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Pharmacological evaluation of 5-{2-[4-(2-methoxy-phenyl)-piperazin-1-yl]-ethyl}-1,3-dihydro-benzimidazole-2-thione as a potential atypical antipsychotic agent

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Antipsychotic drugs (*i.e.* neuroleptics) constitute a diverse group of chemical compounds with common pharmacological action on brain dopamine (DA) receptors and potency to reduce psychotic symptoms in both schizophrenia and forms of toxic psychosis [1]. A group of atypical antipsychotics represents mixed DA-ergic/serotonergic ligands with usually lower affinity for the D_2 receptor than conventional neuroleptics [2, 3]. They have a lower propensity to cause extrapyramidal side effects and a potency to treat both positive and negative schizophrenia symptoms [3]. Examinations on a notable contribution of serotonin (5-HT) dysfunction in the etiology of schizophrenia indicated a significant function of specific 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C} and 5-HT_3 receptors [2, 4]. Important and specific role of 5-HT_{1A} autoreceptors in etiology of schizophrenia [4–6], as well as in depressive disorders [7], suggests them to be a prospective target in the therapeutic approaches [2, 4–7]. Some atypical neuroleptics, acting as antagonists on D_2/D_3 and 5-HT_{2A} receptors, express a partial agonist effect on 5-HT_{1A} receptor [5, 6]. Besides, drugs like roxindole, with agonist action on D_2-like and 5-HT_{1A} receptors and inhibitory potency on 5-HT uptake, have beneficial effects in the treatment of both negative schizophrenia symptoms and depressive disorders [8].

We have found it of considerable interest to identify a drug design strategy and to perform synthesis of mixed DA-ergic/5-HT-ergic compounds with a possible atypical neuroleptic potential. Among the explored novel compounds, 2-(1*H*-benzoimidazol-5-yl)-ethyl-dipropylamine expressed the highest binding affinity at the D_2 receptor, while ligands with arylpiperazine as a lipophylic part of the molecule expressed mixed DA-ergic/5-HT-ergic affinity [9, 10]. From an array of previously synthesized and tested substituted arylpiperazines [9], the most active (at bovine brain D_2 and 5-HT_{1A} receptors) compound **1**, representing 5-{2-[4-(2-methoxy-phenyl)-piperazin-1-yl]-ethyl}-1,3-dihydro-benzoimidazole-2-thione, was selected for further pharmacological screening. Compound **1** was evaluated by *in vitro* assays for its binding affinity at specific DA (D_1, D_2) and 5-HT receptors (5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C} and 5-HT_3) of rat brain striatal or cortical synaptosomes. In a search for a potential antidepressive potency of **1**, an inhibitory effect of the compound on synaptosomal 5-HT reuptake and the activity of MAO enzymes was estimated *in vitro* [11, 12]. The atypical neuroleptic potential of **1** *in vivo* was examined by dose-response behavioral tests [11] for cataleptogenic potency and by evaluating its influence on the total locomotor activity and stereotypy in simple animal models of amphetamine-(AMPH) and MK-801-induced psychoses.

In vitro studies showed (Table) that compound **1** expressed the highest binding affinity at the D_2 and HT_{1A} receptors (6.1 and 11.8 nM, respectively), and moderate affinities for the binding at the 5-HT_{2A} and 5-HT_{2C} receptors. In addition, it sparsely affected synaptosomal 5-HT reuptake and the activity of rat hepatic MAO enzymes *in vitro*. No changes either in total locomotor activity or stereotypy were observed in rats i.p. injected with **1** (Fig. 1). Also, **1** did not express cataleptogenic effects (0/6, 1.0 or 4.0 mg/kg b.w. after 30, 45 or 60 min), while haloperidol induced catalepsy at 1.0 mg/kg (5/6 after 30 and 45 min; 6/6 after 60 min). Compound **1** prevented hyperlocomotion and attenuated stereotypy in rats treated with MK-801 only after the dose of 4.0 mg/kg, while being without effect at the dose of 1.0 mg/kg (Fig. 1). Upon testing the influence of **1** on AMPH-induced hyperactivity and stereotypy, a significant attenuation of hyperlocomotion with all three doses applied was observed (Fig.). Although, it was the most evident for the highest dose of **1** (4.0 mg/kg), no clear dose-response relation was recorded. The attenuation of AMPH-induced stereotypy by pretreatment with **1** was not very pronounced (about 10–20%) and associated mainly to the highest dose used. All these *in vivo* effects of **1** are similar by pattern to the behavioral effects of atypical neuroleptic clozapine in similar tests [13, 14]. Although **1** exhibited D_2/5-HT_{2A} binding ratio (in pKi) of 1.12, proposed as a

Table: Binding affinities (K_i) at the DA and 5-HT receptors, and inhibitory potency (IC_{50}) on synaptosomal 5-HT reuptake and enzymatic MAO activity of compound 1 *in vitro*

Receptor/activity	D_1	D_2	5-HT_{1A}	5-HT_{2A}	5-HT_{2C}	5-HT_3 /	5-HT reuptake	MAO A	MAO B[3]
H-Radioligand (Ci/mmol)	SCH23390 (91)	Spiperone (25)	8-OH-DPAT (245)	Spiperone (25)	Mezulergin (86)	Zacoprid (83)	5-HT (128)	–	–
Conc. in assay (nM)	0.4	0.2	1.5	1	1	1	50		
Values*	K_i (μM) 2.10 (± 0.55)	K_i (nM) 6.1 (± 1.2)	K_i (nM) 11.8 (± 1.5)	K_i (nM) 48.0 (± 2.1)	K_i (nM) 70.6 (± 15.2)	K_i (μM) >10	IC_{50} (μM) 3.86 (± 1.02)	IC_{50} (μM) >10	IC_{50} (μM) >1

* Values are the means of 3–4 independent experiments (for MAO enzymes 2 experiments were performed); standard errors of mean (S.E.M.) are given in parentheses

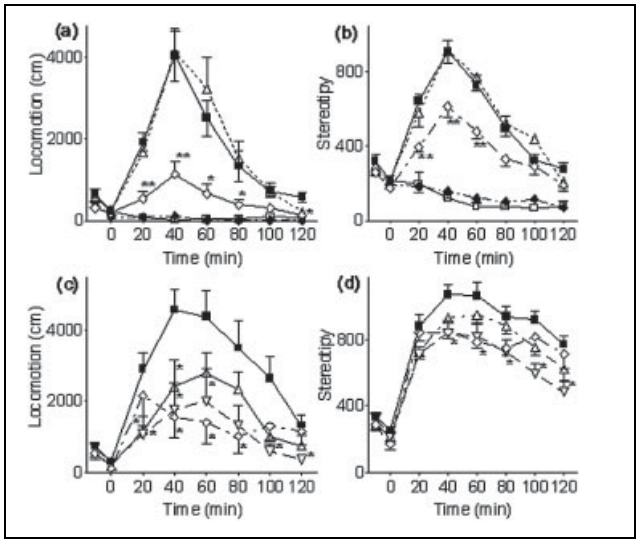

Fig.: Effect of compound **1** on locomotor activity (a, c) and stereotypy (b, d) in animal models of psychosis. Doses of **1**, administered 20 min before d-AMPH (c, d; 5 mg/kg, i.p.) or MK-801 (a, b; 0.3 mg/kg, i.p.): △, 1 mg/kg; ▽, 2 mg/kg; ◇, 4 mg/kg; ■, controls were given saline/DMSO. Groups treated only with 4 mg of **1** (□), or saline/DMSO (◆). Each plot represents the mean ± S.E.M. of 6 to 8 animals. Significance (Mann-Witney U-test): * $p < 0.05$; ** $p < 0.005$, as compared to the control values

profile of classical and not of atypical antipsychotics [15], it neither reduced spontaneous motor activity, nor induced a cataleptogenic effect, like typical neuroleptics. At the same time, the compound diminished hyperlocomotion and stereotypy in animal models of psychosis. Such behavioral effects suggest some clozapine-like atypical neuroleptic potential of **1**, indicating, that at the receptor level, it may act as a possible antagonist or reverse agonist at the $D_{2/3}$, 5-HT_{2A} and 5-HT_{2C} receptors, and a partial agonist at the 5-HT_{1A} receptors, as have already been reported for atypical neuroleptics [3–6]. However, these assumptions should be exactly proved by further studies, as for example, particular arylpiperazines have been diversely reported to be agonists, partial agonists or antagonists at the 5-HT_{1A} receptors [2]. In addition, there is no hint that **1** may have an extra antidepressive potential, as it exhibited minor effects on 5-HT reuptake and MAO enzyme activity. The interesting biological profile of **1** suggests that additional research of this compound would be justified, but our further efforts will be also focused on the design and synthesis of more active aryl-piperazines with D_2/5-HT_{1A} ligand properties and promising atypical neuroleptic potential.

Experimental

Adult male Mill-Hill hooded rats (b.w. 200–250 g) were used in all *in vitro* and *in vivo* experiments. Their brain cortices were used for synaptosome preparations applied for determination of 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C} receptor binding and 5-HT reuptake experiments, the entorhinal cortices for 5-HT_3 and the striata for D_1 and D_2 receptor binding; rat liver microsomal fraction was a source of MAO enzymes [12]. The methods of brain synaptosome preparation, receptor binding assays and 5-HT uptake were slightly modified comparing to the original procedures [11]. Specific radioligands (Table) were products of either NEN or Amersham. Competition binding assays were performed (10 min, 37 °C) with 6 concentrations of compound **1** (10 μM–0.1 nM) in duplicate, non-specific binding was determined using 1.0 μM (+)butaclamol (for DA receptors) or 10 μM 5-HT (for 5-HT receptors) and competition binding curves were constructed and analyzed by "GraphPad Prism" (v. 2.0.) software. Equal concentrations of **1** were used in two other *in vitro* tests. Compound **1** was originally dissolved in DMSO (final conc. up to 2%), diluted with saline and i.p. injected in 2 or 3 doses (1.0–4.0 mg, in 2.0 ml saline per kg b.w.) in all behavioral experiments. Catalepsy was scored by the horizontal bar test [11], performed 30, 45 and 60 min after after i.p. administration of saline/DMSO, **1** (1.0 or 4.0 mg/kg) or haloperidol (1.0 mg/kg), in three trials (six animals per group). Animal models of psychosis were induced by d-AMPH sulphate (5.0 mg/kg) or MK-801 (0.3 mg/kg) administered 20 min after the compound. The controls received saline/DMSO or saline alone. Motor activity in an open field test was monitored automatically with a Columbus Auto-Track System (Version 3.0 A, Columbus Institute, OH, U.S.A.), starting immediately after the first injection, ending 120 min after the second treatment. Statistical analysis was performed by non-parametric Kruskal-Wallis test followed by Mann-Witney U-test.

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