Synthesis, in silico, and in vitro studies of novel dopamine D2 and D3 receptor ligands

Milica Elek1 | Nemanja Djokovic2 | Annika Frank1 | Slavica Oljacic2 | Aleksandra Zivkovic1 | Katarina Nikolic2 | Holger Stark1

1Institute of Pharmaceutical and Medicinal Chemistry, Heinrich Heine University Düsseldorf, Universitaetsstr. 1, Duesseldorf, NRW, Germany
2Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia

Abstract
Dopamine is an important neurotransmitter in the human brain and its altered concentrations can lead to various neurological diseases. We studied the binding of novel compounds at the dopamine D2 (D2R) and D3 (D3R) receptor subtypes, which belong to the D2-like receptor family. The synthesis, in silico, and in vitro characterization of 10 dopamine receptor ligands were performed. Novel ligands were docked into the D2R and D3R crystal structures to examine the precise binding mode. A quantum mechanics/molecular mechanics study was performed to gain insights into the nature of the intermolecular interactions between the newly introduced pentafluorosulfanyl (SF5) moiety and D2R and D3R. A radioligand displacement assay determined that all of the ligands showed moderate-to-low nanomolar affinities at D2R and D3R, with a slight preference for D3R, which was confirmed in the in silico studies. N-{4-[4-(2-Methoxyphenyl)piperazin-1-yl]butyl}-4-(pentafluoro-λ6-sulfanyl)benzamide (7i) showed the highest D3R affinity and selectivity (pKi values of 7.14 [D2R] and 8.42 [D3R]).

KEYWORDS
D2 receptor, D3 receptor, ligands, pentafluorosulfanyl, QM/MM

1 | INTRODUCTION

Disorders as a result of neurodegenerative diseases, such as schizophrenia, Parkinson's disease, Alzheimer's disease, affective disorder, and addictive behavior, have been connected to altered concentrations of neurotransmitters in the human brain, especially dopamine. During the 1960s, dopamine was recognized as an independent neurotransmitter in addition to other well-known catecholamines.[1] Biosynthesis of dopamine, epinephrine, and norepinephrine starts from tyrosine. Dopaminergic neurons do not contain dopamine β-hydroxylase enzymes that convert dopamine to norepinephrine. Catabolic reactions of dopamine include degradation by monoamine oxidase B,[5] catecholamine-O-methyl transferase, as well as partly by monoamine oxidase A.[2-5] On the basis of amino-acid sequences and similarity in signal transduction, dopamine receptors are divided into two different classes: dopamine D1-like receptors that include D1 and D5 receptors and dopamine D2-like receptors that include D2, D3, and D4 receptors.[6-8] All dopamine receptor subtypes belong to the rhodopsin-like A class of the largest group of G-protein-coupled receptors (GPCR), characterized by the presence of seven transmembrane domains. D1-like receptors express a long carboxyl and D2-like receptors express a short carboxyl tail, which is located intracellularly in both receptor subtypes.[9] D1-like receptors signal through Gs protein and enhance the production of cAMP, whereas D2-like receptors activate Gαi that inhibits the production cAMP.
Dopamine D2 receptor (D2R) has also been implicated in the G protein-independent GPCR signaling, involving mediation with β-arrestin 1 and β-arrestin 2, which scaffold the different pathways.  

D1-like receptors are mainly located in the corpus striatum, nucleus accumbens, substantia nigra, olfactory bulb, and frontal cortex. D2-like receptors are mostly found in substantia nigra, hypothalamus, amygdala, and hippocampus, and their density, regional distribution, and synaptic response are affected by various neurological diseases, stress, or drug abuse. Majority of commercially available D2-like receptor ligands have severe side effects due to their low selectivity to the receptors of interest and high affinity toward other off-target receptors. To counter this, efforts have been centered around the design and synthesis of selective D2-like receptor ligands. The main challenges in developing novel ligands as potent pharmacological tools in the treatment of diseases with altered concentration of dopamine are high homology between receptors subtypes (up to 88% of D2R and D3 receptor [D3R] in structurally conserved regions) and almost identical orthosteric binding site (OBS) interaction within two receptors subtypes. Since its revelation, the D3R has been a target of interest in potential pharmacotherapy of addiction and schizophrenia due to its relatively focal localization and its expression in drug-exposed brains. In addition, it has also emerged as a new potential target in the treatment of Parkinson's disease. Although all of the dopamine receptor subtypes show a high level of similarity, it has been shown that dopamine itself has a 100-fold higher affinity at D2R when compared with those at D1R or D3R. In addition, D3R messenger RNA, which is localized predominantly in the islands of Calleja and nucleus accumbens in healthy humans, could be a potential biomarker in early-stage Parkinson's patients. Therefore, serious efforts have been made to find potent, novel, and selective D3R ligands.

A general pharmacophore of D3R antagonists has been described in the early 2000s. It contains four regions: The aromatic, the H-bond acceptor, the linker, and the amine regions. Piperazine has been described as a promising moiety for binding and positioning into OBS of D3R and therefore, is a structural part not only of many commercially available drugs (e.g., lurasidone and cariprazine) but also of preclinical and clinical candidates. The prototype of D3R partial agonists is BP897 (Figure 1), which was developed for the treatment of cocaine abuse, is a potent D3R ligand (Ki = 0.92 nM) that served as a lead compound for many synthesized ligands in this study. In addition, it has been established that ligands containing 4-(2-methoxyphenyl)piperazine and 4-(2,3-dichlorophenyl)piperazine moieties could have beneficial properties for D3R-like receptor binding and lead to the development of new potent selective ligands. These moieties have, therefore, been used in the synthesis of all 10 reported compounds. A novel thermally and chemically stable pentafluorosulfanyl (SF₅) moiety that displays high values of electronegativity and lipophilicity was introduced to compare its effects on affinity and selectivity toward receptors of interest. Due to these beneficial chemical properties, SF₅ can be used as a valuable bioisosteric replacement of the trifluoromethyl and under special circumstances of tert-butyl or nitro group.

To counter this, efforts have been centered around the design and synthesis of selective D2-like receptor ligands. The main challenges in developing novel ligands as potent pharmacological tools in the treatment of diseases with altered concentration of dopamine are high homology between receptors subtypes (up to 88% of D2R and D3 receptor [D3R] in structurally conserved regions) and almost identical orthosteric binding site (OBS) interaction within two receptors subtypes. Since its revelation, the D3R has been a target of interest in potential pharmacotherapy of addiction and schizophrenia due to its relatively focal localization and its expression in drug-exposed brains. In addition, it has also emerged as a new potential target in the treatment of Parkinson's disease. Although all of the dopamine receptor subtypes show a high level of similarity, it has been shown that dopamine itself has a 100-fold higher affinity at D2R when compared with those at D1R or D3R. In addition, D3R messenger RNA, which is localized predominantly in the islands of Calleja and nucleus accumbens in healthy humans, could be a potential biomarker in early-stage Parkinson's patients. Therefore, serious efforts have been made to find potent, novel, and selective D3R ligands.

A general pharmacophore of D3R antagonists has been described in the early 2000s. It contains four regions: The aromatic, the H-bond acceptor, the linker, and the amine regions. Piperazine has been described as a promising moiety for binding and positioning into OBS of D3R and therefore, is a structural part not only of many commercially available drugs (e.g., lurasidone and cariprazine) but also of preclinical and clinical candidates. The prototype of D3R partial agonists is BP897 (Figure 1), which was developed for the treatment of cocaine abuse, is a potent D3R ligand (Ki = 0.92 nM) that served as a lead compound for many synthesized ligands in this study. In addition, it has been established that ligands containing 4-(2-methoxyphenyl)piperazine and 4-(2,3-dichlorophenyl)piperazine moieties could have beneficial properties for D3R-like receptor binding and lead to the development of new potent selective ligands. These moieties have, therefore, been used in the synthesis of all 10 reported compounds. A novel thermally and chemically stable pentafluorosulfanyl (SF₅) moiety that displays high values of electronegativity and lipophilicity was introduced to compare its effects on affinity and selectivity toward receptors of interest. Due to these beneficial chemical properties, SF₅ can be used as a valuable bioisosteric replacement of the trifluoromethyl and under special circumstances of tert-butyl or nitro group. Therefore, our main aim is to develop, synthesize, and in vitro and in silico characterize potent dopamine D2R and D3R ligands, which could be further optimized and in vivo evaluated.

FIGURE 1 Chemical structure of the lead compound BP897

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

To obtain amines 6a–e, two different synthetic methods have been used. In the first synthetic approach (Route I), compound 1a has been alkylated with N-(ω-bromoalkyl)phthalimide derivatives 2a–c to obtain protected amines 4a–c. Consequently, hydrazine as a cleaving reagent has been used for the deprotection of amines. To obtain higher yields as well as to decrease costs of the synthesis, the second synthetic approach (Route II) was introduced, where compounds 1a,b have been alkylated with 4-bromobutanenitrile (3a) and 5-bromovaleronitrile (3b), respectively. Reduction of obtained nitriles led to crude amines 6c–e. Then, the primary amines 6a–e were coupled with a corresponding activated carboxylic acid to amides 7a–j. Both approaches are shown in Scheme 1.

2.2 | Pharmacology

The affinity at human isoform dopamine D2shortR and D3R was determined by radioligand displacement assays, as described before. In brief, radioligand displacement studies on membranes prepared from CHO-K1 cells expressing human dopamine D2shortR or D3R have been performed using [³H]piperone as a radioligand and haloperidol as a standard for unspecific binding. The binding affinities of the synthesized compounds with the corresponding confidence intervals (CIs) as well as the selectivity index (SI) are shown in Table 1.

2.3 | Molecular docking

Molecular docking was used to access the binding modes of the synthesized ligands and to obtain atomistic insight into the observed inhibitory activities. All synthesized compounds were docked into binding pockets of the cocrystal structures of D2R and D3R. In addition, docking scores were evaluated in terms of correlation with observed binding affinities.
2.3.1 | Docking in D3R active site

All ligands were docked into the D3R cocrystal structure (PDB ID: 3PBL) in complex with eticlopride\textsuperscript{[21,51]} (a potent D2R/D3R antagonist) according to the protocol described in Section 4. The docking protocol was validated through redocking of eticlopride and calculation of heavy atoms RMSD (root mean square deviation), which typically should not exceed 2 Å\textsuperscript{[52]} (RMSD eticlopride = 0.67 Å, Figure S1). Pearson's correlation coefficient ($R^2$) and Spearman's rank correlation coefficient ($r_s$) were used to access the correlation between docking and experimental results. Calculated docking scores for our set of ligands significantly correlated with experimental affinity ($pK_i$) measurements for D3R ($R^2 = 0.92$, $r_s = 0.97$, Table S1). High correlation coefficient values justified the reliability of obtained binding modes for ligands.

Predicted binding modes indicate that phenylpiperazine moiety (arylamine head) binds to OBS, with the rest of the structures (arylamide/coumarine tails) extending to the extracellular vestibule/second binding pocket (SBP)\textsuperscript{[21]} (Figures 2a and S2). This is in agreement with previous docking studies of structurally related compounds.\textsuperscript{[52,53]} For all studied ligands, highly conserved residue Asp 110\textsuperscript{3.32} (OBS) formed a salt bridge with positively charged nitrogen from piperazine. This salt bridge interaction is believed to be crucial in binding of ligands at the OBS of D3R, which is in agreement with our docking results.\textsuperscript{[21]}

Comparison of interacting modes between eticlopride and ligand 7i (Figure 1a), as the representative with the highest affinity to the D3R, indicates a similar interaction profile in OBS. Interestingly, $\pi$–alkyl interaction between arylamine heads of the studied ligands and Cys 114\textsuperscript{3.36}, absent in the cocrystal structure with eticlopride (Figure 1a), signifies the importance of this residue in the binding of our series of phenylpiperazine ligands. Residue Cys 114\textsuperscript{3.36} (OBS) has been characterized as important for binding of haloperidol in recent mutagenesis experiments. According to the predicted poses of docked ligands, arylamide/coumarine tails are bound to the extracellular vestibule shaped by Tyr 361\textsuperscript{.39}, Val 86\textsuperscript{2.60}, Leu 89\textsuperscript{2.63}, Glu 90\textsuperscript{2.64}, Gly 93, and Ser 366\textsuperscript{7.35} (Figures 1a and S2). Tyr 361\textsuperscript{3.9} and Glu 90\textsuperscript{2.64} have been characterized in recent combined large-scale high-throughput molecular dynamics study and mutagenesis study as important for binding of GSK598809 (dual D3R/D2R antagonist), which further validates predicted binding modes.\textsuperscript{[54]}
| Name                      | Structure | MW   | $K_i$ (D2R) (nM) (95% CI) | $K_i$ (D3R) (nM) (95% CI) | SI (D2/D3) |
|---------------------------|-----------|------|--------------------------|--------------------------|------------|
| Haloperidol               | ![structure](image1) | 375.9 | 2.61 (2.02; 3.39) | 13.5 (10.4; 17.4) | 0.2 |
| **7a**                    | ![structure](image2) | 448.4 | 105.1 (76.5; 144) | 184 (99.7; 339) | 0.6 |
| **7b**                    | ![structure](image3) | 462.4 | 152 (68.2; 338); 127 (28.1; 570) | 1.2 |
| **7c**                    | ![structure](image4) | 476.4 | 9.45 (4.71; 19.0) | 5.67 (1.88; 17.0) | 1.7 |
| **7d**                    | ![structure](image5) | 490.4 | 13.4 (9.16; 19.7) | 30.7 (12.6; 75.3) | 0.4 |
| **7e**                    | ![structure](image6) | 435.5 | 65.5 (42.6; 101) | 9.04 (6.87; 11.9) | 7.2 |
| **7f**                    | ![structure](image7) | 465.6 | 63.4 (36.5; 110) | 3.90 (1.57; 9.70) | 16.3 |
| **7g**                    | ![structure](image8) | 493.5 | 54.3 (28.2; 105) | 4.96 (2.51; 9.79) | 10.9 |
| **7h**                    | ![structure](image9) | 532.2 | 62.3 (25.5; 152) | 9.34 (5.45; 16.0) | 13.3 |
| **7i**                    | ![structure](image10) | 493.5 | 72.3 (31.3; 167) | 3.52 (1.46; 8.74) | 20.5 |
| **7j**                    | ![structure](image11) | 532.2 | 163 (90.2; 293) | 12.2 (6.69; 22.4) | 6.7 |

*Abbreviations: MW, molecular weight; SI, selectivity index.*
2.3.2 | Docking in the D2R active site

Atypical antipsychotic risperidone was correctly redocked in PDB ID: 6CM4 using the docking protocol described in Section 4 (heavy atoms RMSD 0.62 Å, Figure S3). A correlation between docking scores and experimental affinity (pKᵢ) \( R^2 = 0.32, r_s = 0.73 \), Table S1) was not as good as for D3R docking, but still under the range of medium correlations expected for docking studies. A possible explanation for better correlation found in D3R docking study could be found in recently reported higher flexibility of D2R's extended binding pocket (EBP) as compared with the related part of D3R. This flexibility was previously seen as the main reason why structure-based drug discovery campaigns were less successful in the case of D2R. All the studied ligands showed a similar binding mode to cocrystallized risperidon: arylamino head was docked into OBS, whereas arylamido/coumarine tail was docked into EBP (Figures 2b and S4). A salt bridge was observed between Asp1143.32 and positively charged nitrogen from piperazine. This interaction was previously characterized as fundamental for binding to the OBS of D2R. In addition, two recently reported docking studies on D2R cocrystal structure further support our results regarding predicted binding modes of ligands.

*FIGURE 2* Docking results of 7i into the binding site of (a) D3R and (b) D2R. The left side of the figure corresponds to the three-dimensional (3D) representation of binding sites, whereas the right side of the figure corresponds to the comparative 2D interaction plots obtained for 7i and cocrystal ligands (a, eticlopride; b, risperidone). Encircled residues on 2D interaction plots represent residues that are engaged in interactions with both ligands.
m-substitution is responsible for achieving an optimal interaction with Tyr 408.34 (Figure S5). Our results indicate that p-substitution with a voluminous substituent (e.g., SF5 moiety) in series of similar compounds (four-methylene groups linker) tends to decrease affinity toward D2R due to steric hindrance, whereas it does not affect, to a larger extent, affinity at D3R. This is in accordance with experimental findings regarding the distances between OBS and EBS in D2R and D3R, where this distance is longer in D3R.[21,56]

2.4 | Quantum mechanics/molecular mechanics (QM/MM) calculations

Pentafluorosulfanyl moiety is a relatively novel moiety in medicinal chemistry and only a limited number of compounds containing this moiety have been studied in interaction with biological systems.[47] Furthermore, atomistic details on the interaction between the SF5 group and biological target molecules remain enigmatic, as no co-crystal/nuclear magnetic resonance (NMR) structures have been described so far. Also, there is a lack of detailed molecular modeling studies on SF5 ligands. To the best of the authors’ knowledge, the most common force fields used for biomolecular simulations (e.g., CHARMM and AMBER sets of force fields) do not recognize this moiety or this specific hypervalent sulfur atom type, which hinders the application of classical molecular dynamics simulations. Hybrid QM/MM approaches, where ligand and interacting residues are treated quantum mechanically, whereas the rest of the system (e.g., membrane, solvent, noninteracting residues, etc.) is treated classically, represent viable alternative to overcome limitations of current force fields in studying noncovalent interactions between SF5 and the biomolecule of interest.

To validate predicted docking poses of 7i and to provide more details on nature of SF5 intermolecular interactions with D2R and D3R, we designed a multilevel QM/MM approach. The approach presented here combines a semiempirical level of theory (PM3) with more advanced and computationally demanding DFT calculations (M06-2X functional with def2-TZVP basis set).[59–61] PM3 calculations are inherently faster and can access tens to hundreds of picoseconds (ps) of dynamics. However, M06-2X level of theory has an advantage in implementing higher accuracy in dealing with intermolecular interactions. M06-2X functional has been shown through benchmarking studies to produce a good representation of noncovalent interactions.[59,62]

According to our results, poses of 7i obtained through molecular docking remained stabilized during 100 ps of QM/MM simulations (Figure 3a,b). Compound 7i was stabilized in D2R and D3R through an equilibrium between repulsive and attractive noncovalent interactions (NCIs) (Figure S6a,b). Comparing the initial docking poses and the ones obtained after QM/MM protocol, 7i in D2R slightly moved SF5 moiety toward Pro3627.31 residue and established interactions with it (Figures 3d and S7). This interaction was unseen through molecular

![Figure 3](image-url) Root mean square deviation (RMSD) of atomic positions during 100 ps of QM/MM (quantum mechanics/molecular mechanics) (PM3) simulations, (a) D2R:7i system and (b) D3R:7i system and electrostatic potential (ESP) maps calculated on the M06-2X level of theory for the QM region after QM/MM minimizations, (c) D2R:7i system and (d) D3R:7i system, and after single-point calculations. The blue line in (a) and (b) indicates the RMSD calculated for the ligand atoms, whereas the red line represents the RMSD calculated for the protein backbone.
docking. Contrary to the D3R:7i complex, the D2R:7i complex remained in a similar pose during the course of QM/MM simulation (Figure S7). However, the hydrogen bond between Ser4097.35 of D2R and fluorine of SF₅ moiety predicted by molecular docking (Figure 2b) disappeared after QM/MM, and it was replaced with C–H···F interaction (see below). After QM/MM simulations, we also may note that SF₅ moiety of 7i was encircled with interacting D3R residues, whereas upper fluorine of SF₅ was free and solvent-exposed. Contrary to this, SF₅ moiety of 7i was buried in the sub-pocket of D2R consisting of Tyr4087.34, Pro4057.31, and Ser4097.35 (Figure 3c,d), which was in accordance with molecular docking results (Figure 2). Taken together, results indicate that molecular docking was reasonably accurate in predicting the pose of SF₅ moiety. Nevertheless, QM/MM protocol is indispensable in precise characterization of intermolecular interactions of SF₅.

Electrostatic potentials (ESP) were derived from electron densities and plotted on molecular van der Waals surfaces, to elucidate how protein environment affected electronic density in the ligand. ESP maps obtained after QM/MM calculations of QM regions of protein/ligand complexes (M06-2X level of theory) were compared with ESP maps obtained after single-point calculations of solo ligands in the same conformations (Figure 3c,d, cf. Supporting Information Materials). Results revealed that the protein environment affected the charge distribution of SF₅ moiety, indicating possible intramolecular interactions. Fluorine atoms closer to protein residues experienced more negative electrostatic potential, which could be explained with intramolecular interactions between SF₅ moiety and protein residues (Figure 3c,d).

To gain more details of specific spatial regions and the nature of interactions between proteins and SF₅ moiety of 7i, NCI analysis was performed. Self-consistent field (SCF) densities for NCI analysis were obtained from QM/MM calculations on M06-2X level of theory. The NCI analysis indicated that all the intramolecular interactions between SF₅ moiety and D2R/D3R appear to be in the spectrum of delocalized weak interactions, sign(λ²ρ(r)) between ± 0.01 a.u (Figure 4). No strong stabilizing interactions (e.g., hydrogen bonds) were detected. In the case of D2R, C–H···F–S (Figure 4b,c) and S–F···C=O (Figure 4a) were the most prominent interactions (reduced density gradient, s ≤ 0.3), whereas for the D3R, C–H···F–S (Figure 4d) and S–F···O (Figure 4f) intermolecular interactions were observed as most important (reduced density gradient, s ≤ 0.3). A recent empirical study on fluorine–protein interactions and ¹⁹F NMR isotropic chemical shifts indicated that highly deshielded fluorines (also seen in SF₅ moiety) mainly participate in the formation of intermolecular interactions.
F—C=O orthogonal interactions with carbons from carbonyl groups and in interactions with aliphatic carbons, whereas F—O interaction was detected but classified as less common for deshielded fluorines. Our results are in agreement with these empirical findings.

Furthermore, we evaluated a promolecular approach in characterizing NCIs of SF5. The promolecular method (compared with SCF approach) has an advantage in much faster calculations allowing us to analyze interactions between the whole ligand and all interacting residues (not limited only to the SF5 moiety). Comparative 2D NCI plots (Figure 4) indicated that specific intramolecular interactions obtained through SCF calculations are mainly positioned on similar values of electron density and have similar values of reduced density gradients as for promolecular NCI calculations. 3D NCI plots indicated that promolecular approach successfully reproduced the spatial position of the interactions (Figures 4 and S6). However, in the case of the 7i:D2R complex, the promolecular method predicted the existence of stronger interactions (higher values of electron density) in some cases (Figure 4a,b). After visual inspection of 3D and 2D NCI plots, we concluded that the promolecular approach could be a viable and faster alternative in the analysis of noncovalent interactions between SF5 moiety and D2R and D3R, but some caveats regarding bond strengths should be considered.

Finally, the promolecular method was used to access whole intermolecular interactions between 7i and D2R/D3R (Figure S6). Considering results from both approaches (promolecular and SCF), we may conclude that (pentafluorosulfonyl)phenyl moiety of 7i achieved a larger number of interactions with D2R through SF5 moiety (Figure 4), whereas its interaction with D3R was driven mainly through phenyl moiety (Figure S6). This is in accordance with the results of molecular docking where steric effects of -p SF5 moiety of 7i prevented interaction with Tyr 4087.34 in D2R (see above). In addition, convergence of QM/MM (PM3) simulations was accessed by integrating promolecular densities for each frame. Results indicated well-converged simulations (Figure S6).

### 3 | CONCLUSION

All of the 10 synthesized compounds exhibited nanomolar affinities at dopamine D2R and D3R. Most of them expressed a slight preference for D2R. Compound 7c showed the highest affinity at D2R, pKᵢ (D2R) = 8.02, and 7i showed the highest affinity at D3R, pKᵢ (D3R) = 8.42. Our studies on the structure-activity relationship have determined that the prerequisite for the best affinity toward receptors of interest is the four methylene group linker between the amide and the aryl moiety (7c) pKᵢ (D2R) = 8.02; pKᵢ (D3R) = 8.25. The compound containing five-methylene group linker (7d), pKᵢ (D2R) = 7.87 pKᵢ (D3R) = 7.51, displayed a higher affinity when compared to the compounds that contain three-methylene (7b), pKᵢ (D2R) = 6.82 pKᵢ (D3R) = 6.90, or two-methylene linker (7a), pKᵢ (D2R) = 6.98; pKᵢ (D3R) = 6.74. Further optimization was, therefore, performed with four-methylene linkers. In vitro data confirmed that the substitution of benzene ring with the coumarin moiety (7e, 7f) resulted in remaining affinity to both D2R and D3R, whereas substitution with the 6-methoxy group at coumarin moiety (7f) resulted in increased affinities to both D2R and D3R. Compound 7i that contains the novel SF5 moiety showed not only the highest affinity at D3R, but also the highest selectivity (SI = 20.5). Introduction of the SF5 moiety (7i) into the para-position of the western part of the molecule led to increased selectivity, more than 10-fold, toward D3R. When the eastern part was changed to 4-(2,3-dichlorophenyl)piperazino substituents (7j), the selectivity was reduced in comparison to 7i, but was still more than threefold toward D3R (when compared to the parent compound 7c).

Our in silico results have confirmed that the protonated phe- nylpiperazine moiety binds to OBS at both D2R and D3R, forming a crucially important salt bridge between positively charged nitrogen on piperazine and Asp1103. The aryamide moiety binds to SBP at D3R and EBP at D2R, which correlates with previously reported results. The compound with the highest affinity and selectivity toward D3R (7i) was particularly challenging due to the new SF5 moiety that is neither synthetically nor computationally fully characterized. To the best of the authors’ knowledge, for the first time, the QM/MM approach was used to access intermolecular interactions of SF5 with biomacromolecule. The QM/MM approach revealed that the protein environment changed electron density distribution in SF5 moiety, whereas the NCI analysis confirmed that all of intramolecular interactions between SF5 moiety and receptors of interest are in the class of weak delocalized interactions. In addition, it has been shown that m-substituted SF5 derivative was optimal for interaction with the binding site of D3R, whereas p-substitution with this moiety led to decreased affinity at D2R due to steric hindrance, which is in accordance with in vitro results obtained.

On the basis of reported results, we conclude that all of the 10 synthesized compounds represent potent, novel pharmacological tools in the treatment of various neurological diseases. Compound 7i that contains a pentafluorosulfonyl moiety has shown the highest in vitro affinity and interesting binding mode toward receptors of interest in this small series. The SF5 group can be taken as a promising substituent in dopamine GPCR ligands, and therefore will be further investigated in other compound classes of aminergic GPCRs.

### 4 | EXPERIMENTAL

#### 4.1 | Chemistry

**4.1.1 | General**

All starting materials were obtained from Sigma Aldrich and Apollo Scientific and used without further purification. Analytical thin-layer
chromatography was carried out on precoated TLC sheets ALUGRAM® Xtra SIL G/UV254 (Macherey-Nagel) with visualization under UV light. Mass spectra have been determined using Advion Mass Express. Atmospheric-pressure chemical ionization (APCI) was used as a method of ionization, operating in positive mode. Data are shown as [M+H]+. Melting points (mp) were determined by Büchi Schmelzpunkt M-565 (Büchi) with an open capillary tube and were uncorrected. 1H and 13C NMR spectra of compounds of interest were measured at Bruker Avance-III 300 (2010) and Bruker Avance-III 600 (2011). Deuterated dimethyl sulfoxide (DMSO-d6) was used as a solvent for NMR and tetramethylsilane was used as a standard. Chemical shifts are given as parts per million (ppm) and reported as follows: s (singlet), d (doublet), dd (double of doublets), t (triplet), q (quartet), p (pentet), or m (multiplet). The coupling constant (J) is given in Hertz (Hz). Purification of compounds has been accomplished using flash chromatography: BioTage Isolera™ Spectra Systems by ACI™ and Assist (Biotage). SNAP KP-Sil and SNAP KP-Sil ULTRA (Biotage) were used as stationary phase and dichloromethane (DCM) and MeOH were used as mobile phase. Solvents have been evaporated using a Rotavapor R II (Büchi) with a PC 3001 VARIO Chemie-Vacuum pump (Vacuubrand) and CVC 3000 Vacuum controlling system. The compounds have been dried with the high-vacuum pump (Hybrid-Pumpe RC 6; Vacuubrand). Compound purities were determined by an elemental analysis Vario MICRO cube elemental analyzer (Elementar Analysensysteme) and liquid chromatography–mass spectrometry (LC-MS): Elute SP (HPG 700) Bruker Daltonic and amaNes® ion trap LC/MS system (ESI-MS).

Method: Alternating ion polarity: on; scan range: m/z: 80–1200; nebulizer: nitrogen, 15 Psi; dry gas: nitrogen, 8 l/min, 200°C; mass range mode: UltraScan; column: Intensity Solo 2 C18 (100 × 2.1 mm); temperature: 50°C; mobile phase: A: water hypergrade for LC-MS with 0.1% formic acid (v/v) (Merck); B: acetonitrile hypergrade for LC. Method of analysis: 0–4–min 98% A, 4–5 min gradient 95% A, 5–9 min 95% A, 9–16 min gradient 5% A, 16–17 min gradient to 0% A, reconditioning: 17–18 min gradient to 98% A, 18–21 min 98% A (see Supporting Information Materials).

The InChI codes of the investigated compounds, together with some biological activity data, are provided as Supporting Information.

4.1.2 General procedure for the synthesis of N-[[o-[(2-methoxyphenyl)piperazin-1-yl]alkyl]phthalimides (4a–c)

To a stirred solution of suitable N-[[o-bromo-alkyl]phthalimides 2a–c (1.2 eq.) in acetonitrile, 1-[(2-methoxyphenyl)piperazine (1a) (1 eq.) and anhydrous K2CO3 (6–12 eq.) were added. The reaction mixture was stirred at reflux temperature overnight. After cooling down the reaction mixture to room temperature, inorganic salts were filtered off and the filtrate was concentrated to dryness. The crude reaction mixture was partitioned between EtOAc and water. The organic layer was separated and the remaining aqueous layer was extracted with EtOAc (3×) and washed with brine. The combined organic layers were dried over anhydrous MgSO4, filtered and concentrated under reduced pressure. The crude mixture was purified by flash column chromatography (sorbent: SiO2, eluent: DCM/MeOH gradient: 100–95%/0–5%) to obtain 4a–c.

2-[[4-[(2-Methoxyphenyl)piperazin-1-yl]ethy]l]isoindoline-1,3-dione (4a)[64,65]

Yellow solid. Yield: 42%. 1H NMR (300 MHz, DMSO-d6) δ 7.93–7.81 (m, 4H), 6.97–6.79 (m, 4H), 3.75 (s, 3H), 3.72 (t, J = 6.5 Hz, 2H), 2.88–3.0 (m, 4H), and 2.62–2.52 (m, 6H). MS (APCI+:) m/z [M+H]+: calculated for [C12H22N3O3]+: 366.2, found: 366.1.

2-[[3-[(2-Methoxyphenyl)piperazin-1-yl]propyl]isoindoline-1,3-dione (4b)[66]

Yellow solid. Yield: 51%. 1H NMR (300 MHz, DMSO-d6) δ 7.93–7.77 (m, 4H), 6.96–6.75 (m, 3H), 6.68 (dd, J = 7.6, 1.5 Hz, 1H), 3.72 (s, 3H), 3.67 (t, J = 6.7 Hz, 2H), 2.75–2.62 (m, 4H), 2.44–2.33 (m, 6H), and 1.77 (p, J = 6.6 Hz, 2H). MS (APCI+:) m/z [M+H]+: calculated for [C22H26N3O3]+: 380.1, found: 380.3.

2-[[4-[(2-Methoxyphenyl)piperazin-1-yl]butyl]isoindoline-1,3-dione (4c)[64,66]

Yellow solid. Yield: 96%. 1H NMR (300 MHz, DMSO-d6) δ 7.93–7.78 (m, 4H), 6.99–6.79 (m, 4H), 3.75 (s, 3H), 3.59 (t, J = 6.9 Hz, 2H), 2.97–2.87 (m, 4H), 2.48–2.41 (m, 4H), 2.32 (t, J = 7.2 Hz, 2H), 1.61 (p, 2H), and 1.45 (p, J = 7.3 Hz, 2H). MS (APCI+:) m/z [M+H]+: calculated for [C23H26N3O3]+: 394.2, found: 394.2.

4.1.3 General procedure for the synthesis of o-[(2-methoxyphenyl)piperazin-1-yl]alkylamines (6a–c): Route I

To a stirred solution of N-[[o-[(2-methoxyphenyl)piperazin-1-yl]alkyl]phthalimide 4a (0.76 mmol), 4b (2.24 mmol), and 4c (2.82 mmol) in 30 ml of MeOH, 0.5 ml of hydrazine monohydrate (64–65% eq. solution) was added and stirred under reflux for 2h. After 2 h, 5 ml of 2 M HCl was added to the hot solution and the reaction mixture was stirred at reflux temperature for another hour. After cooling down to room temperature, the reaction mixture was filtered, and the filtrate was concentrated to dryness. Then, 20 ml of 2 M NaOH was added to the concentrated filtrate and residues were washed with water. Extraction was performed with EtOAc and water. The organic layer was separated and the remaining aqueous layer was extracted with EtOAc (3×) and washed with brine. The combined organic layers were dried with anhydrous MgSO4, filtered and concentrated under reduced pressure. Crude products were purified by flash column chromatography (sorbent: SiO2, eluent: DCM/MeOH/NH3).

2-[[4-[(2-Methoxyphenyl)piperazin-1-yl]ethanamine (6a)[64,65,67]

Yellow oil. Yield: 46%. 1H NMR (300 MHz, DMSO-d6) δ 7.03–6.76 (m, 4H), 3.76 (s, 3H), 3.02–2.89 (br s, 4H), 2.74 (d, J = 6.7 Hz, 2H), 2.46–2.35 (m, 2H), and 1.81–1.73 (m, 6H). MS (APCI+:) m/z [M+H]+: calculated for [C13H22N3O]+: 236.2, found: 236.4.
Yellow oil. Yield: 35%. \(^1\)H NMR (300 MHz, DMSO-d\(_6\)): 7.12–6.78 (m, 4H), 3.76 (s, 3H), 2.95–3.00 (br s, 4H), 2.84–2.75 (m, 2H), 2.42–2.32 (m, 2H), and 2.01–1.60 (m, 8H). MS (APCI[+]) \(m/z\) [M+H\(^{+}\)]: calculated for [C\(_{14}\)H\(_{22}\)N\(_3\)O\(_2\)]\(^+\): 262.0 and 262.2, found: 262.0 and 262.14.

4-[(2-Dichlorophenyl)piperazin-1-yl]butanitrile (5b)

Yellow solid. Yield: 95%. \(^1\)H NMR (300 MHz, DMSO-d\(_6\)): \(\delta\) 7.34–7.27 (m, 2H), 7.14 (dd, \(J = 6.2, 3.5\) Hz, 1H), 3.04–2.91 (br s, 4H), 2.57–2.52 (m, 4H), 2.50–2.51 (m, 2H) 2.42 (t, \(J = 6.8\) Hz, 2H), and 1.75 (p, \(J = 6.9\) Hz, 2H). MS (APCI[+]) \(m/z\) [M+H\(^{+}\)]: calculated for [C\(_{14}\)H\(_{18}\)Cl\(_2\)N\(_3\)]\(^+\): 298.1 and 300.1, found: 299.0 and 300.0.

5-[(2-Methoxyphenyl)piperazin-1-yl]pentanitrile (5c)

Transparent oil. Yield: 88%. \(^1\)H NMR (300 MHz, DMSO-d\(_6\)): \(\delta\) 7.05–6.80 (m, 4H), 3.77 (s, 3H), 3.00–2.91 (br s, 4H), 2.56–2.52 (m, 2H), 2.46–2.50 (m, 4H), 2.34 (t, \(J = 6.5\) Hz, 2H), and 1.70–1.47 (m, 4H). MS (APCI[+]) \(m/z\) [M+H\(^{+}\)]: calculated for [C\(_{14}\)H\(_{23}\)N\(_3\)O\(_2\)]\(^+\): 274.2 and 275.2, found: 274.1 and 275.1.
7.19 (d, J = 8.7 Hz, 1H), 6.97–6.82 (m, 4H), 3.90 (s, 3H), 3.77 (s, 3H), 3.40 (t, J = 6.5 Hz, 2H), 3.02–2.88 (br s, 4H), and 2.63–2.54 (m, 4H).

13C NMR (75 MHz, DMSO-d6) δ 164.28, 157.47, 151.93, 141.21, 131.75, 128.41, 127.95, 123.21, 120.79, 117.85, 112.07, 111.86, 110.23, 57.01, 56.48, 55.27, 53.01, 50.01, and 36.86. Elemental analysis (calculated: found): %C 56.26/55.75, %H 5.85/5.87, and %N 9.37/9.50; mp = 163.4°C; Rf = 0.30 (eluent: DCM/MeOH 95:5). MS (APCI[+] m/z [M+H]+): calculated for [C22H27BrN3O3]+: 448.1; 450.1; and 449.1, found: 440.0; 450.0; and 451.0.

3-Bromo-4-methoxy-N-((3-[(4-[(2-methoxyphenyl)piperazin-1-yl]propyl]benzamide (7b)
White solid. Yield: 30%. 1H NMR (300 MHz, DMSO-d6) δ 8.49 (t, J = 5.5 Hz, 1H), 8.08 (d, J = 2.2 Hz, 1H), 7.88 (dd, J = 8.6, 2.2 Hz, 1H), 7.18 (d, J = 8.7 Hz, 1H), 6.99–6.81 (m, 4H), 3.90 (s, 3H), 3.76 (s, 3H), 3.32–3.24 (br s, 4H), 3.00–2.90 (m, 4H), 2.50–2.60 (m, 4H), 2.38 (t, J = 7.0 Hz, 2H), and 1.70 (p, J = 7.0 Hz, 2H). 13C NMR (75 MHz, DMSO-d6) δ 164.25, 157.42, 151.93, 141.21, 131.67, 128.41, 128.05, 122.30, 120.79, 117.86, 112.04, 111.86, 110.21, 56.47, 55.79, 55.27, 53.02, 50.04, 37.96, and 26.14. Elemental analysis (calculated: found): %C 57.15/56.70, %H 6.10/6.06, and %N 9.09/8.82; mp = 127.6°C; Rf = 0.33 (eluent: DCM/MeOH 95:5). MS (APCI[+] m/z [M+H]+): calculated for [C22H23BrN3O3]+: 462.1 and 464.1, found: 462.1 and 464.1.

6-Methoxy-N-[(4-[(2-methoxyphenyl)piperazin-1-yl]butyl)-2-oxo-6-chromene-3-carboxamide (7f)
Light orange solid. Yield: 67%. 1H NMR (300 MHz, DMSO-d6) δ 8.82 (s, 1H), 8.73 (t, J = 5.7 Hz, 1H), 7.56 (d, J = 3.0 Hz, 1H), 7.46 (d, J = 9.1 Hz, 1H), 7.34 (dd, J = 9.1, 3.0 Hz, 1H), 6.99–6.81 (m, 4H), 3.82 (s, 3H), 3.76 (s, 3H), 3.36 (s, 2H), 2.95 (br s, 4H), 2.51 (m, 4H), 2.36 (s, 2H), and 1.54 (s, 3H). 13C NMR (75 MHz, DMSO-d6) δ 161.03, 160.53, 155.92, 151.93, 148.31, 147.11, 128.11, 122.30, 121.86, 120.79, 119.24, 117.84, 117.23, 117.05, 111.86, 111.79, 56.65, 55.82, 55.25, 52.95, 49.98, 40.54, 26.90, and 23.60. Elemental analysis (calculated: found): %C 67.08/66.81, %H 6.71/6.67, and %N 9.03/9.80; mp = 140.4°C; Rf = 0.31 (eluent: DCM/MeOH 95:5). MS (APCI[+] m/z [M+H]+): calculated for [C26H35N3O3]+: 466.2 and 467.2, found: 466.3 and 467.1.

N-[4-[(2-Methoxyphenyl)piperazin-1-yl]butyl]-3-(pentafluorool-α6-sulfanyl)benzamide (7g)
White solid. Yield: 42%. 1H NMR (300 MHz, DMSO-d6) δ 8.82 (t, J = 5.6 Hz, 1H), 8.32 (d, J = 1.9 Hz, 1H), 8.19–8.02 (m, 2H), 7.73 (t, J = 8.0 Hz, 1H), 6.99–6.83 (m, 4H), 3.76 (s, 3H), 3.33–3.28 (m, 2H), 3.02–2.88 (br s, 4H), 2.52–2.60 (m, 4H), 2.38 (t, J = 6.8 Hz, 2H), and 1.64–1.45 (m, 4H). 13C NMR (75 MHz, DMSO-d6) δ 164.01, 152.78, 151.92, 141.16, 135.66, 131.06, 129.77, 128.21, 124.28, 122.33, 120.79, 117.82, 111.86, 57.44, 55.25, 52.92, 49.89, 26.86, and 23.64. Elemental analysis (calculated: found): %C 53.54/53.24, %H 5.72/5.87, %N 8.51/8.25, and %S 6.50/6.24; mp = 123.2°C; Rf = 0.49 (eluent: DCM/MeOH 9:1). MS (APCI[+] m/z [M+H]+): calculated for [C22H23F3N3O3S]+: 494.2, found: 494.9.

N-[4-[(2-Dichlorophenyl)piperazin-1-yl]butyl]-3-(pentafluoro-α6-sulfanyl)benzamide (7h)
Beige solid. Yield: 39%. 1H NMR (300 MHz, DMSO-d6) δ 8.80 (t, J = 5.3 Hz, 1H), 8.31 (d, J = 2.1 Hz, 1H), 8.22–8.02 (m, 2H), 7.72 (t, J = 8.0 Hz, 1H), 7.34–7.24 (m, 2H), 7.17–7.06 (m, 1H), 3.30 (t, J = 5.9 Hz, 2H), 3.06–2.89 (br s, 4H), 2.51–2.55 (m, 4H), 2.36 (t, J = 6.7 Hz, 2H), and 1.68–1.41 (m, 4H). 13C NMR (75 MHz, DMSO-d6) δ 164.01, 153.1, 151.18, 135.67, 132.58, 131.06, 129.77, 128.38, 128.21, 125.96, 124.50, 124.29, 119.45, 57.39, 52.77, 50.61, 40.03, 26.88, and 23.75. LC-MS (ESI[+] m/z) = 95.63%; mp = 104.8°C; Rf = 0.33 (eluent: DCM/MeOH 95:5). MS (APCI[+] m/z [M+H]+): calculated for [C22H23Cl2F3N3O3S]+: 532.1; 533.1; and 534.1, found: 532.1; 533.1; and 534.1.
N-4-[4-[2-Methoxyphenyl]piperazin-1-yl]butyl]-4-[pentafluoro-λ6-sulfanyl]benzamide (7)

White solid. Yield: 46%. 1H NMR (300 MHz, DMSO-d6) δ 8.74 (t, J = 5.7 Hz, 1H), 8.02 (s, 4H), 6.95–6.83 (m, 4H), 3.76 (s, 3H), 3.29 (t, J = 6.4, 5.1 Hz, 2H), 3.01–2.88 (br s, 4H), 2.45–2.50 (m, 4H), 2.35 (d, J = 8.5 Hz, 2H), and 1.64–1.45 (m, 4H). 13C NMR (75 MHz, DMSO-d6) δ 164.43, 151.93, 141.24, 138.17, 128.27, 125.94, 122.28, 120.79, 117.82, 111.87, 57.55, 55.27, 52.99, 50.02, 40.03, 26.90, and 23.74.

Elemental analysis (calculated/found): %C 53.54/53.50, %H 5.72/5.68, %N 8.51/8.38, and %S 6.50/6.34; mp = 137.7°C; Rf = 0.33 (eluent: DCM/MeOH 9:1). MS (APCI[+]) m/z [M+H]+: calculated for [C22H29F5N3O2S]+: 494.2, found: 494.9.

4.2 | Pharmacological/biological assays

Radioligand displacement assays hD2R and hD3R have been performed to determine nonspecific binding. As say procedures: For all compounds, selection of dominant microspecies at physiological pH 7.4 was performed using the Marvin Sketch 5.5.10 program. In the next step, the structures of all dominant forms were preoptimized with the semiempirical/PM3 (parameterized model revision 3) method.[59] The minimized structures were then refined by using a more precise quantum chemical Hartree–Fock/3-21G method[82] for geometry optimization employing Gaussian 09 software included in Chem3D Ultra 7 program. Docking procedure: The binding site was defined as residues within 6 Å from cocrysal ligands, and the number of genetic algorithm runs was set to 30, with maximum flexibility accounted for ligands. GoldScore was chosen as the scoring function, according to the lowest RMSD in redocking experiments and 2D interaction plots were generated using LigPlot+ software.[83]

4.3 | Molecular modeling

4.3.1 | Molecular docking

For molecular docking, GOLD 5.6.3 software was used.[75] All ligands were docked into cocrystal structures of D2R (PDB ID: 6CM4) and D3R (PDB ID: 3PBL). Protein preparation included the following steps: Lysozyme residues were removed manually and seven alanine residues were inserted using Modeler software.[76] Hydrogen atoms were added to proteins using the PlayMolecule Protein Prepare procedure.[77] Proteins were inserted in the POPC membrane using a membrane builder from CHARMM-GUI,[78] protein–ligand complexes were subjected to steepest descent energy minimization protocol in sander suite of Amber 2018 software[79] using Amber ff14sb and GAFF2 force fields.[80,81] Ligands for docking were prepared by the following procedure: For all compounds, selection of dominant microspecies at physiological pH 7.4 was performed using the Marvin Sketch 5.5.10 program. In the next step, the structures of all dominant forms were preoptimized with the semiempirical/PM3 (parameterized model revision 3) method.[59] The minimized structures were then refined by using a more precise quantum chemical Hartree–Fock/3-21G method[82] for geometry optimization employing Gaussian 09 software included in Chem3D Ultra 7 program. Docking procedure: The binding site was defined as residues within 6 Å from cocrysal ligands, and the number of genetic algorithm runs was set to 30, with maximum flexibility accounted for ligands. GoldScore was chosen as the scoring function, according to the lowest RMSD in redocking experiments and 2D interaction plots were generated using LigPlot+ software.[83]
Equation (1), represents a simple function of electron density ($\rho$) and its gradient. It reflects local inhomogeneity of the electron density through points of space. In regions far from the molecule, in which the density decays to zero exponentially, the reduced gradient will have very large positive values. However, values of RDS approach zero in the cases of covalent bonds and NCIs. Lower densities and smaller gradients are usually associated with the NCIs, and higher densities and smaller gradients correspond to the covalent bonds:

$$s(r) = \frac{1}{C_4} \frac{\left|\nabla \rho(r)\right|}{\rho(r)^{4/3}}.$$  

(1)

From a practical point of view, NCIplot analyzes only domains of weak electron density and low reduced density gradients (NCIs). Types of NCIs (hydrogen bond, van der Waals interactions, or steric clashes) are determined using Laplacian of the density. Namely, the sign of the second eigenvalues of the Hessian matrix ($\lambda_2$) can discriminate between different NCIs. The negative sign $\lambda_2$ with higher $\rho$ values (>0.01 a.u.) usually corresponds to the strong stabilizing interactions (e.g., hydrogen bonds), whereas positive $\lambda_2$ and $\rho$ values <0.01 a.u. usually correspond to strong repulsive interactions. $\rho$ values around 0 correspond to delocalized weak interactions (e.g., van der Waals interactions). Around zero, the sign of $\lambda_2$ is unstable and does not reflect the stabilizing or destabilizing nature of such interactions. Higher density corresponds to the stronger interactions and does not reflect the stabilizing or destabilizing nature of such interactions. Higher density corresponds to the stronger interactions and does not reflect the stabilizing or destabilizing nature of such interactions.

NCIs were analyzed in the terms of the 2D NCI plots of $s$ versus $\rho \times \text{sign}2$, 3D NCI plots (isosurfaces), and integrals of electron density ($\rho^2$). The cut-off value of $s \leq 0.3$ was used to plot gradients in 3D space and to generate isosurfaces of well-defined density values. Considering higher computational time required for calculation of NCI from SCF calculations (QM/MM), SCF-derived gradients were used to specifically access interactions of SF5 moiety with protein residues and to compare results with a computationally cheaper promolecular approach. The promolecular approach was further used to access interactions of whole ligands with proteins. Integrals of promolecular densities ($\rho^2$) of three specific regions, sign ($\lambda_2$)$\rho(r)$ between −0.05 and 0.01, sign($\lambda_2$)$\rho(r)$ between −0.01 and 0.01, and sign($\lambda_2$)$\rho(r)$ between 0.01 and 0.05, across QM/MM trajectory were used to quantitatively access convergence of QM/MM simulations.[87,88]

ACKNOWLEDGMENTS

The authors would like to express their gratitude to the German Academic Exchange Service (Deutscher Akademischer Austauschdienst) for Milica Elek. They would like to thank the European Cooperation in Science and Technology (COST) COST Actions CA15135, CA18133, CA18240, and German Research Foundation (Deutsche Forschungsgemeinschaft-DFG: INST 208/664-1 FUGG) for financial support. Numerical simulations were run on the PARADOX-IV supercomputing facility at the Scientific Computing Laboratory, National Center of Excellence for the Study of Complex Systems, Institute of Physics Belgrade, supported in part by the Ministry of Education, Science, and Technological Development of the Republic of Serbia. Nemanja Djokovic, Slavica Oljagic, and Katarina Nikolic acknowledge the Ministry of Science and Technological Development of the Republic of Serbia, Faculty of Pharmacy UB Contract No. 451-03-68/2020-14/200161.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

ORCID

Milica Elek https://orcid.org/0000-0002-9094-8991
Nemanja Djokovic https://orcid.org/0000-0001-9972-3492
Annika Frank https://orcid.org/0000-0002-1637-2699
Slavica Oljagic https://orcid.org/0000-0001-9128-6072
Aleksandra Zivkovic https://orcid.org/0000-0001-5034-7916
Katarina Nikolic https://orcid.org/0000-0002-3656-9245
Holger Stark https://orcid.org/0000-0003-3336-1710

REFERENCES

[1] A. Carlsson, M. Lindqvist, T. Magnusson, B. Waldeck, Science 1958, 127, 471.
[2] O. Resnick, F. Elmadjian, J. Clin. Endocrinol. Metab. 1957, 28.
[3] E. H. Labrosse, J. Axerod, S. Kety, Science 1958, 267.
[4] M. Huotari, J. A. Gogos, M. Kraytigou, O. Koponen, M. Forsberg, A. Raasakja, A. Hyttinen, P. T. Ma, Eur. J. Neurosci. 2002, 15, 246.
[5] J. Smythies, Biochim. Biophys. Acta 1998, 1380, 159.
[6] D. R. Sibley, F. J. Monson, Trends Pharmacol. Sci. 1992, 13, 61.
[7] P. H. Andersen, J. A. Gogos, M. D. Bates, A. Deary, P. Falardeau, S. E. Senogles, M. G. Caron, Trends Pharmacol. Sci. 1990, 11, 231.
[8] O. Civelli, J. R. Bunzow, D. K. Grandy, Annu. Rev. Pharmacol. Toxicol. 1993, 33, 281.
[9] S. Maramai, S. Gemma, S. Brogi, G. Campiani, S. Butini, H. Stark, M. Brindisi, Front. Neurosci. 2016, 10, 451.
[10] V. T. Seeman, Trends Pharmacol. Sci. 1994, 15, 4.
[11] D. Vallone, R. Picetti, E. Borrelli, Neurobiol. Behav. Rev. 2000, 24, 125.
[12] N. M. Urs, S. M. Peterson, M. G. Caron, Biol. Psychiatry 2017, 81, 78.
[13] G. R. Beaulieu JM, Pharmacol. Rev. 2008, 50, 143.
[14] Y. Miyamoto, S. Katayama, N. Shimetaka, A. Nishi, T. Fukuda, Brain Struct. Funct. 2018, 223, 4275.
[15] C. de Mei, M. Ramos, C. Itaka, Curr. Opin. Pharmacol. 2009, 9, 53.
[16] D. Gagnon, S. Petryszyn, M. G. Sanchez, C. Bories, J. M. Beaulieu, Y. de Koninck, A. Parent, M. Parent, Sci. Rep. 2017, 7, 1.
[17] D. Elgueta, M. S. Aymerich, F. Contreras, A. Montoya, M. Celorrio, E. Rojo-Bustamante, E. Riquelme, H. González, M. Vásquez, R. Franco, R. Pacheco, Neuropharmacology 2017, 113110.
[18] A. E. Moritz, R. B. Free, D. R. Sibley, Cell. Signal. 2018, 41, 75.
[19] F. Boeckler, H. Lang, P. Gmeiner, J. Med. Chem. 2005, 48, 694.
[20] L. Shi, J. A. Javitch, Annu. Rev. Pharmacol. Toxicol. 2002, 42, 437.
[21] E. Y. T. Chien, W. Liu, Q. Zhao, V. Katrich, G. W. Han, M. A. Hanson, L. Shi, A. H. Newman, J. A. Javitch, V. Cherezov, R. C. Stevens, Science 2010, 330, 1091.
[22] S. Wang, T. Che, A. Levit, B. K. Shoichet, D. Wacker, B. L. Roth, Nature 2018, 555, 269.
[23] P. Sokoloff, B. le Foll, Eur. J. Neurosci. 2017, 45, 2.
[24] A. H. Newman, P. Gmeiner, M. A. Nader, J. Med. Chem. 2005, 48, 366.
[25] G. A. Prieto, J. Cent. Nerv. Syst. Dis. 2017, 9, 177.
[26] P. Yang, J. S. Pehrsson, T. S. Benzer, J. C. Morris, J. Xu, Ageing Res. Rev. 2020, 57, 100994.
[84] F. Neese, Wiley Interdiscip. Rev.: Comput. Mol. Sci. 2018, 8, 4.
[85] An extensible interface for QM/MM molecular dynamics with AMBER-Abstract
[86] J. Contreras-Garcia, E. R. Johnson, S. Keinan, R. Chaudret, J. P. Piquemal, D. N. Beratan, W. Yang, J. Chem. Theory Comput. 2011, 7, 625.
[87] R. Chaudret, B. de Courcy, J. Contreras-Garcia, E. Gloaguen, A. Zehnacker-Rentien, M. Mons, J. P. Piquemal, Phys. Chem. Chem. Phys. 2014, 16, 9876.
[88] R. Laplaza, F. Peccati, R. A. Boto, C. Quan, A. Carbone, J. P. Piquemal, Y. Maday, J. Contreras-Garcia, Wiley Interdiscip. Rev.: Comput. Mol. Sci. 2020, e1497.

SUPPORTING INFORMATION
Additional supporting information may be found online in the supporting information tab for this article.

How to cite this article: M. Elek, N. Djokovic, A. Frank, S. Oljacic, A. Zivkovic, K. Nikolic, H. Stark. Synthesis, in silico, and in vitro studies of novel dopamine D2 and D3 receptor ligands. Arch. Pharm. 2021, 354, e2000486. https://doi.org/10.1002/ardp.202000486