously been observed for [^{123}I]IDAM and [^{11}C]McNeil 5652.²⁵ No reduced uptake of radioactivity was found in the cerebellum in either blocking study, strengthening the view that the cerebellum is nearly devoid of SERT and justifying the use of this region to estimate nonspecific binding. These results, in conjunction with the lack of effect on regional brain uptake of a variety of other pharmacologically active drugs and the in vitro binding data, strongly indicate that regional brain uptake of [^{11}C]-**15** and [^{11}C]-**16** is mediated by binding to SERT. Further evidence that the regional brain uptake of this class of radiotracers is mediated by SERT binding sites is provided by the effect of PCA treatment. Systemic treatment of rats with a dose which has been shown to reduce the number of projection terminals of serotonin neurons by 80–90%²⁶ resulted in reductions of the SB of [^{11}C]-**16** by a similar magnitude. The potential usefulness of these radiotracers as markers of serotonergic neurons is also suggested by this finding.

Since the presence of radiolabeled metabolites in target regions can represent an insurmountable problem in PET imaging studies, whole rat brain extracts were examined by TLC and HPLC after tail-vein injection of both [^{11}C]-**15** and [^{11}C]-**16**. While TLC could not discern any species other than parent radiotracer, the superior resolution of HPLC revealed the presence of small amounts of more hydrophilic metabolites from both radiotracers. However, assuming a one-to-one allometric extrapolation to humans, the small amount of metabolism should not present a problem in human PET studies.

In conclusion, this study has demonstrated that 4-substituted phenyl thioethers possess high affinity and selectivity for the SERT in vitro and can be efficiently radiolabeled with ^{11}C . Ex vivo, the radiotracers demonstrate high SB in regions of rat brain known to be rich in SERT. Ex vivo binding is saturable and pharmacologically specific for SERT over other receptor binding sites, and a good correlation is found between their lipophilicities, pharmacokinetics, and signal-to-noise ratios. Radiolabeled metabolites are found to only a minor extent in rat brain, and destruction of serotonin terminals results in a significant loss of SB. Radiotracers for in vivo imaging of SERT could be applied to research for both existing and new SSRI drugs by measuring their occupancy, pharmacokinetics, and regulation of SERT during treatment of mood and anxiety disorders. Such an approach requires better PET ligands than have yet been reported. Taken together, our results strongly indicate that both **15**, 2-(2-dimethylamino-

methylphenylsulfanyl)-5-methoxyphenylamine or DAPP, and **16**, 3-amino-4-(2-dimethylaminomethylphenylsulfanyl)benzotrile or DASB, possess such potential as radioligands for imaging SERT in the living human brain by PET and merit further investigation. Human trials with both ^{11}C -labeled ligands are currently in progress.

Acknowledgment. The authors thank Jin Li, Armando Garcia, Doug Hussey, Kevin Cheung, and Corey Jones for their assistance with radiochemistry and biodistribution experiments and Dr. Shitij Kapur for helpful discussions. This work was supported by Eli Lilly & Co. (Canada).

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JM000079I