SYNTHESIS AND SAR STUDIES OF 1,2,3,4-TETRAHYDRO- -CARBOLINE DERIVATIVES AS NEW 5-HT$_7$/5-HT$_{1A}$ RECEPTOR LIGANDS

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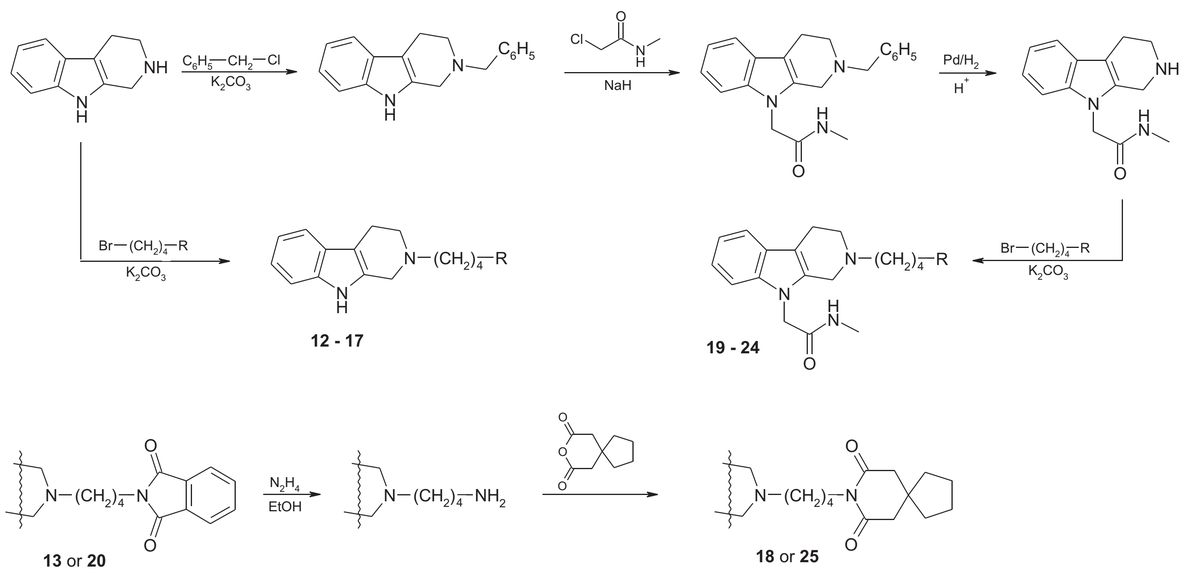
Introduction

Since its discovery in 1993, the 5-HT$_7$ receptor is gaining increasing interest as a potential drug target. Studies utilizing recently developed selective antagonists revealed that 5-HT$_7$ receptors play a role in thermoregulation, learning and memory, hippocampal activity, sleep, circadian rhythms and mood. Due to a relatively limited number of papers describing structure-activity relationship (SAR) studies of 5-HT$_7$ receptor ligands further research in this field is of particular interest.

It is known that pharmacophoric arylpiperazine fragment is well recognized by 5-HT$_{1A}$, 5-HT$_{2A}$ as well as 5-HT$_7$ receptors. Indeed, 1-(2-methoxyphenyl)piperazine (oMPP) derivatives were among the most active 5-HT$_7$ receptor ligands identified by the screening of our compounds library. Those compounds, however, were usually several times more active at 5-HT$_{1A}$ sites (e.g. compounds **1**, **2** and **4**–**6**). Interestingly, indolinone derivative **3** was equally potent, dual 5-HT$_7$/5-HT$_{1A}$ receptor ligand.

In the case of 1,2,3,4-tetrahydroisoquinoline (THIQ) derivatives it was found that they were less active 5-HT$_7$ ligands than the respective oMPP analogues, but more significant decrease was observed for their 5-HT$_{1A}$ affinity. Compounds with indolinone and 8-azaspiro[4.5]decane-7,9-dione fragments (**9** and **11**, respectively) showed the highest 5-HT$_7$ affinity.

In order to search for a new 5-HT$_7$ ligands with increased selectivity for 5-HT$_{1A}$ receptors two new series of compounds were designed based on the results presented by Kikuchi C. et al. [1]. In the structure of oMPP or THIQ derivatives, amine fragment was replaced with a 1,2,3,4-tetrahydro- -carboline (THBC) or 9-methylcarbamoylmethyl-THBC moiety (Scheme, Table). For all those compounds binding affinity for 5-HT$_7$ and 5-HT$_{1A}$ receptors was measured, and next, functional profile at 5-HT$_7$ receptors for two selected derivatives was determined.



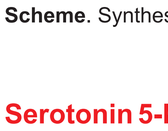
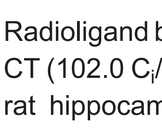
Scheme. Synthesis of compounds **12**–**25**.

Serotonin 5-HT$_7$, and 5-HT$_{1A}$ binding assays

Radioligand binding studies with native 5-HT$_7$ receptors used rat hypothalamic membranes, [^{3}H]-5-CT (102.0 C$_i$/mmol, Amersham) and serotonin for nonspecific binding, whereas for 5-HT$_{1A}$ assays rat hippocampal membranes, [^{3}H]-8-OH-DPAT (170 C$_i$/mmol, NEN Chemicals) and 5-HT for nonspecific binding were used.

The new THBC derivatives exhibited moderate to low 5-HT$_7$ affinity ranging from 80 nM to 2600 nM for **B1013** and **19**, respectively. All the new compounds were less active at 5-HT$_7$ receptors than their oMPP analogues, however, some of them (**12**, **20** and **21**) showed higher affinity than THIQ derivatives. Again, the presence of indolinone and 8-azaspiro[4.5]decane-7,9-dione moieties in the ligand structure was beneficial to 5-HT$_7$ receptor activity.

Table. Structure and 5-HT$_7$ (red) and 5-HT$_{1A}$ (blue) binding affinities[a] of the investigated compounds.

compd No K$_i$ 5-HT$_7$ [nM] K$_i$ 5-HT$_{1A}$ [nM]	R=						
	1 MM77 **90**[b] 6.4[c]	**2** NAN190 **87**[b] 0.6[d]	**3** **28** 27[e]	**4** **70** 10[f]	**5** **308** 17[g]	**6** **102** 4[g]	
	7 **> 10 000** 2920[h]	**8** **135** 140[h]	**9** **112** 145	**10** **208** 120			**11** MM199 **36** 5[h]
	**12** **160** 114	**13** **277** 28	**14** **255** 128	**15** **300**	**16** **320** 139	**17** **1300** 50	**18** **115** 13
	**19** **2590** 164	**20** **117** 55	**21** B1013 **80** 230	**22** **208** 130	**23** **970** 380	**24** **420** 258	**25** B1032 **144** 95

[a]an error of K$_i$ values estimation was < 15%; Data from: [b]Bojarski et al., BMCL 14, 2004, 5863; [c]Mokrosz MJ et al., Med Chem Res 4, 1994, 161; [d]Glennon RA et al., J Med Chem, 31, 1988, 1968; [e]Mokrosz MJ et al., Arch Pharm, 331, 1998, 325; [f]Mokrosz JL et al., Drug Des Discov, 11, 1994, 197; [g]Mokrosz JL et al., J Med Chem, 37, 1994, 2754; [h]Mokrosz JL et al., J Med Chem, 39, 1996, 1125

Regarding 5-HT$_{1A}$ receptors, THBC derivatives always showed higher affinity (K$_i$ = 13–139 nM) than their 9-methylcarbamoylmethyl substituted analogues (K$_i$ = 55–380 nM) and in the case of **B1013** an inverse 5-HT$_7$/5-HT$_{1A}$ selectivity was observed. This compound together with 8-azaspiro[4.5]decane-7,9-dione derivative **B1032** were further tested in functional 5-HT$_7$ adenylate cyclase assay.

Cyclic AMP measurments

5-HT$_7$ receptor is positively coupled to adenylate cyclase and the stimulation of this receptor results in the increase of cAMP level. It was previously shown that 5-CT–(5-carboxamidotryptamine maleate)–stimulated cAMP accumulation in H4 (ATCC HTB-178) human glioblastoma cell line was selectively blocked by SB269970 in a dose dependent manner [2]. Similar experiments were used to determine functional profile of the investigated compounds at 5-HT$_7$ receptor. First it was found that neither **B1013** nor **B1032** used in different concentrations influenced cAMP accumulation.

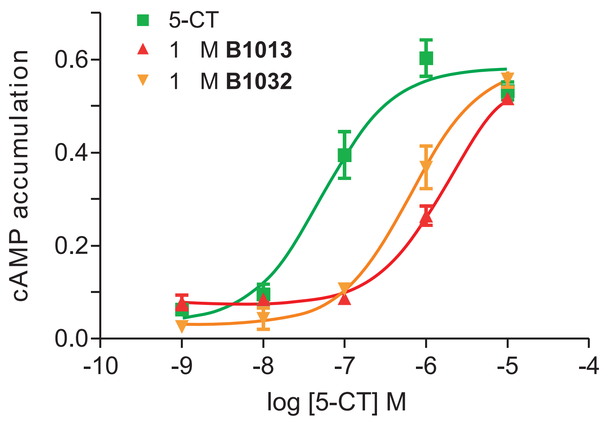


Figure 1. Concentration-response curve of 5-CT in the absence (■) and in the presence B 1013 (▲), B 1032 (▼) for stimulation of cAMP accumulation in H4 cells. Data points represent the mean S.E.M.

Next the ability of **B1013** and **B1032** to inhibit 5-CT (with pEC$_{50}$ 7.27 0.12) stimulated cAMP accumulation in H4 cell line was investigated. The dose–response curves representing 1 M of **B1013** (with pEC$_{50}$ 5.75 0.042) or 1 M of **B1032** (with pEC$_{50}$ 6.18 0.013) in the presence of 5-CT in a concentration of 1 x 10^{-9} to 1 x 10^{-5} M are shown in Figure 1. In accordance with the measured 5-HT$_7$ affinities, antagonistic potency of **B1013** was higher than that of **B1032**.

Electrophysiological studies

In addition to the above experiments, our potent 5-HT$_{1A}$ antagonist with anxiolytic-like activity – **MM77**, displaying comparable 5-HT$_7$ affinty to that of **B1013**, was electophysiologically characterized and compared with a potent 5-HT$_7$ antagonist SB269970.

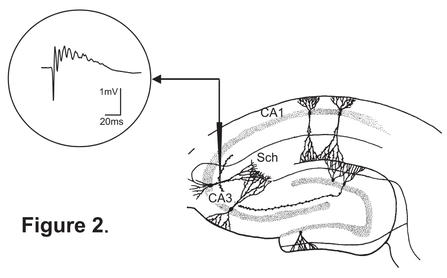


Figure 2.

After dissection, the rat hippocampus was cut into transverse slices (400 m thick) using a vibrating microtome. Spontaneously occurring epileptiform bursts were recorded within 15–30 minutes of perfusion of the slices with nominally Mg^{2+} - free ACSF. Bursting events, representing primary bursts, consisted of a prominent initial population spike-like waveform, reaching 2–3 mV in amplitude, which was followed by smaller afterdischarges, superimposed on a slower, positive-going wave, lasting 60–100 ms (Fig. 2).

The application of 5-CT for 10 min in the presence of 1 M WAY 100635, a selective 5-HT$_{1A}$ receptor antagonist, resulted in an increase in the bursting frequency (Fig. 3). The excitatory effect of 5-CT was dose-dependent. The SB269970, a specific antagonist of the 5-HT$_7$ receptor, in dose dependent manner inhibited the excitatory effect of 5-CT (Fig. 3).

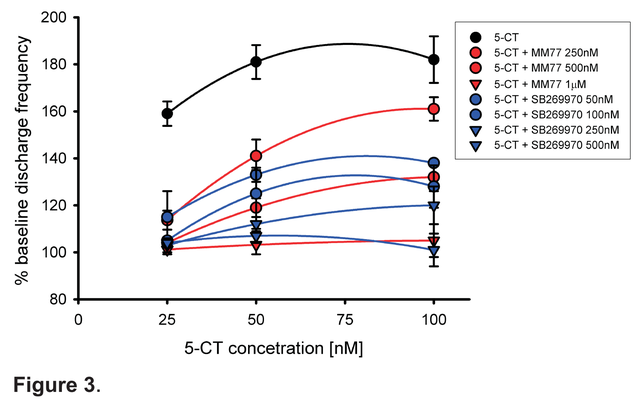


Figure 3.

The application of **MM77** decreased the excitatory action of 5-CT in similar manner to SB269970 but with smaller efficacy (Fig. 3). Neither SB269970 nor **MM77** applied alone exerted any effect on the bursting frequency.

Summary

14 new tetrahydro- -carboline derivatives with tetramethylene linker and different (cyclic imide/amide, benzotriazole) terminals were synthesized and evaluated for 5-HT$_7$ and 5-HT$_{1A}$ receptor affinity. Compounds with indolinone and 8-azaspiro[4.5]decane-7,9-dione fragments were among the best 5-HT$_7$ ligands and two of them (**B1013** and **1032)** showed antagonistic activity in adenylate cyclase assay. Electrophysiological studies with **MM77** revealed that this potent antagonist of postsynaptic 5-HT$_{1A}$ receptors behaved also like antagonist at 5-HT$_7$ receptors, however, of slightly lower efficacy than SB269970.

Acknowledgments

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References

[1] Kikuchi C, Ando T, Watanabe T, Nagaso H, Okuno M, Hiranuma T, Koyama M. 2a-[4-(Tetrahydropyridoindol-2-yl)butyl]tetrahydrobenzindole derivatives: new selective antagonists of the 5-hydroxytryptamine7 receptor. J Med Chem. 2002, 45, 2197.

[2] Mahe C, Bernhard M, Bobirnac I, Keser C, Loetscher E, Feuerbach D, Dev KK, Schoeffter P. Functional expression of the serotonin 5-HT7 receptor in human glioblastoma cell lines. Br J Pharmacol. 2004, 143, 404.