Available on line www.jocpr.com

Journal of Chemical and Pharmaceutical Research

ISSN No: 0975-7384
CODEN(USA): JCPRC5

J. Chem. Pharm. Res., 2010, 2(5); 485-493

Pharmacological screening of novel indolo [2,3-b] quinoxaline derivatives

Nandini R. Pai * and Deeptaunshu Atul Pusalkar

Department of Chemistry, D.G. Ruparel College, Mahim,Mumbai, Maharashtra, INDIA

ABSTRACT

Condensation of o-phenylenediamine with isatine in refluxing glacial acetic acid gives indolo[2,3-b] quinoxaline. This moiety further condensed with n'-(n-Halo aloxy)-3,4-dihydro carbostyril in refluxing acetonitrile using NaI,K_2CO_3 and TBAB as catalyst gave us desire product. Series of all six analogs are taken for their antibacterial and antipsychotic activity. The entire tested compound showed slight antibacterial as well as antipsychotic activity. This slight antimicrobial activity is due to presence of indolo[2,3-b] quinoxaline, and moderate antipsychotic activity due to the structural resemblance with the antipsychotic drug Aripiperazole.

Keywords: Quinoxaline, antibacterial activity, antipsychotic activity.

INTRODUCTION

Heterocycles consisting nitrogen, oxygen moieties constitute the core structures of molecules having various biological activities. The chemistry of indoloquinoxalines is of considerable interest possessing a broad spectrum of biological activities. Derivatives of indolo[2,3-b] quinoxaline posses diverse biological activities such as anticancer, anticonvulsant, antibacterial. These novel derivatives of indolo[2,3-b] quinoxalines also posses structural similarities with antipsychotic drug Aripiperazole.

We have synthesized a series of new compounds with a variety of modifications of lead compound i.e Aripiperazole (antipshychotic) 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. 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. In this paper, we describe the synthesis and the preliminary pharmacology of

EXPERIMENTAL SECTION

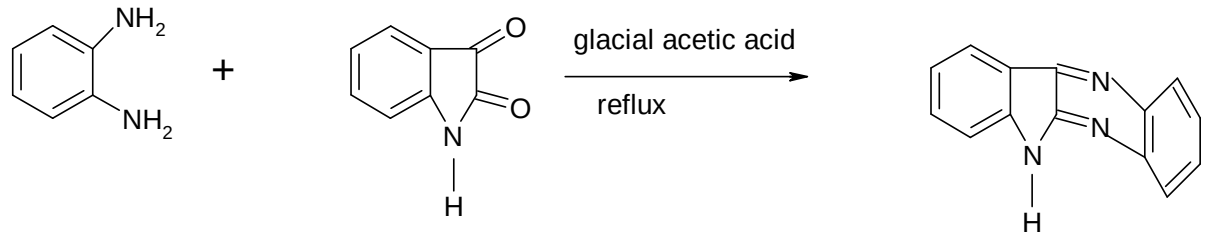
Chemistry

Melting points (mp) were determined using a Thomas Hoover capillary apparatus and are uncorrected. Infrared spectra were acquired on a Perkin Elmer FTIR. A Bruker, 300 MHz spectrophotometer was used to acquire ^{1}H-NMR spectra. All chemicals and laboratory grade (LR) reagents were obtained from Rankem (India), Merck, Sigma, Spectrochem and were used without further purification. 1-bromo -3-chloro-2 methyl propane was purchased from Japan.

Indolo [2,3-b] quinoxaline derivates have been synthesized by following route . These analogs were tested for their antibacterial and antipsychotic activity. The compounds are synthesized by reaction scheme given below.

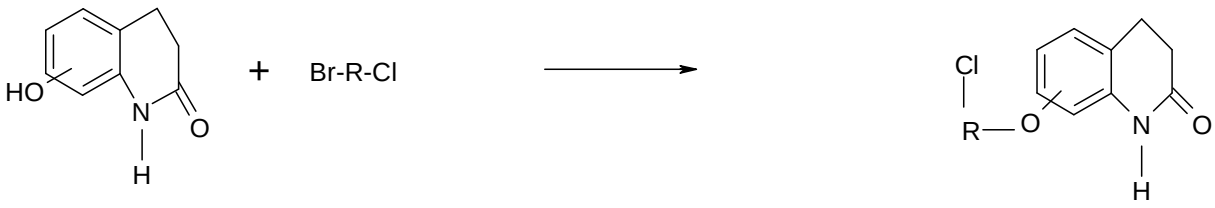
Reaction scheme-

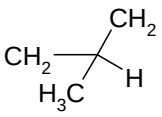
Stage 1-



A

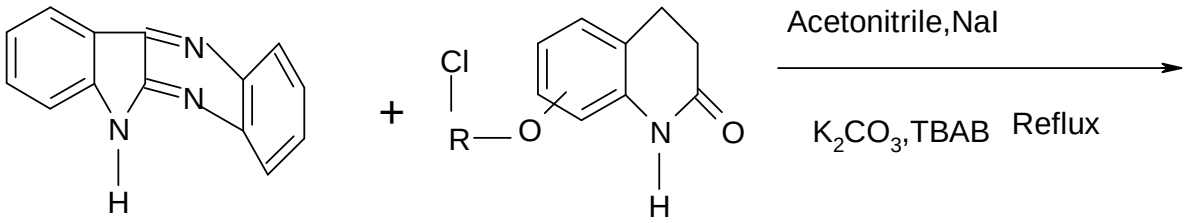
Stage 2-

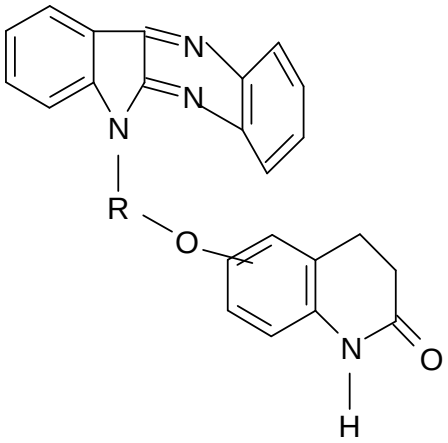


R= $(CH_2)_2$, $(CH_2)_3$, $(CH_2)_4$, $(CH_2)_5$, 

B(1-5)

Stage 3-





C(1-5)

Procedure-

Stage 1- General Synthesis of indolo[2,3-b] quinoxaline[1]

Isatine (1 mol) and o-phenylenediamine (1mol) were refluxed in glacial acetic acid for 4 hrs. Completion of reaction was checked on TLC [n-hexane: ethyl acetate (7:3)]. After completion of reaction it was filtered off and product was recrystallised by using DMF. Overall yield- 95%

Stage 2 - General synthesis of n'-(n-Halo aloxy)-3,4-dihydro carbostyril derivatives-

Stage 2 (a) synthesis of 6-(4-bromo butoxy)-3,4-dihydro carbostyril[2]

In round bottom flask 6-hydoxy carbostyril (1mol) ,in acetone –water(16:1 ml)is taken. To this potassium carbonate (1.1mol) and PEG(equivalent to weight of carbostyril taken) is added. This reaction mixture is stirred at room temperature for 2hrs. To this reaction mixture 1-bromo-4-chloro butane(3 mol) is added. This reaction mixture is stirred at room temperature for 20hrs. Progress of the reaction is checked by TLC Chloroform-Methanol(9:1). After completion of reaction is filtered off. The inorganic materials are washed with chilled acetone. The solvent is removed under vacuum below 40^0C. The thick oily residue was stirred with n-hexane. The solid product obtained is recrystallized using ethanol. Overall yield -90%

Stage 2 (b) synthesis of 7-(5-bromo pentoxy)-3,4-dihydro carbostyril[2]

Same procedure which is mentioned above has been carried out by using 1-bromo-5-chloro pentane for synthesis of above intermediate. Overall yield- 80%

Stage 2 (c)- 7-(2-bromo ethoxy)-3,4-dihydro carbostyril[3]

In round bottom flask 7-hydoxy carbostyril(1 mol) is taken. it is flushed using DMF. To the reaction mixture potassium carbonate (1.1mol) is added. It is stirred for 1 hr at room temperature. To the reaction mixture dibromo ethane(3mol) is added. The reaction mixture is stirred for 20 hrs. The progress of the reaction is checked on TLC Chloroform-Methanol (9:1). After completion of reaction it is filtered off and solvent is removed by vacuum distillation. The oily residue is obtained is stirred with n-hexane. The solid obtained is recrystallised by ethanol.
Overall yield-85%

Stage 2 (d)- 6-(3-chloro propoxy)-3,4-dihydro carbostyril[4]

In round bottom flask 6- hydroxy carbostyril (1mol) is taken in isopropyl alchohol. In reaction mixture NaOH(2 mol) is taken along with 1-bromo-3-chloro propane(3mol) .The reaction mixture is refluxed for 5 hrs. The progress of the reaction is checked on TLC chloroform: methanol(9:1). The reaction mixture is filtered and chilled. The solid obtained is recrystallised by using ethanol.
Overall yield 90%

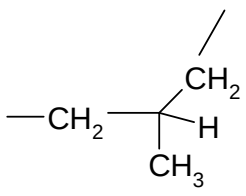
Stage 2 (e)- 7-(2-methyl-3-chloro propoxy)-3,4-dihydro carbostyril[4]

In round bottom flask 7- hydroxy carbostyril (1 mol) is taken in isopropyl alchohol. In reaction mixture NaOH(2 mol) is taken along with 1-bromo-3-chloro 2-methyl propane(3mol) .The reaction mixture is refluxed for 5 hrs. The progress of the reaction is checked on TLC chloroform: methanol(9:1). The reaction mixture is filtered and solvent is removed under vacuum. The oily residue is stirred with n-hexane and solid obtained is recrystallised by using ethanol. Overall yield-70%

Stage 2 (e)- 7-(6-bromohexoxy)-3,4-dihydro carbostyril[1]

Same procedure has been carried for synthesis of this out which is mentioned in stage 2b.

Table No 1: Physical data of the synthesized compounds

Compounds	R	R'	n	m.p	Molecular formula
C1	$(CH_2)_2$	H	7	$>225^0C$	$C_{25}H_{20}N_4O_2$
C2	$(CH_2)_3$	H	7	$>225^0C$	$C_{26}H_{22}N_4O_2$
C3	$(CH_2)_6$	H	7	$>225^0C$	$C_{27}H_{24}N_4O_2$
C4	$(CH2)_4$	H	6	$>225^0C$	$C_{27}H_{24}N_4O_2$
C5	$(CH2)_5$	H	7	$>225^0C$	$C_{28}H_{26}N_4O_2$
C6		H	7	$>225^0C$	$C_{27}H_{24}N_4O_2$

Stage 3- General synthesis of indolo[2,3-b] quinoxaline -n'-(n-Halo aloxy)-3,4-dihydro carbostyril [4]

In round bottom flask stage 2 (1mol) and stage1 (1mol) is taken in acetonitrile along with sodium iodide (1mol) and k_2CO_3(1 mol).the reaction is carried out using TBAB catalyst. The reaction

mixture is refluxed for 15 hrs. The progress of the reaction is checked on TLC Chloroform: Methanol(9:1). After completion of reaction the reaction mixture is filtered off and product is purified by using column chromatography.

These synthesized compounds are characterized by NMR and I.R spectroscopy and screened for their antibacterial and antipsychotic activity.

Table no 2 : I.R data of the synthesized compounds

Compound	I.R (KBr) Cm^{-1}
C1	1188(C=O str), 1589(aromatic C=C stre), 1681(C=O str),2839(Sp^3 C-H str),3055(Aromatic C-H str),3193(N-H str)
C2	1188(C-O str),1589(aroamic C=C str),1647(C=O str),2893(sp3C-H str),3062(aromatic C-H str),3201(N-H str)
C3	1164(C-O str), 1504(aromatic C=C str),1681(C=O str),2962(sp3 C-H str), 3078(aromatic C-H str), 3209(N-H str)
C4	1188(C-O str),1589(aromatic C=C str),1681(C=O str),2839(sp3 C-H str),3055(aromatic C-H str),3193(N-H str)
C5	1200(C-O str),1589.23(aromatic C=C str),1681.81(C=O str),2869(sp3 C-H str),3101(aromatic C-H str),3193(N-H str)
C6	1188(C-O str),1589(aromaric C=C str),1681(C=O str),2831(sp3 C-H str),3055.03(aromatic C-H str),3193.90(N-H str)

Table no 3: NMR data of the synthesized compounds

Compound	NMR (DMSO)
C1	2.20-2.40(t,2H, HN-C=O-CH2),2.80.3.00(Ar-CH2),3.60-3.80(t,2H,N-CH_2),4.40-4.60(t,2H,O-CH_2),6.20-6.40(d,2H,Ar-H),6.60-6.80(d,2H,Ar-H), 7.20-7.40(t,1H,Ar-H),7.60-7.80(m,4H,Ar-H),8.00(t,1H,Ar-H),8.20(d,1H,Ar-H),8.30(d,1h,Ar-H),10.20(s,1H,N-H)
C2	2.20-2.60(m,4H, HN-C=O-CH2, CH_2), 2.80(t,2H,Ar-CH2),4.00(t,2H,N-CH_2),4.60(t,2H,O-CH_2),6.20-6.40(d,2H,Ar-H), 7.00(d,1H,Ar-H),7.15-7.25(t,1H,Ar-H), 7.40-7.80(m,4H,Ar-H), 8.00(d,1H,Ar-H), 8.20(d,1H,Ar-H),8.40(d,1H,Ar-H),10.00(s,1H,N-H)
C3	1.07-1.03(t,2H,CH2), 1.53-1.41(m,2H,CH2),1.70-1.90(m,4H,CH2), 2.41-2.36(t,NH-C=O-CH2),2.89-2.84(t,2H,Ar-CH2),4.03-3.98(t,2H, N-CH_2),4.67-4.62(t,2H, CH_2-C=O),6.38-6.36(t,2H,Ar-H),7.17-7.15(d,1H,Ar-H),7.62-7.57(m,1H,Ar-H),7.99-7.87(m,4H,Ar-H),8.21-8.17(m,1H,Ar-H), 8.36-8.33(q,1H,Ar-H), 8.58-8.56(d,1H,Ar-H), 9.80(s,1H,N-H).
C4	1.88-1.82(q,2H,CH_2), 2.16-2.09(q,2H,CH_2), 2.49-2.44(q,2H,NH-C=O-CH_2),2.85-2.80(t,2H,Ar-CH_2), 4.04-4.00(t,2H,N-CH_2), 4.67-4.62(t,2H,O-CH_2), 6.51-6.48(t,2H,Ar-H), 7.07-7.05(d,1H,Ar-H), 7.52-7.47(m,1H,Ar-H),7.93-7.81(m,4H,Ar-H),8.21-8.17(dd,1H,Ar-H),8.36-8.33(q,1H,Ar-H), 8.48-8.46(d,1H,Ar-H),10.04(s,1H,N-H)
C5	1.49(m,2H,CH_2),1.77-1.75(t,2H,CH_2),1.96(m,2H,CH_2), 2.41-2.36(t,2H,NH-C=O-CH_2),2.76-2.72(t,2H,Ar-CH_2),3.85-3.81(t,2H,N-CH_2), 4.54-4.49(t,2H,O-CH_2), 6.38-6.36(t,2H,ArH), 6.98-6.95(d,1H,Ar-H), 7.43-7.41(t,1H,Ar-H), 7.83-7.72(m,4H,Ar-H), 8.12-8.09(t,1H,Ar-H), 8.28-8.24(q,1H,ArH),8.40-8.37(d,1H,Ar-H),9.50(s,1H,Ar-H)
C6	1.00(s,3H,[CH_2]$_2$-CH-CH_3], 1.87-1.82(t,2H,N-H-C=O-CH2),2.20-2.30(t,2H,Ar-CH_2),2.50(m,1H,C-H),4.00(2dd,2H,N-CH_2),4.30(t,2H,O-CH_2),6.60(t,2H,Ar-H),7.07-7.05(d,1H,Ar-H),7.52-7.47(t,1H,A-H),7.93-7.81(m,4H,Ar-H),8.20-8.17(t,1H,Ar-H),8.36-8.33(t,1h,Ar-H),10.00(s,1H,Ar-H)

Pharmacological Screening

1) Antibacterial activity

Cup plate method using Hi-Media agar medium is employed to study the antibacterial activity of against *Staphylococcus aureus, Bacillus subtilis, Psuedomonas aeruginosa* and *Escherichia*

coli[5]. Preparation of nutrient broth, subculture, base layer medium, agar medium and peptone water is done as per the standard procedure. Each test compound (50mg) is dissolved in 50 mL of Dimethyl Formamide (1000 μg/mL), which is used as sample solution. Sample size for all the compounds is fixed as 0.1 mL.

The cups are made by scooping out agar medium with sterilized cork borer in a petri dish, which is previously inoculated with the microorganisms. The solution of each test compound (0.1 mL) is added in the cups and petri dishes are subsequently incubated at 370 for 48 h. Ampicillin and Streptomycin are used as reference drugs and Dimethyl Formamide as a control. All the newly synthesized compounds show antibacterial activity against *S. aureus, B. subtilis, P. aeruginosa* and *E. coli*, the data of which is presented in Table 1.

Thus a series of analogs of indolo[2,3-b] quinoxaline have been synthesized which shows slight antibacterial activity.

Table 4 represent the antibacterial activity of the synthesized compounds which is given below.

Table No 4: Antibacterial activity of the compounds

Compound	**Gram positive Bacteria** ***S. aureus***	**Gram positive Bacteria** ***B. subtilis***	**Gram negative bacteria** ***P.aeruginosa***	**Gram negative bacteria** ***E.coli***
Ampiciline	+++	++	++	+++
Streptomycine	+++	+++	+++	+++
C1	+	+	-	-
C2	-	-	-	++
C3	+	-	-	-
C4	++	-	-	-
C5	+	-	-	+
C6	-	+	+	_

Meaning of symbols: ; Inactive = - (inhibition zone < 6 mm), slightly active = + (inhibition zone 6-9mm) moderately active = + + (inhibition zone 9-12 mm) highly active = + + + (inhibition zone> 12 mm).

2) Antipsychotic activity

Objectives

1) To evaluate antagonist activity of the synthesize compounds for their ability to inhibit APO-induced stereotypic behavior in mice (anti-APO test).
2) To evaluate DA autoreceptor agonist activity of the selected compounds.
3) To evaluate ability of selected compounds to induce catalapsy in mice.
4) To test compounds for alpha-adrenoceptor antagonist activity.

Research involving investigations using experimental animals adhered to the “Principles of laboratory animal care” (NIH publication # 85-23, revised in 1985).

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[6]. 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 2.

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[9]. DOPA was determined according to the literature method[7-8]. 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 2.

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 no 2.

Anti-epinephrine Test

This test was performed by the method reported[9]. 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 2.

CONCLUSION

Result and structure activity relationship

1) DA receptor antagonist activity

These novel analogs are examined for postsynaptic DA receptor antagonist activity. In cpmpound C1where there is ethyl linker attached shows no potency having ED^{50} >8.0micro mol/kg po, as well as C2 where there is propyl linker is attached shows no potency having ED^{50} >8.0micro mol/kg po. Where as in C3 there is hexyl linker shows better potency than C1 and C2 where its value is 3.10 micro mol/kg po. Where as in C4 there is butyl linker and attachement of the oxygen atom of the carostyril ring is at position number 6^{th} of the benzene ring shows potency 0.9 micro mol/kg po.In next analogs where there is pentyl linker shows no potency with ED^{50} value >8.0micro mol/kg po,and in last analog where there is 2-methyl-propyl linker shows potency with value 3.7 micro mol/kg po which has highest potency.

2) DA autoreceptor agonist activity

These synthesized compounds were tested for their ability to reverse GBL-induced increase in DOPA synthesis in the mouce brain. The compound C1 where there is there is ethyl linker attached shows potency with value ED^{50} 1.38micro mol/kg po. The compound C3 there is hexyl linker shows potency with value of ED^{50} 2.8 micro mol/kg po. Compounds C4 and C6 shows potency with ED^{50} value with1.5 micro mol/kg po and 3.9 micro mol/kg po respectively.

3) Catalepsy

The EPS liability and alpha^1-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.

Compounds C1, C2, C5,C6 have not been tested for catalepsy. Compound C3 shows good potency to induce catalepsy in mice with ED^{50} value 2.1 micro mol/kg po. Compound C4 shows catalepsy value ten times higher then the value of DA receptor antagonist activity i.e 9.1 mol/kg po. The reference drug Chlopromazine is observed to show low activity towards inducing the catalepsy in mice with ED^{50} 24.5 micro mol/kg po.

4) Alpha^1- adrenoceptor antagonist activity

Selected compounds were also tested for their alpha^1- adrenoceptor antagonist activity, since peripheral alpha 1-adrenoreceptor antagonism has been known to cause autonomic side effects Compound C4 and C6 are inactive upto >156 to >256.

The study shows that Compound C3has exhibited consistent activity for all tests. But this compound has very potency in Alpha^1- adrenoceptor antagonist activity which indicates it will have adverse effects as a drug and not recommended.

Table no 5: Antipshychotic activity of synthesized compounds

Compounds	DA receptor antagonist activity[c] (A)	DA autoreceptor agonist activity	Catalepsy (B)	alpha^1 – adrenoceptor antagonist activity	ED_{50} Ratio B/A
Chlorpromazine (Reference Drug)	10.6	IA	19.5	26.8	1.83
C1	>8.0[a]	1.3	NT	NT	-
C2	>7.0[a]	NT	NT	NT	-
C3	3.1	2.8	2.1	7.0	0.67
C4	0.9	1.5	9.1	>256	10.11
C5	>8.0[a]	NT	NT	NT	NT

REFERENCES

[1] Reaction of o-phenylene diamine with isatine. A.V.Ivashchenko, A.G.Drushlyak and V.V.Titov Chemistry Of Heterocyclic Compounds.Vol 20.Number-5, 537-542

[2] WO 2007/094009, PCT/IN 2006/000362 A novel process for preparation of Aripiperazole and its intermediates. Ramkrishnan,arul; Subhash V Damle; Dharmesh G Panchal

[3] Novel antipsychotic agents with Dopamine autoreceptor agonist activity. Yasuo oshiro, seji sato, Takaonishi *J.med.Chem*. **1988**, 41, 658-667.
[4] Yasuo Oshiro,Takashi Himaya, Kazuyuki Nakagawa *Chem.Pharm.Bull*.**1988**, 36(11) 4377
[5] British Pharmacopoeia. **2005**, Vol. IV, p. A300 Appendix XIV
[6] Jaen, J.C.,Wise,L.D.,Heffner,Dugsley,*J.Med.Chem*.,**1988**,31,1621-1625
[7] Carlsson,A.Usdin,E.,Bunny,Marcel Dekker,New York,**1975**,pp 49-65
[8] Jannsen,P.A.,Niemergars,C,J,E,Schellenkens,K.H.K., *Arzneim Forsch (Drug Res.),* **1965**, 15, 104
[9] Simpson,G.M.,Sramek,*Drugs*,**1982**,23,381-393