Development of new 5-HT$_6$R ligand series illustrates the search for selective transmembrane proteins ligands

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How does our drug candidate search work

literature and patent search,
ADMET prediction,
searching for new potential
bioisosteric substitutions,

↓

ideas of new chemical entities,
patentability search/ databases
substructure search to confirm
originality,

↓

retrosynthetic analysis,
synthone availability check,
preliminary experiments,

↓

synthesis of compound series,
purity & structure determination,
binding affinity assay,

↓

SAR study,
exploration of modification possibilities,

structure revision

HIT COMPOUNDS

↓

anti target screening,
ADMET evaluation assays:
genotoxicity,
metabolic stability,
blood brain barrier permeability,
oral absorption potential,

↓

LEAD COMPOUNDS

↓

behavioral tests,
toxicological screening panel,
advanced ADME experiments.

5-HT$_6$ receptor - an emerging drug target for Alzheimer's disease

5-HT$_6$R discovered in the early 90's has proven to be an important target for neurodegenerational disorders.[1] 5-HT$_6$R blockade induces acetylcholine release which might restore function in deteriorated cholinergic system of Alzheimer's Disease (AD) patients.[2] The receptor is expressed almost exclusively in brain which implicates low probability of peripheral side effects. 5-HT$_6$ antagonists administration can signifficantly improve cognitive performance in AD patients.[3] Two 5-HT$_6$ antagonists (Lu AE58054, SB-742457) have completed phase II clinical trials as an augmentation therapy for Alzheimer's disease.

5-HT$_6$receptor ligand series development

Two independent series of selective 5-HT$_6$R ligands consisting of 33 and 37 compounds respectively have been developed.

Pilot compound of serie 1, AH-122 emerged from analysis of pharmacological profile of an ultrapotent 5-HT$_{2A}$R agonist, CIMBI-5[4,5,6] (Fig. 1). Replacement of the benzylic fragment of CIMBI-5 with an 3-indolemethyl scaffold resulted in a steep drop of 5-HT$_{2A}$ activity, while 5-HT$_6$R affinity was enhanced. To reveal the optimal substitution pattern for 5-HT$_6$R binding, 33 compounds have been synthetised till date. This was achieved thanks to the very concise synthetic protocol exploiting commercialy aviable arylacetonitriles developed in ourlab (Fig. 2). It was found thatproper substitution of the phenylethylamine backbone is crucial for 5-HT$_6$R activity (Tab. 1). To our delight, two additional serotonin receptor hit compounds werefound in radioligand binding assays (Fig. 3). A virtual combinatorial library consisting of 3364 structures was generated using Jchem Reactor tool.

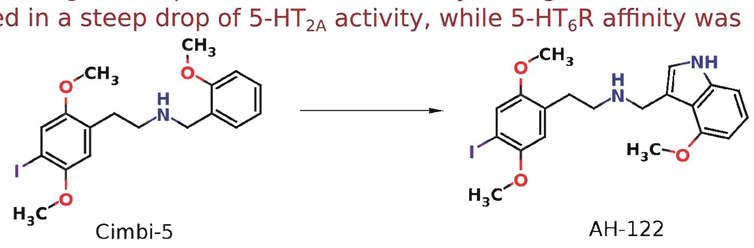


Fig. 1: non-classical bioisosteric substitution yields a hit compound

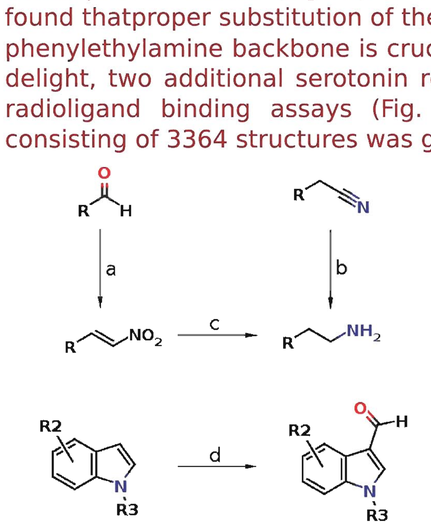
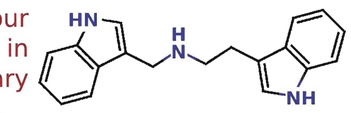


Fig. 2:
Conditions: a) CH_3COONH_4, CH_3NO_2, 100°C b) $LiAlH_4$, $AlCl_3$, Et_2O, reflux c) $LiAlH_4$, THF, reflux d) 1.DMF, $POCl_3$, 0°C, 2.NaOH, heating e) 1. MeOH, 4Å MS, 2. $NaBH_4$
R = Ph, 1-Naph, 2-Naph; R1 = Ph, 1-Naph, 2-Naph, 2-indole, 3-indole; R2 = H, OMe, Br; R3 = H, Me

Virtual docking protocol (5-HT$_6$R, 5-HT$_{2A}$R, 5-HT$_{2C}$R homology models used) was performed using Glide software. Our plan is to investigate binding mode(s) of this serie by analyzing docking results of compounds with determined binding affinities. Next, rigid, conformationally constrained analogues will be synthetised to bring additional evidence.
The synthesis and evaluation of hit compound AH-246 derivatives may bring an insight into 5-HT$_{1A}$R binding mechanisms.



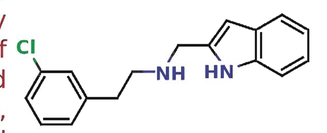
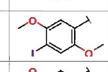
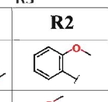
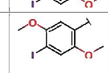
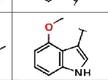
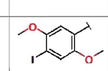
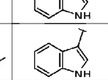
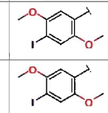
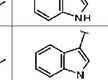
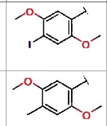
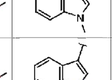
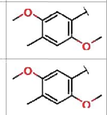
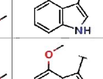
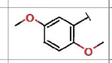
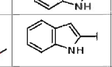
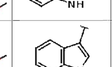
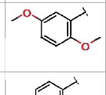
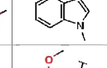


Fig. 3:
5-HT$_{2A}$R and 5-HT$_{1A}$R hit compounds - byproducts of the research

Serie 2, opened with hit compound AH-42 (10nM at 5-HT6R), consists of 37 compounds with 5-HT6R binding affinities in the range of 2 - 455 nM. The selectivity over related targets (5-HT1A, 5-HT2A, 5-HT7 and D2 receptors) is excellent. Further experiments revealed good metabolic stability, lack of genotoxicity (Ames test) and selectivity over wide range of targets (two compounds screened for H1, H3, M1, M5, 5-HT3, alpha1, alpha3,). Functional assay confirmed that the compounds act as antagonists of 5-HT6R. New compounds are synthetised in order to improve ADME properties. Lead structures will go through a panel of additional in-vitro and in-vivo experiments to evaluate drug-likeness.

ID	R1	R2	K_i [nM] 5-HT$_{1A}$	5-HT$_{2A}$	5-HT$_6$	5-HT$_7$	D$_2$
CIMBI-5			3552	1 (0,04 *)	**41 (73 *)**	>10000	-
AH-122			>10000	300	**39**	>10000	1522
AH-120			4214	-	**87**	6224	1488
AH-125			2721	-	**46**	3875	938
AH-189			-	64	**543**	>10000	5223
AH-190			-	1238	**310**	7556	3572
AH-195			398	-	**534**	-	-
AH-196			1895	424	**318**	4257	-
AH-185			>10000	610	**1179**	5338	-
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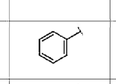
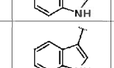
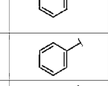
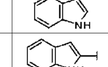
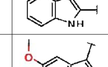
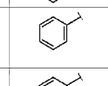
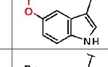
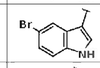
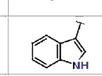
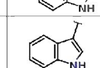
AH-186			-	3180	**39**	4182	2071
AH-199			5730	-	**703**	>10000	-
AH-228			3795	1099	**246**	4166	-
AH-226			1531	**20**	**172**	2861	-
AH-236			173	3422	**1605**	1559	-
AH-246			**19**	2006	**475**	923	-

Tab. 1: Binding affinities of selected compounds from serie 1
** data from ChEMBL database*

Refferences:
[1]Upton, N.;Chuang T. T.; Hunter A. J.; Virley D. J. *Neurotherapeutics*, **2008**, *5*, 458-469,
[2]Holenz, J.; Pauwels P. J.; Diaz J. L.; Merce R.; Codony X.; Buschmann H. *Drug Discovery Today*, **2006**, *11*, 283-299,
[3]Wilkinson, D.; Colding-Jørgensen, E.; Windfeld, K. *Alzheimers Dement*, **2013**, *9*, P529,
[4]Heim, R. Synthese und Pharmakologie potenter 5-HT$_2$A-Rezeptoragonisten mit N-2-Methoxybenzyl-Partialstruktur. Entwicklung eines neuen Struktur-Wirkungskonzepts. Ph.D. Dissertation, Free University of Berlin, 2004,
[5]Braden, M. R.; Parrish, J. C.; Naylor, J. C., Nichols, D. E. *Mol. Pharm.* **2006**, *70*, 1956-1964,
[6]Hansen, M.; Phonekeo, K.; Paine, J., S.; Leth-Petersen, S.; Begtrup, M.; Bräuner-Osborne, H.; Kristensen, J., L. *ACS Chem. Neurosci.*, **2014**, *5*, 243-249.

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