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Synthesis, Characterization and Antimicrobial activity of Azetidine derivatives

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Abstract

In present study azetidine derivatives were synthesized and microbial activity of different derivatives were checked with various microbial stain. Various intermediates was synthesized and characterized in between time to time by chromatography and special methods. Substituted salicylic acid and sulphuric acid were starting material and finally formed2-Hydroxy –N-)2-oxa-4-phenylazet-1(2H)0yl)benzamide(D), while in the intermediate steps(A) Ethylsalicylate react with hydrazine hydrate to get,(B) Salicyl Hydrazide, and this (B) compound reflux with sulphuric acid to get (C) 2 -hydroxy-N-[(Z)- phenylmethylidene]benzohydrazide. Azetidine derivatives have been synthesized from Schiff base of the corresponding hydrazine by using ethanol with reflex. The structure of the entire synthesized compound was established on the basis of element, chromatography and spectral analysis. The synthesized compound was evaluated for their antimicrobial activity. The compound most active be against Staphylococcus aureus ,Bacillus subtillis

Keywords: Azetidine, Antimicrobial, Salicylic acid

Introduction

Azetidines (azacyclobutanes) constitute a well-known class of heterocyclic compounds. Four-membered saturated cyclic amines azacyclobutanes are commonly known as azetidines. Azetidine scaffold is encountered in diverse natural and synthetic compounds exhibiting a broad range of biological activities.The inherent ring strain of azetidines combined with their pres- ence in natural products and biologically important synthetic compounds makes them an appealing target for synthetic chemists. A number of inter- esting synthetic methodologies have been developed in recent years for an easy access to diversely functionalized azetidines. Azetidines, due to ring strain, are also valuable building blocks in organic chemistry. They undergo ring-opening and ring-expansion reactions leading to formation of valuable acyclic products or other heterocyclic compounds. Azetidines give A lot of pharmacological activity like antimicrobial[1],[2],antibacterial[3],[4] ,antifungal[5] antioxidant[6]. Azetidines find applications in medicinal chemistry as pharmacological tools in peptidomimetics as unnatural amino acids.[7]

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Material and Methods

Synthesis of Ethylsalicylate:-First took a round bottom flask of 500ml capacity, 0.2 mol of salicylic acid, 2 mol of ethanol and 8 ml of concentrated sulfuric acid was added in it and also added few small chips of porousporcelain. Then set a reflux condenser to the flask and boil the mixture for 5 hours on a heating mantle. After completion of reaction, Distil off the excess amount of alcohol on a water bath and allowed to cool. The residue was poured into 250 ml of water contained in the separating funnel for 20 minutes. Then compound A was separated. Boiling point is measured and found 232°C. It is colourless liquid ,.Rf value 0.6,

Synthesis of Salicyl Hydrazide:-mol of compound A was taken in a conical flask and then it was stirred magnetically. Thesolution of 0.5 mol of hydrazine hydrate in 40 ml of absolute ethanol was added drop wise in compound A during stirring. When the mixture was stirred for 1 hour at room temperature, thereaction mixture was cooled in an ice bath to complete the crystallization and then filtered. It is off white crystalline powder, and melting point was found between 147-150° C. Rf value 0.43, off whit color

Synthesis of 2 -hydroxy-N-[(Z)-phenylmethylidene]benzohydrazi:- 0.1 mol of Compound B, 1 ml of concentrated sulfuric acid, 10 ml of ethanol andmol of benzaldehyde was mixed in a round bottom flask. Then mixture was refluxed for 1 hour on a heating mantle. Formed compound (compound C) was recrystallized by ethanol and dried. M.p.130-138^{oC},off white color, Rf value0.54.

Synthesis of 2-Hydroxy –N-)2-oxa-4-phenylazet-1(2H)0yl)benzamide:- 0.1 mol Compound C, 0.1 mol Triethylamine and 0.1 mol Dioxane was mixed ina conical flask, then 0.1 mol Chloroacetyl Chloride was added very slowly dropwise with continuous stirring. The reaction mixture was allowed for 24 hours with stirring, the reaction mixture was cooled in an ice bath to complete the crystallization and then filtered. Molecular formula of compound was found that is $C_{16}H_{12}N_2O_3$, and molecular weight was calculated is 264. The Melting Point is between 140-142°C. Compound soluble in Ethanol, DMSO and Acetone. Rf value is 0.684. Newly formed compound's colour is Off white, and percentage yield 62% Infrared Spectral Features (cm^{-1}) 3417 (NH), 1728 (C=O), 1612(NH-C=O), 1581 (C=N).

synthesized of 4-{[2-(2-chlorophenyl)-1-{[2-(2-hydroxyphenyl)- 2-oxoethyl]amino}axet-2(1H)-one:- 0.1 mol of Salicyl hydrazide, 1ml of Concentrated sulfuric acid, 10 ml of ethanol and 0.05 mol of chlorobenzaldehyde was mixed in a round bottom flask, then mixture was refluxed for 1 hour on a heating mantle. Formed compound (2-hydroxy-N-[(Z)- phenylmethylidene] benzohydrazide) was recrystallized, filtered and dried. Then 0.1 mol 2- hydroxy-N-[(Z)-phenylmethylidene] benzohydrazide , 0.1 mol of Triethylamine and 0.1 mol Dioxane was mixed in a conical flask, then 0.1 mol chloroacetyl chloride was added very slowly dropwise in conical flask with continuous stirring. The reaction mixture was allowed for 24 hours with stirring, the mixture was cooled in an ice bath to complete the crystallization and then filtered. The compound with melting point of 145-149°C, Solid State, Molecular weight- 279, Solubility in Ethanol, Acetone. Infrared Spectral Features (cm^{-1})3417 (NH), 1705 (C=O), 1612(NH-N=O), 1584 (Cl).

Synthesis of 1-{[2-(2-hydroxyphenyl)- 2-oxoethyl]amino}- 4-(2-methoxy phenyl)azet-2(1H)one

0.1 mol of Salicyl hydrazide, 1ml of Concentrated sulfuric acid, 10 ml of ethanol and 0.05 mol of methoxybenzaldehyde was mixed in a round bottom flask, then mixture was refluxed for 1 hour on a heating mantle. Formed compound (2-hydroxy-N-[(Z)- phenylmethylidene] benzohydrazide) was recrystallized, filtered and dried. Then 0.1 mol 2- hydroxy-N-[(Z)-phenylmethylidene] benzohydrazide , 0.1 mol of Triethylamine and 0.1 mol Dioxane was mixed in a conical flask, then 0.1 mol chloroacetyl chloride was added very slowly dropwise in conical flask with continuous stirring. The reaction mixture was allowed for 24 hours with stirring, the mixture was cooled in an ice bath to complete the crystallization and then filtered. The compound with melting point of 130-132°C,Solid State, Molecular weight -310, Solubility in Ethanol, Acetone. Infrared Spectral Features (cm^{-1})3417 (NH), 1735 (C=O), 1612(NH-C=O), 1573 (CH_3), 1535 (HO).

Synthesis of 3R1-{[2-(2-hydroxyphenyl)- 2-oxoethyl]amino}- 4-(2-methylphenyl)azet-2(1H)one:--0.1 mol of Salicyl hydrazide, 1ml of Concentrated sulfuric acid, 10 ml of ethanol and 0.05 mol of methylbenzaldehyde was mixed in a round bottom flask, then mixture was refluxed for 1 hour on a heating mantle. Formed compound (2-hydroxy-N-[(Z)- phenylmethylidene] benzohydrazide) was recrystallized, filtered and dried. Then 0.1 mol 2- hydroxy-N-[(Z)-phenylmethylidene] benzohydrazide , 0.1 mol of Triethylamine and 0.1 mol Dioxane was mixed in a conical flask, then 0.1 mol chloroacetyl chloride was added very slowly dropwise in conical flask with continuous stirring. The reaction mixture was allowed for 24 hours with stirring, the mixture was cooled in an ice bath to complete the crystallization and then filtered. The compound with melting point of 135-138°C, Solid State, Molecular weight-294, Solubility in Ethanol, Acetone Infrared Spectral Features (cm^{-1})3417 (NH), 1735 (C=O), 1612(NH-C=O), 1573 (CH_3), 1535 (HO).

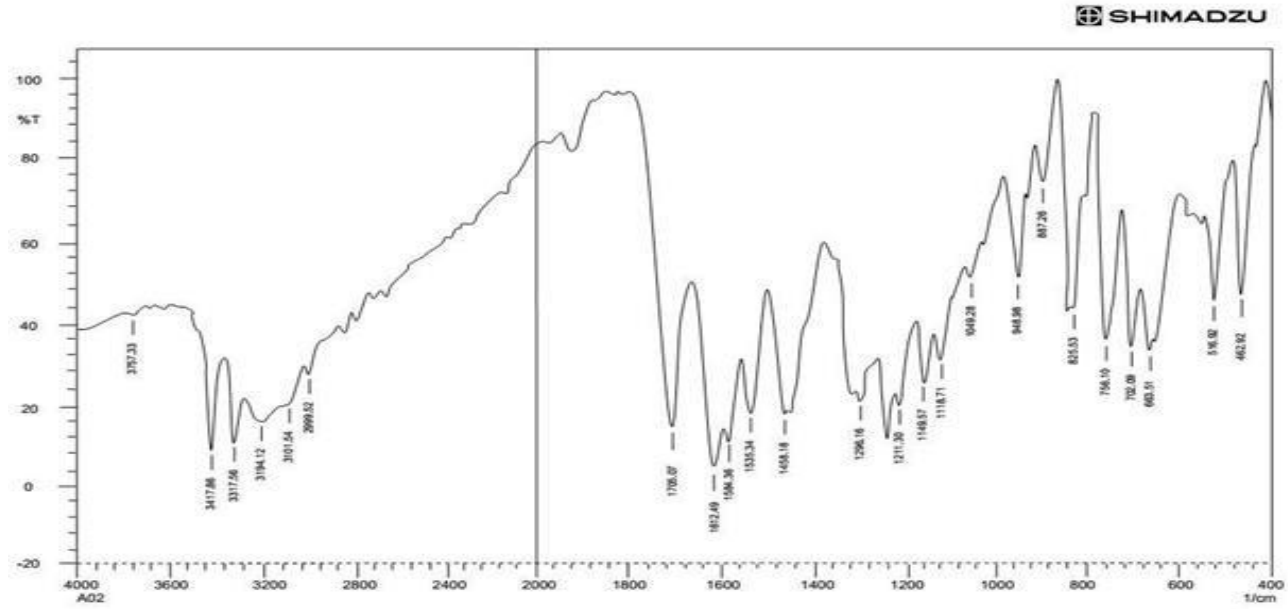

Fig. 1: IR Spectra of 2-Hydroxy –N-)2-oxa-4 phenylazet-1(2H)0yl)benzamid

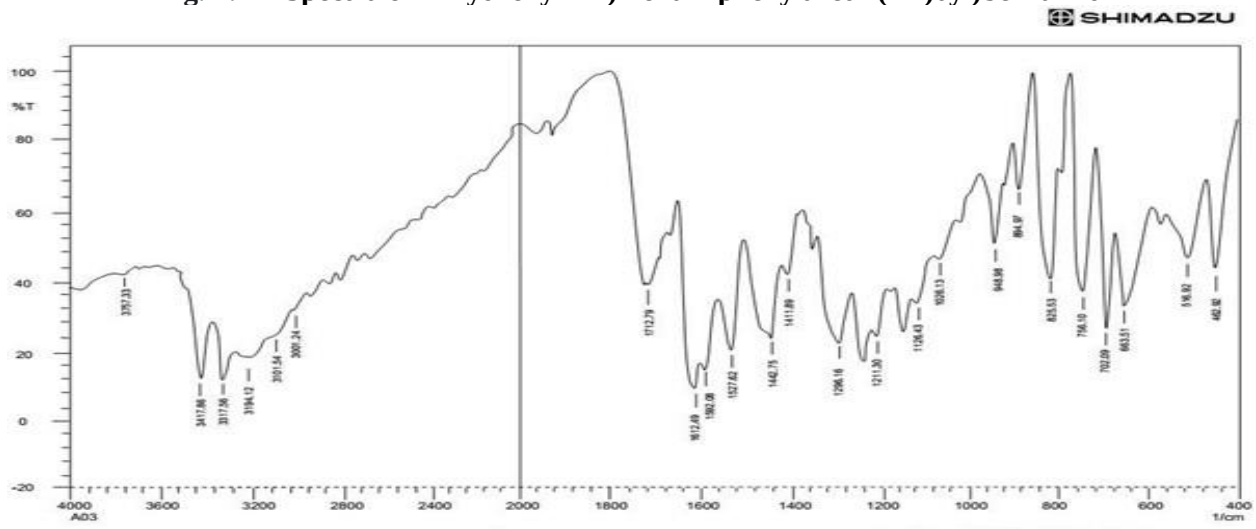

Fig. 2: IR Spectra of 4-{[2-(2-chlorophenyl)-1-{[2-(2-hydroxyphenyl)-2- oxoethyl]amino}axet2(1H)-one

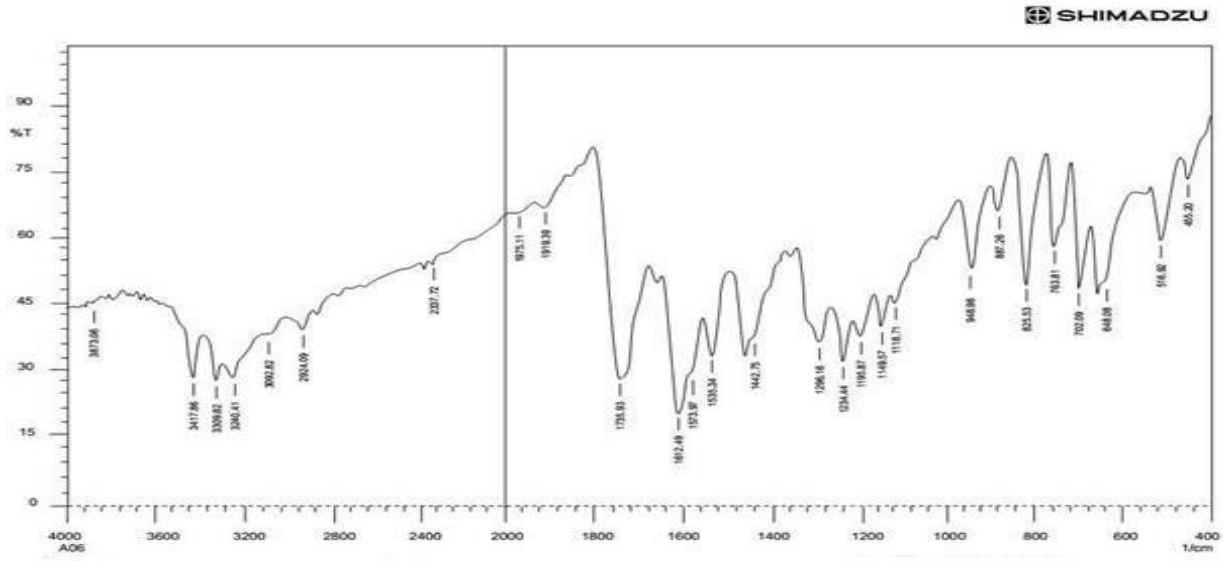

Fig. 3: IR Spectra of 1-{[2)-hydroxyphenyl)-2-oxoethyl}]amino}-4-(-2-methylphenyl)azet-2(1H)one

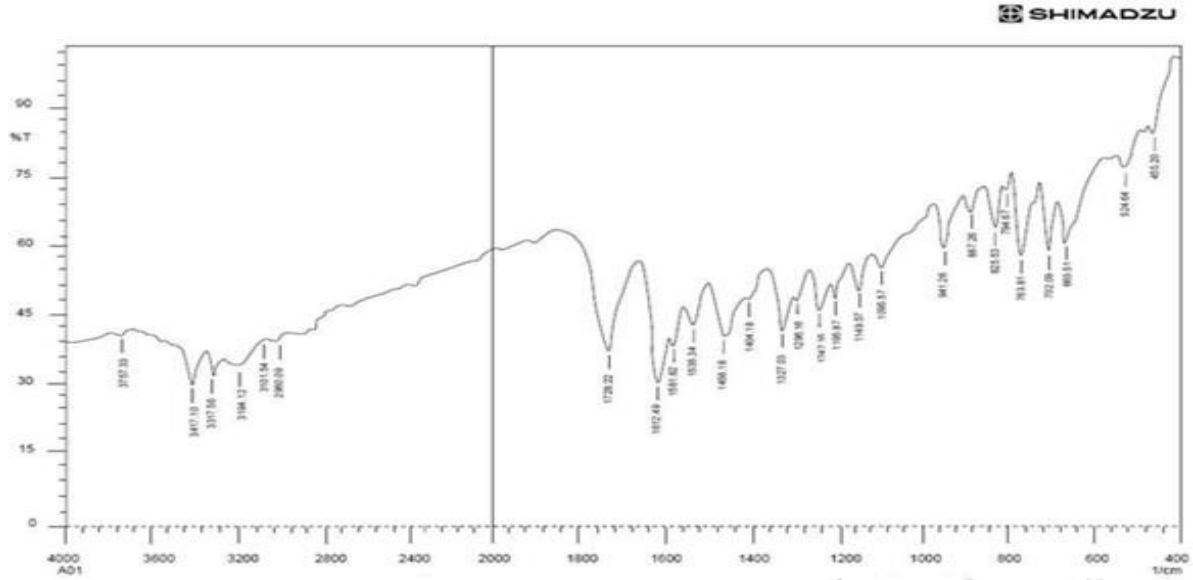

Fig. 4:- IR Spectra of 3R1-{[2-(2-hydroxyphenyl)-2-oxoethyl]amino}-4-(2-methylphenyl)azet-2(1H)one

SCHEME

Compound Code	R
1R	Cl
2R	OCH_3
3R	CH_3

Where R is

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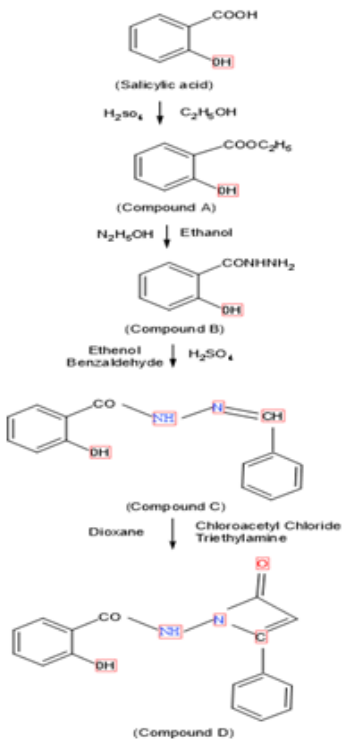

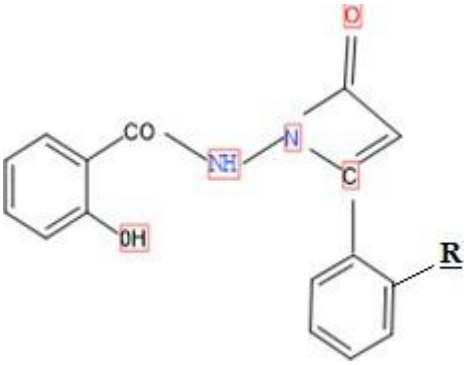

Antimicrobial activity

The antimicrobial activities of synthesized compounds were evaluated by the zone of inhibitionmethod 60. This methods 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[8].

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 100ml of a sterile nutrient broth in a flask, which was shaken thoroughly and incubated at 37^0C for bacteria.

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 (151b psig) for 15 minutes. The contents of tubes were poured aseptically in to sterile Petri plates (90 mm diameter) and allowed to solidity.

Microorgaisms used:-

Staphylococcus aureus (gram positive)
Bacillus subtillis (gram positive)
Pseudomonas aeruginosa (gram negative)
Escherichia coli (gram negative)

Drugs:

Ampicillin (antibacterial)

Preparation of Drug Solution:-

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

Method:

Previously liquefied Muller Hinton agar was inoculated with the requisite quantity of the suspension of the microorganism.

The suspension was added to the medium at a temperature between 40-50^0C and the inoculated medium was poured immediately into dried Petri dish to occupy adepth of 3-4 mm.

The petri dishes were sterilized at 160-170 ^{0}C for 1 hour, before use.

The paper disc (No. 2 Whatman) was cut down into a small disc (6 mm in diameter) and sterilized in the hot air oven, and then impregnated with the test solutions and standard solution.

The dried discs were placed on the surface of the medium.

After addition of all the drugs, Petri dishes were left standing for 1 to 4 hour 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 hour at the required temperature, i.e. 37^0C for bacteria.[9]

After incubation, the diameters of the circular inhibition zones were measured. **Antimicrobial Screening:-** Antimicrobial test was carried out on four bacterial strains, namely Bacillus subtilis

(gram positive), Staphylococcus aureus (gram positive), Escherichia coli (gram negative), Pseudomonas aeruginosa . The results are shown in below table.

Disc Diffusion Method

Antibacterial activity studies: All the synthesized azetidine derivatives have shown moderate to weak antibacterial activity. Compounds D showed potent activity against Bacillus subtillis and pseudomonas aeruginosa. The results are given in below table[10].

Results and Discussion

In the synthesis of Azetidine derivatives Starting with the salicylic acid and compound D and 3 derivatives were synthesized and characterized physiochemically as well as biologically. Based on the results compound D showed better results compared to derivatives which were synthesized and evaluate Antibacterial activity of synthesized compound D and Derivatives

Table 1: ZOI of compounds

Microorganism	Zone of Inhibition(mm)				
	Compound D (100 µg/kg)	Compound 1R(100 µg/kg)	Compound 2R(100 µg/kg)	Compound 3R(100 µg/kg)	Standard (100 µg/kg)
Staphylococcus aureus	10	8	7	8	20
Bacillus Subtillis	15	7	9	8	22
Pseudomonas aeruginosa	12	10	9	7	21
Escherichia coli	8	9	8	8	22

Standard drug- Ampicillin

NA= No activity at this amount of test compound or standard

Table 2: Physicochemical Properties of Compounds

S/No.	Name compound	Mol. Formula	Mol. Wt.	M.P.	Solubility	Rf value	Color	Yield
1	A	C9H10 O3	166.17	232°C	Ethanol	0.6	Colorless liquid	70%
2	B	$C_7H_8N_2O_2$	152.15	147-150°C	Methanol	0.43	Off white	62%
3	C	$C_{14}H_{12}N_2O_2$	240.25	130-138 °C	ethanol	0.52	Off white	65%
4	D	$C_{16}H_{12}N_2O_3$	264	140-142°C	Ethanol, DMSO, Acetone	0.684	Off white	75%
5	E	$C_{16}H_{11}O_3N_2Cl$	279	145-149°C	Ethanol, Acetone	0.51	Off white	70%
6	F	$C_{17}H_{14}O_4N_2$	310	130-132°C	Ethanol, Acetone	0.34	Off white	65%
7	G	$C_{17}H_{14}O_3N_2$	294	135-138°C	Ethanol, Acetone	0.46	Off white	70%

Conclusion

This research work was oriented towards the finding of newer Azetidine derivative with antimicrobial activity. Azetidines are an important class of four-membered monocyclic aza-heterocyclic compounds with remarkable bio-logical activities. Increased activity in the area of azetidine synthesis over the last few decades has been driven by growth in their application in both synthetic and medicinal chemistry.The different substituted azetidine derivative were synthesized followed by cyclization reaction. In the synthesis of Azetidine derivatives, Starting with the salicylic acid and compound D and 3 derivatives were synthesized and characterized physiochemically as well as biologically.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 FTIR spectrometer. The formed compound D (2-hydroxy-N-(2-oxo-4-phenylazet-1(2H)-yl-) benzamide) with melting point of 140- 142^{o}C, Solid State, Molecular Weight-264, Solubility in Ethanol, DMSO, Acetone.

The newly synthesized azetidine derivatives were evaluated for their antimicrobial activity. 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.For antibacterial activity Staphylococcus aureus, Bacillus subtillis, Pseudomonas aeruginosa, Escherichia coli microorganisms used. Compounds D showed potent activity against Bacillus subtillis and pseudomonas aeruginosa, and Ampicillin used as a standard drug.

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