doi.org/10.36721/PJPS.2020.33.2.SUP.871-876.1

Synthesis, characterization, antibacterial, hemolytic and thrombolytic activity evaluation of 5-(3-chlorophenyl)-2-((*N*-(substituted)-2-acetamoyl)sulfanyl)-1,3,4-oxadiazole derivatives

Aziz-ur-Rehman[1], Samreen Gul Khan[2]*, Tanveer Hussain Bokhari[2], Fozia Anjum[2], Naheed Akhter[3], Shahid Rasool[1], Syed Adnan Ali Shah[4,5], Muhammad Shahid[6] and Aneesa Arshad[2]

[1]Department of Chemistry, Government College University, Lahore, Pakistan
[2]Department of Chemistry, Government College University, Faisalabad, Pakistan
[3]College of Allied Health Professional, Directorate of Medical Sciences, Government College University, Faisalabad, Pakistan
[4]Faculty of Pharmacy, Universiti Teknologi MARA, Puncak Alam Campus, Bandar Puncak Alam, Selangor Darul Ehsan, Malaysia
[5]Atta-ur-Rahman Institute for Natural Products Discovery (AuRIns), Universiti Teknologi MARA, Puncak Alam Campus, Bandar Puncak Alam, Selangor Darul Ehsan, Malaysia
[6]Department of Chemistry & Bio-Chemistry, University of Agriculture, Faisalabad, Pakistan

Abstract: A novel series of 5-(3-Chlorophenyl)-2-((N-(substituted)-2-acetamoyl)sulfanyl)-1,3,4-oxadiazole derivatives was efficiently synthesized and screened for antibacterial, hemolytic and thrombolytic activities. The molecule 7c remained the best inhibitor of all selected bacterial strains and furthermore possessed very low toxicity, 8.52±0.31. Compound 7a 7b and 7f showed very good thrombolytic activity relative to Streptokinase employed as reference drug. In addition to low toxicity and moderately good thrombolytic activity, the synthesized compounds possessed excellent to moderate antibacterial activity, relative to ciprofloxacin. All compounds especially 7b and 7f can be consider for further clinical studies and might be helpful in synthesis of new drugs for treatment of cardiovascular diseases.

Keywords: 1,3,4-Oxadiazole, acetamides, antibacterial activity, thrombolytic activity, hemolytic activity.

INTRODUCTION

Arterial thrombo embolism (ATE) is the major cause of death in the world as compared to other diseases like cancer, diabetes and AIDs (Lamberini and Matt, 2018). Various factors, like stress, poor medical facilities and poor diet are also contributing toward different cardiovascular diseases. Currently available thrombolytic drugs have significant side effects with chances of hemorrhage. Development of new thrombolytic agents with least side effects is need of hour for treatment of myocardial infarction. Platelet aggregation plays vital role in atherosclerosis formation and thrombosis leading to heart attack and strokes (Heloise *et al*., 2019; Wohner 2008; Van *et al*., 2019).

In the last 25 years, the prevalence of microbial infectivity has improved in shocking levels worldwide as a consequence of antimicrobial resistance (Malhotra *et al*., 2017). Most of the bacteria developed resistance to existing drugs by altering their gene sequence. Hence, there is a need for such antimicrobials which are structurally modified and that can effectively inhibit the growth of microorganisms having less side effects (Sekhar *et al*., 2018).

The Oxadiazole derivatives have occupied a unique position in heterocyclic compounds due to their diverse biological activities. It has been reported that 1,3,4-oxadiazole based compounds have medicinal importance because of antifungal, antimalarial, antibacterial, anti-mycobacterial, anticancer, anti-HIV, anti-inflammatory, anti-tuberculosis, anticonvulsant, antihypertensive, analgesic, antidepressant and anti-tumor activities (Aziz-ur-Rehman *et al*., 2019; Dabholkar *et al*., 2011; Rashid *et al*., 2012). Compounds bearing acetamoyl group are known to play an active part in a number of drugs. Substituted acetamides are found to possess anthelmintic, anticonvulsant, antioxidant, anti-inflammatory, anti-arthritic, anticancer, antibacterial and antifungal activities (Autore *et al.*, 2010; Ayhan-Kileigil *et al.*, 2012; Jawed *et al.*, 2010; Kanagarajan *et al*., 2010; Sawant *et al*., 2011).

In protraction of our previous projects on 1,3,4-Oxadiazole derivatives (Samreen *et al*., 2017; Samreen *et al*., 2013; Aziz-ur-Rehman *et al*., 2015; Rasool *et al*., 2015; Aziz-ur-Rehman *et al*., 2012), we herein report the synthesis of some new 5-(3-chlorophenyl)-2-((*N*-(substituted)-2-acetamoyl)sulfanyl)-1,3,4-oxadiazole derivatives. All compounds were evaluated for their antibacterial, hemolytic and thrombolytic activities. Antibacterial activities of various 1,3,4-Oxadiazole analogues have been reported but it was the first attempt to report such derivatives with thrombolytic activity.

**Corresponding author:* e-mail: samreengul@gcuf.edu.pk

MATERIALS AND METHODS

Alfa Aesar provided all the chemicals of synthetic grade. The solvents were of analytical grade. Melting points were detected by utilizing Griffin-George apparatus. Progress of reaction and purity of synthesized oxadiazole derivatives were confirmed by silica coated plates F_{256} 20 x 20 cm by using solvent system of different ratios of *n*-Hexane and EtOAc. Bruker spectrometer presented ^{1}H-NMR spectra at 400 MHz in $CDCl_3$. The δ-values were reported as ppm. The *J*-values were reported as Hz. IR spectra of all the derivatives were taken on a model of Jasco-320-A spectrophotometer by KBr pellet method in cm^{-1}. EI-MS spectra were presented by the spectrometer (JMS-HX-110) in *m/z*.

Synthesis of ethyl 3-chlorobenzoate (2)

In a 500mL R.B (round bottom) flask, 3-Chlorobenzoic acid (1; 0.05 mol, 8.0g) was immersed in 40-60mL EtOH. The mixture was aided by 3-5mL of conc. H_2SO_4 as catalyst and subjected to reflux for 4-5 hours. TLC was employed to monitor the reaction. Due to reversibility, upon maximum completion, 15% Na2CO3 solution neutralized the mixture and removed catalyst or unreacted organic acids. Then mixture was transferred in 1000mL separating funnel and chloroform was added to it. Contents in funnel were vigorously shaken for 30 minutes. After shaking, the mixture in funnel was settled to bi-layers. The chloroform was evaporated after separation to acquire title compounds. Pale yellow liquid ester was collected by evaporating chloroform.

Synthesis of 3-chlorobenzohydrazide (3)

In a RB flask (250mL), ethyl 3-chlorobenzoate (2; 0.03 mol, 7.0mL) was stirred with hydrazine hydrate (0.06 mol, 14.0mL) in methanol (17.5mL) for 4-5 hours. Some of the esters were completely converted to corresponding hydrazide by stirring at RT but some after slight heating. TLC confirmed the reaction completion and the precipitates, 3, were quenched by excess distilled water (cold). The title compounds were collected through filtration followed by washing and drying. Re-crystallization was performed using CH_3OH.

Synthesis of 5-(3-chlorophenyl)-2-mercapto-1,3,4-oxadiazole (4)

In 250 mL RB flask a mixture of compound 3 (0.028 mol/6.0 g) and KOH (0.028 mol, 1.58g) in EtOH (35.0 mL) was subjected to reflux for 1.0 hours. Carbon disulphide (0.028mol, 1.68mL) was added and further refluxed for 5.0 hours to furnish 4. Reaction progress was supervised by TLC. Upon completion, the reaction mixture was diluted by adding chilled water. The pH was set to 5-6 by dil. HCl to neutralize the base and get back the acidic form of oxadiazole. Finally, 4 was collected as residue in filtration followed by washing and drying. The title compound was also subjected to recrystallization from CH_3OH.

Synthesis of N-alkyl/aryl-2-bromoacetamide (6a-f)

The alkyl/aryl amines (5a-f; 0.013mol) were dissolved in 12.0mL 5% Na_2CO_3 solution. Bromoacetyl bromide (0.013mol) was poured into the flask and reaction was shaken for 10 minutes till completion. TLC monitored the reaction. The precipitates were quenched by excess distilled water (cold). The electrophiles, 6a-f, were collected through filtration followed by washing and drying.

Synthesis of 5-(3-chlorophenyl)-2-((N-(alkyl/aryl)-2-acetamoyl)sulfanyl)-1,3,4-oxadiazole derivatives (7a-f)

In 100mL R.B flask, compound 4 (0.00047mol, 0.1g) were mixed with 0.001mol NaH in 12-15mL DMF. Reaction was subjected to stir for 20 minutes. Equimolar *N*-alkyl/aryl-2-bromoacetamides (6a-f, 0.00047mol) were added and subjected to stirring for 4-5 hours. TLC monitored the completion of reaction. The precipitates were quenched by dist. water (cold). Title compounds, 7a-f, were collected as white amorphous solids through filtration followed by washing and drying.

5-(3-Chlorophenyl)-2-((N-(2-(methoxycarbonyl)phenyl)-2-acetamoyl)sulfanyl)-1,3,4-oxadiazole (7a)

79%; M.P: 186-188^{o}C; Mol. For.: $C_{18}H_{14}ClN_3O_4S$; Mol. Wt.: 403; IR: 3363, 3095, 1679, 1657, 1583, 1287, 679, 635; ^{1}H-NMR: 11.97 (1H-N, s), 8.77 (1H-6‴, d with 8.4 coupling), 8.45 (1H-2', s), 8.14 (1H-3‴, d, with 7.6 coupling), 8.05 (1H-6', dd with 8.0 & 1.2 coupling), 7.59 (1H-4', dd with 7.6 & 1.2 coupling), 7.55 (1H-5‴, t with 7.6 coupling), 7.43 (1H-5', t with 8.0 coupling), 7.13 (1H-4‴, t with 7.6 coupling), 4.96 (2H-2", s), 3.92 (3H-2‴, s); EIMS: 405 (27%) $[M+2]^+$, 403 (80%) $[M]^+$, 344 (14%), 253 (23%), 226 (100%), 193 (48%), 178 (6%), 179 (22%), 153 (40%), 139 (89%), 137 (44%), 119 (32%), 111 (26%), 76 (10%).

5-(3-Chlorophenyl)-2-((N-cyclohexyl-2-acetamoyl)sulfanyl)-1,3,4-oxadiazole (7b)

93%; M.P: 171-173^{o}C; Mol. For.: $C_{16}H_{18}ClN_3O_2S$; Mol. Wt.: 351; IR: 3349, 3063, 1671, 1667, 1591, 1269, 677, 645; ^{1}H-NMR: 10.32 (1H-N, s), 8.01 (1H-2', s), 7.93 (1H-6', d with 7.6 coupling), 7.59 (1H-4', d with 7.6 coupling), 7.42 (1H-5', t with 8.0 coupling), 4.77 (2H-2", s), 3.85-3.83 (1H-1‴, m), 1.94-1.14 (2H-2‴, 2H-3‴, 2H-4‴, 2H-5‴, 2H-6‴, m); EIMS: 353 (28%) $[M+2]^+$, 351 (81%) $[M]^+$, 253 (21%), 226 (100%), 179 (21.7 %), 139 (87%), 137 (40.3%), 120 (31%).

5-(3-Chlorophenyl)-2-((N-phenyl-2-acetamoyl)sulfanyl)-1,3,4-oxadiazole (7c)

81%; M.P: 180-182^{o}C; Mol. For.: $C_{16}H_{12}ClN_3O_2S$; Mol. Wt.: 345; IR: 3369, 3036, 1679, 1657, 1596, 1294, 677, 644; ^{1}H-NMR: 10.32 (1H-N, s), 7.99 (1H-2', s), 7.91 (1H-6', d with 7.6 coupling), 7.57 (1H-4', d with 7.6 coupling), 7.41 (1H-5', t with 7.6 coupling), 7.33-7.27 (1H-2‴, 1H-3‴, 1H-4‴, 1H-5‴, 1H-6‴, m), 4.86 (2H-2", s); EIMS: 347

Table 1: %age inhibition values of antimicrobial activity of the synthesized compounds

Compound	%age Inhibition					
	Gram negative bacteria			Gram positive bacteria		
	S. typhi	*E. coli*	*K. pneumonae*	*P. aeruginosa*	*B. subtilis*	*S. aureus*
7a	56.08 ±0.50	40.40±0.50	58.58 ±3.19	44.55±3.73	50.10 ±1.12	47.61±0..09
7b	76.67 ±0.50	50.00±1.20	73.82±2.94	60.86±4.05	71.84±3.67	58.82±1.77
7c	81.96 ±2.38	63.55 ±1.85	76.47 ±3.33	65.32±3.68	69.54 ±0.77	74.82±2.31
7d	58.25 ±1.42	37.35 ±3.85	62.79 ±3.87	39.64±4.09	50.20 ±0.51	52.27±3.27
7e	62.29±2.54	46.30±0.50	63.28±2.79	45.00±2.91	48.78 ±3.65	65.42±3.13
7f	61.79±1.62	54.55 ±3.45	68.63±1.67	54.59±3.23	62.24 ±4.10	58.38±0.17
Ciprofloxacin	90.42 ±0.92	90.99 ± 0.15	92.75 ±0.20	91.05±1.18	91.11 ±0.26	91.94±0.86

Table 2: MIC values of antimicrobial activity of the synthesized compounds

Compound	MIC (µg/mL)					
	Gram negative bacteria			Gram positive bacteria		
	S. typhi	*E. coli*	*K. pneumonae*	*P. aeruginosa*	*B. subtilis*	*S. aureus*
7a	15.90 ±2.01	-	14.22 ±1.35	-	17.99 ±3.14	-
7b	10.11 ±2.55	19.23±0.43	10.86±2.15	13.44±2.22	11.61±3.00	14.33±0.98
7c	8.50 ±1.96	11.59 ±1.07	9.10 ±1.50	10.22±2.11	12.42 ±0.93	11.04±0.76
7d	15.21 ±0.49	-	13.98 ±3.10	-	18.92 ±1.57	16.62±1.48
7e	13.06±0.76	-	11.56±1.20	-	-	12.79±0.33
7f	12.75±2.97	18.94±0.07	13.53±0.90	16.85±2.11	12.11±0.36	13.44±1.12
Ciprofloxacin	8.11 ±0.92	9.02 ± 0.47	8.12 ±1.43	8.21±1.24	8.24 ±0.76	8.02±1.01

Table 3: Hemolytic and thrombolytic activities of the synthesized compounds

Compound	Hemolytic activity (Mean % ± S.D)	Thrombolytic activity (Mean % ± S.D)
7a	5.03±0.24	41.03±0.24
7b	9.63±0.23	58.61±0.24
7c	8.52±0.31	2.52±0.37
7d	2.52±0.31	2.56±0.36
7e	6.61±0.12	6.21±0.15
7f	0.19±0.02	35.19±0.02
PBS	0.00±0.0	0.00±0.0
Triton-X-100	100±0.0	-
Streptokinase	-	73.0±0.69

(27%) $[M+2]^+$, 345 (79%) $[M]^+$, 253 (14%), 226 (30%), 179 (20%), 139 (100%), 137 (33%), 120 (13%), 92 (14%), 77 (12%).

5-(3-chlorophenyl)-2-((N-(2,3-dimethylphenyl)-2-acetamoyl)sulfanyl)-1,3,4-oxadiazole (7d)

91%; M.P: 169-171°C; Mol. For.: $C_{18}H_{16}ClN_3O_2S$; Mol. Wt.: 373; IR: 3323, 3083, 1687, 1656, 1590, 1272, 670, 636; ^{1}H-NMR: 9.99 (1H-N, s), 8.07 (1H-2', s), 7.99 (1H-6', d with 7.6 coupling), 7.61 (1H-4', d with 8.0 coupling), 7.57 (1H-6''', d with 7.6 coupling), 7.46 (1H-5', t with 8.0 coupling), 7.11 (1H-5''', t with 8.0 coupling), 7.03 (1H-4''', d with 7.6 coupling), 4.98 (2H-2'', s), 2.34 (3H-2''', s), 2.14 (3H-3''', s); EIMS: 375 (29%) $[M+2]^+$, 373 (71%) $[M]^+$, 253 (7%), 226 (35%), 193 (35%), 179 (22%), 139 (100%), 121 (56%), 111 (23%), 91 (17%), 77 (16%).

5-(3-Chlorophenyl)-2-((N-(2,4-dimethylphenyl)-2-acetamoyl)sulfanyl)-1,3,4-oxadiazole (7e)

93%; M.P: 149-151°C; Mol. For.: $C_{18}H_{16}ClN_3O_2S$; Mol. Wt.: 373; IR: 3339, 3079, 1655, 1643, 1595, 1233, 674, 641; ^{1}H-NMR: 9.98 (1H-N, s), 8.07 (1H-2', s), 7.99 (1H-6', d with 7.6 coupling), 7.72 (1H-6''', d with 8.0 coupling), 7.61 (1H-4', d with 8.0 coupling), 7.46 (1H-5', t with 8.0 coupling), 7.03 (1H-5''', d with 8.4 coupling), 6.97 (1H-3''', s), 4.96 (2H-2'', s), 2.28 (3H-2''', s), 2.20 (3H-4''', s); EIMS: 375 (31%) $[M+2]^+$, 373 (74%) $[M]^+$, 226 (34%), 193 (33%), 179 (19%), 139 (100%), 121 (55%), 111 (23%), 91 (19%), 77 (18%).

5-(3-Chlorophenyl)-2-((N-(2,5-dimethylphenyl)-2-acetamoyl)sulfanyl)-1,3,4-oxadiazole (7f)

87%; M.P: 166-168 °C; Mol. For.: $C_{18}H_{16}ClN_3O_2S$; Mol. Wt.: 373; IR: 3299, 3067, 1667, 1653, 1598, 1198, 670,

642; ^{1}H-NMR: 9.89 (1H-N, s), 8.07 (1H-2', s), 7.99 (1H-6', d with 7.6 coupling), 7.61 (1H-4', d with 7.6 coupling), 7.46 (1H-5', t with 8.0 coupling), 7.15 (1H-6'", s), 7.06 (1H-3'", d with 7.6 coupling), 6.91 (1H-4'", d with 7.6 coupling), 4.96 (2H-2", s), 2.31 (3H-2'", s), 2.20 (3H-5'", s); EIMS: 375 (31%) $[M+2]^+$, 373 (71%) $[M]^+$, 226 (37%), 193 (37%), 179 (23%), 139 (100%), 121 (59%), 111 (27%), 91 (17%), 77 (16%).

STATISTICAL ANALYSIS

All the calculations were executed in triplicate and statistical analysis were performed through SPSS 15.0 (SPSS Inc., Chicago, Illinois, USA).

Antibacterial potential assay

The antibacterial activity assay was according to that of Kaspady *et al.* with minor variations (Kaspady *et al*., 2009).

Hemolytic activity assay

The hemolytic activity assay was according to that of Sharma *et al.* with minute differences for evaluation of toxicity of synthesized compounds (Sharma *et al*., 2001).

Thrombolytic activity assay

The thrombolytic activity assay was according to that of Mannan *et al.* with minor modifications (Mannan *et al*., 2011).

RESULTS

New derivatives of 3-chlorobenzoic acid (1) were efficiently synthesized in search of new potent and less toxic molecules (Scheme-1). Briefly, 3-chlorobenzoic acid (1) was refluxed to acquire respective ester (2) using conc. H_2SO_4 as catalyst. The esters were subjected to nucleophillic substitution by monohydrated hydrazine to acquire hydrazides (3). The hydrazide was cyclized with CS_2 in the presence of KOH to yield corresponding oxadiazole (4). Alkyl/aryl amines, 5a-f, were stepped up to *N*-substituted-2-bromoacetamide, 6a-f, the electrophiles. The title compounds, 7a-f, were afforded by reaction of 4 and 6a-f in DMF and NaH. The molecular structures for 7a-f were corroborated by spectral study including 1D-NMR, IR and EI-MS. These compounds were further screened them for their antibacterial, hemolytic and thrombolytic activities. The results rendered the compounds as excellent to moderate antibacterial, very low hemolytic and moderately good thrombolytic agents. The procedures, reaction conditions and reaction scheme were explicated in experimental section.

Antibacterial activity (in vitro)

Using ciprofloxacin as reference standard, the results of antibacterial activity for all synthesized compounds are presented as mean ± sem in table 1 and table 2 in the form of MIC and %age inhibition value. Overall all compounds executed moderate inhibitory potential. The molecule 7c remained the efficient inhibitor of all the gram-positive and gram-negative bacterial strains taken into account. Its MIC values were noted as, 8.50±1.96, 11.59±1.07, 9.10±1.50, 10.22±2.11, 12.42±0.93 and 11.04±0.76 µM relative to that of ciprofloxacin against S. typhi, E. coli, K. pneumonae, P. aeruginosa, B. subtilis and S. aureus respectively.

Hemolytic activity assay

The hemolytic activity was performed relative to Triton-X-100 as positive control. The results of hemolytic activity are presented in table 3. All the synthesized molecules exhibited very low hemolytic activity.

Thrombolytic activity assay

The thrombolytic activity was performed relative to streptokinase as positive control. The results are presented in table 3. All the synthesized molecules exhibited moderate to good thrombolytic activity and so might be considered as new drug candidates against certain diseases.

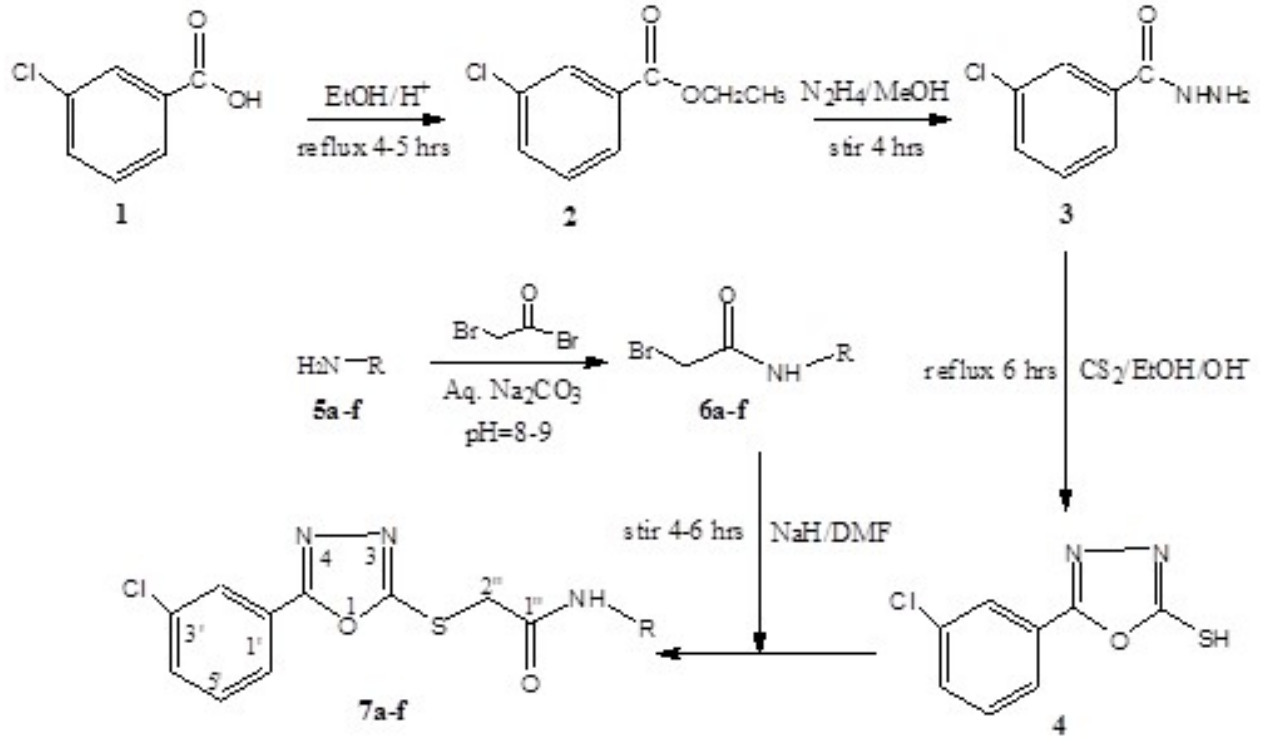
DISCUSSION

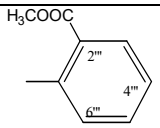
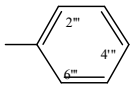
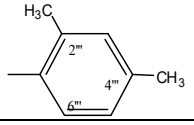
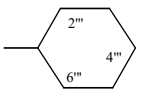
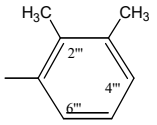
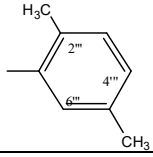
Compound 7a was obtained as a white amorphous solid. The molecular formula of this compound was assigned by ^{1}H-NMR and EI-MS spectra. EI-MS spectrum furnished isotopic and molecular ion peaks at *m/z* 405 and 403. The base peak was observed at *m/z* 226 for 5-(3-chlorophenyl)-2-(methylthio)-1,3,4-oxadiazole and a distinct peak at *m/z* 178 for 2-(methoxycarbonyl) anilinecarbonyl cation. The characteristic IR absorption bands at 3363 and 1657 were attributed to amide functionality; 3095, 1583 and 679 to chlorinated aromatic benzenoid ring; 1679, 1287 and 635 to 2-mercapto-1,3,4-oxadiazole functionality. The ^{1}H-NMR spectrum completely described the protons present in the molecule. Two singlets at 4.96 and 3.92 were assigned to methylene and methoxy groups respectively. A highly deshielded singlet at 11.97 supported amidic proton. The two doublets at 8.77 (with 8.4 coupling) & 8.14 (with 7.6 coupling); and two triplets at 7.55 (with 7.6 coupling) & 7.13 (with 7.6 coupling) were allocated to the di-substituted aromatic ring attached to carbamoyl group. One singlet at 8.45; two doublets of doublet at 8.05 (with 8.0 & 1.2 coupling) and 7.59 (with 7.6 & 1.2 coupling); and one triplet at 7.43 (with 8.0 coupling) were assigned to meta substituted phenyl ring. On the basis of above structural analysis, the molecule was assigned as 5-(3-chlorophenyl)-2-((*N*-(2-(methoxycarbonyl)phenyl)-2-acetamoyl)sulfanyl)-1,3,4-oxadiazole. Likewise the other molecules were corroborated structurally through spectral data.

Antibacterial, hemolytic and thrombolytic activities

The % inhibition and minimum inhibitory concentration (MIC) values for antibacterial activity are presented in table 1 and table 2 along with that of reference standard, ciprofloxacin. The compounds were screened against two gram-positive bacteria and four gram-negative bacteria. Most of the compounds exhibited excellent to moderate activity against the bacterial strains taken into account. Gram-negative strains, *S. typhi* and *K. pneumonae*, were inhibited by all the compounds. The compounds, 7c was best against both but 7b only against *K. pneumonae*. The molecules, 7a, 7d and 7e remained inactive against both *E. coli* and *P. aeruginosa*. The remaining molecules showed moderate activity against these two strains. *B. subtilis* was not inhibited by 7e and *S. aureus* by 7a. The other molecules showed excellent to moderate activity. The molecule 7c remained the efficient inhibitor of all the gram-positive and gram-negative bacterial strains taken into account. Its MIC values were noted as, 8.50±1.96, 11.59±1.07, 9.10±1.50, 10.22±2.11, 12.42±0.93 and 11.04±0.76 µM relative to that of ciprofloxacin against *S. typhi*, *E. coli*, *K. pneumonae*, *P. aeruginosa*, *B. subtilis* and *S. aureus* respectively.

The hemolytic activity was performed relative to Triton-X-100 and thrombolytic activity relative to streptokinase as positive control. The results of both activities are presented in table 3. All the synthesized molecules exhibited very low hemolytic activity and so might be considered as new drug candidates against certain diseases. The molecule, 7c, the best antibacterial inhibitor, also presented very low toxicity as 8.52±0.31 relative to reference Triton-X-100. Moderately good thrombolytic activity was executed by all the compounds. The molecule 7b exhibited the best thrombolytic activity as 58.61±0.24 relative to reference with that of 73.0±0.69. This molecule also showed excellent antibacterial activity and so might be considerable for *in vivo* studies.



C.	R	C.	R	C.	R
7a		7c		7e	
7b		7d		7f	

Scheme 1: Outline for synthesis of *N*-substituted-2-((5-(3-chlorophenyl)-1,3,4-Oxadiazol-2-yl)sulfanyl)acetamide.

CONCLUSION

New derivatives of 3-chlorobenzoic acid (1) were efficiently synthesized in search of new potent and less toxic molecules. All the synthesized compounds executed very excellent to moderate antibacterial, low hemolytic and moderately good thrombolytic activities. The molecules, 7b and7c remained the most efficient with excellent antibacterial activity and low toxicity. Also 7b possessed very high thrombolytic activity. The *in vivo* study of such molecules by the pharmaceutical industries may further elaborate their importance as new drug candidates.

REFERENCES

Autore G, Caruso A, Marzocco S, Nicolaus B, Palladino C, Pinto A, Popolo A, Sinicropi MS, Tommonaro G and Saturnino C (2010). Acetamide derivatives with antioxidant activity and potential, anti-inflammatory activity. *Molecules.,* **15**(3): 2028-2038.

Ayhan-Kılcıgil G, Gurkan S, Coban T, Ozdamar ED and Can-Eke B (2012). Synthesis and evaluation of antioxidant properties of novel 2-[2-(4-chlorophenyl) benzimidazole-1-yl]-N-(2-arylmethylene amino) acetamides and 2-[2-(4-chlorophenyl) benzimidazole-1-yl]-N-(4-oxo-2-aryl-thiazolidine-3-yl)acetamides-I. *Chem. Biol. Drug Des.,* **79**(5): 869-877.

Aziz-ur-Rehman, Aslam SJ, Abbasi MA, Siddiqui SZ, Rasool S and Shah SAA (2019). Synthesis, spectral analysis and biological evaluation of sulfamoyl and 1,3,4-oxadiazole derivatives of 3-pipecoline. *Pak. J. Pharm. Sci.,* **32**(3): 987-996.

Aziz-ur-Rehman**,** Gul S, Abbasi MA, Nafeesa K, Akhtar MN and Afzal S (2015). Synthesis, Structural Analysis and Screening of Some Novel 5-Substituted Aryl/ Aralkyl-1,3,4-Oxadiazol-2-Yl 4-(Morpholin-4-Ylsulfonyl)Benzyl Sulfides as Potential Antibacterial Agents, *Phosphorus, Sulfur, Silicon Relat. Elem,* **190**(7): 1045-1055.

Dabholkar VV, Bhusari NV (2011). Synthesis of 2-substituted-1,3,4-Oxadiazole derivatives. *Int. J. Environ Pharm. Res*., **4**(5): 1-4.

Gul S, Aziz-ur-Rehman, Abbasi MA, Nafeesa K, Malik A, Ashraf M, Islmail T and Ahmad I (2013). Synthesis, characterization and pharmacological evaluation of *N*-substituted derivatives of 5-(4-nitrophenyl)-1,3,4-oxadiazole-2yl-2"-sulfanyl acetamide. *Asian J. Chem.,* **25**(11): 6231-6236.

Jawed H, Shah SUA, Jamall S, Simjee SU (2010). N-(2-hydroxy phenyl) acetamide inhibits inflammation-related cytokines and ROS in adjuvant-induced arthritic (AIA) rats. *Int. Immunopharmacol.,* **10**(8): 900-905.

Kanagarajan V, Thanusu J and Gopalakrishnan M (2010). Synthesis and in vitro microbiological evaluation of an array of biolabile 2-morpholino-N-(4,6-diarylpyrimidin-2-yl)acetamides. *Eur. J. Med. Chem.,* **45**(4): 1583-1589.

Khan SG, Aziz-ur-Rehman, Abbassi MA, Khan KN, Nafeesa K, Siddiqa A, Akhtar M, Shahid M and Subhani Z (2017). Synthesis, antimicrobial evaluation and hemolytic activity of 2-[[5-alkyl/aralkyl substituted-1,3,4-Oxadiazol-2-yl]thio]-N-[4-(4-morpholinyl)phenyl]acetamide derivatives. *J. Saudi Chem. Soc.,* **21**: S425-S433.

Kaspady M, Narayanaswamy VK, Raju M and Rao GK (2009). Synthesis, antibacterial activity of 2,4-disubstituted oxazoles and thiazoles as bioisosteres. *Lett. Drug Des. Discov*., **6**(1): 21-28.

Lambrini T and Matt R (2018).The effect of deprivation on coronary heart disease mortality rate. *Biosci. Horiz.* **11**: 1-6.

Lebas H, Yahiaoui K, Martos R and Boulaftali Y (2019). Platelets are at the nexus of vascular diseases. *Front. cardiovasc. Med.,* **6**: 132-136.

Malhotra M, Rawal RYYK, Malhotra D, Dhingra R, Deep A and Sharma PC (2017). Synthesis, characterization and pharmacological evaluation of (Z)-2-(5-(biphenyl-4-yl)-3-(1-(imino) ethyl)-2, 3-dihydro-1, 3, 4-oxadiazol-2-yl) phenol derivatives as potent antimicrobial and antioxidant agents. *Arab. J. Chem.,* **10**: 1022-1031.

Mannan A, Kawser MJ, Ahmed AMA, Islam NN, Alam SSM, Emon MAEK and Gupta SD (2011). Assessment of antibacterial, thrombolytic and cytotoxic potential of Cassia alata seed oil. *J. Appl. Pharm. Sci.,* **1**(9): 56-59.

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

Sawant R and Kawade D (2011). Synthesis and biological evaluation of some novel 2-phenyl benzimidazole-1-acetamide derivatives as potential anthelmintic agents. *Acta. Pharm.,* **61**(3): 353-361.

Sekhar MM, Nagarjuna U, Padmavathi V, Padmaja A, Reddy NV and Vijaya T (2018). Synthesis and antimicrobial activity of pyrimidinyl 1, 3, 4-oxadiazoles, 1, 3, 4-thiadiazoles and 1, 2, 4-triazoles. *Eur. J. Med. Chem.,* **145**(1): 1-10.

Sharma P and Sharma JD (2001). *In vitro* hemolysis of human erythrocytes by plant extracts with antiplasmodial activity. *J. Ethnopharmacol*., **74**(3): 239-243.

Van der Meijden PE and Heemskerk JW (2019). Platelet biology and functions: New concepts and clinical perspectives. *Natu. Rev. Card.,* **16**(3): 166-179.

Wohner N (2008). Role of cellular elements in thrombus formation and dissolution. *Cardiovasc. Hematol. Agents Med. Chem.,* **6**(3): 224-228.