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Letter

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A pyrano[2,3,4-*cd*]indole as a scaffold for selective non-basic 5-HT$_6$R ligands

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ABSTRACT: In this paper, we report the synthesis of a pyrano[2,3,4-*cd*]indole chemical scaffold designed through a tandem bioisostere generation/virtual screening protocol in search of 5-HT$_6$R ligands. The discovered chemical scaffold resulted in the design of highly active basic and non-basic 5-HT$_6$R ligands (5-HT$_6$R K_i = 1 nM for basic compound **6b** and 5-HT$_6$R K_i = 4 nM for its neutral analog **7b**). Additionally, molecular modeling suggested that the hydroxyl group of non-basic ligands **7a**–**7d** forms hydrogen bonds with aspartic acid D^{3x32} or $D^{7.36x35}$.

KEYWORDS: Serotonin, 5-HT6, non-basic, molecular modeling, bioisosteres, virtual screening

Supporting Information Placeholder

The 5-Hydroxytryptanime receptor 6 (5-HT$_6$R) is one of fourteen identified serotonin receptors belonging to the Class A G-protein coupled receptors (GPCRs) and is positively coupled to adenylate cyclase.[1,2] It has been found that this receptor is distributed almost exclusively in areas of the central nervous system (CNS), which suggests that its ligands are unlikely to cause undesirable peripheral side effects.[3] In situ hybridisation studies of the 5-HT$_6$ receptor in rat brains using mRNA showed a major location in the projection areas of 5-HT neurons, especially in the structures essential for cognitive processes.[4] The location of 5-HT$_6$R clearly suggests application of its ligands as a procognitive drugs, which was confirmed in animal models.[5] Additionally, recent findings showed that 5-HT$_6$R is involved in the development of neural circuits, including neuronal migration and neurite growth.[6,7] As it was shown, blockade of 5-HT$_6$R is a promising new therapeutic perspective in the treatment of cognitive decline in patients with schizophrenia.[7]

So far, no selective 5-HT$_6$R ligand is used in the therapy, however several compounds (all selective antagonists of 5-HT$_6$R) that are currently under phase II or phase III clinical trials for the treatment of moderate Alzheimer disease (e.g.: Idalopirdine – Lundbeck; SUVN-502 – Suven Life Sciences) and for amplifying the effects of antipsychotic drugs (e.g.: AVN-211 – Avineuro Pharm. Inc., RVT-101 – Axovant) indicated promising results.[8] One major drawback of basic 5-HT$_6$R ligands is their possible affinity towards hERG, because they fulfil the hERG pharmacophore model.[9] Although there are examples of non-basic hERG ligands,[10] compounds devoid of a basic nitrogen atom are much less likely to exhibit this kind of activity. Until recently, it was believed that only a compound with a basic nitrogen atom could be an orthosteric ligand of an aminergic GPCR. The validity of this statement was widely acclaimed and independently supported in numerous studies.[11–14] However, the recent emergence of the non-basic ligands has somehow shifted the paradigm of medicinal chemistry.

A very important feature of the non-basic 5-HT$_6$R ligands is their extreme selectivity. Compounds developed by Ivachtchenko et al.[15] were tested against 55 therapeutic targets and achieved 5000- to >50000-fold selectivity towards 5-HT$_6$R. Van Loevezijn et al.[16] profiled their ligands in a panel of 86 receptors, ion channels, transporters and 27 enzymes. The only off-target for which weak affinities were observed was the translocator protein and 5-HT$_{2B}$R.

Our initial attempt was to design a novel 5-HT$_6$R ligands using an automated bioisostere generation from the Pipeline Pilot software[17]. As a basis for the bioisostere generation we utilized 10 structurally diverse, highly active 5-HT$_6$R ligands (parent structures) that were manually selected from 5-HT$_6$R ligands stored in the ChEMBL (version 13) database (see Supplementary Information, page 2).[18]

Each of these chemical entities was processed using the Breed module of Pipeline Pilot,[17] which resulted in 313 bioisosteres that were subsequently visually inspected in terms of novelty and synthetic accessibility (for selected compounds and information about their synthesis see Supplementary Information, page 3). Consequently, one bioisostere (**6a**) was selected for further evaluation, that emerged from reduction of the ring size together with the aromatization of a parent compound A (Figure 1). Here, we present the synthesis of a new pyrano[2,3,4-*cd*]indole scaffold that allowed us to obtain highly active basic and non-basic 5-HT$_6$R ligands.

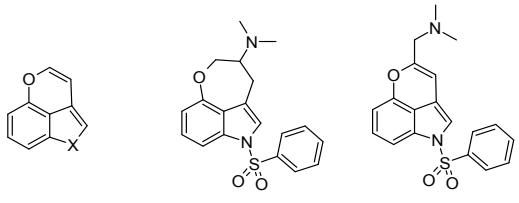


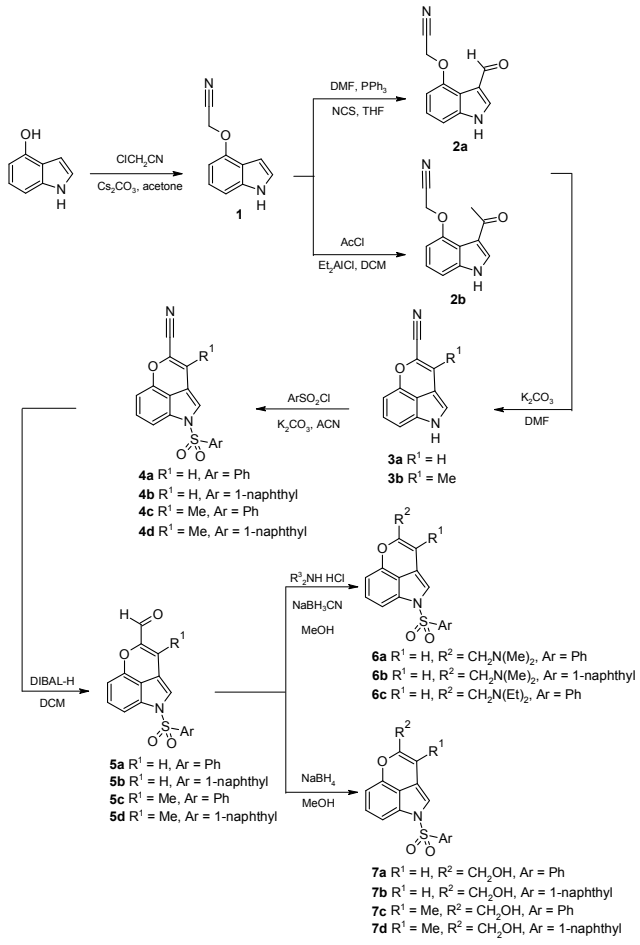
Figure 1. A general formula of a tricyclic scaffold (X = heteroatom), together with bioisosteric substitution (ring size reduction and aromatization) of the parent compound A.

Tricyclic scaffolds containing an indole moiety, as described by a presented general formula (Figure 1), have been barely explored in medicinal chemistry. Only two groups of compounds containing a nitrogen atom can be found: pyrrolo[4,3,2-de]quinolines reported by Balczewski et al.,[19] and marine alkaloids—ammosamides resynthesized by Takayama et al.[20] Ammosamides present significant cytotoxicity against human colon adenocarcinoma and moderately inhibit human quinine reductase II. To date, there is only one analog containing a sulfur atom—chuangxinmycin, a potent and selective inhibitor of bacterial tryptophanyl tRNA synthetase.[21] Surprisingly, no structures containing oxygen in the 6-membered ring have been reported to date.

The synthesis of the designed heterocyclic scaffold was planned as an intramolecular condensation of a carbonyl with the alpha carbon of a nitrile group.

The first step of the synthesis involved the addition of a chloroacetonitrile to a 4-hydroxyindole that resulted in **1** (Scheme 1). Subsequent formylation was performed using DMF with NCS and PPh_3 as a formylating agent because of an unsuccessful standard Vilsmeyer-Haack reaction. Similarly, acetylation of **1** using Vilsmeyer-Haack conditions resulted in a mixture of unidentified products. Finally, the utilization of diethylaluminium chloride combined with acetyl chloride afforded the acetylated product (**2b**) in high yield. The obtained intermediates (**2a**, **2b**) were cyclised in DMF with K_2CO_3 at 150 °C; however, the cyclisation of **2b** required much longer time compared to **2a** (20 min vs. 4 hours).

Scheme 1. Synthesis of designed bioisosteres.



All attempts to reduce the nitrile group to an amine were unsuccessful, and only after substitution of the indole nitrogen with sulfonyl chlorides (compounds **4a**–**4d**) was reduction with DIBAL-H possible, which afforded aldehydes **5a**–**5d**.

Reductive amination or reduction of the obtained aldehydes resulted in the final products (**6a**–**6c**, **7a**–**7d**).

Evaluation of the binding affinities of the synthesized compounds at four serotonin (5-HT_{1A}, 5-HT_{2A}, 5-HT_6, 5-HT_7) and dopamine D_2 receptors using in vitro radioligand displacement revealed that all of the synthesized compounds (except **4b** and **5b**) were potent 5-HT_6R ligands (K_i = 1–52 nM, Table 1). The basic derivatives (**6a**–**6c**) were generally 4- to 7-fold more active than their neutral analogs (**7a**–**7d**). Compounds possessing the 1-naphthylsulfonyl moiety (**6b**, **7b**, **7d**) exhibited 2- to 9-fold higher affinity for 5-HT_6R than the benzenesulfonyl derivatives (**6a**, **6c**, **7a**, **7c**), with **6b** being the highest affinity ligand with 5-HT_6R K_i = 1 nM. Introduction of a methyl group at C3 position of the pyrano[2,3,4-*cd*]indole (compounds **7c** and **7d**) resulted in 2- to 5-fold less active compounds than compounds without methyl group (**7a** and **7b**). Analog **4b** containing nitrile group was found to possess low affinity for 5-HT_6R (266 nM), whereas analog **5b** with carbonyl group was 3-fold less active (K_i = 755 nM).

Neutral analogs possessing a hydroxyl group exhibited much greater selectivity towards other receptors, than their basic counterparts, despite lower affinity for 5-HT_6R. Additionally, their functional antagonist activity was retained though they lost the ability to form a charge reinforced hydrogen bond with aspartic acid D^{3x32} (in GPCRdb notation[22]), which was evidenced by the value of K_b = 16 nM for **7a**.

The binding mode of the synthesized compounds was investigated with docking experiments to the homology models of 5-HT_6R (see Supplementary Information). Both the basic and non-basic analogs exhibited very consistent binding modes (Figure 2), overlapping with the previously reported data: [23] the peripheral aromatic group formed stacking interactions with phenylalanines F^{6x51} and F^{6x52}, and the sulfonyl group of the ligands formed a hydrogen bond with N^{6x55}, in line with the mutagenetic data.[24] In addition, a protonated nitrogen atom of the basic analogs formed a charge-assisted hydrogen bond with D^{3x32}, exhibiting the classical binding mode for the basic compounds.[25] The hydroxyl group formed hydrogen bonds either with aspartic acid D^{3x32}, $D^{7.36x35}$ or tyrosine $Y^{7.43x42}$; however, the hydrogen bond with $D^{7.36x35}$ was the most populated.

The results of molecular modeling suggested that the polar interaction of the basic amine group might be replaced with the interaction with another polar moiety (e.g., hydroxyl), and the *in vitro* tests confirmed that such a substitution did not cause a significant drop of affinity and functional activity. Although nitrile and carbonyl groups can form hydrogen bonds,[26] the nitrile moiety pointed outside of the binding pocket preventing it from forming any interaction. In the case of the carbonyl, the hydrogen bond was formed with a backbone nitrogen of leucine 182 located in the extracellular loop 2. Such an interaction is very weak; nevertheless, the carbonyl derivative **5b** exhibited 3-fold higher affinity than nitrile derivative **4b**.

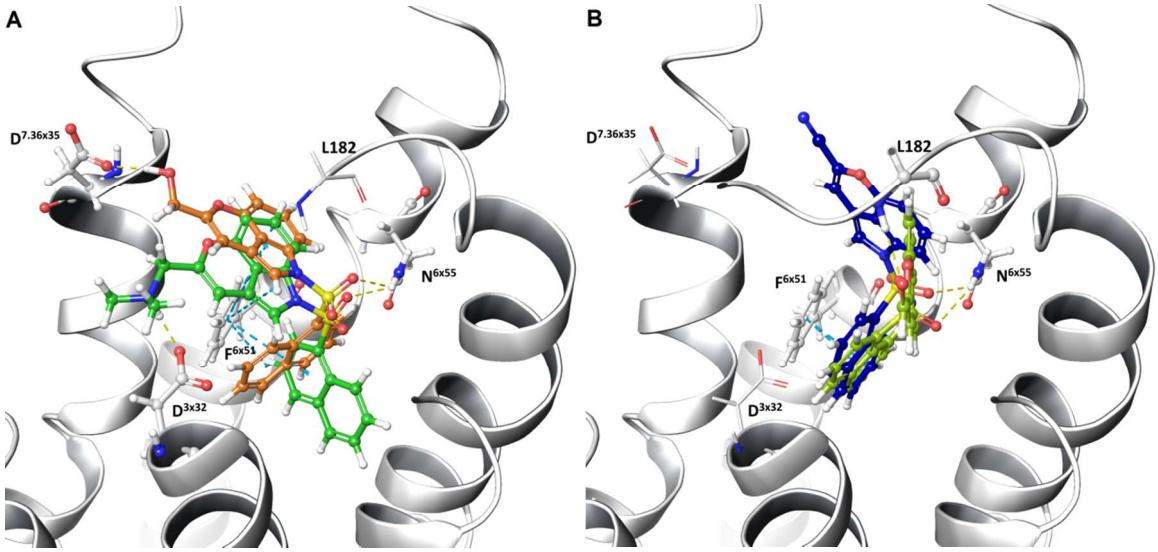
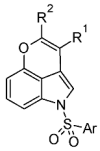


Figure 2. Representative L-R virtual complexes of (**A**) **6b** (5-HT_6R K_i = 1 nM, green) and **7b** (5-HT_6R K_i = 4 nM, orange), and (**B**) **4b** (5-HT_6R K_i = 266 nM, green) and **5b** (5-HT_6R K_i = 755 nM, blue) with a homology model of 5-HT_6R. The protonated nitrogen atom of **6b** formed a charge-assisted hydrogen bond with D^{3x32}, and the hydroxyl group of **7b** formed a hydrogen bond with $D^{7.36x35}$. The carbonyl oxygen of **4b** formed a hydrogen bond with the backbone nitrogen of leucine L182. Sulfonyl groups of every compound formed hydrogen bonds with asparagine N^{6x55}, whereas the peripheral aromatic rings (naphthalene) were placed between phenylalanines F^{6x51} and F^{6x52}, forming stacking interactions. Yellow dotted lines indicate hydrogen bonds, whereas blue dotted lines indicate aromatic interactions.

Table 1. Structure and *in vitro* activity data of compounds 4b, 5b, 6a–c and 7a–d.



Cmpd.	R^1	R^2	Ar	K_i [nM][a] 5-HT_6	5-HT_{1A}	5-HT_{2A}	5-HT_7	D_2
4b	H	CN	1-naphthyl	755	> 10 000	n.d.	> 10 000	> 10 000
5b	H	CHO	1-naphthyl	266	> 10 000	n.d.	> 10 000	5 162
6a	H	$CH_2N(Me)_2$	Ph	5 K_b = 1.5 ± 0.5[b]	> 10 000	65	3 692	6 698
6b	H	$CH_2N(Me)_2$	1-naphthyl	1	5 967	489	438	1 489
6c	H	$CH_2N(Et)_2$	Ph	28	> 10 000	1 703	> 10 000	4 351
7a	H	CH_2OH	Ph	36 K_b = 16 ± 3[b]	> 10 000	> 10 000	> 10 000	> 10 000
7b	H	CH_2OH	1-naphthyl	4	n.d.	> 10 000	> 10 000	4 287
7c	Me	CH_2OH	Ph	52	> 10 000	> 10 000	> 10 000	> 10 000
7d	Me	CH_2OH	1-naphthyl	20	> 10 000	> 10 000	> 10 000	> 10 000

[a] Binding affinity, K_i, expressed as the average of at least two independent experiments; the maximum S.D. did not exceed 32% (see Supplementary Information, page 6); [b] full antagonist, K_b [nM] expressing the functional activity as the average of at least three independent experiments; n.d. – not determined. Affinity of the reference drugs: 5-HT_6R and 5-HT_{2A}R, Olanzapine – K_i = 10.7 ± 2.1 nM and K_i = 6.2 ± 0.9 nM, respectively; 5-HT_{1A}R, Buspirone – K_i = 34.3 ± 4.2 nM; 5-HT_7R, Clozapine – K_i = 45.5 ± 5.1 nM; D_2R, Ziprasidone – K_i = 2.1 ± 0.3 nM.

The presented chemical scaffold of pyrano[2,3,4-*cd*]indole is unique, as no similar compounds have been reported in the literature. The addition of an aromatic-sulfonyl moiety at the indole nitrogen afforded highly active basic and neutral 5-HT_6R ligands. Additionally, the nitrile group of compounds **3a** and **3b** allows them to be utilized in click chemistry, thus providing a valuable building block for the synthesis of biologically active compounds. In addition, the compound **3a** exhibited strong green fluorescence, with a maximum emission of 520 nm (see Supplementary Information), which creates the possibility to use it as a building block for fluorescent markers.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Synthetic procedures and characterization of new compounds, experimental details of *in vitro* assays. Fluorescent spectra of compound **3a** (PDF).

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Author Contributions

J.S. designed and synthesized compounds, S.M. performed the docking to the 5-HT_6R model, D.W. performed the virtual screening protocol, G.S. performed the *in vitro* tests, A.H. performed the spectral analysis of compounds. J.S. and A.J.B. wrote the paper with input from coauthors.

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ABBREVIATIONS

5-HT, 5-hydroxytryptamine; 5-HT_6R, 5-hydroxytryptamine receptor 6; mRNA, messanger RNA; GPCR, G-protein coupled receptor; hERG, **h**uman ***E**ther-à-go-go-***R**elated **G**ene; DMF, dimethylformamide; NCS, *N*-chlorosuccimide; PPh_3, triphenylphosphine; DIBAL-H, diisobutylaluminium hydride; D_2, dopamine receptor 2; GPCRdb, G- protein coupled receptor database, S.D., standard deviation.

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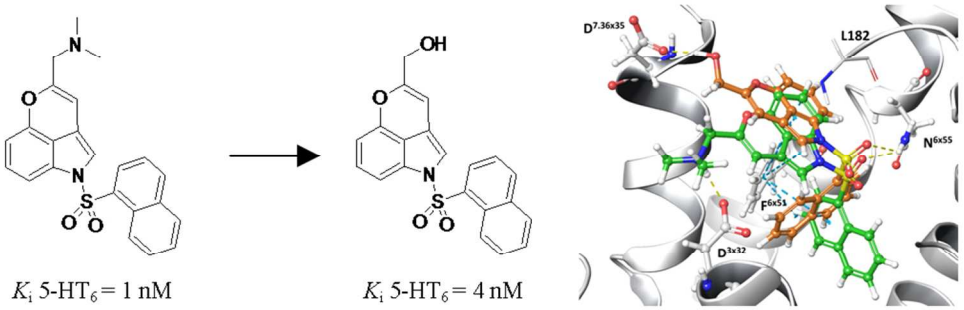

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