Synthesis, spectral analysis and antibacterial activity of some novel 5-substituted-2-((6-bromo-3,4-methylenedioxybenzyl)thio)-1,3,4-oxadiazole derivatives

Aziz-ur-Rehman[1,*], Asia Siddiqa[1], Muhammad Athar Abbasi[1], Sabahat Zahra Siddiqui[1], Samreen Gul Khan[2], Shahid Rasool[1] and Syed Adnan Ali Shah[3,4]

[1]Department of Chemistry, Government College University, Lahore, Pakistan
[2]Department of Chemistry, Government College University, Faisalabad, Pakistan
[3]Faculty of Pharmacy, Universiti Teknologi MARA, Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor Darul Ehsan, Malaysia
[4]Atta-ur-Rahman Institute for Natural Products Discovery (AuRIns), Universiti Teknologi MARA, Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor Darul Ehsan, Malaysia

Abstract: A number of novel 5-substituted-2-((6-bromo-3,4-methylenedioxybenzyl)thio)-1,3,4-Oxadiazole derivatives (6a-l) have been synthesized to evaluate their antibacterial activity. Using aryl/aralkyl carboxylic acids (1a-l) as precursors, 5-substituted-1,3,4-Oxadiazol-2-thiols (4a-l) were yielded in good amounts. The derivatives, 4a-l, were subjected to electrophilic substitution reaction on stirring with 6-bromo-3,4-methylenedioxybenzyl chloride (5) in DMF to synthesize the required compounds. All the synthesized molecules were well characterized by IR, ^{1}H-NMR, ^{13}C-NMR and EIMS spectral data and evaluated for antibacterial activity against some bacterial strains of Gram-bacteria. The molecule, 6d, demonstrated the best activity among all the synthesized molecules exhibiting weak to moderate inhibition potential.

Keywords:1,3,4-Oxadiazoles, antibacterial activity, carboxylic acids,spectral analysis.

INTRODUCTION

Oxadiazoles have been known to possess pharmacological activities with medicinal and agricultural importance (Dabholkar and Bhusari, 2011 and Rashid *et al.*, 2012). Among these molecules, 1,3,4-Oxadiazoles have exhibited radical activities including antibacterial, anti-enzymatic etc (Dabholkar and Bhusari, 2011; Rashid *et al.*, 2012 and Sahin *et al.*, 2002). All the substituted 1,3,4-Oxadiazoles have been synthesized and evaluated for their biological activities (Hui *et al.*, 2002; Mohan *et al.*, 2004 and Omer *et al.*, 1996). 3,4-Methylenedioxyphenyl moiety, attached to 1,3,4-Oxadiazole nucleus through *S*-linkage in the synthesized molecules, have been found to be the part of many antitumor, anticancer and antidepressant drugs such as lycoricidine, narciclasine, pancratistatin (Ingrassia *et al.*, 2008) and paroxetine (Bar-Oz *et al.*, 2007).

1,3,4-Oxadiazoles have been known to possess a broad spectrum of biological activities which prompted us to synthesize derivatives of this class. In continuation of our last projects on synthesis of *S*-substituted derivatives of 1,3,4-Oxadiazole (Aziz-ur-Rehman *et al.*, 2013a; Aziz-ur-Rehman *et al.*, 2016 and Khalid *et al.*, 2016), a series of 5-substituted-2-((6-bromo-3,4-methylenedioxybenzyl) thio)-1,3,4-Oxadiazole derivatives have been synthesized and evaluated for their antibacterial activity. The molecules demonstrated weak to moderate activity against all the bacterial strains.

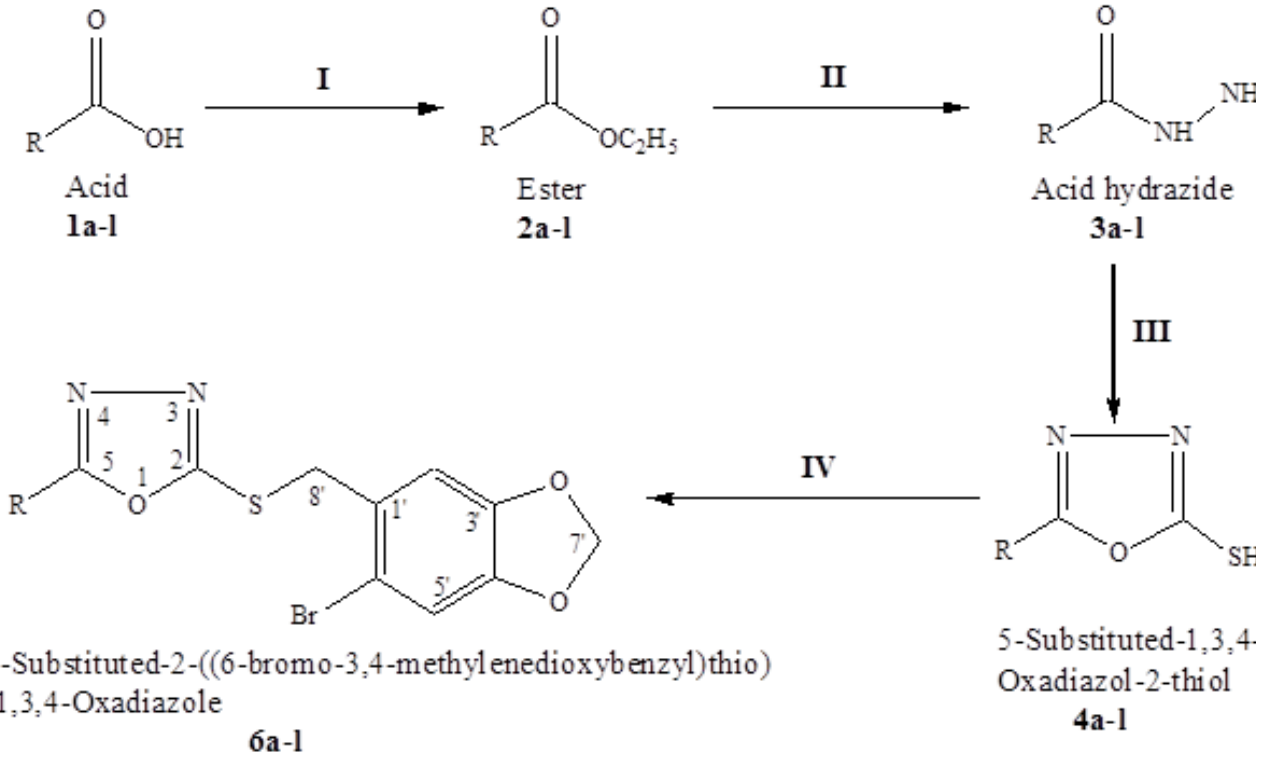
*Corresponding author: e-mail: azizryk@yahoo.com

MATERIALS AND METHODS

The precursors, carboxylic acids, and other reagents of analytical grade were purchased from Alfa Aesar, Merck and Sigma-Aldrich. Griffin-George apparatus was used to visualize melting points with open capillary tubes and all were uncorrected. Thin layer chromatography (TLC) was a source to observe the purity of synthesized molecules and was performed on silica gel G-25-UV_{254} coated plates with EtOAc and *n*-hexane as solvent systems. Jasco-320-A spectrophotometer recorded infrared spectra, with wave number in cm^{-1} on abscissa and absorbance on ordinate, by KBr pellet method. Bruker spectrometers recorded ^{1}H-NMR operating at varying frequency of 300, 400 & 500 MHz and ^{13}C-NMR at 100 MHz in $CHCl_3$-d_1. The *d*-values are given in ppm comparable to TMS, the internal reference, and *J*-values in Hz. JMS-HX-110 spectrometer recorded EIMS with mass number of fragment on abscissa and %age abundance on ordinate.

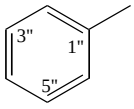
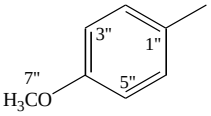
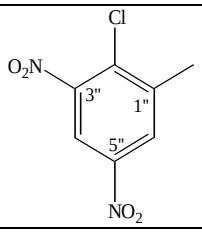
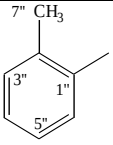
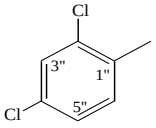
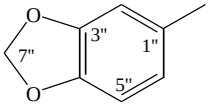
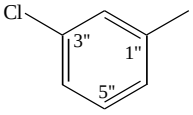
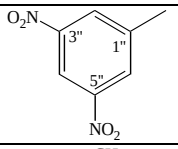
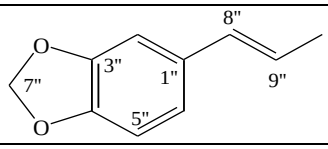
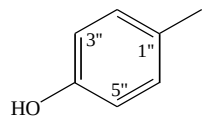
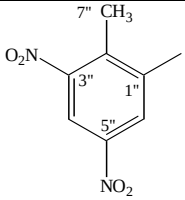
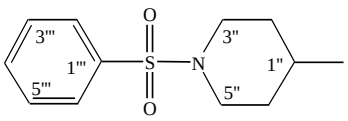
General procedure for the synthesis of ethyl esters (2a-l)

The esterification of substituted organic acids (1a-l; 2.0g) in 8.0mL C_2H_5OH was catalyzed by 1.0mL concentrated H_2SO_4 under reflux for 2-5 hours in a reaction flask. Reaction completion was checked out by TLC. Because of reversibility of reaction, the reaction mixture with maximal completion was transferred to a 250 mL



Scheme 1: Synthesis of 5-substituted-2-((6-bromo-3,4-methylenedioxybenzyl)thio)-1,3,4-Oxadiazole. Reagents and conditions: (I) C_2H_5OH/H^+, refluxing for 2-5 hours (II) $N_2H_4.H_2O/C_2H_5OH$, stirring or refluxing for 3-6 hours (III) $CS_2/KOH/C_2H_5OH$, refluxing for 4-6 hours (IV) 6-Bromo-3,4-methylenedioxybenzyl chloride/LiH/DMF, stirring for 3-5 hours

Table 1: Different 5-substituted aryl/aralkyl groups

Comp.	R	Comp.	R	Comp.	R
6a		6e		6i	
6b		6f		6j	
6c		6g		6k	
6d		6h		6l	

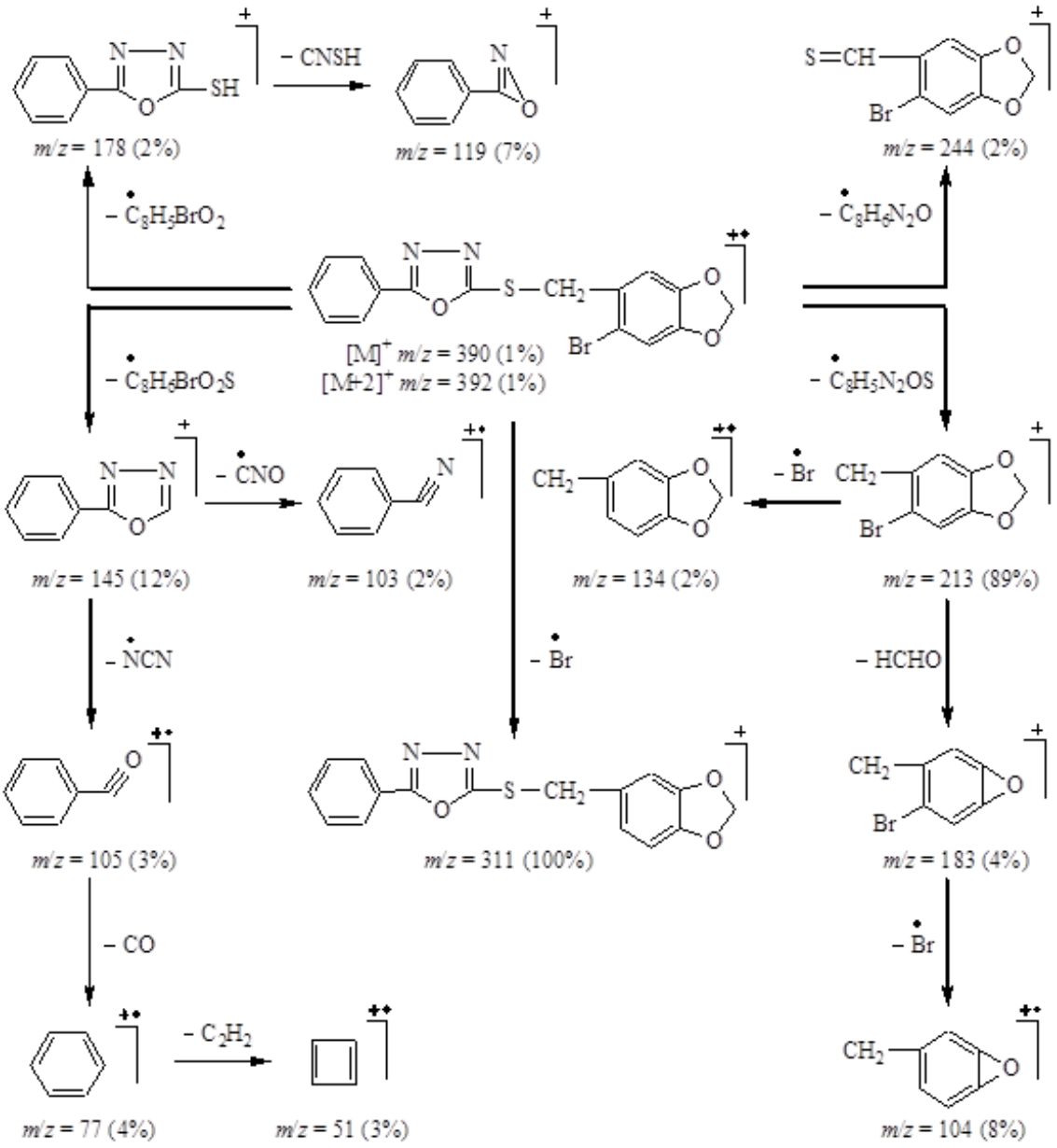


Fig. 1: Mass fragmentation pattern of 5-phenyl-2-((6-bromo-3,4-methylenedioxybenzylthio)-1,3,4-Oxadiazole(6a)

separating funnel followed by 68mL distilled water and 24mL diethyl ether. The mixture was basified by concentrated aqueous Na_2CO_3 solution till constant pH 8-10. The title compounds were extracted from mixture by ether which was separated from products by distillation.

General procedure for the synthesis of carbohydrazides (3a-l)

Various ethyl esters (2a-l; 0.06mol) were converted to corresponding carbohydrazides in 50.0mL ethanol, after continuous stirring with 12.0mL 80% $N_2H_4.H_2O$ in a 100 mL RB flask for 3-6 hours. In some cases, the reaction mixture was set to reflux also. The completion was observed by TLC. Two third of solvent was evaporated and excess of distilled water was added for precipitation on shaking. The precipitates were filtered, washed with distilled water or *n*-hexane and finally dried.

General procedure for the synthesis of 5-substituted-1,3,4-Oxadiazol-2-thiols (4a-l)

The compounds, 3a-l (0.05mol) were taken in a 100mL RB flask, dissolved in 35.0mL ethanol and then basified by solid KOH (0.05 mol). The mixture was refluxed till complete dissolution of solid KOH and cooled to 27ºC, room temperature. Then carbon disulfide (0.10mol) was poured to the flask and refluxed again for 4-6 hours. TLC was employed to verify the reaction completion. The mixture was diluted by excess of distilled water and a pH=4-5 was set by 4-6mL dilute HCl. The system was kept undisturbed at room temperature for 10-15 minutes

and then precipitates were acquired *via* filtration followed by washing and drying.

General procedure for the preparation of 5-substituted-2-((6-bromo-3,4-methylenedioxybenzyl)thio)-1,3,4-Oxadiazole (6a-l)

The synthesized compounds, 4a-l (0.004mol) were taken in a 50mL RB flask and dissolved in 12.0mL DMF on shaking. The molecules were activated by LiH (0.004 mol) after stirring for 35 minutes. Then 6-bromo-3,4-methylenedioxybenzyl chloride (5; 0.004 mol) was added and the stirring was continued for 3-5 hours, monitored by TLC. Excess cold distilled water was added and the products were acquired by filtration or solvent extraction.

Characterization of the synthesized compounds

5-Phenyl-2-((6-bromo-3,4-methylenedioxybenzyl)thio)-1,3,4-Oxadiazole (6a)

White amorphous solid; Yield: 97%; M.P: 153°C; Molecular formula: $C_{16}H_{11}BrN_2O_3S$; Molecular mass: 390; IR (KBr): ν_{max} (cm^{-1}): 3059 (Ar-H str.), 1541 (Ar C=C str.), 1133 (C-O str.), 675 (C-Br str.), 613 (C-S str.); ^{1}H-NMR ($CDCl_3$,500 MHz, δ/ppm): 7.98 (dd, *J*=7.5, 1.0 Hz, 2H, H-2" & 6"), 7.49-7.45 (m, 3H, H-3" to 5"), 7.13 (s, 1H, H-2'), 7.01 (s, 1H, H-5'), 5.94 (s, 2H, H-7'), 4.54 (s, 2H, H-8'); ^{13}C-NMR ($CDCl_3$,100 MHz, δ/ppm): 165.2 (C-5), 164.7 (C-2), 150.8 (C-4'), 148.6 (C-3'), 134.1 (C-3" & 5"), 133.2 (C-2" & 6"), 132.7 (C-4"), 131.2 (C-1"), 129.6 (C-1'), 116.4 (C-6'), 114.3 (C-5'), 110.8 (C-2'), 102.3 (C-7'), 32.5 (C-8'); EIMS (*m/z*): 392 $[M+2]^+$ (1%), 390 $[M]^+$ (1%), 311 (100%), 244 (2%), 213 (89%), 183 (4%), 178 (2%), 145 (12%), 134 (3%), 119 (7%), 105 (3%), 104 (8%), 103 (2%), 77 (4%), 51 (3%).

5-(2-Methylphenyl)-2-((6-bromo-3,4-methylenedioxybenzyl)thio)-1,3,4-Oxadiazole (6b)

Yellow solid; Yield: 98%; M.P: 122°C; Mol formula: $C_{17}H_{13}BrN_2O_3S$; Molecular mass: 404; IR (KBr): ν_{max} (cm^{-1}): 3055 (Ar-H str.), 1534 (Ar C=C str.), 1124 (C-O str.), 654 (C-Br str.), 608 (C-S str.); ^{1}H-NMR ($CDCl_3$,400 MHz, δ/ppm): 7.80 (d, *J*=7.6 Hz, 1H, H-6"), 7.39 (t, *J*= 7.2 Hz,1H, H-4"), 7.31 (d, *J*=7.6 Hz, 1H, H-3"), 7.28 (t, *J* =7.6 Hz, 1H, H-5"), 7.10 (s, 1H, H-2'), 6.85 (s, 1H, H-5'), 5.95 (s, 2H, H-7'), 4.53 (s, 2H, H-8'), 3.78 (s, 3H, H-7"); ^{13}C-NMR ($CDCl_3$,100 MHz, δ/ppm): 164.8 (C-2), 164.3 (C-5), 150.8 (C-4'), 148.2 (C-3'), 133.9 (C-2"), 130.6 (C-4"), 129.8 (C-1'), 128.7 (C-3"), 127.2 (C-1"), 126.5 (C-5"), 125.8 (C-6"), 116.7 (C-6'), 114.2 (C-5'), 110.7 (C-2'), 102.5 (C-7'), 32.8 (C-8'), 20.3 (C-7"); EIMS (*m/z*): 406 $[M+2]^+$ (0.5%), 404 $[M]^+$ (0.5%), 325 (100%), 244 (2%), 213 (76%), 192 (5%), 183 (8%), 160 (2%), 134 (3%), 133 (4%), 119 (2%), 117 (3%), 104 (9%), 91 (30%), 65 (10%), 51 (2%).

5-(3-Chlorophenyl)-2-((6-bromo-3,4-methylenedioxybenzyl)thio)-1,3,4-Oxadiazole (6c)

Golden solid; Yield: 98%; M.P: 130 °C; Mol formula: $C_{16}H_{10}BrClN_2O_3S$; Mol mass: 424; IR (KBr): ν_{max} (cm^{-1}): 3067 (Ar-H str.), 1543 (Ar C=C str.), 1131 (C-O str.), 702 (C-Cl str.), 665 (C-Br str.), 616 (C-S str.); ^{1}H-NMR ($CDCl_3$,400 MHz, δ/ppm): 7.96 (s, 1H, H-2"), 7.88 (d, *J* =7.6 Hz, 1H, H-4"), 7.48 (d, *J*=8.0 Hz, 1H, H-6"), 7.41 (t, *J*=8.0 Hz, 1H, H-5"), 7.08 (s, 1H, H-2'), 6.84 (s, 1H, H-5'), 5.95 (s, 2H, H-7'), 4.53 (s, 2H, H-8'); ^{13}C-NMR ($CDCl_3$,100 MHz, δ/ppm): 165.6 (C-5), 164.2 (C-2), 150.4 (C-4'), 148.7 (C-3'), 133.8 (C-3"), 130.9 (C-2"), 130.3 (C-1"), 129.7 (C-1'), 128.8 (C-4"), 127.5 (C-6"), 126.8 (C-5"), 116.4 (C-6'), 114.8 (C-5'), 110.3 (C-2'), 102.6 (C-7'), 32.3 (C-8'); EIMS (*m/z*): 428 $[M+4]^+$ (1%), 426 $[M+2]^+$ (1.3%), 424 $[M]^+$ (1%), 389 (3%), 345 (100%), 244 (2%), 214 (86%), 213 (23%), 212 (13%), 183 (4%), 179 (19%), 153 (4%), 139 (3%), 137 (3%), 134 (3%), 111 (7%), 104 (9%), 85 (12%), 51 (2%).

5-(4-Hydroxyphenyl)-2-((6-bromo-3,4-methylenedioxybenzyl)thio)-1,3,4-Oxadiazole (6d)

Creamy white amorphous solid; Yield: 98%; M.P: 203°C; Molecular formula: $C_{16}H_{11}BrN_2O_4S$; Molecular mass: 406; IR (KBr): ν_{max} (cm^{-1}): 3069 (Ar-H str.), 1544 (Ar C=C str.), 1133 (C-O str.), 662 (C-Br str.), 613 (C-S str.); ^{1}H-NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.88 (d, *J*=8.4 Hz, 2H, H-2" & 6"), 6.92 (d, *J*=8.8 Hz, 2H, H-3" & 5"), 7.12 (s, 1H, H-2'), 7.00 (s, 1H, H-5'), 5.94 (s, 2H, H-7'), 4.54 (s, 2H, H-8'); ^{13}C-NMR ($CDCl_3$,100 MHz, δ/ppm): 165.7 (C-5), 164.2 (C-2), 162.6 (C-4"), 150.1 (C-4'), 148.5 (C-3'), 129.8 (C-1'), 123.3 (C-1"), 122.6 (C-2" & 6"), 119.2 (C-3" & 5"), 116.5 (C-6'), 114.8 (C-5'), 110.4 (C-2'), 102.1 (C-7'), 32.7 (C-8'); EIMS (*m/z*): 408 $[M+2]^+$ (0.5%), 406 $[M]^+$ (0.5%), 327 (100%), 244 (2%), 213 (78%), 193 (5%), 183 (4%), 161 (2%), 135 (4%), 134 (20%), 121 (6%), 119 (4%), 104 (7%), 93 (15%), 67 (9%), 51 (4%).

5-(4-Methoxyphenyl-2-((6-bromo-3,4-methylenedioxybenzyl)thio)-1,3,4-Oxadiazole (6e)

White amorphous solid; Yield: 95%; M.P: 153°C; Molecular formula: $C_{17}H_{13}BrN_2O_4S$; Molecular mass: 420; IR (KBr): ν_{max} (cm^{-1}): 3071 (Ar-H str.), 1547 (Ar C=C str.), 1138 (C-O str.), 667 (C-Br str.), 616 (C-S str.); ^{1}H-NMR ($CDCl_3$,300 MHz, δ/ppm): 7.92 (d, *J*=8.7 Hz, 2H, H-2" & 6"), 7.12 (s, 1H, H-5'), 7.00 (s, 1H, H-2'), 6.98 (d, *J*=8.7 Hz, 2H, H-3" & 5"), 5.94 (s, 2H, H-7'), 4.52 (s, 2H, H-8'), 3.85 (s, 3H, H-7"); ^{13}C-NMR ($CDCl_3$,100 MHz, δ/ppm): 165.1 (C-5), 164.6 (C-2), 162.2 (C-4"), 150.7 (C-4'), 148.3 (C-3'), 129.8 (C-1'), 124.6 (C-1"), 117.1 (C-2" & 6"), 116.4 (C-3" & 5"), 115.8 (C-6'), 114.4 (C-5'), 110.7 (C-2'), 102.8 (C-7'), 32.3 (C-8'), 54.6 (C-7"); EIMS (*m/z*): 422 $[M+2]^+$ (1%), 420 $[M]^+$ (1%), 341 (100%), 244 (2%), 213 (86%), 208 (16%), 183 (4%), 175 (7%), 149 (2%), 135 (7%), 134 (3%), 133 (4%), 107 (3%), 104 (9%), 81 (40%), 51 (2%).

5-(2,4-Dichlorophenyl)-2-((6-bromo-3,4-methylenedioxybenzyl)thio)-1,3,4-Oxadiazole(6f)

Grey solid; Yield: 91%; M.P: 160°C; Mol formula: $C_{16}H_9BrCl_2N_2O_3S$; Molecular mass: 458; IR (KBr): ν_{max} (cm^{-1}): 3064 (Ar-H str.), 1545 (Ar C=C str.), 1139 (C-O

Table 2:%age inhibition of antibacterial activity

Compound	%age inhibition				
	S. typhi (-)	*E. coli (-)*	*P. aeroginosa (-)*	*B. subtilis (+)*	*S. aureus (+)*
6a	40.06±4.76	51.63±4.58	48.25±0.42	39.67±2.92	42.40±3.40
6b	56.41±4.65	70.11±2.96	54.33±0.67	45.58±1.42	51.10±2.40
6c	75.82±1.00	75.47±1.89	63.42±1.75	52.17±0.25	61.45±3.85
6d	63.47±1.24	76.11±1.42	63.25±0.58	58.63±1.13	61.10±2.10
6e	31.71±1.94	54.11±2.32	53.00±0.42	24.96±1.96	43.85±3.35
6f	48.88±1.82	57.58±0.95	58.25±2.67	51.88±4.29	54.80±0.90
6g	34.12±2.24	54.32±4.00	46.42±0.33	38.63±1.54	43.75±3.65
6h	64.29±5.00	73.47±1.58	58.46±2.46	54.17±2.58	58.70±0.70
6i	35.18±2.65	62.35±3.45	53.34±1.12	52.13±0.87	52.89±2.09
6j	58.41±0.76	62.84±0.84	65.88±1.79	42.46±5.00	51.75±2.55
6k	61.82±1.00	69.37±2.21	57.29±3.38	51.25±1.25	55.85±5.00
6l	24.35±2.59	62.26±4.37	55.46±4.54	39.29±5.00	35.90±3.60
Ciprofloxacin	91.19±2.10	90.44±1.23	92.00±2.76	89.98±2.07	92.21±1.59

Table 3:MIC values of antibacterial activity

Compound	MIC (µM)				
	S. typhi (-)	*E. coli (-)*	*P. aeroginosa (-)*	*B. subtilis (+)*	*S. aureus (+)*
6a	-	18.78±3.43	-	-	-
6b	18.50±1.56	14.62±1.95	18.27±1.83	-	16.29±4.27
6c	14.26±2.00	11.35±1.13	14.48±1.53	18.65±1.32	12.58±2.40
6d	12.10±1.43	10.98±1.85	12.56±2.25	14.39±2.12	12.88±2.40
6e	-	18.00±3.35	18.72±1.67	-	-
6f	-	17.18±2.65	17.25±2.25	19.15±3.50	14.00±2.13
6g	-	18.17±1.35	-	-	-
6h	15.20±2.47	11.69±2.70	15.02±4.71	14.37±3.16	12.80±4.47
6i	-	14.67±1.99	18.56±0.97	17.39±2.19	19.04±1.85
6j	15.89±1.69	12.40±1.50	12.63±2.13	-	15.13±1.79
6k	11.70±1.76	14.36±2.55	15.33±2.21	17.27±1.54	16.49±1.73
6l	-	16.42±2.15	18.36±3.96	-	-
Ciprofloxacin	9.42±1.09	8.02±2.17	8.11±1.32	8.88±2.00	9.23±1.87

Note: 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.

str.), 705 (C-Cl str.), 668 (C-Br str.), 613 (C-S str.); ^{1}H-NMR ($CDCl_3$,400 MHz, δ/ppm): 7.89 (d, J=8.8 Hz, 1H, H-6''), 7.56 (d, J=2.0 Hz, 1H, H-3''), 7.35 (dd, J=8.8, 1.6 Hz, 1H,H-5''), 7.10 (s, 1H, H-2'), 6.85 (s, 1H, H-5'), 5.95 (s, 2H, H-7'), 4.53 (s, 2H, H-8'); ^{13}C-NMR ($CDCl_3$,100 MHz, δ/ppm): 168.2 (C-5), 164.7 (C-2), 150.3 (C-4'), 148.6 (C-3'), 137.2 (C-4''), 133.6 (C-2''), 132.9 (C-6''), 131.2 (C-3''), 129.6 (C-1'), 128.3 (C-5''), 127.1 (C-1''), 116.4 (C-6'), 114.5 (C-5'), 110.2 (C-2'), 102.7 (C-7'), 32.5 (C-8'); EIMS (*m/z*): 464 $[M+6]^+$ (1%), 462 $[M+4]^+$ (1.6%), 460 $[M+2]^+$ (1.3%), 458 $[M]^+$ (1%), 380 (100%), 246 (3%), 244 (2%), 213 (84%), 187 (4%), 183 (4%), 173 (3%), 171 (6%), 145 (5%), 134 (3%), 119 (4%), 105 (9%), 85 (10%).

5-(3,5-Dinitrophenyl)-2-((6-bromo-3,4-methylenedioxybenzyl)thio)-1,3,4-Oxadiazole (6g)

Bright yellow solid; Yield: 98%; M.P: 190^{o}C; Mol formula: $C_{16}H_9BrN_4O_7S$; Molecular mass: 480; IR (KBr): ν_{max} (cm^{-1}): 3063 (Ar-H str.), 1542 (Ar C=C str.), 1126 (C-O str.), 661 (C-Br str.), 617 (C-S str.); ^{1}H-NMR ($CDCl_3$,400 MHz, δ/ppm): 8.30 (s, 1H, H-4''), 8.27 (s, 2H, H-2'' & 6''), 7.12 (s, 1H, H-2'), 6.84 (s, 1H, H-5'), 5.96 (s, 2H, H-7'), 4.62 (s, 2H, H-8'); ^{13}C-NMR ($CDCl_3$,100 MHz, δ/ppm): 171.3 (C-5), 164.7 (C-5), 150.9 (C-4'), 149.2 (C-3'' & C-5''), 147.2 (C-3'), 135.3 (C-1''), 129.7 (C-1'), 127.3 (C-3'' & C-6''), 117.4 (C-4''), 116.8 (C-6'), 114.1 (C-5'), 110.5 (C-2'), 102.9 (C-7'), 32.6 (C-8'); EIMS (*m/z*): 482 $[M+2]^+$ (1%), 480 $[M]^+$ (1%), 401 (40%), 268 (11%), 244 (2%), 235 (19%), 213 (100%), 209 (12%), 195 (10%), 193 (15%), 183 (8%), 167 (5%), 137 (4%), 134 (3%), 107 (6%), 104 (5%).

5-(2-Methyl-3,5-dinitrophenyl)-2-((6-bromo-3,4-methylenedioxybenzyl)thio)-1,3,4-Oxadiazole (6h)

Creamy white amorphous solid; Yield: 97%; M.P: 145 ^{o}C; Mol formula: $C_{17}H_{11}BrN_4O_7S$; Molecular mass: 494; IR (KBr): ν_{max} (cm^{-1}): 3054 (Ar-H str.), 1532 (Ar C=C str.),

1124 (C-O str.), 651 (C-Br str.), 607 (C-S str.); ^{1}H-NMR ($CDCl_3$,400 MHz, δ/ppm): 7.28 (d, *J*=2.4 Hz, 1H, H-4"), 7.14 (d, *J*=2.8 Hz, 1H, H-6"), 7.10 (s, 1H, H-2'), 6.84 (s, 1H, H-5'), 5.96 (s, 2H, H-7'), 4.53 (s, 2H, H-8'); ^{13}C-NMR ($CDCl_3$,100 MHz, δ/ppm): 170.3 (C-5), 164.7 (C-2), 150.8 (C-4'), 149.5 (C-3"), 148.1 (C-3'), 147.6 (C-5"), 136.3 (C-1"), 129.7 (C-1'), 125.8 (C-6"), 124.9 (C-2"), 118.5 (C-4"), 116.8 (C-6'), 114.4 (C-5'), 110.1 (C-2'), 102.6 (C-7'), 32.9 (C-8'), 19.2 (C-7"); EIMS (*m/z*): 496 $[M+2]^+$ (1%), 494 $[M]^+$ (1%), 415 (35%), 282 (6%), 249 (8%), 244 (2%), 223 (9%), 213 (100%), 209 (15%), 207 (8%), 183 (4%), 181 (4%), 134 (3%), 104 (9%).

5-(2-Chloro-3,5-dinitrophenyl)-2-((6-bromo-3,4-methylenedioxybenzyl)thio)-1,3,4-Oxadiazole (6i)

White amorphous solid; Yield: 93%; M.P: 184°C; Mol formula: $C_{16}H_8BrClN_4O_7S$; Molecular mass: 514; IR (KBr): ν_{max} (cm^{-1}): 3069 (Ar-H str.), 1551 (Ar C=C str.), 1137 (C-O str.), 701 (C-Cl str.), 669 (C-Br str.), 617 (C-S str.); ^{1}H-NMR ($CDCl_3$,400 MHz, δ/ppm): 8.34 (s, 1H, H-4"), 8.16 (s, 1H, H-6"), 7.13 (s, 1H, H-2'), 6.85 (s, 1H, H-5'), 5.95 (s, 2H, H-7'), 4.54 (s, 2H, H-8'); ^{13}C-NMR ($CDCl_3$,100 MHz, δ/ppm): 171.7 (C-5), 164.3 (C-2), 152.1 (C-3"), 150.6 (C-4'), 149.0 (C-5"), 148.3 (C-3'), 137.1 (C-1"), 132.7 (C-2"), 129.5 (C-1'), 125.9 (C-6"), 124.2 (C-4"), 116.8 (C-6'), 114.4 (C-5'), 110.5 (C-2'), 102.2 (C-7'), 32.6 (C-8'); EIMS (*m/z*): 518 $[M+4]^+$ (1%), 516 $[M+2]^+$ (1.3%), 514 $[M]^+$ (1%), 435 (22%), 302 (16%), 269 (17%), 244 (2%), 243 (9%), 229 (2%), 227 (2%), 213 (100%), 201 (3%), 183 (4%), 134 (5%), 104 (9%).

5-(3,4-Methylenedioxyphenyl)-2-((6-bromo-3,4-methylenedioxybenzyl)thio)-1,3,4-Oxadiazole (6j)

Grey solid; Yield: 96%; M.P: 176°C; Mol formula: $C_{17}H_{11}BrN_2O_5S$; Molecular mass: 434; IR (KBr): ν_{max} (cm^{-1}): 3067 (Ar-H str.), 1540 (Ar C=C str.), 1133 (C-O str.), 660 (C-Br str.), 610 (C-S str.); ^{1}H-NMR ($CDCl_3$,300 MHz, δ/ppm): 7.51 (dd, *J*=8.1, 1.2 Hz, 1H, H-6"), 7.42 (d, *J*=1.6 Hz, 1H, H-2"), 7.12 (s, 1H, H-2'), 7.00 (s, 1H, H-5'), 6.88 (d, *J*=8.1 Hz, 1H, H-5"), 6.03 (s, 2H, H-7'), 5.94 (s, 2H, H-7"), 4.52 (s, 2H, H-8'); ^{13}C-NMR ($CDCl_3$,100 MHz, δ/ppm): 164.7 (C-2), 164.4 (C-5), 152.1 (C-4"), 150.6 (C-4'), 148.9 (C-3"), 148.1 (C-3'), 129.7 (C-1'), 122.4 (C-1"), 116.5 (C-6'), 115.4 (C-6"), 114.7 (C-5'), 110.4 (C-2'), 109.1 (C-5"), 108.6 (C-2"), 102.3 (C-7'), 101.8 (C-7"), 32.5 (C-8'); EIMS (*m/z*): 436 $[M+2]^+$ (1%), 434 $[M]^+$ (1%), 355 (100%), 244 (2%), 222 (23%), 213 (86%), 189 (16%), 183 (4%), 163 (2%), 149 (2%), 147 (4%), 134 (3%), 121 (3%), 104 (9%), 91 (39%).

5-(2-(3,4-Methylenedioxyphenyl)ethenyl)-2-((6-bromo-3,4-methylenedioxybenzyl)thio)-1,3,4-Oxadiazole (6k)

Grey solid; Yield: 90%; M.P: 175°C; Mol formula: $C_{19}H_{13}BrN_2O_5S$; Molecular mass: 460; IR (KBr): ν_{max} (cm^{-1}): 3067 (Ar-H str.), 1545 (Ar C=C str.), 1134 (C-O str.), 663 (C-Br str.), 611 (C-S str.); ^{1}H-NMR ($CDCl_3$,400 MHz, δ/ppm): 7.35 (d, *J*=16.4 Hz, 1H, H-9"), 7.06 (s, 1H, H-2'), 7.02 (d, *J*=1.6 Hz, 1H, H-2"), 6.98 (dd, *J*=8.0, 1.2 Hz, 1H, H-6"), 6.84 (s, 1H, H-5'), 6.81 (d, *J* = 8.0 Hz, 1H, H-5"), 6.79 (d, *J*=16.4 Hz, 1H, H-8"), 5.99 (s, 2H, H-7"), 5.94 (s, 2H, H-7'), 4.50 (s, 2H, H-8'); ^{13}C-NMR ($CDCl_3$,100 MHz, δ/ppm): 164.7 (C-5), 164.2 (C-2), 150.6 (C-4'), 149.4 (C-4"), 148.9 (C-3"), 148.3 (C-3'), 142.7 (C-8"), 130.9 (C-1"), 129.6 (C-1'), 122.3 (C-9"), 120.1 (C-6"), 116.6 (C-6'), 114.3 (C-5'), 110.8 (C-2'), 108.2 (C-5"), 107.5 (C-2"), 102.8 (C-7'), 101.2 (C-7"), 32.7 (C-8'); EIMS (*m/z*): 462 $[M+2]^+$ (1%), 460 $[M]^+$ (1%), 381 (100%), 244 (7%), 215 (7%), 213 (95%), 189 (10%), 183 (4%), 175 (3%), 173 (2%), 147 (4%), 134 (3%), 121 (7%), 104 (9%), 91 (13%).

5-[1-(Phenylsulfonyl)piperidin-4-yl]-2-((6-bromo-3,4-methylenedioxybenzyl)thio)-1,3,4-Oxadiazole (6l)

White amorphous solid; Yield: 95%; M.P: 120°C; Molecular formula: $C_{21}H_{20}BrN_3O_5S_2$; Molecular mass: 537; IR (KBr): ν_{max} (cm^{-1}): 3069 (Ar-H str.), 1545 (Ar C=C str.), 1387 (S=O str.), 1137 (C-O str.), 668 (C-Br str.), 616 (C-S str.); ^{1}H-NMR ($CDCl_3$,500 MHz, δ/ppm): 7.75 (d, *J*=7.0 Hz, 2H, H-2'" & 6'"), 7.60 (t, *J*=8.0 Hz, 2H, H-3'" & 5'"), 7.59 (t, *J*=7.5 Hz, 1H, H-4'"), 7.04 (s, 1H, H-2'), 6.98 (s, 1H, H-5'), 5.94 (s, 2H, H-7'), 4.43 (s, 2H, H-8'), 3.70 (dt, *J*=7.5, 4.0 Hz, $2H_{eq}$, H-2" & 6"), 2.82 (m, 1H, H-4"), 2.57 (dt, *J*=6.5, 2.0 Hz, $2H_{ax}$, H-2" & 6"), 2.11 (dd, *J*=13.5, 3.0 Hz, $2H_{eq}$, H-3" & 5"), 1.98 (dq, *J*= 11.0, 4.0 Hz, $2H_{ax}$, H-3" & 5"); ^{13}C-NMR ($CDCl_3$,100 MHz, δ/ppm): 171.3 (C-5), 164.8 (C-2), 150.6 (C-4'), 148.3 (C-3'), 144.9 (C-1'"), 134.2 (C-4'"), 129.5 (C-1'), 128.4 (C-3'" & 5'"), 127.1 (C-2'" & 6'"), 116.5 (C-6'), 114.3 (C-5'), 110.5 (C-2'), 102.2 (C-7'), 43.7 (C-2" & 6"), 32.3 (C-8'), 30.8 (C-4"), 28.9 (C-3" & 5"); EIMS (*m/z*): 539 $[M+2]^+$ (1%), 537 $[M]^+$ (1%), 458 (100%), 325 (2%), 213 (4%), 292 (1%), 266 (2%), 252 (3%), 250 (2%), 224 (5%), 183 (2%), 160 (4%), 141 (3%), 104 (8%), 77 (10%), 51 (2%).

Antibacterial activity

The reported assay was employed to evaluate antibacterial activity of all the synthesized molecules (Aziz-ur-Rehman *et al.*, 2013b; Kaspady *et al.*, 2009 and Yang *et al.*, 2006).

STATISTICAL ANALYSIS

Microsoft Excel 2010 was used for Statistical analysis for all the measurements and the results are tabulated as mean ± sem.

RESULTS

Protocol for the synthesis of 5-substituted-2-((6-bromo-3,4-methylenedioxybenzyl)thio)-1,3,4-Oxadiazole derivatives, 6a-l, is outlined in scheme-1 and different 5-stubstituted alky/aryl/aralkyl are mentioned in table 1. A novel series of compounds were synthesized find out new antibacterial agents. The % age inhibition and MIC values for antibacterial activity results are presented in table 2

and table 3. Experimental section is explicating the detail adopted procedures and the characterization of synthesized compounds.

Aryl/aralkyl carboxylic acids (1a-l), the precursors, were converted to ethyl esters (2a-l), carbohydrazides (3a-l), 5-substituted-1,3,4-Oxadiazol-2-thiols (4a-l) and finally 5-substituted-2-((6-bromo-3,4-methylenedioxybenzyl)thio)-1,3,4-Oxadiazoles (6a-l) through a series of steps (scheme-1). All the structures of synthesized molecules were corroborated through spectral analysis. Compound 6a has been discussed to deduce to its molecular and structural formulae.

Antibacterial activity (in vitro)

Using ciprofloxacin as reference standard, the results of antibacterial activity for all the molecules are presented as mean ± sem in table 2 and table 3 in the form of percentage inhibition and MIC values. Overall all the molecules executed very moderate inhibitory potential.

DISCUSSION

Compound 6a was obtained as white amorphous solid with melting point of 153°C. Its molecular formula was deduced as $C_{16}H_{11}BrN_2O_3S$ *via* EIMS and ^{1}H-NMR spectra. The IR spectrum showed the characteristics stretching absorption bands at 3059 (Ar-H), 1541 (Ar C=C), 1133 (C-O), 675 (C-Br) and 613 (C-S). Oxadiazole moiety in all the synthesized molecules was confirmed through stretching bands at 1610-1650 cm^{-1} for C=N bond and at 1030-1060 cm^{-1} for C-O bond. The EIMS showed $[M]^+$ at *m/z* 390 and $[M+2]^+$ at *m/z* 392. The characteristics fragments appeared at *m/z* 178 for 5-phenyl-1,3,4-Oxadiazol-2-thiol cation and at *m/z* 213 for the 6-bromo-3,4-methylenedioxybenzyl group. The noticeable fragments for 6a are given in its mass fragmentation pattern in fig. 1. The ^{1}H-NMR spectrum confirmed phenyl group attached to 5th position of 1,3,4-Oxadiazole by two signals resonating at δ 7.98 (dd, J = 7.5, 1.0 Hz, 2H, H-2" & 6") & 7.49-7.45 (m, 3H, H-3" to 5") and also 6-bromo-3,4-methylenedioxybenzyl group by four signals at δ 7.13 (s, 1H, H-2'), 7.01 (s, 1H, H-5'), 5.94 (s, 2H, H-7') and 4.54 (s, 2H, H-8'). Fourteen signals were shown by ^{13}C-NMR (BB and DEPT) spectrum for seven quaternary, seven methine and two methylene carbons. 5-Phenyl-1,3,4-Oxadiazol-2-thiol was allotted the signals at δ 165.2 (C-5), 164.7 (C-2), 134.1 (C-3" & 5"), 133.2 (C-2" & 6"), 132.7 (C-4") & 131.2 (C-1") and the signals at δ 150.8 (C-4'), 148.6 (C-3'), 129.6 (C-1'), 116.4 (C-6'), 114.3 (C-5'), 110.8 (C-2'), 102.3 (C-7') and 32.5 (C-8') represented 6-bromo-3,4-methylene dioxybenzyl group. The molecule 6a was named, 5-phenyl-2-((6-bromo-3,4-methylenedioxybenzyl)thio)-1,3, 4-Oxadiazole. Likewise the structures of other synthesized compounds were corroborated.

The molecules, 6c, 6d, 6h and 6k remained much efficient against all the bacterial strains but 6d was the best among all the synthesized molecules. The MIC values shown by this molecule were as, 12.10±1.43 relative to 9.42±1.09 for *S. typhi*, 10.98±1.85 relative to 8.02±2.17 for *E. coli*, 12.56±2.25 relative to 8.11±1.32 for *P. aeroginosa*, 14.39±2.12 relative to 8.88±2.00 for *B. subtilis* and 12.88±2.40 relative to 9.23±1.87 for *S. aureus*. Against *S. typhi*, half of the synthesized molecules remained inactive and 6k was the best inhibitor with MIC of 11.70±1.76 µM relative to 9.42±1.09 µM. *E. coli* was inhibited by the whole series with 6d, the best one. *P. aeroginosa* was also best inhibited by 6d and not at all by 6a & 6g. Against *B. subtilis*, 6h executed the most activity with MIC of 14.37±3.16 µM relative to 8.88±2.00µM. The molecule 6c was good against *S. aureus* with MIC of 12.58±2.40 µM relative to 9.23±1.87 µM and 6a, 6e, 6g and 6l remained inactive against this bacterial str.ain.

CONCLUSION

The molecules were synthesized in a series of steps with awesome yields and found to be weak to moderate inhibitors of Gram-bacteria. The compounds, 6c, 6d, 6h and 6k efficiently acted against all the bacterial strains and further 6d was the best one among these four. These molecules can be evaluated for *in vivo* activity and so might be considerable in the drug discovery program for the ailment of various diseases.

ACKNOWLEDGEMENT

The Higher Education Commission (HEC) of Pakistan is acknowledged for support regarding research work and spectral analysis.

REFERENCES

Aziz-ur-Rehman, Fatima A, Abbas N, Abbasi MA, Khan KM, Ashraf M, Ahmad I and Ejaz SA March (2013a). Synthesis, Characterization and Biological Screening of 5-substituted-1,3,4-oxadiazole-2yl-*N*-(2-methoxy-5-chlorophenyl)-2-sulfanyl acetamide. *Pak. J. Pharm. Sci.*,**26**(2): 345-352.

Aziz-ur-Rehman, Fatima A, Abbas N, Abbasi MA, Rasool S, Malik A, Ashraf M, Ahmad I and Ejaz SA (2016). Synthesis of new *N*-(5-Chloro-2-methoxyphenyl)-4-(5-substituted-1,3,4-Oxadiazol-2-ylthio) butanamide derivatives as Suitable Lipoxygenase inhibitors. *J. Saudi Chem. Soc.,* **20**: S488-S494.

Aziz-ur-Rehman, Nafeesa K, Abbasi MA, Kashfa H, Rasool S, Ahmad I and Arshad S (2013b). Synthesis, Characterization and Biological Screening of Various *S*-substituted Derivatives of 5-(3-Nitrophenyl)-1,3,4-Oxadiazole-2-thiol. *Pak. J. Chem.*,**3**(2): 1-8.

Bar-Oz B, Einarson T, Einarson A, Boskovic R, O'Brien L, Malm H, Berard A and Koren G (2007). Paroxetine

and congenital malformations: Meta-Analysis and consideration of potential confounding factors. *Clin. Ther.*,**29**(5): 918-926.

Dabholkar VV and Bhusari NV (2011). Synthesis of 2-Substituted-1,3,4-Oxadiazole Derivatives. *Int. J. Environ. Pharm. Res.*,**4**(1): 1-4.

Hui XP, Chu CH and Zhang ZY (2002). Synthesis and antibacterial activities of 1,3,4 oxadiazole derivatives containing 5-methylisoxazole moiety. *Indian J. Chem.*,**41**B: 2176-2179.

Ingrassia L, Lefranc F, Mathieu V, Darro F and Kiss R (2008). Amaryllidaceae isocarbostyril alkaloids and their derivatives as promising antitumor agents. *Transl. Oncol.*,**1**(1): 1-13.

Kaspady M, Narayanaswamy VK, Raju M and Rao GK (2009). Synthesis, antibacterial activity of 2,4-disubstituted oxazoles and thiazoles as bioesters. *Lett. Drug Des. Discov.*,**6**: 21-28.

Khalid H, Aziz-ur-Rehman, Abbasi MA, Malik A, Rasool S, Nafeesa K, Ahmad I and Afzal S (2013).Synthesis, spectral analysis and anti-bacterial study of N-substituted derivatives of 2-(5-(1-(phenylsulfonyl)piperidin-4-yl)-1,3,4-Oxadiazol-2-ylthio)acetamide. *J. Saudi Chem. Soc.*,**20**: S615-S623.

Mohan TP, Vishalakshi B, Bhat KS, Kendappa GN and Rao KS (2004). Synthesis and insecticidal activity of some 1,3,4-oxadiazole derivatives containing phenoxy-fluoro-phenyl group. *Indian J. Chem.*,**43**B: 1798-1801.

Omar FA, Mahfouz NM and Rahman MA (1996). Design, synthesis and anti-inflammatory activity of some 1,3,4-oxadiazole derivatives. *Eur. J. Med. Chem.*,**31**: 819-825.

Rashid M, Husain A and Mishra R (2012). Synthesis of benzimidazoles bearing oxadiazole nucleus as anticancer agents. *Eur. J. Med. Chem.*,**54**: 855-866.

Sahin G, Palaska E, Ekizoglu M and Ozalp M (2002). Synthesis and antimicrobial activity of some 1,3,4-oxadiazole Derivatives. *Il Farmaco.*,**57**: 539-542.

Yang CR, Zang Y, Melissa RJ, Khan SI, Zhang YJ and Li XC (2006). Antifungal activity of C-27 steroidal saponins. *Antimicrob. Agents Chemother.*,**50**(5): 1710-1714.