Synthesis of 2-{(5-phenyl-1,3,4-Oxadiazol-2-yl)sulfanyl}-*N*-substituted acetamides as potential antimicrobial and hemolytic agents

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Abstract: A new series of *N*-substituted derivatives of 2-{(5-phenyl-1,3,4-Oxadiazol-2-yl)sulfanyl}acetamides was synthesized. The synthesis was carried out by converting benzoic acid (1) into ethyl benzoate (2), benzohydrazide (3) and then 5-pheny-1,3,4-Oxadiazol-2-thiol (4) step by st0ep. The target compounds 6a-p were synthesized by reaction of compound 4 with equimolar ratios of different *N*-alkyl/aryl substituted 2-bromoacetamide (5a-p) in the presence of DMF and sodium hydride (NaH). The spectral (EI-MS, IR, ^{1}H-NMR) characterization of all the synthesized compounds reveal their successful synthesis. The compounds were also screened for antimicrobial & hemolytic activity and most of them were found to be active against the selected microbial species at variable extent relative to reference standards. But 6h was the most active against the selected panel of microbes. This series showed less toxicity and may be considered for further biological screening and application trial except 6m, possessing higher cytotoxicity.

Keywords: Benzoic acid; 1,3,4-Oxadiazole; antimicrobial activity; hemolytic activity; Spectral characterization.

INTRODUCTION

During the last few decades, organic chemist have devoted significant interest to the synthesis of 1,3,4-Oxadiazole derivatives due to their diverse pharmacological and antimicrobial applications (Omar *et al*., 1996; Hui *et al*., 2002 and Mohan *et al*., 2004). Some of these compounds have shown comprehensive biological properties in both agrochemical and pharmaceutical fields such as antimicrobial, antiviral, anti-tumor, antimalarial, anticancer, anti-HIV, anti-inflammatory, anticonvulsant, anti-tuberculosis, anti-analgesic, anti-mycobacterial and antidepressant activities (Zheng *et al*., 2003; Shah *et al*., 1996 and Liszkiewicz *et al.*, 2003) and were utilized successfully (CLSI 2007; Zuhair *et al*., 2008; Hemavathi *et al*., 2011; Rakesh *et al.*, 2010; Omar *et al*., 1996; Priya *et al*., 2007 and Shivarama *et al.*, 2005).

The major challenge in organic synthesis is the development of more efficient and environment friendly chemical process for the preparation of new biologically active molecules that can work more efficiently and effectively against microorganism with less toxicity (Anastas *et al.*, 1998 and Aryanasab *et al.*, 2011).

In continuation of our previous work (Aziz-ur-Rehman *et al.*, 2012a; Aziz-ur-Rehman *et al.*, 2012b; Aziz-ur-Rehman *et al.*, 2013a; Aziz-ur-Rehman *et al.*, 2013b and Aziz-ur-Rehman *et al.*, 2013c), the synthesis and biological screening of *N*-substituted derivatives of 2-{(5-phenyl-1,3,4-Oxadiazol-2-yl)sulfanyl}acetamide were accomplished with an objective to detect the anti-microbial and hemolytic activity of the synthesized compounds. All the synthesized derivatives were screened for anti-microbial and cytotoxic potential and found that the most of compounds were exhibited significant activity against the selected microbial species relative to reference standard drugs.

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MATERIALS AND METHODS

Chemistry

All the starting materials and reagents were purchased from Sigma Aldrich and Alfa Aesar (Germany). The completion of reactions and purity of all synthesized compounds were ascertained by using thin layer chromatography with ethyl acetate & *n*-hexane (30:70) as mobile phase through comparison of R_f values of reactants and products. By using open capillary tube method melting points were determined on Griffin and George melting point apparatus and were uncorrected. IR spectra in KBr were recorded on Jasco-320-A spectrophotometer in cm^{-1}. ^{1}H-NMR spectra were recorded at 400 MHz in $CDCl_3$ using Bruker spectrometers. Chemical shift values were reported in ppm unit using TMS as internal standard. EIMS spectra were recorded by utilizing a JMS-HX-110 spectrometer.

Procedure for the synthesis of ethyl benzoate (2)

Ethyl benzoate was synthesized by refluxing benzoic acid (1; 0.044mol, 8.0g) with absolute ethanol (32.0mL) in the presence of conc. H_2SO_4 (4.0mL) for 4h in round bottom (RB) flask fitted with condenser. Progress of reaction was

monitored time to time by TLC. After maximum completion of reaction, aq. solution of sodium carbonate was added to reaction mixture for neutralization of remaining organic acid and sulfuric acid. Reaction mixture was poured in 150mL distilled water in a separating funnel. Diethyl ether (40.0mL) was used to extract the product from aqueous layer after vigorous shaking in separating funnel. Two layers were separated by allowing the solution to stand for some time. Upper organic layer containing ethyl benzoate was collected from the neck of separating funnel. Liquid ester of light yellow colour was obtained by evaporating diethyl ether (Somani *et al.*, 2011 and Ingale *et al.*, 2012).

Procedure for the synthesis of benzohydrazide (3)

Benzohydrazide was synthesized by allowing ethyl benzoate (2; 7.0mL, 0.01mol.) to react with hydrazine hydrate (15.0mL) in methanol (15.0mL) along with vigorous stirring at room temperature for 3 hrs in a round bottom flask. The progress of reaction was monitored by thin layer chromatography using UV light as visualizing agent. Precipitates of product were quenched by addition of ice cold distilled water. After filtration and washing, product was dried. Finally recrystallization was brought about by using methanol (Somani *et al.*, 2011 and Ingale *et al.*, 2012).

Procedure for the synthesis of 5-phenyl-1,3,4-Oxadiazol-2-thiol (4)

A mixture of compound 3 (5.0g, 0.029mol), carbon disulphide (1.76mL, 0.029mol) and potassium hydroxide (3.4g, 0.058mol.) in ethanol (40.0mL) was set to reflux for 6 hours in 500mL round bottom flask. Reaction progress was monitored by TLC. On completion, ice cold distilled water was added to the reaction mixture and it was then acidified by dilute HCl to set pH of 2-3 to put out the synthesized product in the form of precipitates which were filtered and washed with distilled water. The title product was finally recrystallized from methanol (Somani *et al.*, 2011 and Ingale *et al.*, 2012).

General procedure for synthesis of N-alkyl/aryl-2-bromoacetamides (5a-p)

The *N*-substituted alkyl/aryl amines (13.0mmol.) were dispersed in 12.0mL distilled water in 100mL round bottom flask followed by addition of 5% Na_2CO_3 solution to adjust the pH 8.0 to 9.0. The reaction mass was stirred for 5 minutes at room temperature and then bromoacetyl bromide (13.0mmol) was added gradually drop wise. After that the flask was shaken vigorously till the appearance of precipitates. The reaction mixture was further stirred for 10min. The completion of reaction was monitored by TLC by comparing the R_f values of reacting amine and formed product. The unreacted acyl halides were washed out as sodium salts of corresponding carboxylic acids. At the end of reaction, the solid mass obtained was filtered, washed with distilled water and dried to yield the corresponding electrophiles, *N*-alkyl/aryl-2-bromoacetamides (Aziz-ur-Rehman *et al.*, 2013a and Aziz-ur-Rehman *et al.*, 2013b).

General procedure for the synthesis of 2-{(5-phenyl-1,3,4-Oxadiazol-2-yl)sulfanyl}-N-substituted acetamides (6a-p)

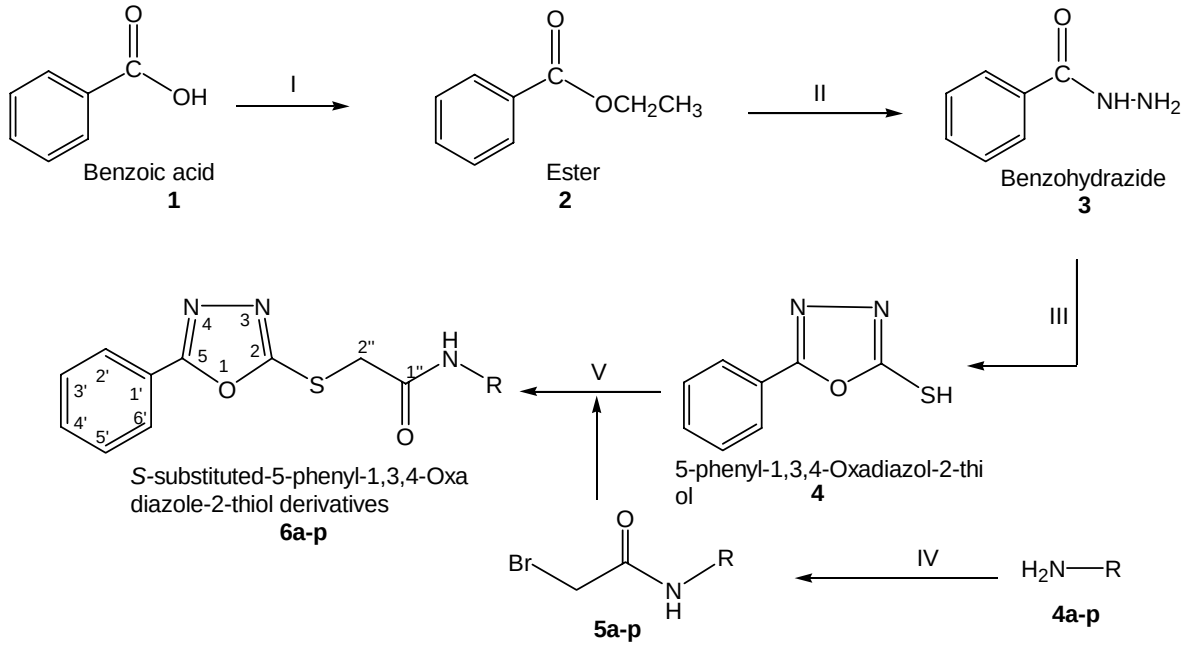
Compound 4 (0.1g, 0.00047mol.) was dissolved in DMF (10-12mL) in 50mL round bottom flask, followed by the addition of NaH (0.002g) acting as a weak base and was stirred at room temperature for 30 minutes. Then it was allowed to react with equimolar ratios of *N*-alkyl/aryl-2-bromoacetamides (5a-p) with constant stirring. The time duration for different *N*-alkyl/aryl-2-bromoacetamides varies from 4-6 hours. Reaction completion was monitored by thin layer chromatography using ethyl acetate & *n*-hexane (30:70) as a mobile phase. Ice cold distilled water was added to the reaction mixture to separate the precipitates. Precipitates so obtained were then filtered, washed and dried for spectral analysis (Aziz-ur-Rehman *et al.*, 2013a and Aziz-ur-Rehman *et al.*, 2013b).

Microbial strains

All the synthesized compounds were tested against microorganisms, including Gram-positive bacteria: *Bacillus subtilis* (*B. subtilis*) & *Staphylococcus aureus* (*S. aureus*) and Gram-negative bacteria: *Pasteurella multocida* (*P. multocida*) & *Escherichia coli* (*E. coli*); and four pathogenic fungi, *Aspergillus flavus* (*A. flavus*), *Aspergillus niger (A. niger), Alternaria alternate (A. alternate)* & *Ganoderma lucidum (G. lucidum).* All bacterial and fungal strains were prevailed from Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan. Purity and identity of the microorganisms were verified by the Department of Microbiology, University of Agriculture, Faisalabad, Pakistan. Bacterial strains were cultured at 37ºC in Nutrient agar (NA) overnight and fungal strains were cultured at 28ºC using Potato dextrose agar (PDA) overnight (Sharma *et al.*, 2001).

Disc diffusion method

Disc diffusion method was used to find out the antimicrobial activity of the synthesized compounds. 100 µL suspension of tested microorganisms was spread on PDA medium for 10^6 spores/mL of fungi and on NA medium for 10^7 colony-forming units/mL of bacteria cells. The filter discs of 6mm diameter were saturated with compound solution and placed on the agar plates inoculated with the tested microorganisms. Filter discs without samples were employed as negative control. Fluconazole (30µg/disk) and Streptomycin (30 µg/dish) were applied as positive reference for bacterial strains and fungal strains, respectively. Plates were placed 4ºC for 2 hours and then incubated at 37ºC for 18 hours for bacterial strains and at 28lºC for 24 hours for fungal strains. Antimicrobial activity was justified after



Scheme 1: Outline for the synthesis of *N*-substituted derivatives of 2-{(5-phenyl-1,3,4-Oxadiazol-2-yl)sulfanyl} acetamides. Reagents & conditions: (I) H_2SO_4/EtOH/refluxing for 4 hours (II) N_2H_4/MeOH/stirring for 3 hours (III) CS_2/KOH/EtOH/refluxing for 6 hours (IV) 2-bromoacetylbromide/H_2O/5% Na_2CO_3 soln./stirring for 1 hour (V) DMF/NaH/stirring for 4-6 hours.

comparison of diameter of growth inhibition zone measured in mm for organisms and the controls (Sharma *et al.*, 2001).

Hemolytic activity

Hemolytic activity was studied by the reported method (Powell *et al.*, 2000). 3.0mL fresh heparin added human blood was obtained from voluntaries after guidance from the Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan. Plasma was disposed off after centrifuging blood at 1000x*g* for 5 min, and blood cells were washed three times by using cold aseptic isosmotic Phosphate-buffered saline (PBS) having pH 7.4. The RBCs for each assay were kept 10^8 cells per mL. 100µL of each compound was poured in each assay. Then the incubation of the assays was carried out at 37°C for 35 min followed by agitation after 10min. All the samples were kept on cold ice for 5 min and then again centrifuged for 5min at 1000x*g*. 100µL was skimmed off from every tube and followed by 10 time dilution with cold PBS. Two controls were employed i.e. PBS as negative control & Triton X-100 (0.1% v/v) as positive control. The % RBCs lysis was computed for every sample by noting the absorbance at 576 nm using UV spectrophotometer.

RESULTS

The *N*-alkyl/aryl-2-bromoacetamides were synthesized according to the protocol sketched in scheme 1 and different alkyl/aryl groups are mentioned in table 1. The general reaction conditions and the structure characterization are illustrated in experimental section. The aim of the described research work was to synthesize a new series of biological active molecules, which may be helpful in drug development program. The structure of all the synthesized compounds were elucidated by spectral (IR, H-NMR, EIMS) data. All spectral data supported the assumed structures assigned to the compounds.

Spectral characterization data

2-{(5-phenyl-1,3,4-Oxadiazol-2-yl)sulfanyl}-N-(2,3-dimethylphenyl)acetamide (6a)

Light Yellow amorphous solid; Yield: 81%; MP: 138-140°C; Molecular Formula: $C_{18}H_{17}N_3O_2S$; Molecular Weight: 339; IR (KBr, cm^{-1}) ν_{max}: 3335(N-H stretching), 3083(C-H str. of aromatic ring), 1675(C=N str. of Oxadiazole ring), 1650(C=O str.), 1557(C=C aromatic str.), 1278(C-O-C bond str.), 631(C-S bond str.); ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 8.80(s,1H,-NH), 7.98(d, *J*=8.0 Hz, 2H, H-2' & H-6'), 7.51-7.49(m, 3H, H-3' to H-5'), 7.10(d, *J*=7.6 Hz 1H, H-6'''), 7.07(t, *J*=7.6 Hz, 1H, H-5'''), 6.97(d, *J*=7.2 Hz 1H, H-4'''), 4.06(s, 2H, CH_2-2"), 2.26(s, 3H, CH_3-2'''), 2.15(s,3H, CH_3-3'''); EIMS (*m/z*): 339(74%) $[M]^+$, 219(34%), 194(33%), 191(29%), 148 (17%), 145(19%), 120(55%), 105(100%), 90(19%), 77(18%).

2-{(5-phenyl-1,3,4-Oxadiazol-2-yl)sulfanyl}-N-(2,4-dimethylphenyl)acetamide (6b)

Yellowish white amorphous solid; Yield: 83%; M.P: 134-136°C; Molecular Formula: $C_{18}H_{17}N_3O_2S$; Molecular Weight: 339; IR (KBr, cm^{-1}) ν_{max}: 3334 (N-H stretching),

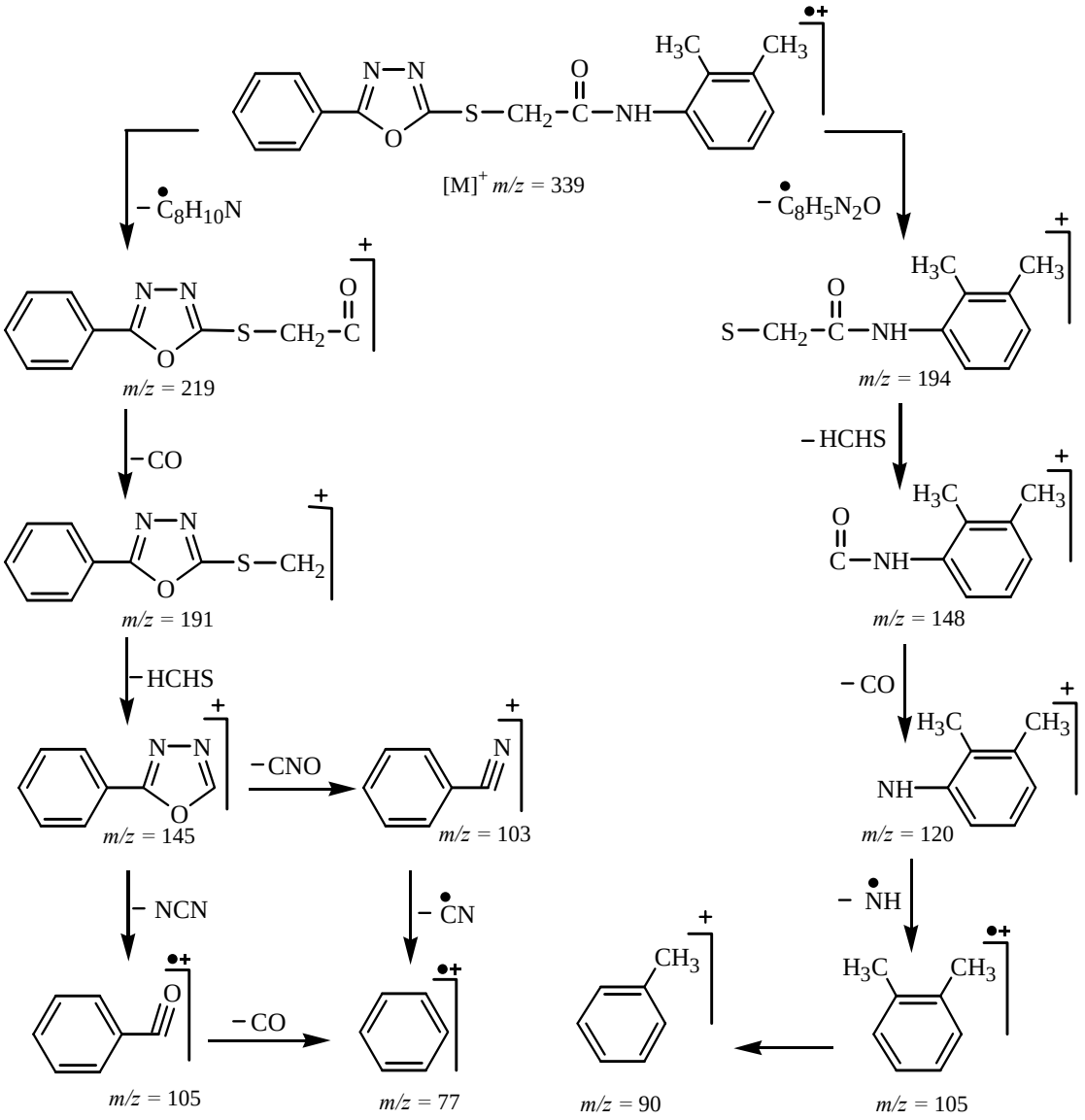


Fig. 1: Mass fragmentation pattern of 2-{(5-phenyl)-1,3,4-Oxadiazol-2-yl)sulfanyl}-*N*-(2,3-dimethylphenyl) acetamide (6a)

3085(C-H str. of aromatic ring), 1669(C=N str. of Oxadiazole ring), 1651(C=O str.), 1561 (C=C aromatic str.), 1282 (C-O-C bond str.), 633 (C-S bond str.); ^{1}H-NMR (400 MHz, $CDCl_3$, δ /ppm): 8.76(s, 1H, -NH), 7.97 (d, 2H, *J*=8.0 Hz, H-2' & H-6'), 7.68(d, *J*=7.6 Hz, 1H, H-6'"), 7.51-7.49(m, 3H, H-3' to H-5'), 6.98(d, *J*=7.6 Hz, 1H, H-5'"), 6.96(s, 1H, H-3'"), 4.05(s, 2H, CH_2-2"), 2.25(s, 3H, CH_3-2'"), 2.22(s, 3H, CH_3-4'"); EIMS(*m/z*): 339 (74%) $[M]^+$, 219(34%), 194(33%), 191(29%), 148(17%), 145(19%), 120(55%), 105(100%), 90(19%), 77(18%).

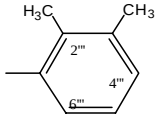
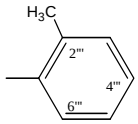
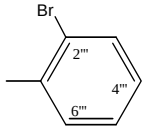
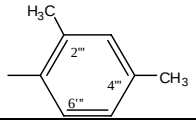
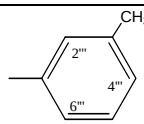
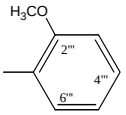
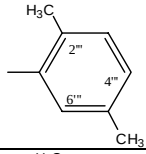
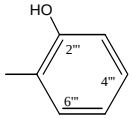
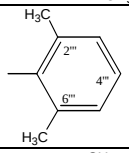
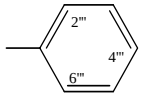
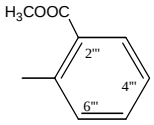
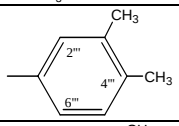
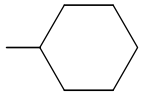
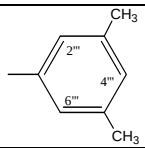
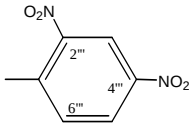
2-{(5-phenyl-1,3,4-Oxadiazol-2-yl)sulfanyl}-N-(2,5-dimethylphenyl)acetamide (6c)

Off white amorphous solid; Yield: 87%; M.P: 144-146^{o}C; Molecular Formula: $C_{18}H_{17}N_3O_2S$; Molecular Weight: 339; IR (KBr, cm^{-1}) ν_{max}: 3311 (N-H stretching), 3061(C-H str. of aromatic ring), 1675(C=N str. of Oxadiazole ring), 1639(C=O str.), 1551(C=C aromatic str.), 12631 (C-O-C bond str.), 641(C-S bond str.); ^{1}H-NMR(400 MHz, $CDCl_3$, δ/ ppm): 8.70(s, 1H, -NH), 7.99(d, *J*=8.0 Hz, 2H, H-2' & H-6'), 7.79(s, 1H, H-6'"), 7.51-7.49(m, 3H, H-3' to H-5'), 7.12(d, *J*=7.6 Hz, 1H, H-3'"), 6.85(d, *J*=7.6 Hz, 1H, H-4'"), 4.03(s, 2H, CH_2-2"), 2.30(s, 3H, CH_3-2'"), 2.21(s, 3H, CH_3-2'"); EIMS (*m/z*): 339(74%) $[M]^+$, 219(34%), 194(33%), 191(29%), 148(17%), 145(19%), 120(55%), 105(100%), 90(19%), 77(18%).

2-{(5-phenyl-1,3,4-Oxadiazol-2-yl)sulfanyl}-N-(2,6-dimethylphenyl)acetamide (6d)

Off White amorphous solid; Yield: 93%; M.P: 167-169^{o}C; Molecular Formula: $C_{18}H_{17}N_3O_2S$; Molecular Weight: 339; IR (KBr, cm^{-1}) ν_{max}: 3311 (N-H stretching), 3061 (C-

Table 1: Different alkyl/aryl groups

C. No	-R	C. No	-R	C. No	-R
6a		6g		6m	
6b		6h		6n	
6c		6i		6o	
6d		6j		6p	
6e		6k			
6f		6l			

H str. of aromatic ring), 1674 (C=N str. of Oxadiazole ring), 1653(C=O str.), 1541(C=C aromatic str.), 1271(C-O-C bond str.), 643(C-S bond str.); ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 8.05(s, 1H,-NH), 7.98(d, *J*=8.0 Hz, 2H, H-2' & H-6'), 7.51-7.49(m, 3H, H-3' to H-5'), 7.06(m, 3H, H-3''' to H-5'''), 4.08(s, 2H, CH_2-2''), 2.16(s, 6H, CH_3-2''' & CH_3-6'''); EIMS (*m/z*): 339(74%) $[M]^+$, 219(34%), 194 (33%), 191(29%), 148(17%), 145(19%), 120(55%), 105 (100%), 90(19%), 77(18%).

2-{(5-phenyl-1,3,4-Oxadiazol-2-yl)sulfanyl}-N-(3,4-dimethylphenyl)acetamide (6e)

Dull brown amorphous solid; Yield: 93%; M.P: 144-146^{o}C; Molecular Formula: $C_{18}H_{17}N_3O_2S$; Molecular Weight: 339;(KBr, cm^{-1}) ν_{max}: 3313(N-H stretching), 3075 (C-H str. of aromatic ring), 1675(C=N str. of Oxadiazole ring), 1645 (C=O str.), 1563(C=C aromatic str.), 1275 (C-O-C bond str.), 639(C-S bond str.); ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 9.0(s, 1H, -NH), 7.98(d, *J*=8.0 Hz, 2H, H-2' & H-6'), 7.52-7.49(m, 3H, H-3' to H-5'), 7.29(d, *J*=8.0 Hz, 1H, H-6'''), 7.26(s, 1H, H-2'''), 7.03(d, *J*=8.0 Hz, 1H, H-5'''), 3.99(s, 2H, CH_2-2''), 2.21(s, 3H, CH_3-3'''), 2.18(s, 3H, CH_3-4'''); EIMS (*m/z*): 339(74%) $[M]^+$, 219(34%), 194(33%), 191(29%), 148(17%), 145(19%), 120(55%), 105(100%), 90 (19%), 77(18%).

2-{(5-phenyl-1,3,4-Oxadiazol-2-yl)sulfanyl}-N-(3,5-dimethylphenyl)acetamide (6f)

White amorphous solid; Yield: 89%; MP: 131-133^{o}C; Molecular Formula: $C_{18}H_{17}N_3O_2S$; Molecular Weight: 339; IR (KBr, cm^{-1}) ν_{max}: 3313(N-H stretching), 3063(C-H str. of aromatic ring), 1661 (C=N str. of Oxadiazole ring), 1651(C=O str.), 1557(C=C aromatic str.), 1287(C-O-C bond str.), 633(C-S bond str.); ^{1}H-NMR(400 MHz, $CDCl_3$, δ/ppm): 9.03(s, 1H, -NH), 7.98(d, *J*=8.0 Hz, 2H, H-2' & H-6'), 7.52-7.49(m, 3H, H-3' to H-5'), 7.16(s, 2H, H-2''' & H-6'''), 6.73(s, 1H, H-4'''), 3.99(s, 2H, CH_2-2''), 2.26(s, 6H, CH_3-3''' & CH_3-5'''); EIMS (*m/z*): 339(74%) $[M]^+$, 219(34%), 194(33%), 191(29%), 148(17%), 145(19%), 120(55%), 105(100%), 90(19%), 77(18%).

2-{(5-phenyl-1,3,4-Oxadiazol-2-yl)sulfanyl}-N-(2-methylphenyl)acetamide (6g)

Buff color amorphous solid; Yield: 88%; M.P: 203^{o}C; Molecular Formula: $C_{17}H_{15}N_3O_2S$; Molecular Weight: 325; IR (KBr, cm^{-1}) ν_{max}: 3344 (N-H stretching), 3053(C-H str. of aromatic ring), 1663 (C=N str. of Oxadiazole ring), 1657(C=O str.), 1575(C=C aromatic str.), 1288(C-O-C bond str.), 639(C-S bond str.); ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 9.13(s, 1H, -NH), 7.92(d, *J*=7.6 Hz, 2H, H-2' & H-6'), 7.63(d, *J*=7.6 Hz, 1H, H-6'''), 7.56-7.49(m, 3H, H-3' to H-5'), 7.36(d, *J*=7.6 Hz, 1H, H-3'''), 7.22(br.t, *J*=7.6, 1.2 Hz, 1H, H-5'''), 7.16(ddd, *J*=6.6, 1.0 Hz, 1H,

H-4'''), 4.31(s, 2H, CH_2-2''), 2.38(s, 3H, CH_3-2'''); EIMS (*m/z*): 325(72%) $[M]^+$, 219(10%), 192(33%), 222(34%), 193(36%), 145(19%), 118(14%), 120(13%), 107(35%), 105(100%), 104(90%), 103(29%), 91(14%), 77(13%), 65(5%).

2-{(5-phenyl-1,3,4-Oxadiazol-2-yl)sulfanyl}-N-(3-methylphenyl)acetamide (6h)

Light grey amorphous solid; Yield: 82%; M.P: 101-103°C; Molecular Formula: $C_{17}H_{15}N_3O_2S$; Molecular Weight: 325; IR (KBr, cm^{-1}) ν_{max}: 3341(N-H stretching), 3063(C-H str. of aromatic ring), 1673(C=N str. of Oxadiazole ring), 1667(C=O str.), 1565(C=C aromatic str.), 1278(C-O-C bond str.), 633(C-S bond str.); ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 9.13(s, 1H, -NH), 7.98(d, *J*=8.0 Hz, 2H, H-2' & H-6'), 7.54-7.47(m, 3H, H-3' to H-5'), 7.36(s, 1H, H-2'''), 7.32(d, *J*=8.0 Hz, 1H, H-6'''), 7.19 (t, *J*=7.6 Hz, 1H, H-5'''), 6.89(d, *J*=7.6 Hz, 1H, H-4'''), 4.03(s, 2H, CH_2-2''), 2.31(s, 3H, CH_3-3'''); EIMS (*m/z*): 325(74%) $[M]^+$, 219(11%), 192(34%), 222(35%), 193(38%), 145(20%), 118(15%), 120(13%), 107(37%), 105(100%), 104(92%), 103(30%), 91(14%), 77(12%), 65 (7%).

2-{(5-phenyl-1,3,4-Oxadiazol-2-yl)sulfanyl}-N-(4-methylphenyl)acetamide (6i)

Off White amorphous solid; Yield: 80%; M.P: 139-141°C; Molecular Formula: $C_{17}H_{15}N_3O_2S$; Molecular Weight: 325; IR (KBr, cm^{-1}) ν_{max}: 3341 (N-H stretching), 3059(C-H str. of aromatic ring), 1639(C=N str. of Oxadiazole ring), 1627(C=O str.), 1533(C=C aromatic str.), 1255(C-O-C bond str.), 623(C-S bond str.); ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 9.11(s, 1H, -NH), 7.98(d, *J*=8.0 Hz, 2H, H-2' & H-6'), 7.51-7.49(m, 3H, H-3' to H-5'), 7.40(d, *J*=8.4 Hz, 2H, H-2''' & H-6'''), 7.08(d, *J*=8.0 Hz, 2H, H-3''' & H-5'''), 3.99(s, 2H, CH_2-2''), 2.28(s, 3H, CH_3-4'''); EIMS (*m/z*): 325(71%) $[M]^+$, 219(10%), 192(32%), 222(34%), 193(37%), 145(20%), 118(14%), 120(12%), 107(36%), 105(100%), 104(91%), 103(30%), 91(13%), 77(12%), 65(6%).

2-{(5-phenyl-1,3,4-Oxadiazol-2-yl)sulfanyl}-N-phenylacetamide (6j)

Off white amorphous solid; Yield: 85%; M.P: 124-126°C; Molecular Formula: $C_{16}H_{15}N_3O_2S$; Molecular Weight: 325; IR (KBr, cm^{-1}) ν_{max}: 3351(N-H stretching), 3045(C-H str. of aromatic ring), 1679(C=N str. of Oxadiazole ring), 1663 (C=O str.), 1575 (C=C aromatic str.), 1277 (C-O-C bond str.), 649(C-S bond str.); ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 10.01(s, 1H,-NH), 7.96(d, *J*=8.0 Hz, 2H, H-2' & H-6'), 7.51-7.48(m, 3H, H-3' to H-5'), 7.38-7.24 (m, 5H, H-2''' to H-6''') 4.46(s, 2H, CH_2-2''); EIMS (*m/z*): 345(79%) $[M]^+$, 219(14%), 192(30%), 145(20%), 120 (13%), 105(100%), 103(33%), 92(14%), 77(12%).

2-{(5-phenyl-1,3,4-Oxadiazol-2-yl)sulfanyl}-N-cyclohexylacetamide (6k)

Light yellow amorphous solid; Yield: 84%; M.P: 139-141°C; Molecular Formula: $C_{16}H_{19}N_3O_2S$; Molecular Weight: 317; IR (KBr, cm^{-1}) ν_{max}: 3343(N-H stretching), 3035 (C-H str. of aromatic ring), 1665(C=N str. of Oxadiazole ring), 1655(C=O str.), 1583 (C=C aromatic str.), 1261(C-O-C bond str.), 653(C-S bond str.); ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm); 10.32(s, 1H, -NH), 7.97(d, *J*=8.0 Hz, 2H, H-2' & H-6'), 7.53-7.49(m, 3H, H-3' to H-5'), 3.84(s, 2H, CH_2-2''), 3.76-3.74(m, 1H, H-1'''), 1.86-1.14(m, 10H, CH_2-2''' to CH_2-6'''); EIMS (*m/z*): 317(71%) $[M]^+$, 220(10%), 192(32%), 145(27%), 118(13%), 105 (100%), 103(30%), 98(12%), 77(12%).

2-{(5-phenyl-1,3,4-Oxadiazol-2-yl)sulfanyl}-N-(2,4-dinitrophenyl)acetamide (6l)

Light brown amorphous solid; Yield: 87%; M.P: 159-161°C; Molecular Formula: $C_{16}H_{11}N_5O_6S$; Molecular Weight: 356; IR (KBr, cm^{-1}) ν_{max}: 3359(N-H stretching), 3041(C-H str. of aromatic ring), 1659(C=N str. of Oxadiazole ring), 1653(C=O str.), 1569(C=C aromatic str.), 1277(C-O-C bond str.), 637(C-S bond str.); ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm); 11.46(s, 1H, -NH), 9.08 (d, *J*=9.2 Hz, 1H, H-3'''), 9.03(d, *J*=9.2 Hz, 1H, H-6'''), 8.48(dd, *J*=8.4 & 2.4 Hz, 1H, H-5'''), 7.97(d, *J*=8.0 Hz, 2H, H-2' & H-6'), 7.53-7.48(m, 3H, H-3' to H-5'), 4.24 (s, 2H, CH_2-2''); EIMS (*m/z*): 401(28.6%) $[M]^+$, 355(10.7%), 219(5.3%), 192(100%), 193(15.5%), 145(29.9%), 118 (7%), 105(82.7%), 103(26.9%), 89 (9.5%).

2-{(5-phenyl-1,3,4-Oxadiazol-2-yl)sulfanyl}-N-(2-bromophenyl)acetamide (6m)

Off white amorphous solid; Yield: 90%; M.P: 189-191°C; Molecular Formula: $C_{16}H_{12}BrN_3O_2S$; Molecular Weight: 388; IR (KBr, cm^{-1}) ν_{max}: 3335(N-H stretching), 3071(C-H str. of aromatic ring), 1657(C=N str. of Oxadiazole ring), 1653(C=O str.), 1555(C=C aromatic str.), 1279(C-O-C bond str.), 644 (C-S bond str.), 569(C-Br bond str.); ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 9.40(s, 1H, -NH), 7.99(d, *J*=8.4 Hz, 2H, H-2' & H-6'), 7.53-7.49(m, 3H, H-3' to H-5'), 7.46-7.38(m, 4H, H-3''' to H-6'''), 3.98(s, 2H, CH_2-2''); EIMS (*m/z*): 389(79%) $[M]^+$, 310(13%), 219 (22%), 192(100%), 145(21%), 105(86%), 103(39%), 77 (10.3%).

2-{(5-phenyl-1,3,4-Oxadiazol-2-yl)sulfanyl}-N-(2-methoxyphenyl)acetamide (6n)

Off white amorphous solid; Yield: 80%; M.P: 119-121°C; Molecular Formula: $C_{17}H_{15}N_3O_3S$; Molecular Weight: 331; IR (KBr, cm^{-1}) ν_{max}: 3329 (N-H stretching), 3063 (C-H str. of aromatic ring), 1653 (C=N str. of Oxadiazole ring), 1645 (C=O str.), 1547 (C=C aromatic str.), 1277 (C-O-C bond str.), 646 (C-S bond str.); ^{1}H-NMR: 9.18 (s, 1H, -NH), 8.28 (d, *J*=7.6 Hz, 1H, H-6'''), 7.98 (d, *J*=8.0 Hz, 2H, H-2' & H-6'), 7.52-7.48 (m, 3H, H-3' to H-5'), 7.01 (t, *J*=7.2 Hz, 1H, H-5''') 6.92(t, *J*=7.6 Hz, 1H, H-4'''), 6.82 (d, *J*=8.0 Hz, 1H, H-3'''), 4.24 (s, 2H, CH_2-2''), 3.85 (s, 3H, CH_3-2'''); EIMS (*m/z*): 341 (73%) $[M]^+$, 310 (16%), 219 (12%), 193 (47.7%), 192 (45%), 145 (24%), 123 (90%), 108 (41), 105 (100%), 103 (44%), 92 (21%), 77 (13%).

2-{(5-phenyl-1,3,4-Oxadiazol-2-yl)sulfanyl}-N-(2-hydroxyphenyl)acetamide (6o)

Red amorphous solid; Yield: 89%; M.P: 153-155°C; Molecular Formula: $C_{17}H_{15}N_3O_3S$; Molecular Weight: 327; IR (KBr, cm^{-1}) ν_{max}: 3355 (N-H stretching), 3047 (C-H str. of aromatic ring), 1675 (C=N str. of Oxadiazole ring), 1661 (C=O str.), 1589 (C=C aromatic str.), 1280 (C-O-C bond str.), 647 (C-S bond str.); ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 10.03 (s, 1H), 7.97 (d, *J*=8.1 Hz, 2H, H-2' & H-6'), 7.56-7.48 (m, 3H, H-3' to H-5'), 7.38 (d, *J*=7.5 Hz, 1H, H-6''') 7.22 (dt, *J*=7.5, 0.9 Hz, 1H, H-5''') 7.18 (dt, *J*=7.5, 0.9 Hz, 1H, H-4'''), 6.82 (d, *J*=8.4 Hz, 1H, H-3'''), 4.84 (s, 2H, CH_2-2''); EIMS (*m/z*): 327 (73%) $[M]^+$, 220 (9%), 192 (36%), 145 (29%), 108 (19%), 105 (100%), 103 (29%), 77 (12%).

2-{(5-phenyl-1,3,4-Oxadiazol-2-yl)sulfanyl}-N-(2-(methoxycarbonyl)phenyl)acetamide (6p)

White amorphous solid; Yield: 90%; M.P: 176-178°C; Molecular Formula: $C_{18}H_{14}ClN_3O_4S$; Molecular Weight: 369; IR (KBr, cm^{-1}) ν_{max}: 3313 (N-H stretching), 3066 (C-H str. of aromatic ring), 1674 (C=N str. of Oxadiazole ring), 1643 (C=O str.), 1593 (C=C aromatic str.), 1267 (C-O-C bond str.), 673 (C-Cl bond str.), 659 (C-S bond str.); ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm); 11.64 (s, 1H, -NH), 8.66 (d, *J*=8.4 Hz, 1H, H-6'''), 7.99 (dd, *J*=8.0, 1.6 Hz, 1H, H-3'''), 7.97 (d, *J*=8.0 Hz, 2H, H-3' & H-5'), 7.53 (dt, *J*= 7.6, 1.6 Hz, 1H, H-5'''), 7.56-7.48 (m, 3H, H-3' to H-5'), 7.11 (t, *J*=7.6 Hz, 1H, H-4'''), 4.22 (s, 2H, CH_2-2''), 3.83 (s, 3H, CH_3OOC-3'''); EIMS (*m/z*): 369 (80%) $[M]^+$, 310 (14%), 219 (23%), 192 (100%), 193 (48.4%), 145 (21.7 %), 151 (40.3%), 105 (89%), 103 (40.3%), 119 (32%), 111 (26.2%), 90 (27.4%), 77 (10%).

DISCUSSION

Chemistry

In this research work, *N*-substituted derivatives of 2-{(5-phenyl-1,3,4-Oxadiazol-2-yl)sulfanyl}acetamides were synthesized in a series of steps and all were screened against gram negative and gram positive bacteria. First ethyl benzoate (2) was prepared by refluxing benzoic acid (1) with ethyl alcohol in the presence of concentrated sulfuric acid for 4 hours. After maximum completion of reaction, conc. solution of base was added to the reaction mixture for neutralization of excess organic acid and sulfuric acid to their respective salts. By using solvent extraction method we get ethyl benzoate in organic layer and both the salts were washed by aqueous layer. Next step is the synthesis of benzohydrazide (3) by allowing ethyl benzoate to react with 80% hydrazine hydrate in methanol at room temperature along with vigorous stirring for 3 hrs. The solid separated out was filtered and washed with *n*-hexane. Further the parent compound 5-phenyl-1,3,4-Oxadiazol-2-thiol (4) was synthesized by refluxing compound 3, with carbon disulphide and potassium hydroxide in ethanol for 6 hours. On completion of reaction, ice cold distilled water was added to the reaction mixture and it was then acidified to set the pH around 2-3 to put out the synthesized product in the form of precipitates which were filtered and washed with distilled water. The product was finally recrystallized from methanol. Last step is the synthesis of title compounds 6a-p, which was afforded by stirring compound 4 with *N*-substituted alkyl/aralkyl/aryl 2-bromoacetamides (5a-p) for 4-6 hours in the presence of DMF and NaH. Ice cold distilled water was added to the reaction mixture to separate the precipitates. Precipitates so obtained were then filtered, washed and dried for spectral analysis.

Compound 6a was obtained as yellowish white amorphous solid with melting point of 138-140°C. The molecular formula $C_{18}H_{17}N_3O_2S$ was established by counting the number of proton in ^{1}HNMR spectrum and from EI-MS which showed $[M]^+$ peak at *m/z* 339. In the EIMS spectrum the two distinct peak were appeared at *m/z* 120 and 105, which were assigned to 2,3-dimethylaniline and carbonyl benzene cation fragments respectively. In IR spectrum, characteristic peaks were appeared at 3335cm^{-1} (N-H stretching), 3083cm^{-1} (C-H str. of aromatic ring), 1675cm^{-1} (C=N str. of Oxadiazole ring), 1650cm^{-1} (C=O str.), 1557cm^{-1} (C=C aromatic str.), 1278cm^{-1} (C-O-C bond str.) and 631 cm^{-1} (C-S bond str.) confirming the presence of Oxadiazole ring and amidic carbonyl group in the molecule. In ^{1}H-NMR spectrum chemical shift values of all protons were seen according to the expectations. In the aromatic region ^{1}H-NMR spectrum, the signals appeared at δ 7.98 (d, *J*=8.0 Hz, 2H, H-2' & H-6') and 7.51-7.49 (m, 3H, H-3' to H-6') were assigned to the phenyl ring. The signals resonated at δ 7.10 (d, *J*=7.6 Hz 1H, H-6'''), 7.07 (t, *J*=7.6 Hz, 1H, H-5''') and 6.97 (d, *J*=7.2 Hz 1H, H-4''') were assigned to the trisubstituted aromatic ring. In the aliphatic region of ^{1}H-NMR spectrum, signals appeared at δ 2.26 (s, 3H, CH_3-2'''), 2.15 (s, 3H, CH_3-3''') and 4.06 (s, 2H, H-2'') were assigned to two methyl and one methylene group in the molecule. The signal at δ 8.80ppm was assigned to N-H proton of amidic group. On the basis of above structural analysis, the structure was assigned as 2{(5-phenyl-1,3,4-Oxadiazol-2-yl)sulfanyl}-*N*-(2,3-dimethylphenyl) acetamide.

Antimicrobial and hemolytic activity

All the synthesized compounds (6a-p) were screened for their antimicrobial activity against selected panel of bacterial and fungal strains. Among the synthesized compounds, 6f, 6i, 6m & 6n showed selective antibacterial and antifungal activity; 6a, 6d & 6j showed antibacterial activity only; and 6b & 6k were inactive against all the microbial strains as evident from table 2. All synthesized compounds were more active against gram –ve bacteria as compared to gram +ve bacteria. The Streptomycin and Fluconazole were used as reference

Table 2: Antibacterial and antifungal activity of all the synthesized derivatives

Compound	Antibacterial activity				Antifungal activity				Hemolytic activity (Mean) % ± S.D
	Zone of inhibition (mm)				Zone of inhibition (mm)				
	Staphylococcus aureus	*Bacillus subtilis*	*Pasturella multocida*	*Escherichia coli*	*Aspergillus niger*	*Aspergillus flavus*	*Ganodea lucidum*	*Alternaria alternate*	
6a	21	22	-	-	-	-	-	-	9.09
6b	-	-	-	-	-	-	-	-	6.51
6c	23	21	27	29	23	21	27	29	6.51
6d	23	22	20	21	-	-	-	-	10.88
6e	24	23	28	29	22	21	24	25	13.72
6f	29	25	-	-	29	27	-	-	16.77
6g	20	21	21	22	19	19	20	21	6.33
6h	27	28	33	34	28	28	32	33	13.46
6i	-	-	30	28	-	-	31	29	5.58
6j	24	22	21	22	-	-	-	-	13.42
6k	-	-	-	-	-	-	-	-	4.12
6l	23	22	27	28	22	21	27	26	6.02
6m	22	23	-	-	-	-	-	-	31.18
6n	20	21	-	-	-	-	-	-	4.88
6o	24	22	22	23	21	20	22	20	6.33
6p	26	25	22	23	25	26	21	22	8.21
Streptomycin	31	32	28	29					
Fluconazole					32	34	32	33	
PBS									0.00±0.0
Triton (toxicity)									100±0.0

standard for all bacterial and fungal strains respectively. Compound 6h was found most active against all bacterial strains i.e. *Bacillus subtilis, Escherichia coli, Pasturella multocida & Staphylococcus aureus* and the all fungal strains i.e. *Aspergillus niger, Aspergillus flavus, Alternaria alternate* & *Ganodea lucidum* in comparison to the other members of its series and that of the reference standards. It may be stated that introduction of alkyl group at *meta* position of substituted phenyl ring is favorable for antimicrobial activity. The highest hemolytic activity was shown by 6n (31.18%) but lower than the positive control (Triton-X-100). The lowest activity was shown by 6k and 6n (4.12% and 4.88% respectively) but higher than the negative controls (PBS). On the basis of all these results we may assume that the synthesized oxadiazole derivatives may be suitable for further improvement to address different targets.

CONCLUSION

The modern organic synthesis deals with the development of new efficient and effective drug candidates with least toxicity. New 2-{(5-phenyl-1,3,4-Oxadiazol-2-yl) sulfanyl}-*N*-sub stitutedacetamides have been developed to inaugurate new drug molecules having anti-microbial potential with least toxicity. The most of synthesized compounds exhibited significant activity. The compound, 6h was the most active against the selected panel of microbes. This series showed less toxicity and may be considered for further biological screening and application trial except 6m, possessing higher cytotoxicity.

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