

Synthesis, spectral analysis and antibacterial evaluation of *N'*-substituted-2-(5-(3-chlorophenyl)-1,3,4-Oxadiazol-2-ylthio)acetohydrazide derivatives

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Abstract: The biological potential of *N'*-substituted-2-(5-(3-chlorophenyl)-1,3,4-Oxadiazol-2-ylthio)acetohydrazide (8a-p) has been evaluated against bacterial strains of Gram-negative and Gram-positive bacteria. The multistep synthesis involved the conversion of 3-chlorobenzoic acid (1) to ethyl 3-chlorobenzoate (2), 3-chlorobenzohydrazide (3), 5-(3-chlorophenyl)-1,3,4-Oxadiazol-2-thiol (4), ethyl 2-(5-(3-chlorophenyl)-1,3,4-Oxadiazol-2-ylthio) acetate (5) and 2-(5-(3-chlorophenyl)-1,3,4-Oxadiazol-2-ylthio)acetohydrazide (6). The last step involved the reaction of 6 and aryl aldehydes, 7a-p, in methanol to synthesize the Schiff bases, 8a-p, with better yields. The structures of all the molecules were corroborated by spectral analysis. The Schiff bases were further evaluated for the antibacterial activity and found to be moderately good inhibitors of bacterial strains of Gram-bacteria.

Keywords: 1,3,4-Oxadiazole, 3-Chlorobenzoic acid, Antibacterial activity, Spectral analysis.

INTRODUCTION

Struggle, to inaugurate new potent molecules, is going on in the world of organic chemistry and pharmacy for new potent antibiotics (Roy *et al.*, 2009). Heterocyclic compounds have gained much attention of synthetic researchers owing to their broad spectrum of biological activities (Rakesh *et al.*, 2009) especially disubstituted-1,3,4-Oxadiazole ring has attracted the synthetic chemists (Jaiswal *et al.*, 2012; Hui *et al.*, 2002 and Mohan *et al.*, 2004). Valuable antibacterial and anti-enzymatic 1,3,4-Oxadiazole inhibitors have been synthesized in good yields by our group (Khalid *et al.*, 2013 and Aziz-ur-Rehman *et al.*, 2013a,b). The 1,3,4-Oxadiazoles also possess antibacterial, antifungal and anti-inflammatory activities (Almasirad *et al.*, 2004; Cansiz *et al.*, 2004; Hemavathi *et al.*, 2011; Koparir *et al.*, 2005; Kumar S, 2011 and Rostom *et al.*, 2003).

In protraction of our previous synthetic projects (Aziz-ur-Rehman *et al.*, 2012a,b), the series of Schiff bases were synthesized from 2-(5-(3-chlorophenyl)-1,3,4-Oxadiazol-2-ylthio) acetohydrazide (6) to evaluate their antibacterial potential against some bacterial strains of Gram-negative and Gram-positive bacteria. This work was conducted with an aim to introduce new molecules with antibacterial inhibition potential.

MATERIAL AND METHODS

General

The chemicals were purchased from Alfa Aesar, Merck and Sigma-Aldrich through local suppliers along with analytical grade solvents and applied for synthesis without further purification. The synthesized molecules were characterized by melting points measured from Griffin-George apparatus with open capillary tube and were uncorrected; I.R. spectra, computed from Jasco-320-A spectrophotometer in KBr pellet; ¹H-NMR spectra, recorded on Bruker spectrometer in DMSO-*d*₆ at 600 MHz; and EIMS spectra, recorded on JMS-HX-110 spectrometer. G-25-UV₂₅₄ plates surfaced with silica gel were employed for thin layer chromatography (TLC) to check out the purity and reaction completion using CH₃COOC₂H₅ and *n*-C₆H₁₄ solvent system in varying ratios.

Procedure for synthesis of ethyl 3-chlorobenzoate (2)

3-Chlorobenzoic acid (1; 3.0g) was added to 12.0mL absolute ethanol followed by the addition of 1.2mL concentrated H₂SO₄ in a 250mL round bottom (RB) flask. The reaction mixture was refluxed for 3 hours for maximal completion, indicated by TLC. The whole mixture was transferred to a 500mL separating funnel along with 120mL distilled water and pH was set to 8-10 by concentrated aqueous Na₂CO₃ solution. The ethyl ester, 2, was extracted using diethyl ether (30mL) and isolated on evaporation. Pale yellow liquid; Yield: 86%; Mol. formula: C₉H₉ClO₂; Mol. mass: 184 gmol⁻¹; IR

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(KBr, ν_{max}/cm^{-1}): 3106 (Ar C-H), 1737 (Ester C=O), 1594 (Ar C=C), 699 (C-Cl); 1H -NMR (600 MHz, DMSO, δ/ppm): 7.93 (dd, $J=7.2, 1.8$ Hz, 1H, H-6'), 7.59 (t, $J=7.8$ Hz, 1H, H-5'), 7.42 (dd, $J=7.8, 1.8$ Hz, 1H, H-4'), 7.41 (d, $J=1.2$ Hz, 1H, H-2'), 4.04 (q, $J=7.2$ Hz, 2H, -OCH₂CH₃), 1.06 (t, $J=7.2$ Hz, 3H, -OCH₂CH₃); EIMS (m/z): 186 [M+2]⁺, 184 [M]⁺, 139 [C₇H₄ClO]⁺, 111 [C₆H₄Cl]⁺, 85 [C₄H₂Cl]⁺, 51 [C₄H₃]⁺.

Procedure for synthesis of 3-chlorobenzohydrazide (3)

Ethyl 3-chlorobenzoate (2; 0.02mol) was mixed with 10.0mL methanol in a 100mL RB flask and stirred for 2 hours at 27°C after the addition of 1.3mL 80% hydrazine hydrate. The activation energy of the reaction can be attained through heating. After completion by single spot on TLC, excess of solvent was evaporated by heating and cold distilled water was added which resulted in precipitation. The precipitates were filtered, washed off by *n*-hexane and dried. Dirty white amorphous solid; Yield: 79%; M.P.: 156-158°C; Mol. formula: C₇H₇ClN₂O; Mol. mass: 170gmol⁻¹; IR (KBr, ν_{max}/cm^{-1}): 3326 (N-H), 3120 (Ar C-H), 1659 (Amide C=O), 1610 (Ar C=C), 706 (C-Cl); 1H -NMR (600 MHz, DMSO, δ/ppm): 9.38 (s, 1H, CONH), 8.75 (s, 2H, N-H), 7.94 (dd, $J=7.2, 1.8$ Hz, 1H, H-6'), 7.57 (t, $J=8.4$ Hz, 1H, H-5'), 7.43 (dd, $J=7.8, 1.8$ Hz, 1H, H-4'), 7.38 (d, $J=1.8$ Hz, 1H, H-2'); EIMS (m/z): 172 [M+2]⁺, 170 [M]⁺, 139 [C₇H₄ClO]⁺, 111 [C₆H₄Cl]⁺, 85 [C₄H₂Cl]⁺, 51 [C₄H₃]⁺.

Procedure for synthesis of 5-(3-chlorophenyl)-1,3,4-Oxadiazol-2-thiol (4)

3-Chlorobenzohydrazide (3; 0.02mol) was dissolved in 30.0mL absolute ethanol in a 250mL RB flask. Potassium hydroxide (0.02mol) was completely dissolved in the reaction mixture on reflux. Then carbon disulfide (0.04 mol) was added gradually and the reaction contents were refluxed for 5 hours till single spot on TLC plate. Cold distilled water was added along with vigorous shaking and then dilute HCl was used to set a pH of 2-3. The resulting precipitates were filtered, washed with distilled water and dried. White amorphous solid; Yield: 84%; M.P.: 168-170°C; Mol. formula: C₈H₅ClN₂OS; Mol. mass: 212 gmol⁻¹; IR (KBr, ν_{max}/cm^{-1}): 3120 (Ar C-H), 1669 (Oxadiazole C=N), 1592 (Ar C=C), 705 (C-Cl); 1H -NMR (600 MHz, DMSO, δ/ppm): 7.92 (dd, $J=7.8, 1.2$ Hz, 1H, H-6'), 7.58 (t, $J=7.8$ Hz, 1H, H-5'), 7.41 (dd, $J=9.0, 1.8$ Hz, 1H, H-4'), 7.39 (d, $J=1.8$ Hz, 1H, H-2'); EIMS (m/z): 214 [M+2]⁺, 212 [M]⁺, 153 [C₇H₄ClNO]⁺, 139 [C₇H₄ClO]⁺, 137 [C₇H₄ClN]⁺, 111 [C₆H₄Cl]⁺, 85 [C₄H₂Cl]⁺, 51 [C₄H₃]⁺.

Procedure for synthesis of ethyl 2-(5-(3-chlorophenyl)-1,3,4-Oxadiazol-2-ylthio)acetate (5)

The compound 4 (0.02 mol) was homogeneously dissolved in 12mL DMF (*N,N*-dimethylformamide) in a 100mL RB flask at 27°C. LiH (lithium hydride, 0.08 g) was added to activate compound 4, for electrophilic substitution, on stirring for about 34 minutes. The

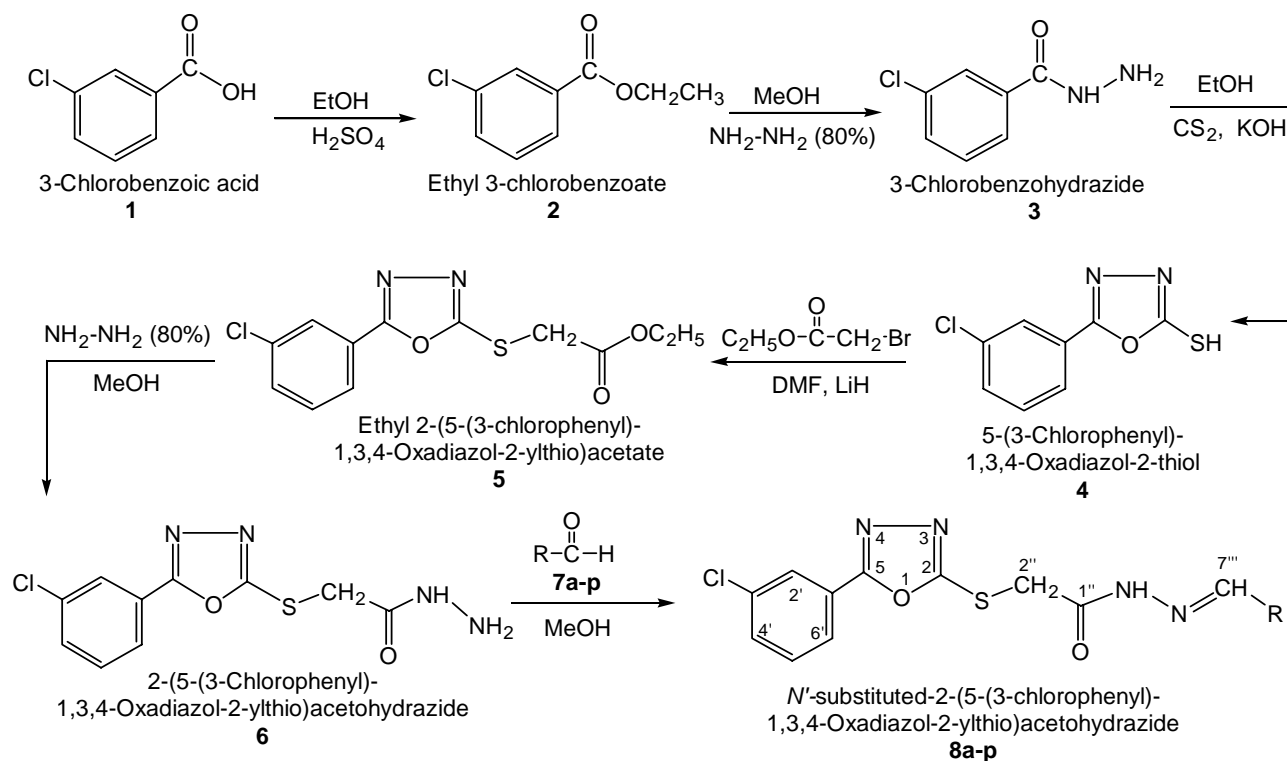
electrophile, ethyl 2-bromoacetate (0.02 mol, 2.2mL) was added followed by further stirring for 3.5 hours. At the end of reaction, managed through TLC, the addition of cold distilled water resulted in precipitation. The precipitates were separated by filtration, washed by distilled water and dried to afford the white amorphous solid. Yield: 83%; M.P.: 172-174°C; Mol. formula: C₁₂H₁₁ClN₂O₃S; Mol. mass: 298 gmol⁻¹; IR (KBr, ν_{max}/cm^{-1}): 3148 (Ar C-H), 1746 (Ester C=O), 1678 (Oxadiazole C=N), 1603 (Ar C=C), 708 (C-Cl); 1H -NMR (600 MHz, DMSO, δ/ppm): 7.92 (dd, $J=8.4, 1.8$ Hz, 1H, H-6'), 7.59 (t, $J=8.4$ Hz, 1H, H-5'), 7.43 (dd, $J=7.8, 1.2$ Hz, 1H, H-4'), 7.40 (d, $J=1.2$ Hz, 1H, H-2'), 4.62 (s, 2H, H-2''), 3.95 (q, $J=7.2$ Hz, 2H, -OCH₂CH₃), 1.01 (t, $J=7.2$ Hz, 3H, -OCH₂CH₃); EIMS (m/z): 300 [M+2]⁺, 298 [M]⁺, 253 [C₁₀H₆ClN₂O₂S]⁺, 225 [C₉H₆ClN₂OS]⁺, 212 [C₈H₅ClN₂OS]⁺, 179 [C₈H₄ClN₂O]⁺, 153 [C₇H₄ClNO]⁺, 139 [C₇H₄ClO]⁺, 137 [C₇H₄ClN]⁺, 111 [C₆H₄Cl]⁺, 85 [C₄H₂Cl]⁺, 51 [C₄H₃]⁺.

Procedure for synthesis of 2-(5-(3-chlorophenyl)-1,3,4-Oxadiazol-2-ylthio)acetohydrazide (6)

The ethyl ester 5 (0.02 mol) was taken in 13.0mL methanol in a 100mL RB flask. Then 1.3mL 80% hydrazine hydrate was added and stirred for 2.5 hours at 27°C. TLC was managed continuously to check out the reaction completion. Stirring at room temperature is strongly recommended avoiding heat because of high sensitivity of molecule. After complete reaction, the product was separated by filtration after addition of cold distilled water. It was washed off by *n*-hexane and dried to get white amorphous solid. Yield: 81%; M.P.: 176-178°C; Mol. formula: C₁₀H₉ClN₄O₂S; Mol. mass: 284 gmol⁻¹; IR (KBr, ν_{max}/cm^{-1}): 3374 (N-H), 3089 (Ar C-H), 1663 (Amide C=O), 1686 (Oxadiazole C=N), 1606 (Ar C=C), 709 (C-Cl); 1H -NMR (600 MHz, DMSO, δ/ppm): 9.85 (s, 1H, CONH), 8.72 (s, 2H, N-H), 7.91 (dd, $J=7.8, 1.8$ Hz, 1H, H-6'), 7.58 (t, $J=8.4$ Hz, 1H, H-5'), 7.41 (dd, $J=7.2, 1.2$ Hz, 1H, H-4'), 7.39 (d, $J=1.8$ Hz, 1H, H-2'), 4.64 (s, 2H, H-2''); EIMS (m/z): 286 [M+2]⁺, 284 [M]⁺, 253 [C₁₀H₆ClN₂O₂S]⁺, 225 [C₉H₆ClN₂OS]⁺, 212 [C₈H₅ClN₂OS]⁺, 179 [C₈H₄ClN₂O]⁺, 153 [C₇H₄ClNO]⁺, 139 [C₇H₄ClO]⁺, 137 [C₇H₄ClN]⁺, 111 [C₆H₄Cl]⁺, 85 [C₄H₂Cl]⁺, 51 [C₄H₃]⁺.

General procedure for synthesis of *N'*-substituted-2-(5-(3-chlorophenyl)-1,3,4-Oxadiazol-2-ylthio)acetohydrazide (8a-p)

The molecule 6 (0.002 mol) was taken in a 50mL RB flask with 16mL methanol at 27°C followed by the addition of few drops of glacial acetic acid. The equimolar aryl aldehydes (7a-p; 0.002 mol) were added along with shaking and stirred for 2 hours. The reaction was supervised by TLC and after completion; the distilled water was added to precipitate the products. The precipitates were collected through filtration and washed with distilled water to yield the title compounds on drying.



Comp.	-R	Comp.	-R	Comp.	-R	Comp.	-R
8a		8e		8i		8m	
8b		8f		8j		8n	
8c		8g		8k		8o	
8d		8h		8l		8p	

Scheme 1: Synthesis of *N'*-substituted-2-(5-(3-chlorophenyl)-1,3,4-Oxadiazol-2-ylthio) acetohydrazide, 8a-p

***N'*-Benzylidene-2-(5-(3-chlorophenyl)-1,3,4-Oxadiazol-2-ylthio) acetohydrazide (8a)**

White amorphous solid; Yield: 76%; M.P.: 152-154°C; Mol. formula: C₁₇H₁₃ClN₄O₂S; Mol. mass: 372 gmol⁻¹; IR (KBr, ν_{max} /cm⁻¹): 3046 (Ar C-H), 1661 (C=N), 1611 (Ar C=C), 692 (C-Cl); ¹H-NMR (600 MHz, DMSO, δ /ppm): 11.81 (s, 1H, CONH), 8.05 (s, 1H, H-7'''), 7.95 (dd, *J*=7.8, 1.8 Hz, 2H, H-2''' & H-6'''), 7.92 (dd, *J*=8.4, 1.2 Hz, 1H, H-6'), 7.71-7.69 (m, 3H, H-3''' to H-5'''), 7.60 (t, *J*=7.8 Hz, 1H, H-5'), 7.43 (dd, *J*=7.2, 1.8 Hz, 1H, H-4'), 7.42 (d, *J*=1.8 Hz, 1H, H-2'), 4.68 (s, 2H, H-2''); EIMS (*m/z*): 374 [M+2]⁺, 372 [M]⁺, 253 [C₁₀H₆ClN₂O₂S]⁺, 225

[C₉H₆ClN₂OS]⁺, 212 [C₈H₅ClN₂OS]⁺, 179 [C₈H₄ClN₂O]⁺, 153 [C₇H₄ClNO]⁺, 147 [C₈H₇N₂O]⁺, 139 [C₇H₄ClO]⁺, 137 [C₇H₄ClN]⁺, 119 [C₇H₇N₂]⁺, 111 [C₆H₄Cl]⁺, 91 [C₇H₇]⁺, 85 [C₄H₂Cl]⁺, 65 [C₅H₅]⁺, 51 [C₄H₃]⁺.

***N'*-(2-Methylbenzylidene)-2-(5-(3-chlorophenyl)-1,3,4-Oxadiazol-2-ylthio) acetohydrazide (8b)**

White amorphous solid; Yield: 81%; M.P.: 160-162°C; Mol. formula: C₁₈H₁₅ClN₄O₂S; Mol. mass: 386 gmol⁻¹; IR (KBr, ν_{max} /cm⁻¹): 3065 (Ar C-H), 1650 (C=N), 1617 (Ar C=C), 704 (C-Cl); ¹H-NMR (600 MHz, DMSO, δ /ppm): 11.69 (s, 1H, CONH), 8.31 (s, 1H, H-7'''), 7.92 (dd, *J*=7.8, 1.8 Hz, 1H, H-6'), 7.75 (ddd, *J*=9.0, 1.8 Hz, 1H, H-

5^{''}), 7.70 (d, *J*=7.8 Hz, 1H, H-6^{''}), 7.61 (t, *J*=8.4 Hz, 1H, H-5^{''}), 7.33 (s, 1H, H-2^{''}), 7.30 (ddd, *J*=7.8, 1.2 Hz, 1H, H-4^{''}), 7.26 (d, *J*=7.2 Hz, 1H, H-4^{''}), 7.23 (d, *J*=7.2 Hz, 1H, H-3^{''}), 4.67 (s, 2H, H-2^{''}), 2.42 (s, 3H, CH₃-2^{''}); EIMS (*m/z*): 388 [M+2]⁺, 386 [M]⁺, 253 [C₁₀H₆ClN₂O₂S]⁺, 225 [C₉H₆ClN₂OS]⁺, 212 [C₈H₅ClN₂OS]⁺, 179 [C₈H₄ClN₂O]⁺, 161 [C₉H₉N₂O]⁺, 153 [C₇H₄ClNO]⁺, 139 [C₇H₄ClO]⁺, 137 [C₇H₄ClN]⁺, 133 [C₈H₉N₂]⁺, 111 [C₆H₄Cl]⁺, 105 [C₈H₉]⁺, 85 [C₄H₂Cl]⁺, 65 [C₅H₅]⁺, 51 [C₄H₃]⁺.

***N'*-(3-Methylbenzylidene)-2-(5-(3-chlorophenyl)-1,3,4-Oxadiazol-2-ylthio)acetohydrazide (8c)**

Dirty white amorphous solid; Yield: 83%; M.P.: 154-156°C; Mol. formula: C₁₈H₁₅ClN₄O₂S; Mol. mass: 386 gmol⁻¹; IR (KBr, *v*_{max}/cm⁻¹): 3058 (Ar C-H), 1677 (C=N), 1605 (Ar C=C), 698 (C-Cl); ¹H-NMR (600 MHz, DMSO, *δ*/ppm): 11.80 (s, 1H, CONH), 8.01 (s, 1H, H-7^{''}), 7.92 (d, *J*=8.4 Hz, 1H, H-6^{''}), 7.59 (d, *J*=8.4 Hz, 1H, H-6^{''}), 7.48 (d, *J*=7.8 Hz, 1H, H-4^{''}), 7.31 (t, *J*=7.8 Hz, 1H, H-5^{''}), 7.24 (t, *J*=9.0 Hz, 1H, H-5^{''}), 7.16 (d, *J*=9.0 Hz, 1H, H-4^{''}), 7.06 (d, *J*=2.4 Hz, 1H, H-2^{''}), 6.73 (s, 1H, H-2^{''}), 4.67 (s, 2H, H-2^{''}), 2.34 (s, 3H, CH₃-3^{''}); EIMS (*m/z*): 388 [M+2]⁺, 386 [M]⁺, 253 [C₁₀H₆ClN₂O₂S]⁺, 225 [C₉H₆ClN₂OS]⁺, 212 [C₈H₅ClN₂OS]⁺, 179 [C₈H₄ClN₂O]⁺, 161 [C₉H₉N₂O]⁺, 153 [C₇H₄ClNO]⁺, 139 [C₇H₄ClO]⁺, 137 [C₇H₄ClN]⁺, 133 [C₈H₉N₂]⁺, 111 [C₆H₄Cl]⁺, 105 [C₈H₉]⁺, 85 [C₄H₂Cl]⁺, 65 [C₅H₅]⁺, 51 [C₄H₃]⁺.

***N'*-(4-Methylbenzylidene)-2-(5-(3-chlorophenyl)-1,3,4-Oxadiazol-2-ylthio)acetohydrazide (8d)**

White amorphous solid; Yield: 78%; M.P.: 164-166°C; Mol. formula: C₁₈H₁₅ClN₄O₂S; Mol. mass: 386 gmol⁻¹; IR (KBr, *v*_{max}/cm⁻¹): 3044 (Ar C-H), 1668 (C=N), 1615 (Ar C=C), 689 (C-Cl); ¹H-NMR (600 MHz, DMSO, *δ*/ppm): 11.75 (s, 1H, CONH), 8.01 (s, 1H, H-7^{''}), 7.93 (d, *J*=8.4 Hz, 1H, H-6^{''}), 7.70 (d, *J*=7.8 Hz, 1H, H-4^{''}), 7.66 (s, 1H, H-2^{''}), 7.63 (d, *J*=8.4 Hz, 2H, H-2^{''} & H-6^{''}), 7.59 (t, *J*=8.4 Hz, 1H, H-5^{''}), 7.20 (d, *J*=8.4 Hz, 2H, H-3^{''} & H-5^{''}), 4.66 (s, 2H, H-2^{''}), 2.34 (s, 3H, CH₃-4^{''}); EIMS (*m/z*): 388 [M+2]⁺, 386 [M]⁺, 253 [C₁₀H₆ClN₂O₂S]⁺, 225 [C₉H₆ClN₂OS]⁺, 212 [C₈H₅ClN₂OS]⁺, 179 [C₈H₄ClN₂O]⁺, 161 [C₉H₉N₂O]⁺, 153 [C₇H₄ClNO]⁺, 139 [C₇H₄ClO]⁺, 137 [C₇H₄ClN]⁺, 133 [C₈H₉N₂]⁺, 111 [C₆H₄Cl]⁺, 105 [C₈H₉]⁺, 85 [C₄H₂Cl]⁺, 65 [C₅H₅]⁺, 51 [C₄H₃]⁺.

***N'*-(2-Hydroxybenzylidene)-2-(5-(3-chlorophenyl)-1,3,4-Oxadiazol-2-ylthio)acetohydrazide (8e)**

White amorphous solid; Yield: 86%; M.P.: 204-206 °C; Mol. formula: C₁₇H₁₃ClN₄O₃S; Mol. mass: 388 gmol⁻¹; IR (KBr, *v*_{max}/cm⁻¹): 3063 (Ar C-H), 1679 (C=N), 1612 (Ar C=C), 698 (C-Cl); ¹H-NMR (600 MHz, DMSO, *δ*/ppm): 11.71 (s, 1H, CONH), 8.36 (s, 1H, H-7^{''}), 7.98 (d, *J*=7.8 Hz, 1H, H-6^{''}), 7.93 (d, *J*=7.8 Hz, 1H, H-6^{''}), 7.70 (d, *J*=8.4 Hz, 1H, H-4^{''}), 7.64 (s, 1H, H-2^{''}), 7.62 (t, *J*=7.8 Hz, 1H, H-5^{''}), 7.57 (dd, *J*=7.8, 1.8 Hz, 1H, H-3^{''}), 7.25 (ddd, *J*=7.8, 1.8 Hz, 1H, H-4^{''}), 6.91 (ddd, *J*=7.2, 1.8 Hz, 1H, H-5^{''}), 4.68 (s, 2H, H-2^{''}); EIMS (*m/z*): 390 [M+2]⁺, 388

[M]⁺, 253 [C₁₀H₆ClN₂O₂S]⁺, 225 [C₉H₆ClN₂OS]⁺, 212 [C₈H₅ClN₂OS]⁺, 179 [C₈H₄ClN₂O]⁺, 163 [C₈H₇N₂O₂]⁺, 153 [C₇H₄ClNO]⁺, 139 [C₇H₄ClO]⁺, 137 [C₇H₄ClN]⁺, 135 [C₇H₇N₂O]⁺, 111 [C₆H₄Cl]⁺, 107 [C₇H₇O]⁺, 85 [C₄H₂Cl]⁺, 65 [C₅H₅]⁺, 51 [C₄H₃]⁺.

***N'*-(3-Hydroxybenzylidene)-2-(5-(3-chlorophenyl)-1,3,4-Oxadiazol-2-ylthio)acetohydrazide (8f)**

White amorphous solid; Yield: 82%; M.P.: 210-212°C; Mol. formula: C₁₇H₁₃ClN₄O₃S; Mol. mass: 388 gmol⁻¹; IR (KBr, *v*_{max}/cm⁻¹): 3037 (Ar C-H), 1680 (C=N), 1610 (Ar C=C), 703 (C-Cl); ¹H-NMR (600 MHz, DMSO, *δ*/ppm): 11.76 (s, 1H, CONH), 8.45 (s, 1H, HO-3^{''}), 8.14 (s, 1H, H-7^{''}), 7.94 (dd, *J*=9.0, 1.2 Hz, 1H, H-6^{''}), 7.70 (dd, *J*=7.8, 1.2 Hz, 1H, H-6^{''}), 7.61 (t, *J*=7.8 Hz, 1H, H-5^{''}), 7.24 (t, *J*=7.8 Hz, 1H, H-5^{''}), 7.13 (s, 1H, H-2^{''}), 7.09 (d, *J*=7.8 Hz, 1H, H-4^{''}), 6.82 (dd, *J*=9.6, 1.2 Hz, 1H, H-4^{''}), 6.78 (s, 1H, H-2^{''}), 4.67 (s, 2H, H-2^{''}); EIMS (*m/z*): 390 [M+2]⁺, 388 [M]⁺, 253 [C₁₀H₆ClN₂O₂S]⁺, 225 [C₉H₆ClN₂OS]⁺, 212 [C₈H₅ClN₂OS]⁺, 179 [C₈H₄ClN₂O]⁺, 163 [C₈H₇N₂O₂]⁺, 153 [C₇H₄ClNO]⁺, 139 [C₇H₄ClO]⁺, 137 [C₇H₄ClN]⁺, 135 [C₇H₇N₂O]⁺, 111 [C₆H₄Cl]⁺, 107 [C₇H₇O]⁺, 85 [C₄H₂Cl]⁺, 65 [C₅H₅]⁺, 51 [C₄H₃]⁺.

***N'*-(4-Hydroxybenzylidene)-2-(5-(3-chlorophenyl)-1,3,4-Oxadiazol-2-ylthio)acetohydrazide (8g)**

Cream white amorphous solid; Yield: 78%; M.P.: 226-228°C; Mol. formula: C₁₇H₁₃ClN₄O₃S; Mol. mass: 388 gmol⁻¹; IR (KBr, *v*_{max}/cm⁻¹): 3041 (Ar C-H), 1676 (C=N), 1604 (Ar C=C), 707 (C-Cl); ¹H-NMR (600 MHz, DMSO, *δ*/ppm): 11.59 (s, 1H, CONH), 9.95 (s, 1H, HO-4^{''}), 8.17 (s, 1H, H-7^{''}), 7.95 (dd, *J*=8.4, 1.2 Hz, 1H, H-6^{''}), 7.60 (t, *J*=7.8 Hz, 1H, H-5^{''}), 7.52 (d, *J*=9.0 Hz, 2H, H-2^{''} & H-6^{''}), 7.14 (d, *J*=8.4 Hz, 1H, H-4^{''}), 6.99 (d, *J*=3.0 Hz, 1H, H-2^{''}), 6.80 (d, *J*=8.4 Hz, 2H, H-3^{''} & H-5^{''}), 4.63 (s, 2H, H-2^{''}); EIMS (*m/z*): 390 [M+2]⁺, 388 [M]⁺, 253 [C₁₀H₆ClN₂O₂S]⁺, 225 [C₉H₆ClN₂OS]⁺, 212 [C₈H₅ClN₂OS]⁺, 179 [C₈H₄ClN₂O]⁺, 163 [C₈H₇N₂O₂]⁺, 153 [C₇H₄ClNO]⁺, 139 [C₇H₄ClO]⁺, 137 [C₇H₄ClN]⁺, 135 [C₇H₇N₂O]⁺, 111 [C₆H₄Cl]⁺, 107 [C₇H₇O]⁺, 85 [C₄H₂Cl]⁺, 65 [C₅H₅]⁺, 51 [C₄H₃]⁺.

***N'*-(2-Nitrobenzylidene)-2-(5-(3-chlorophenyl)-1,3,4-Oxadiazol-2-ylthio)acetohydrazide (8h)**

Light yellow amorphous solid; Yield: 79%; M.P.: 206-208°C; Mol. formula: C₁₇H₁₂ClN₅O₄S; Mol. mass: 417 gmol⁻¹; IR (KBr, *v*_{max}/cm⁻¹): 3081 (Ar C-H), 1670 (C=N), 1611 (Ar C=C), 703 (C-Cl); ¹H-NMR (600 MHz, DMSO, *δ*/ppm): 12.05 (s, 1H, CONH), 8.40 (s, 1H, H-7^{''}), 8.09 (d, *J*=8.4 Hz, 1H, H-6^{''}), 8.02 (d, *J*=7.8 Hz, 1H, H-6^{''}), 7.92 (d, *J*=7.8 Hz, 1H, H-3^{''}), 7.76 (t, *J*=7.2 Hz, 1H, H-5^{''}), 7.69 (d, *J*=7.8 Hz, 1H, H-4^{''}), 7.66 (s, 1H, H-2^{''}), 7.65-7.59 (m, 2H, H-4^{''} & H-5^{''}), 4.66 (s, 2H, H-2^{''}); EIMS (*m/z*): 419 [M+2]⁺, 417 [M]⁺, 253 [C₁₀H₆ClN₂O₂S]⁺, 225 [C₉H₆ClN₂OS]⁺, 212 [C₈H₅ClN₂OS]⁺, 192 [C₈H₆N₃O₃]⁺, 179 [C₈H₄ClN₂O]⁺, 164 [C₇H₆N₃O₂]⁺, 153 [C₇H₄ClNO]⁺, 139 [C₇H₄ClO]⁺, 137 [C₇H₄ClN]⁺, 136 [C₇H₆NO₂]⁺, 111 [C₆H₄Cl]⁺, 85 [C₄H₂Cl]⁺, 65 [C₅H₅]⁺, 51 [C₄H₃]⁺.

Table 1: %age inhibition and MIC values of antibacterial activity

Compound	S. typhi (-)		E. coli (-)		P. aeruginosa (-)		B. subtilis (+)		S. aureus (+)	
	%age inhibition	MIC	%age inhibition	MIC	%age inhibition	MIC	%age inhibition	MIC	%age inhibition	MIC
8a	66.72 ±2.09	14.60 ±5.00	71.89 ±2.37	10.56 ±2.18	54.28 ±1.49	17.93 ±1.85	55.91 ±5.00	15.46 ±4.25	58.15 ±4.95	12.11 ±3.69
8b	62.21 ±1.96	16.80 ±2.17	67.53 ±1.16	10.86 ±1.44	62.89 ±3.40	15.70 ±4.69	62.64 ±0.16	11.06 ±2.44	55.25 ±4.95	13.17 ±1.56
8c	63.28 ±1.44	15.63 ±4.86	64.74 ±2.74	14.44 ±4.87	53.76 ±1.70	18.48 ±4.52	59.50 ±1.44	11.41 ±1.66	61.70 ±1.30	15.27 ±1.94
8d	62.21 ±1.65	16.70 ±1.00	63.42 ±1.68	12.58 ±5.00	44.69 ±1.49	-	51.36 ±2.31	19.40 ±2.13	61.40 ±0.10	13.15 ±2.75
8e	80.59 ±2.20	13.15 ±3.72	73.37 ±3.63	10.69 ±1.67	60.41 ±1.13	13.45 ±1.07	22.36 ±1.81	-	69.40 ±1.33	11.75 ±2.38
8f	70.39 ±2.80	12.21 ±1.58	71.63 ±1.47	11.39 ±2.00	64.33 ±1.54	14.86 ±3.16	66.55 ±3.38	12.95 ±1.14	57.90 ±2.08	15.49 ±4.71
8g	72.21 ±3.26	10.62 ±2.67	78.53 ±1.11	10.28 ±1.07	56.86 ±2.01	14.25 ±1.77	70.05 ±1.52	10.47 ±2.01	51.75 ±3.75	18.08 ±4.88
8h	64.26 ±4.13	10.85 ±3.25	58.00 ±2.16	17.07 ±1.87	62.06 ±2.89	15.20 ±2.77	47.00 ±4.21	-	52.25 ±3.15	16.97 ±5.00
8i	46.13 ±5.00	-	44.95 ±1.47	-	40.88 ±3.76	-	39.59 ±3.12	-	30.70 ±5.00	-
8j	63.33 ±2.13	13.54 ±5.00	67.84 ±1.75	13.16 ±2.00	55.82 ±3.66	15.96 ±5.00	50.55 ±2.41	19.32 ±3.44	60.85 ±2.55	11.25 ±1.19
8k	67.35 ±2.91	15.45 ±5.00	67.95 ±3.63	14.45 ±1.33	55.31 ±1.29	18.02 ±3.00	70.18 ±3.32	14.48 ±1.69	60.75 ±1.95	14.75 ±5.00
8l	41.67 ±1.32	-	59.68 ±5.00	17.85 ±2.93	54.54 ±1.09	19.06 ±1.31	52.55 ±2.50	18.97 ±2.50	60.10 ±1.50	17.44 ±2.72
8m	63.48 ±4.07	13.74 ±0.25	73.37 ±2.11	10.27 ±1.87	53.40 ±4.95	18.35 ±0.23	61.131.65 ±5.00	11.82 ±5.00	56.50 ±0.70	15.59 ±2.50
8n	47.35 ±0.10	-	51.11 ±2.16	19.47 ±2.00	24.54 ±0.72	-	39.91 ±1.19	-	37.85 ±1.05	-
8o	45.44 ±2.01	-	48.84 ±2.05	-	40.26 ±5.00	-	47.73 ±2.55	-	62.45 ±5.00	13.54 ±2.00
8p	61.03 ±1.55	12.89 ±3.67	72.79 ±2.79	10.45 ±1.49	55.36 ±4.02	15.04 ±2.38	66.41 ±1.59	11.11 ±5.00	60.25 ±2.45	11.96 ±2.60
Ciprofloxacin	91.83 ±0.05	9.13 ±2.00	91.65 ±1.47	8.90 ±1.65	90.56 ±1.11	9.01 ±0.13	90.89 ±1.05	8.02 ±0.33	92.05 ±2.32	8.41 ±1.04

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

***N'*-(3-Nitrobenzylidene)-2-(5-(3-chlorophenyl)-1,3,4-Oxadiazol-2-ylthio)acetohydrazide (8i)**

White amorphous solid; Yield: 78%; M.P.: 216-218°C; Mol. formula: C₁₇H₁₂ClN₅O₄S; Mol. mass: 417 gmol⁻¹; IR (KBr, ν_{max}/cm^{-1}): 3074 (Ar C-H), 1657 (C=N), 1603 (Ar C=C), 697 (C-Cl); ¹H-NMR (600 MHz, DMSO, δ/ppm): 12.01 (s, 1H, CONH), 8.54 (t, *J*=1.2 Hz, 1H, H-2'''), 8.35 (s, 1H, H-7'''), 8.21 (d, *J*=8.4 Hz, 1H, H-6'''), 8.13 (d, *J*=7.8 Hz, 1H, H-6''), 8.08 (d, *J*=8.4 Hz, 1H, H-4'''), 7.88 (t, *J*=8.4 Hz, 1H, H-5'''), 7.73 (t, *J*=7.8 Hz, 1H, H-5'), 7.56 (d, *J*=7.8 Hz, 1H, H-4'), 7.51 (s, 1H, H-2''), 4.70 (s, 2H, H-2''); EIMS (*m/z*): 419 [M+2]⁺, 417 [M]⁺, 253 [C₁₀H₆ClN₂O₂S]⁺, 225 [C₉H₆ClN₂OS]⁺, 212 [C₈H₅ClN₂OS]⁺, 192 [C₈H₆N₃O₃]⁺, 179 [C₈H₄ClN₂O]⁺, 164 [C₇H₆N₃O₂]⁺, 153 [C₇H₄ClNO]⁺, 139 [C₇H₄ClO]⁺, 137 [C₇H₄ClN]⁺, 136 [C₇H₆NO₂]⁺, 111 [C₆H₄Cl]⁺, 85 [C₄H₂Cl]⁺, 65 [C₅H₅]⁺, 51 [C₄H₃]⁺.

***N'*-(4-Nitrobenzylidene)-2-(5-(3-chlorophenyl)-1,3,4-Oxadiazol-2-ylthio)acetohydrazide (8j)**

Yellow amorphous solid; Yield: 81%; M.P.: 232-234°C; Mol. formula: C₁₇H₁₂ClN₅O₄S; Mol. mass: 417 gmol⁻¹; IR (KBr, ν_{max}/cm^{-1}): 3083 (Ar C-H), 1662 (C=N), 1616 (Ar C=C), 706 (C-Cl); ¹H-NMR (600 MHz, DMSO, δ/ppm): 11.99 (s, 1H, CONH), 8.33 (s, 1H, H-7'''), 8.29 (d, *J*=8.4 Hz, 1H, H-6'), 8.25 (d, *J*=8.4 Hz, 2H, H-2''' & H-6'''), 7.96 (d, *J*=8.4 Hz, 2H, H-3''' & H-5'''), 7.69 (d, *J*=7.2 Hz, 1H, H-4'), 7.64 (s, 1H, H-2'), 7.61 (t, *J*=7.8 Hz, 1H, H-5'), 4.71 (s, 2H, H-2''); EIMS (*m/z*): 419 [M+2]⁺, 417 [M]⁺, 253 [C₁₀H₆ClN₂O₂S]⁺, 225 [C₉H₆ClN₂OS]⁺, 212 [C₈H₅ClN₂OS]⁺, 192 [C₈H₆N₃O₃]⁺, 179 [C₈H₄ClN₂O]⁺, 164 [C₇H₆N₃O₂]⁺, 153 [C₇H₄ClNO]⁺, 139 [C₇H₄ClO]⁺, 137 [C₇H₄ClN]⁺, 136 [C₇H₆NO₂]⁺, 111 [C₆H₄Cl]⁺, 85 [C₄H₂Cl]⁺, 65 [C₅H₅]⁺, 51 [C₄H₃]⁺.

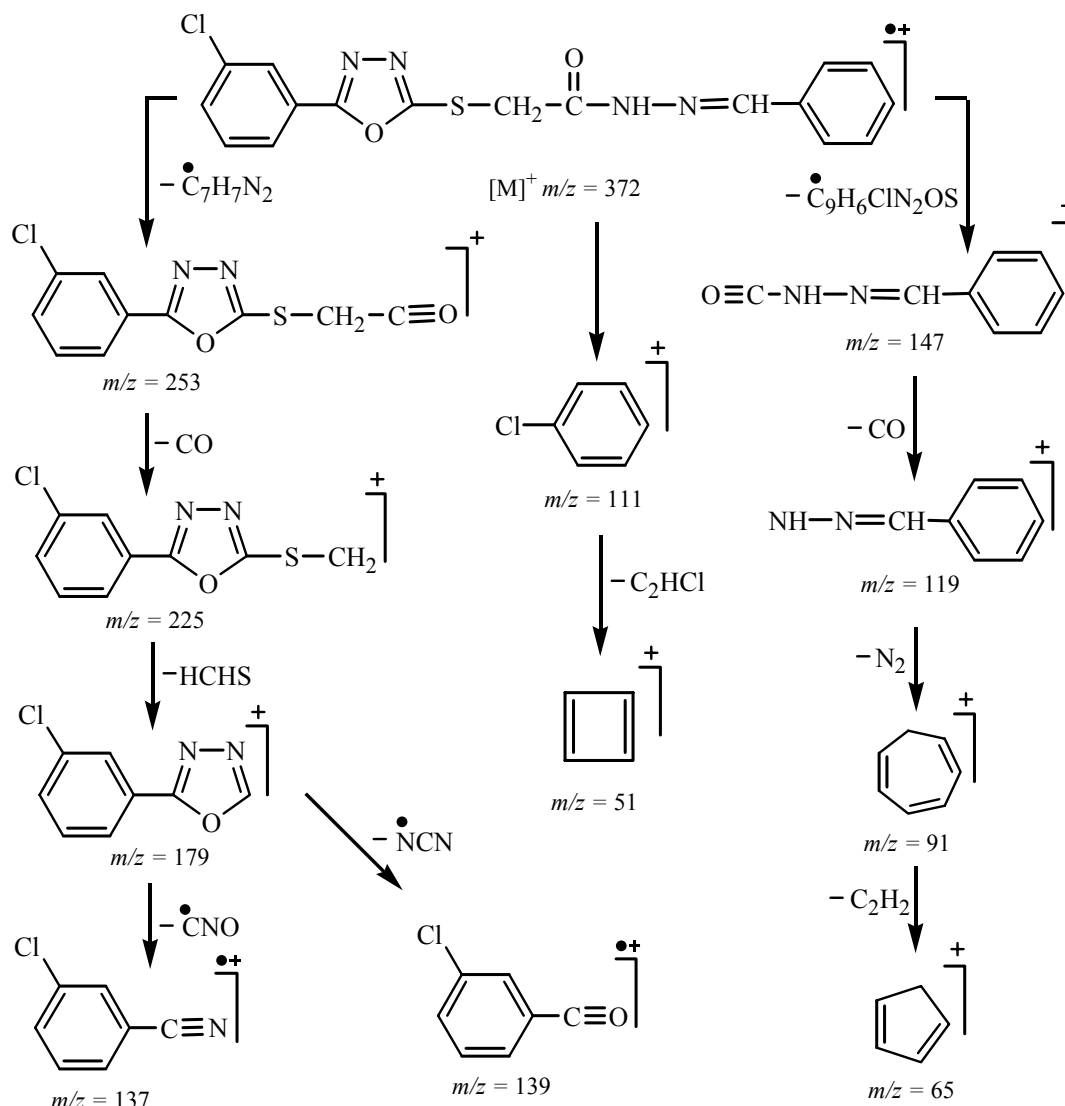


Fig. 1: Mass fragmentation pattern of *N'*-Benzylidene-2-(5-(3-chlorophenyl)-1,3,4-Oxadiazol-2-ylthio) acetohydrazide (8a)

***N'*-(4-(Dimethylamino)benzylidene)-2-(5-(3-chlorophenyl)-1,3,4-Oxadiazol-2-ylthio)acetohydrazide (8k)**

Yellow amorphous solid; Yield: 81%; M.P.: 158-160°C; Mol. formula: $C_{19}H_{18}ClN_5O_2S$; Mol. mass: 415 gmol^{-1} ; IR (KBr, ν_{max}/cm^{-1}): 3047 (Ar C-H), 1681 (C=N), 1619 (Ar C=C), 707 (C-Cl); $^1\text{H-NMR}$ (600 MHz, DMSO, δ/ppm): 11.53 (s, 1H, CONH), 8.06 (s, 1H, H-7'''), 7.93 (dd, $J=7.8, 3.0$ Hz, 1H, H-6'), 7.60 (t, $J=7.8$ Hz, 1H, H-5'), 7.47 (d, $J=9.0$ Hz, 2H, H-2''' & H-6'''), 7.14 (d, $J=8.4$ Hz, 1H, H-4'), 6.97 (s, 1H, H-2'), 6.69 (d, $J=9.0$ Hz, 2H, H-3''' & H-5'''), 4.61 (s, 2H, H-2''), 3.02 (s, 6H, $(\text{CH}_3)_2\text{N-4}''''$); EIMS (m/z): 417 $[M+2]^+$, 415 $[M]^+$, 253 $[\text{C}_{10}\text{H}_6\text{ClN}_2\text{O}_2\text{S}]^+$, 225 $[\text{C}_9\text{H}_6\text{ClN}_2\text{OS}]^+$, 212 $[\text{C}_8\text{H}_5\text{ClN}_2\text{OS}]^+$, 190 $[\text{C}_{10}\text{H}_{12}\text{N}_3\text{O}]^+$, 179 $[\text{C}_8\text{H}_4\text{ClN}_2\text{O}]^+$, 162 $[\text{C}_9\text{H}_{12}\text{N}_3]^+$, 153 $[\text{C}_7\text{H}_4\text{ClNO}]^+$, 139 $[\text{C}_7\text{H}_4\text{ClO}]^+$, 137 $[\text{C}_7\text{H}_4\text{ClN}]^+$, 133 $[\text{C}_9\text{H}_{11}\text{N}]^+$, 111 $[\text{C}_6\text{H}_4\text{Cl}]^+$, 85 $[\text{C}_4\text{H}_2\text{Cl}]^+$, 65 $[\text{C}_3\text{H}_5]^+$, 51 $[\text{C}_4\text{H}_3]^+$.

***N'*-(4-(Diethylamino)benzylidene)-2-(5-(3-chlorophenyl)-1,3,4-Oxadiazol-2-ylthio)acetohydrazide (8l)**

Yellow amorphous solid; Yield: 77%; M.P.: 168-170°C; Mol. formula: $C_{21}H_{22}ClN_5O_2S$; Mol. mass: 443 gmol^{-1} ; IR (KBr, ν_{max}/cm^{-1}): 3059 (Ar C-H), 1636 (C=N), 1613 (Ar C=C), 709 (C-Cl); $^1\text{H-NMR}$ (600 MHz, DMSO, δ/ppm): 11.48 (s, 1H, CONH), 8.04 (s, 1H, H-7'''), 7.94 (d, $J=8.4$ Hz, 1H, H-6'), 7.62 (t, $J=7.8$ Hz, 1H, H-5'), 7.45 (d, $J=9.0$ Hz, 2H, H-2''' & H-6'''), 7.15 (d, $J=7.2$ Hz, 1H, H-4'), 6.96 (s, 1H, H-2'), 6.65 (d, $J=9.0$ Hz, 2H, H-3''' & H-5'''), 4.62 (s, 2H, H-2''), 3.38 (q, $J=7.2$ Hz, 4H, $(\text{CH}_3\text{CH}_2)_2\text{N-4}''''$), 1.10 (t, $J=7.2$ Hz, 6H, $(\text{CH}_3\text{CH}_2)_2\text{N-4}''''$); EIMS (m/z): 445 $[M+2]^+$, 443 $[M]^+$, 253 $[\text{C}_{10}\text{H}_6\text{ClN}_2\text{O}_2\text{S}]^+$, 225 $[\text{C}_9\text{H}_6\text{ClN}_2\text{OS}]^+$, 218 $[\text{C}_{12}\text{H}_{16}\text{N}_3\text{O}]^+$, 212 $[\text{C}_8\text{H}_5\text{ClN}_2\text{OS}]^+$, 190 $[\text{C}_{11}\text{H}_{16}\text{N}_3]^+$, 179 $[\text{C}_8\text{H}_4\text{ClN}_2\text{O}]^+$, 162 $[\text{C}_{11}\text{H}_{15}\text{N}]^+$, 153 $[\text{C}_7\text{H}_4\text{ClNO}]^+$, 139 $[\text{C}_7\text{H}_4\text{ClO}]^+$, 137 $[\text{C}_7\text{H}_4\text{ClN}]^+$, 111 $[\text{C}_6\text{H}_4\text{Cl}]^+$, 85 $[\text{C}_4\text{H}_2\text{Cl}]^+$, 65 $[\text{C}_3\text{H}_5]^+$, 51 $[\text{C}_4\text{H}_3]^+$.

N'-(2,3-Dimethoxybenzylidene)-2-(5-(3-chlorophenyl)-1,3,4-Oxadiazol-2-ylthio)acetohydrazide (8m)

White amorphous solid; Yield: 82%; M.P.: 156-158°C; Mol. formula: C₁₉H₁₇ClN₄O₄S; Mol. mass: 432 gmol⁻¹; IR (KBr, ν_{max}/cm^{-1}): 3069 (Ar C-H), 1635 (C=N), 1605 (Ar C=C), 705 (C-Cl); ¹H-NMR (600 MHz, DMSO, δ/ppm): 11.73 (s, 1H, CONH), 8.32 (s, 1H, H-7'''), 7.91 (dd, $J=9.0, 1.8$ Hz, 1H, H-6'), 7.70 (s, 1H, H-2'), 7.68 (d, $J=7.8$ Hz, 1H, H-4'), 7.60 (t, $J=7.8$ Hz, 1H, H-5'), 7.42 (dd, $J=7.8, 3.0$ Hz, 1H, H-6'''), 7.11 (dd, $J=9.0, 3.0$ Hz, 1H, H-4'''), 7.08 (t, $J=7.8$ Hz, 1H, H-5'''), 4.65 (s, 2H, H-2''), 3.83 (s, 3H, CH₃O-3'''), 3.78 (s, 3H, CH₃O-2'''); EIMS (m/z): 434 [M+2]⁺, 432 [M]⁺, 253 [C₁₀H₆ClN₂O₂S]⁺, 225 [C₉H₆ClN₂OS]⁺, 212 [C₈H₅ClN₂OS]⁺, 207 [C₁₀H₁₁N₂O₃]⁺, 179 [C₉H₁₁N₂O₂]⁺, 179 [C₈H₄ClN₂O]⁺, 151 [C₉H₁₁O₂]⁺, 153 [C₇H₄ClNO]⁺, 139 [C₇H₄ClO]⁺, 137 [C₇H₄ClN]⁺, 111 [C₆H₄Cl]⁺, 85 [C₄H₂Cl]⁺, 51 [C₄H₃]⁺.

N'-(2,4-Dimethoxybenzylidene)-2-(5-(3-chlorophenyl)-1,3,4-Oxadiazol-2-ylthio)acetohydrazide (8n)

Dirty white amorphous solid; Yield: 84%; M.P.: 158-160°C; Mol. formula: C₁₉H₁₇ClN₄O₄S; Mol. mass: 432 gmol⁻¹; IR (KBr, ν_{max}/cm^{-1}): 3064 (Ar C-H), 1645 (C=N), 1606 (Ar C=C), 709 (C-Cl); ¹H-NMR (600 MHz, DMSO, δ/ppm): 11.60 (s, 1H, CONH), 8.27 (s, 1H, H-7'''), 7.92 (d, $J=7.8$ Hz, 1H, H-6'), 7.73 (d, $J=8.4$ Hz, 1H, H-6'''), 7.61 (t, $J=7.2$ Hz, 1H, H-5'), 7.43 (d, $J=7.8$ Hz, 1H, H-4'), 7.37 (s, 1H, H-2'), 6.60 (d, $J=2.4$ Hz, 1H, H-3'''), 6.57 (dd, $J=7.8, 2.4$ Hz, 1H, H-5'''), 4.62 (s, 2H, H-2''), 3.83 (s, 3H, CH₃O-2'''), 3.80 (s, 3H, CH₃O-4'''); EIMS (m/z): 434 [M+2]⁺, 432 [M]⁺, 253 [C₁₀H₆ClN₂O₂S]⁺, 225 [C₉H₆ClN₂OS]⁺, 212 [C₈H₅ClN₂OS]⁺, 207 [C₁₀H₁₁N₂O₃]⁺, 179 [C₉H₁₁N₂O₂]⁺, 179 [C₈H₄ClN₂O]⁺, 151 [C₉H₁₁O₂]⁺, 153 [C₇H₄ClNO]⁺, 139 [C₇H₄ClO]⁺, 137 [C₇H₄ClN]⁺, 111 [C₆H₄Cl]⁺, 85 [C₄H₂Cl]⁺, 51 [C₄H₃]⁺.

N'-(2,5-Dimethoxybenzylidene)-2-(5-(3-chlorophenyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (8o)

White amorphous solid; Yield: 83%; M.P.: 166-168°C; Mol. formula: C₁₉H₁₇ClN₄O₄S; Mol. mass: 432 gmol⁻¹; IR (KBr, ν_{max}/cm^{-1}): 3083 (Ar C-H), 1633 (C=N), 1610 (Ar C=C), 697 (C-Cl); ¹H-NMR (600 MHz, DMSO, δ/ppm): 11.74 (s, 1H, CONH), 8.33 (s, 1H, H-7'''), 7.90 (d, $J=8$ Hz, 1H, H-6'), 7.59 (t, $J=7.8$ Hz, 1H, H-5'), 7.34 (d, $J=9.0$ Hz, 1H, H-4'''), 7.23 (d, $J=7.2$ Hz, 1H, H-4'), 7.19 (s, 1H, H-2'), 7.04 (d, $J=7.8$ Hz, 1H, H-3'''), 6.98 (d, $J=3.6$ Hz, 1H, H-6'''), 4.66 (s, 2H, H-2''), 3.80 (s, 3H, CH₃O-5'''), 3.73 (s, 3H, CH₃O-2'''); EIMS (m/z): 434 [M+2]⁺, 432 [M]⁺, 253 [C₁₀H₆ClN₂O₂S]⁺, 225 [C₉H₆ClN₂OS]⁺, 212 [C₈H₅ClN₂OS]⁺, 207 [C₁₀H₁₁N₂O₃]⁺, 179 [C₉H₁₁N₂O₂]⁺, 179 [C₈H₄ClN₂O]⁺, 151 [C₉H₁₁O₂]⁺, 153 [C₇H₄ClNO]⁺, 139 [C₇H₄ClO]⁺, 137 [C₇H₄ClN]⁺, 111 [C₆H₄Cl]⁺, 85 [C₄H₂Cl]⁺, 51 [C₄H₃]⁺.

N'-(3,4-Dimethoxybenzylidene)-2-(5-(3-chlorophenyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (8p)

White amorphous solid; Yield: 80%; M.P.: 154-156°C; Mol. formula: C₁₉H₁₇ClN₄O₄S; Mol. mass: 432 gmol⁻¹; IR (KBr, ν_{max}/cm^{-1}): 3077 (Ar C-H), 1686 (C=N), 1603 (Ar C=C), 707 (C-Cl); ¹H-NMR (600 MHz, DMSO, δ/ppm): 11.69 (s, 1H, CONH), 8.13 (s, 1H, H-7'''), 7.91 (dd, $J=7.8, 1.2$ Hz, 1H, H-6'), 7.58 (t, $J=7.8$ Hz, 1H, H-5'), 7.43 (d, $J=7.8$ Hz, 1H, H-4'), 7.39 (d, $J=1.2$ Hz, 1H, H-2'), 7.31 (d, $J=1.8$ Hz, 1H, H-2'''), 7.18 (dd, $J=8.4, 1.8$ Hz, 1H, H-6'''), 6.98 (d, $J=8.4$ Hz, 1H, H-5'''), 4.66 (s, 2H, H-2''), 3.80 (s, 3H, CH₃O-3'''), 3.79 (s, 3H, CH₃O-4'''); EIMS (m/z): 434 [M+2]⁺, 432 [M]⁺, 253 [C₁₀H₆ClN₂O₂S]⁺, 225 [C₉H₆ClN₂OS]⁺, 212 [C₈H₅ClN₂OS]⁺, 207 [C₁₀H₁₁N₂O₃]⁺, 179 [C₉H₁₁N₂O₂]⁺, 179 [C₈H₄ClN₂O]⁺, 151 [C₉H₁₁O₂]⁺, 153 [C₇H₄ClNO]⁺, 139 [C₇H₄ClO]⁺, 137 [C₇H₄ClN]⁺, 111 [C₆H₄Cl]⁺, 85 [C₄H₂Cl]⁺, 51 [C₄H₃]⁺.

Antibacterial activity

The antibacterial activity was assayed on sterile 96-wells micro plates by the reported method but with minor modifications (Aziz-ur-Rehman *et al.*, 2013b; Kaspady *et al.*, 2009 and Yang *et al.*, 2006). This method works on the dependence of microbial cell number upon the log phase of microbial growth and also on the absorbance of broth medium.

STATISTICAL ANALYSIS

The results are presented as mean \pm sem after statistical analysis executed by Microsoft Excel 2010 for the calculations attempted in triplicate.

RESULTS

The Schiff bases, 8a-p, derived from 2-(5-(3-chlorophenyl)-1,3,4-oxadiazol-2-ylthio) acetohydrazide (6) were efficiently synthesized in good yields by the protocol sketched in Scheme 1. All the synthesized Schiff bases were processed for the antibacterial activity evaluation against Gram-bacteria. The procedures of various steps, reaction conditions and spectral characterization of all synthesized molecules are explicated in the experimental section.

Chemistry

The structural changes in a compound are subtle for the variation in biological activities. This prompted us to synthesize new molecules related to 1,3,4-Oxadiazoles and to evaluate their antibacterial activities. The MIC (minimum inhibitory concentration) values rendered the synthesized molecules as moderately good inhibitors against the bacterial strains taken into account. In this synthesis, 3-chlorobenzoic acid (1) was treated with ethanol in the presence of concentrated H₂SO₄ to yield ethyl 3-chlorobenzoate (2). The molecule 2 was stepped to 3-chlorobenzohydrazide (3) by nucleophilic reaction of hydrazine through stirring, yet heat can also be applied to attain activation energy. The very next step involves the

intermolecular cyclization of 3 to heterocyclic 1,3,4-Oxadiazole ring by CS₂ in a basic polar organic medium. The product should be isolated in low acidity of mixture for good yield as high acidity has negative effect. The formed product 5-(3-chlorophenyl)-1,3,4-Oxadiazole-2-thiol (4) was reacted with ethyl 2-bromoacetate using DMF as polar aprotic solvent and LiH as an activator to result 5; and further with hydrazine to yield corresponding hydrazide, 6. Only stirring is employed in the synthesis of 6 and heat is avoided because of much sensitivity of this step. The new synthesized Schiff bases, 8a-p, were afforded by the reaction of 6 with aryl aldehydes, 7a-p, from a polar organic medium with low acidity. The proposed structures of all the synthesized molecules were well supported by spectral data, elucidated in experimental section.

DISCUSSION

The synthesized compound, 8a, executed prominent absorption bands at 3046 (Ar C-H), 1661 (C=N), 1611 (Ar C=C) and 692 (C-Cl) in IR spectra. The molecular formula, C₁₇H₁₃ClN₄O₂S was led by EIMS giving [M]⁺ ion peak at *m/z* 372 and by ¹H-NMR spectrum showing integration for protons. EIMS also supported the structure by giving valuable fragments at *m/z* 219 and 148 for 2-(5-(3-chlorophenyl)-1,3,4-Oxadiazol-2-ylthio) acetato and *N'*-benzylideneacetohydrazido cations. The mass fragmentation pattern of 8a was sketched for convenience in fig. 1. The three signals, in ¹H-NMR spectrum, resonating at δ 8.05 (s, 1H, H-7''), 7.95 (dd, J=7.8, 1.8 Hz, 2H, H-2''' & H-6''') and 7.71-7.69 (m, 3H, H-3''' to H-5''') were allocated to six protons of benzylidene ring. The four protons of meta-substituted phenyl ring appeared at δ 7.92 (dd, J=8.4, 1.2 Hz, 1H, H-6'), 7.60 (t, J=7.8 Hz, 1H, H-5'), 7.43 (dd, J=7.2, 1.8 Hz, 1H, H-4') and 7.42 (d, J=1.8 Hz, 1H, H-2'). The signals erecting at δ 11.81 (s, 1H, CONH) and 4.68 (s, 2H, H-2'') affirmed the acetamidic protons and also amidic linkage. All this discussion helped to name 8a as *N'*-benzylidene-2-(5-(3-chlorophenyl)-1,3,4-Oxadiazol-2-ylthio) acetohydrazide. Similar discussion named the other compounds compiled in experimental section.

Antibacterial activity (in vitro)

The *in vitro* %age inhibition and MIC results are offered in table 1. Overall the compounds have shown very moderate antibacterial activity against the bacterial strains of Gram-bacteria taken into account.

The antibacterial activity of all the synthesized molecules was noted in comparison of ciprofloxacin, the reference standard. Most of molecules among the series showed moderate activity against all the bacterial strains. The molecule 8i remained inactive against all the bacterial and 8n & 8o showed activity only against *E. coli* & *S. aureus*, respectively. Against *S. typhi*, 8g & 8h showed almost the

same activity as that of reference standard and 8b & 8d showed 50% activity relative to reference but 8i, 8l, 8n & 8o exhibited no activity at all. The molecules, 8c, 8h, 8k, 8l & 8n executed half activity potential as compared to reference against *E. coli* and remaining were found as moderate inhibitors. The best activity by all the compounds was against *P. aeruginosa*, which was 50% of that for ciprofloxacin. The molecules showed very moderate activity against the both Gram-positive bacteria.

CONCLUSION

All the Schiff bases, 8a-p, were synthesized in good yields and their structures were well supported by spectral data of IR, ¹H-NMR and EIMS. The screening of all the synthesized Schiff bases for antibacterial activity have shown the moderate MIC values against all the bacterial strains of Gram-negative and Gram-positive bacteria with some exceptions. The molecule 8i showed no activity at all against all the bacterial strains and 8n & 8o exhibited activity only against *E. coli* & *S. aureus*, respectively. Overall moderate values might be supporting these molecules as new drug candidates for pharmacological applications.

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