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# Antibacterial and Enzyme Inhibition Study of Hydrazone Derivatives Bearing 1, 3, 4-Oxadiazole

S. Rasool, *Aziz-ur-Rehman, M. A. Abbasi, S. Z. Siddiqi, A. S. Gondal, H. A. Noor, S. Sheral and [1]I. Ahmad
*Department of Chemistry, Government College University, Lahore-54000, Pakistan.
[1]Department of Pharmacy, The Islamia University of Bahawalpur, Bahawalpur-63100, Pakistan.
E-mail: azizryk@yahoo.com, rehman@gcu.edu.pk

## ABSTRACT
The antibacterial and lipoxygenase enzyme inhibition activities of two series of compounds have been investigated in the presented work. The 4-methyl/hydroxy benzoic acids (**1a** & **1b**) were used as starting materials to prepare corresponding esters (**2a** & **2b**), hydrazides (**3a** & **3b**), 5-(4-methylphenyl/4-hydroxyphenyl)-1,3,4-oxadiazol-2-thiols (**4a** & **4b**), *S*-substituted esters (**5a** & **5b**) and acetohydrazides (**6a** & **6b**). The acetohydrazones, **8a-i** & **9a-i**, were synthesized by stirring **6a** & **6b** with mono(di)substituted phenylcarboxaldehydes (**7a-i**) in methanol. The data of IR, $^1$H-NMR and EIMS spectral techniques well confirmed the structural formulae of synthesized compounds. The molecules of 4-methyl series rendered the better results than those of 4-hydroxy series.

**Keywords:** 1, 3, 4-Oxadiazole, carboxylic acids, antibacterial activity, lipoxygenase inhibition activity

## 1. INTRODUCTION
Substituted 1,3,4-oxadiazole[1,2] and acetohydrazone compounds[3,4] individually are known to confront multiple biological activities, that is, antimicrobial, anti-enzymatic, etc. The newly synthesized molecules were assessed for antibacterial and lipoxygenase inhibition. The included bacterial strains were *S. typhi*, *E. coli*, *P. aeruginosa*, *B. subtilis* and *S. aureus* which are responsible for enteric fever[5], food poisoning[6], chronic infection[7], hypersensitivity reactions[8] and pathogenesis[9]. The lipoxygenase enzymes (EC 1.13.11.12) are related to inflammatory drugs[10,11]. The multiple functionality bearing molecules are presented in the current article and such a type of molecules is under study by our group[12-14] looking forward to new drug candidates.

## 2. RESULTS AND DISCUSSION
The two series of acetohydrazone derivatives were accomplished by Scheme 1 and endorsed by spectral study of IR, $^1$HNMR & EIMS and the biological activities, given in Tables 2 to 4.

### 2.1 *Chemistry*
In the second step, carbohydrazide formation can be accompanied by stirring or refluxing if required. The formation of 1,3,4-oxadiazole was performed in basic medium, but the final product should be collected in slight acidic medium, and low pH (about 1-4) has negative effect on the yield. In the fifth step, acetohydrazide formation must be carried out at room temperature. The last step includes the reaction of acetohydrazones with different aldehydes stirring in methanol, which can be catalyzed by a few drops of glacial acetic acid (Scheme 1).
The molecules were structurally finalized through the collective data of spectral analysis of IR, $^1$HNMR and EIMS. The compound **8a** presented molecular formula of $C_{20}H_{21}N_5O_2S$ (mol. mass = 395), obtained from proton integration in $^1$HNMR spectrum and mass fragments based on peaks in EIMS. The significant fragments in EIMS spectrum appeared at *m/z* 395 (molecular ion peak), 233 (the 5-(4-methylphenyl)-1,3,4-oxadiazol-2-thiomethylcarbonyl cationic peak) and 190 (the *N'*-(4-(dimethylamino)benzylidenehydrazinocarbonyl cationic peak). The mass fragmentation pattern of this molecule is sketched in Figure 1. The IR supporting absorption band appeared at $\nu$ (cm$^{-1}$) 1663 (C=N) for imine group. The four doublets of two protons integrated with each other were assigned to two 1,4-disubstituted phenyl rings. The relative positions of these doublets were nominated as $\delta$ 7.88 (d, $J$ = 8.4 Hz, 2H, H-2' & H-6') and 7.22 (d, $J$ = 8.0 Hz, 2H, H-3' & H-5') for methyl substituted ring; and $\delta$ 7.49 (d, $J$ = 8.4 Hz, 2H, H-2''' & H-6''') and 6.64 (d, $J$ = 8.0 Hz, 2H, H-3''' & H-5''') for dimethylamino substituted ring. The two singlets at $\delta$ 2.91 (s, 6H, $(CH_3)_2$N-4''') and 2.37 (s, 3H, $CH_3$-4') resonated for dimethylamino group & methyl group with relative intensity of six & three protons. The one methine proton and two methylene protons resonated at $\delta$ 8.09 (s, 1H, H-7''') and 4.64 (s, 2H, H-2'') as singlets. Finally compound **8a** was written as *N'*-(4-(dimethylamino)benzylidene-2-(5-(4-methylphenyl)-1,3,4-Oxadiazol-2-ylthio)acetohydrazide.

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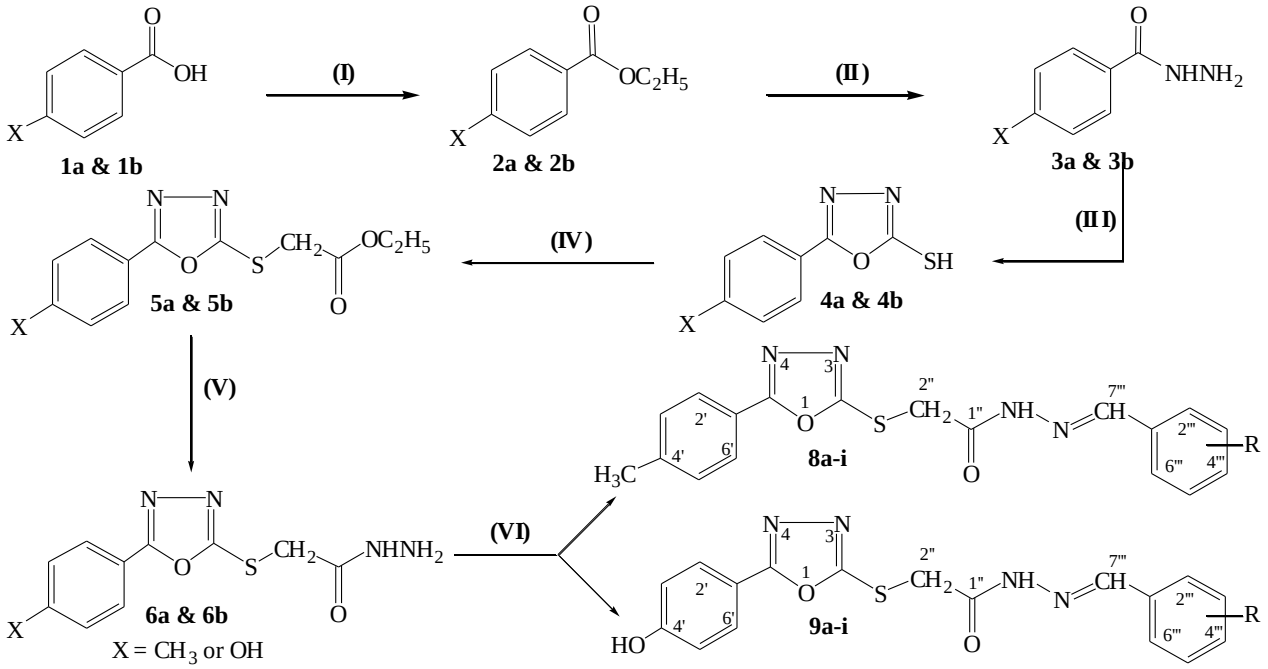


**Scheme-1:** Synthesis of *N'*-substituted-2-(5-(4-methyl/4-hydroxy phenyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (8a-i, 9a-i). Reagents and conditions: (I) EtOH, Conc. $H_2SO_4$, Reflux, 7-8 hours (II) 80% $N_2H_4.H_2O$, MeOH, Stir, 5-6 hours (III) $CS_2$, KOH, EtOH, Reflux, 6-7 hours (IV) EBA (ethyl bromoacetate), LiH, DMF, Stir, 4-5 hours (V) 80% $N_2H_4.H_2O$, MeOH, Stir, 3-4 hours (VI) mono(di)substituted phenylcarboxaldehydes (7a-i), MeOH, Stir, 2-4 hours.

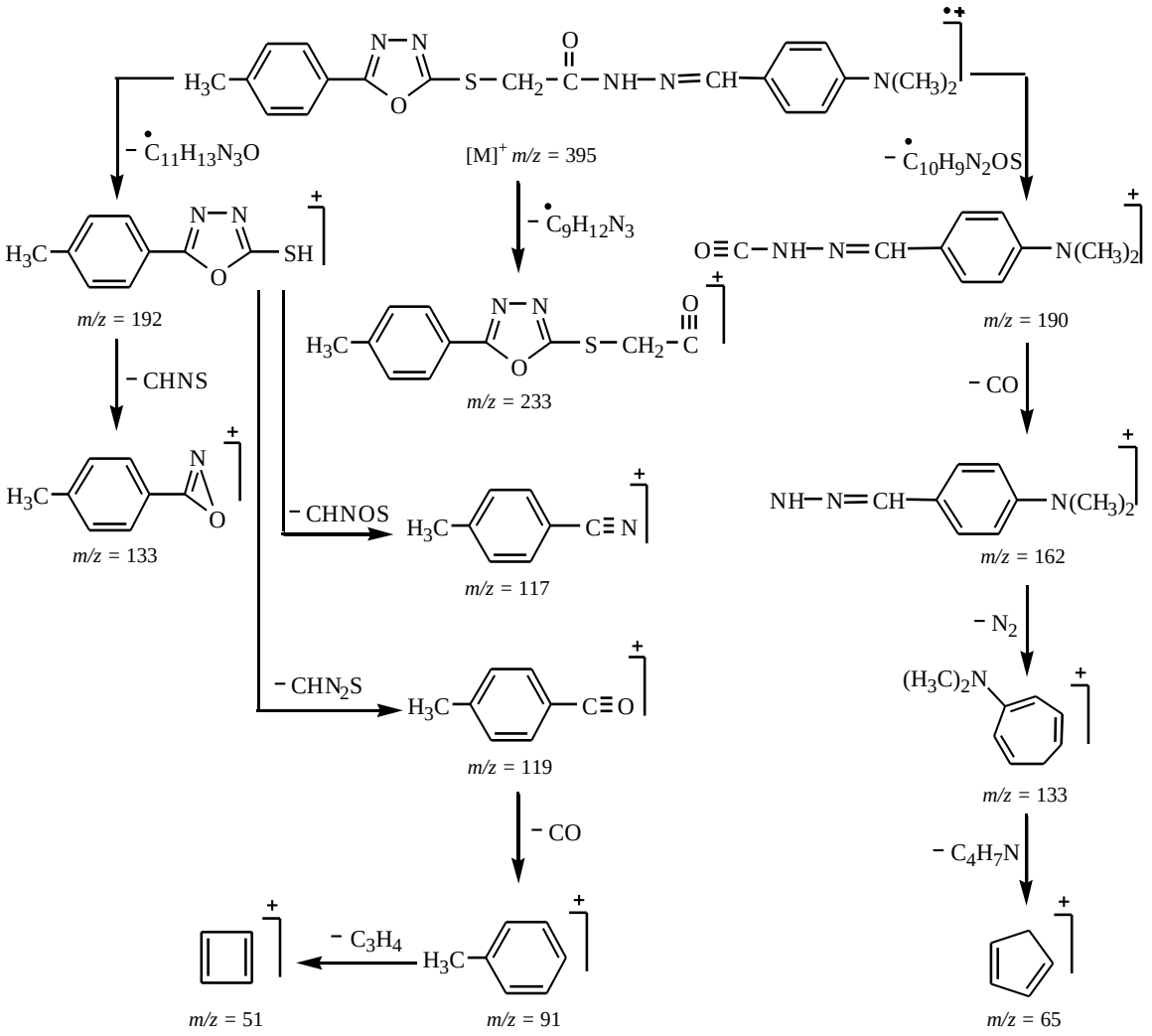


**Figure-1:** Mass fragmentation pattern of *N'*-(4-(Dimethylamino)benzylidene)-2-(5-(4-methylphenyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (8a)

## 2.2 *Antibacterial activity (in vitro)*

The antibacterial activity of the compounds has been checked out in comparison of ciprofloxacin, the routine drug. The results are tabulated as %age inhibition and minimum inhibitory concentration (MIC) values (Table 2 & 3).

**Table-1:** Different substituted R-groups

| Compound | -R | Compound | -R |
|---|---|---|---|
| 8a,9a | 4-$N(CH_3)_2$ | 8f,9f | 2-$OCH_3$, 5-$OCH_3$ |
| 8b,9b | 4-$N(C_2H_5)_2$ | 8g,9g | 3-$OCH_3$, 4-$OCH_3$ |
| 8c,9c | 4-$OCH_3$ | 8h,9h | 2-Cl, 4-Cl |
| 8d,9d | 2-$OCH_3$, 3-$OCH_3$ | 8i,9i | 2-Cl, 6-Cl |
| 8e,9e | 2-$OCH_3$, 4-$OCH_3$ | - | - |

**Table-2:** The %age inhibition for antibacterial activity

| Compound | Percentage Inhibition (%) | | | | |
|---|---|---|---|---|---|
| | Gram negative bacteria | | | Gram positive bacteria | |
| | ***S. typhi*** | ***E. coli*** | ***P. aeruginosa*** | ***B. subtilis*** | ***S. aureus*** |
| 8a | 30.0±2.1 | 42.6±1.0 | 28.4±0.9 | 27.2±2.1 | 70.8±1.5 |
| 8b | 41.5±2.1 | 47.0±0.1 | 50.2±0.4 | 22.2±1.5 | 80.0±0.7 |
| 8c | 64.2±0.8 | 68.2±0.1 | 71.0±0.2 | 58.3±1.4 | 84.0±0.9 |
| 8d | 56.3±0.6 | 54.5±0.8 | 50.6±0.6 | 57.7±0.7 | 46.8±1.0 |
| 8e | 51.3±1.6 | 62.5±0.6 | 61.1±0.5 | 40.9±0.4 | 83.3±0.3 |
| 8f | 75.8±0.6 | 67.8±1.5 | 72.7±1.6 | 61.6±1.0 | 79.4±0.4 |
| 8g | 40.7±1.4 | 64.1±1.4 | 51.5±1.0 | 45.1±1.6 | 79.9±1.3 |
| 8h | 25.6±1.6 | 51.7±0.1 | 40.8±1.5 | 42.6±0.5 | 50.5±0.8 |
| 8i | 49.5±0.3 | 54.6±1.6 | 56.3±1.2 | 27.7±1.0 | 57.0±1.4 |
| 9a | 53.8±0.6 | 42.8±0.5 | 51.8±0.4 | 61.5±0.9 | 65.0±0.6 |
| 9b | 36.6±0.9 | 37.6±0.2 | 37.6±0.2 | 45.8±0.4 | 30.5±0.5 |
| 9c | 45.1±0.1 | 38.6±0.8 | 33.8±0.6 | 40.5±0.4 | 51.8±0.5 |
| 9d | 46.4±1.6 | 33.0±1.1 | 47.2±0.4 | 49.9±0.5 | 61.2±0.5 |
| 9e | 63.6±0.0 | 54.5±1.2 | 69.3±1.0 | 66.0±0.6 | 67.1±0.9 |
| 9f | 76.4±1.2 | 70.4±0.3 | 58.5±0.2 | 51.8±0.8 | 70.6±0.9 |
| 9g | 71.7±1.1 | 64.4±1.2 | 73.3±0.9 | 63.0±1.2 | 42.45±0.6 |
| 9h | 57.0±0.0 | 54.4±0.9 | 55.3±0.5 | 65.6±0.9 | 67.8±0.7 |
| 9i | 49.5±1.0 | 58.6±0.8 | 47.2±0.3 | 58.6±0.3 | 42.1±0.7 |
| Ciprofloxacin | 92.1±0.5 | 91.4±0.8 | 91.0±0.1 | 91.2±1.1 | 92.2±1.0 |

The only two compounds of hydroxy series named **9e** and **9f** rendered relatively better inhibition potential against the encountered five bacterial strains. Overall the methyl series proved to be comparatively better inhibitor of *P aeruginosa* and *S. aureus* but hydroxy series for *B. subtilis* strain. Both of series remained more efficient against *P. aeruginosa* and *S. aureus* as compared to others. Among the weakly active compounds against *S. typhi*, **9g** bearing disubstitued 3,4-dimethoxybenzylidene moiety presented low MIC (µg/mL) of 10.0 ± 0.9 compared with 7.9 ± 0.1 of reference. The molecule, **9f** was proficient against *E. coli* with MIC of 10.4 ± 0.4 compared with 7.3 ± 0.6 of reference. Against *P. aeruginosa*, **8c** and **8f** presented low MICs of 10.9 ± 0.7 and 11.8 ± 0.4 respectively in comparison of 7.9 ± 0.1. *B. subtilis* was inhibited less efficiently by both the series. Against *S. aureus*, **8b**, **8e**, **8f** and **8g** showed low MICs. The molecules of 4-methyl series rendered the better results than those of 4-hydroxy series.

**Table-3:** The MIC values for antibacterial activity

| Compound | MIC (µg/mL) | | | | |
|---|---|---|---|---|---|
| | Gram negative bacteria | | | Gram positive bacteria | |
| | *S. typhi* | *E. coli* | *P. aeruginosa* | *B. subtilis* | *S. aureus* |
| 8a | - | - | - | - | 12.3±0.8 |
| 8b | - | - | 19.9±1.1 | - | 11.1±0.1 |
| 8c | 15.2±0.4 | 15.2±1.4 | 10.9±0.7 | - | 12.1±0.4 |
| 8d | 17.4±1.5 | 18.3±1.3 | 19.6±0.6 | - | - |
| 8e | 19.4+0.0 | 16.8±1.2 | 12.5±0.7 | - | 10.2±0.4 |
| 8f | 14.2±0.2 | 14.2±0.3 | 11.8±0.4 | - | 10.2±1.0 |
| 8g | - | 16.2±1.0 | 19.4±0.6 | - | 10.6±1.4 |
| 8h | - | - | - | 15.9±0.9 | 19.7±0.9 |
| 8i | - | 15.3±0.6 | 18.2±0.6 | - | 17.5±1.5 |
| 9a | 17.5±0.3 | - | 18.9±0.9 | 15.1±0.5 | 13.9±0.9 |
| 9b | - | - | - | - | - |
| 9c | - | - | - | - | 18.3±0.2 |
| 9d | - | - | - | - | 18.1±0.5 |

| | | | | | |
|---|---|---|---|---|---|
| 9e | 15.5±0.4 | 16.9±0.8 | 13.0±0.4 | 13.4±0.5 | 13.4±0.4 |
| 9f | 11.5±0.9 | 10.4±0.4 | 15.4±0.2 | 19.1±0.3 | 15.4±0.9 |
| 9g | 10.0±0.9 | 12.3±0.4 | 13.5±0.8 | 14.0±0.0 | - |
| 9h | 15.4±0.1 | 18.2±0.9 | 17.1±0.9 | 13.9±0.0 | 19.1±0.2 |
| 9i | - | 17.1±0.9 | - | 17.0±0.6 | - |
| Ciprofloxacin | 7.9±0.1 | 7.3±0.6 | 7.9±0.1 | 8.0±0.0 | 8.1±0.1 |

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.

### 2.3 *Enzyme inhibition activity (in vitro)*

The tabulated results of %age inhibition and $IC_{50}$ values (Table 4) for lipoxygenase inhibition relative to Baicalein, the reference standard, showed the compounds of both series remained almost inactive against this enzyme except a few.

**Table-4:** The $IC_{50}$ values of lipoxygenase inhibition activity

| Compound | LOX | | |
|---|---|---|---|
| | Conc. (mM) | Inhibition (%) | $IC_{50}$ (µM) |
| 8a | 0.5 | 54.6±0.1 | >500 |
| 8b | 0.5 | 90.2±0.8 | 288.7±1.5 |
| 8c | 0.5 | 84.6±0.5 | 132.8±0.7 |
| 8d | 0.5 | 52.1±0.6 | >500 |
| 8e | 0.5 | 38.8±0.2 | - |
| 8f | 0.5 | 15.7±0.1 | - |
| 8g | 0.5 | 34.3±0.1 | - |
| 8h | 0.5 | 56.2±0.1 | >500 |
| 8i | 0.5 | 74.6±0.4 | 290.3±1.2 |
| 9a | 0.5 | 28.3±0.1 | - |
| 9b | 0.5 | 53.3±0.1 | >500 |
| 9c | 0.5 | 46.2±0.1 | >500 |
| 9d | 0.5 | 47.3±0.4 | >500 |
| 9e | 0.5 | 10.1±0.1 | - |
| 9f | 0.5 | 32.2±0.1 | - |
| 9g | 0.5 | 28.5±0.1 | - |
| 9h | 0.5 | 70.9±0.9 | 269.6±0.7 |
| 9i | 0.5 | NIL | - |
| Baicalein | 0.5 | 93.7±1.2 | 22.4±1.3 |

NOTE: LOX = Lipoxygenase enzyme. $IC_{50}$ values (concentration for 50% inhibition) of compounds were recorded using EZ–Fit Enzyme kinetics software (Perella Scientific Inc. Amherst, USA).

Their large size, which might be unfit to the active site may attribute to their inactivity. Only compounds **8b**, **8c**, **8i** and **9h** showed weak inhibition potential. The best among these was **8c** with $IC_{50}$ of 132.8 ± 0.7 µM compared with 22.4 ± 1.3 µM of Baicalein.

## 3. CONCLUSION

The presented compounds can be subdivided into two series including 4-methylphenyl and 4-hydroxyphenyl, each incorporating nine molecules. The molecules presented varying activity from weak to moderate antibacterial activity but too much weak against lipoxygenase enzyme. The compounds of 4-methyl series were relatively better for their potential.

## 4. EXPERIMENTAL

### 4.1 *General*

Melting points (M.P.) were measured through Griffin-George apparatus with open capillary tube and were uncorrected. Spectral study included IR, recorded on Jasco-320-A spectrophotometer by KBr pellet method; $^1$HNMR, recorded on Bruker spectrometer in $CDCl_3$ at 400 MHz; and EIMS, recorded on JMS-HX-110 spectrometer. The initial purity of compounds was verified through TLC, performed on Al-plates coated with silica gel G-25-$UV_{254}$ using MeCOOEt and *n*-$C_6H_{14}$ solvent systems. The chemicals of synthetic grade were purchased from Merck, Alfa Aesar & Sigma-Aldrich and the solvents used were of analytical grade.

### 4.2 *Ethyl 4-methyl/4-hydroxy benzoate (2a & 2b) synthesis*

4-Methyl/4-hydroxy benzoic acids (**1a** & **1b**; 0.049 mol) were homogenized in 35 mL EtOH in a 100 mL round bottom (RB) flask and followed by addition of the catalyst, concentrated sulfuric acid (3.0 mL). The flask was set to reflux for 7-8 hours and monitored with TLC. After maximum conversion, reaction mixture was budged to a separating funnel (125 mL) followed by 50 mL ice cold distilled water and aqueous $Na_2CO_3$ solution (10%) to adjust a pH to 9-10. The title compounds were separated through solvent extraction with 25 mL $CHCl_3$.

**4.2.1** ***Ethyl 4-Methylbenzoate (2a)***
Yellow liquid; Yield: 84%; Mol. formula: $C_{10}H_{12}O_2$; Mol. mass: 164 gmol$^{-1}$; IR (KBr, $\nu_{max}$/cm$^{-1}$): 3123 (Ar C-H), 1734 (Ester C=O), 1595 (Ar C=C); $^1$H-NMR (400 MHz, $CDCl_3$, $\delta$/ppm): 7.87 (d, $J$ = 8.0 Hz, 2H, H-2' & H-6'), 7.29 (d, $J$ = 8.0 Hz, 2H, H-3' & H-5'), 4.02 (q, $J$ = 7.2 Hz, 2H, -O$\underline{CH_2}$CH$_3$), 2.42 (s, 3H, $CH_3$-4'), 1.01 (t, $J$ = 7.2 Hz, 3H, -OCH$_2\underline{CH_3}$); EIMS ($m/z$): 164 [M]$^+$, 119 [$C_8H_7O$]$^+$, 91 [$C_7H_7$]$^+$, 65 [$C_5H_5$]$^+$.

**4.2.2** ***Ethyl 4-Hydroxybenzoate (2b)***
White amorphous solid; Yield: 87%; M.P.: 144-116 $^o$C; Mol. formula: $C_9H_{10}O_3$; Mol. mass: 166 gmol$^{-1}$; IR (KBr, $\nu_{max}$/cm$^{-1}$): 3412 (O-H), 3107 (Ar C-H), 1738 (Ester C=O), 1596 (Ar C=C); $^1$H-NMR (400 MHz, $CDCl_3$, $\delta$/ppm): 7.85 (d, $J$ = 8.4 Hz, 2H, H-2' & H-6'), 6.89 (d, $J$ = 8.4 Hz, 2H, H-3' & H-5'), 4.07 (q, $J$ = 7.6 Hz, 2H, -O$\underline{CH_2}$CH$_3$), 1.02 (t, $J$ = 7.6 Hz, 3H, -OCH$_2\underline{CH_3}$); EIMS ($m/z$): 166 [M]$^+$, 121 [$C_7H_5O_2$]$^+$, 93 [$C_6H_5O$]$^+$, 67 [$C_4H_3O$]$^+$.

**4.3** ***4-Methyl/4-Hydroxy benzohydrazide (3a & 3b) synthesis***
The compounds **2a** & **2b** (0.043 mol) were taken in a 100 mL RB flask, already containing 25 mL MeOH. 80% Hydrazine hydrate (0.043 mol) was instantly poured and stirred for 5-6 hours. After all starting material consumed, 50 mL ice cold distilled water was poured followed by gentle shaking to acquire the precipitates of **3a** & **3b**. These were collected through filtration, washed and dried.

**4.3.1** ***4-Methylbenzohydrazide (3a)***
Cream white amorphous solid; Yield: 88%; M.P.: 116-118 $^o$C; Mol. formula: $C_8H_{10}N_2O$; Mol. mass: 150 gmol$^{-1}$; IR (KBr, $\nu_{max}$/cm$^{-1}$): 3337 (N-H), 3119 (Ar C-H), 1662 (Amide C=O), 1596 (Ar C=C); $^1$H-NMR (400 MHz, $CDCl_3$, $\delta$/ppm): 9.39 (s, 1H, CONH), 8.68 (s, 2H, N-H), 7.86 (d, $J$ = 8.4 Hz, 2H, H-2' & H-6'), 7.26 (d, $J$ = 8.4 Hz, 2H, H-3' & H-5'), 2.41 (s, 3H, $CH_3$-4'); EIMS ($m/z$): 150 [M]$^+$, 119 [$C_8H_7O$]$^+$, 91 [$C_7H_7$]$^+$, 65 [$C_5H_5$]$^+$.

**4.3.2** ***4-Hydroxybenzohydrazide (3b)***
White amorphous solid; Yield: 86%; M.P.: 264-266 $^o$C; Mol. formula: $C_7H_8N_2O_2$; Mol. mass: 152 gmol$^{-1}$; IR (KBr, $\nu_{max}$/cm$^{-1}$): 3387 (O-H), 3321 (N-H), 3108 (Ar C-H), 1667 (Amide C=O), 1606 (Ar C=C); $^1$H-NMR (400 MHz, $CDCl_3$, $\delta$/ppm): 9.31 (s, 1H, CONH), 8.72 (s, 2H, N-H), 7.89 (d, $J$ = 8.0 Hz, 2H, H-2' & H-6'), 6.83 (d, $J$ = 8.4 Hz, 2H, H-3' & H-5'); EIMS ($m/z$): 152 [M]$^+$, 121 [$C_7H_5O_2$]$^+$, 93 [$C_6H_5O$]$^+$, 67 [$C_4H_3O$]$^+$.

**4.4** ***5-(4-Methyl/4-Hydroxy phenyl)-1,3,4-oxadiazol-2-thiol (4a & 4b) synthesis***
The compounds, **3a** & **3b** (0.040 mol) were homogenized in 25 mL EtOH in a 100 mL RB flask and basified by solid KOH (0.040 mol) on reflux. The system was cooled to RT and then 0.080 mol $CS_2$ was poured into. After reflux of 6-7 hours, 50 mL cold distilled water was poured into followed by dilute HCl (3-4 mL, pH = 6-7), and the mixture was stirred for 0.25 hours for proper precipitation. Thus obtained products were filtered off, washed, and dried.

**4.4.1** ***5-(4-Methylphenyl)-1,3,4-oxadiazol-2-thiol (4a)***
White amorphous solid; Yield: 78%; M.P.: 172-174 $^o$C; Mol. formula: $C_9H_8N_2OS$; Mol. mass: 192 gmol$^{-1}$; IR (KBr, $\nu_{max}$/cm$^{-1}$): 2548 (S-H), 3137 (Ar C-H), 1667 (C=N), 1609 (Ar C=C); $^1$H-NMR (400 MHz, $CDCl_3$, $\delta$/ppm): 7.89 (d, $J$ = 8.4 Hz, 2H, H-2' & H-6'), 7.23 (d, $J$ = 8.4 Hz, 2H, H-3' & H-5'), 2.42 (s, 3H, $CH_3$-4'); EIMS ($m/z$): 192 [M]$^+$, 133 [$C_8H_7NO$]$^+$, 119 [$C_8H_7O$]$^+$, 117 [$C_8H_7N$]$^+$, 91 [$C_7H_7$]$^+$, 65 [$C_5H_5$]$^+$.

**4.4.2** ***5-(4-Hydroxyphenyl)-1,3,4-oxadiazol-2-thiol (4b)***
White amorphous solid; Yield: 83%; M.P.: 176-178 $^o$C; Mol. formula: $C_8H_6N_2O_2S$; Mol. mass: 194 gmol$^{-1}$; IR (KBr, $\nu_{max}$/cm$^{-1}$): 3398 (O-H), 3133 (Ar C-H), 1661 (C=N), 1599 (Ar C=C); $^1$H-NMR (400 MHz, $CDCl_3$, $\delta$/ppm): 7.88 (d, $J$ = 8.4 Hz, 2H, H-2' & H-6'), 6.63 (d, $J$ = 8.4 Hz, 2H, H-3' & H-5'); EIMS ($m/z$): 194 [M]$^+$, 135 [$C_7H_5NO_2$]$^+$, 121 [$C_7H_5O_2$]$^+$, 119 [$C_7H_5NO$]$^+$, 93 [$C_6H_5O$]$^+$, 67 [$C_4H_3O$]$^+$.

**4.5** ***Ethyl 2-(5-(4-methyl/4-hydroxy phenyl)-1,3,4-oxadiazol-2-ylthio)acetate (5a & 5b) synthesis***
The compounds, **4a** & **4b**, (0.035 mol) were dissolved in 15 mL dimethylformamide (DMF) in a 100 mL RB flask and stirred for 0.5 hours with LiH (0.035 mol). Then ethyl 2-bromoacetate (EBA, 0.035 mol) was added and mixture was further stirred for 4-5 hours. After single spot on TLC, the products were made precipitate after addition of excess cold distilled water and removed from medium by filtration, washing and drying.

**4.5.1** ***Ethyl 2-(5-(4-Methylphenyl)-1,3,4-oxadiazol-2-ylthio)acetate (5a)***
White amorphous solid; Yield: 79%; M.P.: 166-168 $^o$C; Mol. formula: $C_{13}H_{14}N_2O_3S$; Mol. mass: 278 gmol$^{-1}$; IR (KBr, $\nu_{max}$/cm$^{-1}$): 3159 (Ar C-H), 1751 (Ester C=O), 1666 (C=N), 1604 (Ar C=C); $^1$H-NMR (400 MHz, $CDCl_3$, $\delta$/ppm): 7.84 (d, $J$ = 8.4 Hz, 2H, H-2' & H-6'), 7.29 (d, $J$ = 8.0 Hz, 2H, H-3' & H-5'), 4.61 (s, 2H, H-2''), 3.93 (q, $J$ = 7.2 Hz, 2H, -O$\underline{CH_2}$CH$_3$), 2.41 (s, 3H, $CH_3$-4'), 0.99 (t, $J$ = 7.2 Hz, 3H, -OCH$_2\underline{CH_3}$); EIMS ($m/z$): 278 [M]$^+$, 233 [$C_{11}H_9N_2O_2S$]$^+$, 192 [$C_9H_8N_2OS$]$^+$, 133 [$C_8H_7NO$]$^+$, 119 [$C_8H_7O$]$^+$, 117 [$C_8H_7N$]$^+$, 91 [$C_7H_7$]$^+$, 65 [$C_5H_5$]$^+$.

**4.5.2** ***Ethyl 2-(5-(4-Hydroxyphenyl)-1,3,4-oxadiazol-2-ylthio)acetate (5b)***

White amorphous solid; Yield: 76%; M.P.: 180-182 $^{o}$C; Mol. formula: $C_{12}H_{12}N_2O_4S$; Mol. mass: 280 gmol$^{-1}$; IR (KBr, $\nu_{max}$/cm$^{-1}$): 3386 (O-H), 3153 (Ar C-H), 1752 (Ester C=O), 1676 (C=N), 1601 (Ar C=C); $^{1}$H-NMR (400 MHz, $CDCl_3$, $\delta$/ppm): 7.86 (d, $J$ = 8.4 Hz, 2H, H-2' & H-6'), 6.69 (d, $J$ = 8.0 Hz, 2H, H-3' & H-5'), 4.62 (s, 2H, H-2"), 3.90 (q, $J$ = 7.6 Hz, 2H, -O$\underline{CH_2}CH_3$), 0.98 (t, $J$ = 7.6 Hz, 3H, -OCH$_2\underline{CH_3}$); EIMS (*m/z*): 280 $[M]^+$, 235 $[C_{10}H_7N_2O_3S]^+$, 194 $[C_8H_6N_2O_2S]^+$, 135 $[C_7H_5NO_2]^+$, 121 $[C_7H_5O_2]^+$, 119 $[C_7H_5NO]^+$, 93 $[C_6H_5O]^+$, 67 $[C_4H_3O]^+$.

**4.6** ***2-(5-(4-Methyl/4-Hydroxy phenyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (6a & 6b) synthesis***

The compounds **5a** & **5b** (0.031 mol) were mixed with 80% hydrazine hydrate (0.031 mol) in a 100 mL RB flask containing 20 mL MeOH. The reaction mixture was stirred for 3-4 hours at RT. After final TLC with single spot, the precipitates appeared after addition of excess cold distilled water, were filtered and washed by distilled water.

**4.6.1** ***2-(5-(4-Methylphenyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (6a)***

White amorphous solid; Yield: 85%; M.P.: 176-178 $^{o}$C; Mol. formula: $C_{11}H_{12}N_4O_2S$; Mol. mass: 264 gmol$^{-1}$; IR (KBr, $\nu_{max}$/cm$^{-1}$): 3376 (N-H), 3073 (Ar C-H), 1663 (Amide C=O), 1681 (C=N), 1602 (Ar C=C); $^{1}$H-NMR (400 MHz, $CDCl_3$, $\delta$/ppm): 9.41 (s, 1H, CONH), 8.77 (s, 2H, N-H), 7.87 (d, $J$ = 8.0 Hz, 2H, H-2' & H-6'), 7.27 (d, $J$ = 8.0 Hz, 2H, H-3' & H-5'), 4.65 (s, 2H, H-2"), 2.41 (s, 3H, $CH_3$-4'); EIMS (*m/z*): 264 $[M]^+$, 233 $[C_{11}H_9N_2O_2S]^+$, 192 $[C_9H_8N_2OS]^+$, 133 $[C_8H_7NO]^+$, 119 $[C_8H_7O]^+$, 117 $[C_8H_7N]^+$, 91 $[C_7H_7]^+$, 65 $[C_5H_5]^+$.

**4.6.2** ***2-(5-(4-Hydroxyphenyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (6b)***

White amorphous solid; Yield: 81%; M.P.: 208-210 $^{o}$C; Mol. formula: $C_{10}H_{10}N_4O_3S$; Mol. mass: 266 gmol$^{-1}$; IR (KBr, $\nu_{max}$/cm$^{-1}$): 3412 (O-H), 3372 (N-H), 3075 (Ar C-H), 1665 (Amide C=O), 1687 (C=N), 1604 (Ar C=C); $^{1}$H-NMR (400 MHz, $CDCl_3$, $\delta$/ppm): 9.47 (s, 1H, CONH), 8.83 (s, 2H, N-H), 7.87 (d, $J$ = 8.0 Hz, 2H, H-2' & H-6'), 6.67 (d, $J$ = 8.0 Hz, 2H, H-3' & H-5'), 4.64 (s, 2H, H-2"); EIMS (*m/z*): 266 $[M]^+$, 235 $[C_{10}H_7N_2O_3S]^+$, 194 $[C_8H_6N_2O_2S]^+$, 135 $[C_7H_5NO_2]^+$, 121 $[C_7H_5O_2]^+$, 119 $[C_7H_5NO]^+$, 93 $[C_6H_5O]^+$, 67 $[C_4H_3O]^+$.

**4.7** ***N'-Substituted-2-(5-(4-methyl/4-hydroxy phenyl)-1,3,4-oxadiazol-2-ylthio)acetohydrazide (8a-i, 9a-i) synthesis***

The compounds, **6a** & **6b** (0.0036 mol) were mixed with mono(di)substituted phenylcarboxaldehydes (**7a-i**; 0.0036 mol) in a 50 mL RB flask containing 12 mL MeOH. The mixture was simply stirred for 2-4 hours. After reaction completion affirmed *via* TLC, excess cold distilled water was added and aged for precipitate formation. The formed precipitates were filtered off, washed with distilled water, and dried for biological activities.

**4.7.1** ***N'-(4-(Dimethylamino)benzylidene)-2-(5-(4-methylphenyl)-1,3,4-oxadiazol-2-ylthio)aceto hydrazide (8a)***

Yellow amorphous solid; Yield: 81%; M.P.: 182-184 $^{o}$C; Mol. formula: $C_{20}H_{21}N_5O_2S$; Mol. mass: 395 gmol$^{-1}$; IR (KBr, $\nu_{max}$/cm$^{-1}$): 3046 (Ar C-H), 1663 (C=N), 1607 (Ar C=C); $^{1}$H-NMR (400 MHz, $CDCl_3$, $\delta$/ppm): 10.45 (s, 1H, CONH), 8.09 (s, 1H, H-7'"), 7.88 (d, $J$ = 8.4 Hz, 2H, H-2' & H-6'), 7.49 (d, $J$ = 8.4 Hz, 2H, H-2'" & H-6'"), 7.22 (d, $J$ = 8.0 Hz, 2H, H-3' & H-5'), 6.64 (d, $J$ = 8.0 Hz, 2H, H-3'" & H-5'"), 4.64 (s, 2H, H-2"), 2.91 (s, 6H, $(CH_3)_2$N-4'"), 2.37 (s, 3H, $CH_3$-4'); EIMS (*m/z*): 395 $[M]^+$, 233 $[C_{11}H_9N_2O_2S]^+$, 192 $[C_9H_8N_2OS]^+$, 190 $[C_{10}H_{12}N_3O]^+$, 162 $[C_9H_{12}N_3]^+$, 134 $[C_9H_{12}N]^+$, 133 $[C_8H_7NO]^+$, 119 $[C_8H_7O]^+$, 117 $[C_8H_7N]^+$, 91 $[C_7H_7]^+$, 65 $[C_5H_5]^+$, 51 $[C_4H_3]^+$.

**4.7.2** ***N'-(4-(Diethylamino)benzylidene)-2-(5-(4-methylphenyl)-1,3,4-oxadiazol-2-ylthio)aceto hydrazide (8b)***

Light yellow amorphous solid; Yield: 82%; M.P.: 192-194 $^{o}$C; Mol. formula: $C_{22}H_{25}N_5O_2S$; Mol. mass: 423 gmol$^{-1}$; IR (KBr, $\nu_{max}$/cm$^{-1}$): 3049 (Ar C-H), 1669 (C=N), 1604 (Ar C=C); $^{1}$H-NMR (400 MHz, $CDCl_3$, $\delta$/ppm): 10.53 (s, 1H, CONH), 8.06 (s, 1H, H-7'"), 7.86 (d, $J$ = 8.0 Hz, 2H, H-2' & H-6'), 7.45 (d, $J$ = 8.0 Hz, 2H, H-2'" & H-6'"), 7.25 (d, $J$ = 8.0 Hz, 2H, H-3' & H-5'), 6.69 (d, $J$ = 8.0 Hz, 2H, H-3'" & H-5'"), 4.61 (s, 2H, H-2"), 2.63 (q, $J$ = 7.2 Hz, 4H, $(CH_3\underline{CH_2})_2$N-4'"), 2.39 (s, 3H, $CH_3$-4'), 1.04 (t, $J$ = 7.2 Hz, 6H, $(\underline{CH_3}CH_2)_2$N-4'"); EIMS (*m/z*): 423 $[M]^+$, 233 $[C_{11}H_9N_2O_2S]^+$, 218 $[C_{12}H_{16}N_3O]^+$, 192 $[C_9H_8N_2OS]^+$, 190 $[C_{11}H_{16}N_3]^+$, 162 $[C_{11}H_{16}N]^+$, 133 $[C_8H_7NO]^+$, 119 $[C_8H_7O]^+$, 117 $[C_8H_7N]^+$, 91 $[C_7H_7]^+$, 65 $[C_5H_5]^+$, 51 $[C_4H_3]^+$.

**4.7.3** ***N'-(4-Methoxybenzylidene)-2-(5-(4-methylphenyl)-1,3,4-oxadiazol-2-ylthio)aceto hydrazide (8c)***

White amorphous solid; Yield: 79%; M.P.: 202-204 $^{o}$C; Mol. formula: $C_{19}H_{18}N_4O_3S$; Mol. mass: 382 gmol$^{-1}$; IR (KBr, $\nu_{max}$/cm$^{-1}$): 3054 (Ar C-H), 1675 (C=N), 1617 (Ar C=C); $^{1}$H-NMR (400 MHz, $CDCl_3$, $\delta$/ppm): 10.73 (s, 1H, CONH), 8.08 (s, 1H, H-7'"), 7.85 (d, $J$ = 8.0 Hz, 2H, H-2' & H-6'), 7.73 (d, $J$ = 8.4 Hz, 2H, H-2'" & H-6'"), 7.28 (d, $J$ = 8.4 Hz, 2H, H-3' & H-5'), 6.57 (d, $J$ = 8.4 Hz, 2H, H-3'" & H-5'"), 4.63 (s, 2H, H-2"), 3.81 (s, 3H, $CH_3O$-4'"), 2.42 (s, 3H, $CH_3$-4'); EIMS (*m/z*): 382 $[M]^+$, 233 $[C_{11}H_9N_2O_2S]^+$, 192 $[C_9H_8N_2OS]^+$, 177 $[C_9H_9N_2O_2]^+$, 149 $[C_8H_9N_2O]^+$, 133 $[C_8H_7NO]^+$, 121 $[C_8H_9O]^+$, 119 $[C_8H_7O]^+$, 117 $[C_8H_7N]^+$, 91 $[C_7H_7]^+$, 65 $[C_5H_5]^+$, 51 $[C_4H_3]^+$.

**4.7.4** ***N'-(2,3-Dimethoxybenzylidene)-2-(5-(4-methylphenyl)-1,3,4-oxadiazol-2-ylthio)aceto hydrazide (8d)***
White amorphous solid; Yield: 85%; M.P.: 198-200 $^{o}$C; Mol. formula: $C_{20}H_{20}N_4O_4S$; Mol. mass: 412 gmol$^{-1}$; IR (KBr, $\nu_{max}$/cm$^{-1}$): 3067 (Ar C-H), 1658 (C=N), 1594 (Ar C=C); $^{1}$H-NMR (400 MHz, $CDCl_3$, $\delta$/ppm): 10.74 (s, 1H, CONH), 8.19 (s, 1H, H-7'''), 7.89 (d, *J* = 8.4 Hz, 2H, H-2' & H-6'), 7.56 (d, *J* = 8.4 Hz, 1H, H-6'''), 7.44 (dd, *J* = 8.4, 1.2 Hz, 1H, H-4'''), 7.22 (d, *J* = 8.4 Hz, 2H, H-3' & H-5'), 7.14 (t, *J* = 8.4 Hz, 1H, H-5'''), 4.63 (s, 2H, H-2''), 3.82 (s, 3H, $CH_3$O-3'''), 3.80 (s, 3H, $CH_3$O-2'''), 2.40 (s, 3H, $CH_3$-4'); EIMS (*m/z*): 412 $[M]^+$, 233 $[C_{11}H_9N_2O_2S]^+$, 207 $[C_{10}H_{11}N_2O_3]^+$, 192 $[C_9H_8N_2OS]^+$, 179 $[C_9H_{11}N_2O_2]^+$, 151 $[C_9H_{11}O_2]^+$, 133 $[C_8H_7NO]^+$, 119 $[C_8H_7O]^+$, 117 $[C_8H_7N]^+$, 91 $[C_7H_7]^+$, 65 $[C_5H_5]^+$, 51 $[C_4H_3]^+$.

**4.7.5** ***N'-(2,4-Dimethoxybenzylidene)-2-(5-(4-methylphenyl)-1,3,4-oxadiazol-2-ylthio)aceto hydrazide (8e)***
White amorphous solid; Yield: 80%; M.P.: 214-216 $^{o}$C; Mol. formula: $C_{20}H_{20}N_4O_4S$; Mol. mass: 412 gmol$^{-1}$; IR (KBr, $\nu_{max}$/cm$^{-1}$): 3064 (Ar C-H), 1649 (C=N), 1609 (Ar C=C); $^{1}$H-NMR (400 MHz, $CDCl_3$, $\delta$/ppm): 10.63 (s, 1H, CONH), 8.16 (s, 1H, H-7'''), 7.86 (d, *J* = 8.4 Hz, 2H, H-2' & H-6'), 7.75 (d, *J* = 8.0 Hz, 1H, H-6'''), 7.29 (d, *J* = 8.4 Hz, 2H, H-3' & H-5'), 6.63 (d, *J* = 2.4 Hz, 1H, H-3'''), 6.54 (dd, *J* = 8.0, 2.4 Hz, 1H, H-5'''), 4.64 (s, 2H, H-2''), 3.83 (s, 3H, $CH_3$O-2'''), 3.82 (s, 3H, $CH_3$O-4'''), 2.43 (s, 3H, $CH_3$-4'); EIMS (*m/z*): 412 $[M]^+$, 233 $[C_{11}H_9N_2O_2S]^+$, 207 $[C_{10}H_{11}N_2O_3]^+$, 192 $[C_9H_8N_2OS]^+$, 179 $[C_9H_{11}N_2O_2]^+$, 151 $[C_9H_{11}O_2]^+$, 133 $[C_8H_7NO]^+$, 119 $[C_8H_7O]^+$, 117 $[C_8H_7N]^+$, 91 $[C_7H_7]^+$, 65 $[C_5H_5]^+$, 51 $[C_4H_3]^+$.

**4.7.6** ***N'-(2,5-Dimethoxybenzylidene)-2-(5-(4-methylphenyl)-1,3,4-oxadiazol-2-ylthio)aceto hydrazide (8f)***
White amorphous solid; Yield: 83%; M.P.: 222-224 $^{o}$C; Mol. formula: $C_{20}H_{20}N_4O_4S$; Mol. mass: 412 gmol$^{-1}$; IR (KBr, $\nu_{max}$/cm$^{-1}$): 3084 (Ar C-H), 1654 (C=N), 1593 (Ar C=C); $^{1}$H-NMR (400 MHz, $CDCl_3$, $\delta$/ppm): 10.42 (s, 1H, CONH), 8.18 (s, 1H, H-7'''), 7.86 (d, *J* = 8.0 Hz, 2H, H-2' & H-6'), 7.31 (d, *J* = 3.2 Hz, 1H, H-6'''), 7.28 (d, *J* = 8.0 Hz, 2H, H-3' & H-5'), 7.11 (dd, *J* = 9.2, 3.2 Hz, 1H, H-4'''), 6.92 (d, *J* = 9.2 Hz, 1H, H-3'''), 4.59 (s, 2H, H-2''), 3.87 (s, 3H, $CH_3$O-5'''), 3.78 (s, 3H, $CH_3$O-2'''), 2.40 (s, 3H, $CH_3$-4'); EIMS (*m/z*): 412 $[M]^+$, 233 $[C_{11}H_9N_2O_2S]^+$, 207 $[C_{10}H_{11}N_2O_3]^+$, 192 $[C_9H_8N_2OS]^+$, 179 $[C_9H_{11}N_2O_2]^+$, 151 $[C_9H_{11}O_2]^+$, 133 $[C_8H_7NO]^+$, 119 $[C_8H_7O]^+$, 117 $[C_8H_7N]^+$, 91 $[C_7H_7]^+$, 65 $[C_5H_5]^+$, 51 $[C_4H_3]^+$.

**4.7.7** ***N'-(3,4-Dimethoxybenzylidene)-2-(5-(4-methylphenyl)-1,3,4-oxadiazol-2-ylthio)aceto hydrazide (8g)***
White amorphous solid; Yield: 89%; M.P.: 228-230 $^{o}$C; Mol. formula: $C_{20}H_{20}N_4O_4S$; Mol. mass: 412 gmol$^{-1}$; IR (KBr, $\nu_{max}$/cm$^{-1}$): 3078 (Ar C-H), 1659 (C=N), 1599 (Ar C=C); $^{1}$H-NMR (400 MHz, $CDCl_3$, $\delta$/ppm): 10.66 (s, 1H, CONH), 8.17 (s, 1H, H-7'''), 7.86 (d, *J* = 8.0 Hz, 2H, H-2' & H-6'), 7.35 (d, *J* = 1.6 Hz, 1H, H-2'''), 7.29 (d, *J* = 8.0 Hz, 2H, H-3' & H-5'), 7.19 (dd, *J* = 8.4, 1.6 Hz, 1H, H-6'''), 6.92 (d, *J* = 8.4 Hz, 1H, H-5'''), 4.62 (s, 2H, H-2''), 3.81 (s, 3H, $CH_3$O-3'''), 3.80 (s, 3H, $CH_3$O-4'''), 2.40 (s, 3H, $CH_3$-4'); EIMS (*m/z*): 412 $[M]^+$, 233 $[C_{11}H_9N_2O_2S]^+$, 207 $[C_{10}H_{11}N_2O_3]^+$, 192 $[C_9H_8N_2OS]^+$, 179 $[C_9H_{11}N_2O_2]^+$, 151 $[C_9H_{11}O_2]^+$, 133 $[C_8H_7NO]^+$, 119 $[C_8H_7O]^+$, 117 $[C_8H_7N]^+$, 91 $[C_7H_7]^+$, 65 $[C_5H_5]^+$, 51 $[C_4H_3]^+$.

**4.7.8** ***N'-(2,4-Dichlorobenzylidene)-2-(5-(4-methylphenyl)-1,3,4-oxadiazol-2-ylthio)aceto hydrazide (8h)***
White amorphous solid; Yield: 81%; M.P.: 218-220 $^{o}$C; Mol. formula: $C_{18}H_{14}Cl_2N_4O_2S$; Mol. mass: 420 gmol$^{-1}$; IR (KBr, $\nu_{max}$/cm$^{-1}$): 3076 (Ar C-H), 1653 (C=N), 1596 (Ar C=C), 702 (C-Cl); $^{1}$H-NMR (400 MHz, $CDCl_3$, $\delta$/ppm): 10.61 (s, 1H, CONH), 8.41 (s, 1H, H-7'''), 7.88 (d, *J* = 8.4 Hz, 2H, H-2' & H-6'), 7.58 (d, *J* = 8.0 Hz, 1H, H-6'''), 7.43 (dd, *J* = 8.0, 1.2 Hz, 1H, H-5'''), 7.34 (d, *J* = 1.2 Hz, 1H, H-3'''), 7.29 (d, *J* = 8.0 Hz, 2H, H-3' & H-5'), 4.63 (s, 2H, H-2''), 2.39 (s, 3H, $CH_3$-4'); EIMS (*m/z*): 424 $[M+4]^+$, 422 $[M+2]^+$, 420 $[M]^+$, 233 $[C_{11}H_9N_2O_2S]^+$, 215 $[C_8H_5Cl_2N_2O]^+$, 192 $[C_9H_8N_2OS]^+$, 187 $[C_7H_5Cl_2N_2]^+$, 159 $[C_7H_5Cl_2]^+$, 133 $[C_8H_7NO]^+$, 119 $[C_8H_7O]^+$, 117 $[C_8H_7N]^+$, 91 $[C_7H_7]^+$, 65 $[C_5H_5]^+$, 51 $[C_4H_3]^+$.

**4.7.9** ***N'-(2,6-Dichlorobenzylidene)-2-(5-(4-methylphenyl)-1,3,4-oxadiazol-2-ylthio)aceto hydrazide (8i)***
White amorphous solid; Yield: 80%; M.P.: 206-208 $^{o}$C; Mol. formula: $C_{18}H_{14}Cl_2N_4O_2S$; Mol. mass: 420 gmol$^{-1}$; IR (KBr, $\nu_{max}$/cm$^{-1}$): 3071 (Ar C-H), 1664 (C=N), 1601 (Ar C=C), 707 (C-Cl); $^{1}$H-NMR (400 MHz, $CDCl_3$, $\delta$/ppm): 10.57 (s, 1H, CONH), 8.42 (s, 1H, H-7'''), 7.86 (d, *J* = 8.4 Hz, 2H, H-2' & H-6'), 7.54 (d, *J* = 8.4 Hz, 2H, H-3''' & H-5'''), 7.42 (t, *J* = 8.4 Hz, 1H, H-4'''), 7.23 (d, *J* = 8.4 Hz, 2H, H-3' & H-5'), 4.64 (s, 2H, H-2''), 2.38 (s, 3H, $CH_3$-4'); EIMS (*m/z*): 424 $[M+4]^+$, 422 $[M+2]^+$, 420 $[M]^+$, 233 $[C_{11}H_9N_2O_2S]^+$, 215 $[C_8H_5Cl_2N_2O]^+$, 192 $[C_9H_8N_2OS]^+$, 187 $[C_7H_5Cl_2N_2]^+$, 159 $[C_7H_5Cl_2]^+$, 133 $[C_8H_7NO]^+$, 119 $[C_8H_7O]^+$, 117 $[C_8H_7N]^+$, 91 $[C_7H_7]^+$, 65 $[C_5H_5]^+$, 51 $[C_4H_3]^+$.

**4.7.10** ***N'-(4-(Dimethylamino)benzylidene)-2-(5-(4-hydroxyphenyl)-1,3,4-oxadiazol-2-ylthio) acetohydrazide (9a)***
Light orange amorphous solid; Yield: 79%; M.P.: 212-214 $^{o}$C; Mol. formula: $C_{19}H_{19}N_5O_3S$; Mol. mass: 397 gmol$^{-1}$; IR (KBr, $\nu_{max}$/cm$^{-1}$): 3418 (O-H), 3039 (Ar C-H), 1673 (C=N), 1597 (Ar C=C); $^{1}$H-NMR (400 MHz, $CDCl_3$, $\delta$/ppm): 11.45 (s, 1H, CONH), 8.05 (s, 1H, H-7'''), 7.88 (d, *J* = 8.0 Hz, 2H, H-2' & H-6'), 7.46 (d, *J* = 8.4 Hz, 2H, H-2''' & H-6'''), 6.73 (d, *J* = 8.4 Hz, 2H, H-3' & H-5'), 6.67 (d, *J* = 8.0 Hz, 2H, H-3''' & H-5'''), 4.61 (s, 2H, H-2''), 2.93 (s, 6H,

$(CH_3)_2$N-4'''); EIMS (*m/z*): 397 $[M]^+$, 235 $[C_{10}H_7N_2O_3S]^+$, 194 $[C_8H_6N_2O_2S]^+$, 190 $[C_{10}H_{12}N_3O]^+$, 162 $[C_9H_{12}N_3]^+$, 135 $[C_7H_5NO_2]^+$, 134 $[C_9H_{12}N]^+$, 121 $[C_7H_5O_2]^+$, 119 $[C_7H_5NO]^+$, 93 $[C_6H_5O]^+$, 67 $[C_4H_3O]^+$, 51 $[C_4H_3]^+$.

**4.7.11** ***N'-(4-(Diethylamino)benzylidene)-2-(5-(4-hydroxyphenyl)-1,3,4-oxadiazol-2-ylthio) acetohydrazide (9b)***
Light orange amorphous solid; Yield: 84%; M.P.: 216-218 $^oC$; Mol. formula: $C_{21}H_{23}N_5O_3S$; Mol. mass: 425 $gmol^{-1}$; IR (KBr, $\nu_{max}/cm^{-1}$): 3393 (O-H), 3048 (Ar C-H), 1664 (C=N), 1602 (Ar C=C); $^1$H-NMR (400 MHz, $CDCl_3$, $\delta$/ppm): 11.53 (s, 1H, CONH), 8.03 (s, 1H, H-7'''), 7.86 (d, *J* = 8.4 Hz, 2H, H-2' & H-6'), 7.42 (d, *J* = 8.4 Hz, 2H, H-2''' & H-6'''), 6.73 (d, *J* = 8.4 Hz, 2H, H-3' & H-5'), 6.67 (d, *J* = 8.0 Hz, 2H, H-3''' & H-5'''), 4.59 (s, 2H, H-2''), 2.61 (q, *J* = 7.6 Hz, 4H, $(CH_3\underline{CH_2})_2$N-4'''), 1.07 (t, *J* = 7.6 Hz, 6H, $(\underline{CH_3}CH_2)_2$N-4'''); EIMS (*m/z*): 425 $[M]^+$, 235 $[C_{10}H_7N_2O_3S]^+$, 218 $[C_{12}H_{16}N_3O]^+$, 194 $[C_8H_6N_2O_2S]^+$, 190 $[C_{11}H_{16}N_3]^+$, 162 $[C_{11}H_{16}N]^+$, 135 $[C_7H_5NO_2]^+$, 121 $[C_7H_5O_2]^+$, 119 $[C_7H_5NO]^+$, 93 $[C_6H_5O]^+$, 67 $[C_4H_3O]^+$, 51 $[C_4H_3]^+$.

**4.7.12** ***N'-(4-Methoxybenzylidene)-2-(5-(4-hydroxyphenyl)-1,3,4-oxadiazol-2-ylthio)aceto hydrazide (9c)***
White amorphous solid; Yield: 77%; M.P.: 242-244 $^oC$; Mol. formula: $C_{18}H_{16}N_4O_4S$; Mol. mass: 384 $gmol^{-1}$; IR (KBr, $\nu_{max}/cm^{-1}$): 3405 (O-H), 3047 (Ar C-H), 1672 (C=N), 1607 (Ar C=C); $^1$H-NMR (400 MHz, $CDCl_3$, $\delta$/ppm): 11.75 (s, 1H, CONH), 8.05 (s, 1H, H-7'''), 7.83 (d, *J* = 8.0 Hz, 2H, H-2' & H-6'), 7.79 (d, *J* = 8.4 Hz, 2H, H-2''' & H-6'''), 6.68 (d, *J* = 8.0 Hz, 2H, H-3' & H-5'), 6.52 (d, *J* = 8.4 Hz, 2H, H-3''' & H-5'''), 4.62 (s, 2H, H-2''), 3.83 (s, 3H, $CH_3$O-4'''); EIMS (*m/z*): 384 $[M]^+$, 235 $[C_{10}H_7N_2O_3S]^+$, 194 $[C_8H_6N_2O_2S]^+$, 177 $[C_9H_9N_2O_2]^+$, 149 $[C_8H_9N_2O]^+$, 135 $[C_7H_5NO_2]^+$, 121 $[C_8H_9O]^+$, 121 $[C_7H_5O_2]^+$, 119 $[C_7H_5NO]^+$, 93 $[C_6H_5O]^+$, 67 $[C_4H_3O]^+$, 51 $[C_4H_3]^+$.

**4.7.13** ***N'-(2,3-Dimethoxybenzylidene)-2-(5-(4-hydroxyphenyl)-1,3,4-oxadiazol-2-ylthio)aceto hydrazide (9d)***
White amorphous solid; Yield: 86%; M.P.: 230-232 $^oC$; Mol. formula: $C_{19}H_{18}N_4O_5S$; Mol. mass: 414 $gmol^{-1}$; IR (KBr, $\nu_{max}/cm^{-1}$): 3396 (O-H), 3069 (Ar C-H), 1638 (C=N), 1604 (Ar C=C); $^1$H-NMR (400 MHz, $CDCl_3$, $\delta$/ppm): 11.74 (s, 1H, CONH), 8.31 (s, 1H, H-7'''), 7.89 (d, *J* = 8.0 Hz, 2H, H-2' & H-6'), 7.54 (d, *J* = 8.4 Hz, 1H, H-6'''), 7.46 (dd, *J* = 8.0, 1.6 Hz, 1H, H-4'''), 7.13 (t, *J* = 8.4 Hz, 1H, H-5'''), 6.62 (d, *J* = 8.0 Hz, 2H, H-3' & H-5'), 4.65 (s, 2H, H-2''), 3.83 (s, 3H, $CH_3$O-3'''), 3.79 (s, 3H, $CH_3$O-2'''); EIMS (*m/z*): 414 $[M]^+$, 235 $[C_{10}H_7N_2O_3S]^+$, 207 $[C_{10}H_{11}N_2O_3]^+$, 194 $[C_8H_6N_2O_2S]^+$, 179 $[C_9H_{11}N_2O_2]^+$, 151 $[C_9H_{11}O_2]^+$, 135 $[C_7H_5NO_2]^+$, 121 $[C_7H_5O_2]^+$, 119 $[C_7H_5NO]^+$, 93 $[C_6H_5O]^+$, 67 $[C_4H_3O]^+$, 51 $[C_4H_3]^+$.

**4.7.14** ***N'-(2,4-Dimethoxybenzylidene)-2-(5-(4-hydroxyphenyl)-1,3,4-oxadiazol-2-ylthio)aceto hydrazide (9e)***
Cream amorphous solid; Yield: 84%; M.P.: 238-240 $^oC$; Mol. formula: $C_{19}H_{18}N_4O_5S$; Mol. mass: 414 $gmol^{-1}$; IR (KBr, $\nu_{max}/cm^{-1}$): 3398 (O-H), 3059 (Ar C-H), 1647 (C=N), 1605 (Ar C=C); $^1$H-NMR (400 MHz, $CDCl_3$, $\delta$/ppm): 11.63 (s, 1H, CONH), 8.24 (s, 1H, H-7'''), 7.86 (d, *J* = 8.0 Hz, 2H, H-2' & H-6'), 7.71 (d, *J* = 8.4 Hz, 1H, H-6'''), 6.69 (d, *J* = 8.0 Hz, 2H, H-3' & H-5'), 6.61 (d, *J* = 2.0 Hz, 1H, H-3'''), 6.56 (dd, *J* = 8.4, 1.6 Hz, 1H, H-5'''), 4.63 (s, 2H, H-2''), 3.84 (s, 3H, $CH_3$O-2'''), 3.82 (s, 3H, $CH_3$O-4'''); EIMS (*m/z*): 414 $[M]^+$, 235 $[C_{10}H_7N_2O_3S]^+$, 207 $[C_{10}H_{11}N_2O_3]^+$, 194 $[C_8H_6N_2O_2S]^+$, 179 $[C_9H_{11}N_2O_2]^+$, 151 $[C_9H_{11}O_2]^+$, 135 $[C_7H_5NO_2]^+$, 121 $[C_7H_5O_2]^+$, 119 $[C_7H_5NO]^+$, 93 $[C_6H_5O]^+$, 67 $[C_4H_3O]^+$, 51 $[C_4H_3]^+$.

**4.7.15** ***N'-(2,5-Dimethoxybenzylidene)-2-(5-(4-hydroxyphenyl)-1,3,4-oxadiazol-2-ylthio)aceto hydrazide (9f)***
Yellow amorphous solid; Yield: 80%; M.P.: 246-248 $^oC$; Mol. formula: $C_{19}H_{18}N_4O_5S$; Mol. mass: 414 $gmol^{-1}$; IR (KBr, $\nu_{max}/cm^{-1}$): 3413 (O-H), 3082 (Ar C-H), 1634 (C=N), 1603 (Ar C=C); $^1$H-NMR (400 MHz, $CDCl_3$, $\delta$/ppm): 11.74 (s, 1H, CONH), 8.36 (s, 1H, H-7'''), 7.89 (d, *J* = 8.4 Hz, 2H, H-2' & H-6'), 7.35 (d, *J* = 2.4 Hz, 1H, H-6'''), 7.07 (d, *J* = 8.4 Hz, 1H, H-3'''), 7.02 (dd, *J* = 8.0, 2.0 Hz, 1H, H-4'''), 6.68 (d, *J* = 8.0 Hz, 2H, H-3' & H-5'), 4.65 (s, 2H, H-2''), 3.79 (s, 3H, $CH_3$O-5'''), 3.76 (s, 3H, $CH_3$O-2'''); EIMS (*m/z*): 414 $[M]^+$, 235 $[C_{10}H_7N_2O_3S]^+$, 207 $[C_{10}H_{11}N_2O_3]^+$, 194 $[C_8H_6N_2O_2S]^+$, 179 $[C_9H_{11}N_2O_2]^+$, 151 $[C_9H_{11}O_2]^+$, 135 $[C_7H_5NO_2]^+$, 121 $[C_7H_5O_2]^+$, 119 $[C_7H_5NO]^+$, 93 $[C_6H_5O]^+$, 67 $[C_4H_3O]^+$, 51 $[C_4H_3]^+$.

**4.7.16** ***N'-(3,4-Dimethoxybenzylidene)-2-(5-(4-hydroxyphenyl)-1,3,4-oxadiazol-2-ylthio)aceto hydrazide (9g)***
White amorphous solid; Yield: 83%; M.P.: 238-240 $^oC$; Mol. formula: $C_{19}H_{18}N_4O_5S$; Mol. mass: 414 $gmol^{-1}$; IR (KBr, $\nu_{max}/cm^{-1}$): 3399 (O-H), 3074 (Ar C-H), 1653 (C=N), 1592 (Ar C=C); $^1$H-NMR (400 MHz, $CDCl_3$, $\delta$/ppm): 11.66 (s, 1H, CONH), 8.14 (s, 1H, H-7'''), 7.86 (d, *J* = 8.0 Hz, 2H, H-2' & H-6'), 7.31 (d, *J* = 1.6 Hz, 1H, H-2'''), 7.13 (dd, *J* = 8.4, 1.6 Hz, 1H, H-6'''), 6.96 (d, *J* = 8.4 Hz, 1H, H-5'''), 6.67 (d, *J* = 8.0 Hz, 2H, H-3' & H-5'), 4.62 (s, 2H, H-2''), 3.81 (s, 3H, $CH_3$O-3'''), 3.80 (s, 3H, $CH_3$O-4'''); EIMS (*m/z*): 414 $[M]^+$, 235 $[C_{10}H_7N_2O_3S]^+$, 207 $[C_{10}H_{11}N_2O_3]^+$, 194 $[C_8H_6N_2O_2S]^+$, 179 $[C_9H_{11}N_2O_2]^+$, 151 $[C_9H_{11}O_2]^+$, 135 $[C_7H_5NO_2]^+$, 121 $[C_7H_5O_2]^+$, 119 $[C_7H_5NO]^+$, 93 $[C_6H_5O]^+$, 67 $[C_4H_3O]^+$, 51 $[C_4H_3]^+$.

**4.7.17** ***N'-(2,4-Dichlorobenzylidene)-2-(5-(4-hydroxyphenyl)-1,3,4-oxadiazol-2-ylthio)aceto hydrazide (9h)***
White amorphous solid; Yield: 83%; M.P.: 250-252 $^oC$; Mol. formula: $C_{17}H_{12}Cl_2N_4O_3S$; Mol. mass: 422 $gmol^{-1}$; IR (KBr, $\nu_{max}/cm^{-1}$): 3409 (O-H), 3071 (Ar C-H), 1654 (C=N), 1591 (Ar C=C), 704 (C-Cl); $^1$H-NMR (400 MHz, $CDCl_3$, $\delta$/ppm): 11.61 (s, 1H, CONH), 8.71 (s, 1H, H-7'''), 7.89 (d, *J* = 8.0 Hz, 2H, H-2' & H-6'), 7.53 (d, *J* = 8.4 Hz, 1H, H-6'''), 7.45 (dd, *J* = 8.4, 1.6 Hz, 1H, H-5'''), 7.31 (d, *J* = 1.6 Hz, 1H, H-3'''), 6.69 (d, *J* = 8.0 Hz, 2H, H-3' & H-5'), 4.61

(s, 2H, H-2"); EIMS (*m/z*): 426 $[M+4]^+$, 424 $[M+2]^+$, 422 $[M]^+$, 235 $[C_{10}H_7N_2O_3S]^+$, 215 $[C_8H_5Cl_2N_2O]^+$, 194 $[C_8H_6N_2O_2S]^+$, 187 $[C_7H_5Cl_2N_2]^+$, 159 $[C_7H_5Cl_2]^+$, 135 $[C_7H_5NO_2]^+$, 121 $[C_7H_5O_2]^+$, 119 $[C_7H_5NO]^+$, 93 $[C_6H_5O]^+$, 67 $[C_4H_3O]^+$, 51 $[C_4H_3]^+$.

#### 4.7.18 *N'-(2,6-Dichlorobenzylidene)-2-(5-(4-hydroxyphenyl)-1,3,4-Oxadiazol-2-ylthio)aceto hydrazide (9i)*

White amorphous solid; Yield: 84%; M.P.: 244-246 $^oC$; Mol. formula: $C_{17}H_{12}Cl_2N_4O_3S$; Mol. mass: 422 $gmol^{-1}$; IR (KBr, $\nu_{max}/cm^{-1}$): 3394 (O-H), 3078 (Ar C-H), 1659 (C=N), 1597 (Ar C=C), 706 (C-Cl); $^1$H-NMR (400 MHz, $CDCl_3$, $\delta$/ppm): 11.57 (s, 1H, CONH), 8.72 (s, 1H, H-7'"), 7.86 (d, *J* = 8.4 Hz, 2H, H-2' & H-6'), 7.53 (d, *J* = 8.4 Hz, 2H, H-3'" & H-5'"), 7.43 (t, *J* = 8.4 Hz, 1H, H-4'"), 6.73 (d, *J* = 8.0 Hz, 2H, H-3' & H-5'), 4.61 (s, 2H, H-2"); EIMS (*m/z*): 426 $[M+4]^+$, 424 $[M+2]^+$, 422 $[M]^+$, 235 $[C_{10}H_7N_2O_3S]^+$, 216 $[C_8H_5Cl_2N_2O]^+$, 194 $[C_8H_6N_2O_2S]^+$, 188 $[C_7H_5Cl_2N_2]^+$, 160 $[C_7H_5Cl_2]^+$, 135 $[C_7H_5NO_2]^+$, 121 $[C_7H_5O_2]^+$, 119 $[C_7H_5NO]^+$, 93 $[C_6H_5O]^+$, 67 $[C_4H_3O]^+$, 51 $[C_4H_3]^+$.

### 4.8 *Biological assays*

#### 4.8.1 *Antibacterial assay*

The antibacterial activity results were obtained by employing the reported method with minor alterations[12,15].

#### 4.8.2 *Enzyme inhibition assay*

The enzyme inhibition results were obtained by employing the reported method with minor alterations[10,11].

#### 4.8.3 *Statistical analysis*

The presented results are mean ± SEM of calculations obtained after three experiments and statistically analyzed on MS Excel 2010. The MIC and $IC_{50}$ values are reported which are the result of values recorded after varying dilutions of each sample, followed by calculation by EZ-Fit software (Perrella Scientific Inc, Amherst, USA).

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## 6. REFERENCES

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