



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

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ABSTRACT

Forty million Americans suffer from fungal diseases, according to studies. In clinical settings, a range of synthetic antifungal medicines are employed to treat various fungal infections. The present study was based on the synthesis, identification and evaluation of antioxidant and anti-fungal activity of novel derivatives of 1,3,4-oxadiazole. Oxadiazole is heterocyclic compound having 2 nitrogen and 1 oxygen atom together to form a heterocyclic ring with five members. The synthesized 1,3,4-oxadiazole derivatives were identified using through various parameters i.e., physicochemical properties, TLC (R_f values), FTIR, NMR and Mass spectroscopy analysis. The antioxidant activity of synthesized 1,3,4-oxadiazole derivatives were evaluated through DPPH radical scavenging assay. The anti-fungal activity was determined through well diffusion method against *Aspergillus niger*, *Penicillium notatum*, *Candida albicans* and *Rhizopus species* microbial strains. The yield (%) was reported as 26.8 %, 28.4 %, 25.2 %, 29.1 % and 27.3 % in C1, C2, C3, C5 and C6, respectively. However, highest percentage yield was found in F4 as 32.6 %. For 1,3,4-oxadiazole derivatives, the melting point was determined in the range of 175-180 °C, in C1. The highest R_f value in C4 was found to be 0.78. It concluded that at 200µg/ml, % inhibition was found as 89.32%, 91.15%, 88.23%, 91.16%, 87.53% and 90.27% in C1, C2, C3, C4, C5 and C6, respectively. Moreover, ascorbic acid exhibited the 95.23 %. Thus, all the 1,3,4-Oxadiazole derivatives (C1-C6) showed the effective antioxidant agent when tested through DPPH free radical scavenging assay. In *A. niger*, anti-fungal activity was observed as 5.37 mm, 5.11 mm, 4.61 mm, 5.76 mm, 5.32 mm and 5.29 mm, in C1-C6, respectively. This study suggests fellow researchers to identify the mode of action of antioxidant activity that how it prevents the cellular oxidation process as well as anti-fungal activity.

Keywords: 1,3,4-oxadiazole, antioxidant, anti-fungal, DPPH, *A. niger*.

INTRODUCTION

Forty million Americans suffer from fungal diseases, according to studies. In clinical settings, a range of synthetic antifungal medicines are employed to treat various fungal infections. These drugs can be broadly divided into four groups: polyenes, azoles, allylamines, and echinocandins [1]. Antifungal medications are utilized in the treatment and prevention of human fungal infections. Antifungals are categorized into five groups based on their site of action: azoles, which inhibit ergosterol synthesis [2].

Many synthetic antifungal drugs are used in clinical settings to treat various fungal infections. 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].

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 [4].

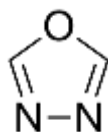


Fig. 1. Structure of 1,3,4-Oxadiazole

Molecular formula: $C_2H_2N_2O$

Molecular weight: $70.051 \text{ g}\cdot\text{mol}^{-1}$

After literature survey, done upon the synthesis, and antioxidant & anti-fungal potential of oxadiazole derivatives, the current study was based on the synthesis, identification and evaluation of antioxidant and anti-fungal activity of novel derivatives of 1,3,4-oxadiazole.

MATERIALS AND METHODS

Experimental requirements

Substituted benzo-hydrazide, KOH, Carbon di-sulfide, ethanol, methanol, DPPH, distilled water and paraffin.

Digital weighing balance, round bottom flask, condenser, clinical thermometer, and digital pH meter.

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

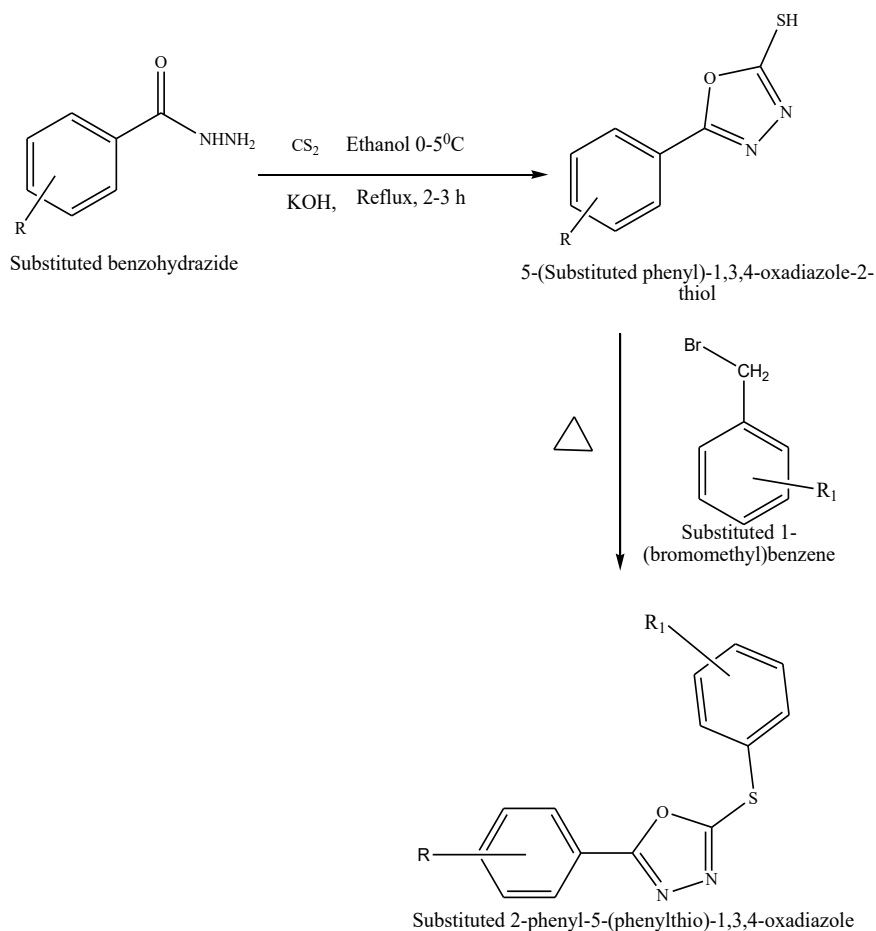


Fig 2. Scheme for synthesis of novel derivatives of 1,3,4-oxadiazole

Upon reaction of 3-hydroxybenzohydrazide with Carbon di-sulfide and Potassium hydroxide at $0-5^{\circ}C$, it produced the 3-(5-mercapto-1,3,4-oxadiazole-2-yl) phenol. All Upon heating it converted into 1-(bromomethyl)benzene which produced the final product 3-(5-phenylthio-1,3,4-oxadiazole-2-yl) phenol.

Synthesis of C1

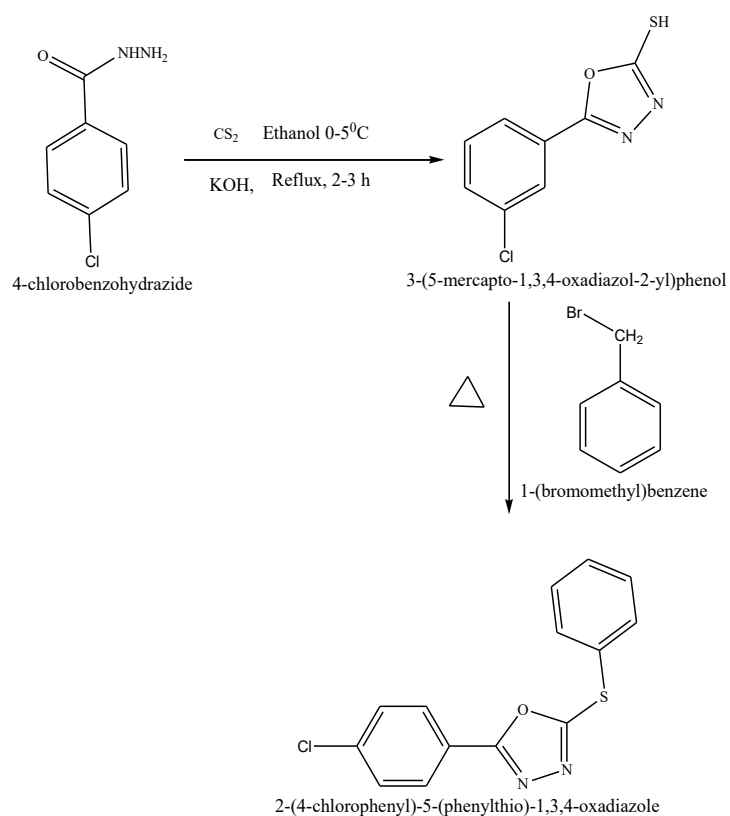


Fig 3. Synthesis of C1

Synthesis of C2

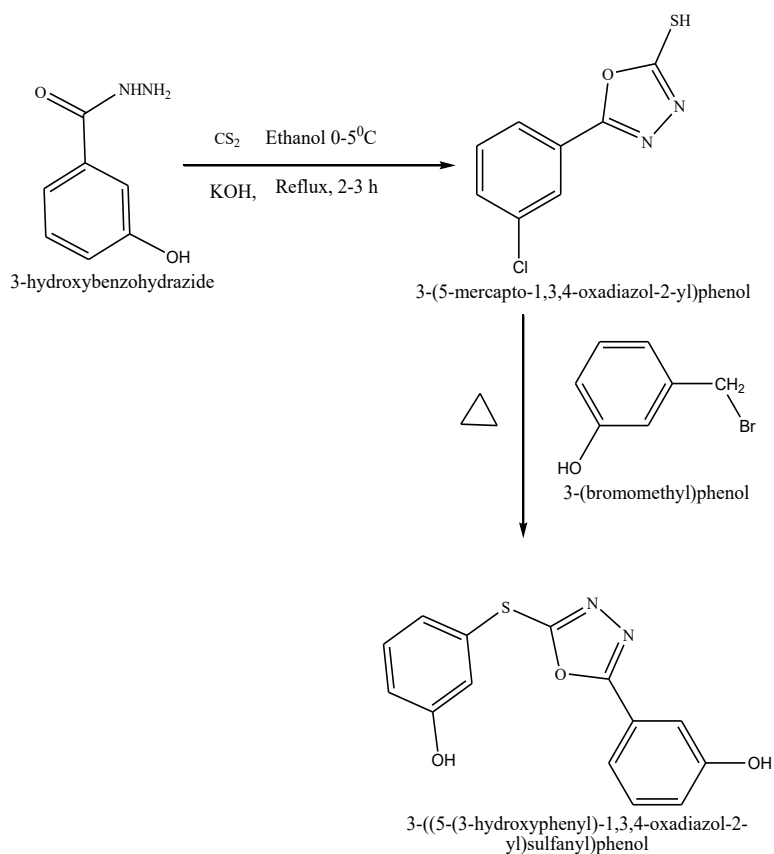


Fig 4. Synthesis of C2

Synthesis of C3

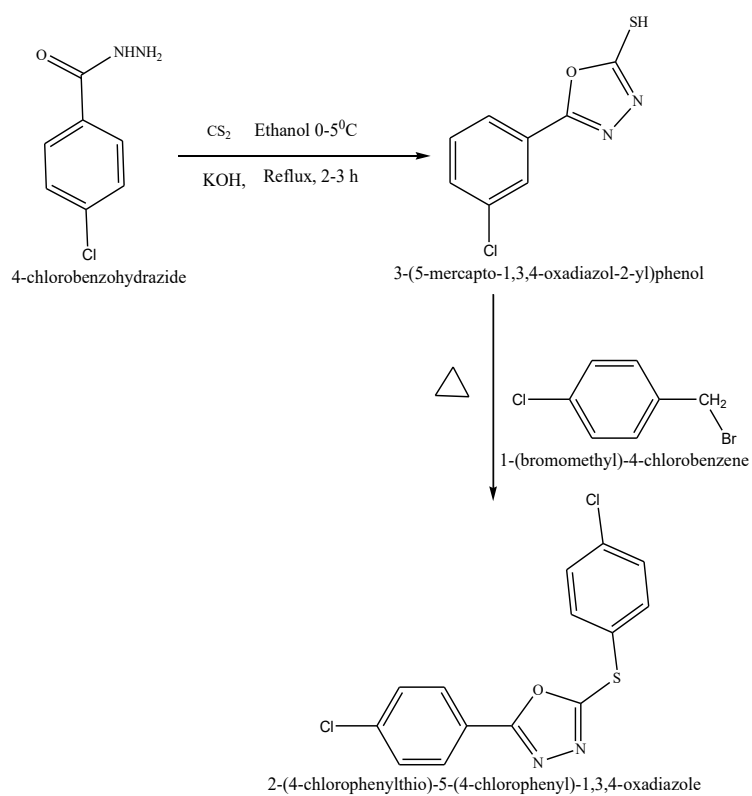


Fig 5. Synthesis of C3

Synthesis of C4

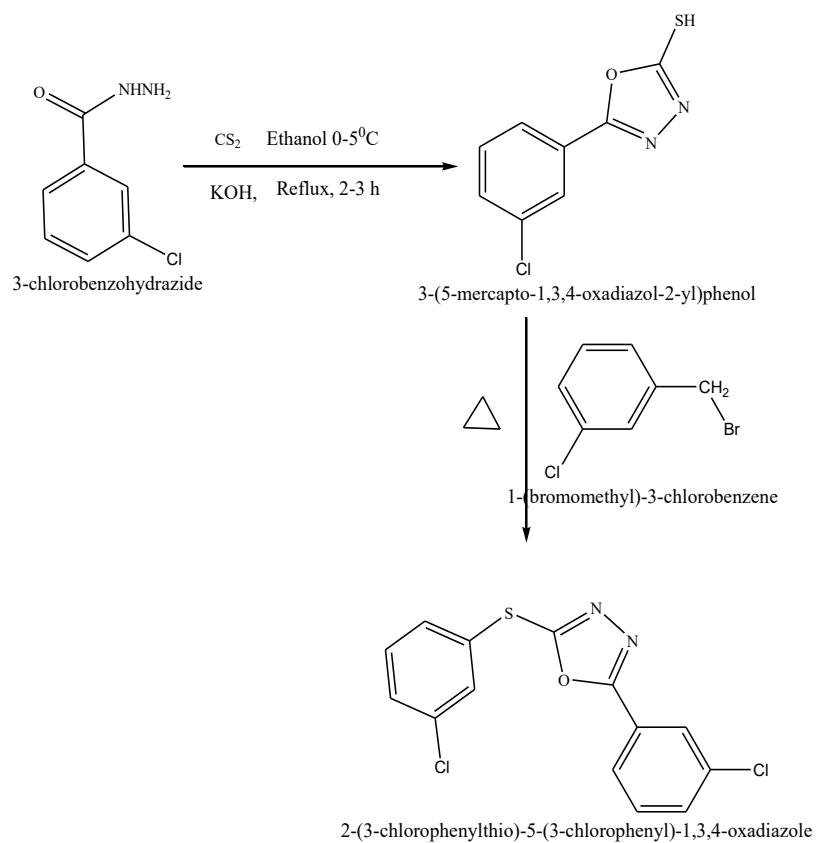


Fig 6. Synthesis of C4

Synthesis of C5

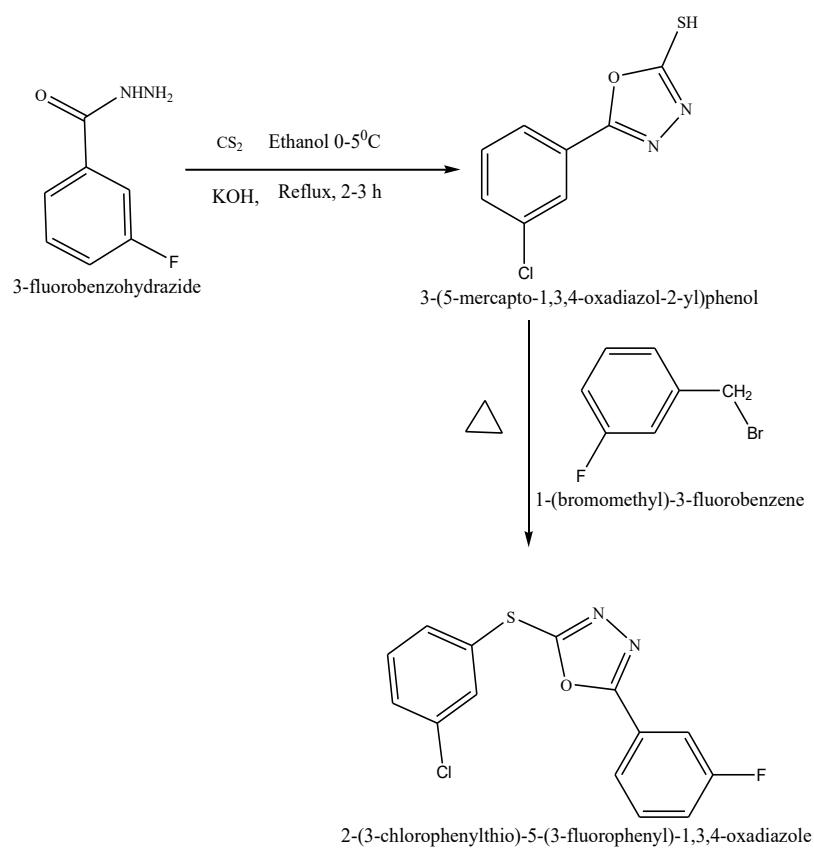


Fig 7. Synthesis of C5

Synthesis of C6

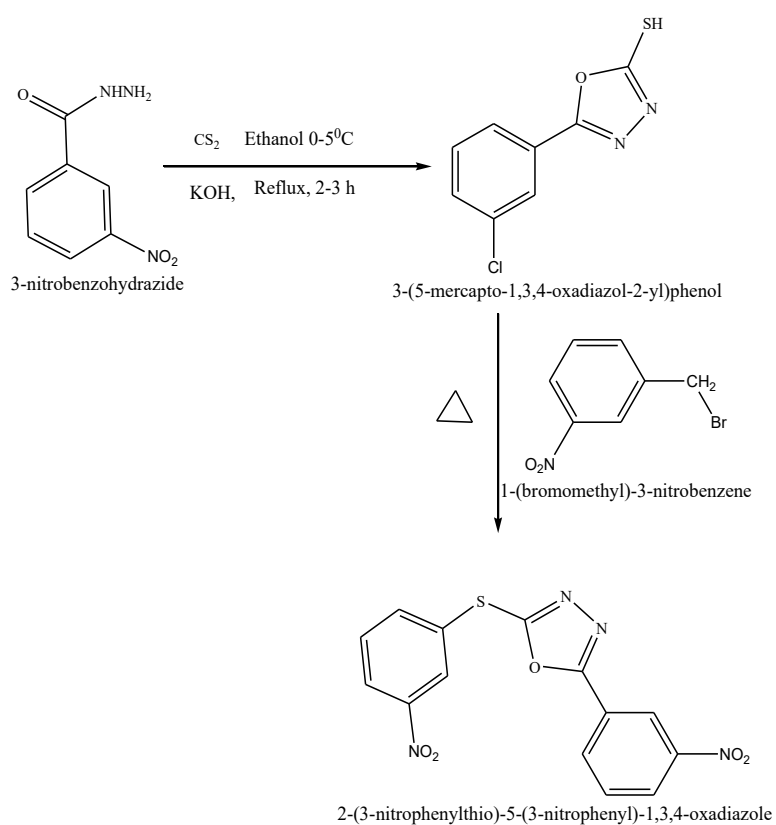


Fig 8. Synthesis of C6

Identification of physicochemical properties

Melting point determination

Thiel's melting point tube was used to determine the melting point of an organic compound (capillary tube method). The most important and straightforward means of distinguishing one compound from another is to determine its melting point.

Determination of R_f value using TLC

TLC stands for thin layer chromatography and was used in synthetic chemistry to infer the production of a molecule based on its R_f value, which varies depending on the compound. It also aids in confirming the reaction's progress [5].

Infrared Spectroscopy

IR spectrum was considered as vibrational-rotational spectra. KBr pellet technique was used for solid compound, for liquid compound Nujol mull method was followed. It is very helpful record which would give information about functional group present in the organic compounds. Mechanism of bond stretching and bending was happened when electromagnetic radiation ranging from 500 cm⁻¹ to 4000 cm⁻¹ passed through sample [6].

NMR Spectroscopy

Most used NMR was Proton NMR, because of the sensitivity and wide range of characteristic information. Range of chemical shift (δ) from 0-14 ppm. Chemical shift of the test unknown compound was compared with TMS protons which are attributed at 0 ppm. But the shift extends for the organic compound range δ 0-14 for the component [7].

Mass Spectroscopy

Mass spectrometry (MS) was an important physicochemical tool applied for structural elucidation of compounds from natural products including medicinal plants. The fundamental principle of MS was the use of different physical means for sample ionization and separation of the ions generated based on their mass (m) to charge (z) ration (m/z). The ionization techniques available techniques include electrospray ionization, atmospheric pressure chemical ionization (APCI), electron ionization (EI), chemical ionization, fast atom bombardment (FAB), and matrix analysis laser desorption ionization (MALDI). Mass spectrometry has high sensitivity with detection limit of femtogram compared to NMR with sensitivity limit of nanogram range and above. The sensitivity and the flexibility for hyphenation with other chromatographic technique made MS a versatile analytical instrument [8].

Pharmacological evaluations

Estimation of DPPH Radical Scavenging Assay

The free radical scavenging potential of derivatives was measured by the 1,1'-diphenyl-1-picrylhydrazyl (DPPH) according to the method by *Tariq et al.* [9]. The assay experimented by reacting 1.6 mL of 0.135 mM DPPH dissolved in 100% v/v methanol with 0.4 mL of various concentrations of synthesized derivatives. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture measured at 517 nm after 2 min. Here, the concentration of the derivatives needed to decrease the absorbance of DPPH radical by 50% was calculated.

The % scavenging activity was determined by the following equation:

$$\text{DPPH scavenging activity (\%)} = [(\text{control absorbance} - \text{sample absorbance}) / (\text{control absorbance})] \times 100$$

Evaluation of anti-fungal potential

By using the well diffusion method, the anti-fungal activity of 1,3,4-oxadiazole derivatives against fungus strains was identified. Nutrient Agar broth was utilized to cultivate a 24-hour-old culture of microbes, which was then used to make a suspension of microorganisms. After sterilization at 121 °C (1.05 kg/cm² pressure) for 20 minutes, nutrient agar solution was added. A sterile spreader was used to cover the whole surface of the agar plates when we inoculated them with 500:1 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 was measured after 24 hours of incubation at 30 °C. As a standard anti-fungal formulation, gentamycin was utilized. Every anti-fungal study was carried out in triplicate. Microbial strains i.e., *Aspergillus niger*, *Penicillium notatum*, *Candida albicans* and *Rhizopus species* was utilized for screening of anti-fungal activity [10].

RESULTS AND DISCUSSION

Identification of physicochemical properties

Physical characteristics i.e., yield (%), melting point, and R_f values were assessed for each of the synthesised 1,3,4-oxadiazole derivatives.

Yield (%)

The yield (%) was reported as 26.8 %, 28.4 %, 25.2 %, 29.1 % and 27.3 % in C1, C2, C3, C5 and C6, respectively. However, highest percentage yield was found in F4 as 32.6 %.

Table 1. Yield (%) of 1,3,4-oxadiazole derivatives

Compound	Yield (%)
C1	26.8
C2	28.4
C3	25.2
C4	32.6
C5	29.1
C6	27.3

Melting point

The compound C4 exhibited the highest melting point as 225-230 °C. The compound's strongest density was indicated by its highest melting point. For 1,3,4-oxadiazole derivatives, the melting point was determined in the range of 175-180 °C, in C1.

Table 2. Melting point of 1,3,4-oxadiazole derivatives

Compound	Melting point (°C)
C1	175-180
C2	195-200
C3	216-220
C4	212-216
C5	225-230
C6	182-186

Rf value

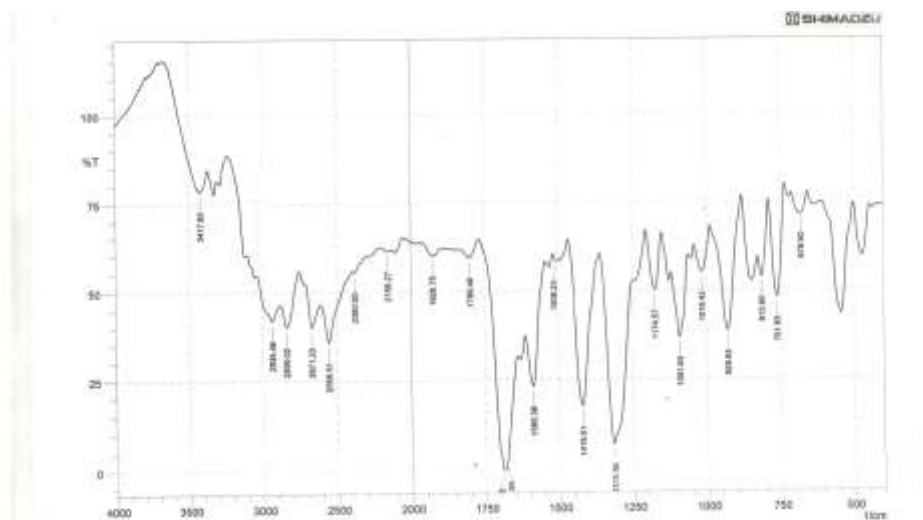
Depending on the chemical, synthetic chemistry uses thin layer chromatography to verify the synthesis of a molecule based on its Rf value. All the derivatives exhibited excellent Rf value when observed in TLC analysis. The highest Rf value in C4 was found to be 0.78.

Table 3. Rf Value of 1,3,4-oxadiazole derivatives

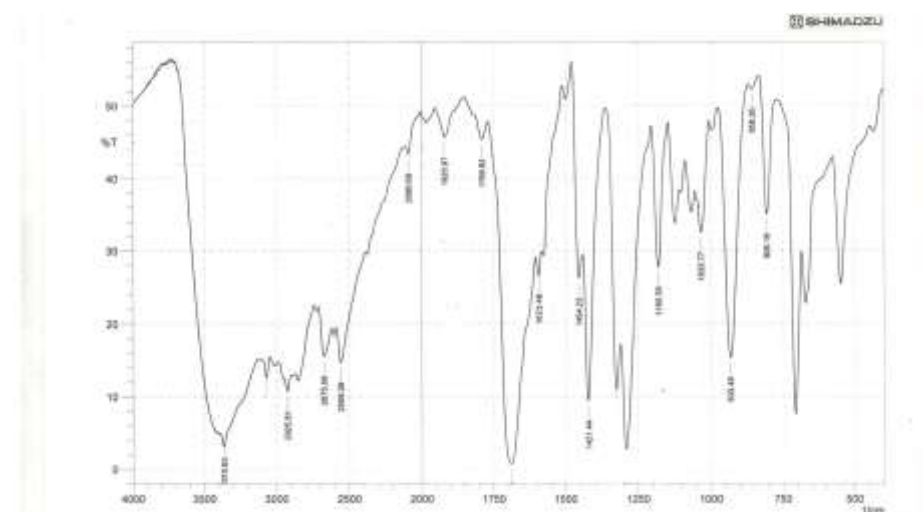
Compound	Rf Value
C1	0.75
C2	0.73
C3	0.71
C4	0.78

C5	0.69
C6	0.72

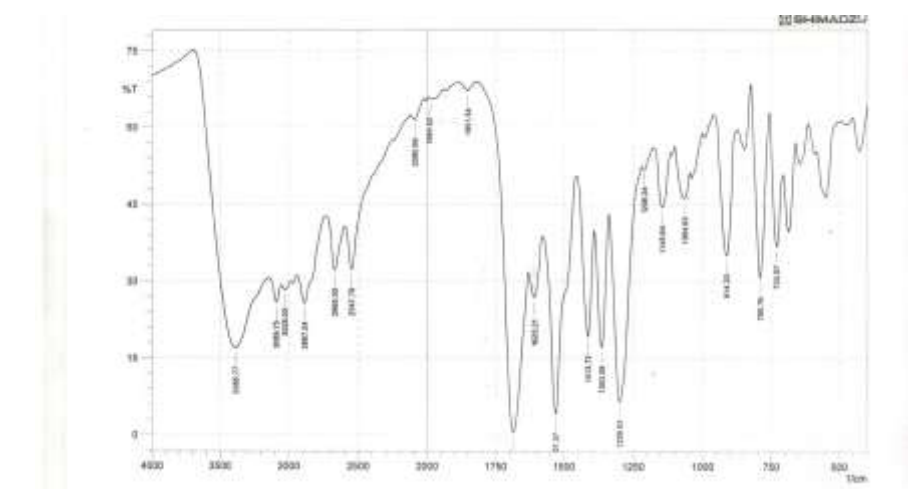
Infrared Spectroscopy



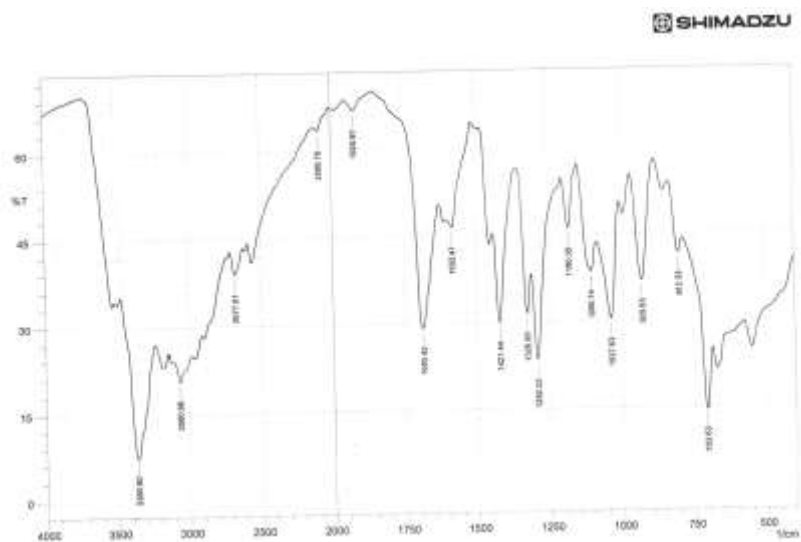
C1



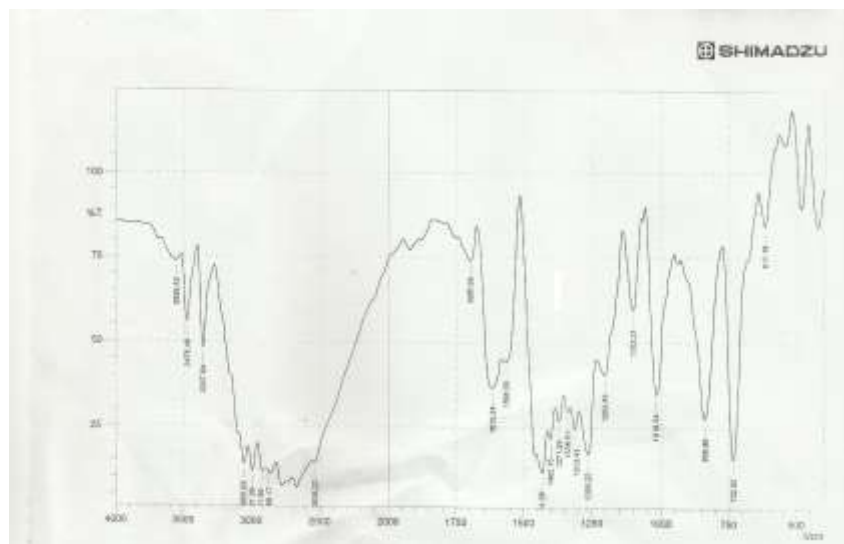
C2



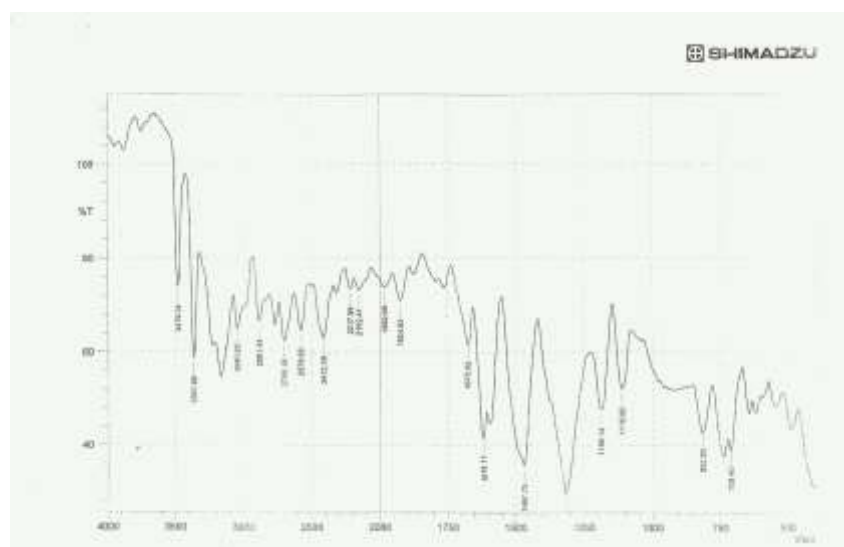
C3



C4

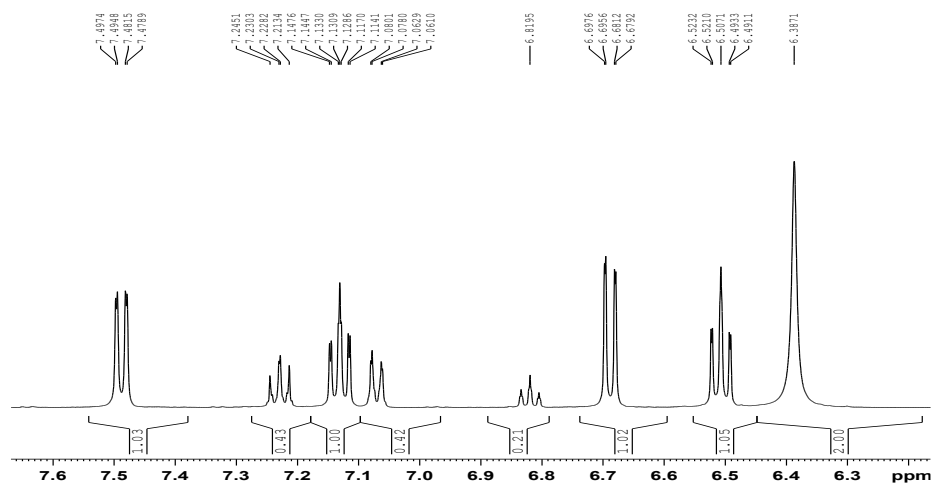


C5

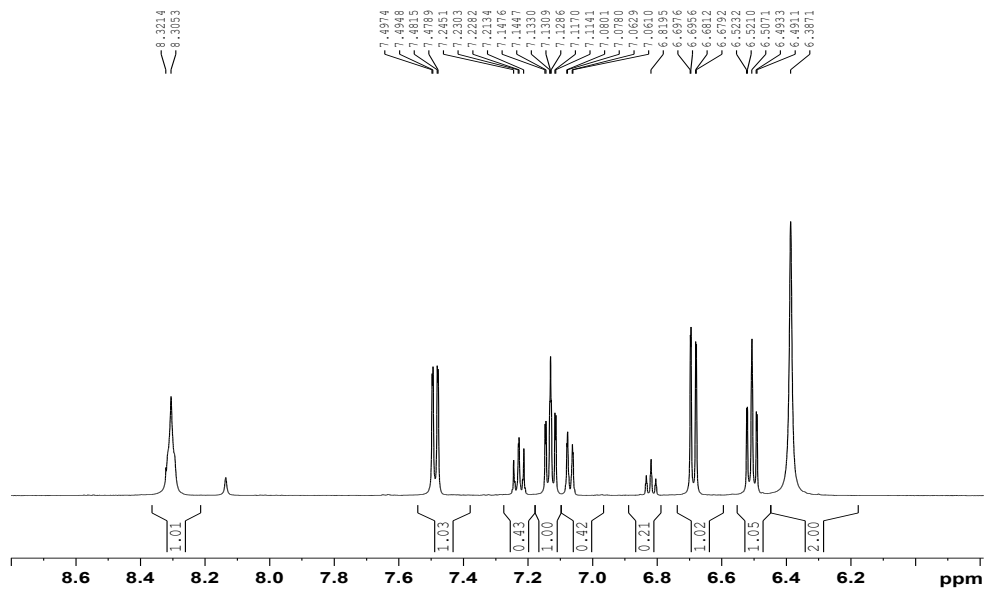


C6

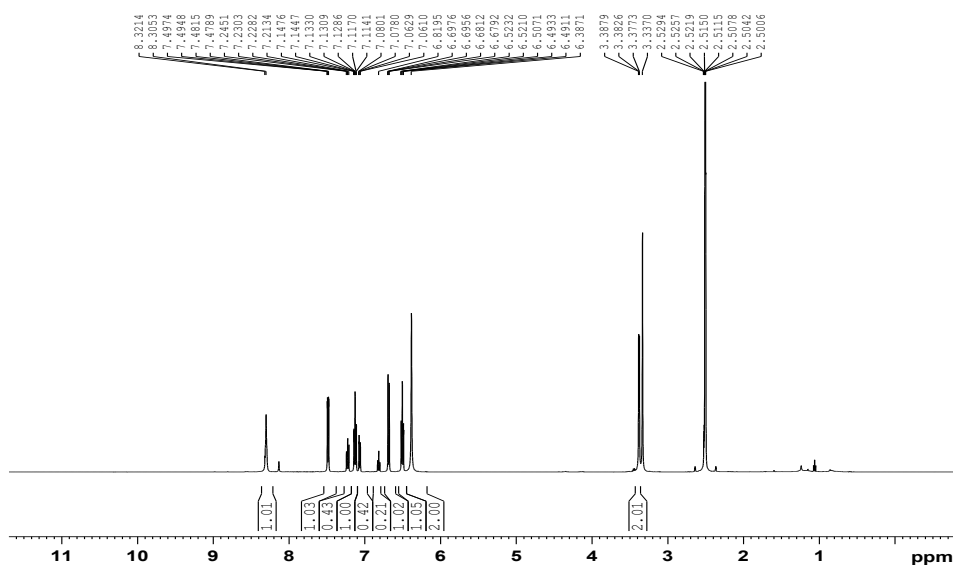
NMR Spectroscopy



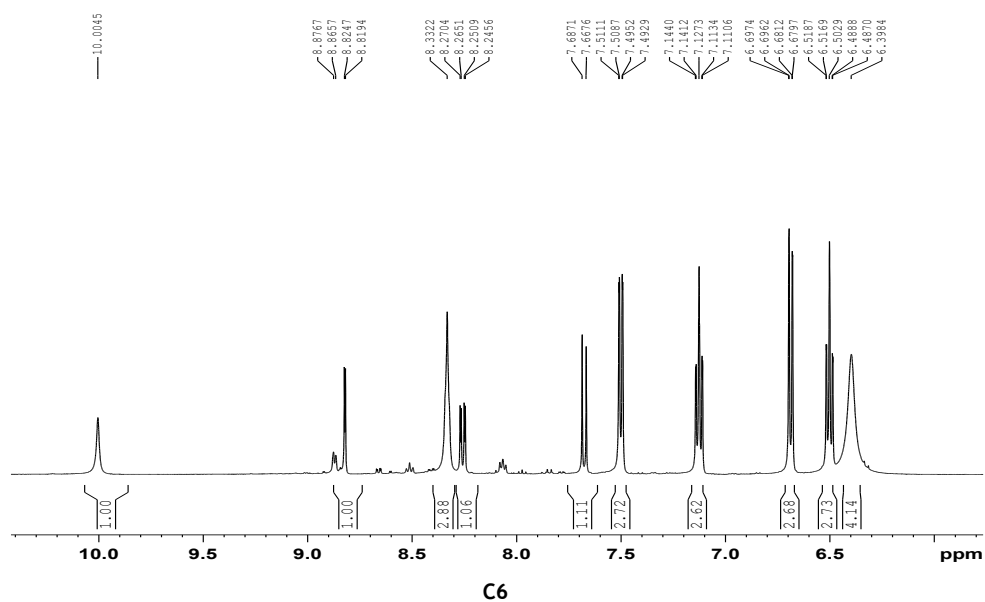
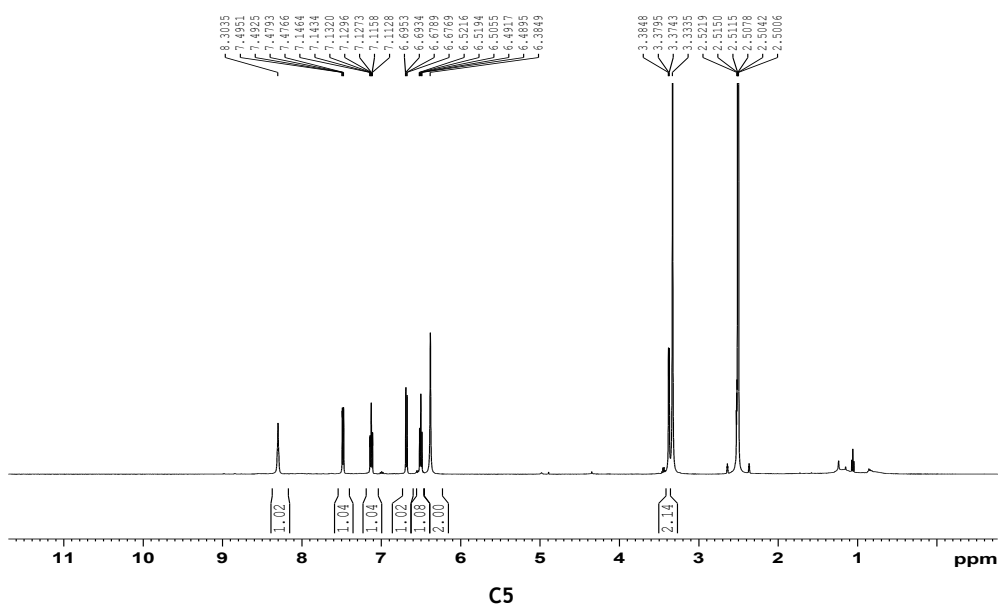
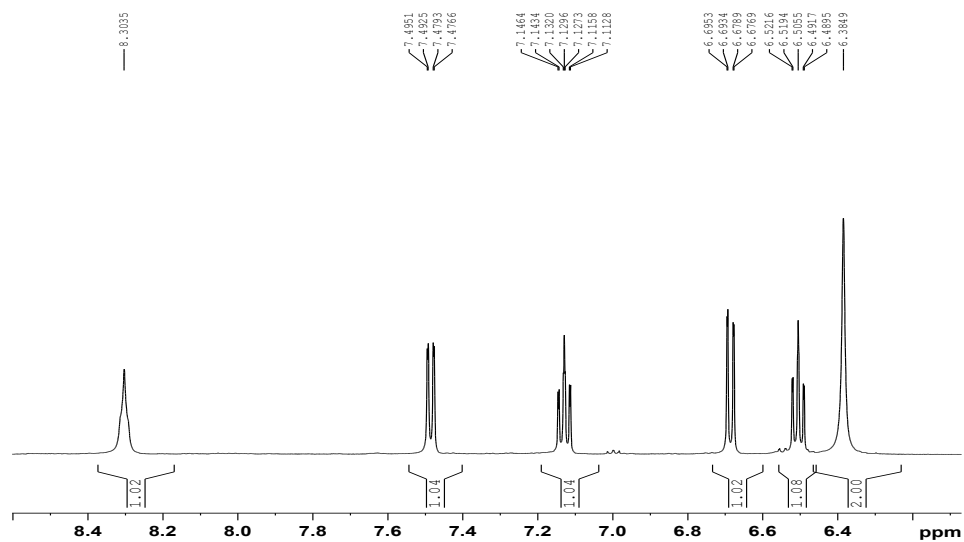
C1



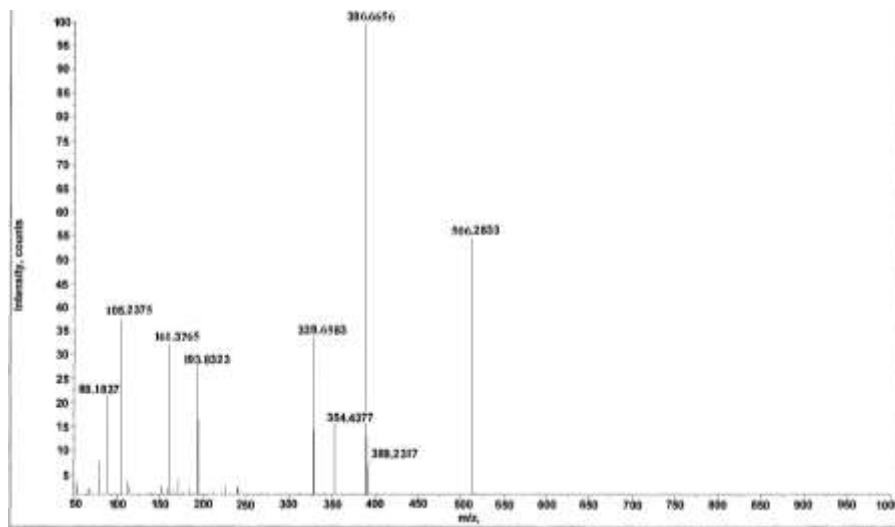
C2



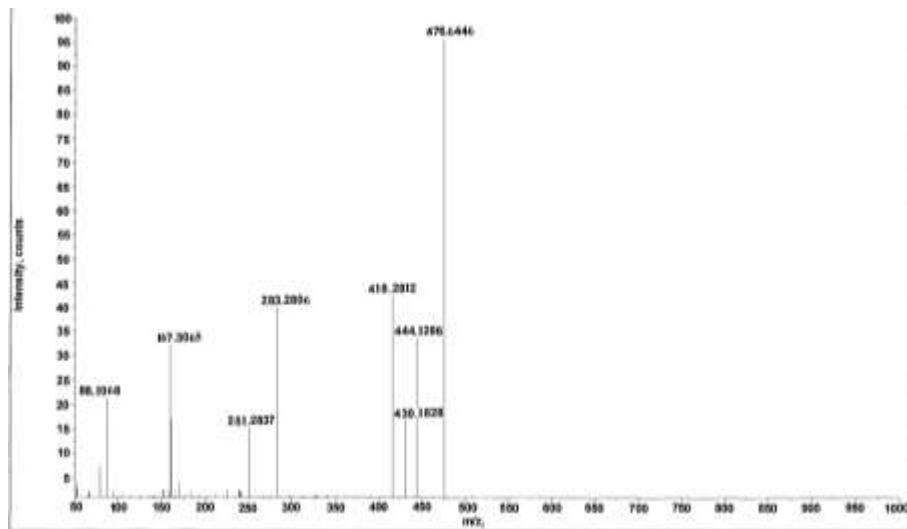
C3



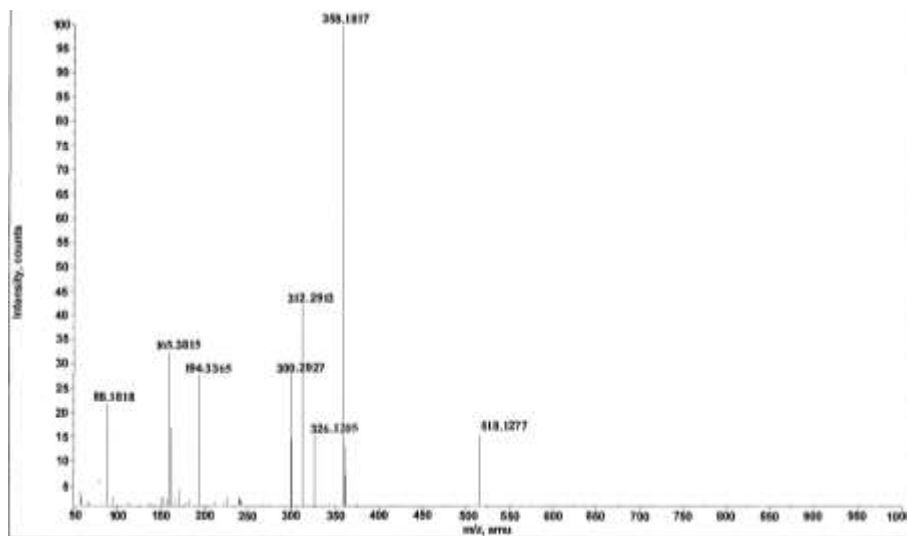
Mass Spectroscopy



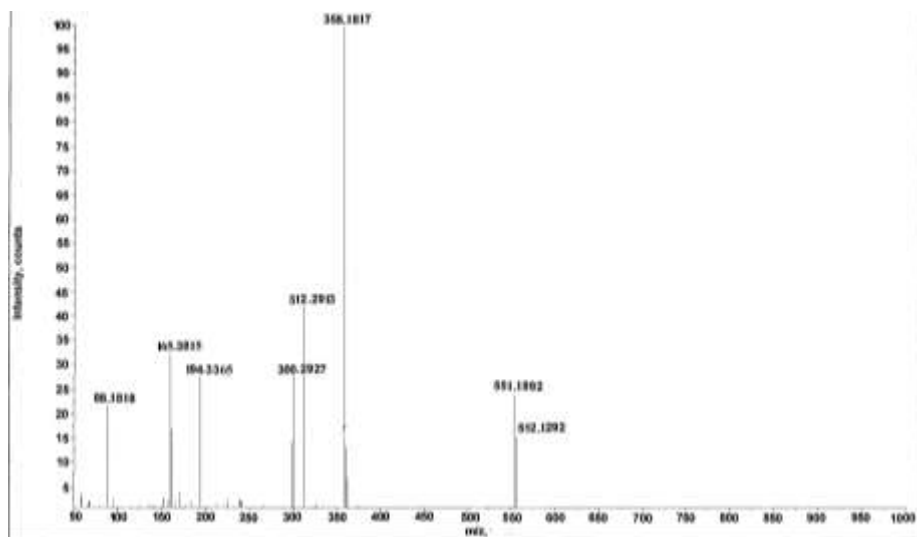
C1



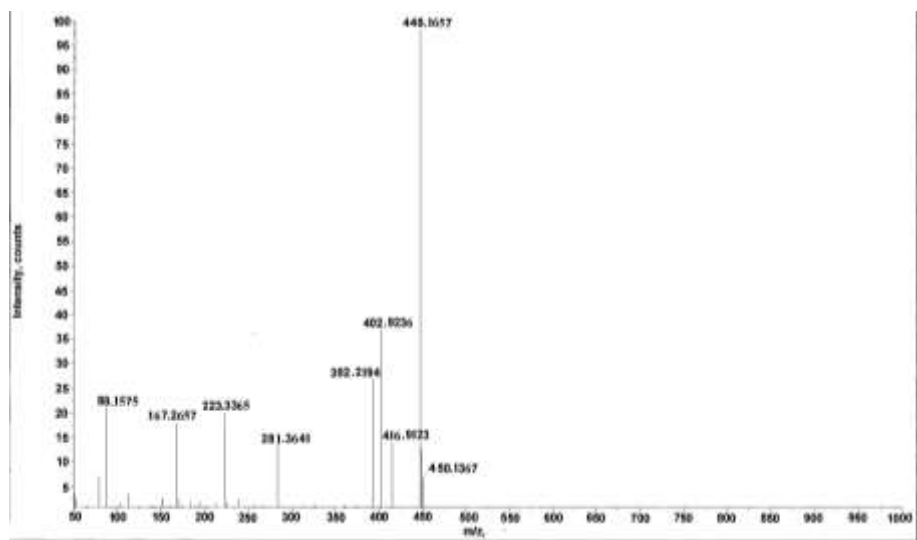
C2



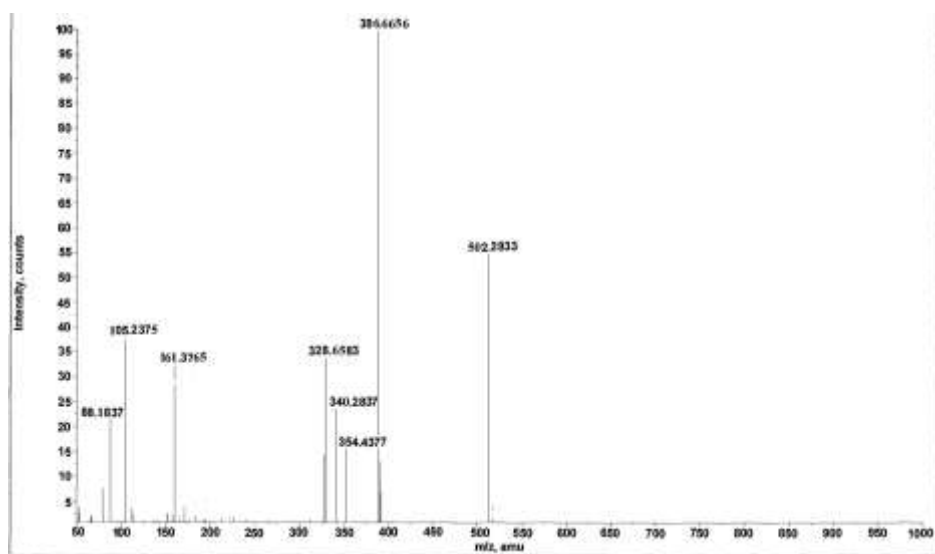
C3



C4



C5



C6

Evaluation of pharmacological activity

DPPH Free-Radical Scavenging assay

In DPPH Free-Radical scavenging activity, different concentrations i.e., 50 µg/ml, 100 µg/ml, 150 µg/ml and 200 µg/ml were tested for ascorbic acid (std.), and 1,3,4-Oxadiazole derivatives (C1-C6). Percent inhibition of novel derivatives of 1,3,4-Oxadiazole was compared with std. antioxidant ascorbic acid.

At 200 µg/ml, % Inhibition was found as 89.32%, 91.15%, 88.23%, 91.16%, 87.53% and 90.27% in C1, C2, C3, C4, C5 and C6, respectively. Moreover, ascorbic acid exhibited the 95.23 %. Thus, all the 1,3,4-Oxadiazole derivatives (C1-C6) showed the effective antioxidant agent when tested through DPPH free radical scavenging assay.

The % inhibition of DPPH Scavenging assay was observed as follow-

Table 4. DPPH Scavenging assay of 1,3,4-Oxadiazole derivatives

Group	DPPH Free-Radical Scavenging assay (% Inhibition)			
	50 µg/ml	100 µg/ml	150 µg/ml	200 µg/ml
Std.	21.34	36.29	63.14	95.23
C1	16.23	31.21	58.14	89.32
C2	18.44	33.12	60.23	91.15
C3	15.64	31.26	57.10	88.23
C4	19.12	34.54	61.21	91.16
C5	17.53	32.14	56.29	87.53
C6	16.28	31.20	59.14	90.27

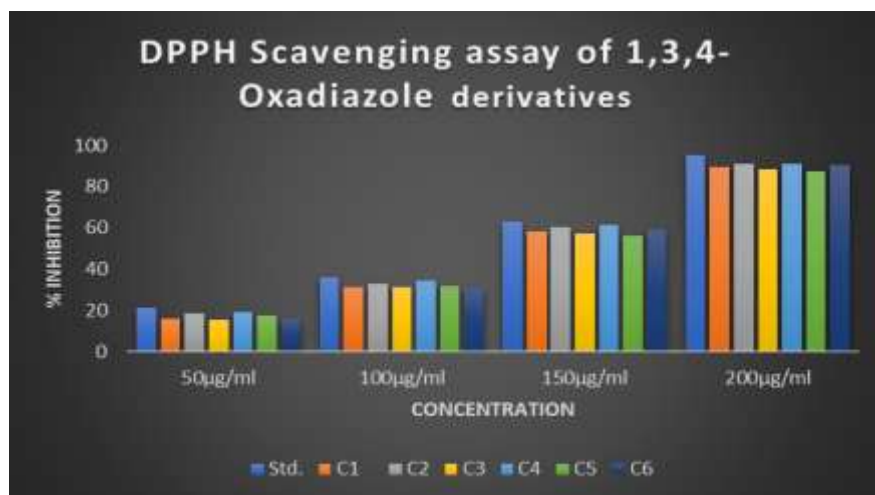


Fig. 9. DPPH Scavenging assay of derivatives of 1,3,4-Oxadiazole

Evaluation of anti-fungal activity

In *A. niger*, anti-fungal activity was observed as 5.37 mm, 5.11 mm, 4.61 mm, 5.76 mm, 5.32 mm and 5.29 mm, in C1-C6, respectively. Similar action was observed in other microbial strains. Moreover, highest disc diffusion was observed against *C. albicans* i.e., C1 (6.41 mm), C2 (6.18 mm), C3 (6.41 mm), C4 (7.53 mm), C5 (7.26 mm), C6 (6.61 mm), respectively.

Std. anti-fungal gentamycin showed the anti-fungal activity through disc diffusion of 6.51 mm, 7.43 mm, 8.30 mm, and 7.62 mm, against *A. niger*, *P. notatum*, *C. albicans* and *Rhizopus sp.*, respectively.

Table 5. Anti-fungal activity of 1,3,4-Oxadiazole derivatives

Treatment	Anti-fungal activity (mm)			
	<i>A. niger</i>	<i>P. notatum</i>	<i>C. albicans</i>	<i>Rhizopus sp.</i>
C1	5.37	5.71	6.41	6.53
C2	5.11	5.26	6.18	6.47
C3	4.61	4.69	6.41	5.62
C4	5.76	6.76	7.53	6.83
C5	5.32	6.45	7.26	6.20
C6	5.29	5.34	6.61	6.18
Gentamycin	6.51	7.43	8.30	7.62

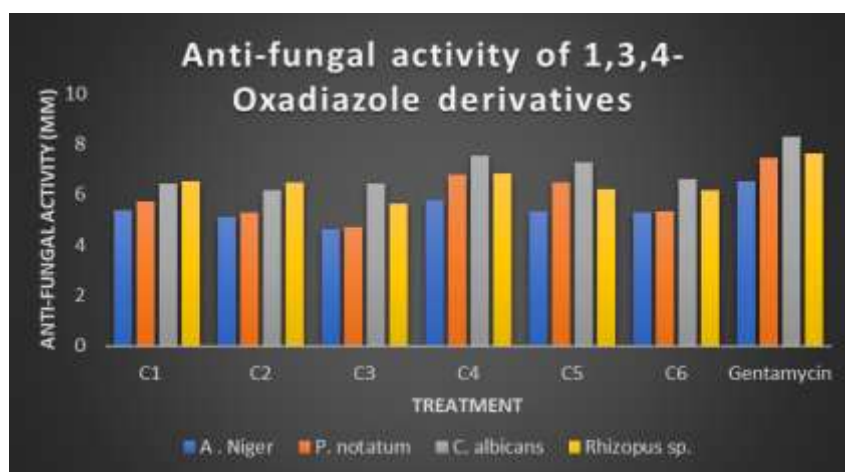


Fig 10. Anti-fungal activity of 1,3,4-Oxadiazole derivatives

Derivatives of 1,2,4-oxadiazole were produced as SDH inhibitors using a multi-step process involving substituted benzaldehyde and derivatives of anisic or cinnamic acid. In the initial assessment of biological efficacy, it was shown that the target compounds derived from anisic acid typically exhibited superior activity and a broader antifungal range. The reactivity of unsubstituted benzaldehyde surpasses that of mono-substituted variants, which in turn exceeds that of multi-substituted compounds. Moreover, the target compounds including phenolic hydroxyl groups, particularly at the 3 and 4 positions of the aromatic ring, have superior antifungal efficacy compared to other substituents. Simultaneously, certain target molecules exhibit remarkable antifungal properties, as exemplified by compounds 4f and 4q. Among the compounds, 4f exhibits the highest growth inhibition rate of 100% against *F. graminearum* at a dose of 50 µg/ml. The suppressive impact of 4f on *E. turcicum* was markedly superior to that of carbendazim. Compounds 4f and 4q exhibited antifungal properties against *C. capsici* in capsicum in vivo [11].

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 β-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 [12].

The identification of innovative leads and fresh targets is a crucial strategy to tackle the problem of fungicide resistance through the development of new fungicides. The indole framework has been extensively employed in agrochemicals owing to its distinctive biological properties. YZK-C22 is a powerful inhibitor of pyruvate kinase exhibiting significant antifungal efficacy. Multiple innovative fungicide candidates were developed from YZK-C22. Motivated by this, the [1,2,4]triazino[4,5-a]indol-1(2H)-one derivatives were formulated employing a skeleton hopping approach; yet, these substances demonstrated limited antifungal efficacy. Surprisingly, 2-(1H-indol-2-yl)-1,3,4-oxadiazole derivatives synthesized under regulated reaction circumstances exhibited

markedly enhanced fungicidal efficacy. Compounds 6c, 6d, 6f, and 6j demonstrated remarkable antifungal efficacy in vitro, with EC₅₀ values between 0.120 and 0.310 µg/mL against *Botrytis cinerea*, surpassing the effectiveness of the commercial fungicide pyrimethanil (EC₅₀ = 0.990 µg/mL). In the experimental trials at 540 and 720 g of active ingredient (ai)/hm², compound 6c demonstrated 81.46% and 86.58% effectiveness against *B. cinerea*, surpassing the efficacy of pyrimethanil at 540 g of ai/hm² (70.46%). The binding affinities of compounds 6c and 6d for pyruvate kinase from *B. cinerea* were inferior to that of YZK-C22. Elevated field efficacy coupled with reduced affinity for pyruvate kinase suggests that these chemicals might function as prodrugs or possess an alternative mechanism of action. Consequently, 2-(1H-indol-2-yl)-1,3,4-oxadiazole derivatives merit additional exploration [13].

CONCLUSION

It concluded that at 200µg/ml, % inhibition was found as 89.32%, 91.15%, 88.23%, 91.16%, 87.53% and 90.27% in C1, C2, C3, C4, C5 and C6, respectively. Moreover, ascorbic acid exhibited the 95.23 %. Thus, all the 1,3,4-Oxadiazole derivatives (C1-C6) showed the effective antioxidant agent when tested through DPPH free radical scavenging assay. In *A. niger*, anti-fungal activity was observed as 5.37 mm, 5.11 mm, 4.61 mm, 5.76 mm, 5.32 mm and 5.29 mm, in C1-C6, respectively.

This study suggests to fellow researchers to identify the mode of action of antioxidant activity that how it prevents the cellular oxidation process as well as anti-fungal activity. Whether 1,3,4-Oxadiazole derivatives show the fungicidal potential through destructions of cell wall or nucleoside/nucleotides.

CONFLICT OF INTEREST

Authors declare for none conflict of interest.

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