2-{[5-(Substituted-phenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-*N*-(1,3-thiazol-2-yl)acetamides: New bi-heterocycles as possible therapeutic agents

Muhammad Shahid Ramzan[1], Muhammad Athar Abbasi[1,*], Aziz-ur-Rehman[1], Sabahat Zahra Siddiqui[1], Syed Adnan Ali Shah[2,3], Muhammad Ashraf[4], Bushra Mirza[5] and Hammad Ismail[5]

[1]Department of Chemistry, Government College University, Lahore, Pakistan
[2]Faculty of Pharmacy & [3]Atta-ur-Rahman Institute for Natural Products Discovery (AuRIns), Level 9, FF3, Universiti Teknologi MARA, Puncak Alam Campus, Bandar, Puncak Alam, Selangor Darul Ehsan, Malaysia
[4]Department of Chemistry, The Islamia University of Bahawalpur, Bahawalpur, Pakistan
[5]Department of Biochemistry, Quaid-i-Azam University, Islamabad, Pakistan

Abstract: An electrophile, *N*-(1,3-thiazol-2-yl)-2-bromoacetamide (3), was synthesized by the reaction of 1,3-thiazole-2-amine (1) and 2-bromoethanoyl bromide (2) in an aqueous medium. A series of carboxylic acids, 7a-j, were converted into 1,3,4-oxadiazole heterocyclic core, through a series of three steps. The final compounds, 8a-j, were synthesized by stirring 7a-j and 3 in an aprotic polar solvent. The structural elucidation of the synthesized compounds was supported by IR, EI-MS, ^{1}H-NMR, and ^{13}C-NMR spectral data. Title compounds were evaluated for enzyme inhibition against cholinesterases and α-glucosidase enzymes and their cytotoxic behavior was monitored using brine shrimp assay. The enzyme inhibitor potential of compounds was supported by molecular docking studies.

Keywords: Bi-heterocycles, thiazole, 1,3,4-oxadiazoles, cholinesterases, α-glucosidase and brine shrimp assay.

INTRODUCTION

Synthetic and natural heterocyclic compounds are the subject of R & D units of many pharmacological, agrochemical and industrial laboratories. Around 90% of new medications contain heterocyclic moieties (Kashyap *et al*., 2011). Thiazole is an imperative heterocyclic moiety, present in vitamin B1 which is a vital co-enzyme of carboxylases enzymes and acts as an intermediate in the manufacturing of synthetic drugs, fungicides and dyes. Thiazole derivatives possess anticancer, antiviral, antibacterial, antifungal, and anti-inflammatory activities (Katrizsky *et al*., 1991). Their derivatives have also been used in the treatment of allergies, hypertension, irritation, schizophrenia, and bacterial and HIV disorders (Reddy *et al*., 2004). The most important molecules are 2-aminothiazole derivatives which possess antitumor activity by inhibiting kinases enzymes (Das *et al*., 2006). In the course of searching for new therapeutic agents, 2-aminothiazole derivatives were recently prepared by varying the 2-amino position and the s-substituted group of the 1,3,4-oxadiazole. The reaction of these moieties have likewise been studied and it is discovered that derivatives of these moieties display wide potential application in chemical, pharmaceutical, biological and material sciences and so on, which indicate extraordinary advancement esteem and draw in progressively uncommon consideration.

Cholinesterases (acetyl cholinesterase, AChE and butyrylcholinesterase, BChE) belong to the class serine hydrolyses. These enzymes play important role in nerve transmission (Gauthier *et al.,* 2001). Acetylcholine is hydrolyzed by these enzymes and therefore is a part of neuromuscular intersections and cholinergic cerebrum neurotransmitters *(*Wang & Tang, 2005). Alzheimer's plaques are known to have BChE in remarkably elevated levels. The inhibitors of these enzymes therefore target in the treatment of Alzheimer's disease (Yoshimizu *et al.,* 2008). α-Glucosidase inhibitors decrease post-prandial increase in blood sugar levels and therefore are used in the treatment of type-2 diabetes mellitus. Acarbose and Miglitol are routinely used marketed drugs which are α-glucosidase inhibitors.

In the present studies, molecules with two heterocyclic rings, 1,3-thiazole and 1,3,4-oxadiazole connected through acetamide linkage were synthesized and their *in vitro* enzyme inhibition activities were carried out in search for new therapeutically important inhibitors. The said data was further supported by *in silico* studies.

MATERIAL AND METHODS

General

All the chemicals were purchased from Sigma Aldrich, Alfa Aesar (Germany) and Merck through local suppliers along with analytical grade solvents. Pre-coated silica gel Al-plates were used for TLC with ethyl acetate and *n*-hexane as solvent system. Spots were detected by UV_{254}. Using open capillary tubes, Gallenkamp apparatus was used to detect melting points. IR spectra were recorded by

**Corresponding author:* e-mail: atrabbasi@yahoo.com

KBr pellet method using on Jasco-320-A spectrometer. ^{1}H-NMR spectra were recorded at 600 MHz in DMSO by Bruker spectrometer. Mass spectra (EIMS) were measured on 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 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 N-(1,3-thiazol-2-yl)-2-bromoacetamide (3)

1,3-Thiazole-2-amino (1;0.038 mol) was dispersed in 25 mL distilled water in iodine flask (100mL) and 20% aq Na_2CO_3 solution was poured to adjust a pH of 9-10. 2-Bromoethanoyl bromide (2; 0.038 mol) was poured in small patches on vigorous shaking and then set to stir for further 2 hours. The completion of the reaction was checked by TLC. Excess ice cold distilled water (40 mL) was added and the precipitates of compound 3 formed were collected through filtration, washed with distilled water and dried.

General procedure for synthesis of ethyl substituted-benzoates (5a-j)

Aryl carboxylic acids (4a-j; 2.5 g) were refluxed with 60 mL EtOH for 4-5 hours in the presence of conc. H_2SO_4 (1.25 mL) in a RB flask (250 mL). TLC plates were used to supervise the reactions. Excess distilled water (150 mL) was added after maximum completion and pH was adjusted to 8-10 by 20% aq. Na_2CO_3 solution. The product was collected through sequential extraction by $CHCl_3$ (50 mL). Chloroform was distilled off to collect products. In some cases, the products were collected through filtration. These obtained esters, 5a-j, were then used further.

General procedure for synthesis of substituted-benzohydrazides (6a-j)

Ethyl esters (5a-j; 4.5mL) were refluxed with 80% $N_2H_4.H_2O$ (7.2 mL) for 3-4 hrs in 20 mL EtOH in a RB flask (100mL). Reaction was monitored by TLC. At the end of the reaction, excess ice cold distilled H_2O (60 mL) was added to get the precipitates which were filtered, washed with distilled H_2O and dried to acquire title compounds, 6a-j.

General procedure for synthesis of 5-(substituted-phenyl)-1,3,4-oxadiazol-2-thiols (7a-j)

Solid KOH (0.029 mol) was dissolved in 25 mL EtOH on reflux in 100 mL RB flask. *substituted-benzohydrazides* (6a-j; 0.029 mol) were refluxed with CS_2 (0.058 mol) in this basified EtOH for 5-6 hrs. Reaction was monitored by TLC. At completion, excess ice cold distilled H_2O (60 mL) was added to form homogeneous solution. A pH of 5-6 was adjusted by pouring dilute HCl and the formed precipitates were filtered, washed with distilled H_2O and dried. The formed products, 7a-j, were also re-crystallized from EtOH.

General procedure for synthesis of 2-{[5-(Substituted-phenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-N-(1,3-thiazol-2-yl)acetamides (8a-j)

5-(Substituted-phenyl)-1,3,4-oxadiazol-2-thiols (**7a-j**; 0.004 mol) were dissolved in DMF (11mL) in a 50mL RB flask. Then LiH (0.004 mol) was added and the mixture was stirred for 0.5 hour. *N*-(1,3-thiazol-2-yl)-2-bromoacetamides (3; 0.004 mol) was poured and further stirred for 4-6 hours. Reaction completion was confirmed by TLC. Then excess ice cold distilled water (25 mL) was poured in patches along with continuous stirring. The reaction mixture was filtered, washed with distilled H_2O and dried. Similar protocol was adopted to get the desired products, 8a-j.

Enzyme inhibition assays

Cholinesterase assay

The method of Ellman was employed for cholinesterase assay as reported earlier (Nisa *et al.,* 2017).

α-Glucosidase assay

Enzyme inhibition assay was carried out as reported earlier (Naureen *et al.,* 2018).

Brine shrimp assay

The standard method for brine shrimp cytotoxicity assay was employed as reported earlier (Ullah *et al.,* 2012).

Computational analysis

The reported MOE-Dock method of MOE 2009-2010 was utilized to study the interactions of molecular recognition (Wadood *et al.,* 2013).

STATISTICAL ANALYSIS

Statistical analysis was performed by Microsoft Excel 2010 for all the thrice measured values and the results are presented as mean ± SEM.

Spectral characterization of synthesized compounds

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

White dull solid; yield: 90%; m.p.:191-192^{o}C; Mol. Formula: $C_{13}H_{10}N_4O_3S_2$; Mol. Mass: 334 g mol^{-1}; IR (KBr, cm^{-1}) ν_{max}: 3320 (N-H), 3150 (C-H stretching of aromatic ring), 2965 (CH_3), 2930 (-CH_2- stretching), 1740 (-CO_2 stretching), 1670 (C=C stretching of aromatic ring), 1170 (C-O-C stretching), 720 (-C-H), 1600 (C=N); ^{1}H-NMR (DMSO-d_6, 600 MHz, δ/ppm): δ 12.54 (s, 1H, CON-H), 7.76 (d, *J*=8.7 Hz, 2H, H-2''' & H-6'''), 7.50 (d, *J* =3.5 Hz, 1H, H-4), 7.26 (d, *J*=3.5 Hz, 1H, H-5), 6.91 (d, *J*=8.7 Hz, 2H, H-3'''& H-5'''), 4.37 (br.s, 2H, CH_2-2'); ^{13}C-NMR (DMSO-d_6, 150 MHz, δ/ppm): δ^{13}C-NMR (DMSO-d_6, 150 MHz, δ/ppm): δ 165.51 (C-1'), 165.46 (C-5''), 162.26 (C-2''), 161.76 (C-4'''), 160.83 (C-2),

137.77 (C-4), 128.35 (C-2'''& C-6'''), 116.12 (C-3'''& C-5'''), 113.60 (C-1'''), 113.60 (C-5), 35.45 (C2'); EI-MS: *m/z* 334 $[M]^+$, 121 $[C_6H_5N_2O]^+$, 100 $[C_3H_3N_2S]^+$, 127 $[C_4H_3N_2OS]^+$, 194 $[C_8H_5N_2O_2S]^+$, 292 $[C_{11}H_8N_4O_2S_2]^+$, 214 $[C_7H_5N_2O_2S_2]^+$.

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

Light yellow solid; yield: 85%; m.p.: 207-208°C; Mol. Formula: $C_{14}H_{12}N_4O_3S_2$; Mol. Mass: 348 g mol^{-1}; IR (KBr, cm^{-1}) ν_{max}: 3360 (N-H), 3170 (C-H stretching of aromatic ring), 2920 (-CH_2- stretching), 2945 (-CH_3), 1700 (-CO_2 stretching),1642 (C=C stretching of aromatic ring), 1150 (C-O), 1160 (C-O-C stretching), 743 (-C-H), 1620 (C=N); ^{1}H-NMR (DMSO-d_6, 600 MHz, δ/ppm): δ 12.54 (s, 1H, CON-H), 7.77 (d, *J* 7.4 Hz, 2H, H-2''' & H-6'''), 7.60 (br.d, *J*=7.3 Hz, 2H, H-3''' & H-5'''), 7.51 (d, *J* = 3.5 Hz, 1H, H-4), 7.26 (d, *J*=3.9 Hz, 1H, H-5), 4.41 (br.s, CH_2, H-2'), 3.85 (br. s, 4-OCH_3); ^{13}C-NMR (DMSO-d_6, 150 MHz, δ/ppm): δ 164.97 (C-1'), 163.99 (C-5''), 162.72 (C-2''), 160.80 (C-2), 134.90 (C-4), 129.45 (C-2''' & C-6'''), 126.30 (C-3''' & C-5'''), 120.69 (C-4'''), 113.85 (C-5), 111.78 (C-1'''), 55.92 (4-OCH_3), 35.33 (C-2'); EIMS: *m/z* 348 $[M]^+$, 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-(1,3-thiazol-2-yl)acetamide (8c)

Dull white solid; yield: 87 %;m.p.: 261-262°C; Mol. Formula: $C_{13}H_9ClN_4O_2S_2$; Mol. Mass: 352 g mol^{-1}; IR (KBr, cm^{-1}) ν_{max}: 3345 (N-H), 3175 (C-H stretching of aromatic ring), 2923 (-CH_2- stretching), 1672 (C=C stretching of aromatic ring), 1590 (C=N), 743 (-C-H), 580 (C-Cl stretching); ^{1}H-NMR (DMSO-d_6, 600 MHz, δ/ppm): δ 12.45 (s, 1H, CON-H), 7.95 (br.d, *J*=7.74 Hz, 1H, H-6'''), 7.69 (br.d, *J* = 8.04 Hz, 1H, H-3'''), 7.63 (br.t, *J* = 7.5 Hz, 1H, H-4'''), 7.53 (br.t *J*=7.6 Hz, 1H, H-5'''), 7.50 (d, *J*=3.4 Hz, 1H, H-4), 7.26 (d, *J*=3.4 Hz, 1H, H-5), 4.40 (br.s, CH_2, H-2'); ^{13}C-NMR (DMSO-d_6, 150 MHz, δ/ppm): δ 165.15 (C-1'), 163.70 (C-5''), 163.32 (C-2''), 160.65 (C-2), 137.76 (C-2'''), 137.69 (C-4), 133.28 (C-4'''), 131.14 (C-3'''), 131.09 (C-6'''), 127.82 (C-5'''), 122.07 (C-1'''), 113.91 (C-5), 35.38 (C-2'),. EIMS: *m/z* 352 $[M]^+$, 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-(1,3-thiazol-2-yl)acetamide (8d)

White amorphous solid; yield: 91%; m.p.: 231-232°C; Mol. Formula: $C_{13}H_9ClN_4O_2S_2$; Mol. Mass: 352 g mol^{-1}; IR (KBr, cm^{-1}) ν_{max}: 3350 (N-H), 3173 (C-H stretching of aromatic ring), 2923 (-CH_2- stretching), 1672 (C=C stretching of aromatic ring), , 743 (-C-H), 1590 (C=N), 584 (C-Cl stretching); ^{1}H NMR (DMSO, 600 MHz, δ/ppm): δ 12.45 (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'''), 7.50 (d, *J*=3.3Hz, 1H, H-4), 7.49 (dd, *J*=2.1, 8.5, Hz, 1H, H-4'''), 7.26 (d, *J*=3.4 Hz, 1H, H-5), 4.40 (br.s, CH_2, H-2'),;^{13}C-NMR (DMSO, 600 MHz): δ 165.15 (C-1'), 163.70 (C-5''), 163.32 (C-2''), 160.65 (C-2), 137.81 (C-4), 137.16 (C-3'''), 133.28 (C-4'''), 131.14 (C-2'''), 131.09 (C-6'''), 127.82 (C-5'''), 122.07 (C-1'''), 113.91 (C-5), 35.38 (C-2'); EI-MS: *m/z* 352 $[M]^+$, 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-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-N-(1,3-thiazol-2-yl)acetamide (8e)

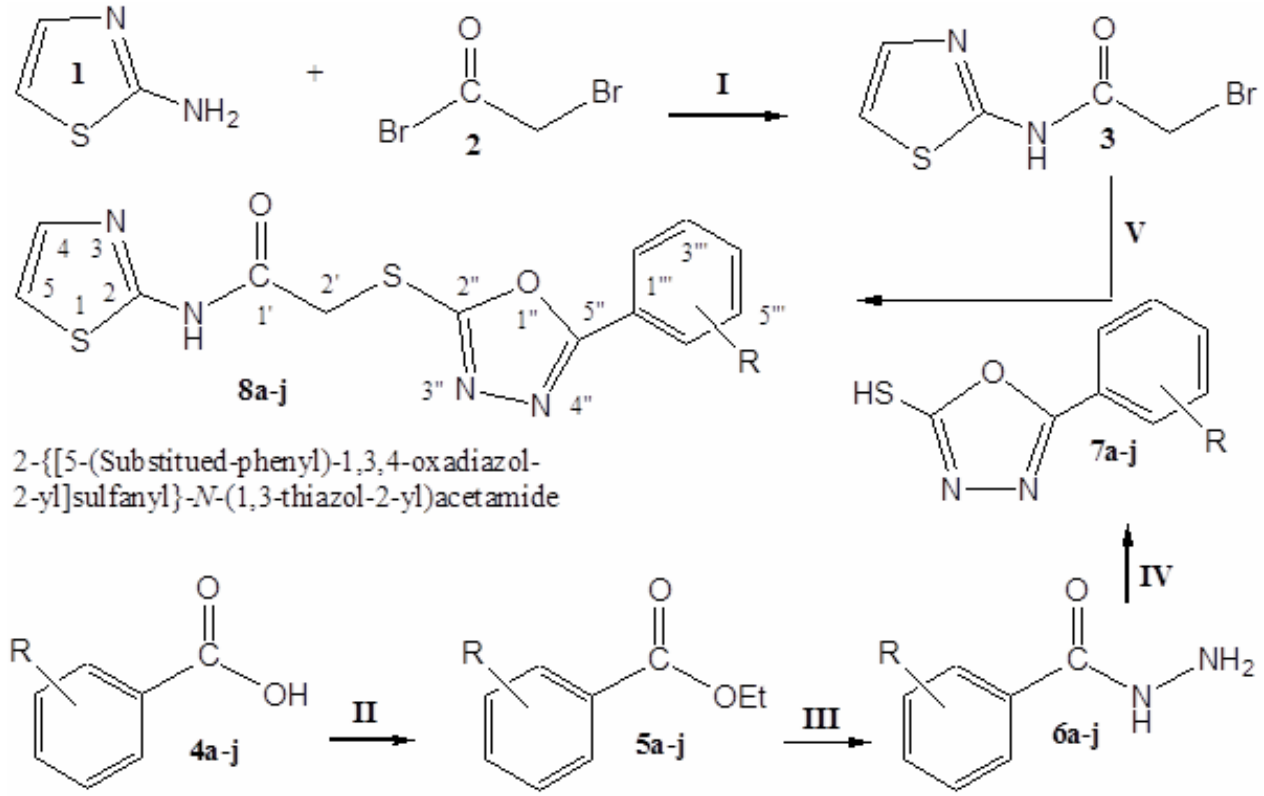
white solid; yield: 86%; m.p.: 205-206°C; Mol. Formula: $C_{13}H_9ClN_4O_2S_2$; Mol. Mass: 352 g mol^{-1}; IR (KBr, cm^{-1}) ν_{max}: 3365 (N-H), 3150 (C-H stretching of aromatic ring), 2923 (-CH_2- stretching), 1672 (C=C stretching of aromatic ring), 735 (-C-H), 1590 (C=N), 590 (C-Cl stretching);^{1}H NMR (DMSO, 600 MHz, δ/ppm): δ 12.45 (s, 1H, CON-H), 7.91 (d, *J*=8.5 Hz, 2H, H-3''' & H-5'''), 7.50 (d, *J*=3.4 Hz, 1H, H-4), 7.49 (d, *J*=8.5 Hz, 2H, H-2''' & H-6'''), 7.26 (d, *J*=3.4 Hz, 1H, H-5), 4.40 (br.s, CH_2, H-2'), ^{13}C-NMR (DMSO, 600 MHz): δ 165.15 (C-1'), 163.70 (C-5''), 163.32 (C-2''), 160.65 (C-2), 137.81 (C-3''' & C-5'''), 137.65 (C-4), 133.28 (C-4'''), 113.91 (C-5), 131.14 (C-2''' & C-6'''), 122.07 (C-1'''), 35.38 (C-2'); EI-MS: *m/z* 352 $[M]^+$, 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-Aminophenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-N-(1,3-thiazol-2-yl)acetamide (8f)

White amorphous solid; yield: 83 %; m.p.:183-184 °C; Mol. Formula: $C_{13}H_{11}N_5O_2S_2$; Mol. Mass: 333 g mol-1; IR (KBr, cm^{-1}) ν_{max}: 3365 (N-H), 3177 (C-H stretching of aromatic ring), 1672 (C=C stretching of aromatic ring), 740 (-C-H), 1520 (C=N), 1380 (C-N-), ^{1}H-NMR (DMSO-d_6, 600 MHz, δ/ppm): δ 12.38 (s, 1H, CON-H), 7.96 (dd, *J* = 1.1, 8.1 Hz, 1H, H-6'''), 7.63 (br.t, *J* = 9.6 Hz, 1H, H-5'''), 7.58 (d, *J* = 2.1 Hz, 1H, H-2'''), 7.47 (dd, *J* = 2.1, 8.5 Hz, 1H, H-4'''), 7.51 (d, *J* = 3.5Hz, 1H, H-4), 7.27 (d, *J* = 3.5Hz, 1H, H-5), 4.42 (br.s, 2H, CH_2-2'); ^{13}C-NMR (DMSO-d_6, 150 MHz, δ/ppm): δ 165.36 (C-1'), 165.24 (C-5''), 162.99 (C-2''), 160.36 (C-2), 137.39 (C-4), 132.04 (C-4'''), 129.38 (C-5'''), 126.35 (C-6'''), 126.02 (C-2'''), 122.88 (C-1'''), 113.89 (C-3'''), 113.19 (C-5), 35.37 (C-2'),; EI-MS: *m/z* 333 $[M]^+$, 234 $[C_{10}H_8N_3O_2S]^+$, 120 $[C_7H_6NO]^+$, 100 $[C_3H_3N_2S]^+$, 92 $[C_6H_6N]^+$.

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

Light brown solid; yield: 77 %; m.p.:198-199°C; Mol. Formula: $C_{13}H_{11}N_5O_2S_2$; Mol. Mass: 333 g mol^{-1}; IR (KBr, cm^{-1}) ν_{max}: 3355 (N-H), 3170 (C-H stretching of



Scheme 1: Protocol for the synthesis of 2-{[5-(substituted-phenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-*N*-(1,3-thiazol-2-yl)acetamide (8a-j). Reagents and conditions: (I) H_2O, 20% Na_2CO_3 solution, stirring for 2 hours. (II) H_2SO_4, EtOH, refluxing for 4-5 hours (III) N_2H_4, EtOH, refluxing for 3-4 hours (IV) CS_2, KOH, EtOH, refluxing for 5-6 hours (V) DMF, LiH, stirring for 4-6 hours.

Table 1: Different substituents (-R) in scheme 1.

Compd.	-R
4a, 5a, 6a, 7a, 8a	4-OH
4b, 5b, 6b, 7b, 8b	4-OCH_3
4c, 5c, 6c, 7c, 8c	2-Cl
4d, 5d, 6d, 7d, 8d	3-Cl
4e, 5e, 6e, 7e, 8e	4-Cl

Compd.	-R
4f, 5f, 6f, 7f, 8f	3-NH_2
4g, 5g, 6g, 7g, 8g	4-NH_2
4h, 5h, 6h, 7h, 8h	2-NO_2
4i, 5i, 6i, 7i, 8i	3- NO_2
4j, 5j, 6j, 7j, 8j	4- NO_2

aromatic ring), 2953 (-CH_2- stretching), 1672 (C=C stretching of aromatic ring), 1125 (C-O-C stretching of ether), 750 (-C-H), 1520 (C=N), 1350 (C-N-), ^{1}H NMR (DMSO, 600 MHz, δ/ppm):): δ 12.54 (s, 1H,CON-H), 7.56 (br.d, *J*=8.6 Hz, 2H, H-2''' & H-6'''), 6.62 (d, *J* = 8.64 Hz, 1H, H-3'' & H-5'') 7.50 (d, *J* = 3.5Hz, 1H, H-4), 7.26 (d, *J*=3.5 Hz, 1H, H-5), 4.40 (br.s, 2H, CH_2-2'),; ^{13}C-NMR (DMSO, 600 MHz): δ165.15 (C-1'), 163.70 (C-5''), 163.32 (C-2''), 160.65 (C-2), 137.81 (C-4), 133.28 (C-4'''), 131.14 (C-2''' & C-6'''), 131.09 (C-6'''), 127.82 (C-3''' & C-5'''), 122.07 (C-1'''), 113.91 (C-5), 35.38 (C-2'). EI-MS: *m/z* 333 $[M]^+$, 234 $[C_{10}H_8N_3O_2S]^+$, 120 $[C_7H_6NO]^+$, 100 $[C_3H_3N_2S]^+$,
92 $[C_6H_6N]^+$.

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

Off white amorphous solid; yield: 87%; m.p.: 183-184°C; Mol. Formula: $C_{13}H_9N_5O_4S_2$; Mol. Mass: 363 g mol^{-1}; ; IR (KBr, cm^{-1}) ν_{max}: 3390 (N-H), 3173 (C-H stretching of aromatic ring), 2930 (CH3), 2915 (-CH_2- stretching), 1650 (C=C stretching of aromatic ring), 1570 (C=N), 1530 (-NO2), 1145 (C-O-C stretching of ether), 740 (-C-H); ^{1}H NMR (DMSO, 600 MHz, δ/ppm): δ 12.53 (s, 1H, CON-H), 8.19-8.17 (m, 1H, H-3'''), 8.01 (m, 1H, H-6''') 7.94-7.90 (m, 2H, H-4''' & H-5'''), 7.51 (d, *J* = 3.3 Hz, 1H, H-4), 7.15 (d, *J* = 3.4 Hz, 1H, H-5), 4.37 (br.s, 2H, CH_2-2'),; ^{13}C-NMR (DMSO, 150 MHz): δ 165.34 (C-1'), 164.13 (C-5''), 163.56 (C-2''), 157.63 (C-2), 137.80 (C-4), 134.05 (C-2'''), 131.84 (C-3'''), 131.41 (C-4'''), 125.88 (C-

Table 2: Inhibition profiles for AChE, BChE and a-glucosidase enzymes

Compound	AChE		BChE		α-glucosidase	
	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	64.17±0.44	179.13±0.30	71.17±0.25	149.23±0.40	65.54±0.23	263.32±1.11
8b	83.19±0.21	39.15±0.19	91.42±0.25	28.64±0.17	86.26±0.18	57.52±0.17
8c	47.74±0.16	117.23±0.44	12.34±0.16	-	75.32±0.19	356.21±0.14
8d	91.71±0.12	88.74±0.25	83.26±0.15	117.25±0.19	77.39±0.19	259.62±0.13
8e	99.17±0.45	69.25±0.29	44.14±0.19	159.15±0.23	67.56±0.21	248.51±0.18
8f	21.67±0.13	-	62.67±0.13	191.23±0.20	68.26±0.21	49.26±0.19
8g	21.89±0.16	-	88.62±0.24	34.52±0.45	89.28±0.19	346.45±0.14
8h	71.31±0.29	59.13±0.45	77.17±0.21	177.13±0.09	57.32±0.11	116.45±0.14
8i	73.74±0.19	187.35±0.09	71.47±0.17	159.23±0.19	85.34±0.26	129.72±0.21
8j	51.11±0.14	101.34±0.44	75.35±0.16	123.25±0.29	63.22±0.17	106.45±0.14
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

Table 3: Cytotoxicity by Brine Shrimp Assay.

Compound	ED_{50} (μg/mL)	Compound	ED_{50} (μg/mL)
8a	65	8f	20
8b	55	8g	1.2
8c	80	8h	1.1
8d	60	8i	3.4
8e	50	8j	2.0

6'''), 125.04 (C-5'''), 124.82 (C-1'''), 113.91 (C-5), 35.35 (C-2'); EI-MS: *m/z* 363 $[M]^+$, 213 $[C_6H_5N_4OS_2]^+$, 199 $[C_6H_5N_3OS_2]^+$, 150 $[C_7H_4NO_3]^+$, 142 $[C_5H_5N_2OS]^+$, 127 $[C_4H_3N_2OS]^+$, 76 $[C_6H_4]^+$.

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

Light yellow solid; yield: 88%; m.p.: 201-202°C; Mol. Formula $C_{13}H_{11}N_5O_2S_2$; Mol. mass: 363 g mol^{-1}; IR (KBr, cm^{-1}) ν_{max}: 3345 (N-H), 3165 (C-H stretching of aromatic ring), 2920 (-CH_2- stretching), 1670 (C=C stretching of aromatic ring), 1570 (C=N), 1530 (-NO2), 1165 (C-O-C stretching), 750 (-CH); ^{1}H-NMR (DMSO-d_6, 600 MHz, *δ*/ppm): *δ* 12.55 (s, 1H,CON-H), 7.92 (br.t, *J*=7.8 Hz, 2H, H-4''' & H-6'''), 7.69 (d, *J*=7.7 Hz, 1H, H-2'''), 7.61 (br.t, *J*=7.8 Hz, 1H, H-5'''), 7.50 (d, *J*=3.4 Hz, 1H, H-4), 7.26 (d, *J*=3.4 Hz, 1H, H-5), 4.43 (br.s, 2H, CH_2-2'); ^{13}C-NMR (DMSO-d_6, 150 MHz, *δ*/ppm): *δ* 165.34 (C-1'), 164.13 (C-5''), 163.56 (C-2''), 160.63 (C-2), 137.80 (C-4), 134.05 (C-3'''), 131.84 (C-2'''), 131.41 (C-4'''), 125.88 (C-6'''), 125.04 (C-5'''), 124.82 (C-1'''), 113.91 (C-5), 35.35 (C-2'); EI-MS: *m/z* 363 $[M]^+$, 213 $[C_6H_5N_4OS_2]^+$, 199 $[C_6H_5N_3OS_2]^+$, 150 $[C_7H_4NO_3]^+$, 142 $[C_5H_5N_2OS]^+$, 127 $[C_4H_3N_2OS]^+$, 76 $[C_6H_4]^+$.

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

Off white solid; yield: 77%; m.p.:221-222°C; Mol. Formula: $C_{13}H_{11}N_5O_2S_2$; Mol. Mass: 363 g mol^{-1}; IR (KBr, cm^{-1}) ν_{max}: 3345 (N-H), 3165 (C-H stretching of aromatic ring), 2945 (CH3), 2920 (-CH_2- stretching), 1725 (-CO_2 stretching), 1670 (C=C stretching of aromatic ring), 1570 (C=N), 1530(-NO2), 1165 (C-O-C stretching of ether), 750 (-C-H);^{1}H-NMR (DMSO-d_6, 600 MHz, *δ*/ppm): *δ* 12.46 (s, 1H,CON-H), 7.93(d, *J*=8.4 Hz, 2H, H-2''' & H-6'''), 7.88 (d, *J* = 8.4Hz, 2H, H-3'''& H-5'''), 7.51 (d, *J*=3.5 Hz, 1H, H-4), 7.26 (d, *J*=3.4 Hz, 1H, H-5), 4.42 (br.s, 2H, CH_2-2'); ^{13}C-NMR (DMSO-d_6, 150 MHz, *δ*/ppm): *δ* 165.26 (C-1'), 162.99 (C-5''), 160.46 (C-2''), 157.68 (C-2), 137.80 (C-4), 132.21 (C-3'''), 129.41 (C-5'''), 129.38 (C-6'''), 126.35 (C-4'''), 126.02 (C-2'''), 122.48 (C-1'''), 113.91 (C-5), 35.36 (C-2); EI-MS: *m/z* 363 $[M]^+$, 213 $[C_6H_5N_4OS_2]^+$, 199 $[C_6H_5N_3OS_2]^+$, 150 $[C_7H_4NO_3]^+$, 142 $[C_5H_5N_2OS]^+$, 127 $[C_4H_3N_2OS]^+$, 76 $[C_6H_4]^+$.

RESULTS

The synthesis of various 2-{[5-(substituted-phenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-*N*-(1,3-thiazol-2-yl)acetamides (8a-j), is sketched in Scheme-1 and varying substituents are given in table 1. The detailed procedures are given in the experimental section. The structural verification of the prepared molecules was performed through spectral data analysis. All the derivatives were subjected to enzyme inhibition against the AChE, BChE and α-glucosidase (table 2) and their cytotoxicity (table 3) was evaluated by brine shrimp assay.

DISCUSSION

Chemistry

The protocol for the synthesis of targeted new bi-heterocyclic molecules, 2-{[5-(substituted-phenyl)-1,3,4-

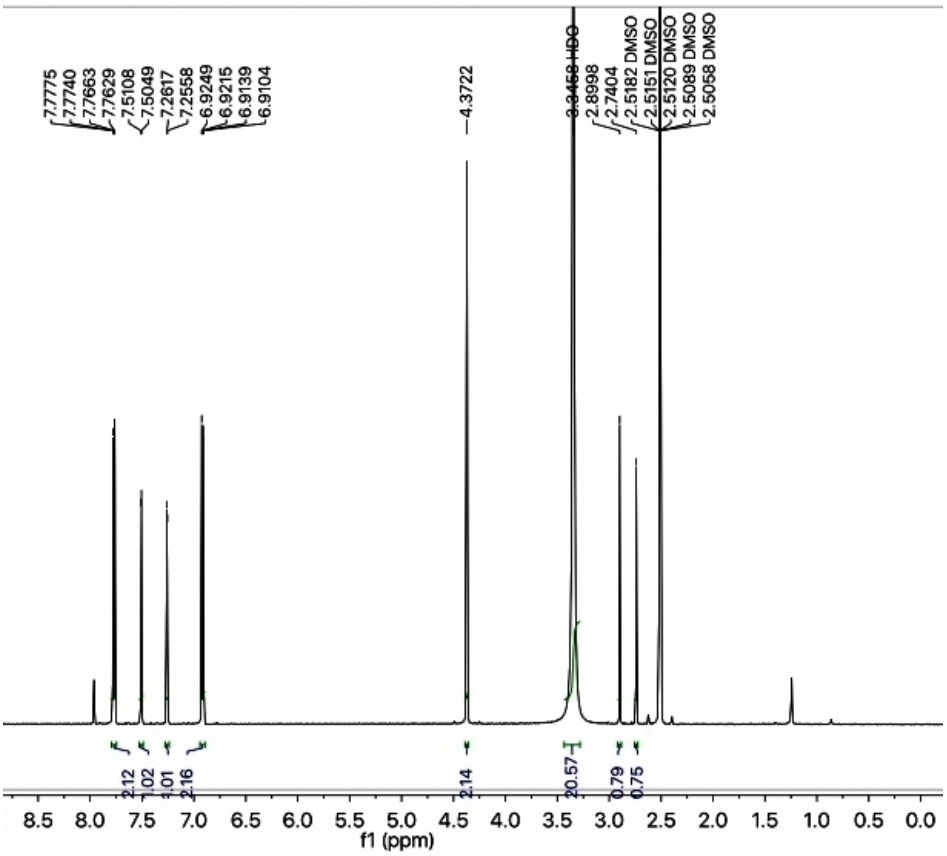


Fig. 1: ^{1}H-NMR spectrum of 8a.

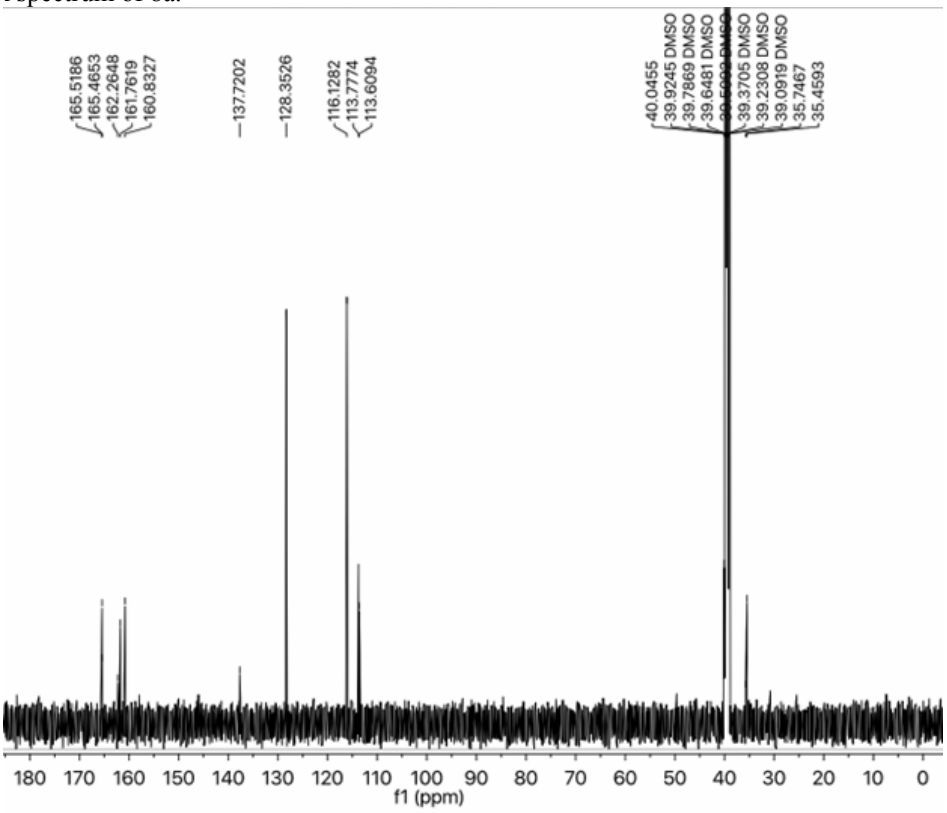


Fig. 2: ^{13}C-NMR spectrum of 8a.

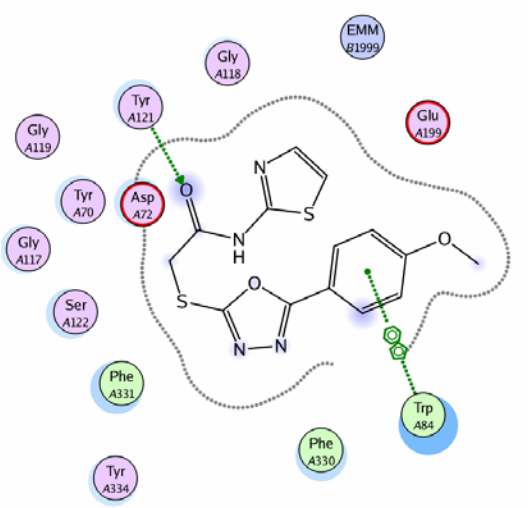


Fig. 3(a): 2D molecular docking study of compound 8b against AChE enzyme.

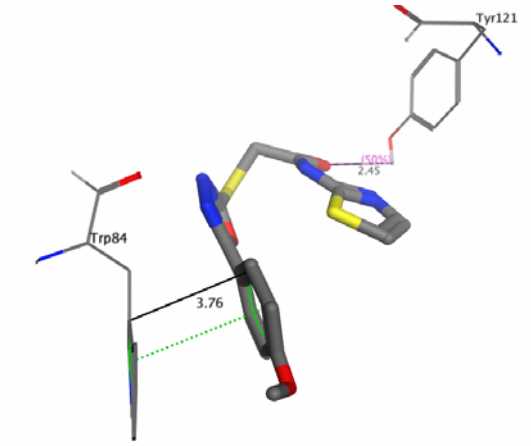


Fig. 3(b): 3D molecular docking study of compound 8b against AChE enzyme.

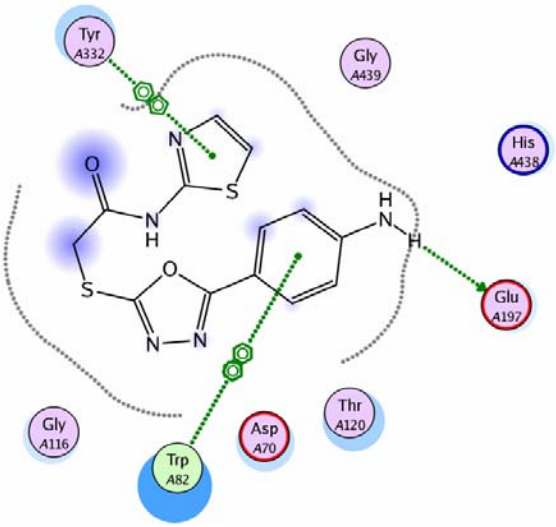


Fig. 4(a): 2D molecular docking study of compound 8g against BChE enzyme.

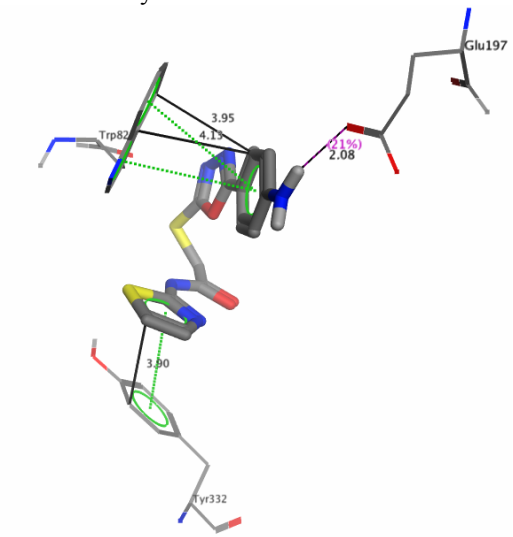


Fig. 4(b): 3D molecular docking study of compound 8g against BChE enzyme.

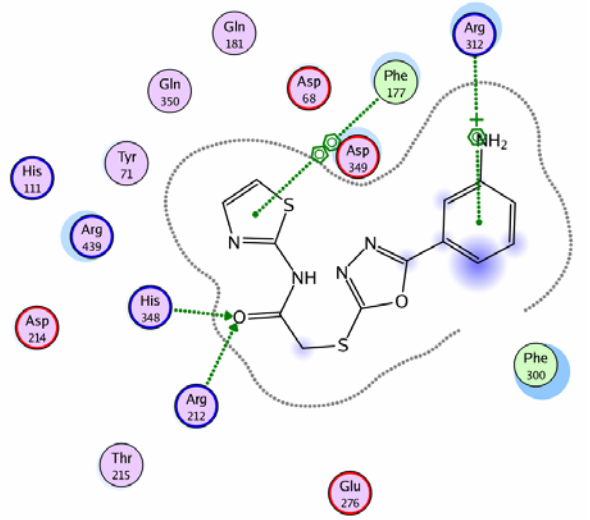


Fig. 5(a): 2D molecular docking study of compound 8f against α-glucosidase enzyme.

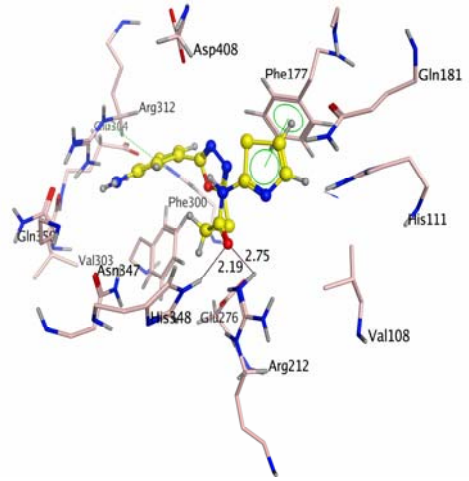


Fig. 5(b): 3D molecular docking study of compound 8f against α-glucosidase enzyme.

oxadiazol-2-yl]sulfanyl}-*N*-(1,3-thiazol-2-yl)acetamides (8a-j), is sketched in scheme-1 and varying groups are listed in table 1. The synthesis was geared up by the reaction of 2-amino-1,3-thiazole (1) with 2-bromoethanoyl bromide (2) in a basic aqueous medium to produce an electrophile, *N*-(1,3-thiazol-2-yl)-2-bromoacetamide (3). In a parallel set of reactions, various substituted-benzoic acids, 4a-j, were converted sequentially to respective esters, 5a-j, hydrazides, 6a-j and then intermolecular cyclization with CS_2 resulted in the formation of corresponding 1,3,4-oxadiazol-2-thiol as nucleophiles. The final step in the synthesis was the coupling of electrophile, 3, with these nucleophiles, 7a-j, one by one, to acquire the targeted molecules, 8a-j. The structures of all the newly synthesized derivatives were confirmed by their spectral data of IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectrometry. One of the compounds, 8a, is discussed hereby in detail for the convenience of the readers. The molecule, 8a, was prepared as white dull solid. The reaction yield was found to be 90% with melting point of 191-92^{o}C. The molecular formula, $C_{13}H_{10}N_4O_3S_2$, of this compound was predicted by the molecular ion peak at *m/z* 334 in its EI-MS spectrum and by counting the number of protons in its ^{1}H-NMR spectrum (fig. 1). The number of carbon resonances in it ^{13}C-NMR spectrum (fig. 2) also supported this assignment. Various functional groups in this molecule were identified by its IR data. Therein, different absorption bands at υ 3320 (N-H), 3250 (OH), 3150 (C-H stretching of aromatic ring), 2930 (-CH_2- stretching), 1740 (-CO_2 stretching), 1670 (C=C stretching of aromatic ring), 1170 (C-O-C stretching), 720 (-C-H), 1600 (C=N). The 4-hydroxyphenyl group in this molecule was ascribed by A_2B_2 spin set of signals in the aromatic region of its ^{1}H-NMR spectrum at *d* 7.76 (d, *J*=8.7Hz, 2H, H-2''' & H-6''') and 6.91 (d, *J*=8.7 Hz, 1H, H-3'''& H-5'''). The structural assignment of this unit was also fully supported by four typical carbon resonances in its ^{13}C-NMR spectrum at *d* 161.76 (C-4'''), 128.35 (C-2''' & C-6'''), 116.12 (C-3''' & C-5''') and 113.60 (C-1'''). The *N*-(1,3-thiazol-2-yl) moiety of the molecule was characterized by two distorted doublets in its ^{1}H-NMR spectrum at *d* 7.50 (d, *J* = 3.5 Hz, 1H, H-4) and 7.26 (d, *J*=3.5 Hz, 1H, H-5). However, in its ^{13}C-NMR spectrum, the appearance of three carbon resonances for this heterocyclic moiety at *d* 160.83 (C-2), 137.72 (C-4) and 113.77 (C-5) was very rational. Similarly, the other heterocyclic core of the molecule i.e. 1,3,4-oxadiazol-2-yl was also affirmed by two quaternary carbon resonances at *d* 165.46 (C-5'') and 162.26 (C-2''). The central *C* & *N*-substituted acetamido group, which was interconnecting the two heterocycles in the molecule, was inferred by two characteristic peaks in both its ^{1}H-NMR and ^{13}C-NMR spectra. In former spectrum, the peaks appeared at δ 12.54 (s, 1H, CON-H) and 4.37 (br.s, CH_2, H-2'), while in latter spectrum, the carbon resonances appeared at δ 165.51 (C-1') and 35.45 (C-2').The mass fragmentation data of this molecule, as described in experimental section, was also in complete agreement with the above discussed structural assignments. So, on the basis of collective spectral evidences, the structure of 8a was confirmed and it was named as 2-{[5-(4-hydroxyphenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-*N*-(1,3-oxazol-2-yl)acetamide. The similar protocol was observed for synthesis and structure elucidation of other derivatives in the series.

Enzyme inhibition and brine shrimp assay

All the synthesized compounds, 8a-j, were subjected to enzyme inhibitions against AChE, BChE and *α*-glucosidase enzymes. All compounds exhibited inhibitions at varying levels as compared to standard used. The results are listed as percent inhibition and IC_{50}values given in table 2.

All the compounds exhibited an inhibitory potential against AChE. Biological analysis of the lead compound 8b with IC_{50} values of 39.15±0.19 µM and a few close analogues established that-OCH_3, electron donor group at the 4-position of phenyl ring was responsible for AChE inhibition. The reference used for this enzyme was eserine with IC_{50} value of 0.04±0.0.0001µM. These bi-heterocycles also contained the substitution of Cl (8c-8e), NH_2 (8f-8g) and NO_2 (8h−8j) in the phenyl ring, and it was noticed that the introduction of -Cl group on the phenyl (8c-8e) caused the inhibition to drop further from *para* to *ortho* position against AChE. Other substituted groups (Cl, NO_2, NH_2) at positions 2, 3 and 4 of the phenyl ring also maintained good activity against AChE. The compounds, 8f and 8g, substituted by amino group at third and fourth positions of phenyl ring, remained inactive against the said enzyme. The overall order for activity for all the synthesized compounds against this enzyme was: 8b > 8h > 8e > 8d > 8j > 8c > 8a > 8i. The IC_{50} results were also supported by molecular docking studies. The docking study of most of the active compounds, 8d, is shown in 2D and 3D images of fig 3. As shown, 8b bound strongly in the active pocket of enzyme by giving a total of three interactions including acidic and arene-arene interactions as is given in 2D and 3D images. The first interaction was between Tyr121 and carbonyl oxygen, while the second weak interaction exists between Trp84 and methoxy phenyl ring of compound showing bond lengths of 2.45 and 3.76 Å, respectively (fig. 3; 2D & 3D).

Against BChE, moderate activity was shown by the synthesized compounds. The most promising compounds were 8b and 8g with IC_{50} values of 28.64±0.17and 34.52±0.45µM, respectively. The better inhibition might be because of 4-methoxyphenyl and 4-aminophenyl groups, respectively. The reference used for BChE was also eserine with IC_{50} value of 0.85±0.0001 µM. The enhanced inhibition activity shown by 8b may be due to the presence of electron donating group at two position of

the phenyl ring. The overall order of activity for all the synthesized compounds against this enzyme was: 8b >8g >8d >8j >8a >8e >8i >8h >8f. The above studies reveal that, the nature of the linkage (substituent on aromatic ring) influences the enzyme inhibition profiles. Compound 8g made three interactions as is seen in 2D and 3D images of fig. 4. From the docking of 8g against BChE, three interactions were demonstrated. Acidic and a couple of π-π interactions were made by Glu197, Tyr332 and Trp82 with the said compound giving bond distances of 2.08Å, 3.90Å and an average of 4.04Å, respectively (fig. 4; 2D & 3D).

The said compounds 2-{[5-(substituted-phenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-*N*-(1,3-thiazol-2-yl) acetamides (8a-j) were also screened against α-glucosidase using acarbose as standard (IC_{50} 37.38±0.12μM). The compounds 8f and 8b showed excellent inhibition against this enzyme with IC_{50} values of 49.26±0.19 and 57.52±0.17μM, respectively. The highest inhibition was shown by 8f (NH_2) which may be attributed to the presence of electron donating group at third position of phenyl ring. The overall order of activity against the said enzyme was: 8f > 8b > 8j > 8h > 8i > 8e > 8d > 8a > 8c. The molecular docking study of 8f revealed four types of interactions (fig. 5 2D & 3D). Two interactions were observed between His348, carbonyl oxygen and Arg212. An interaction was also observed between Phe177 and hydrogen of the thiazol. The hydrogen attached to nitrogen of amino group was involved in hydrogen bonding with Arg332.

The cytotoxicity of these molecules was evaluated by brine shrimp assay and data is shown in table 3. The highest same ED_{50} value of 80μg/mL was observed for two compounds 8c, while 8h exhibited a value of 1.1μg/mL. Doxorubicin was used as reference with ED_{50} value of 5.21μg/mL.

CONCLUSIONS

A series of bi-heterocyclic molecules was synthesized by simple and rapid chemical methods and the compounds were assayed against AChE, BChE and *α*-glucosidase enzymes. The molecules substituted by amino, chloro and methoxy were found to be better inhibitors for studied enzymes than others in the series. Compounds 8b, 8f and 8g were identified as excellent inhibitors of enzymes among the whole series of synthesized molecules. These compounds also rendered least cytotoxicity towards brine shrimps assay.

ACKNOWLEDGEMENTS

The Higher Education Commission (HEC) of Pakistan is highly acknowledged by the authors for financial support regarding purchasing of chemicals and spectral study.

REFERENCES

Das B, Reddy VS and Ramu R (2006). Aapid and high-yielding synthesis of thiazoles and aminothia-zolesusingammonium-12-molybdophosphate. *J. Mol. Catal*., **252**: 235-237.

Gauthier S (2001). Cholinergic adverse effects of cholinesterase inhibitors in Alzheimer's disease. *Drug Aging*, **18**: 853-862.

Kashyap, Tikka B, Sharma S, Kumari S, Verma P, Sharma S and Arya V (2011). Human cancer cell lines- A brief communication. *Chem. Pharm. Res*., **3**: 514-520.

Naureen S, Chaudhry F, Munawar MA, Ashraf M, Hamid S and Khan MA (2018). Biological evaluation of new imidazole derivatives tethered with indole moiety as potent α-glucosidase inhibitors. *Bioorganic Chem*., **76**: 36-369.

Nisa N, Munawar MA, Iqbal A, Ahmed A, Ashraf M, Gardener QAA and Khan MA (2017). Synthesis of novel 5-(aroylhydrazinocarbonyl)escitalopram as cholinesterase inhibitors. *Eur. J. Med. Chem*., **138**: 396-406.

Reddy CS, Suseela K, Kapoor AS, Kaundal R, Krishnaiah NV, Mishra B, Ramakrishna YS, Prasad YG, Reddy DY and Prabhakar (2004). Synthesis, characterization and antimicrobial evaluation of some 5-(substituted)-2-amino-thiadiazoles. *J. Pharm*., **9**: 6-11.

Ullah N, Bibi G and Kanwal S (2012). Antioxidant and cytotoxic activities and phytochemical analysis of *Euphorbia wallichii* root extract and its fractions. *Phytochem. Anal*., **11**: 241-249.

Wadood A, Riaz M, Jamal SB, Shah M and Lodhi MA (2013). Molecular docking study of p4 benzoxaborol ligands as inhibitor of HCV NS3/4A Protease. *Bioinform.,* **9**: 309-314.

Wang R, Tang XC (2005). Neuroprotective effects of huperzine A. A natural cholinesterase inhibitor for the treatment of Alzheimer's disease. *Clin. Chem.,* **14**: 71-82.

Yoshimizu M, Tajima Y, Matsuzawa F, Aikawa S, Iwamoto K, Kobayashi T and Edmunds T (2008). Binding parameters and thermodynamics of the interaction of imino sugars with a recombinant human acid alpha-glucosidase insight into the complex formation mechanism. *Clin. Chim. Acta.,* **9**: 68-73.