Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS

J. Serb. Chem. Soc. 84 (7) 649–661 (2019)
JSCS–5216

UDC 547.7/8+547.245:548.022–188
Original scientific paper

Structure–activity relationship and *in silico* study of unique bi-heterocycles: 5-[(2-amino-1,3-thiazol-4-yl)methyl]-1,3,4--oxadiazole-2-thiol derivatives

MUHAMMAD ATHAR ABBASI[1,2]*, MUHAMMAD SHAHID RAMZAN[1], AZIZ-UR-REHMAN[1], SABAHAT ZAHRA SIDDIQUI[1], MUBASHIR HASSAN[2], HUSSAIN RAZA[2], SYED ADNAN ALI SHAH[3], BUSHRA MIRZA[4] and SUNG-YUM SEO[2]**

[1]Department of Chemistry, Government College University, Lahore-54000, Pakistan, [2]College of Natural Sciences, Department of Biological Sciences, Kongju National University, Gongju, 32588, South Korea, [3]Faculty of Pharmacy and Atta-ur-Rahman Institute for Natural Products Discovery (AuRIns), Level 9, FF3, Universiti Teknologi MARA, Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor Darul Ehsan, Malaysia and [4]Department of Biochemistry, Quaid-i-Azam University, Islamabad, 45320, Pakistan

(Received 2 February, revised 29 October 2018, accepted 4 March 2019)

Abstract: This paper presents the synthesis of some unique bi-heterocyclic hybrid molecules with a thiazole and an oxadiazole ring. The synthesis was initiated by the conversion of ethyl 2-(2-amino-1,3-thiazol-4-yl)acetate (**1**) to the corresponding 2-(2-amino-1,3-thiazol-4-yl)acetohydrazide (**2**) by the reaction with hydrazine hydrate in methanol. The treatment of the acid hydrazide, **2**, with carbon disulfide gave the bi-heterocyclic nucleophile, 5-[(2-amino-1,3--thiazol-4-yl)methyl]-1,3,4-oxadiazole-2-thiol (**3**). Finally, the target compounds, **5a**–**o**, were synthesized by stirring the nucleophile **3** with different electrophiles, **4a**–**o**, in DMF using LiH as a base and an activator. The structures of the newly synthesized molecules were confirmed through spectroscopic techniques, such as IR, EI-MS, ^{1}H-NMR and ^{13}C-NMR. The structure––activity relationship of all these bi-heterocycles was established by evaluating them against four enzymes, namely, acetylcholinesterase, butyrylcholinesterase, urease and α-glucosidase, followed by their *in silico* study. Moreover, their cytotoxicity was also profiled by killing data of brine shrimps at various concentrations.

Keywords: 1,3-thiazole; 1,3,4-oxadiazole; acetylcholinesterase; butyrylcholinesterase; urease; glucosidase; molecular docking; brine shrimps.

*,** Corresponding authors. E-mail: (*)abbasi@gcu.edu.pk; (**)dnalove@kongju.ac.kr
https://doi.org/10.2298/JSC180203019A

INTRODUCTION

Heterocyclic compounds are major division of organic chemistry and are of immense use biologically and industrially. The thiazole nucleus imparts an important function in medicinal chemistry and serves as a key template for the development of various therapeutic agents. A large number of thiazole-containing derivatives have been reported to have a wide variety of biological activities, such as antibacterial, antifungal, antitubercular, anti-mycobacterial, anticancer and antiviral.[1–6] 2-Amino-thiazol acetate esters are present in most organic compounds of interest in biology, pharmacology, and material sciences.[7] Similarly, 1,3,4-oxadiazoles have had appealing attention for the last two decades due to their extensive range of biological activities, such as anti-inflammatory, antifungal, antiparasitic and antimicrobial effects. Such compounds have also demonstrated a very remarkable anti-tumor activity against leukemia, colon and breast cancer.[8,9]

Cholinesterase enzymes, *i.e.*, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) belong to serine hydrolases. Acetylcholine is terminated at cholinergic synapses by these enzymes and these enzymes are also part of neuromuscular junctions and cholinergic brain synapses.[10] Alzheimer's plaque is known to possess BChE in notably elevated quantities. The inhibitors of these enzymes are involved in the treatment of Alzheimer's disease.[11] Urease is known to be involved in different pathogenic processes. It is known to be involved in pyelonephritis, peptic ulceration, kidney stone, hepatic encephalopathy, urolithiasis and urinary catheter incrustation.[12,13] α-Glucosidase inhibitors (AGIs) are drugs that inhibit the absorption of carbohydrates from the gut and may be used in the treatment of patients with type 2 diabetes or impaired glucose tolerance. Inhibitors such as acarbose and miglitol have been approved for clinical use in the management of type 2 diabetes, as well as in the treatment of diabetic complications.[14–16] Molecular docking analysis approximates the ligands regarding their orientation and conformation at binding site of a target protein. The precise forecast of activity and precise structural modeling can be achieved by docking studies. Furthermore, the elaborate interactions the target protein active site and inhibitors.[17]

A literature survey revealed that minor modification in the structure of heterocycles could lead to quantitative as well as qualitative changes in the biological activity. This prompted the present synthesize the various bi-heterocyclic molecules bearing thiazole and oxadiazole rings with the goal of lowering toxicity and improving activity.

EXPERIMENTAL

General

All the chemicals, together with analytical grade solvents, were purchased from Sigma–Aldrich, Alfa Aesar (Germany) or Merck through local suppliers. Pre-coated silica gel Al-

-plates were used for TLC with ethyl acetate and *n*-hexane as the solvent system. Spots were detected by UV_{254}. A Gallonkamp apparatus was used to detect melting points (uncorrected) in capillary tubes. IR spectra (ν / cm^{-1}) were recorded by KBr pellet method in a Jasco-320-A spectrophotometer. Elemental analyses were realized on a Foss Heraeus CHN-O-Rapid instrument and were within±0.4 % of the theoretical values. EI-MS spectra were measured on a JEOL JMS-600H instrument with data processing system. The ^{1}H-NMR spectra (δ / ppm) were recorded at 600 MHz (^{13}C-NMR spectra, at 150 MHz) in DMSO-d_6 using the Bruker Advance III 600 As-cend spectrometer using a BBO probe. The coupling constant (J) is given in Hz and chemical shift (δ / ppm). The abbreviations used in interpretation of ^{1}H-NMR spectra are as follows: *s*, singlet; *d*, doublet; *dd*, doublet of doublets; *t*, triplet; *brt*, broad triplet; *q*, quartet; *quint*, quintet; *sex*, sextet; *sep*, septet; *m*, multiplet; *dist*, distorted. Analytical and spectral data of the investigated compounds are given in Supplementary material to this paper.

*Procedure for the preparation of 2-(2-amino-1,3-thiazol-4-yl)acetohydrazide (**2**)*

Ethyl 2-(2-amino-1,3-thiazol-4-yl)acetate (**1**; 10 g) and methanol (200 mL) were taken in a 500 mL RB flask, then, hydrazine hydrate (2.5 mL, 0.050 mol) was added drop wise and the mixture was allowed to reflux for 30 min. The reaction progress was observed by TLC using *n*-hexane and ethyl acetate as the solvent system (40:60). After completion, the reaction mixture was allowed to cool to room temperature to obtain a white colored precipitate of hydrazide **2**. This product was filtered and washed with methanol.

*5-[(2-Amino-1,3-thiazol-4-yl)methyl]-1,3,4-oxadiazole-2-thiol (**3**)*

2-(2-Amino-1,3-thiazol-4-yl)acetohydrazide (**2**; 4 g, 0.024 mol) was dissolved in C_2H_5OH (20 mL) in a 250 mL RB flask at 28 °C and then solid KOH (1.34 g, 0.024 mol) was added. The mixture was refluxed to dissolve the KOH. Then, carbon disulphide (3.70 mL, 0.048 mol) was added drop wise into the reaction mixture at 28 °C and was allowed to reflux again for 10 h. The reaction completion was checked by TLC using *n*-hexane and ethyl acetate as the solvent system (70:30). The excess of ethanol was evaporated. An excess of ice-cold distilled water was added followed by addition of dilute HCl until a pH of 4–5. The light peach colored precipitate of **3** was filtered and washed with distilled water.

*General procedure for the synthesis of 4-{[5-(substituted-sulfanyl)-1,3,4-oxadiazol-2-yl]-methyl}-1,3-thiazol-2-amines (**5a**–**o**)*

The parent nucleophile (**3**; 0.1 g; 0.00047 mol) was dissolved in *N*,*N*-dimethyl formamide (DMF, 5–10 mL) in a 100 mL RB flask. Solid LiH (0.005 g) was added and mixture was stirred for 30 min. Then, different electrophiles, alkyl/aralkyl halides (**4a**–**o**, one in each reaction) were added in equimolar ratios and further stirred for 3–5 h. The reaction was monitored by TLC using *n*-hexane and ethyl acetate solvent system (80:20). After reaction completion, ice-cold distilled water was added and the respective products, **5a**–**o**, were collected by filtration or solvent extraction.

Cholinesterases assays

The AChE and BChE inhibition activities were obtained according to the Ellman method with slight modifications.[20] The total volume of the reaction mixture was 100 µL. It contained 60 µL Na_2HPO_4 buffer with a concentration of 50 mM and a pH of 7.7. 10 µL test compound (0.5 mM $well^{-1}$) was added, followed by the addition of 10 µL (0.005 unit $well^{-1}$ of AChE or 0.5 unit $well^{-1}$ BChE) enzyme. The contents were mixed and pre-red at 405 nm. Then the contents were pre-incubated for 10 min at 37 °C. The reaction was initiated by the addition of 10

μL of 0.5 mM well^{-1} substrate (acetylthiocholine iodide for AChE or butyrylthiocholine chloride for BChE), followed by the addition of 10 μL DTNB (0.5 mM well^{-1}). After 15 min of incubation at 37 °C, the absorbance was measured at 405 nm using 96-well plate reader Synergy HT, Biotek, USA. All experiments were performed with their respective controls in triplicate. Eserine (0.5 mM well^{-1}) was used as the positive control. The percent inhibition was calculated using the following formula:

$$\text{Inhibition, \%} = 100\frac{\text{Control} - \text{Test}}{\text{Control}}$$

Where Control = total enzyme activity without inhibitor and Test = activity in the presence of a test compound

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

Urease inhibition assay

This enzyme assay was a customized form of the commonly known Berthelot assay.[21] The assay mixture of 85 μL was prepared containing 10 μL of phosphate buffer of pH 7.0 (in each well in a 96-well plate), 10 μL of sample solution and 25 μL of enzyme solution (0.135 units). The contents were pre-incubated at 37 °C for 5 min. 40 μL of urea stock solution (20 mM) was added to each well with incubation for 10 min at 37 °C. This was followed by the addition of 115 μL phenol–hypochlorite reagent (freshly prepared by mixing 45 μL phenol with 70 μL of alkali) per well. For color development, the mixture was incubated for a further 10 min at 37 °C. The absorbance was measured at 625 nm. The percentage enzyme inhibition and IC_{50} values were calculated by the same procedure as mentioned above.

α-*Glucosidase inhibition assay*

The *α*-glucosidase inhibition activity was performed according to a slightly modified literature method.[22] The total volume of the reaction mixture was 100 μL containing 70 μL phosphate buffer saline (50 mM) with a pH of 6.8, 10 μL of test compound (0.5 mM) and 10 μL enzyme (0.057 units). The contents were mixed, pre-incubated for 10 min at 37 °C and pre-red at 400 nm. The reaction was initiated by the addition of 10 μL of 0.5 mM substrate (*p*-nitrophenylglucopyranoside). Acarbose was used as the positive control. After 30 min of incubation at 37°C, the absorbance was measured at 400 nm using Synergy HT microplate reader. All experiments were performed in duplicate. The % inhibition and IC_{50} values were calculated by the same equation as discussed for cholinesterase enzymes.

Cytotoxicity assay

The cytotoxicity was studied by the brine–shrimp cytotoxic assay method.[23,24] Artificial sea water was prepared using sea salt 34 g L^{-1}. Brine shrimp (*Artemia salina*) eggs (Sera, Heidelberg, Germany) were hatched in shallow rectangular dish (22 cm×32 cm) under constant aeration for 48 h at room temperature. After hatching, the active shrimps free from eggs were collected from the brighter portion of the hatching chamber and used for the assay. Ten shrimps were transferred to each vial using a Pasteur pipette vial containing 5 mL of artificial sea water with 200, 20, 2 and 0.2 μg mL^{-1} final concentration of test compound from their stock solution. The vials were maintained under illumination at room temperature 25 to 28 °C. After 24 h, the number of surviving shrimps was counted. Experiment was performed in triplicate. Data was analyzed with Finney computer program to determine LD_{50} (lethal dose that killed 50 % of shrimps) values.

Statistical analysis

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

Grid generation and molecular docking

The AChE, BChE, Urease and α-glucosidase structures were retrieved from Protein Data Bank (PDB, www.rcsb.org) with PDBIDs 4PQE, 4BDS, 4H9M and 4J5T in protein preparation wizard. The selected crystal structures of the proteins were preprocessed and minimized using default parameters in Maestro interface. Bond orders were assigned, and hydrogen atoms were added to the protein. All four structures were minimized separately to reach a converged root mean square deviation (*RMSD*) of 0.30 Å with the OPLS_2005 force field. The active site of the enzyme was defined from the co-crystallized ligands from Protein Data Bank and literature data.[25-28] Furthermore, docking experiment was performed against all synthesized ligands and target protein using the Glide docking protocol.[29] The predicted binding energies (docking scores) and conformational positions of ligands within active region of protein were also defined using Glide experiment. Throughout the docking simulations, both partial flexibility and full flexibility around the active site residues were considered using the Glide/SP/XP and induced fit docking (IFD) approaches.[29]

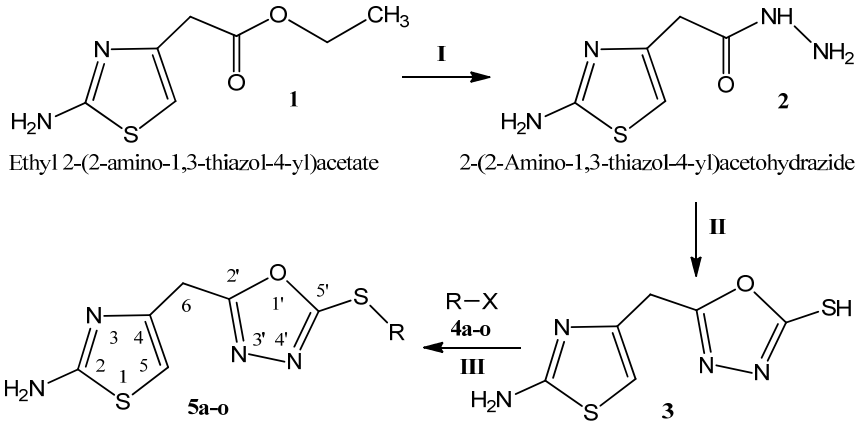
RESULTS AND DISCUSSION

Chemistry

The targeted bi-heterocycles, **5a**–**o**, were acquired in excellent yields by starting the synthesis from ethyl 2-(2-amino-1,3-thiazol-4-yl)acetate (**1**), which was transformed into 2-(2-amino-1,3-thiazol-4-yl)acetohydrazide (**2**) by reaction with hydrazine hydrate in methanol. The cyclization of **2** was realized with carbon disulfide in basic medium to afford the bi-heterocyclic core molecule, 5-[(2--amino-1,3-thiazol-4-yl)methyl]-1,3,4-oxadiazole-2-thiol (**3**) having a light peach color. Finally, its *S*-substituted derivatives, **5a**–**o**, were prepared by reacting the nucleophilic **3** with different aralkyl/alkyl halides, **4a**–**o**, as electrophiles. This synthesis is outlined in Scheme I and the varying substituents are listed in Table I.

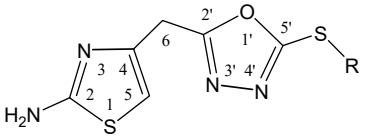
The structures of these synthesized derivatives were corroborated by their IR EI-MS, ^{1}H-NMR and ^{13}C-NMR spectral data and CHN analysis data. The structural assignment of one of the compounds is discussed here in detail for the expediency of the readers. The molecular formula, $C_{13}H_{11}BrN_4OS_2$, of **5g** was established through its CHN analysis data together with its EI-MS data showing $[M+2]^+$ peak at *m*/*z* 384 and a molecular ion peak $[M]^+$ of almost equal intensity at *m*/*z* 382. This assignment was supported by counting the number of protons in its ^{1}H-NMR spectrum (Figs. S-1 and S-2 of the Supplementary material). The number of the carbon atoms resonating in its ^{13}C-NMR spectrum (Fig. S-3 of the Supplementary material) was also in agreement.

The prominent absorption bands in the IR spectrum appeared at 3350 ($-NH_2$ str.), 3173 (C–H str. of the aromatic ring), 2923 ($-CH_2-$ str.), 1672 (C=C str. of aromatic ring) and 1590 cm^{-1} (C=N str.). An A_2B_2 spin system for a 4-bromobenzyl moiety in the molecule was clearly indicated by two *ortho*-coupled doub-



Scheme 1. Outline for the synthesis of 4-{[5-(substituted-sulfanyl)-1,3,4-oxadiazol-2-yl]-methyl}-1,3-thiazol-2-amines. Reagents and conditions: I) MeOH/N_2H_4•H_2O/reflux for 30 min; II) EtOH/CS_2/KOH/refluxing for 10–11 h; III) DMF/LiH/stirring for 3–5 h.

TABLE I. The different –R (aralkyl/alkyl) groups in Scheme I



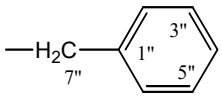
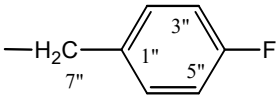
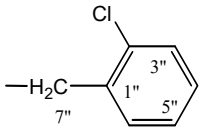
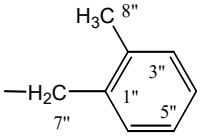
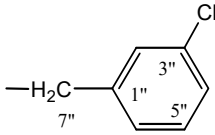
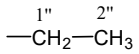
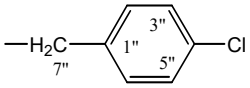
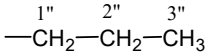
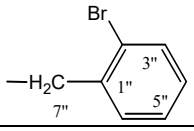
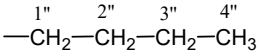
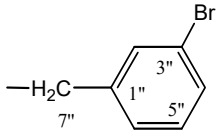
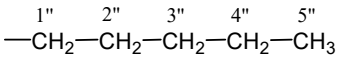
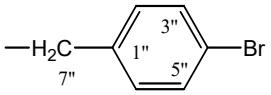
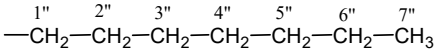
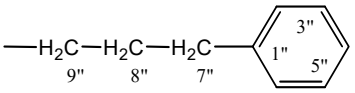
Compd.	–R	Compd.	–R
4a, **5a**		**4h**, **5h**	
4b, **5b**		**4i**, **5i**	
4c, **5c**		**4j**, **5j**	
4d, **5d**		**4k**, **5k**	
4e, **5e**		**4l**, **5l**	

TABLE I. Continued

Compd.	–R	Compd.	–R
4f, **5f**		**4m**, **5m**	
4g, **5g**		**4n**, **5n**	
4o, **5o**			

lets at δ 7.51 (2H, *brd*, J = 8.2 Hz, H-2″ & H-6″) and 7.35 ppm (2H, *brd*, J = 8.2 Hz, H-3″ & H-5″), along with a benzylic methylene signal at δ 4.42 (2H, *s*, CH_2-7″). The 2-amino-1,3-thiazol-4-yl heterocycle was characterized by two singlets at δ 7.02 (2H, $-NH_2$) and 6.40 ppm (1H, H-5), while a singlet at δ 4.03 ppm (2H, CH_2-6) was assignable to a methylene group connecting the two heterocycles in the molecule. All these assignments are also substantiated by its ^{13}C--NMR spectrum that exhibited overall eleven carbon resonances due to some equivalent carbons in the molecule. The 2-amino-1,3-thiazol-4-yl heterocycle was undoubtedly ascribed by two quaternary signals at δ 162.68 (C-2) and 143.79 ppm (C-4), along with a methine signal at δ 103.27 ppm (C-5). Similarly, the other heterocycle, *i.e.*, (5-substituted-1,3,4-oxadiazol-2-yl)sulfanyl was also signified by two quaternary signals at δ 168.78 (C-2′) and 165.85 ppm (C-5′)[30] while a methylene connecting the two heterocycle was obvious at δ 27.52 ppm (C-6).[18] The 4-bromobenzyl moiety was also apparent with two symmetric doublet methine signals 131.39 (C-3″ & C-5″), and 131.28 ppm (C-2″ & C-6″) along with two quaternary signals at δ 136.30 (C-1″) and 120.87 ppm (C-4″). While a methylene signal at δ 34.95 ppm (C-7″) was attributed to the benzylic methylene of this moiety.[18] These structural units of the molecule were also fully coherent with various fragment ion peaks observed in its EI-MS spectrum. Based on the complete consolidated discussion, the deduced structure of **5g** was named as 4-({5-[(4-bromobenzyl)sulfanyl]-1,3,4-oxadiazol-2-yl}methyl)-1,3-thiazol-2-amine. A similar exercise was implemented for the structural analysis of all the other synthesized bi-heterocycles.

Enzyme inhibition and structure–activity relationship

The screening of these synthesized compounds against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes (cholinesterases) revealed that these molecules exhibited variable inhibitory potential as shown by their IC_{50} values (Table S-I of the Supplementary material).

The IC_{50} results against the AChE enzyme demonstrated that 5-({5-[(3--chlorobenzyl)sulfanyl]-1,3,4-oxadiazol-2-yl}methyl)-1,3-thiazol-2-amine (**5c**) was a better inhibitor having an IC_{50} value of 14.27±0.19 μM relative to the other derivatives in the series. Eserine was used as a reference standard, with an IC_{50} value of 0.04±0.01 μM, against this enzyme. The better activity of **5c** might be attributed to the substitution of 3-chlorobenzyl group in the molecule. The synthesized compounds could be arranged in the following order according to their inhibitory activity: **5c** > **5n** > **5g** > **5o** > **5i** > **5l** > **5d** > **5b** > **5a** > **5k** > **5h** > **5j** > **5f** > **5m** > **5e**.

Against the BChE enzyme, only 5-({5-[(4-bromobenzyl)sulfanyl]-1,3,4-oxadiazol-2-yl}methyl)-1,3-thiazol-2-amine (**5g**) exhibited considerable inhibitory potential with an IC_{50} value of 63.51±0.12 μM, as compared to the other derivatives in the series. Here also, the very potent eserine was used the reference standard, having an IC_{50} value of 0.85±0.01 μM. The notable inhibitory potential of **5g** against this enzyme might be an attribute of the substitution of 4-bromobenzyl group in this molecule. The overall order of the active compounds against this enzyme was established as: **5g** > **5d** > **5b** > **5c** > **5o** > **5i** > **5m** > **5a** > **5f** > **5l** > **5j** > **5e** > **5h.** Similarly, against the urease enzyme, most of the molecules also exhibited a very moderate inhibitory potential, relative to the reference thiourea, having an IC_{50} value of 21.11±0.12 μM. In comparison among the synthesized derivatives, **5c** possessed relatively better inhibitory activity, indicating that the incorporation of 3-chlorobenzyl moiety was also adequate for the inhibition of this enzyme. The overall order for the active compound was inferred as: **5c** > **5b** > **5e** > **5g** > **5n** > **5k** > **5i** > **5d** > **5a** > **5f** > **5j** > **5o** > **5c** > **5h**. Their IC_{50} results were also in agreement with molecular docking data.

Against the α-glucosidase enzyme, the reference used was acarbose with an IC_{50} value of 37.38±0.12 μM. Here, **5f** was identified as the most active compound among the series, having an IC_{50} value of 46.17±0.14 μM. The better activity of **5f** against this enzyme might be the outcome of the substitution of 3-bromobenzyl group into the molecule. The inhibitory potential order of the active compounds was observed to be: **5f** > **5l** > **5e** > **5j** > **5b** > **5c** > **5d** > **5h** > **5a** > **5g** > **5i** > **5m** > **5n** > **5k** > **5o**. All these derivatives were docked into the active pocket of the urease enzyme in a similar way.

Docking energy and binding interaction pattern

Molecular docking experiments are the best approach to study the binding conformation of ligands within the active region of target proteins.[31–34] To predict the best-fitted conformational position of synthesized compounds **5a**–**o**, they were docked against AChE, BChE, jack bean urease and α-glucosidase. The generated docked complexes were examined based on the glide docking energy

values (kcal* mol^{-1}) and the bonding interaction (hydrogen/hydrophobic) pattern. The binding energy values depict the conformational positions of ligands within the active region of target proteins. The docking energy values of ligands against different targeted proteins, AChE, BChE, jack bean urease and α-glucosidase, are given in Table II.

TABLE II. Docking energy values of all ligands against selected target proteins

Docking	AChE	BChE	Urease	*α*-Glucosidase
5a	–7.12	–4.00	–4.57	–7.24
5b	–8.09	–4.00	–5.18	–7.22
5c	–8.38	–4.72	–4.37	–7.26
5d	–8.08	–5.13	–4.94	–7.27
5e	–7.87	–4.28	–4.33	–6.82
5f	–8.11	–4.56	–4.76	–7.12
5g	–7.45	–4.27	–4.62	–6.92
5h	–7.31	–4.73	–4.55	–6.05
5i	–7.84	–3.53	–4.59	–7.02
5j	–6.83	–4.14	–4.88	–5.81
5k	–6.63	–3.45	–4.04	–5.61
5l	–5.43	–3.30	–4.40	–6.85
5m	–5.66	–2.60	–3.62	–5.62
5n	–6.85	–3.74	–4.84	–6.39
5o	–8.13	–4.27	–4.94	–5.56

Docking analysis showed that all compounds were confined in the active binding region of the receptor molecules with different conformational poses. In the AChE docking results, three bonds were observed between the most active compound (**5f**) and the target protein. The 2-aminothiazole group of **5f** forms good interaction with Ser293, while the oxadiazole and 3-cholorobenzyl rings were involved in π–π interactions with aromatic residues Tyr341 and Trp86, respectively (Figs. 1A and 2A). The present docking results showed good correlation with already published data.[35]

The synthesized derivatives were computationally docked against BChE to explore their binding modes. Among them, **5g** made a couple of interactions with Asn57 and Trp56 (Figs. 1B and 2B). The amino group of thiazole ring was involved in hydrogen bonding with Asn57, while, the thiazole ring forms π–π interaction with Trp56.

The docking depiction of **5c** against jack bean urease is shown in Figs. 1C and 2C, where it was surrounded by residues in the active binding pocket of the target enzyme. No direct interaction was seen however. Already reported data confirmed its good conformational position within the active region of the target protein.[26,27] From the *α*-glucosidase docking results, it was recognized that com-

* 1 kcal = 4184 J

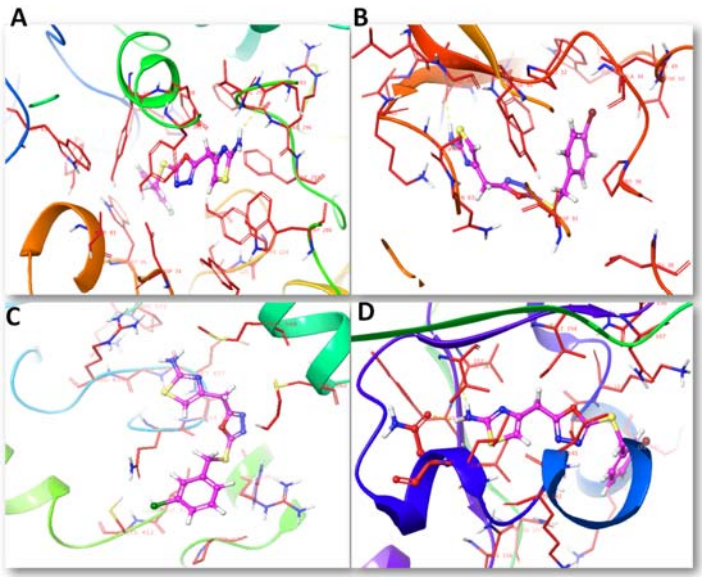


Fig. 1. The 3D interactions depiction of: A) **5c**–AChE, B) **5g**–BChE, C) **5c**–urease and D) **5f**–*α*-glucosidase.

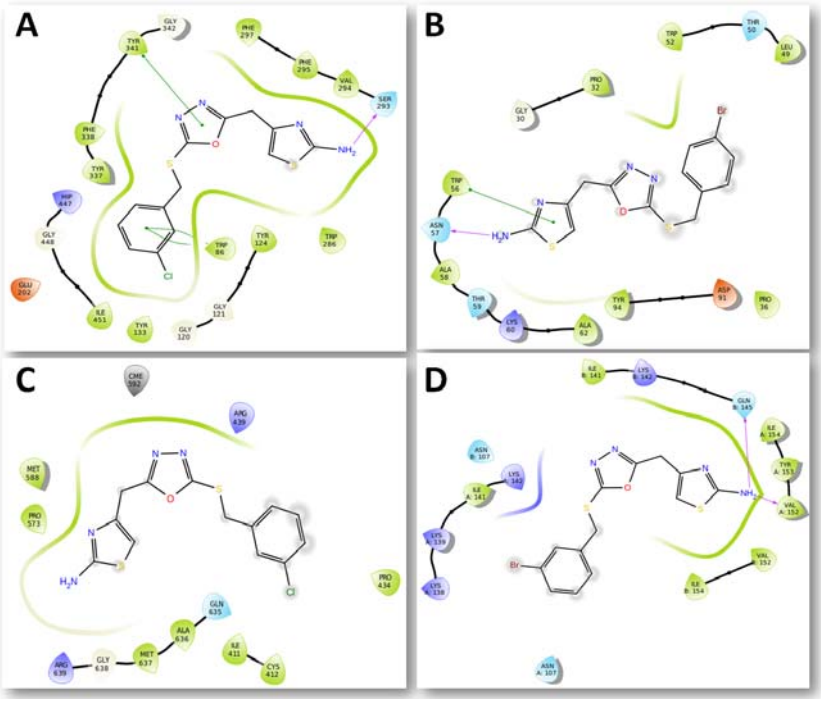


Fig. 2. The depiction of the 2D interactions: A) **5c**–AChE, B) **5g**–BChE, C) **5c**–urease and D) **5f**–*α*-glucosidase.

pound **5f** was bound very strongly in the active pocket of enzyme by making two strong interactions. The 2-aminothiazole group forms interactions with Val152 and Gln145 (Figs. 1D and 2D). All other graphical docking results are mentioned in the Supplementary material to this paper (Figs. S-4–S-63).

Cytotoxicity

The cytotoxicity of the synthesized compounds was evaluated through the brine shrimp lethality assay. Higher ED_{50} values of brine shrimp lethality demonstrated the lower toxicity of the compounds (Table III). Compounds **5i**, and **5k** exhibited higher ED_{50} values, 6.98 and 11.43 mM, respectively, and were less cytotoxic as compared to the standard Doxorubicin having an ED_{50} value of 5.21 mM.

TABLE III. Brine shrimp activity; doxorubicin was used as a standard

Compd.	ED_{50} μg ml^{-1}	Compd.	ED_{50} μg ml^{-1}	Compd.	ED_{50} μg ml^{-1}	Compd.	ED_{50} μg ml^{-1}
5a	1.21	**5e**	2.04	**5i**	6.98	**5m**	1.25
5b	1.45	**5f**	2.43	**5j**	2.32	**5n**	2.00
5c	1.15	**5g**	4.53	**5k**	11.43	**5o**	1.01
5d	3.46	**5h**	2.15	**5l**	2.76	Doxorubicin	5.21

CONCLUSIONS

The targeted series of bi-heterocyclic compounds was synthesized in good yields by a facile strategy in multi-steps and some of the compounds exhibited considerable enzyme inhibitory potential. Therefore, these molecules could be utilized as possible therapeutic agents in drug discovery and design programs.

SUPPLEMENTARY MATERIAL

Analytical and spectral data, as well as other additional data, are available electronically at the pages of journal website: http:// //shd.org.rs/JSCS, or from the corresponding author on request.

Acknowledgment. This work was supported by a research grant of the Kongju National University in 2018.

ИЗВОД

ОДНОС СТРУКТУРЕ И АКТИВНОСТИ И *IN SILICO* АНАЛИЗА БИ-ХЕТЕРОЦИКЛИЧНИХ ЈЕДИЊЕЊА: ДЕРИВАТИ 5-[(2-АМИНО-1,3-ТИАЗОЛ-4-ИЛ)МЕТИЛ]-1,3,4-ОКСАДИАЗОЛ-2-ТИОЛА

MUHAMMAD ATHAR ABBASI[1,2], MUHAMMAD SHAHID RAMZAN[1], AZIZ-UR-REHMAN[1], SABAHAT ZAHRA SIDDIQUI[1], MUBASHIR HASSAN[2], SYED ADNAN ALI SHAH[3], BUSHRA MIRZA[4] и SUNG-YUM SEO[2]

[1]Department of Chemistry, Government College University, Lahore-54000, Pakistan; [2]College of Natural Sciences, Department of Biological Science, Kongju National University, Gongju, 32588, South Korea; [3]Faculty of Pharmacy and Atta-ur-Rahman Institute for Natural Products Discovery (AuRIns), Level 9, FF3, Universiti Teknologi MARA, Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor Darul Ehsan, Malaysia и [4]Department of Biochemistry, Quaid-i-Azam University, Islamabad, 45320, Pakistan

У овом истраживању приказана је синтеза неких би-хетероцикличних хибридних једињења тиазола и оксадиазолског прстена. Синтеза почиње трансформацијом етил 2-(2-амино-1,3-тиазол-4-ил)ацетата (**1**) до одговарајућег 2-(2-амино-1,3-тиазол-4-ил)ацетохидразида (**2**), реакцијом са хидразин-хидратом у метанолу. Реакцијом деривата **2**, са угљен-дисулфидом у киселој средини, добијен је би-хетероциклични производ 5-[(2-амино-1,3-тиазол-4-ил)метил]-1,3,4-оксадиазол-2-тиол (**3**). На крају, циљана једињења, **5a**–**o**, синтетисана су полазећи од једињења **3** са различитим електрофилима, **4a**–**o**, у ДМФ, употребом LiH као базе и активатора. Структуре нових једињења су потврђене IR, EI-MS, ^{1}H-НМР и ^{13}C-НМР спектрима. Однос структуре и активности је анализиран испитивањем активности добијених једињења према ензимима, ацетилхолинестерази, бутирилхолинестерази, уреази α-глукозидази, као и *in silico* испитивању. Осим тога, испитана је и цитотоксичност добијених једињења према рачићима, при различитим концентрацијама.

(Примљено 2. фебруара, ревидирано 29. октобра 2018, прихваћено 4. марта 2019)

REFERENCES

1. A. Foroumadi, S. Mansouri, Z. Kiani, A. Rahmani, *Eur. J. Med. Chem.* **38** (2003) 851
2. J. M. Yatin, M. I. Arun, M. Shridhar, I. Shrikrishna, F. Hoong-Kun, *Arab. J. Chem.* **6** (2013) 177
3. P. Navin, H. K. Imran, *J. Enz. Inhib. Med. Chem.* **26** (2011) 527
4. G. Cihan-Ustundag, B. Simsek, E. Ilhan, G. Capan, *Lett. Drug Design Disc.* **11** (2014) 290
5. S. Holla, B. Veerendra, M. K. Shivananda, B. Poojary, *Eur. J. Med Chem.* **38** (2003) 759
6. F. Abdel-Rahman, D. C. Erik, H. El-Kashef, *Arkivoc* **12** (2006) 137
7. N. Siddiqui, M. F. Arshad, W. Ahsan, M. S. Alam, *Int. J. Pharm. Sci. Drug Res*. (2009) 143
8. S. Sanchit, S. N. Pandeya, *Int. J. Res. Ayurv. Pharm*. **2** (2011) 468
9. Nagaraj, K. C. Chaluvaraju, M. S. Niranjan, S. Kiran, *Int. J. Pharm. Pharm. Sci*. **9** (2012) 16
10. V. Tougu, *Curr. Med. Chem.* **1** (2001) 155
11. S. Gauthier, *Drug Aging* **18** (2001) 853
12. M. A. Lodhi, M. A. Abbasi, M. I. Choudhary, V. U. Ahmad, *Nat. Prod. Res.* **21** (2007) 721
13. M. A. Lodhi, J. Hussain, M. A. Abbasi, A. R. Jassbi, M. I. Choudhary, *J. Enz. Inhib. Med. Chem.* **21** (2006) 531
14. H. E. Lebovitz, *Clin. North. Am.* **26** (1997) 539

15. G. S. Meneilly, E. A. Ryan, J. Radziuk, D. C. Lau, J. F. Yale, J. Morais, *Diabetes care* **23** (2000) 1162
16. M. Nazir, M. A. Abbasi, Aziz-ur-Rehman, S. Z. Siddiqui, K. M. Khan, Kanwal, U. Salar, M. Shahid, M. Ashraf, M. A. Lodhi, F. A. Khan, *Bioorg. Chem.* **81** (2018), 253
17. B. Kitchen, H. Decornez, J. R. Furr, J. Bajorath, *Nat. Rev. Drug Discov*. **3** (2004) 935
18. M. A. Abbasi, H. Raza, Aziz-ur-Rehman, S. Z. Siddiqui, S. A. A. Shah, M. Hassan, S. Y. Seo. *Bioorg. Chem*. **83** (2019) 63
19. K. Rubab, M. A. Abbasi, Aziz-ur-Rehman, S. Z. Siddiqui, and M. N. Akhtar, *Trop. J. Pharm. Res.* **15** (2016) 1515
20. G. L. Ellman, K. D. Courtney, V. Andres, R. M. Featherstone, *Bio. Pharm.* **7** (1961) 88
21. H. L. Mobley, M. J. Cortesia, L. E. Rosenthal, B. D. Jones, *J. Clin. Microbiol.* **26** (1988) 831
22. C. Pierre, R. Roland, *J. Y. Dube. Chem.* **24** (1978) 208
23. V. Modi, P. Modi, *J. Saud. Chem. Soc.* **16** (2012) 332
24. N. Ullah, G. Bibi, S. Kanwal, *Phytochem. Anal.* **11** (2012) 241
25. M. A. Abbasi, M. Hassan, Aziz-ur-Rehman, S. Z. Siddiqui, H. Raza, S. A. A. Shah, S. Y. Seo, *Bioorg. Med. Chem*. **26** (2018) 3791
26. M. Nazir, M. A. Abbasi, Aziz-ur-Rehman, S. Z. Siddiqui, H. Raza, M. Hassan, S. A. A. Shah, M. Shahid, S. Y. Seo, *RSC Adv*. **8** (2018) 25920
27. M. A. Abbasi, H. Raza, A. U. Rehman, S. Z. Siddiqui, M. Nazir, A. Mumtaz, S. A. A. Shah, S. Y. Seo, M. Hassan, *Drug Res (Stuttg.)*, 2018, doi: 10.1055/a-0654-5074
28. Q. Abbas, M. Hassan, H. Raza, S. J. Kim, K. W. Chung, G. H. Kim, S. Y. Seo, *Asian Pac. J. Trop. Med*. **10** (2017) 372
29. R. Farid, T. Day, R. A. Friesner. *Bioorg. Med. Chem.* **14** (2006) 3160
30. M. A. Abbasi, A. Akhtar, Aziz-ur-Rehman, K. Nafeesa, S. Z. Siddiqui, K. M. Khan, M. Ashraf, and S.A. Ejaz, *J. Chil. Chem. Soc.* **58** (2013) 2186
31. M. Hassan, S. Shahzadi, S. Y. Seo, H. Alashwal, N. Zaki, A. A. Moustafa. *Front. Comput. Neurosci.* **12** (2018) 34
32. M. Hassan, Q. Abbas, Z. Ashraf, A. A. Moustafa, S. Y. Seo, *Comput. Biol. Chem*. **68** (2017) 131
33. M. Hassan, Z. Ashraf, Q. Abbas, H. Raza, S. Y. Seo, *Interdiscip. Sci*. **10** (2018) 68
34. M. Hassan, S. Shahzadi, Z. Iqbal, *JSM Biochem. Mol. Biol.* **4** (2017) 1024
35. M. A. Abbasi, M. Hassan, Aziz-ur-Rehman, S.Z. Siddiqui, S. A. A. Shah, H. Raza, S. Y. Seo, *Peer J*. **6** (2018) e4962.