International Journal of Pharmacy and Medicine Sciences
Vol. 2, Issue 5, May 2026 *Indexed Journal*
ISSN: 3108-009X **DOI:** https://doi.org/10.5281/zenodo.20640694

Open Access RESEARCH

Synthesis, Characterization and Antimicrobial Screening of Azetidinone Derivatives

[1]*Vanshika Dixit, [2]Shalini Singh

[1]*Research Scholar, Institute of Pharmaceutical Sciences and Research, Unnao, UP
[2]Associate Professor, Institute of Pharmaceutical Sciences and Research, Unnao, UP

***Submission**: May 19, 2026; **Revised**: May 27, 2026; **Published**: May 31, 2026*

Corresponding author:
Vanshika Dixit
[1]*Research Scholar, Institute of Pharmaceutical Sciences and Research, Unnao, UP
Email id: dixitvanshika2004@gmail.com

ABSTRACT

The study was based on the Synthesis, Characterization and Antimicrobial Screening of Azetidinone Derivatives. After synthesis, the novel Azetidinones derivatives were characterized for following parameters i.e., physical appearance, melting point, TLC (Rf values), FTIR analysis, NMR analysis and Mass spectroscopy. To evaluate the anti-fungal potential of novel Azole (1,3,4-Oxadiazole) derivatives activity through well diffusion method against fungal strains i.e., *S. aureus*, *Aspergillus niger*, *E. coli* and *Candida albicans*. The physical characteristics of every Azetidinones derivative that was synthesized were examined. A2 showed a yield of 0.78 %, which was its highest percentage. A4 had the lowest yield percentage, at 0.65 %. Zone inhibition (anti-microbial activity) was recorded as 4.86 mm, 5.27 mm, 5.49 mm, and 6.34 mm in *S. aureus*, A. *niger*, *E. coli* and *C. albicans*, respectively. It can be concluded that the anti-microbial potential was much significant against *C. albicans*. Its anti-microbial potential might be due to the destruction of cell wall and/ nucleic acid of diverse microbial strains. Gentamycin as std. anti-microbial agent exhibited highest inhibition zone ranging from 13.0 to 26.0 mm. It also showed highest zone inhibition against *C. albicans* as 26.0 mm.

Keywords: Azetidinones derivatives, anti-microbial, Zone inhibition NMR, FTIR.

INTRODUCTION

Bacteria are everywhere and they are essential to maintaining our living environment. Worldwide, just a small percentage of micro-organisms cause disease and infection [1]. Bacteria are distinct among prokaryotes as numerous species constitute common flora that inhabit the host without inducing infection. For an organism to spread, it must survive and find a host that is vulnerable to it. A lot of bacteria have learned how to live in many places, such food, soil, and water [2].

It is also possible to think of the macro- or micro-environments as contributing to the spread of germs. Certain creatures are found in particular environments, such as jails and hospitals. In some geographical areas, some bacteria are rare or non-existent, while in others, they are endemic [3][4].

Drug profile: Azetidinone

2-Azetidinones, sometimes referred to as ß-lactams, are cyclic amides with four members. The nitrogen atom is bonded to the ß carbon in relation to the carbonyl, thus the name [5].

Depending on the number of atoms in the bicyclic system, the fused ß-lactams are called isoheptanam, isooctanam, isononam, and so forth if 2-azetidinones are fused with ring system at positions 3 and 4. The trivial name system refers to ß-lactams having fused bicyclic systems, such as 3, 6-dihydro-2H-1,3 thiazine, as

cepham and ß-lactams fused with thiazolidines as penam. However, this naming scheme was insufficient. Monobactams are ß-lactams that have not been fused with any other ring system [6].

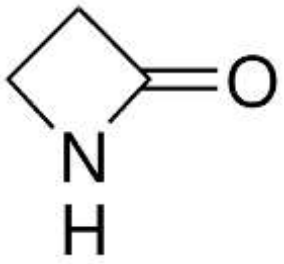

Fig 1. Structure of Azetidinone

IUPAC name : Azetidin-2-one

Molecular formula : C_3H_5NO

Molecular weight : 71.08

Physical state : Colourless solid

Melting point : 73-74°C.

MATERIALS AND METHODS

Experimental requirements

Table 1. List of chemicals

Chemical	Source
Substituted aniline	Sigma-Aldrich
Substituted benzaldehyde	Sigma-Aldrich
Carbon di-sulfide	SD Fine Chemicals
Ethanol	SD Fine Chemicals
Ethanol	SD Fine Chemicals

Table 2. List of Instruments

Chemical	Source
FTIR	Shimadzu Corp.
UV Spectrophotometer	Shimadzu
NMR	Shimadzu
Melting point apparatus	Scientech
Digital pH meter	Citizen scale
Clinical thermometer	Deluxe Scientific Ltd

Synthesis of novel Azetidinones derivatives

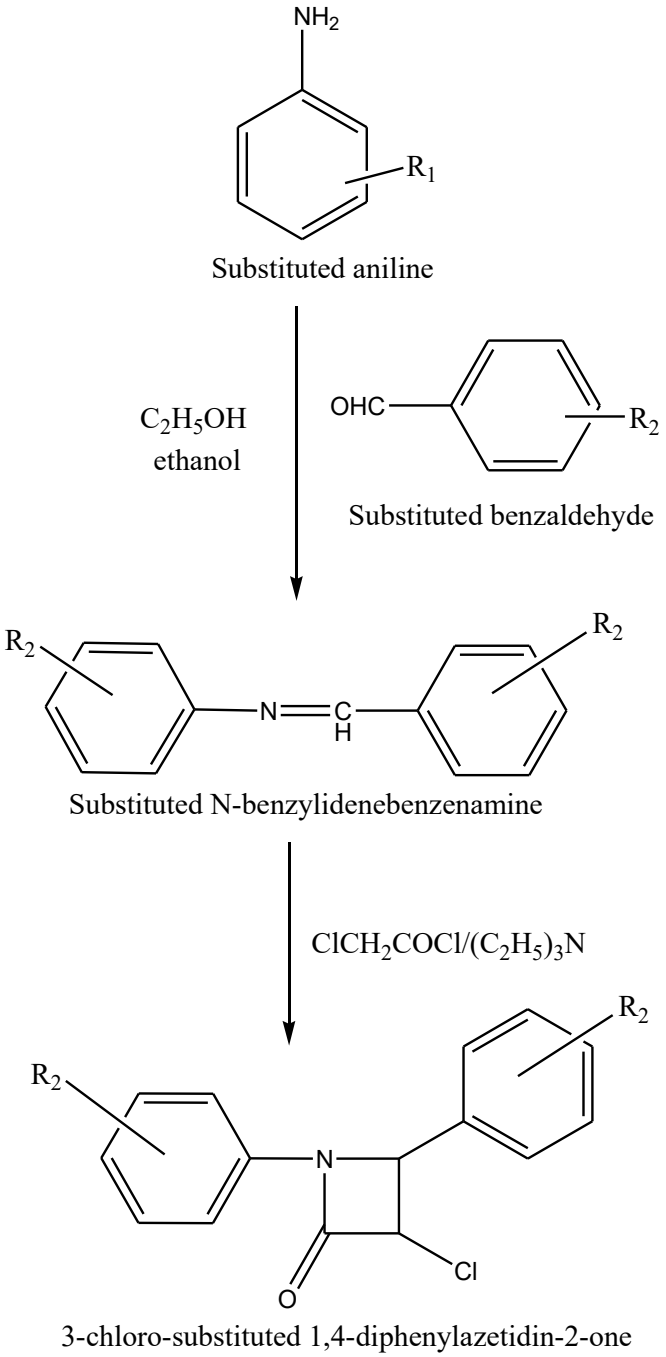

Fig 2. Scheme for synthesis of novel Azetidinones derivatives

Identification of physicochemical properties

Melting point determination

An organic compound's melting point was ascertained using Thiel's melting point tube. Determining a compound's melting point is the most crucial and simple way to differentiate one from another.

Determination of Rf value using TLC

In synthetic chemistry, thin layer chromatography is used to determine a molecule's synthesis based on its Rf value, which changes depending on the substance. Additionally, it helps verify the course of the reaction [7].

Infrared Spectroscopy

The infrared spectrum is seen as a vibrational-rotational spectrum. For solid compounds, the KBr pellet technique is employed; for liquid compounds, the Nujol mull method is utilised. It is a very useful document

that provides details about the functional groups found in organic molecules. When electromagnetic radiation between 500 and 4000 cm-1 passes through a sample, the mechanism of bond stretching and bending occurs [8].

NMR Spectroscopy

Because of its sensitivity and variety of characteristic information, proton NMR is the most often utilised kind of NMR. Chemical 150 shift (δ) range: 0-14 ppm. TMS protons, which are ascribed at 0 ppm, were used to compare the chemical shift of the test unknown substance. However, the shift extends for the component's organic compound range δ 0–14 [9].

Mass Spectroscopy

An essential physicochemical technique for the structural elucidation of molecules from natural products, including medicinal plants, is mass spectrometry (MS). The employment of various physical methods for sample ionisation and the separation of the ions produced according to their mass to charge ratio (m/z) constitute the basic idea of mass spectrometry. Compared to NMR, which has a sensitivity limit of nanograms and higher, mass spectrometry has a high sensitivity with a detection limit of fentograms. MS is a flexible analytical tool due to its sensitivity and ability to be hypenated with other chromatographic techniques [10].

Pharmacological evaluations

Evaluation of anti-microbial potential

By using the well diffusion method, the anti-microbial activity of Azetidinones derivatives against microbial strains is evaluated. Nutrient Agar broth is utilized to cultivate a 24-hour-old culture of microbes, which is then used to make a suspension of microorganisms. After sterilization at 121°C (1.05kg/cm2 pressure) for 20 minutes, nutrient agar solution was added. A sterile spreader is used to cover the whole surface of the agar plates when we inoculated them with 500 l of each fungal suspension. With a sterile cork borer, 5 mm wells were made in the solidified media, and each well was filled with microbes. The diameter (mm) of the inhibitory zone surrounding the well is measured after 24 hours of incubation at 30°C. As a standard anti-microbial formulation, itraconazole gel (Itromed 1%) is utilized. Every anti-fungal study is carried out in triplicate. Microbial strains i.e., *S. aureus*, *Aspergillus niger, E. coli* and *Candida albicans* is utilized for screening of anti-microbial activity [11].

RESULTS AND DISCUSSION

Synthesis of novel Azetidinones derivatives

Azetidinones derivative (1)

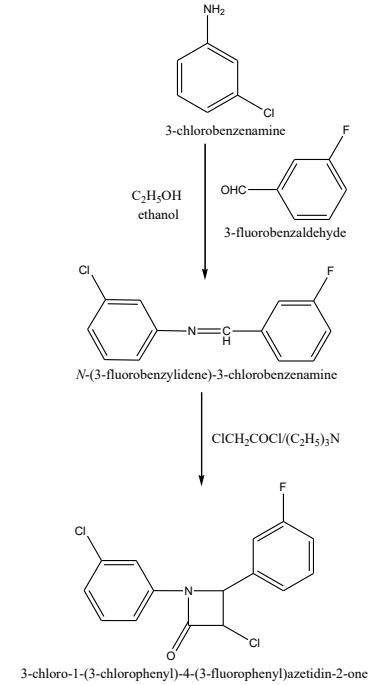

Fig 3. Azetidinones derivative (1)

Azetidinones derivative (2)

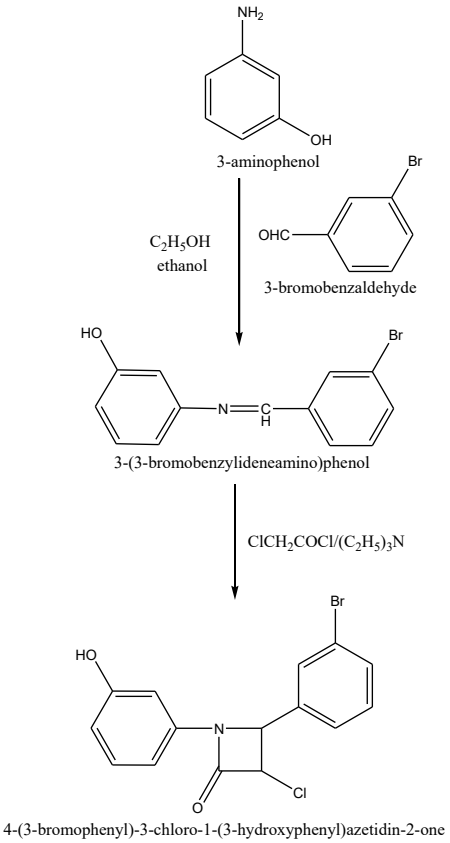

Fig 4. Azetidinones derivative (2)

Azetidinones derivative (3)

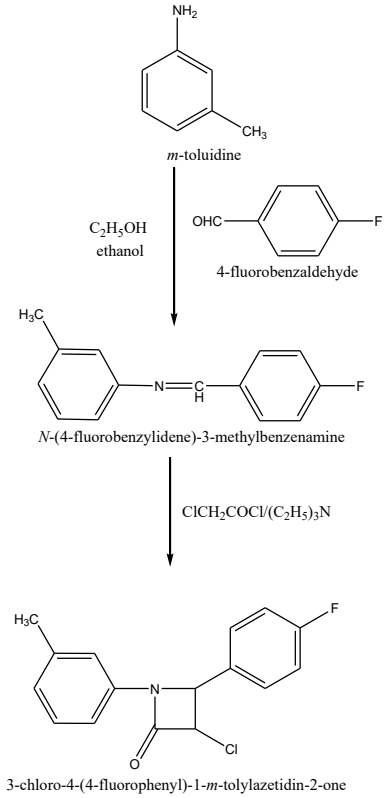

Fig 5. Azetidinones derivative (3)

Azetidinones derivative (4)

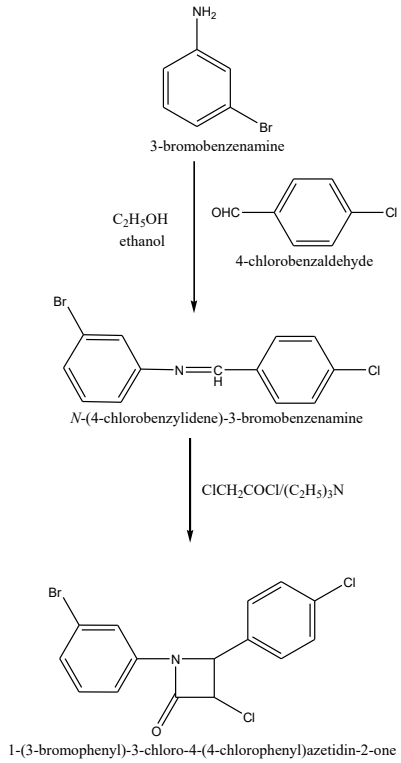

Fig 6. Azetidinones derivative (4)

Azetidinones derivative (5)

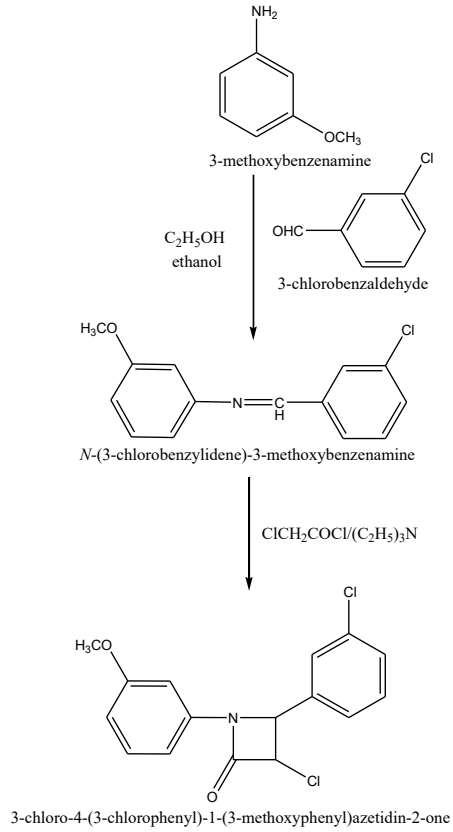

Fig 7. Azetidinones derivative (5)

Azetidinones derivative (6)

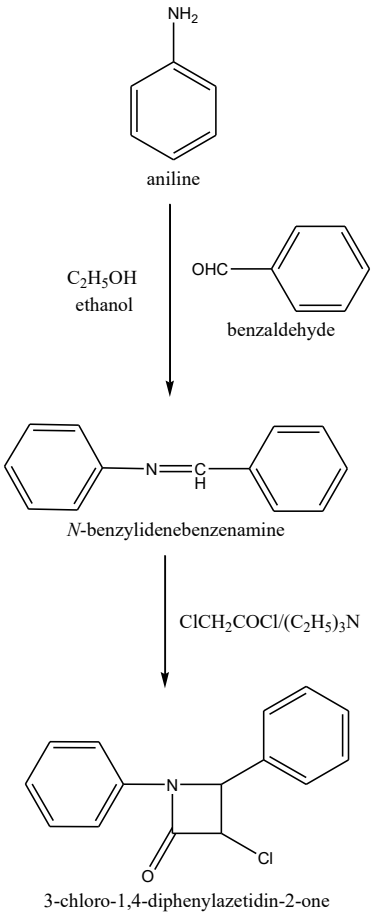

Fig 8. Azetidinones derivative (6)

Identification of physicochemical properties

Melting point determination

Melting point was determined as 74ºC, 76ºC, 75ºC, 74ºC, 76ºC and 76ºC for the Azetidinones derivative (A1), Azetidinones derivative (A2), Azetidinones derivative (A3), Azetidinones derivative (A4), Azetidinones derivative (A5) and Azetidinones derivative (A6), respectively.

Table 3. Melting point

Compound	Melting point
A1	74ºC
A2	76ºC
A3	75ºC
A4	74ºC
A5	76ºC
A6	76ºC

Thin Layer Chromatography

In synthetic chemistry, thin layer chromatography (TLC) was used to determine a molecule's synthesis based on its Rf value, which changes depending on the substance. The corresponding Rf values for A1, A2, A3, A4, A5, and A6 were 0.73, 0.78, 0.73, 0.69, 0.76, and 0.65, respectively.

The physical characteristics of every Azetidinones derivative that was synthesized were examined. A2 showed a yield of 0.78 %, which was its highest percentage. A4 had the lowest yield percentage, at 0.65 %. The compound's strongest density is indicated by its highest melting point. Significant molecular weight was also discovered in the Azetidinones analogues that were produced. The molecular weights of A1, A2 and A6 were determined to be 262.20 g/mol, 254.43 g/mol and 257.74 g/mol, respectively. The physical characteristics of each compound were compiled in the following table.

Table 4. Physico-chemical properties of Azetidinones derivative

Compound	Yield (%)	Rf Value	Mol. weight
A1	26.11	0.73	262.20
A2	29.16	0.78	254.43
A3	24.36	0.73	268.19
A4	27.18	0.69	274.25
A5	23.47	0.76	263.13
A6	47.29	0.65	257.74

FTIR Spectroscopy

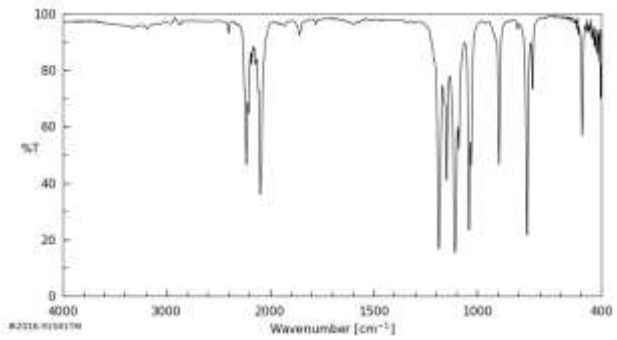

Fig 9. FTIR Spectrum of A1

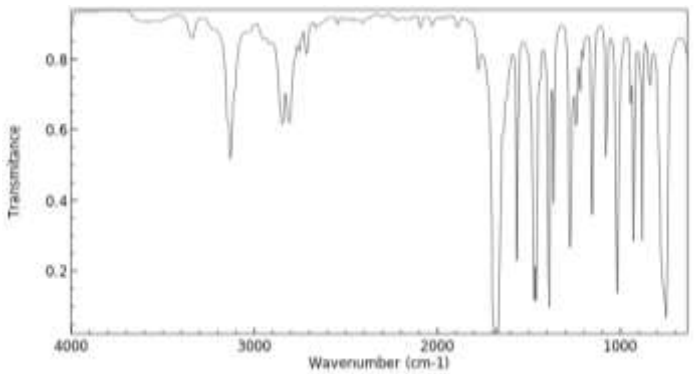

Fig 10. FTIR Spectrum of A2

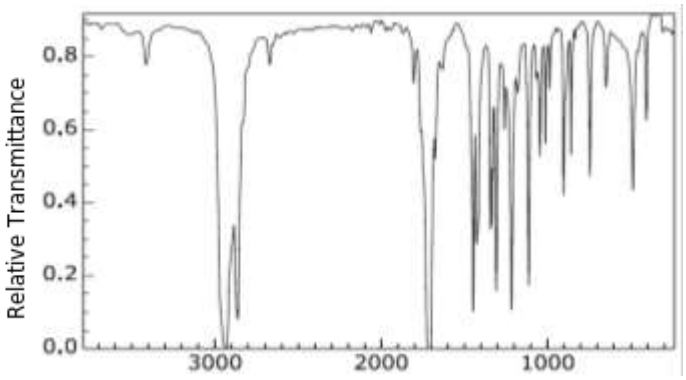

Fig 11. FTIR Spectrum of A3

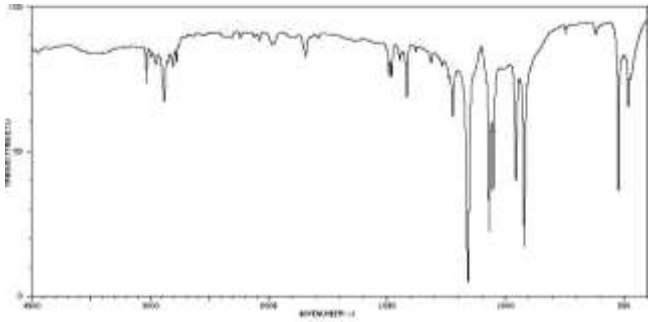

Fig 12. FTIR Spectrum of A4

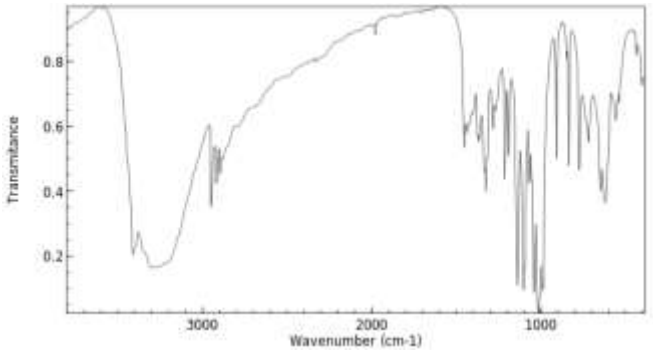

Fig 13. FTIR Spectrum of A5

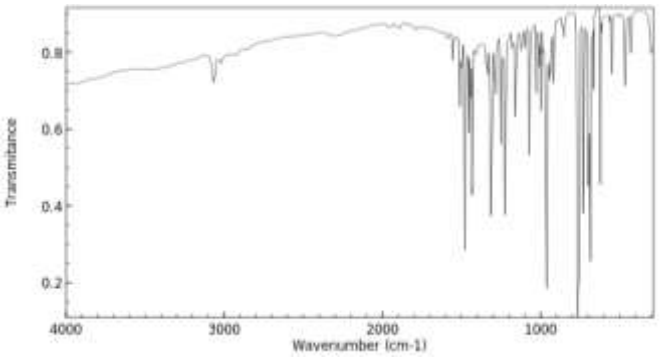

Fig 14. FTIR Spectrum of A6

NMR Spectroscopy

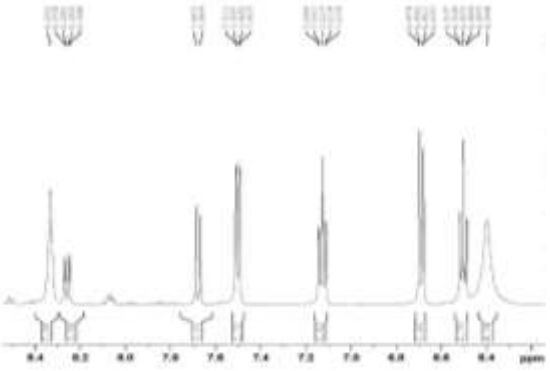

Fig 15. NMR Spectrum of A1

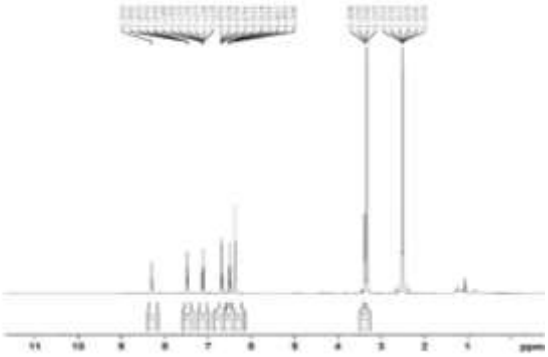

Fig 16. NMR Spectrum of A2

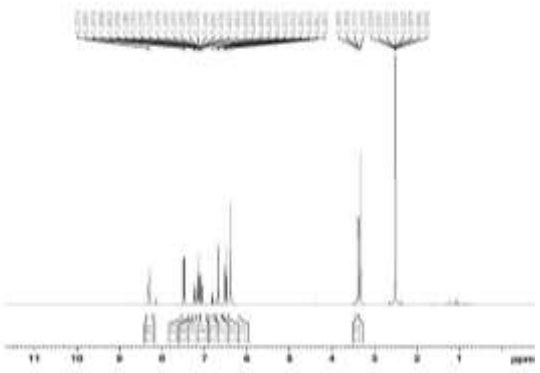

Fig 17. NMR Spectrum of D3

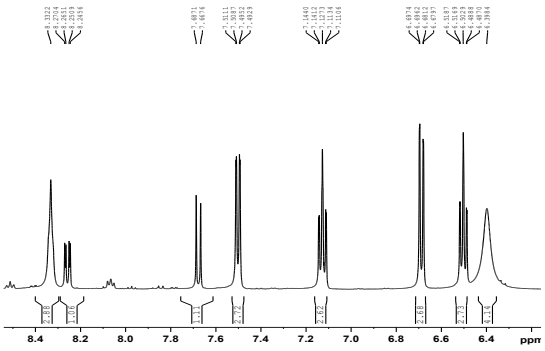

Fig 18. NMR Spectrum of D4

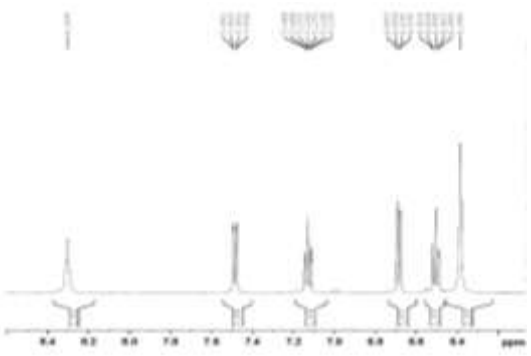

Fig 19. NMR Spectrum of A5

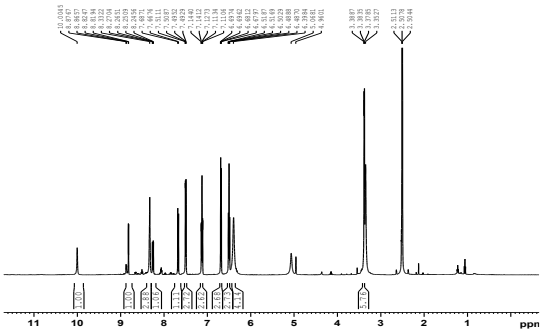

Fig 20. NMR Spectrum of A6

Mass Spectroscopy

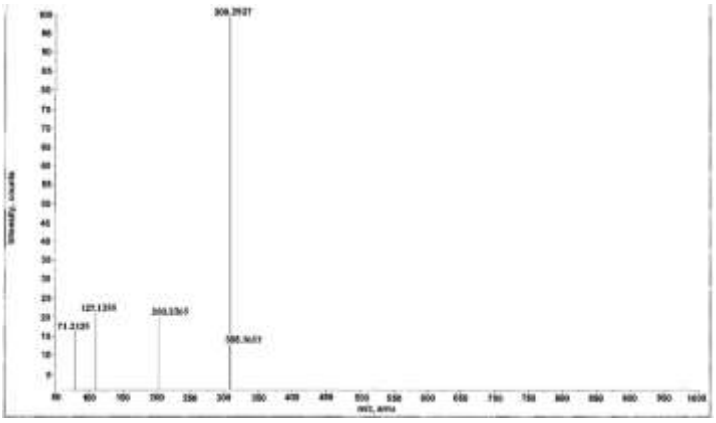

Fig 21. Mass Spectrum of A1

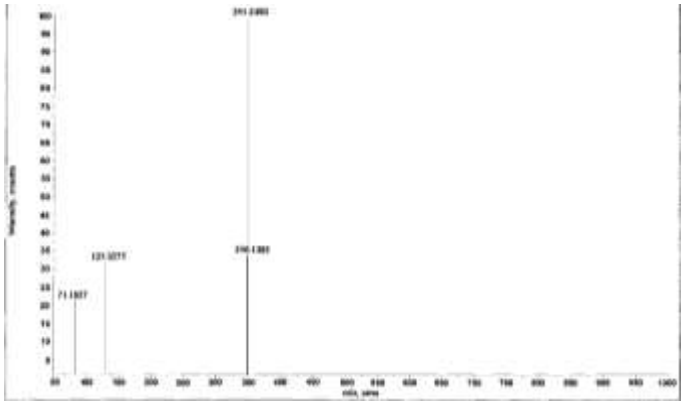

Fig 22. Mass Spectrum of A2

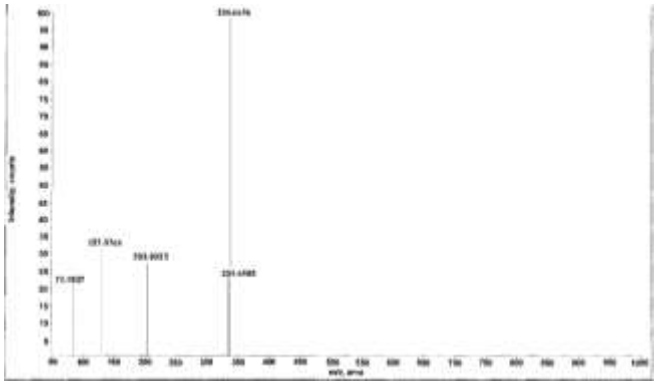

Fig 23. Mass Spectrum of A3

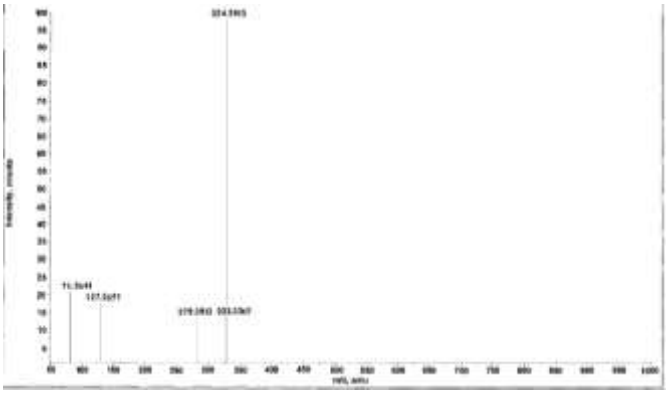

Fig 24. Mass Spectrum of A4

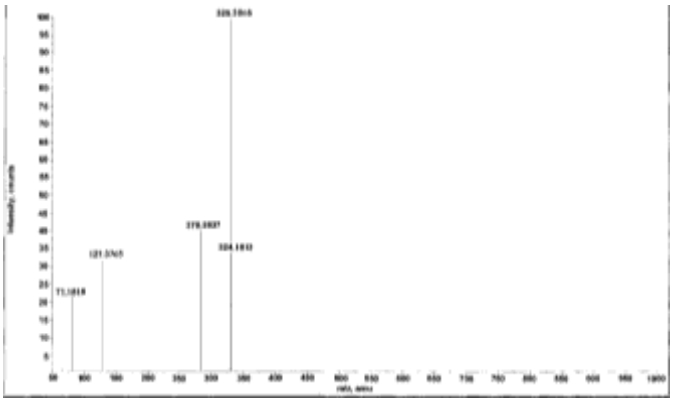

Fig 25. Mass Spectrum of A5

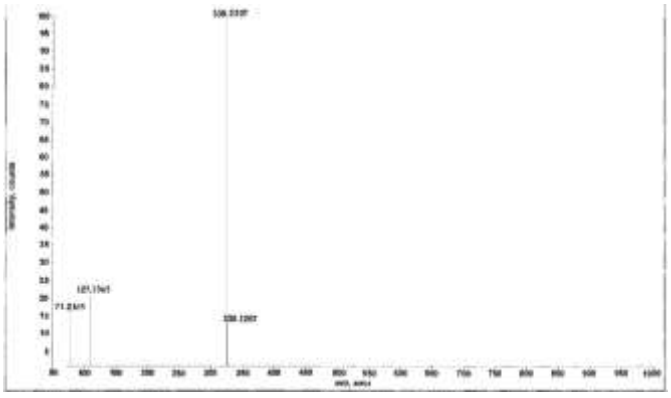

Fig 26. Mass Spectrum of A6

Evaluation of anti-microbial potential

Well diffusion technique

The anti-microbial activity was evaluated using different 4 strains of fungus i.e., *S. aureus*, A. *niger, E. coli* and *C. albicans*. Zone inhibition (anti-microbial activity) was recorded as 4.86 mm, 5.27 mm, 5.49 mm, and 6.34 mm in *S. aureus*, A. *niger, E. coli* and *C. albicans,* respectively. It can be concluded that the anti-microbial potential was much significant against *C. albicans.* Its anti-microbial potential might be due to the destruction of cell wall and/ nucleic acid of diverse microbial strains.

Gentamycin as std. anti-microbial agent exhibited highest inhibition zone ranging from 13.0 to 26.0 mm. It also showed highest zone inhibition against *C. albicans* as 26.0 mm.

Table 5. Anti-microbial potential of Azetidinones derivative

Treatment	Zone inhibition (mm)			
	S. aureus	*A. niger*	*E. Coli*	*C. albicans*
Azetidinones derivative (A6)	4.86	5.27	5.49	6.34
Gentamycin	13.0	16.0	23.0	26.0

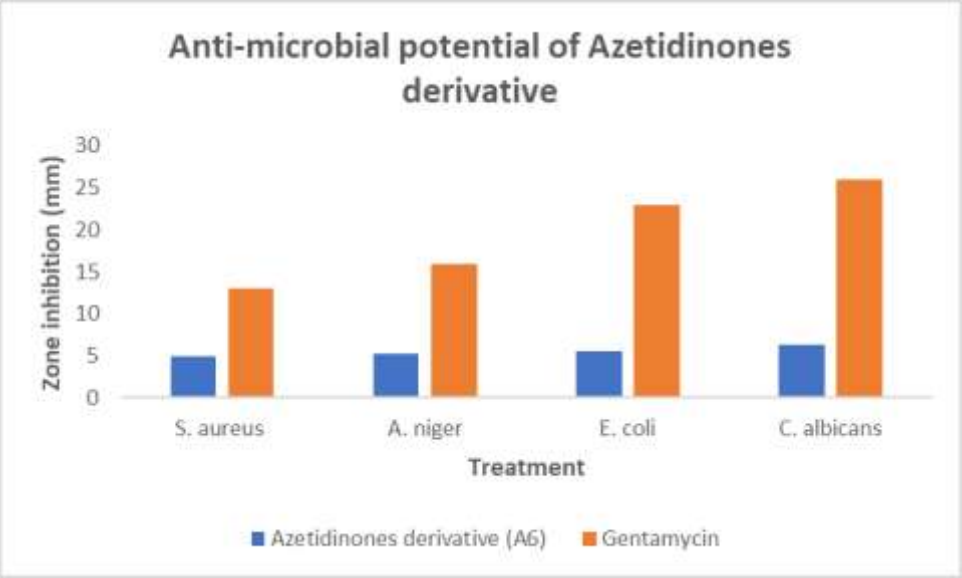

Fig 27. Graphical data of anti-microbial potential of Azetidinones derivative (A6)

Azetidinone hybrids containing 1,2,4 triazoles were discovered to have antitubercular action against the Mycobacterium tuberculosis H37 RV strain on Middle Brook 7H9 agar medium. Several Azetidine-Oxazole hybrids were created and their antioxidant potentials assessed using the free radical scavenging technique; several of these hybrids have demonstrated encouraging outcomes. Additionally, it was discovered that novel Quinazolyl fused azetidinone hybrids were created, described, docked into the energy pockets of enzymes using an in-silico technique, and assessed for antimalarial research, yielding encouraging docking results [12].

Using the proper solvents, each synthesised final chemical was first purified by serial recrystallisation. Melting points and thin-layer chromatography were used to assess the synthetic substances' purity. In order to verify the structures, the synthesised compounds were then submitted to spectrum analysis using IR, NMR, and mass spectra. Every analytical detail reveals outcomes that are favourable. The production of 2-azetidinones was verified by the subsequent peaks. The groups of C=O, C-Cl, and C-N in 2-azetidinones have been identified by the FTIR peaks at 1718-1687 cm-1, 806-804 cm-1, and 1398.30 cm-1. The HNMR spectra of the C-CH-Cl and N-CH-C groups show peaks at 1.4 ppm and 2.12-2.18 ppm have verified the production of 2-azetidinones. Apart

from the fragmentation profile, every mass spectrum displayed the molecular ion peaks for the corresponding molecular weights. Ketoconazole and ampicillin have been employed as benchmarks. Every chemical has demonstrated modest to moderate activity. Among them, 2-azetidinone (compd no. 3g), which has 2, 4 dimethyl amino phenyl at the second position, has demonstrated excellent action in every species [13].

A novel technique for the synthesis of the aforementioned azetidin-2-one derivatives utilising microwave irradiation offers notable advancements over current methods, making it easier to enter the synthesis of a range of azetidin-2-one derivatives. Additionally, this straightforward and repeatable method produces a variety of azetidin-2-one derivatives with quick reaction times, high yields, and no unwanted side product production. The melting point and thin layer, which verify the reaction's completion, were used to characterise the various synthesised compounds, and the yields were determined to be between 70 and 80 percent. Every chemical that was examined had good, moderate, and low biological activity.

CONCLUSION

The synthesized novel Azetidinones derivatives might be very significant in terms of treating microbial infections. It can destroy the microbial growth and development in the living tissues. The Azetidinones derivatives would have a significant impact on microbial infections and leaving the patient healthy.

In conclusion, Azetidinones derivatives might be much significant in destructions of microbial strains which are life-threatening to mankind. Since they are synthesized from the synthetic substituted aniline, their production will be cost-effective, with the worldwide availability.

CONFLICT OF INTEREST

Authors declare for none conflict of interest.

REFERENCES

[1]. Bannister B.A., Begg N.T., Gillespie S.H., editors. Structure and classification of pathogens. 2nd edn. Blackwell Science Ltd; Oxford, UK: 1996. pp. 23-34. (Infectious Disease). ch. 2.

[2]. Engelkirk P.G., Burton G.R., editors. Epidemiology and public health. 8th edn. Lippincott Williams and Wilkins; Baltimore: 2006. (Burton’s Microbiology for the Health Sciences). ch. 11.

[3]. Engleberg N.C., DiRita V., Dermody T.S. Lippincott Williams and Wilkins; Baltimore, MD: 2007. Schaechter's Mechanisms of Microbial Disease.

[4]. S Doron, SL Gorbach . Bacterial Infections: Overview. International Encyclopedia of Public Health. 2008; 273–282.

[5]. Tidwell, Thomas T: Hugo (Ugo) Schiff, Schiff Bases, and a Century of β-Lactam Synthesis. Angewandte Chemie International Edition. 2008; 47(6): 1016.

[6]. Ashokrao A A., Synthesis and Biological Activities Of Some Substituted Azetidinones (dissertation), Rajiv Gandhi University of health sciences, Karnataka, 2006; 23.

[7]. Sanjeet Kumar, K. Jyotirmayee, Monalisa Sarangi. Thin Layer Chromatography: A Tool of Biotechnology for Isolation of Bioactive Compounds from Medicinal Plants. Int. J. Pharm. Sci. Rev. Res., 2013; 18(1) 126-132.

[8]. Griffiths, P.; de Hasseth, J. A. Fourier Transform Infrared Spectrometry (2nd ed.). Wiley-Blackwell. 2007.

[9]. Vandersypen, Lieven M. K.; Steffen, Matthias; Breyta, Gregory; Yannoni, Costantino S.; Sherwood, Mark H.; Chuang, Isaac L. Experimental realization of Shor's quantum factoring algorithm using nuclear magnetic resonance. Nature. 2001; 414 (6866): 883-887.

[10]. Benton HP, Wong DM, Trauger SA, Siuzdak G. XCMS2: processing tandem mass spectrometry data for metabolite identification and structural characterization. Analytical Chemistry. 2008; 80 (16): 6382–9.

[11]. Kaushik K, Sharma RB, Sharma A, Agarwal S. Evaluation of antifungal activity of crude methanolic extract of leaves of Ipomoea carnea and its formulation as a gel. J Res Pharm. 2020; 24(3): 368-379.

[12]. Prakali P and Dinnimath BM: Scope of azetidinone hybrids for diverse pharmacological activities; a review. Int J Pharm Sci & Res 2021; 12(12): 6298-09.

[13]. Jubie Jawahar, Gowramma Byran, N.K. Muthal, Kalirajan Rajagopal. Synthesis and Antimicrobial Evaluation of some2-Azetidinone derivatives. International Journal of ChemTech Research, 2009; 1(2): 153-157