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SYNTHESIS AND EVALUATION OF CARBOXAMIDINE DERIVATIVES AS POTENTIAL ANTIPSYCHOTIC AGENTS

ABSTRACT

A series of carboxamidine derivatives as potential antipsychotic agents was synthesized. These analogues were evaluated in vitro for their binding to the dopamine and serotonin 5-HT receptors. Two derivatives exhibited potent activities comparable to Clozapine (2). Furthermore, these derivatives have exhibited potentiation activity instead of antagonism against 5-HT receptors. Clozapine also potentiate 5-HT because it is having no antagonistic activity on 5-HT, but acts only on dopamine D_4 receptor. So, carboxamidines 16 and 29 were selected for further evaluation as potential backup compounds to clozapine.

Key words: Antipsychotic agents, carboxamidine, clozapine

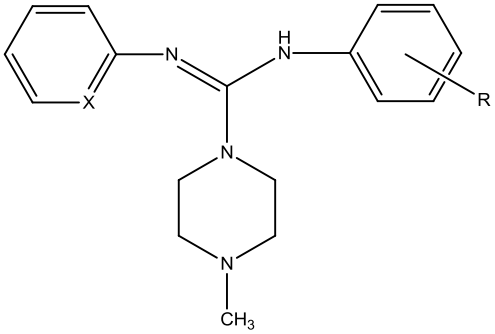
1. INTRODUCTION

Schizophrenia affects about 24 million people worldwide, which is treatable disorder and treatment is being more effective in its initial stages. Several classes of drugs are effective in the symptomatic treatment. Typical antipsychotics like chlorpromazine, haloperidol, droperidol etc. have effect on positive symptoms and induce EPS like parkinsonism, dystonia, akathisia, tardive dyskinesia in up to 75% of patients.[1] Further, these antipsychotics have little or no effect on primary negative symptoms.[2]

Several new agents “Atypical” antipsychotics represent a new generation of antipsychotics with a significantly lower incidence of EPS, as well as little or no effect on prolactin elevation. Clozapine is prototype of atypical antipsychotics, which acts on positive as well as negative symptoms.[3] Unfortunately, clozapine is associated with agranulocytosis, which necessitates costly and inconvenient blood monitoring. Clozapine also induces hypersalivation, hypotension, constipation, and weight gain—all vegetative side effects that can be distressing for the patient.[4]

Future development of antipsychotics should aim to achieve better efficacy than the presently available drugs, not only on positive symptoms but also on negative, cognitive, and psychoaffective signs. Of course, there is a clear need for more efficacious and/or better tolerated compounds, having the best benefit–risk ratio. All side effects that are distressing to the patient should be minimized, not only EPS but also autonomic ones (such as orthostatic hypotension, salivation, weight gain, sexual disturbances, galactorrhea).

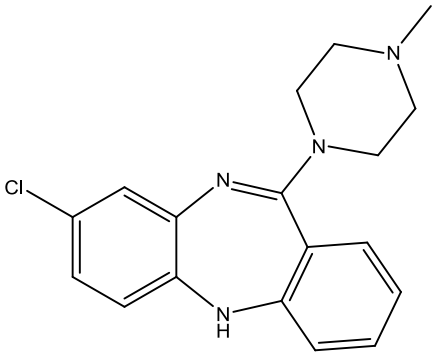
Based on the basic structure of clozapine, two aryl ring and N-methylpiperazine ring in between, we have designed a series of 4-methyl-N-[(un)substituted phenyl]-N’-(phenyl/pyridin-2-yl)piperazin-1-carboxamidine (1).



(1)

R = H, *o*-CH_3, *m*-Cl, *p*-Cl; X = -CH, N

Figure 1: Structure of designed a series of carboxamidine (1)



(2)

Figure 2: Structure of clozapine (2)

It was also thought of interest to check 3D structural similarity between designed molecule (1) and clozapine (2) by indirect type of molecular modeling. Structures were generated, energy minimized, and superimposed, using PC based software Chem office 3D Pro Ultra 8.0 (Cambridge Soft Inc., USA). Both aryl rings carbon and nitrogen, and N-methylpiperazine ring nitrogen and carbon atoms were selected for superimposition.

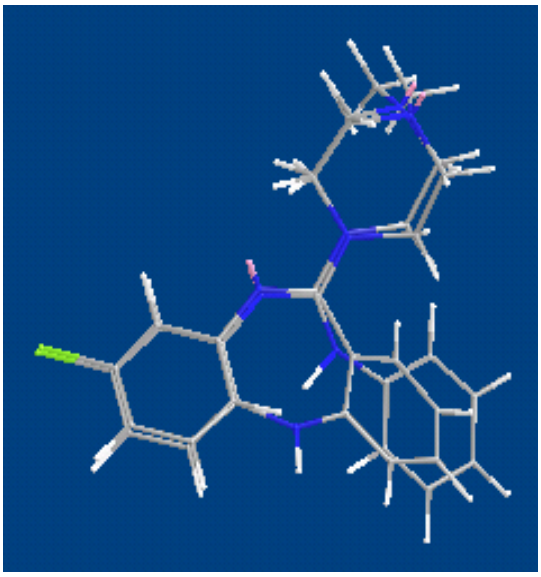
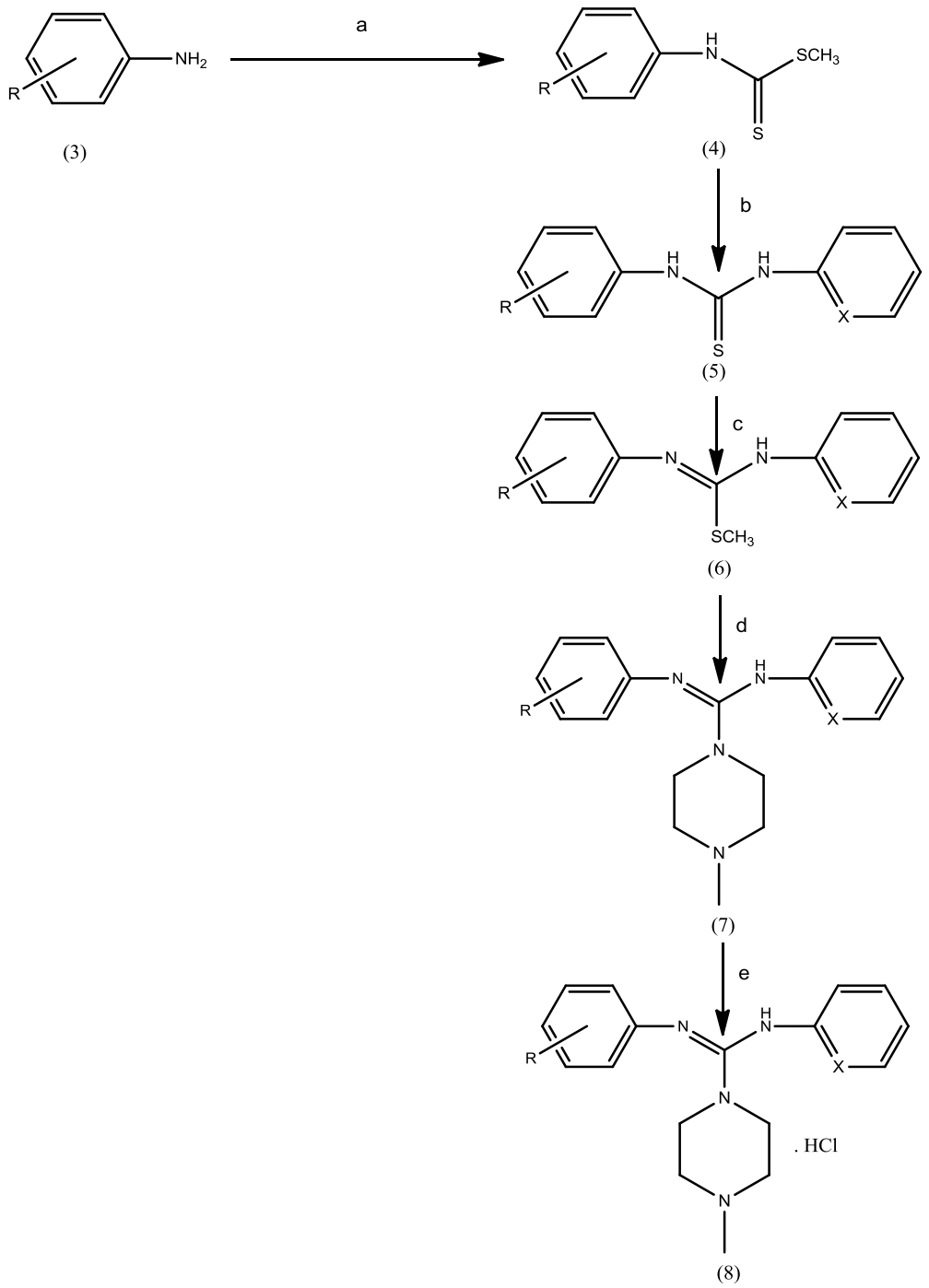


Figure 3: Superimposed structures of clozapine and designed molecule. rmsd (0.268)

RMSD observed on superimposition was 0.268. Low value suggested good 3D structural similarity between designed series and clozapine. This prompted us to synthesize a series of 4-methyl-N-[(un)substituted phenyl]-N'-(phenyl/pyridin-2-yl)- piperazin-1-carboxamidine (1) as potential antipsychotic agents.

In order to synthesize the target compounds (1), the various amines **3** in DMSO, were reacted with CS_2 in presence of NaOH and then DMS was added dropwise to provide compound **4**. Further, compound **4** was refluxed with aniline or 2-amino pyridine in methanol to yield compound **5** and subsequent methylation of compound **5** either with DMS or with MeI in acetone yield compound **6**. Finally, a mixture compound **6** and N-methyl piperazine was subjected to microwave radiation at 100% power (800W) for 1-1.5 hr to provide compound **7**. Oily product of compound **7** was reacted with Dioxane-HCl to yield compound **8**. (Scheme 1)



(3) (4) (5) (6) (7) (8)

Scheme 1. Reagents and conditons: (a) (i) DMSO, 20 M NaOH, CS_2; (ii) DMS; (b) aniline / 2-amno pyridine, methanol, reflux; (c) DMS / MeI, acetone, stirring; (d) N-methyl piperazine, microwave (MW); (e) Dioxane-HCl

2. MATERIAL AND METHODS

2.1. General methods

All chemicals and solvents were commercially available and used without further purification. The reactions were monitored by thin-layer chromatography (TLC) using silica plates (TLC Silica gel 60 F_{254}, Merck KGaA) and spots were visualized in UV-light at λ = 254 nm and 366 nm. Melting points (mp) were determined in open

capillaries and are uncorrected. Mass spectra were obtained on Shimadzu LCMS 2010EV Mass spectrometer. ^{1}H and ^{13}C NMR spectra were obtained in CDCl3 on BRUKER Advance-II 400 MHz instrument and chemical shift were measured as parts per million (ppm) downfield from Tetramethylsilane (TMS) as internal standard.

2.1.1. General preparation of methyl-(substituted phenyl)carbamodithioate (4)[5]

To a vigorously stirred solution of amine (3) (0.2 mol) in dimethylsulfoxide (DMSO, 100 ml) at RT aqueous 20 M NaOH (12 ml) and carbon disulfide (CS_2, 16 ml, 0.26 mol) were added. After 30 min dimethyl sulfate (DMS, 0.25 mol) was added drop wise under cooling condition. After 2 hr the reaction mixture was poured into the water. Solid obtained was filtered. Compound was washed with water thrice. The solids were dried at RT and recrystallized from ethanol. (Yield 90%)

2.1.2. General preparation of 1-[(un)substituted phenyl]-3-(pyridin-2-yl/substituted phenyl)thiourea (5)[6]

To a solution of methyl (substitutedphenyl)carbamodithioate (4) (0.1 mol) in methanol (50 ml), amine (0.1 mol) was added at RT and refluxed for 24-48 hr. Reaction mixture was cooled at 5ºC for 2 hr. The solid was filtered and washed with n- hexane, dried under IR lamp and recrystallized from methanol. (Yield 60%)

2.1.3. General preparation of S-methyl-N-[(un)substituted phenyl]- N'-(pyridin-2-yl)isothiourea (6)

To a solution of 1-[(un)substituted phenyl]-3-(pyridin-2-yl)thiourea (5) (0.01 mol) in acetone (15 ml) were added K_2CO_3 as a catalyst and methyl iodide (MeI, 3.2 ml) at RT. The reaction mixture was stirred for 3 hr at RT, monitored by TLC for completion. A reaction mixture was diluted with water and extracted with ethyl acetate. The organic layer was washed with water thrice, dried over anhydrous Na_2SO_4 and concentrated. The yellow oily residues were obtained. (Yield 65%)

2.1.4. General preparation of S-methyl-N-[(un)substituted phenyl]- N'-phenylisothiourea (6)

To the ice cold suspension of 1-(substituted phenyl)-3-phenylthiourea (5) (0.01 mol) in acetone (15 ml), dimethyl sulphate (0.01 mol) was added drop wise with continuous stirring over a period of half an hr. The mixture was thereafter stirred at room temperature for an additional 1 hour and then refluxed for 3 hr. The reaction was monitored for completion by TLC. Acetone was removed by downward distillation. The heavy oily residue was poured in ice-cold water and basified using Na_2CO_3. The solid separated was filtered, washed with cold water thrice and dried at RT. (Yield 93%)

2.1.5. General preparation of 4-methyl-N-[(un)substituted phenyl]-N'-(pyridin-2-yl/phenyl) piperazin-1-carboxamidine (7)

A mixture of S-methyl-N-[(un)substituted phenyl]-N'-(pyridin-2-yl/phenyl)isothiourea (6) (2.43g, 0.01 mol) and N-methyl piperazine (2.22ml, 0.02 mol) was subjected to microwave radiation in an 150 ml beaker at 100% power (800W) for 1-1.5 hr and was monitored for completion by TLC. Reaction mixture was poured in ice-water and extracted with dichloromethane. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The yellowish oil was obtained, which was then purified with petroleum ether 2-3 times to give colorless solids. The solids were filtered by gravity filtration, dried at RT and recrystallized by petroleum ether. (Yield 28%)

2.1.6. General preparation of 4-methyl-N-(substitutedphenyl)-N'-phenylpiperazin-1-carboxamidine hydrochloride salt (8)

A 4-methyl-N-(substitutedphenyl)-N'-phenylpiperazin-1-carboxamidine (7) was dissolved in 5-10 ml of dichloromethane and approximately 10 ml of dioxane-HCl was added and cooled for 12 hr to prepare salt of the product. After 12 hr solids were obtained which were then filtered by gravity filtration and washed with dry ether, dried and immediately packed. (Yield 29%)

4-methyl-N-phenyl-N'-(pyridin-2-yl)piperazin-1-carboxamidine (9)

IR (KBr, cm-1) 1622-1552 (C=C ring stretch), 2966-2788 (C-H stretch of -CH3, -CH2), 3000-3100(C-H stretch of Ar, pyridine), 3135-3300 (N-H stretch of 2º amine); 1H-NMR (CDCl3, δ ppm): 2.3(s, 3H, -CH3 of N-methylpiperazine), 2.4(t, 4H, N-methylpiperazine), 3.37 (t, 4H, N-methylpiperazine), 6.69-6.98(m, 5H, Ar-H), 7.21-7.36(m, 4H, pyridine); ^{13}C-NMR ($CDCl_3$, δ ppm): 46.13(-CH3), 46.33(2'', 6'' C, N-methylpiperazine), 54.51(3'', 5'' C, N-methylpiperazine), 118.35(6'C, pyridine), 119.85(4''' C, Ar), 122.49(4'C, pyridine), 129.25(3''', 5''' C, Ar), 122.23(2''', 6''' C, Ar), 137.45(5'C, pyridine), 145.94(1'''C, Ar), 150.69(3'C, pyridine), 153.72(1'C, pyridine)

4-methyl-N'-(pyridin-2-yl)-N-o-tolylpiperazin-1-carboxamidine (10)

IR (KBr, cm^{-}1): 1627-1548(C=C ring stretch), 2995-2785(C-H stretch of -CH_3, -CH_2), 3041-3100(C-H stretch of Ar, pyridine), 3199-3244(N-H stretch2º amine); Mass (Methanol) (m/z): 309.9 (M^+); ^{1}H-NMR ($CDCl_3$, δ ppm): 2.28(s, 3H, -C<u>H</u>$_3$ of N-methyl piperazine), 2.37(s, 3H, -CH_3), 2.37-2.39(t, 4H, N-methylpiperazine), 3.44(t, 4H, N-methylpiperazine), 6.78-7.00(m, 5H, Ar-<u>H</u>), 7.05-7.57(m, 4H, pyridine), 11.5 (s, 1H, N<u>H</u> of 2-pyridylamine)

N-(4-chlorophenyl)-4-methyl-N'-(pyridin-2-yl)piperazin-1-carboxamidine (11)

IR (KBr, cm^{-}1): 1614-1552(C=C ring stretch), 2985-2792(C-H stretch of -CH_3, -CH_2), 3002-3078(C-H stretch of Ar, pyridine), 3193-3300(N-H stretch of 2º amine); ^{1}H-NMR ($CDCl_3$, δ ppm): 2.3(s, 3H, -C<u>H</u>$_3$ of N-methylpiperazine), 2.40 (t, 4H, N-methylpiperazine), 3.45(t, 4H, N-methylpiperazine), 6.75-6.99(m, 5H, Ar-<u>H</u>), 7.09-7.21(m, 4H, pyridine), 11.68(s, 1H, N<u>H</u> of 2-pyridylamine); ^{13}C-NMR ($CDCl_3$, δ ppm): 46.22(-CH_3), 46.69(2'', 6''C, N-methylpiperazine), 54.59(3'', 5''C, N-methylpiperazine), 129.16(3''', 5'''C, Ar), 121(2''', 6'''C, Ar), 139.75(1'''C, Ar), 116.65(6'C, pyridine) , 127.44(4'''C, Ar), 137.75(5'C, pyridine), 145.89(3'C, pyridine), 153.57(1'C, pyridine), 161.52(C, carboxamidine)

4-methyl-N'-phenyl-N-o-tolylpiperazin-1-carboxamidine hydrochloride salt (12)

IR (KBr, cm^{-}1): 1611-1494(C=C ring stretch), 2956-2842(C-H stretch of –CH_3, -CH_2), 3000-3100(C-H stretch of Ar), 3390-3417(N-H stretch of 2º amine); Mass (Methanol) (m/z): 309.1 (M+1); ^{1}H-NMR (DMSO-D_6, δ ppm): 2.18(s, 3H, -C<u>H</u>$_3$ of N-methylpiperazine), 2.24(3H, -C<u>H</u>$_3$ of Ar), 6.93-6.98(m, 5H,Ar-<u>H</u>), 7.13-7.20(m, 4H, Ar-<u>H</u>)

N-(4-chlorophenyl)-4-methyl-N'-phenylpiperazin-1-carboxamidine (13)

IR (KBr, cm^{-}1): 1614-1488(C=C ring stretch), 2987-2852(C-H stretch of –CH_3, -CH_2), 3002-3201(C-H stretch of Ar), 3332-3444(N-H stretch of 2º amine); Mass (Methanol) (m/z): 329 (M^+), 331 (M+2)

2.2. Biological Evaluation

2.2.1 Dopamine receptor antagonistic activity[7]

2.2.1.1 Preparation of solutions of compounds

Test compounds were not soluble in water and physiological salt solution. The solutions of the test compounds were prepared by dissolving in 0.01 N HCl solution and further dilutions were made by distilled water.

2.2.1.2 Procedure

The assembly was set up and arrangements were made for experimental conditions mentioned above. Overnight fasted albino male rat of Wistar strain were used for the experiment.

The rat was sacrificed as per CPCSEA recommended guideline by cutting the blood vessels of the neck. The abdominal wall was quickly opened and the two vas deferentia were isolated. Semen from each vas deferens was removed by applying gentle pressure from one end to another. The sheath and connecting tissue were removed from the vas deferens carefully. They were mounted in the isolated organ bath containing 30 ml of Kreb's bicarbonate solution maintained at $37^0 \pm 1^0$C. The solution was continuously bubbled with air. The tissue was allowed to stabilize for 30 min., during that period solution was changed at every 10 min. The dose response curve of dopamine was taken till supramax was obtained. After that 10 µg of compound was added every time in organ tube, after 5 min. the response of dopamine was re-elicited till the maximum response was obtained. The graph thus obtained was used to plot log dose response curve. For each compound, three curves were plotted and the pA_2 value was calculated using the following equation.

pA_2 VALUE:

It is defined as negative logarithm of the molar concentration of antagonist in the presence of which double the dose of agonist was required to produce the same response as produced in absence of antagonist.

$$pA_2 = - \log [M] + \log [(A_2/A_1) -1]$$

Where,

M = Molar concentration of drug solution

A_2 = concentration of agonist at 50% height in presence of antagonist.

A_1 = concentration of agonist at 50% height in absence of antagonist.

2.2.2 5-HT receptor antagonistic activity[8]

2.2.2.1 Preparation of solutions of compounds

Test compounds were not soluble in water and physiological salt solution. The solutions of the test compounds were prepared by dissolving in 0.01 N HCl solution and further dilutions were made by distilled water.

2.2.2.2 Procedure

The assembly was set up and arrangements were made for experimental conditions mentioned above. Overnight fasted albino male rat of Wistar strain were used for the experiment.

The rat was sacrificed as per CPCSEA recommended guideline by cutting the blood vessels of the neck. The abdominal cavity was opened and stomach was taken out. Fundus part of stomach was identified (upper part and grey in color) and was cut away and opened by cutting through lesser curvature. The preparation was placed in petri dish containing Kreb's solution. The fundus strip was mounted in organ bath and allowed to stabilize for 30 min. and during this period PSS was changed after every 10 min. After the stabilization of preparation the dose response curve of 5-HT was taken till supramax was obtained. After that 10 µg of compound was added every time in organ tube, after 5 min. the response of 5-HT was reelicited till the maximum response was obtained. The graph thus obtained was used to plot log dose response curve. For each compound, three curves were plotted and the pA_2 value was calculated using the following equation. Contact time for drug was 90 sec.

pA_2 VALUE:

$$pA_2 = - \log [M] + \log [(A_2/A_1) -1]$$

3. RESULTS AND DISCUSSIONS

All synthesized 4-methyl-N-[(un)substituted phenyl]-N'-(pyridin-2-yl/phenyl) piperazin-1- carboxamidine and clozapine were evaluated *in vitro* for their binding to the dopamine and serotonin 5-HT receptors. The results are summarized in Table 1.

Compounds	R & X	pA_2 (Dopamine receptor antagonistic activity)	pA_2 (5-HT receptor antagonistic activity)
9	R = H, X =N	4.71 ± 0.11	4.56 ± 0.005
10	R = o-CH_3, X = N	5.29 ± 0.18	4.58 ± 0.011
11	R = p-Cl, X = N	4.6 ± 0.058	4.87 ± 0.014
12	R = o-CH_3, X = CH	5.28 ± 0.043	4.57 ± 0.026
13	R = p-Cl, X = CH	4.59 ± 0.003	4.6 ± 0.026
Clozapine*		5.39 ± 0.008	4.79 ± 0.11

* Under experimental conditions

Table 1 – Dopamine receptor and 5-HT receptor antagonistic activity of compounds

All the compounds have exhibited significant Dopamine receptor antagonistic activity with pA_2 value ranging from 4.60-5.29. Standard drug Clozapine has exhibited pA_2 value 5.39.

Out of all the compounds screened, compound 10 and 12 were found to be the most potent with pA_2 value 5.29 and 5.28 respectively, which was found to be comparable to standard drug Clozapine (pA_2= 5.39)

All the compounds have exhibited potentiation activity instead of antagonism against 5-HT receptor. Clozapine also potentiate 5-HT because it is having no antagonistic activity on 5-HT, but acts only on dopamine receptor. So, the compounds are also only dopamine antagonists.

4. CONCLUSION

In summary, we have reported a general route for the preparation of 4-methyl-N-[(un)substituted phenyl]-N'-(pyridin-2-yl/phenyl) piperazin-1- carboxamidine. Two compounds of this series 10 and 12 were shown to have similar *in vitro* activity as Dopamine antagonists as compared to the known antipsychotic agent clozapine.

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Dr. J. M. Amin[a], Dr. M. T. Chhabria[a]
A Department of Pharmaceutical Chemistry,
L. M. College of Pharmacy,
Ahmedabad