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Original Research Article

Synthesis of N'-Substituted-2-(5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide Derivatives as Suitable Antibacterial Agents

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Abstract

Purpose: *To evaluate antibacterial activity of a series of molecules bearing 1,3,4-oxadiazole and azomethine moieties.*
Methods: *The 4-chlorobenzoic acid (**1**) was precursor to N'-substituted-2-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide, **8a-p**, through a multistep synthesis of corresponding ester, **2**, hydrazide, **3** and 1,3,4-oxadiazole, **4**. The molecule, **4**, was subjected to electrophilic substitution by ethyl-2-bromoacetate to yield **5** which was stepped to 2-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (**6**). The target molecules, 8a-p, were synthesized by nucleophilic addition of **6** to arylaldehydes, **7a-p**. The proposed structures of all the synthesized molecules were elucidated by Infra Red (IR), Proton Nuclear Magnetic Resonance (^{1}H-NMR) and Electron Impact Mass Spectrometry (EI-MS) spectral data. Antibacterial activity was evaluated by the principle that microbial growth is in a log phase of growth and so results in increased absorbance of broth medium which is observed.*
Results: *The molecule, **8b**, was active against S. aureus and **8c** against S. typhi only. The molecule, **8p**, was the most active against S. typhi with minimum inhibitory concentration (MIC) value of 10.04 ± 1.25 µM while **8e** was active against E. coli with MIC of 9.45 ± 1.00 µM, both relative to the reference standard, ciprofloxacin, which displayed MIC of 9.13 ± 2.00 and 8.90 ± 1.65 µM, respectively.*
Conclusion: *Most of the synthesized molecules exhibit 50 % antibacterial activity relative to the reference. Molecules **8b** and **8c** are the least active compounds.*

Keywords: *1,3,4-Oxadiazole, 4-Chlorobenzoic acid, Antibacterial activity, Azomethine*

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INTRODUCTION

Bacteria have developed resistance to several antibiotics over time. Scientists have continued for several years to discover new potent molecules with remarkable activities [1]. Heterocyclic compounds have often been synthesized because of their broad spectrum of biological activities [2]. The 2,5-substituted-1,3,4-oxadiazole derivatives, which have often been synthesized in the last decade, exhibit a number of biological activities [3-7] . In continuation of a previous synthetic work using 1,3,4-oxadiazole derivatives [8,9], N-substituted derivatives of 2-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide have been synthesized in this study to determine their antibacterial potential.

EXPERIMENTAL

General

Melting points of the synthesized compounds were carried out in a Griffin-George apparatus with open capillary tube and were uncorrected; I.R. spectral data by KBr pellet on a Jasco-320-A spectrophotometer; ^{1}H-NMR spectral data by Bruker spectrometer in dimethylsulfoxide (DMSO) at 600 MHz; and EIMS spectral data by JMS-HX-110 spectrometer. Purity was verified via thin layer chromatography (TLC) on pre-coated silica gel G-25-UV254 plates with ethyl acetateand n-hexane solvent systems.

Synthesis of ethyl 4-chlorobenzoate (2)

4-Chlorobenzoic acid (**1**; 5.0 g) was refluxed in 20.0 mL absolute ethanol and 2.0 mL concentrated H_2SO_4 in a 250 mL round bottom (RB) flask for 3 h. After completion of the reaction which was verified by TLC, reaction mixture was taken up in a separating funnel followed by the addition of 200 mL distilled water and concentrated aqueous Na_2CO_3 solution (pH = 8-10). 50 mL diethylether was employed to extract the ester from the aqueous layer which was obtained on evaporation.

Synthesis of 4-chlorobenzohydrazide (3)

Ethyl 4-chlorobenzoate (**2**; 0.03 mol) was stirred with 2.0 mL 80 % hydrazine hydrate in 15.0 mL methanol in a 100 mL RB flask for 2 h. After final TLC, the product was precipitated out by the addition of excess cold distilled water, separated by filtration and washed with n-hexane.

Synthesis of 5-(4-chlorophenyl)-1,3,4-oxadiazol-2-thiol (4)

The mixture of 4-chlorobenzohydrazide (**3**; 0.03 mol) and 50.0 mL absolute ethanol in a 250 mL RB flask was basified by KOH (0.03 mol) on reflux. Carbon disulfide (0.06 mol) was added at room temperature. The mixture was refluxed for 5 h till single spot on TLC. Excess cold distilled water was poured into the reaction flask and acidified by dilute HCl (pH of 2-3). The precipitates were filtered and washed with distilled water.

Synthesis of ethyl 2-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-ylthio)acetate (5)

The compound **4** (0.03 mol) was dissolved in 13 mL DMF in a 50 mL RB flask. NaH (0.12 g) was added and stirred for 30 min. Ethyl 2-bromoacetate (3.3 mL) was added and further stirred for 3.5 h. On single spot through TLC, excess ice cold distilled water was poured to flask for precipitation. The precipitates were isolated through filtration, washed and dried.

Synthesis of 2-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (6)

The ester **5** (0.03 mol) in 20.0 mL methanol was stirred with 2.0 mL 80 % hydrazine hydrate strictly at room temperature in a 100 mL RB flask for 2.5 h. After complete reaction, the addition of excess cold distilled water resulted in precipitation. The product was separated by filtration and washed off by n-hexane.

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

The compound 6 (0.001 mol) was taken in 17 mL methanol in a 50 mL RB flask followed by the addition of arylaldehydes (**7a-p**; 0.001 mol). The reaction mixture was stirred for 2 h. After complete reaction, the distilled water was added and the resulting precipitates were filtered, washed with distilled water and dried to afford the title compounds.

Evaluation of antibacterial activity

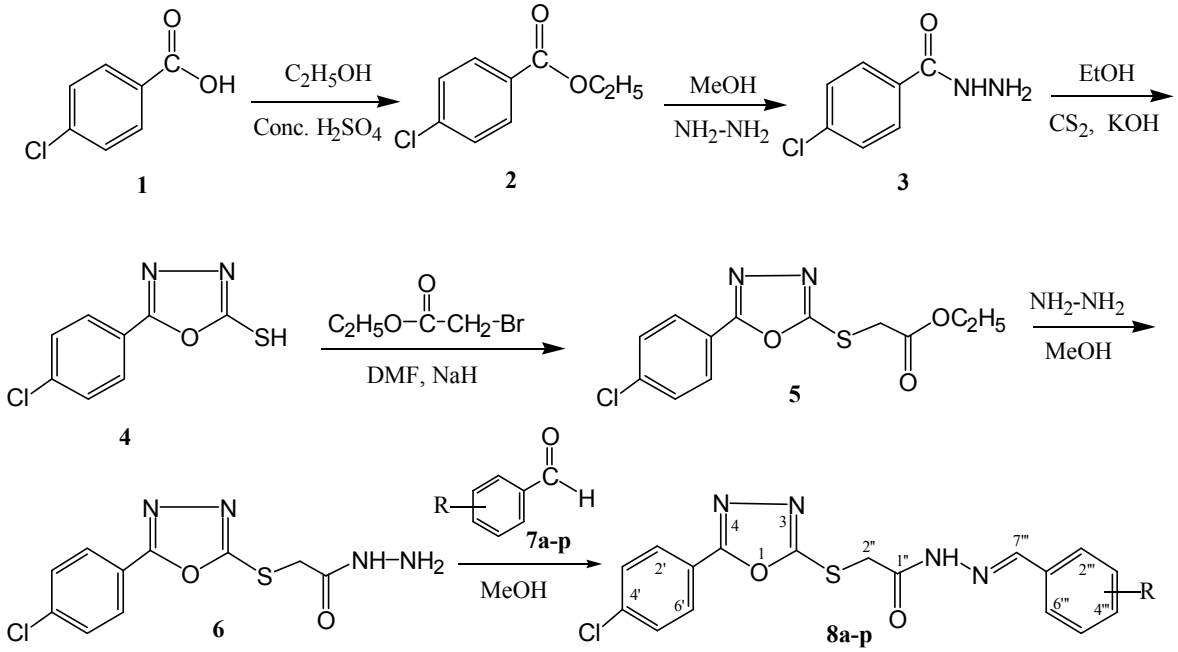
The antibacterial activity assay was executed as reported in the literature [10,11]. Antibacterial activity was performed in sterile 96-wells microplates under aseptic conditions. The variation in absorbance is noted which proportional to the microbial cell number is increasing in a log phase of growth.

Statistical analysis

All computations were executed in triplicate and statistical analysis was performed with Microsoft Excel 2010. The results are expressed as mean ± SEM (n = 3). Minimum inhibitory concentration (MIC) was computed with suitable dilutions (5 - 30 µg/well) for each sample and results calculated using EZ-Fit software (Perrella Scientific Inc, Amherst, USA).

RESULTS

The target molecules, *N′*-substituted-2-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (**8a-p**) were synthesized as shown in Scheme-1. The synthesized compounds were further evaluated for the antibacterial activity.



Comp.	-R	Comp.	-R	Comp.	-R	Comp.	-R
8a	H	**8e**	2-OH	**8i**	3- NO_2	**8m**	2,3-OCH_3
8b	2-CH_3	**8f**	3- OH	**8j**	4- NO_2	**8n**	2,4-OCH_3
8c	3-CH_3	**8g**	4- OH	**8k**	4-$N(CH_3)_2$	**8o**	2,5-OCH_3
8d	4-CH_3	**8h**	2-NO_2	**8l**	4-$N(C_2H_5)_2$	**8p**	3,4-OCH_3

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

Spectral characterization of synthesized molecules (2-6, 8a-p)

Ethyl 4-chlorobenzoate (2)

Yellow liquid; Yield: 84 %; Mol. formula: $C_9H_9ClO_2$; Mol. mass: 184; IR (KBr, ν_{max}/cm^{-1}): 3105, 1735, 1599, 697; ^{1}H-NMR (600 MHz, DMSO, δ/ppm): 7.95 (d, J = 7.2 Hz, 2H, H-2' & H-6'), 7.63 (d, J = 8.4 Hz, 2H, H-3' & H-5'), 4.05 (q, J = 7.2 Hz, 2H, -O$\underline{CH_2}$CH$_3$), 1.10 (t, J = 7.2 Hz, 3H, -OCH$_2\underline{CH_3}$); EIMS (*m/z*): 186 $[M+2]^+$, 184 $[M]^+$, 139 $[C_7H_4ClO]^+$, 111 $[C_6H_4Cl]^+$.

4-Chlorobenzohydrazide (3)

White amorphous solid; Yield: 78 %; M.P.: 162-164 ^{o}C; Mol. formula: $C_7H_7ClN_2O$; Mol. mass: 170; IR (KBr, ν_{max}/cm^{-1}): 3328, 3118, 1657, 1609, 703; ^{1}H-NMR (600 MHz, DMSO, δ/ppm): 9.37 (s, 1H, CONH), 8.76 (s, 2H, N-H), 7.89 (d, J = 7.8 Hz, 2H, H-2' & H-6'), 7.59 (d, J = 7.8 Hz, 2H, H-3' & H-5'); EIMS (*m/z*): 172 $[M+2]^+$, 170 $[M]^+$, 139 $[C_7H_4ClO]^+$, 111 $[C_6H_4Cl]^+$.

5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-thiol (4)

White amorphous solid; Yield: 86 %; M.P.: 170-172 ^{o}C; Mol. formula: $C_8H_5ClN_2OS$; Mol. mass: 212; IR (KBr, ν_{max}/cm^{-1}): 3123, 1665, 1595, 707, 615; ^{1}H-NMR (600 MHz, DMSO, δ/ppm): 7.88 (d, J = 7.2 Hz, 2H, H-2' & H-6'), 7.63 (d, J = 8.4 Hz, 2H, H-3' & H-5'); EIMS (*m/z*): 214 $[M+2]^+$, 212 $[M]^+$, 139 $[C_7H_4ClO]^+$, 137 $[C_7H_4ClN]^+$, 111 $[C_6H_4Cl]^+$.

Ethyl 2-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-ylthio)acetate (5)

White amorphous solid; Yield: 81 %; M.P.: 174-176 ^{o}C; Mol. formula: $C_{12}H_{11}ClN_2O_3S$; Mol. mass: 298; IR (KBr, ν_{max}/cm^{-1}): 3145, 1741, 1679, 1604, 1058, 710; ^{1}H-NMR (600 MHz, DMSO, δ/ppm): 7.96 (d, J = 7.8 Hz, 2H, H-2' & H-6'), 7.69 (d, J = 9.0 Hz, 2H, H-3' & H-5'), 4.64 (s, 2H, H-2''), 3.93 (q, J = 7.2 Hz, 2H, -O$\underline{CH_2}$CH$_3$), 1.03 (t, J = 7.2 Hz, 3H, -OCH$_2\underline{CH_3}$); EIMS (*m/z*): 300 $[M+2]^+$, 298 $[M]^+$, 225 $[C_9H_6ClN_2OS]^+$, 179 $[C_8H_4ClN_2O]^+$, 139 $[C_7H_4ClO]^+$, 137 $[C_7H_4ClN]^+$, 111 $[C_6H_4Cl]^+$.

2-(5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (6)

White amorphous solid; Yield: 87 %; M.P.: 178-180 ^{o}C; Mol. formula: $C_{10}H_9ClN_4O_2S$; Mol. mass: 284; IR (KBr, ν_{max}/cm^{-1}): 3378, 3085, 1661, 1681, 1601, 1065, 703; ^{1}H-NMR (600 MHz, DMSO, δ/ppm): 9.41 (s, 1H, CONH), 8.84 (s, 2H, N-H), 7.97 (d, J = 7.8 Hz, 2H, H-2' & H-6'), 7.67 (d, J = 9.0 Hz, 2H, H-3' & H-5'), 4.61 (s, 2H, H-2''); EIMS

(*m/z*): 286 $[M+2]^+$, 284 $[M]^+$, 225 $[C_9H_6ClN_2OS]^+$, 179 $[C_8H_4ClN_2O]^+$, 139 $[C_7H_4ClO]^+$, 137 $[C_7H_4ClN]^+$, 111 $[C_6H_4Cl]^+$.

_N'_-Benzylidene-2-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (8a)

White amorphous solid; Yield: 73 %; M.P.: 160-162 °C; Mol. formula: $C_{17}H_{13}ClN_4O_2S$; Mol. mass: 372; IR (KBr, v_{max}/cm^{-1}): 3048, 1659, 1609, 1071, 690; ^{1}H-NMR (600 MHz, DMSO, *δ*/ppm): 11.79 (s, 1H, CONH), 8.22 (s, 1H, H-7'''), 7.97 (d, *J* = 8.4 Hz, 2H, H-2' & H-6'), 7.70 (dd, *J* = 7.2, 1.8 Hz, 2H, H-2''' & H-6'''), 7.65 (d, *J* = 9.0 Hz, 2H, H-3' & H-5'), 7.47-7.43 (m, 3H, H-3''' to H-5'''), 4.67 (s, 2H, H-2''); EIMS (*m/z*): 374 $[M+2]^+$, 372 $[M]^+$, 225 $[C_9H_6ClN_2OS]^+$, 179 $[C_8H_4ClN_2O]^+$, 147 $[C_8H_7N_2O]^+$, 139 $[C_7H_4ClO]^+$, 137 $[C_7H_4ClN]^+$, 111 $[C_6H_4Cl]^+$.

_N'_-(2-Methylbenzylidene)-2-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (8b)

White amorphous solid; Yield: 79 %; M.P.: 166-168 °C; Mol. formula: $C_{18}H_{15}ClN_4O_2S$; Mol. mass: 386; IR (KBr, v_{max}/cm^{-1}): 3063, 1651, 1615, 1074, 699; ^{1}H-NMR (600 MHz, DMSO, *δ*/ppm): 11.72 (s, 1H, CONH), 8.31 (s, 1H, H-7'''), 7.97 (d, *J* = 8.4 Hz, 2H, H-2' & H-6'), 7.75 (dd, *J* = 9.0, 1.8 Hz, 1H, H-6'''), 7.65 (d, *J* = 9.0 Hz, 2H, H-3' & H-5'), 7.35-7.30 (m, 2H, H-4''' & H-5'''), 7.26 (d, *J* = 7.2 Hz, 1H, H-3'''), 4.67 (s, 2H, H-2''), 2.44 (s, 3H, CH_3-2'''); EIMS (*m/z*): 388 $[M+2]^+$, 386 $[M]^+$, 225 $[C_9H_6ClN_2OS]^+$, 179 $[C_8H_4ClN_2O]^+$, 161 $[C_9H_9N_2O]^+$, 139 $[C_7H_4ClO]^+$, 137 $[C_7H_4ClN]^+$, 111 $[C_6H_4Cl]^+$.

_N'_-(3-Methylbenzylidene)-2-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (8c)

White amorphous solid; Yield: 80 %; M.P.: 162-164 °C; Mol. formula: $C_{18}H_{15}ClN_4O_2S$; Mol. mass: 386; IR (KBr, v_{max}/cm^{-1}): 3056, 1676, 1602, 1047, 696; ^{1}H-NMR (600 MHz, DMSO, *δ*/ppm): 11.75 (s, 1H, CONH), 8.16 (s, 1H, H-7'''), 7.95 (d, *J* = 9.0 Hz, 2H, H-2' & H-6'), 7.63 (d, *J* = 8.4 Hz, 2H, H-3' & H-5'), 7.48 (d, *J* = 7.2 Hz, 1H, H-6'''), 7.32 (t, *J* = 7.8 Hz, 1H, H-5'''), 7.26 (s, 1H, H-2'''), 7.23 (d, *J* = 7.2 Hz, 1H, H-4'''), 4.66 (s, 2H, H-2''), 2.33 (s, 3H, CH_3-3'''); EIMS (*m/z*): 388 $[M+2]^+$, 386 $[M]^+$, 225 $[C_9H_6ClN_2OS]^+$, 179 $[C_8H_4ClN_2O]^+$, 161 $[C_9H_9N_2O]^+$, 139 $[C_7H_4ClO]^+$, 137 $[C_7H_4ClN]^+$, 111 $[C_6H_4Cl]^+$.

_N'_-(4-Methylbenzylidene)-2-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (8d)

White amorphous solid; Yield: 78 %; M.P.: 170-172 °C; Mol. formula: $C_{18}H_{15}ClN_4O_2S$; Mol. mass: 386; IR (KBr, v_{max}/cm^{-1}): 3046, 1665, 1619, 1056, 692; ^{1}H-NMR (600 MHz, DMSO, *δ*/ppm): 11.72 (s, 1H, CONH), 8.18 (s, 1H, H-7'''), 7.96 (d, *J* = 8.4 Hz, 2H, H-2' & H-6'), 7.64 (d, *J* = 8.4 Hz, 2H, H-3' & H-5'), 7.58 (d, *J* = 7.8 Hz, 2H, H-2''' & H-6'''), 7.24 (d, *J* = 7.8 Hz, 2H, H-3''' & H-5'''), 4.65 (s, 2H, H-2''), 2.34 (s, 3H, CH_3-4'''); EIMS (*m/z*): 388 $[M+2]^+$, 386 $[M]^+$, 225 $[C_9H_6ClN_2OS]^+$, 179 $[C_8H_4ClN_2O]^+$, 161 $[C_9H_9N_2O]^+$, 139 $[C_7H_4ClO]^+$, 137 $[C_7H_4ClN]^+$, 111 $[C_6H_4Cl]^+$.

_N'_-(2-Hydroxybenzylidene)-2-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (8e)

Dirty white amorphous solid; Yield: 87 %; M.P.: 212-214 °C; Mol. formula: $C_{17}H_{13}ClN_4O_3S$; Mol. mass: 388; IR (KBr, v_{max}/cm^{-1}): 3064, 1678, 1611, 1061, 688; ^{1}H-NMR (600 MHz, DMSO, *δ*/ppm): 11.73 (s, 1H, CONH), 8.35 (s, 1H, H-7'''), 7.97 (d, *J* = 9.0 Hz, 2H, H-2' & H-6'), 7.71 (dd, *J* = 7.8, 1.8 Hz, 1H, H-6'''), 7.65 (d, *J* = 8.4 Hz, 2H, H-3' & H-5'), 7.56 (dd, *J* = 7.2, 1.2 Hz, 1H, H-3'''), 7.25 (ddd, *J* = 7.2, 1.8 Hz, 1H, H-4'''), 6.85 (t, *J* = 7.2 Hz, 1H, H-5'''), 4.66 (s, 2H, H-2''); EIMS (*m/z*): 390 $[M+2]^+$, 388 $[M]^+$, 225 $[C_9H_6ClN_2OS]^+$, 179 $[C_8H_4ClN_2O]^+$, 163 $[C_8H_7N_2O_2]^+$, 139 $[C_7H_4ClO]^+$, 137 $[C_7H_4ClN]^+$, 111 $[C_6H_4Cl]^+$.

_N'_-(3-Hydroxybenzylidene)-2-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (8f)

White amorphous solid; Yield: 82 %; M.P.: 218-220 °C; Mol. formula: $C_{17}H_{13}ClN_4O_3S$; Mol. mass: 388; IR (KBr, v_{max}/cm^{-1}): 3035, 1679, 1612, 1072, 698; ^{1}H-NMR (600 MHz, DMSO, *δ*/ppm): 11.74 (s, 1H, CONH), 9.64 (s, 1H, HO-3'''), 8.12 (s, 1H, H-7'''), 7.97 (d, *J* = 7.8 Hz, 2H, H-2' & H-6'), 7.65 (d, *J* = 9.0 Hz, 2H, H-3' & H-5'), 7.24 (t, *J* = 7.8 Hz, 1H, H-5'''), 7.15 (s, 1H, H-2'''), 7.09 (d, *J* = 7.8 Hz, 1H, H-6'''), 6.83 (dd, *J* = 7.8, 2.4 Hz, 1H, H-4'''), 4.66 (s, 2H, H-2''); EIMS (*m/z*): 390 $[M+2]^+$, 388 $[M]^+$, 225 $[C_9H_6ClN_2OS]^+$, 179 $[C_8H_4ClN_2O]^+$, 163 $[C_8H_7N_2O_2]^+$, 139 $[C_7H_4ClO]^+$, 137 $[C_7H_4ClN]^+$, 111 $[C_6H_4Cl]^+$.

_N'_-(4-Hydroxybenzylidene)-2-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (8g)

White amorphous solid; Yield: 77 %; M.P.: 234-236 °C; Mol. formula: $C_{17}H_{13}ClN_4O_3S$; Mol. mass:

388; IR (KBr, v_{max}/cm^{-1}): 3039, 1674, 1601, 1078, 704; ^{1}H-NMR (600 MHz, DMSO, δ/ppm): 11.74 (s, 1H, CONH), 8.09 (s, 1H, H-7'''), 7.96 (d, J = 8.4 Hz, 2H, H-2' & H-6'), 7.64 (d, J = 8.4 Hz, 2H, H-3' & H-5'), 7.52 (d, J = 9.0 Hz, 2H, H-2''' & H-6'''), 6.80 (d, J = 9.0 Hz, 2H, H-3''' & H-5'''), 4.62 (s, 2H, H-2''); EIMS (*m/z*): 390 $[M+2]^+$, 388 $[M]^+$, 225 $[C_9H_6ClN_2OS]^+$, 179 $[C_8H_4ClN_2O]^+$, 163 $[C_8H_7N_2O_2]^+$, 139 $[C_7H_4ClO]^+$, 137 $[C_7H_4ClN]^+$, 111 $[C_6H_4Cl]^+$.

_N'_-(2-Nitrobenzylidene)-2-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (8h)

Yellow amorphous solid; Yield: 73 %; M.P.: 212-214 ^{o}C; Mol. formula: $C_{17}H_{12}ClN_5O_4S$; Mol. mass: 417; IR (KBr, v_{max}/cm^{-1}): 3084, 1673, 1613, 1066, 701; ^{1}H-NMR (600 MHz, DMSO, δ/ppm): 12.09 (s, 1H, CONH), 8.33 (s, 1H, H-7'''), 8.30 (d, J = 9.0 Hz, 1H, H-6'''), 8.26 (d, J = 9.0 Hz, 2H, H-2' & H-6'), 7.98 (dd, J = 9.0, 2.4 Hz, 1H, H-3'''), 7.97-7.95 (m, 2H, H-4''' & H-5'''), 7.65 (d, J = 8.4 Hz, 2H, H-3' & H-5'), 4.70 (s, 2H, H-2''); EIMS (*m/z*): 419 $[M+2]^+$, 417 $[M]^+$, 225 $[C_9H_6ClN_2OS]^+$, 192 $[C_8H_6N_3O_3]^+$, 179 $[C_8H_4ClN_2O]^+$, 139 $[C_7H_4ClO]^+$, 137 $[C_7H_4ClN]^+$, 111 $[C_6H_4Cl]^+$.

_N'_-(3-Nitrobenzylidene)-2-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (8i)

White amorphous solid; Yield: 76 %; M.P.: 222-224 ^{o}C; Mol. formula: $C_{17}H_{12}ClN_5O_4S$; Mol. mass: 417; IR (KBr, v_{max}/cm^{-1}): 3075, 1651, 1606, 1057, 693; ^{1}H-NMR (600 MHz, DMSO, δ/ppm): 12.05 (s, 1H, CONH), 8.52 (t, J = 1.8 Hz, 1H, H-2'''), 8.36 (s, 1H, H-7'''), 8.26 (dd, J = 9.0, 1.8 Hz, 1H, H-6'''), 8.16 (d, J = 7.8 Hz, 1H, H-4'''), 7.96 (d, J = 9.0 Hz, 2H, H-2' & H-6'), 7.74 (t, J = 8.4 Hz, 1H, H-5'''), 7.65 (d, J = 8.4 Hz, 2H, H-3' & H-5'), 4.72 (s, 2H, H-2''); EIMS (*m/z*): 419 $[M+2]^+$, 417 $[M]^+$, 225 $[C_9H_6ClN_2OS]^+$, 192 $[C_8H_6N_3O_3]^+$, 179 $[C_8H_4ClN_2O]^+$, 139 $[C_7H_4ClO]^+$, 137 $[C_7H_4ClN]^+$, 111 $[C_6H_4Cl]^+$.

_N'_-(4-Nitrobenzylidene)-2-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (8j)

Yellow amorphous solid; Yield: 75 %; M.P.: 240-242 ^{o}C; Mol. formula: $C_{17}H_{12}ClN_5O_4S$; Mol. mass: 417; IR (KBr, v_{max}/cm^{-1}): 3081, 1659, 1619, 1069, 691; ^{1}H-NMR (600 MHz, DMSO, δ/ppm): 12.08 (s, 1H, CONH), 8.43 (s, 1H, H-7'''), 8.09 (d, J = 7.8 Hz, 2H, H-3''' & H-5'''), 8.05 (d, J = 9.6 Hz, 2H, H-2''' & H-6'''), 7.96 (d, J = 7.8 Hz, 2H, H-2' & H-6'), 7.66 (d, J = 9.0 Hz, 2H, H-3' & H-5'), 4.67 (s, 2H, H-2''); EIMS (*m/z*): 419 $[M+2]^+$, 417 $[M]^+$, 225 $[C_9H_6ClN_2OS]^+$, 192 $[C_8H_6N_3O_3]^+$, 179 $[C_8H_4ClN_2O]^+$, 139 $[C_7H_4ClO]^+$, 137 $[C_7H_4ClN]^+$, 111 $[C_6H_4Cl]^+$.

_N'_-(4-(Dimethylamino)benzylidene)-2-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (8k)

Yellow amorphous solid; Yield: 71 %; M.P.: 170-172 ^{o}C; Mol. formula: $C_{19}H_{18}ClN_5O_2S$; Mol. mass: 415; IR (KBr, v_{max}/cm^{-1}): 3043, 1679, 1617, 1064, 701; ^{1}H-NMR (600 MHz, DMSO, δ/ppm): 11.48 (s, 1H, CONH), 8.04 (s, 1H, H-7'''), 7.96 (d, J = 9.0 Hz, 2H, H-2' & H-6'), 7.63 (d, J = 8.4 Hz, 2H, H-3' & H-5'), 7.47 (d, J = 8.4 Hz, 2H, H-2''' & H-6'''), 6.69 (d, J = 9.0 Hz, 2H, H-3''' & H-5'''), 4.59 (s, 2H, H-2''), 2.96 (s, 6H, $(CH_3)_2$N-4'''); EIMS (*m/z*): 417 $[M+2]^+$, 415 $[M]^+$, 225 $[C_9H_6ClN_2OS]^+$, 190 $[C_{10}H_{12}N_3O]^+$, 179 $[C_8H_4ClN_2O]^+$, 139 $[C_7H_4ClO]^+$, 137 $[C_7H_4ClN]^+$, 111 $[C_6H_4Cl]^+$.

_N'_-(4-(Diethylamino)benzylidene)-2-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (8l)

Yellow amorphous solid; Yield: 79 %; M.P.: 174-176 ^{o}C; Mol. formula: $C_{21}H_{22}ClN_5O_2S$; Mol. mass: 443; IR (KBr, v_{max}/cm^{-1}): 3058, 1634, 1610, 1059, 689; ^{1}H-NMR (600 MHz, DMSO, δ/ppm): 11.45 (s, 1H, CONH), 8.01 (s, 1H, H-7'''), 7.96 (d, J = 9.0 Hz, 2H, H-2' & H-6'), 7.63 (d, J = 8.4 Hz, 2H, H-3' & H-5'), 7.44 (d, J = 8.4 Hz, 2H, H-2''' & H-6'''), 6.64 (d, J = 9.0 Hz, 2H, H-3''' & H-5'''), 4.58 (s, 2H, H-2''), 2.60 (q, J = 7.2 Hz, 4H, $(CH_3\underline{CH_2})_2$N-4'''), 1.09 (t, J = 7.2 Hz, 6H, $(\underline{CH_3}CH_2)_2$N-4'''); EIMS (*m/z*): 445 $[M+2]^+$, 443 $[M]^+$, 225 $[C_9H_6ClN_2OS]^+$, 212 $[C_8H_5ClN_2OS]^+$, 190 $[C_{11}H_{16}N_3]^+$, 179 $[C_8H_4ClN_2O]^+$, 139 $[C_7H_4ClO]^+$, 137 $[C_7H_4ClN]^+$, 111 $[C_6H_4Cl]^+$.

_N'_-(2,3-Dimethoxybenzylidene)-2-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (8m)

White amorphous solid; Yield: 83 %; M.P.: 164-166 ^{o}C; Mol. formula: $C_{19}H_{17}ClN_4O_4S$; Mol. mass: 432; IR (KBr, v_{max}/cm^{-1}): 3067, 1633, 1602, 1055, 708; ^{1}H-NMR (600 MHz, DMSO, δ/ppm): 11.73 (s, 1H, CONH), 8.32 (s, 1H, H-7'''), 7.96 (d, J = 9.0 Hz, 2H, H-2' & H-6'), 7.64 (d, J = 9.0 Hz, 2H, H-3' & H-5'), 7.55 (d, J = 8.4 Hz, 1H, H-6'''), 7.43 (dd, J = 7.8, 1.8 Hz, 1H, H-4'''), 7.11 (t, J = 7.8 Hz, 1H, H-5'''), 4.65 (s, 2H, H-2''), 3.84 (s, 3H, CH_3O-3'''), 3.77 (s, 3H, CH_3O-2'''); EIMS (*m/z*): 434 $[M+2]^+$, 432 $[M]^+$, 225 $[C_9H_6ClN_2OS]^+$, 179 $[C_8H_4ClN_2O]^+$, 139 $[C_7H_4ClO]^+$, 137 $[C_7H_4ClN]^+$, 111 $[C_6H_4Cl]^+$.

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

White amorphous solid; Yield: 85 %; M.P.: 166-168 °C; Mol. formula: $C_{19}H_{17}ClN_4O_4S$; Mol. mass: 432; IR (KBr, v_{max}/cm^{-1}): 3061, 1643, 1604, 1058, 699; ^{1}H-NMR (600 MHz, DMSO, *δ*/ppm): 11.61 (s, 1H, CONH), 8.27 (s, 1H, H-7'''), 7.96 (d, *J* = 9.0 Hz, 2H, H-2' & H-6'), 7.73 (d, *J* = 8.4 Hz, 1H, H-6'''), 7.65 (d, *J* = 9.0 Hz, 2H, H-3' & H-5'), 6.62 (d, *J* = 2.4 Hz, 1H, H-3'''), 6.57 (dd, *J* = 8.4, 1.8 Hz, 1H, H-5'''), 4.61 (s, 2H, H-2''), 3.85 (s, 3H, CH_3O-2'''), 3.82 (s, 3H, CH_3O-4'''); EIMS (*m/z*): 434 $[M+2]^+$, 432 $[M]^+$, 225 $[C_9H_6ClN_2OS]^+$, 179 $[C_8H_4ClN_2O]^+$, 139 $[C_7H_4ClO]^+$, 137 $[C_7H_4ClN]^+$, 111 $[C_6H_4Cl]^+$.

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

Dirty white amorphous solid; Yield: 80 %; M.P.: 174-176 °C; Mol. formula: $C_{19}H_{17}ClN_4O_4S$; Mol. mass: 432; IR (KBr, v_{max}/cm^{-1}): 3085, 1631, 1608, 1053, 696; ^{1}H-NMR (600 MHz, DMSO, *δ*/ppm): 11.73 (s, 1H, CONH), 8.33 (s, 1H, H-7'''), 7.96 (d, *J* = 8.4 Hz, 2H, H-2' & H-6'), 7.64 (d, *J* = 9.0 Hz, 2H, H-3' & H-5'), 7.36 (d, *J* = 3.0 Hz, 1H, H-6'''), 7.05 (d, *J* = 7.8 Hz, 1H, H-3'''), 7.01 (dd, *J* = 9.0, 3.0 Hz, 1H, H-4'''), 4.66 (s, 2H, H-2''), 3.80 (s, 3H, CH_3O-5'''), 3.75 (s, 3H, CH_3O-2'''); EIMS (*m/z*): 434 $[M+2]^+$, 432 $[M]^+$, 225 $[C_9H_6ClN_2OS]^+$, 179 $[C_8H_4ClN_2O]^+$, 139 $[C_7H_4ClO]^+$, 137 $[C_7H_4ClN]^+$, 111 $[C_6H_4Cl]^+$.

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

White amorphous solid; Yield: 84 %; M.P.: 162-164 °C; Mol. formula: $C_{19}H_{17}ClN_4O_4S$; Mol. mass: 432; IR (KBr, v_{max}/cm^{-1}): 3078, 1689, 1605, 1059, 711; ^{1}H-NMR (600 MHz, DMSO, *δ*/ppm): 11.69 (s, 1H, CONH), 8.12 (s, 1H, H-7'''), 7.94 (d, *J* = 9.0 Hz, 2H, H-2' & H-6'), 7.63 (d, *J* = 9.0 Hz, 2H, H-3' & H-5'), 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'''), 4.64 (s, 2H, H-2''), 3.80 (s, 3H, CH_3O-3'''), 3.79 (s, 3H, CH_3O-4'''); EIMS (*m/z*): 434 $[M+2]^+$, 432 $[M]^+$, 225 $[C_9H_6ClN_2OS]^+$, 179 $[C_8H_4ClN_2O]^+$, 139 $[C_7H_4ClO]^+$, 137 $[C_7H_4ClN]^+$, 111 $[C_6H_4Cl]^+$.

Antibacterial activity

The results of antibacterial activity results are shown in Tables 1 and 2.

Table 1: Antibacterial activity (inhibition) of the synthesized compounds

Compound	Inhibition (%)				
	S. typhi (-)	*E. coli (-)*	*P. aeroginosa (-)*	*B. subtilis (+)*	*S. aureus (+)*
6	76.62±2.00	81.16±1.79	68.04±1.65	64.23±3.06	64.55±2.55
8a	62.79±3.65	58.53±1.42	43.56±1.98	51.95±1.75	43.75±2.35
8b	48.58±1.06	43.53±2.53	20.05±2.84	38.18±2.23	56.70±3.70
8c	53.92±2.13	49.32±2.58	38.76±1.72	46.59±1.77	47.35±3.05
8d	60.64±2.22	57.63±2.14	28.97±5.00	53.23±2.41	56.45±1.55
8e	82.89±2.25	79.84±2.43	76.75±1.60	78.77±2.41	71.45±4.15
8f	69.95±2.87	72.16±1.36	51.60±3.76	58.05±1.79	52.00±1.70
8g	65.59±1.77	53.21±2.16	59.74±0.67	54.18±1.06	66.55±3.45
8h	51.86±3.05	50.63±2.77	51.86±1.75	51.09±1.94	45.60±5.00
8i	54.75±1.55	51.16±4.36	45.88±2.47	53.64±2.59	63.30±4.30
8j	75.25±1.40	74.05±2.49	65.36±1.44	66.55±3.76	70.55±0.65
8k	67.45±1.67	61.74±1.64	53.25±2.01	60.86±2.95	58.10±1.00
8l	64.90±1.32	71.11±2.23	59.02±2.32	57.50±1.18	59.60±2.60
8m	67.65±3.22	64.95±1.33	52.16±3.61	53.09±2.55	56.60±4.30
8n	69.56±1.76	69.16±2.09	55.10±0.15	60.05±1.34	60.95±0.35
8o	75.05±2.08	67.74±2.11	57.73±1.00	60.59±3.14	57.50±2.20
8p	66.76±1.67	60.63±1.88	46.44±1.70	52.14±3.22	55.55±3.35
Ciprofloxacin	**91.83±0.05**	**91.65±1.47**	**90.56±1.11**	**90.89±1.05**	**92.05±2.32**

Table 2: Antibacterial activity (MIC) of the synthesized compounds

Compound	MIC (µM)				
	S. typhi (-)	*E. coli (-)*	*P. aeroginosa (-)*	*B. subtilis (+)*	*S. aureus (+)*
6	11.23±2.08	13.52±2.40	13.49±3.08	13.35±5.00	16.18±1.15
8a	10.37±2.13	13.46±2.50	-	17.54±1.50	-
8b	-	-	-	-	16.51±2.50
8c	18.21±4.83	-	-	-	-
8d	14.29±3.67	16.58±5.00	-	13.59±1.94	16.40±1.15
8e	12.03±4.92	9.45±1.00	10.53±2.97	11.94±5.00	10.65±3.43
8f	10.32±1.42	11.21±1.60	18.52±3.46	14.67±0.55	19.09±1.38
8g	12.93±3.00	13.18±4.07	15.47±1.23	15.44±5.00	13.37±5.00
8h	19.08±5.00	19.66±4.00	19.02±4.08	19.69±1.38	-
8i	17.64±5.00	16.32±1.20	-	15.45±4.37	12.13±5.00
8j	14.26±5.00	12.03±4.13	12.42±1.85	14.68±2.16	10.98±2.64
8k	14.15±0.42	15.36±2.67	18.13±1.12	14.40±3.81	14.15±4.63
8l	17.00±1.87	14.02±5.00	17.68±4.62	16.73±4.75	16.03±3.38
8m	11.41±1.25	12.02±1.87	18.44±3.11	14.41±2.31	15.18±2.38
8n	10.92±1.67	12.76±1.33	15.43±1.46	12.71±4.13	14.31±3.00
8o	12.10±1.00	13.44±4.67	14.91±3.00	11.01±3.94	16.01±5.00
8p	10.04±1.25	11.90±4.20	-	18.10±2.00	17.90±4.00
Ciprofloxacin	**9.13±2.00**	**8.90±1.65**	**9.01±0.13**	**8.02±0.33**	**8.41±1.04**

DISCUSSION

The compound, **8a**, showed characteristic absorption bands of the IR spectra at 3048, 1659, 1609, 1071 and 690. Its molecular formula, $C_{17}H_{13}ClN_4O_2S$ was shown by EIMS with $[M]^+$ peak at *m/z* 372 and number of protons in ^{1}H-NMR spectrum. The prominent fragmentation peaks of this molecule were depicted in Fig. 1. The three signals in ^{1}H-NMR spectrum allotted to six protons of benzylidene moiety were *δ* 8.22 (1H, H-7'''), *δ* 7.70 (*J* = 7.2, 1.8 Hz, 2H, H-2''' & H-6''') and *δ* 7.47-7.43 (3H, H-3''' to H-5'''). Two doublets at *δ* 7.97 with *J* = 8.4 Hz and at *δ* 7.65 with *J* = 9.0 Hz were assigned to H-2' & H-6' and H-3' & H-5', respectively. The signals resonating at *δ* 11.79 (s, 1H, CONH) and 4.67 (s, 2H, H-2'') confirmed the acetamidic protons.

On the basis of spectral analysis, the molecule **8a** was named, *N'*-benzylidene-2-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide. The structures of other synthesized molecules were also corroborated likewise.

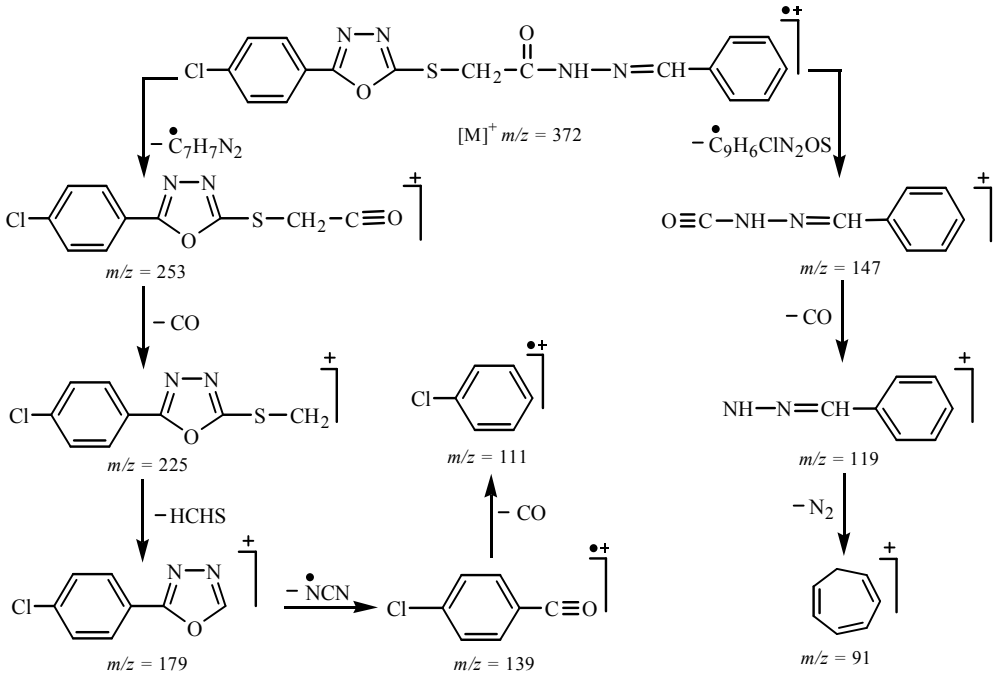


Figure 1: Mass fragmentation pattern of synthesized molecule **8a**

The activities of synthesized molecules were found to be higher against various bacterial strains of gram negative bacteria as compared to such type of other molecules [12-15]. The methylbenzylidenes (**8b**, **8c** & **8d**) and nitrobenzylidene (**8h**, **8i** & **8j**) showed the least activities except for *p*-substituted. Overall **8a**, **8b**, **8c**, **8d**, **8h**, **8i** and **8p** executed a little low activity against some of bacterial strains. Among Gram-negative bacterial strains, **8p** was the most active against *S. typhi* with MIC value of 10.04 ± 1.25 µM relative to the reference standard, ciprofloxacin with MIC value of 9.13 ± 2.00 µM and **8e** against *E. coli* with MIC value of 9.45 ± 1.00 µM relative to 8.90 ± 1.65 µM. The potent activities of **8p** and **8e** may be due to 3,4-dimethoxy and 2-hydroxy benzylidenes respectively.

CONCLUSION

The relatively moderate MIC values indicate that the synthesized molecules possess potentials as antibacterial agents but there is need for further work to develop new drug candidates.

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