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Pharmacological evaluation of selected arylpiperazines with atypical antipsychotic potential

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Abstract—Six active compounds, among previously synthesized and screened arylpiperazines, were selected and evaluated for the binding affinity to rat dopamine, serotonin and α_1 receptors. Two compounds with benztriazole group had a 5-HT_{2A}/D_2 binding ratio characteristic for atypical neuroleptics (>1, pK_i values). Compound **2**, 5-{2-[4-(2,3-dimethyl-phenyl)-piperazin-1-yl]ethyl}1H-benzotriazole, expressed clozapine-like in vitro binding profile at D_2, 5-HT_{2A} and α1 receptors and a higher affinity for 5-HT_{1A} receptors than clozapine. Also, it exhibited the noncataleptic behavioural pattern of atypical antipsychotics and antagonized *d*-amphetamine-induced hyperlocomotion in rats.

1. Introduction

One of the greatest challenges of modern psychopharmacology is a search for new antipsychotic drugs (APDs, i.e., neuroleptics) that would express a higher therapeutic efficiency and a wider spectrum of action on schizophrenia symptoms, with minimized adverse side effects. Conventional APDs, acting by a common mechanism of central dopamine (DA) D_2 receptor blockade, are generally considered to be effective in the treatment of schizophrenics with positive symptoms,[1] while a diversified group of the so-called atypical APDs expresses increased effectiveness in negative, affective and cognitive symptoms, including efficacy in patients resistant to standard therapy.[2] Atypical APDs also have a low incidence of extrapyramidal side effects and prolactinaemia, but may produce other undesirable side effects (e.g., agranulocytosis) that limit their clinical use.[2] The mechanism of their action is still controversial, since there are several models explaining 'atypicality' by specific drug action on the subclasses of serotonergic (5-HT), glutamatergic, muscarinic, or α-adrenergic receptors, by prevalent interaction with DA D_1, D_3 or D_4 receptors, or by loose binding concept and faster drug dissociation from the D_2 receptors.[2–4] A 'dopamine–serotonin hypothesis' of schizophrenia proposed that the essential dysregulation of 5-HT_{2A}-/D_2-mediated neurotransmission may be involved in development of this disease.[2,4] It was hypothesized that antagonism of D_2 and 5-HT_{2A} receptors and enhancement of DA and 5-HT release are critical elements in the action of atypical APDs, which could minimize both positive and negative symptoms without producing significant side effects.[4–6] This model suggests the ratio of 5-HT_{2A} to D_2 receptor affinities to be the major determinant of a drug's possibility to behave as an atypical antipsychotic.[5] Besides the accentuated role of the 5-HT_{2A} receptors in the aetiology of schizophrenia, an important contribution of other specific 5-HT receptors, especially 5-HT_{1A}, and 5-HT_{2C} subclasses, but also of 5-HT_3, 5-HT_6 and 5-HT_7 receptors, is indicated in the literature.[4,6–8] Research data showed that 5-HT_{1A} agonism could be connected to the anticataleptic effect of APDs and improvement of negative schizophrenia symptoms.[4,6–9] The new generation of therapeutically successful atypical APDs that act as a DA-5-HT system stabilizers with a partial agonist activity at the 5-HT_{1A} receptors, strong 5-HT_{2A} antagonism and weak D_2

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antagonism[6] (also suggested to be a partial agonism[9]) promotes such a pattern of interaction for some newly synthesized DA/5-HT ligands that apply for atypical neuroleptic potential.[2,6–9]

During the last decade, we followed the strategy focused on drug design and synthesis of mixed DA-ergic/5-HT-ergic compounds with a possible atypical neuroleptic potential. One of the synthetic approaches was design and synthesis of heterocyclic arylpiperazines, with a specific structure of heteroaryl group, that mimics catechol moiety of the dopamine (benzimidazole, substituted benzimidazoles, benztriazoles and 1,4-dihydroquinoxaline-2,3-diones). Variations of heterocyclic and aryl groups had produced over 100 new compounds that were screened for their affinities at bovine brain D_1, D_2 and 5-HT_{1A} receptors by in vitro radioligand binding assays.[10] The six most active compounds (Table 1) were selected for further pharmacological examinations.

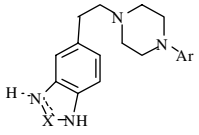
2. Pharmacology

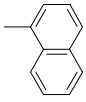
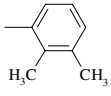
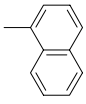
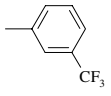
The assorted compounds were evaluated by in vitro assays for binding affinities to the specific DA (D_1, D_2), 5-HT (5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C} and 5-HT_3) and α_1-adrenergic receptors. These receptors were chosen concerning their anticipated role in the action of atypical APDs.[3–8] Specific binding affinities (pK_i values, Table 1) of the new arylpiperazines and clozapine, a prototype of atypical APD, were determined by measuring the extent of displacement of ^{3}H-labelled specific ligands from rat striatal or cortical synaptosomes with a range of concentrations of selected compounds.[11,12] On the premise that drugs with beneficial effects in the treatment of both negative schizophrenia symptoms and depressive disorders may combine partial agonism at 5-HT_{1A} receptors and inhibition of synaptosomal 5-HT reuptake mechanism,[13] we also tested several active compounds (**1**–**3**) for their in vitro inhibitory potency on the 5-HT reuptake.[11] Compound **2** was selected for in vivo tests, regarding its adequate 5-HT_{2A}/D_2 receptor binding ratio, proposed for 'atypicality'[5] (1.14>1, pK_i values). In order to explore atypical neuroleptic potential of **2**, dose–response behavioural tests for its cataleptogenic potency[12,14] and influence on *d*-amphetamine (AMPH) induced changes in open-field behaviour, registered by photocell counts,[12,15] were performed on rats.

3. Results and discussion

All compounds expressed a higher affinity for the 5-HT_{1A} receptors comparing to clozapine for up to one

Table 1. Receptor binding affinity and inhibition of 5-HT uptake for compounds **1**–**6** and clozapine



Compound[a]	X	Ar	pK_i ± SEM							IC_{50} (μM) ± SEM-5-HT reuptake	5-HT_{2A}/D_2 binding ratio
			D_1	D_2	5-HT_{1A}	5-HT_{2A}	5-HT_{2C}	5-HT_3	α_1		
1	CH		5.44 ± 0.10	8.78 ± 0.02	6.90 ± 0.09	7.82 ± 0.05	7.19 ± 0.11	5.28 ± 0.11	7.03 ± 0.13	1.76 ± 0.52	0.89
2	N		5.33 ± 0.11	7.29 ± 0.06	7.08 ± 0.05	8.29 ± 0.14	7.84 ± 0.08	<5.0	7.17 ± 0.14	0.26 ± 0.07	1.14
3	N		5.24 ± 0.12	7.62 ± 0.18	7.41 ± 0.16	7.98 ± 0.12	7.26 ± 0.13	<5.0	—	>10.0	1.05
4	N		6.09 ± 0.09	8.27 ± 0.14	7.10 ± 0.11	6.85 ± 0.12	7.35 ± 0.02	<5.0	—	—	0.83
5	CO–CO		—	7.55 ± 0.20	7.06 ± 0.16	5.81 ± 0.10	—	—	—	—	0.77
6	C–C_6H_5		—	7.50 ± 0.05	6.76 ± 0.13	6.12 ± 0.11	—	—	—	—	0.82
Clozapine			7.50 ± 0.14	7.11 ± 0.05	6.38 ± 0.10	8.57 ± 0.18	8.54 ± 0.02	6.73 ± 0.19	7.32 ± 0.13	>10.0	1.20

[a] Details on complete synthesis and structural analysis of all six compounds are described in Ref. 10.

order of magnitude (Table 1). Only two compounds (**2** and **3**) showed a higher affinity for 5-HT_{2A} than D_2 receptors and this was more prominent for **2** (K_i of 5.10 and 51.3 nM at 5-HT_{2A} and D_2 receptors, respectively). Compound **2** expressed a comparable affinity to clozapine for the D_2, 5-HT_{2A} and α_1-adrenergic receptors, and somewhat lower affinity for the 5-HT_{2C} receptors. It exhibited a poor affinity at the D_1 receptor and no binding potency at the 5-HT_3 receptor. On the other hand, **2** showed a higher affinity for the 5-HT_{1A} receptor than clozapine (K_i values of 90.2 and 415 nM, respectively) and a certain potential to inhibit in vitro synaptosomal 5-HT uptake ($IC_{50} = 260$ nM). However, the inhibitory strength of **2** for 5-HT reuptake, although higher than that of compounds **1** and **3**, seems to be fairly low to take into account its possible antidepressive ability by this mechanism, proposed for some new atypical APDs.[6]

Simple behavioural tests revealed a possible atypical neuroleptic potential of the selected compound **2**. It did not exhibit cataleptogenic effect (0/6 animals after 1.0 or 10.0 mg/kg b.w. upon 30, 60, 90 or 180 min), while haloperidol induced catalepsy at 1.0 mg/kg in rats (4/5 animals after 30 min and 5/5 animals after 60, 90 and 180 min). Also, no significant changes in open field behaviour were observed in rats i.p. injected with compound **2** (1.0 or 10.0 mg/kg b.w.) when compared to saline/DMSO control (Fig. 1). An attenuation of AMPH-induced hyperlocomotion, a measure of antagonism of DA D_2 receptors located in nucleus accumbens,[16] in animals pretreated with compound **2**, suggested a dose–response relation (Fig. 1a). This inhibitory effect was slight after the lowest dose of **2** (1.0 mg/kg), moderate—following its middle dose (3.0 mg/kg), and the most prominent (up to 8-fold reduction of locomotion) after the highest dose of **2** applied, (10.0 mg/kg) throughout the 2 h period after AMPH injection. The reduction of AMPH-induced stereotypy by pretreatment with **2** was not so extensive and expressed exclusively after the highest dose of **2** (Fig. 1b), that is not completely in line with reported failure of clozapine to antagonize AMPH generated stereotypy.[17] In fact, AMPH induces a behavioural syndrome in mammals that includes a variety of repetitive behaviours, where some forms are either suppressed, augmented or without a change upon the action of atypical APDs.[18] The procedures of automated recordings, used to assess the stereotype response to AMPH treatment, reduce these variations and oversimplify the evidence of these changes. However, our unpublished data suggest that clozapine, given to rats at 10.0 mg/kg dose, may moderately reduce such AMPH-induced stereotypy score by similar pattern of compound **2** observed in the present study.

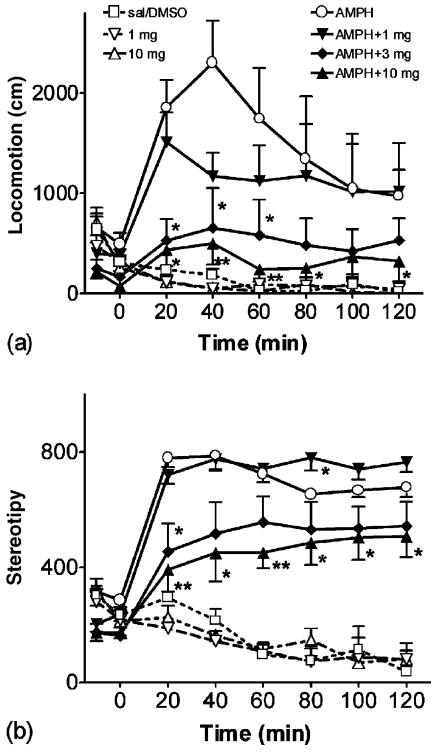


Figure 1. Effect of pretreatment with **2** (1–10 mg/kg b.w.) or saline/DMSO on the (a) locomotor activity (in cm) and (b) stereotypy score in rats treated with *d*-amphetamine (AMPH, 5 mg/kg b.w.) or saline. Each point represents mean ± SEM of 6–8 animals. Significance (Mann–Witney U-test): *, $p < 0.05$; **, $p < 0.01$, in comparison with the values of AMPH group.

In summary, this communication promotes the interaction of some previously synthetized arylpiperazines with a number of rat brain receptors. It seems that some ligands with benztriazole group may express the most favourable interactions with the specific DA-D_2, 5-HT_{1A} and 5-HT_{2A} receptors, regarding atypical antipsychotic potential from the view of the 'DA–5-HT hypothesis',[4,5] where compound **2**, 5-{2-[4-(2,3-dimethyl-phenyl)-piperazin-1-yl]ethyl}1H-benztriazole, exhibited the most suitable HT_{2A}/D_2 binding ratio, among compounds selected here. A proper affinity of **2** for the 5-HT_{1A} receptor, but also for 5-HT_{2C} and α_1 receptors, may be an additional factor strengthening the atypical neuroleptic potential of the compound, although there is a controversy about the role the latter receptors play in the antipsychotic action of clozapine-like drugs.[3] However, the nature of these interactions has not been elucidated yet, as, for example, particular arylpiperazines had been diversely reported to be agonists, partial agonists or antagonists at the 5-HT_{1A} receptor.[19] In vivo tests may suggest that the type of interactions anticipated for some other atypical APDs (partial agonism or antagonism at the D_2 receptors, strong 5-HT_{2A} receptor antagonism, partial 5-HT_{1A} receptor agonism[2,6–9]) is also appropriate for compound **2**, but this needs further clarification. Also, the selected compounds should be tested in the light of the loose binding concept for atypical APDs action[3b] and subjected to further preclinical tests.

Acknowledgements

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11. For experimental details on preparation of brain synaptosomes, in vitro receptor binding assays and synaptosomal 5-HT uptake, see Ref. 12. The only difference in this study was the use of 1.0 nM ^{3}H-ketanserin instead of ^{3}H-spiperone in 5-HT_{2A} receptor competition binding assays and 1.0 nM ^{3}H-prazosine in α_1-adrenergic receptor binding assays.
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14. Test for catalepsy: All behaviour experiments were done with adult Mill–Hill hooded male rats (b.w. 200–250 g). Catalepsy was scored by the horizontal bar test.[12] The animals (5–6 per group) were i.p. injected with saline/DMSO, compound **2** (1.0 or 10.0 mg/kg) or haloperidol (1.0 mg/kg). The forepaws were placed on a 10 cm high horizontal bar, while the hind paws remained on the floor. An amount of time spent when at least one forepaw on bar was determined. The animal was considered to be cataleptic if it remained on the bar for at least 60 s. The test was performed 30, 60, 90 and 180 min upon the treatment in three trials. Percentage of cataleptic animals was calculated.
15. Test on open field behaviour: Two series of four and three experimental groups (6–8 animals per group) were i.p. given *d*-AMPH sulfate (5.0 mg/kg) or physiological saline. Compound **2** was originally dissolved in DMSO (final conc. up to 2%), diluted with saline and i.p. injected 1.0, 3.0 mg (only AMPH group) or 10.0 mg in 2.0 mL volume per kg b.w., 20 min before the *d*-AMPH (or saline) application; control group received only saline/DMSO. Motor activity in an open field test and stereotypy score were monitored automatically in photo-beam activity cages with a Columbus Auto-Track System (Version 3.0 A, Columbus Institute, OH, USA), starting immediately after the first injection, ending 120 min upon the second treatment. The individual amounts of locomotion (in cm) and stereotypic activities were expressed at successive 10 min (before the second injection) or 20 min periods. All experiments were done on habituated animals (20 min before the first injection). Statistical analysis was performed by nonparametric Kruskal–Wallis test followed by Mann–Witney U-test (Statistica for Windows, 5.0, StatSoft).
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