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Synthesis, Characterization And Anti-Microbial Evaluation Of Some Novel 1,3,4-Oxadiazoles Containing Piperazine Moiety

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Abstract: Ethyl 2-(4-(2,3-dichlorophenyl)piperazin-1-yl)acetate 2 was prepared by the condensation of ethyl bromoacetate with 1-(2,3-dichlorophenyl)piperazine 1. The reaction of 2 with hydrazine hydrate furnished 2-[4-(2,3-dichlorophenyl)piperazin-1-yl]acetohydrazide 3, which on cyclisation with substituted aromatic carboxylic acids in the presence of phosphorous oxychloride give 1-(2,3-dichlorophenyl)-4-((5-aryl-1,3,4-oxadiazol-2-yl)methyl)piperazine 4. The newly synthesized compounds have been characterized by IR, 1H NMR, mass spectral data and elemental analysis. The synthesized compounds were screened for in vitro antimicrobial activity. The purity of synthesized compounds was confirmed by TLC.
Keywords: Piperazine, 1,3,4-oxadiazoles, anti-microbial activity, IR, ^{1}H NMR, Mass.

Introduction

Derivatives of 1,3,4-oxadiazole constitute an important family of heterocyclic compounds[1]. Since many of them exhibit a remarkable biological activity[2, 3] and find wide usage as dyes, photosensitive electrical materials, polymer precursors, stabilizers, their synthesis, and transformations have received great attention for a long time. Particularly, the 2-aryl-5-substituted-1,3,4-oxadiazoles have been reported to show antibacterial[4], antifungal[5,6], analgesic, anti-inflammatory[7], and hypoglycemic activities. Similarly a number of piperazine derivatives have been shown to possess a variety of pharmacological properties like antihistamanic[8], analgesic[9], anti-inflammatory[10], anti-HIV[11], antimalarial[12], antitubercular[13] and antimicrobial[14] activity, and hence piperazine is found to be an important structural feature in some synthetic drugs. Keeping this in view, it was thought worth-while to design the synthesis of title compounds wherein the biologically active piperazine is linked to potent 1,3,4-oxadiazole moiety, through methylene bridge. The present communication reports the multistep synthesis of novel 1-(2,3-dichlorophenyl)-4-((5-aryl-1,3,4-oxadiazol-2-yl)methyl)piperazine **4a-j** and their antimicrobial activity.

Experiment

General

Melting points were determined in open capillary tubes in a Thomas Hoover melting point apparatus and are uncorrected. The purity of the compounds was confirmed by thin layer chromatography using silica gel glass plates. The spots were developed in iodine chamber and visualized under ultraviolet lamp. Infrared (IR) and ^{1}H nuclear magnetic resonance (^{1}H NMR) spectra were recorded for the compounds in SHIMADZU FTIR 8400 Spectrophotometer and BRUKER Spectrometer (300 MHz) respectively.

Chemical shifts are reported in parts per million (ppm) using tetramethylsilane (TMS) as an internal standard. Elemental analysis was undertaken with Perkin Elmer 2400 instrument and the measured values agreed with the calculated.

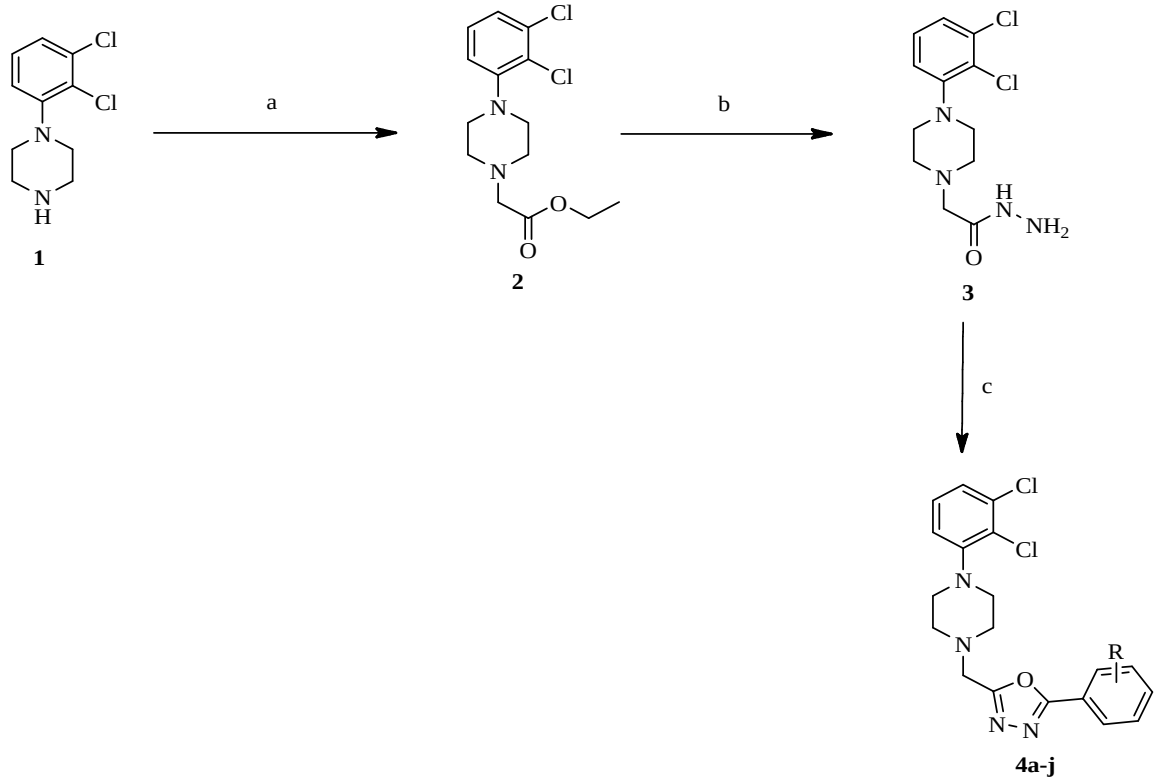
Chemistry

The target compounds were synthesized according to the representative Scheme I. The reaction of 1-(2,3-dichlorophenyl)piperazine **1** with ethyl bromoacetate, in the presence of potassium carbonate under refluxing condition yielded ethyl 2-(4-(2,3-dichlorophenyl) piperazin-1-yl)acetate **2**, which on reaction with hydrazine hydrate in ethanol at reflux temperature to 2-(4-(2,3-dichlorophenyl)piperazin-1-yl)acetohydrazide **3**. The compound **3** was reacted with different aromatic carboxylic acid in the presence of phosphorous oxychloride give the title compounds 1-(2,3-dichlorophenyl)-4-((5-aryl-1,3,4-oxadiazol-2-yl)methyl)piperazine **4a-j**

The structural assignments of all the newly synthesised compounds were made on the basis of elemental analysis, ^{1}H NMR, IR and mass spectral data.

Synthesis of **3** was confirmed by its IR and ^{1}H NMR spectral data. Its FTIR spectrum showed strong peaks at 3292 and 1668 cm^{-1} indicating the presence of -$NHNH_2$ and >C=O groups respectively, while its ^{1}H-NMR spectrum showed a broad peak at 3.89 δ ppm and a sharp peak at 8.17 δ ppm confirming the presence of –$CONHNH_2$ group. The mass spectrum of it showed a molecular ion peak at m/z 303.2 which matches with its molecular formula $C_{12}H_{16}Cl_2N_4O$. Formation of oxadiazoles from **3** was confirmed by IR, ^{1}H NMR and mass spectral studies. ^{1}H NMR spectrum of **4a** showed two triplet at 2.83 δ ppm and 3.10 δ ppm, singlet at 3.98 δ ppm, double doublet at 6.94 δ ppm, multiplet at 7.11 δ ppm, multiplet at 7.49 δ ppm and multiplet at 8.07 δ ppm which confirmed structural requirement of 4a Further the mass spectrum of 4a showed a (M+1) peak at m/z 389.2 (100%), which is in agreement with its molecular formula $C_{19}H_{18}Cl_2N_4O$.

Reaction Scheme:



Scheme 1. Reagents and conditions: (a) Ethyl bromoacetate, K_2CO_3, Acetone, reflux, 15 h; (b) $NH_2NH_2H_2O$, Ethanol, reflux, 6 h; (c) phosphorous oxychloride, substituted carboxylic acid, reflux, 8-15 h.

Procedure for the synthesis of ethyl 2-(4-(2,3-dichlorophenyl) piperazin-1-yl)acetate (2). To the stirred solution of 1-(2,3-dichlorophenyl)piperazine (0.1 mole, 23.1 gm) and potassium carbonate (0.15 mole, 20.7 gm) in 100 ml acetone was added ethyl bromo acetate (0.11 mole, 18.37 gm) drop wise at 10°C then stirred the reaction at room temperature for 14 hr. Completion of reaction was checked by TLC using mobile phase ethyl acetate / Hexane (6/4). After removal of acetone under reduced pressure, the residue was added to chilled water (500 ml) and stirred for overnight. The solid separated was filtered, washed with water, dried and finally crystallized from ethanol to give 2.4 gm cream solid.

ethyl 2-(4-(2,3-dichlorophenyl) piperazin-1-yl)acetate (2). Yield 76 %, ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.05 (t, 3H, Ester-CH_3), 2.79 (t, 4H, Piperazine CH_2), 3.12 (t, 4H, Piperazine CH_2), 3.17 (s, 2H, CH_2), 4.07 (q, 2H, Ester-CH_2), 7.01 (dd, 1H, *J* = 3.0 & 6.3 Hz, Ar-H), 7.23 (m, 2H, Ar-H). IR:, 3005, 2978, 2830 (CH str.), 1736, 1580, 1560, 1477 (C=C str.), 1338, 1276, 1026, 945, 868, 781, 711 cm^{-1}. Mass (*m/z*): 317.3 ([M+H]$^+$), Anal. Calcd. For $C_{14}H_{18}Cl_2N_2O_2$: C, 53.01; H, 5.72; N, 8.83. Found: C, 52.88; H, 5.83; N, 8.77.

Procedure for the synthesis of 2-(4-(2,3-dichlorophenyl)piperazin-1-yl)acetohydrazide (3). A mixture of 2-(4-(2,3-dichlorophenyl)piperazin-1-yl)acetate (3.16 gm, 0.01 mole) and hydrazine hydrate 99 % (1 gm, 0.02 mole) in 10 ml ethanol was stirred and refluxed for 8 hr. Completion of reaction was checked by TLC using mobile phase ethyl acetate / Hexane (8/2). The reaction mixture was then kept in deep-freezer overnight. The precipitated product was filtered off, washed with hexane (10 ml) and crystallized from isopropyl alcohol (IPA) to give 2.44 gm white solid.

2-(4-(2,3-dichlorophenyl)piperazin-1-yl)acetohydrazide (3). Yield 81 %, ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.71 (t, 4H, Piperazine CH_2), 3.06 (bs, 4H, Piperazine CH_2), 3.17 (s, 2H, CH_2), 3.89 (broad doublet, 2H, -NH_2), 6.91 (dd, 1H, *J* = 3.0 & 6.0 Hz, Ar-H), 7.12 (m, 2H, Ar-H), 8.17 (bs, 1H, -CONH-). IR: 3292, 3207, 3005, 2949, 2821 (CH str.), 1668, 1614, 1577, 1556, 1487 (C=C str.), 1307, 1236, 1004, 960, 819, 779 cm^{-1}. Mass (*m/z*): 303.2 ([M+H]$^+$), Anal. Calcd. For $C_{12}H_{16}Cl_2N_4O$: C, 47.54; H, 5.32; N, 18.48. Found: C, 47.59; H, 5.44; N, 18.33.

General procedure for the synthesis of 1-(2,3-dichlorophenyl)-4-((5-aryl-1,3,4-oxadiazol-2-yl)methyl)piperazine 4(a-j). A mixture of 2-(4-(2,3-dichlorophenyl)piperazin-1-yl)acetohydrazide (1.51g, 0.005 mol) and substituted carboxylic acid (0.005 mol) in phosphorous oxychloride (5 ml) was refluxed for 8-15 hrs. The content was cooled, poured into crushed ice and neutralized with sodium bicarbonate solution. Crude product was isolated and crystallized from ethanol. Yield: 60-80 %

1-(2,3-dichlorophenyl)-4-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)piperazine (4a). Yield 69%, mp. 192 °C, ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.83 (t, 4H, Piperazine CH_2), 3.10 (t, 4H, Piperazine CH_2), 3.98 (s, 2H, CH_2), 6.94 (dd, 1H, *J* = 2.7 & 6.6 Hz, Ar-H), 7.11 (m, 2H, Ar-H), 7.49 (m, 3H, Ar-H), 8.07 (m, 2H, Ar-H). IR: 3049, 2960, 1606 (C=N str.), 1523, 1489, 1454, 1346, 1116, 1062 (C-O-C str.), 974, 850, 746, 709, 675 cm^{-1}. Mass (*m/z*): 389.2 ([M+H]$^+$), Anal. Calcd. For $C_{19}H_{18}Cl_2N_4O$: C, 58.62; H, 4.66; N, 14.39. Found: C, 58.54; H, 4.72; N, 14.51.

1-(2,3-dichlorophenyl)-4-((5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl)methyl)piperazine(4b). Yield 76%, mp. 205 °C, ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.69 (t, 4H, Piperazine CH_2), 3.00 (t, 4H, Piperazine CH_2), 3.84 (s, 3H, Ar-OCH_3), 3.94 (s, 2H, CH_2), 7.12 (m, 3H, Ar-H), 7.28 (m, 2H, Ar-H), 7.92 (d, 2H, *J* = 8.7 Hz, Ar-H). IR: 3008, 2948, 1594 (C=N str.), 1498, 1462, 1418, 1322, 1240, 1085 (C-O-C str.), 996, 846, 744, 728 cm^{-1}. Mass (*m/z*): 419.2 ([M+H]$^+$), Anal. Calcd. For $C_{20}H_{20}Cl_2N_4O_2$: C, 63.32; H, 5.31; N, 6.71. Found: C, 63.45; H, 5.23; N, 6.55.

1-(2,3-dichlorophenyl)-4-((5-(4-methylphenyl)-1,3,4-oxadiazol-2-yl)methyl)piperazine (4c). Yield 72%, mp. 256 °C, ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.11 (s, 3H, Ar-CH3), 2.75 (t, 4H, Piperazine CH_2), 3.08 (t, 4H, Piperazine CH_2), 3.91 (s, 2H, CH_2), 6.92 (dd, 1H, *J* = 2.7 & 6.6 Hz, Ar-H), 7.12 (m, 2H, Ar-H), 7.23 (d, 2H, *J* = 8.7 Hz, Ar-H), 7.47 (d, 2H, *J* = 8.7 Hz, Ar-H). IR: 3022, 2930, 2853, 1584 (C=N str.), 1483, 1413, 1375, 1203, 1133, 1095 (C-O-C str.), 1011, 855, 834, 732 cm^{-1}. Mass (*m/z*): 403.2 ([M+H]$^+$), Anal. Calcd. For $C_{20}H_{20}Cl_2N_4O$: C, 59.56; H, 5.00; N, 13.89. Found: C, 59.33; H, 5.16; N, 13.96.

1-(2,3-dichlorophenyl)-4-((5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl)methyl)piperazine (4d). Yield 66%, mp. 217 °C, ^{1}H NMR (300 MHz,

$CDCl_3$): δ 2.71 (t, 4H, Piperazine CH_2), 3.06 (t, 4H, Piperazine CH_2), 4.05 (s, 2H, CH_2), 6.96 (dd, 1H, *J* = 2.7 & 6.6 Hz, Ar-H), 7.15 (m, 2H, Ar-H), 7.81 (d, 2H, *J* = 8.4 Hz, Ar-H), 8.32 (d, 2H, *J* = 8.4 Hz, Ar-H). IR: 3079, 2936, 1607 (C=N str.), 1554, 1461, 1422, 1261, 1043 (C-O-C str.), 839, 720 cm^{-1}. Mass (*m/z*): 434.1 ($[M+H]^+$), Anal. Calcd. For $C_{19}H_{17}Cl_2N_5O_3$: C, 52.55; H, 3.95; N, 16.13. Found: C, 52.68; H, 3.82; N, 16.18.

1-(2,3-dichlorophenyl)-4-((5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl)methyl)piperazine (4e). Yield 71%, mp. 230 ºC, ^1H NMR (300 MHz, $CDCl_3$): δ 2.63 (t, 4H, Piperazine CH_2), 2.95 (t, 4H, Piperazine CH_2), 3.01 (s, 2H, CH_2), 7.11 (m, 3H, Ar-H), 7.32 (m, 2H, Ar-H), 7.56 (d, 2H, *J* = 8.7 Hz, Ar-H). IR: 3024, 2957, 1596 (C=N), 1492, 1410, 1255, 1109, 1011 (C-O-C str.), 942, 835, 752 cm^{-1}. Mass (*m/z*): 407.1 ($[M+H]^+$), Anal. Calcd. For $C_{19}H_{17}Cl_2FN_4O$: C, 56.03; H, 4.21; N, 13.76. Found: C, 56.15; H, 4.29; N, 13.97.

1-(2,3-dichlorophenyl)-4-((5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)methyl)piperazine (4f). Yield 79%, mp. 221 ºC, ^1H NMR (300 MHz, $CDCl_3$): δ 2.88 (t, 4H, Piperazine CH_2), 3.11 (t, 4H, Piperazine CH_2), 3.95 (s, 2H, CH_2), 7.14 (m, 3H, Ar-H), 7.31 (m, 2H, Ar-H), 7.47 (d, 2H, *J* = 8.1 Hz, Ar-H). IR: 3017, 2962, 1608 (C=N str.), 1550, 1474, 1429, 1331, 1234, 1091 (C-O-C str.), 996, 851, 764, 739 cm-1. Mass (*m/z*): 423.0 ($[M+H]^+$), Anal. Calcd. For $C_{19}H_{17}Cl_3N_4O$: C, 53.86; H, 4.04; N, 13.22. Found: C, 53.72; H, 4.21; N, 13.01.

1-((5-(4-bromophenyl)-1,3,4-oxadiazol-2-yl)methyl)-4-(2,3-dichlorophenyl)piperazine (4g). Yield 75%, mp. 233 ºC, ^1H NMR (300 MHz, $CDCl_3$): δ 2.67 (t, 4H, Piperazine CH_2), 3.05 (t, 4H, Piperazine CH_2), 3.91 (s, 2H, CH_2), 6.98 (dd, 1H, *J* = 2.7 & 6.6 Hz, Ar-H), 7.15 (m, 2H, Ar-H), 7.38 (d, 2H, *J* = 8.7 Hz, Ar-H), 7.76 (d, 2H, *J* = 8.4 Hz, Ar-H). IR: 3037, 2938, 2851, 1612 (C=N str.), 1576, 1467, 1407, 1353, 1203, 1133, 1024 (C-O-C str.), 834, 780, 732, 680 cm^{-1}. Mass (*m/z*): 467.0 ($[M+H]^+$), Anal. Calcd. For $C_{19}H_{17}BrCl_2N_4O$: C, 48.74; H, 3.66; N, 11.97. Found: C, 48.79; H, 3.58; N, 12.15.

4-(5-((4-(2,3-dichlorophenyl)piperazin-1-yl)methyl)-1,3,4-oxadiazol-2-yl)phenol (4h). Yield 63%, mp. 195 ºC, ^1H NMR (300 MHz, $CDCl_3$): δ 2.63 (t, 4H, Piperazine CH_2), 3.03 (t, 4H, Piperazine CH_2), 3.87 (s, 2H, CH_2), 6.89 (m, 3H, Ar-H), 7.18 (m, 2H, Ar-H), 7.41 (d, 2H, *J* = 8.7 Hz, Ar-H), 9.63 (s, 1H, Ar-OH). IR: 3529, 3207, 3064, 2945, 2825, 1614 (C=N str.), 1577, 1448, 1421, 1238, 1093 (C-O-C str.), 954, 819, 779, 711 cm^{-1}. Mass (*m/z*): 404.2 ($[M+H]^+$), Anal. Calcd. For $C_{19}H_{18}Cl_2N_4O_2$: C, 56.31; H, 4.48; N, 13.82. Found: C, 56.27; H, 4.54; N, 13.71.

1-(2,3-dichlorophenyl)-4-((5-(3-methylphenyl)-1,3,4-oxadiazol-2-yl)methyl)piperazine (4i). Yield 74%, mp. 181 ºC, ^1H NMR (300 MHz, $CDCl_3$): δ 2.16 (s, 3H, Ar-CH3), 2.71 (t, 4H, Piperazine CH_2), 3.03 (t, 4H, Piperazine CH_2), 3.96 (s, 2H, CH_2), 6.87 (dd, 1H, *J* = 2.7 & 6.6 Hz, Ar-H), 7.08 (m, 2H, Ar-H), 7.23 (m, 1H, Ar-H), 7.39 (m, 3H, Ar-H). IR: 3028, 2922, 2868, 1596 (C=N str.), 1476, 1439, 1381, 1216, 1126, 1088 (C-O-C str.), 1034, 834, 783, 712 cm^{-1}. Mass (*m/z*): 403.2 ($[M+H]^+$), Anal. Calcd. For $C_{20}H_{20}Cl_2N_4O$: C, 59.56; H, 5.00; N, 13.89. Found: C, 59.49; H, 5.18; N, 13.97.

1-((5-(5-chloro-2-nitrophenyl)-1,3,4-oxadiazol-2-yl)methyl)-4-(2,3-dichlorophenyl)piperazin e (4j). Yield 61%, mp. 208 ºC, ^1H NMR (300 MHz, $CDCl_3$): δ 2.84 (t, 4H, Piperazine CH_2), 3.14 (t, 4H, Piperazine CH_2), 3.91 (s, 2H, CH_2), 6.91 (dd, 1H, *J* = 2.7 & 6.6 Hz, Ar-H), 7.14 (m, 2H, Ar-H), 7.67 (d, 1H, *J* = 8.4 Hz, Ar-H), 8.21 (m, 2HAr-H). IR: 3068, 2945, 2825, 1588 (C=N str.), 1531, 1450, 1421, 1240, 1107, 1059 (C-O-C str.), 954, 839, 783, 736 cm^{-1}. Mass (*m/z*): 468.0 ($[M+H]^+$), Anal. Calcd. For $C_{19}H_{16}Cl_3N_5O_3$: C, 48.69; H, 3.44; N, 14.94. Found: C, 48.86; H, 3.21; N, 14.89.

Results And Discussion

Antimicrobial activity

The minimum inhibitory concentrations (MICs) of synthesized compounds were carried out by broth microdilution method as described by Rattan[18]. Antibacterial activity was screened against two gram positive (*Staphylococcus aureus* MTCC 96, *Streptococcus pyogenus* MTCC 443) and two gram negative (*Escherichia coli* MTCC 442, *Pseudomonas aeruginosa* MTCC 441) bacteria, ampicillin was used as a standard antibacterial agent. Antifungal activity was screened against three fungal species *Candida albicans* MTCC 227, *Aspergillus niger* MTCC 282 and *Aspergillus clavatus* MTCC 1323, Griseofulvin was used as a standard antifungal agent.

All MTCC cultures were collected from Institute of Microbial Technology, Chandigarh and Mueller Hinton broth was used as nutrient media

to grow and diluted the drug suspension for the test. Inoculum size for test strain was adjusted to 108 CFU (Colony Forming Unit) per milliliter by comparing the turbidity. DMSO was used to dilute to get desired concentration of drugs to test upon standard bacterial strains. Serial dilutions were prepared in primary and secondary screening. The control tube containing no antibiotic was immediately sub cultured (before inoculation) by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at 37 °C overnight. The tubes were then incubated overnight. The MIC of the control organism was read to check the accuracy of the drug concentrations. The lowest concentration inhibiting growth of the organism was recorded as the MIC. All the tubes not showing visible growth (in the same manner as control tube described above) were sub cultured and incubated overnight at 37 °C. The amount of growth from the control tube before incubation (which represents the original inoculum) was compared. Subcultures might show similar number of colonies indicating bacteriostatic; a reduced number of colonies indicating a partial or slow bactericidal activity and no growth if the whole inoculum has been killed. The test must include a second set of the same dilutions inoculated with an organism of known sensitivity. Each synthesized drug was diluted for obtaining 2000 µg/ml concentration, as a stock solution. In primary screening 500 µg/ml, 250 µg/ml and 125 µg/ml concentrations of the synthesized drugs were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution against all microorganisms. The drugs found active in primary screening were similarly diluted to obtain 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.12 µg/ml and 1.56 µg/ml concentrations. The highest dilution showing at least 99 % inhibition is taken as MIC. Results obtained are given in Table 1.

Antibacterial activity

The minimum inhibitory concentrations (MICs) of the tested compounds are shown in Table 1. The different compounds **4(a-j)** were tested for *in vitro* against two gram positive (*S. aureus* MTCC 96, *S. pyogenus* MTCC 443) and two gram negative (*E. coli* MTCC 442, *P. aeruginosa* MTCC 441) bacteria. From the screening data, most of the compounds possessed very good antibacterial activity (MBC, 50-250 µg/ml) against gram positive *S. aureus*, some of them possessed excellent activity compared to ampicillin. Compound **4b**, **4e, 4f** and **4h** showed MBC value in the range between 50-200 µg/ml while ampicillin has standard MBC value of 100 µg/ml against gram negative *E. coli* which indicates that this compounds have excellent activity, while other Compound **4a, 4c,** and **4j** possessed MBC value in the range of 250-350 µg/ml against gram negative *E. coli* while **4b and 4e** exhibited very good activity against *P. aeruginosa*. Compounds **4a, 4d** and **4j** displayed comparable activity in the range of 350-500 µg/ml while remaining **4b, 4e, 4f** and **4i** were equivalent activity against gram positive *S. aureus* compared with ampicillin. Compound **4b and 4e** have MBC of 50-100 µg/ml against *S. pyogenus* which was comparatively good while compound **4d, 4f, 4h, 4i** and **4j** displayed moderate activity in the range of 150-250 µg/ml against *S. pyogenus.* The remaining piperazine derivatives possessed moderate to poor activity against all four bacterial species.

Table 1: Antimicrobial activity of Compounds 4(a-j)

Comp.	Minimal bactericidal concentration µg/ml				Minimal fungicidal concentration µg/ml		
	Gram negative		Gram positive				
	E. coli	*P. aeruginosa*	*S. aureus*	*S. pyogenus*	*C. albicans*	*A. niger*	*A. clavatus*
4a	300	200	500	500	1000	250	500
4b	150	100	250	50	250	150	100
4c	350	500	1000	500	500	250	500
4d	1000	250	350	200	>1000	200	1000
4e	50	150	250	100	250	150	100
4f	100	200	200	250	500	100	200
4g	500	500	500	500	1000	500	250
4h	200	250	500	150	1000	1000	>1000
4i	1000	1000	200	250	>1000	500	500
4j	250	250	500	200	1000	250	500
ampicillin	100	100	250	100	-	-	-
Griseoful-vin	-	-	-	-	500	100	100

Antifungal activity

The minimum inhibitory concentrations (MICs) of the synthesized compounds are shown in Table 1. For *in vitro* antifungal activity, three fungal species *C, albicans* MTCC 227, *A. niger* MTCC 282 and *A. clavatus* MTCC 1323 were used and compared with standard drug griseofulvin. Most of the compounds possessed very good antifungal activity against *C. albicans*; their MFC values were in the range between 100-500 µg/ml. Compounds **4b** and **4e** showed excellent activity of 250 µg/ml; **4c** and **4f** possessed very good activity of 500 µg/ml which is similar to griseofulvin (500 µg/ml) against *C. albicans* whereas remaining compounds possessed moderate to poor activity against *A. niger* and *A. clavatus* compared with griseofulvin.

Conclusion

A synthetic method has been developed for substituted 1,3,4-oxadiazole derivatives using conventional route, It can be concluded that formation of oxadiazoles via cyclocondensation depends on time period. So the variation found in required reaction time period to yield the concern product with great significance and it was due to steric hinderanance of substituents.

The antimicrobial screening results for compounds **4a-j** showed that the substitution pattern on 5-position of 1,3, 4-oxadiiazole moiety appears to be vital for broad-specturm activity. From the screening results, 1,3,4-oxadiazole derivative which contains 4-fluorophenyl or 4-chlorophenyl group on 5-position was more active while 1,3,4-oxadiazole derivative containing 4-methoxyphenyl group on 5-position was also active against the microbial species. The remaining of oxadiazole derivative exhibited moderate to poor activity. From the above discussions we concluded that the some of newly synthesized compounds displayed excellent antibactarial and antifungal activity. These results put the novel oxadiazoles into class of interesting lead molecules for further synthetic and biological evaluation. It can be concluded that this class of compounds certainly holds great promise towards the pursuit to discover novel classes of antimicrobial agents.

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