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Novel and High Affinity 2-[(Diphenylmethyl)sulfinyl]acetamide (Modafinil) Analogues as Atypical Dopamine Transporter Inhibitors

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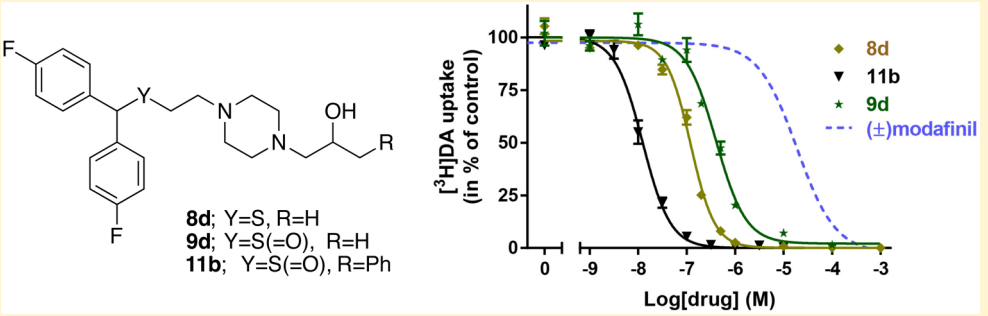
Supporting Information



ABSTRACT: The development of pharmacotherapeutic treatments of psychostimulant abuse has remained a challenge, despite significant efforts made toward relevant mechanistic targets, such as the dopamine transporter (DAT). The atypical DAT inhibitors have received attention due to their promising pharmacological profiles in animal models of cocaine and methamphetamine abuse. Herein, we report a series of modafinil analogues that have an atypical DAT inhibitor profile. We extended SAR by chemically manipulating the oxidation states of the sulfoxide and the amide functional groups, halogenating the phenyl rings, and/or functionalizing the terminal nitrogen with substituted piperazines, resulting in several novel leads such as **11b**, which demonstrated high DAT affinity (K_i = 2.5 nM) and selectivity without producing concomitant locomotor stimulation in mice, as compared to cocaine. These results are consistent with an atypical DAT inhibitor profile and suggest that **11b** may be a potential lead for development as a psychostimulant abuse medication.

INTRODUCTION

With millions of people worldwide suffering from substance use disorders, the development of pharmacotherapeutics for the treatment of addiction is imperative. While medications exist for treating alcohol and opiate addiction, there remains no FDA-approved medication to treat psychostimulant use disorder.[1,2] Psychostimulant drugs of abuse, such as cocaine and methamphetamine, bind to the dopamine transporter (DAT), inhibiting the reuptake of dopamine (DA) into the presynaptic neuron, increasing extracellular dopamine levels, and resulting in the behavioral activation and euphoria that can lead to addiction.[3−7] Although tremendous efforts have been made to develop dopamine uptake inhibitors to block the effects of cocaine and methamphetamine, most of these compounds share the psychostimulant effects and addictive liability of cocaine and have not been developed beyond preclinical studies.[8] One DAT inhibitor, GBR 12909 [**1**, 1-(2-(bis(4-fluorophenyl)methoxy)ethyl)-4-(3-phenylpropyl)-piperazine, Figure 1] was taken on into a Phase 1 clinical trial but failed before being tested for efficacy in the cocaine-abusing population.[9] These results dampened interest in the DAT as a potential therapeutic target for psychostimulant abuse and, indeed, negatively impacted the development of any drugs toward this target, despite the great success of methylphenidate, for example, a DAT inhibitor that has been used for decades to treat Attention Deficit Hyperactivity Disorder (ADHD).[10,11]

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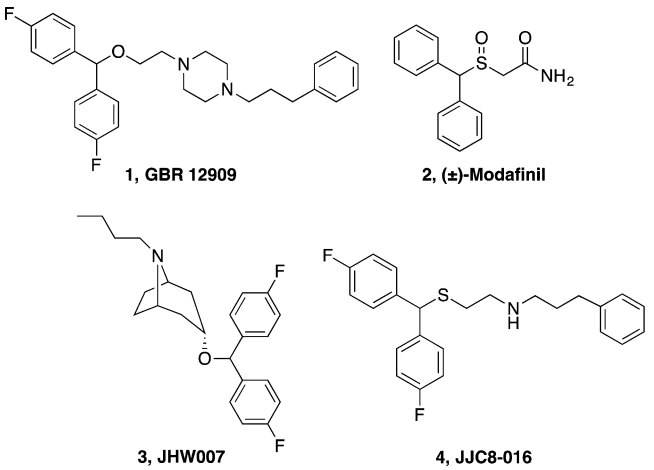


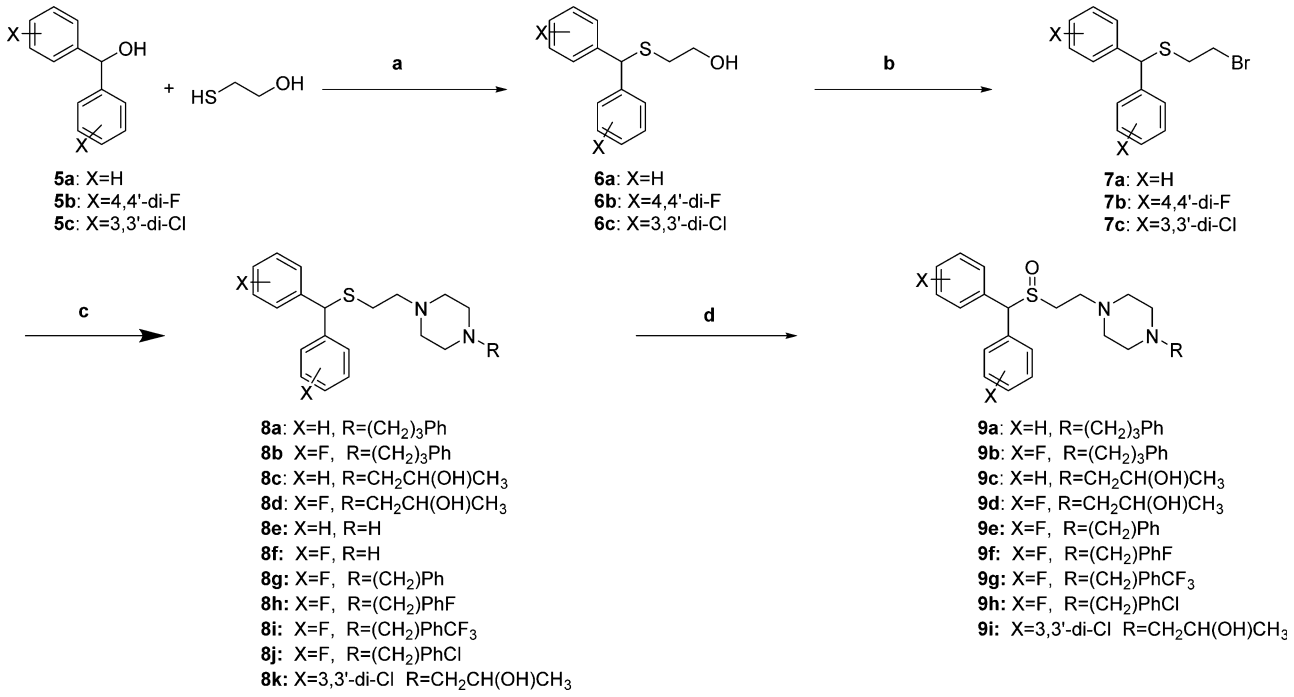
Figure 1. Chemical structures of known DAT inhibitors.

Another DAT inhibitor, (±)-modafinil (**2**, 2-[(diphenylmethyl)sulfinyl]acetamide, Figure 1) and its (*R*)-enantiomer (armodafinil) are FDA-approved to treat narcolepsy and other sleep disorders; however, they are also used off-label as cognitive enhancers, with little evidence of abuse liability in humans.[12] Though modafinil primarily targets the DAT, it does not share the same psychostimulant and abuse liability profile of cocaine.[13] Parallels to its atypical behavior in both humans[14−16] and psychostimulant abuse animal models,[17] along with its unique DAT binding profile have piqued interest for further drug development. In addition, as sleep and cognitive disorders have been linked to chronic drug abuse and the inability to remain abstinent,[18,19] modafinil has additional therapeutic attributes that may be useful in preventing relapse. Nevertheless, in clinical trials, modafinil has demonstrated limited effectiveness in treating cocaine abuse,[14−16,20,21] although a recent clinical study showed promising results in nonalcohol-dependent cocaine abusers.[22]

(±)-Modafinil and its (*R*)-enantiomer display unique pharmacological profiles, suggesting they may bind to a conformation of the DAT dissimilar to the cocaine-bound conformation and more similar to a class of "atypical" DAT inhibitors, based on benztropine (e.g., JHW 007, **3**, (3-(bis(4-fluorophenyl)methoxy)-8-butyl-8-azabicyclo[3.2.1]octane,[23] Figure 1).[13] An independent study on modafinil[24] supported these findings and led us to further investigate (*R*)-modafinil in rodent models of nicotine abuse.[25] These studies and previous investigations of several benztropine analogues suggested that atypical DAT inhibitors might attenuate the psychostimulant effects of cocaine and methamphetamine but may not have abuse liability, thereby leading us to further explore (*R*)-modafinil for the treatment of psychostimulant abuse.

Modafinil is essentially insoluble in water and has relatively low affinity for DAT (K_i = 2 μM), which in some ways makes it an excellent therapeutic, as it cannot be illicitly diverted into an injectable and/or abused drug; however, clinical studies in cocaine addicts who also abuse other substances suggest that modafinil is not an ideal medication for this patient population, especially for those who also abuse alcohol.[20−22] One possibility for its lack of effectiveness in reducing cocaine-taking in this patient population might be due to modafinil's low affinity for the DAT relative to cocaine, precluding it from efficiently blocking cocaine's rapid inhibition of dopamine uptake. Moreover, its low DAT affinity and poor water solubility make modafinil a challenging compound to study preclinically, and thus, our previous efforts[26,27] were aimed at improving its pharmacological profile via a structure−activity relationship (SAR) analyses at the DAT, norepinephrine transporter (NET), and serotonin transporter (SERT). Several compounds

Scheme 1. Synthesis of Compounds 8a−8k and 9a−9i[a]



[a]Reagents and conditions: (a) (i) TFA, CH_2Cl_2, r.t. overnight; (ii) K_2CO_3, H_2O/acetone, overnight; (b) PPh_3, CBr_4, CH_3CN, r.t. overnight; (c) appropriate piperazine, K_2CO_3, acetone, reflux, overnight; (d) H_2O_2, AcOH/MeOH, 40 °C, overnight.

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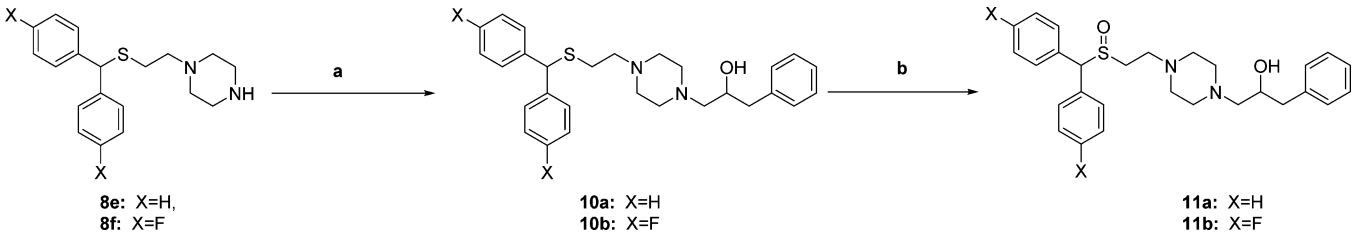
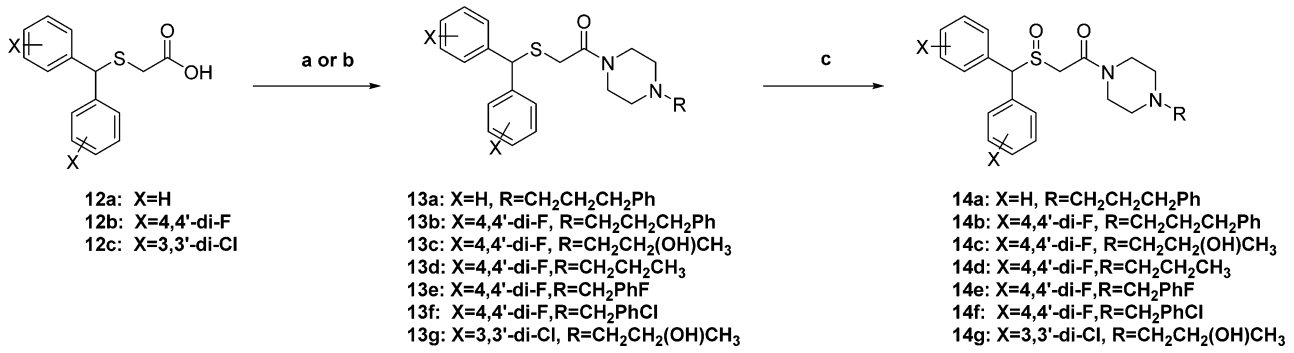
Scheme 2. Synthesis of Compounds 10a,b and 11a,b[a]



[a]Reagents and conditions: (a) 2-benzyloxirane, isopropanol, reflux, overnight; (b) H_2O_2, AcOH/MeOH, 40 °C, overnight.

Scheme 3. Synthesis of Compounds 13a−g and 14 a−g[a]



[a]Reagents and conditions: (a) (i) THF, CDI, r.t. 2 h; (ii) substituted piperazine analogue, THF, r.t. overnight; (b) (i) $SOCl_2$, reflux, 2 h; (ii) appropriate piperazine; (c) H_2O_2, AcOH/MeOH, 40 °C, overnight.

from those libraries showed improved water solubility and higher affinity for DAT (K_i < 1 μM) relative to modafinil; one lead compound arose and is currently being evaluated in behavioral models of cocaine and methamphetamine abuse (**4**, JJC8-016, *N*-(2-((bis(4-fluorophenyl)methyl)thio)ethyl)-3-phenylpropan-1-amine, Figure 1).[27]

In order to further improve DAT binding affinity and selectivity, we designed a series of compounds that extended SAR by chemically manipulating the oxidation states of the sulfoxide and the amide functional groups, halogenating the phenyl rings, and/or functionalizing the terminal nitrogen, borrowing the piperazine ring from **1**, as part of the scaffold.[28−34] All final compounds were evaluated for DAT, NET, and SERT binding in rat brains, and a subset was tested for binding to the σ1 receptor, as it has been previously posited that a dual DAT/σ1 profile may have therapeutic advantages over a highly DAT selective compound.[35−41] This subset was also tested for binding affinities at the dopamine D_2, D_3, and D_4 receptor subtypes and for efficacy in a D_2 Gi_1 Bioluminescence Resonance Energy Transfer (BRET) assay. In addition, we tested this subset of analogues for inhibition of [^{3}H]DA uptake and in a binding assay previously shown to determine if a compound possesses a classical or an atypical DAT binding profile, as reported for (±)-modafinil and its (*R*)-enantiomer.[13] These data suggest that the analogues do indeed appear to bind the DAT in this desired atypical fashion like modafinil and the benztropines, but unlike cocaine. This subset of compounds was also evaluated for metabolic stability in mouse liver microsomes, and the compound with highest DAT affinity and selectivity in this series, **11b**, was tested for its effects on locomotor activity in mice.

CHEMISTRY

Syntheses of novel sulfenylethanamine (**8a−8k**, **10a,b**) and sulfinylethanamine analogues (**9a−9i**, **11a,b**) were achieved as depicted in Schemes 1 and 2. Dehydration of commercially available benzhydrols **5a−c** with 2-mercaptoethan-1-ol in the presence of trifluoroacetic acid followed by treatment with K_2CO_3 in H_2O/acetone provided sulfide alcohols **6a−6c** in 61−95% yield. Appel reaction on **6a−6c** with triphenylphosphine and tetrabromomethane gave bromides **7a−7c** in 61−82% yield. Next, alkylation with the appropriate piperazine provided the sulfenylethanamine analogues **8a−8k** in 50−87% yield. Epoxide ring opening of 2-benzyloxirane using unsubstituted piperazine analogues **8e** and **8f** gave alcohols **10a** and **10b**, respectively (84% and 86% yield, respectively). Lastly, oxidation of the appropriate sulfenylethanamine was achieved using hydrogen peroxide in an acetic acid/methanol solution to give sulfinylethanamine (**9a−9i**, **11a,b**) in 43−78% yield.

Piperazine-substituted sulfenylacetamides **13a−13g** were generated via either synthetic routes a or b, outlined in Scheme 3. Compounds **13a−13c** and **13g** were synthesized through route a by amidation of carboxylic acids **12a−12c** using 1,1′-carbonyldiimidazole (CDI) and the appropriate amines in 71−90% yield. Synthetic route b utilized thionyl chloride to form the acid chloride intermediate of carboxylic acids **12a,b**, followed by coupling with the respective amine to afford **13d−13f** in 54−67% yield. Oxidation of the sulfenyl moiety gave the desired sulfinylacetamide **14a−14g** in 11−72% yield. All final compounds were purified and characterized in their free base form and then converted to the oxalate salts for testing. Note that all final compounds with sulfoxide groups and/or hydroxylated linking chains are the racemic and/or diastereomeric mixtures.

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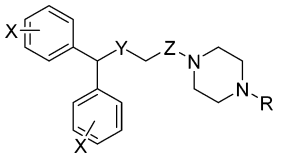
Table 1. Binding Data for Sulfenyl- and Sulfinylacetamide, Sulfenyl-, and Sulfinylethanamine Analogues[a]



compound	X	Y	Z	R	clogP	K_i (nM) ± SEM DAT	NET	SERT
GBR12909, **1**						1.77 ± 0.181[b]	497 ± 17.0[b]	104 ± 11.4[b]
modafinil, **2**						2520 ± 204[c]	IA[d]	IA[d]
3						10.5 ± 0.748	1670 ± 232	IA[d]
4					6.43	116 ± 16.3	3,848 ± 21.7	360 ± 48.3
8a	H	S	C	3-phenylpropyl	5.87	3.58 ± 0.157	988 ± 22.8	1,050 ± 152
8b	4,4′-diF	S	C	3-phenylpropyl	6.12	4.50 ± 0.344	1,890 ± 116	285 ± 35.4
8c	H	S	C	2-OH-propyl	3.13	49.6 ± 4.31	44,500 ± 2400	26,700 ± 2,630
8d	4,4′-diF	S	C	2-OH-propyl	3.42	16.7 ± 1.22	17,800 ± 885	1,770 ± 234
8g	4,4′-diF	S	C	benzyl	6.86	7.40 ± 1.04	8,240 ± 1,240	949 ± 122
8h	4,4′-diF	S	C	4-F-benzyl	6.92	4.98 ± 0.716	6,850 ± 633	463 ± 69.1
8i	4,4′-diF	S	C	4-CF_3-benzyl	7.74	26.6 ± 2.09	15,400 ± 1,950	2,020 ± 266
8j	4,4′-diF	S	C	4-Cl-benzyl	7.57	19.3 ± 1.91	7,410 ± 102	905 ± 121
8k	3,3′-diCl	S	C	2-OH-propyl	4.56	143 ± 19.2	31,300 ± 2,980	10,800 ± 1,030
9a	H	S=O	C	3-phenylpropyl	3.91	3.17 ± 0.112	5,540 ± 808	7,360 ± 881
9b	4,4′-diF	S=O	C	3-phenylpropyl	4.20	2.92 ± 0.383	4281 ± 343	678 ± 66.1
9c	H	S=O	C	2-OH-propyl	1.21	636 ± 14.7	ND[e]	ND[e]
9d	4,4′-diF	S=O	C	2-OH-propyl	1.49	289 ± 43.0	ND[e]	50,300 ± 5,760
9e	4,4′-diF	S=O	C	benzyl	4.93	27.2 ± 1.29	45,700 ± 8,480	11,200 ± 195
9f	4,4′-diF	S=O	C	4-F-benzyl	5.00	9.67 ± 1.37	>50 μM	8,520 ± 349
9g	4,4′-diF	S=O	C	4-CF_3-benzyl	5.81	39.4 ± 1.45	>50 μM	6,800 ± 468
9h	4,4′-diF	S=O	C	4-Cl-benzyl	5.64	7.62 ± 0.900	>50 μM	4,940 ± 665
9i	3,3′-diCl	S=O	C	2-OH-propyl	2.63	403 ± 23.8	>50 μM	58,900 ± 7,310
10a	H	S	C	2-OH-3-phenylpropyl	4.70	2.54 ± 0.233	1,430 ± 118	1,630 ± 169
10b	4,4′-diF	S	C	2-OH-3-phenylpropyl	5.00	6.72 ± 0.977	1,950 ± 227	213 ± 13.2
11a	H	S=O	C	2-OH-3-phenylpropyl	2.78	3.43 ± 0.499	25,300 ± 2,040	21,700 ± 2,020
11b	4,4′-diF	S=O	C	2-OH-3-phenylpropyl	3.06	2.53 ± 0.250	15,000 ± 575	4,610 ± 562
13a	H	S	C=O	3-phenylpropyl	6.37	47.2 ± 5.56	22,600 ± 3,010	9,320 ± 932
13b	4,4′-diF	S	C=O	3-phenylpropyl	6.66	28.6 ± 1.52	20,200 ± 1,410	3,170 ± 275
13c	4,4′-diF	S	C=O	2-OH-propyl	4.03	214 ± 20.7	ND[e]	11,400 ± 902
13d	4,4′-diF	S	C=O	propyl	5.36	277 ± 12.5	ND[e]	5,970 ± 497
13e	4,4′-diF	S	C=O	4-F-benzyl	6.16	53.7 ± 1.89	ND[e]	6,210 ± 579
13f	4,4′-diF	S	C=O	4-Cl-benzyl	6.74	58.7 ± 5.05	ND[e]	2,200 ± 177
13g	3,3′-diCl	S	C=O	2-OH-propyl	5.17	915 ± 84.5	>50 μM	ND[e]
14a	H	S=O	C=O	3-phenylpropyl	4.67	33.0 ± 2.83	54,300 ± 3,210	15,200 ± 1,100
14b	4,4′-diF	S=O	C=O	3-phenylpropyl	5.00	37.6 ± 1.86	12,000 ± 1,430	1,320 ± 152
14c	4,4′-diF	S=O	C=O	2-OH-propyl	2.33	752 ± 87.4	ND[e]	32,800 ± 4,430
14d	4,4′-diF	S=O	C=O	propyl	3.66	279 ± 23.4	ND[e]	14,500 ± 1,430
14e	4,4′-diF	S=O	C=O	4-F-benzyl	4.47	74.3 ± 9.64	ND[e]	23,600 ± 1,030
14f	4,4′-diF	S=O	C=O	4-Cl-benzyl	5.04	56.3 ± 6.60	63,500 ± 5,290	9,460 ± 386
14g	3,3′-diCl	S=O	C=O	2-OH-propyl	3.47	1,380 ± 191	>50 μM	ND[e]

[a]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 Experimental Procedures. [b]Previously reported in Cao et al.[35] [c]Previously reported in Cao et al.[26] [d]IA; less than 50% inhibition at 100 μM. [e]ND; not determined.

BIOLOGICAL RESULTS AND DISCUSSION

SAR at DAT, SERT, and NET. All final compounds (**8**, **9**, **11**, **13**, and **14**) were evaluated for binding at the monoamine transporters (DAT, SERT, and NET) in rat brain membranes and compared to known DAT inhibitors **1**–**4** (Figure 1). Changes in DAT binding affinity and selectivity as a result of modifications to the modafinil structure were evaluated; SAR analyses explored the effects of (1) the sulfur oxidation state, (2) reducing the terminal amide to a secondary amine, (3) halogenating the benzhydrol, and (4) varying the amide/amine substituent. The binding affinities (K_i values) are presented in Table 1, where, in general, these novel analogues displayed low nanomolar DAT affinities and showed >100-fold selectivity for DAT over NET and/or SERT.

In general, the sulfoxides had similar affinities at DAT relative to their sulfide counterparts; however, affinities at SERT and NET generally decreased, resulting in more DAT-selective sulfoxides. The largest sulfide to sulfoxide improvement in

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Table 2. Off Target Binding Affinities for a Selected Subset of Analogues[a]

	K_i ± SEM (nM)					
compd	DAT	D_2R[a]	D_3R[a]	D_4R[a]	σ_1	σ_1/DAT
(*R*)-**2**	3050 ± 258	>100,000	39,000 ± 1050	>100,000	>100,000	>300
3	10.5 ± 0.748	68.1 ± 7.81	34.4 ± 6.47	583 ± 128	2.4	5
4	116 ± 16.3	228 ± 27.6	65.9 ± 12.1	28.1 ± 5.22	159 ± 22.7	1.4
8d	16.7 ± 1.22	693 ± 159	424 ± 117	6300 ± 1460	4.03 ± 0.22	0.24
9d	289 ± 43.0	298 ± 61.1	480 ± 184	3820 ± 876	1010 ± 134	3.5
11b	2.53 ± 0.25	78.6 ± 16.6	652 ± 105	1750 ± 593	336 ± 42.2	133
eticlopride	ND[b]	0.26 ± 0.06	0.11 ± 0.03	70.4 ± 2.33	ND[b]	ND[b]

[a]Each K_i value represents data from at least three independent experiments, each performed in duplicate. K_i values were analyzed by PRISM. Binding assays are described in detail in Experimental Procedures. [b]ND; not determined.

DAT selectivities was seen for the *N*-bearing 2-hydroxypropyl and 2-hydroxyphenylpropyl derivatives (compared to their deshydroxy counterparts). In particular, oxidation of hydroxyl-containing sulfide **10b** to its sulfoxide **11b** gave nominal improvement in DAT affinity (K_i = 6.72 to 2.53 nM, respectively), but a 7- and 20-fold decrease in SERT and NET affinities, respectively, was observed for sulfoxide **11b**, improving DAT selectivity. In contrast, the deshydroxy counterparts sulfide **8b** and sulfoxide **9b** showed no significant difference in DAT K_i values, while SERT/NET selectivities decreased only ∼2-fold for the oxidized compound.

To prevent potential metabolism on the benzhydrol moiety, 4,4′-difluorination was probed, resulting in varying effects on the monoamine transporter binding affinities. When the unsubstituted **8a**, **9a**, **10a**, and **11a** were 4,4′-difluoro-substituted to give **8b**, **9b**, **10b**, and **11b**, respectively, no appreciable change was observed in DAT and SERT K_i values, although NET affinity improved between 5- and 10-fold. DAT affinity (Ki = 143−1380 nM) was lost when the benzhydrol was 3,3′-dichloro-substituted in **8k**, **9i**, **13g**, and **14g**.

The phenylpropyls (**8a**, **8b**, **9a**, **9b**, **13a**, **13b**, **14a**, and **14b**) and 2-hydroxyphenylpropyls (**10a**, **10b**, **11a**, and **11b**) generally demonstrated high binding affinities for DAT, with DAT K_i = 2.5−47 nM. The removal of the phenyl group resulted in a loss of affinity at DAT, implying that an *N*-arylalkyl substituent may be optimal. For example, when the phenyl group is removed from **10a** and **11a** (K_i = 2.5 and 3.4, respectively) to give **8c** and **9c**, respectively, DAT affinity decreases by 25- and 187-fold. In general, the *N*-benzyl analogues (e.g., **8g**−**8j** and **9e**−**9h**) also demonstrated high DAT affinities, but metabolic instability was problematic in this subset (see below).

The 2-hydroxy analogues were prepared based on previous SAR[42] to give compounds with lower cLogP values (Table 1). While 2-hydroxylation of the propyl and phenylpropyl substitutions did not appreciably change K_i values at DAT, the 2-hydroxy sulfoxides **9d**, **11a**, **11b**, and **14c** resulted in a significant decrease in NET and SERT affinities. In contrast, 2-hydroxylation of the linker in sulfides **10a** and **10b** gave only nominal loss in NET or SERT K_i values. Of note, cLogP values were typically in the >5-range for 4,4′-difluoro substituted-analogues without the sulfoxide function or hydroxylated terminal *N*-substituents.

Off-Target Binding Profiles: Dopamine D_2, D_3, D_4, and σ1 Receptors. On the basis of the DAT, SERT, and NET binding results and cLogP values, a subset of analogues (**8d**, **9d**, and **11b**) was then evaluated for binding affinities at dopamine D_2, D_3, and D_4 receptors in HEK 293 cells, as well as σ1 receptors in rat brains and compared to known compounds (*R*)-modafinil [(***R***)-**2**], **3**, and **4**. As can be seen in Table 2, (*R*)-modafinil is highly selective for DAT compared to these off-target receptors but has relatively low affinity for the DAT. In contrast, the atypical DAT inhibitor in the benztropine class, **3**, has relatively high affinities for dopamine D_2 and D_3 receptors and has higher affinity for σ1 than for DAT (σ1/DAT = 0.2). Likewise, our previous lead compound **4**[27] had low affinity for D_2 receptors but moderate affinities for D_3 and D_4 and had comparable affinities at σ1 and DAT (σ1/DAT = 1.4). Compound **8d** was selective for DAT over the D_2-like receptors but had the highest affinity for σ1 in the series (K_i = 4 nM) resulting in a σ1/DAT ratio similar to **3**. Compound **9d** had the lowest affinity for DAT in the group (although ∼10-fold higher than (***R***)-**2**), and its selectivity across the off-target sites was relatively low. Nevertheless, compared to that of the classic D_2 antagonist, eticlopride, the D_2-like receptor affinities are very low. Indeed, in Table 3, it can be seen that although all of these compounds were inhibitors of quinpirole-stimulated D_2 receptor activation of Gi_1, all of them had very low potency. Hence, we conclude that they are very weak antagonists at D_2 receptors and that this off-target activity would likely not affect their behavioral profiles. Finally, in this series, compound **11b** emerged as the highest affinity and most selective DAT inhibitor (Tables 2 and 3).

Table 3. Inhibition of Dopamine D_2 Receptor Gi_1 Activation Using 1 μM Quinpirole[a]

compound	IC_{50} (nM)
(*R*)-**2**	>10,000
3	2000 ± 730
4	>10,000
8d	>10,000
9d	>10,000
11b	>10,000
eticlopride	2.1 ± 0.8 nM

[a]Results were obtained from G protein activation-BRET experiments in HEK-293T cells transiently transfected with Gαi1-RLuc8 and γ2-GFP10 in cells expressing D_2R. Data were fit by nonlinear regression to a sigmoidal dose−response relationship against the ligand concentration (prestimulated with 1 μM quinpirole). The IC_{50} values are the mean ± SEM of 5 experiments performed in triplicate.

Molecular Pharmacology and Mutagenesis Studies. To assess the effect on DAT function, this subset of analogues (**4**, **8d**, **9d**, and **11b**) was tested for inhibition of [^{3}H]DA uptake. Accordingly, COS7 cells transiently expressing DAT were employed. The analogues were added in increasing concentrations followed by a fixed concentration of [^{3}H]DA to

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allow transport. The reaction was stopped after 5 min, and the amount of [^{3}H]DA taken up by the COS7 cells was determined by scintillation counting and plotted as a function of the concentration of added analogue (Figure 2). All analogues from this subset possessed higher inhibition potency than (±)-modafinil. In line with the binding data from rat brain membranes, compound **11b** emerged as the DA uptake inhibitor with the highest potency (K_i = 12 [10;14] nM, mean [SEM interval], n = 3). Compound **8d** had an inhibitory potency similar to that of cocaine (K_i = 200 [140;290] nM and 200 [110;350] nM, respectively, mean [SEM interval], n = 3−4), whereas both compounds **4** and **9d** had significantly lower K_i values than cocaine (890 [810;980] nM and 1730 [1370;2170] nM, respectively, mean [SEM interval], n = 6−8). Although all DAT inhibitors by definition block DA uptake, cocaine's binding preference to an outward facing conformation of DAT has been described experimentally, computationally,[43,44] and more recently demonstrated in the X-ray crystal structure of the drosophila DAT.[45] It has been proposed that the stabilization of the outward facing DAT conformation by cocaine is, at least in part, mediated by its interference with a H-bond that forms between the OH-group of Tyr156 in TM3 and Asp79 in TM1. This has been substantiated experimentally by showing that the binding mode of cocaine is independent of the OH-group in Tyr156;[44] however, when the DAT moves to a more outward occluded and ultimately closed conformation, a critical H-bond forms between Y156 and D79, closing the outer "gate." This conformation seems to be preferred by substrates, such as dopamine, amphetamine, and MDMA and also the atypical DAT inhibitors, such as the benztropines[44,46] [e.g., **3**]. Accordingly, in contrast to cocaine, the binding of modafinil and other atypical DAT inhibitors does depend on the presence of the OH-group since the Y156F mutation affects their binding affinity.[13,43] To assess whether or not compounds **4**, **8d**, **9d**, and/or **11b** demonstrated an atypical DAT inhibitor binding mode, their binding affinities were evaluated in wild type (WT) DAT and the Y156F mutant (Figure 3 and Table 4). The results were compared to the effects of (±)-modafinil (**2**), **3**, and cocaine (Figure 3 and Table 4). Inhibition of [^{3}H]-WIN35,428 binding on COS7 cells transiently expressing WT DAT or Y156F was determined. In contrast to cocaine, (±)-modafinil (**2**) and all the tested analogues showed a significant decrease in binding affinity for Y156F relative to WT DAT. Remarkably, **11b** was particularly affected with a Y156F/WT ratio of ∼15, similar to that found for [(***R***)-**2**] (Figure 3 and Table 4). Also, **9d** shows a marked change (7.6-fold, Table 4), whereas **4** and **8d** were affected to a lesser degree (2.8 and 2.6-fold, respectively, Figure 4 and Table 4). Interestingly, the sulfoxide function was present in the three compounds [(***R***)-**2**, **9d** and **11b**)] that were most impacted by the Y156F mutation. For comparison, the classical atypical DAT inhibitor, **3**, had a Y156F/WT ratio of 5.5.

Metabolic Stability in Mouse Microsomes. On the basis of their in vitro profiles, compounds **8d**, **9d**, and **11b** were tested for phase I metabolism following procedures previously described[47] to predict the susceptibility to metabolism following in vivo administration. As depicted in Figure 4, compounds **8d** and **9d** exhibited good stability with approximately 50% of both compounds remaining in mouse microsomes fortified with NADPH. Corresponding in vitro half-lives for **8d** and **9d** were calculated to be ∼60 min confirming compounds stable to CYP dependent phase I metabolism. In contrast, compound **11b** undergoes substantial phase I metabolism with only ∼4% remaining in microsomes fortified with NADPH at 1 h, suggesting CYP dependent metabolism of the compound and a relatively short in vitro half-life of 13 min. Of note, compounds **9f**, **9g**, **9h**, **11a**, **13c**, **14b**, and **14e** were all metabolized in <30 min in the mouse liver microsomes.

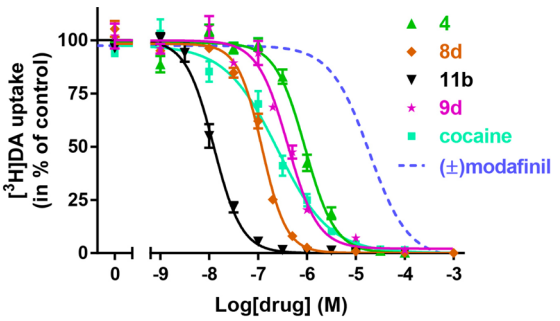

Figure 2. Inhibition potency of [^{3}H]DA uptake by modafinil analogues and cocaine. COS7 cells transiently expressing DAT wild type were incubated with [^{3}H]DA at the indicated concentrations of **4** (green), **8d** (brown), **9d** (magenta), **11b** (black), and cocaine (cyan). The inhibition potency of **2** as assessed previously[13] is inserted for comparison (dotted line). Data are the means ± SEM of 3−10 experiments performed in triplicate.

Locomotor Activity in Mice Compared to Cocaine. Figure 5 shows the effects of (*R*)-modafinil and its analogues **4** and **11b** on locomotor activity compared to cocaine. Systemic administration (i.p.) of cocaine (3, 10 mg/kg) produced a robust dose-dependent increase in locomotion (Figure 5A, cocaine treatment main effect, $F_{2,21}$ = 10,82, p < 0.001, two-way ANOVA), while (*R*)-modafinil and compounds **4** and **11b**, at the same dose (10 mg/kg), produced no significant or a very mild locomotor response. When the dose was increased to 30 mg/kg, (*R*)-modafinil and **11b** produced a significant dose-dependent increase, while **4** did not. Two-way ANOVA for repeated measures over time revealed a statistically significant treatment main effect for (*R*)-modafinil (Figure 5B, $F_{2,21}$ = 10.01, p < 0.001) and for compound **11b** (Figure 5D, $F_{2,21}$ = 4.35, p < 0.005) but not for compound **4** (Figure 5C, $F_{2,21}$ = 0.65, p = ns). Although the assay did not show a significant treatment main effect for compound **4**, it revealed a significant time main effect ($F_{11,231}$ = 5.11, p < 0.001) and treatment × time interactions ($F_{21,231}$ = 1.73, p < 0.05). Therefore, posthoc individual group comparisons revealed statistically significant differences between the different dose groups at certain time points (Figure 5C).

Most typical DAT inhibitors increase locomotor activity in mice; indeed, this is one behavioral hallmark of this class of agents. In contrast, we and others have reported "atypical" DAT inhibitors that, despite binding with high affinity and selectivity to the DAT and inhibiting DA uptake, are not efficacious locomotor stimulants and do not exhibit other cocaine-like behaviors. (±)-Modafinil and its (*R*)-enantiomer display unique pharmacological profiles[12,13,24,48] that suggest they may be atypical. Herein, compound **11b** displayed the highest DAT affinity in the series with good selectivity over other off-targets (Tables 2 and 3). As seen in Figure 5, compound **11d**, does increase locomotor activity in mice, but it is far less efficacious than cocaine suggesting that this modafinil analogue is an atypical DAT inhibitor. In addition, **11b** demonstrated the highest Y156F/WT ratio in the mutagenesis study (Table 4,

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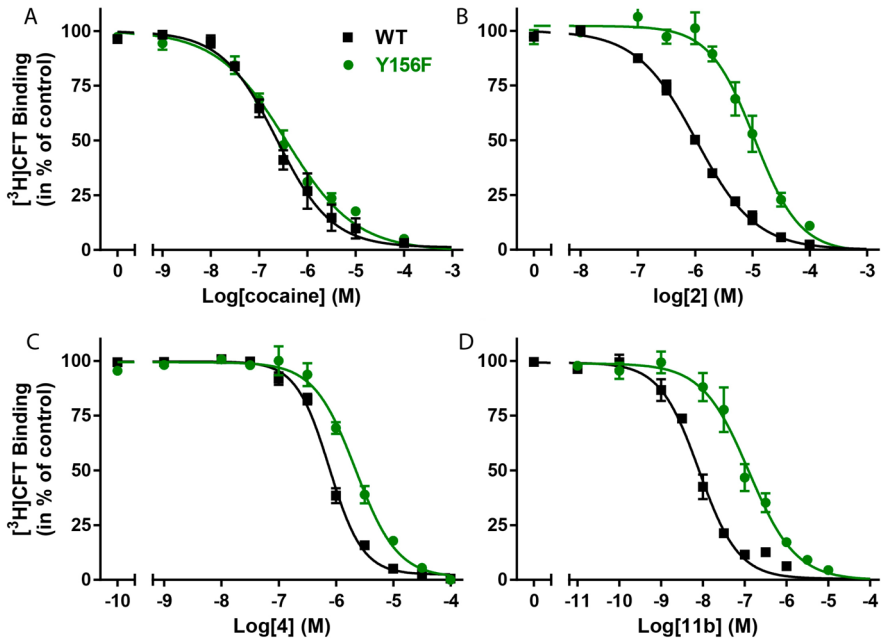

Figure 3. Assessment of DAT binding profiles by modafinil analogues. As demonstrated previously,[13,43] a characteristic feature for atypical DAT inhibitors is that, in contrast to cocaine, their binding depends on the presence of the OH-group on Tyr156. Inhibition of [^{3}H]WIN35,428 binding to DAT WT (black) or the Y156F (green) mutant by (A) cocaine, (B) **2**, (C) **4**, and (D) **11b**. The fold change in IC_{50} values between WT and Y156F is 1.4, 10, 2.8, and 14.9 for cocaine, **2**, **4**, and **11b**, respectively. Binding assays are performed as triplicates on COS7 cells transiently expressing DAT WT or Y156F (n = 3−6). Data are shown as the means ± SEM.

Table 4. Assessment of Atypical Binding Properties for Selected Analogues[a]

	WT	Y156F	Y156F/WT
compd	K_i (nM)	K_i (nM)	affinity ratio
2	927 [834; 1030]	9250 [7350; 11700]	10.0
(*R*)-**2**	647 [593;704]	8920 [6930;11500]	13.8
(*S*)-**2**	1960 [1700;2270]	5910 [4640; 7520]	3.0
3	47 [33;66]	260 [229;296]	5.5
4	720 [661;784]	2050 [1840;2280]	2.8
8d	101 [82;125]	267 [227; 314]	2.6
9d	1200 [824;1760]	9170 [7680; 10900]	7.6
11b	6.5 [5.1;8.3]	97 [67;140]	14.9
cocaine	223 [160;309]	321 [235; 438]	1.4

[a]Inhibition of [^{3}H] WIN35,428 binding to DAT WT or the Y156F mutant. COS7 cells transiently expressing DAT WT or Y156F were assessed for affinity (K_i) for the indicated compounds. Data were analyzed by nonlinear regression analysis using Prism 6.0 (GraphPad). The IC_{50} values were calculated from means of pIC_{50} values and the SEM interval from pIC_{50} ± SEM. The K_i values were calculated from the IC_{50}-values using the equation $K_i = IC_{50}/(1 + (L + K_d)$, where K_d is the affinity for WIN35,428, and L is the concentration of added [^{3}H]WIN35,428). All data are performed in triplicate, n = 3−6.

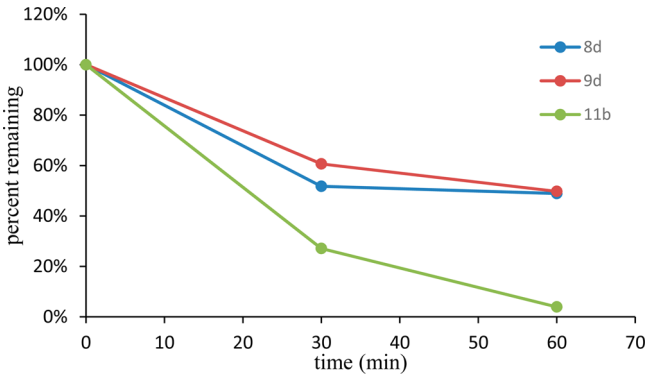

Figure 4. Phase I metabolic stability of selected analogues in mouse liver microsomes. Compounds **8d**, **9d**, and **11b** were tested in mouse liver microsomes fortified with NADPH. Compounds **8d** and **9d** both showed slow metabolism with a half-life of approximately 60 min suggesting the compounds to be stable. Compound **11b**, on the contrary, had a much shorter half-life of 13 min suggesting rapid metabolism. Note: control experiments without cofactors were conducted in parallel, and in all cases, >95% compound remained at 60 min suggesting that the compounds were undergoing Cyp dependent metabolism. Testosterone was used as a positive control.

Figure 3) suggesting that it binds the DAT in a more occluded conformation, unlike cocaine.

CONCLUSION

In summary, we have designed and synthesized a series of modafinil analogues that have higher DAT affinity than the parent molecule and have extended SAR at the DAT by manipulating the oxidation states of the sulfoxide and the amide, halogenating the phenyl rings, and/or functionalizing the terminal nitrogen with *N*-substituted piperazines. Compounds **8d**, **9d**, and **11b** were selected as lead compounds from this series and were tested for binding to σ1, as well as dopamine D_2, D_3, and D_4 receptor subtypes, and for efficacy in a D_2 Gi_1 BRET assay. In addition, we tested this subset of analogues in cell-based [^{3}H]DA uptake and binding assays for affinities at both the WT and the Y156F DAT mutant, in order to determine if they demonstrated an atypical binding profile, as previously reported for (±)-modafinil and its (*R*)-enantiomer.[13] These data suggest that all three lead compounds did indeed bind the DAT in a conformation that was more like the atypical benztropines and unlike cocaine, with **11b** having the highest DAT affinity and selectivity in this series. We

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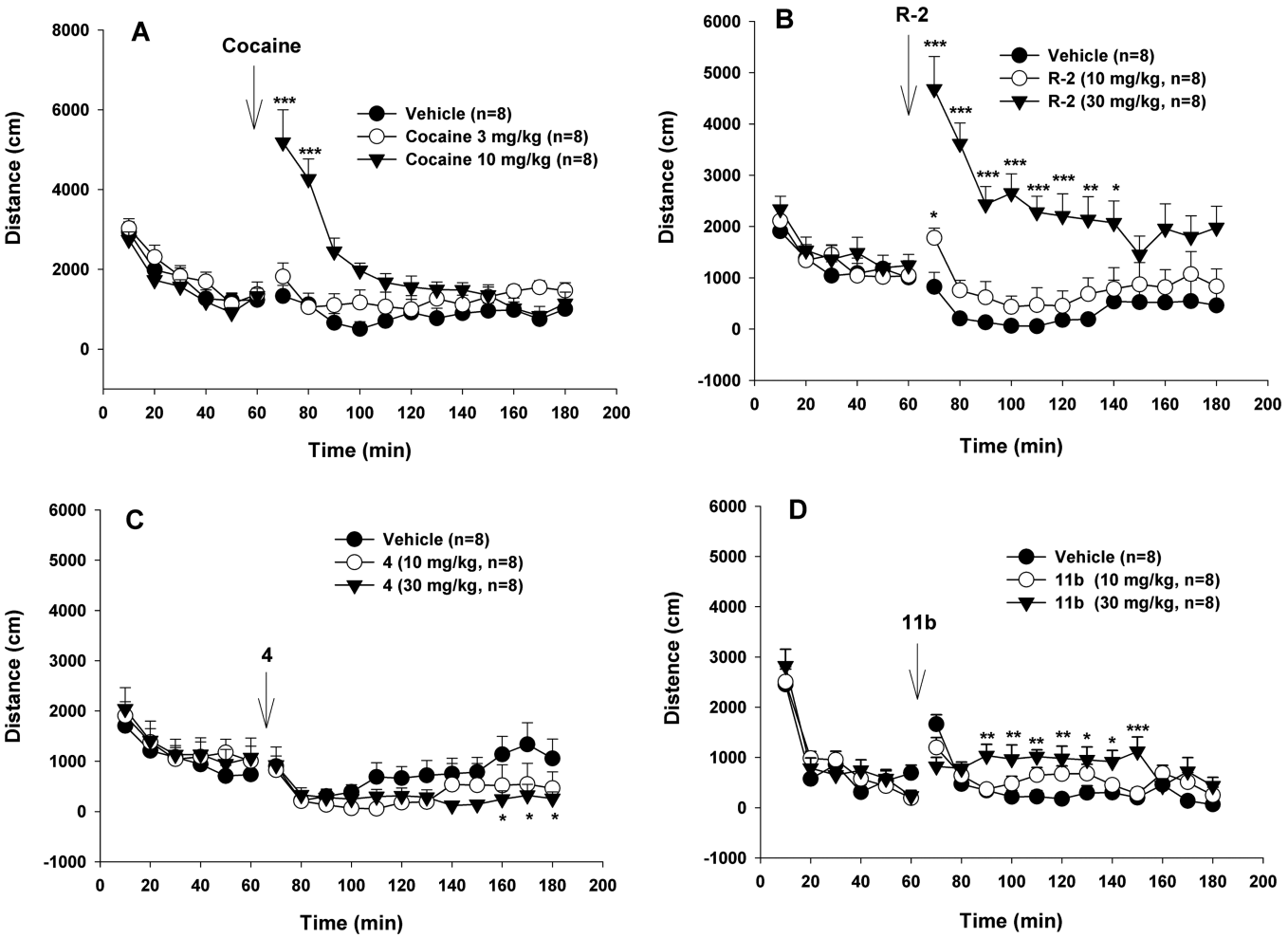

Figure 5. Locomotor effects of (*R*)-modafinil [(***R***)-**2**] and its analogues **4** and **11b** compared to cocaine in mice. Systemic administration of cocaine (3, 10 mg/kg, i.p.) produced a robust dose-dependent increase in locomotion (A). However, (***R***)-**2** and its analogues **4** and **11b**, at the same dose of 10 mg/kg, did not (C,D) or produced a very mild increase (B) in locomotion. When the dose was increased to 30 mg/kg, (***R***)-**2** and **11b** produced a significant dose-dependent increase in locomotion, while its analogue **4** did not. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$, compared to the vehicle control group at each time point marked.

discovered, that similar to previously reported atypical DAT inhibitors,[49−53] compound **11b** produced only moderate locomotor stimulation in mice and was substantially less efficacious than cocaine. These results are consistent with an atypical DAT inhibitor profile and suggest that **11b** may be a potential lead for development as a psychostimulant abuse therapeutic. Further investigations of this compound in rodent models of cocaine and methamphetamine abuse, along with compounds **4**, **8d**, and **9d**, are underway to extend testing of the atypical DAT inhibitor hypothesis, as well as to further investigate the role of σ1 receptors in the behavioral profile of these agents. Finally, although **11b** showed significant metabolism in mouse microsomes, preliminary data show a rapid and high brain to plasma ratio, suggesting that **11b** can penetrate the blood−brain barrier sufficiently to block DA uptake via the DAT. Nevertheless, efforts to modify this structural template in order to improve metabolic stability are also ongoing and will be reported in due course.

EXPERIMENTAL PROCEDURES

Synthesis. ^{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 NMR spectra ($CDCl_3$, 7.26 or DMSO-d_6, 2.50) and ^{13}C NMR spectra ($CDCl_3$, 77.2 or DMSO-d_6, 39.5). Gas chromatography−mass spectrometry (GC/MS) data were acquired (where obtainable) using an Agilent Technologies (Santa Clara, CA) 6890N GC 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. Combustion analysis was performed by Atlantic Microlab, Inc. (Norcross, GA), and the results agree within ±0.5% of calculated values. Melting point determination was conducted using a Thomas-Hoover melting point apparatus and is uncorrected. On the basis of NMR and combustion data, all final compounds are >95% pure.

*2-(Benzhydrylthio)ethan-1-ol (**6a**).* 2-Mercaptoethan-1-ol (7.8 g, 100 mmol) was added to commercially available diphenylmethanol (3.7 g, 20 mmol) in TFA (40 mL) and CH_2Cl_2 (40 mL) at 0 °C. The solution was warmed to room temperature and stirred overnight. The solvent was removed, K_2CO_3 (11 g, 80 mmol), H_2O (7 mL) and acetone (25 mL) were added to the reaction residue, and the mixture stirred at room temperature overnight. The solvent was removed, H_2O (100 mL) was added to the residue obtained, and the aqueous mixture was extracted with ethyl acetate (3 × 100 mL). The organic layer was dried over $MgSO_4$, and the solvent was removed *in vacuo*. The crude product was purified by flash column chromatography (hexane/ethyl

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acetate = 6:4) to give **6a** (3.0 g, 61% yield) as a clear oil. GC/MS (EI) m/z 244 (M^+). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43−7.45 (m, 4H), 7.30−7.34 (m, 4H), 7.21−7.26 (m, 2H), 5.21 (s, 1H), 3.64−3.69 (m, 2H), 2.62−2.65 (m, 2H).

*2-((Bis(4-fluorophenyl)methyl)thio)ethan-1-ol (**6b**).* Compound **6b** was prepared as described for **6a** using bis(4-fluorophenyl)-methanol (6.6 g, 30 mmol) to give the product (6.9 g, 82% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35−7.39 (m, 4H), 6.99−7.03 (m, 4H), 5.20 (s, 1H), 3.68−3.70 (m, 2H), 2.59−2.62 (m, 2H).

*2-((Bis(3-chlorophenyl)methyl)thio)ethan-1-ol (**6c**).* Compound **6c** was synthesized as described for compound **6a** using bis(3-chlorophenyl)methanol (2.84 g, 11.2 mmol) to give the product (3.34 g, 95% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (s, 2H), 7.31−7.22 (m, 6H), 5.14 (s, 1H), 3.71 (t, J = 5.8 Hz, 2H), 2.63 (t, J = 6.0 Hz, 2H), 1.93 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.5, 134.6, 130.0, 128.3, 127.9, 126.4, 60.6, 52.8, 35.3.

*Benzhydryl(2-bromoethyl)sulfane (**7a**).* Triphenylphosphine (1.4 g, 5.3 mmol) was added to a solution of **6a** (890 mg, 3.64 mmol) in CH_3CN (12 mL), followed by the addition of carbon tetrabromide (1.77 g, 5.34 mmol). The reaction was stirred at room temperature overnight. The solvent was removed, and the crude product was purified by flash column chromatography (hexane/ethyl acetate = 5:1) to give **6a** (850 mg, 76% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23−7.45 (m, 10H), 5.23 (s, 1H), 3.33−3.39 (m, 2H), 2.80−2.90 (m, 2H); GC/MS (EI) m/z 307 (M^+).

*(Bis(4-fluorophenyl)methyl)(2-bromoethyl)sulfane (**7b**).* Compound **7b** was prepared as described for **7a** using **6b** (6.9 g, 25 mmol) to give the product (7.0 g, 83% yield) as a light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34−7.37 (m, 4H), 6.00−7.04 (m, 4H), 5.21 (s, 1H), 3.36−3.40 (m, 2H), 2.81−2.85 (m, 2H); GC/MS (EI) m/z 343 (M^+).

*(Bis(3-chlorophenyl)methyl)(2-bromoethyl)sulfane (**7c**).* **7c** was prepared as described for **7a** using **6c** (3.23 g, 10.3 mmol) to give product as colorless oil (3.03 g, 78% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (s, 2H), 7.28−7.22 (m, 6H), 5.14 (s, 1H), 3.39 (t, J = 8.0 Hz, 2H), 2.86 (t, J = 8.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.3, 134.9, 130.2, 128.5, 128.2, 126.5, 53.6, 34.5, 30.2.

*1-(2-(Benzhydrylthio)ethyl)-4-(3-phenylpropyl)piperazine (**8a**).* A mixture of **7a** (850 mg, 2.76 mmol), commercially available 1-(3-phenylpropyl)piperazine (564 mg, 2.76 mmol), K_2CO_3 (1.52 g, 11.0 mmol), and KI (catalytic) in acetone (30 mL) was refluxed overnight. The solvent was removed, H_2O (50 mL) was added to the residue, and the aqueous mixture was extracted with ethyl acetate (3 × 50 mL). The organic layer was dried over $MgSO_4$, the solvent was removed *in vacuo*, and the crude product was purified by flash column chromatography [ethyl acetate/triethylamine = 95:5] to give **8a** (810 mg, 61% yield) as a yellow oil. The free base was converted to the oxalate salt and recrystallized from methanol to give a white solid. Mp 210 °C (dec.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16−7.43 (m, 15H), 5.22 (s, 1H), 2.33−2.64 (m, 16H), 1.78−1.82 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.1, 141.4, 128.5, 128.4, 128.3, 127.2, 125.7, 58.0, 54.5, 54.4, 53.1, 53.0, 33.7, 29.3, 28.6; Anal. ($C_{28}H_{34}N_2S\cdot 2C_2H_2O_4\cdot 0.25H_2O$) C, H, N.

*1-(2-((Bis(4-fluorophenyl)methyl)thio)ethyl)-4-(3-phenylpropyl)-piperazine (**8b**).* Compound **8b** was prepared as described for **8a** using **7b** (950 mg, 2.76 mmol) to give the product (940 mg, 73% yield) as a yellow oil. The free base was converted to the oxalate salt and recrystallized from methanol to give a white solid. Mp 216−217 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34−7.37(m, 4H), 7.24−7.29(m, 2H), 7.15−7.19(m, 3H), 6.97−7.01(m, 4H), 5.20 (s, 1H), 2.33−2.64 (m, 16H), 1.67−1.82 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.1, 160.7, 142.1, 137.0, 129.8, 129.7, 128.4, 128.3, 125.7, 115.6, 115.3, 58.0, 53.1, 52.9, 52.8, 33.7, 29.4, 28.6; Anal. ($C_{28}H_{32}F_2N_2S\cdot 2C_2H_2O_4$) C, H, N.

*1-(4-(2-(Benzhydrylthio)ethyl)piperazin-1-yl)propan-2-ol (**8c**).* Compound **8c** was prepared as described for (**8a**) using **7a** (848 mg, 2.76 mmol) and 1-(piperazin-1-yl)propan-2-ol (398 mg, 2.76 mmol) to give the product (850 mg, 83% yield) as a yellow oil. The free base was converted to the oxalate salt and recrystallized from methanol to give a white solid. Mp 209−210 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41−7.43 (m, 4H), 7.19−7.32 (m, 6H), 5.21 (s, 1H), 3.77−3.82 (m, 1H), 3.41 (br, 1H), 2.17−2.67 (m, 14H), 1.11−1.14 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 141.4, 128.6, 128.5, 128.3, 127.2, 65.6, 62.2, 57.9, 54.5, 54.4, 53.1, 29.3, 20.0; Anal. ($C_{22}H_{30}N_2OS\cdot 2C_2H_2O_4\cdot 0.25H_2O$) C, H, N.

*1-(4-(2-((Bis(4-fluorophenyl)methyl)thio)ethyl)piperazin-1-yl)-propan-2-ol (**8d**; JJC 8-089).* Compound **8d** was prepared as described for **8a** using **7b** (950 mg, 2.76 mmol) and 1-(piperazin-1-yl)propan-2-ol (398 mg, 2.76 mmol) to give the product (880 mg, 79% yield) as a yellow oil. The free base was converted to the oxalate salt and recrystallized from methanol to give a white solid. Mp 205−206 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33−7.37 (m, 4H), 6.96−7.02 (m, 4H), 5.19 (s, 1H), 3.77−3.82 (m, 1H), 3.41 (br,1H), 2.18−2.69 (m, 14H), 1.11−1.13 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.1, 160.7, 137.0, 129.8, 129.7, 115.7, 115.6, 115.5, 115.4, 65.5, 62.2, 57.8, 53.1, 52.9, 29.4, 20.0; Anal. ($C_{22}H_{28}F_2N_2OS\cdot 2C_2H_2O_4$) C, H, N.

*1-(2-(Benzhydrylthio)ethyl)piperazine (**8e**).* A mixture of **7a** (1.4 g, 4.6 mmol), piperazine (2.35 g, 27.3 mmol), and K_2CO_3 (1.05 g, 9.12 mmol) in acetonitrile (25 mL) was refluxed overnight. The solvent was removed, H_2O (100 mL) was added to the residue, and the aqueous mixture was extracted with ethyl acetate (3 × 100 mL). The organic layer was dried over $MgSO_4$, and solvent was removed *in vacuo*. The crude product was purified by flash column chromatography ($CHCl_3$/MeOH/NH_4OH = 90/10/0.5) to give **8e** (710 mg, 50% yield) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41−7.43 (m, 4H), 7.19−7.32 (m, 6H), 5.22 (s, 1H), 2.83−2.85 (m, 4H), 2.52−2.54 (m, 4H), 2.34−2.37 (m, 4H).

*1-(2-((Bis(4-fluorophenyl)methyl)thio)ethyl)piperazine (**8f**).* Compound **8f** was prepared as described for **8e** using **7b** (1.03 g, 3.00 mmol) to give the product (910 mg, 87% yield) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34−7.37 (m, 4H), 6.97−7.26 (m, 4H), 5.20 (s, 1H), 2.84−2.86 (m, 4H), 2.50−2.52 (m, 4H), 2.35−2.37 (m, 4H).

*1-Benzyl-4-(2-((bis(4-fluorophenyl)methyl)thio)ethyl)piperazine (**8g**).* Compound **8g** was prepared as described as **8a** using **7b** (687 mg, 2.00 mmol) and 1-benzylpiperazine (353 mg, 2.00 mmol) to give the product as a yellow oil (530 mg, 60%). The free base was converted to the oxalate salt and recrystallized from methanol to give a white solid. Mp 224−226 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22−7.38 (m, 9H), 6.96−7.02 (m, 4H), 5.20 (s, 1H), 3.45−3.51 (m, 2H), 2.43−2.68 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.1, 160.4, 138.0, 137.0, 129.8, 129.7, 129.2, 128.2, 127.0, 115.7, 115.6, 115.5, 115.4, 63.0, 57.9, 53.1, 53.0, 52.9, 29.4. Anal. Calc.: C, 58.24, H, 5.21, N, 4.53. Found: C, 57.98, H, 5.16, N, 4.63. Anal. ($C_{26}H_{28}F_2N_2S\cdot 2C_2H_2O_4$) C, H, N.

*1-(2-((Bis(4-fluorophenyl)methyl)thio)ethyl)-4-(4-fluorobenzyl)-piperazine (**8h**).* Compound **8h** was prepared as described as **8a** using **7b** (515 mg, 1.50 mmol) and 1-(4-fluorobenzyl)piperazine (194 mg, 1.50 mmol) to give the product as a yellow oil (450 mg, 65.7%). The free base was converted to the oxalate salt and recrystallized from methanol to give a white solid Mp 221−223 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33−7.37 (m, 4H), 7.23−7.27 (m, 2H), 6.95−7.00 (m, 6H), 5.18 (s, 1H), 3.44−3.46 (m, 2H), 2.41−2.56 (m, 12H); ^{13}C NMR (100 MHz, CDCl3) δ 163.1, 160.4, 137.0, 133.8,130.6, 130.5, 129.8, 115.6, 115.4, 115.1, 114.9, 62.2, 57.9, 53.0, 52.9, 29.4; GC/MS (EI) m/z 456 (M^+). Anal. Calc.: C, 56.6, H, 4.91, N, 4.40. Found: C, 56.35, H, 4.91, N, 4.38. Anal. ($C_{26}H_{27}F_3N_2S\cdot 2C_2H_2O_4$) C, H, N.

*1-(2-((Bis(4-fluorophenyl)methyl)thio)ethyl)-4-(4-(trifluoromethyl)benzyl)piperazine (**8i**).* Compound **8i** was prepared as described as **8a** using **7b** (515 mg, 1.50 mmol) and commercially available 1-(4-(trifluoromethyl)benzyl)piperazine (367 mg, 1.50 mmol) to give the product as yellow oil (500 mg, 66%). The free base was converted to the oxalate salt and recrystallized from methanol to give a white solid Mp 223−224 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55−7.57 (m, 2H), 7.41−7.43 (m, 2H), 7.33−7.37 (m, 4H), 6.97−7.01 (m, 4H), 5.19 (s, 1H), 3.53−3.54 (m, 2H), 2.43−2.55 (m, 12H); ^{13}C NMR (100 MHz, CDCl3) δ 163.1, 160.7, 142.4, 137.0, 129.8, 129.7, 129.2, 125.2, 125.1, 115.6, 115.4, 62.4, 57.8, 53.0, 52.9, 29.4; GC/MS (EI) m/z 506 (M^+). Anal. Calc.: C, 53.87, H, 4.59, N, 4.05.

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Found: C, 53.78, H, 4.69, N, 4.05. Anal. ($C_{27}H_{27}F_5N_2S\cdot 2C_2H_2O_4\cdot 0.25H_2O$) C, H, N.

*1-(2-((Bis(4-fluorophenyl)methyl)thio)ethyl)-4-(4-chlorobenzyl)-piperazine (**8j**).* Compound **8j** was prepared as described as **8a** using **7b** (515 mg, 1.50 mmol) and commercially available1-(4-chlorobenzyl)piperazine (316 mg, 1.50 mmol) to give the product as a yellow oil (500 mg, 67.7%). The free base was converted to the oxalate salt and recrystallized from methanol to give a white solid Mp 224−225 °C; GC/MS (EI) *m/z* 473 (M^+); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33−7.37 (m, 4H), 7.22−7.28 (m, 4H), 6.97−7.01 (m, 4H), 5.19 (s, 1H), 3.44−3.45 (m, 2H), 2.41−2.54 (m, 12H); ^{13}C NMR (100 MHz, CDCl3) δ 163.1, 160.6, 137.0, 136.6, 132.8, 130.4, 129.8, 129.7, 128.3, 115.7, 115.6, 115.5, 115.4, 62.2, 57.8, 52.9, 29.4. Anal. Calc.: C, 54.80, H, 4.83, N, 4.26. Found: C, 54.61, H, 4.80, N, 4.28. Anal. ($C_{26}H_{27}ClF_2N_2S\cdot 2C_2H_2O_4\cdot 0.25H_2O$) C, H, N.

*1-(4-(2-((Bis(3-chlorophenyl)methyl)thio)ethyl)piperazin-1-yl)-propan-2-ol (**8k**).* Compound **8k** was prepared as described for **8a** using **7c** (866 mg, 2.30 mmol) and commercially available 1-(piperazin-1-yl)propan-2-ol (335 mg, 2.32 mmol) to give the product as a yellow oil (650 mg, 64% yield). The free base was converted to the oxalate salt in a 2-propanol/acetone solvent mixture and recovered as a white solid. Mp 119−121 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (s, 2H), 7.29−7.18 (m, 6H), 5.15 (s, 1H), 3.85−3.76 (m, 1H), 2.72−2.62 (br m, 2H), 2.59−2.18 (m, 13H), 1.13 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.8, 134.5, 129.96, 128.4, 127.7, 126.4, 65.5, 62.2, 60.4, 57.8, 53.5, 53.1, 29.5, 19.9; Anal. ($C_{22}H_{28}Cl_2N_2OS\cdot 2C_2H_2O_4$) C, H, N.

*1-(2-(Benzhydrylsulfinyl)ethyl)-4-(3-phenylpropyl)piperazine (**9a**).* Compound **9a** was prepared as previously described[8] using **8a** (431 mg, 1.00 mmol) to give the product (250 mg, 56% yield) as a yellow oil. The free base was converted to the hydrochloride salt and recrystallized from methanol to give a white solid. Mp 210 °C (dec.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.15−7.50 (m, 15H), 4.96 (s, 1H), 2.33−2.82 (m, 16H), 1.76−1.84 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.1, 136.0, 135.2, 129.3, 129.2, 128.7, 128.4, 128.3, 128.2, 125.8, 72.1, 57.9, 53.4, 53.0, 51.0, 48.2, 33.7, 28.6; Anal. ($C_{28}H_{34}N_2OS\cdot 2HCl\cdot 0.5H_2O$) C, H, N.

*1-(2-((Bis(4-fluorophenyl)methyl)sulfinyl)ethyl)-4-(3-phenylpropyl)piperazine (**9b**).* Compound **9b** was prepared as described for **9a** using **8b** (466 mg, 1.00 mmol) to give the product (290 mg, 60% yield) as a yellow oil. The free base was converted to the oxalate salt and recrystallized from methanol to give a white solid. Mp 204 °C (dec.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37−7.40(m, 4H), 7.24−7.28(m, 2H), 7.16−7.19(m, 3H), 7.05−7.11(m, 4H), 4.95 (s, 1H), 2.34−2.79 (m, 16H), 1.76−2.04 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.0, 163.8, 161.6, 142.1, 131.0, 130.6, 130.3, 128.4, 128.3, 125.8, 116.4, 116.2, 115.9, 115.6, 68.8, 68.2, 65.8, 57.9, 53.0, 50.9, 48.1, 33.7, 28.5; Anal. ($C_{28}H_{32}F_2N_2OS\cdot 2C_2H_2O_4\cdot H_2O$) C, H, N.

*1-(4-(2-(Benzhydrylsulfinyl)ethyl)piperazin-1-yl)propan-2-ol (**9c**).* Compound **9c** was prepared as described for **9a** using **8c** (556 mg, 1.50 mmol) to give the product (450 mg, 78% yield) as a white solid. The free base was converted to the oxalate salt and recrystallized from methanol to give a white solid. Mp 195−197 °C (dec.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30−7.49 (m, 10H), 4.95 (s, 1H), 3.78−3.82 (m, 1H), 3.39 (br s, 1H), 2.18−2.83 (m, 14H), 1.11−1.12 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 136.0, 135.1, 129.3, 129.2, 128.7, 128.6, 128.3, 72.2, 65.5, 62.2, 53.1, 50.9, 48.3, 20.0; Anal. ($C_{22}H_{30}N_2O_2S\cdot 2C_2H_2O_4\cdot 0.25H_2O$) C, H, N.

*1-(4-(2-((Bis(4-fluorophenyl)methyl)sulfinyl)ethyl)piperazin-1-yl)-propan-2-ol (**9d**; JJC8-091).* Compound **9d** was prepared as described for **9a** using **8d** (610 mg, 1.50 mmol) to give the product (340 mg, 54% yield) as a yellow oil. The free base was converted to the oxalate salt and recrystallized from methanol to give a white solid. Mp 190−191 °C (dec.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36−7.43 (m, 4H), 7.04−7.12 (m, 4H), 4.92 (s, 1H), 3.77−3.83 (m, 1H), 2.22−2.84 (m, 14H), 1.11−1.12 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.0, 163.8, 161.6, 161.3, 131.7, 131.0, 130.6, 130.5, 130.4, 130.3, 116.4, 116.2, 115.9, 115.6, 69.9, 69.8, 65.5, 62.3, 53.1, 50.8, 48.3, 20.0; Anal. ($C_{22}H_{28}F_2N_2O_2S\cdot 2C_2H_2O_4$) C, H, N.

*1-Benzyl-4-(2-((bis(4-fluorophenyl)methyl)sulfinyl)ethyl)-piperazine (**9e**).* Compound **5e** was prepared as described for **9a** using **8g** (307 mg, 0.700 mmol) to give the product (150 mg, 47.1% yield) as a yellow oil. The free base was converted to the oxalate salt and recrystallized from methanol to give a white solid. Mp 219−220 °C (dec.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37−7.44 (m, 4H), 7.24−7.33 (m, 5H), 7.05−7.11 (m, 4H), 5.30 (s, 1H), 3.50 (s, 2H), 2.45−2.80 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.9, 163.7, 161.5, 159.3, 138.0, 131.8, 129.2, 127.1, 115.6, 69.7, 63.0, 52.9, 50.9, 48.1; Anal. ($C_{26}H_{28}F_2N_2OS\cdot 2C_2H_2O_4$) C, H, N.

*1-(2-((Bis(4-fluorophenyl)methyl)sulfinyl)ethyl)-4-(4-fluorobenzyl)piperazine (**9f**).* Compound **9f** was prepared as described for **9a** using **8h** (251 mg, 0.549 mmol) to give the product (100 mg, 38.5% yield) as a yellow oil. The free base was converted to the oxalate salt and recrystallized from methanol to give a white solid. Mp 216−218 °C (dec.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37−7.43 (m, 4H), 7.24−7.27 (m, 2H), 6.96−7.11 (m, 6H), 4.94 (s, 1H), 3,46 (s, 2H), 2.44−2.78 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.0, 163.7, 163.2, 161.5, 161.3, 160.8, 133.5, 131.8, 131.7, 131.0, 130.7, 130.6, 130.5, 130.4, 130.3, 116.4, 116.2, 115.9, 115.6, 115.1, 114.9, 69.7, 62.1, 53.0, 52.7, 50.8, 48.1; Anal. ($C_{26}H_{27}F_3N_2OS\cdot 2C_2H_2O_4\cdot 0.5H_2O$) C, H, N.

*1-(2-((Bis(4-fluorophenyl)methyl)sulfinyl)ethyl)-4-(4-(trifluoromethyl)benzyl)piperazine (**9g**).* Compound **9g** was prepared as described for **9a** using **8i** (304 mg, 0.601 mmol) to give the product (100 mg, 47.8% yield) as a yellow oil. The free base was converted to the oxalate salt and recrystallized from methanol to give a white solid. Mp 216−218 °C (dec.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55−7.57 (m, 2H), 7.37−7.43 (m, 6H), 7.04−7.11 (m, 4H), 4.94 (s, 1H), 3.54 (s, 2H), 2.45−2.83 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.0, 163.8, 161.6, 161.3, 142.3, 131.8, 131.0, 130.5, 130.4, 130.3, 129.5, 129.2, 128.6, 125.6, 125.2, 125.1, 122.9, 116.4, 116.2, 115.9, 115.669.8, 62.3, 53.0, 52.9, 50.8, 48.2; Anal. ($C_{27}H_{27}F_5N_2OS\cdot 2C_2H_2O_4$) C, H, N.

*1-(2-((Bis(4-fluorophenyl)methyl)sulfinyl)ethyl)-4-(4-chlorobenzyl)piperazine (**9h**).* Compound **9h** was prepared as described for **9a** using **8j** (360 mg, 0.761 mmol) to give the product (160 mg, 43.0% yield) as a yellow oil. The free base was converted to the oxalate salt and recrystallized from methanol to give a white solid. Mp 217−219 °C (dec.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37−7.43 (m, 4H), 7.22−7.26 (m, 4H), 7.04−7.10 (m, 4H), 4.94 (s, 1H), 3.45 (s, 2H), 2.44−2.80 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$ δ 164.4, 164.0, 163.8, 161.5, 161.3, 136.4, 132.8, 131.7, 131.7, 131.0, 130.5, 130.4, 130.3, 128.4, 116.4, 116.2, 115.9, 115.6, 69.8, 62.1, 52.9, 52.8, 50.8, 48.1; Anal. ($C_{26}H_{27}ClF_2N_2OS\cdot 2C_2H_2O_4\cdot 0.25H_2O$) C, H, N.

*1-(4-(2-((Bis(3-chlorophenyl)methyl)sulfinyl)ethyl)piperazin-1-yl)-propan-2-ol (**9i**).* Compound **9i** was prepared as described for **9a** using **8k** (400 mg, 1.00 mmol) The free base (190 mg, 46% yield) was obtained as a yellow oil, which was converted to the oxalate salt in a 2-propanol/acetone solvent mixture and recovered as a cream-colored solid. Mp 192−194 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42−7.31 (m, 8H), 4.91 (s, 1H), 3.85−3.80 (m, 1H), 2.84−2.21 (m, 15H), 1.12 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.5, 136.4, 135.3, 134.7, 130.6, 130.1, 129.3, 128.80, 128.77, 127.5, 126.7, 70.4, 65.5, 62.3, 53.2, 50.7, 48.3, 19.9; Anal. ($C_{22}H_{28}Cl_2N_2O_2S\cdot 2C_2H_2O_4$) C, H, N.

*1-(4-(2-(Benzhydrylthio)ethyl)piperazin-1-yl)-3-phenylpropan-2-ol (**10a**).* A solution of compound **8e** (710 mg, 2.27 mmol) and 2-benzyloxirane (304.6 mg, 2.27 mmol) in isopropanol (24 mL) was refluxed overnight. Solvent was removed, and the reaction residue was purified by flash column chromatography (hexane/ethyl acetate/triethylamine = 49:49:2) to give **10a** (850 mg, 84% yield) as a yellow oil. The free base was converted to the oxalate salt and recrystallized from hot isopropanol to give a white solid. Mp 210−211 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40−7.43 (m, 4H), 7.20−7.32 (m, 11H), 5.22 (s, 1H), 3.88−3.93 (m, 1H), 3.45 (br s, 1H), 2.27−2.83 (m, 16H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 141.4, 138.3, 129.3, 128.6, 128.5, 128.3, 127.3, 127.2, 126.3, 67.2, 63.4, 57.9, 54.4, 53.1, 41.3, 29.3; Anal. ($C_{28}H_{34}N_2OS\cdot 2C_2H_2O_4\cdot 0.5H_2O$) C, H, N.

*1-(4-(2-((Bis(4-fluorophenyl)methyl)thio)ethyl)piperazin-1-yl)-3-phenylpropan-2-ol (**10b**).* Compound **10b** was prepared as described

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for **10a** using **8f** (455 mg, 1.31 mmol) to give the product (540 mg, 86% yield) as a yellow oil. The free base was converted to the oxalate salt and recrystallized from hot acetone to give a white solid. Mp 206−207 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19−7.37 (m, 9H), 6.96−7.02 (m, 4H), 5.18 (s, 1H), 3.88−3.93 (m, 1H), 3.45 (br s, 1H), 2.31−2.81 (m, 16H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.1, 160.7, 138.2, 137.0, 129.8, 129.7, 129.3, 128.3, 126.3, 115.6, 115.4, 67.2, 63.4, 57.8, 53.1, 52.9, 41.3, 29.4; Anal. ($C_{28}H_{32}F_2N_2OS\cdot 2C_2H_2O_4\cdot 0.25H_2O$) C, H, N.

*1-(4-(2-(Benzhydrylsulfinyl)ethyl)piperazin-1-yl)-3-phenylpropan-2-ol (**11a**).* Compound **11a** was prepared as described for **9a** using **10a** (534 mg, 1.20 mmol) to give the product (340 mg, 61% yield) as a yellow oil. The free base was converted to the oxalate salt and recrystallized from hot methanol to give a white solid. Mp 198−200 °C (dec.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20−7.49 (m, 15H), 4.95 (s, 1H), 3.88−3.92 (m, 1H), 2.31−2.83 (m, 16H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.2, 136.0, 135.2, 129.3, 129.2, 128.9, 128.7, 128.6, 128.4, 128.3, 126.3, 72.1, 67.3, 63.4, 53.0, 50.8, 48.2, 41.3; Anal. ($C_{28}H_{34}N_2O_2S\cdot 2C_2H_2O_4\cdot 0.25H_2O$) C, H, N.

*1-(4-(2-((Bis(4-fluorophenyl)methyl)sulfinyl)ethyl)piperazin-1-yl)-3-phenylpropan-2-ol (**11b**; JJC 8-088).* Compound **11b** was prepared as described for **11a** using **10b** (400 mg, 0.83 mmol) to give the product (280 mg, 68% yield) as a yellow oil. The free base was converted to the oxalate salt and recrystallized from hot methanol to give a white solid. Mp 198−200 °C (dec.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20−7.43 (m, 9H), 7.05−7.11 (m, 4H), 4.93 (s, 1H), 3.88−3.92 (m, 1H), 2.29−2.84 (m, 16H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.6, 161.3, 138.2, 131.8, 131.0, 130.9, 130.5, 130.3, 129.3, 128.4, 126.3, 116.4, 116.2, 115.9, 115.6, 69.4, 67.3, 63.4, 53.1, 50.8, 48.2, 41.3, Anal. ($C_{28}H_{32}F_2N_2O_2S\cdot 2C_2H_2O_4$) C, H, N.

*2-(Benzhydrylthio)-1-(4-(3-phenylpropyl)piperazin-1-yl)ethan-1-one (**13a**).* A mixture of CDI (583 mg, 3.60 mmol) and **12a**[27] (775 mg, 3.00 mmol) in THF (24 mL) was stirred at room temperature under argon. After 2 h of reaction time, 1-(3-phenylpropyl)piperazine (613 mg, 3.00 mmol) in THF (15 mL) was added, and the reaction mixture was stirred overnight. Solvent was removed, and the reaction residue was purified by flash column chromatography (ethyl acetate/triethylamine 95:5) to give **13a** (1.2 g, 90% yield) as a yellow oil. The free base was converted to the oxalate salt and recrystallized from hot isopropanol to give a white solid. Mp 92−95 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42−7.45 (m, 4H), 7.17−7.33(m, 11H), 5.34 (s, 1H), 3.57−3.60 (t, 2H, J = 5.0 Hz), 3.37−3.40 (t, 2H, J = 5.2 Hz), 3.18 (s, 2H), 2.62−2.66 (m, 2H), 2.34−2.39 (m, 6H), 1.78−1.84 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.2, 141.9, 140.7, 128.6, 128.5, 128.4, 128.3, 127.3, 125.8, 57.7, 54.1, 53.2, 52.7, 46.3, 41.9, 33.5, 28.4; Anal. ($C_{28}H_{32}N_2OS\cdot C_2H_2O_4\cdot 0.5H_2O$) C, H, N.

*2-((Bis(4-fluorophenyl)methyl)thio)-1-(4-(3-phenylpropyl)-piperazin-1-yl)ethan-1-one (**13b**).* Compound **13b** was prepared as described for **13a** using **12b**[27] (588 mg, 2.00 mmol) and 1-(3-phenylpropyl)piperazine (408 mg, 2.00 mmol) to give the product (650 mg, 71% yield) as a yellow oil. The free base was converted to the oxalate salt and recrystallized from hot methanol to give a white solid. Mp 123−125 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36−7.39 (m, 4H), 7.26−7.30 (m, 2H), 7.17−7.20 (m, 3H), 6.98−7.02 (m, 4H), 5.34 (s, 1H), 3.58−3.60 (t, 2H, J = *5.0 Hz*), 3.39−3.42 (t, 2H, J = 4.8 Hz), 3.15 (s, 2H), 2.62−2.66 (m, 2H), 2.34−2.41 (m, 6H), 1.79−1.83 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.1, 163.2, 160.7, 141.9, 136.3, 130.0, 128.4, 125.9, 115.6, 115.4, 57.7, 53.1, 52.7, 52.4, 46.4, 41.9, 33.5, 33.3, 28.4; Anal. ($C_{28}H_{30}F_2N_2OS\cdot C_2H_2O_4\cdot 0.25H_2O$) C, H, N.

*2-((Bis(4-fluorophenyl)methyl)thio)-1-(4-(2-hydroxypropyl)-piperazin-1-yl)ethan-1-one (**13c**).* Compound **13c** was prepared as described for **13a** using **12b** (588 mg, 2.00 mmol) and 1-(piperazin-1-yl)propan-2-ol (288 mg, 2.00 mmol) to give the product (630 mg, 75% yield) as a yellow oil. The free base was converted to the oxalate salt and recrystallized from hot methanol to give a white solid. Mp 163−165 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36−7.37 (m, 4H), 6.99−7.03 (m, 4H), 5.33 (s, 1H), 3.82−3.87 (m, 1H), 3.57−3.61 (m, 2H), 3.40−3.43 (m, 2H), 3.22 (s, 1H), 3.16 (s, 2H), 2.59−2.60 (m, 2H), 2.23−2.38 (m, 4H), 1.13−1.27 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.2, 163.2, 160.7, 136.2, 130.0, 115.6, 115.4, 65.5, 62.4, 53.2, 52.7, 52.4, 46.4, 41.9, 33.2, 21.1; Anal. ($C_{22}H_{26}F_2N_2O_2S\cdot C_2H_2O_4$) C, H, N.

*2-((Bis(4-fluorophenyl)methyl)thio)-1-(4-propylpiperazin-1-yl)-ethan-1-one (**13d**).* Compound **12b** (294 mg, 1.00 mmol) was refluxed in $SOCl_2$ (3 mL) for 2 h. The solvent was removed. The reaction mixture was added to 1-propylpiperazine (128 mg, 1 mmol), $NaHCO_3$ (500 mg, 6.00 mmol) in amylene-stabilized $CHCl_3$ (10 mL) and H_2O (5 mL) at 0 °C and stirred at room temperature for 2 h. H_2O (5 mL) was added to the reaction mixture, and the aqueous mixture was extracted with chloroform (3 × 10 mL). The organic layer was dried over $MgSO_4$, and the solvent was removed *in vacuo*. The crude product was purified by flash column chromatography (ethyl acetate/triethylamine = 95:5) to give **13d** (220 mg, 54% yield) as a yellow oil. The free base was converted to the oxalate salt and recrystallized from methanol and acetone to give a white solid. Mp 167−168 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36−7.40 (m, 4H), 6.98−7.03 (m, 4H), 5.34 (s, 1H), 3.58−3.60 (m, 2H), 3.40−3.42 (m, 2H), 3.16 (s, 2H), 2.37−2.41 (m, 4H), 2.28−2.31 (m, 2H), 1.47−1.52 (m, 2H), 1.13−1.27 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.1, 163.2, 160.7, 136.3, 130.0, 115.6, 115.4, 60.4, 53.2, 52.7, 52.4, 46.3, 41.9, 33.3, 19.9, 11.9; Anal. ($C_{22}H_{26}F_2N_2OS\cdot C_2H_2O_4$) C, H, N.

*2-((Bis(4-fluorophenyl)methyl)thio)-1-(4-(4-fluorobenzyl)-piperazin-1-yl)ethan-1-one (**13e**).* Compound **13e** was prepared as described for **13d** using **12b** (589 mg, 2.00 mmol) and 1-(4-fluorobenzyl)piperazine (389 mg, 2.00 mmol) to give the product (600 mg, 64% yield) as a yellow oil. The free base was converted to the oxalate salt and recrystallized from acetone to give a white solid. Mp 168−170 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35−7.39 (m, 4H), 7.25−7.28 (m, 2H), 6.98−7.03 (m, 6H), 5.34 (s, 1H), 3.57−3.60 (m, 2H), 3.46 (s, 2H), 3.38−3.41 (m, 2H), 3.15 (s, 2H), 2.37−2.40 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.1, 163.3, 163.2, 160.9, 160.7, 136.3, 133.3, 133.2, 130.6, 130.5, 130.0, 115.6, 115.4, 115.3, 115.1, 62.0, 52.8, 52.5, 52.4, 46.3, 41.9, 33.3; Anal. ($C_{26}H_{25}F_3N_2OS\cdot C_2H_2O_4\cdot 1.25H_2O$) C, H, N.

*2-((Bis(4-fluorophenyl)methyl)thio)-1-(4-(4-chlorobenzyl)-piperazin-1-yl)ethan-1-one (**13f**).* Compound **13f** was prepared as described for **13d** using **12b** (589 mg, 2.00 mmol) and 1-(4-chlorobenzyl)piperazine (411 mg, 2.00 mmol) to give the product (650 mg, 67% yield) as a yellow oil. The free base was converted to the oxalate salt and recrystallized from acetone/ethyl ether to give a white solid. Mp 165−167 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36−7.40 (m, 4H), 7.25−7.28 (m, 4H), 6.98−7.02 (m, 4H), 5.33 (s, 1H), 3.58−3.59 (m, 2H), 3.38−3.46 (m, 4H), 3.15 (s, 2H), 2.37−2.40 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.1, 163.2, 160.7, 136.3, 136.1, 133.0, 130.3, 130.0, 128.5, 115.6, 115.4, 62.0, 52.9, 52.6, 52.4, 46.3, 41.8, 33.3; Anal. ($C_{26}H_{25}ClF_2N_2OS\cdot C_2H_2O_4$) C, H, N.

*2-((Bis(3-chlorophenyl)methyl)thio)-1-(4-(2-hydroxypropyl)-piperazin-1-yl)ethan-1-one (**13g**).* Compound **13g** was prepared as described for **13a** using **12c**[27] (654 mg, 2.00 mmol) and 1-(piperazin-1-yl)propan-2-ol (288 mg, 2.00 mmol) to give the product (770 mg, 75% yield) as a yellow oil. The free base was converted to the oxalate salt and recrystallized from hot methanol to give a white solid. Mp 160−161 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28−7.41 (m, 2H), 7.22−7.24 (m, 6H), 5.30 (s, 1H), 3.82−3.87 (m, 1H), 3.56−3.62 (m, 2H), 3.40−3.43 (m, 2H), 3.20−3.22 (m, 3H), 2.59−2.65 (m, 2H), 2.23−2.40 (m, 4H), 1.13−1.15 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.9, 142.1, 134.6, 130.0, 128.4, 127.9, 126.7, 65.5, 62.3, 53.1, 52.9, 52.7, 46.4, 41.9, 33.2, 19.9; Anal. ($C_{22}H_{26}Cl_2N_2O_2S\cdot C_2H_2O_4$) C, H, N.

*2-(Benzhydrylsulfinyl)-1-(4-(3-phenylpropyl)piperazin-1-yl)ethan-1-one (**14a**).* Compound **14a** was prepared as described for **9a** using **13a** (667 mg, 1.50 mmol) to give the product (500 mg, 72%) as a yellow oil. The free base was converted to the oxalate salt and recrystallized from hot acetone to give a white solid. Mp 180−182 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49−7.55 (m, 4H), 7.16−7.43 (m, 11H), 5.30 (s, 1H), 3.27−3.70 (m, 6H), 2.61−2.65 (m, 2H), 2.33−2.47 (m, 6H), 1.75−1.83 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.1, 141.9, 136.0, 133.6, 130.0, 129.1, 129.0, 128.7, 128.5, 128.4,

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128.3, 128.2, 125.8, 70.1, 70.0, 57.5, 53.1, 52.6, 46.4, 42.0, 33.5, 28.4; Anal. ($C_{28}H_{32}N_2O_2S·C_2H_2O_4·0.25H_2O$) C, H, N.

*2-((Bis(4-fluorophenyl)methyl)sulfinyl)-1-(4-(3-phenylpropyl)-piperazin-1-yl)ethan-1-one (**14b**).* Compound **14b** was prepared as described for **9a** using **13b** (480 mg, 1.00 mmol) to give the product (200 mg, 40%) as a yellow oil. The free base was converted to the oxalate salt and recrystallized from hot acetone to give a white solid. Mp 135−136 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45−7.52 (m, 4H), 7.06−7.30 (m, 9H), 5.36 (s, 1H), 3.54−3.69 (m, 3H), 3.27−3.45 (m, 3H), 2.61−2.65 (m 2H), 2.35−2.45 (m, 6H), 1.76−1.84 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.2, 163.8, 162.8, 162.7, 161.7, 161.3, 160.2, 141.8, 131.8, 131.7, 130.7, 130.6, 129.0, 128.4, 125.9, 116.2, 116.0, 115.9, 115.7, 67.7, 57.5, 53.1, 52.7, 52.6, 46.4, 42.0, 33.5, 28.3; Anal. ($C_{28}H_{30}F_2N_2O_2S·2C_2H_2O_4$) C, H, N.

*2-((Bis(4-fluorophenyl)methyl)sulfinyl)-1-(4-(2-hydroxypropyl)-piperazin-1-yl)ethan-1-one (**14c**).* Compound **14c** was prepared as described for **9a** using **13c** (600 mg, 1.43 mmol) to give the product (70 mg, 16%) as a yellow oil. The free base was converted to the oxalate salt and recrystallized from hot acetone to give a white solid. Mp 162−164 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44−7.51 (m, 4H), 7.06−7.13 (m, 4H), 5.36 (s, 1H), 3.83−3.88 (m, 1H), 3.24−3.67 (m, 7H), 2.60−2.67 (m, 2H), 2.26−2.42 (m, 4H), 1.13−1.14 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.2, 163.8, 162.9, 161.7, 161.3, 131.7, 131.6, 131.5, 130.7, 129.1, 116.3, 116.1, 116.0, 65.4, 62.4, 53.3, 53.2, 52.7, 52.6, 52.4, 52.3, 46.4, 42.0, 20.0; Anal. ($C_{22}H_{26}F_2N_2O_3S·C_2H_2O_4$) C, H, N.

*2-((Bis(4-fluorophenyl)methyl)sulfinyl)-1-(4-propylpiperazin-1-yl)-ethan-1-one (**14d**).* Compound **14d** was prepared as described for **9a** using **13d** (120 mg, 0.297 mmol) to give the product (40 mg, 32%) as a yellow oil. The free base was converted to the oxalate salt and recrystallized from hot acetone and methanol to give a white solid. Mp 170−171 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45−7.52 (m, 4H), 7.06−7.13 (m, 4H), 5.36 (s, 1H), 3.36−3.67 (m, 6H), 2.30−2.45 (m, 6H), 1.47−1.53 (m, 2H), 0.90−0.92 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.2, 163.8, 162.8, 161.7, 161.3, 131.8, 131.7, 130.7, 130.6, 129.0, 116.2, 116.0, 115.9, 115.7, 67.7, 60.2, 53.1, 52.6, 46.3, 41.9, 19.8, 11.8; Anal. ($C_{22}H_{26}F_2N_2O_2S·C_2H_2O_4$) C, H, N.

*2-((Bis(4-fluorophenyl)methyl)sulfinyl)-1-(4-(4-fluorobenzyl)-piperazin-1-yl)ethan-1-one (**14e**).* Compound **14e** was prepared as described for **9a** using **10e** (510 mg, 1.09 mmol) to give the product (120 mg, 23%) as a yellow oil. The free base was converted to the oxalate salt and recrystallized from hot acetone and methanol to give a white solid. Mp 158−159 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44−7.48 (m, 4H), 7.24−7.26 (m, 2H), 6.98−7.13 (m, 6H), 5.36 (s, 1H), 3.27−3.68 (m, 8H), 2.34−2.45 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.2, 164.2, 163.8, 162.8, 161.7, 161.3, 160.9, 133.1, 131.7, 130.7, 130.6, 130.5, 129.0, 116.2, 116.0, 115.9, 115.7, 115.3, 115.1, 67.7, 61.8, 52.9, 52.7, 52.4, 46.4, 42.0; Anal. ($C_{26}H_{25}F_3N_2O_2S·C_2H_2O_4·1.5H_2O$) C, H, N.

*2-((Bis(4-fluorophenyl)methyl)sulfinyl)-1-(4-(4-chlorobenzyl)-piperazin-1-yl)ethan-1-one (**14f**).* Compound **14f** was prepared as described for **9a** using **13f** (487 mg, 1.00 mmol) to give the product (200 mg, 40%) as a yellow oil. The free base was converted to the oxalate salt and recrystallized from hot acetone and methanol to give a white solid. Mp 157−158 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44−7.52 (m, 4H), 7.22−7.30 (m, 4H), 7.06−7.13 (m, 4H), 5.36 (s, 1H), 3.30−3.65 (m, 8H), 2.37−2.43 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.2, 163.8, 162.8, 161.7, 161.3, 136.0, 133.1, 131.7, 130.7, 130.3, 129.1, 129.0, 128.5, 116.2, 116.0, 115.9, 115.7, 67.7, 61.9, 52.9, 52.7, 52.4, 46.4, 42.0; Anal. ($C_{26}H_{25}ClF_2N_2O_2S·C_2H_2O_4·1.5H_2O$) C, H, N.

*2-((Bis(3-chlorophenyl)methyl)sulfinyl)-1-(4-(2-hydroxypropyl)-piperazin-1-yl)ethan-1-one (**14g**).* Compound **14g** was prepared as described for **9a** using **13g** (590 mg, 1.15 mmol) to give the product (70 mg, 11%) as a yellow oil. The free base was converted to the oxalate salt and recrystallized from hot acetone and methanol to give a white solid. Mp 169−170 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31−7.43 (m, 8H), 5.33 (s, 1H), 3.82−3.84 (m, 1H), 3.59−3.67 (m, 3H), 3.33−3.47 (m, 3H), 3.16−3.19 (m, 1H), 2.61−2.66 (m, 2H), 2.24−2.42 (m, 4H), 1.13−1.14 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.7, 137.4, 135.1, 134.8, 134.7, 130.4, 130.1, 130.0, 129.1, 129.0, 128.7, 128.1, 127.1, 68.1, 65.4, 62.4, 53.2, 53.1, 52.8, 52.7, 52.6, 46.4, 46.2, 42.0, 19.9; Anal. ($C_{22}H_{26}Cl_2N_2O_3S·C_2H_2O_4$) C, H, N.

Radioligand Binding Assays. *DAT Binding Assay.* Striata were dissected from male Sprague−Dawley rat brains (supplied on ice from Bioreclamation (Hicksville, NY) and prepared by homogenizing tissues in 20 volumes (w/v) of ice cold modified sucrose phosphate buffer (0.32 M sucrose, 7.74 mM Na_2HPO_4, and 2.26 mM NaH_2PO_4, pH adjusted to 7.4) using a Brinkman Polytron (Setting 6 for 20 s) and centrifuged at 20,000 rpm for 10 min at 4 °C. The resulting pellet was resuspended in buffer, recentrifuged, 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 [^{3}H]WIN 35,428[54] (K_d value = 5.53, specific activity = 84 ci/mmol; PerkinElmer Life Sciences, Waltham, MA), 1.0 mg of tissue OWW, and various concentrations of the inhibitor. The reaction was started with the addition of tissue, and tubes were incubated for 120 min on ice. Nonspecific binding was determined using 100 μM cocaine HCl.

SERT Binding Assay. Membranes from frozen brain stems dissected from male Sprague−Dawley rat brains (supplied on ice from Bioreclamation, Hicksville, NY) were homogenized in 20 volumes (w/v) of 50 mM Tris buffer (120 mM NaCl and 5 mM KCl, adjusted to pH 7.4) at 25 °C using a Brinkman Polytron (at setting 6 for 20 s). The tissue was centrifuged at 20,000 rpm for 10 min at 4 °C. The resulting pellet was suspended in buffer and centrifuged again. The final pellet 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 [^{3}H]citalopram (K_d value = 1.94 nM, specific activity = 83 ci/mmol; PerkinElmer Life Sciences, Waltham, MA), 1.5 mg of brain stem tissue, and various concentrations of the 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) were homogenized in 20 volumes (w/v) of 50 mM Tris buffer (120 mM NaCl and 5 mM KCl, adjusted to pH 7.4) at 25 °C using a Brinkman Polytron (at setting 6 for 20 s). The tissue was centrifuged at 20,000 rpm for 10 min at 4 °C. The resulting pellet was suspended in buffer and centrifuged again. The final pellet was resuspended in cold buffer to a concentration of 80 mg/mL OWW. Experiments were conducted in assay tubes containing 0.5 mL of buffer, 0.5 nM [^{3}H]nisoxetine (K_d value = 1.0 nM, specific activity = 82 ci/mmol; PerkinElmer Life Sciences, Waltham, MA), 8 mg of frontal cortex tissue, and various concentrations of the inhibitor. The reaction was started with the addition of the tissue, and the tubes were incubated for 180 min at 0−4 °C. Nonspecific binding was determined using 1 μM desipramine.

The solvent used to dissolve the various analogues of modafinil was typically methanol and was present at a final concentration of 5%. Extensive studies previously in this and other laboratories determined that methanol has no effect on binding at the DAT and SERT. However, there is an effect of methanol on binding at the NET, and therefore, methanol concentration was controlled in all tubes in that assay. When compounds were not soluble in methanol, we used either ethanol or DMSO at final concentrations of no greater than 5 or 6%, respectively. Previous studies found no effect of either of these solvents at these concentrations on binding at any of the sites. For all three MAT binding assays, incubations were terminated by rapid filtration through Whatman GF/B filters, presoaked in 0.3% (SERT) or 0.05% (DAT, NET) polyethylenimine, using a Brandel R48 filtering manifold (Brandel Instruments Gaithersburg, Maryland). The filters were washed twice with 5 mL of cold buffer and transferred to scintillation vials. Cytoscint (MP Biomedicals, OH) (3.0 mL) was added, and the vials were counted the next day using a Beckman 6000 liquid scintillation counter (Beckman Coulter Instruments, Fullerton, California) or a Tri-Carb 2910-B liquid scintillation counter (PerkinElmer Life Sciences, MA). The K_i values for the modafinil derivatives were obtained using nonlinear least-squares regression

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(using GraphPad Prism Software, San Diego, CA) of the displacement data giving IC_{50} values, from which affinities (K_i values) were calculated using the Cheng–Prusoff equation.[55]

σ_1 Receptor Binding Assay. σ_1 receptor binding was performed as previously reported.[56] Briefly, frozen whole-guinea pig brains (minus cerebellum) were thawed on ice, weighed, and homogenized (with a glass and Teflon homogenizer) in 10 mM Tris-HCl with 0.32 M sucrose at pH 7.4 (10 mL/g tissue). The homogenate was centrifuged at 1000*g* for 10 min at 4 °C. The supernatant was collected into a clean centrifuge tube, and the remaining pellet was resuspended by vortex in 10 mL of buffer (tissue) and centrifuged again at 50,000*g* for 15 min at 4 °C. The resulting pellet was resuspended in experimental buffer to 80 mg/mL original wet weight (OWW).

Ligand binding experiments were conducted in polypropylene assay tubes containing 0.5 mL of 50 mM Tris-HCl buffer at pH 8.0. Each tube contained 3 nM [^{3}H](+)-pentazocine (PerkinElmer Life and Analytical Sciences, Waltham, MA) and 8.0 mg of tissue OWW. Nonspecific binding was determined using 10 mM haloperidol. The reaction was started with the addition of tissue, and the tubes were incubated for 120 min at room temperature.

Incubations for all binding assays were terminated by rapid filtration through Whatman GF/B filters, presoaked in polyethylenimine, using a Brandel R48 filtering manifold (Brandel Instruments, Gaithersburg, MD). The filters were washed twice with 5 mL of ice-cold buffer and transferred to scintillation vials. Beckman Ready Safe (3.0 mL) was added, and the vials were counted the next day using a Beckman 6000 liquid scintillation counter (Beckman Coulter Instruments, Fullerton, CA) at 50% efficiency. Assays were typically conducted in at least three independent experiments, each performed in triplicate.

For the displacement of radioligand binding, IC_{50} values were computed using a nonlinear, least-squares regression analysis (Prism; GraphPad Software Inc., San Diego, CA). Affinities (K_i values) were calculated using the concentration of radioligand used in the assay.

D_2-Like Binding Assay. Binding at dopamine D_2-like receptors was determined using previously described methods.[57] Membranes were prepared from HEK293 cells stably expressing human D_2R, D_3R, or D_4R, grown in a 50:50 mix of DMEM and Ham's F12 culture media, supplemented with 20 mM HEPES, 2 mM L-glutamine, 0.1 mM nonessential amino acids, 1× antibiotic/antimycotic, 10% heat-inactivated fetal bovine serum, and 200 μg/mL hygromycin (Life Technologies, Grand Island, NY) and kept in an incubator at 37 °C and 5% CO_2. Upon reaching 80–90% confluence, cells were harvested using premixed Earle's Balanced Salt Solution (EBSS) with 5 mM EDTA (Life Technologies) and centrifuged at 3000 rpm for 10 min at 21 °C. The supernatant was removed, and the pellet was resuspended in 10 mL of hypotonic lysis buffer (5 mM $MgCl_2 \cdot 6\ H_2O$, 5 mM Tris, pH 7.4 at 4 °C) and centrifuged at 20,000 rpm for 30 min at 4 °C. The pellet was then resuspended in fresh EBSS buffer made from 8.7 g/L Earle's Balanced Salts without phenol red (US Biological, Salem, MA) and 2.2 g/L sodium bicarbonate, pH to 7.4. A Bradford protein assay (Bio-Rad, Hercules, CA) was used to determine the protein concentration, and membranes were diluted to 500 μg/mL and stored in a −80 °C freezer for later use. Radioligand competition binding experiments were conducted using thawed membranes. Test compounds were freshly dissolved in 30% DMSO and 70% H_2O to a stock concentration of 1 mM or 100 μM. To assist the solubilization of free-base compounds, 10 μL of glacial acetic acid was added along with the DMSO. Each test compound was then diluted into 11 half-log serial dilutions using 30% DMSO vehicle; final test concentrations ranged from 100 μM to 100 pM or from 10 μM to 10 pM. Previously frozen membranes were diluted in fresh EBSS to a 200 μg/mL (for hD_2R or hD_3R) or 300 μg/mL (hD_4R) stock for binding. Radioligand competition experiments were conducted in 96-well plates containing 300 μL of fresh EBSS buffer with 0.2 mM sodium metabisulfite, 50 μL of diluted test compound, 100 μL of membranes (20 μg total protein for hD_2R or hD_3R, 30 μg of total protein for hD_4R), and 50 μL of [^{3}H] *N*-methylspiperone (0.4 nM final concentration; PerkinElmer). Nonspecific binding was determined using 10 μM butaclamol (Sigma-Aldrich, St. Louis, MO), and total binding was determined with 30% DMSO vehicle. The reaction was incubated for 1 h at room temperature and then terminated by filtration through PerkinElmer UniFilter-96 GF/B filters, presoaked for 1 h in 0.5% polyethylenimine, using a Brandel 96-Well Plates Harvester Manifold (Brandel Instruments, Gaithersburg, MD). The filters were washed 3 times with 3 mL (3 × 1 mL/well) of ice cold EBSS buffer. 65 μL of PerkinElmer MicroScint 20 Scintillation Cocktail was added to each well, and filters were counted using a PerkinElmer MicroBeta Microplate Counter. IC_{50} values for each compound were determined from dose–response curves, and K_i values were calculated using the Cheng–Prusoff equation.[55] These analyses were performed using GraphPad Prism version 6.00 for Macintosh (GraphPad Software, San Diego, CA). Reported K_i values were determined from at least three independent experiments, each with duplicate determinations.

Bioluminescence Resonance Energy Transfer (BRET) Assay. The Gα–γ protein activation assay uses the RLuc8-fused Gα protein subunit and GFP10-fused Gγ protein for a resonance energy transfer pair. Flag-tagged receptor and untagged Gβ constructs were cotransfected. The BRET assays were performed as described previously.[58] Briefly, human embryonic kidney cells (HEK-293T) were transfected, using a constant amount of plasmid DNA but various ratios of plasmids encoding the fusion protein partners. Expression of GFP10 fusion proteins was estimated by measuring fluorescence at 515 nm following excitation at 405 nm. Expression of RLuc8 fusion proteins was estimated by measuring the luminescence of the cells after incubation with 5 μM coelenterazine 400a. In parallel, BRET was measured as the fluorescence of the cells at 535 nm at the same time points using a Mithras LB940 reader (Berthold). In order to measure the antagonist activity, 10 min of preincubation of 1 μM quinpirole precedes the 10 min of incubation of tested compounds before the sample reading.

Molecular Pharmacology. *Site-Directed Mutagenesis.* Synthetic cDNA encoding the human DAT (synDAT) was subcloned into pcDNA3 (Invitrogen, Carlsbad, CA). The Y156F mutation was introduced using QuickChange (adapted from Stratagene, La Jolla, CA) and confirmed by restriction enzyme mapping and DNA sequencing. DAT WT and Y156F cDNA containing plasmids were amplified by transformation into XL1 blue competent cells (Stratagene) 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 (Qiagen) according to the manufacturer's manual.

Cell Culture and Transfection. COS7 cells were grown in Dulbecco's modified Eagle's medium 041 01885 supplemented with 10% fetal calf serum, 2 mM L-glutamine, and 0.01 mg/mL gentamicin at 37 °C in 10% CO_2. DAT WT and Y156F were transiently transfected into COS7 cells with Lipo2000 (Invitrogen) according to the manufacturer's manual using a cDNA/Lipo2000 ratio of 3:6.

[^{3}H]DA Uptake Experiments. Uptake assays were performed on intact COS7 cells essentially as described[59] using 3,4-[Ring-2,5,6-^{3}*H*]-dihydroxyphenylethylamine ([^{3}H]DA) (30–60 Ci/mmol) (PerkinElmer). Briefly, transfected COS7 cells were plated in 24-well dishes (10^5 cells/well) coated with poly ornithine (Sigma) to achieve an uptake level of no more than 10% of total added [^{3}H]DA. The uptake assays were carried out 2 days after transfection in uptake buffer (UB) (25 mM HEPES, 130 mM NaCl, 5.4 mM KCl, 1.2 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1 mM L-ascorbic acid, 5 mM D-glucose, and 1 μM of the catechol-*O*-methyltransferase inhibitor Ro 41-0960 (Sigma), pH 7.4). Prior to the experiment, the cells were washed once in 500 μL of UB, and the nonlabeled compound was added to the cells in the indicated concentrations in a total volume of 500 μL. The assay was initiated by the addition of 6–10 nM [^{3}H]DA. Nonspecific uptake was determined with 1 μM nomifensine (Sigma-Aldrich). After 5 min of incubation at room temperature, the cells were washed twice with 500 μL of ice cold UB, lysed in 250 μL (24 well) of 1% SDS and left for >30 min at 37 °C on gentle shaking. All samples were transferred to 24-well counting plates (PerkinElmer, Waltham, MA), 500 μL (24 well) of Opti-phase Hi Safe 3 scintillation fluid (PerkinElmer) was added followed by counting of the plates in a Wallac Tri-Lux β-scintillation counter (PerkinElmer). All experiments were carried out with 12 determinations of DA or inhibitor concentrations ranging from 1 nM to 1 mM performed in triplicate.

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[^{3}H]WIN35,428 Binding Experiments. Binding assays were carried out essentially as described for the uptake experiments on whole cells only using [^{3}H]2β-carbomethoxy-3β-(4-fluorophenyl)tropane ([^{3}H]-WIN35,428) (76−87 Ci/mmol) (PerkinElmer). Previous to the binding experiment, cells were washed once in ice cold UB, and after the addition of unlabeled compound in the indicated concentrations and [^{3}H]WIN35,428, the reactions were incubated at 5 °C until equilibrium were obtained (>90 min). All experiments were carried out with 12 determinations with inhibitor concentration range from 1 nM to 1 mM, performed in triplicate.

Mouse Microsomal Stability Assay. The phase I metabolic stability assay was conducted in mouse liver microsomes as described previously[47] with minor modifications. Briefly, the reaction was carried out using potassium phosphate buffer (100 mM, pH 7.4), in the presence of an NADPH regenerating system, (compound final concentration was 10 μM; and 0.5 mg/mL microsomes). Testosterone was used as a positive control. Compound disappearance was monitored over time using a liquid chromatography and tandem mass spectrometry (LC/MS/MS) method.

Chromatographic analysis was performed using an Accela ultra high-performance system consisting of an analytical pump and an autosampler coupled with a TSQ Vantage mass spectrometer (Thermo Fisher Scientific Inc., Waltham, MA). Separation of analyte was achieved at ambient temperature using Agilent Eclipse Plus column (100 × 2.1 mm i.d.) packed with a 1.8 μm C18 stationary phase. The mobile phase used was composed of 0.1% formic acid in acetonitrile and 0.1% formic acid in H_2O with gradient elution, starting with 10% (organic) linearly increasing to 99% up to 2 min, maintaining at 99% (2−2.5 min) and reequlibrating to 10%. The total run time was 4.5 min. The mass transitions used for compounds for LC/MS/MS analysis are given in Table S1 (Supporting Information).

Locomotor Activity Studies in Mice. Thirty-two male mice with a C57BL/6J genetic background and body weight of 22−25 g were purchased from the Charles River Laboratories (Raleigh, NC). After arrival, they were group housed (4 per cage) in the animal facility under a reversed 12 h light-dark cycle (light on at 7:00 PM) with free access to food and water, and allowed to acclimate to the new environment for 7 days prior to initiating the experiment. All procedures were in accordance with the "Principles of Laboratory Animal Care" outlined by National Institutes of Health (NIH publication 86−23, 1996).

Locomotor activity tests were conducted in open-field chambers (43 × 43 × 30 cm^3) (Accuscan Instruments, Inc., Columbus, OH, USA). Before testing, the mice were habituated to the locomotor chamber (2 h/day for 3 consecutive days) and then were randomly divided into 4 groups (n = 8 per group) to examine the effects of cocaine, (*R*)-modafinil [(**R**)-**2**], and compounds **4** and **11b** on locomotor activity, respectively. On habituation days, the animals were moved to the test room, acclimated there for about 30 min, and then placed in their assigned locomotor chambers. On test day 1, each group of mice was first placed in the locomotor chambers for 1 h of habituation, and then they received either vehicle (25% of 2-hydroxypropyl-β-cyclodextrin) or one dose of the corresponding test compound. The animals were then immediately placed into the test chambers. Their locomotor activities were recorded every 10 min for 2 h. Each animal was tested 3 times with 3 different doses, with the intertest interval of 3−5 days. The order of the drug doses was counterbalanced.

The locomotor behavioral data are expressed as means (±SEM). The traveled distance (cm) every 10 min (Figure 5) was used to evaluate the locomotor effects of each test compound in mice. Two-way ANOVA with repeated measures over time was used to evaluate the statistical significance of the changes in locomotor activity after each drug administration. Posthoc Fisher's least significant difference was used for multiple comparisons. $p < 0.05$ was considered statistically significant.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.6b01373.

Elemental analysis results (PDF)
SMILES data (CSV)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

DA, dopamine; DAT, dopamine transporter; SERT, serotonin transporter; NET, norepinephrine transporter; CDI, *N*,*N*′-carbonyldiimidazole; IA, inactive; ND, not determined; NADPH, nicotinamide adenine dinucleotide phosphate

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