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Synthesis, characterization and antimicrobial screening of thiazole based 1,3,4-oxadiazoles heterocycles

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Abstract In an effort to find a new pharmacologically dynamic molecule, we report here the synthesis and in vitro antimicrobial activity of various *N*-(4-methyl-5-(4-((substituted phenylamino) methyl)-5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)thiazol-2-yl) benzamides. The structures of newly synthesized compounds were assigned on the bases of IR, ^{1}H NMR, ^{13}C NMR, and mass spectral data. Synthesized compounds were screened for their antibacterial and antifungal activities on gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus pyogenes*), gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and three fungi (*Candida albicans*, *Aspergillus niger* and *Aspergillus clavatus*) by serial broth dilution method. Compounds **5e** and **5k** were associated with considerable potential antibacterial and compounds **5a** and **5b** antifungal activities.

Keywords Thiazole · 1,3,4-Oxadiazole · Potential antimicrobial activity · MIC

Introduction

The thiazole ring system has nitrogen and sulfur heteroatoms at positions 1 and 3, respectively. The thiazole ring has been widely used in several bioactive compounds like thiamin (Formula I) and penicillin G (Formula II) [1, 2]. Due to this reason and medicinal significance of thiazole and 1,3,4-oxadiazole, we have incorporated both the moieties in one structure for the development of more potential antimicrobial agents (Fig. 1).

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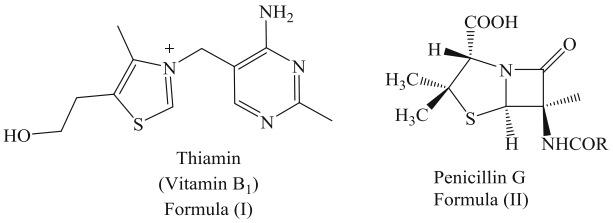

Fig. 1 Thiazole containing important motifs

The search for biologically active substances led us for the investigation of condensed sulfur- and nitrogen-containing heterocycles. Thiazole derivatives have been reported to possess significant and diverse biological activities such as antimicrobial [3], analgesic [4], anti-inflammatory [5], antioxidant [6], anti HIV [7] and antitubercular [8, 9] activities. Moreover, thiazoles are also synthetic intermediates and substructures in several biologically active compounds [10–16]. The substituted thiazole-based compounds are associated with various pharmacological functions such as relatively stable molecules, enhanced lipophilicity and easy metabolism.

Oxadiazole is an important class of heterocyclic compounds with a broad spectrum of biological activities. Out of its four theoretical isomers, 1,3,4-oxadiazole is screened for various biological activities including anti-microbial [17], anticancer [18], anti-inflammatory [19], anti-infective [20] and anti-HIV [21]. Hence, 1,3,4-oxadiazoles have occupied a unique place in the field of medicinal chemistry particularly in anti-edema and anti-inflammatory activity [22–26]. This moiety is an important bioactive class of anti-HIV and angiogenesis inhibitor [27]. Nesapidil [Formula (III)] is useful as an antihypertensive agent while furamizole [Formula (IV)] is useful as an antibiotic. Both the drugs possess an oxadiazole nucleus in the structural framework (Fig. 2).

In continuation of our previous work [28–31] and because of the medicinal importance of thiazole and 1,3,4-oxadiazole, we report herein synthesis of a new class of *N*-(4-methyl-5-(4-((substituted phenylamino)methyl)-5-thioxo-4,5-dihydro-

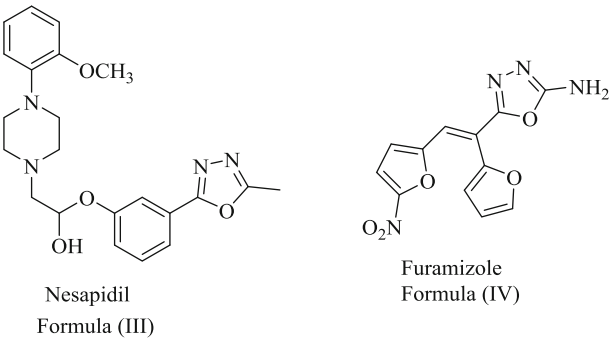

Fig. 2 Oxadiazole containing marketed drugs

1,3,4-oxadiazol-2-yl)thiazol-2-yl)benzamides (**5a–k**). We also tried to develop potential antimicrobials. The structures of newly synthesized compounds were assigned on the basis of IR, ^{1}H NMR, ^{13}C NMR, and Mass spectral analysis. These compounds were evaluated for their antimicrobial screening on different strains of bacteria and fungi.

Experimental

Materials and methods

All reactions except those in aqueous media were carried out by standard techniques for the exclusion of moisture. Melting points were determined on an electro thermal melting point apparatus and were reported uncorrected. TLC on silica gel plates (Merck, $^{60}F_{254}$) was used for purity checking and reaction monitoring. Elemental analysis (% C, H, N) was carried out on a Perkin-Elmer 2400 CHN analyzer. IR spectra of all compounds were recorded on a Perkin-Elmer FT-IR spectrophotometer in KBr. ^{1}H NMR spectra were recorded on a Varian Gemini 300 MHz, and ^{13}C NMR spectra are recorded with a Varian Mercury-400 (100 MHz) NMR spectrometer, using tetramethylsilane as the internal reference, with dimethyl sulfoxide (DMSO-d_6) as solvent. All chemical shifts were expressed in ppm. Mass spectra were scanned on a Shimadzu LCMS 2010 spectrometer. Anhydrous reactions were carried out in oven-dried glassware in a nitrogen atmosphere.

Chemistry

Synthetic strategies adopted to obtain the target compounds are depicted in Fig. 3. The series of title compounds (**5a–k**) were synthesized in four steps. Compound (**1**) was prepared by a mixture of thiourea and ethyl acetoacetate heated at 60–70 °C and a catalytic amount of benzoyl peroxide was added and treated with *N*-bromosuccinamide using benzene as solvent to give ethyl 2-amino-4-methylthiazole-5-carboxylate (**1**). In the first step, compound (**1**) was treated with benzoyl chloride at 0–5 °C to give the intermediate ethyl 4-methyl-2-(phenylcarbonylamino)-1,3-thiazole-5-carboxylate (**2**). In the second step, intermediate (**2**) was refluxed with hydrazine hydrate in the presence of ethanol (99 %) as a solvent to give compound *N*-(5-(*N*-aminocarbamoyl)-4-methyl(1,3-thiazol-2-yl))benzamide (**3**). In the third step, compound (**3**) was refluxed with carbon disulfide (CS_2) in ethanol as a solvent in the presence of potassium hydroxide to achieve *N*-(4-methyl-5-(2-thioxo(1,3,4-oxadiazolin-5-yl))(1,3-thiazol-2-yl))benzamide (**4**). In the final step, compounds (**5a–k**) were generated by Mannich condensation of the intermediate (**4**) with 36 % formaldehyde and different aniline derivatives in ethanol (95 %) used as solvent.

*Synthesis of ethyl 4-methyl-2-(phenylcarbonylamino)-1,3-thiazole-carboxylate (**2**)*

In a round bottom flask, a mixture of 2-amino-4-methyl-1,3-thiazole-5-carboxylate (0.01 mol) and pyridine (10 mL) was placed and benzoyl chloride (0.01 mol) was added dropwise at 0–5 °C. The mixture was stirred at a temperature not exceeding 5 °C for 3 h. The solution was poured into ice cold water. The solid product was filtered and washed with cold dilute hydrochloric acid solution to remove access 2-amino-4-methyl-1,3-thiazole-5-carboxylate. The resulting solid was recrystallized from ethanol (99 %). Yield: 23 %, mp 128–130 °C. IR (KBr, cm^{-1}): 3220 (N–H stretching, secondary amide), 2891 (C–H stretching, CH_3 group), 1753–1740 (–CO stretching, aliphatic ester), 1704 (C=O stretching, secondary amide). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 12.80 (*s*, 1H, Het–NH–CO–Ar), 8.15–7.60 (*m*, 5H, Ar–H), 4.25 (*q*, –C<u>H</u>$_2$–CH_3), 2.54 (*s*, 3H, –CH_3), 1.30 (*t*, –CH_2–C<u>H</u>$_3$). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 165.5, 162.7, 162.3, 156.7, 134.5, 132.3, 128.6, 128.6, 127.3, 127.3, 116.5, 60.4, 16.2, 14.3. LCMS (*m*/*z*): 290.07 (M^+). Anal. calcd. for $C_{14}H_{14}N_2O_3S$: C, 57.65; H, 5.02; N, 9.56 %. Found: C, 57.69; H, 5.09; N, 9.63 %.

*Synthesis of N-(5-(N-aminocarbamoyl)-4-methyl(1,3-thiazol-2-yl))benzamide (**3**)*

Compound (**2**) (0.01 mol) and 99 % hydrazine hydrate (0.015 mol) were placed in a round bottom flask and the mixture was refluxed for 10 min. Alcohol was added till both the layers were miscible and refluxing was continued for 5 h. Excess of alcohol and unreacted hydrazine hydrate was distilled out and the contents were poured into a beaker. The solid was recrystallized from ethanol to get pure crystalline product. Yield: 63 %, mp 141–143 °C. IR (KBr, cm^{-1}): 3424 (–NH stretching, primary amine), 3310 (–NH stretching, secondary amine), 3224 (N–H stretching, secondary amide), 2889 (C–H stretching, CH_3 group), 1707 (C=O stretching, secondary amide). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 12.83 (*s*, 1H, Het–NH–CO–Ar), 9.37 (*t*, –N<u>H</u>–NH_2), 8.14–7.58 (*m*, 5H, Ar–H), 4.57 (*d*, –NH–N<u>H</u>$_2$), 2.52 (*s*, 3H, –CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 165.5, 162.7, 160.5, 156.3, 134.5, 132.6, 132.3, 128.6, 128.6, 127.3, 127.3, 16.7. LCMS (*m*/*z*): 276.07 (M^+). Anal. Calcd. for $C_{12}H_{12}N_4O_2S$: C, 51.95; H, 4.22; N, 20.06 %. Found: C, 51.89; H, 4.18; N, 20.10 %.

*Synthesis of N-(4-methyl-5-(2-thioxo(1,3,4-oxadiazolin-5-yl))(1,3-thiazol-2-yl)) benzamide (**4**)*

A mixture of compound (**3**) (0.01 mol), potassium hydroxide (0.01 mol), carbon disulfide (0.02 mol), and absolute ethanol (10 mL) was refluxed for 12 h. The excess solvent was removed by vacuum evaporation, and the residue was dissolved in water and acidified with acetic acid to get a solid product. It was filtered, dried, and recrystallized from water–ethanol (60–40 %). Yield: 72 %, mp 171–175 °C. IR (KBr, cm^{-1}): 3348 (N–H stretching, secondary amine), 3220 (N–H stretching, secondary amide), 2982 (C–H stretching, aromatic ring), 2891 (C–H stretching, CH_3 group), 1704 (C=O stretching, secondary amide), 1554 (C=C stretching, aromatic

ring), 1292 (C–H bending), 1191, 1090 (C–O–C stretching). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 13.86 (*s*, N–NH–CS), 12.81 (*s*, 1H, Het–NH–CO–Ar), 8.17–7.52 (*m*, 5H, Ar–H), 2.51 (*s*, 3H, –CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 189.7, 165.6, 162.8, 157.2, 156.7, 134.4, 132.3, 132.1, 128.9, 128.9, 127.7, 127.7, 17.3. LCMS (*m*/*z*): 318.02 (M^+). Anal. Calcd. for $C_{13}H_{10}N_4O_2S_2$: C, 48.85; H, 3.32; N, 17.46 %. Found: C, 48.79; H, 3.27; N, 17.51 %.

*General procedure for the synthesis of N-(4-methyl-5-(4-((arylamino)methyl)-5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)thiazol-2-yl)benzamides (**5a–k**)*

A mixture of *N*-(4-methyl-5-(5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)thiazol-2-yl)benzamide [compound (**4**)] (0.01 mol) and aryl amines (0.01 mol) was refluxed in ethanol (30 mL) with 36 % formaldehyde (0.02 mol) for 3 h. The solid product generated was filtered off, washed with water, and crystallized from ethanol (99 %), yield: 58–74 %. All compounds (**5a–k**) of this series were prepared by using the same method.

*N-(5-(4-(((2-fluorophenyl)amino)methyl)-5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)-4-methylthiazol-2-yl)benzamide (**5a**)*

Yield: 62 %, mp 139–143 °C. IR (KBr, cm^{-1}): 3352 (N–H stretching, secondary amine), 3220 (N–H stretching, secondary amide), 2891 (C–H stretching, CH_3 group), 2982 (C–H stretching, aromatic ring), 2772 (C–H stretching, –CH_2 group), 1704 (C=O stretching, secondary amide), 1552 (C=C stretching, aromatic ring), 1290 (C–H bending), 1194, 1088 (C–O–C stretching), 1118 (C–F stretching), 775 (1:2-substituted benzene ring). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 9.17 (*s*, 1H, Het–NH–CO–Ar), 8.03–6.58 (*m*, 9H, Ar–H), 4.44 (*s*, 2H, Het–CH_2), 4.02 (*s*, 1H, Ar–NH–CH_2), 2.47 (*s*, 3H, –N–C–(CH_3)–C–). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 177.8, 166.3, 163.2, 156.2, 155.1, 154.2, 133.9, 132.5, 130.9, 130.9, 129.2, 128.0, 125.7, 120.4, 116.3, 115.1, 104.1, 70.2, 17.4. LCMS (*m*/*z*): 441.07 (M^+). Anal. Calcd. for $C_{20}H_{16}FN_5O_2S_2$; C, 54.41; H, 3.65; N, 15.86 %. Found: C, 54.44, H-3.60, N-15.80 %.

*N-(5-(4-(((4-fluorophenyl)amino)methyl)-5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)-4-methylthiazol-2-yl)benzamide (**5b**)*

Yield: 74 %, mp 148–152 °C. IR (KBr, cm^{-1}): 3358 (N–H stretching, secondary amine), 3222 (N–H stretching, secondary amide), 2893 (C–H stretching, CH_3 group), 2980 (C–H stretching, aromatic ring), 2774 (C–H stretching, –CH_2 group), 1705 (C=O stretching, secondary amide), 1551 (C=C stretching, aromatic ring), 1292 (C–H bending), 1193, 1086 (C–O–C stretching), 1115 (C–F stretching), 810 (1:4-substituted benzene ring). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 9.15 (*s*, 1H, Het–NH–CO–Ar), 8.03–7.02 (*m*, 9H, Ar–H), 4.42 (*s*, 2H, Het–CH_2), 4.0 (*s*, 1H, Ar–NH–CH_2), 2.45 (*s*, 3H, –N–C-(CH_3)–C–). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 177.7, 166.1, 163.1, 156.2, 155.5, 154.8, 144.2, 133.9, 132.4, 129.1, 127.8, 119.0, 116.3, 103.7, 70.0, 17.2. LCMS (*m*/*z*): 441.09 (M^+). Anal. Calcd. for

$C_{20}H_{16}FN_5O_2S_2$; C, 54.40; H, 3.64; N, 15.85 %. Found: C, 54.46; H, 3.59; N, 15.78 %.

*N-(5-(4-(((2-methoxyphenyl)amino)methyl)-5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)-4-methylthiazol-2-yl)benzamide (**5c**)*

Yield: 70 %, mp 139-144 °C. IR (KBr, cm^{-1}): 3357 (N–H stretching, secondary amine), 3220 (N–H stretching, secondary amide), 2987 (C–H stretching, aromatic ring), 2896 (C–H stretching, CH_3 group), 2778 (C–H stretching, $–CH_2$ group), 2840 (C–OCH_3 stretching), 1705 (C=O stretching, secondary amide), 1553 (C=C stretching, aromatic ring), 1296 (C–H bending), 1191, 1088 (C–O–C stretching), 773 (1:2-substituted benzene ring). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 9.13 (*s*, 1H, Het–NH–CO–Ar), 8.04–6.89 (*m*, 9H, Ar–H), 4.42 (*s*, 2H, Het–CH_2), 4.13 (*s*, 1H, Ar–NH–CH_2), 3.83 (*s*, 3H, –OCH_3), 2.41 (*s*, 3H, –N–C–(CH_3)–C–). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 177.4, 166.2, 163.0, 156.6, 155.1, 144.4, 137.9, 134.1, 132.1, 129.0, 127.9, 121.9, 121.2, 114.3, 113.2, 103.6, 69.9, 56.1, 17.1. LCMS (*m/z*): 453.09 (M^+). Anal. Calcd. for $C_{21}H_{19}N_5O_3S_2$; C, 55.61; H, 4.22; N, 15.44 %. Found: C, 55.65; H, 4.16; N, 15.40 %.

*N-(5-(4-(((3-methoxyphenyl)amino)methyl)-5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)-4-methylthiazol-2-yl)benzamide (**5d**)*

Yield: 66 %, mp 153–156 °C. IR (KBr, cm^{-1}): 3356 (N–H stretching, secondary amine), 3222 (N–H stretching, secondary amide), 2987 (C–H stretching, aromatic ring), 2894 (C–H stretching, CH_3 group), 2840 (C–OCH_3 stretching), 2777 (C–H stretching, $–CH_2$ group), 1703 (C=O stretching, secondary amide), 1554 (C=C stretching, aromatic ring), 1298 (C–H bending), 1190, 1086 (C–O–C stretching), 705 (1:3-substituted benzene ring). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 9.13 (*s*, 1H, Het–NH–CO–Ar), 7.95–6.35 (*m*, 9H, Ar–H), 4.44 (*s*, 2H, Het–CH_2), 4.14 (*s*, 1H, Ar–NH–CH_2), 3.83 (*s*, 3H, –OCH_3), 2.43 (*s*, 3H, –N–C-(CH_3)–C–). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 177.2, 166.0, 162.8, 161.0, 156.5, 155.2, 148.7, 134.2, 132.2, 128.9, 127.8, 110.5, 108.8, 105.6, 105.2, 103.4, 69.7, 55.9, 17.1. LCMS (*m/z*): 453.09 (M^+). Anal. Calcd. for $C_{21}H_{19}N_5O_3S_2$; C, 55.61; H, 4.22; N, 15.44 %. Found: C, 55.67; H, 4.28; N, 15.38 %.

*N-(5-(4-(((4-methoxyphenyl)amino)methyl)-5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)-4-methylthiazol-2-yl)benzamide (**5e**)*

Yield: 58 %, mp 153–157 °C. IR (KBr, cm^{-1}): 3359 (N–H stretching, secondary amine), 3221 (N–H stretching, secondary amide), 2986 (C–H stretching, aromatic ring), 2895 (C–H stretching, CH_3 group), 2842 (C–OCH_3 stretching), 2775 (C–H stretching, $–CH_2$ group), 1704 (C=O stretching, secondary amide), 1554 (C=C stretching, aromatic ring), 1294 (C–H bending), 1194, 1087 (C–O–C stretching), 807 (1:4-substituted benzene ring). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 9.13 (*s*, 1H, Het–NH–CO–Ar), 8.04–6.89 (*m*, 9H, Ar–H), 4.42 (*s*, 2H Het–CH_2), 4.12 (*s*, 1H, Ar–NH–CH_2), 3.83 (*s*, 3H, –OCH_3), 2.43 (*s*, 3H, –N–C–(CH_3)–C–). ^{13}C NMR

(100 MHz, DMSO-d_6, δ ppm): 177.2, 166.0, 162.7, 156.6, 155.1, 152.0, 140.1, 134.1, 132.0, 128.9, 127.7, 115.6, 115.2, 103.3, 69.5, 56.0, 17.1. LCMS (*m/z*): 453.09 (M^+). Anal. Calcd. for $C_{21}H_{19}N_5O_3S_2$; C, 55.61; H, 4.22; N, 15.44 %. Found: C, 55.64; H, 4.18; N, 15.40 %.

*N-(4-methyl-5-(4-(((2-nitrophenyl)amino)methyl)-5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)thiazol-2-yl)benzamide (**5f**)*

Yield: 68 %, mp 155–160 °C. IR (KBr, cm^{-1}): 3357 (N–H stretching, secondary amine), 3224 (N–H stretching, secondary amide), 2988 (C–H stretching, aromatic ring), 2896 (C–H stretching, CH_3 group), 2774 (C–H stretching, –CH_2 group), 1702 (C=O stretching, secondary amide), 1554 (C=C stretching, aromatic ring), 1543 (C=N stretching, aromatic ring), 1520 (N=O stretching), 1295 (C–H bending), 1195, 1086 (C–O–C stretching), 780 (1:2-di substituted benzene ring). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 9.12 (*s*, 1H, Het–NH–CO–Ar), 8.14–6.78 (*m*, 9H, Ar–H), 4.49 (*s*, 2H, Het–CH_2–NH–), 4.14 (*s*, 1H, Ar–NH–CH_2), 2.46 (*s*, 3H, –N–C–(CH_3)–C–). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 177.0, 165.8, 162.8, 156.5, 155.3, 146.2, 135.8, 134.2, 132.1, 131.5, 128.9, 127.5, 125.7, 118.2, 114.6, 103.0, 68.9, 16.9. LCMS (*m/z*): 468.07 (M^+). Anal. Calcd. for $C_{20}H_{16}N_6O_4S_2$; C, 51.27; H, 3.44; N, 17.94 %. Found: C, 51.24; H, 3.41; N, 17.90 %.

*N-(4-methyl-5-(4-(((3-nitrophenyl)amino)methyl)-5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)thiazol-2-yl)benzamide (**5g**)*

Yield: 66 %, mp 164–168 °C. IR (KBr, cm^{-1}): 3356 (N–H stretching, secondary amine), 3223 (N–H stretching, secondary amide), 2985 (C–H stretching, aromatic ring), 2893 (C–H stretching, –CH_3 group), 2773 (C–H stretching, –CH_2 group), 1701 (C=O stretching, secondary amide), 1556 (C=C stretching, aromatic ring), 1541 (C=N stretching, aromatic ring), 1522 (N=O stretching, aromatic ring), 1293 (C–H bending), 1196, 1085 (C–O–C stretching), 700 (1:3-di substituted benzene ring). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 9.10 (*s*, 1H, Het–NH–CO–Ar), 8.15–7.20 (*m*, 9H, Ar–H), 4.50 (*s*, 2H, Het–CH_2–NH–), 4.15 (*s*, 1H, Ar–NH–CH_2), 2.40 (*s*, 3H, –N–C–(CH_3)–C–). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 176.9, 165.4, 162.9, 156.6, 155.2, 148.9, 148.4, 134.0, 132.1, 130.5, 128.8, 127.6, 119.7, 112.2, 106.6, 103.2, 69.2, 17.0. LCMS (*m/z*): 468.07 (M^+). Anal. Calcd. for $C_{20}H_{16}N_6O_4S_2$; C, 51.27; H, 3.44; N, 17.94 %. Found: C, 51.31; H, 3.48; N, 17.88 %.

*N-(4-methyl-5-(4-(((4-nitrophenyl)amino)methyl)-5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)thiazol-2-yl)benzamide (**5h**)*

Yield: 65 %, mp 172–177 °C. IR (KBr, cm^{-1}): 3358 (N–H stretching, secondary amine), 3225 (N–H stretching, secondary amide), 2986 (C–H stretching, aromatic ring), 2894 (C–H stretching, –CH_3 group), 2775 (C–H stretching, –CH_2 group), 1703 (C=O stretching, secondary amide), 1558 (C=C stretching, aromatic ring), 1542 (C=N stretching, aromatic ring), 1523 (N=O stretching, aromatic ring), 1295

(C–H bending), 1198, 1088 (C–O–C stretching), 806 (1:4-di substituted benzene ring). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 9.12 (*s*, 1H, Het–NH–CO–Ar), 7.06–6.98 (*m*, 9H, Ar–H), 4.48 (*s*, 2H, Het–CH_2–NH–), 4.16 (*s*, 1H, Ar–NH–CH_2), 2.44 (*s*, 3H, –N–C–(CH_3)–C–). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 177.0, 165.4, 163.0, 156.6, 155.2, 153.8, 135.9, 134.1, 132.0, 128.9, 127.6, 127.4, 114.6, 103.1, 69.1, 17.0. LCMS (*m*/*z*): 468.07 (M^+). Anal. Calcd. for $C_{20}H_{16}N_6O_4S_2$; C, 51.28; H, 3.47; N, 17.91 %. Found: C, 51.25; H, 3.42; N, 17.97 %.

*N-(4-methyl-5-(5-thioxo-4-((o-tolylamino)methyl)-4,5-dihydro-1,3,4-oxadiazol-2-yl)thiazol-2-yl)benzamide (**5i**)*

Yield: 59 %, mp 143–148 °C. IR (KBr, cm^{-1}): 3355 (N–H stretching, secondary amine), 3223 (N–H stretching, secondary amide), 2985 (C–H stretching, aromatic ring), 2892 (C–H stretching, –CH_3 group), 2848 (C–CH_3 stretching), 2773 (C–H stretching, –CH_2 group), 1703 (C=O stretching, secondary amide), 1558 (C=C stretching, aromatic ring), 1294 (C–H bending), 1196, 1087 (C–O–C stretching), 775 (1:2-di substituted benzene ring). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 9.13 (*s*, 1H, Het–NH–CO–Ar), 7.92–6.63 (*m*, 9H, Ar–H), 4.48 (*s*, 2H, Het–CH_2–NH–), 4.13 (*s*, 1H, Ar–NH–CH_2), 2.41 (*s*, 3H, –N–C–(CH_3)–C–), 2.12 (*s*, 3H, C–CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 177.1, 165.4, 163.1, 156.5, 155.0, 146.7, 134.0, 132.2, 128.9, 127.6, 127.1, 126.6, 122.2, 121.8, 110.4, 103.1, 68.8, 17.8, 17.1. LCMS (*m*/*z*): 437.10 (M^+). Anal. Calcd. for $C_{21}H_{19}N_5O_2S_2$; C, 57.65; H, 4.38; N, 16.01 %. Found: C, 57.69; H, 4.34; N, 16.0 %.

*N-(4-methyl-5-(5-thioxo-4-((m-tolylamino)methyl)-4,5-dihydro-1,3,4-oxadiazol-2-yl)thiazol-2-yl)benzamide (**5j**)*

Yield: 60 %, mp 159–164 °C. IR (KBr, cm^{-1}): 3354 (N–H stretching, secondary amine), 3225 (N–H stretching, secondary amide), 2983 (C–H stretching, aromatic ring), 2894 (C–H stretching, –CH_3 group), 2850 (C–CH_3 stretching), 2771 (C–H stretching, –CH_2 group), 1700 (C=O stretching, secondary amide), 1553 (C=C stretching, aromatic ring), 1291 (C–H bending), 1194, 1083 (C–O–C stretching), 703 (1:3-di substituted benzene ring). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 9.09 (*s*, 1H, Het–NH–CO–Ar), 8.02–6.65 (*m*, 9H, Ar–H), 4.48 (*s*, 2H, Het–CH_2–NH–), 4.14 (*s*, 1H, Ar–NH–CH_2), 2.42 (*s*, 3H, –N–C–(CH_3)–C–), 2.33 (*s*, 3H, C–CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 177.0, 165.5, 162.8, 156.2, 155.3, 147.7, 139.4, 134.2, 132.1, 129.3, 128.8, 127.5, 117.2, 113.6, 110.4, 103.0, 68.5, 21.5, 17.1. LCMS (*m*/*z*): 437.10 (M^+). Anal. Calcd. for $C_{21}H_{19}N_5O_2S_2$; C, 57.65; H, 4.38; N, 16.01 %. Found: C, 57.69; H, 4.42; N, 16.06 %.

*N-(4-methyl-5-(5-thioxo-4-((p-tolylamino)methyl)-4,5-dihydro-1,3,4-oxadiazol-2-yl)thiazol-2-yl)benzamide (**5k**)*

Yield: 60 %, mp 152–157 °C. IR (KBr, cm^{-1}): 3357 (N–H stretching, secondary amine), 3224 (N–H stretching, secondary amide), 2984 (C–H stretching, aromatic ring), 2896 (C–H stretching, –CH_3 group), 2850 (C–CH_3 stretching), 2772 (C–H

stretching, $–CH_2$ group), 1702 (C=O stretching, secondary amide), 1555 (C=C stretching, aromatic ring), 1292 (C–H bending), 1195, 1082 (C–O–C stretching), 818 (1:4-di substituted benzene ring). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 9.08 (*s*, 1H, Het–NH–CO–Ar), 8.01–6.60 (*m*, 9H, Ar–H), 4.49 (*s*, 2H, Het–CH_2–NH–), 4.16 (*s*, 1H, Ar–NH–CH_2), 2.39 (*s*, 3H, –N–C–(CH_3)–C–), 2.35 (*s*, 3H, C–CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 177.2, 165.9, 162.8, 156.4, 155.2, 144.8, 134.3, 132.2, 129.8, 129.4, 128.7, 127.6, 113.1, 102.8, 68.7, 21.4, 17.1. LCMS (*m*/*z*): 437.10 (M^+). Anal. Calcd. for $C_{21}H_{19}N_5O_2S_2$; C, 57.65; H, 4.38; N, 16.01 %. Found: C, 57.68; H, 4.45; N, 16.06 %.

Result and discussion

Spectral characterization

Characterization of newly synthesized compounds of the series was carried out by IR, NMR, and mass spectra, and the data with a detailed discussion are given in the experimental section (Fig. 3).

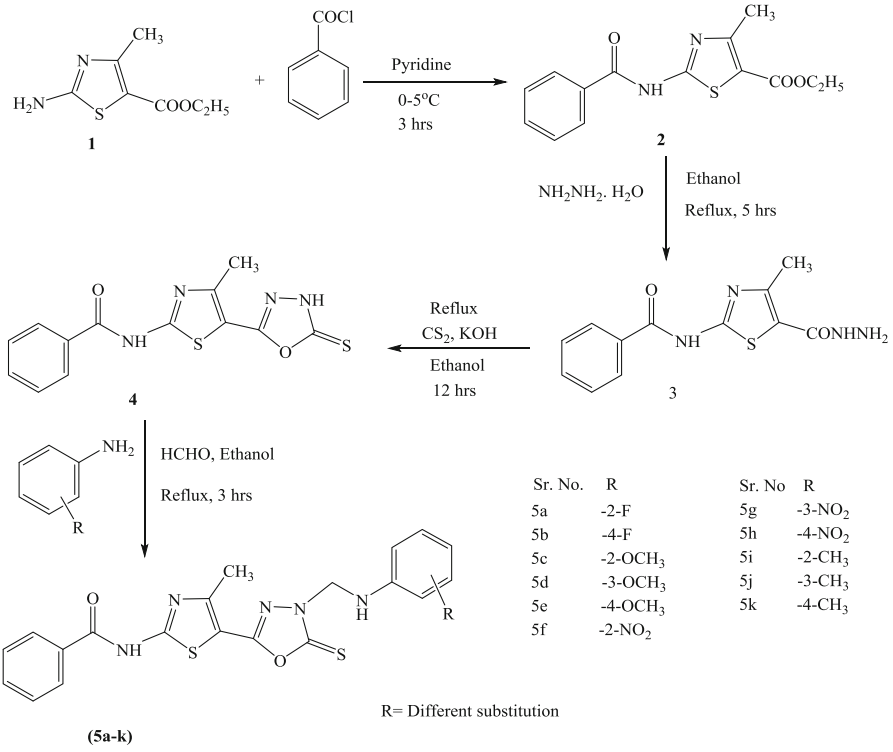

Fig. 3 Synthetic pathway of compounds **(5a–k)**

*IR DATA of compound (**5g**)*

IR spectrum of compound **5g** (molecular formula $C_{20}H_{16}N_6O_4S_2$, M.W. 468.51 gm/mol) has a stretching vibration at 3356 cm^{-1} indicating the presence of N–H stretching of secondary amine. Stretching vibration at 3223 cm^{-1} is an indication of the presence of N–H stretching of secondary amide. The absorption band at 3016 cm^{-1} indicates the presence of Ar–H stretching vibrations. C–H stretching at 2893 cm^{-1} shows the presence of the $–CH_3$ group of the thiazole ring. Stretching at 2773 cm^{-1} is the proof of a $–CH_2$ group in compound **5g**. Absorption bands at 1556, 1541, and 1522 cm^{-1} showed the presence of C=C, C=N, and N=O stretching of the aromatic ring. Also C–H bending at 1293 cm^{-1} showed conformation of the aromatic ring. Stretching at 1196 and 1085 cm^{-1} represent C–O–C bending vibration. The deformation vibration at 812 and 670 is due to presence of nitro group at the third position in our final compound **5g** (Fig. 4).

*^{1}H-NMR spectra of compound (**5g**)*

The ^{1}H-NMR spectra of the final compound **5g** showed that protons were attached at C-15, giving a shift at $\delta = 2.40$ (–N–C–($C\underline{H}_3$)–C–) with a singlet peak and proton of (Ar–N$\underline{H}$–CH_2) appearing as a singlet at $\delta = 4.15$. In the heterocyclic ring, two protons showed a chemical shift at $\delta = 4.50$ due to heterocyclic ring –$C\underline{H}_2$–NH, while protons of the phenyl ring gave multiples at $\delta = 7.20$-8.15 (*m*, 9H, Ar–$\underline{H}$) and heterocyclic protons at position C-8 gave a singlet at $\delta = 9.10$ ppm (*s*, 1H, Het–N$\underline{H}$–CO) (Fig. 4).

*^{13}C-NMR spectra of compound (**5g**)*

The chemical shifts of the final compound **5g** has carbons that varied from $\delta = 176.9$–17.0 ppm. The carbon nuclei under the influence of a strong electronegative environment appeared downfield. Carbon of the amide (Ar–$\underline{C}$ONH) group has a chemical shift at $\delta = 165.4$. The carbon of phenyl group at C-5 attached with amide group has a chemical shift value at $\delta = 132.1$. The carbons of the thiazole ring of carbon C-9, C-11, and C-12 showed chemical shifts at $\delta = 162.9$, 103.2, and 156.6, respectively. The methyl group attached with the C-12 carbon

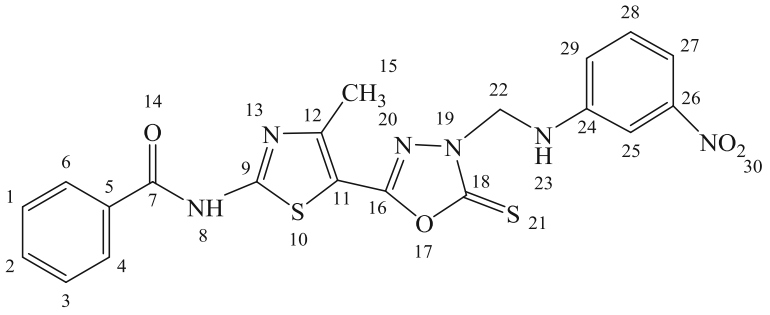

Fig. 4 Compound **5g**

atom in the thiazole ring system showed a chemical shift at 17.0 because of its deshielding effect. 1,3,4-oxadiazole ring carbons C-16 and C-18 appeared with chemical shifts at $\delta = 155.2$ and 176.9, respectively. Methylene carbon C-22, which is attached on both sides to nitrogen atoms, appeared as a chemical shift at $\delta = 69.2$. Aromatic carbon (C-24 to C-29) showed a chemical shift value between 106.6 and 148.9. The nitro group at C-26 in the phenyl ring gave a chemical shift at $\delta = 148.9$.

Discussion of antimicrobial screening

All the newly synthesized compounds **(5a–k)** were initially evaluated for in vitro antibacterial activity against gram- positive bacteria [*Staphylococcus aureus* (MTCC-96), *Streptococcus pyogenes* (MTCC-442)] and gram-negative bacteria [*Escherichia coli* (MTCC-443), *Pseudomonas aeruginosa* (MTCC-1688)] using the conventional broth dilution method. The individual minimal inhibitory concentration (MIC, μg/mL) values of test compounds are listed in Table 1 along with MIC values of the reference compound ampicillin. From screening results, it was observed that final compounds **5c** (–2–OCH_3–C_6H_4), **5i** (2–CH_3–C_6H_4) exhibited good activity against *E. coli* and *S. aureus*. Compound **5d** (–3–OCH_3–C_6H_4) showed good activity against *P. aeruginosa.* Compound **5e** (–4–OCH_3–C_6H_4), showed excellent activity against *E. coli, P. aeruginosa* and *S. aureus* with a two to fourfold higher MIC value (12.5–25 μg/mL) than the reference drug. Very good activity was shown by compound **5j** (3–CH_3–C_6H_4) against *P. aeruginosa* and *S. aureus*. Compound **5k** (4–CH_3–C_6H_4) displayed excellent activity against *S. aureus* and *S. pyogenes*. The remaining compounds of the series possessed moderate antibacterial activity. On the other hand, the presence of similar functional groups at the meta position resulted in minor changes in antibacterial activity as compared to **5c**, **5i**, **5e** and **5k**.

Compounds **(5a–k)** were also evaluated for their in vitro antifungal activity against three fungal strains, *C. albicans, A. niger, and A. clavatus* at various concentrations of 1000, 500, 200, and 100 μg/mL as shown in (Table 1). The data of antimicrobial evaluation of compounds **(5a–k)** were collected in Table 1. The results of antifungal activity indicated that compound **5a** (–2–F–C_6H_4) showed good activity against *C. albicans,* and *A. clavatus.* Compound **5a** (–2–F–C_6H_4) exhibited very good activity against *A. niger*. While compound **5b** (–4–F–C_6H_4) showed excellent activity against *C. albicans*, *A. niger* and *A. clavatus* with a twofold higher MIC value (12.5–25 μg/mL) than the reference drug. The remaining compounds of the series showed feeble antifungal activity. Thus, we have discussed and compared antibacterial and antifungal activities based on standard drugs ampicillin and griseofulvin, respectively.

Determination of antibacterial activity

Antibacterial studies of newly synthesized compounds **(5a–k)** were carried out against the representative panel of gram-positive [*S. aureus* (MTCC-96), *S. pyogenes* (MTCC-442)] and gram-negative [*E. coli* (MTCC-443), *P. aeruginosa*

Table 1 Antibacterial and antifungal activity of compounds **(5a–k)**

S. no.	–R	Minimum inhibitory concentrations for bacteria (MIC_B) in μg/mL				Minimum inhibitory concentration for fungi (MIC_F) in μg/mL		
		E. coli MTCC 443	*P. aeruginosa* MTCC 1688	*S. aureus* MTCC 96	*S. pyogenes* MTCC 442	*C. albicans* MTCC 227	*A. niger* MTCC 282	*A. clavatus* MTCC 1323
5a	–2–F	500	100	1000	500	500	50	100
5b	–4–F	500	1000	500	1000	25	25	12.5
5c	–2–OCH_3	100	500	250	>1000	1000	1000	100
5d	–3–OCH_3	250	100	500	250	1000	500	250
5e	–4–OCH_3	12.5	25	25	100	>1000	250	500
5f	–2–NO_2	250	>1000	250	>1000	1000	>1000	>1000
5g	–3–NO_2	1000	>1000	1000	>1000	1000	>1000	>1000
5h	–4–NO_2	250	>1000	250	250	1000	500	500
5i	–2–CH_3	100	500	250	1000	>1000	500	250
5j	–3–CH_3	250	50	100	250	>1000	1000	250
5k	–4–CH_3	250	100	50	12.5	500	500	>1000
Ampicillin		100	100	250	100	–	–	–
Griseofulvin		–	–	–	–	500	100	100

(MTCC-1688)] bacteria. All MTCC cultures were collected from the Institute of Microbial Technology, Chandigarh. The activity of compounds was determined as per the National Committee for Clinical Laboratory Standards (NCCLS) protocol using Mueller–Hinton Broth (Becton–Dickinson, USA) [32–35]. Primary screening was done first for antibacterial activity in six sets against *E. coli*, *S. aureus*, *P. aeruginosa* and *S. pyogenes* at different concentrations of 1000, 500, and 250 μg/mL. The compounds found to be active in primary screening were similarly diluted to obtain 200, 125, 100, 62.5, 50, 25, and 12.5 μg/mL concentrations for secondary screening to test in a second set of dilutions against all microorganisms. Inoculum size for test strains was adjusted to 10^6 CFU/mL (Colony Forming Units per milliliter) by comparing the turbidity (turbidimetric method). Mueller–Hinton Broth was used as a nutrient medium to grow and dilute the compound suspension for test organisms. Then 2 % DMSO was used as a diluent/vehicle to obtain the desired concentration of synthesized compounds and standard drugs to test upon standard microbial strains. Synthesized compounds were diluted to 1000 μg/mL concentration as stock solution. The control tube containing no antibiotic was immediately subcultured (before inoculation) by spreading a loopful evenly over quarter of a plate of medium suitable for the growth of test organisms. The culture tubes were then incubated for 24 h at 37 °C and the growth was monitored visually and spectrophotometrically. The 10 μg/mL suspensions were further inoculated on an appropriate media, and growth was noted after 24 and 48 h. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as the minimum inhibitory concentration (MIC), i.e., the amount of growth from the control tube before incubation (which represents the original inoculum) that was compared. Solvent had no influence on strain growth. The result of this was greatly affected by the size of the inoculum. The test mixture contained 10^6 CFU/mL organisms. DMSO and sterilized distilled water were used as a negative control while ampicillin antibiotic (1 U strength) was used as a positive control. The standard drug used in the present study was 'ampicillin' for evaluating antibacterial activity which showed 100, 100, 250, and 100 μg/mL MIC against *E. coli*, *P. aeruginosa*, *S. aureus* and *S. pyogenes*, respectively.

Determination of antifungal activity

The newly prepared compounds **(5a–k)** were screened for their antifungal activity as primary screens in six sets against *C. albicans*, *A. niger,* and *A. clavatus* at various concentrations of 1000, 500, and 250 μg/mL. The primary active compounds were similarly diluted to obtain 200, 125, 100, 62.5, 50, 25, and 12.5 μg/mL concentrations for secondary screening to test in a second set of dilutions against all fungi. The fungal activity of each compound was compared with 'griseofulvin' as a standard drug, which showed 500, 100, and 100 μg/mL MIC against *C. albicans*, *A. niger,* and *A. clavatus*, respectively. For fungal growth, in the present protocol, we have used Sabouraud's dextrose broth at 28 °C in aerobic condition for 48 h. DMSO and sterilized distilled water were used as negative control while griseofulvin (1 U strength) was used as a positive control.

SAR studies

The structure–activity relationships (SAR) of compounds **(5a–k)** were determined on the basis of results presented in Table 1. The substitution pattern on the thiazole based 1,3,4-oxadiazole ring system derivatives was carefully selected to confer a different electronic environment of the molecules. SAR studies revealed that the presence of the thiazole ring was essential for a broad range of antimicrobial activity. From the activity data, compounds containing electron-donating substitutions in a skeleton led to an increase in antimicrobial activity. Compound **5e** and **5k** containing 4–OCH_3 and 4–CH_3 groups showed the highest inhibition at MIC = 12.5–25 μg mL^{-1} against bacterial strain *S. aureus* and *S. pyogenes,* and compound **5e** 4–OCH_3 group showed the highest inhibition at MIC = 12.5 μg mL^{-1} against *E. coli*. On the basis of the screening results, it has been observed that the compound **5b** exhibited excellent inhibitory action against all fungal strains while compound **5a** also showed reasonably good inhibition against *C. albicans, A. niger,* and *A. clavatus*. The SAR study indicates that compounds containing electron releasing groups at the para position increases bacterial activity while the presence of electron withdrawing group at the same position lead to enhanced fungal activity.

Conclusion

We have synthesized new thiazole-based 1,3,4-oxadiazoles derivatives with potential antimicrobial activity from available the starting material ethyl 2-amino-4-methylthiazole-5-carboxylate by routine methods. The new compounds were tested for their antimicrobial activity and majority of the compounds showed significant antimicrobial activity. The antimicrobial activity data of the prepared compounds indicated that electron donating groups such as **5e** (methoxy) and **5k** (methyl) groups increased antibacterial activity and electron withdrawing groups such as fluoro, and nitro groups at the para position **5b** increased antifungal activity. Electron withdrawing groups such as fluoro, nitro on the aromatic ring, and the electron donating group such as methoxy and methyl that were taken as substitutes in the chemical structure of the target compounds increased activity against antibacterial and antifungal strains. Results of biological activities of novel thiazole based 1,3,4-oxadiazoles derivatives are interesting for optimization of these leading molecules for further generation of antimicrobial agents.

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