The impact of spacer modifications on 5-HT$_{1A}$/5-HT$_7$ receptor selectivity in the group of arylpiperazine ligands

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INTRODUCTION

Cloned in 1993, the 5-HT$_7$ subtype is the last member of the serotonin receptor family. Its discovery stimulated studies on the role of that new biological target within the central nervous system (CNS). For this reason, 5-HT$_7$ receptor-selective ligands have been urgently sought. More than ten years of investigations resulted in finding a few selective antagonists like SB-269970-A and SB-656104-A, however, substances possessing clear agonistic activity towards the 5-HT$_7$ subtype only are still to be discovered. Studies with those compounds indicated that the above-mentioned receptor played a role in several CNS disorders including sleep disturbances, anxiety and depression and suggested that agents acting selectively or ligands displaying a multireceptoral profile (with a marked interaction with the 5-HT$_7$ receptor) may have promising therapeutic potential.

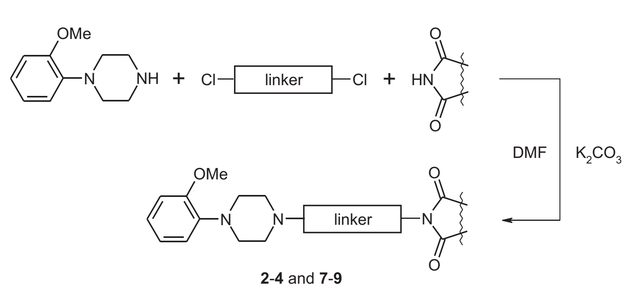
Additionally, it was found that many of the previously described serotonin ligands showed a high level of 5-HT$_7$ receptor activity. The latter was observed for 8-OH-DPAT and some other 5-HT$_{1A}$ agents, in particular in the group of long-chain arylpiperazine derivatives (LCAPs) [1,2]. The same was also found during the screening of our compounds library for 5-HT$_7$ receptor affinity, and among 1-(2-methoxyphenyl)piperazine derivatives (oMPP), compounds displaying distinct 5-HT$_7$ potency were identified.

Examination of 5-HT$_7$ receptor binding site, performed by Wilcox et al., demonstrated close similarities of this region shared with the 5-HT$_{1A}$ receptor, what can explain such dual activity and difficulties in developing selective ligands [3].

The results obtained by screening our compounds library revealed that only flexible derivatives showed significant 5-HT$_7$ affinity, whereas conformationally constrained ligands - still potent at 5-HT$_{1A}$ binding site - were practically inactive towards the 5-HT$_7$ receptor. This phenomenon was exemplified by two pairs of compounds: MM-77 (**1**; a potent 5-HT$_{1A}$ receptor antagonist [4]) and NAN-190 [5] (**6**), and their 1e,4e-disubstituted cyclohexane derivatives **5** [6] and **10** [7] (Table 1). The replacement of a tetramethylene chain with a cyclohexane ring caused following consequences: imposition of significant conformational constraints, freezing of the extended T3-gauche conformation (Figs. 1, 2B), and enlargement of the volume occupied by the spacer. In order to further investigate the influence of the spacer structure on 5-HT$_7$ and 5-HT$_{1A}$ receptor affinities, new MM-77 and NAN-190 analogues were designed. Current modifications of the spacer involved partial limitation of its conformational freedom by introducing a double bond in *trans* (like fully extended; **2** and **7**) and *cis* (similar to T3-gauche; **3** and **8**) configuration, and an increase of its volume by incorporating a benzene ring (**4** and **9**). To better characterize the geometry of the obtained ligands, a conformational analysis was also carried out.

CHEMISTRY

The synthesis of the target compounds (**2-4** and **7-9**) is shown below. To a mixture of *cis* or *trans*-1,4-dichloro-2-butene or 1,2-bis(chloromethyl)benzene (0.01 mole), powdered K_2CO_3 (0.01 mole) and a catalytic amount of KI in DMF (30 mL), an appropriate imide was slowly added at a room temperature. Next, a new portion of anhydrous K_2CO_3 (0.02 mole), and a 1-(2-methoxyphenyl)piperazine hydrochloride were added and the reaction mixture was stirred for 10 h and then poured into water (100 ml). A crude product was filtered off and separated from the symmetrically substituted by-products by extraction with acetone and subsequent crystallization, which was repeated if necessary. The yielded compounds were characterized by ^{1}H NMR spectra. For biochemical studies free bases were converted into hydrochloride salts and their molecular formulae were established on the basis of an elemental analysis.



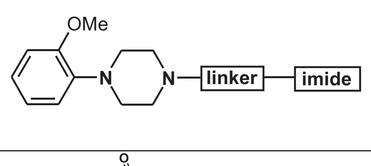
2-4 and **7-9**

RESULTS AND DISCUSSION

All the new compounds were examined in vitro for their ability to displace [^{3}H]-5-CT and [^{3}H]-OH-DPAT binding to rat 5-HT$_7$ and 5-HT$_{1A}$ receptors, respectively. The results are presented in Table 1, in which the 5-HT$_{1A}$ receptor affinities of the parent compounds **1** [4] and **6** [7] and the previously reported rigid ligands **5** [6] and **10** [7] were also included.

An increase in 5-HT$_7$ affinity, in relation to **1** and **6**, was observed for both *trans* derivatives **2** and **7**. The *cis* isomers and both bismethylbenzene derivatives were about 10-fold less active than the respective *trans* analogues. The rigid compounds containing cyclohexane moiety were devoid of 5-HT$_7$ activity but, as had been previously reported, they were potent 5-HT$_{1A}$ ligands. Both *trans* derivatives also bound to 5-HT$_{1A}$ sites with high affinity, but they were several times less active than the parent compounds. The *cis* configuration of a 2-butene spacer and the presence of a benzene ring were unfavorable for the 5-HT$_{1A}$ binding site. The same binding preferences of *trans* vs *cis*-2-butene derivatives for 5-HT$_{1A}$ receptor had already been reported for arylpiperazines with 1-isoindolinone [8] and 3-(diphenylmethylene)-2,5-pyrrolidinedione [9] terminals.

Table 1. The structure and binding affinity data on serotonin 5-HT$_{1A}$, and 5-HT$_7$ receptors of the investigated compounds.



Linker	Cmpd	K_i SEM [nM] 5-HT$_7$		5-HT$_{1A}$		Cmpd	K_i SEM [nM] 5-HT$_7$		5-HT$_{1A}$	
	1	**90**	**5**	6.4	0.3[a]	**6**	**87**	**2**[b]	0.6	0.1[c]
	2	**63**	**4**	29	2	**7**	**36**	**1**	5	0.7
	3	**790**	**58**	157	6	**8**	**353**	**20**	77	2
	4	**910**	**28**	415	50	**9**	**367**	**28**	405	3
	5	**11500**	**2550**	15.2	3.2[d]	**10**	**2045**	**120**	8	2[e]

[a] data from ref [4]; [b] IC50 = 145 nM according to Lovenberg T. et al. [10]; [c] Ki = 0.55 nM according to Glennon R.A. et al. [5]; [d] data from ref [6]; [e] data from ref [7].

The extended conformation of long-chain arylpiperazines is generally accepted as bioactive at the 5-HT$_{1A}$ receptor. Recent investigations showing the high affinity and selectivity of rigid, 1,4-disubstituted cyclohexane derivatives conclusively proved that statement [6,7,11]. Since such linearly constrained compounds do not bind to the 5-HT$_7$ receptor, in this case the bent conformation of flexible LCAPs should be regarded as bioactive. Following this reasoning, the partly constrained *trans* derivatives (most active at 5-HT$_7$ sites) should be able to adopt a bent conformation during an interaction with this receptor. On the other hand, *trans* derivatives display the same high level of affinity for 5-HT$_{1A}$ receptors as do cyclohexane analogues, and should bind to this receptor in a linear, extended conformation.

To examine the conformational behavior of the investigated ligands, a conformational analysis was performed by systematic rotation of torsions T1, T5 and T2, T4 (Fig. 1) every 30 or 10 deg, respectively. All the generated conformations were optimized using a PM5 quantum semi-empirical method with the COnductorlike Screening MOdel (COSMO) approach to simulate water environment (MOPAC 2002, implemented in the CAChe Worksystem Pro 6.1.). That method, previously applied for structurally related LCAPs, was found to produce results which corresponded to the geometries determined by 2D NOESY ^{1}H NMR as well as crystallographic experiments (unpublished data).

In general, the conformation of long-chain arylpiperazines depends on the mutual arrangement of an arylpiperazine and the second terminal fragment (here: an imide moiety). The family of conformations where the terminal imide is located near the axis determined by the phenylpiperazine fragment are classified as extended. Due to differences in torsion T3, they can be further subdivided into fully extended (T3-anti or *trans,* Fig. 2A) and extended T3-gauche or *cis* (Fig. 2B). If an imide moiety is deviated from the axis of a phenylpiperazine, such conformations are bent or folded (Fig. 2C). The differences in the spatial arrangement of both these terminal fragments are determined mainly by torsions T2 and T4, though, the former seems to play a more pivotal role.

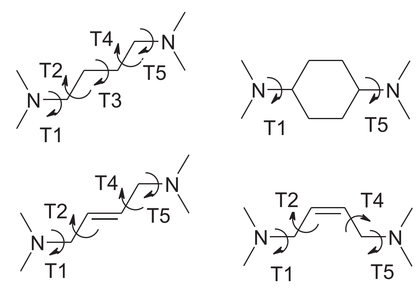


Figure 1. Rotatable bonds in the spacer of the investigated compounds.

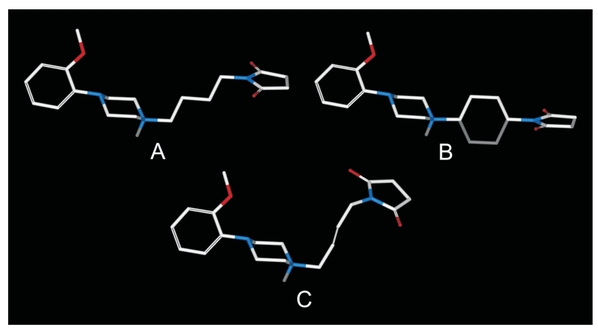


Figure 2. The main types of LCAPs conformers: fully extended (A), extended T3-gauche, frozen in cyclohexane derivatives **5** and **10** (B), bent conformation - here a global energy minimum of compound **2** (C). For simplification, hydrogen atoms have been omitted.

Cyclohexane derivatives are obviously the most rigid structures among these discussed in the paper, since three (T2, T3, T4) out of the five torsion angles present in the flexible butyl spacer are frozen, which reduces conformational freedom to an extended T3-gauche conformation only.

In the case of the 2-butenyl spacer, only torsion T3 is frozen, whereas torsions T2 and T4 remain rotatable. An analysis of T2 and T4 showed high rotational freedom at a range of 90 to 270 deg, hence either extended (T2~180) or bent (T2~90) conformations are allowed for *trans* and *cis*-2-butenyl analogues at a low-energy range (Fig. 3). However, *trans* analogues **2** and **7** show a preference for bent conformations (the global energy minimum at T2-100, Fig. 3A), which - along with their highest 5-HT$_7$ affinity - is in line with our assumption about the conformational requirements of the 5-HT$_7$ binding site. On the other hand, these compounds can also adopt an extended conformation (1.5 kcal/mol over the global energy minimum), thus at the same time are potent 5-HT$_{1A}$ receptor ligands. Since a significant decrease in the affinity for *cis* derivatives for both the receptor subtypes was observed, it seems that the configuration of T3 is important for a proper ligand-5-HT$_7$/5-HT$_{1A}$ receptor interaction.

A global energy minimum for *cis* isomers was found at T2-160 (Fig. 3B) and the energy paid (ca. 3 kcal/mol) when fit to bent conformation of **2**, presumed as bioactive, may explain their lower 5-HT$_7$ receptor activity.

Since the *cis* isomers are geometrically similar to cyclohexane derivatives, their lower affinity for the 5-HT$_{1A}$ receptor may rather be due to the negative influence of the exposed -electrons in the spacer region (defined as hydrophobic for 5-HT$_{1A}$ receptor), than to conformational requirements. The enlargement of the -electron system to benzene ring caused a further decrease in 5-HT$_{1A}$ affinity (**3** vs **4**, and **8** vs **9**), but had no effect in the case of the 5-HT$_7$ receptor.

In conclusion, based on the results discussed above, bioactive conformations of LCAPs for the 5-HT$_7$ receptor, different from those established for 5-HT$_{1A}$, were proposed. The low-energy conformers of the investigated ligands, superimposed on the rigid template of **5** illustrate their geometry at the 5-HT$_{1A}$ receptor (Fig. 4A).

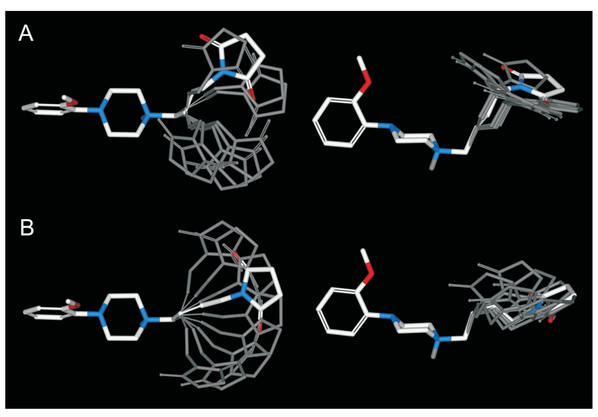


Figure 3. The rotational freedom of torsion T2, shown for *trans*- (A) and *cis*-2-butenyl (B) analogues in an orthogonal view. The conformations at a range of 1 kcal/mol (gray) over the global minimum (colored by atom type) are shown.

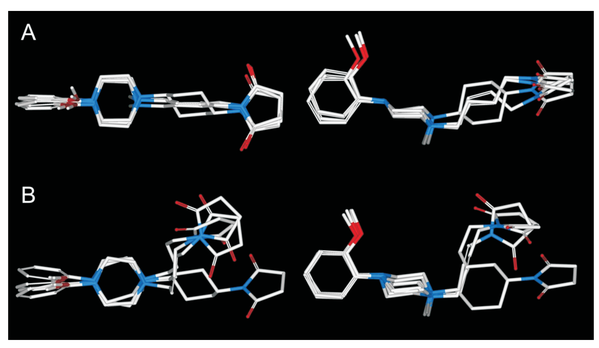


Figure 4. Superimposition of **1**-**3** in the extended conformation on rigid cyclohexane derivative **5** (orthogonal view) - a bioactive LCAP conformation for the 5-HT1A receptor (A). Superposition of **1** and **3** on the global energy minimum conformation of **2** - a proposed geometry of the investigated ligands during interaction with the 5-HT7 receptor; the rigid compound **5** added for comparison (B).

In the case of the 5-HT$_7$ receptor, a global energy minimum of **2** was used as a template (Fig. 4B). Flexible butyl derivatives can easily adopt a similarly bent geometry (E < 1 kcal/mol), whereas the superposition of the partially constrained *cis* isomers is connected with a higher increase in internal energy, thus results in their lower 5-HT$_7$ affinity. It is worth to note that the proposed bioactive conformation of LCAPs is close to the pharmacophore model for 5-HT$_7$ receptor antagonism, developed recently by Lopez-Rodriguez et al. using a CATALYST approach [1].

ACKNOWLEDGMENTS

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