2-(2′-((Dimethylamino)methyl)-4′-(fluoroalkoxy)-phenylthio)benzenamine Derivatives as Serotonin Transporter Imaging Agents

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A novel series of ligands with substitutions at the 5-position on phenyl ring A and at the 4′-position on phenyl ring B of 2-(2′-((dimethylamino)methyl)-4′-(fluoroalkoxy)phenylthio)benzenamine (4′-2-fluoroethoxy derivatives **28–31** and 4′-3-fluoropropoxy derivatives **40–42**) were prepared and tested as serotonin transporter (SERT) imaging agents. The new ligands displayed high binding affinities to SERT (K_i ranging from 0.03 to 1.4 nM). The corresponding ^{18}F labeled compounds, which can be prepared readily, showed excellent brain uptake and retention after iv injection in rats. The hypothalamus region showed high uptake values between 0.74% and 2.2% dose/g at 120 min after iv injection. Significantly, the hypothalamus to cerebellum ratios (target to nontarget ratios) at 120 min were 7.8 and 7.7 for [^{18}F]**28** and [^{18}F]**40**, respectively. The selective uptake and retention in the hypothalamus, which has a high concentration of SERT binding sites, demonstrated that [^{18}F]**28** and [^{18}F]**40** are promising positron emission computed tomography imaging agents for mapping SERT binding sites in the brain.

Introduction

The association between serotonin and depression has been well documented. Patients with major depression show distinct alterations in serotonergic neuronal function.[1–3] Selective serotonin reuptake inhibitors (SSRIs) targeting serotonin transporter (SERT[a]) binding sites in the brain are currently being prescribed to treat millions of patients with depression.[2,4] There is an urgent need to find a simple method to measure the drug occupancy (or the lack thereof) of the target sites in the brains of nonresponders. This could be accomplished by developing PET and SPECT imaging agents to target SERT in the brain. These imaging agents could be used to study the interaction of psychoactive drugs with specific binding sites and therefore to monitor their effectiveness in occupying the targeted binding sites in the living human brain. SERT may also play an important role as a surrogate marker for changes in serotonin transmission in Parkinson's disease, drug abuse, and other mental illnesses. A simple method of imaging SERT would also further our understanding of these conditions.

The ability to image SERT binding sites could significantly improve the management of patients on SSRIs. First, PET imaging techniques could provide a direct measurement of drug occupancy at the target site. This would enable physicians to reduce side effects by titrating SSRI dosage to reach >60–70% occupancy.[5–11] A minimum necessary dose could be individually estimated; personalized medicine can be achieved. Second, it has been observed that there is a lag time of 2−6 weeks before the antidepressant onset of action of SSRIs and that at least 30% of patients are nonresponders. It is believed that SSRIs directly inhibit serotonin reuptake in the synapse, thus leading to an increase in serotonin neurotransmission.[12] Yet the lag time and the percentage of nonresponders indicate that there is no single mechanism that is responsible for depression. There are many alternative explanations, and new treatment targets have been suggested to overcome the lack of drug response.[3,4,13–15] By estimation of the drug occupancy of SERT binding sites by the antidepressant used for treatment, in vivo PET or SPECT imaging studies could assist in the development of more effective approaches to the treatment of depression.

There are several types of core structures that have been employed in developing SERT imaging agents, i.e., nitroquipazines,[16–19] (+)-McN5652,[12,20,21] tropanes,[22–26] and biphenylthiols.[27–36] Because of a higher serotonin transporter binding selectivity and ease of synthesis, in the past few years a large number of biphenylthiol derivatives have been prepared and tested as potential SERT imaging agents.[27–33] Several analogues of biphenylthiol derivatives, such as [^{123}I]ADAM (**2**), have shown excellent binding affinity and selectivity and have been successfully used for SPECT imaging in humans[37] (see Chart 1). Comparable PET imaging agents for SERT, based on the same biphenylthiol core, have also been reported (see Table 1). Wilson's group developed [^{11}C]DASB(**1**),[27] which has now become a standard tracer for PET imaging of SERT in humans.[8,10,38–40] Yet the practical challenges of using a ^{11}C tracer ($T_{1/2}$ = 20 min) on a routine basis have limited its application to major medical centers that possess an on-site cyclotron and a radiochemistry team. In contrast, ^{18}F ($T_{1/2}$ = 109 min) labeled tracers can be prepared by regional radiopharmacies and distributed within any major metropolitan area. Thus, ^{18}F labeled SERT imaging agents could potentially serve many more patients. In the past few years, several ^{18}F derivatives of biphenylthiols have been reported including AFM(**4**),[41] ACF(**5**),[36] and 5-FADAM(**3**)[42,43] (Chart 1). The most promising candidate is 5-FADAM (**3**). However, because of radiochemical limitations, which require a nucleophilic substitution of [^{18}F]fluoride on a less activated aromatic ring, the radiochemical yield

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[a]Abbreviations: SPECT, single photon emission computed tomography; PET, positron emission tomography; FDG, 2-fluoro-2-deoxy-D-glucose; SERT, serotonin transporter; DAT, dopamine transporter; NET, norepinephrine transporter; (+)-McN5652, (+)-*trans*-1,2,3,5,6,10-*β*-hexahydro-6-[4-(methylthio)phenyl[pyrrolo[2,1-*a*]isoquinoline; DASB, 3-amino-4-(2-dimethylaminomethylphenylsulfanyl)benzonitrile; ADAM, 5-iodo-2′-(2-((dimethylamino)methyl)phenylthio)benzenamine; 5-FADAM, *N*,*N*-dimethyl-2-(2-amino-4-[^{18}F]fluorophenylthio)benzylamine; AFM, 2-[(2-amino-4-fluoromethylphenyl)thio]-*N*,*N*-dimethylbenzenemethanamine; ACF, 2-[(2-amino-4-chloro-5-fluorophenyl)thio]-*N*,*N*-dimethyl-benzenmethanamine.

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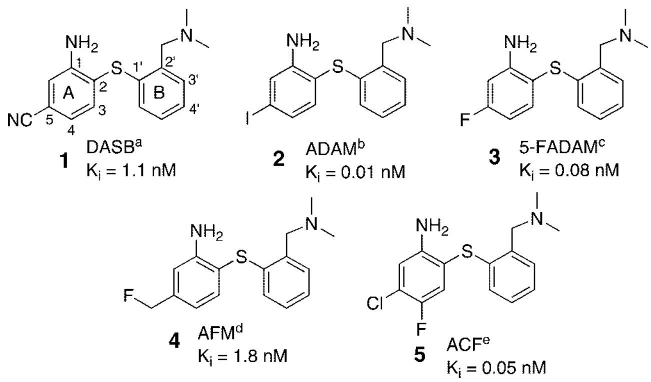
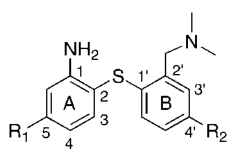
Chart 1. Chemical Structures and Binding Affinities of Serotonin Transporter Ligands (**1–5**)[f]



[a] Wilson et al.[10,27] [b] Choi et al.[35,37,47] [c] Shiue et al.[42] [d] Huang et al.[33] [e] Oya et al.[36] [f] It is important to note that there are at least two different nomenclatures for the same molecule **3**. Depending on the method for counting the positions on ring A, 5-FADAM (**3**) could also be named as 4-FADAM.[42,43,46,54]

Table 1. Binding Affinities of New Biphenylthiols for Monoamine Transporters[a]



compd	R_1	R_2	K_i (nM) SERT	NET	DAT
28	H	$O(CH_2)_2F$	0.25 ± 0.02	7.5 ± 0.7	340 ± 64
29	F	$O(CH_2)_2F$	0.10 ± 0.01	37 ± 5	>1000
30	Cl	$O(CH_2)_2F$	0.03 ± 0.001	97 ± 3	847 ± 73
31	Br	$O(CH_2)_2F$	0.05 ± 0.01	114 ± 8	>1000
40	H	$O(CH_2)_3F$	1.4 ± 0.2	12 ± 2	299 ± 9
41	F	$O(CH_2)_3F$	0.95 ± 0.13	95 ± 9	>1000
42	Br	$O(CH_2)_3F$	0.15 ± 0.02	>1000	>1000
47	H	$O(CH_2)_2OH$	1.1 ± 0.04	11 ± 1	351 ± 49
48	F	$O(CH_2)_2OH$	1.2 ± 0.1	56 ± 5	>1000
49	H	$O(CH_2)_3OH$	1.3 ± 0.3	7 ± 0.2	443 ± 68
50	Br	$O(CH_2)_3OH$	0.21 ± 0.02	279 ± 25	>1000
43	H	OCH_3	5 ± 0.4	14 ± 1	576 ± 31
44	H	OH	17 ± 2	33 ± 3	659 ± 38

[a] In vitro binding assays ($n = 3$) were employed to determine inhibition constants ($K_i \pm$ SEM) with membrane preparations of three different groups of LLC-PK$_1$ cells, each expressing one specific monoamine transporter, SERT, DAT, or NET.[47]

of [^{18}F]**3** is relatively low (<5%).[44] In order to improve the yield and study the structure–activity relationship of this series of biphenylthiol derivatives, we evaluated 4′-(2-fluoroethoxy) and 4′-(3-fluoropropoxy) derivatives with 4′-substitutions on phenyl ring B. These compounds provide an alternative approach for introduction of [^{18}F]fluoride via a simple nucleophilic reaction readily achievable in high yields and in a short time period. The reaction could be performed in an automated radiolabeling synthesizer that is commonly used in centralized radiopharmacies for making [^{18}F]FDG. In this paper, we report the preparation and characterization of the first examples of ^{18}F labeled 4′-substituted (ring B substituted) biphenylthiol derivatives targeting SERT for PET imaging.

Chemistry

A wealth of information on the synthesis of biphenylthiols (**1–5**) has been reported in the past few years.[27,28,30–33,45,46] The new series of biphenylthiols **28−42** were successfully prepared using Schemes 1–3. The synthesis of fluorinated biphenylthiols **28–31** is illustrated in Scheme 1. Commercially available 2-amino-5-methoxybenzoic acid **6** was readily converted to the corresponding thiol **7** via diazotization followed by replacement of the diazonium group by a sulfur atom. The unstable thiol **7** (due to a rapid oxidation of the thiol group) was immediately coupled with various nitrobenzenes **8–11** to give benzoic acids **12–15** in moderate to good yields (ranging from 55% to 77% yield). The carboxylic acids were converted to the amides **16–19** by first treating with thionyl chloride to produce the acid chlorides and the subsequent reaction of acid chlorides with *N*,*N*-dimethylamine. The methoxy group of amides **16–19** were readily demethylated with BBr_3 to produce the corresponding phenolic compounds **20–23**. The free OH groups of phenolic compounds **20–23** were alkylated with 1-bromo-2-fluoroethane to provide 2-fluorethoxy compounds **24–27**. The simultaneous reduction of nitro and amide groups yielded the desired nonradioactive compounds **28–31**.

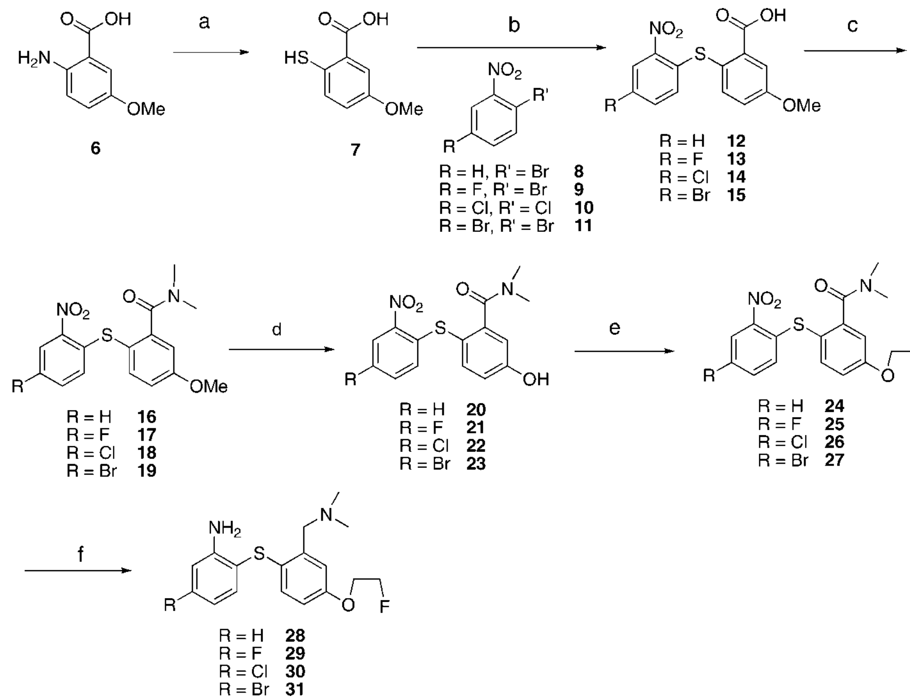
Similarly, three nonradioactive 3-fluorpropoxy compounds **40–42** were synthesized from intermediate phenolic compounds **20–23** (Scheme 2). Alkylation of the free 4′-OH group with 3-bromopropanol yielded compounds **32–34**. The amide **34** was directly converted to the fluoride **39** by treatment with DAST. Alternatively, the amides **32** and **33** were first transformed into their corresponding mesylates **35** and **36** by reacting with methanesulfonyl chloride. These mesylates were further converted to the fluorides **37** and **38** by microwave irradiation with TBAF. The simultaneous reduction of amide and nitro groups furnished the desired 3-fluoropropoxy compounds **40–42**.

Compounds **43** and **44** were obtained by reduction of both amide and nitro groups of intermediate compounds **16** and **20** (Scheme 3). Similarly compounds **47** and **48** were synthesized in two steps from **20** and **21** by alkylation with 2-bromoethanol followed by reduction. Similarly, reduction of **32** and **34** yielded the desired compounds **49** and **50**. The purity of all nonradioactive compounds used for the bioassay was confirmed from HPLC analysis using two different systems. All bioassay involved compounds reported in this paper showed greater than 95% purity in both systems (see Supporting Information).

We have also prepared the *O*-mesylate derivatives for ^{18}F radiolabeling. Methanesulfonate precursors **57–60** were prepared as shown in Scheme 4. The phenolic compounds **20–23** were alkylated with 2-bromoethanol to give compounds **45–46** and **51–52**. Selective reduction of amide group by BH_3−THF followed by an introduction of methanesulfonyl group provided the desired precursors **57–60**. The synthesis of three more methanesulfonate precursors **64–66** in two similar steps is shown in Scheme 4.

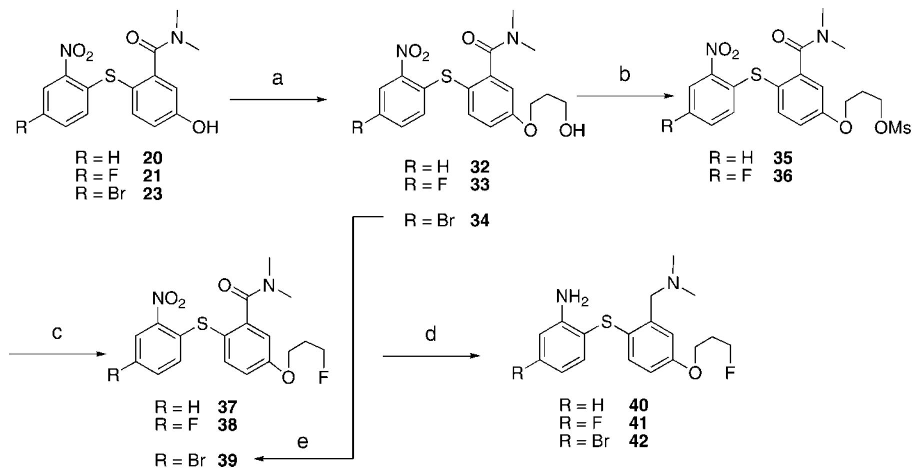
A similar radiolabeling approach, as reported previously, was used for 2-fluoroethoxy and 3-fluoropropoxy derivatives (Scheme 4). The radiolabeling was accomplished by starting with mesylate precursors (**57–60** or **64–66**) reacting with [^{18}F]fluoride in the presence of Kryptofix 222 and potassium carbonate in DMSO at 100 °C for 5–10 min. The resulting ^{18}F labeled nitro intermediates were directly reduced with tin chloride in hydrochloric acid/EtOH to provide desired labeled compounds [^{18}F]**28–31** or [^{18}F]**40–42** (Scheme 4). The crude product was purified by HPLC. The procedure took 60–120 min, and the specific activity at the end of synthesis was 280–1050 mCi/μmol ($n = 3$) (radiochemical purity of >99%, radiochemical yield of 10–35% decay-corrected). The purified product showed

Scheme 1. Preparation of 2-Fluoroethoxy Derivatives[a]



[a] Reagents and conditions: (a) (1) $NaNO_2$, (2) KS_2COEt, (3) HCl; (b) Na/EtOH, (c) (1) $SOCl_2$, (2) $(CH_3)_2NH$, $CHCl_3$; (d) 1.0 M BBr_3 in DCM, MW, 100 or −78 °C to room temp, 8 h; (e) 1-bromo-2-fluoroethane, DMF, K_2CO_3, MW; (f) BH_3−THF, THF, 4–5 h.

Scheme 2. Preparation of 3-Fluoropropoxy Derivatives[a]



[a] Reagents and conditions: (a) 3-bromo-1-propanol, K_2CO_3, DMF, MW, 15 min; (b) MsCl, TEA, DCM, 1 h; (c) TBAF, THF, MW, 20 min; (d) BH_3−THF, THF, 4−5 h; (e) DAST, DCM, −78 °C to room temp, 1 h.

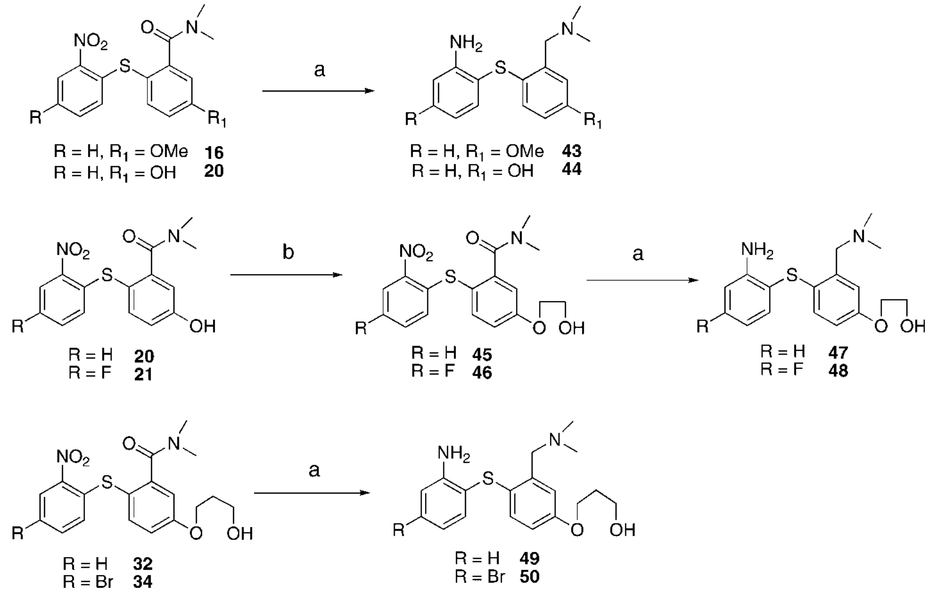
an HPLC profile consistent with the "cold" carrier (a typical example of an HPLC profile is shown in Supporting Information).

When the *O*-mesylates were used as the starting material, the ^{18}F labeling was successfully achieved for [^{18}F]**28–31** and [^{18}F]**40–42** in reasonable radiochemical yield and high radiochemical purity. The labeling reactions have not been optimized further; however, it is likely that the two-step one-pot reaction can be adapted for automation in a higher level (in multi-Ci of ^{18}F) of preparation.

Biological Studies

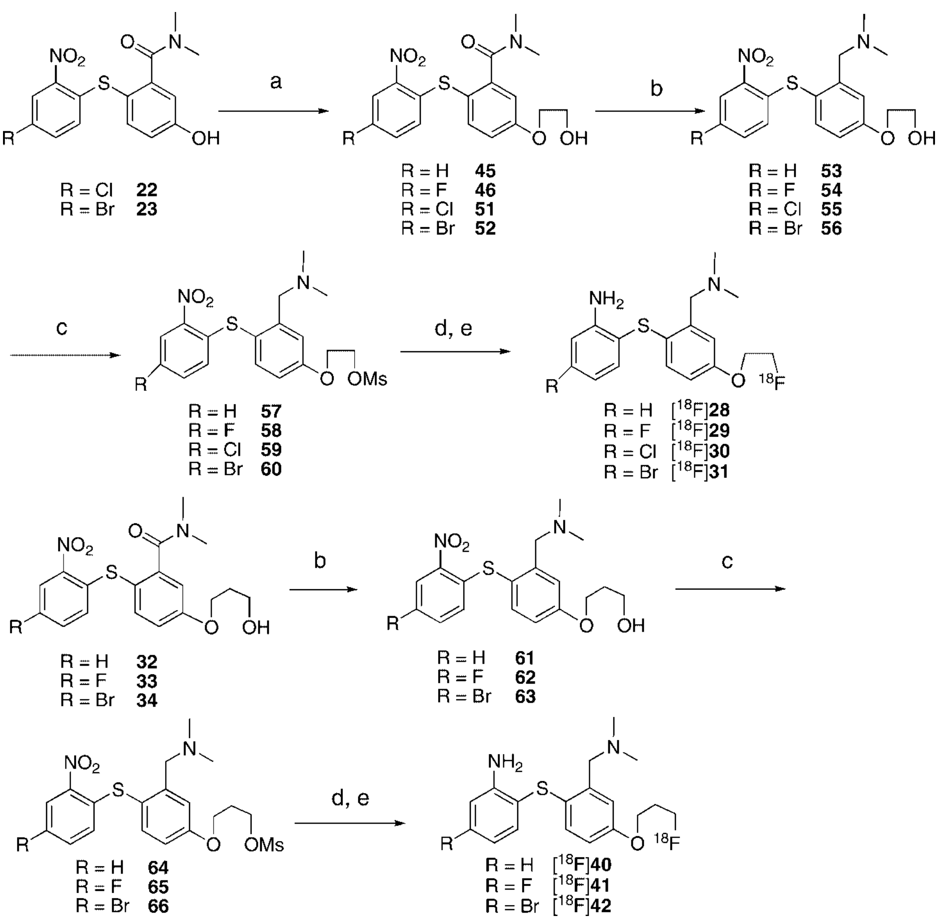
In vitro binding assays were carried out using membrane homogenates prepared from three different LLC-PK_1 cell lines, each overexpressing one of the monoamine transporters. As expected, the binding affinities of the 5-halogen and 4′-2-fluoroethoxy or 4′-3-fluoropropoxy substituted derivatives to SERT were excellent (K_i = 0.03−0.95 nM) with a rank of potency of Br ≥ Cl > F (Table 1). Interestingly, an opposite trend in NET affinity was seen. The binding affinities toward NET decreased with the size of the halogen atom (F > Cl > Br). The analogues that were substituted with heavier halogens (Cl or Br), i.e., **30, 31**, and **42**, showed the weakest NET affinities (K_i = 97, 114, and >1000 nM, respectively). The 5-H substituted analogues **28** and **40**, compared to the 5-halogen substituted analogues, displayed slightly lower SERT binding affinities and showed some NET binding (K_i = 0.25 and 1.4 nM for SERT and 7.5 and 12 nM for NET, respectively). This, however, is not believed to be a significant impediment toward their potential as SERT selective radiotracers because it is known that the concentration of the NET binding sites (B_{max} for NET) is at least 4-fold less than that of SERT in the brain.[48,49] Thus,

Scheme 3. Preparation of 4′-Methoxy, 4′-Hydroxy, 4′-(2-Hydroxyethoxy), and 4′-(3-Hydroxypropoxy) Derivatives[a]



[a] Reagents and conditions: (a) BH_3−THF, THF, 4–5 h; (b) 2-bromo-1-ethanol, K_2CO_3, DMF, MW, 150 °C, 15 min.

Scheme 4. Preparation of O-Mesylated Precursors and ^{18}F Radiolabeling[a]



[a] Reagents and conditions: (a) 2-bromoethanol, K_2CO_3, DMF, MW; (b) BH_3−THF, THF, 1.5 h; (c) methanesulfonyl chloride, TEA, DCM, 1–2 h; (d) K[^{18}F]F, K222, DMSO; (e) $SnCl_2$.

the new 5-H substituted analogues **28** and **40** will likely be sufficiently selective for imaging SERT binding sites and will be further evaluated. It is also apparent that the addition of a carbon atom to form the 4′-3-fluoropropoxy substituted derivatives results in lower SERT and NET binding affinities compared to the corresponding 4′-2-fluoroethoxy derivatives. Binding affinities of these derivatives toward DAT compared to SERT were much lower (K_i > 300 nM). The results were consistent with the binding profiles reported for other 5-methyl or 5-halogen substituted biphenylthiol derivatives reported previously (ring A substituted derivatives).[27,31–33,35,36,47] To our knowledge, this is the first time such a structure–activity

Table 2. Biodistribution of [^{18}F] Labeled SERT Tracers Evaluated in Rats after an Intravenous Injection: Brain Uptake, Hypothalamus Uptake, and Hypothalamus to Cerebellum Ratio

compd	30 min	120 min
Brain Uptake (% Dose/Organ)		
28	1.85 ± 0.15	0.63 ± 0.15
29	2.23 ± 0.22	2.57 ± 0.18
30	1.72 ± 0.35	1.46 ± 0.16
31	1.88 ± 0.18	2.16 ± 0.14
40	1.23 ± 0.18	0.59 ± 0.10
41	1.43 ± 0.06	1.32 ± 0.01
42	1.12 ± 0.06	1.15 ± 0.10
Hypothalamus Uptake (% Dose/g)		
28	1.62 ± 0.10	0.74 ± 0.18
29	1.76 ± 0.16	2.22 ± 0.28
30	1.31 ± 0.28	1.19 ± 0.11
31	1.19 ± 0.10	1.78 ± 0.24
40	1.22 ± 0.16	0.75 ± 0.18
41	1.05 ± 0.12	1.09 ± 0.09
42	0.85 ± 0.08	1.01 ± 0.21
Hypothalamus to Cerebellum Ratio		
28	3.79 ± 0.30	7.83 ± 2.96
29	2.78 ± 0.32	4.34 ± 0.60
30	2.36 ± 0.68	3.77 ± 0.40
31	2.04 ± 0.07	3.70 ± 0.58
40	3.50 ± 0.67	7.67 ± 2.60
41	2.66 ± 0.56	4.36 ± 0.45
42	1.93 ± 0.82	4.17 ± 0.48

relationship has been observed for the biphenylthiol series of compounds. It also suggests that relative binding affinities between SERT and NET are highly dependent on the substitution groups on ring A and ring B. Further exploration of this structure–activity relationship could enhance the selectivity between SERT and NET.

To test whether these potential imaging agents could bind to SERT binding sites in the brain, we measured brain uptake and regional brain distribution of the [^{18}F] labeled compounds in rats after an iv injection. The whole-body biodistribution data, an example of which can be seen in the Supporting Information, were relatively similar to data of previously reported labeled biphenylthiols, and the in vivo defluorination for these agents was relatively low (based on the relatively low bone uptake). The brain uptake between 30 and 120 min provides information on the relative brain washout. Biodistribution of all the [^{18}F] labeled compounds, **28**−**31** and **40**−**42**, in normal rats showed that they readily penetrated the intact blood−brain barrier with initial brain uptakes between 1.12 and 2.23 % dose/organ at 30 min after iv injection (Table 2). We were surprised at the high brain uptake and persistent retention of [^{18}F]**29**−**31**, **41**, and **42** at 120 min after injection (Table 2). It is unlikely that this relatively prolonged retention is caused by any single factor, but rather it is most likely caused by a combination of factors such as lipophilicity, affinity, molecular weight, metabolism, and/or presence of a selective efflux mechanism for the tracer. Ring A 5-halogen (F, Cl, and Br) substituted derivatives all displayed high brain uptake but slow brain washout. In contrast, ring A 5-H substituted compounds, [^{18}F]**28** and **40**, displayed a significant washout from the brain (1.85 vs 0.63 % dose/organ for **28** and 1.23 vs 0.59 % dose/organ for **41** at 30 vs 120 min, respectively). Such high brain uptake and washout have not been observed in any other ^{18}F labeled biphenylthiol derivatives reported in the literature.[28,30,33,36,42] Clearly, the 4′-2-fluoroethoxy or 4′-3-fluoropropoxy substitution on phenyl ring B has led to different in vivo brain kinetics.

Regional brain distribution of [^{18}F]**28**−**31** and [^{18}F]**40**−**42** showed that the hypothalamus, an area containing a high density of SERT binding sites, had the highest uptake (Table 2). The hypothalamus to cerebellum ratios displayed a significant increase from 30 to 120 min, and there was a more pronounced change for the ring A 5-H substituted compounds [^{18}F]**28** and **40**. [^{18}F]**28** displayed a hypothalamus to cerebellum ratio of 3.79 vs 7.83 at 30 vs 120 min, respectively, while [^{18}F]**40** showed a ratio of 3.5 vs 7.67 (Table 2). Although [^{18}F]**28** and **40** had lower hypothalamus uptake compared to [^{18}F]**29**−**31**, **41**, and **42**, the hypothalamus to cerebellum ratios of [^{18}F]**28** and **40** were higher because of faster washout from the cerebellum compared to washout from the hypothalamus. Although [^{18}F]**28** and **40** possess slightly lower in vitro binding affinities than the other compounds in their respective series, it is not clear why [^{18}F]**28** or **40** is able to dissociate more quickly from the nontarget region. It is known that in vitro experiments cannot always predict the behavioral kinetics and success of a tracer. Nevertheless, the favorable in vitro and in vivo kinetic properties of [^{18}F]**28** and **40** make them more desirable compared to other derivatives in the series as potential SERT imaging agents. The absolute values of hypothalamus to cerebellum ratios for [^{18}F]**28** and **40** were comparable to those of [^{11}C]**1**[27] and [^{18}F]**3**[42] (hypothalamus uptake at 60 min was 0.62 and 0.73 % dose/g and hypothalamus to cerebellum ratio at 60 min was 8.89 and 5.98, respectively). The absolute hypothalamus uptake values in the brain were also comparable to those of the above two tracers.[27,42]

In order to demonstrate that the uptake in the hypothalamus region of the brain for this series of ^{18}F labeled derivatives was indeed due to SERT specific binding, we performed a blocking study using several of the [^{18}F] labeled compounds including the most promising ligands, [^{18}F]**28** and [^{18}F]**40** (data not shown). Rats were pretreated with 2 mg/kg IDAM, GBR-12909, or 10 mg/kg nisoxetine iv 5 min prior to the tracer administration. At 2 h after the tracer injection, uptake in each brain region was compared between saline-pretreated (control) and drug-pretreated rats. Only IDAM, a selective SERT ligand, was able to significantly block the uptake in the hypothalamus region, an area containing a high density of SERT binding sites. As expected, the other drugs, nisoxetine, a NET ligand, and GBR-12909, a DAT ligand, showed no significant inhibition of selective uptake in the hypothalamus region (data not shown). The results will be published in the future as part of the full characterization of these promising SERT imaging agents.

Discussion

The biphenylthiol core has several unique advantages for developing new SERT imaging agents. First, the biphenylthiol core is relatively easy to prepare. It is also a relatively simple molecule containing no optical center. Finally, biphenylthiol derivatives show excellent binding affinities to SERT (in the nanomolar or subnanomolar range), while their affinities to the two other monoamine transporters, DAT and NET, are generally lower. Up to this point, most of the reported structure–activity studies of biphenylthiols have focused on the 4- or 5- position of the 2-aminophenyl ring (ring A) (Chart 1). The effects of adding substitution groups on phenyl ring B have not been fully explored. We developed the 4′-2-fluoroethoxy or 4′-3-fluoropropoxy substituted derivatives in order to provide a simple site for ^{18}F labeling. We have demonstrated that the substitution groups described in this paper successfully preserved SERT binding affinity and the respective derivatives showed selective SERT binding with favorable in vivo kinetics in the hypothalamus of the rat brain. We were surprised to find that biodistribution was greatly improved, resulting in better SERT localization (higher target to nontarget, i.e., hypothalamus/cerebellum ratios) and the opportunity to fine-tune the retention time in the brain. The changes in the in vivo pharmacokinetics were novel and unexpected. This is the first time that these types of 4′-2-fluoroethoxy or 4′-3-fluoropropoxy substituted biphenylthiol derivatives have been prepared as PET imaging agents targeting SERT binding sites.

It is important to balance between the brain uptake and washout out rates for SERT binding sites in the brain and selectivity between SERT and NET binding sites. It appears that the 5-H ring A substituted compounds, [^{18}F]**28** and **40**, showed the best kinetic properties. Even though these two 5-H ring A substituted compounds, [^{18}F]**28** and **40**, displayed reasonable binding affinities to SERT, they displayed a lower selectivity between SERT and NET ($K_i = 0.25$ and 1.4 nM for SERT and 7.5 and 12 nM for NET, respectively). We are currently carrying out additional studies of the in vivo binding selectivity by using PET imaging studies in rats. Initial data suggest that the binding in the brain is reversible and the hypothalamus uptake can be selectively displaced by a challenge dose of IDAM during imaging studies of SERT binding sites in the rat brain (data not shown).

Recently, we have reported two other novel ligands with a 4′-substitution (iodo or methoxy group) on the phenyl ring B of biphenylthiol: 5-chloro-2-(2′-((dimethylamino)methyl)-4′-iodophenylthio)benzenamine and 2-(2′-((dimethylamino) methyl)-4′-methoxyphenylthio)-5-iodobenzenamine as potential serotonin transporter (SERT) imaging agents.[45] These new iodinated ligands also 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 DAT and NET. They showed good brain uptakes and prolonged retention after iv injection in rats. Significantly, they also showed excellent uptake and prolonged retention in the hypothalamus where the SERT concentration is the highest. The hypothalamus/cerebellum ratios for the latter ^{125}I labeled agents were 3.97, 5.57, and 5.06 at 1, 2, and 4 h, respectively. Adding the 4′-iodo- group to phenyl ring B appeared to reduce the rate of clearance from the brain, and the kinetics favored uptake and retention in the hypothalamus. These observations are very consistent with what we have observed for the 4′-2-fluoroethoxy and 4′-3-fluoropropoxy derivatives reported in this paper. More recently, an abstract was presented describing the synthesis and testing of [^{11}C]5-hydroxymethyl and 4′-bromo derivatives of the same core structure as SERT imaging agent.[50] The results also confirm our observation that substitution at the 4′-position on the B ring in general changes the in vivo kinetics of SERT binding in the brain.

In conclusion, seven novel biphenylthiol derivatives, [^{18}F]**28**−**31**, **40**−**42**, were prepared and tested as serotonin transporter imaging agents for PET. These new ligands have a 4′-2-fluoroethoxy or 4′-3-fluoropropoxy substitution group on phenyl ring B that maintains the binding affinity and selectivity. These substitutions also dramatically changed the kinetics of in vivo biodistribution and improved the hypothalamus uptake and retention, a desirable property for a PET imaging agent. These novel ligands, especially the 5-H ring A substituted compounds, [^{18}F]**28** and **40**, showed excellent kinetic properties. Considering this, we are confident that these ligands and their analogues with similar substitutions would be good potential candidates for studying serotonin transporters with in vivo PET imaging.

Experimental Section

I. Chemistry. General Procedure A for the Preparation of Thiodiarylbenzoic Acid. To a solution of 2-amino-5-methoxybenzoic acid (**6**) (2.5 g, 15 mmol) in 1.6 mL of 50% sodium hydroxide, water (22 mL) and $NaNO_2$ (1.03 g, 15 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 (∼1–2 g). The mixture was slowly added with vigorous stirring to a solution of *O*-ethylxanthic acid, potassium salt (7 g, 43.7 mmol) in 25 mL of water at 80 °C. The temperature was maintained at 80 °C during the addition, and stirring was continued until the evolution of N_2 gas had subsided. After cooling down to room temperature by external cooling by an ice bath, the mixture was acidified by an addition of concentrated HCl 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-methoxybenzoic acid (**7**), was quickly dissolved in 20 mL of hot ethanol and added to a mixture of nitrobenene (**8**–**11**) (16.5 mmol) and sodium ethoxide (0.76 g of sodium in 35 mL of ethanol). The resulting solution was heated to reflux for 2 h. After the mixture was cooled, 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. The 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 (ethylacetate/methanol 10%) to give a yellow solid.

5-Methoxy-2-(2-nitrophenylthio)-benzoic acid (12) was prepared from **8** as a yellow solid in 77% yield according to general procedure A. ^{1}H NMR (CD_3OD) δ 8.16 (dd, $J = 8.0$, 1.5 Hz, 1H), 7.53 (d, $J = 8.5$ Hz, 1H), 7.45 (d, $J = 2.9$ Hz, 1H), 7.43–7.37 (m, 1H), 7.32–7.24 (m, 1H), 7.17 (dd, $J = 8.5$, 2.9 Hz, 1H), 6.90 (dd, $J = 8.0$, 1.4 Hz, 1H), 3.90 (s, 3H). HRMS calcd for $C_{14}H_{11}NO_5SNa$ [M^+ + Na] 328.0256, obsd 328.0253.

2-(4-Fluoro-2-nitrophenylthio)-5-methoxybenzoic acid (13), as the crude material, was used for the amidation reaction.

2-(4-Chloro-2-nitrophenylthio)-5-methoxybenzoic acid (14) was prepared from **10** as a yellow solid in 57% yield according to general procedure A. ^{1}H NMR (CD_3OD) δ 8.17 (d, $J = 1.9$ Hz, 1H), 7.57–7.50 (m, 2H), 7.31 (d, $J = 2.0$ Hz, 1H), 7.11 (dd, $J =$ 5.9, 2.7 Hz, 1H), 6.79 (d, $J = 8.7$ Hz, 1H), 3.90 (s, 3H).

2-(4-Bromo-2-nitrophenylthio)-5-methoxybenzoic acid (15) was prepared from **11** as a yellow solid in 55% yield according to general procedure A. ^{1}H NMR ($CDCl_3$) 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, $J = 8.6$, $J = 2$ Hz, 1H), 6.86 (d, $J = 8.8$ Hz, 1H), 3.91 (s, 3H).

General Procedure B for the Preparation of Amides. A solution of benzoic acid (**12**–**15**) (5.2 mmol) and thionyl chloride (1.0 mL, 14 mmol) in chloroform (50 mL) was heated to reflux for 2 h. The solvent and excess thionyl chloride were removed in vacuo. The residue was dissolved in chloroform (20 mL) and added to the 2 M solution of dimethylamine in tetrahydrofuran (10 mL) at 0 °C. The mixture was stirred at room temperature for 3 h. 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, eluting with ethyl acetate to give the products as yellow oils.

5-Methoxy-2-(2-nitrophenylthio)-*N*,*N*-dimethylbenzamide (16) was prepared from **12** as a yellow oil in 71% yield according to general procedure B. ^{1}H NMR ($CDCl_3$) δ 8.18 (dd, J = 8.2, 1.5 Hz, 1H), 7.5 (d, $J = 8.5$ Hz, 1H), 7.41–7.32 (m, 1H), 7.23–7.14 (m, 1H), 7.02–6.93 (m, 3H), 3.88 (s, 3H), 3.02 (s, 3H), 2.86 (s, 3H). HRMS calcd for $C_{16}H_{16}N_2NaO_4S$ [M^+ + Na] 355.0728, obsd 355.0738.

2-(4-Fluoro-2-nitrophenylthio)-5-methoxy-*N,N*-dimethylbenzamide (17) was prepared from **13** as a yellow oil in 70% yield according to general procedure B. ^{1}H NMR ($CDCl_3$) δ 7.90 (dd, J = 8.5 Hz, 2.7 Hz, 1H), 7.49 (d, J = 8.5 Hz, 1H), 7.17–7.08 (m, 1H), 7.07–6.90 (m, 3H), 3.88 (s, 3H), 3.03 (s, 3H), 2.86 (s, 3H). HRMS calcd for $C_{16}H_{15}FN_2NaO_4S$ [M^++ Na] 373.0634, obsd 373.0625.

2-(4-Chloro-2-nitrophenylthio)-5-methoxy-*N,N*-dimethylbenzamide (18) was prepared from **14** as a yellow oil in 72% yield according to general procedure B. ^{1}H NMR ($CDCl_3$) δ 8.17 (d, J = 2.1 Hz, 1H), 7.49 (d, J = 8.5 Hz, 1H), 7.32 (m, 1H), 7.03–6.86 (m, 3H), 3.88 (s, 3H), 3.03 (s, 3H), 2.86 (s, 3H). HRMS calcd for $C_{16}H_{15}ClN_2NaO_4S$ [M^+ + Na] 389.0339, obsd 389.0359.

2-(4-Bromo-2-nitrophenylthio)-5-methoxy-*N,N*-dimethylbenzamide (19) was prepared from **15** as a yellow oil in 68% yield according to general procedure B. ^{1}H NMR ($CDCl_3$) 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).

General Procedure C for the Deprotection of Methoxy Group. To a solution of **16–19** (0.78 mmol) in dichloromethane (3.0 mL) was added 1.0 M solution of BBr_3 in dichloromethane (1.1 mL). The mixture was irradiated with microwaves at 100 °C for 20 min and washed with water (2 mL). The organic layer was dried (Na_2SO_4) and purified by silica gel column, eluting with either ethyl acetate or methanol/dichloromethane (1/19) to yield a yellow oil. Alternatively, a solution of amide was added to a 1.0 M solution of BBr_3 in dichloromethane at − 78 °C. The temperature was raised slowly to room temperature, and the mixture was stirred overnight. The workup described earlier gave the product as a yellow oil.

5-Hydroxy-2-(2-nitrophenylthio)-*N,N*-dimethylbanzamide (20) was prepared from **16** as a yellow oil in 85% yield according to general procedure C (eluting with ethyl acetate). ^{1}H NMR ($CDCl_3$) δ 9.01 (s, 1H), 8.19 (dd, J = 8.2, 1.5 Hz, 1H), 7.48–7.36 (m, 2H), 7.22–7.18 (m, 1H), 6.96–6.91 (m, 2H), 6.85 (dd, J = 8.4, 2.7 Hz, 1H), 3.06 (s, 3H), 2.90 (s, 3H). HRMS calcd for $C_{15}H_{15}N_2O_4S$ [M^+ + H] 319.0753, obsd 319.0761.

2-(4-Fluoro-2-nitrophenylthio)-5-hydroxy-*N,N*-dimethylbanzamide (21) was prepared from **17** as a yellow oil in 83% yield according to general procedure C (eluting with methanol/dichloromethane mixture). ^{1}H NMR (CD_3OD) δ 7.97 (dd, J = 8.5, 2.7 Hz, 1H), 7.51 (d, J = 8.5 Hz, 1H), 7.35–7.26 (m, 1H), 7.06–6.97 (m, 2H), 6.84 (d, J = 2.6 Hz, 1H), 3.00 (s, 3H), 2.85 (s, 3H). HRMS calcd for $C_{15}H_{14}FN_2O_4S$ [M^+ + H] 337.0658, obsd 337.0663.

2-(4-Chloro-2-nitrophenylthio)-5-hydroxy-*N,N*-dimethylbanzamide (22) was prepared from **18** as a yellow oil in 80% yield according to general procedure C (eluting with methanol/dichloromethane mixture). ^{1}H NMR (CD_3OD) δ 8.21 (d, J = 2.3 Hz, 1H), 7.53–7.45 (m, 2H), 7.03–6.94 (m, 2H), 6.85 (d, J = 2.6 Hz, 1H), 3.00 (s, 3H), 2.86 (s, 3H).

2-(4-Bromo-2-nitrophenylthio)-5-hydroxy-*N,N*-dimethylbenzamide (23) was prepared from **19** as a yellow oil in 93% yield according to general procedure C (eluting with methanol/dichloromethane mixture). ^{1}H NMR ($CDCl_3$/CD_3OD 10%) δ 8.23 (d, J = 2.2 Hz, 1H), 7.38 (dd, J = 8.4, 2.2 Hz, 1H), 7.28 (d, J = 8.4 Hz, 1H), 6.83 (dd, J = 8.4, 2.6 Hz 1H), 6.70–6.75 (m, 2H), 2.91 (s, 3H), 2.77 (s, 3H).

General Procedure D for the Alkylation with 1-Bromo-2-fluoroethane. To a solution of **20–23** (0.126 mmol) and 1-bromo-2-fluoroethane (25 mg, 0.197 mmol) in DMF (2 mL) was added potassium carbonate (400 mg, 2.9 mmol). The mixture was irradiated with microwaves at 150 °C for 15 min and added to water (10 mL) and extracted with ethyl acetate (5 mL × 2). The combined organic layers were dried (Na_2SO_4) and purified by silica gel column (ethyl acetate or methanol/dichloromethane, 1/19) to yield yellow oil.

5-(2-Fluoroethoxy)-2-(2-nitrophenylthio)-*N,N*-dimethylbenzamide (24) was prepared from **20** as a yellow oil in 95% yield according to general procedure D (eluting with ethyl acetate). ^{1}H NMR ($CDCl_3$) δ 8.18 (dd, J = 8.2, 1.5 Hz, 1H), 7.51 (d, J = 8.5 Hz, 1H), 7.40–7.32 (m, 1H), 7.20 (m, 1H), 7.04–6.93 (m, 3H), 4.77 (dt, J = 47.4, 4.0 Hz, 2H), 4.28 (dt, J = 27.5, 4.0 Hz, 2H), 3.03 (s, 3H), 2.86 (s, 3H). HRMS calcd for $C_{17}H_{18}FN_2O_4S$ [M^+ + H] 365.0971, obsd 365.0982.

5-(2-Fluoroethoxy)-2-(4-fluoro-2-nitrophenylthio)-*N,N*-dimethylbenzamide (25) was prepared from **21** as a yellow oil in 91% yield according to general procedure D (eluting with ethyl acetate). ^{1}H NMR ($CDCl_3$) δ 7.90 (dd, J = 8.5, 2.7 Hz, 1H), 7.51 (d, J = 8.5 Hz, 1H), 7.18–6.84 (m, 4H), 4.79 (dt, J = 47.3, 4.0 Hz, 2H), 4.28 (dt, J = 27.6, 4.0 Hz, 2H), 3.03 (s, 3H), 2.86 (s, 3H). HRMS calcd for $C_{17}H_{17}F_2N_2O_4S$ [M^+ + H] 383.0877, obsd 383.0858.

2-(4-Chloro-2-nitrophenylthio)-5-(2-fluoroethoxy)-*N,N*-dimethylbenzamide (26) was prepared from **22** as a yellow oil in 96% yield according to general procedure D (eluting with methanol/dichloromethane mixture). ^{1}H NMR ($CDCl_3$) δ 8.18 (d, J = 2.3 Hz, 1H), 7.50 (d, J = 8.5 Hz, 1H), 7.32 (dd, J = 8.8, 2.3 Hz, 1H), 7.04 (dd, J = 8.5, 2.8 Hz, 1H), 6.96 (d, J = 2.7 Hz, 1H), 6.88 (d, J = 8.8 Hz, 1H), 4.79 (dt, J = 47.5, 4.0 Hz, 2H), 4.29 (dt, J = 27.6, 4.0 Hz, 2H), 3.02 (s, 3H), 2.86 (s, 3H). HRMS calcd for $C_{17}H_{17}ClFN_2O_4S$ [M^+ + H] 399.0582, obsd 399.0564.

2-(4-Bromo-2-nitrophenylthio)-5-(2-fluoroethoxy)-*N,N*-dimethylbenzamide (27) was prepared from **23** as a yellow oil in 89% yield according to general procedure D (eluting with methanol/dichloromethane mixture). ^{1}H NMR ($CDCl_3$) δ 8.32 (d, J = 2.1 Hz, 1H), 7.50 (d, J = 8.6 Hz, 1H), 7.44 (dd, J = 8.6, 2.1 Hz, 1H), 7.03 (dd, J = 8.4, 2.6 Hz, 1H), 6.95 (d, J = 2.6 Hz, 1H), 6.80 (d, J = 8.6 Hz, 1H), 4.79 (dt J = 47.4, 4.05 Hz, 2H), 4.30 (dt J = 27.6, 4.0 Hz, 2H), 3.02 (s, 3H), 2.85 (s, 3H).

General Procedure E for Simultaneous Reduction of Nitro and Amide Groups. To a solution of **24–27**, **37–39**, and other starting materials having both nitro and amide groups (0.1 mmol) in THF at 0 °C, 1.0 M BH_3−THF (10–15 equiv) was added. The mixture was heated to reflux for 4.5–5 h. After the mixture was cooled, 0.5 mL of 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. To the mixture 1 N NaOH was added to adjust the pH to basic (pH 10–11), and it was extracted with ethyl acetate (5 mL × 2). The organic layer was dried (Na_2SO_4) and purified by silica gel column (2% methanol in dichloromethane to 5% methanol in dichloromethane).

2-(2-((Dimethylamino)methyl)-4-(2-fluoroethoxy)phenylthio)-benzenamine (28) was prepared from **24** as a colorless oil in 63% yield according to general procedure E. ^{1}H NMR ($CDCl_3$) δ 7.38 (dd, J = 7.7, 1.5 Hz, 1H), 7.19–7.11 (m, 1H), 6.96–6.92 (m, 2H), 6.72–6.66 (m, 3H), 4.71 (dt, J = 47.4, 4.0 Hz, 2H), 4.51 (brs, 2H), 4.17 (dt, J = 27.8, 4.0 Hz, 2H), 3.57 (s, 2H), 2.30 (s, 6H). HRMS calcd for $C_{17}H_{22}FN_2OS$ [M^+ + H] 321.1437, obsd 321.1442. HPLC >98%.

2-(2-((Dimethylamino)methyl)-4-(2-fluoroethoxy)phenylthio)-5-fluoro-benzenamine (29) was prepared from **25** as a colorless oil in 62% yield according to general procedure E. ^{1}H NMR ($CDCl_3$) δ 7.45–7.38 (m, 1H), 6.91 (d, J = 8.6 Hz, 1H), 6.86 (d, J = 2.6 Hz, 1H), 6.69 (dd, J = 8.6, 2.8 Hz, 1H), 6.45–6.36 (m, 2H), 4.85 (brs, 2H), 4.71 (dt, J = 47.2, 4.1 Hz, 2H), 4.16 (dt, J = 27.7, 4.0 Hz, 2H), 3.53 (s, 2H), 2.30 (s, 6H). HRMS calcd for $C_{17}H_{21}F_2N_2OS$ [M^+ + H] 339.1343, obsd 339.1342. HPLC >98%.

5-Chloro-2-(2-((dimethylamino)methyl)-4-(2-fluoroethoxy)phenylthio)benzenamine (30) was prepared from **26** as a colorless oil in 52% yield according to general procedure E. ^{1}H NMR ($CDCl_3$) δ 7.33 (d, J = 8.8 Hz, 1H), 6.95 (d, J = 8.8 Hz, 1H), 6.87(d, J = 2.8 Hz), 6.73–6.62 (m, 3H), 4.72 (dt, J = 47.3, 4.1 Hz, 2H), 4.16 (dt, J = 27.8, 4.0 Hz, 2H), 3.52 (s, 2H), 2.29 (s, 6H). HRMS calcd for $C_{17}H_{21}ClFN_2OS$ [M^+ + H] 355.1047, obsd 355.1054. HPLC >96%.

5-Bromo-2-(2-((dimethylamino)methyl)-4-(2-fluoroethoxy)phenylthio)benzenamine (31) was prepared from **27** as a colorless oil in 51% yield according to general procedure E. ^{1}H NMR ($CDCl_3$) δ 7.23 (d, J = 8.0 Hz, 1H), 6.65–6.97 (m, 5H), 4.74 (br s, 2H), 4.73 (dt J = 47.2, 4.0 Hz, 2H), 4.14 (dt J = 27.8, 4.0 Hz, 2H), 3.53 (s, 2H), 2.33 (s, 6H). HRMS calcd for $C_{17}H_{20}BrFN_2OS$ [M^+] 398.0464, obsd 398.0457. HPLC >98%.

2-(2-((Dimethylamino)methyl)-4-(3-fluoropropoxy)phenylthio)-benzenamine (40) was prepared from **37** as a colorless oil in 54% yield according to general procedure E. ^{1}H NMR ($CDCl_3$) δ 7.38 (dd, J = 8.0, 1.7 Hz, 1H), 7.19–7.10 (m, 1H), 6.94 (d, J = 8.6 Hz, 1H), 6.88 (d, J = 2.7 Hz, 1H), 6.72 (m, 1H), 6.69–6.63 (m, 2H), 4.62 (dt, J = 47.0, 5.8 Hz, 2H), 4.50 (brs, 2H), 4.05 (t, J = 6.1 Hz, 2H), 3.54 (s, 2H), 2.30 (s, 6H), 2.13 (dt, J = 26.0, 5.9 Hz, 2H). HRMS calcd for $C_{18}H_{24}FN_2OS$ [M^+ + H] 335.1593, obsd 335.1590. HPLC >99%.

2-(2-((Dimethylamino)methyl)-4-(3-fluoropropoxy)phenylthio)-5-fluorobenzenamine (41) was prepared from **38** as a colorless oil in 57% yield according to general procedure E. ^{1}H NMR ($CDCl_3$) δ 7.45–7.37 (m, 1H), 6.91 (d, J = 8.6 Hz, 1H), 6.82 (d, J = 2.7 Hz, 1H), 6.67 (dd, J = 8.6, 2.7 Hz, 1H), 6.45–6.35 (m, 2H), 4.83 (brs, 2H), 4.62 (dt, J = 47.0, 5.8 Hz, 2H), 4.04 (t, J = 6.1 Hz, 2H), 3.53 (s, 2H), 2.30 (s, 6H), 2.13 (dt, J = 26.0, 6.0 Hz, 2H). HRMS calcd for $C_{18}H_{23}F_2N_2OS$ [M^+ + H] 353.1499, obsd 353.1494. HPLC >98%.

5-Bromo-2-(2-((dimethylamino)methyl)-4-(3-fluoropropoxy)phenylthio)benzenamine (42) was prepared from **39** as a colorless oil in 39% yield according to general procedure E. ^{1}H NMR ($CDCl_3/CD_3OD$) δ 7.34 (d, J = 2.2 Hz, 1H), 7.08 (d, J = 8.6 Hz, 1H), 6.90–6.79 (m, 3H) 6.72 (dd, J = 8.6, 1.4 Hz, 1H), 4.55 (dt, J = 44, 5.8 Hz 2H), 4.35 (s, 2H), 4,09 (t, J = 6.1 Hz, 2H), 2.79 (s, 6H), 2.19–2.00 (m, 2H). HRMS calcd for $C_{18}H_{23}BrFN_2OS$ [M^+ + H] 413.0698, obsd 413.0705. HPLC >95%.

2-(2-((Dimethylamino)methyl)-4-(methoxy)phenylthio)benzenamine (43) was prepared from **16** as a colorless oil in 61% yield according to general procedure E. ^{1}H NMR ($CDCl_3$) δ 7.38 (dd, J = 8.0, 1.7 Hz, 1H), 7.19–7.10 (m, 1H), 6.95 (d, J = 8.5 Hz, 1H), 6.87 (d, J = 2.7 Hz, 1H), 6.72–6.64 (m, 3H), 4.53 (brs, 2H), 3.76 (s, 3H), 3.55 (s, 2H), 2.30 (s, 6H). HRMS calcd for $C_{16}H_{21}N_2OS$ [M^+ + H] 289.1375, obsd 289.1376. HPLC >98%.

4-(2-Aminophenylthio)-3-((dimethylamino)methyl)phenol (44) was prepared from **20** as a colorless oil in 57% yield according to general procedure E. ^{1}H NMR ($CDCl_3$) δ 7.32 (dd, J = 8.0, 1.6 Hz, 1H), 7.18–7.10 (m, 1H), 6.89 (d, J = 8.5 Hz, 1H), 6.81 (d, J = 2.7 Hz, 1H), 6.71 (d, J = 7.6 Hz, 2H), 6.58 (dd, J = 8.5, 2.8 Hz, 1H), 4.29 (brs, 2H), 3.58 (s, 2H), 2.32 (s, 6H). HRMS calcd for $C_{15}H_{19}N_2OS$ [M^+ + H] 275.1218, obsd 275.1211. HPLC >97%.

2-(4-(2-Aminophenylthio)-3-((dimethylamino)methyl)phenoxy)-ethanol (47) was prepared from **45** as a colorless oil in 50% yield according to general procedure E. ^{1}H NMR ($CDCl_3$) δ 7.39 (dd, J = 8.0, 1.6 Hz, 1H), 7.19–7.11 (m, 1H), 6.96–6.90 (m, 2H), 6.73–6.65 (m, 3H), 4.51 (brs, 2H), 4.06–4.01 (m, 2H), 3.94–3.90 (m, 2H), 3.54 (s, 2H), 2.30 (s, 6H). HRMS calcd for $C_{17}H_{23}N_2O_2S$ [M^+ + H] 319.1480, obsd 319.1479. HPLC >97%.

2-(4-(2-Amino-4-fluoro-phenylthio)-3-((dimethylamino)methyl)phenoxy)ethanol (48) was prepared from **46** as a colorless oil in 47% yield according to general procedure E. ^{1}H NMR ($CDCl_3$) δ 7.45–7.37 (m, 1H), 6.94–6.87 (m, 2H), 6.69 (dd, J = 8.5, 2.7 Hz, 1H), 6.44–6.36 (m, 2H), 4.81 (brs, 2H), 4.04 (m, 2H), 3.93 (m, 2H), 3.54 (s, 2H), 2.30 (s, 6H). HRMS calcd for $C_{17}H_{22}F_2N_2O_2S$ [M^+ + H] 337.1386, obsd 337.1401. HPLC >95%.

3-(4-(2-Aminophenylthio)-3-((dimethylamino)methyl)phenoxy)-1-propanol (49) was prepared from **32** as a colorless oil in 49% yield according to general procedure E. ^{1}H NMR ($CDCl_3$) δ 7.37 (dd, J = 8.0, 1.6 Hz, 1H), 7.19–7.11 (m, 1H), 6.96–6.89 (m, 2H), 6.72–6.60 (m, 3H), 4.52 (brs, 2H), 4.08 (t, J = 5.9 Hz, 2H), 3.84 (t, J = 5.9 Hz, 2H), 3.56 (s, 2H), 2.30 (s, 6H), 2.01 (quintet, J = 5.9 Hz, 2H). HPLC >96%.

3-(4-(2-Amino-4-bromophenylthio)-3-((dimethylamino)methyl)phenoxy)propan-1-ol (50) was prepared from **34** as a colorless oil in 70% yield according to general procedure E. ^{1}H NMR ($CDCl_3/CD_3OD$) δ 7.23 (m, 1H), 6.95 (d, J = 8.6 Hz, 1H), 6.87–6.66 (m, 4H) 4.8 (br s, 2H), 4.08 (t, J = 6.0 Hz, 2H), 3.84 (t, J = 6.0 Hz, 2H), 3.54 (s, 2H), 2.30 (s, 6H), 2.07–1.95 (m, 2H).

General Procedure F for Alkylation with Bromoethanol and Bromopropanol. To a solution of **20–23** (0.239 mmol) and 2-bromoethananol or 3-bromopropanol (0.32 mmol) in DMF (10 mL) was added potassiun carbonate (80 mg). The mixture was heated at 100 °C overnight and added to water (50 mL) and extracted with ethyl acetate (20 mL × 2). The organic layer was dried (Na_2SO_4) and purified by silica gel column (ethyl acetate) to yield a yellow oil. Alternatively, the above mixture irradiated with microwaves at 150 °C for 15 min followed by the workup described earlier gave the desired product.

5-(3-Hydroxypropoxy)-2-(2-nitrophenylthio)-*N,N*-dimethylbenzamide (32) was prepared from **20** as a yellow oil in 87% yield according to general procedure F. ^{1}H NMR ($CDCl_3$) δ 8.18 (dd, J = 8.2, 1.5 Hz, 1H), 7.49 (d, J = 8.5 Hz, 1H), 7.40–7.32 (m, 1H), 7.23–7.15 (m, 1H), 7.03–6.93 (m, 3H), 4.19 (t, J = 6.0 Hz, 2H), 3.88 (m, 2H), 3.02 (s, 3H), 2.86 (s, 3H), 2.08 (m, 2H). HRMS calcd for $C_{18}H_{21}N_2O_5S$ [M^+ + H] 377.1171, obsd 377.1156.

2-(4-Fluoro-2-nitrophenylthio)-5-(3-hydroxypropoxy)-*N,N*-dimethylbenzamide (33) was prepared from **21** as a yellow oil in 85% yield according to general procedure F. ^{1}H NMR ($CDCl_3$) δ 7.90 (dd, J = 8.4, 2.7 Hz, 1H), 7.49 (d, J = 8.5 Hz, 1H), 7.13–7.08 (m, 1H), 7.03–6.90 (m, 3H), 4.18 (t, J = 6.0 Hz, 2H), 3.87 (m, 2H), 3.02 (s, 3H), 2.86 (s, 3H), 2.07 (m, 2H). HRMS calcd for $C_{18}H_{20}FN_2O_5S$ [M^+ + H] 395.1077, obsd 395.1051.

2-(4-Bromo-2-nitrophenylthio)-5-(3-hydroxypropoxy)-*N,N*-dimethylbenzamide (34) was prepared from **23** as a yellow oil in 70% yield according to general procedure F. ^{1}H NMR ($CDCl_3$) δ 8.31 (d, J = 2.2 Hz, 1H), 7.49–7.42 (m, 2H), 7.01–6.93 (m, 2H), 6.80 (d, J = 8.4 Hz, 1H), 4.21 (t, J = 5.6 Hz, 2H), 3.87 (dd, J = 5.8, 5.6 Hz, 2H), 3.01 (s, 3H), 2.85 (s, 3H).

5-(2-Hydroxyethoxy)-2-(2-nitrophenylthio)-*N,N*-dimethylbenzamide (45) was prepared from **20** as a yellow oil in 94% yield according to general procedure F. ^{1}H NMR ($CDCl_3$) δ 8.18 (dd, J = 8.2, 1.4 Hz, 1H), 7.50 (d, J = 8.5 Hz, 1H), 7.40–7.32 (m, 1H), 7.19–7.15 (m, 1H), 7.01 (dd, J = 8.5, 2.8 Hz, 1H), 6.96–6.92 (m, 2H), 4.17–4.10 (m, 2H), 4.03–3.93 (m, 2H), 3.02 (s, 3H), 2.86 (s, 3H). HRMS calcd for $C_{15}H_{19}N_2O_5S$ [M^+ + H] 363.1015, obsd 363.1045.

2-(4-Fluoro-2-nitrophenylthio)-5-(2-hydroxyethoxy)-*N,N*-dimethylbenzamide (46) was prepared from **21** as a yellow oil in 93% yield according to general procedure F. ^{1}H NMR ($CDCl_3$) δ 7.90 (dd, J = 8.2, 2.6 Hz, 1H), 7.50 (d, J = 8.5 Hz, 1H), 7.18–7.08 (m, 1H), 7.05–6.90 (m, 3H), 4.15 (m, 2H), 4.01 (m, 2H), 3.03 (s, 3H), 2.88 (s, 3H). HRMS calcd for $C_{17}H_{18}FN_2O_5S$ [M^+ + H] 381.0920, obsd 381.0912.

2-(4-Chloro-2-nitrophenylthio)-5-(2-hydroxyethoxy)-*N,N*-dimethylbenzamide (51) was prepared from **22** as a yellow oil in 89% yield according to general procedure F. ^{1}H NMR ($CDCl_3$) δ 8.17 (d, J = 2.1 Hz, 1H), 7.50 (d, J = 8.5 Hz, 1H), 7.31 (dd, J = 8.5, 2.2 Hz, 1H), 7.03 (dd, J = 8.5, 2.7 Hz, 1H), 6.95 (d, J = 2.4 Hz, 1H), 6.88 (d, J = 8.8 Hz, 1H), 4.17–4.13 (m, 2H), 4.01–3.99 (m, 2H), 3.02 (s, 3H), 2.86 (s, 3H).

2-(4-Bromo-2-nitrophenylthio)-5-(2-hydroxyethoxy)-*N,N*-dimethylbenzamide (52) was prepared from **23** as a yellow oil in 76% yield according to general procedure F. ^{1}H NMR ($CDCl_3$) δ 8.31 (d, J = 2.2 Hz, 1H), 7.49 (d, J = 8.7 Hz, 1H), 7.65 (dd, J = 8.7, 2.2 Hz, 1H), 7.02 (dd, J = 8.7, 2.8 Hz, 1H), 6.95 (d, J = 2.8 Hz, 1H), 8.81 (d, J = 8.7 Hz, 1H), 4.15 (m, 2H), 3.99 (m, 2H), 3.02 (s, 3H), 2.85 (s, 3H).

General Procedure G for Selective Reduction of Amide Group. To a solution of amides **45–52** and **32–34** (0.18 mmol) in THF (10 mL) at 0 °C, 1.0 M BH_3−THF (5 equiv) was added. The mixture was heated to reflux for 1.5–2 h. To the mixture, 0.5 mL of concentrated HCl was cautiously added. The solvent was removed in vacuo. Water (5 mL) was added to the residue, and the mixture was heated to reflux for 30 min. To the mixture, 1 N NaOH was added to adjust the pH to basic (pH 10) and extracted with ethyl acetate (5 mL × 2). The organic layer was dried (Na_2SO_4) and purified by silica gel column (2% methanol in dichloromethane to 5% methanol in dichloromethane) to provide the product as a yellow oil.

2-(3-(Dimethylamino)methyl)-4-(2-nitrophenylthio)phenoxy)-ethanol (53) was prepared from **45** as a pale-yellow oil in 70% yield according to general procedure G. ^{1}H NMR ($CDCl_3$) δ 8.25 (dd, J = 8.0, 1.6 Hz, 1H), 7.49 (d, J = 8.5 Hz, 1H), 7.34–7.30 (m, 2H), 7.22- 7.14 (m, 1H), 6.90 (dd, J = 8.5, 2.8 Hz, 1H), 6.66 (dd, J = 8.0, 1.4 Hz, 1H), 4.18 (t, J = 4.0 Hz, 2H), 4.00 (t, J = 4.0 Hz, 2H), 3.50 (s, 2H), 2.19 (s, 6H). HRMS calcd for $C_{17}H_{21}N_2O_4S$ [M^+ + H] 349.1222, obsd 349.1215.

2-(3-((Dimethylamino)methyl)-4-(4-fluoro-2-nitrophenylthio)phenoxy)ethanol (54) was prepared from **46** as a pale-yellow oil in 69% yield according to general procedure G. ^{1}H NMR ($CDCl_3$) δ 7.97 (dd, J = 8.5, 2.7 Hz, 1H), 7.48 (d, J = 8.5 Hz, 1H), 7.29 (d, J = 2.7 Hz, 1H), 7.11–7.02 (m, 1H), 6.91 (dd, J = 8.5, 2.7 Hz, 1H), 6.64 (m, 1H), 4.18 (t, J = 4.0 Hz, 2H), 4.00 (t, J = 4.0 Hz, 2H), 3.48 (s, 2H), 2.18 (s, 6H). HRMS calcd for $C_{17}H_{20}FN_2O_4S$ [M^+ + H] 367.1128, obsd 367.1121.

2-(4-(4-Chloro-2-nitrophenylthio)-3-((dimethylamino)methyl)phenoxy)ethanol (55) was prepared from **51** as a pale-yellow oil in 66% yield according to general procedure G. ^{1}H NMR ($CDCl_3$) δ 8.24 (d, J = 2.3 Hz, 1H), 7.47 (d, J = 8.5 Hz, 1H), 7.29 (d, J = 2.3 Hz, 1H), 7.23 (d, J = 2.3 Hz, 1H), 6.91 (dd, J = 8.5, 2.8 Hz, 1H), 6.59 (d, J = 8.8 Hz, 1H), 4.17 (m, 2H), 4.00 (m, 2H), 3.47 (s, 3H), 2.18 (s, 3H). HRMS calcd for $C_{17}H_{20}ClN_2O_4S$ [M^+ + H] 383.0832, obsd 383.0817.

2-(4-(4-Bromo-2-nitrophenylthio)-3-((dimethylamino)methyl)phenoxy)ethanol (56) was prepared from **52** as a pale-yellow oil in 71% yield according to general procedure G. ^{1}H NMR ($CDCl_3$) δ 8.38 (d, J = 2.2 Hz, 1H), 7.60 (d, J = 8.6 Hz, 1H), 7.38 (dd, J = 8.6, 2.2 Hz, 1H), 7.29 (d, J = 2.8 Hz, 1H), 6.91 (dd, J = 8.8, 2.8 Hz, 1H), 6.52 (d, J = 8.8 Hz, 1H), 4.17 (t, J = 4.3 Hz, 2H), 4.00 (t, J = 4.3 Hz, 2H), 3.47 (s, 2H), 2.23 (s, 6H).

3-(3-(Dimethylamino)methyl)-4-(2-nitrophenylthio)phenoxy)propanol (61) was prepared from **32** as a pale-yellow oil in 61% yield according to general procedure G. ^{1}H NMR ($CDCl_3$) δ 8.25 (dd, J = 8.0, 1.5 Hz, 1H), 7.48 (d, J = 8.5 Hz, 1H), 7.34–7.29 (m, 2H), 7.22–7.11 (m, 1H), 6.89 (dd, J = 8.5, 2.8 Hz, 1H), 6.66 (dd, J = 8.0, 1.2 Hz, 1H), 4.21 (t, J = 6.0 Hz, 2H), 3.89 (t, J = 5.9 Hz, 2H), 3.49 (s, 2H), 2.19 (s, 6H), 2.08 (quintet, J = 6.0 Hz, 2H). HRMS calcd for $C_{18}H_{23}N_2O_4S$ [M^+ + H] 363.1379, obsd 363.1406.

3-(3-(Dimethylamino)methyl)-4-(4-flouro-2-nitrophenylthio)phenoxy)propanol (62) was prepared from **33** as a pale-yellow oil in 63% yield according to general procedure G. ^{1}H NMR ($CDCl_3$) δ 7.97 (dd, J = 8.4, 2.7 Hz, 1H), 7.47 (d, J = 8.4 Hz, 1H), 7.27 (m, 1H), 7.12–7.02 (m, 1H), 6.89 (dd, J = 8.5, 2.8 Hz, 1H), 6.65 (dd, J = 9.0, 5.2 Hz, 1H), 4.21 (t, J = 6.0 Hz, 2H), 3.90 (t, J = 5.9 Hz, 2H), 3.47 (s, 2H), 2.18 (s, 6H), 2.08 (quintet, J = 5.9 Hz, 2H). HRMS calcd for $C_{18}H_{22}FN_2O_4S$ [M^+ + H] 381.1284, obsd 381.1262.

3-(4-(4-Bromo-2-nitrophenylthio)-3-((dimethylamino)methyl)phenoxy)propan-1-ol (63) was prepared from **34** as a pale-yellow oil in 73% yield according to general procedure G. ^{1}H NMR ($CDCl_3/CD_3OD$) δ 8.37 (d, J = 2.2 Hz, 1H), 7.50 (d, J = 8.5 Hz, 1H), 7.38 (dd, J = 8.5, 2.2 Hz, 2H), 7.28 (d, J = 3.0 Hz, 1H), 6.89 (dd, J = 8.8, 3.0 Hz, 1H), 6.52 (d, J = 8.8 Hz, 1H), 4.20 (t, J = 6.0 Hz, 2H), 3.86 (t, J = 6.0 Hz, 2H), 3.47 (s, 2H), 2.18 (s, 6H), 2.14–2.05 (m, 2H).

General Procedure H for Mesylation. To a solution of **32**–**33**, **53**–**56**, and **61**–**63** (0.11 mmol) in dichloromethane (5 mL) and triethylamine (45 mg, 0.44 mmol) was added methanesulfonyl chloride (30 mg, 0.26 mmol). The solution was stirred at room temperature for 2 h and washed with water (2 mL) and brine (2 mL). The organic layer was dried (Na_2SO_4), and the solvent was removed in vacuo. Purification by silica gel column (dichloromethane/methanol 19/1) gave the product as yellow oils.

3-(3-(Dimethylcarbamoyl)-4-(2-nitrophenylthio)phenoxy)propyl methanesulfonate (35) was prepared from **32** as a yellow oil in 88% yield according to general procedure H. ^{1}H NMR ($CDCl_3$) δ 8.18 (dd, J = 8.2, 1.4 Hz, 1H), 7.50 (d, J = 8.4 Hz, 1H), 7.41–7.32 (m, 1H), 7.23–7.15 (m, 1H), 7.02–6.93 (m, 3H), 4.46 (t, J = 6.0 Hz, 2H). 4.16 (t, J = 5.8 Hz, 2H), 3.04 (s, 3H), 3.02 (s, 3H), 2.86 (s, 3H), 2.28 (quintet, J = 5.9 Hz, 2H).

3-(3-(Dimethylcarbamoyl)-4-(4-fluoro-2-nitrophenylthio)phenoxy)propyl methanesulfonate (36) was prepared from **33** as a yellow oil in 85% yield according to general procedure H. ^{1}H NMR ($CDCl_3$) δ 7.90 (dd, J = 8.4, 2.7 Hz, 1H), 7.50 (d, J = 8.5 Hz, 1H), 7.18–7.09 (m, 1H), 7.01–6.90 (s, 3H), 4.47 (t, J = 6.0 Hz, 2H), 4.16 (t, J = 5.8 Hz, 2H), 3.03 (s, 3H), 3.02 (s, 3H), 2.86 (s, 3H), 2.27 (quintet, J = 6.0 Hz, 2H).

2-(3-((Dimethylamino)methyl)-4-(2-nitrophenylthio)phenoxy)ethyl methanesulfonate (57) was prepared from **53** as a yellow oil in 94% yield according to general procedure H. ^{1}H NMR ($CDCl_3$) δ 8.25 (dd, J = 8.0, 1.5 Hz, 1H), 7.50 (d, J = 8.5 Hz, 1H), 7.35–7.27 (m, 2H), 7.22–7.17 (m, 1H), 6.89 (dd, J = 8.5, 2.9 Hz, 1H), 6.65 (dd, J = 8.0, 1.3 Hz, 1H), 4.63–4.59 (m, 2H), 4.36–4.31 (m, 2H), 3.50 (s, 2H), 3.12 (s, 3H), 2.19 (s, 6H).

2-(3-((Dimethylamino)methyl)-4-(4-fluoro-2-nitrophenylthio)phenoxy)ethyl methanesulfonate (58) was prepared from **54** as a yellow oil in 93% yield according to general procedure H. ^{1}H NMR ($CDCl_3$) δ 7.97 (dd, J = 8.5, 2.7 Hz, 1H), 7.48 (d, J = 8.5 Hz, 1H), 7.29 (d, J = 2.7 Hz, 1H), 7.13–7.03 (m, 1H), 6.89 (dd, J = 8.5, 2.7 Hz, 1H), 6.61 (dd, J = 9.0 Hz, 5.2 Hz, 1H), 4.60 (t, J = 4.0 Hz, 2H), 4.33 (t, J = 4.0 Hz, 2H), 3.47 (s, 2H), 3.12 (s, 3H), 2.17 (s, 6H). HRMS calcd for $C_{18}H_{22}FN_2O_6S_2$ [M^+ + H] 445.0903, obsd 445.0920.

2-(4-(4-Chloro-2-nitrophenylthio)-3-((dimethylamino)methyl)phenoxy)ethyl methanesulfonate (59) was prepared from **55** as a yellow oil in 93% yield according to general procedure H. ^{1}H NMR ($CDCl_3$) δ 8.24 (d, J = 2.2 Hz, 1H), 7.48 (d, J = 8.5 Hz, 1H), 7.29 (d, J = 2.3 Hz, 1H), 7.23 (d, J = 2.3 Hz, 1H), 6.89 (dd, J = 8.5, 2.6 Hz, 1H), 6.58 (d, J = 8.7 Hz, 1H), 4.61 (m, 2H), 4.33 (m, 2H), 3.47 (s, 2H), 3.12 (s, 3H), 2.18 (s, 6H). HRMS calcd for $C_{18}H_{22}ClN_2O_6S_2$ [M^+ + H] 461.0608, obsd 461.0641.

2-(4-(4-Bromo-2-nitrophenylthio)-3-((dimethylamino)methyl)phenoxy))ethyl methanesulfonate (60) was prepared from **56** as a yellow oil in 70% yield according to general procedure H. ^{1}H NMR ($CDCl_3$) δ 8.38 (d, J = 2.2 Hz, 1H), 7.48 (d, J = 8.5 Hz, 1H), 7.38 (dd, J = 8.5, 2.2 Hz, 1H), 7.29 (d, J = 2.8 Hz, 1H), 6.89 (dd, J = 8.6, 2.8 Hz, 1H), 6.51 (d, J = 8.6 Hz, 1H), 4.60 (t, J = 4.5 Hz, 2H), 4.35 (t, J = 4.5 Hz, 2H), 3.48 (s, 2H), 3.11 (s, 3H), 2.18 (s, 6H).

3-(3-((Dimethylamino)methyl)-4-(2-nitrophenylthio)phenoxy) propyl methanesulfonate (64) was prepared from **61** as a yellow oil in 90% yield according to general procedure H. ^{1}H NMR ($CDCl_3$) δ 8.25 (dd, J = 8.0, 1.5 Hz, 1H), 7.48 (d, J = 8.5 Hz, 1H), 7.35–7.14 (m, 3H), 6.88 (dd, J = 8.5, 2.8 Hz, 1H), 6.66 (dd, J = 8.0, 1.1 Hz, 1H), 4.48 (t, J = 6.1 Hz, 2H), 4.18 (t, J = 5.8 Hz, 2H), 3.49 (s, 2H), 3.03 (s, 3H), 2.27 (quintet, J = 6.0 Hz, 2H), 2.19 (s, 6H).

3-(3-((Dimethylamino)methyl)-4-(4-fluoro-2-nitrophenylthio) phenoxy)propyl methanesulfonate (65) was prepared from **62** as a yellow oil in 87% yield according to general procedure H. ^{1}H NMR ($CDCl_3$) δ 7.97 (dd, J = 8.4, 2.7 Hz, 1H), 7.47 (d, J = 8.5 Hz, 1H), 7.27 (m, 1H), 7.12–7.03 (m, 1H), 6.88 (dd, J = 8.5, 2.7 Hz, 1H), 6.69–6.62 (m, 1H), 4.48 (t, J = 6.0 Hz, 2H), 4.18 (t, J = 5.8 Hz, 2H), 3.47 (s, 2H), 3.03 (s, 3H), 2.27 (quintet, J = 5.9 Hz, 2H), 2.18 (s, 6H). HRMS calcd for $C_{19}H_{24}FN_2O_6S_2$ [M^+ + H] 459.1080, obsd 459.1066.

3-(4-(4-Bromo-2-nitrophenylthio)-3-((dimethylamino)methyl)phenoxy)propyl methanesulfonate (66) was prepared from **63** as a yellow oil in 85% yield according to general procedure H. ^{1}H NMR ($CDCl_3/CD_3OD$) δ 8.37 (d, J = 2.2 Hz, 1H), 7.47 (d, J = 8.8 Hz, 1H), 7.39 (dd, J = 8.8, 2.2 Hz, 2H), 7.28 (br s, 1H), 6.88 (dd, J = 8.8, 2.8 Hz, 1H), 6.52 (d, J = 8.8 Hz, 1H), 4.47 (t, J = 6.0 Hz, 2H), 4.17 (t, J = 6.0 Hz, 2H), 3.56 (s, 2H), 3.00 (s, 3H), 2.32–2.18 (m, 2H), 2.10 (s, 6H).

General Procedure I for Fluorination Reaction. A mixture of mesylates (0.14 mmol), 1.0 M TBAF in THF (5 eqiuv, 0.7 mL), and THF (1.3 mL) was irradiated under microwave at 150 °C for 15 min. The solvents were evaporated in vacuo, diluted with 20 mL of dichloromethane, and washed with 2 N HCl. The organic layer was dried (Na_2SO_4), and the solvent was removed. The crude mixture was purified by silica gel chromatography (1/30 methanol/dichloromethane) to give products as yellow oils.

5-(3-Fluoropropoxy)-2-(2-nitrophenylthio)-*N*,*N*-dimethylbenzamide (37) was prepared from **35** as a pale-yellow oil in 71% yield according to general procedure I. ^{1}H NMR ($CDCl_3$) δ 8.18

(dd, $J = 8.2$, 1.4 Hz, 1H), 7.50 (d, $J = 8.4$ Hz, 1H), 7.40–7.32 (m, 1H), 7.25–7.10 (m, 1H), 7.02–6.93 (m, 3H), 4.66 (dt, $J = 47.0$, 5.7 Hz, 2H), 4.17 (t, $J = 6.0$ Hz, 2H), 3.02 (s, 3H), 2.86 (s, 3H), 2.21 (dt, $J = 26.2$, 5.9 Hz, 2H). HRMS calcd for $C_{18}H_{20}FN_2O_4S_2$ $[M^+ + H]$ 379.1128, obsd 379.1112.

2-(4-Fluoro-2-nitrophenylthio)-5-(3-fluoropropoxy)-*N,N*-dimthylbenzamide (38) was prepared from **36** as a pale-yellow oil in 67% yield according to general procedure I. ^{1}H NMR ($CDCl_3$) δ 7.90 (dd, $J = 8.3$, 2.6 Hz, 1H), 7.49 (d, $J = 8.5$ Hz, 1H), 7.18–7.08 (m, 1H), 7.02–6.90 (m, 3H), 4.66 (dt, $J = 47.0$, 5.7 Hz, 2H), 4.17 (t, $J = 6.0$ Hz, 2H), 3.03 (s, 3H), 2.86 (s, 3H), 2.21 (dt, $J = 26.2$, 5.9 Hz, 2H). HRMS calcd for $C_{18}H_{19}F_2N_2O_4S_2$ $[M^+ + H]$ 397.1034, obsd 397.1036.

2-(4-Bromo-2-nitrophenylthio)-5-(3-fluoropropoxy)-*N,N*-dimethylbenzamide (39) was prepared as follows. To a solution of **34** (15 mg, 0.033 mmol) in dichloromethane (1 mL), DAST (10 mg) was added at −78 °C. The mixture was slowly warmed to room temperature (1 h). The mixture was directly placed on a preparatory TLC plate (silica gel) for purification (dichloromethane/ethyl acetate, 1/1). The product was a colorless oil (12 mg, 80%). ^{1}H NMR ($CDCl_3$) δ 8.31 (d, $J = 2.2$ Hz, 1H), 7.48 (d, $J = 8.6$ Hz, 1H), 7.05–6.89 (m, 2H), 6.80 (d, $J = 8.6$ Hz, 1H), 4.65 (dt, $J = 47.0$, 5.7 Hz, 2H), 4,16 (t, $J = 6.0$ Hz, 2H), 3.10 (s, 3H), 2.85 (s, 3H), 2.33–2.03 (m, 2H).

II. Radiochemistry. General Procedure for F-18 Radiolabeling ([^{18}F]28–31, 40–42). [^{18}F]Fluoride was produced by a cyclotron using the $^{18}O(p,n)^{18}F$ reaction and passed through a Sep-Pak Light QMA cartridge as an aqueous solution in [^{18}O]-enriched water. The cartridge was dried by air flow, and the ^{18}F activity was eluted with 2 mL of Kryptofix 222 (K222)/K_2CO_3 solution (13.2 mg of K222 and 3.0 mg of K_2CO_3 in CH_3CN/H_2O, 1.12/0.18). The solvent was removed at 100 °C under an argon stream. The residue was azeotropically dried with 1 mL of anhydrous CH_3CN twice at 100 °C under an argon stream. A solution of mesylate precursor (**57**–**60**, **64**–**66**) (2−3 mg) in DMSO (0.3 mL) was added to the reaction vessel containing the dried ^{18}F activities. The mixture was heated at 100 °C for 3 min. Water (5 mL) was added, and the mixture was passed through a preconditioned Waters OASIS HLB (3 mL) cartridge. The cartridge was washed with water (10 mL), and labeled compound was eluted with ethanol (2 mL). To the ethanol solution, 0.5 mL of 2 N HCl and $SnCl_2$ (50 mg) was added, and the mixture was heated at 80 °C for 4−6 min. Water (5 mL) was added, and the mixture was passed through preconditioned Waters OASIS HLB (3 mL) cartridge. The cartridge was washed with water (20 mL), and labeled compound was eluted with CH_3CN (2 mL). The eluted compound was purified by HPLC [Phenonemex Gemini C-18 semipreparative column (10 mm × 250 mm, 5 μm), CH_3CN/ammonium formate buffer (50 mM), 6/4, flow rate of 4 mL/min, $t_R = 6-15$ min]. To determine radiochemical purity (RCP) and specific activity, analytical HPLC was used [Phenomenex Gemini C18 analytical column (4.6 mm × 250 mm, 5 μm), CH_3CN/ammonium formate buffer (10 mM), 8/2, flow rate of 1 mL/min, $t_R = 4.0-6.5$ min]. Specific activity was estimated by comparing the UV peak intensity of purified F-18 labeled compound with the reference nonradioactive compound of known concentration.

III. Pharmacology. In Vitro Binding Assays. Membrane homogenates containing one of the three monoamine transporters (referred to as LLC-DAT, LLC-NET, and LLC-SERT, which were expressed in a common parental cell line LLC-PK_1) were prepared and used for the binding assays.[51] Competitive binding assays were performed in a final volume of 0.5 mL. Aliquots of membrane suspensions (100 μL, corresponding to 30–80 μg of protein) were mixed with 50 mM Tris-HCl, pH 7.4, 120 mM NaCl, 5 mM KCl, 0.1% bovine serum albumin, radioligand (0.06 nM [^{125}I]*N*-(3′-iodopropen-2′-yl)-2β-carbomethoxy-3β-(4-chlorophenyl)tropane (DAT ligand),[52] 0.09 nM [^{125}I]5-iodo-2-[[2,2-[(dimethylamino)methyl]phenyl]thio]benzyl alcohol (SERT ligand),[53] or 0.06 nM [^{125}I]-iodonisoxetine (NET ligand)[49]), and 8–10 concentrations ($10^{-10}-10^{-5}$M) of competing drugs. Nonspecific binding was defined with 2.5 μM (+)McN5652 for SERT, 1.9 μM GBR-12909 for DAT, and 3.2 μM nisoxetine for NET assays. Incubation was carried out for 1 h 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 γ counter (Packard 5000, Downers Grove, IL). Results of competition experiments were subjected to nonlinear regression analysis using Microsoft Excel.

Biodistribution in Rats. Three rats per group were used for each biodistribution study. While under isoflurane anesthesia, 0.2 mL of sterile water with 1 mg/mL BSA and 10–100 μCi of radioactive tracer was injected into the femoral vein. The rats were sacrificed at the time indicated by cervical dislocation 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. 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 percentage dose/g of each region by that of the cerebellum [region/CB]. The cerebellum (CB) was used as a background region because it contains relatively few serotonin transporters. The standard deviation for the target to nontarget ratios was calculated by the following equation: $(\text{mean}_1/\text{mean}_2)[(SD_1/\text{mean}_1)^2 + (SD_2/\text{mean}_2)^2]^{1/2}$.

In vivo competitive binding in the regional uptake of [^{18}F]**42** was investigated by pretreating animals with competing drugs (2 mg/kg, each, iv at 5 min prior to injection of a tracer dose of [^{18}F]**42**). The competing drugs included GBR-12909, nisoxetine, and (+)McN5652. Regional brain distributions were determined at 120 min after [^{18}F]**42** injection as described above. The reduction of regional specific binding in the drug-pretreated rats was compared to that of control animals that had been pretreated with saline (data not shown).

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Supporting Information Available: ^{1}H spectra of some bioassay involved compounds, HPLC purity analysis data for all bioassay involved compounds in two different HPLC systems, radiochemistry data, and full sets of biodistribution data in normal mice for [^{18}F]**28** and [^{18}F]**42**. This material is available free of charge via the Internet at http://pubs.acs.org.

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