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Design, Synthesis, and Evaluation of Bitopic Arylpiperazine-phthalimides as Selective Dopamine D_3 Receptor Agonists

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Abstract

The dopamine D_3 receptor (D_3R) is a proven therapeutic target for the treatment of neurological and neuropsychiatric disorders. In particular, D_3R-selecitve ligands that can eliminate side effects associated with dopamine D_2R receptor (D_2R) therapeutics have been validated. However, the high homology in signaling pathways and sequence similarity between D_2R and D_3R have rendered the development of D_3R-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 D_3R ligand among these bitopic ligands. Its selectivity improved reference compounds **1** and **2** by 9- and 2-times, 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 at D_3R over D_2R. Functional evaluation revealed that D_3R-selective ligand **9i** displayed subpicomole agonist property at D_3R within a 199-fold increase in potency than quinpirole. These results may be useful for the fragment-based design of bitopic compounds as selective D_3R ligands.

Keywords: Dopamine D_3 receptor; bitopic arylpiperazines; selective ligand; structure-activity relationship; molecular modeling

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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. D_1-like receptors (D_1R and D_5R) primarily couple to stimulatory Gs-proteins, activating adenylyl cyclase, while D_2-like receptors (D_2R, D_3R, and D_4R) principally couple to inhibitory Gi/o-proteins, inhibiting adenylyl cyclase.[1] D_2-like receptor ligands that mainly target for D_2R and D_3R 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 D_2R antagonism.[6,7] D_3Rs are heavily expressed in the brain mesolimbic areas, and are responsible for emotional, motivational, and cognitive functions.[8] Thus, D_3R-selective ligands can avoid these side effects and are expected to treat neuropsychiatric disorders, and D_3R-selective agents may also ameliorate negative symptoms of psychiatric disorders. Interestingly, the D_3R-selective agonists, but not D_2R-selective agonists, can reverse PD-related motivational deficits. Additionally, D_3R-selective agonists can attenuate anxiety- and depressive-like behaviors. Therefore, the development of a selective and biased D_3R ligand is critically important.

D_2R and D_3R share ~46% overall sequence homology, 78% sequence identity in transmembrane domains,[9] and the near-identical binding site residues.[1] Indeed, this has impeded the development of D_3R-selective compounds. Although extensive efforts from medicinal chemists have devoted, and a number of promising D_3R-selective ligands have been developed, few truly selective or biased ligands have approved by Food and Drug Administration (FDA) or progressed to the clinic trials.[10-15] The compound BP897 has been shown to display subnanomolar affinity at D_3R as well as moderate selectivity (Figure 1). However, it acts *in vivo* as either an agonist or an antagonist, and did not indicate clues for achieving selectivity over the D_2R.[6,10] The pramipexole bearing aminothiazolyl group also binds to presynaptic D_2R.[11] It has been reported that the specificity of SB-277011A is still not apparent at D_3R (<100-fold over D_2R). Although GSK598809, a D_3R antagonist, exhibited high D_3R selectivity compared to D_2R, it induced significant hypertension in dogs in the

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presence of cocaine.[12] Compounds **1** and **2** displayed sub-nanomolar affinity at D3R and striking selectivity (4682-fold and 55556-fold, respectively, Figure 1).[13,14] However, to our knowledge, no continued investigation on their preclinical evaluation has been reported. Therefore, on-going efforts to design more novel D_3R-selective ligands is necessary because none of the FDA-approved drugs have selectively targeted D_3R.[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 D_2R crystal structure facilitates the more rational design of D_3R-selective ligands.[16] Bitopic ligands that linked orthosteric and allosteric pharmacophores have been proven to be of particular strategy of 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, the compound **9i** presented 9- and 2-times 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 at the D_3R were also analyzed based on the molecular docking. Importantly, functional evaluation demonstrated that D_3R-selective ligand **9i** exhibited subpicomole agonist activity at D_3R within subpicomolar and 199-fold increase in efficacy compared with quinpirole.

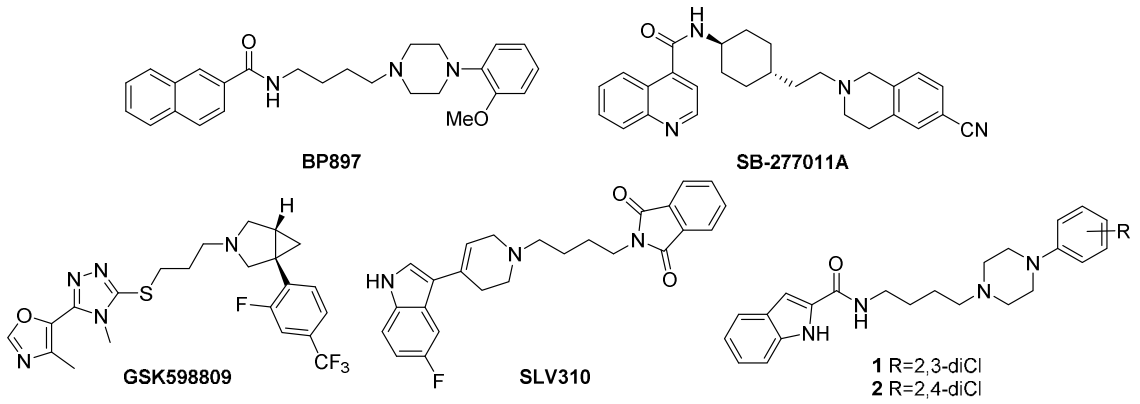

Figure 1. Representative D_3R-selective ligands

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2. Results and Discussion

2.1 Molecular docking inspired design

Buspirone is a bitopic preferential D_3R 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, Figure 2).[20] The former metabolite, 5-hydroxybuspirone, is essentially inactive,[21,22] while the affinity of the latter metabolite, 6'-hydroxybuspirone, to D_2-like receptors decreased significantly (Ki_{D3R}=795 nM, Ki_{D2R}=5390 nM).[20] To obtain high affinity D_3R ligands, we first investigated the pharmacophoric features of D_3R ligands. Both buspirone and compound **1** were docked into the binding cavity of D_3R (PDB code: 3PBL) by 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 (Figure 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, 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 antipyschotics without inducing catalepsy.[24] SLV310, an antipsychotic candidate bearing phthalimide fragment, displayed high D_3R affinity[25] with moderate D_2R binding,[26] and was predicted to be devoid of EPS, weight gain, and hyperprolactinaemia.[25] Molecular docking demonstrated that aryl-3,6-dihydro-2*H*-pyridine from SLV310 was bound in essentially the same OBS of compound **1**, while phthalimide of SLV310 was superimposed with indol-2-yl-carboxamide of compound **1** (Figure 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

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activities,[27] in particular for D_3R affinity, molecular conformations, and crystal packing.[28] As such, arylpiperazine-phthalimides derivatives were designed as potentially novel D_3R ligands.

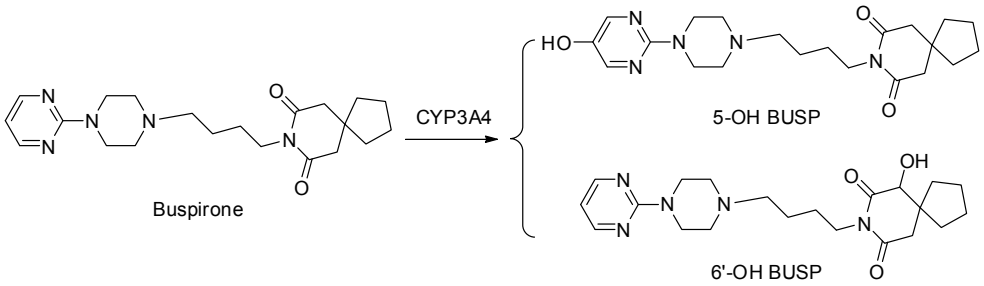

Figure 2. The metabolic pathway of buspirone by CYP3A4

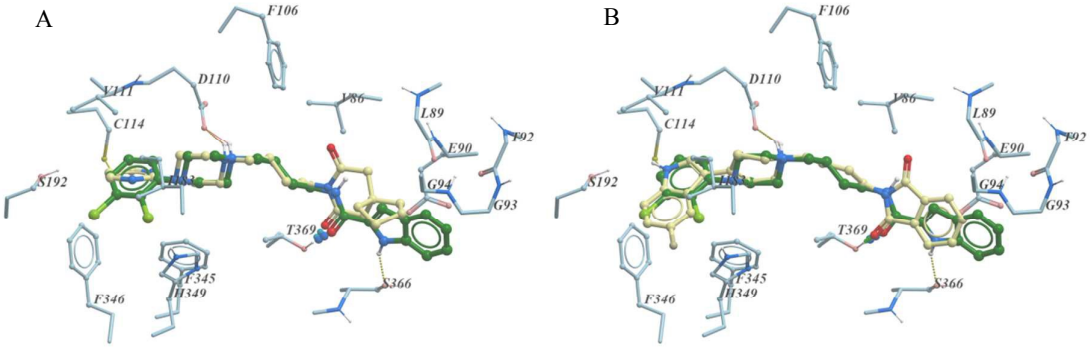

Figure 3. Docking poses of compound **1** (carbon in green, A and B), buspirone (yellow, A), and SLV310 (yellow, B) in D_3R.

2.2 Chemistry

The target arylpiperazine-phthalimides were prepared as shown in Scheme 1, while arylpiperidine-phthalimiedes were synthesized as Scheme 2. All phenylpiperazines were obtained from substituted anilines and bis(2-chloroethyl)amine hydrochloride according to the 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 furnished bitopic arylpiperidine-phthalimides **11**.

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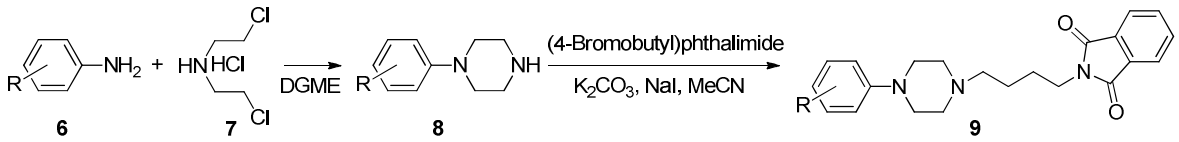
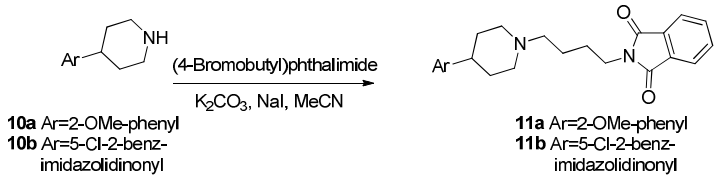


Scheme 1. Synthetic process of target phenylpiperazine-phthalimide compounds



Scheme 2. Synthesis of desired 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 D_2R and D_3R.[17,18] Human embryonic kidney-293 (HEK-293) cells were purchased from the American Type Culture Collection (Manassas, VA, USA). HEK-293 cells stably expressing human D_2R or D_3R were used in competition experiments to evaluate the affinity and selectivity of the target compound for D_3R over D_2R. The displacement of [^{3}H]-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 D_3R-selective ligands and discriminate their signal transduction. The linker is also a major contributing factor of D_3R 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 D_3R selectivity. Therefore, we investigated the serial head group variations presented in Table 1 and Figure 4.

A previous quantitative structure-selectivity relationship (QSSR) demonstrated that the electron-withdrawing group attached to the orthosteric phenyl group favored D_3R selectivity

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over D_2R, 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 D_3R affinity than those of D_2R, while the *para*-chloro analogue **9a** showed a lower affinity for D_3R than D_2R. However, the protonation effect of **9a** reduced D_2R and D_3R affinity, which may be due to a destructive conformational variation, even though the trend of D_3R selectivity versus D_2R is consistent with the base formation of the **9a.** The *meta*-chloro derivative **9b** displayed the most potent activity for both D_2R and D_3R. 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 D_3R. 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 D_3R affinity, whereas *para*-chloro substitution is not tolerated for D_3R affinity and selectivity over D_2R.

Compound **9f**, with a trifluoromethyl group attached to the *meta* position of the head phenyl group, exhibited moderate affinity but no discrimination between D_3R and D_2R. 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 D_3R selectivity compared to D_2R. In contrast, the *ortho*-fluoro analogue **9h** preferentially bound to D_3R rather than to D_2R with the most potent D_3R activity. Grafting a fluoro group onto both the *ortho* and *meta* position of the head group yielded **9i**. Compound **9i** induced D_3R activity and selectivity comparable to **9h,** indicating that the *meta*-fluoro substituent may contribute to D_3R affinity but not to D_3R 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 D_3R 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.

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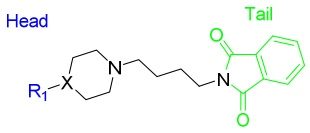
Incorporating the 2-fluoro and 4-chloro substitutions resulted in a dramatic deactivation at both D_3R and D_2R compared with the 2-fluoro derivative **9h** and the 2,3-difluoro substitution **9j**. Based on these findings, we postulated that, whether it is a sterically bulky or slim group, the 4-substituent is not tolerated or beneficial for D_3R 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 D_3R affinity and selectivity over D_2R.

In addition, the bioisosteric replacement of aryl piperazine with aryl piperidine yielded **11a** and **11b**. The *ortho*-methoxyl phenyl piperidine derivate **11a**, bearing an electron-donating group, exhibited pronounced affinity for both D_2R and D_3R, 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 at D_3R but diminished selectivity over D_2R.

Table 1. Binding affinities of butyl phthalimides



Compound	R_1	X	D_3R Displacement (%)	D_2R 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_3phenyl	N	51.5 ± 3.3	44.0 ± 1.6
9f·HCl	3-CF_3phenyl	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

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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

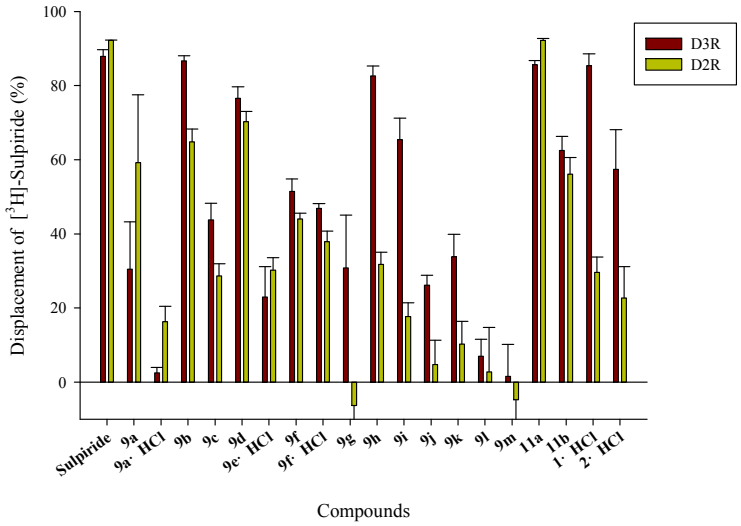

Figure 4. Graphic binding affinities of butyl phthalimides at D2R and D3R

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 D_3R selectivity and specificity. We subsequently specified the head group as an *ortho*-fluoro group, and then investigated the tail group (Table 2 and Figure 5). The introduction of a reverse amide, as well as the bioisosteric replacement of phthalimide with benzothiazole, yielded **12a**, which decreased both the D_2R and D_3R affinity but maintained the 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 D_3R and no selectivity. Unexpectedly, the linker with

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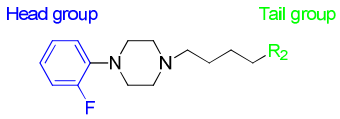
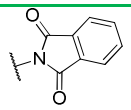
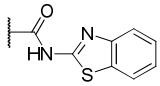
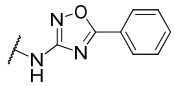
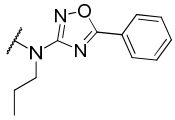
three carbons connecting the piperazine and 1,2,4-oxadiazole destroyed the affinity for D_2R and D_3R.

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



Compound	R_2	D_3R Displacement (%)	D_2R 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

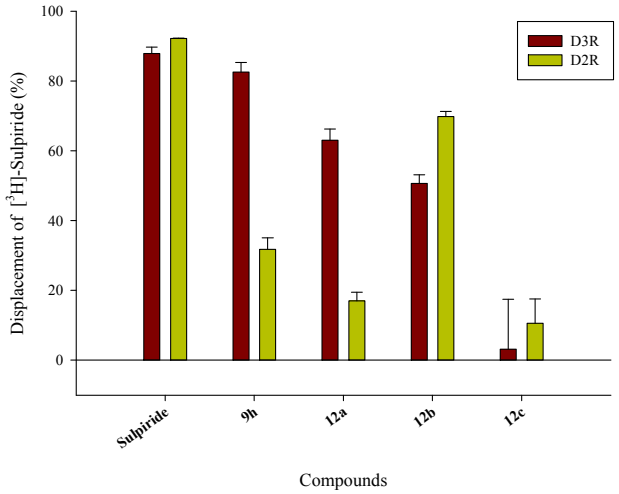


Figure 5. Graphic binding affinities of *ortho*-fluorophenyl piperazines at D2R and D3R

After establishing binding profiles and structure-activity relationships, we further characterized several promising compounds in detail by determining their inhibition constant (Ki) values; we then compared these compounds with two reference compounds known to be potent D_3R-selective ligands (Table 3 and Figure 6). Because phthalimide **9i** and reverse

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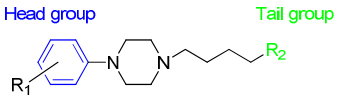
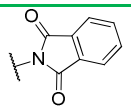
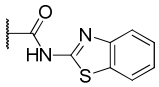
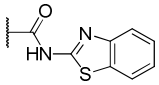
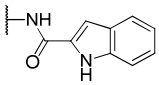
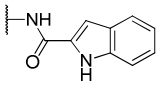
amide **12a** displayed equivalent potentiation and affinity differentiation between D_3R and D_2R, their binding affinities were profiled with Ki values and compared with reference compounds **1** and **2** which are hydrochloride salts. Interestingly, **9i** showed slightly lower binding affinity at D_3R than **12a,** but it had markedly higher selectivity than D_2R. In fact, compound **9i** displayed more preferential affinity for D_3R than the most selective D_3R ligand, **12d**, among the arypiperazine-reverse amides identified. However, **1**·HCl exhibited moderate D_3R affinity and selectivity over D_2R, whereas **2**·HCl showed lower D_3R affinity and more than 59-fold selectivity for D_3R compared to D_2R. The selectivity of **9i** was elevated by 9-, 2- and 2.5-times more than 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 D_3R-selective ligand among the phthalimides, carboxamides, and reverse amides that were synthesized and screened in this study.

Table 3. Ki values of selected compounds and reference compounds



Compound	R_1	R_2	D_3R Ki (nM)	D_2R Ki (nM)	D_2R/D_3R
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	>23889*	>58.7

*Ki value could not be estimated exactly because the dose-response curve did not pass through of the remaining 50% radioligand even at 30 μM concentration.

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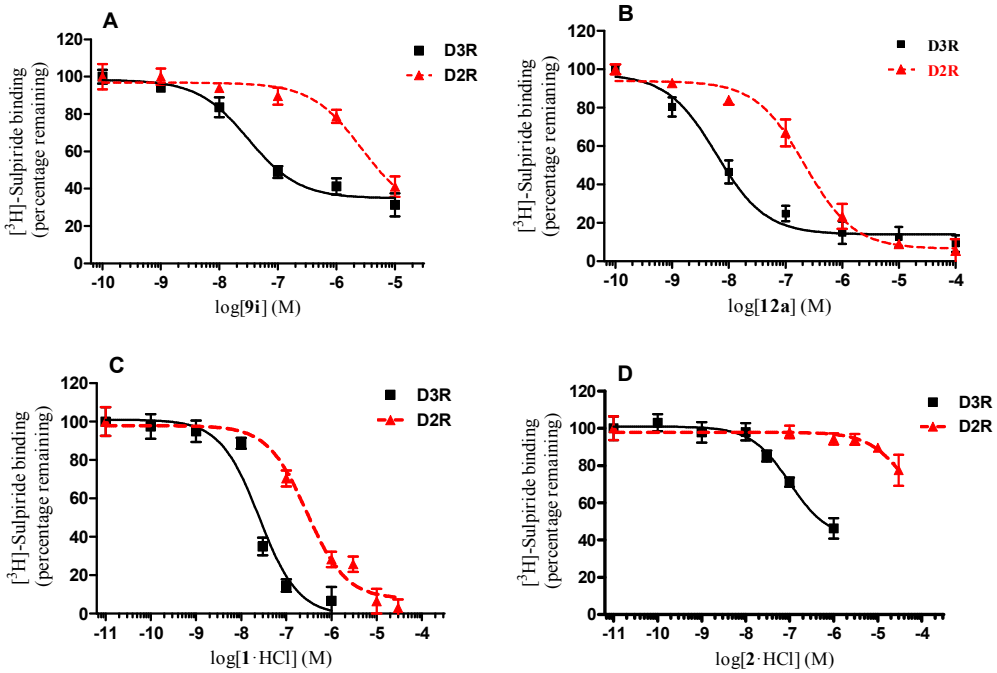

Figure 6. Dose-response curves of compounds **9i** and **12a,** and reference compounds **1**·HCl and **2**·HCl

2.4 Molecular basis of selectivity over D_2R

To shed light on the structural basis of selective ligands at D_3R over D_2R, compound **9i**, the most selective D_3R ligand among the identified compounds, was docked into D_3R (PDB code: 3PBL) and D_2R (PDB code: 6C38), respectively, with the program LeDock (www.lephar.com).[23] Docking poses were further minimized with the CHARMM force field.[30] Briefly, binding of compound **9i** in D_3R 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, His342) deep in the seven trans-membrane bundle, and extension to the extracellular pocket by the phthalimide terminus (Figure 7A). Upon binding in D_2R, its piperazine linker is well overlaid on the piperidine linker of the co-crystalized antipsychotic drug risperidone, establishing a salt bridge to Asp114 (Figure 7B). Similar to risperidone, its head was inserted in the orthosteric site and its tail extends to the extracellular pocket.

Although residues delineating the binding site in D_3R and D_2R are nearly identical, their orientations are significantly different, as revealed by superposition of both structures (Figure 8). Notably, the different orientation of Leu89/94 and Phe365/429 put the phthalimide terminus in distinct regions in the extracellular pocket. The phthalimide terminus in D_3R has

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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 D_2R, 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 pocket in the D_2R and D_3R. As a result, the hydrophobic head of **9i** inserts a bit deeper in the orthosteric pocket of D_2R, 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 (Figure 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 D_2R,[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 pocket in the D_2R and D_3R, leading to distinct interaction features in both sites.

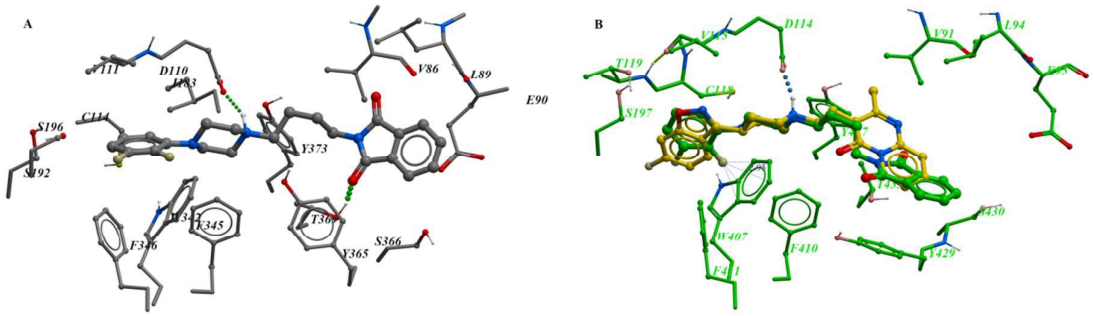

Figure 7. Predicted binding mode of compound **9i** in D_3R (A) and D_2R (B), respectively. For clarity, the co-crystalized ligand Eticlopride in D_3R was not shown. Hydrogen bonds were illustrated by dashed lines.

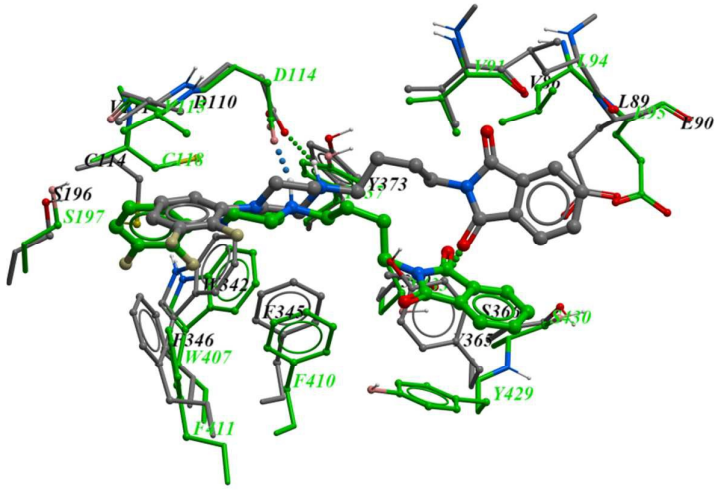

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Figure 8. Superposition of compound **9i** in the binding site of D_3R (carbon shown in gray) and D_2R (carbon shown in green).

2.5 Functional evaluation

To characterize the functional properties of D_3R-selective ligand **9i** and reference compounds **1** and **2**, reporter gene assay based cAMP production assay was conducted as previously described [33-34]. Briefly, Cells stably expressing D_3R were transfected with firefly luciferase reporter genes; after seeding, the cells were treated with 2 μM forskolin and varying concentrations of D_3R-selective ligands (quinpirole as a positive control); finally, the cells were harvested and the relative luciferase expression was measured (Supporting information).

Compared with quinpirole, a full agonist of D_3R, the relative efficacies (the maximal inhibition of the forskolin-induced cAMP production) of reference compounds **1** and **2** were 32.2% and 51.1%, respectively (Figure 9). Thus, reference compounds **1** and **2** were identified as partial agonists. EC_{50} (the concentration of half maximal effect) of qupinpirole 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 shifted to the left, resulting in about 200-fold increase in potency (0.48 pM).

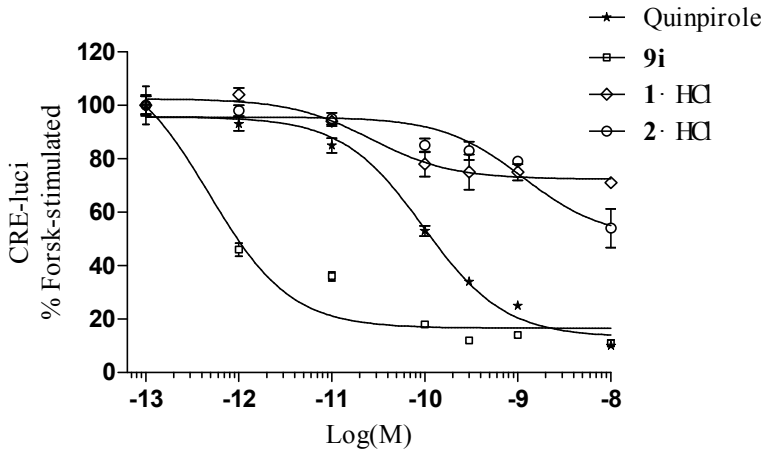

Figure 9. Normalized dose-response curves of the inhibition of forskolin-induced cAMP production by quinpirole, compounds **9i**, **1**·HCl, and **2**·HCl

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3. Conclusion

In this study, a series of bitopic ligands with preferential affinity for dopamine subtype receptor D_3R over D_2R were identified based on molecular docking aided design. The radioligand binding revealed that **9i** was the most potent D_3R-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** improved 21-fold compared to 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 D_3R specificity. The phthalimide moiety in the tail group tolerated D_3R selectivity over D_2R with carboxamide fragments and its reverse amide. Docking of the most promising D_3R-selective ligand, **9i**, into the human D_3R and D_2R crystal structure, provided insights into the molecular determinants of D_3R selectivity. The different orientation of $Leu^{2.64}$ and $Phe^{7.39}$ resulted in a divergent secondary binding site of compound **9i** which may contribute to D_3R selectivity over D_2R. The different packing of D_3R and D_2R 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 D_3R selectivity over D_2R. Functional evaluation demonstrated that D_3R-selective ligand **9i** displayed subpicomole agonist property at D_3R within equivalent efficacy while 199-fold increase in potency as quinpirole.

Acknowledgments

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Conflict Of Interest

The authors declare no competing interests**.**

Supplementary data

The synthesis procedure, NMR data, and biological evaluations are available from the online version.

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Graphical Abstract

Design, Synthesis and Evaluation of Bitopic Arylpiperazine-phthalimide Derivatives as Selective Dopamine D3 Receptor Agonists

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