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The impact of spacer structure on 5-HT₇ and 5-HT_{1A} receptor affinity in the group of long-chain arylpiperazine ligands

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Abstract—New *cis-*, *trans-*2-butene and 1,2-bismethylbenzene analogues of MM77 and NAN-190 (1-{4-[4-(2-methoxyphenyl)-piperazin-1-yl]-butyl}-pyrrolidine-2,5-dione and isoindole-1,3-dione, respectively) were synthesized. The differences in their in vitro affinity for serotonin 5-HT₇ and 5-HT_{1A} receptors were explained using a conformational analysis. A bioactive conformation of those compounds for the 5-HT₇ receptor, different from that established for 5-HT_{1A}, was proposed. © 2004 Elsevier Ltd. All rights reserved.

Cloned in 1993, the 5-HT₇ subtype is the last member of the serotonin receptor family.¹⁻³ 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 10 years of investigations resulted in finding a few selective antagonists like SB-269970-A⁴ and SB-656104-A,⁵ however, substances possessing clear agonistic activity towards the 5-HT₇ subtype only are still to be discovered.⁶ 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-HT₇ receptor) may have promising therapeutic potential.7

Additionally, it was found that many of the previously described serotonin ligands showed a high level of 5-HT₇ receptor activity.² The latter was observed for

8OH-DPAT and some other 5-HT_{1A} agents, in particular in the group of long-chain arylpiperazine derivatives (LCAPs).^{8,9} The same was also found during the screening of our compounds library for 5-HT₇ receptor affinity, and among 1-(2-methoxyphenyl)piperazine derivatives (*o*MPP), compounds displaying distinct 5-HT₇ potency were identified.

Examination of 5-HT₇ 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.¹⁰

The results obtained by screening our compounds library revealed that only flexible derivatives showed significant 5-HT₇ affinity, whereas conformationally constrained ligands—still potent at 5-HT_{1A} binding site—were practically inactive towards the 5-HT₇ receptor. This phenomenon was exemplified by two pairs of compounds: MM-77 (1; a potent 5-HT_{1A} receptor antagonist¹¹) and NAN-190¹² (6), and their le,4e-disubstituted cyclohexane derivatives 5¹³ and 10¹⁴ (Table 1). The replacement of a tetramethylene chain with a cyclohexane ring caused following consequences: imposition of significant conformational constraints, freezing of the extended T_3 -gauche conformation (Figs. 1 and 2B),

Keywords: Long-chain arylpiperazines; 5-HT₇ receptor ligands; Conformational constraints.

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| Table 1. | The structure and | binding affinity | y data on serotonin | 5-HT _{1A} , and 5-HT ₇ | receptors of the | investigated compounds |
|----------|-------------------|------------------|---------------------|--|------------------|------------------------|
| | | | | 1111 | | |

014-

| N N linker imide | | | | | | | | | | | |
|------------------|---------|--|-----------------------|-----------|--------------------|-----------------------|--|--|--|--|--|
| Imide | | -N 0 | \geq | | | | | | | | |
| Linker | No. | $K_{\rm i} \pm {\rm SEM} \ ({\rm nM})$ | | No. | $K_i \pm SEM (nM)$ | | | | | | |
| | | 5-HT ₇ | 5-HT _{1A} | | 5-HT ₇ | 5-HT _{1A} | | | | | |
| \sim | 1 MM-77 | 90 ± 5 | $6.4 \pm 0.3^{\rm a}$ | 6 NAN-190 | 87 ± 2^{b} | $0.6 \pm 0.1^{\circ}$ | | | | | |
| \sim | 2 | 63 ± 4 | 29 ± 2 | 7 | 36 ± 1 | 5 ± 0.7 | | | | | |
| <u> </u> | 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. 11.

 b IC₅₀ = 145 nM according to Lovenberg et al.²

^c $K_i = 0.55 \,\text{nM}$ according to Glennon et al.¹²

^d Data from Ref. 13.

^e Data from Ref. 14.



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



Figure 2. The main types of LCAPs conformers: fully extended (A), extended T_3 -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.

and enlargement of the volume occupied by the spacer. In order to further investigate the influence of the spacer structure on 5-HT₇ 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 T_3 -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.

The synthesis of the target compounds (2-4 and 7-9) is shown in Scheme 1. To a mixture of cis- or trans-1,4-dichloro-2-butene or 1,2-bis(chloromethyl)benzene (0.01 mol), powdered K₂CO₃ (0.01 mol) and a catalytic amount of KI in DMF (30mL), an appropriate imide was slowly added at a room temperature. Next, a new portion of anhydrous K₂CO₃ (0.02mol), and a 1-(2methoxyphenyl)piperazine hydrochloride were added and the reaction mixture was stirred for 10h 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 ¹H NMR spectra. For biochemical studies free bases were converted into hydrochloride salts and their molecular



Scheme 1.

formulae were established on the basis of an elemental analysis.

All the new compounds were examined in vitro for their ability to displace [³H]-5-CT and [³H]-OH-DPAT binding to rat 5-HT₇¹⁵ and 5-HT_{1A}¹⁶ receptors, respectively. The results are presented in Table 1, in which the 5-HT_{1A} receptor affinities of the parent compounds MM-77¹¹ and NAN-190¹⁴ and the previously reported rigid ligands **5**¹³ and **10**¹⁴ were also included.

An increase in 5-HT₇ affinity, in relation to MM-77 and NAN-190, was observed for both trans derivatives 2 and 7. The cis isomers 3 and 8 and both bismethylbenzene derivatives 4 and 9 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 unfavourable for the 5-HT_{1A} binding site. The same binding preferences of trans versus cis-2-butene derivatives for 5-HT1A receptor had already been reported for arylpiperazines with 1isoindolinone¹⁷ and 3-(diphenylmethylene)-2,5-pyrrolidinedione¹⁸ terminals.

The extended conformation of long-chain arylpiperazines is generally accepted as bioactive at the $5-HT_{1A}$ receptor.^{18,19} Recent investigations showing the high affinity and selectivity of rigid, 1,4-disubstituted cyclohexane derivatives conclusively proved that statement.13,14,19 Since such linearly constrained compounds do not bind to the 5-HT₇ 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 behaviour of the investigated ligands, a conformational analysis was performed by systematic rotation of torsions T_1 , T_5 and T_2 , T_4 (Fig. 1) every 30° or 10°, respectively. All the generated conformations were optimized using a PM5 quantum semi-empirical method with the COnductorlike Screening MOdel (COSMO) approach to simulate water environment²⁰ (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 ¹H 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 T_3 , they can be further subdivided into fully extended (T_3 anti or trans, Fig. 2A) and extended T_3 -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 T_2 and T_4 , though, the former seems to play a more pivotal role.

Cyclohexane derivatives are obviously the most rigid structures among these discussed in the paper, since three (T_2, T_3, T_4) out of the five torsion angles present in the flexible butyl spacer are frozen, which reduces conformational freedom to an extended T_3 -gauche conformation only.

In the case of the 2-butenyl spacer, only torsion T_3 is frozen, whereas torsions T_2 and T_4 remain rotatable. An analysis of T_2 and T_4 showed high rotational freedom at a range of 90°-270°, hence either extended $(T_2 \sim 180)$ or bent $(T_2 \sim 90)$ 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 T_2 -100, Fig. 3A), which—along with their highest 5-HT₇ 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 (1.5kcal/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 T_3 is important for a proper ligand—5-HT₇/ 5-HT_{1A} receptor interaction.

A global energy minimum for *cis* isomers was found at T_2 -160 (Fig. 3B) and the energy paid (ca. 3kcal/mol)







Figure 4. Superimposition of MM-77, 2 and 3 in the extended conformation on rigid cyclohexane derivative 5 (orthogonal view)—a bioactive LCAP conformation for the 5-HT_{1A} receptor (A). Superposition of MM-77 and 3 on the global energy minimum conformation of 2—a proposed geometry of the investigated ligands during interactions with the 5-HT₇ receptor; the rigid compound 5 added for comparison (B).

when fit to bent conformation of 2, presumed as bioactive, may explain their lower 5-HT₇ 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 π -electrons in the spacer region (defined as hydrophobic for 5-HT_{1A} receptor), than to conformational requirements. The enlargement of the π -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-HT₇ receptor.

In conclusion, based on the results discussed above, bioactive conformations of LCAPs for the 5-HT₇ 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).

In the case of the 5-HT₇ receptor, a global energy minimum of **2** was used as a template (Fig. 4B). Flexible butyl derivatives can easily adopt a similarly bent geometry ($\Delta E < 1$ 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-HT₇ affinity. It is worth to note that the proposed bioactive conformation of LCAPs is close to the pharmacophore model for 5-HT₇ receptor antagonism, developed recently by Lopez-Rodriguez et al. using a CATALYST approach.⁸

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl. 2004.09.029.

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