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Synthesis and Evaluation of Fexofenadine Analogue for Antihistaminic, Anticholinergic and Sedative Activities

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A novel α-[4-isopropyl phenyl]-4-(hydroxy diphenyl methyl)-1-piperidinyl)butan-1-one (**II**) has been synthesized by Friedel Craft's acylation and condensation reaction. The structures of the synthesized compounds were elucidated by (IR, PMR and Mass) spectral analysis. The compound synthesized was screened for anti-histaminic activity by histamine induced contractions using guinea pig ileum, anticholinergic by guinea pig tracheal chain and sedative activity. The compound exhibited significant anti-histaminic activity.

Key Words: Synthesis, Evaluation, Fexofenadine, Antihistaminic, Anticholinergic, Sedative activities.

INTRODUCTION

Histamine is one of the most frequently studied local hormones and first clinical recognized as a mediator of immediate allergic response in humans. Despite the compelling evidence that suggests histamine playing an important pathological role in asthma[1,2]. Up to now, four of its target proteins have been cloned (H_1, H_2, H_3 and H_4 receptors) from among which H_1 and H_2 receptors are assigned to an over riding pharmaceutical importance. The H_1 receptor is mainly responsible for the inflammatory effect of histamine, example smooth muscle contraction, increasing blood vessel permeability, releasing other local hormones[3]. Therefore, it is the target of most drugs developed against allergic rhinitis. By means of the H_2 receptors, histamine is responsible for the stomach's HCl production, which makes it possible to treat ulcer with H_2 antagonists. Several effective and selective H_2 antagonists are available, that can be used without any serious side effect[3]. The H_3 receptor can enhance some neurotransmitter's release.

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A few H_3 antagonists have already been developed for the treatment of neurological and cognitive disorder, but the efficiency of these compounds has been confirmed reassuringly yet[4]. The H_4 receptor was cloned just a few years ago[5] and there is not sufficient information about its physiological effects, but it seems to play a role in histaminergic receptor mediated chemotoxis[6]. Histamine is performed in cells, has a rapid onset of action and is therefore mainly responsible for the phase of allergic reactions. The first and second generation antihistamines are in use, but these agents exert non negligible adverse effects on the central nerves system such as drowsiness[7] new antihistamines which lack this defect therefore represent attractive target for the drug discovery in the field of antiallergic agents. Some such desired antihistamines terfenadine[8] and astemizole[9] have already been discovered, but we independently focused on the search for new antihistamines using fexofenadine as a chemical lead. Hence, there is a need to develop molecules for the treatment of allergy symptoms with fewer side effects. In this regard because fexofenadine is a widely used drug in the treatment of allergic symptoms, we made an approach to synthesize some relatively complex novel fexofenadine analogues with intention to widen synthetic approaches to synthesize these compounds.

Terfenadine was a selective H_1-receptor antagonist with weak anticholinergic activity, which was originally developed as a selective dopamine receptor antagonist belonging to the haloperidol and azacyclonol class. However, it led to the development of terfenadine, a peripherally acting H_1-receptor antagonist with minimal CNS depression as side effect[10]. A structurally related product of terfenadine, ebastine has also been claimed to have less sedative effects[11]. The novel substituted piperidine derivatives were screened for antihistaminic activity by histamine induced isolated guinea pig ileal muscle contraction[12].

From the literature, it is clear that Friedel Craft's acylation is used, but desired *para*-substituted product however could not be separated from the regioisomers[13]. An alternative strategy employed palladium oxidation catalyzed coupling of terminal alkyne and aromatic bromide followed by regioselective hydration proved very successful[14].

Herewith, we report the synthesis of α-[4-isopropyl phenyl]-4-(hydroxy diphenyl methyl)-1- piperidinyl)butan-1-one (**II**) by Friedel Craft's acylation and condensation with good yield.

EXPERIMENTAL

The melting points were found out using open capillary tubes and were uncorrected. FTIR spectra were recorded using FTIR (Shimadzu-8000) by KBr pellet technique. ^{1}H NMR spectra were recorded by using $CDCl_3$ as a solvent and TMS as an internal standard (AMX 400 MHz). To obtain

molecular weight information, the analogues were analyzed by EI Mass spectroscopy. Purity of the compounds synthesized was monitored by TLC using aluminium plates precoated with silica gel. All the chemicals were obtained from R.L.Fine Chemicals, Bangalore.

The intermediate 4-chloro-1-(4-isopropyl phenyl)butan-1-one (**I**) was prepared by the following method. An equimolar (0.01 mol) mixture of isopropyl benzene, 4-chlorobutaroyl chloride and aluminium chloride was stirred for 4 h at 0-5ºC in 10 mL of dichloromethane. The yield of obtained compound was 80 %. IR (KBr, cm^{-1}): 3010 ν(Ar-CH), 2950 ν(CH), 1735 ν(C=O), 1600 ν(C=C), 830 ν(C-Cl).

The α-[4-isopropyl phenyl]-4-(hydroxy diphenyl methyl)-1-piperidinyl)butan-1-one (**II**) was prepared by following method. An equimolar (0.006 mol) mixture of (**I**), azacyclonol and sodium carbonate was refluxed for 8 h in toluene. The mixture was poured into water; the organic layer was distilled off to get the product. Yield: 80 %, m.p. 190-196ºC. IR (KBr, cm^{-1}): 3404 ν(OH), 3010 ν(Ar-CH), 2951 ν(CH), 1735 ν(C=O), 1600 ν(C=C), 1442 ν(CH), 1049 ν(CN), 750 ν(CH); PMR ($CDCl_3$) δ ppm: 2.7 (b, 1H, -CH-), 1.2 (s, 6H, CH_3), 7.4-7.5 (q, 4H, ArH), 7.1-7.4 (m, 10H, ArH), 2.3-2.4 (m, 6H, CH_2-CH_2-CH_2), 2.2-2.5 (m, 5H, -CH_2-N-CH_2-, OH), 1.9-2.1 (m, 5H, -CH_2-CH-CH_2); EI-MS (m/e): 455.631.

In vitro inhibition of histamine induced contraction on guinea pig ileum

In this method, a piece[15] of ileum about 2 cm length isolated from guinea pigs was trimmed, tied at both ends and mounted in a 20 mL organ bath containing Krebs-buffer (37ºC, constantly bubbled with 95 % O_2/5 % CO_2) the first three dose-response experiments were performed by adding histamine cumulatively to the organ bath. After adequate washing, the ileal strip was incubated with the testing compound for 0.5 h. The dose-response experiment was then conducted again. The dissociation constant (K_b) of the receptors-antagonist complex was used as the parameters to indicate the potency of the testing compound and is calculated according to the cheng-prusoff equation.

In vitro inhibition of histamine induced contraction on guinea pig Trachea

Male guinea pigs (400-700 g) were killed by a blow on the neck and then trachea was removed. Each trachea was cut into 10 rings (each containing 2-3 cartilaginous rings). All the rings were then cut open opposite the trachealis muscle and sutured together to form tracheal chain[16].

Tissue was suspended in 20 mL organ bath containing Krebs-Hense leit solution of the following composition mM: NaCl, $NaHCO_3$, $MgSO_4$, KH_2PO_4, KCl, $CaCl_2$ and dextrose.

The Krebs solution was maintained at 37ºC and constantly bubbled with 95 % O_2/5 % CO_2. The tissue was suspended under an isotonic tension of 1 g and allowed to equilibrate for atleast 1 h while it was washed with Krebs solution for every 15 min.

The inhibitory effect of compound **II** on histamine H_1 receptors was examined by producing cumulative log concentration response curve of histamine acid phosphate induced contraction of tracheal chains 10 min after exposing tissue to one solution of compound **II** in 20, 60 and 200 µM and histamine 0.001 µM, (Sigma Chemical Ltd) the consecutive concentrations of histamine were added for every 2 min (range 0.1-1000 µM) and the percentage of contraction due to each concentration in proportion to the maximum contraction obtained was plotted against log concentration of histamine.

The concentration response curve to histamine was constructed by cumulative addition of histamine in absence and in presence of antagonists. The antagonist was added to the bath after 0.5 h of histamine response alone, antagonist was incubated with the tracheal chain preparation for 0.5 h before addition of cumulative concentration of histamine.

Isolated tracheal muscles of guinea pig relaxant effect of this compound **II** was demonstrated on isolated guinea pig tracheal chains.

***In vitro* inhibition of acetyl choline induced contraction on guinea pig trachea**

Tissue preparation: Male guinea pig 400-700 g was sacrificed by a blow to the neck and the trachea were removed. Each trachea was cut in to 10 rings (each ring contained 2-3 cartilaginous rings). The rings were then cut open opposite the tracheal muscle and sutured together to form a tracheal chain (n = 7)[16].

Tracheal chain tissue was then suspended in a 10 mL organ bath containing Krebs-Hense Leit solution with the following composition (mM): NaCl, $NaHCO_3$, $CaCl_2$, $MgSO_4$, KH_2PO_4, KCl and dextrose.

The Krebs solution was maintained at 37ºC and aerated with 95 % O_2 and 5 % CO_2. Tissue was suspended under an isotonic force of 1 g and allowed to come to equilibrium for at least 1 h while washing with Krebs solution every 15 min.

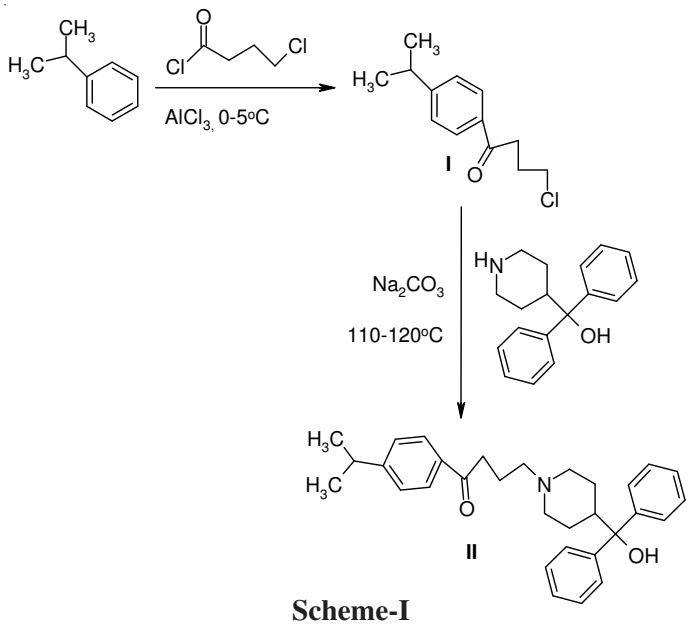
The effect of different concentrations of compound **II** was tested on each trachea, the concentration response curve to Ach was constructed by cumulative addition of Ach in absence and in presence of antagonists. The antagonist was added to the bath after 0.5 h of Ach response alone. Antagonist was incubated with the tracheal chain preparation for 0.5 h before addition of cumulative concentration of Ach.

Spontaneous motor activity (SMA): Spontaneous motor activity was performed using Actophotometer (Techno LE3806, India). Mice were

grouped of six each and treated with saline or the compound **II** (10, 30 and 100 mg/Kg i.p.) or received Mepyramine 10 mg/kg i.p. Activity was automatically recorded 0.5 h after treatment and at every 10 min. The experiments were repeated at an interval of 0.5 h, for a total of 2 h. Results of the treated groups were compared with those of control group at each time interval[17]. SMA measurements started 0.5 h after the administration of the compound and the results were compared with those of control.

RESULTS AND DISCUSSION

Isopropyl benzene undergoes Friedel Craft's acylation reaction with 4-chlorobutaroyl chloride and aluminum chloride in dichloromethane to form **I**. The obtained compound was condensed with azacyclonol, which was synthesized from 4-benzoyl pyridine to afford **II** (**Scheme-I**). The **II** was prepared in salt form by passing dry HCl in acetone for activity. IR, PMR and mass spectra were consistent with the assigned structure.



Scheme-I

Isolated guinea pig ileum: Compound **II** were tested *in vitro* for their inhibitory activity against histamine H_1 by contraction of guinea-pig ileum. The antihistaminic activity of the tested compound at different doses showed significant activity when compared to that of standard drug, surprisingly, however there is a significant difference of antihistamine potency between the tested compounds with the standard drug. The results of the tested compound against the standard drug are shown in the Table-1.

TABLE-1
DATA SHOWING THE DETERMINATION OF PERCENTAGE INHIBITION VALUES OF COMPOUND **II** USING GUINEA PIG ILEUM

Compd.	Trial no.	Histamine (μg/mL)	Height of contraction due to histamine (mm)	Antagonist (μg/mL)	Height of contraction (Histamine + Antagonist) mm	Inhibition (%)
II	1	4.0	40.0	0.1	14.0	65.0
	2	4.0	40.0	0.2	9.0	77.5
	3	4.0	40.0	0.4	0.0	100.0
Fexofen-adine	1	4.0	40.0	0.1	13.0	67.5
	2	4.0	40.0	0.2	8.0	80.0
	3	4.0	40.0	0.3	0.0	100.0

Isolated guinea pig tracheal chain

Shift in cumulative log concentration-response curves: Cumulative log concentration-response curves of histamine obtained in the presence of compound **II** and mepyramine in these experimental groups showed clear rightward shift compared to histamine-response curves produced (Fig. 1).

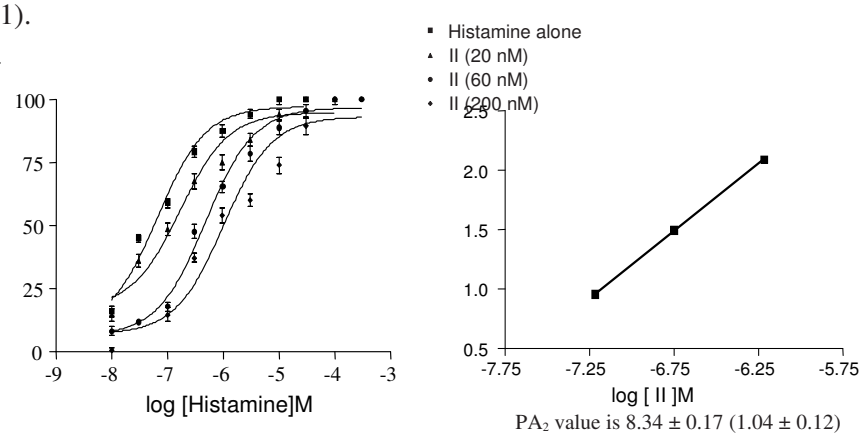


Fig. 1. Cumulative log concentration-response curves of histamine induced contraction of guinea pig tracheal chains, in the presence of compounds on incubated preparations with three different concentrations. The shifts of histamine-response curves obtained in the presence of chlorpheniramine in all three sets of experiments were also parallel

The relaxant effect of different concentration of compound **II** on tracheal chains of guinea pigs might be due to several different mechanisms including stimulation of β-adrenergic receptors, inhibition of histamine H_1 receptors or an anticholinergic property. This compound **II** showed

the relaxant effect due to β_2 stimulatory[18,19] and histamine H_1 receptors inhibitory[20] and anticholinergic property[21].

Shift in cumulative concentration-response curves: Cumulative concentration-response curves of compound **II** obtained with three different concentrations showed clear rightward shifts compared to the standard drug atropine (Fig. 2).

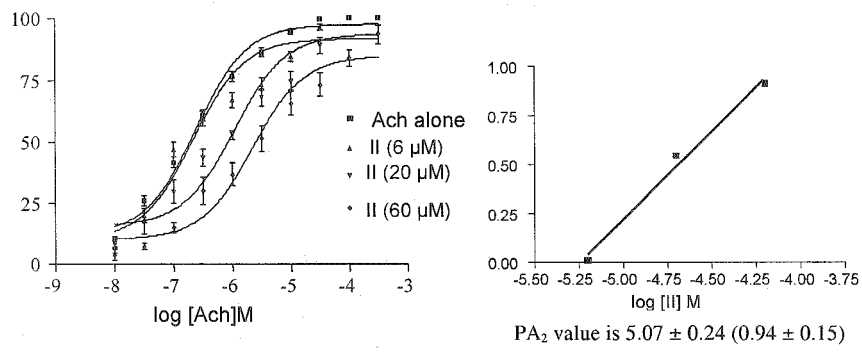


Fig. 2. Cumulative log concentration-response curves of acetyl choline induced contraction of guinea pig tracheal chains, in the presence of compounds on incubated preparations with three different concentrations. The shifts of histamine-response curves obtained in the presence of atropine in all three sets of experiments were also parallel

Spontaneous motor activity compound **II** produced significant decrease in the spontaneous motor activity in mice. This effect was dose dependent and the effect was observed within 0.5 h of drug administration and persisted for 2 h (Table-2). The compound was found to produced alteration in general behaviour pattern, significant reduction of spontaneous motor motility. The present findings suggest that compound possesses CNS-depressant action. The significantly reduced spontaneous motor activity is a measure of the level of excitability of the CNS[22] and this decrease may be closely related to sedation resulting from depression of the central nervous system[23]. The compound possessed central nervous system depressant activity. It also showed a marked sedative effect as indicated by the reduction in motor activity. It is generally accepted that the sedative effect of drugs can be evaluated by measurement of spontaneous motor activity and pentobarbitone induced sleeping time in laboratory animal model[24]. These results corroborate those of[25] who proposed that the enhancement of barbital hypnosis is a good index of CNS depressant activity.

Results of the exploratory behaviour test (Table-2) further support the neuro sedative activity and its possible application in anxiety condition[17]. Further studies are planned to establish mechanism of CNS-depressant action of compound by using various agonists and antagonists.

TABLE-2
SEDATIVE ACTIVITY OF THE COMPOUND **II**

Compound	Dose (mg/kg)	Activity of animal
Distilled water	–	145.7 ± 3.42
II	10	148.86 ± 4.84
II	30	142.06 ± 3.91
II	100	129.21 ± 6.41
Mepyramine	10	104.23 ± 2.89

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