Synthesis, Characterization and Antimicrobial Activity of Novel 3,5-Disubstituted-1,3,4-oxadiazole-2-ones

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A series of novel 5-(3,4-dichlorophenyl)-3-[(4-substitutedpiperazino)methyl]-1,3,4-oxadiazole-2(3H)-one have been synthesized starting from 3,4-dichlorobenzoic acid. Structure identification of the compounds was done by IR, ^{1}H-NMR, ^{13}C-NMR spectra and elemental analyses. The compounds were screened in vitro for their antimicrobial activity against Aspergillus sp., Fusarium oxysporum, Botrytis cinerea, Penicillium sp., Bacillus megaterium, Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa by disc-diffusion method. According to the activity studies, all compounds possess selective antifungal effect. Among the synthesized compounds, ***5a-5d****,* ***5h****,* ***5j*** *and* ***5p*** *were found to be the most effective derivatives with higher zone inhibition values than standard drug nystatin against variable fungal species.*

Keywords: 1,3,4-Oxadiazole; piperazine; antibacterial; antifungal; Mannich base

Antimicrobial agents inhibit or kill the growth of microorganisms such as bacteria or fungus. By means of antimicrobial drug discovery, it is believed that microbial infections will end up. However, rapid increases of microorganism originated diseases make it difficult to happen. Furthermore, senseless usage of antimicrobials exposed another big problem, drug resistance [1, 2]. As a result of this, the need for the synthesis and development of new antimicrobial agents has emerged [3-8].

According to the literature, the importance of nitrogen containing 1,3,4-oxadiazole ring systems has been elevated recently and the 1,3,4-oxadiazole nucleus has emerged as one of the potential pharmacophore that is responsible for diverse pharmacological properties. A wide variety of heterocyclic compounds bearing this moiety has been reported as significant molecules with broad spectrum of biological activities such as antimicrobial [9-14], anticancer [15-19], antitubercular [20-22], anti-inflammatory [11, 23, 24], analgesic [12], and antiviral activities [25, 26].

In the light of consequent literature survey, in this study, we described the synthesis of some new 5-(3,4-diclorophenyl)-3-(4-substitutedpiperazino)methyl-1,3,4-oxadiazol-2(3H)-ones and focused on their potential antimicrobial activity. The modification pattern was performed in a manner with respect to examine SAR.

Experimental part

Materials and methods

All chemicals and reagents used in current study were of analytical grade. The reactions were monitored by thin layer chromatography (TLC) on Merck pre-coated silica GF254 plates. Melting points were determined by using a Mettler Toledo FP62 capillary melting point apparatus (Mettler-Toledo, Greifensee, Switzerland) and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer Spectrum One series FT-IR apparatus (Version 5.0.1) (Perkin Elmer, Norwalk, CT, USA), using potassium bromide pellets, the frequencies were expressed in cm^{-1}. The ^{1}H- and ^{13}C-NMR spectra were recorded with a Varian Mercury-400 FT-NMR spectrometer (Varian, Palo Alto, CA, USA), using tetramethylsilane as the internal reference, with chloroform-$CDCl_3$ as solvent, the chemical shifts were reported in parts per million (ppm) and coupling constants (J) were given as hert (Hz). Elemental analyses were performed on LECO 932 CHNS instrument (Leco-932, St. Joseph, MI, USA) and were within ± 0.4 % of the theoretical values.

General procedure for the preparation of 5-(3,4-dichlorophenyl)-1,3,4-oxadiazol-2(3H)-one ***(4)***

To a 0°C solution of 3,4-dichlorobenzohydrazide (2.2 mmol, 0.451 g) and triethylamine (TEA) (2.2 mmol) in THF (10 mL), 1,1-carbonyldiimidazole (CDI) was added. The resulting mixture was stirred for 20 h at room temperature and concentrated in vacuo. The residue was dissolved in diethyl ether (15 mL), washed with 2 M hydrochloric acid (5 mL) and saturated aqueous sodium bicarbonate and then dried with sodium sulphate. Filtration and concentration in vacuo gave compound **4**, which was recrystallized from ethanol: water.

Yield: 78%, m.p. 193.3°C; IR (KBr) cm^{-1}: 3059 (C-H, aromatic), 2811 (N-H), 1843 (C=O), 1615 (C=N), 1553 (C=C, aromatic), 1263 (C-N), 1242 (C-O); ^{1}H NMR ($CDCl_3$) δ: 12.78 (bs, 1H, -NH); 7.96 (d, 1H, dichlorophenyl H_2 J= 1.2); 7.82 (dd, 1H, dichlorophenyl H_5, J= 8.4, J'= 1.2); 7.76 (d, 1H, dichlorophenyl H_6, J= 8.4).

General procedure for the preparation of 5-(3,4-dichlorophenyl)-3-[(4-substituted-piperazine-1-yl)methyl]-1,3,4-oxadiazol-2(3H)-one ***(5a-5v)***

To a solution of 5-(3,4-dichlorophenyl)-1,3,4-oxadiazol-2(3*H*)-one **4** (1 mmol, 0.231 g) in ethanol (10 mL), a mixture of formaldehyde (1.5 mmol) and piperazine derivative (1 mmol) in ethanol was added by stirring. After complete addition, the mixture was refluxed for 4 h. The solution was precipitated by cooling, the formed compound was filtered and crystallized with ethanol/water.

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5-(3,4-Dichlorophenyl)-3-[(4-phenylpiperazin-1-yl)methyl]-1,3,4-oxadiazol-2(3H)-one ***(5a)***

Yield: 58%, m.p. 142.3°C; IR (KBr) cm^{-1}: 3092 (C-H, aromatic), 2944 (C-H, aliphatic), 1782 (C=O), 1601 (C=N), 1579 (C=C, aromatic), 1237 (C-N), 1218 (C-O); ^1H NMR ($CDCl_3$) δ: 7.95 (d, 1H, dichlorophenyl H_2, *J*= 2.0); 7.68 (dd, 1H, dichlorophenyl H_5, *J*= 8.4, *J'*= 2.0); 7.56 (d, 1H, dichlorophenyl H_6, *J*= 8.4); 7.24-7.28 (m, 3H, phenyl H_2, H_5, H_6); 6.85-6.93 (m, 2H, phenyl H_3, H_4); 4.79 (s, 2H, N-CH_2-N); 3.22 (t, 4H, piperazine H_3, H_5, *J*= 4.8); 2.93 (t, 4H, piperazine H_2, H_6, *J*= 4.8). Anal. calcd. for $C_{19}H_{18}Cl_2N_4O_2$ (405.28): C, 56.31; H, 4.48; N, 13.82%. Found C, 56.18; H, 3.91; N, 13.83%.

5-(3,4-Dichlorophenyl)-3-{[4-(2-fluorophenyl)piperazin-1-yl]methyl}-1,3,4-oxadiazol-2(3H)-one ***(5b)***

Yield: 72%, m.p. 153.1°C; IR (KBr) cm^{-1}: 3093 (C-H, aromatic), 2944 (C-H, aliphatic), 1782 (C=O), 1612 (C=N), 1577 (C=C, aromatic), 1242 (C-N), 1217 (C-O); ^1H NMR ($CDCl_3$) δ: 7.96 (d, 1H, dichlorophenyl H_2, *J*= 2.0); 7.68 (dd, 1H, dichlorophenyl H_5, *J*= 8.4, *J'*= 1.6); 7.57 (d, 1H, dichlorophenyl H_6, *J*= 8.0); 6.94-7.08 (m, 4H, phenyl H_3, H_4, H_5, H_6); 4.79 (s, 2H, N-CH_2-N); 3.12 (t, 4H, piperazine H_3, H_5, *J*= 5.2); 2.96 (t, 4H, piperazine H_2, H_6, *J*= 5.2). Anal. calcd. for $C_{19}H_{17}Cl_2FN_4O_2$ (423.27): C, 53.91; H, 4.05; N, 13.24%. Found C, 53.77; H, 3.73; N, 13.07%.

5-(3,4-Dichlorophenyl)-3-{[4-(4-fluorophenyl)piperazin-1-yl]methyl}-1,3,4-oxadiazol-2(3H)-one ***(5c)***

Yield: 57%, m.p. 138.4°C; IR (KBr) cm^{-1}: 3087 (C-H, aromatic), 2948 (C-H, aliphatic), 1765 (C=O), 1615 (C=N), 1591 (C=C, aromatic), 1239 (C-N), 1227 (C-O); ^1H NMR ($CDCl_3$) δ: 7.95 (d, 1H, dichlorophenyl H_2, *J*= 2.0); 7.68 (dd, 1H, dichlorophenyl H_5, *J*= 8.0, *J'*= 2.0); 7.57 (d, 1H, dichlorophenyl H_6, *J*= 8.0); 6.93-6.98 (m, 2H, phenyl H_2, H_6), 6.85-6.88 (m, 2H, phenyl H_3, H_5); 4.78 (s, 2H, N-CH_2-N); 3.13 (t, 4H, piperazine H_3, H_5, *J*= 5.2); 2.93 (t, 4H, piperazine H_2, H_6, *J*= 5.2). Anal. calcd. for $C_{19}H_{17}Cl_2FN_4O_2$ (423.27): C, 53.91; H, 4.05; N, 13.24%. Found C, 54.12; H, 4.03; N, 13.16%.

3-{[4-(2-Chlorophenyl)piperazin-1-yl]methyl}-5-(3,4-dichlorophenyl)-1,3,4-oxadiazol-2(3H)-one ***(5d)***

Yield: 18%, m.p. 131.8°C; IR (KBr) cm^{-1}: 3068 (C-H, aromatic), 2959 (C-H, aliphatic), 1789 (C=O), 1617 (C=N), 1588 (C=C, aromatic), 1257 (C-N), 1227 (C-O); ^1H NMR ($CDCl_3$) δ: 7.97 (d, 1H, dichlorophenyl H_2, *J*= 2.0); 7.69 (dd, 1H, dichlorophenyl H_5, *J*= 8.0, *J'*= 2.0); 7.57 (d, 1H, dichlorophenyl H_6, *J*= 8.4); 7.34 (dd, 1H, phenyl H_3, *J*= 8.0, *J'*= 1.6); 7.22 (t, 1H, phenyl H_4, *J*= 7.6); 7.05 (dd, 1H, phenyl H_6, *J*= 8.0, *J'*= 1.6); 6.99 (t, 1H, phenyl H_5, *J*= 7.6); 4.79 (s, 2H, N-CH_2-N); 3.09 (t, 4H, piperazine H_3, H_5, *J*= 4.4); 2.96 (t, 4H, piperazine H_2, H_6, *J*= 4.4). Anal. calcd. for $C_{19}H_{17}Cl_3N_4O_2$ (439.72): C, 51.90; H, 3.90; N, 12.74%. Found C, 51.89; H, 3.96; N, 12.59%.

3-{[4-(3-Chlorophenyl)piperazin-1-yl]methyl}-5-(3,4-dichlorophenyl)-1,3,4-oxadiazol-2(3H)-one ***(5e)***

Yield: 29%, m.p. 146.2°C; IR (KBr) cm^{-1}: 3092 (C-H, aromatic), 2945 (C-H, aliphatic), 1779 (C=O), 1595 (C=N), 1564 (C=C, aromatic), 1260 (C-N), 1233 (C-O); ^1H NMR ($CDCl_3$) δ: 7.95 (d, 1H, dichlorophenyl H_2, *J*= 2.0); 7.67 (dd, 1H, dichlorophenyl H_5, *J*= 8.2, *J'*= 2.0); 7.56 (d, 1H, dichlorophenyl H_6, *J*= 8.0); 7.15 (t, 1H, phenyl H_5, *J*= 8.4); 6.75-6.86 (m, 3H, phenyl H_2, H_4, H_6); 4.78 (s, 2H, N-CH_2-N); 3.21 (t, 4H, piperazine H_3, H_5, *J*= 5.2); 2.90 (t, 4H, piperazine H_2, H_6, *J*= 5.6). ^{13}C NMR ($CDCl_3$) d: 154.18 (1,3,4-oxadiazole C=O); 152.38 (1,3,4-oxadiazole C_5); 151.63 (3-chlorophenyl C_1); 136.39 (3,4-dichlorophenyl C_3); 135.18 (3,4-dichlorophenyl C_4); 133.97 (3-chlorophenyl C_3); 131.44 (3,4-dichlorophenyl C_2); 130.29 (3,4-dichlorophenyl C_1); 127.73 (3,4-dichlorophenyl C_5); 124.96 (3,4-dichlorophenyl C_6); 123.76 (3-chlorophenyl C_2); 119.93 (3-chlorophenyl C_6); 116.33 (3-chlorophenyl C_4); 114.53 (3-chlorophenyl C_5); 67.75 (N-CH_2-N); 49.92 (piperazine C_2, C_6); 49.02 (piperazine C_3, C_5). Anal. calcd. for $C_{19}H_{17}Cl_3N_4O_2$ (439.72): C, 51.90; H, 3.90; N, 12.74%. Found C, 51.87; H, 3.79; N, 12.63%.

3-{[4-(4-Chlorophenyl)piperazin-1-yl]methyl}-5-(3,4-dichlorophenyl)-1,3,4-oxadiazol-2(3H)-one ***(5f)***

Yield: 92%, m.p. 244.7°C; IR (KBr) cm^{-1}: 3081 (C-H, aromatic), 2955 (C-H, aliphatic), 1767 (C=O), 1615 (C=N), 1600 (C=C, aromatic), 1248 (C-N), 1232 (C-O); ^1H NMR ($CDCl_3$) δ: 7.95 (d, 1H, dichlorophenyl H_2, *J*= 2.0); 7.67 (dd, 1H, dichlorophenyl H_5, *J*= 8.4, *J*= 2.0); 7.56 (d, 1H, dichlorophenyl H_6, *J*= 8.4); 7.2 (d, 2H, phenyl H_2, H_6, *J*= 8.4); 6.84 (d, 2H, phenyl H_3, H_5, *J*= 8.4); 4.78 (s, 2H, N-CH_2-N); 3.18 (t, 4H, piperazine H_3, H_5, *J*= 4.8); 2.93 (t, 4H, piperazine H_2, H_6, *J*= 4.8). Anal. calcd. for $C_{19}H_{17}Cl_3N_4O_2$ (439.72): C, 51.90; H, 3.90; N, 12.74%. Found C, 51.92; H, 3.90; N, 12.60%.

5-(3,4-Dichlorophenyl)-3-{[4-(2-methoxyphenyl)piperazin-1-yl]methyl}-1,3,4-oxadiazol-2(3H)-one ***(5g)***

Yield: 89%, m.p. 140.2°C; IR (KBr) cm^{-1}: 3073 (C-H, aromatic), 2940 (C-H, aliphatic), 1775 (C=O), 1615 (C=N), 1590 (C=C, aromatic), 1261 (C-N), 1240 (C-O); ^1H NMR ($CDCl_3$) δ: 7.95 (d, 1H, dichlorophenyl H_2, *J*= 2.0); 7.67 (dd, 1H, dichlorophenyl H_5, *J*= 8.0, *J*= 2.0); 7.56 (d, 1H, dichlorophenyl H_6, *J*= 8.0); 6.91-7.03 (m, 3H, phenyl H_3, H_4, H_5); 6.85 (d, 1H, phenyl H_6, *J*= 8.0); 4.80 (s, 2H, N-CH_2-N); 3.83 (s, 3H, -OCH_3); 3.09 (t, 4H, piperazine H_3, H_5); 2.98 (t, 4H, piperazine H_2, H_6, *J*= 4.8). Anal. calcd. for $C_{20}H_{20}Cl_2N_4O_3$ (435.30): C, 55.18; H, 4.63; N, 12.87%. Found C, 55.05; H, 4.69; N, 12.78%.

5-(3,4-Dichlorophenyl)-3-{[4-(3-methoxyphenyl)piperazin-1-yl]methyl}-1,3,4-oxadiazol-2(3H)-one ***(5h)***

Yield: 59%, m.p. 107.0°C; IR (KBr) cm^{-1}: 3087 (C-H, aromatic), 2942 (C-H, aliphatic), 1780 (C=O), 1601 (C=N), 1586 (C=C, aromatic), 1257 (C-N), 1226 (C-O); ^1H NMR ($CDCl_3$) δ: 7.94 (d, 1H, dichlorophenyl H_2, *J*= 2.0); 7.67 (dd, 1H, dichlorophenyl H_5, *J*= 8.0, *J'*= 2.0); 7.55 (d, 1H, dichlorophenyl H_6, *J*= 8.8); 7.16 (t, 1H, phenyl H_5, *J*= 8.0); 6.52 (dd, 1H, phenyl H_4, *J*= 8.0, *J*= 1.6); 6.41-6.45 (m, 2H, phenyl H_2, H_6); 4.78 (s, 2H, N-CH_2-N); 3.78 (s, 3H, -OCH_3); 3.21 (t, 4H, piperazine H_3, H_5, *J*= 4.8); 2.91 (t, 4H, piperazine H_2, H_6, *J*= 4.8). Anal. calcd. for $C_{20}H_{20}Cl_2N_4O_3$ (435.30): C, 55.18; H, 4.63; N, 12.87%. Found C, 55.17; H, 4.52; N, 12.96%.

5-(3,4-Dichlorophenyl)-3-{[4-(4-methoxyphenyl)piperazin-1-yl]methyl}-1,3,4-oxadiazol-2(3H)-one ***(5i)***

Yield: 45%, m.p. 163.6°C; IR (KBr) cm^{-1}: 3095 (C-H, aromatic), 2943 (C-H, aliphatic), 1773 (C=O), 1606 (C=N), 1584 (C=C, aromatic), 1244 (C-N), 1230 (C-O); ^1H NMR ($CDCl_3$) δ: 7.95 (d, 1H, dichlorophenyl H_2, *J*= 2.0); 7.68 (dd, 1H, dichlorophenyl H_5, *J*= 8.4, *J'*= 2.0); 7.56 (d, 1H, dichlorophenyl H_6, *J*= 8.4); 6.90 (dd, 2H, phenyl H_3, H_5, *J*= 6.8, *J*= 2.4); 6.83 (dd, 2H, phenyl H_2, H_6, *J*= 6.4, *J*= 2.0); 4.78 (s, 2H, N-CH_2-N); 3.76 (s, 3H, OCH_3); 3.10 (t, 4H, piperazine H_3, H_5, *J*= 4.8); 2.93 (t, 4H, piperazine H_2, H_6, *J*= 4.8). Anal. calcd. for $C_{20}H_{20}Cl_2N_4O_3$ (435.30): C, 55.18; H, 4.63; N, 12.87%. Found C, 54.94; H, 4.42; N, 13.05%.

5-(3,4-Dichlorophenyl)-3-{[4-(4-trifluoromethylphenyl) piperazin-1-yl]methyl}-1,3,4-oxadiazol-2(3H)-one ***(5j)***

Yield: 79%, m.p. 154.8°C; IR (KBr) cm^{-1}: 3083 (C-H, aromatic), 2970 (C-H, aliphatic), 1769 (C=O), 1620 (C=N), 1588 (C=C, aromatic), 1248 (C-N), 1210 (C-O); ^1H NMR ($CDCl_3$) δ: 7.95 (d, 1H, dichlorophenyl H_2, *J*= 2.0); 7.67 (dd, 1H, dichlorophenyl H_5, *J*= 8.0, *J'*= 2.0); 7.56 (d, 1H, dichlorophenyl H_6, *J*= 8.0); 7.47 (d, 2H, phenyl H_3, H_5, *J*= 8.8); 6.91 (d, 2H, phenyl H_2, H_6, *J*= 8.8); 4.79 (s, 2H, N-CH_2-N); 3.31 (t, 4H, piperazine H_3, H_5, *J*= 5.2); 2.92 (t, 4H, piperazine H_2, H_6, *J*= 5.2). Anal. calcd. for $C_{20}H_{17}Cl_2F_3N_4O_2$ (473.28): C, 50.76; H, 3.62; N, 11.84%. Found C, 50.74; H, 3.48; N, 12.03%.

5-(3,4-Dichlorophenyl)-3-{[4-(4-methylphenyl)piperazin-1-yl]methyl}-1,3,4-oxadiazol-2(3H)-one ***(5k)***

Yield: 81%, m.p. 153.2°C; IR (KBr) cm^{-1}: 3079 (C-H, aromatic), 2949 (C-H, aliphatic), 1763 (C=O), 1615 (C=N), 1587 (C=C, aromatic), 1251 (C-N), 1232 (C-O); ^1H NMR ($CDCl_3$) δ: 7.95 (d, 1H, dichlorophenyl H_2, *J*= 2.0); 7.68 (dd, 1H, dichlorophenyl H_5, *J*= 8.4, *J'*= 2.0); 7.56 (d, 1H, dichlorophenyl H_6, *J*= 8.4); 7.07 (d, 2H, phenyl H_3, H_5, *J*= 8.0); 6.84 (d, 2H, phenyl H_2, H_6, *J*= 6.4); 4.78 (s, 2H, N-CH_2-N); 3.16 (bs, 4H, piperazine H_3, H_5); 2.93 (bs, 4H, piperazine H_2, H_6); 2.26 (s, 3H, 3-CH_3). Anal. calcd. for $C_{20}H_{20}Cl_2N_4O_2$ (419.30): C, 27.29; H, 4.81; N, 13.36%. Found C, 57.09; H, 4.75; N, 13.55%.

5-(3,4-Dichlorophenyl)-3-{[4-(2,3-dimethylphenyl)piperazin-1-yl]methyl}-1,3,4-oxadiazol-2(3H)-one ***(5l)***

Yield: 20%, m.p. 168.3°C; IR (KBr) cm^{-1}: 3076 (C-H, aromatic), 2944 (C-H, aliphatic), 1789 (C=O), 1608 (C=N), 1581 (C=C, aromatic), 1250 (C-N), 1233 (C-O); ^1H NMR ($CDCl_3$) δ: 7.98 (d, 1H, dichlorophenyl H_2, *J*= 2.0); 7.71 (dd, 1H, dichlorophenyl H_5, *J*= 8.4, *J'*= 2.0); 7.57 (d, 1H, dichlorophenyl H_6, *J*= 8.4); 7.07 (t, 1H, phenyl H_6, *J*= 7.2); 6.90 (dd, 2H, phenyl H_4, H_5, *J*= 8.0, *J'*= 3.6); 4.79 (s, 2H, N-CH_2-N); 2.92 (s, 8H, piperazine H_2, H_3, H_5, H_6); 2.25 (s, 3H, 2-CH_3); 2.18 (s, 3H, 3-CH_3). Anal. calcd. for $C_{21}H_{22}Cl_2N_4O_2$ (433.33): C, 58.21; H, 5.12; N, 12.93%. Found C, 58.72; H, 5.01; N, 12.62%.

5-(3,4-Dichlorophenyl)-3-{[4-(4-nitrophenyl)piperazin-1-yl]methyl}-1,3,4-oxadiazol-2(3H)-one ***(5m)***

Yield: 61%, m.p. 183.5°C; IR (KBr) cm^{-1}: 3091 (C-H, aromatic), 2881 (C-H, aliphatic), 1790 (C=O), 1602 (C=N), 1588 (C=C, aromatic), 1288 (C-N), 1255 (C-O); ^1H NMR ($CDCl_3$) δ: 8.12 (d, 2H, p-nitrophenyl H_3, H_5, *J*= 9.2); 7.94 (d, 1H, dichlorophenyl H_2, *J*= 1.6); 7.67 (dd, 1H, dichlorophenyl H_5, *J*= 8.4, *J'*= 2.0); 7.56 (d, 1H, dichlorophenyl H_6, *J*= 8.0); 6.81 (d, 2H, p-nitrophenyl H_2, H_5, *J*= 9.2); 4.79 (s, 2H, N-CH_2-N); 3.46 (t, 4H, piperazine H_3, H_5, *J*= 5.2); 2.91 (t, 4H, piperazine H_2, H_6, *J*= 5.2). Anal. calcd. for $C_{19}H_{17}Cl_2N_5O_4$ (450.27): C, 50.98; H, 3.81; N, 15.55%. Found C, 50.54; H, 3.62; N, 15.77%.

5-(3,4-Dichlorophenyl)-3-{[4-(4-hydroxyphenyl)piperazin-1-yl]methyl}-1,3,4-oxadiazol-2(3H)-one ***(5n)***

Yield: 85%, m.p. 214.9°C (dec.); IR (KBr) cm^{-1}: 3530 (O-H), 3059 (C-H, aromatic), 2951 (C-H, aliphatic), 1772 (C=O), 1616 (C=N), 1592 (C=C, aromatic), 1274 (C-N), 1226 (C-O); ^1H NMR ($CDCl_3$) δ: 7.95 (d, 1H, dichlorophenyl H_2, *J*= 2.0); 7.68 (dd, 1H, dichlorophenyl H_5, *J*= 8.8, *J'*= 2.0); 7.57 (d, 1H, dichlorophenyl H_6, *J*= 8.4); 7.26 (s, 1H, hydroxyl); 6.83 (dd, 2H, phenol H_3, H_5, *J*= 6.8, *J'*= 2.4); 6.76 (dd, 2H, phenol H_2, H_6, *J*= 6.8, *J'*= 2.4); 4.78 (s, 2H, N-CH_2-N); 3.09 (t, 4H, piperazine H_3, H_5, *J*= 4.8); 2.93 (t, 4H, piperazine H_2, H_6, *J*= 4.0). Anal. calcd. for $C_{19}H_{18}Cl_2N_4O_3$ (421.28): C, 54.17; H, 4.31; N, 13.30%. Found C, 53.93; H, 4.33; N, 13.26%.

5-(3,4-Dichlorophenyl)-3-{[4-(2-cyanophenyl)piperazin-1-yl]methyl}-1,3,4-oxadiazol-2(3H)-one ***(5o)***

Yield: 84%, m.p. 154.9°C; IR (KBr) cm^{-1}: 3071 (C-H, aromatic), 2942 (C-H, aliphatic), 2219 (Ca≡N), 1793 (C=O), 1616 (C=N), 1553 (C=C, aromatic), 1261 (C-N), 1235 (C-O); ^1H NMR ($CDCl_3$) δ: 7.96 (d, 1H, dichlorophenyl H_2, *J*= 2.0); 7.70 (dd, 1H, dichlorophenyl H_5, *J*= 8.4, *J'*= 2.0); 7.57 (d, 1H, dichlorophenyl H_6, *J*= 8.0); 7.56 (dd, 2H, cyanophenyl H_3, H_6, *J*= 8.0, *J'*= 1.6); 7.49 (t, 1H, cyanophenyl H_4, *J*= 8.0); 7.03 (t, 1H, cyanophenyl H_5, *J*= 8.2); 4.78 (s, 2H, N-CH_2-N); 3.25 (t, 4H, piperazine H_3, H_5, *J*= 4.4); 2.99 (t, 4H, piperazine H_2, H_6, *J*= 4.8). Anal. calcd. for $C_{20}H_{17}Cl_2N_5O_2$ (430.29): C, 55.83; H, 3.98; N, 16.28%. Found C, 55.58; H, 4.03; N, 16.17%.

5-(3,4-Dichlorophenyl)-3-{[4-(4-cyanophenyl)piperazin-1-yl]methyl}-1,3,4-oxadiazol-2(3H)-one ***(5p)***

Yield: 73%, m.p. 145.8°C; IR (KBr) cm^{-1}: 3089 (C-H, aromatic), 2941 (C-H, aliphatic), 2218 (Ca≡N), 1772 (C=O), 1605 (C=N), 1552 (C=C, aromatic), 1249 (C-N), 1222 (C-O); ^1H NMR ($CDCl_3$) δ: 7.94 (d, 1H, dichlorophenyl H_2, *J*= 1.6); 7.67 (dd, 1H, dichlorophenyl H_5, *J*= 8.8, *J'*= 2.0); 7.56 (d, 1H, dichlorophenyl H_6, *J*= 8.4); 7.48 (d, 2H, cyanophenyl H_3, H_5, *J*= 8.8); 6.84 (d, 2H, cyanophenyl H_2, H_6, *J*= 9.2); 4.78 (s, 2H, N-CH_2-N); 3.36 (t, 4H, piperazine H_3, H_5, *J*= 5.2); 2.90 (t, 4H, piperazine H_2, H_6, *J*= 5.2). Anal. calcd. for $C_{20}H_{17}Cl_2N_5O_2$ (430.29): C, 55.83; H, 3.98; N, 16.28%. Found C, 55.69; H, 3.86; N, 16.30%.

5-(3,4-Dichlorophenyl)-3-[4-(2-pyridinopiperazin-1-yl)methyl]-1,3,4-oxadiazol-2(3H)-one ***(5q)***

Yield: 72%, m.p. 148.9°C; IR (KBr) cm^{-1}: 3092 (C-H, aromatic), 2931 (C-H, aliphatic), 1771 (C=O), 1594 (C=N), 1559 (C=C, aromatic), 1244 (C-N), 1220 (C-O); ^1H NMR ($CDCl_3$) δ: 8.17 (d, 1H, 2-pyridyl H_3, *J*= 5.6); 7.93 (d, 1H, dichlorophenyl H_2, *J*= 2.0); 7.66 (dd, 1H, dichlorophenyl H_5, *J*= 8.2, *J'*= 2.,); 7.55 (d, 1H, dichlorophenyl H_6, *J*= 8.8); 7.47 (t, 1H, 2-pyridyl H_6, *J*= 6.0); 6.62 (m, 2H, 2-pyridyl H_4, H_5, *J*= 8.8); 4.78 (s, 2H, N-CH_2-N); 3.58 (t, 4H, piperazine H_3, H_5, *J*= 5.2); 2.87 (t, 4H, piperazine H_2, H_6, *J*= 5.2). ^{13}C NMR ($CDCl_3$) δ: 159.43 (2-pyridyl C_1); 154.14 (1,3,4-oxadiazole C=O); 151.54 (1,3,4-oxadiazole C_5); 148.17 (d, 2-pyridyl C_3); 137.75 (2-pyridyl C_5); 136.29 (3,4-dichlorophenyl C_3); 133.91 (3,4-dichlorophenyl C_4); 131.40 (3,4-dichlorophenyl C_2); 127.68 (3,4-dichlorophenyl C_1); 124.94 (3,4-dichlorophenyl C_5); 123.76 (3,4-dichlorophenyl C_6); 113.73 (2-pyridyl C_4); 107.40 (2-pyridyl C_6); 77.30 (N-CH_2-N); 49.92 (piperazine C_2, C_6); 45.29 (piperazine C_3, C_5). Anal. calcd. for $C_{18}H_{17}Cl_2N_5O_2$ (406.27): C, 53.21; H, 4.22; N, 17.24%. Found C, 52.90; H: 3.97; N: 17.16%.

5-(3,4-Dichlorophenyl)-3-[4-(4-pyridinopiperazin-1-yl)methyl]-1,3,4-oxadiazol-2(3H)-one ***(5r)***

Yield: 18%, m.p. 136.3°C; IR (KBr) cm^{-1}: 3082 (C-H, aromatic), 2848 (C-H, aliphatic), 1781 (C=O), 1601 (C=N), 1542 (C=C, aromatic), 1256 (C-N), 1231 (C-O); ^1H NMR ($CDCl_3$) δ: 8.25 (d, 2H, 4-pyridyl H_3, H_5, *J*= 6.0); 7.94 (d, 1H, dichlorophenyl H_2, *J*= 2.0); 7.67 (dd, 1H, dichlorophenyl H_5, *J*= 8.0, *J*= 2.0); 7.56 (d, 1H, dichlorophenyl H_6, *J*= 8.0); 6.64 (t, 2H, 4-pyridyl H_2, H_6, *J*= 5.2); 4.77 (s, 2H, N-CH_2-N); 3.37 (t, 4H, piperazine H_3, H_5, *J*= 5.2); 2.88 (t, 4H, piperazine H_2, H_6, *J*= 5.2). Anal. calcd. for $C_{18}H_{17}Cl_2N_5O_2$ (406.27): C, 53.21; H, 4.22; N, 17.24%. Found C, 52.97; H, 4.22; N, 17.21%.

5-(3,4-Dichlorophenyl)-3-[4-(2-pyrimidopiperazin-1-yl)methyl]-1,3,4-oxadiazol-2(3H)-one ***(5s)***

Yield: 49%, m.p. 158.8°C; IR (KBr) cm^{-1}: 3093 (C-H, aromatic), 2931 (C-H, aliphatic), 1771 (C=O), 1606 (C=N), 1583 (C=C, aromatic), 1255 (C-N), 1219 (C-O); ^1H NMR ($CDCl_3$) δ: 8.28 (d, 2H, 2-pyrimidyl H_3, H_5, *J*= 4.8); 7.92 (d, 1H, dichlorophenyl H_2, *J*= 2.0); 7.65 (dd, 1H, dichlorophenyl H_5, *J*= 8.4, *J'*= 2.0); 7.55 (d, 1H, dichlorophenyl H_6, *J*= 8.4); 6.47 (t, 1H, 2-pyrimidyl H_4, *J*= 4.8); 4.78 (s, 2H, N-CH_2-N); 3.87 (t, 4H, piperazine H_3, H_5, *J*= 4.8); 2.82 (t, 4H, piperazine H_2, H_6, *J*= 4.8). Anal. calcd. for $C_{17}H_{16}Cl_2N_6O_2$ (407.25): C, 50.14; H, 3.96; N, 20.64%. Found C, 49.95; H, 3.93; N, 20.58%.

5-(3,4-Dichlorophenyl)-3-{[4-(1,3-benzodioxole-5-ylmethyl)piperazin-1-yl]methyl}-1,3,4-oxadiazol-2(3H)-one ***(5t)***

Yield: 69%, m.p. 137.9°C; IR (KBr) cm^{-1}: 3073 (C-H, aromatic), 2945 (C-H, aliphatic), 1779 (C=O), 1609 (C=N), 1554 (C=C, aromatic), 1247 (C-N), 1203 (C-O); ^1H NMR ($CDCl_3$) δ: 7.93 (d, 1H, dichlorophenyl H_2, *J*= 2.4); 7.66 (dd, 1H, dichlorophenyl H_5 *J*= 8.4, *J'*= 2.0); 7.56 (d, 1H, dichlorophenyl H_6, *J*= 8.4); 6.81 (s, 1H, benzodioxole H_4); 6.71 (d, 2H, benzodioxole H_6 , H_7, *J*= 2.4); 5.92 (s, 2H, benzodioxole H_2); 4.72 (s, 2H, N-CH_2-N); 3.40 (s, 2H, -CH_2-); 2.78 (t, 4H, piperazine H_3, H_5, *J*= 4.8); 2.46 (bs, 4H, piperazine H_2, H_6). Anal. calcd. for $C_{21}H_{20}Cl_2N_4O_4$ (463.31): C, 54.44; H, 4.35; 12.09%. Found C, 54.15; H, 4.36; N, 12.14%.

5-(3,4-Dichlorophenyl)-3-[(4-benzoylpiperazin-1-yl)methyl]-1,3,4-oxadiazol-2(3H)-one ***(5u)***

Yield: 31%, m.p. 135.9°C; IR (KBr) cm^{-1}: 3084 (C-H, aromatic), 2959 (C-H, aliphatic), 1776 (C=O), 1640 (C=N), 1552 (C=C, aromatic), 1277 (C-N), 1235 (C-O); ^1H NMR ($CDCl_3$) δ: 7.95 (d, 1H, dichlorophenyl H_2, *J*= 2.0); 7.68 (dd, 1H, dichlorophenyl H_5, *J*= 8.0, *J'*= 1.6); 7.58 (d, 1H, dichlorophenyl H_6, *J*= 8.4); 7.38 (m, 5H, benzoyl H_2-H_6); 4.75 (s, 2H, N-CH_2-N); 3.81 (bs, 2H, piperazine H_3); 3.47 (bs, 2H, piperazine H_5); 2.85 (bs, 2H, piperazine H_2); 2.70 (bs, 2H, piperazine H_6). Anal. calcd. for $C_{20}H_{18}Cl_2N_4O_3$ (433.29): C, 55.44; H, 4.19; N, 13.93%. Found C, 55.29; H, 4.43; N, 13.70%.

5-(3,4-Dichlorophenyl)-3-[4-(2-furoylpiperazin-1-yl)methyl]-1,3,4-oxadiazol-2(3H)-one ***(5v)***

Yield: 43%, m.p. 136.4°C; IR (KBr) cm^{-1}: 3092 (C-H, aromatic), 2937 (C-H, aliphatic), 1786 (C=O), 1623 (C=N), 1565 (C=C, aromatic), 1283 (C-N), 1265 (C-O); ^1H NMR ($CDCl_3$) δ: 7.94 (d, 1H, dichlorophenyl H_2, *J*= 2.0); 7.66 (dd, 1H, dichlorophenyl H_5, *J*= 8.8, *J'*= 2.0); 7.56 (d, 1H, dichlorophenyl H_6, *J*= 8.4); 7.46 (d, 1H, 2-furoyl H_3, *J*= 1.6); 6.99 (d, 1H, 2-furoyl H_5, *J*= 3.2); 6.47 (t, 1H, 2-furoyl H_4, *J*= 2.8); 4.76 (s, 2H, N-CH_2-N); 3.83 (bs, 4H, piperazine H_3, H_5); 2.83 (t, 4H, piperazine H_2, H_6, *J*= 5.2). Anal. calcd. for $C_{18}H_{16}Cl_2N_4O_4$ (423.25): C, 51.08; H, 3.81; N, 13.24%. Found C, 50.75; H, 3.65; N, 13.15%.

Antimicrobial activity

Dimethylsulfoxide (DMSO) was used to dissolve and prepare the synthesized compounds with a concentration of 10 mg mL^{-1}. The lyophilized compounds sterilized by filtration via 0.45 mm millipore filters. Disc diffusion method was performed by using 100 mL of suspension containing 108 CFU mL^{-1} of bacteria, 106 CFU mL^{-1} of yeast and 104 spore mL^{-1} of fungi spread on nutrient agar (NA), sabourand dextrose agar (SDA) and potato dextrose agar (PDA) medium, in sequence. 15 mL of each synthesized compounds (300 mg/disc) at the concentration of 10 mg mL^{-1} were impregnated to the discs (6 mm in diameter). DMSO impregnated discs were used for negative controls. The compounds and negative controls were located in the inoculated agar. In order to determine the sensitivity of one strain/isolate standard nystatin was used as positive reference. The incubation at 37°C of inoculated plates took 24 h for bacterial strains, 48 h for yeast and 72 h for fungi isolates. The incubation of plant related microorganisms were held at 27°C, differently. Anti-microbial activity was screened by measuring the zone of inhibition against the test organisms in disc diffusion assay. The assays were repeated twice in this study.

Results and discussions

Chemistry

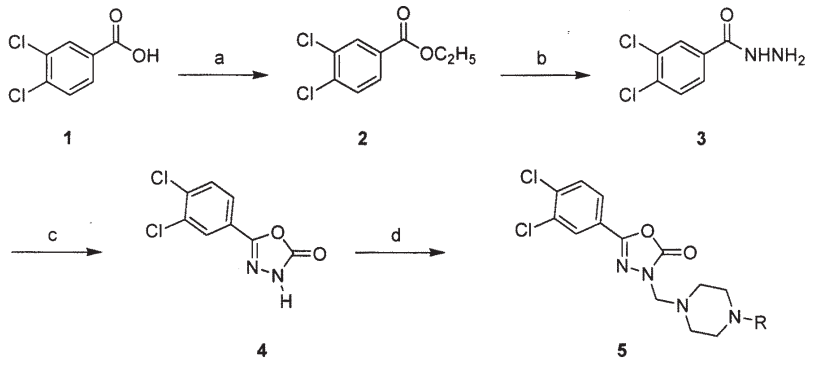
The synthetic route for the preparation of new 3,5-disubstituted-1,3,4-oxadiazole-2-one derivatives (**5a-5v)** is outlined in Scheme 1. The compounds were prepared as the *Mannich* bases of 5-(3,4-dichlorophenyl)-1,3,4-oxadiazol-2(3*H*)-one (**4**). The key intermediate **4** [27] was synthesized in three steps. Esterification of the 3,4-dichlorobenzoic acid (**1**) with ethanol and concentrated sulfuric acid afforded the corresponding ester **2**. The aroyl hydrazide **3** was obtained by the reaction of ethyl 3,4-dichlorobenzoate **2** with hydrazine hydrate monohydrate (85%) in ethanol. Then the treatment of hydrazide **3** with 1,1-carbonyldiimidazole (CDI) in presence of triethylamine (TEA) and tetrahydrofurane (THF) by stirring at room temperature gave the intermedate **4**. The synthesis of compounds **5a-5v** were accomplished by refluxing compound **4** with appropriate substituted piperazine derivatives and formaldehyde in ethanol. All the target compounds **5a-5v** were reported for the first time by our research group.

All of the compounds gave satisfactory analytical and spectroscopic data, which were in full accordance with their depicted structures. IR spectra of the synthesized compounds are similar to the IR values of which were stated in the literature [28-30]. For the compounds, no absorption band was detected at 3100-3400 cm^{-1}, indicating the absence of an NH group as an evidence for the substitution reaction to 5-(3,4-dichlorophenyl)-1,3,4-oxadiazol-2-one with substituted piperazine. In the ^1H-NMR spectra of the all compounds, the methylene protons representing the *Mannich* base formation were seen at about 4.72-4.80 ppm as a singlet. The protons of the 3,4-dichlorophenyl group were seen approximately at 7.95 (1H, d, H^2, *J*= 2 Hz), 7.65 (1H, dd, H^5, *J*= 8.2-8.8 Hz, *J'*= 2.0 Hz) and 7.55 (1H, d, H^6, *J*= 8.0-8.8 Hz) ppm, respectively. As $H^{3'}$ and $H^{5'}$ protons of the piperazine ring are overlapped and seen as a triplet peak at 3.09-3.87 ppm (*J*= 4.8 Hz), $H^{2'}$ and $H^{6'}$ protons are seen at 2.46-2.98 ppm (*J*= 4.8 Hz) likewise. In ^{13}C-NMR spectra of the compounds **5e** and **5q,** characteristic peaks were seen at 45.29, 49.02 (C_3, C_5) and 49.92 (C_2, C_6) ppm for piperazine moiety, 67.75, 77.30 ppm for methylene and 154.10 ppm for oxadiazole carbonyl groups. Other carbon atoms of the aromatic rings of the structures have been similar peak values indicating in the reference books and literature.

Antimicrobial activity

All of the synthesized compounds were evaluated for *in vitro* antimicrobial activity by disc diffusion method. The results were measured as a function of their zone of inhibition in mm and shown in table 1.

It is interesting that all screened compounds only showed antifungal activity. These antifungal selective compounds



Scheme 1. Synthesis of compounds **5a-5v**. Reagents and conditions: (a) H_2SO_4 (concd), ethanol, reflux 24 h; (b) $H_2NNH_2.H_2O$ (85%), ethanol, 24 h; (c) CDI, TEA, THF, rt, 20 h; (d) HCHO, substitutedpiperazine, ethanol, 4 h.

5a: R= C_6H_5-
5b: R= 2-F-C_6H_4-
5c: R= 4-F-C_6H_4-
5d: R= 2-Cl-C_6H_4-
5e: R= 3-Cl-C_6H_4-
5f: R= 4-Cl-C_6H_4-
5g: R= 2-MeO-C_6H_4-
5h: R= 3-MeO-C_6H_4-
5i: R= 4-MeO-C_6H_4-
5j: R= 4-CF_3-C_6H_4-
5k: R= 4-Me-C_6H_4-
5l: R= 2, 3-2Me-C_6H_4-
5m: R= 4-NO_2-C_6H_4-
5n: R= 4-OH-C_6H_4-
5o: R= 2-CN-C_6H_4-
5p: R= 4-CN-C_6H_4-
5q: R= 2-Pyridyl
5r: R= 4-Pyridyl
5s: R= 2-Pyrimidyl
5t: R= Piperonyl
5u: R= Benzoyl
5v: R= 2-Furoyl

Table 1
ANTIMICROBIAL ACTIVITY OF THE SYNTHESIZED COMPOUNDS **5a-5v**

Compd. no	Diameter of the zone inhibition(mm)							
	Antibacterial activity				Antifungal activity			
	Bm	***Ec***	***Sa***	***Pa***	***As***	***Fo***	***Bc***	***P***
5a	-	-	-	-	-	12	5	4
5b	-	-	-	-	18	13	14	2
5c	-	-	-	-	12	6	20	14
5d	-	-	-	-	10	6	13	3
5e	-	-	-	-	8	9	5	-
5f	-	-	-	-	6	4	4	5
5g	-	-	-	-	6	2	4	8
5h	-	-	-	-	16	7	3	10
5i	-	-	-	-	12	7	1	-
5j	-	-	-	-	4	18	-	6
5k	-	-	-	-	8	8	1	6
5l	-	-	-	-	10	6	2	4
5m	-	-	-	-	-	8	5	4
5n	-	-	-	-	10	8	4	11
5o	-	-	-	-	12	10	11	4
5p	-	-	-	-	12	6	15	2
5q	-	-	-	-	12	10	3	7
5r	-	-	-	-	-	10	4	3
5s	-	-	-	-	-	9	6	4
5t	-	-	-	-	9	10	8	2
5u	-	-	-	-	-	5	9	8
5v	-	-	-	-	7	9	9	1
Nystatin	-	-	-	-	14	10	12	14
Ofloxacin	18	20	13	10	-	-	-	-

As: *Aspergillus sp.*, ***Fo***: *Fusarium oxysporum*, ***Bc***: *Botrytis cinerea*, ***P***: *Penicillium*, ***Bm***: *Bacillus megaterium*, ***Ec***: *Escherichia coli*, ***Sa***: *Staphylococcus aureus*, ***Pa***: *Pseudomonas aeroginosa*

were examined against four different fungi and nystatin was used as reference for comparison. It was observed that some of the compounds revealed moderate to significant antifungal activity. Considering the results, it is noteworthy to mention that tested compounds had promising activity especially against *Fusarium oxysporum*. In comparison with nystatin, the compounds **5a**, **5b**, **5j**, **5o**, **5q**, **5r** and **5t** showed equal or better activity, in addition, compounds **5e** and **5s** activity showed comparable activities against this fungus. Among the compounds, compound **5j** was the notable one with 18 mm inhibitory zone. The results against *Aspergillus sp.* displayed that most of the compounds had moderate activity but compounds **5b** and **5h** had more powerful activity than nystatin with 18 and 16 mm inhibitory zones. Although compounds **5b-5d** and **5o** showed stronger activities against *Botrytis cinerea*, other compounds of the set possessed moderate or weak activities. In comparison with nystatin, except compound **5c**, all compounds showed weak activities against *Penicillium sp*.

When structure activity relationships are concerned, there is not a direct relationship between the substituents and activity. But, it is also clear that compounds having aryl substituents on the fourth position of the piperazine ring possessed better activity than acyl substituted structures. Also, the most potent compounds were especially the ones that had electron rich groups (F, Cl, OCH_3 and CF_3) as substituents at piperazine ring.

Conclusions

In conclusion, we have prepared some new 2,6-disubstituted-1,3,4-oxadiazol-2(3*H*)-onesunder environmentally mild conditions and their *in vitro* antimicrobial activities were evaluated. Compounds were identified as selective antifungal agents. Among the synthesized compounds, **5a-5d**, **5h**, **5j** and **5p** were found to be the most effective derivatives with higher zone inhibition values than standard drug nystatin against different fungal species. The active compounds represented in this study deserve to be studied further, since the present results shown here are significant because they can reveal new potent compounds for the antifungal treatment that is still a major worldwide health problem due to the rapid resistance development.

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