ABSTRACT

Background and objectives: Antibiotic resistance is a major public health challenge. The pervasive antibiotic misuse can lead to increased antibiotic resistance. Thus, there is a need for discovery of new compounds against drug-resistant microorganisms. We synthesized new series of 1, 3, 4-oxadiazole derivatives (4a-4d) and evaluated the antibacterial and antifungal activity of the derivatives against Staphylococcus aureus, Staphylococcus epidermidis, Acinetobacter baumannii, Klebsiella pneumoniae, Aspergillus fumigatus and Aspergillus flavus.

Methods: The new derivatives of 1, 3, 4-oxadiazole were synthesized using a single-stage, high-yield method. The structure of the new compounds was confirmed by infrared spectroscopy, carbon-nuclear magnetic resonance and hydrogen-nuclear magnetic resonance. Then, antibacterial and antifungal activities of the prepared derivatives (1 mg/ml) were evaluated by determining minimum inhibitory concentration and minimum bactericidal/fungicidal concentration using the agar well diffusion method.

Results: All synthesized compounds, especially (4d) with methoxyphenyl group, exhibited powerful antibacterial activity against the tested bacteria. However, the compounds had no antifungal effect.

Conclusion: Our findings indicate the antibacterial potential of the novel synthetic 1, 3, 4-oxadiazole compounds.

Keywords: Anti-bacterial agents, Antifungal agents, Oxadiazoles.
INTRODUCTION
Antibiotics are powerful medicines that fight certain infections and can save lives when used properly (1). They either stop bacteria from reproducing or destroy them (2). However, the pervasive antibiotic misuse can lead to antibiotic resistance (3). Therefore, it is necessary to seek novel antibacterial agents (4). Gram-positive pathogens exhibit an immense genetic repertoire to adapt and develop resistance to virtually all available antimicrobials (5). In 2017, the World Health Organization (WHO) has published a list of antibiotic-resistant priority pathogens, which present a great threat to humans. The list is categorized based on the urgency of need for new antibiotics as critical, high and medium priority, in order to guide and promote research and development of new antibiotics. Due to their distinctive structure, gram-negative bacteria are more resistant than gram-positive bacteria, and cause significant morbidity and mortality worldwide (6). Some species of fungi are also naturally resistant to antifungal drugs. For example, fluconazole is not effective against infections caused by Aspergillus (7). Resistance can also develop over time when fungi are exposed to antifungal drugs. So far, different classes of oxadiazoles have been synthesized (8). Oxadiazoles are non-β-lactam class of antibiotics with five-membered heterocyclic aromatic rings containing two carbon, two nitrogen and one oxygen atoms. Among their different structures, 1,3,4-oxadiazole is known for high reactivity and the possibility of adding more functional groups (9). This structure also has antibacterial (10), antifungal (11), antitubercular (12), antiviral (13), anticancer (14) and antimalarial properties (15). The purpose of this study is to synthesize new series of oxadiazoles structures and evaluate antibacterial and antifungal activity of 1,3,4-oxadiazole derivatives against some gram-positive (S. aureus PTCC 1189, S. epidermidis PTCC 1436) and gram-negative (A. baumannii PTCC1855, K. pneumonia PTCC1290, A. fumigates PTCC5009, A. flavus PTCC5006) bacteria as well as some fungi (A. fumigates PTCC5009, A. flavus PTCC5006).

MATERIALS AND METHODS
This experimental research was conducted in the microbiology laboratory of Islamic Azad University, Tehran branch in 2020. Starting materials, solvents and culture media (nutrient agar/broth and sabouraud dextrose agar/broth) were purchased from Merck Co., Germany. Infrared (IR) spectrum was measured using the Shimadzu IR-460 spectrometer. Nuclear magnetic resonance (NMR) spectrum was obtained using the Bruker DRX-300 AVANCE spectrometer (1H NMR at 300 Hz, 13C NMR at 75 Hz) in acetone. Chromatography columns were prepared using silica gel powder (Merck, Germany). All bacterial and fungal strains (S. aureus PTCC 1189, S. epidermidis PTCC 1436, A. baumannii PTCC1855, K. pneumonia PTCC1290, A. fumigates PTCC5009, A. flavus PTCC5006) were obtained from the Iranian Industrial Microorganisms Collection Center in lyophilized form.

Structure synthesis
1. 3. 4-oxadiazole compounds were synthesized using a single-stage, high yield method. The chemical structure of all synthesized compounds was investigated using IR spectroscopy, H-NMR and C-NMR. First, N-Iso-cyan-imino-triphenyl-phosphoran (1mmol, 0.3 g) was dissolved in dichloromethane (7 ml) with 2-pyridine carbaldehyde. Next, carboxylic acid derivatives and cinnamic acid derivatives (1mmol) were added. The solution was stirred for 24 hours on a magnetic stirrer at 37°C. The solvent was removed by evaporation, and the viscous residue was purified by flash column chromatography [silica gel powder: petroleum ether–ethyl acetate (5:1)]. Thin-layer chromatography and NMR indicated that there was no side product.

Preparation of compound concentrations
One mg/ml of synthesized compounds powder (1:10 ratios) was dissolved in dimethyl sulfoxide (99%). The resulting solution was kept at -18°C in sterile test tubes.

Antibacterial activity
Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)/minimum fungicidal concentration (MFC) of the synthesized compounds were evaluated using agar well diffusion method according to the Clinical & Laboratory Standards Institute standards (16).

Preparation of bacterial and fungal suspensions
The lipophilic ampoules containing S. aureus, S. epidermidis, A. baumannii and K.
The antibacterial and antifungal activity of the compounds was evaluated by broth dilution method. First, 10 µl of inoculums containing $1.5 \times 10^9$ CFU/ml of tested microorganism was added to sterile test tubes. Different concentrations (1.95-1000 µg/ml) of the synthesized compounds were added to the test tubes. Lowest concentration of the compounds that inhibited growth of bacteria was recorded as the MIC. Lowest concentration that reduced the viability of bacteria and fungi by ≥99.9% was recorded as the MBC and MFC, respectively.

**RESULTS**

**Chemistry**

Results of IR spectroscopy, C-NMR and H-NMR of all compounds are shown in figure 2. The synthesis of derivatives was performed in a single step with high efficiency (4a-4d) and the structures were determined after purification.

**Agar well diffusion method**

To perform this experiment, wells of 5 mm in diameter were created by a sterile pipette in agar media containing bacterial or fungal suspension. The wells were then filled with the synthesized compounds (4a-4d). Ciprofloxacin and fluconazole were used as the positive controls. The plates were incubated at 37°C for 24 hours. The experiment was performed in triplicate.

**Broth dilution method**

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>K. pneumonia PTCC1290</th>
<th>A. baumannii PTCC1855</th>
<th>S. epidermidis PTCC 1436</th>
<th>S. aureus PTCC 1189</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBC</td>
<td>MIC</td>
<td>IZ</td>
<td>MBC</td>
<td>MIC</td>
</tr>
<tr>
<td>NA</td>
<td>NA</td>
<td>11.33 ± 0.5</td>
<td>1000</td>
<td>100</td>
</tr>
<tr>
<td>NA</td>
<td>NA</td>
<td>14.66 ± 0.5</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>NA</td>
<td>NA</td>
<td>17.33 ± 1.15</td>
<td>125</td>
<td>62.50</td>
</tr>
<tr>
<td>1000</td>
<td>500</td>
<td>30.5±0.3</td>
<td>62.50</td>
<td>31.25</td>
</tr>
</tbody>
</table>

Table 1: Antibacterial and antifungal activities of 1, 3, 4-oxadiazol derivatives by agar well diffusion method.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>A. flavus PTCC5006</th>
<th>A. fumigatus PTCC5009</th>
</tr>
</thead>
<tbody>
<tr>
<td>MFC</td>
<td>MIC</td>
<td>IZ</td>
</tr>
<tr>
<td>NA</td>
<td>NA</td>
<td>11.33±0.5</td>
</tr>
<tr>
<td>1000</td>
<td>1000</td>
<td>11.33±0.5</td>
</tr>
<tr>
<td>NA</td>
<td>NA</td>
<td>11.66±0.5</td>
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<tr>
<td>NA</td>
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<td>11.66±0.5</td>
</tr>
<tr>
<td>NA</td>
<td>NA</td>
<td>11.33±0.5</td>
</tr>
</tbody>
</table>

Results are related to 1 mg/ml of each compound.

NA: no activity
Cip: Ciprofloxacin
Fl: Fluconazole

Figure 1- Inhibition zone of compounds (1 mg/ml) against S. aureus PTCC 1189, S. epidermidis PTCC 1436 and A. baumannii PTCC1855
Spectral information

White powder, m.p. 131 °C, yield 84% (0.21g).
IR (KBr) (νmax, cm⁻¹): 3435, 3066, 2924, 2847, 1668, 1583, 1537, 1512, 1493, 1434, 1379, 1289, 1226, 1047, 921.

1H NMR (300.13 MHz, CDCl₃): δH=5.87 (s, 1H, OH), 5.21 (s, H, CH, Aliphatic), 7.46-7.48 (m, 1H, CH, Arom), 7.53-7.7 (m, 3H, CH, Arom), 7.79 (t, 3JHH= 7.4 Hz, 1C, Arom), 8.24 (d, 3JHH=7.1 Hz, 2H, CH Arom), 8.47 (d, 3JHH= 7.7 Hz, 1H, CH Arom), 8.91-8.92 (m, 1H, Arom).
13C NMR (75.467 MHz, CDCl₃): δC= 67.22 (CH-Aliphatic), 7.49 (m, 1H, CH, Arom), 7.54 (m, 1CH, CH, Arom), 7.59 (t, 3JHH= 7.6 Hz, 1C, Arom), 8.26 (d, 3JHH=7.3 Hz, 2H, CH, Arom), 8.49 (d, 3JHH= 7.9 Hz, 1H, CH Arom), 8.93-8.95 (m, 1H, Arom).

Spectral information

White powder, m.p. 131 °C, yield 84% (0.21g).
IR (KBr) (νmax, cm⁻¹): 3423, 3101, 2823, 2742, 1602, 1301, 1010, 1018.
1H NMR (300.13 MHz, CDCl₃): δH=5.88 (s, 1H, OH), 5.22 (s, H, CH, Aliphatic), 7.48-7.50 (m, 1H, CH, Arom), 7.55-7.72 (m, 3H, CH, Arom), 8 (t, 3JHH= 7.6 Hz, 1C, Arom), 8.26 (d, 3JHH=7.3 Hz, 2H, CH Arom), 8.41 (d, 3JHH= 7.7 Hz, 1H, CH Arom), 8.93-8.95 (m, 1H, Arom).
13C NMR (75.467 MHz, CDCl₃): δC= 67.23 (CH-CH(OH)), 127.91, 128.06, 129.26, 131.90, 131.94 (SC, Phenyl), 125.8, 133.28, 137.25, 150.22 (4CH, Arom, Pyridine), 122.82, 151.45 (2C), 160.86, 164.06.

Spectral information

White powder, m.p. 131 °C, yield 84% (0.21g).
IR (KBr) (νmax, cm⁻¹): 3423, 3101, 2823, 2742, 1601, 1398, 1101, 1016.
1H NMR (300.13 MHz, CDCl₃): δH=5.86 (s, 1H, OH), 5.52 (s, H, CH, Aliphatic), 7.46-7.48 (m, 1H, CH, Arom), 7.53-7.7 (m, 3H, CH, Arom), 7.79 (t, 3JHH= 7.4 Hz, 1C, Arom), 8.24 (d, 3JHH=7.1 Hz, 2H, CH Arom), 8.47 (d, 3JHH= 7.7 Hz, 1H, CH Arom), 8.91-8.92 (m, 1H, Arom).
13C NMR (75.467 MHz, CDCl₃): δC= 67.21 (CH-CH(OH)), 127.85, 128.01, 129.21, 131.85, 131.92 (SC, Phenyl), 125.71, 133.21, 137.25, 150.25 (4CH, Arom, Pyridine), 122.83, 151.45 (2C), 160.85, 164.05.

Spectral information

White powder, m.p. 135 °C, yield 79% (0.22g).
IR (KBr) (νmax, cm⁻¹): 3435, 3066, 2924, 2847, 1668, 1583, 1537, 1512, 1493, 1434, 1379, 1289, 1226, 1047, 921.
1H NMR (300.13 MHz, CDCl₃): δH=3.94 (3H, OCH₃), 6.45 (s, 1H, CH aliphatic), 7.46-7.18 (m, 1H, CH, Arom), 7.49 (t, 3JHH= 8.2 Hz, Arom), 7.64-7.87 (m, 1H, CH, Arom), 7.74-7.76 (m, 1H, CH, Arom), 7.8 (t, 3JHH= 8.2 Hz, CH Arom), 7.9 (t, 3JHH= 8.2 Hz, CH Arom), 8.478 (d, 3JHH= 8.2 Hz, CH Arom), 8.917-8.931 (m, 1H, CH, Arom).
13C NMR (75.467 MHz, CDCl₃): δC= 55.65 (CH), 68.42 (CH-CH(OH)), 112.22, 119.58, 120.34, 125.77, 128.08, 130.45, 137.24, 150.25 (8CH, Arom), 123.95, 151.54, 160.09, 160.88, 164.04.

Figure 2: Structural and spectral information of new derivatives of 1, 3, 4-oxadiazoles
**Determination of the in vitro antibacterial and antifungal activity**

Antibacterial and antifungal activities of the prepared 1, 3, 4-oxadiazole derivatives (4a-4d) moieties were evaluated. The diameters of inhibition zone (IZ) for each compound are reported in **Table 1**. Compound (4d) with methoxyphenyl group showed powerful antibacterial activity against *S. aureus*, *S. epidermidis* and *A. baumannii* (**Figure 1**). Other compounds also showed acceptable antibacterial effects. However, the compounds showed no notable antifungal activity.

**DISCUSSION**

The objective of this study was to evaluate the 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 has been introduced as potential antimicrobial agents (17). The latest study about 1, 3, 4-oxadiazole with methoxyphenyl group reported that methoxyphenyl group in 1, 3, 4-oxadiazole structure have favorable antibacterial effect against gram-positive and gram-negative bacteria (18), which is in line with our findings. In our study, (Z)-N-(2-(4-methoxyphenyl)-1-(5-hydroxyl (pyridin-2-yl) methyl)-1, 3, 4-oxadiazol-2-yl) vinyl) acetamide (4d) showed comparable antibacterial activity to that of the reference drug. This may be due to the presence of methoxyphenyl along with hydroxyl and (pyridin-2-yl) methyl. This finding is similar to results of some previous studies (19, 20). In our study, we synthesized new 1, 3, 4-oxadiazole derivatives with inhibitory properties against gram-positive and gram-negative bacteria. However, the compound exhibited no antifungal activity. In our previous study, the two-component derivatives of 1, 3, 4-oxadiazole with methoxyphenyl group showed acceptable antibacterial effect against *A. baumannii*. It seems that increasing the number of side chains attached to 1, 3, 4-oxadiazole contributed to the increased antibacterial properties (21). In the present study, the presence of chlorophenyl, bromophenyl, fluorophenyl and methoxyphenyl rings caused a considerable decrease in the antifungal potential of the compound while increasing the antibacterial properties. Therefore, it can be inferred that in similar structures, the presence of these groups, especially methoxy phenyl group, enhances the antimicrobial activity. It is suggested to use other functional groups of carboxylic acids in the synthesis of new derivatives. It seems that the ability of oxadiazole structures is influenced by addition of different functional groups.

**CONCLUSION**

Based on our findings, the synthesized compounds, especially compound 4d (methoxy phenyl group), could be utilized in designing more potent antibacterial agents, particularly against *S. aureus*, *S. epidermidis* and *A. baumannii*. Further studies are required to assess cytotoxicity of such compounds.

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We would like to thank all those who helped us in this research.

**CONFLICT OF INTERESTS**

The authors declare that there is no conflict of interest.

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