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Brief Article

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Tranylcypromine Substituted *cis*-Hydroxycyclobutylnaphthamides as Potent and Selective Dopamine D_3 Receptor Antagonists

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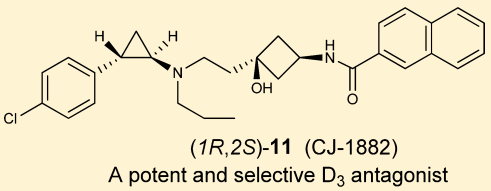
Supporting Information

ABSTRACT: We report a class of potent and selective dopamine D_3 receptor antagonists based upon tranylcypromine. Although tranylcypromine has a low affinity for the rat D_3 receptor (K_i = 12.8 μM), our efforts have yielded (1*R*,2*S*)-**11** (CJ-1882), which has K_i values of 2.7 and 2.8 nM at the rat and human dopamine D_3 receptors, respectively, and displays respective selectivities of >10000-fold and 223-fold over the rat and human D_2 receptors. Evaluation in a β-arrestin functional assay showed that (1*R*,2*S*)-**11** is a potent and competitive antagonist at the human D_3 receptor.



INTRODUCTION

The dopamine-3 (D_3) receptor subtype has been identified as an important target for agents currently in clinical use for the treatment of a variety of neurological diseases, including schizophrenia, Parkinson's disease, and depression. However, all of the clinically approved drugs targeting the D_3 receptor have a very limited selectivity over D_2 receptors and other off-targets.[1,2] Considerable effort has been devoted in the past decade to the design of potent and selective D_3 ligands.[1−16] Although it was initially challenging to design highly selective D_3 ligands, due to the high degree of sequence homology between the D_2 and D_3 receptors, recent SAR studies have demonstrated the feasibility of such a design. For example, upon the basis of pramipexole (**1**), a potent D_3 agonist with only modest selectivity over the D_2 receptor, we designed and prepared compounds CJ-1638 (**2**) and CJ-1639 (**3**), which are potent and selective D_3 agonists (Figure 1).[14] Compounds **2** and **3** bind to the D_3 receptor, with K_i values <1 nM and display >1000-fold selectivity over both the D_1 and D_2 receptors. Further, with the determination of the D_3 receptor crystal structure and derived computational models, the small molecule SARs have been fortified.[17] Not only have highly D_3 receptor selective ligands been discovered, but the roles of the orthosteric site and a secondary binding pocket have further defined the drug−protein interactions responsible for affinity, selectivity, and efficacy.[18,19]

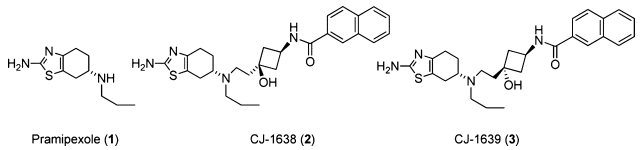


Figure 1. Chemical structures of pramipexole and two previously reported selective D_3 agonists.

Potent and selective D_3 antagonists may have a therapeutic potential for the treatment of drug addictions and relate disorders.[2] Herein we report the design and SAR study of a new class of D_3 antagonists. Analysis of several classes of known D_3 antagonists shows that their structures can be divided into three regions as shown in Figure 2: the headgroup (in blue), the linker (in black), and the tail (in red). The headgroup consists of a basic amine tethered to an aromatic group (typically phenyl), substituted with a hydrogen bonding acceptor (a

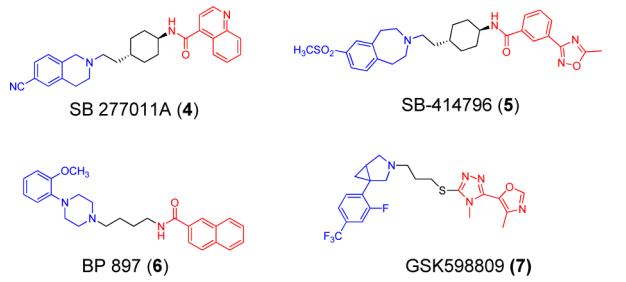


Figure 2. Chemical structures of previous dopamine D_3 receptor antagonists.

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nitrile in **4**[3] or a methoxyl group in **6**[20]), or one or two small hydrophobic groups in **5**[21,22] and **7**.[13] We first selected a new "head" group with these features for the design and development of a new class of D_3 antagonists.

Among potential "head" groups under consideration, we found that tranylcypromine (**8**, Figure 3) was attractive

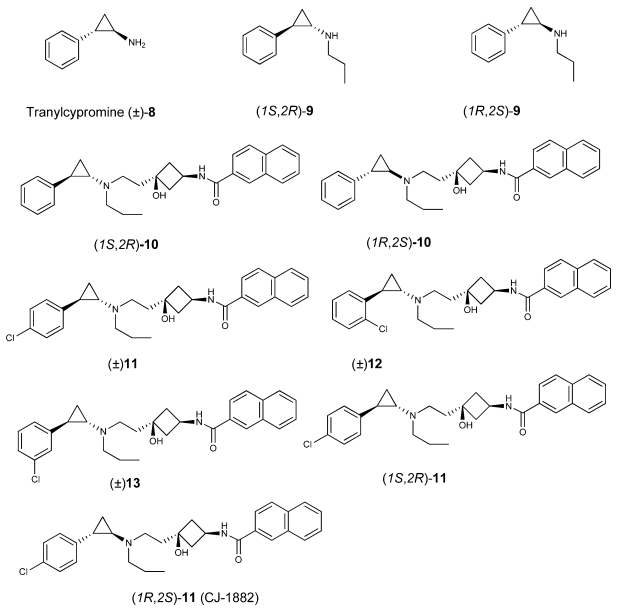

Figure 3. Chemical structures and absolute configurations of the novel compounds.

because, like pramipexole (**1**) and **7**, it contains a structurally rigid phenethylamine moiety. Because the affinities of tranylcypromine for the dopamine receptors are not known, we first tested the commercially available, racemic tranylcypromine in our dopamine receptor binding assays using rat brain.[23,24] Our data showed that tranylcypromine has a weak affinity for the D_3 receptor with a K_i value = 12.8 μM and displays 4-fold selectivity over the D_2 receptor (Table 1). Although tranylcypromine is a weak D_3 ligand and has a very limited selectivity over the D_2 receptor, it was used as a starting point for our SAR study, which has ultimately yielded a class of potent and selective D_3 antagonists.

RESULTS AND DISCUSSION

Our previous study showed that the propyl substituent on the amine group in pramipexole enhances the binding affinity to the D_3 receptors by at least 1 order of magnitude.[14] We therefore investigated whether substitution of a propyl group on the primary amine in tranylcypromine would improve the binding affinity to the D_3 receptor. Chiral resolution of racemic 2-phenyl-cyclopropanamine according to the reported method,[25] followed by addition of the propyl group afforded two stereoisomers (1*S*,2*R*)-**9** and (1*R*,2*S*)-**9**. Binding data showed that both (1*S*,2*R*)-**9** and (1*R*,2*S*)-**9** bind to the rat D_3 receptor with K_i values of 1.2 μM and display approximately 80-fold selectivity over the D_2 receptor (Table 1). Thus, addition of a propyl group to the primary amine in tranylcypromine indeed improves the binding affinity for the D_3 receptor, as well as the selectivity over the D_2 receptor.

Our previous study showed that introduction of appropriate linker and tail groups in compounds **2** and **3** onto the amine group in pramipexole significantly enhanced its selectivity for the rat D_3 receptor over that for the D_2 receptor.[14] Accordingly, we synthesized (1*S*,2*R*)-**10** and (1*R*,2*S*)-**10** with the same linker and tail groups used in compound **2** appended onto the amine group in compounds (1*S*,2*R*)-**9** and (1*R*,2*S*)-**9**. Compounds (1*S*,2*R*)-**10** and (1*R*,2*S*)-**10** have K_i values of 108 and 44 nM to the rat D_3 receptor, respectively, representing a 10−20-fold improvement over (1*S*,2*R*)-**9** and (1*R*,2*S*)-**9**. However, in contrast to the marked improved selectivity of compound **2** over pramipexole for the D_3 receptor over the D_2 receptor observed in our previous study,[14] both (1*S*,2*R*)-**10** and (1*R*,2*S*)-**10** only have modest 20−30-fold selectivity over the rat D_2 receptor.

Addition of a hydrophobic group such as Cl to the phenyl ring has been shown to enhance the binding affinity of antagonists to the D_3 receptor. We have therefore synthesized three compounds (**11**, **12**, and **13**) in which a Cl substitution was installed in the *para*-, *ortho*-, or *meta*-position of the phenyl ring of **10**. Because (1*S*,2*R*)-**10** and (1*R*,2*S*)-**10** do not differ markedly in their binding affinity to the rat D_3 receptor or their selectivity over the rat D_2 receptor, we first synthesized and evaluated the racemic forms of **11**−**13**. Compounds (±)-**11**, (±)-**12**, and (±)-**13** have K_i values of 4.6, 19, and 28 nM, respectively, to the rat D_3 receptor. (±)-**11** and (±)-**13** also display high (>10000 times) selectivity over the rat D_2 receptor, neither compound showing measurable binding to the rat D_2

Table 1. Binding Affinities at Rat Dopamine D_1-Like, D_2-Like, and D_3 Receptors

	$K \pm$ SEM (nM)			selectivity	
compd	D_3	D_2-like	D_1-like	D_3/D_2	D_3/D_1
2	0.40 ± 0.087	725 ± 45	1616 ± 167	1827	4074
6	1.2 ± 0.10	468 ± 51	1042 ± 101	390	868
7	46 ± 5.6	18417 ± 2251	38807 ± 3038	400	484
(±)-**8**	12793 ± 1870	58737 ± 5908	116250 ± 15103	4.3	9.1
(1*S*,2*R*)-**9**	1171 ± 91	95593 ± 5877	122700 ± 10120	82	105
(1*R*,2*S*)-**9**	1195 ± 71	96648 ± 9225	90587 ± 3531	81	76
(1*S*,2*R*)-**10**	108 ± 7.5	2137 ± 247	3088 ± 33	20	29
(1*R*,2*S*)-**10**	44 ± 5.8	1312 ± 170	2825 ± 297	30	64
(±)-**11**	4.6 ± 0.40	>100000	2201 ± 150	>21000	479
(±)-**12**	19 ± 1.8	503 ± 63	743 ± 71	26	39
(±)-**13**	28 ± 2.1	>300000	1804 ± 126	>29000	64
(1*S*,2*R*)-**11**	457 ± 34	>100000	20653 ± 2035	>218	45
1*R*,2*S*)-**11**	2.7 ± 0.30	>300000	25810 ± 1867	>100000	9559

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receptor at 100 μM. However, both (±)-**11** and (±)-**13** have significant affinity for the rat D_1-like receptors with K_i values of 2.2 and 1.8 μM, respectively.

Because (±)-**11** showed high affinity for the rat D_3 receptor, we resolved the stereoisomers, (1*S*,2*R*)-**11** and (1*R*,2*S*)-**11**). (1*R*,2*S*)-**11** has a K_i value of 2.7 nM to the rat D_3 receptor and is >100 times more potent than (1*S*,2*R*)-**11** (K_i = 457 nM). Furthermore, (1*R*,2*S*)-**11** has no appreciable binding to the rat D_2 receptor at concentrations as high as 300 μM and consequently has >100,000-fold selectivity for the rat D_3 receptor over the rat D_2 receptor. (1*R*,2*S*)-**11** shows weak binding affinity to the D_1-like receptors, with a K_i value of 25.8 μM, and has >9000-fold selectivity for the rat D_3 receptor over the rat D_1-like receptors.

We next assessed the binding affinities of (±)-**11**, (±)-**12**, (±)-**13**, and (1*R*,2*S*)-**11** to the human D_2 and D_3 receptors using transfected cell lines and included several previously reported D_2/D_3 antagonists as controls. The data are provided in Table 2.

Table 2. Binding Affinities at Human D_2 and D_3 Receptors

	K_i ± SEM (nM)		selectivity
compd	D_3	D_2	D_3/D_2
(±)-**11**	2.61 ± 0.19	667 ± 230	256
(±)-**12**	22.1 ± 1.87	923 ± 213	42
(±)-**13**	37.9 ± 3.57	1,220 ± 33.7	32
(1*R*,2*S*)-**11**	2.80 ± 0.556	623 ± 105	223
N-methylspiperone	0.265 ± 0.008	0.133 ± 0.009	0.5
eticlopride	0.134 ± 0.004	0.086 ± 0.001	0.6
raclopride	13.4 ± 0.695	12.7 ± 1.21	1
butaclamol	6.39 ± 0.58	2.58 ± 0.473	0.4
PG619	6.70 ± 0.77	1,090 ± 21	163
PG648	1.88 ± 0.11	746 ± 123	397

Racemic compounds (±)-**11**, (±)-**12**, (±)-**13**, and the pure enantiomer (1*R*,2*S*)-**11** have K_i values to the human D_3 receptor of 2.61, 22.1, 37.9, and 2.80 nM, respectively. These values are similar to their respective K_i values of 4.6, 19, 26, and 2.7 nM to the rat D_3 receptor. Compounds (±)-**11**, (±)-**12**, (±)-**13**, and (1*R*,2*S*)-**11** have K_i values, respectively, of 667, 923, 1220, and 623 nM, to the human D_2 receptor. Thus, with the exception of (±)-**12**, these compounds have higher binding affinities to the human D_2 receptor than to the rat D_2 receptor. The selectivities of (±)-**11** and (±)-**13** and the pure enantiomer (1*R*,2*S*)-**11** for the human D_3 receptor over the human D_2 receptor are 256-, 32-, and 223-fold, respectively. These are lower than the selectivities observed for these compounds for the rat D_3 receptor over the rat D_2 receptor. The known D_3 antagonists *N*-methylspiperone,[26] eticlopride,[27] raclopride,[28] and butaclamol,[29] all bind to the human D_3 receptor with high affinities but show no selectivity between the human D_2 and D_3 receptors (Table 2). In comparison, PG619[22] and PG648,[12] two previously reported selective D_3 antagonists, bind to the human D_3 receptor with K_i values of 6.70 and 1.88 nM, respectively, displaying selectivities of 163- and 397-fold respectively, for the human D_3 receptor over the human D_2 receptor.

To assess the functional activity of (1*R*,2*S*)-**11** at the D_3 receptor, we tested it in a DiscoveR$_X$ D_3 functional assay using the U2OS cell line transfected with human dopamine D_3 receptor.[30] In this assay, a D_3 agonist, such as pramipexole, stimulates β-arrestin binding to the D_3 receptor, while a D_3 antagonist, such as **6** (BP 897),[20] blocks the association of β-arrestin induced by a D_3 agonist. Assessed in this manner, pramipexole has an agonist activity with an EC_{50} value of 3.7 ± 0.65 nM. (1*R*,2*S*)-**11** has no agonist activity (EC_{50} > 100 μM), but it dose-dependently inhibits the binding of β-arrestin to the D_3 receptor induced by 100 nM of pramipexole and has an IC_{50} value of 327 ± 126 nM (Table 3). In comparison, **6** shows no agonist activity but has potent antagonist activity, with an IC_{50} value of 13 ± 1.7 nM (Table 3). Hence, (1*R*,2*S*)-**11** is a potent D_3 antagonist, albeit less potent than **6**. We performed a Schild regression analysis for (1*R*,2*S*)-**11** in the human D_3 functional assay (Figure 4). In these experiments, D_3 activity, measured on the basis of β-arrestin binding to the receptor, was stimulated by pramipexole in the presence of three concentrations of (1*R*,2*S*)-**11**. The data were analyzed according to the methods of Kenakin.[31] In the Schild plot (the inset in Figure 4), it can be seen that increasing concentrations of (1*R*,2*S*)-**11** shift the pramipexole dose–response curve to the right, consistent with antagonist activity, with a K_B value of 20 nM. The slope of the Schild plot was −0.83, close to unity, indicating that (1*R*,2*S*)-**11** is a competitive D_3 antagonist.

Table 3. Efficacy at the Human D_3 Receptor in the DiscoveR$_x$ PathHunter eXpress β-Arrestin Assay[a]

	agonist activity		Antagonist Activity
compd	ED_{50} (nM)	E_{max}	IC_{50} (nM)
1	3.7 ± 0.65	100	ND
6	>100000		13 ± 1.7
(1*R*,2*S*)-**11**	>100000		327 ± 126

[a]Data are the mean ± SEM of 3–6 independent determinations (2 determinations if inactive). ND = not determined.

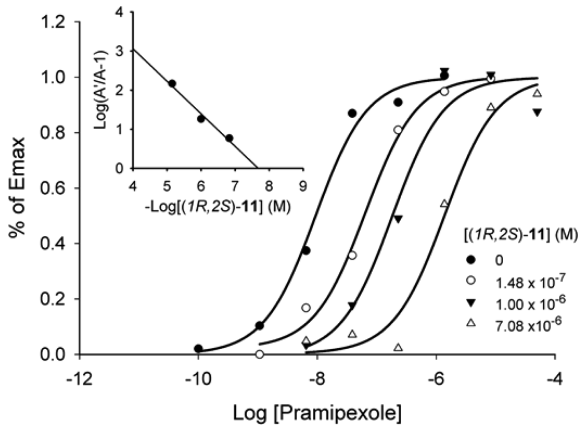

Figure 4. Schild analysis of (1*R*,2*S*)-**11** at the human D_3 receptor in the DiscoveRx PathHunter eXpress β-arrestin assay. D_3 activity was stimulated by pramipexole. Schild transformation (insert) indicated a pA2 of −7.96, corresponding to a KB value of 20 nM. The slope was −0.83. Data shown are representative of two independent determinations.

The synthesis of compounds **9**–**13** is shown in Scheme 1. Enantiomerically pure (1*R*,2*S*)-2-phenylcyclopropanamine and (1*S*,2*R*)-2-phenylcyclo-propanamine were obtained by resolution of commercially available (±)-*trans*-2-phenylcyclopropylamine (**8**) according to the reported method.[25] Reductive amination of (1*R*,2*S*)-**8** and (1*S*,2*R*)-**8** using propionaldehyde and $NaBH_4$ gave (1*R*,2*S*)-**9** and (1*S*,2*R*)-**9** in good yield.

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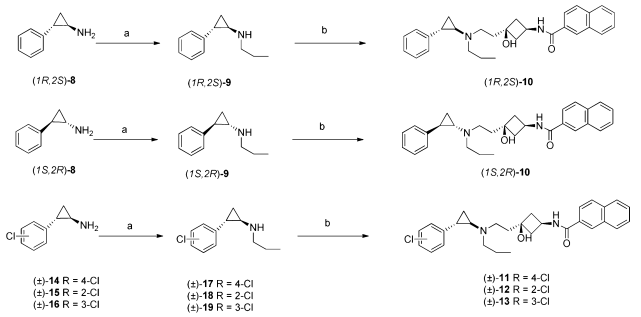
Scheme 1. Synthesis of 9−13[a]

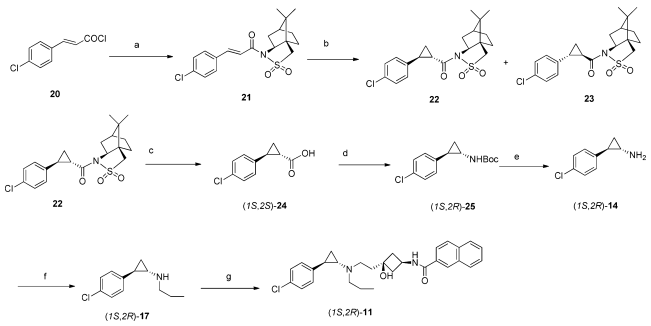


[a]Reagents and conditions: (a) propionaldehyde, $NaBH_4$, MeOH, RT; (b) *N*-(*cis*-3-hydroxy-3-(2-oxoethyl)cyclobutyl)-2-naphthamide, $NaBH(OAc)_3$, HOAc, DCM, RT, 4 h.

Reductive amination of (1*R*,2*S*)-**9** and (1*S*,2*R*)-**9** with *N*-(*cis*-3-hydroxy-3-(2-oxoethyl)cyclobutyl)-2-naphthamide using sodium triacetoxyborohydride as a reductant gave (1*R*,2*S*)-**10** and (1*S*,2*R*)-**10**. Compounds (±)-**11**−(±)-**13** were prepared similarly. Reductive amination of the appropriate commercially available racemic chloro-substituted *trans*-2-phenylcyclopropanamine (±)-**14**−**16** afforded (±)-**17**−**19**. The final compounds (±)-**11**−**13** were prepared by reductive amination of (±)-**17**−**19** with *N*-(*cis*-3-hydroxy-3-(2-oxoethyl)cyclobutyl)-2-naphthamide. *N*-(*cis*-3-Hydroxy-3-(2-oxo-ethyl)cyclobutyl)-2-naphthamide was prepared as described previously.[14]

The synthesis of (1*S*,2*R*)-**11** is shown in Scheme 2. The optically pure key intermediate (1*S*,2*S*)-2-(4-chlorophenyl)-

Scheme 2. Synthesis of (1*S*,2*R*)-11[a]



[a]Reagents and conditions: (a) (1*R*)-(+)-2,10-camphorsultam, NaH, THF, 0 °C, 30 min, then RT overnight; (b) CH_2N_2, $Pd(OAc)_2$, DCM, RT, 10 h; (c) (i)$Ti(iOPr)_4$, BzOH, 150 °C, 30 min, (ii) 2 M LiOH, MeOH, RT, 2 h, (iii) 4 M HCl; (d) (i) ethyl chloroformate. Et_3N, acetone, 0 °C, 2 h, (ii) NaN_3,1 h, (iii) 90 °C, toluene, 3 h, (iv) ButOH, reflux, 16 h. (e) TFA, DCM, RT, 12 h; (f) propionaldehyde, $NaBH_4$, MeOH, RT; (g) *N*-(*cis*-3-hydroxy-3-(2-oxoethyl)cyclobutyl)-2-naprittiamlde, $NaBH(OAc)_3$, HOAc, DCM, RT, 4 h.

cyclopropane-carboxylic acid **24** was prepared according to a reported method.[32] Treatment of the acid chloride **20** with sodium (1*R*)-(+)-2,10-camphorsultam afforded the enoyl sultams **21** in excellent yield (>93%). Reaction of **21** with diazomethane in the presence of a catalytic amount of palladium acetate gave a cyclopropanated diastereomeric mixture of (1*R*)-(+)-2,10-camphorsultam-(1*S*,2*S*)-2-phenylcyclopropane-carboxamide **22** and (1*R*)-(+)-2,10-camphorsultam-(1*R*,2*R*)-2-phenyl-cyclopropane-carboxamide **23** in a ratio of 7:1. Recrystallization from ethanol afforded pure cyclopropanoyl sultams **22**. Treatment of **22** with titanium isopropoxide in benzyl alcohol, followed by lithium hydroxide hydrolysis and acidification, afforded the cyclopropanecarboxylic acid (1*S*,2*S*)-**24**. Boc-protected (1*S*,2*R*)-2-phenylcyclopropylamine **25** was obtained from the carboxylic acid using a Curtius rearrangement of the corresponding acyl azide followed by addition of *t*-butanol to the isocyanate intermediate. Removal of the Boc group, followed by reductive amination, gave (1*S*,2*R*)-**17**. The final compound (1*S*,2*R*)-**11** was obtained by reductive-amination of (1*S*,2*R*)-**17** with *N*-(*cis*-3-hydroxy-3-(2-oxoethyl)cyclo-butyl)-2-naphthamide. (1*R*,2*S*)-**11** was made by a method similar to that used for (1*S*,2*R*)-**11**, except that (1*S*)-(−)-2,10-camphorsultam was used as a chiral auxiliary.

SUMMARY

Starting from tranylcypromine, we have designed and synthesized a class of potent and selective dopamine D_3 receptor ligands. The best compound, (1*R*,2*S*)-**11**, had a K_i value of 2.7 nM to the rat D_3 receptor and displayed a selectivity of >100000-fold over the rat D_2 receptor and >9000-fold over the rat D_1-like receptors. (1*R*,2*S*)-**11** binds to the human D_3 receptor with a K_i value of 2.8 nM and displays 223-fold selectivity over the human D_2 receptor. Functional data and Schild analysis showed that (1*R*,2*S*)-**11** is a potent and competitive antagonist at the human D_3 receptor. (1*R*,2*S*)-**11** (CJ-1882) is being further evaluated for its pharmacokinetics, brain bioavailability, and therapeutic potential for the treatment of drug abuse.

EXPERIMENTAL SECTION

General Methods. Solvents and reagents were obtained commercially and used without further purification. Reactions were monitored by TLC carried out on 250 μm silica gel plates 60F-254 (E. Merck) using UV light as visualizing agent. Silica gel 60, particle size 15−40 μm (E. Merck), was used for flash column chromatography. NMR spectra were recorded on a Bruker Avance 300 spectrometer (300 MHz). Chemical shifts (δ) are reported as δ values (ppm) downfield relative to TMS as an internal standard, with multiplicities reported in the standard manner. All final compounds have purities >95%, as determined by HPLC (UV detection at 254 nm).

(1*S*,2*R*)-2-Phenyl-*N*-propylcyclopropanamine ((1*S*,2*R*)-9). Propionaldehyde (82 mg, 1.41 mmol) was added to a solution of (1*S*,2*R*)-2-phenylcyclopropanamine (188 mg, 1.41 mmol) in MeOH (10 mL), and the reaction mixture was stirred at room temperature for 2 h. Sodium borohydride (79 mg, 2.12 mmol) was then added, and the mixture was stirred at room temperature for 1 h. The reaction was quenched with H_2O (30 mL) and extracted with ethyl acetate (40 mL). The residue was chromatographed (hexane:EtOAc = 50:50) to give (1*S*,2*R*)-**9** (178 mg, 72% yield) as a colorless oil. ^1H NMR (CD_3OD, 300 MHz) δ 7.37−7.12 (m, 5H), 3.20−3.08 (m, 2H), 2.97−2.90 (m, 1H), 2.56−2.45 (m, 1H), 1.82−1.70 (m, 2H), 1.57−1.45 (m, 1H), 1.36 (dd, J = 6.7, 14.4 Hz, 1H), 1.04 (t, J = 7.4 Hz). ^{13}C NMR (CD_3OD, 75 MHz) δ 139.42, 129.68, 127.91, 127.56, 50.88, 39.04, 22.32, 20.55, 13.35, 11.19.

(1*R*,2*S*)-2-Phenyl-*N*-propylcyclopropanamine ((1*R*,2*S*)-9). Propionaldehyde (82 mg, 1.41 mmol) was added to a solution of (1*R*,2*S*)-2-phenylcyclopropanamine (188 mg, 1.41 mmol) in MeOH (10 mL), and the reaction mixture was stirred at room temperature for 2 h. Sodium borohydride (79 mg, 2.12 mmol) was then added, and the mixture was stirred at room temperature for 1 h. The reaction was quenched with H_2O (30 mL) and extracted with EtOAc (40 mL). The residue was chromatographed (hexane:EtOAc = 50:50) to give (1*R*,2*S*)-**9** (171 mg, 69% yield) as a colorless oil. ^1H NMR (CD_3OD, 300 MHz) δ 7.37−7.12 (m, 5H), 3.20−3.08 (m, 2H), 2.97−2.90 (m, 1H), 2.56−2.45 (m, 1H), 1.82−1.70 (m, 2H), 1.57−1.45 (m, 1H), 1.36 (dd, J = 6.7, 14.4 Hz, 1H), 1.04 (t, J = 7.4 Hz). ^{13}C

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NMR (CD_3OD, 75 MHz) δ 139.42, 129.68, 127.91, 127.56, 50.88, 39.04, 22.32, 20.55, 13.35, 11.19.

***N*-(*cis*-3-Hydroxy-3-(2-(((1*S*,2*R*)-2-phenylcyclopropyl)-(propyl)-amino)ethyl)cyclobutyl)-2-naphthamide ((1*S*,2*R*)-10).** *N*-(*cis*-3-Hydroxy-3-(2-oxoethyl)cyclobutyl)-2-naphthamide (80 mg, 0.28 mmol), sodium triacetoxyborohydride (90 mg, 0.43 mmol), and AcOH (26 mg, 0.43 mmol) were added to a solution of (1*S*,2*R*)-**9** (50 mg, 0.28 mmol) in CH_2Cl_2 (20 mL). The mixture was stirred at room temperature for 4 h and then quenched by addition of H_2O (30 mL). The mixture was extracted with CH_2Cl_2 (30 mL × 3). The organic solvent was removed under vacuum, and the residue was chromatographed (MeOH:EtOAc = 10:90) to give (1*S*,2*R*)-**10** (43 mg, 34% yield) as a colorless oil. ^{1}H NMR (CD_3OD, 300 MHz) δ 8.39 (s, 1H), 8.00−7.80 (m, 4H), 7.60−7.50 (m, 2H), 7.40−7.20 (m, 5H), 4.25−4.00 (m, 1H), 3.60−3.25 (m, 4H), 3.20−3.02 (m, 1H), 2.75−2.60 (m, 3H), 2.40−2.09 (m, 4H), 1.90−1.65 (m, 3H), 1.53 (dd, J = 7.1, 14.4 Hz, 1H), 1.05 (t, J = 7.4 Hz, 3H). ^{13}C NMR (CD_3OD, 75 MHz) δ 169.90, 164.83, 138.89, 136.25, 133.99, 132.65, 129.99, 129.91, 129.31, 128.85, 128.76, 128.21, 127.86, 127.18, 124.91, 68.81, 58.31, 53.29, 46.48, 44.19, 44.09, 38.24, 23.66, 18.84, 14.49, 11.23.

***N*-(*cis*-3-Hydroxy-3-(2-(((1*R*,2*S*)-2-phenylcyclopropyl)-(propyl)amino)-ethyl)cyclobutyl)-2-naphthamide ((1*R*,2*S*)-10).** *N*-(*cis*-3-Hydroxy-3-(2-oxoethyl)cyclobutyl)-2-naphthamide (80 mg, 0.28 mmol), sodium triacetoxyborohydride (90 mg, 0.43 mmol), and AcOH (26 mg, 0.43 mmol) were added to a solution of (1*R*,2*S*)-**9** (50 mg, 0.28 mmol) in CH_2Cl_2 (20 mL), and the mixture was stirred at room temperature for 4 h. The reaction was quenched by addition of H_2O (30 mL), and the mixture was extracted with CH_2Cl_2 (30 mL × 3). The organic solvent was removed under vacuum, and the residue was chromatographed (MeOH:EtOAc = 10:90) to give (1*R*,2*S*)-**10** (36 mg, 29% yield) as a colorless oil. ^{1}H NMR (CD_3OD, 300 MHz) δ 8.39 (s, 1H), 8.00−7.80 (m, 4H), 7.60−7.50 (m, 2H), 7.40−7.20 (m, 5H), 4.25−4.00 (m, 1H), 3.60−3.25 (m, 4H), 3.20−3.02 (m, 1H), 2.75−2.60 (m, 3H), 2.40−2.09 (m, 4H), 1.90−1.65 (m, 3H), 1.53 (dd, J = 7.1, 14.4 Hz, 1H), 1.05 (t, J = 7.4 Hz, 3H). ^{13}C NMR (CD_3OD, 75 MHz) δ 169.90, 164.83, 138.89, 136.25, 133.99, 132.65, 129.99, 129.91, 129.31, 128.85, 128.76, 128.21, 127.86, 127.18, 124.91, 68.81, 58.31, 53.29, 46.48, 44.19, 44.09, 38.24, 23.66, 18.84, 14.49, 11.23.

***N*-(*cis*-3-(2-((((±)-*trans*)-2-(2-Chlorophenyl)cyclopropyl)-(propyl)-amino)ethyl)-3-hydroxycyclobutyl)-2-naphthamide ((±)-12).** *N*-(*cis*-3-Hydroxy-3-(2-oxoethyl)cyclobutyl)-2-naphthamide (82 mg, 0.29 mmol), sodium triacetoxyborohydride (91 mg, 0.43 mmol), and AcOH (26 mg, 0.43 mmol) were added to a solution of (±)-**18** (60 mg, 0.29 mmol) in CH_2Cl_2 (20 mL), and the mixture was stirred at room temperature for 4 h. The reaction was quenched by addition of H_2O (30 mL), and the mixture was extracted with CH_2Cl_2 (30 mL × 3). The organic solvent was removed under vacuum, and the residue was chromatographed (MeOH:EtOAc = 10:90) to give (±)-**12** (49 mg, 36% yield) as a colorless oil. ^{1}H NMR (CD_3OD, 300 MHz) δ 8.39 (s, 1H), 8.00−7.85 (m, 4H), 7.70−7.52 (m, 2H), 7.47 (dd, J = 1.6, 7.4 Hz, 1H), 7.40−7.20 (m, 2H), 7.09 (dd, J = 2.0, 7.4 Hz, 1H), 4.25−4.02 (m, 1H), 3.70−3.30 (m, 4H), 3.30−3.20 (m, 1H), 2.75−2.60 (m, 2H), 2.40−2.10 (m, 4H), 2.00−1.50 (m, 4H), 1.07 (t, J = 7.3 Hz, 3H). ^{13}C NMR (CD_3OD, 75 MHz) δ 170.16, 136.48, 136.40, 135.76, 134.23, 132.87, 130.91, 130.19, 129.95, 129.52, 129.05, 128.97, 128.91, 128.06, 127.59, 125.09, 69.09, 58.35, 53.41, 46.50, 44.48, 44.29, 38.48, 34.56, 21.52, 18.85, 14.44, 11.42.

***N*-(*cis*-3-(2-((((±)-*trans*)-2-(4-Chlorophenyl)cyclopropyl)-(propyl)-amino)ethyl)-3-hydroxycyclobutyl)-2-naphthamide ((±)-11).** (±)-**11** was prepared in a manner similar to that used for (±)-**12** in 35% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.28 (s, 1H), 7.90−7.75 (m, 4H), 7.60−7.50 (m, 2H), 7.23 (d, J = 8.5 Hz, 2H), 6.94 (d, J = 8.5 Hz, 2H), 6.53 (d, J = 7.7 Hz, 1H), 4.40−4.20 (m, 1H), 3.00−2.40 (m, 6H), 2.25−1.70 (m, 6H), 1.60−1.50 (m, 2H), 1.25−1.00 (m, 2H), 0.89 (t, J = 7.3 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 167.21, 140.12, 134.93, 132.82, 131.83, 131.72, 129.14, 128.71, 128.67, 127.95, 127.84, 127.58, 127.25, 126.96, 123.75, 71.19, 57.69, 53.27, 48.76, 44.73, 44.57, 37.51, 34.44, 24.94, 20.15, 17.02, 12.23.

***N*-(*cis*-3-(2-((((±)-*trans*)-2-(3-Chlorophenyl)cyclopropyl)-(propyl)-amino)ethyl)-3-hydroxycyclobutyl)-2-naphthamide ((±)-13).** (±)-**13** was prepared in a manner similar to that used for (±)-**12** in 32% yield. ^{1}H NMR (CD_3OD, 300 MHz) δ 8.39 (s, 1H), 8.00−7.80 (m, 4H), 7.70−7.50 (m, 2H), 7.40−7.10 (m, 4H), 4.25−4.10 (m, 1H), 3.60−3.10 (m, 5H), 2.75−2.65 (m, 3H), 2.35−2.10 (m, 4H), 1.90−1.50 (m, 4H), 1.05 (t, J = 7.3 Hz, 3H). ^{13}C NMR (CD_3OD, 75 MHz) δ 170.14, 141.61, 136.45, 136.01, 134.20, 132.86, 131.60, 130.18, 129.50, 129.04, 128.95, 128.51, 128.04, 127.45, 126.01, 125.09, 69.05, 58.49, 53.47, 46.64, 44.42, 44.27, 38.47, 34.50, 23.42, 19.02, 14.99, 11.41.

***N*-(*cis*-3-(2-(((1*S*,2*R*)-2-(4-Chlorophenyl)cyclopropyl)(propyl)-amino)ethyl)-3-hydroxycyclobutyl)-2-naphthamide ((1*S*,2*R*)-11).** *N*-(*cis*-3-Hydroxy-3-(2-oxoethyl)cyclobutyl)-2-naphthamide (191 mg, 0.67 mmol), sodium triacetoxyborohydride (212 mg, 1.01 mmol), and AcOH (60 mg, 1.01 mmol) were added to a solution of (1*S*,2*R*)-**17** (140 mg, 0.67 mmol) in CH_2Cl_2 (20 mL), and the mixture was stirred at room temperature for 4 h. The reaction was quenched by addition of H_2O (30 mL), and the mixture was extracted with CH_2Cl_2 (30 mL × 3). The organic solvent was removed under vacuum, and the residue was chromatographed (MeOH:EtOAc = 10:90) to give (1*S*,2*R*)-**11** (79 mg, 25% yield) as a colorless oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.28 (s, 1H), 7.90−7.75 (m, 4H), 7.60−7.50 (m, 2H), 7.23 (d, J = 8.5 Hz, 2H), 6.94 (d, J = 8.5 Hz, 2H), 6.53 (d, J = 7.7 Hz, 1H), 4.40−4.20 (m, 1H), 3.00−2.40 (m, 6H), 2.25−1.70 (m, 6H), 1.60−1.50 (m, 2H), 1.25−1.00 (m, 2H), 0.89 (t, J = 7.3 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 167.21, 140.12, 134.93, 132.82, 131.83, 131.72, 129.14, 128.71, 128.67, 127.95, 127.84, 127.58, 127.25, 126.96, 123.75, 71.19, 57.69, 53.27, 48.76, 44.73, 44.57, 37.51, 34.44, 24.94, 20.15, 17.02, 12.23.

***N*-(*cis*-3-(2-(((1*R*,2*S*)-2-(4-Chlorophenyl)cyclopropyl)(propyl)-amino)ethyl)-3-hydroxycyclobutyl)-2-naphthamide ((1*R*,2*S*)-11).** (1*R*,2*S*)-**11** was prepared in 35% yield in a manner similar to that used for (1*S*,2*R*)-**11**, except that (1*S*)-(−)-2,10-camphorsultam was used as a chiral auxiliary. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.28 (s, 1H), 7.90−7.75 (m, 4H), 7.60−7.50 (m, 2H), 7.23 (d, J = 8.5 Hz, 2H), 6.94 (d, J = 8.5 Hz, 2H), 6.53 (d, J = 7.7 Hz, 1H), 4.40−4.20 (m, 1H), 3.00−2.40 (m, 6H), 2.25−1.70 (m, 6H), 1.60−1.50 (m, 2H), 1.25−1.00 (m, 2H), 0.89 (t, J = 7.3 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 167.21, 140.12, 134.93, 132.82, 131.83, 131.72, 129.14, 128.71, 128.67, 127.95, 127.84, 127.58, 127.25, 126.96, 123.75, 71.19, 57.69, 53.27, 48.76, 44.73, 44.57, 37.51, 34.44, 24.94, 20.15, 17.02, 12.23.

In Vitro Dopamine Receptor Binding Assays at the Rat Dopamine Receptors. The binding affinities of all the synthetic compounds were determined at the D_3, D_2, and D_1-like receptors in membranes prepared from the brains of adult, male Sprague−Dawley rats (Pel-Freez, Rogers, AR). All compounds were dissolved in 100% EtOH at a concentration of 5 mM.

[^{3}H]R(+)-7-OH-DPAT Binding Assay. The [^{3}H]R-(+)-7-OH-DPAT binding assay for the rat D_3 dopamine receptors was performed as described.[23] A rat ventral striatum (nucleus accumbens and olfactory tubercles) was prepared in assay buffer (50 mM Tris, 1 mM EDTA; pH 7.4 at 23 °C) to yield a final concentration of 10 mg original wet weight (oww)/mL. Membranes were incubated with [^{3}H]R-(+)-7-OH-DPAT (0.15 nM, SA = 163 Ci/mmol; GE Healthcare) and different concentrations of the test compounds (10^{-10} to 10^{-4} M). Nonspecific binding was defined by 1 μM spiperone (Sigma-Aldrich). Assay tubes were incubated at 23 °C for 90 min. The reaction was terminated by rapid vacuum filtration. Data were analyzed using SigmaPlot 10. K_i values were calculated using K_D = 0.15 nM for [^{3}H]7-OH-DPAT[23] and are expressed as the mean ± SEM of 3−5 independent determinations.

[^{3}H]Spiperone Binding Assay. [^{3}H]Spiperone binding assays for rat D_2-like receptors were performed as described[24] for [^{3}H]R-(+)-7-OH-DPAT with the following modifications. Assays were performed using membranes prepared from rat caudate-putamen, which expresses D_2 receptors in high density but with very low levels of D_3 receptors, and the final membrane homogenate concentration was 1.5 mg oww/mL. The assay buffer was 50 mM Tris-HCl, 5 mM KCl, 2 mM $MgCl_2$, and 2 mM $CaCl_2$, pH 7.4 at 23 °C; the concentration of [^{3}H]spiperone (60−96 Ci/mmol; GE Healthcare, PerkinElmer, or American Radiolabeled Chemicals) was 0.2 nM, and the incubation

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time was 90 min at 23 °C. Nonspecific binding was defined in the presence of 1 μM (+)-butaclamol (Sigma-Aldrich). K_i values were calculated using the experimentally determined K_D value for [^{3}H]spiperone of 0.4 nM.

[^{3}H]SCH 23390 Binding Assay. [^{3}H] SCH 23390 binding assay for rat D_1-like dopamine receptors was performed as described[33] for [^{3}H]spiperone binding except the concentration of [^{3}H]SCH 23390 (60 Ci/mmol; American Radiolabeled Chemicals) was 0.3 nM. K_i values were calculated using the K_D value for [^{3}H]SCH 23390 of 0.3 nM.

In Vitro Dopamine Receptor Binding Assays at the Human Dopamine Receptors. HEK293 cells stably expressing human dopamine D_2 and D_3 receptors were grown in a 1:1 mixture 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 μM 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 $MgCl_2 \cdot 6H_2O$, 5 mM Tris, pH 7.4 at 4 °C) and centrifuged at 20000 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), 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.

Immediately prior to testing, all test compounds were freshly dissolved in 30% DMSO and 70% H_2O to a stock concentration of 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 13 half-log serial dilutions using 30% DMSO vehicle; final test concentrations ranged from 10 μM to 10 pM. Previously frozen membranes were diluted in fresh EBSS to a 100 μg/mL stock for binding. Radioligand competition experiments were conducted in glass tubes containing 300 μL of fresh EBSS buffer with 0.2 mM sodium metabisulfite, 50 μL of diluted test compound, 100 μL of membranes (10 μg total protein), 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. All compound dilutions were tested in triplicate and the reaction incubated for 1 h at room temperature. The reaction was terminated by filtration through Whatman GF/B filters, presoaked for 1 h in 0.5% polyethylenimine, using a Brandel R48 filtering manifold (Brandel Instruments, Gaithersburg, MD). The filters were washed 3 times with 3 mL of ice-cold EBSS buffer and transferred to scintillation vials. Then 3 mL of CytoScint liquid scintillation cocktail (MP Biomedicals, Solon, OH) was added and vials were counted using a PerkinElmer Tri-Carb 2910 TR liquid scintillation counter (Waltham, MA). IC_{50} values for each compound were determined from dose−response curves, and K_i values were calculated using the Cheng−Prusoff equation.[34] These analyses were performed using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA). Reported K_i values were determined from least three independent experiments.

Dopamine D_3 β-Arrestin Functional Assay. Functional activity at the D_3 receptor was determined using a PathHunter eXpress β-arrestin assay kit (DiscoveR$_x$, Fremont, CA) for human D_3 receptors transfected in U20S cells.[30] Compounds were screened for agonist and antagonist activity according to the manufacturer's instructions. Pramipexole (100 nM) was used as the reference agonist. Data were analyzed using SigmaPlot 10 and are presented as the mean ± SEM of 4−6 independent determinations. For the Schild analysis, D_3 activity was stimulated by pramipexole in the presence of 3 concentrations of (1*R*,2*S*)-**11** and analyzed according to the methods of Kenakin.[31]

■ ASSOCIATED CONTENT

Supporting Information

Experimental details of chemical synthesis and chemical data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Newman, A. H.; Grundt, P.; Nader, M. A. Dopamine D_3 receptor partial agonists and antagonists as potential drug abuse therapeutic agents. *J. Med. Chem.* **2005**, *48*, 3663−79.

(2) Heidbreder, C. A.; Newman, A. H. Current perspectives on selective dopamine D(3) receptor antagonists as pharmacotherapeutics for addictions and related disorders. *Ann. N. Y. Acad. Sci.* **2010**, *1187*, 4−34.

(3) Reavill, C.; Taylor, S. G.; Wood, M. D.; Ashmeade, T.; Austin, N. E.; Avenell, K. Y.; Boyfield, I.; Branch, C. L.; Cilia, J.; Coldwell, M. C.; Hadley, M. S.; Hunter, A. J.; Jeffrey, P.; Jewitt, F.; Johnson, C. N.; Jones, D. N.; Medhurst, A. D.; Middlemiss, D. N.; Nash, D. J.; Riley, G. J.; Routledge, C.; Stemp, G.; Thewlis, K. M.; Trail, B.; Vong, A. K.; Hagan, J. J. Pharmacological actions of a novel, high-affinity, and selective human dopamine D(3) receptor antagonist, SB-277011-A. *J. Pharmacol. Exp. Ther.* **2000**, *294*, 1154−65.

(4) Hackling, A.; Ghosh, R.; Perachon, S.; Mann, A.; Holtje, H. D.; Wermuth, C. G.; Schwartz, J. C.; Sippl, W.; Sokoloff, P.; Stark, H. *N*-(omega-(4-(2-Methoxyphenyl)piperazin-1-yl)alkyl)carboxamides as dopamine D_2 and D_3 receptor ligands. *J. Med. Chem.* **2003**, *46*, 3883−99.

(5) Macdonald, G. J.; Branch, C. L.; Hadley, M. S.; Johnson, C. N.; Nash, D. J.; Smith, A. B.; Stemp, G.; Thewlis, K. M.; Vong, A. K.; Austin, N. E.; Jeffrey, P.; Winborn, K. Y.; Boyfield, I.; Hagan, J. J.; Middlemiss, D. N.; Reavill, C.; Riley, G. J.; Watson, J. M.; Wood, M.; Parker, S. G.; Ashby, C. R., Jr. Design and synthesis of *trans*-3-(2-(4-((3-(3-(5-methyl-1,2,4-oxadiazolyl))- phenyl)carboxamido)-cyclohexyl)ethyl)-7-methylsulfonyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (SB-414796): a potent and selective dopamine D_3 receptor antagonist. *J. Med. Chem.* **2003**, *46*, 4952−64.

(6) Campiani, G.; Butini, S.; Trotta, F.; Fattorusso, C.; Catalanotti, B.; Aiello, F.; Gemma, S.; Nacci, V.; Novellino, E.; Stark, J. A.; Cagnotto, A.; Fumagalli, E.; Carnovali, F.; Cervo, L.; Mennini, T. Synthesis and pharmacological evaluation of potent and highly selective D_3 receptor ligands: inhibition of cocaine-seeking behavior and the role of dopamine D_3/D_2 receptors. *J. Med. Chem.* **2003**, *46*, 3822−39.

(7) Bettinetti, L.; Schlotter, K.; Hubner, H.; Gmeiner, P. Interactive SAR studies: rational discovery of super-potent and highly selective dopamine D_3 receptor antagonists and partial agonists. *J. Med. Chem.* **2002**, *45*, 4594−7.

(8) Chen, J.; Ding, K.; Levant, B.; Wang, S. Design of novel hexahydropyrazinoquinolines as potent and selective dopamine D_3

receptor ligands with improved solubility. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 443−6.
(9) Ji, M.; Chen, J.; Ding, K.; Wu, X.; Varady, J.; Levant, B.; Wang, S. Design, synthesis and structure−activity relationship studies of hexahydropyrazinoquinolines as a novel class of potent and selective dopamine receptor 3 (D_3) ligands. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1701−5.
(10) Varady, J.; Wu, X.; Fang, X.; Min, J.; Hu, Z.; Levant, B.; Wang, S. Molecular modeling of the three-dimensional structure of dopamine 3 (D_3) subtype receptor: discovery of novel and potent D_3 ligands through a hybrid pharmacophore- and structure-based database searching approach. *J. Med. Chem.* **2003**, *46*, 4377−92.
(11) Ehrlich, K.; Gotz, A.; Bollinger, S.; Tschammer, N.; Bettinetti, L.; Harterich, S.; Hubner, H.; Lanig, H.; Gmeiner, P. Dopamine D_2, D_3, and D_4 selective phenylpiperazines as molecular probes to explore the origins of subtype specific receptor binding. *J. Med. Chem.* **2009**, *52*, 4923−35.
(12) Newman, A. H.; Grundt, P.; Cyriac, G.; Deschamps, J. R.; Taylor, M.; Kumar, R.; Ho, D.; Luedtke, R. R. *N*-(4-(4-(2,3-Dichloro- or 2-methoxyphenyl)piperazin-1-yl)butyl)heterobiarylcarboxamides with functionalized linking chains as high affinity and enantioselective D_3 receptor antagonists. *J. Med. Chem.* **2009**, *52*, 2559−70.
(13) Micheli, F.; Arista, L.; Bertani, B.; Braggio, S.; Capelli, A. M.; Cremonesi, S.; Di-Fabio, R.; Gelardi, G.; Gentile, G.; Marchioro, C.; Pasquarello, A.; Provera, S.; Tedesco, G.; Tarsi, L.; Terreni, S.; Worby, A.; Heidbreder, C. Exploration of the amine terminus in a novel series of 1,2,4-triazolo-3-yl-azabicyclo[3.1.0]hexanes as selective dopamine D_3 receptor antagonists. *J. Med. Chem.* **2010**, *53*, 7129−39.
(14) Chen, J.; Collins, G. T.; Levant, B.; Woods, J.; Deschamps, J. R.; Wang, S. CJ-1639: A Potent and Highly Selective Dopamine D_3 Receptor Full Agonist. *ACS Med. Chem. Lett.* **2011**, *2*, 620−5.
(15) Song, R.; Yang, R. F.; Wu, N.; Su, R. B.; Li, J.; Peng, X. Q.; Li, X.; Gaal, J.; Xi, Z. X.; Gardner, E. L. YQA14: a novel dopamine D_3 receptor antagonist that inhibits cocaine self-administration in rats and mice, but not in D_3 receptor-knockout mice. *Addict. Biol.* **2012**, *17*, 259−73.
(16) Micheli, F.; Heidbreder, C. Dopamine D_3 receptor antagonists: a patent review (2007−2012). *Expert Opin. Ther. Pat.* **2013**, *23*, 363−81.
(17) Chien, E. Y.; Liu, W.; Zhao, Q.; Katritch, V.; Han, G. W.; Hanson, M. A.; Shi, L.; Newman, A. H.; Javitch, J. A.; Cherezov, V.; Stevens, R. C. Structure of the human dopamine D_3 receptor in complex with a D_2/D_3 selective antagonist. *Science* **2010**, *330*, 1091−5.
(18) Newman, A. H.; Beuming, T.; Banala, A. K.; Donthamsetti, P.; Pongetti, K.; LaBounty, A.; Levy, B.; Cao, J.; Michino, M.; Luedtke, R. R.; Javitch, J. A.; Shi, L. Molecular determinants of selectivity and efficacy at the dopamine D_3 receptor. *J. Med. Chem.* **2012**, *55*, 6689−99.
(19) Keck, T. M.; Burzynski, C.; Shi, L.; Newman, A. H. Beyond small-molecule SAR: using the dopamine D_3 receptor crystal structure to guide drug design. *Adv. Pharmacol.* **2014**, *69*, 267−300.
(20) Pilla, M.; Perachon, S.; Sautel, F.; Garrido, F.; Mann, A.; Wermuth, C. G.; Schwartz, J. C.; Everitt, B. J.; Sokoloff, P. Selective inhibition of cocaine-seeking behaviour by a partial dopamine D_3 receptor agonist. *Nature* **1999**, *400*, 371−5.
(21) Grundt, P.; Carlson, E. E.; Cao, J.; Bennett, C. J.; McElveen, E.; Taylor, M.; Luedtke, R. R.; Newman, A. H. Novel heterocyclic trans olefin analogues of *N*-{4-[4-(2,3-dichlorophenyl)piperazin-1-yl]butyl}-arylcarboxamides as selective probes with high affinity for the dopamine D_3 receptor. *J. Med. Chem.* **2005**, *48*, 839−848.
(22) Grundt, P.; Prevatt, K. M.; Cao, J.; Taylor, M.; Floresca, C. Z.; Choi, J. K.; Jenkins, B. G.; Luedtke, R. R.; Newman, A. H. Heterocyclic analogues of *N*-(4-(4-(2,3-dichlorophenyl)piperazin-1-yl)butyl)-arylcarboxamides with functionalized linking chains as novel dopamine D_3 receptor ligands: potential substance abuse therapeutic agents. *J. Med. Chem.* **2007**, *50*, 4135−46.
(23) Bancroft, G. N.; Morgan, K. A.; Flietstra, R. J.; Levant, B. Binding of [^{3}H]PD 128907, a putatively selective ligand for the D_3 dopamine receptor, in rat brain: a receptor binding and quantitative autoradiographic study. *Neuropsychopharmacology* **1998**, *18*, 305−16.
(24) Levant, B.; Grigoriadis, D. E.; DeSouza, E. B. Characterization of [^{3}H]quinpirole binding to D_2-like dopamine receptors in rat brain. *J. Pharmacol. Exp. Ther.* **1992**, *262*, 929−35.
(25) Kinzel, O.; Alfieri, A.; Altamura, S.; Brunetti, M.; Bufali, S.; Colaceci, F.; Ferrigno, F.; Filocamo, G.; Fonsi, M.; Gallinari, P.; Malancona, S.; Hernando, J. I.; Monteagudo, E.; Orsale, M. V.; Palumbi, M. C.; Pucci, V.; Rowley, M.; Sasso, R.; Scarpelli, R.; Steinkuhler, C.; Jones, P. Identification of MK-5710 ((8a*S*)-8a-methyl-1,3-dioxo-2-[(1*S*,2*R*)-2-phenylcyclo- propyl]-*N*-(1-phenyl-1*H*-pyrazol-5-yl)hexahydro-imidazo[1,5-*a*]pyrazine-7(1*H*)-carbox amide), a potent smoothened antagonist for use in Hedgehog pathway dependent malignancies, part 2. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 4429−35.
(26) Andree, B.; Nyberg, S.; Ito, H.; Ginovart, N.; Brunner, F.; Jaquet, F.; Halldin, C.; Farde, L. Positron emission tomographic analysis of dose-dependent MDL 100,907 binding to 5-hydroxytryptamine-2A receptors in the human brain. *J. Clin. Psychopharmacol.* **1998**, *18*, 317−23.
(27) Claytor, R.; Lile, J. A.; Nader, M. A. The effects of eticlopride and the selective D_3-antagonist PNU 99194-A on food- and cocaine-maintained responding in rhesus monkeys. *Pharmacol., Biochem. Behav.* **2006**, *83*, 456−64.
(28) Kohler, C.; Hall, H.; Ogren, S. O.; Gawell, L. Specific in vitro and in vivo binding of ^{3}H-raclopride. A potent substituted benzamide drug with high affinity for dopamine D_2 receptors in the rat brain. *Biochem. Pharmacol.* **1985**, *34*, 2251−9.
(29) Chrzanowski, F. A.; McGrogan, B. A.; Maryanoff, B. E. The pK_a of butaclamol and the mode of butaclamol binding to central dopamine receptors. *J. Med. Chem.* **1985**, *28*, 399−400.
(30) Olson, K. R.; Eglen, R. M. Beta-galactosidase complementation: a cell-based luminescent assay platform for drug discovery. *Assay Drug Dev. Technol.* **2007**, *5*, 137−44.
(31) Kenakin, T. P. *Pharmacologic Analysis of Drug−Receptor Interaction*; Raven Press: New York, 1987.
(32) Vallgarda, J.; Appelberg, U.; Csoregh, I.; Hacksell, U. Stereoselectivity and Generality of the Palladium-Catalyzed Cyclopropanation of Alpha,Beta-Unsaturated Carboxylic Acids Derivatized with Oppolzers Sultam. *J. Chem. Soc., Perkin Trans. 1* **1994**, 461−70.
(33) Levant, B. Characterization of Dopamine Receptors. In *Current Protocols in Pharmacology*; John Wiley & Sons, Inc.: New York, 2001.
(34) Cheng, Y.; Prusoff, W. H. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50% inhibition (IC_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099−3108.