# Studies of Biologically Active Heterocycles: Synthesis, Characterization and Antimicrobial Activity of Some 2,5-Disubstituted-1,3,4-Oxadiazoles

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**ABSTRACT:** 1,3,4-oxadiazoles are important because of its versatile biological actions. In the present study, several 2,5-disubstituted-1,3,4-oxadiazoles (**3a-o**) have been synthesized by the condensation of 4-hydroxybenzohydrazide (**1**) with various aromatic acids (**2a-o**) in presence of phosphorus oxychloride. The structures of the newly synthesized compounds have been established on the basis of elemental analysis, UV, IR and $^1$H NMR spectral data. The synthesized compounds were screened for their *in vitro* growth inhibiting activity against different strains of bacteria and fungi viz., *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus megaterium*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella dysenteriae*, *Candida albicans*, *Aspergillus niger* and *Aspergillus flavus* and the results were compared with the standard antibiotics such as chloramphenicol (50µg/ml) and Griseofulvin (50µg/ml) using agar diffusion technique. Compounds **3b**, **3e**, **3g**, **3h**, **3j**, **3m** and **3n** exhibited strong antibacterial activity and compounds **3a**, **3d**, **3g**, **3h** and **3i** showed good antifungal activity.

**Key words:** 2,5-disubstituted-1,3,4-oxadiazoles, 4-hydroxybenzohydrazide, phosphorus oxychloride, antimicrobial evaluation, agar well diffusion method.

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## INTRODUCTION

1,3,4-Oxadiazole is a versatile lead molecule for designing potential bioactive agents. The 1,3,4-oxadiazole derivatives have been found to exhibit diverse biological activities such as antimicrobial[1], anti-HIV[1], antitubercular[2], antimalarial[3], analgesic[4], anti-inflammatory[5], anticonvulsant[6], hypoglycemic[7] and other biological properties such as genotoxic studies[8] and lipid peroxidation inhibitor.[9]

Aryl alkanoic acids provide one of the most fascinating class of compounds recognized for various pharmacological actions like antipyretic, analgesic and anti-inflammatory activity[10], and are used extensively in the symptomatic treatment of rheumatic fever, arthritis[11] (rheumatoid, osteo and Jaundice arthritis), myocardial infarctions and management of primary dysmenorrheal.[12] The major side effects in the use of aryl alkanoic acids is their gastric irritation, which is partly due to the corrosive nature of carboxylic acid group present in them. In order to reduce or mask the side effects of carboxylic moiety we planned to synthesize different 2,5-disubstituted-1,3,4-oxadiazoles (**3a-o**) via the condensation of 4-hydroxybenzohydrazide with various aromatic acids in presence of phosphorus oxychloride respectively in the hope of getting potent biodynamic agents and evaluate their antimicrobial activity.

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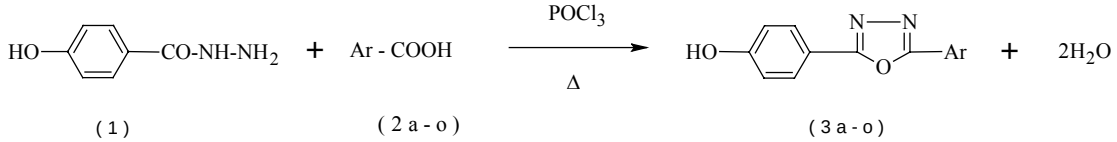
## MATERIALS AND METHODS

**General experimental procedure.** Column chromatography was carried out by using Merck silica gel 60. The solvents used for elution were petroleum ether and ethyl acetate (4:1% v/v). The purity of all the compounds were checked by TLC on precoated Silica-60$F_{254}$ plates (Merck Mumbai) using Iodine vapours and UV light as detecting agents with the help of mobile phase ethyl acetate: acetone (9:1). The $R_f$ value of the synthesized compounds were determined and the melting points of the synthesized compounds were recorded by open capillaries in a liquid paraffin bath and are uncorrected. The absorbance maxima ($\lambda_{max}$) of the synthesized compounds were determined on a Systronics UV-Visible double beam spectrophotometer (2201) in methanol. While the IR spectra of the synthesized compounds were recorded on a Perkin Elmer Spectrum RX I, FTIR spectrophotometer using potassium bromide (anhydrous IR grade) pellets. $^1$H NMR spectra were acquired on AMX-400, NMR spectrometer using DMSO-$d_6$ as solvent and TMS as an internal standard (chemical shift in δ ppm). The structures of the newly synthesized compounds were assigned on the basis of elemental analysis and were recorded on a Carlo Erba 1108 Heraeus at Regional Sophisticated Instrumentation Centre, CDRI, Lucknow. Carbon, hydrogen and nitrogen analyses were within ± 0.4% of the theoretical values. All the chemicals used were of synthetic and AR grade and was procured from Agros-Organics, USA, S.D. Fine Chem. Ltd., Mumbai and Merck, Mumbai, India.

**Synthesis of 2,5-disubstituted-1,3,4-oxadiazoles** (**3a-o**). A mixture of different aromatic acid(s) (0.01mol) with 4-hydroxybenzohydrazide (1.5215g, 0.01mol) in phosphorus oxychloride (15ml) was refluxed over a steam bath for 5-6 h. The progress of the reaction was monitored by TLC (on precoated silica 60$F_{254}$ plates Merck, Mumbai) using ethylacetate: acetone (9:1) as developing solvents. The reaction mixture was cooled and poured onto crushed ice (~200 g) with continuous stirring. The solid mass separated was neutralized with sodium bicarbonate solution (10% w/v). The resulting solid thus obtained was collected by filtration, washed well with cold water, dried in vaccum and crystallized from absolute ethanol (95%) and analyzed spectroscopically. Adopting the above procedure fifteen different 2,5-disubstituted-1,3,4-oxadiazoles (**3a-o**) were synthesized and their characterization data are presented in Table 1. Yield and melting point of the product(s) were determined and summarized in below.

**Scheme 1. Synthetic route for the preparation of some 2,5-disubstituted-1,3,4-oxadiazoles (3a-o).**




( 1 ) ( 2 a - o ) ( 3 a - o )

| Compound | Ar | Compound | Ar |
|---|---|---|---|
| **3a** | **$C_6H_5$** | **3i** | **$C_6H_5CONHC_6H_4$** |
| **3b** | **4-$CH_3C_6H_4$** | **3j** | **4-$OCH_3C_6H_4$** |
| **3c** | **CH=CH-$C_6H_5$** | **3k** | **3,4,5-$(OCH_3)_3C_6H_2$** |
| **3d** | **4-$NH_2C_6H_4$** | **3l** | **$C_5H_4N$** |
| **3e** | **4-$NO_2C_6H_4$** | **3m** | **2,4-$(OH)_2C_6H_3$** |
| **3f** | **3,5-$(NO_2)_2C_6H_3$** | **3n** | **3-$NH_2C_6H_4$** |
| **3g** | **2,4-$(NO_2)_2C_6H_3NHC_6H_4$** | **3o** | **2-OH3-$CH_3C_6H_3$** |
| **3h** | **2-$NO_2C_6H_4NHC_6H_4$** | | |

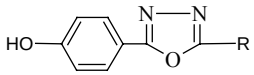
**4-(5-phenyl-1,3,4-oxadiazol-2-yl)phenol** (**3a).** Yield: 72.41% (1.68 g); mp: 212°C; $R_f$ value: 0.762; UV ($\lambda_{max}$, nm): 342.3; IR (KBr, cm$^{-1}$): 3601 (O-H stretch), 3045 (aromatic C-H stretch), 1602, 1498, 1470 (aromatic C=C ring stretch), 1634 (C=N stretch), 1220 (C-O stretch), 1247 (asymmetric C-O-C ring stretch), 1040 (symmetric C-O-C stretch), 1339, 1360 (in-plane O-H bend); $^1$H NMR (DMSO-

$d_6$, δ ppm): 6.87-7.12 (4H, m, aromatic protons), 7.27-7.41 (5H, m, aromatic protons), 5.57 (1H, s, Ar-OH).

**4-[5-(4-methylphenyl)-1,3,4-oxadiazol-2-yl]phenol (3b).** Yield: 76.66% (1.98 g); mp: 220°C; $R_f$ value: 0.851; UV ($\lambda_{max}$, nm): 270.2; IR (KBr, $cm^{-1}$): 3056 (aromatic C-H stretch), 3594 (O-H stretch), 1224 (C-O stretch), 1602, 1493, 1456 (aromatic C=C ring stretch), 1247 (asymmetric C-O-C stretch), 1040 (symmetric C-O-C stretch), 1642 (C=N stretch), 2960 (methyl C-H stretch γas $CH_3$), 2872 (methyl C-H stretch γs $CH_3$); $^1$H NMR (DMSO-$d_6$, δ ppm): 6.85-7.07 (4H, m, aromatic protons), 7.27-7.42 (4H, m, aromatic protons), 2.39 (3H, s, Ar-$CH_3$), 5.59 (1H, s, Ar-OH).

**4-{5-[(E)-2-phenylvinyl]-1,3,4-oxadiazol-2-yl}phenol (3c).** Yield: 79.46% (2.1 g); mp: 242°C; $R_f$ value: 0.842; UV ($\lambda_{max}$, nm): 261.2; IR (KBr, $cm^{-1}$): 3606 (O-H stretch), 3042 (aromatic C-H stretch), 1220 (C-O stretch), 1609, 1493, 1466 (aromatic C=C ring stretch), 1633 (C=N stretch), 3049 (alkene C-H stretch), 1252 (asymmetric C-O-C ring stretch), 1045 (symmetric C-O-C stretch), 1642 (C=C stretch, alkene), 1345, 1365 (in-plane O-H bend), 690 (out-of-plane C=C bend); $^1$H NMR (DMSO-$d_6$, δ ppm): 6.87-7.07 (4H, m, aromatic protons), 7.22-7.48 (5H, m, aromatic protons), 5.60 (1H, s, Ar-OH), 4.8-6.0 (2H, d, CH=CH).

**Table 1. Physical and analytical data of 2,5-disubstituted-1,3,4-oxadiazoles (3a-o).**




( 3a - o )

| Compound | R | Mol. for / Mol. wt | Elemental analysis found (calcd)% | | |
|---|---|---|---|---|---|
| | | | C | H | N |
| 3a | $C_6H_5$ | $C_{14}H_{10}N_2O_2$/238.24 | 70.54 (70.58 | 4.21 4.23 | 11.71 11.76) |
| 3b | 4-$CH_3C_6H_4$ | $C_{15}H_{12}N_2O_2$/252.26 | 71.38 (71.42 | 4.76 4.79 | 11.07 11.10) |
| 3c | CH=CH-$C_6H_5$ | $C_{16}H_{12}N_2O_2$/264.27 | 72.68 (72.72 | 4.54 4.58 | 10.55 10.60) |
| 3d | 4-$NH_2C_6H_4$ | $C_{14}H_{11}N_3O_2$/253.25 | 66.36 (66.40 | 4.34 4.38 | 16.55 16.59) |
| 3e | 4-$NO_2C_6H_4$ | $C_{14}H_9N_3O_4$/283.24 | 59.33 (59.36 | 3.18 3.20 | 14.81 14.83) |
| 3f | 3,5-$(NO_2)_2C_6H_3$ | $C_{14}H_8N_4O_6$/328.23 | 51.18 (51.23 | 2.42 2.46 | 17.02 17.07) |
| 3g | 2,4-$(NO_2)_2C_6H_3NHC_6H_4$ | $C_{20}H_{13}N_5O_6$/419.34 | 57.25 (57.28 | 3.10 3.12 | 16.67 16.70) |
| 3h | 2-$NO_2C_6H_4NHC_6H_4$ | $C_{20}H_{14}N_4O_4$/374.34 | 64.13 (64.17 | 3.72 3.77 | 14.94 14.97) |
| 3i | $C_6H_5CONHC_6H_4$ | $C_{21}H_{15}N_3O_3$/357.36 | 70.57 (70.58 | 4.21 4.23 | 11.72 11.76) |
| 3j | 4-$OCH_3C_6H_4$ | $C_{15}H_{12}N_2O_3$/268.26 | 67.13 (67.16 | 4.48 4.51 | 10.42 10.44) |
| 3k | 3,4,5-$(OCH_3)_3C_6H_2$ | $C_{17}H_{16}N_2O_5$/328.31 | 62.16 (62.19 | 4.89 4.91 | 8.51 8.53) |
| 3l | $C_5H_4N$ | $C_{13}H_9N_3O_2$/239.22 | 65.24 (65.27 | 3.78 3.79 | 17.54 17.56) |
| 3m | 2,4-$(OH)_2C_6H_3$ | $C_{14}H_{10}N_2O_4$/270.24 | 62.20 (62.22 | 3.72 3.73 | 10.35 10.37) |
| 3n | 3-$NH_2C_6H_4$ | $C_{14}H_{11}N_3O_2$/253.25 | 66.37 (66.40 | 4.36 4.38 | 16.56 16.59) |
| 3o | 2-OH3-$CH_3C_6H_3$ | $C_{15}H_{12}N_2O_3$/268.26 | 67.12 (67.16 | 4.50 4.51 | 10.42 10.44) |

**4-[5-(4-aminophenyl)-1,3,4-oxadiazol-2-yl]phenol (3d).** Yield: 84.89% (2.15 g); mp: 192°C; $R_f$ value: 0.823; UV ($\lambda_{max}$, nm): 263.6; IR (KBr, $cm^{-1}$): 3610 (O-H stretch), 3049 (aromatic C-H stretch), 1224 (C-O stretch), 1607, 1495, 1465 (aromatic C=C ring stretch), 1248 (asymmetric C-O-C ring stretch),

1040 (symmetric C-O-C stretch), 1644 (C=N stretch), 3520 (N-H stretch for aromatic primary amine, asymmetric), 3425 (N-H stretch, primary amine, symmetric), 1296 (aromatic C-N stretch for primary amine); $^1$H NMR (DMSO-$d_6$, δ ppm): 6.76-7.08 (4H, m, aromatic protons), 7.23-7.52 (4H, m, aromatic protons), 5.58 (1H, s, Ar-OH), 4.42 (2H, s, $NH_2$).

**4-[5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl]phenol (3e).** Yield: 74.14% (2.1 g); mp: 228°C; $R_f$ value: 0.726; UV ($\lambda_{max}$, nm): 273.2; IR (KBr, $cm^{-1}$): 3052 (aromatic C-H stretch), 3588 (O-H stretch), 1226 (C-O stretch), 1602, 1495, 1470 (C=C stretch), 1250 (asymmetric C-O-C stretch), 1045 (symmetric C-O-C stretch), 1636 (C=N stretch), 1523 (asymmetric $ArNO_2$, $NO_2$ stretch), 1347 (symmetric $ArNO_2$, $NO_2$ stretch), 852 (C-N stretch of $ArNO_2$); $^1$H NMR (DMSO-$d_6$, δ ppm): 6.78-7.01 (4H, m, aromatic protons), 7.27-7.62 (4H, m, aromatic protons), 5.57 (1H, s, Ar-OH).

**4-[5-(3,5-dinitrophenyl)-1,3,4-oxadiazol-2-yl]phenol (3f).** Yield: 73.78% (2.420 g); mp: 256°C; $R_f$ value: 0.812; UV ($\lambda_{max}$, nm): 245.6; IR (KBr, $cm^{-1}$): 3065 (aromatic C-H stretch), 3592 (Ar-OH stretch), 1224 (C-O stretch), 1602, 1493, 1455 (C=C ring stretch), 1256 (asymmetric C-O-C stretch), 1050 (symmetric C-O-C stretch), 1640 (C=N stretch), 1542 (asymmetric $ArNO_2$, $NO_2$ stretch), 1358 (symmetric $ArNO_2$, $NO_2$ stretch), 858 (C-N stretch of $ArNO_2$); $^1$H NMR (DMSO-$d_6$, δ ppm): 6.87-7.05 (4H, m, aromatic protons), 7.28-7.48 (3H, m, aromatic protons), 5.59 (1H, s, Ar-OH).

**4-(5-{4-[(2,4-dinitrophenyl)amino]phenyl}-1,3,4-oxadiazol-2-yl)phenol (3g).** Yield: 82.27% (3.45 g); mp: 248°C; $R_f$ value: 0.762; UV ($\lambda_{max}$, nm): 263.4; IR (KBr, $cm^{-1}$): 3030 (aromatic C-H stretch), 3595 (O-H stretch), 1226 (C-O stretch), 1596, 1492, 1458 (C=C ring stretch), 1248 (asymmetric C-O-C stretch), 1047 (symmetric C-O-C stretch), 1647 (C=N stretch), 1525 (asymmetric $ArNO_2$, $NO_2$ stretch), 1342 (symmetric $ArNO_2$, $NO_2$ stretch), 854 (C-N stretch of $ArNO_2$), 3325 (N-H stretch, aromatic secondary amine), 1298 (C-N stretch for secondary aromatic amine), 1358 (in-plane O-H bend); $^1$H NMR (DMSO-$d_6$, δ ppm): 6.81-7.04 (4H, m, aromatic protons), 7.18-7.61 (7H, m, aromatic protons), 5.58 (1H, s, Ar-OH), 2.18 (1H, s, NH).

**4-(5-{4-[(2-nitrophenyl)amino]phenyl}-1,3,4-oxadiazol-2-yl)phenol (3h).** Yield: 75.86% (2.84 g); mp: 218°C; $R_f$ value: 0.824; UV ($\lambda_{max}$, nm): 261.6; IR (KBr, $cm^{-1}$): 3070 (aromatic C-H stretch), 3590 (O-H stretch), 1230 (C-O stretch), 1601, 1495, 1474 (C=C ring stretch), 1242 (asymmetric C-O-C stretch), 1041 (symmetric C-O-C stretch), 1636 (C=N stretch), 1538 (asymmetric $ArNO_2$, $NO_2$ stretch), 1350 (symmetric $ArNO_2$, $NO_2$ stretch), 852 (C-N stretch of $ArNO_2$), 3330 (N-H stretch, aromatic secondary amine), 1293 (C-N stretch for secondary aromatic amine), 1338, 1418 (in-plane O-H bend); $^1$H NMR (DMSO-$d_6$, δ ppm): 6.79-7.0 (4H, m, aromatic protons), 7.21-7.48 (8H, m, aromatic protons), 5.59 (1H, s, Ar-OH), 2.21 (1H, s, NH).

**N-{4-[5-(4-hydroxyphenyl)-1,3,4-oxadiazol-2-yl]phenyl}benzamide (3i).** Yield: 81.15% (2.9 g); mp: 250°C; $R_f$ value: 0.782; UV ($\lambda_{max}$, nm): 268.6; IR (KBr, $cm^{-1}$): 3048 (aromatic C-H stretch), 3589 (O-H stretch), 1227 (C-O stretch), 1609, 1494, 1460 (C=C ring stretch), 1258 (asymmetric C-O-C stretch), 1049 (symmetric C-O-C stretch), 1645 (C=N stretch), 3432 (N-H stretch, secondary amide), 1285 (aromatic C-N stretch for secondary amide), 1642 (C=O stretch, amide), 1346, 1420 (in-plane O-H bend); $^1$H NMR (DMSO-$d_6$, δ ppm): 6.87-7.07 (4H, m, aromatic protons), 7.23-7.48 (9H, m, aromatic protons), 5.57 (1H, s, Ar-OH), 8.48 (1H, s, CONH).

**4-[5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl]phenol (3j).** Yield: 78.28% (2.1 g); mp: 232°C; $R_f$ value: 0.794; UV ($\lambda_{max}$, nm): 275.6; IR (KBr, $cm^{-1}$): 3048 (aromatic C-H stretch), 3604 (O-H stretch), 1227 (C-O stretch), 1595, 1499, 1472 (aromatic C=C ring stretch), 1249 (asymmetric C-O-C stretch), 1042 (symmetric C-O-C stretch), 1648 (C=N stretch), 2950 (C-H stretch γas $CH_3$), 2835 (C-H stretch γsy $CH_3$), 1224 (C-O stretch); $^1$H NMR (DMSO-$d_6$, δ ppm): 6.87-7.18 (4H, m, aromatic protons), 3.85 (3H, s, $OCH_3$), 7.23-7.48 (4H, m, aromatic protons), 5.57 (1H, s, Ar-OH).

**4-[5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-yl]phenol (3k).** Yield: 88.82% (2.90 g); mp:

244°C; $R_f$ value: 0.813; UV ($\lambda_{max}$, nm): 302.2; IR (KBr, $cm^{-1}$): 3060, 3039 (aromatic C-H stretch), 3591 (O-H stretch), 1598, 1493, 1455 (aromatic C=C ring stretch), 1236 (asymmetric C-O-C stretch), 1038 (symmetric C-O-C stretch), 1636 (C=N stretch), 2954 (C-H stretch γas $CH_3$), 2840 (C-H stretch γs $CH_3$), 1219 (C-O stretch), 830 (out-of-plane aromatic C-H bend); $^1$H NMR (DMSO-$d_6$, δ ppm): 6.37-6.87 (4H, m, aromatic protons), 6.92-7.21 (2H, m, aromatic protons), 3.86 (9H, s, $OCH_3$), 5.56 (1H, s, OH).

**4-(5-pyridinyl-3-yl-1,3,4-oxadiazol-2-yl)phenol (3l).** Yield: 64.85% (1.55 g); mp: 254°C; $R_f$ value: 0.782; UV ($\lambda_{max}$, nm): 279.0; IR (KBr, $cm^{-1}$): 3080 (aromatic C-H stretch), 3605 (O-H stretch), 1222 (C-O stretch), 1604, 1499, 1472 (aromatic C=C ring stretch), 1252 (asymmetric C-O-C stretch), 1049 (symmetric C-O-C stretch), 1647 (C=N stretch), 750 (out-of-plane aromatic C-H bend); $^1$H NMR (DMSO-$d_6$, δ ppm): 6.92-7.07 (4H, m, aromatic protons), 7.11-7.42 (4H, m, aromatic protons), 5.55 (1H, s, OH).

**4-[5-(4-hydroxyphenyl)-1,3,4-oxadiazol-2-yl]benzene-1,3-diol (3m).** Yield: 75.85% (2.050 g); mp: 292°C; $R_f$ value: 0.826; UV ($\lambda_{max}$, nm): 277.0; IR (KBr, $cm^{-1}$): 3046 (aromatic C-H stretch), 3596 (O-H stretch), 1224 (C-O stretch), 1598, 1497, 1473 (aromatic C=C ring stretch), 1249 (asymmetric C-O-C stretch), 1036 (symmetric C-O-C stretch), 1635 (C=N stretch); $^1$H NMR (DMSO-$d_6$, δ ppm): 6.92-7.03 (4H, m, aromatic protons), 7.27-7.32 (3H, m, aromatic protons), 5.59 (3H, s, OH).

**4-[5-(3-aminophenyl)-1,3,4-oxadiazol-2-yl]phenol (3n).** Yield: 70.28% (1.78 g); mp: 210°C; $R_f$ value: 0.698; UV ($\lambda_{max}$, nm): 286.0; IR (KBr, $cm^{-1}$): 3602 (O-H stretch), 3042 (aromatic C-H stretch), 1230 (C-O stretch), 1604, 1490, 1462 (aromatic C=C ring stretch), 1252 (asymmetric C-O-C ring stretch), 1039 (symmetric C-O-C stretch), 1646 (C=N stretch), 3515 (N-H stretch for aromatic primary amine, asymmetric), 3421 (N-H stretch, primary amine, symmetric), 1290 (C-N stretch for aromatic primary amine); $^1$H NMR (DMSO-$d_6$, δ ppm): 6.92-7.13 (4H, m, aromatic protons), 7.13-7.4 (4H, m, aromatic protons), 4.40 (2H, s, $NH_2$), 5.60 (1H, s, OH).

**2-[5-(4-hydroxyphenyl)-1,3,4-oxadiazol-2-yl]-6-methylphenol (3o).** Yield: 68.96% (1.85 g); mp: 221°C; $R_f$ value: 0.782; UV ($\lambda_{max}$, nm): 282.0; IR (KBr, $cm^{-1}$): 3045 (aromatic C-H stretch), 3599 (O-H stretch), 1228 (C-O stretch), 1604, 1491, 1462 (aromatic C=C ring stretch), 1246 (asymmetric C-O-C stretch), 1038 (symmetric C-O-C stretch), 1654 (C=N stretch), 2962 (methyl C-H stretch γas $CH_3$), 2872 (methyl C-H stretch γs $CH_3$), 1450 (C-H bend δas $CH_3$), 1378 (C-H bend δs $CH_3$), 3342 (N-H stretch, secondary amide); $^1$H NMR (DMSO-$d_6$, δ ppm): 6.67-7.03 (3H, m, aromatic protons), 7.11-7.38 (4H, m, aromatic protons), 5.59 (2H, s, Ar-OH), 2.38 (3H, s, Ar-$CH_3$).

**Screening for Antimicrobial activity**. The antimicrobial activity of all the newly synthesized compounds (3a-o) was determined by well plate method[13] in nutrient agar (Hi-Media) (antibacterial activity) and Sabouraud dextrose agar (SDA) (Hi-Media) (antifungal activity). The *in vitro* antimicrobial activity was carried out against 24h old cultures of bacterial and 72h old cultures of fungal strain. The bacterial and fungal strains for the study are listed in Tables 2 and 3.

Pure cultures of the test microorganisms were procured from Universal supplies, Coimbatore, Tamilnadu. The compounds were tested at a concentration of 100 µg/ml and solutions were prepared were prepared in dimethyl formamide (DMF). The petridishes used for antibacterial screening were incubated at 37±1º C for 24h, while those used for antifungal activity were incubated at 28ºC for 48-72h. The results were compared to Chloramphenicol (50µg/ml) and Griseofulvin (50µg/ml) for antibacterial and antifungal activity respectively by measuring zone of inhibition in mm. The antibacterial and antifungal screening results were presented in Table 2 and Table 3.

**Table 2. Antibacterial screening results of 2,5-disubstituted-1,3,4-oxadiazoles (3a-o).**

| Compound | Antibacterial activity | | | | | |
|---|---|---|---|---|---|---|
| | Zone of inhibition (mm) | | | | | |
| | *Staphylococcus aureus* (ATCC 25923) | *Bacillus subtilis* (ATCC 6633) | *Bacillus megaterium* (ATCC 1327) | *Escherichia coli* (ATCC 25922) | *Pseudomonas aeruginosa* (ATCC 27853) | *Shigella dysenteriae* (ATCC 13313) |
| 3a | 14 | 13 | 13 | 10 | 15 | 12 |
| 3b | 18 | 21 | 17 | 21 | 17 | 15 |
| 3c | 14 | 13 | 15 | 13 | 12 | 14 |
| 3d | 15 | 17 | 16 | 20 | 15 | 14 |
| 3e | 19 | 24 | 20 | 22 | 18 | 15 |
| 3f | 15 | 16 | 16 | 16 | 14 | 12 |
| 3g | 19 | 23 | 21 | 23 | 18 | 16 |
| 3h | 17 | 21 | 19 | 21 | 17 | 15 |
| 3i | 15 | 17 | 16 | 17 | 14 | 15 |
| 3j | 16 | 22 | 17 | 21 | 17 | 14 |
| 3k | 14 | 16 | 14 | 18 | 14 | 15 |
| 3l | 11 | 12 | 10 | 09 | 12 | 11 |
| 3m | 17 | 22 | 16 | 22 | 17 | 15 |
| 3n | 19 | 21 | 17 | 21 | 15 | 15 |
| 3o | 15 | 19 | 16 | 17 | 14 | 15 |
| Chloramphenicol (50 µg/ml) | 20 | 24 | 21 | 24 | 19 | 17 |

**Table 3. Antifungal screening results of 2,5-disubstituted-1,3,4-oxadiazoles** (**3a-o**).

| Compound | Antifungal activity | | |
|---|---|---|---|
| | Zone of inhibition (mm) | | |
| | *Candida albicans* (ATCC 10231) | *Aspergillus niger* (ATCC 16404) | *Aspergillus flavus* (ATCC 22547) |
| 3a | 18 | 17 | 16 |
| 3b | 15 | 14 | 13 |
| 3c | 14 | 13 | 12 |
| 3d | 16 | 17 | 17 |
| 3e | 18 | 18 | 15 |
| 3f | 14 | 16 | 14 |
| 3g | 19 | 19 | 17 |
| 3h | 17 | 18 | 17 |
| 3i | 17 | 18 | 18 |
| 3j | 15 | 14 | 16 |
| 3k | 16 | 17 | 16 |
| 3l | 12 | 12 | 13 |
| 3m | 15 | 14 | 13 |
| 3n | 17 | 17 | 16 |
| 3o | 15 | 14 | 14 |
| Griseofulvin (50µg/ml) | 23 | 21 | 19 |

## RESULTS AND DISCUSSION

The results of antimicrobial activity indicated that compound **3b**, **3e**, **3g**, **3h**, **3j**, **3m** and **3n** were found to be active against *Bacillus subtilis*, *Staphylococcus aureus* and *Bacillus megaterium* whereas compound **3b**, **3d**, **3e**, **3g**, **3h**, **3j** and **3m** exhibit significant antibacterial activity against *Escherichia coli* and *Pseudomonas aeruginosa* and *Shigella dysenteriae* at 100µg/ml concentration as compared to chloramphenicol (50µg/ml). The screening results revealed that compounds **3a**, **3d**, **3e**, **3g**, **3h**, **3i**, **3k** and **3n** displayed better antifungal activity against *Candida albicans*, *Aspergillus niger* and *Aspergillus flavus*.

The antimicrobial activity of the compounds varied upon the type and position of the substituents at 5-(4-hydroxyphenyl)-1,3,4-oxadiazole moiety. It can be concluded from the antimicrobial screening results that when 5-(4-hydroxyphenyl)-1,3,4-oxadiazole were substituted with p-tolulyl, 4-nitrophenyl, 4-methoxyphenyl, 4-aminophenyl, 2-nitrophenyl anilinyl, 2,4-dinitrophenyl anilinyl, 2,4-

dihydroxy phenyl and 3-aminophenyl at 2nd position, the antimicrobial activity was altered to an appreciable extent.

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