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Synthesis of Novel Bi-Heterocycles as Valuable Anti-Diabetic Agents: 2-({5-((2-Amino-1,3-Thiazol-4-yl)methyl)-1,3,4-Oxadiazol-2-yl}sulfanyl)-*N*-(Substituted)acetamides

Muhammad Athar Abbasi[a, 1], Muhammad Shahid Ramzan[a], Aziz-ur-Rehman[a], Sabahat Zahra Siddiqui[a], Syed Adnan Ali Shah[b], Muhammad Arif Lodhi[c], Farman Ali Khan[c], and Bushra Mirza[d]

[a] *Department of Chemistry, Government College University, Lahore, 54000 Pakistan*

[b] *Faculty of Pharmacy and Atta-ur-Rahman Institute for Natural Products Discovery (AuRIns), Level 9, FF3, Universiti Teknologi MARA, Puncak Alam Campus, Bandar Puncak Alam, Selangor Darul Ehsan, 42300 Malaysia*

[c] *Department of Biochemistry, Abdul Wali Khan University, Mardan, 23200 Pakistan*

[d] *Department of Biochemistry, Quaid-i-Azam University, Islamabad, 45320 Pakistan*

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Abstract—The synthesis of a new series of *S*-substituted acetamides derivatives of 5-[(2-amino-1,3-thiazol-4-yl)methyl]-1,3,4-oxadiazol-2-thiol were synthesized and evaluated for enzyme inhibition study along with cytotoxic behavior. Ethyl 2-(2-amino-1,3-thiazol-4-yl)acetate was converted to corresponding acid hydrazide by hydrazine hydrate in ethanol. The reflux of acid hydrazide with carbon disulfide resulted to 5-[(2-amino-1,3-thiazol-4-yl)methyl]-1,3,4-oxadiazol-2-thiol. Different electrophiles were synthesized by the reaction of respective anilines (one in each reaction) and 2-bromoacetylbromide in an aqueous medium. The targeted bi-heterocyclic compounds were synthesized by stirring nucleophilic 5-[(2-amino-1,3-thiazol-4-yl)methyl]-1,3,4-oxadiazol-2-thiol with different acetamides electrophiles (one after another), in DMF using LiH as base and activator. The proposed structures of newly synthesized compounds were deduced by spectroscopic techniques such as ^{1}H NMR, ^{13}C NMR, EI MS and elemental analysis. These novel bi-heterocycles were tested for their anti-diabetic potential via the in vitro inhibition of α-glucosidase enzyme. The in silico study of these molecules was also coherent with their enzyme inhibition data. Furthermore, these molecules were analyzed for their cytotoxic behavior against brine shrimps. It was inferred from the results that most of them exhibited very potent inhibitory potential against the studied enzyme and can be utilized as valuable anti-diabetic agent.

Keywords: ethyl 2-(2-amino-1,3-thiazol-4-yl)acetate, 1,3,4-oxadiazole, acetamide, enzyme inhibition, molecular docking, cytotoxicity

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INTRODUCTION

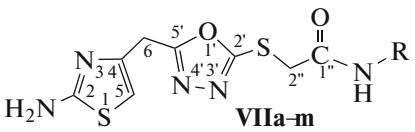
Thiazole derivatives are used as drugs in treatment of cancer, lowering blood pressure and treatment of infection [1]. The in vitro potency of some thiazole derivatives has been proven to inhibit the bacterial pathogens [2]. A large number of thiazole derivatives have been incorporated into a wide variety of biological activities such as antibacterial, antifungal, antitubercular, anti-mycobacterial, anticancer, antiviral ones [3–7]. 1,3,4-Oxadiazole is an important heterocycle and its different derivatives possess an extensive spectrum of pharmacological activities such as antiviral, antibacterial, antitumor, antituberculosis, anti-inflammatory, anticonvulsant and anti-Alzheimer activities [8–13]. Oxadiazoles have played an important role in medicinal chemistry, pesticide chemistry, polymer and display remarkable biological activities, such as antimicrobial, anti-HIV, antitubercular, antimalarial, analgesic, anti-inflammatory [14–20].

Alpha-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 [21]. AGIs 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.

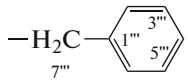
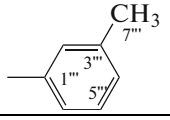
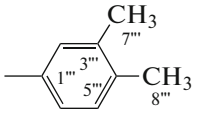
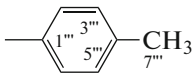
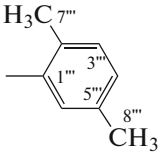
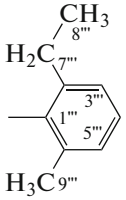
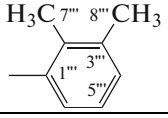
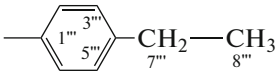
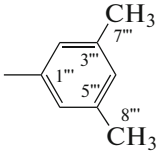
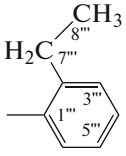
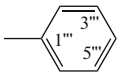
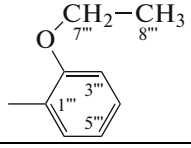
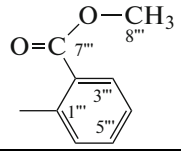
One of the key objectives of organic and medicinal chemists is to design and synthesize the molecules having potent therapeutic values. The rapid development of resistance to existing drugs generated a serious

[1] Corresponding author: phone: (+92)-42-111000010; e-mail: atrabbasi@yahoo.com; abbasi@gcu.edu.pk

Table 1. Different substituents (–R) in Scheme 1



VIIa–m

Compound	–R	Compound	–R
IVa, **VIa**, **VIIa**		**IVg**, **VIg**, **VIIg**	
IVb, **VIb**, **VIIb**		**IVh**, **VIh**, **VIIh**	
IVc, **VIc**, **VIIc**		**IVi**, **VIi**, **VIIi**	
IVd, **VId**, **VIId**		**IVj**, **VIj**, **VIIj**	
IVe, **VIe**, **VIIe**		**IVk**, **VIk**, **VIIk**	
IVf, **VIf**, **VIIf**		**IVl**, **VIl**, **VIIl**	
IVm, **VIm**, **VIIm**			

challenge to the scientific community. Consequently, there is a vital need for the development of new therapeutic agents having potent activity. So, it prompted us to synthesize some new bi-heterocycles by the amalgamation of thiazole and 1,3,4-oxadiazole heterocyclic cores to serve as possible lead compounds.

RESULTS AND DISCUSSION

Chemistry

Different thiazole derivatives bearing 1,3,4-oxadiazole were synthesized (Scheme 1 and Table 1) in a series of steps and evaluated for their biological potential. The synthesis was initiated with ethyl 2-(2-amino-1,3-thiazol-4-yl)acetate (**I**) which was refluxed with hydrazine hydrate in methanol to acquire corresponding acid hydrazide, **II**. This nucleophilic substitution reaction was completed in two hours. The acid hydrazide, **II**, was made to react with carbon disulfide in the presence of KOH, an activator for cyclization, to yield 5-[(2-amino-1,3-thiazol-4-yl)methyl]-1,3,4-oxadiazol-2-thiol (**III**). The different acetamides, **VIa**–**m**, were synthesized as electrophiles by stirring substituted amines, **IVa**–**m**, with 2-bromoacetylbromide (**V**) in a weak basic aqueous medium. Finally, the target compounds, **VIIa**–**m**, were synthesized by stir-

Table 2. Percent inhibition at 0.5 mM and IC_{50} values against α-glucosidase enzyme

Compd.	α-Glucosidase inhibition	
	inhibition, %	IC_{50}, μM
VIIa	89.34 ± 0.17	28.52 ± 0.11
VIIb	91.56 ± 0.18	14.38 ± 0.12
VIIc	26.25 ± 0.14	–
VIId	91.78 ± 0.16	15.47 ± 0.11
VIIe	92.57 ± 0.17	18.29 ± 0.13
VIIf	51.48 ± 0.14	>500
VIIg	91.23 ± 0.15	16.29 ± 0.12
VIIh	71.42 ± 0.21	57.45 ± 0.16
VIIi	87.82 ± 0.17	35.62 ± 0.12
VIIj	75.36 ± 0.24	37.67 ± 0.18
VIIk	76.25 ± 0.22	45.42 ± 0.17
VIIl	87.42 ± 0.17	23.25 ± 0.12
VIIm	77.67 ± 0.13	26.52 ± 0.21
Acarbose	92.23 ± 0.16	37.38 ± 0.12

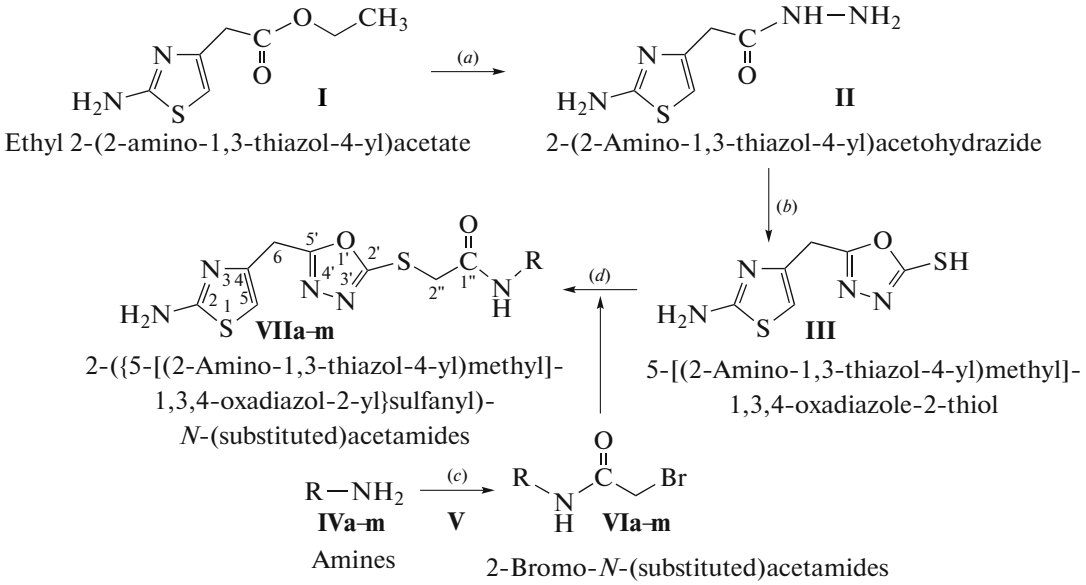
All compounds were dissolved in methanol and experiments were performed in triplicate (mean ± sem, $n = 3$).

ring **III** with different electrophiles, **VIa**–**m**, in DMF using LiH as base and activator. The molecular structures of these derivatives were well corroborated by IR, EI MS, ^{1}H NMR and ^{13}C NMR data. The structural analysis of one of the compounds is discussed hereby in detail for the benefit of the reader. The molecular formula, $C_{16}H_{17}N_5O_2S_2$, of **VIIb** was established through EI MS spectrum showing molecular ion peak at m/z 375, and by counting the number of protons in its ^{1}H NMR spectrum. The number of the carbon atoms resonating in its ^{13}C NMR spectrum was also in agreement with the deduced molecular formula. The prominent absorption bands in IR spectrum appeared at ν 3360 (N–H stretching), 3045 (C–H of aromatic ring), 2920 (–CH_2– stretching), 1660 (C=O stretching), 1570 (C=C stretching of aromatic ring) and 1545 (C=N stretching) cm^{-1}. The 3,4-dimethylphenyl moiety of the molecule was ascribed in its ^{1}H NMR spectrum by three signals in aromatic region at δ 7.34 (br.s, 1H, H-2'''), 7.26 (br.d, J = 8.1 Hz, 1H, H-6'''), and 7.06 (br.d, J = 8.1, H-5''') along with two methyl singlets in aliphatic region at δ 2.18 (s, 3H, CH_3-8''') and 2.16 (s, 3H, CH_3-7'''). The 2-amino-1,3-thiazol-4-yl heterocycle was characterized by two signals at δ 6.99 (br.s, 2H, $-NH_2$), and 6.38 (s, 1H, H-5). A signal at δ 4.03 (br.s, 2H, CH_2-6) was assignable to a methylene group connecting the two heterocycles in the molecule [22]. The remaining two signals δ 10.21 (s, 1H, –CONH), and 4.23 (br.s, 2H, CH_2-2'') were characteristic of the *C*- and *N*-substituted acetamidic moiety in the molecule [23]. All these assignments are also substantiated by its ^{13}C NMR spectrum which exhibited overall sixteen carbon resonances. The 2-amino-1,3-thiazol-4-yl heterocycle was clearly indicated by two quaternary signals at δ 163.03 (C-2), and 143.90 (C-4), along with a methine signal at δ 103.12 (C-5) [24]. Similarly, the other heterocycle i.e. (5-substituted-1,3,4-oxadiazol-2-yl)sulfanyl was also signified by two quaternary signals at δ 168.74 (C-2') and 165.66 (C-5') while a methylene connecting the two heterocycle was obvious at δ 27.52 (C-6) [22]. The 3,4-dimethylphenyl moiety was also apparent with three quaternary signals at δ 138.34 (C-1'''), 136.41 (C-3'''), and 131.41 (C-4'''), along with three methine signals at δ 129.62 (C-5'''), 120.35 (C-2'''), and 116.68 (C-6''') while the two methyl carbon resonances appeared at δ 19.57 (C-7''') and 18.74 (C-8'''). The *C*- and *N*-substituted acetamidic moiety in the molecule was corroborated by a quaternary signal at δ 164.34 (C-1'') and a methylene signal at δ 36.64 (C-2'') [24]. These structural units of the molecule were also fully coherent with various fragment ion peaks observed in its EI-MS spectrum. On the basis of whole consolidated discussion, the structure of **VIIb** was confirmed and it was named as 2-({5-[(2-amino-1,3-thiazol-4-yl)methyl]-1,3,4-oxadiazol-2-yl}sulfanyl)-*N*-(3,4-dimethylphenyl)acetamide. Similar approach was exercised for the structural analysis of all the synthesized bi-heterocycles.

Table 3. Brine shrimp activity

Compd.	LD_{50}, mM	Compd.	LD_{50}, mM	Compd.	LD_{50}, mM
VIIa	350.9	**VIIf**	458.1	**VIIk**	151.7
VIIb	443.2	**VIIg**	498.8	**VIIl**	245.7
VIIc	213.5	**VIIh**	344.1	**VIIm**	287.4
VIId	398.4	**VIIi**	199.1	Doxorubicin	5.21
VIIe	155.4	**VIIj**	21.8		

Doxorubicin was used as standard.



Scheme 1. Outline for the synthesis of 2-({5-[(2-amino-1,3-thiazol-4-yl)methyl]-1,3,4-oxadiazol-2-yl}sulfanyl)-*N*-(substituted)acetamides (**VIIa–m**). Reagents and conditions: (*a*) $MeOH/N_2H_4 \cdot H_2O$/refluxing for 2 hours. (*b*) $EtOH/CS_2/KOH$/refluxing for 3 hours. (*c*) Aq. 5% Na_2CO_3 soln./stirring for 1–2 hours after addition of 2-bromoacetylbromide (**V**). (*d*) DMF/LiH/stirring for 3–5 hours.

α-*Glucosidase Inhibition and Structure–Activity Relationship*

The synthesized molecules were screened for α-glucosidase inhibition. The results of percentage inhibition and IC_{50} values are presented in Table 2, as mean ± SEM. From the results, it was obvious that eight molecules in the series exhibited IC_{50} values lower than the Acarbose, a reference standard against this enzyme. The most potent compound was **VIIb**, with IC_{50} value of 14.38 ± 0.12 μM, relative to Acarbose, having an IC_{50} value of 37.38 ± 0.12 μM. The most excellent inhibitory potential of this compound might be attributed to the presence of 3,4-dimethylphenyl group at the acetamidic nitrogen atom in the molecule. Similarly, very comparable inhibitory potentials were revealed by **VIId** and **VIIg**, bearing IC_{50} values of 15.47 ± 0.11 and 16.29 ± 0.12 μM, respectively. Hereby, their valuable potential can be an outcome of the presence of 2,3-dimethylphenyl and 3-methylphenyl groups in these molecules. The inhibition order of

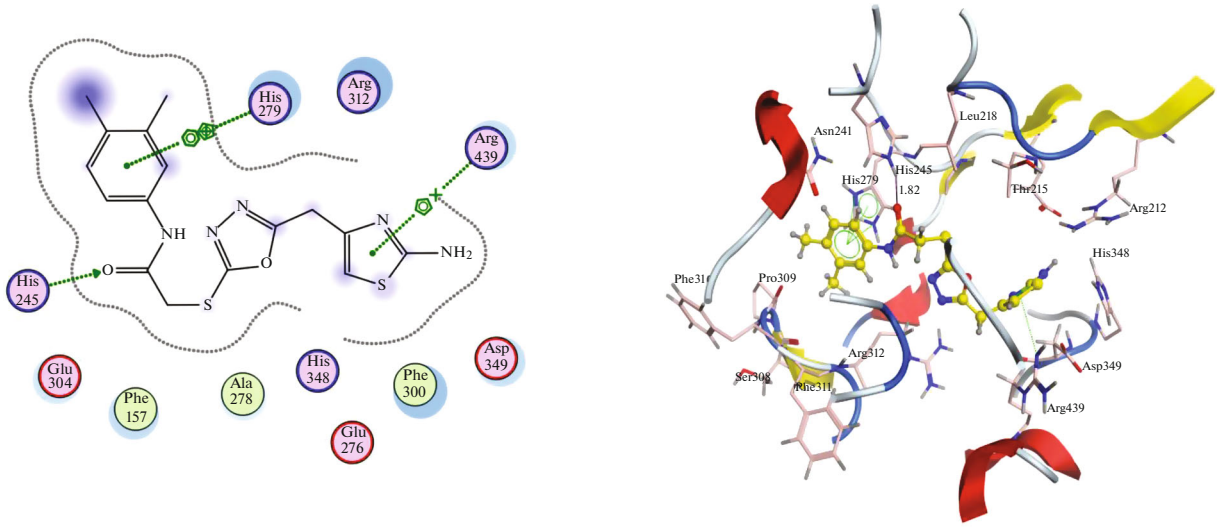


Fig. 1. The 2D and 3D interaction analysis of compound (**VIIb**) against α-glucosidase enzyme.

synthesized compounds was found to be **VIIb > VIIg > VIIe >VIIl > VIIm > VIIa >VIIi > VIIj >VIIh**. So, in general, it was lucid to infer that most of the compounds exhibited very excellent activities against the α-glucosidase enzyme and might serve as valuable anti-diabetic agents.

Molecular Docking (in silico) Study

All these bi-heterocycles, **VIIa–m**, were docked into the active pocket of this enzyme. Whereby, the most potent compound, **VIIb**, inhibited alpha-glucosidase by making three interactions From the Fig. 1 (2D and 3D), it was clear that His245 has made a strong acidic interaction with acetamoyl mioety of the compound showing bond length of 1.82 Å. The Arg439 and His279 have also made somewhat weak arene-cation and a couple of π-π interactions with terminal thiazol-2-amine ring and with phenyl ring of the compound, respectively. Similarly, the in silico results of other molecules were also coherent with their in vitro enzyme inhibition data.

Brine Shrimps Activity

The cytotoxicity of the synthesized compounds was evaluated through brine shrimp lethality. The higher LD_{50} values of brine shrimp lethality analysis demonstrated the lowest toxicity of these compounds (Table 3). It was rational from the results that the synthesized derivatives were not highly toxic and hence can be utilized as safe therapeutic agents.

EXPERIMENTAL

General

All the chemical reagents were purchased from Alfa Aesar, Merck and Sigma Aldrich through local suppliers. The solvents were of analytical grade and used without further purification. The reaction completion and purity were confirmed by TLC performed on aluminum plates coated with silica gel G-25-UV254, run by ethyl acetate : *n*-hexane solvent system and visualized under UV at 254 nm. Melting points were recorded on Griffin-George melting point apparatus by using open capillary tubes and were uncorrected. ^{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 BBO probe. EI-MS spectra were measured on a JEOL JMS-600H instrument with data processing system. IR spectra were recorded by KBr pellet method using on Jasco-320-A spectrometer.

Procedure for the Synthesis of 5-[(2-Amino-1,3-thiazol-4-yl)acetohydrazide (II)

Ethyl 2-(2-amino-1,3-thiazol-4-yl)acetate (**I**; 10 g, 0.050 mol) and methanol (200 mL) were taken in a 500 mL RB flask. Hydrazine hydrate (2.5 mL, 0.050 mol) was added drop wise and the mixture was allowed to reflux for 2 hours. The reaction progress was observed by TLC using *n*-hexane and ethyl acetate solvent system (40 : 60). After complete reaction, the reaction mixture was allowed to cool at room temperature to attain white colored precipitates of hydrazide (**II**). Product was filtered and washed with methanol [22].

Procedure for the Synthesis of 5-[(2-Amino-1,3-thiazol-4-yl)methyl]-1,3,4-oxadiazol-2-thiol (III)

5-[(2-Amino-1,3-thiazol-4-yl)acetohydrazide (**II**; 4 g, 0.024 mol) was dissolved in C_2H_5OH (70 mL) in a 250 mL RB flask at 28°C and then solid KOH (1.34 g, 0.024 mol) was dissolved on reflux. Carbon disulfide (3.70 mL, 0.048 mol) was poured drop-wise at 28°C and then the reaction mixture was allowed to reflux again for 3 hours. The completion of reaction was observed by TLC using *n*-hexane and ethyl acetate solvent system (70 : 30). The excess of ethanol was distilled off. Excess of ice cold distilled water was added followed by addition of dilute HCl till constant pH of 4–5. Light peach colored precipitates of **III** were filtered and washed with distilled water [22].

General Procedure for the Preparation of 2-Bromo-N-(substituted)acetamides (VIa–m)

Substituted amines (**IVa–m**; 0.038 mol; one in each reaction) were suspended in 30 mL distilled water in an iodine flask (100 mL). Aqueous Na_2CO_3 solution (10%, 2–3 mL) was poured to keep the reaction continued. 2-Bromoacetylbromide (**V**; 0.038 mol) was added gradually on vigorous shaking and then stirred for further 1–2 h. Reaction completion was monitored by TLC. Excess ice cold distilled water (60 mL) was added. The resulting precipitates were collected through filtration, washed with distilled water and dried [24].

General Procedure for the Synthesis of 2-({5-[(2-Amino-1,3-thiazol-4-yl)methyl]-1,3,4-oxadiazol-2-yl}sulfanyl)-N-(substituted)acetamides (VIIa–m)

5-[(2-Amino-1,3-thiazol-4-yl)methyl]-1,3,4-oxadiazol-2-thiol (3; 0.1 g, 0.47 mmol) 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 the mixture was stirred for half an hour. Then, different electrophiles, *N*-substituted-2-bromoacetamide (**VIa–m**; 0.47 mmol; one in each reaction), were added and set to stir for 3–5 h. The reaction was monitored by TLC using *n*-hexane and ethyl acetate solvent system (80 : 20). Excess ice cold distilled water was added

and the products (**VIIa**–**m**) were filtered, washed with distilled water and dried for further use.

2-({5-[(2-Amino-1,3-thiazol-4-yl)methyl]-1,3,4-oxadiazol-2-yl}sulfanyl)-*N*-benzylacetamide (VIIa). Light brown solid; Yield: 89%; mp: 229–230°C; Mol. Formula: $C_{15}H_{15}N_5O_2S_2$; Mol. Mass.: 361 g mol^{-1}; IR: 3345 (N–H stretching), 3050 (C–H of aromatic ring), 2915 (–CH_2– stretching), 1675 (C=O stretching), 1570 (C=C stretching of aromatic ring), 1515 (C=N stretching); ^{1}H NMR: 10.37 (s, 1H, N–H), 7.56 (br.d, J = 8.0, 2H, H-2''' & H-6'''), 7.32 (br.t, J = 7.8, 2H, H-3''' and H-5'''), 7.07 (br.t, J = 7.2, 1H, H-4'''), 6.99 (2H, –NH_2), 6.39 (s, 1H, H-5), 4.26 (s, 2H, CH_2-2''), 4.04 (s, 2H, CH_2-6); ^{13}C NMR: 168.75 (C-2'), 165.69 (C-5'), 164.71 (C-1''), 163.05 (C-2), 143.90 (C-4), 138.61 (C-1'''), 128.70 (C-3''' and C-5'''), 123.64 (C-4'''), 119.15 (C-2''' and C-6'''), 103.13 (C-5), 36.67 (C-2''), 27.53 (C-6); EI MS: *m/z* 361 [M]$^+$, 287 [$C_{14}H_{13}N_3O_2S$]$^+$, 248 [$C_{11}H_{10}N_3O_2S$]$^+$, 228 [$C_7H_8N_4OS_2$]$^+$, 221 [$C_{10}H_9N_2O_2S$]$^+$, 194 [$C_9H_{10}N_2OS$]$^+$, 181 [$C_9H_{11}NOS$], 141 [$C_5H_5N_2OS$]$^+$, 114 [$C_4H_6N_2S$]$^+$, 91 [C_7H_7].

2-({5-[(2-Amino-1,3-thiazol-4-yl)methyl]-1,3,4-oxadiazol-2-yl}sulfanyl)-*N*-(3,4-dimethylphenyl)acetamide (VIIb). Dark brown gummy solid; Yield: 84%; mp: 278–279°C; Mol. Formula: $C_{16}H_{17}N_5O_2S_2$; Mol. Mass: 375 g mol^{-1}; IR: 3360 (N–H stretching), 3045 (C–H of aromatic ring), 2920 (–CH_2 stretching), 1660 (C=O stretching), 1570 (C=C stretching of aromatic ring), 1545 (C=N stretching); ^{1}H NMR: 10.21 (s, 1H, N–H), 7.34 (br.s, 1H, H-2'''), 7.26 (br.d, J = 8.1 Hz, 1H, H-6'''), 7.06 (br.d, J = 8.1, H-5'''), 6.99 (br.s, 2H, –NH_2), 6.38 (s, 1H, H-5), 4.23 (br.s, 2H, CH_2-2''), 4.03 (br.s, 2H, CH_2-6), 2.18 (s, 3H, CH_3-8'''), 2.16 (s, 3H, CH_3-7'''); ^{13}C NMR: 168.74 (C-2'), 165.66 (C-5'), 164.37 (C-1''), 163.03 (C-2), 143.90 (C-4), 138.34 (C-1'''), 136.41 (C-3'''), 131.41 (C-4'''), 129.62 (C-5'''), 120.35 (C-2'''), 116.68 (C-6'''), 103.12 (C-5), 36.64 (C-2''), 27.52 (C-6), 19.57 (C-7'''), 18.74 (C-8'''); EI MS: *m/z* 375 [M]$^+$, 301 [[$C_{15}H_{15}N_3O_2S$]$^+$, 262 [$C_{12}H_{12}N_3O_2S$]$^+$, 235 [$C_{11}H_{11}N_2O_2S$]$^+$, 195 [$C_{10}H_{13}NOS$]$^+$, 163 [$C_{10}H_{13}NO$]$^+$, 141 [$C_5H_5N_2OS$]$^+$, 121 [$C_8H_{11}N$]$^+$, 114 [$C_4H_6N_2S$]$^+$.

2-({5-[(2-Amino-1,3-thiazol-4-yl)methyl]-1,3,4-oxadiazol-2-yl}sulfanyl)-*N*-(2,5-dimethylphenyl)acetamide (VIIc). Dull white amorphous solid; Yield: 83%; mp: 207–208°C; Mol. Formula: $C_{16}H_{17}N_5O_2S_2$; Mol. Mass: 375 g mol^{-1}; IR: 3340 (N–H stretching), 3060 (C–H of aromatic ring), 2915 (–CH_2 stretching), 1570 (C=C stretching of aromatic ring), 1520 (C=N stretching); ^{1}H NMR: 10.37 (s, 1H, N–H), 7.56 (br.d, J = 8.0 Hz, 2H, H-2''' and H-6'''), 7.32 (br.t, J = 7.8 Hz, 2H, H-3''' and H-5'''), 7.07 (br.t, J = 7.2 Hz, 1H, H-4'''), 6.99 (s, 2H, –NH_2), 6.39 (s, 1H, H-5), 4.26 (s, 2H, CH_2-2''), 4.04 (s, 2H, CH_2-6); ^{13}C NMR: 168.75 (C-2'), 165.69 (C-5'), 164.71 (C-1''), 163.05 (C-2), 143.90 (C-4), 138.61 (C-1'''), 128.70 (C-3'' and C-5''), 123.64 (C-4'''), 119.15 (C-2''' and C-6'''), 103.13 (C-5), 36.67 (C-2''), 27.53 (C-6); EI MS: *m/z* 375 [M]$^+$, 301 [[$C_{15}H_{15}N_3O_2S$]$^+$, 262 [$C_{12}H_{12}N_3O_2S$]$^+$, 235 [$C_{11}H_{11}N_2O_2S$]$^+$, 195 [$C_{10}H_{13}NOS$]$^+$, 163 [$C_{10}H_{13}NO$]$^+$, 141 [$C_5H_5N_2OS$]$^+$, 121 [$C_8H_{11}N$]$^+$, 114 [$C_4H_6N_2S$]$^+$.

2-({5-[(2-Amino-1,3-thiazol-4-yl)methyl]-1,3,4-oxadiazol-2-yl}sulfanyl)-*N*-(2,3-dimethylphenyl)acetamide (VIId). Brown solid; Yield: 87%; mp: 241–242°C; Mol. Formula: $C_{16}H_{17}N_5O_2S_2$; Mol. Mass: 375 g mol^{-1}; IR: 3352 (N–H stretching), 3050 (C–H of aromatic ring), 2930 (–CH_2 stretching), 1675 (C=O stretching), 1575 (C=C stretching of aromatic ring), 1520 (C=N stretching); ^{1}H NMR: 10.21 (s, 1H, N–H), 7.26 (br.d, J = 8.1 Hz, 1H, H-4'''), 7.06 (br.d, J = 8.1, 1H, H-5'''), 7.26 (br.d, J = 8.1 Hz, 1H, H-6'''), 6.99 (br.s, 2H, –NH_2), 6.38 (s, 1H, H-5), 4.23 (br.s, 2H, CH_2-2''), 4.03 (br.s, 2H, CH_2-6), 2.18 (s, 3H, CH_3-8'''), 2.16 (s, 3H, CH_3-7'''); ^{13}C NMR: 168.74 (C-2'), 165.66 (C-5'), 164.37 (C-1''), 163.03 (C-2), 143.90 (C-4), 138.34 (C-1'''), 136.41 (C-3'''), 131.41(C-4'''), 129.62 (C-5'''), 120.35 (C-2'''), 116.68 (C-6'''), 103.12 (C-5), 36.64 (C-2''), 27.52 (C-6), 19.57 (C-7'''), 18.74 (C-8'''); EI MS: *m/z* 375 [M]$^+$, 301 [$C_{15}H_{15}N_3O_2S$]$^+$, 262 [$C_{12}H_{12}N_3O_2S$]$^+$, 235 [$C_{11}H_{11}N_2O_2S$]$^+$, 195 [$C_{10}H_{13}NOS$]$^+$, 163 [$C_{10}H_{13}NO$]$^+$, 141 [$C_5H_5N_2OS$]$^+$, 121 [$C_8H_{11}N$]$^+$, 114 [$C_4H_6N_2S$]$^+$.

2-({5-[(2-Amino-1,3-thiazol-4-yl)methyl]-1,3,4-oxadiazol-2-yl}sulfanyl)-*N*-(3,5-dimethylphenyl)acetamide (VIIe). Light brown amorphous solid; Yield: 82%; mp: 297–298°C; Mol. Formula: $C_{16}H_{17}N_5O_2S_2$; Mol. Mass: 375 g mol^{-1}; IR: 3367 (N–H stretching), 3040 (C–H of aromatic ring), 2940 (–CH_2 stretching), 1665 (C=O stretching), 1570 (C=C stretching of aromatic ring), 1530 (C=N stretching); ^{1}H NMR: 10.21 (s, 1H, N–H), 7.34 (br.s, 1H, H-2'''), 7.26 (br.d, J = 8.1 Hz, 1H, H-6'''), 7.06 (d, J = 8.1, H-4'''), 6.99 (br.s, 2H, –NH_2), 6.38 (s, 1H, H-5), 4.23 (br.s, 2H, CH_2-2''), 4.03 (br.s, 2H, CH_2-6), 2.18 (s, 3H, CH_3-8'''), 2.16 (s, 3H, CH_3-7'''); ^{13}C NMR: 168.75 (C-2'), 165.67 (C-5'), 164.39 (C-1''), 163.05 (C-2), 143.90 (C-4), 138.34 (C-1'''), 136.41 (C-3'''), 131.41 (C-4'''), 129.62 (C-5'''), 120.35 (C-2'''), 116.68 (C-6'''), 103.12 (C-5), 36.64 (C-2''), 27.52 (C-6), 19.57 (C-7'''), 18.74 (C-8'''); EI MS: *m/z* 375 [M]$^+$, 301 [[$C_{15}H_{15}N_3O_2S$]$^+$, 262 [$C_{12}H_{12}N_3O_2S$]$^+$, 235 [$C_{11}H_{11}N_2O_2S$]$^+$, 195 [$C_{10}H_{13}NOS$]$^+$, 163 [$C_{10}H_{13}NO$]$^+$, 141 [$C_5H_5N_2OS$]$^+$, 121 [$C_8H_{11}N$]$^+$, 114 [$C_4H_6N_2S$]$^+$.

2-({5-[(2-Amino-1,3-thiazol-4-yl)methyl]-1,3,4-oxadiazol-2-yl}sulfanyl)-*N*-phenylacetamide (VIIf). Brick brown amorphous solid; Yield: 87%; mp: 190–191°C; Mol. Formula: $C_{14}H_{13}N_5O_2S_2$; Mol. Mass: 347 g mol^{-1}; IR: 3350 (N–H stretching), 3052 (C–H of aromatic ring), 2923 (–CH_2 stretching), 1576 (C=C

stretching of aromatic ring), 1518 (C=N stretching); ^{1}H NMR: 10.37 (s, 1H, N–H), 7.56 (br.d, J = 8.0 Hz, 2H, H-2''' and H-6'''), 7.32 (br.t, J = 7.8 Hz, 2H, H-3''' and H-5'''), 7.07 (br.t, J = 7.2 Hz, 1H, H-4'''), 6.99 (s, 2H, $-NH_2$), 6.39 (s, 1H, H-5), 4.26 (s, 2H, CH_2-2''), 4.04 (s, 2H, CH_2-6); ^{13}C-NMR: 168.74 (C-2'), 165.69 (C-5'), 164.71 (C-1''), 163.05 (C-2), 143.90 (C-4), 138.61 (C-1'''), 128.70 (C-3''' and C-5'''), 123.64 (C-4'''), 119.15 (C-2''' and C-6'''), 103.13 (C-5), 36.67 (C-2''), 27.53 (C-6); EI MS: m/z 347 $[M]^+$, 273 $[C_{13}H_{11}N_3O_2S]^+$, 234 $[C_{13}H_{11}N_3O_2S]^+$, 207 $[C_9H_7N_2O_2S]^+$, 193 $[C_9H_9N_2OS]^+$, 141 $[C_5H_5N_2OS]^+$, 120 $[C_7H_6NO]^+$, 92 $[C_6H_6N]^+$.

2-({5-[(2-Amino-1,3-thiazol-4-yl)methyl]-1,3,4-oxadiazol-2-yl}sulfanyl)-*N*-(3-methylphenyl)acetamide (VIIg). Light brown solid; Yield: 87%; mp: 245–246°C; Mol. Formula: $C_{15}H_{15}N_5O_2S_2$; Mol. Mass: 361 g mol^{-1}; IR: 3370 (N–H stretching), 3050 (C–H of aromatic ring), 2930 ($-CH_2$ stretching), 1680 (C=O stretching), 1550 (C=C stretching of aromatic ring), 1545 (C=N stretching); ^{1}H NMR: 10.21 (s, 1H, N–H), 7.34 (br.s, 1H, H-2'''), 7.26 (br.d, J = 8.1 Hz, 1H, H-6'''), 7.06 (d, J = 8.1, H-5'''), 6.99 (br.s, 2H, $-NH_2$), 6.38 (s, 1H, H-5), 4.23 (br.s, 2H, CH_2-2''), 4.03 (br.s, 2H, CH_2-6), 2.18 (s, 3H, CH_3-8'''), 2.16 (s, 3H, CH_3-7'''); ^{13}C NMR: 168.74 (C-2'), 165.66 (C-5'), 164.77 (C-1''), 163.03 (C-2), 143.90 (C-4), 138.34 (C-1'''), 136.41 (C-3'''), 131.41 (C-4'''), 129.62 (C-5'''), 120.35 (C-2'''), 116.68 (C-6'''), 103.12 (C-5), 36.64 (C-2''), 27.52 (C-6), 19.57 (C-7'''), 18.74 (C-8'''); EI MS: m/z 361 $[M]^+$, 287 $[C_{14}H_{13}N_3O_2S]^+$, 248 $[C_{11}H_{10}N_3O_2S]^+$, 228 $[C_7H_8N_4OS_2]^+$, 221 $[C_{10}H_9N_2O_2S]^+$, 194 $[C_9H_{10}N_2OS]^+$, 181 $[C_9H_{11}NOS]$, 141 $[C_5H_5N_2OS]^+$, 114 $[C_4H_6N_2S]^+$, 91 $[C_7H_7]$.

2-({5-[(2-Amino-1,3-thiazol-4-yl)methyl]-1,3,4-oxadiazol-2-yl}sulfanyl)-*N*-(4-methylphenyl)acetamide (VIIh). Light brown amorphous solid; Yield: 80%; mp: 237–238°C; Mol. Formula: $C_{15}H_{15}N_5O_2S_2$; Mol. Mass.: 361 g mol^{-1}; IR: 3340 (N–H stretching), 3060 (C–H of aromatic ring), 2915 ($-CH_2-$ stretching), 1570 (C=C stretching of aromatic ring), 1540 (C=N stretching); ^{1}H NMR: 10.21 (s, 1H, N–H), 7.42 (d, J = 7.0 Hz, 2H, H-2''' and H-6'''), 7.12 (d, J = 7.0 Hz, 2H, H-3''' and H-5'''), 6.99 (br.s, 2H, $-NH_2$), 6.38 (s, 1H, H-5), 4.23 (br.s, 2H, CH_2-2''), 4.03 (br.s, 2H, CH_2-6), 2.21 (s, 3H, CH_3-7'''); ^{13}C NMR: 168.74 (C-2'), 165.66 (C-5'), 164.37 (C-1''), 163.03 (C-2), 143.90 (C-4), 136.39 (C-1'''), 132.10 (C-4'''), 129.05 (C-3''' and C-5'''), 119.06 (C-2''' and C-6'''), 103.12 (C-5), 36.64 (C-2''), 27.52 (C-6), 20.39 (C-7'''); EI MS: m/z 361 $[M]^+$, 287 $[C_{14}H_{13}N_3O_2S]^+$, 248 $[C_{11}H_{10}N_3O_2S]^+$, 228 $[C_7H_8N_4OS_2]^+$, 221 $[C_{10}H_9N_2O_2S]^+$, 194 $[C_9H_{10}N_2OS]^+$, 181 $[C_9H_{11}NOS]$, 141 $[C_5H_5N_2OS]^+$, 114 $[C_4H_6N_2S]^+$, 91 $[C_7H_7]$.

2-({5-[(2-Amino-1,3-thiazol-4-yl)methyl]-1,3,4-oxadiazol-2-yl}sulfanyl)-*N*-(2-ethyl-6-methylphenyl)-acetamide (VIIi). Light brown amorphous solid; Yield: 89%; mp: 237–238°C; Mol. Formula: $C_{17}H_{19}N_5O_2S_2$; Mol. Mass: 389 g mol^{-1}; IR: 3355 (N–H stretching), 3050 (C–H of aromatic ring), 2923 ($-CH_2$ stretching), 1570 (C=C stretching of aromatic ring), 1545 (C=N stretching); ^{1}H NMR: 7.31 (br.s, 1H, H-5'''), 7.04 (d, J = 7.68 Hz, 1H, H-3'''), 6.89 (s, 2H, $-NH_2$), 6.38 (br.d, J = 7.38 Hz, 1H, H-4'''), 6.27 (s, 1H, H-4), 4.26 (s, 2H, CH_2-2''), 4.04 (s, 2H, CH_2-6), 2.43 (q, J = 7.5, 2H, CH_2-7'''), 2.09 (s, 3H, CH_3-9'''), 1.06 (t, J = 7.5, 3H, CH_3-8'''); ^{13}C NMR: 168.75 (C-2'), 165.69 (C-5'), 164.71 (C-1''), 163.05 (C-2), 143.91 (C-4), 141.05 (C-1'''), 135.59 (C-6'''), 134.19 (C-2'''), 127.64 (C-4'''), 126.84 (C-3'''), 125.99 (C-5'''), 103.11 (C-5), 36.62 (C-2'') 27.54 (C-6), 24.25 (C-9'''), 17.99 (C-7'''), 14.63 (C-8'''); EI MS: m/z 389 $[M]^+$, 347 $[C_{16}H_{17}N_3O_2S]^+$, 276 $[C_{13}H_{14}N_3O_2S]^+$, 234 $[C_{13}H_{11}N_3O_2S]^+$, 209 $[C_{11}H_{15}NOS]^+$, 176 $[C_{11}H_{14}NO]^+$, 162 $[C_{10}H_{12}NO]^+$, 141 $[C_5H_5N_2OS]^+$, 134 $[C_9H_{12}N]^+$.

2-({5-[(2-Amino-1,3-thiazol-4-yl)methyl]-1,3,4-oxadiazol-2-yl}sulfanyl)-*N*-(4-ethyl-phenyl)acetamide (VIIj). Light brown amorphous solid; Yield: 84%; mp: 113–14°C; Mol. Formula: $C_{16}H_{17}N_5O_2S_2$; Mol. Mass: 375 g mol^{-1}; IR: 3345 (N–H stretching), 3050 (C–H of aromatic ring), 2940 ($-CH_2$ stretching), 1580 (C=C stretching of aromatic ring), 1518 (C=N stretching); ^{1}H NMR: 10.30 (s, 1H, HN–CO), 7.32 (d, J = 9.0 Hz, 2H, H-2''' & H-6'''), 6.82 (d, J = 9.0 Hz, 2H, H-3''' & H-5'''), 7.00 (br.s, 2H, $-NH_2$), 6.39 (s, 1H, H-5), 4.24 (s, 2H, CH_2-2''), 4.03 (s, 2H, CH_2-6), 2.52 (q, J = 7.1, 2H, CH_2-7'''), 1.15 (t, J = 7.1, 3H, CH_3-8'''); ^{13}C NMR: 168.74 (C-2'), 165.68 (C-5'), 164.44 (C-1''), 163.07 (C-2), 143.89 (C-4), 138.10 (C-1'''), 136.41 (C-4'''), 128.98 (C-3''' & C-5'''), 119.15 (C-2''' & 6'''), 103.12 (C-5), 37.01 (C-7'''), 27.55 (C-6), 27.52 (C-2''), 15.61 (C-8'''); EI MS: m/z 375 $[M]^+$, 301 $[[C_{15}H_{15}N_3O_2S]^+$, 262 $[C_{12}H_{12}N_3O_2S]^+$, 235 $[C_{11}H_{11}N_2O_2S]^+$, 195 $[C_{10}H_{13}NOS]^+$, 163 $[C_{10}H_{13}NO]^+$, 141 $[C_5H_5N_2OS]^+$, 121 $[C_8H_{11}N]^+$, 114 $[C_4H_6N_2S]^+$.

2-({5-[(2-Amino-1,3-thiazol-4-yl)methyl]-1,3,4-oxadiazol-2-yl}sulfanyl)-*N*-(2-ethyl-phenyl)acetamide (VIIk). Light brown amorphous solid; Yield: 81%; mp: 189–190°C; Mol. Formula: $C_{16}H_{17}N_5O_2S_2$; Mol. Mass: 375 g mol^{-1}; IR: 3350 (N–H stretching), 3052 (C–H of aromatic ring), 2923 ($-CH_2$ stretching), 1570 (C=C stretching of aromatic ring), 1540 (C=N stretching); ^{1}H NMR: 10.21 (s, 1H, N–H), 7.27 (br.d, J = 7.0 Hz, 1H, H-6'''), 7.18 (br.d, J = 7.5 Hz, 1H, H-3'''), 7.15–7.13 (m, 1H, H-5'''), 7.12–7.10 (m, 1H, H-4''') 6.99 (br.s, 2H, $-NH_2$), 6.38 (s, 1H, H-5), 4.23 (br.s, 2H, CH_2-2''), 4.03 (br.s, 2H, CH_2-6), 2.51 (q,

J = 7.3, 2H, CH_2-7'''), 1.14 (t, J = 7.3, 3H, CH_3-8'''); ^{13}C NMR: 168.74 (C-2'), 165.66 (C-5'), 164.37 (C-1''), 163.03 (C-2), 143.90 (C-4), 137.34 (C-1'''), 135.22 (C-2'''), 128.58 (C-3'''), 126.14 (C-5'''), 125.88 (C-4'''), 125.13 (C-6'''), 103.12 (C-5), 36.64 (C-2''), 27.56 (C-6), 24.68 (C-7'''), 14.96 (C-8'''); EI MS: m/z 375 $[M]^+$, 301 $[[C_{15}H_{15}N_3O_2S]^+$, 262 $[C_{12}H_{12}N_3O_2S]^+$, 235 $[C_{11}H_{11}N_2O_2S]^+$, 195 $[C_{10}H_{13}NOS]^+$, 163 $[C_{10}H_{13}NO]^+$, 141 $[C_5H_5N_2OS]^+$, 121 $[C_8H_{11}N]^+$, 114 $[C_4H_6N_2S]^+$.

2-({5-[(2-Amino-1,3-thiazol-4-yl)methyl]-1,3,4-oxadiazol-2-yl}sulfanyl)-*N*-(2-ethoxyphenyl)acetamide (VIIl). Light brown amorphous solid; Yield: 77%; mp: 181–182°C; Mol. Formula: $C_{16}H_{17}N_5O_3S_2$; m.m.: 391 g mol^{-1}; IR: 3350 (N–H stretching), 3052 (C–H of aromatic ring), 2923 (–CH_2– stretching), 1576 (C=C stretching of aromatic ring), 1518 (C=N stretching); ^{1}H NMR: 10.21 (s, 1H, N–H), 7.92 (d, J = 7.2 Hz, 1H, H-6'''), 7.05-7.03 (m, 2H, H-3''' and H-5'''), 6.99 (br.s, 2H, –NH_2), 6.87 (dt, J = 1.2, 7.4 Hz, 1H, H-4'''), 6.38 (s, 1H, H-5), 4.23 (br.s, 2H, CH_2-2''), 4.08 (q, J = 6.8 Hz, 2H, CH_2-7'''), 4.03 (br.s, 2H, CH_2-6), 1.38 (t, J = 6.8 Hz, 3H, CH_3-8'''); ^{13}C NMR: 168.77 (C-2'), 165.65 (C-5'), 164.35 (C-1''), 163.04 (C-2), 148.52 (C-2'''), 143.90 (C-4), 127.07 (C-1'''), 124.68 (C-4'''), 121.39 (C-6'''), 120.35 (C-5'''), 112.31 (C-3'''), 103.12 (C-5), 63.98 (C-7'''), 36.64 (C-2''), 27.52 (C-6), 14.49 (C-8'''); EI MS: m/z 391 $[M]^+$, 317 $[C_{15}H_{15}N_3O_3S]$, 278 $[C_{12}H_{12}N_3O_3S]$, 211 $[C_{10}H_{12}NO_2S]$, 114 $[C_4H_6N_2S]^+$.

2-({5-[(2-Amino-1,3-thiazol-4-yl)methyl]-1,3,4-oxadiazol-2-yl}sulfanyl)-*N*-(2-methoxycarbonylphenyl)acetamide (VIIm). Light brown amorphous solid; Yield: 85%; mp: 243–244°C; Mol. Formula: $C_{16}H_{15}N_5O_4S_2$; Mol. Mass: 405 g mol^{-1}; IR: 3380 (N–H stretching), 3065 (C–H of aromatic ring), 2950 (–CH_2 stretching), 1570 (C=C stretching of aromatic ring), 1540 (C=N stretching); ^{1}H NMR: 11.04 (s, 1H, N–H), 8.23 (br.d, J = 8.2, 1H, H-3'''), 7.92 (dist. d, J = 7.1, 1H, H-4'''), 7.62 (br.t, J = 7.9, 1H, H-5'''), 7.23 (br.d, J = 7.7, 1H, H-6'''), 6.98 (2H, –NH_2), 6.38 (s, 1H, H-5), 4.27 (s, 2H, CH_2-2''), 4.05 (s, 2H, CH_2-6), 3.83 (s, 3H, CH_3O-8'''); ^{13}C NMR: 168.74 (C-2'), 167.25 (C-7'''), 165.85 (C-5'), 165.30 (C-1''), 162.70 (C-2), 143.85 (C-4), 138.83 (C-1'''), 133.84 (C-5'''), 130.57 (C-3'''), 123.80 (C-4'''), 121.31 (C-6'''), 118.17 (C-2'''), 52.43 (C-8'''), 36.48 (C-2'') 27.53 (C-6); EI MS: m/z 405 $[M]^+$, 265 $[C_{11}H_9N_2O_4S]$, 228 $[C_7H_8N_4OS_2]$, 193 $[C_{10}H_{11}NO_3]$, 114 $[C_4H_6N_2S]^+$.

α-*Glucosidase Inhibition Assay*

The α-glucosidase inhibition activity was performed according to the slightly modified method [25]. Total volume of the reaction mixture was made 100 μL containing 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 minutes of incubation at 37°C, absorbance was measured at 400 nm using Synergy HT microplate reader. All experiments were carried out in triplicates. The percent inhibition was calculated by the help of following formula:

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

Control = Total enzyme activity without inhibitor.

Test = Activity in the presence of test compound.

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

Molecular Docking

To predict the bioactive conformations, various ligands were docked into the binding pockets of the enzymes by using the default parameters of MOE-Dock program.

Ligands preparation. The three dimensional (3D) structures of synthesized compounds were made by using ChemDraw Ultra 12.0 (Cambridge Soft, 2001) and saved in MDL Mol file format which were then opened in Molecular Operating Environment (MOE 2009–2010). The energies of the compounds were minimized by using the default parameter of MOE energy minimization algorithm (gradients: 0.05, force field: MMFF94X). Database was created in which all the compounds were saved in the mdb file format for the next step of docking.

Receptor protein preparation. The 3D structures of receptor protein molecules of yeast α-glucosidase (PDB Code: 3NO4) were retrieved from Protein Data Bank. All water molecules were released from the receptor proteins and 3D protonation was carried out by using Protonate 3D Option [26]. Protein molecules were energy minimized by using the default parameters of MOE 2009–2010 energy minimization algorithm (gradient: 0.05, Force Field: MMFF94X). By using default parameters of MOE-Dock Program, all the compounds were docked into binding pockets of the above proteins. Re-docking procedure was also used to increase the validity of docking protocol [27].

Cytotoxicity Assay

The cytotoxicity was studied by the brine-shrimp cytotoxic assay method [28]. 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 × 32 cm)

under constant aeration for 48 hours 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^{-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 hours, the number of surviving shrimp 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

The targeted series of bi-heterocyclic compounds was synthesized in good yields by a convergent strategy and most of the compounds exhibited potent enzyme inhibitory potential against α-glucosidase and also possessed very mild cytotoxicity. Therefore, these molecules can be utilized as safe anti-diabetic agents in drug discovery and designing program.

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COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any studies involving animals or human participants performed by any of the authors.

Conflict of Interests

The authors declare that they have no conflict of interests.

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