FLEXIBLE VS PARTLY CONSTRAINED LINKERS IN NAN-190 AND PK-13 ANALOGS INVESTIGATED AS 5-HT$_{1A}$/5-HT$_7$ RECEPTOR LIGANDS

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Introduction

It was proposed that binding modes of arylpiperazine ligands for 5-HT$_{1A}$ and 5-HT$_7$ receptors are similar. Previous investigation with *cis*-, *trans*-2-butene and 1,2-bismethylbenzene analogues of MM77 and NAN-190 showed that spacer structure may influenced the level of 5-HT$_{1A}$ and 5-HT$_7$ affinity and selectivity. The results of conformational analysis revield that the linear geometry of studied compounds was preferred by 5-HT$_{1A}$ receptor while partly bent conformations were more optimal for interactions with 5-HT$_7$ site [1].

Continuing our study on the influence of linker modifications on the affinity at serotonin 5-HT$_{1A}$ and 5-HT$_7$ receptors in the group of long-chain arylpiperazines (LCAPs; [1]), new flexible and partly constrained derivatives were designed. All compounds were synthesized at the Department of Organic Chemistry, Cracow University of Technology, and their affinities for 5-HT$_{1A}$ and 5-HT$_7$ receptors were measured at the Deptartment of Medicinal Chemistry Institute of Pharmacology PAS. Conformations of selected derivatives were additionaly studied in the binding pocket of homology model of 5-HT$_7$ receptor, using docking methodology described previously [2].

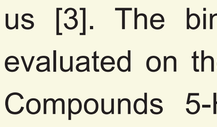
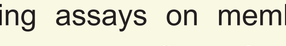
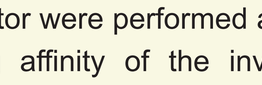
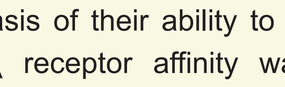
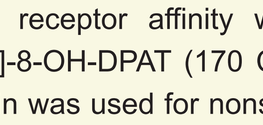
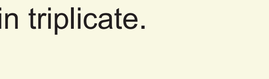
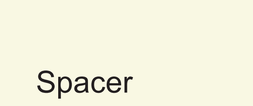
Chemistry

The synthesis of the compounds under study where prepared by a two-step procedure. At first, N-alkylation of both imides with 1,5-dibromopentane, 1,6-dibromobutane, α,α'-dichloro-m-xylene and α,α'-dichloro-p-xylene were performed. The reactions, carried out at ambient temperature in the presence of K_2CO_3 and DMF as a solvent, give the efficient yield of N-(ω-haloalkyl)imides. The second step, i.e. condensation of N-(ω-alkyl)imides with 1-(2-methoxyphenyl)piperazine or 1,2,3,4-tetrahydroisoquinoline afforded final compounds (Table). Condensation was carried out at ambient temperature in the presence K_2CO_3, using DMF as a solvent. For biological experiments, free bases were converted into hydrochloride salts with ethanol saturated with HCl, and their molecular weights were established on the basis of elemental analysis.

Binding experiments

Radioligand binding assays on membranes from HEK293 cells stably expressing human 5-HT$_{7b}$ receptor were performed according to the methods previously described by us [3]. The binding affinity of the investigated compounds for 5-HT$_7$ receptor was evaluated on the basis of their ability to displace [^{3}H]-5-CT (93.0 Ci/mmol. Amersham). Compounds 5-HT$_{1A}$ receptor affinity was determined using native rat hippocampal membranes and [^{3}H]-8-OH-DPAT (170 Ci/mmol, NEN Chemical) as radioligand. In both experiments serotonin was used for nonspecific binding. Ki values were calculated on the basis of at least three independent experiments with the use of 7 - 8 compound concentrations, run in triplicate.

Table. Structure and 5-HT1A and 5-HT7 affinities of new compounds.

Amine A:	Spacer	Imide B:			
				K_i nM	
		5-HT$_{1A}$	5-HT$_7$	5-HT$_{1A}$	5-HT$_7$
		7.2	80	20	106
		22	118	18	112
		68	292	69	159
		16	50	34	83
		324	245	746	342
		108	324	62	290
		278	768	1141	1060
		459	781	572	1097

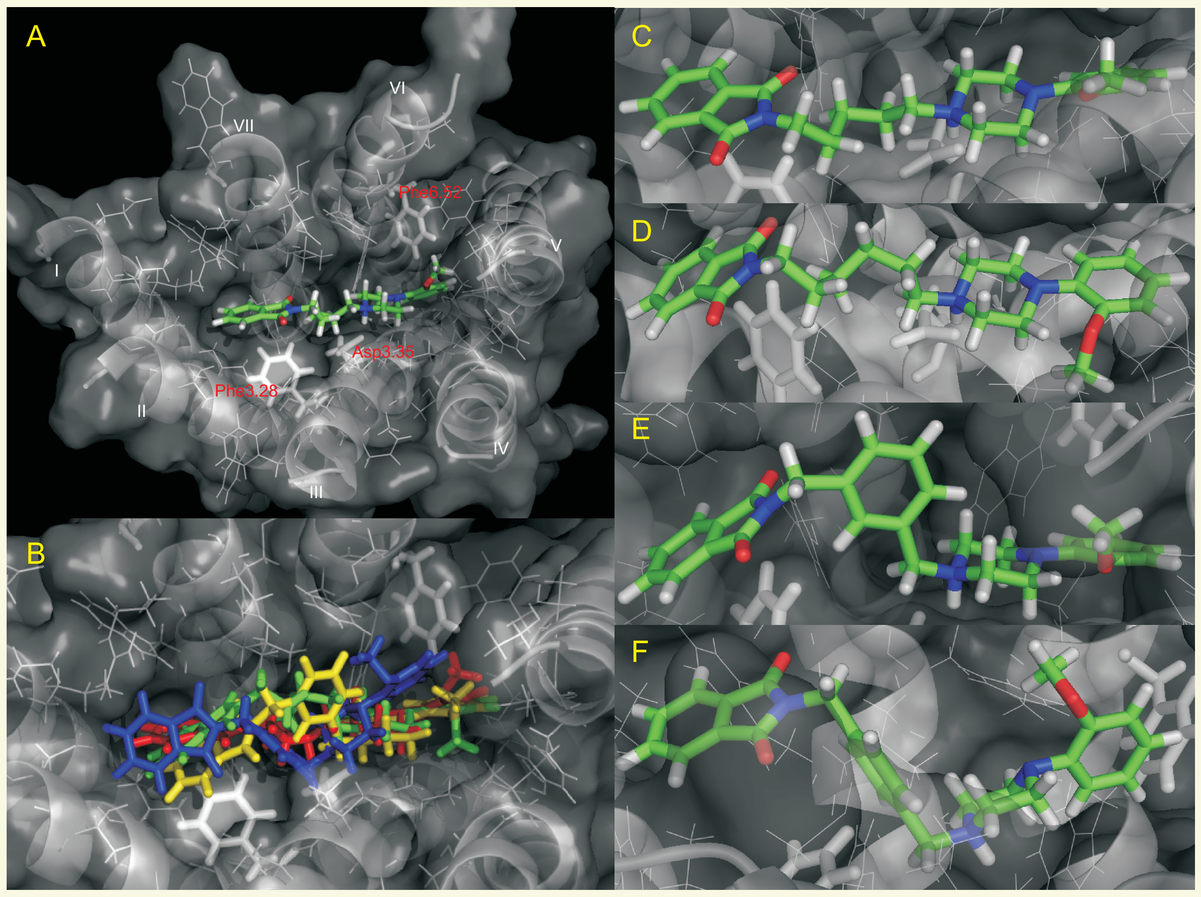


Figure. Docking results of o-methoxyphenylpiperazine derivaives with phthalimide terminal to 5-HT$_7$R model. A) Binding mode for compound with pentamethylene spacer, B) Top-scored solutions for all four docked compounds, C-F) Conformation of individual compounds within 5-HT$_7$R binding site.

Results

Two series of derivatives were evaluated: one containing o-methoxyphenylpiperazine (*o*-OMePhP) fragment, and the second with isosteric tetrahydroisoquinoline (THIQ) moiety. In relation to previous study, four new spacers were introduced into the ligand structure: *n*-pentyl, *n*-hexyl, *m*-xylyl and *p*-xylyl. Generally *o*-OMePhP derivatives displayed high 5-HT$_{1A}$, and lower but still significant 5-HT$_7$ affinities. Compounds within the THIQ series were less active at both receptors.

Dockings to in silico model of 5-HT$_7$R showed that all selected *o*-OMePhP derivatives occupied similar position in the the binding pocket. For both compounds with aliphatic spacers, a linear molecule geometry was predicted. Xylene linkers, keeping ligands in a partly bent conformations, caused only slight diferences in binding interactions. The most significant change concerned *p*-xylene derivative, which lost common aromatic constact with Phe6.52 and instead an interaction between Phe3.28 with and aromatic part of a spacer was formed.

Conclusions

1. *o*-methoxyphenylpiperazine series showed better affinity especially at 5-HT$_{1A}$ binding site; their activity at 5-HT$_7$ receptors were within the range of K_i = 50 - 292 nM, i.e. 2-10 times lower than that found at 5-HT$_{1A}$ receptor;

2. the influence of spacer modification was only visible, when *m*-xylene was introduced as a spacer, each time decreasing the affinity;

3. in the THIQ series, the best 5-HT$_{1A}$ results were obtained for derivatives with *n*-hexyl linker; while in the case of 5-HT$_7$ affinity, ligands with both polimethylene spacers had comparable affinity, which was app. 2-3 times higher than that obtained for their xylene analogues;

4. the applied spacer modifications did not significantly influenced the preference of 5-HT$_{1A}$ binding over 5-HT$_7$ one, as it was observed in the previous investigation;

5. the interactions of *o*-OMePhP derivatives found in the 5-HT$_7$R binding site generally reflected the obtained affinity results.

Acknowledgements

This study was partly supported by the Ministry of Science and Higher Education (MNiSW), Grant No. 2 P05F 019 30

References

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