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Synthesis, Characterization and Biological Screening of Various *S*-substituted Derivatives of 5-(3-Nitrophenyl)-1,3,4-Oxadiazole-2-thiol

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ABSTRACT
In the presented research work, a series of *S*-substituted derivatives of 5-(3-nitrophenyl)-1,3,4-Oxadiazole-2-thiol was synthesized from 3-nitrobenzoic acid. The synthesis was carried out by converting 3-nitrobenzoic acid correspondingly into ester, hydrazide and 5-(3-nitrophenyl)-1,3,4-Oxadiazole-2-thiol **(4)**. Finally, the *S*-substituted derivatives **(6a-m)** were brought about by reacting 5-(3-nitrophenyl)-1,3,4-Oxadiazole-2-thiol with different electrophiles **(5a-m)** in the presence of NaH and *N*,*N*-dimethylformamide. Structure elucidation for all the synthesized compounds was executed by different spectroscopic analysis like IR, ^{1}H-NMR and EI-MS. All the synthesized derivatives were screened for antibacterial activity. Antibacterial potential was sorted out for four gram-negative bacteria including *Shigella sonnei, Escherichia coli, Pseudomonas aeruginosa* & *Salmonella typhi*; and two gram-positive bacteria including *Bacillus subtilis* & *Staphylococcus aureus* and found results that most of the compounds exhibit significant activity relative to the standard drugs Ciprofloxacin and Gentamycin.

Keywords: 3-Nitrobenzoic acid, Oxadiazole, Antibacterial potential, ^{1}H-NMR and EI-MS.

1. INTRODUCTION
Oxadiazoles constitute four different classes but the most active and potent class is 1,3,4-Oxadiazole which comprises a large number of biologically active molecules of various pharmacological classes[1]. Due to susceptibility of 1,3,4-Oxadiazole ring to undergo variety of electrophilic and nucleophilic substitution reactions, a large number of derivatives have been synthesized, and still in progress, that have splendid medicinal, pharmacological and biological activities like antimicrobial[2,3], anti-inflamatory[4,5], cytotoxic[6], hypoglycemic[7], anticancer[8,9], anti-hypertensive agents, anticonvulsive, anti-tubercular, fungicidal and insecticidal[10]. 1,3,4-oxadiazoles can be used as a skeleton in medicinal chemistry to synthesize large number of bioactive agents. Several 2,5-disubstituted-1,3,4-Oxadiazole derivatives have a powerful effect against 60 malignant tumor cell lines. Biological effects demonstrate a very significant anti-tumor activity against leukemia, breast cancer and colon[11, 12]. 1,3,4-oxadiazole is extensively used in the treatment of arthritis (jaundice, rheumatoid and osteoarthritis), myocardial infections and controlling of primary dysmenorrhea[13]. Oxadiazoles also exhibit herbicidal, pesticidal, analgesic and plant growth regulatory activities[14, 15].

In continuation of our previous work[16-19], the synthesis and biological screening of *S*-substituted derivatives of 5-(3-nitrophenyl)-1,3,4-Oxadiazole-2-thiols with an objective to detect the antimicrobial activity of all the synthesized compounds. The synthesis was carried out through the intermolecular cyclization of organic acid hydrazide to the 5-(3-nitrophenyl)-1,3,4-Oxadiazole-2-thiols and finally to *S*-substituted 5-(3-nitrophenyl)-1,3,4-oxadiazole-2-thiol products.

2. RESULTS AND DISCUSSION
2.1 *Chemistry*
In the undertaken research, a number of *S*-alkyl/aralkyl substituted derivatives of 5-(3-nitrophenyl)-1,3,4-oxadiazol-2-thiol was synthesized according to the protocol sketched in scheme-1. The general reaction conditions and the structure characterization are described in experimental section.

Our objective was to synthesize some new *S*-substituted 1,3,4-Oxadiazole compounds and to find out their antimicrobial activity. Starting from 3-nitrobenzoic acid **(1)**, 3-nitrobenzoate **(2)** was prepared by esterification of the acid in the presence of conc. H_2SO_4 and ethanol. The mixture was refluxed for 2-3 hrs. The liquid product was separated from the reaction mixture by solvent extraction using ether after the addition of aq. Na_2CO_3 solution. Sodium carbonate was added to remove the unreacted organic and inorganic acids in the form of salts. The synthesized ethyl ester **(2)** was converted into corresponding acid hydrazide **(3)** by refluxing it with 80% hydrated hydrazine in the presence of methanol as solvent for 2 hrs. The precipitated product **(3)** was filtered and washed with water. The compound **(3)** was brought to intermolecular cyclization into oxadiazole ring by refluxing it with KOH, CS_2 and ethanol for 3-4 hrs. Solid KOH was used to balance the charge during cyclization, CS_2 provided electrophilic carbon for oxadiazole ring and ethanol is used as solvent. At the last stage of synthesis, the *S*-substituted derivatives **6a-m** of oxadiazole were geared up by coupling 5-(3-nitrophenyl)-1,3,4-oxadiazole-2-thiol **(4)** with different alkyl/aralkyl halides **(5a-m)** in the comportment of NaH as base and DMF as aprotic solvent. The protic solvent should be obviated because it decrements the rate of reaction and yield by decreasing the nucleophilic character of thiol group. The products were collected by filtration after the addition of cold distilled water. In some cases, the solvent extraction was also used for the collection of products. The parent compound 5-(3-nitrophenyl)-1,3,4-

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oxadiazole-2-thiol **(4)** was synthesized in good yield and obtained as yellow colored amorphous solid. EI-MS established the molecular formula $C_8H_5N_3O_3S$ by giving molecular ion peak at *m/z* 223 while other characteristic peaks appeared at 177, 163, 148, 150, 122 and 63. Base peak appeared at *m/z* 148 with 100% intensity characterized for *p*-nitrocyanobenzene moiety. Characteristic bands in IR spectrum appeared at 3031, 1523, 1635, 1227, 1059, 1257 and 615 for C-H stretching of aromatic ring, C=C stretching of aromatic ring, C=N str. of oxadiazole ring, C-O-C bond stretching,C=S bond stretching and stretching of C-S bond respectively. ^{1}H-NMR spectra recorded at 300 MHz using C_5D_5N as solvent revealed aromatic protons at δ (ppm) 10.87 as singlet integrated for one proton, a doublet of doublet at 8.31 with coupling constant value of 8.4 & 1.2 Hz having integration of one proton, a doublet with *J* value of 7.8 Hz indicating ortho coupling appeared at 8.16 ppm having the integration of one proton and another signal revealed at 7.58 as triplet with coupling constant of 8.1 Hz with single proton integration. All these signals collectively corroborated the presence of meta disubstituted benzene ring that is corresponding to 3-nitrophenyl group of parent 5-(3-nitrophenyl)-1,3,4-oxadiazol-2-thiol. In ^{13}C-NMR broad band and DEPT spectra eight signals appeared indicating four quaternary carbons and four methine carbons. Most downfield signals at δ (ppm) 180.1 and 159.4 were attributed to the quaternary carbons of oxadiazole ring attached to thiol and substituted phenyl group. The aromatic quaternary carbon attached to nitro group gave signal at δ (ppm) 148.8 and the other quaternary carbon attached to oxadiazole ring appeared at δ (ppm) 125.5. All the methine signals appeared at 131.8, 130.8, 126.2 and 121.1 for C-6', C-5', C-4' and C-2' respectively. Thus, the structure of parent compound established as 5-(3-nitrophenyl)-1,3,4-oxadiazole-2-thiol **(4)**. The mass fragmentation pattern of 5-(3-Nitrophenyl)-2-(allylthio)-1,3,4-oxadiazole (**6i**) was clearly described in figure-1.Similarly, on the basis of spectral evidences from EI-MS and ^{1}H-NMR, the structures of other derivatives **6a-m** were elucidated.

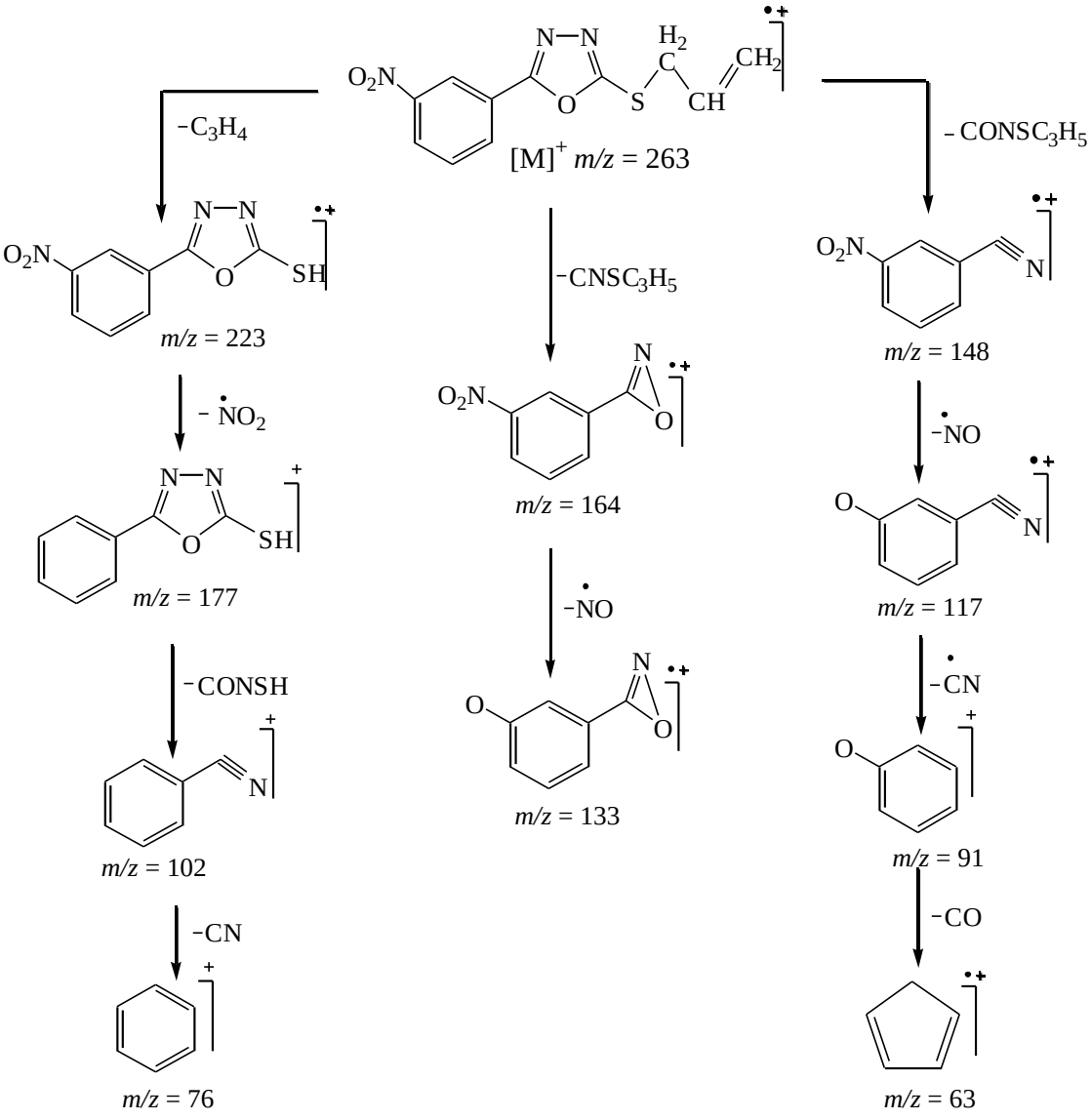


Fig-1: Mass fragmentation pattern of 5-(3-Nitrophenyl)-2-(allylthio)-1,3,4-oxadiazole (**6i**)

2.2 *Antibacterial Activity*

All the synthesized *S*-substituted derivatives of 5-(3-nitrophenyl)-1,3,4-oxadiazole-2-thiol were screened against clinically isolated two Gram-positive bacteria *Bacillus subtilis, Staphylococcus aureus* and four Gram-negative *Shigella sonnei, Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi* using Ciprofloxacin and Gentamycin as reference standard. The MIC values of *in vitro* antibacterial activity of the titled compounds are presented in table-1.

Table-1: Bioactivity studies of *S*-substituted derivatives of 5-(3-nitrophenyl)-1,3,4-oxadiazole-2-thiol.

Sample Code	MIC (µg/ml)					
	B.subtilis (+)	***S.aureus* (+)**	***E.coli (-)***	***S.sonnei (-)***	***S.typhi (-)***	***P.aureginosa (-)***
6a	-	-	-	-	-	-
6b	11.36±0.31	19.47 ± 0.09	8.73±0.42	13.37±0.27	11.01±0.22	7.52±0.24
6c	-	-	-	-	-	-
6d	12.12±0.21	-	-	14.88±0.34	-	-
6e	12.19±0.26	19.04 ± 0.07	9.11±0.08	14.17±0.26	13.16±0.52	7.48±0.11
6f	-	15.92 ± 0.79	11.31±0.24	-	10.31±0.31	7.39±0.08
6g	-	22.6 ± 0.98	8.84±0.41	11.29±0.31	8.412±0.02	8.412±0.18
6h	13.11±0.18	-	11.75±0.10	15.00±0.27	17.36±0.31	8.88±0.24
6i	-	-	-	-	-	-
6j	13.61±0.11	15.18 ± 0.27	13.92±0.52	12.36±0.46	17.91±0.21	7.44±0.41
6k	14.26±0.22	18.3 ± 0.35	10.00±0.61	13.83±0.33	8.48±0.19	6.50±0.29
6l	-	17.94 ± 0.46	11.33±0.09	-	11.92±0.22	6.97±0.12
6m	-	14.42 ± 0.25	-	-	12.36±0.18	-
Ciprofloxacin	9.42 ± 0.14	23.49±0.21	8.36± 0.18	20.07±0.22	7.31± 0.18	23.95±0.09
Gentamycin	8.42± 0.18	23.94±0.05	10.36±0.73	22.07±0.21	9.31 ± 0.08	28.87±0.11

Note: MIC = Minimum Inhibitory Concentration

The most of the derivatives exhibit greater potential than Ciprofloxacin and Gentamycin used as reference standards against *S. aureus* of Gram-positive family and *S. sonnei* & *P. aeruginosa* of Gram-negative family. Some derivatives like **6b**, **6e**, **6j** and **6k** revealed good inhibitory potential against all the strains of Gram-positive and Gram-negative bacteria but only three compounds **6a**, **6c** and **6i** were found to be inactive against all bacterial strains. This might because of very small alkyl group in case of **6a** and **6c** that have not shown any activity while in **6i** the presence of allyl group change the trend and did not show any activity. For Gram-positive bacteria, compounds **6b**, **6d**, **6e**, **6h**, **6j** and **6k** were active against *B. subtilis* only but the compounds **6b**, **6e**, **6f**, **6g**, **6j**, **6k**, **6l** and **6m** were active against *S. aureus* only. The compounds **6b**, **6e**, **6j** and **6k** showed good zone of inhibition against both *B. subtilis* and *S. aureus*. For Gram-negative bacteria, the compounds **6b** and **6g** showed the almost same activity as that for Ciprofloxacin and more activity than that of Gentamycin against *E. coli*. The compounds **6b**, **6d**, **6e**, **6g**, **6h**, **6j** and **6k** were more active than the reference standards and almost showed double activity against *S. sonnei*. The most of the compounds were active against *S. typhi* showing good potential. The synthesized compounds **6b**, **6e**, **6f**, **6g**, **6h**, **6j**, **6k** and **6l** were almost three times more active than that of the reference standards against *P. aureginosa*, as clear from their MIC values. The discussion revealed that the substitution by the long chain aliphatic alkyl halides and some substituted aralkyl halides showed good inhibitory potential.

3. CONCLUSION

It can be concluded that the enzyme inhibition, antibacterial and antifungal activities varied upon the type and position of the substituent at 5-(3-Nitrophenyl)-1,3,4-oxadiazole-2-thiol core. It can also be inferred from the results of these bioactivity studies that when the thiol group at 2-position of this core was substituted with different alkyl/aralkyl groups, the bioactivity was altered to an appreciable extent. Thus this series of the synthesized compounds is valuable for the pharmaceutical industries for the design of the new antibacterially applicable medicine.

4. EXPERIMENTAL

4.1 *General*

Reaction coordinates and purity of the compounds was confirmed by TLC plates pre-coated with silica gel $60F_{254,}$ developed by using *n*-hexane and EtOAc as solvent system. UV lamp at 254 nm and ceric sulfate soln. for UV inactive compounds was utilized to visualize the plates. Melting points taken by open capillary tube method using Griffin & George melting point apparatus and were uncorrected. IR spectra were recorded by KBr pellet method. ^{1}H-NMR spectra and^{13}C-NMR spectra were commemorated at 300 MHz and 75 MHz Bruker NMR spectrometers respectively, in pyridine demonstrating chemical shifts in ppm values taking TMS as reference standard. EI-MS spectra were taken using JMS-HX-110 spectrometer, with data system.

4.2 *Procedure for the synthesis of Ethyl 3-nitrobenzoate (2)*

3-Nitrobenzoic acid (**1**; 6g, 35.4mmol) was taken in a 250mLround bottom flask and absolute C_2H_5OH (24.0 mL) was introduced to dissolve the acid. 3.0 mL of conc.H_2SO_4 was also added drop wise as a catalyst and the reaction assembly was set to reflux by fitting with a condenser for 2-3 hrs. Reaction progress was checked by thin layer chromatography developed by *n*-hexane and EtOAc as solvent system time by time. On completion, reaction contents were transferred to 250 mL separating funnel containing distilled water (50.0 mL) along with the addition of Na_2CO_3 soln. to remove unwanted acid contents while diethyl ether was introduced to separating funnel as organic layer to extract ethyl 3-nitrobenzoate. The contents of separating funnel were shaken vigorously and then allowed to settle down for some time to collect the upper organic layer containing our product. The lower aqueous layer was discarded. Diethyl ether was distilled off and ethyl 3-nitrobenzoate was obtained as pure white crystalline solid.

4.3 *Procedure for the preparation of 3-nitrobenzohydrazide (3)*

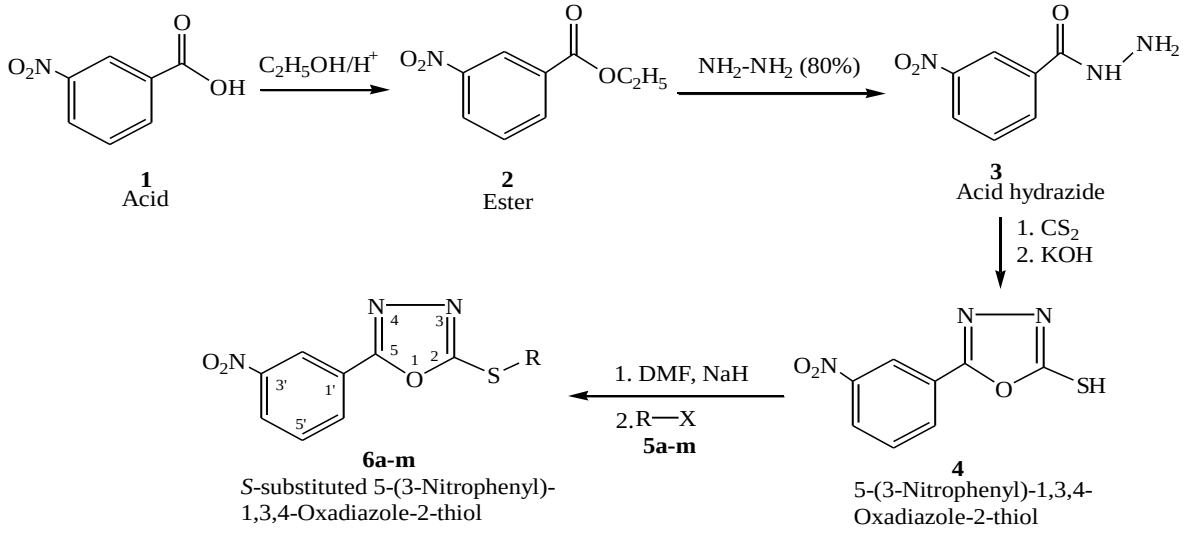
Ethyl 3-nitrobenzoate (**2**; 4g, 20.0mmol) taken in 250 mL round bottom flask was dissolved in methanol (30 mL) simply by stirring at room temperature. On complete dissolution of compound **2**, hydrazine hydrate (80%, 50.0 mmol) was introduced into the flask drop wise and the reaction apparatus was set to stir at room temperature for 2 hrs. Reaction progress was confirmed by thin layer chromatography developed by *n*-hexane and ethyl acetate as solvent system and UV lamp was used to visualize the TLC. At the end of reaction, excess of solvent was evaporated and residue was poured into the ice cold water. Precipitates were filtered, washed with water and dried. Product was recrystallized using methanol.

4.4 *Synthesis of 5-(3-nitrophenyl)-1,3,4-oxadiazole-2-thiol (4)*

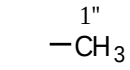
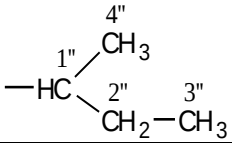
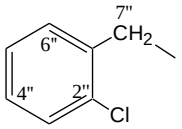
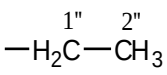
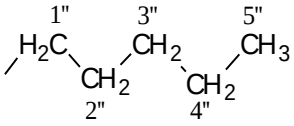
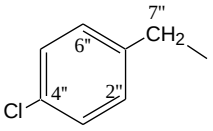
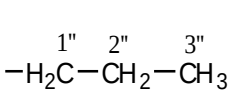
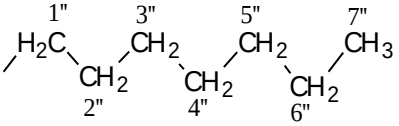
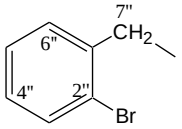
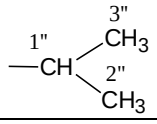
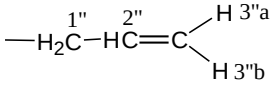
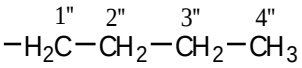
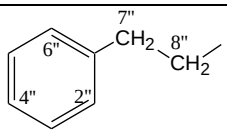
Compound **3** (6g, 33.0mmol) was dissolved in 30.0 mL of absolute C_2H_5OH in a 250 mL round bottom flask, 3.7g (66.0mmol) KOH and 1.98 mL carbon disulphide (33.0mmol) was added to reaction flask. KOH was added to provide the basic media that facilitated the nucleophilic attack on electophilic carbon of carbon disulfide. The reaction assembly was set to reflux for 3-4 hrs with continuous stirring. Reaction coordinates were monitored by thin layer chromatography time by time. After single spot on TLC plate, cold distilled water was added to the flask contents along with the addition of conc. HCl. But the highly acidic pH was avoided because of lowering of yield. Light yellow colored precipitates were produced and were filtered, washed with distilled water and dried. To obtain the purified product, precipitates were recrystallized from methanol.

4.5 *General procedure for the synthesis of S-substituted derivatives of 5-(3-nitrophenyl)-1,3,4-oxadiazole-2-thiol (6a-m)*

Parent compound **4** (0.1g, 4.5 mmol.) was taken in a 100 mL round bottom flask and an aprotic solvent DMF was utilized to dissolve the parent compound by stirring at room temperature. Solid NaH (0.002g) was added as a strong base, enhancing the nucleophilic character of sulfur attached to oxadiazole ring and stirring was continued for half an hour. Then different electrophiles i.e. alkyl/aralkyl halides **(5a-m**) were introduced in equimolar ratios to the parent compound to synthesize the respective *S*-substituted derivatives **(6a-m)** of **4**.



The reaction duration for different electrophiles varied from 3-5 hrs. Reaction completion was supervised by TLC using *n*-hexane and ethyl acetate solvent system (4:1). On completion, the contents were slightly basified to remove unreacted oxadiazole. Cold distilled water was added to quench the precipitates. Precipitates were filtered, washed with distilled water and dried. Dried samples were then sent for different spectroscopic analysis and enzyme inhibition activity.

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

Scheme1: Outline for the synthesis of *S*-substituted derivatives of 5-(3-Nitrophenyl)-1,3,4-oxadiazole-2-thiol

5. SPECTRAL CHARACTERIZATION OF THE SYNTHESIZED COMPOUNDS

5.1 *5-(3-Nitrophenyl)-1,3,4-oxadiazole-2-thiol (4)*

Light yellow amorphous solid; Yield: 81%; M.P. 144-146^{o}C; Mol. formula: $C_8H_5N_3O_3S$; Mol. Wt. 223; IR (KBr, ν_{max}cm^{-1}): 3031 (C-H str. of ar. ring), 2830 (S-H bond str.), 1635 (C=N str. of oxadiazole ring), 1523 (C=C ar. str.), 1227, 1059 (C-O-C bond str.), 1257 (C=S bond str.), 615 (C-S bond str.); ^{1}H-NMR (C_5D_5N, 300 MHz, δ ppm): 10.87 (s, 1H, H-2'), 8.31 (dd, *J* = 8.4, 1.2 Hz, 1H, H-4'), 8.16 (d, *J* = 7.8 Hz, 1H, H-6'), 7.58 (t, *J* = 8.1 Hz, 1H, H-5'); ^{13}C-NMR (C_5D_5N, 75 MHz, δ ppm): 180.1 (C-2), 159.4 (C-5), 148.8 (C-3'), 131.8 (C-6'), 130.8 (C-5'), 126.2 (C-4'), 125.5 (C-1'), 121.1 (C-2'); EIMS (*m/z*): 223 (20%)[M^{+}], 177 (23%), 163 (37%), 148 (100%), 150 (35%), 122 (45%), 63 (75%).

5.2 *5-(3-Nitrophenyl)-2-(methylthio)-1,3,4-oxadiazole(6a)*

Cream amorphous solid; Yield: 73%; M.P. 162-164^{o}C; Mol. formula: $C_9H_7N_3O_3S$; Mol. Wt. 237; IR (KBr, ν_{max} cm^{-1}): 3029 (C-H str. of ar. ring), 1639 (C=N str. of oxadiazole ring), 1527 (C=C ar. str.), 1229, 1063 (C-O-C bond str.), 1254 (C=S bond str.), 613 (C-S bond str.); ^{1}H-NMR (C_5D_5N, 300 MHz, δ ppm): 8.76 (s, 1H, H-2'), 8.30 (dd, *J* = 8.4, 1.2 Hz, 1H, H-4'), 8.28 (d, *J* = 7.8 Hz, 1H, H-6'), 7.60 (t, *J* = 8.1 Hz, 1H, H-5'), 2.72 (s, 3H, H-1''); EIMS (*m/z*): 237 (17%)[M^{+}], 222 (13%), 177 (25%), 163 (33%), 148 (100%), 150 (32%), 122 (43%), 63 (67%).

5.3 *5-(3-Nitrophenyl)-2-(ethylthio)-1,3,4-oxadiazole(6b)*

Light yellow sticky solid; Yield: 76%; M.P. 150-152 ^{o}C; Mol. formula: $C_{10}H_9N_3O_3S$;Mol. Wt. 251; IR (KBr, ν_{max} cm^{-1}): 3019 (C-H str. of ar. ring), 1649 (C=N str. of oxadiazole ring),1537 (C=C ar. str.), 1233,1067 (C-O-C bond str.), 617 (C-S bond str.);^{1}H-NMR (C_5D_5N, 300 MHz, δ ppm): 8.74 (s, 1H, H-2'), 8.31 (dd, *J* = 8.4, 1.2 Hz, 1H, H-4'), 8.29 (d, *J* = 7.8 Hz, 1H, H-6'), 7.62 (t, *J* = 8.1 Hz, 1H, H-5'), 3.30 (q, *J* = 7.2 Hz, 2H, H-1''), 1.50 (t, *J* = 7.2 Hz, 3H, H-2''); EIMS (*m/z*): 251 (19%)[M^{+}], 222 (13%), 177 (25%), 163 (33%), 148 (100%), 150 (32%), 122(43%), 63 (67%).

5.4 *5-(3-Nitrophenyl)-2-(n-propylthio)-1,3,4-oxadiazole(6c)*

Light yellow amorphous solid; Yield: 79%; M.P. 136-138 ^{o}C; Mol. formula: $C_{11}H_{11}N_3O_3S$; Mol. Wt. 265; IR (KBr, ν_{max} cm^{-1}): 3023 (C-H str. of ar. ring), 1641 (C=N str. of oxadiazole ring), 1533 (C=C ar. str.), 1239, 1061 (C-O-C bond str.), 615 (C-S bond str.); ^{1}H-NMR (C_5D_5N, 300 MHz, δ ppm): 8.78 (s, 1H, H-2'), 8.32 (dd, *J* = 7.8, 1.5 Hz, 1H, H-4'), 8.28 (d, *J* = 7.5 Hz, 1H, H-6'), 7.61 (t, *J* = 8.1 Hz, 1H, H-5'), 3.26 (t, *J* = 7.2 Hz, 2H, H-1''), 1.75 (q_{uin}, *J* = 7. Hz, 2H, H-2''), 0.91 (t, *J* = 7.2 Hz, 3H, H-3''); EIMS (*m/z*): 265 (17%)[M^{+}], 223 (14%), 177 (25%), 163 (33%), 148 (100%), 150 (32%), 122 (43%), 63 (67%), 43 (76%).

5.5 *5-(3-Nitrophenyl)-2-(isopropylthio)-1,3,4-oxadiazole(6d)*

Yellowish brown amorphous solid; Yield: 83%; M.P. 102-104 ^{o}C; Mol. formula: $C_{11}H_{11}N_3O_3S$; Mol. Wt. 265; IR (KBr, ν_{max} cm^{-1}): 3027 (C-H str. of ar. ring), 1647 (C=N str. of oxadiazole ring), 1539 (C=C ar. str.), 1232, 1061 (C-O-

C bond str.), 1255 (C=S bond str.), 614 (C-S bond str.); ^{1}H-NMR (C_5D_5N, 300 MHz, δ ppm): 8.79 (s, 1H, H-2'), 8.33 (dd, J = 6.9, 1.8 Hz, 1H, H-4'), 8.31 (d, J = 7.2 Hz, 1H, H-6'), 7.61 (t, J = 8.1 Hz, 1H, H-5'), 3.95 (sep, J = 6.6 Hz, 1H, H-1''), 1.39 (d, J = 6.6 Hz, 6H, CH_3-2'' &CH_3-3''); EIMS (*m/z*): 265 (13%)[M^+], 223 (19%), 177 (27%), 163 (35%), 148 (100%), 150 (29%), 122 (41%), 63 (67%), 43 (80%).

5.6 *5-(3-Nitrophenyl)-2-(n-butylthio)-1,3,4-oxadiazole(6e)*

Yellowish cream amorphous solid; Yield: 76%; M.P. 108-110^{o}C; Mol. formula: $C_{12}H_{13}N_3O_3S$;Mol. Wt. 279; IR (KBr, ν_{max} cm^{-1}): 3025 (C-H str. of ar. ring), 1648 (C=N str. of oxadiazole ring), 1539 (C=C ar. str.), 1245, 1067 (C-O-C bond str.), 611 (C-S bond str.); ^{1}H-NMR (C_5D_5N, 300 MHz, δ ppm): 8.77 (s, 1H, H-2'), 8.33 (dd, J = 7.8, 1.5 Hz, 1H, H-4'), 8.27 (d, J = 7.5 Hz, 1H, H-6'), 7.63 (t, J = 8.1 Hz, 1H, H-5'), 3.27 (t, J = 7.2 Hz, 2H, H-1''), 1.73 (t, J = 7.2 Hz, 2H, H-2''), 1.04-1.01 (m, 2H, H-3''), 0.89 (t, J = 7.2 Hz, 3H, CH_3-4''); EIMS (*m/z*): 279 (15%)[M^+], 223 (16%), 177 (27%), 163 (37%), 148 (100%), 150 (36%), 122 (47%), 63 (71%), 57 (79%).

5.7 *5-(3-Nitrophenyl)-2-(butan-2-ylthio)-1,3,4-oxadiazole(6f)*

Brown sticky solid; Yield: 73%; M.P. 140-142 ^{o}C; Mol. formula: $C_{12}H_{13}N_3O_3S$; Mol. Wt. 279; IR (KBr, ν_{max} cm^{-1}):3027 (C-H str. of ar. ring), 1646 (C=N str. of oxadiazole ring), 1537 (C=C ar. str.), 1247, 1063 (C-O-C bond str.), 615 (C-S bond str.); ^{1}H-NMR (C_5D_5N, 300 MHz, δ ppm): 8.75 (s, 1H, H-2'), 8.32 (dd, J = 7.8, 1.5 Hz, 1H, H-4'), 8.26 (d, J = 7.5 Hz, 1H, H-6'), 7.64 (t, J = 8.1 Hz, 1H, H-5'), 3.21 (m, 1H, H-1''), 1.73 (quin, J = 7.2 Hz, 2H, H-2''), 1.04 (d, J = 7.2 Hz, 3H, H-4''), 0.99 (t, J = 7.2 Hz, 3H, H-3''); EIMS (*m/z*): 279 (14%)[M^+], 223 (17%), 177 (29%), 163 (35%), 148 (100%), 150 (31%), 122 (43%), 63 (69%), 57 (81%).

5.8 *5-(3-Nitrophenyl)-2-(n-pentylthio)-1,3,4-oxadiazole(6g)*

Yellowish brown amorphous solid; Yield: 75%; M.P. 97-99 ^{o}C; Mol. formula: $C_{13}H_{15}N_3O_3S$; Mol. Wt. 293; IR (KBr, ν_{max} cm^{-1}): 3023 (C-H str. of ar. ring), 1649 (C=N str. of oxadiazole ring), 1535 (C=C ar. str.), 1243, 1068 (C-O-C bond str.), 1254 (C=S bond str.), 617 (C-S bond str.); ^{1}H-NMR (C_5D_5N, 300 MHz, δ ppm): 8.78 (s, 1H, H-2'), 8.33 (dd, J = 7.8, 3.0 Hz, 1H, H-4'), 8.29 (d, J = 7.5 Hz, 1H, H-6'), 7.61 (t, J = 7.8 Hz, 1H, H-5'), 3.31 (t, J= 7.2 Hz, 2H, H-1''), 1.76 (quin, J = 7.5 Hz, 2H, H-2''), 1.32-1.16 (m, 4H, H-3'' & H-4''), 0.79 (t, J = 7.2 Hz, 3H, CH_3-5''); EIMS (*m/z*): 293 (14%)[M^+], 223 (17%), 177 (29%), 163 (35%), 148 (100%), 150 (31%), 122 (43%), 63 (69%), 57 (81%).

5.9 *5-(3-Nitrophenyl)-2-(n-heptylthio)-1,3,4-oxadiazole(6h)*

Yellow sticky solid; Yield: 81%; M.P. 120-122 ^{o}C; Mol. formula: $C_{15}H_{19}N_3SO_3$; Mol. Wt. 321; IR (KBr, ν_{max} cm^{-1}): 3028 (C-H str. of ar. ring), 1651 (C=N str. of oxadiazole ring), 1539 (C=C ar. str.), 1247, 1071 (C-O-C bond str.), 1257 (C=S bond str.), 612 (C-S bond str.); ^{1}H-NMR (C_5D_5N, 300 MHz, δ ppm): 8.73 (s, 1H, H-2'), 8.37 (dd, J = 7.8, 3.0 Hz, 1H, H-4'), 8.26 (d, J = 7.5 Hz, 1H, H-6'), 7.57 (t, J = 7.8 Hz, 1H, H-5'), 3.29 (t, J= 7.2 Hz, 2H, H-1''), 1.73 (quint, J = 7.5 Hz, 2H, H-2''), 1.32-1.16 (m, 4H, H-3'' & H-4''), 1.08-1.04 (m, 2H, H-5''), 0.84 (t, J = 7.2 Hz, 3H, CH_3-6''); EIMS (*m/z*): 321 (13%)[M^+], 223 (19%), 177 (27%), 163 (31%), 148 (100%), 150 (35%), 122 (41%), 63 (71%), 57 (81%).

5.10 *5-(3-Nitrophenyl)-2-(allylthio)-1,3,4-oxadiazole(6i)*

Brown amorphous solid; Yield: 78%; M.P. 188-190 ^{o}C; Mol. formula: $C_{11}H_9N_3O_3S$; Mol. Wt. 263; IR (KBr, ν_{max} cm^{-1}): 3025 (C-H str. of ar. ring), 1657 (C=N str. of oxadiazole ring), 1537 (C=C ar. str.), 1245, 1075 (C-O-C bond str.), 1253 (C=S bond str.), 609 (C-S bond str.); ^{1}H-NMR (C_5D_5N, 300 MHz, δ ppm): 8.74 (s, 1H, H-2'), 8.35 (dd, J = 7.8, 3.0 Hz, 1H, H-4'), 8.23 (d, J = 7.5 Hz, 1H, H-6'), 7.56 (t, J = 7.8 Hz, 1H, H-5'), 5.97 (m, 1H, H-2''), 5.35 (brd, J = 17.2 Hz, 1H, H_b-3''), 5.19 (brd, J = 9.6 Hz, 1H, H_a-3''), 3.89 (d, J = 7.2 Hz, 2H, H-1''); EIMS (*m/z*): 263 (13%)[M^+], 223 (18%), 177 (25%), 164 (33%), 148 (100%), 117 (40%), 63 (80%).

5.11 *5-(3-Nitrophenyl)-2-(2-phenylethylthio)-1,3,4-oxadiazole(6j)*

White amorphous solid; Yield: 83%; M.P. 118-120 ^{o}C; Mol. formula: $C_{16}H_{13}N_3O_3S$; Mol. Wt. 327; IR (KBr, ν_{max} cm^{-1}): 3029 (C-H str. of ar. ring), 1659 (C=N str. of oxadiazole ring), 1531 (C=C ar. str.), 1241, 1079 (C-O-C bond str.), 1257 (C=S bond str.), 612 (C-S bond str.); ^{1}H-NMR (C_5D_5N, 300 MHz, δ ppm): 8.64 (s, 1H, H-2'), 8.24-8.21 (m, 2H, H-4' & H-6'), 7.51 (t, J = 8.1 Hz, 1H, H-5'), 7.19-7.12 (m, 5H, H-2'' to H-6''), 3.55 (t, J = 6.9 Hz, 2H, H-8''), 3.11 (t, J = 6.9 Hz, 2H, H-7''); EIMS (*m/z*): 327 (11%)[M^+], 223 (17%), 177 (23%), 163 (29%), 148 (100%), 150 (31%), 122 (43%), 91 (71%).

5.12 *5-(3-Nitrophenyl)-2-(2-Chlorobenzylthio)-1,3,4-oxadiazole(6k)*

Brown sticky solid; Yield: 80%; M.P. 112-114 ^{o}C; Mol. formula: $C_{15}H_{10}ClN_3O_3S$; Mol. Wt. 347; IR (KBr, ν_{max} cm^{-1}): 3037 (C-H str. of ar. ring), 1653 (C=N str. of oxadiazole ring), 1535 (C=C ar. str.), 1247, 1073 (C-O-C bond str.), 1252 (C=S bond str.), 616 (C-S bond); ^{1}H-NMR (C_5D_5N, 300 MHz, δ ppm): 8.74 (s, 1H, H-2'), 8.29 (dd, J = 8.4, 1.2 Hz, 1H, H-4'), 8.24 (d, J = 7.8 Hz, 1H, H-6'), 7.60 (t, J = 8.1 Hz, 1H, H-5'), 7.45-7.20 (m, 4H, H-3'' to H-6''), 4.75 (s,

2H, H-7"); EIMS (*m/z*): 347 (13%)[M^+], 223 (19%), 177 (27%), 163 (34%), 148 (100%), 150 (39%), 122 (47%), 91 (61%).

5.13 *2-(4-Chlorophenyl)-5-(4-chlorobenzylthio)-1,3,4-oxadiazole(6l)*

Light brown amorphous solid; Yield: 80%; M.P. 122-124 ^oC; Mol. formula: $C_{15}H_{10}ClN_3O_3S$; Mol. Wt. 347; IR (KBr, ν_{max} cm^{-1}): 3039 (C-H str. of ar. ring), 1656 (C=N str. of oxadiazole ring), 1534 (C=C ar. str.), 1242, 1078 (C-O-C bond str.), 1259 (C=S bond str.), 623 (C-S bond str.); ^{1}H-NMR (C_5D_5N, 300 MHz, δ ppm): 8.71 (s, 1H, H-2'), 7.49 (t, *J* = 8.1 Hz, 1H, H-5'), 7.34 (d, *J* = 9.0 Hz, 2H, H-2" & H-6"), 7.31-7.27 (m, 2H, H-4' & H-6'), 7.24 (d, *J* = 9.0 Hz, 2H, H-3" & H-5"), 4.49 (s, 2H, H-7"); EIMS (*m/z*): 347.5 (17%)[M^+], 223 (23%), 177 (31%), 163 (37%), 148 (100%), 150 (32%), 122 (49%), 91 (61%).

5.14 *5-(4-Chlorophenyl)-2-(2-Bromobenzylthio)-1,3,4-oxadiazole(6m)*

Dark green amorphous solid; Yield: 85%; M.P. 116-118 ^oC; Mol. formula: $C_{15}H_{10}BrN_3O_3S$; Mol. Wt. 392; IR (KBr, ν_{max} cm^{-1}): 3021 (C-H str. of ar. ring), 1649 (C=N str. of oxadiazole ring), 1527 (C=C ar. str.), 1253, 1067 (C-O-C bond str.), 1259 (C=S bond str.), 613 (C-S bond str.); ^{1}H-NMR (C_5D_5N, 300 MHz, δ ppm): 9.67 (s, 1H, H-2'), 7.69 (dd, *J* = 8.1, 2.1 Hz, 1H, H-4'), 7.65 (d, *J* = 8.1 Hz, 1H, H-6'), 7.57 (t, *J* = 8.1 Hz, 1H, H-5'), 7.48 (dt,*J* = 8.1, 2.1 Hz,1H, H-4"), 7.38 (dd, *J* = 8.1, 2.1 Hz, 1H, H-3"), 7.23 (dd, *J* = 8.1, 2.1 Hz, 1H, H-6"), 7.09 (dt, *J* = 8.1, 2.1 Hz, 1H, H-5"), 4.71 (s, 2H, H-7"); EIMS (*m/z*): 392 (10%)[M^+], 223 (17%), 177 (32%), 163 (47%), 148 (100%), 150 (31%), 122 (49%), 91 (57%).

5.15 *Antibacterial assay*

The antibacterial assay was executed in sterile 96-wells microplates under aseptic conditions. The method worked on the principle that microbial cell number enhances as the microbial growth proceeds in a long phase of growth resulting in raised absorbance of broth medium[18, 19]. For the antibacterial study, clinically isolated two gram-positive bacteria (*Bacillus subtilis and Staphylococcus aureus*) and four gram-negative (*Shigella sonnei, Escherichia coli, Pseudomonas aeruginosa and Salmonella typhi*) were utilized. The organisms were kept on stock culture agar medium. The total volume per well was 200 µL including 20 µg test samples (with suited solvents & dilutions) and 180 µL an overnight maintained fresh bacterial culture after reasonable dilution with fresh nutrient broth. The initial absorbance of the culture was solely maintained between 0.12-0.19 at 540 nm. The incubation was executed at 37 ^oC for 16-24 hrs with lid on the micro plate. The absorbance was measured at 540 nm using micro plate reader before and after incubation and the difference was noted as an index of bacterial growth. The percent inhibition was calculated using the formula:

$$\textbf{Inhibition } \% = \frac{\textbf{Control} - \textbf{Test}}{\textbf{Control}} \times \textbf{100}$$

Where Control = Absorbance in control with bacterial culture
Test = Absorbance in test sample

Results are mean of triplicate (n=3, ± sem). Ciprofloxacin and Gentamycin were taken as standard. Minimum inhibitory concentration (MIC) was measured with suitable dilutions (5-30 µg/well) and results were calculated using EZ-Fit Perrella Scientific Inc. Amherst USA software, and data was expressed as MIC.

5.16 *Statistical Analysis*

All the measurements were performed in triplicate and statistical analysis was performed by Microsoft Excel 2010. Results are presented as mean ± sem.

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