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ORIGINAL ARTICLE **OPEN ACCESS**

Synthesis, Anti-oxidant and Anti-bacterial activity of the 2-azetidinone derivatives from phenyl acetic acid

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ABSTRACT
In the present study new azetidinone derivatives moiety have been prepared by cyclocondensation of the Schiff bases derived from phenyl acetic acid via a series of reactions in the presence of triethylamine. The structure of the azetidinons were confirmed by elemental analysis (C. H. N.) and FT-IR, 1H-NMR spectroscopy. The compounds were screened for their antimicrobial activity and antioxidant activity. The compounds exhibited good antimicrobial activity and antioxidant activity in comparison with standard drugs.
Keywords: azetidinone, Schiff base, β-lactam, sulfadrugs and antimicrobial activity.

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INTRODUCTION

Antibiotic discovery and development is one of modern science and technology's most powerful and successful achievements in the fight against infectious illnesses. However, the advent of antimicrobial resistance and its spread across bacterial strains has diminished the efficacy and success of a significant number of medications. The identification of novel active chemicals is a matter of urgency in order to tackle this worrying challenge. Many compounds with the -lactam ring have a wide range of biological activitiesv [1]. Because of the relevance of this structural unit in penicillin and related antibiotics, the synthesis of 2-azetidinone is still a very active research area[2]. Azetidinones, which are part of the structure of antibiotics, have been shown to have interesting biological properties[3, 4].Many 3- chloro monocyclic -lactams have potent antibacterial, antimicrobial, anti-inflammatory, anticonvulsant, and antitubercular properties. They are also effective on the central nervous system (CNS) and act as enzyme inhibitors.

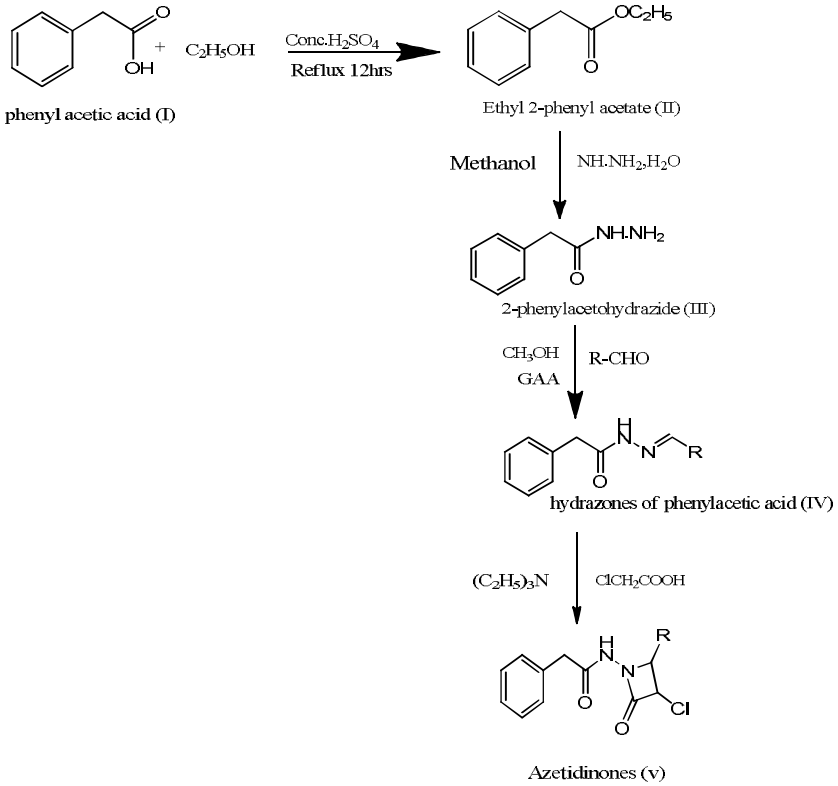
The -lactam ring, also known as the 2-azetidinone skeleton, has long been recognised as a significant building block in the synthesis of biologically relevant molecules. Antifungal, antibacterial, antitubercular, analgesic, anti-inflammatory [8], chymase inhibitory [9], antitumoral [10,11,12], antiviral, antidiabetic, and cholesterol absorption inhibitory effects [13] are some of the biological actions of azetindin-2-one derivatives. The presence of a 2-azetidinone ring is responsible for the activity of well-known antibiotic classes such as penicillins, cephalosporins, carumonam, aztreonam, thienamicine, nocardicins, and carbapenems [2]. Unfortunately, the most extensively used ones create selective pressure on bacteria, allowing resistant organisms to thrive.Due to the increasing resistance of bacteria to traditional -lactam antibiotics and the need for medicines with more specific antibacterial action, several synthetic and semi-synthetic -lactam antibiotics were created [1]. Sulfonamides' biological action has also been widely documented. Antibacterial, antifungal, anticancer drugs, diuretics, carbonic anhydrase inhibitors, hypoglycemic medicines, thyroid inhibitors, anticonvulsants, and protease inhibitors have all been discovered to be beneficial [14,15]. Sulfadiazine and its silver and cerium salts are among the most effective antibacterial sulfonamides. They're commonly used as topical treatments to treat burns, preventing infections and promoting quick healing with minimum scarring [15].

Wounds are physical injuries that cause the skin to open. The repair of compromised anatomical continuity and impaired functional status of the skin requires proper wound healing [16]. Normal wound healing is a three-phase process that overlaps in time: inflammation, granulation (tissue creation), and re-epithelization (tissue remodelling) [17]. Due to their adverse effects on cells and tissues, reactive oxygen species (ROS) and bacterial infections have been shown to be detrimental to wound healing [18]. As a defence mechanism against invading microorganisms, wound areas create large levels of ROS.At the same

time, the presence of free radicals, which can damage the cells surrounding the wound, or microbial infection can impede wound healing [19], and new research has demonstrated the positive benefits of antioxidants in the wound healing process [20,21,22].The goal of this research is to create azetidinones by cycloaddition of -chloroacetyl chloride with Schiff bases produced from phenyl acetic acid, resulting in the production of a 2-azetidinone ring (-lactam).

MATERIAL AND METHODS

EXPERIMENTAL PROCEDURE



R=$C_6H_4NO_2$,$C_6H_4N(CH_3)_2$,C_6H_4OH,$C_6H_4(OCH_3)_2$,C_6H_5-CH=CH,C_3H_6CHO

Scheme

Synthesis of Ethyl2-phenyl acetate(II):

A mixture of phenyl acetic acid (0.15 M) and excess of methanol (50 ml) with 1 ml of sulfuric acid was refluxed for 6hrs in round bottom flask. The reaction mixture was poured onto ice cold water and the precipitated solid was separated by filtration,dried and recrystallized from methanol to yield ethyl 2-phenyl acetate.(Yield 80%,m.p147-150^{0}C).

Synthesis of 2-Phenyl acetohydrazide(III):

Ethyl 2-phenyl acetate(0.16M) and excess of hydrazine hydrate (0.30 M) and ethanol (50 ml) was refluxed for about 12hrs and cooled. The solid was separated by filtration and recrystallized from ethanol to afford 2-phenyl acetohydrazide(Yield 78% m.p:147^{0}C).

Synthesis of hydrazones of Phenyl acetic acid(IV):

A mixture of 2-phenylacetohydrazide (0.025 M) and required aromatic aldehydes (0.025 M) was refluxed in methanol (50 ml) in the presence of a catalytic amount of glacial acetic acid for about12 hrs. The mixture was cooled the solid was separated by filtration and recrystallized from methanol to give the corresponding hydrazones of phenylaceti acid (Yield69%m.p:143^{0}C).

Synthesis of Azetidinones(V):

A mixture of hydrazone of phenyl acetic acid (0.015 M) and required amount of triethylamine (0.02 M) and chloroaceticacid (0.02 M) in ethanol (50 ml) was refluxed for 12-24hrs on water bath to yield 2-azetidinone derivatives (Va-Vf). After cooling, the solution was poured on crushed ice to precipitate the product. The product was recrystallized from methanol.(Yield 65%,m.p:140^{0}C).

Evaluation for Antibacterial Activity:

The antibacterial activity of the azetidinone derivatives (Va-Vf) had been assayed against four different strains of bacteria by cup-plate agar diffusion method by measuring the zone of inhibition in mm.

Organisms selected:

Gram –positive bacteria Gram-negative bacteria

Staphylococcus aureus, Escherichia coli, Bacillus cereus and Pseudomonas auruginosa were selected.

Generally, the antibacterial activity of a compound is expressed in terms of its ability to inhibit the growth of bacteria in nutrient broth or agar media. The bacterial growth inhibition can be measured by two methods, Cup plate method and Serial dilution method

Bacterial Culture medium

Nutrient broth has been used for the preparation of inoculums of the bacteria and nutrient agar is used for the evaluation of antibacterial activity.

Procedure: The test organisms were sub cultured using nutrient broth medium.The tubes containing sterilized medium were inoculated with respective bacterial strain. After incubation at37 ± 1^0C for 24 hrs, They were stored in refrigerator. The stock cultures were maintained. Bacterial inoculum was prepared by transferring a loopful of culture to nutrient broth in conical flask. The flask were incubated at 37± 1^0Cfor 48 hrs before the experimentation.

Test sample preparation:

The test compounds were prepared for assay by dissolving them in DMSO in required in concentrations making 50µg/ml,100µg/ml.A reference streptomycin as Gram-positive and Gram-negative bacteria was made in same concentrations as test compounds. The nutrient agar medium was sterilized by autoclaving at 121^0c for 15 min. The petriplates, tubes and flasks plugged with cotton were sterilized in hot air oven at 160^0c for an hour. Into each sterilized petriplate, about 25ml of molten nutrient agar medium inoculated with the respective strains of bacteria was transferred, aseptically. The plates were left at room temperature to allow solidification. In each plate ,cups of 10mm diameter were made with sterile borer.Then 100µg/ml of the test solution was added to the respective cups aseptically and labeled accordingly. The plates were kept undisturbed for at least 2 hours in refrigerator to allow diffusion of the solution properly into the nutrient agar medium. After the incubation of the plates at 37 ± 1^0 c for 24 hours, the diameter of the zone of inhibition surrounding each of the cup was measured with the help of scale and tabulated (Table-1&2).

ANTIOXIDANT ACTIVITY

The synthesized Azetidinone derivatives were evaluated for antioxidant and antimicrobial activities.

Procedure

Preparation of standard solution of Ascorbic acid:

Required amount of ascorbic acid was accurately weighed and dissolved in distilled water to prepare 1mM stock solution. Solutions of different concentrations of ascorbic acid 1nM, 3nM, 10nM, 30nM, 100nM, 1µM,3µM, 1µM, 3µM,10µM,30µM,100µM,300µMwere prepared from 1mM stock solution.

Preparation of DPPH solution

0.5Mm of DPPH was prepared by dissolving 19.71 mg of DPPH in 100ml of methanol.The solution was protected from sunlight to prevent the oxidation of DPPH.

Preparation of Test compounds

Required amount of test compounds (Va,Vb,Vc,Vd,Ve,Vf) were dissolved in methanol and 1mM stock solution was prepared. Solutions of different concentrations of test compounds 1nM to 1mM were prepared.

Estimation of antioxidant activity of Ascorbic acid

0.2 ml of DPPH solution was added to 2.8 ml of ascorbic acid solution in a test tube wrapped with aluminium foil, incubated for 30 min at room temperature and its absorbance was read out at 517 nm using UV-Visible double beam spectrophotometer. The results were plotted on a graph and the IC_{50} values were determined.

Estimation of antioxidant activity of Test compounds:

The IC_{50} values of the test compounds were determined by a procedure similar to that described under ascorbic acid.

RESULT AND DISCUSSION

In this study, we have synthesized a new series of Azetidinone derivatives **(Va-Vf).** Yields of all synthesized compounds were good. Azetidinones has been subject of numerous investigations. The compounds were confirmed by TLC, melting point and spectral studies such as FTIR, MS, and HNMR.

All the derivatives were subjected to antioxidant activity (DPPH method) and antibacterial activity. The synthesized derivatives Va-Vf has been evaluated for their antimicrobial activity. The results of the derivatives were compared with standard drug streptomycin. All the compounds were less active than standard.The compound Vd showed maximum activity against *Bacillus cereus*, at 50µg/ml with zone of inhibition 12mm (Table 1 & Table 2 and Figure 1, Figure 2).

All the new Azetidinone derivatives employed in the investigation have been found tohave antioxidant activity. ll the compounds were tested at 10nM to 1mM concentrations and results were compared with standard drug (Ascorbic acid) at the same concentrations. Among all the compounds, Ve and Vd are effective antioxidant activity 41.3□M and 46.28 □M respectively. The compound Va was found to be posses least antioxidant activity than all the tested compounds with 82.41 □M (Table 3 & Figure 3).

Table1:Antibacterial activity against *Bacillus cereus*-(Zone of inhibition in mm).

Compound	50µg/ml	100µg/ml
Va	9mm	8mm
Vb	8mm	9mm
Vc	0	0
Vd	12mm	9mm
Ve	11mm	8mm
Vf	11mm	8mm
Streptomycin	24mm	22mm

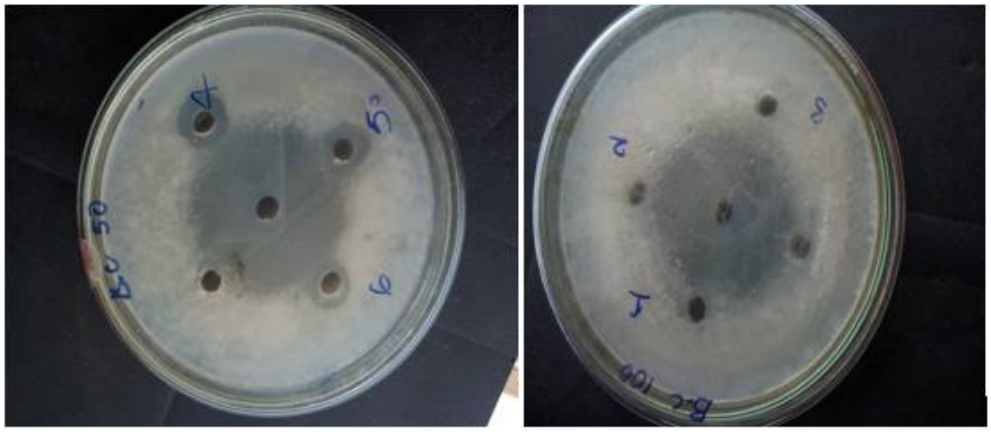


Figure 1. Antibacterial activity against *Bacillus cereus*-(Zone of inhibition in mm).

Table2:Antibacterial activity against*Pseudomonas aeruginosa*-(Zone of inhibition in mm).

Compound	50µg/ml	100µg/ml
Va	9mm	8mm
Vb	8mm	9mm
V1c	-	-
Vd	8mm	9mm
Ve	7mm	8mm
Vf	-	-
Streptomycin	24mm	22mm

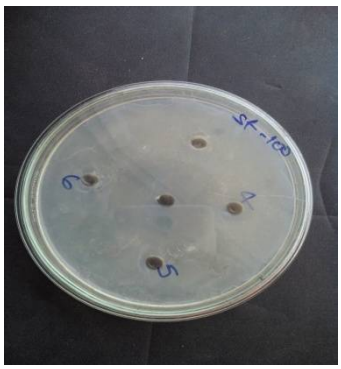


Figure 2. Antibacterial activity against *Pseudomonas aeruginosa*-(Zone of inhibition in mm).

ANTIOXIDANT ACTIVITY

Table3:Anti-oxidant activity of Azetidinone derivatives(V).

S.NO	Compound	R	IC50(µM)
1	Va	$C_6H_4NO_2$	82.41
2	Vb	$C_6H_4N(CH_3)_2$	58.5
3	Vc	C_6H_4OH	55.37
4	Vd	$C_6H_3(OCH_3)_2$	46.28
5	Ve	C_6H_5-CH=CH	41.3
6	Vf	C_3H_6CHO	73.28
7	Standard	Ascorbic acid	8.65

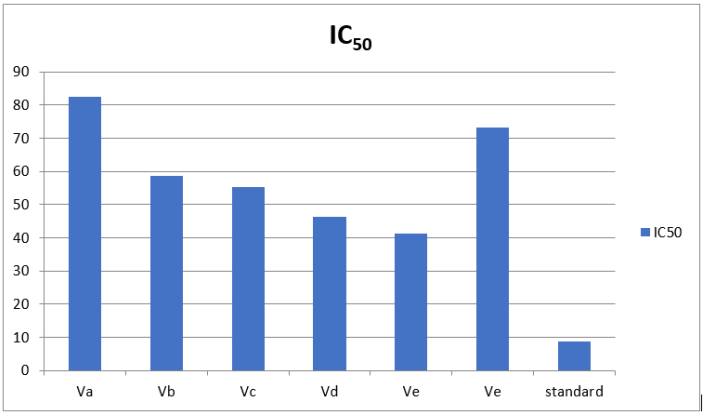


Figure 3. Graph showing Anti-oxidant activity of Azetidinone derivatives(V).

CONCLUSION

Synthetic work of these studies have positively undergone as per the plan and as such in all the reactions carried, the compounds alone could be obtained. Compounds were synthesized and were analyzed by physical and spectral data (FT-IR, NMR, Mass).Synthesized Azetidinone derivatives gave satisfactory results for various evaluations like TLC, melting point, spectral data, antibacterial and antioxidant activities. Characterization of the synthesized compounds was done by FT-IR, 1HNMR and Mass spectral data. The structures, functional groups and molecular weights of the compounds were confirmed. All the compounds were screened for antibacterial studies. The results of derivatives. were compared with standard drug Streptomycin. All the compounds were less active than standard. All the series of the compounds showed antioxidant activity. Compound Ve with IC50value of 41.3µM and Vd with IC50 value of46.28 µM was found to be more effective antioxidant.

CONFLICT OF THE INTEREST

The authors declared no conflict of the interest

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