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Design, synthesis, and evaluation of bitopic arylpiperazine-phthalimides as selective dopamine D₃ receptor agonists†

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The dopamine D₃ receptor (D₃R) is a proven therapeutic target for the treatment of neurological and neuropsychiatric disorders. In particular, D₃R-selective ligands that can eliminate side effects associated with dopamine D₂ receptor (D₂R) therapeutics have been validated. However, the high homology in signaling pathways and the sequence similarity between D₂R and D₃R have rendered the development of D₃R-selective ligands challenging. Herein, we designed and synthesized a series of piperazine-phthalimide bitopic ligands based on a fragment-based and molecular docking inspired design. Compound **9i** was identified as the most selective D₃R ligand among these bitopic ligands. Its selectivity was improved compared to reference compounds **1** and **2** by 9- and 2-fold, respectively, and it was 21-fold more potent than compound **2**. Molecular docking demonstrated that the orientation of Leu^{2.64} and Phe^{7.39} and the packing at the junction of helices may affect the specificity for D₃R over D₂R. Functional evaluation revealed that D₃R-selective ligand **9i** displayed a subpicomolar agonist activity at D₃R with a 199-fold increase in potency compared to quinpirole. These results may be useful for the fragment-based design of bitopic compounds as selective D₃R ligands.

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1. Introduction

Dopamine, a catecholamine neurotransmitter, exerts its biological effects by binding to five dopamine receptors, which can be divided into two subfamilies. D₁-like receptors (D₁R and D₅R) primarily couple to stimulatory Gs-proteins, activating adenylyl cyclase, while D₂-like receptors (D₂R, D₃R, and D₄R) principally couple to inhibitory Gi/o-proteins, inhibiting adenylyl cyclase.¹ D₂-like receptor ligands that mainly target D₂R and D₃R are approved for the treatment of schizophrenia, Parkinson's disease (PD), drug addiction, and substance abuse.^{2–5} However, these therapies have adverse effects such as hyperprolactinemia, metabolic syndrome, and extrapyramidal symptoms (EPS), which are believed to arise from D₂R an-

tagonism.^{6,7} D₃Rs are heavily expressed in the brain mesolimbic areas, and are responsible for emotional, motivational, and cognitive functions.⁸ Thus, D₃R-selective ligands can avoid these side effects and are expected to treat neuropsychiatric disorders, and D₃R-selective agents may also ameliorate negative symptoms of psychiatric disorders. Interestingly, D₃R-selective agonists, but not D₂R-selective agonists, can reverse PD-related motivational deficits. Additionally, D₃R-selective agonists can attenuate anxiety- and depressive-like behaviors. Therefore, the development of a selective and biased D₃R ligand is critically important.

D₂R and D₃R share ~46% of the overall sequence homology, 78% of the sequence identity in transmembrane domains,⁹ and near-identical binding site residues.¹ Indeed, these have impeded the development of D₃R-selective compounds. Although extensive efforts have been made by medicinal chemists and a number of promising D₃R-selective ligands have been developed, few truly selective or biased ligands have been approved by the Food and Drug Administration (FDA) or have progressed to clinical trials.^{10–15} Compound BP897 has been shown to display subnanomolar affinity for D₃R as well as moderate selectivity (Fig. 1). However, it acts *in vivo* as either an agonist or an antagonist, and did not indicate clues for achieving selectivity over D₂R.^{6,10} The pramipexole bearing aminothiazolyl group also binds to presynaptic D₂R.¹¹ It has been reported that the specificity of SB-

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Fig. 1 Representative D₃R-selective ligands.

277011A is still not apparent for D₃R (<100-fold over D₂R). Although GSK598809, a D₃R antagonist, exhibited high D₃R selectivity compared to D₂R, it induced significant hypertension in dogs in the presence of cocaine.¹² Compounds 1 and 2 displayed subnanomolar affinity for D₃R and striking selectivity (4682-fold and 55 556-fold, respectively, Fig. 1).^{13,14} However, to our knowledge, no continued investigation on their preclinical evaluation has been reported. Therefore, ongoing efforts to design more novel D₃R-selective ligands are necessary because none of the FDA-approved drugs have selectively targeted D₃R.^{6,15}

Additional selective compounds would provide a better understanding of the physiological role and the distribution of these two receptor subtypes, and would offer the potential for improved therapeutics without the above-mentioned side effects of hyperprolactinemia, metabolic syndrome, and EPS. Recently, the elucidated D₂R crystal structure facilitates the more rational design of D₃R-selective ligands.¹⁶ Bitopic ligands that linked orthosteric and allosteric pharmacophores have been proven to be a particular strategy for enhancing the selectivity of ligands for dopamine receptors. In the current study, a fragment-based and molecular docking inspired design was used to conceive a novel set of bitopic ligands based on molecular modelling. The radioligand binding assay demonstrated that, among the arylpiperazine-phthalimides, compound 9i presented 9- and 2-fold improvement in selectivity compared to reference compounds 1 and 2, respectively, in the testing system which had been validated previously.^{17,18} The molecular determinants of selectivity towards D₃R were also analyzed based on the molecular docking. Importantly, functional evaluation demonstrated that D₃R-selective ligand 9i exhibited subpicomolar agonist activity at D₃R with a subpicomolar and 199-fold increase in efficacy compared with quinpirole.

2. Results and discussion

2.1 Molecular docking inspired design

Buspirone is a bitopic preferential D₃R antagonist approved by the FDA for the treatment and short-term relief of anxiety.¹⁹ However, it is subjected to first-pass metabolism and can be

metabolized to 5-hydroxybuspirone and 6'-hydroxybuspirone by cytochrome P450 3A4 (CYP3A4, Fig. 2).²⁰ The former metabolite, 5-hydroxybuspirone, is essentially inactive,^{21,22} while the affinity of the latter metabolite, 6'-hydroxybuspirone, to D₂-like receptors decreased significantly ($K_{i,D_2R} = 795$ nM, $K_{i,D_3R} = 5390$ nM).²⁰ To obtain high affinity D₃R ligands, we first investigated the pharmacophoric features of D₃R ligands. Both buspirone and compound 1 were docked into the binding cavity of D₃R (PDB code: 3PBL) using the program LeDock2 (<http://lephar.com>).²³ The tertiary amine in the piperazine ring of both compounds forms a salt bridge to the carboxylate of the strongly conserved Asp110 (Fig. 3). This salt bridge is pharmacologically critical for high-affinity ligand binding to the dopaminergic receptors.¹ The pyrimidine motif of buspirone, and particularly the 2,3-dichlorophenyl of compound 1, fit tightly within a hydrophobic cavity, with the orthosteric binding site (OBS) delineated by Phe345, Phe346, Ser192, Val111, and Ile183. Considering that halogen substitution on the phenyl group can achieve good metabolic stability, the halogen substituted phenyl piperazine was adopted as a primary pharmacophore. Additionally, phthalimides represent a promising scaffold for antipsychotics without inducing catalepsy.²⁴ SLV310, an antipsychotic candidate bearing a phthalimide fragment, displayed high D₃R affinity²⁵ with moderate D₂R binding,²⁶ and was predicted to be devoid of side effects such as EPS, weight gain, and hyperprolactinaemia.²⁵ Molecular docking demonstrated that aryl-3,6-dihydro-2H-pyridine from SLV310 was bound in essentially the same OBS as that of compound 1, while the phthalimide of SLV310 was superimposed with indol-2-yl-carboxamide of compound 1 (Fig. 3). Namely, both pyrrolidine-2,5-dione and carboxamide form a hydrogen bond with Thr369; the phenyl fragment from phthalimide was positioned in a hydrophobic cavity of the allosteric binding pocket occupied by the indole in compound 1. In this regard, the phthalimide moiety was used as a secondary pharmacophore. As reported previously, a flexible alkyl linker, such as a butyl spacer, is more beneficial for pronounced dopaminergic activities,²⁷ in particular for D₃R affinity, molecular conformations, and crystal packing.²⁸ As such, arylpiperazine-phthalimide derivatives were designed as potentially novel D₃R ligands.

Fig. 2 The metabolic pathway of buspirone by CYP3A4.

Fig. 3 Docking poses of compound 1 (carbon in green, A and B), buspirone (yellow, A), and SLV310 (yellow, B) in D₃R.

2.2 Chemistry

The target arylpiperazine-phthalimides were prepared as shown in Scheme 1, while arylpiperidine-phthalimides were synthesized as shown in Scheme 2. All phenylpiperazines were obtained from substituted anilines and bis(2-chloroethyl)amine hydrochloride according to procedures described in the literature.²⁹ Subsequently, the phenylpiperazines were alkylated with (4-bromobutyl)-phthalimide to afford the desired compounds. 5-Chloro-1-(4-piperidinyl)-2-benzimidazolidinone and 4-(2-methoxyphenyl)-piperidine were purchased from Alfa Aesar and Sigma-Aldrich, respectively. Nucleophilic substitution of the piperidine was then performed with the (4-bromobutyl)phthalimide furnishing bitopic arylpiperidine-phthalimides 11.

2.3 *In vitro* binding and structure–activity relationship (SAR) studies

The target compounds were initially screened at a concentration of 100 nM in cell-based assays with both D₂R and D₃R.^{17,18} Human embryonic kidney-293 (HEK-293) cells were purchased from the American Type Culture Collection (Ma-

nassas, VA, USA). HEK-293 cells stably expressing human D₂R or D₃R were used in competition experiments to evaluate the affinity and selectivity of the target compound for D₃R over D₂R. The displacement of [³H]-sulpiride binding was assessed for each compound using sulpiride as a positive control.

Generally, the orthosteric binding site is primarily responsible for the affinity and efficacy of a ligand, whereas the allosteric binding site is associated with selectivity.¹³ Bitopic or dualsteric ligands that engage both binding sites are expected to increase selectivity and retain affinity. Indeed, this strategy has been a proven and validated model to develop D₃R-selective ligands and discriminate their signal transduction. The linker is also a major contributing factor of D₃R selectivity, because the spacer effect and odd-even effect influence the divergent conformation and packing of a ligand second binding pocket (SBP, generally aryl amide).²⁸ Furthermore, the protonation of piperazine or piperidine, or even subtle variations of the head group, can affect SBP and D₃R selectivity. Therefore, we investigated the serial head group variations presented in Table 1 and Fig. 4.

A previous quantitative structure–selectivity relationship (QSSR) study demonstrated that the electron-withdrawing

Scheme 1 Synthetic process of target phenylpiperazine-phthalimide compounds.

Scheme 2 Synthesis of desired arylpiperidine-phthalimides 11.

group attached to the orthosteric phenyl group favored D₃R selectivity over D₂R, but the electron-donating group did not.¹⁴ Consequently, in this study we focused on the electron-withdrawing substituent of phenyl piperazines. Among the monochloro substituents, the *meta*- and *ortho*-occupied derivatives exhibited higher D₃R affinity than that for D₂R, while the *para*-chloro analogue 9a showed a lower affinity for D₃R than D₂R. However, the protonation effect of 9a reduced the D₂R and D₃R affinity, which may be due to a destructive conformational variation, even though the trend of D₃R selectivity *versus* D₂R is consistent with the base formation of 9a. The *meta*-chloro derivative 9b displayed the most potent activity for both D₂R and D₃R. Decorating the ligand with both *ortho*-chloro and *meta*-chloro yielded a 2,3-dichloro hybrid 9d, which exhibited high activity at both targets, but no preference for D₃R. Similarly, while the incorporation of *ortho*-chloro and *para*-chloro afforded the 2,4-dichloro hybrid 9e, this compound could not be dissolved in the test solvent system, even with dimethyl sulfoxide (DMSO); we therefore converted 9e to the corresponding salt, the most convenient being a hydrochloride salt. 9e then

showed diminished affinity compared with 9d but slightly greater activity compared with 9a·HCl. This indicated that *ortho*-chloro and *meta*-chloro substitutions contribute to D₃R affinity, whereas *para*-chloro substitution is not tolerated for D₃R affinity and selectivity over D₂R.

Compound 9f, with a trifluoromethyl group attached to the *meta* position of the head phenyl group, exhibited moderate affinity but no discrimination between D₃R and D₂R. We then investigated the sterically less bulky fluoro group attached to the phenyl group. The *para*-fluorinated derivative 9g exhibited relatively lower affinity and moderate D₃R selectivity compared to D₂R. In contrast, the *ortho*-fluoro analogue 9h preferentially bound to D₃R rather than to D₂R with the most potent D₃R activity. Grafting a fluoro group onto both the *ortho* and *meta* positions of the head group yielded 9i. Compound 9i induced D₃R activity and selectivity comparable to 9h, indicating that the *meta*-fluoro substituent may contribute to the D₃R affinity but not to the D₃R selectivity. However, changing the fluoro substituent from the *meta* position to the *para* position led to a reduction in both the potency and selectivity, which suggests that the *para*-fluoro substituent is not tolerated for D₃R affinity and selectivity, nor is it compatible with the *ortho*-fluorinated substituent. In contrast, the 2,6-difluoro derivative 9k exhibited relatively low activity and moderate selectivity. Incorporating the 2-fluoro and 4-chloro substitutions resulted in a dramatic deactivation of both D₃R and D₂R compared with the 2-fluoro derivative 9h and the 2,3-difluoro substituted 9j. Based on these findings, we postulated that, whether it is a sterically bulky or slim

Table 1 Binding affinities of butyl phthalimides

Compound	R ₁	X	D ₃ R displacement (%)	D ₂ R displacement (%)
9a	4-Clphenyl	N	30.5 ± 12.8	59.2 ± 18.3
9a·HCl	4-Clphenyl	N	2.5 ± 1.5	16.3 ± 4.2
9b	3-Clphenyl	N	86.7 ± 1.4	64.8 ± 3.5
9c	2-Clphenyl	N	43.8 ± 4.5	28.7 ± 3.3
9d	2,3-DiClphenyl	N	76.6 ± 3.1	70.3 ± 2.7
9e	2,4-DiClphenyl	N	N.D.	N.D.
9e·HCl	2,4-DiClphenyl	N	23.0 ± 8.2	30.2 ± 3.4
9f	3-CF ₃ phenyl	N	51.5 ± 3.3	44.0 ± 1.6
9f·HCl	3-CF ₃ phenyl	N	46.9 ± 1.3	37.9 ± 2.8
9g	4-Fphenyl	N	30.8 ± 14.3	-6.3 ± 4.0
9h	2-Fphenyl	N	82.6 ± 2.7	31.8 ± 3.3
9i	2,3-DiFphenyl	N	65.4 ± 5.8	17.7 ± 3.7
9j	2,4-DiFphenyl	N	26.2 ± 2.7	4.8 ± 6.5
9k	2,6-DiFphenyl	N	33.9 ± 6.0	10.3 ± 6.1
9l	4-Cl-2-Fphenyl	N	7.0 ± 4.6	2.8 ± 12.0
9m	5-CF ₃ -2-Fphenyl	N	1.6 ± 8.6	-4.7 ± 7.8
11a	2-OMephenyl	C	85.6 ± 1.2	92.2 ± 0.5
11b	5-Cl-2-Benzimidazolidinonyl	C	62.5 ± 3.8	56.1 ± 4.5
1-HCl	—	—	85.4 ± 3.2	29.6 ± 4.2
2-HCl	—	—	57.4 ± 10.7	22.7 ± 8.5
Sulpiride	—	—	87.9 ± 1.8	92.2 ± 0.1

N.D. = not determined because the compound cannot be dissolved in the test system, even with DMSO.

Fig. 4 Graph of the binding affinities of butyl phthalimides at D₂R and D₃R.

group, the 4-substituent is not tolerated or beneficial for D₃R affinity and selectivity. Moreover, the combination of a 2-fluoro substituent and a 5-trifluoromethylphenyl head group yielded **9m**, which had almost no activity at either target, illustrating that the 2- and 5-positions are not compatible with D₃R affinity and selectivity over D₂R.

In addition, the bioisosteric replacement of aryl piperazine with aryl piperidine yielded **11a** and **11b**. The *ortho*-methoxy phenyl piperidine derivative **11a**, bearing an electron-donating group, exhibited pronounced affinity for both D₂R and D₃R, resulting in diminished selectivity. In contrast, the extension of the phenyl group with a benzimidazolidinonyl

moiety along with an electron-withdrawing group produced **11b**, which displayed moderate affinity for D₃R but diminished selectivity for D₂R.

After screening and exploring the head group, we found that 3-fluoro/2,3-difluoro phenyl derivatives as orthosteric modulators were compatible with the phthalimide group as an allosteric scaffold for D₃R selectivity and specificity. We subsequently specified the head group as an *ortho*-fluoro group, and then investigated the tail group (Table 2 and Fig. 5). The introduction of a reverse amide, as well as the bioisosteric replacement of phthalimide with benzothiazole, yielded **12a**, which decreased both the D₂R and D₃R affinity but maintained the

Table 2 Binding profiles of *ortho*-fluorophenyl piperazine derivatives

Compound	R ₂	Head group		Tail group	
Compound	R ₂	D ₃ R displacement (%)		D ₂ R displacement (%)	
9h		82.6 ± 2.7		31.8 ± 3.3	
12a		63.1 ± 3.2		17.0 ± 2.5	
12b		50.7 ± 2.5		69.8 ± 1.5	
12c		3.2 ± 14.3		10.6 ± 7.0	

differentiation between the two targets. Splitting the benzo moiety and incorporating a carbonyl group afforded 1,2,4-oxadiazoles **12b** and **12c**. Compound **12b** displayed slightly reduced affinity for D₃R and no selectivity. Unexpectedly, the linker with three carbons connecting the piperazine and 1,2,4-oxadiazole destroyed the affinity for D₂R and D₃R.

After establishing the binding profiles and structure–activity relationships, we further characterized several promising compounds in detail by determining their inhibition constant (K_i) values; we then compared these compounds with two reference compounds known to be potent D₃R-selective ligands (Table 3 and Fig. 6). Because phthalimide **9i** and reverse amide **12a** displayed equivalent potentiation and affinity differentiation between D₃R and D₂R, their binding affinities were profiled with K_i values and compared with reference compounds **1** and **2** which are hydrochloride salts. Interestingly, **9i** showed slightly lower binding affinity for D₃R than **12a**, but it had markedly higher selectivity for D₂R. In fact, compound **9i** displayed more preferential affinity for D₃R than the most selective D₃R ligand, **12d**, among the arylpiperazine–reverse amides identified. However, **1-HCl** exhibited moderate D₃R affinity and selectivity over D₂R, whereas **2-HCl** showed lower D₃R affinity and more than 59-fold selectivity for D₃R compared to D₂R. The selectivity of **9i** was elevated by 9-, 2- and 2.5-fold higher than that of reference compound **1**, reference compound **2**, and compound **12a**, respectively; compound **9i** is 21-fold more potent than reference compound **2**, but showed equivalent potency to that of compound **1**. As such, **9i** was the most potent D₃R-selective ligand among the phthalimides, carboxamides, and reverse amides that were synthesized and screened in this study.

2.4 Molecular basis of selectivity over D₂R

To shed light on the structural basis of selective ligands at D₃R over D₂R, compound **9i**, the most selective D₃R ligand

among the identified compounds, was docked into D₃R (PDB code: 3PBL) and D₂R (PDB code: 6C38), respectively, with the program LeDock (www.lephar.com).²³ Docking poses were further minimized with the CHARMM force field.³⁰ Briefly, the binding of compound **9i** in D₃R is characterized by a salt bridge to the conserved Asp110, hydrophobic burial of the 2,3-difluorophenyl in the orthosteric site (Val111, Cys114, Ile183, Ser192, Ser196, Phe345, Phe346, and His342) deep in the seven trans-membrane bundle, and extension to the extracellular pocket by the phthalimide terminus (Fig. 7A). Upon binding in D₂R, its piperazine linker is well overlaid on the piperidine linker of the co-crystallized antipsychotic drug risperidone, establishing a salt bridge to Asp114 (Fig. 7B). Similar to risperidone, its head was inserted into the orthosteric site and its tail extends to the extracellular pocket.

Although the residues delineating the binding sites in D₃R and D₂R are nearly identical, their orientations are significantly different, as revealed by the superposition of both structures (Fig. 8). Notably, the different orientations of Leu89/94 and Phe365/429 put the phthalimide terminus in distinct regions in the extracellular pocket. The phthalimide terminus in D₃R has a tighter interaction with the three residues Val86, Leu89 and Glu90 from the first extracellular loop (ECL1), and forms a H-bond with Thr369. When it is bound to D₂R, this hydrogen bond was not formed due to a different orientation of the corresponding Thr433. The different packing at the junction of helices leads to a subtle yet critical difference in the relative disposition between the orthosteric and extracellular pockets in D₂R and D₃R. As a result, the hydrophobic head of **9i** inserts a bit deeper into the orthosteric pocket of D₂R, with the fluorine at the *ortho*-position facing the aromatic ring of Trp407 at a distance of about 3 Å, slightly shorter than the sum of van der Waals radii (Fig. 7B). Fluorine, which does not typically feature a σ -hole,³¹ thus experiences electrostatic repulsion with the π -electrons of the aromatic ring. This observation is consistent with the previous SAR analysis that *ortho*-fluoro substitution confers selectivity over D₂R,³² which is further confirmed in the current study. Taken together, the selectivity of **9i** originates from the subtle but critical difference in the relative disposition between the orthosteric and extracellular pockets in D₂R and D₃R, leading to distinct interaction features in both sites.

2.5 Functional evaluation

To characterize the functional properties of D₃R-selective ligand **9i** and reference compounds **1** and **2**, a reporter gene assay-based cAMP production assay was conducted as previously described.^{33,34} Briefly, cells stably expressing D₃R were transfected with firefly luciferase reporter genes; after seeding, the cells were treated with 2 μ M forskolin and varying concentrations of D₃R-selective ligands (quinpirole as a positive control); finally, the cells were harvested and the relative luciferase expression was measured (ESI†).

Compared with that of quinpirole, a full agonist of D₃R, the relative efficacies (the maximal inhibition of the

Fig. 5 Graph of the binding affinities of *ortho*-fluorophenyl piperazines at D₂R and D₃R.

Table 3 K_i values of selected compounds and reference compounds

<div><div>Head group</div><div> </div><div>Tail group</div></div>					
Compound	R ₁	R ₂	D ₃ R K _i (nM)	D ₂ R K _i (nM)	D ₂ R/D ₃ R
9i	2,3-diF		19.3	2163.1	112
12a	2-F		3.9	175	45
12d·HCl	2,4-diCl		87.6	5586	63.8
1·HCl	2,3-diCl		15.6	202.3	13
2·HCl	2,4-diCl		407.2	>23 889 ^a	>58.7

^a The K_i value could not be estimated exactly because the dose-response curve did not pass through the remaining 50% of the radioligand even at 30 μ M concentration.

Fig. 6 Dose-response curves of compounds 9i (A) and 12a (B), and reference compounds 1·HCl (C) and 2·HCl (D).

forskolin-induced cAMP production) of reference compounds 1 and 2 were 32.2% and 51.1%, respectively (Fig. 9). Thus, reference compounds 1 and 2 were identified as partial agonists. EC_{50} (the concentration of half maximal effect) of

quinpirole was 97 pM, whereas those of reference compounds 1 and 2 were 26 pM and 1.1 nM, respectively. The efficacy of compound 9i was similar to that of quinpirole but the dose-response curve of compound 9i was drastically

Fig. 7 Predicted binding mode of compound **9i** in D₃R (A) and D₂R (B), respectively. For clarity, the co-crystallized ligand eticlopride in D₃R is not shown. Hydrogen bonds are illustrated by dashed lines.

Fig. 8 Superposition of compound **9i** in the binding site of D₃R (carbon shown in gray) and D₂R (carbon shown in green).

Fig. 9 Normalized dose-response curves of the inhibition of forskolin-induced cAMP production by quinpirole, compounds **9i**, 1-HCl, and 2-HCl.

shifted to the left, resulting in an about 200-fold increase in potency (0.48 pM).

3. Conclusion

In this study, a series of bitopic ligands with preferential affinity for dopamine subtype receptor D₃R over D₂R were identified based on a molecular docking aided design. The

radioligand binding revealed that **9i** was the most potent D₃R-selective ligand among our reverse amides, phthalimides, and carboxamides. The selectivity of **9i** is 9- and 2-times higher than that of reference compounds **1** and **2**; the binding affinity of **9i** was improved by 21-fold compared to that of reference compound **2**. SAR studies demonstrated that an electron-withdrawing group and a sterically less bulky substituent at the *ortho* and *para* position of the head phenyl group were favorable for D₃R specificity. The phthalimide moiety in the tail group tolerated D₃R selectivity over D₂R with carboxamide fragments and its reverse amide. Docking of the most promising D₃R-selective ligand, **9i**, into the human D₃R and D₂R crystal structure, provided insights into the molecular determinants of D₃R selectivity. The different orientations of Leu^{2.64} and Phe^{7.39} resulted in a divergent secondary binding site of compound **9i** which may contribute to D₃R selectivity over D₂R. The different packings of D₃R and D₂R at the junction of helices gave rise to a distinctly relative disposition between the orthosteric and allosteric pockets, which may also be responsible for the D₃R selectivity over D₂R. Functional evaluation demonstrated that D₃R-selective ligand **9i** displayed a subpicomolar agonist activity at D₃R with equivalent efficacy while exhibiting a 199-fold increase in potency compared to quinpirole.

Conflicts of interest

The authors declare no competing interests.

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