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CJ-1639: A Potent and Highly Selective Dopamine D3 Receptor Full Agonist

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Abstract

We have identified several ligands with high binding affinities to the dopamine D3 receptor and excellent selectivity over the D2 and D1 receptors. CJ-1639 (**17**) binds to the D3 receptor with a K_i value of 0.50 nM and displays a selectivity of >5,000 times over D2 and D1 receptors in binding assays using dopamine receptors expressed in the native rat brain tissues. CJ-1639 binds to human D3 receptor with a K_i value of 3.61 nM and displays over >1000-fold selectivity over human D1 and D2 receptors. CJ-1639 is active at 0.01 mg/kg at the dopamine D3 receptor in the rat and only starts to show a modest D2 activity at doses as high as 10 mg/kg. CJ-1639 is the most potent and selective D3 full agonist reported to date.

Keywords

Dopamine receptors; ligands; agonists; drug abuse

Dopamine is an essential neurotransmitter in the central nervous system and exerts its effects through activation of five distinct dopamine receptor subtypes that belong to the G protein-coupled receptor superfamily. The receptors are grouped into the D1-like (D1 and D5) and D2-like (D2, D3, and D4) receptor subtypes. Numerous studies have provided strong evidence that the D3 receptor is a promising therapeutic target for a variety of conditions, including drug abuse, restless legs syndrome, schizophrenia, Parkinson's disease, and depression.(1)-(3) Extensive efforts have been devoted to the discovery and development of potent and selective D3 ligands, including agonists, partial agonists and antagonists.(5)-(6)

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Supporting Information Available: Experimental details of chemical synthesis, chemical data, *in vitro* and *in vivo* characterizations of the ligands. This material is free of charge via the Internet at http://pubs.acs.org.

Due to the high degree of sequence identity within the transmembrane helices between D2 and D3 receptors, and the near-identity of the residues inferred to form the binding site in these receptors,[4] it has been a challenge to design highly selective D3 ligands with excellent aqueous solubility and bioavailability.[5],[6] Pramipexole (**1**), Quinpirole (**2**) and 7-hydroxy dipropylaminotetralin (7-OH-DPAT, **3**), three widely used D3 agonists, all have modest selectivity (<100-fold) over the D2 receptor based upon their *in vitro* binding data[4] and a narrow range of selectivity at the D3 receptor over the D2 receptor *in vivo*.[7],[8] SB-277011A (**4**),[9] SB-414796 (**5**),[10] NGB 2904 (**6**) [11] and BP 897(**7**)[12] are four commonly used D3 antagonists but they also have a selectivity of 100-fold or less for D3 over D2. Furthermore, these four ligands have limited aqueous solubility. *R*-PG648 (**8**), whose design is based upon the core structure of NGB 2904, is a potent D3 antagonist with a selectivity of >400-fold over D2 and also has a much improved aqueous solubility compared to NGB 2904.[13] Recently, compound **9** was reported to be a potent and orally active D3 antagonist with a 500-fold selectivity over D2 in *in vitro* functional assays. [14] The *in vivo* selectivity for compounds **8** and **9** at the D3 receptor over the D2 receptor has not been reported.

In our effort to identify D3 ligands with high selectivity and good solubility, we have previously reported the design of a set of new compounds based upon pramipexole (**1**),[15] which included CJ-1037 (**10**) and CJ-998 (**11**) as the two best compounds. Both **10** and **11** bind to D3 with high affinities, have a good selectivity for D3 over D2 and excellent aqueous solubility (>50 mg/ml). Unlike pramipexole, which is a D3 full agonist, compounds **10** and **11** appear to behave as partial agonists with low intrinsic agonist activity in rats.[15] However, despite their high binding affinities to D3, both compounds **10** and **11** only show *in vivo* activity at a very high dose (32 mg/kg), indicating their poor bioavailability in the brain. Here we report further modifications based upon compound **11**. These efforts have now yielded a set of new D3 ligands that are highly potent and selective, not only *in vitro*, but also *in vivo*.

A recent publication by Newman's group showed that introduction of a hydroxyl group in the linker region in their D3 ligands improves D3 selectivity and aqueous solubility.[13] Accordingly, we designed and synthesized compounds **12** and **13** by placing a hydroxyl group in the cyclohexane ring of compound **11** to improve its solubility and to also explore the effect of this hydroxyl group for binding and selectivity at the D3 receptor (Figure 2). Compound **12** with a *cis*-hydroxycyclohexyl group and compound **13**, the corresponding *trans* isomer, have K_i values of 1.6 nM and 0.9 nM to D3, respectively, in our *in vitro* binding assays using dopamine receptors expressed in the native rat brain tissues. Thus, they are as potent as compound **11**, indicating that introduction of a hydroxyl group at the bridge carbon atom of the cyclohexyl group is not detrimental to D3 binding. Both compounds **12** and **13**, however, have a reduced binding affinity to D2 as compared to pramipexole. As a result, **12** and **13** display a selectivity of >1000-times and >3000-times, respectively, for D3 over D2.

We next synthesized compounds **14** and **15** with a 4-membered ring in the linker region to replace the 6-membered ring in compound **11**. Compound **14**, with a *trans*-cyclobutyl group, has a K_i value of 0.41 nM to D3 and is thus 2 times more potent than **11**. Compound **14** binds to D2 with a K_i value of 584 nM and is 6 times less potent than **11**. Hence, compound **14** is a highly potent D3 ligand and displays >1000-fold selectivity for D3 over D2. Compound **15** with a *cis*-cyclobutyl group in the linker region binds to D3 with a K_i value of 0.35 nM, very similar to that for compound **14**. Compound **15** also shows a 1000-fold selectivity for D3 over D2. Hence, replacement of the *trans*-cyclohexyl group in compound **11** with either a *trans*- or *cis*-cyclobutyl group improves both the binding affinity for D3 and selectivity over D2.

Encouraged by the promising binding and selectivity data observed for compounds **12-15**, we next synthesized compounds **16** and **17**, in which a hydroxyl group is introduced to the bridge carbon atom of the 4-membered ring in compounds **14** and **15**, respectively. Compound **16** binds to D3 with a K_i value of 0.40 nM and displays a selectivity of 1827-times over D2. Compound **17** binds to D3 with a K_i of 0.50 nM and displays a selectivity of >5000-times over D2. In addition to their high binding affinities to D3 and excellent selectivity over D2, compounds **16** and **17** also have excellent aqueous solubility (>100 mg/ml) in their HCl salt form.

The synthesis of compounds **12** and **13** were outlined in Scheme 1. Basically 2-naphthoyl chloride was reacted with trans-4-aminocyclohexanol in the presence of triethylamine, followed by oxidation with PCC in DCM to give ketone **18**. Ketone **18** was treated with allylmagnesium bromide at - 78 °C to afford cis-cyclohexanol **20** and trans-cyclohexanol **21**. Compounds **20** and **21** were separated by silica gel chromatography (Hexane:Ethyl acetate=1:1). *Cis*-aldehyde **22** was obtained by the treatment of cis-cyclohexanol **20** with Osmium tetraoxide followed by sodium periodate. *Trans*-aldehyde **23** was obtained in a similar manner. Reductive amination of pramipexole **1** with **22** and **23** gave designed compounds **12** and **13** respectively. Details of the synthesis of compounds **12-17** are provided in Supporting Information (SI).

The stereochemistry of *cis*-conformation of compound **12** was confirmed by x-ray crystallographic analysis of key intermediate **20** (Figure 3). The stereochemistry of *cis*-conformation of compound **16** was confirmed by x-ray crystallographic analysis of a key intermediate (see supporting information).

Our previous studies have shown that the induction of yawning by D2-like agonists is a sensitive and well validated measure of agonist activity at the dopamine D3 receptor,[7],[8],[17] whereas the inhibition of yawning, and induction of hypothermia by D2-like agonists is indicative of an agonist activation at the D2 receptor.[17]-[19] We have evaluated compounds **16** and **17** for their *in vivo* function in the yawning and hypothermia assays in the rat and included pramipexole as the reference compound in the evaluations. The results are shown in Figure 4.

Pramipexole produced dose-dependent increases in yawning at low doses and inhibition of yawning and induction of hypothermia at higher doses (Figure 4A, and 4D). The data is consistent with its full agonist activity profile at the D3 and D2 receptors and a narrow *in vivo* selectivity at the D3 receptor.[7],[8] Like pramipexole, both **16** and **17** produced a dose-dependent increase in yawning (Figure 4A). Unlike pramipexole, which induced yawning over a very narrow dose range, compound **16** and **17** produced increases in yawning over a very wide range of doses, with significant increases in yawning observed at a dose range of 0.1 to 10.0 mg/kg for compound **16** (CJ-1638), and 0.01 to 10.0 mg/kg for compound **17** (CJ-1639) (Figure 4A). Although both compounds have very similar binding affinities to D3 based upon our *in vitro* binding data, our yawning assay showed that compound **17** is more active than **16** in activation of the D3 receptor in the rat and has a significant yawning activity at a dose of 0.01 mg/kg. These data suggest that compound **17** has a better bioavailability in the brain than compound **16**. Both compounds produced peak levels of yawning at a dose of 1.0 mg/kg (Figure 4A).

Because **17** is more potent *in vivo* in the yawning assay, we focused our further evaluation on this compound. Consistent with previous reports, significant decreases in core body temperature were observed with doses of 0.32 and 1.0 mg/kg pramipexole (Figure 4D). In comparison, compound **17** had no significant effect on core body temperature at 3.2 mg/kg and had a significant but a modest effect at 10 mg/kg (Figure 4D). These data suggest that

compound **17** readily crosses the blood-brain barrier, and functions as a highly selective, full efficacy agonist at the D3 receptor.

The *in vivo* profiles of activity for compound **17** and pramipexole were assessed by the antagonist interaction studies with the known D3 antagonist SB-277011A[9] and the D2 antagonist L-741,626[20] in both yawning and hypothermia assays. The selective D3 antagonist SB-277011A effectively inhibited the induction of yawning by both 0.1 and 0.32 mg/kg of pramipexole and 1 and 10 mg/kg of **17** (Figure 4B and 4C). In comparison, although the selective D2 antagonist L-741,626 failed to modulate the yawning effect induced by the lower doses of pramipexole and **17** (0.1 and 1.0 mg/kg, respectively), a significant increase in the yawning effect was observed when L-741,626 was administered prior to the higher doses of pramipexole and **17** (0.32 and 10.0 mg/kg, respectively; Figure 4B and 4C). These data further confirm the agonist activity for pramipexole and compound **17** at the D3 receptor, and suggest that D2 agonist effects may be observed with high doses of pramipexole (~1.0 mg/kg) and 17 (~10.0 mg/kg). In the hypothermia assay, although the D2 antagonist L-741,626 significantly reversed the hypothermia effect induced by 0.32 mg/kg of pramipexole, it did not have a significant effect on the modest hypothermia induced by 10 mg/kg of compound **17** (Figure 4E). Of note, both pramipexole at 0.32 mg/kg and compound **17** at 10 mg/kg produced similar modest decreases in core body temperature (approximately 0.75°C) with pretreatment of L-741,626 at 1 mg/kg (Figure 4E). Taken together, these *in vivo* data show that **17** functions as a potent, highly selective, full efficacy agonist at the D3 receptor, with the onset of modest D2 agonist activity observed at 10 mg/kg for compound **17** and at 0.32 mg/kg for pramipexole. Pramipexole was one of the most selective D3 agonists identified to date[7] and has been extensively used in *in vitro* and *in vivo* studies. The profiles of activity obtained in the current studies have shown that compound **17** functions as a full agonist at the D3 receptor at a dose of 0.01 mg/kg, but has minimal D2 activity at a dose of 10.0 mg/kg, displaying not only higher potency but also much better selectivity than pramipexole.

To further assess the binding affinity, selectivity and functional activity of compound **17**, we evaluated this compound in the Addiction Treatment Discovery Programs of the National Institute on Drug Abuse (NIDA) using cloned human dopamine receptors (SI). Compound **17** binds to human D3 receptor with a K_i value of 3.61 ± 0.53 nM and to human D2 receptor with a K_i value of >4000 nM. Furthermore, **17** shows no detectable binding to human D1 receptor at 10,000 nM. Functional testing using a mitogenesis functional assay for the D3 receptor in CHO cells showed that **17** is a potent and full agonist with an EC_{50} value of 1.70 ± 0.53 nM and 103.4% of maximum stimulation of quinpirole as the standard D3 agonist control. Therefore, compound **17** is also a highly potent D3 full agonist in the functional assays using the cloned human D3 receptor and displays a >1000-fold selectivity for D3 over D2 and D1 in the binding assays using cloned human dopamine receptors.

In summary, through exploration of the linker region of our previously reported D3 ligand **11**, we have identified a number of new D_3 ligands with high binding affinities to the D_3 receptor and a selectivity of >1000-times over the D2 and D1 receptors based upon our *in vitro* binding data. Compound **17** binds to D3 with a K_i value of 0.50 nM and shows a selectivity of >5000-times over D2 and >10,000 over D1 in our binding assays using rat brain. The high binding affinity and selectivity of **17** for the D3 receptor was further confirmed using cells transfected with cloned human dopamine receptors. Our *in vivo* functional evaluation demonstrated that compound **17** is a highly potent and selective D3 full agonist. While it is active at the D3 receptor at doses as low as 0.01 mg/kg, it has a minimal activity at the D2 receptor in the rat at doses as high as 10 mg/kg. Its full agonist profile at the D3 receptor was also confirmed using cells transfected with cloned human D3 receptor in the mitogenesis assay. To the best of our knowledge, compound **17** is the most

active and selective D_3 full agonist reported to date. Furthermore, compound **17** also has good aqueous solubility. Taken together, our data show that compound **17** (CJ-1639) is an excellent pharmacological tool with which to elucidate the role of the D3 receptor in different neurological conditions in animal models. Extensive *in vivo* studies are in progress to evaluate its therapeutic potential for the treatment of drug abuse and other neurological conditions in which the D_3 receptor may play a role, and the results will be reported in due course.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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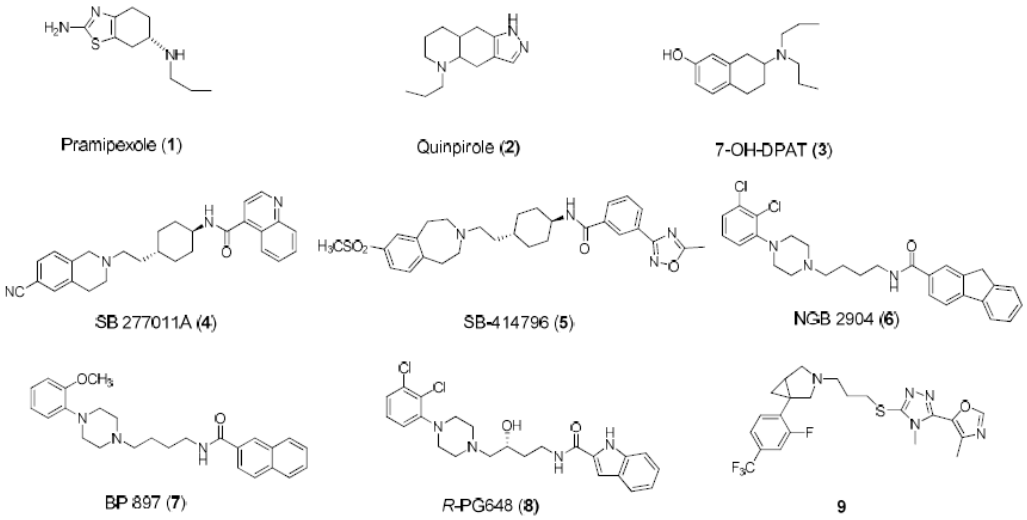


Figure 1.
Chemical structures of representative known D3 agonists (**1-3**) and antagonists (**4-9**).

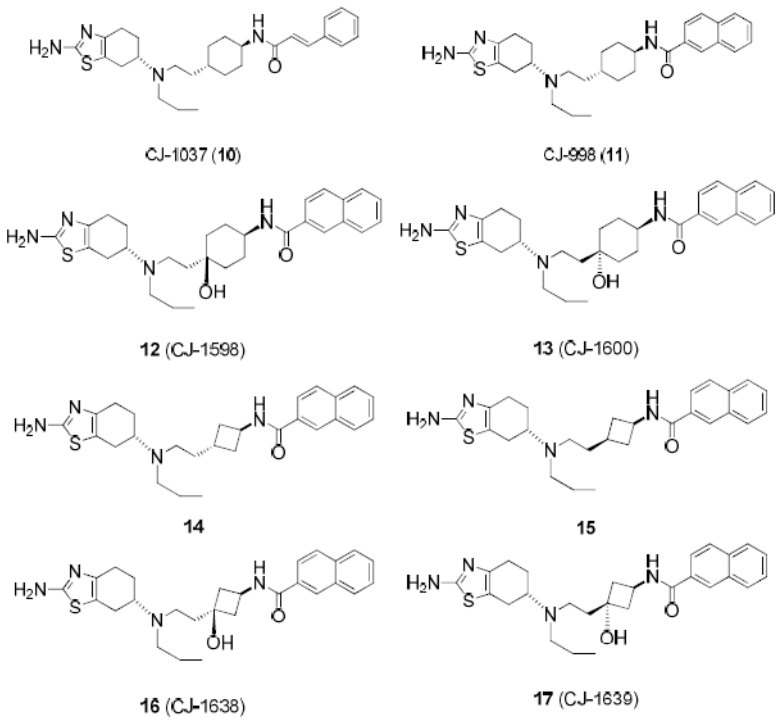


Figure 2.
Chemical structures of two previously reported D_3 ligands, **10** and **11**, and designed new analogues **12-17**.

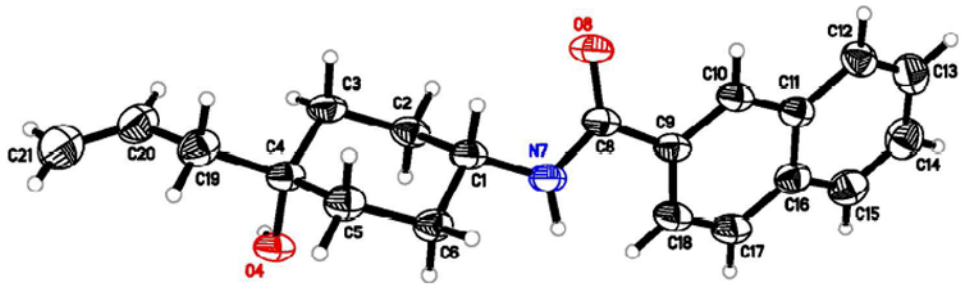


Figure 3.
Crystal structure of intermediate **20**.

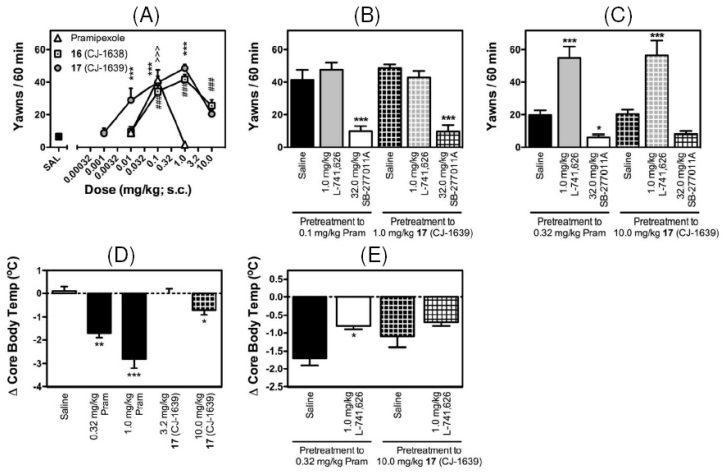
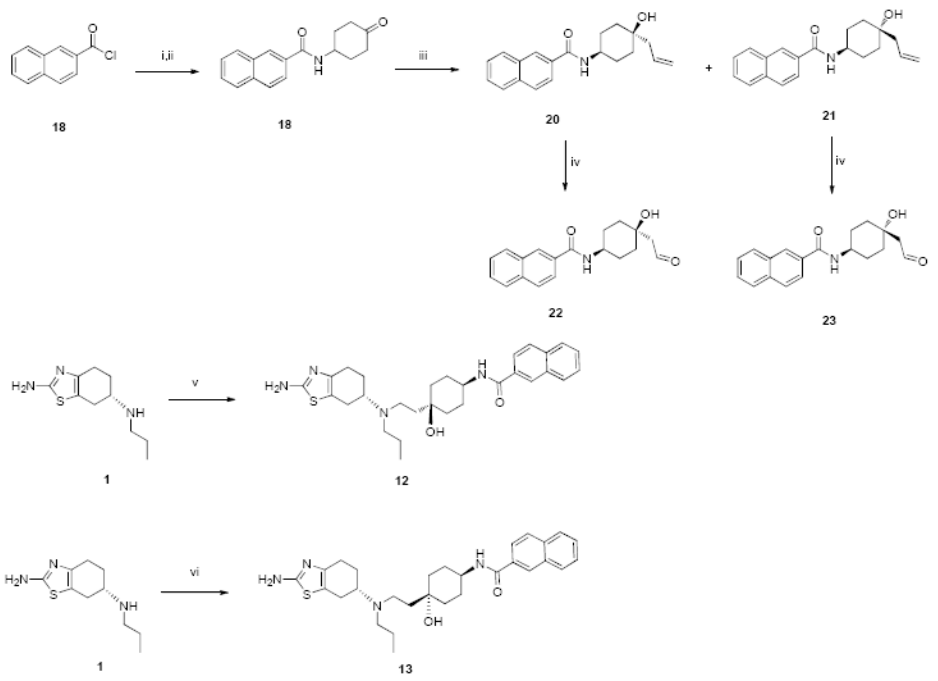


Figure 4.

Functional evaluations of the D3 and D2 activity of pramipexole and compounds **16** and **17** in yawning and hypothermia assays in rats. *, P <0.05; **, P<0.005; ***, P<0.001.



Scheme 1.

Synthesis of compounds **12** and **13**. Conditions and reagents: (i) 2-naphthoyl chloride, trans-4-aminocyclohexanol, triethylamine, DCM; (ii) PCC, DCM, RT, 12 hr; (iii) allylmagnesium bromide, THF, -78 °C, 2 hr; (iv) OsO_4, $NaIO_4$, THF-H_2O, RT, 30 min; (v) **22**, $NaBH(OAc)_3$, CH_3CO_2H, DCM; (vi) **23**, $NaBH(OAc)_3$, CH_3CO_2H, DCM.

Table 1

Binding Affinities at the D_1-like, D_2-like, and D_3 Receptors in Binding Assays Using Rat Brain. Binding affinities for all the compounds in this Table were evaluated under the same assay conditions.

	K_i ± SEM (nM)			Selectivity	
ligand	**D_3 ([^{3}H]-*R*(+)-7-OH-DPAT)**	**D_2-like [^{3}H]Spiperone**	**D_1-like [^{3}H]SCH23390**	**D_2-like/D_3**	**D_1-like/D_3**
1	0.45±0.034	39± 4	>10,000	87	>10,000
11	0.80 ± 0.05	92 ± 9	>5,000	115	>5,000
12	1.6±0.25	1,628±234	11,557±996	1,039	7,373
13	0.93±0.094	3,549±421	14,057±1,095	3,816	15,115
14	0.41±0.038	584±72	6,125±271	1,416	14,848
15	0.35±0.019	564±52	3,058±198	1,627	8,822
16	0.40±0.087	725±45	1,616±167	1,827	4,074
17	0.50±0.080	2,568±302	10,127±1,002	5,136	20,254