

# In Vitro Evaluation of Antibacterial and Antifungal Properties of Some New 1, 3, 4-Oxadiazole Derivatives Containing Phenyl Group

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## ABSTRACT

**Aims:** Antibiotic resistance is recognized as one of the most challenging public health problems in the world. The need for new antibacterial and antifungal drugs is justified because many pathogens are currently resistant to available drugs. Several components of 1, 3, 4-oxadiazoles have been shown to pose a wide range of antibacterial activities.

**Materials & Methods:** The new derivatives of 1, 3, 4-oxadiazole were synthesized using a single-stage method. The structure of derivatives was evaluated by IR, H-NMR, C-NMR, and GC-Mass methods. Then to measure the antibacterial and antifungal activities of the prepared derivatives at a concentration of 0.5 mg/mL, agar well diffusion method was employed, and the minimum inhibitory concentration (MIC) and the minimum bactericidal/fungicidal concentration (MBC/MFC) were determined with three replications.

**Findings:** The study of antibacterial properties of the prepared derivatives showed the highest activity of the compounds 4b-g against *Enterococcus faecalis* strains, among which the compound 4g with  $IZ = 55.66 \pm 0.5$  mm and  $MIC = 31.25$  mg/mL had the greatest effect compared to the others. Also, the compound 4f with  $MIC = 125$  mg/mL had a powerful effect against *E. faecalis* strains. In the case of fungal samples, the highest activity of the compound 4b was with  $IZ = 12.33 \pm 0.5$  mm against *Candida glabrata* and with  $IZ = 13.33 \pm 0.5$  mm against *C. krusei* strains.

**Conclusion:** The new 1, 3, 4-oxadiazole derivatives (4b, 4d, and 4g) with tolyl, dimethylphenyl, and methoxyphenyl groups were shown to be a promising compounds for pharmaceutical applications so that by adding other functional groups to their structure, it is possible to increase the destructive power of these compounds.

**Keywords:** Oxadiazole, *Enterococcus faecalis*, Drug resistance.

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## Introduction

The trend of drug resistance in bacteria and fungi has been growing in recent decades, and the first case of drug resistance was reported in 1947 [1]. Antibiotic resistance is a type of drug resistance in which disease-causing microorganisms (bacteria, fungi, etc.) do not respond to antibiotic treatment [2]. The *Streptococcaceae* family members, especially *S. mutans* species which is a Gram-positive bacterium in the mouth, create an acidic environment by metabolizing various carbohydrates; Extracellular glucan formation is the main cause of pathogenicity of this bacterium and the cause of dental caries in humans and animals [3]. Nowadays, there are many reports about the relative resistance of normal oral microflora, especially streptococci, to biocides and antibiotics like beta lactam, penicillin, erythromycin, macrolides, and tetracycline [4]. The *Enterococcus* family members, including *E. faecalis* which is a Gram-positive bacterium, have been identified as major causative agents of infections, especially nosocomial infections, and the epidemiology of enterococci infections has received considerable attention. These bacteria are known as the fourth leading cause of nosocomial infections, the third leading cause of bacteremia infections, and the second leading cause of urinary tract infections [5]. Various studies have shown antibiotic resistance of this bacterial species [6]. *Proteus* bacilli are also optional Gram-negative bacilli belonging to the group of *Proteobacteria* and aerobic anaerobes [7]. *Proteus* in humans sometimes causes urinary tract and wound infections. Today, there are numerous reports about the resistance of this group of bacteria to ampicillin and cephalosporins [8]. *Neisseria meningitidis* or *meningococcus* is a bacterium causing meningitis. This bacterium could also cause meningococemia (infection of the blood). *Meningococcus* is one of the leading

causes of death in children in developed countries, sometime causing epidemics in Asia and Africa. Rifampin is the drug of choice for the treatment of infections caused by this bacterium, but its use may be harmful [9] because there are several reports about antibiotic resistance after treatment [10]. *Candida* yeasts are microorganisms that are widely distributed and naturally coexist in humans. The prevalence of candida infections, especially in patients with impaired immune systems, has been significant and increasingly challenging over the past three decades [11]. Different strains of *Candida* are known to cause infections. Recent epidemiological studies have shown that drug-resistant species of *Candida*, like *C. glabrata*, *C. krusei*, *C. tropicalis*, and *C. parapsilosis*, are gradually replaced *C. albicans* in diseases [12]. Due to the spread of fungal infections and the increase in the use of antifungal drugs such as azoles, a significant increase in the resistance of *Candida* species to antifungal compounds is expected [13]. 1, 3, 4-oxadiazole compounds are one of the 4 subsets of oxadiazoles. A ring contains one oxygen atom and two nitrogen atoms in a five-membered ring. Among the ring compounds, 1, 3, 4-oxadiazole is a main component suitable for the production of new drugs [14]. Various 1, 3, 4-oxadiazole derivatives have been shown to have antimicrobial [15], antifungal [16], anti-cancer [17], and etc. [18] properties so far. In our previous study, some of the structures of these compounds were synthesized and shown to have desirable antibacterial properties against Gram-positive samples [19]. In addition, due to the high reactivity of the main ring containing different functional groups, different functional groups could be synthesized, and their biological properties could be investigated [20].

**Objectives:** As the synthesis and evaluation of antibacterial and antifungal activities of the new 1, 3, 4-oxadiazole derivatives were

an important part of this research program [21]; herein, antibacterial and antifungal activities of 1, 3, 4-oxadiazole derivatives containing tolyl, phenyl, dimethylphenyl, aniline, naphthalene, and methoxyphenyl groups were evaluated against *S. mutans* PTCC1683, *E. faecalis* PTCC1778, *P. vulgaris* PTCC1861, *N. meningitidis* PTCC1507, *C. glabrata* PTCC5297, and *C. krusei* PTCC5295. All organic solvents used in this study were new and different from those used in other studies; the one-step method of synthesizing new derivatives was different too.

### Materials and Methods

This research was conducted in the microbiology laboratory of Islamic Azad University, Tehran branch in 2020. Starting materials, solvents, and culture environments (Nutrient agar/broth, Sabouraud dextrose agar/broth) were obtained from Merck, Germany and used moving forward without any more filtration. The infrared spectrum was measured by a Shimadzu IR-460 spectrometer. Nuclear magnetic resonance (NMR) spectrum was obtained by a Bruker DRX-300 AVANCE spectrometer ( $^1\text{H}$  NMR at 300 Hz,  $^{13}\text{C}$  NMR at 75 Hz) in acetone. Chromatography columns were prepared using silica gel powder (Merck, Germany). Gas Chromatography–Mass Spectrometry spectra were obtained by Shimadzu triple-quad GCMS-TQ8050 NX. All bacterial and fungal strains were prepared from the Iranian Industrial Microorganisms Collection Center (Lyophilized). Microbiological tests were

performed using a Memmert- INC153T2T3 incubator.

**Chemistry:** 1, 3, 4-oxadiazole compounds were synthesized using a single-stage high yield method (Figure 1). The chemical structure of all the synthesized compounds was investigated using IR spectroscopy, H-NMR, C-NMR, and GC-Mass.

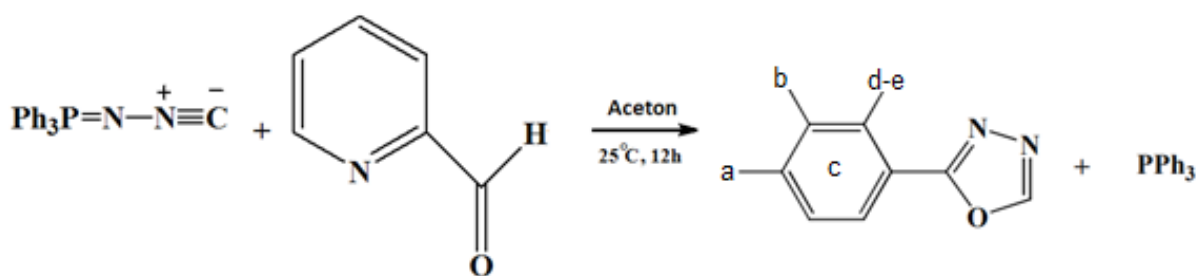
Single-stage method was performed as follows:

1. First, (N-Isocyanimino)triphenylphosphorane (1mmol, 0.3g) was dissolved in dichloromethane (7 mL).

2- In the next step, p-tolyl (4a), m-tolyl (4b), phenyl (4c), dimethyl-phenyl (4d), aniline (4e), naphthalene (4f), and methoxy-phenyl (4g) (1mmol) were added to the previous solution (Table 1, Figure 1) [19]. The final solution was stirred for 24 hrs by a magnetic stirrer at 37°C. The solvent was picked up by vaporization, and the viscous residue was purified by flash column chromatography (silica gel powder: petroleum ether–ethyl acetate (5:1)) [22]. Infrared, nuclear magnetic resonance, and gas chromatography analysis showed that there was no side product (Figure 2).

**Table 1)** Functional groups

<b>4a</b>	Para-tolyl
<b>4b</b>	Meta-tolyl
<b>4c</b>	Phenyl
<b>4d</b>	Dimethyl-phenyl
<b>4e</b>	Aniline
<b>4f</b>	Naphthalene
<b>4g</b>	Methoxy-phenyl



**Figure 1)** Single-stage synthesis of 1, 3, 4-oxadiazole

**Preparation of the compounds with intended concentrations:** Dimethyl sulfoxide (99%) (DMSO) was used to dissolve all the compounds. Initially, a concentration of 0.5 mg/mL was prepared from the powders of the synthesized compounds (1:9 ratio). Afterwards, they were kept at -18° C in sterile test tubes until the tests were performed.

**Antibacterial activity:** Agar well diffusion (Inhibition Zone), MIC (Minimum Inhibitory Concentration), and MBC (Minimum Bactericidal Concentration)/MFC (Minimum Fungicidal Concentration) methods were used to evaluate antibacterial and antifungal properties of the new synthesized compounds. All antibacterial tests were conducted according to CLSI [23].

**Preparation of bacterial and fungal suspension:** The lipophilic ampoules containing *S. mutans*, *E. faecalis*, *P. vulgaris*, and *N. meningitides* strains were first opened under sterile conditions and transferred to the nutrient broth culture medium and incubated for 24 hours at 37°C. Also, for fungi samples, the ampoules containing *C. glabrata* and *C. krusei* strains were first opened under sterile conditions and transferred to the sabouraud dextrose broth and incubated for 24 hours at 37°C. Using a sampler, 0.5 mL of 24-hour culture of microbial suspension was transferred to a tube containing sterile nutrient broth, and then the turbidity of the microbial suspension was visually compared to the McFarland standard set with a spectrophotometer at 625 nm and absorption rate of  $1.5 \times 10^8$  CFU/mL. For bacterial strains, the nutrient agar culture medium was applied for agar well diffusion test, and the nutrient broth culture medium was used to test the dilution in the tubes. For fungi strains, the sabouraud dextrose agar culture medium was used for agar well diffusion test, and sabouraud dextrose broth culture medium was used to test the dilution in the tubes [23].

**Agar well diffusion method:** To perform this experiment, wells of 5 mm in diameter

were created by a sterile pipette in NA culture media containing cultured bacterial suspension as well as in SDA culture media containing cultured fungal suspension. The wells were then filled with the synthesized compounds (4a-g) and put inside the incubator for 24 hours at 37 °C. It is worth noting that all the steps were done near the flame and in a sterile environment [19].

This experiment was performed in triplicate, and the average results were reported.

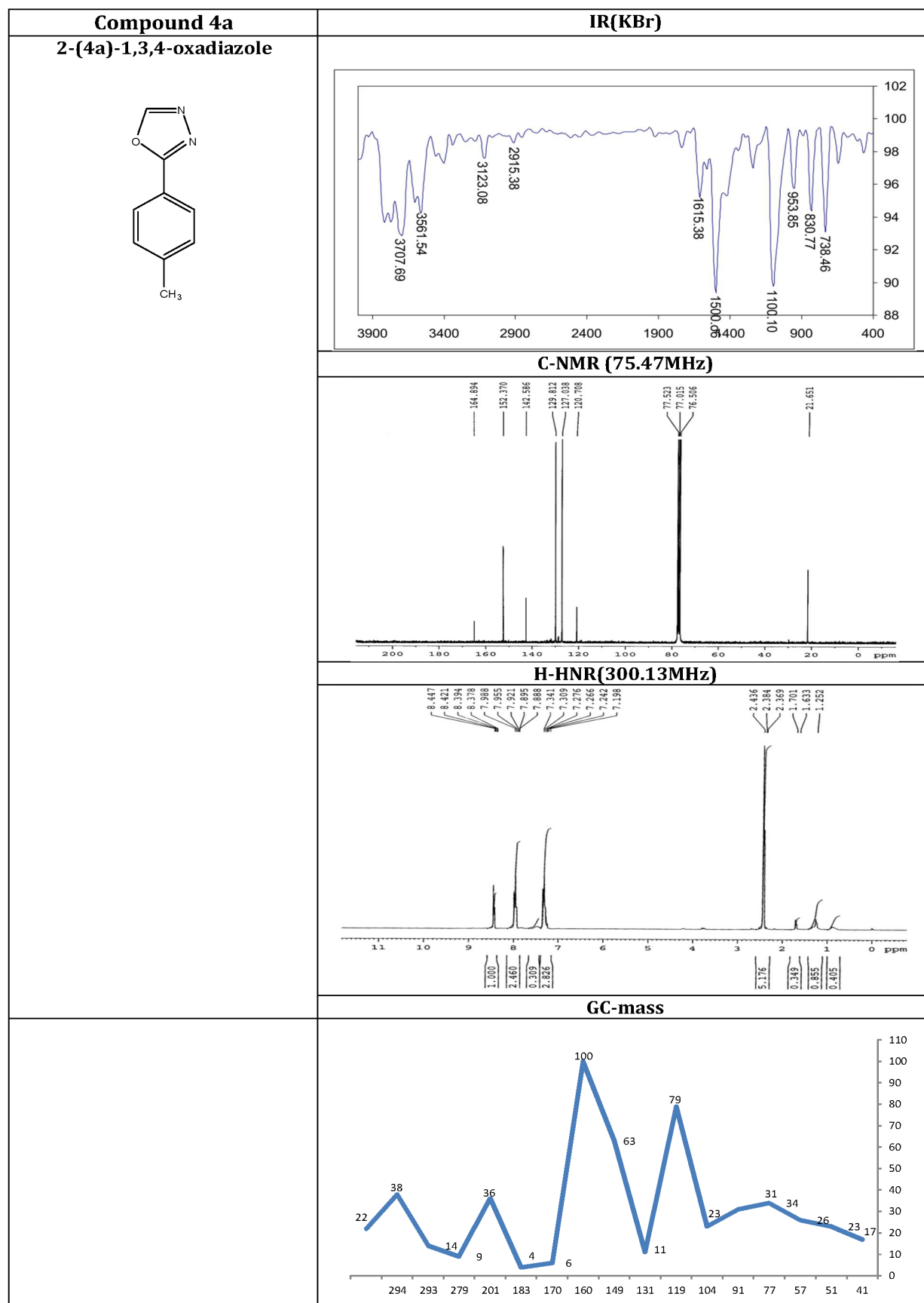
**Broth dilution method:** In the next stage, antibacterial and antifungal activities of the compounds were appraised by broth dilution method. The goal of this method is to characterize the minimum concentration of an antimicrobial agent that, under specific situation, is able to prevent the visible growth of the tested organisms which are thought-out by the lack of darkness in a broth medium. Minimum inhibitory concentration (MIC) is used in order to specify the capacity of an organism against antibiotics and new antibacterial effects. In this research, sterile glass test tubes containing nutrient broth were used. About 10 µL of inoculum containing  $1.5 \times 10^9$  CFU/mL of tested microorganism was added to each test tube. The minimum inhibitory concentration of the tested compounds was between 1000-1.95 µg/mL. For bacteria, minimum bactericidal concentration (MBC) was determined by sub culturing of the test tubes on NA media with no antibacterial factor. For fungi, minimum fungicidal concentration (MFC) was determined by sub culturing of the test tubes on SDA media with no antifungal factor [24].

**Statistical methods:** All the inhibition zones diameters obtained in three repetitions were reported as the mean ± standard deviation. SPSS statistical software Version 22 was used for data analysis.

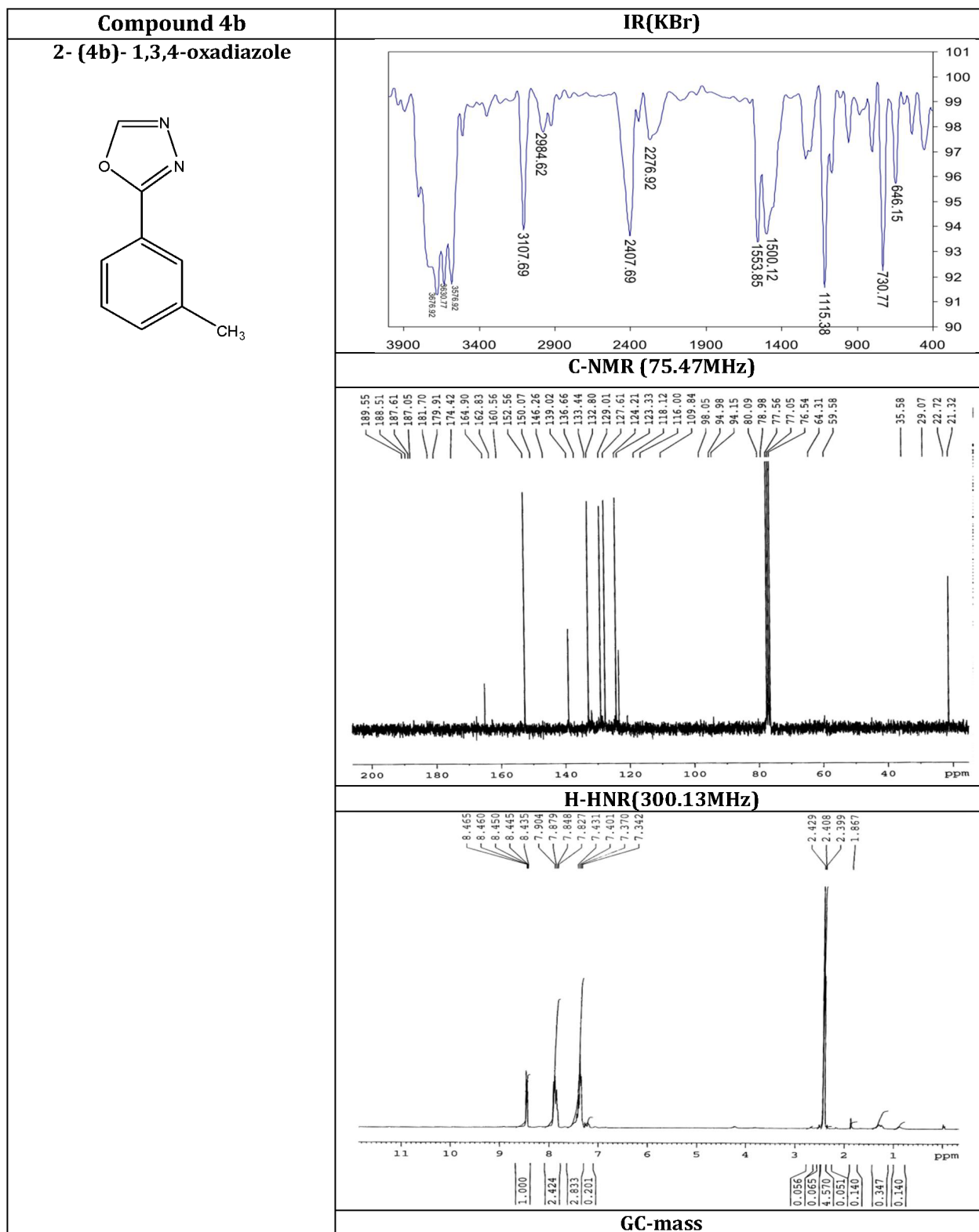
## Findings

**Chemistry:** Infrared, C-NMR, H-NMR, and GC-mass spectra of all the compounds were obtained (Figure 2).

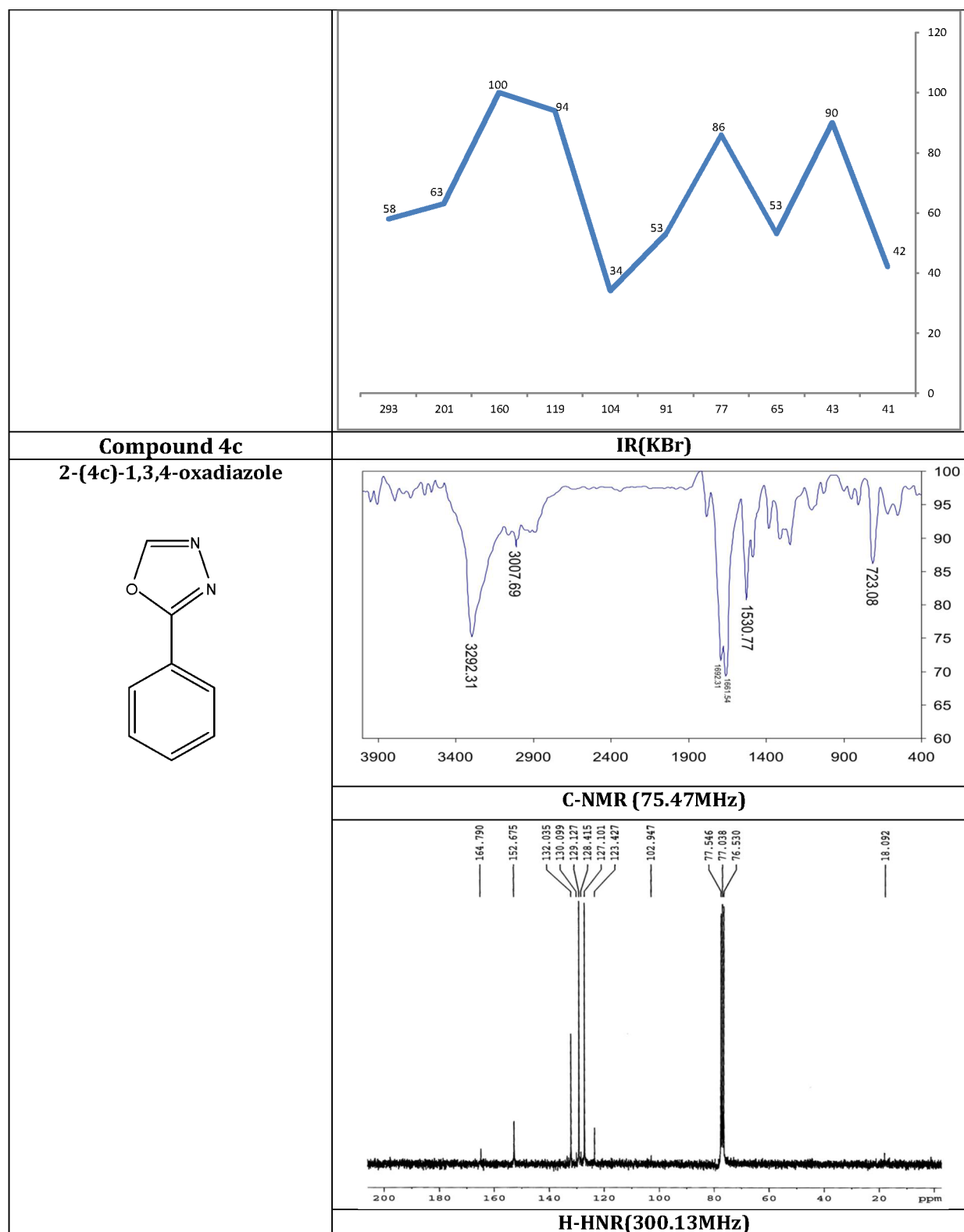




**Figure 2)** Structural and spectral information of the new synthesized derivatives of 1, 3, 4-oxadiazole



**Figure 2) continued**



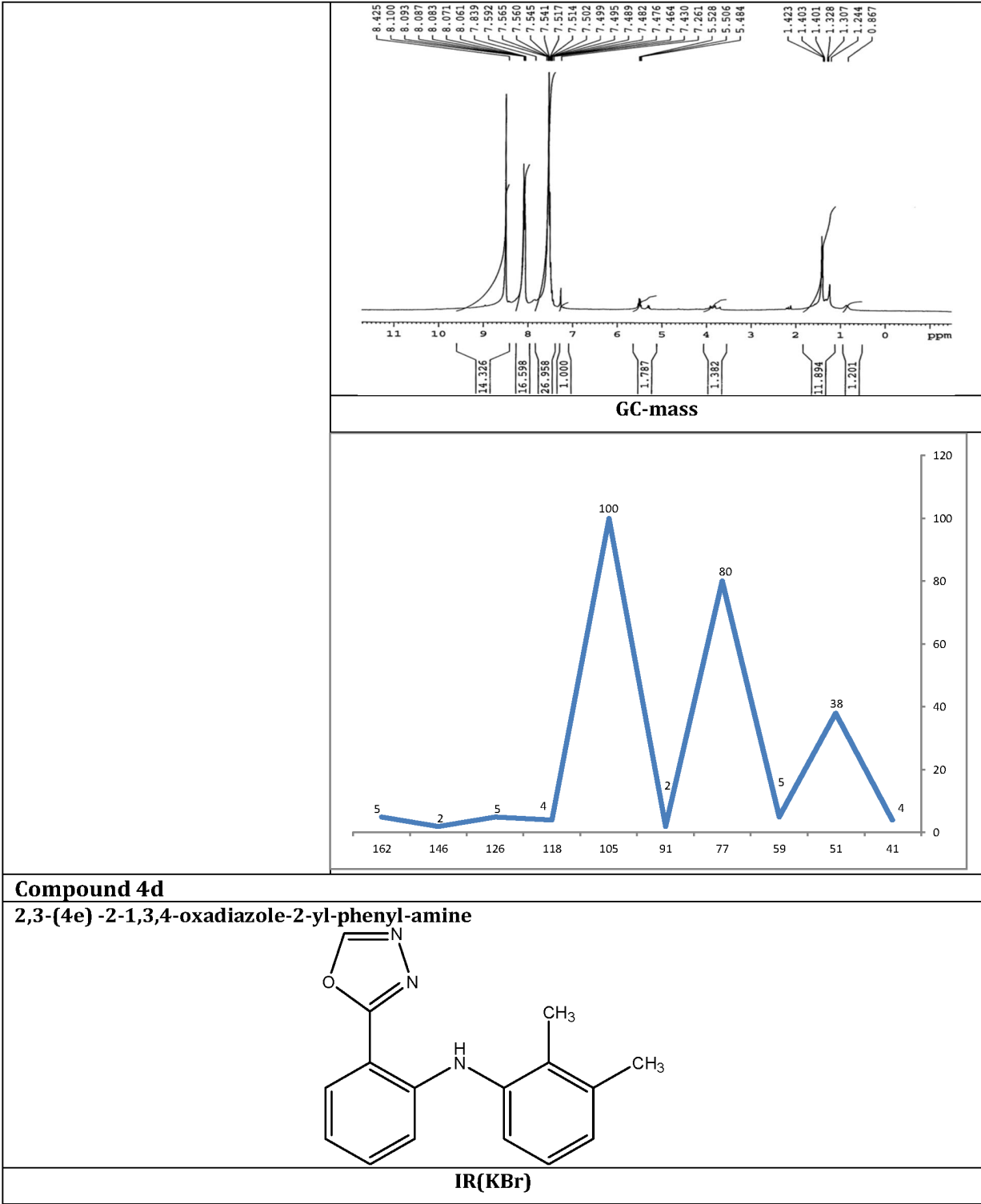


Figure 2) continued



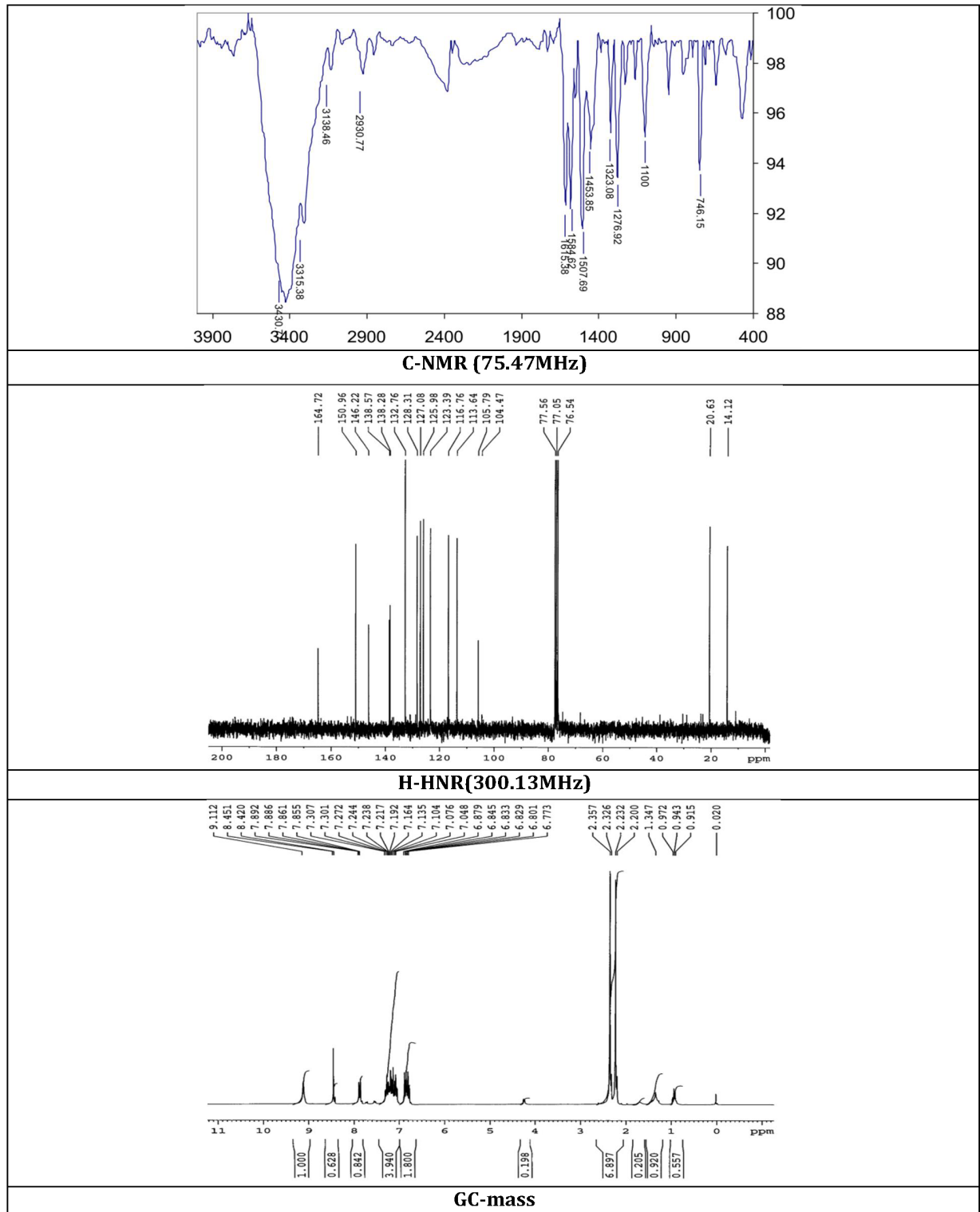
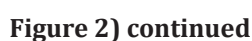


Figure 2) continued



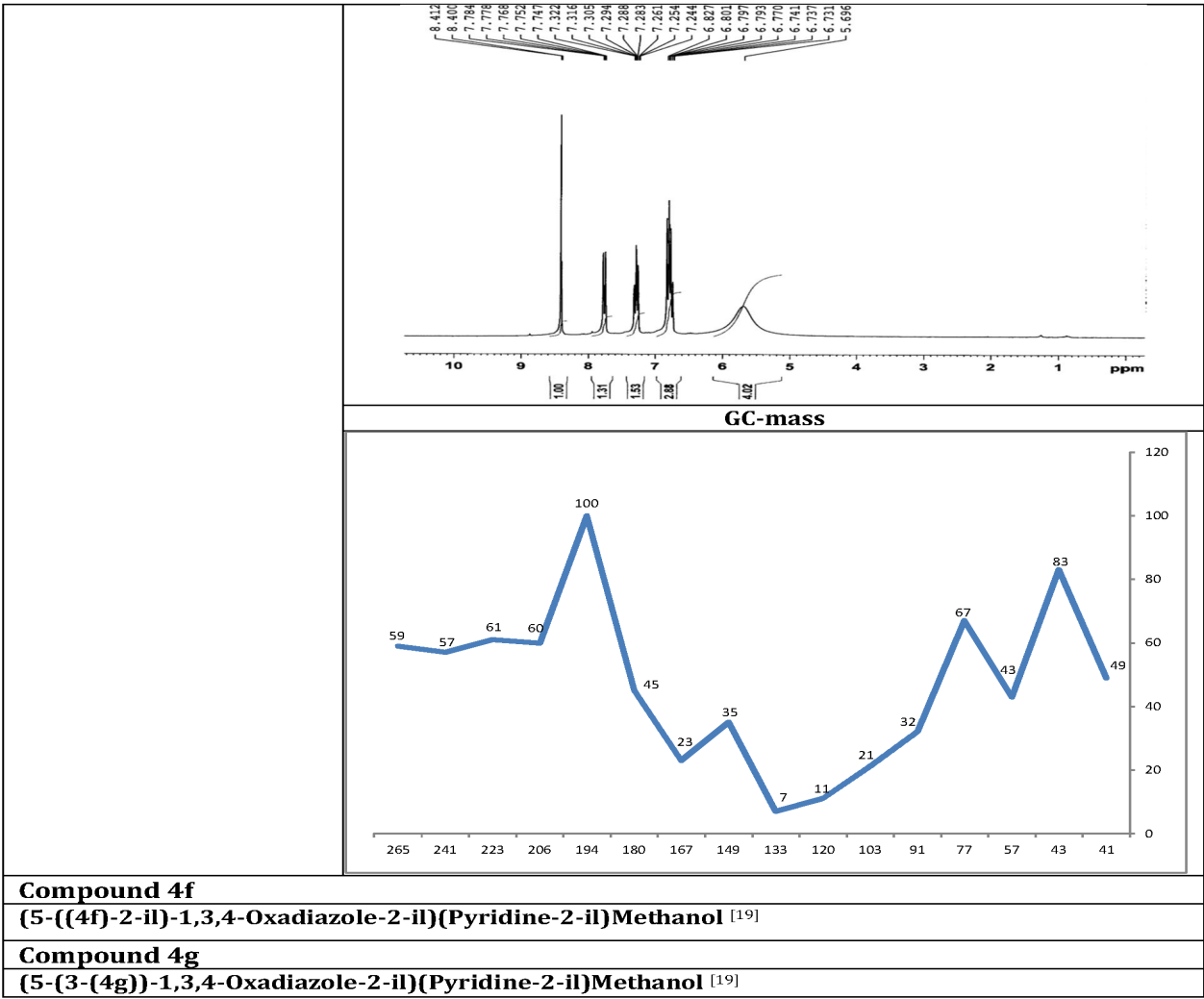


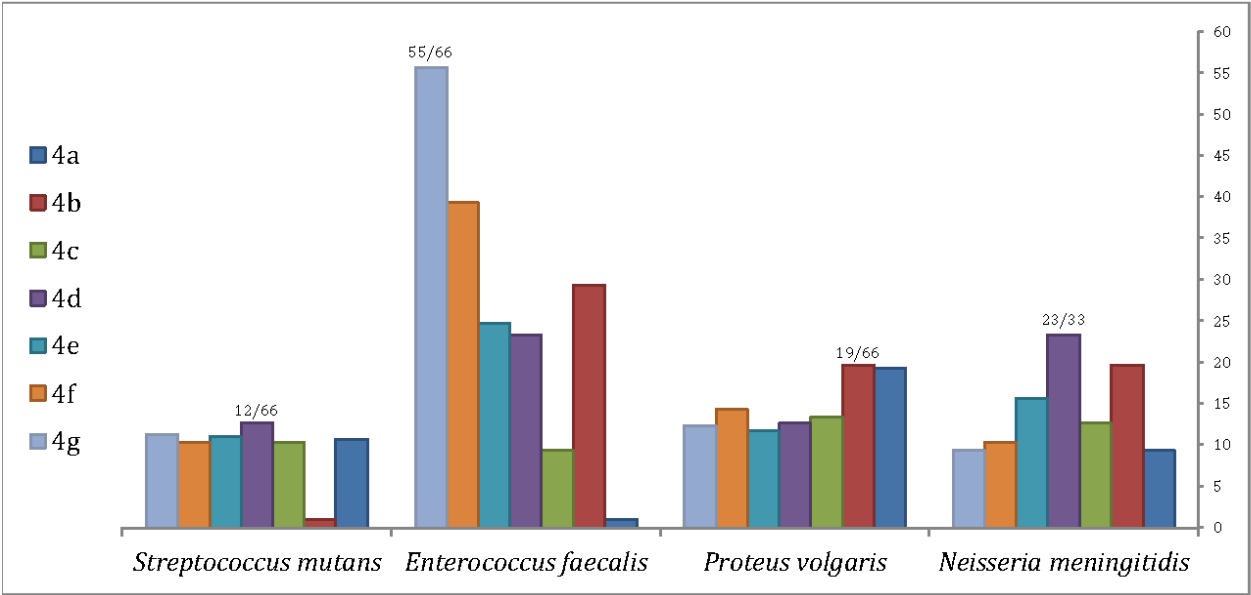
Figure 2) continued

**Determination of the *in vitro* antibacterial and antifungal activities:** Antibacterial and antifungal activities of the prepared 13, 4-oxadiazole derivatives (4a-4g) moieties were evaluated in terms of their structure (Figure 2). The inhibition zones diameters of the synthesized compounds against the tested bacteria and fungi are presented in Table 2 and 3. As shown in Table 2, the compound 4d showed high antibacterial activity against *S. mutans* with IZ=12.66±0.5 mm, MIC=500 mg/mL, and MBC=1000 mg/mL and against *N. meningitidis* with IZ=23.33±1.15 mm, MIC=500 mg/mL, and MBC=1000 mg/mL; also, the compound 4g showed high antibacterial activity against *E. faecalis* with IZ=55.66±0.5 mm, MIC=31.25

mg/mL, and MBC=62.50 mg/mL, and the compound 4b showed high antibacterial activity against *P. vulgaris* with IZ=19.66±0.5 mm, MIC=500 mg/mL, and MBC=500 mg/mL. As shown in Table 3, the compound 4b showed high antifungal activity against *C. glabrata* with IZ=12.33±0.5 mm, MIC=1000 mg/mL, and MFC=1000 mg/mL, and against *C. krusei* with IZ=13.33±0.5 mm, MIC=1000 mg/mL, and MFC=1000 mg/mL. As shown in Figure 5, the significant results obtained in this study indicated that most of the synthesized compounds performed very well against the Gram-positive samples (*E. faecalis*); so that the compound 4g showed acceptable inhibitory effects with high IZ, MIC, and MBC.

**Table 2)** Antibacterial activity of 1, 3, 4-oxadiazol derivatives, evaluated by Agar well diffusion (IZ), MIC, and MBC methods (0.5 mg/mL). ±: Averaged three times. -: No activity

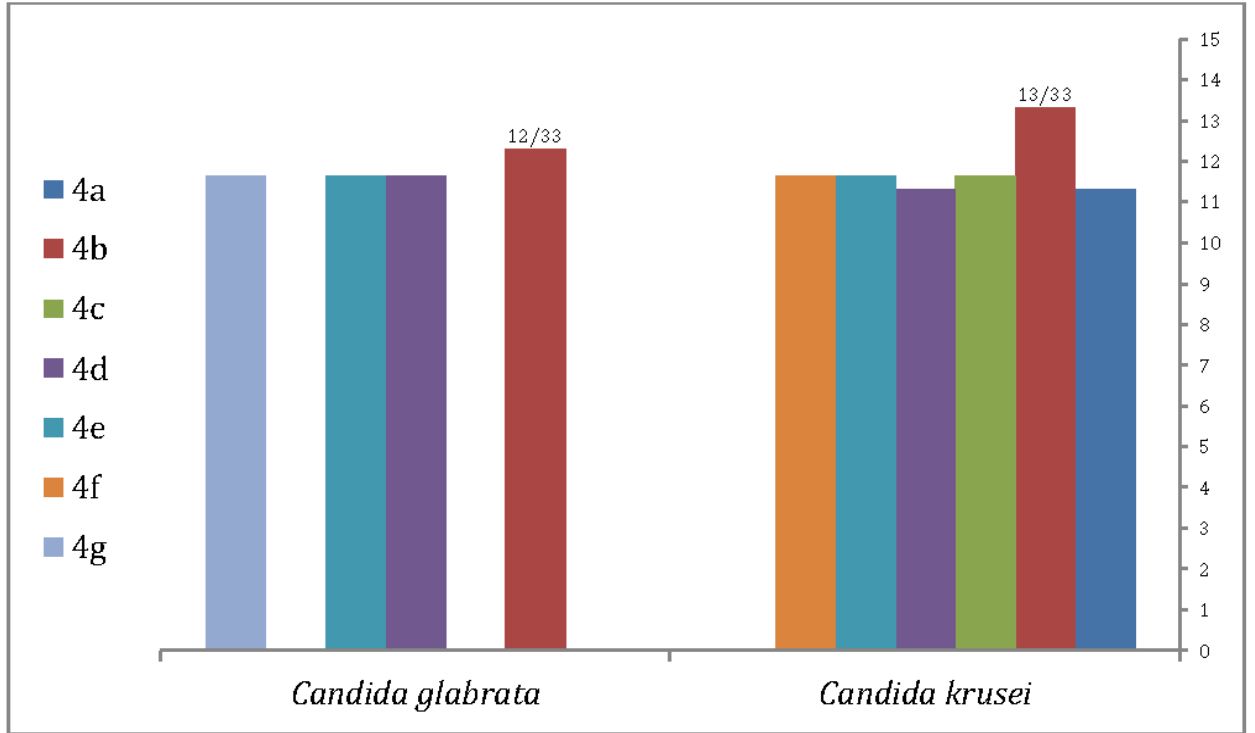
Microorganism												Compounds
<i>Neisseria meningitidis</i> PTCC1507			<i>Proteus vulgaris</i> PTCC1861			<i>Enterococcus faecalis</i> PTCC1778			<i>Streptococcus mutans</i> PTCC1683			
MBC	MIC	IZ	MBC	MIC	IZ	MBC	MIC	IZ	MBC	MIC	IZ	
-	-	9.33 ± 0.5	1000	500	19.33 ± 1.15	-	-	-	-	-	10.66± 0.5	4a
-	-	19.66 ± 0.5	500	500	19.66± 0.5	1000	500	29.33± 0.5	-	-	-	4b
-	-	12.66 ± 0.5	-	-	13.33 ± 1.15	-	-	9.33 ± 0.5	-	-	10.33 ± 0.5	4c
1000	500	23.33 ± 1.15	-	-	12.66± 1.15	1000	500	23.33 ± 1.15	1000	500	12.66 ± 0.5	4d
-	-	15.66 ± 0.5	-	-	11.66 ± 0.5	500	500	24.66 ± 1.15	-	-	11.00 ± 0.5	4e
-	-	10.33 ± 0.5	-	-	14.33 ± 1.15	500	125	39.33 ± 1.15	-	-	10.33±0.5	4f
-	-	9.33 ± 0.5	-	-	12.33 ± 1.15	62.50	31.25	55.66 ± 0.5	-	-	11.33± 0.5	4g



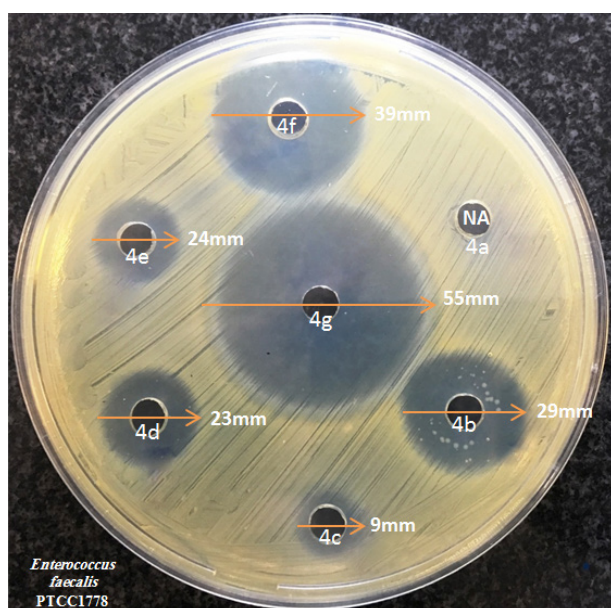
**Figure 3)** Comparison of the inhibition zone diameters of the compounds against the bacterial samples

**Table 3)** Antifungal activity of 1, 3, 4-oxadiazol derivatives, evaluated by Agar well diffusion (IZ), MIC, and MFC methods (0.5 mg/mL). ±: Averaged three times. -: No activity

Microorganism						Compounds
<i>Candida krusei</i> PTCC5295			<i>Candida glabrata</i> PTCC5297			
MFC	MIC	IZ	MFC	MIC	IZ	
-	-	11.33 ±0.5	-	-	-	4a
1000	1000	13.33 ±0.5	1000	1000	12.33 ±0.5	4b
-	-	11.66 ±0.5	-	-	-	4c
-	-	11.66 ±0.5	-	-	11.66 ±0.5	4d
-	-	11.33 ±0.5	-	-	11.66 ±0.5	4e
-	-	11.66 ±0.5	-	-	-	4f
-	-	-	-	-	11.66 ±0.5	4g



**Figure 4)** Comparison of the inhibition zone diameters of the compounds against the fungal samples



**Figure 5)** Inhibition zone of the compounds against *Enterococcus faecalis* at 0.5 mg/mL concentration

## Discussion

Antimicrobial resistance has the potential to influence individuals at any stage of life, healthcare system, veterinary, and horticulture businesses, making it one of the most important public health problems in the world. Each year in the United States, at least 2.8 million individuals are infected by antibiotic-resistant microbes or parasites, and more than 35,000 individuals die as a result. No one could totally maintain a safe strategic distance from the risk of diseases, but a few individuals are at higher risk than others. If antimicrobial agents lose their efficiency, at this point the healthcare system loses the capacity to treat contaminations and control public health risks [25]. One way to solve this crisis is to find new antimicrobial compounds to replace current antibiotics. 1, 3, 4-oxadiazole derivatives are novel antibacterial compounds, which are good replacements for a few antibacterial drugs. The objective of this study was to evaluate in vitro antibacterial and antifungal activities of 1, 3, 4-oxadiazole derivatives against some pathogenic bacteria and fungi. In recent

years, a number of new 1, 3, 4-oxadiazole analogues have been introduced as antimicrobial agents [26]. The latest study on 1, 3, 4-oxadiazole with methoxyphenyl group reported that this group in 4, 3, 1-oxadiazole structure has a good antibacterial effect against Gram-positive and Gram-negative bacteria [27], which is in line with the present study results. In the present investigation, (5-(3-methoxyphenyle)-1, 3, 4-oxadiazole-2-yl) (pyridine-2-yl) methanol (4g) was the most active antibacterial compound with inhibition zone diameters similar or comparable to that of the reference drug. This may be due to the presence of methoxyphenyl with (pyridine-2-yl) and methanol together. These findings are in line with the findings of other studies by Guo et al. (2019) [28], Bhaumik et al. (2019) [18], and Tresse et al. (2019) [29] on 1, 3, 4-oxadiazole derivatives. In this study, all the derivatives of 1, 3, 4-oxadiazole were synthesized for the first time. They showed inhibitory properties against Gram positive and Gram negative bacteria; however, they did not show inhibitory properties against fungal strains except 4b. This study showed more promising results than previous studies to introduce these structures as antibacterial agents. In the present study, the presence of tolyl, phenyl, and naphthalene rings caused a considerable decrease in the antifungal potential of the compounds, but they were able to increase antibacterial properties. Therefore, it could be inferred that the presence of these groups in similar structures, especially the methoxy-phenyl group, enhances the antimicrobial activity.

## Conclusion

In this study, 1, 3, 4-oxadiazole derivatives exhibited a narrow-spectrum antibacterial activity, especially against *E. faecalis*. The obtained results showed that dimethylphenyl and 3-methoxyphenyl could



be considered as a useful structure for the possible development of new antibacterial drugs. Also, tolyl group could be considered as a useful structure for the possible development of new antifungal drugs. Further studies are required to assess the cytotoxicity of such compounds.

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**Ethical Permissions:** There are no ethical permissions.

**Conflicts of Interests:** There are no conflicts of interest.

**Author Contributions:** Conceptualization: AS, YSA; Data curation and formal analysis: NZA, AS, YSA; Investigation: YSA; Methodology and project administration: YSA, NZA; Supervision: NZA, AS, YSA; Validation: YSA, NZA; Writing of original draft: YSA; Writing, reviewing, and editing: YSA.

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**Consent to participate:** Not applicable.

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