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Studies of Antipsychotic drugs as potential schizophrenia agents

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Abstract

To study describe the actions of antipsychotics or neuroleptics on the behavioral effects elicited by ketamine on open-field, rota rod and tail suspension tests in mice. A series of novel analogs of 2-[3-{4-(3-chlorophenyl)-1-piperazinyl} propyl]-1,2,4-triazolo [4,3-a] pyridine-3- (2H)-one hydrochloride, a potential psychoactive drug of the piperazine and triazolopyridine chemical classes that has antidepressant, anxiolytic, and hypnotic properties were synthesized. Male swiss albino mice (25–30g) were used for the study and compounds were administered alone (0.1 or 0.2 mg/kg) or thirty minutes before ketamine (10 mg/Kg, ip). ketamine increased (63.3 ± 4.2) the locomotor activity compared to control, while neuroleptics decreased it (25.5 ± 4.2). Pretreatment with neuroleptics, in both doses, blocked hyperlocomotion caused by ketmine.

In rota rod test, ketamine decreased (Ketamine: 15 ± 4.1) the permanence time of the animals compared to control (Control: 59 ± 0.6), but this effect was not observed when antipsychotics were administered alone. Pretreatment with antipsychotics reverted the effect of ketamine only in the rota rod. While ketamine (17.3 ± 5.6) decreased the time of immobility in the tail suspension test compared to the control (80.2 ± 10.2), the pretreatment with neuroleptics reverted this mobility. The action of antipsychotics or neuroleptics tested have exhibited encouraging results in the behavioral model induced by ketamine in mice and can be further evaluated as potential candidates for treatment of schizophrenia

Keywords: Antipsychotic drug • Neuroleptic • Ketamine • Locomotor activity • Schizophrenia

Introduction

Schizophrenia is a heterogeneous syndrome with no pathognomonic features that commonly begins in late adolescence. The syndrome has a poor outcome and is present in 0.85% of individuals worldwide [1]. There are many theories attempting to explain the physiopathology of this illness, including the dopamine hypothesis. This hypothesis postulates that the dopaminergic hyperfunction is based on the following evidences: 1) psychotic symptoms presented by patients using drugs that induce dopamine release; 2) efficacy of typical antipsychotics in many patients [2] via action on dopamine D2-like receptors [3, 4]. However, the basis of the dopaminergic hypothesis has been questioned in some studies which demonstrated that a certain level (> 65%) of receptor blockade is necessary [5, 6], but not sufficient to cause clinical results[3]. Some atypical antipsychotics are efficient in the schizophrenia treatment, although they block a lower number of dopamine receptors (< 60%) [7, 8]. The glutamate model, however, became more accepted in the late 1980s [9]. Current researches have indicated that dysfunctions in the neurotransmission modulated by the excitatory amino acid glutamate may play a central role in the physiopathology of schizophrenia [10].

The glutamatergic system has several receptors that are activated by glutamate [11]. Among these, the N-methyl-D-aspartate (NMDA) receptors are especially important for the understanding of the illness[3]. Ketamine is one derivative of the phencyclidine hydrochloride (PCP) [12]. It is referred in literature as a dissociative anesthetic, since it induces strong sensory loss and analgesia, as well as amnesia and paralysis, without real loss of consciousness [13].

Ketamine, a competitive antagonist of NMDA receptor, induces behavioral effects in healthy humans that mimic positive, negative and cognitive schizophrenic symptoms [14-16]. Schizophrenic patients using ketamine present symptoms similar to that experienced during the active phase of the illness [17-19]. These data provide support for the hypothesis that reduced NMDA receptor function could contribute to the physiopathology of schizophrenia [20].

Antipsychotics or neuroleptics, drugs clinically used for the schizophrenia treatment, are categorized as dopaminergic antagonists, although many also act in other targets, particularly in the serotonin 5-HT2 receptors [21]. Vasconcelos [22] reported the importance of the establishment of animal models in order to study schizophrenia and possible development of new antipsychotic drugs. The purpose of this work is to understand the interaction between the dopaminergic and glutamatergic systems, analyzing the effects of series of antipsychotics of piperazine and triazolopyridine chemical classes in the behavioral model induced by ketamine in mice.

Materials and Methods

Chemistry

Our new target compounds, 2-[3-{4-(3-chlorophenyl)-1-piperazinyl} propyl]-1, 2, 4-triazolo [4, 3-a] pyridine-3- (2H)-one hydrochloride derivatives [6a-6z] listed in Table 1, were prepared using the process described in Figure 1.

Melting points (mp) were determined using a Thomas Hoover capillary apparatus and are uncorrected (Table 4). Infrared spectra were acquired on a Perkin Elmer FTIR (Table 5). Mass spectra were acquired with a Shimadzu Qp-2010 Mass spectrometer (Table 5). A Bruker, 300 MHz spectrophotometer was used to acquire 1H-NMR spectra; chloroform-d, DMSO-d6 and methanol-d4 were used as solvents (Table 6). Elemental analyses were carried out with a Perkin Elmer Model 240-C apparatus. The results of the elemental analyses (C, H, and N) were within ± 0.4% of the calculated amounts (Table 4). All chemicals and laboratory grade (LR) reagents were obtained from Rankem (India) and were used without further purification.

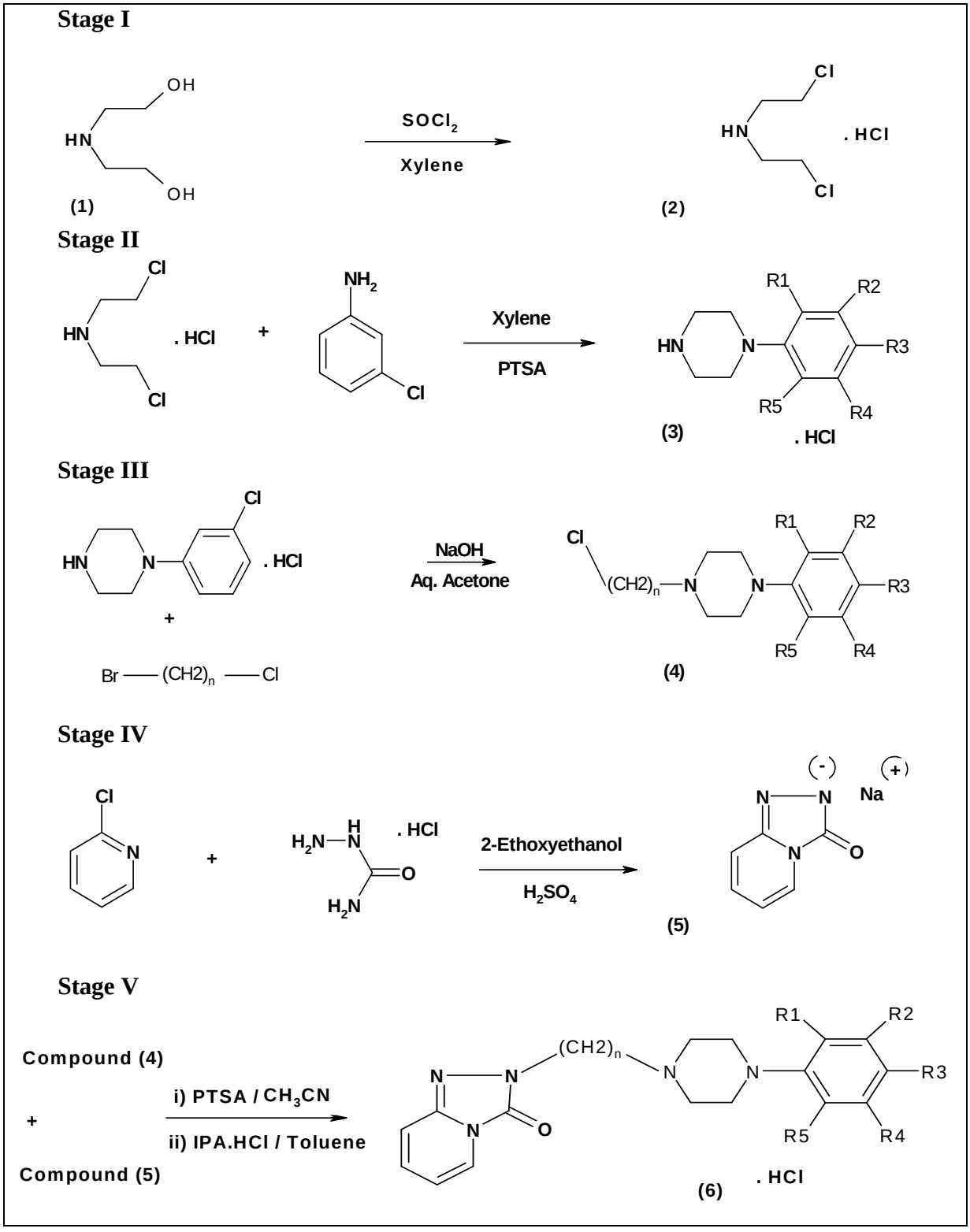
Table 1: Different derivatives of lead compound "6"

Different derivatives of the lead compound	R1	R2	R3	R4	R5	n
"6" Lead Compound	H	Cl	H	H	H	3
6A	Cl	Cl	H	H	H	3
6B	H	Cl	Cl	H	H	3
6C	H	Br	H	H	H	3
6D	H	H	F	H	H	3
6E	Cl	H	Cl	Cl	H	3
6F	CH_3	H	CH_3	H	H	3
6G	C_2H_5	H	H	H	H	3
6H	H	Cl	H	H	H	2
6I	H	Cl	H	H	H	4

Detailed synthetic process

General procedure for the preparation of bis-(2-chloroethylamine) hydrochloride [2].
To the mixture of diethanolamine (100 gm, 0.9523 mol), para toluenesulphonic acid (PTSA) (3 gm, 3%) and Chloroform (250 mL) was added thionyl chloride (104.7 gm, 1.42 mol) at 25-30^{o}C under stirring. After complete addition, the reaction mass is heated to 75-80^{o}C when a mild reflux was observed. The reaction continued for 2 hours to ensure completion and cooled to 25^{o}C when product crystallizes out of solution. The white crystalline product is isolated by filtration and dried under vacuum at 30^{o}C.
Product Yield: 94.10 gm, 94.1 %

Figure 1: Synthetic reaction scheme



General procedure for the preparation of 1-(3-chlorophenyl)-piperazine hydrochloride and its derivatives [3].

The mixture of bis-(2-chloroethylamine) hydrochloride [2] (100 gm, 0.56 mol), 3-chloro-aniline (78.54 gm, 0.61 mol), *para* toluenesulphonic acid (PTSA) (3 gm, 3%) in xylene (300 mL) was heated to reflux (140-145^{o}C) and progress of the reaction was monitored by TLC. On completion the reaction mass was cooled to 30^{o}C and further chilled to 0-5^{o}C when product crystallizes as off-white crystals. The product is isolated by filtration and washed with chilled xylene (5^{o}C, 75 mL) followed by acetone (5^{o}C, 75 mL) before drying in oven under reduced pressure (100 mm/Hg) at 40^{o}C for 8 hours.

Product Yield: 110 gm, 84.6 %

General procedure for preparation of 1-(3-chlorophenyl)-4-(3-chloropropyl) piperazine and its derivatives [4].

To the mixture of 1-(3-chlorophenyl)-piperazine hydrochloride [3] (100 gm, 0.43 mol) in acetone (300 mL) and water (500 mL) was added sodium hydroxide (46 gm, 1.15 mol) followed by 1-bromo-3- chloropropane (143.6 gm, 0.911 mol) under stirring at 25-30^{o}C. The reaction was further stirred for 15 hours at same temperature and progress was monitored by TLC. On completion the stirring was stopped and reaction mass was settled when two layers were obtained. The lower organic layer was separated and evaporated to isolate product as pale yellow oily product.

Product Yield: 85.0 gm, 72.6 %

General procedure for preparation of sodium salt of 1, 2, 4-triazolo [4, 3-a] pyridine-3-(2H)-one [5].

A mixture of 2-chloropyridine (100 gm, 0.88 mol) and semicarbazide hydrochloride (200 gm, 1.79 mol) in 2-ethoxyethanol (200 mL) was heated to 145-150^{o}C for 12 hours. Progress of the reaction was monitored by TLC. On completion the reaction mass was cooled to 60 ^{o}C and water (400 mL) was added. The solution further cooled to 0^{o}C and stirred for 0.5 hours. The precipitated product was isolated by filtration.

Product Yield: 112.0 gm, 94.3 %

The above solid was then dissolved in 30 % sodium hydroxide solution (100 mL) and warmed to 40^{o}C when a clear solution was obtained. The solution was then slowly cooled to 0^{o}C when product crystallizes as sodium salt and thick slurry was obtained. The sodium salt of the product was isolated by filtration and washed with chilled water (0^{o}C, 200 mL) prior to drying at 70^{o}C under reduced pressure (10 mm/Hg) for 12 hours.

Product Yield: 127.2 gm, 97.0 %

Stage V: General procedure for preparation of 2-[3-{4-(3-chlorophenyl)-1-piperazinyl} propyl]-1, 2, 4-triazolo [4, 3-a] pyridine-3- (2H)-one hydrochloride and its derivatives [6a-6z].

The mixture of 1-(3-chlorophenyl)-4-(3-chloropropyl) piperazine [4] (100 gm, 0.36 mol), 1, 2, 4-triazolo [4, 3-a] pyridine-3- (2H)-one [5] (66.1 gm, 1.15 mol) and para toluenesulphonic acid (PTSA) (3 gm, 3%) in acetonitrile (300 mL) was refluxed at 80-82^{o}C for 20 hours. Progress of the reaction was monitored by TLC to ensure formation of product and complete conversion of

starting 2-[3-{4-(3-chlorophenyl)-1-piperazinyl} propyl]-1. On completion the reaction mass was cooled to 50^{o}C and filtered. The acetonitrile was recovered by atmospheric distillation (~80 %) and toluene (300 mL) was added to residual reaction mass when a clear solution was obtained. The toluene solution was further washed twice with 20% sodium hydroxide solution (2x 50 mL) followed by 2% brine solution (2x 50 mL) at 50^{o}C.

To the toluene solution containing product as base, was added IPA HCl solution (15%, 80 mL) and pH adjusted between 2-2.5 when salt starts precipitating. The precipitated hydrochloride salt of target molecule was isolated by filtration and recrystallised from methanol (200 mL) to achieve white crystalline compound.
Product Yield: 126.0 gm, 85.0 %

Pharmacology

Animals
The experiments were carried out on male Swiss albino mice (*Mus musculus)* (25–30 g). They were maintained at a controlled temperature (23 ± 1 °C) with a 12h dark/light cycle and free access to water and food. All the animal experimental procedures were performed in accordance with the opinion of Institutional Ethics Committee designed for the purpose.

Drugs and treatment
Ketamine hydrochloride (50 mg/mL, ampoules) were used. Ketamine and all the drug analogs under test (6 - 6I) were dissolved in distilled water and administered intraperitoneally (ip) in volumes of 10 mL/Kg body weight. All the compounds (0.1 mg/Kg or 0.2 mg/Kg) were administered alone or thirty minutes before ketamine (10 mg/Kg). Control animals received distilled water in the same period.

Procedure
Animals were tested during the light period and observed in a closed room, poorly illuminated, at a constant temperature of 25 ± 1 ºC. Immediately after treatment with ketamine or water, the tests were performed. First, animals were placed in the open field arena where the locomotor activities, such as number of grooming, rearing and stereotyped activity (repetitive movements) were measured. Subsequently, the same animals were placed on rota rod and on the tail suspension device straight afterward.

Open-field test (OF)
The OF area was made of acrylic (transparent walls and black floor, 30 cm x 30 cm x 20 cm) divided into nine squares of equal area. The OF was used to evaluate the animals exploratory activity [23]. The observed parameters were: number of squares crossed (with the four paws) during three minutes after one minute for acclimatization (locomotor activity) and number of grooming and rearing. In this apparatus, behavioral changes, such as stereotyped behaviors (striking or preservative behaviors), walking in circles and ataxia were also observed and recorded.

Rota rod (RR)
The method of Dunham and Miya [24] was used on rota rod test. Animals were placed with the paws on a 2, 5 cm diameter bar, 25 cm above the floor, which rotates 12 times per minute. The number of falls (up to three falls) and the time of permanence on the bar for one minute were registered.

Tail suspension test (TS)
For the tail suspension test, the method described by Porsolt et al. [25] was used. Mice were suspended by tail on the edge of a shelf 58 cm above a table top by adhesive tape placed approximately 1 cm from the tip of the tail. The duration of immobility was recorded during a period of five minutes.

Statistical analyses
All analyses were performed using one-way analysis of variance (ANOVA), at Prism 3.0 software. For significant results, multiple comparisons were made using Tukey as the post hoc test. Results were considered significant at $p < 0.05$, and presented as mean ± E.P.M.

Results

Chemistry
As stated earlier the target compounds, 2-[3-{4-(3-chlorophenyl)-1-piperazinyl} propyl]-1, 2, 4-triazolo [4, 3-a] pyridine-3- (2H)-one hydrochloride derivatives (6A-6I) as listed in Table 1, were prepared using the process described in Figure 1. To examine structure-activity relationships on the nucleus portion in the 2-[3-{4-(3-chlorophenyl)-1-piperazinyl} propyl]-1, 2, 4-triazolo [4,3-a] pyridine-3- (2H)-one derivatives, respective analogs of process intermediate 1-(3-chlorophenyl)-piperazine hydrochloride and further 1-(3-chlorophenyl)-4-(3-chloropropyl) piperazine were prepared by employing similar process and then reacted with 1, 2, 4-triazolo [4, 3-a] pyridine-3- (2H)-one to achieve novel derivatives of the lead compound desired for the study.

In first part of the synthetic process bis-(2-chloroethylamine) hydrochloride is prepared by chlorination of diethanolamine with thionyl chloride in xylene, which is then condensed with various substituted anilines to get different derivatives of 1-(3-chlorophenyl)-piperazine hydrochloride intermediate.

Alkylation of these using 1-bromo-3-chloropropane in alkaline aqueous acetone (50 %) gave various analogs of 1-(3-chlorophenyl)-4-(3-chloropropyl) piperazine intermediate. Using similar process, compounds 4H and 4I were prepared by alkylation using dibromoethane and dibromobutane respectively where replacement of propyl linker with ethyl and butyl linker is achieved. In second part of the process these various derivatives of 1-(3-chlorophenyl)-4-(3-chloropropyl) piperazine intermediate were condensed with sodium salt of 1, 2, 4-triazolo [4, 3-a] pyridine-3- (2H)-one prepared in a single step process by simple reaction of 2-chloropyridine and semicarbazide hydrochloride in 2-ethoxyethanol afforded structurally diverse target compounds 6A-6I.

Table 2: Physical data of Synthesized Compound (6A-6I)

Product	Mol. Formula	Molecular Weight	M.P ^{0}C
"6"	$C_{19}H_{22}ClN_5O$	372	223
6A	$C_{19}H_{21}Cl_2N_50$	406	>250
6B	$C_{19}H_{21}Cl_2N_50$	406	>250
6C	$C_{19}H_{22}BrN_5O$	415	211-213
6D	$C_{19}H_{22}FN_5O$	356	240-242
6E	$C_{19}H_{20}Cl_3N_5O$	440.5	>250
6F	$C_{21}H_{27}N_5O$	366	230-232
6G	$C_{21}H_{27}N_50$	366	204-206
6H	$C_{18}H_{20}ClN_5O$	357.5	218-220
6I	$C_{20}H_{24}Cl_2N_50$	386	227-230

Table 3: MS and IR spectral data of Synthesized Compound (6A-6I)

Product	MS (m/z)	IR (cm^{-1})
"6"	372	3000 (aromatic C-H stretching), 2954 (aliphatic C-H stretching), 1704 (C=O stretching), 1650 (C=N stretching), 1600 (aromatic C=C stretching), 1350.80 (C=N stretching), 750 (C-Cl stretching)
6A	406	3000 (aromatic C-H stretching), 2850 (aliphatic C-H stretching), 1700 (C=O stretching), 1650 (C-N stretching), 1600 (aromatic C-H stretching), 1350 (C-N stretching), 750 (C-Cl stretching)
6B	406.3	3050,3100 (aromatic C-H stretching), 2862,2947(aliphatic C-H stretching), 1704.96 (C=O stretching), 1643.24 (C-N stretching), 1635.23 (aromatic C-H stretching), 1350.08 (C-N stretching), 750 (C-Cl stretching)
6C	415	3000 (aromatic C-H stretching), 2870 (aliphatic C-H stretching), 1710 (C=O stretching), 1635 (C=N stretching), 1610 (aromatic C=C stretching), 1350 (C=N stretching), 575 (C-Br stretching)
6D	356	3050 (aromatic C-H stretching), 2850,2950 (aliphatic C-H stretching), 1720 (C=0 stretching), 1650 (C=N stretching), 1500 (aromatic C=C stretching), 1325 (C-N stretching), 1164.92 (C-F stretching)

6E	441	3010 (aromatic C-H stretching), 2875 (aliphatic C-H stretching), 1715 (C=O stretching), 1650 (C-N stretching), 1550 (aromatic C-H stretching), 1350.45 (C-N stretching), 775 (C-Cl stretching)
6F	366	3000 (aromatic C-H stretching), 2947.03 (aliphatic C-H stretching), 1710 (C=O stretching), 1650 (C=N stretching), 1500 (aromatic C=C stretching), 1350 (C-N stretching)
6G	366.53	3050 (aromatic C-H stretching), 2850 (aliphatic C-H stretching), 1700 (C=O stretching), 1650 (C=N stretching), 1600 (aromatic C=C stretching), 1347 (C-N stretching)
6H	358	3050 (aromatic C-H stretching), 2862 (aliphatic C-H stretching), 1710 (C=O stretching), 1650 (C-N stretching), 1500 (aromatic C-H stretching), 1350 (C-N stretching), 750 (C-Cl stretching)
6I	386	3000 (aromatic C-H stretching), 2850 (aliphatic C-H stretching), 1700 (C=O stretching), 1650 (C-N stretching), 1600 (aromatic C-H stretching), 1350 (C-N stretching), 750 (C-Cl stretching)

Table 4: ^{1}H NMR spectral data of Synthesized Compound (6A-6I)

Product	**^{1}H NMR (ð)**
"6"	ð 2.16-2.12 ppm (t, 2H, N-CH_2-CH_2-CH_2-N), 2.64-2.60 (t, 2H,-N CH_2), 2.73 (s, 4H, -CH_2-N-CH_2), 3.09 (s, 4H, CH_2-N-CH_2), 4.12-4.07 (t, 2H,-CH_2-N), 6.51-6.46 (m,1H,-ArH), 7.02-6.93 (m, 2H,-ArH), 7.09-7.08 (d, 2H,-ArH), 7.26-7.17 (m, 1H,-ArH), 7.34-7.31 (d, 1H,-ArH) 7.76-7.74 (d, 1H,-ArH)
6A	ð 2.50-2.49 (m, 2H, -CH_2), 3.21-3.15 (m, 8H, CH_2- piperazine), 3.60 (t, 2H -CH_2), 4.01(t, 2H, -CH_2) ,6.65-6.63 (m,1H, -Ar H), 7.25-7.21(m, 3H, -Ar H), 7.36-7.34 (t, 2H, -Ar H), 7.88-7.86 (d,1H, -Ar H)
6B	ð 2.26-2.19 (m, 2H, CH_2) , 3.23-3.01(m, 6H, CH_2- piperazine), 3.40-3.50 (d, 2H, CH_2-piperazine), 3.90-3.86 (d, 2H, CH_2), 4.01-3.97 (t, 2H, CH_2), 6.67-6.60 (m,1H,Ar H), 7.01-6.97 (m, 1H, -Ar H), 7.21-7.20 (m, 3H, -Ar H), 7.40 (d,1H, -Ar H), 7.88-7.859 (d,1H, -Ar H)
6C	ð 2.28-2.26 (t, 2H, CH_2), 3.09 (t, 2H, CH_2-piperazine), 3.23-3.20 (d, 4H, CH_2-piperazine), 3.53-3.51 (t,2H,CH_2-Piperazine), 3.87-3,84 (d, 2H, CH_2), 4.02-3.98 (t, 2H, CH_2), 6.64-6.63 (t,1H, -ArH), 6.86-6.84 (m,1H, -Ar H), 6.96-6.95 (d,1H, -Ar H), 7.04-7.03 (t,1H, -Ar H), 7.26-7.22 (m, 3H, -Ar H), 7.87-7.85 (d,1H, -Ar H)
6D	ð 2.31-2.24 (m, 2H, CH_2), 3.20-3.12 (m, 6H, CH_2-piperazine), 3.56-3.53 (d, 2H, CH_2-piperazine), 3.72-3.69 (d, 2H, CH_2), 4.03-3.99 (t, 2H, CH_2), 6.66-6.61(m,1H, -Ar H), 7.13-7.00 (m, 4H, -Ar H), 7.25-7.24 (d, 2H, -Ar H), 7.88-7.86 (d,1H, -Ar H)
6E	ð 1.94-1.88 (m, 2H, CH_2), 2.38-2.28 (m, 2H, CH_2), 2.44-2.40 (s, 4H, CH_2-piperazine), 3.07-3.04 (t, 4H, CH_2-piperazine), 3.97-3.93 (t, 2H, CH_2), 6.61-6.65 (m,1H, -Ar H), 6.90-6.97 (m, 2H, -Ar H), 7.12-7.09 (s,1H, -Ar H), 7.25-7.15

	(m,1H, -Ar H), 7.84-7.82 (d,1H, -Ar H)
6F	ð 1.92-1.87 (t, 2H, CH_2), 2.09-2.03 (d, 6H, -Ar-CH_3), 2.50-2.36 (m, 6H, CH_2-piperazine), 2.66 (s, 4H, CH_2-piperazine), 3.98-3.94 (t, 2H, CH_2), 6.62-6.58 (t, 1H, -Ar H), 6.82-6.80 (d,1H, -Ar H), 6.93-6.90 (d, 2H, -Ar H), 7.25-7.10 (m, 2H, -Ar H), 7.86-7.84 (d,1H, -Ar H)
6G	ð 1.16-1.13 (t, 3H, CH_3), 1.93-1.89 (m, 2H, CH_2), 2.29 (t, 2H, CH_2), 2.99-2.77 (m, 6H, CH_2, CH_2-piperazine), 3.17 (t, 2H, CH_2), 4.12-3.93 (m, 4H, CH2-piperazine), 5.20 (m, 1H, -ArH), 5.77 (m, 1H, -ArH), 6.59-6.57 (m, 2H, ArH), 6.8 (d,1H, -ArH), 7.07 (d,1H, -ArH), 7.27-7.17 (d, 2H, -ArH)
6H	ð 3.23-3.01 (m, 6H, CH_2- piperazine), 3.50-3.40 (d, 2H, CH_2-piperazine), 3.90-3.86 (d, 2H, CH_2), 4.01-3.97 (t, 2H, CH_2), 6.67-6.60 (m, 2, -Ar H), 7.01-6.97 (m, 1, ArH), 7.21-7.20 (m, 3H, -Ar H), 7.40 (d,1H, -Ar H,), 7.88-7.85 (d,1H, -Ar H)
6I	1.20 (m, 2H, CH_2), 1.40 (m, 2H, CH_2), 2.15 (t, 2H, CH_2), 2,75 (m, 4H, CH_2-piperazine), 3.10 (t, 2H, CH_2), 4.10 (m, 4H, CH_2-piperazine), 6.10 (d, 1H, ArH), 6.76 (t, 1H, ArH), 7.10 (d, 1H, ArH), 7.75 (t, 1H, ArH), 8.15 (s, 1H, ArH), 8.30 (d, 1H, ArH), 8.45 (t, 1H, ArH), 9.11 (d, 1H, ArH)

Pharmacology

Table 5: Effects of antipsychotic drugs and ketamine on the open-field test in mice

Group	Locomoter activity	Rearing	Grooming
Control	25.5 ± 4.2 (10)	13.45 ± 1.6 (11)	3.3 ± 0.3 (10)
Ketamine 10	63.3 ± 4.2 (10)	1.1 ± 0.7 (10)	0.2 ± 0.1(10)
6	7.38 ± 1.5 (13)	5.1 ± 1.4 (11)	2.3 ± 0.4 (11)
6A	5.9 ± 2.8 (12)	0.4 ± 0.3 (12)	0.6 ± 0.25 (12)
6B	3.5 ± 1.2 (12)	0.7 ± 0.2 (12)	0.6 ± 0.1 (12)
6C	2.2 ± 0.8 (10)	0.25 ± 0.2 (12)	0.08 ± 0.08 (12)
6D	11.4 ± 4.1(10)	0.7 ± 0.4 (9)	0.6 ± 0.2 (10)
6E	29.9 ± 6.3 (9)	0.0 ± 0 (10)	0.4 ± 0.2 (10)
6F	4.2 ± 2 (10)	1.6 ± 0.6 (10)	1.2 ± 0.3 (9)
6G	2.2 ± 0.8 (10)	0 ± 0 (10)	0.08 ± 0.08 (12)
6H	2.2 ± 0.9 (10)	0 ± 0 (10)	1.2 ± 0.08 (12)
6I	15.2 ± 0.8 (9)	3.5 ± 0.4 (9)	0.7 ± 0.2 (10)

Values are reported as means ± e.p.m. for the number of mice shown in parentheses.
Analysis of variance and Tukey as the post-hoc test.

Open-field test

The number of crossing was increased by ketamine (10mg/Kg: 63.3 ± 4.2) and significantly decreased by test compounds "6" (0.1 mg/Kg: 7.38 ± 1.5), "6B" (0.1 mg/Kg: 3.5 ± 1.2), "6D" (0.1 mg/Kg: 11.4 ± 4.1), "6F" (0.1 mg/Kg: 4.2 ± 2) and "6H" (0.1 mg/Kg: 2.2 ± 0.9) when compared to control (25.5 ± 4.2). The hyper motility (seen using the same parameter) induced by ketamine was significantly decreased by the test compounds at all doses of pretreatment ["6A" 0.1 mg/Kg: 5.9 ± 2.8; '6C" 0.1 mg/Kg: 2.2 ± 0.8; '6E" 0.1 mg/Kg: 29.9 ± 6.3; "6G" 0.2 mg/Kg: 2.2 ± 0.8; "6I" 0.1 mg/Kg: 15.2 ± 0.8]. However, animals treated with these compounds (0.1 mg/Kg) before injection of Ket reduced their motility to control levels [$F(9,105) = 39.09$; $p < 0001$], as shown in Table 5. Ketamine (Ket: 1.1 ± 0.7) as well as the test compounds "6" (0.1 mg/Kg: 5.1 ± 1.4), "6B" (0.1 mg/Kg: 0.7 ± 0.2), "6D" (0.1 mg/Kg: 0.7 ± 0.4), "6F" (0.1 mg/Kg: 1.6 ± 0.6) and "6H" (0.1 mg/Kg: 0 ± 0) decreased the number of rearing compared to control (13.45 ± 1.6) [$F(9,106)= 30.69$; $p < 0001$]. All drugs decreased the number of grooming compared to control (3.3 ± 0.3), except haloperidol (2.3 ± 0.4) at the 0.1 mg/Kg dose [$F(9,107) = 19.26$; $p < 0001$] (Table 1).

Rota rod

At the Rota rod test, ketamine (Ket: 15.06 ± 4.1) significantly decreased the time of animals permanence on the bar compared to control (59.01 ± 0.6) (Table 6). The pretreatment with test compounds alone induced no changes. However, animals that received ketamine after have being treated with test compounds at 0.2 mg (Test Compound 0.2 mg/Kg + Ket: 41 ± 5.5) increased the time of permanence on the bar compared to the Ketamine alone group [$F(9,110) = 9.101$; $p < 0001$]. Ketamine (Ket: 2.9 ± 0.1) increased the number of falls (Table 2) compared to control (control: 0.14 ± 0.01), and this effect was not changed by the test compounds pretreatment [$F(9,120) = 12.15$; $p < 0001$].

Tail suspension test (TS)

In TS, ketamine (Ket: 17.3 ± 5.6) significantly decreased the time of immobility in mice compared to the control group (80.2 ± 10.2). However, the pretreatment of animals with neuroleptics (risperidone e haloperidol) blocked the effect of ketamine. Haloperidol at the highest dose (Hal 0.2 mg/Kg: 176.1 ±8) and risperidone in the two doses (Risp 0.1 mg/Kg: 189.7 ±19; Risp 0.2 mg/Kg: 175.2 ± 12) significantly increased the time of immobility when compared to control [$F(9,123) = 19.96$; $p < 0001$].

Table: 6. Effects of antipsichotics drugs and ketamine on the Rota rod test in mice.

Group	Time of permanence (s)		N° falls
	Pretreatment with test compounds alone	Receipt of ketamine after treating with test compounds	
Control	59.01 ± 0.6 (10)		0.14 ± 0.01 (14)
Ketamine	15.06 ± 4.1 (10)a		2.9 ± 0.1(10)
6	55.06 ± 1.3 (14)	35.06 ± 1.3 (14)	0.86 ± 0.2 (14)

6A	50.03 ± 5.6 (14)	28.03 ± 5.2 (14)	2.6 ± 0.2 (20)
6B	49 ± 2.7 (12)	26.09 ± 3.7 (12)	1.6 ± 0.3 (12)
6C	51 ± 3.5 (12)	41 ± 5.5 (12)	2.2 ± 0.3 (12)
6D	54 ± 1.8 (09)	34 ± 1.8 (09)	0.7 ± 0.2 (09)
6E	48.8 ± 5.4 (10)	22.8 ± 6.3 (10)	1.6 ± 0.5 (10)
6F	51.1 ± 3.3 (10)	37.1 ± 4.3 (10)	1.7 ± 0.4 (10)
6G	52.2 ± 6.4 (10)	32.2 ± 6.4 (10)	2.6 ± 0.2 (10)
6H	47.2 ± 6.4 (10)	22.2 ± 5.3 (10)	2.6 ± 0.2 (10)
6I	50.2 ± 4.1 (10)	21.2 ± 2.5 (10)	2.7 ± 0.6 (10)

Values are reported as means ± e.p.m. for the number of mice shown in parentheses.
Analysis of variance and Tukey as the post-hoc test.

Discussion

Pharmacological experiments have demonstrated that sub anesthetic doses of ketamine induce schizophrenia-like symptoms in humans [18] as well as behavioral activation in experimental animals [26]. The exact mechanism of this functional activation remains unknown. Duncan et al [27] suggested that relatively low doses of this drug produce several excitatory effects after systemic administration, and that these effects might result either from disinhibitory actions (e.g.: reduced activity of inhibitory neurons), or from disruption of the negative feedback regulation of excitatory amino acid-secreting neurons. This hypothesis can explain some studies which have revealed a lower density of glutamatergic receptors in brains of schizophrenic patients [28, 29]. In accord with this, latter finding [30] showed decreased glutamate binding in frontal cortex of subchronically ketamine-treated rats and suggested the use of this animal model for the study of this disease.

Similar to Yamamoto et al. [31], in this paper, we demonstrated that ketamine, acutely administered at low doses in mice, induces hyperactivity. It is well known that dopaminergic mechanisms play important role in the mediation of the locomotor activity, and ketamine may influence dopamine transmission and receptor activation via multiple mechanisms [32].
Regarding to neuroleptic test compounds, a decrease on locomotor activity showed an acute depressant effect of this class of drugs (Table 1). Among the pretreated groups, compound “6E” showed the most satisfactory results, reversing the locomotor hyperactivity to similar levels of the control group. The hypomotility caused by neuroleptics may result from a reduced excitability of the central nervous system or sedation [33]. Many antipsychotic drugs, including agents of low potency, present prominent sedative effects. This is particularly conspicuous early in treatment, although some typical tolerance can be developed [21].

An antipsychotic drug that presents antagonist properties on dopamine D2, serotonin 5HT2 and α1 adrenoreceptors, has been the focus of several clinical studies [34, 35]. The study carried out

by Su et al. [36], using MK-801 (ketamine-like NMDA antagonist), showed that they have an inhibitory effect on MK-801-induced hyperactivity in mice, at doses in which it caused no alteration in spontaneous activity when administered alone. This study also suggested that the inhibitory effect was mostly caused by the blockage of serotonin 5-HT2A receptors and secondarily by the attenuation of dopamine D2 and α1 adrenoreceptors.

In respect to rearing and grooming (Table 1), the animals treated with ketamine and test compounds, except for compound "6" (grooming), showed decreased responses in comparison to controls. In mice pretreated with neuroleptics, the depressant effect seen in the locomotor activity test could have been possibly caused by sedation. Curiously, in Hal 0.1 mg group, the number of grooming did not differ from control.

Results from the RR test showed that animals which received ketamine presented a decrease in the time of permanence on the bar and an increase in the number of fall (Table 2), possibly due to the lack of motor coordination presented by this group. The test compounds '6" increased the time of animal permanence in the bar, reflecting improvement in the motor coordination, however not sufficient to reduce the ketamine-increased number of falls. These data led us to suggest that such effect could be the result of an acute blockade of dopamine D2 receptors in the striatum [37, 38]. The time of permanence on the bar of the groups treated exclusively with neuroleptics was not different from the controls, suggesting that the sedative effect of these drugs could contribute to the increased number of falls particularly at the higher doses, thus not being able to reverse the ketamine-increased number of falls in this test.

Conclusion

The results obtained in this work showed that antipsychotics or neuroleptic test compounds, under the mentioned experimental conditions, attenuated the increase of locomotor activity and stereotyped behavior, reversed the motor incoordination and blocked the hypermobility induced by acute administration of ketamine. The present results suggest that the ketamine mechanism of action may involve the dopaminergic system. Based on the study it is concluded that the test compounds have exhibited encouraging results in the behavioral model induced by ketamine in mice and can be further evaluated as potential candidates for treatment of schizophrenia.

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