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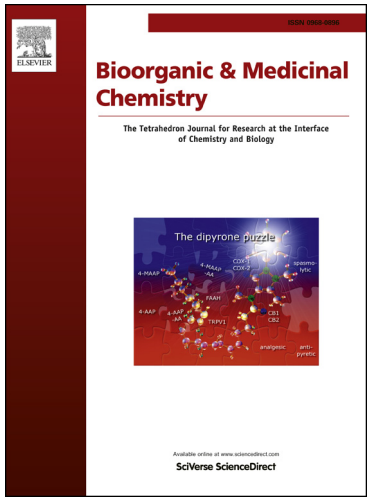

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Graphical Abstract

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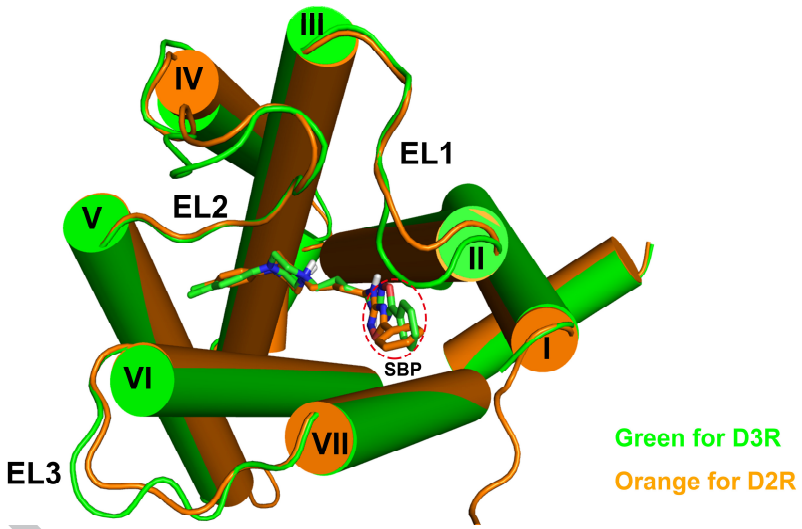

Design, synthesis and evaluation of bitopic arylpiperazinephenyl-1,2,4-oxadiazoles as preferential dopamine D3 receptor ligands

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Abstract: The dopamine D3 receptor (D3R) was proposed as a therapeutic target for drug development to treat drug abuse and addiction and neuropsychiatric disorders. Several D3R-selective modulators over the dopamine D2 receptor (D2R) can avoid extrapyramidal symptoms (EPS) and hyperprolactinemia. However, few biased D3R ligands were identified or showed a narrow range of selectivity at the D3R over D2R because of their high sequence homology. Herein, we designed, synthesized and evaluated the binding affinity of a series of bitopic ligands: arypiperazine-phenyl-1,2,4-oxadiazoles. Compound **9e**·HCl was the most potent and selective D3R modulator among these bitopic ligands. Molecular modeling revealed that D3R selectivity depends on the divergence of secondary binding pocket (SBP) in D3R and D2R. Specifically, non-conserved Tyr36, EL1 especially non-conserved Thr92 and Gly94, and EL2 Val180, Cys181 and Ser182 of D3R may contribute to D3R specificity over D2R.

Key words: Dopamine D3 receptor; phenyl-1,2,4-oxadiazoles; structure-activity relationship; molecular modeling

1. Introduction

The neurotransmitter dopamine modulates movement, cognition, emotion, and affect through diverse signal transductions in the central nervous system.[1] Dopamine is functionalized by facilitating the coupling of stimulatory protein α subunits (Gs) to D1-like receptors to sequentially stimulate adenyl cyclase, while enhancing inhibitory G-protein α subunits (Gi/0) coupling to D2-like receptors and the consequent inhibition of adenyl cyclase.[2-4] Dopamine D2 and D3 receptors (D2R and D3R) of the D2-like subfamily are therapeutic targets for neurological and neuropsychiatric disorders.[5-7] However, some side effects, such as extrapyramidal symptoms (EPS) and prolactin elevation, have also been observed, which arise from antagonism of D2R in the striatum of the brain.[8] Interestingly, the highest levels of D3Rs are expressed in the limbic areas of the brain.[2,9] This supports the view that D3R might be related to the etiology of schizophrenia and selective D3R modulator may avoid EPS and hyperprolactinemia.[10] Furthermore, D3R plays an important role in modulating the cholinergic levels at the prefrontal cortex and D3R blockade can enhance dopamine and acetylcholine release in the frontal cortex without muscarinic effects.[11] In this regard, D3R was proposed as a valuable target for development of antipsychotic drugs. Moreover, several D3R-specific compounds can reduce opiate-, cocaine-, nicotine-, and ethanol-seeking behaviors demonstrating the potential of D3R for treatment of substance addiction and dependence.[12,13] As well, D3R may also have therapeutic potential for other neuropsychiatric disorders, such as Parkinson's disease and depression.[12,14,15] Therefore, development of a selective and biased D3R modulator is needed.

Despite many studies of selective D3R agents, obstacles remain because of the high sequence identity and similarity between D3R and D2R. Generally, selective D3R ligands are bitopic or allosteric molecules with a primary pharmacophore (PP, 4-phenylpiperazine), secondary pharmacophore (SP, aryl amide), and a butyl linker.[1,16] This has made the development of D3R preferential ligands with high aqueous solubility and bioavailability challenging. Consequently, herein we split the benzo moiety of tail group and incorporated it into the carbonyl group, which can sometimes improve solubility. Further bioisosteric replacement was utilized to yield the target

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compounds, 1,2,4-oxadiazoles (**Figure 1**). Additionally, the selective D3R antagonist SB-414796, which has a 1,2,4-oxadiazole moiety, has high oral bioavailability and is central nervous system (CNS) penetrant in the rat (**Figure 2**).[17] Interestingly, two marine natural products, phidianidines A and B (**Figure 2**), the first natural products characterized that contain a 1,2,4-oxadiazole moiety, are selective inhibitors of dopamine transporter (DAT) over serotonin transporter (SERT)/norepinephrine transporter (NET), and are selective partial agonists of μ-opioid receptor over δ-/κ-opioid receptors.[18] Accordingly, a series of 4-phenylpiperazine-1,2,4-oxadiazole were designed and synthesized.

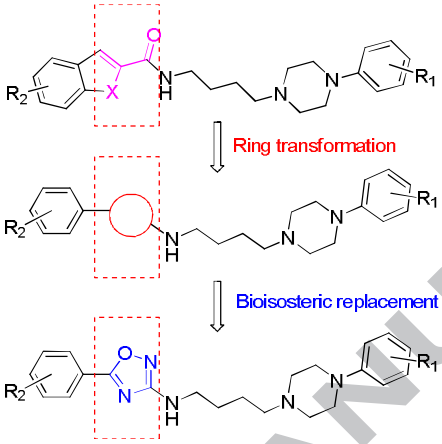


Figure 1. Rational development of novel selective D3R ligands

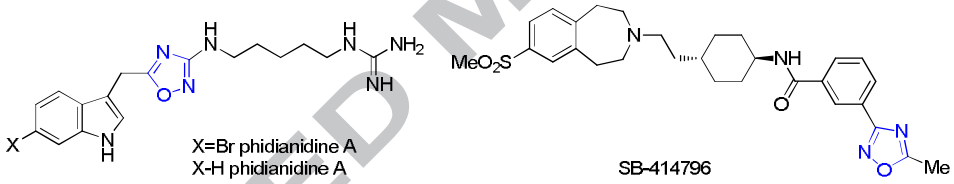
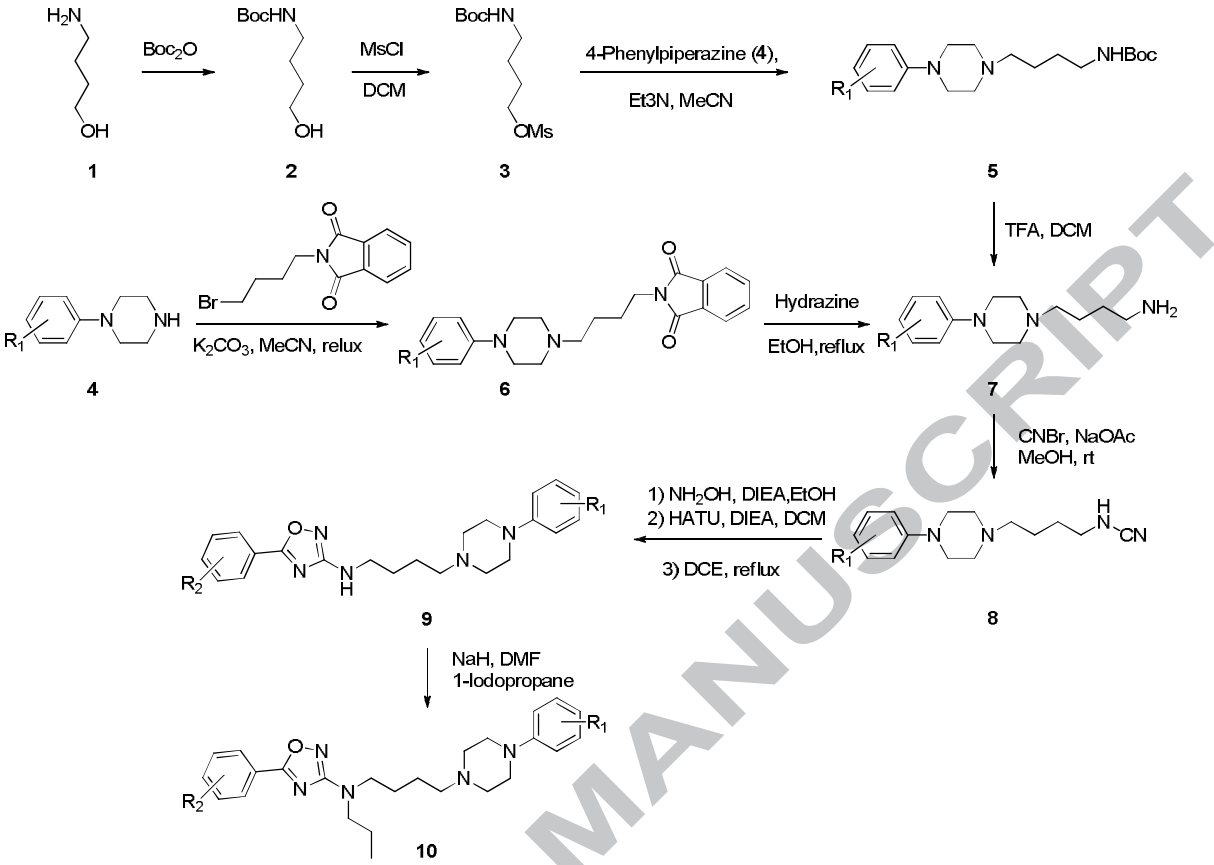


Figure 2. Some key 1,2,4-oxadiazoles in the design of biased D3R modulators

2. Results and Discussion

2.1. Chemistry

The synthetic route of target compounds is described in the **Scheme 1**. 4-Aminobutanol was protected with Boc group, followed by activation of a hydroxyl group with methanesulfonyl chloride. The mesylate **3** was coupled with appropriate 4-phenylpiperazine, sequentially deprotecting the Boc group to furnish the corresponding 4-piperazinylbutanamine **7**. Alternatively compound **7** was prepared starting from substituted 4-phenylpiperazine that was alkylated with *N*-(4-bromobutyl)phthalimide to afford the corresponding phthalimides **6**. Sequentially, **6** was deprotected by hydrazine hydrate to yield amine **7**. Grafting a cyan group onto **7** with cyanogen bromide yielded substantial intermediate **8**. The following three steps were performed in one-pot reaction, cyanide **8** was transformed to hydroxylguanidine, followed by amidation and cyclization to give the target compounds.



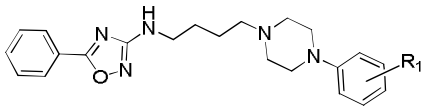
Scheme 1. General synthetic procedure of target compounds

2.2. *In vitro* binding studies and structure-activity relationship analysis

Human embryonic kidney (HEK) 293 cells stably transfected with the human D2 or D3 receptor were used to evaluate the target compounds' activity through competition binding experiment at 4 °C using [^{3}H]-sulpiride as a radioligand and sulpiride as a positive control. The target compounds were initially screened at one concentration (100 nM or 1 μM) to investigate affinity and selectivity at D3R over D2R in the cell level. Even though D3R selectivity arise from the interaction of the secondary binding pocket, a subtle variation of substitute at PP changes the head group's conformation and the consequent affecting SBP and D3R selectivity. Moreover, PP plays an essential role in receptor activation. Accordingly, we herein, explored the serial head group variations presented in **Table 1**.

Among these 4-phenylpiperazine free base derivatives, introduction of a chloro group at the *para*-position of the phenyl group gave compound **9e**, which showed no affinity at both D2R and D3R. Similarly, no binding affinity at D2R and D3R with 2,3-dichloro and 2,4-dichloro substitutions. Grafting a trifluoromethyl group onto the *meta*-position of the head phenyl group yielded **9d**, which had moderate D3R affinity and selectivity. 2-Fluorinated analog **9a** displayed most potent at two targets. However, a small difference in displacement was apparent between D2R and D3R. A small hydrophobic substitution at *ortho*-position may be critical to dopaminergic receptors' affinity, consistent with a previous CoMFA/CoMSIA model and our SAR assay. In order to improve target compound's physicochemical profiles, free bases were converted to their corresponding hydrochloride salts. Interestingly, the binding affinities of all the salts increased dramatically. Strikingly, **9e**·HCl displayed 18% biased displacement at D3R versus D2R, and **9c**·HCl 30% difference. By contrast, 4-chloro substitution, **9e**·HCl, exhibited higher D3R affinity than 2,4-dicloro substitution **9c**·HCl.

Table 1. Binding affinities of substituted 4-phenylpiperazines

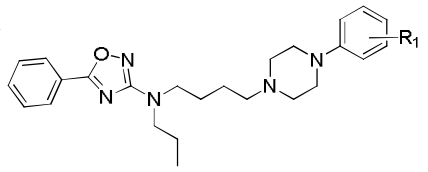


Compound	R1	D3R Displacement (%)*	D2R Displacement (%)*
9a	2-F	50.7 ± 2.5	69.8 ± 1.5
9b	2,3-diCl	-2.8 ± 16.2	4.9 ± 2.8
9b·HCl	2,3-diCl	54.1 ± 3.4	42.6 ± 7.9
9c	2,4-diCl	-4.1 ± 12.1	-2.6 ± 23.2
9c·HCl	2,4-diCl	38.2 ± 4.5	8.3 ± 1.4
9d	3-CF_3	35.3 ± 4.7	19.8 ± 2.7
9e	4-Cl	-0.7 ± 8.2	-5.4 ± 5.3
9e·HCl	4-Cl	49.4 ± 1.4	31.1 ± 1.1

* Test concentration: 0.1 μM

Given that propyl substituent was proved to be an important part of the binding pocket, we introduced a propyl group at the linker amine of 5-phenyl-1,2,4-oxadiazole scaffold (**Table 2**). Unexpectedly, the binding affinities of all the alkylated ligands were substantially reduced even at 1 μM, and the unmet biological profiles were observed for both D2R and D3R. Interestingly, the propyl analogue **10b** presented some selectivity at D2R but not at D3R.

Table 2. Binding affinities of propyl analogs



Compound	R1	D3R Displacement (%)*	D2R Displacement (%)*
10a	2-F	3.2 ± 14.3	10.6 ± 7.0
10b	2,3-diCl	22.1 ± 6.4	50.1 ± 3.0
10c	2,4-diCl	15.1 ± 9.8	-0.6 ± 10.0
10d	3-CF_3	9.7 ± 3.1	17.8 ± 1.2

* Test concentration: 1 μM

Based on the aforementioned structure-activity relationship, we found that the 2,4-dichlorophenyl head group well tolerated D3R selectivity and specificity. After fixing the head group, diverse tail groups were explored (**Table 3**). Among these phenyl-1,2,4-oxidazole free base, compared with **9c**, the affinity of 4-chloro substitution **9f**

increased slightly, the displacement of 3-chloro surrogate **9g** improved strikingly, while 2-chloro substitution **9h** did not show activity at 100 nM. Similarly, the introduction of a bulkier electron-withdrawing bromo group at *para*-position of tail group enhanced the binding affinity slightly. Additionally, an electron-donating group was also incorporated onto the phenyl group to modify the physicochemical properties and facilitate the binding affinity of D3R. Indeed, introduction of a methoxyl group at *meta*- and *para*- position enhanced the bioactivity dramatically compared with **9c**. Substituting methoxyl group at *ortho*-position of the phenyl tail group failed to improve binding affinity. Similarly, grafting a cyan group onto the tail phenyl group furnished **9l** along with somewhat higher affinity than **9c**. In this regard, substitution of either electron-withdrawing or donating group at the *para*-position of tail phenyl group slightly increased the binding affinity of D3R and D2R; incorporation of an electron-withdrawing or donating group at *meta*-position of phenyl group strikingly improved the affinity of two targets; and attachment of a group at the *ortho*-position of phenyl did not raise biological activity. Moreover, replacement of the phenyl group of **9c** with pyridinyl group yielded **9n**, along with diminished binding affinity. Unfortunately, these modifications cannot facilitate the selectivity of D3R over D2R. Compared with **9c**·HCl, the hydrochloride salts of chloro substituted compounds showed improved affinities at both D2R and D3R. Unexpectedly, the affinities of HCl salts of methoxyl substituted analogues decreased at both D3R and D2R, in agreement with our previous SAR analysis.[19] In this regard, salt formation is not beneficial to the hydrophobic interaction between methoxyl group and SP of the target.

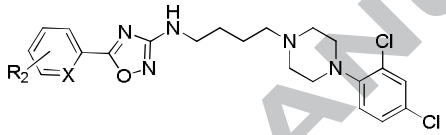


Table 3. Binding affinities of aryl-1,2,4-oxadiazoles

Compound	R2	X	D3R Displacement (%)*	D2R Displacement (%)*
9c	H	C	-4.1 ± 12.1	-2.6 ± 23.2
9c·HCl	H	C	38.2 ± 4.5	8.3 ± 1.4
9f	4-Cl	C	16.6 ± 8.6	15.2 ± 6.8
9f·HCl	4-Cl	C	33.8 ± 5.8	40.0 ± 3.3
9g	3-Cl	C	41.5 ± 6.3	28.3 ± 6.8
9g·HCl	3-Cl	C	37.0 ± 3.5	40.4 ± 1.8
9h	2-Cl	C	-0.2 ± 7.4	3.7 ± 5.2
9h·HCl	2-Cl	C	45.8 ± 5.0	50.0 ± 5.2
9i	4-OMe	C	42.6 ± 5.8	33.4 ± 7.6
9i·HCl	4-OMe	C	-0.2 ± 0.7	2.9 ± 3.3
9j	3-OMe	C	37.3 ± 4.0	37.0 ± 0.7
9j·HCl	3-OMe	C	10.6 ± 0.6	2.4 ± 2.3
9k	2-OMe	C	7.7 ± 2.0	5.6 ± 8.3

9k·HCl	2-OMe	C	4.2 ± 3.3	5.4 ± 3.1
9l	4-CN	C	11.8 ± 9.2	8.8 ± 4.8
9l·HCl	4-CN	C	36.6 ± 3.1	22.8 ± 5.9
9m	4-Br	C	14.2 ± 3.1	12.7 ± 5.2
9n	H	N	9.5 ± 7.7	7.8 ± 5.6
9n·HCl	H	N	14.8 ± 1.5	17.9 ± 3.4

* Test concentration: 0.1 μM

With knowledge of the binding profiles and SAR results, we further characterized some promising compounds in detail with Ki values (**Table 4** and **Figure 3**). All candidates and standard compound **11** displayed submicromolar binding affinities, however, **9c**·HCl and **9e**·HCl are equipotent and more potent than standard and **9c**. Even though salt formation of **9c** increased affinity of two dopaminergic receptors slightly, this failed to exert effort on improvement of D3R selectivity over D2R. Standard **11** hydrochloride salt exhibited at least 58-fold selectivity at D3R versus D2R. In contrast, **9c**·HCl, 2,4-dichlorophenylpiperazine analog, showed low selectivity for D3R. Interestingly, **9e**·HCl, 4-chlorophenylpiperazine analog, turned out to be a high selective D3R ligands over D2R among arylpiperazine-phenyl-1,2,4-oxadiazole derivatives. As such, *para*-chloro of phenyl head group may be critical to D3R specificity over D2R, however, introduction of one more *ortho*-chloro onto phenyl head group led to loss preference for D3R versus D2R.

Table 4. Biological Ki values of selected compounds at human D2R and D3R

Compound	D3R Ki (μM)	D2R Ki (μM)	D2R/D3R
9c	0.81	6.23	7.7
9c·HCl	0.29	1.29	4.4
9e·HCl	0.27	79.63	297.3
11·HCl	0.41	>23.89*	>58.7

*Ki value cannot be estimated because the concentration of DMSO is larger than 0.1% in the highest dose of curve.

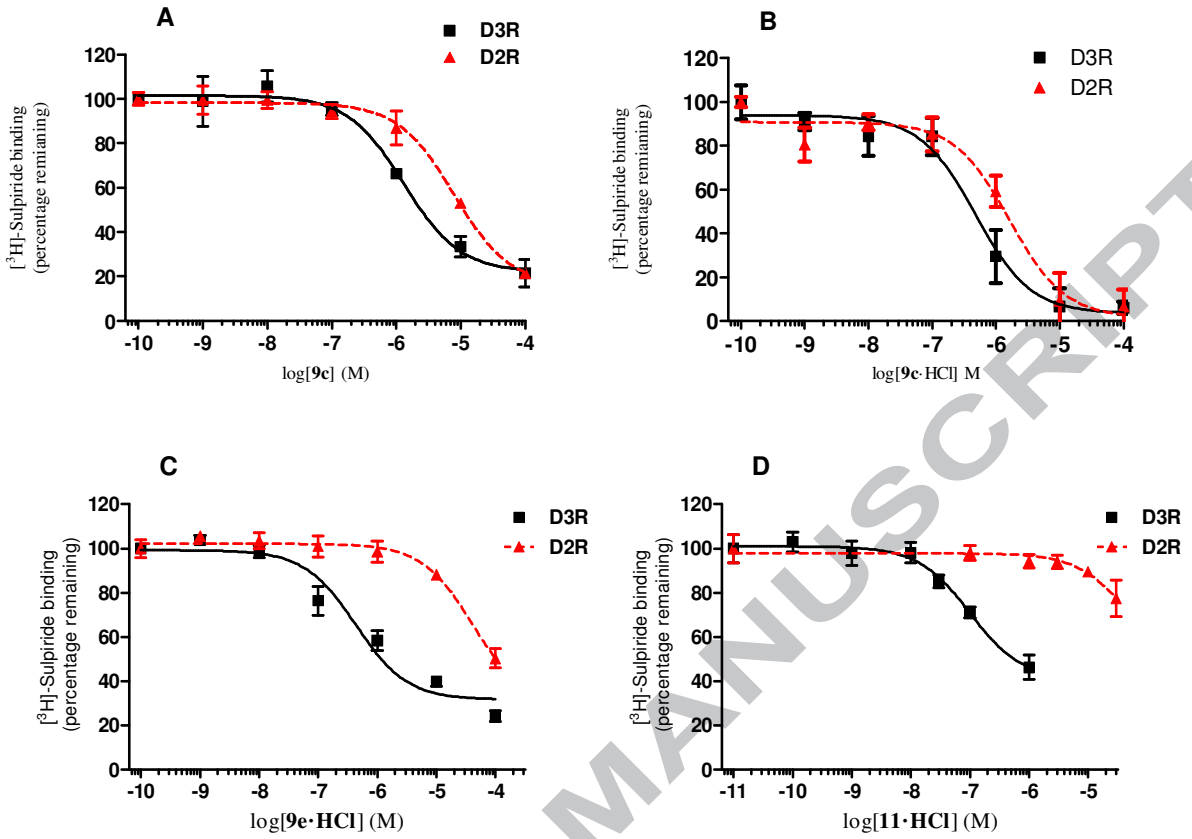


Figure 3. Dose-response curves of compounds **9c** (A), **9c**·HCl (B), **9e**·HCl (C) and standard (D)

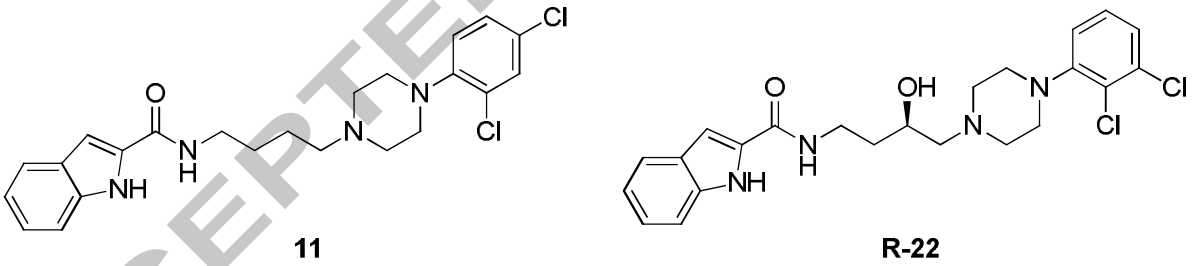


Figure 4. Representative D3R-selective antagonist

2.3. Molecular modeling studies

The dopamine D2R model was constructed based on homology modeling using the crystal structure of the human D3R as the template and the human D2R sequence as query sequence. Because the extracellular loop 1 (EL1) and loop 2 (EL2) are determinants of D3R specificity over D2R, we manually refined the loops based on DOPE scores in order to get a perfect D2R model. The resulting Ramachandran plot showed more than 99% residues distributed in allowed regions except Gly173 because it also located in the disallowed position in homology protein D3R. In docking simulation, a small root-mean-square deviation (RMSD) of 0.5129 and overlapping conformation between redocked and native eticlopride were observed (see Figure S3), indicating that the generated binding pocket is reasonable and the software is reliable.

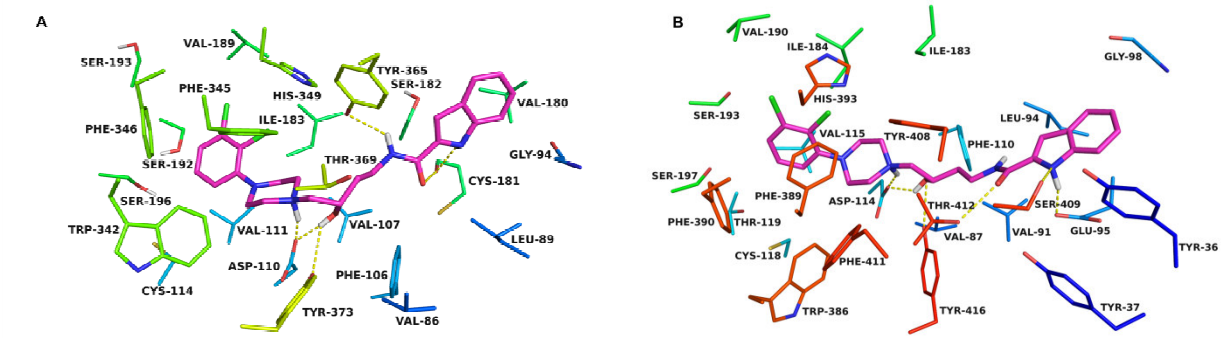


Figure 5. Docking conformations of R-22 with D3R (A) and D2R (B). H-bonds are represented by yellow dashed lines.

In order to explore the real molecular determinants of D3R selectivity, we first docked and analyzed R-22, a D3R-selective antagonist, into the D3R and D2R model (**Figure 5**). The PP of R-22, 2,3-dichlorophenylpiperazinyl moiety, occupies the orthosteric binding site (OBS) of D2R and D3R, essentially same as bound eticlopride and dopamine (Asp$^{3.32}$, His$^{6.55}$, Ser$^{5.42}$, Ser$^{5.46}$, Phe$^{6.52}$, Phe$^{6.51}$). The pronated piperazines forms a key ionic lock and salt bridge with the conserved Asp$^{3.32}$ of both D2R and D3R, which is essential for binding affinity and intrinsic activity of ligands. This residue also interacts with the hydroxyl group of R-22 by a hydrogen bond. The hydroxyl group of R-22 forms hydrogen bonding interaction with Tyr$^{7.43}$ of both two dopaminergic receptors. Indeed, the 2,3-dichlorophenyl moiety is perpendicular to the piperaznyl ring, and the substitutions pack toward extracellular direction and create hydrophobic interactions with both D2R and D3R by Pi-alkyl (Val$^{3.32}$) and alkyl interactions (Ile$^{5.33}$ (EL2), Val$^{5.39}$, His$^{6.55}$). On the other hand, the SP carbonyl group of R22 forms a hydrogen bond with the conserved Cys181 and van der Waals interaction with Ser182 of D3R, while it interacts with the opposite direction Thr412 of D2R because Ile183 of D2R is more lipophilic than Ser182 of D3R. Furthermore, the indole moiety was stabilized in D3R by hydrogen bond with Cys181 and van der Waals interaction with Val180 of D3R, while in D2R by hydrogen bonds with Glu95 and Ser409 because Glu181 is more lipophobic than D3R corresponding Val180. Additionally, the interaction between amidic NH and Tyr365 facilitate R22 D3R affinity. Strikingly, R-22 hydrophobically interacts with Gly94 of EL1 in D3R, while the SP of R22 failed to interact with corresponding EL1 of D2R because EL1 is shorter in D2R than D3R. In this regard, the hydrogen bond and van der Waals interaction between R-22 and EL2 of D3R and the hydrophobic interaction between R-22 and EL1 of D3R may exert an important role to D3R selectivity over D2R.

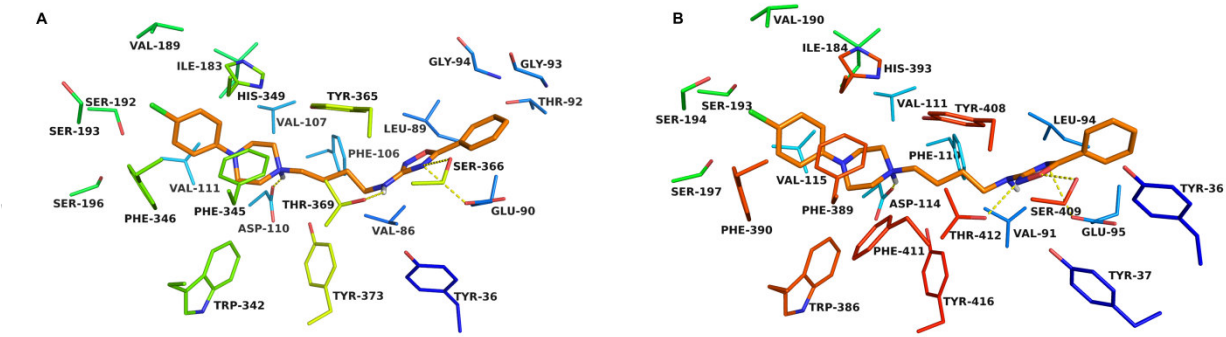


Figure 6. Docking conformations of **9e** with D3R (A) and D2R (B). H-bonds are represented by yellow dashed lines.

Based on the biological assay, we also docked our promising compound **9e** into the D3R and D2R model (**Figure 6**). **9e** is also buried the same OBS of both D2R and D3R (Asp$^{3.32}$, His$^{6.55}$, Ser$^{5.42}$, Ser$^{5.46}$, Phe$^{6.52}$, Phe$^{6.51}$) and PP of **9e** captures establishes interactions with two dopaminergic receptors, including Pi-alkyl, alkyl interactions (Ile$^{5.33}$,

Val[5.39], Phe[6.51], His[6.55]) with phenyl of PP and salt bridge with Asp[3.32] (**Figure 4** and **Figure S4**). The allosteric binding sites of D2R and D3R are divergent. The SP of **9e** is closer to helix II and EL1 in D3R, while it approaches closer to helix VI and VII, which may unravel the real molecular determinant of D3R specificity. The linker NH forms a hydrogen bond with conserved Thr[7.39] of both D2R and D3R. Interestingly, it weakly hydrogen bonds with Tyr36 of D3R (distance=3.5 Å), whereas no hydrogen bond occurs between the corresponding Leu41 of D2R and NH because Leu41 of D2R is shorter than Tyr36 of D3R and is a hydrophobic contribution rather than a hydrogen bonding donor. Five member ring, 1,2,4-oxadiazole moiety, generates hydrogen bonding interaction with both the Glu2.65 and Ser7.36 at two targets, but it orients differently towards SP of D2R and D3R. Moreover, Glu90 of D3R has Pi-anion interaction with 1,2,4-oxadiazole moiety, while corresponding Glu 95 of D2R forms Pi-anion interaction with phenyl group of SP. Of note, SP of **9e** also interacts with EL1 (Thr92, Gly93, Gly94) of D3R, but there are no corresponding interaction between EL1of D2R and **9e**, in agreement with previous result.[19]

3. Conclusion

Ring transformation and bioisosteric replacement strategies were used to design novel bitopic ligands, phenylpiperazine-1,2,4-oxadiazoles, as selective D3R modulators and improve their physicochemical property. SAR studies indicated that a small hydrophobic substitution at *ortho*-position of phenyl head group facilitates D3R affinity, while the *para*-chloro surrogate tolerates D3R specificity. Substituents, an electron-withdrawing or donating group, at the *para*-position of the tail group slightly increased the receptor affinity of both dopaminergic receptors and substitutions at the *meta*-position improved dramatically the affinity of the two targets. However, they failed to contribute to the D3R versus D2R selectivity. Molecular modeling illustrated that selectivity of ligands at D3R over D2R arise from the divergence of ligand SP. The SP of R22 was stabilized by hydrogen bond between carbonyl group and indole moiety with Cys181 (El2) as well as amidic NH with Tyr365 of D3R. In contrast, the SP of R22 preferentially binds to Glu95, Ser409 and Thr412 of D2R. The selective ligand **9e** forms a weak hydrogen bond with Tyr36 of D3R, while they do not show any interaction with corresponding Leu41 of D2R. Most strikingly, EL1 of D3R participates in the hydrophobic interaction between SP of both R-22 and **9e**, while no interactions were observed, especially in the non-conserved residues Val97 and Glu99. All these differences may contribute to the D3R over D2R selectivity.

4. Materials and Methods

4.1. Chemistry

All extra pure grade solvents were purchased from OCI, and chemicals were supplied either by Sigma-Aldrich or Alfa Aesar and used without purification. Thin-layer chromatography (TLC) was developed on silica gel 60 F254 glass plates (Merck, Germany). The conjugated compounds were visualized using an ultraviolet filtered lampshade and TLC dark room. Melting points were determined on a Thomas-Hoover melting point apparatus and were uncorrected. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded with a Varian Unity Plus NMR spectrometer at 300 and 75 MHz, respectively. Deuterated chloroform ($CDCl_3$) was used as deuterated solvent. Chemical shifts were presented in parts per million (ppm) relative to internal standard tetramethylsilane (TMS). Coupling constants *J* were expressed in MHz and splitting patterns in s (singlet), d (doublet), t (triplet), q (quartet), br (broad), dd (doublet of doublets), and dt (doublet of triplets), m (multiple).

4.1.1. Procedure of Boc protection

To the solution of 4-aminobutanol (100 µL) in 2 mL triethylamine/methanol (Et_3N/MeOH, v/v, 1:7) di-*tert*-butyl dicarbonate (278 µL) in 2.5 mL methanol was added dropwise at 0 °C. The solution was stirred at the same temperature overnight and allowed to warm to room temperature during 2 hours and kept at ambient temperature for another 6 hours. Upon completion of the reaction monitored by TLC visualized by iodine and ninhydin, excess Boc_2O and solvent were removed under vacuum. The residue was partitioned between dichloromethane (CH_2Cl_2)

and brine and extracted with CH_2Cl_2 (3×50 mL). The combined organic layers were washed by brine, then dried over $MgSO_4$, filtered and concentrated *in vacuo* to provide *tert*-butyl *N*-(4-hydroxybutyl)carbamate **2** as colorless oil (152 mg, 100%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.02 (s, 1H), 3.63 (t, *J* = 6.1 Hz, 2H), 3.40 (s, 1H), 3.13 (d, *J* = 5.9 Hz, 2H), 1.56 (dd, *J* = 7.2, 4.1 Hz, 4H), 1.44 (s, 9H).

4.1.2. Procedure of mesylation

Methanesulfonyl chloride (135 μL) was added dropwise at 0 °C under an argon atmosphere to a solution of alcohol **2** (661.4 mg) and Et_3N (4.9 mL) in 10 mL CH_2Cl_2. The reaction mixture was stirred at 0 °C for 1 hour, warmed to room temperature slowly and stirred for 12 hours. After completion of reaction monitored by TLC visualized by iodine, the reaction mixture was quenched by 15 mL of 5% NaOH aqueous solution. The aqueous layer was extracted with CH_2Cl_2 (3×40 mL), washed by brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by silica column eluting with CH_2Cl_2/ethyl acetate (EtOAc) (10:1) to afford *tert*-butyl N-(4-methanesulfonyloxybutyl)carbamate **3** (453 mg, 70%) as colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.64 (s, 1H), 4.25 (t, *J* = 6.3 Hz, 2H), 3.17 (dd, *J* = 13.1, 6.6 Hz, 2H), 3.02 (s, 3H), 1.86 – 1.73 (m, 3H), 1.62 (dt, *J* = 8.0, 7.0 Hz, 2H), 1.45 (d, *J* = 6.1 Hz, 9H).

4.1.3. General procedure of *N*-alkylation

The 4-phenylpiperazines were prepared followed the literature method. *N*-(4-Bromobutyl)phthalimide was added to a suspension of 4-phenylpiperazine **4** and K_2CO_3 in acetonitrile. The reaction mixture was allowed to reflux overnight and monitored by TLC (CH_2Cl_2/MeOH, 10:1). After completion of reaction, K_2CO_3 was removed by filtration and washed by acetone. The resulting filtrate was concentrated under reduced pressure. The crude product was purified by flash column chromatography eluting with EtOAc/hexane (1:4→1:3) to furnish corresponding phthalimides **6**.

2-(4-(4-(2-Fluorophenyl)piperazin-1-yl)butyl)isoindoline-1,3-dione (6a)

Compound **6a** was obtained as pale-yellow crystals. Yield: 80%. Mp: 120-122 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (dd, *J* = 5.5, 3.0 Hz, 2H), 7.71 (dd, *J* = 5.4, 3.1 Hz, 2H), 7.09 – 6.86 (m, 4H), 3.73 (t, *J* = 7.0 Hz, 2H), 3.18 – 3.00 (m, 4H), 2.71 – 2.54 (m, 4H), 2.51 – 2.35 (m, 2H), 1.75 (dt, *J* = 14.3, 6.9 Hz, 2H), 1.65 – 1.48 (m, 2H).

2-(4-(4-(2,3-Dichlorophenyl)piperazin-1-yl)butyl)isoindoline-1,3-dione (6b)

Compound **6b** was obtained as white solid. Yield: 77.7%. Mp: 121-123 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (dd, *J* = 5.5, 3.0 Hz, 2H), 7.71 (dd, *J* = 5.5, 3.0 Hz, 2H), 7.13 (dd, *J* = 8.4, 5.8 Hz, 2H), 6.94 (dd, *J* = 6.2, 3.5 Hz, 1H), 3.83 – 3.61 (m, 2H), 3.05 (s, 4H), 2.62 (s, 4H), 2.52 – 2.36 (m, 2H), 2.05 (s, 1H), 1.75 (dt, *J* = 14.4, 7.3 Hz, 2H), 1.57 (dt, *J* = 10.1, 7.5 Hz, 2H), 1.26 (t, *J* = 7.1 Hz, 1H).

2-(4-(4-(2,4-Dichlorophenyl)piperazin-1-yl)butyl)isoindoline-1,3-dione (6c)

Compound **6c** was obtained as withe solid. Yield: 57.6%. Mp: 106-107 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (dd, *J* = 5.5, 3.0 Hz, 2H), 7.71 (dd, *J* = 5.5, 3.0 Hz, 2H), 7.35 (d, *J* = 2.4 Hz, 1H), 7.17 (dd, *J* = 8.6, 2.5 Hz, 1H), 6.95 (d, *J* = 8.6 Hz, 1H), 3.73 (dd, *J* = 8.3, 5.8 Hz, 2H), 3.02 (s, 4H), 2.61 (s, 4H), 2.49 – 2.39 (m, 2H), 1.82 – 1.67 (m, 2H), 1.65 – 1.49 (m, 2H).

2-(4-(4-(3-(Trifluoromethyl)phenyl)piperazin-1-yl)butyl)isoindoline-1,3-dione (6d)

Compound **6d** was obtained as pale-yellow solid. Yield: 86.9%. Mp: 96-98 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (dt, *J* = 7.0, 3.5 Hz, 2H), 7.71 (dd, *J* = 5.4, 3.1 Hz, 2H), 7.33 (t, *J* = 8.0 Hz, 1H), 7.14 – 6.98 (m, 3H), 3.73 (t, *J* = 7.0 Hz, 2H), 3.33 – 3.10 (m, 4H), 2.69 – 2.51 (m, 4H), 2.50 – 2.36 (m, 2H), 1.83 – 1.66 (m, 2H), 1.66 – 1.47 (m, 2H).

2-(4-(4-(4-Chlorophenyl)piperazin-1-yl)butyl)isoindoline-1,3-dione (6e)

Compound **6e** was obtained as pale-yellow solid. Yield: 73.8%. Mp: 139-141 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (td, *J* = 5.3, 2.1 Hz, 2H), 7.72 (td, *J* = 5.2, 2.1 Hz, 2H), 7.24 – 7.13 (m, 2H), 6.88 – 6.72 (m, 2H), 3.73 (t, *J* = 7.0 Hz, 2H), 3.25 – 3.04 (m, 4H), 2.73 – 2.49 (m, 4H), 2.51 – 2.31 (m, 2H), 1.73 (dd, *J* = 14.3, 7.1 Hz, 3H), 1.58 (dd, *J* = 14.9, 8.4 Hz, 2H).

4.1.4. Procedure of deprotection

Method A: Trifluoroacetic acid was added dropwise at 0 °C under argon atmosphere to a solution of 4-piperazinyl carbamate in 10 mL CH_2Cl_2. The reaction mixture was allowed to stir for 15 hours and was monitored by TLC. Upon completion of the reaction, the reaction was quenched with saturated aqueous $NaHCO_3$. The reaction mixture was extracted with CH_2Cl_2, washed by brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude produce was further purified by silica gel chromatography eluted with CH_2Cl_2/EtOAc/MeOH (1:1:2, +0.5%Et_3N) to afford desired compound **7** as colorless liquid.

Method B: Hydrazine monohydrate was added to a solution of intermediate phthalimide **6**. The reaction mixture was heated at 55 °C overnight and cooled by ice-bath. The resulting mixture was filtered and washed with cold ethanol and then filtrate was concentrated in vocuo. The crude product was purified by silica gel chromatography eluted with CH_2Cl_2/MeOH (10:1, +0.5% Et_3N) to provide amine **7** as colorless liquid.

4.1.5. General procedure for preparation of cyanamides

A solution of cyanogen bromide (1.5 equiv.) in 2 mL MeOH was added dropwise into a solution of amine **7** (1 equiv.) and sodium acetate (2.8 equiv.) in 2 mL MeOH at 0 °C, while maintaining reaction 0 °C overnight. Upon completion, the reaction mixture was concentrated under reduce pressure, partitioned between CH_2Cl_2 and water and extracted with CH_2Cl_2 (3×50 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The resulting crude product was purified by flash column chromatography eluted with EtOAc/MeOH (v/v, 100:1) to afford cyanamide **8** as colorless liquid.

***N*-(4-(4-(2-Fluorophenyl)piperazin-1-yl)butyl)cyanamide (8a)**

Compound **8a** was prepared from **7a**. Yield: 42.3%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.12 – 6.87 (m, 4H), 3.12 (dd, *J* = 11.1, 6.1 Hz, 6H), 2.77 – 2.59 (m, 4H), 2.51 – 2.37 (m, 2H), 1.69 (ddt, *J* = 13.5, 7.8, 4.0 Hz, 4H).

***N*-(4-(4-(2,3-Dichlorophenyl)piperazin-1-yl)butyl)cyanamide (8b)**

Compound **8b** was prepared from **7b**. Yield: 40.4%.^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16 (dd, *J* = 9.4, 5.4 Hz, 2H), 6.98 (dd, *J* = 6.7, 2.9 Hz, 1H), 3.11 (dd, *J* = 10.9, 4.9 Hz, 6H), 2.69 (s, 4H), 2.53 – 2.41 (m, 2H), 1.84 – 1.57 (m, 4H).

***N*-(4-(4-(2,4-Dichlorophenyl)piperazin-1-yl)butyl)cyanamide (8c)**

Compound **8c** was prepared from **7c**. Yield: 42.4%.^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36 (d, *J* = 2.4 Hz, 1H), 7.20 (dd, *J* = 8.6, 2.4 Hz, 1H), 6.98 (d, *J* = 8.6 Hz, 1H), 3.10 (dd, *J* = 14.2, 8.2 Hz, 6H), 2.67 (s, 4H), 2.56 – 2.37 (m, 2H), 1.83 – 1.54 (m, 4H).

***N*-(4-(4-(3-(Trifluoromethyl)phenyl)piperazin-1-yl)butyl)cyanamide (8d)**

Compound **8d** was prepared from **7c**. Yield: 44.5% ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (t, *J* = 7.8 Hz, 1H), 7.14 – 7.02 (m, 3H), 3.35 – 3.21 (m, 4H), 3.18 – 3.07 (m, 2H), 2.72 – 2.58 (m, 4H), 2.46 (t, *J* = 5.9 Hz, 2H), 1.81 – 1.58 (m, 4H).

***N*-(4-(4-(4-Chlorophenyl)piperazin-1-yl)butyl)cyanamide (8e)**

Compound **8e** was prepared from **7e**. Yield: 46.2%.^{1}H NMR (300 MHz, $CDCl_3$) δ 7.21 (d, *J* = 8.9 Hz, 2H), 6.84 (d, *J* = 8.9 Hz, 2H), 3.29 – 3.15 (m, 4H), 3.12 (t, *J* = 5.7 Hz, 2H), 2.76 – 2.65 (m, 4H), 2.50 (t, *J* = 5.9 Hz, 2H), 1.77 – 1.64 (m, 4H).

4.1.6. General procedure for preparation of 1,2,4-oxadiazoles

Hydroxylamine HCl (1.2 equiv.) and DIEA (2 equiv.) were added to a solution of cyanamide **8** (1 equiv.) in 2 mL absolute ethanol The reaction mixture was allowed to warm to room temperature and was kept at ambient temperature overnight. Upon completion, the solvent was removed *in vacuo*. The residue was used in the next step without purification and dissolved in 2.5 mL CH_2Cl_2. HATU (1.1 equiv.), DIEA (1.1 equiv.), and benzoic acid or substituted benzoic acid (1 equiv.) were sequentially added to the reaction solution at 0 °C. The reaction mixture was allowed to warm to room temperature and maintained at the same temperature overnight. Upon consumption of the starting material, the solvent was removed again and DIEA (2.2 equiv.) and 2.5 mL DCE were added. The resulting reaction mixture was refluxed for 5 hours and monitored by TLC. Upon completion, the reaction mixture was partitioned between CH_2Cl_2 and water and extracted with CH_2Cl_2 (4×40 mL). The combined organic layers were washed by brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by flash column chromatography and eluted with CH_2Cl_2/EtOAc (v/v, 1:1) to provide the target compound **9**.

***N*-(5-Phenyl-1,2,4-oxadiazol-3-yl)-4-(4-(2-fluorophenyl)piperazin-1-yl)butan-1-amine (9a)**

Compound **9a** was prepared from **8a** as colorless wax and benzoic acid. Yield: 56.4%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.06 – 7.98 (m, 2H), 7.60 – 7.41 (m, 3H), 7.10 – 6.87 (m, 4H), 5.70 (s, 1H), 3.32 (dd, *J* = 11.7, 6.1 Hz, 2H), 3.25 – 3.10 (m, 4H), 2.76 – 2.59 (m, 4H), 2.47 (t, *J* = 6.7 Hz, 2H), 1.89 – 1.56 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.88, 169.24, 157.35, 154.09, 140.17 (d, J_{CF}=8.66), 132.25, 128.84, 127.81, 124.65, 124.38 (d, J_{CF}=3.70 Hz), 122.37 (d, J_{CF}=7.91 Hz), 118.97 (d, J_{CF}=3.25 Hz), 116.04 (d, J_{CF}=20.88 Hz), 58.31, 53.28, 50.34, 50.29, 43.38, 27.79, 24.54.

***N*-(5-Phenyl-1,2,4-oxadiazol-3-yl)-4-(4-(2,3-dichlorophenyl)piperazin-1-yl)butan-1-amine (9b)**

Compound **9b** was prepared as colorless wax from **8b** and benzoic acid. Yield: 83.9%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03 (dd, *J* = 8.3, 1.4 Hz, 2H), 7.61 – 7.39 (m, 3H), 7.19 – 7.08 (m, 2H), 7.00 (dd, *J* = 7.5, 2.1 Hz, 1H), 5.79 (s, 1H), 3.32 (d, *J* = 4.9 Hz, 2H), 3.15 (t, *J* = 4.5 Hz, 4H), 2.68 (s, 4H), 2.49 (t, *J* = 6.6 Hz, 2H), 1.84 – 1.62 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.89, 169.25, 151.31, 133.98, 132.30, 128.87, 127.83, 127.55, 127.39, 124.67, 124.56, 118.73, 58.29, 53.30, 51.08, 43.40, 27.83, 24.61.

***N*-(5-Phenyl-1,2,4-oxadiazol-3-yl)-4-(4-(2,4-dichlorophenyl)piperazin-1-yl)butan-1-amine (9c)**

Compound **9c** was prepared as colorless wax from **8c** and benzoic acid. Yield: 37.0%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.08 – 7.96 (m, 2H), 7.50 (dt, *J* = 8.6, 7.2 Hz, 3H), 7.37 (d, *J* = 2.4 Hz, 1H), 7.16 (dd, *J* = 8.6, 2.4 Hz, 1H), 7.00 (d, *J* = 8.6 Hz, 1H), 5.77 (s, 1H), 3.32 (s, 2H), 3.13 (t, *J* = 4.6 Hz, 4H), 2.68 (s, 4H), 2.49 (t, *J* = 6.6 Hz, 2H), 1.88 – 1.64 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.89, 169.25, 148.11, 132.31, 130.25, 129.47, 128.86, 128.14, 127.81, 127.55, 124.66, 121.25, 58.26, 53.24, 50.95, 43.39, 27.80, 24.56.

***N*-(5-Phenyl-1,2,4-oxadiazol-3-yl)-4-(4-(3-trifluorophenyl)piperazin-1-yl)butan-1-amine (9d)**

Compound **9d** was prepared as colorless wax from **8d** and benzoic acid. Yield: 56.5%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.06 – 7.90 (m, 2H), 7.53 (ddd, *J* = 6.5, 3.8, 1.4 Hz, 1H), 7.50 – 7.39 (m, 2H), 7.34 (t, *J* = 7.9 Hz, 1H), 7.13 (s, 1H), 7.08 (d, *J* = 8.0 Hz, 2H), 5.62 (s, 1H), 3.33 (dd, *J* = 10.2, 5.1 Hz, 6H), 2.72 – 2.57 (m, 4H), 2.47 (t, *J* = 6.7 Hz, 2H),

1.85 – 1.60 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.92, 169.22, 151.42, 132.29, 131.42 (d, J_{CF}=31.90 Hz), 129.49, 128.86, 127.77, 124.59, 124.23 (dd, J_{CF}=272.74 Hz), 118.67, 115.73 (dd, J_{CF}=3.75 Hz), 112.14 (dd, J_{CF}=3.75 Hz), 58.24, 53.00, 48.46, 43.34, 27.76, 24.52.

***N*-(5-Phenyl-1,2,4-oxadiazol-3-yl)-4-(4-(4-chlorophenyl)piperazin-1-yl)butan-1-amine (9e)**

Compound **9e** was prepared as white solid from **8e** and benzoic acid. Yield: 32.3%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96 (d, *J* = 7.2 Hz, 2H), 7.54 (t, *J* = 7.3 Hz, 1H), 7.45 (t, *J* = 7.5 Hz, 2H), 7.21 (d, *J* = 9.0 Hz, 2H), 6.86 (d, *J* = 9.0 Hz, 2H), 5.74 (s, 1H), 3.39 – 3.19 (m, 6H), 2.81 – 2.54 (m, 4H), 2.46 (t, *J* = 6.6 Hz, 2H), 1.90 – 1.48 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.87, 169.19, 149.96, 132.31, 128.89, 128.84, 127.77, 124.55, 124.46, 117.24, 58.29, 53.04, 48.89, 43.33, 27.78, 24.54.

***N*-(5-(4-Chlorophenyl)-1,2,4-oxadiazol-3-yl)-4-(4-(2,4-dichlorophenyl)piperazin-1-yl)butan-1-amine (9f)**

Compound **9f** was prepared as colorless solid from **8c** and 4-chlorobenzoic acid. Yield: 38.4%. Mp: 120-122 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.06 – 7.89 (m, 2H), 7.54 – 7.40 (m, 2H), 7.37 (d, *J* = 2.4 Hz, 1H), 7.17 (dd, *J* = 8.6, 2.4 Hz, 1H), 6.99 (d, *J* = 8.6 Hz, 1H), 5.81 (s, 1H), 3.32 (s, 2H), 3.12 (s, 4H), 2.67 (s, 4H), 2.48 (t, *J* = 6.6 Hz, 2H), 1.85 – 1.60 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.94, 169.20, 148.03, 138.68, 130.26, 129.42, 129.26, 129.08, 128.14, 127.53, 123.06, 121.15, 77.42, 77.00, 76.57, 58.21, 53.20, 50.91, 43.35, 27.73, 24.54.

***N*-(5-(3-Chlorophenyl)-1,2,4-oxadiazol-3-yl)-4-(4-(2,4-dichlorophenyl)piperazin-1-yl)butan-1-amine (9g)**

Compound **9g** was prepared as colorless liquid from **8c** and 3-chlorobenzoic acid. Yield: 35.7%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01 (t, *J* = 1.7 Hz, 1H), 7.90 (d, *J* = 7.7 Hz, 1H), 7.60 – 7.47 (m, 1H), 7.47 – 7.30 (m, 2H), 7.18 (dd, *J* = 8.6, 2.4 Hz, 1H), 7.00 (d, *J* = 8.6 Hz, 1H), 6.01 (s, 1H), 3.31 (d, *J* = 4.2 Hz, 2H), 3.13 (s, 4H), 2.68 (s, 4H), 2.48 (t, *J* = 6.5 Hz, 2H), 1.88 – 1.56 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.57, 169.20, 148.03, 134.99, 132.33, 130.24, 129.40, 128.15, 127.76, 127.63, 126.22, 125.85, 121.14, 58.30, 53.22, 50.89, 43.37, 38.58, 27.82, 24.60.

***N*-(5-(2-Chlorophenyl)-1,2,4-oxadiazol-3-yl)-4-(4-(2,4-dichlorophenyl)piperazin-1-yl)butan-1-amine (9h)**

Compound **9h** was prepared as colorless liquid from **8c** and 2-chlorobenzoic acid. Yield: 36.6%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.49 (dtd, *J* = 9.8, 8.1, 1.6 Hz, 2H), 7.41 – 7.30 (m, 2H), 7.14 (dd, *J* = 8.6, 2.4 Hz, 1H), 6.98 (d, *J* = 8.6 Hz, 1H), 5.87 (s, 1H), 3.33 (t, *J* = 6.1 Hz, 2H), 3.12 (t, *J* = 4.4 Hz, 4H), 2.68 (s, 4H), 2.49 (t, *J* = 6.6 Hz, 2H), 1.84 – 1.57 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.33, 168.96, 147.99, 133.51, 132.71, 131.51, 131.31, 130.21, 129.39, 128.11, 127.53, 126.90, 123.88, 121.27, 58.17, 53.19, 50.89, 43.34, 27.68, 24.50.

***N*-(5-(4-Methoxylphenyl)-1,2,4-oxadiazol-3-yl)-4-(4-(2,4-dichlorophenyl)piperazin-1-yl)butan-1-amine (9i)**

Compound **9i** was prepared as colorless solid from **8c** and 4-methoxylbenzoic acid. Yield: 32.3%. Mp: 111-113 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 – 7.85 (m, 2H), 7.36 (d, *J* = 2.4 Hz, 1H), 7.16 (dd, *J* = 8.6, 2.4 Hz, 1H), 7.06 – 6.85 (m, 3H), 5.65 (s, 1H), 3.88 (s, 3H), 3.31 (t, *J* = 6.0 Hz, 2H), 3.12 (t, *J* = 4.3 Hz, 4H), 2.67 (s, 4H), 2.48 (t, *J* = 6.7 Hz, 2H), 1.84 – 1.57 (m, 4H). ^{13}C NMR (151 MHz, $cdcl_3$) δ 173.76, 169.14, 162.79, 148.06, 130.23, 129.67, 129.41, 128.09, 127.55, 121.22, 117.16, 114.23, 58.24, 55.44, 53.20, 50.90, 43.34, 27.76, 24.51.

***N*-(5-(3-Methoxylphenyl)-1,2,4-oxadiazol-3-yl)-4-(4-(2,4-dichlorophenyl)piperazin-1-yl)butan-1-amine (9j)**

Compound **9j** was prepared as colorless liquid from **8c** and 3-methoxylbenzoic acid. Yield: 47.2%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.62 (d, *J* = 7.7 Hz, 1H), 7.53 (s, 1H), 7.38 (dd, *J* = 10.9, 5.2 Hz, 2H), 7.13 (ddd, *J* = 21.6, 8.4, 2.4 Hz, 2H), 7.00 (d, *J* = 8.6 Hz, 1H), 5.81 (s, 1H), 3.85 (s, 3H), 3.32 (s, 2H), 3.12 (s, 4H), 2.67 (s, 4H), 2.48 (t, *J* = 6.6 Hz, 2H), 1.87 – 1.55 (m, 4H). ^{13}C NMR (151 MHz, $cdcl_3$) δ 173.76, 169.17, 159.71, 148.04, 130.19, 129.99, 129.38, 128.09, 127.59, 125.67, 121.21, 120.21, 118.59, 112.45, 77.20, 76.99, 76.78, 58.28, 55.39, 53.21, 50.91, 43.35, 27.78, 24.54.

***N*-(5-(2-Methoxylphenyl)-1,2,4-oxadiazol-3-yl)-4-(4-(2,4-dichlorophenyl)piperazin-1-yl)butan-1-amine (9k)**

Compound **9k** was prepared as colorless liquid from **8c** and 2-methoxylbenzoic acid. Yield: 37.9%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.94 (dd, *J* = 8.0, 1.8 Hz, 1H), 7.52 (ddd, *J* = 8.5, 7.4, 1.8 Hz, 1H), 7.36 (d, *J* = 2.4 Hz, 1H), 7.15 (dd, *J* = 8.6, 2.5 Hz, 1H), 7.07 – 7.00 (m, 2H), 6.98 (d, *J* = 8.6 Hz, 1H), 5.38 (s, 1H), 3.93 (s, 3H), 3.32 (d, *J* = 5.7 Hz, 2H), 3.18 – 3.00 (m, 4H), 2.66 (s, 4H), 2.48 (t, *J* = 6.8 Hz, 2H), 1.85 – 1.59 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.01, 168.92, 158.26, 148.04, 133.67, 131.21, 130.21, 129.42, 128.12, 127.55, 121.26, 120.59, 113.71, 112.01, 58.17, 56.00, 53.19, 50.93, 43.34, 27.65, 24.36.

***N*-(5-(4-Cyanophenyl)-1,2,4-oxadiazol-3-yl)-4-(4-(2,4-dichlorophenyl)piperazin-1-yl)butan-1-amine (9l)**

Compound **9l** was prepared as pale-yellow solid from **8c** and 4-cyanobenzoic acid. Yield: 28.9%. Mp: 142-144 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.18 – 8.07 (m, 2H), 7.82 – 7.72 (m, 2H), 7.37 (d, *J* = 2.4 Hz, 1H), 7.17 (dd, *J* = 8.6, 2.4 Hz, 1H), 6.99 (d, *J* = 8.6 Hz, 1H), 6.03 (s, 1H), 3.32 (d, *J* = 4.9 Hz, 2H), 3.13 (t, *J* = 4.4 Hz, 4H), 2.68 (s, 4H), 2.49 (t, *J* = 6.6 Hz, 2H), 1.84 – 1.58 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.99, 169.29, 148.00, 132.65, 130.30, 129.43, 128.32, 128.24, 128.19, 127.50, 121.09, 117.82, 115.69, 58.19, 53.20, 50.89, 43.36, 27.72, 24.57.

***N*-(5-(4-Bromophenyl)-1,2,4-oxadiazol-3-yl)-4-(4-(2,4-dichlorophenyl)piperazin-1-yl)butan-1-amine (9m)**

Compound **9m** was prepared as pale-yellow wax from **8c** and 4-bromobenzoic acid. Yield: 45.0%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96 – 7.81 (m, 2H), 7.69 – 7.55 (m, 2H), 7.37 (d, *J* = 2.4 Hz, 1H), 7.17 (dd, *J* = 8.6, 2.4 Hz, 1H), 6.99 (d, *J* = 8.7 Hz, 1H), 5.79 (s, 1H), 3.32 (d, *J* = 5.1 Hz, 2H), 3.12 (s, 4H), 2.67 (s, 4H), 2.48 (t, *J* = 6.6 Hz, 2H), 1.84 – 1.61 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.06, 169.23, 132.24, 130.28, 129.44, 129.21, 128.17, 127.55, 127.23, 123.50, 121.17, 58.21, 53.21, 50.91, 43.36, 29.68, 27.74, 24.53.

***N*-(5-Pyridin-2-yl-1,2,4-oxadiazol-3-yl)-4-(4-(2,4-dichlorophenyl)piperazin-1-yl)butan-1-amine (9n)**

Compound **9n** was prepared as yellowish wax from **8c** and picolinic acid. Yield: 56.4%. Yield: 25.6%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.80 (d, *J* = 4.1 Hz, 1H), 8.09 (d, *J* = 7.9 Hz, 1H), 7.87 (td, *J* = 7.8, 1.7 Hz, 1H), 7.49 (ddd, *J* = 7.6, 4.8, 1.1 Hz, 1H), 7.36 (d, *J* = 2.4 Hz, 1H), 7.18 (dd, *J* = 8.6, 2.4 Hz, 1H), 7.00 (d, *J* = 8.6 Hz, 1H), 3.37 (d, *J* = 6.1 Hz, 2H), 3.15 (s, 4H), 2.81 (s, 4H), 2.63 (d, *J* = 6.6 Hz, 2H), 2.03 (d, *J* = 5.1 Hz, 2H), 1.75 (m, 4H).

4.1.7. General procedure of propylation

Iodopropane was added at 0 °C under argon atmosphere to a 1 mL solution of 1,2,4-oxadiazole and NaH. The reaction mixture was stirred for 5 hours at the same temperature. Upon completion, the reaction was quenched by saturated aqueous NH_4Cl and extracted with CH_2Cl_2 (3×50 mL). The combined organic layers were washed by brine, dried over MgSO4, filtered, and concentrated *in vacuo*. The crude product was purified by flash column chromatography and eluted with CH_2Cl_2/EtOAc (v/v, 5:1) to furnish the desired compound **10** as colorless liquid.

***N*-(5-Phenyl-1,2,4-oxadiazol-3-yl)-*N*-propyl-4-(4-(2-fluorophenyl)piperazin-1-yl)butan-1-amine (10a)**

Yield: 51.1%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.13 – 8.00 (m, 2H), 7.61 – 7.41 (m, 3H), 7.11 – 6.84 (m, 4H), 3.56 – 3.31 (m, 4H), 3.23 – 3.01 (m, 4H), 2.74 – 2.56 (m, 4H), 2.54 – 2.36 (m, 2H), 1.78 – 1.51 (m, 6H), 0.95 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.66, 170.15, 157.31, 154.06, 140.19, 140.08, 132.09, 128.78, 127.83, 124.98, 124.42, 124.37, 122.38, 122.28, 118.87, 118.83, 116.17, 115.89, 58.34, 53.27, 50.53, 50.48, 48.60, 25.68, 24.10, 20.87, 11.28.

***N*-(5-Phenyl-1,2,4-oxadiazol-3-yl)-*N*-propyl-4-(4-(2,3-dichlorophenyl)piperazin-1-yl)butan-1-amine (10b)**

Yield: 66.7%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.14 – 7.99 (m, 2H), 7.61 – 7.43 (m, 3H), 7.19 – 7.07 (m, 2H), 6.95 (dd, *J* = 6.4, 3.2 Hz, 1H), 3.54 – 3.33 (m, 4H), 3.07 (s, 4H), 2.65 (s, 4H), 2.54 – 2.40 (m, 2H), 1.79 – 1.53 (m, 6H),

0.95 (t, J = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.93, 170.43, 151.53, 134.24, 132.37, 129.06, 128.10, 127.72, 127.66, 125.25, 124.76, 118.82, 58.56, 53.55, 51.55, 50.75, 48.85, 25.95, 24.38, 21.14, 11.56, 0.25.

***N*-(5-Phenyl-1,2,4-oxadiazol-3-yl)-*N*-propyl-4-(4-(2,4-dichlorophenyl)piperazin-1-yl)butan-1-amine (10c)**

Yield: 82.2%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.14 – 7.95 (m, 2H), 7.61 – 7.42 (m, 3H), 7.35 (d, J = 2.4 Hz, 1H), 7.17 (dd, J = 8.6, 2.5 Hz, 1H), 6.94 (d, J = 8.6 Hz, 1H), 3.55 – 3.31 (m, 4H), 3.04 (s, 4H), 2.63 (s, 4H), 2.53 – 2.39 (m, 2H), 1.64 (ddd, J = 28.1, 15.3, 8.0 Hz, 6H), 0.95 (t, J = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.65, 170.14, 148.06, 132.09, 130.23, 129.36, 128.78, 128.04, 127.81, 127.54, 124.96, 121.05, 58.27, 53.23, 51.17, 50.46, 48.57, 25.65, 24.11, 20.86, 11.28.

***N*-(5-Phenyl-1,2,4-oxadiazol-3-yl)-*N*-propyl-4-(4-(3-trifluorohenyl)piperazin-1-yl)butan-1-amine (10d)**

Yield: 45.5%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.13 – 8.03 (m, 2H), 7.61 – 7.43 (m, 3H), 7.33 (t, J = 8.0 Hz, 1H), 7.14 – 6.99 (m, 3H), 3.56 – 3.32 (m, 4H), 3.30 – 3.16 (m, 4H), 2.68 – 2.54 (m, 4H), 2.52 – 2.38 (m, 2H), 1.66 (ddd, J = 17.9, 8.9, 5.7 Hz, 6H), 0.95 (t, J = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.69, 170.18, 151.36, 132.12, 131.42 (dd, J_{CF}=32.03 Hz), 129.48, 128.80, 127.83, 125.57 (d, J_{CF}=273.20 Hz), 124.98, 118.54, 115.68 (dd, J_{CF}=3.94 Hz), 112.05 (dd, J_{CF}=3.97 Hz), 58.24, 53.02, 50.49, 48.62, 48.58, 25.64, 24.11, 20.89, 11.29.

4.2. Biological assays

Ligand binding assays were carried out with HEK 293 cells based on competition-binding experiment. Cells were cultured in fresh minimal essential medium (MEM) containing 10% fetal bovine serum (FBS), 100 units of penicillin, and 100 g/ml streptomycin. Cells were transfected with different plasmids (D2R/D3R) using polyethylenimine (PEI). After transfection for 4 hours, the medium was replaced by fresh MEM containing FBS, and the cells were grown for 24 hours. Subsequently, cells were seeded in 24-well plates and incubated overnight or 14-16 hours. After discarding the medium, cells were rinsed once with ice-cold serum-free MEM. [^{3}H]-Sulpiride (2.2 nM for D2R and 4.4 nM for D3R) and varying concentrations of synthesized ligands were incubated with the cells at 4ºC for 150 minutes. Unbound ligands were removed by rinsing three times with ice-cold MEM containing 10 mM HEPES at pH 7.4. After drying the harvest plates, the cells were lysed in 1% sodium dodecyl sulfate and the remaining radioligand was measured using a liquid scintillation counter. The resulting dose-response curves were obtained by fitting nonlinear regression using Graphpad Prism, and IC_{50} values were estimated from the fitting curves. Ki values were converted from IC_{50} values according to the equation of the Cheng-Prusoff.

4.3. Molecular modeling

Since human dopamine D2 and D3 receptors share more than 50% overall sequence identity and more than 70% homology within the transmembrane domains, chain A of human D3 receptor was selected as template (PDB code: 3PBL). The D2 receptor homology model was constructed through Modeller 9.15[20] using human D2 receptor sequence (SWISS-PROT code: P14416.2) as the starting point. The homology modellings were carried out 10 times and generated 9 models every time. The top 10 models ranked by a Ramachandran plot and DOPE scores were further refined loops. In each step, 9 variations were generated for every model, and the resulting best 10 models were selected and subjected to the next step. Extracellular loop (EL, 96-99) 1, EL2 (178-181, 175-180, 185-186), EL3 (371-376), and helix 1 (33-37) were refined in serial order.[1] Ramachadran plot evaluation and comparison between D3R were performed to provide the best D2R model.

The SYBYL-X 2.0 program was used for ligand sketching, hydrogen addition, and minimization with the Trips force field and Gasteriger-Huckel charges. The modeled dopaminergic receptors, D3R (PDB code: 3PBL) and D2R, were refined using the Biopolymer module implemented in SYBYL-X 2.0. After the removal of all the ligands and water molecules, the N- and C-termini were treated with charges. Subsequently, hydrogens were added and staged minimizations were performed using AMBER7_FF99 force field and Gasteriger-Marsili charges.[21] A rectangular box was generated in the LeDockGUI, a free graphics user interface of VMD, based on the binding pocket of

eticlopride (ETQ) in D3R. The synthesized ligands were docked into the dopaminergic receptors using LeDock program and for each ligands, 30 poses were generated and clustered by a RMSD cutoff of 1 Å. The docking results were analyzed by VMD frame by frame. The final reasonable binding conformations were accomplished by docking energy and poses.

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Supplementary data

Sequence alignment, homology model, docking evaluation and 2D diagram of interactions are available.

References and notes

1. Chien, E.Y.; Liu, W.; Zhao, Q.; Katritch, V.; Han, G.W.; Hanson, M.A.; Shi, L.; Newman, A.H.; Javitch, J.A.; Cherezov, V.; Stevens, R.C. Science. **2010**, *330*, 1091-1095.
2. Missale, C.; Nash, S.R.; Robinson, S.W.; Jaber, M.; Caron, M.G. Physiol. Rev. **1998**, *78*, 189-225.
3. Romanelli, R.J.; Williams, J.T.; Neve, K.A. In The Dopamine Receptors; Neve, K.A.; Eds. Humana Press: New York, **2010**; pp. 137-173.
4. Beaulieu, J.M.; Gainetdinov, R.R. Pharmacol. Rev. **2011**, *63*, 182-217.
5. Miyamoto, S.; Miyake, N.; Jarskog, L.F.; Fleischhacker, W.W.; Lieberman, J.A. Mol. Psychiatry. **2012**, *17*, 1206-1227.
6. Ginovart, N.; Kapur, S. In The Dopamine Receptors; Neve, K.A.; Eds. Humana Press: New York, **2010**; pp. 431-477.
7. Gurevich, E.V.; V.gurevich, V. In The Dopamine Receptors; Neve, K.A.; Eds. Humana Press: New York, **2010**; pp. 525-584.
8. Sokoloff, P.; Giros, B.; Martres, M.P.; Bouthenet, M.L.; Schwartz, J.C. Nature. **1990**, *347*, 146-151.
9. Gurevich, E.V.; Joyce, J.N. Neuropsychopharmacology. **1999**, *20*, 60-80.
10. Tu, Z.; Li, S.; Cui, J.; Xu, J.; Taylor, M.; Ho, D.; Luedtke, R.R.; Mach, R.H. J. Med. Chem. **2011**, *54*, 1555-1564.
11. Millan, M.J.; Di Cara, B.; Dekeyne, A.; Panayi, F.; De Groote, L.; Sicard, D.; Cistarelli, L.; Billiras, R.; Gobert, A. J. Neurochem. **2007**, *100*, 1047-1061.
12. Newman, A.H.; Grundt, P.; Nader, M.A. J. Med. Chem. **2005**, *48*, 3663-3679.
13. Keck, T.M.; John, W.S.; Czoty, P.W.; Nader, M.A.; Newman, A.H. J. Med. Chem. **2015**, *58*, 5361-5380.
14. Joyce, J.N.; Millan, M.J. Drug Discovery Today. **2005**, *10*, 917-925.
15. Micheli, F.; Heidbreder, C. Expert Opin. Ther. Pat. **2013**, *23*, 363-381.
16. Lober, S.; Hubner, H.; Tschammer, N.; Gmeiner, P. Trends Pharmacol. Sci. **2011**, *32*, 148-157.
17. Macdonald, G.J.; Branch, C.L.; Hadley, M.S.; Johnson, C.N.; Nash, D.J.; Smith, A.B.; Stemp, G.; Thewlis, K.M.; Vong, A.K.; Austin, N.E.; Jeffrey, P.; Winborn, K.Y.; Boyfield, I.; Hagan, J.J.; Middlemiss, D.N.; Reavill, C.; Riley, G.J.; Watson, J.M.; Wood, M.; Parker, S.G.; Ashby Jr, C.R. J. Med. Chem. **2003**, *46*, 4952-4964.
18. Brogan, J.T.; Stoops, S.L.; Lindsley, C.W. ACS Chem. Neurosci. **2012**, *3*, 658-664.
19. Cao, Y.; Paudel, S.; Zhang, X.; Kim, K.M.; Cheon, S.H. Bioorg. Med. Chem. **2015**, *23*, 5264-5272.
20. Eswar, N.; Webb, B.; Marti-Renom, M.A.; Madhusudhan, M.S.; Eramian, D.; Shen, M.Y.; Pieper, U.; Sali, A. Curr. Protoc. Bioinformatics. **2006**, *Chapter 5*, Unit 5.6.
21. St. Louis, M., Sybyl-x, Tripos International, USA.