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.20641312

Open Access RESEARCH

Design Synthesis and Evaluation of New Azole as Anti-fungal Agents

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***Submission**: May 03, 2026; **Revised**: May 27, 2026; **Published**: May 31, 2026*

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ABSTRACT

The study was based on the design, synthesis and evaluation of new azole as antifungal agents. After design and synthesis, the novel Azole derivatives, these were characterized the synthesized novel Azole derivatives using 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., *Aspergillus niger, Penicillium notatum, Candida albicans* and *Rhizopus species.* Zone inhibition (anti-fungal activity) of Oxadiazole (C4) 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-fungal potential was much significant against *Rhizopus species* which proves its anti-fungal action. Its anti-fungal potential might be due to the destruction of cell wall and/ nucleic acid of diverse fungal species. Ketoconazole as std. anti-fungal agent exhibited highest inhibition zone ranging from 12.0 to 24.0 mm. It also showed highest zone inhibition against *Rhizopus species* as 24.0 mm.

Keywords: 1,3,4-Oxadiazole derivatives, anti-fungal, NMR, FTIR.

INTRODUCTION

Fungal skin infections are one of the most prevalent dermatological issues nowadays. According to research, 40 million people have fungal infections [1]. Antifungal medications are utilized in the treatment and prevention of human fungal infections [2]. Antifungals are categorized into five groups based on their site of action: azoles, which inhibit ergosterol synthesis [3][4][5]. Many synthetic antifungal drugs are used in clinical settings to treat various fungal infections. Azoles, allylamines, echinocandins, and polyenes are the four general groups into which these drugs can be divided [6][7].

The use of antibacterial medicines for viral illnesses, infections that go away on their own without treatment and have little chance of serious side effects, and infections brought on by organisms that are resistant to the antibiotic are all examples of inappropriate use. Using broad-spectrum antibiotics, which kill or stop a lot of organisms at once, when a medicine with a narrower spectrum will work is another example of misuse [8].

Drug profile: 1,3,4-oxadiazole

Oxadiazole is heterocyclic compound having 2 nitrogen and 1 oxygen atom together to form a heterocyclic ring with five members. These substances have a broad range of biological activity, allowing for their use as active agents in pharmacology and medicine including blood pressure lowering, antibacterial, antiviral, antifungal, anticancer, and anti-inflammatory & anti-nociceptive. As a result, both the molecule's stability and quantum

yield of fluorescence can be raised. Additionally, products like thermal insulation polymers include these molecules [9].

Fig 1. Isomeric structures of oxadiazoles

MATERIALS AND METHODS

Experimental Requirements

Potassium hydroxide, Substituted benzo-hydrazide, Carbon di-sulfide, ethanol, distilled water and paraffin, Weight balance, RBM, condenser, thermometer, and pH meter.

Synthesis of novel derivatives of 1,3,4-oxadiazole

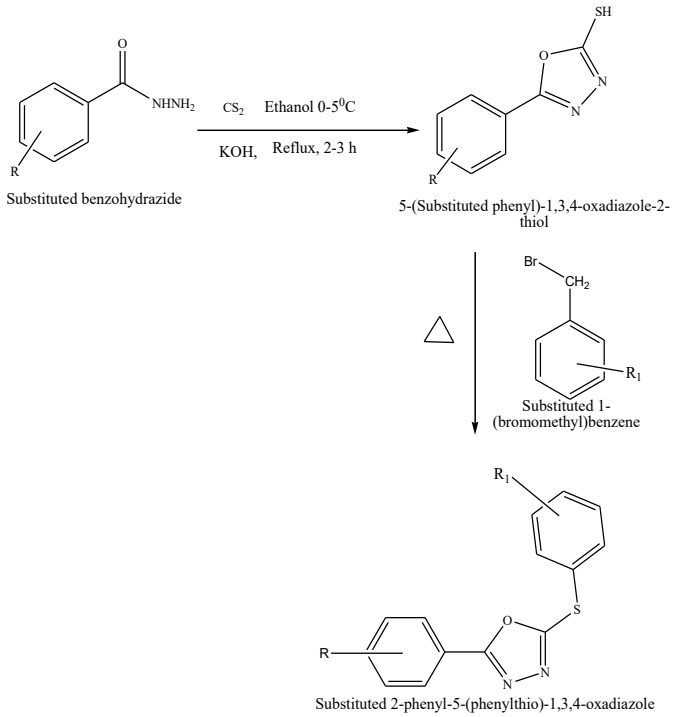

Fig 2. Scheme for synthesis of 1,3,4-oxadiazole derivatives

Identification of physicochemical properties

Melting point

The melting point of an organic compound was ascertained using Thiel's melting point tube. Finding a compound's melting point is the most crucial and direct way to differentiate one from another [10].

Rf value

Thin layer chromatography (TLC) is a technique in synthetic chemistry that uses a compound's variable Rf value to deduce the molecule's synthesis. It also helps to validate the reaction's advancement [11].

Infrared Spectroscopy

One classifies the infrared spectrum as a vibrational-rotational spectrum. For solid compounds, the KBr pellet technique is utilized; for liquid compounds, the Nujol mull method is employed. It is a very useful document that provides details about the functional groups found in organic molecules. When electromagnetic radiation

with a wavelength spanning from 500 cm-1 to 4000 cm^{-1} passes through a sample, the mechanism of bond stretching and bending occurs [12].

NMR Spectroscopy

Proton NMR is the most widely utilized NMR method due to its high sensitivity and extensive characteristic information. The chemical shift (δ) range is 0–14 ppm. The test unknown compound's chemical shift was compared to TMS protons, which had an attribution of 0 ppm. However, the shift extends to the component for the organic compound range δ 0 – 14 [13].

Mass Spectroscopy

An essential physicochemical tool for determining the structures of chemicals found in natural goods, such as medicinal herbs, is mass spectrometry. The application of various physical techniques for sample ionization and ion generation based on mass to charge ratio (m/z) is the fundamental idea of mass spectrometry. Electrospray ionization, air pressure chemical ionization, electron ionization, chemical ionization, rapid atom bombardment, and matrix analysis laser desorption ionization are among the ionization techniques that are accessible. Compared to NMR, which has a sensitivity limit of the nanogram range and above, mass spectrometry has a high sensitivity with a detection limit of the fentogram. MS is a versatile analytical tool because to its sensitivity and versatility for hypenation with other chromatographic techniques [14].

Pharmacological evaluations

Evaluation of anti-microbial potential

The anti-fungal activity of Azole derivatives against fungal strains is evaluated using the well diffusion method. 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 is 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-fungal, itraconazole gel is utilized. Every anti-fungal study is carried out in triplicate.

Fungal strains i.e., *Aspergillus niger, Penicillium notatum, Candida albicans* and *Rhizopus species* is utilized for screening of anti-fungal activity [15].

RESULTS AND DISCUSSION

Synthesis of Azole derivatives

Synthesis of C1

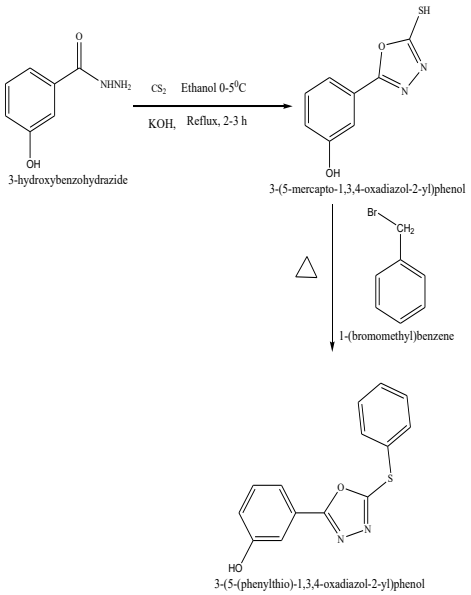

Fig 3. Synthesis of C1

Synthesis of C2

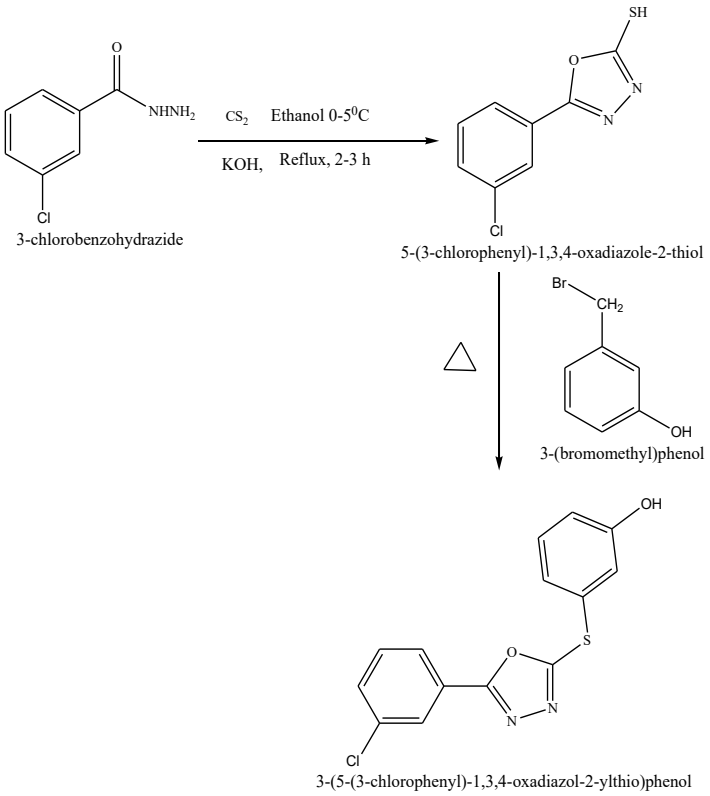

Fig 4. Synthesis of C2

Synthesis of C3

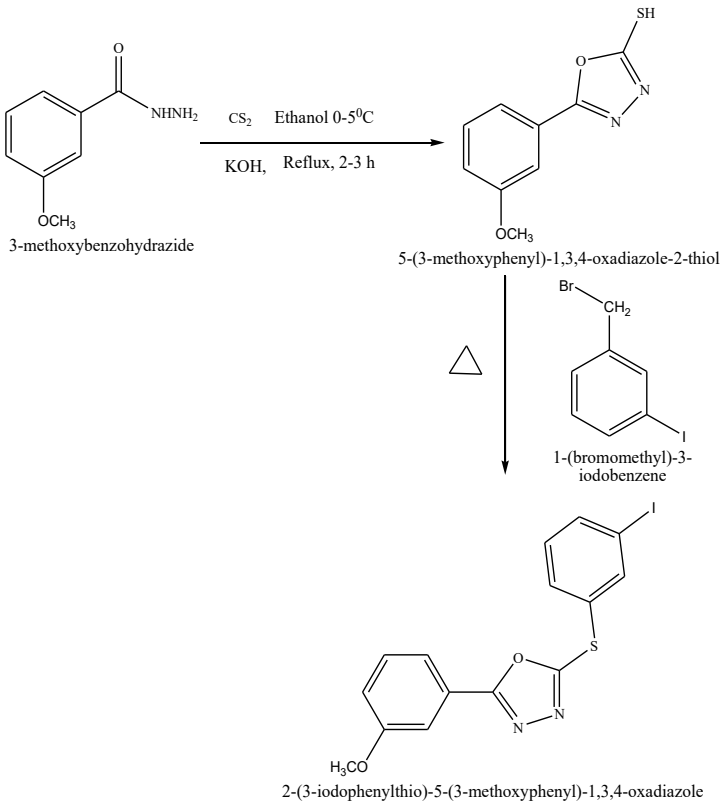

Fig 5. Synthesis of C3

Synthesis of C4

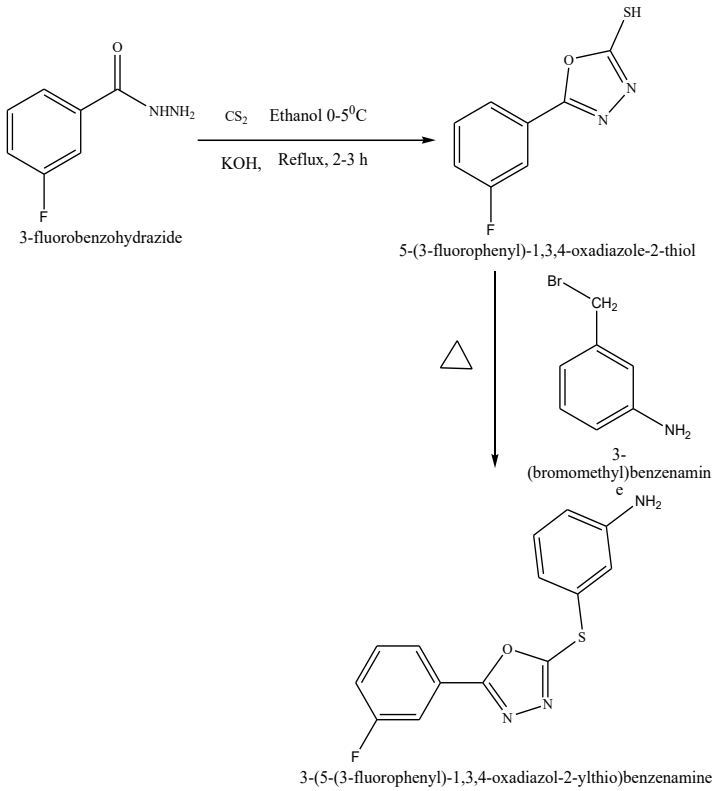

Fig 6. Synthesis of C4

Synthesis of C5

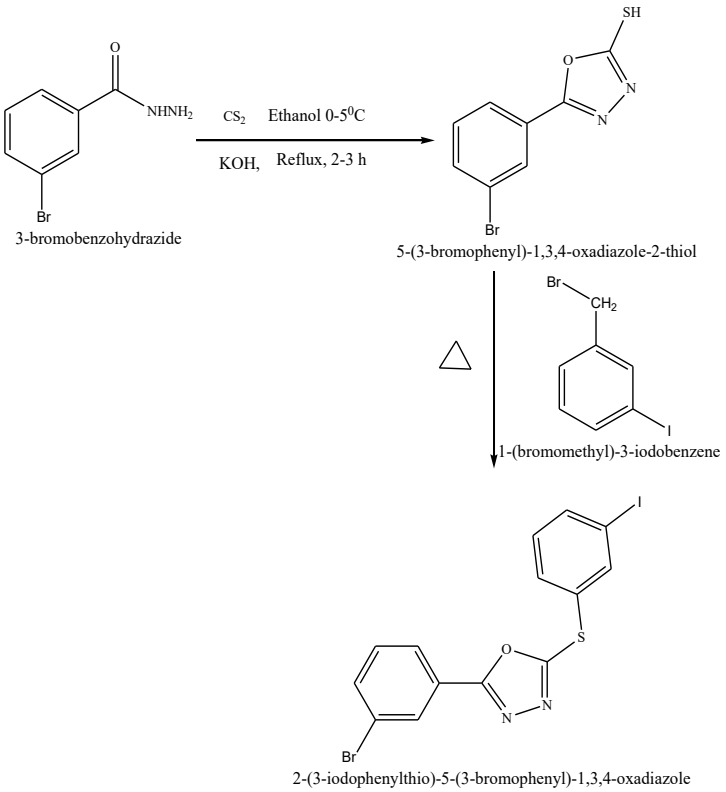

Fig 7. Synthesis of C5

Synthesis of C6

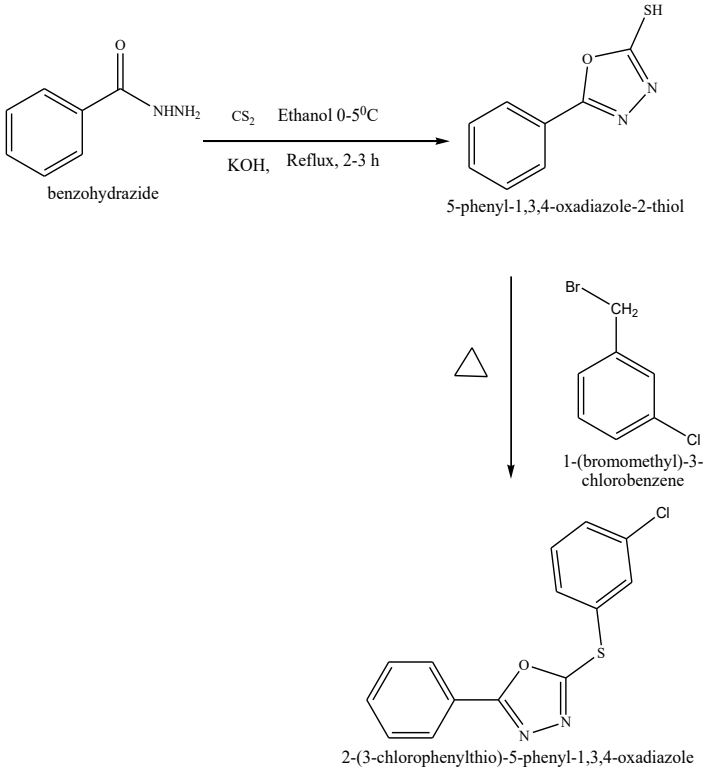

Fig 8. Synthesis of C6

Identification of physicochemical properties

Physical appearance

Table 1. Physical appearance

Compound	Physical appearance
C1	Pale-yellow
C2	Pale-yellow
C3	Pale-yellow
C4	Pale-yellow
C5	Pale-yellow
C6	Pale-yellow

Melting point determination

Table 2. Melting point

Compound	Melting point (°C)
C1	178-182
C2	184-186
C3	204-208

C4	194-198
C5	216-220
C6	174-178

Thin Layer Chromatography

Thin layer chromatography was used to determine a molecule's synthesis based on its Rf value, which changes depending on the substance. The Rf values for C1, C2, C3, C4, C5, and C6 were 0.79, 0.73, 0.76, 0.74, 0.78, and 0.72, respectively.

C4 showed a yield of 29.41 %, which was its highest percentage. C6 had the lowest yield percentage, at 19.25 %. The compound's strongest density is indicated by its highest melting point.

Significant molecular weight was also discovered in the 1,3,4-oxadiazole analogues that were produced. The molecular weights of C3, C4 and C5 were determined to be 268.19 g/mol, 274.25 g/mol and 263.13 g/mol, respectively.

The physicochemical characteristics of each compound were compiled in the following table.

Table 3. Physicochemical properties of 1,3,4-oxadiazole derivative

Compound	Yield (%)	Rf Value	Mol. weight
C1	24.16	0.79	262.20
C2	21.34	0.73	254.43
C3	26.11	0.76	268.19
C4	29.41	0.74	274.25
C5	23.47	0.78	263.13
C6	19.25	0.72	257.74

FTIR Spectroscopy

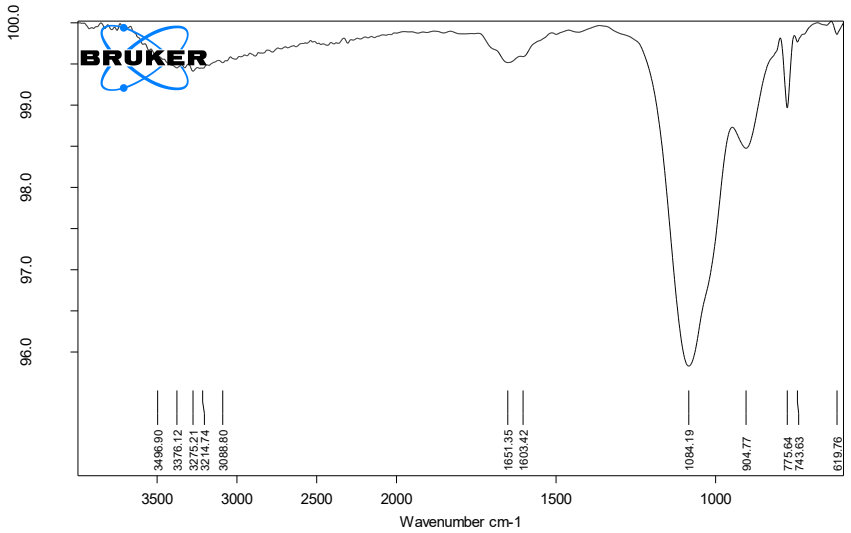

Fig 9. FTIR Spectrum of C1

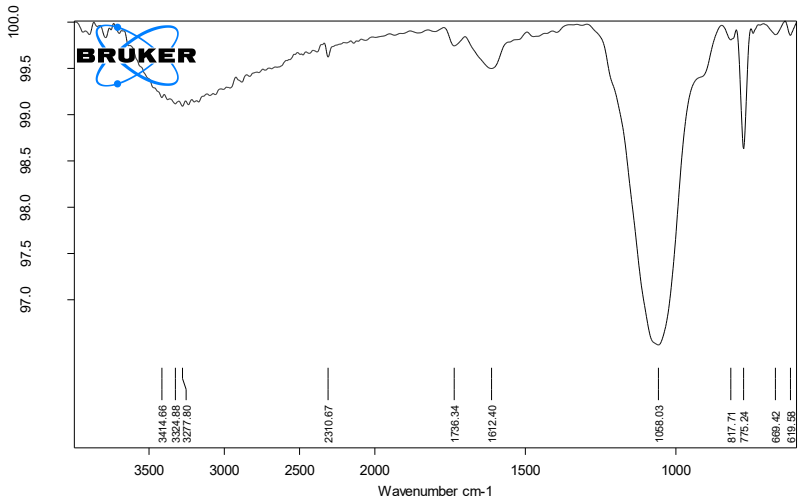

Fig 10. FTIR Spectrum of C2

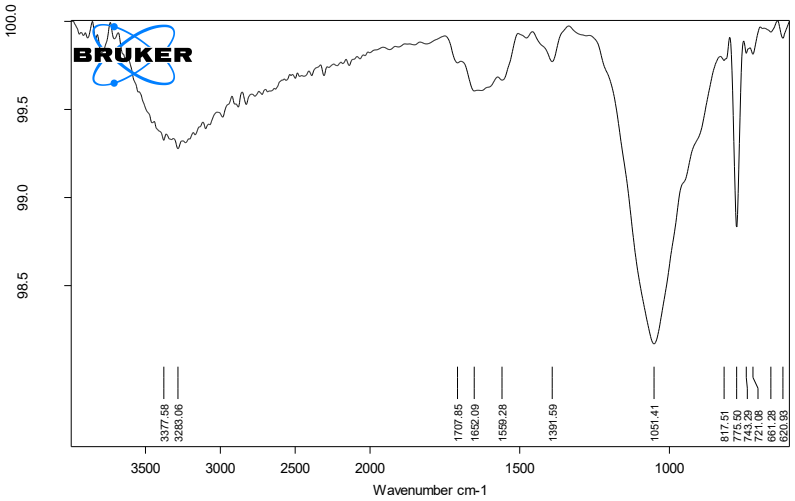

Fig 11. FTIR Spectrum of C3

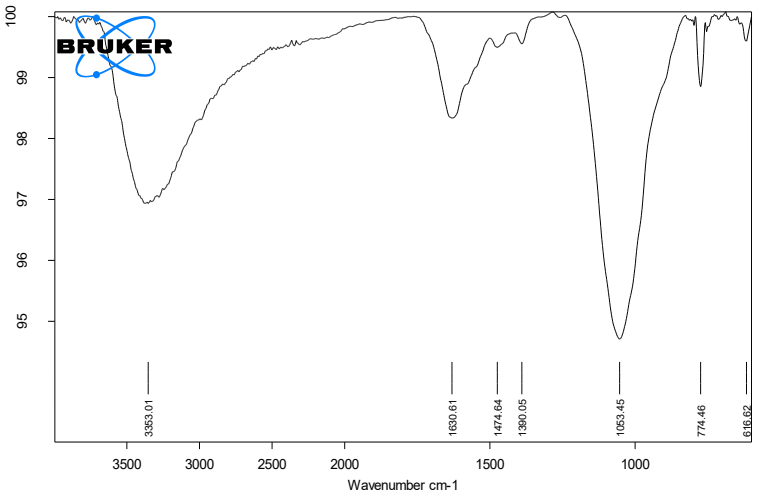

Fig 12. FTIR Spectrum of C4

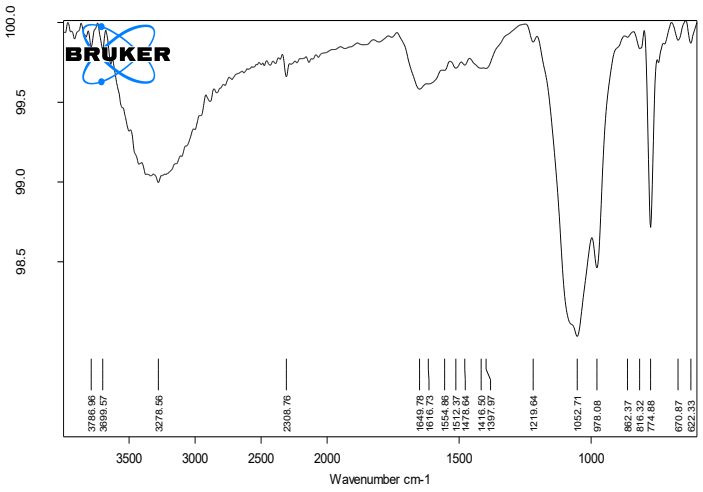

Fig 13. FTIR Spectrum of C5

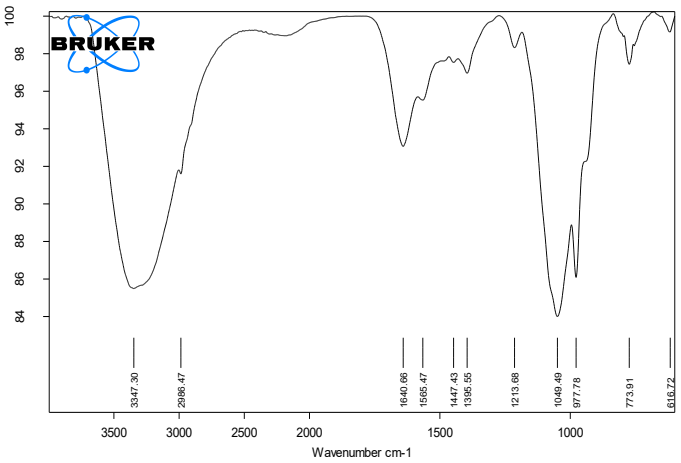

Fig 14. FTIR Spectrum of C6

NMR Spectroscopy

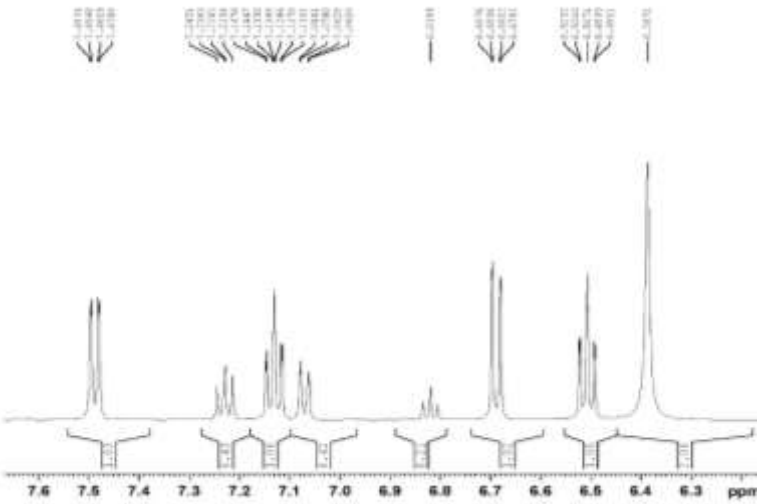

Fig 15. NMR Spectrum of C1

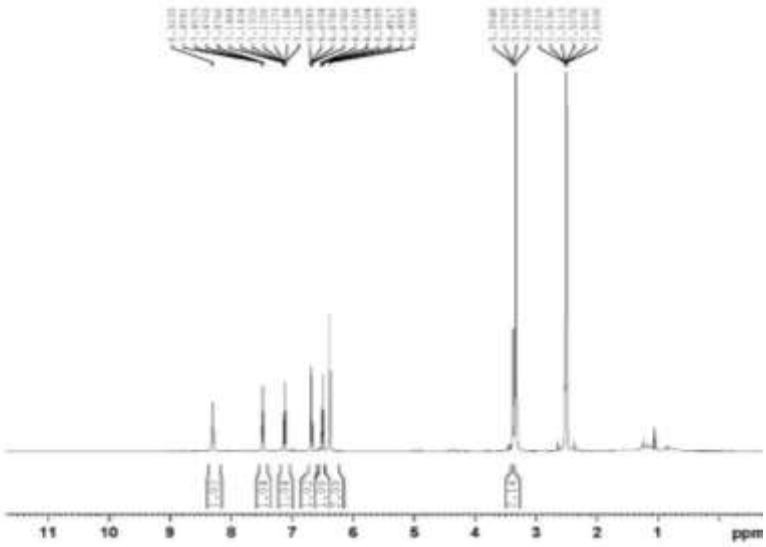

Fig 16. NMR Spectrum of C2

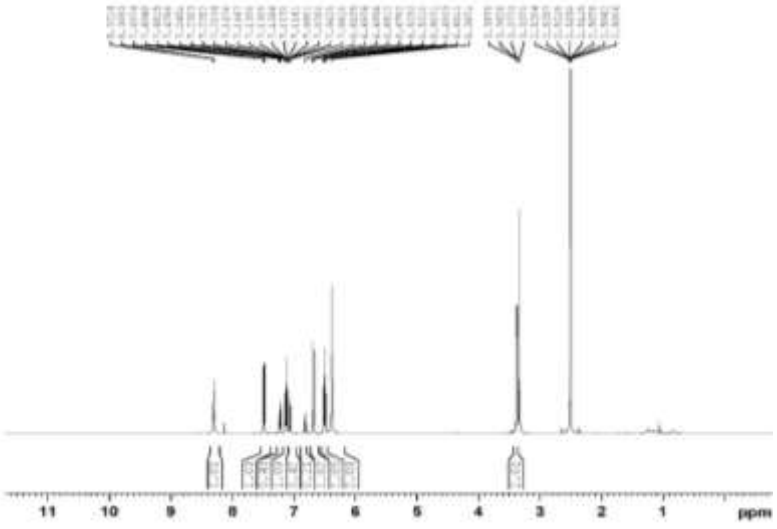

Fig 17. NMR Spectrum of C3

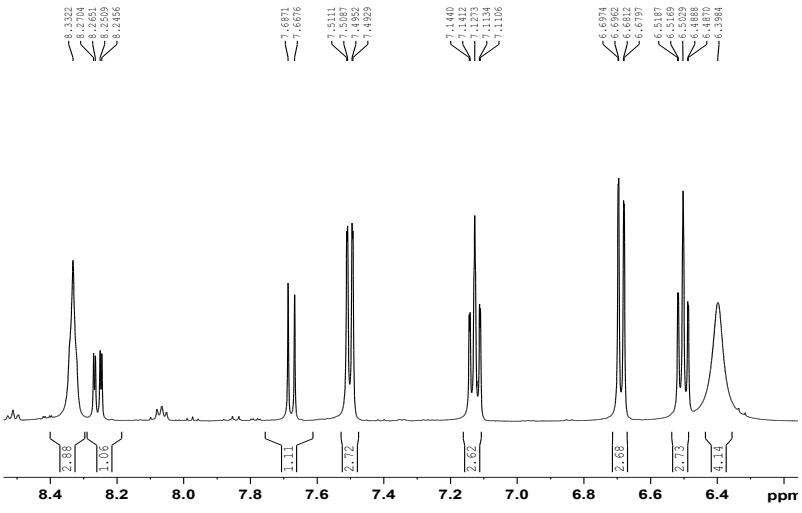

Fig 18. NMR Spectrum of C4

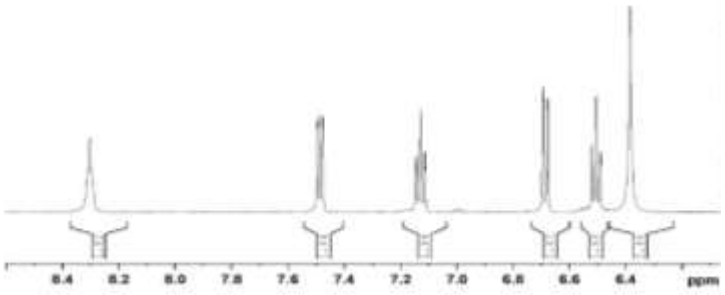

Fig 19. NMR Spectrum of C5

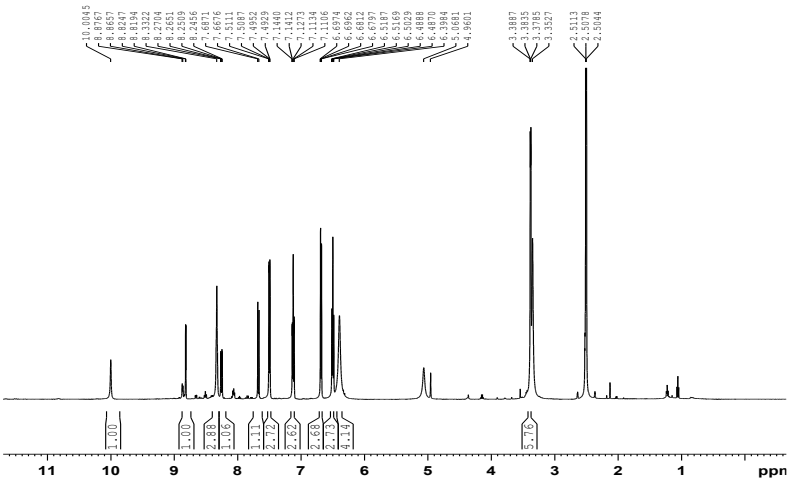

Fig 20. NMR Spectrum of C6

Mass Spectroscopy

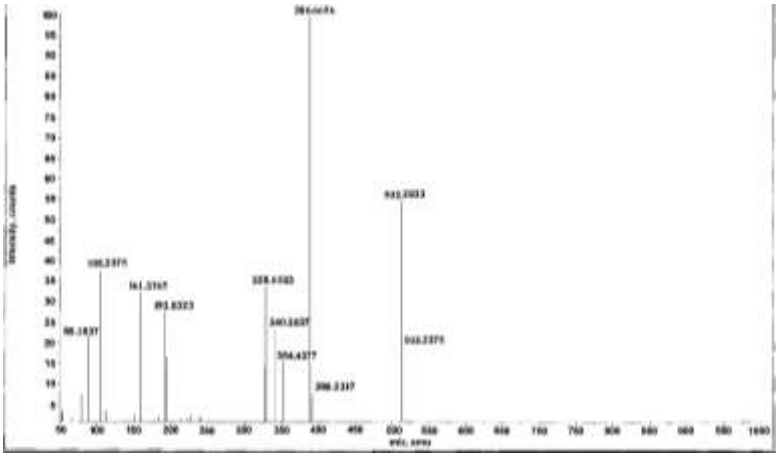

Fig 21. Mass Spectrum of C1

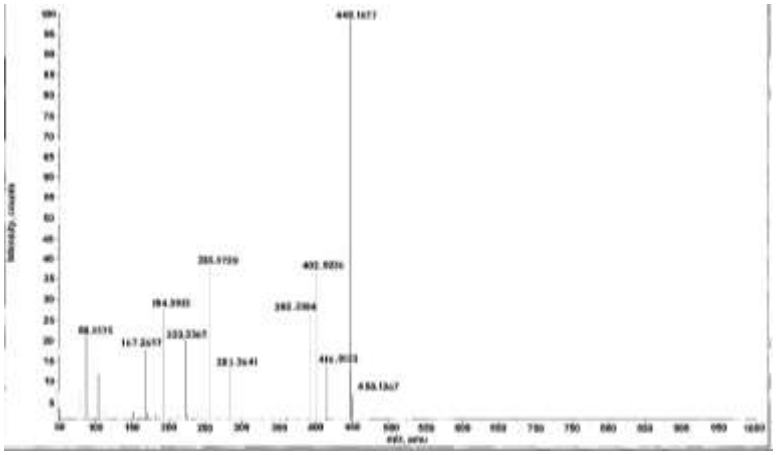

Fig 22. Mass Spectrum of C2

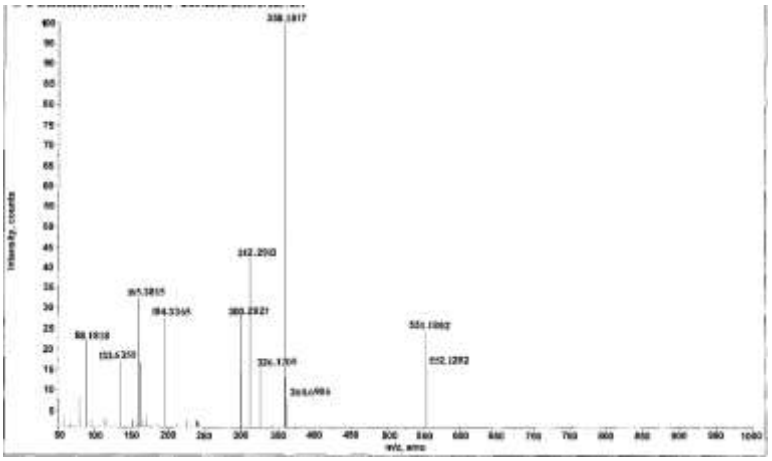

Fig 23. Mass Spectrum of C3

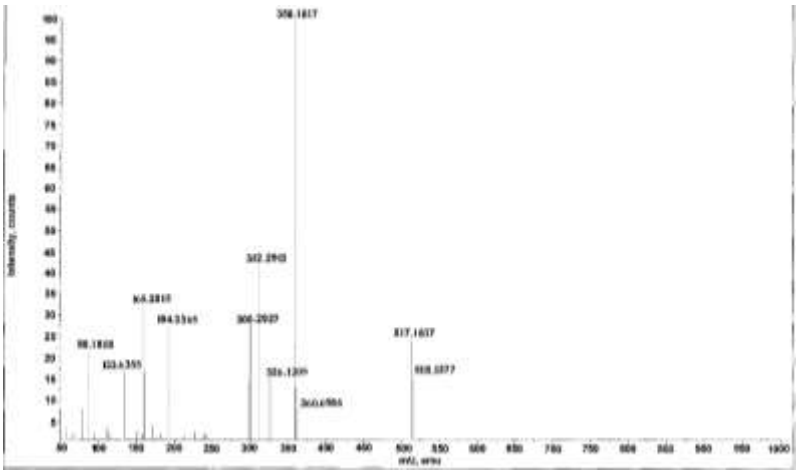

Fig 24. Mass Spectrum of C4

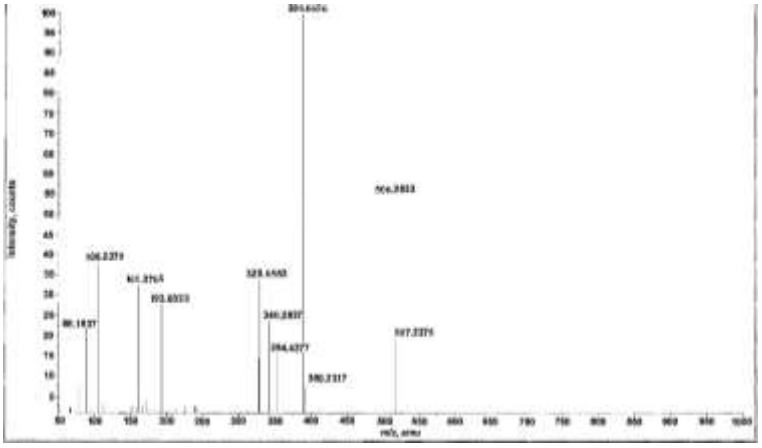

Fig 25. Mass Spectrum of C5

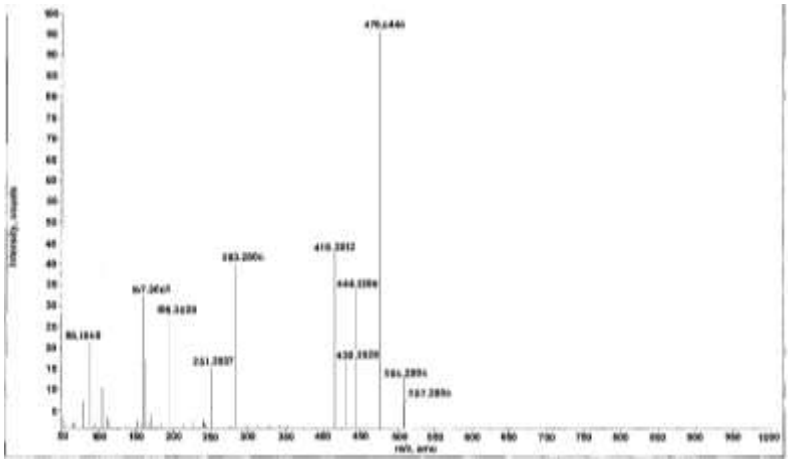

Fig 26. Mass Spectrum of C6

Evaluation of anti-microbial potential

Well diffusion technique

The anti-fungal activity was evaluated using different 4 strains of fungus i.e., A. *niger, P. notatum, C. albicans* and *Rhizopus species*. Zone inhibition (anti-fungal activity) of Oxadiazole (C4) 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-fungal potential was much significant against *Rhizopus species* which proves its anti-fungal action. Its anti-fungal potential might be due to the destruction of cell wall and/ nucleic acid of diverse fungal species.

Ketoconazole as std. anti-fungal agent exhibited highest inhibition zone ranging from 12.0 to 24.0 mm. It also showed highest zone inhibition against *Rhizopus species* as 24.0 mm.

Table 4. Anti-fungal potential of 1,3,4-oxadiazole derivative

Treatment	Zone inhibition (mm)			
	A. niger	*P. notatum*	*C. albicans*	*Rhizopus species*
1,3,4-oxadiazole derivatives	4.18	4.92	5.61	6.18

Ketoconazole	12.0	14.0	21.0	24.0

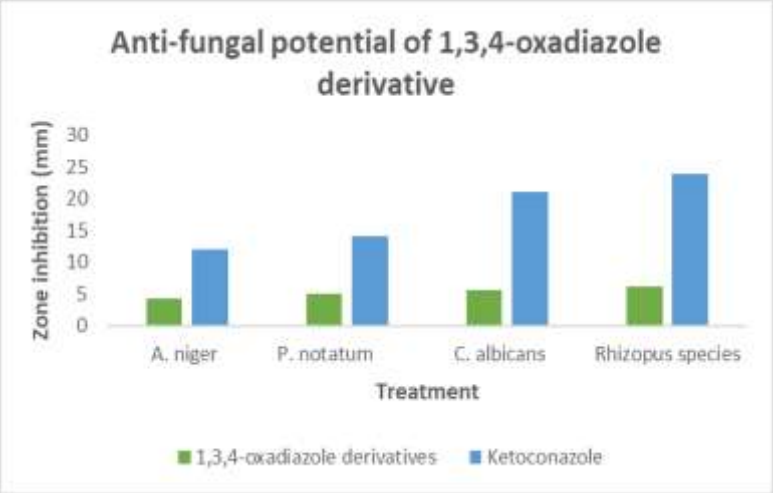

Fig 28. Graphical data of anti-fungal potential of 1,3,4-oxadiazole

According to published research, lipophilicity-the ability of only lipid-soluble substances to readily pass through a microorganism's cell membrane-is one of the main elements that significantly influences antibacterial activity [16]. Our main emphasis during the synthetic process was this important element as well as the critical role of the chloro group in azetidinones. Because lipophilicity is a key component, it is important to note that we have avoided the hydroxyl group in our synthesised molecules. As a result, every synthetic molecule has a halogen substituent and is highly lipophilic without hydroxyl groups. The classic cup-plat diffusion technique was used to assess the antibacterial activity of five newly synthesised compounds (1A-5A) against both Gram-positive and Gram-negative bacterial strains. Two Gram-positive and Gram-negative bacterial strains were employed: Bacillus subtilis (MTCC-736) and Pseudomonas (MTCC-1688). When compared to the conventional procaine penicillin and streptomycin, all of the novel synthesised compounds exhibited poor to very good inhibitory power against both Gram-positive and Gram-positive bacteria, according to the zone width of growth inhibition (mm) findings. In comparison to the standard, compounds 1A and 5A showed noticeably strong activity against B. subtilis and pseudomonas [17].

The antifungal bioassay uses griseoflavin as a standard. We took into consideration these important structural characteristics, which are also present in our synthesised derivatives, in the hopes that our molecules will similarly exhibit antifungal activity. Using the cup-plate diffusion technique, all compounds' inhibitory antifungal activity was assessed against two species: Aspergillus flavus and Candida albicans. By measuring the fungal colony's diameter after a week, the linear growth of every fungus was determined. The data are summarised as zone diameter of growth inhibition (mm). Every compound exhibited mild to moderate effectiveness against A. flavus and Candida albicans, respectively [18].

When compared to the typical medication Sparfloxacin, it demonstrates that test compounds E and F have good efficacy while the remaining compounds have modest anti-bacterial action. The most often administered antibiotics in medicine are still lactams. The 2-azetidinone ring is responsible for the action of well-known antibiotics as penicillin, cephalosporins, and carbapenems. The antibacterial activity of the 2-azetidinone moiety is confirmed by the literature. Numerous synthetic techniques were developed to create the suitably substituted 2-azetidinones due to the significance and structural variety of physiologically active ß-lactam. Thus, it can be said that 2-azetidinone derivatives have a high potential for becoming bioactive compounds. The same line of more recent derivatives and their biological screening for the different pharmacological properties require more research [19].

CONCLUSION

The 3,4-Oxadiazole derivatives might be much significant in destructions of fungus 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.

In conclusion, Azole derivatives might be much significant in destructions of fungal strains which are life-threatening to the mankind. Their action would be significant, with the worldwide availability.

CONFLICT OF INTEREST

Authors declare for none conflict of interest.

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