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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design and antiproliferative and antioxidant activities of furan-based thiosemicarbazides and 1,2,4-triazoles: their structure-activity relationship and SwissADME predictions

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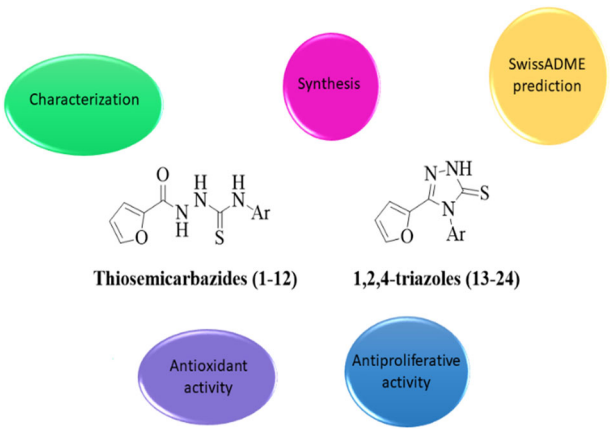
Abstract

Due to the limited number of drugs in current clinical use, the diverse biological applications of furan have encouraged the preparation of a wide variety of thiosemicarbazide and triazole derivatives for the purpose of developing new drug agents. This study aimed to investigate the antiproliferative and antioxidant activities of thiosemicarbazides (**1**–**12**) and 1,2,4-triazoles (**13**–**24**). Out of the synthesized target compounds, **3**, **4**, **6**, **7**, **8**, **9**, **10**, **11**, **12**, **15**, **16**, **18**, **19**, **20**, **21**, **22**, **23**, and **24** are novel while the synthesis of the remaining compounds is present in the literature. Compound **15** (IC_{50}: 8.81 ± 0.28 µM) showed the highest antiproliferative activity against the cervical (HeLa) cancer cell line among the compounds. In the lipid peroxidation inhibitory activity, thiosemicarbazide derivatives **3**, **10**, and **9** showed highest activity with IC_{50} of 21.80 ± 0.69, 26.49 ± 0.61, and 29.07 ± 0.52 µM, respectively, while triazole derivatives **15**, **18**, **19**, **20**, **21**, and **22** exhibited the highest activity. Moreover, physicochemical properties, pharmacokinetic properties, and drug-likeness of all synthesized products were calculated using SwissADME. In addition, the effect of the structure–activity relationships of the 1,2,4-triazole derivatives (**13**–**24**) on the results of antiproliferative and antioxidant activity assays was evaluated.

Graphical Abstract



Keywords Thiosemicarbazide · 1,2,4-Triazole · HeLa cell line · Antioxidant activity · SwissADME

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Introduction

Cancer, which has been one of the world's biggest health problems for many years, is a malignant disease of the cell cycle that involves uncontrollable mitosis of abnormal cells, which invade surrounding tissues and often spread to other parts of the body [1, 2]. About 11 million cases of cancer are diagnosed each year. Cancer, if not treated correctly, is likely to become widespread in a large proportion of the world's population [3, 4]. The cervical cancer cell line known as HeLa, which was taken from Henrietta Lack, who passed away in 1951, is the oldest and most common cell line affecting women worldwide and cervical cancer ranks fourth in terms of both incidence and mortality [5, 6]. The main treatments of the disease are surgery and radiotherapy. Surgery is performed if the disease is caught in the early stage of the disease, while radiotherapy is used for advanced stages. In the many clinical studies on cervical cancer, satisfactory results have been obtained with chemotherapy such as increasing the five-year survival rate of patients [7, 8]. Since the incidence of cervical cancer is at an undeniable level in young women, chemotherapeutic agents are needed [9]. However, despite superior studies on the design of effective chemotherapeutic drugs, there are still drawbacks involving toxicity and selectivity [10, 11]. The problem of toxicity and resistance of cancer cells to anticancer agents has led to a continuous search for new chemotherapy agents. High amounts of reactive oxygen species (ROS) have been reported to promote many aspects of tumor development and progression in almost all types of cancer [12].

ROS produced by the cellular metabolism in living cells, while essential for life, can adversely cause the destruction of tissues or affect their normal functioning [13]. By attacking healthy cells, ROS can change cell structure or cause the cell to lose its ability to function [14]. All ROS form strand breaks or damaged bases because they have the potential to interact with DNA cellular components found in the genetic material to match its unstable free electron [13]. ROS-induced oxidative damage plays an important role in the development of diseases such as neurodegenerative disorders, arthritis, arteriosclerosis, inflammation, weakening of the immune system, liver disease, brain dysfunction, cardiovascular events, diabetes, and kidney failure [15]. Furthermore, many researchers suggest that ROS damage plays a vital role particularly in the development of malignant cancer and the initiation of proliferation of cancerous cells [13]. Antioxidant defense system agents developed for the elimination or cleaning of ROS in order to prevent permanent diseases caused by ROS can prevent oxidative chain reactions, direct quenching of reactive oxygen species, enzyme inhibition, reactions responsible for chelating metal ions such as Fe^{+2} and Cu^{+}, and free radical-mediated oxidative damage of biomolecules such as proteins, nucleic acids, polyunsaturated lipids, and sugars even at low concentrations [16]. Therefore, there is a need to develop radical scavenging antioxidant agents to control the harmful effects of free radicals in the human body.

Heterocycles are compounds where the cyclic ring contains heteroatoms i.e., nitrogen, oxygen, and sulfur. They are capable of various supramolecular interactions including hydrogen bonding, self-assembly, pi-stacking, ability to bind to enzymes, forming coordination bonds with metals and Van der Waals and hydrophobic forces [17, 18]. Many enzyme binding pockets undergo interactions with heterocycles as they possess versatile functionalities and can plays important roles in the biological pathways. There are various studies where heterocycles have been found to treat cancer or disrupt the continuity of the cancer progression [18]. In particular, furan-derived rings are an important class of heterocyclic compounds with very important biological properties. Many researchers have shown intense interest in the synthesis of a new furan-derived scaffold with different pharmacological activities for the discovery of new drugs in recent decades [19]. On the other hand, 1,2,4-triazole ($C_2N_3H_3$) derivatives containing three nitrogen atoms from five-membered ring systems, which were firstly described by Blodin, have become the focus of attention of many research teams for obtaining synthetic agents with high bioavailability due to the known antidepressant, anticancer, anti-inflammatory, analgesic, antiviral, and antioxidant effectiveness of these compounds [20–22].

To date, all triazoles recorded in the literature have been of synthetic origin, and there is no study showing that a triazole and its derivative isolated from natural products have been detected [23]. The synthesis strategy of the present study, inspired by the known anticancer therapeutic effect of 1,2,4-triazole, due to the need to discover alternative agent(s) for cancer, which is the biggest health problem of the age, started with the synthesis of some thiosemicarbazide derivatives (**1**–**12**) from heterocyclic-based 2-furanoylhydrazide, followed by the synthesis of the activity potential agent 1,2,4-triazole derivatives (**13**–**24**). After the structures of all the derivatives synthesized were elucidated, their in vitro antiproliferative activity against the HeLa cancer cell line and in vitro antioxidant activity were tested. In addition, the physicochemical properties (including Lipinski), pharmacokinetics, drug-likeness, and medicinal chemistry properties of products synthesized (**1**–**24**) were calculated using the program SwissADME.

Results and discussion

Chemistry

This study is the first to determine the in vitro antiproliferative activity against HeLa cancer cells and

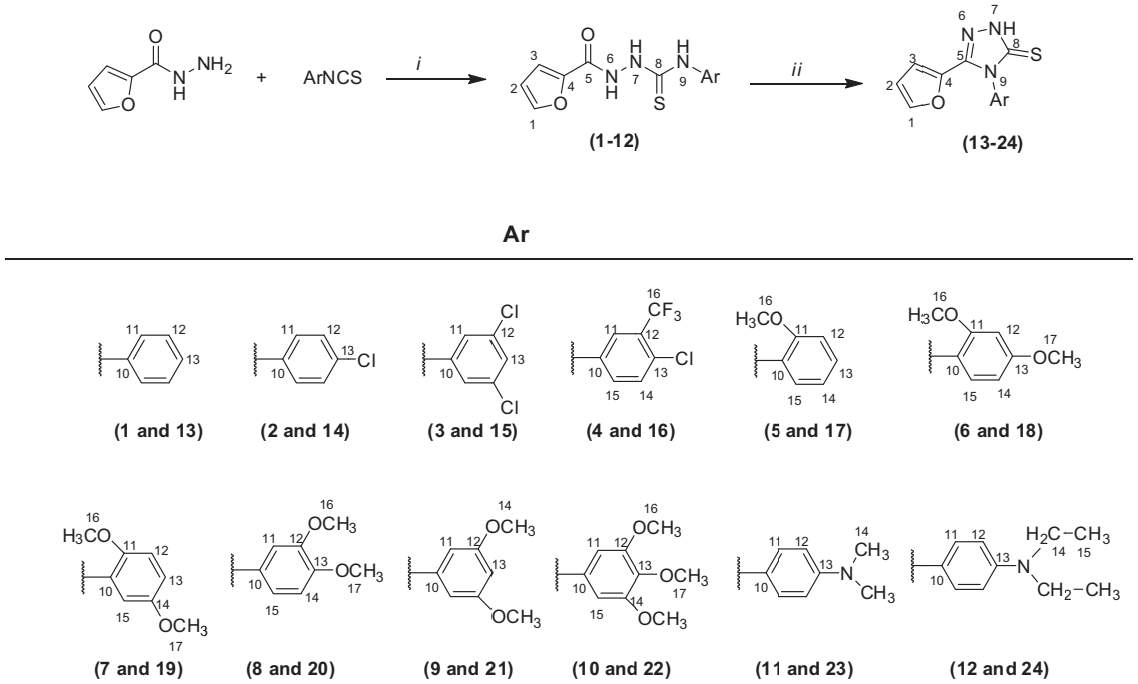

Fig. 1 Synthetic pathway of thiosemicarbazides **(1–12)** and 1,2,4-triazoles **(13–24)**. Reagents and conditions: **a** DCM, rt.; **b** EtOH, reflux

antioxidant activity of thiosemicarbazides (**1–12**) and triazoles (**13–24**). The synthetic route to prepare the thiosemicarbazides (**1–12**) and triazoles (**13–24**), the target molecules, is given in Fig. 1.

In the IR spectra, ν(N-H) stretching bands of thiosemicarbazide derivates **1–12** for CONH, PhNH, and CSNH were observed at 3748–3629 cm^{-1}, 3351–3211 cm^{-1}, and 3211–3117 cm^{-1}, respectively [24]. In addition, characteristic ν(C = 0) and ν(C = S) stretching bands were determined at 1689–1647 cm^{-1} and 1118–1012 cm^{-1}, respectively. Moreover, the stretching bands of the N-H group was determined to be absorption at 2600–2550 cm^{-1}. The ν(N-H) stretching band for CSNH of triazole derivates **13–24** was observed at 3276–3056 cm^{-1}. The ν(N-H) stretching band of CONH and PhNH observed for thiosemicarbazide derivates **1–12** disappeared in triazoles **13–24**.

In the ^{1}H NMR spectra of the thiosemicarbazides **1–12** and 1,2,4-triazole compounds **13–24**, the protons of the phenyl ring and its substituents in compounds **1–24** appeared to be resonance in the expected regions. Moreover, the protons (H-1, H-2, and H-3) of the furan ring in compounds **1–12** were found to have resonance at 7.78–8.02, 6.31–6.70, and 7.23–7.29 ppm, respectively, while the protons (H-1, H-2, and H-3) of the furan ring in compounds **13–24** were found to have resonance at 7.73–8.14, 6.31–6.70, and 5.86–6.30 ppm, respectively. In general, the aromatic protons (H-11, H-12, and H-13) were observed between δ 6.60 and 7.87 ppm for thiosemicarbazides **1–12**. The NH (H-6, H-7, and H-9) protons of thiosemicarbazide (-NH-NH-C = S-NH-) derivatives **1–12** were detected at δ 9.11–10.03, 10.34–11.29, and 9.55–10.52 ppm, respectively. ^{1}H NMR protons of the thiosemicarbazide scaffold were found to be compatible with the reported chemical shift values reported earlier [25]. The resonance of proton H-7 in the 1,2,4-triazole derivatives at 13.97–14.22 ppm and the disappearance of the H-6 and H-9 protons in the thiosemicarbazide derivatives in the cyclization products were definite evidence of the synthesis of 1,2,4-triazole derivatives. It has been reported in the literature that the NH proton of the 1,2,4-triazole ring resonates at 13.99–14.01 ppm [26].

In the ^{13}C NMR spectra of thiosemicarbazides **1–12**, carbon resonances of the C = O and C = S groups were determined at 158.02–159.13 cm^{-1} and 180.63–182.27 cm^{-1}, respectively. In the study conducted by [27], it was determined that the ^{13}C NMR spectra of C = O and C = S chemical shift values in the thiosemicarbazide scaffold were in similar ranges with the C = O and C = S chemical shift values of thiosemicarbazide derivatives **(1–12)** in this study. In the ^{13}C NMR spectra, the C-5 carbon of azomethine (-C = N-) and the C_8 carbon of thioxo (-C = S-) of 1,2,4-triazole derivatives **13–24** resonated at δ 145.69–162.28 ppm and 168.82–169.57 ppm. The aromatic carbons were observed between δ 102.07 and 156.66 ppm for 1,2,4-triazole derivatives **13–24**, while C-1, C-2, C-3, and C-4 of the furan ring of 1,2,4-triazole derivatives **13–24** were observed at δ 140.17–144.25 ppm, 111.51–112.43 ppm, 111.82–113.31 ppm, and 143.37–145.97 ppm, respectively. The aromatic

protons and carbons were shifted up or down by the influence of electron-withdrawing or electron-donating groups connected to the aromatic ring.

Three spin systems were observed in the COSY spectrum (H-14-H-15, H-11-H-12) of compound **24**, which was chosen as a model compound for the 2D NMR spectrum (Fig. S1).

The HMQC spectrum of compound **24** showed that H-1 was in correlation with C-1, and H-2 with C-2, H-3 with C-3, H-5 with C-8, H-6 with C-9, H-7 with C-11, and H-8 with C-12 (Fig. S2).

The values obtained from the elemental analysis of thiosemicarbazides **1-12** and 1,2,4-triazole derivatives **13–24** were compatible with the calculated values.

Pharmacology

Antiproliferative activity of the synthesized compounds

The antiproliferative activities of compounds **1–24** against HeLa cell lines were investigated at four concentrations (100, 50, 25, and 5 μM). The IC_{50} values of compounds **1–24** against HeLa are given at Table 1. The anticancer activity of **1–24** increased with the increasing concentration against the HeLa cell lines. The antiproliferative activity of 1,2,4-triazole derivatives **13–24** against the HeLa cells was almost twice as high as that of thiosemicarbazide derivatives **1–12** obtained by ring closure of thiosemicarbazide derivatives. Compound **3** (IC_{50}: 19.83 ± 0.42 μM) from thiosemicarbazide derivatives **1–12** showed the highest antiproliferative activity against HeLa cancer cells. Among the 1,2,4-triazole derivatives **13–24** compound **15** (IC_{50}: 8.81 ± 0.28 μM) exhibited the highest activity. Additionally, compound **18** displayed moderate activity with an IC_{50} value of 27.31 ± 0.05 μM, followed by compound **16** (IC_{50}: 30.54 ± 0.19 μM).

Table 1 IC_{50} values against HeLa cancer cells of synthesized compounds **1–24**

Thiosemicarbazide derivatives		1,2,4-Triazole derivatives	
Compounds	IC_{50} (μM)	Compounds	IC_{50} (μM)
1	76.93 ± 0.51	13	42.50 ± 0.81
2	74.64 ± 0.19	14	47.87 ± 0.74
3	19.83 ± 0.42	15	8.81 ± 0.28
4	62.73 ± 0.25	16	30.54 ± 0.65
5	86.37 ± 0.33	17	45.07 ± 0.68
6	61.10 ± 0.84	18	27.31 ± 0.59
7	75.16 ± 0.31	19	48.87 ± 0.27
8	69.69 ± 0.96	20	44.08 ± 0.66
9	97.62 ± 0.55	21	51.44 ± 0.40
10	89.57 ± 0.73	22	42.67 ± 0.67
11	52.32 ± 0.61	23	53.55 ± 0.38
12	68.17 ± 0.27	24	49.12 ± 0.16
5-FU	4.93 ± 0.24	5-FU	4.93 ± 0.24

Antioxidant activity of the synthesized compounds

The in vitro antioxidant activities of thiosemicarbazides (**1–12**) and 1,2,4-triazoles (**13–24**) were determined by four complimentary assays, namely β-carotene-linoleic acid, $ABTS^{\cdot+}$ scavenging, CUPRAC, and $DPPH^{\cdot}$ scavenging methods. Generally, the antioxidant activity of 1,2,4-triazoles (**13–24**) was higher than that of the thiosemicarbazides derivatives (**1–12**). The antioxidant activity results of compounds **1–24** are shown in Table 2. According to these assay results, compounds **3**, **10**, and **9** of the thiosemicarbazide derivatives (**1–12**) demonstrated the highest lipid peroxidation inhibitory activity IC_{50} values of 21.80 ± 0.69, 26.49 ± 0.61, and 29.07 ± 0.52 μM, respectively, while compounds **15**, **18**, **19**, **20**, **21**, and **22** of the 1,2,4-triazole derivatives (**13–24**) exhibited the highest lipid peroxidation inhibitory activity. Among all the synthetic products (**1–24**), compounds **3**, **9**, and **10** of the thiosemicarbazide series and compounds **15** (IC_{50}: 9.38 ± 0.93 μM), **22** (IC_{50}: 14.79 ± 0.26 μM), **21** (IC_{50}: 19.88 ± 0.75 μM), **20** (IC_{50}: 21.35 ± 0.14 μM), **18** (IC_{50}: 23.96 ± 0.24 μM), and **19** (IC_{50}: 26.74 ± 0.66 μM) of the 1,2,4-triazole series showed the best cation radical scavenging activity against $ABTS^{\cdot+}$. Compound **3** of the thiosemicarbazide series (**1–12**) and compound **15** of the 1,2,4-triazole derivatives (**13–24**) showed excellent antioxidant activity in all four assays. In addition, while the CUPRAC activity of all synthesized compounds was higher than that of α-TOC used as pharmaceutical standard in the assay, $DPPH^{\cdot}$ scavenging activity showed it was higher than that of BHT used as positive standard.

SwissADME Prediction of synthesized compounds

The data predicted for the physicochemical characteristics, lipophilicity, solubility, pharmacokinetics, drug likeness, and medicinal chemistry of synthesized compounds evaluated by SwissADME are given in Table S1 (see: supplementary file). According to Lipinski's rule of five, the molecular weights of thiosemicarbazide and 1,2,4-triazole derivatives were 261.30–363.74 and 243.28–345.73 Da, respectively, and within the limits of 200–600 Da. The logP values of the thiosemicarbazide (**1–12**) and 1,2,4-triazole (**13–24**) derivatives were less than 5, in the range of 2.24–3.11. The HBA number of all synthesis products was between 2 and 5 and less than 10; the number of HBD atoms for the thiosemicarbazide (**1–12**) and 1,2,4-triazole (**13–24**) derivatives was 3 and 1, respectively, less than 5 [28].

Table 2 Antioxidant activity results of thiosemicarbazide **1–12** and 1,2,4-triazole derivatives **13–24**[a]

Compound	β-carotene/ linoleic acid assay IC_{50} (μM)	$DPPH^{\cdot}$ assayIC_{50} (μM)	$ABTS^{\cdot+}$ assay IC_{50} (μM)	CUPRAC $A_{0.5}$ (μM)
1	52.96 ± 0.74	49.31 ± 0.48	56.03 ± 0.31	37.68 ± 0.02
2	40.39 ± 0.80	41.43 ± 0.77	43.19 ± 0.84	29.94 ± 0.01
3	21.80 ± 0.69	18.21 ± 0.75	19.20 ± 0.25	10.11 ± 0.00
4	44.37 ± 0.17	43.94 ± 0.11	45.92 ± 0.87	31.32 ± 0.01
5	39.02 ± 0.66	36.17 ± 0.89	39.21 ± 0.34	26.39 ± 0.02
6	32.58 ± 0.31	28.94 ± 0.52	32.27 ± 0.06	21.77 ± 0.00
7	35.07 ± 0.55	32.19 ± 0.08	35.80 ± 0.72	24.28 ± 0.02
8	30.14 ± 0.33	25.81 ± 0.33	30.03 ± 0.76	19.22 ± 0.00
9	29.07 ± 0.52	24.09 ± 0.36	27.06 ± 0.49	15.09 ± 0.03
10	26.49 ± 0.61	22.16 ± 0.51	23.20 ± 0.37	13.47 ± 0.01
11	47.34 ± 0.19	47.16 ± 0.20	51.37 ± 0.44	35.64 ± 0.00
12	43.09 ± 0.73	46.44 ± 0.34	49.54 ± 0.63	34.03 ± 0.01
13	41.86 ± 0.52	38.29 ± 0.99	40.17 ± 0.65	34.61 ± 0.00
14	32.67 ± 0.11	29.34 ± 0.95	32.38 ± 0.15	27.82 ± 0.00
15	16.28 ± 0.30	8.39 ± 0.87	9.38 ± 0.93	5.75 ± 0.01
16	34.07 ± 0.89	33.29 ± 0.23	34.92 ± 0.16	29.13 ± 0.00
17	30.12 ± 0.27	25.65 ± 0.18	30.71 ± 0.55	22.29 ± 0.01
18	25.87 ± 0.30	19.31 ± 0.66	23.96 ± 0.24	16.50 ± 0.03
19	26.32 ± 0.18	22.92 ± 0.44	26.74 ± 0.66	19.36 ± 0.00
20	22.39 ± 0.79	18.49 ± 0.37	21.35 ± 0.14	14.79 ± 0.01
21	21.26 ± 0.08	16.70 ± 0.51	19.88 ± 0.75	11.13 ± 0.00
22	19.17 ± 0.34	13.39 ± 0.07	14.79 ± 0.26	9.47 ± 0.00
23	39.78 ± 0.51	37.62 ± 0.43	36.21 ± 0.61	33.07 ± 0.02
24	37.55 ± 0.60	36.81 ± 0.32	35.99 ± 0.30	31.37 ± 0.03
BHT[b]	2.34 ± 0.09	54.97 ± 0.99	2.91 ± 0.55	3.80 ± 0.02
α-TOC[b]	4.50 ± 0.09	12.26 ± 0.07	4.87 ± 0.45	40.48 ± 0.02

$p < 0.05$, significantly different with student's *t*-test

[a]Values expressed are means ± S.E.M. of three parallel measurements

[b]Reference compound

Brain Or IntestinaL EstimateD permeation (BOILED-Egg) method is a graphical model that works by calculating the polarity and lipophilicity of small molecules. This prediction provides a visual clue to the synthesis design of new compounds in terms of the oral absorption potential of drug candidates [29]. Graphical estimations of gastrointestinal absorption and blood-brain barrier (BBB) penetration of the synthesized thiosemicarbazide and 1,2,4-triazole derivatives are shown in Fig. 2. According to the BOILED-Egg plot, none of the compounds synthesized were located in the BBB (except compound **13** of the 1,2,4-triazole derivatives) with the yellow circle expressing good intestinal absorption and the gray region representing poor intestinal absorption. All compounds (except compound **13**) are contained within the while ellipse representing human intestinal absorption. Compound **10** was found to be the blue spot, evidence of its good bioavailability. Thus, compound **10** demonstrated that it could be a substrate for P-glycoprotein and it could reduce its absorption and penetration in the brain. All compounds except compound **13** can be promising agents that can very easily be absorbed by the gastrointestinal tract without potential BBB permeability. Since these compounds cannot cross the BBB, they do not cause central nervous system depression or drowsiness as side effects.

Conclusions

The current research focused mainly on the synthesis of some 1,2,4-triazole derivatives (**13–24**) and determination of the antiproliferative and antioxidant activities of the synthesized compounds. Based on the finding that the compounds can increase the absorption power of free radicals with their high nitrogen content, 1,2,4-triazole derivatives (**13–24**) were synthesized in order to prevent various disorders caused by free radicals. The synthetic route to the 1,2,4-triazoles (**13–24**) was based on cyclization of thiosemicarbazides (**1–12**). Of the series synthesized, compound **15** exhibited the best antiproliferative activity against cervical cell lines. According to the results of antioxidant capacity, compounds **15**, **20**, **21**, and **22**, which protect against lipid peroxidation and radical damage, may be drug candidates as antioxidant agents. It is observed that chemical structural variation in the synthesized molecules leads to different bioactivity and of course structural modification of the molecules changes the biological activity in a regular trend [30]. This situation, especially the substitution of chlorine or methoxy groups in the aromatic ring system, has been found to vary in anticancer and antioxidant activity. Based on these results, new studies in the field of medicinal chemistry on the modification of structures bearing 1,2,4-triazole core can be pioneered. Consequently, the presence of 2,4-dichloro and 2,4 or 3,4-dimethoxy substituents in the phenyl ring attached to position 4 in the 1,2,4-triazole nucleus was found to increase antioxidant and anticancer activity.

Materials and methods

General

Analytical grade chemicals and solvents were purchased from Acros, Alfa Aesar, Sigma-Aldrich, and Merck. Thin layer chromatography (TLC, Merck 60 F_{254}) was used to monitor the chemical reactions. The melting points were examined using an SMP20 melting point apparatus and were uncorrected. The FTIR spectra were acquired using a

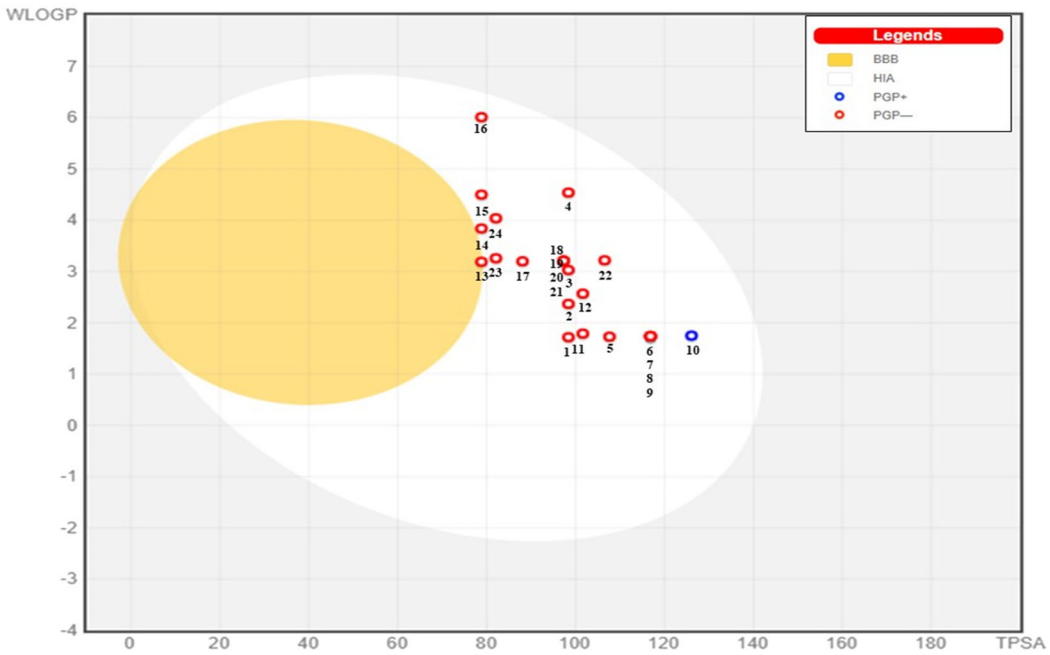

Fig. 2 Graphical distribution of synthesized thiosemicarbazides **1**–**12** and 1,2,4-triazoles **13**–**24** and enzyme inhibitor standards according to the BOILED-EGG predictive model Main text paragraph

Frontier spectrometer by attenuated total reflectance (ATR) apparatus (PerkinElmer, Waltham, Massachusetts, USA). Elemental analyses (CHNS) were performed on a Thermo Scientific Flash 2000 elemental analyzer (Finnigan MAT, USA). The ^{1}H-NMR, ^{13}C-NMR, COSY, and HMQC spectra were recorded on an Agilent Technologies 400 MHz NMR spectrometer (Agilent, USA). The cell proliferation BrdU ELISA kits were supplied by Roche (Roche Diagnostic GmbH, Germany). The FTIR and NMR data of compounds **1**–**24** are presented in the supplementary data section.

Synthesis

General synthetic procedure for compounds 1–12

2-Furoic acid hydrazide (0.01 mol) and substituted phenylisothiocyanate (0.01 mol) were dissolved in methanol and the mixture was refluxed overnight. After completion of the reaction, the mixture was left to cool down in order to precipitate the solids. Finally, ethanol was used to purify the precipitate [31].

1-[(furan-2-carbonyl)]-4-[phenyl]thiosemicarbazide (1) [32]

White solid. Yield: 73%; m.p. 205–206 °C. FTIR νmax (cm^{-1}): 3659, 3317, 3135 (NH stretching), 3057 (ArCH stretching), 1670 (C = O), 1294 (C-N), 1068 (C = S). ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.88 (d, 1H, $J_1 = 3.2$ Hz, $J_2 = 1.6$ Hz, H-2), 7.16 (t, 1H, $J = 7.2$ Hz, H-13), 7.25 (d, 1H, $J = 3.2$ Hz, H-3), 7.33 (t, 2H, $J = 7.6$ Hz, H-12), 7.45 (d, 2H, $J = 7.2$ Hz, H-11), 7.92 (brs, 1H, H-1), 9.67 (brs, 1H, H-6), 9.82 (brs, 1H, H-9), 10.42 (brs, 1H, H-7). ^{13}C NMR (150 MHz, DMSO-d_6): δ 112.33 (C-2), 115.26 (C-3), 125.49 (C-13), 126.38 (C-11), 128.35 (C-12), 139.65 (C-10), 146.09 (C-1), 146.87 (C-4), 158.07 (C-5), 181.53 (C-8). Anal. calc. for ($C_{12}H_{11}N_3O_2S$): C, 55.16; H, 4.24; N, 16.08; S: 12.27%; found: C, 55.10; H, 4.16; N, 16.10; S: 11.98%.

1-[(furan-2-carbonyl)]-4-[4-chlorophenyl]thiosemicarbazide (2) [32]

White solid. Yield: 71%; m.p. 218–220 °C. FTIR νmax (cm^{-1}): 3671, 3293, 3180 (NH stretching), 2989 (ArCH stretching), 1668 (C = O), 1300 (C-N), 1090 (C = S), 1013 (C-Cl). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 6.68 (dd, 1H, $J_1 = 3.6$ Hz, $J_2 = 1.6$ Hz, H-2), 7.25 (d, 1H, $J = 3.2$ Hz, H-3), 7.38 (d, 2H, $J = 8.8$ Hz, H-11), 7.49 (d, 2H, $J = 8.0$ Hz, H-12), 7.92 (d, 1H, $J = 0.8$ Hz, H-1), 9.77 (s, 1H, H-6), 9.86 (brs, 1H, H-9), 10.44 (s, 1H, H-7). ^{13}C NMR (150 MHz, DMSO-d_6): δ 112.36 (C-2), 115.35 (C-3), 127.93 (C-11), 128.26 (C-13), 129.51 (C-12), 138.66 (C-10), 146.15 (C-1), 146.81 (C-4), 158.09 (C-5), 181.53 (C-8). Anal. calc. for ($C_{12}H_{10}ClN_3O_2S$): C, 48.73; H.3.41; N, 14.21; S: 10.84%; found: C, 47.99; H, 3.40; N, 14.17; S: 10.75%.

1-[(furan-2-carbonyl)]-4-[3,5-(dichloro)phenyl] thiosemicarbazide (3)

White solid. Yield: 90%; m.p. 211–213 °C. FTIR νmax (cm^{-1}): 3667, 3316, 3209 (NH stretching), 2988 (ArCH stretching), 1672 (C = O), 1298 (C-N), 1118 (C = S), 1015 (C-Cl). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 6.68 (dd, 1H, $J_1 = 3.2$ Hz, $J_2 = 1.6$ Hz, H-2), 7.26 (d, 1H, $J = 3.2$ Hz, H-3), 7.37 (brs, 1H, H-13), 7.71 (s, 2H, H-11), 7.94 (brs, 1H, H-1), 9.94 (brs, 1H, H-6), 10.02 (brs, 1H, H-9), 10.49 (brs, 1H, H-7). ^{13}C NMR (150 MHz, DMSO-d_6): δ 112.44 (C-2), 115.57 (C-3), 123.69 (C-13), 124.48 (C-11), 133.40 (C-12), 142.13 (C-10), 146.27 (C-1), 146.69 (C-4), 158.11 (C-5), 181.20 (C-8). Anal. calc. for ($C_{12}H_9Cl_2N_3O_2S$): C, 43.65; H, 2.75; N, 12.73; S: 9.71%; found: C, 44.01; H, 2.70; N, 12.76; S: 9.88%.

1-[(furan-2-carbonyl)]-4-[{4-chloro-3-(trifluoromethyl) phenyl}]thiosemicarbazide (4)

White solid. Yield: 84%; m.p. 222–224 °C. FTIR νmax (cm^{-1}): 3671, 3315, 3211 (NH stretching), 3084 (ArCH stretching), 1672 (C = O), 1321 (C-N), 1111 (C-F), 1080 (C = S), 1015 (C-Cl). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 6.70 (d, 1H, $J = 1.6$ Hz, H-2), 7.27 (d, 1H, $J = 2.8$ Hz, H-3), 7.68 (d, 1H, $J = 8.4$ Hz, H-14), 7.87–7.96 (m, 2H, H-11 and H-15), 8.02 (d, 1H, $J = 0.8$ Hz, H-1), 10.03 (s, 1H, H-6), 10.52 (s, 1H, H-9), 11.29 (s, 1H, H-7). ^{13}C NMR (150 MHz, DMSO-d_6): δ 112.45 (C-2), 112.89 (C-11), 113.33 (C-3), 122.42 (C-15), 130.79 (C-13), 131.59 (C-16), 132.96 (C-12), 139.08 (C-14), 146.28 (C-10), 146.72 (C-1), 151.82 (C-4), 159.13 (C-5), 181.41 (C-8). Anal. calc. for ($C_{13}H_9ClF_3N_3O_2S$): C, 42.93; H, 2.49; N, 11.55; S: 8.82%; found: C, 42.86; H, 2.39; N, 11.62; S: 8.75%.

1-[(furan-2-carbonyl)]-4-[{5-chloro-2-(methoxy)phenyl}] thiosemicarbazide (5) [32]

White solid. Yield: 59%; m.p. 209–211 °C. FTIR νmax (cm^{-1}): 3672, 3264, 3143 (NH stretching), 2989 (ArCH stretching), 1668 (C = O), 1315 (C-N), 1086 (C = S), 1020 (C-Cl). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 3.76 (s, 3H, H-16), 6.69 (d, 1H, $J = 1.3$ Hz, H-2), 7.08 (d, 1H, $J = 7.8$ Hz, H-12), 7.21 (d, 1H, $J = 7.8$ Hz, H-13), 7.29 (d, 1H, $J = 3.3$ Hz, H-3), 7.94 (brs, 2H, H-1 and H-15), 9.26 (brs, 1H, H-6), 9.93 (brs, 1H, H-9), 10.57 (brs, 1H, H-7). ^{13}C NMR (150 MHz, DMSO-d_6): δ 56.57 (C-16), 112.38 (C-2), 113.45 (C-12), 115.62 (C-3), 123.16 (C-15), 123.50 (C-13), 126.27 (C-14), 129.47 (C-10), 146.44 (C-1), 149.90 (C-4), 151.88 (C-11), 158.09 (C-5), 182.27 (C-8). Anal. calc. for ($C_{13}H_{12}ClN_3O_3S$): C, 47.93; H, 3.71; N, 12.90; S: 9.84%; found: C, 47.83; H, 3.61; N, 12.93; S: 9.82%.

1-[(furan-2-carbonyl)]-4-[2,4-(dimethoxy)phenyl] thiosemicarbazide (6)

Cream solid. Yield: 37%; m.p. 199–201 °C. FTIR νmax (cm^{-1}): 3285, 3186 (NH stretching), 2988 (ArCH stretching), 2836 (R-CH), 1647 (C = O), 1269 (C-N), 1027 (C = S). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 3.74–3.83 (m, 6H, H-16 and H-17), 6.51 (d, 1H, $J = 8.4$ Hz, H-14), 6.60 (brs, 1H, H-2), 6.67 (brs, 1H, H-12), 7.26 (d, 1H, $J = 2.8$ Hz, H-3), 7.44 (d, 1H, $J = 8.4$ Hz, H-15), 7.91 (brs, 1H, H-1), 9.11 (brs, 1H, H-6), 9.59 (brs, 1H, H-9), 10.43 (s, 1H, H-7). ^{13}C NMR (150 MHz, DMSO-d_6): δ 55.74 (C-16), 56.09 (C-17), 99.28 (C-12), 104.46 (C-14), 112.27 (C-2), 115.32 (C-3), 121.42 (C-15), 129.16 (C-10), 146.19 (C-1), 146.69 (C-4), 151.42 (C-11), 157.08 (C-13), 158.12 (C-5), 182.00 (C-8). Anal. calc. for ($C_{14}H_{15}N_3O_4S$): C, 52.33; H, 4.70; N, 13.08; S: 9.98%; found: C, 52.28; H, 4.69; N, 13.13; S: 9.95%.

1-[(furan-2-carbonyl)]-4-[2,5-(dimethoxy)phenyl] thiosemicarbazide (7)

White solid. Yield: 77%; m.p. 204–206 °C. FTIR νmax (cm^{-1}): 3658, 3211, 3117 (NH stretching), 2997 (ArCH stretching), 2832 (R-CH), 1668 (C = O), 1280 (C-N), 1012 (C = S). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 3.70 (s, 6H, H-16 and H-17), 6.70 (d, 1H, $J = 2.4$ Hz, H-2), 6.71 (d, 1H, $J = 9.2$ Hz, H-13), 6.96 (d, 1H, $J = 9.2$ Hz, H-12), 7.29 (d, 1H, $J = 3.2$ Hz, H-3), 7.78–7.94 (m, 2H, H-1 and H-15), 9.17 (brs, 1H, H-6), 9.86 (brs, 1H, H-9), 10.55 (brs, 1H, H-7). ^{13}C NMR (150 MHz, DMSO-d_6): δ 55.81(C-17), 56.75 (C-16), 110.45 (C-13), 111.94 (C-15), 112.38 (C-2), 112.69 (C-12), 115.59 (C-3), 128.90 (C-10), 146.38 (C-1), 146.48 (C-4), 148.45 (C-11), 152.87 (C-14), 158.09 (C-5), 180.63 (C-8). Anal. calc. for ($C_{14}H_{15}N_3O_4S$): C, 52.33; H, 4.70; N, 13.08; S: 9.98%; found: C, 52.30; H, 4.72; N, 13.11; S: 9.93%.

1-[(furan-2-carbonyl)]-4-[3,4-(dimethoxy)phenyl] thiosemicarbazide (8)

White solid. Yield: 66%; m.p. 200–202 °C. FTIR νmax (cm^{-1}): 3629, 3310, 3125 (NH stretching), 2957 (ArCH stretching), 2836 (R-CH), 1686 (C = O), 1280 (C-N), 1023 (C = S). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 3.73 (s, 3H, H-16), 3.75 (s, 3H, H-17), 6.68 (dd, 1H, $J_1 = 3.6$ Hz, $J_2 = 1.6$ Hz, H-2), 6.89–6.86 (m, 2H, H-14 and H-15), 7.08 (brs, 1H, H-11), 7.25 (d, 1H, $J = 3.2$ Hz, H-3), 7.92 (d, 1H, $J = 0.8$ Hz, H-1), 9.58 (brs, 1H, H-6), 9.70 (brs, 1H, H-9), 10.38 (s, 1H, H-7). ^{13}C NMR (150 MHz, DMSO-d_6): δ 55.89 (C-17), 56.05 (C-16), 110.98 (C-11), 111.40 (C-2), 112.32 (C-14), 115.24 (C-3), 118.36 (C-15), 132.65 (C-10), 146.07 (C-1), 146.70 (C-13), 146.87 (C-4), 148.25 (C-12), 158.10

(C-5), 181.44 (C-8). Anal. calc. for ($C_{14}H_{15}N_3O_4S$): C, 52.33; H, 4.70; N, 13.08; S, 9.98%; found: C, 52.26; H, 4.61; N, 13.35; S, 9.86%.

1-[(furan-2-carbonyl)]-4-[3,5-(dimethoxy)phenyl] thiosemicarbazide (9)

White solid. Yield: 65%; m.p. 203–205 °C. FTIR νmax (cm^{-1}): 3654, 3320, 3145 (NH stretching), 2961 (ArCH stretching), 2813 (R-CH), 1689 (C = O), 1264 (C-N), 1057 (C = S). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 3.72 (s, 6H, H-14), 6.31 (brs, 1H, H-2), 6.65 (s, 1H, H-13), 6.79 (brs, 2H, H-11), 7.25 (d, 1H, $J = 2.4$ Hz, H-3), 7.92 (brs, 1H, H-1), 9.69 (s, 2H, H-6 and H-9), 10.40 (s, 1H, H-7). ^{13}C NMR (150 MHz, DMSO-d_6): δ 55.63 (C-14), 97.32 (C-13), 104.00 (C-11), 112.34 (C-2), 115.31 (C-3), 141.23 (C-10), 146.13 (C-1), 146.82 (C-4), 158.10 (C-5), 160.15 (C-12), 181.01 (C-8). Anal. calc. for ($C_{14}H_{15}N_3O_4S$): C, 52.33; H, 4.70; N, 13.08; S, 9.98%; found: C, 52.31; H, 4.76; N, 13.09; S, 9.94%.

1-[(furan-2-carbonyl)]-4-[3,4,5-(trimethoxy)phenyl] thiosemicarbazide (10)

White solid. Yield: 50%, m.p. 222–224 °C. FTIR νmax (cm^{-1}): 3748, 3241, 3136 (NH stretching), 2988 (ArCH stretching), 2842 (R-CH), 1681 (C = O), 1263 (C-N), 1013 (C = S). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 3.65 (s, 3H, H_{15}), 3.75 (s, 6H, H_{14}), 6.68 (dd, 1H, $J_1 = 3.2$ Hz, $J_2 = 1.6$ Hz, H_2), 6.89 (brs, 2H, H_{11}), 7.26 (d, 1H, $J = 3.2$ Hz, H_3), 7.92 (brs, 1H, H_1), 9.66 (brs, 2H, H_6 and H_9), 10.38 (brs, 1H, H_7). ^{13}C NMR (150 MHz, DMSO-d_6): δ 55.25 (C_{14}), 60.51 (C_{15}), 103.58 (C_{11}), 112.35 (C_2), 115.34 (C_3), 135.06 (C_{10}), 135.38 (C_{13}), 146.14 (C_1), 146.81 (C_4), 152.45 (C_{12}), 158.16 (C_5), 181.02 (C_8). Anal. calc. for ($C_{15}H_{17}N_3O_5S$): C, 51.27; H, 4.88; N, 11.96; S: 9.13%; found: C, 51.23; H, 4.84; N, 11.90; S: 9.10%.

1-[(furan-2-carbonyl)]-4-[4-(dimethylamino)phenyl] thiosemicarbazide (11)

White solid. Yield: 67%; m.p. 214–216 °C. FTIR νmax (cm^{-1}): 3658, 3351, 3132 (NH stretching), 2961 (ArCH stretching), 2805 (R-CH), 1683 (C = O), 1263 (C-N), 1056 (C = S). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 2.88 (s, 6H, H-14), 6.67–6.69 (m, 3H, H-2 and H-11), 7.17 (d, 2H, $J = 8.8$ Hz, H-12), 7.24 (d, 1H, $J = 3.2$ Hz, H-3), 7.91 (d, 1H, $J = 0.8$ Hz, H-1), 9.45 (s, 1H, H-6), 9.59 (s, 1H, H-9), 10.35 (s, 1H, H-7). ^{13}C NMR (150 MHz, DMSO-d_6): δ 40.79 (C-14), 112.17 (C-12), 112.26 (C-2), 115.12 (C-3), 127.36 (C-11), 128.77 (C-10), 146.00 (C-1), 146.93 (C-4), 148.69 (C-13), 158.05 (C-5), 181.71 (C-8). Anal. calc. for ($C_{14}H_{16}N_4O_2S$): C, 55.25; H, 5.30; N, 18.41; S, 10.53%; found: C, 55.21; H, 5.28; N, 18.35; S, 10.42%.

1-[(furan-2-carbonyl)]-4-[4-(diethylamino)phenyl] thiosemicarbazide (12)

White solid. Yield: 62%; m.p. 215-217 °C. FTIR νmax (cm^{-1}): 3672, 3346, 3203 (NH stretching), 3114 (ArCH stretching), 2967 (R-CH), 1686 (C = O), 1265 (C-N), 1079 (C = S). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.08 (t, 6H, $J = 6.8$ Hz, H-15), 3.30 (q, 4H, H-14), 6.60 (d, 2H, $J = 8.8$ Hz, H-11), 6.70 (dd, 1H, $J_1 = 3.2$ Hz, $J_1 = 1.6$ Hz, H-2), 7.12 (d, 2H, $J = 8.4$ Hz, H-12), 7.23 (d, 1H, $J = 3.2$ Hz, H-3), 7.90 (brs, 1H, H-1), 9.42 (s, 1H, H-6), 9.55 (s, 1H, H-9), 10.34 (s, 1H, H-7). ^{13}C NMR (150 MHz, DMSO-d_6): δ 12.88 (C-15), 44.16 (C-14), 111.25 (C-2), 112.26 (C-12), 115.10 (C-3), 127.63 (C-11), 127.70 (C-10), 145.68 (C-13), 146.00 (C-1), 146.93 (C-4), 158.02 (C-5), 181.55 (C-8). Anal. calc. for ($C_{16}H_{20}N_4O_2S$): C, 57.81; H, 6.06; N, 16.85; S, 9.65%; found: C, 57.86; H, 6.03; N, 16.77; S, 9.62%.

General synthetic procedure for compounds 13–24

A mixture of thiosemicarbazides **1**–**12** (0.45 mmol) and 2 N NaOH solution (10 mL) was refluxed for 4 h. The reaction mixture was filtered, allowed to cool, and then brought to pH 5.0–6.0 with a dilute solution of HCl. The crude product was dried, washed with water, and recrystallized from ethanol [31].

5-(2-furyl)-4-phenyl-2,4-dihydro-1,2,4-triazole-3-thione (13) [32] Cream solid. Yield: 67%; m.p. 209–211 °C. FTIR νmax (cm^{-1}): 3094 (NH stretching), 2986 (ArCH stretching), 1272 (C-N), 1015 (C = S). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 5.89 (d, 1H, $J = 3.6$ Hz, H-3), 6.50 (dd, 1H, $J_1 = 3.6$ Hz, $J_2 = 1.6$ Hz, H-2), 7.43–7.45 (m, 2H, H-12), 7.59–7.61 (m, 3H, H-11 and H-13), 7.81 (d, 1H, $J = 1.2$ Hz, H-1), 14.15 (s, 1H, H-7). ^{13}C NMR (100 MHz, DMSO-d_6): δ 112.07 (C-2), 113.02 (C-3), 129.09 (C-11), 130.40 (C-13), 134.81 (C-12), 140.31 (C-10), 143.61 (C-1), 145.74 (C-4), 158.84 (C-5), 169.18 (C-8). Anal. calc. for ($C_{12}H_9N_3OS$): C, 59.24; H, 3.73; N, 17.27; S, 13.18%; found: C, 59.21; H, 3.75; N, 17.12; S, 13.11%.

5-(2-furyl)-4-(4-chlorophenyl)-2,4-dihydro-1,2,4-triazole-3-thione (14) [32] White solid. Yield: 52%; m.p. 224–226 °C. FTIR νmax (cm^{-1}): 3092 (NH stretching), 2982 (ArCH stretching), 1265 (C-N), 1091 (C = S), 1024 (C-Cl). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 6.11 (d, 1H, $J = 3.6$ Hz, H-3), 6.54 (dd, 1H, $J_1 = 3.6$, $J_2 = 1.2$ Hz, H-2), 7.51 (d, 2H, $J = 8.8$ Hz, H-11), 7.67 (d, 2H, $J = 8.8$ Hz, H-12), 7.82 (d, 1H, $J = 1.2$ Hz, H-1), 14.02 (s, 1H, H-6). ^{13}C NMR

(100 MHz, DMSO-d_6): δ 112.25 (C-2), 113.21 (C-3), 130.13 (C-11), 131.17 (C-12), 133.71 (C-13), 135.12 (C-10), 140.17 (C-1), 143.45 (C-4), 145.89 (C-5), 168.97 (C-8). Anal. calc. for ($C_{12}H_8ClN_3OS$): C, 51.90; H, 2.90; N, 15.13; S, 11.55%; found: C, 51.82; H, 2.81; N, 15.16; S, 11.57%.

5-(2-furyl)-4-(3,5-dichlorophenyl)-2,4-dihydro-1,2,4-triazole-3-thione (15) Cream solid. Yield: 85%; m.p. 217–219 °C. FTIR νmax (cm^{-1}): 3056 (NH stretching), 2971 (ArCH stretching), 1278 (C-N), 1104 (C = S), 980 (C-Cl). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 6.28 (d, 1H, J = 3.6 Hz, H-3), 6.58 (dd, 1H, J_1 = 3.6 Hz, J_2 = 1.6 Hz, H-2), 7.73 (d, 1H, J = 1.6 Hz, H-1), 7.83 (brs, 1H, H-13), 7.87–7.90 (m, 2H, H-11), 14.22 (s, 1H, H-7). ^{13}C NMR (100 MHz, DMSO-d_6): δ 112.38 (C-2), 113.30 (C-3), 128.71 (C-13), 134.93 (C-11), 137.05 (C-12), 140.09 (C-10), 143.20 (C-1), 145.97 (C-4), 154.28 (C-5), 168.86 (C-8). Anal. calc. for ($C_{12}H_7Cl_2N_3OS$): C, 46.17; H, 2.26; N, 13.46; S. 10,27%; found: C, 46.23; H, 2.34; N, 13.35; S, 10.29%.

5-(2-furyl)-4-(4-chloro-3-(trifluoromethyl)phenyl)-2,4-dihydro-1,2,4-triazole-3-thione (16) White solid. Yield: 72%; m.p. 229–231 °C. FTIR νmax (cm^{-1}): 3080 (NH stretching), 2971 (ArCH stretching), 1275(C-N), 1131 (C-F), 1073 (C-Cl), 1071 (C = S). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 6.30 (d, 1H, J = 3.6 Hz, H-3), 6.56 (dd, 1H, J_1 = 3.6, J_2 = 1.6 Hz, H-2), 7.78–8.01 (m, 2H, H-11 and H-15), 7.97 (d, 1H, J = 8.4 Hz, H-14), 8.14 (d, 1H, J = 2.4 Hz, H-1), 14.21 (brs, 1H, H-7). ^{13}C NMR (100 MHz, DMSO-d_6): δ 112.35 (C-2), 113.31 (C-3), 124.17 (C-11), 129.31 (C-13), 132.70 (C-16), 134.33 (C-15), 135.27 (C-12), 140.19 (C-14), 142.34 (C-10), 143.27 (C-1), 145.91 (C-4), 155.48 (C-5), 168.90 (C-8). Anal. calc. for ($C_{13}H_7ClF_3N_3OS$): C, 45.16; H, 2.04; N, 12.15; S, 9.27%; found: C, 45.07; H, 2.11; N, 12.01; S, 9.30%.

5-(2-furyl)-4-(5-chloro-2-methoxyphenyl)-2,4-dihydro-1,2,4-triazole-3-thione (17) [32] White solid. Yield: 88%; m.p. 216–218 °C. FTIR νmax (cm^{-1}): 3094 (NH stretching), 2971 (ArCH stretching), 2773 (R-CH), 1281 (C-N), 1028 (C = S), 980 (C-Cl). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 3.69 (s, 3H, H-16), 6.14 (d, 1H, J = 3.6 Hz, H-3), 6.54 (dd, 1H, J_1 = 3.6, J_2 = 1.6 Hz, H-2), 7.30 (d, 1H, J = 8.8 Hz, H-13), 7.61–7.66 (m, 2H, H-12 and H-15), 7.81 (d, 1H, J = 1.2 Hz, H-1), 14.02 (brs, 1H, H-7). ^{13}C NMR (150 MHz, DMSO-d_6): δ 56.96 (C-16), 112.10 (C-2), 112.21 (C-3), 115.16 (C-12), 124.39 (C-15), 124.58 (C-13), 130.56 (C-10), 131.87 (C-14), 140.49 (C-11), 143.70 (C-1), 145.69 (C-4), 154.63 (C-5), 169.22 (C-8). Anal. calc. for ($C_{13}H_{10}ClN_3O_2S$): C, 50.73; H, 3.28; N, 13.65; S, 10.42%; found: C, 50.59; H, 3.23; N, 13.58; S, 10.29%.

5-(2-furyl)-4-(2,4-dimethoxyphenyl)-2,4-dihydro-1,2,4-triazole-3-thione (18) Brown solid. Yield: 65%; m.p. 205–207 °C. FTIR νmax (cm^{-1}): 3286 (NH stretching), 2918 (ArCH stretching), 1211 (C-N), 1048 (C = S). ^{1}H-NMR (400 MHz, DMSO-d_6): 3.67 (s, 3H, H-17), 3.86 (s, 3H, H-16), 5.96 (brs, 1H, H-3), 6.51 (brs, 1H, H-2), 6.70 (d, 1H, J = 7.2 Hz, H-14), 6.79 (brs, 1H, H-12), 7.29 (d, 1H, J = 8.0 Hz, H-15), 7.81 (brs, 1H, H-1), 14.02 (s, 1H, H-7). ^{13}C NMR (150 MHz, DMSO-d_6): δ 56.10 (C-16), 56.54 (C-17), 100.20 (C-12), 106.14 (C-14), 111.97 (C-2), 112.19 (C-3), 115.83 (C-15), 131.22 (C-10), 144.21 (C-1), 145.67 (C-4), 156.36 (C-11), 162.28 (C-5), 169.46 (C-8). Anal. calc. for ($C_{14}H_{13}N_3O_3S$): C, 55.43; H, 4.32; N, 13.85; S, 10.57%; found: C, 55.40; H, 4.31; N, 13.76; S, 10.55%.

5-(2-furyl)-4-(2,5-dimethoxyphenyl)-2,4-dihydro-1,2,4-triazole-3-thione (19) Cream solid. Yield: 85%; m.p. 210–212 °C. FTIR νmax (cm^{-1}): 3082 (NH stretching), 2913 (ArCH stretching), 2776 (R-CH), 1224 (C-N), 1043 (C = S). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 3.61 (s, 3H, H-16), 3.75 (s, 3H, H-17), 5.94 (d, 1H, J = 3.6 Hz, H-3), 6.50 (q, 1H, H-2), 7.03 (d, 1H, J = 2.8 Hz, H-15), 7.13 (d, 1H, J = 9.2, H-13), 7.19 (d, 1H, J = 9.2 Hz, H-12), 7.79 (brs, 1H, H-1), 14.00 (s, 1H, H-7). ^{13}C NMR (150 MHz, DMSO-d_6): δ 56.30 (C-17), 56.97 (C-16), 111.51 (C-2), 112.07 (C-3), 114.67 (C-15), 116.58 (C-13), 116.87 (C-12), 124.17 (C-10), 140.92 (C-11), 149.63 (C-14), 143.91 (C-1), 145.32 (C-4), 153.76 (C-5), 169.27 (C-8). Anal. calc. for ($C_{14}H_{13}N_3O_3S$): C, 55.43; H, 4.32; N, 13.85; S, 10.57%; found: C, 55.40; H, 4.39; N, 13.75; S, 10.36%.

5-(2-furyl)-4-(3,4-dimethoxyphenyl)-2,4-dihydro-1,2,4-triazole-3-thione (20) Cream solid. Yield: 93%; m.p. 206–208 °C. FTIR νmax (cm^{-1}): 3276 (NH stretching), 2974 (ArCH stretching), 2770 (R-CH), 1221 (C-N), 1043 (C = S). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 3.72 (s, 3H, H-17), 3.85 (s, 3H, H-16), 5.92 (d, 1H, J = 2.8 Hz, H-3), 6.51 (dd, 1H, J_1 = 3.2, J_2 = 2.8 H-2), 6.95 (d, 1H, J = 8.4, H-14), 7.07–7.13 (m, 2H, H-11 and H-15), 7.82 (brs, 1H, H-1), 14.05 (brs, 1H, H-7). ^{13}C NMR (150 MHz, DMSO-d_6): δ 56.28 (C-17), 56.50 (C-16), 112.43 (C-2), 112.11 (C-11), 112.91 (C-3), 113.06 (C-14), 121.48 (C-15), 127.34 (C-10), 140.51 (C-13), 149.74 (C-12), 143.92 (C-1), 145.57 (C-4), 150.35 (C-5), 169.37 (C-8). Anal. calc. for ($C_{14}H_{13}N_3O_3S$): C, 55.43; H, 4.32; N, 13.85; S, 10.57%; found: C, 55.42; H, 4.30; N, 13.81; S, 10.48%.

5-(2-furyl)-4-(3,5-dimethoxyphenyl)-2,4-dihydro-1,2,4-triazole-3-thione (21) Yellow solid. Yield: 91%; m.p. 209–211 °C. FTIR νmax (cm^{-1}): 3218 (NH stretching), 2970 (ArCH stretching), 2771 (R-CH), 1207 (C-N), 1159 (C = S). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 3.76 (s, 6H,

H-14), 6.03 (d, 1H, $J = 3.2$ Hz, H-3), 6.53 (dd, 1H, $J_1 = 3.2$, $J_2 = 1.6$ Hz, H-2), 6.63 (d, 2H, $J = 2.0$ Hz, H-11), 6.71 (d, 1H, $J = 2.0$, H-13), 7.83 (d, 1H, $J = 1.6$ Hz, H-1), 13.97 (brs, 1H, H-7). ^{13}C NMR (100 MHz, DMSO-d_6): δ 56.15 (C-14), 102.07 (C-13), 112.21 (C-2), 112.88 (C-3), 107.54 (C-11), 136.38 (C-10), 140.29 (C-1), 143.51 (C-4), 145.69 (C-5), 161.35 (C-12), 168.82 (C-8). Anal. calc. for ($C_{14}H_{13}N_3O_3S$): C, 55.43; H, 4.32; N, 13.85; S, 10.57%; found: C, 55.40; H, 4.28; N, 13.81; S, 10.51%.

5-(2-furyl)-4-(3,4,5-trimethoxyphenyl)-2,4-dihydro-1,2,4-triazole-3-thione (22) Cream solid. Yield: 47%; m.p. 228–230 °C. FTIR νmax (cm^{-1}): 3242 (NH stretching), 2972 (ArCH stretching), 2769 (R-CH), 1235 (C-N), 1128 (C = S). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 3.74 (s, 6H, H-14), 3.77 (s, 3H, H-15), 5.98 (d, 1H, $J = 3.6$ Hz, H-3), 6.54 (dd, 1H, $J_1 = 3.6$ Hz, $J_2 = 1.6$ Hz, H-2), 6.84 (brs, 2H, H-11), 7.92 (d, 1H, $J = 1.2$ Hz, H-1), 14.00 (s, 1H, H-7). ^{13}C NMR (100 MHz, DMSO-d_6): δ 56.81 (C-15), 60.69 (C-14), 112.28 (C-2), 112.95 (C-3), 107.06 (C-11), 130.18 (C-10), 138.93 (C-13), 140.31 (C-1), 143.37 (C-4), 145.67 (C-12), 153.80 (C-5), 168.99 (C-8). Anal. calc. for ($C_{15}H_{15}N_3O_4S$): C, 54.04; H, 4.54; N, 12.60; S, 9.62%; found: C, 54.07; H, 4.56; N, 12.57; S, 9.58%.

5-(2-furyl)-4-(4-(dimethylamino)phenyl)-2,4-dihydro-1,2,4-triazole-3-thione (23) Cream solid. Yield: 89%; m.p. 221–223 °C. FTIR νmax (cm^{-1}): 3196 (NH stretching), 2972 (ArCH stretching), 2770 (R-CH), 1327 (C-N), 1230 (C = S). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 3.00 (s, 6H, H-14), 5.86 (d, 1H, $J = 3.2$ Hz, H-3), 6.51 (dd, 1H, $J_1 = 3.6$, $J_2 = 3.6$ Hz, H-2), 6.82 (d, 2H, $J = 8.8$ Hz, H-11), 7.15 (d, 2H, $J = 8.8$ Hz, H-12), 7.83 (brs, 1H, H-1), 14.02 (brs, 1H, H-7). ^{13}C NMR (100 MHz, DMSO-d_6): δ 40.72 (C-14), 112.05 (C-2), 112.56 (C-3), 112.81 (C-12), 122.59 (C-11), 129.32 (C-10), 140.64 (C-13), 144.14 (C-1), 145.56 (C-4), 151.32 (C-5), 169.51 (C-8). Anal. calc. for ($C_{14}H_{14}N_4OS$): C, 58.72; H, 4.93; N, 19.57; S, 11.20%; found: C, 58.66; H, 4.95; N, 19.48; S, 11.14%.

5-(2-furyl)-4-(4-(diethylamino)phenyl)-2,4-dihydro-1,2,4-triazole-3-thione (24) Cream solid. Yield: 80%; m.p. 219–220 °C. FTIR νmax (cm^{-1}): 3076 (NH stretching), 2977 (ArCH stretching), 2767 (R-CH), 1270 (C-N), 1190 (C = S). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.15 (t, 6H, $J = 6.8$ Hz, H-15), 3.41 (q, 4H, H-14), 5.87 (d, 1H, $J = 3.6$ Hz, H-3), 6.53 (dd, 1H, $J_1 = 3.6$, $J_2 = 1.6$ Hz, H-2), 6.77 (d, 2H, $J = 9.2$ Hz, H-11), 7.12 (d, 2H, $J = 9.2$ Hz, H-12), 7.85 (d, 1H, $J = 0.8$ Hz, H-1), 14.02 (s, 1H, H-7). ^{13}C NMR (100 MHz, DMSO-d_6): δ 12.81 (C-15), 44.22 (C-14), 111.82 (C-2), 112.07 (C-3), 112.84 (C-12), 121.58 (C-11), 129.55 (C-10), 140.64 (C-13), 144.25 (C-1), 145.56 (C-4), 148.73 (C-5), 169.57 (C-8). Anal. calc. for ($C_{16}H_{18}N_4OS$): C, 61.12; H, 5.77; N, 17.82; S, 10.20%; found: C, 61.10; H, 5.65; N, 17.71; S, 10.24%.

Biological studies

Antiproliferative activity assay

The antiproliferative assessment of the compounds was conducted on HeLa (cervical) cells using the BrdU ELISA [33–36]. The results of this in vitro investigation were given as means ± SEM of six parallel measurements ($p < 0.01$). IC_{50} values were calculated using the online software ED_{50} plus v1.0.

Antioxidant activity assays

Solutions of thiosemicarbazides **1**–**12** and 1,2,4-triazole **23**–**24** were prepared at four different concentrations (25, 50, 100, 200 µM) in DMSO. Activity was compared using control (DMSO) and antioxidant standards (BHA and α-TOC). The results were presented as 50% inhibition versus concentration (IC_{50}) for the $ABTS^{\cdot+}$ scavenging activity, β-carotene-linoleic acid, and $DPPH^{\cdot}$ assays, while in the CUPRAC assay the results were expressed as 0.5 absorbance versus concentration ($A_{0.5}$). As given in the literature, $ABTS^{\cdot+}$ scavenging activity [37], lipid peroxidation inhibitory activity (β-carotene-linoleic acid assay) [38], cupric reducing antioxidant capacity (CUPRAC assay) [39], and DPPH radical scavenging activity [40] assays were used to determine the antioxidant activity of the thiosemicarbazides **1**–**12** and 1,2,4-triazoles **23**–**24**.

In silico ADME prediction

Computational studies of the synthesized compounds **1**–**24** were performed to predict molecular properties using the SwissADME online server [41]. The molecular volume (Mv), molecular weight (Mw), logarithm of partition coefficient (milog P), number of hydrogen-bond donors (HBDs), number of hydrogen-bond acceptors (HBAs), topological polar surface area (TPSA), number of rotatable bonds (Nrotbs), and Lipinski's rule of five of the synthesized compounds were determined.

Statistical analysis

The antiproliferative and antioxidant activity data were given as the means of six and three parallel measurements, respectively. All biological activity assays were carried out at four different concentrations, and the results were presented as IC_{50} values. The data were recorded as mean ± SEM (standard error of the mean); $p < 0.01$.

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Compliance with ethical standards

Conflict of interest The author declares no competing interest.

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