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### RESEARCH ARTICLE

#### IN VITRO ANTIMICROBIAL ACTIVITY OF NEW SUBSTITUTED 1,3,4-OXADIAZOLE DERIVATIVES.

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#### Abstract

**Background:** The need of new antimicrobial agents is justified because more microorganisms are being resistance to the currently available antibacterial drugs and this is bringing alarming threat to public health and causing growing concern among people across the globe. 1,3,4-Oxadiazole have shown a wide spectrum of antimicrobial activity. Some of the marketed drugs also possessed this heterocyclic moiety.

**Objectives:** We have previously synthesized new series of (5-aryl-1,3,4-oxadiazol-2-yl)(pyridin-2-yl)methanol derivatives (4a-4d) in fairly high yields. Here, we evaluated the antimicrobial activity of these derivatives against the following microorganisms: *Staphylococcus epidermidis* ATCC14990, *Bacillus cereus* PTCC 1015, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Candida tropicalis* PTCC 5028 and *Candida albicans* ATCC10239.

**Materials and Method:** The broth macrodilution and well agar diffusion methods were used for determination of inhibition zone (IZ) and minimum inhibitory concentration (MIC) during preliminary evaluation of antimicrobial activity.

**Results:** All of the synthesized compounds exhibited promising antibacterial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis*.

**Conclusions:** In this study, data suggest that compound (4a-4d) is a potent antibacterial compound with excellent MICs and acceptable selectivity index against Staphylococcal infections.

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#### Implication:

Methicillin-resistant *Staphylococcus aureus* (MRSA) are a serious global public-health concern, as MRSA has become broadly resistant to many classes of antibiotics. We disclose herein the discovery of a new class of non- $\beta$ -lactam antibiotics, the oxadiazoles, which inhibit *Staphylococcus* species.

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The discovery of new substances with proven antimicrobial activity is the current study goal of various researchers. Usage of synthetic products has grown considerably in the past few years due to processing agility, and capability of going through previous chemical modifications in order to enhance its biological activity. Widespread careless use of antimicrobials has made the number of resistant microorganisms rise significantly, thus demanding more efficient drugs to fight them.

Compounds containing 1,3,4-oxadiazole rings have a broad biological activity spectrum including antibacterial (1), antifungal (2), analgesic (3), anti-inflammatory (4), antiviral(5), anticancer(6), antihypertensive(7), anticonvulsant (8), and anti-diabetic properties(9). They have also attracted interest in medicinal chemistry as surrogates (bioisosteres) for carboxylic acids, esters and carboxamides (10). Two examples of compounds containing the 1,3,4-oxadiazole unit currently used in clinical are Raltegravir(11), antiretroviral drug(12) and Zibotent(13) an anticancer agent (14). In recent years, a number of new 1,3,4-oxadiazole derivatives as antibacterial agents have been reported (15). The main modifications of these compounds were focused on the naphthylxymethyl groups. In our previous study, we synthesized (5-aryl-1,3,4-oxadiazol-2-yl)(pyridin-2-yl)methanol, under mild conditions in a one-pot reaction to afford four novel (5-aryl-1,3,4-oxadiazol-2-yl)(pyridin-2-yl)methanol derivatives in good yields (16).

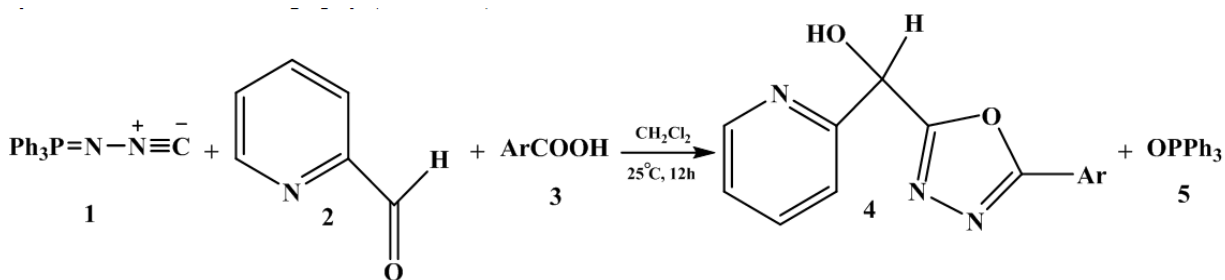
### Objectives:

As synthesis and evaluation of antimicrobial activity is an important part of our research program (17-19); herein, we have evaluated antibacterial and antifungal activity of these derivatives against gram positive (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus cereus*), gram negative (*Pseudomonas aeruginosa*, *Escherichia coli*) bacteria and fungal (*Candida albicans* and *Candida tropicalis*).

### Material and Methods:

#### Chemistry:

A group of (5-aryl-1,3,4-oxadiazol-2-yl)(pyridin-2-yl)methanol derivatives (4a-d) were synthesized using previously published methods. Briefly, solution of *N*-isocyaniminotriphenylphosphorane (1) and 2-pyridinecarbaldehyde (2) in  $\text{CH}_2\text{Cl}_2$  was added dropwise to a solution of a carboxylic acid (3) in  $\text{CH}_2\text{Cl}_2$  at room temperature over 15 min. The mixture was stirred for 12 h. The solvent was removed under reduced pressure and the viscous residue was purified by flash column chromatography (Scheme 1).



	Ar
4a	4-Br-Ph
4b	3-CH <sub>3</sub> -Ph
4c	5-Naphthalen
4d	4-CH <sub>3</sub> -Ph

**Scheme 1:-** Synthesis of (5-aryl-1,3,4-oxadiazol-2-yl)(pyridine-2-yl)methanol derivatives.

### Antibacterial Activity:

#### Agar Diffusion Method-Individual compounds Solutions:

Antibacterial activity of synthesized compounds were tested against several gram positive and gram negative bacteria including: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus cereus* PTCC 1015, *Staphylococcus aureus* ATCC 25923 and *Staphylococcus epidermidis* ATCC14990. These microorganisms were chosen because they are commonly isolated pathogens from hospitalized patient with intestinal ailments, blood and skin infections. All microorganisms were obtained from Urmia University of Medical Sciences.

The standardization of each bacterial inoculum was done following the National Committee for Clinical Laboratory Standards (NCLS). Briefly, each bacterial strain inoculated into Mueller Hinton broth (MHB; Oxoid) was incubated to a turbidity equivalent to 0.5 McFarland units. Bacterial strains were incubated for 12 hours at 35°C to achieve the turbidity of 0.5 McFarland units. A standardized bacterial inoculum containing  $5 \times 10^5$  cfu/mL (forming units per millimeter) colony were used for antibacterial screening. The antibacterial activity of the prepared compounds were screened using the well diffusion method (20). In short, each compound was dissolved in DMSO and a solution with 1 mg/ml concentration was prepared. 200 ml of Muller Hinton Agar (MHA, Merck, Germany) was melted over a boiling water bath then was stabilized at 45°C and aseptically seeded with 100  $\mu$ l inoculum, containing  $0.5 \times 10^6$  cells/ml of bacteria, transferred into a sterile Petri dish. Wells were made in agar using a sterile glass tube and 50  $\mu$ l of compounds was transferred to each well. 50  $\mu$ l of DMSO was inoculated into another well as a negative control. The antibacterial activity of compounds was determined by measuring the zones around each well against defined bacteria after incubation for 24h. Ceftizoxime and Ciprofloxacin used with the same method as standard antibacterial agents. Experiments were performed at least three times and the moral values were selected.

#### **Broth dilution Method:**

In the next step antimicrobial activity of compounds was evaluated by broth dilution method(21). The aim of this method is determining the lowest concentration of an antimicrobial agent that, under defined conditions, inhibits the visible growth of the tested organism which is considered by the lack of turbidity in a broth medium. Minimum Inhibitory Concentration (MIC) values are used in order to determining the susceptibility of the organism to antibiotics and new antibacterial agents. In this study sterile glass test tubes containing Muller Hinton Broth((MHB, Merck, Germany) was used. 10  $\mu$ L of inoculum contained  $1.5 \times 10^6$  C.F.U/ml of tested microorganism was added to each test tube. Minimum Bactericidal Concentration (MBC) values were determined by sub culturing of the tested tubes on agar media that do not contain the antibacterial agent. The MBC is determined by detecting the lowest concentration of compounds that reduces the viability of the bacterial inoculums by  $\geq 99.9$ . Ceftizoxime and ciprofloxacin (Sigma, Aldrich, US) were used with the same method as standard antimicrobial agents.

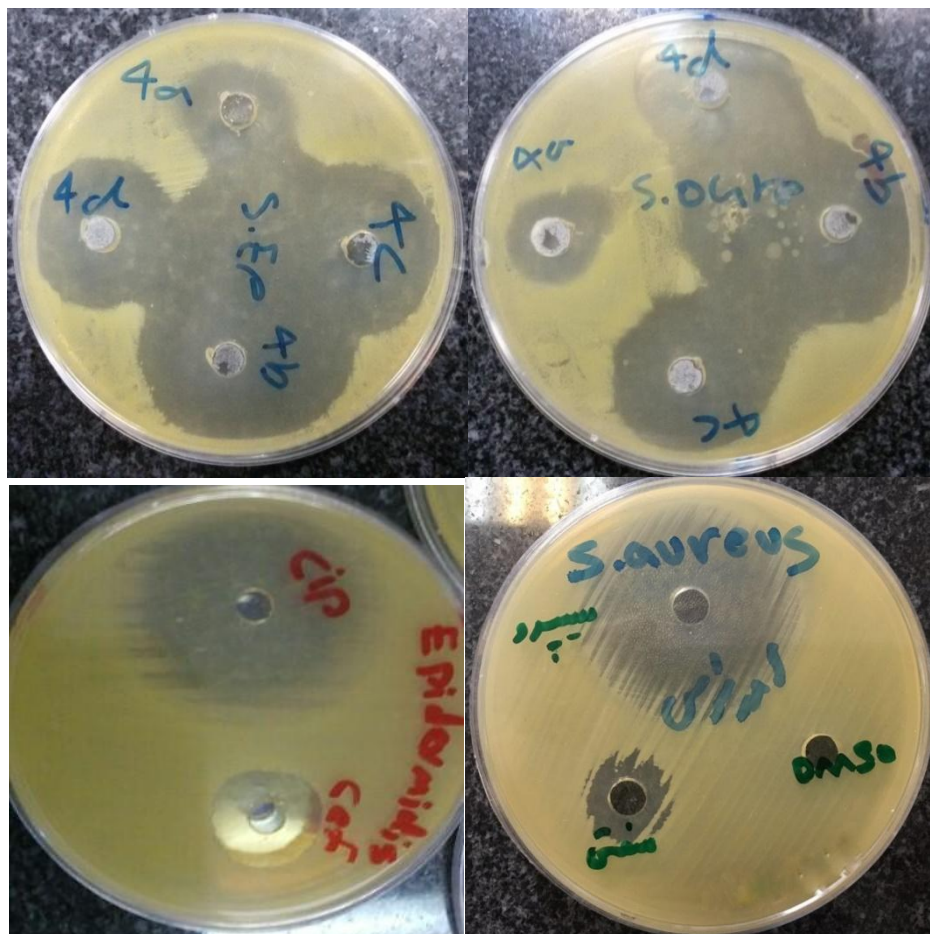
#### **Antifungal Activity:**

The in vitro antifungal activity of the (5-aryl-1,3,4-oxadiazol-2-yl)(pyridin-2yl)methanol derivatives (4a-4d) were evaluated against standardized clinically important fungi, including: *Candida tropicalis* and *Candida albicans* ATCC 10239. The antifungal assay was performed by the agar well diffusion and Broth macrodilution methods(22, 23). In brief, fungal suspension containing  $1.5 \times 10^6$  cell/mL of yeast was swabbed and spread on Sabouraud dextrose agar (SDA, (Merck, Germany). A well was cut at the centre of each inoculated medium using a standard cork borer of 6 mm diameter and 100  $\mu$ g/mL of the test compounds dissolved in DMSO were introduced into the wells. Following addition of solutions, these Petri plates were incubated for 48 h at a temperature of  $28 \pm 2^\circ\text{C}$  and then growth and zones of inhibition (in mm) were recorded.

In the next steps the Minimum Inhibitory concentrations (MICs) were determined by broth dilution technique. The Sabouraud dextrose broth (SDB, (Merck, Germany) tubes containing test compounds were serially diluted. The 48 h grown cultures of fungi was inoculated in each tube. The tubes were incubated at room temperature for 24h. The lowest concentration required to arrest the growth of fungi was regarded as MIC. To get the minimum fungicidal concentration (MFC), a loopful was taken from the MIC tubes and streaked on SDA plates. The growth was observed after incubation at 37°C at 24h. The lowest concentration which showed no growth was recorded as MFC(24).

#### **Antibacterial Activity:**

Herein, we have evaluated antibacterial and antifungal activity of some of previously prepared 1,3,4-oxadiazol derivatives containing 4-bromophenyl(4a), 3-methylphenyl(4b), Naphthalene (4c) and 4-methylphenyl (4d) moieties in their structure (Scheme 1). Results of the inhibition zone values for synthesized compounds against tested bacteria presented in Table 1. According to Figure 2 all of the compounds showed high antibacterial activity against *S. epidermidis* and *S. aureus*. No zone of inhibition was seen around the well containing DMSO (Figure 1).



**Fig1:**Inhibition zone of compounds against *Staphylococcus aureus* and *Staphylococcus epidermidis* in 1mg/ml concentration

**Table 1:** Antibacterial activity of 5-aryl-1,3,4-oxadiazol derivatives by Agar well diffusion (1mg/ml)

N	<i>S.aureus</i> ATCC.25923	<i>S.epidermidis</i> ATCC.14990	Mean zone of inhibition(mm) <i>P.aeruginosa</i> ATCC.27853	<i>E.coli</i> ATCC.25922	<i>Bacillus cereus</i> PTCC 1015
4a	17±0.6	17±0.28	NA	13±0.23	NA
4b	27±0.16	30±0.8	NA	14±0.8	NA
4c	24±0.18	27±0.23	NA	14±0.18	NA
4d	25±0.8	27±0.18	NA	14±0.23	NA
Ceftizoxim	15±.03	14±0.8	20±0.8	17±0.3	11±0.3
Ciprofloxacin	34±0.6	35±0.8	42±0.6	44±0.3	34±0.8

NA: No activity

#### Determination of MIC:

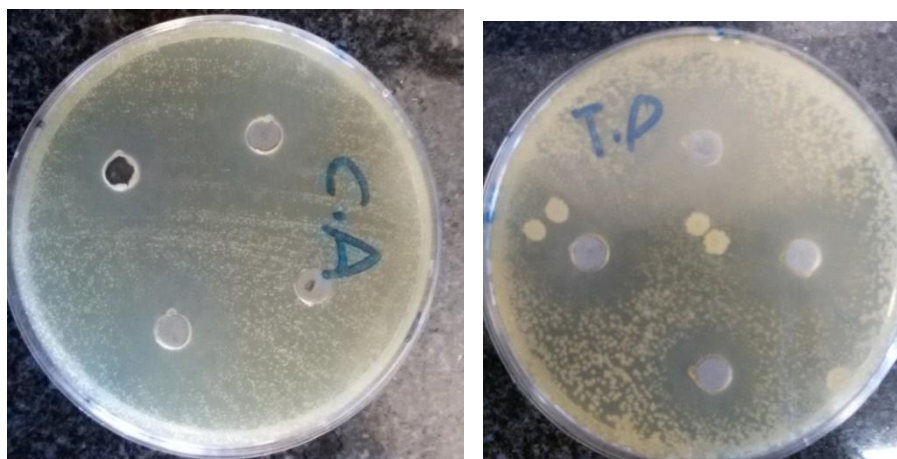
The minimum Inhibitory concentrations (MIC) of tested compounds (4a-4d) are shown in (Table2). Our results revealed that all compounds were active against *S.aureus* and *S.epidermidis*.The MBC of compounds was the same or three fold higher than the corresponding MIC results.

**Table 2:-** Minimum inhibitory concentrations of synthesized compounds ( $\mu\text{g}/\text{mL}$ ) for tested bacteria

N	<i>S.aureus</i> ATCC.25923	MIC( $\mu\text{g}/\text{ml}$ ) <i>S.epidermidis</i> ATCC.14990	<i>P.aeruginosa</i> ATCC.27853	<i>E.coli</i> ATCC.25922	<i>Bacilluscereus</i> PTCC 1015
4a	62	31	1000	500	1000
4b	15	250	1000	250	1000
4c	32	250	1000	500	1000
4d	32	500	1000	500	1000
Cefizoxim	62	50	62	32	50
Ciprofloxacin	0.029	15	0.23	0.23	15

**Antifungal Activity:**

The antifungal activity of (5-aryl-1,3,4-oxadiazol-2-yl)(pyridin-2-yl) methanol derivatives (4a-4d) against two kinds of pathogenic fungi was investigated and summarized in Table3. Figure 2 presents the results for antifungal activities of all the title compounds.

**Fig.2:-** Inhibition zone of compounds against *Candida tropicalis* and *Candida albicans* in 1mg/ml concentration.**Table 3:-** Antifungal activity of 5-aryl-1,3,4-oxadiazol

N	<i>Candida albicans</i>		<i>Candida tropicalis</i>	
	IZ(mm)	MIC( $\mu\text{g}$ )	IZ(mm)	MIC( $\mu\text{g}$ )
4a	16 $\pm$ 0.8	500 $\pm$ 0.6	16 $\pm$ 0.18	500 $\pm$ 0.8
4b	15 $\pm$ 0.12	500 $\pm$ 0.23	20 $\pm$ 0.8	250 $\pm$ 0.26
4c	16 $\pm$ 0.23	500 $\pm$ 0.8	17 $\pm$ 0.6	500 $\pm$ 0.6
4d	N	N	17 $\pm$ 0.28	500 $\pm$ 0.34

**Discussion:-**

In vitro antibacterial activity of the synthesized compounds was performed against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus cereus*, *Pseudomonas aeruginosa* and *Escherichia coli*. The activity potentials were qualitatively assessed by the presence or absence of inhibition zones, zone diameters, MIC and MBC values. As shown in Table 1 the minimum Inhibitory concentrations (MIC) of tested compounds revealed that all compounds were active against *S. aureus* and *S. epidermidis* (MIC = 15-62 $\mu\text{g}/\text{mL}$ ) and exhibited excellent antibacterial activity in comparison to ceftizoxim (MIC = 6.25  $\mu\text{g}/\text{mL}$ ) however they showed poor activity compared with Ciprofloxacin (MIC = 0.39  $\mu\text{g}/\text{mL}$ ). From the screening data, The Oxadiazol having Naphthalenesubstituent(4b) showed better activity against *E. coli* (MIC = 15 $\mu\text{g}/\text{mL}$ ). The lowest activity of compounds (4a-4d) was observed against *Bacillus cereus* and *P. aeruginosa* (MIC = 1000 $\mu\text{g}/\text{mL}$ ). A comparative study of MIC values indicates that the inhibitory activity of these compounds on Gram positive bacteria is better than on Gram negative bacteria. Antifungal potential of synthesized compounds (4a-4d) were evaluated according to their zone of inhibition against two pathogens and the MIC and MFC values were determined in the range of 250

to 1000 µg/ml. The results revealed that all the compounds are potent antimicrobials against *Candida tropicalis* and *Candida albicans*. The zones inhibition diameters and the MIC values indicate that all synthesized compounds 4a-d have moderate inhibition activities. Compound (4c) showed the maximum activity with MIC value of 250 µg/ml against *Candida albicans* and *Candida tropicalis* but the (5-Phenyl-1,3,4-oxadiazol-2-yl)(pyridin-2-yl)methanol (4d) showed no antifungal activity against *Candida albicans*. MFC values for title compounds were similar to MIC results. As a result of our previous work, the antimicrobial activity of 2-aryl-1,3,4-oxadiazole against Gram-positive bacteria was proved (18). The data found in the literature matching our research claims that the compounds with halogen substituent are the most efficient against Gram-positive bacteria, particularly against *S. aureus* [23, 24]. A comparison between the results of this study and our previous report, revealed that all of our 1,3,4-oxadiazole derivatives with pyridine ring at the C-2 position on the linker of 1,3,4-oxadiazole, can be identified as the most promising antibacterial agents against *S. aureus* and *S. epidermidis* with a MIC value of 7.5 to 15 µg/mL. In this study, data suggest that compound (4a-4b) is a potent antibacterial compound with excellent MICs and acceptable selectivity index against Staphylococcal infections. Furthermore the easy workup, high yield, and short reaction times makes the method a useful addition for preparing modern pharmaceutical synthetics.

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