



HAL
open science

Impact of the Substitution Pattern at the Basic Center and Geometry of the Amine Fragment on 5-HT₆ and D₃R Affinity in the 1H-pyrrolo[3,2-c]quinoline Series

Katarzyna Grychowska, Wojciech Pietruś, Ludmila Kulawik, Ophélie Bento, Grzegorz Satala, Xavier Bantreil, Frédéric Lamaty, Andrzej J Bojarski, Joanna Golebiowska, Agnieszka Nikiforuk, et al.

► To cite this version:

Katarzyna Grychowska, Wojciech Pietruś, Ludmila Kulawik, Ophélie Bento, Grzegorz Satala, et al.. Impact of the Substitution Pattern at the Basic Center and Geometry of the Amine Fragment on 5-HT₆ and D₃R Affinity in the 1H-pyrrolo[3,2-c]quinoline Series. *Molecules*, 2023, 28, pp.1096. 10.3390/molecules28031096 . hal-03956220

HAL Id: hal-03956220

<https://hal.science/hal-03956220v1>

Submitted on 25 Jan 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

Article

Impact of the Substitution Pattern at the Basic Center and Geometry of the Amine Fragment on 5-HT₆ and D₃R Affinity in the 1*H*-pyrrolo[3,2-*c*]quinoline Series

Katarzyna Grychowska ^{1,*}, Wojciech Pietruś ², Ludmiła Kulawik ¹, Ophélie Bento ³, Grzegorz Satała ², Xavier Bantreil ^{4,5}, Frédéric Lamaty ⁴, Andrzej J. Bojarski ², Joanna Gołębiowska ², Agnieszka Nikiforuk ², Philippe Marin ³, Séverine Chaumont-Dubel ³, Rafał Kurczab ² and Paweł Zajdel ¹

¹ Faculty of Pharmacy, Jagiellonian University Medical College, 9 Medyczna Str., 30-688 Kraków, Poland

² Maj Institute of Pharmacology, Polish Academy of Sciences, 12 Smętna Str., 31-324 Kraków, Poland

³ Institut de Génomique Fonctionnelle, Université de Montpellier, CNRS INSERM, 34094 Montpellier, France

⁴ IBMM, Université de Montpellier, CNRS, ENSCM, 34094 Montpellier, France

⁵ Institut Universitaire de France (IUF), 75005 Paris, France

* Correspondence: katarzyna.grychowska@uj.edu.pl

Abstract: Salt bridge (SB, double-charge-assisted hydrogen bonds) formation is one of the strongest molecular non-covalent interactions in biological systems, including ligand–receptor complexes. In the case of G-protein-coupled receptors, such an interaction is formed by the conserved aspartic acid (D3.32) residue and the basic moiety of the aminergic ligand. This study aims to determine the influence of the substitution pattern at the basic nitrogen atom and the geometry of the amine moiety at position 4 of 1*H*-pyrrolo[3,2-*c*]quinoline on the quality of the salt bridge formed in the 5-HT₆ receptor and D₃ receptor. To reach this goal, we synthesized and biologically evaluated a new series of 1*H*-pyrrolo[3,2-*c*]quinoline derivatives modified with various amines. The selected compounds displayed a significantly higher 5-HT₆R affinity and more potent 5-HT₆R antagonist properties when compared with the previously identified compound **PZ-1643**, a dual-acting 5-HT₆R/D₃R antagonist; nevertheless, the proposed modifications did not improve the activity at D₃R. As demonstrated by the *in silico* experiments, including molecular dynamics simulations, the applied structural modifications were highly beneficial for the formation and quality of the SB formation at the 5-HT₆R binding site; however, they are unfavorable for such interactions at D₃R.

Keywords: 5-HT₆R antagonists; D₃R ligands; dual-acting compounds; molecular dynamics; salt bridge formation



Citation: Grychowska, K.; Pietruś, W.; Kulawik, L.; Bento, O.; Satała, G.; Bantreil, X.; Lamaty, F.; Bojarski, A.J.; Gołębiowska, J.; Nikiforuk, A.; et al. Impact of the Substitution Pattern at the Basic Center and Geometry of the Amine Fragment on 5-HT₆ and D₃R Affinity in the 1*H*-pyrrolo[3,2-*c*]quinoline Series. *Molecules* **2023**, *28*, 1096. <https://doi.org/10.3390/molecules28031096>

Academic Editors: Lee Wei Lim and Luca Aquili

Received: 19 December 2022

Revised: 17 January 2023

Accepted: 18 January 2023

Published: 21 January 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Serotonin type 6 receptor (5-HT₆R) belongs to the family of G-protein-coupled receptors (GPCRs), which has emerged as a promising target for the treatment of cognitive decline associated with neurodegenerative (e.g., Alzheimer's disease and Parkinson's disease) and psychiatric disorders (e.g., depression and schizophrenia).

Apart from coupling to the G_s protein, 5-HT₆R participates in other signaling pathways [1], including the mechanistic target of rapamycin (mTOR) [2], which accounts for the impact of the receptor in some cognition paradigms in rodents and cyclin-dependent kinase 5 (Cdk5) [3], which is involved in the neurogenesis process. One of the characteristic features of this receptor is its high level of constitutive activity, defined as the spontaneous activity of the receptor in the absence of an agonist [4]. In the hippocampus and the frontal cortex, 5-HT₆R are localized on the neuronal dendrites and primary neuronal cilia of glutamatergic, GABA-ergic, and cholinergic neurons [5,6]. The ciliary location is of particular interest, as these sensory organelles are implicated in the neurodevelopmental process.

The pharmacological blockade of 5-HT₆R enhances cholinergic and glutamatergic neurotransmission, indicating that this mechanism is engaged in the improvement of cognitive functions displayed by 5-HT₆R antagonists in animal models [7,8].

Recently, the development of dual-acting agents, which not only could relieve cognitive decline but may also produce antidepressant and anxiolytic effects [9] and antipsychotic [10] and neuroprotective properties [11], has gained considerable attention. A number of compounds combine antagonism at the 5-HT₆R with serotonin type 2A receptor (5-HT_{2A}R) antagonism [12,13], serotonin type 3 receptor (5-HT₃R) antagonism [10], serotonin type 4 receptor (5-HT₄R) agonism [14,15], GABA-A agonism [16] acetylcholinesterase inhibition [17], or monoaminoxidase type B (MAO-B) inhibition [18].

Simultaneous blockade of the dopamine D₃ receptor (D₃R) is one of the promising strategies in the elaboration of 5-HT₆R antagonism-based dual-acting compounds for improved treatment of Alzheimer's disease and other neurodegenerative disorders [19,20]. D₃R is a GPCR localized in the limbic areas of the brain [21]. In addition to coupling to the G_i/o protein, it additionally engages the Cdk5 [22] and mTOR [23] pathways, leading to the enhancement of acetylcholine and glutamate signaling [19,24].

A quest for dual-acting 5-HT₆/D₃R antagonists has been initiated by the identification of compound SB737050 (Figure 1); however, it was burdened with a relatively high affinity for D₂Rs [25]. Subsequently, a 2,3,4,7-tetrahydro-1*H*-pyrrolo[2,3-*h*]isoquinoline derivative **I** displaying a more balanced profile for 5-HT₆ and D₃Rs was described [19].

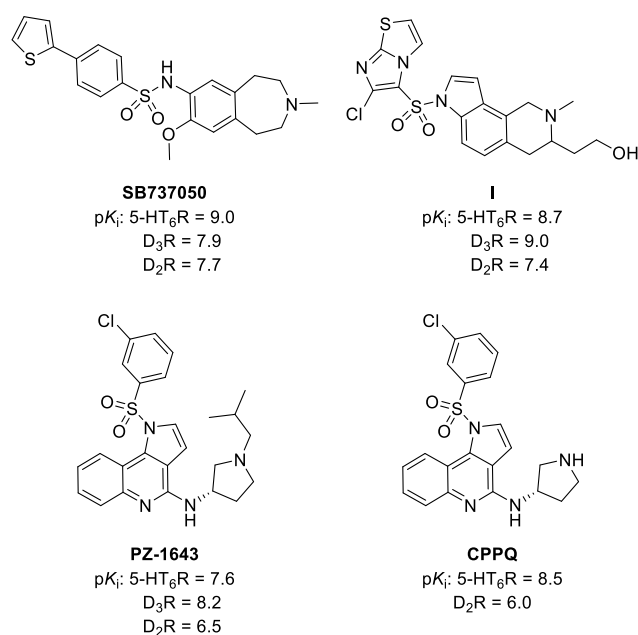


Figure 1. Structures of dual-acting 5-HT₆/D₃R antagonists and 5-HT₆R antagonist CPPQ.

Recently disclosed compound **PZ-1643**, assigned as derivative 19 in [26], a dual-acting 5-HT₆/D₃R antagonist, was designed as a merged ligand in which the selective 5-HT₆R neutral antagonist CPPQ ((*S*)-1-((3-chlorophenyl)sulfonyl)-*N*-(pyrrolidin-3-yl)-1*H*-pyrrolo[3,2-*c*]quinolin-4-amine, disclosed as compound 14 in [27], was combined with an alkyl chain, representing a characteristic structural feature of D₃R antagonists.

To further investigate the impact of the kind of the substituent at the basic nitrogen atom and the geometry of the amine fragment on 5-HT₆R and D₃R affinity, we designed a small series of compound **PZ-1643** analogs. Structural modifications comprised the introduction of various alkyl-derived chains on the basic nitrogen atom and replacement of (*S*)-3-amino-1-Boc-pyrrolidine with enantiomers of 2-(aminomethyl)pyrrolidines and 3-(aminomethyl)pyrrolidines (Figure 2). The affinity for both targets was assessed in the binding experiments at 5-HT₆R and D₃R and was confirmed by molecular dynamics (MD)

evaluation, which determined the quality of the salt bridge (SB) formed with D3.32 of 5-HT₆R and D₃R.

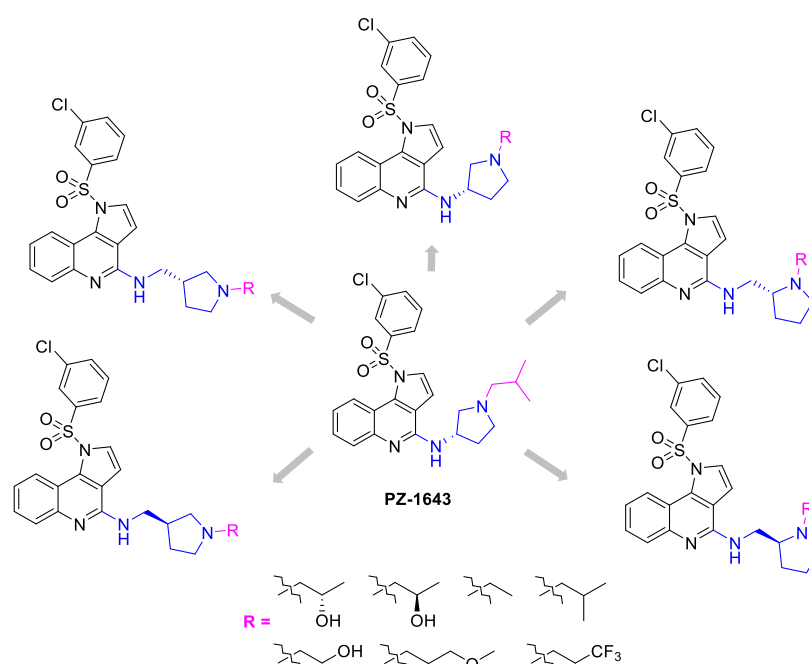
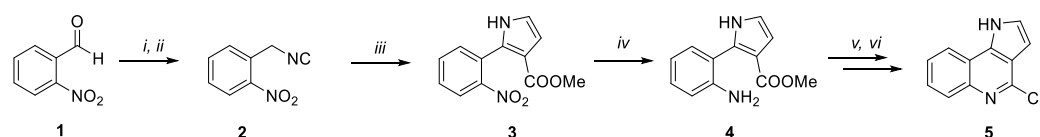


Figure 2. Structural modifications in the amine fragment of novel 1H-pyrrolo[3,2-c]quinoline derivatives.

2. Results and Discussion

2.1. Chemistry

The key 1H-pyrrolo[3,2-c]quinoline synthon **5** was obtained in a multistep synthesis route, following the previously reported protocol (Scheme 1) [28].

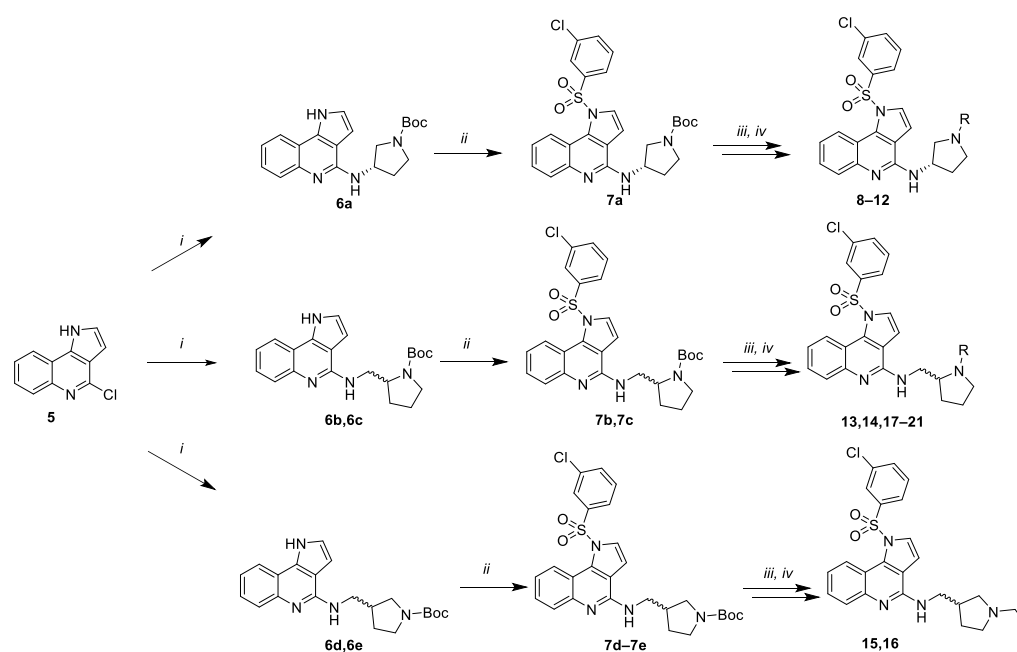


Scheme 1. Synthetic pathway leading to 1H-pyrrolo[3,2-c]quinoline **5**: (i) Formamide, HCOOH, 120 °C, 12 h; (ii) TEA, POCl₃, 0 °C, 30 min; (iii) Methyl propiolate, Ag₂CO₃, dioxane, 80 °C, 30 min; (iv) H₂, Pd/C, MeOH, rt, 2 h; (v) AcOH, *sec*-BuOH, 60 °C, 3 h; (vi) POCl₃, 105 °C, 4 h.

Heating of compound **5** with the excess of respective amine under microwave-assisted conditions yielded Boc-protected amine derivatives, **6a–6e**, which were further coupled with 3-chlorobenzenesulfonyl chloride in the presence of a phosphazene base yielding sulfonyl derivatives **7a–7e** (Scheme 2). Treatment with 1M HCl in methanol afforded secondary amines, for which reductive amination was carried out with respective aldehydes.

2.2. Determination of Affinity of Compounds for 5-HT₆R and D₃R and Assessment of the Impact of the Selected Compounds on 5-HT₆R-Dependent Gs Signaling

The biological evaluation was initiated by assessing the affinity of the compounds for 5-HT₆R in the [³H]-LSD radioligand binding assay. Experiments were performed in a stable HEK293 cell line expressing the human 5-HT₆R [10]. The selected compounds, which showed the highest affinity for the serotonergic target, were further tested for their affinity for D₃R in the screening procedure using [³H]-methylspiperone as the radioligand. Experiments were performed in Chinese hamster ovary (CHO) cells with the stable expression of human D₃R (Eurofins, Celle-L'Escault, France) [29].



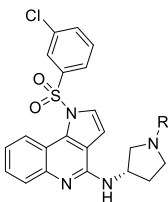
Scheme 2. Synthetic pathway leading to final compounds 8–21: (i) (*S*)-3-amino-1-Boc-pyrrolidine or (*S*)-2-(aminomethyl)pyrrolidine or (*R*)-2-(aminomethyl)pyrrolidine or (*S*)-3-(aminomethyl)pyrrolidine or (*R*)-3-(aminomethyl)pyrrolidine, acetonitrile, 140 °C, 7 h MW; (ii) 3-chlorobenzyl sulfonyl chloride, BTPP, DCM, 0 °C-rt; (iii) 1M HCl/MeOH; (iv) aldehyde: (*S*)-2-hydroxypropanal or (*R*)-2-hydroxypropanal or glycoaldehyde or 3-methoxypropanal or 3,3,3-trifluoropropanal or acetaldehyde or isobutyraldehyde, NaBH₃CN, EtOH, rt, 12h.







Antagonist properties of the most active compounds at 5-HT₆R were evaluated in cAMP cellular assays, and their impact on cAMP production induced by 5-CT was studied [30]. The experiments were performed in 1321N1 cells expressing the human serotonin 5-HT₆R. Finally, the impact of the selected derivatives on agonist-independent 5-HT₆R-operated G_s signaling was tested in NG108-15 cells transiently expressing 5-HT₆R, a cellular model in which 5-HT₆R exhibits a high level of constitutive activity [5].

2.3. Structure–Activity Relationship Analysis

Designing dual-acting 5-HT₆/D₃R ligands in a group of 1*H*-pyrrolo[3,2-*c*]quinolines revealed that the introduction of an isobutyl chain on the nitrogen atom of pyrrolidine present in compound **PZ-1643** maintained the high affinity for 5-HT₆R and was beneficial for the affinity for D₃R [26]. The analysis of molecular dynamics (MD) of the pairs of *R* and *S* enantiomers indicated that the *S* counterpart showed beneficial parameters for the distance and angle of the SB in 5-HT₆R.

In the present study, initial efforts comprised the replacement of the isobutyl chain of the lead compound **PZ-1643** (Figure 2) [26], with more polar substituents (Table 1). Encouragingly, the introduction of 2-hydroxyprop-1-yl enantiomers significantly increased the affinity for 5-HT₆R (**8**, **9** vs. **PZ-1643**). Additionally, a preference for the *R* enantiomer was observed. The introduction of the 2-hydroxyethyl moiety was unfavorable for the interaction with 5-HT₆R and decreased the affinity by threefold (**10** vs. **PZ-1643**). Replacement of the hydroxyl group of **10** with a more hydrophobic trifluoromethyl substituent further decreased the 5-HT₆R affinity (**12** vs. **10**). However, the introduction of 3-methoxyprop-1-yl was well tolerated (**11**). Being the most active compound from the evaluated series, **9** displayed a moderate affinity for D₃R in the screening procedure.

Table 1. Binding data of compounds 8–12 and reference compound **PZ-1643** for 5-HT₆ and D₃ receptors.


Compd	R	K _i 5-HT ₆ R [nM] ^a	%inh Binding to D ₃ R @1μM ^b
PZ-1643		27 ^c	98% (7 nM) ^c
8		11	–
9		6	54%
10		84	–
11		17	25%
12		556	–

^a Mean K_i values based on at least three independent binding experiments (SEM ≤ 18%) performed in HEK293 cells with stable expression of human 5-HT₆R. ^b Percentage displacement values at 10^{−6} M; performed at Eurofins in CHO cells with stable expression of human D₃R. ^c Data taken from [26].

Further studies focusing on the modifications of the geometry of the amine fragment (Table 2) revealed that R enantiomers were preferred for binding to 5-HT₆R (**13** vs. **14**, **15** vs. **16**, **17** vs. **18**, **19** vs. **20**).

Our recent studies revealed that the introduction of an alkyl substituent on the basic nitrogen atom could be beneficial for dual 5-HT₆R/D₃R activity. In the evaluated series the ethyl chain on the basic center of (R)-2-(aminomethyl)pyrrolidiny maintained the affinity for 5-HT₆R when compared with **PZ-1643** (**13** vs. **PZ-1643**). The same modification applied in the 3-(aminomethyl)pyrrolidiny derivatives decreased the affinity for 5-HT₆R (**15**, **16** vs. **PZ-1643**) and indicated the preference of the (R)-2-(aminomethyl)-congener in the interaction with a serotonergic target. Therefore, 2-(aminomethyl)pyrrolidiny was selected as the amine fragment for further diversification and evaluation for 5-HT₆R and D₃R.

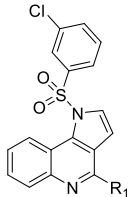
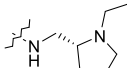
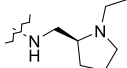
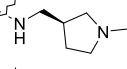
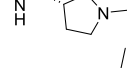
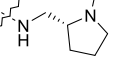
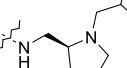
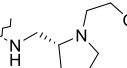
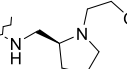
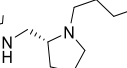
Further structural diversification at the basic nitrogen atom, which involved the replacement of ethyl with a more bulky isobutyl substituent in the R series of 2-(aminomethyl)pyrrolidine (**17**), maintained the affinity for 5-HT₆R compared with compound **PZ-1643**. Subsequent modification, which comprised the introduction of 2-hydroxyethyl and 3-methoxyprop-1-yl, was unfavorable for the interaction with 5-HT₆R (**19**, **20**, **21** vs. **PZ-1643**).

Most active compounds of the evaluated series were functionalized with (R)-2-(aminomethyl)pyrrolidine at position 4 of the 1H-pyrrolo[3,2-c]quinoline core. Among them, derivatives containing an amine fragment substituted with ethyl (**13**) or isobutyl chains (**17**) displayed moderate affinity for D₃R. Therefore, the applied structural modifications were favorable for the 5-HT₆R affinity compared with **PZ-1643**; however, they did not improve the affinity for D₃R.

Taking into account the high affinity of **13** and **17** for 5-HT₆R and the most potent affinity for D₃R among the evaluated compounds, these derivatives were further tested for their antagonist properties at 5-HT₆R in cellular assays performed in recombinant 1321N1 cells expressing the human serotonin 5-HT₆R. The results of these assays were in line with those obtained from the binding experiments, since the evaluated compounds displayed nanomolar antagonist properties at 5-HT₆R (**13**, K_b = 1.2 nM; **17**, K_b = 3.8 nM). Further

evaluation of the impact of the selected derivatives on 5-HT₆R-operated Gs signaling revealed their neutral antagonist properties in this pathway (Figure 3) [10].

Table 2. Binding data of compounds 13–21 for 5-HT₆ and D₃ receptors.

Compd	R ₁	K _i 5-HT ₆ R [nM] ^a	%inh Binding to D ₃ R @1μM ^b
			
13		27	58%
14		72	–
15		38	–
16		64	–
17		27	57%
18		65	–
19		58	–
20		77	–
21		87	–

^a Mean K_i values based on at least three independent binding experiments (SEM ≤ 18%) performed in HEK293 cells with stable expression of human 5-HT₆R. ^b Percentage displacement values at 10^{−6} M; performed at Eurofins in CHO cells with stable expression of human D₃R.

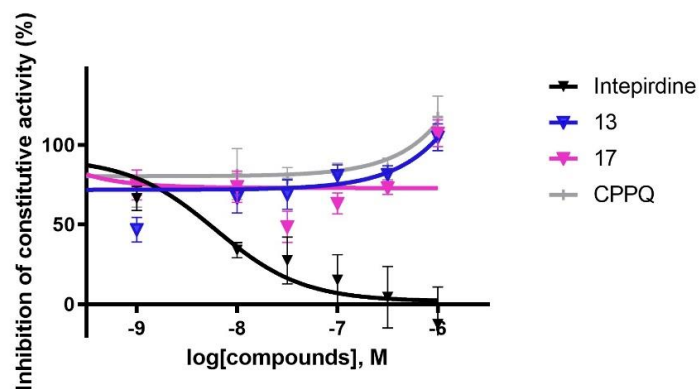


Figure 3. Influence of 13, 17, CPPQ, and intepirdine on 5-HT₆R constitutive activity at Gs signaling in NG108-15 cells.

2.4. In Silico Studies

To further investigate the influence of the kind of the substituent at the basic nitrogen atom and geometry of the amine fragment on the SB parameters (i.e., distance and angle) and receptor affinity, the molecular mechanism of action of selected structurally diverse compounds **9**, **12**, **13**, and **PZ-1643** was evaluated by flexible molecular docking (IFD procedure) to 5-HT₆R (PDB ID: 7XTB) and D₃R (PDB ID: 3PBL). The binding modes were coherent with our previously reported results [26] but did not show clear explanation of the structure–activity relationships (Figure S1). Therefore, a series of MD simulations was performed to determine the features responsible for changes in the potency.

A closer inspection of the MD trajectories showed that various alkyl chains on the nitrogen atom penetrated the narrow hydrophobic subpocket formed by transmembrane (TM) helices 2, 3, and 7 in both receptors. As we proposed in our previous study, the higher binding activity of different derivatives as well as enantiomers originated from the quality of the SB formed with D3.32 [26,31,32]. Thus, the geometric parameters for the interaction with D3.32 observed during molecular dynamics simulations were analyzed (Table 3).

Table 3. The mean geometric parameters of the salt bridge (SB) between basic nitrogen and carboxylic group of D3.32, observed during the molecular dynamics simulations for compounds **9**, **12**, **13**, and **PZ-1643**.

Compound	D ₃ R				Freq. (%)	5-HT ₆ R				Freq. (%)
	SB angle (°)		SB distance (Å)			SB angle (°)		SB distance (Å)		
	N-H...O=	N-H...O [−]	N...O=	N...O [−]		N-H...O=	N-H...O [−]	N...O=	N...O [−]	
PZ-1643	145.7	146.9	3.8	3.8	84	149.8	144.1	3.5	3.9	98
9	135.8	129.6	5.2	4.1	30	131.2	145.7	3.8	3.4	89
12	142.2	143.0	4.8	5.8	0	139.6	129.9	5.3	3.6	77
13	145.0	145.5	4.2	4.3	44	155.9	161.5	3.8	3.2	98

Regarding the interaction with 5-HT₆R, compounds **PZ-1643**, **9**, and **13** displayed the most favorable mean geometric parameters of the SB with D3.32 (i.e., both the distance and the angle of the SB lie in the favorable area of the interaction energy) [33]. In addition, **9** showed a hydrogen bond of the -OH group with T3.29. Molecular dynamics results further showed that the SB is a highly stable interaction, with a frequency of occurrence of more than 89% in complexes (Table 3). However, compound **12**, bearing a 3,3,3-trifluoropropyl chain, showed an acceptable geometry of an SB, but in this case, a part of the salt bridge geometry might be distorted by the unfavorable polar interactions formed by the -CF₃ group and the side chain of Y7.43 and T3.29 (the most distorted side chain among all of Y7.43; Figure 4A). In addition, significant stabilization of all derivatives by the hydrogen bond between S5.44 and the oxygen of sulfonamide groups and the halogen bond formed between the chlorine substituent and the carbonyl oxygen of S4.57 were noted. These interactions are not depicted in Figure 4, since their contribution to the MD trajectory, depending on the derivative, was between 20–40%.

In the case of D₃R, only **PZ-1643** displayed the favorable geometric parameters of the SB. As revealed by MD simulations, the introduction of a 2-hydroxypropyl or 3,3,3-trifluoropropyl chain on the basic nitrogen atom led to the higher distortion during molecular dynamics, and thus less stable complexes (frequencies of the SB occurrence were substantially lower than for **PZ-1643**; Table 3). The conformations of the side chains of the respective amino acids in the D₃R binding side were more distorted than in 5-HT₆R.

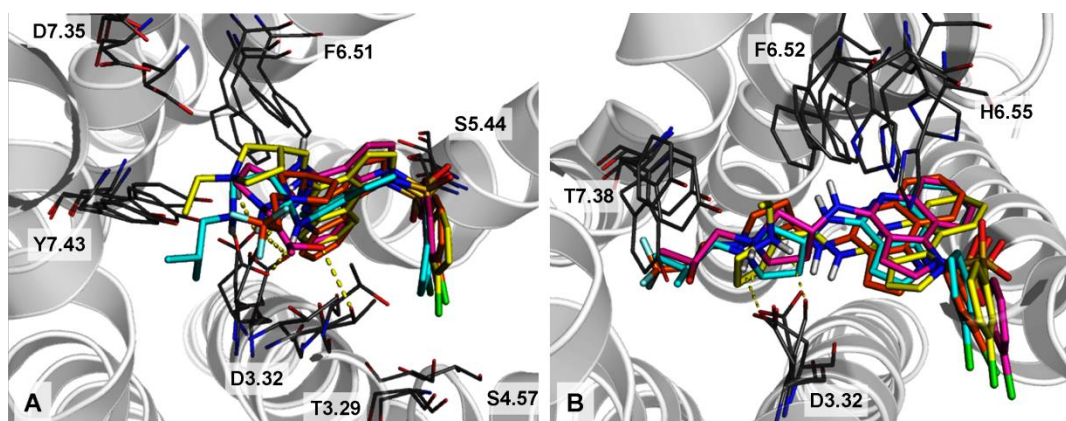


Figure 4. Superposition of the binding modes of **9** (magenta), **12** (orange), **13** (yellow), and **PZ-1643** (cyan) in the 5-HT₆ (A) and D₃R (B) binding sites. Complexes were selected as the most populated conformations obtained by the clustering of the MD trajectories.

3. Experimental Methods

3.1. Chemistry

General Method

The synthesis was conducted at room temperature, unless indicated otherwise. Organic solvents (from Chempur, Piekary Śląskie, Poland) were of reagent grade and were used without purification. All reagents (Sigma-Aldrich (Saint Louis, MI, USA), Fluorochem (Glossop, UK) and TCI (Zwijndrecht, Belgium) were of the highest purity. Column chromatography was performed on silica gel Merck 60 (70–230 mesh ASTM, Darmstadt, Germany).

UPLC and MS were carried out on a system consisting of a Waters Acquity UPLC coupled (Waters Corporation, Milford, MA, USA) to a Waters TQD mass spectrometer. All the analyses were carried out using an Acquity UPLC BEH C18 100 × 2.1 mm² column at 40 °C. A flow rate of 0.3 mL/min and a gradient of (0–100) % B over 10 min was used: eluent A, water/0.1% HCOOH and eluent B, acetonitrile/0.1% HCOOH. Retention times, t_R , were given in minutes. The UPLC/MS purity of all the test compounds and key intermediates was determined to be >95%.

The ¹H NMR and ¹³C NMR spectra were recorded using JEOL JNM-ECZR 500 RS1 (ECZR version) at 500 and 126 MHz, respectively, as well as Bruker Advance III HD at 400 and 100 MHz, respectively. Chemical shifts are reported in parts per million using deuterated solvent for calibration (CD₃OD). The J values are given in Hertz (Hz).

Compounds **1–5**, **6a**, and **7** were obtained according to the previously reported procedure and the analytical data are in accordance with the literature [26,28]. Compounds **13–21** were converted to hydrochloride salts.

(S)-1-((S)-3-((1-((3-chlorophenyl)sulfonyl)-1H-pyrrolo[3,2-c]quinolin-4-yl)amino)pyrrolidin-1-yl)propan-2-ol 8

Pale oil, 53% yield, t_R = 3.84, C₂₄H₂₅ClN₄O₃S, MW 485.00, ¹H NMR (400 MHz, CD₃OD) δ (ppm) 1.17 (d, J = 6.1, 3H), 1.81–1.96 (m, 1H), 2.35–2.50 (m, 2H), 2.52–2.58 (m, 2H), 2.75–2.88 (m, 1H), 2.90–3.09 (m, 2H), 3.26–3.37 (m, 2H), 3.86–3.99 (m, 1H), 4.77–4.85 (m, 1H), 7.12–7.21 (m, 2H), 7.36–7.47 (m, 2H), 7.51–7.59 (m, 1H), 7.60–7.66 (m, 1H), 7.67–7.73 (m, 1H), 7.75–7.79 (s, 1H), 7.86–7.97 (d, J = 3.4 Hz, 1H), 8.71 (d, J = 8.3 Hz, 1H); ¹³C NMR (100 MHz, CD₃OD) δ (ppm) 20.50, 31.44, 49.63, 53.61, 60.66, 63.50, 65.15, 105.92, 114.34, 116.12, 121.49, 122.77, 125.00, 126.5, 127.5, 128.23, 131.01, 134.26, 135.18, 139.60, 146.47, 151.22. Monoisotopic mass: 484.13, [M + H]⁺ = 485.1.

(R)-1-((S)-3-((1-((3-chlorophenyl)sulfonyl)-1H-pyrrolo[3,2-c]quinolin-4-yl)amino)pyrrolidin-1-yl)propan-2-ol 9

Pale oil, 57% yield, $t_R = 3.85$, $C_{24}H_{25}ClN_4O_3S$, MW 485.00, 1H NMR (400 MHz, CD_3OD) δ (ppm) 1.18 (d, $J = 6.26$ Hz, 3H), 1.81–1.94 (m, 1H), 2.36–2.48 (m, 2H), 2.48–2.64 (m, 2H), 2.79 (dd, $J = 9.98, 3.72$ Hz, 1H), 2.92–3.09 (m, 2H), 3.33 (dt, $J = 3.28, 1.59$ Hz, 1H), 3.88–3.99 (m, 1H), 4.80–4.85 (m, 1H), 7.14–7.20 (m, 2H), 7.35–7.44 (m, 2H), 7.54 (d, $J = 8.02$ Hz, 1H), 7.60–7.65 (m, 1H), 7.68–7.73 (m, 1H), 7.75–7.80 (m, 1H), 7.92 (d, $J = 3.72$ Hz, 1H), 8.68–8.76 (m, 1H), 8.75 (d, $J = 8.3$ Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ (ppm) 20.56, 31.51, 49.56, 52.89, 61.25, 63.46, 65.13, 105.96, 114.34, 116.15, 121.47, 122.77, 125.00, 126.48, 127.54, 128.21, 131.00, 134.25, 135.17, 135.28, 139.58, 146.50, 151.25. Monoisotopic mass: 484.13, $[M + H]^+ = 485.1$.

(S)-2-(3-((1-((3-chlorophenyl)sulfonyl)-1H-pyrrolo[3,2-c]quinolin-4-yl)amino)pyrrolidin-1-yl)ethan-1-ol 10

Pale oil, 45% yield, $t_R = 3.86$ min, $C_{23}H_{23}ClN_4O_3S$, MW 470.97, 1H NMR (400 MHz, CD_3OD) δ (ppm) 2.23–2.38 (m, 1H), 2.52–2.62 (m, 4H), 3.27–3.37 (m, 2H), 3.72–3.89 (m, 4H), 7.19 (t, $J = 7.73$ Hz, 1H), 7.22–7.28 (m, 1H), 7.37 (q, $J = 8.15$ Hz, 2H), 7.51 (dd, $J = 8.22, 0.98$ Hz, 1H), 7.60 (d, $J = 8.02$ Hz, 1H), 7.70 (s, 1H), 7.81–7.91 (m, 2H), 8.64 (d, $J = 8.41$ Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD) 29.06, 39.05, 49.95, 53.38, 56.33, 58.53, 106.28, 114.01, 123.05, 125.25, 126.56, 128.37, 129.06, 131.20, 134.61, 134.61, 135.36, 139.29, 149.81. Monoisotopic mass: 470.12, $[M + H]^+ = 471.1$.

(S)-1-((3-chlorophenyl)sulfonyl)-N-(1-(3-methoxypropyl)pyrrolidin-3-yl)-1H-pyrrolo[3,2-c]quinolin-4-amine 11

Pale oil, 30% yield, $t_R = 3.95$, $C_{25}H_{27}ClN_4O_3S$, MW 499.03, 1H NMR (400 MHz, CD_3OD) δ (ppm) 1.76–1.99 (m, 2H), 2.15–2.30 (m, 1H), 2.41–2.58 (m, 1H), 3.03 (s, 2H), 3.11–3.19 (m, 2H), 3.21–3.24 (m, 3H), 3.24–3.29 (m, 1H), 3.41–3.57 (m, 2H), 3.63–3.80 (m, 1H), 4.78–4.83 (m, 1H), 7.02–7.12 (m, 1H), 7.13–7.21 (m, 1H), 7.36 (dt, $J = 10.86, 8.07$ Hz, 2H), 7.46–7.54 (m, 1H), 7.54–7.65 (m, 2H), 7.66–7.70 (m, 1H), 7.83–7.89 (m, 1H), 8.62–8.72 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) 26.13, 29.22, 49.88, 52.89, 53.64, 57.61, 59.11, 69.62, 105.86, 110.01, 114.48, 116.08, 122.25, 122.96, 125.13, 126.46, 127.86, 128.63, 131.08, 134.39, 135.24, 139.48, 150.52. Monoisotopic mass 498.15, $[M + H]^+ = 499.1$.

(S)-1-((3-chlorophenyl)sulfonyl)-N-(1-(3,3,3-trifluoropropyl)pyrrolidin-3-yl)-1H-pyrrolo[3,2-c]quinolin-4-amine 12

Pale oil, 30% yield, $t_R = 4.05$, $C_{24}H_{22}ClF_3N_4O_2S$, MW 522.97, 1H NMR (400 MHz, CD_3OD) δ (ppm) 2.10 (dd, $J = 12.89, 5.15$ Hz, 1H), 2.45–2.55 (m, 1H), 2.54–2.54 (m, 1H), 2.56–2.65 (m, 2H), 2.90–2.99 (m, 1H), 3.06–3.13 (m, 1H), 3.15–3.22 (m, 2H), 3.37 (ddd, $J = 10.02, 8.45, 5.30$ Hz, 1H), 4.76–4.83 (m, 1H), 7.15–7.22 (m, 2H), 7.35–7.43 (m, 2H), 7.51 (ddd, $J = 8.16, 2.00, 1.00$ Hz, 1H), 7.58–7.62 (m, 1H), 7.64 (dd, $J = 8.31, 0.86$ Hz, 1H), 7.72 (t, $J = 1.86$ Hz, 1H), 7.89 (d, $J = 3.72$ Hz, 1H), 8.68 (dd, $J = 8.59, 1.15$ Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD) 21.64, 30.28, 50.03, 53.00, 59.36, 106.17, 114.24, 116.03, 122.34, 122.98, 125.16, 126.47, 128.66, 131.22, 134.49, 135.27, 139.37, 144.72, 150.53, 175.74, 177.46. Monoisotopic mass 522.11, $[M + H]^+ = 523.1$.

(R)-1-((3-chlorophenyl)sulfonyl)-N-((1-ethylpyrrolidin-2-yl)methyl)-1H-pyrrolo[3,2-c]quinolin-4-amine hydrochloride 13

White solid, 47% yield, $t_R = 4.07$, $C_{24}H_{26}Cl_2N_4O_2S$, MW 505.46, 1H NMR (400 MHz, CD_3OD) δ (ppm) 1.09 (t, $J = 7.24$ Hz, 3H), 1.69 (s, 3H), 1.82–1.98 (m, 1H), 2.36 (d, $J = 1.56$ Hz, 1H), 2.48 (d, $J = 1.57$ Hz, 1H), 2.94–3.09 (m, 2H), 3.18 (d, $J = 2.54$ Hz, 1H), 3.46 (dd, $J = 13.69, 5.48$ Hz, 1H), 3.80 (dd, $J = 13.89, 4.89$ Hz, 1H), 7.00–7.11 (m, 2H), 7.21–7.33 (m, 2H), 7.34–7.43 (m, 1H), 7.47–7.56 (m, 2H), 7.64 (s, 1H), 7.76–7.83 (m, 1H), 8.60 (dd, $J = 8.41, 0.78$ Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD) 11.55, 22.29, 28.00, 43.04, 49.18, 53.35, 64.59, 105.95,

114.42, 115.99, 121.63, 122.89, 125.03, 126.27, 128.39, 131.01, 134.28, 135.18, 135.27, 139.54, 146.19, 151.92. Monoisotopic mass 468.14, $[M + H]^+ = 469.4$.

(S)-1-((3-chlorophenyl)sulfonyl)-N-((1-ethylpyrrolidin-2-yl)methyl)-1H-pyrrolo[3,2-c]quinolin-4-amine hydrochloride 14

White solid, 50% yield, $t_R = 4.08$, $C_{24}H_{26}Cl_2N_4O_2S$, MW 505.46, 1H NMR (500 MHz, CD_3OD) δ (ppm) 1.26 (dt, $J = 11.0, 5.5$ Hz, 3H), 1.25–1.30 (s, 1H), 1.82–1.95 (s, 3H), 2.00–2.15 (s, 1H), 2.60–2.72 (s, 1H), 3.12–3.32 (m, 2H), 3.33–3.44 (s, 1H), 3.55–3.70 (m, 1H), 3.90 (dd, $J = 14.0, 5.1$ Hz, 1H), 4.53–4.60 (s, 1H), 7.12–7.17 (s, 1H), 7.19 (dd, $J = 16.8, 9.0$ Hz, 1H), 7.40–7.51 (m, 2H), 7.55–7.70 (m, 3H), 7.77–7.80 (s, 1H), 7.95 (d, $J = 2.6$ Hz, 1H), 8.75 (d, $J = 8.5$ Hz, 1H); ^{13}C NMR (126 MHz, CD_3OD) 11.57, 22.31, 28.02, 43.07, 49.22, 53.34, 64.62, 105.96, 114.42, 116.00, 121.66, 122.89, 125.05, 126.29, 128.41, 131.02, 134.30, 135.21, 135.30, 139.56, 146.21, 151.94. Monoisotopic mass 468.14, $[M + H]^+ = 469.4$.

(R)-1-((3-chlorophenyl)sulfonyl)-N-((1-ethylpyrrolidin-3-yl)methyl)-1H-pyrrolo[3,2-c]quinolin-4-amine hydrochloride 15

White solid, 52% yield, $t_R = 4.09$, $C_{24}H_{26}Cl_2N_4O_2S$, MW 505.46, 1H NMR (500 MHz, CD_3OD) δ (ppm) 1.22–1.29 (m, 1H), 1.31–1.41 (m, 3H), 1.85–2.12 (m, 1H), 2.46 (dd, $J = 6.73, 5.30$ Hz, 1H), 2.90–3.05 (m, 1H), 3.06–3.19 (m, 1H), 3.63 (dd, $J = 5.87, 3.87$ Hz, 1H), 3.66–3.74 (m, 1H), 3.74–3.83 (m, 1H), 3.83–3.90 (m, 1H), 3.83–3.90 (m, 1H), 3.91–4.04 (m, 1H), 3.92–3.95 (m, 1H), 7.45–7.51 (m, 1H), 7.51–7.57 (m, 2H), 7.62–7.71 (m, 2H), 7.83 (dd, $J = 8.02, 1.15$ Hz, 1H), 7.94 (t, $J = 2.00$ Hz, 1H), 8.17 (d, $J = 3.72$ Hz, 2H), 8.79–8.87 (m, 1H); ^{13}C NMR (126 MHz, CD_3OD) δ (ppm) 40.57, 44.46, 50.21, 56.29, 106.54, 115.09, 123.99, 125.68, 126.92, 130.12, 130.60, 131.56, 135.29, 138.78. Monoisotopic mass 468.14, $[M + H]^+ = 469.4$.

(S)-1-((3-chlorophenyl)sulfonyl)-N-((1-ethylpyrrolidin-3-yl)methyl)-1H-pyrrolo[3,2-c]quinolin-4-amine hydrochloride 16

White solid, 52% yield, $t_R = 4.10$, $C_{24}H_{26}Cl_2N_4O_2S$, MW 505.46, 1H NMR (500 MHz, CD_3OD) δ (ppm) 1.22–1.30 (m, 1H), 1.30–1.31 (m, 1H), 1.32–1.41 (m, 3H), 1.34–1.35 (m, 1H), 1.85–2.10 (m, 1H), 2.28–2.52 (m, 1H), 2.52–2.54 (m, 1H), 2.53–2.53 (m, 1H), 2.90–3.05 (m, 1H), 3.05–3.18 (m, 1H), 3.57–3.66 (m, 1H), 3.71 (dd, $J = 5.30, 0.72$ Hz, 1H), 3.75–3.87 (m, 1H), 3.76–3.86 (m, 1H), 3.87–4.02 (m, 2H), 7.46–7.52 (m, 1H), 7.52–7.56 (m, 2H), 7.62–7.71 (m, 2H), 7.80–7.86 (m, 1H), 7.94 (t, $J = 1.86$ Hz, 1H), 8.17 (d, $J = 3.72$ Hz, 2H), 8.10–8.16 (m, 1H), 8.83 (d, $J = 8.59$ Hz, 1H); ^{13}C NMR (126 MHz, CD_3OD) δ (ppm) 40.42, 44.90, 50.19, 56.30, 106.48, 113.13, 115.09, 119.07, 124.00, 126.92, 130.11, 130.60, 131.55, 135.29, 135.76, 138.78. Monoisotopic mass 468.14, $[M + H]^+ = 469.4$.

(R)-1-((3-chlorophenyl)sulfonyl)-N-((1-isobutylpyrrolidin-2-yl)methyl)-1H-pyrrolo[3,2-c]quinolin-4-amine hydrochloride 17

White solid, 34% yield, $t_R = 4.43$, $C_{26}H_{30}Cl_2N_4O_2S$, MW 533.51, 1H NMR (500 MHz, CD_3OD) δ (ppm) 0.95–1.07 (m, 6H), 1.23–1.30 (m, 1H), 1.26–1.30 (m, 1H), 1.98–2.12 (m, 2H), 2.12–2.13 (m, 1H), 2.12–2.22 (m, 2H), 2.42 (dd, $J = 12.74, 6.44$ Hz, 1H), 3.03–3.15 (m, 1H), 3.79–3.89 (m, 1H), 4.00–4.11 (m, 1H), 4.12–4.23 (m, 1H), 4.34–4.47 (m, 1H), 7.45–7.57 (m, 2H), 7.61–7.70 (m, 3H), 7.83 (d, $J = 7.73$ Hz, 1H), 7.93 (s, 1H), 8.17 (d, $J = 3.72$ Hz, 2H), 8.86 (d, $J = 8.02$ Hz, 1H); ^{13}C NMR (126 MHz, CD_3OD) δ (ppm) 19.83, 22.03, 25.51, 27.09, 29.43, 42.61, 54.79, 63.49, 106.83, 115.20, 123.94, 125.68, 126.89, 130.62, 131.57, 135.27, 135.72, 138.77. Monoisotopic mass 496.17, $[M + H]^+ = 497.4$.

(S)-1-((3-chlorophenyl)sulfonyl)-N-((1-isobutylpyrrolidin-2-yl)methyl)-1H-pyrrolo[3,2-c]quinolin-4-amine hydrochloride 18

White solid, 34% yield, $t_R = 4.41$, $C_{26}H_{30}Cl_2N_4O_2S$, MW 533.51, 1H NMR (500 MHz, CD_3OD) δ (ppm) 0.95–1.06 (m, 6H), 1.24–1.31 (m, 1H), 1.96–2.09 (m, 2H), 1.96–2.10 (m, 1H), 2.08–2.22 (m, 2H), 2.09–2.23 (m, 1H), 2.38 (dd, $J = 12.74, 6.44$ Hz, 1H), 3.09 (dd, $J = 12.89, 6.01$ Hz, 1H), 3.74–3.86 (m, 1H), 3.91–4.00 (m, 1H), 4.03–4.14 (m, 1H), 4.21–4.36 (m, 1H),

7.45–7.56 (m, 3H), 7.67 (d, $J = 7.73$ Hz, 2H), 7.83 (d, $J = 8.02$ Hz, 1H), 7.92 (s, 1H), 8.18 (d, $J = 3.72$ Hz, 2H), 8.88 (d, $J = 8.31$ Hz, 1H); ^{13}C NMR (126 MHz, CD_3OD) δ (ppm) 19.81, 22.02, 25.54, 27.12, 29.46, 42.63, 54.82, 63.52, 106.81, 115.22, 123.97, 125.69, 126.90, 130.64, 131.59, 135.29, 135.75, 138.80. Monoisotopic mass 496.17, $[\text{M} + \text{H}]^+ = 497.4$.

(R)-2-(2-(((1-((3-chlorophenyl)sulfonyl)-1H-pyrrolo[3,2-*c*]quinolin-4-yl)amino)methyl)pyrrolidin-1-yl)ethan-1-ol hydrochloride 19

White solid, 60% yield, $t_{\text{R}} = 3.86$, $\text{C}_{24}\text{H}_{26}\text{Cl}_2\text{N}_4\text{O}_3\text{S}$, MW 521.46, ^1H NMR (500 MHz, CD_3OD) δ (ppm) 1.78–1.94 (m, 3H), 1.97–2.15 (s, 1H), 2.60–2.76 (s, 1H), 2.80–2.95 (s, 1H), 3.17–3.30 (m, 1H), 3.35–3.47 (m, 2H), 3.59–3.74 (m, 1H), 3.80–3.86 (m, 2H), 3.87–3.92 (m, 1H), 4.50–4.74 (s, 1H), 7.18 (q, $J = 6.2$ Hz, 2H), 7.41 (t, $J = 7.9$ Hz, 2H), 7.56 (d, $J = 8.0$ Hz, 1H), 7.64 (d, $J = 7.9$ Hz, 1H), 7.72–7.76 (m, 1H), 7.77–7.80 (s, 1H), 7.92 (d, $J = 3.7$ Hz, 1H), 8.70 (d, $J = 8.4$ Hz, 1H); ^{13}C NMR (126 MHz, CD_3OD) δ (ppm) 22.53, 29.01, 43.12, 49.99, 52.28, 54.67, 56.29, 106.88, 114.12, 124.75, 125.87, 127.02, 130.12, 130.76, 131.63, 135.31, 139.79. Monoisotopic mass 484.13, $[\text{M} + \text{H}]^+ = 485.5$.

(S)-2-(2-(((1-((3-chlorophenyl)sulfonyl)-1H-pyrrolo[3,2-*c*]quinolin-4-yl)amino)methyl)pyrrolidin-1-yl)ethan-1-ol hydrochloride 20

White solid, 60% yield, $t_{\text{R}} = 3.83$, $\text{C}_{24}\text{H}_{26}\text{Cl}_2\text{N}_4\text{O}_3\text{S}$, MW 521.46, ^1H NMR (500 MHz, CD_3OD) δ (ppm) 1.77–1.95 (m, 3H), 1.99–2.16 (s, 1H), 2.61–2.75 (s, 1H), 2.81–2.94 (s, 1H), 3.15–3.31 (m, 1H), 3.32–3.45 (m, 2H), 3.59–3.75 (m, 1H), 3.81–3.88 (m, 2H), 3.89–3.91 (m, 1H), 4.51–4.75 (s, 1H), 7.19 (q, $J = 6.2$ Hz, 2H), 7.42 (t, $J = 7.9$ Hz, 2H), 7.55 (d, $J = 8.0$ Hz, 1H), 7.65 (d, $J = 7.9$ Hz, 1H), 7.73–7.77 (m, 1H), 7.79–7.81 (s, 1H), 7.95 (d, $J = 3.7$ Hz, 1H), 8.73 (d, $J = 8.4$ Hz, 1H); ^{13}C NMR (126 MHz, CD_3OD) δ (ppm) 22.51, 28.98, 42.13, 49.89, 52.31, 54.87, 56.53, 106.70, 114.15, 124.83, 125.92, 127.01, 130.31, 130.82, 131.61, 135.77, 139.82. Monoisotopic mass 484.13, $[\text{M} + \text{H}]^+ = 485.5$.

(R)-1-((3-chlorophenyl)sulfonyl)-*N*-((1-(3-methoxypropyl)pyrrolidin-2-yl)methyl)-1H-pyrrolo[3,2-*c*]quinolin-4-amine 21

White solid, 38% yield, $t_{\text{R}} = 4.25$, $\text{C}_{26}\text{H}_{30}\text{Cl}_2\text{N}_4\text{O}_3\text{S}$, MW 549.51, ^1H NMR (500 MHz, CD_3OD) δ (ppm) 1.80–1.98 (m, 5H), 2.05–2.18 (m, 1H), 2.58–2.73 (s, 1H), 2.73–2.90 (s, 1H), 3.16–3.20 (s, 3H), 3.20–3.27 (m, 1H), 3.33–3.47 (m, 4H), 3.48–3.59 (m, 1H), 3.60–3.79 (m, 1H), 3.91 (dd, $J = 14.2, 5.5$ Hz, 1H), 7.16–7.26 (m, 2H), 7.46 (t, $J = 8.0$ Hz, 2H), 7.56–7.62 (m, 1H), 7.68 (d, $J = 8.2$ Hz, 2H), 7.81 (t, $J = 1.9$ Hz, 1H), 7.97 (d, $J = 3.7$ Hz, 1H), 8.76 (d, $J = 8.4$ Hz, 1H); ^{13}C NMR (126 MHz, CD_3OD) δ (ppm) 22.95, 27.22, 42.79, 53.88, 57.42, 69.30, 101.97, 106.09, 114.61, 115.92, 120.53, 122.26, 123.10, 124.03, 125.22, 126.55, 127.38, 128.72, 129.83, 131.18, 133.80, 134.48, 135.31, 139.54, 152.31. Monoisotopic mass: 512.16, $[\text{M} + \text{H}]^+ = 513.4$.

3.2. In Silico Evaluation

3.2.1. Structures of the Receptors

The structure of D_3R in the complex with antagonist eticlopride (PDB code: 3PBL) and $5\text{-HT}_6\text{R}$ in the complex with agonist serotonin (PDB code: 7XTB) were retrieved from the Protein Data Bank [34].

3.2.2. Molecular Docking

The 3-dimensional structures of the ligands were prepared using LigPrep v3.6 [35], and the appropriate ionization states at $\text{pH} = 7.4 \pm 1.0$ were assigned using Epik v3.4 [36,37]. The Protein Preparation Wizard was used to assign the bond orders and appropriate amino acid ionization states and to check for steric clashes. The receptor grid was generated (OPLS4 force field) by centering the grid box with a size of 12 Å on the D3.32 side chain. Automated flexible docking was performed using Glide v6.9 [38,39] at the SP level, and ten poses per ligand were generated. All ligands were docked using the induced fit docking (IFD) [40] protocol with SP with an OPLS4 force field [41]. The L-R complexes selected in the IFD procedure were next used in molecular dynamics simulations.

3.2.3. Molecular Dynamics

A 100 ns long molecular dynamics (MD) simulation was performed using Schrödinger Desmond software [42]. Each ligand–receptor complex was immersed into a POPC (309.5 K) membrane bilayer, the position of which was calculated using the PPM web server (https://opm.phar.umich.edu/ppm_server, accessed 20 May 2022) [43]. The system was solvated by water molecules described by the TIP4P potential and the OPLS4 force field was used for all atoms. An amount of 0.15 M NaCl was added to mimic the ionic strength inside the cell. The output trajectories were hierarchically clustered into 10 groups according to the ligand using the trajectory analysis tool from Schrödinger Suite. Based on obtained trajectories, the mean geometrical parameters of the salt bridge (distance and angle) with D3.32 were calculated using the Simulation Event Analysis tool in Maestro Schrödinger Suite.

3.3. *In Vitro* Pharmacological Evaluation

3.3.1. The 5-HT₆Rs Affinity Evaluation

Cell Culture and Preparation of Cell Membranes for Radioligand Binding Assays

HEK293 cells with stable expression of human 5-HT₆ receptors (prepared with the use of Lipofectamine 2000) were maintained at 37 °C in a humidified atmosphere with 5% CO₂ and grown in Dulbecco's modified Eagle medium containing 10% dialyzed fetal bovine serum and 500 µg/mL G418 sulfate. For membrane preparation, cells were sub-cultured in 150 cm² flasks, grown to 90% confluence, washed twice with phosphate buffered saline (PBS), prewarmed to 37 °C, and pelleted by centrifugation (200× *g*) in PBS containing 0.1 mM EDTA and 1 mM dithiothreitol. Prior to membrane preparation, pellets were stored at −80 °C.

Radioligand Binding Assays

The cell pellets were thawed and homogenized in 10 volumes of assay buffer using an Ultra Turrax tissue homogenizer (IKA, Warsaw, Poland), centrifuged twice at 35,000× *g* for 15 min at 4 °C, and incubated for 15 min at 37 °C between centrifugation rounds [10]. The composition of the assay buffers was 50 mM Tris HCl, 0.5 mM EDTA, and 4 mM MgCl₂. The assays were incubated in a total volume of 200 µL in 96-well microtiter plates for 1 h at 37 °C. The process of equilibration was terminated by rapid filtration through Unifilter plates with a 96-well cell harvester, and radioactivity retained on the filters was quantified on a Micro-beta plate reader (PerkinElmer, Waltham, MA, USA). For displacement studies, the assay samples contained as radioligands (PerkinElmer, USA) 2 nM [³H]-LSD (83.6 Ci/mmol). Nonspecific binding was defined with 10 µM methiothepine. Each compound was tested in triplicate at 7 concentrations (10^{−10} to 10^{−4} M). The inhibition constants (*K_i*) were calculated from the Cheng–Prusoff equation [30]. Results were expressed as means of at least two independent experiments.

3.3.2. Evaluation of Antagonism at Functional Activity on 5-HT₆Rs

The functional properties of compounds on 5-HT₆R were evaluated using its ability to inhibit cAMP production induced by 5-CT (1000 nM), a 5-HT₆R agonist [10]. The compound was tested in triplicate at 8 concentrations (10^{−11} to 10^{−4} M). The level of adenylyl cyclase activity was measured using frozen recombinant 1321N1 cells expressing the human serotonin 5-HT₆R (PerkinElmer). Total cAMP was measured using the LANCE cAMP detection kit (PerkinElmer), according to the manufacturer's directions. For quantification of cAMP levels, cells (5 µL) were incubated with a mixture of compounds (5 µL) for 30 min at room temperature in 384-well white opaque microtiter plates. After incubation, the reaction was stopped, and cells were lysed by the addition of 10 µL of working solution (5 µL of Eu-cAMP and 5 µL of ULIGHT-anti-cAMP). The assay plate was incubated for 1 h at room temperature. Time-resolved fluorescence resonance energy transfer (TR-FRET) was detected by an Infinite M1000 Pro (Tecan, Männedorf, Switzerland) using instrument settings from LANCE cAMP detection kit manual (PerkinElmer, Waltham, MA, USA).

3.3.3. Determination of 5-HT₆R Constitutive Activity at Gs Signaling

Neuroblastoma cells (NG108-15) were grown in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% heat-inactivated fetal bovine serum, 2% HAT (hypoxanthine/aminopterin/thymidine, Life technologies), glutamine, and antibiotics at 37 °C under 5% of CO₂. cAMP measurement was performed in cells transiently transfected with a construct expressing the CAMYEL bioluminescence resonance energy transfer (BRET) sensor for cAMP [44] (3 µg DNA/million cells) alone or in combination with a plasmid encoding the human 5-HT₆R (0.5 µg DNA/million cells). Transfection of the NG108-15 cells was conducted in suspension using Lipofectamine 2000, according to the manufacturer's protocol. Plasmids and lipofectamine were diluted in Opti-MEM Reduced Serum Media (Gibco) and incubated at room temperature for 20 min before being added to the cells. Transfected cells were subsequently plated in white 96-well plates (Greiner) at a density of 50,000 cells per well. Then, 48 h after transfection, cells were washed with PBS containing calcium and magnesium. A triplicate of well was treated with the tested compound diluted in PBS containing calcium and magnesium at concentrations ranging from 0.1 nM to 10 µM. Intepirdine was used as a control for inverse agonist activity. Coelanterazine H (Molecular Probes) was added in each well at a final concentration of 5 µM and incubated at room temperature for 5 min before measuring BRET in a Mithras LB 940 plate reader (Berthold Technologies, Bad Wildbad, Germany). The decrease in CAMYEL BRET induced by the coexpression of the probe with the 5-HT₆R as compared to the BRET measured in cells expressing the probe alone was used as an index of the 5-HT₆R constitutive activity.

4. Conclusions

To investigate the impact of structural diversification of the amine fragment of the previously reported compound **PZ-1643**, a dual 5-HT₆R/D₃R antagonist, the new series of 1*H*-pyrrolo[3,2-*c*]quinolines modified at position 4 with various pyrrolidine-derived moieties was evaluated for the affinity for 5-HT₆ and D₃Rs. The selected compounds displayed a higher affinity and more potent antagonist properties for 5-HT₆R than the previously reported lead compound; however, their affinity for D₃R was not improved. As observed in the subsequent molecular dynamics simulations, the structural modifications applied, which were favorable for the interaction with 5-HT₆R, showed a negative impact on the interactions with D₃R. These effects result from the differences in the distance and angles formed between the basic center of the molecule and the respective residues of aspartic acid in the receptor binding sites. These changes in the geometry parameters affected the quality of the formed SB. The outcomes of this study provide structural hints for designing of dual-acting 5-HT₆R/D₃R antagonists to evaluate a contribution of the combination of 5-HT₆R antagonism and D₃R antagonism to the neurodegenerative processes.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/molecules28031096/s1>, Figure S1: Superposition of the binding modes of compounds 9, 12 and 13; Figures S2–S7: 1H NMR and 13C NMR spectra of representative compounds

Author Contributions: Conceptualization: K.G. and P.Z.; Methodology: W.P. and R.K.; Software: W.P. and R.K.; Investigation: K.G., W.P., L.K., O.B., G.S., X.B., J.G., A.N., S.C.-D. and R.K.; Writing—original draft: K.G.; Writing—review & editing: S.C.-D., R.K. and P.Z.; Supervision: F.L., A.J.B., P.M., S.C.-D., R.K. and P.Z.; Project administration: K.G. and P.Z. All authors have read and agreed to the published version of the manuscript.

Funding: The study was financially supported by the National Science Center, Grant No. DEC-2019/03/X/NZ7/01894, the Priority Research Area qLife under the program "Excellence Initiative Research University" at the Jagiellonian University in Krakow, the Université de Montpellier, Centre National de la Recherche Scientifique (CNRS), the French National Research Agency ("Investissements d'avenir" programme with the reference ANR-16-IDEX-0006).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in the Supplementary Materials.

Acknowledgments: L.K. thanks the Erasmus program for the possibility of international exchange between Faculty of Pharmacy, Jagiellonian University Medical College and Université de Montpellier.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Chaumont-Dubel, S.; Dupuy, V.; Bockaert, J.; Bécamel, C.; Marin, P. The 5-HT₆ receptor interactome: New insight in receptor signaling and its impact on brain physiology and pathologies. *Neuropharmacology* **2020**, *172*, 107839. [[CrossRef](#)] [[PubMed](#)]
2. Meffre, J.; Chaumont-Dubel, S.; Mannoury la Cour, C.; Loiseau, F.; Watson, D.J.G.; Dekeyne, A.; Séveno, M.; Rivet, J.M.; Gaven, F.; Délérès, P.; et al. 5-HT₆ Receptor recruitment of mTOR as a mechanism for perturbed cognition in schizophrenia. *EMBO Mol. Med.* **2012**, *4*, 1043. [[CrossRef](#)] [[PubMed](#)]
3. Duhr, F.; Délérès, P.; Raynaud, F.; Séveno, M.; Morisset-Lopez, S.; Mannoury La Cour, C.; Millan, M.J.; Bockaert, J.; Marin, P.; Chaumont-Dubel, S. Cdk5 Induces constitutive activation of 5-HT₆ receptors to promote neurite growth. *Nat. Chem. Biol.* **2014**, *10*, 590. [[CrossRef](#)] [[PubMed](#)]
4. De Deurwaerdere, P.; Bharatiya, R.; Chagraoui, A.; Di Giovanni, G. Constitutive activity of 5-HT receptors: Factual analysis. *Neuropharmacology* **2020**, *168*, 107967. [[CrossRef](#)] [[PubMed](#)]
5. Deraredj Nadim, W.; Chaumont-Dubel, S.; Madouri, F.; Cobret, L.; De Tauzia, M.L.; Zajdel, P.; Benedetti, H.; Marin, P.; Morisset-Lopez, S. Physical interaction between neurofibromin and serotonin 5-HT₆ receptor promotes receptor constitutive activity. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 12310. [[CrossRef](#)]
6. Lesiak, A.J.; Brodsky, M.; Cohenca, N.; Croicu, A.G.; Neumaier, J.F. Restoration of physiological expression of 5-HT₆ receptor into the primary cilia of null mutant neurons lengthens both primary cilia and dendrites. *Mol. Pharmacol.* **2018**, *94*, 731–742. [[CrossRef](#)]
7. Codony, X.; Vela, J.M.; Ramírez, M.J. 5-HT₆ Receptor and cognition. *Curr. Opin. Pharmacol.* **2011**, *11*, 94. [[CrossRef](#)] [[PubMed](#)]
8. de Jong, I.E.M.; Mørk, A. Antagonism of the 5-HT₆ receptor—Preclinical rationale for the treatment of Alzheimer’s disease. *Neuropharmacology* **2017**, *125*, 50. [[CrossRef](#)]
9. Partyka, A.; Jastrzębska-Więsek, M.; Antkiewicz-Michaluk, L.; Michaluk, J.; Wąsik, A.; Canale, V.; Zajdel, P.; Kołaczkowski, M.; Wesółowska, A. Novel antagonists of 5-HT₆ and/or 5-HT₇ receptors affect the brain monoamines metabolism and enhance the anti-immobility activity of different antidepressants in rats. *Behav. Brain Res.* **2019**, *359*, 9. [[CrossRef](#)]
10. Zajdel, P.; Grychowska, K.; Mogilski, S.; Kurczab, R.; Satała, G.; Bugno, R.; Kos, T.; Gołębiowska, J.; Malikowska-Racia, N.; Nikiforuk, A.; et al. Structure-based design and optimization of FPPQ, a dual-acting 5-HT₃ and 5-HT₆ receptor antagonist with antipsychotic and procognitive properties. *J. Med. Chem.* **2021**, *64*, 18. [[CrossRef](#)]
11. Vanda, D.; Canale, V.; Chaumont-Dubel, S.; Kurczab, R.; Satała, G.; Koczurkiewicz-Adamczyk, P.; Krawczyk, M.; Pietruś, W.; Blicharz, K.; Pękala, E.; et al. Imidazopyridine-based 5-HT₆ receptor neutral antagonists: Impact of N1-benzyl and N1-phenylsulfonyle fragments on different receptor conformational states. *J. Med. Chem.* **2021**, *64*, 1180. [[CrossRef](#)] [[PubMed](#)]
12. Staroń, J.; Kurczab, R.; Warszycki, D.; Satała, G.; Krawczyk, M.; Bugno, R.; Lenda, T.; Popik, P.; Hogendorf, A.S.; Hogendorf, A.; et al. Virtual screening-driven discovery of dual 5-HT₆/5-HT_{2A} receptor ligands with pro-cognitive properties. *Eur. J. Med. Chem.* **2020**, *185*, 111857. [[CrossRef](#)]
13. Kucwaj-Brysz, K.; Ali, W.; Kurczab, R.; Sudol-Tałaj, S.; Wilczyńska-Zawal, N.; Jastrzębska-Więsek, M.; Satała, G.; Mordyl, B.; Żesławska, E.; Olejarz-Maciej, A.; et al. An exit beyond the pharmacophore model for 5-HT₆R agents—A new strategy to gain dual 5-HT₆/5-HT_{2A} action for triazine derivatives with procognitive potential. *Bioorg. Chem.* **2022**, *121*, 105695. [[CrossRef](#)] [[PubMed](#)]
14. Yahiaoui, S.; Hamidouche, K.; Ballandonne, C.; Davis, A.; Sopkova de Oliveira Santos, J.; Freret, T.; Boulouard, M.; Rochais, C.; Dallemagne, P. Design, synthesis, and pharmacological evaluation of multitarget-directed ligands with both serotonergic subtype 4 receptor (5-HT₄R) partial agonist and 5-HT₆R antagonist activities, as potential treatment of Alzheimer’s disease. *Eur. J. Med. Chem.* **2016**, *121*, 283. [[CrossRef](#)]
15. Claeysen, S.; Bockaert, J.; Giannoni, P. Serotonin: A New Hope in Alzheimer’s Disease? *ACS Chem. Neurosci.* **2015**, *6*, 940. [[CrossRef](#)] [[PubMed](#)]
16. Marcinkowska, M.; Mordyl, B.; Fajkis-Zajczkowska, N.; Siwek, A.; Karcz, T.; Gawalska, A.; Bucki, A.; Żmudzki, P.; Partyka, A.; Jastrzębska-Więsek, M.; et al. Hybrid molecules combining GABA-A and serotonin 5-HT₆ receptors activity designed to tackle neuroinflammation associated with depression. *Eur. J. Med. Chem.* **2023**, *247*, 115071. [[CrossRef](#)] [[PubMed](#)]
17. Wichur, T.; Pasieka, A.; Godyń, J.; Panek, D.; Góral, I.; Latacz, G.; Honkisz-Orzechowska, E.; Buski, A.; Siwek, A.; Głuch-Litwin, M.; et al. Discovery of 1-(phenylsulfonyle)-1H-indole-based multifunctional ligands targeting cholinesterases and 5-HT₆ receptor with anti-aggregation properties against amyloid-beta and tau. *Eur. J. Med. Chem.* **2021**, *225*, 113783. [[CrossRef](#)]
18. Canale, V.; Grychowska, K.; Kurczab, R.; Ryng, M.; Raheem Keeri, A.; Satała, G.; Olejarz-Maciej, A.; Koczurkiewicz, P.; Drop, M.; Blicharz, K.; et al. A dual-acting 5-HT₆ receptor inverse agonist/MAO-B inhibitor displays glioprotective and pro-cognitive properties. *Eur. J. Med. Chem.* **2020**, *208*, 112765. [[CrossRef](#)]

19. Millan, M.J.; Dekeyne, A.; Gobert, A.; Brocco, M.; Mannoury la Cour, C.; Ortuno, J.-C.; Watson, D.; Fone, K.C.F. Dual-acting agents for improving cognition and real-world function in Alzheimer's disease: Focus on 5-HT₆ and D₃ receptors as hubs. *Neuropharmacology* **2020**, *177*, 108099. [[CrossRef](#)]
20. Saavedra, O.M.; Karila, D.; Brossard, D.; Rojas, A.; Dupuis, D.; Gohier, A.; Mannoury la Cour, C.; Millan, M.J.; Ortuno, J.-C.; Hanessian, S. Design and synthesis of novel *N*-sulfonyl-2-indoles that behave as 5-HT₆ receptor ligands with significant selectivity for D₃ over D₂ receptors. *Bioorg. Med. Chem.* **2017**, *25*, 38. [[CrossRef](#)]
21. Chagraoui, A.; Di Giovanni, G.; De Deurwaerdère, P. Neurobiological and pharmacological perspectives of D₃ receptors in Parkinson's disease. *Biomolecules* **2022**, *12*, 243. [[CrossRef](#)] [[PubMed](#)]
22. Chen, P.-C.; Lao, C.-L.; Chen, J.-C. The D₃ dopamine receptor inhibits dopamine release in PC-12/hD3 cells by autoreceptor signaling via PP-2B, CK1, and Cdk-5. *J. Neurochem.* **2009**, *110*, 1180. [[CrossRef](#)] [[PubMed](#)]
23. Salles, M.J.; Hervé, D.; Rivet, J.M.; Longueville, S.; Millan, M.J.; Girault, J.; Mannoury la Cour, C. Transient and rapid activation of Akt/GSK-3 β and mTORC1 signaling by D₃ dopamine receptor stimulation in dorsal striatum and nucleus accumbens. *J. Neurochem.* **2013**, *125*, 532. [[CrossRef](#)]
24. Sokoloff, P.; Leriche, L.; Diaz, J.; Louvel, J.; Pumain, R. Direct and indirect interactions of the dopamine D₃ receptor with glutamate pathways: Implications for the treatment of schizophrenia. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2013**, *386*, 107. [[CrossRef](#)] [[PubMed](#)]
25. Dupuis, D.S.; Mannoury la Cour, C.; Verrièle, C.L.; Lavielle, G.; Millan, M.J. Actions of novel agonists, antagonists and antipsychotic agents at recombinant rat 5-HT₆ receptors: A comparative study of coupling to G α s. *Eur. J. Pharmacol.* **2008**, *588*, 170. [[CrossRef](#)] [[PubMed](#)]
26. Grychowska, K.; Chaumont-Dubel, S.; Kurczab, R.; Koczurkiewicz, P.; Deville, C.; Krawczyk, M.; Pietruś, W.; Satała, G.; Buda, S.; Piska, K.; et al. Dual 5-HT₆ and D₃ receptor antagonists in a group of 1H-pyrrolo[3,2-c]quinolines with neuroprotective and procognitive activity. *ACS Chem. Neurosci.* **2019**, *10*, 3183. [[CrossRef](#)] [[PubMed](#)]
27. Grychowska, K.; Satała, G.; Kos, T.; Partyka, A.; Colacino, E.; Chaumont-Dubel, S.; Bantreil, X.; Wesołowska, A.; Pawłowski, M.; Martinez, J.; et al. Novel 1H-pyrrolo[3,2-c]quinoline based 5-HT₆ receptor antagonists with potential application for the treatment of cognitive disorders associated with Alzheimer's disease. *ACS Chem. Neurosci.* **2016**, *7*, 972. [[CrossRef](#)]
28. Grychowska, K.; Olejarz-Maciej, A.; Blicharz, K.; Pietruś, W.; Karcz, T.; Kurczab, R.; Koczurkiewicz, P.; Doroz-Płonka, A.; Latacz, G.; Raheem Keeri, A.; et al. Overcoming undesirable hERG affinity by incorporating fluorine atoms: A case of MAO-B inhibitors derived from 1H-pyrrolo[3,2-c]quinolines. *Eur. J. Med. Chem.* **2022**, *236*, 114329. [[CrossRef](#)]
29. MacKenzie, R.G.; Van Leeuwen, D.; Pugsley, T.A.; Shih, Y.H.; Demattos, S.; Tang, L.; Todd, R.D.; O'Malley, K.L. Characterization of the human dopamine D₃ receptor expressed in transfected cell lines. *Eur. J. Pharmacol.* **1994**, *266*, 79. [[CrossRef](#)]
30. Cheng, Y.; Prusoff, W.H. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50% inhibition (I₅₀) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099.
31. Grychowska, K.; Kurczab, R.; Śliwa, P.; Satała, G.; Dubiel, K.; Matłoka, M.; Moszczyński-Pętkowski, R.; Pieczykolan, J.; Bojarski, A.J.; Zajdel, P. Pyrroloquinoline scaffold-based 5-HT₆R ligands: Synthesis, quantum chemical and molecular dynamic studies, and influence of nitrogen atom position in the scaffold on affinity. *Bioorg. Med. Chem.* **2018**, *26*, 3588. [[CrossRef](#)] [[PubMed](#)]
32. Hogendorf, A.S.; Hogendorf, A.; Kurczab, R.; Kalinowska-Tluscik, J.; Popik, P.; Nikiforuk, A.; Krawczyk, M.; Satała, G.; Lenda, T.; Knutelska, J.; et al. 2-Aminoimidazole-based antagonists of the 5-HT₆ Receptor—A new concept in aminergic GPCR ligand design. *Eur. J. Med. Chem.* **2019**, *179*, 1. [[CrossRef](#)] [[PubMed](#)]
33. Kurczab, R.; Śliwa, P.; Rataj, K.; Kafel, R.; Bojarski, A.J. The salt bridge in ligand-protein complexes—Systematic theoretical and statistical investigations. *J. Chem. Inf. Model.* **2018**, *58*, 2224. [[CrossRef](#)] [[PubMed](#)]
34. Chien, E.Y.T.; Liu, W.; Zhao, Q.; Katrich, V.; Won Han, G.; Hanson, M.H.; Shi, L.; Newman, A.H.; Javitch, J.A.; Cherezov, V.; et al. Structure of the human dopamine D₃ receptor in complex with a D₂/D₃ selective antagonist. *Science* **2010**, *30*, 1091. [[CrossRef](#)] [[PubMed](#)]
35. Murphy, R.B.; Philipp, D.M.; Friesner, R.A. A Mixed Quantum Mechanics/Molecular Mechanics (QM/MM) Method for Large-Scale Modeling of Chemistry in Protein Environments. *J. Comput. Chem.* **2000**, *21*, 1442–1457. [[CrossRef](#)]
36. Anighoro, A.; Bajorath, J.; Rastelli, G. Polypharmacology: Challenges and opportunities in drug discovery. *J. Med. Chem.* **2014**, *57*, 7874. [[CrossRef](#)]
37. Shelley, J.C.; Cholleti, A.; Frye, L.L.; Greenwood, J.R.; Timlin, M.R.; Uchimaya, M. Epik: A software program for pK(a) prediction and protonation state generation for drug-like molecules. *J. Comput. Aided. Mol. Des.* **2007**, *21*, 681. [[CrossRef](#)]
38. Halgren, T. New method for fast and accurate binding-site identification and analysis. *Chem. Biol. Drug Des.* **2007**, *69*, 146. [[CrossRef](#)]
39. Friesner, R.A.; Banks, J.L.; Murphy, R.B.; Halgren, T.A.; Klicic, J.J.; Mainz, D.T.; Repasky, M.P.; Knoll, E.H.; Shelley, M.; Perry, J.K.; et al. Glide: A new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. *J. Med. Chem.* **2004**, *47*, 1749. [[CrossRef](#)]
40. Sherman, W.; Day, T.; Jacobson, M.P.; Friesner, R.A.; Farid, R. Novel procedure for modeling ligand/receptor induced fit effects. *J. Med. Chem.* **2006**, *49*, 534–553. [[CrossRef](#)]
41. Harder, E.; Damm, W.; Maple, J.; Wu, C.; Reboul, M.; Xiang, J.Y.; Wang, L.; Lupyan, D.; Dahlgren, M.K.; Knight, J.L.; et al. OPLS3: A force field providing broad coverage of drug-like small molecules and proteins. *J. Chem. Theor. Comput.* **2016**, *12*, 281. [[CrossRef](#)] [[PubMed](#)]

42. Bowers, K.J.; Chow, D.E.; Xu, H.; Dror, R.O.; Eastwood, M.P.; Gregersen, B.A.; Klepeis, J.L.; Kolossvary, I.; Moraes, M.A.; Sacerdoti, F.D.; et al. Scalable Algorithms for Molecular Dynamics Simulations on Commodity Clusters. In Proceedings of the ACM/IEEE SC 2006 Conference (SC'06), Tampa, FL, USA, 11–17 November 2006; p. 43.
43. Lomize, M.A.; Pogozheva, I.D.; Joo, H.; Mosberg, H.I.; Lomize, A.L. OPM database and PPM web server: Resources for positioning of proteins in membranes. *Nucleic Acids Res.* **2011**, *40*, 370. [[CrossRef](#)] [[PubMed](#)]
44. Jiang, L.I.; Collins, J.; Davis, R.; Lin, K.-M.; DeCamp, D.; Roach, T.; Hsueh, R.; Rebres, R.A.; Ross, E.M.; Taussig, R.; et al. Use of a cAMP BRET sensor to characterize a novel regulation of cAMP by the sphingosine 1-phosphate/G13 pathway. *J. Biol. Chem.* **2007**, *282*, 10576. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.