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Synthesis and Antimicrobial Screening of Some Derivatives of Novel 2-Substituted-5-Phenyl-1,3,4-Oxadiazole Derivatives

Valarmathy Joshua*[1], L. Samuel Joshua[2], Sherlyn Joshua[1] and Vinolyn Joshua[1]

[1] *Medicinal Research Laboratory, Department of Pharmaceutical Chemistry, Sri Venkateswaraa Faculty of Pharmacy, Sri Venkateswaraa University, Ettayapuram -628902., Tamil Nadu, India*
[2] *UV Gullas College of Medicine, 6014 Mandaue City, Cebu, Philippines*

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Corresponding Author: **Valarmathy Joshua**
E-Mail: **valarmathyjoshua@gmail.com**

ABSTRACT

1,3,4-Oxadiazole derivatives constitute an important class of nitrogen- and oxygen-containing heterocyclic compounds that have attracted considerable attention owing to their broad spectrum of biological activities and pharmaceutical relevance. In the present investigation, a series of novel 2-substituted-5-phenyl-1,3,4-oxadiazole derivatives were successfully synthesized through an efficient synthetic route and obtained in satisfactory to excellent yields. The structures of the synthesized compounds were established and confirmed using spectroscopic techniques, including Fourier-transform infrared (FT-IR) spectroscopy, proton nuclear magnetic resonance (^1H NMR) spectroscopy, and gas chromatography–mass spectrometry (GC–MS). The antimicrobial potential of the synthesized derivatives was evaluated against selected Gram-positive and Gram-negative bacterial strains as well as pathogenic fungal species. Several compounds exhibited noteworthy antimicrobial activity, indicating that structural modification of the oxadiazole scaffold can significantly influence biological efficacy. The findings suggest that these newly synthesized oxadiazole derivatives may serve as promising lead molecules for the development of novel antimicrobial agents and warrant further pharmacological investigation.

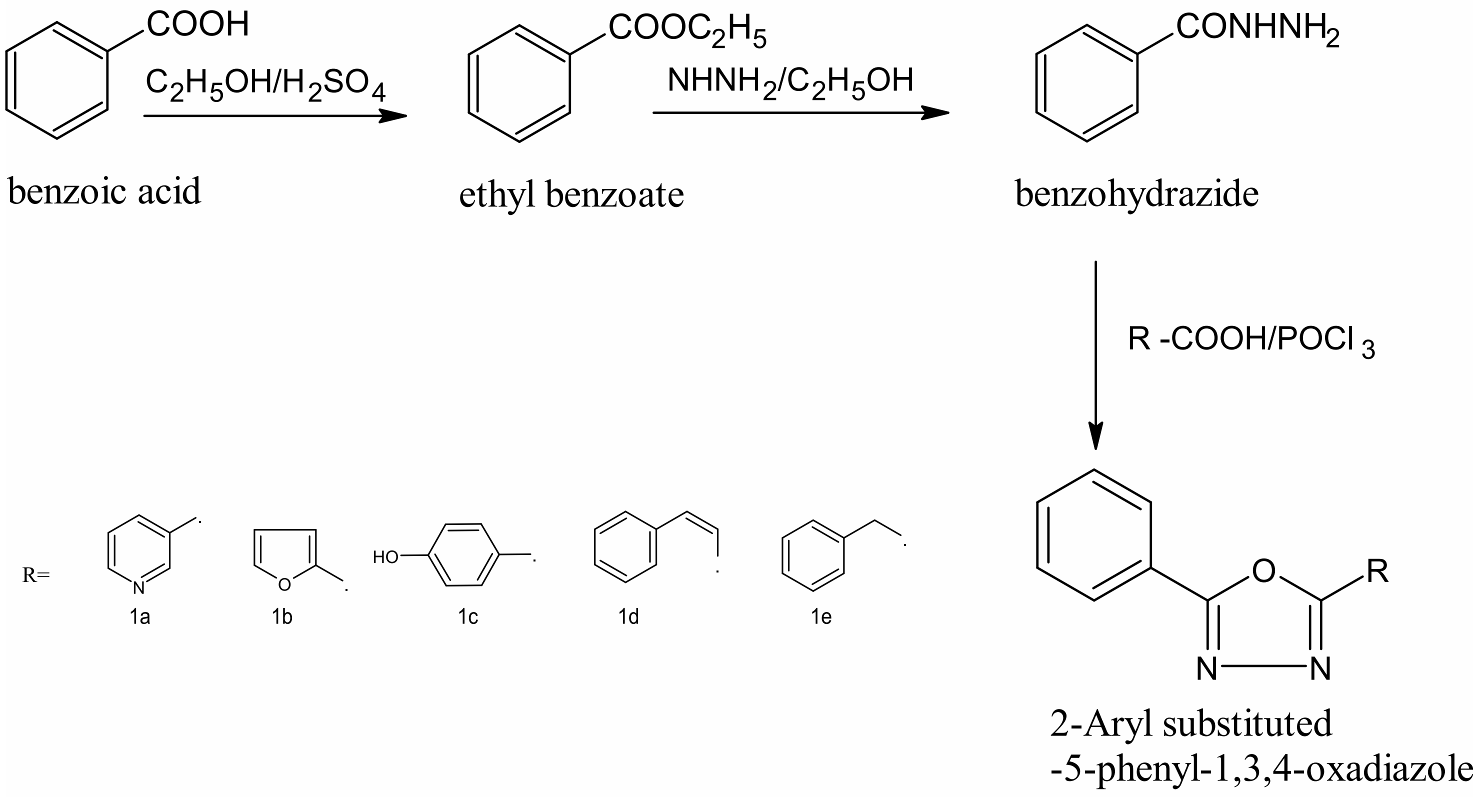
Keywords: *1,3,4-Oxadiazole, heterocyclic compounds, antimicrobial activity, antibacterial activity, antifungal activity, spectral characterization.*

1. Introduction

Heterocyclic compounds occupy a central role in medicinal chemistry and pharmaceutical sciences, forming the structural basis of countless biologically active molecules. The incorporation of heteroatoms such as nitrogen, oxygen, or sulfur into cyclic frameworks imparts unique chemical reactivity and biological properties, making these scaffolds indispensable in the design of therapeutic agents. Their versatility allows them to interact with diverse biological targets, and they are frequently employed in the development of agents with applications ranging from anti-infective to anti-inflammatory therapies. Within this broad class, oxadiazoles represent an especially important group of heterocycles [1]. Structurally, oxadiazoles are five-membered rings containing one oxygen atom and two nitrogen atoms. Their derivatives have been widely investigated and reported to exhibit a remarkable spectrum of pharmacological activities, including antibacterial, antifungal, anti-inflammatory, anticancer, anticonvulsant, and insecticidal properties [2-3]**.** Based on the arrangement of heteroatoms, oxadiazoles are classified into four distinct types: 1,2,3-oxadiazoles, 1,2,4-oxadiazoles, 1,2,5-oxadiazoles, and 1,3,4-oxadiazoles.

Among these, the 1,3,4-oxadiazole nucleus has emerged as particularly significant due to its broad therapeutic potential and adaptability in drug design. The importance of oxadiazoles is further reinforced by extensive literature, which highlights their role in multiple pharmacological domains. Reported activities include antimicrobial[1-4], anticonvulsant[5], anti-tubercular[6], anti-inflammatory[7], antileprotic[8] and analgesic[9] effects [4-6]. Their ability to act on diverse biological pathways underscores their relevance in modern medicinal chemistry and justifies continued exploration of novel derivatives, the present study was undertaken to synthesize a series of 2-substituted-5-phenyl -1,3,4-oxadiazole derivatives**.** The synthesized compounds were structurally characterized using IR, ^{1}H NMR, and GC-MS analyses, ensuring accurate confirmation of their chemical identity. Following characterization, the compounds were subjected to antimicrobial screening against selected bacterial and fungal strains to evaluate their therapeutic potential. The findings of this work contribute to the growing body of evidence supporting oxadiazoles as promising scaffolds for the development of new antimicrobial agents.

Scheme 1:



2. Experimental Work

2.1 Materials and Methods

All chemicals, reagents, and solvents used in the present study were of analytical grade and were utilized without further purification unless otherwise specified. Prior to use, all glassware and laboratory apparatus were thoroughly cleaned, dried, and maintained under appropriate experimental conditions. The melting points of the synthesized compounds were determined using the open capillary tube method and are reported uncorrected. The purity of the synthesized compounds was monitored by thin-layer chromatography (TLC) performed on silica gel G-coated glass plates. A benzene (9:1, v/v) solvent system was employed as the mobile phase, and the chromatograms were visualized using iodine vapour. The crude products obtained after synthesis were purified by recrystallization using suitable solvents, and their purity was further confirmed by consistent melting point measurements. Infrared (IR) spectra were recorded on a PerkinElmer IR 283 aspectrophotometer using KBr pellet methodology, and absorption frequencies are reported in cm^{-1}. Proton nuclear magnetic resonance (^1H NMR) spectra were obtained using a Bruker 200 MHz spectrophotometer with $CDCl_3$ as the solvent and tetramethylsilane (TMS) as the internal reference standard. Chemical shifts are expressed as δ values in parts per million (ppm). Mass spectral analyses were carried out using a Shimadzu GC–MS instrument under standard operating conditions to confirm the molecular weights and structural characteristics of the synthesized compounds.

The antimicrobial activity of the synthesized derivatives was evaluated against selected Gram-positive and Gram-negative bacterial strains, as well as a representative fungal strain. Antimicrobial efficacy was assessed using the zone of inhibition method and minimum inhibitory concentration (MIC) determination, providing a comprehensive evaluation of the antibacterial and antifungal potential of the synthesized compounds.

2.2. Synthesis of Ethyl Benzoate

Ethyl benzoate was synthesized through the esterification of benzoic acid with ethanol. Benzoic acid (0.01 mol) was placed in a clean round-bottom flask, and absolute ethanol (25 mL) was added. A few drops of concentrated sulfuric acid were carefully introduced as a catalyst, and the reaction mixture was refluxed for 3 h with continuous stirring. After completion of the reaction, the mixture was poured into distilled water and allowed to stand overnight. The product was extracted with diethyl ether (50 mL), and the organic layer was separated and concentrated under reduced pressure. The crude ester obtained was filtered, washed with cold water to remove residual impurities, and recrystallized from ethanol to afford pure ethyl benzoate as a white crystalline solid.

2.3. Synthesis of Benzohydrazide

Benzohydrazide was prepared by the reaction of ethyl benzoate with hydrazine hydrate. Ethyl benzoate (1.5 g, 0.01 mol) and hydrazine hydrate (0.32 g, 0.01 mol) were dissolved in dry ethanol (20 mL) and refluxed for 5 h. Upon completion of the reaction, the mixture was cooled and maintained in an ice bath overnight to facilitate crystallization. The reaction mixture was then treated with 10% sodium bicarbonate solution and poured onto crushed ice (100 g). After standing for approximately 10 min, the precipitated solid was collected by filtration, washed thoroughly with cold water, and recrystallized from ethanol to obtain pure benzohydrazide as a white crystalline product.

2.4. Synthesis of 2-Substituted-5-Phenyl-1,3,4-Oxadiazole Derivatives (1a–e)

A series of 2-substituted-5-phenyl-1,3,4-oxadiazole derivatives were synthesized via cyclodehydration of benzohydrazide with various aromatic carboxylic acids. Benzohydrazide (1.37 g, 0.01 mol) and the appropriate substituted aromatic acid (0.01 mol) were dissolved in phosphorus oxychloride ($POCl_3$, 50 mL) and refluxed on a water bath for 6 h.

After completion of the reaction, the mixture was cooled to room temperature and carefully poured into crushed ice-cold water. The reaction mixture was neutralized using aqueous sodium bicarbonate solution until effervescence ceased. The resulting precipitate was filtered, washed with water, dried, and recrystallized from ethanol to yield the corresponding 2-substituted-5-phenyl-1,3,4-oxadiazole derivatives (1a–e) in good yields.

2.5. Physical and Spectral Characterization of Representative Compound

3-(5-Phenyl-1,3,4-oxadiazol-2-yl)pyridine (1a)

Yield: 81%

Molecular Formula: $C_{13}H_9N_3O$

Molecular Weight: 223.23 g/mol

Elemental Composition: C, 69.95%; H, 4.06%; N, 18.82%; O, 7.17%.

FT-IR (KBr, νmax cm^{-1}): 3100.20 (aromatic C–H stretching), 1660.41 (C=N stretching), 1600.62 and 1560.46 (aromatic C=C stretching), 1440 (C–N stretching), 1255.50 (C–O–C stretching).

^1H NMR ($CDCl_3$, δ ppm): 7.19–8.45 (multiplet, 5H, aromatic protons), 8.66–8.93 (multiplet, 4H, heteroaromatic pyridyl protons).

GC–MS (m/z): 223 [M^+], corresponding to the molecular ion peak of the target compound.

The spectral data obtained were in good agreement with the proposed molecular structure, confirming the successful synthesis of the desired 1,3,4-oxadiazole derivative.

2-(furan-2-yl)-5-phenyl-1,3,4-oxadiazole 1b: Yield: 88, Molecular Formula: $C_{12}H_8N_2O_2$
Molecular Weight: 212.20 g/mol, Elemental Composition: C (67.92%), H (3.79%), N (13.20%), O (15.09%).IR (KBr, νmax cm^{-1}): 3100 (Aromatic C–H stretching),1665 (C=N stretching),1600 (Aromatic C=C stretching),1250 (C–O–C stretching),1440 (C–N stretching) 1020 (C–O stretching).^1H NMR ($CDCl_3$, δ ppm):6.40–6.60 (m, 2H, Ar-H),7.10–7.60 (m, 5H, Ar-H).MS (m/z): 212

4-(5-phenyl-1,3,4-oxadiazol-2-yl) phenol 1C: Yield: 84%, Molecular Formula: $C_{14}H_{10}N_2O_2$
Molecular Weight: 238.24 g/mol, Elemental Composition: C (70.58%), H (4.23%), N (11.76%), O (13.43%).IR (KBr, νmax cm^{-1})3400 (O–H stretching)3090 (Aromatic C–H stretching)1669 (C=N stretching)1590 (Aromatic C=C stretching)1250 (C–O–C stretching),1432 (C–N stretching).^1H NMR ($CDCl_3$, δ ppm)6.80–7.20 (m, 4H, Ar-H),7.30–7.70 (m, 5H, Ar-H),9.80(s, 1H, phenolic –OH).MS (m/z):238.

2-phenyl-5-[(Z)-2-phenylethenyl]-1,3,4-oxadiazole 1d: Yield:70 %Molecular Formula: $C_{16}H_{12}N_2O$,Molecular Weight: 248.28 g/mol,Elemental Composition: C (77.41%), H (4.87%), N (11.28%), O (6.44%).IR (KBr, νmax cm^{-1}):3089 (Aromatic C–H stretching),2806 (–CH=CH– stretching (vinyl group),1667 (C=N stretching),1239 (C–O–C stretching).^1H NMR ($CDCl_3$, δ ppm):4.82–5.12 (d, 2H, –CH=CH–),7.19–8.45 (m, 9H, Ar-H).MS (m/z): 248

2-benzyl-5-phenyl-1,3,4-oxadiazole 1e:Yield: Molecular Formula: $C_{15}H_{12}N_2O$,Molecular Weight: 236.27 g/mol,Elemental Composition: C (76.26%), H (5.12%), N (11.86%), O (6.76%).IR (KBr, νmax cm^{-1}):3095 (Aromatic C–H stretching),1668 (C=N stretching),1248(C–O–C stretching).^1H NMR ($CDCl_3$, δ ppm):7.21–7.60 (m, 10H, Ar-H),4.52 (s, 2H, –CH_2– of benzyl).MS (m/z): 236

2.6. Antimicrobial Screening

A compound is considered to possess antibacterial **or** antifungal activity when it effectively inhibits the growth, proliferation, or metabolic activity of bacterial or fungal microorganisms, respectively. Such compounds may act by interfering with essential cellular processes, thereby preventing microbial multiplication. Agents that primarily suppress microbial growth without causing cell death are classified as bacteriostatic **or** fungistatic. However, the distinction between static and cidal activity is often influenced by factors such as drug concentration, exposure time, microbial species, and environmental conditions. Consequently, compounds that exhibit bactericidal or fungicidal effects under certain conditions may demonstrate only bacteriostatic or fungistatic activity under others. The antimicrobial activity depends on several factors, including inoculum size, metabolic state of the organisms, pH, temperature, duration of exposure, concentration of the inhibitor, and presence of interfering substances. The modified Kirby–Bauer method was employed to evaluate the sensitivity of the synthesized compounds against selected organisms. The diameters of the zones of inhibition were compared with those produced by standard drugs.

For MIC determination, graded concentrations of the test compounds were prepared by serial dilution and incorporated into appropriate agar media. Three bacterial strains (*Staphylococcus aureus, Pseudomonas aeruginosa,* and *Escherichia coli*) and one fungal strain (*Candida albicans*) were used as test organisms. Mueller–Hinton agar was used for bacterial strains, while Sabouraud's dextrose agar was used for fungal strains. Ampicillin (30 µg) and Griseofulvin (10 µg) served as standard drugs. All solutions were prepared under aseptic conditions.

Table 1: Data of Minimum Inhibitory Concentration (MIC) for Antibacterial activity

Compound No:	*Bacillus subtilis*						*Staphylococcus aureus*						*Escherichia coli*						*Klebsiella species*					
	Concentration (µg/ml)						Concentration (µg/ml)						Concentration (µg/ml)						Concentration (µg/ml)					
	1000	500	250	125	62.5	31.25	1000	500	250	125	62.5	31.25	1000	500	250	125	62.5	31.25	1000	500	250	125	62.5	31.25
1a	–	–	–	+	+	+	–	–	–	+	+	+	–	+	+	+	+	+	–	+	+	+	+	+
1b	–	–	–	–	+	+	–	–	–	–	+	+	–	–	+	+	+	+	–	–	–	+	+	+
1c	–	+	+	+	+	+	–	–	–	–	–	+	–	+	+	+	+	+	–	+	+	+	+	+
1d	–	–	–	+	+	+	–	–	–	–	+	+	–	–	+	+	+	+	–	–	–	+	+	+
1e	–	–	–	–	+	+	–	–	–	–	+	+	–	–	+	+	+	+	–	–	–	+	+	+
+ve control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
-ve control	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+
Ampicillin	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

(+) indicates presence of growth. (-) indicates absence of growth

Table 2: Data of Minimum Inhibitory Concentration for Antifungal Activity

Compound No.	*Candida albicans*					
	Concentration (μg/ ml)					
	1000	500	250	125	62.5	31.25
1a	-	+	+	+	+	+
1b	-	+	+	+	+	+
1c	+	+	+	+	+	+
1d	+	+	+	+	+	+
1e	-	+	+	+	+	+
+ve control	+	+	+	+	+	+
-ve control	-	-	-	-	-	-
Griesofulvin	-	-	-	-	-	-

(+) indicates the presence of growth. (-) indicates absence of growth

Compounds 1a, 1b, and 1e showed moderate antifungal activity against *Candida albicans* at higher concentrations (1000 μg/ml). Compounds 1c and 1d were inactive against *Candida albicans*, but 1c displayed notable antibacterial activity against *Staphylococcus aureus* at a low MIC (31.25 mg/ml).1b and 1e demonstrated consistent antibacterial activity across multiple strains at MIC values between 62.5–250 mg/ml. Ampicillin showed no inhibition under the tested conditions, while Griseofulvin was highly effective against fungal strains.

3. Results and Discussion

Synthesis and Characterization

A series of 2-substituted-5-phenyl-1,3,4-oxadiazole derivatives (1a–1e) were synthesized in good yields (67–88%) via cyclization of benzohydrazide with various aromatic acids in $POCl_3$. The reactions proceeded smoothly, affording crystalline products after recrystallization. The compounds were characterized by melting point determination, IR, ^1H NMR, and mass spectrometry.

The IR spectra confirmed the presence of characteristic functional groups: C=N stretching (1660–1670 cm^{-1}) validating the oxadiazole nucleus; C–O–C stretching (1240–1255 cm^{-1}) indicating the heterocyclic linkage. Aromatic C–H stretching (~3090–3100 cm^{-1}) consistent across all derivatives. Additional absorptions (e.g., O–H at 3400 cm^{-1} in 1c, vinyl –CH=CH– at 2806 cm^{-1} in 1d, and furan C–O at 1020 cm^{-1} in 1b) confirmed the nature of substituents.

^1H NMR spectra revealed multiplets in the aromatic region (δ 7.0–8.5 ppm), consistent with phenyl and heteroaryl protons. Distinct signals such as δ 9.8 ppm (phenolic –OH in 1c), δ 4.82–5.12 ppm (vinyl protons in 1d), and δ 4.52 ppm (benzyl $-CH_2-$ in 1e) further validated structural assignments. Mass spectra showed molecular ion peaks corresponding to the expected molecular weights (m/z 212–248), confirming purity and identity.

Antimicrobial Activity

The synthesized compounds were screened against Gram-positive (*Bacillus subtilis, Staphylococcus aureus*) and Gram-negative (*Escherichia coli, Klebsiella species)* bacteria, as well as the fungal strain *Candida albicans*. Compound 1c exhibited the strongest antibacterial activity, particularly against *Staphylococcus aureus* (MIC 31.25 mg/ml), suggesting the phenolic substituent enhances activity. Compounds 1b and 1e showed consistent inhibition across multiple bacterial strains (MIC 62.5–250 mg/ml), indicating that furan and benzyl groups contribute to broad-spectrum activity. Compound 1a displayed moderate antibacterial activity (MIC 125–250 mg/ml), while 1d was less potent but still active against *E. coli and Klebsiella*. Against *Candida albicans*, 1a, 1b, and 1e showed weak activity at high concentrations (1000 μg/ml), whereas 1c and 1d were inactive. Griseofulvin served as the positive control and completely inhibited fungal growth at all tested concentrations.

Discussion

The results highlight the influence of substituents on antimicrobial potency: The phenolic group (1c) significantly improved antibacterial activity, likely due to enhanced hydrogen bonding and cell wall penetration. The furan ring (1b) and benzyl group (1e) contributed to moderate, broad-spectrum antibacterial effects. The pyridyl derivative (1a) showed balanced but less pronounced activity, while the vinyl-phenyl derivative (1d) was comparatively weaker. Antifungal activity was generally poor, suggesting that further structural modification is required to enhance efficacy against fungal strains [7-15], the study demonstrates that 1,3,4-oxadiazole derivatives are promising scaffolds for antibacterial drug development, with compound 1c emerging as the most potent candidate against *Staphylococcus aureus*.

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References

1. Dhall, E., Sain, S., Jain, S., & Dwivedi, J. (2018). Synthesis of triazole derivatives manifesting antimicrobial and anti-tubercular activities. *Mini-Reviews in Organic Chemistry*, *15*(4), 291-314.
2. Ayyash, A. N. (2020). Synthesis and Antimicrobial Studies of New [Tetrakis (1, 2, 4-Triazole/1, 3, 4-Oxadiazole/1, 3, 4-Thiadiazole][Bis-(Benzene-1, 3, 5-Triyl)] Dioxymethylene Compounds. *Iraqi Journal of Science*, 234-245.
3. Ayyash, A. N., Juwair, K. A., Najeeb, O. G., & Jasim, N. M. (2021). Synthesis of New Bioactive Compounds of Pyrazino [2, 3-e][1, 3] Oxazepines and Pyrazino [2, 3-e][1, 3] Diazepines Bearing 1, 3, 4-Oxadiazole Moieties. *Egyptian Journal of Chemistry*, *64*(6), 2945-2952.
4. Şahin, G., Palaska, E., Ekizoğlu, M., & Özalp, M. (2002). Synthesis and antimicrobial activity of some 1, 3, 4-oxadiazole derivatives. *Il Farmaco*, *57*(7), 539-542.
5. Bakht, M. A., Yar, M. S., Abdel-Hamid, S. G., Al Qasoumi, S. I., & Samad, A. (2010). Molecular properties prediction, synthesis and antimicrobial activity of some newer oxadiazole derivatives. *European journal of medicinal chemistry*, *45*(12), 5862-5869.
6. Islam, M., Siddiqui, A. A., Rajesh, R., Bakht, A., & Goyal, S. (2008). Synthesis and antimicrobial activity of some novel oxadiazole derivatives. *Acta Pol Pharm*, *65*, 441-447.
7. Sadek, B., & Fahelelbom, K. M. S. (2011). Synthesis, characterization, and antimicrobial evaluation of oxadiazole congeners. *Molecules*, *16*(6), 4339-4347.
8. Zhu, L., Zeng, H., Liu, D., Fu, Y., Wu, Q., Song, B., & Gan, X. (2020). Design, synthesis, and biological activity of novel 1, 2, 4-oxadiazole derivatives. *BMC chemistry*, *14*(1), 68.
9. Ghelani, S. M., Khunt, H. R., & Naliapara, Y. T. (2017). Design, synthesis, characterization, and antimicrobial screening of novel indazole bearing oxadiazole derivatives. *Journal of Heterocyclic Chemistry*, *54*(1), 65-70.
10. Jha, K. K., Samad, A., Kumar, Y., Shaharyar, M., Khosa, R. L., Jain, J., ... & Singh, P. (2010). Design, synthesis and biological evaluation of 1, 3, 4-oxadiazole derivatives. *European Journal of Medicinal Chemistry*, *45*(11), 4963-4967.
11. Kaplancikli, Z. A. (2011). Synthesis of some oxadiazole derivatives as new anticandidal agents. *Molecules*, *16*(9), 7662-7671.

12. Bala, S., Kamboj, S., Kajal, A., Saini, V., & Prasad, D. N. (2014). 1, 3, 4-oxadiazole derivatives: synthesis, characterization, antimicrobial potential, and computational studies. *BioMed research international*, *2014*(1), 172791.
13. Kaplancikli, Z. A., Altintop, M. D., Turan-Zitouni, G., Ozdemir, A., Ozic, R., & Akalın, G. (2012). Synthesis, antimicrobial activity and cytotoxicity of novel oxadiazole derivatives. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *27*(1), 51-57.
14. Jafari, E., Mohammadi, T., Jahanian-Najafabadi, A., & Hassanzadeh, F. (2017). Synthesis and antimicrobial evaluation of some 2, 5 disubstituted 1, 3, 4-oxadiazole derivatives. *Research in pharmaceutical sciences*, *12*(4), 330-336.
15. Li, P., Hu, D., Xie, D., Chen, J., Jin, L., & Song, B. (2018). Design, synthesis, and evaluation of new sulfone derivatives containing a 1, 3, 4-oxadiazole moiety as active antibacterial agents. *Journal of agricultural and food chemistry*, *66*(12), 3093-3100.