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RESEARCH ARTICLE

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SYNTHESIS CHARACTERIZATION AND BIOLOGICAL EVALUATION OF 1,3,4 OXADIAZOLE DERIVATIVES

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ABSTRACT: Ten novel 1,3,4-oxadiazole analogs (A-J) were prepared and tested in terms of their antibacterial and antioxidant properties. The obtained compounds were identified by the determination of melting points, thin-layer chromatography, and the confirmation of molecular formulas, whereas the identification of representative compounds proceeded by adding the UV- Visible spectroscopy, Fourier-transform infrared spectroscopy (FT-IR), and nuclear magnetic resonance (^{1}H- and ^{1}C-NMR) methods. The spectral analyses proved the successful construction of the oxadiazole nucleus and the existence of the typical functional groups. The synthesized derivatives were evaluated with respect to the Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*) and Gram-positive bacterium (*Staphylococcus aureus* and *Micrococcus luteus*) regarding their antibacterial activity based on the agar diffusion test with 100 µg/mL, 500 µg/mL, and 1000 µg/mL concentrations of the derivatives. In a concentration-dependent fashion, several compounds showed a significant level of antibacterial activity. Out of them, methyl-substituted (B) and chloro-substituted (D) counterparts exhibited similar inhibition zones as the standard drug ciprofloxacin. The DPPH free radical scavenging assay was used to determine the antioxidant activity. These findings revealed moderate-strong antioxidant potential of most compounds, with some halogenated derivatives having lower IC50 values compared to ascorbic acid. Possible structure-activity relationship analysis showed that electron-withdrawing and electron-donating substituents have a significant impact on biological activity. All in all, the results demonstrate substituted 1,3,4-oxadiazole derivatives as promising candidates in the further development as antimicrobial and antioxidant agents.

Keywords: 1,3,4-Oxadiazole; Antibacterial activity; Antioxidant activity; DPPH assay

I. INTRODUCTION

Compounds with the oxadiazole ring system that are heterocyclic in nature have received immense interest in medicinal chemistry because of their various pharmacological effects among them being antimicrobial, anti-inflammatory, antioxidant and anticancer effects. Of particular interest are those isomers that are 1,3,4-oxadiazoles due to their attractive physicochemical characteristics, metabolic characteristics, and capability to serve as bioisomers of ester and amide groups [1]. The growing rate of microbial resistance to the current antibiotics makes the need to develop new antimicrobial agents that are more effective and have different modes of action. Simultaneously, oxidative stress is directly involved in the pathogenesis of a number of chronic diseases, which explains the necessity of compounds with antimicrobial and antioxidant properties [2].

With this respect, the current work aimed to prepare a range of structurally related derivatives of 1,3,4-oxadiazole that have several different substituents on the aromatic ring and the evaluation of their antibacterial and antioxidant properties. Preliminary analysis of structure to activity was also done to investigate the effect of the type of substituent on biological activity [3].

2. MATERIALS AND METHODS

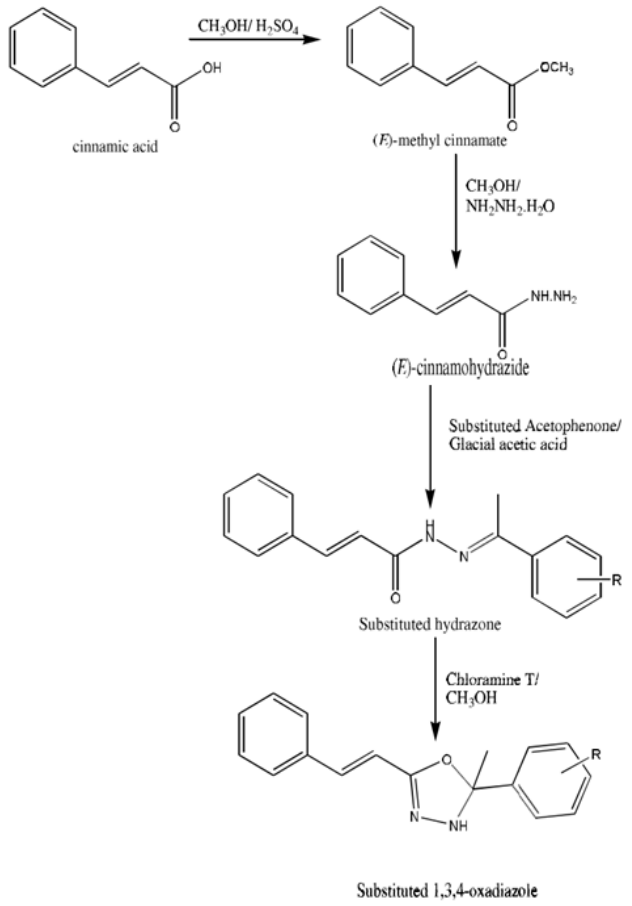
2.1 Chemicals and Instruments

Reagents of analytical grade were used without any additional purification. The digital melting point apparatus was used to determine the melting points. Silica gel plates were subjected to TLC and views were obtained under UV light. The FT-IR spectra were taken through the KBr pellet method. NMR spectra were acquired with the help of DMSO-d_6 or $CDCl_3$ [4].

2.2 General Synthetic Procedure for 1,3,4-Oxadiazole Derivatives

The 1,3,4-oxadiazole analogs that were substituted were prepared by the cyclization of the corresponding acyl hydrazides with substituted aromatic acids under reaction conditions. The flask was then boiled owing to a given period after which the mixture was cooled and the product was precipitated. The raw product was filtered, washed and re-crystallized to get pure compounds [5].

Reaction Scheme



2.3 Antibacterial Activity

The agar diffusion method was used in conducting the antibacterial screening. Test solutions were made in DMSO and tested on 100, 500 and 1000 µg/mL. The standard reference drug was Ciprofloxacin. Measurement of zones of inhibition was done after incubation [6].

2.4 Antioxidant Activity

The antioxidant activity of DPPH radical scavenging assay was carried out using ascorbic acid as a reference. The absorbance was obtained at 517 nm and IC_{50} obtained using dose response curves [7].

3. RESULT

3.1 Synthesis and Physical Characterization

Ten (1,3,4-oxadiazole) derivatives (A-J) were prepared successfully using the given synthetic procedure. All the products were of solid form and yielded between 52 percent and 72 percent. The derivatives obtained by the synthesis displayed sharp melting points of 90-115 ºC, which implies reasonably good purity and cyclization of the oxadiazole ring.

The homogeneity of the synthesized derivatives was verified by Thin-layer chromatography in which each product appeared as a single spot in the hexane:ethyl acetate (7:3) system as the solvent. Table 1 summarizes the physical properties of compounds A–J such as molecular formula, molecular weight, the melting point and the percentage yield. The derivatives may differ in substituent nature and steric effects in minor changes in their melting points and yields.

Table 1: Physical Data of Synthesized Compounds

S. no.	*Compound*	*R*	*Mol. Formula*	*Mol. Weight*	*Melting point*	*% Yield*
1	a	H	$C_{15}H_{10}N_2O_2$	250.26	112ºC	67%
2	b	CH_3	$C_{16}H_{12}N_2O_2$	264.28	115ºC	72%
3	c	F	$C_{15}H_9FN_2O_2$	268.25	95ºC	70.0%
4	d	Cl	$C_{15}H_9ClN_2O_2$	284.70	90ºC	69.10%
5	e	Br	$C_{15}H_9BrN_2O_2$	329.15	92ºC	52%
6	f	I	$C_{15}H_9IN_2O_2$	376.15	92ºC	66%
7	g	CH_2F	$C_{16}H_{11}FN_2O_2$	282.27	90ºC	61%
8	h	CH_2Br	$C_{16}H_{11}BrN_2O_2$	343.18	92ºC	57.8%
9	i	CH_2Cl	$C_{16}H_{11}ClN_2O_2$	298.73	108ºC	69%
10	j	CH_2I	$C_{16}H_{11}IN_2O_2$	390.18	100ºC	58%

3.2 Spectral Characterization

The synthesized oxadiazole derivatives were structurally confirmed by the UV-Visible, FT-IR, and NMR spectroscopic analysis. One of them, representative compounds B, were chosen to be characterized in more detail in terms of their spectral properties since they are all part of the same structural series. The UV-Visible spectrums of the representative compounds were typical with characteristic absorption peaks within the wavelength range of 200-250nm confirming the presence of conjugated aromatic systems. Figure 1 displays the UV spectrum of compound B.

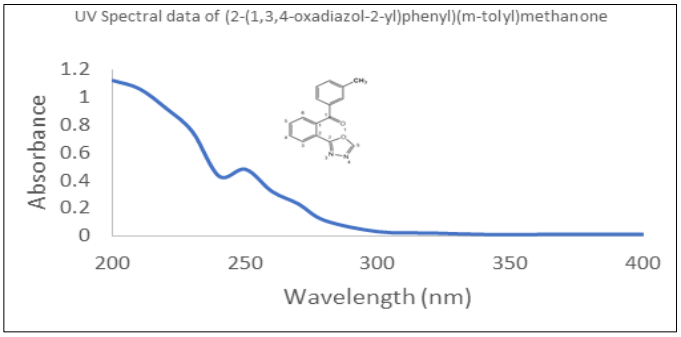


Figure 1: UV–Visible spectrum of compound B

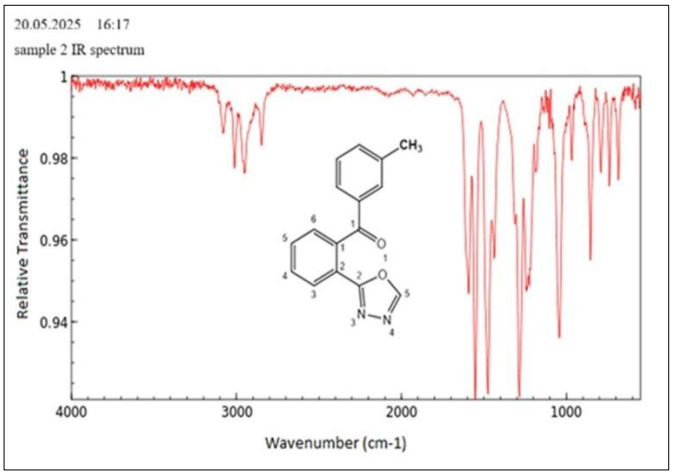


Figure 2: FT-IR spectrum of compound B

The FT-IR spectrums had characteristic absorption bands related to important functional groups of the oxadazine structure. Close bands that were observed in the area of 16001700 cm^{-1} were attributed to the C=N and C=O vibrations thus showing the formation of oxadiazole rings. C-H aromatic vibration was observed within the range of 3000 cm^{-1}, whereas C-N aromatic vibration was observed within the range of 1000-1100 cm^{-1}. In halogen substituted compounds, other bands were also noted and those occupied the positions of carbon-halogen vibration. **Figure 2** below show the FT-IR spectra of compound B, respectively.

The ^{1}H-NMR spectrums revealed that in the 7.277.9 ppm region of the spectrum, there were multiplets with the aromatic protons. A singlet at a frequency of 2.3 ppm in compound B also proved the presence of a methyl substituent. The ^{13} C-NMR spectra showed typical carbonyl carbon 79 at 195 ppm, oxadiazole ring carbons 88 at 150-165 ppm, and aromatic carbons 98 at 125-140 ppm. Figure 3 to 6 depict the NMR spectra of the representative compounds which affirm the suggested molecular structures.

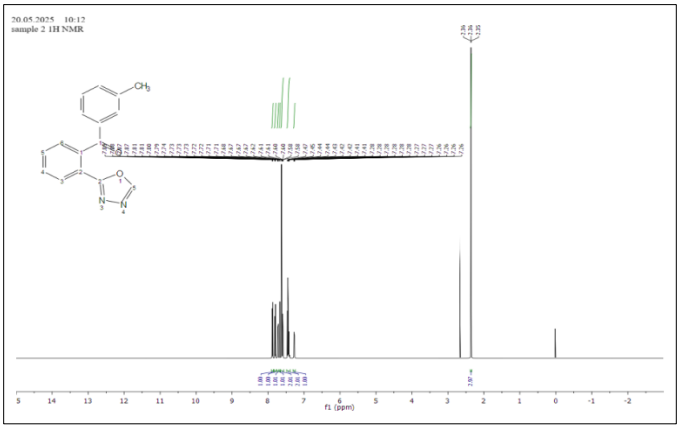


Figure 3: ^{1}H-NMR spectrum of compound B

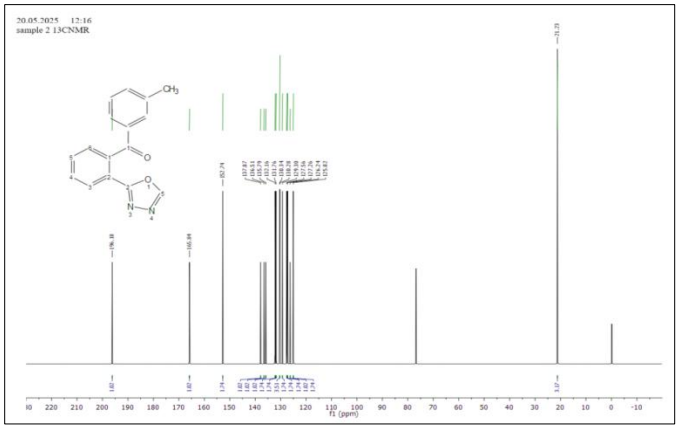


Figure 4: ^{13}C-NMR spectrum of compound B

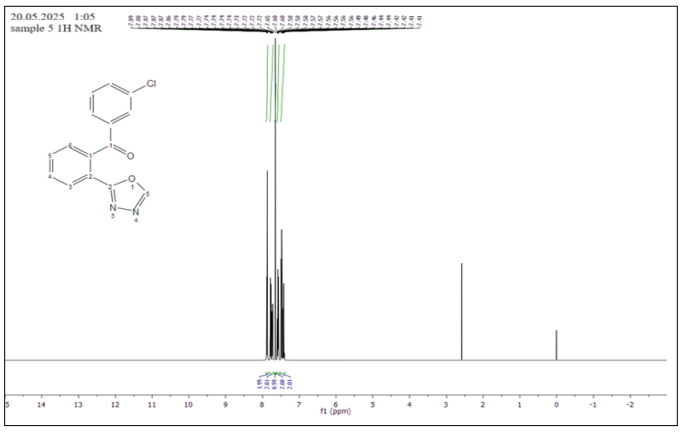


Figure 5: ^{1}H-NMR spectrum of compound D

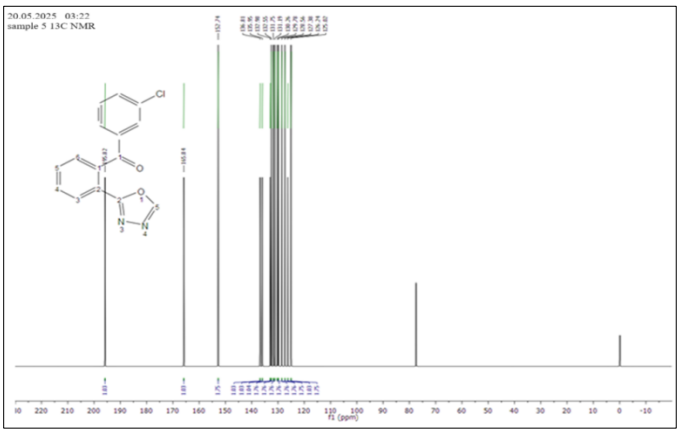


Figure 6: ^{13}C-NMR spectrum of compound D

3.3 Antibacterial Activity

The 1,3,4-oxadiazole derivatives synthesized were tested on the Gram-positive bacteria (Staphylococcus aureus and Micrococcus luteus) and Gram-negative bacteria (Escherichia coli and Klebsiella pneumoniae) on the agar diffusion technique. The compounds were used at the concentration level of 100, 500, and 1000 µg/mL and the standard drug was ciprofloxacin. Table 2 (Gram-negative bacteria) and table 3 (Gram-positive bacteria) show the results of the antibacterial activity. Compounds all had a concentration-dependent antibacterial effect, with the broader the concentrations used, the greater the zone of inhibition. Of all the tested derivatives, B (methyl-substituted) and D (chloro-substituted) were found

to be relatively more active as the antibacterial agents towards Gram-positive and Gram-negative bacterial strains. Specifically compound D showed similar inhibition zone with the standard drug against *Klebsiella pneumoniae* and *Escherichia coli* at higher concentrations.

Table 2: Antibacterial Activity of Synthesized 1,3,4-Oxadiazole Derivatives Against Gram-Negative Bacteria

Compound	*Klebsiella pneumoniae*			*Escherichia coli*		
	100 µg/mL	500 µg/mL	1000 µg/mL	100 µg/mL	500 µg/mL	1000 µg/mL
A	2	–	8	2	7	9
B	3	7	13	2	2	13
C	2	4	8	4	6	8
D	4	9	14	1	2	15
E	1	2	5	1	1	2
F	4	–	10	5	12	8
G	2	4	9	1	2	5
H	1	6	10	1	6	8
I	1	4	7	1	5	9
J	2	4	7	2	2	5
Ciprofloxacin	5	7	15	5	10	16

Table 3: Antibacterial Activity of Synthesized 1,3,4-Oxadiazole Derivatives Against Gram-Positive Bacteria

Compound	*Micrococcus luteus*			*Staphylococcus aureus*		
	100 µg/mL	500 µg/mL	1000 µg/mL	100 µg/mL	500 µg/mL	1000 µg/mL
A	2	5	7	2	5	9
B	5	10	13	2	5	12
C	3	7	10	2	5	10
D	4	8	13	3	7	13
E	1	1	2	1	1	2
F	4	7	10	1	2	4
G	1	2	3	1	2	4
H	2	5	7	2	5	7
I	1	5	8	1	5	6
J	2	2	3	2	5	8
Ciprofloxacin	5	9	18	6	9	15

3.4 Antioxidant Activity

The antioxidant capacity of the oxadiazole derivatives produced were determined by the DPPH free radical scavenging test. The samples were added in 10 µg/mL and 50 µg/mL concentration and the standard antioxidant was ascorbic acid.

Table 4: DPPH Free Radical Scavenging Activity (IC_{50} Values) of Synthesized 1,3,4-Oxadiazole Derivatives

S. No.	*Compound*	*IC_{50} at 10 µg/mL*	*IC_{50} at 50 µg/mL*
1	A	45.10	60.25
2	B	59.37	93.67
3	C	37.92	57.92
4	D	57.15	86.36
5	E	27.50	41.92
6	F	30.59	58.37
7	G	28.13	49.15
8	H	27.69	49.30
9	I	31.76	48.24
10	J	23.45	48.20
Standard	Ascorbic acid	65.24	99.37

The inhibition percentage and IC_{50} of the obtained compounds are presented in Table 4, whereas the relative antioxidant activity profile is shown in Figure 7. Free radical scavenging activity was detected in all the synthesized derivatives. The derivatives of some of them containing halogen substitutions displayed lower values of $IC_{50}$and this is indicative of high antioxidant activity.

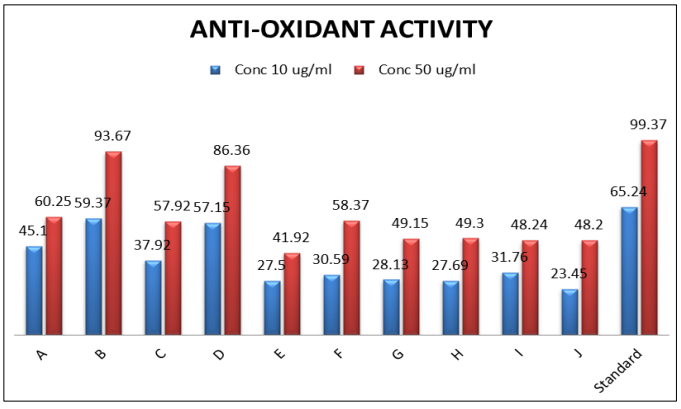


Figure 7: Comparative DPPH radical scavenging activity of synthesized compounds

3.5 Overall Interpretation

A combination of physical characterization, spectral analysis, antibacterial screening, and antioxidant analysis prove the successful production of the biologically active 1,3, 4-oxadiazole derivatives. The effects of substituent on the aromatic ring were also found to play a big role in the antibacterial and antioxidant action, and therefore the structure was important in terms of optimizing biological performance.

4. DISCUSSION

The current paper was aimed at the synthesis, structural analysis and biological tests of a set of substituted derivatives of 1,3,4-oxadiazole, in particular their antibacterial and antioxidation activity. The synthesis of all the target compounds was successfully achieved as indicated by good yields, sharp melting points and recurrent TLC profiles, indicating the presence of chemically stable yet relatively pure compounds. The nature and the size of the substituents bonded to the aromatic ring, mostly the massive size of the halogen atom, were the main factors that led to variation of the yield and melting points of the derivatives.

The formation of the oxadiazole structure was successful due to spectral characterization. The UV–visible spectra revealed the existence of conjugated aromatic systems whereas, FT-IR revealed typical absorption bands that define C=N, C=O, C-N functionalities, which are characteristic of 1,3,4-oxadiazole nucleus. The emergence of the halogen-specific stretching bands in the FT-IR spectra were also an additional confirmation of successful substitution. The ^{1}H and ^{13}C-NMR spectrum of selected compounds was a clear indicator of the proposed molecular structures, and the signals of the aromatic protons, carbonyl carbons, and heterocyclic carbons would be expected to give a signal in the corresponding regions of the chemical shift.

The antimicrobial testing of the synthesized derivatives indicated that there was moderate to high activity of the synthesized derivatives against Gram-positive and Gram-negative bacterial strains. Most of the compounds had a clear concentration-dependent rise in antibacterial action, which indicates effective contact between the produced molecules and cellular targets of bacteria. The most promising derivatives of the tested factors, compounds B (methyl-substituted) and D (chloro-substituted), always had a high-quality antibacterial effect in several different strains. The similar increased activity of these compounds could be explained by the optimal combination of electronic effects and lipophilicity which could help to penetrate through bacterial cell membranes. Conversely, derivatives with more bulky halogen substituents had a relatively lower antibacterial activity which could be because of steric hindrance and low membrane permeability.

The antioxidant ability determined by DPPH radical scavenging assay revealed that all the compounds synthesized had a quantifiable free radical scavenging ability. A number of halogen-substituted derivatives had lower IC_{50} values, indicating high antioxidant activity. This effect can be explained by the fact that these substituents can stabilize free radicals by means of the electron delocalization. Ascorbic acid had the highest antioxidant activity, though, certain of its synthetic counterparts demonstrated similar radical scavenging ability, which underlines their use as synthetic antioxidants.

In general, these biological findings indicate that structural alteration of the oxadiazole ring is an important factor that affects the antibacterial and antioxidant properties. The occurrence of the electron-donating and moderately electron-withdrawing substituents seems to be especially conducive to the improvement of the biological performance.

CONCLUSION

Finally, an array of 10 novel oxadiazole derivatives 1,3,4-oxadiazoles were successfully prepared and characterized systematically with the help of physicochemical and spectroscopic methods. The quality of the synthesized compounds was established via UV Visible, FT-IR and NMR tests that ensured the formation of the oxadiazole scaffold was successful.

It was shown by biological assessment that a number of the synthesized derivatives had excellent antibacterial properties against both Gram-positive and Gram-negative bacteria with compounds B and D being the most effective ones. The antioxidant investigations also disclosed that the products formed had good free radical scavenging capacity and some halogen derivatives of the products had lower IC_{50} values similar to the antioxidant used.

The overall findings point to the significance of substituent changes to control the biological activity of 1, 3,4-oxadiazole analogs. The results of the given research indicate that the chosen members of this series can be used as the possible lead molecules to be further optimized and developed into the new antimicrobial and antioxidant agents. Their therapeutic potential can be further clarified in future research including mechanism-based studies and sophisticated biological tests.

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CONFLICT OF INTEREST: Nil

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