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Structural Insights into 5-HT$_{1A}$/D$_4$ Selectivity of WAY-100635 Analogues: Molecular Modeling, Synthesis, and in Vitro Binding

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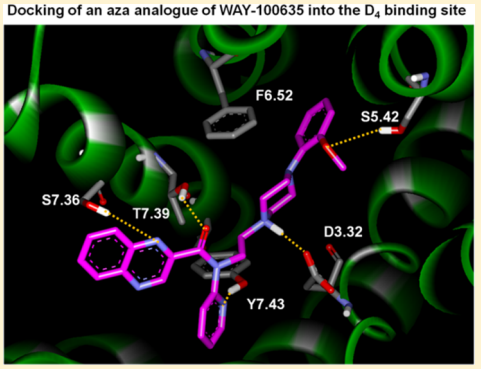
Supporting Information

ABSTRACT: The resurgence of interest in 5-HT$_{1A}$ receptors as a therapeutic target requires the existence of highly selective 5-HT$_{1A}$ ligands. To date, WAY-100635 has been the prototypical antagonist of these receptors. However, this compound also has significant affinity for and activity at D$_4$ dopamine receptors. In this context, this work was aimed at better understanding the 5-HT$_{1A}$/D$_4$ selectivity of WAY-100635 and analogues from a structural point of view. In silico investigations revealed two key interactions for the 5-HT$_{1A}$/D$_4$ selectivity of WAY-100635 and analogues. First, a hydrogen bond only found with the Ser 7.36 of D$_4$ receptor appeared to be the key for a higher D$_4$ affinity for newly synthesized aza analogues. The role of Ser 7.36 was confirmed as the affinity of aza analogues for the mutant D$_4$ receptor S7.36A was reduced. Then, the formation of another hydrogen bond with the conserved Ser 5.42 residue appeared to be also critical for D$_4$ binding.



1. INTRODUCTION

As shown by some recent studies,[1−4] 5-HT$_{1A}$ receptors are a re-emerging therapeutic target for various diseases as different as central nervous system disorders and cancer. Therefore, their pharmacological investigations require the existence of highly selective ligands. Up to now, WAY-100635 (compound **1**), *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]-ethyl]-*N*-(2-pyridinyl)-cyclohexane carboxamide (Table 1), has been the prototypical antagonist of 5-HT$_{1A}$ receptors.[5] Regarding pharmacological studies and pharmacological tools, such a reference molecule is expected to be highly selective for the selected target. It is reported that this molecule shows affinity for and activity at D$_4$ dopamine receptors that could hamper its use as a pharmacological tool, especially when both receptors are close together.[6] Moreover, in a behavioral model, WAY-100635 leads to discriminative stimulus effects in rats mediated by dopamine D$_4$ receptor activation.[7] In this context, diverse chemical changes of the WAY-100635 structure were carried out with the aim of improving its 5-HT$_{1A}$ versus D$_4$ selectivity.[8−13] In particular, the selectivity significantly increased when the basic side chain of WAY-100635 was replaced by a 4-phenylpiperazine (compound **3** in Table 1) or a 4-phenyl-1,2,3,6-tetrahydropyridine moiety (compound **5** in Table 1).[12] In the same study,[12] interestingly, the presence of nitrogen atoms in the acyl group both reduced the affinity for 5-HT$_{1A}$ receptors and increased the affinity for D$_4$ receptors, thus reducing the 5-HT$_{1A}$/D$_4$ selectivity (compound **2**, **4**, and **6** in Table 1). For the purpose of understanding these changes in 5-HT$_{1A}$/D$_4$ selectivity, this present work explores the binding modes of the compounds by docking analysis on homology models of the two receptors. The structural features identified by the in silico investigations as influencing the selectivity are then evaluated by a mutagenesis approach and in vitro binding assays. Furthermore, new compounds were also synthesized and tested to validate our assumptions.

2. MATERIALS AND METHODS

2.1. Molecular modeling. *2.1.1. Receptor Modeling.* The two receptor models were built by homology modeling by means of the SYBYL 8.0 molecular modeling package[14] and according to several stages.

First, the human sequences of the 5-HT$_{1A}$ and D$_4$ receptors obtained from the Universal Protein Resource[15] (code entries P08908[16] and P21917,[17] respectively) were aligned with the sequence of the turkey β1 adrenergic G-protein coupled receptor (GPCR) (code entry P07700)[18] by using the FUGUE sequence alignment module.[19] An adequate alignment of the highly conserved residues of the GPCR superfamily according to Baldwin et al.[20] was carefully checked. For 5-HT$_{1A}$, the sequence alignment indicated homology rates of 83 and 73%, respectively, with the full-length sequence and the transmembrane (TM) regions of the 5-HT$_{1A}$ sequence. For D$_4$, these homology rates were 80 and 66%, respectively.

The next step was the copy of a set of constraints derived from ligand-biased crystal structures of the turkey β1 adrenergic GPCR to the corresponding residues of the sequences to be modeled using the ORCHESTRAR protein structure modeling module.[21] Since the compounds are 5-HT$_{1A}$ antagonists,[12] the 5-HT$_{1A}$ receptor model was built in its inactive form from a

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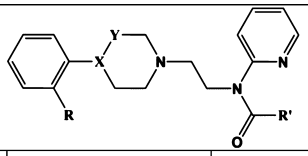
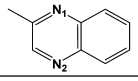
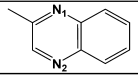
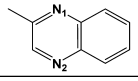
Table 1. In Vitro Binding Evaluation of Compounds 1−6 on Cloned 5-HT_{1A} and D_4 Receptors[12]



Compounds	X-Y	R	R'	5-HT_{1A}[a]	D_4[a]	Selectivity ratio[b]
1	N-CH_2	O-CH_3		0.25 ± 0.04	52 ± 9.4	208
2	N-CH_2	O-CH_3		2.48 ± 0.46	15 ± 1.5	6
3	N-CH_2	H		1.21 ± 0.21	946 ± 144	782
4	N-CH_2	H		36 ± 5.1	56 ± 2.9	1.6
5	C=CH	H		0.51 ± 0.04	385 ± 28	755
6	C=CH	H		8.27 ± 1.14	35 ± 7	4

[a] K_i in nanomolar; mean ± SEM, $n \geq 3$. [b] The selectivity ratio is calculated by dividing D_4 affinity by 5-HT_{1A} affinity.

crystal structure of the inactivated turkey β1 adrenergic receptor (PDB code 2VT4).[22] In the modeling protocol, the cocrystallized antagonist cyanopindolol in the inactivated β1 crystal structure was replaced by WAY-100635. To this end, an exhaustive analysis of conformational space (391 conformations) for WAY-100635 was carried out using the program Random Search[23] of SYBYL (including an energy cutoff of 10 kcal/mol). All the conformations were then aligned according two common structural features for the ligand binding in biogenic amine receptors by using a SPL (SYBYL programming language) program developed by our team. The first key binding feature is the protonated amine which is involved in a hydrogen bond with Asp 3.32 (Ballesteros and Weinstein numbering),[24] an important residue for ligand binding among all mammalian monoamine receptors.[25] The second key binding feature is the aromatic ring which interacts with aromatic residues in helices V and VI.[26−28] In the transfer process, ORCHESTRAR treated WAY-100635 as a rigid group by transferring it from the template into the model protein. In the case of the D_4 receptor, the compounds act as agonists.[12] Thus, the D_4 receptor model was generated in its active form from a crystal structure of the activated turkey β1 adrenergic receptor (PDB code 2Y00).[29] The cocrystallized agonist dobutamine in the activated β1 crystal structure was replaced by WAY-100635 according the same protocol as previously described in the 5HT_{1A} model generation. In the generation of both models, N- and C-terminal parts that are not aligned with the template structures along with the very variable IL3 region were removed and not regarded since they do not participate in the ligand binding. Moreover, the conserved disulfide bond between the cysteine Cys 3.25 of helix 3 and the residue cysteine in the center of extracellular loop ECL2 was added and was maintained as a constraint in the model refinement.

The resulting WAY-100635−receptor complexes were then optimized by energy minimization by means of the MMFF94 force field[30] and the same protocol described in our previous work.[31]

Lastly, the robustness of the complex models was assessed by observing their Ramachandran plot using the RAMPAGE program.[32] High quality parameters with a very good distribution of φ and ψ angles for both models were found. Indeed, more than 100% and 98% of the residues were located in the favored and allowed regions for 5-HT_{1A} and D_4 receptor models, respectively. The residues composing the binding site of the D_4 receptor model do not fall into the 2% disallowed regions.

2.1.2. Ligands Modeling. The protonated compounds were modeled under the Sybyl 8.0 software[14] by using a library of standard fragments. Their geometry was then optimized using the MMFF94 force field.[30] A conformational analysis was performed with the program Random Search[23] of Sybyl to identify the low-energy conformer for each compound.

2.1.3. Binding Modes of the Ligands. The binding mode of the ligands for the two receptors was explored by flexible docking simulations using the GOLD 5.1 program.[33] A sphere of 15 Å centered on the centroid of WAY-100635 was defined as the receptor binding pockets. Flexibility of the ligands was applied by applying torsion angle distributions extracted from the Cambridge Structural Database (CSD) (http://www.ccdc.cam.ac.uk/products/csd/). These distributions help GOLD to find the correct solution by orienting the search toward ligand torsion-angle values that are frequently found in crystal structures. Thirty docking runs were performed. The best docking models were selected on the basis of the high-scoring conformation given by ChemPLP.[34] The docking protocols were validated by redocking WAY-100635 into the rigid 5-HT_{1A} and D_4 models. The resulting positions of the redocked WAY-100635 were found to be close to the initial positions with a RMSD score of 0.75 Å for 5HT_{1A} and 0.52 Å for D_4. The ligand−receptor complexes were further optimized by energy

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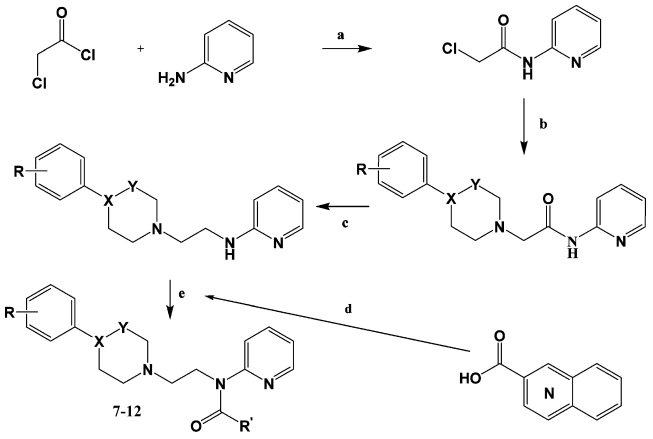
minimization using the MMFF94 force field,[30] and the same protocol defined in our previous work.[31]

2.2. Chemistry. A general procedure for the preparation of the target compounds (**7**−**12**) is summarized below and illustrated in Scheme 1. The corresponding amide (∼1.5 mmol) is dissolved in 25 mL anhydrous diethyl ether. Carefully, an excess of $LiAlH_4$ is added in one time under stirring with cooling. The mixture is stirred for 5 min at 0 °C. After that, the suspension is heated at reflux for 1 h. The end of the reaction is checked by TLC. Then, the mixture is poured on ice with stirring. The precipitate is removed by filtration and washed with methylene chloride. The crude product is extracted with methylene chloride. The organic layer is dried with anhydrous $MgSO_4$ and evaporated under reduced pressure. The residue is used immediately in the next step without further purification. In parallel, the acid chloride (≈1.5 mmol) is prepared by treatment of the appropriate acid with an excess of $SOCl_2$ in freshly distilled acetonitrile at reflux. After removal of the reagent, the crude acid chloride is used immediately.

The crude amine obtained in the previous step is dissolved in ethyl acetate (20 mL) and Et_3N (3 mL) is added. The corresponding acid chloride is dissolved in a mixture of methylene chloride and ethyl acetate and added dropwise. The end of the reaction is checked by TLC. Then, the mixture is evaporated under reduced pressure and the residue is mixed with a 10% aqueous potassium carbonate solution. The suspension is extracted with methylene chloride. The organic layer is dried with anhydrous $MgSO_4$ and evaporated under reduced pressure. The product is purified on kieselgel with ethyl acetate or acetone (or mixture of methanol in ethyl acetate) as mobile phase and further recrystallized mostly in diisopropylether.

Scheme 1. Synthesis of Compounds 7−12[a]



[a]Reagents and conditions: (a) Et_3N, dioxane, 2−3 h, rt, (b) 1-phenylpiperazine or 1-(2-methoxyphenyl)piperazine or 4-phenyl-1,2,3,6-tetrahydropyridine, Et_3N, dioxane, 1−2 h, reflux, (c) $LiAlH_4$, Et_2O, 5 min at 0 °C and 1 h at reflux, (d) $SOCl_2$, ACN, reflux, (e) R′-COCl, Et_3N, EtOAc, 2 h, rt.

2.3. Cell Culture, Cell, and Membrane Preparation. sf9 cells expressing wildtype human cloned $D_{4.2}$ receptors were employed as membrane preparations (purchased from PerkinElmer 6110112). Mutant D_4 receptor DNAs was subcloned into the mammalian plasmid expression vector pCDNA3.1/hygro (+) (Eurogentec, Seraing, Belgium). Mutant D_4 receptors were then transiently expressed in HEK293 cells. Experimental procedures can be found in our previous reports.[12]

2.4. In Vitro Binding. The procedure has been already applied in the work of Graulich et al.[35] and is described in the Supporting Information section.

2.5. Data Analysis. Statistical analysis was carried out by means of PRISM 5.02 (GraphPad Software, San Diego, CA, USA). Data were analyzed with a Kruskal−Wallis test followed by post hoc Mann−Whitney *U* test.

3. RESULTS AND DISCUSSION

3.1. Molecular Docking Studies. *3.1.1. 5-HT$_{1A}$−Ligand Binding Modes.* As presented in Figure 1A, the binding site of WAY-100635 (compound 1) is located in the top of receptor helix bundle between TMD III, V, VI, VII and the extracellular loop 2 and is found to be coherent with data from the literature.

Indeed, in addition to the expected charge reinforced hydrogen bond between the protonated nitrogen atom and the residue Asp 116 (Asp 3.32 according to the Ballesteros and Weinstein numbering[24])[25] and the edge-to-face CH−π interaction between the phenyl ring and the residue Phe 362 (Phe 6.52),[26] three hydrogen bonds (HB) are detected. First, a hydrogen bond is found between the methoxy oxygen atom of the ligand and the hydroxyl group of Ser 199 (Ser 5.42), thus confirming the involvement of this residue in the binding process.[36] Then, a second hydrogen bond is detected between the pyridine nitrogen atom and the hydroxyl group of Tyr 390 (Tyr 7.43).

An interaction with this residue was also found in the ligand binding of other aryl piperazine derivatives.[37] The last hydrogen bond is detected between the carbonyl oxygen atom of the ligand and the amide group of Asn 386 (Asn 7.39). This is coherent with site directed mutagenesis data indicating that the replacement of Asn 386 (Asn 7.39) by a valine leads to a decrease in the 5-HT$_{1A}$ binding affinity of WAY100635 (compound **1**).[38] The geometrical parameters of the interactions are indicated in Table S1.

The hypothetical binding site of WAY-100635 (compound **1**) was then used for docking simulations of compounds **2**−**6**.

The key interactions found for WAY-100635 (compound **1**) are maintained for compound **2** (Figure 1B and Table S1). However, some interactions are less favorable, which could explain the lower affinity found for the receptor (10-fold lower than WAY-100635). Indeed, first, the charge reinforced hydrogen bond formed with Asp 116 (Asp 3.32) appears weaker with HB lengths and angles of 1.93 Å and 161° (versus 1.64 Å and 174° for WAY-100635). Second, the hydrogen bonds formed with Asn 386 (Asn 7.39) and Tyr 390 (Tyr 7.43) are also weaker due to poor HB angles (86° and 96°, respectively). The presence of the bulky quinoxaline group seems to prevent the molecule to adopt a conformation favorable for optimal interactions with the binding site.

For compound **3** (Figure 1C), the removal of the methoxy group obviously leads to an absence of interaction with Ser 199 (Ser 5.42) but has little impact on the other key interactions with a good quality of their parameters (Table S1). It is

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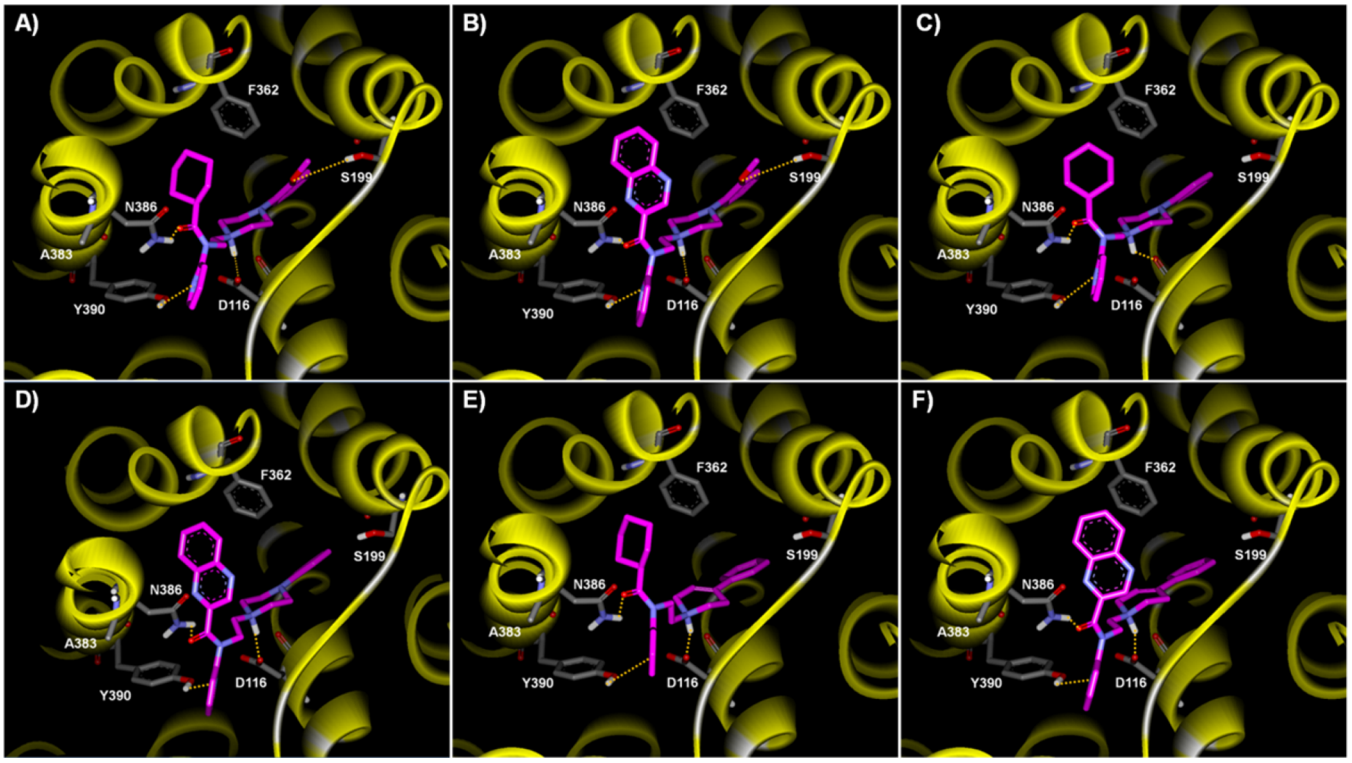

Figure 1. Binding modes of WAY-100635 (A) and compounds **2**–**6** (B–F) in the 5-HT_{1A} receptor model. C atoms are colored gray for the receptor and magenta for the ligands. O, N, and H are colored red, blue, and white, respectively. The hydrogen and ionic bonds are highlighted by orange dashed lines.

probably due to the conserved CH-π interaction with Phe 362 (Phe 6.52) that stabilizes the binding.

For compounds **4** (Figure 1D), the absence of the methoxy group coupled with the presence of the bulky quinoxaline group leads to less favorable interactions, particularly for Asp 116 (Asp 3.32) (L = 1.85 Å) and Asn 386 (Asn 7.39; A = 63°). This could explain the lower affinity for the receptor.

For compounds **5** (Figures 1E), the absence of the methoxy group also reduces the affinity for the receptor, but in a lesser degree than the other compounds. Indeed, as proposed in previous work[39] in the THP analogues, the phenyl ring presents in preference an almost coplanar orientation relative to the THP moiety, which is more favorable for an edge-to-face CH-π interaction with Phe 362 (Phe 6.52).[40] The dihedral angle measured between the ring planes (92°) is very close to the optimal value of 90°.[41] On the contrary, for the piperazine analogue **3**, the more perpendicular orientation of the phenyl ring is less favorable for the CH–π interaction with a dihedral angle of 59°. The same observation can be made for compound **6** that has a better affinity than its piperazine analogue **4**. Indeed, a dihedral angle of 85° is found for compound **6** whereas compound **4** presents a value of 61°.

3.1.2. D_4–Ligand Binding Modes. As shown in Figure 2A, WAY-100635 (compound **1**) is located in the top of receptor helix bundle between TMD III, V, VI, VII, and the extracellular loop 2 and is found to interact with the same conserved residues between D_4 and 5-HT_{1A} receptor binding sites, i.e. Asp 115 (Asp 3.32), Ser 196 (Ser 5.42), Phe 410 (Phe 6.52), Tyr 438 (Tyr 7.43).

The replacement of Asn 434 (Asn 7.39) by a threonine residue in D4 receptor does not seem unfavorable since a hydrogen bond is formed between the carbonyl oxygen atom of the ligand and the hydroxy group of Thr 434 (Thr 7.39). However, some interactions are less favorable than those for 5-HT_{1A} (Table S2), especially the hydrogen bond with Asp 115 (Asp 3.32) (L = 1.93 Å) and the aromatic stacking with Phe 410 (Phe 6.52) (A = 48°). This could explain the lower affinity of WAY-100635 for this receptor.

For compound **2**, two binding modes are detected. In the first one (Figure 2B1 and Table S2), the interactions found with WAY-100635 (compound **1**) are maintained. In addition, a hydrogen bond is formed between the nitrogen N1 of the quinoxaline group and the hydroxy group of Ser 431 (Ser 7.36). In the second binding mode (Figure 2B2 and Table S2), the same interactions are found but the residue Ser 431 (Ser 7.36) interact with the ligand through a hydrogen bond formed with the nitrogen N2 of the quinoxaline group. This interaction could explain the higher affinity found for the receptor.

For compound **3**, the absence of interaction with Ser 196 (Ser 5.42) due to the removal of the methoxy group (Figure 2C and Table S2) coupled to a weak aromatic interaction with Phe 362 (Phe 6.52) (A = 47°) could explain the low D_4 affinity found for this molecule.

For compound **4**, two binding modes are also detected. As with compound **2**, a hydrogen bond is found between the hydroxy group of Ser 431 (Ser 7.36) and either the nitrogen N1 or the nitrogen N2 of the quinoxaline group (Figure 2D1 and D2 and Table S2). This interaction seems compensate the absence of interaction with Ser 196 (Ser 5.42) due to the removal of the methoxy group and thus stabilize the ligand in its binding site. This could explain the good D_4 affinity found for this ligand.

For compound **5**, the removal of the methoxy group coupled to the absence of interaction with the residue Ser 431 (Ser

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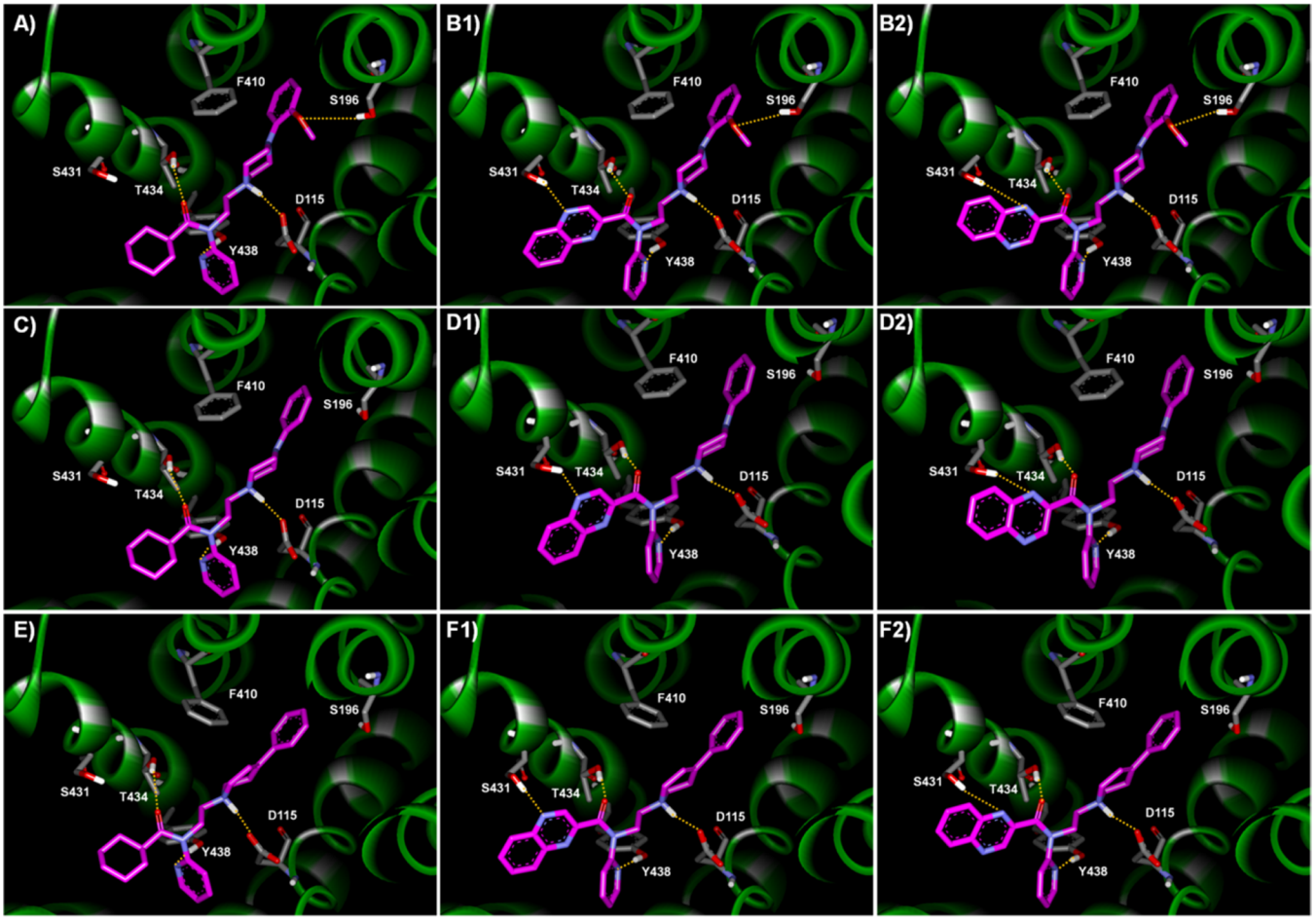

Figure 2. Binding modes of WAY-100635 (A) and compounds **2** (two binding modes B1 and B2), **3** (C), **4** (two binding modes D1 and D2), **5** (E), and **6** (two binding modes F1 and F2) in the D_4 receptor model. C atoms are colored gray for the receptor and magenta for the ligands. O, N, and H are colored red, blue, and white, respectively. The hydrogen and ionic bonds are highlighted by orange dashed lines.

7.36) (Figure 2E) leads to a lower affinity for D4 receptor, but in a lesser extent than its piperazine analogue (compound **3**). According to the geometrical parameters (Table S2), it is probably due to the THP moiety that leads to a better CH-π interaction with Phe 410 (Phe 6.52) (A = 82°) and a stronger reinforced hydrogen bond formed with Asp 115 (Asp 3.32) (L = 1.79 Å).

For compound **6**, like for compounds **2** and **4**, the residue Ser 431 (Ser 7.36) can form a hydrogen bond either the nitrogen N1 or the nitrogen N2 of the quinoxaline group (Figure 2F1 and F2). As compound **5**, the THP moiety also leads to a stronger CH-π interaction with Phe 410 (Phe 6.52) (A = 80°). These favorable interactions can explain the relatively good affinity for the receptor despite the absence of interaction with Ser 196 (Ser 5.42).

In summary, these docking studies first reveal that the absence of interaction with Ser 5.42 due to the removal of the methoxy group is much more unfavorable for the binding to D4 receptor than for the binding to 5-HT_{1A} receptors, thus explaining the higher selectivity for compounds **3** and **5** (782 and 755, respectively). Furthermore, the presence of nitrogen in the acyl group leads to the formation of an additional hydrogen bond with only the D_4 receptors due to the presence of the polar residue Ser 7.36 in the immediate vicinity of the nitrogenous heterocycle (versus an alanine residue in 5HT_{1A} receptors). Thus, this discriminant hydrogen bond appears as the key of the observed increase of D_4 affinity and consequently the reduced of 5-HT_{1A}/D_4 selectivity for compounds **2**, **4**, and **6**.

3.2. Experimental Validation of in Silico Results.

3.2.1. Mutation of Serine 7.36 of D_4 Receptors Decreases the Affinity of Aza Derivatives. In order to validate our assumption about the importance of the residue Ser 7.36 in 5-HT_{1A}/D_4 selectivity, a modified D_4 receptor was prepared by replacing this serine residue by an alanine. The affinity of WAY-100635 and compounds **2**−**6** was determined in in vitro binding assays. As presented in Table 2, WAY-100635 and its related cyclohexyl analogues (**3**, **5**) have a similar affinity for both the native and the S7.36A mutant receptors (≈1−1.5-fold;

Table 2. In Vitro Binding Affinity of Compounds 1−6 for Native and Mutant D_4 Receptors

compounds	D_4[a]	D_4 S7.36A[a]	change ratio[b]
1	52 ± 9.4	80 ± 12	1.5
2	15 ± 1.5	65 ± 11	4.3
3	946 ± 144	1026 ± 167	1.1
4	56 ± 2.9	496 ± 87	8.8
5	385 ± 28	436 ± 75	1.1
6	35 ± 7	230 ± 50	6.5

[a]K_i in nanomolar, mean ± SEM, $n \geq 3$. [b]The change ratio is calculated by dividing D_4 S7.36A affinity by D_4 affinity.

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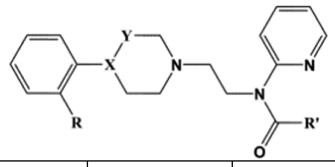
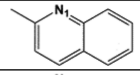
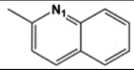
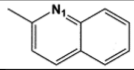
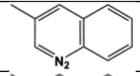
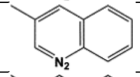
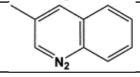
Table 3. In Vitro Binding Affinity of WAY-100635 (1) and Compounds 7−12 for 5-HT_{1A} Receptors, Native, and Mutant D_4 Receptors



Compounds	X-Y	R	R'	5-HT_{1A} [a]	D_4 [a]	Selectivity ratio [b]	D_4 S7.36A [a]	Change ratio [c]
WAY-100635	N-CH_2	O-CH_3		0.25 ± 0.04	52 ± 9.4	208	80 ± 12	1.5
7	N-CH_2	O-CH_3		1.69 ± 0.14	74 ± 10.4	43	347 ± 88.5	4.6
8	N-CH_2	H		27 ± 2.5	278 ± 35	10	1610 ± 188	5.7
9	C=CH	H		9.78 ± 0.82	97 ± 7.5	10	556 ± 113	5.7
10	N-CH_2	O-CH_3		5.86 ± 0.56	191 ± 27.5	32	554 ± 85	2.9
11	N-CH_2	H		85 ± 8.5	1186 ± 86	13	NT	-
12	C=CH	H		28 ± 0.87	415 ± 15.6	14	692 ± 95	1.6

[a] K_i in nanomolar; mean ± SEM, $n \geq 3$. [b] The 5-HT_{1A}/D_4 selectivity is obtained by dividing D_4 affinity by 5-HT_{1A} affinity. [c] The change ratio is calculated by dividing D_4 S7.36A affinity by D_4 affinity. NT: not tested due to a low D_4 affinity.

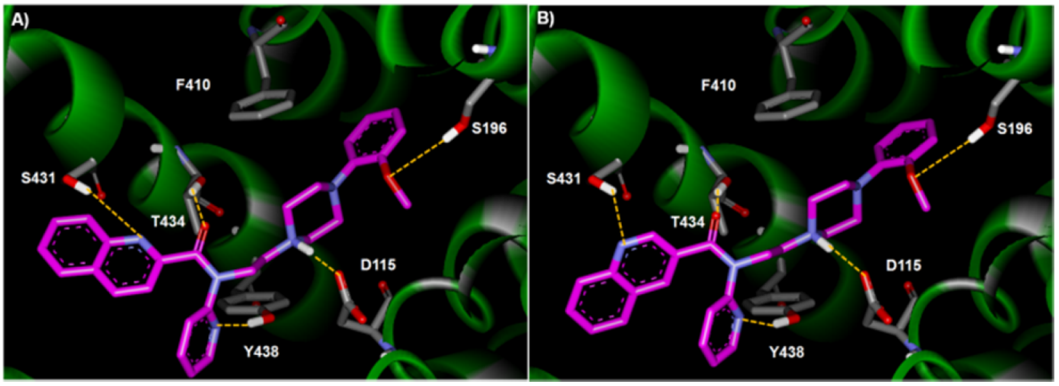


Figure 3. Binding mode of compound 7 (A) and compound **10** (B) in D_4 receptor. C atoms are colored gray for the receptor and magenta for the ligands. O, N, and H are colored red, blue, and white, respectively. The hydrogen and ionic bonds are highlighted by orange dashed lines.

$n \geq 3$, $P > 0.05$, Mann−Whitney test) while the affinity of quinoxaline analogues (**2**, **4**, **6**) is significantly reduced for the S7.36A mutant receptor (≈4−9; $n \geq 3$, $P < 0.05$, Mann−Whitney test). These changes may appear quite low but similar impacts are reported in the literature.[42] Indeed, although some reported mutations had a strong effect on the interactions with small molecules studied in this work such as dopamine or norepinephrine, the impact on bigger molecules is lower and interestingly in the same range than those observed in our paper. This is probably due to the fact that bigger molecules have more interactions, thus stabilizing their binding. In our case, besides the hydrogen bond found with Ser 7.36, other interactions are present (H bonds, salt bridge, CH-π interaction).

Therefore, as previously proposed by in silico data, the polar residue serine 7.36 plays a major role in the higher D_4 affinity found for aza analogues of WAY-100635.

3.2.2. Identification of the Nitrogen Atom Involved in the Interaction with Ser 7.36. According to the docking studies of aza analogues, the residue Ser 7.36 can form a hydrogen bond either the nitrogen atom N1 or the nitrogen atom N2. In order to identify the correct binding mode, i.e. to determine which nitrogen atom is involved in the interaction, 2-quinoline, and 3-quinoline analogues were synthesized (Scheme 1).

All synthesized compounds were subjected to ^1H and ^{13}C NMR, IR, and elemental analysis before biological testing to confirm their chemical structure and/or purity. Yields are not optimized and are between 50 and 70%. Target compounds (**7**−**12**) are evaluated in in vitro binding assays to get the

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affinity for native and mutant D_4 receptors and also for 5-HT_{1A} receptors to determine 5-HT_{1A}/D_4 selectivity (Table 3).

For 2-quinoline analogues (compounds **7**−**9** in Table 3), a significant reduction of the D_4 S7.36A affinity is observed (≈4−6 fold; $n \geq 3$, $P < 0.05$, Mann−Whitney test), thus supporting the role of the nitrogen in the interaction with the residue Ser 431 (Ser 7.36). The binding mode of compound **7** is presented in Figure 3A. However, the D_4 affinity of these compounds is lower than that of their quinoxaline analogues (Table 1), thus suggesting that the presence of a nitrogen atom that position (N1) is a necessary but not sufficient condition for an optimal interaction with the D_4 binding site.

For 3-quinoline analogue **10** (Table 3), a significant reduction of the D_4 S7.36A affinity is observed but in a lesser extent than that for their 2-quinoline analogues **7** ($n \geq 3$, $P < 0.05$, Mann−Whitney test). For 3-quinoline analogue **12** (Table 3), a reduction is also found but it is not significant ($n \geq 3$, $P = 0.100$, Mann−Whitney test). Compound **11** (Table 3) was not tested due to a low D_4 affinity. These data show that an interaction is possible between the nitrogen N2 and Ser 431 (Ser 7.36) (e.g., compounds 7 and 10 in Figure 3B). However, the geometrical parameters of the hydrogen bond formed with Ser 431 (Ser 7.36) appears less favorable than those of analogue **7** (L = 3.70 Å and A = 152° versus L = 2.60; A = 170°, respectively). This could explain why the affinity of compound **10** is less affected by the mutation than that of its 2-quinoline analogue.

In summary, these results indicate that two binding modes are possible, but the one involving the nitrogen N1 appears more favorable.

4. CONCLUSION

This work aimed at explaining the impact of some chemical modifications of WAY-100635 on 5-HT_{1A}/D_4 selectivity. Our investigations identified two interactions with a significant role in that selectivity. The absence of hydrogen bond with Ser 5.42 combined to the presence of an acyl group capable of forming a hydrogen bond with Ser 7.36 appeared as the key for the observed increase of selectivity of WAY-100635 analogues. These results are a step forward for understanding the 5-HT_{1A}/D_4 selectivity. In addition, through this work, we generated and validated robust models of 5-HT_{1A} and D_4 receptors that will be useful for a future virtual screening to identify new ligands with a high 5-HT_{1A}/D_4 selectivity.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jcim.5b00753.

Ramachandran plots and 3D coordinates of the 5-HT_{1A} and D_4 receptor models; additional tables containing the geometrical parameters of the interactions found between compounds **1**−**6** and the receptor models; details about spectroscopic data, elemental analysis results, and melting points for compounds **7**−**12**; details about in vitro binding procedure (PDF)

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Notes

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■ ABBREVIATIONS

TMD, transmembrane domain; HB, hydrogen bond; THP, tetrahydropyridine; GPCR, G protein coupled receptor

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