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ORIGINAL RESEARCH

Synthesis and antitumor effects of a new class of 1,2,4-triazole derivatives

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Abstract

In order to obtain more effective antitumor agents, a new class of 1,2,4-triazole derivatives bearing disulfide bond were designed and synthesized. All the final compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR and HR-ESI-MS. The in vitro cytotoxicity of the compounds on the SMMC-7721, Hela, A549 cancer cell lines and the L929 normal cell lines were assessed by cell counting kit-8 (CCK-8). Many of tested compounds **8a–h**, **9a–h**, **10a–h** had better cytotoxic activity on various cancer cell lines than positive control 5-fluorouracil, and they were less cytotoxic to normal cell line L929 than cancer cells. Among them, compounds **9e**, **9g**, and **10h** showed better cytotoxic activity on SMMC-7721 cells with IC_{50} values 4.12, 2.92, and 4.53 μM, respectively. Compounds **8a**, **9g**, **10g** and **10h** displayed high antiproliferative activity against Hela cells with IC_{50} values 6.31, 4.31, 6.31 and 3.97 μM, respectively. Compounds **8c**, **10a** and **10h** revealed effective biological potency on A549 cells with IC_{50} values 4.75, 4.92 and 3.73 μM, respectively. Moreover, a great majority of tested compounds revealed low cytotoxicity on normal cell line L929.

Keywords 1,2,4-Triazole · Disulfides · Synthesis · Antiproliferative activity · Cytotoxicity

Introduction

Cancer is a disease with high mortality characterized by abnormal cell proliferation and spread, and has become a disease that needs to be solved urgently [1]. Therefore, designing and synthesizing new anticancer drugs with high efficiency and selectivity has important modern significance [2].

Five-membered heterocyclic compounds, such as 1,3,4-oxadiazoles, 1,3,4-thiadiazoles, and 1,2,4-triazoles, have been found to possess a wide array of biological properties and been a most referenced building blocks in the field of pharmaceutical research [3–7]. Particularly, further literature survey revealed that 1,2,4-triazoles and their fused heterocyclic derivatives have expressed a high level of selectivity with versatile biological activities, such as antioxidant [3, 8, 9], anticonvulsant [10, 11], anticancer [12, 13], antibacterial [14–19], anti-inflammatory [20–23], analgesic [24, 25], antifungal [26], antitubercular [27, 28], antimalarial [29], antidepressant [30], hypoglycemic [31] and thymidine phosphorylase inhibitory properties [32–34]. In addition to this, as a nitrogen-containing heterocyclic compound, 1,2,4 triazole has high metabolic stability and unique ability to interact with biomolecular targets [35–37]. In recent years, more and more reports on the synthesis of 1,2,4-triazole derivatives have also been reported [38–41], which provides many ideas for the design of our subject. Meanwhile, disulfide derivatives are reported to show extensive pharmacological activities including targeting antitumor activity [42–44]. Consequently, the importance of disulfide group in making up biologically active compounds has received much attention.

In view of the above observations, some derivatives of 1,2,4-triazole bearing disulfide bond were synthesized in this research work and found to be good cytotoxic agents against some cancer cell. Therefore, we researched for their

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in vitro cytotoxic activities on a panel of the selected human cancer cell in lines, including SMMC-7721, Hela, A549 and L929 normal cell lines.

Materials and methods

Chemistry

All reagents and solvents used in this study were supplied from Tianjin Hengshan (P.R. China), Tianjin Jiangtiantongyi (P.R. China) and Energy Chemical (P.R. China), and used without further purification. In addition, some biological reagents were purchased from Dojindo.

Thin-layer chromatography (TLC) analysis was accomplished with Silica gel plates GF254 and visualization on TLC was achieved by UV light. Melting points (m.p.) were determined on an X-6 microscope melting point apparatus and were given uncorrected. Infrared spectra were recorded on a Nicolet Avatar 370 spectrometer with KBr as diluents. The high-resolution mass spectra were taken with a Waters Xevo G2. ^{13}C NMR and ^{1}H NMR spectra were collected at frequencies of 100 and 400 MHz. NMR spectra were obtained by a Bruker Avance III 400 MHz spectrometer using $CDCl_3$ as solvent and tetramethylsilane as internal. The chemical shifts are illustrated in values (ppm) and the coupling constant values (J) are shown in Hertz. Signal multiplicities are reported by the following abbreviations: s (singlet), d (doublet), t (triplet), dd (double doublet), q (quartet), m (multiplet), and brs (broad signal).

Tumor cell growth inhibitory assay

The various cells used in the following cell experiments were obtained from the Tumor Cell Resources Bank of the Chinese Academy of Medical Sciences. Cell counting kit-8 was purchased from Dojindo (Japan). The in vitro human cancer cell lines SMMC-7721, A549, Hela, and normal cell lines L929 were maintained and cultured in proper medium in a 5% CO_2 at 37 °C during the experiment. In the antitumor cell test, we repeated the biological test three times, and used the Origin software for statistical analysis. The inhibition (IC_{50}) of the selected cells proliferation by target compounds **8a–h**, **9a–h**, **10a–h** and reference drug was assessed by CCK-8 assay as described in the literature [45].

General procedure for the synthesis of intermediates 7a–c

The compound 1-phenyl-1-(4-substituted phenyl)-3-thiosemicarbazide **6** (17 mmol) was dissolved in a mixed solution of 60 mL 10% NaOH aqueous solution and methanol with a volume ratio of 5:1, the reaction mixture was stirred at room temperature for 30 min, and then refluxed for 3 h. After monitoring by thin layer chromatography until the end of the reaction, hydrochloric acid is slowly dropped into the reaction mixture at 0–5 °C until the solids precipitate out, and recrystallized from ethanol to afford pure compounds.

2,3-diphenyl-5-sulfhydryl-1,2,4-triazole(7a)

Yellow solid, Yield 61%, m.p.: 176–177 °C. IR (KBr) cm^{-1}: 2360, 1733, 1500, 1257, 688. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.53–7.49 (m, 2H, Ar-H), 7.45–7.40 (m, 4H, Ar-H), 7.40–7.33 (m, 4H, Ar-H), 5.09 (s, 1H, SH). MS-ESI (m/z): $C_{14}H_{11}N_3S$: $[M+H]^+$ 254.0.

2-phenyl-3-(4-methylphenyl)-5-sulfhydryl-1,2,4-triazole(7b)

Yellow solid, Yield 77%, m.p.: 235–236 °C. IR (KBr) cm^{-1}: 2360, 1637, 1455, 1239, 771. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.45–7.41 (m, 3H, Ar-H), 7.40 (s, 1H, Ar-H), 7.38 (d, $J = 4.0$ Hz, 3H, Ar-H), 7.15 (d, $J = 8.0$ Hz, 2H, Ar-H), 5.07 (s, 1H, SH), 2.38 (s, 3H, CH_3). MS-ESI (m/z): $C_{15}H_{13}N_3S$: $[M+H]^+$ 268.0.

2-phenyl-3-(4-chlorophenyl)-5-sulfhydryl-1,2,4-triazole(7c)

White solid, Yield 55%, m.p.: 168–169 °C. IR (KBr) cm^{-1}: 2360, 1596, 1454, 1276, 736, 690. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.49–7.44 (m, 4H, Ar-H), 7.44 (d, $J =$ 4.0 Hz, 1H, Ar-H), 7.39 – 7.35 (m, 2H, Ar-H), 7.34 (d, $J =$ 4.0 Hz, 1H, Ar-H), 7.32 (d, $J = 4.0$ Hz, 1H, Ar-H), 5.06 (s, 1H, SH). MS-ESI (m/z): $C_{14}H_{10}N_3SCl$: $[M+H]^+$ 288.0.

General procedure for the synthesis of compounds 8a–h, 9a–h and 10a–h

S-Alkyl-thioisothiourea hydrochloride **2** (2.2 mmol) and 2-phenyl-3-(4-substituted phenyl)-5- sulfhydryl-1,2,4-triazole **7a–h** (2 mmol) were dissolved in ethanol (15 mL) and water (5 mL). Then, a solution of saturated $NaHCO_3$ (20 mL) was added dropwise with stirring for 4 h at 0-5 °C. After monitoring by thin layer chromatography until the end of the reaction, the reaction mixture was extracted with dichloromethane and water, the organic phase was collected and concentrated. The final products were obtained by filtration and purification of crude solids by silica gel column chromatography eluting with petroleum ether/ethyl acetate (24:1, by volume).

2,3-diphenyl-5-ethyldisulfanyl-1,2,4-triazole(8a)

Yellow solid, Yield 85%, m.p.: 87–88 °C. IR (KBr) cm^{-1}: 2920, 1592, 1496, 1246, 696, 511. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.51 (t, $J = 4.0$ Hz, 1H, Ar-H), 7.49 (t,

$J = 4.0$ Hz, 1H, Ar-H), 7.46–7.39 (m, 4H, Ar-H), 7.38–7.31 (m, 4H, Ar-H), 3.03 (m, 2H, CH_2), 1.45 (t, $J = 8.0$ Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 161.44 (C-5), 155.37 (C-3), 137.84 (C-4"), 130.29 (C-1"), 129.41 (C-2', C-6'), 129.09 (C-4'), 128.96 (C-3", C-5"), 128.55 (C-3', C-5'), 127.31 (C-1'), 125.39 (C-2", C-6"), 33.23 (-S$\underline{C}H_2CH_3$), 14.17 (-SCH$_2\underline{C}H_3$). HR-ESI-MS (m/z): Calcd. for $C_{16}H_{15}N_3S_2$: 314.0786 $[M+H]^+$, Found 314.0797.

2,3-diphenyl-5-(*n*-propyldisulfanyl)-1,2,4-triazole(8b)

White solid, Yield 80%, m.p.: 50–51 °C. IR (KBr) cm^{-1}: 2960, 1595, 1501, 1255, 697, 470. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.49 (t, $J = 4.0$ Hz, 1H, Ar-H), 7.47 (t, $J = 4.0$ Hz, 1H, Ar-H), 7.42–7.35 (m, 4H, Ar-H), 7.36–7.28 (m, 4H, Ar-H), 2.97 (t, $J = 8.0$ Hz, 2H, CH_2), 1.83 (m, 2H, CH_2), 1.02 (t, $J = 8.0$ Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 161.38 (C-5), 155.33 (C-3), 137.79 (C-4"), 130.32 (C-1"), 129.43 (C-2', C-6'), 129.11 (C-4'), 128.93 (C-3", C-5"), 128.57 (C-3', C-5'), 127.25 (C-1'), 125.35 (C-2", C-6"), 41.29 (-S$\underline{C}H_2CH_2CH_3$), 22.04 (-SCH$_2\underline{C}H_2CH_3$), 13.11 (-SCH$_2$CH$_2\underline{C}H_3$). HR-ESI-MS (m/z): Calcd. for $C_{17}H_{17}N_3S_2$: 328.0942 $[M+H]^+$, Found 328.0956.

2,3-diphenyl-5-(2-propyldisulfanyl)-1,2,4-triazole(8c)

White solid, Yield 71%, m.p.: 90–91 °C. IR (KBr) cm^{-1}: 2958, 1594, 1501, 1262, 697, 495. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.52 (t, $J = 4.0$ Hz, 1H, Ar-H), 7.50 (t, $J = 4.0$ Hz, 1H, Ar-H), 7.47–7.40 (m, 4H, Ar-H), 7.40–7.32 (m, 4H, Ar-H), 3.40 (m, 1H, CH), 1.44 (d, $J = 8.0$ Hz, 6H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 161.70 (C-5), 155.26 (C-3), 137.85 (C-4"), 130.28 (C-1"), 129.40 (C-2', C-6'), 129.06 (C-4'), 128.97 (C-3", C-5"), 128.55 (C-3', C-5'), 127.31 (C-1'), 125.39 (C-2", C-6"), 41.72 (-S$\underline{C}H_2(CH_3)_2$), 22.39 (-SCH$_2$($\underline{C}H_3$)$_2$). HR-ESI-MS (m/z): Calcd. for $C_{17}H_{17}N_3S_2$: 328.0942 $[M+H]^+$, Found 328.0946.

2,3-diphenyl-5-(*n*-butyldisulfanyl)-1,2,4-triazole(8d)

Yellow oily liquid, Yield 77%. IR (KBr) cm^{-1}: 2956, 1595, 1498, 1300, 692, 509. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.52 (t, $J = 4.0$ Hz, 1H, Ar-H), 7.50 (t, $J = 4.0$ Hz, 1H, Ar-H), 7.47–7.40 (m, 4H, Ar-H), 7.40–7.32 (m, 4H, Ar-H), 3.05–3.00 (m, 2H, CH_2), 1.85–1.75 (m, 2H, CH_2), 1.49 (t, $J = 4.0$ Hz, 2H, CH_2), 0.95 (t, $J = 8.0$ Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 161.48 (C-5), 155.36 (C-3), 137.86 (C-4"), 130.27 (C-1"), 129.40 (C-2', C-6'), 129.07 (C-4'), 128.96 (C-3", C-5"), 128.55 (C-3', C-5'), 127.33 (C-1'), 125.39 (C-2", C-6"), 39.16 (-S$\underline{C}H_2CH_2CH_2CH_3$), 30.78 (-SCH$_2\underline{C}H_2CH_2CH_3$), 21.57 (-SCH$_2CH_2\underline{C}H_2CH_3$), 13.71 (-SCH$_2CH_2CH_2\underline{C}H_3$). HR-ESI-MS (m/z): Calcd. for $C_{18}H_{19}N_3S_2$: 342.1099 $[M+H]^+$, Found 342.1104.

2,3-diphenyl-5-(2-butyldisulfanyl)-1,2,4-triazole(8e)

Yellow-brown solid, Yield 74%, m.p.: 64–65 °C. IR (KBr) cm^{-1}: 2956, 1595, 1501, 1264, 696, 495. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.52 (t, $J = 4.0$ Hz, 1H, Ar-H), 7.50 (t, $J = 4.0$ Hz, 1H, Ar-H), 7.47–7.40 (m, 4H, Ar-H), 7.40–7.33 (m, 4H, Ar-H), 2.92 (d, $J = 8.0$ Hz, 2H, CH_2), 2.13 (m, 1H, CH), 1.07 (d, $J = 4.0$ Hz, 6H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 161.46 (C-5), 155.36 (C-3), 137.87 (C-4"), 130.28 (C-1"), 129.40 (C-2', C-6'), 129.07 (C-4'), 128.96 (C-3", C-5"), 128.55 (C-3', C-5'), 127.33 (C-1'), 125.39 (C-2", C-6"), 48.75 (-S$\underline{C}H_2CH_2(CH_3)_2$), 27.90 (-SCH$_2\underline{C}H_2(CH_3)_2$), 21.78 (-SCH$_2CH_2$($\underline{C}H_3$)$_2$). HR-ESI-MS(m/z): Calcd. for $C_{18}H_{19}N_3S_2$: 342.1099 $[M+H]^+$, Found 342.1102.

2,3-diphenyl-5-(*i*-butyldisulfanyl)-1,2,4-triazole(8f)

Yellow oily liquid, Yield 65%. IR (KBr) cm^{-1}: 2964, 1596, 1498, 1259, 692, 505. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.51 (t, $J = 4.0$ Hz, 1H, Ar-H), 7.49 (t, $J = 4.0$ Hz, 1H, Ar-H), 7.46–7.39 (m, 4H, Ar-H), 7.39–7.32 (m, 4H, Ar-H), 3.15 (m, 1H, CH), 1.93–1.81 (m, 1H, CH), 1.69-1.58 (m, 1H, CH), 1.43 (d, $J = 8.0$ Hz, 3H, CH_3), 1.03 (t, $J = 8.0$ Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 161.74 (C-5), 155.22 (C-3), 137.86 (C-4"), 130.26 (C-1"), 129.40 (C-2', C-6'), 129.05 (C-4'), 128.95 (C-3", C-5"), 128.55 (C-3', C-5'), 127.33 (C-1'), 125.36 (C-2", C-6"), 48.58 (-S$\underline{C}H(CH_3)CH_2CH_3$), 28.67 (-SCH($\underline{C}H_3$)$CH_2CH_3$), 19.90 (-SCH($CH_3$)$\underline{C}H_2CH_3$), 11.43 (-SCH($CH_3$)CH$_2\underline{C}H_3$). HR-ESI-MS (m/z): Calcd. for $C_{18}H_{19}N_3S_2$: 342.1099 $[M+H]^+$, Found 342.1106.

2,3-diphenyl-5-(*n*-pentyldisulfanyl)-1,2,4-triazole(8g)

Colorless oily liquid, Yield 77%. IR (KBr) cm^{-1}: 2925, 1595, 1505, 1259, 695, 505. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.52 (t, $J = 4.0$ Hz, 1H, Ar-H), 7.50 (t, $J = 4.0$ Hz, 1H, Ar-H), 7.46–7.39 (m, 4H, Ar-H), 7.39–7.32 (m, 4H, Ar-H), 3.01 (t, $J = 8.0$ Hz, 2H, CH_2), 1.82 (m, 2H, CH_2), 1.47–1.39 (m, 2H, CH_2), 1.38–1.30 (m, 2H, CH_2), 0.90 (t, $J = 8.0$ Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 161.48 (C-5), 155.35 (C-3), 137.85 (C-4"), 130.28 (C-1"), 129.40 (C-2', C-6'), 129.08 (C-4'), 128.96 (C-3", C-5"), 128.55 (C-3', C-5'), 127.31 (C-1'), 125.38 (C-2", C-6"), 39.48 (-S$\underline{C}H_2CH_2CH_2CH_2CH_3$), 30.58 (-SCH$_2\underline{C}H_2$ $CH_2CH_2CH_3$), 28.41 (-SCH$_2$CH$_2\underline{C}H_2CH_2CH_3$), 22.33 (-SCH$_2CH_2CH_2\underline{C}H_2CH_3$), 13.98 (-SCH$_2CH_2CH_2CH_2\underline{C}H_3$).

HR-ESI-MS (m/z): Calcd. for $C_{19}H_{21}N_3S_2$: 356.1255 $[M+H]^+$, Found 356.1271.

2,3-diphenyl-5-(benzyldisulfanyl)-1,2,4-triazole(8h)

Yellow solid, Yield 71%, m.p.: 73–74 °C. IR (KBr) cm^{-1}: 3028, 2912, 1601, 1541, 695, 468. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.53 (t, $J = 4.0$ Hz, 1H, Ar-H), 7.51 (t, $J = 4.0$ Hz, 1H, Ar-H), 7.47–7.43 (m, 4H, Ar-H), 7.42 (m, 2H, Ar-H), 7.40–7.36 (m, 3H, Ar-H), 7.36–7.33 (m, 1H, Ar-H), 7.32 (d, $J = 4.0$ Hz, 1H, Ar-H), 7.31 (d, $J = 4.0$ Hz, 1H, Ar-H), 7.29–7.24 (m, 1H, Ar-H), 4.29 (s, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 161.16 (C-5), 155.25 (C-3), 137.86 (C-4"), 136.65 (-$SCH_2\underline{C}(CH)_2(CH)_2CH$), 130.34 (C-1"), 129.69 (-$SCH_2C(\underline{CH})_2(CH)_2CH$), 129.44 (C-2', C-6'), 129.12 (C-4'), 128.99 (C-3", C-5"), 128.61 (-$SCH_2C(CH)_2(\underline{CH})_2CH$), 128.45 (C-3', C-5'), 127.50 (-$SCH_2C(CH)_2(CH)_2\underline{CH}$), 127.35 (C-1'), 125.36 (C-2", C-6"), 43.92 (-$S\underline{C}H_2Ph$). HR-ESI-MS (m/z): Calcd. for $C_{21}H_{17}N_3S_2$: 376.0942 $[M+H]^+$, Found 376.0952.

2-phenyl-3-(4-methylphenyl)-5-ethyldisulfanyl-1,2,4-triazole(9a)

White solid, Yield 76%, m.p.: 91–92 °C. IR (KBr) cm^{-1}: 2957, 1592, 1493, 1257, 693, 519. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.44 (m, 3H, Ar-H), 7.40 (d, $J = 4.0$ Hz, 1H, Ar-H), 7.40–7.35 (m, 3H, Ar-H), 7.15 (d, $J = 8.0$ Hz, 2H, Ar-H), 3.06–3.01 (m, 2H, CH_2), 2.37 (s, 3H, CH_3), 1.45 (t, $J = 8.0$ Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 161.29 (C-5), 155.52 (C-3), 140.57 (C-4"), 137.98 (C-4'), 129.38 (C-2', C-6'), 129.25 (C-3", C-5"), 129.01 (C-1'), 128.86 (C-3', C-5'), 125.45 (C-2", C-6"), 124.40 (C-1"), 33.22 (-$S\underline{C}H_2CH_3$), 21.44 (Ph$\underline{C}H_3$), 14.16 (-$SCH_2\underline{C}H_3$). HR-ESI-MS (m/z): Calcd. for $C_{17}H_{17}N_3S_2$: 328.0942 $[M+H]^+$, Found 328.0942.

2-phenyl-3-(4-methylphenyl)-5-(*n*-propyldisulfanyl)-1,2,4-triazole(9b)

White solid, Yield 72%, m.p.: 79–80 °C. IR (KBr) cm^{-1}: 2960, 1541, 1496, 1259, 768, 508. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.44 (m, 3H, Ar-H), 7.41 (d, $J = 4.0$ Hz, 1H, Ar-H), 7.40–7.36 (m, 3H, Ar-H), 7.15 (d, $J = 8.0$ Hz, 2H, Ar-H), 2.99 (t, $J = 8.0$ Hz, 2H, CH_2), 2.38 (s, 3H, CH_3), 1.86 (m, 2H, CH_2), 1.05 (t, $J = 8.0$ Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 161.27 (C-5), 155.51 (C-3), 140.58 (C-4"), 137.96 (C-4'), 129.39 (C-2', C-6'), 129.26 (C-3", C-5"), 129.02 (C-1'), 128.85 (C-3', C-5'),125.44 (C-2", C-6"), 124.38 (C-1"), 41.33 (-$S\underline{C}H_2CH_2CH_3$), 22.06 (-$SCH_2\underline{C}H_2CH_3$), 21.46 (Ph$\underline{C}H_3$), 13.09 (-$SCH_2CH_2\underline{C}H_3$). HR-ESI-MS (m/z): Calcd. for $C_{18}H_{19}N_3S_2$: 342.1099 $[M+H]^+$, Found 342.1107.

2-phenyl-3-(4-methylphenyl)-5-(2-propyldisulfanyl)-1,2,4-triazole(9c)

White solid, Yield 68%, m.p.: 90–91 °C. IR (KBr) cm^{-1}: 2960, 1595, 1499, 1260, 773, 497. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.46–7.42 (m, 3H, Ar-H), 7.40 (d, $J = 4.0$ Hz, 1H, Ar-H), 7.39–7.35 (m, 3H, Ar-H), 7.15 (d, $J = 8.0$ Hz, 2H, Ar-H), 3.39 (m, 1H, CH), 2.38 (s, 3H, CH_3), 1.43 (d, $J = 4.0$ Hz, 6H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 161.54 (C-5), 155.41 (C-3), 140.55 (C-4"), 137.97 (C-4'), 129.37 (C-2', C-6'), 129.24 (C-3", C-5"), 128.99 (C-1'), 128.86 (C-3', C-5'), 125.43 (C-2", C-6"), 124.41(C-1"), 47.71 (-$S\underline{C}H_2(CH_3)_2$), 22.39 (-$SCH_2(\underline{C}H_3)_2$), 21.46 (Ph$\underline{C}H_3$). HR-ESI-MS (m/z): Calcd. for $C_{18}H_{19}N_3S_2$: 342.1099 $[M+H]^+$, Found 342.1100.

2-phenyl-3-(4-methylphenyl)-5-(*n*-butyldisulfanyl)-1,2,4-triazole(9d)

Yellow solid, Yield 67%, m.p.: 74–75 °C. IR (KBr) cm^{-1}: 2922, 1595, 1499, 1264, 691, 501. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.46–7.42 (m, 3H, Ar-H), 7.41 (d, $J = 4.0$ Hz, 1H, Ar-H), 7.40–7.35 (m, 3H, Ar-H), 7.15 (d, $J = 8.0$ Hz, 2H, Ar-H), 3.02 (t, $J = 8.0$ Hz, 2H, CH_2), 2.38 (s, 3H, CH_3), 1.80 (m, 2H, CH_2), 1.48 (m, 2H, CH_2), 0.95 (t, $J = 8.0$ Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 161.32 (C-5), 155.51 (C-3), 140.56 (C-4"), 137.99 (C-4'), 129.37 (C-2', C-6'), 129.24 (C-3", C-5"), 128.99 (C-1'), 128.85 (C-3', C-5'), 125.44 (C-11,C-14), 124.42 (C-1"), 39.15 (-$S\underline{C}H_2CH_2CH_2CH_3$), 30.78 (-$SCH_2\underline{C}H_2CH_2CH_3$), 21.57 (-$SCH_2CH_2\underline{C}H_2CH_3$), 21.44 (Ph$\underline{C}H_3$), 13.71 (-$SCH_2CH_2CH_2\underline{C}H_3$). HR-ESI-MS (m/z): Calcd. for $C_{19}H_{21}N_3S_2$: 356.1255 $[M+H]^+$, Found 356.1272.

2-phenyl-3-(4-methylphenyl)-5-(2-butyldisulfanyl)-1,2,4-triazole(9e)

Yellow solid, Yield 75%, m.p.: 65–66 °C. IR (KBr) cm^{-1}: 2954, 1596, 1500, 1266, 762, 503. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.47–7.42 (m, 3H, Ar-H), 7.41 (d, $J = 4.0$ Hz, 1H, Ar-H), 7.40–7.36 (m, 3H, Ar-H), 7.15 (d, $J = 8.0$ Hz, 2H, Ar-H), 2.92 (d, $J = 4.0$ Hz, 2H, CH_2), 2.38 (s, 3H, CH_3), 2.14 (m, 1H, CH), 1.06 (d, $J = 8.0$ Hz, 6H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 161.30 (C-5), 155.50 (C-3), 140.55 (C-4"), 138.01 (C-4'), 129.37 (C-4,C-8), 129.25 (C-3", C-5"), 128.98 (C-1'), 128.85 (C-3', C-5'), 125.43 (C-11, C-14), 124.44 (C-1"), 48.74 (-$S\underline{C}H_2CH_2(CH_3)_2$), 27.89 (-$SCH_2\underline{C}H_2(CH_3)_2$), 21.78 (-$SCH_2CH_2(\underline{C}H_3)_2$), 21.44 (Ph$\underline{C}H_3$). HR-ESI-MS (m/z): Calcd. for $C_{19}H_{21}N_3S_2$: 356.1255 $[M+H]^+$, Found 356.1248.

2-phenyl-3-(4-methylphenyl)-5-(*i*-butyldisulfanyl)-1,2,4-triazole(9f)

Colorless oily liquid, Yield 72%. IR (KBr) cm^{-1}: 2923, 1615, 1505, 1269, 690, 507. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.46–7.42 (m, 3H, Ar-H), 7.40 (d, $J = 4.0$ Hz, 1H, Ar-H), 7.39–7.34 (m, 3H, Ar-H), 7.15 (d, $J = 8.0$ Hz, 2H, Ar-H), 3.21–3.10 (m, 1H, CH), 2.37 (s, 3H, CH_3), 1.92–1.80 (m, 1H, CH), 1.69–1.58 (m, 1H, CH), 1.42 (d, $J = 4.0$ Hz, 3H, CH_3), 1.02 (t, $J = 8.0$ Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 161.58 (C-5), 155.36 (C-3), 140.54 (C-4"), 138.00 (C-4'), 129.36 (C-2', C-6'), 129.24 (C-3", C-5"), 128.97 (C-1'), 128.84 (C-3', C-5'), 125.41 (C-11, C-14), 124.43 (C-1"), 48.58 (-S<u>C</u>H(CH_3)CH_2CH_3), 28.67 (-SCH(<u>C</u>H_3)CH_2CH_3), 21.45 (Ph<u>C</u>H_3), 19.89 (-SCH(CH_3)<u>C</u>H_2CH_3), 11.42 (-SCH(CH_3)CH_2<u>C</u>H_3). HR-ESI-MS (m/z): Calcd. for $C_{19}H_{21}N_3S_2$: 356.1255 $[M+H]^+$, Found 356.1252.

2-phenyl-3-(4-methylphenyl)-5-(*n*-pentyldisulfanyl)-1,2,4-triazole(9g)

White solid, Yield 63%, m.p.: 54–55 °C. IR (KBr) cm^{-1}: 2951, 1594, 1497, 1257, 693, 503. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.46–7.42 (m, 3H, Ar-H), 7.40 (d, $J = 4.0$ Hz, 1H, Ar-H), 7.40–7.35 (m, 3H, Ar-H), 7.15 (d, $J = 8.0$ Hz, 2H, Ar-H), 3.01 (t, $J = 8.0$ Hz, 2H, CH_2), 2.37 (s, 3H, CH_3), 1.85–1.78 (m, 2H, CH_2), 1.47–1.39 (m, 2H, CH_2), 1.38–1.29 (m, 2H, CH_2), 0.91 (t, $J = 8.0$ Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 161.32 (C-5), 155.50 (C-3), 140.57 (C-4"), 137.98 (C-4'), 129.37 (C-2', C-6'), 129.25 (C-3", C-5"), 129.00 (C-1'), 128.85 (C-3', C-5'), 125.43 (C-2", C-6"), 124.40 (C-1"), 39.47 (-S<u>C</u>$H_2CH_2CH_2CH_2CH_3$), 30.59 (-SCH_2<u>C</u>H_2CH_2 CH_2CH_3), 28.42 (-SCH_2CH_2<u>C</u>$H_2CH_2CH_3$), 22.34 (-$SCH_2CH_2CH_2$<u>C</u>H_2 CH_3), 21.45 (Ph<u>C</u>H_3), 13.99 (-$SCH_2CH_2CH_2CH_2$<u>C</u>H_3). HR-ESI-MS (m/z): Calcd. for $C_{20}H_{23}N_3S_2$: 370.1412 $[M+H]^+$, Found 370.1423.

2-phenyl-3-(4-methylphenyl)-5-(benzyldisulfanyl)-1,2,4-triazole(9h)

Pink solid, Yield 70%, m.p.: 86–87 °C. IR (KBr) cm^{-1}: 3054, 2916, 1592, 1496, 706, 509. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.47–7.42 (m, 4H, Ar-H), 7.42 (s, 2H, Ar-H), 7.40 (d, $J = 4.0$ Hz, 1H, Ar-H), 7.39–7.35 (m, 2H, Ar-H), 7.35–7.30 (m, 2H, Ar-H), 7.29–7.24 (m, 1H, Ar-H), 7.17 (d, $J = 8.0$ Hz, 2H, Ar-H), 4.28 (s, 2H, CH_2), 2.39 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 161.00 (C-5), 155.40 (C-3), 140.62 (C-4"), 137.98 (C-4'), 136.67 (-SCH_2<u>C</u>$(CH)_2(CH)_2CH$), 129.69 (-SCH_2C(<u>C</u>H)$_2$(CH)$_2$CH), 129.41 (C-2', C-6'), 129.32 (C-3", C-5"), 129.05 (C-1'), 128.89 (C-3', C-5'), 128.45 (-$SCH_2C(CH)_2$(<u>C</u>H)$_2$CH), 127.49 (-$SCH_2C(CH)_2(CH)_2$<u>C</u>H), 125.40 (C-2", C-6"), 124.44 (C-1"), 43.89 (-SCH_2Ph), 21.51 (Ph<u>C</u>H_3). HR-ESI-MS (m/z): Calcd. for $C_{22}H_{19}N_3S_2$: 390.1099 $[M+H]^+$, Found 390.1113.

2-phenyl-3-(4-chlorophenyl)-5-ethyldisulfanyl-1,2,4-triazole (10a)

Yellow solid, Yield 79%, m.p.: 86–87 °C. IR (KBr) cm^{-1}: 2919, 1596, 1497, 1267, 778, 698, 494. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.46–1.43 (m, 4H, Ar-H), 7.43 (s, 1H, Ar-H), 7.38–7.33 (m, 2H, Ar-H), 7.32 (d, $J = 4.0$ Hz, 1H, Ar-H), 7.30 (d, $J = 4.0$ Hz, 1H, Ar-H), 3.01 (m, 2H, CH_2), 1.44 (t, $J = 8.0$ Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 161.64 (C-5), 154.35 (C-3), 137.63 (C-4"), 136.59 (C-1"), 130.23 (C-2', C-6'), 129.58 (C-3", C-5"), 129.37 (C-4'), 128.92 (C-2", C-6"), 125.71 (C-1'), 125.44 (C-3', C-5'), 33.22 (-S<u>C</u>H_2CH_3), 14.15 (-SCH_2<u>C</u>H_3). HR-ESI-MS (m/z): Calcd. for $C_{16}H_{14}N_3S_2Cl$: 348.0396 $[M+H]^+$, Found 348.0404.

2-phenyl-3-(4-chlorophenyl)-5-(*n*-propyldisulfanyl)-1,2,4-triazole(10b)

White solid, Yield 73%, m.p.: 88–89 °C. IR (KBr) cm^{-1}: 2956, 1596, 1498, 1268, 778, 700, 491. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.49–7.46 (m, 4H, Ar-H), 7.45 (s, 1H, Ar-H), 7.39-7.35 (m, 2H, Ar-H), 7.34 (d, $J = 4.0$ Hz, 1H, Ar-H), 7.33 (d, $J = 4.0$ Hz, 1H, Ar-H), 2.99 (t, $J = 8.0$ Hz, 2H, CH_2), 1.90–1.81 (m, 2H, CH_2), 1.06 (t, $J = 8.0$ Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 161.65 (C-5), 154.34 (C-3), 137.64 (C-4"), 136.59 (C-1"), 130.23 (C-2', C-6'), 129.58 (C-3", C-5"), 129.36 (C-4'), 128.92 (C-2", C-6"), 125.72 (C-1'), 125.43 (C-3', C-5'), 41.34 (-S<u>C</u>$H_2CH_2CH_3$), 22.06 (-SCH_2<u>C</u>H_2CH_3), 13.08 (-SCH_2CH_2<u>C</u>H_3). HR-ESI-MS (m/z): Calcd. for $C_{17}H_{16}N_3S_2Cl$: 362.0552 $[M+H]^+$, Found 362.0566.

2-phenyl-3-(4-chlorophenyl)-5-(2-propyldisulfanyl)-1,2,4-triazole(10c)

White solid, Yield 75%, m.p.: 107–108 °C. IR (KBr) cm^{-1}: 2960, 1595, 1497, 1270, 776, 696, 489. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.49–7.44 (m, 4H, Ar-H), 7.43 (s, 1H, Ar-H), 7.38–7.34 (m, 2H, Ar-H), 7.33 (s, 1H, Ar-H), 7.31 (d, $J = 4.0$ Hz, 1H, Ar-H), 3.42–3.35 (m, 1H, CH), 1.42 (d, $J = 4.0$ Hz, 6H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 161.89 (C-5), 154.23 (C-3), 137.64 (C-4"), 136.58 (C-1"), 130.24 (C-2', C-6'), 129.56 (C-3", C-5"), 129.34 (C-4'), 128.91 (C-2", C-6"), 125.72 (C-1'), 125.43 (C-3', C-5'), 41.75 (-S<u>C</u>$H_2(CH_3)_2$), 22.37 (-SCH_2 (<u>C</u>$H_3)_2$). HR-ESI-MS (m/z): Calcd. for $C_{17}H_{16}N_3S_2Cl$: $[M+H]^+$ 362.0552, Found 362.0540.

2-phenyl-3-(4-chlorophenyl)-5-(*n*-butyldisulfanyl)-1,2,4-triazole(10d)

White solid, Yield 78%, m.p.: 62–63 °C. IR (KBr) cm^{-1}: 2924, 1596, 1499, 1272, 769, 695, 494. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.49–7.45 (m, 4H, Ar-H), 7.45 (d, $J = 4.0$ Hz, 1H, Ar-H), 7.39-7.35 (m, 2H, Ar-H), 7.35 (s, 1H, Ar-H), 7.32 (d, $J = 4.0$ Hz, 1H, Ar-H), 3.02 (t, $J = 8.0$ Hz, 2H, CH_2), 1.80 (m, 2H, CH_2), 1.48 (m, 2H, CH_2), 0.95 (t, $J = 8.0$ Hz, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 161.66 (C-5), 154.34 (C-3), 137.63 (C-4"), 136.58 (C-1"), 130.23 (C-2', C-6'), 129.58 (C-3", C-5"), 129.36 (C-4'), 128.92 (C-2", C-6"), 125.72 (C-1'), 125.43 (C-3', C-5'), 39.13 (-S$\underline{C}H_2CH_2CH_2CH_3$), 30.77 (-$SCH_2\underline{C}H_2CH_2CH_3$), 21.57 (-$SCH_2CH_2\underline{C}H_2CH_3$), 13.72 (-$SCH_2CH_2CH_2\underline{C}H_3$). HR-ESI-MS (m/z): Calcd. for $C_{18}H_{18}N_3S_2Cl$: 376.0709 $[M+H]^+$, Found 376.0720.

2-phenyl-3-(4-chlorophenyl)-5-(2-butyldisulfanyl)-1,2,4-triazole(10e)

White solid, Yield 64%, m.p.: 83–84 °C. IR (KBr) cm^{-1}: 2951, 1595, 1455, 1271, 775, 696, 489. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.49–7.46 (m, 4H, Ar-H), 7.45 (d, $J = 4.0$ Hz, 1H, Ar-H), 7.39–7.36 (m, 2H, Ar-H), 7.34 (s, 1H, Ar-H), 7.32 (d, $J = 4.0$ Hz, 1H, Ar-H), 2.90 (d, $J = 4.0$ Hz, 2H, CH_2), 2.12 (m, 1H, CH), 1.06 (d, $J = 8.0$ Hz, 6H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 161.62 (C-5), 154.33 (C-3), 137.62 (C-4"), 136.58 (C-1"), 130.23 (C-2', C-6'), 129.59 (C-3", C-5"), 129.36 (C-4'), 128.93 (C-2", C-6"), 125.71 (C-1'), 125.41 (C-3', C-5'), 48.69 (-S$\underline{C}H_2CH_2(CH_3)_2$), 27.88 (-$SCH_2\underline{C}H_2(CH_3)_2$), 21.79 (-$SCH_2CH_2(\underline{C}H_3)_2$). HR-ESI-MS (m/z): Calcd. for $C_{18}H_{18}N_3S_2Cl$: 376.0709 $[M+H]^+$, Found 376.0702.

2-phenyl-3-(4-chlorophenyl)-5-(*i*-butyldisulfanyl)-1,2,4-triazole(10f)

White solid, Yield 70%, m.p.: 76–77 °C. IR (KBr) cm^{-1}: 2962, 1594, 1455, 1270, 775, 696, 489. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.46–7.44 (m, 4H, Ar-H), 7.43 (d, $J = 4.0$ Hz, 1H, Ar-H), 7.37-7.33 (m, 2H, Ar-H), 7.32 (d, $J = 4.0$ Hz, 1H, Ar-H), 7.30 (d, $J = 4.0$ Hz, 1H, Ar-H), 3.16–3.09 (m, 1H, CH), 1.90–1.78 (m, 1H, CH), 1.69–1,58 (m, 1H, CH), 1.41 (d, $J = 8.0$ Hz, 3H, CH_3), 1.01 (t, $J = 8.0$ Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 161.93 (C-5), 154.19 (C-3), 137.64 (C-4"), 136.56 (C-1"), 130.22 (C-2', C-6'), 129.57 (C-3", C-5"), 129.33 (C-4'), 128.91 (C-2", C-6"), 125.73 (C-1'), 125.40 (C-3',C-5'), 48.59 (-S$\underline{C}H(CH_3)CH_2CH_3$), 28.66 (-$SCH(\underline{C}H_3)CH_2CH_3$), 19.88 (-$SCH(CH_3)\underline{C}H_2CH_3$), 11.44 (-$SCH(CH_3)CH_2\underline{C}H_3$). HR-ESI-MS (m/z): Calcd. for $C_{18}H_{18}N_3S_2Cl$: 376.0709 $[M+H]^+$, Found 376.0695.

2-phenyl-3-(4-chlorophenyl)-5-(*n*-pentyldisulfanyl)-1,2,4-triazole(10g)

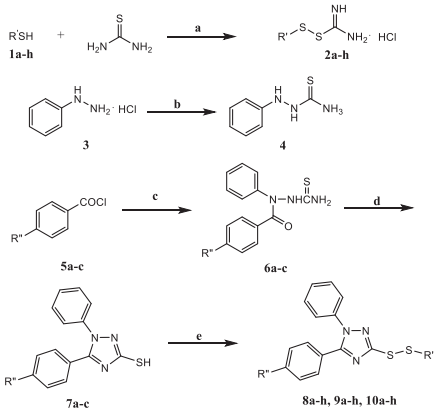
White solid, Yield 65%, m.p.: 72–73 °C. IR (KBr) cm^{-1}: 2926, 1595, 1453, 1266, 768, 692, 491. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.46–7.42 (m, 3H, Ar-H), 7.41 (d, $J = 4.0$ Hz, 2H, Ar-H), 7.35–7.30 (m, 2H, Ar-H), 7.30 (d, $J = 4.0$ Hz, 1H, Ar-H), 7.28 (d, $J = 4.0$ Hz, 1H, Ar-H), 2.98 (t, $J = 8.0$ Hz, 2H, CH_2), 1.82–1.74 (m, 2H, CH_2), 1.45–1.36 (m, 2H, CH_2), 1.35–1.25 (m, 2H, CH_2), 0.87 (t, $J = 8.0$ Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 161.64 (C-5), 154.29 (C-3), 137.61 (C-4"), 136.55 (C-1"), 130.22 (C-2', C-6'), 129.58 (C-3", C-5"), 129.36 (C-4'), 128.91 (C-2", C-6"), 125.70 (C-1'), 125.40 (C-3', C-5'), 39.44 (-S$\underline{C}H_2CH_2CH_2CH_2CH_3$), 30.57 (-$SCH_2\underline{C}H_2CH_2$ CH_2CH_3), 28.40 (-$SCH_2CH_2\underline{C}H_2CH_2CH_3$), 22.34 (-$SCH_2CH_2CH_2\underline{C}H_2CH_3$), 14.02 (-$SCH_2CH_2$ $CH_2CH_2\underline{C}H_3$). HR-ESI-MS (m/z): Calcd. for $C_{19}H_{20}N_3S_2Cl$: 390.0865 $[M+H]^+$, Found 390.0874.

2-phenyl-3-(4-chlorophenyl)-5-(benzyldisulfanyl)-1,2,4-triazole(10h)

White solid, Yield 61%, m.p.: 85–86 °C. IR (KBr) cm^{-1}: 3045, 2933, 1595, 1454, 774, 698, 488. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.49–7.46 (m, 4H, Ar-H), 7.43 (d, $J = 4.0$ Hz, 2H, Ar-H), 7.41 (d, $J = 4.0$ Hz, 1H, Ar-H), 7.38-7.35 (m, 3H, Ar-H), 7.33 (d, $J = 4.0$ Hz, 1H, Ar-H), 7.31 (d, $J = 4.0$ Hz, 1H, Ar-H), 7.30 (d, $J = 4.0$ Hz, 1H, Ar-H), 7.27 (m, 1H, Ar-H), 4.26 (s, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 161.31 (C-5), 154.18 (C-3), 137.60 (C-4"), 136.60 (C-1"), 136.56 (-$SCH_2\underline{C}(CH)_2(CH)_2CH$), 130.24 (C-2', C-6'), 129.66 (-$SCH_2C(\underline{C}H)_2(CH)_2CH$), 129.59 (C-3", C-5"), 129.37 (C-4'), 128.94 (C-2", C-6"), 128.43 (-$SCH_2C(CH)_2(\underline{C}H)_2CH$), 127.49 (-$SCH_2C(CH)_2(CH)_2\underline{C}H$), 125.70 (C-1'), 125.38 (C-3', C-5'), 43.87 (-S$\underline{C}H_2Ph$). HR-ESI-MS (m/z): Calcd. for $C_{21}H_{16}N_3S_2Cl$: 410.0552 $[M+H]^+$, Found 410.0570.

Results and discussion

According to the reaction described in Scheme 1, the 24 novel 1,2,4-triazole derivatives bearing disulfide bond **8a–h**, **9a–h**, **10a–h** were synthesized. The synthesis of 1-phenylthiosemicarbazide **4** was performed as outlined in Scheme 1 following the reported literature [46]. Under the protection of nitrogen, the compound 1-phenyl-1-(4-substituted phenyl)-3-thiosemicarbazide **6** was synthesized by treatment of 1-phenylthiosemicarbazide **4** with 4-substituted benzoyl chloride in dry acetone. And then 1-phenyl-1-(4-substituted phenyl)-3-thiosemicarbazide **6** was refluxed in the presence of methanol and NaOH in aqueous solution to



R'(R" = H): ethyl (**8a**), n-propyl (**8b**), i-propyl(**8c**), n-butyl(**8d**), i-butyl(**8e**), sec-butyl(**8f**), n-pentyl(**8g**), benzyl(**8h**); R'(R" =CH_3): ethyl (**9a**), n-propyl (**9b**), i-propyl(**9c**), n-butyl(**9d**), i-butyl(**9e**), sec-butyl(**9f**), n-pentyl(**9g**), benzyl(**9h**); R'(R" =Cl): ethyl (**10a**), n-propyl (**10b**), i-propyl(**10c**), n-butyl(**10d**), i-butyl(**10e**), sec-butyl(**10f**), n-pentyl(**10g**), benzyl(**10h**)

Scheme 1 Synthesis of target compounds **8a–h**, **9a–h**, **10a–h**. Reaction conditions and reagents: **a** Conc. HCl, H_2O_2 (30%), 0–5 °C, 8–10 h; **b** NH_4SCN, EtOH, reflux, 12 h; **c** Substituted benzoyl chloride, dry acetone, triethylamine, reflux, 4–6 h; **d** NaOH, MeOH, reflux, 3–5 h; **e** Compound **2**, EtOH, $NaHCO_3/H_2O$, rt, 4–6 h

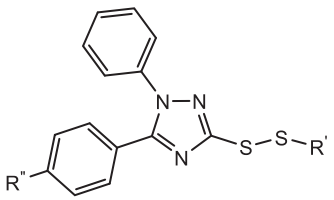


Fig. 1 The structure of target compounds **8a–h**, **9a–h**, **10a–h**

form **7a–c**. In the presence of $NaHCO_3$ in water and ethanol at 0–5 °C, the final products **8a–h**, **9a–h** and **10a–h** were prepared by the reaction of intermediates **7a–c** and compound **2**. All final products **8a–h**, **9a–h** and **10a–h** were extracted and then purified by column chromatography.

The structure of all the final compounds **8a–h**, **9a–h** and **10a–h** was confirmed by IR, ^{1}H NMR, ^{13}C NMR and HR-ESI-MS (Fig. 1). The IR spectrum of compounds **8a–h**, **9a–h** and **10a–h** revealed absorption bands between 519 and 468 cm^{-1} corresponding to S-S, 1615-1592 cm^{-1} corresponding to C=N function. For the ^{1}H-NMR spectra of compound **9a**, a triplet at δ 1.45 ppm and a multiplet at δ 3.01–3.06 ppm that corresponded to S-CH_2CH_3 functionality, a singlet at δ 2.37 ppm revealed the presence of the methyl group was attached to the benzene ring. In the ^{13}C-NMR spectra of compound **9a**, the methyl carbon signal on the benzene ring appeared at 21.44 ppm. The structure of **9a** was also confirmed by its mass spectral data. In its mass spectrum, the molecular ion peak was noticed m/z at 328.0942 $[M+H]^+$ corresponding to its molecular weight.

Our laboratory team has done previous studies on the antitumor activity of triazole compounds [41]. On the basis of previous studies, this project is designed. Compared with the previous research, there are some differences in the structure characteristics and pharmacological activities of the compounds studied in this research, and a few compounds have certain selectivity to tumor cells.

Table 1 In vitro antiproliferative activities of compounds **8a–h**, **9a–h**, **10a–h** against three cancer cell lines

Compounds	IC_{50} (μM)		
	SMMC-7721	Hela	A549
8a	21.72 ± 0.81	6.31 ± 0.28	8.92 ± 0.87
8b	40.52 ± 2.31	11.73 ± 0.33	7.73 ± 0.61
8c	18.11 ± 1.18	18.53 ± 0.96	4.75 ± 0.22
8d	8.53 ± 0.34	25.33 ± 1.41	28.91 ± 1.68
8e	17.31 ± 0.27	32.91 ± 1.51	28.52 ± 1.65
8f	19.72 ± 0.92	31.72 ± 1.24	23.72 ± 1.01
8g	21.33 ± 0.88	45.32 ± 2.36	33.72 ± 1.08
8h	46.71 ± 2.76	42.90 ± 2.16	38.13 ± 2.07
9a	38.10 ± 1.99	38.13 ± 2.07	31.33 ± 1.92
9b	8.12 ± 0.46	9.73 ± 0.41	13.72 ± 0.37
9c	9.31 ± 0.31	23.72 ± 0.88	5.12 ± 0.28
9d	26.91 ± 0.76	37.33 ± 1.99	29.72 ± 1.22
9e	4.12 ± 0.37	16.53 ± 1.08	32.11 ± 1.06
9f	9.73 ± 0.41	21.33 ± 1.02	35.72 ± 1.89
9g	2.92 ± 0.08	4.31 ± 0.28	25.72 ± 1.12
9h	30.11 ± 2.01	8.92 ± 0.46	29.72 ± 1.22
10a	6.12 ± 0.37	9.73 ± 0.41	4.92 ± 0.37
10b	8.53 ± 0.43	32.52 ± 0.98	26.52 ± 1.66
10c	7.31 ± 0.36	43.54 ± 2.36	7.31 ± 0.37
10d	30.52 ± 2.02	43.32 ± 2.86	21.72 ± 0.79
10e	35.72 ± 1.79	29.72 ± 1.59	6.53 ± 0.38
10f	42.13 ± 2.46	42.90 ± 2.58	43.31 ± 0.89
10g	10.53 ± 0.27	6.31 ± 0.15	6.53 ± 0.12
10h	4.53 ± 0.09	3.97 ± 0.06	3.73 ± 0.04
5-FU	5.62 ± 0.28	17.21 ± 0.27	8.13 ± 0.34

Pharmacology and discussion

The final products **8a–h**, **9a–h** and **10a–h** were evaluated for their in vitro antiproliferative activity against Hela, SMMC-7721, A549 and L929 cell lines by CCK-8 assay. The proliferation inhibitory activity of these compounds was measured at different concentrations and their IC_{50} values (concentration leading to 50% cell proliferation inhibition) were calculated. The results are presented in Table 1.

Antitumor activity

As exhibited in Table 1, the final products **8a–h**, **9a–h** and **10a–h** revealed different degrees of antitumor activity, and

the antitumor effect of most compounds on different cancer cell lines was superior to the 5-fluorouracil positive control. The majority of the compounds showed moderate to good activity on all the tested cancer cell lines. In SMMC-7721 cells, compounds **9e**, **9g**, and **10h** showed high antitumor activity with IC_{50} values 4.12, 2.92, and 4.53 μM, respectively. Obviously, As compared with compound **9g**, which has 4-methyl substitution at the phenyl ring while R' is n-pentyl group, the other compounds showed reduced antitumor activity. Compared with compounds **8a** and **10a**, R' is the same, compound **9g** electron-donating group substituted displayed better activity. In Hela cells, compounds **8a**, **9g**, **10g** and **10h** exhibited more potent antiproliferative activity with IC_{50} values 6.31, 4.31, 6.31, and 3.97 μM, respectively. It is clear that other compounds have lower antiproliferative activity than compound **10h** which has a 4-chloro substitution on the phenyl ring while R' is a benzyl group. The compound **10h** carrying electron-withdrawing substituents has better activity than compounds of **8h** and **9h** which have the same R' group. In A549 cells, compounds **8c**, **10a** and **10h** displayed high antitumor activity with IC_{50} values 4.75, 4.92 and 3.73 μM, respectively. It is apparent that other compounds exhibited lower antitumor activity than compound **10h** which has a 4-chloro substitution on the phenyl ring while R' is a benzyl group. While R_2 is the same substituent, the activity of the benzyl derivative **10h** is higher than that of the ethyl derivative **10a**. Moreover, the cytotoxicity of all compounds on L929 cells was inferior to the 5-fluorouracil. The above results illustrate that the change of substituent can affect anticancer activity to some extent, but it also does not show apparent regularity Table 2.

Table 2 In vitro antiproliferative activities of compounds **8a–h**, **9a–h**, **10a–h** against normal cell line L929

Compounds	L929 IC_{50} (μM)	Compounds	L929 IC_{50} (μM)
8a	21.33 ± 0.91	**9e**	15.72 ± 1.09
8b	18.11 ± 1.13	**9f**	>50
8c	22.92 ± 0.86	**9g**	15.32 ± 0.91
8d	42.13 ± 2.36	**9h**	40.52 ± 1.99
8e	>50	**10a**	15.31 ± 1.21
8f	>50	**10b**	39.50 ± 2.89
8g	41.71 ± 0.66	**10c**	47.30 ± 2.87
8h	>50	**10d**	44.11 ± 2.26
9a	49.72 ± 2.89	**10e**	37.71 ± 1.99
9b	9.31 ± 0.29	**10f**	>50
9c	40.91 ± 2.01	**10g**	>50
9d	47.51 ± 2.77	**10h**	49.2 ± 2.97
5-FU	2.98 ± 0.15		

Conclusion

In this study, the 24 target compounds **8a–h**, **9a–h** and **10a–h**, which are 1,2,4-triazole derivatives bearing disulfide bond, were synthesized and evaluated for their in vitro antiproliferative activities against human cancer cell lines SMMC-7721, Hela, A549 and normal cell lines L929 by CCK-8 assay. Among these compounds, compound **10h** was found to exhibit better selectivity against all three cancer cells at IC_{50} values of 4.53, 3.97, and 3.73 μM, respectively. In addition, compounds **9e**, **9g**, and **10h** revealed better cytotoxic activity on against SMMC-7721 cells with IC_{50} values 4.12, 2.92, and 4.53 μM, respectively. Compounds **8a**, **9g**, **10g** and **10h** showed effective biological potency on Hela cells with IC_{50} values 6.31, 4.31, 6.31, and 3.97 μM, respectively. Compounds **8c**, **10a** and **10h** revealed excellent antitumor activity on A549 cells with IC_{50} values 4.75, 4.92 and 3.73 μM, respectively. Moreover, most of the final products revealed low cytotoxicity on normal cell line L929. Therefore, the above findings will be of great significance for the development of potential antitumor agents.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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References

1. Çoruh I, Çevik Ö, Yelekçi K, Djikic T, Küçükgüzel ŞG. Synthesis, anticancer activity, and molecular modeling of etodolac-thioether derivatives as potent methionine aminopeptidase (type II) inhibitors. Arch Pharm Chem Life Sci. 2018;351:1700195.
2. Mahanti S, Sunkaraa S, Bhavanib R. Synthesis, biological evaluation and computational studies of fused acridine containing 1,2,4-triazole derivatives as anticancer agents. Synth Commun. 2019;49:1729–40.
3. Beyzaei H, Khosravi Z, Aryan R, Ghasemi B. A green one-pot synthesis of 3(5)-substituted 1,2,4-triazol-5(3)-amines as potential antimicrobial agents. J Iran Chem Soc. 2019;16:2565–73.
4. Abu-Hashem AA, Hussein HAR, Abu-zied KM. Synthesis of novel 1,2,4-triazolopyrimidines and their evaluation as antimicrobial agents. Med Chem Res. 2016;26:120–30.
5. Hkiri S, Hafidh A, Cavalier JF, Touil S, Samarat A. Design, synthesis, antimicrobial evaluation, and molecular docking studies of novel symmetrical 2,5-difunctionalized 1,3,4-oxadiazoles. J Heterocyl Chem. 2019;57:1044–54.
6. Sekhar MM, Yamini G, Divya KRG, Padmavathi V, Padmaja A. Synthesis and bioassay of a new class of disubstituted 1,3,4-

oxadiazoles, 1,3,4-thiadiazoles and 1,2,4-triazoles. Med Chem Res. 2019;28:1049–62.
7. Bektaş H, Sökmen BB, Aydın S, Menteşe E, Bektaş A, Dilekçi G. Design, synthesis, and characterization of some new benzimidazole derivatives and biological evaluation. J Heterocyl Chem. 2019;57::2234–42.
8. Menteşe E, Karaali N, Yılmaz F, Ülker S, Kahveci B. Microwave-assisted synthesis and biological evaluation of some benzimidazole derivatives containing a 1,2,4-triazol ring. Arch Pharm Chem Life Sci. 2013;346:556–61.
9. Ozdemir SB, Demirbas N, Demirbas A, Ayaz FA, Çolak NJ. Microwave-assisted synthesis, antioxidant, and antimicrobial evaluation of piperazine-azole-fluoroquinolone based 1,2,4-triazole derivatives. J Heterocyl Chem. 2018;55:2744–59.
10. Doğan IS, Özdemir Z, Sari S, Bozbey I, Karakurt A, Saraç S. Synthesis, anticonvulsant activity, and molecular modeling studies of novel 1-phenyl/1-(4-chlorophenyl)-2-(1H-triazol-1-yl)ethanol ester derivatives. Med Chem Res. 2018;27:2171–86.
11. Kahveci B, Menteşe E, Akkaya E, Yılmaz F, Doğan İS, Özel A. Synthesis of some novel 1,2,4-triazol-3-one derivatives bearing the salicyl moiety and their anticonvulsant activities. Arch Pharm Chem Life Sci. 2014;347:449–55.
12. Holla BS, Veerendra B, Shivananda MK, Poojary B. Synthesis characterization and anticancer activity studies on some Mannich bases derived from 1,2,4-triazoles. Eur J Med Chem. 2003;38:759–67.
13. Al-Soud YA, Al-Masoudi NA, Ferwanah AE. Synthesis and properties of new substituted 1,2,4-triazoles: potential antitumor agents. Bioorg Med Chem. 2003;11:1701–8.
14. Ceylan S. Synthesis and biological evaluation of new Mannich and Schiff bases containing 1,2,4-triazole and 1,3,4-oxadiazole nucleus. Med Chem Res. 2016;25:1958–70.
15. Jalilian AR, Sattari S, Bineshmarvasti M, Shafiee A, Daneshtalab M. Synthesis and in vitro antifungal and cytotoxicity evaluation of thiazolo-4H-1,2,4-triazoles and 1,2,3-thiadiazolo -4H-1,2,4-triazoles. Arch Pharm. 2000;333:347–54.
16. Li BC, Zhang DW, Zhang YM, Jiang D, Li S, Lei W, et al. Synthesis and evaluation of novel benzene-ethanol bearing 1,2,4-triazole derivatives as potential antimicrobial agents. Med Chem Res. 2016;26:44–51.
17. Lingappa B, Girisha KS, Kalluraya BN, Rai S, Kumari NS. Regioselective reaction: synthesis of novel mannich bases derived from 3-(4,6-disubstituted-2-thiomethylpyrimidyl) -4-amino-5-mercapto-1,2,4-tiiazoles and their antimicrobial properties. Ind J Chem. 2008;47B:1858–64.
18. Zeydi MM, Montazeri N, Fouladi M. Synthesis and evaluation of novel [1,2,4]Triazolo [1,5-c] quinazoline derivatives as antibacterial agents. J Heterocyl Chem. 2017;54:3549–53.
19. Almajan GL, Barbuceanu SF, Almajan ER, Draghici C, Saramet G. Synthesis, characterization and antibacterial activity of some triazole Mannich bases carrying diphenylsulfone moieties. Eur J Med Chem. 2009;44:3083–9.
20. Abdel-Megeed AM, Abdel-Rahman HM, Alkaramany GES, El-Gendy MA. Design, synthesis and molecular modeling study of acylated 1,2,4-triazole-3-acetates with potential anti-inflammatory activity. Eur J Med Chem. 2009;44:117–23.
21. Sujith KV, Rao JN, Shetty P, Kalluraya B. Regioselective reaction: synthesis and pharmacological study of mannich bases containing ibuprofen moiety. Eur J Med Chem. 2009;44:3697–702.
22. Tozkoparan B, Aytaç SP, Aktay G. Novel 3,6-disubstituted 7H-1,2,4-triazolo[3,4-b] [1,3,4]thiadiazines: synthesis, characterization, and evaluation of analgesic/antiinflammatory, antioxidant activities. Arch Pharm Chem Life Sci. 2009;40:291–8.
23. Akhter MW, Hassan MZ, Amir M. Synthesis and pharmacological evaluation of 3-diphenylmethyl-6-substituted-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazoles: a condensed bridgehead nitrogen Heterocyl system. Arab J Chem. 2014;7:955–63.
24. Hunashal RD, Satyanarayana D, Maddi VS. Synthesis and antimicrobial activity of 2-[4-(substituted benzylidenamino)-5-(substituted phenoxymethyl)-4H-1,2,4-triazol-3-yl thio] acetic acid derivatives. E-J Chem. 2012;9:1206–12.
25. Ahirwar J, Ahirwar D, Lanjhiyana S, Jha AK, Dewangan D, Badwaik H. Analgesic and anti-inflammatory potential of merged pharmacophore containing 1,2,4-triazoles and substituted benzyl groups via thio linkage. J Heterocycl Chem. 2018;55:2130–41.
26. Miniyar PB, Mahajan AA, Mokale SN, Shah MU, Kumar AS, Chaturbhuj GU. Triazole hybrids as new type of anti-fungal agents. Arab J Chem. 2017;10:295–9.
27. Khanage SG, Mohite PB, Pandhare RB, Raju SA. Investigation of pyrazole and tetrazole derivatives containing 3,5 disubstituted-4H-1,2,4-triazole as a potential antitubercular and antifungal agent. Bioint Res Appl Chem. 2012;2:277–83.
28. Küçükgüzel I, Küçükgüzel SG, Rollas S, Kiraz M. Some 3-thioxo/alkylthio-1,2,4-triazoles with a substituted thiourea moiety as possible antimycobacterials. Bioorg Med Chem Lett. 2001;11:1703–7.
29. Xiao Z, Waters NC, Woodard CL, Li PK. Design and synthesis of pfmrk inhibitors as potential antimalarial agents. Bioorg Med Chem Lett. 2001;11:2875–78.
30. Chelamalla R, Akena V, Manda S. Synthesis of N′-arylidene-2-(5-aryl-1H-1,2,4-triazol -3-ylthio)acetohydrazides as antidepressants. Med Chem Res. 2017;26:1359–66.
31. Hichri F, Omri A, Hossan ASM, Jannet HB. Alpha-glucosidase and amylase inhibitory effects of eruca vesicaria subsp. longirostris essential oils: synthesis of new 1,2,4-triazole-thiol derivatives and 1,3,4-thiadiazole with potential inhibitory activity. Pharm Biol. 2019;57:564–70.
32. Bera H, Dolzhenko AV, Sun L, Gupta SD, Chui WK. Synthesis and in vitro evaluation of 1,2,4-triazolo[1,5-a][1,3,5]triazine derivatives as thymidine phosphorylase inhibitors. Chem Biol Drug Des. 2013b;82:351–60.
33. Hassan GS, El-Sherbeny MA, El-Ashmawy MB, Bayomi SM, Maarouf AR, Badria FA. Synthesis and antitumor testing of certain new fused triazolopyrimidine and triazoloquinazoline derivatives. Arab J Chem. 2017;10:S1345–55.
34. Bera H, Chui WK, Gupta SD. Synthesis, in vitro evaluation of thymidine phosphorylase inhibitory activity, and in silico study of 1,3,5-triazin-2,4-dione and its fused analogues. Med Chem Res. 2013a;22:6010–21.
35. Dhall E, Jain S, Mishra A, Dwivedi J, Sharma S. Synthesis and evaluation of some phenyl substituted azetidine containing 1,2,4-triazole derivatives as antibacterial agents. J Heterocyl Chem. 2018;55:2859–68.
36. Sahin D, Bayrak H, Demirbas A, Alpaykaraoglu S. Design and synthesis of some azole derivatives as potential antimicrobial agents. Med Chem Res. 2012;21:4485–98.
37. Vatmurge NS, Hazra BG, Pore VS, Shirazi F, Chavan PS, Deshpande MV. Synthesis and antimicrobial activity of b-lactam-bile acid conjugates linked via triazole. Bioorg Med Chem Lett. 2008;18:2043–7.
38. Chernyshev VM, Pyatakov DA, Sokolov AN, Astakhov AV, Gladkov ES, Shishkina SV, et al. Partially hydrogenated 2-amino [1,2,4]triazolo[1,5-a]pyrimidines as synthons for the preparation of polycondensed heterocycles: reaction with chlorocarboxylic acid chlorides. Tetrahedron. 2014;70:684–701.
39. Abu-Hashem AA, Gouda MA. Synthesis and antimicrobial activity of some novel quinoline, chromene, pyrazole derivatives bearing triazolopyrimidine moiety. J Heterocyl Chem. 2017;54:850.
40. Tokala R, Bale S, Janrao IP, Vennela A, Kumar NP, Senwar KR, et al. (2018) Synthesis of 1,2,4-triazole-linked urea/thiourea

conjugates as cytotoxic and apoptosis inducing agents. Bioorg Med Chem Lett. 2018;28:1919–24
41. Wang XF, Zhang S, Li BL, Zhao JJ, Liu YM, Zhang RL, et al. Synthesis and biological evaluation of disulfifides bearing 1,2,4-triazole moiety as antiproliferative agents. Med Chem Res. 2017;26:3367–74.
42. Branowska D, Ławecka J, Sobiczewski M, Karczmarzyk Z, Wysocki W, Wolin´ska E, et al. Synthesis of unsymmetrical disulfanes bearing 1,2,4-triazine scaffold and their in vitro screening towards anti-breast cancer activity. Monatsh Chem. 2018;149:1409–20.
43. Cincinelli R, Musso L, Artali R, Guglielmi M, Bianchino E, Cardile F, et al. Camptothecin-psammaplin A hybrids as topoisomerase I and HDAC dual-action inhibitors. Eur J Med Chem. 2018;143:2005–14.
44. Zhu SJ, Ying HZ, Wu Y, Qiu N, Liu T, Yang B, et al. Design, synthesis and biological evaluation of novel podophyllotoxin derivatives bearing 4β-disulfide/trisulfide bond as cytotoxic agents. RSC Adv. 2015;5:103172–83.
45. Liu HY, Wang HX, Li X, Wu Z, Li CW, Liu YM, et al. Synthesis, antitumor and antimicrobial evaluation of novel 1,3,4-thiadiazole derivatives bearing disulfide bond. Med Chem Res. 2018;27:1929–40.
46. Blanchard S, Bill E, Weyhermuller T, Wieghardt K. *N,N*-coordinated π radical anions of S-methyl-1-phenyl-isothiosemicarbazide in two five-coordinate ferric Complexes $[Fe^{III}(L^{Me\cdot})_2X]$ ($X = CH_3S^-$, Cl^-). Inorg Chem. 2004;43:2324–9.