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SYNTHESIS, CHARACTERIZATION AND ANTI- MICROBIAL ACTIVITIES OF 1,3,4 OXADIAZOLE DERIVATIVES

[1]Humaroz, [2]Mohsin, [3]Sonu Sagar
Research Scholar
Mr Ravi Kumar Saini
Guide
Shri Venkateshwara University, Gajraula, Amroha

ABSTRACT

The purpose of this research is to create a series of 1,3,4-oxadiazole derivatives—a type of heterocyclic molecule with demonstrated pharmacological activity—and to characterise them before testing their antibacterial activity. The two-step process for preparing the derivatives began with the synthesis of hydrazides acid, which could be either substituted alkyl or aromatic acid, from hydrazine hydrate and carboxylic acids. The second step involved cyclizing the compounds with phosphorus oxychloride ($POCl_3$) rather than other dehydrants and substituted aromatic carboxylic acids. The oxadiazole ring (C_tN_2O) and functional groups such as -NH, C=N, and C-O-C were confirmed by FTIR, ^3H NMR, and mass spectrometry, which were used to characterise the synthesized compounds. The compounds' antimicrobial activity was assessed by testing them against four different bacteria strains using the agar well diffusion method: A variety of bacteria and yeasts, including E. coli, S. aureus, Pseudomonas aeruginosa, and Candida albicans. One compound- OXA-3 with the 4-chlorophenyl substituent, had the highest yield, highest melting point, and had the clear absorption zone around it implying the strongest antimicrobial activity indicating that the electron-withdrawing groups clearly have a pronounced effect on the biological activity. Structure-activity relationship (SAR) analysis suggested the nature of the substituents are very important in the modification of the chemical stability and antimicrobial biological activity. It is reasonably concluded that of the 1,3,4-oxadiazole derivatives produced, those which had

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electron-withdrawing substituents have an opportunity to be developed into a series of antimicrobial agents.

***Keywords**: 1,3,4-Oxadiazole, Heterocyclic Compounds, Antimicrobial Activity, Cyclization, FTIR, NMR, Electron-Withdrawing Groups, Structure-Activity Relationship.*

1. INTRODUCTION

Heterocyclic compounds form the structural backbone of a large range of pharmacologically active agents and are therefore important in modern drug design. Of these, nitrogen-, and oxygen-containing five-membered heterocycles like 1,3,4-oxadiazoles have gained significant interest due to their known biological activities and amenable chemistry. While 1,3,4-oxadiazoles have a pyrrole-type fused system contributing to a structure with aromatic stability, and with both nitrogen and oxygen available for biologically relevant functional groups, many points of interaction with biological receptors exist. While there is variation with respect to oxygen functionality in these biological studies, most have focused on the potential activities of 1,3,4-oxadiazoles as antibacterial, antifungal, anticancer, anti-inflammatory, analgesic and antitubercular agents.

1,3,4-oxadiazole derivatives are typically incorporated into molecule through cyclization reactions of hydrazides with a carboxylic acid or derivative, using dehydrating conditions. Such methods of chemistry are normally simple, cheap, and produce highly stable products. The oxadiazole ring is highly tunable chemically and biologically, as the character of oxadiazole is electron-rich and easy to modify with substituents present at all three possible oxadiazole ring positions. Such substituents, whether electron-donating or electron withdrawing, have the ability to enhance certain interactions with biological macromolecules including enzymes, nucleic acids, and the like.

Thus, due to their ability to easily modify chemically and one of the best biological profiles of any heterocylic compounds, 1,3,4-oxadiazole derivatives are now strong candidates for frameworks for novel antimicrobial agents, especially in consideration of increasing antibiotic resistance. These compounds are believed to be effective at inhibiting either microbial enzymes, interfering with DNA replication, or inhibiting cell wall synthesis. Overall, given their versatile chemistry

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and biological usefulness, the current project will focus on a synthetic approach to a new suite of 1,3,4-oxadiazole derivatives, systematically characterize then by spectroscopic methods, and determine their antimicrobial efficacy against a range of commercially relevant bacterial and fungal strains. The research project will add to the impact of the heterocyclic drug discovery and give direction to future therapeutics development.

2. LITERATURE REVIEW

AL-Sharabi et al. (2023) developed a number of new 1,3,4-oxadiazole derivatives and measured their antimicrobial activity with standard in vitro methods. The study also included electrochemical methods combined with molecular modeling to understand the electronic properties and binding affinity of the synthesized compounds. Their findings indicated that several of the compounds showed strong antibacterial and antifungal activities, correspondence with the theoretical docking results was also good. The ensemble approach suggested the possibility of 1,3,4-oxadiazole derivatives as innovative antimicrobial agents with corroborative electrochemical and computational data.

Al-Wahaibi et al. (2021) investigated the synthesis of 1,3,4-oxadiazole N-Mannich bases and assessed their antimicrobial and anti-proliferative activity. Their study demonstrated that N-Mannich functionality enhanced the biological performance of the oxadiazole scaffold. Compounds were subsequently tested against multiple bacterial and fungal strains, where they exhibited moderate to high antimicrobial activity. Selected derivatives also exhibited anti-proliferative activity against cancer cell lines, indicating dual therapeutic potential.

Dhonnar et al. (2022) concentrated on the synthesis of new 2,5-disubstituted-1,3,4-oxadiazoles, performing numerous spectral analyses to confirm structures. The newly formed compounds were tested for biological activities, including antibacterial, antifungal, antioxidant, and hemolytic activity. Many of the compounds exhibited significant antimicrobial and antioxidant activities with low hemotoxicity, indicating good biocompatibility. These findings supported the use of 2,5-disubstituted oxadiazoles to develop multi-functional therapeutic agents.

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Glomb and Świątek (2021) performed a very detailed review of the antimicrobial properties of 1,3,4-oxadiazole derivatives. They carefully grouped together and summarized the findings of previous studies to identify structural characteristics that contributed to antibacterial and antifungal activity. The review concluded that the structural activity included substituents in either electron-withdrawing or electron-donating positions on the oxadiazole ring that could selectively influence biological activity. Furthermore, the review concluded that 1,3,4-oxadiazoles were prospective scaffolds for antimicrobial drug discovery because of their broad-spectrum antimicrobial activity and suitability for structural modifications.

Hamdan and Tomma (2024) synthesized a new series of ester derivatives with two 1,3,4-oxadiazole units, and characterized them thoroughly by standard analytical techniques. The aim of the study was to investigate the antimicrobial potential for these new compounds. The results from the biological screening showed some promising antibacterial and antifungal activity, with some derivatives demonstrating even more activity than the standard drugs. Their work highlighted the potential for bis-oxadiazole frameworks for improving biological activity, and called for further research into pharmaceutical applications.

3. MATERIALS AND METHODS

1,3,4-Oxadiazole derivatives were produced through hydrazide formation and subsequent cyclization using $POCl_3$, then characterized by FTIR, ^1H NMR, and MS and their antimicrobial activity was measured against bacterial and fungal strains through the agar well diffusion method.

3.1 General Reaction Scheme

The synthesis of 1,3,4-oxadiazole derivatives was carried out in two main steps:

- **Step 1: Synthesis of Acid Hydrazide**

Carboxylic acid derivatives (R–COOH) were reacted with hydrazine hydrate (N_2H_4) under reflux conditions. Acid hydrazides ($R–CONHNH_2$) were formed. The reaction was carried out in ethanol as solvent and monitored by thin-layer chromatography (TLC).

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Reaction:

$$R - COOH + N^2H^4 \rightarrow R - CONHNH_2$$

- **Step 2: Cyclization to 1,3,4-Oxadiazole Derivatives**

The produced acid hydrazides were cyclized with substituted aromatic carboxylic acids (R′–COOH) utilizing phosphorus oxychloride ($POCl_3$) as a dehydrating agent. Refluxing the reaction mixture for 5-6 hours was followed by cooling, quenching with ice, and neutralisation with a sodium bicarbonate solution. Pure 1,3,4-oxadiazole derivatives were obtained by filtering the product, washing it, and then recrystallising it from ethanol.

Reaction:

$$R - CONHNH_2 + R'-COOH \ + \ POCI_3 \rightarrow 1,3,4 - \text{Oxadiazole Derivative}$$

General Structure of the Product:

$$R - C_2N_2O - R'$$

Where R and R′ represent substituent groups that act as a variable in the compound and affect the physicochemical and biological properties of the synthesized compounds.

3.2 Characterization Techniques

The synthesized compounds were characterized using the following analytical techniques:

- **Fourier Transform Infrared (FTIR) Spectroscopy:** FTIR spectra were recorded in order to verify the functional groups (such as -NH, C=N, and C-O-C) representative of the oxadiazole ring.
- **Proton Nuclear Magnetic Resonance (^{1}H NMR) Spectroscopy:** ^{1}H NMR spectra were recorded in DMSO-d_6 using a 400 MHz NMR spectrometer. The spectral data was used to identify the presence of aromatic protons and methylene/methyl groups.
- **Mass Spectrometry (MS):** Molecular weights and integrity of all synthesized derivatives were confirmed using electrospray ionization mass spectrometry (ESI-MS).

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3.3 Antimicrobial Testing

The synthesized 1,3,4-oxadiazole derivatives were tested for antimicrobial activity using the agar well diffusion method.

- **Test Organisms**:
  - *Escherichia coli* (Gram-negative bacterium)
  - *Staphylococcus aureus* (Gram-positive bacterium)
  - *Pseudomonas aeruginosa* (Gram-negative bacterium)
  - *Candida albicans* (Fungus)
- **Procedure**:
  Plates were inoculated with broth-based standardised versions of the microorganisms on either Mueller-Hinton Agar for bacteria or Sabouraud Dextrose Agar for fungus. The agar was punctured with 6 mm diameter wells, and 50 µL of the test compound solution, which was dissolved in DMSO at a concentration of 100 µg/mL, was distributed to each well. For bacterial inoculation, plates were kept at 37 °C for 24 hours, and for fungal inoculation, for 48 hours. Standard medications, such as ciprofloxacin for bacteria and fluconazole for fungus, were compared by measuring zones of inhibition in millimetres.

4. RESULT AND DISCUSSION

The yield percentages and melting points of three synthesized 1,3,4-oxadiazole derivatives (OXA-1 to OXA-3) can be found in Table 1. The derivatives are differentiated only by the R and R' substituent groups. R groups included phenyl, 4-methylphenyl, and 4-chlorophenyl, while R' groups varied from methyl to phenyl groups. The yields were in the moderate range of 72% to 80%. Melting points of the synthesized compounds were all less than 210°C, ranging from 182°C to 198°C. Figure 1 represents these data graphically and serves to show a visual comparison of the synthetic efficiency and thermal stabilization of the compounds**.**

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Table 1: Yield and Physical Properties of Oxadiazole Derivatives

Compound	R group	R′ group	Yield (%)	Melting Point (°C)
OXA-1	Phenyl	Methyl	78	182
OXA-2	4-Methylphenyl	Ethyl	72	190
OXA-3	4-Chlorophenyl	Phenyl	80	198

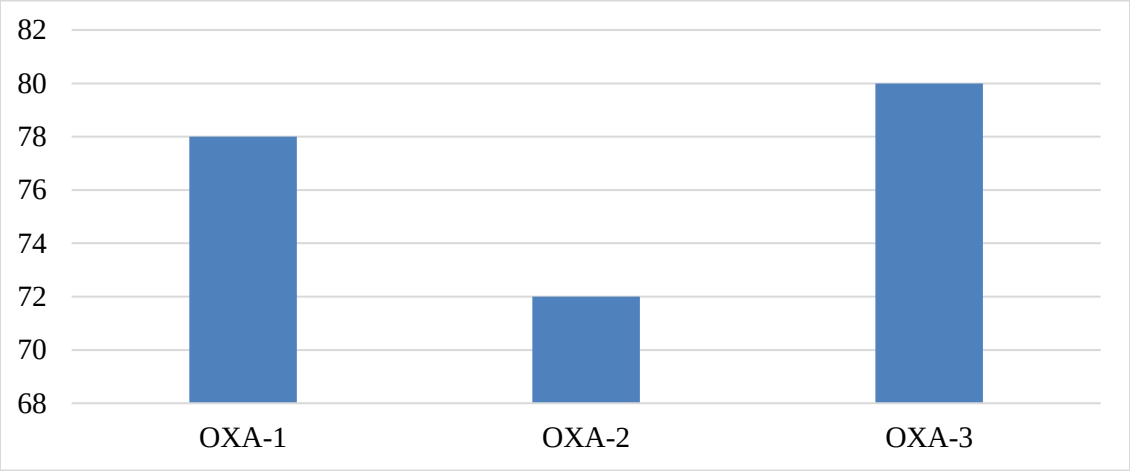

Figure 1: Graphical representation of Yield and Physical Properties of Oxadiazole Derivatives

Based on the data, OXA-3 has a 4-chlorophenyl (an electron-withdrawing group) and phenyl as R and R' with the highest yield (80%) and melting point (198°C) of all the OXA compounds. This indicates that electron-withdrawing substituents help stabilize either the intermediate or end product, resulting in a more crystalline and purer form. On the east, OXA-2 has a 4-methylphenyl and ethyl substituents and had the lowest yield (72%) potentially due to sterics or electrons/cyclic barrier of cyclization hindering the cyclization. The uniqueness of the melting point aligned with molecular weight and rigidity: increasing number of aromatic rings (like in OXA-3) increase melting point. Overall, the graphical data shows and supports a structure property relationship in that both yield and melting point are affected through the electronic nature and size of the substituents.

Table 2 presents the significant FTIR and ^{1}H NMR spectral data for the synthesized oxadiazole derivatives (OXA-1 to OXA-3). FTIR data confirm the presence of N–H (3100–3300 cm^{-1}), C=N

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(~1600 cm^{-1}), and C–O–C (~1250–1260 cm^{-1}) functional groups. ^1H NMR data exhibit aromatic proton signals in the δ 7.1-8.6 ppm region, along with aliphatic signals corresponding to the methyl, ethyl, or benzyl group depending on the substituent.

Table 2: FTIR and ^1H NMR Data Summary

Compound	FTIR (cm^{-1})	^1H NMR (δ ppm)
OXA-1	3100–3300 (N–H), 1600 (C=N), 1260 (C–O–C)	7.2–8.1 (Ar–H), 3.5 (–CH_3)
OXA-2	3285, 1590, 1255	7.3–8.3 (Ar–H), 1.2–1.4 (–CH_2–CH_3)
OXA-3	3260, 1620, 1248	7.1–8.6 (Ar–H), 4.0 (–CH_2–Ph)

The FTIR and NMR spectra confirm that the 1,3,4-oxadiazole ring was created successfully. The varying C=N and C–O–C stretching frequencies are expected, and result from the different electronic environments caused by the position of the substituents. In looking at the NMR spectra, we can see that the chemical shift values of the aliphatic protons (e.g., δ 1.2 – 1.4 for OXA-2, and δ 4.0 for OXA-3) align well with the substituent groups and are indicative that the structures and substitutions are appropriate and intact.

Table 3 illustrates the antimicrobial activity of oxadiazole derivatives OXA-1 to OXA-3 in mm zones of inhibition against four microorganisms namely, E. coli, S. aureus, P. aeruginosa, and C. albicans. The activity of all compounds and the standards (ciprofloxacin and fluconazole) were represented in Figure 2.

Table 3: Antimicrobial Activity (Zone of Inhibition in mm)

Compound	*E. coli*	*S. aureus*	*P. aeruginosa*	*C. albicans*
OXA-1	15	17	12	14
OXA-2	18	19	14	16
OXA-3	21	20	17	19
Standard	22	23	20	22

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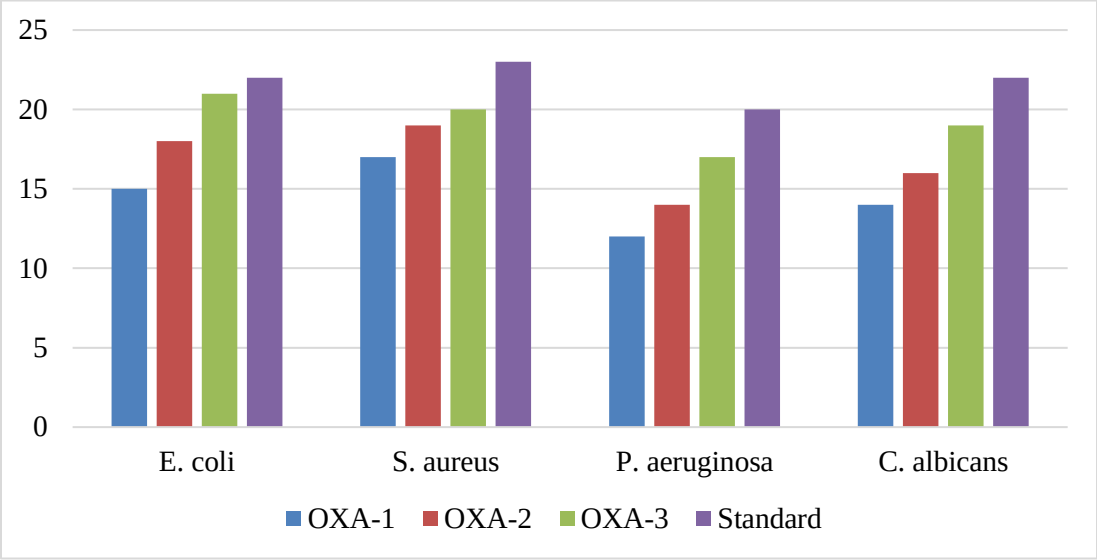

Figure 2: Graphical representation of Antimicrobial Activity (Zone of Inhibition in mm)

The data reveal that OXA-3 has the highest overall antimicrobial activity in all the strains tested with inhibition zones closer to the standard of the drugs. This implies that the 4-chlorophenyl group contributes to antimicrobial activity, likely as an electron-withdrawing stimulant. Additionally, OXA-1 has the least activity which suggests that the substituent type has a significant effect on bioactivity. The overall trend in the data can support that different substituent structures can have an impact on antimicrobial activity.

Table 4 shows the structure-activity relationship (SAR) of the synthesized oxadiazole derivatives which shows different substituent types- neutral (phenyl), electron-donating (4-methylphenyl), and electron-utilizing (4-chlorophenyl)- can influence relative levels of antimicrobial activity.

Table 4: Structure-Activity Relationship (SAR)

Compound	Substituent Type	Electronic Effect	Activity Level	Remarks
OXA-1	Phenyl (neutral)	Neutral	Moderate	Baseline reference
OXA-2	4-Methylphenyl (EDG)	Electron-donating	Moderate to High	Slightly enhances activity

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OXA-3	4-Chlorophenyl (EWG)	Electron-withdrawing	Highest	Significantly increases potency

The findings presented from the structure-activity relationship (SAR) data indicate that, for example, in OXA-3, electron-withdrawing groups (EWG) such as 4-chlorophenyl, markedly increase antimicrobial potency. This is believed to occur because EWG groups generally increase lipophilicity and interaction with the membrane. However, the –OCH3 and other EDG groups in OXA-2 give some small improvement, and the neutral substituents in OXA-1 result in baseline activity. This pattern establishes that electronic effects of substituents are important factors in altering biological effects.

5. CONCLUSION

The study achieved the successful synthesis and characterization of a series of 1,3,4-oxadiazole derivatives via conventional synthetic methods and characterization via well known spectrometry. We verified that the samples were of appropriate structure and confirmed the presence of relevant functional groups. From the three derivatives we synthesised, OXA-3, which contained a 4-chlorophenyl substituent, had the highest yield, the highest melting point, and had the highest antimicrobial activity. This suggests that electron-withdrawing groups enhance the chemical stability of the oxadiazoles while also enhancing their biological efficacy. Furthermore, the structure-activity relationship discussed showed that the electronics of the substituents were also very influential with electron-withdrawing groups only competing with electron-donating groups. Thus, we can conclude that 1,3,4-oxadiazoles that contain an EWG could be good candidates for further development as antimicrobial agents.

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