Elucidation of Structural Elements for Selectivity across Monoamine Transporters: Novel 2-[(Diphenylmethyl)sulfinyl]acetamide (Modafinil) Analogues

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Supporting Information

ABSTRACT: 2-[(Diphenylmethyl)sulfinyl]acetamide (modafinil, (±)-1) is a unique dopamine uptake inhibitor that binds the dopamine transporter (DAT) differently than cocaine and may have potential for the treatment of psychostimulant abuse. To further investigate structural requirements for this divergent binding mode, novel thio- and sulfinylacetamide and ethanamine analogues of (±)-1 were synthesized wherein (1) the diphenyl rings were substituted with methyl, trifluoromethyl, and halogen substituents and (2) substituents were added to the terminal amide/amine nitrogen. Halogen substitution of the diphenyl rings of (±)-1 gave several amide analogues with improved binding affinity for DAT and robust selectivity over the serotonin transporter (SERT), whereas affinity improved at SERT over DAT for the p-halo-substituted amine analogues. Molecular docking studies, using a subset of analogues with DAT and SERT homology models, and functional data obtained with DAT (A480T) and SERT (T497A) mutants defined a role for TM10 in the substrate/inhibitor S1 binding sites of DAT and SERT.

Inhibition of dopamine (DA) reuptake is proposed to be the mechanism underlying the reinforcing effects of abused psychostimulant drugs such as cocaine and methamphetamine. Modafinil (2-[(diphenylmethyl)sulfinyl]acetamide, (±)-1; Figure 1) is used clinically for the treatment of sleep disorders and inhibits DA reuptake, with no evidence of abuse liability in humans.1,2 Recent attention has focused on a distinctive binding mode at the dopamine transporter (DAT) to explain this curious pharmacological profile of (±)-1 and particularly its R-enantiomer (armodafinil, R-(−)-1).3,5 These studies independently demonstrated that (±)-1 binds the DAT in a unique fashion compared to cocaine, which may be related to its distinct behavioral profile. However, there are other reports suggesting additional mechanisms underlying the pharmacological actions of (±)-1 and in particular its effectiveness in attenuating psychostimulant drug seeking in animal models.4–9 Nevertheless, direct interaction with these other targets has not been demonstrated.2 One potential contribution to the preclinical pharmacology of (±)-1 is that it is a nonaminergic compound with limited water solubility, which can complicate investigation due to the large concentration of drug needed for in vitro and in vivo studies. The high doses of (±)-1 used in

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Figure 1. (±)-1, its enantiomers, and the DAT-selective amino analogue 2.
preclinical studies may indeed have direct or downstream interactions with numerous targets, including histaminergic, GABAergic, orexinergic, glutamatergic, adrenergic, and serotonergic neurons. However, whether or not these targets are related to therapeutic or behavioral outcomes remains unknown.

In a previous study, we began to explore the structure–activity relationships (SARs) of (+)-1 analogues at the DAT, serotonin transporter (SERT), and norepinephrine transporter (NET) and identified one analogue, compound 2 (Figure 1), in which the terminal amide was replaced with a 3-phenylpropyl-substituted amine group, with enhanced DAT affinity. The DAT affinity for 2 was improved by 10-fold, compared to that for (+)-1, as was its water solubility. In addition, 2 demonstrated low micromolar binding affinities for SERT and NET, which prompted a systematic and comparative exploration of the SAR of the (+)-1 scaffold at all three monoamine transporters (MATs) with a series of novel analogues. Specifically, we wanted to further investigate the role of the terminal amide or substituted amine functions on DAT vs SERT and NET binding and also determine how additional diphenyl substitutions on the sulfinylethanamine or reduced thioethanamine template affected the binding affinities and modes. To this end, a series of thioacetamide and sulfinylacetamide analogues were prepared and compared to a set of thioethanamine and sulfinylethanamine analogues of (+)-1.

**CHEMISTRY**

Scheme 1 outlines the synthesis of novel thioacetamide (compounds 4a−4z) and sulfinylacetamide (compounds 5a−5g) analogues of (+)-1. The thioacetamide analogues 4a−4z were generated via three different synthetic routes.

The first route (procedure A) affords the thioacetamides in one step and was employed in the synthesis of compound 4a and the N-methylthioacetamides 4g−4i. As opposed to the previously reported two-step synthesis, thioacetamide 4a was synthesized in one step by coupling 2-mercaptoacetamide with diphenylmethanol in trifluoroacetic acid (TFA) at room temperature. Similarly, N-methylthioacetamides 4g−4i were synthesized via the coupling reaction between 2-mercapto-N-methylacetamide and diphenylmethanol (for 4g) or the corresponding bis(halophenyl)methanol (for 4h and 4i) in TFA. To improve product yields, the reactions for compounds 4h and 4i required heating to 60 °C.

The second synthetic route (procedure B), used for the synthesis of thioacetamides 4b−4f, required three steps, similar to previously described methods. Mono- or disubstituted diphenylmethanol was coupled with thioglycolic acid in trifluoroacetic acid (TFA) at room temperature. Similarly, N-methylthioacetamides 4g−4i were synthesized via the coupling reaction between 2-mercapto-N-methylacetamide and diphenylmethanol (for 4g) or the corresponding bis(halophenyl)methanol (for 4h and 4i) in TFA. To improve product yields, the reactions for compounds 4h and 4i required heating to 60 °C.
Scheme 2. Synthesis of Thioethanamine and Sulfonylthioethanamine Analogues of (+)-1a

Reagents and conditions: (a) cysteamine hydrochloride, BF3·OEt2, glacial AcOH, 80–90 °C, ~20 min (40–50 min for substituted analogues (procedure D)); (b) (i) procedure D; (ii) cyclopropane carboxaldehyde, NaBH3CN, MeOH, 1,2-dichloroethane, room temperature, overnight; (c) (i) procedure D; (ii) BuBr, CsOH; (d) LiAlH4, H2SO4, THF; (e) BH3·THF, THF, reflux, overnight; (f) NaIO4, H2O, EtOH, 0 °C to room temperature, overnight; (g) H2O2 (30%), AcOH, 80 °C to 90 °C, 20 h; (h) LiAlH4, H2SO4, THF; (i) BH3·THF, THF, reflux, overnight; (j) NaIO4, H2O, EtOH, 0 °C to room temperature, overnight; (k) H2O2 (30%), AcOH–MeOH (1:3), 40 °C, 24 h.

were then synthesized by coupling the carboxylic acid to the corresponding primary amine via an in situ N,N'-carbonimidazole coupling reaction. Oxidation of the appropriate thioacetamide (4b–4g and 4x) using hydrogen peroxide (H2O2; 30%) in an acetic acid methanol solution mixture gave sulfonylacetamides 5a–5g.

Scheme 2 outlines the synthesis of the thioethanamine (6a–6i) and sulfonylthioethanamine (7a–7e) analogues of (+)-1. Thioethanamines 6a–6c were synthesized by coupling diphenylmethanol or the appropriate bis(halophenyl)methanol with cysteamine hydrochloride in glacial acetic acid in the presence of the Lewis acid catalyst boron trifluoride dimethyl etherate (BF3·OEt2) (procedure D).12,13 The N-substituted thioethanamine 6d was synthesized by a reductive amination reaction between the hydrochloride salt of compound 6a and cyclopropane carboxaldehyde using sodium cyanoborohydride as the reducing agent. Similarly, N-substituted thioethanamine 6e was synthesized by coupling n-butyl bromide to the free base of compound 6a in the presence of Cs2SO4·H2O (Cs+ ions served as templating catalysts).14 N-substituted thioethanamines 6f–6h were synthesized by the reduction of thioacetamides 4u–4w using alane (LiAlH4–sulfuric acid mixture).15 Lastly, N-substituted thioethanamine 6i was prepared from thioacetamide 4x by reduction with borane in THF (BH3·THF). Sulfonylthioethanamines 7a–7e were synthesized from the appropriate thioethanamines (6a, 6e, or 6g–6i) by oxidation of the thioether function using either sodium periodate (NaIO4) in an ethanol–water solution (compounds 7a and 7b) or H2O2 (30%) in an acetic acid–methanol solution (compounds 7c–7e).

RESULTS AND DISCUSSION

SARs at DAT, SERT, and NET. In a previous study, we showed that, in general, p-halogen substitution of the diphenylmethyl moiety of the (±)-1 structure gave analogues with improved binding affinities for the DAT over SERT and NET.10 Additionally, we confirmed that enantioselectivity at DAT for the R- and S-enantiomers was only ~3-fold and that replacement of the sulfoxide (S=O) with a sulfide function may have minimal effects on DAT binding. Importantly, we discovered that reducing the terminal amide and appending a 3-phenylpropyl substituent resulted in compound (Figure 1), which was identified as having higher binding affinities than the amide analogues at all three MATs. This result was particularly encouraging, as salts of amine analogues present a solubility advantage over the parent amide (±)-1. In the current study, we further explored the effect of reducing the S=O, while adding increasingly bulky substituents at the amide nitrogen. Additionally, we expanded the library of amine analogues with and without the S=O motif. Our hypothesis was that SARs within this class of (±)-1 analogues would help unravel SAR differences between the MATs and also identify binding motifs related to the unique binding mode of this class of DAT inhibitors.

Binding affinities of all novel (±)-1 analogues were evaluated at the DAT, SERT, and NET in rat brain membranes using a slightly modified version of previously described methods13 and are detailed in the Experimental Methods. The results of the in vitro assays, grouped by functionality into amides and amines, are presented in Tables 1 and 2, respectively. All sulfinyl compounds were tested as racemic mixtures.

In Table 1, most of the thioacetamide and sulfinylacetamide analogues displayed micromolar affinities at the DAT, within ±10-fold of that of (±)-1 (Kd = 2600 nM). Reducing the S=O to the thioether 4a decreased DAT binding by ~5-fold, while improving SERT affinity. When the diphenyl rings were unsubstituted, alkyl substitution of the terminal amide nitrogen decreased binding affinity at the DAT with or without the S=O motif (e.g., compounds 4a, 4g, 4j, 4k, 4o, 4r, and 5f). The exception to this trend was observed with compounds 4u
and 4y, which displayed similar or nominally improved binding affinities ($K_i = 2020$ and 1150 in nM, respectively) in comparison to (±)-1. Within each series of N-substituted thioacetamides, binding affinity at the DAT generally increased with halogen substitution at the para-position of the diphenylmethyl moiety in the order $H < F < Cl \leq Br$. This order is in agreement with previously reported data, and applies to both the thioacetamides and sulfinylacetamides with or without substitution on the amide nitrogen. It has been proposed that if a ligand can establish a halogen bond interaction with a receptor in an optimal orientation, the CI to Br to I substitution may result in an increase of affinity. Thus, the order we observed might be consistent with the halogen substituent forming a halogen bond with a polar residue of DAT. Additionally, substitution at other positions of the diphenyl rings followed this halogen substitution order, for example, compounds 5c–5e with halogen substituents in the meta-positions of the diphenyl rings. In general, the novel acetamides were selective for the DAT over the SERT and NET, except for compounds 4a and 5g, both of which displayed roughly equal affinities at the DAT and SERT (DAT:SERT affinity ratios of 1 and 1.4, respectively). Five amide analogues—4e, 4w, 4x, 4z, and 5e—were identified as the most DAT-selective compounds in the series (e.g., SERT:DAT affinity ratios of >2900 for 4e and 249 for 4x, with no displacement at the SERT for 4w, 4z, or 5e). The pronounced selectivity observed with compound 4e for DAT over SERT is remarkable, especially in comparison to its regioisomer, compound 8t (Table 1), which is only modestly selective for DAT over SERT (SERT:DAT affinity ratio = 18).

As shown in Table 2, removal of the amide carbonyl (C=O) function resulted in improved affinities at the DAT, SERT, and NET (compare compounds 6a and 7a to (±)-1), with several of the novel amino analogues having nanomolar binding affinities at the DAT in comparison to the micromolar affinity of compound (±)-1. With the thioethanamines, in contrast to the thioacetamides, DAT affinity generally increased with increasingly bulky substitution on the terminal amine nitrogen for analogues with unsubstituted diphenyl rings (see compounds 6d, 6e, and 6f). For analogues with halogen
Table 2. MAT Binding Data for Thio- and Sulfinylethanamine Analogues

<table>
<thead>
<tr>
<th>compd</th>
<th>X</th>
<th>Y</th>
<th>R</th>
<th>DAT (K_i [SE interval] nM)</th>
<th>SERT (K_i [SE interval] nM)</th>
<th>NET (K_i [SE interval] nM)</th>
</tr>
</thead>
</table>

Each K_i value represents data from at least three independent experiments, each performed in triplicate. K_i values were analyzed by PRISM. Binding assays are described in detail in the Experimental Methods. Previously reported by Cao et al.10

It is clear from the SARs described herein that reduction of the amide to a secondary or primary amine significantly improves binding affinities at all three MATs (e.g., 4a vs 6a). This effect appears to be most consistent at DAT, as all but a few analogues in Table 2 have submicromolar affinities. Interestingly, when the diphenyl ring system is substituted with either p-Cl or p-Br groups, the binding affinities at SERT are more improved than at DAT in all cases and most dramatically with compounds 6b and 6c, which bind with K_i values of ≤30 nM at SERT, suggesting a specific interaction at the para-position that may differ between these two transporters. To investigate this further, we carried out molecular docking studies with a group of representative compounds using the homology models of DAT and SERT based on the crystal structure of LeuT11,19 to compare the differences in their binding modes for these targets.

Previously, we proposed that the sulfoxide O interacted with the conserved Y156 in DAT.1 Interestingly, the residue immediately before Y156 is divergent among MATs: whereas in DAT this residue is phenylalanine (F155), the aligned position in SERT/NET is a tyrosine. Molecular docking studies revealed that while both F155 in DAT and Y175 in SERT directly interact with (±)-1, this molecule differentially affects how Y156 of DAT and Y176 of SERT are positioned when bound. Thus, the O=N is optimally positioned to interact with Y156 of DAT but not Y176 of SERT. If the S=O cannot properly interact with the conserved Tyr in SERT, the S=O contributes negatively to the binding affinity, and as a result (±)-1 has higher affinity for DAT than SERT. Conversely, absence of the sulfoxide oxygen should increase the affinity for SERT. Consistent with this prediction, in the presence of the carbonyl oxygen of the amide ([±]-1 vs compound 4a, Table 1), reducing the S=O decreased the binding affinity for DAT but increased the affinity for SERT. Nevertheless, when either of the phenyl rings was substituted with halogens (4e vs 5d) or the terminal amide was substituted (5f vs 4g), this trend was

\[ Y \]
not obvious, underscoring the influence of these additional substituents on the binding mode in both DAT and SERT. Note the binding affinities at SERT are so low for these analogues it is impossible to determine a specific trend.

By reducing the amide carbonyl, the N becomes positively charged, resulting in an increase in affinity for all three MATs as described above [compare (+)-1 to 7a]. An interpretation is that the positive charge facilitates direct interaction between the N and the conserved negatively charged Asp involved in the Na₁ binding site for all three transporters. Additionally, the combined effect of a global reduction of both the amide carbonyl and sulfide oxygens is even higher affinities at the DAT, SERT, and NET, suggesting that the impact of the charged N is dominant compared to removal of the sulfide O, especially for DAT and SERT (compare (+)-1 to 4a vs (±)-1 to 6a).

To test the hypothesis that these residues in TM10 are part of the primary substrate/inhibitor (S1)₁⁻²⁰ binding site and play different roles in DAT vs SERT binding for para-halogenated analogues in this series, we created two chimera mutants in DAT and SERT in which the residues are interchanged, resulting in DAT-A480T and SERT-T497A. The effect of the mutations on uptake inhibition potency for compounds with a Cl substituent in the para-position were measured on intact COS-7 cells transiently expressing WT’s or the Ala- and Thr-substituted mutants (Tables 3 and 4). While this paper was being prepared, the crystal structure of Drosophila melanogaster DAT (dDAT) bound with the tricyclic antidepressant norriptyline became available.²¹ The core of the dDAT structure “closely resembles that of LeuT,”²¹ which we used as the template to build the DAT and SERT homology models for this study, and shows the aligned Ala479 of TM10 is indeed in direct contact with the edge of one of the norriptyline phenyl rings. Therefore, the dDAT structure supports our prediction that this TM10 position faces the S1 binding sites of SERT and DAT. Interestingly, the affinity of (±)-1 is increased in DAT-A480T (~5-fold) and perhaps slightly in the SERT-T497A mutant, compared to those of their corresponding WT’s. In addition, whereas the affinity of the p-Cl-substituted thioacetamide 4h (a secondary amide) is significantly decreased at SERT-T497A compared to SERT-WT (Table 4), suggesting a direct interaction between the p-Cl and the side chain of T497 (Figure 2), the affinity of 4h at DAT-A480T is essentially the same as that at DAT-WT, similar to 4g, which does not possess the p-Cl substituent (Table 3). The observed affinity is also consistent with an alternative explanation that the hydroxyl group of the Thr497 side chain forms an intrahelical H-bond with the protein backbone,²² while the α-methyl group is exposed to the binding site as a hydrophobic contact to accommodate the halogen substitution, especially for amide analogues (e.g., 4h; Table 4).

These results support our hypothesis that halogen bond interactions at SERT T497 affect the binding affinities of these analogues. We also hypothesized that a change of affinity might result for the A480T DAT mutant; however, we found that the binding affinity was not affected by this mutation. Hence, these data suggest that the binding sites of DAT and SERT obviously have other divergences beyond this single residue position—the ways in which the rest of the binding sites of DAT and SERT change in response to the mutations are different—and simply switching the residues at this position is not enough to interconvert the specificity of the compounds. For example, in both DAT and SERT, the affinities of 6a and 6b (both primary amines) remain unchanged at the mutants, suggesting that the exact configuration near the terminal nitrogen, either amide or charged nitrogen, has a strong impact on the orientation of the diphenylmethyl moiety in both transporters. Taken together, this residue position of TM10 appears to be more important for binding of the amide derivatives of (±)-1 with a p-Cl substituent at the SERT, compared to binding at the DAT. If the amide function is reduced to an amine, the relative importance of interaction at these residues is diminished.

Consistent with this understanding, at the SERT, the difference in binding affinities for halogen-substituted analogues of (±)-1 is more pronounced in compounds lacking a charged N (e.g., >50-fold increase in SERT affinity for amide 4i vs 4g in Table 1 and only a 9-fold increase in SERT affinity for amine 6c vs 6a in Table 2). In both cases, improvements in DAT affinity were diminished compared to those in SERT affinity, with only a 6-fold improvement in DAT affinity between 4i and 4g and only a 3-fold improvement for DAT affinity between 6c and 6a. In contrast, moving the halogen substituent to the meta-position as in compounds 4e and 5d has little if any effect on SERT binding; hence, a decrease in or no change in binding affinity resulted compared to those of compound 4a and (±)-1, respectively. However, the halogen substituent in the meta-position appears to generate new interactions that favor binding to the DAT, further supporting the influence of other residue divergences in the binding sites of DAT and SERT on compound affinity. Thus, we propose that substitution at the meta-position may be more favorable for designing DAT-over-SERT-selective analogues of (±)-1 and may warrant further exploration.

### Conclusion

A series of novel thio- and sulfinylacetamide and -ethanamine analogues of (±)-1, with or without substituents on the diphenyl rings, were synthesized to investigate the contributions of structural variations to selectivity across MATs. Previous SARs had suggested that the sulfinyl (S=O) function was not critical for binding to the DAT, but differential interactions with Y156 of DAT and Y176 of SERT may affect selectivity for SERT.²³ In addition, we showed that reduction of the amide function to the amine not only improved water

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### Table 3. [3H]DA Uptake Inhibition Potency for Selected Analogues Measured in Intact COS7 Cells Expressing the Human DAT Wild Type or the A480T Mutant²⁴

<table>
<thead>
<tr>
<th>compd</th>
<th>hDAT-WT Kᵢ (μM)</th>
<th>n</th>
<th>hDAT-A480T Kᵢ (μM)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>(±)-1</td>
<td>1300 [1000–17000]</td>
<td>6</td>
<td>3090 [2300–4200]</td>
<td>3</td>
</tr>
<tr>
<td>4g</td>
<td>5500 [4000–7600]</td>
<td>4</td>
<td>3600 [2010–6300]</td>
<td>3</td>
</tr>
<tr>
<td>4h</td>
<td>3700 [2700–5100]</td>
<td>5</td>
<td>2300 [1700–3100]</td>
<td>3</td>
</tr>
<tr>
<td>6a</td>
<td>390 [280–540]</td>
<td>3</td>
<td>720 [620–830]</td>
<td>4</td>
</tr>
<tr>
<td>6b</td>
<td>1210 [960–1510]</td>
<td>3</td>
<td>1370 [1240–1510]</td>
<td>3</td>
</tr>
</tbody>
</table>

“*The inhibition potency for [3H]dopamine (DA) uptake was calculated from nonlinear regression analysis of uptake experiments performed on COS7 cells transiently transfected with DNA of the human dopamine transporter (hDAT) wild type (WT) or the Ala480 to Thr mutant (A480T). The IC₅₀ values used in the calculation of Kᵢ and Kᵢ were calculated from the means of pIC₅₀, and the indicated SE intervals were calculated from pIC₅₀ ± SE. Nonspecific uptake was determined using 50 μM nomifensine.*
solubility, but also enhanced DAT affinity.\textsuperscript{10} In the current study, the earlier SARs were expanded. \textit{para}- or \textit{meta}-substitution of the phenyl rings of (±)-1 with Cl or Br gave several amide analogues with improved selectivity for DAT over SERT and NET, whereas selectivity was improved at SERT over DAT and NET for the amine analogues. Overall, we identified five highly DAT-selective amide analogues (e.g., 4e, 2900-fold over SERT) and two SERT-selective amine analogues with high affinity ($K_i \leq 30 \text{ nM}$). Computational modeling of DAT and SERT led to the identification of key amino acid residues in TM10 that form part of the S1 binding pocket in both DAT and SERT. By switching the T497 in SERT to Ala and the A480 in DAT to Thr and then testing a selected subgroup of analogues, the role of TM10 in DAT and SERT binding was further defined. Moreover, we propose that this TM10 position faces the S1 binding site and plays a role in propagating the conformational changes of the homologous LeuT from the S1 binding site to the intracellular gate, such divergent interactions with TM10 are likely to have an impact on the overall transporter conformation\textsuperscript{25} and may contribute to the mechanism underlying the unique pharmacology of (±)-1 and its analogues at DAT.

\section*{EXPERIMENTAL METHODS}

\textbf{Synthesis.} 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 benzenophene ketyl. All other chemicals and reagents were purchased from Sigma-Aldrich Co. LLC, Combi-Blocks, TCI America, OChem Incorporation, Acros Organics, Maybridge, and Alfa Aesar. The diphenylmethanols (3a–c, 3e–i) were commercially available, except 3d, which was synthesized as outlined below. Unless otherwise stated, amine final products were converted into oxide salts, typically by treating the free base in 2-propanol with a 1:1 molar ratio of oxalic acid in aceton. As described, some of the oxalate salts were recrystallized from hot methanol or a methanol–acetone solvent mixture. Spectroscopic data and yields refer to the free base, except for compounds 6b and 6c, which were synthesized as the hydrochloride salts. Flash chromatography was performed using silica gel (EMD Chemicals, Inc., 230–400 mesh, 60 Å). Compounds 4a and 6f were purified using a Teledyne ISCO.
CombiFlash® Rf instrument. 1H and 13C 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 1H spectra (CDCl3, 7.26 ppm, or DMSO-d6, 2.50 ppm) and 13C spectra (CDCl3, 77.2 ppm, or DMSO-d6, 39.5 ppm). 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, then increased to 295 °C at 15 °C/min over 13 min, and finally maintained at 295 °C for 10 min. Combustion analysis was performed by Atlantic Microlab, Inc. (Norcross, GA), and the results agree within ±0.5% of the calculated values. Melting point determination was conducted using a Thomas-Hoover melting point apparatus, and the melting points are uncorrected. On the basis of NMR and combustion data, all final compounds are >95% pure.

**Bis(4-bromophenyl)methanol (3d).** Compound 3d was synthesized by adapting a literature method33 from bis(4-bromophenyl)-methanol (10.2 g, 30.0 mmol) and NaBH4 (2.55 g, 67.4 mmol) in anhydrous ethanol (65 mL) at 0 °C under argon. The product 3d (9.8 g, 52% yield) was recovered as a white solid. Mp: 109–111 °C. 1H NMR (CDCl3): δ 7.66 (d, J = 8.6 Hz, 4H), 7.22 (d, J = 8.6 Hz, 4H), 5.76 (s, J = 3.5 Hz, 1H), 2.21 (s, J = 3.5 Hz, 1H). 13C NMR (CDCl3): δ 142.4, 131.9, 128.3, 121.9, 75.2.

**Thioacetamides. General Thioacetamide Synthesis Procedures.** Procedure A. A solution of 2-mercapto-N-methylacetamide (10 mmol) and diphenylmethanol, 3a or the appropriate substituted diphenylmethanol, 3c or 3d (10 mmol), in trifluoroacetic acid (TFA; 200 mmol) was stirred at room temperature (60 °C for substituted analogues) for 20 h. The solvent was removed in vacuo, and the thick oily residue was washed with water (30 mL). After the water was decanted, a crude solid product was isolated by addition of disopropyl ether (20 mL) to the oily residue and vigorous mixing. The crude solid was filtered and purified by flash column chromatography using 5% MeOH/CH2Cl2 to give the pure, desired product.

**Procedure B.** Thioacetamides 4b–4f were synthesized34 in three steps. Step 1: Thioglycolic acid (1 mmol) was reacted with the appropriate substituted diphenylmethanol, 3e–3i (1 mmol), in TFA (14 mmol) overnight at room temperature. After solvent removal in vacuo, the residue obtained was washed with water (5 mL) and hexanes (15 mL) to give the carboxylic acid product, which was carried to the next step without further purification. Step 2: The acid product (3 mmol) from step 1 was reacted with K2CO3 (4.5 mmol) and iodomethane (CH3I, 4.5 mmol) in acetone (50 mL) overnight under reflux conditions. After solvent removal in vacuo, the residue was suspended in water (20 mL) and extracted with CH2Cl2 (3 × 20 mL). The combined organic layer was dried over MgSO4 and concentrated to give the methyl ester, which was carried to the next step without further purification. Step 3: A mixture of the ester (3 mmol), NH4Cl (4.2 mmol), concentrated NH4OH (28.0–30.0%, 20 mL), and MeOH (5.7 mL) was stirred at 50 °C for 72 h. MeOH was removed in vacuo, and the reaction mixture was diluted with water (50 mL), extracted with ethyl acetate (3 × 50 mL), and dried over Na2SO4. The solvent was evaporated, and the recovered crude product was purified by flash column chromatography using 1:1 ethyl acetate/hexanes to afford the pure product.

**Procedure C.** Thioacetamides 4i, 4j, 4k, 4l, and 4m were synthesized in two steps according to a published procedure,17 while compounds 4j, 4k, 4m–4o, 4q, 4r, 4t, 4u, and 4x–4z were synthesized in two steps with slight modifications to the published procedure in the second step. Step 1 is the same as step 1 for procedure B. Step 2: CDI (11 mmol) was added to a solution of the carboxylic acid product (10 mmol) from step 1 in anhydrous THF (25 mL). The reaction mixture was stirred at room temperature for 2 h and then cooled to 0 °C. Water (0.1–0.2 mL) was added to the reaction mixture (to quench excess CDI), followed by the dropwise addition of the appropriate amine (10 mmol, dissolved in THF). The reaction mixture was left to warm to room temperature and stir overnight. The solvent was removed under vacuum to give a crude residue, which was dissolved in diethyl ether or ethyl acetate. The organic solution was washed with aqueous 1.0 M HCl solution (55 mL), water (80 mL), dilute aqueous NaHCO3 solution (36 mL, 1:6 dilution of saturated NaHCO3 solution), and water (2 × 30 mL). The organic layer was dried over MgSO4 and concentrated in vacuo to give the pure product. The bromo-substituted analogues 4q, 4t, 4x, and 4z required further purification by flash column chromatography as indicated.
31°C NMR (CDCl3): δ 168.0, 138.6, 133.8, 129.7, 129.2, 53.6, 41.7, 36.1, 22.9, 11.5. Anal. (C19H21F4NOS) C, H, N.

2-(Bis(4-fluorophenyl)hydroxy)methylthio)-N-butyramide (4r). Compound 4r was synthesized from 2-(bis(4-fluorophenyl)methylthio)acetic acid and cyclopropylmethylamine according to the modified general procedure C. The product 4r (80 mg, 88% yield) was obtained as a yellow oil. 1H NMR (CDCl3): δ 7.34 (d, J = 8.4, 2.0 Hz, 4H), 7.24 (d, J = 8.8, 2.0 Hz, 4H), 6.69 (br s, 1H), 6.59–7.05 (m, 4H), 5.13 (s, 1H), 3.24 (q, J = 6.6 Hz, 2H), 3.07 (s, 2H), 2.80 (m, 2H), 2.43–2.57 (m, 4H), 1.91 (s, 3H), 1.52 (s, 3H), 0.94 (t, J = 7.4 Hz, 3H). 13C NMR (CDCl3): δ 167.9, 162.1 (1JCF = 247 Hz), 136.0 (1JCF = 3.7 Hz), 129.8 (1JCF = 8.1 Hz), 115.7 (1JCF = 21.4 Hz), 53.2, 41.5, 36.0, 22.8, 11.4. Anal. (C19H21F4NOS) C, H, N.

2-(Bis(4-fluorophenyl)methylthio)-N-butyramide (4s). Compound 4s was synthesized from 2-(bis(4-fluorophenyl)methylthio)acetic acid and n-butyramide according to the modified general procedure C. The product 4s (350 mg, 100% yield) was obtained as a white solid. Mp: 117–118.5°C. 1H NMR (CDCl3): δ 7.34 (d, J = 8.4, 2.0 Hz, 4H), 7.24 (d, J = 8.8, 2.0 Hz, 4H), 6.69–7.05 (m, 4H), 4.97 (s, 1H), 3.17 (s, 1H), 3.24 (q, J = 6.6 Hz, 2H), 2.80 (s, 2H), 2.73 (m, 2H), 2.43–2.57 (m, 4H), 1.91 (s, 3H), 1.52 (s, 3H), 0.94 (t, J = 7.4 Hz, 3H). 13C NMR (CDCl3): δ 167.9, 162.1 (1JCF = 247 Hz), 136.0 (1JCF = 3.7 Hz), 129.8 (1JCF = 8.1 Hz), 115.7 (1JCF = 21.4 Hz), 53.3, 39.5, 36.0, 31.6, 20.1, 13.7. Anal. (C19H21F4NOS) C, H, N.

2-(Bis(4-fluorophenyl)methylthio)-N-butyramide (4t). Compound 4t was synthesized from 2-(bis(4-fluorophenyl)methylthio)acetic acid and n-butyramide according to the modified general procedure C. Purification by flash column chromatography using 1:1 ethyl acetate/hexanes gave the pure product 4t (0.5 g, 88% yield) as a yellow oil. 1H NMR (CDCl3): δ 7.45 (d, J = 8.4, 2.0 Hz, 4H), 7.24 (d, J = 8.8, 2.0 Hz, 4H), 6.69 (br s, 1H), 6.59–7.05 (m, 4H), 5.13 (s, 1H), 3.24 (q, J = 6.6 Hz, 2H), 3.07 (s, 2H), 1.91 (s, 3H), 1.52 (s, 3H), 0.94 (t, J = 7.4 Hz, 3H). 13C NMR (CDCl3): δ 167.9, 162.1 (1JCF = 247 Hz), 136.0 (1JCF = 3.7 Hz), 129.8 (1JCF = 8.1 Hz), 115.7 (1JCF = 21.4 Hz), 53.3, 39.5, 36.0, 31.6, 20.1, 13.7. Anal. (C19H21F4NOS) C, H, N.
1.88 (m, 2H). 13C NMR (CDCl₃): δ 168.0, 162.1 (JCF = 247 Hz), 141.1, 135.9 (JCF = 2.9 Hz), 129.8 (JCF = 8.1 Hz), 128.4 (JCF = 21.4 Hz), 126.1, 115.8, 115.6, 53.3, 39.6, 36.0, 32.1. Anal. (C₈H₁₂F₃NO₂S) C, H, N.

2-(4-Bromophenyl)ethylthio)-N-(3-phenylpropyl)acetamide (4x). Compound 4x was synthesized as previously described from 2-(4-(4-bromophenyl)ethylthio)acetic acid and 3-phenyl-1-propylamine according to general procedure C. The product 4w was synthesized as described for 4x using compound 4w (500 mg, 2.45 mmol) to give the product 5x (600 mg, 71%) as a white solid. Mp: 115–116 °C. 1H NMR (CDCl₃): δ 7.43–7.43 (m, 2H), 7.31–7.37 (m, 6H), 7.05 (br s, 1H), 6.63 (br s, 1H), 5.36 (s, 1H), 3.47 (d, J = 13.6 Hz, 1H), 3.27 (d, J = 13.6 Hz, 1H). 13C NMR (CDCl₃): δ 166.0, 163.0 (JCF = 248 Hz), 162.8 (JCF = 248 Hz), 136.5, 135.8, 131.2 (JCF = 81 Hz), 130.5 (JCF = 81 Hz), 125.3, 124.5 (JCF = 3.0 Hz), 116.5 (JCF = 22.8 Hz), 115.9 (JCF = 22.1 Hz), 69.7, 52.2. Anal. (C₁₄H₁₉BrNOS) C, H, N.

2-(Benzhydrylo)ethylthio)-N-(4-phenylbutyl)acetamide (4y). Compound 4y was synthesized from (benzhydrylo)acetic acid and 4-phenyl-1-butanamine according to the modified general procedure C. The product 4y (0.793 g, 96% yield) was obtained as a yellow oil. 1H NMR (CDCl₃): δ 7.35–7.38 (m, 4H), 7.28–7.32 (m, 6H), 7.16–7.26 (m, 5H), 6.61 (br s, 1H), 5.08 (s, 1H), 3.22 (q, J = 6.7 Hz, 2H), 3.10 (s, 2H), 2.64 (t, J = 7.4 Hz, 2H), 1.61–1.69 (m, 2H), 1.48–1.55 (m, 2H). 13C NMR (CDCl₃): δ 168.2, 142.1, 140.4, 128.9, 128.2, 125.0, 128.3, 127.7, 126.6, 55.2, 39.7, 36.2, 35.6, 29.2, 28.8. Anal. (C₂₀H₂₁NO₂S) C, H, N.

Sulfinylacetamides. 2-(4i-Propyl)methylsulfinylacetamide (5a). Compound 5a was synthesized following a literature procedure. 10 Briefly, H₂O₂ (0.11 mL, 1 mmol, 1 equiv) was added to a solution of compound 4b (310 mg, 1.1 mmol, 1 equiv) in a solvent mixture of acetic acid (1.1 mL) and MeOH (3.3 mL). The reaction mixture was stirred at 40 °C overnight. The solvent was removed in vacuo, and the isolated crude residue was purified by flash column chromatography using a gradient solvent system, viz., 1:1 ethyl acetate/CH₂Cl₂ to 5% MeOH/CH₂Cl₂. The pure product 5a (310 mg, 72%) was obtained as a white solid. Mp: 138–139 °C. 1H NMR (CDCl₃): δ 7.43 (d, J = 8.3 Hz, 2H), 7.28 (t, J = 7.4 Hz, 2H), 7.20 (d, J = 8.4 Hz, 4H), 7.15–7.19 (m, 3H), 6.39 (br s, 1H), 5.04 (s, 1H), 3.23 (q, J = 6.7 Hz, 2H), 3.05 (s, 2H), 2.64 (t, J = 7.6 Hz, 2H), 1.61–1.69 (m, 2H), 1.48–1.55 (m, 2H). 13C NMR (CDCl₃): δ 168.0, 142.0, 139.0, 132.1, 130.0, 128.54, 128.52, 126.1, 121.9, 53.6, 39.8, 36.0, 35.6, 29.2, 28.8. Anal. (C₅H₁₃NO₂S) C, H, N.

2-(4-Propyl)methylsulfinylacetamide (5b). Compound 5b was synthesized as described for 5a using compound 4c (680 mg, 1.73 mmol) to give the product 5b (510 mg, 72%) as a white solid. Mp: 75–77 °C. 1H NMR (CDCl₃): δ 7.70 (d, J = 8.0, 6.0 Hz, 4H), 7.60 (d, J = 8.6, 2.6 Hz, 4H), 6.70 (br s, 1H), 5.71 (br s, 1H), 5.40 (s, 1H), 3.56 (d, J = 14.0 Hz, 1H), 3.12 (d, J = 14.2 Hz, 1H). 13C NMR (CDCl₃): δ 165.3, 137.8, 130.7, 131.4 (JCF = 33.2 Hz), 131.2 (JCF = 33.2 Hz), 129.9, 129.3, 126.6 (JCF = 3.7 Hz), 126.0 (JCF = 3.7 Hz), 123.8 (JCF = 272 Hz), 123.6 (JCF = 273 Hz), 69.6, 51.8. Anal. (C₅H₁₃NO₂S) C, H, N.
was evaporated in vacuo to give the free base 6a (7.90 g, 90% yield) as a yellow oil. Some of the isolated free base was converted into the oxalate salt. Mp: 177–179 °C.1H NMR (CDCl3): δ 7.43 (d, J = 8.0 Hz, 4H), 7.31 (t, J = 7.4 Hz, 4H), 7.22 (tt, J = 7.4, 1.5 Hz, 2H), 5.16 (s, 1H), 2.81 (t, J = 6.2 Hz, 2H), 2.51 (t, J = 6.4 Hz, 2H).13C NMR (CDCl3): δ 141.5, 128.7, 128.4, 127.4, 54.0, 41.0, 36.7. Anal. (C19H23NS·H2O·HCl) C, H, N.

2-(Bis[4-chlorophenyl]methyl)[thio]ethan-1-amine (6b). Compound 6b was synthesized from bis-[4-chlorophenyl]methanol, 3c, according to general procedure D with a reaction time of 50 min. The hydrochloride salt product 6b (1.06 g, 62% yield) was obtained as an off-white solid. Mp: 179–181 °C.1H NMR (HCl salt, DMSO-d6): δ 8.12 (br s, 1H), 7.48 (d, J = 8.4 Hz, 4H), 7.41 (d, J = 8.8, 2.2 Hz, 4H), 5.56 (s, 1H), 2.94 (t, J = 7.6 Hz, 2H), 2.59 (t, J = 7.2 Hz, 2H).13C NMR (HCl salt, DMSO-d6): δ 140.2, 131.6, 130.2, 120.5, 50.2, 54.7, 54.3, 48.1, 32.9, 11.4, 3.5. Anal. (C19H23Cl2NS·H2O·HCl) C, H, N.

58.7% yield) was obtained as a yellow oil. Some of the isolated free base was converted into the oxalate salt. Mp: 211–213 °C.1H NMR (CDCl3): δ 7.42 (d, J = 7.7 Hz, 4H), 7.30 (t, J = 7.6 Hz, 4H), 7.22 (tt, J = 7.2, 1.6 Hz, 2H), 5.17 (s, 1H), 2.74 (t, J = 6.4 Hz, 2H), 2.58 (t, J = 6.2 Hz, 2H), 2.53 (t, J = 7.2 Hz, 2H), 1.40–1.47 (m, 2H), 1.27–1.37 (m, 2H), 0.90 (s, J = 6.4 Hz, 3H).13C NMR (CDCl3): δ 141.6, 128.7, 128.4, 127.3, 54.2, 49.3, 48.3, 32.8, 32.3, 20.6, 14.1. Anal. (C19H18Cl2NS·HCl) C, H, N.

The filtrate was evaporated in vacuo to give a liquid residue, which was taken up in aqueous 1 M NaOH (30 mL) and extracted with ethyl acetate (2×25 mL). The organic extract was washed with brine (50 mL), dried over Na2SO4/MgSO4 mixture, and concentrated in vacuo. The crude product was purified by flash column chromatography using 5% diethyl ether/hexanes (with 0.5% NEt3) to give the free base 6e (0.22 g, 44% yield) as a yellow oil. Some of the isolated free base was converted into the oxalate salt. Mp: 209–211 °C.1H NMR (CDCl3): δ 7.42 (d, J = 7.2 Hz, 4H), 7.30 (t, J = 7.6 Hz, 4H), 7.22 (tt, J = 7.2, 1.6 Hz, 2H), 5.17 (s, 1H), 2.74 (t, J = 6.4 Hz, 2H), 2.58 (t, J = 6.2 Hz, 2H), 2.53 (t, J = 7.2 Hz, 2H), 1.40–1.47 (m, 2H), 1.27–1.37 (m, 2H), 0.90 (s, J = 6.4 Hz, 3H).13C NMR (CDCl3): δ 141.6, 128.7, 128.4, 127.3, 54.2, 49.3, 48.3, 32.8, 32.3, 20.6, 14.1. Anal. (C19H18NS·H2O·HCl) C, H, N.

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Compound 7a was synthesized with slight modifications to a published procedure.2 Briefly, a solution of sodium periodate ([NaO₄] charge, 2.25 g, 10.5 mmol) in water (50 mL) was added in a dropwise manner to a solution of the hydrochloride salt of compound 6a (2.80 g, 10.0 mmol) in ethanol (150 mL) at 0 °C. The reaction was allowed to stir and warm to room temperature for ~20 h under an argon atmosphere. The reaction mixture, which contained a white precipitate, was cooled, filtered, and washed twice with 5 mL of cold bu. The resulting fi was dissolved in CHCl₃, washed with an aqueous NaHCO₃ solution, distilled water, and aqueous brine, and dried over Na₂SO₄. After filtration, solvent was removed in vacuo to give the crude, free base of compound 7a. The crude product was purified by flash column chromatography using a MeOH/CHCl₃ (with 0.1% NH₄OH gradient (from 0% to 1% MeOH) to give pure 7a (1.12 g, 43% yield) as a yellow oil. Some of the isolated free base was converted to the oxalate salt. Mp: 161–163 °C. H NMR (CDCl₃): δ 7.50 (d, J = 7.8 Hz, 2H), 7.31–7.44 (m, 3H), 4.90 (s, 1H), 3.10–3.23 (m, 2H), 2.53–2.65 (m, 2H). 13C NMR (CDCl₃): δ 135.8, 135.2, 132.4, 128.7, 128.5, 128.3, 73.1, 54.4, 36.5. Anal. (C₁₉H₂₅NOS·H₂O) C, H, N.

**Sulfinylethanes.** 2-(Benzhydrylsulfinyl)ethan-1-amine (7b). Compound 7b was synthesized as described for 7a from compound 6e (0.070 g, 0.23 mmol) and NaO₄ (0.053 g, 0.25 mmol) in an ethanol/water (1:3 dilution in water of saturated NaHCO₃ solution), filtered. The resulting fi was centrifuged at 30000 g for 10 min at 4 °C. The fi was resuspended in buffer and centrifuged again. The final fi was resuspended in cold buffer to a concentration of 15 mg/mL OWW. Experiments were conducted in assay tubes containing 0.5 mL of buffer, 1.4 nM [3H]citalopram (Kᵦ = 1.94 nM, specific activity = 83 Ci/mmol; Perkin-Elmer Life Sciences), 1.5 mg of brain stem tissue, and various concentrations of inhibitor. The reaction was started with the addition of the tissue, and the tubes were incubated for 60 min at room temperature. Nonspecific binding was determined using 10 μM fluoxetine.

**NET Binding Assay.** Membranes from frozen frontal cortex dissected from male Sprague–Dawley rat brains (supplied on ice from Bioreclamation (Hicksville, NY), prepared by homogenizing tissues in 20 volumes (w/v) of ice cold modified sucrose phosphate buffer (0.32 M sucrose, 7.74 mM NaHPO₄, 2.26 mM NaH₂PO₄, pH adjusted to 7.4) using a Brinkman Polytron (setting 6 for 20 s), and centrifuged at 30000 g for 10 min at 4 °C. The resulting fi was resuspended in buffer, centrifuged, and suspended in buffer again to a concentration of 10 mg/mL, original wet weight (OWW). Experiments were conducted in assay tubes containing 0.5 mL of sucrose phosphate buffer, 0.5 nM [3H]WIN 35,428 (Kᵦ = 5.3, specific activity 84 Ci/mmol; Perkin-Elmer Life Sciences), 1.0 mg of tissue OWW, and various concentrations of inhibitor. The reaction was started at the addition of tissue, and the tubes were incubated for 120 min on ice. Nonspecific binding was determined using 100 μM cocaine hydrochloride.**
software, GraphPad Software, Inc., San Diego, CA) of the displacement data, giving IC_{50} values, from which affinities (K_i values) were calculated using the Cheng–Prusoff equation.\cite{10}

**Molecular Pharmacology. Site-Directed Mutagenesis.** Synthetic cDNA encoding the human DAT (synDAT) was subcloned into pcDNAs (Invitrogen, Carlsbad, CA). cDNA encoding the human SERT (hSERT) was cloned into the pUbIz expression vector. Mutations herein were generated by the QuickChange method (adapted from Stratagene, La Jolla, CA) and confirmed by restriction enzyme mapping and DNA sequencing. Positive clones were amplified by transformation into XL1 blue competent cells (Stratagene), positive colony picked, and grown in LB media overnight at 37 °C in an orbital incubator (Infors) at 200 rpm. Plasmids were harvested using the maxi prep kit provided by Qiagen according to the manufacturer’s manual.

**Cell Culture and Transfection.** COS-7 cells were grown in Dulbecco’s modified Eagle’s medium 041 0188S supplemented with 10% fetal calf serum, 2 mM t-glutamine, and 0.01 mg/mL gentamicin at 37 °C in 10% CO_2. Wild-type and mutant constructs were transiently transfected into COS-7 cells with Lipo2000 (Invitrogen) according to the manufacturer’s manual using cDNA:Lipo2000 ratios of 3:6 and 2:6 for hDAT and hSERT, respectively.

[^1]: [H]Dopamine and [3H]-5-HT Uptake Experiments. Uptake assays were performed essentially as previously described\cite{11} using [2,5,6,7,8-3H](dihydroxyphenyl)ethyamine ([3H]DA; 94.4 Ci/mmol, Perkin-Elmer) or 5-[1,2-3H(N)]hydroxytryptamine ([3H]-5-HT; 28 Ci/mmol, Perkin-Elmer) for hDAT- and hSERT-expressing cells, respectively. Transiently transfected COS-7 cells were plated in 12-well (3 x 10^4 cells/well) or 24-well (10^4 cells/well) dishes coated with polyornithine to achieve an uptake level of no more than 10% of the total added radioligand. The uptake assays were carried out 2 days after transfection. Prior to the experiment, the cells were washed once in 500 μL of uptake buffer (25 mM HEPES, 130 mM NaCl, 5.4 mM KCl, 1.2 mM CaCl_2, 1.2 mM MgSO_4, 1 mM L-glutamine, and 1 μM catechol O-methyltransferase inhibitor Ro 41-0960 (Sigma), pH 7.4) at room temperature. All the tested ligands were solubilized in DMSO to obtain a stock concentration of 10 mM. From here the compounds were further diluted 10-fold in H_2O followed by consecutive dilutions in uptake buffer. The trace amounts of DMSO (maximum 1% for the highest added concentration) did not influence the binding affinity (Loland et al., unpublished experiments). The unlabeled ligand (e.g., modafinil ([±]-1) or analogues) was added to the cells in 10 concentrations from 1 nM to 0.1 mM equally distributed around the expected IC_{50} value, and uptake was initiated by addition of ~10 nM radioligand in a final volume of 500 μL. After 3 (for the hSERT) or 5 (hDAT) min of incubation, the reaction was stopped by rapid washing with 2 x 500 μL of ice cold uptake buffer, lysed in 250 μL (300 μL for 12-well plates) of 1% SDS, and left for 30 min at 37 °C with gentle shaking. All samples were transferred to 24-well counting plates, and 500 μL (or 600 μL) of Opti-phase Hi Safe 3 scintillation fluid (Perkin-Elmer) was added followed by counting of the plates in a Wallac Tri-Lux β-scintillation counter (Perkin-Elmer). Nonspecific uptake was determined in the presence of 5 μM paroxetine for hSERT-expressing cells and 50 μM nomifensine for hDAT-expressing cells. All determinations were performed in triplicate. Uptake data were analyzed by nonlinear regression analysis using Prism 5.0 from GraphPad Software. The IC_{50} values used in the estimation of K_i for uptake were calculated from the means of PIC_{50} values and the SE intervals from the PIC_{50} ± SE. The K_i values were calculated from the IC_{50} values using the equation K_i = IC_{50}/(1 + (L/K_m)) (L = concentration of [3H]DA or [3H]-5-HT).

**Molecular Modeling.** We docked the modafinil derivative compound 4h in our LeuT-based SERT model. The preparation and MD equilibration of the homology model of SERT were previously described.\cite{12} The compound was constructed and prepared for docking using LigPrep (Schrodinger Inc., Portland, OR). Docking of the compound was carried out with Glide (Schrodinger Inc.). The binding modes shown in Figure 2 were chosen on the basis of both the docking scores and the consistency with the (±)-1 pose in the previously modeled DAT–(±)-1 complexes.\cite{13}

**REFERENCES**


