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Synthesis and Antimicrobial Evaluation of 1,3,4-Oxadiazole bearing Schiff Base Moiety

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ABSTRACT

New series of 4-((5-(2-chlorophenyl)-1,3,4-oxadiazol-2-yl)methoxy)-*N*-(benzylidene derivatives)benzenamine **(5a-k)** have been synthesized and were screened for their *in vitro* antibacterial activity against Gram-positive bacteria (*Pseudomonas aeruginosa, Streptococcus pyogenes*), Gram-negative bacteria (*Escherichia coli, Staphylococcus aureus*) and antifungal activity (*Candida albicans, Aspergillus niger, Aspergillus clavatus*). Synthesized compounds were characterized by IR, mass (MS), ^{1}H NMR and ^{13}C NMR spectra. The synthesized compounds **5b**, **5c**, **5g** and **5i** showed potency in terms of antimicrobial activity against tested microorganisms.

KEYWORDS

1,3,4-Oxadiazole, Schiff base, Antimicrobial evaluation, MIC.

INTRODUCTION

There is a growing interest in recent years in the synthesis of oxadiazole based heterocycles because of the significant role of the oxadiazole unit [1]. The 1,3,4-oxadiazole scaffold is a useful structural motif for displaying chemical functionality in biologically active compounds [2]. Furthermore, 1,3,4-oxadiazole have been used as "honored" scaffolds to produce substances of interest in numerous therapeutic areas, such as antibacterial [3], anti-inflammatory [4], antioxidant [5], analgesic [6], antituberculosis [7], anticonvulsant [8], antiviral [9], anticancer [10] and many others [11]. Hence, 1,3,4-oxadiazole have occupied an exclusive place in the field of medicinal chemistry and literature review suggest to generate novel scaffolds to increase the potency. The well-known example of 1,3,4-oxadiazole based drug is "Nesapidil" (Fig. 1), which is used as an antihypertensive agent [12].

As an effort to formulate and generate variety of novel heterocycles based on nitrogen and oxygen [13], we aimed in

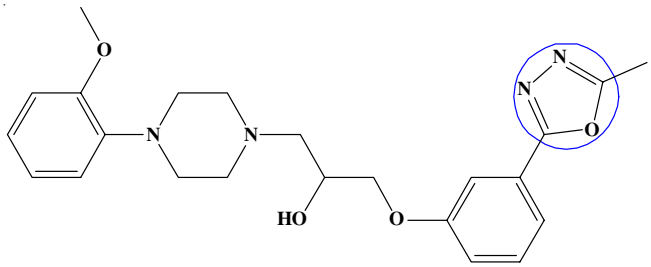


Fig. 1. Drug with 1,3,4-oxadiazole nucleus available in market (Nesapidil)

the paper on medicinal importance 1,3,4-oxadiazole. We report herein the synthesis of a new class of 4-((5-(2-chlorophenyl)-1,3,4-oxadiazol-2-yl)methoxy)-*N*-(benzylidenederivatives) benzenamine **(5a-k)** and try to develop potential antimicrobials. The structure of newly synthesized compounds was elucidated based on various spectral analyses. The synthesized molecules were evaluated for their antimicrobial screening on various strains of bacteria and fungi.

EXPERIMENTAL

All the chemicals and reagents were purchased from Sigma-Aldrich and HIMEDIA. The completion of the reaction was monitored by TLC using various solvent systems and visualized under ultraviolet (UV) light or iodine vapour. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker instrument in DMSO-d_6 as solvent and tetramethylsilane (TMS) as an internal standard. Chemical shifts are reported in parts per million (δ ppm). Mass spectrometer GCMS-QP 2010 (Shimadzu) was used to resolute the mass spectra of compounds and Bookie Rota vapor was used for drying the compounds. Melting point of all the synthesized compounds was carried in open capillaries and is uncorrected.

General procedure

Synthesis of 2-chlorobenzohydrazide (2): In a 100 mL conical flask, a solution of methyl 2-chlorobenzoate (**1**) (0.0058 mol, 1.0 g) and hydrazine hydrate (0.024 mol, 1.19 g, 4 eq.) in methanol were refluxed for 5 h. The progress of the reaction was monitored by thin layer chromatography (TLC). After completion of the reaction, the methanol was distilled off and then cooled to room temperature. Filtration of isolated solid was carried out on Whatman filter paper and washed with ice-cold water. The product obtained was dried and recrystallized from alcohol. The obtained product was used directly for the next step. Confirmation of the intermediate 2-chlorobenzohydrazide **(2)** was carried out using ^{1}H NMR spectrum of compound. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 3.18 (s, 2H, -NH_2 proton), 6.47-7.87 (m, 4H, Ar-H proton) 9.27 (s, 1H, -NH proton).

Synthesis of 2-(chloromethyl)-5-(2-chlorophenyl)-1,3,4-oxadiazole (3): A mixture containing 2-chlorobenzohydrazide **(2)** (0.0058 mol, 1.0 g) and chloroacetic acid (0.016 mol, 1.09 g, 2 eq.) in phosphorous oxychloride (7 mL) was refluxed for 4 h and monitored by TLC using mobile phase ethyl acetate:*n*-hexane (3:7). The final product thus obtained were poured into ice cold water and stirred for 30 min. The separated products were filtered using vaccum filtration apparatus and washed with cold water. The compound was recrystallized from alcohol. The intermediate 2-(chloromethyl)-5-(2-chlorophenyl)-1,3,4-oxadiazole (**3**) was confirmed by ^{1}H NMR spectrum of compound. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 4.84 (s, 2H, -CH_2 proton), 6.88-7.64 (m, 4H, Ar-H proton).

Synthesis of 4-((5-(2-chlorophenyl)-1,3,4-oxadiazol-2-yl)methoxy)benzenamine (4): In a round bottom flask (moisture free) containing 2-(chloromethyl)-5-(2-chlorophenyl)-1,3,4-oxadiazole **(3)** (0.0044 mol, 1.0 g) in dimethyl formamide and 4-aminophenol (0.0044 mol, 0.47 g) were added. Dry powder of K_2CO_3 (0.0088 mol, 1.2 g) was added to neutralize the liberated hydrochloric acid during the reaction. This mixture was allowed to be refluxed for 3 h. The resulting material was poured onto crushed ice and stirred well for 30 min. The solid separated out was filtered and washed with cold water. The product obtained was dried and recrystallized from ethyl acetate. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 4.14 (s, 2H, -NH_2 proton), 5.36 (s, 2H, -CH_2 proton), 6.38-7.63 (m, 8H, Ar-H proton).

General procedure for the synthesis of 4-((5-(2-chlorophenyl)-1,3,4-oxadiazol-2-yl)methoxy)-*N*-(benzylidene derivatives)benzenamine (5a-k): Intermediate **4** and substituted aromatic amine (0.005 mol) in methanol (20 mL) were taken in a round-bottom flask and refluxed for 12 h. The progress of the reaction was carried out by TLC. The separated solid was filtered, dried and recrystallized from ethyl acetate. All other compounds of this series were synthesized using the same route (**Scheme-I**).

Physical and analytical data

4-((5-(2-Chlorophenyl)-1,3,4-oxadiazol-2-yl)methoxy)-*N*-benzylidenebenzenamine (5a): Yield: 82 %; m.p.: 162 °C; IR (ATR, ν_{max}, cm^{-1}): 3053.15 (C-H, aromatic), 1637.53 (C=C), 1561.82 (C=N), 1324.25 (C-O), 1281.45, 1030.67 (C-O-C, oxadiazole ring), 769.60 (C–Cl); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 4.56 (s, 2H, -CH_2), 6.78-7.80 (m, 13H, Ar-H), 9.25 (s, 1H, CH=N); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 173.07, 169.23, 165.13, 160.25, 156.13, 141.30, 138.35, 135.08, 134.13, 133.78, 131.21, 131.21, 130.46, 130.46, 128.52, 127.65, 125.50, 120.54, 120.54, 118.31, 118.31, 53.23; MS (*m/z*): 389.25.

4-((5-(2-Chlorophenyl)-1,3,4-oxadiazol-2-yl)methoxy)-*N*-(4-fluorobenzylidene)benzenamine (5b): Yield: 72 %; m.p.: 174 °C; IR (ATR, ν_{max}, cm^{-1}): 3120.25 (C-H, aromatic), 1687.18 (C=C), 1578.57 (C=N), 1280.91 (C-O), 1275.30, 1081.17 (C-O-C, oxadiazole ring), 1085.46 (C-F), 749.19 (C–Cl); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 4.14 (s, 2H, -CH_2), 6.45-7.67 (m, 12H, Ar-H), 9.65 (s, 1H, CH=N); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 175.13, 172.78, 168.28, 162.15, 158.90, 150.78, 142.18, 138.11, 138.11, 135.70, 133.37, 132.12, 129.60, 128.13, 126.65, 124.23, 124.23, 118.91, 118.91, 114.73, 114.73, 54.18; MS (*m/z*): 407.48.

4-((5-(2-Chlorophenyl)-1,3,4-oxadiazol-2-yl)methoxy)-*N*-(4-nitrobenzylidene)benzenamine (5c): Yield: 78 %; m.p.: 166 °C; IR (ATR, ν_{max}, cm^{-1}): 3187.51 (C-H, aromatic), 1621.20 (C=C), 1545.73 (C=N), 1488.35, 1349.68 (C-NO_2) 1210.45 (C-O), 1245.44, 1056.47 (C-O-C, oxadiazole ring), 749.19 (C–Cl); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 4.38 (s, 2H, -CH_2), 6.53-7.83 (m, 12H, Ar-H), 9.32 (s, 1H, CH=N); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 173.14, 168.28, 162.78, 159.16, 155.79, 150.46, 147.19, 145.89, 141.13, 141.13, 136.56, 135.48, 132.62, 131.13, 127.34, 126.18, 126.18, 120.63, 120.63, 117.90, 117.90, 53.48; MS (*m/z*): 434.65.

4-((5-(2-Chlorophenyl)-1,3,4-oxadiazol-2-yl)methoxy)-*N*-(3-nitrobenzylidene)benzenamine (5d): Yield: 73 %; m.p.: 192 °C; IR (ATR, ν_{max}, cm^{-1}): 3296.15 (C-H, aromatic), 1613.48 (C=C), 1522.77 (C=N), 1481.58, 1353.08 (C-NO_2) 1178.96 (C-O), 1273.19, 1022.61 (C-O-C, oxadiazole ring), 679.71 (C–Cl); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 4.27 (s, 2H, -CH_2), 6.67-7.48 (m, 12H, Ar-H), 9.47 (s, 1H, CH=N); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 173.78, 165.13, 162.47, 160.45,

157.88, 152.63, 148.18, 146.70, 143.18, 141.20, 137.65, 136.84, 131.69, 128.06, 126.19, 124.78, 123.89, 120.20, 120.20, 117.95, 117.95, 53.78; MS (*m/z*): 434.78.

4-((5-(2-Chlorophenyl)-1,3,4-oxadiazol-2-yl)methoxy)-*N*-(2-nitrobenzylidene)benzenamine (5e): Yield: 88 %; m.p.: 212 °C; IR (ATR, ν_{max}, cm^{-1}): 3155.36 (C-H, aromatic), 1674.62 (C=C), 1558.92 (C=N), 1475.17, 1337.41 (C-NO_2) 1190.77 (C-O), 1210.34, 1079.27 (C-O-C, oxadiazole ring), 715.25 (C–Cl); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 4.18 (s, 2H, -CH_2), 6.81-7.79 (m, 12H, Ar-H), 9.86 (s, 1H, CH=N); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 173.45, 162.78, 160.30, 158.41, 153.83, 152.74, 141.30, 140.41, 139.67, 135.58, 133.63, 133.63, 130.78, 128.61, 127.63, 126.35, 125.28, 122.68, 122.68, 116.36, 116.36, 53.69; MS (*m/z*): 434.13.

4-((5-(2-Chlorophenyl)-1,3,4-oxadiazol-2-yl)methoxy)-*N*-(3-chlorobenzylidene)benzenamine (5f): Yield: 83 %; m.p.: 230 °C; IR (ATR, ν_{max}, cm^{-1}): 3147.61 (C-H, aromatic), 1689.18 (C=C), 1514.21 (C=N), 1281.72 (C-O), 1263.23, 1023.85 (C-O-C, oxadiazole ring), 673.87 (C–Cl); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 4.68 (s, 2H, -CH_2), 6.48-7.69 (m, 12H, Ar-H), 9.49 (s, 1H, CH=N); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 174.15, 169.29, 160.12, 156.94, 151.13, 140.16, 139.39, 135.44, 134.68, 133.36, 132.21, 130.68, 130.79, 128.61, 127.83, 127.45, 125.43, 120.73, 120.73, 116.58, 116.58, 54.30; MS (*m/z*): 424.68.

4-((5-(2-Chlorophenyl)-1,3,4-oxadiazol-2-yl)methoxy)-*N*-(4-chlorobenzylidene)benzenamine (5g): Yield: 70 %; m.p.: 180 °C; IR (ATR, ν_{max}, cm^{-1}): 3215.52 (C-H, aromatic), 1677.26 (C=C), 1559.49 (C=N), 1279.57 (C-O), 1243.33, 1017.20 (C-O-C, oxadiazole ring), 695.38 (C–Cl); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 4.20 (s, 2H, -CH_2), 6.55-7.78 (m, 12H, Ar-H), 9.63 (s, 1H, CH=N); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 174.68, 168.29, 159.15, 155.35, 147.37, 139.63, 135.45, 134.51, 133.38, 132.51, 132.51, 130.12, 128.53, 129.53, 125.63, 125.95, 126.48, 122.43, 122.43, 117.68, 117.68, 54.19; MS (*m/z*): 424.71.

4-((5-(2-Chlorophenyl)-1,3,4-oxadiazol-2-yl)methoxy)-*N*-(4-methylbenzylidene)benzenamine (5h): Yield: 75 %; m.p.: 214 °C; IR (ATR, ν_{max}, cm^{-1}): 3251.12 (C-H, aromatic), 2945.96 (C-CH_3), 1637.63 (C=C), 1563.70 (C=N), 1221.44 (C-O), 1213.36, 1087.18 (C-O-C, oxadiazole ring), 715.46 (C–Cl); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 3.12 (s, 3H, -CH_3), 4.35 (s, 2H, -CH_2), 6.34-7.57 (m, 12H, Ar-H), 9.41 (s, 1H, CH=N); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 173.54, 165.36, 162.11, 155.94, 149.51, 145.65, 139.51, 135.87, 134.63, 133.21, 131.91, 131.91, 128.21, 128.58, 127.16, 127.91, 125.43, 122.18, 122.18, 115.30, 115.30, 52.51, 23.38; MS (*m/z*): 403.12.

4-((5-(2-Chlorophenyl)-1,3,4-oxadiazol-2-yl)methoxy)-*N*-(3-methylbenzylidene)benzenamine (5i): Yield: 86 %; m.p.: 248 °C; IR (ATR, ν_{max}, cm^{-1}): 3287.52 (C-H, aromatic), 2968.71 (C-CH_3), 1673.61 (C=C), 1522.18 (C=N), 1281.07 (C-O), 1267.74, 1038.43 (C-O-C, oxadiazole ring), 761.17 (C–Cl); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 3.47 (s, 3H, -CH_3), 4.48 (s, 2H, -CH_2), 6.47-7.79 (m, 12H, Ar-H), 9.56 (s, 1H, CH=N); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 173.67, 166.63, 161.12, 155.53, 150.13, 145.76, 138.11, 135.56, 134.83, 133.63, 132.82, 131.67, 129.20, 128.62, 127.76, 125.11, 125.86, 120.23, 120.23, 116.38, 116.38, 53.68, 23.44; MS (*m/z*): 403.49.

4-((5-(2-Chlorophenyl)-1,3,4-oxadiazol-2-yl)methoxy)-*N*-(2-methylbenzylidene)benzenamine (5j): Yield: 69 %; m.p.: 240 °C; IR (ATR, ν_{max}, cm^{-1}): 3251.63 (C-H, aromatic), 2911.48 (C-CH_3), 1648.16 (C=C), 1515.74 (C=N), 1219.13 (C-O), 1248.63, 1012.16 (C-O-C, oxadiazole ring), 710.35 (C–Cl); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 3.35 (s, 3H, -CH_3), 4.67 (s, 2H, -CH_2), 6.66-7.83 (m, 12H, Ar-H), 9.73 (s, 1H, CH=N); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 172.30, 164.51, 160.13, 156.39, 148.35, 139.51, 138.86, 137.51, 135.68, 133.63, 132.21, 131.51, 130.73, 127.21, 127.88, 126.12, 125.41, 120.53, 120.53, 114.51, 114.51, 53.11, 22.45; MS (*m/z*): 403.71.

4-((5-(2-Chlorophenyl)-1,3,4-oxadiazol-2-yl)methoxy)-*N*-(4-methoxybenzylidene)benzene amine (5k): Yield: 74 %; m.p.: 206 °C; IR (ATR, ν_{max}, cm^{-1}): 3289.15 (C-H, aromatic), 2887.51 (C-H, -OCH_3), 1615.71 (C=C), 1571.13 (C=N), 1235.70 (C-O), 1277.11, 1083.41 (C-O-C, oxadiazole ring), 748.63 (C–Cl); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 3.56 (s, 3H, -OCH_3), 4.18 (s, 2H, -CH_2), 6.78-7.48 (m, 12H, Ar-H), 9.81 (s, 1H, CH=N); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 173.18, 168.35, 165.92, 160.13, 156.39, 147.13, 139.83, 135.23, 135.23, 133.37, 132.61, 129.13, 128.64, 127.13, 126.43, 122.81, 122.81, 118.16, 118.16, 113.49, 113.49, 53.45, 52.11; MS (*m/z*): 419.46.

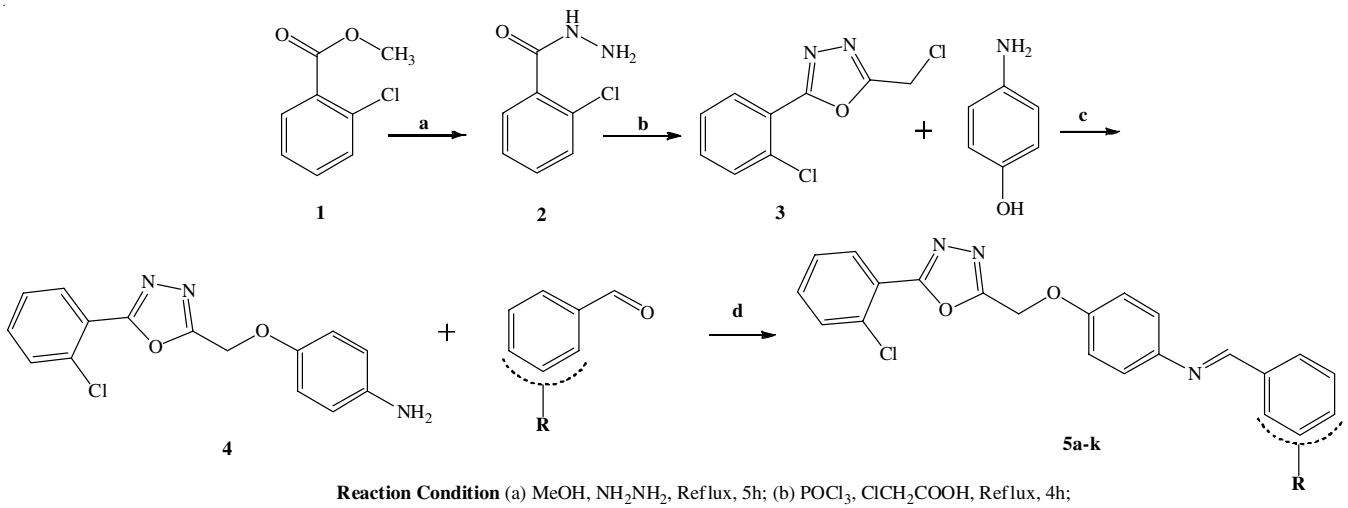
RESULTS AND DISCUSSION

The novel series of 4-((5-(2-chlorophenyl)-1,3,4-oxadiazol-2-yl)methoxy)-*N*-(benzylidene derivatives)benzenamine were achieved by sequence of reactions exhibited in **Scheme-I**.

Methyl 2-chlorobenzoate was selected as starting material for the synthesis of target compounds. The ester was converted into hydrazide **2** (85 %), using hydrazine followed by treatment of intermediate 2 with $POCl_3$ and chloroacetylchloride to afford 2-(chloromethyl)-5-(2-chlorophenyl)-1,3,4-oxadiazole (79 %), core molecule **3**. Oxadiazole intermediate **3** gave the 4-((5-(2-chlorophenyl)-1,3,4-oxadiazol-2-yl)methoxy)benzenamine (**4**) in 92 % yield by reaction with *p*-hydroxyaniline. RT stirring of intermediate compounds **4** with substituted benzaldehyde without any catalyst/reagents furnished 1,3,4-oxadiazole derivatives **5a-k** in 69-88 % yield. The synthetic reactions are summarized in **Scheme-I**.

Spectral discussion: The synthesis of **2** was confirmed by ^{1}H NMR spectra. The ^{1}H NMR spectrum exhibited two singlets at 3.18 and 9.27 δ ppm for –NH_2 and –NH protons respectively. The same can also concluded on the basis of IR spectra which show characteristics –NH peak at 3350 cm^{-1}. Spectral support for the formation of intermediate **3** was achieved by ^{1}H NMR data which shows sharp singlet at 4.84 δ *ppm* for -CH_2 proton and absence of –NH and –NH_2 protons peak. The intermediate **2** and **3** was also confirmed by MS analysis and gave depicted molecular ion peak and fragmentation pattern.

The structure of compound **4** was also established using NMR spectroscopy and the molecular mass confirmed by MS. In the ^{1}H NMR of compound **4**, a broad singlet observed at δ 4.14 and sharp singlet observed at δ 5.36 was assigned to proton of -NH_2 and –CH_2 group correspondingly. The structures of **5a-k** were confirmed by the MS, IR, PMR and CMR spectra. The IR spectra were characterized by the C-O absorptions in



Reaction Condition (a) MeOH, NH_2NH_2, Reflux, 5h; (b) $POCl_3$, $ClCH_2COOH$, Reflux, 4h; (c) DMF, K_2CO_3, $HOC_6H_4NH_2$, Reflux, 3h; (d) MeOH, RC_6H_5CHO, Stirring, 12h.

Scheme-I: Route of the synthesis for the compounds **5a-k**

the range ν_{max} ~1235 cm^{-1}, an indicative for the 1,3,4-oxadiazole ring formation and C=N stretching of arylidene group at ~1550 cm^{-1}. In the ^1H NMR spectra, four aromatic protons were appeared in the range of δ 6.5-7.9 ppm. The singlets in the range δ 9.2-9.4 ppm were assigned to methine proton of arylidene group in highly desheilded region due to presence of nitrogen and aromatic ring in the environment. In the ^{13}C NMR spectra, the resonances in the region δ ~154.0 and δ ~162.0 were assigned to C-2 and C-5 of the oxadiazole ring, respectively. The carbons of the methylene and methine were located at the region δ 56-58 and 163-166 ppm respectively. Substituents used in the synthesized compounds (**5a-k**) were in good agreement in MS, IR, PMR and CMR.

Biological evaluation

Antibacterial screening: All the newly synthesized compounds **5a-k** were screened for their *in vitro* antibacterial activity against Gram-positive bacteria *Staphylococcus aureus* and *Staphylococcus pyogenes* and Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* by conventional broth micro dilution method using Ampicillin as a standard drug for antibacterial activity at different concentrations of 1000, 500, 250, 100, 50, 25 and 12.5 μg mL^{-1} as shown in Table-1 [14]. Among the synthesized compounds **5a-k**, many of them had verified their antimicrobial potential which varies from moderate to excellent. Compound **5c** (-4-NO_2) had excellent activity against *P. aeruginosa* and *S. pyogenes*. It is noteworthy that compound **5c** (-4-NO_2) showed the greatest inhibition at MIC = 12.5 μg mL^{-1}, while compound **5g** (-4-Cl) showed inhibition at MIC = 12.5 μg mL^{-1} against *E. coli* and *S. aureus*. These data revealed that compound **5g** (-4-Cl) was highly active against both organisms. Compounds **5c** (-4-NO_2) and **5g** (-4-Cl) showed very good activity at MIC = 50 μg mL^{-1}. Compound **5c** (-4-NO_2) display very good activity against *E. coli* while compound **5g** (-4-Cl) showed very good activity against *P. aeruginosa*. Moreover, compound **5e** (-2-NO_2) exhibited very good activity against *S. aureus*. Compounds **5d** (-3-NO_2) and **5f** (-3-Cl) displayed good activity against *E. coli* and *S. aureus* while compound **5i** (-3-CH_3) showed good activity against *P. aeruginosa* and *S. aureus* at MIC = 100 μg mL^{-1}. The remaining compounds of the series possessed delicate antibacterial activity. On the other hand, the presence of similar fictional

TABLE-1
in vitro RESULTS OF ANTIBACTERIAL AND ANTIFUNGAL SCREENING OF COMPOUNDS **5a-k**

Compd. No.	-R	Minimum inhibitory concentration (MIC) (μg mL^{-1})						
		Bacteria				Fungi		
		E.c.	*P.a.*	*S.a.*	*S p.*	*C.a.*	*A.n.*	*A.c.*
5a	-H	250	> 1000	500	250	> 1000	500	> 1000
5b	-4-F	500	1000	1000	500	25	12.5	250
5c	-4-NO_2	50	12.5	50	12.5	500	1000	1000
5d	-3-NO_2	100	250	100	250	250	> 1000	> 1000
5e	-2-NO_2	250	500	50	> 1000	1000	250	1000
5f	-3-Cl	100	250	100	500	500	250	250
5g	-4-Cl	12.5	50	12.5	250	> 1000	500	1000
5h	-4-CH_3	250	1000	500	1000	500	1000	> 1000
5i	-3-CH_3	1000	100	100	500	12.5	25	100
5j	-2-CH_3	100	250	1000	500	100	> 1000	100
5k	-4-OCH_3	500	250	> 1000	500	> 1000	100	> 1000
	Ampicillin	100	100	250	100	–	–	–
	Griseofulvin	–	–	–	–	500	100	–

E.c., Escherichia coli MTCC 443; *P.a., Pseudomonas aeruginosa* MTCC 1688; *S.a., Staphylococcus aureus* MTCC 96; *S.p., Staphylococcus pyogenes* MTCC 442; *C.a., Candida albicans* MTCC 227; *A.n., Aspergillus niger* MTCC 282. *A.c., Aspergillus clavatus* MTCC 1323

groups at the *para* position resulted in minor increase in antibacterial activity as compared to **5c** (-4-NO_2) and **5g** (-4-Cl).

Antifungal screening: Minimum inhibitory concentration (MIC) values of antifungal activity were observed against *Candida albicans, Aspergillus niger* and *Aspergillus clavatus* by conventional broth microdilution method [15]. Antifungal activity showed that compound **5i** (-3-CH_3) exhibited very good activity against *A. clavatus* at MIC = 100 µg mL^{-1}. When we replaced hydrogen by group like (-4-OCH_3) in compound **5k**, the activity was slightly decreased against *A. niger.* The same result exhibited in compound **5j** (-2-CH_3) possessed good activity against *C. albicans* and *A. clavatus* respectively. When hydrogen was replaced in compound **5b** (-4-F) and **5i** (-3-CH_3), both display excellent activity against *C. albicans* and *A. niger* with twofold greater MIC (12.5-25 µg mL^{-1}) than the reference drug. The remaining compounds of the series showed feeble antifungal activity. Thus, we have discussed and compared antifungal activity based on the standard drug griseofulvin shown in Table-1.

Conclusion

The research confirms that the most convenient way to synthesize 4-((5-(2-chlorophenyl)-1,3,4-oxadiazol-2-yl)-methoxy)-*N*-(benzylidenederivatives)benzenamine (**5a-k**) is simple hydrazide formation from key starting material **1** followed by cyclization to furnish oxadiazole **3** and in the last step using various aromatic aldehyde, it gives variety of potent antimicrobial agents. Out of synthesized molecules, four molecules were found most active in anti-bacterial screening as compared to standard drug (ampicillin) *i.e.* **5b**, **5c**, **5g** and **5i** which may due to the presence of electron withdrawing effect of substituents. The antifungal data of the compounds is found moderate as compared to standard drug (griseofulvin) and only **5b** and **5i** shows comparable potency. Overall it seems that the combination of *in silico* and *in vitro* study may enhance the potency of such derivatives by choosing appropriate functionalities.

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