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Synthesis and pharmacological evaluation of 2-substituted 1,3,4-oxadiazole derivatives as antimicrobial agents

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ABSTRACT

The present communication deals with the synthesis of some N´-alkylidene/arylidene-2-(4-substituted-5-phenyl-1,3,4-oxadiazol-2-ylthio) aceto/arylhydrazides, their characterization and antimicrobial activity against medically important microbes. The final compounds were synthesized by reaction of 2-(4-substituted-5-phenyl-1,3,4-oxadiazol-2-ylthio) acetohydrazide with various aliphatic and aromatic aldehydes in presence of glacial acetic acid in ethanol. The structures of the synthesized compounds were established by IR, NMR and Mass spectral studies. The antimicrobial activity was studied against *Staphylococcus aureus* (MTCC 87), *Bacillus subtilis* (MTCC 121), *Pseudomonas aeruginosa* (MTCC 424), *Escherichia coli* (MTCC 40), *Candida albicans* (MTCC 183), *Fusarium solani* (MTCC 2935) using agar well diffusion method. Some of the tested compounds exhibited activity against all the microorganisms. In particular, compounds 12, 19 and 22 were found to be active against all the tested bacterial strains and fungal strain *Candida albicans*. It was concluded that number and position of electron withdrawing substituents affects the activity.

Keywords: 1, 3, 4-oxadiazole, disc diffusion method, acetohydrazides and imines.

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INTRODUCTION

Heterocyclic system has gained importance as they constitute the structural features of many bioactive compounds[1]. Among them 1,3,4-oxadiazole is of significant interest in medicinal chemistry because of the presence of toxophoric –N=C-O- linkage[2]. Literature reveals that 1,3,4-oxadiazole is a highly privileged structure the derivatives of which exhibit a wide range of biological activities including antibacterial[3], antitubercular[4], vasodilatory[5], antifungal[6], cytotoxic[7], anti-inflammatory and analgesic [8-9], hypolipidemic[10], anticancer[11] and ulcerogenic[12] activities. The five membered 1,3,4-oxadiazole heterocycles are also useful intermediates in organic synthesis[13] and widely employed as electron transporting materials[14]. The widespread use of 1,3,4-oxadiazoles as a scaffold in medicinal chemistry establishes this moiety as a member of privileged structures class[15]. Furamizole (Figure 1) is a compound which is based upon 1,3,4-oxadiazole ring and has strong antibacterial activity. Schiff bases have also been shown to exhibit a broad range of biological activities including antifungal, antibacterial, antimalarial, antiproliferative, anti-inflammatory, antiviral and antipyretic[16-17]. Imine or azomethine groups are present in various natural, natural-derived and non-natural compounds. The imine group present in such compounds has been shown to be critical to their biological activities [18-20].

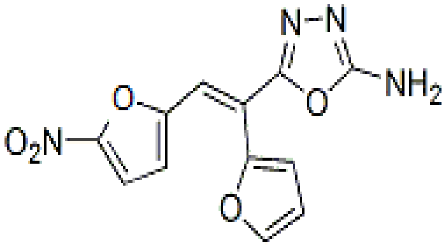
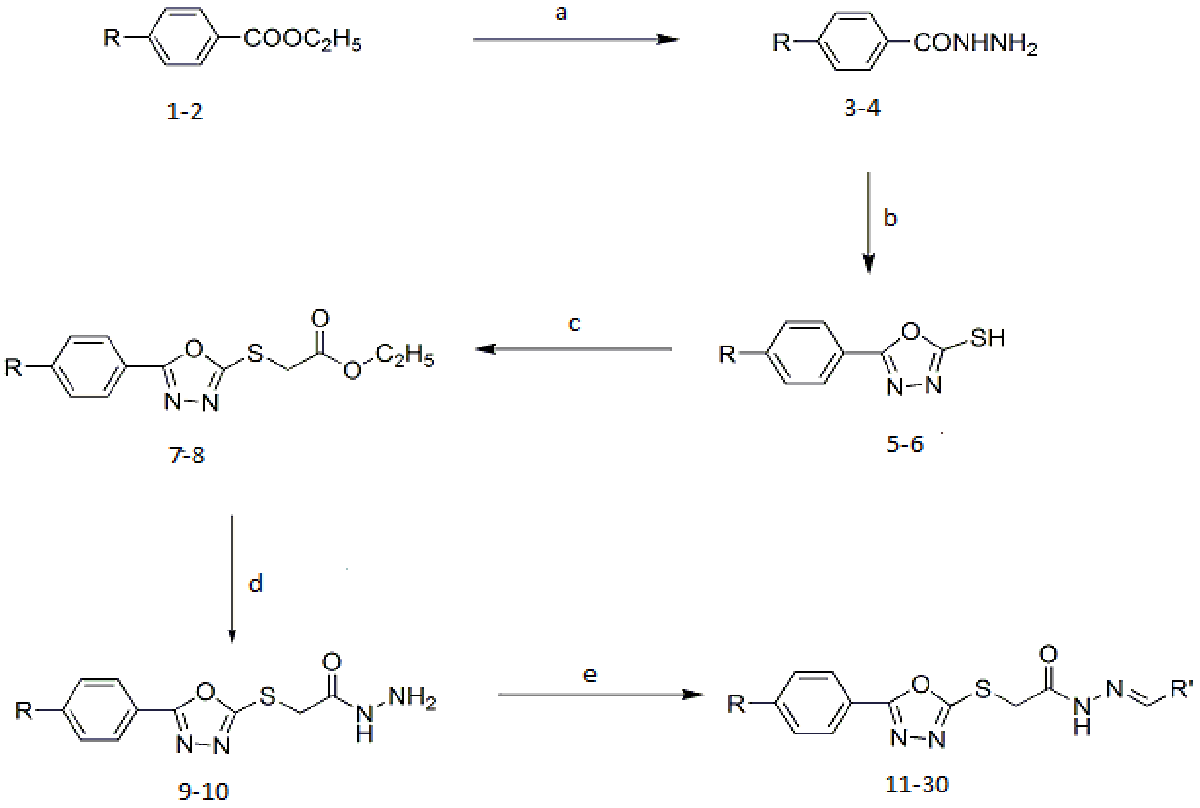


Figure: 1. Structure of Furamizole

The synthesis of heterocyclic compounds containing multi-structure in a molecule has received much attention. Encouraged by these observations and in continuation of our research work, it was considered valuable to integrate one heterocyclic ring and one imine linkage together in a molecular frame work to investigate the additive effect of these rings toward the antibacterial and antifungal activity. The present work deals with the synthesis of N´-alkylidene/arylidene-2-(4-substituted-5-phenyl-1,3,4-oxadiazol-2-ylthio)acetohydrazide **(11-30)** by appropriate methods. The newly synthesized compounds **(11-30)** were further subjected to antimicrobial activity against Gram positive bacteria *Staphylococcus aureus* (MTCC 87), *Bacillus subtilis* (MTCC 121), two Gram negative bacteria *Pseudomonas aeruginosa* (MTCC 424), *Escherichia coli* (MTCC 40), and fungal strains *Candida albicans* (MTCC 183), *Fusarium solani* (MTCC 2935).

MATERIALS AND METHOD

The commercial chemicals employed for the present work were purchased from Sigma-Aldrich, Merck India and spectrochem. All the solvents were used after distillation. Thin layer chromatography was performed on E Merck silica gel GF-254 precoated plates (Merck, Darmstadt, Germany) and the identification was done with UV light and iodine vapours. Various solvent systems used for developing the chromatograms were Petroleum ether: ethyl acetate (2:1), benzene: acetone (7:3), toluene: ethyl acetate: formic acid (5:4:1), chloroform: methanol (9:1). Melting points were determined in open capillaries on a Mel-Temp apparatus and are uncorrected. The identification and characterization of the compounds were carried out by determining melting point, IR, ^{1}H NMR, ^{13}C NMR and Mass spectrometry. All the infra red (IR) spectra (KBr) were recorded on an FT-IR Perkin-Elmer spectrometer (4000-400 cm^{-1}). ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Avance II 400 (400 MHz) spectrometer. Chemical shifts were recorded in parts per million using tetramethylsilane (TMS) as standard. Mass spectra were run on micromas Q-T of micro spectrometer rometer using EI and the m/z values are indicated in Dalton.



Scheme 1: Synthetic pathway for compounds (11-20). (a) $NH_2NH_2.H_2O$, C_2H_5OH, reflux, 10 hrs (b) CS_2, KOH, C_2H_5OH, reflux, 4 hrs (c) $ClCH_2COOC_2H_5$, K_2CO_3, CH_3COCH_3, reflux, 2 hrs (d) $NH_2NH_2.H_2O$, C_2H_5OH, reflux, 5 hrs (e) ArCHO/RCHO, C_2H_5OH, reflux, 8-10 hrs

1: R=H	3: R=H	5: R=H	7: R=H	9: R=H	11-20:R=H 11:R´= Ph
2: R= CH_3	4: R= CH_3	6: R= CH_3	8: R= CH_3	10: R= CH_3	12:R´=4-OCH_3Ph
					13:R´= C_3H_7
					14:R´= $C_6H_{13,}$
					15:R´= 4-OHPh
					16:R´= 3-BrPh
					17:R´= 4-CH_3Ph
					18:R´=2,5-OCH_3Ph
					19:R´= 4-ClPh
					20:R´= 2-OHPh
					21-30:R= CH_3 21: R´= Ph
					22:R´=4-OCH_3Ph
					23:R´= C_3H_7
					24:R´= $C_6H_{13,}$
					25:R´= 4-OHPh
					26:R´= 3-BrPh
					27:R´= 4-CH_3Ph
					28:R´=2,5-OCH_3Ph
					29:R´= 4-ClPh
					30:R´= 2-OHPh

Synthesis of 4-substituted benzohydrazide (3-4):

Compounds 3-4 were prepared by the procedure given in the literature[21]. Yield: 70%; Melting Point: 80-84°C.

Synthesis of 4-substituted-5-phenyl-1,3,4-oxadiazol-2-thiol (5-6):

Compounds 5-6 were prepared by the procedure given in the literature[22]. Yield: 83%; Melting Point: 200-204°C; ^{1}H NMR (400MHz, DMSO-d^6, ppm): ^{1}H NMR (400MHz, $CDCl_3$, ppm): 10.93(s, 1H, SH), 7.26-7.95 (m, 5H, Ar-H).

Synthesis of ethyl 2-(4-substituted-5-phenyl-1,3,4-oxadiazol-2-ylthio) acetate (7-8): Compounds 7-8 were prepared by the procedure given in the literature[23]. Yield: 83%; Melting Point: 64-66°C; ^{1}H NMR (400MHz, DMSO-d^6, ppm): 1.27-1.30 (t, 3H,CH_3), 4.11 (s, 2H, S-CH_2), 4.22-4.27 (q, 2H,CH_2), 7.31-7.99 (m, 5H, Ar-H)

Synthesis of 2-(4-substituted-5-phenyl-1,3,4-oxadiazol-2-ylthio)acetohydrazide (9-10): Compounds 9-10 were prepared by the procedure given in the literature[24]. Yield: 63%; Melting Point: 80-84°C; ^{1}H NMR (400MHz, DMSO-d^6, ppm): 2.58-2.59 (s, 1H,NH), 4.02 (s, 2H, S-CH_2), 4.13-4.14 (s, 2H,NH_2), 7.49-7.99 (m, 5H, Ar-H)**.**

Synthesis of N´-alkylidene/arylidene-2-(4-substituted-5-phenyl-1,3,4-oxadiazol-2-ylthio)acetohydrazide (11-30):

The compounds were prepared by dissolving 5 mmoles of 2-(4-substituted-5-phenyl-1,3,4-oxadiazol-2-ylthio) acetohydrazide and 5 mmoles of appropriate alkyl or aryl aldehyde in 20 ml of ethanol and few drops (4-5) of glacial acetic acid were added. The resulting reaction mixture was refluxed on water bath for 8-10 h. After the completion of reaction, solvent was removed on rotaevaporator and residue was recrystallized from ethanol.

4.5.1 N'-benzylidene-2-(5-phenyl 1,3,4-oxadiazol-2-ylthio)acetohydrazide (11):

Yield: 52%; Melting Point: 158-162°C; IR (KBr, ν, cm^{-1}): 1676 (C=O), 1613 (N=CH), 3176 (N-H), 2943 (C-H), 3077 (Ar), 1061 (oxadiazole ring); ^{1}H NMR (400MHz, DMSO-d^6, ppm): 4.46 (s, 2H, S-CH_2), 11.67 (s, 1H, N-H), 8.22 (s, 1H, N=CH), 7.38-8.01 (m, 10H, Ar-H); MS (m/z %): 338.40 [M^+], 339.08 $[M+ 1]^+$.

4.5.2 N'-(4-methoxybenzylidene)-2-(5-phenyl-1,3,4-oxadiazol-2-ylthio)acetohydrazide (12):

Yield: 70%; Melting Point: 138-142°C; IR (KBr, ν, cm^{-1}): 1676 (C=O), 1606 (N=CH), 3175 (N-H), 2940 (C-H), 3078 (Ar), 1061 (oxadiazole ring); ^{1}H NMR (400MHz, DMSO-d^6, ppm): 4.60 (s, 2H, S-CH_2), 11.58 (s, 1H, N-H), 8.15 (s, 1H, N=CH), 6.88-7.97 (m, 9H, Ar-H), 3.82 (s, 3H,OCH_3); MS (m/z %): 368.52 [M^+].

4.5.3 N'-butylidene-2-(5-phenyl-1,3,4-oxadiazol-2-ylthio)acetohydrazide (13):

Yield: 43%; Melting Point: 110-115°C; IR (KBr, ν, cm^{-1}): 1656 (C=O), 1630 (N=CH), 3213 (N-H), 2956 (C-H), 3072 (Ar), 1064 (oxadiazole ring); ^{1}H NMR (400MHz, DMSO-d^6, ppm): 4.49 (s, 2H, S-CH_2), 11.15 (s, 1H, N-H), 7.34-7.37 (t, J = 6 Hz, 1H, N=CH), 7.50-7.99 (m, 5H, Ar-H), 0.93-0.96 (t, J=6 Hz, 3H,CH_3), 1.50-1.59 (m, 2H, CH_2), 2.18-2.58 (m, 2H, CH_2); MS (m/z %): 304.12 [M^+].

4.5.4 N'-heptylidene-2-(5-phenyl-1,3,4-oxadiazol-2-ylthio)acetohydrazide (14):

Yield: 40%; Melting Point: 100-104°C; IR (KBr, ν, cm^{-1}): 1657 (C=O), 1630 (N=CH), 3214 (N-H), 2955 (C-H), 3072 (Ar), 1064 (oxadiazole ring); ^{1}H NMR (400MHz, DMSO-d^6, ppm): 4.46 (s, 2H, S-CH_2), 11.26 (s, 1H, N-H), 7.34-7.37 (t, J = 6 Hz, 1H, N=CH), 7.51-7.97 (m, 5H, Ar-H), 0.85-0.88 (t, J= 6 Hz, 3H, CH_3), 1.45-1.50 (m, 2H, CH_2), 1.24-1.29 (m, 6H, C_3H_6), 2.18-2.58 (m, 2H, CH_2); MS (m/z %): 346.15 [M^+], 347.10 $[M+1]^+$.

4.5.5 N'-(4-hydroxybenzylidene)-2-(5-phenyl-1,3,4-oxadiazol-2-ylthio)acetohydrazide (15):

Yield: 50%; Melting Point: 206-210°C; IR (KBr, ν, cm^{-1}): 1670 (C=O), 1621 (N=CH), 3169 (N-H), 2962 (C-H), 3075 (Ar), 1070 (oxadiazole ring), 3419 (O-H); ^{1}H NMR (400MHz, DMSO-d^6, ppm): 4.59 (s, 2H, S-CH_2), 11.55 (s, 1H, N-H), 8.10 (s, 1H, N=CH), 6.80-8.01 (m, 9H, Ar-H), 9.67 (s, 1H, O-H); MS (m/z %): 354.78 [M^+] .

4.5.6 N'-(3-bromobenzylidene)-2-(5-phenyl-1,3,4-oxadiazol-2-ylthio)acetohydrazide (16):

Yield: 75%; Melting Point: 180-184°C; IR (KBr, ν, cm^{-1}): 1676 (C=O), 1610 (N=CH), 3173 (N-H), 2971 (C-H), 3075 (Ar), 1062 (oxadiazole ring), 652 (C-Br); ^{1}H NMR (400MHz, DMSO-d^{6}, ppm): 4.58 (s, 2H, S-CH$_2$), 11.65 (s, 1H, NH), 8.10 (s, 1H, N=CH), 7.01-7.98 (m, 9H, Ar-H); MS (m/z %): 415.89 [M^{+}].

4.5.7 N'-(4-methylbenzylidene)-2-(5-phenyl-1,3,4-oxadiazol-2-ylthio)acetohydrazide (17):

Yield: 71%; Melting Point: 152-155°C; IR (KBr, ν, cm^{-1}): 1670 (C=O), 1608 (N=CH), 3181 (N-H), 2975 (C-H), 3057 (Ar), 1063 (oxadiazole ring); ^{1}H NMR (400MHz, DMSO-d^{6}, ppm): 4.61 (s, 2H, S-CH$_2$), 11.69 (s, 1H, NH), 8.17 (s, 1H, N=CH), 7.18-7.99 (m, 9H, Ar-H), 2.36 (s, 3H, CH$_3$); MS (m/z %): 353.4 [M+1]$^{+}$.

4.5.8 N'-(2,5-dimethoxybenzylidene)-2-(5-phenyl-1,3,4-oxadiazol-2 ylthio)acetohydrazide (18):

Yield: 57%; Melting Point: 166-170°C; IR (KBr, ν, cm^{-1}): 1681 (C=O), 1600 (N=CH), 3176 (N-H), 2963 (C-H), 3006 (Ar), 1065 (oxadiazole ring); ^{1}H NMR (400MHz, DMSO-d^{6}, ppm): 4.63 (s, 2H, S-CH$_2$), 11.73 (s, 1H, N-H), 8.55 (s, 1H, N=CH), 6.94-8.35 (m, 8H, Ar-H), 3.81 (s, 3H,OCH$_3$), 3.76 (s, 3H,OCH$_3$); MS (m/z %): 398.49 [M^{+}].

4.5.9 N'-(4-chlorobenzylidene)-2-(5-phenyl-1,3,4-oxadiazol-2-ylthio)acetohydrazide (19):

Yield: 54%; Melting Point: 158-162°C; IR (KBr, ν, cm^{-1}): 1677 (C=O), 1623 (N=CH), 3183 (N-H), 2965 (C-H), 3087 (Ar), 1061 (oxadiazole ring), 768 (C-Cl); ^{1}H NMR (400MHz, DMSO-d^{6}, ppm): 4.61 (s, 2H, S-CH$_2$), 11.82 (s, 1H, N-H), 8.21 (s, 1H, N=CH), 7.38-8.07 (m, 9H, Ar-H); MS (m/z %): 372.03 [M^{+}]

4.5.10 N'-(2-hydroxybenzylidene)-2-(5-phenyl-1,3,4-oxadiazol-2-ylthio)acetohydrazide (20):

Yield: 52%; Melting Point: 182-185°C; IR (KBr, ν, cm^{-1}): 1637 (C=O), 1613 (N=CH), 3146 (N-H), 2971 (C-H), 3043 (Ar), 1053 (oxadiazole ring), 3446 (O-H); ^{1}H NMR (400MHz, DMSO-d^{6}, ppm): 4.11 (s, 2H, S-CH$_2$), 10.87 (s, 1H, N-H), 8.04 (s, 1H, N=CH), 6.92-8.04 (m, 9H, Ar-H), 9.43 (s, 1H, OH); MS (m/z %): 354.70 [M^{+}], 355.68 [M+1]$^{+}$.

4.5.11 N'-benzylidene-2-(5-*p*-tolyl-1,3,4-oxadiazol-2-ylthio)acetohydrazide (21):

Yield: 55%; Melting Point: 162-166°C; IR (KBr, ν, cm^{-1}): 1675 (C=O), 1612 (N=CH), 3183 (N-H), 2969 (C-H), 3084 (Ar), 1064 (oxadiazole ring), 1449, 1372 (CH$_3$); ^{1}H NMR (400MHz, DMSO-d^{6}, ppm): 4.61 (s, 2H, S-CH$_2$), 11.75 (s, 1H, NH), 8.03 (s, 1H, N=CH), 7.30-7.86 (m, 9H, Ar-H), 2.41 (s, 3H, CH$_3$); MS (m/z %): 353.41 [M+ 1]$^{+}$.

4.5.12 N'-(4-methoxy benzylidene)-2-(5-*p*-tolyl-1,3,4-oxadiazol-2-ylthio)acetohydrazide (22):

Yield: 75%; Melting Point: 158-162°C; IR (KBr, ν, cm^{-1}): 1661 (C=O), 1605 (N=CH), 3177 (N-H), 2970 (C-H), 3079 (Ar), 1068 (oxadiazole ring), 1415, 1388 (CH_3); ^{1}H NMR (400MHz, DMSO-d^6, ppm): 4.58 (s, 2H, S-CH_2), 11.61 (s, 1H, N-H), 8.15 (s, 1H, N=CH), 6.90-8.05 (m, 8H, Ar-H), 3.88 (s, 3H, OCH_3), 2.41 (s, 3H, CH_3); MS (m/z %): 382.63 [M^+].

4.5.13 N'-butylidene-2-(5-*p*-tolyl-1,3,4-oxadiazol-2-ylthio)acetohydrazide (23):

Yield: 41%; Melting Point: 116-120°C; IR (KBr, ν, cm^{-1}): 1657 (C=O), 1628 (N=CH), 3209 (N-H), 2955 (C-H), 3072 (aromatic), 1069 (oxadiazole ring), 1407, 1370 (CH_3); ^{1}H NMR (400MHz, DMSO-d^6, ppm): 4.45 (s, 2H, S-CH_2), 11.30 (s, 1H, N-H), 7.51-7.54 (t, J = 6 Hz, 1H, N=CH), 7.29-7.86 (m, 4H, Ar-H), 0.92-0.97 (m, 3H, CH_3), 1.48-1.57 (m, 3H, OCH_2), 2.16-2.28 (m, 2H, CH_2), 2.41 (s, 3H, CH_3); MS (m/z %): 318.52 [M^+].

4.5.14 N'-heptylidene-2-(5-*p*-tolyl-1,3,4-oxadiazol-2-ylthio)acetohydrazide (24):

Yield: 38%; Melting Point: 120-124°C; IR (KBr, ν, cm^{-1}): 1660 (C=O), 1629 (N=CH), 3214 (N-H), 2923 (C-H), 3071 (Ar), 1069 (oxadiazole ring), 1406, 1367 (CH_3); ^{1}H NMR 400MHz, DMSO-d^6, ppm): 4.44 (s, 2H, S-CH_2), 11.28 (s, 1H, N-H), 7.50-7.53 (t, J = 6 Hz, 1H, N=CH), 7.18-7.85 (m, 4H, Ar-H), 0.86-0.89 (t, J=6 Hz, 3H, CH_3), 1.44-1.50 (m, 3H, OCH_2), 1.28-1.34 (m, 2H, CH_2),2.17-2.29 (m, 2H, CH_2), 2.42 (s, 3H, CH_3); MS (m/z %): 360.71 [M^+]

4.5.15 N'-(4-hydroxy benzylidene)-2-(5-*p*-tolyl-1,3,4-oxadiazol-2-ylthio)acetohydrazide (25):

Yield: 55%; Melting Point: 206-210°C; IR (KBr, ν, cm^{-1}): 1664 (C=O), 1608 (N=CH), 3193 (N-H), 2886 (C-H), 3002 (Ar), 1070 (oxadiazole ring), 1443, 1375 (CH_3), 3416 (O-H); ^{1}H NMR (400MHz, DMSO-d^6, ppm): 4.58 (s, 2H, S-CH_2), 11.43 (s, 1H, N-H), 8.10 (s, 1H, N=CH), 6.81-7.91 (m, 8H, Ar-H), 2.42 (s, 3H, CH_3), 9.55 (s, 1H, O-H); MS (m/z %): 368.49 [M^+], 369.56[M+1]$^+$.

4.5.16 N'-(3-bromo benzylidene)-2-(5-*p*-tolyl-1,3,4-oxadiazol-2-ylthio)acetohydrazide (26):

Yield: 79%; Melting Point: 155-158°C; IR (KBr, ν, cm^{-1}): 1677 (C=O), 1607 (N=CH), 3179 (N-H), 2964 (C-H), 3082 (Ar), 1066 (oxadiazole ring), 1445, 1379 (CH_3), 655 (C-Br); ^{1}H NMR (400MHz, DMSO-d^6, ppm): 4.60 (s, 2H, S-CH_2), 11.47 (s, 1H, N-H), 8.12 (s, 1H, N=CH), 6.89-7.90 (m, 8H, Ar-H), 2.42 (s, 3H, CH_3); MS (m/z %): 430.33 [M^+].

4.5.17 N'-(4-methylbenzylidene)-2-(5-*p*-tolyl-1,3,4-oxadiazol-2-ylthio)acetohydrazide (27):

Yield: 70%; Melting Point: 150-154°C; IR (KBr, ν, cm^{-1}): 1674 (C=O), 1612 (N=CH), 3175 (N-H), 2942 (C-H), 3079 (Ar), 1065 (oxadiazole ring), 1408, 1381 (CH_3); ^{1}H NMR (, 400MHz, $CDCl_3$, ppm): 4.63 (s, 2H, S-CH_2), 10.42 (s, 1H, N-H), 8.10 (s, 1H, N=CH), 7.12-7.88 (m, 8H, Ar-H), 2.35 (s, 3H, CH_3), 2.39 (s, 3H, CH_3); MS (m/z %): 366.68 [M^+].

4.5.18 N'-(2,5-dimethoxybenzylidene)-2-(5-*p*-tolyl-1,3,4-oxadiazol-2-ylthio)acetohydrazide (28):

Yield: 53%; Melting Point: 178-180°C; IR (KBr, ν, cm^{-1}): 1676 (C=O), 1613 (N=CH), 3178 (N-H), 2971 (C-H), 3075 (Ar), 1064 (oxadiazole ring), 1442, 1368 (CH_3); ^{1}H NMR (400MHz, DMSO-d^6, ppm): 4.59 (s, 2H, S-CH_2), 11.60 (s, 1H, N-H), 8.36 (s, 1H, N=CH), 6.87-7.86 (m, 7H, Ar-H), 2.42 (s, 3H, CH_3), 3.79 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3); MS (m/z %): 338.40 [M^+].

4.5.19 N'-(4-chloro benzylidene)-2-(5-*p*-tolyl-1,3,4-oxadiazol-2-ylthio)acetohydrazide (29): Yield: 54%; Melting Point: 180-183°C; IR (KBr, ν, cm^{-1}): 1671 (C=O), 1609 (N=CH), 3187 (N-H), 2978 (C-H), 3092 (Ar), 1068 (oxadiazole ring), 1399, 1367 (CH_3), 720 (C-Cl); ^{1}H NMR (400MHz, DMSO-d^6, ppm): 4.63 (s, 2H, S-CH_2), 11.59 (s, 1H, N-H), 8.27 (s, 1H, N=CH), 6.98-7.91 (m, 8H, Ar-H), 2.34 (s, 3H, CH_3); MS (m/z %): 386.57 [M^+].

4.5.20 N'-(2-hydroxy benzylidene)-2-(5-*p*-tolyl-1,3,4-oxadiazol-2-ylthio)acetohydrazide (30): Yield: 50%; Melting Point: 140-144 °C; IR (KBr, ν, cm^{-1}): 1649 (C=O), 1624 (N=CH), 3178 (N-H), 2986 (C-H), 3028 (Ar), 1047 (oxadiazole ring), 1396, 1379 (CH_3), 3396 (O-H); ^{1}H NMR (400MHz, DMSO-d^6, ppm): 4.65 (s, 2H, S-CH_2), 11.64 (s, 1H, N-H), 8.21 (s, 1H, N=CH), 6.92-7.88 (m, 8H, Ar-H), 2.30 (s, 3H, CH_3), 9.45 (s, 1H, O-H); MS (m/z %): 368.49 [M^+].

ANTIMICROBIAL ACTIVITY

Test microorganisms

For the determination of antimicrobial activities Agar well diffusion method was employed for all the synthesized compounds. All the tested bacterial strains (*Staphylococcus aureus* MTCC 87, *Bacillus subtilis* MTCC 121, *Pseudomonas aeruginosa* MTCC 424, *Escherichia coli* MTCC 40) and fungal strains (*Candida albicans* MTCC 183, *Fusarium solani* MTCC 2935) were obtained from IMTECH, Chandigarh. Nutrient agar medium and potato dextrose agar (PDA) media were used for bacteria and fungi respectively. Freshly prepared media were sterilized by autoclaving at 15 lb/psi pressure for 15 minutes. Bacterial and fungal cultures of test organisms were maintained at refrigerated conditions on Nutrient agar slants.

Antibacterial and antifungal screening by Agar well diffusion method

20 compounds (11-30) were tested for *in vitro* antimicrobial activity. The synthesized compounds and standard drugs (Ciprofloxacin and ketoconazole) were dissolved DMSO to get a concentration of 200 µg/ml and then further diluted to 100 µg/ml. Approximately 30 ml of agar media was poured into sterile petri plates and transferred 0.5 ml of test microorganisms. The plates were allowed to dry. Wells were bored into the plates with a sterile cork borer (5.0 mm diameter). Each well received 0.l ml of the test compound and standard drug in the concentrations of 100µg/ml and 200µg/ml. These plates were then incubated at 37°C for 24 h for bacterial strains and 48 h for

fungal strains. After incubation, the resulting inhibition zone (IZ) was recorded[25-26]. DMSO was used as a negative control and showed no antimicrobial activity.

RESULTS AND DISCUSSION

N´-alkylidene/arylidene-2-(4-substituted-5-phenyl-1,3,4-oxadiazolylthio)aceto/arylhydrazide **(11-30)** described in the study were prepared as depicted in **scheme 1**. Intermediate 4-substituted benzoyl hydrazide **(3-4)** was synthesized by refluxing 4-substituted ethyl benzoate **(1-2)** with hydrazine hydrate in ethanol. Then mixture of arylhydrazide **(3-4)** and carbon disulphide in the presence of KOH was refluxed in ethanol and then acidified with dilute HCl to give 4-substituted-5-phenyl-1,3,4-oxadiazol-2-thiol **(5-6)**. Ester derivatives i.e ethyl 2-(4-substituted-5-phenyl-1,3,4-oxadiazol-2-ylthio) acetate **(7-8)** were synthesized by the reaction of ethylchloroacetate with 5-substituted-4-phenyl-1,3,4-oxadiazol-2-thiol **(5-6)** in presence of anhydrous potassium carbonate. The thioacetate derivative of mercaptoheterocyclic compound **(7-8)** was reacted with hydrazine hydrate in ethanol resulted in the formation of 2-(4-substituted-5-phenyl-1,3,4-oxadiazol-2-ylthio) acetohydrazide **(9-10)**. The final compounds **(11-30)** were synthesized by reaction of 2-(4-substituted-5-phenyl-1,3,4-oxadiazol-2-ylthio) acetohydrazide **(9-10)** with various aliphatic and aromatic aldehydes in presence of glacial acetic acid in ethanol. Some physical properties of the synthesized compounds were shown in Table 1. The synthesized compounds were characterized by FT-IR, ^{1}H-NMR, Mass spectrometry and melting point analysis. Compounds **(11-30)** were confirmed by its IR spectrum which showed the presence of characteristic strong absorption band 1670 cm^{-1} of carbonyl group (C-O stretch), absorption band at 1608 cm^{-1} of imine (N=C stretch), absorption band at 1063 cm^{-1} of oxadiazole ring, absorption band at 3181 cm^{-1} of N-H stretching, absorption band at 2975 cm^{-1} of C-H stretching, absorption band at 3057 cm^{-1} of aromatic C-H stretch. In ^{1}H NMR spectra of the compounds the imine group (–N=CH) was observed as a singlet at δ 8.17 ppm, the presence of aromatic protons was confirmed by multiplet at δ 7.18-7.99. ^{13}C NMR spectra of the compounds confirmed the number and types of carbon atoms. The mass spectrum of compounds showed molecular ion peak $(M+H)^+$ confirming their purity and molecular weight. The R_f value of all synthesized compounds was calculated in ethyl acetate (EtOAc) and hexane (3:7) and ranges between 0.4- 0.7. The analytical data were fully consistent with the proposed structures.

Table: 1: Some characteristics of synthesized compounds (11-30)

Compound	R	R´	Molecular Formula	Melting Point (°C)	Yield (%)	R_f
11	H	Ph	$C_{17}H_{14}N_4O_2S$	158-162	52	0.58
12	H	4-OCH_3Ph	$C_{18}H_{16}N_4O_3S$	138-142	70	0.59
13	H	C_3H_7	$C_{14}H_{16}N_4O_2S$	110-115	43	0.46
14	H	C_6H_{13}	$C_{17}H_{22}N_4O_2S$	100-104	40	0.41
15	H	4-OHPh	$C_{17}H_{14}N_4O_3S$	206-210	50	0.53
16	H	3-BrPh	$C_{17}H_{13}BrN_4O_2S$	180-184	75	0.61
17	H	4-CH_3Ph	$C_{18}H_{16}N_4O_2S$	152-155	71	0.59
18	H	2,5-OCH_3Ph	$C_{19}H_{18}N_4O_4S$	166-170	57	0.6
19	H	4-ClPh	$C_{17}H_{13}ClN_4O_2S$	158-162	54	0.53
20	H	2-OHPh	$C_{17}H_{14}N_4O_3S$	182-185	52	0.56
21	CH_3	Ph	$C_{18}H_{16}N_4O_2S$	162-166	55	0.6
22	CH_3	4-OCH_3Ph	$C_{19}H_{18}N_4O_3S$	158-162	75	0.62
23	CH_3	C_3H_7	$C_{15}H_{18}N_4O_2S$	116-120	41	0.48
24	CH_3	C_6H_{13}	$C_{18}H_{24}N_4O_2S$	120-124	38	0.43
25	CH_3	4-OHPh	$C_{18}H_{16}N_4O_3S$	206-210	55	0.54
26	CH_3	3-BrPh	$C_{18}H_{15}BrN_4O_2S$	155-158	79	0.64
27	CH_3	4-CH_3Ph	$C_{19}H_{18}N_4O_2S$	150-154	70	0.6
28	CH_3	2,5-OCH_3Ph	$C_{20}H_{20}N_4O_4S$	178-180	53	0.62
29	CH_3	4-ClPh	$C_{18}H_{15}ClN_4O_2S$	180-183	54	0.53
30	CH_3	2-OHPh	$C_{18}H_{16}N_4O_3S$	140-144	50	0.59

Antimicrobial activity

All synthesized compounds were preliminary evaluated for their *in vitro* antimicrobial activity by the Agar well diffusion. Antimicrobial activity was measured against tested microorganisms (*Staphylococcus aureus* MTCC 87, *Bacillus subtilis* MTCC 121, *Pseudomonas aeruginosa* MTCC 424, *Escherichia coli* MTCC 40) and fungal strains (*Candida albicans* MTCC 183, *Fusarium solani* MTCC 2935). Standard antibacterial drug, ciprofloxacin and antifungal drug, ketoconazole were used to compare the antibacterial and antifungal activities shown by tested compounds. The zone of inhibition was observed in mm. The mean ± SD (n=3) of inhibition zone was taken for evaluating the antimicrobial activity of test and standard compounds. All the compounds exhibited the antibacterial activity against the growth of tested Gram-positive and Gram-negative bacterial strains with diameter of zone of inhibition ranging from 7-32 mm The most potent antibacterial activity was exhibited by compounds 12, 18, 19, 22, 28 and 29 (32 mm) against all the bacterial strains, at the concentration of 200 µg/ml. The highest antifungal effect was obtained in the cases of compounds 12, 16, 19, 22, 26 and 29 (25mm), against *C. Albicans* at the concentration of 200µg/ml. The order of activity was observed to be 2,5-OCH_3Ph > 4-ClPh > 4-OCH_3Ph> 3-Br > H>4-CH_3. The screening results are summarized in **Table 2**. Good antimicrobial activities of compounds 12, 16, 18, 19, 22, 26, 28 and 29 may be attributed to the presence of various electron withdrawing groups (-Cl, -Br, $-OCH_3$). However, the activity varies with the number and position of substitution of electron withdrawing groups. Substitutions with electron donating substituents lead to the significant fall in the activity

Table: 2. Results of antibacterial and antifungal screening of the synthesized compounds

Compound Conc. (µg/ml)	***Zone of inhibition (mm)****											
	Gram-positive bacteria				**Gram-negative bacteria**				**Fungal strains**			
	S. aureus		***B. subtilis***		***P. aeruginosa***		***E. coli***		***C. albicans***		***F. solani***	
	100	**200**	**100**	**200**	**100**	**200**	**100**	**200**	**100**	**200**	**100**	**200**
11	14±0.5	18±0.4	16±0.6	22±0.5	10±0.7	12±0.6	12±0.3	14±0.4	08±0.3	10±0.3	07±0.3	11±0.2
12	26±0.6	31±0.4	25±0.5	32±0.6	14±0.4	22±0.5	16±0.3	25±0.4	16±0.5	25±0.3	15±0.0.4	22±0.5
13	08±0.6	10±0.5	07±0.3	11±0.4	10±0.8	11±0.6	12±0.3	09±0.8	08±0.4	10±0.3	06±0.3	10±0.6
14	10±0.4	11±0.3	08±0.4	11±0.3	07±0.5	11±0.4	10±0.5	10±0.4	10±0.1	12±0.2	11±0.3	12±0.2
15	15±0.4	20±0.3	20±0.4	27±0.3	13±0.5	18±0.4	15±0.2	21±0.4	14±0.2	16±0.4	10±0.5	14±0.3
16	18±0.4	25±0.5	21±0.4	29±0.3	15±0.3	19±0.4	16±0.3	21±0.4	17±0.4	23±0.6	15±0.5	22±0.3
17	16±0.4	22±0.3	15±0.3	24±0.4	14±0.3	21±0.3	13±0.4	22±0.3	12±0.3	15±0.7	11±0.3	14±0.5
18	21±0.4	28±0.5	24±0.3	32±0.3	20±0.3	26±0.5	19±0.4	27±0.6	16±0.5	23±0.5	15±0.4	22±0.3
19	23±0.4	32±0.7	24±0.4	31±0.6	19±0.5	25±0.4	22±0.6	29±0.5	20±0.6	30±0.7	18±0.5	26±0.6
20	15±0.4	19±0.5	18±0.6	24±0.4	10±0.7	13±0.7	12±0.6	15±0.6	10±0.3	14±0.4	09±0.2	12±0.3
21	16±0.6	22±0.4	15±0.5	24±0.6	14±0.5	21±0.3	13±0.2	22±0.2	11±0.3	15±0.3	12±0.6	14±0.3
22	19±0.3	25±0.6	21±0.5	30±0.4	19±0.6	24±0.5	22±0.5	28±0.4	18±0.5	27±0.4	16±0.4	24±0.4
23	12±0.3	14±0.7	11±0.5	15±0.5	10±0.5	14±0.2	12±0.4	13±0.5	11±0.3	13±0.3	10±0.6	12±0.3
24	11±0.7	13±0.3	12±0.5	13±0.3	12±0.5	14±0.5	11±0.3	13±0.3	09±0.3	12±0.2	08±0.1	11±0.6
25	14±0.5	21±0.5	16±0.6	23±0.4	12±0.4	14±0.6	14±0.4	16±0.4	14±0.3	22±0.4	13±0.3	20±0.3
26	16±0.3	24±0.4	20±0.5	26±0.3	18±0.3	13±0.4	16±0.5	15±0.5	21±0.6	28±0.5	20±0.4	26±0.6
27	14±0.3	23±0.4	15±0.4	23±0.5	14±0.3	15±0.2	14±0.3	22±0.4	13±0.4	21±0.5	12±0.3	15±0.4
28	20±0.3	26±0.4	22±0.4	31±0.6	25±0.5	32±0.7	25±0.3	32±0.5	17±0.4	23±0.3	22±0.5	26±0.3
29	22±0.5	28±0.5	23±0.5	30±0.6	22±0.6	29±0.5	23±0.4	32±0.3	18±0.4	25±0.3	19±0.5	27±0.5
30	15±0.4	19±0.5	16±0.3	22±0.3	11±0.3	13±0.4	12±0.3	21±0.3	13±0.3	18±0.4	12±0.5	14±0.3
Ciprofloxacin	34±0.2	38±0.2	36±0.2	42±0.3	32±0.3	37±0.3	40±0.2	45±0.2				
Ketoconazole									25±0.2	33±0.2	26±0.3	31±0.2

*± SD (n=3) mean of zone of inhibition in mm

CONCLUSION

A series of N´-alkylidene/arylidene-2-(4-substituted-5-phenyl-1,3,4-oxadiazol-2-ylthio) acetohydrazide derivatives with moderate to high yields. The *in vitro* antimicrobial activity of these compounds was also determined by Agar well diffusion and compared with standard drugs. In general, most of the compounds synthesized during the present work exhibited promising activity against all tested bacteria and fungi except *Fusarium solani*. The present work adds new data in the relationship with oxadiazole, imine and their antimicrobial activities.

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