THE SCREENING OF A LIBRARY OF ARYLPIPERAZINE DERIVATIVES FOR 5-HT$_7$ RECEPTOR AFFINITY

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INTRODUCTION

Discovery of 5-HT$_7$ serotonin receptor subtype, which has a high affinity to a lot of antipsychotic and antidepressant drugs, stimulated several research groups to study this new biological target. The main attention was focused on clarification of its role in affective disorders, thus ligands selective to 5-HT$_7$ receptor have been urgently needed. Ten years of explorations resulted in identification of a few selectively blocking agents, but substances possessing clear agonistic activity only to 5-HT$_7$ subtype are still not available [1].

On the other hand, considering therapeutic applications, selectivity of action is not a definitely required feature, and multireceptoral ligands constitute a valuable source of potential psychotropic agents.

It was reported that some high affinity ligands among long chain arylpiperazines (in particular 1-(2-methoxy-phenyl)piperazine derivatives, *o*-OMePhP) [2, 3] and tetrahydroisoquinolines (THIQ) [4] were found. Since compounds of this type were investigated in our laboratory as 5-HT$_{1A}$ and 5-HT$_{2A}$ receptor agents, we decided to launch a new project - searching for a novel 5-HT$_7$ receptor ligands. Our efforts are focused on finding the selective substances (agonists or antagonists), as well as compounds interacting also with another therapeutically strategic serotonin receptor subtypes mainly 5-HT$_{1A}$ and 5-HT$_{2A}$.

RESULTS

In a first stage of investigation we were screening our compounds library against the native 5-HT$_7$ receptor from rat hypothalamic membranes.

As a starting point we selected compounds demonstrating significant affinity for 5-HT$_{1A}$ receptors (K_i < 100 nM), since the similarity in binding sites for both 5-HT$_7$ and 5-HT$_{1A}$ receptors was reported [5]. We supposed that among those 5-HT$_{1A}$ ligands, activity for 5-HT$_7$ receptors is more probable to detect. Here we present the results for 40 compounds, mainly *o*-OMePhP and THIQ derivatives.

Initial tests were performed at two compound concentrations: 1 μM and 0.1 μM. The ligands showing an inhibition lower than 30% at the concentration of 0.1 μM were considered as inactive. Compounds in which inhibition of more than 30% (0.1 μM) was detected, were selected for the determination of affinity constants (K_i) for 5-HT$_7$ receptors, measured by a standard competition binding procedure. Structures of all the derivatives examined and screening results obtained are presented in the Table. Additionally, affinities for 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors (data previously reported), and first K_i values for 5-HT$_7$ receptors from ongoing binding experiments are also shown.

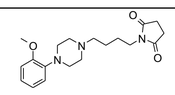
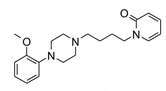
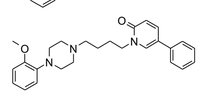
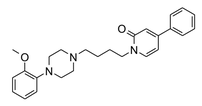
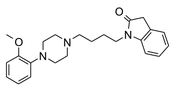
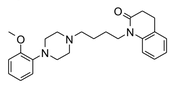
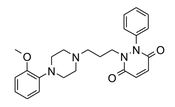
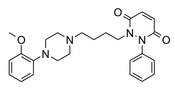
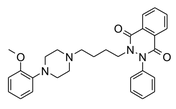
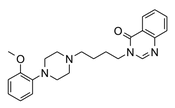
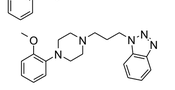
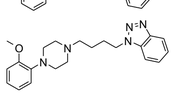
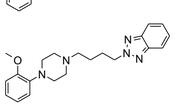
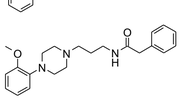
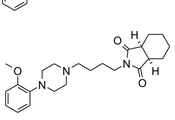
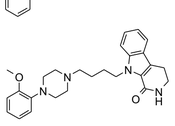
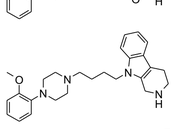
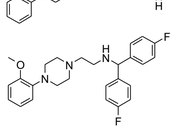
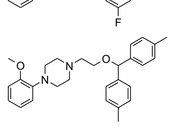
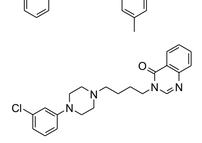
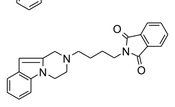
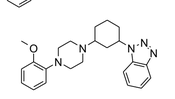
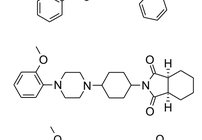
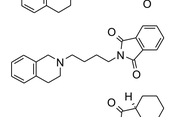
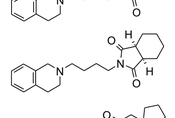
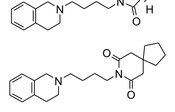
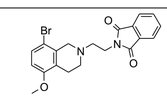
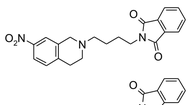
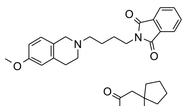
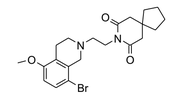
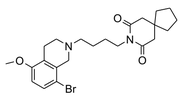
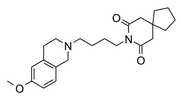
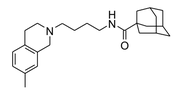
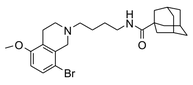
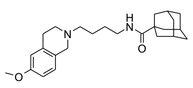
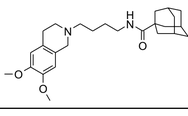
It was found that 14 compounds (7 *o*-OMePhP, 1 *m*-ClPhP and 6 THIQ derivatives) demonstrated satisfactory activity in the applied preliminary screening. Indeed, recent results of competition binding assays for 6 of them confirmed their distinct activity for 5-HT$_7$ receptors (K_i = 25-70 nM).

Although at this early stage of investigation we are not able to discuss real structure-activity relationships, it is clear that the presence of a longer linker and THIQ moiety are beneficial for 5-HT$_7$ binding. On the other hand, rigid alkyl spacer is an unfavorable structural feature, as comes out from the comparison of the respective pairs of compounds: **1** vs **24** and **15** vs **23**. Compound **23**, the most active from the prescreened rigid derivatives, evaluated in a full binding experiment, displayed low 5-HT$_7$ affinity (K_i = 720 nM) what additionally validate discriminative utility of the applied screening method.

CONCLUSIONS

Exploration of our compounds library towards 5-HT$_7$ activity, led to the identification of several ligands having good 5-HT$_7$ receptor affinity, but poor selectivity over 5-HT$_{1A}$ receptor. Interestingly, 50% of the analyzed THIQ derivatives were qualified for full binding evaluation, thus we consider them as initial leads for future structural optimization. In the next stage, compounds with low 5-HT$_{1A}$ affinity will be screened in order to find selective 5-HT$_7$ receptor ligands.

Table. Structures, percentage of inhibition at 10^{-7}M and affinity constants for 5-HT$_7$ receptors of the investigated compounds. K_i values for 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors added for comparison.

Compd.	Structure	% of inhib. at 10^{-7}M	K_i [nM]		
		5-HT$_7$	5-HT$_7$	5-HT$_{1A}$	5-HT$_{2A}$
1		40.0	37	6.4	1510
2		32.3		18	717
3		36.0	73	15	296
4		11.9		40	522
5		50.0	25	27	66
6		36.1	70	10	40
7		26.9		54	2125
8		26.9		11	1456
9		26.9		43	374
10		16.1		36	566
11		24.1		15	1040
12		23.7		17	160
13		33.7		4	214
14		12.0		90	1120
15		30.0		4	109
16		0.0		0.3	0.9
17		0.0		30	343
18		0.0		3	235
19		0.0		154	735
20		35.0		50	68
21		0.0		74	3680
22		10.0		47	300
23		13.3	720	72	1900
24		12.0		15	11500
25		0.0		8	2600
26		0.0		3000	
27		38.0	62	140	
28		17.9		50	
29		42.0	35	5	
30		20.0		0.95	450
31		20.0		15870	4273
32		34.3		1468	1843
33		31.4		316	3092
34		0.0		1041	837
35		46.1		31	43
36		19.6		192	1250
37		53.8		4.9	99
38		6.0		6	40
39		0.0		34	1450
40		0.0		42	1140

5-HT$_7$ Receptor Binding Assay

The serotonin 5-HT$_7$ receptor binding assay was performed using rat hypothalamic membranes, according to the method described by Aguirre et al. [6] with minor modifications. In brief, hypothalami dissected out from male Wistar rats (200-250 g) were frozen at -80ºC 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 23ºC) and centrifuged at 48 000g for 10 min at 4ºC. The supernatant was removed, resulting pellet rehomogenized and incubated at 37ºC 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 23ºC 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.

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