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SYNTHESIS PHARMACOLOGICAL EVALUATION, MOLECULAR DOCKING AND CYTOTOXICTY STUDIES ON SOME *N*-SUBSTITUTED 5-[(4-CHLOROPHENOXY)METHYL]-1,3,4-OXADIAZOLE-2YL-2-SULFANYL ACETAMIDES

Sabahat Zahra Siddiqui[1], Muhammad Athar Abbasi[1,*], Aziz-ur-Rehman[1], Misbah Irshad[1], Babar Shahzad[1], Muhammad Ashraf[2], Irshad Ahmad[3], Muhammad A. Lodhi[4], Bushra Mirza[5]Hammad Ismail[5], Muhammad N. Akhtar[6]

[1]*Department of Chemistry, Government College University, Lahore-54000, Pakistan.*
[2]*Department of Biochemistry and Biotechnology, The Islamia University of Bahawalpur, Bahawalpur-63100, Pakistan.*
[3]*Department of Pharmacy, The Islamia University of Bahawalpur, Bahawalpur-63100, Pakistan.*
[4]*Department of Biochemistry, Abdul Wali Khan University, Mardan-23200, Pakistan.*
[5]*Department of Biochemistry, Quaid-i-Azam University, Islamabad, 45320, Pakistan.*
[6]*Faculty of Industrial Sciences & Technology, University Malaysia Pahang, Leburaya TunRazak 26300, Kuantan Pahang, Malaysia.*

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ABSTRACT

The framework of our systematic efforts focuses on the synthesis of *N*-substituted 5-[(4-chlorophenoxy) methyl]-1,3,4-oxadiazole-2yl-2-sulfanyl acetamides. 4-Chlorophenoxyacetic acid (1) was utilized as a precursor for the synthesis of parent 1,3,4-oxadiazole moiety. Esterification of 1in the presence of catalytic amount of concentrated sulfuric acid and absolute alcohol generated ethyl 2-(4-chlorophenoxy)acetate (2) which was treated with hydrazine hydrate to yield 2-(4-chlorophenoxy)acetohydrazide (3). Ring closure reaction of 3 with carbon disulfide and alcoholic potassium hydroxide afforded [5-(4-chlorophenoxy)methyl)]-1,3,4-oxadiazole-2-thiol (4). Finally, substitution at thiol position of 4 with electrophiles, *N*-substituted-2-bromoacetamides (6a-p) in polar aprotic solvent and LiH yielded various *N*-substituted 5-[(4-chlorophenoxy) methyl]-1,3,4-oxadiazole-2yl-2-sulfanyl acetamides (7a-p). IR, ^{1}H-NMR and EI-MS spectral analysis data unequivocally confirmed all the substitutions on 1,3,4-oxadiazole-2-thiol core. It was recognized that the synthesized derivatives are potential anti-bacterial agents against both gram negative and gram positive bacteria and moderate inhibitors of α-chymotrypsin enzyme. *In vitro* screening against various bacterial strains unleashed their anti-bacterial potential, especially 5-[(4-chlorophenoxy)methyl]-1,3,4-oxadiazol-2yl-*N*-(3,4-dimethylphenyl)-2-sulfanyl acetamide (7o) exhibited marvelous activity when compared with standard ciprofloxacin against *S.typhi* (-), *K.pneumonae* (-) and *S. aureus* (+).Compounds were computationally docked with the α-chymotrypsin enzyme protein to unravel the active binding sites which displayed significant correlation with the bioactivity data. It can be envisioned that the amalgamation of 5-[(4-chlorophenoxy)methyl]-1,3,4-oxadiazole-2-thiol with *N*-substituted-2-bromoacetamides generated *N*-substituted 5-[(4-chlorophenoxy)methyl]-1,3,4-oxadiazole-2yl-2-sulfanyl acetamides having tremendous antibacterial activity and moderate anti-enzymatic potential. Moreover, substitutions on the oxadiazole moiety lead to the discovery of less cytotoxic compounds as evident from the cytotoxicity data.

Corresponding author
Dr. Muhammad Athar Abbasi,
Department of Chemistry, Government College University,
Lahore-54000, Pakistan.
atrabbasi@yahoo.com ; abbasi@gcu.edu.pk

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INTRODUCTION:

1,3,4-Oxadiazole, a versatile molecule for designing potential bioactive agents. The 1,3,4-oxadiazole derivatives have been found to exhibit diverse biological activities such as anti-HIV [1], antitubercular [2], antimalarial [3], analgesic [4], anticonvulsant [5], hypoglycemic [6], and other biological properties such as genotoxic studies [7] and lipid peroxidation inhibitor [8]. The diverse biological effects are associated with 2,5-disubstituted-1,3,4-oxadiazole-2-thiol and their derivatives. These significantly include antimicrobial, anti-inflammatory and antiviral activities. They also are important intermediates in organic synthesis, because of the presence of nitrogen and exocyclic sulfur atoms which are nucleophilic in nature and are readily attacked by electrophilic reagents [9-17].

In literature a number of methodologies have been illustrated for the preparation of substituted 1,3,4-oxadiazoles [18]. The most appropriate methods apply acid-catalyzed cyclization of 1,2-diacylhydrazines [19-21] and oxidative cyclization of acyl hydrazones [22-24].These reactions advance by the application of heat and anhydrous reagents e.g. thionyl chloride [25], phosphorous oxychloride [26],phosphorous pentoxide [27], triphenylphosphene and triflic anhydride [28]. Alternatively synthetic methods comprise of reacting carboxylic hydrazides with keteneylidenetriphenyl phosphorane [29] and base-promoted cyclization reaction of trichloroacetic acid hydrazones [30]. Oxadiazoles have occupied a unique place in the field of medicinal chemistry due to its wide range of activities. The oxdiazole derivatives have been reported to have various biological activities. 1,3,4-oxadiazole based Schiff's bases possessed *in-vitro* anti-bacterial, anti-fungal and anti-tubercular activities. The QSAR study suggested that polarity, lipophilicity and thermodynamic stability were the major contributing parameters for exhibiting anti-fungal activity [31].

In prolongation of our preceding research efforts [32-33] and based on the recent advances on the discovery of correlated bioactive compounds have prompted us to synthesize*N*-substituted-5-[(4-chlorophenoxy)methyl]-1,3,4-oxadiazole-2yl-2-sulfanyl acetamides. Anti-bacterial screening against clinically isolated gram positive and negative bacteria revealed that the synthesized compounds depicted good to excellent activity even better than the standard in some cases. Based on the literature data, alpha chymotrypsin enzyme belongs to class of serine protease have protease specificity for 1,3,4-oxadiazoles substituted with aromatic and large hydrophobic residues [33] so compounds, (**7a-p)**were evaluated for their anti-enzymatic potential and were further correlated with molecular modeling studies against chymotrypsin enzyme to understand the mode of interactions and to generate more effective and potential derivatives via ligand-based drug designing approaches. The molecular modeling and anti-enzymatic analysis confirmed that they are moderate inhibitors of α-chymotrypsin enzyme. Moreover, the brine shrimp protocol revealed that most of the compounds are less cytotoxic in comparison to the standard, Doxorubicin.

MATERIALS AND METHOD:

Measurements:

The chemicals employed in the current research work were attained from Sigma/Fluka and all were of analytical grade. All solvents consumed herein were distilled before use. Melting points of the synthesized compounds were determined in open capillary tubes on electro-thermal Griffin and George melting point apparatus. The homogeneity of the synthesized compounds and the advancement in the reactions were monitored by ascending thin layer chromatographic technique which was executed on pre coated silica gel 60 F_{254} plates as adsorbent and UV light was used as visualizing agent at 254 nm. Various percentages of *n*-hexane and ethyl acetate were used as mobile phase. IR spectra were recorded on a Jasco-320-A spectrometer by KBr disc method and absorption bands are expressed in wave number (cm^{-1}). ^{1}H-NMR was acquired in deuterated chloroform on a Bruker spectrometer operating at 400 MHz frequency and chemical shifts are expressed in parts per million (δ ppm) while coupling constants (*J*) are mentioned in Hertz (Hz). Mass spectra (EI-MS) were recorded on a JMS-HX-110 spectrometer, with a data system.

Synthesis:

***Step 1:* Synthesis of ethyl 2-(4-chlorophenoxy)acetate (2)**

2-(4-Chlorophenoxy)acetic acid (18.6 g; 0.1 moles; **1**) was taken in a 500mL round bottomed flask outfitted with a reflux condenser. Ethyl alcohol (70 mL) and concentrated sulfuric acid (4.9 mL, 0.05 moles) was added in the reaction mixture and was refluxed under stirring for 6 h till the reaction reached completion. The reaction mixture was neutralized with aqueous 10 % sodium carbonate till pH 8. The product was isolated using solvent extraction technique using dichloromethane (40 mL × 3) as the organic phase. The lower organic phase containing the ester was decanted and washed a number of times with distilled water and dried over anhydrous $MgSO_4$.Complete removal of organic solvent resulted in an off-white solid i.e. Ethyl 2-(4-chlorophenoxy)acetate (16 g, 75 %, **2**). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.22 (br.d, *J* = 7.0 Hz, 2H, H-3′& H-5′), 6.81 (br.d, *J* = 7.0 Hz, 2H, H-2′ & H-6′), 4.56 (s, 2H, CH_2-2), 4.24 (q, *J* = 7.2 Hz, 2H, CH_2-1″), 1.27 (t, *J* = 7.2 Hz, 3H, CH_3-2″).

***Step 2:* Synthesis of 2-(4-chlorophenoxy)acetohydrazide (3)**

Ethyl 2-(4-chlorophenoxy)acetate, (15.0 g, 0.07 moles, **2**) in 30 mL methanol was added in a 250 mL round bottomed flask. Hydrazine hydrate (80 %, 15 mL) was added in the reaction mixture at 0-5 °C and was further stirred at room temperature for 1h. Absolute conversion was examined by thin layer chromatographic technique, after which the precipitates of the crude product were achieved by distilling excess of methyl alcohol from the reaction mixture. The precipitates were filtered, washed with cold *n*-hexane and were air dried to achieve 2-(4-chlorophenoxy)acetohydrazide (9.76 g, 71%, **3**). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.64 (s, 1H, NH), 7.24 (br.d, *J* = 9.0 Hz, 2H, H-3′& H-5′), 6.82 (br.d, *J* = 9.0 Hz, 2H, H-2′ & H-6′), 4.52 (s, 2H, CH_2-2), 3.90 (br.s, 2H, NH_2).

Step 3: **Synthesis of 5-[(4-chlorophenoxy)methyl]-1,3,4-oxadiazole-2-thiol (4)**

2-(4-chlorophenoxy)acetohydrazide, (9.0 g, 0.044 moles, **3**) was dissolved in 30 mL of absolute ethanol in a 500mL round bottom flask equipped with a reflux condenser. Potassium hydroxide (2.46 g, 0.044 moles) and carbon disulfide (5.3 mL, 0.088 moles) was added in the reaction flask which was refluxed for 6 h. The completion of reaction was examined by TLC which was found to be a single spot. Precipitates of the crude product were obtained by distilling ethanol from the mixture, and were further dissolved in 20 mL distilled water and pure product was re-precipitated by addition of dilute hydrochloric acid till pH 2. The precipitates were filtered, washed with cold distilled water and air dried to afford pure 5-[(4-chlorophenoxy)methyl]-1,3,4-oxadiazole-2-thiol (9.7g, 91.5 %, **4**).^{1}H-NMR (400 MHz, $CDCl_3$, Fig. 1): δ10.2 (br.s, 1H, SH), 7.25 (br.d, J = 9.0 Hz, 2H, H-3' & H-5'), 6.89 (br.d, J = 9.0 Hz, 2H, H-2' & H-6'), 5.00 (s, 2H, CH_2-6); EI-MS: *m/z* 246 $[M+4]^+$, 244 $[M+2]^+$and242 $[M^+]$, 183 $(C_8H_6ClNO_2)^{\cdot+}$, 169 $(C_8H_6ClO_2)^+$, 167 $(C_8H_6ClNO)^{\cdot+}$, 143 $(C_7H_6ClO + 2)^+$, 141 $(C_7H_6ClO)^+$, 113 $(C_6H_4Cl + 2)^+$, 111 $(C_6H_4Cl)^+$, 76 $(C_6H_4)^{\cdot+}$, 51 $(C_4H_3)^+$, 50 $(C_4H_2)^{\cdot+}$.

Step 5: **Synthesis of *N*-substituted-5-[(4-chlorophenoxy)methyl]-1,3,4-oxadiazole-2yl-2-sulfanyl acetamides (7a-p)**

N-substituted acetamides, **7a-p** were synthesized by reacting equimolar quantities of **4** with *N*-substituted-2-bromoacetamides (0.001 moles, **6a-p**) in *N*-*N*-dimethylformamide (7 mL) and lithium hydride (0.002 moles) in a 25 mL round bottom flask. The reaction mixture was stirred for 3 h at room temperature. After absolute conversion, the reaction mixture was poured on crushed ice, acquired precipitates were filtered, washed with distilled water and dried to afford pure *N*-substituted-5-[(4-chlorophenoxy)methyl]-1,3,4-oxadiazole-2yl-2-sulfanyl acetamides(87-98 %, **7a-p**).

1. 5-[(4-Chlorophenoxy)methyl]-1,3,4-oxadiazol-2yl-N-(cyclohexyl)-2-sulfanyl acetamide (7a)

^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.91 (d, J = 6.8 Hz, 2H, H-3' & H-5'), 6.38 (d, J = 6.8 Hz, 2H, H-2' & H-6'), 5.18 (s, 2H, CH_2-6), 3.80 (s, 2H, CH_2-2"), 3.73 (m, 1H, CH-1'"), 1.82-1.11 (m, 10H, CH_2-2'" to CH_2-6'"); HR-MS: 381.8776 $[M]^+$ (Calcd. for $C_{17}H_{20}ClN_3O_3S$; 381.9771); EI-MS: *m/z* 383 $(C_{17}H_{20}ClN_3O_3S)$ $[M + 2]^+$, 381 $(C_{17}H_{20}ClN_3O_3S)$ $[M]^+$, 254 $(C_{11}H_{10}N_3O_2S)^+$, 240 $(C_{10}H_{14}N_3O_2S)^+$, 200 $(C_9H_{14}NO_2S)^+$, 198 $(C_9H_{14}N_2OS)^{\cdot+}$, 183 $(C_8H_6ClNO_2)^{\cdot+}$, 172 $(C_8H_{14}NOS)^+$, 169 $(C_8H_6ClO_2)^+$, 167 $(C_8H_6ClNO)^{\cdot+}$, 143 $(C_7H_6ClO + 2)^+$, 141 $(C_7H_6ClO)^+$, 140 $(C_8H_{14}NO)^+$, 129 $(C_6H_4ClO + 2)^+$, 127 $(C_6H_4ClO)^+$, 126 $(C_7H_{12}NO)^+$, 113 $(C_6H_4Cl +2)^+$, 111 $(C_6H_4Cl)^+$, 101 $(C_5H_4Cl + 2)^+$, 99 $(C_5H_4Cl)^+$, 98 $(C_6H_{12}N)^+$, 83 $(C_6H_{11})^+$, 76 $(C_6H_4)^{\cdot+}$, 55 $(C_4H_7)^+$, 51 $(C_4H_3)^+$, 50 $(C_4H_2)^{\cdot+}$.

2. 5-[(4-Chlorophenoxy)methyl]-1,3,4-oxadiazol-2yl-N-(benzyl)-2-sulfanyl acetamide (7b)

^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.28-7.25 (m, 5H, H-2'"- H-6'"), 7.21 (d, J = 8.0 Hz, 2H, H-3' & H-5'), 6.90 (d, J = 8.0 Hz, 2H, H-2' & H-6'), 5.16 (s, 2H, CH_2-6), 4.44 (br.s, 2H, CH_2-7'"), 3.89 (s, 2H, CH_2-1"); HR-MS: 389.8559 $[M]^+$ (Calcd. for $C_{17}H_{14}ClN_3O_3S$; 389.9559); EI-MS: *m/z* 391 $(C_{18}H_{16}ClN_3O_3S)$ $[M + 2]^+$, 389 $(C_{18}H_{16}ClN_3O_3S)$ $[M]^+$, 262 $(C_{12}H_{12}N_3O_2S)^+$, 248 $(C_{11}H_{10}N_3O_2S)^+$, 208 $(C_{10}H_{10}NO_2S)^+$, 206 $(C_{10}H_{10}N_2OS)^{\cdot+}$, 183 $(C_8H_6ClNO_2)^{\cdot+}$, 180 $(C_9H_{10}NOS)^+$, 169 $(C_8H_6ClO_2)^+$, 167 $(C_8H_6ClNO)^{\cdot+}$, 148 $(C_9H_{10}NO)^+$, 143 $(C_7H_6ClO + 2)^+$, 141 $(C_7H_6ClO)^+$, 134 $(C_8H_8NO)^+$, 129 $(C_6H_4ClO + 2)^+$, 127 $(C_6H_4ClO)^+$, 113 $(C_6H_4Cl + 2)^+$, 111 $(C_6H_4Cl)^+$, 106 $(C_7H_8N)^+$, 101 $(C_5H_4Cl + 2)^+$, 99 $(C_5H_4Cl)^+$, 91 $(C_7H_7)^+$, 76 $(C_6H_4)^{\cdot+}$, 65 $(C_5H_5)^+$, 51 $(C_4H_3)^+$, 50 $(C_4H_2)^{\cdot+}$.

3. 5-[(4-Chlorophenoxy)methyl]-1,3,4-oxadiazol-2yl-N-(phenyl)-2-sulfanyl acetamide (7c)

^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.41 (s, 1H, NH), 7.50-7.47 (m, 2H, H-3'" & H-5'"), 7.43-7.41 (m, 1H, H-4'"), 7.33 (br.d, J = 7.2 Hz, 2H, H-2'" & H-6'"), 7.25 (d, J = 9.0 Hz, 2H, H-3' & H-5'), 6.98 (d, J = 9.0 Hz, 2H, H-2' & H-6'), 4.56 (s, 2H, CH_2-6), 4.02 (s, 2H, CH_2-2"); HR-MS: 375.8293 $[M]^+$ (Calcd. for $C_{17}H_{14}ClN_3O_3S$; 375.9289); EI-MS: *m/z* 377 $(C_{17}H_{14}ClN_3O_3S)$ $[M + 2]^+$, 375 $(C_{17}H_{14}ClN_3O_3S)$ $[M]^+$, 248 $(C_{11}H_{10}N_3O_2S)^+$, 234 $(C_{10}H_8N_3O_2S)^+$, 194 $(C_9H_8NO_2S)^+$, 192 $(C_9H_8N_2OS)^{\cdot+}$, 183 $(C_8H_6ClNO_2)^{\cdot+}$, 169 $(C_8H_6ClO_2)^+$, 167 $(C_8H_6ClNO)^{\cdot+}$, 143 $(C_7H_6ClO + 2)^+$, 141 $(C_7H_6ClO)^+$, 134 $(C_8H_8NO)^+$, 129 $(C_6H_4ClO + 2)^+$, 127 $(C_6H_4ClO)^+$, 120 $(C_7H_6NO)^+$, 113 $(C_6H_4Cl + 2)^+$, 111 $(C_6H_4Cl)^+$, 101 $(C_5H_4Cl + 2)^+$, 99 $(C_5H_4Cl)^+$, 92 $(C_6H_6N)^+$, 77 $(C_6H_5)^+$, 76 $(C_6H_4)^{\cdot+}$, 51 $(C_4H_3)^+$, 50 $(C_4H_2)^{\cdot+}$.

4. 5-[(4-Chlorophenoxy)methyl]-1,3,4-oxadiazol-2yl-N-(4-ethylphenyl)-2-sulfanyl acetamide (7d)

^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.41 (s, 1H, NH), 7.30 (d, J = 8.8 Hz, 2H, H-2'" & H-6'"), 7.26 (d, J = 8.0 Hz, 2H, H-3' & H-5'), 7.23 (d, J = 8.8 Hz, 2H, H-3'" & H-5'"), 6.84 (d, J = 8.0 Hz, 2H, H-2' & H-6'),4.56 (s, 2H, CH_2-6), 4.01 (s, 2H, CH_2-2"), 2.60 (q, J = 7.0 Hz, 2H, CH_2-4'"), 1.22 (t, J = 7.0 Hz, 3H, $\underline{CH_3}CH_2$-4'"); HR-MS: 403.8825 $[M]^+$ (Calcd. for $C_{19}H_{18}ClN_3O_3S$; 403.9825); EI-MS: *m/z* 405 $(C_{19}H_{18}ClN_3O_3S)$ $[M + 2]^+$, 403 $(C_{19}H_{18}ClN_3O_3S)$ $[M]^+$, 276 $(C_{13}H_{14}N_3O_2S)^+$, 262 $(C_{12}H_{12}N_3O_2S)^+$, 222 $(C_{11}H_{12}NO_2S)^+$, 220 $(C_{11}H_{12}N_2OS)^{\cdot+}$, 194 $(C_{10}H_{12}NOS)^+$, 183 $(C_8H_6ClNO_2)^{\cdot+}$, 169 $(C_8H_6ClO_2)^+$, 167 $(C_8H_6ClNO)^{\cdot+}$, 162 $(C_{10}H_{12}NO)^+$, 148 $(C_9H_{10}NO)^+$, 143 $(C_7H_6ClO + 2)^+$, 141 $(C_7H_6ClO)^+$, 129 $(C_6H_4ClO + 2)^+$, 127 $(C_6H_4ClO)^+$, 120 $(C_8H_{10}N)^+$, 113 $(C_6H_4Cl + 2)^+$, 111 $(C_6H_4Cl)^+$, 105 $(C_8H_9)^+$, 101 $(C_5H_4Cl + 2)^+$, 99 $(C_5H_4Cl)^+$, 90 $(C_7H_6)^{\cdot+}$, 76 $(C_6H_4)^{\cdot+}$, 64 $(C_5H_4)^{\cdot+}$, 51 $(C_4H_3)^+$, 50 $(C_4H_2)^{\cdot+}$.

5. 5-[(4-Chlorophenoxy)methyl]-1,3,4-oxadiazol-2yl-N-(2-methylphenyl)-2-sulfanyl acetamide (7e)

^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.68 (s, 1H, NH), 7.87 (br.d, J = 7.6 Hz, 1H, H-6'"), 7.24 (br.d, J = 8.0 Hz, 2H, H-3' & H-5'), 7.18-7.13 (m, 2H, H-3'" & H-5'"), 7.05 (br.t, J = 7.2 Hz,1H, H-4'"), 6.91 (br.d, J = 8.0 Hz, 2H, H-2' & H-6'), 5.19 (s, 2H, CH_2-6), 4.01 (s, 2H, CH_2-2"), 2.21 (s, 3H, CH_3-2'"); HR-MS: 389.8559 $[M]^+$ (Calcd. for $C_{18}H_{16}ClN_3O_3S$; 389.9559); EI-MS: *m/z* 391 $(C_{18}H_{16}ClN_3O_3S)$ $[M + 2]^+$, 389 $(C_{18}H_{16}ClN_3O_3S)$ $[M]^+$, 262 $(C_{12}H_{12}N_3O_2S)^+$, 248 $(C_{11}H_{10}N_3O_2S)^+$, 208 $(C_{10}H_{10}NO_2S)^+$, 206

$(C_{10}H_{10}N_2OS)^{\cdot+}$, 183 $(C_8H_6ClNO_2)^{\cdot+}$, 180 $(C_9H_{10}NOS)^+$, 169 $(C_8H_6ClO_2)^+$, 167 $(C_8H_6ClNO)^{\cdot+}$, 148 $(C_9H_{10}NO)^+$, 143 $(C_7H_6ClO + 2])^+$, 141 $(C_7H_6ClO)^+$, 134 $(C_8H_8NO)^+$, 129 $(C_6H_4ClO + 2)^+$, 127 $(C_6H_4ClO)^+$, 113 $(C_6H_4Cl + 2)^+$, 111 $(C_6H_4Cl)^+$, 106 $(C_7H_8N)^+$, 101 $(C_5H_4Cl + 2)^+$, 99 $(C_5H_4Cl)^+$, 91 $(C_7H_7)^+$, 76 $(C_6H_4)^{\cdot+}$, 65 $(C_5H_5)^+$, 51 $(C_4H_3)^+$, 50 $(C_4H_2)^{\cdot+}$.

6. 5-[(4-Chlorophenoxy)methyl]-1,3,4-oxadiazol-2yl-N-(3-methylphenyl)-2-sulfanyl acetamide (7f)

^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.88 (s, 1H, NH), 7.32 (br.s, 1H, H-2'''), 7.24 (br.d, J = 8.2 Hz, 2H, H-3' & H-5'), 7.17 (t, J = 8.0 Hz, 1H, H-5'''), 7.07-6.99 (m, 2H, H-4'''& H-6'''), 6.91 (br.d, J = 8.2 Hz, 2H, H-2' & H-6'), 5.19 (s, 2H, CH_2-6), 3.93 (s, 2H, CH_2-2''), 2.31 (s, 3H, CH_3-3'''); HR-MS: 389.8559 $[M]^+$ (Calcd. for $C_{18}H_{16}ClN_3O_3S$; 389.9559); EI-MS: *m/z* 391 $(C_{18}H_{16}ClN_3O_3S)$ $[M + 2]^+$, 389 $(C_{18}H_{16}ClN_3O_3S)$ $[M]^+$, 262 $(C_{12}H_{12}N_3O_2S)^+$, 248 $(C_{11}H_{10}N_3O_2S)^+$, 208 $(C_{10}H_{10}NO_2S)^+$, 206 $(C_{10}H_{10}N_2OS)^{\cdot+}$, 183 $(C_8H_6ClNO_2)^{\cdot+}$, 180 $(C_9H_{10}NOS)^+$, 169 $(C_8H_6ClO_2)^+$, 167 $(C_8H_6ClNO)^{\cdot+}$, 148 $(C_9H_{10}NO)^+$, 143 $(C_7H_6ClO + 2)^+$, 141 $(C_7H_6ClO)^+$, 134 $(C_8H_8NO)^+$, 129 $(C_6H_4ClO + 2)^+$, 127 $(C_6H_4ClO)^+$, 113 $(C_6H_4Cl + 2)^+$, 111 $(C_6H_4Cl)^+$, 106 $(C_7H_8N)^+$, 101 $(C_5H_4Cl + 2)^+$, 99 $(C_5H_4Cl)^+$, 91 $(C_7H_7)^+$, 76 $(C_6H_4)^{\cdot+}$, 65 $(C_5H_5)^+$, 51 $(C_4H_3)^+$, 50 $(C_4H_2)^{\cdot+}$.

7. 5-[(4-Chlorophenoxy)methyl]-1,3,4-oxadiazol-2yl-N-(2-methoxyphenyl)-2-sulfanylacetamide (7g)

^{1}H-NMR (400 MHz, $CDCl_3$): δ 9.04 (s, 1H, NH), 8.28 (dd, J = 1.6, 8.0 Hz, 1H, H-6'''), 7.23 (br.d, J = 8.0 Hz, 2H, H-3' & H-5'), 7.04 (ddd, J = 1.2, 7.8, 8.0, Hz, 1H, H-5'''), 6.92 (ddd, J = 1.0, 7.8, 8.0, Hz, 1H, H-4'''), 6.89 (br.d, J = 7.8 Hz, 1H, H-3'''), 6.83 (br.d, J = 8.0, 2H, H-2' & H-6'), 5.19 (s, 2H, CH_2-6), 4.06 (s, 2H, CH_2-2''), 3.82 (s, 3H, OCH_3-2'''); HR-MS: 405.8553 $[M]^+$ (Calcd. for $C_{18}H_{16}ClN_3O_3S$; 405.9551); EI-MS: *m/z* 407 $(C_{18}H_{16}ClN_3O_3S)$ $[M + 2]^+$, 405 $(C_{18}H_{16}ClN_3O_4S)$ $[M]^+$, 278 $(C_{12}H_{12}N_3O_3S)^+$, 264 $(C_{11}H_{10}N_3O_3S)^+$, 224 $(C_{10}H_{10}NO_3S)^+$, 222 $(C_{10}H_{10}N_2O_2S)^{\cdot+}$, 196 $(C_9H_{10}NO_2S)^+$, 183 $(C_8H_6ClNO_2)^{\cdot+}$, 169 $(C_8H_6ClO_2)^+$, 167 $(C_8H_6ClNO)^{\cdot+}$, 164 $(C_9H_{10}NO_2)^+$, 150 $(C_8H_8NO_2)^+$, 143 $(C_7H_6ClO + 2)^+$, 141 $(C_7H_6ClO)^+$, 129 $(C_6H_4ClO + 2)^+$, 127 $(C_6H_4ClO)^+$, 122 $(C_7H_8NO)^+$, 111 $(C_6H_4Cl)^+$, 107 $(C_7H_7O)^+$, 92 $(C_6H_4O)^{\cdot+}$, 76 $(C_6H_4)^{\cdot+}$, 64 $(C_5H_4)^{\cdot+}$, 51 $(C_4H_3)^+$, 50 $(C_4H_2)^{\cdot+}$.

8. 5-[(4-Chlorophenoxy)methyl]-1,3,4-oxadiazol-2yl-N-(2-ethoxyphenyl)-2-sulfanyl acetamide (7h)

^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.97 (s, 1H, NH), 8.30 (d, J = 7.6 Hz, 1H, H-6'''), 7.22 (br.d, J = 8.0 Hz, 2H, H-3' & H-5'), 7.02 (t, J = 7.6 Hz, 1H, H-5'''), 6.93-6.89 (m, 2H, H-3''' & H-4'''), 6.83 (br.d, J = 8.0 Hz, 2H, H-2' & H-6'), 5.17 (s, 2H, CH_2-6), 4.08-4.04 (m-overlapped, 4H, CH_2-2'' & $O\underline{CH_2}CH_3$-2'''), 1.43 (t, J = 7.2 Hz, 3H, $OCH_2\underline{CH_3}$-2'''); HR-MS: 419.8819 $[M]^+$ (Calcd. for $C_{19}H_{18}ClN_3O_4S$; 419.9818); EI-MS: *m/z* 421 $(C_{19}H_{18}ClN_3O_4S)$ $[M + 2]^+$, 419 $(C_{19}H_{18}ClN_3O_4S)$ $[M]^+$, 292 $(C_{13}H_{14}N_3O_3S)^+$, 278 $(C_{12}H_{12}N_3O_3S)^+$, 238 $(C_{11}H_{12}N_2O_2S)^+$, 236 $(C_{11}H_{12}N_2O_2S)^+$, 210 $(C_{10}H_{12}NO_2S)^+$, 183 $(C_8H_6ClNO_2)^{\cdot+}$, 178 $(C_{10}H_{12}NO_2)^+$, 169 $(C_8H_6ClO_2)^+$, 167 $(C_8H_6ClNO)^{\cdot+}$, 164 $(C_9H_{10}NO_2)^{\cdot+}$, 143 $(C_7H_6ClO + 2)^+$, 141 $(C_7H_6ClO)^+$, 136 $(C_8H_{10}NO)^+$, 129 $(C_6H_4ClO + 2)^+$, 127 $(C_6H_4ClO)^+$, 121 $(C_8H_9O)^+$, 113 $(C_6H_4Cl + 2)^+$, 111 $(C_6H_4Cl)^+$, 101 $(C_5H_4Cl + 2)^+$, 99 $(C_5H_4Cl)^+$, 92 $(C_6H_4O)^{\cdot+}$, 76 $(C_6H_4)^{\cdot+}$, 64 $(C_5H_4)^{\cdot+}$, 51 $(C_4H_3)^+$, 50 $(C_4H_2)^{\cdot+}$.

9. 5-[(4-Chlorophenoxy)methyl]-1,3,4-oxadiazol-2yl-N-(3-ethoxyphenyl)-2-sulfanyl acetamide (7i)

^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.93 (s, 1H, NH), 7.26 (br.s, 1H, H-2'''), 7.18 (br.d, J = 8.4 Hz, 2H, H-3'& H-5'), 7.17 (t, J = 8.4 Hz, 1H, H-5'''), 6.96 (d, J = 8.4 Hz, 1H, H-6'''), 6.92 (br.d, J = 8.4 Hz, 2H, H-2' & H-6'), 6.64 (dd, J = 2.0, 8.4 Hz, 1H, H-4'''), 5.19 (s, 2H, CH_2-6), 4.02-3.96 (m-overlapped, 4H, CH_2-2'' & $O\underline{CH_2}CH_3$-3'''), 1.37 (t, J = 6.8 Hz, 3H, $OCH_2\underline{CH_3}$-3'''); HR-MS: 419.8819 $[M]^+$ (Calcd. for $C_{19}H_{18}ClN_3O_4S$; 419.9818); EI-MS: *m/z* 421 $(C_{19}H_{18}ClN_3O_4S)$ $[M + 2]^+$, 419 $(C_{19}H_{18}ClN_3O_4S)$ $[M]^+$, 292 $(C_{13}H_{14}N_3O_3S)^+$, 278 $(C_{12}H_{12}N_3O_3S)^+$, 238 $(C_{11}H_{12}N_2O_2S)^+$, 236 $(C_{11}H_{12}N_2O_2S)^+$, 210 $(C_{10}H_{12}NO_2S)^+$, 183 $(C_8H_6ClNO_2)^{\cdot+}$, 178 $(C_{10}H_{12}NO_2)^+$, 169 $(C_8H_6ClO_2)^+$, 167 $(C_8H_6ClNO)^{\cdot+}$, 164 $(C_9H_{10}NO_2)^{\cdot+}$, 143 $(C_7H_6ClO)^+[M + 2]$, 141 $(C_7H_6ClO)^+$, 136 $(C_8H_{10}NO)^+$, 129 $(C_6H_4ClO + 2)^+$, 127 $(C_6H_4ClO)^+$, 121 $(C_8H_9O)^+$, 113 $(C_6H_4Cl + 2)^+$, 111 $(C_6H_4Cl)^+$, 101 $(C_5H_4Cl + 2)^+$, 99 $(C_5H_4Cl)^+$, 92 $(C_6H_4O)^{\cdot+}$, 76 $(C_6H_4)^{\cdot+}$, 64 $(C_5H_4)^{\cdot+}$, 51 $(C_4H_3)^+$, 50 $(C_4H_2)^{\cdot+}$.

10. 5-[(4-Chlorophenoxy)methyl]-1,3,4-oxadiazol-2yl-N-(4-ethoxyphenyl)-2-sulfanyl acetamide (7j)

^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.40 (s, 1H, NH), 7.27 (d, J = 6.4 Hz, 1H, H-2''' & H-6'''), 7.23 (d, J = 8.0 Hz, 2H, H-3'& H-5'), 6.95 (d, J = 8.0 Hz, 2H, H-2' & H-6'), 6.86 (br.d, J = 6.4 Hz, 2H, H-3''' & H-5'''), 4.56 (s, 2H, CH_2-6), 4.08-4.00 (m-overlapped, 4H, CH_2-2'' & $O\underline{CH_2}CH_3$-4'''), 1.40 (t, J = 7.4 Hz, 3H, $OCH_2\underline{CH_3}$-4'''); HR-MS: 419.8819 $[M]^+$ (Calcd. for $C_{19}H_{18}ClN_3O_4S$; 419.9818); EI-MS: *m/z* 421 $(C_{19}H_{18}ClN_3O_4S)$ $[M + 2]^+$, 419 $(C_{19}H_{18}ClN_3O_4S)$ $[M]^+$, 292 $(C_{13}H_{14}N_3O_3S)^+$, 278 $(C_{12}H_{12}N_3O_3S)^+$, 238 $(C_{11}H_{12}N_2O_2S)^+$, 236 $(C_{11}H_{12}N_2O_2S)^+$, 210 $(C_{10}H_{12}NO_2S)^+$, 183 $(C_8H_6ClNO_2)^{\cdot+}$, 178 $(C_{10}H_{12}NO_2)^+$, 169 $(C_8H_6ClO_2)^+$, 167 $(C_8H_6ClNO)^{\cdot+}$, 164 $(C_9H_{10}NO_2)^{\cdot+}$, 143 $(C_7H_6ClO + 2)^+$, 141 $(C_7H_6ClO)^+$, 136 $(C_8H_{10}NO)^+$, 129 $(C_6H_4ClO + 2)^+$, 127 $(C_6H_4ClO)^+$, 121 $(C_8H_9O)^+$, 113 $(C_6H_4Cl + 2)^+$, 111 $(C_6H_4Cl)^+$, 101 $(C_5H_4Cl + 2)^+$, 99 $(C_5H_4Cl)^+$, 92 $(C_6H_4O)^{\cdot+}$, 76 $(C_6H_4)^{\cdot+}$, 64 $(C_5H_4)^{\cdot+}$, 51 $(C_4H_3)^+$, 50 $(C_4H_2)^{\cdot+}$.

11. 5-[(4-Chlorophenoxy)methyl]-1,3,4-oxadiazol-2yl-N-(2,3-dimethylphenyl)-2-sulfanyl acetamide (7k)

^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.59 (s, 1H, NH), 7.53 (br.d, J = 8.0 Hz, 1H, H-6'''), 7.21 (d, J = 8.0 Hz, 2H, H-3' & H-5'), 7.07 (t, J = 8.0 Hz, 1H, H-5'''), 6.97 (br.d, J = 7.6 Hz, 1H, H-4'''), 6.91 (br.d, J = 8.0 Hz, 2H, H-2' & H-6'), 5.19 (s, 2H, CH_2-6), 4.00 (s, 2H, CH_2-2''), 2.25 (s, 3H, CH_3-3'''), 2.15 (s, 3H, CH_3-2'''); HR-MS: 403.8825 $[M]^+$ (Calcd. for $C_{19}H_{18}ClN_3O_4S$; 403.9820); EI-MS: *m/z* 405 $(C_{19}H_{18}ClN_3O_3S)$ $[M + 2]^+$, 403 $(C_{19}H_{18}ClN_3O_3S)$ $[M]^+$, 276 $(C_{13}H_{14}N_3O_2S)^+$, 262 $(C_{12}H_{12}N_3O_2S)^+$, 222 $(C_{11}H_{12}NO_2S)^+$, 220 $(C_{11}H_{12}N_2OS)^{\cdot+}$, 194 $(C_{10}H_{12}NOS)^+$, 183 $(C_8H_6ClNO_2)^{\cdot+}$, 169 $(C_8H_6ClO_2)^+$, 167 $(C_8H_6ClNO)^{\cdot+}$, 162 $(C_{10}H_{12}NO)^+$, 148 $(C_9H_{10}NO)^+$, 143 $(C_7H_6ClO + 2)^+$, 141 $(C_7H_6ClO)^+$, 129 $(C_6H_4ClO + 2)^+$, 127 $(C_6H_4ClO)^+$, 120 $(C_8H_{10}N)^+$, 113 $(C_6H_4Cl + 2)^+$, 111 $(C_6H_4Cl)^+$, 105 $(C_8H_9)^+$, 101 $(C_5H_4Cl + 2)^+$, 99 $(C_5H_4Cl)^+$, 90 $(C_7H_6)^{\cdot+}$, 76 $(C_6H_4)^{\cdot+}$, 64 $(C_5H_4)^{\cdot+}$, 51 $(C_4H_3)^+$, 50 $(C_4H_2)^{\cdot+}$.

12. 5-[(4-Chlorophenoxy)methyl]-1,3,4-oxadiazol-2yl-N-(2,4-dimethylphenyl)-2-sulfanyl acetamide (7l)

^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.56 (s, 1H, NH), 7.67 (br.d, *J* = 8.0 Hz, 1H, H-6'"), 7.23 (br.d, *J* = 8.0 Hz, 2H, H-3' & H-5'), 6.98-6.97 (m, 2H, H-3'" & H-5'"), 6.92 (br.d, *J* = 8.0 Hz, 2H, H-2' & H-6'), 5.19 (s, 2H, CH_2-6), 4.00 (s, 2H,CH_2-2"), 2.25 (s, 3H, CH_3-2'"), 2.15 (s, 3H, CH_3-4'"); HR-MS: 403.8825 $[M]^+$ (Calcd. for $C_{19}H_{18}ClN_3O_4S$; 403.9820); EI-MS: *m/z* 405 ($C_{19}H_{18}ClN_3O_3S$) [M + 2]$^+$, 403 ($C_{19}H_{18}ClN_3O_3S$) $[M]^+$, 276 ($C_{13}H_{14}N_3O_2S)^+$, 262 ($C_{12}H_{12}N_3O_2S)^+$, 222 ($C_{11}H_{12}NO_2S)^+$, 220 ($C_{11}H_{12}N_2OS)^{\cdot+}$, 194 ($C_{10}H_{12}NOS)^+$, 183 ($C_8H_6ClNO_2)^{\cdot+}$, 169 ($C_8H_6ClO_2)^+$, 167 ($C_8H_6ClNO)^{\cdot+}$, 162 ($C_{10}H_{12}NO)^+$, 148 ($C_9H_{10}NO)^+$, 143 ($C_7H_6ClO$ + 2)$^+$, 141 ($C_7H_6ClO)^+$, 129 (C_6H_4ClO + 2)$^+$, 127 ($C_6H_4ClO)^+$, 120 ($C_8H_{10}N)^+$, 113 (C_6H_4Cl + 2)$^+$, 111 ($C_6H_4Cl)^+$, 105 ($C_8H_9)^+$, 101 (C_5H_4Cl + 2)$^+$, 99 ($C_5H_4Cl)^+$, 90 ($C_7H_6)^{\cdot+}$, 76 ($C_6H_4)^{\cdot+}$, 64 ($C_5H_4)^{\cdot+}$, 51 ($C_4H_3)^+$, 50 ($C_4H_2)^{\cdot+}$.

13. 5-[(4-Chlorophenoxy)methyl]-1,3,4-oxadiazol-2yl-N-(2,5-dimethylphenyl)-2-sulfanyl acetamide (7m)

^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.60 (s, 1H, NH), 7.70 (br.s, 1H, H-6'"), 7.23 (br.d, *J* = 8.6 Hz, 2H, H-3' & H-5'), 7.02 (d, *J* = 7.0 Hz, 1H, H-3'"), 6.91 (br.d, *J* = 8.6, 2H, H-2' & H-6'), 6.88 (d, *J* = 7.0 Hz, H-4'"), 5.19 (s, 2H, CH_2-6), 4.01 (s, 2H, CH_2-2"), 2.29 (s, 3H, CH_3-2'"), 2.16 (s, 3H, CH_3-5'"); HR-MS: 403.8825 $[M]^+$ (Calcd. for $C_{19}H_{18}ClN_3O_4S$; 403.9820); EI-MS: *m/z* 405 ($C_{19}H_{18}ClN_3O_3S$) [M + 2]$^+$, 403 ($C_{19}H_{18}ClN_3O_3S$) $[M]^+$, 276 ($C_{13}H_{14}N_3O_2S)^+$, 262 ($C_{12}H_{12}N_3O_2S)^+$, 222 ($C_{11}H_{12}NO_2S)^+$, 220 ($C_{11}H_{12}N_2OS)^{\cdot+}$, 194 ($C_{10}H_{12}NOS)^+$, 183 ($C_8H_6ClNO_2)^{\cdot+}$, 169 ($C_8H_6ClO_2)^+$, 167 ($C_8H_6ClNO)^{\cdot+}$, 162 ($C_{10}H_{12}NO)^+$, 148 ($C_9H_{10}NO)^+$, 143 ($C_7H_6ClO$ + 2)$^+$, 141 ($C_7H_6ClO)^+$, 129 (C_6H_4ClO + 2)$^+$,127 ($C_6H_4ClO)^+$, 120 ($C_8H_{10}N)^+$, 113 (C_6H_4Cl + 2)$^+$, 111 ($C_6H_4Cl)^+$, 105 ($C_8H_9)^+$, 101 (C_5H_4Cl + 2)$^+$, 99 ($C_5H_4Cl)^+$, 90 ($C_7H_6)^{\cdot+}$, 76 ($C_6H_4)^{\cdot+}$, 64 ($C_5H_4)^{\cdot+}$, 51 ($C_4H_3)^+$, 50 ($C_4H_2)^{\cdot+}$.

14. 5-[(4-Chlorophenoxy)methyl]-1,3,4-oxadiazol-2yl-N-(2,6-dimethylphenyl)-2-sulfanyl acetamide (7n)

^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.25 (s, 1H, NH), 7.26 (br.d, *J* = 8.8 Hz, 2H, H-3' & H-5'), 7.09-7.02 (m, 3H, H-3'"- H-5'"), 6.92 (br.d, *J* = 8.8 Hz, 2H, H-2' & H-6'), 5.21 (s, 2H, CH_2-6), 4.04 (s, 2H, CH_2-2"), 2.11 (s, 6H, CH_3-2'" & CH_3-6'"); HR-MS: 403.8825 $[M]^+$ (Calcd. for $C_{19}H_{18}ClN_3O_4S$; 403.9820); EI-MS: *m/z* 405 ($C_{19}H_{18}ClN_3O_3S$) [M + 2]$^+$, 403 ($C_{19}H_{18}ClN_3O_3S$) $[M]^+$, 276 ($C_{13}H_{14}N_3O_2S)^+$, 262 ($C_{12}H_{12}N_3O_2S)^+$, 222 ($C_{11}H_{12}NO_2S)^+$, 220 ($C_{11}H_{12}N_2OS)^{\cdot+}$, 194 ($C_{10}H_{12}NOS)^+$, 183 ($C_8H_6ClNO_2)^{\cdot+}$, 169 ($C_8H_6ClO_2)^+$, 167 ($C_8H_6ClNO)^{\cdot+}$, 162 ($C_{10}H_{12}NO)^+$, 148 ($C_9H_{10}NO)^+$, 143 ($C_7H_6ClO$ + 2)$^+$, 141 ($C_7H_6ClO)^+$, 129 (C_6H_4ClO + 2)$^+$, 127 ($C_6H_4ClO)^+$, 120 ($C_8H_{10}N)^+$, 113 (C_6H_4Cl + 2)$^+$, 111 ($C_6H_4Cl)^+$, 105 ($C_8H_9)^+$, 101 (C_5H_4Cl + 2)$^+$, 99 ($C_5H_4Cl)^+$, 90 ($C_7H_6)^{\cdot+}$, 76 ($C_6H_4)^{\cdot+}$, 64 ($C_5H_4)^{\cdot+}$, 51 ($C_4H_3)^+$, 50 ($C_4H_2)^{\cdot+}$.

15. 5-[(4-Chlorophenoxy)methyl]-1,3,4-oxadiazol-2yl-N-(3,4-dimethylphenyl)-2-sulfanyl acetamide (7o)

^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.39 (s, 1H, NH), 7.27 (br.s, 1H, H-2'"), 7.23 (br.d, *J* = 8.8 Hz, 2H, H-3' & H-5'), 7.06-7.03 (m, 2H, H-5'" & H-6'"), 6.84 (br.d, *J* = 8.8, 2H, H-2' & H-6'), 4.55 (s, 2H, CH_2-6), 4.00 (s, 2H, CH_2-2"), 2.26 (s, 3H, CH_3-3'"), 2.25 (s, 3H, CH_3-4'"); HR-MS: 403.8825 $[M]^+$ (Calcd. for $C_{19}H_{18}ClN_3O_4S$; 403.9820); EI-MS: *m/z* 405 ($C_{19}H_{18}ClN_3O_3S$) [M + 2]$^+$, 403 ($C_{19}H_{18}ClN_3O_3S$) $[M]^+$, 276 ($C_{13}H_{14}N_3O_2S)^+$, 262 ($C_{12}H_{12}N_3O_2S)^+$, 222 ($C_{11}H_{12}NO_2S)^+$, 220 ($C_{11}H_{12}N_2OS)^{\cdot+}$, 194 ($C_{10}H_{12}NOS)^+$, 183 ($C_8H_6ClNO_2)^{\cdot+}$, 169 ($C_8H_6ClO_2)^+$, 167 ($C_8H_6ClNO)^{\cdot+}$, 162 ($C_{10}H_{12}NO)^+$, 148 ($C_9H_{10}NO)^+$, 143 ($C_7H_6ClO$ + 2)$^+$, 141 ($C_7H_6ClO)^+$, 129 (C_6H_4ClO + 2)$^+$, 127 ($C_6H_4ClO)^+$, 120 ($C_8H_{10}N)^+$, 113 (C_6H_4Cl + 2)$^+$, 111 ($C_6H_4Cl)^+$, 105 ($C_8H_9)^+$, 101 (C_5H_4Cl + 2)$^+$, 99 ($C_5H_4Cl)^+$, 90 ($C_7H_6)^{\cdot+}$, 76 ($C_6H_4)^{\cdot+}$, 64 ($C_5H_4)^{\cdot+}$, 51 ($C_4H_3)^+$, 50 ($C_4H_2)^{\cdot+}$.

16. 5-[(4-Chlorophenoxy)methyl]-1,3,4-oxadiazol-2yl-N-(3,5-dimethylphenyl)-2-sulfanyl acetamide (7p)

^{1}H-NMR (400 MHz, $CDCl_3$, Fig. 2): δ 7.65 (br.d, *J* = 8.8 Hz, 2H, H-3' & H-5'), 7.16 (br.s, 2H, H-2'" & H-6'"), 6.99 (br.d, *J* = 8.8, 2H, H-2' & H-6'), 6.73 (br.s, 1H, H-4'"), 4.98 (s, 2H, CH_2-6), 3.86 (s, 2H, CH_2-2"), 2.26 (s, 6H, CH_3-3'" & CH_3-5'"); HR-MS: 403.8825 $[M]^+$ (Calcd. for $C_{19}H_{18}ClN_3O_4S$; 403.9820); EI-MS: *m/z* 405 ($C_{19}H_{18}ClN_3O_3S$) [M + 2]$^+$, 403 ($C_{19}H_{18}ClN_3O_3S$) $[M]^+$, 276 ($C_{13}H_{14}N_3O_2S)^+$, 262 ($C_{12}H_{12}N_3O_2S)^+$, 222 ($C_{11}H_{12}NO_2S)^+$, 220 ($C_{11}H_{12}N_2OS)^{\cdot+}$, 194 ($C_{10}H_{12}NOS)^+$, 183 ($C_8H_6ClNO_2)^{\cdot+}$, 169 ($C_8H_6ClO_2)^+$, 167 ($C_8H_6ClNO)^{\cdot+}$, 162 ($C_{10}H_{12}NO)^+$, 148 ($C_9H_{10}NO)^+$, 143 ($C_7H_6ClO$ + 2)$^+$, 141 ($C_7H_6ClO)^+$, 129 (C_6H_4ClO + 2)$^+$, 127 ($C_6H_4ClO)^+$, 120 ($C_8H_{10}N)^+$, 113 (C_6H_4Cl + 2)$^+$, 111 ($C_6H_4Cl)^+$, 105 ($C_8H_9)^+$, 101 (C_5H_4Cl + 2)$^+$, 99 ($C_5H_4Cl)^+$, 90 ($C_7H_6)^{\cdot+}$, 76 ($C_6H_4)^{\cdot+}$, 64 ($C_5H_4)^{\cdot+}$, 51 ($C_4H_3)^+$, 50 ($C_4H_2)^{\cdot+}$.

BIOLOGICAL ASSAYS:

Antibacterial assay:

The antibacterial activity was performed in sterile 96-wells microplates under aseptic environments. The method is based on the principle that microbial cell number increases as the microbial growth proceeds in a log phase of growth which results in increased absorbance of broth medium [35,36]. Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*) (clinical isolate) and gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*) (clinical isolate) were included in the study. The organisms were maintained on stock culture agar medium. The test samples with suitable solvents and dilutions were pipetted out into wells (20 µg/well). Overnight maintained fresh bacterial culture after suitable dilution with fresh nutrient broth was poured into wells (180 µL). The initial absorbance of the culture was strictly maintained between 0.12-0.19 at 540 nm. The total volume in each well was kept to 200 µL. The incubation was done at 37 °C for 16-24 hours with lid on the micro plate. The absorbance was measured at 540 nm using micro plate reader, before and after incubation and the difference was noted as an index of bacterial growth. The percent inhibition was calculated using the formula:

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

Where, X = Absorbance in control with bacterial culture

Y = Absorbance in test sample.

Results are mean of triplicate (n = 3, ± SEM). Ciprofloxacin was taken as a standard. Minimum inhibitory concentration (MIC) was measured with suitable dilutions (5-30 µg/ well) and results were calculated using EZ-Fit5 Perrella Scientific Inc. Amherst USA software, and data expressed as *MIC*.

Brine shrimp assay:

The assay was employed by the reported method [37]. Sea salt $34gL^{-1}$ was used to prepare artificial sea water. A shallow rectangular dish (22×32 cm) containing brine shrimps was maintained under constant aeration for 48h at RT for hatching brine shrimp (*Artemia salina*) eggs (Sera, Heidelberg, Germany). After hatching, active shrimps were collected without eggs from brighter portion of the dish for analysis. 10 shrimps were transferred to each vial via pasture pipette vial containing 5 mL of artificial sea water with 200, 20, 2 and 0.2 (μgmL^{-1}) of test compound from their stock solution. The vials were maintained at 25-28 °C under illumination for 24 h. After 24 h, the number of survived shrimps was counted. Finney computer program was used to analyze data and for determining LD_{50} (lethal dose that killed 50% shrimps) values.

α-Chymotrypsin inhibition assay:

The assay was performed according to the cited methods [38,39]. 100 µL of reaction mixture comprising of 60 µL (50 mM) of Tris-HCl buffer (pH 7.6), 10 µL (0.5 mM) of test compound and 15 µL (0.9 units) of enzyme was pre-incubated for 15 min. at 37 ^{o}C. Moreover, it was pre-read at 410 nm. The reaction was started by the addition of 15 µL (1.3 mM) of *N*-succinyl phenylalanine-*p*-nitroanilide (Sigma, USA). Absorbance was measured at 410 nm using Synergy HT microplate reader. After 30-60 minutes absorbance values of uninhibited enzyme assay reached 0.7-0.9. The assay included positive and negative controls. The percent inhibition was calculated by following equation:

$$\%\ age\ Inhibition\ = \frac{Control - Test}{Control} \times 100$$

IC_{50}values (concentration at which enzyme inhibition is 50 %) were calculated using EZ-Fit Enzyme Kinetics Software (Perrella Scientific Inc. Amherst, USA).

Computational methodology

The molecular docking method was used to approximate the conformation of ligands and orientation within the binding site of target protein. There are two aims of docking studies: specific structural modeling and accurate prediction of activity [40]. Molecular Docking protocol can be applied to find out the interactions between inhibitors and active site of the target protein. To study the interactions of molecular recognition MOE-Dock method was utilized [41] which allow the ligands to be flexible during docking so that the ligands can adjust their different conformations in the binding pocket of the receptor. This method has been used in our study to find the best binding modes between the synthesized ligands and α-chymotrypsin enzyme.

Protein preparation:

The protein (α-chymotrypsin) molecules were retrieved from Protein Data Bank. Water molecules were removed and the 3D protonation of the protein molecule was performed using MOE applications. The energy of the protein molecules were minimized via energy minimization algorithm of MOE tool. The following parameters were used for energy minimization; gradient: 0.05, Force Field: MMFF94X + Solvation, Chiral Constraint: Current Geometry. Energy minimization was terminated when the root mean square gradient falls below the 0.05. The minimized structure was used as the template for docking.

Molecular docking: The binding mode of the ligands into the binding pocket of protein molecule was predicted by MOE-Dock implemented in MOE. After the completion of docking we analyze the best poses for hydrogen bonding/π-π interactions by using MOE applications.

Statistical Analysis:

All the measurements were done in triplicate and statistical analysis was performed by Microsoft Excel 2010. Results are presented as mean ± SEM.

RESULTS AND DISCUSSION:

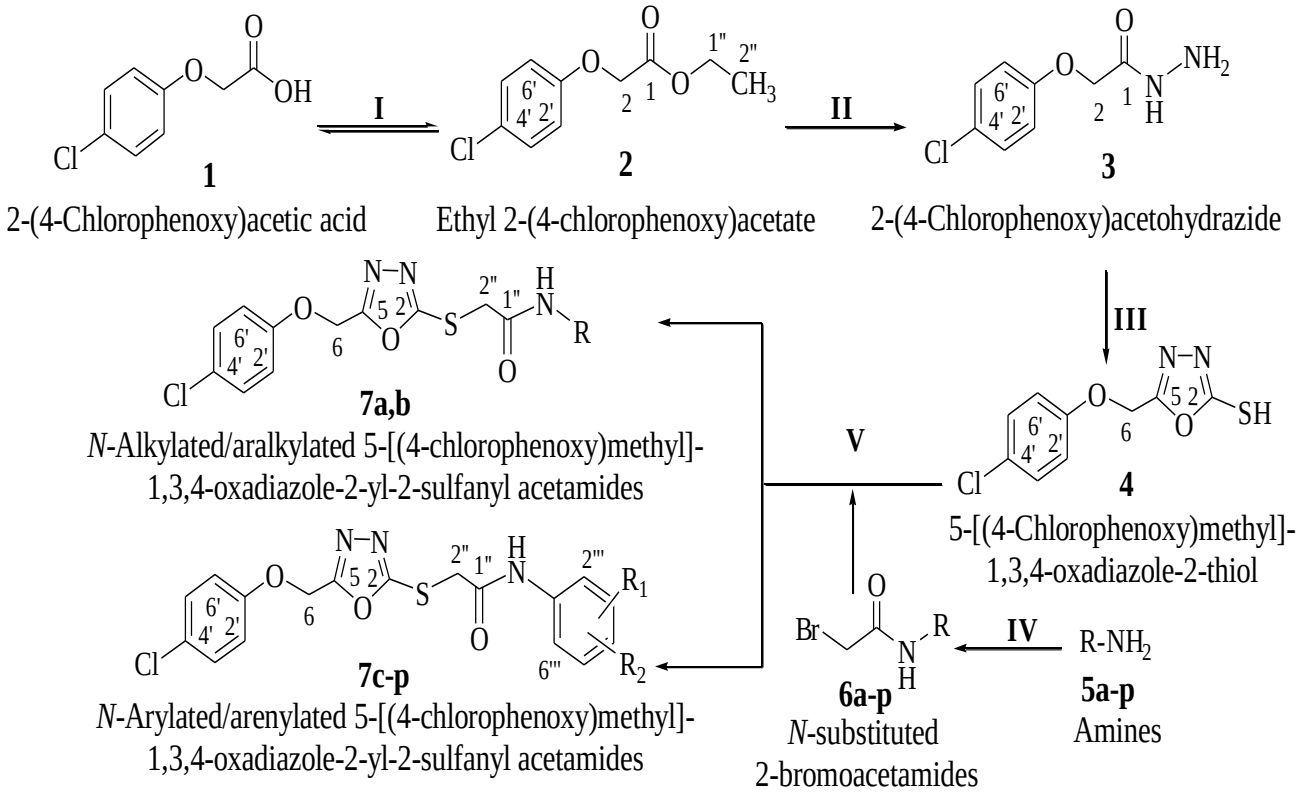
Chemistry:

4-Chlorophenoxyacetic acid (**1**)was used as a precursor to synthesize various *N*-substituted-5-[(4-chlorophenoxy)methyl]-1,3,4-oxadiazole-2yl-2-sulfanylacetamides.The first step in the synthesis involved preparation of ethyl 2-(4-chlorophenoxy)acetate (**2**) by acid-catalyzed esterification of 4-chlorophenoxyacetic acid (**1**) in absolute alcohol which was then converted to2-(4-chlorophenoxy)acetohydrazide (**3**) upon reaction with hydrazine hydrate in methanol under stirring at 0 °C to room temperature. The ring closure reaction on **3** underwent in the presence of carbon disulfide to a stirred solution of alcoholic potassium hydroxide after which the reaction was refluxed under stirring for 6 h to yield 5-[(4-Chlorophenoxy)methyl]-1,3,4-oxadiazole-2-thiol (**4**). The nucleophilic substitution reaction of **4** with various *N*-substituted-2-bromoacetamides (**6a-p**)was carried out under stirring at room temperature for 3h in *N*,*N*-dimethylformamide and LiH which acts as a catalyst and base. The reaction mixture was quenched with cold distilled water and the precipitates obtained were filtered and dried to yield the targeted *N*-substituted-5-[(4-chlorophenoxy)methyl]-1,3,4-oxadiazole-2yl-2-sulfanyl acetamides (**7a-p**,Scheme 1, Table 1). The structures of the synthesized compounds were elucidated by IR (Table 2),^{1}H-NMR and mass spectral data as illustrated in experimental section. The ^{1}H-NMR

spectra of compounds **4** and **7p** are shown as **Figure 1** and **Figure 2** respectively, while mass fragmentation pattern of the compounds**7 k-p** is sketched as **Figure 3**.

Structure determination:

The compound **7a** was obtained as a white solid; yield: 90 % and m.p. 134 °C. The molecular formula $C_{17}H_{20}ClN_3O_3S$ was established by HR-MS showing molecular ion peak at *m/z* 381.8776 and by counting the number of protons in the ^{1}H-NMR spectrum. The EI-MS spectrum showed molecular ion peak at *m/z* 381 M^+ $[C_{17}H_{20}ClN_3O_3S]^{\cdot+}$ (Figure 3).The Infrared spectrum demonstrated absorption bands at 2294 (C-H aromatic stretching), 1641 (C=O stretching of amide), 1574 (C=N imine stretching), 1430 (C=C aromatic ring stretching), 1066 (C-Cl stretching) (Table 2). In the aromatic region of the ^{1}H-NMR spectrum, appearance of typical A_2B_2 spin system by two *ortho*-coupled doublets, one at δ 6.91, J = 6.8 Hz having integration for 2 protons (H-3' & H-5') and other at δ 6.38, J = 6.8 Hz for two protons (H-2' & H-6') revealed that the aromatic ring is disubstituted at *para* (1,4) positions. A singlet at δ 5.18 was due to a methylene group (CH_2-6) flanked in between the oxadiazole core and an electronegative oxygen atom. Similarly, another singlet at δ 3.80 was due to a methylene group (CH_2-1″) linking the amide and sulfanyl groups. In the aliphatic region of the spectrum, a multiplet at δ 3.73-3.70 was assigned to a methine proton (CH-1‴) while the multiplet at δ 1.82-1.11 was integrated for 10 protons (five methylene groups) and hence these two corroborated signals were characteristic of a cyclohexyl group substituted at nitrogen atom in the molecule. Therefore, on the basis of above cumulative evidences, the structure of **7a**was confirmed as 5-[(4-chlorophenoxy)methyl]-1,3,4-oxadiazol-2yl-*N*-(cyclohexyl)-2-sulfanyl acetamide. The structures of other *N*-substituted acetamides were also characterized in a similar manner. All the signals in ^{1}H-NMR spectrum of **7a-p** thoroughly affirmed the successful substitutions on the parent 1,3,4-oxadiazole core.



Scheme 1: Outline for the synthesis of*N*-substituted-5-[(4-chlorophenoxy)methyl]-1,3,4-oxadiazole-2yl-2-sulfanyl acetamides (7a-p). Reagents & conditions: (I) H_2SO_4, ethanol, reflux for 6 h; (II) $N_2H_4.H_2O$, methanol, stirring for 0.5 h at 0 °C to RT; (III) CS_2, KOH, ethanol, reflux for 6 h; (IV) bromoacetyl bromide, amine (5a-p, one in each case), H_2O, 10% Na_2CO_3, stirring for 20 min, 0 °C to RT; (V) *N*-substituted-2-bromoacetamide (6a-p,one in each case), DMF, LiH, stirring for 3 h at RT.

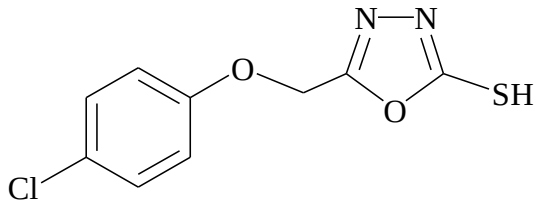
Table 1: Different substitutions in 7a-p.

Codes	R
7a	Cyclohexyl
7b	Benzyl

Codes	7c	7d	7e	7f	7g	7h	7i	7j	7k	7l	7m	7n	7o	7p
R_1	H	H	H	H	H	H	H	H	2-CH_3	2-CH_3	2-CH_3	2-CH_3	3-CH_3	3-CH_3
R_2	H	4-C_2H_5	2-CH_3	3-CH_3	2-OCH_3	2-OC_2H_5	3-OC_2H_5	4-OC_2H_5	3-CH_3	4-CH_3	5-CH_3	6-CH_3	4-CH_3	5-CH_3

Table 2: Physical parameters of *N*-substituted 5-[(4-chlorophenoxy)methyl]-1,3,4-oxadiazole-2yl-2-sulfanyl acetamides (7a-p).

Code	Appearance	M.P. (°C)	Yield (%)	Mol. For./Wt. ($gmol^{-1}$)	IR KBr, $\nu_{max}(cm^{-1})$
7a	White solid	134	90	$C_{17}H_{20}ClN_3O_3S$ 381	2924 (C-H aromatic str.), 1641 (C=O amide str.), 1574 (C=N imine str.), 1430 (C=C aromatic ring str.), 1066 (C-Cl str.)
7b	Cream solid	203	96	$C_{17}H_{14}ClN_3O_3S$ 375	2922 (C-H aromatic str.), 1630 (C=O C=O amide str.), 1580 (C=N imine str.), 1479 (C=C aromatic ring str.), 1435 (-CH_2 bend), 1021 (C-Cl str.)
7c	White powder	178	91	$C_{18}H_{16}ClN_3O_3S$ 389	2769 (C-H aromatic str.), 1626 (C=O amide str.), 1490 (C=N imine str.), 1478 (C=C aromatic ring str.), 1440 (-CH_2 bend), 1161 (C-Cl str.)
7d	Light yellow powder	180	89	$C_{19}H_{18}ClN_3O_3S$ 403	2844 (C-H aromatic str.), 1640 (C=O amide str.), 1489 (C=N imine str.), 1468 (C=C aromatic ring str.), 1452 (-CH_2 bend), 1060 (C-Cl str.)
7e	White pallets	135	98	$C_{18}H_{16}ClN_3O_3S$ 389	2844 (C-H aromatic str.), 1639 (C=O amide str.), 1588 (C=N imine str.), 1386 (C=C aromatic ring str.), 1470 (-CH_2 bend), 1281 (-CH_3 bend),
7f	Greyish powder	140	87	$C_{18}H_{16}ClN_3O_3S$ 389	2861 (C-H aromatic str.), 1624 (C=O amide str.),1491 (C=N imine str.), 1376 (C=C aromatic ring str.), 1401 (-CH_2 bend), 1250 (-CH_3 bend)
7g	White solid	198	93	$C_{18}H_{16}ClN_3O_4S$ 405	2930 (C-H aromatic str.), 1629 (C=O amide str.), 1491 (C=N imine str.), 1467 (C=C aromatic ring str.), 1064 (C-Cl str.)
7h	Off-white solid	172	89	$C_{19}H_{18}ClN_3O_4S$ 419	2935 (C-H aromatic str.), 1621 (C=O amide str.), 1533 (C=N imine str.), 1463 (C=C aromatic ring str.), 1237 (-CH_3 str.), 1152 (C-Cl str.)
7i	Cream colored solid	173	87	$C_{19}H_{18}ClN_3O_4S$ 419	2940 (C-H aromatic str.), 1644 (C=O amide str.), 1572 (C=N imine str.), 1479 (C=C aromatic ring str.), 1333 (-CH_2 bend), 1152 (C-Cl str.)
7j	Light brown powder	175	89	$C_{19}H_{18}ClN_3O_4S$ 419	2910 (C-H aromatic str.), 1647 (C=O amide str.), 1492 (C=N imine str.), 1380 (C=C aromatic ring str.), 1380 (-CH_2 bend), 1162 (C-Cl str.)
7k	White pallets	168	95	$C_{19}H_{18}ClN_3O_3S$ 403	2938 (C-H aromatic str), 1647 (C=O amide str.), 1595 (C=N imine str.), 1482 (C=C aromatic ring str.), 1063 (C-Cl str.)
7l	White solid	130	92	$C_{19}H_{18}ClN_3O_3S$ 403	2878 (C-H aromatic str.), 1638 (C=O amide str.), 1647 (C=N imine str.), 1512 (C=C aromatic ring str.), 1063 (C-Cl str.)
7m	White pallets	130	97	$C_{19}H_{18}ClN_3O_3S$ 403	2940 (C-H aromatic str.), 1643 (C=O amide str.), 1598 (C=N imine str.), 1479 (C=C aromatic ring str.), 1078 (C-Cl str.)
7n	White solid	148	94	$C_{19}H_{18}ClN_3O_3S$ 403	2940 (C-H aromatic str.), 1645 (C=O amide str.), 1600 (C=N imine str.), 1479 (C=C aromatic ring str.), 1078 (C-Cl str.)
7o	Yellow powder	184	96	$C_{19}H_{18}ClN_3O_3S$ 403	2928 (C-H aromatic str.), 1647 (C=O amide str.), 1610 (C=N imine str.), 1575 (C=C aromatic ring str.), 1147 (C-Cl str.)
7p	White solid	181	92	$C_{19}H_{18}ClN_3O_3S$ 403	2894 (C-H aromatic str.), 1642 (C=O amide str.), 1600 (C=N imine str.), 1512 (C=C aromatic ring str.), 1178 (C-Cl str.)



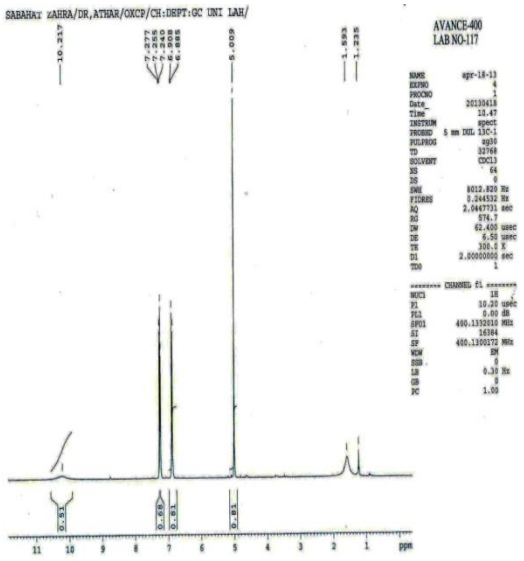
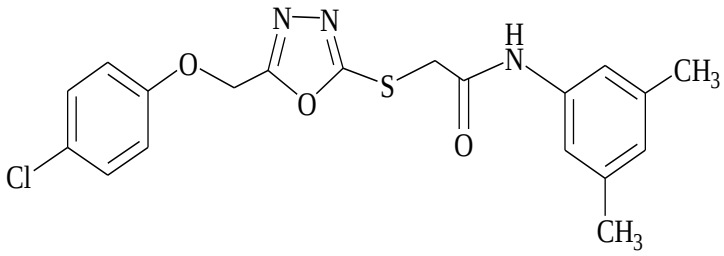


Figure1: ^{1}H-NMR Spectrum of 5-[(4-chlorophenoxy)methyl]-1,3,4-oxadiazole-2-thiol 4.



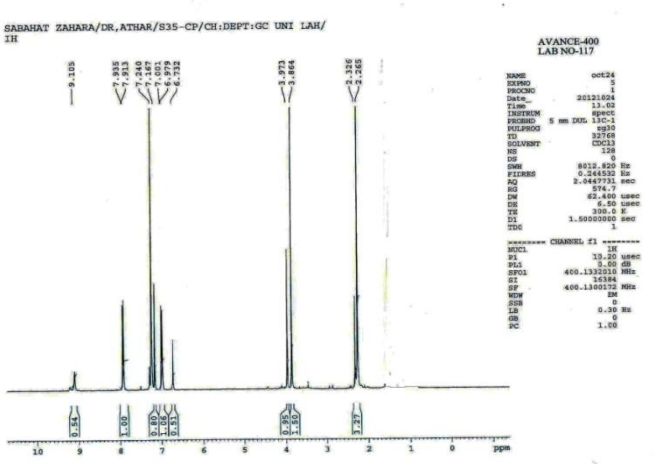


Figure2: ^{1}H-NMR Spectrum of 7p.

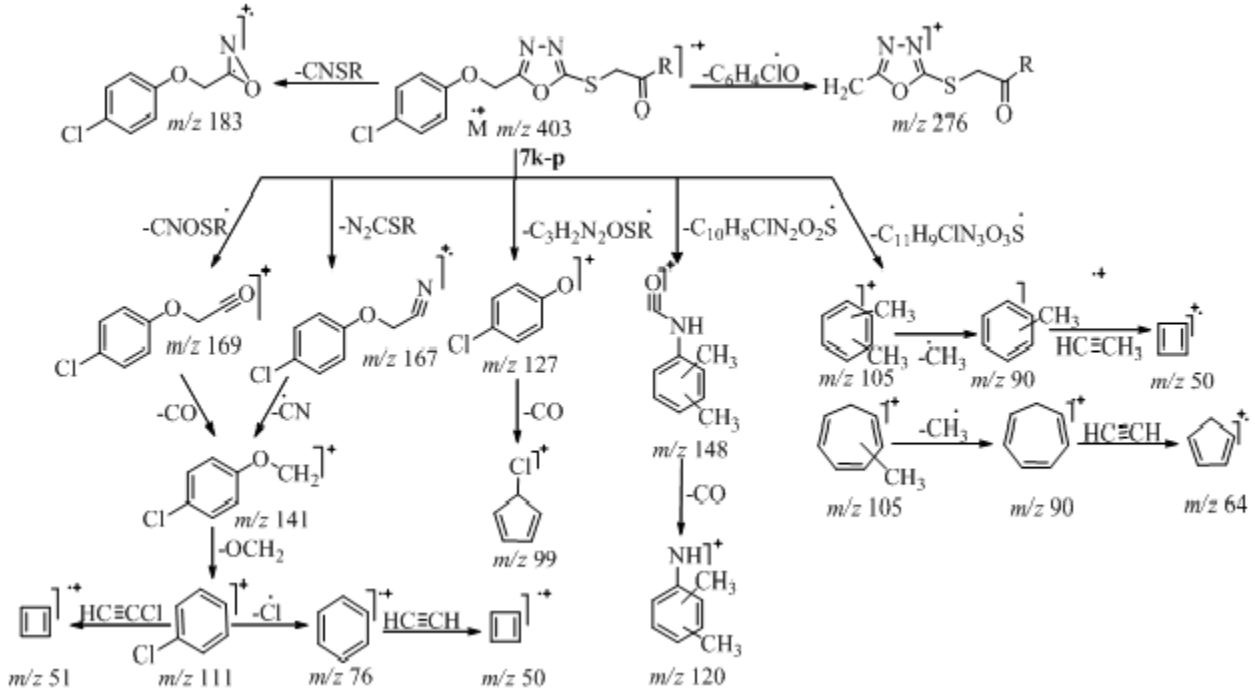


Figure 3: Mass fragmentation pattern of *N*-substituted-5-[(4-chlorophenoxy)methyl]-1,3,4-oxadiazole-2yl-2-sulfanyl acetamides(7k-p).

BIOLOGICAL SCREENING:

Antibacterial activity (in vitro):

All the synthesized derivatives displayed excellent to moderate minimal inhibitory concentration against *S. typhi* (-), *E. coli* (-), *P. areoginosa* (-), *K. pneumonae* (-), *B. subtilis* (+) and *S. aureus* (+). The molecule **7o** displayed excellent MIC value i.e. 8.78±0.20µg/well against *K. pneumonae* (-) which is even lower than the standard ciprofloxacin having 9.01±1.34µg/ well.This exceptional antibacterial potential of this molecule might be attributed to the presence of *N*-(3,4-dimethylphenyl) acetamide moiety. This molecule also exhibited very comparable MICs against *S. typhi* (-)and *E. coli* (-) with values of 9.50±2.59 and 10.70±4.00 µg/well respectively, relative to the standard ciprofloxacin having values of 8.43±0.98 and 8.71±1.20 µg/well respectively. In general, the synthesized derivatives depicted admirable antibacterial activity against studied gram positive and gram negative strains as it is evident from their *MIC* values with exception of compound **7p** which showed no minimal inhibitory concentration against all strains. The data is tabulated in **Table 3** & **Table 4**.

Table 3: Antibacterial activity of *N*-substituted 5-[(4-chlorophenoxy)methyl]-1,3,4-oxadiazole-2yl-2-sulfanyl acetamides (7a-p).

Bacterial Strains	***S. typhi (-)***		***E. coli (-)***		***K. pneumonae (-)***		***P. aeroginosa (-)***	
Codes	***% age Inhibition***	***MIC***	***% age Inhibition***	***MIC***	***% age Inhibition***	***MIC***	***% age Inhibition***	***MIC***
7a	55.22±0.94	17.10±1.21	60.22±1.33	14.15±1.67	46.33 ±2.87	-	56.98±2.02	16.74±3.19
7b	69.44±1.94	15.06±1.47	69.56±3.05	13.36±2.14	50.35±2.27	19.84±2.65	64.53±2.51	15.10±3.01
7c	72.33±2.73	12.43±2.31	73.44±1.49	12.03±2.25	74.30±2.66	13.11±1.38	70.12±3.11	13.32±3.00
7d	79.29±2.71	13.99±1.63	65.85±2.77	15.75±2.43	62.76±3.32	16.45±0.43	79.20±0.83	11.78±1.71
7e	60.13±2.38	16.24±2.35	72.96±3.81	12.07±1.09	70.33 ±1.76	15.02±2.25	63.35±0.94	15.69±2.31
7f	56.00±1.87	16.36±1.58	71.80±3.61	13.65±0.25	54.42±2.46	18.18±1.81	70.93±1.52	13.01±2.87
7g	65.72±1.83	16.09±2.97	72.00±3.00	14.44±1.58	63.83±3.81	16.93±1.94	74.47±0.17	10.01±1.63
7h	58.71±3.63	15.08±1.62	67.89±3.12	13.29±1.41	50.01±2.55	19.95±2.41	53.87±2.98	18.23±1.04
7i	69.61±4.71	16.65±2.46	69.77±3.41	13.12±1.70	45.35±2.05	-	76.51±2.04	10.35±1.46
7j	64.89±3.78	17.37±1.56	57.22±2.13	16.69±2.71	30.58±3.69	-	58.37±1.56	15.64±1.55
7k	68.94±1.16	17.02±1.15	59.72±0.28	15.82±1.88	49.36±2.74	-	63.78±0.52	14.48±1.98
7l	68.55±2.77	15.75±2.32	53.05±3.51	16.00±0.58	63.02±2.09	15.49±2.75	72.38±0.52	12.44±0.49
7m	79.44±3.71	14.82±0.26	76.00±1.44	13.87±3.21	70.11±3.44	13.23±1.97	78.02±4.65	12.12±1.62
7n	62.92±2.25	16.21±1.75	75.04±1.12	12.83±1.77	64.32±1.88	15.02±0.98	66.83±1.45	14.35±1.23
7o	80.33±1.11	9.50±2.59	81.83±4.16	10.70±4.00	77.12±0.84	8.78±0.20	75.76±3.29	13.08±2.21
7p	40.89±1.72	-	32.89±2.63	-	34.19±1.62	-	36.74±2.81	-

Ciprofloxacin	**90.23±1.87**	**8.43±0.98**	**89.93±2.43**	**8.71±1.20**	**91.76±1.54**	**9.01±1.34**	**91.48±1.50**	**9.13±2.31**

Table 4: Antibacterial activity of *N*-substituted 5-[(4-chlorophenoxy)methyl]-1,3,4-oxadiazole-2yl-2-sulfanyl acetamides(7a-p).

Bacterial Strains	***B. subtilis (+)***		***S. aureus (+)***	
Codes	***% age Inhibition***	***MIC***	***% age Inhibition***	***MIC***
7a	57.41±2.21	17.21±1.87	56.20±3.98	16.74±3.19
7b	68.61±2.79	14.33±3.01	75.40±3.62	15.10±3.01
7c	65.30±1.93	14.18±2.73	67.50±1.18	13.32±3.00
7d	69.54±0.88	14.77±2.44	53.67±3.17	11.78±1.71
7e	76.25±1.25	12.34±2.33	69.56±3.94	15.69±2.31
7f	63.46±1.66	16.80±4.77	68.70±3.11	13.01±2.87
7g	62.50±1.00	16.61±1.55	72.40±0.00	10.01±1.63
7h	56.60±1.31	17.69±0.59	62.30±2.21	18.23±1.04
7i	67.52±1.27	14.59±2.35	75.00±0.34	10.35±1.46
7j	58.30±2.20	17.45±1.55	62.50±3.41	15.64±1.55
7k	52.75±3.21	18.96±0.68	69.90±0.70	14.48±1.98
7l	56.86±0.23	16.30±2.06	70.75±1.65	12.44±0.49
7m	65.02±1.77	15.43±2.64	75.25±0.35	12.12±1.62
7n	65.13±3.96	16.24±0.64	59.02±2.06	14.35±1.23
7o	73.20±1.80	14.10±1.77	85.80±0.20	13.08±2.21
7p	11.61±3.23	-	52.60±2.28	-
Ciprofloxacin	**90.95±1.69**	**8.99±2.12**	**88.17±2.96**	**9.13±2.31**

Brine shrimp assay:

N-Substituted-5-[(4-chlorophenoxy)methyl]-1,3,4-oxadiazole-2yl-2-sulfanyl acetamides (**7a-p**) were evaluated for LD_{50} level and compounds **7c**, **7e** and **7l** showed cytotoxicity having LD_{50} level of 3.8, 2.4 and 2.2 as compared to the standard Doxorubicin having value of 5.21, as depicted in **Figure 4**.

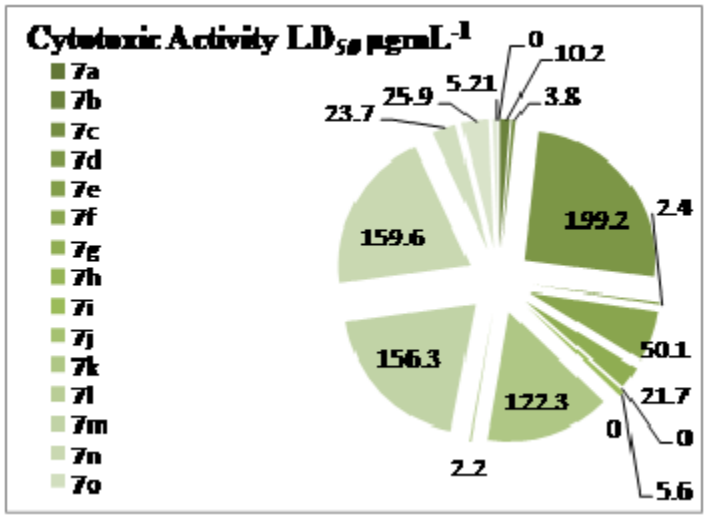


Figure 4: Cytotoxic analysis of *N*-substituted-5-[(4-chlorophenoxy)methyl]-1,3,4-oxadiazole-2yl-2-sulfanyl acetamides (7a-p).

α-Chymotrypsin assay:

All the synthesized derivatives were screened for anti-enzymatic potential against α-chymotrypsin. Most of the derivatives were found inactive but few of them showed good to moderate activity as presented in **Figure 5**. Amongst the series,5-[(4-chlorophenoxy)methyl]-1,3,4-oxadiazol-2yl-*N*-(cyclohexyl)-2-sulfanyl acetamide (**7a**) displayed good inhibitory potential with IC_{50} value of 36.94 ± 0.13 M relative to chymostatin havingIC_{50} of 8.24± 0.02 M.

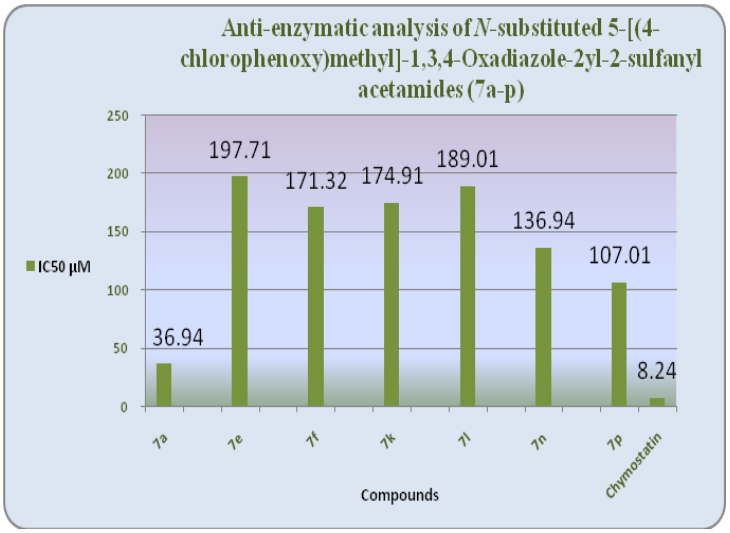
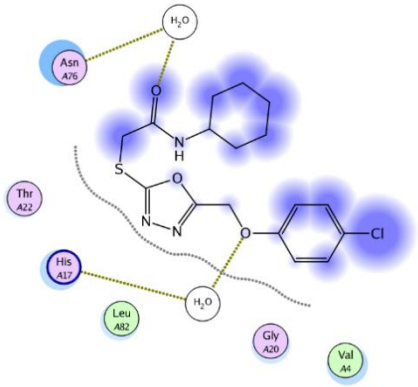


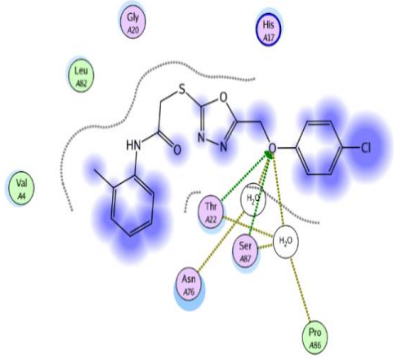
Figure 5: Chymotrypsin Enzyme Inhibition of *N*-substituted-5-[(4-chlorophenoxy)methyl]-1,3,4-oxadiazole-2yl-2-sulfanyl acetamides (7a-p).

Molecular docking:

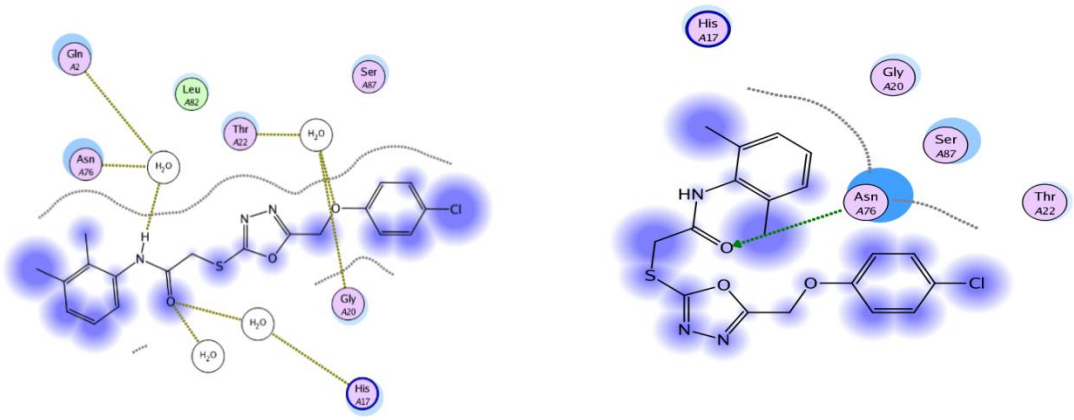
The molecular modeling studies correlate well with the anti-enzymatic potential against *α*-chymotrypsin enzyme and the important correlations of some molecules are shown in Figure 6 and Figure 7. In 2D analysis (a), the molecule 7a showed interactions with 2 residues of the binding site of *α*-chymotrypsin protein, one with the oxygen atom of the chlorophenoxy moiety and other with the oxygen atom of acetamide linkage to the HisA17 and AsnA75 respectively, via H_2O molecule. The analysis (b) showed binding modes of the *O*-atom of chlorophenoxy ring in 7e with ThA22, SerA87 connected directly and to AsnA76 and ProA86 via H_2O molecule. The analysis (c) illustrated the interactions of *N*-atom of substituted acetamide moiety in 7k on the binding pocket of enzyme with GlnA2 and AsnA76 residues via H_2O molecule, while *O*-atom of the chlorophenoxy linkage shows interactions with ThrA22 and GlnA20 via H_2O.The analysis (d) depicted the direct interaction with AsnA76 residue by the *O*-atom of acetamide portion 7n molecule. The 3D analysis (e) revealed interactions by 7p with two residues Pro86 & Thr22 of the target protein and (f) showed for the molecule 7f, two amino acid residues Thr22E & Pro86 are major contributors in interactions.



(a, 7a)

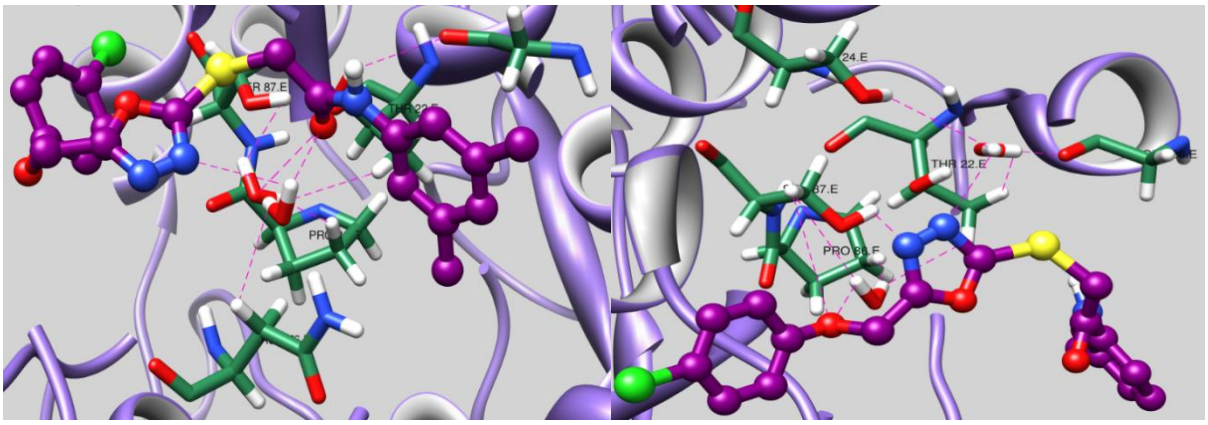


(b, 7e)



(c, 7k) **(d, 7n)**

Figure 6: 2D molecular analysis of the binding modes of 7a, 7e, 7kand 7n.



(e, 7p) **(f, 7f)**

Figure 7: 3D molecular analysis of the binding modes of 7p and 7f.

CONCLUSION

5-[(4-Chlorophenoxy)methyl]-1,3,4-oxadiazole-2-thiolwas synthesized by ring closure reaction of the corresponding acetohydrazide in CS_2 in ethanol and potassium hydroxide as a base, which was further transformed to a series of *N*-substituted derivatives by reaction with *N*-substituted-2-bromoacetamides in DMF/LiH with an ambition to explore novel therapeutic agents with elevated antibacterial and anti-enzymatic potential. It can be concluded that based on our aim and literature data this class of compounds certainly holds great potential towards the pursuit in discovery of superior antibacterial agents with less cytotoxicity. The synthesized derivatives were also found to be moderate inhibitors of α-chymotrypsin enzyme as depicted by anti-enzymatic analysis which was further supported by computational analysis. Further modifications in the parent oxadiazole core may lead to expand our research horizons with enhanced and marvelous biological activities.

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