ISSN 1068-1620, Russian Journal of Bioorganic Chemistry, 2018, Vol. 44, No. 6, pp. 801–811. © Pleiades Publishing, Ltd., 2018.

N-(5-Methyl-1,3-Thiazol-2-yl)-2-{[5-((Un)Substituted-Phenyl)1,3,4-Oxadiazol-2-yl]Sulfanyl}acetamides. Unique Biheterocycles as Promising Therapeutic Agents[1]

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Received June 7, 2018; in final form, July 10, 2018

Abstract—An electrophile, 2-bromo-*N*-(5-methyl-1,3-thiazol-2-yl)acetamide, was synthesized by the reaction of 5-methyl-1,3-thiazol-2-amine and bromoacetyl bromide in an aqueous medium. In a parallel scheme, a series of (un)substituted benzoic acids was converted sequentially into respective esters, acid hydrazides, and then into 1,3,4-oxadiazole heterocyclic cores. The electrophile was coupled with the aforementioned 1,3,4-oxadiazoles to obtain the targeted bi-heterocyles. Structural analysis of the synthesized compounds was performed by IR, EI-MS, ^{1}H NMR, and ^{13}C NMR. The enzyme inhibition study of these molecules was carried out against four enzymes, namely, acetylcholinesterase, butyrylcholinesterase, α-glucosidase, and urease. The interactions of these compounds with respective enzymes were recognized by their in silico study. Moreover, their cytotoxicity was also determined to find out their utility as possible therapeutic agents.

Keywords: 5-methyl-1,3-thiazol-2-amine, 1,3,4-oxadiazole, acetamide, cholinesterases, glucosidase, urease, brine shrimps

DOI: 10.1134/S106816201806002X

INTRODUCTION

Heterocyclic compounds containing five-membered heterocyclic units have a diversity of valuable biological effects. In medicinal chemistry, heterocyclic nucleus imparts an important function and serves as a key template for the development of various therapeutic agents. Different classes of heterocyclic compounds possess a broad range of pharmacological activities. Compounds bearing thiazole ring are used as drugs in treatment of cancer, lowering blood pressure, and treatment of infection [1]. Researchers have proven the in vitro potency of some thiazole derivatives to inhibit the bacterial pathogens [2]. 1,3,4-Oxadiazoles belong to a group of heterocyclic compounds that have enjoyed attention of researchers for the last two decades; numerous derivatives have been synthesized and evaluated for an extensive range of biological properties. Molecules with 1,3,4-oxadiazole nucleus are known to exhibit unique anti-edema [3] and anti-inflammatory activities [4, 5]. Literature survey revealed that minor modification in the structure of 1,3,4-oxadiazole can lead to quantitative, as well as qualitative, changes in biological activity. These heterocyclic derivatives have inhibitory effect against HIV replication, leukemia, colon, and breast cancer [6, 7]. 1,3,4-Oxadiazole has two extendable positions at second and fifth carbons; therefore a large number of 2,5-disubstituted derivatives have been synthesized and attracted attention due to their broad range of biological activity, such as anti-inflammatory, antifungal [8], antiparasitic [9], and antimicrobial effects [10].

Abbreviations: AChE, acetylcholinesterase; AGIs, α-glucosidase inhibitors ; BChE, butyrylcholinesterase.

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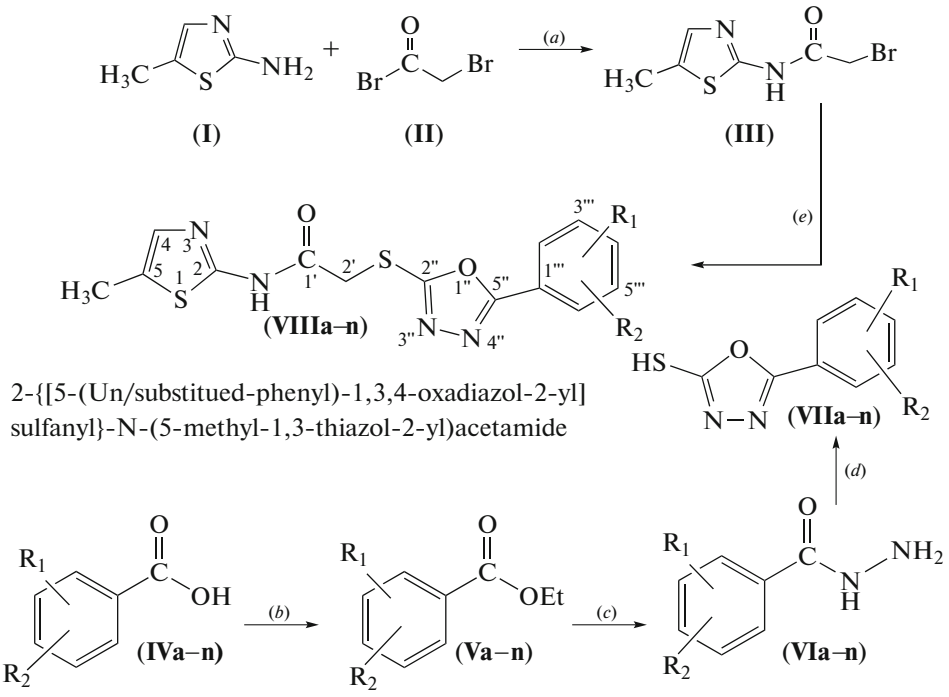

Scheme 1. Protocol for the synthesis of 2-{[5-((un)substituted-phenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-*N*-(5-methyl-1,3-thiazol-2-yl)acetamide (**VIIIa–n**). Reagents and conditions: (*a*) H_2O, 20% Na_2CO_3 solution, stirring for 2 h. (*b*) H_2SO_4, EtOH, refluxing for 4–5 h; (*c*) N_2H_4, EtOH, refluxing for 3–4 h; (*d*) CS_2, KOH, EtOH, refluxing for 5–6 h; (*e*) DMF, LiH, stirring for 4–6 h.

Acetylcholinesterase (AChE, or acetylhydrolase; EC 3.1.1.7) is the primary cholinesterase in the body, which catalyzes breakdown of acetylcholine and some other choline esters functioning as neurotransmitters. Its activity serves to terminate synaptic transmission. It belongs to carboxylesterase family of enzymes. **Butyrylcholinesterase** (BChE) is of pharmacological and toxicological importance, because it hydrolyzes ester-containing drugs and scavenges cholinesterase inhibitors, including potent organophosphorus nerve agents, before they reach their synaptic targets [10, 11]. It has been found that BChE inhibition is an effective tool to cure Alzheimer's disease and dementias. For the treatment of Alzheimer's and related diseases, it is of great importance to search for new cholinesterase inhibitors as possible drug candidates [12].

Urease (urea amidohydrolase; EC 3.5.1.5) occurs throughout the animal and plant kingdom. Many microorganisms use this enzyme to provide a source of nitrogen for growth; it plays an important role in plant nitrogen metabolism during the germination process. Of medical and veterinary interest, urease is a virulence factor in certain human and animal pathogens; it participates in the development of kidney stones, pyelonephritis, peptic ulcers, and other disease states. Meanwhile, the obvious remedy for treating bacterial infection by *Helicobacter pylori* with antimicrobials has often been proven futile and only a few combination regimens have reached clinical practice. Thus, the need for alternative or novel treatment is evident [13] and controlling urease activity is a promising approach.

Diabetes mellitus is an endocrine disorder characterized by hyperglycemia and associated with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both. **α-Glucosidase** inhibitors (AGIs) are drugs that inhibit the absorption of carbohydrates from the gut and may be used in treatment of patients with diabetes type 2 or impaired glucose tolerance [14, 15]. α-Glucosidase inhibitors, such as acarbose and miglitol, have been approved for clinical use in management of diabetes type 2, as well as the treatment of diabetic complications. A main drawback of the currently used α-glucosidases, such as acarbose, are the side effects such as abdominal distention, flatulence, meteorism, and possible diarrhea [16].

Molecular docking analysis approximates the synthesized ligands regarding their orientation and conformation at binding site of target protein. Precise forecast of activity and structural modeling can be achieved by docking studies. Furthermore, it elucidates interactions between the target protein active site and an inhibitor [17].

Based upon the aforementioned biological activities of various heterocyclic molecules, in the present research, amalgamation of two heterocyclic moieties, i.e. 1,3-thiazole and 1,3,4-oxadiazole, was carried out

to impart the designed molecules with possible therapeutic properties.

RESULTS AND DISCUSSION

Chemistry

Various biheterocycles were obtained by starting the synthesis from 5-methyl-1,3-thiazol-2-amine (**I**), which was reacted with 2-bromoethanoyl bromide (**II**) in a basic aqueous medium to obtain an electrophile, 2-bromo-*N*-(5-methyl-1,3-thiazol-2-yl)acetamide (**III**). In a parallel series of reactions, different nucleophiles were synthesized starting from (un)substituted-benzoic acids (**IVa**–**n**) and converting them sequentially into respective esters (**Va**–**n**), acid hydrazides (**VIa**–**n**), and 5-(un/substituted-phenyl)-1,3,4-oxadiazole-2-thiols (**VIIa**–**n**). The thiols, serving as nucleophiles, were finally coupled one by one with compound (**III**), in an aprotic polar solvent, i.e. DMF, using LiH as a base, to obtain the targeted biheterocyclic molecules (**VIIIa**–**n**). This synthesis is outlined in Scheme and the substituents are listed in Table 1. Structures of synthesized derivatives are corroborated by their IR, EI-MS, ^{1}H NMR, and ^{13}C NMR spectral data. Structural assignment of one of the compounds is discussed hereby in detail for the convenience of the readers. Compound (**VIIId**) was obtained as dull white solid. The reaction yield was found to be 77% and the melting point of the compound was 205–206°C. Molecular formula of this compound, $C_{15}H_{14}N_4O_3S_2$, was predicted by the molecular ion peak at *m/z* 362 in its EI-MS spectrum and by counting the number of protons in its ^{1}H NMR spectrum. The number of carbon resonances in its ^{13}C NMR spectrum also supported the assignment. Various functional groups in this molecule were identified by the IR data. Therein, different absorption bands at ν 3358 (NH stretching), 2977 (CH stretching of aromatic ring), 2823 (OCH_3 stretching, 1674 (C=N stretching), 1644 (C=O stretching), 1575 (C=C stretching of aromatic ring), 1163 (C–O–C stretching) were observed for the speculated functionalities. The 2-methoxyphenyl group in this molecule was ascribed by a set of discrete signals in the aromatic region of its ^{1}H NMR spectrum at δ 7.77 (dd, J = 1.5, 7.7 Hz, 1H, H-6'''), 7.59 (br.t, J = 8.6 Hz, 1H, H-5'''), 7.24 (br.d, J = 8.5 Hz, 1H, H-3'''), and 7.09 (br.t, J = 7.6 Hz, 1H, H-4''') along with a methoxy group signal at 3.85 (br.s, 3H, CH_3-7'''). Structural assignment of this unit was also fully supported by seven typical carbon resonances in its ^{13}C NMR spectrum at δ 157.26 (C-2'''), 133.60 (C-5'''), 130.0 (C-6'''), 120.69 (C-4'''), 112.62 (C-3'''), 111.78 (C-1'''), and 55.92 (C-7'''). *N*-(5-Methyl-1,3-thiazol-2-yl) moiety of the molecule was characterized by two distorted doublets in its ^{1}H NMR spectrum at δ 7.16 (distorted d, J = 1.1 Hz, 1H, H-4) and 2.34 (distorted d, J = 0.6 Hz, 3H, CH_3-6). The unique multiplicity of both these signals was an attribute of the mutual allylic coupling in them. However, in its ^{13}C NMR spectrum, the appearance of four carbon resonances for this methylated heterocyclic moiety at δ 155.82 (C-2), 134.90 (C-4), 126.67 (C-5), and 11.07 (C-6) 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 δ 163.99 (C-5'') and 162.73 (C-2''). Central C- and N-substituted acetamido group, which connects the two heterocycles in the molecule, was inferred by two characteristic peaks in both its ^{1}H NMR and ^{13}C NMR spectra. In the former spectrum, the peaks appeared at δ 12.36 (s, 1H, CONH) and 4.38 (br.s, 2H, CH_2-2') while in the latter spectrum, the carbon resonances appeared at δ 164.97 (C-1') and 35.29 (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 (**VIIId**) was confirmed and it was named 2-{[5-(2-methoxyphenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-*N*-(5-methyl-1,3-thiazol-2-yl)acetamide (**VIIId**). A similar pattern was adopted for the structural characterization of other derivatives in the synthesized series.

Table 1. Different substituents (R_1 and R_2) in Scheme 1

Compds.	$-R_1$	$-R_2$
(IV–VIIIa)	–H	–H
(IV–VIIIb)	4-CH_3	–H
(IV–VIIIc)	4-OH	–H
(IV–VIIId)	2-OCH_3	–H
(IV–VIIIe)	2-Cl	–H
(IV–VIIIf)	3-Cl	–H
(IV–VIIIg)	4-Cl	–H
(IV–VIIIh)	2-Cl	4-Cl
(IV–VIIIi)	3-NH_2	–H
(IV–VIIIj)	4-NH_2	–H
(IV–VIIIk)	2-NO_2	–H
(IV–VIIIl)	3-NO_2	–H
(IV–VIIIm)	4-NO_2	–H
(IV–VIIIn)	3-NO_2	5-NO_2

Enzyme Inhibition, Molecular Docking, and Structure-Activity Relationship

All the synthesized compounds (**VIIIa**–**n**) were assessed for their enzyme inhibition activity against four enzymes, namely acetylcholinesterase, butyrylcholinesterase, urease, and α-glucosidase. All compounds exhibited enzyme inhibition activity but differed in their potency compared to the standard reference used. The outcomes are recorded as percent

Table 2. Percent inhibition at 0.5 mM and IC_{50} values for cholinesterases, urease, and α-glucosidase

Compd.	AChE		BChE		Urease		α-Glucosidase	
	inhibition, %	IC_{50}, μM	inhibition, %	IC_{50}, μM	inhibition, %	IC_{50}, μM	inhibition, %	IC_{50}, μM
(VIIIa)	49.19 ± 0.15	43.15 ± 0.13	55.15 ± 0.15	157.35 ± 0.30	63.27 ± 0.25	117.22 ± 0.45	71.15 ± 0.45	297.29 ± 0.26
(VIIIb)	89.29 ± 0.21	57.51 ± 0.17	49.27 ± 0.26	121.17 ± 0.55	71.55 ± 0.15	220.31 ± 0.22	73.55 ± 0.15	198.12 ± 0.21
(VIIIc)	87.29 ± 0.35	55.17 ± 0.07	77.14 ± 0.25	141.15 ± 0.19	81.35 ± 0.15	245.15 ± 0.19	89.17 ± 0.25	52.11 ± 1.2
(VIIId)	79.37 ± 0.30	50.41 ± 0.19	89.62 ± 0.34	74.52 ± 0.25	91.15 ± 0.45	84.17 ± 0.23	75.17 ± 0.35	235.33 ± 0.22
(VIIIe)	63.43 ± 0.29	51.15 ± 0.19	77.23 ± 0.35	115.05 ± 0.21	87.17 ± 0.25	222.30 ± 0.35	83.35 ± 0.15	24.23 ± 0.29
(VIIIf)	75.55 ± 0.21	59.25 ± 0.21	49.27 ± 0.35	105.29 ± 0.15	79.43 ± 0.35	245.13 ± 0.23	64.17 ± 0.71	291.15 ± 0.17
(VIIIg)	89.29 ± 0.19	83.17 ± 0.09	53.14 ± 0.35	89.17 ± 0.35	43.19 ± 0.30	149.45 ± 0.17	70.49 ± 0.19	232.55 ± 0.19
(VIIIh)	93.27 ± 0.44	11.49 ± 0.13	73.42 ± 0.45	215.64 ± 0.29	49.29 ± 0.25	199.35 ± 0.55	50.35 ± 0.17	272.44 ± 0.21
(VIIIi)	89.49 ± 0.37	81.55 ± 0.19	89.74 ± 0.63	69.27 ± 0.35	67.15 ± 0.45	145.21 ± 0.17	50.29 ± 0.25	289.27 ± 0.24
(VIIIj)	88.77 ± 0.39	19.35 ± 0.10	61.26 ± 0.15	113.85 ± 0.45	90.23 ± 0.17	76.26 ± 0.25	81.43 ± 0.35	270.33 ± 0.25
(VIIIk)	83.76 ± 0.30	35.34 ± 0.12	77.47 ± 0.16	163.23 ± 0.17	77.17 ± 0.35	105.25 ± 0.15	75.15 ± 0.45	140.25 ± 0.26
(VIIIl)	81.41 ± 0.19	29.19 ± 0.04	55.47 ± 0.27	83.35 ± 0.41	51.29 ± 0.22	97.15 ± 0.29	73.29 ± 0.22	242.11 ± 0.29
(VIIIm)	70.87 ± 0.21	43.35 ± 0.01	71.35 ± 0.36	171.25 ± 0.17	61.17 ± 0.71	249.11 ± 0.17	69.23 ± 0.17	310.17 ± 0.11
(VIIIn)	59.19 ± 0.35	77.60 ± 0.50	73.27 ± 0.29	113.21 ± 0.49	69.35 ± 0.17	217.29 ± 0.45	73.25 ± 0.29	326.21 ± 0.35
Eserine	91.27 ± 1.17	0.04 ± 0.01	82.82 ± 1.09	0.85 ± 0.01				
Thiourea					98.12 ± 0.18	21.11 ± 0.12		
Acarbose							92.23 ± 0.16	37.38 ± 0.12

inhibition and IC_{50} (50% inhibitory concentration) in Table 2. Cytotoxicity of these molecules was also evaluated and LD_{50} values for brine shrimps are reported in Table 3.

All the molecules are noticeable inhibitors of **AChE**. The most promising results against this enzyme were shown by compounds **(VIIIh)** and **(VIIIj)** with IC_{50} values of 11.49 ± 0.13 and 19.35 ± 0.10 μM, respectively. This good inhibitory potential of **(VIIIh)** might be attributed to the presence of 2,4-dichloro groups, while that of **(VIIIj)** might be related to the presence of 4-amino group in this molecule. Eserine with IC_{50} value of 0.04 ± 0.01 μM was used as standard inhibitor against this enzyme. Among mono-chloro compounds **(VIIIe–g)**, the order of activity was found to be as *ortho* > *meta* > *para*. However, for amino bearing compounds **(VIIIi)** and **(VIIIj)**, the observed order was *para* > *meta*. For the mono-nitro compounds **(VIIIk–m)**, the order of activity was found to be *meta* > *ortho* > *para*. Derivatives, **(VIIIa–n)**, were docked into the active pocket of this enzyme. From the docking results, we have identified that compound **(VIIIh)** created a couple of π–π and arene–cation interactions with Trp82, Phe329, and His438 residues, with bond lengths of 3.39, 3.28, and 4.73 Å, respectively (Fig. 1).

Table 3. Brine shrimp activity assay

Compd.	LD_{50}, mM	Compd.	LD_{50}, mM	Compd.	LD_{50}, mM
(VIIIa)	1.9	**(VIIIf)**	1.8	**(VIIIk)**	–
(VIIIb)	4.7	**(VIIIg)**	255.8	**(VIIIl)**	5.7
(VIIIc)	275.5	**(VIIIh)**	1.3	**(VIIIm)**	9.1
(VIIId)	2.1	**(VIIIi)**	184.5	**(VIIIn)**	4.3
(VIIIe)	1.9	**(VIIIj)**	1.4	Doxorubicin	5.21

Doxorubicin was used as reference standard.

Against **BChE**, a noticeable inhibition was observed for compounds **(VIIId)** and **(VIIIi)** with IC_{50} values of 74.52 ± 0.25 and 69.27 ± 0.35 μM, respectively. This inhibition might be due to the incorporation of 2-methoxy and 2-amino groups, respectively. The reference used for BChE was also eserine with IC_{50} value of 0.85 ± 0.01 μM. Synthesized compounds can be arranged in the following row according to their inhibitory activity: **(VIIIi)** > **(VIIId)** > **(VIIIl)** > **(VIIIg)** > **(VIIIf)** > **(VIIIn)** > **(VIIIj)** > **(VIIIe)** > **(VIIIb)** > **(VIIIc)** > **(VIIIm)** > **(VIIIk)** > **(VIIIh)** (see Table 2 for IC_{50} values). As follows from Fig. 2, arene–arene interactions have been established for compound **(VIIId)** with Tyr121 and Trp279, with bond lengths of 2.14 and 3.54 Å, respectively.

Against **urease**, all the active compounds displayed moderate inhibitory potential when contrasted with the reference, thiourea, with IC_{50} value of 21.11 ± 0.12 μM. The most active urease inhibitors were compounds **(VIIIj)** and **(VIIId)** with IC_{50} values of 76.26 ± 0.25

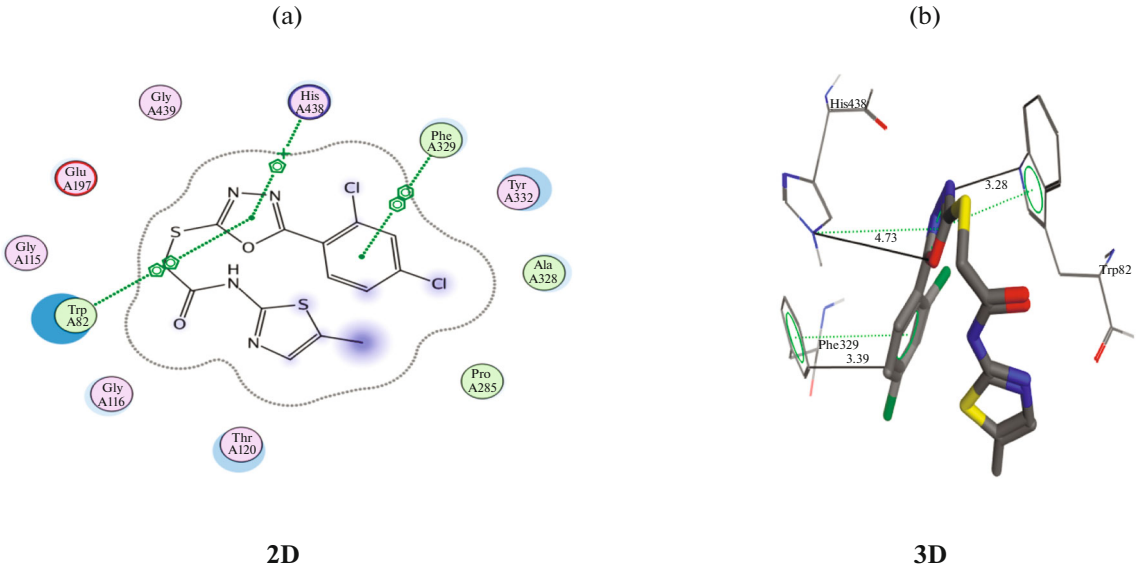

Fig. 1. The 2D (a) and 3D (b) interaction analysis of compound (**VIIIh**) against AChE.

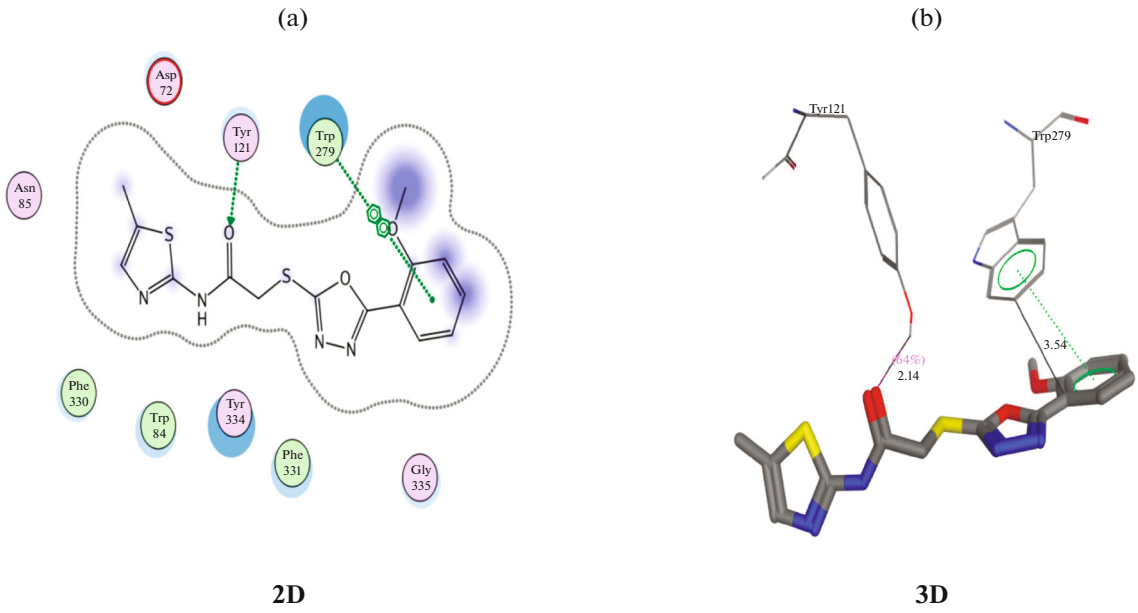

Fig. 2. The 2D (a) and 3D (b) interaction analysis of compound (**VIIId**) against BChE.

and 84.17 ± 0.23 μM, respectively. Synthesized compounds can be arranged in the following row according to their inhibitory activity: **(VIIIj)** > **(VIIId)** > **(VIIIl)** > **(VIIIk)** > **(VIIIa)** > **(VIIIi)** > **(VIIIg)** > **(VIIIh)** > **(VIIIn)** > **(VIIIb)** > **(VIIIe)** > **(VIIIf)** > **(VIIIc)** > **(VIIIm)** (see Table 2 for IC_{50} values). Conclusions drawn from the inhibitory activity experiments were supported by molecular docking studies. Figure 3 depicts docking results for the most active compound **(VIIIj)**. It demonstrates two very strong polar interactions through 5-methyl-1,3-thiazol-2-yl bearing acetamidic nitrogen and 4-aminophenyl group with Asp224 and Glu-166 at distances of 1.52 and 1.85 Å, respectively. It also exhibited strong acidic interaction through its acetamidic carbonyl oxygen with His323 showing a bond length of 1.58 Å.

Against **α-glucosidase**, the reference used was acarbose with IC_{50} value of 37.38 ± 0.12 μM. Compound **(VIIIe)** with IC_{50} value of 24.11 ± 0.29 μM was the most active one against α-glucosidase. Synthesized compounds can be arranged in the following row according to their inhibitory activity: **(VIIIe)** > **(VIIIc)** >

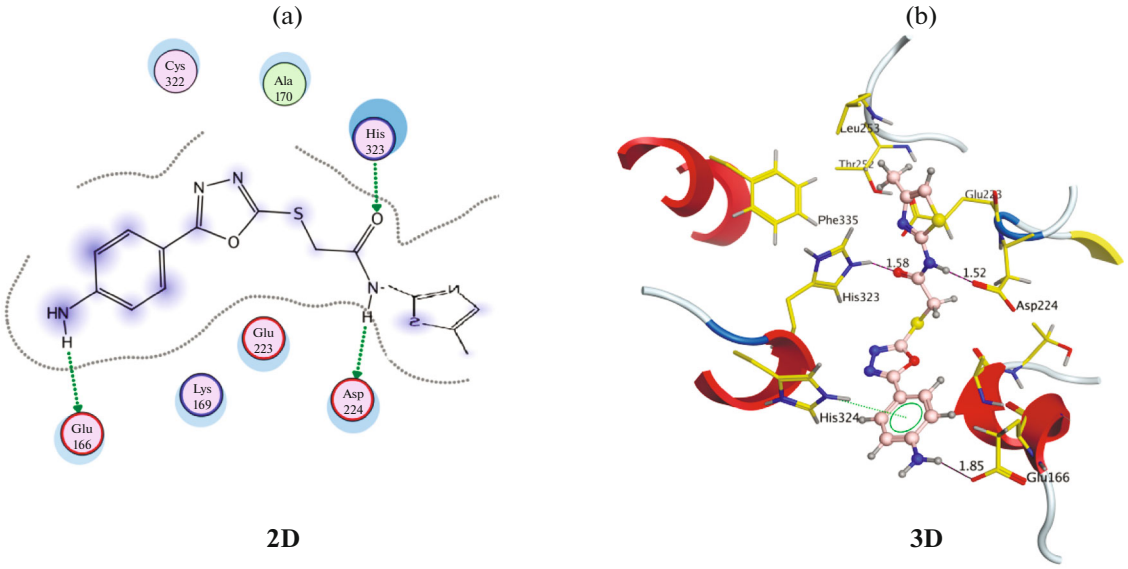

Fig. 3. The 2D (a) and 3D (b) interaction analysis of compound (**VIIIj**) against urease.

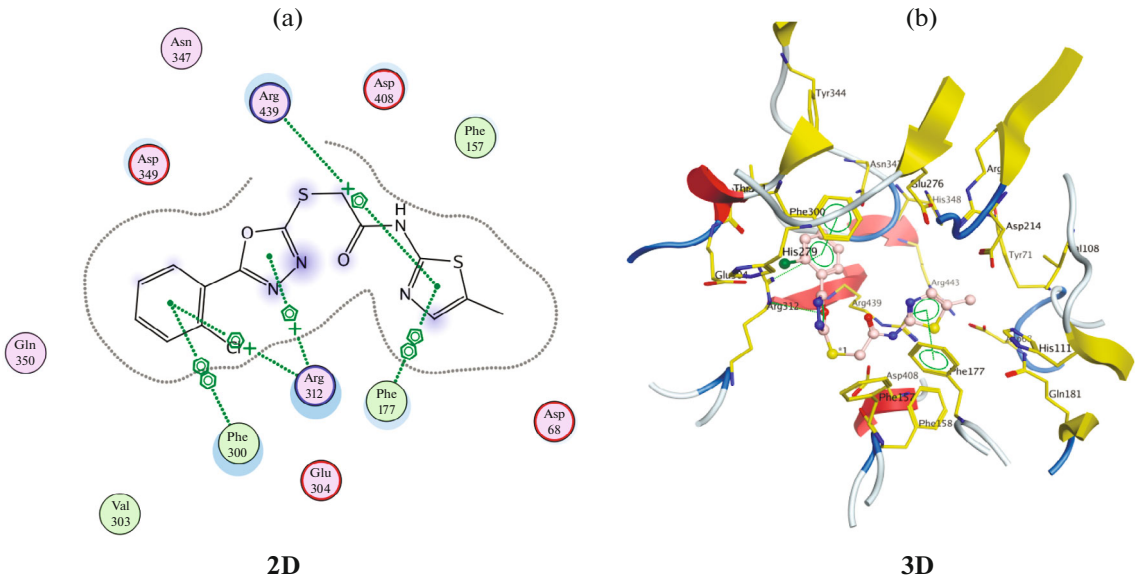

Fig. 4. The 2D (a) and 3D (b) interaction analysis of compound (**VIIIe**) against α-glucosidase.

(**VIIIn**) > (**VIIIk**) > (**VIIIb**) > (**VIIIg**) > (**VIIId**) > (**VIIIl**) > (**VIIIj**) > (**VIIIh**) > (**VIIIi**) > (**VIIIf**) > (**VIIIa**) > (**VIIIm**) > (**VIIIn**) (see Table 2 for IC_{50} values). The inhibitory activity experiment results were likewise helped by molecular docking. Figure 4 shows the docking results for the most active compound (**VIIIe**), which was strongly bound with the active pocket of α-glucosidase through five interactions. Phe177 and Phe300 were found to be involved in a separate π–π interactions with 5-methyl-1,3-thiazol-2-yl and 2-chlorophenyl moieties. Similarly, Arg312 and Arg439 have also given three arene–cation interactions with 2-chlorophenyl, 1,3,4-oxadiazole, and 5-methyl-1,3-thiazol-2-yl units of the ligand, respectively.

Inhibitory activity of the newly synthesized compounds against the four enzymes showed that molecules bearing chloro, amino, hydroxy, and methoxy groups were found to be better inhibitors, probably because they fit well into the active sites of the enzymes. Also, these functional groups are able to generate better π–π interactions with the active site. Thus, compounds (**VIIId**), (**VIIIe**), (**VIIIh**), (**VIIIc**), and (**VIIIj**) were significantly more efficient among the whole series of synthesized molecules.

Brine Shrimp Activity

Cytotoxicity of the compounds was analyzed by brine shrimp activity assay. The high LD_{50} values of compounds proved their low toxicity. Therefore, these compounds might be suitable for use as drugs. Doxorubicin was used as reference with LD_{50} value of 5.21 mM. Compounds (**VIIIc**), (**VIIIg**), (**VIIIi**), and (**VIIIp**) with LD_{50} values of 275.5, 255.8, 184.5, and 186.5 mM were the most suitable ones.

EXPERIMENTAL

General

All the chemicals, along with analytical grade solvents, were purchased from Sigma Aldrich, Alfa Aesar (Germany), or Merck through local suppliers. Precoated silica gel Al-plates were used for TLC with ethyl acetate and *n*-hexane as solvent system. Spots were detected by UV_{254}. Gallonkamp apparatus was used to detect melting points in capillary tubes. IR spectra (ν_{max}, cm^{-1}) were recorded by KBr pellet method in the Jasco-320-A spectrophotometer. ^1H NMR spectra (δ, ppm) were recorded at 600 MHz and ^{13}C NMR spectra, at 150 MHz, in DMSO-d_6 using the Bruker Advance III 600 Ascend spectrometer using the BBO probe. EI-MS spectra were measured on a JEOL JMS-600H instrument with native data processing system.

Synthesis

***N*-(5-Methyl-1,3-thiazol-2-yl)-2-bromoacetamide (III).** 2-Amino-5-methyl-1,3-thiazole (**I**) (0.038 mol) was dispersed in 25 mL distilled water in iodine flask (100 mL) and 20% aq. Na_2CO_3 solution was poured to adjust pH to 9–10. 2-Bromoethanoyl bromide (**II**) (0.038 mol) was poured in small patches upon vigorous shaking and then set to stir for further 2 h. Reaction completion was monitored by TLC. Excess ice-cold distilled water (40 mL) was added and the formed precipitate was collected by filtration. The precipitate of compound (**III**) was washed with distilled water and dried.

General procedure for synthesis of ethyl (un)substituted benzoates (Va–n). (Un)Substituted benzoic acid (**IVa–n**) (0.02 mol) was refluxed with 60 mL EtOH for 4–5 h in the presence of conc. H_2SO_4 (1.25 mL) in a round-bottom flask (250 mL). TLC plates were used to monitor the reaction. Excess distilled water (150 mL) was added after reaction completion and pH was adjusted to 8–10 by 20% aq. Na_2CO_3 solution. The product was collected through sequential extraction with $CHCl_3$ (20 mL × 3). Chloroform was distilled off to collect the product. In some cases, the product was collected through filtration. Esters (**Va–n**) were used in further synthesis [18, 19].

General procedure for synthesis of (un)substituted-benzohydrazides (VIa–n). Ethyl ester (**Va–n**) (0.04 mol) was refluxed with 80% $N_2H_4 \cdot H_2O$ (7.2 mL) for 3–4 h in 20 mL EtOH in a round-bottom flask (100 mL). The reaction was monitoredby TLC. At completion, excess ice-cold distilled water (60 mL) was added to get the precipitate, which was filtered, washed with distilled water, and dried to acquire title compounds (**VIa–n**) [18, 19].

General procedure for synthesis of 5-((un)substituted-phenyl)-1,3,4-oxadiazol-2-thiols (VIIa–n). Solid KOH (0.03 mol) was dissolved in 25 mL EtOH on reflux in a 100 mL round-bottom flask. (Un)Substituted benzohydrazide (**VIa–n**) (0.03 mol) was refluxed with CS_2 (0.06 mol) in this basified EtOH for 5–6 h. The reaction was monitored by TLC. At completion, excess ice-cold distilled water (60 mL) was added to form homogeneous solution. pH was adjusted to 5–6 by pouring dilute HCl; precipitate thus formed was filtered, washed with distilled water and dried. The formed products (**VIIa–n**) were also re-crystallized from EtOH [18, 19].

General procedure for synthesis of 2-{[5-((un)substituted-phenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-*N*-(5-methyl-1,3-thiazol-2-yl)acetamides (VIIIa–n). 5-((Un)substituted-phenyl)-1,3,4-oxadiazol-2-thiol (**VIIa–n**) (0.004 mol) was dissolved in DMF (11 mL) in a 50-mL round-bottom flask. Then, LiH (0.004 mol) was added and the mixture was stirred for 0.5 h. *N*-(5-Methyl-1,3-thiazol-2-yl)-2-bromoacetamide (**III**) (0.004 mol) was poured in and the mixture was further stirred for 4–6 h. Completion of the reaction was confirmed by TLC. Then, excess ice-cold distilled water (25 mL) was poured in small portions along with continuous stirring. Aqueous NaOH was added drop-wise upon gentle shaking to adjust the pH to 8–10. The reaction mixture was aged for 1 h and the precipitate of (**VIIIa–n**) was filtered, washed with distilled water, and dried.

***N*-(5-Methyl-1,3-thiazol-2-yl)-2-[(5-phenyl-1,3,4-oxadiazol-2-yl)sulfanyl]acetamide (VIIIa).** Yellowish amorphous solid; yield 71%; mp 187–188°C. Mol. formula $C_{14}H_{12}N_4O_2S_2$; mol. mass 332 g mol^{-1}; IR: 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); ^1H NMR: 12.38 (s, 1H, CONH), 7.95 (dd, J = 1.6, 8.6 Hz, 2H, H-2''' and H-6'''), 7.63 (br.t, J = 7.4 Hz, 1H, H-4'''), 7.59 (br.t, J = 7.8 Hz, 2H, H-3''' and H-5'''), 7.17 (distorted d, J = 1.3 Hz, due to allylic coupling, 1H, H-4), 4.43 (br.s, 2H, CH_2-2'), 2.34 (distorted d, J = 1.3 Hz, due to allylic coupling, 3H, CH_3-6); ^{13}C NMR: 165.26 (C-1'), 165.01 (C-5''), 162.98 (C-2''), 156.86 (C-2), 136.88 (C-4), 132.06 (C-4'''), 129.38 (C-3''' and C-5'''), 126.36 (C-2''' and C-6'''), 122.89 (C-1'''), 113.91 (C-5), 35.37 (C-2'), 11.07 (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*-(5-methyl-1,3-thiazol-2-yl)acetamide (VIIIb). Light brown solid; yield 73%; mp 229–230°C. Mol. formula $C_{15}H_{14}N_4O_2S_2$; mol. mass 346 g mol^{-1}; IR: 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: 12.38 (s, 1H, CONH), 7.82 (br.d, J = 7.9 Hz, 2H, H-2''' and H-6'''), 7.38 (br.d, J = 7.8 Hz, 2H, H-3''' and H-5'''), 7.17 (br.s, 1H, H-4), 4.37 (br.s, 2H, CH_2-2'), 2.38 (s, 3H, CH_3-4'''), 2.34 (s, 3H, CH_3-6); ^{13}C NMR: 165.34 (C-1'), 165.04 (C-5''), 162.61 (C-2''), 156.01 (C-2), 142.26 (C-4'''), 134.94 (C-4), 129.94 (C-2''' and C-6'''), 126.71 (C-5), 126.32 (C-3''' and C-5'''), 120.15 (C-1'''), 35.30 (C-2'), 21.07 (CH_3-4'''), 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*-(5-methyl-1,3-thiazol-2-yl)acetamide (VIIIc). Brown solid; yield 85%; mp 231–232°C. Mol. formula $C_{14}H_{12}N_4O_3S_2$; mol. mass 348 g mol^{-1}; IR: 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: 12.38 (s, 1H, CONH), 7.76 (d, J = 8.7 Hz, 2H, H-3''' and H-5'''), 7.17 (distorted d, J = 1.4 Hz, due to allylic coupling, 1H, H-4), 6.91 (d, J = 8.7 Hz, 2H, H-2''' and H-6'''), 4.34 (br.s, 2H, CH_2-2'), 2.34 (distorted d, J = 1.3 Hz, due to allylic coupling, 3H, CH_3-6); ^{13}C NMR: 165.47 (C-1'), 161.70 (C-5''), 160.81 (C-2''), 156.01 (C-2), 134.92 (C-4), 133.28 (C-4'''), 128.35 (C-2''' and C-6'''), 113.62 (C-1'''), 116.12 (C-3''' and C-5'''), 113.62 (C-5), 35.32 (C-2'), 11.06 (C-6). EI-MS: m/z 348 $[M]^+$, 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 $[C_7H_5O_2]^+$, 93 $[C_6H_5O]^+$.

2-{[5-(2-Methoxyphenyl)-1,3,4-oxadiazol-2-yl]-sulfanyl}-*N*-(5-methyl-1,3-thiazol-2-yl)acetamide (VIIId). Dull white solid; yield 77%; mp 205–206°C. Mol. formula $C_{15}H_{14}N_4O_3S_2$; mol. mass 362 g mol^{-1}; IR: 3358 (N–H stretching), 2977 (C–H stretching of aromatic ring), 2823 (O–CH_3 stretching), 1674 (C=N stretching), 1644 (C=O stretching), 1575 (C=C stretching of aromatic ring), 1163 (C–O–C stretching); ^{1}H NMR: 12.36 (s, 1H, CONH), 7.77 (dd, J = 1.5, 7.7 Hz, 1H, H-6'''), 7.59 (br.t, J = 8.6 Hz, 1H, H-5'''), 7.24 (br.d, J = 8.5 Hz, 1H, H-3'''), 7.16 (distorted d, J = 1.1 Hz, due to allylic coupling, 1H, H-4), 7.09 (br.t, J = 7.6 Hz, 1H, H-4'''), 4.38 (br.s, 2H, CH_2-2'), 3.85 (br.s, 3H, CH_3O-2'''), 2.34 (distorted d, J = 0.6 Hz, due to allylic coupling, 3H, CH_3-6); ^{13}C NMR: 164.97 (C-1'), 163.99 (C-5''), 162.73 (C-2''), 157.26 (C-2'''), 155.82 (C-2), 134.90 (C-4), 133.60 (C-5'''), 130.0 (C-6'''), 126.67 (C-5), 120.69 (C-4'''), 112.62 (C-3'''), 111.78 (C-1'''), 55.92 (CH_3O-2'''), 35.29 (C-2'), 11.07 (C-6). EI-MS: m/z 362 $[M]^+$, 249 $[C_{11}H_9N_2O_3S]^+$, 222 $[C_{10}H_9N_2O_2S]^+$, 189 $[C_6H_9N_2OS_2]^+$, 175 $[C_9H_7N_2O_2]^+$, 141 $[C_5H_5N_2OS]^+$, 135 $[C_8H_7O]^+$, 114 $[C_3H_2N_2OS]^+$, 92 $[C_7H_8]^+$, 77 $[C_6H_5]^+$.

2-{[5-(2-Chlorophenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-*N*-(5-methyl-1,3-thiazol-2-yl)acetamide (VIIIe). Light brown amorphous solid; yield 89%; mp 236–237°C. Mol. formula $C_{13}H_{11}ClN_4O_2S_2$; mol. mass 366 g mol^{-1}; IR: 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: 12.37 (s, 1H, CONH), 7.95 (br.d, J = 7.5 Hz, 1H, H-6'''), 7.71 (br.d, J = 7.9 Hz, 1H, H-3'''), 7.64 (br.t, J = 7.3 Hz, 1H, H-5'''), 7.52 (br.t, J = 7.5 Hz, 1H, H-4'''), 7.17 (br.s, 1H, H-4), 4.41 (br.s, 2H, CH_2-2'), 2.34 (s, 3H, CH_3-6); ^{13}C NMR: 164.86 (C-1'), 163.71 (C-5''), 163.30 (C-2''), 155.80 (C-2), 149.15 (C-4'''), 134.91 (C-4), 133.28 (C-4'''), 131.65 (C-2'''), 131.13 (C-3'''), 131.10 (C-6'''), 127.82 (C-5'''), 126.67 (C-5), 122.05 (C-1'''), 35.36 (C-2'), 11.07 (C-6); EI-MS: m/z 366 $[M]^+$, 257 $[C_8H_9N_4O_2S_2]^+$, 226 $[C_9H_6ClN_2OS]^+$, 179 $[C_8H_4ClN_2OS]^+$, 141 $[C_5H_5N_2OS]^+$, 75 $[C_6H_3]^+$.

2-{[5-(3-Chlorophenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-*N*-(5-methyl-1,3-thiazol-2-yl)acetamide (VIIIf). Off-white amorphous powder; yield 83%; mp 213–214°C. Mol. formula $C_{13}H_{11}ClN_4O_2S_2$; mol. mass 366 g mol^{-1}; IR: 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: 12.37 (s, 1H, CONH), 7.99 (br.d, J = 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 (distorted dd, J = 2.1, 8.5 Hz, 1H, H-4'''), 7.17 (br.s, 1H, H-4), 4.41 (br.s, 2H, CH_2-2'), 2.34 (s, 3H, CH_3-6); ^{13}C NMR: 165.15 (C-1'), 163.70 (C-5''), 163.32 (C-2''), 157.65 (C-2), 137.81 (C-3'''), 134.81 (C-4), 133.28 (C-4'''), 131.14 (C-2'''), 131.09 (C-6'''), 127.82 (C-5'''), 126.91 (C-5), 122.07 (C-1'''), 35.38 (C-2'), 11.07 (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*-(5-methyl-1,3-thiazol-2-yl)acetamide (VIIIg). Brown solid; yield 88%; mp 207–208°C; Mol. formula $C_{14}H_{11}ClN_4O_2S_2$; mol. mass 366 g mol^{-1}; IR: 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: 12.38 (s, 1H, CONH), 7.91 (br.d, J = 8.5 Hz, 2H, H-3''' and H-5'''), 7.49 (br.d, J = 8.5 Hz, 2H, H-2''' and H-6'''), 7.17 (br.s, 1H, H-4), 4.43 (br.s, 2H, CH_2-2'), 2.34 (s, 3H, CH_3-6); ^{13}C NMR: 165.15 (C-1'), 163.70 (C-5''), 163.32 (C-2''), 157.65 (C-2), 137.81 (C-3''' and C-5'''), 134.81 (C-4), 133.28 (C-4'''), 131.14 (C-2''' and C-6'''), 126.91 (C-5), 122.07 (C-1'''), 35.38 (C-2'), 11.07 (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*-(5-methyl-1,3-thiazol-2-yl)acetamide (VIIIh). Brown greasy liquid; yield 79%; Mol. formula $C_{13}H_9Cl_2N_4O_2S_2$; mol. mass 400 g mol^{-1}; IR: 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: 12.35 (s, 1H, CONH), 7.98 (d, J = 8.5 Hz, 1H, H-6'''), 7.91 (d, J = 2.1 Hz, 1H H-3'''), 7.65 (dd, J = 1.2, 8.5 Hz, 1H, H-5'''), 7.17 (distorted d, J = 1.2 Hz, due to allylic coupling, 1H, H-4), 4.41 (br.s, 2H, CH_2-2'), 2.34 (distorted d, J = 1.3 Hz, due to allylic coupling, 3H, CH_3-6); ^{13}C NMR: 165.01 (C-1'), 163.88 (C-5''), 162.64 (C-2''), 156.01 (C-2), 137.18 (C-4'''), 134.90 (C-4), 132.72 (C-1'''), 132.23 (C-6'''), 130.71 (C-3'''), 128.13 (C-5'''), 126.65 (C-5), 121.0 (C-2'''), 35.40 (C-2'), 11.0 (C-6). EI-MS: *m/z* 400 $[M]^+$, 260 $[C_9H_5ClN_2O_2S]^+$, 173 $[C_7H_3Cl_2N]^+$, 141 $[C_5H_5N_2OS]^+$, 114 $[C_3H_2N_2OS]^+$.

2-{[5-(3-Aminophenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-*N*-(5-methyl-1,3-thiazol-2-yl)acetamide (VIIIi). White solid; yield: 77%; mp 206–207°C; Mol. formula $C_{14}H_{13}N_5O_2S_2$; Mol. mass g mol^{-1}; IR: 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: 12.38 (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'''), 7.17 (br.s, 1H, H-4), 4.43 (br.s, 2H, CH_2-2'), 2.34 (s, 3H, CH_3-6); ^{13}C NMR: 165.26 (C-1'), 162.99 (C-5''), 160.46 (C-2''), 157.71 (C-2), 134.80 (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'''), 126.01 (C-5), 35.36 (C-2'), 11.07(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*-(5-methyl-1,3-thiazol-2-yl)acetamide (VIIIj). Brown amorphous powder; yield 81%; mp 225–226°C; Mol. formula $C_{14}H_{13}N_5O_2S_2$; mol. mass 347 g mol^{-1}; IR: 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: 12.70 (s, 1H, CONH), 7.56 (br.d, J = 8.6 Hz, 2H, H-2''' and H-6'''), 7.16 (d, J = 1.1 Hz, due to allylic coupling, 1H, H-4), 6.62 (br.d, J = 8.6 Hz, 1H, H-3''' and H-5'''); 4.30 (br.s, 2H, CH_2-2'), 2.34 (d, J = 0.7 Hz, due to allylic coupling, 3H, CH_3-6); ^{13}C-NMR: 166.09 (C-1'), 165.22 (C-5''), 160.57 (C-2''), 156.01 (C-2), 152.40 (C-4'''), 134.92 (C-4), 127.89 (C-2''' and C-6'''), 126.68 (C-5), 113.47 (C-3''' and C-5'''), 109.13 (C-1'''), 35.28 (C-2'), 11.07 (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 $[C_5H_5]^+$.

2-{[5-(2-Nitrophenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-*N*-(5-methyl-1,3-thiazol-2-yl)acetamide (VIIIk). Light yellow solid; yield 79%; mp 217–218°C; Mol. formula $C_{14}H_{11}N_5O_4S_2$; mol. mass 377 g mol^{-1}; IR: 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 of ether); ^{1}H NMR: 12.36 (s, 1H, CONH), 8.19-8.17 (m, 1H, H-3'''), 8.01 (m, 1H, H-6'''), 7.94-7.90 (m, 2H, H-4''' and H-5'''), 7.16 (distorted d, J = 1.2 Hz, due to allylic coupling, 1H, H-4), 4.40 (br.s, 2H, CH_2-2'), 2.34 (d, J = 0.9 Hz, due to allylic coupling, 3H, CH_3-6); ^{13}C NMR: 165.0 (C-1'), 164.3 (C-5''), 161.61 (C-2''), 156.0 (C-2), 147.5 (C-2'''), 134.85 (C-4), 133.61 (C-4'''), 133.43 (C-5'''), 131.09 (C-3'''), 124.70 (C-6'''), 116.43 (C-1'''), 113.91 (C-5), 35.33 (C-2'), 11.07 (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 $[C_6H_4]^+$.

2-{[5-(3-Nitrophenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-*N*-(5-methyl-1,3-thiazol-2-yl)acetamide (VIIIl). Light yellow solid; yield 84%; mp 223–224°C; Mol. formula $C_{14}H_{11}N_5O_4S_2$; mol. mass 377 g mol^{-1}; IR: 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: 12.37 (s, 1H, CONH), 8.62 (distorted d, J = 1.8 Hz, 1H, H-2'''), 8.45 (dd, J = 1.3, 8.2 Hz, 1H, H-6'''), 8.38 (br.d, J = 7.8 Hz, 1H, H-4'''), 7.89 (br.t, J = 7.3 Hz, 1H, H-5'''), 7.16 (distorted d, J = 1.1 Hz, due to allylic coupling, 1H, H-4), 4.43 (br.s, 2H, CH_2-2'), 2.34 (distorted d, J = 0.84 Hz, due to allylic coupling, 3H, CH_3-6); ^{13}C NMR: 164.93 (C-1'), 164.06 (C-5''), 163.77 (C-2''), 155.0 (C-2), 148.21 (C-3'''), 134.90 (C-4), 132.41 (C-6'''), 131.31 (C-5'''), 126.37 (C-4'''), 124.40 (C-1'''), 120.88 (C-2'''), 113.91 (C-5), 35.41 (C-2'), 11.07 (C-6). EI-MS: *m/z* 377 $[M]^+$, 150$[C_7H_4NO_3]^+$, 141 $[C_5H_5N_2OS]^+$, 114 $[C_3H_2N_2OS]^+$, 76 $[C_6H_4]^+$.

2-{[5-(4-Nitrophenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-*N*-(5-methyl-1,3-thiazol-2-yl)acetamide (VIIIm).

Light yellow solid; yield 81%; mp 216–217°C; Mol. formula $C_{14}H_{11}N_5O_4S_2$; mol. mass 377 g mol^{-1}; IR: 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 of ether); ^{1}H NMR: 12.38 (s, 1H, CONH), 8.42 (br.d, J = 7.0 Hz, 2H, H-3''' and H-5'''), 8.21 (br.d, J = 7.0 Hz, 2H, H-2''' and H-6'''), 7.17 (distorted d, J = 1.2 Hz, due to allylic coupling, 1H, H-4), 4.43 (br.s, 2H, CH_2-2'), 2.34 (d, J = 0.9 Hz, due to allylic coupling, 3H, CH_3-6); ^{13}C NMR: 165.00 (C-1'), 164.51 (C-5''), 163.93 (C-2''), 156.01 (C-2), 149.15 (C-4'''), 134.95 (C-4), 127.74 (C-2''' and C-6'''), 124.58 (C-3''' and C-5'''), 122.01 (C-1'''), 113.91 (C-5), 35.38 (C-2'), 11.07(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*-(5-methyl-1,3-thiazol-2-yl)acetamide (VIIIn). Light yellow amorphous solid; yield 83%; mp 273–274°C; Mol. formula $C_{14}H_{10}N_6O_6S_2$; mol. mass 422 g mol^{-1}; IR: 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: 12.38 (s, 1H, CONH), 8.92 (d, J = 2.4 Hz, 2H, H-2''' and H-6'''), 8.66 (d, J = 2.4 Hz, 1H, H-4'''), 7.17 (br.s, 1H, H-4), 4.43 (br.s, 2H, CH_2-2'), 2.34 (s, 3H, CH_3-6); ^{13}C NMR: 165.12 (C-1'), 163.88 (C-5''), 162.67 (C-2''), 157.71 (C-2), 144.97 (C-3''' and C-5'''), 134.88 (C-4), 132.60 (C-1'''), 128.14 (C-2''' and C-6'''), 118.19 (C-4'''), 113.91 (C-5), 35.40 (C-2'), 11.07 (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 $[C_6H_4]^+$.

Enzyme Inhibition Assays

Cholinesterases. AChE and BChE inhibition activities were evaluated according to Ellman method with slight modifications [20, 21]. Total volume of the reaction mixture was 100 μL. It contained 60 μL Na_2HPO_4 buffer with concentration of 50 mM and pH 7.7; 10 μL test compound (0.5 mM per well) was added, followed by the addition of 10 μL (0.005 unit per well AChE or 0.5 unit per well BChE) enzyme. The contents were mixed and pre-read at 405 nm. Then contents were pre-incubated for 10 min at 37°C. The reaction was initiated by the addition of 10 μL of 0.5 mM substrate (acetylthiocholine iodide for AChE or butyrylthiocholine chloride for BChE) per well, followed by the addition of 10 μL DTNB (0.5 mM per well). After 15 min of incubation at 37°C absorbance was measured at 405 nm using a Synergy HT (Biotek, US) 96-well plate reader. All experiments were carried out with their respective controls in triplicate. Eserine (0.5 mM per well) was used as a positive control. The percent inhibition was calculated using the following formula:

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

where Control is the total enzyme activity without inhibitor and Test, the activity in the presence of test compound.

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

Urease. This enzyme assay is the customized form of the commonly known Berthelot assay [22]. The assay mixture (85 μL per well of a 96-well plate) contains 10 μL of phosphate buffer, pH 7.0, 10μL 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. Then, 115 μL phenol hypochlorite reagents (freshly prepared by mixing 45 μL phenol with 70 μL of alkali) were added into each well. For color development, incubation was carried out for further 10 min at 37°C. Absorbance was measured at 625 nm. Percentage enzyme inhibition and IC_{50} values were calculated by the same procedure as mentioned above.

α-Glucosidase. α-Glucosidase inhibition activity was performed according to the slightly modified method [23]. Total volume of the reaction mixture was 100 μL; it contained 70 μL phosphate buffer saline (50 mM) with 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-read at 400 nm. The reaction was initiated by the addition of 10 μL of 0.5 mM substrate (*p*-nitrophenylglucopyranoside). Acarbose was used as positive control. After 30 min of incubation at 37°C, absorbance was measured at 400 nm using the Synergy HT microplate reader. All experiments were carried out in duplicates. The percent inhibition and IC_{50} values were calculated by the same equation as described for cholinesterase enzymes above.

Molecular Docking

To prove the bioactive conformations, the synthesized compounds were docked into the binding pockets of the proteins under study using the default parameters of the MOE-Dock method [24]. Before docking, the molecules of acetylcholinesterase, butyrylcholinesterase, urease, and α-glucosidase were retrieved from the Protein Data Bank (PDB ID codes 1GQR, 1POP, 4UBP, and 3NO4, respectively). MOE applications were used for performing 3D protonation of the protein molecules. Energy minimization algorithm of MOE tool was used to minimize the protein molecule energy in the gradient of 0.05 Force Field of MMFF94X with the default parameters of MOE

2009-10. Then all the ligands (3D protonated and energy minimized) were docked into the binding pockets (selective residues/amino acids are shown in the docking images in Figs. 1–4) of the above-mentioned proteins using the Triangular Matching docking method. Re-docking procedure was also applied in order to validate the docking protocol. Each complex was analyzed for the type of interactions; bond distances and their 3D images were taken.

Cytotoxicity assay. Cytotoxicity was studied by the brine shrimp cytotoxic assay [25]. Artificial sea water was prepared using sea salt, 34 g/L. Brine shrimp (*Artemia salina*) eggs (Sera, Heidelberg, Germany) were hatched in shallow rectangular dish (22 × 32 cm) under constant aeration for 48 h at room temperature. After hatching, active shrimps free from eggs were collected from brighter portion of the hatching chamber and used for the assay. Ten shrimps were transferred to each vial using Pasteur pipette vial containing 5 mL of artificial sea water with 200, 20, 2 and 0.2 μg/mL 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.

CONCLUSION

We can conclude that the incorporation of electron donating groups (OCH_3, NH_2, OH) in the thiazole–oxadiazole biheterocyclic core renders good inhibitory potential against AChE, BChE, urease, and α-glucosidase. Similarly, from the cytotoxicity study, it was inferred that most of the compounds possess low toxicity and hence, these molecules can find their utility as promising therapeutic agents.

ACKNOWLEDGMENTS

The present study was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2017R1D1A1B03034948).

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