

Synthesis, spectral analysis and pharmacological study of *N'*-substituted-2-(5-((2,4-dimethylphenoxy)methyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazides

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A series of molecules bearing multiple functional groups were synthesized to study their antibiotic effect against Gram-positive and Gram-negative bacteria and lipoxygenase activity as well. 2,4-Dimethylcarboic acid (**1**) was refluxed with ethyl 2-bromoacetate to synthesize ethyl 2-(2,4-dimethylphenoxy)acetate (**2**). Compound **2** was converted to the corresponding hydrazide **3**, again on refluxing with hydrazine. The compound 5-((2,4-dimethylphenoxy)methyl)-1,3,4-oxadiazol-2-thiol (**4**) was synthesized by the reaction of **3** and CS₂ in the presence of KOH. Compound **4** was further converted to the corresponding ester **5** and then 2-(5-((2,4-dimethylphenoxy)methyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (**6**). The final molecules *N'*-substituted-2-(5-((2,4-dimethylphenoxy)methyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide, **8a-m**, bearing ether, 1,3,4-oxadiazole, thioether, hydrazone and azomethine functional groups were synthesized by stirring the aryl carboxaldehydes **7a-m** with **6** in methanol at room temperature. The depicted structures of all synthesized molecules were corroborated by IR, ¹H-NMR and EIMS spectral data analysis. **8m** and **8i** showed substantial antibacterial activity and lipoxygenase inhibitory activity, respectively.

Uniterms: Acetohydrazides/ Synthesis. Acetohydrazides/Antibacterial activity/ Acetohydrazides/ Lipoxygenase/inhibition activity.

INTRODUCTION

1,3,4-Oxadiazole heterocycle and azomethine derivatives are known to be involved in a variety of biological activities, and a few examples of them are described here. Regarding 1,3,4-oxadiazole heterocycle, antibacterial activity has been found for 2-aryl-7-alkyl/aryl-[1,3,4]-oxadiazolo[3,2-a][1,3,5]triazin-5-one/thione (Deshmukh *et al.*, 2011), anticonvulsant activity for 2-(2-phenoxy)phenyl-1,3,4-oxadiazole derivatives (Tabatabai *et al.*, 2013), antitumor activity for salicylic acid-based 1,3,4-oxadiazole derivatives (Murty *et al.*, 2014), and anticancer activity for 1-(1*H*-benzo[d]imidazol-2-yl)-3-(1,3,4-oxadiazol-5-substituted derivatives-2-yl)

propan-1-ones (Rashid, Husain, Mishra, 2012). The 1,3,4-oxadiazole moiety has also shown preferable binding to the active site of an enzyme through its oxygen (Zhang *et al.*, 2011). With regard to azomethine derivatives, antibacterial and antifungal activities have been found for 2-(5-benzoyl-1*H*-benzo[d][1,2,3]triazol-1-yl)-*N'*-arylidene acetohydrazide (Narsibhai *et al.*, 2012), antifungal activity (against *Aspergillus niger* and *A. flavus*), antioxidant activities for azomethine-*N*-oxides (Salman, 2013), and antibacterial along with antifungal and antiviral activities for azomethine derivatives of isoniazid bearing a 1,3,4-oxadiazole nucleus (Somani *et al.*, 2011). Catalytic enantioselective 1,3-dipolar cycloadditions of azomethine ylides have been evaluated for biological activity (Narayan *et al.*, 2014). Kamel *et al.* (2010) synthesized azomethine compounds and studied their interaction through molecular docking. The results proved them to possess higher binding energies with 1-3

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hydrogen bonds. Thus, this functional group would allow considerable binding with the amino acid residues at the active site of an enzyme. All these findings were also supported by Aslam *et al.* (2011).

All synthesized compounds were evaluated for antibacterial activity against certain strains of Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative (*Escherichia coli*, *S. typhi* and *Pseudomonas aeruginosa*) bacteria. *B. subtilis* is believed to be harmless but produces subtilisin, which causes dermal allergic or hypersensitivity reactions with long exposure (Barbe *et al.*, 2009), and *S. aureus* is pathogenic because it can adhere to the extracellular matrix and plasma proteins on biomaterials (Harris, Foster, Richards, 2002). *E. coli* is also thought to be harmless but causes food poisoning (Vogt, Dippold, 2005). The other strains, *S. typhi* and *P. Aeruginosa*, are associated with enteric fever (Bhattacharya, Das, Choudhury, 2011) and chronic infection (Pressler *et al.*, 2011), respectively. The synthesized compounds were also tested for enzyme inhibitory activity against lipoxygenase. Lipoxygenases (EC 1.13.11.12) are widely distributed in animals and plants. They are involved in arachidonic acid metabolism and also generate various biologically active lipids that have a primary role in inflammation. Therefore, these are targets for drug development for the treatment of a variety of disorders such as bronchial asthma, inflammation etc. (Abbasi *et al.*, 2005; Alitonou *et al.*, 2006).

Focusing on the future of these moieties in drug development owing to the notable biological activities and the results of previous work by our group (Aziz-ur-Rehman *et al.*, 2013a; Aziz-ur-Rehman *et al.*, 2013b; Gul *et al.*, 2013; Khalid *et al.*, 2013), some new molecules of 1,3,4-oxadiazole-bearing azomethine derivatives were synthesized and evaluated for their antibacterial and enzyme inhibition potential. A literature review has shown these moieties to have enzyme inhibitory and antibacterial activity. Thus, the synthesized molecules were tested for lipoxygenase inhibitory activity, in search of new anti-inflammatory agents, and for antibacterial potential.

MATERIAL AND METHODS

General

The chemicals were purchased through local suppliers from Alfa Aesar, Merck and Sigma-Aldrich. Analytical grade solvents were used without further purification. Thin layer chromatography (TLC) was the supporting tool for evaluation of purity of compounds and reaction completion, and carried out using silica gel

G-25-UV₂₅₄-coated aluminum plates and solvent systems of CH₃COOC₂H₅ and *n*-C₆H₁₄ in varying proportions. The melting points of synthesized molecules were determined on a Griffin-George apparatus with an open capillary tube and were uncorrected. The spectral data included IR spectra using a Jasco-320-A spectrophotometer and KBr pellet method, ¹H-NMR spectra obtained with a Bruker spectrometer in CDCl₃ at 400 MHz, and EIMS spectra determined with a JMS-HX-110 spectrometer.

Procedure for synthesis of ethyl 2-(2,4-dimethylphenoxy)acetate (2)

2,4-Dimethylcarboic acid (**1**; 0.05 mol) was added to 20 mL absolute ethanol in a 100-mL round bottom (RB) flask followed by solid KOH (0.05 mol). The reaction contents were refluxed for 0.5 hour. Ethyl 2-bromoacetate (0.05 mol) was then added and further refluxed for 6 hours. The reaction was monitored by TLC. The reaction mixture was brought to room temperature and transferred to a 250-mL separatory funnel followed by 40 mL ice cold distilled water and 30 mL chloroform. The funnel was shaken vigorously and allowed to stand until there were two layers. The lower chloroform layer was separated and evaporated to afford **2**. Light brown liquid; Yield: 86%; Molecular formula: C₁₂H₁₆O₃; Molecular weight: 208 gmol⁻¹; IR (KBr, ν_{max}/cm^{-1}): 3057 (Ar C-H), 1733 (ester C=O), 1583 (Ar C=C), 1121 (C-O); ¹H-NMR (400 MHz, CDCl₃, δ/ppm): 6.95 (s, 1H, H-3'), 6.85 (d, $J = 8.0$ Hz, 1H, H-5'), 6.60 (d, $J = 8.0$ Hz, 1H, H-6'), 4.58 (s, 2H, H-2), 4.24 (q, $J = 7.2$ Hz, 2H, H-1''), 2.23 (s, 3H, CH₃-4'), 2.20 (s, 3H, CH₃-2'), 1.28 (t, $J = 7.2$ Hz, 3H, CH₃-2''); EIMS (m/z): 208 [M]⁺, 163 [C₁₀H₁₁O₂]⁺, 135 [C₉H₁₁O]⁺, 121 [C₈H₉O]⁺, 105 [C₈H₉]⁺, 93 [C₇H₉]⁺; Anal. Calcd for C₁₂H₁₆O₃: C 69.21, H 7.74; found C 69.11, H 7.49.

Procedure for synthesis of 2-(2,4-dimethylphenoxy)acetohydrazide (3)

The ester **2** (0.045 mol) and 80% hydrazine hydrate (0.045 mol) were added to 15 mL absolute ethanol in a 100-mL RB flask and refluxed for 4 hours. After a final TLC check, the solvent was reduced to about 5 mL by distillation followed by the addition of 30 mL cold distilled water. A precipitate appeared on gentle shaking and was acquired after filtration, washing and drying. Pink amorphous solid; Yield: 80%; Melting point: 164-166 °C; Molecular formula: C₁₀H₁₄N₂O₂; Molecular weight: 194 gmol⁻¹; IR (KBr, ν_{max}/cm^{-1}): 3412 (N-H), 3071 (Ar C-H), 1667 (amide C=O), 1595 (Ar C=C), 1155 (C-O); ¹H-NMR (400 MHz, CDCl₃, δ/ppm): 6.96 (s, 1H, H-3'), 6.93 (d, $J =$

8.4 Hz, 1H, H-5'), 6.63 (d, $J = 8.4$ Hz, 1H, H-6'), 4.52 (s, 2H, H-2), 2.24 (s, 3H, CH₃-4'), 2.21 (s, 3H, CH₃-2'); EIMS (m/z): 194 [M]⁺, 192 [C₁₀H₁₂N₂O₂]⁺, 163 [C₁₀H₁₁O₂]⁺, 135 [C₉H₁₁O]⁺, 121 [C₈H₉O]⁺, 105 [C₈H₉]⁺, 93 [C₇H₉]⁺; Anal. Calcd for C₁₀H₁₄N₂O₂: C 61.84, H 7.27, N 14.42; found C 61.63, H 7.09, N 14.25.

Procedure for synthesis of 5-((2,4-dimethylphenoxy)methyl)-1,3,4-oxadiazol-2-thiol (4)

Compound **3** (0.04 mol) was mixed with 50 mL absolute ethanol in a 250-mL RB flask. Solid KOH (0.04 mol) was added and the mixture refluxed until complete dissolution. The liquid CS₂ (0.08 mol) was added strictly at RT. Again refluxing was continued for another 6 hours along with TLC guidance. The solvent was distilled off to one-third and added to excess cold distilled water. Dilute HCl (4-5 mL) was added to adjust to pH 5-6 and allowed to stand for 15 minutes. The precipitate was filtered, washed with distilled water and dried. Cream white amorphous solid; Yield: 86%; Melting point: 104-106 °C; Molecular formula: C₁₁H₁₂N₂O₂S; Molecular weight: 236 gmol⁻¹; IR (KBr, ν_{max}/cm^{-1}): 3231 (Ar C-H), 2553 (S-H), 1673 (C=N), 1587 (Ar C=C), 1092 (C-O); ¹H-NMR (400 MHz, CDCl₃, δ/ppm): 6.95 (d, $J = 8.4$ Hz, 1H, H-5'), 6.92 (d, $J = 1.6$ Hz, 1H, H-3'), 6.75 (d, $J = 8.4$ Hz, 1H, H-6'), 4.99 (s, 2H, H-7'), 2.24 (s, 3H, CH₃-4'), 2.19 (s, 3H, CH₃-2'); EIMS (m/z): 236 [M]⁺, 203 [C₁₁H₁₁N₂O₂]⁺, 177 [C₁₀H₁₁NO₂]⁺, 163 [C₁₀H₁₁O₂]⁺, 161 [C₁₀H₁₁NO]⁺, 135 [C₉H₁₁O]⁺, 121 [C₈H₉O]⁺, 105 [C₈H₉]⁺, 93 [C₇H₉]⁺; Anal. Calcd for C₁₁H₁₂N₂O₂S: C 55.91, H 5.12, N 11.86, S 13.57; found C 55.68, H 5.07, N 11.65, S 13.42.

Procedure for synthesis of ethyl 2-(5-((2,4-dimethylphenoxy)methyl)-1,3,4-oxadiazol-2-ylthio)acetate (5)

Compound **4** (0.035 mol) was dissolved in 15 mL dimethylformamide (DMF) in a 100-mL RB flask at RT followed by the addition of NaH (0.035 mol) and stirred for 30 minutes. Ethyl 2-bromoacetate (0.035 mol) was added and stirring continued for 4 hours. The reaction was checked by TLC. Excess cold distilled water was added with gentle shaking until precipitation. The precipitated product was filtered, washed with distilled water and dried. White amorphous solid; Yield: 81%; M.P.: 110-112 °C; Mol. formula: C₁₅H₁₈N₂O₄S; Mol. mass: 322 gmol⁻¹; IR (KBr, ν_{max}/cm^{-1}): 3046 (Ar C-H), 1752 (Ester C=O), 1681 (C=N), 1596 (Ar C=C), 1119 (C-O); ¹H-NMR (400 MHz, CDCl₃, δ/ppm): 6.94 (d, $J = 8.0$ Hz, 1H, H-5'), 6.90 (d, $J = 1.2$ Hz, 1H, H-3'), 6.72 (d, $J = 8.0$ Hz, 1H, H-6'), 4.97

(s, 2H, H-7'), 3.93 (q, $J = 7.2$ Hz, 2H, -OCH₂CH₃), 4.67 (s, 2H, H-2'), 2.22 (s, 3H, CH₃-4'), 2.18 (s, 3H, CH₃-2'), 1.02 (t, $J = 7.2$ Hz, 3H, -OCH₂CH₃); EIMS (m/z): 322 [M]⁺, 203 [C₁₁H₁₁N₂O₂]⁺, 177 [C₁₀H₁₁NO₂]⁺, 163 [C₁₀H₁₁O₂]⁺, 161 [C₁₀H₁₁NO]⁺, 135 [C₉H₁₁O]⁺, 121 [C₈H₉O]⁺, 105 [C₈H₉]⁺, 93 [C₇H₉]⁺, 73 [C₃H₅O₂]⁺; Anal. Calcd for C₁₅H₁₈N₂O₄S: C 55.88, H 5.63, N 8.69, S 9.95; found C 55.57, H 5.49, N 8.54, S 9.82.

Procedure for synthesis of 2-(5-((2,4-dimethylphenoxy)methyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (6)

The ethyl ester **5** (0.03 mol) and 80% hydrazine hydrate (0.03 mol) were dissolved in 30 mL methanol in a 100-mL RB flask and stirred for 3 hours strictly at RT. TLC was used to monitor the reaction. The product was acquired after addition of excess cold distilled water and separated by filtration and washed and dried. Cream white amorphous solid; Yield: 83%; M.P.: 128-130 °C; Mol. formula: C₁₃H₁₆N₄O₃S; Mol. mass: 308 gmol⁻¹; IR (KBr, ν_{max}/cm^{-1}): 3389 (N-H), 3071 (Ar C-H), 1673 (Amide C=O), 1695 (C=N), 16041 (Ar C=C), 1115 (C-O); ¹H-NMR (400 MHz, CDCl₃, δ/ppm): 9.41 (s, 1H, CONH), 8.72 (s, 2H, N-H), 6.94 (d, $J = 8.4$ Hz, 1H, H-5'), 6.91 (d, $J = 1.2$ Hz, 1H, H-3'), 6.74 (d, $J = 8.4$ Hz, 1H, H-6'), 4.95 (s, 2H, H-7'), 4.66 (s, 2H, H-2'), 2.21 (s, 3H, CH₃-4'), 2.19 (s, 3H, CH₃-2'); EIMS (m/z): 308 [M]⁺, 203 [C₁₁H₁₁N₂O₂]⁺, 177 [C₁₀H₁₁NO₂]⁺, 163 [C₁₀H₁₁O₂]⁺, 161 [C₁₀H₁₁NO]⁺, 135 [C₉H₁₁O]⁺, 121 [C₈H₉O]⁺, 105 [C₈H₉]⁺, 93 [C₇H₉]⁺; Anal. Calcd for C₁₃H₁₆N₄O₃S: C 50.64, H 5.23, N 18.17, S 10.40; found C 50.53, H 5.19, N 18.11, S 10.22.

General procedure for the synthesis of *N'*-Substituted-2-(5-((2,4-dimethylphenoxy)methyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (8a-m)

The hydrazide **6** (0.004 mol) was dissolved in 10 mL methanol in a 5- mL RB flask at RT. The aryl carboxaldehydes **7a-m** (0.004 mol) were added and the mixture was stirred for 2 hours. The reaction was guided by TLC. Excess cold distilled water was added for precipitation. The precipitate formed was filtered, washed with distilled water and dried. All synthesized compounds were recrystallized from chloroform and tested for biological activity.

N'-Benzylidene-2-(5-((2,4-dimethylphenoxy)methyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (8a)

Light grey amorphous solid; Yield: 81%; M.P.: 136-138 °C; Mol. formula: C₂₀H₂₀N₄O₃S; Mol. mass: 396

gmol⁻¹; IR (KBr, ν_{max}/cm^{-1}): 3057 (Ar C-H), 1659 (C=N), 1614 (Ar C=C), 1093 (C-O); ¹H-NMR (400 MHz, CDCl₃, δ/ppm): 11.74 (s, 1H, CONH), 8.18 (s, 1H, H-7'''), 7.74 (dd, $J = 7.6, 1.2$ Hz, 2H, H-2''' & H-6'''), 7.47-7.43 (m, 3H, H-3''' to H-5'''), 6.95 (d, $J = 8.0$ Hz, 1H, H-5'), 6.92 (d, $J = 1.6$ Hz, 1H, H-3'), 6.71 (d, $J = 8.4$ Hz, 1H, H-6'), 4.91 (s, 2H, H-9'), 4.65 (s, 2H, H-2''), 2.23 (s, 3H, CH₃-8'), 2.19 (s, 3H, CH₃-7'); EIMS (m/z): 396 [M]⁺, 203 [C₁₁H₁₁N₂O₂]⁺, 177 [C₁₀H₁₁NO₂]⁺, 163 [C₁₀H₁₁O₂]⁺, 161 [C₁₀H₁₁NO]⁺, 147 [C₈H₇N₂O]⁺, 135 [C₉H₁₁O]⁺, 121 [C₈H₉O]⁺, 119 [C₇H₇N₂]⁺, 105 [C₈H₉]⁺, 105 [C₇H₅O]⁺, 93 [C₇H₉]⁺, 91 [C₇H₇]⁺, 77 [C₆H₅]⁺, 65 [C₅H₃]⁺, 51 [C₄H₃]⁺; Anal. Calcd for C₂₀H₂₀N₄O₃S: C 60.59, H 5.08, N 14.13, S 8.09; found C 60.41, H 5.02, N 14.05, S 8.02.

N'-(2-Methylbenzylidene)-2-(5-((2,4-dimethylphenoxy)methyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (8b)

Dirty white amorphous solid; Yield: 81%; M.P.: 148-150 °C; Mol. formula: C₂₁H₂₂N₄O₃S; Mol. mass: 410 gmol⁻¹; IR (KBr, ν_{max}/cm^{-1}): 3071 (Ar C-H), 1657 (C=N), 1603 (Ar C=C), 1091 (C-O); ¹H-NMR (400 MHz, CDCl₃, δ/ppm): 11.71 (s, 1H, CONH), 8.36 (s, 1H, H-7'''), 7.70 (dd, $J = 8.4, 1.2$ Hz, 1H, H-6'''), 7.37-7.33 (m, 2H, H-4''' & H-5'''), 7.26 (d, $J = 7.6$ Hz, 1H, H-3'''), 6.92 (d, $J = 8.4$ Hz, 1H, H-5'), 6.92 (d, $J = 1.2$ Hz, 1H, H-3'), 6.73 (d, $J = 8.4$ Hz, 1H, H-6'), 4.94 (s, 2H, H-9'), 4.66 (s, 2H, H-2''), 2.44 (s, 3H, CH₃-8'''), 2.23 (s, 3H, CH₃-8'), 2.21 (s, 3H, CH₃-7'); EIMS (m/z): 410 [M]⁺, 203 [C₁₁H₁₁N₂O₂]⁺, 177 [C₁₀H₁₁NO₂]⁺, 163 [C₁₀H₁₁O₂]⁺, 161 [C₁₀H₁₁NO]⁺, 161 [C₉H₉N₂O]⁺, 135 [C₉H₁₁O]⁺, 133 [C₈H₉N₂]⁺, 121 [C₈H₉O]⁺, 119 [C₇H₅NO]⁺, 105 [C₈H₉]⁺, 93 [C₇H₉]⁺, 91 [C₇H₇]⁺; Anal. Calcd for C₂₁H₂₂N₄O₃S: C 61.44, H 5.40, N 13.65, S 7.81; found C 61.34, H 5.31, N 13.57, S 7.67.

N'-(3-Methylbenzylidene)-2-(5-((2,4-dimethylphenoxy)methyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (8c)

Light brown amorphous solid; Yield: 82%; M.P.: 140-142 °C; Mol. formula: C₂₁H₂₂N₄O₃S; Mol. mass: 410 gmol⁻¹; IR (KBr, ν_{max}/cm^{-1}): 3053 (Ar C-H), 1682 (C=N), 1614 (Ar C=C), 1083 (C-O); ¹H-NMR (400 MHz, CDCl₃, δ/ppm): 11.67 (s, 1H, CONH), 8.16 (s, 1H, H-7'''), 7.41 (d, $J = 8.0$ Hz, 1H, H-6'''), 7.33 (t, $J = 8.0$ Hz, 1H, H-5'''), 7.27 (s, 1H, H-2'''), 7.20 (d, $J = 8.0$ Hz, 1H, H-4'''), 6.93 (d, $J = 8.4$ Hz, 1H, H-5'), 6.93 (d, $J = 1.2$ Hz, 1H, H-3'), 6.72 (d, $J = 8.4$ Hz, 1H, H-6'), 4.93 (s, 2H, H-9'), 4.67 (s, 2H, H-2''), 2.32 (s, 3H, CH₃-8'''), 2.23 (s, 3H, CH₃-8'), 2.21 (s, 3H, CH₃-7'); EIMS (m/z): 410 [M]⁺, 203 [C₁₁H₁₁N₂O₂]⁺, 177 [C₁₀H₁₁NO₂]⁺, 163 [C₁₀H₁₁O₂]⁺, 161 [C₁₀H₁₁NO]⁺, 161 [C₉H₉N₂O]⁺, 135 [C₉H₁₁O]⁺, 133 [C₈H₉N₂]⁺, 121 [C₈H₉O]⁺, 119 [C₇H₅NO]⁺, 105 [C₈H₉]⁺, 93 [C₇H₉]⁺, 91 [C₇H₇]⁺; Anal. Calcd for C₂₁H₂₂N₄O₃S: C

61.44, H 5.40, N 13.65, S 7.81; found C 61.34, H 5.31, N 13.57, S 7.67.

N'-(4-Methylbenzylidene)-2-(5-((2,4-dimethylphenoxy)methyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (8d)

Light green amorphous solid; Yield: 84%; M.P.: 152-154 °C; Mol. formula: C₂₁H₂₂N₄O₃S; Mol. mass: 410 gmol⁻¹; IR (KBr, ν_{max}/cm^{-1}): 3047 (Ar C-H), 1656 (C=N), 1608 (Ar C=C), 1080 (C-O); ¹H-NMR (400 MHz, CDCl₃, δ/ppm): 11.71 (s, 1H, CONH), 8.15 (s, 1H, H-7'''), 7.61 (d, $J = 8.4$ Hz, 2H, H-2''' & H-6'''), 7.26 (d, $J = 8.4$ Hz, 2H, H-3''' & H-5'''), 6.91 (d, $J = 8.0$ Hz, 1H, H-5'), 6.88 (d, $J = 1.6$ Hz, 1H, H-3'), 6.72 (d, $J = 8.0$ Hz, 1H, H-6'), 4.93 (s, 2H, H-9'), 4.64 (s, 2H, H-2''), 2.34 (s, 3H, CH₃-8'''), 2.23 (s, 3H, CH₃-8'), 2.20 (s, 3H, CH₃-7'); EIMS (m/z): 410 [M]⁺, 203 [C₁₁H₁₁N₂O₂]⁺, 177 [C₁₀H₁₁NO₂]⁺, 163 [C₁₀H₁₁O₂]⁺, 161 [C₁₀H₁₁NO]⁺, 161 [C₉H₉N₂O]⁺, 135 [C₉H₁₁O]⁺, 133 [C₈H₉N₂]⁺, 121 [C₈H₉O]⁺, 119 [C₇H₅NO]⁺, 105 [C₈H₉]⁺, 93 [C₇H₉]⁺, 91 [C₇H₇]⁺; Anal. Calcd for C₂₁H₂₂N₄O₃S: C 61.44, H 5.40, N 13.65, S 7.81; found C 61.34, H 5.31, N 13.57, S 7.67.

N'-(2-Hydroxybenzylidene)-2-(5-((2,4-dimethylphenoxy)methyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (8e)

Light yellow amorphous solid; Yield: 88%; M.P.: 158-160 °C; Mol. formula: C₂₀H₂₀N₄O₄S; Mol. mass: 412 gmol⁻¹; IR (KBr, ν_{max}/cm^{-1}): 3069 (Ar C-H), 1677 (C=N), 1607 (Ar C=C), 1092 (C-O); ¹H-NMR (400 MHz, CDCl₃, δ/ppm): 11.73 (s, 1H, CONH), 8.26 (s, 1H, H-7'''), 7.72 (dd, $J = 8.0, 1.6$ Hz, 1H, H-6'''), 7.54 (dd, $J = 8.4, 1.2$ Hz, 1H, H-3'''), 7.23 (dt, $J = 8.4, 1.6$ Hz, 1H, H-4'''), 6.90 (d, $J = 8.4$ Hz, 1H, H-5'), 6.86 (d, $J = 1.6$ Hz, 1H, H-3'), 6.84 (t, $J = 8.0$ Hz, 1H, H-5'''), 6.74 (d, $J = 8.4$ Hz, 1H, H-6'), 4.93 (s, 2H, H-9'), 4.65 (s, 2H, H-2''), 2.21 (s, 3H, CH₃-8'), 2.19 (s, 3H, CH₃-7'); EIMS (m/z): 412 [M]⁺, 203 [C₁₁H₁₁N₂O₂]⁺, 177 [C₁₀H₁₁NO₂]⁺, 163 [C₁₀H₁₁O₂]⁺, 163 [C₈H₇N₂O]⁺, 161 [C₁₀H₁₁NO]⁺, 135 [C₉H₁₁O]⁺, 135 [C₇H₇N₂O]⁺, 121 [C₈H₉O]⁺, 107 [C₇H₇O]⁺, 105 [C₈H₉]⁺, 93 [C₇H₉]⁺; Anal. Calcd for C₂₀H₂₀N₄O₄S: C 58.24, H 4.89, N 13.58, S 7.77; found C 58.11, H 4.73, N 13.41, S 7.59.

N'-(3-Hydroxybenzylidene)-2-(5-((2,4-dimethylphenoxy)methyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (8f)

Light brown amorphous solid; Yield: 79%; M.P.: 150-152 °C; Mol. formula: C₂₀H₂₀N₄O₄S; Mol. mass: 412 gmol⁻¹; IR (KBr, ν_{max}/cm^{-1}): 3043 (Ar C-H), 1675 (C=N), 1609 (Ar C=C), 1079 (C-O); ¹H-NMR (400 MHz, CDCl₃, δ/ppm): 11.79 (s, 1H, CONH), 8.14 (s, 1H, H-7'''), 7.21 (t, $J = 8.8$ Hz, 1H, H-5'''), 7.16 (s, 1H, H-2'''), 7.09 (d,

$J = 8.8$ Hz, 1H, H-6'''), 6.94 (d, $J = 8.4$ Hz, 1H, H-5'), 6.90 (d, $J = 1.6$ Hz, 1H, H-3'), 6.85 (dd, $J = 8.4, 2.0$ Hz, 1H, H-4'''), 6.73 (d, $J = 8.4$ Hz, 1H, H-6'), 4.93 (s, 2H, H-9'), 4.64 (s, 2H, H-2''), 2.20 (s, 3H, CH₃-8'), 2.17 (s, 3H, CH₃-7'); EIMS (m/z): 412 [M]⁺, 203 [C₁₁H₁₁N₂O₂]⁺, 177 [C₁₀H₁₁NO₂]⁺, 163 [C₁₀H₁₁O₂]⁺, 163 [C₈H₇N₂O₂]⁺, 161 [C₁₀H₁₁NO]⁺, 135 [C₉H₁₁O]⁺, 135 [C₇H₇N₂O]⁺, 121 [C₈H₉O]⁺, 107 [C₇H₇O]⁺, 105 [C₈H₉]⁺, 93 [C₇H₉]⁺; Anal. Calcd for C₂₀H₂₀N₄O₄S: C 58.24, H 4.89, N 13.58, S 7.77; found C 58.11, H 4.73, N 13.41, S 7.59.

N'-(4-Hydroxybenzylidene)-2-(5-((2,4-dimethylphenoxy)methyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (8g)

Brown crystalline solid; Yield: 81%; M.P.: 166-168 °C; Mol. formula: C₂₀H₂₀N₄O₄S; Mol. mass: 412 gmol⁻¹; IR (KBr, ν_{max}/cm^{-1}): 3037 (Ar C-H), 1671 (C=N), 1603 (Ar C=C), 1095 (C-O); ¹H-NMR (400 MHz, CDCl₃, δ/ppm): 11.75 (s, 1H, CONH), 8.13 (s, 1H, H-7'''), 7.51 (d, $J = 8.4$ Hz, 2H, H-2''' & H-6'''), 6.92 (d, $J = 8.0$ Hz, 1H, H-5'), 6.89 (d, $J = 1.6$ Hz, 1H, H-3'), 6.81 (d, $J = 8.8$ Hz, 2H, H-3''' & H-5'''), 6.74 (d, $J = 8.0$ Hz, 1H, H-6'), 4.94 (s, 2H, H-9'), 4.65 (s, 2H, H-2''), 2.24 (s, 3H, CH₃-8'), 2.21 (s, 3H, CH₃-7'); EIMS (m/z): 412 [M]⁺, 203 [C₁₁H₁₁N₂O₂]⁺, 177 [C₁₀H₁₁NO₂]⁺, 163 [C₁₀H₁₁O₂]⁺, 163 [C₈H₇N₂O₂]⁺, 161 [C₁₀H₁₁NO]⁺, 135 [C₉H₁₁O]⁺, 135 [C₇H₇N₂O]⁺, 121 [C₈H₉O]⁺, 107 [C₇H₇O]⁺, 105 [C₈H₉]⁺, 93 [C₇H₉]⁺; Anal. Calcd for C₂₀H₂₀N₄O₄S: C 58.24, H 4.89, N 13.58, S 7.77; found C 58.11, H 4.73, N 13.41, S 7.59.

N'-(2-Nitrobenzylidene)-2-(5-((2,4-dimethylphenoxy)methyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (8h)

Yellow amorphous solid; Yield: 79%; M.P.: 146-148 °C; Mol. formula: C₂₀H₁₉N₅O₅S; Mol. mass: 441 gmol⁻¹; IR (KBr, ν_{max}/cm^{-1}): 3089 (Ar C-H), 1677 (C=N), 1613 (Ar C=C), 1115 (C-O); ¹H-NMR (400 MHz, CDCl₃, δ/ppm): 12.01 (s, 1H, CONH), 8.35 (s, 1H, H-7'''), 8.31 (d, $J = 8.4$ Hz, 1H, H-6'''), 7.95 (dd, $J = 8.4, 1.6$ Hz, 1H, H-3'''), 7.95-7.93 (m, 2H, H-4''' & H-5'''), 6.91 (d, $J = 8.8$ Hz, 1H, H-5'), 6.88 (d, $J = 2.0$ Hz, 1H, H-3'), 6.76 (d, $J = 8.8$ Hz, 1H, H-6'), 4.93 (s, 2H, H-9'), 4.65 (s, 2H, H-2''), 2.23 (s, 3H, CH₃-8'), 2.21 (s, 3H, CH₃-7'); EIMS (m/z): 441 [M]⁺, 203 [C₁₁H₁₁N₂O₂]⁺, 192 [C₈H₆N₃O₃]⁺, 177 [C₁₀H₁₁NO₂]⁺, 164 [C₇H₆N₃O₂]⁺, 163 [C₁₀H₁₁O₂]⁺, 161 [C₁₀H₁₁NO]⁺, 136 [C₉H₁₁O]⁺, 135 [C₉H₁₁O]⁺, 121 [C₈H₉O]⁺, 105 [C₈H₉]⁺, 93 [C₇H₉]⁺; Anal. Calcd for C₂₀H₁₉N₅O₅S: C 54.41, H 4.34, N 15.86, S 7.26; found C 54.27, H 4.22, N 15.68, S 7.19.

N'-(3-Nitrobenzylidene)-2-(5-((2,4-dimethylphenoxy)methyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (8i)

Light brown amorphous solid; Yield: 87%; M.P.:

142-144 °C; Mol. formula: C₂₀H₁₉N₅O₅S; Mol. mass: 441 gmol⁻¹; IR (KBr, ν_{max}/cm^{-1}): 3086 (Ar C-H), 1652 (C=N), 1606 (Ar C=C), 1093 (C-O); ¹H-NMR (400 MHz, CDCl₃, δ/ppm): 12.02 (s, 1H, CONH), 8.44 (t, $J = 1.6$ Hz, 1H, H-2'''), 8.38 (s, 1H, H-7'''), 8.21 (dd, $J = 8.4, 1.6$ Hz, 1H, H-6'''), 8.12 (d, $J = 8.8$ Hz, 1H, H-4'''), 7.69 (t, $J = 8.4$ Hz, 1H, H-5'''), 6.93 (d, $J = 8.4$ Hz, 1H, H-5'), 6.91 (d, $J = 1.2$ Hz, 1H, H-3'), 6.71 (d, $J = 8.4$ Hz, 1H, H-6'), 4.92 (s, 2H, H-9'), 4.65 (s, 2H, H-2''), 2.24 (s, 3H, CH₃-8'), 2.22 (s, 3H, CH₃-7'); EIMS (m/z): 441 [M]⁺, 203 [C₁₁H₁₁N₂O₂]⁺, 192 [C₈H₆N₃O₃]⁺, 177 [C₁₀H₁₁NO₂]⁺, 164 [C₇H₆N₃O₂]⁺, 163 [C₁₀H₁₁O₂]⁺, 161 [C₁₀H₁₁NO]⁺, 136 [C₇H₆NO₂]⁺, 135 [C₉H₁₁O]⁺, 121 [C₈H₉O]⁺, 105 [C₈H₉]⁺, 93 [C₇H₉]⁺; Anal. Calcd for C₂₀H₁₉N₅O₅S: C 54.41, H 4.34, N 15.86, S 7.26; found C 54.27, H 4.22, N 15.68, S 7.19.

N'-(4-Nitrobenzylidene)-2-(5-((2,4-dimethylphenoxy)methyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (8j)

Brown amorphous solid; Yield: 79%; M.P.: 154-156 °C; Mol. formula: C₂₀H₁₉N₅O₅S; Mol. mass: 441 gmol⁻¹; IR (KBr, ν_{max}/cm^{-1}): 3087 (Ar C-H), 1665 (C=N), 1614 (Ar C=C), 1083 (C-O); ¹H-NMR (400 MHz, CDCl₃, δ/ppm): 12.03 (s, 1H, CONH), 8.43 (s, 1H, H-7'''), 8.13 (d, $J = 8.0$ Hz, 2H, H-3''' & H-5'''), 8.05 (d, $J = 8.0$ Hz, 2H, H-2''' & H-6'''), 6.96 (d, $J = 8.0$ Hz, 1H, H-5'), 6.93 (d, $J = 1.6$ Hz, 1H, H-3'), 6.72 (d, $J = 8.4$ Hz, 1H, H-6'), 4.93 (s, 2H, H-9'), 4.62 (s, 2H, H-2''), 2.23 (s, 3H, CH₃-8'), 2.21 (s, 3H, CH₃-7'); EIMS (m/z): 441 [M]⁺, 203 [C₁₁H₁₁N₂O₂]⁺, 192 [C₈H₆N₃O₃]⁺, 177 [C₁₀H₁₁NO₂]⁺, 164 [C₇H₆N₃O₂]⁺, 163 [C₁₀H₁₁O₂]⁺, 161 [C₁₀H₁₁NO]⁺, 136 [C₇H₆NO₂]⁺, 135 [C₉H₁₁O]⁺, 121 [C₈H₉O]⁺, 105 [C₈H₉]⁺, 93 [C₇H₉]⁺; Anal. Calcd for C₂₀H₁₉N₅O₅S: C 54.41, H 4.34, N 15.86, S 7.26; found C 54.27, H 4.22, N 15.68, S 7.19.

N'-(4-(Dimethylamino)benzylidene)-2-(5-((2,4-dimethylphenoxy)methyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (8k)

Brown crystalline solid; Yield: 77%; M.P.: 178-180 °C; Mol. formula: C₂₂H₂₅N₅O₃S; Mol. mass: 439 gmol⁻¹; IR (KBr, ν_{max}/cm^{-1}): 3061 (Ar C-H), 1654 (C=N), 1614 (Ar C=C), 1111 (C-O); ¹H-NMR (400 MHz, CDCl₃, δ/ppm): 11.47 (s, 1H, CONH), 8.04 (s, 1H, H-7'''), 7.47 (d, $J = 8.4$ Hz, 2H, H-2''' & H-6'''), 6.97 (d, $J = 8.4$ Hz, 1H, H-5'), 6.92 (d, $J = 1.6$ Hz, 1H, H-3'), 6.77 (d, $J = 8.4$ Hz, 1H, H-6'), 6.61 (d, $J = 8.8$ Hz, 2H, H-3''' & H-5'''), 4.94 (s, 2H, H-9'), 4.56 (s, 2H, H-2''), 2.94 (s, 6H, CH₃-8''' & CH₃-9'''), 2.20 (s, 3H, CH₃-8'), 2.17 (s, 3H, CH₃-7'); EIMS (m/z): 439 [M]⁺, 203 [C₁₁H₁₁N₂O₂]⁺, 190 [C₁₀H₁₂N₃O]⁺, 177 [C₁₀H₁₁NO₂]⁺, 163 [C₁₀H₁₁O₂]⁺, 162 [C₉H₁₂N₃]⁺, 161 [C₁₀H₁₁NO]⁺, 135 [C₉H₁₁O]⁺, 134 [C₉H₁₂N]⁺, 121 [C₈H₉O]⁺, 105 [C₈H₉]⁺, 93 [C₇H₉]⁺; Anal. Calcd for C₂₂H₂₅N₅O₃S: C

60.12, H 5.73, N 15.93, S 7.30; found C 60.07, H 5.66, N 15.84, S 7.16.

N'-(4-(Diethylamino)benzylidene)-2-(5-((2,4-dimethylphenoxy)methyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (8l)

Light brown amorphous solid; Yield: 78%; M.P.: 186-188 °C; Mol. formula: C₂₄H₂₉N₅O₃S; Mol. mass: 467 gmol⁻¹; IR (KBr, ν_{max}/cm^{-1}): 3063 (Ar C-H), 1642 (C=N), 1614 (Ar C=C), 1081 (C-O); ¹H-NMR (400 MHz, CDCl₃, δ/ppm): 11.43 (s, 1H, CONH), 8.04 (s, 1H, H-7'''), 7.41 (d, $J = 8.4$ Hz, 2H, H-2''' & H-6'''), 6.93 (d, $J = 8.0$ Hz, 1H, H-5'), 6.91 (d, $J = 2.0$ Hz, 1H, H-3'), 6.75 (d, $J = 8.0$ Hz, 1H, H-6'), 6.63 (d, $J = 8.4$ Hz, 2H, H-3''' & H-5'''), 4.91 (s, 2H, H-9'), 4.61 (s, 2H, H-2''), 2.63 (q, $J = 7.6$ Hz, 4H, H-8''' & H-10'''), 2.19 (s, 3H, CH₃-8'), 2.17 (s, 3H, CH₃-7'), 1.05 (t, $J = 7.6$ Hz, 6H, CH₃-9''' & CH₃-11'''); EIMS (m/z): 467 [M]⁺, 218 [C₁₂H₁₆N₃O]⁺, 203 [C₁₁H₁₁N₂O₂]⁺, 190 [C₁₁H₁₆N₃]⁺, 177 [C₁₀H₁₁NO₂]⁺, 163 [C₁₀H₁₁O₂]⁺, 162 [C₁₁H₁₆N]⁺, 161 [C₁₀H₁₁NO]⁺, 135 [C₉H₁₁O]⁺, 121 [C₈H₉O]⁺, 105 [C₈H₉]⁺, 93 [C₇H₉]⁺; Anal. Calcd for C₂₄H₂₉N₅O₃S: C 61.65, H 6.25, N 14.98, S 6.86; found C 61.52, H 6.12, N 14.84, S 6.71.

N'-(4-Methoxybenzylidene)-2-(5-((2,4-dimethylphenoxy)methyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (8m)

Light green amorphous solid; Yield: 79%; M.P.: 164-166 °C; Mol. formula: C₂₁H₂₂N₄O₄S; Mol. mass: 426 gmol⁻¹; IR (KBr, ν_{max}/cm^{-1}): 3066 (Ar C-H), 1646 (C=N), 1610 (Ar C=C), 1084 (C-O); ¹H-NMR (400 MHz, CDCl₃, δ/ppm): 11.41 (s, 1H, CONH), 8.15 (s, 1H, H-7'''), 7.82 (d, $J = 8.4$ Hz, 2H, H-2''' & H-6'''), 6.94 (d, $J = 8.4$ Hz, 1H, H-5'), 6.91 (d, $J = 1.6$ Hz, 1H, H-3'), 6.74 (d, $J = 8.4$ Hz, 1H, H-6'), 6.55 (d, $J = 8.4$ Hz, 2H, H-3''' & H-5'''), 4.92 (s, 2H, H-9'), 4.63 (s, 2H, H-2''), 3.83 (s, 3H, CH₃O-8'''), 2.23 (s, 3H, CH₃-8'), 2.20 (s, 3H, CH₃-7'); EIMS (m/z): 426 [M]⁺, 203 [C₁₁H₁₁N₂O₂]⁺, 177 [C₁₀H₁₁NO₂]⁺, 177 [C₉H₉N₂O₂]⁺, 163 [C₁₀H₁₁O₂]⁺, 161 [C₁₀H₁₁NO]⁺, 149 [C₈H₉N₂O]⁺, 135 [C₉H₁₁O]⁺, 121 [C₈H₉O]⁺, 107 [C₇H₇O]⁺, 105 [C₈H₉]⁺, 93 [C₇H₉]⁺; Anal. Calcd for C₂₁H₂₂N₄O₄S: C 59.14, H 5.20, N 13.14, S 7.52; found C 59.03, H 5.11, N 13.01, S 7.41.

BIOLOGICAL ACTIVITY ASSAYS

Antibacterial activity assay

Antibacterial activity was assayed as reported elsewhere, with minor modifications (Aziz-ur-Rehman *et al.*, 2013c; Kaspady *et al.*, 2009; Yang *et al.*, 2006). The assay was performed in sterile 96-well microplates under aseptic conditions. The method is based on the

principle that microbial cell number increases during log phase growth, resulting in increased absorbance of broth medium. The microorganisms used in this study included three Gram-negative bacteria, namely *Salmonella typhi*, *Escherichia coli* and *Pseudomonas aeruginosa*, and two Gram-positive bacteria, namely *Bacillus subtilis* and *Staphylococcus aureus*. The strains used were grown on stock agar culture medium. The test samples were diluted in suitable solvents and 20 μL of each sample was pipetted into every well. Fresh bacterial culture grown overnight was suitably diluted with fresh nutrient broth and 180 μL of this bacterial culture were added to every well. The starting absorbance of the culture at 540 nm was strictly maintained at 0.12-0.19. The total volume in each well was 200 μL . These microplates were incubated for 16-24 hours at 37°C. Before and after incubation, the absorbance was measured at 540 nm using a microplate reader. Bacterial growth rate was determined by the difference in absorbance before and after incubation. The formula for calculating the percentage inhibition was:

$$\text{Inhibition (\%)} = \frac{X - Y}{X} \times 100$$

where X = absorbance in control, containing bacterial culture without test sample; Y = absorbance of bacterial culture with test sample.

Results are given as the mean of three sets of test samples ($n = 3$, \pm SEM). The reference standard used was ciprofloxacin. Suitable dilutions ranging from 5-30 $\mu\text{g}/\text{well}$ were used to measure the minimum inhibitory concentration (MIC).

Enzyme inhibition activity assay

Lipoxygenase enzyme inhibition activity was determined by a previously reported method, with minor modifications (Abbasi *et al.*, 2005; Alitonou *et al.*, 2006). A 200- μL reaction mix consisted of 150 μL 100 mM sodium phosphate buffer (pH 8.0), 10 μL test compound and 15 μL purified lipoxygenase enzyme (600 units well⁻¹). After mixing, pre-reading at 234 nm and pre-incubation for 10 min at 25 °C (room temperature), 25 μL substrate solution were added to initiate the reaction. The results were based on the change in absorbance, observed after 6 min at 234 nm using a microplate reader (Synergy HT, Biotek, USA). Assays were done in triplicate, and positive and negative controls were included in the assay. Baicalein (0.5 mM) was used as a positive control. The results were calculated according to the following formula.

$$\text{Inhibition (\%)} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

where Control is the absorbance in the presence of reference and Test is the absorbance in the presence of the test compound.

Statistical analysis

The results are presented as mean \pm SEM for triplicate calculations after statistical analysis executed by MS Excel 2010. Minimum inhibitory concentration (MIC) for antibacterial activity and IC₅₀ (concentration with 50% inhibition) for enzyme inhibition was computed with suitable dilutions of each sample, and the results were obtained using EZ-Fit software (Perrella Scientific Inc, Amherst, USA).

RESULTS AND DISCUSSION

The *N*-substituted derivatives **8a-m** were synthesized by the protocol given in Figure 1 and substituents in Table I. The multiple functional groups were synthesized to evaluate the combined effect of these moieties against some strains of Gram-negative and Gram-positive bacteria and against lipoxygenase activity.

Chemistry

The synthesis of ethyl 2-(2,4-dimethylphenoxy)acetate (**2**) was carried out with reflux in the presence of a strong base. The harsh conditions were applied

because of low acidity of 2,4-dimethylcarboxylic acid (**1**) owing to two electron-donating methyl groups. 2-(2,4-dimethylphenoxy)acetohydrazide (**3**) was also synthesized with reflux, because of the low electrophilic character of carbonyl carbon; otherwise, most of such reactions were performed with stirring. After synthesis of 5-((2,4-dimethylphenoxy)methyl)-1,3,4-oxadiazol-2-thiol (**4**) with reflux in basic medium, it was filtered out from slightly acidic medium (pH 5-6). Such low pH is necessary to get maximum yield. The yield is much lower on filtration in basic medium or strong acidic medium because of salt formation. Molecule **4** possessed a more acidic proton as a thiol, so ethyl 2-(5-((2,4-dimethylphenoxy)methyl)-1,3,4-oxadiazol-2-ylthio)acetate (**5**) was prepared simply by stirring in the presence of a weak base. 2-(5-((2,4-Dimethylphenoxy)methyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (**6**) was also obtained with stirring at RT. The target molecules **8a-m** were synthesized by stirring compound **6** with the aryl carboxaldehydes **7a-m** in methanol. Although this step has reasonable speed, it can be catalyzed by a few drops of glacial acetic acid. All the protocols with necessary conditions are explained in the experimental section.

Compound **8a** was a light grey amorphous solid with a melting point of 136-138°C. Its molecular formula, C₂₀H₂₀N₄O₃S, was established with the aid of ¹H-NMR and EIMS spectra. The prominent peaks in the

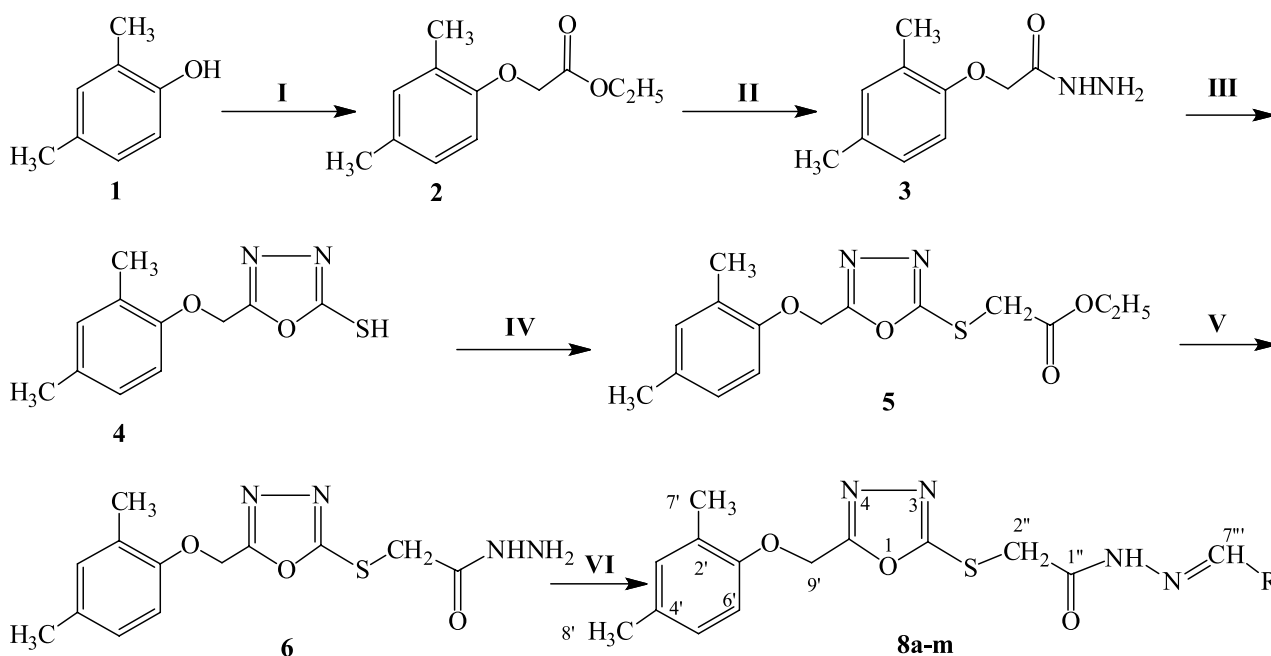


FIGURE 1 - Synthesis of *N*'-substituted-2-(5-((2,4-dimethylphenoxy)methyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (**8a-m**). **Results and Conditions:** (I) Ethyl 2-bromoacetate (EBA), KOH, EtOH, Reflux, 6 hours (II) 80% N₂H₄·H₂O, EtOH, Reflux, 4 hours (III) CS₂, KOH, EtOH, Reflux, 6 hours (IV) EBA, NaH, DMF, Stir, 4 hours (V) 80% N₂H₄·H₂O, MeOH, Stir, 3 hours (VI) Aryl carboxaldehydes (**7a-m**), MeOH, Stir, 2 hours.

TABLE I - Different aryl groups

Comp	-R	Comp	-R	Comp	-R
8a	Phenyl	8f	3-Hydroxyphenyl	8k	4-Dimethylaminophenyl
8b	2-Methylphenyl	8g	4-Hydroxyphenyl	8l	4-Diethylaminophenyl
8c	3-Methylphenyl	8h	2-Nitrophenyl	8m	4-Methoxyphenyl
8d	4-Methylphenyl	8i	3-Nitrophenyl		
8e	2-Hydroxyphenyl	8j	4-Nitrophenyl		

EIMS spectrum were at m/z 396 (molecular ion), 203 (5-((2,4-dimethylphenoxy)methyl)-1,3,4-oxadiazol-2-thio cation) and 147 (N' -benzylidenehydrazinocarbonyl cation). The mass fragmentation pattern of **8a** is elaborated in Figure 2. The major absorptions in the IR spectrum appeared at (cm^{-1}) 3057 (Ar C-H), 1659 (C=N), 1614 (Ar C=C) and 1093 (C-O). The three aromatic protons of the phenoxy group showed three doublets, two with ortho coupling and one with meta coupling, resonating at δ 6.95 (d, $J = 8.0$ Hz, 1H, H-5'), 6.92 (d, $J = 1.6$ Hz, 1H, H-3') and 6.71 (d, $J = 8.4$ Hz, 1H, H-6'). The six protons of two methyl groups resonated at δ 2.23 (s, 3H, CH_3 -4') and 2.19 (s, 3H, CH_3 -2'). The five aromatic and one methine protons of the benzylidene moiety were confirmed by one singlet, one double doublet and one multiplet at δ 8.18 (s, 1H, H-7'''), 7.74 (dd, $J = 7.6, 1.2$ Hz, 2H, H-2''' & H-6''') and 7.47-7.43 (m, 3H, H-3''' to H-5'''). The other three resonating peaks at δ 11.74 (s, 1H, CONH), 4.91 (s, 2H, H-7') and 4.65 (s, 2H, H-2'') were allocated to one carbamoyl proton and two methylene groups. The above structural demonstration justified **8a** as N' -benzylidene-2-(5-((2,4-dimethylphenoxy)methyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide. The other compounds were likewise corroborated.

Biological activities

Subtle structural changes in molecules are known to have a great influence on the pharmacological behavior (Liu *et al.*, 2012). Therefore, a series of compounds were synthesized that varied in nature and position of the substituent present on the phenyl ring attached to the methine carbon of the azomethine moiety.

Antibacterial activity (in vitro)

The antibacterial activity results of the synthesized compounds are presented as % inhibition and minimum inhibitory concentration (MIC) values in Table II and Table III, respectively.

Overall, the whole series exhibited moderate antibacterial activity with IC_{50} comparable to

ciprofloxacin, the reference drug. The bacterial strains *E. coli*, *P. aeruginosa* and *S. aureus* were inhibited by all the synthesized compounds but some were inactive against *S. typhi* and *B. subtilis*. Half of the series was moderately active against all the bacterial strains. Against *S. typhi*, compounds **8g**, **8k** and **8l** were inactive at all dilutions and the rest were moderate inhibitors. All compounds showed moderate activity against *E. coli*, where **8g** and **8m** were the most effective against this strain bearing an *O*-substituted group at the 4th position of the phenyl ring of the benzylidene moiety, *p*-hydroxyphenyl and *p*-methoxyphenyl, respectively. *P. aeruginosa* was moderately inhibited by all compounds. Although five compounds were inactive against *B. subtilis*, the others displayed substantial activity, except **8g**. Moderate to high inhibitory effect was exerted by all synthesized compounds against *S. aureus*, but **8m** was the best. The un-substituted benzylidene-bearing molecule, **8a**, was moderately effective against all strains, but the substituted ones exhibited notable variation in their antibacterial activity. Among the *o*- and *m*-substituted ones, molecules bearing methyl/nitro-substituted benzylidene moieties (**8b**, **8h**, **8c** and **8i**) were better than those bearing hydroxyl-substituted benzylidene moieties (**8e** and **8f**). The molecules bearing *p*-substituted benzylidene moieties showed considerable variation in their activity. Compound **8m** demonstrated a prominent activity against all strains except *B. subtilis* and most probably because of the *para* methoxy substituent on benzylidene. The low MIC values ($\mu\text{g/mL}$) shown by **8m** were 9.48 ± 0.00 (*S. typhi*), 7.37 ± 0.23 (*E. coli*), 13.69 ± 1.66 (*P. aeruginosa*) and 10.88 ± 1.21 (*S. aureus*), compared to MIC of ciprofloxacin, 7.83 ± 0.78 , 8.01 ± 0.12 , 7.98 ± 0.89 and 7.00 ± 0.54 respectively. *E. coli* was best inhibited by **8g**, also bearing a *para* hydroxy benzylidene, with MIC of $10.78 \pm 1.21 \mu\text{g/mL}$ relative to $8.01 \pm 0.12 \mu\text{g/mL}$. After **8m**, the molecule **8j** bearing *p*-nitrobenzylidene also exhibited notable activity against all strains. Overall, the nitro-substituted benzylidenes exerted greater inhibitory effect against all bacterial strains relative to the other substituted benzylidenes. The

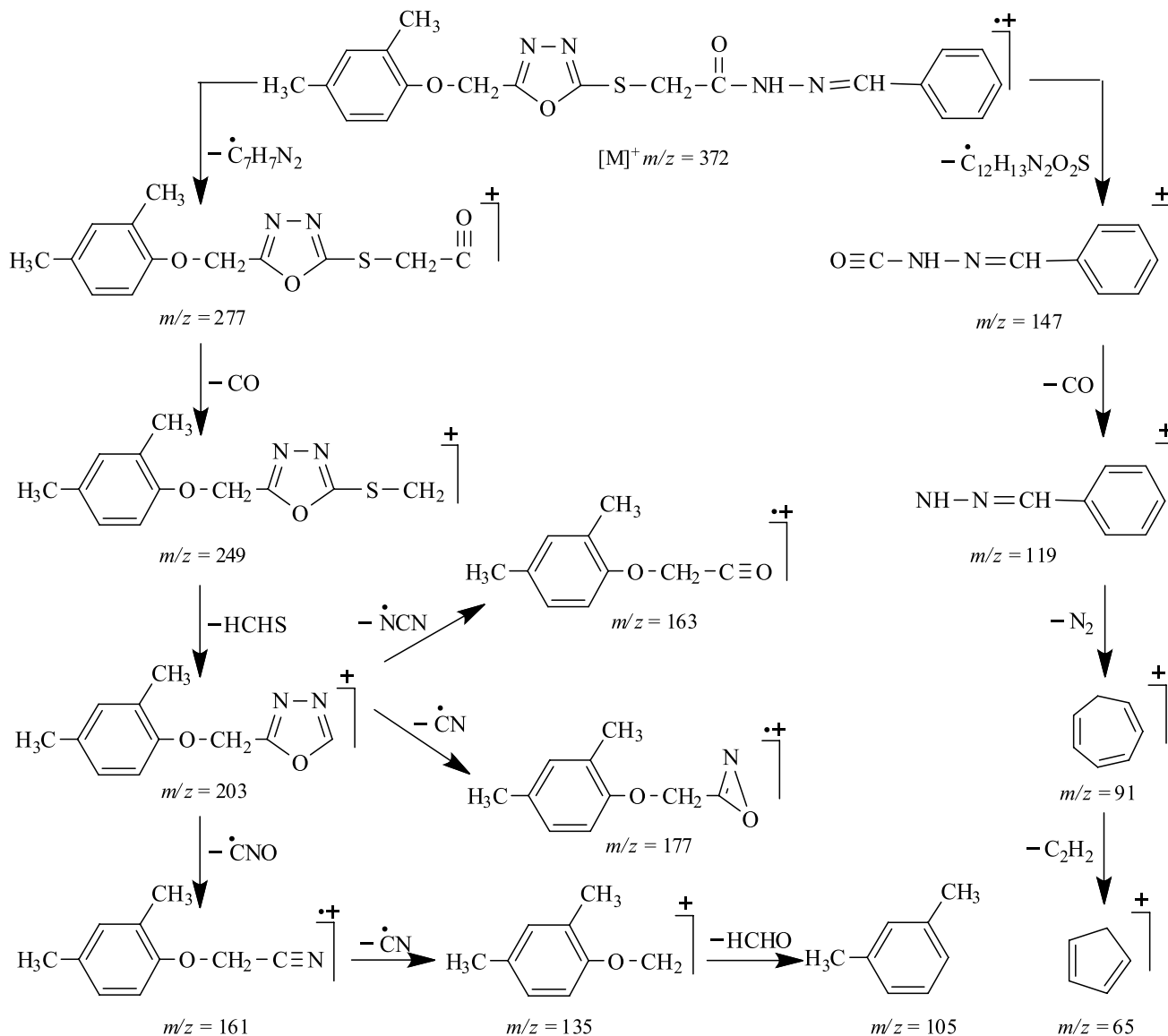


FIGURE 2 - Proposed mass fragmentation pattern of *N*'-benzylidene-2-(5-((2,4-dimethylphenoxy)methyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (**8a**).

hydroxy-substituted benzylidenes were the least active. Compounds **8k** and **8l** showed no activity at all or weakly moderate activity.

Enzyme inhibition activity (in vitro)

The results of screening the compounds against lipoxygenase activity are given as % inhibition and concentration for 50% inhibition (IC_{50}) values in Table IV.

More than half of the synthesized molecules were inactive at all dilutions, and only five compounds, **8b**, **8c**, **8f**, **8h** and **8i**, showed inhibitory potential varying from high to moderate. The lower activity of these molecules might have been attributed to the large molecular size,

resulting in hindrance to binding with the active site of the enzyme. But unexpectedly, molecule, **8i**, bearing a 3-nitrobenzylidene moiety, exerted the highest inhibitory effect with IC_{50} value of $5.21 \pm 0.011 \mu\text{M}$ with respect to that of baicalein, $22.4 \pm 1.3 \mu\text{M}$, the reference standard. This better inhibitory effect could be ascribed to some additional interaction modes shown by this molecule with the amino acid residues at the active site of the enzyme. Overall, the results demonstrated that the molecules bearing *p*-substituted benzylidenes were inactive at all dilutions. The *ortho* and *meta* substituted benzylidenes were more effective. The nitro-substituted benzylidenes (*ortho* and *meta*) were the most active ones among all molecules.

TABLE II - The % inhibition of antimicrobial activity of the synthesized compounds

Compound	% Inhibition				
	Gram negative bacteria			Gram positive bacteria	
	<i>S. typhi</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>B. subtilis</i>	<i>S. aureus</i>
8a	78.75±0.75	76.80±0.68	77.55±0.25	72.50±1.21	77.33±1.11
8b	76.25±1.22	67.05±1.37	80.25±1.59	79.29±0.57	70.61±1.43
8c	66.75±0.72	63.90±1.23	59.90±0.58	63.00±1.60	69.83±0.84
8d	68.85±1.22	56.45±1.73	61.10±0.20	60.71±1.30	67.11±0.53
8e	58.85±0.28	61.50±2.17	52.20±0.30	43.21±1.31	71.33±1.00
8f	57.25±0.85	65.30±1.38	52.30±0.59	43.57±1.25	75.28±1.37
8g	47.95±0.72	73.45±1.27	77.50±1.30	66.36±1.67	75.00±0.63
8h	75.90±1.67	76.95±1.14	78.45±0.48	73.43±0.50	77.11±0.61
8i	75.56±1.37	75.05±1.56	71.38±1.87	72.15±1.78	76.89±1.03
8j	75.30±1.89	75.85±1.55	54.65±0.80	70.43±1.42	79.50±0.28
8k	46.75±1.57	60.95±0.78	55.95±0.20	45.72±1.05	71.56±1.58
8l	47.80±1.62	66.55±1.03	54.06±1.35	41.71±0.65	75.06±1.09
8m	80.65±0.94	84.45±0.48	82.35±0.60	81.21±0.90	83.28±1.02
Ciprofloxacin	91.54±0.39	90.98±0.83	91.04±0.65	91.93±0.78	92.20±0.49

TABLE III - The MIC values of antimicrobial activity of the synthesized compounds

Compound	MIC (µg/ mL)				
	Gram negative bacteria			Gram positive bacteria	
	<i>S. typhi</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>B. subtilis</i>	<i>S. aureus</i>
8a	12.62±0.11	13.32±0.68	12.83±1.11	10.20±0.05	12.85±0.51
8b	13.26±0.90	15.01±0.10	14.43±0.78	10.01±0.55	13.59±1.03
8c	14.53±1.21	16.19±1.30	16.63±1.44	10.51±0.82	13.78±0.19
8d	14.66±0.44	17.34±0.80	16.68±0.11	11.37±0.40	14.69±1.05
8e	17.02±1.22	16.04±0.45	19.13±1.00	-	14.05±0.62
8f	17.42±0.22	16.33±0.60	18.26±0.67	-	13.43±0.54
8g	-	10.78±1.21	13.02±0.45	16.44±1.65	13.87±2.34
8h	13.30±1.33	14.33±0.80	12.88±1.33	10.87±0.09	12.70±1.68
8i	13.25±1.67	14.42±0.46	13.18±1.29	10.54±1.54	12.98±2.08
8j	13.22±0.48	14.57±0.07	14.42±0.76	10.08±0.91	12.55±0.05
8k	-	15.49±0.70	17.87±1.67	-	14.13±0.92
8l	-	15.98±1.10	18.42±0.88	-	13.21±0.98
8m	9.48±0.00	7.37±0.23	13.69±1.66	-	10.88±1.21
Ciprofloxacin	7.83±0.78	8.01±0.12	7.98±0.89	7.22±0.67	7.00±0.54

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

CONCLUSION

A series of molecules bearing 1,3,4-oxadiazole and azomethine moieties were synthesized with good yields. The structures were corroborated using spectral data, and these molecules were screened for their antibacterial and lipoxigenase inhibitory potentials. High to moderate

antibacterial activity was demonstrated against certain strains of Gram-positive and Gram-negative bacteria. These molecules were found to be weak enzyme inhibitors except **8i**, which showed remarkable inhibition of lipoxigenase, as evidenced by its low IC₅₀ value. The molecule **8m** showed high antibacterial activity might be due to *p*-methoxybenzylidene moiety. This study could be

TABLE IV - The IC₅₀ values of enzyme inhibition activity of the synthesized compounds

Compound	LOX		
	Conc. (mM)	Inhibition (%)	IC ₅₀ (μM)
8a	0.5	37.47±0.21	-
8b	0.5	75.47±0.49	259.9±1.79
8c	0.5	71.69±0.61	231.5±1.83
8d	0.5	8.53±0.01	-
8e	0.5	8.11±0.023	-
8f	0.5	81.58±0.65	395.3±1.56
8g	0.5	44.53±0.15	>500
8h	0.5	78.11±0.64	54.8±0.32
8i	0.0625	75.11±0.47	5.21±0.011
8j	0.5	45.73±0.13	>500
8k	0.5	-	-
8l	0.5	35.37±0.18	-
8m	0.5	49.61±0.21	>500
Baicalein	0.5	93.79±1.27	22.4±1.3

NOTE: LOX = Lipoxygenase. IC₅₀ values (concentration for 50% inhibition) of compounds were recorded using EZ-Fit Enzyme kinetics software (Perella Scientific Inc. Amherst, USA).

extended to synthesize more derivatives with variation in substituted benzylidenes for better results to advance our drug discovery programs.

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REFERENCES

- ABBASI, M.A.; AHMAD, V.U.; ZUBAIR, M.; RASHID, M.A.; FAROOQ, U.; NAWAZ, S.A.; LODHI, M.A.; MAKHMOOR, T.; CHOUDHARY, M.I.; ATTA-UR-RAHMAN. Benzoylsalireposide an antioxidant, lipoxygenase and chymotrypsin inhibitor. *Proc. Pak. Acad. Sci.*, v.42, p.121-124, 2005.
- ALITONOU, G.A.; AVLESSI, F.; SOHOUNHLOUE, D.K.; AGNANIET, H.; BESSIERE, J.M.; MENUT, C. Investigations on the essential oil of *Cymbopogon giganteus* from benin for its potential use as an anti inflammatory agent. *Int. J. Aromather.*, v.16, p.37-41, 2006.
- ASLAM, M.A.S.; MAHMOOD, S.; SHAHID, M.; SAEED, A.; IQBAL, J. Synthesis, biological assay *in vitro* and molecular docking studies of new Schiff base derivatives as potential urease inhibitors. *Eur. J. Med. Chem.*, v.46, p.5473-5479, 2011.
- AZIZ-UR-REHMAN; FATIMA, A.; ABBAS, N.; ABBASI, M.A.; KHAN, K.M.; ASHRAF, M.; AHMAD, I.; EJAZ, S.A. 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.*, v.26, p.345-352, 2013a.
- AZIZ-UR-REHMAN; FATIMA, A.; ABBASI, M.A.; RASOOL, S.; MALIK, A.; ASHRAF, M.; AHMAD, I.; EJAZ, S.A. 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.*, 2013b. DOI: <<http://dx.doi.org/10.1016/j.jscs.2013.02.006>>. [In Press].
- AZIZ-UR-REHMAN; NAFEESA, K.; ABBASI, M.A.; KASHFA, H.; RASOOL, S.; AHMAD, I.; ARSHAD, S. Synthesis, characterization and biological screening of various *S*-substituted derivatives of 5-(3-nitrophenyl)-1,3,4-oxadiazole-2-thiol. *Pak. J. Chem.*, v.3, p.1-8, 2013c.
- BARBE, V.; CRUVEILLER, S.; KUNST, F.; LENOBLE, P.; MEURICE, G.; SEKOWSKA, A.; VALLENET, D.; WANG, T.; MOSZER, I.; MEDIGUE, C.; DANCHIN, A. From a consortium sequence to a unified sequence: the *Bacillus subtilis* 168 reference genome a decade later. *Microbiol.*, v.155, p.1758-1775, 2009.
- BHATTACHARYA, S.S.; DAS, U.; CHOUDHURY, B.K. Occurrence & antibiogram of *Salmonella typhi* & *S. paratyphi* A isolated from Rourkela, Orissa. *Indian J. Med. Res.*, v.133, p.431-433, 2011.
- DESHMUKH, R.; JHA, A.K.; THAKUR, A.S.; DEWANGAN, D. Synthesis and antibacterial activity of some 1,3,4-oxadiazole derivatives and their thione analogues. *Int. J. Res. Pharm. Biomed. Sci.*, v.2, p.215-219, 2011.
- GUL, S.; AZIZ-UR-REHMAN; ABBASI, M.A.; NAFEESA, K.; MALIK, A.; ASHRAF, M.; ISLMAIL, T.; AHMAD, I. Synthesis, characterization and pharmacological evaluation of *N*-substituted derivatives of 5-(4-nitrophenyl)-1,3,4-oxadiazole-2yl-2-sulfanyl acetamide. *Asian J. Chem.*, v.25, p.6231-6236, 2013.

- HARRIS, L.G.; FOSTER, S.J.; RICHARDS, R.G. An introduction to *Staphylococcus aureus*, and techniques for identifying and quantifying *S. aureus* adhesins in relation to adhesion to biomaterials: review. *Eur. Cells Mater.*, v.4, p.39-60, 2002.
- KAMEL, M.M.; ALI, H.I.; ANWAR, M.M.; MOHAMEDA, N.A.; SOLIMAN, A.M. Synthesis, antitumor activity and molecular docking study of novel sulfonamide-Schiff's bases, thiazolidinones, benzothiazinones and their C-nucleoside derivatives. *Eur. J. Med. Chem.*, v.45, p.572-580, 2010.
- KASPADY, M.; NARAYANASWAMY, V.K.; RAJU, M.; RAO, G.K. Synthesis, antibacterial activity of 2,4-disubstituted oxazoles and thiazoles as bioesters. *Lett. Drug Des. Discov.*, v.6, p.21-28, 2009.
- KHALID, H.; AZIZ-UR-REHMAN; ABBASI, M.A.; MALIK, A.; RASOOL, S.; NAFEESA, K.; AHMAD, I.; AFZAL, S. 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.*, 2013. DOI: <<http://dx.doi.org/10.1016/j.jscs.2013.05.001>>. [In Press].
- LIU, J.; WU, F.; CHEN, L.; ZHAO, L.; ZHAO, Z.; WANG, M.; LEI, S. Biological evaluation of coumarin derivatives as mushroom tyrosinase inhibitors. *Food Chem.*, v.135, p.2872-2878, 2012.
- MURTY, M.S.R.; PENTHALA, R.; NATH, L.R.; ANTO, R.J. Synthesis of salicylic acid-based 1,3,4-oxadiazole derivatives coupled with chiral oxazolidinones: novel hybrid heterocycles as antitumor agents. *Lett. Drug Des. Discov.*, v.11, p.1133-1142, 2014.
- NARAYAN, R.; POTOWSKI, M.; JIA, Z.; ANTONCHICK, A.P.; WALDMANN, H.; Catalytic enantioselective 1,3-dipolar cycloadditions of azomethine ylides for biology-oriented synthesis. *Acc. Chem. Res.*, v.47, p.1296-1310, 2014.
- NARSIBHAI, B.D.; MISHRA, D.; VYAVAHARE, L.V.; SINGH, A. Thiazolidinone: synthesis and biological studies. *Arch. Appl. Sci. Res.*, v.4, p.1816-1820, 2012.
- PRESSLER, T.; BOHMOVA, C.; CONWAY, S.; DUMCIUS, S.; HJELTE, L.; HØIBY, N.; KOLLBERG, H.; TÜMMLER, B.; VAVROVA, V. Chronic *Pseudomonas aeruginosa* infection definition: EuroCareCF Working Group report. *J. Cyst. Fibros.*, v.10, p.S75-S78, 2011.
- RASHID, M.; HUSAIN, A.; MISHRA, R. Synthesis of benzimidazoles bearing oxadiazole nucleus as anticancer agents. *Eur. J. Med. Chem.*, v.54, p.855-866, 2012.
- SALMAN, H.H. Synthesis of new azomethine-*N*-oxide compounds and study of their antifungal and antioxidant activities. *J. Basrah Res. (Sci.)*, v.39, p.91-99, 2013.
- SOMANI, R.R.; AGRAWAL, A.G.; KALANTRI, P.P.; GAVARKAR, P.S.; CLERCQ, E.D. Investigation of 1,3,4-oxadiazole scaffold as potentially active compounds. *Int. J. Drug Des. Discov.*, v.2, p.353-360, 2011.
- TABATABAI, S.A.; LASHKARI, S.B.; ZARRINDAST, M.R.; GHOLIBEIKIAN, M.; SHAFIEE, A. Design, synthesis and anticonvulsant activity of 2-(2-phenoxy)phenyl- 1,3,4-oxadiazole derivatives. *Iran. J. Pharm. Res.*, v.12, Suppl, p.105-111, 2013.
- VOGT, R.L.; DIPPOLD, L. *Escherichia coli* O157:H7 outbreak associated with consumption of ground beef, June-July 2002. *Public Health Rep.*, v.120, p.174-178, 2005.
- YANG, C.R.; ZANG, Y.; JACOB, M.R.; KHAN, S.I.; ZHANG, Y.J.; LI, X.C. Antifungal activity of C-27 steroidal saponins. *Antimicrob. Agents Ch.*, v.50, p.1710-1714, 2006.
- ZHANG, X.; QIU, M.; SUN, J.; ZHANG, Y.; YANG Y.; WANG, X.; TANG, J.; ZHU, H. Synthesis, biological evaluation and molecular docking studies of 1,3,4-oxadiazole derivatives possessing 1,4-benzodioxan moiety as potential anticancer agents. *Bioorg. Med. Chem.*, v.19, p.6518-6524, 2011.

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