



Original article

Synthesis and biological activities of some novel aminomethyl derivatives of 4-substituted-5-(2-thienyl)-2,4-dihydro-3H-1,2,4-triazole-3-thiones



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ARTICLE INFO

Article history:

Received 15 November 2012

Received in revised form

19 February 2013

Accepted 21 February 2013

Available online 1 March 2013

Keywords:

4-Substituted-5-(2-thienyl)-2,4-dihydro-3H-1,2,4-triazole-3-thiones

Mannich bases

Biological activity

ABSTRACT

A novel series of compounds were synthesized by cyclic condensation reaction of substituted isothiocyanate (**2a–c**) with 2-thiophenecarboxylic acid hydrazide (**1**) in the presence of ethyl alcohol, to obtain intermediate thiosemicarbazides (**3a–c**), which were further treated with sodium hydroxide in the presence of ethanol to obtain triazole derivatives (**4a–c**). The latter were refluxed with substituted secondary amines and formaldehyde for 6–10 h to afford Mannich bases (**5a–k**). The synthesized compounds were characterized on the basis of their spectral (IR, ¹³C and ¹H NMR) data and evaluated for biological activities. Some of the compounds were found to exhibit significant antimicrobial and antioxidant activity.

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1. Introduction

The ring-closure reactions of carbohydrazides are well-known and have been thoroughly studied. In these reactions five-membered heterocycles with three heteroatoms such as are formed, 1,3,4-oxadiazoles, 1,3,4-thiadiazoles, and 1,2,4-triazoles. 1,3,4-oxadiazole, 1,3,4-thiadiazole, and 1,2,4-triazole ring systems are typical planar six- π -electron partially aromatic systems, and are used, along with their derivatives, as starting materials for the synthesis of many heterocycles. The 1,2,4-triazole and its derivatives were reported to exhibit various pharmacological activities such as antimicrobial, analgesic, anti-inflammatory, anticancer and antioxidant properties [1–4]. Some present day drugs such as Ribavirin (antiviral agent), Rizatriptan (antimigraine agent), Alprazolam (anxiolytic agent), Fluconazole and Itraconazole (antifungal agents) are examples of potent molecules possessing a triazole nucleus [5–7]. Many studies have shown that Mannich bases have possess potent biological characteristics such as antibacterial, antifungal, anti-inflammatory, antimalarial and pesticide

properties [8–11]. Few Mannich bases derived from 1,2,4-triazoles carrying *N*-methylpiperazine substituent were biologically active [12,13]. 4,5-Substituted products containing 1,2,4-triazole in their molecules seem to be suitable candidates for further chemical modifications and might be of interest as pharmacologically active compounds and ligands useful in coordination chemistry [14]. Derivatives of 4,5-substituted 1,2,4-triazole were synthesized by intramolecular cyclization of 1,4-disubstituted thiosemicarbazides [15]. In addition there are some studies on electronic structures and thiol–thione tautomeric equilibrium of heterocyclic thione derivatives [16–19].

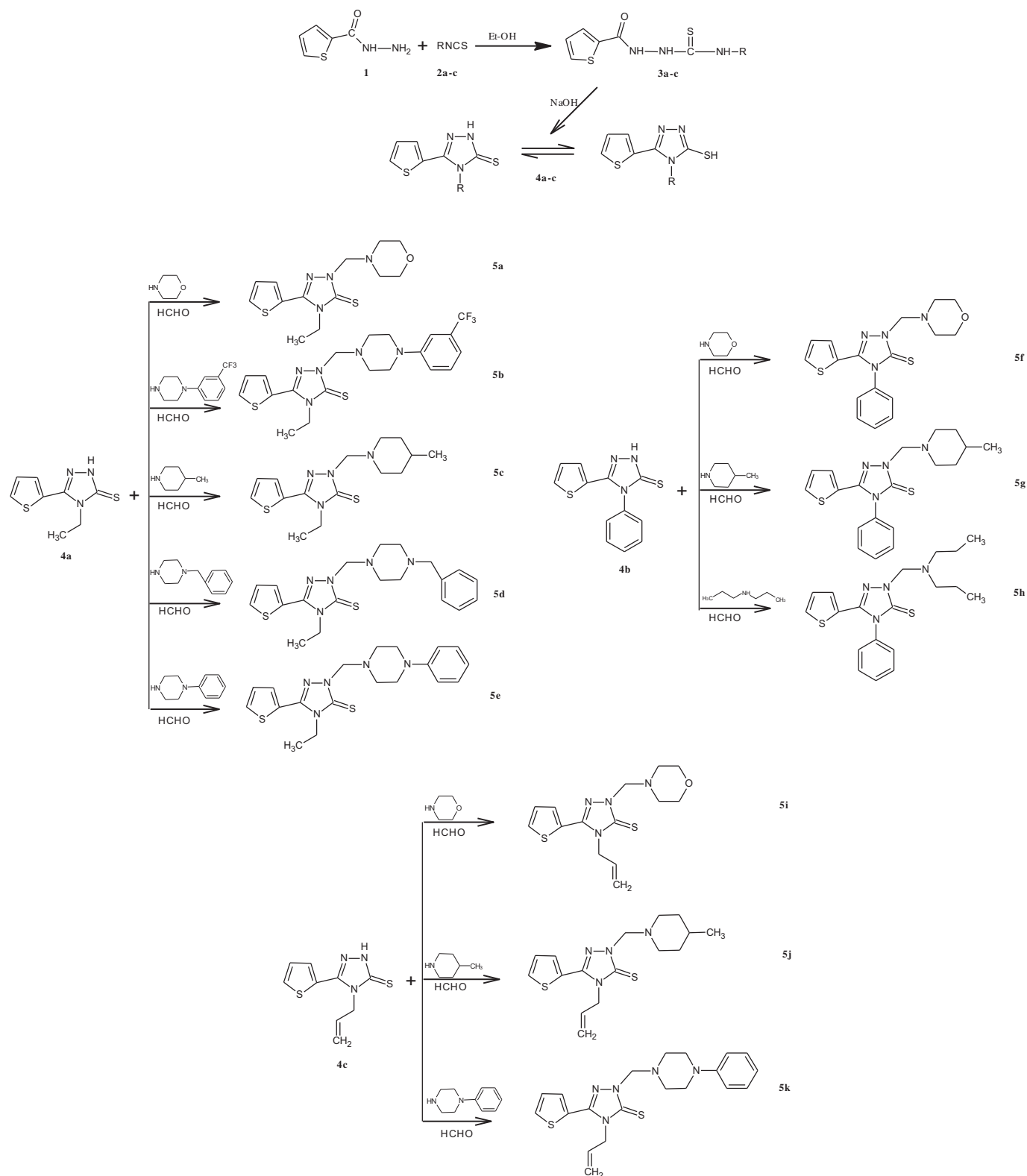
In view of these findings, we report on the synthesis, characterization and antibacterial, antifungal and antioxidant activities some of 4-substituted-5-(2-thienyl)-1,2,4-triazole 3-thione and their Mannich bases.

2. Results and discussion

2.1. Chemistry

The reaction sequences employed for synthesis of title compounds are shown in Scheme 1. In the present work,

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Scheme 1. Synthesis and the structures of the compounds.

thiosemicarbazides (**3a-c**) were synthesized by reacting 2-thiophenecarboxylic acid hydrazide (**1**) with substituted isothiocyanate (**2a-c**) in the presence of ethanol at reflux temperature by condensation method. The synthesized thiosemicarbazides

were reacted with sodium hydroxide in the presence of ethanol to obtain 4-substituted-5-(2-thienyl)-2,4-dihydro-3H-1,2,4-triazole-3-thiones (**4a-c**). The synthesized triazoles were reacted with formaldehyde and secondary amines to afford Mannich bases (**5a-**

k). All compounds displayed IR, ^1H and ^{13}C NMR spectra and elemental analyzes consistent with the assigned structures. In the ^1H NMR spectrum of compounds (**4a–c**), the signal due to $-\text{SH}$ group appeared at 13.98–14.08 ppm. The aromatic protons appeared as a multiplet at 6.71–7.85 ppm. Moreover, the $\text{C}=\text{S}$ group resonated at 167.3–169.6 ppm in the ^{13}C NMR spectra of compounds (**4a–c**). In the IR spectrum of (**4a–c**) the most characteristic absorptions are at 3015 and 3109 cm^{-1} aromatic bands, 1577 and 1645 cm^{-1} ($\text{C}=\text{N}$), 1261 and 1277 cm^{-1} , ($\text{C}=\text{S}$) and ($\text{C}-\text{S}-\text{C}$) stretching bands at 713–736 cm^{-1} . When compounds (**4a–c**) were converted to Mannich bases of 4-substituted-5-(2-thienyl)-2,4-dihydro-3H-1,2,4-triazole-3-thiones in acidic media, $-\text{NH}$ peaks disappeared, while new signals due to the $-\text{N}-\text{CH}_2-\text{N}-$ group were observed at 5.04–5.21 ppm in the ^1H NMR spectra of compounds (**5a–k**). In the ^1H NMR spectrum of compounds (**5a–e**), signal due to $\text{Ar}-\text{H}$ appeared at 6.73–7.92 ppm. The $\text{N}-\text{CH}_2-\text{CH}_3$ appeared as a quartet at 4.24–4.30 ppm and $\text{N}-\text{CH}_2-\text{CH}_3$ appeared as a triplet at 1.25–1.41 ppm. Moreover, the $\text{C}=\text{S}$ group resonated at 168.1–168.7 ppm in the ^{13}C NMR spectra of compounds (**5a–e**). In the IR spectrum of (**5a–e**), the most characteristic absorptions are at 3022 and 3107 cm^{-1} aromatic bands, 1572 and 1612 cm^{-1} ($\text{C}=\text{N}$), 1276 and 1285 cm^{-1} , and ($\text{C}=\text{S}$) and ($\text{C}-\text{S}-\text{C}$) stretching bands at 740–782 cm^{-1} . In the ^1H NMR spectrum of compounds (**5f–h**), signal due to $\text{Ar}-\text{H}$ appeared at 6.66–7.72 ppm. Moreover, the $\text{C}=\text{S}$ group resonated at 169.6–169.8 ppm in the ^{13}C NMR spectra of compounds (**5f–h**). In the IR spectrum of (**5f–h**) the most characteristic absorptions are at 3040 and 3114 cm^{-1} aromatic bands, 1578 and 1593 cm^{-1} ($\text{C}=\text{N}$), 1277 and 1280 cm^{-1} , and ($\text{C}=\text{S}$) and ($\text{C}-\text{S}-\text{C}$) stretching bands at 710–783 cm^{-1} . ^1H NMR spectrum of compounds (**5i–k**), a signal due to $\text{Ar}-\text{H}$ appeared at 6.83–7.88 ppm. The $\text{N}-\text{CH}_2-\text{CH}=\text{CH}_2$ appeared as a twelve peaks at 5.90–6.04 ppm, $\text{N}-\text{CH}_2-\text{CH}=\text{CH}_2$ and trans protons of CH_2 in the allyl group appeared as a multiple signals at 4.85–4.92 ppm; and $\text{N}-\text{CH}_2-\text{CH}=\text{CH}_2$ cis protons of CH_2 in the allyl group appeared as a doublet at 5.20 ppm. Moreover, the $\text{C}=\text{S}$ group resonated at 168.9–169.3 ppm in the ^{13}C NMR spectra of compounds (**5i–k**). In the IR spectrum of (**5i–k**) the most characteristic absorptions are at 3073 and 3090 cm^{-1} aromatic bands, 1506 and 1665 cm^{-1} ($\text{C}=\text{N}$), 1268 and 1271 cm^{-1} , and ($\text{C}=\text{S}$) and ($\text{C}-\text{S}-\text{C}$) stretching bands at 721–729 cm^{-1} . The data for all compounds are given in the Experimental section.

2.2. Biological evaluation

2.2.1. Antibacterial and antifungal activity

The new synthesized compounds were evaluated for their antibacterial and antifungal activities against the Gram-positive bacteria: *Staphylococcus aureus* (ATCC 25923) the Gram negative bacteria: *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Klebsiella pneumoniae* (recultured) and four fungal strains *Aspergillus flavus* (NCIM No. 524), *Aspergillus fumigatus* (NCIM No. 902), *Penicillium marneffei* (recultured) and *Trichophyton mentagrophytes* (recultured). Both microbial studies were assessed by minimum inhibitory concentration (MIC) by serial plate dilution method. MIC is the highest dilution of the compound, which shows clear fluid with no development of turbidity.

Antibacterial and antifungal screening revealed that some of the tested compounds showed good inhibition at 1.56–25 $\mu\text{g/mL}$ in DMSO. The compounds **4a–c** showed moderate activity against all the fungal strains. Among the screened compounds, **5c**, **5g**, **5h** and **5j** showed comparatively good activity against all the fungal strains. Compounds **5a**, **5b**, **5d–f**, **5i** and **5k** showed good antifungal activity against all the fungal strains. Of all the synthesized derivatives, compounds **5a**, **5b**, **5d–f**, **5i** and **5k** were the most active against the investigated strains, compared to the standard

drug. Compounds **5b**, **5d**, **5e** and **5k** exhibited good antifungal activity almost equivalent to that of the standard. Antifungal screening revealed that compounds, **5a**, **5f** and **5i** showed excellent activity against all the tested fungal strains, namely *P. marneffei*, *T. mentagrophytes*, *A. flavus* and *A. fumigatus* at 1.56–3.12 $\mu\text{g/mL}$ concentration. The remaining compounds were found to be active at higher concentrations, e.g., 6.25 and 25 $\mu\text{g/mL}$. It was therefore concluded that the presence of morpholine and piperazine moiety, in addition to thienyl, phenyl, ethyl and benzyl groups, was found to be essential for their high antifungal activity of these compounds. The results of antifungal screening of newly compounds 1,2,4-triazole (**4a–c**) and Mannich bases (**5a–k**) as the MIC values, are summarized in Table 1.

Compounds **4a–c** showed moderate activity against all the bacterial strains. All the synthesized derivatives, compounds **5d**, **5e**, **5h** and **5k** showed comparatively good activity against all the bacterial strains. Compounds **5a**, **5f** and **5i** exhibited good antibacterial activity almost equivalent to that of the standard drug. The antibacterial screening revealed that of the tested compounds, **5b**, **5c**, **5g** and **5j** showed excellent activity against all the tested bacterial strains, *S. aureus*, *K. pneumoniae*, *E. coli* and *P. aeruginosa* at 3.12–1.56 $\mu\text{g/mL}$ concentration. The remaining compounds found to be active at higher concentrations, e.g., 6.25 and 25 $\mu\text{g/mL}$. Compounds **5b**, **5c**, **5g** and **5j** were also the most active against the investigated strains as compared to the standard drug. It was therefore concluded that the presence of 4-methyl piperidine and trifluoromethylphenylpiperazine moiety were essential for their high antibacterial activity of these compounds. The results of antibacterial screening of newly prepared compounds **5a–k** expressed as the MIC values, compared with the starting triazoles (**4a–c**), and control (ciprofloxacin), are summarized in Table 2.

2.2.2. Antioxidant activity

Since antioxidants are gaining attention as a potential means of treating a large number of lifestyle diseases like aging, cancer, diabetes, cardiovascular and other degenerative diseases, it is of immense significance to establish some new antioxidants via a convenient synthetic methodology. Although a number of methods are available, including ORAC, ABTS, DMPD, FRAP, TRAP, TBA, superoxide radical scavenging, hydroxyl radical scavenging, nitric oxide radical scavenging, xanthine oxidase, cytochrome C, reducing power method, etc. DPPH method is very common and has been shown to be the most appropriate method [20].

Table 1
Antifungal activity of compounds (**4a–c**) and (**5a–k**).

Compound	MIC in $\mu\text{g/mL}$			
	<i>P. marneffei</i>	<i>T. mentagrophytes</i>	<i>A. flavus</i>	<i>A. fumigatus</i>
4a	25	12	12	25
4b	25	12	12	25
4c	25	12	12	25
5a	3.12	1.56	1.56	3.12
5b	6.25	3.12	3.12	6.25
5c	12	6.25	6.25	12
5d	6.25	3.12	3.12	6.25
5e	6.25	3.12	3.12	6.25
5f	3.12	1.56	1.56	3.12
5g	12	6.25	6.25	12
5h	12	6.25	6.25	12
5i	3.12	1.56	1.56	3.12
5j	12	6.25	6.25	12
5k	6.25	3.12	3.12	6.25
Cicloprioxolamine ^a	6.25	3.12	3.12	6.25
DMSO (control)	0	0	0	0

The MIC values were evaluated at concentration range, 1.56–25 $\mu\text{g/mL}$.

^a Cicloprioxolamine was used as a standard.

Table 2
Antibacterial activity of compounds (**4a–c**) and (**5a–k**).

Compound	MIC in µg/mL			
	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>
4a	12	12	12	12
4b	12	12	12	12
4c	12	25	12	25
5a	6.25	6.25	6.25	3.12
5b	3.12	3.12	3.12	1.56
5c	3.12	3.12	3.12	1.56
5d	12	6.25	12	6.25
5e	12	6.25	12	6.25
5f	6.25	6.25	6.25	12
5g	3.12	3.12	3.12	1.56
5h	12	6.25	12	6.25
5i	6.25	6.25	6.25	3.12
5j	3.12	3.12	3.12	1.56
5k	12	6.25	12	6.25
Ciprofloxacin ^a	6.25	6.25	6.25	3.12
DMSO (control)	0	0	0	0

The MIC values were evaluated at concentration range, 1.56–25 µg/mL.

^a Ciprofloxacin was used as a standard.

Compounds **4a–c** showed moderate antioxidant activity. Of all the synthesized derivatives, compounds **5b**, **5d**, **5e**, **5h** and **5k** showed very good activities which were similar to reference antioxidant compound. In addition compounds **5c**, **5g** and **5j** showed good scavenging activities. From all the synthesized derivatives, compounds **5a**, **5f** and **5i** exhibited the highest radical scavenging activities which were better than the reference antioxidant compound.

Compounds **5a**, **5f** and **5i** with morpholine moiety showed the best DPPH radical scavenging activity at all concentration, followed by compound **5d**, **5e** and **5k** with piperazine moiety, **5h** with dipropylamine and **5b** with having trifluoromethyl phenyl moiety.

As shown in Table 3, the title compounds have scavenging activity between 36.1% and 95.0% within the investigated concentration range. The antioxidant activity of the title compounds are obvious that the scavenging activity increases with increasing sample concentration in the range tested.

3. Conclusions

The research study reports the successful synthesis and antimicrobial activity of new 1,2,4-triazole and Mannich bases bearing

Table 3
Antioxidant scavenging activity of compounds (**4a–c**) and (**5a–k**) on DPPH[•] free radical at different concentrations.

Tested Compound	DPPH scavenging activity (%)				
	62.5 µM	125 µM	187.5 µM	250 µM	312.5 µM
4a	36.4 ± 0.7	47.1 ± 0.1	49.6 ± 0.1	53.3 ± 0.5	62.1 ± 0.2
4b	36.1 ± 0.6	45.7 ± 0.6	47.9 ± 0.1	51.2 ± 0.6	65.1 ± 1.0
4c	40.7 ± 0.3	45.2 ± 0.4	47.4 ± 0.3	55.5 ± 0.1	68.8 ± 0.5
5a	60.4 ± 0.6	69.8 ± 0.2	82.8 ± 0.4	88.6 ± 0.5	93.6 ± 0.3
5b	52.0 ± 0.1	61.1 ± 0.4	71.3 ± 0.3	80.2 ± 0.2	81.6 ± 0.2
5c	49.2 ± 0.2	52.5 ± 0.4	65.2 ± 0.4	67.6 ± 0.6	71.3 ± 0.4
5d	53.5 ± 0.4	63.2 ± 0.6	74.5 ± 0.2	83.1 ± 0.2	90.9 ± 0.2
5e	52.2 ± 0.1	63.1 ± 0.2	72.7 ± 0.2	82.5 ± 0.3	90.2 ± 0.1
5f	59.1 ± 0.2	68.4 ± 0.2	79.2 ± 0.1	87.2 ± 0.1	92.0 ± 0.2
5g	44.7 ± 0.4	51.1 ± 0.8	61.1 ± 0.4	66.9 ± 0.3	70.0 ± 0.5
5h	52.1 ± 0.6	62.6 ± 0.2	72.1 ± 0.1	81.4 ± 0.4	89.0 ± 0.2
5i	62.3 ± 0.1	71.2 ± 0.2	83.1 ± 0.2	89.9 ± 0.2	95.0 ± 0.1
5j	50.1 ± 0.3	53.5 ± 0.4	68.1 ± 0.3	69.7 ± 0.2	72.4 ± 0.2
5k	54.0 ± 0.1	64.4 ± 0.3	75.0 ± 0.1	84.1 ± 0.2	91.6 ± 0.2
Ascorbic acid ^a	55.1 ± 0.2	65.0 ± 0.2	75.2 ± 0.2	85.8 ± 0.4	91.7 ± 0.2

^a Ascorbic acid (reference antioxidant compounds) was used as a standard. The scavenging capacities were represented as percentage inhibition and values were the means of three replicates (mean ± SD, *n* = 3).

thienyl moiety. The antimicrobial activity study revealed that all the compounds tested showed good antibacterial and antifungal activities against pathogenic strains. The structure and biological activity relationship of title compounds showed that the presence of thienyl groups and biologically active groups like morpholine, 4-benzylpiperazine, *N*-methylpiperidine and trifluoromethylphenyl-piperazine groups attached to the triazole ring of the title compounds are responsible for good antimicrobial activity. A similar correlation was observed for antioxidant activity. All compounds greatly improved their activity compared to precursors. Therefore, the significant antifungal, antibacterial and antioxidant activity of compounds may be due to the presence of morpholine, *N*-methylpiperidine and piperazine moiety in addition to thienyl, phenyl, methyl, ethyl and allyl group. Finally, our biological activity results agree with the results reported in literature, take into 1,2,4-triazole, phenyl, piperazine, morpholine and benzyl the groups [21–26]. Hence it is concluded that there is ample scope for further study.

4. Experimental

4.1. Chemistry assays

Melting points were determined on a Thomas Hoover melting point apparatus and uncorrected, but checked by differential scanning calorimeter (DSC). The IR spectra were measured with Perkin–Elmer spectrum one FT–IR spectrophotometer. Electronic spectral studies were conducted on a Shimadzu model UV–1700 spectrophotometer in the wavelength 1100–200 nm. The ¹H and ¹³C spectra were taken on Bruker AC-400 NMR spectrometer operating at 400 MHz for ¹H, 100 MHz for ¹³C NMR. Compounds were dissolved in DMSO and chemical shifts were referenced to TMS (¹H and ¹³C NMR). Starting chemicals were obtained from Merck or Aldrich.

4.1.1. General procedure for the synthesis of **4a–c**

A mixture of 2-thienyl thiosemicarbazides (**3a–c**) (0.01 mol) and 10% potassium hydroxide solution (10 mL) was refluxed for 3 h. The mixture was then cooled to room temperature and filtered. The filtrate was neutralized by the gradual addition of glacial acetic acid. The resulting solid was collected by filtration, dried and recrystallized from ethyl alcohol.

4.1.1.1. 4-Ethyl-5-(2-thienyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (4a). This compound was obtained as shining white solid, yield: 85%; mp: 256–257 °C; IR (KBr, cm^{−1}) 3015–3102 (Ar–H), 2868–2938 (CH str.), 1577 (C=N), 1277 (C=S), 713 (C–S–C); ¹H NMR (DMSO-*d*₆) δ: 1.23 (t, 3H, N–CH₂–CH₃, *J* = 7.2 Hz), 4.21 (q, 2H, –N–CH₂–CH₃, *J* = 7.2 Hz), 7.26 (dd, 1H, Ar–H, *J* = 4.0, 4.8 Hz), 7.67 (d, 1H, Ar–H, *J* = 3.2 Hz), 7.85 (d, 1H, Ar–H, *J* = 4.8 Hz), 14.0 (s, 1H, SH); ¹³C NMR (DMSO-*d*₆) δ: 13.6, 39.6, 126.7, 128.7, 129.4, 130.1, 146.1, 167.3. MW: C₈H₉N₃S₂ (211). Elemental analysis: calcd C, 45.47; H, 4.29; N, 19.89; S, 30.35; found, C, 45.43; H, 4.26; N, 19.90; S, 30.33.

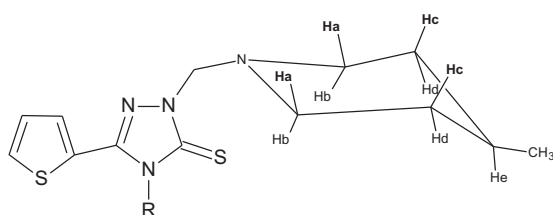
4.1.1.2. 4-Phenyl-5-(2-thienyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (4b). This compound was obtained as shining white solid, yield: 79%; mp: 164–165 °C; IR (KBr, cm^{−1}) 3065–3109 (Ar–H), 1579 (C=N), 1267 (C=S), 715 (C–S–C); ¹H NMR (DMSO-*d*₆) δ: 6.66–7.70 (m, 8H, Ar–H), 13.98 (s, 1H, SH); ¹³C NMR (DMSO-*d*₆) δ: 126.6, 128.1, 129.2, 129.4, 130.1, 130.3, 130.6, 135.0, 144.7, 169.6. MW: C₁₂H₉N₃S₂ (259). Elemental analysis: calcd C, 55.57; H, 3.50; N, 16.20; S, 24.73; found, C, 55.56; H, 3.49; N, 16.19; S, 24.74.

4.1.1.3. 4-Allyl-5-(2-thienyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (4c). This compound was obtained as shining claret red solid, yield: 75%; mp: 157–158 °C; IR (KBr, cm^{−1}) 3070–3086 (Ar–H), 2967 (CH

str.), 1645 (C=N), 1261 (C=S), 736 (C–S–C); ^1H NMR (DMSO- d_6) δ : 4.83–4.89 (m, 3H, N–CH₂–CH=CH₂ and N–CH₂–CH=CH₂, (trans)), 5.17 (d, 1H, N–CH₂–CH–CH₂, $J_{\text{cis}} = 10.4$ Hz), 5.87–5.95 (ddt, 1H, N–CH₂–CH–CH₂, $J_{\text{trans}} = 17.1$ Hz, $J_{\text{cis}} = 10.5$ Hz, $J_{\text{CH}_2} = 4.8$ Hz), 7.21 (dd, 1H, Ar–H, $J = 2.7, 3.0$ Hz), 7.58 (d, 1H, Ar–H, $J = 1.8$ Hz), 7.81 (d, 1H, Ar–H, $J = 3.9$ Hz), 14.08 (s, 1H, SH); ^{13}C NMR (DMSO- d_6) δ : 46.1, 117.0, 126.6, 128.6, 129.1, 130.1, 131.8, 146.5, 167.9. MW: C₉H₉N₃S₂ (223). Elemental analysis: calcd C, 48.40; H, 4.06; N, 18.82; S, 28.72; found, C, 48.38; H, 4.09; N, 18.80; S, 28.74.

4.2. General procedure for the synthesis of **5a–k**

A solution of appropriate triazoles (**4a–c**) (0.01 mol), formaldehyde (40%, 1.5 mL) and suitably substituted secondary amines (0.01 mol) in ethanol (20 mL) was stirred for an hour and left overnight at room temperature. The solid mass thus separated was collected by filtration, dried and recrystallized from ethyl alcohol.



Note: the structure for NMR (**5c**, **5g** and **5j**).

4.2.1. 4-Ethyl-2-(morpholin-4-ylmethyl)-5-(2-thienyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**5a**)

This compound was obtained as shining white solid, yield: 69%; mp: 96–97 °C; IR (KBr, cm^{−1}) 3070 (Ar–H), 2839–2962 (CH str.), 1574 (C=N), 1279 (C=S), 779 (C–S–C); ^1H NMR (DMSO- d_6) δ : 1.25 (t, 3H, N–CH₂–CH₃, $J = 7.2$ Hz), 2.66 (t, 4H, N–CH₂ morpholine), 3.54 (t, 4H, OCH₂ morpholine), 4.24 (q, 2H, N–CH₂–CH₃, $J = 7.2$ Hz), 5.06 (s, 2H, N–CH₂–N), 7.28–7.89 (m, 3H, ArH); ^{13}C NMR (DMSO- d_6) δ : 13.5, 40.7, 50.5, 66.4, 69.2, 126.6, 128.7, 129.1, 130.1, 146.0, 167.3. MW: C₁₃H₁₈N₄OS₂ (310). Elemental analysis: calcd C, 50.30; H, 5.84; N, 18.05; S, 20.66; found, C, 50.29; H, 5.79; N, 18.03; S, 20.68.

4.2.2. 4-Ethyl-5-(2-thienyl)-2-([4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)methyl]-2,4-dihydro-3H-1,2,4-triazole-3-thione (**5b**)

This compound was obtained as shining white solid, yield: 70%; mp: 80–81 °C; IR (KBr, cm^{−1}) 3105 (Ar–H), 2840–2948 (CH str.), 1612 (C=N), 1282 (C=S), 782 (C–S–C); ^1H NMR (DMSO- d_6) δ : 1.27 (t, 3H, N–CH₂–CH₃, $J = 7.2$ Hz), 2.86 (t, 4H, N–CH₂ piperazine ring), 3.22 (t, 4H, ArN CH₂ piperazine ring), 4.26 (q, 2H, N–CH₂–CH₃, $J = 7.2$ Hz), 5.17 (s, 2H, NCH₂N), 7.03–7.92 (m, 7H, ArH); ^{13}C NMR (DMSO- d_6) δ : 13.5, 48.1, 50.1, 51.2, 68.9, 111.4, 111.6, 115.0, 123.7, 126.6, 129.0, 129.6, 130.1, 130.4, 130.6, 145.1, 151.7, 168.4. MW: C₂₀H₂₂F₃N₅S₂ (453). Elemental analysis: calcd C, 52.96; H, 4.89; N, 15.44; S, 14.14; found, C, 52.94; H, 4.86; N, 15.46; S, 14.12.

4.2.3. 4-Ethyl-2-[(4-methylpiperidin-1-yl)methyl]-5-(2-thienyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**5c**)

This compound was obtained as shining white solid, yield: 69%; mp: 77–78 °C; IR (KBr, cm^{−1}) 3069–3107 (Ar–H), 2819–2941 (CH str.), 1572 (C=N), 1276 (C=S), 770 (C–S–C); ^1H NMR (DMSO- d_6) δ : 0.82 (d, 3H, –CH–CH₃, $J = 6.4$ Hz), 1.02–1.07 (ddt, 2H, H_d, $J = 7.2$ Hz), 1.08–1.25 (m, 4H, N–CH₂–CH₃ and H_e), 1.53 (d, 2H, H_c, $J = 12$ Hz), 2.28 (t, 2H, H_b, $J = 11.6$ Hz), 3.00 (d, 2H, H_a, $J = 11.2$ Hz), 4.24 (q, 2H, N–CH₂–CH₃, $J = 6.8$ Hz), 5.04 (s, 2H, N–CH₂–N), 7.26–7.88 (m, 3H, Ar–H); ^{13}C NMR (DMSO- d_6) δ : 13.4, 22.1, 29.9, 34.1, 40.7, 50.7, 69.6, 126.1, 128.8, 129.6, 130.4, 144.7, 168.1. MW:

C₁₅H₂₂N₄S₂ (322). Elemental analysis: calcd C, 55.86; H, 6.88; N, 17.37; S, 19.89; found, C, 55.84; H, 6.89; N, 17.35; S, 19.87.

4.2.4. 2-[(4-Benzylpiperazin-1-yl)methyl]-4-ethyl-5-(2-thienyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**5d**)

This compound was obtained as shining white solid, yield: 68%; mp: 92–93 °C; IR (KBr, cm^{−1}) 3022–3083 (Ar–H), 2812–2937 (CH str.), 1574 (C=N), 1285 (C=S), 740 (C–S–C); ^1H NMR (DMSO- d_6) δ : 1.41 (t, 3H, N–CH₂–CH₃, $J = 9.6$ Hz), 2.50 (t, 4H, –CH₂–N–CH₂ piperazine ring), 2.90 (t, 4H, N–CH₂ piperazine ring), 3.48 (s, 2H, N–CH₂–Ar), 4.30 (q, 2H, N–CH₂–CH₃, $J = 9.6$ Hz), 5.21 (s, 2H, N–CH₂–N) 7.18–7.56 (m, 8H, ArH); ^{13}C NMR (DMSO- d_6) δ : 13.6, 30.9, 40.9, 50.4, 53.0, 63.1, 67.1, 69.4, 126.4, 127.1, 127.9, 128.2, 128.8, 129.3, 144.9, 168.7. MW: C₂₀H₂₅N₅S₂ (399). Elemental analysis: calcd C, 60.12; H, 6.31; N, 17.53; S, 16.05; found, C, 60.09; H, 6.29; N, 17.50; S, 16.02.

4.2.5. 4-Ethyl-2-[(4-phenylpiperazin-1-yl)methyl]-5-(2-thienyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**5e**)

This compound was obtained as shining white solid, yield: 69%; mp: 126–127 °C; IR (KBr, cm^{−1}) 3083 (Ar–H), 2826–2961 (CH str.), 1599 (C=N), 1279 (C=S), 761 (C–S–C); ^1H NMR (DMSO- d_6) δ : 1.27 (t, 3H, N–CH₂–CH₃), 2.85 (t, 4H, –CH₂–N–CH₂ piperazine ring), 3.11 (t, 4H, N–CH₂ piperazine ring), 4.25 (q, 2H, N–CH₂–CH₃), 5.16 (s, 2H, N–CH₂–N), 6.73–7.91 (m, 8H, ArH); ^{13}C NMR (DMSO- d_6) δ : 13.5, 40.9, 48.7, 50.3, 69.0, 116.1, 119.4, 126.7, 129.0, 129.3, 129.9, 130.6, 145.1, 151.5, 168.4. MW: C₁₉H₂₃N₅S₂ (385). Elemental analysis: calcd C, 59.19; H, 6.01; N, 18.16; S, 16.63; found, C, 59.21; H, 6.03; N, 18.14; S, 16.65.

4.2.6. 2-(Morpholin-4-ylmethyl)-4-phenyl-5-(2-thienyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**5f**)

This compound was obtained as shining yellow solid, yield: 66%; mp: 197–198 °C; IR (KBr, cm^{−1}) 3040–3114 (Ar–H), 2852–2961 (CH str.), 1579 (C=N), 1278 (C=S), 783 (C–S–C); ^1H NMR (DMSO- d_6) δ : 3.34 (t, 4H, N–CH₂ morpholine), 3.58 (t, 4H, O–CH₂ morpholine), 5.13 (s, 2H, N–CH₂–N), 6.71–7.72 (m, 8H, Ar–H); ^{13}C NMR (DMSO- d_6) δ : 50.4, 66.3, 69.2, 126.6, 128.1, 129.2, 129.4, 130.1, 130.3, 130.6, 135.0, 144.9, 169.8. MW: C₁₇H₁₈N₄OS₂ (358). Elemental analysis: calcd C, 56.96; H, 5.06; N, 15.63; S, 17.89; found, C, 56.94; H, 5.09; N, 15.61; S, 17.88.

4.2.7. 2-[(4-Methylpiperidin-1-yl)methyl]-4-phenyl-5-(2-thienyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**5g**)

This compound was obtained as shining white solid, yield: 65%; mp: 153–154 °C; IR (KBr, cm^{−1}) 3051–3106 (Ar–H), 2818–2943 (CH), 1593 (C=N), 1277 (C=S), 712 (C–S–C); ^1H NMR (DMSO- d_6) δ : 0.85 (d, 3H, –CH–CH₃, $J = 6.0$ Hz), 1.03–1.25 (m, 3H, H_e and H_d), 1.57 (d, 2H, H_c, $J = 12$ Hz), 2.41 (t, 2H, H_b, $J = 11.6$ Hz), 3.05 (d, 2H, H_a, $J = 11.2$ Hz), 5.10 (s, 2H, N–CH₂–N), 6.68–7.70 (m, 8H, Ar–H); ^{13}C NMR (DMSO- d_6) δ : 22.2, 30.0, 34.2, 50.8, 69.9, 126.6, 128.1, 129.2, 129.4, 130.1, 130.3, 130.6, 135.0, 144.7, 169.6. MW: C₁₉H₂₂N₄S₂ (370). Elemental analysis: calcd C, 61.59; H, 5.98; N, 15.12; S, 17.31; found, C, 61.62; H, 6.01; N, 15.11; S, 17.29.

4.2.8. 2-[(Dipropylamino)methyl]-4-phenyl-5-(2-thienyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**5h**)

This compound was obtained as shining white solid, yield: 62%; mp: 128–129 °C; IR (KBr, cm^{−1}) 3043–3098 (Ar–H), 2870–2955 (CH str.), 1578 (C=N), 1280 (C=S), 710 (Ar–H); ^1H NMR (DMSO- d_6) δ : 0.87 (t, 6H, N–CH₂–CH₂–CH₃, $J = 7.2$ Hz), 1.50–1.56 (m, 4H, N–CH₂–CH₂–CH₃, $J = 7.2$ Hz), 2.68 (t, 4H, N–CH₂–CH₂–CH₃, $J = 6.8$ Hz), 5.17 (s, 2H, N–CH₂–N), 6.66–7.70 (m, 8H, Ar–H); ^{13}C NMR (DMSO- d_6) δ : 13.19, 25.3, 50.2, 69.7, 126.7, 128.0, 129.1, 129.4, 130.1, 130.3, 130.6, 135.1, 144.7, 169.6. MW: C₁₉H₂₄N₄S₂ (372).

Elemental analysis: calcd C, 61.25; H, 6.49; N, 15.04; S, 17.21; found, C, 61.22; H, 6.46; N, 15.06; S, 17.19.

4.2.9. 4-Allyl-2-(morpholin-4-ylmethyl)-5-(2-thienyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (5i)

This compound was obtained as shining orange solid, yield: 55%; mp: 78–79 °C; IR (KBr, cm^{-1}) 3085–3090 (Ar–H), 2853–2966 (CH str.), 1665 (C=N), 1270 (C=S), 721 (C–S–C); ^1H NMR (DMSO- d_6) δ : 2.69 (t, 4H, N–CH₂ morpholine, J = 4.8 Hz), 3.56 (t, 4H, O–CH₂ morpholine, J = 4.5 Hz), 4.87–4.92 (m, 3H, N–CH₂–CH=CH₂ and N–CH₂–CH=CH₂ (trans H)), 5.12 (s, 2H, N–CH₂–N), 5.20 (d, 1H, N–CH₂–CH=CH₂, J_{cis} = 10.2 Hz), 5.90–6.03 (ddt, 1H, CH₂–CH=CH₂), 7.25–7.88 (m, 3H, Ar–H); ^{13}C NMR (DMSO- d_6) δ : 47.4, 50.7, 66.5, 69.4, 117.3, 126.2, 128.8, 130.0, 130.7, 131.9, 145.5, 169.2. MW: C₁₄H₁₈N₄OS₂ (322). Elemental analysis: calcd C, 52.15; H, 5.63; N, 17.38; S, 19.89; found, C, 52.13; H, 5.61; N, 17.40; S, 19.94.

4.2.10. 4-Allyl-2-[(4-methylpiperidin-1-yl)methyl]-5-(2-thienyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (5j)

This compound was obtained as shining pink solid, yield: 50%; mp: 60–61 °C; IR (KBr, cm^{-1}) 3073 (Ar–H), 2838–2919 (CH str.), 1506 (C=N), 1268 (C=S), 729 (C–S–C); ^1H NMR (DMSO- d_6) δ : 0.86 (d, 3H, CHCH₃), 1.03–1.24 (m, 3H, H_d and H_e), 1.56 (d, 2H, H_c, J = 10.5 Hz), 2.33 (t, 2H, H_b, J = 11.7 Hz), 3.03 (d, 2H, H_a, J = 11.7 Hz), 4.85–4.91 (m, 3H, N–CH₂–CH=CH₂ and N–CH₂–CH=CH₂ (trans H)), 5.10 (s, 2H, N–CH₂–N), 5.20 (d, 1H, N–CH₂–CH=CH₂, J_{cis} = 10.5 Hz), 5.90–6.00 (ddt, 1H, N–CH₂–CH=CH₂), 7.24–7.87 (m, 3H, Ar–H); ^{13}C NMR (DMS- d_6) δ : 22.3, 30.1, 34.3, 47.3, 50.9, 70.2, 117.1, 126.3, 128.8, 129.9, 130.6, 131.9, 145.3, 168.9. MW: C₁₆H₂₂N₄S₂ (334). Elemental analysis: calcd C, 57.45; H, 6.63; N, 16.75; S, 19.17; found, C, 57.43; H, 6.61; N, 16.72; S, 19.18.

4.2.11. 4-Allyl-2-[(4-phenylpiperazin-1-yl)methyl]-5-(2-thienyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (5k)

This compound was obtained as shining pink solid, yield: 54%; mp: 143–144 °C; IR (KBr, cm^{-1}) 3084 (Ar–H), 2821–2947 (CH str.), 1648 (C=N), 1271 (C=S), 721 (C–S–C); ^1H NMR (DMSO- d_6) δ : 3.03 (t, 4H, –CH₂–N–CH₂ piperazine ring), 3.20 (t, 4H, N–CH₂ piperazine ring), 4.87–4.89 (m, 2H, N–CH₂–CH=CH₂), 5.11 (d, 2H, N–CH₂–CH=CH₂ (trans H)), 5.29–5.30 (m, 3H, N–CH₂–N and N–CH₂–CH=CH₂ (cis H)), 5.97–6.04 (ddt, 1H, N–CH₂–CH=CH₂), 6.83–7.54 (m, 8H, Ar–H); ^{13}C NMR (DMS- d_6) δ : 47.6, 49.4, 50.4, 69.5, 116.3, 118.2, 119.9, 126.1, 128.0, 129.1, 129.3, 129.4, 130.6, 145.4, 151.3, 168.9. MW: C₂₀H₂₃N₅S₂ (397). Elemental analysis: calcd C, 60.42; H, 5.83; N, 17.62; S, 16.13; found, C, 60.44; H, 5.85; N, 17.65; S, 16.15.

4.3. Biological assays

4.3.1. Antibacterial activity

The newly synthesized compounds were screened for antibacterial activity against *E. coli* (ATTC 25922), *S. aureus* (ATTC 25923), *P. aeruginosa* (ATCC 27853) and *K. pneumoniae* (recultured) bacterial strains by serial plate dilution method [27,28]. Serial dilutions of the drug in Mullere-Hinton broth were taken in tubes and their pH was adjusted to 5.0 using phosphate buffer. Standardized suspension of the test bacterium was inoculated and incubated for 16–18 h at 37 °C. The minimum inhibitory concentration (MIC) was noted by observing the lowest concentration of the drug at which there was no visible growth. Agar media were poured into each Petri dish. Excess suspension was decanted and plates were dried by placing in incubator at 37 °C for 1 h using an agar punch, wells were made on these seeded agar plates and minimum inhibitory concentrations of the test compounds in dimethylsulfoxide (DMSO) were added into each labeled well. A control was also prepared for the plates in the same way using solvent DMSO. The Petri dishes

were prepared in triplicate and maintained at 37 °C for 3–4 days. Antibacterial activity was determined by measuring the minimum inhibitory concentration (MIC) values. The activity of each compound was compared with ciprofloxacin as standard [29,30].

4.3.2. Antifungal activity

Newly prepared compounds were screened for their antifungal activity against *A. flavus* [NCIM No. 524], *A. fumigatus* [NCIM No. 902], *P. marneffeii* [recultured] and *T. mentagrophytes* [recultured] in DMSO by serial plate dilution method [31,32]. Agar media were prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 mL) and adjusting the pH to 5.7. Normal saline was used to make a suspension of the spores of fungal strains for lawning. A loopful of particular fungal strain was transferred to 3 mL of saline to obtain a suspension of corresponding species. Agar media of 20 mL were poured into each Petri dish. Excess suspension was decanted and plates were dried by placing in an incubator at 37 °C for 1 h. An agar punch was used to produce wells on these seeded agar plates and minimum inhibitory concentrations of the test compounds in dimethylsulfoxide (DMSO) were added into each labeled well. A control was also prepared for the plates in the same way using solvent DMSO. Petri dishes were prepared in triplicate and maintained at 37 °C for 3–4 days. Antifungal activity was determined by measuring the minimum inhibitory concentration (MIC) values. The activity of each compound was compared with ciclopiroxolamine as standard.

4.3.3. DPPH free radical scavenging activity

The free radical-scavenging activity of the title compound was determined by spectrophotometric measurement of the change in the absorbance of DPPH• (1,1-diphenyl-2-picrylhydrazyl radical) at 517 nm. Stock solutions (500 μM) of the tested sample and DPPH• were prepared in DMSO. DPPH• solution (400 μM) was added to the sample solution at different concentrations (500, 1000, 1500, 2000 and 2500 μL) and appropriately diluted with DMSO to a total volume of 4.0 mL. A control was produced by diluting 400 μL from DPPH• stock solution was also diluted to 4.0 mL using DMSO solvent. For the control, only solvent was added. Ascorbic acid was used as a standard (using the reference antioxidant) for this test. For the standard, the sample was replaced with the same amount of ascorbic acid. The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging method was chosen to assess the antioxidant potential of the target compounds in comparison with the commercially available antioxidant ascorbic acid at the same concentrations. The reaction mixtures were thoroughly mixed by shaking the test tubes vigorously and incubated at 25 °C for 60 min in a water bath in the dark. Absorbance at 517 nm was measured and the solvent was corrected throughout. The scavenging effect was calculated using the following equation [33]:

$$\text{Scavenging activity(\%)} = \frac{A_0 - A_s}{A_0} \times 100$$

where A_s is the absorbance of the DPPH• in the presence of the tested compounds and standard and A_0 is the absorbance of the DPPH• in the absence of the tested compound and standard (control).

Acknowledgments

This study was supported by FUBAP (grant number FF.11.38), Elazig, Turkey.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.ejmech.2013.02.025>.

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