SYNTHESIS AND PHARMACOLOGY OF NOVEL ANTIDEPRESSANT AGENTS WITH DOPAMINE AUTORECEPTOR AGONIST PROPERTIES

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ABSTRACT

To develop a novel antipsychotic agent which is an agonist of dopamine (DA) autoreceptors and an antagonist of postsynaptic DA receptors, a series of 2-[3-{4-(3-chlorophenyl)-1-piperazinyl} propyl]-1,2,4-triazolo [4,3-a] pyridine-3- (2H)-one hydrochloride was synthesized and their dual activities were examined. The reaction between 1, 2, 4-triazolo [4, 3-a] pyridine-3- (2H)-one and 1-(substituted-phenyl)-4-(3-halo-aryl) piperazine obtained from reaction of 1-(substituted-phenyl)-piperazine hydrochloride and dihalo-alkanes in two step process gave novel analogs of 2-[3-{4-(3-chlorophenyl)-1-piperazinyl} propyl]-1,2,4-triazolo [4,3-a] pyridine-3- (2H)-one hydrochloride has been achieved. These structures were established by IR, Mass and ^{1}HNMR spectral data. The postsynaptic DA receptor antagonistic activities of the compounds were evaluated by their ability to inhibit stereotypy induced by apomorphine in mice, and the autoreceptor agonist activities were determined by their effects on the ç-butyrolactone (GBL) – induced increase in L-dihydroxyphenylalanine (DOPA) synthesis in the mouse brain. Many compounds inhibited the stereotypic behavior, and several compounds reversed the GBL induced increase in the DOPA synthesis.

Keywords: Dopamine (DA) autoreceptors, Postsynaptic DA receptors, Autoreceptor agonist, Catalepsy, α_1 –adrenoceptor antagonist activity.

INTRODUCTION

According to the "dopamine hypothesis of schizophrenia", a functional hyperactivity of the dopamine (DA) neuronal systems of the brain is involved as a major aspect of the disease [1]. All clinically available antipsychotic agents inhibit DA neurotransmission by blocking the postsynaptic DA receptors. Unfortunately, DA antagonism is also responsible for the most serious side effects of these agents, e.g., extrapyramidal syndrome (EPS), a parkinsonian-like syndrome caused by DA receptor blocking, tardive dyskinesia (TD), a syndrome of involuntary movements that has been linked to supersensitivity of brain DA receptors after long-term DA receptor blockage, and hyperprolactinemia, which is caused by blocking the pituitary DA receptors [2, 3]. Reducing dopaminergic neurotransmission via stimulation of the DA autoreceptors has become of interest in the search for effective therapeutic agents that lack the side effects of available antipsychotic agents. The hypothesis underlying this approach stems from evidence that DA autoreceptors serve as an inhibitory feedback function of neurotransmission [4-8]. The clinical results with other DA autoreceptor agonists strongly suggest that a DA autoreceptor agonist is effective in treating the negative symptoms and a potent DA postsynaptic receptor antagonism might be necessary for treatment of the positive symptoms in patients [9-11]. To find a more effective agent for treating both negative and positive symptoms in schizophrenia and with fewer side effects than the standard agents, we have searched for a compound which is an agonist of the DA autoreceptors and a potent antagonist of the postsynaptic DA receptors. An ideal compound would be a potent and effective agent for treatment of both the positive and negative symptoms of schizophrenia with less adverse effects than clinically available agents. We have synthesized a series of new compounds with a variety of modifications of lead compound "6" and examined the postsynaptic DA receptor antagonist activity of all compounds synthesized by evaluation of their ability to antagonize the DA agonist apomorphine (APO) in the stereotypy test[12]. Selected compounds which showed a potent postsynaptic DA receptor antagonist activity were evaluated for their DA autoreceptor agonist activity by testing their reversing effects on the ç-butyrolactone (GBL) - induced increase in L-dihydroxyphenylalanine (DOPA) synthesis in the mouse brain [13, 14]. In this paper, we describe the synthesis and the preliminary pharmacology of 2-[3-{4-(3-chlorophenyl)-1-piperazinyl} propyl]-1, 2, 4-triazolo [4, 3-a] pyridine-3- (2H)-one hydrochloride derivatives. Structure-activity relationships (SAR) are also discussed.

MATERIALS AND METHODS

Chemistry

The synthetic process followed for the preparation of novel analogs of the lead compound is described below. All the compounds were characterized by physical data and structures were established by using spectroscopy techniques. Melting points (M. P.) of the compounds were determined using a Thomas Hoover capillary apparatus and are uncorrected (Table 2). Infrared spectral data was acquired on a Perkin Elmer FTIR (Table 3). Mass spectra were acquired with a Shimadzu Qp-2010 Mass spectrometer (Table 3). A Bruker, 300 MHz spectrophotometer was used to acquire 1H-NMR spectra; chloroform-d, DMSO-d6 and methanol-d4 were used as solvents (Table 4).

All chemicals and laboratory grade (LR) reagents were obtained from Rankem (India) and were used without further purification.

Detailed synthetic process

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] as listed in Table 1, were prepared following the process presented in Scheme 1.

Standard process for the preparation of bis-(2-chloroethylamine) hydrochloride [2]

To the mixture of diethanolamine (100 gm, 0.9523 mol), *p*- toluenesulphonic acid (3 gm, 3%) and Chloroform (250 mL) was added thionyl chloride (104.7 gm, 1.42 mol) at 25-30°C under stirring. After complete addition, the reaction mass is heated to 75-80°C when a mild reflux was observed. The reaction continued for 2 hours to ensure completion and cooled to 25°C when product crystallizes out of solution. The white crystalline product is isolated by filtration and dried under vacuum at 30°C.

Product Yield: 94.10 gm, 94.1 %

Scheme I: Reaction scheme for the preparation of lead compound and its analogs

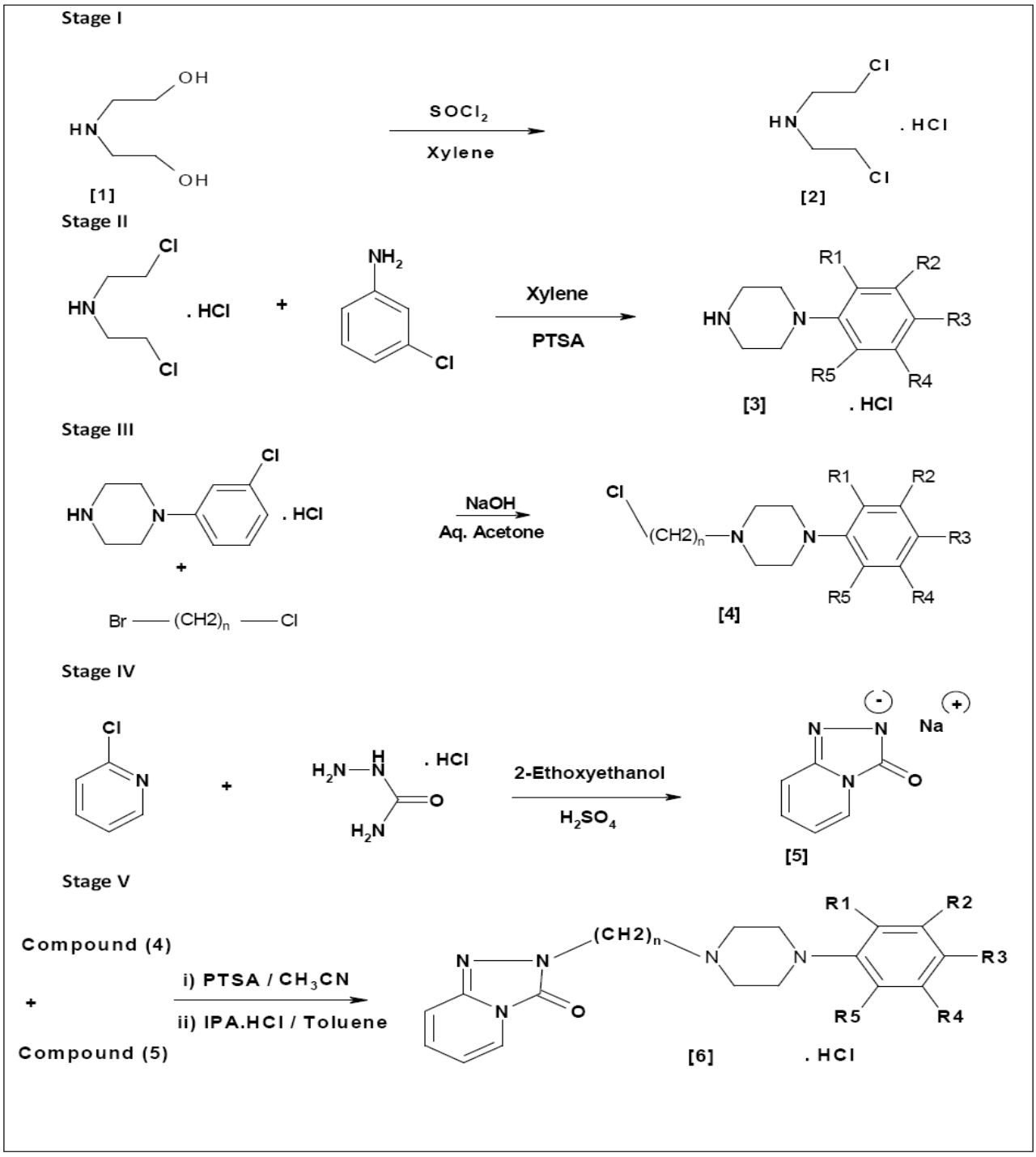

Table 1: Derivatives of lead compound (6a-6z) synthesized for study

Derivatives of the lead compound	R1	R2	R3	R4	R5	n
"6" Lead Compound	H	Cl	H	H	H	3
6a	Cl	H	H	H	H	3
6b	H	H	Cl	H	H	3
6c	Cl	Cl	H	H	H	3
6d	H	Cl	Cl	H	H	3
6e	H	Br	H	H	H	3
6f	H	Br	Br	H	H	3
6g	H	F	H	H	H	3
6h	H	H	F	H	H	3
6i	H	Cl	F	H	H	3
6j	Cl	H	Cl	Cl	H	3
6k	F	H	Br	H	F	3
6l	CH_3	CH_3	H	H	H	3
6m	CH_3	H	CH_3	H	H	3
6n	CH_3	H	H	CH_3	H	3
6o	CH_3	H	H	H	CH_3	3
6p	H	CH_3	CH_3	H	H	3
6q	C_2H_5	H	H	H	H	3
6r	C_2H_5	H	H	H	C_2H_5	3
6s	H	H	H	H	H	3
6t	H	Cl	H	H	H	2
6u	H	H	Cl	Cl	H	2
6v	F	H	Br	H	F	2
6w	CH_3	H	H	CH_3	H	2
6x	H	Cl	H	H	H	4
6y	Cl	Cl	H	H	H	4
6z	1-naphthyl		H	H	H	3

Standard process 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), *p*- toluenesulphonic acid (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) for removal of aniline traces before drying in oven under reduced pressure (100 mm/Hg) at 40^{o}C for 8 hours.

Product Yield: 110 gm, 84.6 %

Standard process 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- chloro-propane (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 under reduced pressure to isolate product as pale yellow oily product.

Product Yield: 85.0 gm, 72.6 %

Standard process 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 %

Standard process 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

Research involving investigations' using experimental animals was carried out by adhering to the "Principles of laboratory animal care" was carried out on approval of Institutional Animal Ethics Committee constituted for the purpose.

Male ICR mice weighing 20-30 g. and male Wistar rats weighing 148-250 g. were used. The test compounds were suspended in 0.5 % gum Arabic-0.9 % saline, Trazodone (serenace, NPIL), GBL (sigma), chlorpromazine (contomin, NPIL), and 3-hydroxybenzylhydrazine 2HCl (NSD-1015, Nakarai) were diluted with0.9 % saline, APO HCl (Sigma) was dissolved in 0.9 % saline.

Inhibition of APO-induced Stereotypy of Behavior (Anti-APO test)

Mice and Rats were fasted overnight (16-20 h.). Test compounds were orally administered to groups of 10 mice or 06 rats, 1 h. before APO (1.5 mg/Kg sc) injection. Stereotypy of behavior was observed for 1 min. at 10-min. intervals for 40 min. starting 20 min. after APO injection and scored according to the method reported [12]. The ED_{50} values and 95 % confidence limits were calculated using the linear regression analysis method, and the values are presented as m mol/Kg po in Table 5.

Inhibition of GBL-induced Increase in DOPA Synthesis

Mice and Rats were fasted overnight (16-20 h.). Test compounds were orally administered 1 h. before sacrifice. GBL (750 mg./Kg. ip) and NSD-1015 (100 mg/Kg. ip) were given to animals 35 and 30 min before sacrifice, respectively, according to the method reported [13]. DOPA was determined according to the literature method [11, 15]. A Chemocosorb 5-ODS (20-x4.6-mm i.d.) separation column was used. The mobile phase contained 50 mM KH_2PO_4, 8 Mm H_3PO_4, and 2.5 mM EDTA.Na in 0.7 % acetonitrile (pH 3). The ED_{50} values and 95 % confidence limits were calculated using the linear regression analysis method, and the values are presented as m mol/Kg po in Table 5.

Catalepsy Test

The Test compounds and reference drugs were orally administered to groups of 10 mice or 06 rats, and catalepsy was observed at 0, 1, 2, 4, 6 and 8 h. after administration. The animals were put in an unnatural posture with their forelimbs on a vertical plate. When this posture was maintained for over 30 sec, the animal was judged to have catalepsy. The ED_{50} values and 95 % confidence limits were calculated by the probit method, and the values are presented as m mol/Kg po in Table 5.

Anti-epinephrine Test

This test was performed by the method reported [16]. The Test compounds and reference drugs were orally administered to groups of 10 mice or 06 rats. Epinephrine was injected at 40-mg. /Kg. ip 60 min. after administration of the compounds or reference drugs. The 24-h. survival rate was observed. The ED_{50} values and 95 % confidence limits were calculated by the probit method, and the values are presented as m mol/Kg po in Table 5.

RESULTS AND DISCUSSION

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. 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 using 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. The derivatives 6a, 6b and 6i are reported in the literature[1], but their dopamine (DA) autoreceptors and an antagonist of postsynaptic DA receptors properties are not stated.

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 4t, 4u, 4v, 4w, 4x and 4y 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-6z. All the analogs synthesized were characterized by physical data (Table 2) and structure elucidation techniques like FTIR (Table 3), Mass spectroscopy (Table 3) and H^1NMR spectroscopy (Table 4). The results are presented in respective tables.

Table 2: Physical data of Synthesized Compound (6a-6z)

Product	Mol. Formula	Molecular Weight	M.P. ^{0}C
“6”	$C_{19}H_{22}ClN_5O$	372	223
“6a”	$C_{19}H_{22}ClN_5O$	372	>250
“6b”	$C_{19}H_{22}ClN_5O$	372	>250
“6c”	$C_{19}H_{21}Cl_2N_50$	406	>250
“6d”	$C_{19}H_{21}Cl_2N_50$	406	>250
“6e”	$C_{19}H_{22}BrN_5O$	415	211-213
“6f”	$C_{19}H_{21}Br_2N_5O$	493	220-222
“6g”	$C_{19}H_{22}FN_5O$	356	215-217
“6h”	$C_{19}H_{22}FN_5O$	356	240-242
“6i”	$C_{19}H_{21}Cl\ FN_5O$	390	240-242
“6j”	$C_{19}H_{20}Cl_3N_5O$	440.5	>250
“6k”	$C_{18}H_{21}BrClFN_5O$	457.5	230-232
“6l”	$C_{21}H_{27}N_5O$	366	>250
“6m”	$C_{21}H_{27}N_5O$	366	230-232
“6n”	$C_{21}H_{27}N_5O$	366	>250
“6o”	$C_{21}H_{27}N_5O$	366	242-244
“6p”	$C_{21}H_{27}N_5O$	366	>250
“6q”	$C_{21}H_{27}N_50$	366	204-206
“6r”	$C_{21}H_{27}N_5O$	393	222-224
“6s”	$C_{19}H_{24}N_50$	337	>250
“6t”	$C_{18}H_{20}ClN_5O$	357.5	218-220
“6u”	$C_{18}H_{19}Cl_2N_5O$	392	212-214
“6v”	$C_{17}H_{19}BrClFN_5O$	443.5	250-252
“6w”	$C_{20}H_{25}N_50$	339	202-204
“6x”	$C_{20}H_{24}Cl_2N_50$	386	227-230
“6y”	$C_{20}H_{23}Cl_2N_50$	420	>250
“6z”	$C_{23}H_{25}N_50$	387	225-227

Table 3: MS and IR data of Synthesized Compound (6a-6z)

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"	372	3050 (aromatic C-H stretching), 2960 (aliphatic C-H stretching), 1710 (C=O stretching), 1650 (C=N stretching), 1600(aromatic C=C stretching), 1350.80 (C=N stretching), 750 (C-Cl stretching)
"6b"	372	3050 (aromatic C-H stretching), 2950 (aliphatic C-H stretching), 1700 (C=O stretching), 1650 (C=N stretching), 1650 (aromatic C=C stretching), 1350 (C=N stretching), 800 (C-Cl stretching)
"6c"	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)
"6d"	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)
"6e"	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)
"6f"	494	3060 (Aromatic C-H stretching), 2870 (aliphatic C-H stretching), 1710 (C=O stretching), 1635 (C=N stretching), 1600 (aromatic C=C stretching), 1350 (C=N stretching), 590 (C-Br stretching)
"6g"	356.3	3050 (aromatic C-H stretching), 2854.45,2954.74 (aliphatic C-H stretching), 1712.67 (C=0 stretching), 1625 (C=N stretching), 1500 (aromatic C=C stretching), 1350 (C-N stretching), 1103 (C-F stretching)
"6h"	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)
"6i"	390	3070 (aromatic C-H stretching), 2854.45,2923.88 (aliphatic C-H stretching), 1704.96 (C=O stretching), 1643.24 (C=N stretching), 1512.09 (aromatic C=C stretching), 1350 (C-N stretching),748.33 (C-Cl stretching), 948 (C-F stretching)
"6j"	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)
"6k"	458.5	3060 (aromatic C-H stretching), 2870 (aliphatic C-H stretching), 1710 (C=O stretching), 1635 (C=N stretching), 1600 (aromatic C=C stretching), 1350 (C=N stretching), 570 (C-Br stretching), 1146 (C-F stretching)
"6l"	366	3075 (aromatic C-H stretching), 2947.03 (aliphatic C-H stretching), 1712.67 (C=O stretching), 1643.24 (C=N stretching), 1535.23 (aromatic C=C stretching), 1334.65 (C-N stretching)
"6m"	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)
"6n"	366.45	3000 (aromatic C-H stretching), 2950 (aliphatic C-H stretching), 1700 (C=O stretching), 1650 (C=N stretching), 1550 (aromatic C=C stretching), 1350 (C-N stretching)
"6o"	366	3075 (aromatic C-H stretching), 2947.03 (aliphatic C-H stretching), 1712.67 (C=O stretching), 1643.24 (C=N stretching), 1535.23 (aromatic C=C stretching), 1334.65 (C-N stretching)
"6p"	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)
"6q"	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)
"6r"	393	3075 (aromatic C-H stretching), 2947.03 (aliphatic C-H stretching), 1712.67 (C=O stretching), 1643.24 (C=N stretching), 1535.23 (aromatic C=C stretching), 1334.65 (C-N stretching)
"6s"	337	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)
"6t"	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)
"6u"	392	3050 (aromatic C-H stretching), 2960 (aliphatic C-H stretching), 1710 (C=O stretching), 1650 (C=N stretching), 1600 (aromatic C=C stretching), 1350.80 (C=N stretching), 750 (C-Cl stretching)
"6v"	444.5	3060 (aromatic C-H stretching), 2870 (aliphatic C-H stretching), 1710 (C=O stretching), 1635 (C=N stretching), 1600 (aromatic C=C stretching), 1350 (C=N stretching), 595 (C-Br stretching), 1150 (C-F stretching)
"6w"	339	3075 (aromatic C-H stretching), 2947.03 (aliphatic C-H stretching), 1712.67 (C=O stretching), 1643.24 (C=N stretching), 1535.23 (aromatic C=C stretching), 1334.65 (C-N stretching)
"6x"	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)
"6y"	420	3050 (aromatic C-H stretching), 2850 (aliphatic C-H stretching), 1715 (C=O stretching), 1650 (C-N stretching), 1600 (aromatic C-H stretching), 1350 (C-N stretching), 800 (C-Cl stretching)
"6z"	388	3050 (aromatic C-H stretching), 2850,2925 (aliphatic C-H stretching), 1700 (C=O stretching), 1650 (C=N stretching), 1535.23 (aromatic C=C stretching), 1325 (C-N stretching)

Table 4: ^{1}H NMR data of Synthesized Compound 6a-6z

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 2.31-2.21 (m, 2H, CH_2), 3.13-3.04 (m, 6H, CH_2-piperazine), 3.78-3.61 (m, 4H, CH_2, piperazine) , 3.96-3.93 (d, 2H, CH_2), 6.66-6.57 (m, 1H, -Ar H), 7.42-6.98 (m, 4H, -Ar H),7.72-7.52 (d,2H,Ar-H), 7.96 (s,1H, -Ar H)
"6b"	ð 1.94-1.85 (m, 2H, -CH_2), 2.47-2.36 (t, 2H, -CH_2), 2.73-2.72 (s, 4H, CH_2-piperazine), 3.98 (s, 4H, CH_2-Piperazine), 4.14-3.98 (m, 2H, -CH_2), 6.62-6.57 (m, 1H, -Ar H), 7.08-7.01 (m, 2H, -Ar H), 7.29-7.18 (m, 3H, -Ar-H), 7.38-7.35 (m, 1H, -Ar H), 7.85-7.83 (t, 1H, -Ar H)
"6c"	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)
"6d"	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)
"6e"	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,CH2-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, -ArH), 7.04-7.03 (t,1H,-Ar H), 7.26-7.22 (m, 3H,-Ar H), 7.87-7.85 (d,1H, -Ar H)
"6f"	ð 2.30-2.21 (m, 2H, CH_2), 3.18-3.08 (m, 6H, CH_2-piperazine), 3.54-3.45 (d, 2H, CH_2-piperazine), 3.80-3.76 (d, 2H, CH_2), 4.01-3.97 (t, 2H, CH_2), 6.66-6.60 (m,1H, -Ar H), 7.04-6.95 (m,1H, -Ar H),7.30-7.16 (m, 4H, -Ar H), 7.86-7.84 (d,1H, -Ar H)
"6g"	ð 2,25 (s, 2H, CH_2), 3.37-3.08 (m, 6H, CH_2-piperazine), 3.61-3.49 (d, 2H, CH_2-piperazine), 3.81-3.74 (d, 2H, CH_2), 3.96-3.85 (s, 2H, CH_2), 6.62-6.56 (t, 2H, -Ar H), 6.82 (d, 2H, -Ar H), 7.26-7.23 (s, 3H, -Ar H), 7.83-7.81(d, 1H, -Ar H)
"6h"	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)
"6i"	ð 2.30-2.21 (m, 2H, CH_2), 3.18-3.08 (m, 6H, CH_2-piperazine), 3.54-3.45 (d, 2H, CH_2-piperazine), 3.80 (d, 2H, CH_2), 4.01 (t, 2H, CH_2), 6.66-6.60 (m,1H, -Ar H), 7.04-6.95 (m,1H, -Ar H), 7.30-7.16 (m, 4H, -Ar H), 7.86-7.84 (d,1H, -Ar H)
"6j"	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)
"6k"	ð 1.65 (m, 2H, CH2) 2.36 (t, 2H, CH2), 2.60 (m, 4H, CH2-piperazine), 3.15 (t, 2H, CH2) , 3.45 (m, 4H, CH2-piperazine), 6.15 (d, 1H, ArH), 6.75 (t, 1H, ArH), 7.20 (t, 1H, ArH), 7.45 (d, 1H, ArH), 8.20 (s, 2H, ArH)
"6l"	ð 2.20-2.14 (m, 8H, Ar-CH_3,CH_2), 3.28-3.07 (m, 8H, CH_2- piperazine), 3.53 (d, 2H, CH_2), 4.03 (t, 2H, CH_2), 6.64-6.62 (t,1H, -Ar H), 6.93-6.87 (m, 2H, -Ar H), 7.06-7.03 (m, 1H, -Ar H), 7.24-7.23 (d, 2H, -Ar H), 7.88-7.86 (d, 1H, -Ar H)
"6m"	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)
"6n"	ð 2.24-2.17 (m, 8H, -Ar-CH_3,CH_2), 3.18-3.11 (m, 8H, CH_2-piperazine), 3.49 (d, 2H, CH_2), 4.02-3.98 (t, 2H, CH_2), 6.64-6.61 (t, 1H, -Ar H), 6.81-6.79 (d, 2H, -Ar H), 7.05-7.03 (d,1H, -Ar H), 7.24-7.23 (d, 2H, -Ar H), 7.87-7.85 (d,1H, -Ar H)
"6o"	ð 2.19-1.85 (m, 2H, CH_2), 2.36 (s, 6H, Ar-CH_3), 2.50 (s, 6H, CH_2, piperazine-CH_2), 2.88-2.85 (t, 4H, CH_2), 4.05-3.95 (m, 2H, CH_2), 6.62-6.58 (m,1H, -Ar H), 6.95-6.90 (m, 4H, -Ar H), 7.25-7.17 (m, 1H, -Ar H), 7.86-7.80 (d, 1H, -Ar H)
"6p"	ð 1.92-1.87 (m, 2H, CH_2), 2.19-2.16 (d, 6H, Ar-CH_2), 2.41-2.28 (m, 2H, CH_2), 2.51-2.50 (s, 2H, CH_2), 3.99-3.94 (m, 2H, CH_2), 4.15-4.09 (m, 6H, CH_2-piperazine), 6.60 (s,1H, -Ar H), 6.82-6.80 (d,1H, -Ar H), 6.93-6.90 (d, 2H, -Ar H), 7.22-7.20 (m, 2H, -Ar H), 7.86-7.84 (d,1H, -Ar H)
"6q"	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)
"6r"	ð 1.96-1.50 (m, 10H, -Ar-C_2H_5), 2.31-2.21 (m, 2H, CH_2), 3.18-3.11 (d, 8H, CH_2-piperazine), 3.96-3.93 (d, 2H, CH_2), 4.14-4.01 (m, 2H, -CH_2), 6.62-6.57 (m, 1H, -Ar H), 7.08-7.01 (m, 2H, -Ar H), 7.04-7.03-(t, 1H, -Ar H), 7.26-7.22 (m, 2H, -Ar H), 7.87-7.85 (d, 1H, -Ar H)
"6s"	ð 1.96-1.89 (m, 2H, CH_2), 3.04-3.01(t, 4H, CH_2-piperazine), 3.04 (s, 4H, CH_2-piperazine), 3.96-3.94 (t, 2H, CH_2), 4.17-4.12 (m, 2H, CH_2), 6.61-6.56 (m,1H, -Ar H), 6.78-6.73 (t,1H, -Ar H), 6.90-6.84 (d, 2H, -Ar H), 7.25-7.16 (m, 4H, -Ar H), 7.85-7.83 (d,1H, -Ar H)
"6t"	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)
"6u"	2.49 (t, 2H, CH2), 2.56 (t, 4H, piperazine), 3.13 (t, 4H, piperazine), 4.09 (t, 2H, CH2), 6.46 (m,1H, ArH), 6.76 (m, 2H, ArH), 6.84 (m, 1H, ArH), 7.11 (m, 2H, ArH), 7.75 (d, 1H, ArH)
"6v"	2.59-1.90 (m, 4H, CH2-piperazine), 2.62 (t, 2H, CH2), 3.26-2.80 (t, 2H, CH2), 4.13-3.45 (m, 4H, CH2-piperazine), 6.30-6.15 (d, 1H, ArH), 6.45-6.36 (t, 1H, ArH), 7.10-6.55 (t, 1H, ArH), 7.45-7.15 (s, 2H, ArH), 8.10-7.83 (d, 1H, ArH)
"6w"	ð 2.27-2.22 (m, 2H, CH_2), 3.28-3.08 (m, 8H, CH_2-piperazine), 3.38 (s, 3H, Ar-CH_3), 3.60-3.45 (m, 3H, -Ar-CH_3), 4.01-3.97(d, 2H, CH_2), 6.66-6.59 (m,1H, -Ar H), 7.03-6.98 (m,1H, -Ar H), 7.28-7.20 (m, 4H, -Ar H),7.86-7.83 (d,1H, -Ar H)
"6x"	1.20 (m, 2H, CH2), 1.40 (m, 2H, CH2), 2.15 (t, 2H, CH2), 2,75 (m, 4H, CH2-piperazine), 3.10 (t, 2H, CH2), 4.10 (m, 4H, CH2-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)
"6y"	1.75 (m, 4H, CH2), 2.36 (t, 2H, CH2), 3.10 (m, 4H, CH2-piperazine), 3.45 (m, 4H, CH2-piperazine), 4.10 (t, 2H, CH2), 6.10 (d, 1H, ArH), 6.40 (t, 1H, ArH), 7.10 (d, 1H, ArH), 7.35 (t, 1H, ArH), 8.10 (t, 1H, ArH), 8.33 (d, 1H, ArH), 8.46 (d, 1H, ArH)
"6z"	ð 2.65-2.63 (t, 2H, CH_2), 3.24-3.22 (m, 4H, CH_2-piperazine), 3.41-3.38 (t, 2H, CH_2), 3.77-3.68 (m, 4H, CH_2-piperazine), 4.21-4.18 (t, 2H, CH_2), 6.55 (t, 1H, -Ar H), 7.21-7.11(m, 6H, -Ar H), 7.42 (s,1H, -Ar H), 7.49-7.47 (m,1H, -Ar H), 7.63-7.62 (d,1H, -Ar H), 7.78-7.76 (d,1H, -Ar H)

Table 5: Pharmacology test results of test compounds 6a-6z

Compounds	DA receptor antagonist activity[c] (A)	DA autoreceptor agonist activity	Catalepsy (B)	α_1 –adrenoceptor antagonist activity	ED_{50} Ratio (B/A)
Lead Compound-6	0.24	IA	0.9	>341	3.75
Chlorpromazine	12.3	IA	24.5	19.7	2.0
6a	2.8	3.9	NT	>299	-
6b	>8.0[a]	3.1	NT	NT	-
6c	0.9	1.5	9.1	>256	10.11
6d	>7.0[b]	>22	1.2	>256	-
6e	3.7	3.5	NT	156	-
6f	2.9	>21	1.3	>256	0.44
6g	3.6	NT	NT	NT	-
6h	>8.0[a]	3.8	10.4	156	-
6i	1.5	1.8	15.8	>256	10.53
6j	>7.0[b]	NT	NT	NT	-
6k	2.0	1.3	1.3	7.1	-
6l	41.3	3.5	73.2	151	1.77
6m	19.0	3.5	10.0	152	-
6n	29.0	3.6	12.9	NT	-
6o	26.0	8.9	73.1	153	2.81
6p	43.0	NT	NT	NT	-
6q	27.0	9.1	14.1	7.3	0.52
6r	43.0	8.3	NT	7.3	-
6s	>8.0[a]	NT	NT	7.6	-
6t	42.1	>20	2.7	7.0	0.67
6u	>7.0[a]	IA	NT	7.1	-
6v	33.1	IA	2.1	7.0	0.67
6w	>8.0[a]	>22	NT	NT	-
6x	40.6	>26	4.1	8.0	0.67
6y	32.5	>21	3.8	7.1	
6z	16.3	IA	19.4	7.0	

a: Inactive at below given dose; b: Inactive at below given dose

c: Inhibition of the apomorphine-induced stereotyped behavior in mice. Test compound was administered orally to 10 mice 1h before subcutaneous injection of apomorphine (1.5 mg/Kg sc.). The ED_{50} values and 95 % confidence limits were calculated by the linear regression analysis and are presented in micro mol./Kg. po.CL represents 95 % confidence limits.

IA: Inactive; NT: Not tested

Table 6: Comparison of Biological Activities of selected Compounds and reference agents

Compound	ED_{50} (micro mol/Kg po)		Catalepsy (B)	Ratio of ED_{50} (B/A)
	Inhibition of GBL-induced increase in DOPA synthesis	Inhibition of APO-induced stereotyped behavior (A)		
Haloperidol	inactive	1.1 (0.5-1.3)	1.9 (1.6-2.1)	1.7
Chlorpromazine	inactive	28.4 (24.5-33.2)	0.8 (0.8-0.9)	0.03
Compound "6c"	15.1 (10.2-22.0)	1860 (1250-3730)	>2600	>1.4
Compound "6f"	6.9 (4.5-10.7)	11.8 (8.5-15.4)	149 (137-161)	12.6
Compound "6i"	8.6 (5.7- 11.2)	15.7 (12.5-19.6)	201 (189-215)	12.8

[a] Numbers in parentheses represent 95 % confidence limits.

Pharmacology

The postsynaptic DA receptor antagonist activity of all compounds synthesized was evaluated by the ability to inhibit APO-induced stereotypic behavior in mice (anti-APO test) [12]. The clinically available standard antipsychotic agent chlorpromazine was also examined in the test as reference drugs, and the results are summarized in Table 5. The DA autoreceptor agonist activity of selected compounds was determined by their reversal effects on the GBL-induced increase in DOPA synthesis in the mouse brain [13]. The EPS liability of selected compounds was examined by measuring their ability to induce catalepsy in mice. Several compounds were tested for their α_1-adrenoceptor antagonist activity since peripheral R1-adrenoceptor antagonism has been reported to cause autonomic side effects [2]. The results are summarized in Table 5. Furthermore, to evaluate its potential as an antipsychotic agent, the activities of the selected compound in behavioral tests with rats were compared with those of chlorpromazine and haloperidol. The results are summarized in Table 6.

DA receptor antagonist activity

Initially in the search of potent postsynaptic DA receptor antagonist, we examined the postsynaptic DA receptor antagonist activity of all the analogs i.e. 6a-6z of lead molecule 2-[3-{4-(3-chlorophenyl)-1-piperazinyl} propyl]-1, 2, 4-triazolo [4, 3-a] pyridine-3- (2H)-one hydrochloride. The compound "6s" i.e. 2-[3-{4-phenyl-1-piperazinyl} propyl]-1, 2, 4-triazolo [4, 3-a] pyridine-3- (2H)-one hydrochloride with no replacement on phenyl ring exhibited no potency with ED^{50} >8.0 micro mol/Kg po. Replacement of propyl side chain with ethyl and butyl is also studied. The compounds "6t", "6u", "6v", 6w", "6x" and compound "6y" with respective side chain replacements and with diverse functional group substitution on phenylpiperazinyl in lead molecule was observed to exhibit reduced potency with ED^{50} 42.1 micro mol/Kg po, ED^{50} >7.0 micro mol/Kg po, ED^{50} 33.1micro mol/Kg po, ED^{50} > 8.0 micro mol/Kg po, ED^{50} 40.6 micro mol/Kg po and ED^{50} 32.5 micro mol/Kg po respectively when compared with lead compound with ED^{50} 0.24 micro mol/Kg po.

Structure-Activity relationship

The results indicated superiority of propyl side chain to ethyl and butyl side chain in producing potent DA antagonist activity in 2-[3-{4-(3-chlorophenyl)-1-piperazinyl} propyl]-1, 2, 4-triazolo [4, 3-a] pyridine-3-(2H)-one hydrochloride and also indicated that appropriate substitution in the phenylpiperazinyl moiety is required in order to display activity in this series of compounds.

Thus we selected compound 6, having the propyl side chain and 3-chlorophenyl-1-piperazinyl moiety, as a lead compound, and a variety of modifications on it were carried out to synthesize compounds 6a-6x.

First, we examined the effects of the substituent of the aromatic ring in the phenylpiperazinyl moiety in compound "6" on the postsynaptic DA receptor antagonist activity. As shown in the Table 5, altering the chlorine substituent from position 3- in lead compound "6" to position R2 and R4 as in the compound "6a" and "6b" greatly reduced the potency with ED^{50} equal to 2.8 micro mol/Kg po and ED^{50} >8.0 micro mol/Kg po. This indicates, in the series of compounds with one chloro functional group substitution on phenylpiperazinyl moiety, the compound having substitution at position R2 showed highest potency (0.24 > 2.8 > 8.0).

Of the compounds with two chlorine substituents are adjusted to each other at the 2- and R3 positions in the phenylpiperazinyl moiety as in "6c" showed much higher potency with ED^{50} 0.9 micro mol/Kg po which is close to the lead compound "6" but when the substituent positions are altered to R3 and R4 as in compound "6d" shows no potency with ED^{50} >7.0 micro mol/Kg po. In the compound "6e" the introduction of more bulky bromine atom at position 3- instead of chlorine exhibited decreased potency (ED^{50} 3.7 micro mol/Kg po) but showed marginal increase with two bromine substituents as in compound "6f" with ED^{50} 2.9 micro mol/Kg po. The similar observation was when chlorine is replaced with a smaller fluorine substituent on the position R3 as in compound "6g" that showed much less potency with ED^{50} 3.6 micro mol/Kg po similar to compound "6e". This proves the superiority of chlorine over bromine and fluorine atom in producing potent DA antagonist activity in lead molecule when substituted at position R3 as in lead molecule. It is surprising to see that the compound "6h" with fluorine atom at position R4 exhibit no potency with ED^{50} >8.0 micro mol/Kg po. It is concluded based on test results of compound "6b", "6d" and "6h" that the compound exhibits much reduced DA receptor antagonist activity with halogen substituent at position R4 on phenylpiperazinyl moiety.

Further, substitution of multiple halogen atoms and relative effect on DA receptor antagonist activity of the compound was studied with compound "6i" where chlorine and fluorine substituent are introduced at position R3 and R4 respectively on phenylpiperazinyl moiety. The test result indicated increased DA antagonist activity of the compound in "6i" with ED^{50} 1.5 micro mol/Kg po, when compared to the compounds "6d" and "6f" which has two chlorine and two bromine substituent respectively and exhibited reduced activity with ED^{50} >7.0 micro mol/Kg po and ED^{50} 2.9 micro mol/Kg po respectively. Replacement of three chlorine substituent as in compound "6j" leads compound inactive with ED^{50} >7.0 micro mol/Kg po but surprisingly three different halogen atoms as in "6k" showed enhanced activity with ED^{50} 2.0 micro mol/Kg po close to "6i".

Also, the effects of structural modifications on their DA antagonist activity were examined with respect to introduction of electron donating groups like two methyl substituent on phenylpiperazinyl moiety. In the

compound "6l", and "6p" where methyl groups are *ortho* to each other showed much less potency (ED^{50} 41.3 micro mol/Kg po, and ED^{50} 43.0 micro mol/Kg po) compare to compound '6m", "6n" and "6o" (ED^{50} 19.0 micro mol/Kg po, ED^{50} 29.0 micro mol/Kg po and ED^{50} 26.0 micro mol/Kg po) where methyl substituents are at *meta* and *para* position. The compounds "6q" and "6r" with strong electron donating ethyl and diethyl substitution at position R2 and R6 in phenylpiperazinyl moiety has shown the DA antagonist activity similar to compounds with electron donating methyl substitution with ED^{50} 27.0 micro mol/Kg po and ED^{50} 43.0 micro mol/Kg po respectively.

The compound "6z" with napthyl substitution on piperazine showed moderate DA antagonist activity with ED^{50} 16.3 micro mol/Kg po but close to reference standard chlorpromazine.

In the structure activity relationships, the structural requirements for the postsynaptic DA receptor antagonist activity found in this series are as follows:

- In the series of compounds with one chloro substituent on phenylpiperazinyl moiety, the compound having chloro substituent at the 2-position showed the highest potency.
- Compounds with halogen substituent at position 4- of the phenylpiperazinyl moiety exhibited no DA receptor antagonist activity.
- The compound with two chlorine substituent has shown greatly enhanced DA receptor antagonist activity which is reduced with replacement of chloro by bulky bromine substituent in compound.
- Introduction of multiple halogen substituents on phenylpiperazinyl moiety has improved the activity of the compound but three halogen substitution led compound inactive.
- The DA receptor antagonist activity of the compounds with replacement using electron donating methyl or ethyl substituent on phenylpiperazinyl moiety was observed to be much reduced than the lead compound.
- Replacement of propyl side chain with ethyl and butyl has shown diminished DA receptor antagonist activity of the compounds against the lead compound. No improvements are observed with two methyl or halogen substitutions on phenylpiperazinyl moiety.
- All the novel analogs prepared were observed to be less active and therefore has less potency than the lead compound and reference drug used for comparison i.e. Chlorpromazine.

DA autoreceptor agonist activity

The selected compounds were tested for their ability to reverse GBL-induced increase in DOPA synthesis in the mouse brain. This effect reflects DA autoreceptor agonist activity. The selected compounds "6c" and "6i" dose-independently reversed the GBL-induced increase in DOPA synthesis (0.3, 1,3,10 mg/Kg po), but these two compounds did not completely antagonize GBL even at the highest dose in this model (70 % inhibition at 10 mg/Kg po). The compounds "6c" and "6i" showed the activity almost well comparable to their activity for DA receptor antagonist activity with ED^{50} 1.5 micro mol/Kg po and ED^{50} 1.8 micro mol/Kg po respectively.

The compound "6k" which showed good DA receptor antagonist activity at given dose has also exhibited higher DA autoreceptor agonist activity with ED^{50} 1.3 micro mol/Kg po. The compounds "6d", "6f" and "6w" with dichloro, dibromo and dimethyl with butyl linker replacements respectively were inactive with ED^{50} >22 micro mol/Kg po, ED^{50} >21 micro mol/Kg po and ED^{50} >22 micro mol/Kg po correspondingly.

The compounds "6a", "6b", "6e", and "6h" with one halogen substitution on phenyl ring were observed to be equipotent with ED^{50} 3.9 micro mol/Kg po, ED^{50} 3.1 micro mol/Kg po, ED^{50} 3.5 micro mol/Kg po and ED^{50} 3.8 micro mol/Kg po respectively.

The activities of compounds "6m" "6n" and "6o" having two methyl groups attached to the phenyl ring shows some distinct pattern. The compounds "6m" and "6n" where methyl group are at *meta* and *para* position to each other exhibit higher potency with ED^{50} 3.5 micro mol/Kg po and ED^{50} 3.6 micro mol/Kg po respectively compare to compound "6n" where the two methyl groups are at R2 *and* R6 position to each other with ED^{50} 8.9 micro mol/Kg po. The compounds "6r" and "6s" having mono and diethyl ethyl group attached to the phenyl ring were observed to be equipotent with ED^{50} 9.1 micro mol/Kg po, ED^{50} 8.3 micro mol/Kg po respectively.

The compounds "6t", '6u', "6v", "6w", "6x" and "6y" which exhibited no potency for DA receptor antagonist activity also shows similar low or no DA autoreceptor agonist activity with ED^{50} >20 micro mol/Kg po. The compounds "6u" and "6v" were found to be inactive.

The compound "6z" with napthyl substitution on piperazine which showed moderate DA antagonist activity has shown no DA autoreceptor agonist activity.

Catalepsy

The EPS liability and alpha1-adrenoceptor antagonist activity of selected compounds were examined. Typical antipsychotic agents induce catalepsy. Selected compounds were also examined for their ability to induce catalepsy in mice.

The compounds "d" and "f" which showed reduced DA autoreceptor agonistic activity are observed to be the potent cataleptogenic compounds (ED^{50} 1.2 micro mol/Kg po and ED^{50} 1.3 micro mol/Kg po) in the series of compounds examined.

The compounds "6c" and "6i" also induced catalepsy but with 10 times higher ED^{50} values then that of anti-APO

test (ED^{50} 0.9 micro mol/Kg po and ED^{50} 1.5 micro mol/Kg po) in mice with ED^{50} 9.1 micro mol/Kg po and ED^{50} 15.8 micro mol/Kg po respectively suggesting their lower propensity to induce EPS then the typical antipsychotic agent (lead compound "6" with ED^{50} 0.9 micro mol/Kg po) examined. Thus the weak catalaptogenic effects of compounds "6c" and "6i" may contribute to its DA autoreceptor agonistic activity at present. The reference drug Chlorpromazine is observed to show low activity towards inducing the catalepsy in mice with ED^{50} 24.5 micro mol/Kg po. The compound "6k" which is a DA receptor antagonist and with good DA autoreceptor agonist activity showed the strong catalaptogenic effect with ED^{50} 1.3 micro mol/Kg po

The compounds "6h", "6l", "6m", "6n", "6o" and "6r" showed the weak catalaptogenic effects with ED^{50} 10.4 micro mol/Kg po, ED^{50} 73.2 micro mol/Kg po, ED^{50} 73.2 micro mol/Kg po, ED^{50} 10.0 micro mol/Kg po, ED^{50} 12.9 micro mol/Kg po, ED^{50} 73.1 micro mol/Kg po and ED^{50} 14.1 micro mol/Kg po respectively.

Surprisingly the compound "6t" and "6v" has shown a good potency to induce catalepsy in mice with ED^{50} 2.7 micro mol/Kg po and ED^{50} 2.1 micro mol/Kg po respectively. Compound "6 x" and "6y" exhibited cataleptogenic effect in lesser potency to these compounds with ED^{50} 4.1 micro mol/Kg po and ED^{50} 3.8 micro mol/Kg po respectively.

The compound "6z" which showed no DA receptor antagonist activity and DA autoreceptor agonist activity has shown weak catalaptogenic effects with ED^{50} 19.4 micro mol/Kg po.

α_1 –adrenoceptor antagonist activity

Selected compounds were also tested for their α_1-adrenoreceptor antagonist activity since peripheral α_1-adrenoreceptor antagonism has been known to cause autonomic side effects [2]. An abnormality of noradrenaline neurotransmission in schizophrenia patients has been reported [17]. Although a beneficial effect of this activity in treatments of these patients has been suggested [18]. As shown in Table 6, the reference lead compound and Chlorpromazine showed α_1-adrenoreceptor antagonist activity with ED^{50} 150.7 micro mol/Kg po and ED^{50} 23.2 micro mol/Kg po respectively.

The compounds "6k", "6r", "6s", "6t", "6u", "6v", "6x" and "6y" showed the highest activity with ED^{50} 7.1 micro mol/Kg po, ED^{50} 7.3 micro mol/Kg po, ED^{50} 7.6 micro mol/Kg po, ED^{50} 7.0 micro mol/Kg po, ED^{50} 7.1 micro mol/Kg po, ED^{50} 7.0 micro mol/Kg po, ED^{50} 8.0 micro mol/Kg po and ED^{50} 7.1 micro mol/Kg po respectively. This indicates they will have very high level of adverse effects as a drug.

The compounds "6a", "6c", "6d", "6f" and "6i" were found inactive up to >256 - >299.Simmilerely the compounds "6e", "6h", "6l", "6m" and "6o" showed the moderate potency with ED^{50} values in the range of ED^{50} 151 micro mol/Kg po to ED^{50} 156 micro mol/Kg po.

The compound "6z" which showed no DA receptor antagonist activity, DA autoreceptor agonist activity and weak catalaptogenic effects was found to be potent α_1-adrenoreceptor antagonist with ED^{50} 7.0 micro mol/Kg po. Data represented in the Table 5 show that the compounds with high potency in DA receptor antagonist activity and DA autoreceptor agonist activity exhibited very weak α_1-adrenoreceptor antagonist activity.

As shown in Table 6, the DA autoreceptor agonist and postsynaptic DA receptor antagonist activities of "6c", "6f", "6i" and reference agents were also confirmed in two tests in rats. Compound "6f", "6i", chlorpromazine, and haloperidol inhibited the APO induced stereotypic behavior in rats with ED_{50} values of 11.8, 15.7, 28.4, and 1.1 micro mol/kg po, respectively. Compounds "6c", "6f" and "6i" antagonized the GBL-induced increase in DA synthesis in the rat brain with ED50 values of 15.1, 6.9 and 8.6 micro mol/kg po, respectively. Compounds showed lower activities in rats than in mice after oral administration. These differences in potencies between mice and rats may be attributable to the difference in first-pass metabolism between the two species. Compound "6f" and "6i" induced catalepsy in rats with an ED_{50} value of 149.2 and 201 micro mol/kg po respectively which is about 10 times higher than that for APO-induced stereotypic behavior test as seen in mice. Thus, compounds "6f" and "6i" were confirmed in mice and rats to have these dual activities and a low potential to induce the EPS. Because of their attractive profile and its minimal adverse effect after toxicological studies, compound "6f" and "6i" were recommended as candidates for further evaluation.

CONCLUSION

The course of above studies indicated that the compounds "6c", "6f", and "6i" are an agonist of the DA autoreceptors, and also acts as antagonist of the postsynaptic DA receptors almost comparable in potency to the reference standards. The compound "6c", "6i" have shown lower potential to induce catalepsy than the reference standard except compounds "6f" which is seen almost equipotent with the standard. These compounds also did not show α_1-adrenoreceptor antagonist activity which indicates they will have less adverse effect as a drug.

This indicated further that the compounds with either two similar halogen atoms (compound "6c" and "6f") or two different halogen substitution (compound "6i") at *ortho* position to each other on phenyl ring were compounds those given desired results.

It is also a fact that the compounds "6k" which has three halogen atom substitution on the phenyl ring has exhibited consistent activity in all the tests but has shown very high potency in α_1-adrenoreceptor antagonist activity which indicates it will have adverse effects as a drug and is not recommended

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