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EFFECT OF SYNTHETIC PIPERIDINE ON HOSPITAL BACTERIAL ISOLATES

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ABSTRACT

Piperidine-4-one was synthesized by the condensing hexane-2-one, 4-fluoro benzaldehydes and ammonium acetate in 1:2:1 ratio. Synthesized compound was characterized by ^{1}H NMR spectra. In spectral studies, the observed carbonyl stretching frequency at 1715 cm^{-1}, secondary amine stretching frequency at 3300 cm^{-1} and aliphatic and aromatic C-H stretching frequencies appeared at 3075-2804 cm^{-1} were being the supporting evidence for the formation of target compound. On the basis of chemical shift and coupling constant value, it has been confirmed that compound 3-allyl 2,6-bis(4-fluorophenyl)piperidine-4-one adopt chair confirmation with equatorial orientation of phenyl rings. Results of present study demonstrate that a new class of piperidine was synthesized and evaluated for its pharmacological study as antibacterial agent. The newly synthesized heterocyclic piperidine exhibited efficient, shorter reaction times and simple purification procedures. It is economic for the synthesis of 3-allyl 2,6-bis(4-fluorophenyl) piperidine-4-one. It was shown that the piperidine rings of compound adopt chair confirmations. Analytical and spectral data of 3-allyl 2,6-bis(4-fluorophenyl) piperidine-4-one revealed a new horizon towards the pharmacological actions against the bacterial pathogens of hospital environment. The promising effect of synthetic piperidine from 2mg/ml to 5mg/ml concentrations against bacterial pathogens of hospital effluents was evident from the results of three different sites. There was no activity less than 1 mg/ml. Moderate activity was observed from 1.2mg/ml to 2mg/ml.Hence it can be concluded that this synthetic piperidine at concentrations from 3mg/ml to 5mg /ml certainly holds great potential towards good active compound leads in antimicrobal study.

KEYWORDS: Synthetic piperidine, spectral study, hospital effluent, pathogenic bacteria.

1. INTRODUCTION

Many organic compounds are used in our daily lives in medicine, agriculture, general life and many new synthetic methods, reaction mechanism, structural theories, analytical techniques have been developed and expended to the study of biological system such as protein, DNA, etc.[1-3]

Heterocyclic ring systems having piperidin-4-one nucleus have aroused great interest in the past and recent years due to their wide variety of biological properties and their presence in biologically active pharmaceutical ingredients[4] Particularly, 3-substituted 2, 6-diarylpiperidin-4-one compounds have also attracted much attention as they display diverse biological and pharmacological properties.[5-7] Hence this eventually formed a new basis and opened up a horizon to synthesis of 3-allyl 2,6-bis(4-fluorophenyl)piperidin-4-one (Fig.1).

With this background the synthesized compound piperidine was performed antibacterial activity against pathogenic bacterial isolates of hospital effluents.

2. MATERIALS AND METHODS

2.1. Synthesis of 3-allyl 2,6-diphenylpiperidin-4-one

A mixture of hexene-2-one (0.05 mol), benzaldehyde (0.1 mol), ammonium acetate (0.05 mol) and ethanol (40 ml) was heated gently and poured into ether (50 ml) and treated with concentrated hydrochloric acid (25 ml). The precipitated hydrochloride was washed with ethanol-ether mixture. The base was liberated by suspending strong ammonia till the hydrochloride dissolved. Dilution with water afforded the free base. After recrystallization from benzene-petroleum ether the compound melted at 56-58°C.

The reagents used were purchased from commercial suppliers without further purification. Melting points

were determined by using an open capillary method and are uncorrected. Thin layer chromatography (TLC) was performed with Aluminium sheet–silica gel 60F254 purchased from Merck. The column chromatography with silica gel (100-200 mesh) using Benzene: pet ether (9:1) as eluent and spots were visualized under iodine chamber (Scheme is given in Fig-2).

2.2. SPECTRAL MEASUREMENTS

The reagents used were purchased from commercial suppliers without further purification. Melting points were determined by using an open capillary method and are uncorrected. Thin layer chromatography (TLC) was performed with Aluminium sheet–silica gel 60F254 purchased from Merck. The column chromatography with silica gel (100-200 mesh) using Benzene: petroleum ether (9:1) as eluent and spots were visualized under iodine chamber. Data was collected from ^{1}H NMR spectra were recorded on BRUKER 400MHz using DMSO as solvent at 296K.

For this synthesized compound, the effect of substituent on the ring conformation and orientation of the substituent and the chemical shift of the carbon and their associated protons are discussed with the help of NMR Spectral data.[8-11]

2.3. Determination of antibacterial activity by Disc Diffusion Method

2.3.1. Preparation of Culture Media

The following media were used for the bacterial growth.

a) Nutrient agar medium
b) Nutrient broth medium

The media were sterilized by autoclaving at a pressure of 15psi at 121˚C for 20min.

Nutrient agar plates were prepared under sterile condition and incubated overnight to detect contamination about 0.2mL of working stock culture was transferred into separate nutrient agar plates and spreaded thoroughly using a glass spreader. Whatman No.1 discs (6mm in diameter) were impregnated in the test compound dissolved in DMSO (2,2.5,3,3.5,4,4.5 and 5 mg/ml) for about half an hour. Commercially available drug disc (Ciprofloxacin 10mg/disc) was used as positive reference standard. Negative controls were also prepared by impregnating the disc of same size on the inoculated agar plates and incubated at ±37˚C for about 18-24h.

2.3.2. Isolation and culture of hospital bacterial isolates

Pathogenic bacteria isolated from the hospital effluents collected from three different sites were tested for the susceptibility of Piperidine at 2,2.5,3,3.5,4,4.5 and 5 mg/ml of concentrations. The antibiotic sensitivity of the isolates was determined using the disc diffusion method. Microbiological assay was conducted for Site No.1,2 and 3.Bacterial pathogens were identified using standard procedures and references.[12-15] Isolates were maintained on standardized inocula under asceptic conditions.

Pathogenic bacteria were spread on Mueller-Hinton agar plates using sterile swabs. The plates were dried at room temperature for 20 min.Different concentrations at 2,2.5,3,3.5,4,4.5 and 5mg/mL of synthetic piperidine was loaded on their respective wells and allowed to diffuse. The plates were incubated for 24 h at 37°C. All the tests were conducted in triplicates. The diameter of zone of inhibition was measured in mm.

All the data obtained from the present study were analysed by SPSS -IBM for the statistical significance.

3. RESULTS AND DISCUSSION

3.1. SPECTRAL ANALYSIS

The synthesized compound has been characterized by ^{1}H NMR spectra revealed the following structure (Figure-3).

Generally ketones, aldehyde, carboxylic acid and amide carbonyl stretching vibration shown in the region of 1870-1540 cm^{-1}. In the target compound, the ring carbonyl band was appeared around 1715 cm^{-1}. NH stretching band was appeared around 3300 cm^{-1}.Aliphatic and aromatic CH stretching frequency was found from 2804 to 3075 cm^{-1}.

^{1}H NMR chemical shift values are given in Table 1. In the substituted electron withdrawing fluoro group at phenyl ring site of 2,6-diphenyl piperidine-4-one ring is known to exert a minor change in the chemical shifts of the ring carbons and their attached protons.

^{1}H NMR signals of the target compound is assigned based on their position, multiplicity, and integral values. In general, the aromatic CH protons absorbed in the downfield region of about 7 ppm due to the ring current effect. Similarly, the target compound, a multiplet observed in the region of 5.61 ppm with one proton integral was assigned to H-8 proton of the allylic group.

In ^{1}H NMR spectrum, two doublets are observed at 4.72 and 4.86 ppm with each one proton integral value is assigned for axial and equatorial protons present in the C-9 carbon. A doublet observed at 2.08 and 2.55ppm with two protons integral was assigned for H-7 proton and H-5 proton. Two broad signals appeared in the downfield region with one proton and two protons integral value were assigned to H-6 and H-2, H-3 protons of the piperidones ring. A signal with minimum intensity is appeared at 2.00 ppm was characteristic for NH proton. The aromatic ring protons were appeared between the region of 7.19-7.71ppm (Table-2).

The present method is practically efficient, involves shorter reaction times, simple purification procedures and is economic for the synthesis of piperidin-4-one oxime esters. From the results, it was shown that the piperidine rings of compound adopt chair confirmations. Chemical synthesis of 3-allyl 2,6-bis(4-fluorophenyl)

piperidine-4-one and its characterization study is in accordance with earlier reports.[16-20] Synthesis and characterization of pharmacological compounds such as N-Methyl Piperidone Oxime Ethers, 1-(Substituted-benzoyl)-piperidin-4-yl, piperidin-4-one oxime esters, 3'-Methyl-2',6'-diphenyl1,3-dihydrospiro[benzo[D]imidazole-2,4'-piperidine and 3,4,5-substituted piperidine derivatives were recorded.[16-20]

Table 1: Chemical shift values for the synthesized compound.

Compound	NH	H-7/ H-5	H-2/ H-3	H-6	H-9a /H9b	H-8	Aromatic protons
33	2.00	2.08/2.55	3.19	4.22	4.72/4.86	5.61	7.19-7.71

Table 2: Analytical and spectral data of 3-allyl 2,6-bis(4-fluorophenyl) piperidine-4-one.

M.F.: $C_{20}H_{19}F_2NO$	**m.p. (^{0}C) :**56-58	**Yield (%):**70	**Structure**
IR(KBr, cm^{-1}); 1715 (C=O), 3300 (N-H), 3075-2804 (C-H aromatic and aliphatic)			
^{1}H NMR(DMSO, ppm); δ: 2.00(s,1H, NH), 2.08 (d,2H,H-7), 2.55 (d,2H,H-5),3.19 (s,2H,H-2 and H-3),4.2 2(s,1H,H-6), 4.72 (d,1H,H-9a),4.86 (d,1H,H-9b)5.61(m,1H,H-8), 7.19-7.71(m, 8H, aromatic protons)			

Table 3: Antibacterial activity of piperidine (5.0mg/ml) against hospital effluent pathogens at Site No.1 and 2 (Zone of inhibition as mm).

Bacterial Pathogens	SIte No.1	Control	SIte No.2	Control
	5.0mg/ml	10 mg/ml	5.0 mg/ml	10 mg/ml
Escherichia coli	25.2 ±0.01	25.0	26.9 ±0.03	25.0
Enterococcus faecalis	16.1 ±0.04	20.1	14.4 ±0.07	22.1
Bacillus subtilis	23.0 ±0.01	22.0	25.9 ±0.05	22.0
Pseudomonas sp	26.8 ±0.07	25.0	18.2 ±0.03	25.1
Streptococcus sp	28.1 ±0.04	26.0	25.0 ±0.04	24.9
Staphylococcus sp	24.8 ±0.04	24.2	26.5 ±0.04	24.3
Klebsiella sp	20.0 ±0.08	25..5	17.1 ±0.01	25.5

* Data represented as mean values ± standard derivation, Significance level at p<0.05

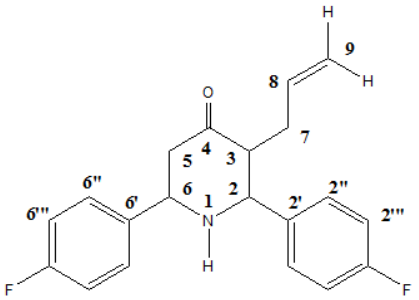

Fig. 1

3-allyl-2,6-bis(4-fluorophenyl)piperidin-4-one

Figure 1: Structure of Synthetic Piperidine.

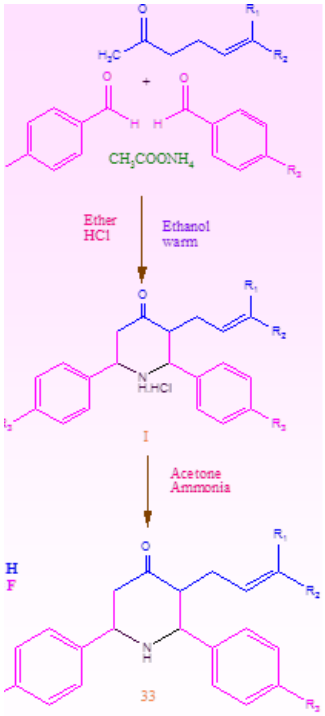

Figure 2: Scheme of synthesis of Piperidine.

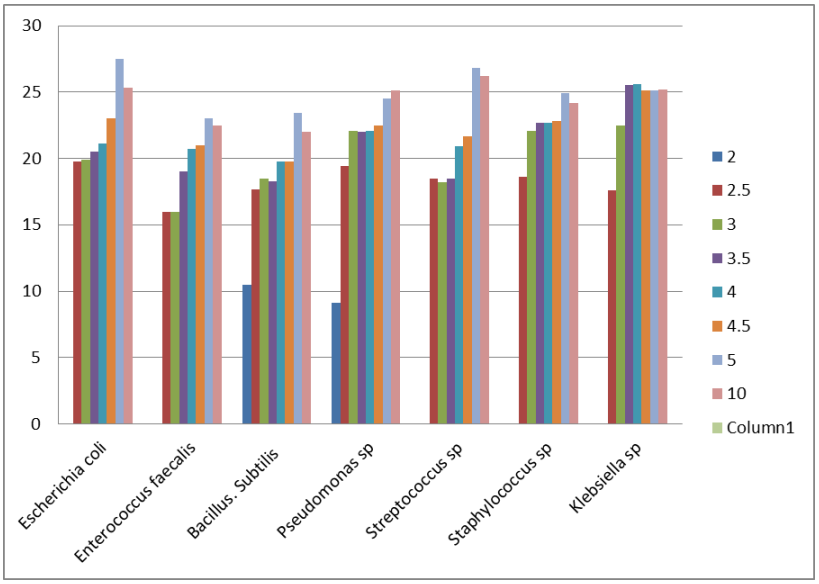

Figure 3: Antibacterial activity of synthetic piperidine at various concentrations against bacterial pathogens of Site No.3 (zone of inhibition -mm).

1.2. ANTIBACTERIAL ACTIVITTY

The following bacterial pathogens were isolated from the hospital effluents collected from three different sites of Chennai City of Tamilnadu, India.

Bacillus subtilis, Escherichia coli, Enterococcus faecalis Staphylococcus sp, Streptococcus sp, Pseudomonas sp and Klebsiella sp.

These bacterial pathogens were tested for the susceptibility against the synthetic piperidine in terms of zone of inhibition(diameter in mm).Thus the minimum concentration of synthetic piperidine was identified to kill the bacterial pathogens at site number 1,2 and 3.

Chemical synthesis and pharmacological evaluation of various derivatives of piperidine are well documented. The results of present study revealed that synthetic piperidine is a potential pharmacological backbone for exploration of new drugs against antibiotic resistant and other bacterial flora. Similar kind of experiments were performed by different researchers with different substitutions to arrive the piperidine derivatives.[21-25]

Ramalingan et al. prepared a string of piperidine-4-one oxime derivatives that was investigated for their in vitro antimicrobial activity. This compound exhibited strong antifungal activity against *Aspergillus flavus* and *Candida-51*, respectively. Compound 15b was found to be more effective than the reference drug; amphotericin B regarding MIC.[26]

From the results of present study, it is obvious that the synthetic piperidine with minimum concentration range 3-5mg/ml recorded excellent pharmacological activity for the hospital effluent pathogens of bacterial flora at site no.1(figure-4). Whereas from site no.2 the bacterial pathogens viz., *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas sp* and *Staphylococcus sp* registered good response.

At site no.3 only *Escherichia coli*, *Streptococcus sp*, *Staphylococcus sp* and *Klebsiella sp* were recorded good activity. There was a moderate response for the synthetic piperidine at 5mg/ml.exhibited by *Bacillus subtilis*, *Enterococcus faecalis* and *Pseudomonas sp*. This might be due to the release of antibiotics in the hospital effluent in which *Bacillus subtilis Enterococcus faecalis* and *Pseudomonas sp.* Showed their multidrug resistance nature.

Susceptibility of bacterial test organisms was studied with different concentrations of synthetic piperidines and their derivatives reported in earlier investigations.[27-38]

Response of pathogenic bacteria from site no.3 such as *Escherichia coli, Bacillus subtilis, Pseudomonas sp and Staphylococcus sp* against the synthetic piperidine was good compared to the response of bacterial flora from site no.1 and 2(Table-3).

Generally hospital effluents are discharged directly without pre-treatment, to municipal sewage. Last few decades hospital effluents gained much scientific and public attention because of undesirable constituents such as antibiotics, disinfectants, heavy metals and multi drug resistant bacteria.[39-44]

CONCLUSION

The present investigation involved an efficient, economic and simple purification procedures. 3-allyl 2,6-bis(4-fluorophenyl) piperidine-4-one was synthesized at shorter reaction times. It was shown that the piperidine rings of compound adopt chair confirmations. The NMR spectral studies of the present investigation revealed that the compound formation through their structure

confirmations. Analytical and spectral data of 3-allyl 2,6-bis(4-fluorophenyl) piperidine-4-one revealed a new horizon towards the pharmacological actions against the microbial flora. Compared to the commercial antibiotic, half of the concentration of synthetic piperidine could be added to the pharmacological formulations. Hence it is suggested that the minimum quantity of 3-allyl 2,6-bis(4-fluorophenyl) piperidine-4-one ranging from 3-5mg/ml is sufficient to kill the pathogenic bacteria of hospital environment. Hence the organically synthesized piperidine (3-allyl 2,6-bis(4-fluorophenyl) piperidine-4-one) is recommended for the pre-treatment processes of the hospital effluents.Further research would be focused on the effect of piperidines and its derivatives on the metabolic profile and genetic mechanism of the various pathogens.

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