BIOISOSTERIC APPROACH TO INVESTIGATION OF LIGAND BINDING MODE AT 5-HT6R

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Introduction

5-HT6 receptor was recognized as promising target, among other, for novel antidepressat and procognitive drugs.

Up to date, several thousands of chemical compounds acting at this target were acquired. Almost all of them contain basic nitrogen atom and about 85% of them contain sulphonyl group as a pharmacophore feature. These moieties were commonly recognized as essential for ligand-5-HT$_6$R interactions.[1-4] However, the discovery of highly active ligands without sulphonyl moiety, together with the discovery of non-basic ligands, made current knowledge necessary to be updated. Several scientific groups tried to provide explenation for activity of novel ligands, but there is still much to be discovered.[5,6]

Compounds design

The basis for compound design was provided by bioisistere database generated in Open Eye Pipeline-Pilot programme generated on 5-HT$_6$R ligands stored in ChEMBL. Three methods of ligand design were implemented;

- virtual screening protocol of bioisostere database,
- comparison of bioisostere database with commercially available compounds,
- comparison of bioisostere database with database of D2R ligands (taken from ChEMBL).

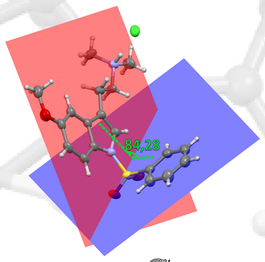
All three methods provided finally 22 compounds representing eight bioisosteric substitutions (**table 1**).

Crystal structures

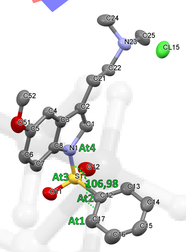
Crystal structures of 14 compounds (**1**, **2**, **6**, **7**, **8**, **10**, **11**, **12**, **13**, **14**, **16**, **18**, **19**, **21** - **table 1**) were obtained. All of them were next described by four geometrical parameters (**table 2**).

Geometrical paramaters compound **6** as an axample

Angle 1 - a plane angle defined by two aromatic systems



Angle 2 - a torsion angle defined by atoms At1-At2-At3-At4



Distances between centroids of aromatic rings (dist. 1) and between basic nitrogen atom and peripheral aromatic ring (dist. 2).

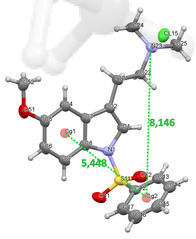


Table 2. Geometrical parameters of crystallized compounds.

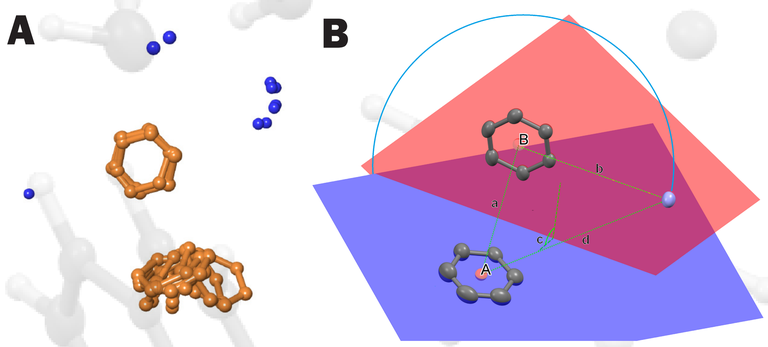
Compd. No.	K_i [nM]	angle 1 [°]	angle 2 [°]	dist. 1 [Å]	dist. 2 [Å]
1	1	83.14	93.82	5.322	9.138
2	1280	54.00	135.41	6.452	9.785
6	11	84.28	106.98	5.448	8.146
7	44	53.62	45.43	6.540	7.344
8	23	74.46	67.85	6.010	7.829
10	21	89.92	136.80	5.059	6.771
11	245	21.43	165.32	6.209	10.004
12	62	22.61	163.76	6.428	9.956
13	6	59.21	62.01	5.523	7.335
14	4	89.17	104.28	5.224	9.054
16	18	83.10	35.81	5.889	8.663
16[a]	18	82.27	146.29	5.163	9.604
18	2204	55.29	139.72	6.487	10.676
18[b]	2204	56.06	136.91	6.496	10.775
18[b]	2204	72.56	136.04	6.485	10.845
18[b]	2204	67.93	122.08	6.474	10.565
19	24	86.76	158.78	5.412	10.569
21	63	79.38	108.39	6.633	8.527

[a] crystal structure of **16** contains two compound molecules in the assymetric unit.

[b] crystal structure of **18** contains four compound molecules in the assymetric unit.

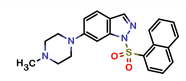
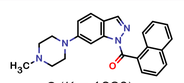
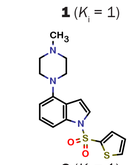
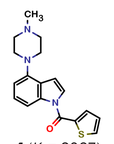
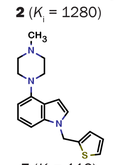
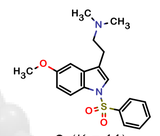
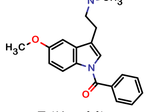
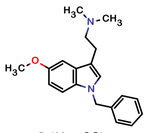
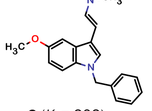
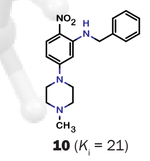
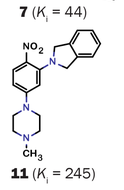
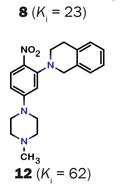
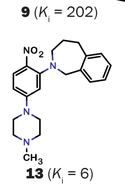
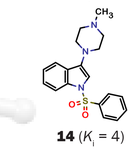
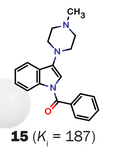
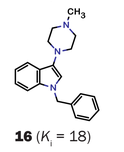
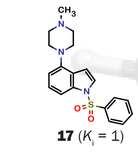
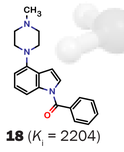
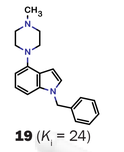
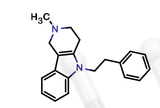
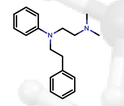
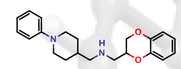
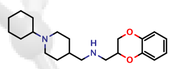
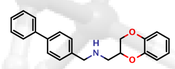
Average geometrical parameters for most active compounds (K_i < 30 nM) are equal: angle 1 = 81.37 ± 9.11°, angle 2 = 101.40 ± 38.93°, dist. 1 = 5.450 ± 0.241 Å, dist. 2 = 8,534 ± 1.038 Å.

Superposition of crystal structures of most the active compounds (**A**) revealed very conserved location of aromatic moieties, while position of basic nitrogen atom is unrestricted. This led to a new comprehensive pharmacophore model (**B**), with conserved aromatic features and area of possible nitrogen atom position (blue line). The geometrical parameters **a**, **b**, **c** and **d** are equal to average parameters listed above with range of values: **a = 5.450 ± 0.241 Å**, **b = 6.159 ± 0.463 Å**, **c = 81.37 ± 9.11°**, **d = 8.534 ± 1.038°**.



Bioisosteric pairs

Table 1. Affinity values (K_i) for 5-TH$_6$R in [nM] measured in our laboratory.

Ligand	Bioisostere	Type of substitution
 **1** (K_i = 1)	 **2** (K_i = 1280)	sulphonyl - carbonyl
 **3** (K_i = 1)	 **4** (K_i = 2067)  **5** (K_i = 116)	sulphonyl - carbonyl, methanediyl
 **6** (K_i = 11)	 **7** (K_i = 44)  **8** (K_i = 23)  **9** (K_i = 202)	sulphonyl - carbonyl, methanediyl
 **10** (K_i = 21)	 **11** (K_i = 245)  **12** (K_i = 62)  **13** (K_i = 6)	noncyclic - cyclic ring expanding
 **14** (K_i = 4)	 **15** (K_i = 187)  **16** (K_i = 18)	sulphonyl - carbonyl, methanediyl
 **17** (K_i = 1)	 **18** (K_i = 2204)  **19** (K_i = 24)	sulphonyl - carbonyl, methanediyl
 **20** (K_i = 22)	 **21** (K_i = 63)	cyclic - noncyclic
 **22** (K_i = 2280)	 **23** (K_i = 2675)  **24** (K_i = 3760)	phenyl - cyclohexane piperidine - phenyl

SIFt representation

For each compound, only the best docking pose per receptor model was considered and 100 the best scored complexes were transformed into bitstring applying SIFt formalism statistically describing interactions between ligand and receptor.[7,8]

Residue	[%] compounds interacting with residue		average SIFt	
	active K_i < 100 nM	inactive K_i > 100 nM	active K_i < 100 nM	inactive K_i > 100 nM
W3.28	42	50	0.69	0.72
T3.29	**83**	75	0.68	0.62
D3.32	**100**	83	**0.88**	0.78
V3.33	100	100	**0.90**	0.83
C3.36	**100**	83	0.81	0.76
L4.61	50	50	0.62	0.61
G146	0	**25**	-	**0.60**
R162	0	**17**	-	**0.57**
L163	100	92	0.98	0.92
L164	67	67	0.71	0.76
A165	100	92	**0.82**	0.74
F5.38	100	100	0.81	0.76
V5.39	100	100	0.85	0.83
A5.42	92	83	0.72	0.70
S5.43	33	**50**	**0.82**	0.64
T5.46	**75**	42	0.61	0.65
W6.48	**75**	50	**0.64**	0.57
F6.51	100	100	0.99	0.95
F6.52	**100**	83	0.79	0.73
N6.55	100	92	0.85	0.82
V6.58	**58**	17	0.66	0.71
F7.35	100	100	0.99	0.99
D7.36	17	**50**	0.62	**0.72**
T7.39	100	100	**0.92**	0.84
Y7.43	100	83	0.73	0.70

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Docking studies

In order to measure the position of a ligand in a binding pocket of 5-HT$_6$R, all obtained compounds were docked to 200 homology models of 5-HT$_6$R. For 100 the best scored complexes, distances between ligand and different amino acid residues were calculated.

„Classical'' position

Anchor points for measurement: Amino acid residue	Ligand	Average distance [Å] for active ligands (K_i < 30 nM)	Average distance [Å] for inactive ligands (K_i > 100 nM)
W6.48	Nearest ligand atom	5.55 ± 0.04	5.94 ± 0.05
Nearest aa. in a loop between 2nd and 3rd helix	Nearest ligand atom	2.06 ± 0.01	2.03 ± 0.01
D3.32	Ionised basic nitrogen atom	3.26 ± 0.02	3.33 ± 0.02
F6.51	Nearest aromatic moiety	6.15 ± 0.03	6.68 ± 0.06
F6.52	Nearest aromatic moiety	6.46 ± 0.04	6.96 ± 0.07

„Alternative' position

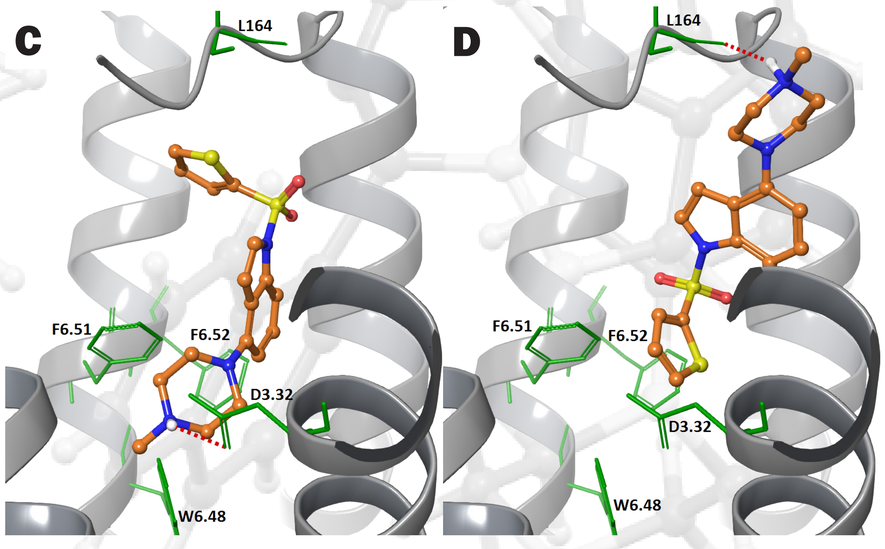
Anchor points for measurement: Amino acid residue	Ligand	Average distance [Å] for active ligands (K_i < 30 nM)	Average distance [Å] for inactive ligands (K_i > 100 nM)
W6.48	Nearest ligand atom	6.71 ± 0.06	8.05 ± 0.13
Nearest aa. in a loop between 2nd and 3rd helix	Nearest ligand atom	1.95 ± 0.01	1.98 ± 0.03
D3.32	Ionised basic nitrogen atom	12.07 ± 0.05	12.40 ± 0.15
F6.51	Nearest aromatic moiety	5.64 ± 0.03	6.32 ± 0.07
F6.52	Nearest aromatic moiety	6.64 ± 0.04	7.41 ± 0.12

Conclusions

The performed studies stressed the importance of aromatic/hydrophobic interactions in the 5-HT$_6$R-ligand complexes together with reduction of basic nitrogen atom importance. Additionally, a new ligand position in receptors binding pocket was proposed, though its potential significance need to be further confirmed.

Virtual comlexes

Docking studies revealed two possible positions of a ligand in the binding pocket. The „classical'' position with basic nitrogen atom heading towards the bottom of the binding pocket (**C**) and „alternative'' position with basic nitrogen atom pointing towards the entrance to the binding pocket (**D**). Different complexes of compound **3** are shown.



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(5) Ivachtchenko, A. V et al. Synthesis of Cycloalkane-Annelated 3-Phenylsulphonyl-pyrazolo[1,5-A]pyrimidines and Their Evaluation as 5-HT 6 Receptor Antagonists. Bioorgan. Med. Chem. Lett. 2010, 20 (7), 2133–2136.
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(8) Witek, J. et al. An Application of Machine Learning Methods to Structural Interaction Fingerprints-a Case Study of Kinase Inhibitors. Bioorg. Med. Chem. Lett. 2013, 24 (2), 580–585.