N-Substituted Derivatives of 5-(4-chlorophenyl)-1,3,4-Oxadiazol-2-yl-2-sulfanyl acetamide as Valuable Bioactive Compounds

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Summary: In the presented work, a new series of *N*-substituted derivatives of 5-(4-chlorophenyl)-1,3,4-Oxadiazol-2-yl-2-sulfanyl acetamide has been synthesized. The synthesis was carried out by converting 4-chlorobenzoic acid **(1)** into ethyl 4-chlorobenzoate (**2**), 4-chlorobenzohydrazide (**3**) and then 5-(4-chlorophenyl)-1,3,4-Oxadiazol-2-thiol **(4)** respectively. The target compounds **6a-o** were synthesized by reaction of compound **4** with equimolar ratios of different *N*-alkyl/aryl substituted 2-bromoacetamide **(5a-o)** in the presence of DMF and sodium hydride (NaH**)**. The structure elucidation of all the synthesized compounds was carried out by EI-MS, IR and ^{1}H-NMR. The compounds were also screened for antimicrobial & hemolytic activity and most of them were found active against the selected microbial species at variable extent relative to reference standards. But **6f** and **6o** were the active against the selected panel of microbes and former was most potent one. This series showed less toxicity and may consider for further biological screening and application trial except **6g** and **6j**, possessing higher cytotoxicity.

Keywords: 4-Chlorobenzoic acid, 1,3,4-Oxadiazoles, antimicrobial activity, hemolytic activity,^{1}H-NMR and EI-MS.

Introduction

Synthetic organic chemists have focused considerable attention on 1,3,4-Oxadiazoles and related compounds due to their diverse pharmacological and antimicrobial applications [1-3]. Some of these compounds have shown significant antimicrobial, anti-tumor, antimalarial, anticancer, anti-HIV, anti-inflammatory, anticonvulsant, anti-tuberculosis, anti-analgesic, anti-mycobacterial and antidepressant activities [4-8].

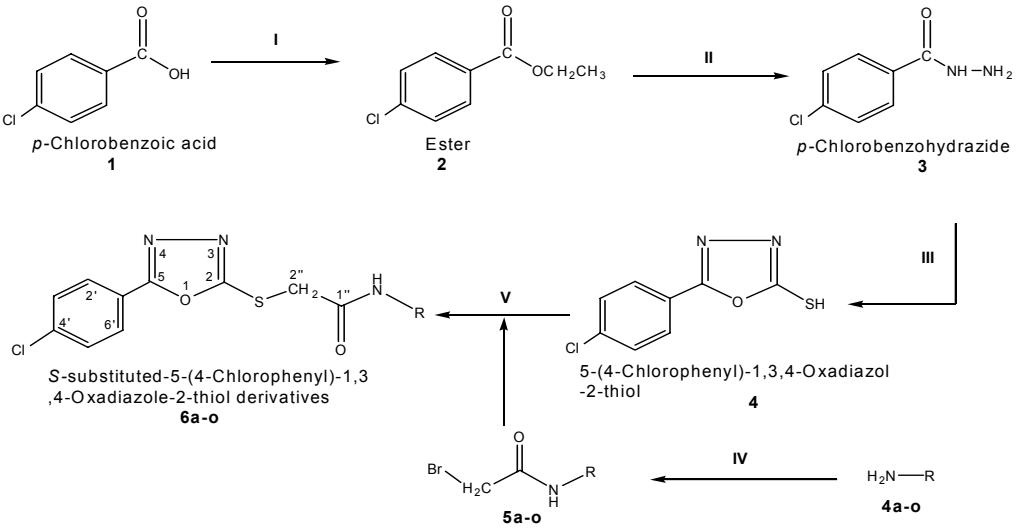
Due to increase in resistance of microorganisms against the drugs in use, it is the need of hour to design new compounds which can work more efficiently and effectively but with less toxicity. Due to medicinal importance and in continuation of our previous research work on 1,3,4-Oxadiazoles [9-12], the synthesis of 2,5-disubstituted-1,3,4-Oxadiazole derivatives with an objective to detect the biological activities. We have synthesized a new series of *N*-alkyl/aryl substituted 5-(4-chlorophenyl)-1,3,4-Oxadiazol-2-yl-2-sulfanyl acetamide compounds. The synthesis was carried out through the intermolecular cyclization of 4-chlorobenzohydrazide to the 5-(4-chlorophenyl)-1,3,4-oxadiazol-2-thiols and finally to *N*-substituted-5-(4-chlorophenyl)-1,3,4-Oxadiazol-2-ylthio)acetamide products. Further, the synthesized compounds were screened for the antimicrobial and hemolytic activities and found that the most of compounds were exhibited significant activity against the selected microbial species relative to reference standard drugs.

Results and Discussion

The substituted 1,3,4-Oxadiazole derivatives **6a-o** were synthesized according to the protocol sketched in scheme-1 and different *N*-substituted alkyl/aryl groups are mentioned in Table-1. The general reaction conditions and the structure characterization are described in experimental section.

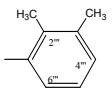
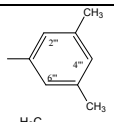
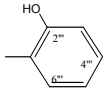
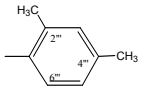
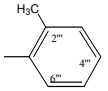
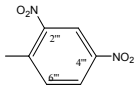
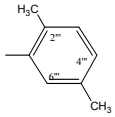
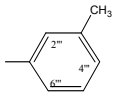
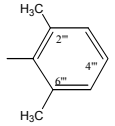
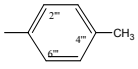
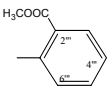
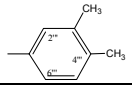
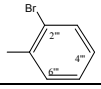
The aim of the present research work was to synthesize new compounds that have great potential as antimicrobial agent. The need of hour is to introduce pharmacologically active molecules to help pharmacy against the increasing resistance of microorganisms for existing drugs. In the described research work, *N*-alkyl/aryl substituted-5-(4-chlorophenyl)-1,3,4-Oxadiazol-2-yl-2-sulfanyl acetamide derivatives (**6a-o**) were synthesized in a series of steps and all were screened for antimicrobial and hemolytic activities. The starting compound, 4-chlorobenzoic acid (**1**) was converted into ethyl 4-chlorobenzoate (**2**) by refluxing it with ethanol in the presence of concentrated sulfuric acid as a catalyst. Due to reversibility of esterification, 100% completion is not achieved. So after maximum completion, the compound **2** was collected by solvent extraction after the addition of distilled water & sodium carbonate solution. Next step comprises the

synthesis of 4-chlorobenzohydrazide (**3**) from compound **2** by stirring with 80% hydrated hydrazine in methanol for 2-4 hrs. Compound **3** was then cyclized intermolecularly to afford 5-(4-chlorophenyl)-1,3,4-oxadiazol-2-thiol (**4**) through reflux in ethanol after the addition of CS_2/KOH for 3-6 hrs. The product **4** was isolated after acidification of the reaction mixture. The last step is the synthesis of **6a-o** which was afforded through the reaction of compound **4** with different electrophiles **5a-o** in the presence of DMF and NaH. Reactions of the electrophiles were completed within 2-3 hours by stirring at RT. The products were isolated after adding cold distilled water *via* filtration or solvent extraction. The structures of the synthesized compounds **2**, **3**, **4** and *N*-substituted derivatives, **6a-o** were corroborated by spectral data as presented in experimental section.



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

Table-1: Different *N*-substituted aryl/aralky groups.

Compd	R	Compd	R	Compd	R
6a		**6f**		**6k**	
6b		**6g**		**6l**	
6c		**6h**		**6m**	
6d		**6i**		**6n**	
6e		**6j**		**6o**	

Compound **6a** was obtained as pink colour amorphous solid with melting point of 160-162 ^{o}C and molecular formula of $C_{18}H_{16}ClN_3O_2S$ was established by HR-MS showing $[M]^+$ at *m/z* 373.834 (cald for $C_{18}H_{16}ClN_3O_2S$, 373.3294). The EI-MS gave two distinct peaks at *m/z* 120 and 137 which were attributed to the 2,3-dimethylaniline ion and 4-chlorophenyl cyanide fragment respectively. In IR spectrum, characteristic peaks appeared at 3337 (N-H bond), 3085 (C-H str. of aromatic ring), 1677 (C=N str. of Oxadiazole ring), 1652 (C=O str.), 1561 (C=C aromatic str.), 1280 (C-O-C bond str.), 683 (C-Cl bond str.), 633 (C-S bond str.) confirming the presence of amidic carbonyl group and Oxadiazole ring. In ^{1}H-NMR spectrum, signals of aromatic proton appearing at δ 7.91 (d, J = 8.4 Hz, 2H, H-3', 5') & 7.46 (d, J = 8.4 Hz, 2H, H-2', 6') were typical for 1,4-disubstituted aromatic ring. Dimethyl disubstituted aromatic ring showed two peaks in aromatic region at δ 7.54 and 6.97 which showed doublet with coupling constant 7.6 Hz each having integration of one proton. The signal at δ 7.07 appeared as triplet indicating integration for one proton and *J*-coupling of 7.6 Hz that is characteristic of H-5 protons. Two peaks in aliphatic region appeared at δ 2.32 (s, 3H, CH_3-2''') & 2.14 (s, 3H, CH_3-3''') for two methyl groups at 2nd & 3rd position of aromatic ring. In the aliphatic region, one peak at δ 4.06 (s, 2H, H-2'') was assigned to methylene group of acetamide moiety attached to sulfur of Oxadiazole ring. On the basis of these spectral evidences, the structure was appointed as 2-[{5-(4-Chlorophenyl)-1,3,4-Oxadiazol-2-yl}sulfanyl]-*N*-(2,3-dimethylphenyl)acetamide. The mass fragmentation pattern of **6a** is clearly sketched in Fig. 1 and also discussed as representative compound. All the synthesized derivatives, **6a-o** were characterized by IR, ^{1}H-NMR and EI-MS spectral analysis.

As far as the antimicrobial activity is concerned, all the synthesized compounds were screened against different bacterial and fungal strains *in vitro*. Among the synthesized compounds, **6a**, **6f** and **6o** showed the antimicrobial activity against the selected panel of both bacterial and fungal species; **6g** & **6n** showed only antibacterial activity; and **6b**, **6k**, **6l** & **6m** were inactive against all the strains as evident from Table-2. The reference standards used for the bacterial & fungal strains were streptomycin & fluconazole respectively. Compound **6o** was the most active against the all bacterial strains i.e. *Staphylococcus aureus*, *Bacillus subtilis*, *Pasturella multocida* & *Escherichia coli* and the all fungal strains i.e. *Aspergillus niger*, *Aspergillus flavus*, *Ganodea lucidum* & *Alternaria alternate* in comparison to the rest of the members of its series and that of the reference standards. The significant activity of this compound was most probably because of aryl group bearing halogen i.e. bromine to the nitrogen of amide group. The highest hemolytic activity was shown by **6g** (69.40%) but lower than the positive control (Triton-X-100). The lowest activity was shown by **6k** and **6l** (4.93% and 4.46% respectively) but higher than the negative controls (PBS). On the basis of the previous results we may assume that the synthesized oxadiazole derivatives may be suitable for further improvement to address different targets.

Table-2: Antimicrobial activity of the synthesized compounds.

Compd No.	Zone of inhibition (mm)								Hemolytic activity (Mean) % ± S.D
	Antibacterial activity				Antifungal activity				
	S. aureus	*B. subtilis*	*P. multocida*	*E. coli*	*A. niger*	*A. flavus*	*G. lucidum*	*A. alternate*	
6a	**29**	**28**	**28**	**23**	**27**	**29**	**26**	**24**	**22.33%**
6b	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**39.30%**
6c	**32**	**31**	**-**	**-**	**32**	**30**	**23**	**22**	**34.07%**
6d	**31**	**31**	**-**	**-**	**31**	**31**	**-**	**-**	**65.58%**
6e	**35**	**38**	**-**	**-**	**34**	**36**	**32**	**32**	**34.61%**
6f	**29**	**31**	**22**	**23**	**30**	**32**	**23**	**24**	**49.44%**
6g	**24**	**23**	**21**	**23**	**-**	**-**	**-**	**-**	**69.40%**
6h	**29**	**32**	**-**	**-**	**29**	**32**	**29**	**31**	**32.12%**
6i	**25**	**26**	**-**	**-**	**26**	**24**	**-**	**-**	**24.95%**
6j	**33**	**32**	**-**	**-**	**33**	**32**	**-**	**-**	**66.07%**
6k	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**4.93%**
6l	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**4.46%**
6m	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**7.39%**
6n	**22**	**23**	**21**	**22**	**-**	**-**	**-**	**-**	**11.60%**
6o	**35**	**34**	**25**	**27**	**33**	**32**	**25**	**27**	**23.05%**
Streptomycin	**31**	**32**	**28**	**29**					
Fluconazole					**33**	**34**	**35**	**33**	
PBS									**0.00±0.0**
Triton (toxicity)									**100±0.0**

Note: PBS = Phosphate buffered saline

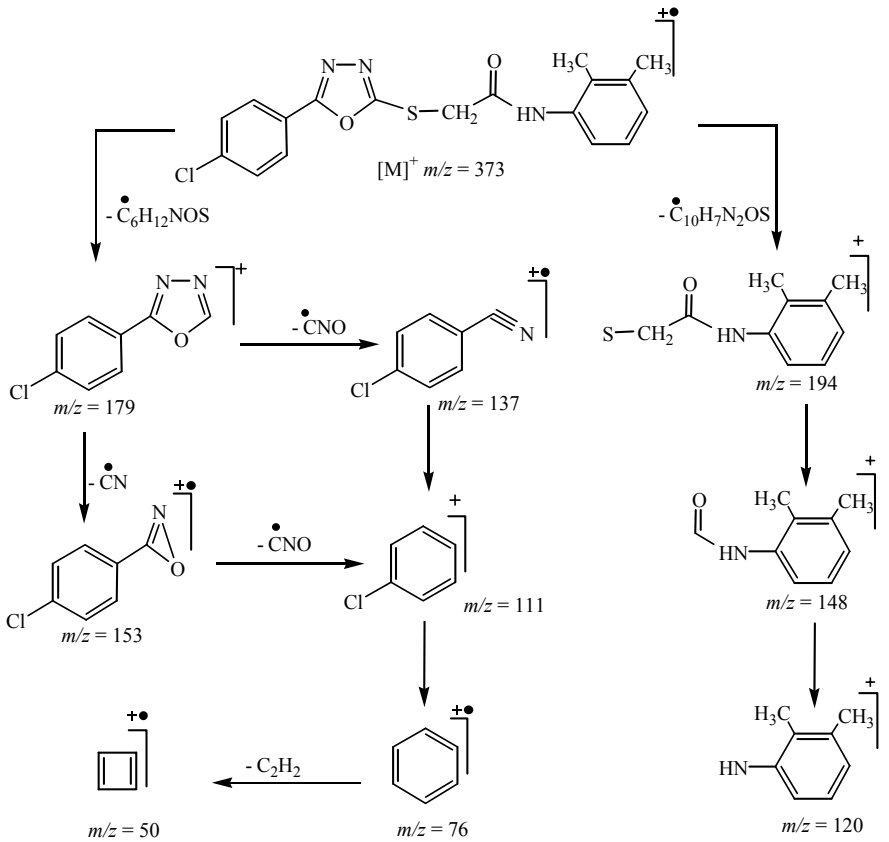


Fig. 1: Mass fragmentation pattern of 2-[{5-(4-Chlorophenyl)-1,3,4-Oxadiazol-2-yl}sulfanyl]-*N*-(2,3-dimethylphenyl) acetamide (6a).

Experimental

General

Chemicals were purchased from Sigma Aldrich and Alfa Aesar (Germany) and solvents of analytical grade were supplied by local suppliers. By using open capillary tube method melting points of all synthesized compounds were taken on Griffin and George melting point apparatus and were uncorrected. By using thin layer chromatography (with ethyl acetate & *n*-hexane (30:70) as mobile phase), purity of the synthesized compounds was detected at 254 nm. IR peaks were recorded on a Jasco-320-A spectrophotometer by using KBr pellet method. ^{1}H-NMR signals were recorded at 400 MHz in $CDCl_3$ using Bruker spectrometers with chemical shift values in ppm unit. EIMS signals were recorded by utilizing a JMS-HX-110 spectrometer.

Procedure for the Synthesis of ethyl 4-chlorobenzoate **(2)**

Synthesis of ethyl 4-chloro benzoate was afforded by refluxing 4-chlorobenzoic acid (**1**; 0.044 mole, 7.0 g) with absolute ethanol (28.0 mL) in the presence of conc. H_2SO_4 (3.5 mL) for 2.0 to 3.0 h in round bottom flask fitted with condenser. TLC was the basic tool for reaction monitoring. Because of reversibility of reaction, after maximum completion of reaction, concentrated solution of sodium carbonate was added to the reaction mixture for neutralization of excess organic acid and also sulfuric acid. Reaction mixture was poured in 100 mL distilled water in a separating funnel. Diethyl ether (50.0 mL) 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. To avoid contamination of upper organic layer containing ethyl 4-chlorobenzoate, it was collected from the neck of separating funnel. Yellow colored liquid ester was collected by evaporating diethyl ether.

Procedure for the preparation of 4-chlorobenzohydrazide **(3)**

4-Chlorobenzohydrazide was synthesized by allowing ethyl 4-chlorobenzoate (**2**; 6.0 mL, 0.01 moles) to react with hydrazine hydrate (13.0 mL, 0.03 moles) in methanol (15.0 mL) along with

vigorous stirring at room temperature for 3 hours in a round bottom flask. The completion of reaction was checked by thin layer chromatography and UV light to visualize the spots. Precipitates of product were quenched by addition of distilled water, filtration and washing. Finally recrystallization was brought about by using methanol.

Synthesis of 5-(4-chlorophenyl)-1,3,4-oxadiazol-2-thiol **(4)**

5-(4-Chlorophenyl)-1,3,4-Oxadiazol-2-thiol was synthesized by mixing compound **3** (5.0 g, 0.029 mole), carbon disulphide (1.76 mL, 0.029 mole) and potassium hydroxide (3.4 g, 0.058 mole) in ethanol (40.0 mL) as solvent in 500 mL round bottom flask. Reaction assembly was set to reflux reaction contents for 6 hours with continuous stirring. Reaction coordinates were monitored by TLC. On completion, the reaction mixture was acidified to set the pH of 2-3 after treating with distilled water to put out the synthesized product in the form of precipitates which was filtered and washed with distilled water. The product was finally recrystallized from methanol.

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

The *N*-substituted alkyl/aryl amines (0.013 moles) were dispersed in 12.0 mL distilled water in 100 mL RB 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 min at RT and then bromoacetyl bromide (0.013 moles) was added gradually drop wise. After that the flask was shaken vigorously till the appearance of precipitates. The reaction mixture was further stirred for 15 min. The reaction progress was monitored by TLC. At the end of reaction, the obtained solids were filtered, washed with distilled water and dried to yield the corresponding electrophiles, *N*-alkyl/aryl-substituted-2-bromoacetamides (**5a**–**o**).

General procedure for the synthesis of N-substituted-5-(4-chlorophenyl)-1,3,4-Oxadiazol-2-yl-2-sulfanyl acetamide derivatives **(6a-o)**

Compound **4** (0.1 g, 0.00047 mole) was dissolved in DMF (8-10 mL) in 100 mL round bottom flask, followed by the addition of NaH (0.002 g) acting as a weak base and was stirred at room temperature for 0.5 hour. Then it was allowed to react with equimolar ratios of *N*-substituted alkyl/aralkyl/aryl 2-bromoacetamides (**5a-o**) by stirring. The time duration for different *N*-alkyl/aralkyl/aryl 2-bromoacetamides varies from 3-5 hours. Thin layer chromatography by using ethyl acetate and *n*-hexane (30:60) as a mobile phase was carried out to monitor the reaction completion. Chilled distilled water was added to the reaction mixture to separate the precipitates. Precipitates so obtained were then filtered, washed and dried for spectral analysis.

Spectral Characterization of the Synthesized Compounds

2-[{5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl}sulfanyl]-N-(2,3-dimethylphenyl)acetamide **(6a):**

Pink amorphous solid; Yield: 90%; M.P: 160-162 ^{o}C; Molecular Formula: $C_{18}H_{16}ClN_3O_2S$; Molecular Weight: 373.8; IR (KBr, cm^{-1}) ν_{max}: 3337 (N-H stretching), 3085 (C-H str. of aromatic ring), 1677 (C=N str. of Oxadiazole ring), 1652 (C=O str.), 1561 (C=C aromatic str.), 1280 (C-O-C bond str.), 683 (C-Cl bond str.), 633 (C-S bond str.); ^{1}H-NMR (400 MHz, $CDCl_3$, δ / ppm): 8.74 (s, 1H, -NH), 7.91 (d, J = 8.4 Hz, 2H, H-3' & H-5'), 7.54 (d, J = 7.6 Hz, 1H, H-6'''), 7.46 (d, J = 8.4 Hz, 2H, H-2' & H-6'), 7.07 (t, J = 7.6 Hz, 1H, H-5'''), 6.97 (d, J = 7.6 Hz, 1H, H-4'''), 4.06 (s, 2H, CH_2-2''), 2.32 (s, 3H, CH_3-2'''), 2.14 (s, 3H, CH_3-3'''). EIMS (*m/z*): 375 (5%) $[M+2]^+$, 373 (15%) $[M^+]$, 179 (23%), 137 (100%), 120 (35%), 111 (69%), 76 (17%).

2-[{5-(4-Chlorophenyl)-1,3,4-Oxadiazol-2-yl}sulfanyl]-N-(2,4-dimethylphenyl)acetamide **(6b):**

Light yellow amorphous solid; Yield: 92%; M.P: 158-160 ^{o}C; Molecular Formula: $C_{18}H_{16}ClN_3O_2S$; Molecular Weight: 373.8; IR (KBr, cm^{-1}) ν_{max}: 3336 (N-H stretching), 3087 (C-H str. of aromatic ring), 1671 (C=N str. of Oxadiazole ring), 1653 (C=O str.), 1563 (C=C aromatic str.), 1283 (C-O-C bond str.), 687 (C-Cl bond str.), 635 (C-S bond str.); ^{1}H-NMR (400 MHz, $CDCl_3$, δ / ppm): 8.69 (s, 1H, -NH), 7.91 (d, J = 8.4 Hz, 2H, H-3' & H-5'), 7.68 (d, J = 7.6 Hz, 1H, H-6'''), 7.46 (d, J = 8.4 Hz, 2H, H-2' & 6'), 6.97 (d, J = 7.6 Hz, 1H, H-5'''), 6.96 (s, 1H, H-3'''), 4.04 (s, 2H, CH_2-2''), 2.25 (s, 3H, CH_3-2'''), 2.20 (s, 3H, CH_3-4'''); EIMS (*m/z*): 375 (4%) $[M+2]^+$, 373 (12%) $[M^+]$, 253 (17%), 139 (100%), 120 (71%), 111 (37%), 77 (21%).

2-[{5-(4-Chlorophenyl)-1,3,4-Oxadiazol-2-yl}sulfanyl]-N-(2,5-dimethylphenyl)acetamide **(6c):**

Light brown amorphous solid; Yield: 94%; M.P: 168-170 ^{o}C; Molecular Formula: $C_{18}H_{16}ClN_3O_2S$; Molecular Weight: 373.8; IR (KBr, cm^{-1}) ν_{max}: 3313 (N-H stretching), 3063 (C-H str. of

aromatic ring), 1677 (C=N str. of Oxadiazole ring), 1641 (C=O str.), 1551 (C=C aromatic str.), 1263 (C-O-C bond str.), 679 (C-Cl bond str.), 643 (C-S bond str.); ^{1}H-NMR (400 MHz, $CDCl_3$, δ / ppm): 8.73 (s, 1H, -NH), 7.91 (d, *J* = 8.4 Hz, 2H, H-3' & H-5'), 7.46 (d, *J* = 8.4 Hz, 2H, H-2' & H-6'), 7.12 (s, 1H, H-6'''), 7.01 (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''), 2.28 (s, 3H, CH_3-2'''), 2.21 (s, 3H, CH_3-5'''); EIMS (*m/z*): 375 (6%) $[M+2]^+$, 373 (18%) $[M^+]$, 253 (22%), 137 (100%), 120 (67%), 111 (31%), 77 (13%).

2-[{5-(4-Chlorophenyl)-1,3,4-Oxadiazol-2-yl}sulfanyl]-N-(2,6-dimethylphenyl)acetamide **(6d):**

Off white amorphous solid; Yield: 90%; M.P: 182-184 ^{o}C; Molecular Formula: $C_{18}H_{16}ClN_3O_2S$; Molecular Weight: 373.8; IR (KBr, cm^{-1}) ν_{max}: 3313 (N-H stretching), 3063 (C-H str. of aromatic ring), 1676 (C=N str. of Oxadiazole ring), 1655 (C=O str.), 1543 (C=C aromatic str.), 1273 (C-O-C bond str.), 683 (C-Cl bond str.), 645 (C-S bond str.); ^{1}H-NMR (400 MHz, $CDCl_3$, δ / ppm): 8.42 (s, 1H, -NH), 7.92 (d, *J* = 8.4 Hz, 2H, H-3' & H-5'), 7.47 (d, *J* = 8.4 Hz, 2H, H-2' & H-6'), 7.06-7.02 (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*): 375 (5%) $[M+2]^+$, 373 (16%) $[M^+]$, 253 (19%), 137 (100%), 120 (73%), 111 (41%), 77 (21%).

2-[{5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl}sulfanyl]-N-(3,4-dimethylphenyl)acetamide **(6e):**

Light brown amorphous solid; Yield: 89%; M.P: 175-177 ^{o}C; Molecular Formula: $C_{18}H_{16}ClN_3O_2S$; Molecular Weight: 373.8; (KBr, cm^{-1}) ν_{max}: 3315 (N-H stretching), 3077 (C-H str. of aromatic ring), 1677 (C=N str. of Oxadiazole ring), 1647 (C=O str.), 1565 (C=C aromatic str.), 1277 (C-O-C bond str.), 677 (C-Cl bond str.), 641 (C-S bond str.); ^{1}H-NMR (400 MHz, $CDCl_3$, δ / ppm): 8.92 (s, 1H, -NH), 7.91 (d, *J* = 8.4 Hz, 2H, H-3' & H-5'), 7.46 (d, *J* = 8.4 Hz, 2H, H-2' & H-6'), 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*): 375 (5%) $[M+2]^+$, 373 (15%) $[M^+]$, 253 (17%), 137 (100%), 120 (69%), 111 (41%), 77 (29%).

2-[{5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl}sulfanyl]-N-(3,5-dimethylphenyl)acetamide **(6f):**

Light brown amorphous solid; Yield: 93%; M.P: 136-138 ^{o}C; Molecular Formula: $C_{18}H_{16}ClN_3O_2S$; Molecular Weight: 373.8; IR (KBr, cm^{-1}) ν_{max}: 3315 (N-H stretching), 3065 (C-H str. of aromatic ring), 1663 (C=N str. of Oxadiazole ring), 1653 (C=O str.), 1559 (C=C aromatic str.), 1289 (C-O-C bond str.), 687 (C-Cl bond str.), 635 (C-S bond str.); ^{1}H-NMR (400 MHz, $CDCl_3$, δ / ppm): 8.95 (s, 1H, -NH), 7.91 (d, *J* = 8.4 Hz, 2H, H-3' & H-5'), 7.47 (d, *J* = 8.4 Hz, 2H, H-2' & H-6'), 7.15 (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*): 375 (6%) $[M+2]^+$, 373 (18%) $[M^+]$, 253 (17%), 137 (100%), 120 (73%), 111 (31%), 77 (27%).

2-[{5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl}sulfanyl]-N-(2-methylphenyl)acetamide **(6g):**

Off white amorphous solid; Yield: 94%; M.P: 198-200 ^{o}C; Molecular Formula: $C_{17}H_{14}ClN_3O_2S$; Molecular Weight: 359.8; IR (KBr, cm^{-1}) ν_{max}: 3323 (N-H stretching), 3035 (C-H str. of aromatic ring), 1633 (C=N str. of Oxadiazole ring), 1613 (C=O str.), 1523 (C=C aromatic str.), 1231 (C-O-C bond str.), 677 (C-Cl bond str.), 619 (C-S bond str.); ^{1}H-NMR (400 MHz, $CDCl_3$, δ / ppm): 10.32 (s, 1H, -NH), 7.92 (d, *J* = 8.4 Hz, 2H, H-3' & H-5'), 7.46 (d, *J* = 8.1 Hz, 2H, H-2' & H-6'), 7.39 (d, *J* = 7.6 Hz, 1H, H-6'''), 7.24 (d, *J* = 7.6 Hz, 1H, H-3'''), 7.14 (br.t, *J* = 7.6, 1.2 Hz, 1H, H-5'''), 7.11 (dt, *J* = 6.6, 1.0 Hz, 1H, H-4'''), 4.31 (s, 2H, CH_2-2''), 3.30 (s, 3H, CH_3-2'''); EIMS (*m/z*): 361 (4%) $[M+2]^+$, 359 (12%) $[M^+]$, 253 (17%), 137 (95%), 111 (35%), 106 (67%), 91 (71%), 77 (21%), 51 (100%).

2-[{5-(4-Chlorophenyl)-1,3,4-Oxadiazol-2-yl}sulfanyl]-N-(3-methylphenyl)acetamide **(6h):**

Off white amorphous solid; Yield: 92%; M.P: 152-154 ^{o}C; Molecular Formula: $C_{17}H_{14}ClN_3O_2S$; Molecular Weight: 359.8; IR (KBr, cm^{-1}) ν_{max}: 3343 (N-H stretching), 3065 (C-H str. of aromatic ring), 1671 (C=N str. of Oxadiazole ring), 1667 (C=O str.), 1567 (C=C aromatic str.), 1280 (C-O-C bond str.), 676 (C-Cl bond str.), 631 (C-S bond str.); ^{1}H-NMR (400 MHz, $CDCl_3$, δ / ppm): 9.04 (s, 1H, -NH), 7.92 (d, *J* = 8.4 Hz, 2H, H-3' & H-5'), 7.47 (d, *J* = 8.4 Hz, 2H, H-2' & H-6'), 7.36 (s, 1H, H-2'''), 7.31 (d, *J* = 8.0 Hz, 1H, H-6'''), 7.17 (t, *J* = 8.0 Hz, 1H, H-5'''), 6.89 (d, *J* = 7.2 Hz, 1H, H-4'''), 4.00 (s, 2H, CH_2-2''), 2.31 (s, 3H, CH_3-3'''); EIMS (*m/z*): 361 (5%) $[M+2]^+$, 359 (15%) $[M^+]$, 253 (16%), 137 (97%), 111 (37%), 106 (62%), 91 (69%), 77 (29%), 51 (100%).

2-[{5-(4-Chlorophenyl)-1,3,4-Oxadiazol-2-yl}sulfanyl]-N-(4-methylphenyl)acetamide **(6i):**

Off white amorphous solid; Yield: 94%; M.P: 204-206 ^{o}C; Molecular Formula:

$C_{17}H_{14}ClN_3O_2S$; Molecular Weight: 359.8; IR (KBr, cm^{-1}) ν_{max}: 3343 (N-H stretching), 3058 (C-H str. of aromatic ring), 1641 (C=N str. of Oxadiazole ring), 1628 (C=O str.), 1535 (C=C aromatic str.), 1250 (C-O-C bond str.), 679 (C-Cl bond str.), 621 (C-S bond str.); ^{1}H-NMR (400 MHz, $CDCl_3$, δ / ppm): 10.32 (s, 1H, -NH), 7.92 (d, *J* = 8.4 Hz, 2H, H-3' & H-5'), 7.63 (d, *J* = 8.8 Hz, 2H, H-2''' & H-6'''), 7.46 (d, *J* = 8.4 Hz, 2H, H-2' & H-6'), 7.13 (d, *J* = 8.8 Hz, 2H, H-3''' & H-5'''), 4.31 (s, 2H, CH_2-2''), 2.49 (s, 3H, CH_3-4'''); EIMS (*m/z*): 361 (6%) $[M+2]^+$, 359 (17%) $[M^+]$, 253 (15%), 137 (100%), 111 (34%), 91 (71%), 77 (35%).

2-[{5-(4-Chlorophenyl)1,3,4-Oxadiazol-2-yl}sulfanyl]-N-(cyclohexyl)acetamide **(6j):**

Off white amorphous solid; Yield: 94%; M.P: 175-177 ^oC; Molecular Formula: $C_{16}H_{18}ClN_3O_2S$; Molecular Weight: 351.8; IR (KBr, cm^{-1}) ν_{max}: 3347 (N-H stretching), 3033 (C-H str. of aromatic ring), 1661 (C=N str. of Oxadiazole ring), 1657 (C=O str.), 1581 (C=C aromatic str.), 1259 (C-O-C bond str.), 687 (C-Cl bond str.), 655 (C-S bond str.); ^{1}H-NMR (400 MHz, $CDCl_3$, δ / ppm); 10.32 (s, 1H, -NH), 7.91 (d, *J* = 8.4 Hz, 2H, H-3' & H-5'), 7.46 (d, *J* = 8.4 Hz, 2H, H-2' & H-6'), 3.84 (s, 2H, CH_2-2''), 3.75-3.69 (m, 1H, H-1'''), 1.86-1.14 (m, 10H, H-2''' to H-6'''); EIMS (*m/z*): 353 (3%) $[M+2]^+$, 351 (9%) $[M^+]$, 253 (19%), 137 (100%), 111 (33%), 98 (75%).

2-[{5-(4-Chlorophenyl)-1,3,4-Oxadiazol-2-yl}sulfanyl]-N-(2-hydroxyphenyl)acetamide **(6k):**

Brown colour solid; Yield: 68%; M.P: 221-223 ^oC; Molecular Formula: $C_{16}H_{12}ClN_3O_3S$; Molecular Weight: 361.8; IR (KBr, cm^{-1}) ν_{max}: 3357 (N-H stretching), 3049 (C-H str. of aromatic ring), 1673 (C=N str. of Oxadiazole ring), 1663 (C=O str.), 1577 (C=C aromatic str.), 1282 (C-O-C bond str.), 693 (C-Cl bond str.), 649 (C-S bond str.); ^{1}H-NMR (400 MHz, $CDCl_3$, δ / ppm): 10.32 (s, 1H, -NH), 7.94 (d, *J* = 8.8 Hz, 2H, H-3' & H-5'), 7.54 (d, *J* = 8.4 Hz, 2H, H-2' & H-6'), 7.38 (d, *J* = 7.6 Hz, 1H, H, H-6'''), 7.33 (d, *J* = 7.6 Hz, 1H, H-3'''), 7.20 (dt, *J* = 7.6, 0.8 Hz, 1H, H-5'''), 7.14 (dt, *J* = 7.8, 1.2 Hz, 1H, H-4'''), 3.31 (s, 2H, CH_2-2''); EIMS (*m/z*): 363 (5%) $[M+2]^+$, 361 (16%) $[M^+]$, 253 (15%), 137 (87%), 111 (41%), 108 (67%), 77 (25%), 51 (100%).

2-[{5-(4-Chlorophenyl)-1,3,4-Oxadiazol-2-yl}sulfanyl]-N-(2,4-dinitrophenyl)acetamide **(6l):**

Lemon yellow amorphous solid; Yield: 85%; M.P: 210-212 ^oC; Molecular Formula: $C_{16}H_{10}ClN_5O_6S$; Molecular Weight: 435.8; IR (KBr, cm^{-1}) ν_{max}: 3361 (N-H stretching), 3043 (C-H str. of aromatic ring), 1660 (C=N str. of Oxadiazole ring), 1655 (C=O str.), 1567 (C=C aromatic str.), 1279 (C-O-C bond str.), 690 (C-Cl bond str.), 633 (C-S bond str.); ^{1}H- NMR (400 MHz, $CDCl_3$, δ / ppm); 11.43 (s, 1H, -NH), 9.08 (d, *J* = 2.8 Hz, 1H, H-3'''), 9.00 (d, *J* = 9.6 Hz, 1H, H-6'''), 8.47 (dd, *J* = 9.6, 2.4 Hz, 1H, H-5'''), 7.91 (d, *J* = 8.4 Hz, 2H, H-3' & H-5'), 7.46 (d, *J* = 8.4 Hz, 2H, H-2' & H-6'), 4.24 (s, 2H, CH_2-2''); EIMS (*m/z*): 437 (5%) $[M+2]^+$, 435 (16%) $[M^+]$, 253 (22%), 182 (57%), 137 (100%), 111 (35%), 77 (31%).

2-[{5-(4-Chlorophenyl)-1,3,4-Oxadiazol-2-yl}sulfanyl]-N-(2-methoxyphenyl)acetamide **(6m):**

Off white amorphous solid; Yield: 90%; M.P: 145-147 ^oC; Molecular Formula: $C_{17}H_{14}ClN_3O_3S$; Molecular Weight: 365.8; IR (KBr, cm^{-1}) ν_{max}: 3325 (N-H stretching), 3065 (C-H str. of aromatic ring), 1651 (C=N str. of Oxadiazole ring), 1647 (C=O str.), 1545 (C=C aromatic str.), 1273 (C-O-C bond str.), 686 (C-Cl bond str.), 642 (C-S bond str.); ^{1}H-NMR: 9.12 (s, 1H, -NH), 8.28 (d, *J* = 8.0 Hz, 1H, H-6'''), 7.91 (d, *J* = 8.4 Hz, 2H, H-3' & H-5'), 7.45 (d, *J* = 8.4 Hz, 2H, H-2' & H-6'), 7.01 (t, *J* = 7.2 Hz, 1H, H-5'''), 6.92 (t, *J* = 7.2 Hz, 1H, H-4'''), 6.82 (d, *J* = 8.4 Hz, 1H, H-3'''), 4.10 (s, 2H, CH_2-2''), 3.85 (s, 3H, CH_3O-2'''); EIMS (*m/z*): 367 (6%) $[M+2]^+$, 365 (19%) $[M^+]$, 253 (13%), 137 (100%), 122 (45%), 111 (38%), 77 (27%).

2-[{5-(4-Chlorophenyl)-1,3,4-Oxadiazol-2-yl}sulfanyl]-N-(2-(methoxycarbonyl)phenyl) acetamide **(6n):**

White amorphous solid; Yield: 90%; M.P: 176-178 ^oC; Molecular Formula: $C_{18}H_{14}ClN_3O_4S$; Molecular Weight: 403.8; IR (KBr, cm^{-1}) ν_{max}: 3323 (N-H stretching), 3062 (C-H str. of aromatic ring), 1655 (C=N str. of Oxadiazole ring), 1649 (C=O str.), 1553 (C=C aromatic str.), 1277 (C-O-C bond str.), 693 (C-Cl bond str.), 649 (C-S bond str.); ^{1}H-NMR (400 MHz, $CDCl_3$, δ / ppm); 11.6 (s, 1H, -NH), 8.63 (d, *J* = 8.4 Hz, 1H, H-6'''), 8.00 (dd, *J* = 8.0, 1.2 Hz, 1H, H-3'''), 7.91 (d, *J* = 8.4 Hz, 2H, H-3' & H-5'), 7.55 (dt, *J* = 8.8, 1.2 Hz, 1H, H-5'''), 7.43 (d, *J* = 8.4 Hz, 2H, H-2' & H-6'), 7.12 (dt, *J* = 7.6 Hz, 1H, H-4'''), 4.22 (s, 2H, CH_2-2''), 3.75 (s, 3H, $\underline{CH_3}OOC$-3'''); EIMS (*m/z*): 405 (7%) $[M+2]^+$, 403 (20%) $[M^+]$, 253 (22%), 150 (49%), 137 (100%), 111 (31%), 77 (33%).

2-[{5-(4-Chlorophenyl)-1,3,4-Oxadiazol-2-yl}sulfanyl]-N-(2-bromophenyl)acetamide **(6o):**

Yellow colour; Yield: 79%; M.P: 208-210 ^oC; Molecular Formula: $C_{16}H_{11}BrClN_3O_2S$;

Molecular Weight: 424.7; IR (KBr, cm^{-1}) ν_{max}: 3337 (N-H stretching), 3073 (C-H str. of aromatic ring), 1659 (C=N str. of Oxadiazole ring), 1654 (C=O str.), 1549 (C=C aromatic str.), 1277 (C-O-C bond str.), 686 (C-Cl bond str.), 643 (C-S bond str.), 567 (C-Br bond str.); ^{1}H-NMR (400 MHz, $CDCl_3$, δ / ppm): 9.29 (s, 1H, -NH), 7.94 (d, J = 8.4 Hz, 2H, H-3' & H-5'), 7.49 (d, J = 8.4 Hz, 2H, H-2' & H-6'), 7.45-7.39 (m, 4H, H-3''' to H-6'''), 3.98 (s, 2H, CH_2-2''); EIMS (*m/z*): 428 (4%) $[M+4]^+$, 426 (7%) $[M+2]^+$, 424 (21%) $[M^+]$, 253 (14%), 137 (87%), 171 (57%), 111 (43%), 77 (35%), 51 (100%).

Antimicrobial activity
Microbial strains

All the synthesized compounds were tested against microorganisms, including Gram-positive bacteria: *Staphylococcus aureus* (*S. aureus*) & *Bacillus subtilis* (*B. subtilis*) and Gram-negative bacteria: *Escherichia coli* (*E. coli*) & *Pasteurella multocida* (*P. multocida*); and four pathogenic fungi, *Aspergillus niger (A. niger)*, *Aspergillus flavus* (*A. flavus*), *Ganoderma lucidum (G. lucidum)* & *Alternaria alternate (A. alternate)*. The bacterial and fungal strains were prevailed from Department of Plant Pathology and Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan. Bacterial strains were cultured at 37 ºC on Nutrient agar (Oxoid, UK) overnight and fungal strains were cultured at 28 ºC using Potato dextrose agar (Oxoid, UK) for 48 hours [13].

Disc diffusion method

Disc diffusion method was used to find out the antimicrobial activity of the synthesized compounds. A total 100 µL suspension of tested microorganisms was spread on NA medium for 10^7 colony-forming units/mL (CFU/mL) of bacteria cells and on PDA medium for 10^6 spores/mL of fungi. The filter discs of 6 mm 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. Streptomycin (30 µg/dish) and Fluconazole (30 µg/disk) were applied as positive reference for bacterial strains and fungal strains, respectively. Plates were placed at 4 ºC for 2 h and then incubation was carried out at 37 ºC for 18 h for bacterial strains and at 28 ºC for 24 h for fungal strains. Antimicrobial activity was justified after comparison of diameter of growth inhibition zone measured in mm against microorganisms and the controls [13].

Hemolytic activity

Hemolytic activity was studied by the reported method [14,15]. Fresh heparin added human blood (3.0 mL) was obtained from voluntaries after guidance from the Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan. After centrifuging blood at 1000x*g* for 5 min, plasma was disposed off and cold aseptic isosmotic Phosphate-buffered saline (PBS) having pH 7.4 was utilized for washing of the blood cells three times. The RBCs for each assay were kept 10^8 cells per mL. The 100 µL of each compound was poured in each assay and then incubation was carried out at 37 ^{o}C for 35 min followed by agitation after 10 min. The samples were kept on cold ice for 5 min and then again centrifuged for 5 min at 1000x*g*. The 100 µL 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 microplate reader spectrophotometer.

Conclusion

The projected structures of the synthesized compounds are well supported by spectroscopic data. From the antibacterial activity data (Table-2), it is obvious that the compounds were found to possess significant inhibitory action for the different bacterial strains. In short, we have inaugurated a series of compounds with handsome biological activity and the synthesized compounds can be assistive for the pharmaceutical industries in designing of medicines. Synthesis of some new analogues of similar kind, investigating of their biological activities and estimation of their SAR is under investigation.

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