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Design, Synthesis and Evaluation of Anti-Fungal Activity of Novel Derivatives of 1,3,4-Oxadiazole

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ABSTRACT:

Oxadiazole is heterocyclic compound having 2 nitrogen and 1 oxygen atom together to form a heterocyclic ring with five members. The current research was based on the design, synthesis and evaluation of anti-fungal activity of novel derivatives of 1,3,4-oxadiazole. The 1,3,4-oxadiazole derivatives based on physicochemical properties i.e., melting point, Rf value, FTIR, NMR and Mass spectroscopy and evaluated for the anti-fungal potential through well diffusion method against fungus strains i.e., Aspergillus niger, Penicillium notatum, Candida albicans and Rhizopus species. In results, the highest Rf values in C2, C3, C4, and C6 were found to be 0.73, 0.72, 0.73, and 0.71, respectively. The compound C4 exhibited the highest melting point, measuring between 184 and 188ºC. Highest anti-fungal activity was observed against the C. albicans as 7.59, 6.28, 7.11 and 7.38, in the C3, C4, C5 and C6, respectively. It can be said that 1,3,4-oxadiazole derivatives has more anti-fungal potential on C. albicans as compared to A. niger. It might be due to destruction of cell wall and/ nucleic acid of fungal strains. In conclusion, C3 and C6 were most prominent synthetic derivative among others. It also showed optimum physicochemical properties and anti-fungal potential when tested on the selected fungal strains. It showed a remarkable anti-fungal potential against different 4 fungal strains used.

Keywords: 1,3,4-oxadiazole, synthesis, evaluation, anti-fungal, FTIR.

1. INTRODUCTION

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-cociceptive [1][2]. As a result, both the molecule's stability and quantum yield of fluorescence can be raised [3]. Additionally, products like thermal insulation polymers include these molecules [4][5].

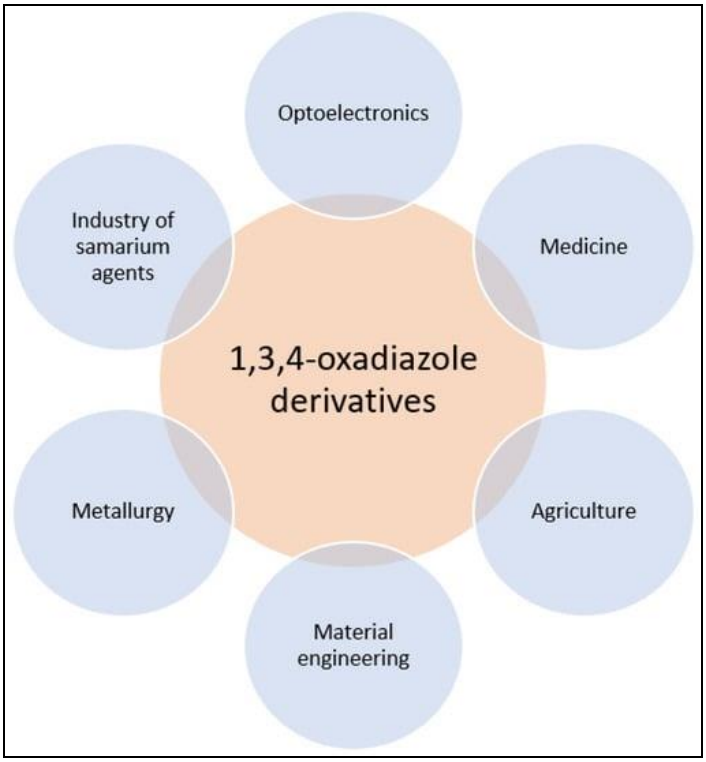


Fig 1. Different uses of 1,3,4-oxadiazole derivatives [1]

Oxadiazoles are a class of substances having effective electron transport and electron hole blocking properties. Polymers, dendritic forms, and low-molecular-weight derivatives all exhibit this kind of action [6]. Because of this, a large class of chemicals known as 1,3,4-oxadiazole derivatives has found successful applications in organic photovoltaics, optoelectronics, and organic light emitting diodes [7]. The aim of research was the design, synthesis and evaluation of anti-fungal activity of novel derivatives of 1,3,4-oxadiazole

2. MATERIALS AND METHODS

Experimental Requirements

- Benzoic acid
- Salicylic acid
- Cinnamic acid
- Anthranilic acid
- Methanol
- Hydrazine hydrate
- Benzyl alcohol
- Succinic anhydride

- Phosphorous oxychloride
- Anhydrous $AlCl_3$
- Concentrated sulphuric acid
- Sodium hydroxide
- Sodium bicarbonate
- Ethanol
- 4-Chloro benzoic acid
- 4-nitro benzoic acid
- 4-amino benzoic acid
- Nicotinic acid
- Benzylic acid
- P-toluene sulfonic acid
- Distilled water
- Diclofenac

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

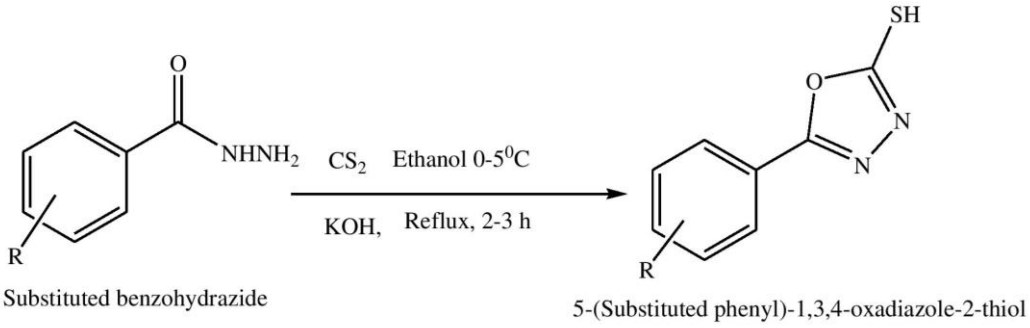


Fig 1. Scheme for synthesis of 1,3,4-oxadiazole derivatives

Upon reaction of substituted benzohydrazide with Carbon di-sulfide and Potassium hydroxide at 0-5ºC, it produced the 5-(substituted phenyl)-1,3,4-oxadiazole-2-thiol. All the 6 novel derivatives of 1,3,4-oxadiazole were synthesized using above scheme. These will be evaluated for physiochemical parameters- spectroscopy analysis and anti-diabetic activity further.

Synthesis of C1

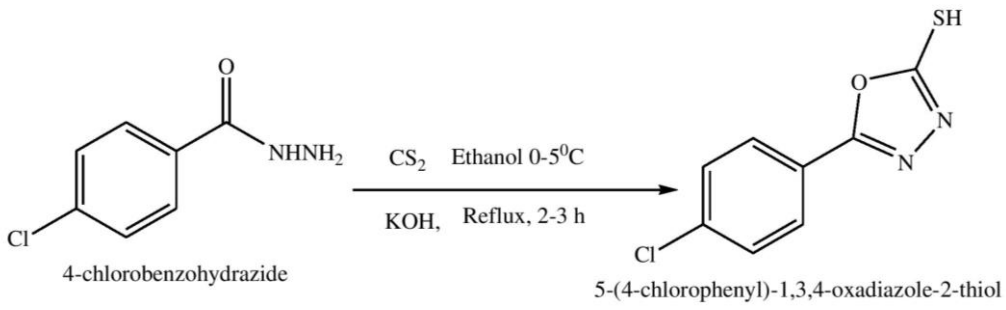
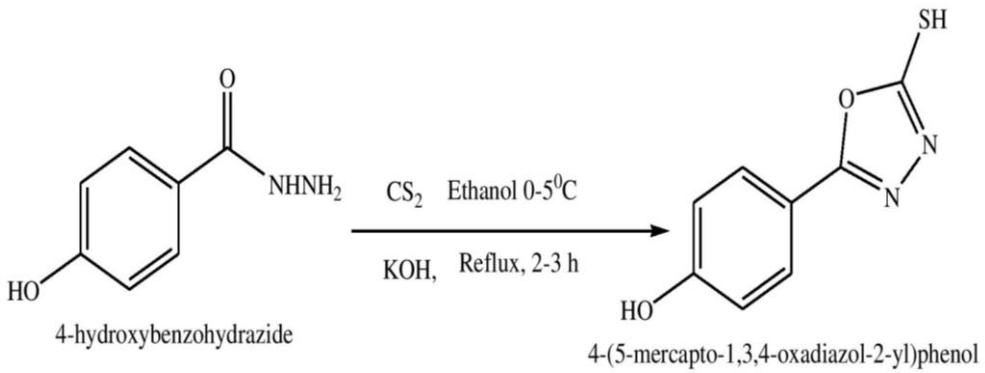


Fig 2. Synthesis of C1

Synthesis of C2



4-hydroxybenzohydrazide

4-(5-mercapto-1,3,4-oxadiazol-2-yl)phenol

Fig 3. Synthesis of C2

Synthesis of C3

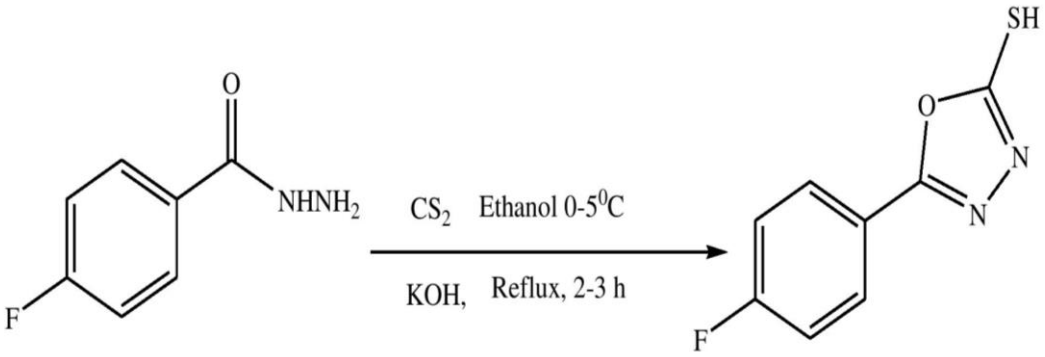
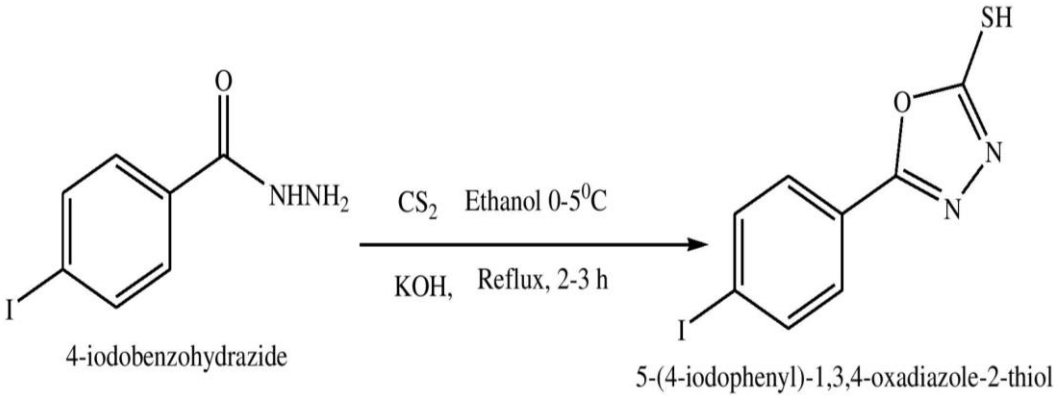


Fig 4. Synthesis of C3

Synthesis of C4

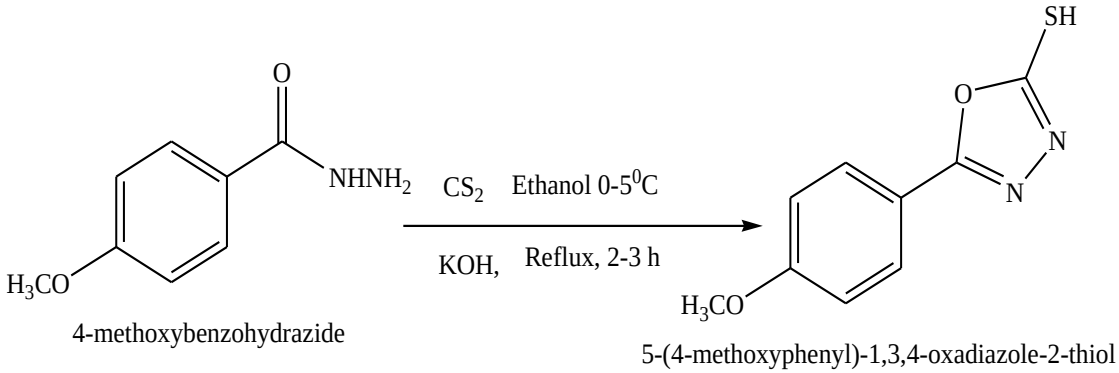


4-iodobenzohydrazide

5-(4-iodophenyl)-1,3,4-oxadiazole-2-thiol

Fig 5. Synthesis of C4

Synthesis of C5

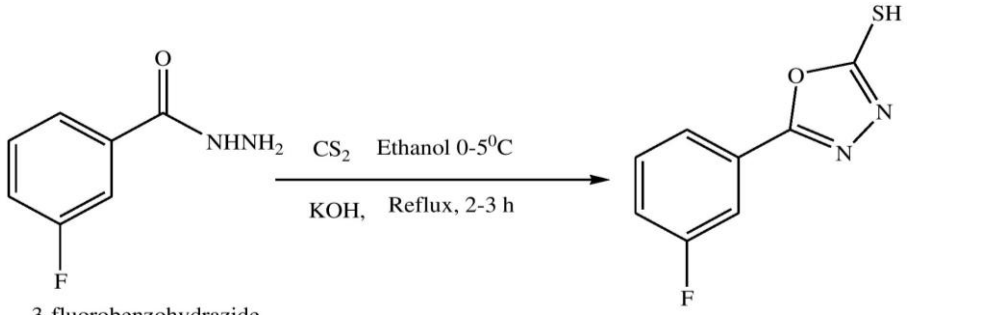


4-methoxybenzohydrazide

5-(4-methoxyphenyl)-1,3,4-oxadiazole-2-thiol

Fig 6. Synthesis of C5

Synthesis of C6



3-fluorobenzohydrazide

5-(3-fluorophenyl)-1,3,4-oxadiazole-2-thiol

Fig 7. Synthesis of C6

A. 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 [8].

- **Rf value**

Thin layer chromatography, or TLC for short, 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 [9].

- **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 [10].

- **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 [11] for the organic compound range δ 0 – 14.

- **Mass Spectroscopy**

An essential physico-chemical 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 [12].

B. Evaluation of anti-fungal activity

By using the well diffusion method, the antifungal activity of 1,3,4-oxadiazole derivatives against fungus strains will be identified. Nutrient Agar broth is utilized to cultivate a 24-hour-old culture of fungus, which is then used to make a suspension of fungi. 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 ml of each fungal suspension. With a sterile cork borer, 5 mm wells are made in the solidified media, and each well is filled with the derivatives. The diameter (mm) of the inhibitory zone surrounding the well was measured after 24 hours of incubation at 30°C. As a standard antifungal formulation, itraconazole gel (Itromed 1%) is utilized. Every antifungal study will be carried out in triplicate. Fungus strains i.e., *Aspergillus niger, Penicillium notatum, Candida albicans and Rhizopus species* will be used for screening of anti-fungal activity [13].

3. RESULTS AND DISCUSSION

Identification of physicochemical properties

Melting point

For 1,3,4-oxadiazole derivatives, the melting point was determined in the range of 142-146ºC, 168-172ºC, 120-124ºC, 184-188ºC, 146-150ºC and 128-132ºC for compounds C1 to C6, respectively.

Rf value

Depending on the chemical, synthetic chemistry uses thin layer chromatography to verify the creation of a molecule based on its Rf value. The highest Rf values in C2, C3, C4, and C6 were found to be 0.73, 0.72, 0.73, and 0.71, respectively.

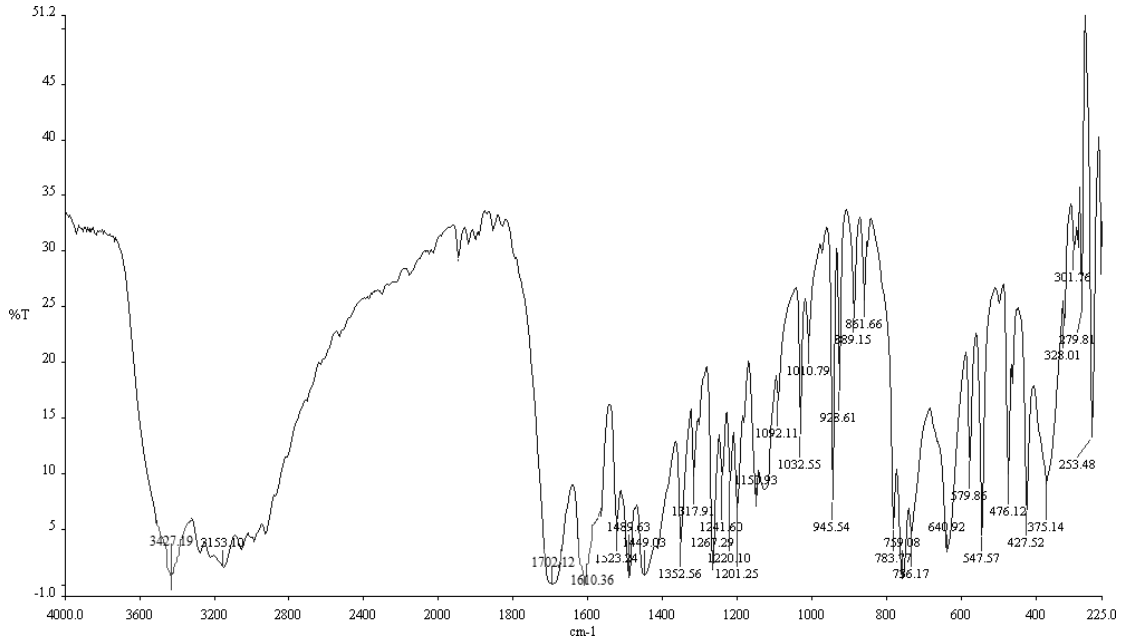
Physical characteristics such as yield percentage, melting point, and functional groups attached were assessed for each of the synthesised 1,3,4-oxadiazole derivatives. The greatest yield percentages of 68.26% and 67.20% were shown for C2 and C6. The lowest yield, 62.42%, was seen in C3. The compound C4 exhibited the highest melting point, measuring between 184 and 188ºC. The compound's strongest density is indicated by its highest melting point. The physical characteristics of each chemical are listed in the following table.

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

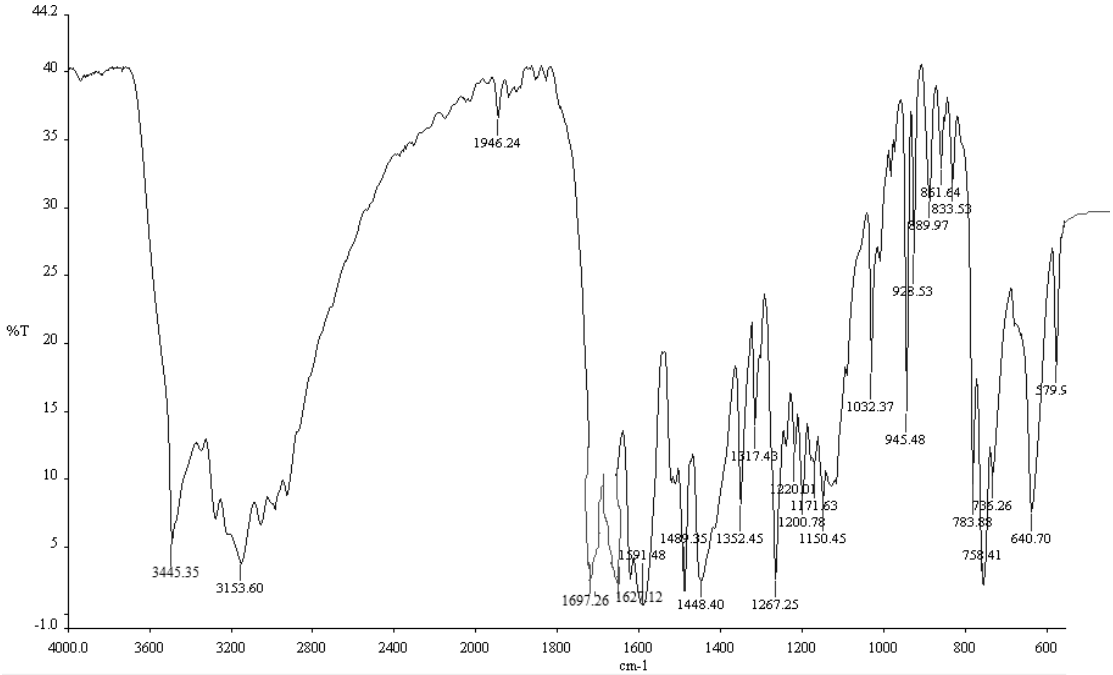
Compound	Yield (%)	Rf Value	Melting point
C1	64.32	0.68	142-146ºC
C2	68.26	0.73	168-172ºC
C3	62.42	0.72	120-124ºC

C4	64.29	0.73	184-188ºC
C5	64.28	0.69	146-150ºC
C6	67.20	0.71	128-132ºC

Infrared Spectroscopy

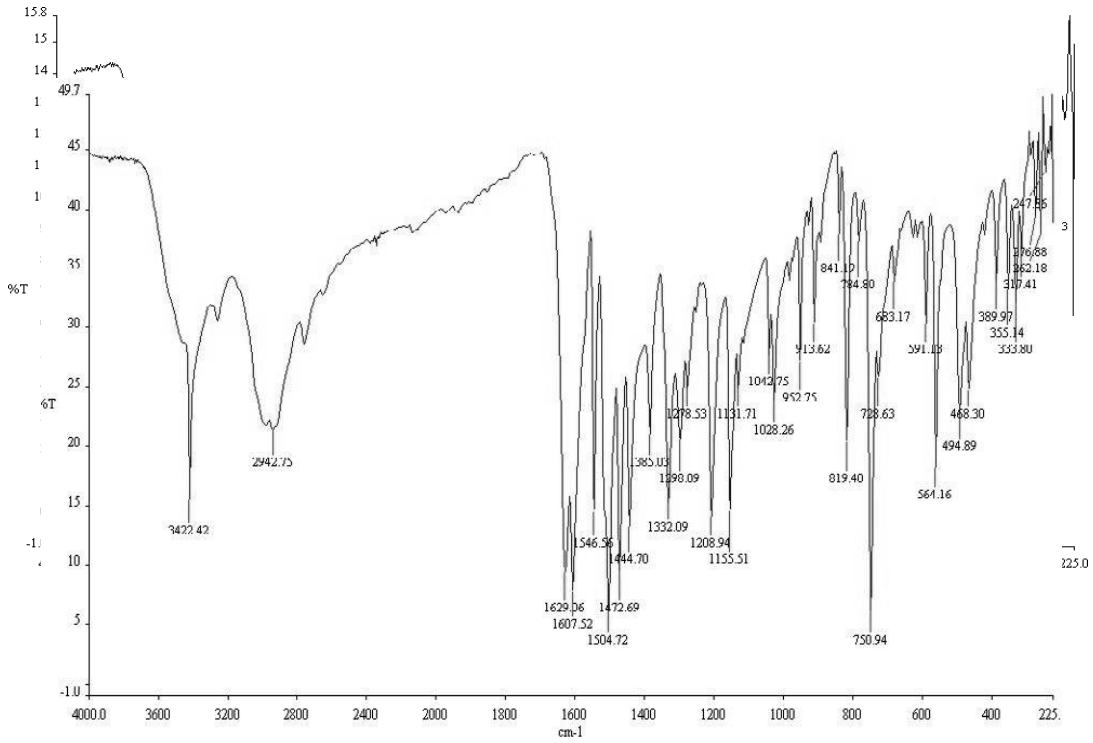


FTIR Spectrum of C1

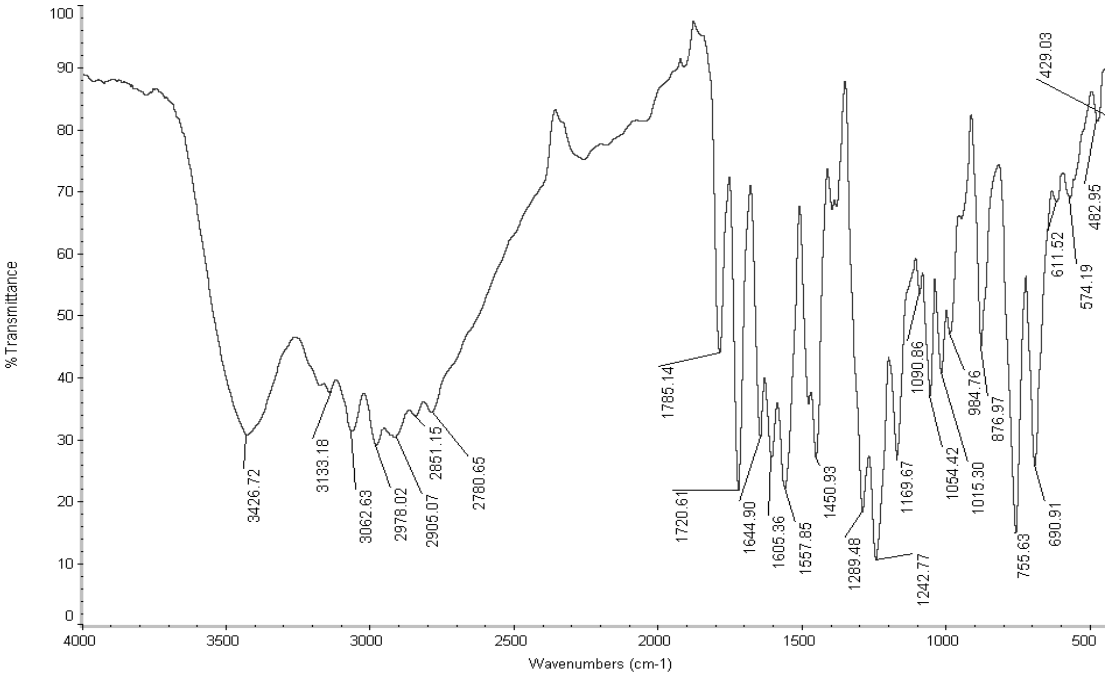


FTIR Spectrum of C2

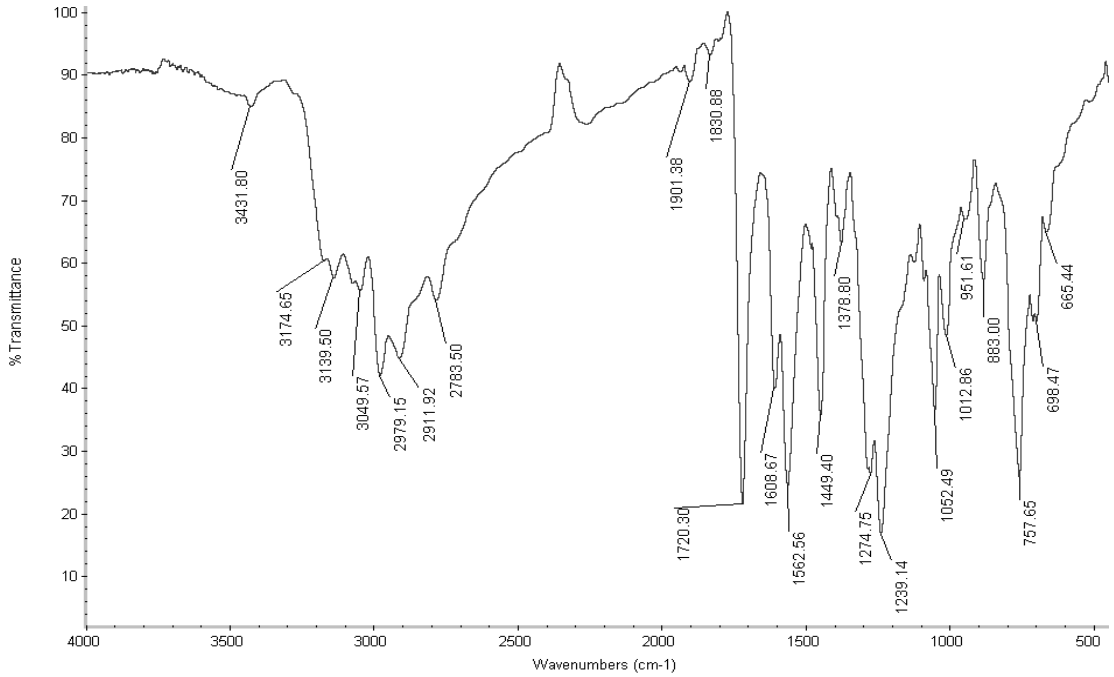
FTIR Spectrum of C3



FTIR Spectrum of C4

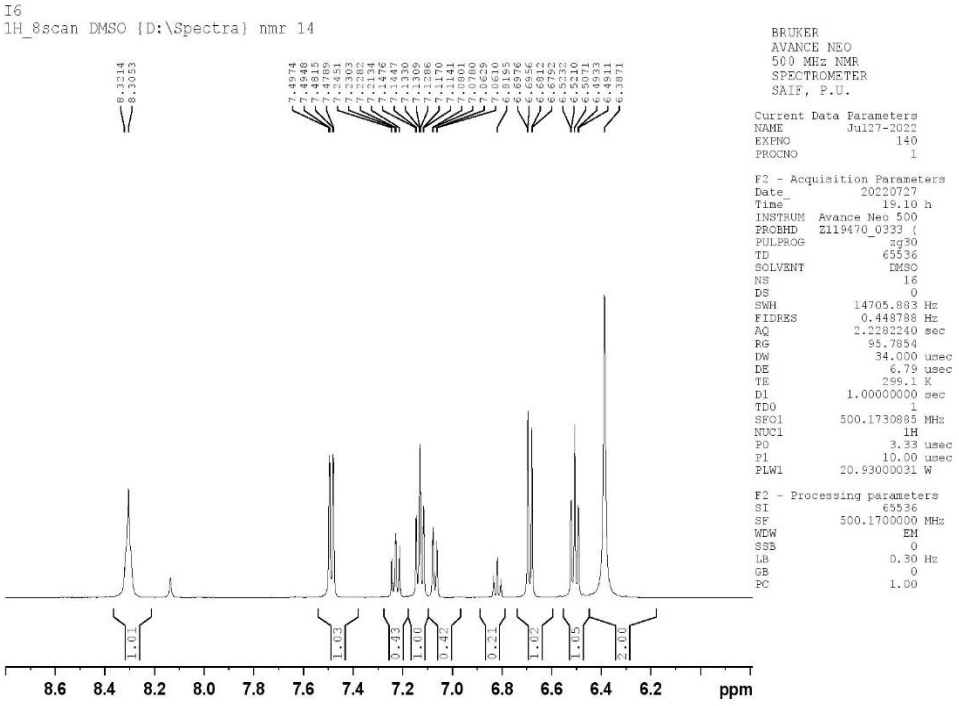


FTIR Spectrum of C5

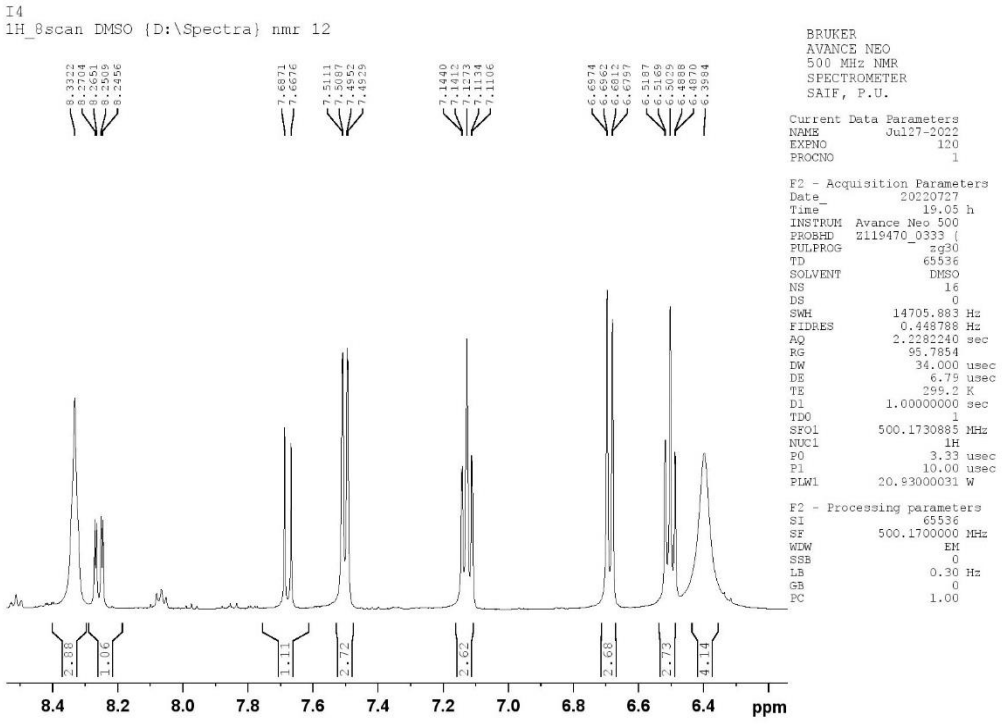


FTIR Spectrum of C6

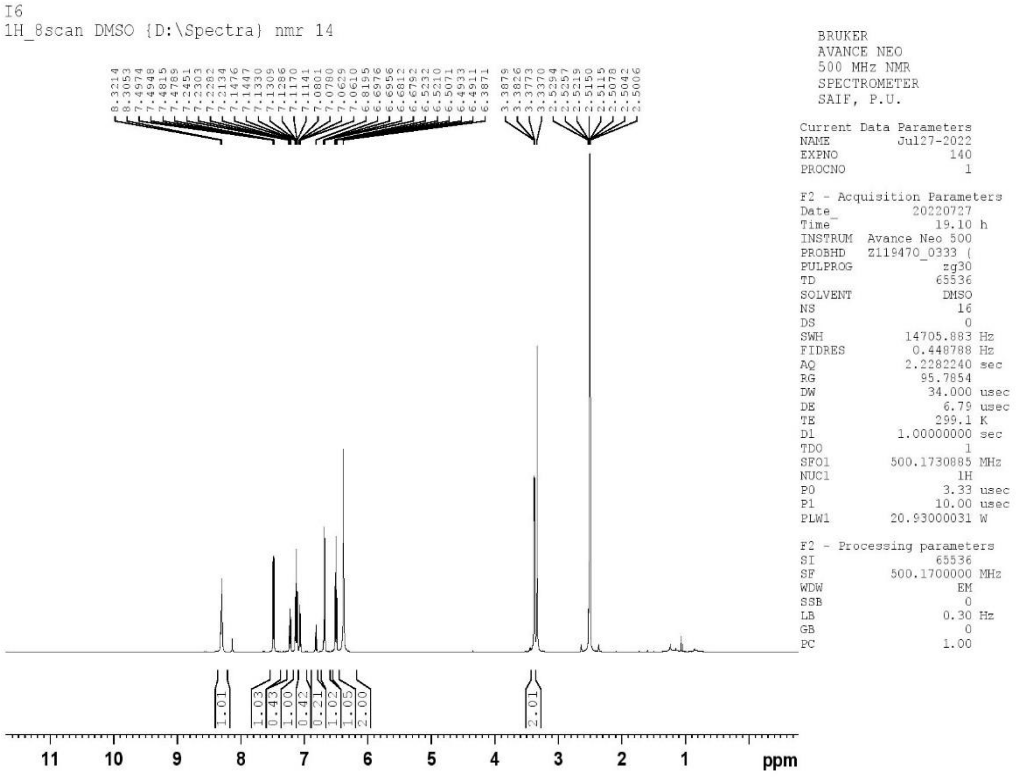
NMR Spectroscopy



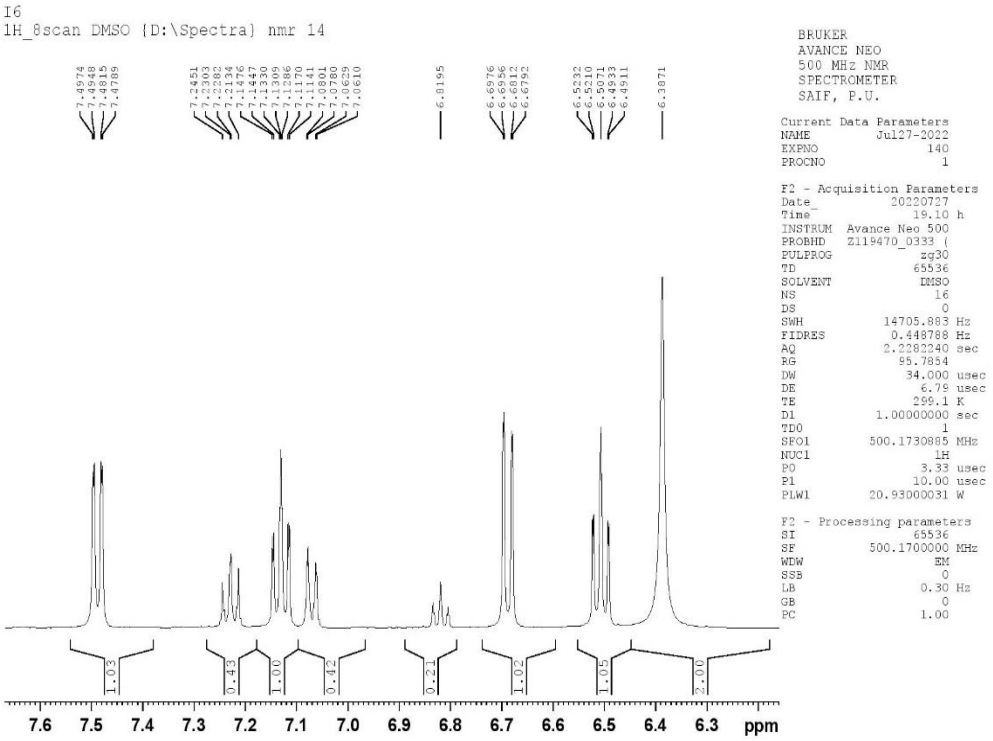
NMR Spectrum of C1



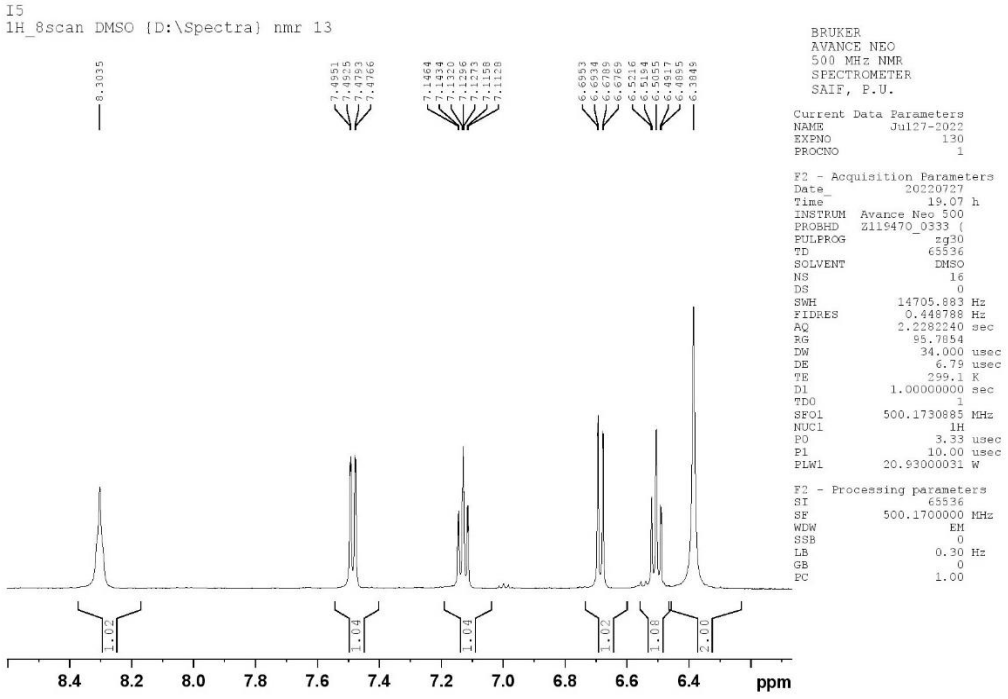
NMR Spectrum of C2



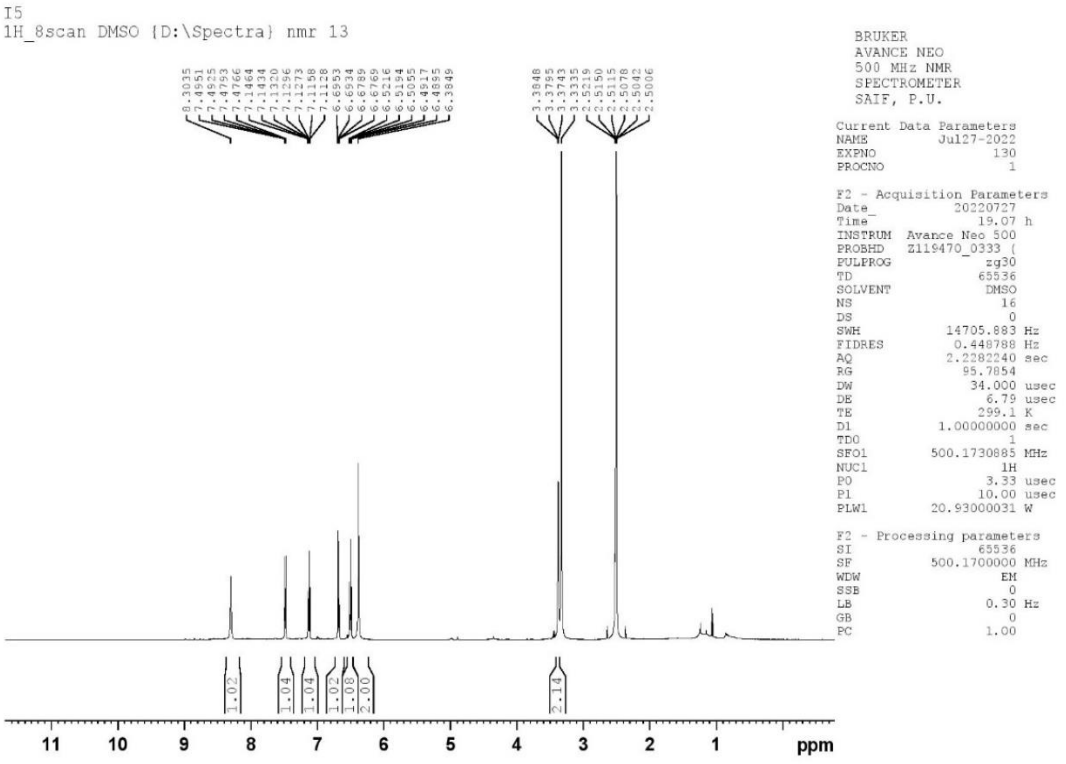
NMR Spectrum of C3



NMR Spectrum of C4

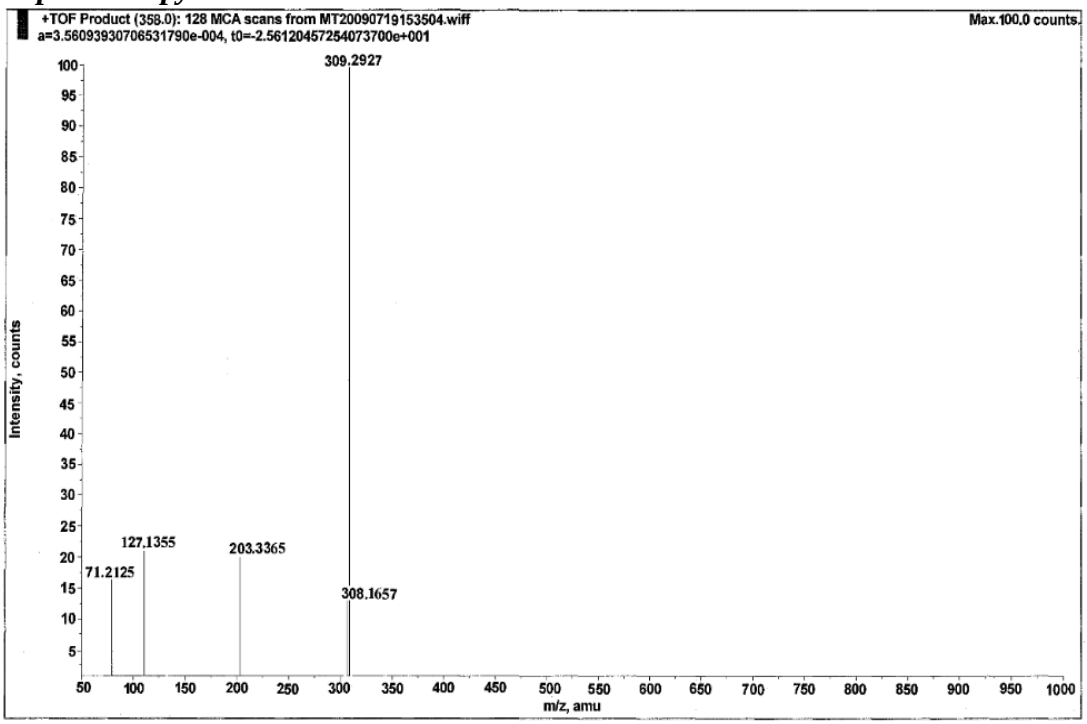


NMR Spectrum of C5



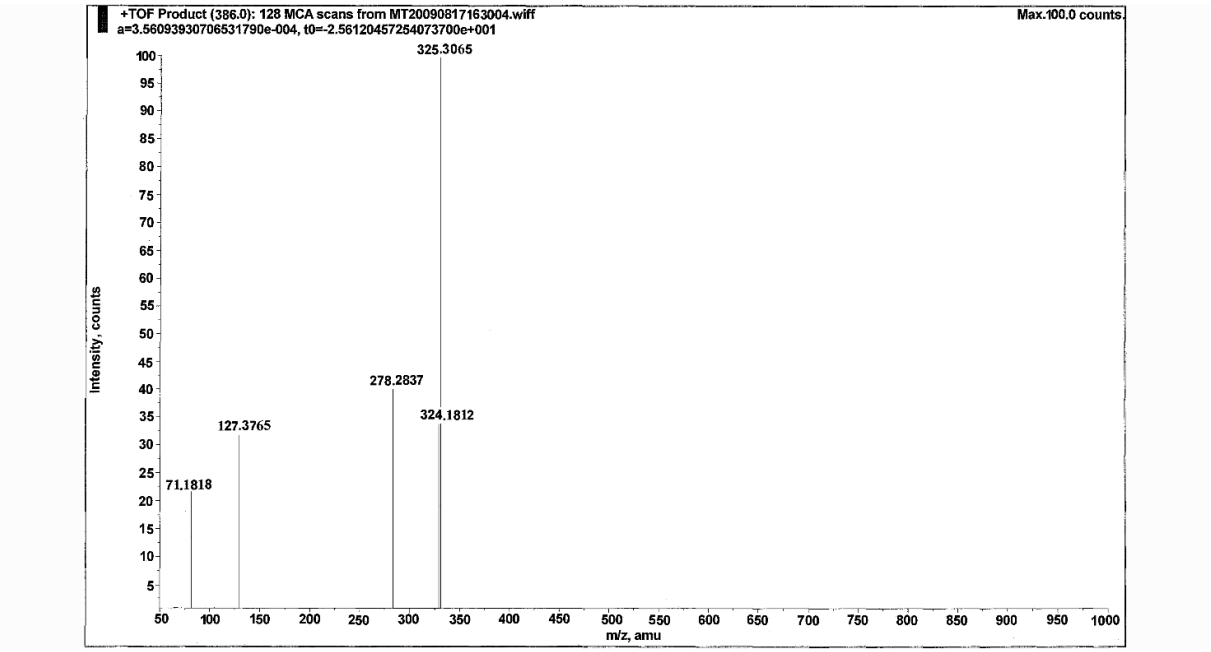
NMR Spectrum of C6

Mass Spectroscopy



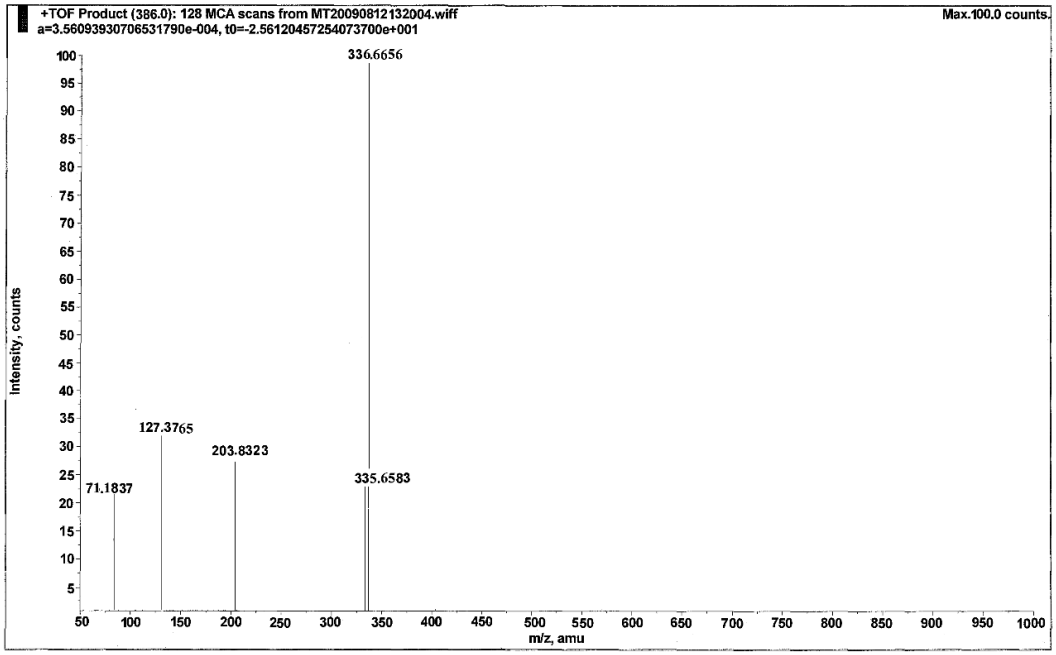
Mass Spectrum of C1

Interpretation: MS (m/z): 308 (100) $[M]^+$, 309 (15) $[M+1]^+$
Fragments: 203 (22), 127 (23), 71 (18).



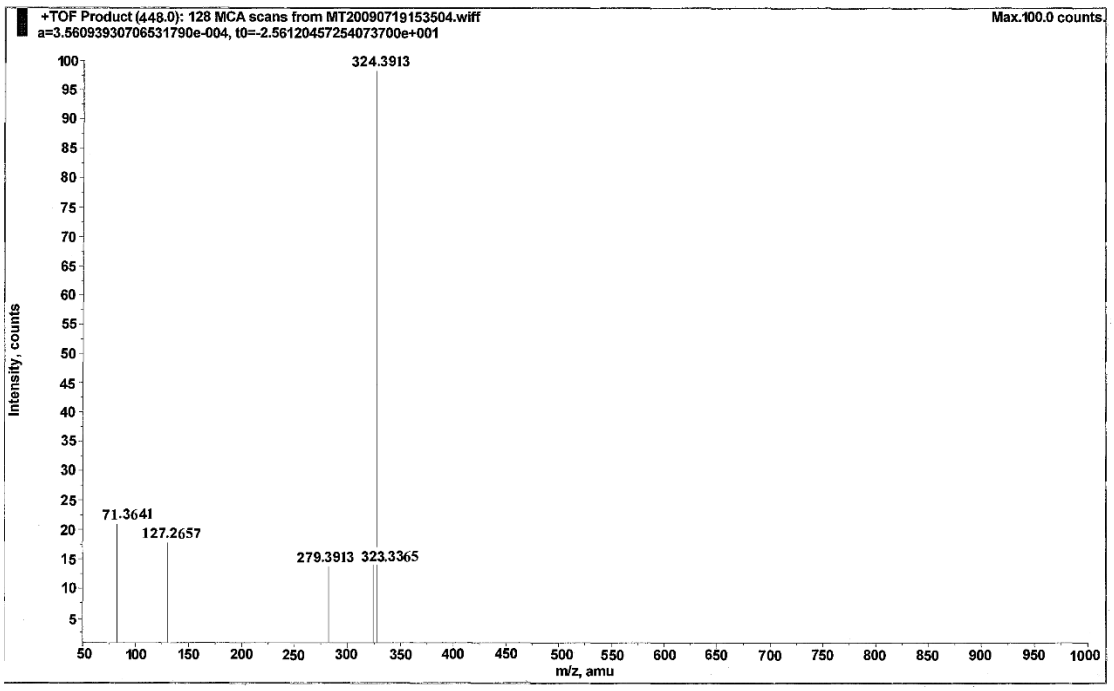
Mass Spectrum of C2

Interpretation: MS (m/z): 324 (100) [M]+, 325 (35) [M+1]+
Fragments: 278 (42), 127 (33), 71 (22).



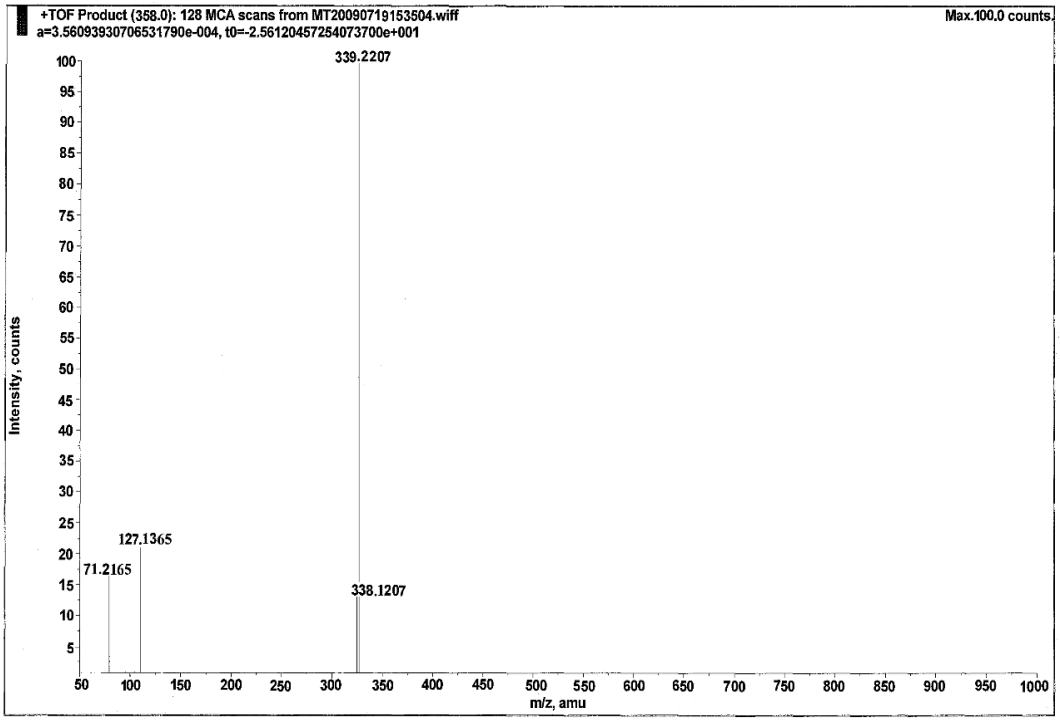
Mass Spectrum of C3

Interpretation: MS (m/z): 335 (100) [M]+ , 336 (25) [M+1]+
Fragments: 203 (28), 127 (34), 71 (23)



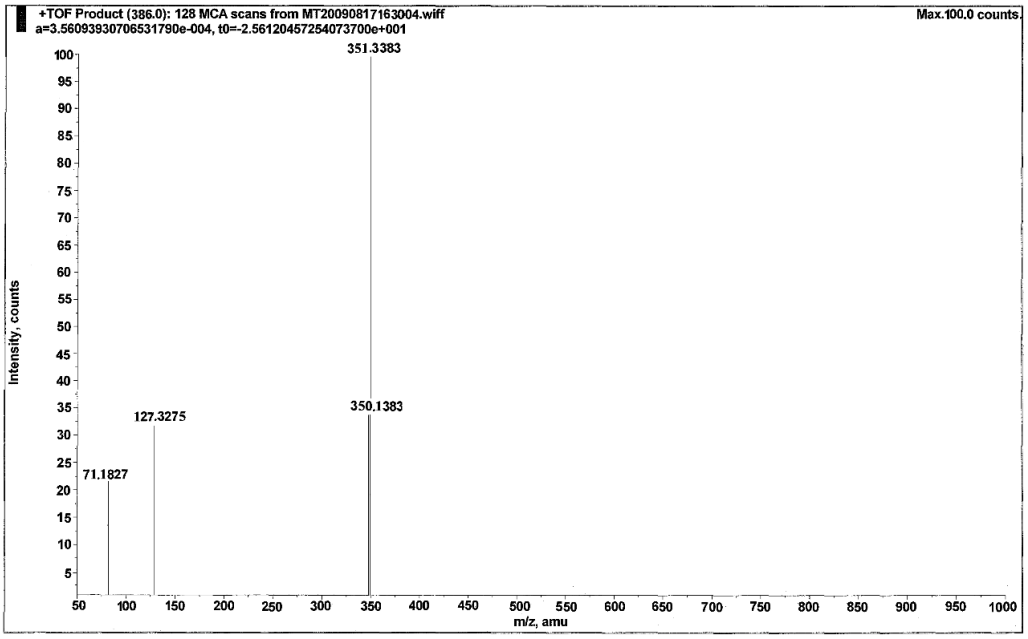
Mass Spectrum of C4

Interpretation: MS (m/z): 323 (100) [M]+, 324 (17) [M+1]+
Fragments: 279 (15), 127 (19), 71 (22)



Mass Spectrum of C5

Interpretation: MS (m/z): 338 (100) $[M]^+$, 339 (15) $[M+1]^+$
Fragments: 127 (23), 71 (18).



Mass Spectrum of C6

Interpretation: MS (m/z): 350 (100) $[M]^+$, 351 (35) $[M+1]^+$
Fragments: 127 (33), 71 (23).

5.3 Evaluation of anti-fungal activity

Different 4 strains species of fungus i.e., *A. niger*, *P. notatum, C. albicans and Rhizopus species* were included for the evaluation of anti-fungal activity. Antifungal activity of 1,3,4-oxadiazole derivatives was recorded as 4.43mm, 5.35mm, 7.38mm, and 4.94mm in *A. niger*, *P. notatum, C. albicans and Rhizopus species*, respectively. However, the synthesized derivatives of oxadiazoles demonstrated antifungal response in a significant manner.
Highest anti-fungal activity was observed against the *C. albicans* as 7.59, 6.28, 7.11 and 7.38, in the C3, C4, C5 and C6, respectively. It can be said that 1,3,4-oxadiazole derivatives has more anti-fungal potential on *C. albicans* as compared to *A. niger*. It might be due to destruction of cell wall and/ nucleic acid of fungal strains.

Table 2. Antifungal activity of 1,3,4-oxadiazole derivatives

Derivatives	Anti-fungal activity (mm)			
	A. niger	*P. notatum*	*C. albicans*	*Rhizopus sp.*
C1	4.24	4.31	6.59	6.34
C2	4.39	4.82	6.24	6.49
C3	5.14	6.78	7.59	6.20
C4	4.19	5.64	6.28	5.24
C5	5.68	6.59	7.11	6.46
C6	4.43	5.35	7.38	4.94

In previous study, the inhibition activity of the synthesized 1,3,4- oxadiazoles against phytopathogenic fungi was studied. Fusarium oxysporum, a typical fungus that is often present in the agro-ecosystem, was chosen for screening the anti-fungicide activity based on mycelial growth inhibitory bioassay. In a preliminary test, all tested compounds inhibited F. oxysporum, in which compounds 4a, 7a, and 7f exerted clear inhibition after 2 days of treatment at the concentration ranging from 12.5 to 100 µg/mL and somewhat remained after 3 days. Interestingly, all the compounds inhibited this fungal strain in a dose-dependent manner.

When the concentration of 100 µg/mL was applied, all the tested compounds displayed moderate inhibition activity against F. oxysporum. Among them, compounds 4a, 7a and 7f showed the inhibition activity after 3 days, with the control efficacies of 16.0%, 11.0%, and 18.0%, respectively. These initial results have suggested the potential uses of 1,3,4-oxadiazole containing compounds treating fungal diseases in plants. Further investigation of the inhibition activities of these compounds against F. oxysporum at higher concentrations has been continued in our lab in order to calculate their IC50 and IC90. In addition, the mode of action of these compounds against F. oxysporum has also been conducted for designing more potent 1,3,4-oxadiazole-based antifungal agents in the future. Oxadiazoles are a significant class of drugs with a variety of biological characteristics that are being developed.

Structure-activity connections have demonstrated that the antibacterial properties of phenyl rings can be amplified by the addition of electronegative groups, such as Cl or NO2. Significant antibacterial activity was demonstrated when nitro furan or furan ring was substituted for oxadiazole. In comparison to other compounds examined, the phenyl ring exhibited reasonable antifungal activity upon substitution of a nitro or chlorine group. Conversely, the furan derivatives had impressive antibacterial properties. The compounds with the highest activity against S. aureus at 8, 4, and 4 µg/mL concentrations were furan and nitro furan derivatives, according to the results of the antibacterial screening. It appears that substances containing the hydroxyl group of mandelate residue may enhance the activity of gram-positive bacteria. The highest activity against E. coli were demonstrated by these compounds at doses of 16, 16, and 8 (µg/mL), respectively. When compared to oxadiazole's furan derivative, the compounds' fungistatic and fungicidal properties against Candida albicans may be attributed to the presence of a para-substituted-phenyl group at position 5.

4. CONCLUSION

Moving towards allopathic treatments would be a huge step towards combating the late and widespread effects on millions of lives. It might also be made better so that production costs would be low and its usefulness would be high. It would be highly advantageous for the synthetic derivatives' stability. Inhibiting the growth and multiplication of bacteria will make it easier to offer the long-lasting effect. One crucial method that increases the 1,3,4-oxadiazole derivatives bioavailability and yields a strong therapeutic impact to cure a variety of medical. These days, synthetic moieties are made to deliver medications by various modes of administration. Synthetic medications have sudden and unpredictable effects on the human body, in some cases.

In conclusion, C3 and C6 were most prominent synthetic derivative among others. It also showed optimum physicochemical properties and anti-fungal potential when tested on the selected fungal strains. It showed a remarkable anti-fungal potential against different 4 fungal strains used. Derivatives of 1,3,4-oxadiazole may have significant effects on the management of bacterial infections and other stability requirements.

As a result, medications for dermatitis, pruritis, and other ailments affecting the epidermis may be distributed and treated using 1,3,4-oxadiazole derivatives.

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