Published in final edited form as:
J Med Chem. 2008 October 9; 51(19): 5905–5908. doi:10.1021/jm800471h.

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[1], **Gregory T. Collins**[2], **Jian Zhang**[1], **Chao-Yie Yang**[1], **Beth Levant**[4], **James Woods**[2], and **Shaomeng Wang**[1,2,3,*]

1*Department of Internal Medicine, University of Michigan, 1500 East Medical Center Drive, Ann Arbor, Michigan 48109*

2*Department of Pharmacology, University of Michigan, 1500 East Medical Center Drive, Ann Arbor, Michigan 48109*

3*Department of Medicinal Chemistry, University of Michigan, 1500 East Medical Center Drive, Ann Arbor, Michigan 48109*

4*Department of Pharmacology, Toxicology, and Therapeutics, University of Kansas Medical Center, Kansas City, Kansas 66160*

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 >30,000- 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) 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. 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 whom correspondence should be addressed. Phone: 734-615-0362. Fax: 734-647-9647. E-mail: shaomeng@umich.edu.

To overcome this major limitation, we investigated other core structures for the design of potent and selective D_3 ligands. 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. 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 since these two proteins belong to the same GPCR sub-family [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 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 π-π 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 11,000 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 nM 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 2-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 5-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 3-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 >30,000-fold selectivity over the 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 inuction 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 2-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 non-selective 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

$D_{1–5}$, dopamine 1–5 receptor subtypes; β2AD, the human β2 adrenergic receptor; GPCR, G-protein coupled receptor..

Acknowledgment

This work was supported by a grant from the National Institute of Drug Abuse, National Institutes of Health (R01DA020669).

References

1. Joyce JN. Dopamine D_3 receptor as a therapeutic target for antipsychotic and antiparkinsonian drugs. Pharmacol. Ther 2001;90:231–259. [PubMed: 11578658]
2. Pilla M, Perachon S, Sautel F, Garrido F, Mann A, Wermuth CG, Schwartz J-C, Everitt BJ, Sokoloff P. Selective inhibition of cocaine-seeking behaviour by a partial dopamine D_3 receptor agonist. Nature 1999;400:371–375. [PubMed: 10432116]
3. Koob GF, Caine SB. Cocaine addition therapy- Are we partially there? Nature Medicine 1999;5:993–995.
4. Montplaisir J, Nicolas A, Denesle R, Gomez-Mancilla B. Restless legs syndrome improved by pramipexole: a double-blind randomized trial. Neurology 1999;52:938–943. [PubMed: 10102409]
5. Levant B. The D_3 dopamine receptor: neurobiology and potential clinical relevance. Pharmacol. Rev 1997;49:231–252. [PubMed: 9311022]
6. Newman AH, Grundt P, Nader MA. Dopamine D_3 Receptor Partial Agonists and Antagonists as Potential Drug Abuse Therapeutic Agents. J. Med. Chem 2005;48:3663–3679. [PubMed: 15916415]
7. Grundt P, Prevatt KM, Cao J, Taylor M, Floresca CZ, Choi J-K, Jenkins BG, Luedtke RR, Newman AH. Heterocyclic Analogues of N-(4-(4-(2,3-Dichlorophenyl)piperazin-1-yl)butyl)arylcarboxamides with Functionalized Linking Chains as Novel Dopamine D3 Receptor Ligands: Potential Substance Abuse Therapeutic Agents. J. Med. Chem 2007;50:4135–4146. [PubMed: 17672446]
8. Wustrow DJ, Wise LD, Cody DM, MacKenzie RG, Georgic LM, Pugsley TA, Heffner TG. Studies of the active conformation of a novel series of benzamide dopamine D_2 agonists. J. Med. Chem 1994;37:4251–4257. [PubMed: 7990124]
9. Wustrow D, Belliotti T, Glase S, Kesten SR, Johnson D, Colbry N, Rubin R, Blackburn A, Akunne H, Corbin A, Davis MD, Georgic L, Whetzel S, Zoski K, Heffner T, Pugsley T, Wise L. Aminopyrimidines with high affinity for both serotonin and dopamine receptors. J. Med. Chem 1998;41:760–771. [PubMed: 9513604]
10. Belliotti TB, Kesten SR, Wustrow DJ, Geogric LM, Zoski KT, Akunne HC, Wise LD. Novel cyclohexyl amides as potent and selective D_3 dopamine receptor ligands. Bioorg. Med. Chem. Letts 1997;7:2403–2408.
11. Robarge MJ, Husbands SM, Kieltyka A, Brodbeck R, Thurkauf A, Newman AH. Design and synthesis of [(2,3-dichlorophenyl)piperazin-1-yl] alkylfluorenylcarboxamides as novel ligands selective for the dopamine D_3 receptor subtype. J. Med. Chem 2001;44:3175–3186. [PubMed: 11543687]
12. Bettinetti L, Schlotter K, Hubner H, Gmeiner P. Interactive SAR studies: rational discovery of super-potent and highly selective D_3 receptor antagonistsand partial agonists. J. Med. Chem 2002;45:4594–4597. [PubMed: 12361386]
13. Hackling A, Ghosh R, Perachon S, Mann A, Holtje HD, Wermuth CG, Schwartz JC, Sippl W, Sokoloff P, Stark H. N-(ö-(4-(2-Methoxyphenyl)piperazin-1-yl)alkyl) carboxamides as dopamine D_2 and D_3 receptor ligands. J. Med. Chem 2003;46:3883–3899. [PubMed: 12930150]
14. Leopoldo M, Berardi F, Colabufo NA, De Giorgio P, Lacivita E, Perrone R, Tortorella V. Structure-affinity relationship study on N-[4-(4-arylpiperazin-1-yl)butyl] arylcarboxamides as potent and selective dopamine D_3 receptor ligands. J. Med. Chem 2002;45:5727–5735. [PubMed: 12477356]
15. Campiani G, Butini S, Trotta F, Fattorusso C, Catalanotti B, Aiello F, Gemma S, Nacci V, Novellino E, Stark JA, 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;46:3822–3839. [PubMed: 12930145]
16. Campiani G, Butini S, Fattorusso C, Catalanotti B, Gemma S, Nacci V, Morelli E, Cagnotto A, Mereghetti I, Mennini T, Carli M, Minetti P, Di Cesare MA, Mastroianni D, Scafetta N, Galletti B, Stasi MA, Castorina M, Pacifici L, Vertechy M, Serio SD, Ghirardi O, Tinti O, Carminati P. Pyrrolo [1,3]benzothiazepine-based serotonin and dopamine receptor antagonists: molecular modeling, further structure activity relationship studies, and identification of novel atypical antipsychotic agents. J. Med. Chem 2004;47:143–157. [PubMed: 14695828]
17. Grundt P, Carlson EE, Cao J, Bennett CJ, McElveen E, Taylor M, Luedtke RR, Newman AH. Novel Heterocyclic Trans Olefin Analogues of N-{4-[4-(2,3-Dichloro phenyl)piperazin-1-yl]butyl}

arylcarboxamides as Selective Probes with High Affinity for the Dopamine D_3 Receptor. J. Med. Chem 2005;48:839–848. [PubMed: 15689168]

18. Varady J, Wu X, Fang X, Ji M, Hu Z, Levant B, Wang S. Molecular modeling of the three-dimensional structure of dopamine 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;46:4377–4392. [PubMed: 14521403]
19. Haadsma-Svensson SR, Cleek KA, Dinh MD, Duncan JN, Haber CL, Huff RM, Lajiness ME, Nichols NF, Smith MW, Sevensson KA, Zaya MJ, Carlsson A, Lin C-H. Dopamine D_3 receptor antagonists. 1. Synthesis and structure-activity relationship of 5,6-dimethoxyl-N-alkyl- and N-alkylaryl-substituted 2-aminoindans. J. Med. Chem 2001;44:4716–4732. [PubMed: 11741489]
20. 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;15:1701–1705. [PubMed: 15745825]
21. Ding K, Chen J, Ji M, Wu X, Varady J, Yang C, Lu Y, Deschamps JR, Levant B, Wang S. Enantiomerically Pure Hexahydropyrazinoquinolines as Potent and Selective Dopamine 3 Subtype Receptor Ligands. J. Med. Chem 2005;48:3171–3181. [PubMed: 15857123]
22. 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;16:443–446. [PubMed: 16290142]
23. Millan MJ, Maiofiss L, Cussac D, Audinot V, Boutin JA, Newman-Tancredi A. Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor. I. A multivariate analysis of the binding profiles of 14 drugs at 21 native and cloned human receptor subtypes. J. Pharmacol. Exp. Ther 2002;303:791–804. [PubMed: 12388666]
24. Collins GT, Witkin JM, Newman AH, Svensson KA, Grundt P, Cao J, Woods JH. Dopamine agonist-induced yawning in rats: a dopamine D_3 receptor-mediated behavior. J. Pharmacol. Exp. Ther 2005;314:310–319. [PubMed: 15833897]
25. Collins GT, Newman AH, Grundt P, Rice KC, Husbands SM, Chauvignac C, Chen J, Wang S, Woods JH. Yawning and hypothermia in rats: effects of dopamine D_3 and D_2 agonists and antagonists. Psychopharmacology (Berl) 2007;193:159–170. [PubMed: 17393143]
26. Cherezov V, Rosenbaum DM, Hanson MA, Rasmussen SG, Thian FS, Kobilka TS, Choi HJ, Kuhn P, Weis WI, Kobilka BK, Stevens RC. High resolution crystal structure of an engineered human beta2-adrenergic G protein-coupled receptor. Science 2007;318:1258–1265. [PubMed: 17962520]
27. Rasmussen SG, Choi HJ, Rosenbaum DM, Kobilka TS, Thian FS, Edwards PC, Burghammer M, Ratnala VR, Sanishvili R, Fischetti RF, Schertler GF, Weis WI, Kobilka BK. Crystal structure of the human beta2 adrenergic G-protein-coupled receptor. Nature 2007;450:383–387. [PubMed: 17952055]
28. Kenakin T. The classification of seven transmembrane receptors in recombinant expression systems. Pharmacol. Rev 1996;48:413–463. [PubMed: 8888308]
29. Levant B, Grigoriadis DE, De Souza EB. Characterization of [^{3}H]quinpirole binding to D_2-like dopamine receptors in rat brain. J. Pharmacol. Exp. Ther 1992;262:929–935. [PubMed: 1356154]
30. Bancroft GN, Morgan KA, Flietstra RJ, Levant B. Binding of [3H]PD 128907, a putatively selective ligand for the D_3 dopamine receptor, in rat brain: a receptor binding and quantitative autoradiographic study. Neuropsychopharmacology 1998;18:305–316. [PubMed: 9509498]
31. Levant, B. Characterization of dopamine receptors. In: Enna, SJ.; Williams, M.; Ferkany, JW.; Kenakin, T.; Porsolt, RD.; Sullivan, JP., editors. Current Protocols in Pharmacology. New York: John Wiley & Sons; 1998. p. 1.6.1-1.6.16.
32. Boulay D, Depoortere R, Perrault G, Borrelli E, Sanger DJ. Dopamine D_2 receptor knock-out mice are insensitive to the hypolocomotor and hypothermic effects of dopamine D_2/D_3 receptor agonists. Neuropharmacology 1999;38:1389–1396. [PubMed: 10471093]
33. Chaperon F, Tricklebank MD, Unger L, Neijt HC. Evidence for regulation of body temperature in rats by dopamine D_2 receptor and possible influence of D_1 but not D_3 and D_4 receptors. Neuropharmacology 2003;44:1047–1053. [PubMed: 12763098]

34. Fujikawa M, Nagashima M, Inoue T, Yamada K, Furukawa T. Partial agonistic effects of OPC-14597, a potential antipsychotic agent, on yawning behavior in rats. Pharmacol. Biochem. Behav 1996;53:903–909. [PubMed: 8801596]

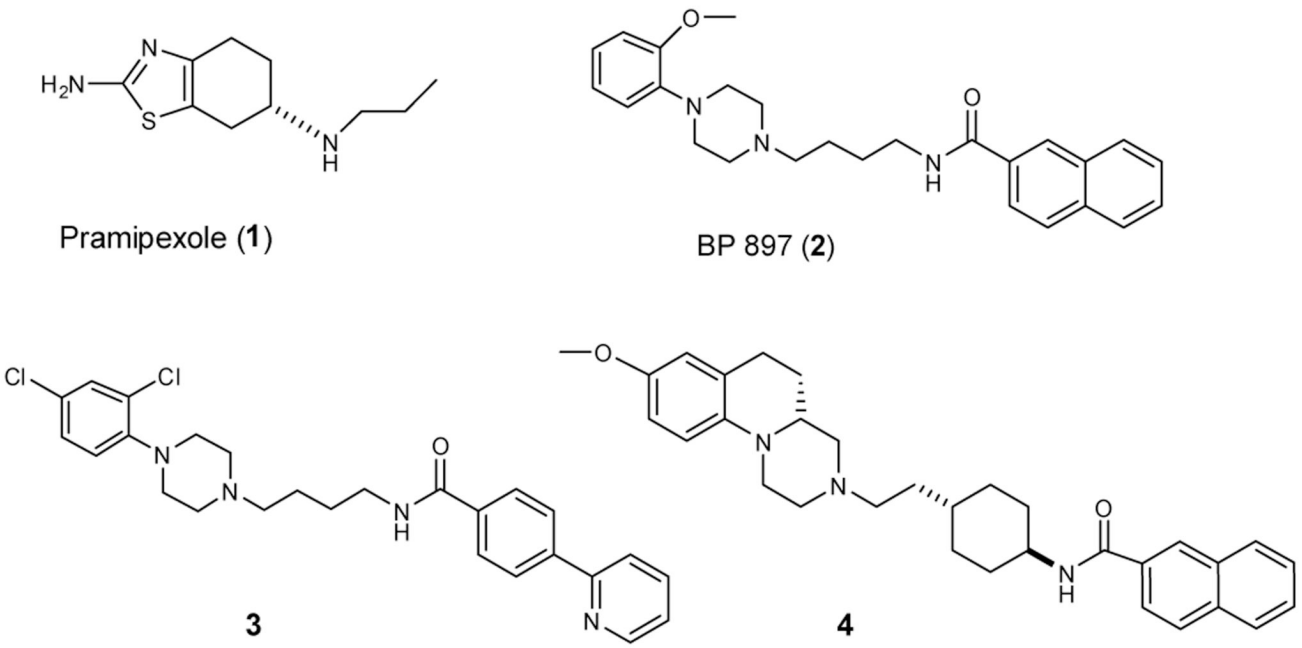

Figure 1.
Chemical structures of representative D_3 ligands.

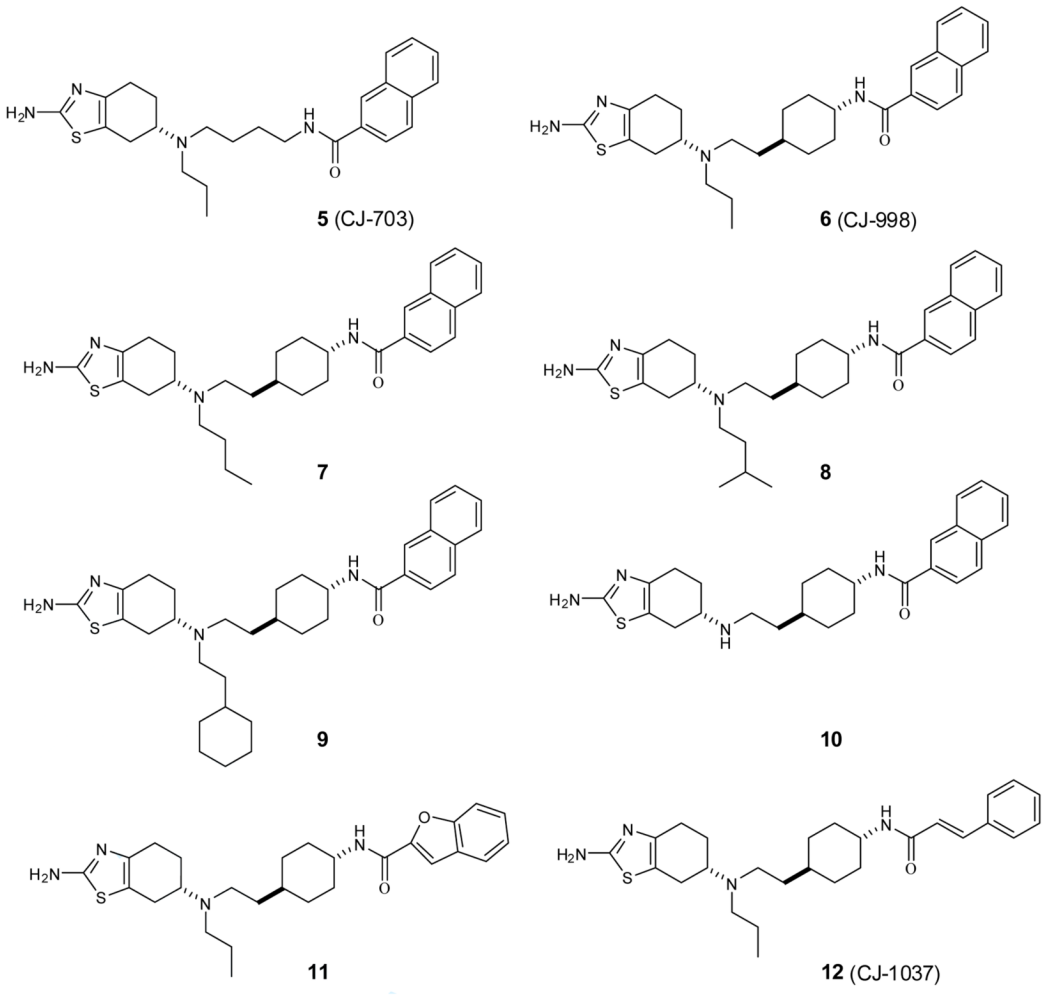

Figure 2.
New analogues structurally related to pramipexole.

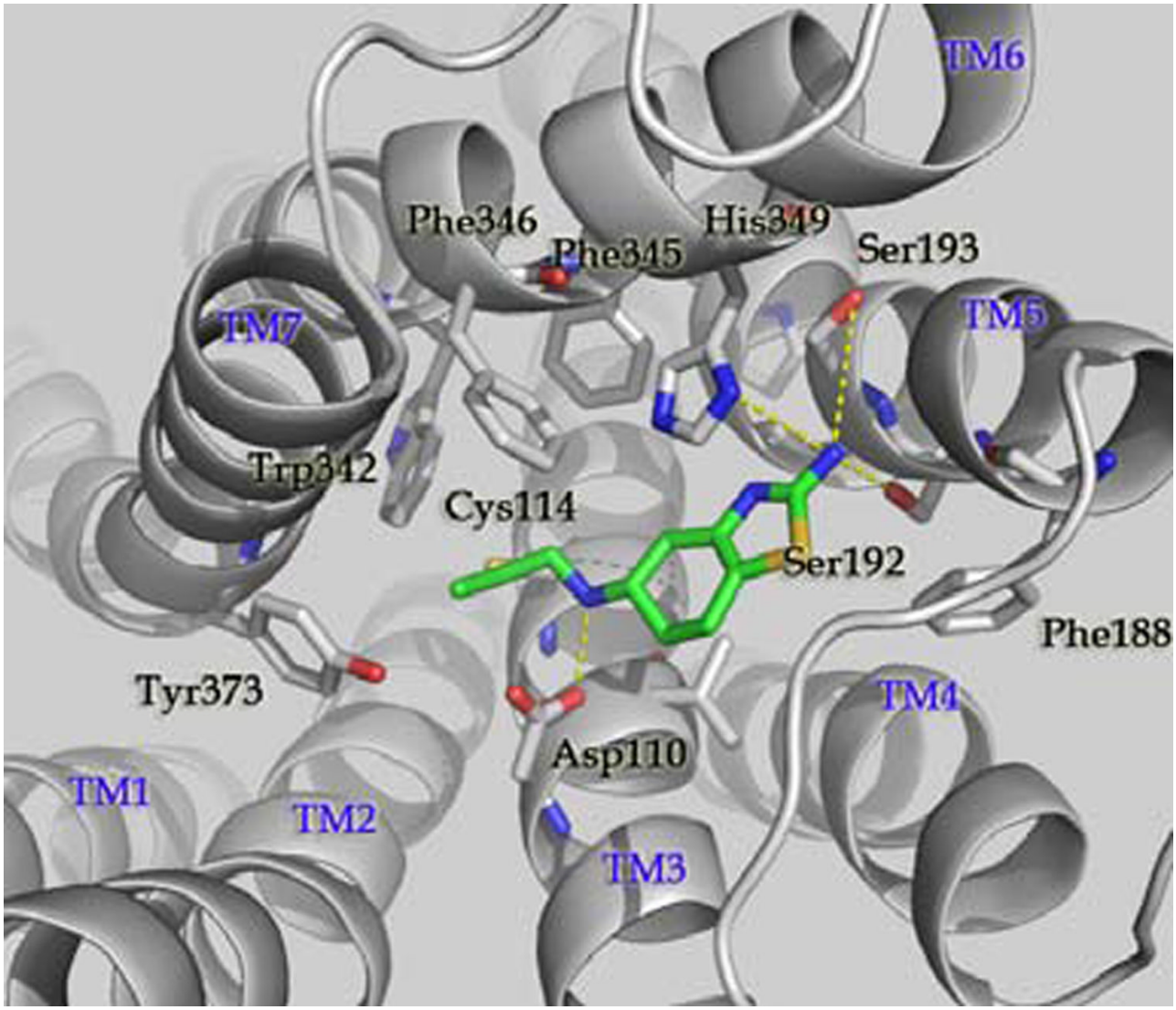

Figure 3.

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.

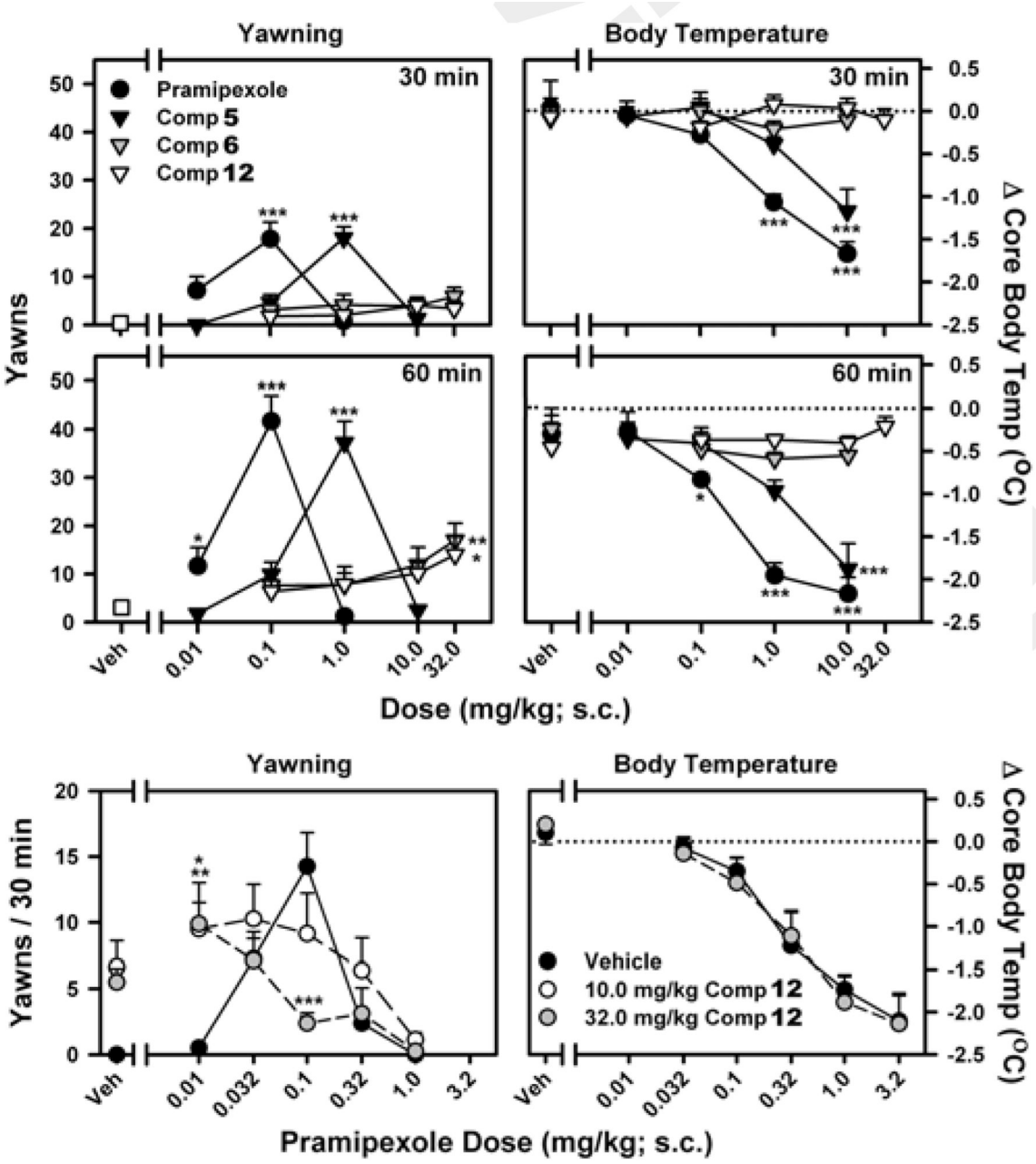

Figure 4.

Functional evaluations of the D_3 and D_2 activity of pramipexole, 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.

Table 1

Binding affinities at the D_1-like, D_2 and D_3 receptors in binding assays using rat brain. Data represent the mean ± SEM of 3–5 independent determinations. For compounds producing a 2-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.

	K_i ± SEM (nM)			Selectivity	
Ligand	**D_3[^{3}H]PD128 907**	**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)	>100,000	4.0	>100,000
4	5.7 ± 0.4	>10000	>50000	>1000	>5000
5	0.043 ± 0.006	2.7 ± 0.4 (h) 6700 ± 1500 (l)	11,000 ± 500	62	>100,000
6	0.40 ± 0.057	307 ± 38	3,400 ± 300	763	>7,000
7	0.74 ± 0.083	55 ± 12 (h) 1300 ± 180 (l)	5,400 ± 500	74	>7,000
8	2.2 ± 0.10	345 ± 33	13,000 ± 1,000	157	>5,000
9	23 ± 2.7	1,200 ± 170	4,400 ± 800	53	194
10	7.6 ± 0.87	670 ± 140	64,000 ± 7,000	88	>8,000
11	0.51 ± 0.10	68 ± 4.6	4,900 ± 600	133	>9,000
12	0.41 ± 0.031	330 ± 69	13,000 ± 1,700	800	>30,000