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ORIGINAL ARTICLE

Synthesis and biological evaluation of newer analogues of 2,5-disubstituted 1,3,4-oxadiazole containing pyrazole moiety as antimicrobial agents

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Abstract A series of 2,5-disubstituted-1,3,4-oxadiazole derivatives bearing pyrazole moiety were synthesized by reacting various substituted pyrazole-4-carboxylic acids with different hydrazides in $POCl_3$. All the synthesized compounds **(4a–n)** were characterized by IR, NMR, mass spectra and elemental analyses. Synthesized 1,3,4-oxadiazole derivatives were screened for their antibacterial activity against three different strains, namely *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, while antifungal activity was determined against three different strains *Aspergillus flavus*, *Chrysosporium keratinophilum* and *Candida albicans*. The investigation of antimicrobial screening revealed that compounds **4i** and **4j** exhibited excellent activity when compared with the standard drugs.

1. Introduction

Infectious diseases are one of the leading causes of death worldwide. During the past few decades, new infectious diseases have appeared and old ones previously thought to be controlled have reemerged (Sharma and Jain, 2008). Despite the critical need for new antimicrobial agents, the development of these agents is declining. Solutions encouraging and facilitating the development of new antimicrobial agents are needed.

1,3,4-Oxadiazoles constitute an important family of heterocyclic compounds as they have attracted significant interest in

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medicinal chemistry, pesticide chemistry and polymer science (Bostrom et al., 2012; Mohan et al., 2004; Schulz et al., 1997). Since many of 1,3,4-oxadiazoles display a remarkable biological activity (Zoumpoulakis et al., 2012; Ingale et al., 2012), their synthesis and transformations have been receiving particular interest for a long time (Bondock et al., 2012). Most of the marketed antihypertensive agents such as Tiodazosin (Vardan et al., 1983) and Nesapidil (Schlecker and Thieme, 1988) as well as antibiotics such as Furamizole (Ogata et al., 1971) contain oxadiazole nucleus. During the past years, considerable evidences have accumulated to demonstrate the efficacy of 1,3,4-oxadiazole including antimicrobial (Zoumpoulakis et al., 2012), anti-inflammatory, analgesic (Ingale et al., 2012), anti-HIV (Sriram et al., 2009), antimycobacterial (Macaev et al., 2005), cathepsin K inhibitors (Palmer et al., 2006), tyrosinase inhibitors (Khan et al., 2005), monoamine oxidase (MAO) inhibitors (Ke et al., 2008) and anticonvulsant (Almasirad et al., 2004) properties.

The pyrazole motif makes up the core structure of numerous biologically active compounds (Elguero et al., 2002). Thus, some representatives of this heterocycle have an affinity for the human CRF-1 receptor (Wustrow et al., 1998), exhibit antiviral/anti-tumor (Manfredini et al., 1992; Sunil et al., 2013; Park et al., 2005), antimicrobial (Isloor et al., 2009; Vijesh et al., 2010) antipyretic (Eid et al., 1978), anti-inflammatory (Bekhit et al., 2003), analgesic (Menozzi et al., 1997), fungistatic (Sridhar et al., 2004), fungicidal (Rich and Horsfall, 1952) and anti-hyperglycemic activity (Kees et al., 1996; Bebernitz et al., 2001). Apart from this, pyrazole entity may also be used as nonlinear optical materials (Chandrakantha et al., 2013). Against this background, to extend our research work in heterocyclic synthesis coupled with the significant biological importance of oxadiazoles and pyrazole derivatives, prompted us to undertake the synthesis of 2,5-disubstituted-1,3,4-oxadiazoles. We report herein the synthesis of 2,5-disubstituted-1,3,4-oxadiazoles with an expectation to find new and more potent antimicrobial agents.

2. Experimental

2.1. Materials and methods

All the laboratory grade reagents were obtained commercially. The reaction was monitored by thin layer chromatography, which was performed on Merck precoated plates (silica gel. 60 F_{254}, 0.25 mm) and was visualized by fluorescence quenching under UV light (254 nm). Melting points were determined by the open capillary method and were uncorrected. The IR spectra were recorded on a Thermo Nicolet avatar 330-FT-IR spectrophotometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded (DMSO-d_6) on a Bruker (400 and 100 MHz). Chemical shift values are given in δ scales. The mass spectra were recorded on LC–MS-Agilent 1100 series. Elemental analyses were performed on a Flash EA 1112 series CHNS–O Analyzer.

2.2. Synthesis

2.2.1. General procedure for the synthesis of 3-(4-substitutedphenyl)-1H-pyrazole-4-carboxylic acid (3)

3-Substituted-pyrazole-4-carbaldehydes (0.01 mol) were dissolved by stirring in a solution of 2 g of NaOH in 40 ml of water. The mixture was cooled to 15 °C, and a solution of $KMnO_4$ (0.0088 mol) in 40 ml of water was quickly added. The mixture was stirred for 30 min at 20 °C and then heated to 100 °C until the solution becomes completely decolorized. The solution was cooled and filtered to remove MnO_2 precipitate. Then the filtrate was acidified with Conc. HCl to pH 3. The resulting solid was filtered off, washed with water and dried (Lebedev et al., 2005).

2.2.2. General procedure for the synthesis of 2,5-disubstituted-1,3,4-oxadiazoles (4a–n)

An equimolar mixture of respective substituted acid hydrazides **(2)** (0.001 mol) and 3-(4-substituted phenyl-1H-pyrazole-4-carboxylic acids **(3)** (0.001 mol) was dissolved in 5 ml of dry phosphorous oxychloride. The resulted solution was further refluxed for 8–9 h. Excess of phosphorous oxychloride was then distilled off and the mixture was gradually poured into crushed ice with stirring. The separated solid was filtered, washed thoroughly with cold water, 20% $NaHCO_3$ solution and recrystallized from a mixture of DMF and water.

2.3. Characterization of synthesized compounds

2.3.1. 2-(4-Chlorophenyl)-5-(3-phenyl-1H-pyrazol-4-yl)-1,3,4-oxadiazole (4a)

Color: off white amorphous solid. Yield 66%, m.p. 223–225 °C. IR (KBr, υ_{max} cm^{-1}): 3143 (N–H-str), 3053 (C–H-str), 1593 (C═N), 1531 (C═C), 1087 (C–O–C); ^{1}H-NMR (DMSO-d_6): δ 13.79 (s, 1H, pyrazole-NH), 8.7 (s, 1H, pyrazole-5H), 7.55–8.0 (m, 9H, Ar–H). MS: m/z = 321 (M-1). Anal. calcd. for $C_{17}H_{11}ClN_4O$: C, 63.26; H, 3.44; N, 17.36. Found: C, 63.23; H, 3.49; N, 17.30%.

2.3.2. 2-(4-Chlorophenyl)-5-(3-(4-chlorophenyl)-1H-pyrazol-4-yl)-1,3,4-oxadiazole (4b)

Color: off white amorphous solid. Yield 68%, m.p. 126–128 °C. IR (KBr, υ_{max} cm^{-1}): 3160 (N–H-str), 3021 (C–H-str), 1603 (C═N), 1554 (C═C), 1090 (C–O–C); ^{1}H-NMR (DMSO-d_6): δ 13.71 (s, 1H, pyrazole-NH), 8.67 (s, 1H, pyrazole-5H), 7.52–8.23 (m, 8H, Ar–H). MS: m/z = 357 (M +), 359 (M + 2), 361 (M + 4). Anal. calcd. for $C_{17}H_{10}Cl_2N_4O$: C, 57.16; H, 2.82; N, 15.69. Found: C, 57.11; H, 2.86; N, 15.65%.

2.3.3. 2-(4-Chlorophenyl)-5-(3-(4-fluorophenyl)-1H-pyrazol-4-yl)-1,3,4-oxadiazole (4c)

Color: off white amorphous solid. Yield 75%, m.p. 273–275 °C. IR (KBr, υ_{max} cm^{-1}): 3182 (N–H-str), 3077 (C–H-str), 1609 (C═N), 1542 (C═C), 1089 (C–O–C); ^{1}H-NMR (DMSO-d_6): δ 13.73 (s, 1H, pyrazole-NH), 8.5 (s, 1H, pyrazole-5H), 7.32–7.98 (m, 8H, Ar–H). ^{13}C-NMR: 162.59, 160.78, 136.97, 131.38, 131.30, 130.04, 129.87, 128.60, 122.78, 115.77, 115.55, 103.14. MS: m/z = 341(M + 1), 343 (M + 2). Anal. calcd. for $C_{17}H_{10}ClFN_4O$: C, 59.92; H, 2.96; N, 16.44. Found: C, 59.96; H, 2.90; N, 16.49%.

2.3.4. 2-(4-Chlorophenyl)-5-(3-(4-methoxyphenyl)-1H-pyrazol-4-yl)-1,3,4-oxadiazole (4d)

Color: off white amorphous solid. Yield 73%, m.p. 176–178 °C. IR (KBr, υ_{max} cm^{-1}): 3143 (N–H-str), 3014 (C–H-str), 1605 (C═N), 1516 (C═C), 1088 (C–O–C); ^{1}H-NMR (DMSO-d_6):

δ 13.76 (s, 1H, pyrazole-NH), 8.39 (s, 1H, pyrazole-5H), 7.05–7.97 (m, 8H, Ar–H), 3.82 (s, 3H, $-OCH_3$). ^{13}C-NMR: 162.5, 160.34, 136.92, 130.50, 130.12, 130.01, 129.87, 129.28, 128.56, 122.81, 122.63, 114.21, 102.63, 55.74. MS: m/z = 353 (M + 1), 355 (M + 2). Anal. calcd. for $C_{18}H_{13}ClN_4O_2$: C, 61.28; H, 3.71; N, 15.88. Found: C, 61.25; H, 3.75; N, 15.84%.

2.3.5. 2-(4-Chlorophenyl)-5-(3-(2,4-dichlorophenyl)-1H-pyrazol-4-yl)-1,3,4-oxadiazole ***(4e)***

Color: off white amorphous solid. Yield 67%, m.p. 234–236 °C. IR (KBr, υ_{max} cm^{-1}): 3155 (N–H-str), 3078 (C–H-str), 1599 (C═N), 1543 (C═C), 1093 (C–O–C); ^{1}H-NMR (DMSO-d_6): δ 13.81 (s, 1H, pyrazole-NH), 8.64 (s, 1H, pyrazole-5H), 7.56–7.85 (m, 7H, Ar–H). MS: m/z = 391 (M +). Anal. calcd. for $C_{17}H_9Cl_3N_4O$: C, 52.14; H, 2.32; N, 14.31. Found: C, 52.18; H, 2.38; N, 14.36%.

2.3.6. 2-(4-Methoxyphenyl)-5-(3-phenyl-1H-pyrazol-4-yl)-1,3,4-oxadiazole ***(4f)***

Color: off white amorphous solid. Yield 64%, m.p. 133–135 °C. IR (KBr, υ_{max} cm^{-1}): 3143 (N–H-str), 3063 (C–H-str), 1604 (C═N), 1490 (C═C), 1077 (C–O–C); ^{1}H-NMR (DMSO-d_6): δ 13.71 (s, 1H, pyrazole-NH), 8.66 (s, 1H, pyrazole-5H), 7.12–8.21 (m, 9H, Ar–H), 3.83 (s, 3H, $-OCH_3$). MS: m/z = 319(M + 1). Anal. calcd. for $C_{18}H_{14}N_4O_2$: C, 67.91; H, 4.43; N, 17.60. Found: C, 67.95; H, 4.47; N, 17.64%.

2.3.7. 2-(3-(4-Chlorophenyl)-1H-pyrazol-4-yl)-5-(4-methoxyphenyl)-1,3,4-oxadiazole ***(4g)***

Color: off white amorphous solid. Yield 71%, m.p. 238–240 °C. IR (KBr, υ_{max} cm^{-1}): 3143 (N–H-str), 3064 (C–H-str), 1608 (C═N), 1543 (C═C), 1086 (C–O–C); ^{1}H-NMR (DMSO-d_6): δ 13.82 (s, 1H, pyrazole-NH), 8.71 (s, 1H, pyrazole-5H), 7.12–8.24 (m, 8H, Ar–H), 3.84 (s, 3H, $-OCH_3$). MS: m/z = 353 (M + 1), 354 (M + 2). Anal. calcd. for $C_{18}H_{13}ClN_4O_2$: C, 61.28; H, 3.71; N, 15.88. Found: C, 61.24; H, 3.75; N, 15.83%.

2.3.8. 2-(3-(4-Fluorophenyl)-1H-pyrazol-4-yl)-5-(4-methoxyphenyl)-1,3,4-oxadiazole ***(4h)***

Color: off white amorphous solid. Yield 62%, m.p. 240–242 °C. IR (KBr, υ_{max} cm^{-1}): 3144 (N–H-str), 3073 (C–H-str), 1610 (C═N), 1546 (C═C), 1087 (C–O–C); ^{1}H-NMR (DMSO-d_6): δ 13.79 (s, 1H, pyrazole-NH), 8.67 (s, 1H, pyrazole-5H), 7.15–8.04 (m, 8H, Ar–H), 3.84 (s, 3H, $-OCH_3$). MS: m/z = 337 (M + 1). Anal. calcd. for $C_{18}H_{13}FN_4O_2$: C, 64.28; H, 3.90; N, 16.66. Found: C, 64.24; H, 3.93; N, 16.69%.

2.3.9. 2-(2-Chlorophenyl)-5-(3-phenyl-1H-pyrazol-4-yl)-1,3,4-oxadiazole ***(4i)***

Color: off white amorphous solid. Yield 69%, m.p. 139–141 °C. IR (KBr, υ_{max} cm^{-1}): 3163 (N–H-str), 3102 (C–H-str), 1611 (C═N), 1536 (C═C), 1020 (C–O–C); ^{1}H-NMR (DMSO-d_6): δ 13.73 (s, 1H, pyrazole-NH), 8.64 (s, 1H, pyrazole-5H), 7.52–7.96 (m, 9H, Ar–H). MS: m/z = 323 (M + 1). Anal. calcd. for $C_{17}H_{11}ClN_4O$: C, 63.26; H, 3.44; N, 17.36. Found: C, 63.23; H, 3.47; N, 17.39%.

2.3.10. 2-(2-Chlorophenyl)-5-(3-(4-chlorophenyl)-1H-pyrazol-4-yl)-1,3,4-oxadiazole ***(4j)***

Color: off white amorphous solid. Yield 65%, m.p. 241–243 °C. IR (KBr, υ_{max} cm^{-1}): 3247 (N–H-str), 3138 (C–H-str), 1602 (C═N), 1559 (C═C), 1087 (C–O–C); ^{1}H-NMR (DMSO-d_6): δ 13.84 (s, 1H, pyrazole-NH), 8.65 (s, 1H, pyrazole-5H), 7.54–7.99 (m, 8H, Ar–H). MS: m/z = 357 (M +). Anal. calcd. for $C_{17}H_{10}Cl_2N_4O$: C, 57.16; H, 2.82; N, 15.69. Found: C, 57.13; H, 2.86; N, 15.64%.

2.3.11. 2-(2-Chlorophenyl)-5-(3-(4-fluorophenyl)-1H-pyrazol-4-yl)-1,3,4-oxadiazole ***(4k)***

Color: off white amorphous solid. Yield 61%, m.p. 214–216 °C. IR (KBr, υ_{max} cm^{-1}): 3248 (N–H-str), 3137 (C–H-str), 1601 (C═N), 1557 (C═C), 1086 (C–O–C); ^{1}H-NMR (DMSO-d_6): δ 13.79 (s, 1H, pyrazole-NH), 8.61 (s, 1H, pyrazole-5H), 7.33–7.98 (m, 8H, Ar–H). MS: m/z = 341 (M + 1). Anal. calcd. for $C_{17}H_{10}ClFN_4O$: C, 59.92; H, 2.96; N, 16.44. Found: C, 59.95; H, 2.93; N, 16.41%.

2.3.12. 2-(2-Chlorophenyl)-5-(3-(4-methoxyphenyl)-1H-pyrazol-4-yl)-1,3,4-oxadiazole ***(4l)***

Color: off white amorphous solid. Yield 67%, m.p. 185–187 °C. IR (KBr, υ_{max} cm^{-1}): 3272 (N–H-str), 3129 (C–H-str), 1602 (C═N), 1543 (C═C), 1100 (C–O–C); ^{1}H-NMR (DMSO-d_6): δ 13.71 (s, 1H, pyrazole-NH), 8.59 (s, 1H, pyrazole-5H), 7.05–7.98 (m, 8H, Ar–H), 3.82 (s, 3H, $-OCH_3$). MS: m/z = 353 (M + 1). Anal. calcd. for $C_{18}H_{13}ClN_4O_2$: C, 61.28; H, 3.71; N, 15.88. Found: C, 61.24; H, 3.74; N, 15.83%.

2.3.13. 2-(4-Nitrophenyl)-5-(3-phenyl-1H-pyrazol-4-yl)-1,3,4-oxadiazole ***(4m)***

Color: yellow amorphous solid. Yield 65%, m.p. 131–133 °C. IR (KBr, υ_{max} cm^{-1}): 3120 (N–H-str), 3070 (C–H-str), 1596 (C═N), 1519 (C═C), 1075 (C–O–C); ^{1}H-NMR (DMSO-d_6): δ 13.76 (s, 1H, pyrazole-NH), 8.72 (s, 1H, pyrazole-5H), 7.52–8.43 (m, 8H, Ar–H). MS: m/z = 334 (M + 1). Anal. calcd. for $C_{17}H_{11}N_5O_3$: C, 61.26; H, 3.33; N, 21.01. Found: C, 61.22; H, 3.37; N, 21.06%.

2.3.14. 2-(3-(4-Chlorophenyl)-1H-pyrazol-4-yl)-5-(4-nitrophenyl)-1,3,4-oxadiazole ***(4n)***

Color: yellow amorphous solid. Yield 63%, m.p. 146–148 °C. IR (KBr, υ_{max} cm^{-1}): 3157 (N–H-str), 3111 (C–H-str), 1603 (C═N), 1558 (C═C), 1093 (C–O–C); ^{1}H-NMR (DMSO-d_6): δ 13.76 (s, 1H, pyrazole-NH), 8.58 (s, 1H, pyrazole-5H), 7.57–8.44 (m, 8H, Ar–H). MS: m/z = 368 (M + 1). Anal. calcd. for $C_{17}H_{10}ClN_5O_3$: C, 55.52; H, 2.74; N, 19.04. Found: C, 55.56; H, 2.71; N, 19.01%.

2.4. Antimicrobial activity

The following bacteria and fungi were used for the experiment. Bacteria: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853. All bacterial strains were maintained on nutrient agar medium at ±37 °C. Fungi: *Aspergillus flavus, Chrysosporium keratinophilum* and *Candida albicans MTCC 227* are used in this study. These cultures are obtained from the Department of Microbiology, Kuvempu University, Shimoga, India. All

fungus strains were maintained on potato dextrose agar (PDA) at ±25 °C. After measuring the zone of inhibition, the values for Dimethylsulfoxide (DMSO) were subtracted to get the actual values.

The antibacterial activity of newly synthesized compounds (**4a–n**) was determined by the well plate method in Mueller–Hinton Agar (Arthington-Skaggs et al., 2000; Rocha et al., 1995). The compounds were tested against a panel of pathogenic microorganisms, including *E. coli*, *S. aureus* and *P. aeruginosa*. Microorganism strains were maintained on nutrient agar medium at ±37 °C. The cultures were inoculated in fresh 10 ml Nutrient Broth to yield an initial suspension of approximately 10–100 cfu/ml. All broths were then incubated statically at the aforementioned temperatures for microorganisms, for 18–24 h so that all cells were in the stationary phase. Susceptibility of the test organism to the compounds was determined by employing in the well plate technique. The bacterial suspensions were diluted ten times with distilled water, and 0.1 ml diluted culture was spread plated on nutrient agar in order to give a population of approximately 10^6 cfu/plate. Six millimeter diameter well was then punched carefully using a sterile cork borer and 30 µl of test solutions of different concentrations (1000 µg/ml and 500 µg/ml) was added into each labeled well. The same procedure was repeated for different micro-organisms. Each experiment was carried out in triplicate. After the inoculation of the organism and compound, the Petri plates were incubated for 24 h at ± 37 °C. After the incubation, the inhibition zone was measured and the values for DMSO were subtracted to get the actual values. Streptomycin was used as standard drug.

Antifungal studies of newly synthesized compounds **4a-n** were determined by the well plate method (Mac. Lowry et al., 1970; Portillo et al., 2001) against *A. flavus*, *C. keratinophilum* and *C. albicans*. Sabourands agar media were prepared by dissolving peptone (10 g), D-glucose (40 g) and agar (20 g) in distilled water (1000 ml) and adjusting the pH to 5.7. Normal saline was used to make a suspension of spore of fungal strains for lawning. A loopful of particular fungal strain was transferred to 3 ml saline to get a suspension of corresponding species. Twenty milliliters of agar media was poured into each petri dish. Excess of suspension was decanted and plates were dried by placing in an incubator at ±37 °C for 1 h. Six millimeter diameter well were then punched carefully using a sterile cork borer and 30 µl of test solutions of different concentrations (1000 µg/ml and 500 µg/ml) were added into each labeled well. A control was also prepared for the plates in the same way using solvent DMSO. The Petri dishes were prepared in triplicate and maintained at ±25 °C for 72 h. Antifungal activity was determined by measuring the diameter of inhibition zone. The activity of each compound was compared with fluconazole as standard.

3. Results and discussion

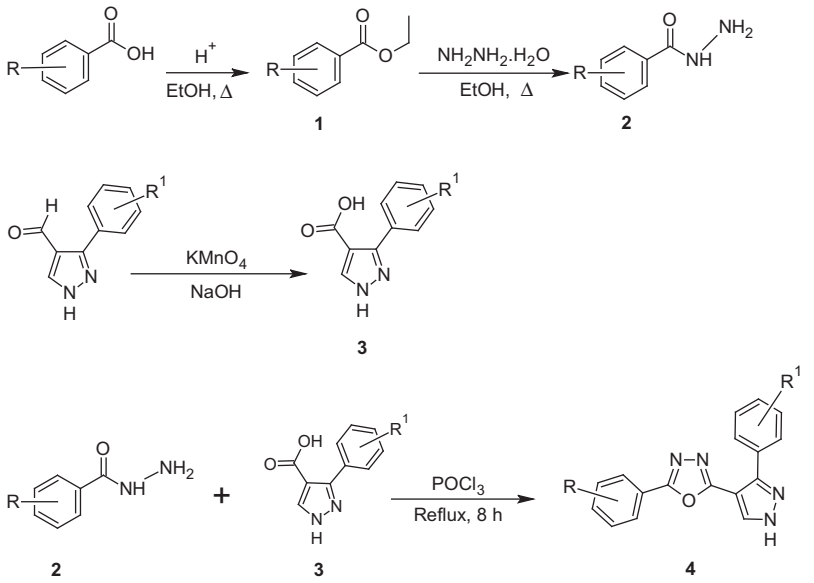
3.1. Chemistry

The synthetic route has been outlined in Scheme 1. In the current work, aromatic esters (**1**) were synthesized from the appropriate aromatic acids by treating with ethanol in the presence of catalytic amount of sulfuric acid. Reaction of compound (**1**) with hydrazine hydrate yielded corresponding acid hydrazides (**2**) (Husain and Ajmal, 2009). Similarly, 3-substituted-pyrazole-4-carboxylic acids were synthesized as per the reported procedure (Lebedev et al., 2005). Subsequently condensation of substituted acid hydrazides (**2**) with various 3-substituted-pyrazole-4-carboxylic acids **(3)** in the presence of phosphorous oxychloride afforded a series of 2,5-disubstituted-1,3,4-oxadiazoles **(4a-n)**. Newly synthesized compounds (**4a–n**) were characterized by IR, NMR, mass spectral and C, H, N analyses. Analytical and spectral data of all synthesized compounds were in full agreement with the proposed structures. IR spectrum of compound **4a** showed absorption bands at 3143, 3053, 1593, 1531, 1087 cm^{-1} which was due to the N–H, C–H, C═N, C═C and C–O–C groups, respectively. In ^{1}H-NMR spectra, all protons were seen according to the expected chemical shift and integral values. The ^{1}H-NMR spectrum of **4a** showed a singlet at δ 13.79 corresponds to pyrazole NH proton. A singlet at δ 8.7 was due to pyrazole 5H proton. Also, at δ 7.55–8.0 a multiplet was observed which was due to aromatic protons. The mass spectrum of **4a** showed a molecular ion peak at m/z = 321 (M-1), which is in agreement with the molecular formula $C_{17}H_{11}ClN_4O$. When the para position of the phenyl ring attached to pyrazole nucleus was substituted by electron donating and electron withdrawing groups, a change in delta values of aromatic protons was observed. In the presence of the electron donating group, delta value shifted to lower wavelength side whereas in the presence of electron withdrawing groups, delta value shifted to higher end. In compound **4d**, due to the presence of the $-OCH_3$ group an additional singlet peak was observed at δ 3.82. Due to the presence of two chlorine atoms in mass spectrum of compound **4b,** M+, M+2 and M+4 chlorine patterns were clearly observed. Similarly, the presence of one chorine atom in compound **4d** showed M+1 and M+2 chlorine pattern which further confirms the structure. Similarly the spectral values for all the compounds and C, H, N analyses are given in the experimental part.

3.2. Antimicrobial activity

E. coli is a common micro-organism which causes secondary infection, food poisoning in human. *S. aureus* causes septic arthritis, staphylococcal endocarditis and pneumonia. On the other hand *P. aeruginosa* causes skin and soft tissue infection, gastrointestinal infection, urinary tract infection and septic shock pneumonia. These pathogens are commonly causing harmful effects on human life. The evaluation of antimicrobial activity of 1,3,4-oxadiazole moiety against *E. coli*, *S. aureus* and *P. aeruginosa* has been carried out by many researchers and reported good results with respect to the above mentioned microorganisms (Sahin et al., 2002; de Oliveira et al., 2012; Li et al., 2012). Hence it was thought worthwhile to evaluate antimicrobial activity using these microorganisms.

The newly synthesized compounds **4a–n** were tested for their antibacterial activity (*in vitro*) against *E. coli*, *S. aureus* and *P. aeruginosa* and their activity was compared to a well-known commercial antibiotic, streptomycin. Antibacterial activity was carried out by the well plate method by measuring its zone of inhibition. The compounds **4a–n** were screened for their antibacterial activity in triplicate against *E. coli*, *S. aureus* and *P. aeruginosa* at two different concentrations of 1000, 500 µg/ml as shown in Table 1. The investigation of



Scheme 1 Schematic diagram showing the synthesis of 1,3,4-oxadiazole derivatives (**4a–n**) where, R = 4-Cl and R^1 = H (**4a**); R = 4-Cl and R^1 = 4-Cl (**4b**); R = 4-Cl and R^1 = 4-F (**4c**); R = 4-Cl and R^1 = 4-OCH_3 (**4d**); R = 4-Cl and R^1 = 2,4-Cl_2 (**4e**); R = 4-OCH_3 and R^1 = H (**4f**); R = 4-OCH_3 and R^1 = 4-Cl (**4g**); R = 4-OCH_3 and R^1 = 4-F (**4h**); R = 2-Cl and R^1 = H (**4i**); R = 2-Cl and R^1 = 4-Cl (**4j**); R = 2-Cl and R^1 = 4-F (**4k**); R = 2-Cl and R^1 = 4-OCH_3 (**4l**); R = 4-NO_2 and R^1 = H (**4m**); R = 4-NO_2 and R^1 = Cl (**4n**).

antibacterial screening data revealed that most of the tested compounds showed moderate to good bacterial inhibition. Compound **4i** exhibited equipotent activity as that of standard against *E. coli* and *S. aureus* at 500 μg/ml. Compound **4j** exhibited equipotent activity as that of standard against *E. coli* whereas slightly less than that of standard against *S. aureus* and *P. aeruginosa* at 500 μg/ml. **4l** also inhibited the growth of *S. aureus* similarly as that of standard whereas slightly less than that of standard against *E. coli* and *P. aeruginosa at* 500 μg/ml. Compounds **4e** and **4f** were found to be active against all the tested bacterial strains. Compound **4b** showed moderate activity against *E. coli* and *S. aureus*. Remaining compounds showed fair or poor activity against tested bacterial strains.

All the synthesized compounds were also tested for their antifungal activity (*in vitro)* against *A. flavus*, *C. keratinophilum* and *C. albicans* by measuring their average zone of inhibition (Table 2). Fluconazole was used as standard for antifungal activity. Among the tested compounds, **4i** and **4j** showed a good antifungal profile against *A. flavus*, and *C. keratinophilum* at a concentration of 500 μg/ml when compared with the standard. **4e**, **4f** and **4l** showed moderate activity against *A. flavus* but showed poor activity against rest of two microorganisms. Remaining compounds showed poor activity against the tested microorganisms.

The enhanced activity of **4i** and **4j** can be attributed to the presence of 2-chlorophenyl substituent attached to the 5th position of the oxadiazole ring and the presence of phenyl

Table 1 Antibacterial activity of compounds **4a–n**.

Compound code	Zone of inhibition (mm)					
	Escherichia coli		*Staphylococcus aureus*		*Pseudomonas aeruginosa*	
Conc. in μg/ml	1000	500	1000	500	1000	500
4a	04 ± 0.2	01 ± 0.1	02 ± 0.1	00	03 ± 0.2	01 ± 0.2
4b	07 ± 0.1	05 ± 0.2	08 ± 0.2	06 ± 0.2	04 ± 0.2	03 ± 0.1
4c	00	00	00	00	00	00
4d	00	00	00	00	00	00
4e	09 ± 0.1	07 ± 0.2	09 ± 0.2	06 ± 0.1	10 ± 0.2	08 ± 0.1
4f	07 ± 0.1	06 ± 0.1	09 ± 0.1	07 ± 0.2	08 ± 0.2	06 ± 0.1
4g	04 ± 0.2	02 ± 0.1	04 ± 0.1	02 ± 0.2	05 ± 0.1	02 ± 0.1
4h	00	00	00	00	00	00
4i	12 ± 0.2	10 ± 0.1	11 ± 0.1	10 ± 0.1	12 ± 0.2	09 ± 0.1
4j	13 ± 0.1	10 ± 0.2	12 ± 0.1	09 ± 0.2	11 ± 0.1	09 ± 0.2
4k	11 ± 0.1	08 ± 0.1	10 ± 0.1	08 ± 0.2	12 ± 0.1	07 ± 0.1
4l	11 ± 0.2	09 ± 0.1	12 ± 0.1	10 ± 0.2	11 ± 0.1	08 ± 0.2
4m	03 ± 0.2	02 ± 0.1	04 ± 0.1	03 ± 0.1	04 ± 0.2	01 ± 0.1
4n	00	00	00	00	00	00
Streptomycin (Std.)	16 ± 0.2	10 ± 0.1	15 ± 0.2	10 ± 0.2	16 ± 0.2	13 ± 0.2

Table 2 Antifungal activity of compounds **4a–n**.

Compound code	Zone of inhibition (mm)					
	Aspergillus flavus		*Chrysosporium keratinophi*lum		*Candida albicans*	
Con in μg/ml	1000	500	1000	500	1000	500
4a	00	00	00	00	00	00
4b	03 ± 0.2	01 ± 0.1	04 ± 0.1	03 ± 0.1	04 ± 0.1	03 ± 0.1
4c	00	00	00	00	00	00
4d	00	00	00	00	00	00
4e	08 ± 0.2	06 ± 0.1	06 ± 0.1	04 ± 0.1	06 ± 0.2	03 ± 0.2
4f	06 ± 0.1	04 ± 0.1	05 ± 0.1	03 ± 0.2	04 ± 0.2	02 ± 0.2
4g	04 ± 0.1	03 ± 0.2	06 ± 0.1	05 ± 0.2	04 ± 0.1	02 ± 0.1
4h	00	00	00	00	00	00
4i	10 ± 0.2	08 ± 0.1	09 ± 0.1	07 ± 0.1	09 ± 0.2	06 ± 0.1
4j	09 ± 0.1	08 ± 0.2	07 ± 0.2	06 ± 0.1	04 ± 0.2	02 ± 0.1
4k	05 ± 0.1	03 ± 0.1	04 ± 0.2	03 ± 0.1	05 ± 0.1	02 ± 0.1
4l	06 ± 0.1	05 ± 0.2	04 ± 0.1	03 ± 0.1	05 ± 0.1	04 ± 0.1
4m	04 ± 0.2	01 ± 0.1	04 ± 0.1	02 ± 0.1	03 ± 0.2	01 ± 0.2
4n	00	00	00	00	00	00
Fluconazole (Std.)	13 ± 0.2	10 ± 0.1	17 ± 0.2	15 ± 0.2	22 ± 0.2	20 ± 0.2

and p-chlorophenyl substituents on the pyrazole ring which is attached to the 2nd position of the oxadiazole ring. The presence of 2-chlorophenyl substituent (5th position of oxadiazole) along with 4-fluorophenyl, 4-methoxyphenyl substituent on the pyrazole ring in **4k** and **4l** respectively may be the reason for its enhanced activity. Compounds **4e** and **4f** contain 4-chlorophenyl and 4-methoxyphenyl substituent (5th position of oxadiazole) along with 2,4-dichlorophenyl and phenyl substituents on a pyrazole ring which is attached to the 2nd position of the oxadiazole ring which may be the reason for its activity.

It can be concluded that the compounds **4i**, **4j, 4k** and **4l** which contain 2-chlorophenyl substituent on the 5th position of the oxadiazole ring may increase the antimicrobial profile of the compound.

4. Conclusion

In summary, we have synthesized a new series of 2,5-disubstituted-1,3,4-oxadiazoles and screened for their antimicrobial activity against few microorganisms. Among the synthesized compounds, **4i** and **4j** showed excellent antimicrobial activity against various tested microorganisms. Hence it can be concluded that the compounds **4i** and **4j** are identified as the most potent antimicrobial agents in the present series and deserve further investigation in order to clarify the mode of action at molecular level responsible for the activity observed.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.arabjc.2013.12.020.

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