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Journal of Chemical and Pharmaceutical Research, 2012, 4(11):4946-4952

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Application of click chemistry in the synthesis of coumarin based glycoconjugates

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ABSTRACT

Click chemistry refers to a group of reactions that are fast, simple to use, easy to purify,versatile, regiospecific, and give high product yields. While there are a number of reactions that fulfill the criteria, the Huisgen 1, 3-dipolar cycloaddition of azides and terminal alkynes has emerged as the frontrunner. It has found applications in a wide variety of research areas, including materials sciences, polymer chemistry, and pharmaceutical sciences.

Keywords: Glycoconjugates, coumarin, click chemistry, antibacterial activity, antipsychotic activity.

INTRODUCTION

Recently 1, 3-Dipolar cycloadditions (1,3 – dcr) of azides and alkynes (Click Chemistry) has attracted great attention of many synthetic chemists as the process allows coupling two or more complex molecules resulting into new molecular entity containing triazole ring as linker. Considering the involvement of various glycoconjugates in biological events, various glycoconjugates such as neoglycoconjugates, ferrocene glycoconjugates and many other glycoconjugates have been synthesized employing click chemistry protocol. As a contribution to this area, it appeared of interest to prepare another class of glycoconjugates by the incorporation of sugar residue into substrates endowed with biologically important activites such as coumarins; the derived molecular entity might improve their bioavailability while retaining their activity and selectivity.

Coumarin was chosen as the profluorophore since it is small in size and also the coumarin and its derivatives represent one of the most active classes of compounds possessing a wide spectrum of biological activity[1-9] Many of these compounds have proved to be active as antitumor[1-2], antibacterial[3,4], antifungal[5-7],anticoagulant[8] and antiinflammatory[9]. In addition, these compounds are used as additives to food and cosmetics [10], dispersed fluorescent and laser [11]. Various analogues of 3-substituted coumarins such as 3-aminocoumarins exhibit antimicrobial activity [12, 13].

In view of this, some novel coumarin-sugar conjugates have been synthesized under the click condition utilizing azido coumarins and sugar propargyl ethers.Azido coumarins were made to react with propargyl ethers of sugars in the presence of $CuSO_4.5H_2O$ or Cu $(OAc)_2$, sodium ascorbate in tertiary butanol and water at room temperature to afford regioselectively 1, 4-disubstituted triazole linked coumarin based glycoconjugates.

In a way the methodology described in this work offers an alternative approach of generating coumarin-based glycoconjugates in facile manner

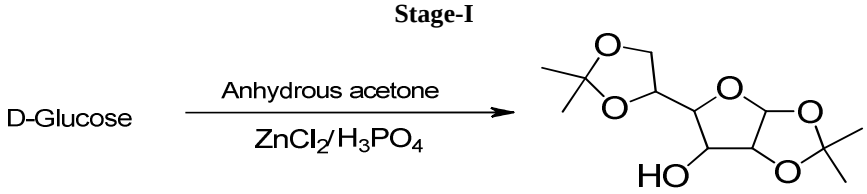
EXPERIMENTAL SECTION

Chemistry

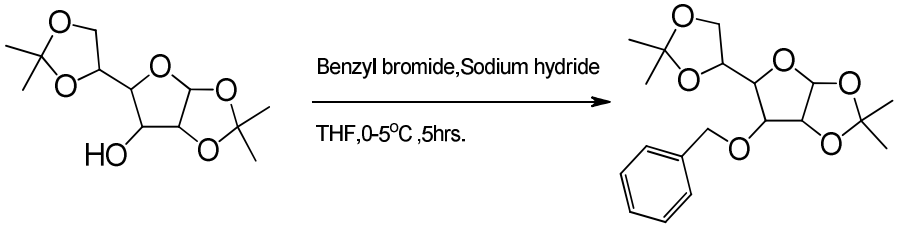
Melting points (mp) were determined using a Thomas Hoover capillary apparatus and are uncorrected. Infrared spectra were acquired on a Perkin Elmer FTIR. A Bruker, 300 MHz spectrophotometer was used to acquire 1H-NMR spectra. All chemicals and laboratory grade.

Reaction Scheme

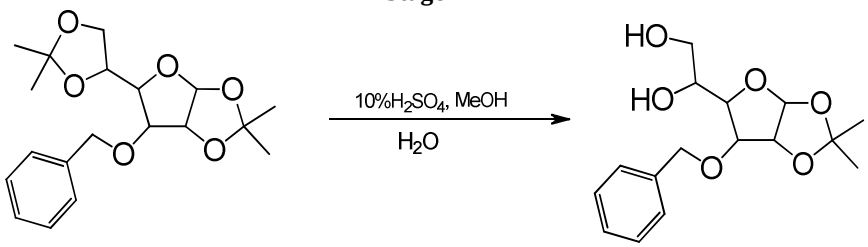
Stage-I



Stage-II



Stage-III



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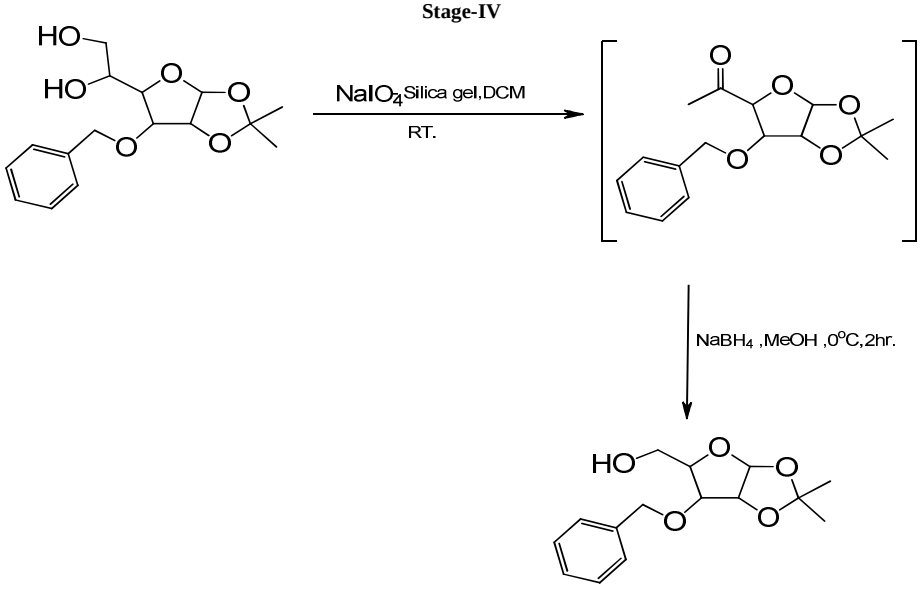
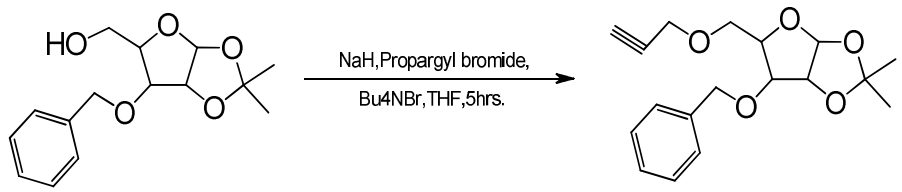
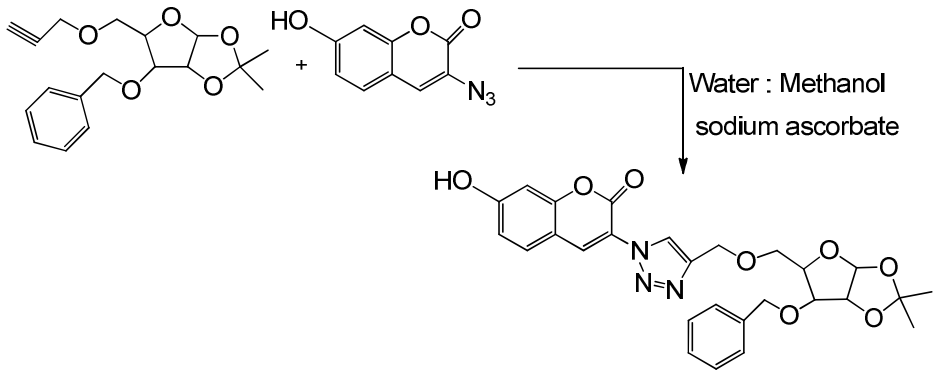


Stage-V



Stage-VI



PROCEDURE-

Stage 1-

To a suspension of dry D-glucose (500g) in acetone (3000 ml) was added pulverized anhydrous zinc chloride (400g) and 30 ml of phosphoric acid. The reaction mixture was stirred for 36 hours.Unreacted glucose was filtered and inorganic salt were precipitated by the addition of solution of sodium hydroxide (330g) in water (600 ml).The resulting suspension filtered ,the residue was washed with acetone and the acetone was evaporated. The mass was dissolved in water (1500 ml) and extracted with dichloromethane. The collected organic phase were dried over anhydrous Na_2SO_4 and evaporated. The resultant mass was then dissolved in hexane and decolorized with charcoal and recrystallized to give compound (505g 70%)

Stage 2 –

Hexane washed sodium hydride (60 %, 58 g,) was suspended in dry THF (250 mL) at 0^oC. A solution of 1, 2:5, 6-di-*O*-isopropylidene-D-glucofuranose from stageNo.1 (250 g) in 1250 mL THF was added drop wise using dropping funnel and the mixture was stirred for 0.5 h at rt. Benzyl bromide (145 mL) was added slowly at 0^oC. The mixture was stirred for 5 h at rt. Saturated NH_4Cl was added slowly at 0^oC. The solution was extracted with $CHCl_3$, dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated to give yellow syrup. The crude syrup was purified by column chromatography to benzyl ether as yellow liquid (268 g, 80 %)

Stage 3

Benzyl protected diacetonide from stageNo.2 (35 g,) was dissolved in methanol (543 mL), water (109 mL) and 10% H_2SO_4 (25 mL) and stirred at room temperature. After 5 hours, saturated potassium carbonate was added to neutralized the reaction mixture to pH = 7-8. Methanol was evaporated and the residue was extracted with $CHCl_3$ (175 ml X 4). Organic layer was dried over sodium sulphate and evaporated to give syrup as Compound 3 (29 g, 93 %).

Stage 4

To vigorously stirred slurry of silica gel (130 g, 60-120 mesh) in DCM (500 mL) a solution of $NaIO_4$ (31 g in 100 mL H_2O) was slowly added and continued stirring at room temperature. After 10 minutes, a solution of compound from stageNo.3 (25 g,) in MeOH (10 mL) was added at room temperature and continued stirring for additional 3 h. The mixture was filtered and the silica gel was thoroughly washed with DCM. Removal of the solvent from the filtrate afforded crude aldehyde as light liquid that was used in the next stage without purification

Aldehyde was dissolved in 15 mL MeOH. The solution was cooled at 0^oC and $NaBH_4$ (0.5 g,) was added in portions. The mixture was stirred for 2 h before quenching by the addition of water and dichloromethane. The

organic layers were washed with brine and dried over sodium sulphate and concentrated under reduced pressure to give product. The crude product was purified by column chromatography on silica gel to afford pure product (20 g, 88.5 %).

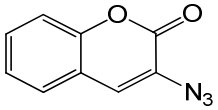
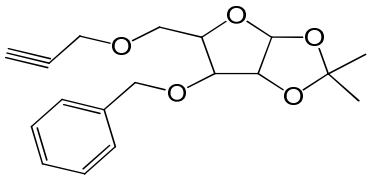
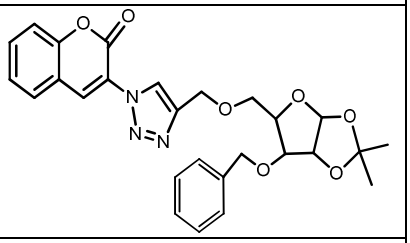
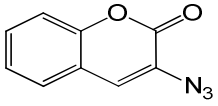
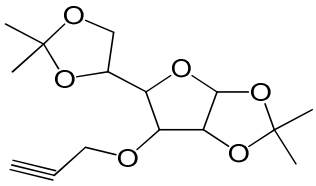
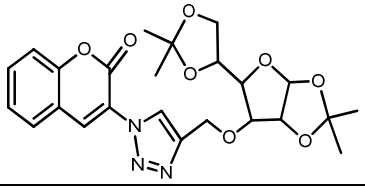
Stage 5

Hexane washed sodium hydride (60%, 6.8g,) was suspended in dry THF (150 mL) at 0^{o}C. A solution of alcohol from stageNo.4 (15g,) in 50 mL THF was added drop wise using dropping funnel and the mixture was stirred for 0.5 h at rt. Propargyl bromide (12.5 mL, 96 mmol) was added slowly at 0^{o}C followed by Bu_4NBr in catalytic amount. The mixture was stirred for 5 h at rt. Saturated NH_4Cl was added slowly at 0^{o}C. The solution was extracted with $CHCl_3$, dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated to give yellow liquid. The crude syrup was purified by column chromatography to propargyl ether as yellow syrup (13.5 g, 79 %).

Stage 6

In a mixture of acetylene from stageNo.5 (10g) and 7-hydroxy-3-azidocoumarin (25g) in water and methanol(v/v = 1:1,150 ml),sodium ascorbate (2ml) of freshly prepared 1 M solution in water was added,followed by the addition of copper(II) sulfate pentahydrate 7.5% in water (1.2ml).The heterogeneous mixture was stirred vigorously overnight at room temperature.TLC analysis indicated complete consumption of the reactants in 12 h.The methanol was removed and the residue was diluted with water (150 ml),cooled in ice and then precipitate was collected by filtration .After washing the precipitate with cold water (50 ml),it was dried under vacuum to afford (12.0g,75.94%) of pure product.

Table No 1: Structurally diverse novel analogs of lead Molecule

Compound No.	Azides	Propargyl Ethers	Cycloadducts
1			
2			

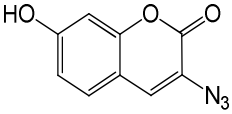
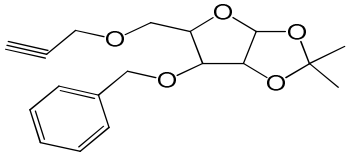
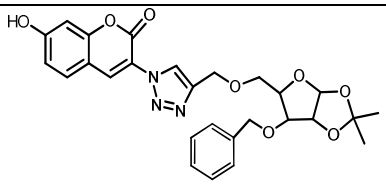
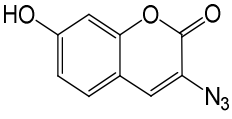
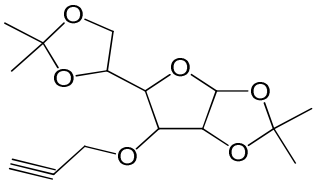
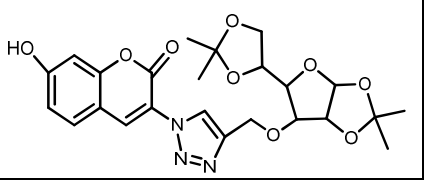
Compound No.	Azides	Propargyl Ethers	Cycloadducts
3			
4			

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

Compound No.	IR
1	3063.80 cm^{-1} (aromatic C-H stretching), 2923.35 cm^{-1} (aliphatic C-H stretching), 1763.10 cm^{-1} (C=O stretching), 1548.31-1472.05 cm^{-1} (aromatic region), 1364.55 cm^{-1} (C-N stretching).
2	3058.67 cm^{-1} (aromatic C-H stretching), 2935.67 cm^{-1} (aliphatic C-H stretching), 1795.04 cm^{-1} (C=O stretching), 1578.81-1456.96 cm^{-1} (aromatic region), 1369.58 cm^{-1} (C-N stretching).
3	3356.11 cm^{-1} (O-H stretching), 3054.98 cm^{-1} (aromatic C-H stretching), 2916.90 cm^{-1} (aliphatic C-H stretching), 1694.13 cm^{-1} (C=O stretching),1595.45-1487.36 cm^{-1} (aromatic region), 1362.95 cm^{-1} (C-N stretching).
4	3356.11 cm^{-1} (O-H stretching), 3058.67 cm^{-1} (aromatic C-H stretching), 2935.67 cm^{-1} (aliphatic C-H stretching), 1795.04 cm^{-1} (C=O stretching), 1578.81-1456.96 cm^{-1} (aromatic region), 1369.58 cm^{-1} (C-N stretching).

Table no 3: NMR data of the synthesized compounds

Compound No.	^{1}H NMR
1	δ 1.33 (s ,3H) , δ 1.50 (s ,3H),δ 3.87 (d ,2H), δ 4.01 (m ,1H), δ 4.42 (m ,1H), δ 4.51 (t ,1H), δ 4.67 (s,2H), δ 4.72 (s ,2H), δ 5.95 (s ,1H), δ 7.26 (d ,1H), δ 7.32 (m ,3H), δ 7.37(m ,1H), δ 7.64 (m ,2H) , δ 7.70 (m ,2H), δ 8.57 (s ,1H) , δ 8.7 (s ,1H).
2	δ 1.33 (d ,6H) , δ 1.44 (d ,6H),δ 3.99 (t ,1H), δ 4.16 (m ,3H), δ 4.36 (t ,1H), δ 4.67 (t ,1H), δ 4.88 (s,2H), δ 5.92(s ,1H), δ 7.48 (q ,2H), δ 7.69 (q ,2H), δ 8.62 (s ,1H) , δ 8.75 (s ,1H).
3	δ 1.33 (s ,3H) , δ 1.50 (s ,3H),δ 3.87 (d ,2H), δ 4.01 (m ,1H), δ 4.42 (m ,1H), δ 4.51 (t ,1H), δ 4.67 (s,2H), δ 4.72 (s ,2H), δ 5.95 (s ,1H), δ 5.97 (s ,1H), δ 7.26 (d ,1H), δ 7.32 (m ,3H), δ 7.37(m ,1H), δ 7.64 (m ,2H) , δ 7.70 (m ,2H), δ 8.57 (s ,1H) , δ 8.7 (s ,1H).
4	δ 1.33 (d ,6H) , δ 1.44 (d ,6H),δ 3.99 (t ,1H), δ 4.16 (m ,3H), δ 4.36 (t ,1H), δ 4.67 (t ,1H), δ 4.88 (s,2H), δ 5.91(s ,1H),δ 5.92(s ,1H), δ 7.28 (s ,1H), δ 7.41 (d ,1H), δ 7.43 (d ,1H), δ 8.62 (s ,1H) , δ 8.75 (s ,1H).

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 [14]. 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.*Tables 4 represent the antibacterial activity of the synthesized compounds which is given below.

Table No 4: Antibacterial activity of the compounds

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

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 APOinduced 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 [15]. The ED50 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 [16]. DOPA was determined according to the literature method [17-18]. A Chemocosorb 5-ODS (20-x4.6-mm i.d.) separation column was used. The mobile phase contained 50 mM KH2PO4, 8 Mm H3PO4, and 2.5 mM EDTA. Na in 0.7 % acetonitrile (pH 3). The ED50 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 ED50 values and 95 % confidence limits were calculated by the probit method, and the values are presented as m mol/Kg po in Table no 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 ED50 values and 95 % confidence limits were calculated by the probit method, and the values are presented as m mol/Kg po in Table 5.

CONCLUSION

Result and structure activity relationship
1) DA receptor antagonist activity
These novel analogs are examined for postsynaptic DA receptor antagonist activity. In compound C2 as well as C4shows no potency having ED50 >8.0micro mol/kg po,Where as in C1 and C3 there is benzyl group and hydroxyl group attached shows better potency than C2 and C4 where its value is 3.10 micro mol/kg po and 0.9 micro mol/kg po.

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 shows potency with value ED50 2.8 micro mol/kg po. The compound C3 shows potency with value of ED 50 1.5 micro mol/kg po. Compounds C2 shows potency with ED 50 value with1.3 micro mol/kg po and compound C4 is not tested.

3) 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.Compounds C2, C4 have not been tested for catalepsy. Compound C1 shows good potency to induce

catalepsy in mice with ED50 value 2.1 micro mol/kg po. Compound C3 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 ED50 19.5 micro mol/kg po.

4) Alpha1- adrenoceptor antagonist activity

Selected compounds were also tested for their alpha1- adrenoceptor antagonist activity, since peripheral alpha 1-adrenoreceptor antagonism has been known to cause autonomic side effects Compound C3 is inactive upto >156 to >256.

The study shows that Compound C3has exhibited consistent activity for all tests. But this compound has very potency in Alpha1- 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	ED50 Ratio B/A
Chlorpromazine (Reference Drug)	10.6	IA	19.5	26.8	1.83
C1	3.1	2.8	2.1	7.0	0.67
C2	>8.0[a]	1.3	NT	NT	-
C3	0.9	1.5	9.1	>256	10.11
C4	>7.0[a]	NT	NT	NT	-

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