Examination of 5-HT$_6$ receptor affinity in the group of arylsulfonamide derivatives

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Introduction

The 5-HT$_6$ serotonin receptor (5-HT$_6$R) has become an attractive and promising therapeutic target for the development of new CNS agents. There are evidence suggesting its role in cognition and learning, certain types of neuropsychological and neuropsychiatric diseases such as affective disorders, schizophrenia, Alzheimer's disease, anxiety/depression, the treatment of obesity and related metabolic disorders.[1] Recently the discovery of ligands with affinity and selectivity for this receptor has become an area of intense research in medicinal chemistry. To date a number of selective antagonists, mostly identified by high-throughput screening, are known.[2] Since the arylsulfonyl moiety was a common feature of nearly all published structures, it was proposed that it constitutes an important pharmacophoric element (mHBA - multiple hydrogen bond acceptor group), which strongly influences 5-HT$_6$R affinity. Apart of the basic ionizable group, PI which is a common pharmacophoric element of the compounds interacting with aminergic receptors,[2] 5-HT$_6$R ligands usually contain also a two hydrophobic sites HYD (e.g. phenyl). Due to high similarity of pharmacophore features between majority of 5-HT$_6$ antagonists and a series of arylsulfonamide derivatives (**1**–**34**), recently developed in our laboratory as 5-HT$_7$ ligands, we examined them at 5-HT$_6$ receptors.

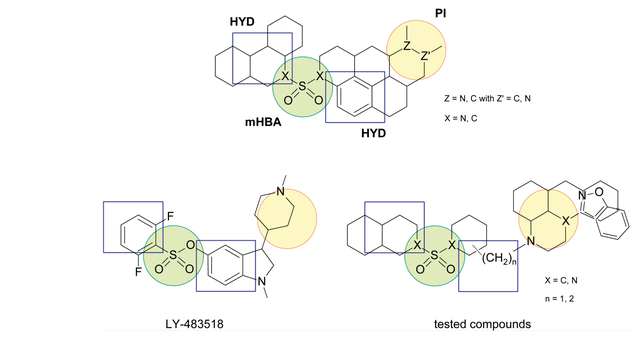
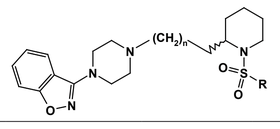


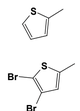
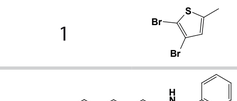
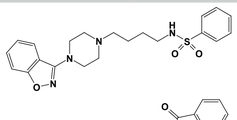
Figure 1. Schematic representation of a 5-HT$_6$R pharmacophore model, PI - positive ionizable group; mHBA - multiple hydrogen bond acceptor group; HYD - hydrophobic site.[2]

Results of affinity experiments

The compounds were divided on three groups according to their chemical structure and the binding results are presented in Tables 1–4.
The affinity for the 5-HT$_6$R for the series of ***benzo[d]isoxazole derivatives*** **1**–**10** is shown in Table 1. Except **2**, all the new compounds displayed high to moderate affinity at 5-HT$_6$R (43–528 nM). Compound **1** with the 3-(piperazin-1-yl)benzo[*d*]isoxazole fragment revealed the moderate affinity (K_i = 393 nM,), but the substitution of the phenyl ring (**3**–**5**) in position C-3, caused the increase of 5-HT$_6$R activity, and *m*-Br derivative (**4**) displayed K_i = 62 nM. Analogically, ligand **7** with unsubstituted thienyl group was found less active then its 5,6-dibromosubstituted derivative **8** which presented the highest affinity (K_i = 43 nM).
Increasing the distance between the aromatic ring and the sulfonic group (**6** *vs* **1**) caused a four-fold enhancement in the affinity. The flexible compound **9** with *n*-butyl chain revealed nearly the same K_i values as its partly constrained analogue – compound **1**. The replacement of the phenylsulfonamide fragment in compound **9** by phthalimide moiety (**10**) revealed over 2.5-fold decrease in the affinity for 5-HT$_6$R.

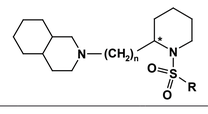
Table 1. Series of benzo[*d*]isoxazole piperazine derivatives.

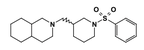
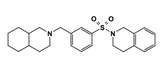
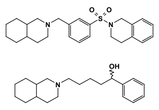
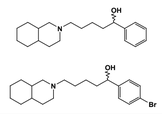


Compd	n	R	K_i [nM] 5-HT$_6$
1	1		393
2	0		5626
3	1		123
4	1		62
5	1		199
6	1		92
7	1		528
8	1		43
9			342
10			874

The compounds from the second group, i.e. ***perhydroisoquinoline derivatives*** **11**–**25** (Table 2) – displayed moderate to very low affinity (K_i = 397 – > 10 000 nM) at 5-HT$_6$R. When compared to their structural analogues in the series of benzo[*d*]isoxazole derivatives, all compounds were significantly less active. Among the tested derivatives, the highest activity to 5-HT$_6$R binding site was observed for the racemic mixture of compound **19** (K_i = 625 nM) with the 5,6-dibromothienyl substituent. Its *R* isomer **21** revealed even higher affinity (K_i = 397 nM) that was also six-fold more active than its counterpart **20** with *S* conformation of the 2-ethylenepiperidyl spacer.

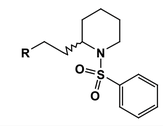
Table 2. Series of perhydroisoquinoline derivatives.

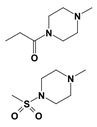
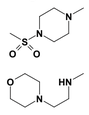
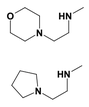


Compd	Structure			K_i [nM]
	n	R	*	5-HT$_6$
11	2		*RS*	> 10 000
12	1		*RS*	> 10 000
13	2		*RS*	> 10 000
14	2		*RS*	2999
15	2		*RS*	9287
16	2		*RS*	7917
17	2		*RS*	> 10 000
18	1		*RS*	> 10 000
19	2		*RS*	625
20	2		*S*	2394
21	2		*R*	397
22				4818
23				4869
24				> 10 000
25				> 10 000

In the third group of compounds **26**–**34** different modifications of ***terminal amine moiety*** were applied (Table 3). All compounds, i.e. derivatives of: perhydroquinoline (**24)** and *N*-cyclohexane- (**27**), *N*-acyl- (**29** and **30**), *N*-mesyl- (**31**) piperazines, and secondary amines **28, 32, 33,** as well as 1,2,3,4-tetrahydroquinoline (**34**), were practically inactive at 5-HT$_6$R (the K_i > 5400 nM).

Table 3. Series of compounds with modified terminal amine moiety.



Compd	R	K_i [nM] 5-HT$_6$
26		> 10 000
27		5468
28		7837
29		> 10 000
30		> 10 000
31		> 10 000
32		> 10 000
33		> 10 000
34		> 10 000

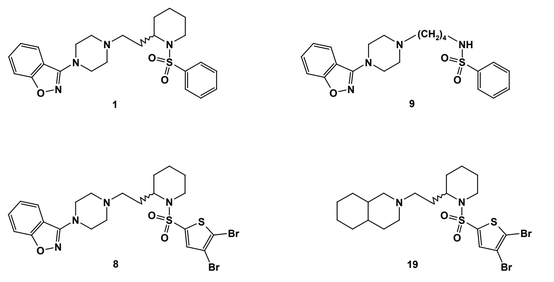
Methods of binding experiments

Membrane preparation and general assay procedures for 5-HT$_{1A}$,[4] 5-HT$_{2A}$,[4] 5-HT$_7$,[5, 6] 5-HT$_6$,[7] D$_2$ [8] and α_1 [9] receptors were performed exactly as previously described.
For binding experiments 7–9 sample concentrations, each run in triplicate, were used to determine inhibition constant (K_i) on the base of Cheng-Prusoff'a equation: $K_i = IC_{50} / (1 + L/K_D)$. Values are means of three experiments run in triplicate, SEM ≤ 16%.

Extended receptor profile of selected arylsulfonylpiperidine derivatives

Since amino acid sequence of the 5-HT$_6$R is related to other monoaminergic receptors, serotoninergic ligands frequently present high affinity to several closely related targets. Therefore, selected ligands (**1**, **8**, **9**, **19**) were examined additionally at 5-HT$_{1A}$, 5-HT$_{2A}$, 5-HT$_7$, and dopaminergic D$_2$ receptors. It was found that benzisoxazole piperazine derivatives **1**, **8**, **9**, indeed showed significant activity to all tested receptors, whereas in the case of perhydroisoquinoline derivative **19**, it showed selective profile towards 5-HT$_7$R population.

Table 4. Extended receptor profile of selected arylsulfonamide 5-HT$_6$R ligands from tested series of compounds.



Compd	5-HT$_{1A}$	5-HT$_{2A}$	5-HT$_6$	5-HT$_7$	α_1	D$_2$
1	105	19	393	11	20	12
9	300	44	342	13	3.3	691
8	112	45	43	1,5	NT	35
19	612	1621	625	8	NT	1547

NT – not tested

Docking studies to the serotonin 5-HT$_6$ receptor homology model

Models of 5-HT$_6$ receptor were generated as previously described for 5-HT$_7$R using β_1-adrenergic template. Selection of models was based on docking results of a set of 106 ligands (65 referenced ligands from publication[10] and 41 ligand-like compounds with K_i > 1000 nM). The four models with the highest discrimination ratio were next used in docking of the whole set of 34 studied compounds. Majority of active ligands (K_i < 1000 nM) were successfully docked to the best model (60%), which recognized also only 5% of inactive compounds.

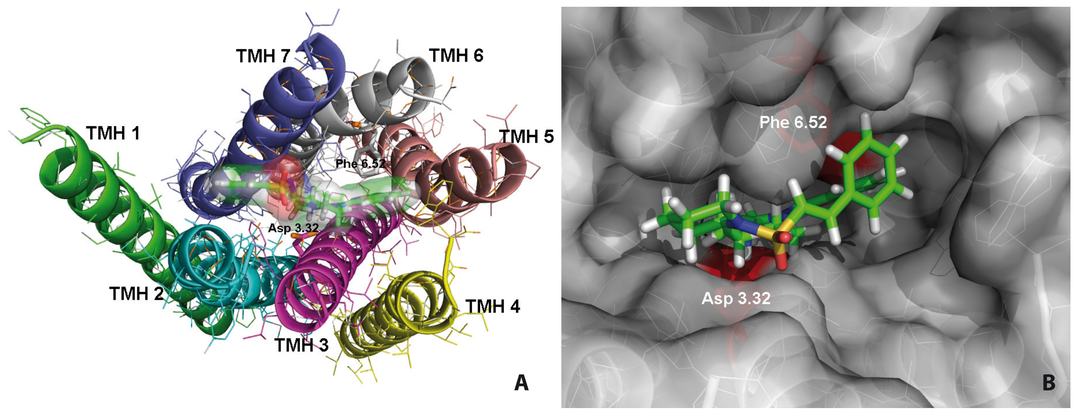


Figure 2. Representative binding mode - compounds **9** (**A**) and **6** (**B**) - within the 5-HT$_6$ receptor model

Conclusions

- The quality of terminal amine fragment is significant for affinity to 5-HT$_6$R
- The type of aryl fragment of phenylsulfonamide terminal is important for interactions with 5-HT$_6$ receptor
- Ligands with *R* conformation of 2-ethylenepiperidyl spacer are preferred at 5-HT$_6$R binding site.

References

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Acknowledgments

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