Gangadhara Seelolla and Venkateswarlu Ponneri*

Department of Chemistry, Sri Venkateswara University, Tirupati 517502, Andhra Pradesh, India
*E-mail: ponneri.chem@gmail.com

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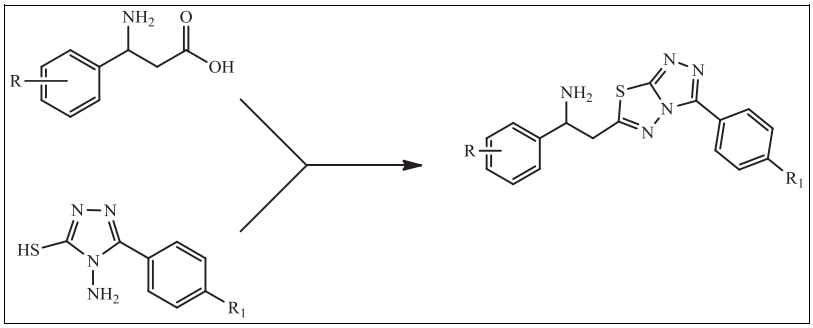

In the present work, we synthesized a series of [1,2,4]triazolo[3,4-b][1,3,4]thiadiazole derivatives (**6a–f** and **7a–f**) by using simple starting materials, namely, β-amino acids and different aromatic acid hydrazides. The newly synthesized compounds were characterized by mass, IR, ^{1}H, and^{13}C-NMR spectral data analysis. The newly synthesized compounds were tested for their antimicrobial activities and antioxidant properties. Compound **6c** was a potent microbial agent particularly against *Staphylococcus aureus* (MIC 3.12 μg/mL) and *Candida albicans* (MIC 6.25 μg/mL) when compared with the reference drugs ciprofloxacin and fluconazole, respectively. The antioxidant activity of the synthesized compounds was also evaluated by 1,1-diphenyl-2-picryl hydrazyl, nitric oxide, and hydrogen peroxide radical scavenging methods. Compounds **6c**, **6f, 7c,** and **7f** showed good radical scavenging activity due to the presence of electron-donating group on phenyl ring.

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INTRODUCTION

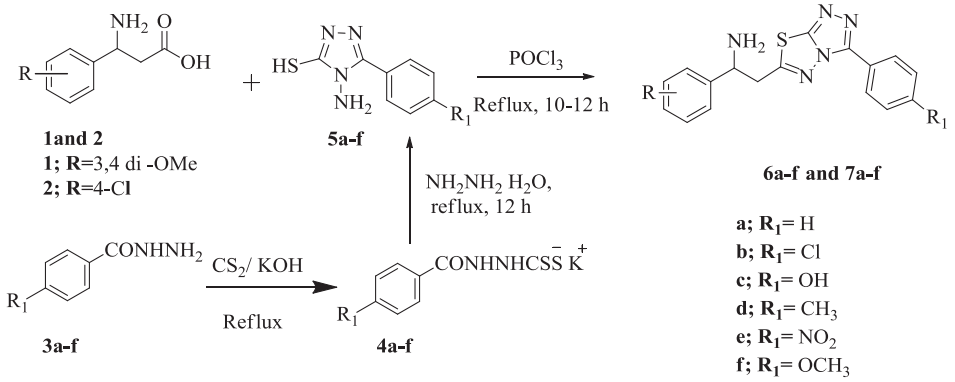
The high resistance acquired by microbes against the antimicrobial drugs existing in the market is of a great challenge to the scientific organization, involved in the development of novel and effective drugs against human diseases. It is, therefore, necessary to develop therapeutic agents with improved potential for treating broad spectrum microbial infections. For the last few decades, there has been a tremendous growth of research in the synthesis of nitrogen and sulfur containing heterocyclic derivatives because of their utility in various pharmaceuticals applications.

1,2,4-Triazole derivatives are an important group of heterocyclic compounds and have been the subject of extensive study in the recent years [1]. It has been reported in the literature that certain compounds bearing 1,2,4-triazole nucleus possess significant antimicrobial activities [2–4]. Recently, triazole–thiadiazole-fused compounds have been frequently found to display a broad spectrum of biological activities [5,6]. Further, triazolo–hiadiazole system may be viewed as a cyclic analog of two important components, namely, thiosemicarbazide and biguanide [7,8], which often displays diverse biological activities including antimicrobial [9,10], antifungal [11], antiinflammatory [12], anticancer [13], analgesic [14], and antitumor [15] activities in continuation to our efforts directed toward the synthesis of heterocyclic compounds containing nitrogen, sulfur, and bicyclic systems with anticipated antibacterial activities [9,10,16].

In addition, β-amino acids (BAA) are not as common in nature as their α-analogs. Some of the BAA were found in more complex structures such as peptides, dipeptides, lactones, alkaloids, and other natural products, and in free form, they show interesting pharmacological effects. These are currently of growing interest not only because of their natural roles but also because of their use in the synthesis of peptide mimetics and certain quite new biologically active substances [17]. BAA is a constituent of some toxins and a special class of pore-forming lipopeptides, which exhibit antibacterial and antifungal activities [18]. Peptidomimetics containing BAA represent one of the strategies in developing therapeutic candidates with increased oral bioavailability and resistance to metabolic degradation [19]. Recently, the chemistry of hybrid heterocyclic derivatives has received considerable attention owing to their synthetic and effective biological importance.

By finding the antimicrobial and antioxidant activities of heterocyclic compounds, we decided to synthesis of some fused triazol thiadizol derivatives such as 1-(3,4-dimethoxyphenyl)-2-(3-(para-substitutedphenyl)[1,2,4]triazolo[3,4-b][1,3,4] thiadiazol-6-yl)ethnamine derivatives and 1-(4-chlorophenyl)-2-(3-(para-substitutedphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6yl)ethanamine derivatives and to evaluate their antimicrobial activities and antioxidant properties.

Scheme 1



Compound	R	R_1
6a	3,4 di-OMe	H
6b		Cl
6c		OH
6d		CH_3
6e		NO_2
6f		OCH_3
7a	4-Cl	H
7b		Cl
7c		OH
7d		CH_3
7e		NO_2
7f		OCH_3

RESULTS AND DISCUSSION

Chemistry. The general synthetic pathway for the preparation of [1,2,4]triazolo[3,4-b][1,3,4] thiadiazole derivatives is depicted in Scheme 1. Compounds **5a–f** were prepared according to a methodology [20,21] involving condensation of different para-substituted benzoic acid hydrazides (**3a–f**) with carbon disulfide and potassium hydroxide under reflux condition that gave the potassium dithiocarbazate salt (**4a–f**). Potassium dithio carbazate salt reaction with hydrazine hydrate under reflux condition to produce the amino thiols (**5a–f**). Finally, [1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles were prepared by cyclic condensation of the SH and NH_2 functions of **5a–f** compounds with aromatic carboxylic acids **1** and **2** in the presence of phosphorous oxychloride ($POCl_3$), under reflux condition that afforded compounds **6a–f** and **7a–f** with good yield.

The structures of the newly synthesized compounds were established on the basis of their mass, IR, ^{1}H, and ^{13}C-NMR spectral data. The $(M+H)^+$ molecular ion peak of all compounds was confirmed by their molecular weights. The IR spectra of **6a–f** and **7a–f** compounds which showed characteristic absorption band in the range between 1062 and 1138 cm^{-1} attributed to N=C–S–C=N vibrations, which were indicating the formation of 1,3,4-thiadiazole. The other absorptions band appear in the range between 1551–1651 cm^{-1} and 3338–3392 cm^{-1} for C=N and N–H bonds, respectively. ^{1}H-NMR spectra of compounds **6a–f** and **7a–f** showed the signals in the region of δ 3.35–4.35 and 4.25–5.85 ppm that correspond to chiral –CH group and $–NH_2$, respectively. ^{13}C-NMR further confirmed the structures of the desired products.

Antimicrobial activity. A broad panel of microbes was used for testing *in vitro* antibacterial and antifungal properties of the synthesized molecules. The results obtained were reproduced in Table 1 as MIC values. The antimicrobial values showed that most of the synthesized derivatives exhibited excellent activities against different bacterial strains and moderate activity against fungal strains. Ciprofloxacin and fluconazole were used as the standard drugs for antimicrobial and antifungal testing, respectively. When ciprofloxacin was used as a standard drug for testing the MIC values against the Gram-positive bacterial strains, it exhibited MIC values of 6.25 and 12.5 μg/mL against *Staphylococcus aureus* and *Bacillus subtilis,* respectively. The MIC values of the standard ciprofloxacin were found to be 6.25 μg/mL against Gram-negative bacterial strains (*Klebsiella pneumoniae* and

Table 1

Antimicrobial activity (MIC profiles) of the synthesized compounds (**6a–f** and **7a–f**).

	Minimum inhibitory concentration (MIC) (μg/mL)					
	Antibacterial activity					
	Gram-positive bacteria		Gram-negative bacteria		Antifungal activity	
Compound	*Staphylococcus aureus*	*Bacillus subtilis*	*Klebsiella pneumoniae*	*Escherichia coli*	*Candida albicans*	*Aspergillus flavus*
6a	12.5	12.5	25	12.5	12.5	50
6b	25	25	25	50	–	–
6c	3.12	6.25	6.25	3.12	6.25	50
6d	6.25	6.25	12.5	12.5	25	50
6e	50	50	100	–	>100	–
6f	6.25	6.25	3.12	6.25	12.5	12.5
7a	12.5	100	12.5	6.25	50	–
7b	50	50	25	50	–	50
7c	3.12	12.5	3.12	3.12	6.25	6.25
7d	6.25	50	12.5	12.5	50	>100
7e	>100	>100	50	–	–	–
7f	6.25	6.25	6.25	3.12	12.5	25
Ciprofloxacin	6.25	12.5	6.25	6.25	–	–
Fluconazole	–	–	–	–	12.5	12.5

(–), No activity.

Escherichia coli). The standard drug fluconazole displayed an MIC value of 12.5 μg/mL against fungal strains *Candida albicans* and *Aspergillus flavus*.

Antibacterial activity. All the newly synthesized compounds (**6a–f** and **7a–f**) were screened for their *in vitro* antimicrobial activity determined by well plate method [22,23]. All compounds were screened against Gram-positive bacterial strains such as *S. aureus* and *B, subtilis* and Gram-negative bacterial strains such as *K. pneumoniae* and *E. coli*. Compounds **6c** and **7c** exhibited excellent activity with the value of MIC 3.12 μg/mL against the Gram-positive bacteria strain *S. aureus*. Compounds **6d**, **6f**, **7d,** and **7f** showed an MIC value of 6.25 μg/mL, which is equivalent to the standard ciprofloxacin. The remaining compounds **6a**, **6b**, **6e**, **7a**, **7b,** and **7e** showed low activity (≥25 μg/mL) when compared with the standard. *B. subtilis* was introduced as another Gram-positive strain for conducting the antibacterial test, it was found that **6b**, **6e**, **7a**, **7b**, **7d,** and **7e** compounds exhibited poor activity compared with the standard. The derivatives **6c**, **6d**, **6f,** and **7f** showed excellent activity with the value of MIC 6.25 μg/mL against *B. subtilis*. The remaining compounds **6a** and **7c** showed an MIC value of 12.5 μg/mL, which was equivalent to the standard ciprofloxacin. All prepared compounds were tested for their antibacterial property against Gram-negative bacterial strains such as *K. pneumoniae* and *E. coli*. When compounds were exposed against Gram-negative bacteria *K. pneumoniae*, it was observed that compounds **6f** and **7c** exhibited excellent activity with the value of MIC 3.12 μg/mL compared with the standard drug ciprofloxacin. Other derivatives **6c** and **7f** also showed good MIC value of 6.25 μg/mL, equivalent to the standard ciprofloxacin. The remaining compounds **6a**, **6b**, **6d**, **6e**, **7a**, **7b**, **7d,** and **7e** were found to exhibit low activity. When *E. coli* was introduced as another Gram-negative strain for conducting the antibacterial test, it was found that **6c**, **7c,** and **7f** compounds exhibited better activity (3.12 μg/mL) compared with the standard. The remaining compounds **6f** and **7a** showed an MIC value (6.25 μg/mL) equivalent to that reported by the standard ciprofloxacin. The remaining compounds **6a**, **6b**, **6d,** and **7b** were found to exhibit low activity, and **6e** and **7e** did not showed any activity compared with the standard.

Antifungal activity. The synthesized molecules were tested against *C. albicans* and *A. flavus* for their antifungal activities. It was found that compared with the antibacterial activity, very few derivatives exhibited good activity. When molecules **6a–f** and **7a–f** were tested against *C. albicans*, derivatives **6c** and **7c** showed good activity (6.25 μg/mL) compared with the standard fluconazole. A few molecules **6a**, **6f,** and **7f** exhibited an MIC value (12.5 μg/mL) equivalent to that of the standard drug fluconazole. The remaining molecules **6d**, **6e**, **7a,** and **7d** showed less activity when compared with the standard drug. Further, *A. flavus* was also used as a fungal strain for checking the antifungal potency of the derived bioactive compounds. It was observed that one of the compounds **7c** showed good activity (6.25 μg/mL) and

another compound **6f** showed good activity equivalent to the standard (12.5 μg/mL). The remaining synthesized compounds displayed poor activity.

Antioxidant testing. Compounds **6a–f** and **7a–f** were tested for their antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, nitric oxide (NO), and hydrogen peroxide (H_2O_2) [24–28] methods. The obtained results were summarized in Tables 2–4. All synthesized compounds were tested for their antioxidant activities compared with the standard ascorbic acid by DPPH method. Among the synthesized compounds, **6f**, **6c**, **7c,** and **7f** were found to have moderate antioxidant activities when compared with the standard (ascorbic acid 15.57 ± 0.01) with the IC_{50} values of 16.83 ± 0.02, 17.29 ± 0.03, 17.25 ± 0.02, and 17.53 ± 0.01, respectively (Table 2). The remaining compounds **6a**, **6b**, **6d**, **6e**, **7a**, **7b**, **7d,** and **7e** showed moderate activity. Compounds **6c** and **7c** displayed greater NO radical scavenging activity with the IC_{50} values of 16.81 ± 0.04 and 16.99 ± 0.08, respectively, compared with standard ascorbic acid (16.61 ± 0.02). The remaining compounds **6f** and **7f** showed moderate activities of 17.26 ± 0.02 and 17.33 ± 0.06, respectively (Table 3). All the other derivatives were found to exhibit less activity. The good scavenging effect by H_2O_2 was detected in compounds **6c**, **6f**, **7c,** and **7f**

Table 2

The *in vitro* antioxidant activity of **6a–f** and **7a–f** in DPPH method.

	Concentration (μg/mL)				
Compound	25	50	75	100	IC_{50}
6a	60.19 ± 0.84	61.39 ± 0.72	62.46 ± 0.63	63.09 ± 0.94	20.76 ± 0.30
6b	59.25 ± 0.27	60.09 ± 0.93	61.18 ± 0.07	62.04 ± 0.97	21.09 ± 0.93
6c	78.28 ± 0.23	73.42 ± 0.19	74.16 ± 0.14	75.24 ± 0.78	17.29 ± 0.03
6d	62.16 ± 0.99	63.10 ± 0.95	64.24 ± 0.96	65.28 ± 0.82	20.10 ± 1.05
6e	58.34 ± 0.64	59.08 ± 0.96	60.19 ± 1.08	61.07 ± 0.98	21.42 ± 0.33
6f	74.24 ± 0.07	75.28 ± 0.07	71.86 ± 0.03	77.38 ± 0.10	16.83 ± 0.02
7a	60.46 ± 0.10	61.09 ± 0.06	62.29 ± 0.89	63.07 ± 1.06	20.67 ± 0.18
7b	61.36 ± 0.08	62.29 ± 0.07	63.46 ± 0.04	64.49 ± 1.26	20.37 ± 1.03
7c	72.46 ± 0.06	73.28 ± 0.03	74.24 ± 0.06	75.29 ± 0.02	17.25 ± 0.02
7d	62.40 ± 0.30	63.09 ± 0.92	64.23 ± 0.12	65.10 ± 0.90	20.03 ± 0.97
7e	57.20 ± 0.85	59.26 ± 0.08	60.16 ± 0.92	61.07 ± 1.04	21.85 ± 0.47
7f	71.28 ± 0.05	72.14 ± 0.03	73.46 ± 0.01	74.29 ± 0.03	17.53 ± 0.01
Ascorbic acid	80.28 ± 0.03	81.16 ± 0.02	82.25 ± 0.01	83.20 ± 0.05	15.57 ± 0.01
Blank	–	–	–	–	–

(−), Showed no scavenging activity. Values were the means of three replicates ± SD.

Table 3

The *in vitro* antioxidant activity of **6a–f** and **7a–f** in NO method.

	Concentration (μg/mL)				
Compound	25	50	75	100	IC_{50}
6a	60.38 ± 0.84	62.13 ± 0.88	64.36 ± 0.34	65.19 ± 0.98	20.70 ± 0.70
6b	60.27 ± 1.02	61.21 ± 0.17	63.52 ± 0.47	65.91 ± 0.21	20.74 ± 0.76
6c	74.36 ± 0.10	75.21 ± 0.09	76.45 ± 0.05	78.16 ± 0.09	16.81 ± 0.04
6d	61.42 ± 0.52	63.45 ± 0.35	64.72 ± 0.43	67.17 ± 0.90	20.35 ± 0.61
6e	60.24 ± 0.86	60.78 ± 0.43	62.46 ± 1.11	63.59 ± 0.54	20.75 ± 0.90
6f	72.39 ± 0.52	74.25 ± 0.13	76.19 ± 0.09	77.13 ± 0.03	17.26 ± 0.02
7a	61.27 ± 1.07	62.10 ± 0.95	64.27 ± 0.19	66.19 ± 0.10	20.40 ± 0.35
7b	60.46 ± 0.46	61.68 ± 0.90	63.17 ± 0.93	64.76 ± 1.07	20.67 ± 0.48
7c	73.56 ± 0.05	74.52 ± 0.04	76.48 ± 0.30	78.29 ± 0.06	16.99 ± 0.08
7d	63.46 ± 0.33	63.91 ± 0.41	64.68 ± 1.08	65.27 ± 1.02	19.69 ± 0.96
7e	60.24 ± 0.86	60.78 ± 0.43	62.46 ± 1.11	63.59 ± 0.54	20.75 ± 0.90
7f	72.12 ± 0.08	7.164 ± 0.06	75.44 ± 0.22	78.10 ± 0.04	17.33 ± 0.06
Ascorbic acid	75.22 ± 0.05	78.26 ± 0.05	80.44 ± 0.02	81.26 ± 0.03	16.61 ± 0.02
Blank	–	–	–	–	–

(−), Showed no scavenging activity. Values were the means of three replicates ± SD.

Table 4

The *in vitro* antioxidant activity of **6a–f** and **7a–f** in H_2O_2 method.

	Concentration (μg/mL)				
Compound	25	50	75	100	IC_{50}
6a	59.33 ± 0.09	62.25 ± 0.87	64.37 ± 0.81	66.19 ± 0.94	21.06 ± 0.98
6b	56.44 ± 0.66	57.32 ± 0.85	59.14 ± 0.96	61.56 ± 1.15	22.14 ± 0.91
6c	73.29 ± 0.02	74.16 ± 0.06	75.42 ± 0.07	76.33 ± 0.24	17.05 ± 0.04
6d	60.43 ± 0.18	61.29 ± 1.07	62.34 ± 0.96	66.29 ± 0.18	20.68 ± 0.48
6e	57.14 ± 0.96	60.15 ± 0.87	62.46 ± 0.26	64.59 ± 0.36	21.87 ± 1.10
6f	72.16 ± 0.06	74.19 ± 0.04	77.35 ± 0.01	80.25 ± 0.03	17.32 ± 0.03
7a	60.15 ± 0.87	62.48 ± 0.92	64.62 ± 0.36	65.29 ± 0.90	20.78 ± 0.55
7b	59.19 ± 0.98	62.08 ± 0.96	64.16 ± 0.05	65.29 ± 0.87	21.11 ± 0.26
7c	72.42 ± 0.04	74.62 ± 0.02	76.48 ± 0.08	78.21 ± 0.02	17.26 ± 0.02
7d	61.28 ± 0.23	64.12 ± 0.93	65.26 ± 1.04	67.48 ± 0.20	20.39 ± 0.35
7e	58.32 ± 0.90	59.16 ± 0.84	60.29 ± 0.21	64.16 ± 0.88	21.43 ± 0.55
7f	74.31 ± 0.03	76.18 ± 0.04	78.29 ± 0.06	80.33 ± 0.02	16.82 ± 0.03
Ascorbic acid	76.27 ± 0.07	78.44 ± 0.04	80.32 ± 0.04	83.46 ± 0.03	16.38 ± 0.02
Blank	–	–	–	–	–

(−), Showed no scavenging activity. Values were the means of three replicates ± SD.

Table 5

Analytical data of synthesized compounds (**6a–f** and **7a–f**).

			Elemental analysis						
			Calculated (%)			Found (%)			
S. No	Molecular formula	Melting point	C	H	N	C	H	N	Mass M + 1
6a	$C_{19}H_{19}N_5SO_2$	183-185	59.82	5.02	18.36	59.73	5.00	18.30	382
6b	$C_{19}H_{18}ClN_5SO_2$	178-180	54.87	4.36	16.84	54.80	4.33	16.78	416
6c	$C_{19}H_{19}N_5SO_3$	193-195	57.42	4.82	17.62	57.35	4.80	17.57	398
6d	$C_{20}H_{21}N_5SO_2$	181-183	60.74	5.35	17.71	60.68	5.31	17.65	396
6e	$C_{19}H_{18}N_6SO_4$	213-215	53.51	4.25	19.71	53.46	4.22	19.65	427
6f	$C_{20}H_{21}N_5SO_3$	198-200	58.38	5.14	17.02	58.30	5.12	17.96	412
7a	$C_{17}H_{14}ClN_5S$	164-166	57.38	3.97	19.68	57.32	3.94	19.60	356
7b	$C_{17}H_{13}Cl_2N_5S$	179-181	52.32	3.36	17.94	52.27	3.34	17.86	390
7c	$C_{17}H_{14}ClN_5SO$	161-163	54.91	3.79	18.83	54.83	3.75	18.75	372
7d	$C_{18}H_{16}ClN_5S$	163-165	58.45	4.36	18.93	58.39	4.34	18.88	370
7e	$C_{17}H_{13}ClN_6SO_2$	185-187	50.94	3.27	20.97	50.88	3.25	20.91	401
7f	$C_{18}H_{16}ClN_5SO$	163-165	56.03	4.18	18.15	55.94	4.15	18.15	386

shown as 17.05 ± 0.04, 17.32 ± 0.03, 17.26 ± 0.02, and 16.82 ± 0.03, respectively, (Table 4). Analytical data of the target compounds also were displayed in Table 5.

Structure activity relationship study helped to reveal the effect of different substituents on the microbial strains, depending upon the different electronic environments developed on the aromatic ring by substituting both electron-withdrawing and electron-donating groups. It was observed that compounds **6c** (4–OH), **6f** (4–OCH_3), **7c** (4–OH), and **7f** (4–OCH_3) with electronic-donating groups executed good antifungal results, displaying much lesser MIC values than the standard drugs. The remaining compounds, namely, **6b** (4–Cl), **6e** (4–NO_2), **7b** (4–Cl), and **7e** (4–NO_2) showed lesser activity compared with the standard due to the presence of electron-withdrawing group on the aromatic ring.

CONCLUSION

In the conclusion of this work, a new series of [1,2,4] triazolo[3,4-b][1,3,4]thiadiazole was synthesized from simple starting materials, namely, β-amino acids and para-substituted aromatic acid hydrazides. The newly synthesized compounds were screened for their antimicrobial and antioxidant activity studies. The investigation of antioxidant activity screening data reveals that, among all compounds, only few of them exhibited good activity. Compounds **6c, 6f, 7c,** and **7f** showed an excellent, almost equivalent to that of the standard, the rest of the compounds showed moderate to mild inhibition activity. The presence of electron-donating substituent on the phenyl ring enhances the activity, whereas electron-withdrawing group decreases the activity.

EXPERIMENTAL

Chemistry. Melting points were determined in open capillaries on a Mel-Temp apparatus and are uncorrected. All reactions were monitored by TLC on precoated silica gel 60 F254 (mesh); spots were visualized with UV light. Merck (Merck KGaA, Darmstadt, Germany) silica gel (60–120 mesh) was used for column chromatography. The IR spectra were recorded on a Perkin Elmer (Waltham, MA) BX1 FTIR Spectrophotometer using KBr pellets, and the wave numbers were given in cm^{-1}. ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker (Bruker GmbH, Karlsruhe, Germany) AMX 400 MHz NMR spectrometer in $CDCl_3$/DMSO-d_6 solution using TMS as an internal standard. All chemical shifts were reported in δ (ppm) using TMS as an internal standard. The mass spectra were recorded on Agilent 1100 LC/MSD instrument (Agilent, Japan) with method API–ES at 70 eV. The microanalyses were performed on a Perkin Elmer 240C elemental analyzer. The antioxidant property was carried out by using Shimadzu UV-2450 s spectrophotometer.

General procedure for the synthesis of 1-(3,4-dimethoxyphenyl)-2-(3-(para-substituted phenyl)-[1,2,4]triazolo[3,4-b] [1,3,4] thiadiazol-6-yl)ethanamine (6a–f). An equimolar mixture of 3-amino-3-(3,4-dimethoxyphenyl) propanoic acid with 4-amino-5-phenyl-4H-[1,2,4]triazole-3-thiol (a)/4-amino-5-(4-chlorophenyl)-4H[1,2,4] triazole-3-thiol (b)/4-(4-amino-5-mercapto-4H-[1,2,4] triazol-3-yl)-phenol (c)/4-amino-5-p-tolyl-4H-[1,2,4]triazole-3-thiol (d)/4-amino-5-(4-nitrophenyl)-4H-[1,2,4]-triazole-3-thiol (e)/ 4-amino-5-(4-methoxyphenyl)-4H-[1,2,4]-triazole-3-thiol (f) in $POCl_3$ (7 mL) was refluxed over a steam bath for 10–12 h. The progress of reaction was monitored by TLC. The excess $POCl_3$ was removed under reduced pressure, and the residue was poured in to crushed ice. The resulting precipitate was filtered, washed with saturated sodium bicarbonate solution and then with water. It was dried and recrystallized from ethanol.

1-(3,4-Dimethoxyphenyl)-2-(3-phenyl-[1,2,4]triazolo[3,4-b] [1,3,4]thiadiazol-6-yl)ethanamine (6a). Pale yellow solid in 67%; mp: 183–185°C; IR (KBr, υ cm^{-1}): 3350 (N–H), 2937 (C–H), 1579 (C=N), 1230 (N–N=C), 1084 (N=C–S–C=N), 695 (C–S–C); ^1H–NMR (DMSO-*d6*) *δ*: 8.12 (d, 2H, –ArH, *J* = 8.0 Hz), 7.60 (d, 2H, –ArH, *J* = 8.0 Hz), 7.32 (t, 1H, –ArH, *J* = 8.0 Hz), 6.87 (s, 1H, –ArH, *J* = 8.0 Hz), 6.63 (d, 1H, –ArH, *J* = 8.0 Hz), 6.56 (dd, 1H, –ArH, *J* = 4.0 Hz), 4.75 (s, 2H, $–NH_2$), 4.05 (t, 1H, –CH), 3.78 (s, 6H, $–OCH_3$), 3.09 (d, 2H, $–CH_2$); ^{13}C–NMR (DMSO-*d6*) δ (ppm): 164.2, 163.5, 151, 147.3, 144.2, 141., 132.9, 127.4, 127.1, 126.2, 122.9, 115.4, 111.3, 55.9, 55.6, 43; MS: *m/z* 382 $(M+H)^+$ for $C_{19}H_{19}N_5O_2S$.

2-(3-(4-Chlorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)-1-(3,4dimethoxy-phenyl)ethanamine (6b). Pale brown solid in 69%; mp: 178–180°C; IR (KBr, υ cm^{-1}): 3365 (N–H), 2996 (C–H), 1590 (C=N), 1262 (N–N=C), 1082 (N=C–S–C=N), 683 (C–S–C); ^1H–NMR (DMSO-*d6*) *δ*: 8.17 (d, 2H, –ArH, *J* = 8.0 Hz), 7.52 (d, 2H, –ArH, *J* = 8.0 Hz), 6.94 (s, 1H, –ArH, *J* = 8.0 Hz), 6.61 (d, 1H, –ArH, *J* = 8.0 Hz), 6.43 (dd, 1H, –ArH, *J* = 4.0 Hz), 5.65 (s, 2H, $–NH_2$), 4.35 (t, 1H, –CH), 3.63 (s, 6H, $–OCH_3$), 3.26 (d, 2H, $–CH_2$);^{13}C–NMR (DMSO-*d6*) δ (ppm): 167.2, 153.5, 151, 149.1, 144.5, 141.4, 134.9, 129.2, 127.4, 127.2, 124.4, 122.9, 115.6, 55.6, 55.2, 44.2; MS: *m/z* 416 $(M+H)^+$ for $C_{19}H_{18}ClN_5O_2S$.

4-(6-(2-Amino-2-(3,4-dimethoxyphenyl)ethyl)-[1,2,4]triazolo [3,4-b][1,3,4]thiadiazol-3-yl)phenol (6c). Yellowish brown solid in 72%; mp: 193–195°C; IR (KBr, υ cm^{-1}): 3345 (N–H), 2986 (C–H), 1580 (C=N), 1252 (N–N=C), 1062 (N=C–S–C=N), 710 (C–S–C); ^1H–NMR (DMSO-*d6*) *δ*: 7.69 (d, 2H, –ArH, *J* = 8.0 Hz), 6.95 (d, 2H, –ArH, *J* = 8.0 Hz), 7.34 (s, 1H, –ArH, *J* = 8.0 Hz), 6.73 (d, 1H, –ArH, *J* = 8.0 Hz), 6.45 (dd, 1H, –ArH, *J* = 4.0 Hz), 8.75 (s, 1H, –OH), 4.45 (s, 2H, $–NH_2$), 4.35 (t, 1H, –CH), 3.48 (s, 6H, $–OCH_3$), 3.10 (d, 2H, $–CH_2$); ^{13}C–NMR (DMSO-*d6*) δ (ppm): 164.7, 164.2, 157.3, 152.3, 146.2, 144.5, 134.2, 132.1, 126.2, 124.3, 118.4, 112.2, 109.3, 55.6, 55.5, 42.1; MS: *m/z* 398 $(M+H)^+$ for $C_{19}H_{19}N_5O_3S$.

1-(3,4-Dimethoxyphenyl)-2-(3-(p-tolyl)-[1,2,4]triazolo[3,4-b] [1,3,4]thiadiazol-6-yl)ethanamine (6d). Ash color solid in 68%; mp: 181–183°C; IR (KBr, υ cm^{-1}): 3361 (N–H), 2922 (C–H), 1593 (C=N), 1264 (N–N=C), 1083 (N=C–S–C=N), 715 (C–S–C); ^1H–NMR (DMSO-*d6*) *δ*: 8.09 (d, 2H, –ArH, *J* = 8.0 Hz), 7.78 (d, 2H, –ArH, *J* = 8.0 Hz), 6.87 (s, 1H, –ArH, *J* = 8.0 Hz), 6.36 (d, 1H, –ArH, *J* = 8.0 Hz), 6.30 (dd, 1H, –ArH, *J* = 4.0 Hz), 4.96 (s, 2H, $–NH_2$), 4.35 (t, 1H, –CH), 3.98 (s, 6H, $–OCH_3$), 3.04 (d, 2H, $–CH_2$), 2.45 (s, 3H, $–CH_3$); ^{13}C–NMR (DMSO-*d6*) δ (ppm): 164.1, 163.8, 150.4, 148.6, 146.2, 135.5, 130.5, 128.9, 126.7, 124.5, 120.6, 116.2, 107.2, 59.1, 55.7, 41.8, 22.6; MS: *m/z* 396 $(M+H)^+$ for $C_{20}H_{21}N_5O_2S$.

1-(3,4-Dimethoxyphenyl)-2-(3-(4-nitrophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)ethanamine (6e). Yellowish brown solid in 68%; mp: 213–215°C; IR (KBr, υ cm^{-1}): 3362 (N–H), 2933 (C–H), 1598 (C=N), 1263(N–N=C), 1072 (N=C–S–C=N), 693 (C–S–C); ^1H–NMR (DMSO-*d6*) *δ*: 8.22 (d, 2H, –ArH, *J* = 8.0 Hz), 8.16 (d, 2H, –ArH, *J* = 8.0 Hz), 6.97 (s, 1H, –ArH, *J* = 8.0 Hz), 6.73 (d, 1H, –ArH, *J* = 8.0 Hz), 6.46 (dd, 1H, –ArH, *J* = 4.0 Hz), 5.15 (s, 2H, $–NH_2$), 4.15 (t, 1H, –CH), 3.46 (s, 6H, $–OCH_3$), 3.15 (d, 2H, $–CH_2$); ^{13}C–NMR (DMSO-*d6*) δ (ppm)**:** 162.1, 161.4, 154.8, 149.1, 146.9, 146.1, 133.9, 137.8, 127.5, 124.5, 122.5, 114.6, 110.1, 54.9, 53.6, 40.4; MS: *m/z* 427 $(M+H)^+$ for $C_{19}H_{18}N_6O_4S$.

1-(3,4-Dimethoxyphenyl)-2-(3-(4-methoxyphenyl)-[1,2,4]triazolo [3,4-b][1,3,4]thiadiazol-6-yl)ethanamine (6f). Orange solid in 74%; mp: 198–200°C; IR (KBr, υ cm^{-1}): 3370 (N–H), 2943 (C–H), 1605 (C=N), 1252 (N–N=C), 1138 (N=C–S–C=N), 683 (C–S–C); ^1H–NMR (DMSO-*d6*) *δ*: 7.82 (d, 2H, –ArH, *J* = 8.0 Hz), 7.36 (d, 2H, –ArH, *J* = 8.0 Hz), 7.22 (s, 1H, –ArH, *J* = 8.0 Hz), 6.87 (d, 1H, –ArH, *J* = 8.0 Hz), 6.56 (dd, 1H, –ArH, *J* = 4.0 Hz), 4.65 (s, 2H, $–NH_2$), 4.35 (t, 1H, –CH), 3.79 (s, 9H, $–OCH_3$), 3.10 (d, 2H, $–CH_2$); ^{13}C NMR (DMSO-*d6*) δ (ppm) : 166.6, 160.7, 152.6, 151, 149.1, 145.3, 141.1, 127.4, 127.2, 122.8, 118, 115.8, 114.4, 111.6, 110.1, 55.6, 55.5, 55.3; MS: *m/z* 412 $(M+H)^+$ for $C_{20}H_{21}N_5O_3S$.

General procedure for the synthesis of 1-(4-chlorophenyl)-2-(3-(para-substituted phenyl)-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazol-6-yl) ethanamine (7a–f). An equimolar mixture of 3-amino-3-(4-chlorophenyl) propanoic acid with 4-amino-5-phenyl-4H-[1,2,4]triazole-3-thiol (a)/4-Amino-5-(4-chlorophenyl)-4H [1,2,4] triazole-3-thiol (b)/4-(4-amino-5-mercapto-4H-[1,2,4] triazol-3-yl)-phenol (c)/4-amino-5-p-tolyl-4H-[1,2,4] triazole-3-thiol (d)/4-amino-5-(4-nitrophenyl)-4H-[1,2,4]-triazole-3-thiol (e)/4-amino-5-(4-methoxyphenyl)-4H-[1,2,4]-triazole-3-thiol (f) in $POCl_3$ (7 mL) was refluxed over a steam bath for 10–12 h. The progress of reaction was monitored by TLC. The excess $POCl_3$ was removed under reduced pressure, and the residue was poured on to crushed ice. The resulting precipitate was filtered, washed with saturated sodium bicarbonate solution, and then with water. It was dried and recrystallized from ethanol.

1-(4-Chlorophenyl)-2-(3-phenyl-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazol-6-yl)ethanamine (7a). Pale brownish yellow solid in 69%; mp: 164–166°C; IR (KBr, υ cm^{-1}): 3370 (N–H), 2930 (C–H), 1569 (C=N), 1230 (N–N=C), 1090 (N=C–S–C=N), 693 (C–S–C); ^{1}H–NMR (DMSO-*d6*) δ: 8.45 (d, 2H, –ArH, *J* = 8.0 Hz), 7.68 (d, 2H, –ArH, *J* = 8.0 Hz), 7.46 (d, 2H, –ArH, *J* = 8.0 Hz), 7.30 (d, 2H, –ArH, *J* = 8.0 Hz), 7.19 (t, 1H, –ArH, *J* = 8.0 Hz), 5.85 (s, 2H, $–NH_2$), 3.90 (t, 1H, –CH), 3.10 (d, 2H, $–CH_2$); ^{13}C–NMR (DMSO-*d6*) δ (ppm): 165.1, 162.6, 150.8, 141.9, 133.2, 131.6, 130.2, 128.6, 127.4, 126.7, 126.1, 53.8, 40.5; MS: *m/z* 356 $(M + H)^+$ for $C_{17}H_{14}ClN_5S$.

1-(4-Chlorophenyl)-2-(3-(4-chlorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)ethanamine (7b). Greenish brown solid in 66%; mp:179–181°C; IR (KBr, υ cm^{-1}): 3340 (N–H), 2885 (C–H), 1588 (C=N), 1238 (N–N=C), 1087 (N=C–S–C=N), 714 (C–S–C); ^{1}H–NMR (DMSO-d_6) δ: 8.24 (d, 2H, –ArH, *J* = 8.0 Hz), 7.85 (d, 2H, –ArH, *J* = 8.0 Hz), 7.70 (d, 2H, –ArH, *J* = 8.0 Hz), 7.52 (d, 2H, –ArH, *J* = 8.0 Hz), 5.67 (s, 2H, $–NH_2$), 3.35 (t, 1H, –CH), 2.50 (d, 2H, $–CH_2$); ^{13}C–NMR (DMSO-*d6*) δ (ppm): 164.2, 163.1, 150.5, 140.2, 136.2, 134.2, 128.6, 128.4, 127.1, 125.8, 124.6, 56.4, 41.1; MS: *m/z* 390 $(M + H)^+$ for $C_{17}H_{13}Cl_2N_5S$.

4-(6-(2-Amino-2-(4-chlorophenyl)ethyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3yl) phenol (7c). Pale yellow solid in 72%; mp: 161–163°C; IR (KBr, υ cm^{-1}): 3300 (O–H), 3354 (N–H), 2882 (C–H), 1551 (C=N), 1240 (N–N=C), 1120 (N=C–S–C=N), 693 (C–S–C); ^{1}H–NMR (DMSO-*d6*) δ: 7.52 (d, 2H, –ArH, *J* = 8.0 Hz), 7.42 (d, 2H, –ArH, *J* = 8.0 Hz), 7.29 (d, 2H, –ArH, *J* = 8.0 Hz), 6.99 (d, 2H, –ArH, *J* = 8.0 Hz), 6.98 (s, 1H, –OH); 4.76 (s, 2H, $–NH_2$), 3.91 (t, 1H, –CH), 3.14 (d, 2H, $–CH_2$); ^{13}C–NMR (DMSO-*d6*) δ (ppm):163.2**,** 161.4, 155**.**4, 150**.**2**,** 142.7, 134.9, 131.5, 129.4, 128.9, 119.4, 117.4, 51.4, 42.5; MS: *m/z* 372 $(M + H)^+$ for $C_{17}H_{14}ClN_5OS$.

1-(4-Chlorophenyl)-2-(3-(p-tolyl)-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazol-6-yl)ethanamine (7d). Pale brown solid in 68%; mp: 163–165°C; IR (KBr, υ cm^{-1}): 3340 (N–H), 2882 (C–H), 1651 (C=N), 1294 (N–N=C), 1088 (N=C–S–C=N), 717 (C–S–C); ^{1}H–NMR (DMSO-*d6*) δ: 8.16 (d, 2H, –ArH, *J* = 8.0 Hz), 7.88 (d, 2H, –ArH, *J* = 8.0 Hz), 7.64 (d, 2H, –ArH, *J* = 8.0 Hz), 7.42 (d, 2H, –ArH, *J* = 8.0 Hz), 5.80 (s, 2H, $–NH_2$), 3.48 (t, 1H, –CH), 3.23(d, 2H, $–CH_2$), 2.42 (s, 3H,$–CH_3$); ^{13}C–NMR (DMSO-*d6*) δ (ppm): 161**.**8, 160.2, 149.4, 137.4, 134.3, 130.7, 127.9, 126.5, 124.2, 122.3, 121.6, 57.5, 42.4, 20.9; MS: *m/z* 370 $(M + H)^+$ for $C_{18}H_{16}ClN_5S$.

1-(4-Chlorophenyl)-2-(3-(4-nitrophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)ethanamine (7e). Yellowish brown solid in 68%; mp: 185–187°C; IR (KBr, υ cm^{-1}): 3338 (N–H), 2883 (C–H), 1607 (C=N), 1256 (N–N=C), 1065 (N=C–S–C=N), 691 (C–S–C); ^{1}H–NMR (DMSO-*d6*) δ: 7.93 (d, 2H, –ArH, *J* = 8.0 Hz), 7.86 (d, 2H, –ArH, *J* = 8.0 Hz), 7.53 (d, 2H, –ArH, *J* = 8.0 Hz), 6.73 (d, 2H, –ArH, *J* = 8.0 Hz), 5.85 (s, 2H,$–NH_2$), 4.10 (s, 1H, –CH), 3.04 (d, 2H, $–CH_2$); ^{13}C–NMR (DMSO-*d6*) δ (ppm): 172.4, 171.1, 154.9, 147.1, 143.3, 139.4, 134.9, 128.3, 126.4, 115.7, 114.8, 54.2, 40.8; MS: *m/z* 401 $(M + H)^+$ for $C_{17}H_{13}ClN_6O_2S$.

1-(4-Chlorophenyl)-2-(3-(4-methoxyphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)ethanamine (7f). Pale yellow solid in 73%; mp:163–165°C; IR (KBr, υ cm^{-1}): 3392 (N–H), 2894 (C–H), 1612 (C=N), 1252 (N–N=C), 1089 (N=C–S–C=N), 682 (C–S–C); ^{1}H–NMR (DMSO-*d6*) δ: 8.19 (d, 2H, –ArH, *J* = 8.0 Hz), 7.87 (d, 2H, –ArH, *J* = 8.0 Hz), 7.52 (d, 2H, –ArH, *J* = 8.0 Hz), 7.06 (d, 2H, –ArH, *J* = 8.0 Hz), 4.25 (s, 2H, $–NH_2$), 3.65 (s, 1H, –CH), 3.40 (s, 3H, $–OCH_3$), 2.95 (d, 2H, $–CH_2$); ^{13}C–NMR (DMSO-*d6*) δ (ppm): 162.3, 161.4, 159.5, 150.7, 141.3, 136.4, 133.9, 126.3, 124.5, 120.3 115.4, 55.3, 54.2, 41.8; MS: *m/z* 386 $(M + H)^+$ for $C_{18}H_{16}ClN_5OS$.

PHARMACOLOGICAL SCREENING

Antioxidant screening (*in vitro*). Compounds **6a–f** and **7a–f** are tested for antioxidant property by DPPH, NO, and H_2O_2 methods.

DPPH radical scavenging activity. The hydrogen atom or electron donation ability of the compounds was measured from the bleaching of the purple-colored methanol solution of 1,1-diphenyl-1-picrylhydrazyl. The spectrophotometric assay uses the stable radical DPPH as a reagent. About 1 mL of various concentrations of the test compounds (25, 50, 75, and 100 μg/mL) in methanol was added to 4 mL of 0.004% (w/v) methanol solution of DPPH. After a 30-min incubation period at room temperature, the absorbance was read against blank at 517 nm. The percentage of inhibition (I%) of free radical production from DPPH was calculated by the following equation

$$\%\ \text{of scavenging} = [(\text{A control} - \text{A sample}) / \text{A blank}] \times 100 \rightarrow \quad (1)$$

where A control is the absorbance of the control reaction (containing all reagents except the test compound), and A sample is the absorbance of the test compound. Tests were carried at in triplicate.

Nitric oxide scavenging activity. Nitric oxide scavenging activity was measured by slightly modified methods of Green et al. and Marcocci *et al*. NO radicals were generated from sodium nitro prusside. About 1 mL of sodium nitroprusside (10 mM) and 1.5 mL of phosphate buffer saline (0.2 M, pH 7.4) were added to different concentrations (25, 50, 75, and 100 μg/mL) of the test compounds and incubated for 150 min at 25°C, and 1 mL of the reaction mixture was treated with 1 mL of Griess reagent (1% sulfanilamide, 2% H_3PO_4, and 0.1% naphthyl ethylenediamine dihydrochloride). The absorbance of the chromatophore was measured at 546 nm. NO scavenging activity was calculated using Equation (1).

Hydrogen peroxide scavenging activity. The H_2O_2 scavenging ability of the test compound was determined according to the method of Ruch *et al*. A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). A total of 25, 50, 75, and 100 μg/mL concentrations of the test compounds in 3.4 mL phosphate buffer were added to H_2O_2 solution (0.6 mL, 40 mM). The absorbance value of the reaction mixture was recorded at 230 nm. The percentage of scavenging of H_2O_2 was calculated using Equation 1.

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