doi.org/10.36721/PJPS.2021.34.1.SP.441-446.1

Synthesis, spectral analysis and biological evaluation of 2-{[(morpholin-4-yl)ethyl]thio}-5-phenyl/aryl-1,3,4-oxadiazole derivatives

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Abstract: A series of new derivatives of 4-(2-chloroethyl)morpholine hydrochloride (5) were efficiently synthesized. Briefly, different aromatic organic acids (1a-f) were refluxed to acquire respective esters (2a-f) using conc. H_2SO_4 as catalyst. The esters were subjected to nucleophillic substitution by monohydrated hydrazine to acquire hydrazides (3a-f). The hydrazides were cyclized with CS_2 in the presence of KOH to yield corresponding oxadiazoles (4a-f). Finally, the derivatives, 6a-f, were prepared by reacting oxadiazoles (4a-f) with 5 using NaH as activator. Structures of all the derivatives were elucidated through 1D-NMR EI-MS and IR spectral data. All these molecules were subjected to antibacterial and hemolytic activities and showed good antibacterial and hemolytic potential relative to the reference standards.

Keywords: Hemolytic activity, antibacterial activity, morpholine, 1,3,4-oxadiazole.

INTRODUCTION

Development of drug recalcitrance and resistance in pathogens against existing antimicrobial drugs has posed a challenge to scientific community. So for the treatment of different infections and other malfunctions, there is a need for continuous research in designing, modification and development of molecules bearing attractive therapeutic activity (Naqvi and Drlica, 2017; Naqvi *et al.*, 2018; Somani and Shirodkar, 2009). Oxadiazole ring is nitrogen and oxygen containing heterocyclic compound. Out of four isomers of oxadiazoles, the di-substituted 1,3,4-oxadiazole ring exhibits excellent bioactivities (Manjunath *et al.*, 2009). The presence of -N=C-S and -N=C-O linkages in oxadiazole ring is associated with a large number of pharmacological applications. -N=C-O linkage is reported to react with nucleophilic centres present in microbial cells. The heterocyclic 1,3,4-oxadiazole has been reported to be used extensively for drug designing.

Literature has reported the antibacterial, antitubercular, anticancer, anti-inflammatory, antiviral, antitumor, antihypertensive, anti-HIV, anticonvulsant, insecticidal and analgesic activities of oxadiazole class of heterocyclic compounds (Bhatt *et al.*, 2020; Selvaraj *et al.*, 2017; Vaidya *et al.*, 2020). Tiodazosin, Raltegravir, Nesapidil and Furamizole are some oxadiazole bearing commercial drugs available in market having good biological activities.

Morpholine is a heterocyclic moiety containing one nitrogen and one oxygen. It has been found to exhibit high antimicrobial and enzyme inhibition potential (Padmavath *et al.*, 2009). Drug development methods have shown the C-substituted morpholine units as biologically active compounds (Hanif *et al.*, 2010). Chiral and non-chiral derivatives of morpholine are effective antagonists for γ-amino butyric acid sub-class ($GABA_B$) receptors. Because of therapeutic properties, synthesis of new morpholine derivatives is under consideration by the scientists (Zou *et al.*, 2002).

In protraction of research projects conducted on 1,3,4-oxadiazole (Samreen *et al.*, 2013; Samreen *et al.*, 2017; Aziz-ur-Rehman *et al.*, 2015; Aziz-ur-Rehman *et al.*, 2020), the molecules bearing submerged 1,3,4-oxadiazole and morpholine moieties were successfully synthesized to investigate their combined effect on bioactivity potential. All the derivatives were analyzed for antibacterial and hemolytic activities and showed reasonably considerable potential.

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MATERIALS AND METHODS

Material

Sigma Aldrich provided all the chemicals. 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 and that at 75 MHz for ^{13}C-NMR spectra of synthesized compounds. IR spectra of all the derivatives were taken on a model of Jasco-320-AIR spectrophotometer. EI-MS spectra were presented by the spectrometer (JMS-HX-110).

Synthesis of ethyl esters (2a-f)

In a 500 mL R.B (round bottom) flask, 0.1 mol phenyl/aryl carboxylic acids (1a-f) were dissolved in 40-60 mL EtOH. The reaction mixture was catalyzed by 5-7 mL of conc. H_2SO_4 and subjected to be refluxed for 4-5 hours. TLC was employed to monitor the reaction. Upon maximal completion, 15% Na_2CO_3 solution neutralized the mixture and removed catalyst or unreacted organic acids. Then mixture was transferred in 1000 mL separating funnel and chloroform was added to it. Contents in funnel were vigorously shaken for 20 minutes. After shaking, the mixture in funnel was settled to bi-layers. The chloroform was evaporated after separation to acquire title compounds.

Synthesis of hydrazides (3a-f)

In a 250mL R.B flask, 0.05 mol the ethyl esters (2a-f) were mixed in 30mL methanol. 12-15mL hydrazine hydrate was also mixed and stirred at 25-30°C for 3-4 hours. Some of the esters were completely converted to corresponding hydrazides by stirring at RT but some after slight heating. TLC confirmed the reaction completionand the precipitates, 3a-f, were quenched by excess distilled water (cold). The title compounds were collected through filtration followed by washing and drying.

Synthesis of 1,3,4-oxadiazoles (4a-f)

In 250 mL R.B flask, 0.05 mol hydrazides (3a-f) were mixed with 50 mL ethanol, 0.1 mol solid KOH and then 0.05 mol CS_2. The mixture was subjected to be refluxed for 6-7 hours. TLC monitored the reaction. Upon completion, the excess distilled water was poured. The pH was set to 5-6 by dil. HCl to neutralize the base and get back the acidic form of oxadiazoles. The title compounds, 4a-f, were collected through filtration followed by washing and drying. The title compounds were also subjected to recrystallization from methanol.

Synthesis of 2-{[(morpholin-4-yl)ethyl]thio}-5-phenyl/aryl-1,3,4-oxadiazoles(6a-f)

In 100mL R.B flask, 0.001 mol 1,3,4-oxadiazoles (4a-h) were mixed with 0.001 mol NaH in 15mL DMF and stirred for 20 minutes. 4-(2-Chloroethyl)morpholine hydrochloride (5, 0.001 mol) was added and subjected to be stirred for 4-5 hours. TLC monitored the reaction. The precipitates were quenched by excess distilled water (cold). The title compounds, 6a-f, were collected through filtration followed by washing and drying.

2-{[(Morpholin-4-yl)ethyl]thio}-5-phenyl-1,3,4-oxadiazole (6a)

Mol. For.: $C_{14}H_{17}N_3O_2S$; Mol. Wt.: 291 gmol^{-1}; Yield: 87 %; Brown sticky solid; IR (υ_{max}/cm^{-1}, KBr): 3033, 1614, 1471, 1232, 1058, 653; ^{1}H-NMR ($CDCl_3$): *δ*, ppm, 7.96 (H-2' & H-6', 2H, dd, *J* = 7.6, 1.2 Hz, Ar-H), 7.50-7.46 (H-3' to H-5', 3H, m, Ar-H), 3.76-3.68 (H-3" & H-5", 4H, m), 3.47 (H-8", 2H, t, *J* = 6.8 Hz, Ar-H), 2.82 (H-7", 2H, t, *J* = 6.8 Hz, Ar-H), 2.59-2.54 (H-2" & H-6", 4H, m); ^{13}C-NMR (*δ*/ppm, 75 MHz, $CDCl_3$): 166.3 (C-2), 164.8 (C-5), 133.6 (C-1'), 128.9 (C-3' & C-5'), 128.8 (C-2' & C-6'), 127.6 (C-4'), 66.3 (C-3" & C-5"), 56.8 (C-7"), 53.0 (C-2" & C-6"), 31.8 (C-8"); EI-MS (*m/z*): 291 $[C_{14}H_{17}N_3O_2S]^{\bullet+}$, 178 $[C_8H_6N_2OS]^{\bullet+}$, 145 $[C_8H_5N_2O]^{+}$,119 $[C_7H_5NO]^{\bullet+}$, 113 $[C_6H_{11}NO]^{\bullet+}$, 103 $[C_7H_5N]^{\bullet+}$, 100 $[C_5H_{10}NO]^{+}$, 85 $[C_4H_7NO]^{\bullet+}$, 77 $[C_6H_5]^{+}$.

2-{[(Morpholin-4-yl)ethyl]thio}-5-(4-methylphenyl)-1,3,4-oxadiazole (6b)

Mol. For.: $C_{15}H_{19}N_3O_2S$; Mol. Wt.: 305 gmol^{-1}; Yield: 84 %; Light brown powder; M. Pt.: 58 °C; IR (υ_{max}/cm^{-1}, KBr): 3037, 1611, 1473, 1229, 1070, 657; 1H-NMR (CDCl3): δ, ppm, 7.87 (H-2' & H-6', 2H, d, *J* = 8.0 Hz, Ar-H), 7.28 (H-3' to H-5', 2H, d, *J* = 7.6 Hz, Ar-H), 3.75-3.71 (H-3" & H-5", 4H, m), 3.52 (H-8", 2H, br.t, *J* = 7.2 Hz), 2.92 (H-7", 2H, t, *J* = 7.2 Hz,), 2.63-2.59 (H-2" & H-6", 4H, m), 2.23 (CH_3-7', 3H, s); ^{13}C-NMR (*δ*/ppm, 75 MHz, $CDCl_3$): 165.0 (C-2), 164.8 (C-5), 142.2 (C-4'), 129.7 (C-2' & C-6'), 126.5 (C-3' & C-5'), 120.8 (C-1'), 66.0 (C-3" & C-5"), 56.9 (C-7"), 53.1 (C-2" & C-6"), 29.6 (C-8"), 21.6 (C-7'); EI-MS (*m/z*): 305 $[C_{15}H_{19}N_3O_2S]^{\bullet+}$, 192 $[C_9H_8N_2OS]^{\bullet+}$, 159 $[C_9H_7N_2O]^{+}$,133 $[C_8H_7NO]^{\bullet+}$, 117 $[C_8H_7N]^{\bullet+}$, 113 $[C_6H_{11}NO]^{\bullet+}$, 100 $[C_5H_{10}NO]^{+}$, 85 $[C_4H_7NO]^{\bullet+}$, 91 $[C_7H_7]^{+}$.

2-{[(Morpholin-4-yl)ethyl]thio}-5-(4-hydroxyphenyl)-1,3,4-oxadiazole (6c)

Mol. For.: $C_{14}H_{17}N_3O_3S$; Mol. Wt.: 307 gmol^{-1}; Yield: 81 %; Yellow sticky solid; IR (υ_{max}/cm^{-1}, KBr): 3027, 1617, 1477, 1238, 1068, 663; 1H-NMR (CDCl3): δ, ppm, 7.90 (H-2' & H-6', 2H, d, *J* = 8.8 Hz, Ar-H), 6.97 (H-3' & H-5', 2H, d, *J* = 8.8 Hz, Ar-H), 3.93 (H-8", 2H, t, *J* = 7.2 Hz), 3.90-3.54 (H-3" & H-5", 4H, m), 3.53 (H-7", 2H, t, *J* = 7.2 Hz), 2.94-2.66 (H-2" & H-6", 4H, m); ^{13}C-NMR (*δ*/ppm, 75 MHz, $CDCl_3$): 168.2 (C-2), 164.8 (C-5), 161.2 (C-4'), 124.8 (C-1'), 124.2 (C-3' & C-5'), 120.5 (C-2' & C-6'), 66.5 (C-3" & C-5"), 56.7 (C-7"), 53.3 (C-2" & C-6"), 29.3 (C-8"); EI-MS (*m/z*): 307 $[C_{14}H_{17}N_3O_3S]^{\bullet+}$, 194 $[C_8H_6N_2O_2S]^{\bullet+}$, 161 $[C_8H_5N_2O_2]^{+}$, 135 $[C_7H_5NO_2]^{\bullet+}$, 113

$[C_6H_{11}NO]^{\bullet+}$, 119 $[C_7H_5NO]^{\bullet+}$, 100 $[C_5H_{10}NO]^+$, 85 $[C_4H_7NO]^{\bullet+}$, 93 $[C_6H_5O]^+$.

2-{[(Morpholin-4-yl)ethyl]thio}-5-(4-nitrophenyl)-1,3,4-oxadiazole (6d)

Mol. For.: $C_{14}H_{16}N_4O_4S$; Mol. Wt.: 336 gmol^{-1}; Yield: 85 %; Golden yellow sticky solid; IR (υ_{max}/cm^{-1}, KBr): 3037, 1617, 1475, 1230, 1069, 650; 1H-NMR (CDCl3): δ, ppm, 7.76 (H-3' & H-5', 2H, d, *J* = 8.4 Hz, Ar-H), 6.68 (H-2' & H-6', 2H, d, *J* = 8.4 Hz, Ar-H), 4.03-3.81 (H-3" & H-5", 4H, m), 3.80 (H-8", 2H, t, *J* = 7.2 Hz), 2.98 (H-7", 2H, t, *J* = 7.2 Hz), 2.81-2.76 (H-2" & H-6", 4H, m); ^{13}C-NMR (δ/ppm, 75 MHz, $CDCl_3$): 168.1 (C-2), 164.5 (C-5), 147.8 (C-4'), 137.3 (C-1'), 126.3 (C-2' & C-6'), 125.5 (C-3' & C-5'), 66.3 (C-3" & C-5"), 53.3 (C-7"), 53.1 (C-2" & C-6"), 34.5 (C-8"); EI-MS (*m/z*): 336 $[C_{14}H_{16}N_4O_4S]^{\bullet+}$, 222 $[C_8H_5N_3O_3S]^{\bullet+}$, 190 $[C_8H_4N_3O_3]^+$, 164 $[C_7H_4N_2O_3]^{\bullet+}$, 148 $[C_7H_4N_2O_2]^{\bullet+}$, 113 $[C_6H_{11}NO]^{\bullet+}$, 100 $[C_5H_{10}NO]^+$, 85 $[C_4H_7NO]^{\bullet+}$.

2-{[(Morpholin-4-yl)ethyl]thio}-5-(4-aminophenyl)-1,3,4-oxadiazole (6e)

Mol. For.: $C_{14}H_{18}N_4O_2S$; Mol. Wt.: 306 gmol^{-1}; Yield: 80 %; Light pink amorphous solid; M. Pt.: 130 °C; IR (υ_{max}/cm^{-1}, KBr): 3039, 1617, 1470, 1230, 1069, 654; 1H-NMR (CDCl3): δ, ppm, 7.76 (H-2' & H-6', 2H, d, *J* = 8.4 Hz, Ar-H), 6.68 (H-3' & H-5', 2H, d, *J* = 8.8 Hz, Ar-H), 4.03-3.96 (H-3" & H-5", 4H, m), 3.86 (H-8", 2H, t, *J* = 7.2 Hz), 3.47 (H-7", 2H, t, *J* = 7.2 Hz), 2.88-2.82 (H-2" & H-6", 4H, m); ^{13}C-NMR (δ/ppm, 75 MHz, $CDCl_3$): 168.1 (C-2), 164.7 (C-5), 153.7 (C-4'), 125.8 (C-1'), 125.3 (C-2' & C-6'), 114.5 (C-3' & C-5'), 66.4 (C-3" & C-5"), 56.4 (C-7"), 53.1 (C-2" & C-6"), 34.3 (C-8"); EI-MS (*m/z*): 306 $[C_{14}H_{18}N_4O_2S]^{\bullet+}$, 194 $[C_8H_7N_3OS]^{\bullet+}$, 160 $[C_8H_6N_3O]^+$,134 $[C_7H_6N_2O]^{\bullet+}$, 118 $[C_7H_6N_2]^{\bullet+}$, 113 $[C_6H_{11}NO]^{\bullet+}$, 100 $[C_5H_{10}NO]^+$, 85 $[C_4H_7NO]^{\bullet+}$.

2-{[(Morpholin-4-yl)ethyl]thio}-5-(2-nitrophenyl)-1,3,4-oxadiazole (6f)

Mol. For.: $C_{14}H_{16}N_4O_4S$; Mol. Wt.: 336 gmol^{-1}; Yield: 93 %; Dark brown sticky solid; IR (υ_{max}/cm^{-1}, KBr): 3039, 1619, 1478, 1236, 1068, 649; 1H-NMR (CDCl3): δ, ppm, 7.66 (H-3', 1H, dd, *J* = 7.6, 0.8 Hz, Ar-H), 7.21 (H-6', 1H, dd, *J* = 7.6, 0.8 Hz, Ar-H), 6.75 (H-5', 1H, t, *J* = 8.4 Hz, Ar-H), 6.70 (H-4', 1H, t, *J* = 7.6 Hz, Ar-H), 3.83-3.71 (H-3" & H-5", 4H, m), 3.47 (H-8", 2H, t, *J* = 7.2 Hz), 2.98 (H-7", 2H, t, *J* = 7.2 Hz), 2.85-2.57 (H-2" & H-6", 4H, m); ^{13}C-NMR (δ/ppm, 75 MHz, $CDCl_3$): 168.1 (C-2), 163.7 (C-5), 147.7 (C-2'), 131.8 (C-4'), 130.9 (C-5'), 128.1 (C-3'), 125.5 (C-6'), 124.9 (C-1'), 66.1 (C-3" & C-5"), 56.6 (C-7"), 53.2 (C-2" & C-6"), 34.1 (C-8"); EI-MS (*m/z*): 336 $[C_{14}H_{16}N_4O_4S]^{\bullet+}$, 222 $[C_8H_5N_3O_3S]^{\bullet+}$, 190 $[C_8H_4N_3O_3]^+$,164 $[C_7H_4N_2O_3]^{\bullet+}$, 148 $[C_7H_4N_2O_2]^{\bullet+}$, 113 $[C_6H_{11}NO]^{\bullet+}$, 100 $[C_5H_{10}NO]^+$, 85 $[C_4H_7NO]^{\bullet+}$.

Antibacterial potential assay

Antibacterial potential was assayed through the reported method (Kaspady *et al*., 2009) by noting the absorbance change with and without the synthesized compounds. Ciprofloxacin was taken as standard and all results were measured in triplicate. EZ-Fitz Perrella Scientific Inc. Amherst USA software was used for calculation of results.

Hemolytic potential assay

Hemolytic potential to assess the toxicity of derivatives was assayed through reported method (Sharma and Sharma, 2001) by noting the absorbance change at 576 nm under UV spectrophotometer.

STATISTICAL ANALYSIS

Statistical analysis of all readings was finished by ME 2010 and findings are offered as mean ± SEM.

RESULTS

A new series of molecules, 6a-f, bearing 1,3,4-oxadiazole and morpholine moieties were proficiently synthesized and analyzed for their antibacterial and hemolytic potentials. The 1,3,4-oxadiazol-2-thiols (4a-f) were synthesized by refluxing hydrazides, prepared from corresponding ester, with CS_2 and KOH in ethanol. All these compounds were treated with 4-(2-chloroethyl)morpholine hydrochloride (5) to synthesize the target compounds, 2-{[(morpholin-4-yl)ethyl]thio}-5-phenyl/aryl-1,3,4-oxadiazole (6a-f). The synthetic outline is given in scheme 1 and varying groups are given in table 3. All these molecules were characterized by IR, EI-MS, ^{1}H-NMR and ^{13}C-NMR spectral data. All these molecules were subjected to antibacterial and hemolytic activities and showed moderately good antibacterial and hemolytic potential relative to the reference standards.

Antibacterial activity

Antibacterial activity of the newly synthesized compounds were tested using most frequently reported method to test the bacterial sensitivity of the compounds. The results of the antibacterial study are summarized in table 1. The bacteriostatic study was also explained in term of minimum inhibitory concentration values which are also shown in table 2.

Hemolytic potential

The hemolytic activity was performed reported by Sharma and Sharma (2001). The results of hemolytic activity of all the synthesized compounds are tabulated in table 2.

DISCUSSION

IR spectra revealed the appearance of definite functionalities present in the compounds at wave number of 3030, 1610, 1460, 1260 and 1060cm^{-1}. In ^{1}H-NMR, the adjacent methylene groups of ethyl chain presented two

Table 1: % Inhibition of antibacterial potential

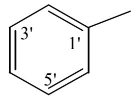
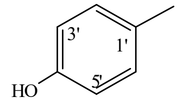
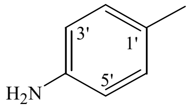
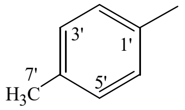
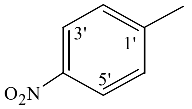
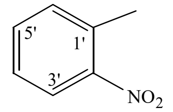
Compound	% Inhibition					
	Gram-negative bacteria				Gram-positive bacteria	
	S. typhi	*E. coli*	*K. pneumonae*	*P. aeruginosa*	*B. subtilis*	*S. aureus*
6a	68.81±2.50	52.52±3.90	70.18±1.18	67.59±0.41	63.81±1.79	67.60±1.80
6b	58.28±0.72	28.62±3.43	46.14±3.59	67.53±2.00	70.12±0.60	60.75±2.95
6c	68.89±1.56	36.65±0.41	52.41±0.96	76.88±0.41	76.73±1.01	77.05±2.35
6d	61.22±4.01	30.55±3.43	47.27±2.91	67.24±0.53	72.92±3.04	68.50±2.60
6e	65.72±1.06	42.80±0.14	52.23±1.86	75.41±2.00	76.13±1.96	69.65±4.00
6f	64.22±3.22	30.50±3.11	48.73±1.55	70.35±3.36	75.83±0.00	70.35±0.15
Ciprofloxacin	91.14±0.84	88.94±1.21	90.65±1.29	90.36±1.86	91.33±1.99	91.87±2.51

Table 2: MIC values of antibacterial potential and hemolytic potential

Compound	MIC (μM)						
	Gram-negative bacteria				Gram-positive bacteria		Hemolytic potential
	S. typhi	*E. coli*	*K. pneumonae*	*P. aeruginosa*	*B. subtilis*	*S. aureus*	% (Mean)
6a	12.86±0.69	13.62±2.90	10.09±1.18	10.10±2.91	11.97±2.10	13.54±1.00	6.67
6b	15.41±2.62	-	-	11.38±0.91	13.04±1.40	14.51±1.14	7.02
6c	12.49±0.54	-	17.13±0.96	10.45±1.64	9.90±0.00	10.04±0.00	5.84
6d	14.09±0.46	-	-	11.98±2.09	9.30±2.50	13.11±1.36	6.30
6e	13.28±1.69	-	19.08±1.86	11.56±2.36	10.61±1.10	10.28±0.86	6.14
6f	11.74±0.77	-	-	12.46±3.73	10.81±1.30	11.49±2.57	7.09
Ciprofloxacin	8.26±0.92	9.14±0.28	8.92±0.41	8.56±1.18	9.11±1.30	8.48±1.43	
PBS							0.00±0.0
Triton-X							100±0.0

Note: PBS = Phosphate buffered saline

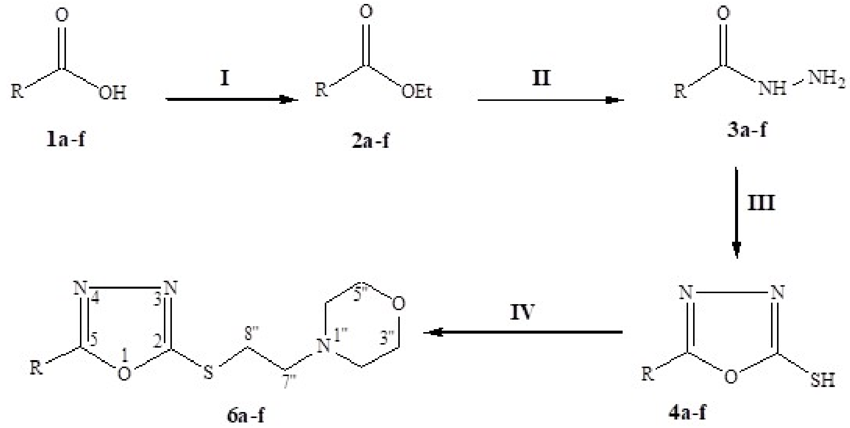
Table 3: Different varying phenyl/aryl groups

Compound	R	Compound	R	Compound	R
6a		6c		6e	
6b		6d		6f	

triplets at 3.50 and 2.80 ppm with integration of two protons. Methylenes of morpholine ring appeared as multiplet in these ranges 3.65-3.75 and 2.57-2.65 ppm. Aromatic protons of varying group resonated as two doublets with two proton integration at 6.65-7.50 & 7.58-7.95 ppm for 4-substituted ring; as doublet of doublet with two proton integration at 7.96 ppm & multiplet with three proton integration at 7.50-7.46 ppm for phenyl ring; and two doublets of doublet with one proton integration at 7.66 & 7.21 ppm and two triplets with one proton integration at 6.75 & 6.70 ppm for the 2-substituted ring. In ^{13}C-NMR, quaternary carbons of oxadiazole nucleus resonated around 166.1-169.9 and 161.1-165.9 while methylenes of morpholine ring appeared around 65.1-66.5 due to vicinity of electronegative oxygen atom and 53.1-56.5 ppm under the electronegative effect of vicinal nitrogen atom. Methylene attached to thio group of oxadiazole ring appeared around 32.1-32.9 ppm. Similarly methylene group that have one nitrogen atom of morpholine ring and one methylene attached to sulphur gave signals around 53.1-53.9 ppm. In EI-MS, the definite peaks appeared at *m/z* 113, 100 and 85 corroborating the presence of morpholine ring. The detailed spectroscopic data of synthesized compounds is presented in the experimental section.

Antibacterial Activity

Lifesaving chemically synthesized compounds play quite important role in strengthening and increasing the health and age of plants and animals (Naqvi et. al., 2020; Naqvi



Scheme 1: Synthesis of 2-{[(morpholin-4-yl)ethyl]thio}-5-phenyl/aryl-1,3,4-oxadiazole derivatives. Reagents & conditions: (I) Reflux for 4-5 hours, Conc. H_2SO_4, EtOH (II) Stir for 3-4 hours, MeOH, Hydrazine hydrate (III) Reflux for 6-7 hours, EtOH, KOH, CS_2 (IV) 4-(2-chloroethyl)morpholine hydrochloride (5), NaH, DMF, Reflux for 4-5 hours.

et al., 2020; Melesse *et al*., 2020). Prior to modernization, plant biomass was used to treat infections which was then translated to modified compounds for the betterment of human beings either in the field of health or social (Li *et al.,* 2020; Teong *et al*. 2020). Four Gram-negative and two Gram-positive bacterial strains were taken into account for the evaluation of antibacterial potential. The findings are tabulated in table 2 and table 3 as inhibition (%) and MIC. The reference standard was ciprofloxacin which presented % inhibition of 91.87±2.51, 91.33±1.99, 90.36±1.86, 90.65±1.29, 88.94±1.21and 91.14±0.84against *S. aureus*, *B. subtilis*, *P. aeruginosa*, *K. pneumonae*, *E. coli* and *S. typhi* respectively. Compound 6a bearing unsubstituted phenyl ring showed relatively good inhibition of *S. typhi*, *E. coli* and *K. pneumonae* with % inhibition of 68.81±2.56, 52.52±3.90 and 70.18±1.18 respectively relative to the ciprofloxacin. Similarly compound 6c bearing 4-hydroxyphenyl ring showed good inhibition of *S. typhi*, *B. subtilis* and *S. aureus* with % inhibition of 68.89±1.56, 76.73±1.01 and 77.05±2.35 respectively relative to the standard. Compounds 6e and 6f bearing 4-aminophenyl and 2-nitrophenyl showed relatively good inhibition for only *B. subtilis* at 76.13±1.96 and 75.83±0.00 respectively.

Minimum inhibitory concentrations analysis was also checked out for all these compounds. Compounds 6a, 6c and 6f revealed almost 1.5 times less activity against *S. typhi* as compared to ciprofloxacin with MIC of 8.26±0.92 μM. Against *E. coli*, only 6a compound showed MIC of 13.62±2.90 that was also 1.5 times less active as compared to reference with that of 9.14±0.28 μM. For *K. pneumonae*, compound 6a bearing phenyl ring had relatively good results with MIC of 10.09±1.18 μM with reference to 8.91±0.41 μM. Compounds 6a, 6c and 6f, bearing phenyl, 4-hydroxyphenyl and 2-nitrophenyl respectively, showed relatively better inhibition but still lesser than that of standard. Compound 6c and 6d, bearing 4-hydroxyphenyl and 4-nitrophenyl respectively, showed excellent MIC values of 9.90±0.00 and 9.30±2.50 μM respectively against *B. subtilis*, as these values were very close to that of ciprofloxacin as 9.11±1.30 μM. Overall the compound 6a was the most efficient against the taken bacterial strains.

Hemolytic potential

The hemolytic activity results (table 3) presented compound 6c bearing 4-hydroxyphenyl ring as the least toxic one owing to its lowest hemolytic percentage, 5.84% relative to Triton-X used as standard with %lysis of 100, a positive control. Overall, all the compounds may be rendered as the least toxic ones. Because of the least toxic effects, these compounds may be utilizable in pharmaceutical industries in drug designing or development programs.

CONCLUSION

The good yields of the final products confirmed the affordability of the synthetic procedures at commercial level. The purity and complete synthesis of the compounds were fully justified by the spectral data of ^{13}C-NMR,^{1}H-NMR, IR and EI-MS. The antibacterial evaluation showed that compound 6a bearing unsubstituted phenyl ring showed relatively good inhibition of *S. typhi, E. coli* and *K. pneumonae* with % inhibition of 68.81±2.56, 52.52±3.90 and 70.18±1.18 respectively relative to the ciprofloxacin. Similarly compound 6c bearing 4-hydroxyphenyl ring showed good inhibition of *S. typhi, B. subtilis* and *S. aureus* with % inhibition of 68.89±1.56, 76.73±1.01 and 77.05±2.35 respectively relative to the standard. So these molecules can be used as moderately good inhibitors. The hemolytic potential results marked these compounds much less toxic relative to Triton-X. So these molecules may be considerable for the pharmacists for further process in drug development program.

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