



Synthesis and Evaluation of Antibacterial Activity of 1,3,4-Oxadiazoles Derivatives Containing Pyridine Ring

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ABSTRACT

In the present article synthesis and evaluation for antibacterial activity of a new series of (5-aryl-1,3,4-oxadiazol-2-yl) (pyridin-2-yl) methanol derivatives were described. The compounds were characterized by IR, NMR, and mass spectroscopy. All the synthesized compounds were screened for their antimicrobial activity against *Staphylococcus epidermidis* ATCC14990, *Bacillus cereus* PTCC1050, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922. The broth macrodilution and agar well diffusion methods were used for determination of inhibition zone (IZ) and minimum inhibitory concentration (MIC) during preliminary evaluation of antimicrobial activity. All of the synthesized compounds exhibited promising antibacterial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis*.

Keywords: Antibacterial activity; 1,3,4-Oxadiazoles; Minimum inhibitory concentration; *In vitro*

INTRODUCTION

Due to the changes in the culture and life style, new diseases are emerging among the human population such as cancers and AIDS which increase the risk of bacterial infections [1]. Increasing resistance of microorganisms to currently available antimicrobial drugs is the major cause of morbidity and mortality throughout the world. Thus, development of novel antimicrobial drugs is still in demand [2]. Among several classes of antimicrobial agents, oxadiazoles have very good antibacterial properties and nitroimidazoles are the most known among them. Oxadiazole derivatives had been reported to exhibit several biological activities like antibacterial [3], anti HIV [4], antifungal [5], antitubercular [6], virucidal [7], antimalarial [8], anti-epileptic [9], herbicidal [10], analgesic [11], anti-inflammatory [12], anticancer [13], anti-epileptic [14], anthelmintic [15]. They have also attracted interest in medicinal chemistry as bioisosteres for carboxylic acids, esters and carboxamides [16-18].

EXPERIMENTAL SECTION

Chemistry

Synthesis of compound:

^1H (300.13 MHz) and ^{13}C (75.47 MHz) NMR measurements were recorded on a Bruker 250 spectrometer in CDCl_3 with tetramethylsilane as internal standard. IR spectra were measured on a Shimadzu IR-460 spectrometer. Elemental analyses for C and H were performed using a Heraeus CHN-O-Rapid analyzer. Mass spectra were recorded on a FINNIGAN-MATT 8430 mass spectrometer operating at an ionization potential of 20 eV. Melting points were measured on an Electrothermal 9100 apparatus and are uncorrected. (*N*-Isocyanimino) triphenylphosphorane **2** was prepared based on a reported procedure [19,20]. Other starting materials and solvents were obtained from Merck (Germany) and Fluka (Switzerland) and were used without further purification. Flash chromatography columns were prepared from Merck silica gel powder. The structures of the products were deduced from their IR, ^1H NMR and ^{13}C NMR spectra. The mass spectra of these compounds displayed molecular ion peaks at the appropriate m/z values.

General Procedure for the preparation of products (4a-d):

To a magnetically stirred solution of (*N*-isocyanimino) triphenylphosphorane (1) (0.302 g, 1 mmol) in CH₃CN (8 ml) was added drop-wise a solution of 2 (1 mmol) in CH₃CN (7 ml) over 15 min. The mixture was stirred for 12 h at room temperature. The solvent was removed under reduced pressure and the viscous residue was purified by flash column chromatography (silica gel; petroleum ether–ethyl acetate (10:3). The solvent was removed under reduced pressure and the products (4a–d) were obtained.

(5-Phenyl-1,3,4-oxadiazol-2-yl) (pyridin-2-yl) methanol:

White powder, m.p. 131 °C, yield 84% (0.21g). IR (KBr) (ν_{\max} , Cm⁻¹): 3433, 3053, 2924, 2855, 1669, 1439, 1187, 1117. ¹HNMR (300.13 MHz, CDCl₃): δ_{H} =6.78 (s, 1H, OH), 6.20 (s, H, CH Aliphatic), 7.47-7.49 (m, 1H, CH, Arom), 7.54-7.71 (m, 3H, CH, Arom), 7.99 (t, ³J_{HH}= 7.5Hz, 1C, Arom), 8.25 (d, ³J_{HH}=7.2 Hz, 2H, CH Arom), 8.48 (d, ³J_{HH}= 7.8Hz, 1H, CH Arom), 8.92-8.93 (m, 1H, Arom). ¹³CNMR (75.467 MHz, CDCl₃): δ_{C} = 67.22 (CH-OH), 127.90, 128.05, 129.25, 131.89, 131.93 (5CH, Phenyl), 125.79, 133.27, 137.24, 150.21 (4CH, Arom, Pyridine), 122.81, 151.43 (2C), 160.86, 164.06 (2C, Oxadiazole). Anal. Calcd for C₁₄H₁₁N₃O₂: C, 66.40; H, 4.38; N, 16.59 found C, 66.18; H, 4.11; N, 16.42%. MS (EI, 20 eV): *m/z* (%)= 253.26 (M⁺, 66), 235.34 (58), 176.15 (61), 175.16 (32), 145.14(57).

(5-(Naphthalen-2-yl)-1,3,4-oxadiazol-2-yl) (pyridin-2-yl) methanol:

White powder, m.p. 134 °C, yield 81% (0.24g). IR (KBr) (ν_{\max} , cm⁻¹): 3434, 3052, 2925, 2856, 1686, 1582, 1547, 1491, 1437, 1187, 1118, 997. ¹HNMR (300.13 MHz, CDCl₃): δ_{H} =6.11 (s, 1H, CH aliphatic), 6.61 (s, 1H, OH), 7.47-7.49 (m, 1H, CH Arom), 7.53-7.58 (m, 1H, CH Arom), 7.65-7.71 (m, 3H, CH Arom), 7.92-7.94 (m, 1H, CH Arom), 8.01-8.03 (m, 1H, CH Arom), 8.27-8.30 (m, 1H, CH Arom), 8.49-8.52 (m, 1H, CH Arom), 8.93-8.95 (m, 2H, CH Arom). ¹³CNMR (75.467 MHz, CDCl₃): δ_{C} =67.77 (CH aliphatic), 123.51, 124.44, 127.37, 128.51, 128.58, 129.39, 132.47 (7CH, Naphthalene ring), 130.00, 134.82, 128.42 (3C, Naphthalene ring), 125.69, 128.16, 137.24, 150.20 (4CH, Pyridine ring), 158.2 (C, Pyridine ring), 162.34, 166.81 (2C, Oxadiazole). Anal. Calcd for C₁₈H₁₃N₃O₂: C, 71.28; H, 4.32; N, 13.85 found: C, 70.91; H, 4.11; N, 13.78%. MS (EI, 20 eV): *m/z* (%)=303.28 (M⁺, 64), 302.09 (45), 285.09 (38), 225.07 (44), 208.06 (27), 176.05 (38), 158.04 (51), 127.05 (19), 78.03 (44).

(5-(3-bromophenyl)-1,3,4-oxadiazol-2-yl) (pyridin-2-yl) methanol:

White powder, m.p. 162 °C, yield 78% (0.26g). IR (KBr) (ν_{\max} , Cm⁻¹): 3419 (br), 3054, 2924, 2855, 1666, 1573, 1401, 1379, 1187, 1117, 992. ¹HNMR (300.13 MHz, CDCl₃): δ_{H} = 6.64 (s, 1H, OH), 6.18 (s, 1H, CH aliphatic), 7.47-7.56 (m, 2H, CH, Arom), 7.65-7.77 (m, 2H, CH Arom), 8.00 (t, ³J_{HH}= 7.5 Hz, 1H, CH Arom), 8.18 (d, 1H, ³J_{HH}= 8.1 Hz, CH, Arom), 8.45 (d, 1H, ³J_{HH}= 7.8 Hz, CH, Arom), 8.90-8.96 (m, 1H, CH, Arom). ¹³CNMR (75.467 MHz, CDCl₃): δ_{C} = 67.3 (CH-OH), 124.7, 128.4, 128.6, 130.6, 131.9, 125.7, 135.8, 137.3, 150.2 (9CH, Arom.), 122.4, 123.3 152.8 (3C, Arom.), 162.4, 164.7 (2C, Oxadiazole). Anal. Calcd for C₁₄H₁₀BrN₃O₂: C, 50.62; H, 3.03; N, 12.65 found: C, 50.59; H, 3.00; N, 12.60%. MS (EI, 20 eV): *m/z* (%)= 332.15 (M⁺, 76), 254.96 (18), 252.25 (15), 234.23 (20), 224.03 (22), 156.00 (17).

(5-(3-Chlorophenyl)-1,3,4-oxadiazol-2-yl) (pyridin-2-yl) methanol:

White powder, m.p. 139 °C, yield 77% (0.22g). IR (KBr) (ν_{\max} , cm⁻¹): 3444, 2926, 2856, 1673, 1578, 1513, 1377, 1239, 1120, 1022, 927. ¹HNMR (300.13 MHz, CDCl₃): δ_{H} =6.71 (s, 1H, OH), 6.19 (s, 1H, CH aliphatic), 7.38-7.56 (m, 2H, CH, Arom), 7.58-7.94 (m, 1H, CH, Arom), 7.94-7.99 (m, 1H, CH, Arom), 8.12-8.14 (m, 1H, CH, Arom), 8.12-8.25 (m, 1H, CH, Arom), 8.43-8.46 (m, 1H, CH, Arom), 8.87-8.93 (m, 1H, CH, Arom). ¹³CNMR (75.467 MHz, CDCl₃): δ_{C} = 68.91 (CH-OH), 127.80, 128.41, 128.57, 130.61, 131.91, 132.03, 137.26, 150.196 (8CH, Arom), 128.179, 135.474, 158.338 (3C, Arom), 161.8, 164.1 (2C, Oxadiazole). Anal. Calcd for C₁₄H₁₀ClN₃O₂ (287.70): C, 58.45; H, 3.50; N, 14.61 found: C, 58.42; H, 3.31; N, 14.60%. MS (EI, 20 eV): *m/z* (%)= 287.64(M⁺, 68), 269.69 (10), 254.25 (15), 179.58 (22), 176.05 (21), 111.55 (19).

Antibacterial evaluation**Agar well diffusion method:**

Antibacterial activity of the new 1,3,4-Oxadiazole compounds was evaluated by well diffusion method [19] as well as broth dilution method [20]. In the first method, Mueller-Hinton agar (MHA) medium was used to prepare MHA agar plates which were inoculated with the test bacterium. Then, six millimeter holes were punched in the MHA plates. Test compounds were prepared the following final concentrations: 5000, 2500 and 1000 µg mL⁻¹ in DMSO (Dimethylsulfoxid). After that, wells were filled with 50 µL of the test compounds (1 mg mL⁻¹). Ceftizoxime and Ciprofloxacin was used as a positive control and DMSO was used as a negative control. After incubation, the average diameter of inhibition zone (IZ) around each well was measured to the nearest millimeter [21].

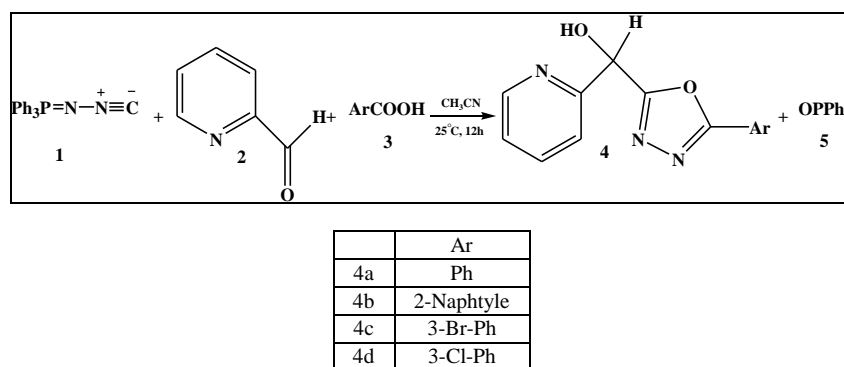
Determination of minimum inhibitory concentration

For each compound, a test tube containing specific media (8 mL) and the test compound stock solution (1 mL) was prepared. A positive control tube containing the media (8 mL) and inoculant (1 mL) and DMSO (1 mL) was also prepared as a negative control, Ceftizoxime and Ciprofloxacin stock solution (200 μ L, 1 mg/mL) was added to a test tube containing media (8 mL) and inoculants (1 mL). The mixture of the inoculated media containing compound (9 mL in each tube) were serially diluted in 8 more tubes by the addition of 1 mL of first tube and specific media (8 mL) and finally to each tube, inoculants bacteria (1 mL) was added, while all tubes were shaken for 30 seconds before each dilution and/or inoculants addition. The transparency of all test tubes was checked carefully before incubation. The tube caps were sealed by Parafilm and kept at 37°C in incubator overnight. After the incubation the tubes were checked for the minimum concentration [22]. The positive and negative controls were also observed for the accuracy of the tests.

RESULTS AND DISCUSSION

Chemistry

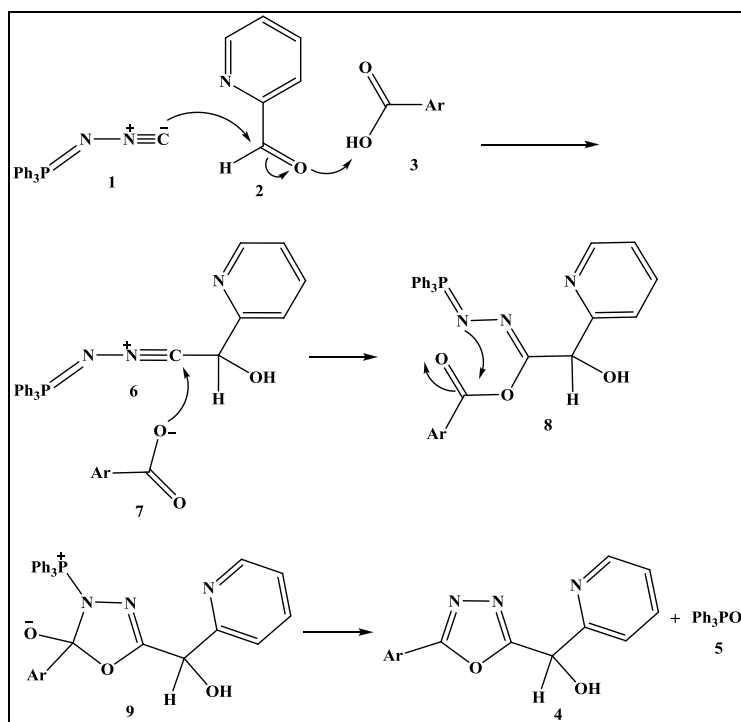
We found that benzoic acid 3a reacted with 2-pyridinecarbaldehyde 2 and (*N*-isocyanimino) triphenylphosphorane 1 in CH₃CN react in a 1 : 1 : 1 ratio at room temperature to produce (5-aryl-1,3,4-oxadiazol-2-yl) (pyridin-2-yl) methanol 4a and, as a by-product, Ph₃P=O 5 (Scheme 1). The reaction proceeded smoothly and cleanly under mild conditions in 84% yield, and no side reactions were observed. The other aromatic carboxylic acids also reacted smoothly to give similar products 4b-d in yields of 77-81% (Scheme1). We tried using the simple ketone analogous, 1-(2-pyridyl)-1-ethanone instead of 2-pyridinecarbaldehyde 2 in this reaction, but no corresponding products of type 4 were observed.



Scheme 1: Synthesis of (5-aryl-1,3,4-oxadiazol-2-yl) (pyridin-2-yl) methanol (4a-4d)

The structures of the products were deduced from their IR, MS, and ¹H- and ¹³C-NMR data. The mass spectra of these compounds displayed molecular-ion peaks at the appropriate m/z values. The ¹H-NMR spectrum of 4a consisted of a singlet for CH aliphatic (δ = 6.20 ppm), a singlet for OH (δ = 6.78 ppm), exchangeable by D₂O and multiple at δ = 7.47-8.93 ppm for the aromatic hydrogen atoms. The ¹H-decoupled ¹³C-NMR spectrum of 4a showed fourteen distinct resonances, partial assignment of these resonances is given in the experimental. The ¹H- and ¹³C-NMR spectra of compounds 4b-4d were similar to those of 4a, except for the aromatic moiety, which exhibited characteristic signals with appropriate chemical shifts.

A mechanistic rationalization for this reaction is depicted in Scheme 2. On the basis of the chemistry of isocyanides, it is reasonable to assume that the first step involves nucleophilic addition of 1 to 2-pyridinecarbaldehyde 2, facilitated by its protonation with the acid 3, leading to nitrilium intermediate 6. This intermediate may be attacked by the conjugate base of the acid 7 to form the 1 : 1 adduct 8. This adduct may undergo an intramolecular aza-Wittig reaction of the iminophosphorane moiety with the ester C=O group to afford the isolated (5-aryl-1,3,4-oxadiazol-2-yl) (pyridin-2-yl) methanol 4 by removal of Ph₃P=O 5 from intermediate 9. In this reaction, the first two reaction steps are analogous to the well-known Passerini reaction, and the final step is analogous to the well-known intramolecular aza-Wittig reaction (tandem Passerini/intramolecular aza-Wittig sequence) (Scheme 2).



Scheme 2: Plausible mechanism for the (5-aryl-1,3,4-oxadiazol-2-yl) (pyridin-2-yl) methanols

Measurement of antimicrobial activity using agar well diffusion method

The antimicrobial potential of compounds was evaluated according to their zone of inhibition against various pathogens and the results (zone of inhibition) were compared with the activity of the standards, Ceftriaxone (1.0 mg/ml) and Ciprofloxacin (1.0 mg/ml). The results revealed that all the compounds are significant antimicrobials activity against *Staphylococcus aureus* and *Staphylococcus epidermidis* and their antibacterial activity are notably more in compare with Ceftriaxone (positive control). No zone of inhibition was seen around the well containing DMSO (Negative control). (Figure 1 and Graph 1-3)

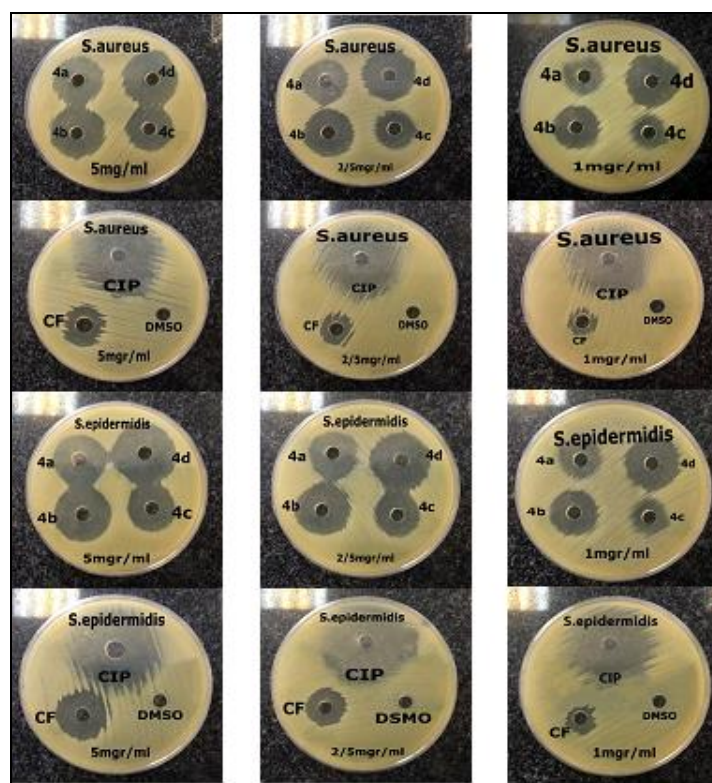
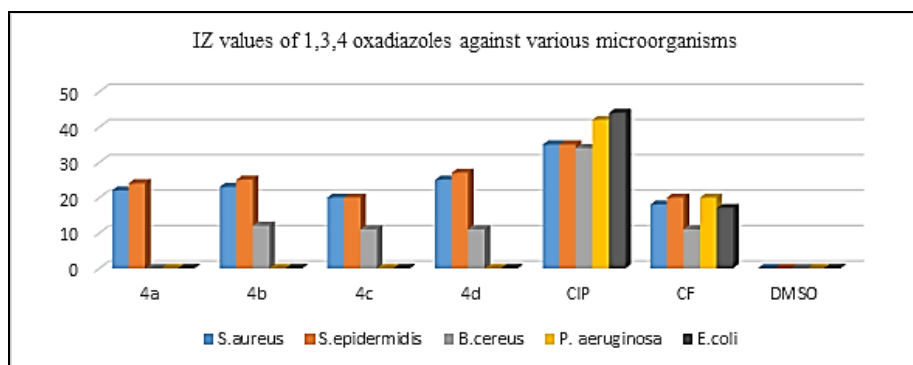
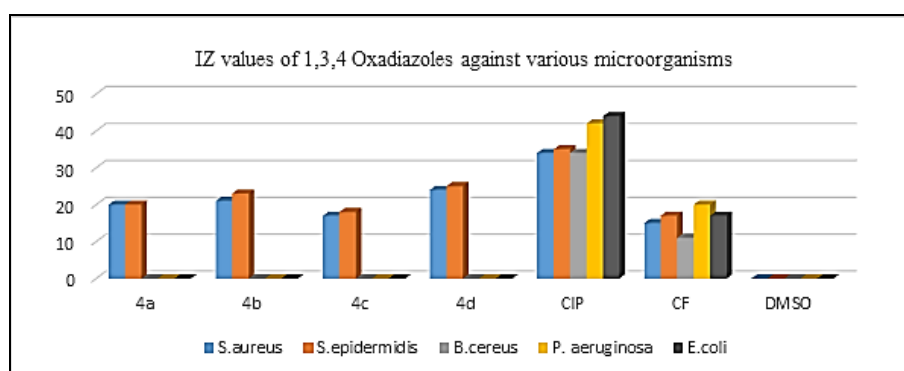


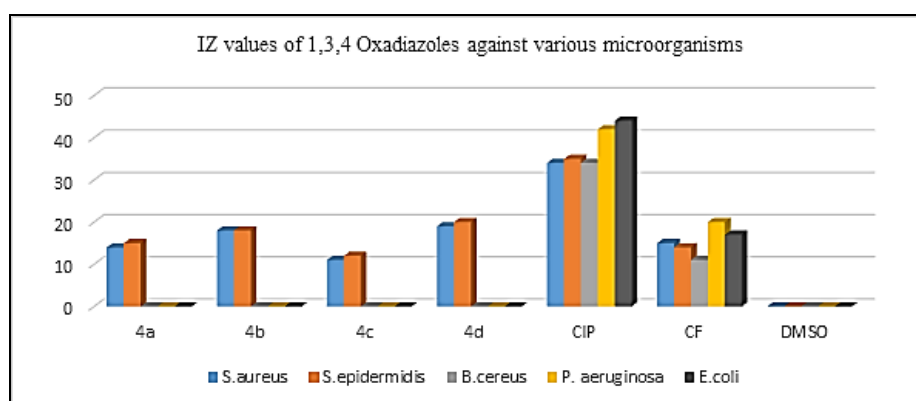
Figure 1: Anti-microbial activity of compounds against *Staphylococcus aureus* and *Staphylococcus epidermidis* in three different concentrations (5, 2.5 and 1 mg/ml)



Graph 1: Activation index against various microorganisms in concentration of 5mg/ml



Graph 2: Activation index against various microorganisms in concentration of 2.5mg/ml



Graph3: Activation index against various microorganisms in concentration of 1mg/ml

Table 1: In vitro antibacterial activity of (5-aryl-1,3,4-oxadiazol-2-yl) (pyridin-2-yl) methanol derivatives by broth dilution method($\mu\text{g/mL}$)

Compounds code	<i>E. coli</i> ATCC 2921	Microorganism MIC <i>P. aeruginosa</i> ATCCT 27853	<i>S. aureus</i> ATCC 25923	<i>S. epidermidis</i> ATCC 23074	<i>B. cereus</i> PTCC 1050
4a	500	1000	125	62.5	500
4b	500	1000	31.25	31.25	500
4c	1000	1000	62.5	250	500
4d	1000	1000	31.25	31.25	500
ceftizoxime	10	10	0.15	0.15	0.07
ciprofloxacin	0.07	10	0.039	0.039	0.039

MIC = minimum inhibitory concentration. Each experiment was performed in triplicate.

The antibacterial effects of compounds were compared with ceftizoxime and Ciprofloxacin as a reference. As can be deduced from tables, most of the synthesized compounds exhibited noticeable antibacterial activity

against gram-positive bacteria including *Staphylococcus epidermidis* and *Staphylococcus aureus* (figure 2). A comparative study of MIC values indicates that the inhibitory activity of these compounds on Gram positive bacteria is better than that on Gram-negative bacteria. (Table1). In this study, data suggest that compound (4a-4b) is a potent antibacterial compound with excellent MICs and acceptable selectivity index against Staphylococcal infections. Further focused analog synthesis and antitubercular/ antifungal activity studies are underway for compounds 4a-4d which may lead to new derivatives with enhanced efficiency against M. tuberculosis and fungal species.

CONCLUSION

The results of the determining MIC and IZ values revealed that all the synthesized compounds were fairly active against *Staphylococcus aureus* and *Staphylococcus epidermidis*.

The important point in this study is the synthesis of anti-bacterial effective oxadiazoles in a single-step, easy, highly effective and by using a fast reaction. So by some modifications, this method could be introduced as a cost-effective one in modern medicine and suitable molecular modification of these compounds that can generate potent antimicrobial agents in future.

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