Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 16 (2008) 783–793
Bioorganic & Medicinal Chemistry

Synthesis, in vitro characterization, and radiolabeling of reboxetine analogs as potential PET radioligands for imaging the norepinephrine transporter

Fanxing Zeng,[a] Nachwa Jarkas,[a] Jeffrey S. Stehouwer,[a] Ronald J. Voll,[a] Michael J. Owens,[b] Clinton D. Kilts,[b] Charles B. Nemeroff[b] and Mark M. Goodman[a,*]

[a]*Department of Radiology, Division of Radiological Sciences, Emory University, Atlanta, GA 30322, USA*
[b]*Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine, Atlanta, GA 30322, USA*

Received 26 July 2007; revised 2 October 2007; accepted 10 October 2007
Available online 13 October 2007

Abstract—Six new (*S*, *S*)-enantiomers of reboxetine derivatives were synthesized and their binding affinities were determined via competition binding assays in cells expressing the human norepinephrine transporter (NET), serotonin transporter (SERT) or dopamine transporter (DAT). All six compounds prepared exhibit high affinity for the NET ($K_i \leqslant 2$ nM) and selectivity versus the SERT and DAT. Radiolabeling methods were also developed to prepare these ligands in moderate to high radiochemical yield with high radiochemical purity via O- or S-methylation with [^{11}C]CH_3I, or O-alkylation with [^{18}F]fluoroethyl brosylate or [^{18}F]fluoropropyl brosylate, and their log $P_{7.4}$ was measured. These new C-11- and F-18-labeled tracers will be utilized in comparative microPET studies to evaluate their potential as PET radioligands for imaging brain NET in nonhuman primates.

1. Introduction

The norepinephrine transporter (NET) is a 12-membrane-spanning protein located on the nerve terminals as well as the cell bodies of noradrenergic neurons.[1,2] The NET plays a critical role in regulating norepinephrine (NE) concentration at noradrenergic synapses by reclaiming uptake of NE from the extracellular space into presynaptic terminals.[1,3] Evidence from animal model, postmortem human, and molecular neuroscience studies support a brain regional and complex dysregulation of noradrenergic function in mood and anxiety disorders.[4,5] The NET is also an established molecular target for the treatment of attention-deficit/hyperactivity disorder (ADHD), substance abuse, neurodegenerative disorders, and depression. Therefore, A NET imaging agent would be a unique tool to determine the role of noradrenergic mechanisms in neuropsychiatric disorders as well as the in vivo pharmacokinetic and pharmacodynamic properties of potential medications with affinity for the NET.

Several potent NET-selective antidepressants, such as desipramine,[6–8] nisoxetine,[9–12] oxaprotiline,[11] lortalamine,[11] and analogs of tomoxetine[13] have been labeled for in vitro or in vivo mapping of brain NET. Unfortunately, the high in vivo nonspecific binding of these radioligands excluded their utility as NET imaging agents.

Reboxetine (2-[α-(2-ethoxyphenoxy)benzyl]morpholine), a racemic mixture of (*R*, *R*) and (*S*, *S*) enantiomers, is a potent and relatively selective NE reuptake inhibitor and commercially available in several countries outside the United States as an antidepressant. Reboxetine has recently been widely chosen as a molecular model for the design of NET radioligands and several ^{11}C-, ^{18}F- or ^{123}I-labeled derivatives of reboxetine have been developed and evaluated as putative PET (positron emission tomography) or SPECT (single photon emission computed tomography) imaging agents for the NET.[11,14–21] These radioligands, including *O*-methyl ((*S*, *S*)-[^{11}C]-MeNER),[14–16] *O*-fluoromethyl ((*S*,*S*)-[^{18}F]FMeNER-D_2),[17,18] and 2-iodo ((*S*, *S*)-[^{123}I]IPBM)[19,20] analogs, showed many desired in vivo properties for imaging non-

Keywords: Norepinephrine transporter; Positron Emission Tomography; Carbon-11; Fluorine-18; (*S*, *S*)Reboxetine analogs.
* Corresponding author. Tel.: +1 404 727 9366; fax: +1 404 727 3488; e-mail: mgoodma@emory.edu

0968-0896/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2007.10.025

human primates that have not been observed with any other NET ligands. (*S*, *S*)-[^{11}C]MeNER and (*S*, *S*)-[^{18}F]FMeNER-D$_2$ are probably the most promising candidates reported so far for imaging NET by PET with the highest thalamus/striatum ratio of ~1.5 in cynomolgus monkey. However, these two radioligands do not appear to be ideal. Previous evaluation of (*S*, *S*)-[^{11}C]MeNER has shown that specific binding to NET did not reach peak equilibrium during a 90-min PET measurement. This, together with its relatively noisy signal at later time points, might hamper its utility in the quantitative assessment of NET binding occupancy in brain. (*S*, *S*)-[^{18}F]FMeNER-D$_2$ undergoes a moderate degree of defluorination resulting in skull uptake of radioactivity which was found to contaminate images in cortical areas in PET studies.

In an effort to develop NET-specific PET imaging agents, the promising results from reboxetine motif served as the impetus for our group to continuously explore reboxetine analogs to further optimize this ligand by, for example, significantly decreasing the time to reach a specific binding peak equilibrium while maintaining the favorable specific-to-nonspecific binding ratios in NET-rich tissues, and reducing the defluorination to an unobservable level for fluorine-18 radioligands. Because the most active reboxetine isomer always corresponds to the *S*, *S* isomer, we decided to focus our efforts on the *S*, *S* isomer. We report here the synthesis, in vitro binding affinities, and the radiolabeling with carbon-11 or fluorine-18 of new reboxetine analogs (**1**–**6,** Fig. 1).[22] Results of comparative microPET studies of these tracers in non-human primate brain are currently in preparation and will be published in a separate report.

2. Results and discussion

2.1. Synthesis of reference compounds and precursors

The stereoselective synthesis of new ligands **1**–**6** is shown in Scheme 1. The key intermediates (2*S*, 3*S*)-*N*-Boc-2-[α-hydroxy(phenyl)methyl]morpholine (**7**) and (2*S*, 3*R*)-*N*-Boc-2-[α-hydroxy(phenyl)methyl]morpholine (**8**) were synthesized in good yields and 99% enatiomeric excess in six linear steps starting from commercially available (*S*)-3-amino-1,2-propanediol according to a recently reported procedure.[23] **7** was the major isomer and **8** was the minor isomer. Ligands **1** and **2** were prepared in 29% and 44% two-step yields by reacting **8** with 2-(methylthio)phenol and methyl salicylate under Mitsunobu conditions[24] followed by standard deprotection of *N*-Boc group with excess trifluoroacetic acid, respectively. The (*R*) configuration of the benzylic carbon in compound **8** was inverted after Mitsunobu coupling reaction to furnish the (*S*) form of aryl benzyl ethers, as demonstrated by Tamagnan and colleagues.[23] The structures of target compounds (**3**–**6**) were successfully built by a double inversion strategy[25]: conversion of alcohol **7** to the corresponding benzyl bromide **11** using carbon tetrabromide and triphenylphosphine with inversion of configuration at the benzylic position from (*S*) to (*R*); reaction of benzyl bromide **11** with thiophenols under nucleophilic substitution S_N2 mechanism to provide arylthiomethyl morpholines (**12**, **13**, and **16**) with inversion of (*R*) configuration of benzylic carbon to (*S*). Therefore, **3** and **6** were prepared in 42% and 65% two-step yields by reacting (2*S*, 3*R*)-*N*-Boc-2-[α-bromo(phenyl)methyl]-morpholine (**11**) with 2-methoxybenzenethiol and methyl thiosalicylate followed by deprotection of *N*-Boc group with excess trifluoroacetic acid, respectively. The reference compounds **4** and **5** were prepared in the following two steps from (2*S*, 3*S*)-*N*-*Boc*-2-[α-(2-hydroxyphenylthio)benzyl]morpholine (**13**) which was obtained in 91% yield by the reaction of **11** with 2-hydroxythiophenol. First, **13** was fluoroethylated and fluoropropylated at the phenolic oxygen using 2-fluoroethyl tosylate[26] and 3-fluoropropyl tosylate[27] to give **14** and **15**, respectively. The subsequent Boc removal of **14** and **15** with CF_3COOH yielded **4** and **5** in 44% and 57% (two-step) yields, respectively.

The synthesis of precursors **18**–**20** for radiolabeling is shown in Scheme 2. The preparation of [^{11}C]**1** through an S-methylation reaction required preparation of the corresponding thiol. Considering the fact that thiol is generally unstable, we need to prepare *S*-protected precursor that would be stable to storage and directly used for radiolabeling. In view of a previous paper, in which an *S*-acetyl ester was used as a precursor for S-methylation,[28] we attempted to protect the thiol group of the starting material, 2-hydroxy-thiophenol, as a thioester by treatment with acetyl chloride or acetic anhydride. Unfortunately, very low regioselectivity was observed under all attempted conditions. While we were in the process of exploring synthetic methods of the precursor, Schou et al. reported an effective precursor containing sulfanyl γ-propionic acid methyl ester protecting group for ^{11}C-methylation of (*R*)-thionisoxetine.[29] They claimed that this protective group could be removed in situ under mild basic condition to generate free thiolate ion for radiolabeling. We therefore decided to use this strategy. First, the thiol moiety of 2-hydroxy-thiophenol was regioselectively protected by reaction with methyl acrylate to give S-alkylation compound **17**.[29] Then, **8** was coupled with **17** under Mitsunobu conditions to give **18** as the labeling precursor for [^{11}C]**1** with complete inversion of configuration. Esters **10** and **16** were hydrolyzed under basic conditions to afford corresponding carboxylic acids **19** and **20** which were used as radiolabeling precursors for [^{11}C]**2** and [^{11}C]**6**, respectively. Compound **13** was used as the precursor in the radiosynthesis of [^{11}C]**3**, [^{18}F]**4**, and [^{18}F]**5**.

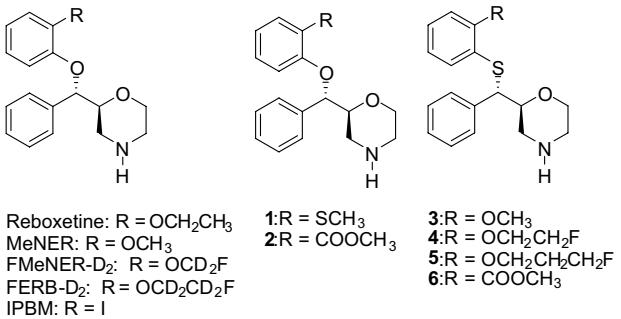
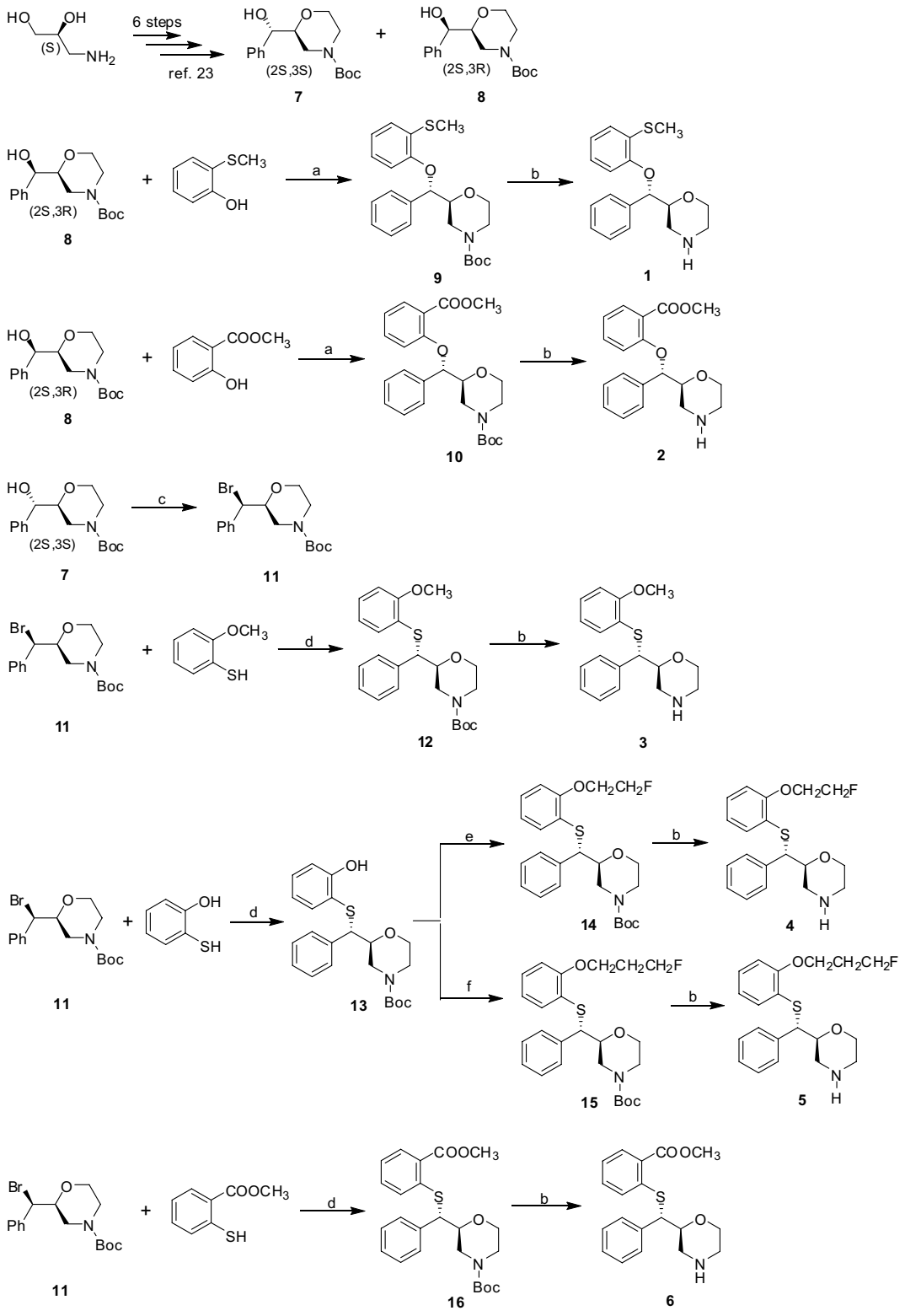


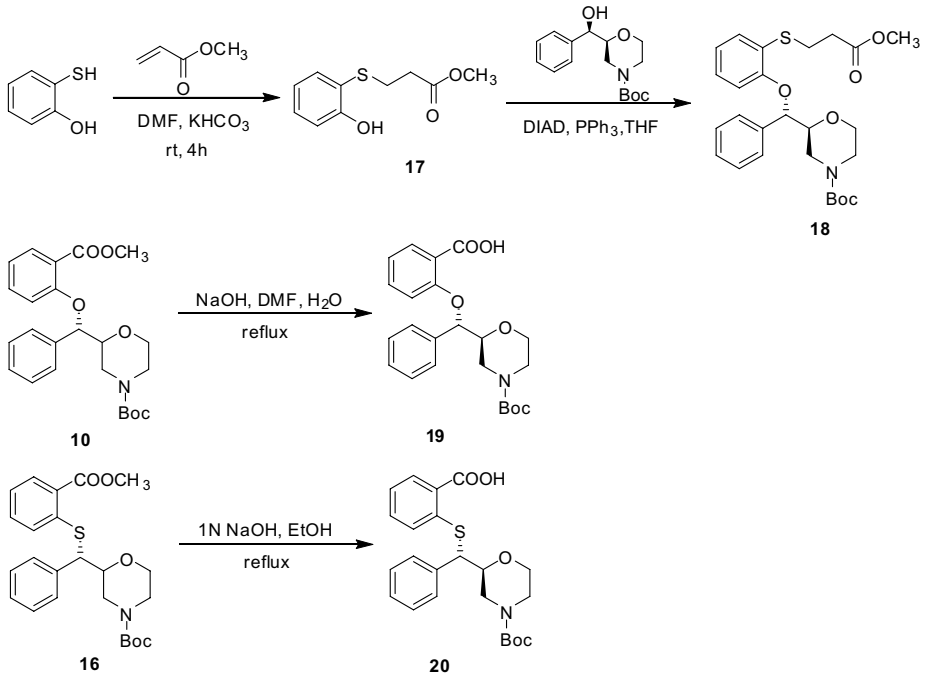
Figure 1. (2*S*, 3*S*) Enantiomers of reboxetine derivatives.



Scheme 1. Reagents and conditions: (a) DIAD, PPh_3, THF, 0 °C–rt, 48 h; (b) TFA, CH_2Cl_2, 0 °C–rt, 2 h; (c) CBr_4, PPh_3, CH_2Cl_2, 0 °C–rt, 30 min; (d) Cs_2CO_3, DMF, rt, 18 h; (e) FCH_2CH_2OTs, Bu_4NOH, DMF; (f) $FCH_2CH_2CH_2OTs$, Bu_4NOH, DMF.

2.2. Radiolabeling

The radiosynthesis of [^{11}C]**1**, [^{11}C]**2**, [^{11}C]**6**, [^{11}C]**3**, [^{18}F]**4**, and [^{18}F]**5** is shown is Scheme 3, and the results are listed in Table 1. [^{11}C]**1** was obtained in quantitative decay-corrected radiochemical yield through S-alkylation of the intermediate thiol **21** with [^{11}C]CH_3I in THF, followed by deprotection with 6 M HCl and HPLC purification. KO^{t}Bu-promoted in situ β-elimination of the sulfanyl γ-propionic acid methyl ester moiety in **18** generated the free



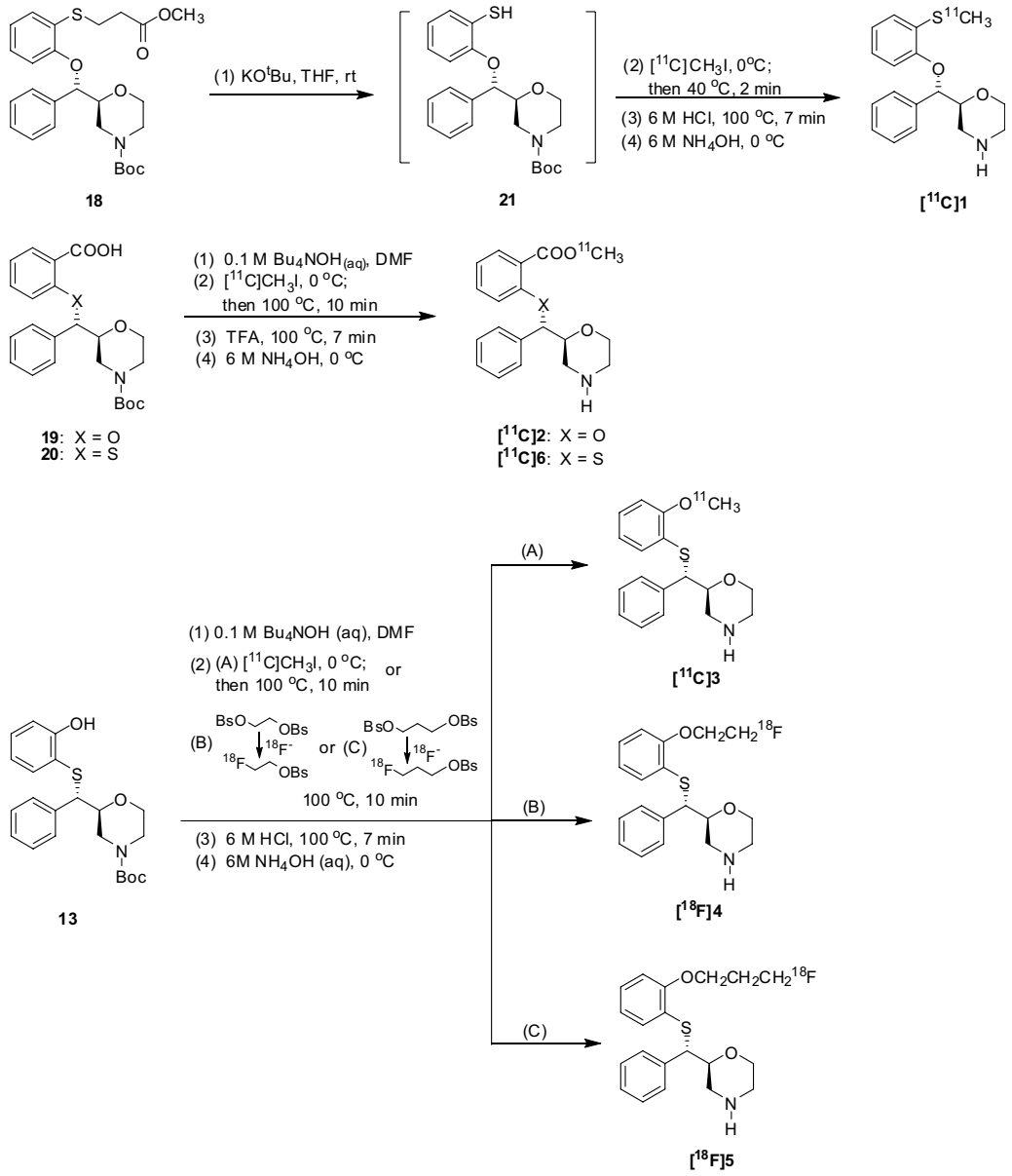
Scheme 2. Synthesis of precursors for radiolabeling.

thiolate ion (**21**), which was then effective for accomplishing ^{11}C-methylation. The radiolabeling of [^{11}C]**2** and [^{11}C]**6** was accomplished through O-methylation of *N*-Boc carboxylic acid precursors **19** and **20** with [^{11}C]CH_3I in the presence of 0.1 M Bu_4NOH, followed by removal of *N*-Boc group under acidic conditions. The radiosynthesis of [^{11}C]**3** was conducted via O-methylation of deprotonated *N*-Boc phenol **13** with [^{11}C]CH_3I. The preparation of [^{18}F]**4** and [^{18}F]**5** was accomplished via a three-step procedure using **13** as the precursor. First, 2-[^{18}F]fluoroethyl brosylate and 3-[^{18}F]fluoropropyl brosylate were prepared as a secondary radiolabeling synthon by the nucleophilic substitution of 1,2-dibrosylethane and 1,3-dibrosylpropane with [^{18}F]F^- in acetonitrile, respectively. The subsequent coupling of **13** with 2-[^{18}F]fluoroethyl brosylate or 3-[^{18}F]fluoropropyl brosylate was carried out in DMF at 100 °C in the presence of 0.1 M Bu_4NOH. Last, the Boc group was cleaved by treating *N*-Boc-[^{18}F]**4** or *N*-Boc-[^{18}F]**5** with 6 M HCl. After HPLC purification, [^{18}F]**4** and [^{18}F]**5** were obtained in 6–8% decay-corrected radiochemical yields from [^{18}F]F^- in a synthesis time of 120 min with a radiochemical purity of >98% and a specific activity of 1.7–2.3 Ci/μmol at time of injection. The lipophilicities of radioligands **1**–**6** were measured by traditional extraction with octanol and pH 7.4 phosphate buffer according to a previously reported procedure,[30] and log $P_{7.4}$ values are given in Table 1. The log $P_{7.4}$ values of all the radiotracers measured are in the optimal range (1.0–3.0) for compounds expected to enter the brain readily.[31]

2.3. Biological results: in vitro competition assays

New reboxetine derivatives **1**–**6** were screened for binding to human monoamine transporters using in vitro competition binding assays in transfected HEK-293 cells stably expressing the human NET (hNET), serotonin transporter (hSERT), or dopamine transporter (hDAT) according to a previously reported procedure.[32,33] (*S*,*S*)-MeNER was also screened under the same assay system for comparison.

[^{3}H]nisoxetine (NET ligand), [^{3}H]citalopram (SERT ligand), and [^{125}I]RTI-55 (DAT ligand) were used as radiotracers during the in vitro displacement experiments. Data in Table 2 indicate that all new reboxetine derivatives displayed high affinities for the hNET comparable to the value for (*S*,*S*)-MeNER. The rank of order of hNET affinities (K_i in nM) was **3** (0.76) > (*S*,*S*)-MeNER (0.95) > **4** (1.0) > **5** (1.21) > **2** (1.32) ⩾ **6** (1.35) > **1** (2.06). The reboxetine derivatives tested exhibited very low affinities for the DAT (K_i > 3000) as well as moderate to low affinities for the SERT (91 ⩽ K_i ⩽ 498), except for **3** with K_i = 9.69 nM for the SERT. Replacing the methoxy group on the benzene ring of MeNER with a methylthio group afforded **1**, whose affinity for the NET decreased 2-fold, while the affinity for the SERT increased 2-fold compared to those affinities for MeNER. Substitution of the oxygen atom with sulfur as the linker of the three rings in MeNER slightly improved the affinity for the NET, while increasing the affinity for the SERT 20-fold as seen in compound **3**. Consequently, **3** exhibited a lower selectivity for the hNET versus hSERT than MeNER, with a SERT/NET ratio of 13. Incorporation of ω-fluoroethyl and ω-fluoropropyl groups as in analogs **4** and **5** retained the NET potency about the same compared to **3**, while SERT affinity significantly decreased. Compound **5** had the highest selectivity (~700-fold) for the NET over the SERT. Two 2-(α-(2-carbomethoxy)) analogs of reboxetine (**2** and **6**) have roughly the same affinities toward hNET, hSERT, and hDAT, respectively.



Scheme 3. Radiosynthesis of reboxetine analogs.

Table 1. Overall synthesis times from EOB, average decay-corrected radiochemical yields (RCY), radiochemical purities, specific activities at time of injection, and log $P_{7.4}$ values of radioligands

Radioligands	Synthesis time (min)	RCY (%)	Radiochemical purity (%)	Specific activity (Ci/μmol)	Log $P_{7.4}$
[^{11}C]**1**	50	~100[a]	>98	0.4–0.8	1.91
[^{11}C]**2**	58	24[a]	>98	0.3–0.9	1.38
[^{11}C]**6**	60	23[a]	>98	0.2–0.6	1.64
[^{11}C]**3**	55	35[a]	>98	0.5–1.0	1.81
[^{18}F]**4**	120	8[b]	>98	1.7–2.2	1.83
[^{18}F]**5**	120	6[b]	>98	1.9–2.3	2.30

[a] From end of [^{11}C]CH$_3$I synthesis.
[b] From [^{18}F]F$^-$ produced.

3. Conclusions

A series of reboxetine analogs have been prepared and their in vitro affinities to the human monoamine transporters, NET, SERT, and DAT have been evaluated. Competition binding assays showed that all of the compounds tested exhibited high affinity and selectivity for the NET over the SERT and DAT. [^{11}C]**1**–**3**, and **6**, and [^{18}F]**4**–**5** were prepared in moderate to high radiochemical yields with high radiochemical purity and specific activity, and their lipophilicity was measured. These new C-11- and F-18-labeled tracers will permit compar-

Table 2. In vitro evaluation of candidate NET ligands in competition assays with human monoamine transporters[a]

Compound	K_i for hNET[b]	K_i for hSERT[c]	K_i for hDAT[d]	NET selectivity	
				SERT/NET	DAT/NET
1	2.06 ± 0.13	90.88 ± 4.63	>6000	44	>3000
2	1.32 ± 0.06	614 ± 9	>3000	465	>2000
3	0.76 ± 0.06	9.69 ± 1.34	>4000	13	>4000
4	1.00 ± 0.10	733 ± 101	>10,000	733	>10,000
5	1.21 ± 0.37	121 ± 18	>9000	100	>7000
6	1.35 ± 0.17	498 ± 49	>30,000	369	>20,000
(*S*,*S*)-MeNER	0.95 ± 0.03	185 ± 3	>10,000	195	>10,000

[a] All K_i values are reported with nanomolar (nM) units. Data are expressed as geometric means ± standard deviation of at least three separate experiments performed in triplicate.
[b] Competitive binding vs [^{3}H]nisoxetine in HEK-293 cells transfected with human norepinephrine transporters.
[c] Competitive binding vs [^{3}H]citalopram in HEK-293 cells transfected with human serotonin transporters.
[d] Competitive binding vs [^{125}I]RTI-55 in canine kidney cells transfected with human dopamine transporters.

ative microPET studies to evaluate their potential as PET radioligands for imaging NET in the central nervous system of nonhuman primates.

4. Experimental

4.1. General methods

2-Fluoroethyl tosylate,[26] 3-fluoropropyl tosylate,[27] and methyl 3-(2-hydroxyphenylthio)propanoate (**17**)[29] were prepared according to the published procedures. All other reagents used were obtained from commercially available sources and were used without further purification. All reactions were performed in oven glassware fitted with rubber septa under argon. ^{1}H NMR spectra were recorded on a Varian spectrometer at 400 MHz and referenced to the NMR solvent (chemical shifts in ppm values, *J* values in Hz). High-resolution mass spectra were acquired on a VG 70-S double focusing mass spectrometer using electron ionization (EI). Thin-layer chromatography (TLC) was performed using 250 μm layers of F-254 silica on aluminum plates obtained from Whatman (Clifton, NJ) and visualized by UV light. Flash chromatography was carried out using Merck silica gel 60 (40–63 μm particle size). No carrier-added [^{11}C]CO_2 was produced through the bombardment of $^{14}N_2$ gas containing 1% $^{16}O_2$ by a Siemens 11 MeV RDS 112 cyclotron at Emory University Hospital through the ^{14}N[p,α]^{11}C reaction. A GE MicroLab methyl iodide system was employed for the conversion of [^{11}C]CO_2 to [^{11}C]CH_3I.

4.2. (2*S*,3*S*)-*N*-*tert*-Butoxycarbonyl-2-[α-(2-(methylthio) phenoxy)benzyl]morpholine (9)

To a mixture of (2*S*,3*R*)-*N*-Boc-2-[α-hydroxy(phenyl) methyl]morpholine (**8**) (38 mg, 0.13 mmol), 2-(methylthio)phenol (36 mg, 0.26 mmol), and triphenylphosphine (68 mg, 0.26 mmol) in dry THF (2 mL) was added diisopropyl azodicarboxylate (52 μL, 0.26 mmol) at 0 °C. The reaction mixture was allowed to reach room temperature and stirred for 24 h. The crude product was concentrated under reduced pressure and was purified by flash chromatography on silica eluted with hexane/EtOAc (80:20) to afford **9** as colorless oil (20 mg, 37%): ^{1}H NMR ($CDCl_3$, 400 MHz), δ 7.25–7.39 (m, 5H), 7.09 (dd, *J* = 1.9, 7.3 Hz, 1H), 6.85–6.93 (m, 2H), 6.60–6.62 (m, 1H), 5.25 (br s, 1H), 3.92 (dd, *J* = 2.2, 11.4 Hz, 1H), 3.83–3.86 (m, 3H), 3.52–3.58 (m, 1H), 2.73–2.88 (m, 2H), 2.44 (s, 3H), 1.42 (s, 9H).

4.3. (2*S*,3*S*)-2-[α-(2-(Methylthio)phenoxy)benzyl]morpholine (1)

Trifluoroacetic acid (0.07 mL, 0.91 mmol) was added dropwise to a solution of **9** (20 mg, 0.048 mmol) in CH_2Cl_2 (1.5 mL) at 0 °C. The reaction mixture was allowed to reach room temperature and stirred for another 2 h. Two milliliters of 1 M NaOH solution was then slowly added at 0 °C and the mixture was extracted with CH_2Cl_2. The extracts were combined, dried over Na_2SO_4, and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica eluted with MeOH/CH_2Cl_2 (10:90) to afford **1** as colorless oil (12 mg, 79%): ^{1}H NMR ($CDCl_3$, 400 MHz), δ 7.25–7.38 (m, 5H), 7.08 (dd, *J* = 1.9, 7.6 Hz, 1H), 6.83–6.93 (m, 2H), 6.63 (dd, *J* = 1.2, 8.2 Hz, 1H), 5.20 (d, *J* = 5.7 Hz, 1H), 3.92–3.96 (m, 2H), 3.65 (td, *J* = 3.5 10.8 Hz, 1H), 2.73–2.82 (m, 3H), 2.60–2.66 (m, 1H), 2.44 (s, 3H), 2.10 (s, br, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 154.5, 137.4, 128.6, 128.3, 127.3, 125.9, 125.6, 121.6, 113.3, 81.6, 79.1, 68.3, 47.3, 45.8, 14.8. HRMS $[MH]^+$ Calcd for $C_{18}H_{22}NO_2S$: 316.1366, found: 316.1367.

4.4. (2*S*,3*S*)-*N*-*tert*-Butoxycarbonyl-2-[α-(2-(carbomethoxy)phenoxy)benzyl]morpholine (10)

To a mixture of **8** (40 mg, 0.136 mmol), methyl salicylate (35 μL, 0.27 mmol), and triphenylphosphine (71 mg, 0.27 mmol) in dry THF (3 mL) was added diisopropyl azodicarboxylate (53 μL, 0.27 mmol) at 0 °C. The reaction mixture was allowed to reach room temperature and stirred for 24 h. The crude product was concentrated under reduced pressure and was purified by flash chromatography on silica eluted with hexane/EtOAc (80:20) to afford **10** as colorless oil (43 mg, 74%): ^{1}H NMR ($CDCl_3$, 400 MHz), δ 7.75 (d, *J* = 1.9, 7.6 Hz, 1 H), 7.21–7.39 (m, 6 H), 6.88 (t, *J* = 7.3 Hz, 1 H), 6.75

(d, J = 8.6 Hz, 1H), 5.25 (br s, 1H), 3.92 (s, 3H), 3.84–3.90 (m, 4H), 3.54 (td, J = 2.5, 11.4 Hz, 1H), 2.87(br s, 1H), 2.72 (br s, 1H), 1.41 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 157.3, 154.9, 136.6, 133.3, 131.9, 128.7, 128.5, 127.4, 121.3, 120.7, 115.2, 81.7 (br), 80.2, 77.9, 67.0, 52.2, 44.2 (br), 43.2 (br), 28.5. HRMS $[MH]^+$ Calcd for $C_{24}H_{30}NO_6$: 428.2068, found: 428.2067.

4.5. (2*S*,3*S*)-2-[α-(2-(Carbomethoxy)phenoxy)benzyl]morpholine (2)

Trifluoroacetic acid (0.11 mL, 1.44 mmol) was added dropwise to a solution of **10** (41 mg, 0.096 mmol) in CH_2Cl_2 (2 mL) at 0 °C. The reaction mixture was allowed to reach room temperature and stirred for another 2 h. 3 mL of 1 M NaOH solution was then slowly added at 0 °C and the mixture was extracted with CH_2Cl_2. The extracts were combined, dried over Na_2SO_4, and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica eluted with MeOH/CH_2Cl_2 (10:90) to afford **2** as colorless oil (19 mg, 60%): ^{1}H NMR ($CDCl_3$, 400 MHz), δ 7.75 (*dd*, J = 1.6, 7.6 Hz, 1H), 7.21–7.37 (m, 6H), 6.88 (td, J = 1.0, 7.6 Hz, 1H), 6.75 (d, J = 8.2 Hz, 1H), 5.24 (d, J = 5.4 Hz, 1H), 3.97–4.02 (m, 2H), 3.91 (s, 3H), 3.66–3.72 (m, 1H), 2.84–2.88 (m, 3H), 2.68–2.73 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 167.1, 157.3, 136.7, 133.4, 131.9, 128.7, 128.5, 127.3, 121.1, 120.8, 115.2, 81.8, 78.2, 67.4, 52.2, 46.3, 45.1. HRMS $[MH]^+$ calcd for $C_{19}H_{22}NO_4$: 328.1543, found: 328.1541.

4.6. (2*S*,3*R*)-*N*-*tert*-Butoxycarbonyl-2-[α-bromo(phenyl)methyl]morpholine (11)

To a solution of (2*S*,3*S*)-*N*-Boc-2-[α-hydroxy(phenyl)methyl]morpholine (**7**) (118 mg, 0.4 mmol) in CH_2Cl_2 (3 mL) was added PPh_3 (210 mg, 0.8 mmol) at room temperature. The mixture was then cooled down to 0 °C, and a solution of CBr_4 (266 mg, 0.8 mmol) in CH_2Cl_2 (1 mL) was then added dropwise at this temperature. The reaction mixture was warmed up to room temperature and stirring was continued for 30 min. The solvent was evaporated and the crude product was purified by flash chromatography on silica eluted with hexane/EtOAc (80:20) to afford **11** as colorless oil (107 mg, 75%): ^{1}H NMR ($CDCl_3$, 400 MHz), δ 7.26–7.41 (m, 5 H), 4.84 (d, J = 7.6 Hz, 1H), 4.36 (br s, 1H), 3.79–3.86 (m, 3H), 3.46 (td, J = 2.8, 11.4 Hz, 1H), 2.92–2.96 (m, 2H), 1.46 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 154.8, 138.7, 128.8, 128.6, 80.5, 77.9, 66.9, 53.3, 47.0 (br), 43.2 (br), 28.6. HRMS $[MH]^+$ calcd for $C_{16}H_{23}O_3N^{79}Br$: 356.0856, found: 356.0854.

4.7. (2*S*,3*S*)-*N*-*tert*-Butoxycarbonyl-2-[α-(2-methoxyphenylthio)phenylmethyl]morpholine (12)

A reaction mixture of **11** (68 mg, 0.19 mmol), 2-methoxybenzenethiol (46 μL, 0.38 mmol), and Cs_2CO_3 (124 mg, 0.38 mmol) in DMF (3 mL) was stirred at room temperature for 18 h. The crude product was directly loaded on a short silica column eluted with hexane/EtOAc (80:20) to afford **12** as colorless oil (45 mg), which was used without any further purification.

4.8. (2*S*,3*S*)-2-[α-(2-Methoxyphenylthio)phenylmethyl]morpholine (3)

Trifluoroacetic acid (0.13 mL, 1.68 mmol) was added dropwise to a solution of **12** (45 mg, 0.11 mmol) obtained as above in CH_2Cl_2 (2 mL) at 0 °C. The reaction mixture was allowed to reach room temperature and stirred for another 2 h. Three milliliters of 1 M NaOH solution was then slowly added at 0 °C and the mixture was extracted with CH_2Cl_2. The extracts were combined, dried over Na_2SO_4, and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica eluted with MeOH/CH_2Cl_2 (10:90) to afford **3** as colorless oil (25 mg, 42% in two-step): ^{1}H NMR ($CDCl_3$, 400 MHz), δ 7.06–7.19 (m, 7 H), 6.65–6.69 (m, 2H), 4.39 (d, J = 8.2 Hz, 1H), 3.97–4.01 (m, 1H), 3.79–3.84 (m, 1H), 3.78 (s, 3H), 3.63 (td, J = 2.8, 11.1 Hz, 1H), 2.86 (td, J = 3.2, 11.1 Hz, 1H), 2.77 (d, J = 12.0 Hz, 1H), 2.61–2.64 (m, 2H), 1.92 (s, br, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 159.0, 139.1, 134.3, 128.8, 128.3, 127.4, 122.4, 120.7, 110.5, 79.8, 68.6, 55.8, 54.7, 49.7, 45.8. HRMS $[MH]^+$ Calcd for $C_{18}H_{22}NO_2S$: 316.1366, found: 316.1363.

4.9. (2*S*,3*S*)-*N*-*tert*-Butoxycarbonyl-2-[α-(2-hydroxyphenylthio)benzyl]morpholine (13)

A reaction mixture of **11** (71 mg, 0.2 mmol), 2-hydroxythiophenol (50 mg, 0.4 mmol), and Cs_2CO_3 (130 mg, 0.4 mmol) in DMF (2 mL) was stirred at room temperature for 18 h. The crude product was directly purified by flash chromatography on silica eluted with hexane/EtOAc (80:20) to afford **13** as colorless oil (73 mg, 91%): ^{1}H NMR ($CDCl_3$, 400 MHz), δ 7.52 (s, 1H), 7.21–7.25 (m, 3H), 6.98–7.02 (m, 3H), 6.91–6.94 (m, 1H), 6.68 (td, J = 1.2, 7.6 Hz, 1H), 4.06–4.11 (m, 1H), 3.83–3.86 (m, 1H), 3.66–3.74 (m, 4H), 2.95–3.02 (m, 1H), 2.52 (m, 1H), 1.35 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 158.4, 154.5, 138.6, 138.2, 132.1, 129.0, 128.1, 128.0, 120.3, 115.4, 80.4, 76.5, 67.1, 57.8 (br), 47.0 (br), 43.8 (br), 28.5. HRMS $[MH]^+$ calcd for $C_{22}H_{28}NO_4S$: 402.1734, found: 402.1739.

4.10. (2*S*,3*S*)-*N*-*tert*-Butoxycarbonyl-2-[α-(2-(2-fluoroethoxy)phenylthio)benzyl]morpholine (14)

A stirred mixture of **13** (30 mg, 0.075 mmol) and 2-fluoroethyl tosylate (22 mg, 0.097 mmol) in DMF (2 mL) was treated with tetrabutylammonium hydroxide (1 M in methanol, 89 μL, 0.089 mmol). After 20 min at room temperature, the solution was stirred at 80 °C for 2 h, cooled, and quenched with aqueous Na_2CO_3 (0.1 M, 8 mL). The mixture was extracted with ethyl acetate, the organic layer washed with water, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was loaded on a short silica column eluted with hexane/EtOAc (80:20), and 100 mL fractions was collected. After evaporating the solvent, colorless oil (28.5 mg) was obtained, and NMR shows

it is the mixture of product and 2-fluoroethyl tosylate (3.5:1), which was used without any further purification.

4.11. (2*S*,3*S*)-2-[α-(2-(2-Fluoroethoxy)phenylthio)benzyl] morpholine (4)

Trifluoroacetic acid (70 μL, 0.83 mmol) was added dropwise to a solution of compounds (28.5 mg) obtained as above in CH_2Cl_2 (2 mL) at 0 °C. The reaction mixture was allowed to reach room temperature and stirred for another 2 h. Two milliliters of 1 M NaOH solution was then slowly added at 0 °C and the mixture was extracted with CH_2Cl_2. The extracts were combined, dried over Na_2SO_4, and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica eluted with MeOH/CH_2Cl_2 (10:90) to afford **4** as colorless oil (12 mg, 44% in two-step): ^{1}H NMR ($CDCl_3$, 400 MHz), δ 7.06–7.22 (m, 7 H), 6.68–6.73 (m, 2H), 4.90–4.95 (m, 1/2H), 4.72–4.84 (m, 1H), 4.68–4.71 (m, 1/2H), 4.52 (d, J = 7.9 Hz, 1H), 4.09–4.27 (m, 2H), 3.98–4.02 (m, 1H), 3.79–3.84 (m, 1H), 3.64 (td, J = 2.8, 11.1 Hz, 1H), 2.84–2.91 (m, 1H), 2.76–2.80 (m, 1H), 2.67 (d, J = 6.3 Hz, 2H), 1.75 (s, 1H). HRMS $[MH]^+$ calcd for $C_{19}H_{23}FNO_2S$: 348.1428, found: 348.1418.

4.12. (2*S*,3*S*)-*N*-*tert*-Butoxycarbonyl-2-[α-(2-(3-fluoropropoxy)phenylthio)benzyl]morpholine (15)

A stirred mixture of **13** (33 mg, 0.082 mmol) and 3-fluoropropyl tosylate (26.6 mg, 0.11 mmol) in DMF (2 mL) was treated with tetrabutylammonium hydroxide (1 M in methanol, 98 μL, 0.098 mmol). After 20 min at room temperature, the solution was stirred at 80 °C for 2 h, cooled, and quenched with aqueous Na_2CO_3 (0.1 M, 10 mL). The mixture was extracted with ethyl acetate, the organic layer washed with water, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was loaded on a short silica column eluted with hexane/EtOAc (80:20), and 100 mL fractions were collected. After evaporating the solvent, colorless oil (31 mg) was obtained, and NMR shows it is the mixture of product and 3-fluoropropyl tosylate (3.8:1), which was used without any further purification.

4.13. (2*S*,3*S*)-2-[α-(2-(3-Fluoropropoxy)phenylthio)benzyl] morpholine (5)

Trifluoroacetic acid (75 μL, 0.97 mmol) was added dropwise to a solution of compounds (31 mg) obtained as above in CH_2Cl_2 (2 mL) at 0 °C. The reaction mixture was allowed to reach room temperature and stirred for another 2 h. Two milliliters of 1 M NaOH solution was then slowly added at 0 °C and the mixture was extracted with CH_2Cl_2. The extracts were combined, dried over Na_2SO_4, and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica eluted with MeOH/CH_2Cl_2 (10:90) to afford **5** as colorless oil (17 mg, 57% in two-step): ^{1}H NMR ($CDCl_3$, 400 MHz), δ 7.05–7.23 (m, 7 H), 6.67–6.73 (m, 2H), 4.73–4.81 (m, 1H), 4.62–4.69 (m, 1H), 4.35 (d, J = 8.0 Hz, 1H), 4.02–4.11 (m, 2H), 3.98 (d, J = 10.8 Hz, 1H), 3.78–3.83 (m, 2H), 3.64 (td, J = 2.8, 11.1Hz, 1H), 2.76–2.88 (m, 2H), 2.59–2.68 (m, 2H), 2.23 (t, J = 6.0 Hz, 1H), 2.17 (t, J = 6.0 Hz, 1H), 1.81 (br s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 158.1, 139.3, 134.2, 128.7, 128.6, 128.3, 127.4, 123.1, 121.1, 111.8, 81.9, 80.0 (J = 49.6 Hz), 68.5, 64.3 (J = 5.3 Hz), 54.8, 49.7, 45.8, 30.6 (J = 19.8 Hz). HRMS $[MH]^+$ calcd for $C_{20}H_{25}FNO_2S$: 362.1584, found: 362.1573.

4.14. (2*S*,3*S*)-*N*-*tert*-Butoxycarbonyl-2-[α-(2-(carbomethoxy)phenylthio)benzyl]morpholine (16)

A reaction mixture of **11** (31 mg, 0.087 mmol), methyl thiosalicylate (24 μL, 0.174 mmol), and Cs_2CO_3 (57 mg, 0.174 mmol) in DMF (2 mL) was stirred at room temperature for 18 h. The crude product was directly purified by flash chromatography on silica eluted with hexane/EtOAc (80:20) to afford **16** as colorless oil (33 mg, 85%): ^{1}H NMR ($CDCl_3$, 400 MHz), δ 7.75–7.77 (m, 1H), 7.35–7.37 (m, 2H), 7.17–7.24 (m, 5H), 7.05–7.09 (m, 1H), 4.40 (d, J = 7.6 Hz, 1H), 3.91–3.97 (m, 1H), 3.89 (s, 3H), 3.72–3.75 (m, 3H), 3.50–3.55 (m, 1H), 2.93–2.98 (m, 1H), 2.77 (br s, 1H), 1.37 (s, 9H).

4.15. (2*S*,3*S*)-2-[α-(2-(Carbomethoxy)phenylthio)benzyl] morpholine (6)

Trifluoroacetic acid (85 μL, 1.11 mmol) was added dropwise to a solution of **16** (33 mg, 0.074 mmol) in CH_2Cl_2 (2 mL) at 0 °C. The reaction mixture was allowed to reach room temperature and stirred for another 2 h. Three milliliters of 1 M NaOH solution was then slowly added at 0 °C and the mixture was extracted with CH_2Cl_2. The extracts were combined, dried over Na_2SO_4, and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica eluted with MeOH/CH_2Cl_2 (10:90) to afford **6** as colorless oil (20 mg, 78%): ^{1}H NMR ($CDCl_3$, 400 MHz), δ 7.75 (dd, J = 1.6, 8.0 Hz, 1 H), 7.35 (d, J = 6.7 Hz, 1H), 7.16–7.26 (m, 5H), 7.05 (td, J = 1.3, 7.6 Hz, 1H), 4.39 (d, J = 7.9 Hz, 1H), 3.89–3.98 (m, 1H), 3.88 (s, 3H), 3.77–3.83 (m, 1H), 3.62 (dd, J = 2.9, 11.1 Hz, 1H), 2.84 (td, J = 3.5, 12.4 Hz, 1H), 2.74–2.77 (m, 1H), 2.61 (d, J = 6.7 Hz, 2H), 1.97 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 167.3, 139.2, 138.7, 131.8, 130.8, 130.3, 129.5, 128.8, 128.6, 127.7, 124.9, 79.5, 68.6, 55.2, 52.4, 49.7, 45.7. HRMS $[MH]^+$ calcd for $C_{19}H_{22}NO_3S$: 344.1315, found: 344.1311.

4.16. (2*S*,3*S*)-*N*-*tert*-Butoxycarbonyl-2-[α-(2-(3-methoxy-3-oxopropylthio)phenoxy)benzyl]morpholine (18)

To a mixture of **8** (40 mg, 0.136 mmol), methyl 3-(2-hydroxyphenylthio)propanoate (58 mg, 0.27 mmol), and triphenylphosphine (71 mg, 0.27 mmol) in dry THF (3 mL) was added diisopropyl azodicarboxylate (53 μL, 0.27 mmol) at 0 °C. The reaction mixture was allowed to reach room temperature and stirred for 24 h. The crude product was concentrated under reduced pressure and was purified by flash chromatography on silica eluted with hexane/EtOAc (80:20) to afford **18** as colorless oil (40 mg, 60%): ^{1}H NMR ($CDCl_3$, 400 MHz), δ 7.24–7.38 (m, 6H), 6.96–7.00 (td, J = 1.6, 8.3 Hz, 1H), 6.80–6.84 (m, 1H), 6.63–6.65 (m, 1H),

5.25 (br s, 1H), 3.74–3.93 (m, 4H), 3.67 (s, 3H), 3.52–3.65 (m, 1H), 3.15–3.27 (m, 2H), 2.92 (m, 1H), 2.72–2.78 (m, 1H), 2.65 (t, $J = 7.6$ Hz, 2H), 1.41 (s, 9H). HRMS $[MH]^+$ calcd for $C_{26}H_{34}NO_6S$: 488.2101, found: 488.2090.

4.17. (*S*,*S*)-2((*N*-(*tert*-Butoxycarbonyl)morpholin-2-yl)(phenyl)methoxyl)benzoic acid (19)

A mixture of **10** (17 mg, 0.04 mmol) and NaOH (16 mg, 0.4 mmol) in DMF (1.5 mL) and H_2O (0.5 mL) was heated at 110 °C overnight. After cooling to room temperature, the crude product was directly purified by flash chromatography on silica eluted with CH_2Cl_2/MeOH (95:5) to afford **19** as colorless oil (11 mg, 67%), which was solidified by cooling in a freezer: ^{1}H NMR ($CDCl_3$, 400 MHz), δ 8.11 (dd, $J = 1.7$, 7.8 Hz, 1 H), 7.31–7.42 (m, 4 H), 7.28 (td, $J = 0.9$, 2.0 Hz, 2 H), 7.05 (td, $J = 0.9$, 7.9 Hz, 1H), 6.71 (d, $J = 8.3$ Hz, 1H), 5.06 (d, $J = 7.3$ Hz, 1H), 3.98–4.02 (m, 1H), 3.82 (m, 2H), 3.53–3.64 (m, 2H), 2.98–3.04 (m, 1H), 2.80 (m, 1H), 1.39 (m, 9H). HRMS $[MH]^+$ calcd for $C_{23}H_{28}NO_6$: 414.1911, found: 414.1914.

4.18. (*S*,*S*)-2((*N*-(*tert*-Butoxycarbonyl)morpholin-2-yl)(phenyl)methylthio)benzoic acid (20)

A solution of **16** (15 mg, 0.03 mmol) and 1 M NaOH (0.3 mL, 0.3 mmol) in ethanol (1.5 mL) was refluxed overnight. After cooling to room temperature, the crude product was directly purified by flash chromatography on silica eluted with CH_2Cl_2/MeOH (95:5) to afford **20** as colorless oil (8 mg, 57%): ^{1}H NMR ($CDCl_3$, 400 MHz), δ 7.92 (d, $J = 7.3$ Hz, 1 H), 7.12–7.33 (m, 8H), 4.35(d, $J = 7.6$ Hz, 1H), 3.95 (d, $J = 11.4$ Hz, 1H), 3.78 (m, 3H), 3.51–3.58 (m, 1H), 2.91–2.98 (m, 2H), 1.36 (s, 9H). HRMS $[MH]^+$ calcd for $C_{23}H_{28}NO_5S$: 430.1683, found: 430.1688.

4.19. Radiosynthesis of [^{11}C]1

To a suspension of **18** (1 mg, 2.0 μmol) in THF (0.3 mL) was added potassium *tert*-butoxide (4 μL, 1.0 M in THF) at room temperature. The reaction vial was sealed, mixed, and kept at room temperature for 30 min until $[^{11}C]CH_3I$ was delivered as a gas and bubbled through the solution with ice bath cooling. After delivery of $[^{11}C]CH_3I$, the reaction vial was warmed to 40 °C for 2 min, 6 M HCl (0.21 mL) was added, and the solution was heated at 100 °C for 7 min, cooled to 0 °C, and neutralized by addition of 6 M NH_4OH (0.22 mL). The solution was purified by semipreparative HPLC (Water XTerra Prep RP_{18} 5 μm, 19 × 100 mm; 56:44:0.1 v/v/v MeOH/H_2O/Et_3N; 9 mL/min; t_R (range) = 10.5–12.5 min). The desired fractions were combined, diluted with double volume of water and loaded onto a Waters C_{18} SepPak cartridge that was washed with saline (0.9% NaCl, 40 mL) and ethanol (0.5 mL). The radioactive product was washed out of the cartridge by absolute ethanol (1.5 mL) into a sealed sterile vial containing 3.5 mL of saline. The resulting solution was transferred under argon pressure through a Millipore filter (pore size 1.0 μm) followed by a smaller one (pore size 0.2 μm), to a 30 mL sterile vial containing 10 mL of saline and is ready for PET study.

4.20. Radiosynthesis of [^{11}C]2 and [^{11}C]6

(*S*,*S*)-2((*N*-(*tert*-Butoxycarbonyl)morpholin-2-yl) (phenyl)-methoxyl)benzoic acid (**19**) or (*S*,*S*)-2((*N*-(*tert*-Butoxycarbonyl)morpholin-2-yl)(phenyl)methylthio)benzoic acid (**20**) (1 mg) was dissolved in DMF (0.26 mL) followed by addition of 0.1 M Bu_4NOH (aq) (23 μL). The solution was placed in a sealed conical vial and cooled to 0 °C, and $[^{11}C]CH_3I$ was bubbled through the solution at 0 °C. The solution was heated at 100 °C for 10 min, trifluoroacetic acid (0.21 mL) was added, and the solution was heated at 100 °C for 7 min, cooled to 0 °C, and neutralized by addition of 6 M NH_4OH (0.6 mL). The solution was purified by semipreparative HPLC (Water XTerra Prep RP_{18} 5 μm, 19 × 100 mm). The retention time of [^{11}C]**2** was 11 min with buffered mobile phase consisting 52:48:0.1 v/v/v MeOH/H_2O/Et_3N at a 8 mL/min flow rate. The retention time of [^{11}C]**6** was 12.5 min with buffered mobile phase consisting 53:47:0.1 v/v/v MeOH/H_2O/Et_3N at a 9-mL/min flow rate. The dose formulation was conducted by the same procedure described above.

4.21. Radiosynthesis of [^{11}C]3

(2*S*,3*S*)-*N*-*tert*-Butoxycarbonyl-2-[α-(2-hydroxyphenylthio)benzyl]morpholine (**13**) (1 mg) was dissolved in DMF (0.25 mL) followed by addition of 0.1 M Bu_4NOH (aq) (22 μL). The solution was placed in a sealed conical vial and cooled to 0 °C, and $[^{11}C]CH_3I$ was bubbled through the solution at 0 °C. The solution was heated at 100 °C for 10 min, 6 M HCl(aq) (0.21 mL) was added, and the solution was heated at 100 °C for 7 min, cooled to 0 °C, and neutralized by addition of 6 M NH_4OH (0.22 mL). The solution was purified by semipreparative HPLC (Water XTerra Prep RP_{18} 5 μm, 19 × 100 mm; 55:45:0.1 v/v/v MeOH/H_2O/Et_3N; 9 mL/min; t_R = 10 min). The dose formulation was conducted by the same procedure described above as for [^{11}C]**1** .

4.22. Radiosynthesis of 2-[^{18}F]fluoroethyl brosylate and 3-[^{18}F]fluoropropyl brosylate

[^{18}F]HF was produced with a Siemens 11 MeV RDS 112 cyclotron by employing the $^{18}O(p,n)^{18}F$ reaction in $H_2{}^{18}O$. The [^{18}F]HF was transferred to a chemical processing control unit (CPCU), collected on a trap/release cartridge (DW-TRC, D&W Inc., Oakdale, TN), released with K_2CO_3(aq) (0.9 mg in 0.6 mL H_2O), and added to a CH_3CN solution of Kryptofix 222 (5 mg, in 1 mL). The solution was placed in a 110 °C oil bath, the solvent was evaporated under a $N_{2(g)}$ flow, and CH_3CN (3 mL) was added and evaporated in order to azeotropically dry the Kryptofix 222/$K^{18}F$. 1,2-Dibrosylethane or 1,3-dibrosylpropane (4 mg in 1 mL CH_3CN) was added, the reaction mixture was heated at 90 °C for 10 min, and the 2-[^{18}F]fluoroethyl brosylate or 3-[^{18}F]fluoropropyl brosylate trapped on a silica Sep-Pak (previously prepped with 10 mL Et_2O). The 2-[^{18}F]flu-

oroethyl brosylate or 3-[^{18}F]fluoropropyl brosylate was eluted with Et_2O, and Et_2O solution was transferred to a hot cell under $N_{2(g)}$ pressure and collected in a V-tube to give 2-[^{18}F]fluoroethyl brosylate or 3-[^{18}F]fluoropropyl brosylate in 74% or 67% radiochemical yield (decay-corrected from transfer of $H^{18}F_{(aq)}$ to the CPCU). The V-tube was placed in an 80 °C oil bath and the Et_2O evaporated with an Ar flow.

4.23. Radiosynthesis of [^{18}F]4 and [^{18}F]5

A solution of (2*S*,3*S*)-*N*-*tert*-butoxycarbonyl-2-[α-(2-hydroxyphenylthio)benzyl]morpholine (**13**) (1 mg) and 0.1 M Bu_4NOH (aq) (23 μL) in DMF (0.26 mL) was added to 2-[^{18}F]fluoroethyl brosylate or 3-[^{18}F]fluoropropyl brosylate. The reaction mixture was heated at 100 °C for 10 min, 6 M HCl (0.21 mL) was added, and the solution was heated at 100 °C for another 7 min, cooled to 0 °C, and neutralized by addition of 6 M NH_4OH (0.22 mL). The solution was purified by semi-preparative HPLC (Water XTerra Prep RP_{18} 5 μm, 19 × 100 mm). The retention time of [^{18}F]**4** was t_R (range) = 23–27 min with buffered mobile phase consisting 50:50:0.1 v/v/v MeOH/H_2O/Et_3N at a 5.5-mL/min flow rate. The retention time of [^{18}F]**5** was t_R (range) = 19–22 min with buffered mobile phase consisting 55:45:0.1 v/v/v MeOH/H_2O/Et_3N at a 7-mL/min flow rate. The dose formulation was conducted by the same procedure described above as for [^{11}C]**1**. The total synthesis time was 120 min from EOB with an 11% radiochemical yield for [^{18}F]**4** and a 9% radiochemical yield for [^{18}F]**5** (decay-corrected from transfer of [^{18}F]fluoroethyl brosylate and [^{18}F]fluoropropyl brosylate to the hot cell).

4.24. Quality control of purified radioligands

The radiochemical purities of radioligands were determined by an analytical radio-HPLC (waters Nova-Pak C_{18} 3.9 × 150 mm) using a UV detector and a radioactivity detector with or without coinjection with unlabeled reference compounds. The specific activities were determined by the UV absorbance of the radioactive peaks as compared with standard curves of unlabeled reference compounds. The analytical HPLC column was eluted with 70:30:0.1 v/v/v MeOH/H_2O/Et_3N for [^{11}C]**1**, [^{18}F]**4**, and [^{18}F]**5**, and 65:35:0.1 v/v/v MeOH/H_2O/Et_3N for [^{11}C]**2**, [^{11}C]**3**, and [^{11}C]**6** at a 1 mL/min flow rate. The retention times of radioligands were 4.08 min for [^{11}C]**1**, 3.53 min for [^{11}C]**2**, 4.41 min for [^{11}C]**3**, 3.55 min for [^{18}F]**4**, 3.70 min for [^{18}F]**5**, and 3.70 min for [^{11}C]**6**, respectively.

4.25. Lipophilicity measurements

Measurement of distribution coefficients of radiolabeled compounds was performed according to a previously reported procedure.[30] Briefly, the test tubes containing 2 mL of 1-octanol, 2 mL of 0.02 M sodium phosphate buffer (pH 7.4), and ~5–10 μCi portion of the radiotracer were vortexed for 10 min at room temperature and then centrifuged for 5 min. Samples (0.5 mL) from the 1-octanol and buffer layers were counted in a Packard Cobra II automated gamma-counter and decay corrected. The measurement was repeated three times. The $\log P_{7.4}$ values were calculated for each replicate with the following equation: $\log P_{7.4} = \log[(\text{counts in octanol phase})/(\text{counts in buffer phase})]$. The $\log P_{7.4}$ measurements from each replication were averaged to give the $\log P_{7.4}$ values for the radiotracer.

4.26. In vitro competition assays

Competition assays were performed based on methods reported previously.[31,32] Cell membranes prepared from a human embryonic kidney cell line (HEK-293) stably expressing hNET or hSERT (a gift from Randy Blakely, Ph.D., Vanderbilt University) and Madin Darby canine kidney cells stably expressing hDAT (a gift from Dr. Gary Rudnick, Yale University) were used in these assays. Cells were grown to confluency in DMEM containing 10% fetal bovine serum and Geneticin sulfate and then harvested using pH 7.4 phosphate-buffered saline (PBS) containing 0.53 mM ethylenediaminetetraacetic acid at 37 °C. Cell pellets were prepared through centrifugation at 400*g* for 10 min, the supernatant was decanted, and the pellets were homogenized with a Polytron PT3000 (Brinkman, Littau, Switzerland) at 11,000 rpm for 12 s in 30 vol of PBS. The resulting cell membrane suspensions were centrifuged at 43,000*g* for 10 min, the supernatants were decanted, and the resulting pellets were stored at −70 °C until used in the assays.

Competition assays were performed in 13 × 100 mm polystyrene tubes in a 2.0 mL final volume consisting of 1.7 mL of assay buffer, 100 μL of competing ligand in assay buffer, 100 μL of radioligand in assay buffer, and 100 μL of cell membrane suspension (corresponding to 30–70 μg of protein) in assay buffer. Cell membrane pellets were characterized prior to competition assays to determine membrane concentrations that gave optimal signal while not significantly affecting the concentration of the free radioligand. The cell membrane pellets were resuspended in the appropriate volume of assay buffer through brief homogenization using a Polytron PT3000. Competing ligands were assayed in triplicate at 12 concentrations ranging from 10^{-13} to 10^{-5} M. To ensure solubility, the competing ligands were dissolved in 1:1 ethanol/5 mM HCl and then serially diluted in 5 mM HCl.

For NET assays, the assay buffer consisted of 53 mM Tris buffer, 320 mM NaCl, and 5.3 mM KCl (pH 7.4 at 4 °C) and the equilibrium incubation time was 4 h at 4 °C. The radioligand used for NET assays was [^{3}H]nisoxetine obtained from Dupont NEN (3000 GBq/mmol). For SERT assays, the assay buffer consisted of 53 mM Tris buffer, 126 mM NaCl, and 5.3 mM KCl (pH 7.9 at room temperature) and the equilibrium incubation time was 2 h at room temperature. The radioligand used for SERT assays was [^{3}H]citalopram obtained from Dupont NEN (Boston, MA, 3100 GBq/mmol). For the DAT assay, the assay buffer consisted of 42 mM sodium phosphate buffer and 320 mM sucrose (pH 7.4 at room temperature) and the equilibrium incubation time was 1 h at room tempera-

ture. The radioligand used for DAT assays was [^{125}I]RTI-55 obtained from Dupont NEN (Boston, MA, 2200 Ci/mmol, 2.0 nM final concentration).

All of the assays were initiated by the addition of the cell membrane suspension. At the end of the incubation, the assays were terminated by the addition of ~5 mL of assay buffer at 4 °C followed by rapid vacuum filtration with 3× 5 mL washes with assay buffer at 4 °C through GF/B filters (Whatman Inc., Clifton, NJ) presoaked in assay buffer containing 0.3% polyethyleneimine. Data from the competition curves were analyzed, and K_i values were calculated using GraphPad Prism software (GraphPad software, San Diego, CA). The K_i values were reported as the geometric mean of at least three separate assays, each in duplicates, for each compound.

Acknowledgments

This project was supported by an investigator initiated research grant from Wyeth Laboratories. The authors are supported by NIH 1-R21-MH-66622-01, MH-42088, RR-000039, MH-39415, and MH-52899.

References and notes

1. Blakely, R. D.; DeFelice, L. J.; Hartzell, H. C. *J. Exp. Biol.* **1994**, *196*, 263.
2. Pacholcyzk, T.; Blakely, R. D.; Amara, S. G. *Nature* **1991**, *350*, 350.
3. Galli, A.; DeFelice, L. J.; Duke, B. J.; Moore, K. R.; Blakely, R. D. *J. Exp. Biol.* **1995**, *198*, 2197.
4. Ordway, G. A.; Klimek, V. *CNS Spectrums* **2001**, *6*, 697.
5. Ressler, K. J.; Nemeroff, C. B. *Biol. Psychiatry* **1999**, *46*, 1219.
6. Lee, C. M.; Snyder, S. H. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 5250.
7. Biegon, A.; Rainbow, T. *J. Neurosci.* **1983**, *3*, 1069.
8. Van Dort, M. E.; Kim, J. H.; Tluczek, L.; Wieland, D. M. *Nucl. Med. Biol.* **1997**, *24*, 707.
9. Tejani-Butt, S. M. *J. Phar. Exp. Ther.* **1992**, *260*, 427.
10. Haka, M. S.; Kilbourn, M. R. *Nucl. Med. Biol.* **1989**, *16*, 771.
11. Ding, Y.-S.; Lin, K.-S.; Logan, J.; Benveniste, H.; Carter, P. *J. Neurochem.* **2005**, *94*, 337.
12. Kung, M.-P.; Choi, S.-R.; Hou, C.; Zhuang, Z.-P.; Foulon, C.; Kung, H. F. *Nucl. Med. Biol.* **2004**, *31*, 533.
13. McConathy, J.; Owen, M. J.; Kilts, C. D.; Malveaux, E. J.; Camp, V. M.; Votaw, J. R.; Nemeroff, C. B.; Goodman, M. M. *Nucl. Med. Biol.* **2004**, *31*, 705.
14. Ding, Y.-S.; Lin, K. S.; Gaza, V.; Carter, P.; Alexoff, D.; Logan, J., et al. *Synapse* **2003**, *50*, 345.
15. Wilson, A. A.; Johnson, D. P.; Mozley, D.; Hussey, D.; Ginovart, N.; Nobrega, J.; Garcia, A.; Meyer, J.; Houls, S. *Nucl. Med. Biol.* **2003**, *30*, 85.
16. Schou, M.; Halldin, C.; Sovago, J.; Pike, V. W.; Gulyas, B.; Mozley, P. D.; Johnson, D. P.; Hall, H.; Innis, R. B.; Farde, L. *Nucl. Med. Biol.* **2003**, *30*, 707.
17. Schou, M.; Halldin, C.; Sovago, J.; Pike, V. W.; Hall, H.; Gulyas, B.; Mozley, P. D.; Dobson, D.; Shchukin, E.; Innis, R. B.; Farde, L. *Synapse* **2004**, *53*, 57.
18. Seneca, N.; Gulyas, B.; Varrone, A.; Schou, M.; Airaksinen, A.; Tauscher, J.; Vandenhende, F.; Kielbasa, W.; Farde, L.; Innis, R. B.; Halldin, C. *Psychopharmacology* **2006**, *188*, 119.
19. Kanegawa, N.; Kiyono, Y.; Kimura, H.; Sugita, T.; Kajiyama, S.; Kawashima, H.; Ueda, M.; Kuge, Y.; Saji, H. *Eur. J. Nucl. Med. Mol. Imaging* **2006**, *33*, 639.
20. Tamagnan, G. D.; Brenner, E.; Alagille, D.; Staley, J. K.; Haile, C.; Koren, A.; Early, M.; Baldwin, R. M.; Tarazi, F. I.; Baldessarini, R. J.; Jarkas, N.; Goodman, M. M.; Seibyl, J. P. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 533.
21. Lin, K.-S.; Ding, Y.-S.; Kim, S.-W.; Kil, K.-E. *Nucl. Med. Biol.* **2005**, *32*, 415.
22. In a paper, see Ref. 25, J. Boot et al., reported that the racemic mixture of compound **3** containing (*S*,*S*) and (*R*,*R*) enantiomers was prepared and resolved using chiral HPLC. One enantiomer was found to be potent and selective toward NET, whereas the opposite enantiomer was potent for both the NET and SERT. However, the assignment of absolute stereochemistry of resolved enantiomers was not reported in this paper.
23. Brenner, E.; Baldwin, R. M.; Tamagnan, G. *Org. Lett* **2005**, *7*, 937.
24. Mitsunobu, O. *Synthesis* **1981**, 1.
25. Boot, J.; Cases, M.; Clark, B. P.; Findlay, J.; Gallagher, P. T.; Hayhurst, L.; Man, T.; Montalbetti, C.; Rathmell, R. E.; Rudyk, H.; Walter, M. W.; Whatton, M.; Wood, V. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 699.
26. Wilson, A. A.; DaSilva, J. N.; Houle, S. *Appl. Radiat. Isot.* **1995**, *46*, 765.
27. Millington, J. E.; Pattison, F. L. M. *Can. J. Chem.* **1956**, *34*, 1532.
28. Zessin, J.; Gucker, P.; Ametamey, S. M.; Steinbach, J.; Brust, P.; Vollenweider, F. X.; Johannsen, B.; Schubiger, P. A. *J. Label. Compd. Radiopharm.* **1999**, *42*, 1301.
29. Schou, M.; Pike, V. W.; Varrone, A.; Gulyás, B.; Farde, L.; Halldin, C. *J. Label. Compd. Radiopharm.* **2006**, *49*, 1007.
30. Wilson, A. A.; Houle, S. *J. Label. Compd. Radiopharm.* **1999**, *42*, 1277.
31. Dischino, D. D.; Welch, M. J.; Kilbourn, M. R.; Raichle, M. E. *J. Nucl. Med.* **1983**, *24*, 1030.
32. Owens, M. J.; Morgan, W. N.; Plott, S. J.; Nemeroff, C. B. *J. Pharmacol. Exp. Ther.* **1997**, *283*, 1305.
33. Plisson, C.; McConathy, J.; Martarello, L.; Malveaux, E. J.; Camp, V. M.; Williams, L.; Votaw, J. R.; Goodman, M. M. *J. Med. Chem.* **2004**, *47*, 1122.