Computational Elucidation of the Structural Basis of Ligand Binding to the Dopamine 3 Receptor through Docking and Homology Modeling

Judith Varady Hobrath and Shaomeng Wang*

Departments of Internal Medicine, Pharmacology, and Medicinal Chemistry, University of Michigan, 1500 E. Medical Center Drive, Ann Arbor, Michigan 48109-0934

Received February 20, 2005

The dopamine subtype 3 receptor (D_3) is a promising therapeutic target for the treatment of cocaine addiction, schizophrenia, Parkinson's disease, and other disorders, but little is known about the binding of ligands to D_3 at the atomic level. In the present study, binding of 29 known ligands to the D_3 receptor was modeled computationally using four D_3 receptor models which were obtained from homology modeling. The predicted binding models were validated with experimental data from site-directed mutagenesis, structure−activity relationship studies, and affinity labeling studies. Docking scores calculated for these 29 ligands correlate reasonably well with the experimentally determined binding affinities. A pharmacophore model is proposed that describes the binding of ligands at a single D_3 receptor binding site and offers insights into the binding of structurally diverse D_3 ligands to this receptor.

Introduction

The dopamine-3 (D_3) receptor, cloned in 1990, has 52% sequence homology to the D_2 receptor and a similar, but distinct, pharmacological profile.[1] Compared to the D_1 and D_2 receptors, the D_3 receptor is much less abundant and concentrated almost exclusively in limbic brain regions such as the nucleus accumbens, olfactory tubercle, and islands of Calleja.[2−4] These are regions associated with emotions, motivated behaviors, cognitive functions, and reward mechanisms,[5−13] and implicated in schizophrenia,[14−16] Parkinson's disease,[17] and drug addiction.[18−20] The D_3 receptor is therefore considered to be a promising therapeutic target for the treatment of such disorders,[5−20] and selective D_3 ligands may have therapeutic potential. Accordingly, there is strong research interest in the design of potent and selective D_3 ligands.[21,22]

Accurate three-dimensional (3D) structural information for the dopamine receptors is not available, and the structural basis of ligand binding and selectivity to the D_3 receptor is poorly understood. The dopamine receptors are membrane-bound proteins, and experimental determination of their 3D structures is an extremely difficult task. The lack of accurate structural information on D_3 and other dopamine receptors is a significant impediment to the development of highly selective D_3 ligands.

Dopamine receptors belong to the family of G-protein coupled receptors (GPCRs), whose structures are characterized by seven transmembrane helices (TM1−TM7). In our previous study,[23] we employed a computational homology modeling approach to model the 3D structure of the D_3 receptor based upon a high-resolution crystal structure of another GPCR, rhodopsin.[24] With modeled structures for the D_3 receptor, we discovered a number of structurally diverse D_3 ligands,[23] suggesting that our modeled structures may be used to study the interaction between other D_3 ligands and the D_3 receptor.

In this study, we performed computational docking studies to investigate the interaction of the D_3 receptor with 29 known D_3 ligands with diverse chemical structures (Figure 1, **1**−**29**). Predicted binding models for these ligands were validated with experimental data from site-directed mutagenesis, structure−activity relationship studies, and affinity labeling studies. Analysis of these results suggests a pharmacophore model that describes the binding of ligands at a single D_3 receptor binding site. Docking scores for these 29 ligands correlate well with experimentally determined binding affinities. This study provides insights into the binding of structurally diverse D_3 ligands to the D_3 receptor, and our results may be used to aid the design of potent and selective D_3 ligands.

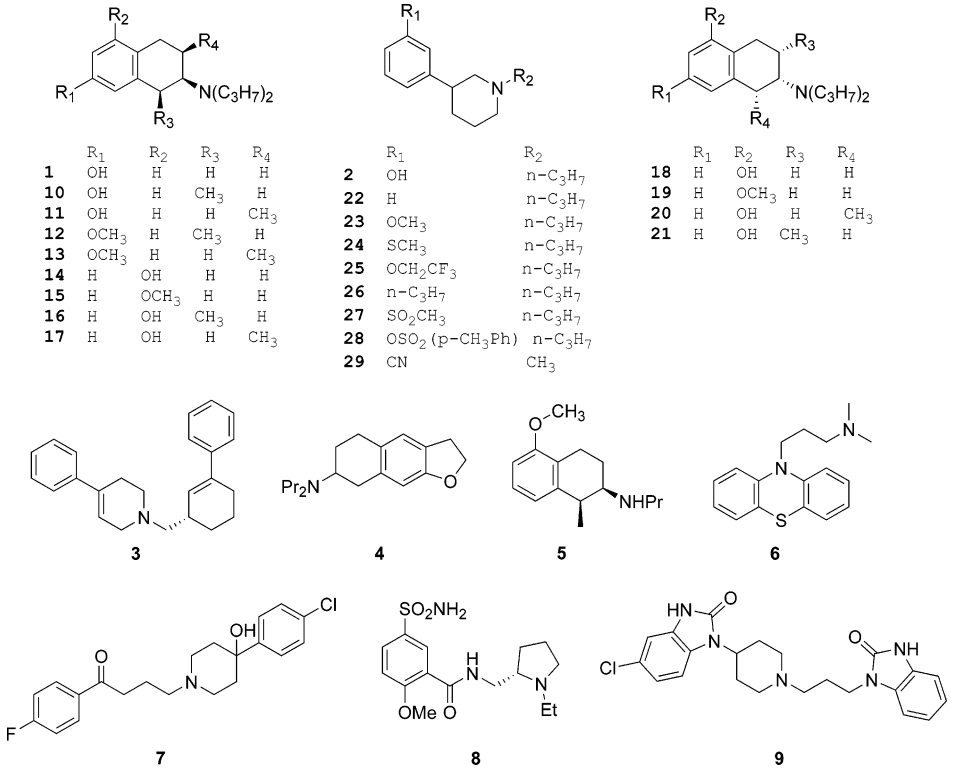
Methods

1. Modeling of the D_3 Receptor. Modeling and refinement of the D_3 dopamine receptor have been reported previously.[23] The D_3 receptor was homology-modeled using the crystal structure of rhodopsin[24] at 2.8 Å resolution as the template. The sequence alignment used was based on sequence analysis of 493 members of the rhodopsin family of GPCR proteins.[25] The membrane−water environment is important for structure and folding of these proteins,[26] so for modeling, the receptor was inserted into a 2-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine membrane model[27] in a water environment. After equilibration, an MD simulation run of 2.0 ns was performed using CHARMM (version 27),[28] with an all-atom representation except for the hydrocarbon tails of lipid molecules for which a united atom representation was used. Coordinates were saved every picosecond. Receptor conformations saved during the last 1.5 ns of the simulation were clustered on the basis of the kink angles of helices TM5 and TM6 and the side chain dihedral angles of residues implicated in ligand binding to various GPCR proteins.[29−39] These were χ_1 for V78, D110, V111, C114, S192, S193, S196, and T369 and χ_1, χ_2 for the side chains of F197, W342, F345, F346, and Y373. In this way, these protein conformers fell into four major clusters (1−4), containing respectively 30%, 16%, 13%, and 10% of the total of 1500 receptor structures. The center conformer from each of these four conformational clusters was used for docking studies, and the coordinates of these four models are available in Supporting Information.

2. Ligand Binding Model Predictions. The structure of each ligand **1**−**29** was built using Quanta[40] (version 2000), energy-minimized using 1000 steps of the Adopted-Basis Newton Raphson method and then further optimized by the application of semi-empirical energy minimization using Mopac (version 6.0) PM3 as implemented in Insight II.[41] The resulting structures were used in the docking experiments. The D_3 models represent the inactive state of the D_3 receptor.[23] It is known that full agonists bind to the active

* To whom all correspondence and request for reprints should be sent. (734) 615-0362 (phone), (734) 647-9647 (fax), shaomeng@umich.edu (e-mail).

10.1021/jm0501634 CCC: $33.50
Published on Web 06/28/2006



	R_1	R_2	R_3	R_4
1	OH	H	H	H
10	OH	H	CH_3	H
11	OH	H	H	CH_3
12	OCH_3	H	CH_3	H
13	OCH_3	H	H	CH_3
14	H	OH	H	H
15	H	OCH_3	H	H
16	H	OH	CH_3	H
17	H	OH	H	CH_3

	R_1	R_2
2	OH	$n\text{-}C_3H_7$
22	H	$n\text{-}C_3H_7$
23	OCH_3	$n\text{-}C_3H_7$
24	SCH_3	$n\text{-}C_3H_7$
25	OCH_2CF_3	$n\text{-}C_3H_7$
26	$n\text{-}C_3H_7$	$n\text{-}C_3H_7$
27	SO_2CH_3	$n\text{-}C_3H_7$
28	$OSO_2(p\text{-}CH_3Ph)$	$n\text{-}C_3H_7$
29	CN	CH_3

	R_1	R_2	R_3	R_4
18	H	OH	H	H
19	H	OCH_3	H	H
20	H	OH	H	CH_3
21	H	OH	CH_3	H

Figure 1. Compounds **1**−**29**.

Table 1. Binding Affinities Obtained from Literature of 29 Compounds to the D_3 Receptor

ligand	K_i (nM)	ref	ligand	K_i (nM)	ref	ligand	K_i (nM)	ref
1	0.61 ± 0.052 (1.6 ± 0.4)	51 (72)	**11**	112 ± 18	51	**21**	3.66 ± 0.36	70
2	132 ± 16	51	**12**	79.9 ± 5.0		**22**	777 ± 39	
3	16.6 ± 3.8	74	**13**	867 ± 130		**23**	1328 ± 57	
4	13.0 ± 0.7	75	**14**	7.90 ± 1.8		**24**	139 ± 12	
5	35.0 ± 4.9 (70 ± 7)	51 (16)	**15**	2.95 ± 0.60		**25**	1414 ± 202	
6	3.0 ± 0.7	72	**16**	14.2 ± 2.4		**26**	86 ± 10	
7	2.2 ± 0.3	75	**17**	597 ± 140		**27**	1305 ± 249	
8	8.0 ± 0.6	72	**18**	0.89 ± 0.051		**28**	92 ± 10	
9	3.5 ± 0.7	71	**19**	10.0 ± 3.2		**29**	1759 ± 92	
10	0.57 ± 0.14	51	**20**	11.1 ± 1.1				

receptor conformation significantly more effectively than to the inactive conformation, and partial agonists are less discriminatory while antagonists bind both states with similar affinities.[42−45] We thus selected antagonists and partial agonists but not full agonists in the present docking studies. The experimentally determined binding affinities for these ligands are provided in Table 1.

Ligands were docked into each of the four D_3 receptor models using the Ligandfit module in Cerius[2] (version 4.6)[46] or Autodock (version 3.0).[47,48] Ligands were allowed to be flexible during docking. Definition of the binding site grid was based on the shape of the protein with default parameters. All atoms were assigned a radius of 2.0 Å. Previous experimental and computational studies have strongly suggested that for dopamine receptors, the ligand binding site is located in the transmembrane region near the extracellular side.[29−39,49] Thus, for our docking studies, we have excluded the cytoplasmic side or loops that, from our calculations, are not part of the binding site.

The CFF force field (version 1997) implemented into Cerius[2] was used. The number of Monte Carlo-based conformational searching trials was set to 0.99×10^6. Clustering of ligand conformations was accomplished with default parameters. A united atom representation was applied in Autodock to the protein with polar hydrogen atoms added and Kollman united-atom partial charges were assigned. A Lamarckian genetic algorithm method was chosen with a maximum number of energy evaluations of 25×10^6. A total 10 docking simulations were performed for each ligand using each receptor model.

3. Docking Models. Binding models of compounds **1**−**9** predicted by Ligandfit or Autodock were evaluated using the following considerations. For **1**, [*R*-(+)-7-hydroxy-2-(di-*n*-propylamino)tetralin, *R*-(+)-7-OH-DPAT], the predicted binding model was validated against experimental data. For compounds **2**−**9**, there being insufficient experimental data to support a specific binding model, the following criteria were used to select the most plausible binding models from those predicted. Each of these ligands contains a protonated amine. It has been proposed[30] that a salt bridge forms between this amine in ligands and the highly conserved D110 in TM3. We have set the distance between the cationic nitrogen in the ligand and an oxygen in D110 to be less than 4.0 Å and used this as an acceptance criterion; binding models that lacked this common salt bridge were excluded.

Structures of the receptor−ligand complex predicted by Ligandfit were ranked with built-in scoring functions in Cerius[2].[46] Each scoring function may rank binding models differently, and so a consensus scoring approach was used with six different scoring functions: (i) Dock score; (ii) Ligscore; (iii) Plp1; (iv) Plp2; (v) Potential mean force; and (vi) Ludi. Each predicted binding model was selected for further consideration in the next step if it was ranked in the top 10% by at least three of the six scoring functions. Models selected by Ligandfit in this way were compared to those predicted by Autodock. With a single D_3 receptor model, the docked structure obtained by Ligandfit was compared to that from Autodock. If the root-mean-square-deviation (RMSD) between the corresponding heavy atoms for the ligand in two predicted binding

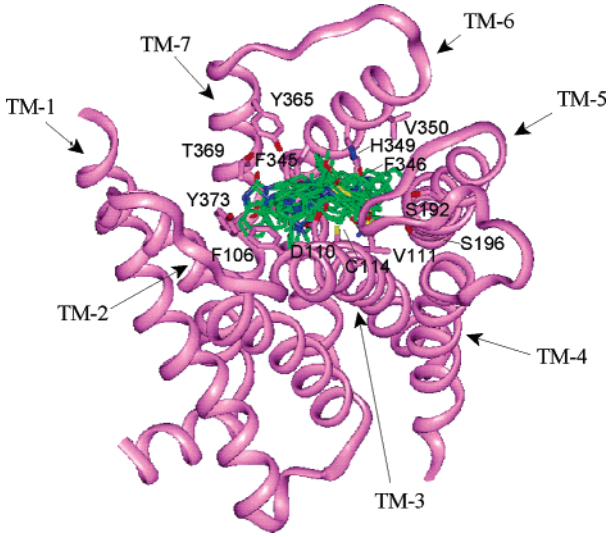


Figure 2. Predicted binding models for compounds **1**−**9**. Only the most populated D_3 cluster center conformer is displayed. Only heavy atoms for ligands are displayed for clarity. Ligand atoms are colored by atom type: carbons green, oxygens red, nitrogen blue, sulfur yellow. The same coloring scheme is used for the D_3 receptor as the ligands except that carbons and backbone ribbon are colored pink.

models was less than 2 Å, these two binding models were considered to be in good agreement and retained for further evaluation.

Docking studies were carried out with compounds **10**−**21**, related to *R*-(+)-7-OH-DPAT (**1**) and compounds **22**−**29**, analogues of *S*-(−)-3-(3-hydroxyphenyl)-*N*-(*n*-propyl)piperidine (*S*-(−)-3-PPP) (**2**). The D_3 conformer used was that which showed optimum binding with either **1** or **2**. All docking was with Ligandfit under the same protocols used for compounds **1**−**9**. The predicted binding models for these ligands must meet two criteria to be accepted: (1) the salt bridge is observed between a ligand and D110 residue in TM3 of the receptor; (2) the binding model is ranked on the top 10% based on Cerius[2] Dock scores.

For evaluation of the interaction between the receptor and ligands, we used simple distance criteria as follows. The cutoff for hydrogen bonding/salt bridge was 4 Å between the donor and acceptor heavy atoms. For hydrophobic contacts involving a group consisting of three carbons or fewer in the ligand, the cutoff distance was 5 Å and for larger groups, 6 Å. Distances were calculated between centers of mass of heavy atoms of the specified interacting groups in the predicted models. For compounds **1**−**9** for which two binding models were obtained using Cerius[2] and Autodock methods, the average distance was used in evaluation of the interactions between two groups.

Results and Discussion

1. Binding Models of Compounds 1−9. Nine ligands (**1**−**9**) were docked on each of the four conformers of the D_3 receptor. For each ligand except for **7** and **9**, there was only a single binding pose in good agreement between Ligandfit and AutoDock methods, which was selected as the predicted binding model for the specific ligand. For compound **7** and **9**, there were four predicted binding poses that were in good agreement between these two docking methods. For these two ligands, each of the predicted binding poses was validated using available experimental data. The binding pose that had the best agreement with experimental data was selected as the predicted binding model for the ligand.

The predicted binding models for these nine ligands are shown in Figure 2. All the ligands **1**−**9** occupy the same D_3 binding site formed by TM3, TM5, TM6, and TM7. Residues D110, F345, and H349 in the receptor were found to interact

Table 2. Pharmacophore Model Based on Predicted Binding Modes of Ligands

average deviations of atom groups from site center (Å)	sites	distance between site centers (Å)				
		Ar	Non-Ar	Hb-1	Hb-2	Hb-3
1.67 ± 0.92	Ar		2.92	2.61	3.46	10.00
1.65 ± 0.66	Non-Ar			4.95	2.80	7.18
1.45 ± 0.69	Hb-1				6.08	11.54
0.45 ± 0.00	Hb-2					8.64
0.40 ± 0.00	Hb-3					
1.76 ± 0.89	N−s	5.03	2.18	6.70	4.40	5.09

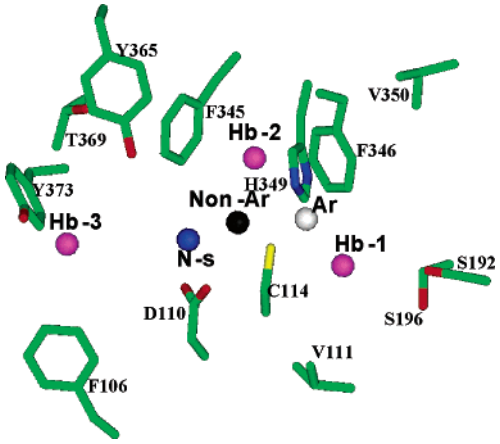


Figure 3. Pharmacophore of the dopamine D_3 receptor. Ar is the preferred site for an aromatic ring, non-Ar for an alicyclic ring, three hydrogen bonding (Hb) centers and a salt bridging site (N−s).

with every ligand while residues F346, Y373, C114, S192, S196, V111, and T369 make contact with some but not all of the ligands examined. Some of the binding interactions, for example in **7** and **8**, are similar to interactions seen at the D_2 receptor.[49]

Our docking studies suggest that there are five important features in the pharmacophore: a salt bridge, an aromatic and a nonaromatic pharmacophore site, and donors/acceptors for three hydrogen bonds (Figure 4). A hydrogen bond, Hb-1, is found between the ligands and S192 and S196 in TM5. The two other hydrogen bonding centers are Hb-2 near the H349 and F345 backbone atoms in TM6 and Hb-3, which is close to Y373 in TM7. The salt bridge interaction with D110 in TM3 is formed via a protonated amine in all the ligands. A partial view of the receptor emerges from these data and is summarized in Table 2, which lists the distances between each of the five binding points of the receptor. These binding points are mapped onto the model of the D_3 binding site, as shown in Figure 3. It should be noted that not all the ligands use all five of the pharmacophore sites in interacting with the D_3 receptor.

2. Binding Models of Analogues of (*R*)-7-OH-DPAT (compounds 10−21). Docking of compounds **10**−**21**, all analogues of (*R*)-7-OH-DPAT (**1**), was studied in the same way. These DPAT analogues have D_3 binding affinities between <1 nM and >0.8 μM (Table 1)[51] and were grouped according to their D_3 binding affinities.

Compounds **1**, **10**, and **18** all have sub-nanomolar binding affinities and are the most potent ligands in this series (Table 1). The docked structures for these three ligands possess a number of common features. All form a salt bridge involving D110. In the models of **1** and **10** bound to the receptor (Figure 4), there is a hydrogen bond between the phenolic hydroxyl of the ligands and S192. Indeed, mutagenesis results[50] combined with SAR data[51] strongly support a hydrogen bonding interaction between **1** and S192. For ligand **18**, a hydrogen bond is predicted to form between the ligand hydroxyl and H349. The tetralin

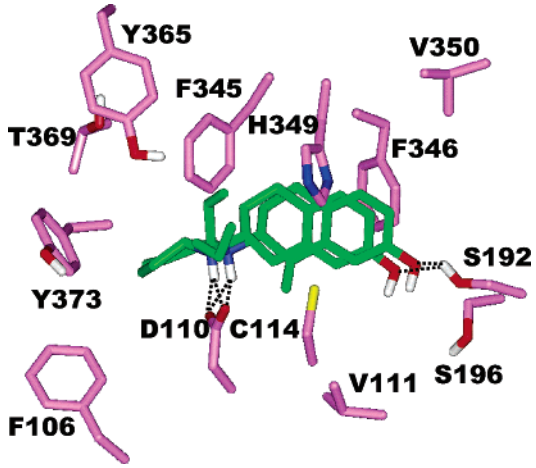


Figure 4. Predicted binding models for compounds **1** and **10**. Same color schemes are used as those in Figure 3.

ring of each compound lies in approximately the same plane and participates in nonpolar interactions with F345, F346, and H349.

Ligands **12**, **14**−**16**, and **19**−**21** have binding affinities between 1 and 100 nM (Table 1). Their docked structures occupy approximately the same space in the D_3 receptor ligand binding pocket as the more potent ligands, but their tetralin rings do not all lie in a single plane. Compounds **11**, **13**, and **17**, the least potent analogues, while forming the salt bridge with D110, lack the favorable interactions with F345, F346, and H349, which were seen with the more potent ligands. In addition, none of these three compounds shows any hydrogen bonding with the serines in TM5.

3. Binding models of 3-substituted *S*-phenylpiperidine analogues (compounds **22**−**29**).

These compounds are all analogues of compound **2**. Introduction of various substituents at position 3 of the phenyl ring in these compounds modulates the binding affinities of ligands **22**−**29** as follows. The unsubstituted analogue (**22**) has a K_i value of 777 nM. Compounds **2**, **24**, **26**, and **28** have better affinities than **22** (K_i <150 nM) while compounds **23**, **25**, **27**, and **29** all have $K_i > 1.3\ \mu$M. Thus, with reference to compound **22**, these ligands may be grouped into a more potent group (**2**, **24**, **26**, **28)** and a less potent group (**23**, **25**, **27**, **29**).

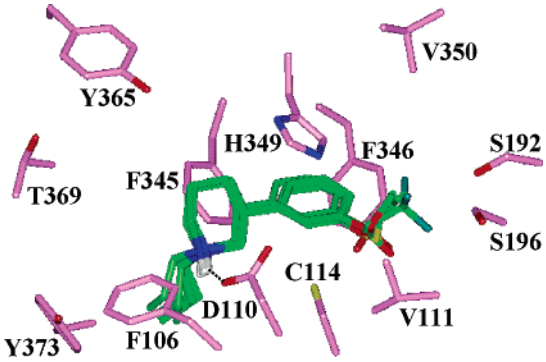


Figure 5. Predicted binding models of compound **2** and its analogues **22**−**27**.

Upon the basis of our predicted binding models, the 3-phenyl substituent of the phenylpiperidine occupies a cavity formed by the D_3 side chains V111, H349, C114, and F346. Thus, a small nonpolar phenyl substituent, as in **24** and **26**, may be accommodated favorably in such a cavity. If the side chain of H349 is unprotonated, then hydrogen bond donors such as the hydroxyl group of **2** will be more favorable than hydrogen bond acceptors, e.g. those in **23**, **25**, and **27**, which may explain why **2** is more potent than **23**, **25**, and **27**. The predicted binding models for several compounds in this series are shown in Figure 5.

4. Validation of Binding Model Predictions. Our docking studies allowed the identification of a set of residues in the D_3 receptor involved in ligand binding, which are listed in Table 3. We have attempted to validate these predictions using experimental data.

Several residues are implicated in the binding for the majority of the 29 ligands, including D110, C114, F345, F346, and H349. Although there is no experimental data to directly support the involvement of D110 in the D_3 receptor for ligand binding, site-directed mutagenesis study for the corresponding aspartate residue in the D_2 receptor has provided clear evidence for the importance of this highly conserved aspartate residue in the binding of dopamine receptors to their ligands.[56] F345 and F346 in TM6 are very close to ligands with which they have hydrophobic interactions. Mutation of the corresponding residue for F345 in the D_2 receptor has indeed established its importance in ligand binding.[62] The possible involvement of F346 in ligand

Table 3. Residues Found to Interact with Ligands Based upon Predicted Models and Direct or Indirect Experimental Supporting Evidence

D_3 residue	helix	type of interaction	binding models that contain the interaction (%) **1**−**9**	**10**−**21**	**22**−**29**	all	experimental evidence
F106	TM3	hydrophobic/steric	11	10	11	11	mutation data of the corresponding D_2 residue.[55]
D110	TM3	salt bridge	100	100	100	100	mutation data of the corresponding D_2 residue.[56]
V111	TM3	nonpolar	22	19	44	27	mutations of the corresponding D_2 residue.[32]
C114	TM3	steric/nonpolar	56	48	100	62	mutation data of C114 to Serine at D_3.[57]
S192	TM5	hydrogen bonding	22	14	0	11	mutation data at the D_3 and SAR data.[50,58−61]
S196	TM5	hydrogen bonding	33	10	11	16	mutation data of the corresponding D_2 residue.[56,58,61,62]
F345	TM6	hydrophobic	100	100	100	100	mutations of the corresponding D_2 residue.[62]
	TM6	backbone hydrogen bonding	11	5	0	5	
F346	TM6	hydrophobic	89	86	78	84	mutation data of the corresponding residues in other GPCR members.[63, 64, 65]
	TM6	backbone hydrogen bonding	0	14	0	8	
H349	TM6	steric/nonpolar	100	100	100	100	mutation data of the corresponding D_2 residue.[66]
	TM6	hydrogen bonding	22	48	11	32	Mutation data.[67]
V350	TM6	steric/nonpolar	11	5	0	5	cysteine substitution of the corresponding residue at the D_2 receptor.[34]
Y365	TM7	steric/nonpolar	0	5	22	8	mutation data of the corresponding D_2 residue.[55]
	TM7	hydrogen bonding	0	0	11	3	
T369	TM7	steric	22	14	0	14	mutation data.[67]
Y373	TM7	steric/nonpolar	56	0	22	16	SCAM[37] and mutation data at D_2[69]
	TM7	hydrogen bonding	11	14	0	11	

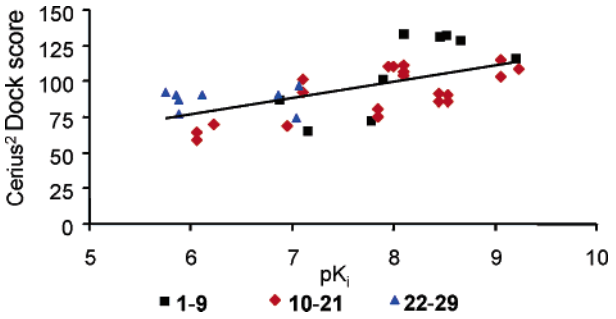


Figure 6. Calculated Dock scores for compounds **1**−**29** using the predicted binding models and experimentally determined binding affinities (pK_i). The Pearson's correlation coefficient is 0.61.

binding is supported by site-directed mutagenesis studies of the corresponding residues in other GPCR members, including the V1a vasopressin receptor,[63] the serotonin $5HT_{1B}$ receptor,[64] and the histamine 1 receptor.[65] The residue H349 has been implicated in ligand binding based upon the site-directed mutagenesis analysis of the corresponding residue in the D_2 receptor[66,67] and C114 has been directly implicated in ligand binding by mutation of this residue to serine in the D_3 receptor.[57]

Our modeling studies also indicate that S192 and S196 may play a role in ligand binding for a subset of ligands, through hydrogen bonding. The involvement of S192 has been strongly suggested through extensive mutation studies at the D_3 receptor and structure−activity relationship studies of ligands,[30,50,58−61] and the importance of S196 in ligand binding has been indirectly suggested by mutation studies of the corresponding serine residue at the D_2 receptor.[30,56,58,61,62] The involvement of other residues in ligand binding also finds experimental support, as indicated in Table 3.

It should be noted that many of the mutagenesis analyses were performed on the D_2 receptor. The D_2 and D_3 receptors are highly homologous proteins and most ligands bind to these two receptors with no or very little selectivity, suggesting very similar 3D structures for these two receptors. Nevertheless, direct site-directed mutagenesis studies with the D_3 receptor are clearly needed to confirm these predictions which are based upon molecular models.

5. Calculated Scores and Experimental Binding Affinities. Figure 6 shows a plot of the calculated Cerius^2 Dock scores for compounds **1**−**29** against pK_i. The docking scores for compounds **1**−**29** correlate reasonably well with ligand binding affinities, giving a correlation coefficient of 0.61. Although the correlation coefficient of 0.61 appears to be lower than those obtained from ligand-based modeling approaches, it is important to note that our study includes structurally diverse ligands. In addition, various assay conditions were used in the determination of the binding affinities for these ligands. The reasonable correlation between the calculated docking scores and the experimentally determined binding affinities for these ligands further suggests that our predicted binding models may be useful for the understanding of ligand binding to the D_3 receptor and for the design of new D_3 ligands.

6. Reliability and Quality of Ligand Binding Model Predictions. The reliability and quality of binding model predictions depend on many factors, including the correctness of the protein structure(s), knowledge of the binding site, reliability of the docking method, quality of the force field employed, accounting of the flexibility of both protein and ligand during docking, and accuracy of the scoring functions used for ranking and selection of binding models. In this study, we have attempted to improve the reliability of the predicted binding models for these 29 ligands in the following ways.

The receptor models we used in our study were refined in an explicit lipid bilayer−water environment through a 2 ns MD simulation. Refinement of D_3 receptor structures via MD simulation leads to structures in which the distances between D110 in TM3 and the three residues S192, S193, and S196 in TM5 are less than in the rhodopsin crystal structure. Many of the ligand models include interactions with these residues and thus the closer TM3−TM5 distances could make an important difference in ligand docking predictions. Evidence presented in the case of $5\text{-}HT_{1A}$[52] suggests that the distances between these residues are crucial for the binding of neurotransmitters.

Four distinct D_3 receptor structures were used in this study. Each of these is representative of a cluster of receptor structures. Such use of multiple receptor structures accounts at least partially for protein flexibility and generally gives results superior to those obtained by docking with a single rigid protein conformer.[53,54]

We used two different docking methods, Ligandfit and Autodock, in our study. We felt that the use of two different docking methods may provide a validation to each other, and we required agreement between these methods when selecting binding models.

In addition, we have validated the predicted binding models using available experimental data. Finally, we have shown that there is a good correlation between the calculated docking scores and experimentally determined binding affinities for these 29 ligands.

Summary

In this study we have performed binding model predictions for 29 antagonist and partial agonist ligands to the D_3 receptor. Four different D_3 receptor structures obtained from our previous homology modeling and MD refinement in an explicit lipid−water environment were each used for docking. Selection of 'best' binding models was based on scoring functions and agreement between different docking methods. The obtained binding models for ligands were validated using experimental data wherever available. The results lead to a proposed pharmacophore model, consisting of an aromatic region, a nonaromatic area, three hydrogen bonding sites, and a salt bridging interaction site. However, not all the D_3 ligands use all the five pharmacophore sites. The docking score for these 29 ligands correlates reasonably well with experimentally determined ligand binding affinities. This study provides an improved understanding for the structural basis of ligand binding to the D_3 receptor, which may be used for the structure-based design of potent and novel D_3 ligands.

Acknowledgment. We would like to thank Dr. G. W. A. Milne for careful reading and editing of this manuscript.

Supporting Information Available: Coordinates of the four dopamine 3 receptor models used in this study are included. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Sokoloff, P.; Giros, B.; Martres, M.-P.; Bouthenet, M.-L.; Schwartz, J.-C. Molecular cloning and characterization of a novel dopamine receptor (D_3) as a target for neuroleptics. *Nature* **1990**, *347*, 146−151.

(2) Landwhermeyer, B.; Mengod, G.; Palacios, J. M. Dopamine D_3 receptor mRNA and binding sites in human brain. *Brain Res. Mol. Brain Res.* **1993**, *18*, 187−192.

(3) Murray, A. M.; Ryoo, H. L.; Gurevich, E.; Joyce, J. N. Localization of dopamine D_3 receptors to mesolimbic and D_2 receptors to mesostriatal regions of human forebrain. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 11271−5.

(4) Bouthenet, M. L.; Souil, E.; Martres, M. P.; Sokoloff, P.; Giros, B.; Schwartz, J. C. Localization of dopamine D_3 receptor mRNA in the rat brain using in situ hybridization histochemistry: comparison with dopamine D_2 receptor mRNA. *Brain Res.* **1991**, *564*, 203−219.

(5) Fibiger, H. C.; Phillips, A. G. Dopamine systems and reward. *Ann. N. Y. Acad. Sci.* **1988**, *537*, 206−215.

(6) McBride, W. J.; Murphy, J. M.; Ikemoto, S. Localization of brain reinforcement mechanisms: intracranial self-stimulation and intracranial place-conditioning studies. *Behav. Brain Res.* **1999**, *101*, 129−152.

(7) Emilien, G.; Maloteaux, J.-M.; Geurts, M.; Hoogenberg, K.; Cragg, S. Dopamine receptors − physiological understanding to therapeutic intervention potential. *Pharmacol. Ther.* **1999**, *84*, 133−156.

(8) Schwartz, J.-C.; Diaz, J.; Pilon, C.; Sokoloff, P. Possible implications for the dopamine D3 receptor in schizophrenia and in antipsychotic drug actions. *Brain Res. Brain Res. Rev.* **2000**, *31*, 277−287.

(9) Strange, P. G. Antipsychotic drugs: Importance of dopamine receptors for mechanisms of therapeutic actions and side effects. *Pharmacol. Rev.* **2001**, *53*, 119−133.

(10) Richtand, N. M.; Woods, S. C.; Berger, S. P.; Strakowski, S. M. D_3 dopamine receptor, behavioral sensitization and psychosis. *Neurosci. Biobehav. Rev.* **2001**, *25*, 427−443.

(11) Carroll, F. I.; Howell, L. L.; Kuhar, M. J. Pharmacotherapies for treatment of cocaine abuse: preclinical aspects. *J. Med. Chem.* **1999**, *42*, 2721−2731.

(12) Levant, B. The D_3 dopamine receptor: neurobiology and potential clinical relevance. *Pharmacol. Rev.* **1997**, *49*, 231−252.

(13) Zhang, J.; Xu, M. Toward a molecular understanding of psychostimulant actions using genetically engineered dopamine receptor knockout mice as model systems. *J. Addict. Dis.* **2001**, *20*, 7−18.

(14) Shapiro, R. M. Regional neuropathology in schizophrenia: where are we? Where are we going? *Schizophr. Res.* **1993**, *10*, 187−239.

(15) Gurevich, E. V.; Bordelon, Y.; Shapiro, R. M.; Arnold, S. E.; Gur, R. E.; Joyce, J. N. Mesolimbic dopamine D_3 receptors and use of antipsychotics in patients with schizophrenia. A postmortem study. *Arch. Gen. Psychiatry* **1997**, *54*, 225−232.

(16) Joyce, J. N.; Gurevich, E. V. D_3 receptors and the actions of neuroleptics in the ventral striatopallidal system of schizophrenics. *Ann. N. Y. Acad. Sci.* **1999**, *877*, 595−613.

(17) Morissette, M.; Goulet, M.; Grondin, R.; Blanchet, P.; Bedard, P. J.; Di Paolo, T.; Levesque, D. Associative and limbic regions of monkey striatum express high levels of dopamine D_3 receptors: effects of MPTP and dopamine agonist replacement therapies. *Eur. J. Neurosci.* **1998**, *10*, 2565−2573.

(18) Caine, S. B.; Koob, G. F. Modulation of cocaine self-administration in the rat through D_3 dopamine receptors. *Science* **1993**, *260*, 1814−6.

(19) 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*, 371−375.

(20) McBride, W. J.; Murphy, J. M.; Ikemoto, S. Localization of brain reinforcement mechanisms: intracranial self-stimulation and intracranial place-conditioning studies. *Behav. Brain Res.* **1999**, *101*, 129−152.

(21) Hackling, A. E.; Stark, H. Dopamine D_3 receptor ligands with antagonist properties. *ChemBioChem.* **2002**, *3*, 946−961.

(22) Newman, A. H.; Grundt, P.; Nader, M. A. Dopamine D3 Receptor Partial Agonists and Antagonists as Potential Drug Abuse Therapeutic Agents, *J. Med. Chem.* **2005**, *48*, 3663−3679.

(23) Varady, J.; Wu, X.; Fang, X.; Min, J.; Hu, Z.; Levant, B. 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*, 4377−4392.

(24) Palczewski, K.; Kumasaka, T.; Hori, T.; Behnke, C. A.; Motoshima, H.; Fox, B. A.; Trong, I. L.; Teller, D. C.; Okada, T.; Stenkamp, R. E.; Yamamoto, M.; Miyano, M. Crystal structure of rhodopsin: a G protein-coupled receptor. *Science* **2000**, *289*, 739−745.

(25) Baldwin, J. M.; Schertler, G. F. X.; Unger, V. M. An Alpha-carbon template for the transmembrane helices in the rhodopsin family of G-protein-coupled receptors. *J. Mol. Biol.* **1997**, *272*, 144−164.

(26) Efremov, R. G.; Nolde, D. E.; Vergoten, G.; Arseniev, A. S. A solvent model for simulations of peptides in bilayers. II. Membrane-spanning α-helices. *Biophys. J.* **1999**, *76*, 2460−2471.

(27) Heller, H.; Schaefer, M.; Schulten, K. Molecular dynamics simulation of a bilayer of 200 lipids in the gel and in the liquid-crystal phases. *J. Phys. Chem.* **1993**, *97*, 8343−8360.

(28) Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. CHARMM: A Program for Macromolecular Energy, Minimization, and Dynamics Calculations. *J. Comput. Chem.* **1983**, *4*, 187−217.

(29) Ballesteros, J. A.; Shi, L.; Javitch, J. A. Structural mimicry in G protein-coupled receptors: implications of the high-resolution of rhodopsin for structure−function analysis of rhodopsin-like receptors. *Mol. Pharmacol.* **2001**, *60*, 1−19.

(30) Shi, L.; Javitch, J. The binding site of aminergic G protein-coupled receptors: the transmembrane segments and second extracellular loop. *Annu. Rev. Pharmacol. Toxicol.* **2002**, *42*, 437−467.

(31) Javitch, J. A.; Fu, D.; Chen, J. Residues in the fifth membrane-spanning segment of the dopamine D_2 receptor exposed in the binding-site crevice. *Biochemistry* **1995**, *34*, 16433−39.

(32) Javitch, J. A.; Fu, D.; Chen, J.; Karlin, A. Mapping the binding-site crevice of the dopamine D_2 receptor by the substituted-cysteine accessibility method. *Neuron* **1995**, *14*, 825−831.

(33) Shi, L.; Simpson, M. M.; Ballesteros, J. A.; Javitch, J. A. The first transmembrane segment of the dopamine D_2 receptor: Accessibility in the binding site crevice and position in the transmembrane bundle. *Biochemistry* **2001**, *40*, 12339−12348.

(34) Javitch, J. A.; Ballesteros, J. A.; Weinstein, H.; Chen, J. A cluster of aromatic residues in the sixth membrane-spanning segment of the dopamine D_2 receptor is accessible in the binding-site crevice. *Biochemistry* **1998**, *37*, 998−1006.

(35) Javitch, J. A.; Ballesteros, J. A.; Chen, J.; Chiappa, V.; Simpson, M. M. Electrostatic and aromatic microdomains within the binding-site crevice of the D_2 receptor: contributions of the second membrane-spanning segment. *Biochemistry* **1999**, *38*, 7961−68.

(36) Javitch, J. A.; Shi, L.; Simpson, M. M.; Chen, J.; Chiappa, V.; Visiers, I.; Weinstein, H.; Ballesteros, J. A. The fourth transmembrane segment of the dopamine D_2 receptor: accessibility in the binding-site crevice and position in the transmembrane bundle. *Biochemistry* **2000**, *39*, 12190−99.

(37) Fu, D.; Ballesteros, J. A.; Weinstein, H.; Chen, J.; Javitch, J. A. Residues in the seventh membrane-spanning segment of the dopamine D_2 receptor accessible in the binding-site crevice. *Biochemistry* **1996**, *35*, 11278−85.

(38) Javitch, J. A.; Li, X.; Kaback, J.; Karlin, A. A cysteine residue in the third membrane-spanning segment of the human D_2 dopamine receptor is exposed in the binding-site crevice. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 10355−59.

(39) Javitch, J. A.; Fu, D.; Chen, J. Differentiating dopamine D_2 ligands by their sensitivities to modification of the cysteine exposed in the binding-site crevice. *Mol Pharmacol.* **1996**, *49*, 692−698.

(40) Quanta, a molecular modeling system, supplied by Accelrys., San Diego, CA.

(41) Insight II, a molecular modeling system, supplied by Accelrys, San Diego, CA.

(42) Burris, K. D.; Pacheco, M. A.; Filts, T. M.; Kung, M. P.; Kung, H. F.; Molinoff, P. B. Lack of discrimination by agonists for D_2 and D_3 dopamine receptors. *Neuropsychopharmacology* **1995**, *12*, 335−345.

(43) Malmberg, A.; Mohell, N.; Hook, B. B.; Johansson, A. M.; Hacksell, U.; Nordvall, G. Interactions of ligands with active and inactive conformations of the dopamine D_2 receptor. *Eur. J. Pharmacol.* **1998**, *346*, 299−307.

(44) Vanhauwe, J. F.; Fraeyman, N.; Francken, B. J.; Luyten, W. H.; Leysen, J. E. Comparison of the ligand binding and signaling properties of human dopamine D(2) and D(3) receptors in Chinese hamster ovary cells. *J. Pharmacol. Exp. Ther.* **1999**, *290*, 908−916.

(45) Malmberg, M.; Mohell, N. Agonist and inverse agonist activity at the dopamine D_3 receptor measured by guanosine 5′−gamma-thio-triphosphate−35S− binding. *J. Pharmacol. Exp. Ther.* **1998**, *285*, 119−126.

(46) Cerius^2, a molecular modeling system, supplied by Accelrys Inc., San Diego, CA.

(47) Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olson, A. J. Automated docking using Lamarckian genetic algorithm and an empirical binding free energy function. *J. Comput. Chem.* **1998**, *19*, 1639−1662.

(48) Goodsell, D. S.; Morris, G. M.; Olson, A. J. Docking of flexible ligands: Applications of AutoDock. *J. Mol. Recognit.* **1996**, *9*, 1−5.

(49) Kalani M. Y. S.; Vaidehi, N.; Hall, S. E.; Trabanino, R. J.; Freddolino, P. L.; Kalani, M. A.; Floriano, W. B.; Kam, V. W. T.; Goddard, W. A., III The predicted 3D structure of the human D2 dopamine receptor and the binding site and binding affinities for agonists and antagonists. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 3815−20.

(50) Sartania, N.; Strange, P. G. Role of conserved serine residues in the interaction of agonists with D_3 dopamine receptors. *J. Neurochem.* **1999**, *72*, 2621−2624.

(51) Malmberg, A.; Nordvall, G.; Johansson, A. M.; Mohell, N.; Hacksell, U. Molecular basis for the binding of 2-aminotetralins to human dopamine D_{2A} and D_3 receptors. *Mol. Pharmacol.* **1994**, *46*, 299−312.
(52) Lopez-Rodriguez, M. L.; Vicente, B.; Deupi, X.; Barrondo, S.; Olivella, M.; Morcillo, M. J.; Behamu, B.; Ballesteros, J. A.; Salles, J.; Pardo, L. Design, synthesis and pharmacological evaluation of 5-Hydroxytryptamine$_{1a}$ receptor ligands to explore the three-dimensional structure of the receptor. *Mol. Pharmacol.* **2002**, *62*, 15−21.
(53) Carlson, H. A.; McCammon, J. A. Accommodating protein flexibility in computational drug design. *Mol. Pharmacol.* **2000**, *57*, 213−218.
(54) Knegtel, R. M. A.; Kuntz, I. D.; Oshiro, C. M. Molecular docking to ensembles of protein structures. *J. Mol. Biol.* **1997**, *266*, 424−440.
(55) Simpson, M. M.; Ballesteros, J. A.; Chiappa, V.; Chen, J.; Suehiro, M.; Hartman, D.; Godel, T.; Snyder, L. A.; Sakmar, T. P.; Javitch, J. A. Dopamine D_4/D_2 receptor selectivity is determined by a divergent aromatic microdomain contained within the second, third and seventh membrane-spanning segments. *Mol. Pharmacol.* **1999**, *56*, 1116−1126.
(56) Mansour, A.; Meng, F.; Meador-Woodruff, J. H.; Taylor, L. P.; Civelli, O.; Akil, H. Site-directed mutagenesis of the human dopamine D_2 receptor. *Eur. J. Pharmacol.* **1992**, *227*, 205−214.
(57) Alberts, G. L.; Pregenzer, J. F.; Im, W. B. Contributions of cysteine 114 of the human D_3 dopamine receptor to ligand binding and sensitivity to external oxidizing agents. *Br. J. Pharmacol.* **1998**, *125*, 705−710.
(58) Coley, C.; Woodward, R.; Johansson, A. M.; Strange, P. G.; Naylor, L. H. Effect of multiple serine/alanine mutations in the transmembrane spanning region V of the D_2 dopamine receptor on ligand binding. *J. Neurochem.* **2000**, *74*, 358−366.
(59) Wiens, B. L.; Nelson, C. S.; Neve, K. A. Contribution of serine residues to constitutive and agonist-induced signaling via the D_{2S} dopamine receptor: evidence for multiple, agonist-specific active conformations. *Mol. Pharmacol.* **1998**, *54*, 435−444.
(60) Cox, B. A.; Henningsen, R. A.; Spanoyannis, A.; Neve, R. L.; Neve, K. A. Contributions of conserved serine residues to the interactions of ligands with dopamine D_2 receptors. *J. Neurochem.* **1992**, *59*, 627−635.
(61) Wilcox, R. E.; Huang, W. H.; Brusniak, M. Y.; Wilcox, D. M.; Pearlman, R. S.; Teeter, M. M.; DuRand, C. J.; Wiens, B. L.; Neve, K. A. CoMFA-based prediction of agonist affinities at recombinant wild type versus serine to alanine point mutated D_2 dopamine receptors. *J. Med. Chem.* **2000**, *43*, 3005−19.
(62) Cho, W.; Taylor, L. P.; Mansour, A.; Akil, H. Hydrophobic residues of the D_2 dopamine receptor are important for binding and signal transduction. *J. Neurochem.* **1995**, *65*, 2105−15.
(63) Cotte, N.; Balestre, M. N.; Aumelas, A.; Mahe, E.; Phalipou, S.; Morin, D.; Hibert, M.; Manning, M.; Durroux, T.; Barberis, C.; Mouillac, B. Conserved aromatic residues in the transmembrane region VI of the V1a vasopressin receptor differentiate agonist vs antagonist ligand binding. *Eur. J. Biochem.* **2000**, *267*, 4253−63.
(64) Granas, C.; Nordvall, G.; Larhammar, D. Mutagenesis of the human 5-HT1B receptor: differences from the closely related 5-HT1A receptor and the role of residue F331 in signal transduction. *J. Recept. Signal. Transduct. Res.* **1998**, *18*, 225−241.
(65) Wieland, K.; Zuurmond, H. M.; Krasel, C.; Ijzerman, A. P., Lohse, M. J. Involvement of Asn-293 in stereospecific agonist recognition and in activation of the β2-adrenergic receptor. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 9276−81.
(66) Woodward, R.; Daniell, S. J.; Strange, P. G.; Naylor, L. H. Structural studies on D_2 dopamine receptors: mutation of a histidine residue specifically affects the binding of a subgroup of substituted benzamide drugs. *J. Neurochem.* **1994**, *62*, 1664−1669.
(67) Lundstrom, K.; Turpin, M. P.; Large, C.; Robertson, G.; Thomas, P., Lewell, X.-Q. Mapping of dopamine D_3 receptor binding site by pharmacological characterization of mutants expressed in CHO cells with the Semliki Forest virus system *J. Recept. Signal Transduct. Res.* **1998**, *18*, 133−150.
(68) Alberts, G. L.; Pregenzer, J. F.; Im, W. B. Identification of transmembrane regions critical for ligand binding to the human D_3 dopamine receptor using various D_3/D_1 transmembrane chimeras. *Mol. Pharmacol.* **1998**, *54*, 379−388.
(69) Daniell, S. J.; Strange, P. G.; Naylor, L. H. Site-directed mutagenesis of Tyr417 in the rat D_2 dopamine receptor. *Biochem. Soc. Trans.* **1994**, *22*, 144S.
(70) Sonesson, C.; Lin, C.-H.; Hansson, L.; Waters, N.; Svensson, K.; Carlsson, A.; Smith, M. W.; Wikstrom, H. Substituted (*S*)-Phenylpiperidines and rigid congeners as preferential dopamine autoreceptor antagonists: synthesis and structure−activity relationships. *J. Med. Chem.* **1994**, *37*, 2735−53.
(71) Millan, M. J.; Peglion, J. L.; Rivet, J. M.; Brocco, M.; Gober, A.; Newman-Tancredi, A.; Dacquet, C.; Bervoets, K.; Girardon, S. et al. Functional correlates of dopamine D_3 receptor activation in the rat in vivo and their modulation by the selective antagonist, (+)-S14297: 1. Activation of postsynaptic D_3 receptors mediates hypothermia, whereas blockade of D_2 receptors elicits prolactin secretion and catalepsy. *J. Pharmacol. Exp. Ther.* **1995**, *275*, 885−898.
(72) Freedman, S. B.; Patel, S.; Marwood, R.; Emms, F.; Seabrook, G. R.; Knowles, M. R.; McAllister, G. Expression and pharmacological characterization of the human D_3 dopamine receptor. *J. Pharmacol. Exp. Ther.* **1994**, *268*, 417−426.
(73) Vanhauwe, J. F.; Josson, K.; Luyten, W. H.; Driessen, A. J.; Leysen, J. E. G-protein sensitivity of ligand binding to human dopamine D(2) and D(3) receptors expressed in *Escherichia coli*: clues for a constrained D(3) receptor structure. *J. Pharmacol. Exp. Ther.* **2000**, *295*, 274−283.
(74) Pugsley, T. A.; Davis, M. D.; Akunne, H. C.; Cooke, L. W.; Whetzel, S. Z.; MacKenzie, R. G.; Shih, Y. Y.; van Leeuwen, D. H.; DeMattos, S. B.; Georgic, L. M. CI-1007, a dopamine partial agonist and potential antipsychotic agent. I. Neurochemical effects. *J. Pharmacol. Exp. Ther.* **1995**, *274*, 898−911.
(75) Newman-Tancredi, A.; Audinot, V.; Jacques, V.; Peglion, J. L.; Millan, M. J. [^{3}H](+)S14297: A novel, selective radioligand at cloned human dopamine D_3 receptors. *Neuropharmacology* **1995**, *34*, 1693−1696.

JM0501634