Searching of novel leads for 5-HT$_7$ receptor antagonists - selectivity hints from molecular modeling studies

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Objective

To identify new 5-HT$_7$R antagonists as potential antidepressant agents, a pilot set of structurally diversified compounds was designed, synthesized, and binding affinities for 5-HT$_7$R and other therapeutically important: 5-HT$_{1A}$R, 5-HT$_{2A}$R, 5-HT$_6$R as well as opamine D$_2$ receptors, were assessed. In addition, docking studies using the previously developed 5-HT$_7$R homology models,[1] were used to interpret affinity and selectivity data.

Design and synthesis

New compounds have been designed based on structural features, present in model ligands **1–5**, which might be important for both high affinity for 5-HT$_7$R and selectivity. As a starting point, the dual antagonist of 5-HT$_{1A}$ and 5-HT$_7$R – NAN-190, was used (Table 1). Firstly, 1,2-benzoxazole fragment was introduced into arylpiperazine part of compound according to Perrone at al.[2] who reported it as improving 5-HT$_7$R *vs* 5-HT$_{1A}$R selectivity (e.g. **5**). This fragment is also present in the structure of multireceptor antipsychotic drug risperidone (**1**), which has a particularly high affinity for 5-HT$_7$R. Next, in derivatives **7–12**, phthalimide portion of **6** was replaced by arylsulfonyl group, (a common motif of many selective 5-HT$_7$R antagonists (e.g. **2**, **4**), and subsequently, in a place of flexible alkyl chain, 2-ethyl-1-piperidine fragment, was introduced. In the structures of compounds **9–12** the amine pharmacophore was also modified, and among others, the perhydroisoquinoline moiety (**4**) was used, following the results of Raubo et al.[3], who found this fragment important for selectivity.

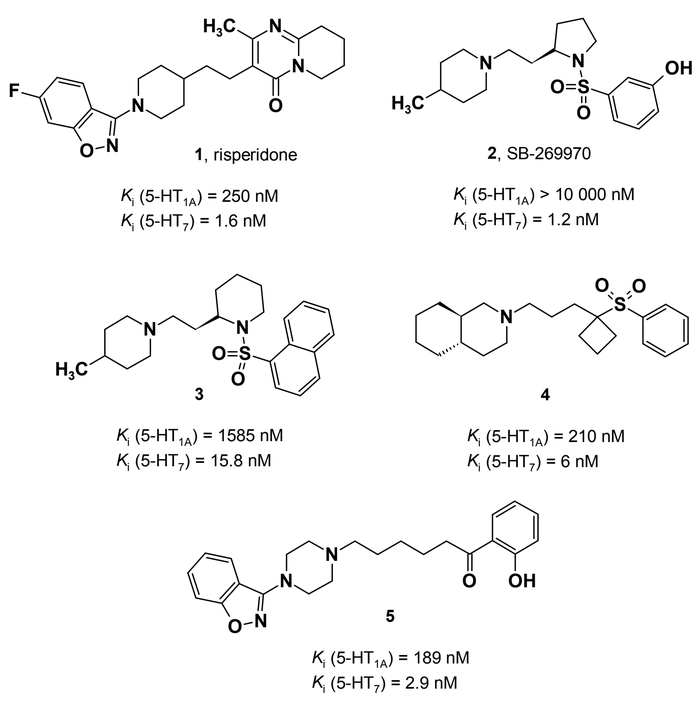
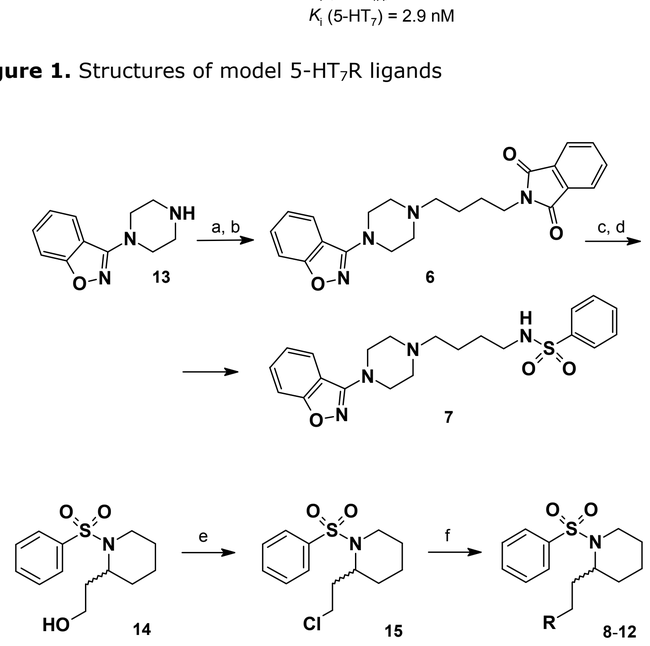


Figure 1. Structures of model 5-HT$_7$R ligands



R: 3-(piperazin-1-yl)-1,2-benzoxazole (**8**), 2-(morpholin-4-yl)ethan-1-amine (**9**), 2-(pyrrolidin-1-yl)ethan-1-amine (**10**), *trans*-decahydroisoquinoline (**11**), *cis/trans*-decahydroquinoline (**12**)

Scheme 1. Synthesis of target compounds **6–12**. (a): 1,4-dibromobutane, K_2CO_3, 18-crown-6, 95% ethanol, 24h; (b): phthalimide, K_2CO_3, 18-crown-6, xylene, 22h; (c): 1). $N_2H_4 \cdot H_2O$, ethanol, 1h; 2). HCl, 4h; (d): $PhSO_2Cl$, Et_3N, $CHCl_3$, 20°C, 4h; (e): $PhSO_2Cl$, DIPEA, $CHCl_3$, reflux, 6h; (f): amine, NaI, K_2CO_3, CH_3CN, reflux, 2h.

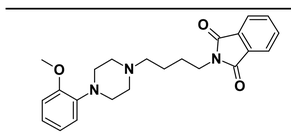
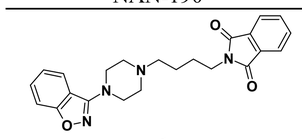
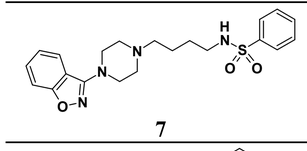
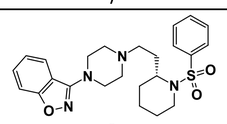
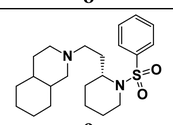
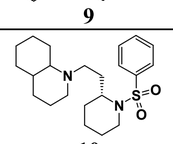
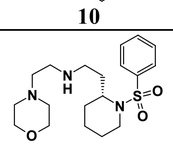
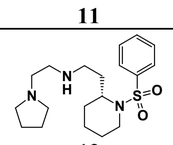
In vitro results

The affinities of compounds **6–12** for 5-HT$_7$, 5-HT$_{1A}$ 5-HT$_{2A}$, 5-HT$_6$, and dopamine D$_2$ receptors were determined using competitive radioligand binding assays according to previously published procedures (table 1).

Based on presented affinity and selectivity, compounds **8** and **9** were selected for testing their functional behaviour using a LANCE cAMP assay, where both displayed antagonistic properties (pEC$_{50}$ = 9.13 and 5.78, respectively).

References: [1] Kołaczkowski M. et al. *J. Med. Chem.* **2006**, *49(23)*, 6732-41. [2] Perrone R. et al. *J. Med. Chem.* **2003**, *46(4)*, 646-9. [3] Raubo P. et al. *Bioorg. Med. Chem. Lett.* **2006**, *16(5)*, 1255-8.

Table 1. The receptor binding affinity of NAN-190 and test set of compounds **6**–**12**.

Structure	K_i [nM]				
	5-HT$_7$	5-HT$_{1A}$	5-HT$_{2A}$	5-HT$_6$	D$_2$
 NAN-190[a]	128	0.6	182	NT	47
 **6**	128	24	NT	874	195
 **7**	13	299	44	341	691
 **8**	11	104	19	393	12
 **9**	44	2966	6000	>10 000	6970
 **10**	407	10 050	9000	>10 000	>10 000
 **11**	>5000	>10 000	NT	>10 000	NT
 **12**	>5000	>10 000	NT	>10 000	NT

In silico results

Compounds **6** and **7** with flexible *n*-butyl alkyl chain were anchored in analogous mode to that found in our earlier studies for non-selective derivatives, and accepted slightly bent conformation (fig. 3 A, B).[1] The amine part, i.e. the 3-(piperazin-1-yl)-1,2-benzoxazole fragment, occupied the pocket I, interacting with the amino acids of helices 3 and 6. An opposite terminals, the phthalimide or the phenylsulfonamide moieties penetrated the pocket II, and the oxygen atom of carbonyl group in **5** was able to form a strong hydrogen bond with Tyr7.43. Partial rigidification of **8** by 2-piperidine ring, forced more bent conformation, causing phenyl substituent of sulfonamide group, docked nearby 3-(piperazin-1-yl)-1,2-benzoxazole fragment, to interact with Phe6.51 (fig. 3 C).

The docking results of compound **9** showed, that the molecule was considerably shifted toward pocket II and there were all main ineractions. The sulfonamide oxygen was in vicinity to Tyr7.43, and phenyl substituent could form the interactions of type p-p with Phe3.28, whereas perhydroisoquinoline moiety was in vicinity of Trp6.48 and Phe6.52 (fig. 3 D). The mode of binding identified for compound **9** was analogous to that, described earlier for highly-active arylsulfonamidalkylamine ligands (fig. 2)[1] and reflected real high affinity and the selectivity of **9** to 5-HT$_7$R (fig. 3 D).

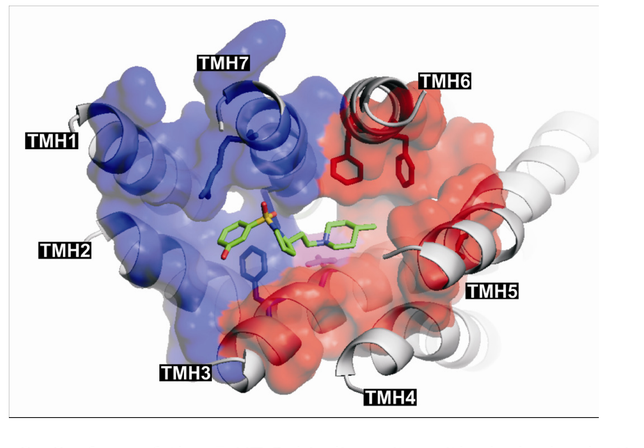


Figure 2. Surface of the 5-HT$_7$R binding site with docked selective antagonist SB 269970 (**2**). Two binding pockets: I – between TMHs 3–6 (red) and II – between TMHs 7–3 (blue) are, indicated. Common anchoring point (Asp3.32) in magenta.

The perhydroquinoline derivative **10**, was anchored nearby the central part of receptor binding site with the oxygen atom from sulfonamide moiety turned in side of Tyr7.43 (fig. 3 E). The geometry of perhydroquinoline moiety caused its different arrangement in binding pocket, making possible only few, unspecific interactions of hydrophobic nature. This results may explain lower 5-HT$_7$R affinity of **10** than that found for **9**, however prediction of activity/selectivity of **10** based on docking solutions was not so obvious as in the case of derivatives **6–9**.

Although the docking results of compounds **11** and **12** indicated the possibilities of forming complexes with 5-HT$_7$R model mainly in pocket I, their binding modes were quite opposite (fig. 3 F, G) in relation to solutions found for SB 269970 as well as for others compounds investigated in the present study. In both cases the sulfonamide part was directed in right, hydrogen bond with Ser5.42 was formed by sulfonamide oxygen, and phenyl substituent interacted with Phe6.52. These differences caused that the *ex ante* prognosis of activity/selectivity for **11** and **12** was doubtful, nevertheless both derivatives were found inactive in our binding experiments.

Conclusions

On the basis of obtained results perhydroisoquinoline **9** was chosen as a lead compound for further development and optimization of 5-HT$_7$R selective ligands.

Moreover, since the recent data have already provided interesting insights regarding 5-HT7$_7$R antagonism as beneficial component of therapeutic action of numerous antipsychotic drugs (amisulpride and lurasidone) we also selected benzisoxazole piperazine derivative **8** to develop the second series of potential multireceptoral agents with high 5-HT$_7$R affinity.

The present investigation has also confirmed the usefulness of our 5-HT$_7$R model in designing active 5-HT$_7$R ligands with purposeful selectivity showing supporting role of docking results during lead identification.

Acknowledgments

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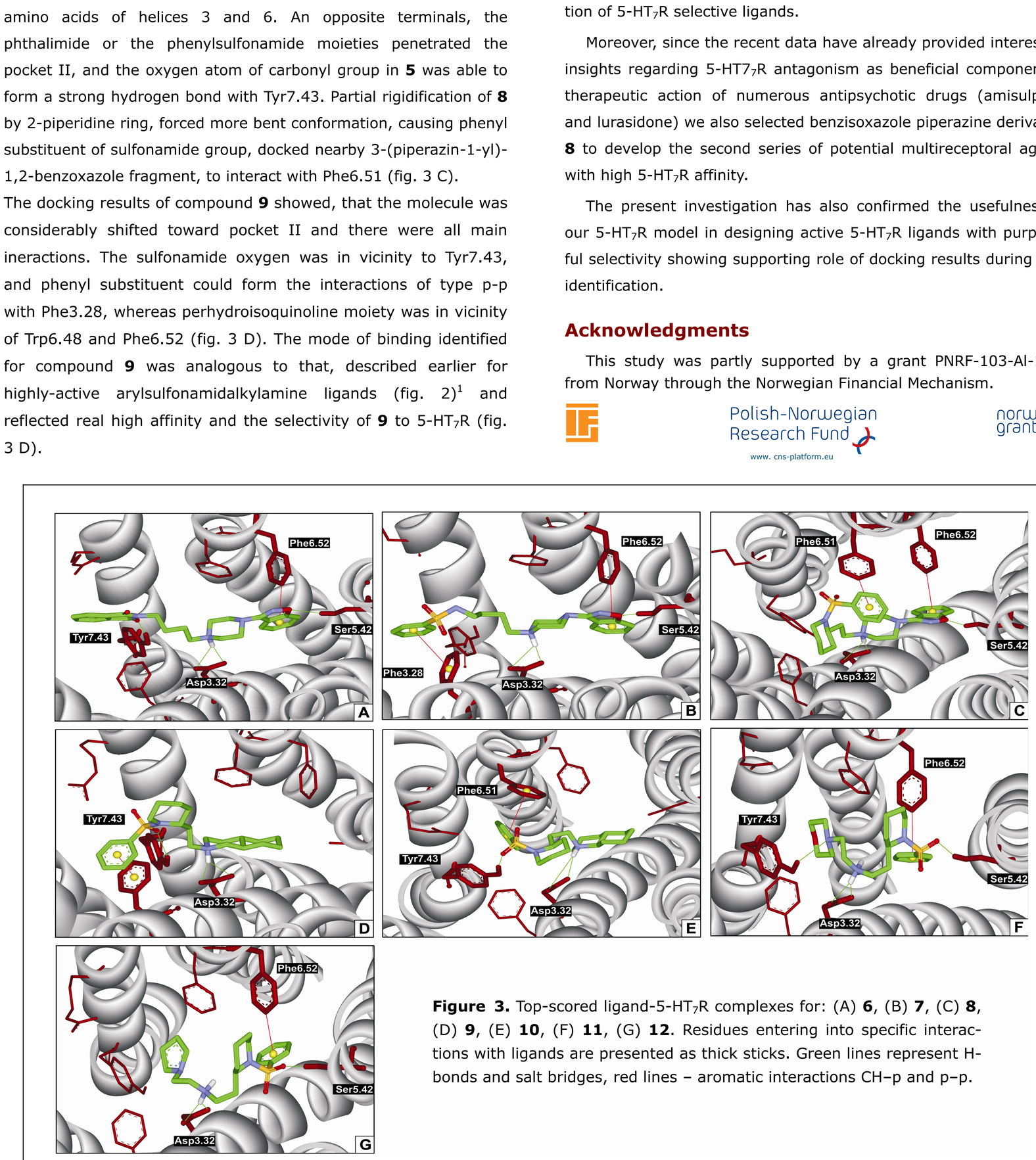

Figure 3. Top-scored ligand-5-HT$_7$R complexes for: (A) **6**, (B) **7**, (C) **8**, (D) **9**, (E) **10**, (F) **11**, (G) **12**. Residues entering into specific interactions with ligands are presented as thick sticks. Green lines represent H-bonds and salt bridges, red lines – aromatic interactions CH–p and p–p.