

Original Research Article

Synthesis and Evaluation of some New 5-Substituted-1,3,4-oxadiazol-2-yl-4-(morpholin-4-yl Sulfonyl)benzyl Sulfides as Antibacterial Agent

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Abstract

Purpose: To synthesise a new series of 5-substituted-1,3,4-Oxadiazol-2-yl-4-(morpholin-4-yl sulfonyl)benzyl sulfide and evaluate their antibacterial activity.

Methods: Different organic acids were converted consecutively into corresponding esters, hydrazides and 5-substituted-1,3,4-Oxadiazol-2-thiols (**4a-e**). The targets, **6a-e** were synthesized by stirring **4a-e** with 4-(4-(bromomethyl)phenylsulfonyl) morpholine (**5**) in the presence of *N,N*-dimethylformamide (DMF) and sodium hydride (NaH). All the structures were elucidated by modern spectroscopic techniques and screened against bacteria using standard procedure and ciprofloxacin drug as positive control.

Results: The yield of the synthesized compounds (**4a-e** and **6a-e**) were moderate (65 - 90 %). Compounds **6a-e** had antibacterial activity against *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus* while some had activity against the other bacteria used. One of the compounds, **6b**, exhibited significant activity against all the bacterial strains, i.e., *S. typhi* (-), *E. coli* (-), *K. pneumoniae* (-), *P. aeruginosa* (-), *B. subtilis* (+) and *S. aureus* (+) with MIC (μM) values of 11.01 ± 0.31 , 15.37 ± 3.33 , 16.11 ± 1.14 , 9.70 ± 1.96 , 10.01 ± 2.70 and 9.15 ± 0.29 , respectively. However, none of the compounds had any inhibitory activity against any bacteria as high as that of ciprofloxacin.

Conclusion: Five new compounds with antibacterial activities have been synthesized. Their potential as therapeutic agents is, however, yet to be evaluated.

Keywords: 1,3,4-Oxadiazole, Benzyl sulfide, 4-(4-(bromomethyl)phenylsulfonyl)morpholine, Spectral analysis, Antibacterial activity

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INTRODUCTION

Organic chemists are interested in designing and synthesizing fresh and therapeutically active compounds, useful to alleviate different disorders and diseases. Scientists are facing a serious problem of increased resistance of microbes

against the traditional antimicrobial drugs and so are interested in developing new biologically active compounds with excellent therapeutic activity. Oxadiazole is a class of heterocyclic organic compounds that is known because of its magnificent therapeutic potential. Thousands of oxadiazole and their derivatives have been

synthesized and evaluated for different antimicrobial and enzyme inhibition activities. Oxadiazole possesses splendid anti-inflammatory [1,2], fungicidal [3], insecticidal [4], herbicidal [5], antibacterial [6], antitumor [7], antitubercular [8], antiviral, anticonvulsant and analgesic activities [9]. Furthermore, morpholine and its analogues are important in pharmaceuticals due to their great enzyme inhibition potential, antimicrobial and antioxidant activity [10,11].

Synthesis of new morpholine containing compounds is the area of interest for the pharmacists because therapeutically potent compounds are needed to cure different malfunctions and diseases [12]. This current work (an extension of previous work, [13-15]) aimed to synthesize poly-functional compounds having 1,3,4-oxadiazole and sulfa morpholine moiety together in an individual molecule. It was expected that 2,5-disubstituted 1,3,4-oxadiazole ring along with the potential morpholine moiety will boost up the antibacterial activity of the molecule and hence the synthesized compounds were screened against clinically isolated bacteria.

EXPERIMENTAL

All the chemicals and analytical grade solvents were purchased through local suppliers. Melting points were taken on Griffin and George melting point apparatus by open capillary tube method. TLC plate F₂₅₆ 20 x 20 cm coated with silica gel was used to detect the purity of synthesized compounds, developed by different ratios of n-Hexane and EtOAc as solvent system. With the help of KBr pellet method, IR spectrum was recorded by using Jasco-320-A spectrophotometer. ¹H-NMR spectra were recorded on Bruker spectrometers at frequency of 400 MHz, taken in deuterated chloroform, indicating chemical shifts value in ppm taking TMS as reference standard.

Synthesis of ethyl esters of different substituted carboxylic acids (2a-e)

The organic acids [(5 g, Piperonylic acid (**1a**); 3,4-(Methylenedioxy)cinnamic acid (**1b**); Nicotinic acid (**1c**); 2-naphthyl acetic acid (**1d**); 3,5-dinitro-2-methyl benzoic acid (**1e**)] were taken in 250 mL round bottom flask and dissolved in 20 mL ethanol along with 2.5 mL concentrated H₂SO₄. The mixture was refluxed for 2-3 h. TLC was used to monitor the reaction completion by using n-hexane and EtOAc as solvent system. On completion, reaction contents were shifted to a separating funnel containing distilled H₂O (20 mL). The contents were neutralized using

concentrated Na₂CO₃ solution (15 %). Diethyl ether was added to the separating funnel followed by shaking, allowed to separate into two layers and the ethyl esters (**2a-e**) were recovered following by evaporating the diethyl ether.

Synthesis of different hydrazides (3a-e)

The ethyl esters (25 mmol, **3a-e**) were taken in 250 mL RB flask and dissolved in methanol (60 mL). Hydrazine hydrate (80 %, 0.05-0.07 mol) was introduced to the reaction flask along with stirring for 4-5 h at room temperature. Some of the hydrazides were formed at room temperature while some esters got altered on refluxing with constant stirring. Completion of reaction was monitored by TLC using n-hexane and EtOAc as solvent system. On completion, cold distilled H₂O was added to obtain the precipitates. Precipitates of hydrazides (**3a-e**) were filtered, washed with H₂O and dried.

Synthesis of 5-substituted-1,3,4-oxadiazole-2-thiols (4a-e)

The hydrazides (0.01 mol, **3a-e**) were taken in 250 mL RB flask, dissolved in ethanol and CS₂ (0.01 mol) was added followed by the addition of KOH (0.03 mol) to provide basic media. The mixture was refluxed for 5-6 h and reaction progress was monitored by TLC. The reaction completion was confirmed by TLC and the reaction mixture was acidified (pH = 2) to remove un-reacted hydrazides in the form of salts and also to convert thiol group of oxadiazole into acidic form. Precipitates (**4a-d**) were filtered, washed with H₂O and dried. Precipitates were re-crystallized by methanol.

Synthesis of 4-(4-(bromomethyl)phenyl sulfonyl)morpholine (5)

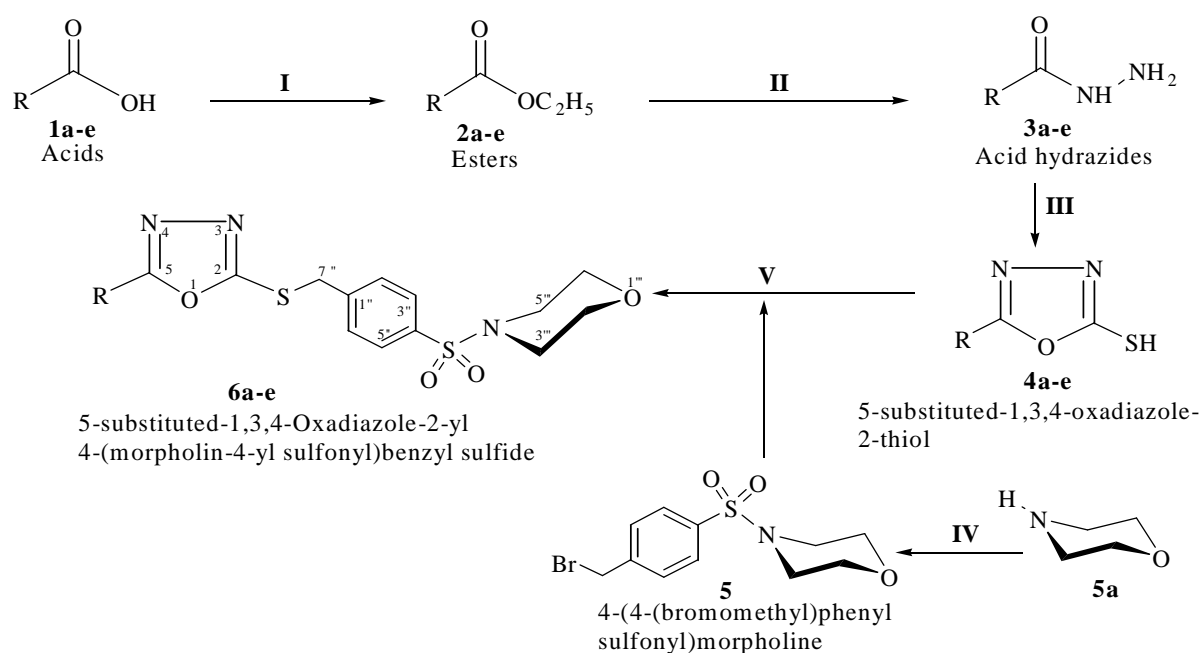
Morpholine (0.005 mol) was added into a round bottom flask containing basic aqueous solution with a pH 9-10. Equimolar 4-(bromomethyl) benzenesulfonyl chloride was introduced to the reaction flask with constant stirring under dynamic pH control. The completion of reaction was monitored via TLC utilizing different ratios of n-hexane and EtOAc. On completion, reaction mixture was acidified (pH = 2) to obtain the product (**5**). Precipitates were filtered, washed with water and dried.

Synthesis of 5-substitued-1,3,4-Oxadiazole-2-yl 4-(morpholin-4yl sulfonyl)benzyl sulfide (6a-e)

5-substitued-1,3,4-oxadiazole-2-thiols (0.1 g, **4a-e**) were taken in 50 mL RB flask and dissolved in

dimethylformamide. On complete dissolution, 2 mg NaH was added and stirred for half an hour. The electrophile, 4-(4-(bromomethyl)phenyl sulfonyl) morpholine (**5**) was added to the reaction mixture, and stirring was continued for further 1-2 h. TLC was used to check the reaction completion by using n-hexane and EtOAc as a solvent system. On completion, cold distilled water was added to get the precipitates which were then filtered, washed with H₂O and dried. The pathway for the synthesis of **6a-e** is provided in scheme 1 while the different 5-substituted aryl/alkyl groups of synthesized compounds are shown in Table 1.

In summary, the synthesis was carried out in different steps. First, the different organic acids (**1a-e**) were converted into subsequent ethyl esters (**2a-e**) by refluxing in concentrated H₂SO₄ and ethanol for 2-3 h. Second, the ethyl esters (**2a-e**) were consequently transformed into hydrazides (**3a-e**) by refluxing with hydrazine hydrate (80 %) using ethanol as a solvent for 4-5 h. Third, the hydrazides (**3a-e**) were cyclized to 5-substituted-1,3,4-oxadiazoles-2-thiols (**4a-e**) by refluxing for 5-6 h with CS₂ in the presence of KOH. The products were acquired by acidifying the reaction mixture. The electrophile **5** was synthesized by the reaction of morpholine with 4-



Scheme-1: Outline for the synthesis of 5-substituted-1,3,4-Oxadiazole-2-yl 4-(morpholin-4-ylsulfonyl)benzyl sulfide. Reagents & conditions: (I) H₂SO₄/EtOH/refluxing for 2-3 h (II) N₂H₄/MeOH/stirring for 4-5 h (III) CS₂/KOH/EtOH/refluxing for 5-6 h (IV) 4-bromomethylbenzenesulfonyl chloride/H₂O/5 % Na₂CO₃ soln./stirring for 1 h (V) DMF/NaH/stirring for 2-3 h

Table-1: Different 5-substituted aryl/alkyl groups of synthesized compounds

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

bromomethylbenzenesulfonyl chloride in the presence basic aqueous media. Fourth, these 5-substituted-1,3,4-Oxadiazoles-2-thiols (**4a-e**) were treated with 4-(4-(bromomethyl)phenylsulfonyl)morpholine (**5**) in the presence of dimethylformamide as solvent and sodium hydride as base. The final products (**6a-e**) were collected by the addition of cold water. The structures of all the synthesized compounds were established by ¹H-NMR, IR and mass spectral data as illustrated in experimental section.

Antibacterial activity assay

The antibacterial activity assay was processed on sterile 96-wells microplates under sterile conditions. Synthesized compounds were screened against four Gram-negative and two Gram-positive bacteria as previously reported [13-15]. Briefly, the test organisms were maintained on stock culture agar medium. Each test sample (in suitable solvent and appropriate dilution) was pipetted into each well (20 µg/well). Freshly maintained overnight bacterial culture (180 µL), after dilution with fresh nutrient broth, was poured into each well which was then incubated at 37 °C for 16-24 h under cover of a microplate. Absorbance was measured at 540 nm using microplate reader, before and after incubation and the difference was noted as an index of bacterial growth using ciprofloxacin as reference standard. Percentage inhibition of each bacteria was calculated and the minimum inhibitory concentration (MIC) was measured using suitable dilutions (5-30 µg/ well) of each compound.

Statistical analysis

All the calculations for MIC values were made after performing the experiments in triplicate. The triplicate calculated values were analyzed statistically through Microsoft Excel 2010 with CL of 85 %. The results are offered as mean ± SEM.

RESULTS

Spectral characteristics of the synthesized compounds

5-(1,3-Benzodioxol-5-yl)-1,3,4-oxadiazol-2-yl-4-(morpholin-4-yl sulfonyl)benzyl sulfide (**6a**)

Light pink amorphous solid; Yield: 88 %; M.P: 140-142 °C; molecular formula: C₂₀H₁₉N₃O₆S₂; Mol. Wt: 461; IR (KBr, cm⁻¹) ν_{max}: 3439 (C-H stretching of benzene ring), 2929 (C-H stretching -CH₂), 1623 & 1610 (C=N stretching of Oxadiazole ring), 1463 (Ar-C=C aromatic

stretching band), 1408 (-SO₂- stretching), 1331 & 1143 (C-O-C bond stretching); ¹H-NMR (400 MHz, CDCl₃, δ / ppm); 7.56 (d, *J* = 8.0 Hz, 2H, H-2" & H-6"), 7.53 (d, *J* = 8.4 Hz, 2H, H-3" & H-5"), 7.34 (d, *J* = 8.0 Hz, 1H, H-6'), 7.24 (d, *J* = 2.0 Hz, 1H, H-4'), 6.76 (d, *J* = 8.4 Hz, 1H, H-7'), 5.89 (s, 2H, CH₂-2'), 4.38 (s, 2H, CH₂-7"), 3.57 (t, *J* = 4.4 Hz, 4H, CH₂-2" & CH₂-6"), 2.81 (t, *J* = 4.4 Hz, 4H, CH₂-3" & CH₂-5"); EIMS (*m/z*): 461 [M]⁺, 311 [M-C₄H₈NO₃S]⁺, 271 [C₁₁H₁₃NO₃S]⁺, 240 [C₁₁H₁₄NO₃S]⁺, 221 [M-C₁₁H₁₄NO₃S]⁺, 189 [M-C₁₁H₁₄NO₃S]⁺, 149 [M-C₁₂H₁₄N₃O₃S₂]⁺, 147 [M-C₁₂H₁₄N₂O₄S₂]⁺, 121 [M-C₁₃H₁₄N₃O₄S₂]⁺, 86 [C₄H₈NO]⁺.

5-[2-(1,3-Benzodioxol-5-yl)ethenyl]-1,3,4-oxadiazol-2-yl-4-(morpholin-4-yl sulfonyl)benzyl sulfide (**6b**)

Light green amorphous solid; Yield: 66 %; M.P: 156-158 °C; molecular formula: C₂₂H₂₁N₃O₆S₂; Mol. Wt: 487; IR (KBr, cm⁻¹) ν_{max}: 3438 (C-H stretching of benzene ring), 2927 (C-H stretching -CH₂), 1622 & 1611 (C=N stretching of Oxadiazole ring), 1462 (Ar-C=C aromatic stretching band), 1407 (-SO₂- stretching), 1330 & 1142 (C-O-C bond stretching); ¹H-NMR (300 MHz, CDCl₃, δ / ppm); 7.63 (d, *J* = 8.0 Hz, 2H, H-2" & H-6"), 7.54 (d, *J* = 8.4 Hz, 2H, H-3" & H-5"), 7.38 (d, *J* = 8.0 Hz, 1H, H-6'), 7.29 (d, *J* = 16.5 Hz, 1H, H-9'), 6.93 (d, *J* = 16.5 Hz, 1H, H-8'), 6.88 (s, 1H, H-4'), 6.70 (d, *J* = 8.4 Hz, 1H, H-7'), 5.90 (s, 2H, CH₂-2'), 4.43 (s, 2H, CH₂-7"), 3.63 (t, *J* = 4.2 Hz, 4H, CH₂-2" & CH₂-6"), 2.90 (t, *J* = 4.2 Hz, 4H, CH₂-3" & CH₂-5"); EIMS (*m/z*): 487 [M]⁺, 337 [M-C₄H₈NO₃S]⁺, 271 [C₁₁H₁₃NO₃S]⁺, 240 [C₁₁H₁₄NO₃S]⁺, 247 [M-C₁₁H₁₄NO₃S]⁺, 215 [M-C₁₁H₁₄NO₃S]⁺, 175 [M-C₁₂H₁₄N₃O₃S₂]⁺, 173 [M-C₁₂H₁₄N₂O₄S₂]⁺, 147 [M-C₁₃H₁₄N₃O₄S₂]⁺, 86 [C₄H₈NO]⁺.

5-(3-pyridin)-1,3,4-oxadiazol-2-yl-4-(morpholin-4-yl sulfonyl)benzyl sulfide (**6c**)

Off white amorphous solid; Yield: 77 %; M.P: 161-163 °C; molecular formula: C₁₈H₁₈N₄O₄S₂; Mol. Wt: 418; IR (KBr, cm⁻¹) ν_{max}: 3437 (C-H stretching of benzene ring), 2925 (C-H stretching -CH₂), 1621 & 1613 (C=N stretching of Oxadiazole ring), 1463 (Ar-C=C aromatic stretching band), 1405 (-SO₂- stretching), 1331 & 1143 (C-O-C bond stretching); ¹H-NMR (400 MHz, CDCl₃, δ / ppm); 8.71 (s, 1H, H-2'), 7.91 (br.s, 1H, H-4'), 7.75 (d, *J* = 7.6 Hz, 1H, H-6'), 7.67 (d, *J* = 8.0 Hz, 2H, H-2" & H-6"), 7.63 (d, *J* = 8.8 Hz, 2H, H-3" & H-5"), 7.58 (t, *J* = 8.4 Hz, 1H, H-5'), 4.54 (s, 2H, CH₂-7") 3.63 (t, *J* = 4.4 Hz, 4H, CH₂-2" & CH₂-6"), 2.92 (t, *J* = 4.8 Hz, 4H, CH₂-3" & CH₂-5"); EIMS (*m/z*): 418 [M]⁺, 268 [M-C₄H₈NO₃S]⁺, 271 [C₁₁H₁₃NO₃S]⁺, 240

[C₁₁H₁₄NO₃S]⁺, 178 [M-C₁₁H₁₄NO₃S]⁺, 146 [M-C₁₁H₁₄NO₃S]⁺, 106 [M-C₁₂H₁₄N₃O₃S₂]⁺, 104 [M-C₁₂H₁₄N₂O₄S₂]⁺, 78 [M-C₁₃H₁₄N₃O₄S₂]⁺, 86 [C₄H₈NO]⁺.

5-(2-naphthalenylmethyl)-1,3,4-oxadiazol-2-yl-4-(morpholin-4-yl sulfonyl)benzyl sulfide (6d)

Off white amorphous solid; Yield: 64 %; M.P: 190-192 °C; molecular formula: C₂₄H₂₃N₃O₄S₂; Mol. Wt: 481; IR (KBr, cm⁻¹) ν_{\max} : 3447 (C-H stretching of benzene ring), 2923 (C-H stretching -CH₂), 1620 & 1611 (C=N stretching of Oxadiazole ring), 1461 (Ar-C=C aromatic stretching band), 1404 (-SO₂- stretching), 1329 & 1141 (C-O-C bond stretching); ¹H-NMR (400 MHz, CDCl₃, δ / ppm); 8.00 (d, J = 8.0 Hz, 1H, H-4'), 7.80 (d, J = 7.6 Hz, 1H, H-8'), 7.77 (d, J = 6.8 Hz, 1H, H-5'), 7.51 (d, J = 8.4 Hz, 1H, H-3'), 7.49 (s, 1H, H-1'), 7.48-7.44 (m, 2H, H-6' & H-7'), 7.38 (d, J = 8.0 Hz, 2H, H-2" & H-6"), 7.36 (d, J = 8.0 Hz, 2H, H-3" & H-5"), 4.53 (s, 2H, CH₂-2'), 4.29 (s, 2H, CH₂-7"), 3.64 (t, J = 4.4 Hz, 4H, CH₂-2" & CH₂-6"), 2.87 (t, J = 4.8 Hz, 4H, CH₂-3" & CH₂-5"); EIMS (m/z): 481 [M]⁺, 331 [M-C₄H₈NO₃S]⁺, 271 [C₁₁H₁₃NO₃S]⁺, 240 [C₁₁H₁₄NO₃S]⁺, 241 [M-C₁₁H₁₄NO₃S]⁺, 209 [M-C₁₁H₁₄NO₃S]⁺, 169 [M-C₁₂H₁₄N₃O₃S₂]⁺, 167 [M-C₁₂H₁₄N₂O₄S₂]⁺, 141 [M-C₁₃H₁₄N₃O₄S₂]⁺, 86 [C₄H₈NO]⁺.

5-(2-methyl-3,5-dinitrophenyl)-1,3,4-oxadiazol-2-yl-4-(morpholin-4-yl sulfonyl)benzyl sulfide (6e)

Yellow amorphous solid; Yield: 79 %; M.P: 247-249 °C; molecular formula: C₂₀H₁₉N₅O₈S₂; Mol. Wt: 521; IR (KBr, cm⁻¹) ν_{\max} : 3441 (C-H stretching of benzene ring), 2933 (C-H stretching -CH₂), 1624 & 1607 (C=N stretching of Oxadiazole ring), 1465 (Ar-C=C aromatic stretching band), 1409 (-SO₂- stretching), 1333 & 1145 (C-O-C bond stretching); ¹H-NMR (300 MHz, CDCl₃, δ / ppm); 7.65 (d, J = 8.1 Hz, 2H, H-2" & H-6"), 7.60 (d, J = 8.1 Hz, 2H, H-3" & H-5"), 7.16 (d, J = 2.7 Hz, 1H, H-4'), 7.08 (d, J = 2.7 Hz, 1H, H-6'), 4.47 (s, 2H, CH₂-7"), 3.65 (t, J = 4.5 Hz, 4H, CH₂-2" & CH₂-6"), 2.88 (t, J = 4.8 Hz, 4H, CH₂-3" & CH₂-5"); EIMS (m/z): 521 [M]⁺, 371

[M-C₄H₈NO₃S]⁺, 271 [C₁₁H₁₃NO₃S]⁺, 240 [C₁₁H₁₄NO₃S]⁺, 281 [M-C₁₁H₁₄NO₃S]⁺, 249 [M-C₁₁H₁₄NO₃S]⁺, 209 [M-C₁₂H₁₄N₃O₃S₂]⁺, 207 [M-C₁₂H₁₄N₂O₄S₂]⁺, 181 [M-C₁₃H₁₄N₃O₄S₂]⁺, 86 [C₄H₈NO]⁺.

Antibacterial activity

The percentage inhibition of each bacteria and the MICs are presented in Tables 2 and 3, respectively. All five compounds synthesized had antibacterial activity against *P. aeruginosa*, *B. subtilis* and *S. aureus* while the growth of *S. typhi*, *E. coli* and *K. pneumoniae* was inhibited by four, one and two of the compounds, respectively. All the compounds, however, had lower levels of inhibition and higher MIC against the bacteria inhibited than the standard ciprofloxacin.

DISCUSSION

The intention of the presented research work was to synthesize biologically active molecules. We have synthesized some new 2,5-disubstituted 1,3,4-oxadiazole molecules and to find out the anti-bacterial activity of all the synthesized molecules. We synthesized 5-substituted-1,3,4-oxadiazol-2-yl-4-(morpholin-4-yl sulfonyl)benzyl sulfide (**6a-e**) derivatives in good yields having significant antibacterial activities. Compound **6a** was synthesized as a light pink amorphous solid having yield 88 % and melting point 140 - 142 °C. In the IR spectrum, the characteristic peaks appeared at 3439 cm⁻¹, 1632 cm⁻¹, 1443 cm⁻¹ and 1408 cm⁻¹ which were corresponding to C-H stretching, C=N stretching, C=C stretching and stretching of sulfonyl group respectively. The molecular formula C₂₀H₁₉N₃O₆S₂ was established by HR-MS showing molecular ion [M]⁺ peak at m/z 461.516 (calcd for C₂₀H₁₉N₃O₆S₂ 461.513). In the EI-MS spectrum, four distinct peaks appeared at m/z 149, 147, 121 and 86 which pointed out the presence of 1,3-benzodioxol-5-yl carbonyl cation, 1,3-benzodioxol-5-yl cyanide cation, 1,3-benzodioxole cation and morpholine cation fragments respectively in the molecule.

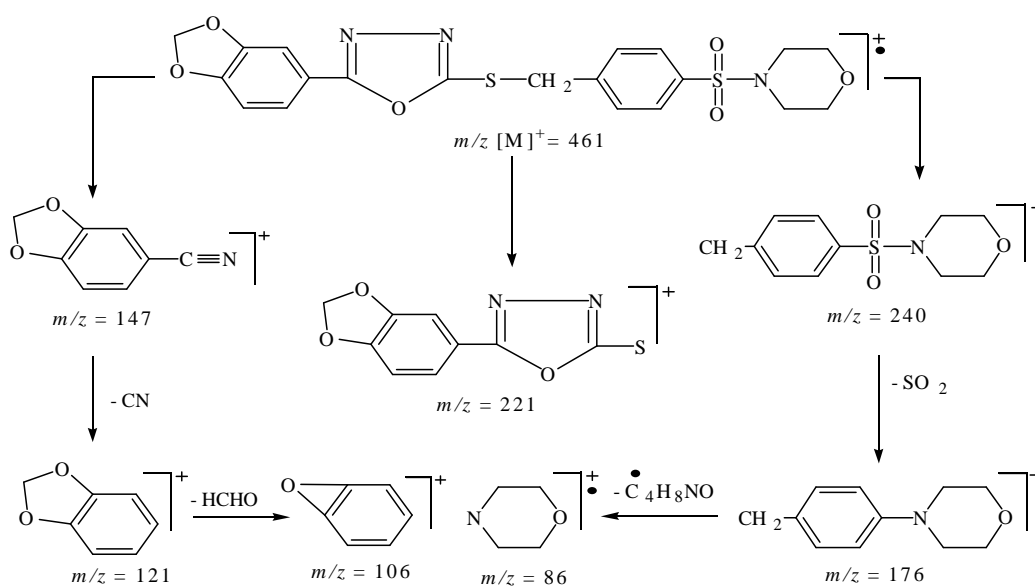
Table 2: Inhibition (%) of selected bacterial strains by five 5-substituted-1,3,4-Oxadiazole-2-yl 4-(morpholin-4-ylsulfonyl)benzyl sulfides (**6a-e**)

Compound	% Inhibition					
	<i>S. typhi</i> (-)	<i>E. coli</i> (-)	<i>K. pneumoniae</i> (-)	<i>P. aeruginosa</i> (-)	<i>B. subtilis</i> (+)	<i>S. aureus</i> (+)
6a	56.33±1.44	-	-	75.35±0.06	60.36±1.31	54.55±2.95
6b	75.00±0.33	56.91±4.12	55.59±1.14	84.59±0.71	82.20±0.54	77.85±3.45
6c	50.94±0.39	-	-	76.94±0.12	61.37±0.42	61.70±0.80
6d	-	-	-	59.71±3.03	50.24±1.43	51.75±1.65
6e	55.22±1.78	-	53.59±1.14	50.32±2.08	60.50±3.40	57.06±0.12
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 3: MIC of five 5-substituted-1,3,4-Oxadiazole-2-yl 4-(morpholin-4-ylsulfonyl)benzyl sulfides (**6a-e**) against selected bacterial strains

Compound	MIC (μM)					
	<i>S. typhi</i> (-)	<i>E. coli</i> (-)	<i>K. pneumoniae</i> (-)	<i>P. aeruginosa</i> (-)	<i>B. subtilis</i> (+)	<i>S. aureus</i> (+)
6a	16.34 \pm 3.00	-	-	12.39 \pm 1.45	12.52 \pm 0.10	17.28 \pm 4.07
6b	11.01 \pm 0.31	15.37 \pm 3.33	16.11 \pm 1.14	9.70 \pm 1.96	10.01 \pm 2.70	9.15 \pm 0.29
6c	18.49 \pm 0.38	-	-	13.16 \pm 3.45	12.50 \pm 2.40	13.99 \pm 0.86
6d	-	-	-	16.79 \pm 4.55	19.87 \pm 2.20	17.16 \pm 0.57
6e	16.56 \pm 2.23	-	16.27 \pm 1.14	18.86 \pm 2.10	11.49 \pm 1.64	17.58 \pm 3.21
Ciprofloxacin	8.26\pm0.92	9.14\pm0.28	8.92\pm0.41	8.56\pm1.18	9.11\pm1.30	8.48\pm1.43

Minimum inhibitory concentration (MIC) was measured with suitable dilutions of test samples (5-30 μg /well) and results were calculated using EZ-Fit Perrella Scientific Inc. Amherst USA software

**Figure 1:** Mass fragmentation pattern of 5-(1,3-benzodioxol-5-yl)-1,3,4-oxadiazol-2-yl-4-(morpholin-4-ylsulfonyl)benzyl sulfide (**6a**)

The $^1\text{H-NMR}$ spectrum showed four signals at δ 7.34 (d, $J = 8.0$ Hz, 1H, H-6'), 7.24 (d, $J = 2.0$ Hz, 1H, H-4'), 6.76 (d, $J = 8.4$ Hz, 1H, H-7') and 5.89 (s, 2H, CH_2 -2') in the downfield region owing to protons of 1,3-benzodioxole moiety attached to the 5-position of oxadiazole ring.

Two signals were resonated at δ 7.56 (d, $J = 8.0$ Hz, 2H, H-2'' & H-6'') and 7.53 (d, $J = 8.4$ Hz, 2H, H-3'' & H-5''), due to higher coupling constant and each signal with integration of two protons, indicating the presence of para substituted aromatic ring. The two signals emerging at δ 9.54 (s, 1H, CON-H) and 4.36 (s, 2H, CH_2 -2) which depicted the presence of acetamide group in the molecule. In the aliphatic region of the spectrum, two triplet were appeared at δ 3.57 (t, $J = 4.4$ Hz, 4H, CH_2 -2''' & CH_2 -6''') and 2.81 (t, $J = 4.4$ Hz, 4H, CH_2 -3''' & CH_2 -5''') each with integration of four protons, showed the presence of morpholine ring and a methylene signal was appeared at 4.38 (s, 2H, CH_2 -7'').

The mass fragmentation pattern of **6a** was also clearly sketched in Fig 1. All the above signals collectively established the structure of compound **6a** and named as 5-(1,3-benzodioxol-5-yl)-1,3,4-oxadiazol-2-yl-4-(morpholin-4-ylsulfonyl)benzyl sulfide. Similarly, the structures of all the other synthesized compounds were elucidated with the help of above mentioned analytical techniques.

Antibacterial activity

Some of the synthesized compounds were moderate inhibitors of three bacterial strains employed in this study. One of the compounds, **6b**, exhibited significant activity against all the bacterial strains i.e. *S. typhi* (-), *E. coli* (-), *K. pneumoniae* (-), *P. aeruginosa* (-), *B. subtilis* (+) and *S. aureus* (+) with MIC (μM) values of 11.01 \pm 0.31, 15.37 \pm 3.33, 16.11 \pm 1.14, 9.70 \pm 1.96, 10.01 \pm 2.70 and 9.15 \pm 0.29 respectively. This compound was found to be more active because of the presence of 1,3-benzodioxol-5-yl ethenyl

group attached to the oxadiazole ring present in the molecule. Against *Pseudomonas aeruginosa*, the order of activity of the compounds were **6b**>**6c**>**6a**>**6d**>**6e** from the highest activity to lowest activity but lower antibacterial activity than the standard compound used in this assay. Overall the activity revealed by the synthesized compounds was good as supported by their MIC values. Such kind of compounds can further be exploited and their derivatives could be synthesized to acquire closer MIC values to the standard, ciprofloxacin.

CONCLUSION

New compounds (**6a-e**) with antibacterial activity have been synthesized. One of the compounds inhibited the growth of four Gram negative (*S. typhi*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*) and two Gram positive (*B. subtilis* and *S. aureus*) bacterial strains. Thus, the compounds are potential lead molecules in the search for potent agents for the treatment of bacterial infections.

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