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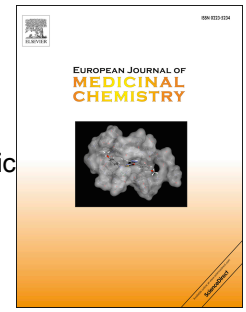

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basic

nonbasic

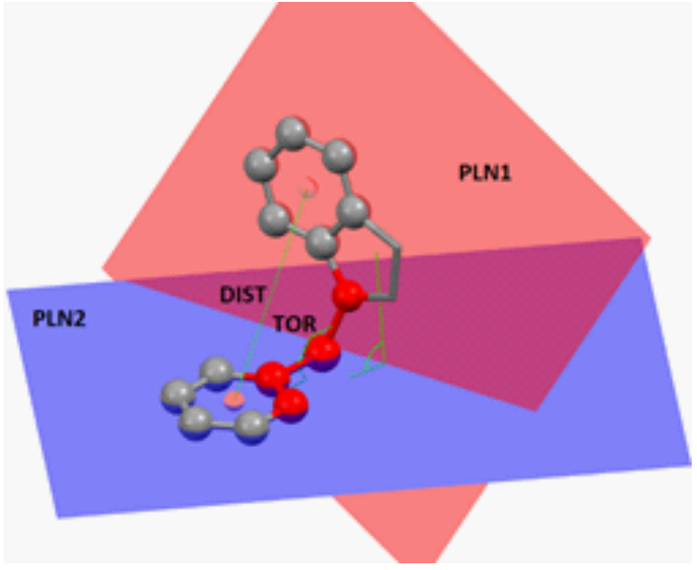
?

5-HT$_6$
K_i = 4 nM

5-HT$_6$
K_i = 1.2 nM

nonbasic
analogs

Simple
Pharmacophore



Rationally designed N-phenylsulfonylindoles as a tool for the analysis of the non-basic 5-HT$_6$R ligands binding mode.

Jakub Staroń[1], Ryszard Bugno[1], Wojciech Pietruś[1], Grzegorz Satała[1], Stefan Mordalski[1], Dawid Warszycki[1], Agata Hogendorf[1], Adam S. Hogendorf[1], Justyna Kalinowska-Tłuścik[2], Tomasz Lenda[1], Bogusław Pilarski[3], Andrzej J. Bojarski[1]

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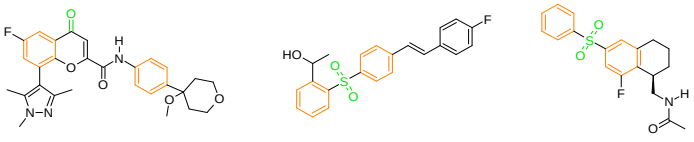
Abstract

Among all of the monoaminergic receptors, the 5-HT$_6$R has the highest number of non-basic ligands (approximately 5% of compounds stored in 25th version of ChEMBL database have the strongest basic pKa below 5, calculated using the Instant JChem calculator plugin). These compounds, when devoid of a basic nitrogen, exhibit high affinity and remarkable selectivity. Despite a decade of research, no clues have been given for explanation of such an intriguing phenomenon. Here, a series of analogs of four known 5-HT$_6$R ligands, has been rationally designed to approach this issue. For each of the synthesized 42 compounds, a binding affinity for 5-HT$_6$R has been measured, together with a selectivity profile against 5-HT$_{1A}$R, 5-HT$_{2A}$R, 5-HT$_7$R and D$_2$R. Performed induced fit docking and molecular dynamics experiments revealed that no particular interaction was responsible for the activity of non-basic compounds. In fact, a plain *N*-phenylsulfonylindole (**1e**) was found to possess a moderate (5-HT$_6$R, K_i = 159 nM) affinity. No other monoaminergic receptor has as simple and selective ligand as this one. Thus, it is stated that it binds to the receptor solely based on its conformation and as such, possesses a minimum amount of features, required for binding. Also, any functional group able to form an additional interaction with the receptor increase the binding affinity, like in the case of two highly active non-basic compounds **3e** and **5g** (5-HT$_6$R, K_i = 65 nM and 38 nM, respectively).

1. Introduction

The aminergic receptors, i.e., G-Protein Coupled Receptors activated by endogenous amines, are targets of ~25% of drugs.[1] The common mechanism of ligand binding in the aminergic receptors involves the formation of an ion pair (charge assisted hydrogen bond) between a highly conserved aminoacid residue D3.32 and a positively ionized atom (fragment) of the ligand.[2] This anchoring interaction is reinforced by several other, in particular, the π-π interactions with residues from the 5th, 6th and 7th helices, formation of hydrogen bonds, van der Waals interactions, halogen bonds as well as by the entropic driving forces.

There have been reports of non-basic antagonists of 5-HT$_{1B}$[3], 5-HT$_{2A}$[4–6] and 5-HT$_6$[7] receptors in the recent years. Despite the lack of basic functionality, some of these compounds show very high binding affinity, even in sub-nanomolar range (Fig. 1). The unique feature of 5-HT$_6$ receptor is the unusually high abundance of its weakly- or non-basic ligands.[7–11]



5-HT$_{1B}$R K_i = 0.43 nM
Nugiel et. al. 2010

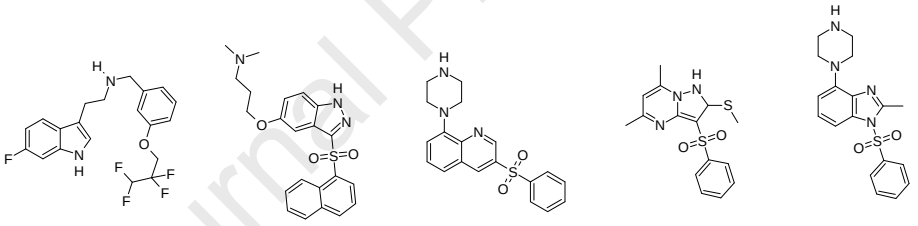
5-HT$_{2A}$R K_i = 0.1 nM
Ladduwahety et. al. 2006 and 2010

5-HT$_6$R K_i = 0.32 nM
Harris et. al. 2010

Figure. 1 Non-basic serotonin receptor antagonists. The pharmacophore features were marked: aromatic ring - orange, hydrogen bond acceptor - green.

The 5-HT$_6$ receptor is a well documented drug target for the treatment of cognitive deficits. The pharmacological blockade of 5-HT$_6$R can potentially restore the deteriorated cholinergic transmission in cognitive impairment.[12,13] There have been several lead compounds targeting 5-HT$_6$R developed for the treatment of dementia, Alzheimer's disease (AD), and to relieve the symptoms of schizophrenia (Tab. 1).[14] The clinical trials conducted so far did not confirm the efficacy of 5-HT$_6$R antagonists as an augmentative therapy for AD. However, AVN-211 has been shown to be effective in the treatment schizophrenia, relieving positive symptoms and adding some procognitive (increased attention) effects to antipsychotic medication.[15]

Table 1 Selected advanced clinical candidates for the treatment of cognitive impairment that reached phase III trials or are chemically similar to compounds presented in this manuscript. There have been no successful phase III trials till date in AD patients, while AVN-211, a non-basic 5-HT$_6$R antagonist was found effective for the treatment of negative symptoms of schizophrenia. A recent review summarizes the ongoing trails.[16]



Idalopirdine (Lu AE58054)
K_i = 0.8 nM

Cerlapirdine (WAY-262,531)
K_i = 1.3 nM

Intepirdine (SB-742457)
K_i = 0.2 nM

AVN-211
K_i = 1.2 nM

PF-05212377
K_i = 0.53 nM

	Idalopirdine	**Cerlapirdine**	**Intepirdine**	**AVN-211**	**PF-05212377**
Current developer	Lundbeck	Pfizer (suspended)	Axovant	Avineuro	Pfizer
Indication	AD/Schizophrenia	AD/Schizophrenia	AD/dementia with Levy bodies	Schizophrenia	AD
Phase I	Well tolerated NCT02415907	Well tolerated NCT00726115	Well tolerated NCT00551772	Well tolerated[15]	Well tolerated NCT00948662
Phase II	Effective for schizophrenia but not for AD patients	Program suspended NCT00481520	Some efficacy reported in AD patients, dose dependent NCT00710684 NCT00708552	Effective vs placebo[17]	Safe and well tolerated but no benefit on cognition[18]
Phase III	No improvement vs placebo in AD patients (lower dosage used than in phase II) NCT02079246	-	No improvement versus placebo in AD patients in test scores; the subjective condition of the patient signifficantly improved vs placebo NCT02585934	Recrutation ongoing	-

Most of the 5-HT$_6$R antagonists fall into the category of basic bis-arylsulfones which, in some cases, may lead to shortcoming in terms of blood-brain barrier permeability, metabolic stability, and hERG binding.[2]

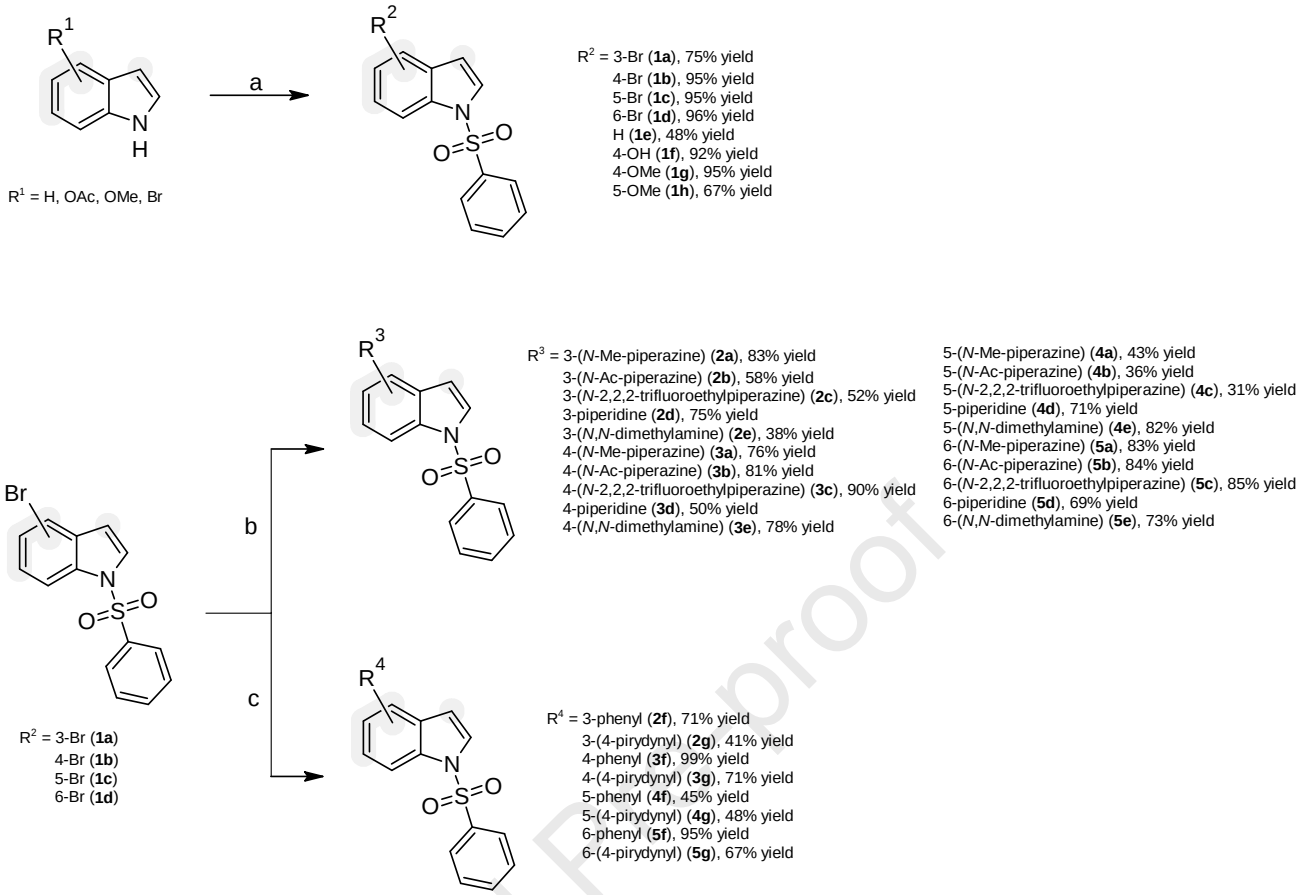
The development of novel, rule-breaking 5-HT$_6$ receptor antagonists, which lack the archetypical basic functionality, emerges as a game changer in the field.[2] A new, unexplored chemical space that

can enable the escape from the dead end of classic aryl-amine classes has been opened. An enhanced blood-brain barrier permeation and better absorption could be expected for weakly basic compounds due to the fact, that they remain in the unionized state under physiological pH, moreover in some cases the lack of basic moiety implicates a lower molecular mass, *i.e*., better diffusion.

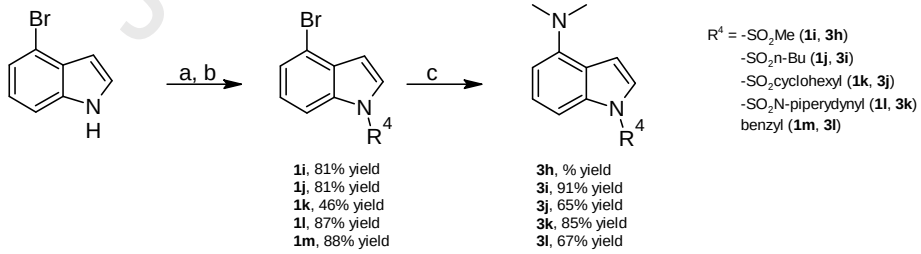
Although the existence of such ligands has questioned the amine-D3.32 residue anchoring paradigm, there has been little research into this uncommon binding mode. Herein, an effort has been made to study the mechanism of action of weakly basic antagonists of the 5-HT_6 receptor. The structures of four active phenylsulfonyl indoles, representing analogs with different position of *N*-methylpiperazinyl substituent (C3, C4, C5 and C6 of indole carbons), were selected as a starting point for further modifications.[19–22] At first, the *N*-methyl substituent of piperazine ring was replaced by *N*-acetyl and *N*-2,2,2-trifluoroethyl group, in order to lower the basicity of the distal nitrogen atom. Next, the *N*-methylpiperazine ring was simplified to piperidine and dimethylamine. These moieties were selected to investigate the effect of a distal nitrogen atom cancellation and its replacement to carbon atom. Finally, aromatic substituents of phenyl and 4-pyridinyl were introduced. Phenyl ring was selected as a planar hydrophobic group, whereas 4-pyridinyl was selected as a surrogate of piperazine, because of its basic distal nitrogen atom. Each of the synthesized compounds was evaluated in terms of activity at 5-HT_6R and its virtual interactions with a homology model of the receptor were comprehensively analyzed.

1. Synthesis

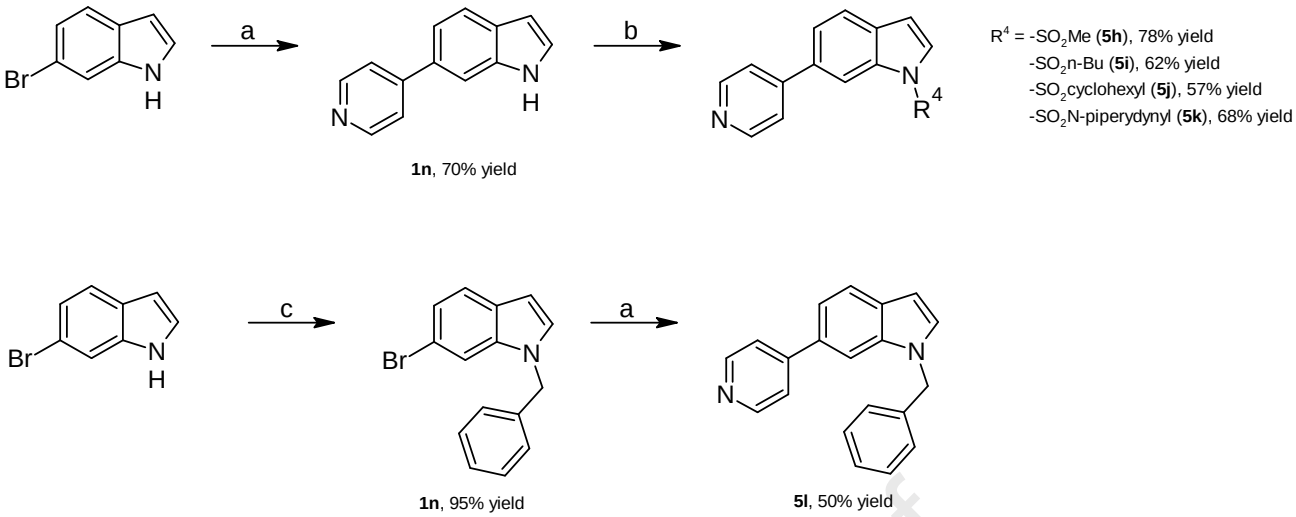
Phenylsulfonyl indole derivatives **1a**-**1h** were synthesized via phase transfer catalyzed reaction between the appropriate indoles and phenylsulfonyl chloride. Final compounds **2(a-g)**-**5(a-g)** were obtained by cross-coupling of intermediates **1a**-**1d** (Scheme 1). The introduction of *N*-Me-piperazine, *N*-Ac-piperazine, *N*-2,2,2-trifluoroethylpiperazine, piperidine and *N,N*-dimethylamine moieties (compounds **2(a-e)**-**5(a-e)**) was carried out via Buchwald-Hartwig reaction, whereas coupling to phenyl and 4-pirydinyl groups (compounds **2(f,g)**-**5(f,g)**) was performed via Suzuki-Miyaura reaction. Synthesis of compounds **3h**-**3k** was accomplished in two steps (Scheme 2), first by substitution of the indole with appropriate sulfonyl chlorides or benzyl bromide and then coupling with a dimethylamine. Compounds **5h**-**5k** were synthesized in a similar, but reversed way (Scheme 3). First by coupling with 4-pirydinyl moiety at C6 of indole, then by substitution at indole -NH with appropriate sulfonyl chlorides. Derivative **2l** was prepared using sodium hydride and benzyl chloride, then coupling with 4-pirydinylboronic acid (Scheme 3).



Scheme 1. Synthesis of *N*-phenylsulfonylindole derivatives substituted at C3, C4, C5 or C6 of indole. a) $PhSO_2Cl$, DCM, 50% NaOH, TBAB; b) R^3H, $Pd(OAc)_2$, Cs_2CO_3, XPhos, toluene; c) $R^4B(OH)_2$, $Pd(PPh_3)_4$, K_2CO_3, toluene, EtOH, H_2O.



Scheme 2. Synthesis of compounds **3h**-**3l**. a) R^4SO_2Cl, DCM, 50% NaOH, TBAB; b) in case of a benzyl derivative BnBr, NaH, DMF; c) $Me_2NH{\cdot}HCl$, $Pd(OAc)_2$, Cs_2CO_3, XPhos, toluene.

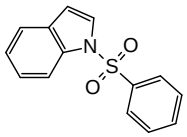
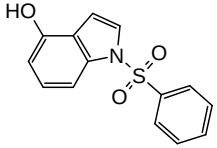
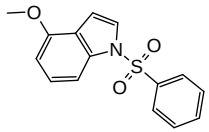
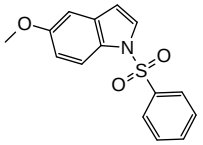


Scheme 3. Synthesis of compounds **5h**-**5l**. a) $R^2B(OH)_2$, $Pd(PPh_3)_4$, K_2CO_3, toluene, EtOH, H_2O; b) R^4SO_2Cl, DCM, 50% NaOH, TBAB; c) BnBr, NaH, DMF.

2. In vitro studies

Radioligand assays were utilized to determine the binding affinity and selectivity profiles of the synthesized compounds in competition binding experiments for human serotonin 5-HT_6R. Selectivity profiles against 5-HT_{2A}R, 5-HT_{1A}R, 5-HT_7R and dopaminergic D_2R were presented in Supplementary Information (SI Table 3). The plain *N*-phenylsulfonylindole (**1e**), together with its three analogs (Table 2), exhibited a moderate binding affinity (**1e**, 5-HT_6R K_i = 159 nM). In turn, among the rest of the obtained compounds, five (out of 24) reached comparable affinity as **1e**, whereas another five exhibited 1.6- to 4-fold higher affinity, with **3e** (Table 3, 5-HT_6R K_i = 65 nM) and **5g** (Table 3, 5-HT_6R K_i = 38 nM) being the best binders. These two substitution patterns were further modified in order to investigate the importance of the phenylsulfonyl moiety. All modifications of this structural fragment were found to be not tolerated by the receptor (Table 4).

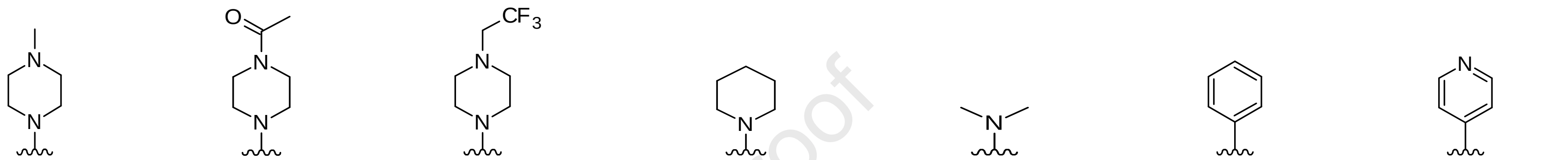
Table 2. Structure and in vitro affinity data for 5-HT$_6$R for the synthesized N-phenylesulfonylindole and its derivatives.

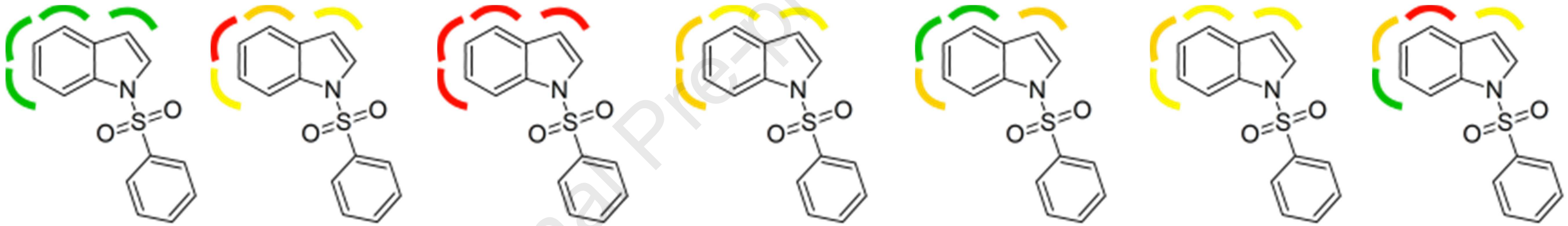
				
Cmpd.	***1e***[23]	***1f***[24]	***1g***[25]	***1h***[26]
5-HT$_6$ K_i [nM][a]	*159*	*108*	*262*	*127*

[a] Binding affinity, K_i, expressed as the average of at least two independent experiments; the maximum S.D. did not exceed 10% (see Supplementary Information, page 9, Table 4); affinity of the reference drugs: 5-HT$_6$R, Olanzapine – K_i = 10.7 ± 2.1 nM.

*Table 3. Structure and in vitro affinity data for 5-HT$_6$R for the synthesized compounds, substituted at 3[rd], 4[th], 5[th], and 6[th] position of indole with either N-Me-piperazine, N-Ac-piperazine, N-2,2,2-trifluoroethylpiperazine, piperidine, N,N-dimethylamine, phenyl or 4-pyridynyl. Unsubstituted N-phenylsulfonylindole (**1e**, K_i = 159 nM), was used as a reference compound. Green color marks the placement of substituent which resulted in 1.6 – 100-fold increase of affinity for 5-HT$_6$R, compared to **1e**. Yellow color marks compounds with comparable affinity; orange, compounds 4.4 – 1.6-fold less active and red, compounds 32 – 7.5-fold less active. A calculated value of pK_a is given in parentheses next to the symbol of a compound.*

R substituent



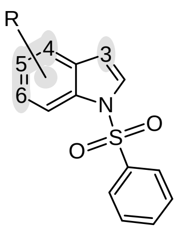


R position	Cmpd (pK_a)	5-HT$_6$ K_i [nM][a]	Cmpd (pK_a)	5-HT$_6$ K_i [nM][a]	Cmpd (pK_a)	5-HT$_6$ K_i [nM][a]	Cmpd (pK_a)	5-HT$_6$ K_i [nM][a]	Cmpd (pK_a)	5-HT$_6$ K_i [nM][a]	Cmpd (pK_a)	5-HT$_6$ K_i [nM][a]	Cmpd (pK_a)	5-HT$_6$ K_i [nM][a]
3	**2a** (7.4)	**4**	**2b** (0.6)	**97**	**2c** (3.6)	**2 029**	**2d** (4.5)	**219**	**2e** (4.2)	**474**	**2f** (-7.5)	**146**	**2g** (5.0)	**124**
4	**3a** (7.3)	**1**	**3b** (0.8)	**714**	**3c** (3.9)	**1 743**	**3d** (5.4)	**128**	**3e** (5.0)	**65**	**3f** (-6.8)	**162**	**3g** (4.9)	**1 508**
5	**4a** (7.4)	**27**	**4b** (0.9)	**4 853**	**4c** (3.4)	**5 252**	**4d** (1.9)	**360**	**4e** (3.3)	**82**	**4f** (-7.6)	**558**	**4g** (4.5)	**409**
6	**5a** (8.2)	**9**	**5b** (-0.4)	**97**	**5c** (3.6)	**1 201**	**5d** (-0.4)	**403**	**5e** (-0.3)	**211**	**5f** (-7.3)	**178**	**5g** (4.9)	**38**

Placement of substituents which resulted in:

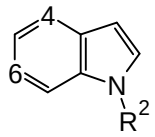
7.5 - 32 decrease
1.6 - 4.4 decrease
comparable
1.6 - 100 increase

5-HT$_6$R affinity *vs* unsubstituted reference compound **1e** (R = H, K_i = 159 nM)

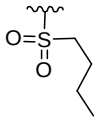


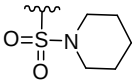
[a] Binding affinity, K_i, expressed as the average of at least two independent experiments; the maximum S.D. did not exceed 10% (see Supplementary Information, page 9, Table 4). Affinity of the reference drugs: 5-HT$_6$R, Olanzapine – K_i = 10.7 ± 2.1 nM.

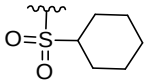
*Table 4. Structure and in vitro affinity data for 5-HT$_6$R for the synthesized derivatives of **3e** and **5g**, substituted with benzyl and different sulfonyl chlorides.*

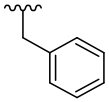


R^2 substituent









	Cmpd	5-HT$_6$ K_i [nM][a]	*Cmpd*	5-HT$_6$ K_i [nM][a]	*Cmpd*	5-HT$_6$ K_i [nM][a]	*Cmpd*	5-HT$_6$ K_i [nM][a]	*Cmpd*	5-HT$_6$ K_i [nM][a]
4-(N,N-dimethylamine)	***3h***	*4 721*	***3i***	*49 900*	***3j***	*39 070*	***3k***	*4 886*	***3l***	*2 563*
6-(4-pyridine)	***5h***	*4 050*	***5i***	*1 151*	***5j***	*1 664*	***5k***	*2 183*	***5l***	*3 262*

[a] Binding affinity, K_i, expressed as the average of at least two independent experiments; the maximum S.D. did not exceed 10% (see Supplementary Information, page 9, Table 4); affinity of the reference drugs: 5-HT$_6$R, Olanzapine – K_i = 10.7 ± 2.1 nM.

3. Molecular modelling

A mass induced fit docking (ifd) of compounds **2(a-g)**-**5(a-g)** to 5-HT_6R homology models was performed. The resulting 28 316 ligand-receptor complexes were filtered regarding the mutual orientation of phenylsulfonyl and indole moieties so that it corresponded to the existence of a weak, intramolecular hydrogen bond, which may be important for 5-HT_6R ligand binding, according to our recent finding.[27] No strong, specific interaction was observed between any of the non-basic ligands and the receptor models within the analyzed complexes. However, superposition of the active (K_i < 500 nM) non-basic compounds revealed that they adopt a markedly different position in the binding pocket than the basic compounds (Figure 2; green vs orange, respectively). A strong interaction between protonated nitrogen of the piperazine ring and the side chain D3.32 imposed the overall binding pose. The phenylsulfonyl moiety was positioned close to the helices 6 and 7, forming interactions with the phenylalanine cluster (F6.51, F6.52), while maintaining contact between sulfonyl group and N6.55 and S5.43. In turn, the non-basic compounds, free from the constraint of hydrogen bond with D3.32, head towards the helix 4, either with phenylsulfonyl moiety or with the non-polar group, the piperazine was substituted for. Despite the different placement, the sulfonyl moiety could still form interactions with N6.55 and S5.43. Interestingly, only for compounds representing the 1,6- substitution – **5f** and **5g** the phenyl ring of the phenylsulfonyl moiety turned in such a way that it was placed similarly to the piperazine ring of basic compounds. While for **5f** such position gave no gain in binding affinity over other phenyl substituted compounds **2f** – **4f**, the pyridine substituted **5g** exhibited the highest affinity for 5-HT_6R, among all non-basic derivatives. Compounds substituted with dimethylamine were found to be positioned in another binding pocket, much closer to the entrance to the receptor binding pocket, surrounded by residues W3.28, V2.57, P2.60 and A2.61 (for representative ligand-receptor complexes see Supplementary Information). Next, to further study the investigated binding modes, molecular dynamics experiments were performed. The best scoring complexes of each synthesized compound, were selected as starting poses. Numerical analysis of the molecular dynamics was based on the measurement of distances between sulfonyl group and neighboring amino acid residues: S5.43 and N6.55. As a result, it was revealed that the average distances calculated for active and non-active compounds did not correspond with the binding affinity (see Supplementary Information). The position of ligands during the whole simulation (100 ns) was stable, as evidenced by the low variation of the measured distances (see Supplementary Information).

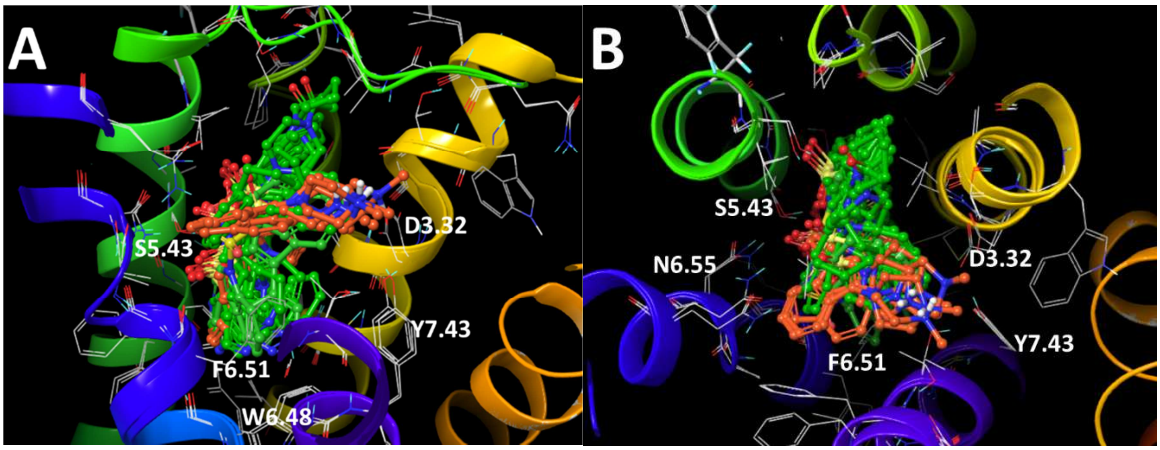


Figure 2. Superposition of active (K_i < 500 nM) non-basic compounds (green) with basic compounds (orange). A – side view; B – view from above. The poses were selected from the top scored ligand-

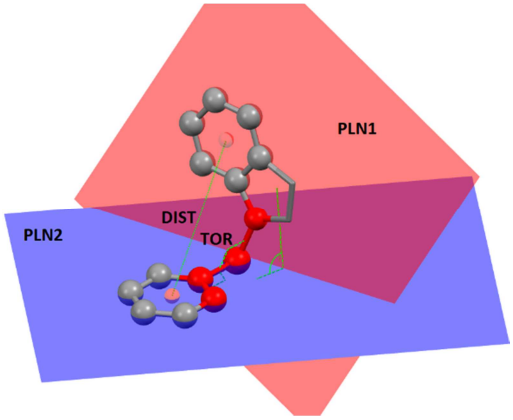
receptor ifd complexes, so that they represent a corresponding binding motif. The green structures comprise of compounds: ***2b****,* ***2d****,* ***2f****,* ***2g****,* ***3f****,* ***4d****,* ***5b****,* ***5d****,* ***5f*** *and* ***5g****. The orange structures comprise of:* ***2a****,* ***3a****,* ***4a*** *and* ***5a****. The basic compounds positioned themselves so that an interaction is formed between the protonated piperazine nitrogen atom and aspartic acid D3.32. Among the non-basic compounds, only* ***5f*** *and* ***5g*** *had a phenyl ring placed in such a way, that it mimicked the position of a piperazine. An additional comparison between* ***3a*** *and* ***5g****, together with comparison between* ***3a*** *and* ***3e*** *is presented in the supplementary information (Figures S4 and S5, respectively).*

4. Discussion

The observed structure-activity relationship is very complex. The simplest compound, *N*-phenylsulfonylindole (**1e**) has no functional groups serving as attachment points, besides sulfonyl moiety, and yet exhibited moderate binding affinity (5-HT_6R K_i = 159 nM). For comparison, this value will be used as a reference 'base' level of affinity. Introduction of a hydroxy and methoxy substituents at C4 and C5 carbons caused only small changes in the binding affinity value (Table 2). Also, addition of large substituents like piperidine, N-acetylpiperazine, phenyl and pyridine surprisingly exhibited a limited influence on binding affinity, in most cases. In turn, introduction of a 2,2,2-trifluoroethylpiperazine was unfavorable in all of the studied substitution patterns, despite the fact that these four compounds exhibited very similar binding mode in the modelling study, as their active non-basic analogs.

No specific interaction responsible for the variable activity of non-basic compounds was identified during a detailed analysis of binding poses obtained from ifd experiments. In a general view, non-basic compounds interacted with phenylalanine cluster of F6.51 and F6.52 either through the phenyl group of the phenylsulfonyl moiety or the opposite hydrophobic group. The only aspect that is common to all of the obtained poses is the relatively stable position of the sulfonyl moiety, which remained close to the residues N6.55 and S5.43, presumably due to hydrogen bonding. However, statistical analysis of the PDB (Protein Data Bank) showed that the sulfonyl group is generally not involved in hydrogen bond formation and usually occupies the hydrophobic pocket of the protein binding site.[28] In turn, mutagenesis studies performed by Harris et al. showed that both mutations N6.55A and S5.43A had a negative (ΔpK_i = -0.4), yet small influence on the binding affinity of a non-basic ligands. The only mutations that markedly affected the binding affinity of the non-basic compounds were of hydrophobic residues of W6.48 and F6.51.[29] Thus the most probable role of the sulfonyl group is keeping the mutual orientation of the aromatic systems, and not being a hydrogen bond acceptor. Thus, the substitution of a sulfonyl with a methylene group should have a limited effect on the binding affinity. In our previous paper we showed that highly basic, methylene linked 5-HT_6R ligands indeed maintain high affinity.[27] However, that was not the case with non-basic compounds. Substitution of sulfonyl with methylene in two most active non-basic derivatives dramatically lowered the binding affinity (**5l** 5-HT_6R K_i = 3262 nM; **3l** 5-HT_6R K_i = 2563 nM, respectively). The sulfonyl group may thus play a crucial role, by stabilizing the conformation of the molecules.[27] In fact, comparative molecular dynamics of weakly- and highly-basic compounds showed that they exerted no marked conformational changes. Next, we assumed that in the case of strong binders, distances to the N6.55 and S5.43 residues should be shorter. In this case, the position of the ligands would be stabilized, which may have been responsible for the increased activity. However, the comparison of the average distances between the sulfonyl groups' oxygen atoms and hydrogen bond donors, did not distinguish actives from non-actives.

Taking into account the importance of a sulfonyl group, we anticipated that crystal structures containing *N*-phenylsulfonylindole moiety might provide a probable most optimal, geometrical parameters of the pharmacophore (Scheme 4). Thus, the average distance between centroids of the phenyl rings is 5.27 Å, the torsion angle (defined as shown on Scheme 4) is equal 91.06° and the angle between the planes of the phenyl rings equals 80.48° (diagrams representing the distribution of the selected values are presented in Supplementary Information page 15). The provided values define a novel pharmacophore for the ligands of 5-HT_6R that contains the indispensable elements needed for the activity at this target.



	ANG (A) [°]	DIST (D) [Å]	TOR [°]
Mean	80.48	5.27	91.06
Median	82.74	5.26	90.87
Min	52.50	4.39	45.25
Max	89.99	6.30	158.74

Scheme 4. On the left the fragment searched within CSD is presented with the defined geometrical parameters: angle between planes PLN1 (marked in red, defined by the benzene ring of the indole moiety) and PLN2 (marked in blue, defined by the benzene ring of the phenylsulfonyl moiety) (ANG), torsion angle N-S-C-C (TOR, marked in red) and distance between centroids of fenyl ring and the six-membered ring of indole moiety (DIST). On the right a table with calculated mean, median, minimum and maximum values is shown.

Among all of the obtained non-basic derivatives two compounds stand out, regarding their binding affinity: **3e**, substituted at C4 with dimethylamine, and **5g** substituted at C6 with 4-pyridyl. Detailed analysis of the ifd generated binding modes of **3e** showed, that it adopted a markedly different position, located at the entrance to the binding pocket with both of its methyl groups staying in the proximity of the W3.28 tryptophan (Figures S2 and S3, Supplementary Information pages 3-4). That interaction was identified only for the two most active dimethylamine compounds **3e** and **4e**. We thus assume that this weak C-H…pi interaction might be partially responsible for the increased affinity of these compounds. However, the most active of the presented non-basic ligands is the 6-pyridinyl derivative **5g**. Comparing its binding mode with the phenyl analog **5f**, shows that in both cases the phenyl/pyridinyl moieties rest near the phenylalanine Phe284, in an almost identical position (Figure S1, Supplementary Information page 2). Still, the pyridinyl nitrogen atom does not point towards any amino acid residue so, it is unable to form any strong interaction. However, it was shown that phenyl-pyridine stacking interaction is much more favorable than the phenyl-phenyl stacking interactions.[30] Thus, in this case, the slightly stronger interaction between the phenyl of Phe284 and piperidine might be responsible for the 4.7-fold increase of binding affinity.

The removal of basic nitrogen from the structure of 5-HT_6R ligand significantly increased the selectivity over other monoaminergic receptors (see Supplementary Information, page 10). Taking

into consideration two most active nonbasics **3e** and **5g**, they exert no activity at 5-HT_{1A}, 5-HT_{2A}, 5-HT_7 and D2 receptors. In comparison, the piperazinyl parent of **3e** (**3a)** have high affinity for 5-HT_{2A} and D_2 receptors (5-HT_{2A}R K_i = 41 nM, D_2R K_i = 31 nM) and moderate affinity for 5-HT_{1A}R (K_i = 164 nM). In turn, the parent for **5g** (**5a**) is characterized by moderate affinities for 5-HT_{1A}R and D_2R (K_i = 247 nM and 241 nM, respectively). Despite higher selectivity, the attenuation of basicity brings some consequences regarding the drugability of a compound, i.e.: lower solubility in water. These feature can considerably undermine the possibility of such compounds becoming successful drugs.

5. Conclusions

The *N*-phenylsulfonylindole seems to possess the minimum number of features that are required for activity at 5-HT_6R. These are: two phenyl rings and a linker that stabilizes them in a defined, optimal position. Substantially, the sulfonyl linker could be changed to a methylene group, as long as the defined mutual orientation of both aromatic rings is kept (an example of a non-sulfonyl rigid linker, stabilizing the mutual orientation of two aromatic ring in 5-HT_6R ligands can be found in our previous paper[31]). Interestingly, changing the sulfonyl linker to a methylene in basic compounds does not have a detrimental effect on the affinity for 5-HT_6R,[27] which is not the case with the non-basic compounds. The switch from a sulfonyl group to a methylene in the case of the studied non-basic compounds resulted in a >60-fold drop in binding affinity. We assume, that the main feature defining the activity at 5-HT_6R is the very specific mutual orientation of two aromatic rings (described by a pharmacophore presented on Scheme 4). The function of the basic nitrogen atom would lie mainly in the stabilization of the position of the rest of the molecule. Our statement is markedly supported by the unusual activity of a plain *N*-phenylsulfonylindole **1e** (Table 2) and its close analogs **1f**-**1h**. No remark of activity at monoaminergic receptors, of such a simple non-basic compound have been reported so far.

Although there have been several groups of rigorously scrutinized 5-HT_6 antagonists, involving several compounds in the clinical trials[32] (Table 1), the success of the future applications may be dependent upon the finding of new compounds, which would not be limited by the classical pharmacophore of the aminergic ligands.

6. Acknowledgements

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7. Methodology

Synthesis

A general procedure for the functionalization of the indole nitrogen

An indole derivative (0.015 mol) and TBAB (0.5 g) were dissolved in a mixture of dichloromethane (100 ml) and 50% NaOH (50 ml) then, sulfonyl chloride (1.2 eq.) was added dropwise. Reaction mixture was vigorously stirred at room temperature for 1 h then, distilled water (200 ml) was slowly added and reaction mixture was extracted with dichloromethane (3x100 ml). Combined organic extracts were washed with distilled water (50 ml), brine (30 ml) dried over anhydrous magnesium sulfate and evaporated under reduced pressure. Product was purified by column chromatography on silica.

A general procedure for the substitution of indole nitrogen with benzyl

To a solution of indole starting material (2.5 mmol) in dry DMF (2 mL) at 0°C was added NaH (60% dispersal in mineral oil, 1.5 eq). The mixture was stirred at 0 °C for 10 min, and then it was allowed to warm to ambient temperature and stirred for 30 min. The solution was cooled to 0 °C, and then benzyl bromide (1.5 eq) was added. The ice bath was removed, and the reaction mixture was warmed to 45°C and stirred for 1 h. The mixture was cooled to room temperature, concentrated, and then diluted with ethyl acetate and saturated NH_4Cl. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography on silica

A general procedure for the Buchwald coupling

Starting bromo derivative (0.74 mmol), amine (3 eq.), palladium acetate (8 mg, 0.05 eq.), cesium carbonate (0.72g, 3 eq.), XPhos (30 mg, 0.083 eq.) and anhydrous toluene (5 ml) were placed in a round bottom flask and sealed under argon. Reaction mixture was heated at 130°C for 20 h. After cooling, distilled water was added (100 ml) and reaction mixture was extracted with ethyl acetate (3x100 ml). Combined organic extracts were washed with distilled water (100 ml), brine (30 ml) dried over anhydrous magnesium sulfate and evaporated under reduced pressure. Product was purified by column chromatography on silica.

A general procedure for the Suzuki coupling

A starting bromo derivative (1.2 mmol), boronic acid (1.6 eq.), tetrakis(triphenylphosphine)palladium(0) (69 mg, 0.016 eq.), 2M aqueous solution of potassium carbonate (1.2 mL, 2.0 eq.), toluene (5.0 ml) were sealed under argon. Reaction mixture was heated at 100°C for 1.5 h in microwave oven (150 W), after cooling to room temperature, distilled water was added (100 ml) and reaction mixture was extracted with ethyl acetate (3x100 ml). Combined organic extracts were washed with distilled water (100 ml), brine (30 ml) dried over anhydrous magnesium sulfate and evaporated under reduced pressure. Product was purified by column chromatography on silica.

Synthetic procedures, together with MS and NMR spectra, were presented in Supplementary Information (pages 18-166).

pK_a calculation

pK_a values calculations were run in Jaguar [33–36] under the default settings (energy change at level of $5x10^{-05}$, no SCF level shift, none termal smearing, accuracy level was set as ultrafine) for the lowest energy conformation for each compound (minimization performed in macroModel[37]) in water as a solvent.

Induced Fit Docking

The protocol of homology modelling consisted of three steps and aimed to mimic the conformational flexibility of the receptor observed in living cells.[38]

In the first step, the raw models of 5-HT_6R were generated based on the available crystal templates of 5-HT_{1B} and 2B subtypes (PDB: 4IAR and 4IB4, respectively). GPCRdb[39] sequence alignments between target and the templates were used, and water molecules as well as the ligand structure

from the crystal structures were preserved. Schrödinger Prime[40] was used to build the model structures.

The raw models underwent the Simulated Annealing protocol with reference compound CHEMBL1642416 (ChEMBL ID) docked, (Schrödinger Desmond, default settings) resulting in a batch of 250 ligand-receptor conformations for each of the templates. Ligand structure was then removed and the set of compounds were docked into the models. The model used in the third step was selected based on the number of compounds docked, average Glide score, and consistency of the obtained docking poses. The latter was evaluated by measuring the distance between fenyl rings of the docked series of compounds and the residues W^{6x48}, F^{6x51} and N^{6x55}.

The last step of the protocol was to generate the conformations of the ligand binding pockets with Induced Fit Docking protocol. Each series of synthesized compounds was used in the IFD. As a result of the ifd, a total number of 28 316 ligand-receptor complexes were obtained. These were filtered regarding the mutual orientation of the phenylsulfonyl and indole moieties, so that it corresponds with the data published in our previous work.[27] 4 317 ligand-receptor complexes fulfilled the applied constraints. From among those, 50 best scored (regarding the Glide Score value) complexes were selected and visually clustered and analyzed. Instant JChem was used as an additional software for the compound database management.[41]

Molecular Dynamics

A Molecular Dynamics (MD) simulations were performed using Schrödinger Desmond software.[42] Each ligand–protein complex was immersed into a POPC (300 K) membrane bilayer, which position was calculated using the System Builder interface. The system was solvated by water molecules described by the TIP4P potential and the OPLS3[43] force field parameters were used for all atoms. 0.15 M NaCl was added to mimic the ionic strength inside the cell. Molecular simulations for 100 ns with a step of 10 ps, using NPAT ensemble class (constant normal pressure, temperature and lateral surface area of membranes) and OPLS3e[44] force field were calculated for each system. Based on obtained trajectories, the mean geometrical distances between amino acids and ligand were calculated using Simulation Event Analysis tools in Maestro Schrödinger Suit.

Radioligand binding assays

Cell pellets were thawed and homogenized in 20 volumes of assay buffer using an Ultra Turrax tissue homogenizer and centrifuged twice at 35 000 g for 20 min at 4°C, with incubation for 15 min at 37°C in between rounds of centrifugation. The composition of the assay buffers was as follows: for 5-HT_{1A}R: 50 mM Tris–HCl, 0.1 mM EDTA, 4 mM $MgCl_2$, 10 µM pargyline and 0.1% ascorbate; for 5-HT_6R: 50 mM Tris–HCl, 0.5 mM EDTA and 4 mM $MgCl_2$, for 5-HT_{7b}R: 50 mM Tris–HCl, 4 mM $MgCl_2$, 10 µM pargyline and 0.1% ascorbate; for dopamine D_{2L}R: 50 mM Tris–HCl, 1 mM EDTA, 4 mM $MgCl_2$, 120 mM NaCl, 5 mM KCl, 1.5 mM $CaCl_2$ and 0.1% ascorbate.

All assays were incubated in a total volume of 200 µl in 96-well microtiter plates for 1 h at 37°C, excluding 5-HT_{1A}R, which was incubated at room temperature for 1 h. The equilibration process was terminated by rapid filtration through Unifilter plates with a 96-well cell harvester, and the radioactivity retained on the filters was quantified using a Microbeta plate reader.

For the displacement studies, the assay samples contained the following as radioligands: 1.5 nM [^{3}H]-8-OH-DPAT (187 Ci/mmol) for 5-HT$_{1A}$R, 2 nM [^{3}H]-LSD (85.2 Ci/mmol for 5-HT$_6$R, 0.6 nM [^{3}H]-5-CT (39.2 Ci/mmol) for 5-HT$_{7b}$R or [^{3}H]-Raclopride (74.4 Ci/mmol) for D$_2$R.

Non-specific binding was defined using 10 μM of 5-HT in the 5-HT$_{1A}$R and 5-HT$_{7b}$R binding experiments, whereas 10 μM methiothepine or 1 μM (+)butaclamol was used in the 5-HT$_6$R and D$_{2L}$ assays, respectively. Each compound was tested in triplicate at 7 – 8 concentrations (10^{-11} – 10^{-4} M). The inhibition constants (K_i) were calculated using the Cheng-Prusoff equation,[45] and the results are expressed as the means of at least two independent experiments.

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HIGHLIGHTS

Rationally designed N-phenylsulfonylindoles as a tool for the analysis of the non-basic 5-HT$_6$R ligands binding mode.

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1) The analysis of activity of non-basic serotonin receptor ligands is performed.
2) Substantially simplified ligands still exhibit affinity for the receptor.
3) N-Phenylsulfonyl indole possess all features necessary for the activity.
4) A slight, weak interaction increases the baseline activity.
5) A new pharmacophore for 5-HT6R is proposed.

Declaration of interests

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

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: