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Original Research Article

Synthesis of 2-{[5-(aralkyl/aryl)-1,3,4-oxadiazol-2-yl]sulfanyl}-N-(4-methyl-1,3-thiazol-2-yl)acetamides: Novel bi-heterocycles as potential therapeutic agents

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Abstract

Purpose: *To evaluate the therapeutic potential of new bi-heterocycles containing a 1,3-thiazole and 1,3,4-oxadiazole in the skeleton against Alzheimer's disease and diabetes, supported by in-silico study.*
Methods: *The synthesis was initiated by the reaction of 4-methyl-1,3-thiazol-2-amine (**1**) with bromoacetyl bromide (**2**) in aqueous basic medium to obtain an electrophile,2-bromo-N-(4-methyl-1,3-thiazol-2-yl)acetamide (**3**). In parallel reactions, a series of carboxylic acids, **4a-r**, were converted through a sequence of three steps, into respective 1,3,4-oxadiazole heterocyclic cores, **7a-r**, to utilize as nucleophiles. Finally, the designed molecules, **8a-r**, were synthesized by coupling 7a-r individually with **3** in an aprotic polar solvent. The structures of these bi-heterocycles were elucidated by infrared (IR), electron ionization-mass spectrometry (EI-MS), proton nuclear magnetic resonance (^{1}H-NMR) and carbon nuclear magnetic resonance (^{13}C-NMR). To evaluate their enzyme inhibitory potential, **8a-r** were screened against acetylcholinesterase (AChE), but brine shrimp lethality bioassay.*
Results: *The most active compound against AChE was **8l** with half-maximal inhibitory concentration (IC_{50}) of 17.25 ± 0.07 µM. Against BChE, the highest inhibitory effect was shown by **8k** (56.23 ± 0.09 µM). Compound **8f** (161.26 ± 0.23µM) was recognized as a fairly good inhibitor of urease. In view of its inhibition of α-glucosidase, **8o** (57.35 ± 0.17µM) was considered a potential therapeutic agent.*
Conclusion: *The results indicate that some of the synthesized products with low toxicity exhibit notable enzyme inhibitory activity against selected enzymes compared with the reference drug, and therefore, are of potential therapeutic interest*

Keywords: *4-Methyl-1,3-thiazol-2-amine,1,3,4-Oxadiazole, Cholinesterases, α-Glucosidase, Urease, Brine shrimp*

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INTRODUCTION

The chemistry and biological study of heterocyclic compounds has been an interesting field for a long time in medicinal chemistry. Thiazoles play a crucial role in the activity of biological compounds. For example, the thiazole ring is a component of vitamin B1 (thiamine), an important coenzyme of carboxylases. This heterocycle is also present in penicillins, which serve as antibiotics [1]. In addition, high antioxidant, anti-inflammatory and inhibitory effects of thiazoles have been observed *in vitro* in their action against parasites (*Plasmodium* and *Trypanosoma*) and fungi (*Candida albicans)* [2]. Similarly, 1,3,4-oxadiazoles have demonstrated a range of bioactivities [3-5].Molecules bearing this moiety are known to possess anti-parasitic, hypoglycemic, anti-inflammatory, antifungal and antibacterial activities [6-8].

Cholinesterases, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), belong to the class of serine hydrolases. Acetylcholine activity is terminated at cholinergic synapses by these enzymes, and these enzymes are also found at neuromuscular junctions and cholinergic brain synapses [9]. It is known that BChE is associated with Alzheimer's plaque in notably elevated quantities. The inhibitors of these enzymes may have future prospects as curative agents for Alzheimer's disease [10]. α-Glucosidase inhibitors (AGIs) such as acarbose and miglitol have been approved for clinical use in controlling the digestion of complex carbohydrates in the gut and may be used in the treatment of patients with type 2 diabetes or impaired glucose tolerance [11]. Urease is involved in different pathogenic processes such as pyelonephritis, peptic ulceration, hepatic encephalopathy, urolithiasis and urinary catheter encrustation [12]. Molecular docking analysis approximates ligands regarding their orientation and conformation at the binding site of target proteins. The precise prediction of activity and structural modeling can be achieved by docking studies. Furthermore, it elucidates the active site of proteins and interactions by inhibitors [13].

New drugs are continually being synthesized, aimed at treating and/or preventing various human diseases. The documented bioactivities of heterocyclic compounds prompted us to synthesize molecules having an amalgamation of two heterocyclic cores, thiazole and 1,3,4-oxadiazole, linked through an acetamide. As it is known that structural modifications have an effect on the therapeutic behavior of drugs [14], the molecules were synthesized with variation of the groups attached to the 5-position of the 1,3,4-oxadiazole core. The whole series of compounds was tested against the four aforementioned enzymes and also cytotoxicity.

EXPERIMENTAL

General

All the chemicals and analytical grade solvents were purchased from Sigma Aldrich, Alfa Aesar (Germany) and Merck through local suppliers. Pre-coated silica gel Al-plates were used for TLC with ethyl acetate and *n*-hexane as solvent system. Spots were detected under UV light at 254 nm. With open capillary tubes, a Gallonkamp apparatus was used to determine the melting point. IR spectra were recorded using the KBr pellet method on a Jasco-320-A spectrometer. ^{1}H-NMR spectra were recorded at 600 MHz in DMSO with a Bruker spectrometer. Mass spectra (EIMS) were obtained on a JEOL JMS-600H instrument with data system. The coupling constant (*J*) is given in Hz and chemical shift (δ) in ppm. The abbreviations used in the interpretation of ^{1}H NMR spectra are as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; br.t, broad triplet; q, quartet; quin, quintet; sex, sextet; sep, septet; m, multiplet.

Procedure for synthesis of 2-bromo-N-(4-methyl-1,3-thiazol-2-yl)acetamide (3)

4-Methyl-1,3-thiazol-2-amine (**1**;0.038 mol) was dissolved in 25 mL of distilled water in an iodine flask (100 mL) and 20 % aq. $Na_2CO_3$was added to adjust the pH to 9-10. 2-Bromoethanoyl bromide (**2**; 0.038 mol; bromoacetyl bromide) was added gradually with vigorous shaking and the mixture then allowed to stir for 2 h. Reaction completion was monitored by TLC. Excess ice-cold distilled water (40 mL) was added, and the precipitate formed was collected by filtration. This precipitate of **3** was washed with distilled water and dried.

General procedure for synthesis of ethyl aralkyl/arylcarboxylates (5a-r)

Aralkyl/arylcarboxylic acids (**4a-r**; 2.5 g each) were refluxed, individually, with 60 mL of EtOH for 4-5 h in the presence of conc. H_2SO_4 (1.25 mL) in a 250-mL round-bottom flask. TLC plates were used to round-bottom monitor the reactions. Excess distilled water (150 mL) was added after maximum completion of the reaction, and the pH was adjusted to 8-10 with 20 % aq. Na_2CO_3. The product was collected through sequential extraction with $CHCl_3$ (50 mL × 3). $CHCl_3$ was distilled off to collect the products. In some cases, the products were collected by filtration.

The esters obtained, **5a-r**, were then used further.

General procedure for synthesis of aralkyl/arylcarbohydrazides (6a-r)

Ethyl esters (**5a-r**; 4.5 mL) were refluxed with 80% $N_2H_4.H_2O$ (7.2 mL) for 3-4 h in 20 mL of EtOH in a round-bottom flask (100 mL). The reaction was monitored by TLC. At completion, excess ice-cold distilled H_2O (60 mL) was added to obtain the precipitate, which was filtered off, washed with distilled H_2O and dried to acquire the compounds, **6a-r**.

General procedure for synthesis of 5-aralkyl/aryl-1,3,4-oxadiazol-2-thiol (7a-r)

Solid KOH (0.029 mol) was dissolved in 25 mL of EtOH on reflux in a 100-mL round-bottomflask. Aralkyl/arylcarbohydrazides (**6a-r**; 0.029 mol,), individually, were refluxed with CS_2 (0.058 mol) in this alkaline EtOH for 5-6 h. Reaction was monitored by TLC. At completion, excess ice-cold distilled H_2O (60 mL) was added to form a homogeneous solution. pH was adjusted to 5-6 by adding dilute HCl, and the precipitate formed was filtered off, washed with distilled H_2O and dried. The products formed, **7a-r**, were also re-crystallized from EtOH.

General procedure for synthesis of 2-{[5-aralkyl/aryl-1,3,4-oxadiazol-2-yl]sulfanyl}-*N*-(4-methyl-1,3-thiazol-2-yl)acetamides (8a-r)

5-Aralkyl/aryl-1,3,4-oxadiazol-2-thiols (**7a-r**; 0.004 mol), individually, were dissolved in DMF (11 mL) in a 50-mL round-bottom flask. LiH (0.004 mol) was then added, and the mixture was stirred for 0.5 h. 2-Bromo-*N*-(4-methyl-1,3-thiazol-2-yl)acetamide (**3**; 0.004 mol) was added and the mixture further stirred for 4-6 h. Reaction completion was confirmed by TLC. Excess ice-cold distilled water (25 mL) was then added gradually to the reaction mixture with continuous stirring. Aqueous NaOH was added dropwise with gentle shaking to adjust the pH to 8-10. The reaction mixture was stirred for 1 h, and the precipitates of **8a-r** were filtered off, washed with distilled H_2O and dried.

Enzyme inhibition assays

Cholinesterase inhibition assay

AChE and BChE inhibitory activity was determined in 96-well microplates according to a reported method [15] with slight modifications. The reaction mixture totaled 100 µL and contained the following: 60µL of 50 mM Na_2HPO_4 buffer, pH 7.7; 10 µL of acetylthiocholine iodide for AChE and butyrylthiocholine bromide for BChE, at final concentrations of acetylthiocholine iodide;10 µL of test compound at 0.5 mM, and 10 µL of enzyme (0.005 U/well AChE or BChE). The microplates were mixed, pre-read, and incubated for 10 min at 37 ºC. The enzyme reaction product was determined by the addition of 0.5 mM Ellman's reagent (5,5'-dithio-bis-[2-nitrobenzoic acid)and further incubation for 15 min at 37ºC, followed by reading absorbance at 405 nm. All experiments were performed with controls and in triplicate. Eserine (0.5 mM) served as the positive control. Inhibition (H) was calculated using Eq 1.

$$H\ (\%) = \{(Ac - At)/Ac\}100 \quad \ldots\ldots\ldots\ldots\ldots\ (1)$$

where Ac = absorbance of control and At = absorbance of test compound.

IC_{50} values were calculated using EZ–Fit Enzyme kinetics software (Perrella Scientific Inc. Amherst, NH, USA).

α-Glucosidase inhibition assay

This enzyme inhibition assay was carried out in 96-well as previously reported [16], with modifications, in which a 100-µL reaction mixture contained 70 µL of phosphate-buffered saline, pH 6.8, 10 µL of test compound and 10 µL of enzyme (*p*-nitrophenyl-alpha-D-glucopyranoside U/well).

The reaction mixture was mixed, incubated for 10 min at 37 ºC and pre-read at 400nm. The reaction was initiated by the addition of 10 µL of 0.5 mM *p*-nitrophenyl-alpha-D-glucopyranoside. Acarbose was used as positive control. Microplates were incubated for 30 min at37 ºC and absorbance read at 400 nm using a Synergy HT microplate reader. All experiments were performed in duplicate. The equation discussed for cholinesterase enzymes was used for the determination of % inhibition and IC_{50} values.

Urease inhibition assay

This enzyme inhibition assay is the customized form of the commonly known Berthelot assay [17]. To each well of a 96-well microplate, 45 µL of reaction mixture containing 10µL of phosphate buffer, pH 7.0, 10µL of test sample and 25µL of urease solution (0.135 U) were added. Contents were pre-incubated at 37 ºC for 5 min. A volume (40µL) of urea stock solution (20 mM) was added to each well, and the microplate incubated for 10 min at 37ºC. This was followed by the addition of

115µL of phenol-hypochlorite reagent (freshly prepared by mixing 45µL phenol with 70 µL of alkali) per well. For color development, the microplate was incubated for another 10 min at 37 ºC, and absorbance measured at 625 nm. The percentage enzyme inhibition and IC_{50} values were determined as mentioned above.

Cytotoxicity assay

Cytotoxicity was evaluated by the brine shrimp lethality bioassay [18]. Artificial sea water was prepared with sea salt at 34g/L.A shallow rectangular dish (22×32 cm) was used for the hatching of brine shrimp (*Artemia salina*) eggs (Sera, Heidelberg, Germany) under constant aeration for 48 h at room temperature. After hatching, active shrimp were collected from the brighter portion of the hatching chamber and used for the assay. Vials containing 5 mL of artificial sea water (with different concentrations of test compounds from the stock) were used. Ten shrimp were transferred to each vial. The temperature of the vials was maintained at 26 °C. The number of surviving shrimp were calculated after one day. The experiment was performed in triplicate and data analyzed with the Finney computer program to determine the LD_{50} (lethal dose that killed 50 % of shrimp).

Molecular docking studies

The reported MOE-Dock method of MOE 2009-2010 was utilized to study molecular recognition [19]. The Protein Data Bank site was used to retrieve protein molecules of acetylcholinesterase (PDB code: 1GQR), butyrylcholinesterase (PDB code: 1POP), α-glucosidase (PDB ID: $3NO_4$) and urease (PDB ID: 4UBP). After removing water molecules, MOE applications were used for performing 3D protonation of the protein molecule. The energy minimization algorithm of the MOE tool was used to minimize the energy of protein molecules using the following parameters; gradient of 0.05, Force Field of MMFF94X & Solvation and Chiral Constraint of Current Geometry. However, minimization of energy was ended for the gradient below 0.05. The templates for docking were the energy minimized protonated structures that were saved in a separate database (using a mdb file format). Finally, all compounds were docked into the binding pockets of enzymes. For the confirmation of validity, a re-docking procedure was applied. After docking analysis of each compound with 30 conformations, the best 2D images were selected for their specific types of interactions and their 3D images were drawn along with their bond lengths.

Statistical analysis

All measurements were carried out in triplicate and statistical analysis was performed by Microsoft Excel 2010. The results are presented as mean ± SEM with 90 % CL. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Chemistry

The protocol for the synthesis of the new molecules, 2-{[5-(aralkyl/aryl)-1,3,4-oxadiazol-2-yl]sulfanyl}-*N*-(4-methyl-1,3-thiazol-2-yl) acetamides (**8a-r**), is sketched in Figure 1, and the various groups are given in Table 1. The first step in the synthesis was the reaction of 4-methyl-1,3-thiazol-2-amine (**1**) with 2-bromoethanoyl bromide (**2**) in a basic aqueous medium to synthesize 2-bromo-*N*-(4-methyl-1,3-thiazol-2-yl)acetamide (**3**), an electrophile. Various nucleophiles were then synthesized in a parallel sequence of reactions, starting from a series of aralkyl/arylcarboxylic acids, **4a-r**, which were converted to respective esters, **5a-r**; acid hydrazides, **6a-r**, which were cyclized to the respective 5-aralkyl/aryl-1,3,4-oxadiazole-2-thiols (**7a-r**). These thiols, serving as nucleophiles, were finally coupled one by one with **3**in an aprotic polar solvent, i.e., DMF, using LiH as a base to obtain the target heterocyclic derivatives, **8a-r**. The structural verification was performed through spectral data analysis. All compounds were tested for enzyme inhibitory activity against cholinesterases, urease, and α-glucosidase, and the results are as shown in Table 2. The brine shrimp lethality bioassay was used to evaluate the cytotoxicity of the synthesized compounds and these results are given in Table 3.

Spectral characteristics of the synthesized molecules

N-(4-Methyl-1,3-thiazol-2-yl)-2-[(5-phenyl-1,3,4-oxadiazol-2-yl)sulfanyl]acetamide (8a)

Dull-white solid; yield: 80%; m.p.: 176-177 ^{o}C Mol. Formula: $C_{14}H_{12}N_4O_2S_2$; Mol. Mass: 332 g/mol; IR (KBr, cm^{-1}) υ_{max}: 3347 (N-H stretching), 2976 (C-H stretching of aromatic ring), 1678 (C=N stretching), 1644 (C=O stretching), 1570 (C=C stretching of aromatic ring), 1154 (C-O-C stretching); ^{1}H-NMR (DMSO-d_6, 600 MHz, *δ*/ppm): *δ* 12.47 (s, 1H, CON-H), 7.93 (d, *J* = 8.5 Hz,2H, H-2''' & H-6'''), 7.62-7.57 (m, 3H, H-3''' to H-5'''), 6.80 (br.s, 1H, H-5), 4.38 (br.s, 2H, CH_2-2'), 2.27 (s, 3H, CH_3-6); ^{13}C-NMR (DMSO-d_6, 150 MHz, *δ*/ppm): *δ* 165.76 (C-1'), 165.26 (C-5''), 162.98 (C-2''), 156.86 (C-2), 146.88 (C-4),

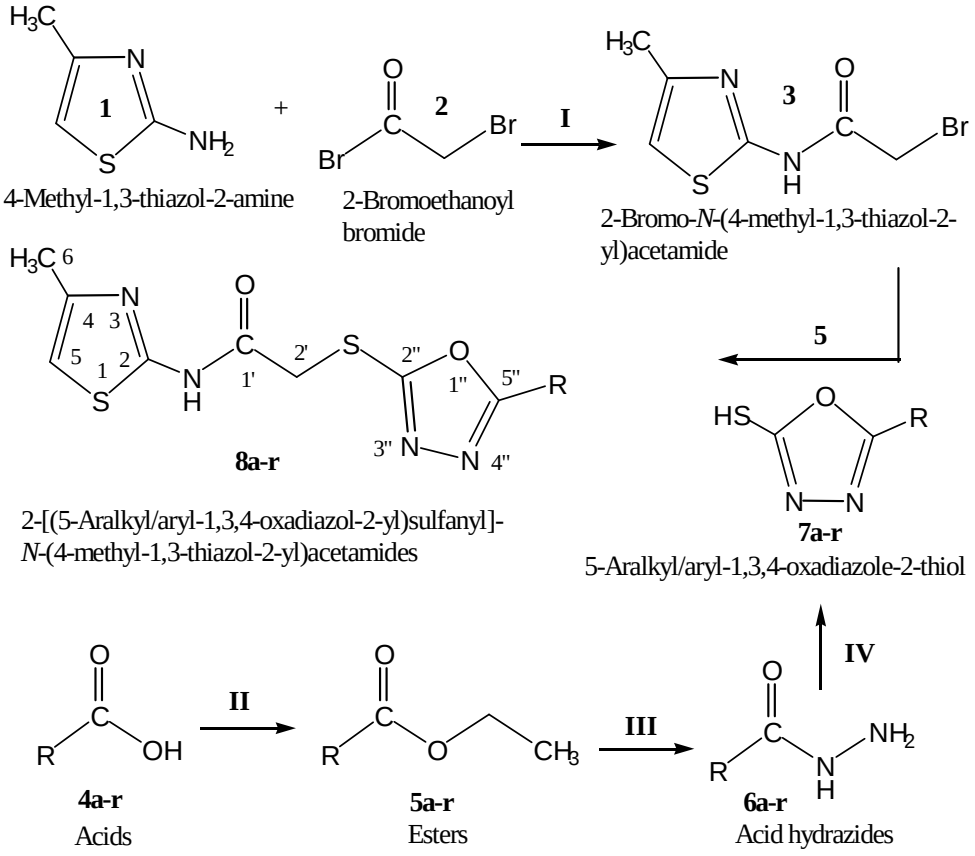


Figure 1: Protocol for synthesis of 2-{[5-(aralkyl/aryl)-1,3,4-oxadiazol-2-yl]sulfanyl}-*N*-(4-methyl-1,3-thiazol-2-yl)acetamides

132.06 (C-4'''), 129.38 (C-3''' & C-5'''), 126.36 (C-2''' & C-6'''), 122.89 (C-1'''), 108.12 (C-5), 35.37 (C-2'), 16.83 (C-6); EI-MS: *m/z* 332 $[M]^+$, 219 $[C_{10}H_7N_2O_2S]^+$, 192 $[C_9H_7N_2OS]^+$, 114 $[C_3H_2N_2OS]^+$, 77 $[C_6H_5]^+$.

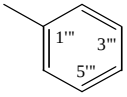
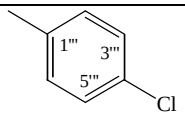
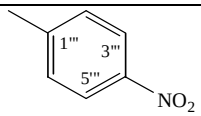
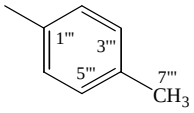
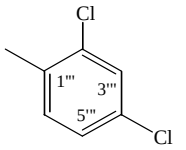
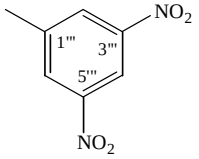
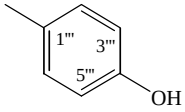
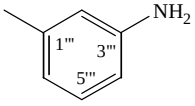
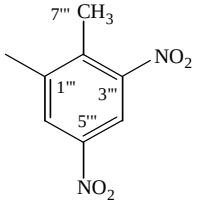
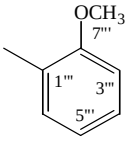
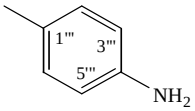
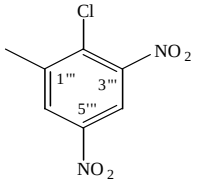
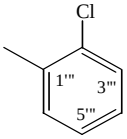
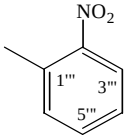
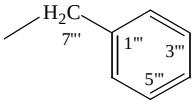
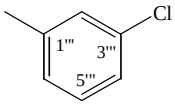
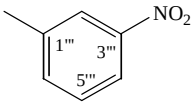
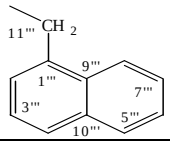
2-{[5-(4-Methylphenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-N-(4-methyl-1,3-thiazol-2-yl)acetamide (8b)

Light-brown solid; yield: 79%; m.p.: 227-228 ^oC; Mol. Formula: $C_{15}H_{14}N_4O_2S_2$; Mol. Mass: 346 g/mol; IR (KBr, cm^{-1}) υ_{max}: 3358 (N-H stretching), 2977 (C-H stretching of aromatic ring), 1675 (C=N stretching), 1645 (C=O stretching), 1576 (C=C stretching of aromatic ring), 1169 (C-O-C stretching); ^{1}H-NMR (DMSO-d_6, 600 MHz, *δ*/ppm): *δ* 12.46 (s, 1H, CON-H), 7.82 (br.d, *J* = 8.1 Hz, 2H, H-2''' & H-6'''), 7.38 (br.d, *J* = 7.8 Hz, 2H, H-3''' & H-5'''), 6.79 (br.s, 1H, H-5), 4.37 (br.s, 2H, CH_2-2'), 2.38 (s, 3H, CH_3-7'''), 2.27 (s, 3H, CH_3-6); ^{13}C-NMR (DMSO-d_6, 150 MHz, *δ*/ppm): *δ* 165.35 (C-1'), 164.35 (C-5''), 162.60 (C-2''), 156.7 (C-2), 142.25 (C-4), 141.0 (C-4'''), 129.92 (C-2''' & C-6'''), 126.32 (C-3''' & C-5'''), 120.15 (C-1'''), 108.06 (C-5), 35.41 (C-2'), 21.09 (C-7'''), 11.07 (C-6) ; EI-MS: *m/z* 346 $[M]^+$, 233 $[C_{11}H_9N_2O_2S]^+$, 206 $[C_{10}H_9N_2OS]^+$, 192 $[C_8H_5N_3OS]^+$, 159 $[C_9H_7N_2O]^+$, 141 $[C_5H_5N_2OS]^+$, 119 $[C_8H_7O]^+$, 104 $[C_7H_4O]^+$, 91 $[C_7H_7]^+$, 65 $[C_5H_5]^+$.

2-{[5-(4-Hydroxyphenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-N-(4-methyl-1,3-thiazol-2-yl)acetamide (8c)

Dull-white solid; yield: 80%; m.p.: 186-187 ^oC; Mol. Formula: $C_{14}H_{12}N_4O_3S_2$; Mol. Mass: 348 g/mol; IR (KBr, cm^{-1}) υ_{max}: 3356 (N-H stretching), 2979 (C-H stretching of aromatic ring), 1667 (C=N stretching), 1646 (C=O stretching), 1571 (C=C stretching of aromatic ring), 1148 (C-O-C stretching); ^{1}H-NMR (DMSO-d_6, 600 MHz, *δ*/ppm): *δ* 12.43 (s, 1H, CON-H), 7.96 (br.d, *J* = 8.5 Hz, 2H, H-3''' & H-5'''), 7.91 (br.d, *J* = 8.5 Hz, 1H, H-2''' & H-6'''), 6.79 (s, 1H, H-5), 4.30 (br.s, 2H, CH_2-2'), 2.27 (s, 3H, CH_3-6); ^{13}C-NMR (DMSO-d_6, 150 MHz, *δ*/ppm): *δ* 165.15 (C-1'), 163.70 (C-5''), 163.32 (C-2''), 157.65 (C-2), 146.81 (C-4), 137.81 (C-3''' & C-5'''), 133.28 (C-4'''), 131.14 (C-2''' & C-6'''), 122.07 (C-1'''), 108.05 (C-5), 35.38 (C-2'), 16.83 (C-6); EI-MS: *m/z* 348 $[M]^+$, 235 $[C_{10}H_7N_2O_3S]^+$, 228 $[C_7H_7N_3O_2S_2]^+$, 208 $[C_9H_8N_2O_2S]^+$, 175 $[C_9H_7N_2O_2]^+$, 161 $[C_9H_9N_2O]^+$, 141 $[C_5H_5N_2OS]^+$, 121 $[C_7H_5O_2]^+$, 114 $[C_4H_5N_2S]^+$, 93 $[C_6H_5O]^+$.

Table 1: Different aralkyl/aryl groups in 2-{[5-(aralkyl/aryl)-1,3,4-oxadiazol-2-yl]sulfanyl}-*N*-(4-methyl-1,3-thiazol-2-yl)acetamides (**8a-r**)

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

Protocol for synthesis of 2-{[5-(aralkyl/aryl)-1,3,4-oxadiazol-2-yl]sulfanyl}-*N*-(4-methyl-1,3-thiazol-2-yl)acetamides (**8a-r**). **Reagents & conditions:** (**1**) H_2O, 20 % Na_2CO_3, stirring for 2 h. (**2**) H_2SO_4, EtOH, refluxing for 4-5 h. (**3**) N_2H_4, EtOH, refluxing for 3 - 4 h. (**4**) CS_2, KOH, EtOH, refluxing for 5 - 6 h. (**5**) DMF, LiH, stirring for 4-6 h

2-{[5-(2-Methoxyphenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-N-(4-methyl-1,3-thiazol-2-yl)acetamide (8d)

Light-brown solid; yield: 75%; m.p.: 157-158 ^oC; Mol. Formula: $C_{15}H_{14}N_4O_3S_2$; Mol. Mass: 362 g/mol; IR (KBr, cm^{-1}) υ_{max}: 3358 (N-H stretching), 2977 (C-H stretching of aromatic ring), 1674 (C=N stretching), 1644 (C=O stretching), 1575 (C=C stretching of aromatic ring), 1163 (C-O-C stretching); ^{1}H-NMR (DMSO-d_6, 600 MHz, δ/ppm): δ 12.46 (s, 1H, CON-H), 7.76 (dd, J = 1.5, 7.6 Hz, 1H, H-6'''), 7.60 (dt, J = 1.5, 8.6 Hz, 1H, H-5'''), 7.24 (br.d, J = 8.4 Hz, 1H, H-3'''), 7.09 (br.t, J = 7.5 Hz, 1H, H-4'''), 6.80 (s, 1H, H-5), 4.38 (br.s, 2H, CH_2-2'), 3.85 (br.s, 3H, CH_3O-7'''), 2.27 (s, 3H, CH_3-6); ^{13}C-NMR (DMSO-d_6, 150 MHz, δ/ppm): δ 165.15 (C-1'), 164.0 (C-5''), 162.71 (C-2''), 157.86 (C-2'''), 156.92 (C-2), 146.85 (C-4), 133.60 (C-5'''), 133.01 (C-6'''), 120.68 (C-4'''), 112.62 (C-3'''), 111.79 (C-1'''), 108.06 (C-5), 55.92 (C-7'''), 35.34 (C-2'), 16.81 (C-6); EI-MS: *m/z* 362 $[M]^+$, 257 $[C_8H_9N_4O_2S_2]^+$, 249 $[C_{11}H_9N_2O_3S]^+$, 222 $[C_{10}H_9N_2O_2S]^+$, 189 $[C_6H_9N_2OS_2]^+$, 175 $[C_9H_7N_2O_2]^+$, 161 $[C_9H_9N_2O]^+$, 141 $[C_5H_5N_2OS]^+$, 135 $[C_8H_7O]^+$, 114 $[C_3H_2N_2OS]^+$, 91 $[C_7H_8]^+$, 77 $[C_6H_5]^+$.

2-{[5-(2-Chlorophenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-N-(4-methyl-1,3-thiazol-2-yl)acetamide (8e)

Light-brown solid; yield: 77%; m.p.: 158-159 ^oC; Mol. Formula: $C_{14}H_{11}ClN_4O_2S_2$; Mol. Mass: 366 g/mol; IR (KBr, cm^{-1}) υ_{max}: 3350 (N-H stretching), 2973 (C-H stretching of aromatic ring), 1670 (C=N stretching), 1640 (C=O stretching), 1572 (C=C stretching of aromatic ring), 1159 (C-O-C stretching), 684 (C-Cl stretching); ^{1}H-NMR (DMSO-d_6, 600 MHz, δ/ppm): δ 12.47 (s, 1H, CON-H), 7.94 (dd, J = 1.6, 7.8 Hz, 1H, H-3'''), 7.69 (br.d, J = 7.9 Hz, 1H, H-6'''), 7.62 (dt, J =

1.6, 8.1 Hz, 1H, H-4'''), 7.53 (dt, *J* = 0.9, 6.6 Hz, 1H, H-5'''), 6.80 (br.s, 1H, H-5), 4.40 (br.s, 2H, CH_2-2'), 2.27 (s, 3H, CH_3-6); ^{13}C-NMR (DMSO-d_6, 150 MHz, *δ*/ppm): *δ* 165.01 (C-1'), 163.39 (C-5''), 163.30 (C-2''), 156.66 (C-2), 146.87 (C-4), 133.30 (C-4'''), 131.66 (C-2'''), 131.14 (C-3'''), 131.10 (C-6'''), 127.82 (C-5'''), 122.06 (C-1'''), 108.12 (C-5), 35.39 (C-2'), 16.84 (C-6); EI-MS: *m/z* 368 $[M+2]^+$, 366 $[M]^+$, 253 $[C_{10}H_6ClN_2O_2S]^+$, 226 $[C_9H_6ClN_2OS]^+$, 193 $[C_8H_6N_3OS]^+$, 179 $[C_8H_4ClN_2OS]^+$, 139 $[C_5H_3N_2OS]^+$, 125 $[C_7H_5Cl]^+$, 114 $[C_3H_2N_2OS]^+$, 75 $[C_6H_3]^+$.

2-{[5-(3-Chlorophenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-N-(4-methyl-1,3-thiazol-2-yl)acetamide (8f)

Light-brown solid; yield: 79%; m.p.: 189-190 ^oC; Mol. Formula: $C_{14}H_{11}ClN_4O_2S_2$; Mol. Mass: 366 g/mol; IR (KBr, cm^{-1}) υ_{max}: 3368 (N-H stretching), 2979 (C-H stretching of aromatic ring), 1674 (C=N stretching), 1646 (C=O stretching), 1571 (C=C stretching of aromatic ring), 1155 (C-O-C stretching), 689 (C-Cl stretching); ^{1}H-NMR (DMSO-d_6, 600 MHz, *δ*/ppm): *δ* 12.47 (s, 1H, CON-H), 7.98 (dd, *J* = 2.1, 8.5 Hz, 1H, H-6'''), 7.96 (br.t, *J* = 8.5 Hz, 1H, H-5'''), 7.52 (d, *J* = 2.1 Hz, 1H, H-2'''), 7.49 (dd, *J* = 2.1, 8.5 Hz, 1H, H-4'''), 6.80 (br.s, 1H, H-5), 4.40 (br.s, 2H, CH_2-2'), 2.27 (s, 3H, CH_3-6); ^{13}C-NMR (DMSO-d_6, 150 MHz, *δ*/ppm): *δ* 165.15 (C-1'), 163.70 (C-5''), 163.32 (C-2''), 157.65 (C-2), 146.81 (C-4), 137.81 (C-3'''), 133.28 (C-4'''), 131.14 (C-2'''), 131.09 (C-6'''), 127.82 (C-5'''), 122.07 (C-1'''), 108.12 (C-5), 35.38 (C-2'), 16.84 (C-6); EI-MS: *m/z* 366 $[M]^+$, 226 $[C_9H_6ClN_2OS]^+$, 193 $[C_8H_6N_3OS]^+$, 179 $[C_8H_4ClN_2OS]^+$, 139 $[C_5H_3N_2OS]^+$, 125 $[C_7H_5Cl]^+$, 114 $[C_3H_2N_2OS]^+$, 75 $[C_6H_3]^+$.

2-{[5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-N-(4-methyl-1,3-thiazol-2-yl)acetamide (8g)

Light-brown solid; yield: 76%; m.p.: 187-188 ^oC; Mol. Formula: $C_{14}H_{11}ClN_4O_2S_2$; Mol. Mass: 366 g/mol; IR (KBr, cm^{-1}) υ_{max}: 3347 (N-H stretching), 2979 (C-H stretching of aromatic ring), 1681 (C=N stretching), 1636 (C=O stretching), 1584 (C=C stretching of aromatic ring), 1165 (C-O-C stretching), 691 (C-Cl stretching); ^{1}H-NMR (DMSO-d_6, 600 MHz, *δ*/ppm): *δ* 12.47 (s, 1H, CON-H), 7.91 (br.d, *J* = 8.5 Hz, 2H, H-2''' & H-6'''), 7.49 (br.d, *J* = 8.5 Hz, 1H, H-3''' & H-5'''), 6.80 (br.s, 1H, H-5), 4.40 (br.s, 2H, CH_2-2'), 2.27 (s, 3H, CH_3-6); ^{13}C-NMR (DMSO-d_6, 150 MHz, *δ*/ppm): *δ* 165.15 (C-1'), 163.70 (C-5''), 163.32 (C-2''), 157.65 (C-2), 146.81 (C-4), 137.81 (C-3''' & C-5'''), 133.28 (C-4'''), 131.14 (C-2''' & C-6'''), 122.07 (C-1'''), 108.09 (C-5), 35.38 (C-2'), 16.80 (C-6); EI-MS: *m/z* 366 $[M]^+$, 226 $[C_9H_6ClN_2OS]^+$, 179 $[C_8H_4ClN_2OS]^+$, 139 $[C_5H_3N_2OS]^+$, 125 $[C_7H_5Cl]^+$, 114 $[C_3H_2N_2OS]^+$, 75 $[C_6H_3]^+$.

2-{[5-(2,4-Dichlorophenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-N-(4-methyl-1,3-thiazol-2-yl)acetamide (8h)

Light-brown solid; yield: 77%; m.p.: 168-169 ^oC; Mol. Formula: $C_{14}H_{10}Cl_2N_4O_2S_2$; Mol. Mass: 400 g/mol; IR (KBr, cm^{-1}) υ_{max}: 3379 (N-H stretching), 2982 (C-H stretching of aromatic ring), 1677 (C=N stretching), 1647 (C=O stretching), 1575 (C=C stretching of aromatic ring), 1164 (C-O-C stretching), 694 (C-Cl stretching); ^{1}H-NMR (DMSO-d_6, 600 MHz, *δ*/ppm): *δ* 12.47 (s, 1H, CON-H), 7.96 (br.d, *J* = 8.5 Hz, 1H, H-6'''), 7.90 (d, *J* = 1.8 Hz, 1H, H-3'''), 7.64 (dd, *J* = 1.6, 8.4 Hz, 1H, H-5'''), 6.80 (br.s, 1H, H-5), 4.41 (br.s, 2H, CH_2-2'), 2.27 (s, 3H, CH_3-6); ^{13}C-NMR (DMSO-d_6, 150 MHz, *δ*/ppm): *δ* 165.01 (C-1'), 163.88 (C-5''), 162.65 (C-2''), 156.89 (C-2), 146.81 (C-4), 137.19 (C-4'''), 132.73 (C-2'''), 132.23 (C-6'''), 130.72 (C-3'''), 128.13 (C-5'''), 121.06 (C-1'''), 108.09 (C-5), 35.43 (C-2'), 16.80 (C-6); EI-MS: *m/z* 404 $[M+4]^+$, 402 $[M+2]^+$, *m/z* 400 $[M]^+$, 260 $[C_9H_5ClN_2O_2S]^+$, 172 $[C_7H_3Cl_2N]^+$, 141 $[C_5H_5N_2OS]^+$, 114 $[C_3H_2N_2OS]^+$, 100 $[C_2N_2OS]^+$.

2-{[5-(3-Aminophenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-N-(4-methyl-1,3-thiazol-2-yl)acetamide (8i)

White amorphous solid; yield: 83%; m.p.: 190-191 ^oC; Mol. Formula: $C_{14}H_{13}N_5O_2S_2$; Mol. Mass: 347 g/mol; IR (KBr, cm^{-1}) υ_{max}: 3378 (N-H stretching), 2962 (C-H stretching of aromatic ring), 1680 (C=N stretching), 1653 (C=O stretching), 1566 (C=C stretching of aromatic ring), 1164 (C-O-C stretching); ^{1}H-NMR (DMSO-d_6, 600 MHz, *δ*/ppm): *δ* 12.43 (s, 1H, CON-H), 7.93 (dd, *J* = 2.1, 8.5 Hz, 1H, H-6'''), 7.88 (br.t, *J* = 8.5 Hz, 1H, H-5'''), 7.48 (d, *J* = 2.1 Hz, 1H, H-2'''), 7.47 (dd, *J* = 2.1, 8.5 Hz, 1H, H-4'''), 6.79 (s, 1H, H-5), 4.30 (br.s, 2H, CH_2-2'), 2.27 (s, 3H, CH_3-6); ^{13}C-NMR (DMSO-d_6, 150 MHz, *δ*/ppm): *δ* 165.26 (C-1'), 162.99 (C-5''), 160.46 (C-2''), 157.71 (C-2), 146.80 (C-4), 132.21 (C-3'''), 132.04 (C-1'''), 129.41 (C-5'''), 129.38 (C-6'''), 126.35 (C-4'''), 126.02 (C-2'''), 108.05 (C-5), 35.36 (C-2'), 16.84 (C-6); EI-MS: *m/z* 347 $[M]^+$, 207 $[C_9H_8N_3OS]^+$, 193 $[C_8H_6N_3OS]^+$, 141 $[C_5H_5N_2OS]^+$, 133 $[C_8H_7NO]^+$, 118 $[C_3HNOS]^+$, 92 $[C_6H_6N]^+$, 65 $[C_5H_5]^+$.

2-{[5-(4-Aminophenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-N-(4-methyl-1,3-thiazol-2-yl) acetamide (8j)

White amorphous solid; yield: 82%; m.p.: 215-216 ^{o}C; Mol. Formula: $C_{14}H_{13}N_5O_2S_2$; Mol. Mass: 347 g/mol; IR (KBr, cm^{-1}) υ_{max}: 3376 (N-H stretching), 2966 (C-H stretching of *aromatic ring), 1683 (C=N stretching), 1657 (C=O stretching), 1563 (C=C stretching of* aromatic ring), 1168 (C-O-C stretching); ^{1}H-NMR (DMSO-d_6, 600 MHz, *δ*/ppm): *δ* 12.43 (s, 1H, CON-H), 7.56 (br.d, *J* = 8.5 Hz, 2H, H-2''' & H-6'''), 6.79 (s, 1H, H-5), 6.63 (br.d, *J* = 8.6 Hz, 2H, H-3''' & H-5'''), 4.30 (br.s, 2H, CH_2-2'), 2.27 (s, 3H, CH_3-6); ^{13}C-NMR (DMSO-d_6, 150 MHz, *δ*/ppm): *δ* 166.11 (C-1'), 165.39 (C-5''), 160.54 (C-2''), 156.88 (C-2), 152.40 (C-4'''), 146.90 (C-4), 127.90 (C-2''' & C-6'''), 113.46 (C-3''' & C-5'''), 109.13 (C-1'''), 108.00 (C-5), 35.31 (C-2'), 16.84 (C-6); EI-MS: *m/z* 347 $[M]^+$, 207 $[C_9H_8N_3OS]$, 193 $[C_8H_6N_3OS]$, 160 $[C_8H_6N_3O]^+$, 141 $[C_5H_5N_2OS]$, 133 $[C_8H_7NO]$, 118 $[C_3HNOS]$, 92 $[C_6H_6N]$, 65 $[C_5H_5]^+$.

N-(4-Methyl-1,3-thiazol-2-yl)-2-{[5-(2-nitrophenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}acetamide(8k)

Light-yellow solid; yield: 77%; m.p.: 170-171 ^{o}C; Mol. Formula: $C_{14}H_{11}N_5O_4S_2$; Mol. Mass: 377 g/mol; IR (KBr, cm^{-1}) υ_{max}: 3364 (N-H stretching), 2982 (C-H stretching of aromatic ring), 1673 (C=N stretching), 1649 (C=O stretching), 1578 (C=C stretching of aromatic ring), 1157 (C-O-C stretching); ^{1}H-NMR (DMSO-d_6, 600 MHz, *δ*/ppm): *δ* 12.47 (s, 1H, CON-H), 8.19-8.17 (m, 1H, H-3'''), 8.01-7.97 (m, 1H, H-6'''), 7.94-7.90 (m, 2H, H-4''' & H-5'''), 6.80 (br.s, 1H, H-5), 4.41 (br.s, 2H, CH_2-2'), 2.27 (s, 3H, CH_3-6); ^{13}C-NMR (DMSO-d_6, 150 MHz, *δ*/ppm): *δ* 164.91 (C-1'), 164.41 (C-5''), 161.69 (C-2''), 156.70 (C-2), 147.66 (C-2'''), 146.84 (C-4), 133.67 (C-4'''), 133.50 (C-5'''), 131.50 (C-3'''), 124.77 (C-6'''), 116.49 (C-1'''), 108.09 (C-5), 35.48 (C-2'), 16.81 (C-6); EI-MS: *m/z* 377 $[M]^+$, 227 $[C_8H_7N_2O_2S_2]^+$, 141 $[C_5H_5N_2OS]^+$, 114 $[C_3H_2N_2OS]^+$, 76 $[C_6H_4]^+$.

N-(4-Methyl-1,3-thiazol-2-yl)-2-{[5-(3-nitrophenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}acetamide (8l)

Light-yellow solid; yield: 78%; m.p.: 194-195 ^{o}C; Mol. Formula: $C_{14}H_{11}N_5O_4S_2$; Mol. Mass: 377 g/mol; IR (KBr, cm^{-1}) υ_{max}: 3360 (N-H stretching), 2963 (C-H stretching of aromatic ring), 1660 (C=N stretching), 1650 (C=O stretching), 1562 (C=C stretching of aromatic ring), 1169 (C-O-C stretching); ^{1}H-NMR (DMSO-d_6, 600 MHz, *δ*/ppm): *δ* 12.48 (s, 1H, CON-H), 8.63 (br.s, *J* = 7.7 Hz, 1H, H-2'''), 8.46 (dd, *J* = 1.8, 8.2 Hz, 1H, H-4'''), 8.37 (br.d, *J* = 7.8 Hz, 1H, H-6'''), 7.88 (br.t, 1H, H-5'''), 6.79 (br.s, 1H, H-5), 4.40 (br.s, 2H, CH_2-2'), 2.26 (s, 3H, CH_3-6); ^{13}C-NMR (DMSO-d_6, 150 MHz, δ/ppm): δ 165.12 (C-1'), 163.97 (C-5''), 163.70 (C-2''), 157.63 (C-2), 148.17 (C-3'''), 148.15 (C-4), 126.30 (C-4'''), 131.23 (C-5'''), 132.34 (C-6'''), 124.33 (C-1'''), 120.82 (C-2'''), 108.91 (C-5), 35.36 (C-2'), 16.73 (C-6); EI-MS: *m/z* 377 $[M]^+$, 227 $[C_8H_7N_2O_2S_2]^+$, 150$[C_7H_4NO_3]^+$, 141 $[C_5H_5N_2OS]^+$, 114 $[C_3H_2N_2OS]^+$, 76 $[C_6H_4]^+$.

N-(4-Methyl-1,3-thiazol-2-yl)-2-{[5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}acetamide (8m)

Light-yellow solid; yield: 77%; m.p.: 173-174 ^{o}C; Mol. Formula: $C_{14}H_{11}N_5O_4S_2$; Mol. Mass: 377 g/mol; IR (KBr, cm^{-1}) υ_{max}: 3364 (N-H stretching), 2987 (C-H stretching of aromatic ring), 1684 (C=N stretching), 1654 (C=O stretching), 1586 (C=C stretching of aromatic ring), 1173 (C-O-C stretching); ^{1}H-NMR (DMSO-d_6, 600 MHz, *δ*/ppm): *δ* 12.48 (s, 1H, CON-H), 8.42 (br.d, *J* = 7.0 Hz, 1H, H-3''' & H-5'''), 8.21 (br.d, *J* = 7.0 Hz, 2H, H-2''' & H-6'''), 6.79 (br.s, 1H, H-5), 4.40 (br.s, 2H, CH_2-2'), 2.26 (s, 3H, CH_3-6); ^{13}C-NMR (DMSO-d_6, 150 MHz, *δ*/ppm): *δ* 165.00 (C-1'), 164.51 (C-5''), 163.93 (C-2''), 156.01 (C-2), 149.15 (C-4'''), 148.15 (C-4), 127.74 (C-2''' & C-6'''), 124.58 (C-3''' & C-5'''), 122.01 (C-1'''), 108.12 (C-5), 35.38 (C-2'), 16.73 (C-6); EI-MS: *m/z* 377 $[M]^+$, 227 $[C_8H_7N_2O_2S_2]^+$, 150$[C_7H_4NO_3]^+$, 141 $[C_5H_5N_2OS]^+$, 114 $[C_3H_2N_2OS]^+$, 76 $[C_6H_4]^+$.

2-{[5-(3,5-Dinitrophenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-N-(4-methyl-1,3-thiazol-2-yl)acetamide(8n)

Light-green solid; yield: 74%; m.p.: 215-216 ^{o}C; Mol. Formula: $C_{14}H_{10}N_6O_6S_2$; Mol. Mass: 422 g/mol; IR (KBr, cm^{-1}) υ_{max}: 3366 (N-H stretching), 2985 (C-H stretching of aromatic ring), 1685 (C=N stretching), 1658 (C=O stretching), 1589 (C=C stretching of aromatic ring), 1192 (C-O-C stretching); ^{1}H-NMR (DMSO-d_6, 600 MHz, *δ*/ppm): *δ* 12.48 (s, 1H, CON-H), 7.98 (br.d, *J* = 1.6 Hz, 2H, H-2''' & H-6'''), 7.66 (br.s, 1H, H-4'''), 6.79 (br.s, 1H, H-5), 4.40 (br.s, 2H, CH_2-2'), 2.26 (s, 3H, CH_3-6); ^{13}C-NMR (DMSO-d_6, 150 MHz, *δ*/ppm): *δ* 165.12 (C-1'), 163.88 (C-5''), 162.67 (C-2''), 157.71 (C-2), 145.23 (C-4), 132.60 (C-1'''), 132.24 (C-2''' & C-6'''), 131.97 (C-3''' & C-5'''), 128.19 (C-4'''), 108.91 (C-5), 35.40 (C-2'), 16.70 (C-6); EI-MS: *m/z* 422 $[M]^+$, 303 $[C_{12}H_8N_4O_2S_2]^+$, 193 $[C_8H_6N_3OS]^+$, 140 $[C_5H_5N_2OS]^+$, 114 $[C_3H_2N_2OS]^+$, 104 $[C_7H_4O]^+$, 76 $[C_6H_4]^+$.

2-{[5-(2-Methyl-3,5-dinitrophenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-N-(4-methyl-1,3-thiazol-2-yl)acetamide (8o)

Light-brown solid; yield: 71%; m.p.: 191-192 ^{o}C; Mol. Formula: $C_{15}H_{12}N_6O_6S_2$; Mol. Mass: 436 g/mol; IR (KBr, cm^{-1}) υ_{max}: 3346 (N-H stretching), 2969 (C-H stretching of aromatic ring), 1665 (C=N stretching), 1636 (C=O stretching), 1568 (C=C stretching of aromatic ring), 1152 (C-O-C stretching); ^{1}H-NMR (DMSO-d_6, 600 MHz, *δ*/ppm): *δ* 12.40 (s, 1H, CON-H), 7.90 (d, *J* = 2.8 Hz, 1H, H-4'''), 7.64 (d, *J* = 2.8 Hz, 1H, H-6'''), 6.79 (br.s, 1H, H-5), 4.24 (br.s, 2H, CH_2-2'), 2.34 (br.s, 3H, CH_3-7'''), 2.27 (s, 3H, CH_3-6); ^{13}C-NMR (DMSO-d_6, 150 MHz, *δ*/ppm): *δ* 165.12 (C-1'), 163.88 (C-5''), 162.67 (C-2''), 157.71 (C-2), 146.88 (C-4), 132.60 (C-1'''), 132.24 (C-6'''), 131.97 (C-5'''), 130.81 (C-3'''), 128.19 (C-4'''), 128.14 (C-2'''), 108.07 (C-5), 35.40 (C-2'), 20.67 (C-7'''), 16.82 (C-6'''); EI-MS: *m/z* 436 $[M]^+$, 303 $[C_{12}H_8N_4O_2S_2]^+$, 281 $[C_9H_5N_4O_5S]^+$, 193 $[C_8H_6N_3OS]^+$, 181 $[C_7H_5N_2O_4]^+$, 140 $[C_5H_5N_2OS]^+$, 114 $[C_3H_2N_2OS]^+$, 104 $[C_7H_4O]^+$.

2-{[5-(2-Chloro-3,5-dinitrophenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-N-(4-methyl-1,3-thiazol-2-yl)acetamide (8p)

Light-brown solid; yield: 77%; m.p.: 177-178 ^{o}C; Mol. Formula: $C_{14}H_9ClN_6O_6S_2$; Mol. Mass: 456 g/mol; IR (KBr, cm^{-1}) υ_{max}: 3354 (N-H stretching), 2978 (C-H stretching of aromatic ring), 1674 (C=N stretching), 1645 (C=O stretching), 1576 (C=C stretching of aromatic ring), 1163 (C-O-C stretching), 693 (C-Cl stretching); ^{1}H-NMR (DMSO-d_6, 600 MHz, *δ*/ppm): *δ* 12.40 (s, 1H, CON-H), 7.91 (d, *J* = 2.6 Hz, 1H, H-4'''), 7.68 (d, *J* = 2.6 Hz, 1H, H-6'''), 6.79 (br.s, 1H, H-5), 4.24 (br.s, 2H, CH_2-2'), 2.27 (s, 3H, CH_3-6); ^{13}C-NMR (DMSO-d_6, 150 MHz, *δ*/ppm): *δ* 165.12 (C-1'), 163.88 (C-5''), 162.67 (C-2''), 157.71 (C-2), 147.88 (C-4), 132.60 (C-1'''), 132.24 (C-6'''), 131.97 (C-5'''), 130.81 (C-3'''), 128.19 (C-4'''), 128.14 (C-2'''), 108.07 (C-5), 35.40 (C-2'), 16.82 (C-6); EI-MS: *m/z* 456 $[M]^+$, 303 $[C_{12}H_8N_4O_2S_2]^+$, 302 $[C_8H_2ClN_4O_5S]^+$, 227 $[C_9H_6ClN_2OS]^+$, 201 $[C_6H_2ClN_2O_4]^+$, 193 $[C_8H_6N_3OS]^+$, 141 $[C_5H_5N_2OS]^+$, 114 $[C_3H_2N_2OS]^+$, 104 $[C_7H_4O]^+$, 76 $[C_6H_4]^+$.

2-[(5-Benzyl-1,3,4-oxadiazol-2-yl)sulfanyl]-N-(4-methyl-1,3-thiazol-2-yl)acetamide(8q)

Dull-white solid; yield: 80%; m.p.: 178-179 ^{o}C, Mol. Formula: $C_{15}H_{14}N_4O_2S_2$; Mol. Mass: 346 g/mol; IR (KBr, cm^{-1}) υ_{max}: 3357 (N-H stretching), 2974 (C-H stretching of aromatic ring), 1682 (C=N stretching), 1646 (C=O stretching), 1582 (C=C stretching of aromatic ring), 1166 (C-O-C stretching); ^{1}H-NMR (DMSO-d_6, 600 MHz, *δ*/ppm): *δ* 12.40 (s, 1H, CON-H), 7.32-7.25 (m, 5H, H-2''' to H-6'''), 6.79 (br.s, 1H, H-5), 4.33 (br.s, 2H, CH_2-7'''), 4.24 (br.s, 2H, CH_2-2'), 2.27 (s, 3H, CH_3-6); ^{13}C-NMR (DMSO-d_6, 150 MHz, *δ*/ppm): *δ* 166.73 (C-1'), 165.01 (C-5''), 162.38 (C-2''), 157.01 (C-2), 147.01 (C-4), 134.13 (C-1'''), 128.79 (C-3''' & C-5'''), 128.69 (C-2''' & C-6'''), 127.22 (C-4'''), 108.07 (C-5), 35.27 (C-2'), 30.67 (C-7'''), 16.82 (C-6); EI-MS: *m/z* 346 $[M]^+$, 255 $[C_8H_7N_4O_2S]^+$, 228 $[C_7H_7N_4OS_2]^+$, 206 $[C_{10}H_9N_2OS]^+$, 192 $[C_8H_5N_3OS]^+$, 141 $[C_5H_5N_2OS]^+$, 114 $[C_3H_2N_2OS]^+$, 91 $[C_7H_7]^+$, 83 $[C_3H_3N_2O]^+$.

N-(4-Methyl-1,3-thiazol-2-yl)-2-{[5-(naphthalen-1-ylmethyl)-1,3,4-oxadiazol-2-yl]sulfanyl}acetamide(8r)

Light-yellow solid; yield: 78%; m.p.: 206-207 ^{o}C; Mol. Formula: $C_{18}H_{14}N_4O_2S_2$; Mol. Mass: 382 g/mol; IR (KBr, cm^{-1}) υ_{max}: 3359 (N-H stretching), 2978 (C-H stretching of aromatic ring), 1677 (C=N stretching), 1646 (C=O stretching), 1579 (C=C stretching of aromatic ring), 1163 (C-O-C stretching); ^{1}H-NMR (DMSO-d_6, 600 MHz, *δ*/ppm): *δ* 12.40 (s, 1H, CON-H), 8.07 (br.d, *J* = 7.9 Hz, 1H, H-6'''), 7.95 (dist.dd, *J* = 1.5, 7.4 Hz, 1H, H-5'''), 7.88 (dist.dd, *J* = 1.5, 7.1 Hz, 1H, H-4'''), 7.57-7.53 (m, 2H, H-7''' & H-8'''), 7.48-7.44 (m, 2H, H-2'''& H-3'''), 6.79 (br.s, 1H, H-5), 4.59 (s, 2H, CH_2-11'''), 4.24 (br.s, 2H, CH_2-2'), 2.27 (s, 3H, CH_3-6); ^{13}C-NMR (DMSO-d_6, 150 MHz, *δ*/ppm): *δ* 166.73 (C-1'), 164.96 (C-5''), 164.76 (C-2''), 155.76 (C-2), 148.56 (C-1'''), 147.0 (C-4), 133.39 (C-7'''), 131.22 (C-6'''), 130.22 (C-3'''), 128.56 (C-4'''), 128.11 (C-5'''), 127.63 (C-8'''), 126.50 (C-2'''), 125.98 (C-9'''), 123.62 (C-10'''), 108.07 (C-5), 35.24 (C-2'), 28.44 (C-11'''), 16.82 (C-6); EI-MS: *m/z* 382 $[M]^+$, 255 $[C_8H_7N_4O_2S]^+$, 228 $[C_7H_7N_4OS_2]^+$, 242 $[C_{13}H_{10}N_2OS]^+$, 241 $[C_{13}H_9N_2OS]^+$, 141 $[C_5H_5N_2OS]^+$, 114 $[C_3H_2N_2OS]^+$, 141 $[C_{11}H_9]^+$, 83 $[C_3H_3N_2O]^+$.

Enzyme inhibition

In the search for new more effective therapeutic agents, the synthesized bi-heterocycles **8a-p** were screened for their inhibitory activity against four pharmacologically relevant enzymes: acetyl*cholinesterase, butyrylcholinesterase, urease* and *α*-glucosidase. The results of enzyme inhibition are represented as % inhibition and IC_{50} for cholinesterases, urease, glucosidase (Table 2**)** and LD_{50} values for brine shrimps (Table 3**)**.

The structural verification was performed by spectral analysis. The structures of these derivatives were deduced based on IR, EI-MS,

Table 2: Inhibition (%) and IC_{50} values for acetylcholinesterase, butyrylcholinesterase, α-glucosidase and urease.

Compd.	AChE inhibition		BChE inhibition		α- Glucosidase inhibition		Urease inhibition	
	Inhibition (%) at 0.5 mM	*IC_{50} (µM)*	*Inhibition (%) at 0.5 mM*	*IC_{50} (µM)*	*Inhibition (%) at 0.5 mM*	*IC_{50} (µM)*	*Inhibition (%) at 0.5 mM*	*IC_{50} (µM)*
8a	36.12±0.19	-	36.52±0.29	-	56.21±0.30	112.13±0.16	46.52±0.19	-
8b	84.31±0.24	43.85±0.12	47.27±0.26	-	67.14±0.19	342.13±0.16	69.31±0.31	254.11±0.12
8c	89.31±0.35	49.25±0.09	58.14±0.25	129.15±0.11	51.32±0.14	> 500	77.13±0.20	291.47±0.19
8d	86.52±0.29	39.47±0.13	54.62±0.34	438.52±0.22	32.25±0.13	-	56.95±0.37	223.15±0.14
8e	86.28±0.36	49.73±0.15	12.23±0.35	-	86.41±0.17	192.27±0.14	76.31±0.50	250.30±0.25
8f	83.74±0.23	53.15±0.25	45.27±0.35	-	79.11±0.21	187.35±0.11	92.35±0.31	161.26±0.23
8g	77.31±0.15	79.25±0.05	45.14±0.35	-	77.23±0.19	201.23±0.21	30.61±0.29	228.13±0.14
8h	71.36±0.64	119.53±0.21	71.42±0.45	238.64±0.18	78.12±0.18	107.42±0.12	41.56±0.15	-
8i	92.53±0.45	72.85±0.24	52.74±0.63	464.27±0.19	87.15±0.19	156.29±0.15	67.28±0.41	212.31±0.12
8j	87.85±0.42	92.74±0.15	79.26±0.15	67.85±0.11	82.11±0.18	278.21±0.15	-	-
8k	87.61±0.27	29.53±0.12	82.47±0.16	56.23±0.09	31.13±0.15	-	-	-
8l	91.74±0.13	17.25±0.07	15.47±0.27	-	91.83±0.15	62.23±0.13	10.26±0.20	-
8m	90.71±0.14	36.84±0.08	67.35±0.36	109.25±0.21	91.48±0.15	61.25±0.11	49.24±0.51	-
8n	87.21±0.32	63.80±0.45	38.27±0.29	-	77.51±0.55	287.13±0.12	47.28±0.15	-
8o	90.12±0.29	49.47±0.13	58.62±0.34	238.52±0.22	91.59±0.23	57.35±0.17	60.54±0.17	249.35±0.15
8p	66.42±0.17	59.47±0.25	78.62±0.34	137.52±0.22	63.48±0.17	293.15±0.19	35.67±0.10	-
8q	89.27±0.15	18.36±0.08	65.15±0.45	292.14±0.16	52.15±0.17	> 500	61.45±0.40	247.35±0.18
8r	37.50±0.23	-	-	-	69.24±0.27	77.35±0.11	27.52±0.19	-
Eserine	91.27±1.17	0.04±0.0001	82.82±1.09	0.85±0.0001				
Acarbose					92.23±0.16	37.38±0.12		
Thiourea							98.12±0.18	21.11±0.12

Note: All compounds were dissolved in methanol and experiments were performed in triplicate (mean ± SEM, n = 3). AChE = acetylcholinesterase, BChE =butyrylcholinesterase.

Table 3: Brine shrimp activity

Compd.	LD_{50} (mM)	Compd.	LD_{50} (mM)	Compd.	LD_{50} (mM)	Compd.	LD_{50} (mM)
8a	45.21	***8f***	243.6	***8k***	358.4	***8p***	201.1
8b	213.5	***8g***	354.4	***8l***	466.7	***8q***	198.5
8c	122.7	***8h***	395.4	***8m***	77.34	***8r***	61.47
8d	498.7	***8i***	459.7	***8n***	171.8	Doxo-rubicin	5.21
8e	521.1	***8j***	365.8	***8o***	189.7		

Note: Doxorubicin was used as standard

^{1}H-NMR and ^{13}C-NMR spectral data. The structural analysis of one of the compounds is discussed here in detail for the convenience of the readers. The molecule, **8e**, was obtained as a light-brown solid with 77 % yield and had a melting point of 158-159 ^oC. Its molecular formula, $C_{14}H_{11}ClN_4O_2S_2$, was established by identifying the $[M]^+$ ion peak at *m/z* 366, in the EI-MS spectrum and counting the number of protons in the ^{1}H-NMR spectrum. The number of carbons in the ^{13}C-NMR spectrum also supported the assignment of its molecular formula. In the EI-MS spectrum, the peak at *m/z* 368 was identified as the $[M+2]^+$ peak, while the molecular ion peak was observed at *m/z* 366 along with some peculiar fragment ion peaks as indicated in the spectral characterization section. The 2-chlorophenyl moiety in the molecule was rationalized by four typical signals in the aromatic region of the ^{1}H-NMR spectrum (Figure 2) at *δ* 7.94 (dd, *J* = 1.6, 7.8 Hz, 1H, H-3'''), 7.69 (br.d, *J* = 7.9 Hz, 1H, H-6'''), 7.62 (dt, *J* = 1.6, 8.1 Hz, 1H, H-4'''), and 7.53 (dt, *J* = 0.9, 6.6 Hz, 1H, H-5'''). Similarly, the 4-methyl-1,3-thiazole moiety was deduced through two peaks at *δ* 6.80 (br.s, 1H,

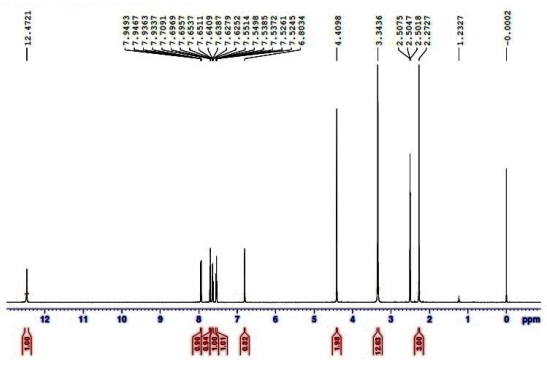


Figure 2: ^{1}H-NMR spectrum of **8e**

H-5) and 2.27 (s, 3H, CH_3-6) while the interconnecting *C* & *N*-substituted acetamido moiety in the molecule was inferred by two characteristic peaks at *δ* 12.47 (s, 1H, CON-H), and 4.40 (br.s, 2H, CH_2-2'). In ^{13}C-NMR spectrum (Figure 3), overall, fourteen signals clearly indicated the successful synthesis of the desired compound. The spectrum identified seven quaternary carbons at *δ* 165.01 (C-1'), 163.39 (C-5''), 163.30 (C-2''), 156.66 (C-2),

146.87 (C-4), 131.66 (C-2'''), & 122.06 (C-1'''), five methines at *δ* 133.30 (C-4'''), 131.10 (C-6'''), 131.14 (C-3'''), 127.82 (C-5'''), & 108.12 (C-5), one methylene at *δ* 35.39 (C-2'), and a methyl carbon resonating at *δ* 16.84 (C-6).

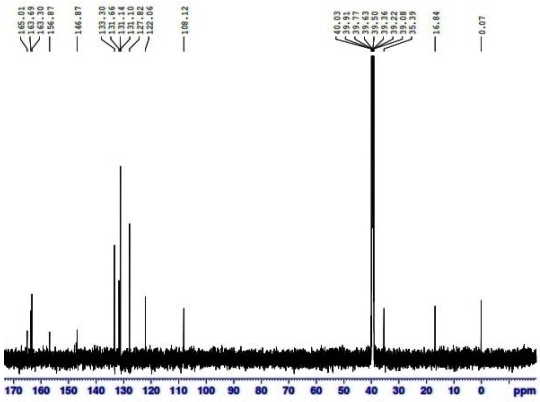


Figure 3: ^{13}C-NMR spectrum of **8e**

Various functional groups were ascribed to this molecule by IR data. The prominent absorption bands were observed at *δ* 3350 (N-H stretching), 2973 (C-H stretching of aromatic ring), 1670 (C=N stretching), 1640 (C=O stretching), 1572 (C=C stretching of aromatic ring), 1159 (C-O-C stretching) and 684 (C-Cl stretching) cm^{-1}. This corroborated the structural confirmation of **8e** as 2-{[5-(2-chlorophenyl)-1,3,4-oxadiazol-2-yl] sulfanyl}-*N*-(4-methyl-1,3-thiazol-2-yl)acetamide. A similar strategy was employed for the structural interpretation of the other compounds in the series.

Enzyme inhibition, molecular docking and structure-activity relationship

To ascertain the therapeutic potential of **8a-r**, these compounds were tested for inhibitory activity against four pharmacologically relevant enzymes, namely, AChE, BChE, α-glucosidase and urease. The results are given as % inhibition and IC_{50} (50% inhibitory concentration) values in Table 2. The modes of interactions of the synthesized molecules (**8a-r**) with these enzymes were also described with *in silico* (molecular docking) study. All compounds except two, **8a** and **8r,** exhibited an inhibitory potential against AChE. The most promising compounds against this enzyme were **8l** and **8q** with IC_{50} values of 17.25 ± 0.07 and 18.36 ± 0.08 µM, respectively, which might have been due to the presence of a substituted nitro group at the three position of the phenyl ring.

Eserine was used as a positive control against this enzyme, having an IC_{50} of 0.04 ± 0.0001 µM. The better activity of **8l** and **8q**, relative to other derivatives in the series, might have been due to the presence of a 3-nitrophenyl group and benzyl group, respectively, in these molecules.

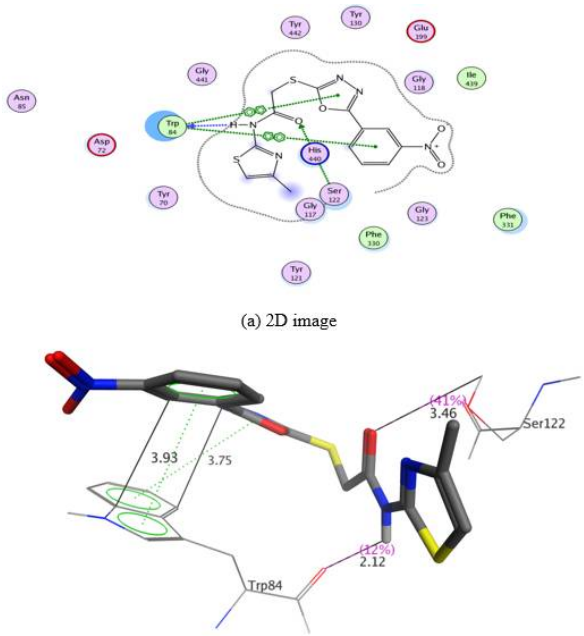
Among the *mono*-chloro substituted analogues, **8e-g**, the order of activity was *ortho* > *meta* > *para*, as per their IC_{50} values: **8e** (49.73 ± 0.15 µM), **8f** (53.15 ± 0.25 µM), and **8g** (79.25 ± 0.05 µM), respectively. It would be expected that the lower the IC_{50} value, the greater the inhibitory potential of any molecule will be. Dichloro-substituted **8h** (119.53±0.21µM), was less active than the mono-chloroderivatives. Similarly, in the case of amino-substituted analogues, the molecule with *meta*-amino group, **8i** (72.85±0.24 µM), was more active than that with a *para*-amino group, **8j** (92.74±0.15 µM). For the mono-nitro regioisomers, **8k-m**, the order of activity was *meta* > *ortho* > *para* as reflected by the IC_{50} v for 8**l** (17.25±0.07 µM), **8k** (29.53±0.12 µM), and **8m** (36.84±0.08 µM), respectively. In the *di*-nitro substituted compounds, **8n-p**, it was observed that substitution of an additional methyl group rendered the molecule more active relative to substitution with a chloro group. Among these derivatives, the order of activity was: 2-methyl-3,5-dinitrophenyl>2-chloro-3,5-dinitrophenyl>3,5-dinitrophenyl.This was obvious from their IC_{50} values of 49.47±0.13 µM (**8o**), 59.47±0.25 µM (**8p**), and 63.80±0.45 µM (**8n**).

Varying trends were observed in other derivatives of the series. All derivatives, **8a-r**, were docked into the active pocket of this enzyme.

All synthesized derivatives were computationally docked against α -glucosidase, AChE and BChE to explore the binding modes of the ligands. Four interactions were shown by the most active compound **8l** in this series. Ser122 makes an acidic interaction with carbonyl oxygen of the ligand, with **8l** giving a bond length of 3.46 Å. Trp84 gave the strongest backbone donor interaction with -HNof thiazol and two arene-arene interactions with oxadiazol ring, having bond distances of 2.12, 3.75 and 3.93 Å , respectively, as identified from the 2D and 3D images of Figure 4.

Against BChE, weak activity was shown by the synthesized compounds, and almost half of the synthesized compound were inactive, where a moderate inhibition potential was shown by the compound **8k**, with an IC_{50} of 56.23±0.09 µM. This might have been attributed to the substitution of a 2-nitrophenyl group in the skeleton. The docking results revealed that the **8k** showed three strong interactions, an acidic interaction between Ser122 and acetyl moiety, a

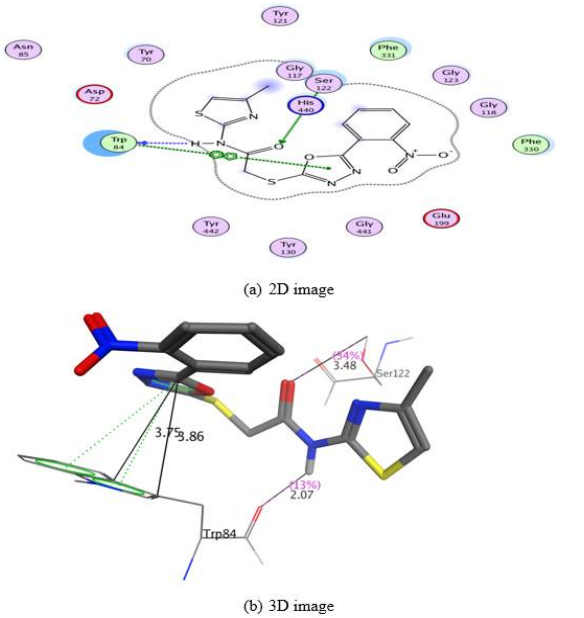
backbone donor and an arene-arene interaction with Trp84, having bond lengths of 3.48, 2.07 and 3.86 Å, respectively, as indicated in Figure 5 (2D & 3D).



(a) 2D image

(b) 3D image

Figure 4: The 2D (a) and 3D (b) interaction analysis of **8l** against AChE



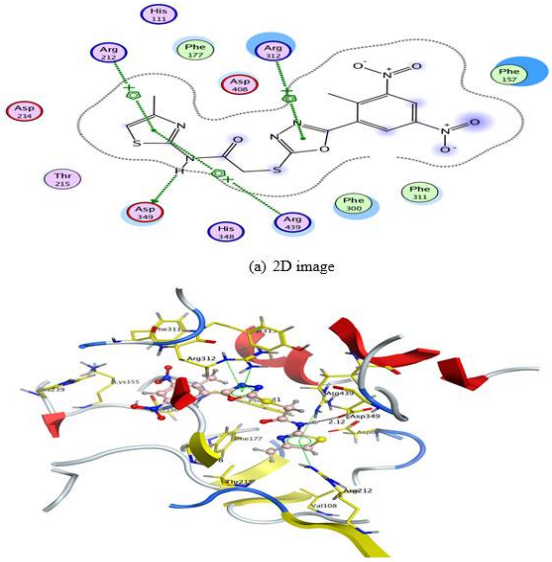
(a) 2D image

(b) 3D image

Figure 5: The 2D (a) and 3D (b) interaction analysis of **8k** against BChE

Similarly, a very moderate inhibitory potential was demonstrated by most of the compounds against α-glucosidase. Acarbose was used as the reference standard (IC_{50} = 37.38 ± 0.12 µM). The molecule **8o (**57.35 ± 0.17µM) exhibited relatively better inhibitory activity in the series studied against this enzyme. This improved activity might have been due to the incorporation of a 2-methyl-3,5-dinitrophenyl ring in the molecule.

The 1,4 glycosidic linkage is hydrolyzed by α-glucosidase. Postprandial hyperglycemia is caused by the inhibition of α-glucosidase [20]. Therefore, inhibition of α-glucosidase is considered important in managing type-2 diabetes whose blood sugar is highest after eating complex carbohydrates. From the *in silico* study, (Figure 6) the hydrogen attached to nitrogen of the acetamoyl group in **8o** was involved in strong polar bonding with Asp349 giving a bond length of 2.12Å. In addition, 1,3,4-oxadiazole and 4-methyl-1,3-thiazol-2-yl groups were also found to show arene-cation interactions with Arg312, Arg212 and Arg439, respectively.



(a) 2D image

(b) 3D image

Figure 6: The 2D (a) and 3D (b) interaction analysis of **8o** against α-glucosidase.

Some compounds were found to be active against urease but displayed feeble inhibition relative to the reference, thiourea (IC_{50} = 21.11±0.12 µM). In comparison with the other synthesized derivatives, **8f** possessed relatively considerable inhibitory activity, indicating that the incorporation of 3-chlorophenyl moiety was adequate for the inhibition of this enzyme. Against urease, the molecular docking study (Figure 7) of **8f** revealed π-π and arene-cation interactions for the 4-methyl-1,3-thiazol-2-yl and 3-chlorophenyl groups with His323 and His324, respectively. The hydrogen attached to nitrogen of the acetamoyl group was involved in polar bonding with Asp224, giving a bond length of 2.16Å.

The cytotoxicity of the synthesized compounds was evaluated by brine shrimp lethality. The

higher LD_{50} values of compounds **8d** (498.7 mM), **8l** (466.7 mM) and **8h** (395.4 mM) of brine shrimp lethality analysis demonstrated the lowest toxicity of these compounds. Thus the synthesized molecules might be used as new drug candidates for the related diseases shown by their enzyme inhibition behavior.

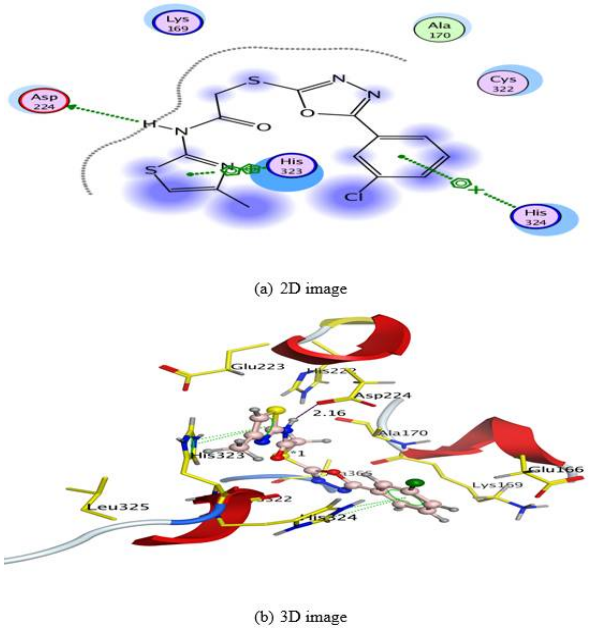


Figure 7: The 2D (a) and 3D (b) interaction analysis of **8f** against urease.

CONCLUSION

Most of the derivatives among the synthesized molecules displayed notable inhibitory activity against the selected enzymes, along with very low cytotoxicity. Therefore, these synthesized compounds may have important roles in drug design and development.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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