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Novel Azido-Iodo Photoaffinity Ligands for the Human Serotonin Transporter Based on the Selective Serotonin Reuptake Inhibitor (*S*)-Citalopram

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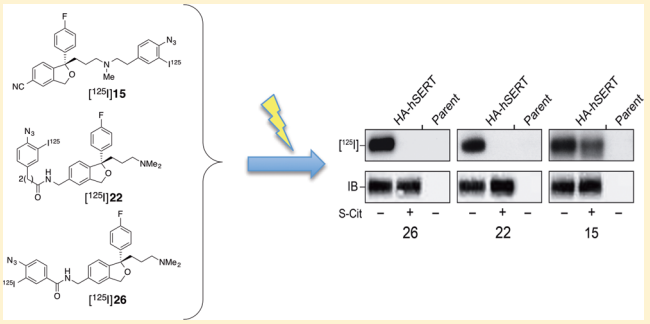
Supporting Information

ABSTRACT: Three photoaffinity ligands (PALs) for the human serotonin transporter (hSERT) were synthesized based on the selective serotonin reuptake inhibitor (SSRI), (*S*)-citalopram (**1**). The classic 4-azido-3-iodo-phenyl group was appended to either the C-1 or C-5 position of the parent molecule, with variable-length linkers, to generate ligands **15**, **22**, and **26**. These ligands retained high to moderate affinity binding (K_i = 24−227 nM) for hSERT, as assessed by [^{3}H]5-HT transport inhibition. When tested against Ser438Thr hSERT, all three PALs showed dramatic rightward shifts in inhibitory potency, with K_i values ranging from 3.8 to 9.9 μM, consistent with the role of Ser438 as a key residue for high-affinity binding of many SSRIs, including (*S*)-citalopram. Photoactivation studies demonstrated irreversible adduction to hSERT by all ligands, but the reduced (*S*)-citalopram inhibition of labeling by [^{125}I]**15** compared to that by [^{125}I]**22** and [^{125}I]**26** suggests differences in binding mode(s). These radioligands will be useful for characterizing the drug−protein binding interactions for (*S*)-citalopram at hSERT.



INTRODUCTION

The serotonin transporter (SERT) is a member of the solute carrier 6 (SLC6) family of transporters that functions to regulate serotonin neurotransmission and homeostasis.[1−3] In particular, selective serotonin reuptake inhibitors (SSRIs) and tricyclic antidepressants (TCAs), widely prescribed medications for treatment of anxiety and major depressive disorders, principally work by binding to SERT and inhibiting serotonin reuptake into presynaptic neurons.[4,5] Notably, (*S*)-citalopram (**1**, Escitalopram, Figure 1) binds with high affinity and selectivity to hSERT. However, despite its well-documented clinical success, the molecular interactions between (*S*)-citalopram and hSERT that determine its reuptake inhibition potency and selectivity over the norepinephrine transporter (NET) and dopamine transporter (DAT) remain poorly understood.

The 3D structure of SERT as well as its structure, function, and regulation, including oligomerization and distribution in cells or brain tissue, has been studied with small molecular probes containing electrophiles (e.g., N_3, NCS) and radiolabels (e.g., ^{3}H, ^{125}I). In particular, the well-known technique of photoaffinity labeling utilizes molecular probes that contain a functional group capable of forming a covalent bond to a biological target upon photoactivation.[6,7] Of the numerous photoreactive functional groups that can be employed in the design of photoaffinity ligands (PALs) (e.g., benzophenones, aliphatic and aromatic diazirines, etc.), aryl azides are frequently used given their relatively small size, ease of synthetic incorporation, and chemical stability.[8]

Numerous [^{125}I]-radiolabeled-arylazido analogues of dopamine transporter (DAT) inhibitors including cocaine,[9−12] GBR12909 (1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazine),[13−16] benztropine,[10,17,18] pyrovaler-

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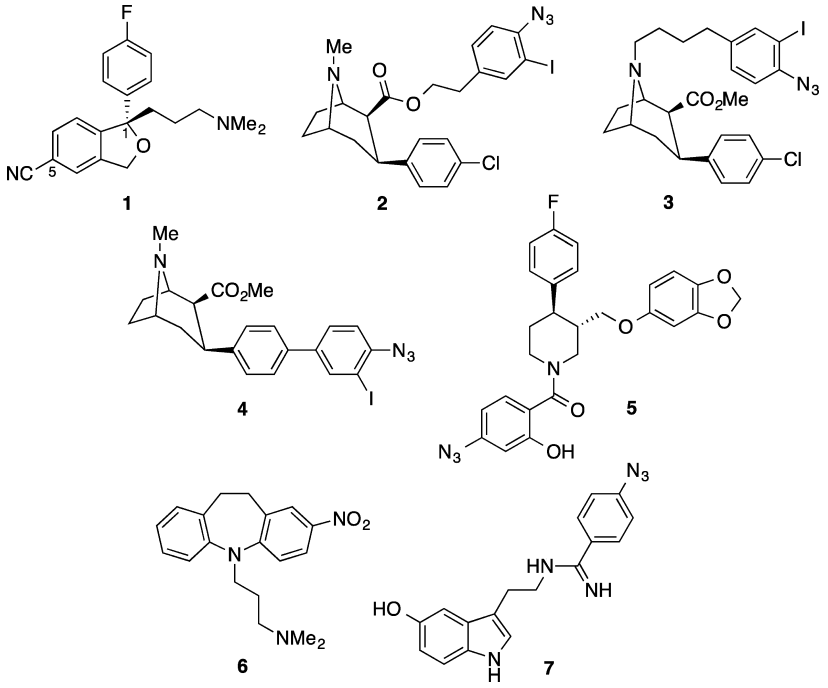
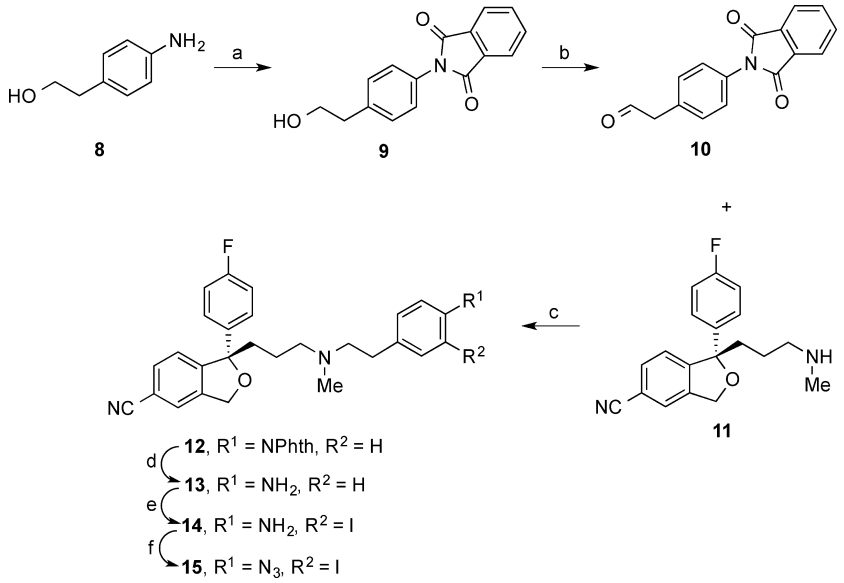


Figure 1. Chemical structures of (*S*)-citalopram (**1**) and known DAT and/or SERT inhibitor PALs (**2**–**7**).

Scheme 1. Synthesis of PAL 15[a]



[a]Reagents and conditions: (a) phthalic anhydride, pyridine, reflux, 16 h; (b) Dess–Martin periodinane, CH_2Cl_2, −78 °C to RT, 3 h; (c) $NaBH(OAc)_3$, HOAc, DCE, RT 18 h; (d) hydrazine, EtOH, reflux, 3 h; (e) ICl (1 M in CH_2Cl_2), 0–5 °C to RT; (f) $NaNO_2$, NaN_3, TFA, 0–5 °C to RT.

one,[19] bupropion,[20] and methylphenidate[21,22] have been developed to elucidate molecular components of the ligand-binding site and mechanisms underlying uptake inhibition. The 4′-azido, 3′-iodo-substituted phenyl ring is a common structural motif found in many PALs that bind DAT irreversibly upon photoactivation (e.g., RTI 82 (**2**), MFZ 2-24 (**3**), and JHC 2-48 (**4**); Figure 1).[9−12] Specifically, tropane-based PALs **2** and **3** have been instrumental in defining drug−protein interactions at the molecular level and thus further defining the structural components of DAT that are critical for transport inhibition by cocaine.[23−25]

In contrast to DAT PALs, the development of SERT-selective PALs has been more limited. To the best of our knowledge, only a few PALs have been synthesized and experimentally validated to label SERT (Figure 1). Although tropane-based PALs **2**–**4** bind to SERT as well as DAT, to date, only probes **3** and **4** have been used to photolabel hSERT.[12,26] Other inhibitor-based ligands that photolabel SERT include an aryl-azido derivative of the SSRI paroxetine (**5**)[27] and a tricyclic antidepressant-based PAL, [^{3}H]-2-nitroimipramine (**6**), that showed covalent incorporation into SERT present in membrane homogenates of rat brain and liver

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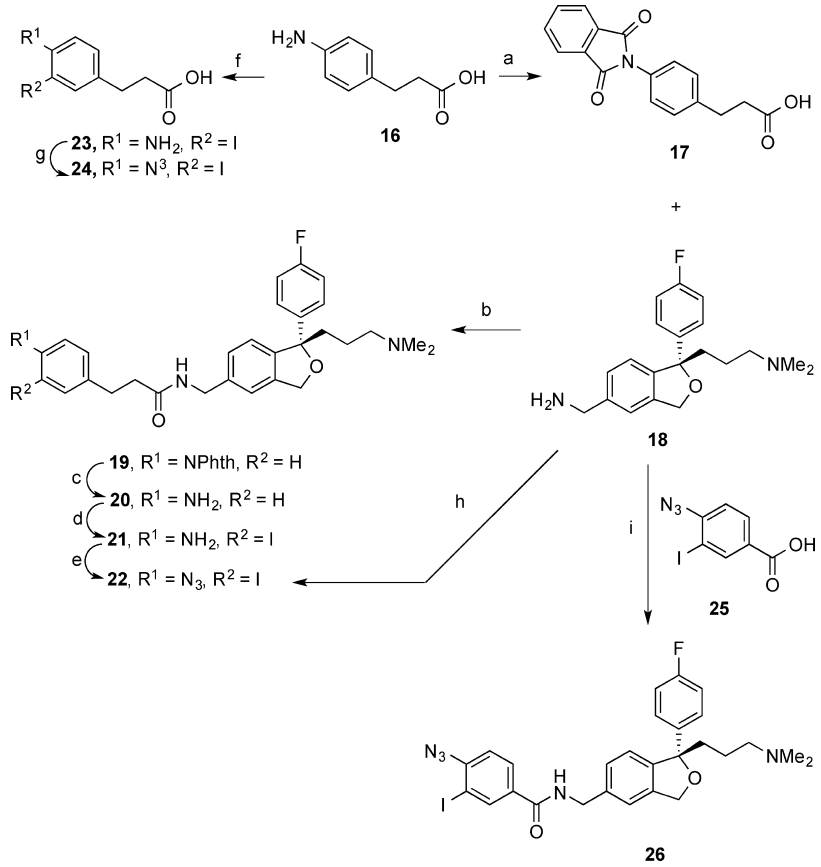
Scheme 2. Synthesis of PALs 22 and 26[a]



[a]Reagents and conditions: (a) phthalic anhydride, pyridine, reflux, 16 h; (b) $SOCl_2$, 3 h; (c) hydrazine, EtOH, reflux, 6 h; (d) ICl, CH_2Cl_2, 0−5 °C to RT; (e) $NaNO_2$, NaN_3, HOAc, H_2O; (f) ICl, HOAc, RT; (g) $NaNO_2$, NaN_3, TFA; (h) **24**, CDI, THF, 0 °C to RT; (i) EDC, HOBT, Et_3N, DMF, 0 °C to RT.

and human platelets.[28,29] Finally, photoinactivation of serotonin uptake has been demonstrated by an azidobenzamidine derivative of serotonin (**7**).[30] However, to date, none of these SERT PALs have been used to define the structural basis of their binding interactions at hSERT.

On the basis of successful application of PALs **2**−**4** in elucidating drug−binding site interactions at DAT,[23−25] our concept for the present study was that appending the 4′-azido, 3′-iodo aryl moiety onto different positions of the (*S*)-citalopram base might reveal different sites of adduction on hSERT that would enable a clearer understanding of the (*S*)-citalopram binding mechanism. Extensive structure−activity relationships have been described with analogues of citalopram that suggest positions C-1 and C-5 can accommodate significant steric bulk without appreciable loss in hSERT binding affinity.[31−34] Herein, we describe the design, synthesis, pharmacological evaluation, and radiosynthesis of three novel C-1- or C-5-substituted 4′-azido, 3′-iodo analogues of (*S*)-citalopram and demonstrate their ability to covalently label hSERT upon photoactivation.

CHEMISTRY

The synthesis of C-1-substituted (*S*)-citalopram PAL **15** is shown in Scheme 1. Phthalimide derivative **9**[35] was synthesized by stirring 2-(4-aminophenyl)ethan-1-ol (**8**) with phthalic anhydride in pyridine, at reflux, and oxidizing to aldehyde **10** using a Dess−Martin periodinane reagent.[36,37] (*S*)-*N*-Desmethylcitalopram (**11**)[31,32] was then coupled to aldehyde **10** using standard reductive amination conditions to provide tertiary amine **12** in 80% yield, followed by phthalimide deprotection to give aniline **13**. Iodination with iodine monochloride (ICl) gave the intermediate **14**, which was then treated with $NaNO_2$ followed by NaN_3 to give 4′-azido, 3′-iodo product **15**.

The synthesis of C-5-substituted (*S*)-citalopram PALs **22** and **26** is shown in Scheme 2. 3-(4-Aminophenyl)propanoic acid (**16**) was initially stirred at reflux with phthalic anhydride to afford the corresponding phthalimide carboxylic acid **17**.[38] Subsequent coupling of **17** to primary amine **18**[31,32] via the acid chloride provided amide **19** in 97% yield. Deprotection of **19** using hydrazine provided aniline intermediate **20**, which was reacted with ICl to give 3′-iodo-substituted product **21**. Conversion to azido PAL **22** was then performed as previously described for **15** via diazotization and azide displacement of aniline **21**. This final step went in poor yield (18%); thus, a second strategy was also developed that featured a decrease in the number of synthetic steps and an increase in the overall yield. This alternative strategy began with electrophilic iodination of aniline **16** *ortho* to the amine to provide the corresponding iodo-substituted aniline **23** in 67% yield, which was subsequently treated with $NaNO_2$ followed by NaN_3 in TFA to give iodo-azide **24**.[39] Primary amine **18** was then coupled to azido-iodo carboxylic acid **24** using 1,1′-carbonyldiimidazole (CDI) in THF to generate amide **22**. Although this route using **24** is preferred for generating nonradioactive **22** in high yield, the initial route that provided **20** was utilized

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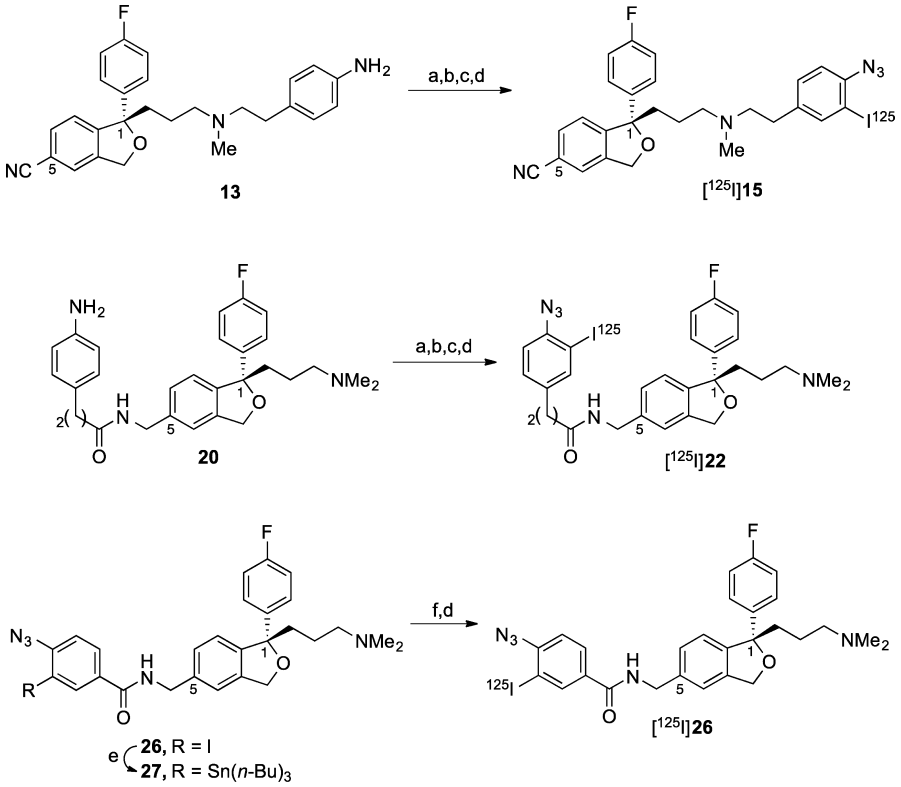
Scheme 3. Radiosynthesis of [^{125}I]15, [^{125}I]22, and [^{125}I]26[a]



[a]Reagents and conditions: (a) [^{125}I]NaI, chloramine-T, NaOAc (0.2 M, pH 4.0), RT, 30 min; (b) HOAc (3.0 M), $NaNO_2$ (0.5 M), −5 °C, 20 min; (c) NaN_3 (0.5 M), RT, 30 min; (d) $Na_2S_2O_5$ (50 mM), RT; (e) $Pd(PPh_3)_2Br_2$, $((n\text{-}Bu)_3Sn)_2$, toluene, 105 °C, 4 h; (f) [^{125}I]NaI, chloramine-T, MeOH, 3% HOAc, RT, 3 min.

in the synthesis of [^{125}I]-labeled **22**. Similarly, **18** was coupled to 4-azido-3-iodobenzoic acid[40] (**25**) using EDC and HOBT to provide PAL **26** in 67% yield.

Radioiodinated PALs [^{125}I]**15**, [^{125}I]**22**, and [^{125}I]**26** were prepared under no-carrier-added conditions as shown in Scheme 3. Electrophilic radioiodination of anilines **13** and **20** was accomplished by treatment with [^{125}I]NaI and chloramine-T at ambient temperature for 30 min. Acidification with HOAc followed by treatment with $NaNO_2$ at −5 °C generated the diazonium salts *in situ*. Decomposition upon warming, in the presence of NaN_3, gave [^{125}I]-labeled aryl azides [^{125}I]**15** and [^{125}I]**22**. This sequence was accomplished in one flask, as previously described for other radioiodinated PALs.[11,41,42] Electrophilic radioiododestannylation of tri-*n*-butylstannyl azide **27** using chloramine-T as the oxidant in methanolic acetic acid for 3 min was employed to prepare [^{125}I]**26**. The organostannane precursor was obtained in 52% yield by treating nonradioactive iodide **26** with bis(tri-*n*-butyltin) and bis(triphenylphosphine)palladium(II) dibromide. Since carry-over of trace amounts of **26** would dramatically lower the specific radioactivity of [^{125}I]**26** prepared from **27**,[43] this material was purified by reversed-phase HPLC prior to radiolabeling.

All three radioligands were purified by reversed-phase HPLC and then formulated as concentrated solutions in MeOH using solid-phase extraction. Isolated radiochemical yields were 46−55%. Specific radioactivities were determined as 2045 to 2098 mCi/μmol, near the theoretical value of 2175 mCi/μmol, using HPLC to determine the mass associated with the UV absorbance peak in samples of known radioactivity. Radiochemical purities were ≥98% by HPLC (Figure 2), and the materials were stable for at least 5 weeks when stored at −20 °C in the dark. The three radioligands proved to be lipophilic, with [^{125}I]**22** (t_R = 13.2 min, k' = 10.4) and [^{125}I]**26** (t_R 13.4 min, k' = 10.6) being substantially less hydrophobic than [^{125}I]**15** (t_R = 22.4 min, k' = 18.3). These findings are in accord with their relative cLogP values (**15**, 7.02; **22**, 6.39; **26**, 6.35; generated in ChemDraw).

RESULTS AND DISCUSSION

Pharmacological Properties of PALs 15, 22, and 26. Inhibition of [^{3}H]5-HT uptake by WT hSERT and the hSERT Ser438Thr mutant was evaluated to determine inhibitory constants for PALs **15**, **22**, **26** and parent compound (*S*)-citalopram (**1**). When tested against WT hSERT, C-5-substituted PALs **26** and **22** showed affinities of 24 and 38 nM, respectively, values that were reduced by 11- and 17-fold compared to that of (*S*)-citalopram (K_i = 2.2 nM) (Figure 3 and Table 1). In contrast, C-1 substituted PAL **15** showed a greater reduction in affinity (K_i = 227 nM, 100-fold) compared to that of (*S*)-citalopram, which is in accord with structure−activity relationships previously described.[32] When tested against the hSERT Ser438Thr mutant, all four compounds showed dramatic rightward shifts in inhibitory potency (Figure 3, dashed lines) with K_i values ranging from 3.8 to 9.9 μM (Table 1). These findings are consistent with previous reports that Ser438 is a key residue for high-affinity binding of many hSERT antagonists and that its mutation to Thr results in a significant loss in potency for (*S*)-citalopram and many other hSERT inhibitors.[44] Interestingly, C-1-substituted PAL **15**

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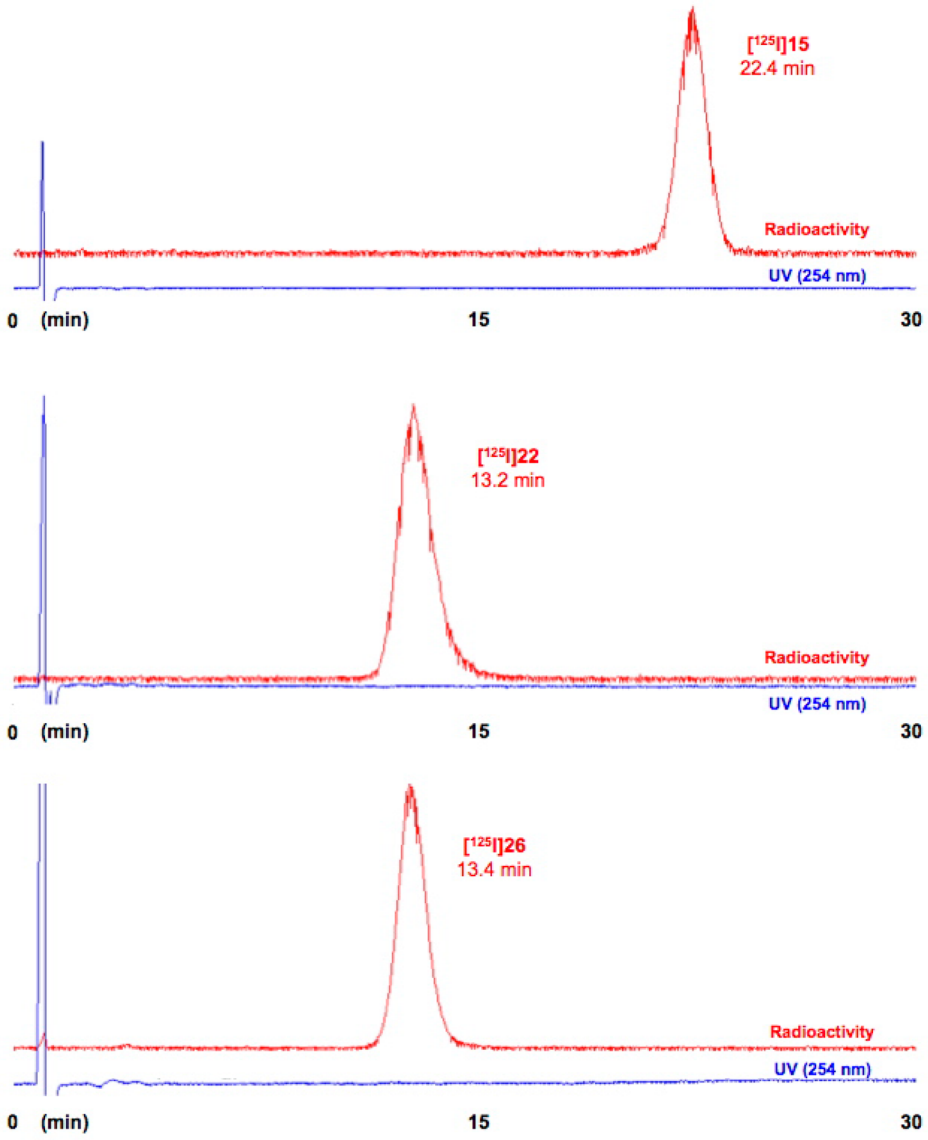

Figure 2. Reversed-phase HPLC chromatograms of isolated radioiodinated ligands illustrating purity and relative lipophilicity. Chromatography performed using 45% MeOH/CH_3CN (1:1, v/v) and 55% water containing Et_3N (2.1% v/v) and HOAc (2.8% v/v) at a flow rate of 3.0 mL/min on a C-18 column.

demonstrated lower affinity than (*S*)-citalopram or the other PALs for WT hSERT but higher affinity for the S438T mutant. Moreover, the difference in binding affinities between the mutant and WT hSERT for **15** was only 17-fold, as compared to a more dramatic shift for parent ligand **1** (~3600-fold).

Photoaffinity Labeling Experiments with hSERT. Next, we tested [^{125}I]**15**, [^{125}I]**22**, and [^{125}I]**26** for irreversible labeling of HA-hSERT (Figure 4). For these studies, HA-hSERT LLCPK$_1$ cells were incubated with the PALs in the presence or absence of (*S*)-citalopram and irradiated with UV light to cross-link the ligand to the protein. Lysates were immunoprecipitated with anti-HA or anti-hSERT antibodies, and samples were analyzed by SDS-PAGE and autoradiography. These methods (described in detail in the Experimental Methods) have been used by our laboratories to verify photolabeling of DAT and SERT by numerous structurally diverse tropane- and non-tropane-based ligands.[11,12,17,19−26] The results (Figure 4) show that when using HA-hSERT expressing cells, [^{125}I]**15**, [^{125}I]**22**, and [^{125}I]**26** all covalently label a protein of the expected hSERT molecular mass of ~100 kDa, whereas photolabeled bands were not obtained from nontransfected parent cells (upper panels), strongly supporting the identity of the photolabeled bands as hSERT. Immunoblotting (lower panels) verified the presence and absence of hSERT protein in transfected and untransfected cells, respectively. The photoaffinity labeled proteins were immunoprecipitated using anti-HA antibody (Figure 4) as well as with the anti-hSERT antibody ST51 (not shown), but they were not precipitated with nonimmune IgG (not shown), also supporting the identity of the bands as hSERT. Similar irreversible labeling and immunoprecipitation results were obtained using tropane PAL **3**, which was previously demonstrated to photolabel hSERT.[26] Together, these results demonstrate that hSERT protein is irreversibly labeled with [^{125}I]**15**, [^{125}I]**22**, and [^{125}I]**26** upon photoactivation, providing the first demonstration of photoaffinity labeling of hSERT by analogues of (*S*)-citalopram.

For pharmacological characterization of irreversible labeling, HA-hSERT-LLCPK$_1$ cells were incubated with vehicle or 10

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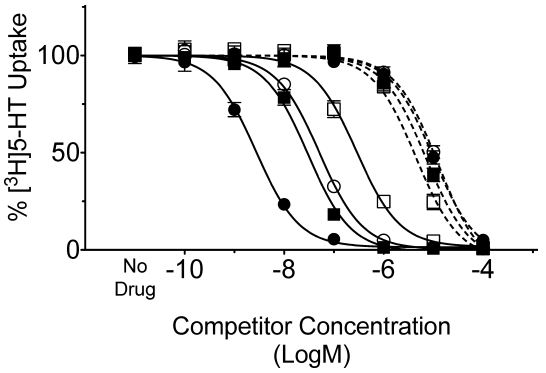

Figure 3. [^{3}H]5-HT uptake inhibition analysis for (*S*)-citalopram, **15**, **22**, and **26**. HEK-293 Griptite cells expressing hSERT (solid lines) or hSERT S438T (dashed lines) were assayed for [^{3}H]5-HT uptake in the presence of the indicated concentrations of (*S*)-citalopram (**1**) (●), **15** (□), **22** (○), or **26** (■). Data shown are the mean ± SEM of transport activity for each form in the absence of competitor, set to 100%. Assays were conducted in triplicate and were repeated at least four times.

Table 1. Inhibitory Constants of (*S*)-Citalopram (1), 15, 22, and 26 for [^{3}H]5-HT Transport by hSERT and hSERT Ser438Thr[a]

	hSERT (K_i, nM)	hSERT S438T (K_i, nM)
(*S*)-citalopram	2.2 ± 0.17	7879 ± 1300*
15	227 ± 23#	3811 ± 590*
22	38 ± 2.9#	9879 ± 1200*
26	24 ± 2.2#	5697 ± 745*

[a]Data are K_i values (nM) (mean ± SEM) for the ability of the (*S*)-citalopram-based PALs to inhibit uptake of [^{3}H]5-HT, as shown in Figure 3. K_i values were calculated using the Cheng−Prusoff equation in GraphPad Prism 5. Data were analyzed by paired *t*-tests for statistical significance, where * indicates that the K_i value obtained with the hSERT S438T mutant is significantly different than the K_i of that compound for WT hSERT ($p < 0.001$) and # indicates that the K_i value for the analogue is significantly different than the K_i for (*S*)-citalopram obtained with WT hSERT ($p < 0.001$). The K_i values obtained with hSERT S438T for the analogues and (*S*)-citalopram were not statistically different from one another.

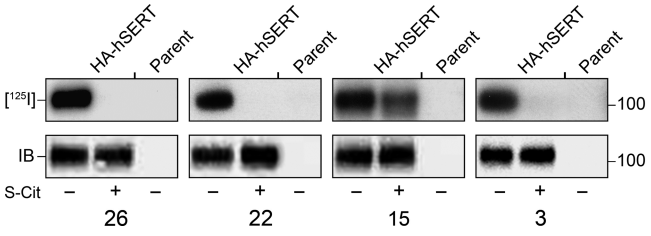

Figure 4. Photoaffinity labeling of hSERT. HA-hSERT LLCPK$_1$ cells or nontransfected (parent) LLCPK$_1$ cells were incubated with the indicated ^{125}I-labeled ligands (**26**, **22**, **15**, **3**) in the absence (−) or presence (+) of 10 μM (*S*)-citalopram (S-Cit) followed by cross-linking to SERT with UV light. Cell lysates were immunoprecipitated with anti-HA antibody followed by SDS-PAGE and autoradiography to detect [^{125}I] radiolabeled proteins (upper panels) or were analyzed by immunblotting (IB) with anti-HA antibody to detect total hSERT (lower panels). Results are representative of three independent experiments.

μM (*S*)-citalopram prior to addition of the radioiodinated PALs. Incorporation of [^{125}I]**22** and [^{125}I]**26** was blocked by >99%, demonstrating that (*S*)-citalopram fully displaces these C-5 substituted radioligands, similar to displacement of hSERT labeling by [^{125}I]**3**.[26] In contrast, 10 μM (*S*)-citalopram inhibited [^{125}I]**15** labeling of hSERT by only ∼30%, suggesting that this C-1-substituted PAL exhibits a different binding mode compared to that of the two C-5-substituted ligands. In a previous SAR study, wherein (*S*)-citalopram was modified by N-substitution at the C-5 position with a second molecule of (*S*)-citalopram to create a homobifunctional ligand, binding data suggested simultaneous interaction of the ligand with the S1 and S2 binding sites.[32] Moreover, the affinity of (*S*)-citalopram for S2 is much lower than that for S1.[45] Thus, it is conceivable that [^{125}I]**15** may be binding to both the S1 and S2 sites and that the latter might not be fully inhibited by 10 μM (*S*)-citalopram.[32,45]

In summary, three novel PALs, [^{125}I]**15**, [^{125}I]**22**, and [^{125}I]**26**, were designed by substituting the parent SSRI, (*S*)-citalopram, with a 4′-azido, 3′-iodophenyl moiety in either the C-1 or C-5 position, with variable-length linker chains. Our results showed that the nonradioiodinated compounds were able to inhibit [^{3}H]5-HT uptake with high to moderate potency and that the radioiodinated PALs displayed requisite characteristics as irreversible probes for molecular characterization of the hSERT (*S*)-citalopram binding site(s). Preincubation of hSERT with (*S*)-citalopram completely eliminated irreversible labeling by C-5-substituted [^{125}I]**22** and [^{125}I]**26**, but it reduced irreversible labeling by C-1-substituted [^{125}I]**15** by only ∼30%. The inability of (*S*)-citalopram to completely block [^{125}I]**15** hSERT photolabeling indicates that this PAL has complex hSERT binding properties compared to those of C-5-substituted PALs [^{125}I]**22** and [^{125}I]**26** that will require further investigation.

EXPERIMENTAL METHODS

General. Reaction conditions and yields were not optimized. Anhydrous solvents were purchased from Aldrich and were used without further purification, except for tetrahydrofuran, which was freshly distilled from sodium benzophenone ketyl. All other chemicals and reagents were purchased from Sigma-Aldrich Co. LLC, Combi-Blocks, TCI America, Acros Organics, and Alfa Aesar. Unless otherwise stated, final amine products were converted into oxalate salts, typically by treating the free base in $CHCl_3$ with a 1:1 molar ratio of oxalic acid in acetone. As described, some of the oxalate salts were recrystallized from hot methanol or a methanol−acetone solvent mixture. Flash chromatography was performed using silica gel (EMD Chemicals, Inc., 230−400 mesh, 60 Å). ^{1}H and ^{13}C NMR spectra were acquired using a Varian Mercury Plus 400 spectrometer at 400 and 100 MHz, respectively. Chemical shifts are reported in parts per million (ppm) and referenced according to deuterated solvent for ^{1}H spectra ($CDCl_3$, 7.26 ppm, or CD_3OD-d_4, 3.31 ppm) and ^{13}C spectra ($CDCl_3$, 77.2 ppm, or CD_3OD-d_4, 49.00 ppm). Infrared (IR) spectra were obtained (neat) on a PerkinElmer Spectrum Two FTIR spectrometer. Gas chromatography/mass spectrometry (GC/MS) data were acquired (where obtainable) using an Agilent Technologies (Santa Clara, CA) 6890N gas chromatograph equipped with an HP-5MS column (cross-linked 5% PH ME siloxane, 30 m × 0.25 mm i.d. × 0.25 μm film thickness) and a 5973 mass-selective ion detector in electron-impact mode. Ultrapure grade helium was used as the carrier gas at a flow rate of 1.2 mL/min. The injection port and transfer line temperatures were 250 and 280 °C, respectively, and the oven temperature gradient used was as follows: the initial temperature (100 °C) was held for 3 min, increased to 295 °C at 15 °C/min over 13 min, and finally maintained at 295 °C for 10 min. To acquire high-resolution mass spectrometry (HRMS) data, 1 μL of sample was mixed with 1 μL of matrix (saturated solution of 2,4,6-trihydroxyacetophenone/2,5-dihydroxybenzoic acid in 50:50 ethanol/water); then, 1 μL was deposited on a stainless steel plate and analyzed in

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J. Med. Chem. 2015, 58, 5609−5619

positive ion mode in a MALDI LTQ-XL Orbitrap (Thermo-Scientific, San Jose, CA) using a laser energy fixed at 6 μJ and a mass resolution of 60 000 at an m/z of 400 Th. Combustion analysis was performed by Atlantic Microlab, Inc. (Norcross, GA), and the results agree within ±0.4% of the calculated values, unless indicated otherwise (S.I.). Melting point determination was conducted using a Thomas-Hoover melting point apparatus; the melting points are uncorrected. On the basis of NMR, HRMS, HPLC, and combustion data, all final compounds are >95% pure.

(*S*)-1-(3-((4-(1,3-Dioxoisoindolin-2-yl)phenethyl)(methyl)-amino)propyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile (12). (*S*)-*N*-Desmethylcitalopram[31,32] (**11**, 0.164 g, 0.53 mmol) and 2-(4-(1,3-dioxoisoindolin-2-yl)phenyl)acetaldehyde[37] (**10**, 0.140 g, 0.53 mmol) were mixed in 1,2-dichloroethane (10 mL) and then treated with sodium triacetoxyborohydride (0.167 g, 0.79 mmol) and HOAc (0.2 mL). The mixture was then stirred at RT for 6 h under an inert atmosphere. The reaction mixture was quenched with 2 N aq NaOH (10 mL) and extracted with EtOAc (3 × 20 mL). The combined organic layers were then dried over Na_2SO_4 and concentrated in vacuo. Purification by flash column chromatography eluting with 2:8 acetone/$CHCl_3$ provided 0.235 g of tertiary amine **12** (80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (dd, J = 5.2, 3.2 Hz, 2H), 7.73 (dd, J = 5.2, 3.2 Hz, 2H), 7.54 (d, J = 7.6 Hz, 1H), 7.45−7.28 (m, 8H), 6.98−6.94 (m, 2H), 5.16 (d, J = 12.8 Hz, 1H), 5.11 (d, J = 12.4 Hz, 1H), 2.76−2.72 (m, 2H), 2.55−2.51 (m, 2H), 2.35 (t, J = 6.8 Hz, 2H), 2.19−2.06 (m, 5H), 1.48−1.30 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.2, 161.9 (d, J = 244.8 Hz), 149.4, 140.6, 140.2, 139.7, 139.6, 134.3, 131.8, 131.7, 129.5, 129.3, 126.7 (d, J = 7.6 Hz), 126.4, 125.1, 123.6, 122.8, 118.6, 115.2 (d, J = 21.2 Hz), 111.5, 91.0, 71.2, 58.9, 57.2, 41.9, 38.9, 33.2, 21.8.

(*S*)-1-(3-((4-Aminophenethyl)(methyl)amino)propyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile (13). Hydrazine (0.092 g, 2.88 mmol) was added to a solution of phthalimide **12** (0.538 g, 0.96 mmol) in EtOH (20 mL). The reaction mixture was subsequently stirred at reflux for 3 h, concentrated, and diluted with 30% aq K_2CO_3. The crude mixture was extracted (3 × 20 mL) with $CHCl_3$, concentrated, and purified by flash column chromatography using 2.5:7.5 acetone/$CHCl_3$ to provide 0.395 g of aniline **13** (96%). ^{1}H NMR of the oxalate salt (400 MHz, CD_3OD) δ 7.70−7.55 (m, 5H), 7.13−7.05 (m, 5H), 6.93 (d, J = 6.8 Hz, 1H), 5.24 (d, J = 13.2 Hz, 1H), 5.17 (d, J = 13.6 Hz, 1H), 3.23−3.21 (m, 4H), 2.92−2.88 (m, 2H), 2.81 (s, 3H), 2.35−2.20 (m, 2H), 1.71−1.62 (m, 2H). ^{13}C NMR of oxalate salt (100 MHz, CD_3OD) δ 164.6 (d, J = 243.3 Hz), 150.3, 141.7, 140.7, 133.24, 130.9, 128.2 (d, J = 8.3 Hz), 126.7, 124.3, 119.5, 116.4 (d, J = 24.3 Hz), 113.1, 92.0, 72.5, 58.2, 57.1, 40.5, 38.5, 30.6, 20.5. The oxalate salt was precipitated from acetone; mp 122−123 °C. Anal. ($C_{27}H_{28}FN_3O\cdot 2C_2H_2O_4\cdot H_2O$) C, H, N.

(*S*)-1-(3-((4-Amino-3-iodophenethyl)(methyl)amino)propyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile (14). A 1 M solution of ICl in CH_2Cl_2 (0.085 g, 0.64 mmol) was added dropwise to a solution of aniline **13** (0.230 g, 0.53 mmol) in CH_2Cl_2 at 0−5 °C. The reaction mixture was allowed to warm to RT and stirred overnight. The reaction mixture was then quenched with 10% aq sodium thiosulfate. The organic layer was collected, dried, concentrated, and purified by flash chromatography using 2:8 acetone/$CHCl_3$ to give 0.095 g of iodide **14** (32%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (d, J = 8.4 Hz, 1H), 7.49−7.41 (m, 5H), 7.04−6.99 (m, 2H), 6.92 (dd, J = 8.0, 2 Hz, 1H), 6.67 (d, J = 8.0 Hz, 1H), 5.20 (d, J = 13.6 Hz, 1H), 5.14 (d, J = 12.8 Hz, 1H), 2.75−2.69 (m, 6H), 2.40 (s, 3H), 2.28−2.15 (m, 2H), 1.61−1.48 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.2 (d, J = 245.6 Hz), 149.3, 145.5, 140.3, 139.3, 139.3, 138.9, 132.2, 129.9, 126.9 (d, J = 8.3 Hz), 125.4, 123.0, 118.8, 115.6 (d, J = 21.3 Hz), 114.9, 112.0, 91.1, 84.3, 71.5, 58.4, 56.6, 41.2, 38.8, 30.9, 20.8. The oxalate salt was precipitated from acetone, mp 60−61 °C. HRMS calcd for $C_{27}H_{27}FIN_3O$ [M + H^+], 556.1251; found, 556.1252.

(*S*)-1-(3-((4-Azido-3-iodophenethyl)(methyl)amino)propyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile (15). To a solution of aniline **14** (0.095 g, 0.17 mmol) in TFA (3 mL) was added $NaNO_2$ (0.023 g, 0.34 mmol) at 0 °C, in the dark. The reaction mixture was then stirred for 45 min at 0−5 °C. NaN_3 (0.111 g, 1.71 mmol) and anhydrous ether (3 mL) was then added to the reaction mixture, followed by stirring for 1 h at 0−5 °C, and the mixture was then diluted with H_2O and ether (10 mL each). The organic layer was separated, dried, concentrated, and purified by flash chromatography using 3:7 acetone/$CHCl_3$ to provide 0.086 g of azide **15** as an oil (87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (d, J = 7.2 Hz, 2H), 7.49 (s, 1H), 7.37 (dd, J = 8.4, 5.6 Hz, 2H), 7.33 (d, J = 8.4 Hz, 1H), 7.16 (d, J = 8.4 Hz, 1H), 7.02−6.98 (m, 3H), 5.18 (d, J = 12.8 Hz, 1H), 5.13 (d, J = 13.2 Hz, 1H), 2.67−2.63 (m, 2H), 2.53−2.49 (m, 2H), 2.39−2.35 (m, 2H), 2.20−2.02 (m, 5H), 1.51−1.26 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.3 (d, J = 245.7 Hz), 148.7, 141.2, 140.0, 138.6, 132.3, 130.2, 126.7 (d, J = 8.3 Hz), 125.5, 122.8, 118.9, 118.6, 115.8 (d, J = 21.2 Hz), 112.3, 90.7, 88.2, 71.4, 57.0, 56.0, 53.9, 39.8, 37.9, 29.4, 19.4. IR: azide, 2112 cm^{-1}; HRMS calcd for $C_{27}H_{25}FIN_5O$ [M + H^+], 582.1160; found, 582.1159. Anal. ($C_{27}H_{25}FIN_5O\cdot 2.5H_2O$): C, H, N.

***(S)*-*N*-((1-(3-(Dimethylamino)propyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-yl)methyl)-3-(4-(1,3-dioxoisoindolin-2-yl)phenyl)propanamide (19).** A mixture of carboxylic acid **17**[38] (0.257 g, 0.87 mmol) and thionyl chloride (2 mL) was stirred at reflux for 3 h and then concentrated in vacuo. The remaining thionyl chloride was removed by azeotropic distillation using benzene. The crude solid was dissolved in the $CHCl_3$ and added dropwise to a vigorously stirred mixture of amine **18**[31,32] (0.395 g, 0.73 mmol), $CHCl_3$ (10 mL), and 0.5 M aq NaOH (10 mL) at 0 °C, followed by stirring for 3 h at RT. The organic layer was separated, dried, and purified by flash chromatography using 6:94 MeOH/$CHCl_3$ eluent to provide 0.425 g of amide **19** as a white solid (97%), mp 114−115 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92−7.88 (m, 2H), 7.78−7.74 (m, 2H), 7.45−7.40 (m, 2H), 7.31−7.21 (m, 5H), 7.08−7.04 (m, 2H), 6.96−6.90 (m, 2H), 6.35 (d, J = 6.0 Hz, 1H), 5.10 (d, J = 12.4 Hz, 1H), 5.06 (d, J = 12.4 Hz, 1H), 4.35 (d, J = 5.6 Hz, 2H), 3.02−2.98 (m, 2H), 2.51−2.47 (m, 2H), 2.25−2.21 (m, 2H), 2.19−2.06 (m, 8H), 1.48−1.29 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.8, 167.3, 161.7 (d, J = 243.3 Hz), 143.4, 141.1, 141.0, 139.6, 138.2, 134.5, 131.7, 129.8, 129.2, 127.2, 126.8 (d, J = 7.6 Hz), 126.7, 123.7, 122.0, 120.6, 114.9 (d, J = 21.2 Hz), 90.7, 71.7, 59.5, 45.1, 43.3, 39.3, 39.0, 31.2, 22.1.

(*S*)-3-(4-Aminophenyl)-*N*-((1-(3-(dimethylamino)propyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-yl)methyl)-propanamide (20). Hydrazine (0.069 g, 2.15 mmol) was added to a solution of phthalimide **19** (0.425 g, 0.72 mmol) in EtOH (15 mL). The reaction mixture was stirred at reflux for 6 h, concentrated, and diluted with a 30% aq K_2CO_3 solution. The organic layer was extracted, dried, and purified by flash chromatography using 8:92 MeOH/$CHCl_3$ to provide 0.301 g of aniline **20** (88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46−7.42 (m, 2H), 7.20 (d, J = 7.6 Hz, 1H), 7.06 (d, J = 8.0 Hz, 1H), 7.00−6.94 (m, 5H), 6.58−6.54 (m, 2H), 5.62 (m, 1H), 5.12 (d, J = 12.4 Hz, 1H), 5.08 (d, J = 12.4 Hz, 1H), 4.37 (d, J = 5.6 Hz, 2H), 3.58 (bs, 2H), 2.88−2.85 (m, 2H), 2.47−2.43 (m, 2H), 2.26−2.06 (10H), 1.49−1.29 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.3, 161.9 (d, J = 244.1 Hz), 144.8, 143.5, 141.2, 141.2, 139.8, 138.2, 130.6, 129.4, 127.3, 126.9 (d, J = 8.3 Hz), 122.1, 120.7, 115.4, 115.1 (d, J = 21.2 Hz), 90.9, 71.9, 59.8, 50.9, 45.5, 43.4, 39.5, 39.0, 31.0, 22.4. The hygroscopic oxalate salt was precipitated from acetone. Anal. ($C_{29}H_{34}Cl_2FN_3O_2\cdot 2C_2H_2O_4\cdot 5H_2O$): C, H, N.

(*S*)-3-(4-Amino-3-iodophenyl)-*N*-((1-(3-(dimethylamino)-propyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-yl)-methyl)propanamide (21). A 1.0 M solution of ICl in CH_2Cl_2 (0.083 g, 0.63 mmol) was added dropwise to a solution of aniline **20** (0.231 g, 0.48 mmol) in CH_2Cl_2 (15 mL) at 0−5 °C over 30 min. The reaction mixture was allowed to warm to RT and stirred overnight. The reaction mixture was then diluted with 10% aq $Na_2S_2O_3$ (25 mL), stirred for 10 min, and extracted in CH_2Cl_2. After discarding the aqueous layer, the black residue was dissolved in MeOH and combined with the previous CH_2Cl_2 solution. The organic layer was concentrated and purified by flash chromatography using 7:93 MeOH/$CHCl_3$ to provide 0.292 g of iodide **21** as an oil (62%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56−7.51 (m, 2H), 7.46 (d, J = 1.6 Hz,

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J. Med. Chem. 2015, 58, 5609−5619

1H), 7.32 (d, J = 7.6 Hz, 1H), 7.07−6.95 (m, 5H), 6.70 (d, J = 8.4 Hz, 1H), 5.14 (d, J = 12.8 Hz, 1H), 5.09 (d, J = 13.2 Hz, 1H), 4.30 (s, 2H), 2.96−2.92 (m, 2H), 2.79−2.75 (m, 2H), 2.64 (s, 6H), 2.48−2.44 (m, 2H), 2.29−2.13 (m, 2H), 1.66−1.51 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.0, 163.3 (d, J = 242.6 Hz), 147.6, 143.8, 142.2, 142.2, 140.6, 140.2, 139.7, 133.0, 130.7, 128.1 (d, J = 7.6 Hz), 128.0, 123.0, 121.1, 116.0 (d, J = 21.2 Hz), 115.9, 91.7, 84.4, 73.0, 59.4, 43.9, 43.7, 39.1, 38.8, 31.3, 21.6. HRMS calcd for $C_{29}H_{33}FIN_3O_2$ [M + H^+], 602.1666; found, 602.1667.

(*S*)-3-(4-Azido-3-iodophenyl)-*N*-((1-(3-(dimethylamino)-propyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-yl)-methyl)propanamide (22). *Method A.* $NaNO_2$ (9.6 mg, 0.14 mmol) was added to a suspension of aniline **21** (60 mg, 0.10 mmol) in a mixture of glacial HOAc (3 mL) and H_2O (1 mL) at 0 °C, in the dark. The mixture was stirred for 30 min; then, NaN_3 (9.7 mg, 0.15 mmol) was added to the reaction mixture. After stirring at 0−5 °C for 30 min, the reaction was diluted with water (10 mL). The mixture was then carefully quenched with saturated aq $NaHCO_3$ to a pH of 9−10. The crude product was extracted with $CHCl_3$, dried over Na_2SO_4, concentrated, and purified by preparative TLC using 15:85 MeOH/$CHCl_3$ to provide 20 mg of azide **22** as a yellow oil (18%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, J = 2.0 Hz, 1H), 7.46−7.41 (m, 2H), 7.23 (d, J = 6.8 Hz, 2H), 7.09 (d, J = 8.0 Hz, 1H), 7.04−6.95 (m, 4H), 6.02 (bt, J = 5.0 Hz, 1H), 5.13 (d, J = 12.4 Hz, 1H), 5.07 (d, J = 12.4 Hz, 1H), 4.37 (d, J = 5.6 Hz, 2H), 2.91 (t, J = 7.2 Hz, 2H), 2.64−2.57 (m, 2H), 2.48 (t, J = 7.2 Hz, 2H), 2.39 (s, 6H), 2.19 (m, 2H),1.66−1.47 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6, 161.9 (d, J = 243.4 Hz), 143.4, 141.1, 139.9, 139.8, 139.6, 138.2, 130.0, 127.6, 126.8 (d, J = 10.6 Hz), 122.1, 120.9, 118.4, 115.1 (d, J = 21.2 Hz), 115.0, 90.9, 87.9, 71.9, 59.5, 45.1, 43.5, 39.4, 37.9, 30.5, 22.3. IR: azide, 2114 cm^{-1}; HRMS calcd for $C_{29}H_{31}FIN_5O_2$ [M + H^+], 628.1579; found, 628.1569. Anal. ($C_{29}H_{31}FIN_5O_2\cdot 2H_2O\cdot 0.5C_3H_6O$): C, H, N.

Method B. CDI (0.064 g, 0.39 mmol) was added to a solution of carboxylic acid **24**[39] (0.125 g, 0.39 mmol) in THF (15 mL) under an argon atmosphere. The reaction mixture was stirred for 6 h and then cooled to 0−5 °C using an ice bath. Primary amine **18**[31,32] (0.129 g, 0.39 mmol) was then added dropwise after dilution with THF (10 mL). The reaction mixture was allowed to warm to RT and stirred overnight, followed by subsequent concentration, dilution with water (20 mL), and extraction with $CHCl_3$ (2 × 20 mL). The organic layer was dried, concentrated, and purified by flash chromatography using 3:97 MeOH/$CHCl_3$ as eluent to provide 0.054 g of amide **22** as a yellow oil (22%).

3-(4-Amino-3-iodophenyl)propanoic Acid (23).[39] A 1 M solution of ICl in HOAc (0.240 g, 1.82 mmol) was added dropwise slowly to a solution of 3-(4-aminophenyl)propanoic acid (**16**, 0.250 g, 1.51 mmol) in glacial HOAc (8 mL) at RT. The reaction mixture was stirred overnight and then quenched with 10% aq Na_2SO_3. The crude product was extracted with CH_2Cl_2 (2 × 20 mL), dried, concentrated, and purified by flash chromatography using 3:97 MeOH/$CHCl_3$ to provide 0.296 g of acid **23** as a pure white solid (67%).

3-(4-Azido-3-iodophenyl)propanoic Acid (24).[39] Azide **24** was prepared using the same method as described for azide **22** (Method A) in TFA. The crude product was purified by flash chromatography using 2:98 MeOH/$CHCl_3$ and used directly in the next step, Method B, to synthesize **22**.

(*S*)-4-Azido-*N*-((1-(3-(dimethylamino)propyl)-1-(4-fluoro-phenyl)-1,3-dihydroisobenzofuran-5-yl)methyl)-3-iodobenza-mide (26). To a solution of 4-azido-3-iodobenzoic acid[40] (**25**, 87 mg, 0.30 mmol) in DMF (2 mL) at 0 °C were added EDC (49 mg, 0.31 mmol) and HOBT (43 mg, 0.31 mmol). The resulting mixture was stirred in the dark for 1 h at 0 °C. To this mixture were added a solution of **18**[31,32] (99 mg, 0.30 mmol) in DMF (1 mL) and Et_3N (0.2 mL), followed by stirring in the dark at RT overnight. The mixture was then diluted with H_2O and extracted with EtOAc. The combined organic extracts were washed with 1 M aq NaOH and brine, dried ($MgSO_4$), filtered, concentrated, and chromatographed using 95:5 EtOAc/Et_3N to provide 120 mg of amide **26** as a colorless semisolid (67%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (d, J = 1.9 Hz, 1H), 7.81 (dd, J = 8.3, 1.9 Hz, 1H), 7.50−7.40 (m, 2H), 7.30−7.20 (m, 2H), 7.16 (s, 1H), 7.10 (d, J = 8.3 Hz, 1H), 6.97 (t, J = 8.7 Hz, 2H), 6.75 (t, J = 5.5 Hz, 1H), 5.11 (dd, J = 17.1, 12.6 Hz, 2H), 4.56 (d, J = 5.7 Hz, 2H), 2.3−2.0 (m, 10H), 1.5−1.2 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.9, 162.9, 144.9, 143.8, 140.9, 139.8, 138.7, 137.6, 131.9, 128.5, 127.4, 126.7, 126.6, 122.1, 120.8, 117.9, 115.1, 114.8, 90.8, 87.5, 71.7, 59.6, 45.4, 43.9, 39.3, 22.3. HRMS calcd for $C_{27}H_{26}FIN_5O_2$ [M + H^+], 600.1266; found, 600.1268. IR: azide, 2117 cm^{-1}. The oxalate salt was precipitated from acetone; mp 83−84 °C.

(*S*)-4-Azido-*N*-((1-(3-(dimethylamino)propyl)-1-(4-fluoro-phenyl)-1,3-dihydroisobenzofuran-5-yl)methyl)-3-(tri-*n*-butylstannyl)benzamide (27). A mixture of iodide **26** (170 mg, 0.28 mmol), $Pd(PPh_3)_2Br_2$ (13.5 mg, 0.02 mmol), and bis(tri-*n*-butyltin) (0.26 mL, 0.51 mmol) in toluene (10 mL) was heated at 105 °C for 4 h. The mixture was then cooled to room temperature, diluted with saturated aq K_2CO_3, and then extracted with EtOAc. The organic layers were then washed with brine, dried ($MgSO_4$), filtered, concentrated, and chromatographed (EtOAc/Et_3N, 95:5) to give 110 mg of the requisite organostannane **27** as a brown oil (52%). Final purification was achieved by reversed-phase HPLC of 3−5 mg portions on a Waters C-18 Nova-Pak column (radial compression module, 8 × 100 mm, 6 μm) using MeOH (42.5%), CH_3CN (42.5%), and an aqueous solution (15%) of Et_3N (2.1% v/v) and HOAc (2.8% v/v) at a flow rate of 3 mL/min with UV detection (254 nm). Material with retention time (t_R) 8.5 min was collected, water was added to lower the organic component to 9% of the total volume, and the mixture was passed through an activated (MeOH/water) solid-phase extraction cartridge (Waters Sep-Pak Plus *t*-C-18) that was flushed with water (5 mL) and then with air. Elution with MeOH (6 mL) and concentration under a stream of argon at 60 °C provided **27** for direct use in the radioiodination reaction. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.83 (d, J = 2.1 Hz, 1H), 7.74 (dd, J = 8.3, 2.1 Hz, 1H), 7.50−7.40 (m, 2H), 7.25 (s, 2H), 7.19 (s, 1H), 7.13 (d, J = 8.3 Hz, 1H), 6.97 (t, J = 8.7, 2H), 6.42 (t, J = 5.6 Hz, 1H), 5.12 (dd, J = 17.4, 12.5 Hz, 2H), 4.62 (d, J = 5.7 Hz, 2H), 2.30−2.0 (m, 10H), 1.60−1.40 (m, 7H), 1.4−1.2 (m, 7H), 1.2−1.0 (m, 6H), 0.9−0.8 (m, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.9, 162.9, 160.5, 149.2, 143.6, 140.9, 139.7, 137.9, 136.3, 135.1, 130.0, 128.5, 127.2, 126.7, 126.6, 121.9, 120.6, 116.5, 114.9, 114.7, 90.7, 71.6, 59.6, 45.3, 43.7, 39.3, 28.9, 27.2, 22.3,13.6, 10.1. IR: azide, 2109 cm^{-1}; HRMS calcd for $C_{39}H_{54}FN_5O_2SnH^+$, 764.3356; found, 764.3369.

Radiochemistry. General. No-carrier-added [^{125}I]NaI was obtained from PerkinElmer, Inc. (Waltham, MA). Radioactivity was measured with a dose calibrator (Capintec CRC-15W; Ramsey, NJ) employing similar counting geometries and attenuation correction factors as necessary. The radio-HPLC system was equipped with flow-through UV absorbance (254 nm) and radioactivity detectors. A Waters Corp. (Milford, MA) C-18 Nova-Pak HPLC column (8 × 100 mm, 6 μm; Radial Compression Module) was used for preparative separations and for analytical work. HPLC mobile phases were composed of varying proportions of an organic component (MeOH/CH_3CN; 1:1, v/v) and an aqueous component containing Et_3N (2.1% v/v) and HOAc (2.8% v/v). Activated Sep-Pak Light *t*-C-18 cartridges (Waters Corp.) were used for solid-phase extraction. Specific radioactivities were calculated by determining the carrier mass associated with the absorbance peak for carrier in purified samples of known radioactivity. The carrier mass in the radioactive samples was assessed by HPLC in reference to linear (r^2 = 1.0) six-point standard curves (0−3000 or 0−6000 pmol) generated using the corresponding nonradioactive materials.

(*S*)-1-(3-((4-Azido-3-[^{125}I]-iodophenethyl)(methyl)amino)-propyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-car-bonitrile ([^{125}I]15). A solution of aniline **13** as the oxalate salt (75 μL, 4.0 mM) in NaOAc buffer (pH 4.0; 0.2 M) was treated with [^{125}I]NaI (2.42 mCi) in NaOH (20 μL, 10 μM) followed by *N*-chloro-4-toluenesulfonamide (chloramine-T) trihydrate (20 μL, 5.0 mM). After 30 min at room temperature, the mixture was chilled in an ice/MeOH bath and treated sequentially with ice-cold HOAc (75 μL, 3.0 M) and $NaNO_2$ (30 μL, 0.5 M). After 20 min in the ice bath, NaN_3 (30 μL, 0.5 M) was added, and the mixture was allowed to warm to room temperature. After 30 min, the reaction was quenched with $Na_2S_2O_5$

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J. Med. Chem. 2015, 58, 5609−5619

(10 μL, 50 mM). Purification was achieved by reverse-phase HPLC (45% organic/55% aqueous; 3.0 mL/min). Radioactive material with retention time (t_R) 22.4 min and capacity factor (k') 18.3 corresponded to the chromatographic profile established for nonradioactive **15** and was collected in a 7.5 mL volume. This sample was diluted to 32 mL with H_2O and then passed through an activated (MeOH/H_2O) solid-phase extraction cartridge that was flushed with H_2O (2.0 mL) and then with air. Elution with MeOH (1.5 mL) gave [^{125}I]**15** (1.20 mCi) in 50% radiochemical yield. A ≥98% radiochemical purity was determined by HPLC, the radioactive material coeluted with nonradioactive **15**, and the specific radioactivity was calculated as 2045 mCi/μmol. The major nonradioactive products observed during HPLC purification are likely to be the aryl azide (t_R = 10.2 min, k' = 7.8) and chlorinated aryl azide (t_R = 16.6 min, k' = 13.3) analogues.

(*S*)-3-(4-Azido-3-[^{125}I]-iodophenyl)-*N*-((1-(3-(dimethylamino)-propyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-yl)-methyl)propanamide ([^{125}I]22). A solution of free-base **20** (75 μL, 4.0 mM) in NaOAc buffer (pH 4.0; 0.2 M) was treated with [^{125}I]NaI (2.14 mCi) in NaOH (20 μL, 10 μM) and chloramine-T trihydrate (15 μL, 5.0 mM) for 30 min. Further treatments with HOAc/$NaNO_2$, NaN_3, and $Na_2S_2O_5$ were performed as described above for [^{125}I]**15**. Purification was accomplished by HPLC (42% organic/58% aqueous; 3.0 mL/min). Radioactive material (t_R = 20.0 min, k' = 16.2) corresponding to **22** was collected in a 12.5 mL volume, diluted to 50 mL with H_2O, and processed by solid-phase extraction as described above to give pure [^{125}I]**22** (1.17 mCi) in 55% radiochemical yield. This material coeluted with nonradioactive **22** on HPLC, and a specific radioactivity of 2098 mCi/μmol was calculated. Nonradioactive products observed during HPLC purification are likely to be the aryl azide (t_R = 9.6 min, k' = 7.3) and chlorinated aryl azide (t_R = 15.1 min, k' = 12.0) analogues.

(*S*)-4-Azido-*N*-((1-(3-(dimethylamino)propyl)-1-(4-fluoro-phenyl)-1,3-dihydroisobenzofuran-5-yl)methyl)-3-[^{125}I]-iodo-benzamide ([^{125}I]26). A solution of organostannane **27** (0.11 mg, 0.15 μmol) in MeOH (25 μL) was treated with [^{125}I]NaI (2.07 mCi) in dilute NaOH (20 μL, 10 μM) followed by a mixture of aqueous chloramine-T trihydrate (15 μL, 5.0 mM) and 3% glacial HOAc in MeOH (85 μL). After 3 min at room temperature, the reaction was quenched with $Na_2S_2O_5$ (10 μL, 50 mM). Purification was accomplished by HPLC (45% organic/55% aqueous; 3.0 mL/min). The major radioactive product (t_R 13.4 min, k' = 10.6) corresponded to **26** and was resolved from minor nonradioactive and radioactive side products. This material was collected in 7.5 mL of mobile phase, diluted to 35 mL with water, and processed by solid-phase extraction as described above for [^{125}I]**22** to give 0.96 mCi (46%) of pure [^{125}I]**26** that coeluted with nonradioactive **26** on HPLC and had a specific radioactivity of 2096 mCi/μmol.

Whole-Cell Competition Uptake Assay. HEK-293 GripTite cells (Invitrogen) were plated in 24-well CulturPlates (PerkinElmer) at 50 000 cells/well and transiently transfected with wild-type hSERT or hSERT S438T-containing pcDNA3-based plasmid constructs using the Trans-IT LTI transfection system (Mirus Bio). Assays were conducted 36−48 h after transfection, where cells were washed with MKRHG buffer (5 mM Tris, 7.5 mM HEPES, 120 mM NaCl, 5.4 mM KCl, 1.2 mM $CaCl_2$, 1.2 mM $MgSO_4$, 10 mM dextrose, pH 7.4) and preincubated with the competitor compounds for 5 min followed by addition of [^{3}H]5-HT at a final concentration of 50 nM. Uptake was allowed to proceed for 10 min and then terminated by washing twice with ice-cold MKRHG. [^{3}H]5-HT uptake was quantified by dissolving cells in Microscint-20 (PerkinElmer) and using the Packard TopCount NXT (PerkinElmer) to measure radioactivity (CPM). Data were normalized to percent activity in the absence of a drug competitor for each form. Assays were carried out in triplicate and were repeated at least four times. K_i values were determined using Prism 5 (GraphPad).

hSERT Photoaffinity Labeling. Photoaffinity labeling of hSERT was performed using methods that have been verified previously for assessing irreversible adduction of a variety of ligands for both DAT and SERT.[11,12,17,19−26] These procedures were performed using Lewis lung carcinoma porcine kidney ($LLCPK_1$) cells stably expressing hemagluttinin (HA)-hSERT (generous gift of Dr. James Foster) and using untransfected parent $LLCPK_1$ cells for negative controls. Cells were grown to 90% confluence in 12-well plates and washed with KRH buffer. [^{125}I]**15**, [^{125}I]**22**, or [^{125}I]**26** was added to a final concentration of 10 nM and incubated for 1.5 h at 4 °C. In some experiments, hSERTs were irreversibly labeled in parallel with 10 nM [^{125}I]**3** as a positive control.[26] For pharmacological displacement, 10 μM (*S*)-citalopram was added 30 min prior to addition of the radioligands. Cells were irradiated with shortwave ultraviolet light (254 nm, Fotodyne UV Lamp model 3-6000) for 5 min at a distance of 15−20 mm to photoactivate the radioligand, washed twice with 1 mL of ice-cold KRH buffer, and lysed by addition of 0.5 mL of RIPA buffer (50 mM NaF, 2 mM EDTA, 125 mM Na_3PO_4, 1.25% Triton X-100, and 1.25% sodium deoxycholate) containing protease inhibitors for 30 min on ice. Lysates were centrifuged at 20 000*g* for 15 min at 4 °C to remove insoluble material and subjected to immunoprecipitation and immunoblotting using anti-HA monoclonal antibody (Covance) or anti-SERT antibody ST51 (mAB Technologies) as previously described.[26] Immunoprecipitated samples were separated on 4−20% SDS-polyacrylamide gels followed by autoradiography using Hyperfilm MP film (GE Healthcare) for 1−3 days at −80 °C. For immunoblotting, cell lysates were separated on 4−20% SDS-polyacrylamide gels transferred to 0.45 μm poly(vinylidene difluoride) membranes and probed for hSERT with anti-HA antibody.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Elemental analysis results, additional HPLC chromatograms, and standard curves. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.5b00682.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

PAL, photoaffinity ligand; hSERT, human serotonin transporter; SSRI, selective serotonin reuptake inhibitors; TCA, tricyclic antidepressant; DAT, dopamine transporter; NET, norepinephrine transporter; LLCPK1 cells, Lewis lung carcinoma porcine kidney cells; HA, hemagluttinin; CDI, 1,1′-carbonyldiimidazole

■ REFERENCES

(1) Pramod, A. B.; Foster, J.; Carvelli, L.; Henry, L. K. SLC6 transporters: structure, function, regulation, disease association and therapeutics. *Mol. Aspects Med.* **2013**, *34*, 197−219.

DOI: 10.1021/acs.jmedchem.5b00682
J. Med. Chem. 2015, 58, 5609−5619

(2) Rudnick, G.; Krämer, R.; Blakely, R. D.; Murphy, D. L.; Verrey, F. The SLC6 transporters: perspectives on structure, functions, regulation, and models for transporter dysfunction. *Pfluegers Arch.* **2014**, *466*, 25−42.
(3) Kristensen, A. S.; Andersen, J.; Jørgensen, T. N.; Sørensen, L.; Eriksen, J.; Loland, C. J.; Strømgaard, K.; Gether, U. SLC6 neurotransmitter transporters: structure, function, and regulation. *Pharmacol. Rev.* **2011**, *63*, 585−640.
(4) Pastoor, D.; Gobburu, J. Clinical pharmacology review of escitalopram for the treatment of depression. *Expert Opin. Drug Metab. Toxicol.* **2014**, *10*, 121−128.
(5) Gillman, P. K. Tricyclic antidepressant pharmacology and therapeutic drug interactions updated. *Br. J. Pharmacol.* **2007**, *151*, 737−748.
(6) Smith, E.; Collins, I. Photoaffinity labeling in target- and binding-site identification. *Future Med. Chem.* **2015**, *7*, 159−183.
(7) Chuang, V. T.; Otagiri, M. Photoaffinity labeling of plasma proteins. *Molecules* **2013**, *18*, 13831−13859.
(8) Lochner, M. Expanding the small molecular toolbox to study big biomolecular machines. *Chimia* **2010**, *64*, 241−246.
(9) Carroll, F. I.; Gao, Y.; Abraham, P.; Lewin, A. H.; Lew, R.; Patel, A.; Boja, J. W.; Kuhar, M. J. Probes for the cocaine receptor. Potentially irreversible ligands for the dopamine transporter. *J. Med. Chem.* **1992**, *35*, 1813−1817.
(10) Zou, M.; Kopajtic, T.; Katz, J. L.; Wirtz, S.; Justice, J. J.; Newman, A. H. Novel tropane-based irreversible ligands for the dopamine transporter. *J. Med. Chem.* **2001**, *44*, 4453−4461.
(11) Lever, J. R.; Zou, M. F.; Parnas, M. L.; Duval, R. A.; Wirtz, S. E.; Justice, J. B.; Vaughan, R. A.; Newman, A. H. Radioiodinated azide and isothiocyanate derivatives of cocaine for irreversible labeling of dopamine transporters: synthesis and covalent binding studies. *Bioconjugate Chem.* **2005**, *16*, 644−649.
(12) Newman, A. H.; Cha, J. H.; Cao, J.; Kopajtic, T.; Katz, J. L.; Parnas, M. L.; Vaughan, R.; Lever, J. R. Design and synthesis of a novel photoaffinity ligand for the dopamine and serotonin transporters based on 2β-carbomethoxy-3β-biphenyltropane. *J. Med. Chem.* **2006**, *49*, 6621−6625.
(13) Sallee, F. R.; Fogel, E. L.; Schwartz, E.; Choi, S. M.; Curran, D. P.; Niznik, H. B. Photoaffinity labeling of the mammalian dopamine transporter. *FEBS Lett.* **1989**, *256*, 219−224.
(14) Grigoriadis, D. E.; Wilson, A. A.; Lew, R.; Sharkey, J. S.; Kuhar, M. J. Dopamine transport sites selectively labeled by a novel photoaffinity probe: ^{125}I-DEEP. *J. Neurosci.* **1989**, *9*, 2664−2670.
(15) Dutta, A. K.; Fei, X. S.; Vaughan, R. A.; Gaffaney, J. D.; Wang, N. N.; Lever, J. R.; Reith, M. E. A. Design, synthesis, and characterization of a novel, 4-[2-(diphenylmethoxy)ethyl]-1-benzyl piperidine-based, dopamine transporter photoaffinity label. *Life Sci.* **2001**, *68*, 1839−1849.
(16) Cao, J.; Lever, J. R.; Kopajtic, T.; Katz, J. L.; Pham, A. T.; Holmes, M. L.; Justice, J. B.; Newman, A. H. Novel azido and isothiocyanato analogues of [3-(4-phenylalkylpiperazin-1-yl)propyl]-bis(4-fluorophenyl)amines as potential irreversible ligands for the dopamine transporter. *J. Med. Chem.* **2004**, *47*, 6128−6136.
(17) Agoston, G. E.; Vaughan, R.; Lever, J. R.; Izenwasser, S.; Terry, P. D.; Newman, A. H. A novel photoaffinity label for the dopamine transporter based on N-substituted 3α[bis(4′-fluorophenyl)methoxy]-tropane. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 3027−3032.
(18) Zou, M. F.; Kopajtic, T.; Katz, J. L.; Newman, A. H. Structure−activity relationship comparison of (*S*)-2β-substituted 3α-(bis[4-fluorophenyl]methoxy)tropanes and (*R*)-2β-substituted 3β-(3,4-dichlorophenyl)tropanes at the dopamine transporter. *J. Med. Chem.* **2003**, *46*, 2908−2916.
(19) Lapinsky, D. J.; Aggarwal, S.; Huang, Y.; Surratt, C. K.; Lever, J. R.; Foster, J. D.; Vaughan, R. A. A novel photoaffinity ligand for the dopamine transporter based on pyrovalerone. *Bioorg. Med. Chem.* **2009**, *17*, 3770−3774.
(20) Lapinsky, D. J.; Aggarwal, S.; Nolan, T. L.; Surratt, C. K.; Lever, J. R.; Acharya, R.; Vaughan, R. A.; Pandhare, A.; Blanton, M. P. (±)-2-(*N*-*tert*-Butylamino)-3′-[^{125}I]-iodo-4′-azidopropiophenone: a dopamine transporter and nicotinic acetylcholine receptor photoaffinity ligand based on bupropion (Wellbutrin, Zyban). *Bioorg. Med. Chem. Lett.* **2012**, *22*, 523−526.
(21) Lapinsky, D. J.; Velagaleti, R.; Yarravarapu, N.; Liu, Y.; Huang, Y.; Surratt, C. K.; Lever, J. R.; Foster, J. D.; Acharya, R.; Vaughan, R. A.; Deutsch, H. M. Azido-iodo-*N*-benzyl derivatives of (±)-*threo*-methylphenidate (Ritalin, Concerta): rational design, synthesis, pharmacological evaluation, and dopamine transporter photoaffinity labeling. *Bioorg. Med. Chem.* **2011**, *19*, 504−512.
(22) Lapinsky, D. J.; Yarravarapu, N.; Nolan, T. L.; Surratt, C. K.; Lever, J. R.; Tomlinson, M.; Vaughan, R. A.; Deutsch, H. M. Evolution of a compact photoprobe for the dopamine transporter based on (±)-*threo*-methylphenidate. *ACS Med. Chem. Lett.* **2012**, *3*, 378−382.
(23) Vaughan, R. A.; Agoston, G. E.; Lever, J. R.; Newman, A. H. Differential binding of tropane-based photoaffinity ligands on the dopamine transporter. *J. Neurosci.* **1999**, *19*, 630−636.
(24) Parnas, M. L.; Gaffaney, J. D.; Zou, M. F.; Lever, J. R.; Newman, A. H.; Vaughan, R. A. Labeling of dopamine transporter transmembrane domain 1 with the tropane ligand *N*-[4-(4-azido-3-[^{125}I]iodophenyl)butyl]-2beta-carbomethoxy-3beta-(4-chlorophenyl)-tropane implicates proximity of cocaine and substrate active sites. *Mol. Pharmacol.* **2008**, *73*, 1141−1150.
(25) Dahal, R. A.; Pramod, A. B.; Sharma, B.; Krout, D.; Foster, J. D.; Cha, J. H.; Cao, J.; Newman, A. H.; Lever, J. R.; Vaughan, R. A.; Henry, L. K. Computational and biochemical docking of the irreversible cocaine analog RTI-82 directly demonstrates ligand positioning in the dopamine transporter central substrate-binding site. *J. Biol. Chem.* **2014**, *289*, 29712−29727.
(26) Henry, L. K.; Field, J. R.; Adkins, E. M.; Parnas, M. L.; Vaughan, R. A.; Zou, M. F.; Newman, A. H.; Blakely, R. D. Tyr-95 and Ile-172 in transmembrane segments 1 and 3 of human serotonin transporters interact to establish high affinity recognition of antidepressants. *J. Biol. Chem.* **2006**, *281*, 2012−2023.
(27) Chudzik, J.; McCarthy, D.; Bakish, D.; Ravindran, A.; Hrdina, P. D. Synthesis and characterization of an aryl-azidoparoxetine—a novel photo-affinity probe for serotonin-transporter. *Biochem. Pharmacol.* **1995**, *50*, 1211−1215.
(28) Rehavi, M.; Tracer, H.; Rice, K.; Skolnick, P.; Paul, S. M. [^{3}H]-Nitroimipramine: a selective "slowly-dissociating" probe of the imipramine binding site ("serotonin transporter") in platelets and brain. *Life Sci.* **1983**, *32*, 645−653.
(29) Wennogle, L. P.; Ashton, R. A.; Schuster, D. I.; Murphy, R. B.; Meyerson, L. R. 2-Nitroimipramine: a photoaffinity probe for the serotonin uptake/tricyclic binding site complex. *EMBO J.* **1985**, *4*, 971−977.
(30) Ransom, R. W.; Lee, J. D.; Bolger, M. B.; Shih, J. C. Photoinactivation of serotonin uptake by an arylazide derivative of 5-hydroxytryptamine. *Mol. Pharmacol.* **1985**, *28*, 185−190.
(31) Kumar, V.; Rahbek-Clemmensen, T.; Billesbølle, C. B.; Jorgensen, T. N.; Gether, U.; Newman, A. H. Novel and high affinity fluorescent ligands for the serotonin transporter based on (*S*)-citalopram. *ACS Med. Chem. Lett.* **2014**, *5*, 696−699.
(32) Banala, A. K.; Zhang, P.; Plenge, P.; Cyriac, G.; Kopajtic, T.; Katz, J. L.; Loland, C. J.; Newman, A. H. Design and synthesis of 1-(3-(dimethylamino)propyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile (citalopram) analogues as novel probes for the serotonin transporter S1 and S2 binding sites. *J. Med. Chem.* **2013**, *56*, 9709−9724.
(33) Zhang, P.; Cyriac, G.; Kopajtic, T.; Zhao, Y.; Javitch, J. A.; Katz, J. L.; Newman, A. H. Structure−activity relationships for a novel series of citalopram (1-(3-(dimethylamino)propyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile) analogues at monoamine transporters. *J. Med. Chem.* **2010**, *53*, 6112−6121.
(34) Bøgesø, K. P.; Sánchez, C. *Analog Based Drug Discovery III*; Wiley-VCH: Weinheim, Germany, 2013; Chapter 11, pp 269−294.
(35) dos Santos, J. L.; Lanaro, C.; Lima, L. M.; Gambero, S.; Franco-Penteado, C. F.; Alexandre-Moreira, M. S.; Wade, M.; Yerigenahally, S.; Kutlar, A.; Meiler, S. E.; Costa, F. F.; Chung, M. Design, synthesis,

DOI: 10.1021/acs.jmedchem.5b00682
J. Med. Chem. 2015, 58, 5609−5619

and pharmacological evaluation of novel hybrid compounds to treat sickle cell disease symptoms. *J. Med. Chem.* **2011**, *54*, 5811−5819.
(36) Dess, D. B.; Martin, J. C. Readily accessible 12-I-5 oxidant for the conversion of primary and secondary alcohols to aldehydes and ketones. *J. Org. Chem.* **1983**, *48*, 4155.
(37) Joossens, J.; Van der Veken, P.; Lambeir, A. M.; Augustyns, K.; Haemers, A. Development of irreversible diphenyl phosphonate inhibitors for urokinase plasminogen activator. *J. Med. Chem.* **2004**, *47*, 2411−2413.
(38) Zhang, P.; Jørgensen, T. N.; Loland, C. J.; Newman, A. H. A rhodamine-labeled citalopram analogue as a high-affinity fluorescent probe for the serotonin transporter. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 323−326.
(39) Lowndes, J. M.; Hokin-Neaverson, M.; Ruoho, A. E. *N*- (3-(*p*-Azido-*m*-[^{125}I]iodophenyl)propionyl)-succinimide—a heterobifunctional reagent for the synthesis of radioactive photoaffinity ligands: synthesis of a carrier-free ^{125}I-labeled cardiac glycoside photoaffinity label. *Anal. Biochem.* **1988**, *168*, 39−47.
(40) Shu, A. Y. L.; Yamashita, D. S.; Holt, D. A.; Heys, J. R. Synthesis of I-125 labeled photoaffinity rapamycin analogs. *J. Labelled Compd. Radiopharm.* **1996**, *38*, 227−237.
(41) Wilson, A. A.; Grigoriadis, D. E.; Dannals, R. F.; Ravert, H. T.; Wagner, H. N., Jr. A one-pot radiosynthesis of [^{125}I]iodoazido photoaffinity labels. *J. Labelled Compd. Radiopharm.* **1989**, *27*, 1299−1305.
(42) Lever, J. R.; Carroll, F. I.; Patel, A.; Abraham, P.; Boja, J.; Lewin, A.; Lew, R. Radiosynthesis of a photoaffinity probe for the cocaine receptor of the dopamine transporter: 3*β*-(*p*-chlorophenyl)tropan-2*β*-carboxylic acid *m*-([^{125}I]-iodo)-*p*-azidophenethyl ester ([^{125}I]-RTI-82). *J. Labelled Compd. Radiopharm.* **1993**, *33*, 1131−1137.
(43) Lever, J. R.; Scheffel, U.; Stathis, M.; Seltzman, H. H.; Wyrick, C. D.; Abraham, P.; Parham, K.; Thomas, B.; Boja, J. W.; Kuhar, M. J.; Carroll, F. I. Radiosynthesis and *in vivo* studies of a selective ligand for the dopamine transporter: 3*β*-(4-[^{125}I]iodophenyl)tropan-2*β*-carboxylic acid isopropyl ester ([^{125}I]RTI-121). *Nucl. Med. Biol.* **1996**, *23*, 277−284.
(44) Andersen, J.; Taboureau, O.; Hansen, K. B.; Olsen, L.; Egebjerg, J.; Strømgaard, K.; Kristensen, A. S. Location of the antidepressant binding site in the serotonin transporter: importance of Ser-438 in recognition of citalopram and tricyclic antidepressants. *J. Biol. Chem.* **2009**, *284*, 10276−10284.
(45) Plenge, P.; Shi, L.; Beuming, T.; Te, J.; Newman, A. H.; Weinstein, H.; Gether, U.; Loland, C. J. Steric hindrance mutagenesis in the conserved extracellular vestibule impedes allosteric binding of antidepressants to the serotonin transporter. *J. Biol. Chem.* **2012**, *287*, 39316−39326.

DOI: 10.1021/acs.jmedchem.5b00682
J. Med. Chem. 2015, 58, 5609−5619