Design, Synthesis, and Evaluation of Potent and Selective Ligands for the Dopamine 3 (D_3) Receptor with a Novel in Vivo Behavioral Profile

Jianyong Chen,[†] Gregory T. Collins,[‡] Jian Zhang,[†] Chao-Yie Yang,[†] Beth Levant,[||] James Woods,[‡] and Shaomeng Wang*[,†,‡,§]

Departments of Internal Medicine, Pharmacology, and Medicinal Chemistry, University of Michigan, 1500 East Medical Center Drive, Ann Arbor, Michigan 48109, Department of Pharmacology, Toxicology, and Therapeutics, University of Kansas Medical Center, Kansas City, Kansas 66160

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Abstract: A series of compounds structurally related to pramipexole were designed, synthesized, and evaluated as ligands for the dopamine 3 (D_3) receptor. Compound **12** has a K_i value of 0.41 nM to D_3 and a selectivity of >30000- and 800-fold over the D_1-like and D_2 receptors, respectively. Our in vivo functional assays showed that this compound is a partial agonist at the D_3 receptor with no detectable activity at the D_2 receptor.

Dopaminergic neurotransmission is mediated by five dopamine receptors (D_1-D_5), which can be grouped into the D_1-like (D_1 and D_5) and D_2-like (D_2, D_3, and D_4[a]) receptor subtypes. Recent studies have suggested that the D_3 receptor is a promising therapeutic target for a variety of conditions, including drug abuse, restless legs syndrome, schizophrenia, Parkinson's disease, and depression.[1−6] Considerable effort has been devoted in recent years to the discovery and development of potent and selective D_3 ligands.[6−22]

Despite intense research efforts, design of truly selective D_3 ligands with good solubility and bioavailability remains a challenge. Compound **1** (pramipexole) is a potent D_3-preferring agonist but has limited selectivity over the D_2 receptor in vitro[23] and in vivo.[24,25] Compound **2** was initially reported as a D_3 partial agonist and has a 67-fold selectivity over the D_2 receptor.[2] A number of potent and selective D_3 ligands, such as **3**, have been designed based upon the core structure of **2**.[17] Our laboratory has reported the design of **4** as a potent and selective D_3 ligand using the hexahydropyrazinoquinoline as the core structure.[21] Despite its relatively high affinity and excellent selectivity for D_3 over other dopamine receptor subtypes, **4** has a poor aqueous solubility, which limits its in vivo evaluations (Figure 1). The poor aqueous solubility is also a major limitation for many recently described potent and selective D_3 ligands and an obstacle for evaluation of these novel agents in behavioral models in animals and their therapeutic potential.

To overcome this major limitation, we investigated other core structures for the design of potent and selective D_3 ligands.

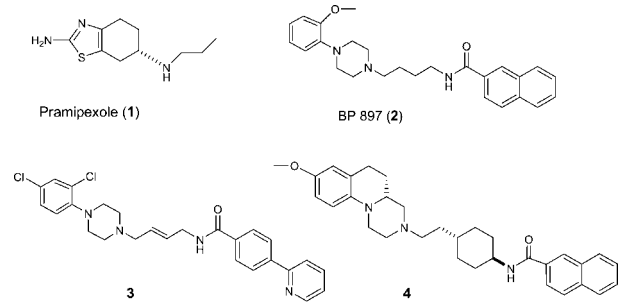

Figure 1. Chemical structures of representative D_3 ligands.

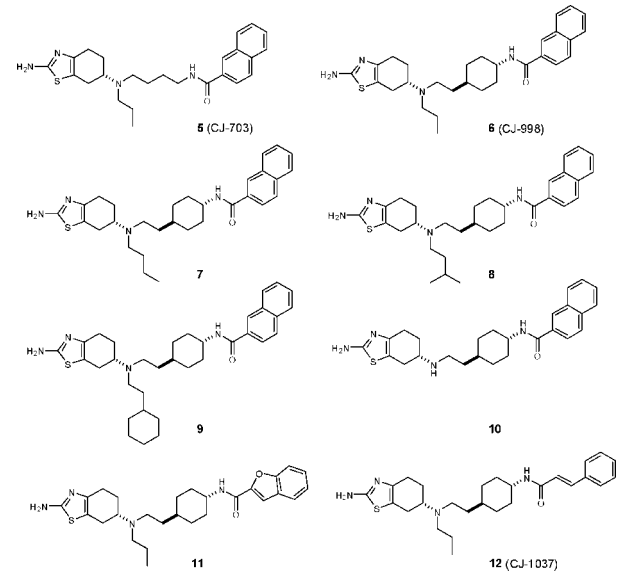

Figure 2. New analogues structurally related to pramipexole.

Among them, the core structure in **1** has a number of very attractive features. First, **1** itself is a very potent D_3 ligand and has a K_i value of 0.78 nM to D_3 in our binding assay (Table 1). Second and importantly, **1** has an excellent aqueous solubility. Third, pramipexole dihydrochloride has been approved for the treatment of Parkinson's disease and restless legs syndrome and has an excellent pharmacological and toxicological profile in humans and in animals. Hence, **1** represents a particularly attractive template for the design of potent and selective D_3 ligands with desirable physiochemical and pharmacological properties (Figure 2). Of note, although **1** has been widely used as a D_3 preferring ligand, it potently binds to the high affinity state of the D_2 receptor with a K_i value of 3.1 nM in our binding assays (Table 1), thus displaying only a 4-fold selectivity for the D_3 receptor over the D_2 receptor.

Recently, the crystal structures for the human β2 adrenergic (β2AD) G-protein coupled receptor (GPCR) were solved.[26,27] We have modeled the human D_3 receptor structure based upon the high-resolution crystal structures of β2AD receptor because these two proteins belong to the same GPCR subfamily[28] and share close sequence homology. Because the crystal structure of β2AD receptor was solved with an inverse agonist bound to it, our modeled D_3 structure likely represents the conformational state bound to either antagonists or inverse agonists. Hence, care must be taken when using the structure to model the interactions of the D_3 receptor with its ligands with different intrinsic functions. Nevertheless, we reasoned the modeled

* To whom correspondence should be addressed. Phone: 734-615-0362. Fax: 734-647-9647. E-mail: shaomeng@umich.edu.
[†] Department of Internal Medicine, University of Michigan.
[‡] Department of Pharmacology, University of Michigan.
[§] Department of Medicinal Chemistry, University of Michigan.
[||] Department of Pharmacology, Toxicology, and Therapeutics, University of Kansas Medical Center.
[a] Abbreviations: D_{1-5}, dopamine 1−5 receptor subtypes; β2AD, the human β2 adrenergic receptor; GPCR, G-protein coupled receptor.

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Table 1. Binding Affinities at the D_1-like, D_2, and D_3 Receptors in Binding Assays Using Rat Brain[a]

	$K_i \pm$ SEM (nM)			selectivity	
ligand	D_3 [^{3}H]PD128907	D_2 [^{3}H]Spiperone	D_1-like [^{3}H]SCH23390	D_2-like/D_3	D_1-like/D_3
1	0.78	3.1 ± 0.3 (h) 6400 ± 1700 (l)	>100000	4.0	>100000
4	5.7 ± 0.4	>10000	>50000	>1000	>5000
5	0.043 ± 0.006	2.7 ± 0.4 (h) 6700 ± 1500 (l)	11000 ± 500	62	>100000
6	0.40 ± 0.057	307 ± 38	3400 ± 300	763	>7000
7	0.74 ± 0.083	55 ± 12 (h) 1300 ± 180 (l)	5400 ± 500	74	>7000
8	2.2 ± 0.10	345 ± 33	13000 ± 1000	157	>5000
9	23 ± 2.7	1200 ± 170	4400 ± 800	53	194
10	7.6 ± 0.87	670 ± 140	64000 ± 7000	88	>8000
11	0.51 ± 0.10	68 ± 4.6	4900 ± 600	133	>9000
12	0.41 ± 0.031	330 ± 69	13000 ± 1700	800	>30000

[a] Data represent the mean ± SEM of 3−5 independent determinations. For compounds producing a two-site fit in competition with [^{3}H]-spiperone, K_i values are presented for the high and low affinity components and are indicated by the designation "(h)" or "(l)". All other K_i values are based on a single-site model.

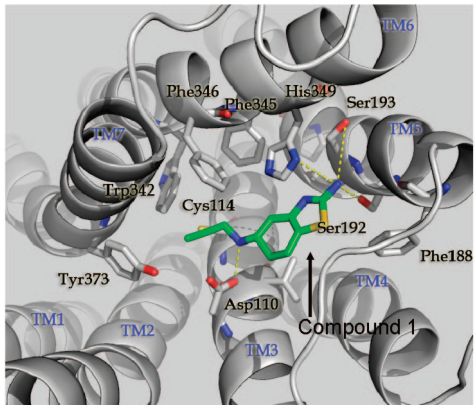

Figure 3. Predicted binding model of compound **1** to the human D_3 receptor. For protein, carbon atoms of human D_3 are shown in white, oxygen atoms in red, and nitrogen atoms in blue. Side chains of crucial residues in the binding site are shown as stick and labeled. Hydrogen bonds between **1** and D_3 are depicted in dotted line in yellow. Figures were generated by Pymol.

human D_3 structure based upon the very first human GPCR structure could be useful to guide the design of novel D_3 ligands.

To this end, we modeled the binding of **1** to the D_3 receptor structure through computational docking, followed by extensive refinement (Supporting Information). The predicted model (Figure 3) showed that the primary amino group in the thiazol ring of **1** forms a hydrogen bonding network with the hydroxyl groups of Ser192 and Ser193. The thiazol ring in **1** is parallel to the imidazole ring in His349, making favorable $\pi-\pi$ stacking interaction. The protonated nitrogen in **1** forms a salt bridge with the negatively charged Asp110. The *n*-propyl group in **1** inserts into a hydrophobic channel formed by Cys114, Phe345, Phe346, Trp342, and Try373.

The predicted model of **1** in complex with the D_3 receptor suggested that there is ample room available to accommodate a much larger hydrophobic group where the *n*-propyl group in **1** binds. Interestingly, in the adjacent area, there is another well-defined but smaller hydrophobic cavity formed by Cys114, Phe197, and Trp342 residues. We have thus designed and synthesized compound **5** to explore the interactions with these two pockets.

Compound **5** was tested for its binding affinities to the dopamine receptors using the same methods as described previously (Table 1).[21] It was found that **5** has a K_i value of 0.043 nM to the D_3 receptor, being 18 times more potent than **1**. Compound **5**, however, also potently binds to the high affinity state of the D_2 receptor with a K_i value of 2.7 nM, thus displaying a 62-fold selectivity for the D_3 receptor over the D_2 receptor. Similar to **1**, **5** has a weak affinity to the D_1-like receptors and has a K_i value of 11000 nM. Hence, although **5** has a very high affinity to the D_3 receptor, its selectivity over the D_2 receptor is modest.

In our previous design of **4**, we have shown that introduction of a *trans*-cyclohexyl group into the linker region yielded new ligands with much improved selectivity for the D_3 receptor over the D_2 receptor as compared to a linear 4-carbon linker.[21] We have thus designed compound **6** to investigate if introduction of this rigid cyclohexyl group into **5** may also improve the selectivity. Compound **6** binds to the D_3 and D_2 receptors with K_i values of 0.40 and 307 nM, respectively. Hence, **6** is a potent D_3 ligand and displays an excellent selectivity of 763-fold for the D_3 receptor over the D_2 receptor.

We next designed and synthesized compounds **7**−**10** to investigate the importance of the *n*-propyl group in **6** for binding and selectivity. Compound **7** with an *n*-butyl group has a slightly weaker affinity for the D_3 receptor than **6** and exhibited a two-site competition curve at the D_2 receptor, with roughly 10-fold less selectivity for the D_3 receptor over the D_2 receptor with the high affinity binding component. Compound **8** with an isopentyl group is five times less potent than **6** to the D_3 receptor but has a similar binding affinity to the D_2 receptor. Compound **9** with a bulky cyclohexylethyl group is 55 times less potent than **6** to the D_3 receptor but is only three times less potent than **6** to the D_2 receptor. Compound **10** with a hydrogen atom at this site has a K_i value of 7.6 nM to the D_3 receptor, being 19 times less potent than **6**, but their binding affinities to the D_2 receptor are essentially the same. Therefore, our binding data clearly showed that the substitution on this nitrogen atom has a major effect on the binding to the D_3 receptor but modest influence on the binding to the D_2 receptor. Our data also showed that the *n*-propyl group in **6** enhances the binding affinity to the D_3-receptor by 19-fold as compared to a hydrogen atom in **10**.

We next investigated the influence of the naphthyl group in **6** for binding and selectivity. Compound **11**, in which the naphthyl group is replaced by a 2-benzofuran, binds to the D_3 receptor with the same affinity (K_i = 0.51 nM) as **6**, but its selectivity over the D_2 receptor is decreased to 133-fold due to its increased binding affinity to the D_2 receptor. Compound **12**, in which a cinnamyl group is used to replace the naphthyl, retains a high binding affinity for the D_3-receptor (K_i = 0.41 nM) and displays 800- and >30000-fold selectivity over the

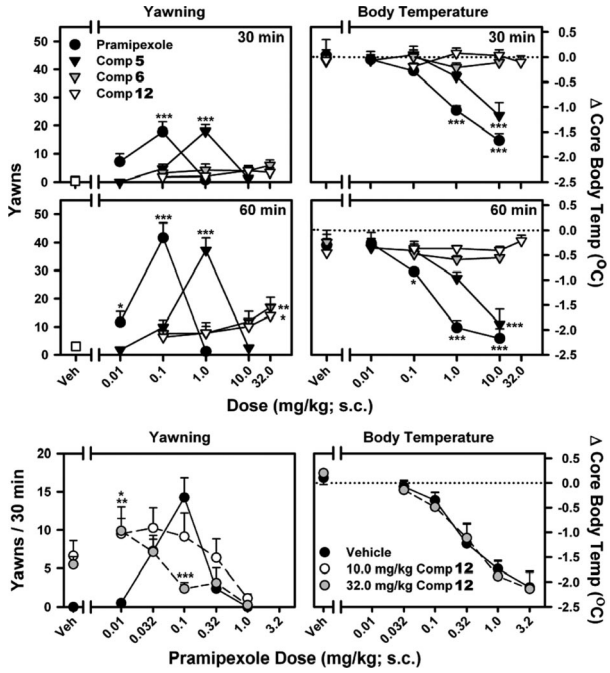

Figure 4. Functional evaluations of the D_3 and D_2 activity of pramipexole and compounds **5**, **6**, and **12** in yawning and hypothermia assays in rats. Top and middle panels: induction of yawning or hypothermia by D_3 ligands. Bottom panels: interactions between pramipexole and compound **12** in yawning and hypothermia assays.

D_2 and D_1-like receptors. These data suggested that the modifications of the naphthyl group can have a significant effect on the selectivity, and this region should be further investigated for the design of potent and selective D_3 ligands.

The synthesis of compounds **5**−**12** is provided in the Supporting Information.

Compounds **5**, **6**, and **12** were found to have good aqueous solubility. For example, the dihydrochloride salt form of **6** has an aqueous solubility greater than 100 mg/mL. Their excellent aqueous solubility provided us with an opportunity to evaluate their in vivo functional profiles in animals.

Another challenge in the development of selective D_3 ligands was that the current in vitro functional assays for the D_3 receptor are not predictive of the in vivo function of D_3 ligands.[6] Furthermore, there was also the lack of a robust in vivo functional assay for the D_3 receptor. To addresses these challenges, we have recently validated in vivo functional assays for the D_3 and D_2 receptors.[24,25] Our studies showed that yawning in rats provides a sensitive measure of in vivo agonist activity at the dopamine D_3 receptor,[24,25] while the induction of hypothermia has been shown to be mediated by agonist activity at the D_2 receptor.[32,33] Employing these well-validated assays, we evaluated **5**, **6**, and **12** for their in vivo functional activity at the D_3 and D_2 receptors. Compound **1**, a known D_3 and D_2 agonist, was used as a control in our evaluations. The results are shown in Figure 4.

Consistent with the data obtained in previous studies,[24,25] increases in yawning were observed over low doses (0.01 to 0.1 mg/kg) of **1** with inhibition of yawning and the induction of hypothermia occurring at higher doses. These data indicate that **1** functions as a preferential D_3 agonist in vivo and a D_2 agonist at higher doses.

Compound **5** induced yawning and produced an inverted U-shaped dose−response curve. The maximum levels of yawning induced by **5** are very similar to that induced by **1**. Furthermore, hypothermia was induced by **5** at higher doses, concurrent with deceases in yawning. These data showed that **5** functions as a full agonist at the D_3 and D_2 receptors in vivo, consistent with the two-site competition curve observed in the [^{3}H]spiperone binding assay for **1** and **5** (Supporting Information). Furthermore, the in vivo data suggested that **5** is bioavailable.

Unlike **1** and **5**, the dose−response curves for **6** and **12** induced yawning were relatively flat and failed to reach significance during the initial 30 min observation period. While significant levels of yawning induced by **6** and **12** were observed after 60 min, the dose−response curves for both compounds remained relatively flat. Moreover, **6** and **12** failed to induce changes in body temperature over the initial hour of observation, an effect that is indicative of D_2 agonist activity. Together, the low levels of yawning, combined with the absence of any hypothermic effect, suggested two possibilities: (1) **6** and **12** function as weak partial agonists at the D_3 receptor, with no detectable agonist activity at the D_2 receptor, or (2) they are simply not bioavailable.

To investigate these two possibilities, we next evaluated the ability of **12** to alter compound **1**-induced yawning and hypothermia and the data are shown in Figure 4. Similar to the effects of **12** alone, but unlike the effects of D_3-selecitve antagonists,[24,25] low levels of yawning were observed during the initial 30 min after administration of either 10.0 or 32.0 mg/kg of **12**. Interestingly, this effect appeared to persist upon administration of low doses of **1** as significant increases in yawning were observed when rats were pretreated with **12** (10.0 or 32.0 mg/kg). However, **12** resulted in a dose-dependent decrease in the amount of yawning observed following the maximally effective dose of **1** at 0.1 mg/kg. No significant effects of **12** were observed at higher doses of **1** (0.32 and 1 mg/kg). These data suggested that **12** is capable of antagonizing the D_3-mediated effects of **1**. However, the profile of activity for **12** is different from that observed for selective D_3 antagonists, which generally produce selective rightward and/or downward shifts of the ascending limb of the yawning dose−response curve for D_3-preferring agonists without increasing the amount of yawning observed at low doses.[24,25] In fact, the effects of **12** alone, and in combination with **1**, suggest that it is more similar to the partial agonist, aripiprazole,[34] than an antagonist. Moreover, **12** failed to alter the induction of hypothermia by **1**, an effect that is indicative of D_2 agonist activity, which can be reliably blocked by both selective and nonselective D_2 antagonists.[32,33] Together, our data provide evidence that **12** is a partial agonist at the D_3 receptor with no detectable agonist or antagonist activity at the D_2 receptor, thus possessing a novel in vivo functional profile.

In summary, a series of enantiomerically pure pramipexole derivatives have been designed, synthesized, and evaluated for their binding and selectivity to the D_3, D_1-like, and D_2 receptor. This led to the identification of several potent and highly selective D_3 ligands with excellent aqueous solubility. Our in vivo functional evaluations showed that while **5** functions as a full D_3 agonist, **12** behaves as a selective D_3 partial agonist with no activity at the D_2 receptor. Further in vivo studies are underway to evaluate the therapeutic potential of **12** for the treatment of drug abuse and other indications. The results will be reported in due course.

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

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