4,6-DISUBSTITUTED 2-(4-METHYLPIPERAZIN -1- YL)PYRIDINES: SYNTHESIS AND THEIR BINDING TO SEROTONIN 5-HT$_{1A}$, 5-HT$_{2A}$, AND 5-HT$_7$ RECEPTORS

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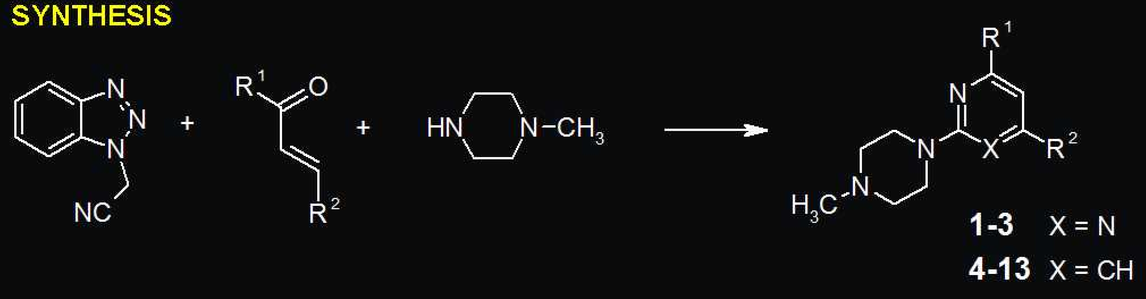
INTRODUCTION

The role of the 5-HT$_7$ receptors in the CNS and the periphery has not been fully clarified since to date there are only limited number of the selective-active ligands [1]. During screening of our compounds library against the 5-HT$_7$ receptor from rat hypothalamic membranes, it was found that a lot of agents, besides 5-HT$_{1A}$ and/or 5-HT$_{2A}$ receptors activity, displayed also a significant affinity towards 5-HT$_7$ sites. The same was also stated for 2-(4-methylpiperazin-1-yl)pyrimidines **1–3** which showed moderate affinity for the 5-HT$_{1A}$ binding site and have marked affinity for the 5-HT$_{2A}$ receptor subtype [2–5]. Since compounds of such structure have not yet been reported as 5-HT$_7$ receptor ligands we extended our studies on a group of the 4,6-diaryl-, and 6-alkyl-4-arylsubstituted 2-(4-methypiperazin-1-yl)pyridines **4–13**.

SYNTHESIS



The described previously [6] pyridines **4–8** as well as the newly designed derivatives **9–13** were synthesized by using the benzotriazole-assisted Katritzky method [7]. The reaction of α,β-unsaturated ketones with 2-(1-benzotriazolyl)acetonitrile and N-methylpiperazine afforded the corresponding 4,6-disubstituted 2-(4-methylpiperazin-1-yl)-pyridines. The starting α,β-unsaturated ketones were synthesized by the reaction of the appropriate aldehyde and acetophenone in ethanol-aqueous NaOH medium at room temperature.

RESULTS

The SAR analysis of described earlier [2–5] pyrimidines and additional derivatives (they are not shown) suggests that

- the piperazinopyrimidines demonstrated a high 5-HT$_7$ receptor affinity,
- the presence of a 3-furyl substituent strongly increases the activity,
- the presence of a 3-thienyl group is also beneficial for that activity,
- piperazinopyrimidines substituted with a 2-furyl or 2-thienyl at the 4 position show little affinity to the 5-HT$_7$ receptor,
- all active agents have relatively small second substituent at the 6 position.

An important conclusion is that piperazinopyrimidines substituted with a 3-furyl group show the highest activity.

Table. Structure and binding data of compounds **1–13**

Comp.	R^1	R^2	K_i [nM]		
			5-HT$_{1A}$	5-HT$_{2A}$	5-HT$_7$
1	Cl	3-furyl	113	41[5]	38
2	Me	3-furyl	265[4]	50[4]	50
3	H	3-thienyl	486[3]	192[3]	209
4	Ph	Ph	2010[6]	144[6]	15800
5	Ph	2-thienyl	3150[6]	34[6]	5980
6	2-thienyl	2-thienyl	3670[6]	64[6]	1830
7	Me	Ph	2130[6]	139[6]	1300
8	Me	2-thienyl	2740[6]	189[6]	480
9	Me	3-thienyl	637	33	97
10	Me	*o*-MeO-Ph	1840	636	4800
11	Me	*m*-MeO-Ph	349	427	1560
12	Me	*p*-MeO-Ph	1889	116	6700
13	Ph	3-furyl	–	–	700

Here we report the 5-HT$_7$ receptor affinity for some previously described 4,6-disubstituted 2-(4-methylpiperazin-1-yl)pyridines as well as a series of newly designed and synthesized analogues structures.
The results of binding experiments (Table) clearly show that

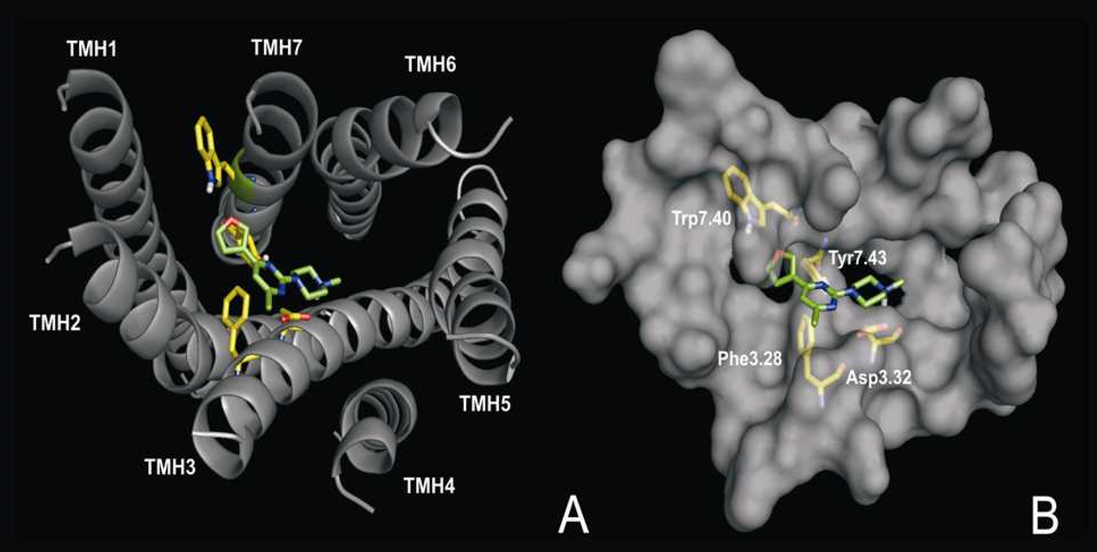
- two aryl substitutents at pyridine ring are not well accommodated by the 5-HT$_7$ receptor binding pocket, and compounds **4–6** remain selective 5-HT$_{2A}$ receptor ligands,
- 4-aryl-6-alkylsubstituted derivatives show higher 5-HT$_7$ receptor affinity, especially when substituted with a heteroaromatic group (c.f. **8** and **9** vs **7**).

Our docking experiments indicate that an H-bond between 3-furyl or 3-thienyl substituent and NH group of Trp7.40 may be formed, which increases the observed affinity. The comparison of K_i values obtained for compounds **4** and **13** (15800 and 700 nM, respectively) additionally confirm the importance of this interaction. Thus as a next step, the methoxy group was introduced in different positions of phenyl moiety of **7** (compounds **10–12**). Since the 5-HT$_7$ receptor affinity of methoxyphenyl derivatives is lower than that of the parent compound **7**, it seems that steric factors and/or size of substituents are also important features limiting ligand-receptor interactions.

DOCKING

The methodology used for studying potential binding mode of the investigated compounds in the 5-HT$_7$ receptor was based on the automated docking (FlexX implemented in SYBYL 7.1) of a ligand to a population of rhodopsin based receptor models (MODELLER 7v7), which explores the conformational space of amino acid side-chains of the binding site. Hence, the information about the flexibility of the receptor and its potential induced fit to the bound ligand was included in the resulting model complexes (flexible docking). Each obtained ligand-receptor complex was subjected to a consensus scoring procedure (CScore module of SYBYL) to select the results that were well scored by five scoring functions simultaneously. Ligand poses having the highest PMF scores of those scored "5" by the consensus scoring procedure (top scored) were used to predict potential interactions with the receptor.
The investigated compounds were docked to the receptor model in a way enabling formation of the salt bridge between the protonated nitrogen of ligand piperazine moiety and carboxylic group of the Asp3.32 side-chain. In such a ligand position, π-electrons of the pyrimidine ring were prone to create an edge-to-face CH-π interaction with Phe3.28 and the pyrimidine nitrogen atom accepted an H-bond from Tyr7.43. Derivatives with the 3-furanyl or 3-thienyl moiety were able to develop another H-bond with the NH group of Trp7.40, which may be an explanation of their significantly higher 5-HT$_7$R affinity, comparing to their analogs.

Figure. A binding site of 5-HT$_7$ receptor with docked compound **1** (a view from the extracellular side); Amino acids entering into specific interactions with ligands are presented as "sticks". (A) "Cartoon" helices; (B) surface of the "activesite" subset.



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