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Research Article

Synthesis, Characterization and Biological Evaluation of Azetidine Analogues and Related Compounds

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ABSTRACT

Three compounds of azetidine derivative was successfully synthesized. Infrared (IR), proton (H) NMR was used to characterize the newly synthesized azetidine derivative. Disc Diffusion method was used to determine zone of inhibition of the azetidine derivative against 4 bacteria (2 Gram Positive,2 Gram Negative) and 2 fungal strains. Results obtained show that the newly synthesized azetidine derivative are very potent antimicrobial agents against Gram negative bacteria Pseudomonas aeruginosa, Escherichia coli and Gram-positive bacteria Bacillus subtills, Staphylococcus aureus. Result show that Compound D1 exhibited same antibacterial potency than Ampicillin reference drug. The test compounds also show high anti-fungal activity against Candida albican Aspergillus Niger. Compounds D1 and D3 also show Potential activity against bacteria and fungi. Development of antimicrobial agents also draw attention to the many researcher because there are lots of area in this field which require improvements. So, there are still need to developed new antimicrobial agents.

Key words: Antimicrobial, Azetidine, Antifungal, Heterocyclic compounds, Nitrogen derivative

INTRODUCTION

Microbial infections remain a leading cause of mortality and morbidity worldwide. Unfortunately, a number of current antimicrobial agent are becoming less effective [1]. With all of this in Mind there is an urgent need for the discovery of novel compounds endowed with antimicrobial activity [2]. The synthesis of heterocyclic compounds has drawn attention of medicinal chemist mainly because of their structural diversity coupled with their important therapeutic properties [3]. Number of heterocyclic derivatives containing nitrogen atom serve as a versatile scaffold for experimental drug design [4]. Microorganisms are at the forefront of the global health challenges as they continue to decrease the potency of many antimicrobial agents [5]. Infections by these microorganisms affect the health of the consumers or the host organisms that may result in loss of life and also, they cause economical loss. This therefore encourages the continuous search for novel compounds with enhanced bioactive properties [6]. Nitrogen-containing heterocyclic compounds such as azetidine and its analogues form a good base of the important class of pharmacophores [7]. Azetidine is a heterocyclic organic compound. It belongs to the class of four membered rings and it contains a nitrogen atom azetidine and its analogues show antimicrobial activity [8]. In our search for more dependable antimicrobial agents to limits microbial invasion and resistance to known drugs we have synthesized azetidine derivative and evaluated their antimicrobial activity against few bacterial strains [9]. Antimicrobial agents also draw attention of number of researchers due to there are lots of opportunity to improve the activity [10], potency [11], safety [12] of antimicrobial agents. We also try to deal with resistance [13] against antimicrobial agents [14-16].

MATERIALS AND METHODS

All reactions were carried out under prescribed laboratory conditions. Solvents and reagents used were of laboratory grade and were purified by distillation and crystallization techniques where ever necessary and their melting point were checked with the available literature. All the reactions requiring anhydrous conditions were conducted in well dried apparatus. The synthesized compounds were purified by recrystallization. Melting points of newly synthesized compounds were determined by

open capillary method and were uncorrected. The final products were purified by recrystallization and purity was checked by TLC. The IR spectra of the compounds were recorded on JASCO FT/IR-5300 spectrometer using KBr pellet. ^{1}H NMR spectra were recorded BRUKER DPX-400 MHz on a spectrometer using TMS as internal standard. The spectra were obtained in chloroform and the chemical shift values are reported as values in ppm relative to TMS as internal standard.

Synthesis

Synthesis of Compound A

0.125 Mol (2.1 g) of p-nitro benzoic acid mix with 0.25mol (1.15 g) of absolute ethanol, 3.8 g of concentrated H_2SO_4 in a round bottom flask fit a condenser and heat the mixture under reflux for 16 hrs [18].

Synthesis of Compound B

0.5 Mol (1.5 g) of compound "A" in a conical flask and add with stirring during slow dropwise of a solution of 0.5 mol (1.5 g) of hydrazine hydrate and 0.5 mol (5ml) of absolute ethanol then further stirring for 1 hrs. and cool the reaction mixture in an ice bath [18].

Synthesis of Compound C

0.1 Mol (1.5 g) of compound "B" mix with 3.8 g of H_2SO_4 and 7ml of absolute ethanol and add 5.3 ml of aldehyde fit a reflux condenser and heat the mixture under reflux for 1hrs [18].

Synthesis of Compound D

2.4 g of compound "C" mix with 1.0 ml of Triethylamine and 0.8 ml of 4-dioxane in a beaker and add with stirring for 1hrs.during slow dropwise addition of 1.13 ml of chloro acetyl chloride [18]. Structure of compound is given into Figure 1.

Synthesis of Compound (P-nitro carboxyamido-4[(2-chloro) phenyl]-2-one azetidine) D1:

A mixture of Compound D (0.005 mol) and 2-chloro -benzaldehyde (0.005 mol) was refluxed in dimethyl formamide (DMF) for 5 hrs then left to cool to room temperature .The reaction mixture was poured into cold water for complete precipitation, and formed precipitate was filtered off dried and recrystallized from ethanol to give compound [17] (D1) Yield:82%.

Synthesis of Compound (P-nitro carboxyamido-4[p-methyl phenyl]-2 one azetidine) D2:

A mixture of Compound D (0.005 mol) and 4-methyl -benzaldehyde (0.005 mol) was refluxed in dimethyl formamide (DMF) for 5hrs then left to cool to room temperature .The reaction mixture was poured into cold water for complete precipitation, and formed precipitate was filtered off dried and recrystallized from ethanol to give compound [17] (D2) Yield:78%,

Synthesis of Compound (P-nitro carboxyamido -4[-formyl phenyl]-2-one azetidine) D3:

A mixture of Compound D (0.005 mol) and benzaldehyde (0.005 mol) was refluxed in dimethyl formamide (DMF) for 5hrs then left to cool to room temperature .The reaction mixture was poured into cold water for complete precipitation, and formed precipitate was filtered off dried and recrystallized from ethanol to give compound D3, Yield: 76% [17].

Reaction Scheme [17] General Synthetic scheme is given into Figure 2. Structures of different derivative of azetidine is given into Table 1.

Antimicrobial Activity

Principle

The antimicrobial activities of synthesized compounds were evaluated by the zone of inhibition method 60. This method is based on the diffusion of an antibiotic from a filter paper disc through the solidified culture media of a Petri dish used for the study. Growth of inoculated microorganism is inhibited entirely in a circular area "zone" around the filter paper disc containing a solution of the antibiotic and test compounds. The organisms were maintained on nutrient ager slants. One loopful of each strain of microorganism was transferred into a suitable ager slant by using a sterile Pasteur loop. These slants were incubated for 24hrs at 37°C for bacteria and 25^{0}C for fungi and were observed for the growth of the organism with naked eye for their turbid nature. The presence of turbidity indicated the growth and stability of the culture for further work [19-20].

Materials

Microorganisms used
Bacterial strains
Staphylococcus aureus (gram positive),
Bacillus subtillis (gram positive),
Pseudomonas aeruginosa (gram negative),
Escherichia coli (gram negative),
Fungal Strains
Candida albican
Apsergillus niger
Drugs (Control)
Ampicillin (antibacterial)
Griseofulvin (antifungal)

Preparation of Stock Culture

From the culture, which were maintained on nutrient agar slants, one loop full of the respective organisms were taken and aseptically transferred to 100 ml of a sterile nutrient broth in a flask, which

was shaken thoroughly and incubated at 37^0 C for bacteria and 25^0 C for fungi.

Preparation of Culture Medium

The medium was prepared by dissolving the specified quantity of the dehydrated medium in purified water and was dispersed in 20 ml volumes in to test tubes. The test tubes were closed with cotton plugs and were sterilized by autoclaving at 121^0C (15lb pigs) for 15 minutes. The contents of tubes were poured aseptically in to sterile Petri plates (90 mm diameter) and allowed to solidity. Muller Hinton agar medium was used to inoculate bacterial cultures and Sabouraud's dextrose agar medium was used for fungal cultures. Composition of Muller Hinton agar medium is given into Table 2. Composition of Sabouraud's dextrose agar medium is given into Table 3.

Preparation of Drug Solution

The drug solutions were prepared by dissolving in dimethyl sulfoxide (DMSO). The solutions of the test drugs and standard drugs ampicillin, and griseofulvin were prepared at the concentration of 100μg/ml in DMSO.

Method

Previously liquefied Muller Hinton agar media was inoculated with the requisite quantity of the suspension of the microorganism. The suspension was added to the medium at a temperature between 40-50 ^{0}C and the inoculated medium was poured immediately into dried Petri dish to occupy a depth of 3-4mm. The Petri dishes were sterilized at 160-170^{0}C for 1 hr before use. Wafers containing antibiotics are placed on an petri dish where bacteria have been placed, after addition of all the drugs, Petri dish were left standing for 1 to 4 hrs at room temperature, as a period of pre-incubation diffusion to minimize the effects of variation in time between the applications of different solutions. All the Petri dishes were incubated for 24 hrs at the required temperature, i.e. 37^{0}C for bacteria and 25^{0}C for fungi. After incubation, the diameters of the circular inhibition zones were measured.

Table 1: Synthesized azetidine derivatives

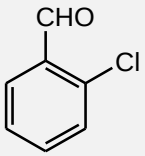
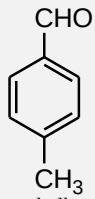
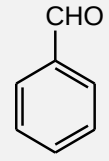
Code	Substitution (R1, R2, R3)	IUPAC Name
D1	R1 -  2-chlorobenzaldehyde	P-nitro carboxyamido-4[(2-chloro) phenyl]-2-one azetidine
D2	R2-  4-methylbenzaldehyde	P-nitro carboxyamido-4[p-methyl phenyl]-2 one azetidine
D3	R3-  benzaldehyde	P-nitro carboxyamido -4[-formyl phenyl]-2-one azetidine

Table 2: Composition of Mueller Hinton agar medium

Condition	Requirements
Beef infusion	300 ml
Casein hydrolysate	16 g
Starch	1.5 g
Agar	1.5 g
Distilled water	1000 ml
pH	7.2 ± 0.2

Table 3: Composition of Seaboard's dextrose agar medium

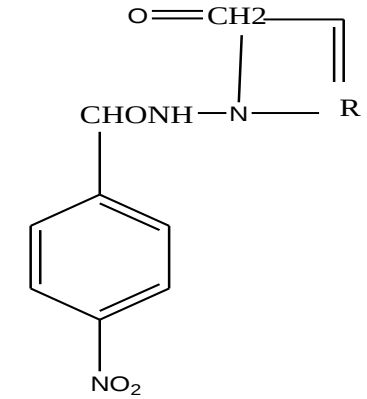
Condition	Requirements
Dextrose	40 g
Peptone	10 g
Agar	20 g
Distilled water	1000 ml
pH	5.6 ± 0.2

Table 4: Antibacterial activity of synthesized compounds D1, D2 &D3

Microorganism	Diameter of Zone of Inhibition (mm)			
	Compound D_1 (100 μg/kg)	Compound D_2 (100 μg/kg)	Compound D_3 (100 μg/kg)	Standard (100 μg/kg)
Staphylococcus aureus	17	10	14	20
Bacillus subtillis	18	9	15	22
Pseudomonas aeruginosa	17	12	12	21
Escherichia coli	18	8	13	22

Table 5: Antifungal activity of synthesized compounds D1, D2 & D3

Microorganism	Diameter of Zone of Inhibition (mm)			
	Compound D_1 (100 μg/kg)	Compound D_2 (100 μg/kg)	Compound D_3 (100 μg/kg)	Standard (100 μg/kg)
Candida albicans	15	17	13	20
Apsergillus niger	16	18	14	20



COMPOUND D

Figure 1: Structure of Compound D

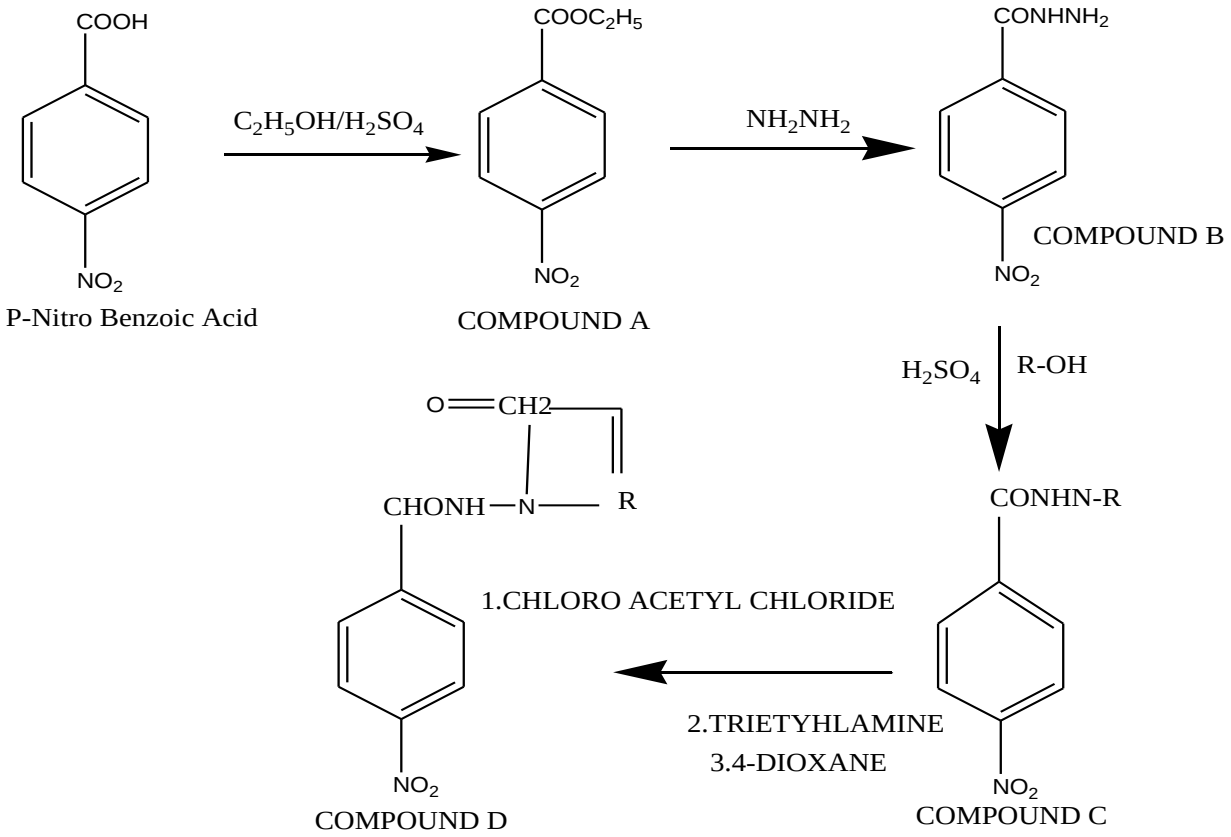


Figure 2: General synthetic scheme for derivative of azetidine

RESULTS & DISCUSSION

According to scheme, the different derivative of azetidine was synthesized with good Percentage yield. The melting points of synthesized compounds were determined by open capillary method and are uncorrected.

CHEMISTRY

Characterization of Compound (P-nitro carboxyamido-4[(2-chloro) phenyl]-2-one azetidine) D1:

Melting point: 111-112°C, Solid State, Molecular Weight: 345 g, Solubility In DMSO, Acetone etc was analyzed for $C_{16}H_{13}O_4N_3Cl$ The TLC method RF value 0.82. The IR spectrum of the compound by KBr methods. It exhibits intense bands at 3428 cm^{-1} (N-H stretching),3009 cm^{-1} (-C-H- stretching), 1687 cm^{-1} (C=O stretching), 1602 cm^{-1} (Ar C-H), 1582 cm-1 (Ar C=C) , 1453 cm^{-1} (C-N), 736 cm^{-1} (C-cl), 1326, 1292 cm^{-1} (Ar C-N). The 1H NMR spectrum in Chloroform, it shows peaks at δ: 6-8.5 (m, 9H, Ar-H), 1.2-1.5 (s, 5H, -CO NH).

Characterization of Compound of Compound (P-nitro carboxyamido-4[p-methyl phenyl]-2 one azetidine) D2:

Melting point: 109-111°C, Solid State, Molecular Weight: 326 g, Solubility In DMSO, Acetone etc was analyzed for $C_{17}H_{16}O_4N_3$ The TLC method RF value 0.89. The IR spectrum of the compound by KBr methods. It exhibits intense bands at 3428 cm^{-1} (N-H stretching),3009 cm^{-1} (-C-H- stretching), 1687 cm^{-1} (C=O stretching), 1602 cm^{-1} (Ar C-H), 1582 cm^{-1} (Ar C=C) , 1453 cm^{-1} (C-N), 1384 cm^{-1} (C-CH3), 1326,1292 cm^{-1} (Ar C-N). The 1H NMR spectrum in Chloroform It shows peaks 6-8.5 (m, 4H, Ar-H), 1.2-1.5 (d, 5H-COO C2H5), 2.1-2.8 (s, 1H, -CH, s,6H, CH3).

Characterization of Compound (P-nitro carboxyamido -4[-formyl phenyl]-2-one azetidine) D3:

Melting point: 104-108°C, Solid State, Molecular Weight: 311 g, Solubility In DMSO, Acetone etc was analyzed for $C_{16}H_{13}O_4N_3$ The TLC method RF value

0.94. The IR spectrum of the compound by KBr method. It exhibits intense bands at 3428 cm^{-1} (N-H stretching), 3009 cm^{-1} (-C-H- stretching), 1687 cm^{-1} (C=O stretching), 1602 cm^{-1} (Ar C-H), 1582 cm^{-1} (Ar C=C) , 1453 cm^{-1} (C-N), 1384 cm^{-1} (C-CH3), 1326,1292 cm^{-1} (Ar C-N). The 1H NMR spectrum in Chloroform. It shows peaks at δ: 6-8.5 (m, 9H, Ar-H), 1.2-1.5 (s,5H, -CO NH).

Antimicrobial Screening

In vitro tests are used as screening procedure for new agents and for testing the susceptibility of individual isolates from infections to determine which of the available drugs might be useful therapeutically. Due to the development of sulphonamides and penicillin, in vitro measurement of susceptibility of microbes to chemotherapeutic agents has been use.

A drug is considered to have bacteriostatic or fungistatic activity when it inhibits the activity of bacteria or fungi respectively and bactericidal or fungicidal activity and its kill bacteria and fungi. Important factors for antimicrobial activity are size of the inoculums, metabolic state of microbe, pH, temperature, duration of interaction, concentration of inhibitor and presence of interference substances.

The development of resistance among various pathogenic microbes towards the antibiotics has increased the impetus for investigating new antimicrobial agent. When a compound was found to have positive therapeutic index, a new series of related compounds are synthesized in the hope that one of them would be more effective than the existing one. a drug, which kills or inhibits the growth of microbes, is known as antimicrobial agent.

Antibacterial test was carried out on four bacterial strains, namely Bacillus subtilis (gram positive), Staphylococcus aureus (gram positive), Eschertia coli (gram negative), Pseudomonas aeruginosa and antifungal test was carried out on Candida albicans and Aspergillus Niger

Disc Diffusion Method

Antibacterial activity studies

All the synthesized azetidine derivatives have shown moderate to weak antibacterial activity. Compounds D1 and D3 showed potent activity against Bacillus subtillis and pseudomonas aeruginosa. Results are given in Table 4 Standard drug used Ampicillin

Antifungal activity studies

From the antifungal activities studies it is evident that the synthesized compound showed potent to moderate antifungal activity. Compounds D1 and D3 shows potent activity against Candida albicans and Aspergillus niger. Compound D2 shows moderate activity compared to the standard. Results are given in Table 5. Standard drug used Griseofulvin.

CONCLUSION

Three Azetidine derivative was successfully synthesized and their chemical structures were confirmed by IR, ^1H NMR analysis. The biological evolution of these compounds was studied against four bacteria, two fungi. The compounds have good antimicrobial activity. So, the overall study show that our newly synthesized compounds show potency against bacteria and fungi. There are chance to develop a new compound which contain azetidine moiety.

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CONFLICT OF INTEREST

Declared None

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