PRELIMINARY SAR STUDIES ON THE SEROTONIN 5-HT$_7$ RECEPTOR ACTIVITY IN THE GROUP OF MM-77 DERIVED COMPOUNDS

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INTRODUCTION

Cloned in 1993, 5-HT$_7$ subtype remains the last member in the serotonin receptor family. Its discovery stimulated studies on the role of this new biological target within the Central Nervous System (CNS). For this reason 5-HT$_7$ receptor-selective ligands have been urgently seeking. More than ten years of investigations resulted in finding a few selective antagonists like SB-269970-A and SB-656104-A, and recently, a first, potent agonist AS19 (as reported on XIV Camerino-Noordwijkerhout Symposium, September 2003). Studies utilizing these compounds supported evidences that this receptor plays a role in the several CNS disorders including sleep disturbances, anxiety and depression. A growing number of results suggest that agents acting either selectively at the 5-HT$_7$ receptor or multireceptoral ligands may have promising therapeutic potential.

Additionally, many of the previously described serotonin ligands showed high level of 5-HT$_7$ receptor activity. That was the case of 8-OH-DPAT and some other 5-HT$_{1A}$ agents, especially in the group of 1-arylpiperazine derivatives (e.g. NAN-190). The same was also observed after the screening 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. Based on these results MM-77 (**1**) and NAN-190 (**5**) were chosen as lead structures for SAR analysis. The preliminary studies concerned the modification of the spacer (Table 1), an amide terminus, and the change of oMPP fragment into isosteric: 1,2,3,4-tetrahydroisoquinoline (THIQ), 1,2,3,4-tetrahydro- -carboline (THBC) or 1,2,3,4-tetrahydropyrazino[1,2-a]indole (Table 2).

The synthesis of compounds **2-4** and **6-8** is presented by Kowalski et al. on the poster entitled "Synthesis and biological evaluation of *cis*- and *trans*-2-butene arylpiperazine derivatives investigated as 5-HT$_{1A}$ receptor ligands". The rest of investigated compounds were either previously reported as 5-HT$_{1A}$ receptor ligands (**1**, **5**, **9-17**) or newly synthesized in our laboratory according to synthesis routes outlined in Scheme 1 (**18-23**).

SCHEME 1

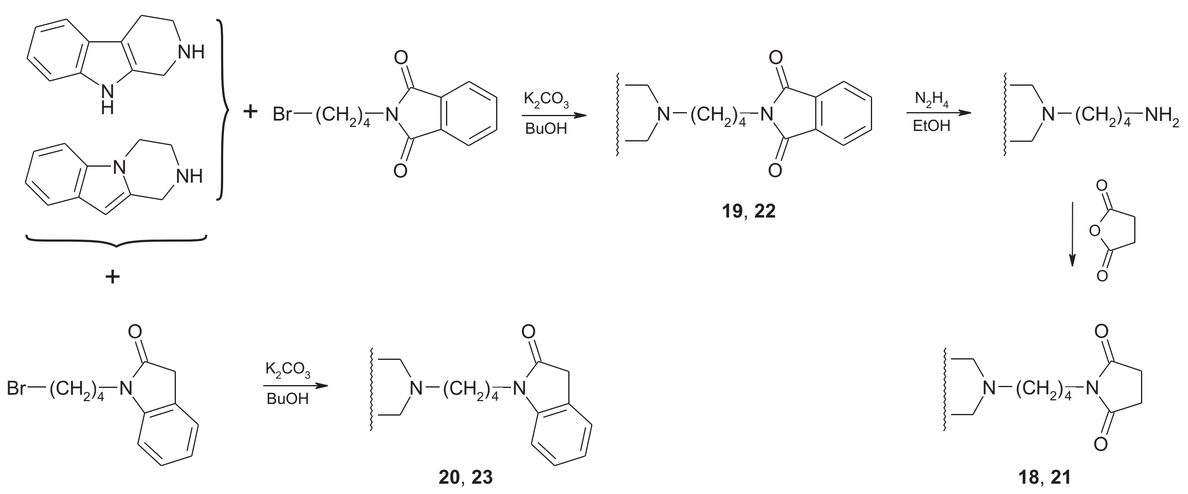
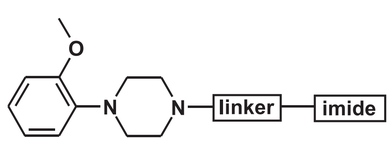
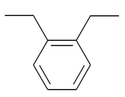


Table 1. Structure and 5-HT$_7$ and 5-HT$_{1A}$ binding affinities of compounds **1-8**.



Linker	Cmpd	K_i SEM [nM] 5-HT$_7$		K_i SEM [nM] 5-HT$_{1A}$		Cmpd	K_i SEM [nM] 5-HT$_7$		K_i SEM [nM] 5-HT$_{1A}$	
Imide:										
	1	**90**	**5**	6.4	0.3[1]	**5**	**87**	**2**	0.6	0.1[2]a
	2	**63**	**4**	29	2	**6**	**36**	**1**	5	0.7
	3	**790**	**58**	157	6	**7**	**353**	**20**	77	2
	**4**	**910**	**28**	415	50	**8**	**367**	**28**	405	3

[a] Ki = 0.55 nM according to Glennon et al., J. Med. Chem. 2003, 46, 2795-812.

RESULTS

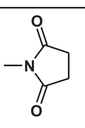
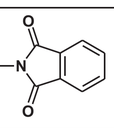
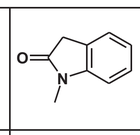
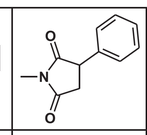
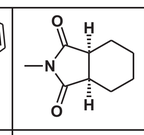
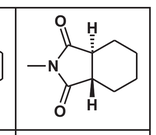
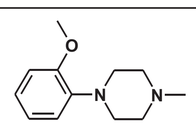
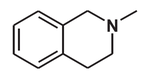
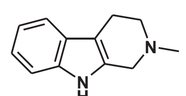
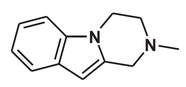
In order to trace the influence of spacer modification on 5-HT$_7$ receptor affinity, the respective *cis*/*trans*-2-butene and 1,2-bismethylbenzene analogues of **1** and **5** are shown in Table 1. The increase of 5-HT$_7$ affinity is observed for both *trans* derivatives **2** and **6**. The *cis* isomers and both bismethylbenzene derivatives were about 10-folds less active than the respective *trans* analogues. These results indicate that a double bond configuration (which determines compound geometry) is important for the interaction with 5-HT$_7$ binding site.

Interestingly, the introduced spacer modifications were unfavorable for 5-HT$_{1A}$ receptors, thus emulation of trans arrangement in the new compounds seems to be a promising strategy in development of more selective 5-HT$_7$ ligands.

In regard to fragment containing a basic nitrogen atom, the pronounced affinity at 5-HT$_7$ receptors can be found mainly for *o*-methoxyphenylpiperazines. THIQ and THBC derivatives generally showed moderate to low activity, whereas 1,2,3,4-tetrahydropyrazino[1,2-a]indoles were not recognized by the 5-HT$_7$ sites.

The impact of modifications within imide terminus is equvocal, and it is not possible to indicate the optimal fragment. It is worth to note that compounds **9-12** showed better 5-HT$_7$ affinity than that of both leads and moreover 5HT$_7$/5HT$_{1A}$ selectivity ratio of **9** is ~1.

Table 2. Structure and 5-HT$_7$ and 5-HT$_{1A}$ binding affinities of compounds **1**, **5** and **9-23**; (linker = –(CH$_2$)$_4$–).

Amine moiety \ I(A)mide						
	**1** **90** **5** 6.4 0.3[1]	**5** **87** **2** 0.6 0.1[2]	**9** **28** **2** 27 3[3]	**10** **40** **7** 6.8 0.5[4]	**11** **46** **1** 4 2[2]	**12** **22** **4** 1.7 0.5[4]
	**13** **> 10 000** 2920 90[5]	**14** **135** **14** 140 10[5]		**15** **418** **36** 153 15[4]	**16** **138** **8** 50 11[4]	**17** **257** **8** 157 17[4]
	**18** **160** **24** 114 12	**19** **277** **25** 28 3	**20** **225** **18** 128 14			
	**21** **> 10 000**	**22** **> 10 000** 74 9	**23** **> 10 000**			

Cmpd
K_i (5-HT$_7$) ± SEM
K_i (5-HT$_{1A}$) ± SEM

Receptor Binding Assays

The in vitro affinity of the investigated compounds for 5-HT$_{1A}$ receptors was assessed on the basis of their ability to displace [^{3}H]-8-OH-DPAT (222 Ci/mmol, Amersham, England) from the rat brain tissue (hippocampus), according to the previously published procedures [6].

The serotonin 5-HT$_7$ receptor binding assay was performed using rat hypothalamic membranes, according to the method described by Aguirre et al. [7] with minor modifications. In brief, hypothalami dissected out from male Wistar rats (200-250 g) were frozen at -80°C prior to the preparation of radioligand binding homogenate. On the day of experiment hypothalami were allowed to defrost, then immediately homogenized in 20 volumes of 50 mM Tris-HCl buffer (pH 7.4 at 23°C) and centrifuged at 48 000 g for 10 min at 4°C. The supernatant was removed, resulting pellet rehomogenized and incubated at 37°C for 15 min, to remove endogenous serotonin. After this incubation, the homogenate was centrifuged twice under the same conditions as before. The final pellet was resuspended in assay buffer (50 mM) Tris-HCl containing 0.01 mM pargyline, 4 mM $CaCl_2$ and 0.1% ascorbate. Aliquots of membranes (10 mg original wet tissue weight) were incubated in the presence of 3 M (+/-)-pindolol (to eliminate binding to 5-HT$_{1A/B}$ receptors) with 0.5 nM [^{3}H]-5-CT (specific activity, 34.5 Ci/mmol; NEN) and two (in prescreening) or eight (in full assay) concentrations of the displacing drug. Non-specific binding was determined using 10 M of serotonin. After incubation at 23°C for 120 min, the reaction was terminated by rapid filtration through Whatman GF/B filter followed by three 4 ml washes with ice-cold 50 nM Tris-HCl buffer, pH 7.4. The radioactivity retained on the filters was measured by liquid scintillation counting (Beckman L SM 6500) in 4 ml of scintillation fluid (Akwascynt, BioCare).

Affinity constants (K_i) were determined using non-linear least-squares regression software (Graphad Inplot, San Diego). Data are expressed as the mean +/- S.E.M. of at least three separate experiments each performed in triplicate.

ACKNOWLEDGMENTS

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