Synthesis and evaluation of a new indole-based series as non-basic 5-HT$_6$ receptor ligands

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Introduction

The 5-HT$_6$ receptor is the most recently identified member of the 5-HT receptor superfamily. During the past 20 years, the 5-HT$_6$R has received increasing attention and become a promising target primarily for improving cognition.[1] Currently, some 5-HT$_6$R ligands are being subjected to clinical development processes for future use as potential anti-dementia, anti-psychotic, and anti-obese drugs (e.g. Idalopirdine, SB-742457, AVN-101).[2]

Since the initial discovery of the first ligands in the late 1990s, a number of highly potent ligands have been reported.[3] The majority of known 5-HT$_6$R ligands, like endogenous agonist – serotonin, possess positively charged at physiological pH basic nitrogen atom, which is considered to be necessary for effective interaction with the receptor. However, in the last years, new generations of 5-HT$_6$R ligands without a protonable nitrogen atom were obtained.

The 5-HT$_6$R ligands with reduced basicity developed so far revealed excellent selectivity over other monoaminergic GPCRs and low hERG affinity. The mechanism of a non-basic ligand-receptor interaction has been studied and some hypotheses were formulated but the phenomenon is still unclear.[4-6]

As a part of our study on the non-basic 5-HT$_6$R ligands, in the first step a consistent series of indole derivatives has been designed in an attempt to describe their specific interactions in the binding pocket (Figure 1). Following the examples of literature ligands with the 1-(phenylsulfonyl)-1H-indole fragment (first column in Table 1) and the basic nitrogen atom, their counterparts with reduced and/or removed basicity were synthesized. Four series of indole derivatives with different basicity and geometry of terminal pharmacophore fragments were synthesized. This resulted in seven groups of geometric isomers with different affinities for the 5-HT$_6$R. In the second step the ligands with the highest affinity were further developed (Table 2).

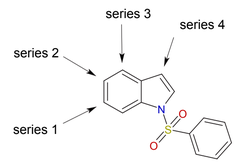
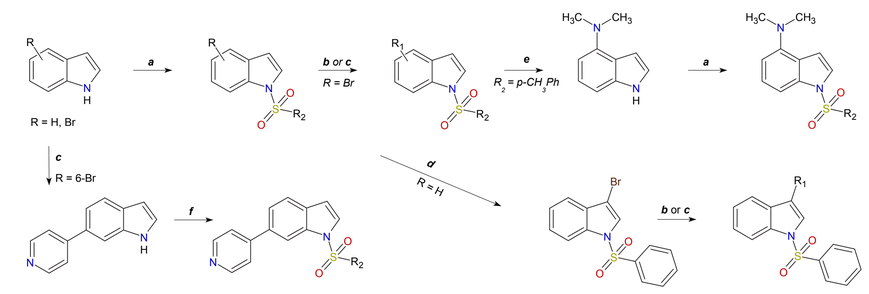


Figure 1

Synthesis



a: R_2SO_2Cl, NaH; ***b***: amine, Cs_2CO_3, XPhOS, $Pd(OAc)_2$; ***c***: $ArB(OH)_2$, K_2CO_3, $Pd[(C_6H_5)_3P]_4$; ***d***: Br_2; ***e***: Mg, ultrasound irradiation; ***f***: R_2SO_2Cl, TBAB, 30% NaOH.

In silico

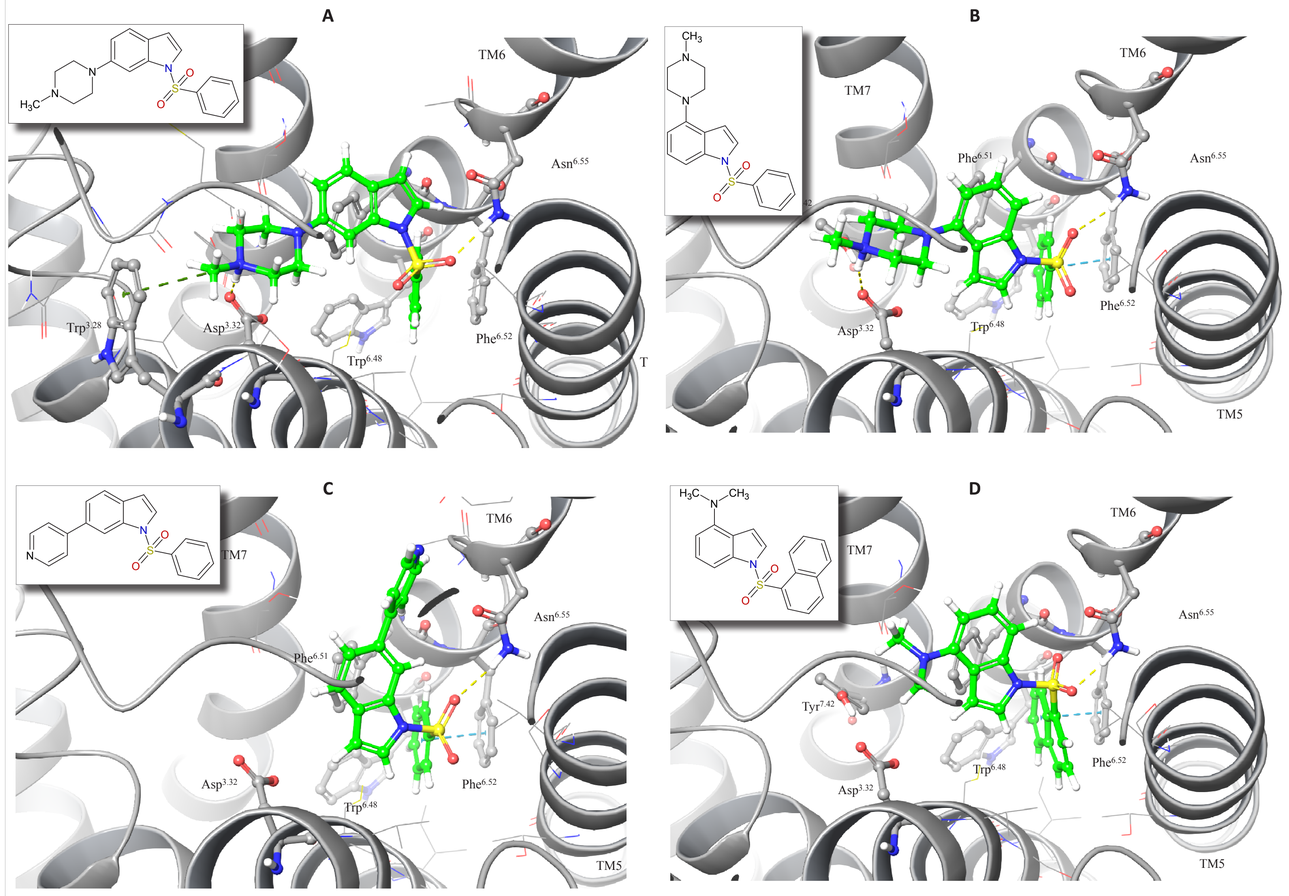


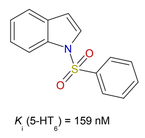
Figure 3. Binding modes for two basic reference compounds (**A**, **B**) and two non-basic 5-HT$_6$R ligands (**C, D**). Hydrogen bonds, pi-stacking and pi-cation interactions labelled as yellow, blue and green dashed lines, respectively.

To examine binding modes of non-basic 5-HT$_6$R ligands, class-specific homology models were generated utilizing previously applied methodology.[12] The analysis of ligand-receptor complexes indicated that the arylsulfonyl moiety in the structures of non-basic as well as in the reference basic ligands are located deep at the aromatic cluster (Phe6.51, Phe6.52 and Trp6.48). This hydrophobic cavity is well filled by the 1-naphthyl group.

Low-basicity ligands were docked close to the TM6 and in contrary to classical 5-HT$_6$R ligands, due to lack of protonated nitrogen in physiological conditions, did not create the charge-assisted hydrogen bond with the Asp3.32. Additionally, the interactions of the sulfonyl group with Asn6.55 were observed.

In vitro

Within the basic reference ligand group (first column in Table 1), in the contrast to presented here less basic compounds, the arrangement of terminal pharmacophore groups did not significantly influence affinity for 5-HT$_6$R. Among the 24 synthesized low-basicity compounds, only 9 (yellow numbers in Table 1) showed comparable or higher affinity for the 5-HT$_6$R than the unsubstituted reference core (K_i = 159 nM, Figure 2). The most favorable for affinity was 4-pyridyl group in the 6 position of the indole scaffold (K_i = 38 nM) and small dimethylamino substituent at the 4 and 5 positions (K_i = 65 and 82 nM, respectively). A bulky 1-acetylpiperazine substituent was well tolerated in the 3 and 6 positions (K_i = 97 nM), however 1-(trifluoromethyl)piperazine moiety in the all positions reduced the affinity.



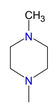
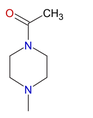
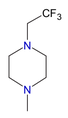
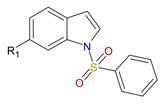
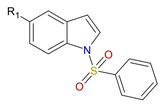
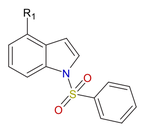
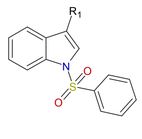
K_i (5-HT$_6$) = 159 nM

Figure 2

In view of these results, for further modifications of the terminal aromatic substituent, two compounds with the highest affinity were selected (Table 2; **1** and **2**). Removal of the aromaticity (**12**–**18**) strongly reduced the affinity, particularly in the case of the structure A. The replacement of the terminal phenyl group, with 5- (**5**) or 4,5-dibromo-2-thienyl (**6**) substituents significantly improved the affinity, but the lowest value of K_i was observed for the derivative with 1-naphthyl group (**10**). Biaryl analogues with 2-naphthyl (**9**) and 2-benzothienyl (**8**) moieties showed five-fold lower affinity, which may indicate the limit of binding pocket shape.

In order to verify the selectivity of the presented compounds, the receptor binding profile was extended (Table 1 and Table 2). As expected, unlike the reference basic compounds, the non- and low-basic ligands revealed the high selectivity for 5-HT$_6$R.

Table 1

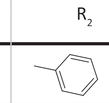
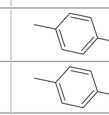
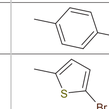
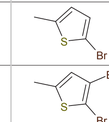
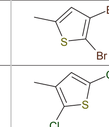
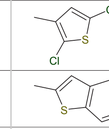
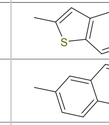
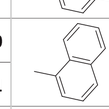
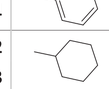
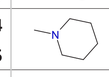
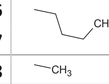
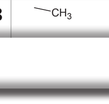
	Reference basic compounds	R$_1$							
									
series 1		7.4	0.6	3.6	4.5	4.2	-7.5	5.0	pK_a
		247	NA	NA	NA	NA	NA	NA	5-HT$_{1A}$
		2809	NA	NA	NA	NA	1039	1050	5-HT$_{2A}$
		9	**97**	**1201**	**403**	**211**	**178**	**38**	K_i [nM] **5-HT$_6$**
		1818	ND	NA	NA	NA	NA	NA	5-HT$_7$
		241	6993	NA	NA	NA	9587	NA	D$_2$
series 2		7.3	0.8	3.9	5.4	5.0	-6.8	4.9	pK_a
		NA	NA	NA	NA	NA	NA	NA	5-HT$_{1A}$
		4197	NA	NA	NA	NA	NA	NA	5-HT$_{2A}$
		27	**4853**	**5252**	**360**	**82**	**558**	**409**	K_i [nM] **5-HT$_6$**
		NA	NA	NA	NA	NA	NA	NA	5-HT$_7$
		802	NA	NA	7767	NA	NA	NA	D$_2$
series 3		7.4	0.9	3.4	1.9	3.3	-7.6	4.5	pK_a
		164	NA	NA	NA	NA	NA	NA	5-HT$_{1A}$
		41	NA	NA	NA	NA	NA	NA	5-HT$_{2A}$
		1	**714**	**1743**	**128**	**65**	**162**	**1508**	K_i [nM] **5-HT$_6$**
		5462	ND	8920	7153	NA	NA	NA	5-HT$_7$
		353	NA	NA	8472	NA	5611	NA	D$_2$
series 4		8.2	-0.4	3.6	-0.4	-0.3	-7.3	4.9	pK_a
		1404	NA	NA	3272	NA	NA	NA	5-HT$_{1A}$
		657	NA	NA	NA	NA	NA	7676	5-HT$_{2A}$
		4	**97**	**2029**	**219**	**474**	**146**	**124**	K_i [nM] **5-HT$_6$**
		NA	NA	ND	NA	NA	ND	ND	5-HT$_7$
		532	ND	NA	NA	NA	5650	8491	D$_2$

NA – Not Active (K_i > 10 000 nM), ND – Not Determined

The aqueuos pK_a were calculated using the Jaguar software of the Schrödinger suite.

Membrane preparation and general assay procedures for 5-HT$_{1A}$,[7] 5-HT$_{2A}$,[7] 5-HT$_7$,[8-9] 5-HT$_6$,[10] and D$_2$,[11] receptors were performed exactly as previously described. For binding experiments 7–9 sample concentrations were used to determine inhibition constant (K_i) on the basis of Cheng-Prusoff equation: $K_i = IC_{50} / (1 + L/K_D)$. Values are means of three experiments run in triplicate, SEM ≤ 16%.

Table 2

No	R$_2$	Structure	K_i [nM] 5-HT$_{1A}$	5-HT$_{2A}$	**5-HT$_6$**	5-HT$_7$	D$_2$
1		A	NA	NA	**65**	NA	NA
2		B	NA	1050	**38**	NA	NA
3		A	ND	7683	**157**	NA	NA
4		A	NA	NA	**83**	NA	5130
5		A	NA	NA	**40**	NA	NA
6		A	NA	6758	**40**	NA	3357
7		A	NA	NA	**413**	NA	NA
8		A	NA	9584	**97**	NA	NA
9		A	NA	NA	**98**	NA	NA
10		A	ND	NA	**17**	NA	NA
11		B	NA	NA	**56**	NA	NA
12		A	NA	NA	**4886**	NA	NA
13		B	ND	ND	**2183**	ND	ND
14		A	NA	NA	**NA**	4806	NA
15		B	ND	ND	**1664**	ND	ND
16		A	NA	NA	**NA**	3129	NA
17		B	ND	ND	**1151**	ND	ND
18		B	ND	ND	**4050**	ND	ND

References

[1] Ramírez M. J. Alzheimers Res. Ther. 2013, 5(2), 15. [2] Wicke K. et al. Expert Opin. Investig. Drugs. 2015, 24, 1515–28. [3] Ivachtchenko A. V. et al. Current Bioactive Compounds, 2013, 9(1), 64–100. [4] Harris R. N. et al. Bioorg. Med. Chem. Lett. 2010, 20, 3436–40. [5] Ivachtchenko A. V. et al. J. Med. Chem. 2011, 54, 8161–73. [6] Van Loevezijn A. et al. Bioorg. Med. Chem. Lett. 2016, 26, 1605–11. [7] Bojarski A. J. et al. Pharmazie. 1993, 48(4), 289–94. [8] Bojarski A. J. et al. Bioorg. Med. Chem. Lett. 2004, 14(23), 5863–6. [9] Paluchowska M. H. et al. Bioorg. Med. Chem. 2007, 15(22), 7116–25. [10] Duszyńska B. et al. poster, 25–29.05.2008 Krasiczyn, Book of Abstract, 23–24. [11] Bojarski A. J. et al. Bioorg. Med. Chem. 2005, 13(6), 2293–303. [12] Rataj K. et al. J. Chem. Inf. Model. 2014, 54, 1661–68.

Acknowledgments

National Science Centre Poland

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Conclusions

Interaction of a non-basic ligand with the 5-HT$_6$ receptor is more demanding in terms of the molecule geometry and the type of terminal pharmacophore groups than in the case of classical basic ligands.

Linking the existing information from the SAR and molecular docking experiments allows to formulate preliminary hypotheses for the anchoring non-basic ligands to the receptor binding pocket, but the completely explanation of all these interactions requires further experiments.