European Journal of Medicinal Chemistry 261 (2023) 115751

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: www.elsevier.com/locate/ejmech

Research paper

Synthesis of bitopic ligands based on fallypride and evaluation of their affinity and selectivity towards dopamine D_2 and D_3 receptors

Gui-Long Tian [a], Chia-Ju Hsieh [a], Michelle Taylor [b], Ji Youn Lee [a], Aladdin A. Riad [a], Robert R. Luedtke [b,1], Robert H. Mach [a,*]

[a] Division of Nuclear Medicine and Clinical Molecular Imaging, Department of Radiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, 19104, USA

[b] Department of Pharmacology and Neuroscience, University of North Texas Health Science Center-Fort Worth, Texas, TX, 76107, USA

ARTICLE INFO

Keywords:
Dopamine 2 receptor
Dopamine 3 receptor
Fallypride
Bitopic ligands
PET imaging

ABSTRACT

The difference in the secondary binding site (SBS) between the dopamine 2 receptor (D_2R) and dopamine 3 receptor (D_3R) has been used in the design of compounds displaying selectivity for the D_3R versus D_2R. In the current study, a series of bitopic ligands based on **Fallypride** were prepared with various secondary binding fragments (SBFs) as a means of improving the selectivity of this benzamide analog for D_3R versus D_2R. We observed that compounds having a small alkyl group with a heteroatom led to an improvement in D_3R versus D_2R selectivity. Increasing the steric bulk in the SBF increase the distance between the pyrrolidine *N* and Asp110, thereby reducing D_3R affinity. The best-in-series compound was **(2*S*,4*R*)-*trans*-27** which had a modest selectivity for D_3R versus D_2R and a high potency in the β-arrestin competition assay which provides a measure of the ability of the compound to compete with endogenous dopamine for binding to the D_3R. The results of this study identified factors one should consider when designing bitopic ligands based on **Fallypride** displaying an improved affinity for D_3R versus D_2R.

1. Introduction

The dopamine 2-like receptors belong to the G-protein-coupled receptors (GPCRs). Various neurological and neuropsychiatric disorders including schizophrenia, Parkinson's disease, dementia, anxiety, and depression are associated with dopamine 2-like receptors [1–3]. The dopamine 2-like receptors include the dopamine 2 receptor (D_2R), dopamine 3 receptor (D_3R), and dopamine 4 receptor (D_4R). The D_2R and D_3R are the most investigated central nervous system (CNS) receptors using PET imaging. Previous studies indicate that these two receptors have a different distribution [4–7] and function [8,9] in the CNS. Therefore, developing a positron emission tomography (PET) radiotracer that can independently image each of the dopamine receptor subtypes could provide an improved method for studying D_2R/D_3R-related CNS disorders as well as aid in the design of therapeutics having fewer side effects. The PET radiotracers currently used to image the $D_{2/3}R$ have a high affinity for both D_2R and D_3R [10,11], with the most prominent representatives being **[^{11}C] Raclopride,** [12] **[^{18}F] Fallypride** [13] and **[^{11}C] PHNO**. (Fig. 1) [14–16]. PET imaging data reports the results of these radiotracers as providing a measure of the $D_{2/3}R$ binding potential.

The crystal structure of D_2R, D_3R [17,18], and related computational studies [19–23] revealed that the secondary binding site (SBS) of D_2R is different from D_3R. Designing a bitopic ligand is becoming an important research topic since this can lead to an increase in the selectivity and affinity for the different receptor subtypes [24,25]. This strategy was successfully used for designing *N*-phenylpiperazine ligands which are the most investigated scaffold leading to high D_3R subtype selectivity (Fig. 2) [1,26–31]. The PET ligand **[^{18}F] LS-3-134** (Fig. 2) has a sub-nM affinity and excellent selectivity for D_3R versus D_2R and showed promise in preclinical imaging studies [32]. However, it failed to be a D_3R selective PET imaging radiotracer in human studies *in vivo* (unpublished results).

Our recent study with *in silico* and *in vitro* (β-arrestin recruitment assays) methods suggested this might be due to phenylpiperazine (**KX-02–065**, IC_{50} = 678.1 nM) analogs having a weaker contact with the

* Corresponding author.
E-mail address: rmach@pennmedicine.upenn.edu (R.H. Mach).
[1] Deceased.

https://doi.org/10.1016/j.ejmech.2023.115751
Received 30 June 2023; Received in revised form 8 August 2023; Accepted 21 August 2023
Available online 9 September 2023

orthosteric binding site (OBS) of the D_3R than that of **Fallypride** (Fig. 3). This results in decreasing the ability of *N*-phenylpiperazine benzamide ligands to compete with endogenous dopamine [33]. A D_3R selective PET radiotracer must have a high potency (i.e., numerically low IC_{50} value) in the β-arrestin assays in order to image the D_3R *in vivo* [33].

The benzamide analog, **Fallypride,** binds with high affinity to both the D_2R and D_3R, because of its high affinity to the OBS for both receptors; however, it does not interact with the SBS in either receptor. This observation prompted us to design a D_2R or D_3R selective bitopic ligand based on **Fallypride** by introducing a fragment that could interact with the SBS. A few examples of bitopic ligands based on **[^{11}C] raclopride** and **[^{18}F] fallypride** have been reported [34,35]. Two decades ago, our group studied a series of naphthamides as D_3R ligands; this apporoach mainly focused on different N-substituents of the pyrrolidine, shifting the position of the *N*- in the pyrrolidine ring, and expanding the pyrrolidine to a piperidine (Fig. 4a) [34]. This approach resulted in a modest improvement in the selectivity for D_3R versus D_2R. While the current study was being conducted, Newman et al. reported **Eticlopride**-based dopamine $D_{2/3}R$ bitopic ligands. They created bitopic ligands by introducing secondary binding fragments (SBFs) on the 4-position of pyrrolidine (Fig. 4b) as well as introducing SBFs on the nitrogen of pyrrolidine ring. Other modifications included shifting the nitrogen in the pyrrolidine and expanding the pyrrolidine to a piperidine ring [32]. Although D_3R-selective compounds were not identified in this study, the authors made suggestions on how one may improve D_3R selectivity of this scaffold. Consequently, the goal of the current study was to build on the structure-activity relationships (SAR) of this scaffold by further exploring substitutions of the pyrrolidine ring. Firstly, previous research [33,35] suggested a long linker or bulker SBFs could increase the selectivity of $D_{2/3}R$. Secondly, the chirality of the orthosteric binding fragments (OBFs) is a critical property that needs to be explored in greater detail [34–38]. Finally, the position of the fragment interacting with the SBS on the pyrrolidine ring as well as the effect of N-substitution of the pyrrolidine needed to be explored.

Fallypride was chosen as our lead compound because of its high affinity for the OBS and its ease of radiolabeling with fluorine-18. Our design involved preparing **Fallypride** analogs with a hydroxyl on the pyrrolidine which could be functionalized to introduce fragments interacting with the SBS. As shown in Fig. 5, we prepared **Fallypride** analogs **1a** with a hydroxyl on the pyrrolidine for the initial SAR study. The SAR study included linkers with various SBFs. Next, the effect of stereochemistry was evaluated with bitopic ligands prepared from **1a**, **2a**, **1b** and **2b**. Further, the bitopic ligands made from **1a** and **3** could elicit a better position for the SBF on the pyrrolidine (3-or 4- position on the pyrrolidine) for increasing the affinity and selectivity. Finally, in comparison with the bitopic ligands originating from **1a** and **4**, we evaluated the effect of an N-substituted pyrrolidine on the affinity of D_2R and D_3R. All bitopic ligands reported here were submitted to radioligand binding assays to measure their affinity for D_2R and D_3R, and molecular modeling studies were conducted to elucidate the *in vitro* binding results.

2. Results and discussion

Chemistry: The synthetic route for the preparation of **1a**, **2a**, and **4** is shown in Scheme 1. Conversion of **5** to **6** and **7** via a 2-step procedure occurred in good yield. The hydroxyl of **6** and **7** were protected by *tert*-butyldimethylsilyl chloride (TBSCl) and generated **8** and **9**. Subsequently, the amidation of the corresponding ester with 7 N NH_3 in methanol (MeOH) afforded amides the **10** and **11**. Reduction of these amides with lithium aluminum hydride ($LiAlH_4$) gave the primary amines **11** and **12**. Coupling these two amines with 5-(3-fluoropropyl)-2,3-dimethoxybenzoic acid using HBTU in dimethylformamide (DMF) afforded **13** and **14**. Removing the TBS with *tetra-n*-butylammonium fluoride (TBAF) delivered **1a** and **4**. **1a** was submitted to the Mitsunobu reaction followed by hydrolysis to generate the diastereomer **2a**.

Compound 3 with a hydroxyl in the 3-position of the pyrrolidine was prepared from **16** using the same route for preparing **1a** (Scheme 2).

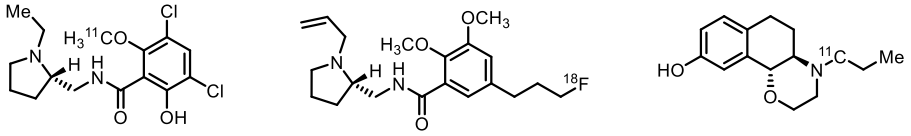
For the synthesis of **1b** and **2b**, which are the enantiomers of **1a** and **2a**, we investigated another synthetic route starting from commercially available 1-(*tert*-butyl) 2-methyl (2*R*,4*S*)-4-hydroxypyrrolidine-1,2-dicarboxylate **22** (Scheme 3). First, the hydroxyl of **22** was protected with TBSCl to generate **23**. This ester was treated with sodium borohydride ($NaBH_4$) to generate primary alcohol **24**. Tosylation of this primary hydroxyl and subsequent displacement by NaN_3 produced **26**. Hydrogeneration of the azide using Pd/C as a catalyst in ethanol generated primary amine **27**, which was directedly used for the coupling reaction with 5-(3-fluoropropyl)-2,3-dimethoxybenzoic acid to afford **28**. **1b** was obtained after deprotection of TBS, *N* and followed by *N*-allylation. For **1b**, the configuration of the chiral center attached to hydroxyl was inverted with the Mitsunobu reaction and followed by hydrolysis to generate compound **2b**.

With the six basic **Fallypride** analogs **1a** to **4** in hand (Fig. 5), we next introduced various SBFs. First, bitopic ligands with aryl, heteroaryl, or allyl as an SBF were prepared as outlined in Scheme 4. A solution of compound **1a**, NaH, and (bromomethyl)benzene or 1-(bromomethyl)-4-fluorobenzene in THF was heated to 60 °C overnight. This method (a) was used to prepare **29a**,**b**. An alternative alkylation method (b) using 50% NaOH as the base, in the presence of NH_4HSO_4 was successfully used for the synthesis of **29c**–**f**.

We next explored aliphatic primary alcohol, primary amine, and amide as an SBF. The synthetic routes for preparing these bitopic ligands were shown in Scheme 5. Alkylation of the secondary hydroxyl with corresponding alkylation reagents afforded the **30a**,**b** in moderate yield. For **30a**,**b**, TBAF removed the TBS of each to give **32a**,**b**. The primary hydroxyl of the two compounds was converted to the mesylate group to afford derivatives **22** and **23** and subsequently treated with NaN_3 to generate **34a**,**b**. The two azido compounds were treated with the Staudinger reaction and afforded the bitopic ligands **35a**,**b** with a primary amine as an SBF. Whereas azide **26** was obtained directly via treating **31** with NaN_3 in DMF at 60 °C. This azide was converted to the primary amine **35c** using the Staudinger reaction. **35b** was reacted with 4-(thiophen-3-yl)benzoic acid using HBTU as the coupling reagent in DMF to give **36** which has an amide group in the SBF.

We next used two methods to prepare the bitopic ligands containing a sterically bulky carbamate as the SBF (Scheme 6). For the first method, **1a** was reacted with commercially available isocyanates and afforded **37a**–**e** and **37i** (Scheme 6). The second method used a modified Curtis reaction which involved heating a solution of compound **1a**, corresponding carboxyl acid, trimethylsilyl azide ($TMSN_3$), and propanephosphonic acid anhydride (T3P) in THF to 70 °C for 16 h to give **37f–h**, **37j–l**.

To study the chirality of OBFs, both the diastereomers and



[^{11}C]Raclopride [^{18}F]Fallypride [^{11}C]-(+)-PHNO

Fig. 1. Representative radiotracers were used for the PET imaging $D_{2/3}R$ study.

enantiomers of **35b** and **37l** were prepared as shown in Scheme 7. A similar method for the preparation of **35b** was also used for the synthesis of **38**, **40,** and **42.** The method shown in the preparation of **37l** was also used to prepare bitopic ligands **39**, **41**, and **43**.

Finally, bitopic ligands were made from **3** and **4** for the evaluation of the SBF position and the alkyl groups on the N-substituted pyrrolidine ring (Fig. 6). The routes for the synthesis of the compounds can be found in SI Scheme S1,2).

SAR study of bitopic ligands based on Fallypride towards D_2R and D_3R. The **Fallypride** bitopic ligands were evaluated by measuring the affinity for D_3R and D_2R. The results of **29a**–**f** are listed in Table 1. Most of the bitopic ligands have high affinities for both D_2R and D_3R. The analogs with a benzyl or substituted benzyl as an SBF (**29a**–**c**) had good affinity for both D_2R and D_3R. An exception was the CF_3 analog (**29b**,**c** vs **a**) which had a lower affinity for both D_2R and D_3R. Compounds with pyridine as an SBF (**29d**,**e**) had a slightly improved affinity over the benzyl analog **29a**. These results suggest that the SBFs including nitrogen atoms could improve affinity for D_2R and D_3R. When a carbon-carbon double bond was introduced as an SBF (**29f**), the affinity of D_2R (K_i = 7.4 ± 0.1 nM) and D_3R (K_i = 2.6 ± 0.2 nM) were slightly increased, leading to a modest selectivity for D_3R over D_2R (D_2R/D_3R = 2.8). Shifting the SBFs to the 3-position on the pyrrolidine **44** (D_2R: K_i = 7.3 ± 0.3 nM; D_3R: K_i = 2.9 ± 0.1 nM), the affinity for both $D_{2/3}R$ and the D_3R versus D_2R selectivity were slightly increased (vs **29a**).

Next, the affinity for D_2R and D_3R was measured for ligands with long-chain primary alcohol, primary amine, and amide as SBFs (Table 2). Most of the compounds in this series had a similar affinity for both D_2R and D_3R. The exception was **35a**, which had an 11-fold higher affinity for D_3R versus D_2R and a D_3R affinity of ~10 nM.

We next explored the steric bulk of the SBF by designing compounds **37a**–**l** with a carbamate linkage with the pyrrolidine ring. The results of the binding assays are shown in Table 3. Most of the compounds had a D_3R affinity ranging from 4 to 15 nM and low selectivity versus D_2R. An exception was **37h**, which had a higher affinity for D_2R versus D_3R. In our previous studies, ligands having 4-(thiophen-3-yl)phenyl as an SBF had excellent D_3R versus D_2R selectivity. It was surprising to see that incorporating this strategy into this scaffold **37l** resulted in only a slight increase in D_3R versus D_2R selectivity (D_2R/D_3R = 3.7).

We next evaluated the chirality of the OBFs using bitopic ligands listed in Scheme 7 which includes both the enantiomers and diastereomers (Table 4). The *cis* substituted bitopic ligand **40**, has higher affinity for D_3R (K_i: 2.5 ± 0.1 nM) versus its affinity for D_2R (K_i = 16.9 ± 0.9 nM) and a reasonable selectivity for D_3R versus D_2R (D_2R/D_3R = 6.8). A modest D_3R versus D_2R selectivity was also observed using the *cis*-substituted bitopic ligand **41**. However, the other isomers in this series had a low affinity for both D_2R and D_3R. These results indicate that the chiral center of the 2-position in these analogs plays a critical role in determining the affinity for D_2R and D_3R.

The ability of a compound to compete with dopamine for binding to the D_3R is critical for determining whether a PET radioligand can be used for imaging D_3R *in vivo* [30]. Therefore, a panel of the compounds described above were evaluated by using a β-arrestin recruitment assay to assess their ability to compete with dopamine for binding to the D_3R (Table 5). The results of this study showed that **35a** (IC_{50} = 11.0 ± 1.9 nM), **35b** (IC_{50} = 1.3 ± 0.2 nM), **40** (IC_{50} = 11.7 ± 4.5 nM), **46** (IC_{50} = 3.3 ± 1.5 nM) and **36** (IC_{50} = 8.1 ± 1.1 nM) had the highest potency in inhibiting dopamine in the β-arrestin recruitment assay. Although the potency in the β-arrestin recruitment assay generally matched the affinity of the compounds in the D_3R binding assays, some exceptions were noted. For example, **37k, 37l,** and **47** had a much lower potency in the β-arrestin competition assay than what was predicted by the affinity in the radioligand binding assay for the D_3R. The reason for this is not clear.

Molecular Modeling: Fig. 7 shows the representative binding pose from the molecular dynamics simulation (MDS) production runs for 5 compounds with structural diversity in the SBFs: **29c**, **35a**, **35c**, **37d**, and **37l**. Hydrogen bonds formed between ASP110 and the protonated nitrogen in the pyrrolidine ring and π-staking interactions between the benzene ring of ligands and PHE345 or HIS349 in the OBS were observed in all 5 compounds. A salt bridge formed between GLU90 in the secondary binding site and **35a** and **35c** was also observed in the MDS for each compound (Fig. 7B and C).

To further investigate the relationship between D_3R binding affinity and the points of contact in the binding sites, the interactions between 23 selected ligands and the protein residues in both the OBS and SBS were calculated. The distance between ASP110 in the D_3R and the protonated nitrogen in the pyrrolidine ring of the 23 ligands is in the range of 3.16–3.84 Å (Table S2). The protonated nitrogen in the pyrrolidine ring of most of the compounds are more distant to the ASP110 than **Fallypride**, indicating that these compounds have weaker interactions with ASP110 as compared to **Fallypride**. The superposition of the representative binding poses from the MDS of each ligand to **Fallypride** revealed that the position of each compound was shifted toward the SBS in the binding pocket (Fig. 7F). The weaker interaction with the ASP110 and the shift in the binding pose resulted in a reduction in affinity for the D_3R for these compounds.

The summary of the frequency of contacts for different interactions of each compound is shown in Fig. 8. All 23 compounds formed a stable hydrogen bound or salt bridge (frequency of contact >0.8) with the key interaction residue, ASP110. The average frequency of hydrophobic interactions for the residues in the OBS including ASP110, CYS114, SER192, and SER196 for the 23 compounds is slightly lower than **Fallypride** (frequency of contact = 0.78 ± 0.04; range: 0.63–0.83 for all the 23 compounds vs. 0.83 for **Fallypride**). Compounds having a primary amine in the SBF including **46**, **35c**, **38**, **35b**, and **35a** display a good probability to form a salt bridge with GLU90 (frequency of contact = 0.28–0.36) in the SBS. Other compounds formed hydrophobic interactions with GLU90. A moderate probability of forming a hydrogen bound with THR369 (frequency of contact = 0.45–0.72) was also observed in the compounds that have a larger functional group for the SBF, such as **37d**, **37f**, **37a**, **37k**, and **39**. These data indicate that introducing an SBF to **Fallypride** increased the probability to interact

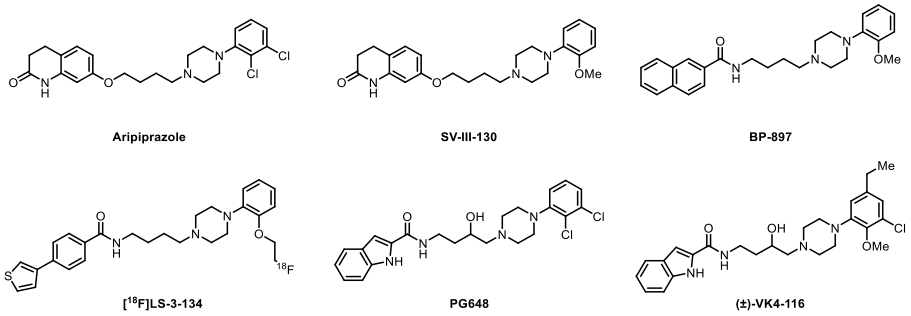


Fig. 2. Representative *N*-phenylpiperazine benzamides favor D_2R or D_3R.

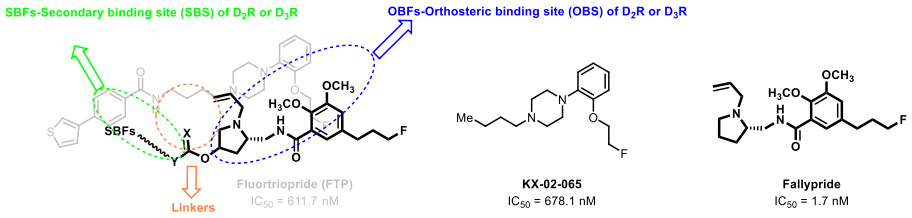


Fig. 3. Comparison of **Fluortriopride** (**FTP**, **LS-3-134**) and **Fallypride** in the β-arrestin assay.

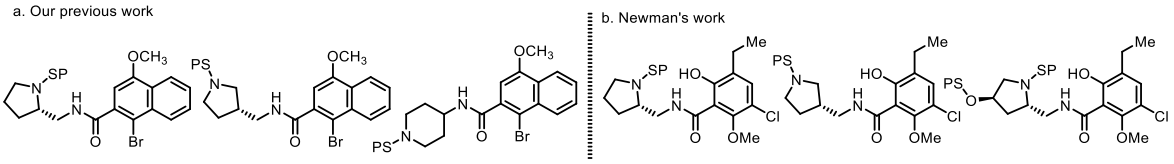


Fig. 4. Our previous work and Newman's work.

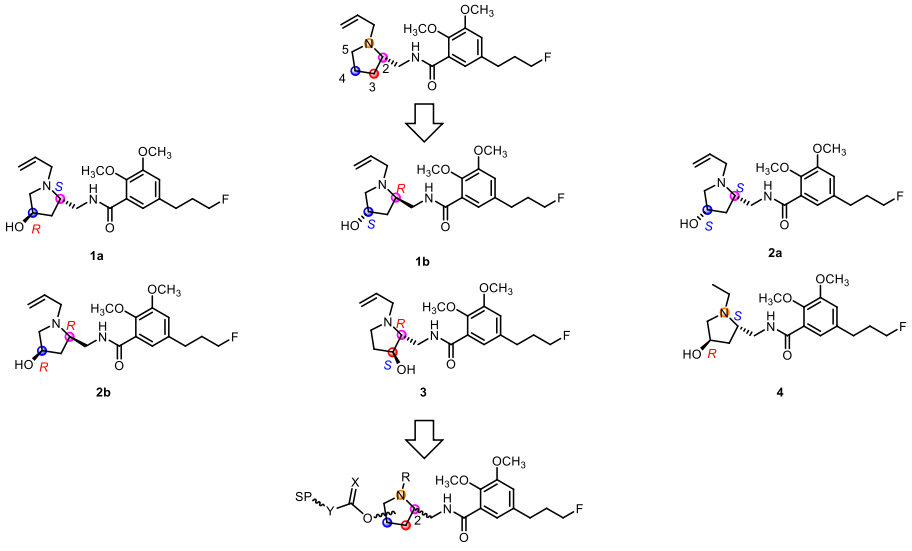
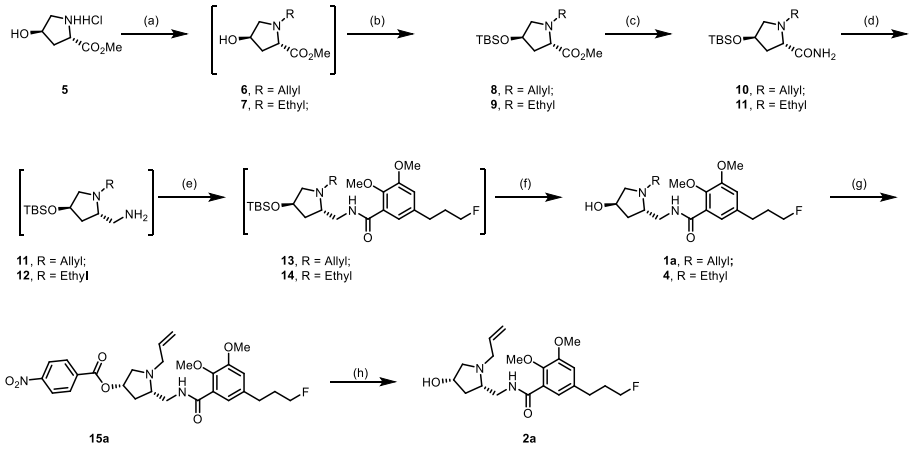


Fig. 5. Six basic **Fallypride** analogs for preparing the bitopic ligands.

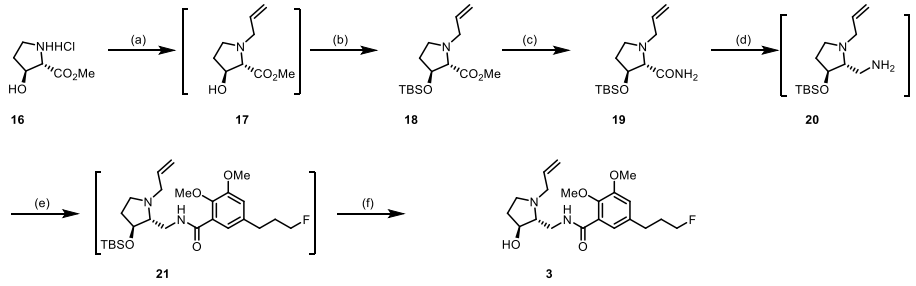


Scheme 1. The route for the synthesis of **1a**, **2a**, and **4**. [a]

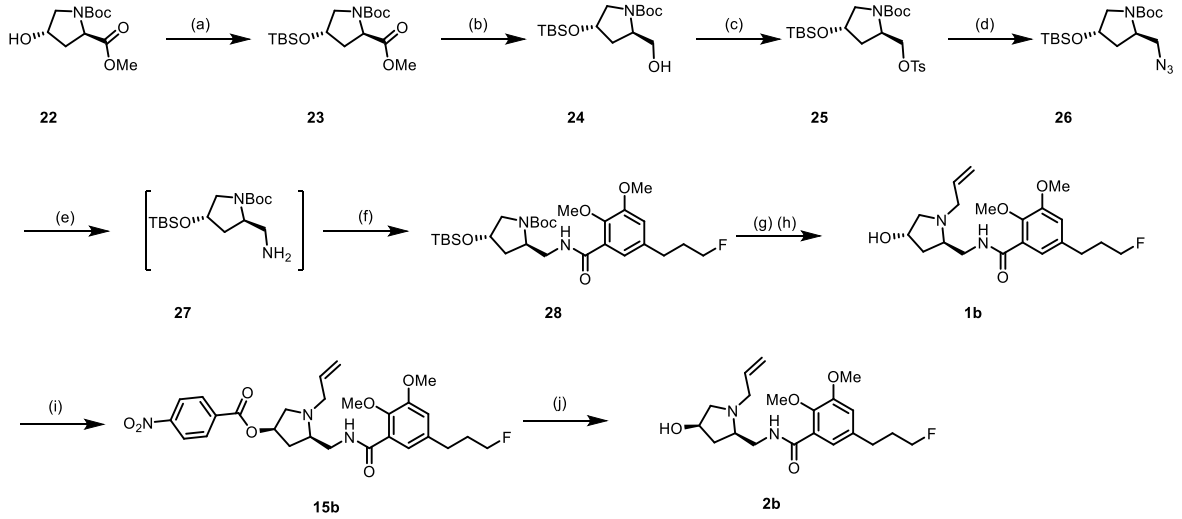
a. Reagents and conditions: (a) Allyl bromide or ethyl iodide, Et_3N, CH_2Cl_2, 40 °C, 24 h; (b) TBSCl, imidazole, THF, 0 °C to rt., 6 h; (c) KI (catalytic amount),7 N NH_3 in MeOH, 40 °C, 48 h; (d) $LiAlH_4$, THF, 0–40 °C, 4 h; (e) HBTU, DIPEA, 5-(3-fluoropropyl)-2,3-dimethoxybenzoic acid, DMF, rt., overnight; (f) TBAF, THF, 0 °C to rt., 4 h; (g) DIAD, PPh_3, 4-nitrobenzoic acid, THF, 0 °C to rt., overnight; (h) NaN_3, MeOH, 45 °C, overnight.

with SBS, but reduced the frequency of contact in the OBS, thereby resulting in a decrease in binding affinity for D_3R.

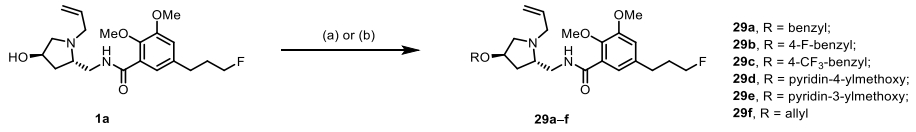
Conclusion: We have developed a series of bitopic ligands based on the structure of **Fallypride**. Many of the compounds were found to have a high affinity for both D_2R and D_3R. The factors related to the affinity and selectivity for $D_{2/3}R$ were investigated. Molecular modeling studies



Scheme 2. The route for the synthesis of **3**.
a. Reagents and conditions: (a) Allyl bromide, Et_3N, CH_2Cl_2, 40 °C, 24 h; (b) TBSCl, imidazole, THF, 0 °C to rt., 4 h; (c) KI (catalytic amount), 7 N NH_3 in MeOH, 40 °C, 48 h; (d) $LiAlH_4$, THF, 0–40 °C, 4 h; (e) HBTU, DIPEA, 5-(3-fluoropropyl)-2,3-dimethoxybenzoic acid, DMF, rt., overnight; (f) TBAF, THF, 0 °C to rt., 4 h.



Scheme 3. The route for the Synthesis of **1b** and **2b**.
a. Reagents and conditions: (a) TBSCl, imidazole, THF0 °C to rt., 4 h; (b)$NaBH_4$, LiCl, EtOH/THF, 0 °C to rt., overnight; (c) TsCl, pyridine, CH_2Cl_2, 0 °C to rt., overnight; (d) NaN_3, 70 °C, overnight; (e) H_2, Pd/C, MeOH, rt., overnight; (f) HBTUDIPEA, 5-(3-fluoropropyl)-2,3-dimethoxybenzoic acid, DMF, rt., overnight; (g) HCl, THF, rt, 4 h; (h) Allyl bromide, Cs_2CO_3, MeCN,rt., 24 h; (i) DIAD, PPh_3, 4-nitrobenzoic acid, THF, 0 °C to rt., overnight; (j) NaN_3, MeOH, 45 °C, overnight.



Scheme 4. The methods for the synthesis of **29c–f**.[a]
a. Reagents and conditions: (a) RBr, NaH, THF, 60 °C, overnight; (b) RBr, (n-Bu)$_4$N(HSO_4), 50% NaOH in H_2O, toluene, rt., 48 h.

revealed substitution of the pyrrolidine ring of **Fallypride** increased the distance and frequency of interaction with ASP110, which likely explains the reduction in D_3R affinity since this interaction is critical for affinity for the D_3R. The compound having the proper affinity and selectivity for D_3R versus D_2R was **35a**, which has a 2-aminoethoxy substituent in the SBS. This observation suggests that small substituents in the SBS may improve the D_3R-selectivity of **Fallypride** and structurally-related benzamides.

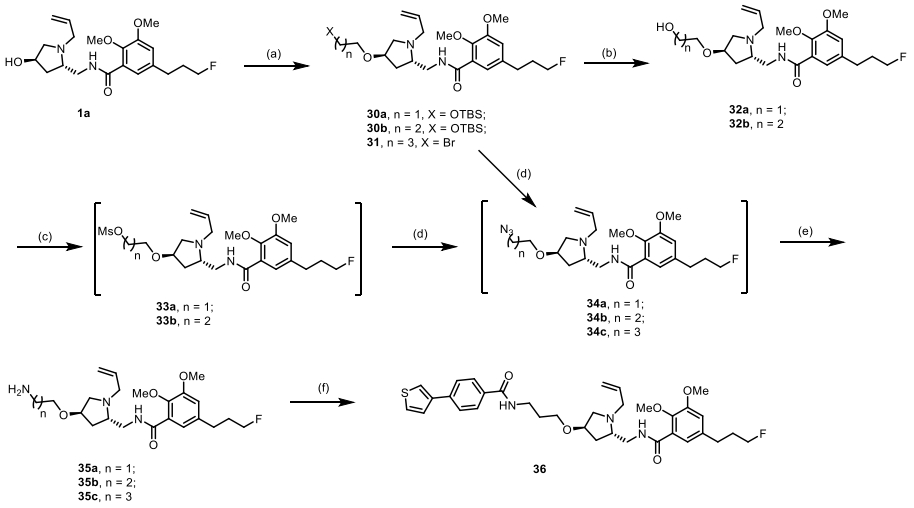
In summary, we conducted a comprehensive study to reveal the factors that could benefit the development of a D_3R-selective bitopic ligand for PET imaging based on **Fallypride**. **35a** is the best-in-series compound since it has a modest selectivity for D_3R versus D_2R and high potency in the β-arrestin competition assay, suggesting that it could compete with endogenous dopamine for binding to the D_3R. We are currently using this compound as a secondary lead for further structure-activity relationship studies of **Fallypride** analogs.

3. Experimental methods

The starting materials and anhydrous solvents except specially mentioned were purchased from Sigma-Aldrich, TCI America, and Alfa Aesar, and Ambeed were used without further purification. 5-(3-fluoropropyl)-2,3-dimethoxybenzoic acid was prepared with a reported method [40]. The NMR spectra were taken on a Bruker DMX 400 MHz. Chemical shifts (δ) in the NMR spectra (^1H and ^{13}C) were referenced by assigning the residual solvent peaks. Purification of organic compounds was carried out on a Biotage Isolera One with a dual-wavelength UV–vis detector (silica gel: 230−400 mesh, 60 Å). Compound structures and identity were confirmed by ^1H and ^{13}C NMR and mass spectrometry.

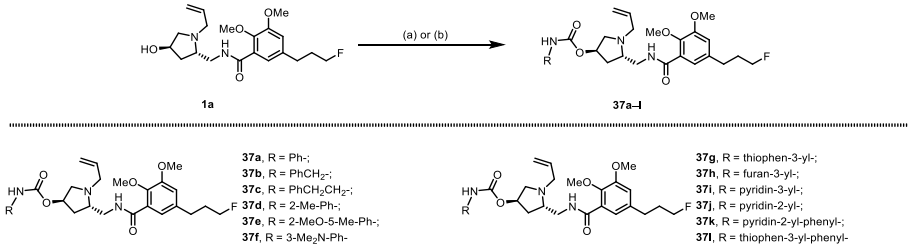
3.1. General procedure for the synthesis of 1a, 4 and 2a

N-alkylation: To a solution of **5** or **16** (2.0 g, 11.0 mmol) and Et_3N

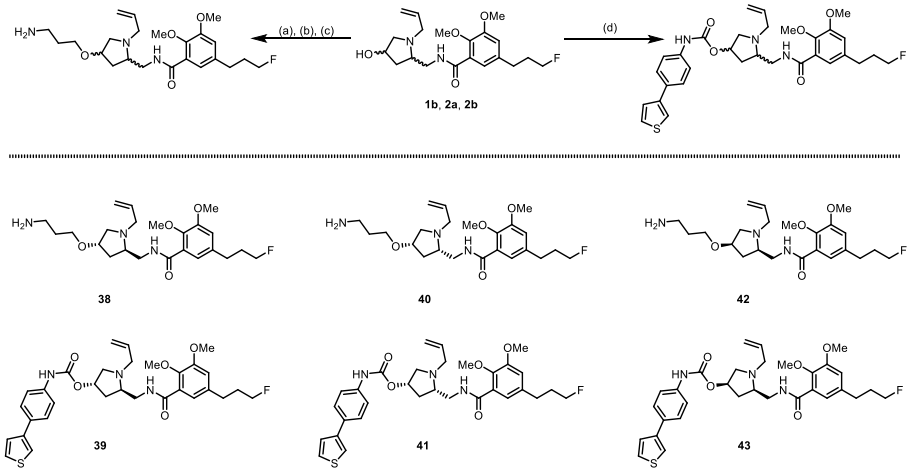


Scheme 5. The routes for the synthesis of bitopic ligands with long linkers. [a]

a. Reagents and conditions: (a) $Br(CH_2)_nOTBS$, (**17**), n = 1; (**18**), n = 2 and (**19**) n = 3, $Br(CH_2)_4Br$, (n-Bu)$_4N(HSO_4)$, 50% NaOH in H_2O, toluene, rt., 48 h; (b) TBAF, THF, 0 °C to rt., 4 h; (c) MsCl, Et_3N, CH_2Cl_2, 0 °C to rt., 6 h; (d) NaN_3, DMF, 60 °C, 6 h; (e) Ph_3P, H_2O, THF, 60 °C, 6 h; (f) HBTU, DIPEA, 4-(thiophen-3-yl)benzoic acid, DMF, rt., overnight.



Scheme 6. Synthesis of **37a–l**. Reagents and conditions: (a) RNCO, THF, 60 °C 16 h; (b) RCO_2H, T_3P, $TMSN_3$, THF, 70 °C 16 h.



Scheme 7. The routes for the synthesis of diastereomers and enantiomers. [a]

a. Reagents and conditions: (a) 2-(2-bromoethyl)-1,3-dioxolane, (n-Bu)$_4N(HSO_4)$, 50% NaOH in H_2O, toluene, rt., 48 h; (b) 4 N HCl, THF, rt., 4 h; (c) NH_2OH, Zn, 6 N HCl, 0 °C to rt., 3 h; (d) 4-(thiophen-3-yl)benzoic acid, T_3P, $TMSN_3$, THF, 70 °C, 16 h.

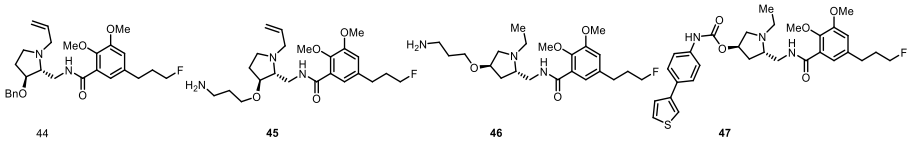
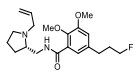
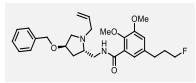
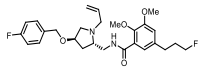
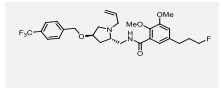
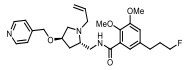
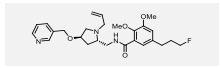
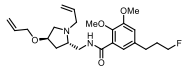
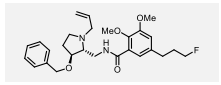


Fig. 6. Bitopic ligands for the evaluation of SBF position and the N-substituted on pyrrolidine.

Table 1
The binding affinity of **29a–f** and **44**[a] for D_2R and D_3R.

ligands	Structure	$K_i \pm$ SEM (nM)[c]		D_2R/D_3R ratio[f]	CLogP[g]
		D_2R[d]	D_3R[e]		
Fallypride[b]		0.02	0.19	0.1	3.18
29a		12.7 ± 3.1	9.5 ± 2.9	1.3	4.53
29b		9.5 ± 1.1	4.7 ± 0.5	2.0	4.67
29c		32.1 ± 7.3	23.7 ± 6.2	1.4	5.41
29d		7.0 ± 0.9	4.0 ± 0.5	1.7	3.03
29e		2.5 ± 0.1	1.7 ± 0.2	1.5	3.03
29f		7.4 ± 0.1	2.6 ± 0.2	2.8	3.41
44		7.3 ± 0.3	2.9 ± 0.1	2.5	4.53

[a] All compounds were tested as HCl salts
[b] Data from reference 39
[c] K_i values were determined by at least three experiments
[d] K_i values for D_2R were measured using human D_2R expressed in HEK cells with [^{125}I]IABN as the radioligand
[e] K_i values for D_3R were measured using human D_3R expressed in HEK cells with [^{125}I]IABN as the radioligand
[f] (K_i for D_2R)/(K_i for D_3R)
[g] Calculated using ChemDraw Professional 15.1.

(1.6 mL, 11.3 mmol) in dry CH_2Cl_2 (50 mL) was added allyl bromide (1.6 g, 13.2 mmol) or iodoethane (13.2 mmol) at room temperature. The mixture was stirred at room temperature for 24 h. The solvents were removed under vacuum and gave out a crude product **6, 7** or **17.**

TBS protection of hydroxyl: A solution of the crude product above (4.9 mmol) and imidazole (733 mg, 10.8 mmol) in THF (15 mL) was cooled to 0 °C under N_2. TBSCl (1.5 g, 9.8 mmol) was added to the solution at 0 °C. The mixture was warmed to room temperature and stirred for 6 h. The reaction was quenched with saturated NH_4Cl and extracted with EtOAc (4 × 10 mL), the organic extracts were dried with Na_2SO_4, filtered, and evaporated to afford the crude product which was purified by flash silica chromatography (EtOAc/Hexanes = 4: 1) to afford **8**, **9** or **18.**

Methyl (2S,4R)-1-allyl-4-((tert-butyldimethylsilyl)oxy)pyrrolidine-2-carboxylate ***(8)***. 1.4 g light yellow oil, 74% yield for 2 steps. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 5.97–5.82 (m, 1H), 5.20 (d, J = 16.7 Hz, 1H), 5.13 (d, J = 9.7 Hz, 1H), 4.44–4.39 (m, 1H), 3.72 (s, 3H), 3.48 (q, J = 8.2 Hz, 1H), 3.41–3.34 (m, 2H), 3.22–3.15 (m, 1H), 2.44–2.38 (m, 1H), 2.20–2.12 (m, 1H), 2.07–2.00 (m, 1H), 0.87 (s, 9H), 0.04 (s, 6H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 174.2, 134.6, 118.2, 70.6, 64.3, 61.9, 58.4, 52.2, 39.8, 25.9, 18.2, −4.7. **HRMS** (ESI) calculated for $C_{15}H_{30}NO_3Si^+$([M + H^+]) 300.1989, found: 300.2003.

Methyl (2S,4R)-4-((tert-butyldimethylsilyl)oxy)-1-ethylpyrrolidine-2-carboxylate ***(9)***. 750 mg light yellow oil, 47% yield for 2 steps. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 4.44–4.38 (m, 1H), 3.70 (s, 3H), 3.43–3.32 (m, 2H), 2.78–2.69 (m, 1H), 2.52–2.43 (m, 1H), 2.28 (dd, J = 9.7, 5.2 Hz, 1H), 2.16–2.09 (m, 1H), 2.02–1.96 (m, 1H), 1.06 (t, J = 7.2 Hz, 3H), 0.85 (s, 9H), 0.02 (d, 6H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 174.5, 70.5, 64.8, 61.6, 52.0, 49.4, 39.7, 25.9, 18.1, 13.3, −4.8. **HRMS** (ESI) calculated for $C_{14}H_{30}NO_3Si^+$ ([M + H^+]) 288.1989, found: 288.2005.

Methyl (2S,3S)-1-allyl-3-((tert-butyldimethylsilyl)oxy)pyrrolidine-2-carboxylate ***(18)***. 1.4 g light yellow oil, 74% yield for 2 steps. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 5.91–5.81 (m, 1H), 5.15 (dd, J = 17.1, 1.7 Hz, 1H), 5.07 (dd, J = 10.1, 1.0 Hz, 1H), 4.38–4.35 (m, 1H), 3.69 (s, 3H), 3.31–3.04 (m, 4H), 2.66–2.59 (m, 1H), 2.12–2.03 (m, 1H), 1.69–1.63 (m, 1H), 0.85 (s, 9H), 0.02 (s, 3H), 0.01 (s, 3H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 173.6, 135.0, 117.9, 76.6, 74.8, 58.3, 52.0, 51.8, 34.5, 25.8, 18.1, −4.9, −4.9. **HRMS** (ESI) calculated for $C_{15}H_{30}NO_3Si^+$([M + H^+]) 300.1989, found: 300.1997.

3.2. Synthesis of 10, 11 and 19

To a solution of **8, 9 or 18** (1.4 mmol) in MeOH (7 N NH_3, 20 mL) was added potassium iodide (30 mg) at room temperature. The mixture was heated to 40 °C for 48 h. The solvents were removed under vacuum, the residue was purified by flash silica chromatography (EtOAc/Hexanes/CH_2Cl_2 = 1: 1: 0.1) to afford **10, 11 or 19**.

(2S,4R)-1-allyl-4-((tert-butyldimethylsilyl)oxy)pyrrolidine-2-carboxamide ***(10)***. 347 mg brown oil, 85% yield. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.18 (s, 1H), 6.04 (s, 1H), 5.88–5.78 (m, 1H), 5.18 (d, J = 16.5 Hz, 1H), 5.11 (d, J = 10.2 Hz, 1H), 4.32–4.27 (m, 1H), 3.40–3.27 (m, 2H), 3.25 (dd, J = 10.4, 4.9 Hz, 1H), 3.14 (dd, J = 13.9, 6.9 Hz, 1H), 2.44 (dd, J = 10.4, 4.6 Hz, 1H), 2.19–2.12 (m, 1H), 1.99–1.92 (m, 1H), 0.85 (s, 9H), 0.03 (s, 3H), 0.02 (s, 3H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 177.9, 135.2, 117.6, 71.4, 66.2, 61.6, 59.2, 40.5, 25.9, 18.1, −4.7, −4.8; **HRMS** (ESI) calculated for $C_{14}H_{29}N_2O_2Si^+$ ([M + H^+]) 285.1998, found: 285.1984.

O-((2S,4R)-4-((tert-butyldimethylsilyl)oxy)-1-ethylpyrrolidine-2-carbonyl)hydroxylamine ***(11)***. 74% for 2 steps 256 mg brown oil, 61% yield. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.18 (s, 1H), 6.12 (s, 1H), 4.31–4.26 (m, 1H), 3.30–3.24 (m, 2H), 2.74–2.66 (m, 1H), 2.56–2.48 (m, 1H), 2.36 (dd, J = 9.9, 5.3 Hz, 1H), 2.15–2.08 (m, 1H), 1.98–1.92 (m, 1H), 1.04 (t, J = 7.2 Hz, 3H), 0.85 (s, 9H), 0.02 (s, 6H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 178.4, 71.1, 66.5, 61.3, 50.5, 40.4, 25.9, 18.1, 14.1, −4.7, −4.8. **HRMS** (ESI) calculated for $C_{13}H_{29}N_2O_2Si^+$ ([M + H^+]) 273.1998, found: 273.1990.

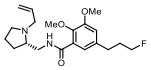
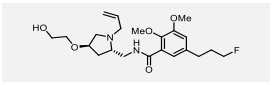
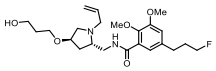
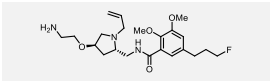
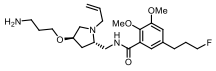
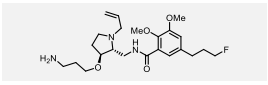
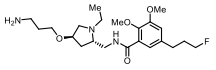
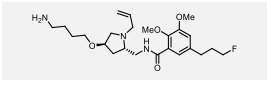
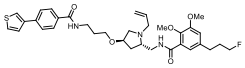
O-((2S,3S)-1-allyl-3-((tert-butyldimethylsilyl)oxy)pyrrolidine-2-carbonyl)hydroxylamine ***(19)***. 200 mg brown oil, 50% yield. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.36 (s, 1H), 6.05 (s, 1H), 5.90–5.85 (m, 1H), 5.19 (d, J = 17.1 Hz, 1H), 5.11 (d, J = 10.2 Hz, 1H), 4.36 (d, J = 4.3 Hz, 1H), 3.41–3.18 (m, 2H), 3.10 (s, 2H), 2.80–2.74 (m, 1H), 1.84–1.67 (m, 2H), 0.88 (d, J = 1.0 Hz, 9H), 0.12 (s, 3H), 0.07 (s, 3H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 175.9, 135.2, 117.7, 77.0, 76.3, 59.0, 51.8, 34.7, 25.9, 18.0, −4.6, −4.8. **HRMS** (ESI) calculated for $C_{14}H_{29}N_2O_2Si^+$ ([M + H^+]) 285.1993, found: 285.1990.

3.3. Reduction of carboxamid compounds for the synthesis of 1a, 4 and 2a

A solution of **10, 11 or 19** (1.2 mmol) in THF (4 mL) was cooled to 0 °C under N_2. $LiAlH_4$ (1 M in THF, 4.6 mL, 4.6 mmol) was added dropwise to the solution. The mixture was heated to 40 °C for 4 h. The mixture was cooled to 0 °C. Saturated NH_4Cl was added dropwise to quench the reaction. The solid was filtered through a Celite pad and the aqueous phase was extracted with CH_2Cl_2 (3 × 20 mL), the organic extracts were combined, dried with Na_2SO_4, filtered, and evaporated to afford crude compound **11, 12 or 20** as a colorless oil.

A solution of 5-(3-fluoropropyl)-2,3-dimethoxybenzoic acid (324 mg, 1.2 mmol), HBTU (455 mg, 1.2 mmol), and DIPEA (155 mg, 1.2 mmol) in DMF (4 mL) was stirred at room temperature for 30 min under N_2. A solution of the crude product above (1.0 mmol) in DMF (2 mL) was added to the mixture. The reaction was stirred overnight and diluted with H_2O. The mixture was extracted with EtOAc (3 × 25 mL), the organic extracts were combined, washed with saturated NaCl and dried with Na_2SO_4, filtered, and evaporated to afford crude product **13**, **14** or **21** for the next step without further purification.

Table 2
The binding affinity of **32a-46**[a] for D_2R and D_3R.

Compounds	Structure	$K_i \pm$ SEM (nM)[c]		D_2R/D_3R ratio[f]	CLogP[g]
		D_2R[d]	D_3R[e]		
Fallypride[b]		0.02	0.19	0.1	3.18
32a		8.4 ± 0.3	2.4 ± 0.1	3.5	2.08
32b		14.2 ± 3.9	7.3 ± 2.2	1.9	2.40
35a		107.0 ± 21.6	9.5 ± 0.7	11.3	2.15
35b		18.6 ± 4.1	8.4 ± 2.4	2.2	2.48
45		21.4 ± 0.6	6.2 ± 0.8	3.5	2.48
46		2.9 ± 0.2	1.4 ± 0.1	2.1	2.33
35c		2.2 ± 0.01	1.5 ± 0.1	1.4	2.34
36		13.4 ± 2.8	4.2 ± 0.8	3.2	5.63

[a] All compounds were tested as HCl salts.
[b] Data from reference 39.
[c] K_i values were determined by at least three experiments.
[d] K_i values for D_2R were measured using human D_2R expressed in HEK cells with [^{125}I]IABN as the radioligand.
[e] K_i values for D_3R were measured using human D_3R expressed in HEK cells with [^{125}I]IABN as the radioligand.
[f] (K_i for D_2R)/(K_i for D_3R).
[g] Calculated using ChemDraw Professional 15.1.

A solution of the crude product above in THF (1 mL) was cooled to 0 °C under N_2. TBAF (1.3 mL, 1 M in THF) was added to the solution. The mixture was warmed to room temperature and stirred for 4 h. The reaction was quenched with saturated NH_4Cl, and extracted with CH_2Cl_2 (3 × 15 mL), the organic extracts were combined, dried with Na_2SO4, filtered, and evaporated to afford the crude product which was purified by flash silica chromatography (10% MeOH/CH_2Cl_2) to afford **1a**, **4** or **3** as a light yellow oil.

N-(((2S,4R)-1-allyl-4-hydroxypyrrolidin-2-yl*)methyl)-5-(3-fluoropropyl)-2,3-dimethoxybenzamide (***1a***)*. 346 mg light yellow oil, 76% yield for 3 steps. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.39 (d, J = 5.7 Hz, 1H), 7.48 (s, 1H), 6.84 (d, J = 2.2 Hz, 1H), 5.89–5.79 (m, 1H), 5.17 (d, J = 17.1 Hz, 1H), 5.07 (d, J = 10.1 Hz, 1H), 4.47 (t, J = 6.0 Hz, 1H), 4.35 (t, J = 6.0 Hz, 1H), 4.31 (s, 1H), 3.85 (s, 3H), 3.82 (s, 3H), 3.74 (dd, J = 13.9, 7.3 Hz, 1H), 3.45 (dd, J = 13.7, 5.4 Hz, 1H), 3.38 (dd, J = 10.4, 5.8 Hz, 1H), 3.30 (d, J = 14.1 Hz, 1H), 3.04 (s, 1H), 2.96 (dd, J = 13.6, 7.5 Hz, 1H), 2.69 (t, J = 7.7 Hz, 2H), 2.33 (dd, J = 10.4, 5.0 Hz, 1H), 2.05–1.77 (m, 4H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 165.6, 152.5, 145.9, 137.5, 135.4, 126.2, 122.2, 117.4, 115.7, 83.0 (d, J = 164.9 Hz), 69.5, 62.1, 61.3, 60.6, 56.7, 56.1, 40.2, 38.6, 31.9 (d, J = 19.7 Hz), 31.2 (d, J = 5.4 Hz). **HRMS** (ESI) calculated for $C_{20}H_{30}FN_2O_4^+$ ([M + H^+]) 381,2190, found: 381.2189.

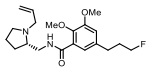
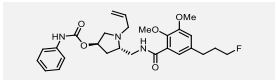
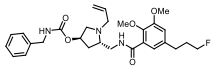
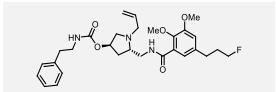
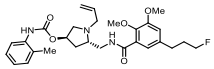
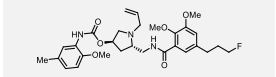
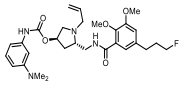
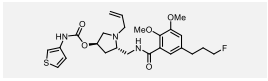
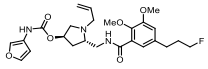
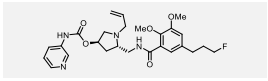
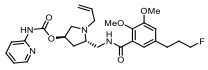
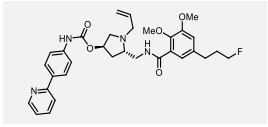
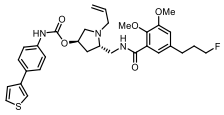
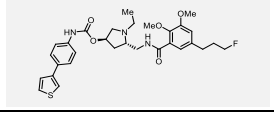
N-(((2S,4R)-1-ethyl-4-hydroxypyrrolidin-2-yl*)methyl)-5-(3-fluoropropyl)-2,3-dimethoxybenzamide (***4***)*. 242 mg light yellow oil, 53% yield for 3 steps. **^{1}H NMR (400 MHz, $CDCl_3$)**). δ 8.39 (dd, J = 7.6, 2.9 Hz, 1H), 7.51 (s, 1H), 6.85 (s, 1H), 4.49 (t, J = 5.9 Hz, 1H), 4.42–4.30 (m, 2H), 3.87 (s, 3H), 3.85 (s, 3H), 3.80–3.74 (m, 1H), 3.50–3.45 (m, 1H), 3.30 (dt, J = 13.8, 2.4 Hz, 1H), 3.00 (s, 1H), 2.92–2.83 (m, 1H), 2.71 (t, J = 7.7 Hz, 2H), 2.37–2.31 (m, 1H), 2.27 (dd, J = 10.2, 5.1 Hz, 1H), 2.07–1.88 (m, 3H), 1.84–1.78 (m, 1H), 1.09 (t, J = 7.2 Hz, 3H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 165.7, 152.5, 145.9, 137.5, 126.4, 122.2, 115.7, 83.1 (d, J = 164.9 Hz), 69.7, 69.6, 61.7, 61.3, 61.0, 56.2, 47.7, 40.3, 38.7, 31.9 (d, J = 19.7 Hz), 31.3 (d, J = 5.3 Hz). **HRMS** (ESI) calculated for $C_{19}H_{30}FN_2O_4^+$ ([M + H^+]) 369.2190, found: 369.2186.

N-(((2R,3S)-1-allyl-3-hydroxypyrrolidin-2-yl*)methyl)-5-(3-fluoropropyl)-2,3-dimethoxybenzamide (***3***)*. 100 mg light yellow oil, 22% yield for 3 steps. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.57 (s, 1H), 7.49 (s, 1H), 6.87 (s, 1H), 6.00–5.86 (m, 1H), 5.27 (d, J = 16.9 Hz, 1H), 5.18 (d, J = 10.2 Hz, 1H), 4.51 (t, J = 5.8 Hz, 1H), 4.39 (t, J = 5.8 Hz, 1H), 4.20 (s, 1H), 3.99–3.80 (m, 8H), 3.59–3.39 (m, 2H), 3.17 (d, 2H), 2.73 (t, J = 6.0 Hz, 2H), 2.13–1.90 (m, 4H), 1.77 (s, 1H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 166.5, 152.6, 146.2, 137.5, 134.0, 126.0, 122.1, 119.1, 116.0, 83.1 (d, J = 165.0 Hz), 74.1, 71.8, 61.6, 57.5, 56.2, 51.7, 39.3, 32.4, 32.0 (d, J = 19.8 Hz), 31.3 (d, J = 5.3 Hz). **HRMS** (ESI) calculated for $C_{20}H_{29}FN_2O_4Na^+$ ([M + Na^+]) 403.2004, found: 403.2026.

3.4. General procedure for the synthesis of 1 b

*1-(tert-Butyl) 2-methyl (2R,4S)-4-((tert-butyldimethylsilyl)oxy)pyrrolidine-1,2-dicarboxylate (**23**)*. A solution of **22** (4.9 g, 20 mmol) and imidazole (2.7 g, 40 mmol) in THF (50 mL) was cooled to 0 °C under N_2. TBSCl (6.0 g, 40 mmol) was added to the solution at 0 °C. The mixture was warmed to room temperature and stirred for 4 h. The reaction was quenched with saturated NH_4Cl, and extracted with Et_2O (3 × 30 mL), the organic extracts were dried with Na_2SO_4, filtered, and evaporated to afford the crude product which was purified by flash silica

Table 3
Binding affinity of **37a–l** and **47**[a] for D_3R and D_2R.

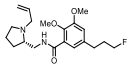
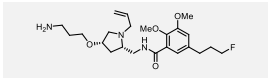
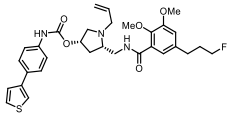
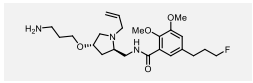
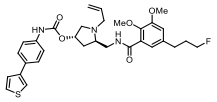
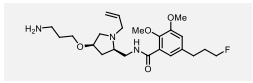
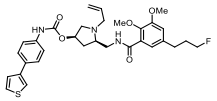
Compounds	Structures	K_i ± SEM (nM)[c]		D_2R/D_3R ratio[f]	CLogP[g]
		D_2R[d]	D_3R[e]		
Fallypride[b]		0.02	0.19	0.1	3.18
37a		29.2 ± 6.5	14.6 ± 3.0	2.0	4.26
37b		10.1 ± 1.9	6.2 ± 0.8	1.6	4.38
37c		30.0 ± 7.8	12.8 ± 3.1	2.3	4.76
37d		12.6 ± 2.3	8.4 ± 1.8	1.5	4.20
37e		18.7 ± 5.7	12.5 ± 3.8	1.5	4.81
37f		14.0 ± 2.6	10.6 ± 1.9	1.3	4.43
37g		7.7 ± 1.3	6.1 ± 1.4	1.3	4.06
37h		8.3 ± 1.6	15.0 ± 3.1	0.6	3.44
37i		8.3 ± 0.5	3.7 ± 0.1	2.2	3.44
37j		10.1 ± 0.7	4.1 ± 0.1	2.4	3.44
37k		22.4 ± 5.2	15.8 ± 4.2	1.4	5.00
37l		28.8 ± 2.7	7.7 ± 1.5	3.7	5.82
47		16.2 ± 1.7	4.3 ± 0.1	3.8	5.67

[a] All compounds were tested as HCl salts.
[b] Data from reference 39.
[c] K_i values were determined by at least three experiments.
[d] K_i values for D_2R were measured using human D_2R expressed in HEK cells with [^{125}I]IABN as the radioligand.
[e] K_i values for D_3R were measured using human D_3R expressed in HEK cells with [^{125}I]IABN as the radioligand.
[f] (K_i for D_2R)/(K_i for D_3R).
[g] Calculated using ChemDraw Professional 15.1.

chromatography (EtOAc/Hexanes = 4: 1) to afford the **23**.7.0 g colorless oil, 97% yield. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 4.43–4.30 (m, 2H), 3.72 (d, 3H), 3.62–3.54 (m, 1H), 3.41–3.29 (m, 1H), 2.20–2.12 (m, 1H), 2.04–1.97 (m, 1H), 1.47–1.37 (m, 9H), 0.88–0.82 (m, 9H), 0.05 (s, 6H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 173.9, 173.7, 154.8, 154.0, 80.2, 80.2, 70.5, 69.9, 58.2, 57.7, 55.0, 54.7, 52.3, 52.1, 40.0, 39.0, 28.5, 28.4, 25.8, 25.8, 18.1, −3.5, −4.7. This compound is a rotamer.

Tert-Butyl (2R,4S)-4-((tert-butyldimethylsilyl)oxy)-2-(hydroxymethyl) pyrrolidine-1-carboxylate ***(24)***. A solution of the product above (3.6 g, 10 mmol) in THF (25 mL) and EtOH (40 mL) was cooled to 0 °C under N_2. $NaBH_4$ (760 g, 20 mmol) and LiCl (860 mg, 20 mmol) were added to the solution at 0 °C. The mixture was warmed to room temperature and stirred overnight. The reaction was quenched with saturated NH_4Cl. The solvent was removed under *vacuum* at room temperature and the residue was extracted with CH_2Cl_2 (3 × 25 mL). The organic extracts were dried with Na_2SO_4, filtered, and evaporated to afford the crude product which was purified by flash silica chromatography (EtOAc/Hexanes = 2: 1) to afford the compound **24**.3.0 g colorless oil, 91% yield. **^{1}H NMR (400**

Table 4
The binding affinity of diastereomers and enantiomers[a] for D_3R and D_2R.

Compounds	Structure	K_i ± SEM (nM)[c]		D_2R/D_3R ratio[f]	CLogP[g]
		D_2R[d]	D_3R[e]		
Fallypride[b]		0.02	0.19	0.1	3.18
38		16.9 ± 0.9	2.5 ± 0.1	6.8	2.48
39		189.0 ± 24.5	28.1 ± 3.4	6.7	5.83
40		797.0 ± 165.0	1313.0 ± 418.0	0.6	2.48
41		9685.0 ± 1842.0	587.0 ± 184.0	16.5	5.83
42		2355.0 ± 171.0	1953.0 ± 665.0	1.2	2.48
43		>41000.0	6785.0 ± 1642.0	>6.0	5.83

[a] All compounds were tested as HCl salts.
[b] Data from reference 39.
[c] K_i values were determined by at least three experiments.
[d] K_i values for D_2R were measured using human D_2R expressed in HEK cells with [^{125}I]IABN as the radioligand.
[e] K_i values for D_3R were measured using human D_3R expressed in HEK cells with [^{125}I]IABN as the radioligand.
[f] (K_i for D_2R)/(K_i for D_3R).
[g] Calculated using ChemDraw Professional 15.1.

MHz, $CDCl_3$) δ 4.27 (s, 1H), 4.12–4.07 (m, 1H), 3.70–3.65 (m, 1H), 3.56–3.50 (m, 1H), 3.45–3.40 (m, 1H), 3.35–3.30 (m, 1H), 1.98–1.89 (m, 1H), 1.63–1.60 (m, 1H), 1.44 (s, 9H), 0.84 (s, 9H), 0.03 (s, 6H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 157.1, 80.3, 69.8, 58.9, 56.0, 38.0, 28.4, 25.7, 17.9, −4.8, −4.9. This compound is a rotamer.

*Tert-Butyl (2R,4S)-4-((tert-butyldimethylsilyl)oxy)-2-((tosyloxy) methyl)pyrrolidine-1-carboxylate (**25**).* A solution of the product above (2.6 g, 7.9 mmol) in CH_2Cl_2 (20 mL) and pyridine (10 mL) was cooled to 0 °C under N_2. TsCl (1.8 g, 9.4 mmol) was added to the solution at 0 °C. The mixture was warmed to room temperature and stirred overnight. The reaction was quenched with saturated NH_4Cl. The mixture was extracted with CH_2Cl_2 (3 × 20 mL). The organic extracts were washed with 1 N HCl and saturated Na_2CO_3, dried with Na_2SO_4, filtered, and evaporated to afford the crude product which was purified by flash silica chromatography (EtOAc/Hexanes = 3: 1) to afford **25**.2.8 g colorless oil, 74% yield. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.75 (d, J = 7.9 Hz, 2H), 7.33 (s, 2H), 4.38–3.96 (m, 4H), 3.42–3.20 (m, 2H), 2.42 (s, 3H), 1.96 (s, 2H), 1.37 (d, 9H), 0.84 (s, 9H), 0.03 (s, 6H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 154.9, 144.8, 132.9, 129.9, 127.9, 79.7, 70.4, 70.0, 69.4, 55.2, 54.8, 38.1, 37.0, 28.3, 25.7, 21.6, 17.9, −4.9. This compound is a rotamer.

*Tert-butyl (2R,4S)-2-(azidomethyl)-4-((tert-butyldimethylsilyl)oxy) pyrrolidine-1-carboxylate (**26**).* A solution of the product above (2.4 g, 5 mmol) and NaN_3 (660 mg, 10 mmol) in DMF (20 mL) was heated to 70 °C overnight under N_2. The reaction was quenched with saturated NH_4Cl. The mixture was extracted with EtOAc (3 × 15 mL). The organic extracts were washed with saturated NaCl, dried with Na_2SO_4, filtered, and evaporated to afford the crude product which was purified by flash silica chromatography (EtOAc/Hexanes = 2: 1) to afford **26**.1.5 g colorless oil, 85% yield. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 4.38–4.34 (m, 1H), 4.14–3.78 (m, 1.5H), 3.65–3.12 (m, 3.5H), 1.93 (s, 2H), 1.45 (s, 9H), 0.85 (s, 9H), 0.05 (s, 6H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 155.0, 79.7, 70.1, 69.6, 55.7, 55.6, 52.8, 39.1, 38.0, 28.4, 25.7, 17.9, −4.9. This compound is a rotamer.

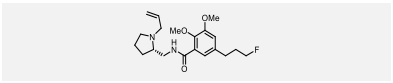
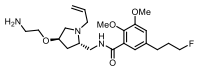
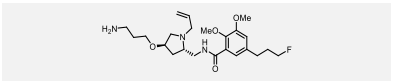
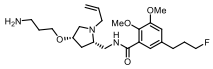
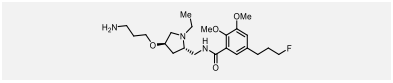
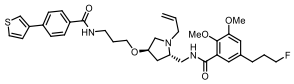
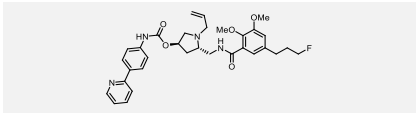
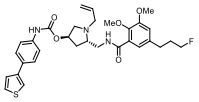
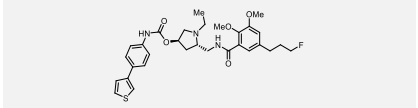
Tert-Butyl (2*R*, 4S)-4-((tert-butyldimethylsilyl)oxy)-2-((5-(3-fluoropropyl)-2,3-dimethoxybenzamido)methyl)pyrrolidine-1-carboxylate (**28**).

A solution of **26** (1.5 g, 4.2 mmol) and Pd/C (10%) (420 mg) in EtOH (15 mL) under N_2. The system was exchanged with H_2 3 times and stirred overnight under an H_2 balloon. The mixture was filtered through a Celite pad. The solvents were removed in *vacuo* and the crude product **27** was used for the next step without further purification.

28 was prepared from **27** and 5-(3-fluoropropyl)-2,3-dimethoxybenzoic acid with the same method to **1**.2.1 g light yellow oil, 91% yield for 2 steps. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.18 (d, 1H), 7.47 (s, 1H), 6.83 (s, 1H), 4.46 (t, J = 5.9 Hz, 1H), 4.34 (t, J = 5.9 Hz, 1H), 4.27 (s, 1H), 4.08 (s, 1H), 3.84 (d, 6H), 3.78–3.27 (m, 4H), 2.69 (t, J = 7.7 Hz, 2H), 2.03–1.84 (m, 4H), 1.43 (s, 9H), 0.80 (s, 9H), −0.05 (m, 6H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 165.7, 165.6, 155.6, 152.5, 145.8, 137.4, 126.5, 123.2, 122.2, 116.6, 115.8, 83.0 (d, J = 165.0 Hz), 79.7, 70.0, 61.3, 56.1, 55.7, 42.2, 38.8, 31.9 (d, J = 19.7 Hz), 31.2 (d, J = 5.3 Hz), 28.5, 25.7, 18.0, −4.8, −4.9. **HRMS** (ESI) calculated for $C_{28}H_{48}FN_2O_6Si^+$ ([M + H^+]) 555.3260, found: 555.3265. This compound is a rotamer.

*N-(((2R,4S)-1-allyl-4-hydroxypyrrolidin-2-yl)methyl)-5-(3-fluoropropyl)-2,3-dimethoxybenzamide (**1b**).* A solution of **28** (1.4 g, 2.5 mmol) in THF (15 mL) was cooled to 0 °C under N_2. To the mixture was added 4 N HCl (4 mL). The mixture was stirred for 4 h and then cooled to 0 °C. 50% Na_2CO_3 (10 mL) solution was added to the mixture. The solvent was removed under *vacuum* at room temperature and the residue was extracted with EtOAc (4 × 25 mL). The organic extracts were

Table 5
Results of the β-arrestin assay.[a]

Compounds	Structure	β-arrestin assay IC_{50} ± SEM(nM)
Fallypride[b]		1.7 ± 0.8
35a		11.0 ± 1.9
35b		1.3 ± 0.2
38		11.7 ± 4.5
46		3.3 ± 1.5
36		8.1 ± 1.1
37k		79.2 ± 16.2
37l		169.0 ± 53.5
47		62.7 ± 22.1

[a] IC_{50} values were determined by at least three experiments, Mean ± standard error of the mean (SEM).
[b] Data from reference 33.

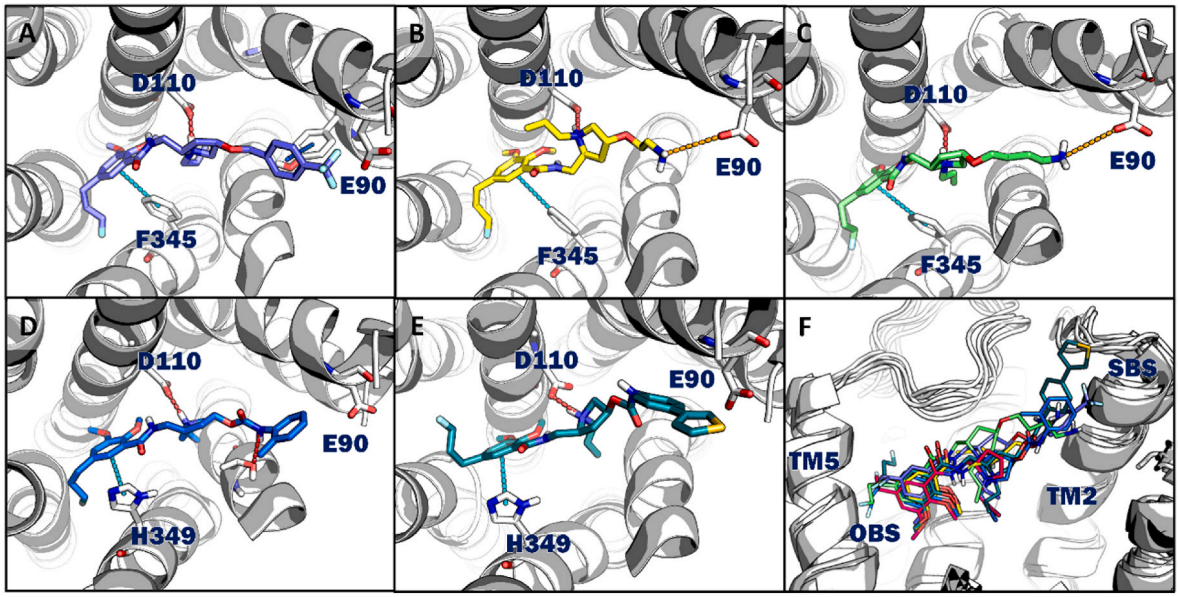


Fig. 7. MDS screenshot of the key interaction residues of D_3R with selective 5 compounds having different secondary binding site substitute. (A) **29c**; (B) **35a**; (C) **35c**; (D) **37d**; (E) **37l**; (F) superposition of 5 compounds with **Fallypride** (red); Red: H-Bond; orange: salt bridge; blue: π-π interaction; cyan: π-staking; OBS: orthosteric binding site; SBS: secondary binding site.

washed with saturated NaCl, dried with Na_2SO_4, filtered, and evaporated to afford the crude product which was used for the next step without further purification.

A solution of the crude product from the last step, Cs_2CO_3 (2.0 g, 6.1 mmol) and ally bromide (460 mg, 3.8 mmol) in CH_3CN (25 mL) was stirred for 24 h under N_2. The mixture was filtered through a Celite pad.

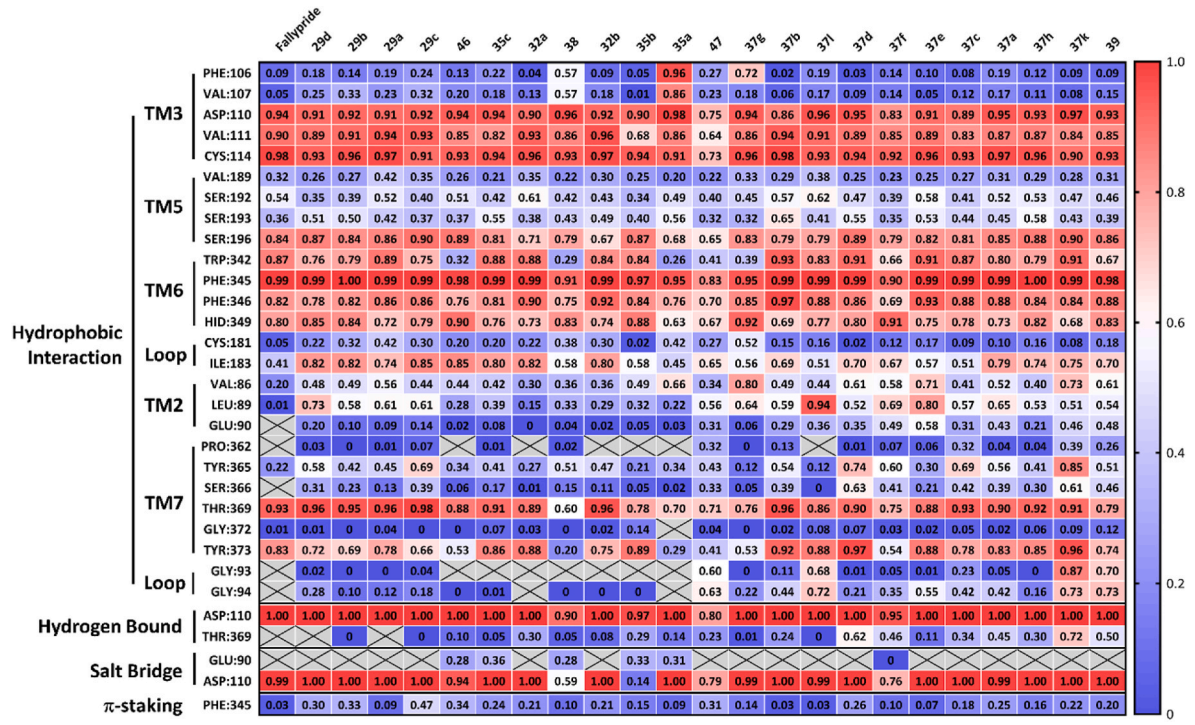


Fig. 8. Summary of frequency of interactions between the residues in the binding pocket of D_3R and ligands.

The solvents were removed in *vacuo* and the crude product was purified by flash silica chromatography (10% MeOH/CH_2Cl_2) to afford 412 mg compound **1b** as a light-yellow oil, 42% yield for 2 steps. The ^{1}H NMR data is the same as **1a**.

3.5. General procedure for the synthesis of 2a and 2 b

(3S,5S)-1-allyl-5-((5-(3-fluoropropyl)-2,3-dimethoxybenzamido) methyl)pyrrolidin-3-yl *4-nitrobenzoate* (**15a**) and *(3R,5R)-1-allyl-5-((5-(3-fluoropropyl)-2,3-dimethoxybenzamido)methyl)pyrrolidin*-3-yl *4-nitrobenzoate* (**15b**). To **1a** or **1b** (760 mg, 2.0 mmol) dissolved in anhydrous THF (8 mL) at 0 °C was added Ph_3P (790 mg, 3.0 mmol) and 4-nitrobenzoic acid (400 mg, 2.4 mmol). DIAD (600 μL, 3.0 mmol) was added dropwise to the reaction mixture and was stirred vigorously for 1 h at 0 °C. The ice bath was removed and stirring was continued overnight at room temperature. The reaction was quenched with 10 mL 5% NH_4Cl and THF was removed under *vacuum*. The crude product was purified by silica gel column chromatography (0–5% CH_3OH/CH_2Cl_2) to yield compound **15a** or **15b**. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.55 (d, J = 7.2 Hz, 1H), 8.07 (s, 4H), 7.50 (d, J = 2.6 Hz, 1H), 6.86 (d, J = 2.1 Hz, 1H), 5.96–5.85 (m, 1H), 5.40 (s, 1H), 5.26 (d, J = 16.8 Hz, 1H), 5.16 (d, J = 10.1 Hz, 1H), 4.48 (t, J = 5.9 Hz, 1H), 4.36 (t, J = 5.9 Hz, 1H), 3.91 (s, 3H), 3.83 (s, 3H), 3.58–3.43 (m, 2H), 3.34 (d, J = 11.5 Hz, 1H), 2.94–2.82 (m, 2H), 2.71 (t, J = 7.7 Hz, 2H), 2.61–2.49 (m, 2H), 2.10–1.87 (m, 4H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 165.8, 164.6, 152.5, 150.4, 145.8, 137.7, 135.7, 135.0, 130.8, 126.5, 123.4, 122.2, 117.9, 115.3, 83.1 (d, J = 165.2 Hz), 74.1, 61.3, 61.1, 60.1, 56.1, 56.0, 39.7, 35.5, 32.0 (d, J = 19.7 Hz), 31.3 (d, J = 5.2 Hz). **HRMS** (ESI) calculated for $C_{27}H_{33}FN_3O_7^+$ ([M + H^+]) 530.2297, found: 530.2297.

N-(((2S,4S)-1-allyl-4-hydroxypyrrolidin-2-yl*)methyl)-5-(3-fluoropropyl)-2,3-dimethoxybenzamide* (**2a**) *and N-(((2R,4R)-1-allyl-4-hydroxypyrrolidin*-2-yl*)methyl)-5-(3-fluoropropyl)-2,3-dimethoxybenzamide* (**2b**). 125 mg light yellow oil, 67% yield. Compound **15** (260 mg, 0.5 mmol) was hydrolyzed by reacting with NaN_3 (96 mg, 1.5 mmol) in methanol (10 mL) at 45 °C overnight. The solvent was removed in *vacuo* at room temperature and the residue was dissolved in CH_2Cl_2 and washed with $NaHCO_3$ and brine. The organic phase was dried with Na_2SO_4, filtered, and evaporated to afford crude product was purified by silica gel column chromatography (0–10% CH_3OH/CH_2Cl_2) to obtain compound **2a** and **2b**. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.33 (d, J = 7.2 Hz, 1H), 7.49 (s, 1H), 6.84 (s, 1H), 5.93–5.82 (m, 1H), 5.19 (d, J = 16.3 Hz, 1H), 5.10 (d, J = 10.2 Hz, 1H), 4.49 (t, J = 6.0 Hz, 1H), 4.37 (t, J = 6.0 Hz, 1H), 4.19 (s, 1H), 3.90 (s, 3H), 3.85 (s, 3H), 3.83–3.77 (m, 1H), 3.48 (dd, J = 13.2, 5.3 Hz, 1H), 3.36 (d, J = 13.9 Hz, 1H), 3.08 (d, J = 10.4 Hz, 1H), 2.86 (dd, J = 13.5, 7.6 Hz, 1H), 2.71 (t, J = 7.6 Hz, 2H), 2.47–2.27 (m, 3H), 2.06–1.91 (m, 2H), 1.64 (dd, J = 14.4, 6.8 Hz, 1H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 165.9, 152.5, 145.8, 137.5, 135.3, 126.5, 122.2, 117.6, 115.8, 83.1 (d, J = 164.9 Hz), 69.7, 62.8, 61.6, 61.4, 56.4, 56.2, 40.6, 39.0, 31.9 (d, J = 19.8 Hz), 31.2 (d, J = 5.3 Hz). **HRMS** (ESI) calculated for $C_{20}H_{30}FN_2O_4^+$ ([M + H^+]) 381,2190, found: 381.2175.

3.6. General procedure for the synthesis of 29a-f and 44

3.6.1. Method A

To a solution of NaH (8 mg, 0.33 mmol) and **1a** (0.10 mmol) in dry THF (2 mL) was added corresponding benzyl bromide (0.12 mmol) at room temperature. The mixture was heated to 60 °C overnight. The reaction was quenched with water, and extracted with EtOAc (3 × 15 mL), the organic extracts were combined, dried with Na_2SO_4, filtered, and evaporated to afford the crude product which was purified by flash silica chromatography (10% MeOH/CH_2Cl_2) to afford the products as a colorless oil.

3.6.2. Method B

50% Sodium hydroxide solution in water (0.7 mL) was added to a vigorously stirred solution of **1a** or **3** (0.10 mmol), corresponding alkyl bromide (0.12 mmol) and tetrabutylammonium hydrogen sulfate (7.0 mg, 0.02 mmol) in toluene (1 mL). The resulting mixture was vigorously stirred at room temperature for 48 h. The reaction mixture was diluted with water (5 mL) and extracted with EtOAc (3 × 15 mL), the organic extracts were combined, dried with Na_2SO_4, filtered, and evaporated to afford the crude product which was purified by flash silica chromatography (10% MeOH/CH_2Cl_2) to afford the products as a colorless oil.

N-(((2S,4R)-1-allyl-4-(benzyloxy)pyrrolidin-2-yl*)methyl)-5-(3-*

*fluoropropyl)-2,3-dimethoxybenzamide (**29a**)*. Method A. 19.0 mg colorless oil, 36% yield. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.34 (br, 1H), 7.50 (d, J = 2.1 Hz, 1H), 7.28–7.18 (m, 5H), 6.82 (d, J = 2.1 Hz, 1H), 5.89–5.79 (m, 1H), 5.16 (dd, J = 17.2, 1.6 Hz, 1H), 5.06 (d, J = 10.2 Hz, 1H), 4.45 (t, J = 5.9 Hz, 1H), 4.41 (d, J = 11.7 Hz, 1H), 4.37 (d, J = 11.7 Hz, 1H), 4.33 (t, J = 5.9 Hz, 1H), 4.03–3.98 (m, 1H), 3.83 (s, 3H), 3.79 (s, 3H), 3.81–3.74 (m, 1H), 3.47–3.27 (m, 3H), 3.02–2.88 (m, 2H), 2.68 (t, J = 8.0 Hz, 2H), 2.40 (dd, J = 10.2, 5.3 Hz, 1H), 2.04–1.84 (m, 4H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 165.6, 152.5, 145.9, 138.3, 137.5, 135.4, 128.5, 127.7, 126.4, 122.3, 117.5, 115.6, 83.0 (d, J = 165.0 Hz), 76.5, 71.2, 61.3, 59.7, 56.7, 56.1, 40.1, 35.5, 31.9 (d, J = 20.1 Hz), 31.3 (d, J = 5.0 Hz). **HRMS** (ESI) calculated for $C_{27}H_{36}FN_2O_4^+$ ([M + H^+]) 471,2659, found: 471.2649.

N-(((2S,4R)-1-allyl-4-((4-fluorobenzyl)oxy)pyrrolidin-2-yl*)methyl)-5-(3-fluoropropyl)-2,3-dimethoxybenzamide (**29b**)*. Method A. 21 mg colorless oil, 41% yield. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.39 (s, 1H), 7.54 (d, J = 2.1 Hz, 1H), 7.29–7.22 (m, 2H), 7.03–6.95 (m, 2H), 6.87 (d, J = 2.1 Hz, 1H), 5.97–5.81 (m, 1H), 5.22 (dd, J = 17.2, 1.7 Hz, 1H), 5.12 (d, J = 10.3 Hz, 1H), 4.51 (t, J = 5.9 Hz, 1H), 4.45–4.35 (m, 3H), 4.08–4.01 (m, 1H), 3.89 (s, 3H), 3.84 (s, 3H), 3.83–3.77 (m, 1H), 3.56–3.30 (m, 3H), 3.09–2.91 (m, 2H), 2.76–2.69 (m, 2H), 2.45 (s, 1H), 2.06–1.90 (m, 4H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 165.7, 162.4 (d, J = 245.7 Hz), 152.6, 146.0, 137.5, 135.1, 129.5 (d, J = 8.0 Hz), 126.4, 122.3, 117.6, 115.7, 115.4 (d, J = 21.4 Hz), 83.1 (d, J = 165.0 Hz), 76.5, 70.5, 61.4, 59.5, 56.9, 56.2, 40.1, 35.4, 32.0 (d, J = 19.8 Hz), 31.3 (d, J = 5.2 Hz). **HRMS** (ESI) calculated for $C_{27}H_{35}F_2N_2O_4^+$ ([M + H^+]) 489,2565, found: 489.2575.

N-(((2S,4R)-1-allyl-4-((4-(trifluoromethyl)benzyl)oxy)pyrrolidin-2-yl*)methyl)-5-(3-fluoropropyl)-2,3-dimethoxybenzamide (**29c**)*. Method B.34 mg colorless oil, 63% yield. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.40 (s, 1H), 7.59–7.54 (m, 3H), 7.41 (d, J = 7.9 Hz, 2H), 6.87 (d, J = 2.1 Hz, 1H), 5.95–5.82 (m, 1H), 5.22 (dd, J = 17.4, 1.8 Hz, 1H), 5.13 (d, J = 10.2 Hz, 1H), 4.54–4.47 (m, 3H), 4.39 (t, J = 5.9 Hz, 1H), 4.09–4.02 (m, 1H), 3.89 (s, 3H), 3.86–3.83 (m, 4H), 3.53–3.34 (m, 3H), 3.12–2.94 (m, 2H), 2.77–2.70 (m, 2H), 2.47 (s, 1H), 2.07–1.91 (m, 4H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 165.7, 152.6, 145.9, 137.6, 135.4, 129.1, 128.3, 127.6, 127.5 (d, J = 3.4 Hz), 125.7 (d, J = 3.9 Hz), 125.4 (q, J = 3.6 Hz), 122.3, 117.4, 83.1 (d, J = 165.0 Hz), 77.0, 70.4, 61.4, 59.6, 56.7, 56.2, 40.1, 35.4, 32.0 (d, J = 19.8 Hz), 31.3 (d, J = 5.3 Hz). **HRMS** (ESI) calculated for $C_{28}H_{35}F_4N_2O_4^+$ ([M + H^+]) 539,2533, found: 539.2512.

N-(((2S,4R)-1-allyl-4-(pyridin-4-ylmethoxy)pyrrolidin-2-yl*)methyl)-5-(3-fluoropropyl)-2,3-dimethoxybenzamide (**29d**)*. Method B. 23 mg colorless oil, 48% yield. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.53 (d, J = 5.0 Hz, 2H), 8.39 (br, 1H), 7.53 (s, 1H), 7.20 (d, J = 5.1 Hz, 2H), 6.86 (s, 1H), 5.94–5.89 (m, 1H), 5.22 (d, J = 17.1 Hz, 1H), 5.13 (d, J = 10.2 Hz, 1H), 4.51–4.48 (m, 1H), 4.46 (s, 1H), 4.43 (s, 1H), 4.40–4.36 (m, 1H), 4.08–4.02 (m, 1H), 3.87 (s, 3H), 3.84 (s, 3H), 3.59–3.32 (m, 3H), 3.07–2.97 (m, 2H), 2.72 (t, J = 7.7 Hz, 2H), 2.49 (s, 1H), 2.11–1.87 (m, 4H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 165.6, 152.4, 149.8, 147.3, 145.8, 137.4, 135.0, 126.2, 122.1, 121.7, 117.8, 115.6, 83.0 (d, J = 165.0 Hz), 77.2, 69.3, 61.3, 60.9, 59.3, 56.8, 56.1, 40.0, 35.3, 31.9 (d, J = 20.1 Hz), 31.2 (d, J = 5.0 Hz). **HRMS** (ESI) calculated for $C_{26}H_{35}FN_3O_4^+$ ([M + H^+]) 472.2612, found: 472.2630.

N-(((2S,4R)-1-allyl-4-(pyridin-3-ylmethoxy)pyrrolidin-2-yl*)methyl)-5-(3-fluoropropyl)-2,3-dimethoxybenzamide (**29e**)*. Method B. 12 mg colorless oil, 24% yield. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.54–8.40 (m, 2H), 8.40 (br, 1H), 7.63 (d, J = 7.6 Hz, 1H), 7.53 (d, J = 2.1 Hz, 1H), 7.27–7.24 (m, 1H), 6.87 (d, J = 2.1 Hz, 1H), 5.94–5.84 (m, 1H), 5.22 (d, J = 17.1 Hz, 1H), 5.13 (d, J = 10.2 Hz, 1H), 4.52–4.37 (m, 4H), 4.07 (s, 1H), 3.88 (s, 3H), 3.86 (s, 3H), 3.54–3.38 (m, 3H), 3.15–2.92 (m, 2H), 2.73 (t, J = 7.7 Hz, 2H), 2.48 (s, 1H), 2.09–1.88 (m, 4H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 165.6, 152.4, 149.1, 149.0, 145.8, 137.4, 135.3, 133.6, 126.3, 123.4, 122.2, 115.6, 83.0 (d, J = 165.0 Hz), 77.0, 68.6, 61.3, 60.7, 59.4, 56.8, 56.1, 40.0, 35.3, 31.9 (d, J = 20.1 Hz), 31.2 (d, J = 5.0 Hz). **HRMS** (ESI) calculated for $C_{26}H_{35}FN_3O_4^+$ ([M + H^+]) 472.2606, found: 472.2612.

N-(((2S,4R)-1-allyl-4-(allyloxy)pyrrolidin-2-yl*)methyl)-5-(3-fluoropropyl)-2,3-dimethoxybenzamide (**29**)*. Method B. 37 mg colorless oil, 88% yield. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.36 (d, J = 5.5 Hz, 1H), 7.53 (d, J = 2.1 Hz, 1H), 6.86 (d, J = 2.1 Hz, 1H), 5.94–5.78 (m, 2H), 5.27–5.05 (m, 4H), 4.50 (t, J = 6.1 Hz, 1H), 4.38 (t, J = 6.0 Hz, 1H), 4.02–3.96 (m, 1H), 3.92–3.90 (m, 1H), 3.88 (s, 3H), 3.85 (s, 3H), 3.83–3.77 (m, 1H), 3.54–3.27 (m, 4H), 2.99–2.92 (m, 2H), 2.72 (t, J = 7.7 Hz, 2H), 2.44–2.28 (m, 1H), 2.05–1.87 (m, 4H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 165.5, 152.4, 145.8, 137.4, 135.3, 134.7, 126.4, 122.2, 117.4, 116.9, 115.5, 83.0 (d, J = 165.0 Hz), 76.4, 70.1, 66.2, 61.3, 60.6, 59.6, 56.6, 56.1, 40.0, 35.4, 31.9 (d, J = 20.1 Hz), 31.2 (d, J = 5.0 Hz). **HRMS** (ESI) calculated for $C_{23}H_{34}FN_2O_4^+$ ([M + H^+]) 421,2503, found: 421.2521.

N-(((2R,3S)-1-allyl-3-(benzyloxy)pyrrolidin-2-yl*)methyl)-5-(3-fluoropropyl)-2,3-dimethoxybenzamide (**44**)*. Method B. 30 mg colorless oil, 64% yield. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.45 (br, 1H), 7.55 (d, J = 2.1 Hz, 1H), 7.30–7.29 (m, 4H), 7.27–7.21 (m, 1H), 6.87 (d, J = 2.2 Hz, 1H), 5.95–5.85 (m, 1H), 5.22 (dd, J = 17.1, 1.7 Hz, 1H), 5.12 (d, J = 10.1 Hz, 1H), 4.54–4.44 (m, 3H), 4.40 (t, J = 5.9 Hz, 1H), 3.95–3.90 (m, 2H), 3.89 (s, 3H), 3.84 (s, 3H), 3.50–3.41 (m, 2H), 3.12–2.99 (m, 2H), 2.84–2.82 (m, 1H), 2.74 (t, J = 7.7 Hz, 2H), 2.61–2.51 (m, 1H), 2.10–1.82 (m, 4H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 165.5, 152.5, 145.9, 138.2, 137.3, 128.4, 127.7, 127.6, 126.3, 122.2, 115.6, 83.0 (d, J = 165.0 Hz), 82.2, 71.3, 61.3, 57.0, 56.1, 52.0, 39.9, 31.9 (d, J = 20.1 Hz), 31.2 (d, J = 5.0 Hz), 30.3. **HRMS** (ESI) calculated for $C_{27}H_{36}FN_2O_4^+$ ([M + H^+]) 471.2659, found: 471.2668.

3.7. General procedure for the synthesis of 32a,b

50% Sodium hydroxide solution in water (1 mL) was added to a vigorously stirred solution of **1a** (220 mg, 0.58 mmol), (2-bromoethoxy) (*tert*-butyl)dimethylsilane or *tert*-butyl (3-iodopropoxy)dimethylsilane (1.74 mmol) and tetrabutylammonium hydrogensulfate (39 mg, 0.12 mmol) in toluene (2 mL). The resulting mixture was vigorously stirred at room temperature for 48 h. The reaction mixture was diluted with water (10 mL) and extracted with EtOAc (3 × 15 mL), the organic extracts were washed with saturated NaCl, dried with Na_2SO_4, filtered, and evaporated to afford the crude product which was used for the next step directly.

A solution of the crude product above in THF (1 mL) was cooled to 0 °C. TBAF (300 μL) (1 M in THF) was added to the solution. The mixture was warmed to room temperature and stirred for 4 h. The reaction was quenched with MeOH (2 mL), and extracted with EtOAc (3 × 15 mL), the organic extracts were combined, dried with Na_2SO_4, filtered, and evaporated to afford the crude product which was purified by flash silica chromatography (10% MeOH/CH_2Cl_2) to afford the product.

N-(((2S,4R)-1-allyl-4-(2-hydroxyethoxy)pyrrolidin-2-yl*)methyl)-5-(3-fluoropropyl)-2,3-dimethoxybenzamide (**32a**)*. 108 mg colorless oil, 44% yield. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.55 (br, 1H), 7.50 (s, 1H), 6.88 (d, J = 2.2 Hz, 1H), 5.98 (br, 1H), 5.40–5.12 (m, 2H), 4.51 (t, J = 5.9 Hz, 1H), 4.39 (t, J = 5.9 Hz, 1H),4.08 (s, 1H), 3.93 (s, 3H), 3.88 (s, 3H), 3.71 (t, J = 4.6 Hz, 2H), 3.56–3.46 (m, 5H), 3.26–3.22 (m, 1H), 2.73 (t, J = 7.7 Hz, 2H), 2.09–1.90 (m, 4H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 166.0, 152.5, 146.1, 137.3, 129.4, 125.9, 122.0, 115.9, 83.0 (d, J = 165.0 Hz), 77.3, 70.3, 61.8, 61.5, 58.9, 56.1, 40.2, 35.4, 31.9 (d, J = 20.1 Hz), 31.2 (d, J = 5.0 Hz). **HRMS** (ESI) calculated for $C_{22}H_{34}FN_2O_5^+$ ([M + H^+]) 425.2452, found: 425.2462.

N-(((2S,4R)-1-allyl-4-(3-hydroxypropoxy)pyrrolidin-2-yl*)methyl)-5-(3-fluoropropyl)-2,3-dimethoxybenzamide (**32b**)*. 117 mg light yellow oil, 46% yield. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.38 (br, 1H), 7.52 (d, J = 2.1 Hz, 1H), 6.86 (d, J = 2.1 Hz, 1H), 5.91–5.86 (m, 1H), 5.20 (d, J = 16.0 Hz, 1H), 5.11 (d, J = 10.2 Hz, 1H), 4.49 (t, J = 5.9 Hz, 1H), 4.37 (t, J = 5.9 Hz, 1H), 3.96–3.90 (m, 1H), 3.87 (s, 3H), 3.85 (s, 3H), 3.82–3.67 (m, 3H), 3.57–3.32 (m, 5H), 2.97–2.91 (m, 2H), 2.71 (t, J = 7.7 Hz, 2H), 2.40 (d, J = 33.2 Hz, 2H), 2.06–1.83 (m, 4H), 1.81–1.75 (m, 2H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 165.6, 152.4, 145.8, 137.4, 135.1, 126.3,

122.2, 117.6, 115.6, 83.8, 82.2, 77.3, 68.1, 61.7, 61.3, 60.7, 59.5, 56.6, 56.1, 40.0, 35.3, 32.2, 32.0, 31.9 (d, J = 20.1 Hz), 31.2 (d, J = 5.0 Hz). **HRMS** (ESI) calculated for $C_{23}H_{36}FN_2O_5^+$ ([M + H^+]) 439.2608, found: 439.2628.

3.8. General procedure for the synthesis of 35a,b

A solution of product **32a** or **32b** (0.1 mmol) and Et_3N (0.3 mmol) in CH_2Cl_2 (2 mL) was cooled to 0 °C. MsCl (5 μL) was added to the mixture. The reaction was warmed to room temperature and stirred for 6 h. The reaction was quenched with H_2O (1 mL) and extracted with CH_2Cl_2 (3 × 10 mL), the organic extracts were combined, dried with Na_2SO_4, filtered, and evaporated to afford crude product **33a** or **33b**.

A solution of the crude product above and NaN_3 (13 mg, 1.0 mmol) in DMF (2 mL) was heated to 70 °C for 6 h. The reaction was quenched with H_2O (10 mL) and extracted with CH_2Cl_2 (3 × 10 mL), the organic extracts were combined, dried with Na_2SO_4, filtered, and evaporated to afford crude product **34a** or **34b**.

A solution of the crude product above in THF (2 mL) and H_2O (10 μL) was cooled to 0 °C. A solution of PPh_3 (36 mg) in THF (2 mL) was added to the solution. The mixture was heated to 50 °C for 6 h. Removed the solvents under vacuum, and the residue was purified by flash silica chromatography (10% MeOH (7 N NH_3)/CH_2Cl_2) to afford the product.

*N-(((2S,4R)-1-allyl-4-(2-aminoethoxy)pyrrolidin-2-yl)methyl)-5-(3-fluoropropyl)-2,3-dimethoxybenzamide **(35a)***. 10 mg colorless oil, 23% yield for 3 steps. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.39 (br, 1H), 7.53 (d, J = 2.2 Hz, 1H), 6.87 (d, J = 2.2 Hz, 1H), 5.94–5.82 (m, 1H), 5.23 (dd, J = 17.2, 1.6 Hz, 1H), 5.12 (d, J = 10.1 Hz, 1H), 4.51 (t, J = 5.9 Hz, 1H), 4.39 (t, J = 5.9 Hz, 1H), 4.01–3.95 (m, 1H), 3.89 (s, 3H), 3.87 (s, 3H), 3.84–3.78 (m, 1H), 3.53–3.29 (m, 6H), 2.91 (t, J = 5.2 Hz, 1H), 2.77–2.69 (m, 2H), 2.07–1.88 (m, 4H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 165.7, 152.5, 145.9, 137.4, 122.2, 115.8, 83.9, 82.2, 77.2, 68.6, 61.4, 61.3, 59.3, 56.1, 41.2, 40.5, 40.0 (d, J = 4.1 Hz), 35.3, 35.3, 31.9 (d, J = 20.1 Hz), 31.2 (d, J = 5.0 Hz). **HRMS** (ESI) calculated for $C_{22}H_{35}FN_3O_4^+$ ([M + H^+]) 424,2612, found: 424.2595.

*N-(((2S,4R)-1-allyl-4-(3-aminopropoxy)pyrrolidin-2-yl)methyl)-5-(3-fluoropropyl)-2,3-dimethoxybenzamide **(35b)** and N-(((2R,4S)-1-allyl-4-(3-aminopropoxy)pyrrolidin-2-yl)methyl)-5-(3-fluoropropyl)-2,3-dimethoxybenzamide **(38)***. 16 mg colorless oil, 38% yield for 3 steps. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.36 (d, J = 6.0 Hz, 1H), 7.54 (d, J = 2.1 Hz, 1H), 6.87 (d, J = 2.1 Hz, 1H), 5.92–5.82 (m, 1H), 5.20 (dd, J = 17.1, 1.6 Hz, 1H), 5.10 (d, J = 11.1 Hz, 1H), 4.51 (t, J = 5.9 Hz, 1H), 4.39 (t, J = 5.9 Hz, 1H), 3.94–3.89 (m, 1H), 3.89 (s, 3H), 3.86 (s, 3H), 3.79 (qd, J = 14.1, 2.2 Hz, 1H), 3.50–3.36 (m, 4H), 3.32 (dq, J = 14.0, 2.6 Hz, 1H), 2.97–2.89 (m, 2H), 2.78–2.71 (m, 4H), 2.31 (dd, J = 10.0, 5.5 Hz, 1H), 2.08–1.94 (m, 2H), 1.88 (dd, J = 8.2, 5.6 Hz, 2H), 1.70–1.64 (m, 2H), 1.38 (br, 2H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 165.5, 152.4, 145.8, 137.4, 135.5, 126.4, 122.2, 117.2, 115.5, 83.0 (d, J = 165.0 Hz), 77.0, 67.3, 61.3, 60.5, 59.7, 56.5, 56.1, 40.0, 39.7, 35.5, 33.7, 31.9 (d, J = 20.1 Hz), 31.2 (d, J = 5.0 Hz). **HRMS** (ESI) calculated for $C_{23}H_{37}FN_3O_4^+$ ([M + H^+]) 438.2768, found: 438.2755.

*Compound **40, 42, 45** and **46** were prepared with a similar procedure to **35b**.*

*N-(((2S,4S)-1-allyl-4-(3-aminopropoxy)pyrrolidin-2-yl)methyl)-5-(3-fluoropropyl)-2,3-dimethoxybenzamide **(40)** and N-(((2R,4R)-1-allyl-4-(3-aminopropoxy)pyrrolidin-2-yl)methyl)-5-(3-fluoropropyl)-2,3-dimethoxybenzamide **(42)***. 17 mg colorless oil, 39% yield for 2 steps. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.40 (d, J = 3.9 Hz, 1H), 7.47 (s, 1H), 6.82 (s, 1H), 5.91–5.81 (m, 1H), 5.16 (d, J = 17.1 Hz, 1H), 5.06 (d, J = 10.1 Hz, 1H), 4.47 (t, J = 5.9 Hz, 1H), 4.35 (t, J = 5.9 Hz, 1H), 3.85 (d, 7H), 3.76–3.70 (m, 1H), 3.44 (dd, J = 13.7, 5.2 Hz, 1H), 3.39–3.31 (m, 3H), 3.14 (d, J = 10.7 Hz, 1H), 2.79 (dd, J = 13.5, 7.7 Hz, 1H), 2.71–2.65 (m, 5H), 2.38–2.29 (m, 3H), 2.21–2.14 (m, 1H), 2.07–1.89 (m, 2H), 1.72–1.66 (m, 1H), 1.58–1.52 (m, 2H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 165.7, 152.5, 146.0, 137.3, 135.6, 126.8, 122.1, 117.2, 115.5, 83.1 (d, J = 164.9 Hz), 77.1, 66.9, 61.6, 61.3, 59.5, 56.6, 56.1, 40.5, 39.5, 35.9, 32.8, 31.9 (d, J = 19.7 Hz), 31.2 (d, J = 5.4 Hz). **HRMS** (ESI) calculated for $C_{23}H_{36}FN_3O_4Na^+$ ([M + Na^+]) 460.2582, found: 460.2581.

*N-(((2R,3S)-1-allyl-3-(3-aminopropoxy)pyrrolidin-2-yl)methyl)-5-(3-fluoropropyl)-2,3-dimethoxybenzamide **(45)***. 16 mg colorless oil, 36% yield for 2 steps. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.45 (d, J = 8.4 Hz, 1H), 7.54 (d, J = 2.2 Hz, 1H), 6.87 (d, J = 2.2 Hz, 1H), 5.93–5.83 (m, 1H), 5.21 (dd, J = 17.1, 1.6 Hz, 1H), 5.10 (dd, J = 10.2, 1.5 Hz, 1H), 4.51 (t, J = 5.9 Hz, 1H), 4.39 (t, J = 5.9 Hz, 1H), 3.93 (dd, J = 7.5, 2.4 Hz, 1H), 3.89 (s, 3H), 3.86 (s, 3H), 3.76–3.72 (m, 1H), 3.54–3.39 (m, 3H), 3.35 (dq, J = 14.1, 2.7 Hz, 1H), 3.05 (t, J = 9.3 Hz, 1H), 2.95 (dd, J = 13.5, 7.5 Hz, 1H), 2.87–2.79 (m, 2H), 2.74 (t, J = 7.7 Hz, 2H), 2.62–2.59 (m, 1H), 2.56–2.43 (m, 3H), 2.10–1.94 (m, 2H), 1.91–1.81 (m, 1H), 1.76–1.70 (m, 3H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 165.4, 152.4, 145.9, 137.4, 135.6, 126.3, 122.2, 117.2, 115.6, 83.0 (d, J = 165.0 Hz), 82.4, 68.7, 67.3, 61.3, 56.8, 56.1, 52.0, 39.6, 39.4, 32.6, 31.9 (d, J = 20.1 Hz), 31.2 (d, J = 5.0 Hz), 30.1. **HRMS** (ESI) calculated for $C_{23}H_{37}FN_3O^+$ ([M + H^+]) 438.2768, found: 438.2766.

*N-(((2S,4R)-4-(3-aminopropoxy)-1-ethylpyrrolidin-2-yl)methyl)-5-(3-fluoropropyl)-2,3-dimethoxybenzamide **(46)***. 14 mg colorless oil, 33% yield for 2 steps. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.36 (d, J = 7.1 Hz, 1H), 7.54 (d, J = 2.2 Hz, 1H), 6.86 (d, J = 2.2 Hz, 1H), 4.50 (t, J = 5.9 Hz, 1H), 4.39 (t, J = 5.9 Hz, 1H), 3.96–3.89 (m, 1H), 3.89 (s, 3H), 3.86 (s, 3H), 3.78 (dd, J = 14.0, 7.5, 2.2 Hz, 1H), 3.52–3.39 (m, 3H), 3.33–3.28 (m, 1H), 2.92–2.78 (m, 4H), 2.73 (m, 2H), 2.55 (s, 2H), 2.33–2.21 (m, 2H), 2.08–1.94 (m, 2H), 1.87 (dd, J = 8.2, 5.5 Hz, 2H), 1.76–1.68 (m, 2H), 1.09 (t, J = 7.1 Hz, 3H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 165.5, 152.5, 145.8, 137.4, 126.4, 122.2, 115.5, 83.0 (d, J = 165.0 Hz), 67.3, 61.3, 60.8, 59.2, 56.1, 47.4, 40.0, 39.6, 35.4, 32.7, 31.9 (d, J = 19.7 Hz), 31.2 (d, J = 5.4 Hz), 13.6. **HRMS** (ESI) calculated for $C_{22}H_{36}FN_3O_4Na^+$ ([M + Na^+]) 448.2582, found: 448.2594.

*N-(((2S,4R)-1-allyl-4-(4-aminobutoxy)pyrrolidin-2-yl)methyl)-5-(3-fluoropropyl)-2,3-dimethoxybenzamide **(35c)***. 50% Sodium hydroxide solution in water (1 mL) was added to a vigorously stirred solution of **1a** (110 mg, 0.29 mmol), tetrabutylammonium hydrogen sulfate (7.0 mg, 0.02 mmol) 1,4-dibromobutane (0.87 mmol) in toluene (2 mL). The resulting mixture was vigorously stirred at room temperature for 48 h. The reaction mixture was diluted with water (10 mL) and extracted with EtOAc (3 × 15 mL), the organic extracts were washed with saturated NaCl, dried with Na_2SO_4, filtered, and evaporated to afford the crude product which was used for the next step directly.

A solution of the crude product above and NaN_3 (38 mg, 0.58 mmol) in DMF (4 mL) was heated to 70 °C for 6 h. The reaction was quenched with H_2O (10 mL) and extracted with CH_2Cl_2 (3 × 10 mL), the organic extracts were washed with saturated NaCl, dried with Na_2SO_4, filtered, and evaporated to afford crude product **34c**.

A solution of the crude product above in THF (2 mL) and H_2O (10 μL) was cooled to 0 °C. A solution of PPh_3 (36 mg) in THF (2 mL) was added to the solution. The mixture was heated to 50 °C for 6 h. The solvents were removed under vacuum, the residue was purified by flash silica chromatography (10% MeOH (7 N NH_3)/CH_2Cl_2) to afford the compound **35c** as a colorless oil. 31 mg colorless oil, 24% yield for 3 steps. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.40 (br, 1H), 7.53 (dd, J = 7.2, 2.2 Hz, 1H), 6.87 (d, J = 2.1 Hz, 1H), 5.94–5.81 (m, 1H), 5.23 (d, J = 18.1 Hz, 1H), 5.12 (d, J = 10.2 Hz, 1H), 4.51 (t, J = 5.9 Hz, 1H), 4.39 (t, J = 5.9 Hz, 1H), 3.96–3.92 (m, 1H), 3.89 (s, 3H), 3.87 (s, 3H), 3.83–3.77 (m, 1H), 3.49 (dd, J = 13.6, 5.3 Hz, 1H), 3.44–3.33 (m, 4H), 3.05–2.91 (m, 3H), 2.74 (t, J = 7.7 Hz, 2H), 2.38–2.36 (m, 1H), 2.08–1.84 (m, 4H), 1.76–1.52 (m, 5H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 165.7, 152.5, 145.9, 137.4, 135.2, 126.2, 122.2, 117.5, 115.6, 83.0 (d, J = 165.0 Hz), 77.2, 68.6, 61.4, 61.3, 60.7, 59.5, 56.6, 56.1, 40.0, 35.4, 35.4, 31.9 (d, J = 20.1 Hz), 31.2 (d, J = 5.0 Hz), 27.2. **HRMS** (ESI) calculated for $C_{24}H_{39}FN_3O_4^+$ ([M + H^+]) 452.2925, found: 452.2928.

*N-(((2S,4R)-1-allyl-4-(3-(4-(thiophen-3-yl)benzamido)propoxy)pyrrolidin-2-yl)methyl)-5-(3-fluoropropyl)-2,3-dimethoxybenzamide **(36)***. Compound **36** was prepared from **35b** and 4-(thiophen-3-yl)benzoic acid with the same method to **13**.44 mg light yellow solid, 71% yield. **^{1}H**

NMR (400 MHz, $CDCl_3$) δ 8.37 (br, 1H), 7.78 (d, J = 8.4 Hz, 2H), 7.64 (d, J = 8.3 Hz, 2H), 7.56–7.52 (m, 2H), 7.41 (d, J = 2.2 Hz, 2H), 6.93 (br, 1H), 6.87 (d, J = 2.1 Hz, 1H), 5.86 (br, 1H), 5.20 (d, J = 17.2 Hz, 1H), 5.11 (d, J = 8.0 Hz, 1H), 4.50 (t, J = 5.9 Hz, 1H), 4.38 (t, J = 5.9 Hz, 1H), 3.98 (s, 1H), 3.88 (s, 3H), 3.86 (s, 3H), 3.62–3.40 (m, 6H), 3.35 (d, J = 1.8 Hz, 1H), 3.08–2.80 (m, 2H), 2.72 (t, J = 7.7 Hz, 2H), 2.35 (s, 1H), 2.09–1.82 (m, 6H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 166.8, 165.7, 152.5, 145.9, 141.2, 138.6, 137.5, 133.2, 132.1, 132.0, 127.4, 126.6, 126.4, 126.2, 122.2, 121.5, 115.6, 83.0 (d, J = 165.0 Hz), 77.3, 68.8, 61.3, 60.4, 59.7, 56.6, 56.1, 40.0, 39.3, 35.4, 31.9 (d, J = 20.1 Hz), 31.2 (d, J = 5.0 Hz), 29.1. **HRMS** (ESI) calculated for $C_{34}H_{43}FN_3O_5S^+$ ([M + H^+]) 624.2907, found: 624.2918.

3.9. General methods for the synthesis of 37a–l, 39, 41, 43 and 47

3.9.1. Method C

To a solution of **1a** (0.2 mmol), Et_3N (25 μL), DMAP (5 mg) in CH_2Cl_2 (2 mL) was added corresponding isocyanate (0.3 mmol) at rt. The mixture was stirred at rt for 16 h. The solvents were removed under vacuum, and the residue was purified by flash silica chromatography (5% MeOH/CH_2Cl_2) to afford the products.

3.9.2. Method D

To a solution of **1a**, **1b**, **2a, 2b or 4** (0.24 mmol), corresponding carboxyl acid (0.20 mmol), Et_3N (0.30 mmol) and $TMSN_3$ (0.22 mmol) in THF (2 mL) was added T_3P (50% in THF) (130 μL, 0.22 mmol) at rt. The mixture was heated to 70 °C for 16 h. Removed the solvents under vacuum, and the residue was purified by flash silica chromatography (5% MeOH/CH_2Cl_2) to afford the products.

(3R,5S)-1-allyl-5-((5-(3-fluoropropyl)-2,3-dimethoxybenzamido) methyl)pyrrolidin-3-yl *phenylcarbamate* ***(37a)***. Method C. 66 mg colorless oil, 66% yield. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.42 (br, 1H), 7.55 (d, J = 2.1 Hz, 1H), 7.37 (d, J = 8.0 Hz, 2H), 7.29 (t, J = 7.9 Hz, 2H), 7.06 (t, J = 7.3 Hz, 1H), 6.88 (d, J = 2.1 Hz, 1H), 6.68 (br, 1H), 5.88 (br, 1H), 5.29–5.07 (m, 3H), 4.51 (t, J = 5.9 Hz, 1H), 4.39 (t, J = 5.9 Hz, 1H), 3.89 (s, 7H), 3.60 (s, 1H), 3.51 (d, J = 13.0 Hz, 1H), 3.35 (d, J = 13.9 Hz, 1H), 3.02–2.92 (m, 2H), 2.73 (t, J = 7.7 Hz, 2H), 2.56–2.42 (m, 1H), 2.14–1.91 (m, 4H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 165.7, 153.1, 152.6, 146.0, 137.9, 137.6, 135.2, 129.2, 126.3, 123.7, 122.3, 118.8, 117.6, 115.8, 83.1 (d, J = 165.0 Hz), 73.4, 61.5, 60.5, 59.7, 56.4, 56.2, 39.7, 35.7, 32.0 (d, J = 20.1 Hz), 31.3 (d, J = 5.0 Hz); **HRMS** (ESI) calculated for $C_{27}H_{35}FN_3O_5^+$ ([M + H^+]) 500.2561, found: 500.2568.

(3R,5S)-1-allyl-5-((5-(3-fluoropropyl)-2,3-dimethoxybenzamido) methyl)pyrrolidin-3-yl *benzylcarbamate* ***(37b)***. Method C. 44 mg colorless oil, 43% yield. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.33 (br, 1H), 7.47 (d, J = 2.1 Hz, 1H), 7.28–7.18 (m, 5H), 6.80 (d, J = 2.1 Hz, 1H), 5.89–5.69 (m, 1H), 5.18–4.90 (m, 4H), 4.44 (t, J = 5.9 Hz, 1H), 4.32 (t, J = 5.9 Hz, 1H), 4.27 (d, J = 6.0 Hz, 2H), 3.82 (s, 3H), 3.81 (s, 3H), 3.55–3.36 (m, 2H), 3.27 (br, 1H), 3.02–2.80 (m, 2H), 2.66 (t, J = 7.7 Hz, 2H), 2.36 (s, 1H), 2.02–1.84 (m, 4H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 165.7, 156.1, 152.6, 146.0, 138.5, 137.5, 135.3, 128.8, 127.7, 126.3, 122.3, 117.5, 115.8, 83.0 (d, J = 165.0 Hz), 73.0, 61.4, 60.5, 59.6, 56.4, 56.2, 45.2, 39.7, 35.8, 32.0 (d, J = 20.1 Hz), 31.3 (d, J = 5.0 Hz); **HRMS** (ESI) calculated for $C_{28}H_{37}FN_3O_5^+$ ([M + H^+]) 514.2717, found: 514.2723.

(3R,5S)-1-allyl-5-((5-(3-fluoropropyl)-2,3-dimethoxybenzamido) methyl)pyrrolidin-3-yl *phenethylcarbamate* ***(37c)***. Method C. 41 mg colorless oil, 39% yield. **^{1}H NMR (400 MHz, Methanol-d_4)** δ 7.30–7.14 (m, 6H), 7.04 (s, 1H), 5.97–5.86 (m, 1H), 5.24 (d, J = 17.2 Hz, 1H), 5.14 (d, J = 8.0 Hz, 1H), 4.97 (t, J = 5.1 Hz, 1H), 4.49 (t, J = 5.9 Hz, 1H), 4.37 (t, J = 5.9 Hz, 1H), 3.88 (s, 3H), 3.86 (s, 3H), 3.71 (dd, J = 13.8, 3.0 Hz, 1H), 3.54 (dd, J = 12.9, 6.2 Hz, 1H), 3.46 (dd, J = 11.1, 5.9 Hz, 1H), 3.34–3.28 (m, 4H), 3.00 (dd, J = 13.7, 7.5 Hz, 2H), 2.77–2.74 (m, 4H), 2.43 (dd, J = 11.1, 4.3 Hz, 1H), 2.03–1.93 (m, 3H); **^{13}C NMR (101 MHz, Methanol-d_4)** δ 166.9, 157.0, 152.8, 145.8, 139.1, 137.7, 135.1, 128.6, 128.4, 128.0, 126.4, 125.9, 121.9, 120.8, 116.7, 115.7, 83.0 (d, J = 165.0 Hz), 72.6, 60.9, 60.5, 59.1, 56.5, 55.2, 41.9, 40.2, 35.7, 31.9 (d, J = 20.1 Hz), 31.2 (d, J = 5.0 Hz); **HRMS** (ESI) calculated for $C_{29}H_{39}FN_3O_5^+$ ([M + H^+]) 528.2874, found: 528.2881.

(3R,5S)-1-allyl-5-((5-(3-fluoropropyl)-2,3-dimethoxybenzamido) methyl)pyrrolidin-3-yl *o-tolylcarbamate* ***(37d)***. Method C. 13 mg colorless oil, 25% yield. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.43 (br, 1H), 7.74 (br, 1H), 7.54 (s, 1H), 7.22–7.13 (m, 2H), 7.02 (t, J = 7.4 Hz, 1H), 6.87 (s, 1H), 6.38 (s, 1H), 5.99–5.80 (m, 1H), 5.23 (d, J = 17.2 Hz, 1H), 5.15 (s, 2H), 4.51 (t, J = 5.9 Hz, 1H), 4.39 (t, J = 5.9 Hz, 1H), 3.89 (s, 7H), 3.62 (s, 1H), 3.53 (d, J = 13.5 Hz, 1H), 3.37 (d, J = 14.1 Hz, 1H), 3.11–2.90 (m, 2H), 2.74 (t, J = 7.7 Hz, 2H), 2.51 (s, 1H), 2.25 (s, 3H), 2.13–1.92 (m, 4H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 165.6, 153.3, 152.5, 145.9, 137.4, 135.7, 135.1, 130.4, 126.9, 126.2, 124.3, 122.2, 121.1, 117.5, 115.7, 83.0 (d, J = 165.0 Hz), 73.3, 61.4, 60.4, 59.5, 56.3, 56.1, 39.6, 35.6, 31.9 (d, J = 20.1 Hz), 31.2 (d, J = 5.0 Hz), 17.7; **HRMS** (ESI) calculated for $C_{28}H_{37}FN_3O_5^+$ ([M + H^+]) 514.2717, found: 514.2693.

(3R,5S)-1-allyl-5-((5-(3-fluoropropyl)-2,3-dimethoxybenzamido) methyl)pyrrolidin-3-yl *(2-methoxy-5-methylphenyl)carbamate* ***(37e)***. Method C. 21 mg yellow oil, 39% yield. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.41 (br, 1H), 7.88 (s, 1H), 7.55 (d, J = 2.0 Hz, 1H), 7.16 (s, 1H), 6.88 (d, J = 2.1 Hz, 1H), 6.81–6.71 (m, 2H), 5.98–5.78 (m, 1H), 5.22 (d, J = 17.3 Hz, 1H), 5.14 (s, 2H), 4.52 (t, J = 5.9 Hz, 1H), 4.40 (t, J = 5.9 Hz, 1H), 3.90 (s, 6H), 3.84 (s, 3H), 3.61 (s, 1H), 3.52 (d, J = 13.3 Hz, 1H), 3.35 (d, J = 14.2 Hz, 1H), 3.11–2.88 (m, 2H), 2.74 (t, J = 5.9 Hz, 2H), 2.49 (d, J = 11.2 Hz, 1H), 2.28 (s, 3H), 2.12–1.95 (m, 4H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 165.5, 152.5, 145.9, 145.5, 137.4, 130.6, 123.0, 122.2, 118.8, 117.5, 115.7, 109.9, 83.0 (d, J = 165.0 Hz), 73.1, 61.4, 60.4, 59.6, 56.2, 56.1, 55.8, 39.6, 35.6, 31.9 (d, J = 20.1 Hz), 31.2 (d, J = 5.0 Hz), 21.0; **HRMS** (ESI) calculated for $C_{29}H_{39}FN_3O_6^+$ ([M + H^+]) 544.2823, found: 544.2822.

(3R,5S)-1-allyl-5-((5-(3-fluoropropyl)-2,3-dimethoxybenzamido) methyl)pyrrolidin-3-yl *(3-(dimethylamino)phenyl)carbamate* ***(37f)***. Method D. 21 mg light yellow oil, 39% yield. **^{1}H NMR (400 MHz, Methanol-d_4)** δ 7.30 (s, 1H), 7.10–7.01 (m, 2H), 6.91 (s, 1H), 6.73 (dd, J = 8.0, 1.9 Hz, 1H), 6.44 (d, J = 8.3 Hz, 1H), 5.98–5.88 (m, 1H), 5.25 (d, J = 17.4 Hz, 1H), 5.14 (d, J = 10.2 Hz, 1H), 5.09 (s, 1H), 4.48 (t, J = 5.9 Hz, 1H), 4.36 (t, J = 5.9 Hz, 1H), 3.88 (s, 6H), 3.73 (dd, J = 13.9, 2.9 Hz, 1H), 3.58–3.51 (m, 2H), 3.38–3.28 (m, 2H), 3.09–3.00 (m, 2H), 2.88 (s, 6H), 2.71 (t, J = 7.8 Hz, 2H), 2.52 (dd, J = 11.1, 4.3 Hz, 1H), 2.10–1.89 (m, 4H); **^{13}C NMR (101 MHz, Methanol-d_4)** δ 166.9, 154.1, 152.8, 151.4, 145.8, 139.3, 137.7, 135.1, 128.8, 126.4, 120.9, 116.7, 115.7, 107.8, 82.5 (d, J = 164.0 Hz), 72.7, 60.9, 60.5, 59.1, 56.5, 55.2, 40.1, 39.6, 35.7, 31.8 (d, J = 19.1 Hz), 30.8 (d, J = 5.0 Hz); **HRMS** (ESI) calculated for $C_{29}H_{40}FN_4O_5^+$ ([M + H^+]) 543.2983, found: 543.2971.

(3R,5S)-1-allyl-5-((5-(3-fluoropropyl)-2,3-dimethoxybenzamido) methyl)pyrrolidin-3-yl *thiophen-3-ylcarbamate* ***(37g)***. Method D. 25 mg light yellow oil, 50% yield. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.47 (d, J = 6.0 Hz, 1H), 7.53 (d, J = 2.1 Hz, 1H), 7.23 (s, 1H), 7.18 (dd, J = 5.2, 3.2 Hz, 1H), 6.99 (d, J = 5.1 Hz, 1H), 6.87 (d, J = 2.1 Hz, 1H), 5.86 (ddd, J = 17.3, 10.1, 7.5, 5.2 Hz, 1H), 5.25–5.16 (m, 1H), 5.12 (d, J = 9.7 Hz, 2H), 4.49 (t, J = 5.9 Hz, 1H), 4.38 (t, J = 5.9 Hz, 1H), 3.88 (s, 3H), 3.87 (s, 3H), 3.84 (d, J = 2.4 Hz, 1H), 3.60 (dd, J = 11.2, 6.0 Hz, 1H), 3.49 (dd, J = 13.8, 5.2 Hz, 1H), 3.36 (d, J = 13.7 Hz, 1H), 3.08–2.89 (m, 2H), 2.72 (t, J = 7.7 Hz, 2H), 2.47 (d, J = 9.3 Hz, 1H), 2.10–1.91 (m, 4H); **^{13}C NMR (101 MHz, Chloroform-d)** δ 165.7, 153.3, 152.5, 145.9, 137.4, 135.8, 134.8, 126.0, 124.7, 122.1, 120.8, 117.6, 115.8, 107.8, 83.0 (d, J = 165.0 Hz), 73.2, 61.3, 60.4, 59.6, 56.3, 56.1, 39.5, 35.5, 31.9 (d, J = 20.1 Hz), 31.2 (d, J = 5.0 Hz); **HRMS** (ESI) calculated for $C_{25}H_{33}FN_3O_5S^+$ ([M + H^+]) 506.2125, found: 506.2105.

(3R,5S)-1-allyl-5-((5-(3-fluoropropyl)-2,3-dimethoxybenzamido) methyl)pyrrolidin-3-yl *furan-3-ylcarbamate* ***(37h)***. Method D. 16 mg light yellow oil, 33% yield. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.41 (br, 1H), 7.70 (s, 1H), 7.54 (s, 1H), 7.28 (s, 1H), 6.88 (d, J = 2.1 Hz, 1H), 6.46 (br, 1H), 6.31 (s, 1H), 5.88 (br, 1H), 5.22 (d, J = 17.4 Hz, 1H), 5.13 (s, 2H), 4.51 (t, J = 5.9 Hz, 1H), 4.40 (t, J = 5.9 Hz, 1H), 3.89 (s, 7H), 3.59 (s, 1H), 3.51 (d, J = 12.9 Hz, 1H), 3.35 (s, 1H), 2.98 (s, 2H), 2.74 (d, J = 7.7 Hz, 2H), 2.46 (s, 1H), 2.11–1.85 (m, 4H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ

165.7, 152.5, 145.7, 141.9, 137.4, 135.0, 131.0, 126.3, 124.4, 122.2, 117.5, 115.7, 104.7, 83.0 (d, J = 165.0 Hz), 77.2, 73.5, 61.3, 60.4, 59.6, 56.2, 56.1, 39.5, 35.6, 31.9 (d, J = 20.1 Hz), 31.2 (d, J = 5.0 Hz); **HRMS** (ESI) calculated for $C_{25}H_{33}FN_3O_6^+$ ([M + H^+]) 490.2353, found: 490.2356.

(3R,5S)-1-allyl-5-((5-(3-fluoropropyl)-2,3-dimethoxybenzamido) methyl)pyrrolidin-3-yl *pyridin-3-ylcarbamate* ***(37i)*****.** Method C. 12 mg colorless oil, 24% yield. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.53 (s, 1H), 8.45 (s, 1H), 8.30 (s, 1H), 7.97 (s, 1H), 7.53 (d, J = 2.1 Hz, 1H), 7.27–7.21 (m, 1H), 6.87 (d, J = 2.1 Hz, 1H), 5.96–5.78 (m, 1H), 5.15 (dd, J = 19.5, 11.6 Hz, 3H), 4.50 (t, J = 5.9 Hz, 1H), 4.38 (t, J = 5.9 Hz, 1H), 3.89 (s, 7H), 3.62 (dd, J = 11.3, 6.0 Hz, 1H), 3.51 (d, J = 13.4 Hz, 1H), 3.40–3.38 (m, 1H), 3.11–2.91 (m, 2H), 2.72 (t, J = 7.7 Hz, 2H), 2.50 (s, 1H), 2.11–1.93 (m, 4H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 165.7, 153.1, 152.5, 145.9, 144.4, 140.4, 137.5, 134.9, 126.0, 125.8, 123.7, 123.2, 122.1, 117.8, 115.8, 83.0 (d, J = 165.0 Hz), 73.6, 61.3, 60.6, 59.4, 56.3, 56.1, 39.6, 35.5, 31.9 (d, J = 20.1 Hz), 31.2 (d, J = 5.0 Hz); **HRMS** (ESI) calculated for $C_{26}H_{34}FN_4O_5^+$ ([M + H^+]) 501.2513, found: 501.2516.

(3R,5S)-1-allyl-5-((5-(3-fluoropropyl)-2,3-dimethoxybenzamido) methyl)pyrrolidin-3-yl *pyridin-2-ylcarbamate* ***(37j)*****.** Method D. 38 mg light yellow oil, 76% yield. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.63 (br, 1H), 8.40 (br, 1H), 8.28 (dd, J = 5.0, 1.1 Hz, 1H), 7.95 (d, J = 8.4 Hz, 1H), 7.74–7.64 (m, 1H), 7.54 (d, J = 2.1 Hz, 1H), 7.04–6.96 (m, 1H), 6.88 (d, J = 2.1 Hz, 1H), 5.89 (s, 1H), 5.31–5.06 (m, 3H), 4.51 (t, J = 5.9 Hz, 1H), 4.40 (t, J = 5.9 Hz, 1H), 3.89 (s, 7H), 3.61 (s, 1H), 3.52 (d, J = 11.1 Hz, 1H), 3.42–3.32 (m, 1H), 3.12–2.91 (m, 2H), 2.74 (t, J = 7.7 Hz, 2H), 2.51 (s, 1H), 2.21–1.87 (m, 4H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 165.6, 152.8, 152.5, 151.8, 147.7, 145.9, 138.5, 137.4, 134.9, 126.2, 122.2, 118.8, 117.4, 115.7, 112.5, 83.0 (d, J = 165.0 Hz), 73.5, 61.4, 60.4, 59.3, 56.3, 56.1, 39.6, 35.6, 31.9 (d, J = 20.1 Hz), 31.2 (d, J = 5.0 Hz); **HRMS** (ESI) calculated for $C_{26}H_{34}FN_4O_5^+$ ([M + H^+]) 501.2513, found: 501.2510.

(3R,5S)-1-allyl-5-((5-(3-fluoropropyl)-2,3-dimethoxybenzamido) methyl)pyrrolidin-3-yl *(4-(pyridin*-2-yl*)phenyl)carbamate* ***(37k)*****.** Method D. 40 mg white solid, 70% yield. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.65 (d, J = 4.7 Hz, 1H), 8.46 (br, 1H), 7.95 (d, J = 8.7 Hz, 2H), 7.74–7.65 (m, 2H), 7.56–7.48 (m, 4H), 7.20–7.17 (m, 1H), 7.04 (br, 1H), 6.88 (d, J = 2.2 Hz, 1H), 5.93–5.83 (m, 1H), 5.26–5.19 (m, 1H), 5.18–5.09 (m, 2H), 4.50 (t, J = 5.9 Hz, 1H), 4.38 (t, J = 5.9 Hz, 1H), 3.89 (s, 7H), 3.62 (dd, J = 11.1, 6.0 Hz, 1H), 3.51 (d, J = 10.8 Hz, 1H), 3.38 (d, J = 14.9 Hz, 1H), 3.05–2.91 (m, 2H), 2.73 (d, J = 7.7 Hz, 2H), 2.50 (s, 1H), 2.11–1.95 (m, 4H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 156.8, 152.5, 149.6, 145.9, 138.7, 137.5, 136.7, 134.5, 132.1, 132.0, 128.7, 128.6, 127.6, 122.1, 121.8, 120.0, 118.6, 117.7, 115.8, 83.0 (d, J = 165.0 Hz), 73.3, 61.4, 60.4, 59.5, 56.1, 39.6, 35.5, 32.0 (d, J = 20.1 Hz), 31.3 (d, J = 5.0 Hz); **HRMS** (ESI) calculated for $C_{32}H_{38}FN_4O_5^+$ ([M + H^+]) 577.2826, found: 577.2814.

(3R,5S)-1-allyl-5-((5-(3-fluoropropyl)-2,3-dimethoxybenzamido) methyl)pyrrolidin-3-yl *(4-(thiophen*-3-yl*)phenyl)carbamate* ***(37l)*** *and (3S,5R)-1-allyl-5-((5-(3-fluoropropyl)-2,3-dimethoxybenzamido)methyl) pyrrolidin*-3-yl *(4-(thiophen*-3-yl*)phenyl)carbamate* ***(39)*****.** Method D. 41 mg light yellow solid, 71% yield. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.45 (br, 1H), 7.57–7.49 (m, 3H), 7.46–7.29 (m, 5H), 6.88 (d, J = 2.1 Hz, 2H), 6.85 (br, 1H), 5.99–5.77 (m, 1H), 5.23 (d, J = 20.0 Hz, 1H), 5.16 (s, 2H), 4.51 (t, J = 5.9 Hz, 1H), 4.39 (t, J = 5.9 Hz, 1H), 3.89 (s, 6H), 3.88–3.83 (m, 1H), 3.63 (s, 1H), 3.54 (d, J = 11.2 Hz, 1H), 3.40 (s, 1H), 3.15–2.89 (m, 2H), 2.74 (d, J = 5.9 Hz, 2H), 2.52 (s, 1H), 2.12–1.93 (m, 4H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 165.8, 153.0, 152.6, 146.0, 141.8, 137.5, 136.9, 135.2, 131.4, 127.2, 126.3, 126.2, 122.3, 119.7, 119.1, 117.7, 115.9, 83.0 (d, J = 165.0 Hz), 73.4, 61.5, 60.6, 59.6, 56.5, 56.2, 39.7, 35.7, 32.0 (d, J = 20.1 Hz), 31.3 (d, J = 5.0 Hz); **HRMS** (ESI) calculated for $C_{31}H_{37}FN_3O_5S^+$ ([M + H^+]) 582.2432, found: 582.2454.

(3S,5S)-1-allyl-5-((5-(3-fluoropropyl)-2,3-dimethoxybenzamido) methyl)pyrrolidin-3-yl *(4-(thiophen*-3-yl*)phenyl)carbamate* ***(41)*** *and (3R,5R)-1-allyl-5-((5-(3-fluoropropyl)-2,3-dimethoxybenzamido)methyl) pyrrolidin*-3-yl *(4-(thiophen*-3-yl*)phenyl)carbamate* ***(43)*****.** Method D. 4o mg light yellow solid, 69% yield. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.51 (s, 1H), 7.71 (s, 1H), 7.61 (s, 1H), 7.54–7.45 (m, 4H), 7.41–7.33 (m, 3H), 6.90 (d, J = 2.2 Hz, 1H), 5.88 (s, 1H), 5.33–5.03 (m, 3H), 4.51 (t, J = 5.9 Hz, 1H), 4.39 (t, J = 5.9 Hz, 1H), 3.98 (s, 3H), 3.92 (s, 3H), 3.80–3.47 (m, 3H), 3.35–3.29 (m, 1H), 3.05–2.84 (m, 2H), 2.75 (dd, J = 8.7, 6.7 Hz, 2H), 2.62–2.41 (m, 2H), 2.12–1.84 (m, 3H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 171.3, 153.2, 152.5, 142.0, 138.1, 137.6, 131.0, 127.0, 126.3, 122.5, 119.6, 119.1, 1155, 83.1 (d, J = 165.0 Hz), 72.8, 61.6, 60.5, 60.0, 55.9, 41.3, 35.1, 32.0 (d, J = 19.7 Hz), 31.4 (d, J = 5.3 Hz); **HRMS** (ESI) calculated for $C_{31}H_{37}FN_3O_5S^+$ ([M + H^+]) 582.2432, found: 582.2421.

(3R,5S)-1-ethyl-5-((5-(3-fluoropropyl)-2,3-dimethoxybenzamido) methyl)pyrrolidin-3-yl *(4-(thiophen*-3-yl*)phenyl)carbamate* ***(47)*****.** Method D. 41 mg light yellow solid, 72% yield. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.45 (s, 1H), 7.57–7.51 (m, 3H), 7.44–7.32 (m, 5H), 6.88 (d, J = 2.1 Hz, 1H), 6.74 (s, 1H), 5.19 (s, 1H), 4.51 (t, J = 5.9 Hz, 1H), 4.40 (t, J = 5.9 Hz, 1H), 3.90 (d, J = 2.4 Hz, 7H), 3.77–3.65 (m, 1H), 3.38 (s, 1H), 3.10–2.83 (m, 2H), 2.77–2.70 (m, 2H), 2.42 (d, J = 29.7 Hz, 2H), 2.15–1.93 (m, 4H), 1.14 (t, J = 7.2 Hz, 3H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 165.8, 153.1, 152.6, 146.1, 141.8, 137.6, 136.9, 131.5, 127.2, 126.4, 126.3, 122.3, 119.7, 119.1, 115.9, 83.2 (d, J = 164.9 Hz), 73.4, 61.5, 61.0, 59.2, 56.2, 47.6, 39.8, 35.7, 32.0 (d, J = 19.7 Hz), 31.3 (d, J = 5.3 Hz), 13.5. **HRMS** (ESI) calculated for $C_{30}H_{37}FN_3O_5S^+$ ([M + H^+]) 570.2432, found: 582.24337.

Receptor Binding Assays: Receptor K_i values were measured using human D_2 (long) and D_3 expressed in HEK cells with [^{125}I]IABN as the radioligand. The binding properties of membrane-associated receptors were characterized by a filtration binding assay [41]. Membrane homogenates were suspended in 50 mM Tris−HCl/150 mM NaCl/10 mM EDTA buffer, pH 7.5, and incubated with [^{125}I]IABN [41] at 37 °C for 60 min, using 20 μM (+)-butaclamol to define the nonspecific binding.

The radioligand concentration was equal to approximately 0.5 ($D_{2/3}$R) times the K_d, and the concentration of the competitive inhibitor ranged over 5 orders of magnitude. For each competition curve, two concentrations of inhibitor per decade were used, and triplicates were performed. Binding was terminated by the addition of ice-cold wash buffer ($D_{2/3}$R, 10 mM Tris−HCl, 150 mM NaCl, pH 7.5; 5-HT1AR, 10 mM Tris−HCl, pH 7.4) and filtration over a glass-fiber filter ($D_{3/2}$R, Pall A/B Glass Fiber Filter). A Packard gamma counter was used to measure the radioactivity. The equilibrium dissociation constant and maximum number of binding sites were generated using unweighted nonlinear regression analysis of data modeled according to the equation describing mass R-binding. The concentration of inhibitor that inhibits 50% of the specific binding of the radioligand (IC_{50}) was determined by using nonlinear regression analysis to analyze the data of competitive inhibition experiments. Competition curves were modeled for a single site, and the IC_{50} values were converted to equilibrium dissociation constants (K_i) using the Cheng and Prusoff [42] correction. Mean K_i ± SEM values are reported for at least three independent experiments.

β-Arrestin Assay: PathHunter™ β-arrestin recruitment assay kit and the Chinese hamster ovary CHO–K1 cell line were purchased from DiscoverX (Fremont, CA). CHO–K1 cells which were over-expressed human D_3 receptor were cultured in assay complete™ cell culture kit 107. Cells were seeded at a density of 25,000 cells per well of a 96-well plate and incubated at 5% CO_2, 37 °C. Two days later, compounds were dissolved in DMSO and diluted with 11-point series in phosphate-buffered saline (PBS). Prepared compounds were added to the cells, and incubated for 30 min at 5% CO_2, 37 °C. Then, cells were treated with 30 nM (EC_{80}) of dopamine, and the plate was incubated for another 90 min. PathHunter™ detection reagent was added to each well, and then the plate was incubated for 80 min at room temperature in the dark. The chemiluminescent signal was measured by a PerkinElmer Enspire plate reader (PerkinElmer, Boston, MA). Data were analyzed by Prism followed by non-linear regression.

Molecular Docking Studies: Method: Docking and molecular dynamic studies were conducted on 23 structurally diverse compounds with D_3R by using the previously reported methods [33,43]. All the

structures were drawn using ChemDraw Professional 15.1 (PerkinElmer Informatics, Inc.). The nitrogen of the pyrrolidine ring of each compound was protonated since the pyrrolidine ring was expected to be protonated at physiological pH. The compound structures were imported to Chem3D Ultra 15.1 (PerkinElmer Informatics, Inc.), and then minimized by using MMFF94 force field calculations. Molecular docking studies were performed via the AutoDock 4.2 plugin on PyMOL (pymol.org) [44]. The X-ray structure of the D_3R (PDB ID 3PBL, Resolution 2.89 Å) was obtained from the RCSB Protein Data Bank (www.rcsb.org). Heteroatoms were removed from the protein structure, followed by adding polar hydrogens. Non-polar hydrogens were removed from the compounds. A grid box with a dimension of 30 × 30 × 28.2 $Å^3$ was applied to the D_3R X-ray structure covering orthosteric and secondary binding sites. The Lamarckian Genetic Algorithm with a maximum of 2,500,000 energy evaluations was used to calculate 100 D_3R-ligand binding poses for each compound. The D_3R−ligand complex that reproduced the crystallographic ligand binding pose and with a good docking score was reported for each compound.

Molecular Dynamics Simulation (MDS): The preparation of molecular dynamics simulation (MDS) was performed on the CHARMM-GUI web-server [45]. The Ligand Reader and Modeler module [46,47] were used to generate the topology and parameter files for each compound. The MDS system with FF19SB force field was built by Bilayer Membrane Builder [48,49]. The protein-ligand complexes obtained from docking studies were aligned to the D_3R structure (PDB ID: 3PBL) obtained from the Orientations of Protein in Membranes (OPM) database [50], and the POPC membrane were placed by the OPM D_3R model. The protein, ligand, and membrane complex were solvated in a TIP3P water box with a volume of 80 × 80 × 112 $Å^3$, and then Monte-Carlo sampling was used to add 0.15 M NaCl to neutralize the simulation system.

The MDS studies were performed via Amber18 [51] on the high-performance computing (HPC) cluster at the Center for Biomedical Image Computing and Analytics at the University of Pennsylvania. The input files of system minimization, 6 steps equilibration, and production run for MDS were generated from the last step of Membrane Builder [48,49] on the CHARMM-GUI web-server [45]. Periodic boundary conditions were used for the MDS studies. SHAKE algorithm was used to constrain bonds involving hydrogen atoms. An energy minimization of 5000 steps was implemented. Then, the minimized system was heated in a 2-step NVT ensemble with constant volume at 310 K for 125 ps with a time step of 1 fs in each step. The system was then equilibrated in a 4-step NPT ensemble at 310 K and 1 atm for a total of 1625 ps (125 ps with 1 fs time step at the first step of NPT ensemble, followed by 500 ps with 2 fs time step at the second to the fourth steps of NPT ensemble). The system minimization and equilibration simulations were performed using pmemd.MPI in Amber18 [51] on 40 CPUs. Five copies of the production simulations were performed for 200 ns with a time step of 2 fs in each copy and computed by using pmemd.cuda Amber18 [51] on NVIDIA P100 GPU.

The 50–200 ns of each production simulation with a total of 7500 frames (1500 frames of each of 5 production simulation copies) for each compound were used for further MDS analysis. The interactions between ligand and protein in the production simulations were calculated by using the software BINANA v2.1 [52].

4. Associated Content

The Supporting Information is available free of charge on the website at DOI:

^1H and ^{13}C NMR spectra.

Author contributions

The manuscript was written through the contributions of all authors. The authors have approved the final version of the manuscript (except for RRL, who passed away during the final editing of this manuscript).

Funding sources

National Institute on Drug Abuse (DA029840) is gratefully acknowledged for financial support.

Notes

The authors declare no competing financial interest.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgment

This work is dedicated to the memory of our longtime friend and collaborator, Robert R. Luedtke. The molecular dynamic simulation studies were conducted on the high-performance computing cluster (https://www.med.upenn.edu/cbica/cubic.html) at the University of Pennsylvania Center for Biomedical Image Computing and Analytics and supported by the National Institutes of Health, Grant Number: 1S10OD023495-01.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2023.115751.

References

[1] T.M. Keck, W.S. John, P.W. Czoty, M.A. Nader, A.H. Newman, Identifying medication targets for psychostimulant addiction: unraveling the dopamine D3 receptor hypothesis, J. Med. Chem. 58 (2015) 5361–5380.
[2] G.M. Leggio, C. Bucolo, C.B.M. Platania, S. Salomone, F. Drago, Current drug treatments targeting dopamine D3 receptor, Pharmacol. Ther. 165 (2016) 164–177.
[3] R.R. Luedtke, C. Rangel-Barajas, M. Malik, D.E. Reichert, R.H. Mach, Bitropic D3 dopamine receptor selective compounds potential antipsychotics, Curr. Pharmaceut. Des. 21 (2015) 3700–3724.
[4] E.V. Gurevich, J.N. Joyce, Distribution of dopamine D3 receptor expressing neurons in the human forebrain: comparison with D2 receptor expressing neurons, Neuropsychopharmacology 20 (1999) 60–80.
[5] M. Morissette, M. Goulet, R. Grondin, P. Blanchet, P.J. Bédard, T.D. Paolo, D. Lévesque, Associative and limbic regions of monkey striatum express high levels of dopamine D_3 receptors: effects of MPTP and dopamine agonist replacement therapies, Eur. J. Neurosci. 10 (1998) 2565–2573.
[6] J. Sun, N.J. Cairns, J.S. Perlmutter, R.H. Mach, J. Xu, Regulation of dopamine D_3 receptor in the striatal regions and substantia nigra in diffuse Lewy body disease, Neuroscience 248 (2013) 112–126.
[7] J. Sun, J. Xu, N.J. Cairns, J.S. Perlmutter, R.H. Mach, Dopamine D1, D2, D3 Receptors, Vesicular monoamine transporter Type-2 (VMAT2) and dopamine transporter (DAT) densities in aged human brain, PLoS One 7 (2012), e49483.
[8] N.P. Visanji, S.H. Fox, T. Johnston, G. Reyes, M.J. Millan, J.M. Brotchie, Dopamine D3 receptor stimulation underlies the development of L-DOPA-induced dyskinesia in animal models of Parkinson's disease, Neurobiol. Dis. 35 (2009) 184–192.
[9] N.D. Volkow, J.S. Fowler, G.J. Wang, J.M. Swanson, Dopamine in drug abuse and addiction: results from imaging studies and treatment implications, Mol. Psychiatr. 9 (2004) 557–569.
[10] R.H. Mach, R.R. Luedtke, Challenges in the development of dopamine D2- and D3-selective radiotracers for PET imaging studies, J. Label. Compd. Radiopharm. 61 (2018) 291–298.
[11] R.K. Doot, J.G. Dubroff, K.J. Labban, R.H. Mach, Selectivity of probes for PET imaging of dopamine D3 receptors, Neurosci. Lett. 691 (2019) 18–25.
[12] L. Farde, E. Ehrin, L. Eriksson, T. Greitz, H. Hall, C.G. Hedstrom, J.E. Litton, G. Sedvall, Substituted benzamides as ligands for visualization of dopamine

receptor binding in the human brain by positron emission tomography, Proc. Natl. Acad. Sci. U.S.A. 82 (1985) 863–3867.
[13] J. Mukherjee, Z.Y. Yang, T. Brown, et al., Preliminary assessment of extrastriatal dopamine D-2 receptor binding in the rodent andnonhuman primate brains using the high affinity radioligand, 18F-fallypride, Nucl. Med. Biol. 26 (1999) 519–527.
[14] S.B. Freedman, et al., Expression and pharmacological characterization of the human D3 dopamine receptor, Pharmacol. Exp. Ther. 268 (1994) 417–426.
[15] P. Seeman, et al., Antiparkinson concentrations of pramipexole and PHNO occupy dopamine D2(high) and D3(high) receptors, Synapse 58 (2005) 122–128.
[16] J.D. Gallezot, et al., Affinity and selectivity of [11C]-(+)-PHNO for the D3 and D2 Receptors in the rhesus monkey brain in vivo, Synapse 66 (2012) 489–500.
[17] S. Wang, T. Che, A. Levit, B.K. Shoichet, D. Wacker, B.L. Roth, Structure of the D2 dopamine receptor bound to the atypical antipsychotic drug risperidone, Nature 555 (2018) 269–273.
[18] E.Y.T. Chien, W. Liu, Q. Zhao, V. Katritch, G. Won Han, M.A. Hanson, L. Shi, A. H. Newman, J.A. Javitch, V. Cherezov, R.C. Stevens, Structure of the human dopamine D3 receptor in complex with a D2/D3 selective antagonist, Science 330 (2010) 1091–1095.
[19] Q. Wang, R.H. Mach, R.R. Luedtke, D.E. Reichert, Subtype selectivity of dopamine receptor ligands: insights from structure and ligand-based methods, J. Chem. Inf. Model. 50 (2010) 1970–1985.
[20] A.H. Newman, T. Beuming, A.K. Banala, P. Donthamsetti, K. Pongetti, A. LaBounty, B. Levy, J. Cao, M. Michino, R.R. Luedtke, Jonathan A. Javitch, L. Shi, Molecular determinants of selectivity and efficacy at the dopamine D3 receptor, J. Med. Chem. 55 (2012) 6689–6699.
[21] M. Michino, T. Beuming, P. Donthamsetti, A.H. Newman, J.A. Javitch, L. Shi, What can crystal structures of aminergic receptors tell us about designing subtype-selective ligands? Pharmacol. Rev. 67 (2015) 198–213.
[22] R.K. Verma, A.M. Abramyan, M. Michino, R.B. Free, D.R. Sibley, J.A. Javitch, J. R. Lane, L. Shi, The E2.65A mutation disrupts dynamic binding poses of SB269652 at the dopamine D2 and D3 receptors, PLoS Comput. Biol. 14 (2018), e1005948.
[23] M. Michino, P. Donthamsetti, T. Beuming, A. Banala, L. Duan, T. Roux, Y. Han, E. Trinquet, A.H. Newman, J.A. Javitch, L. Shi, A single glycine in extracellular loop 1 is the critical determinant for pharmacological specificity of dopamine D2 and D3 receptors, Mol. Pharmacol. 84 (2013) 854–864.
[24] J.R. Lane, P.M. Sexton, A. Christopoulos, Bridging the gap: bitopic ligands of G-protein-coupled receptors, Trends Pharmacol. Sci. 34 (2013) 59–66.
[25] A.H. Newman, F.O. Battiti, A. Bonifazi, Philip S. Portoghese medicinal chemistry lectureship: designing bivalent or bitopic molecules for G-protein coupled receptors. The whole is greater than the sum of its parts, J. Med. Chem. 63 (2016) 1779–1797, 2020.
[26] K.D. Burris, T.F. Molski, C. Xu, E. Ryan, K. Tottori, T. Kikuchi, F.D. Yocca, P. B. Molinoff, Aripiprazole, a novel antipsychotic, is a high-affinity partial agonist at human dopamine D2 receptors, J. Pharmacol. Exp. Therapeut. 2 (2002) 381–389.
[27] S. Vangveravong, Z. Zhang, M. Taylor, M. Bearden, J. Xu, J. Cui, W. Wang, R. R. Luedtke, R.H. Mach, Synthesis and characterization of selective dopamine D_2 receptor ligands using aripiprazole as the lead compound, Bioorg. Med. Chem. 19 (2011) 3502–3511.
[28] Z.D. Tu, S.H. Li, J.Q. Cui, J. Xu, M. Taylor, D. Ho, R.R. Luedtke, R.H. Mach, Synthesis and pharmacological evaluation of fluorine-containing D3 dopamine receptor ligands, J. Med. Chem. 54 (2011) 1555–1564.
[29] A.H. Newman, P. Grundt, G. Cyriac, J.R. Deschamps, M. Taylor, R. Kumar, D. Ho, R.R. Luedtke, *N*-(4-(4-(2,3-Dichloro- or 2- methoxyphenyl)piperazin-1-yl)butyl) heterobiarylcarboxamides with functionalized linking chains as high affinity and enantioselective D3 receptor antagonists, J. Med. Chem. 52 (2009) 2559–2570.
[30] V. Kumar, A. Bonifazi, M.P. Ellenberger, T.M. Keck, E. Pommier, R. Rais, B. S. Slusher, E. Gardner, Z.B. You, Z.X. Xi, A.H. Newman, Highly selective dopamine D3 receptor (D_3R) antagonists and partial agonists based on eticlopride and the D3R crystal structure: new leads for opioid dependence treatment, J. Med. Chem. 59 (2016) 7634–7650.
[31] W. Chu, Z. Tu, E. McElveen, J. Xu, M. Taylor, R.R. Luedtke, R.H. Mach, Synthesis and in vitro binding of *N*-phenyl piperazine analogs as potential dopamine D3 receptor ligands, Bioorg. Med. Chem. 13 (2005) 77–87.
[32] R.H. Mach, Z. Tu, J. Xu, S. Li, L.A. Jones, M. Taylor, R.R. Luedtke, C.P. Derdeyn, J. S. Perlmutter, M.A. Mintun, Endogenous dopamine (DA) competes with the binding of a radiolabeled D3 receptor partial agonist in vivo: a positron emission tomography study, Synapse 65 (2011) 724–732.
[33] C.J. Hsieh, A. Riad, J.Y. Lee, K. Sahlholm, K. Xu, R.R. Luedtke, R.H. Mach, Interaction of Ligands for PET with the dopamine D3 receptor: in silico and in *vitro* methods, Biomolecules 11 (2021) 529.
[34] Y. Huang, R.R. Luedtke, R.A. Freeman, L. Wu, R.H. Mach, Synthesis and structure-activity relationships of naphthamides as dopamine D3 receptor ligands, J. Med. Chem. 44 (2001) 1815–1826.
[35] A.B. Shaik, C.A. Boateng, F.O. Battiti, A. Bonifazi, J. Cao, L. Chen, R. Chitsazi, S. Ravi, K.H. Lee, L. Shi, A.H. Newman, Structure activity relationships for a series of Eticlopride-based dopamine D2/D3 receptor bitopic ligands, J. Med. Chem. 64 (2021) 15313–15333.
[36] J. Chen, B. Levant, C. Jiang, T.M. Keck, A.H. Newman, S. Wang, Tranylcypromine substituted *cis*-hydroxycyclobutylnaphthamides as potent and selective dopamine D3 receptor antagonists, J. Med. Chem. 57 (2014) 4962–4968.
[37] J. Chen, G.T. Collins, Beth Levant, James Woods, J.R. Deschamps, S. Wang, CJ-1639: a potent and highly selective dopamine D3 receptor full agonist, ACS Med. Chem. Lett. 2 (2011) 620–625.
[38] F.O. Battiti, S.A. Zaidi, V. Katritch, A.H. Newman, A. Bonifazi, Chiral cyclic aliphatic linkers as building blocks for selective dopamine D2 or D3 receptor agonists, J. Med. Chem. 64 (2021) 16088–16105.
[39] Z.Y. Yang, J. Mukherjee, N-[(1-Cyclopropylmethyl-2-pyrrolidinyl)methyl]-substituted benzamides: synthesis and dopamine D-2 and D-3 receptor binding affinities, Med. Chem. Res. 9 (1999) 1–8.
[40] M. Gao, M. Wang, B.H. Mock, B.E. Glick-Wilson, K.K. Yoder, G.D. Hutchins, Q. H. Zheng, An improved synthesis of dopamine D2/D3 receptor radioligands [11C] fallypride and[18F]fallypride, Appl. Radiat. Isot. 68 (2010) 1079–1086.
[41] R.R. Luedtke, R.A. Freeman, V.A. Boundy, M.W. Martin, Y. Huang, R.H. Mach, Characterization of 125I-IABN, A novel azabicyclononane benzamide selective for D2-like dopamine receptors, Synapse 38 (2000) 438–449.
[42] Y.C. Cheng, W.H. Prusoff, Relationship between the inhibition constant (KI) and the concentration of inhibitor which causes 50 percent inhibition (I50) of an enzymatic reaction, Biochem. Pharmacol. 22 (1973) 3099–3108.
[43] S.W. Reilly, A.A. Riad, C.-J. Hsieh, K. Sahlholm, D.A. Jacome, S. Griffin, M. Taylor, C.-C. Weng, K. Xu, N. Kirschner, R.R. Luedtke, C. Parry, S. Malhotra, J. Karanicolas, R.H. Mach, Leveraging a low-affinity diazaspiro orthosteric fragment to reduce dopamine D3 receptor (D3R) ligand promiscuity across highly conserved aminergic G-protein-coupled receptors (GPCRs), J. Med. Chem. 62 (2019) 5132–5147.
[44] G.M. Morris, R. Huey, W. Lindstrom, M.F. Sanner, R.K. Belew, D.S. Goodsell, A. J. Olson, AutoDock 4 and AutoDockTools 4: automated docking with selective receptor flexibility, J. Comput. Chem. 30 (2009) 2785–2791.
[45] J. Lee, X. Cheng, J.M. Swails, M.S. Yeom, P.K. Eastman, J.A. Lemkul, S. Wei, J. Buckner, J.C. Jeong, Y. Qi, S. Jo, V.S. Pande, D.A. Case, C.L. Brooks, A. D. MacKerell, J.B. Klauda, W. Im, CHARMM-GUI input generator for NAMD, GROMACS, AMBER, OpenMM, and CHARMM/OpenMM simulations using the CHARMM36 additive force field, J. Chem. Theor. Comput. 12 (2016) 405–413.
[46] S. Jo, T. Kim, V.G. Iyer, W. Im, CHARMM-GUI: a web-based graphical user interface for CHARMM, J. Comput. Chem. 29 (2008) 1859–1865.
[47] S. Kim, J. Lee, S. Jo, C.L. Brooks III, H.S. Lee, W. Im, CHARMM-GUI ligand reader and modeler for CHARMM force field generation of small molecules, J. Comput. Chem. 38 (2017) 1879–1886.
[48] J.B. Klauda, R.M. Venable, J.A. Freites, J.W. O'Connor, D.J. Tobias, C. Mondragon-Ramirez, I. Vorobyov, A.D. MacKerell Jr., R.W. Pastor, Update of the CHARMM all-atom additive force field for lipids: validation on six lipid types, J. Phys. Chem. B 114 (2010) 7830–7843.
[49] R.M. Venable, A.J. Sodt, B. Rogaski, H. Rui, E. Hatcher, A.D. MacKerell, R. W. Pastor, J.B. Klauda, CHARMM all-atom additive force field for sphingomyelin: elucidation of hydrogen bonding and of positive curvature, Biophys. J. 107 (2014) 134–145.
[50] M.A. Lomize, I.D. Pogozheva, H. Joo, H.I. Mosberg, A.L. Lomize, OPM database and PPM web server: resources for positioning of proteins in membranes, Nucleic Acids Res. 40 (2012) D370–D376.
[51] D. Case, Y. Huang, R.C. Walkeret, C. Lin, T.E. Cheatham III, D.J. Mermelstein, C. Simmerling, et al., AMBER 18; 2018, University of California, San Francisco, 2018, p. 47.
[52] J.D. Durrant, J.A. McCammon, BINANA: a novel algorithm for ligand-binding characterization, J. Mol. Graph. Model. 29 (2011) 888–893.