Antimicrobial agents inhibit or kill the growth of microorganisms such as bacteria or fungi. 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-dichlorophenyl)-3-[(4-substituted-piperazino)methyl]-1,3,4-oxadiazole-2(3H)-one derivatives (5a-5p) 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 within ± 0.4 °C. 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 within ± 0.4 °C. 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 within ± 0.4 °C. 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 within ± 0.4 °C. 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 within ± 0.4 °C. 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 within ± 0.4 °C. 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 within ± 0.4 °C. 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 within ± 0.4 °C. The reactions were monitored by thin layer chromatography (TLC) on Merck pre-coated silica G...
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, 1801 (C=N), 157.0 (C=C, aromatic), 1237 (C=C, aromatic), 1118 (C=O); ¹H NMR (CDCl₃) δ: 7.95 (d, 1H, dichlorophenyl H, J = 2.0); 7.67 (dd, 1H, dichlorophenyl H, J = 8.4, J' = 2.0); 7.56 (d, 1H, dichlorophenyl H, J = 8.2, J' = 2.0); 7.53 (d, 1H, dichlorophenyl H, J = 8.0, J' = 2.4); 7.15 (1H, phenyl H, J = 8.4); 6.75-6.86 (m, 3H, 2H, phenyl H, H'); 4.78 (s, 2H, N-CH₂-); 3.21 (t, 4H, piperazine H₃, H₅, J' = 5.2); 2.90 (4H, piperazine H₆, J = 5.2). Anal. calcd. for C₁₉H₁₇Cl₃N₄O₂: C, 51.90; H, 3.90; N, 12.68%. Found C, 51.90; H, 3.90; N, 12.68%.

5-(3,4-Dichlorophenyl)-3-[4-(2-fluorophenyl)piperazin-1-yl]-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), 1342 (C=C, aromatic), 1217 (C=O); ¹H NMR (CDCl₃) δ: 7.96 (d, 1H, dichlorophenyl H, J = 2.0); 7.68 (dd, 1H, dichlorophenyl H, J = 8.4, J' = 1.6); 7.57 (1H, dichlorophenyl H, J = 8.0); 6.94-7.08 (m, 4H, phenyl H, H', H'); 4.79 (s, 2H, N-CH₂-); 3.12 (t, 4H, piperazine H₃, H₅, J = 5.2); 2.96 (4H, piperazine H₆, J = 5.2). Anal. calcd. for C₁₉H₁₆Cl₂N₄O₂: C, 50.79; H, 3.86; N, 13.07%.

5-(3,4-Dichlorophenyl)-3-[4-(4-(3-methoxyphenyl)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), 1349 (C=C, aromatic), 1227 (C=O); ¹H NMR (CDCl₃) δ: 7.95 (d, 1H, dichlorophenyl H, J = 2.0); 7.68 (dd, 1H, dichlorophenyl H, J = 8.0, J' = 2.0); 7.57 (1H, dichlorophenyl H, J = 8.0); 6.93-6.98 (m, 2H, phenyl H, H'); 6.85-6.88 (m, 2H, phenyl H, H'); 4.78 (s, 2H, N-CH₂-); 3.13 (t, 4H, piperazine H₃, H₅, J = 5.2); 2.93 (4H, piperazine H₆, J = 5.2). Anal. calcd. for C₂₀H₂₀Cl₂N₄O₃: 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-(2-methoxyphenyl)piperazin-1-ylmethyl]-1,3,4-oxadiazol-2(3H)-one (5d)

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 (CDCl₃) δ: 7.95 (d, 1H, dichlorophenyl H, J = 2.0); 7.67 (dd, 1H, dichlorophenyl H, J = 8.0, J' = 2.0); 7.56 (1H, dichlorophenyl H, J = 8.0); 6.91-7.03 (m, 3H, phenyl H, H', H'); 6.85 (d, 1H, phenyl H, J = 8.0); 4.80 (s, 2H, N-CH₂-); 3.83 (3H, OCH₃); 2.98 (t, 4H, piperazine H₆, J = 5.2). Anal. calcd. for C₁₉H₁₇Cl₂N₄O₃: 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-(3-methoxyphenyl)piperazin-1-ylmethyl]-1,3,4-oxadiazol-2(3H)-one (5e)

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 (CDCl₃) δ: 7.94 (d, 1H, dichlorophenyl H, J = 2.0); 7.67 (dd, 1H, dichlorophenyl H, J = 8.0, J' = 2.0); 7.55 (1H, dichlorophenyl H, J = 8.0); 7.16 (t, 1H, phenyl H, J = 8.0); 6.52 (dd, 1H, phenyl H, J = 8.0, J' = 1.6); 6.41-6.45 (m, 2H, phenyl H, H'); 4.78 (s, 2H, N-CH₂-); 3.78 (3H, OCH₃); 3.21 (t, 4H, piperazine H₆, J = 4.3); 2.91 (4H, piperazine H₆, J = 4.8). Anal. calcd. for C₁₉H₁₈Cl₂N₄O₃: 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-trifluoromethylphenyl)piperazin-1-yl][methyl]-1,3,4-oxadiazol-2(3H)-one (5f)

Yield: 79%; m.p. 154.8°C; IR (KBr) cm⁻¹: 3083 (C-H, aromatic), 2970 (C-H, aliphatic), 1765 (C=O), 1620 (C-N), 1588 (C=C, aromatic), 1428 (C-N), 1210 (C-O); ¹H NMR (CDCl₃): δ: 7.95 (d, 1H, dichlorophenyl H, J = 2.0); 7.67 (dd, 1H, dichlorophenyl H, J = 8.0, J' = 2.0); 7.56 (d, 1H, dichlorophenyl H, J = 8.0); 7.47 (d, 2H, phenyl H, J = 8.8); 6.91 (d, 2H, phenyl H, J = 8.8); 4.79 (s, 2H, N-CH₂-N); 3.31 (t, 4H, piperazine H, J = 5.2); 2.92 (t, 4H, piperazine H, J = 5.2). Anal. calcld. for C₂₅H₂₁Cl₂N₅O₂ (539.52): C, 56.63; H, 3.90; N, 15.77%. Found C, 56.19; H, 3.83; N, 15.75%.

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), 1593 (C=C, aromatic), 1274 (C-N), 1226 (C-O); ¹H NMR (CDCl₃): δ: 8.25 (d, 2H, 4-pyridyl H, J = 6.0); 7.94 (d, 1H, dichlorophenyl H, J = 2.0); 7.67 (dd, 1H, dichlorophenyl H, J = 8.8, J' = 2.0); 7.57 (s, 1H, dichlorophenyl H, J = 8.4); 7.26 (s, 1H, hydroxyl); 6.83 (dd, 2H, phenol H, J = 6.8, J' = 2.4); 6.76 (dd, 2H, phenol H, J = 6.8, J' = 2.4); 4.78 (s, 2H, N-CH₂-N); 3.09 (t, 4H, piperazine H, J = 4.8); 2.93 (t, 4H, piperazine H, J = 4.0). Anal. calcld. for C₂₅H₂₁Cl₂N₅O₂ (521.28): 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 (CDCl₃): δ: 7.96 (d, 1H, dichlorophenyl H, J = 2.0); 7.70 (dd, 1H, dichlorophenyl H, J = 8.4, J' = 2.0); 7.57 (d, 1H, dichlorophenyl H, J = 8.0); 7.56 (dd, 2H, cyanophenyl H, J = 8.0, J' = 1.6); 7.49 (t, 1H, cyanophenyl H, J = 8.0); 7.03 (t, 1H, cyanophenyl H, J = 8.2); 4.78 (s, 2H, N-CH₂-N); 3.25 (t, 4H, piperazine H, J = 4.4); 2.99 (t, 4H, piperazine H, J = 4.8). Anal. calcld. for C₂₆H₂₂Cl₂N₅O₂ (540.30): C, 55.58; H, 3.98; N, 16.28%. Found C, 55.58; H, 4.03; N, 16.17%.
5-(3,4-Dichlorophenyl)-3-[4-(2-pyrimidopiperazin-1-yl)methyl]-1,3,4-oxadiazol-2(3H)-one (5s)

Yield: 45%, m.p. 138.8°C; IR (KBr) cm\(^{-1}\): 3093 (C-H, aromatic), 2951 (C-H, aliphatic), 1771 (C=O), 1606 (C=N), 1583 (C=C, aromatic), 1535 (C-N), 1219 (C-O); \(\delta\) NMR (CDCl\(_3\)) 8: 8.28 (d, 2H, 2-pyrimidyl H\(_2\), J=4.8); 7.92 (d, 1H, dichlorophenyl H\(_2\), J=2.0); 7.65 (dd, 1H, dichlorophenyl H\(_4\), J=8.4, J=2.0); 7.55 (d, 1H, dichlorophenyl H\(_5\), J=8.4); 6.47 (t, 1H, 2-pyrimidyl H, J=4.8); 4.78 (s, 2H, N-CH\(_2\)-N); 3.81 (bs, 2H, piperazine H\(_3\)); 3.47 (s, 2H, -CH\(_2\)-); 4.72 (s, 2H, N-CH\(_2\)-N); 3.40 (s, 2H, -CH\(_2\)-); 2.78 (t, 4H, piperazine H\(_2\), J=4.8); 2.46 (bs, 4H, piperazine H\(_5\), J=4.8).

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. The 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(3H)-one (4). The key intermediate 4 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 arylo 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 intermediate 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 \(^1\)H-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, 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\(_4\), J\(=8.0-8.8\) Hz) ppm, respectively. As H\(^3\) and H\(^4\) protons of the piperazine ring are overlapped and seen as a triplet peak at 3.09-3.87 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

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, 104 CFU mL\(^{-1}\) of yeast and 104 CFU mL\(^{-1}\) of fungi spread on nutrient agar (NA), sabouraud 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.

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 are promising for the treatment of fungi in medical and agricultural applications.
**Scheme 1.** Synthesis of compounds 5a-5v.
Reagents and conditions: (a) H$_2$SO$_4$ (concd), ethanol, reflux 24 h; (b) H$_2$NNH$_2$H$_2$O (85%), ethanol, 24 h; (c) CDI, TEA, THF, rt, 20 h; (d) HCHO, substituted piperazine, ethanol, 4 h.

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

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 *P. 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 nm 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 alkyl 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, OCH₃ and CF₃) as substituents at piperazine ring.

**Conclusions**

In conclusion, we have prepared some new 2,6-disubstituted-1,3,4-oxadiazol-2(3H)-ones under environmental mild conditions and their in vitro antifungal 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.

**References**


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