Synthesis and bio-evaluation of heterocyclic compound as antimicrobial agents

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DOI 10.51129/ujpah-2024-36-1(7)

Received – 26 December, 2023
Revised – 15 January, 2024
Accepted – 20 January, 2024
Published – 29 June, 2024

Abstract- 1, 4-Dihydropyridines (1, 4-DHPs) are Heterocyclic compounds, an important class of molecules in the field of drugs and pharmaceuticals. Dihydro-pyridine nucleus possess various activities like Vasodilator, bronchodilator, anti-atherosclerotic, antitumor, antidiabetic and hepatoprotective agents. Interest in 1,4-dihydropyridines is due to nicotinamide adenine dinucleotide (NADH), a coenzyme, with unique ability to reduce various functional groups in biological systems. Very recently, dihydropyridines have been reported for resistance reversal properties and antitubercular activities and act as calcium channels blockers. They inhibit the movement of calcium across membrane of myocardial and smooth muscle and their block impulse formation and inhibit conduction velocity and contraction. Further recently such compounds are known to have antibacterial and antifungal activity. Present work is based on the synthesis and bio-evaluation of substituted derivative of 1, 4-Dihydropyridine.

Keywords: 3-nitro Benzaldehyde, Ammonium acetate, antimicrobial activity, Agar nutrient, DMSO

Introduction

Dihydropyridines (DHPs) have very important role in biological activity. The most important biological activity associated with 1,4 Dihydropyridine are calcium channel blocker and there use in treatment of cardiovascular diseases and hypertention.1,2. Calcium channels blockers like 1,4 Di-hydro pyridine, Diphenyl alky amine, Benzothiazepines etc. are developed to inhibit the movement of calcium across membrane of myocardial and smooth muscle and there block impulse formation (automaticity) and inhibit conduction velocity and contraction. As a result of which the Calcium channels blockers have therapeutic utility in angina, arrhythmias, hypertension, antitumer, antidiabetic and other cardio vascular disorder.3,4,5 Calcium channels blockers do not cause fluid retention and can be used equally effective alone for the treatment of mild to moderate hypertension. The efficiency of Calcium channels blockers is enhanced by the concomitant use of β-adrenergic antagonist5, ACEs and methyldopa. Dihydropyridine nucleus is very common in many drugs. DHPs are also known as neuroprotectants, anti-platelet aggregators and are important in Alzheimer's disease

as anti-ischemic agents. They act as Vasodilator, bronchodilator are reported as antioxidants, antitubercular and known to have drug reversal property6-9. These are also known to act as neuroprotectants anti-platelet aggregators. They are also very important in the treatment of Alzheimer's disease as anti-ischemic agents.10. It is interesting to note that an enzyme Nicotinamide Adenine Dinucleotide (NADH) is also related to 1,4 Dihydro-pyridine nucleus. This co-enzyme plays very important role in biological system. NAD+ is an important cofactor used in energy transfer process in all living cells. In plants Dihydropyridine nucleus is mostly found in alkaloids. ref In biological system many metabolic processes involve redox reactions of Nicotinamide adenine dinucleotide (NAD+) reduces its pyridine ring into NADH. The plant Senna spp. is a natural alkaloids of pyridine classes.ref
Several pyridine derivates like pyridinyl thiazyolyazepidinanones, pyridinyl thiazolydiols etc. have been employed for insecticidal, antifungal and antibacterial activity and many compounds have found to exhibit insecticidal and antifungal activity.11-13 The insecticidal and a in vitro antifungal activity was found against different strains of fungi like Aspergilus fumigatus, Candida albicans, etc[14,15]

Review of Literature

Most microbiologists distinguish two groups of antimicrobial agents used in the treatment of infectious disease: antibiotics, which are natural substances produced by certain groups of microorganisms, and chemotherapeutic agents, which are chemically synthesized. A hybrid substance is a semi synthetic antibiotic, where in a molecular version produced by the microbe is subsequently modified by the chemist to achieve desired properties. Furthermore, some antimicrobial compounds, originally discovered as products of micro-organisms, can be synthesized entirely by chemical means. They might be referred to as synthetic antibiotics to distinguish them from the chemotherapeutic agents. Anti-biotics may have a cidal (killing) effect or a static (inhibitory) effect on a range of microbes. The range of bacteria or other microorganisms that is affected by a certain antibiotic is expressed as its spectrum of action. Antibiotics effective against prokaryotes which kill or inhibit a wide range of Gram-positive and Gram-negative bacteria are said to be broad spectrum. If effective mainly against Gram-positive or Gram-negative bacteria, they are narrow spectrum. If effective against a single organism or disease, they are referred to as limited spectrum. Hence, it was thought to prepare 1,4 Dihydropyridine derivatives for its pharmacological importance.14-18 It was reported that compounds having dihydropyridine nucleus are active molecules to fight against infectious diseases. They have been found to have very potent antibacterial activity against gram-negative bacteria and high antifungal activity against Candida albicans. Such compounds have been investigated for various other bacteria like Staphylococcus epi-dermis and found to have binding interaction with its protein. Hence, the scientific community found the 1,4 Dihydropyridine derivatives a new class of compounds as anti-bacterial and antifungal agents[16-18].

Aim of Study

1,4-dihydropyridine is a six membered aromatic ring containing N at 1 position are very important molecules. The hetero-cyclic ring is the common feature for various pharmacological activities such as Ca^{++} channel blockers, vasodilators and their role as drugs for the treatment of cardiovascular diseases. Some drugs like such as, nifedipine and nitrendipine are used for the treatment of hypertension and angina pectoris. In view of this context, the present study was carried to prepare some new antimicrobial agents as possible inhibitor of bacteria and fungi.

Material and Methods

A synthesis of hybrids of 1,4-dihydropyridines was undertaken in the present study. The compound has also been screened against certain strains of bacteria and fungi.

Synthetic Strategy

Since the first report of the Hantzsch synthesis for 1,4-dihydropyridines a number of strategies have been developed for the synthesis of Dihydropyridines[19-22] In this present study the compound has been synthesized by modified Hantzsch synthesis. To the magnetically stirred slurry of 4 Ao molecular sieve (400mg) in ethylene glycol (20ml), Acetyl Acetone 4.1ml, 39.70 milli mole) and Ammonium Acetate (1.530gms, 19.85 milli mole) was added at room temperature, the reaction mixture was heated up to 60°C and continued for 15 minute and followed by addition of 3-nitrobenzaldehyde (3gms, 19.85 milli mole) and Tetra butyl ammonium bromide (500mg) as a catalyst and stirring was continued till the disappearance of aldehyde. Reaction was monitored by TLC. After completion of reaction final compound was obtained by evaporation of solvent. The reaction mixture was then poured into the cold water and the precipitate obtained was the filtered and dissolved in suitable solvent (chloroform) anhydrous Sodium sulphate (Na_2SO_4) was then added to absorb the moisture and filtered. The solvent chloroform was evaporated under reduced pressure and the product obtained was dried and weighed. The product obtained was purified by column chromatography.

Yield	= 5.146 gms
Solubility	= Chloroform and Ethyl acetate
Physical App.	= Solid
Element Present	= Nitrogen
Molecular Formula	= $C_{17}H_{18}N_2O_4$
Formula Weight	= 314.335

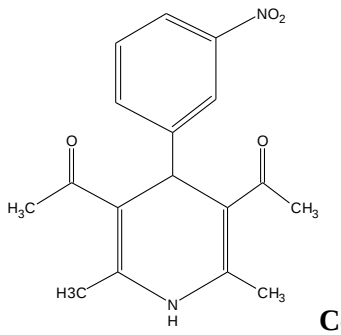
The following reaction shows synthesis of Dihydropyridine based heterocyclic compound.

$C_7H_5NO_3$ + CH_3CONH_4 + $CH_3COCH_2COCH_3$

3-Nitrobenzaldehyde Am. Acetate Acetyl Acetone

Compound [I]

1. 4 A° Molecular Seive
2. Ethylene Glycol
3. TBAB
4. 70°C to 80°C, 3 hrs



Compound I

Bio-evaluation

Synthesized compound was then used for the determination of Antimicrobial activity. 200 mg/ml (in alcohol) dilution was prepared for each strain.

Microorganism Used

Fresh culture of following bacteria & fungi were used in the study:

Bacterial species

1) Bacillus cereus
2) Staphylococcus aureus
3) Proteus mirabilis
4) Escherichia coli

Fungal species

1) Aspergillus niger
2) Aspergillus tereus
3) Penicillium crysogenum
4) Aspergillus japanicus

Culture Media Inoculums

All-purpose Muller Hinton Agar media, (Himedia, No. 173) was used in this study microbial culture, freshly grown at 37oC were appropriately diluted in sterile normal saline solution to obtain the cell suspension at 10^5CFU/ml.

Antimicrobial Assay

The agar well diffusion method was used. 1 ml of diluted inoculums (105FU/ml) of test organism was mixed on the Muller Hinton agar media (for bacteria) and scabbard's agar (for fungus), shacked and pour in sterilized Petri plates. The wells of 8mm diameter were punched into the agar medium. To each well 200mg/ml of compound was added and allowed to diffuse. The compound was tested against each organism in triplicate set. The plate were then incubated aerobically 37oC for the bacteria stains for 24 hrs and at 27oC for the fungus for 72 hrs.

The antimicrobial activities then tested for compound and recorded as the diameter of the resulting growth inhibition in millimeters.

In the present work 1,4 dihydro-pyridine derivative has been synthesised by an efficient method. One mole of 3-Nitrobenzaldehyde 99% on reaction with 2 mole of β-keto compound and 1 mole of ammonium acetate in the presence of ecofriendly solvent ethylene glycol underwent the condensation reaction to give N, N'-disubstituted 1,4-dihydropyridine compound [1] in quantitative yield. In above method 4 A˚ molecular sieves play very important role to carry out the reaction in forward direction by simultaneous absorption of water released during the condensation reaction. TBAB used as phase transfer catalyst to increase nucleophillicity of attacking nucleophile in reaction mechanism. Compound [I] obtained by above mentioned method was characterized with the help of nitrogen element detection and I.R. spectroscopy. The synthesis of compound [I] is evaluated for antimicrobial activity. Since pyridine and its dihydropyridine derivatives are well known to show antibacterial activity. This prompted us to investigated the synthesized compound as possible inhibitor of pathogenic microorganisms.

The compound [I] was screened against two gram positive i.e. Staphylococcus aureus and Bacillus cereus and two gram negative bacterial strains i.e. Escherichia coli and Proteus mirabilis. The compound

[I] was dissolved in DMSO (Dimethyl Sulfoxide) an concentration of 200 mg/ml was made. The activity result of compound [I] against above mentioned strains has been given in table (1).

Table-1 The antibacterial activity of compound I

S. No	Bacterial Species	Kind of Bacteria	Concentration	Inhibition zone in (mm)
1	Bacillus cereus	Gram positive	200mg/ml	26mm
2	Staphylococcus aureus	Gram positive	200mg/ml	29mm
3	Proteus mirabilis	Gram negative	200mg/ml	-
4	Escherichia Coli	Gram negative	200mg/ml	26mm

Results and Discussions

The above activity result shown is very encouraging. Compound [I] was found to have inhibitory activity against both gram positive as well as gram negative bacteria however the synthesized compound was not active against all test organism. Inhibition zone shown by compound (1) are 26 mm,29 mm and 26 mm against Bacillus cereus, Staphylococcus aureus and Escherichia coli respectively.

The antibacterial activity result of compound [I] against the four different strains of bacteria is also shown in shown in pictures 1, 2, 3 and 4.

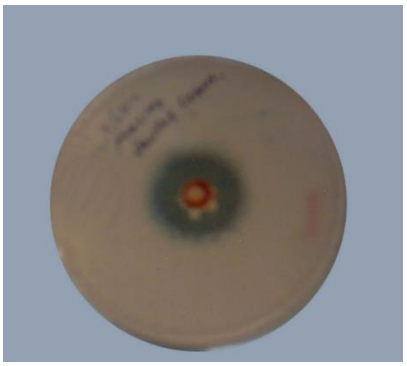


Figure – 1 Bacillus cereus

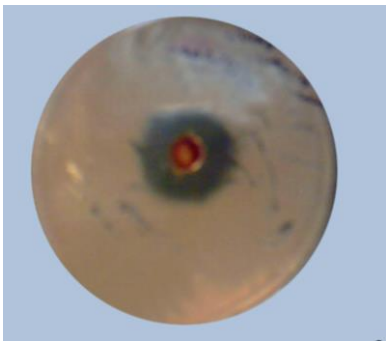


Figure -2 Staphylococcus aureus

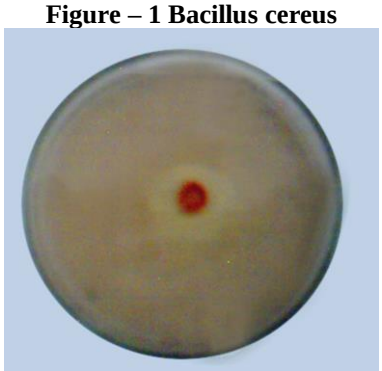


Figure 3 Proteus mirabilis

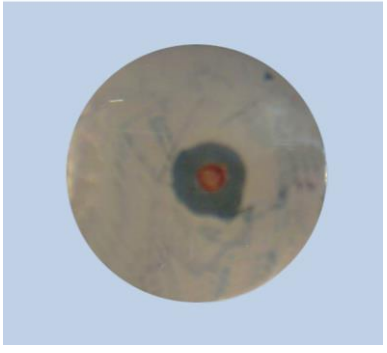


Figure 4 Escherichia coli

Further the synthesized compound [I] was also evaluated for antifungal activity. The fungal strains used as organisms are Aspergillus niger, Aspergillus tereus, Aspergillus japanicus and Penicillium crysogerium. The compound was dissolved in DMSO (Dimethyl sulfoxide) and 200mg/ml concentration was made and inoculated to the test organism, after 72 hours a portion of fungal colony was killed by the test sample and this was appeared as cleaned zone around the test compound [I]. This zone was measured in mm scale and the result obtained are a 16.33 mm (Aspergillus nijer), 14 mm (Aspergil-lustereus), 21mm (Aspergillus japanicus) and 23 mm (Penicillium crysogerium) as given in Table (2).

Table 2: The antifungal activity of compound [1]

S. No.	Test organism	Concentration of compound [I]	Zone of inhibition in (mm)
1.	*Aspergillus nijer*	200mg/ml	16.33mm
2.	*Aspergillus tereus*	200mg/ml	14 mm
3.	*Penicillium crysogerium*	200mg/ml	23mm
4.	*Aspergillus japanicus*	200mg/ml	21mm

Conclusion

Conclusion: The synthesized compound [1] i.e.(3,5-Diacetyl-2,6-dimethyl-4-(3-nitro phenyl-1,4-Dihydropyridine) was synthesized in quantitative yield and it was evaluated for antibacterial and antifungal activity. The maximum inhibition was noticed against the bacterium Staphylococcus aureus and better antifungal activity was observed against Penicillium chrysogenum.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

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