Med Chem Res
DOI 10.1007/s00044-017-2029-0

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and biological evaluation of disulfides bearing 1,2,4-triazole moiety as antiproliferative agents

Xue-Feng Wang[1] · Shuai Zhang[1] · Bao-Lin Li[1] · Ji-Jun Zhao[1] · Yu-Ming Liu[1] · Rui-Lian Zhang[1] · Bo Li[1] · Bao-Quan Chen[1]

Received: 19 June 2017 / Accepted: 5 August 2017

Abstract A series of novel nonsymmetrical disulfides bearing 1,2,4-triazole moiety were designed, 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. The preliminary bioassay results demonstrated that most of the tested compounds **8a–r** exhibited good antiproliferative activities, and some compounds showed better effects than positive control 5-fluorouracil against various cancer cell lines. Among these compounds, compound **8l** showed significant antiproliferative activity against SMMC-7721 cells with IC_{50} value of 2.97 μM. Compound **8f** displayed highly effective biological activity against Hela cells with IC_{50} value of 3.51 μM. Compound **8d** exhibited the best inhibitory effect against A549 cells with IC_{50} value of 2.79 μM. Furthermore, some of the tested compounds showed weak cytotoxic effect against the normal cell lines L929. The pharmacological results suggest that the substituent groups are vital for improving the potency and selectivity of this class of compounds.

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

Introduction

Cancer is considered to be the second most common cause of deaths after cardiovascular diseases in the world. Despite significant progress has been achieved in anticancer therapy, the discovery and development of effective anticancer drugs remain one of the most intractable worldwide health problems (Anand et al. 2008). During recent years, some small molecules containing various azoles were synthesized as potential anticancer agents and have become the subject of considerable growing interest for the treatment of cancer (Li et al. 2016; Wang et al. 2015; Yadagiri et al. 2015; Wang et al. 2011).

1,2,4-Triazole is a versatile lead molecule for designing potential bioactive agent, and its derivatives are associated with many types of biological properties, including antioxidant (Padmaja et al. 2015; Aktas-Yokus et al. 2015), antibacterial (Li et al. 2015; Sokmen et al. 2013), anti-inflammatory (Sarigol et al. 2015; El-Serwy et al. 2013; Li et al. 2016), antifungal (Yu et al. 2013; Li et al. 2014), anticonvulsant (Kahveci et al. 2014; Botros et al. 2013), analgesic (Ashour et al. 2013; Vijesh et al. 2013), antidepressant (Patel et al. 2010; Chelamalla et al. 2017), anticoagulant (Sun et al. 2017), and anti-thymidine phosphorylase effects (Bera et al. 2013). In particular, a great number of 1,2,4-triazole derivatives have been proved to show potent antitumor activities (Nagender et al. 2016; Abdo and Kamel 2015; Kamel and Abdo 2014; Popiołek et al. 2014; Hou et al. 2011; Wang et al. 2015; Zhao et al. 2012; Shi et al. 2013). Thus, the importance of 1,2,4-triazole heterocyclic in the fields of medicinal chemistry has drawn widespread attention during the last few decades. Meanwhile, disulfide derivatives are also known to display a wide spectrum of biological activities because the disulfide group is an important core found in many biologically

Electronic supplementary material The online version of this article (doi:10.1007/s00044-017-2029-0) contains supplementary material, which is available to authorized users.

✉ Bao-Quan Chen
chenbaoquan66@126.com

[1] School of Chemistry and Chemical Engineering, Tianjin Key Laboratory of Organic Solar Cells and Photochemical Conversion, Tianjin University of Technology, 300384 Tianjin, China

Published online: 05 September 2017

active compounds with many types of biological activities including antibacterial (Park et al. 2009; Turos et al. 2008), anti-HIV-1 (Cesarini et al. 2008), antitumor (Ryu et al. 2004; Hunter et al. 2008; Diraimondo et al. 2013; Rubino et al. 2017), and β-glucuronidase inhibition properties (Teha et al. 2016). Particularly, the antitumor character of disulfide derivatives attracts great interests from medicinal chemists in recent years.

Considering the biological significance of 1,2,4-triazole and disulfide, we report herein the synthesis of non-symmetrical disulfides bearing 1,2,4-triazole moiety. Meanwhile, their in vitro antiproliferative activities were evaluated against human cancer cell lines SMMC-7721 (human liver cancer cell), Hela (human cervix adenocarcinoma cell), A549 (human lung cancer cell), and normal cell lines L929 (mouse fibroblast cell) with the ultimate aim of developing novel potent antitumor agents (Fig. 1).

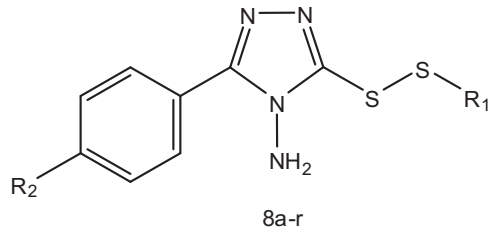


Fig. 1 The structure of target compounds **8a–r**

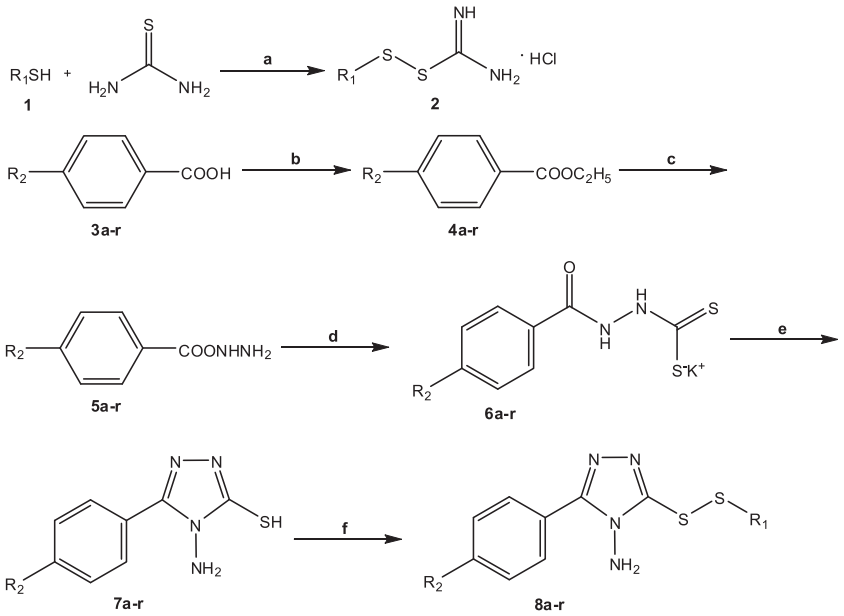
Results and discussion

Chemistry

As depicted in Scheme 1, eighteen nonsymmetrical disulfides bearing 1,2,4-triazole moiety **8a–r** were synthesized and reported for the first time. The preparation of *S*-alkyl-thioisothiourea hydrochloride **2** was carried out by reported literature method (Sirakawa et al. 1970). 3-Substitutedphenyl-4-amino-5-mercapto-1,2,4-triazole **7a–r** were synthesized from substituted benzoic acid in four steps including esterification, hydrazidation, salt formation, and cyclization according to reported literature method (Qiao et al. 2001; Aswathanarayanappa et al. 2013). Finally, the target compounds **8a–r** were successfully obtained by the reaction of intermediate 2 and compounds **7a–r** in the presence of $NaHCO_3$ in ethanol and water at 0–10 °C. All newly synthesized compounds **8a–r** were purified by recrystallization from ethanol/water and their structures were characterized by IR, ^{1}H NMR, ^{13}C NMR, and High Resolution-Electrospray Ionization-Mass Spectrometer (HR-ESI-MS).

Pharmacology and discussion

The antiproliferative activities of the newly synthesized compounds **8a–r** were tested against SMMC-7721, Hela, A549, and L929 cell lines using CCK-8 [2-(2-methoxy-4-nitro-phenyl)-3-(4-nitro-phenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt] assay. Inhibition of cell proliferation by these active compounds at various



Scheme 1 Synthesis of the target compounds **8a–r**. Reagents and conditions: **a** Concd. HCl, H_2O_2(30%), 0–5 °C, 3 h; **b** $SOCl_2$, ethanol, reflux, 4–6 h; **c** $NH_2NH_2{\cdot}H_2O$ (85%), ethanol, reflux, 6–8 h; **d** KOH, CS_2, ethanol, 0–5 °C, 4–6 h; **e** $NH_2NH_2{\cdot}H_2O$ (85%), ethanol, reflux, 6–8 h; **f** Compound **2**, ethanol, $NaHCO_3/H_2O$, 0–10 °C, 6–8 h

R_1 (R_2 = H): C_2H_5 (**8a**), *n*-C_3H_7 (**8b**), *i*-C_3H_7 (**8c**), *n*-C_4H_9 (**8d**), *i*-C_4H_9 (**8e**), *n*-C_5H_{11} (**8f**), *n*-C_6H_{13} (**8g**), $PhCH_2$ (**8h**), 4-Cl-$PhCH_2$ (**8i**); R_1 (R_2 = Cl): C_2H_5 (**8j**), *n*-C_3H_7 (**8k**), *i*-C_3H_7 (**8l**), *n*-C_4H_9 (**8m**), *i*-C_4H_9 (**8n**), *n*-C_5H_{11} (**8o**), *n*-C_6H_{13} (**8p**), $PhCH_2$ (**8q**), 4-Cl-$PhCH_2$ (**8r**).

concentrations was measured, and their IC_{50} (the concentration that causes a 50% cell proliferation inhibition) values were calculated and are summarized in Table 1. 5-Fluorouracil (5-FU) was used as positive control.

As shown in Table 1, the newly synthesized compounds **8a–r** displayed very good antiproliferative effects against SMMC-7721 cells with IC_{50} values ranging from 2.97 to 6.62 μM. Meanwhile, the majority of them showed better activities than positive control 5-fluorouracil.

Especially noteworthy is compound **8l** possessing *i*-propyl group while R_2 is chlorine atom, which showed significant antiproliferative activity with IC_{50} value of 2.97 μM. But, no matter R_2 is hydrogen or chlorine atom, the effects of R_1 substituents on antiproliferative activity against SMMC-7721 cells did not show apparent regularity. In Hela cells, except compounds **8a**, **8o**, and **8p** which showed moderate antitumor activities, the other compounds exhibited good antiproliferative effects with IC_{50} values ranging from 3.51 to 9.15 μM, and displayed higher activities than positive control 5-fluorouracil. In particular, compound **8f** carrying *n*-pentyl substituent while R_2 is hydrogen atom displayed highly effective biological activity with IC_{50} value of 3.51 μM. However, compound **8p** possessing *n*-hexyl substituent while R_2 is chlorine atom showed poor activity with IC_{50} value of 42.92 μM. In A549 cells, most of the newly synthesized compounds exhibited good antiproliferative activities and showed better effects than positive control 5-fluorouracil. Among them, compound **8d** possessing *n*-butyl substituent while R_2 is hydrogen atom exhibited the best inhibitory effect with IC_{50} value of 2.79 μM. With the exception of compound **8d**, while R_2 is hydrogen atom, on the whole the shorter alkyl chain-substituted derivatives, including **8a**, **8b**, and **8c**, showed weaker activities than the longer alkyl chain-substituted derivatives, including **8e**, **8f,** and **8g**. But, while R_2 is chlorine atom, on the whole the shorter alkyl chain-substituted derivatives, including **8j**, **8k,** and **8l**, exhibited better activities than the longer alkyl chain-substituted derivatives, including **8n**, **8o,** and **8p**. These results indicated that the influence of substituents on antiproliferative activity against A549 cells is complex and unexplained at present. These results also suggest that this class of compounds displayed stronger antiproliferative effects against SMMC-7721 cells than Hela and A549 cells on the whole. We also found from the results that compounds **8h** and **8i** possessing benzyl and 4-cholobenzyl substituents, respectively, while R_2 is hydrogen atom, showed comparable activities against Hela and A549 cells, and compounds **8q** and **8r** possessing benzyl and 4-cholobenzyl substituents, respectively, while R_2 is chlorine atom, displayed similar effects against SMMC-7721 and A549 cells. Furthermore, some of the tested compounds showed weak cytotoxic effect against the normal cell lines L929. In particular, as compared with SMMC-7721 cells, all compounds **8a–r** exhibited weaker cytotoxic activity against L929 cells, and most of the compounds showed highly lower cytotoxic effect than 5-fluorouracil. Therefore, the results indicate ample scope for screening hitherto relatively unexplored disulfides bearing 1,2,4-triazole moiety as antiproliferative agents.

Table 1 In vitro antiproliferative activities of compounds 8a–r against various cell lines

Compound	IC_{50}[a] (μM)			
	SMMC-7721	Hela	A549	L929
8a	6.62 ± 0.18	17.31 ± 1.19	13.38 ± 1.28	9.44 ± 0.43
8b	3.80 ± 0.16	7.75 ± 0.27	8.87 ± 0.39	8.94 ± 0.35
8c	4.64 ± 0.17	7.74 ± 0.18	8.30 ± 0.30	8.91 ± 0.26
8d	4.36 ± 0.21	6.34 ± 0.32	2.79 ± 0.09	4.65 ± 0.12
8e	3.80 ± 0.18	7.17 ± 0.41	6.60 ± 0.34	7.11 ± 0.56
8f	4.64 ± 0.24	3.51 ± 0.13	6.62 ± 0.28	5.95 ± 0.32
8g	4.09 ± 0.20	6.05 ± 0.25	5.77 ± 0.29	15.46 ± 1.29
8h	3.79 ± 0.12	7.10 ± 0.38	7.47 ± 0.40	7.07 ± 0.37
8i	4.37 ± 0.09	7.17 ± 0.15	7.17 ± 0.35	7.26 ± 0.22
8j	3.24 ± 0.14	6.62 ± 0.36	4.37 ± 0.22	5.84 ± 0.15
8k	3.24 ± 0.10	5.21 ± 0.17	5.49 ± 0.27	7.37 ± 0.23
8l	2.97 ± 0.07	4.37 ± 0.13	5.59 ± 0.19	8.11 ± 0.48
8m	3.24 ± 0.19	7.74 ± 0.29	4.92 ± 0.23	8.49 ± 0.39
8n	3.79 ± 0.08	7.75 ± 0.34	6.05 ± 0.38	17.60 ± 1.27
8o	5.22 ± 0.23	19.84 ± 1.78	8.30 ± 0.45	26.92 ± 2.05
8p	5.49 ± 0.09	42.92 ± 3.36	37.59 ± 2.88	32.77 ± 2.37
8q	3.79 ± 0.11	6.05 ± 0.29	8.30 ± 0.48	9.87 ± 0.47
8r	3.52 ± 0.14	9.15 ± 0.45	8.02 ± 0.38	8.80 ± 0.39
5-FU[b]	5.62 ± 0.28	17.21 ± 0.67	8.13 ± 0.34	2.98 ± 0.15

Each experiment was independently performed three times and expressed as mean ± standard deviation (SD)

[a] IC_{50} is defined as the concentration that causes a 50 % cell proliferation inhibition

[b] Used as positive control

Conclusion

In summary, a series of novel nonsymmetrical disulfides bearing 1,2,4-triazole moiety were designed, synthesized, and evaluated for their in vitro antiproliferative activities against SMMC-7721, Hela, and A549 human cancer cell lines by CCK-8 assay. The majority of tested compounds **8a–r** inhibited the proliferation better than positive control 5-fluorouracil. In particular, compound **8l** showed significant antiproliferative activity against SMMC-7721 cells with IC_{50} value of 2.97 μM. Compound **8f** displayed highly effective biological activity against Hela cells with IC_{50} value of 3.51 μM. Compound **8d** exhibited the best inhibitory effect against A549 cells with IC_{50} value of 2.79 μM. Furthermore, some of the tested compounds showed weak cytotoxic effect against the normal cell lines L929.

Therefore, the results will be significant in the development of potent antitumor agents.

Experimental

Chemistry

Melting points were determined by a X-6 microscope melting point apparatus and are uncorrected. Infrared spectra were recorded in KBr pellets on a Nicolet Avatar 370 spectrometer. NMR spectra were performed on a Bruker Avance III 400 MHz spectrometer (^{1}H, 400 MHz; ^{13}C, 100 MHz) using DMSO-d_6 as the solvent and tetramethylsilane as the internal standard. Chemical shifts are showed in and values (ppm) and the coupling constants are expressed in *J* values (Hz). Mass spectra were taken with a Waters Xevo G2 QTof (ESI) mass spectrometer. Unless otherwise noted, all solvents and reagents were obtained from commercial suppliers and used without further purification.

*General procedure for the preparation of compounds (**8a–r**)*

S-Alkyl-thioisothiourea hydrochloride **2** (2.0 mmol) and 3-substitutedphenyl-4-amino-5-merca-pto-1,2,4-triazole **7a–r** (2.0 mmol) were dissolved in 15 ml ethanol. A solution of $NaHCO_3$ (4.0 mmol) in 15 ml water was added dropwise with vigorous stirring at 0–10 °C. The mixture was stirred for additional 6–8 h. The insoluble solid was collected and further purified by recrystallization from ethanol/water to afford the desired products.

3-Phenyl-4-amino-5-ethyldisulfanyl-1,2,4-triazole **(8a)** White solid, Yield 50%, m.p.: 120.8–122.5 °C. IR (KBr, cm^{-1}): 3474, 3413, 3134, 2362, 1638, 1618, 1472, 1453, 1401, 1284, 1242, 975, 769, 690. ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 8.03–8.05 (m, 2H, Ar–H), 7.53–7.56 (m, 3H, Ar–H), 6.26 (s, 2H, NH_2), 2.97–3.02 (q, $J = 7.2$ Hz, 2H, SCH_2), 1.36 (t, $J = 7.2$ Hz, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm): 154.86, 153.06, 130.39, 128.97, 128.47, 127.23, 32.74, 14.44. HR-ESI-MS (*m/z*): Calcd for $C_{10}H_{12}N_4S_2$: 253.0582 $[M + H]^+$, Found 253.0590.

3-Phenyl-4-amino-5-(n-propyldisulfanyl)-1,2,4-triazole **(8b)** White solid, Yield 50%, m.p.: 109.6–111.4 °C. IR (KBr, cm^{-1}): 3471, 3413, 3130, 1638, 1618, 1401, 1110, 993, 766, 691. ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 8.03–8.05 (m, 2H, Ar–H), 7.53–7.56 (m, 3H, Ar–H), 6.25 (s, 2H, NH_2), 2.97 (t, $J = 7.2$ Hz, 2H, SCH_2), 1.72–1.81 (m, 2H, CH_2), 0.91 (t, $J = 7.2$ Hz, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm): 154.86, 153.01, 130.39, 128.97, 128.46, 127.24, 41.13, 21.96, 13.19. HR-ESI-MS (*m/z*): Calcd for $C_{11}H_{14}N_4S_2$: 267.0738 $[M + H]^+$, Found 267.0743.

3-Phenyl-4-amino-5-(i-propyldisulfanyl)-1,2,4-triazole (**8c**) White solid, Yield 49%, m.p.: 105.3–106.8 °C. IR (KBr, cm^{-1}): 3464, 3415, 3134, 2360, 2342, 1638, 1619, 1452, 1401, 1284, 1242, 1109, 977, 769, 725, 710, 693. ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 8.03–8.05 (m, 2H, Ar–H), 7.53–7.56 (m, 3H, Ar–H), 6.25 (s, 2H, NH_2), 3.39 (m, 1H, SCH), 1.34 (d, $J = 6.8$ Hz, 6H, CH_3). ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm): 154.79, 153.20, 130.39, 128.97, 128.45, 127.21, 41.64, 22.47. HR-ESI-MS (*m/z*): Calcd for $C_{11}H_{14}N_4S_2$: 267.0738 $[M + H]^+$, Found 267.0741.

3-Phenyl-4-amino-5-(n-butyldisulfanyl)-1,2,4-triazole (**8d**) White solid, Yield 50%, m.p.: 102.4–104.1 °C. IR (KBr, cm^{-1}): 3472, 3413, 3132, 2360, 1637, 1401, 1111, 767, 691. ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 8.03–8.05 (m, 2H, Ar–H), 7.53–7.56 (m, 3H, Ar–H), 6.26 (s, 2H, NH_2), 2.99 (t, $J = 7.2$ Hz, 2H, SCH_2), 1.68–1.75 (m, 2H, CH_2), 1.34–1.43 (m, 2H, CH_2), 0.89 (t, $J = 7.2$ Hz, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm): 154.85, 153.02, 130.39, 128.98, 128.46, 127.23, 38.37, 30.64, 21.36, 13.96. HR-ESI-MS (*m/z*): Calcd for $C_{12}H_{16}N_4S_2$: 281.0895 $[M + H]^+$, Found 281.0900.

3-Phenyl-4-amino-5-(i-butyldisulfanyl)-1,2,4-triazole (**8e**) White solid, Yield 47%, m.p.: 104.2–105.3 °C. IR (KBr, cm^{-1}): 3472, 3413, 3133, 2361, 1638, 1618, 1401, 1110, 990, 767, 712, 692. ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 8.03–8.05 (m, 2H, Ar–H), 7.53–7.56 (m, 3H, Ar–H), 6.26 (s, 2H, NH_2), 2.90 (d, $J = 6.8$ Hz, 2H, SCH_2), 1.98–2.09 (m, 1H, CH), 0.97 (d, $J = 6.8$ Hz, 6H, CH_3). ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm): 154.86, 152.98, 130.40, 128.98, 128.45, 127.22, 47.80, 27.79, 21.83. HR-ESI-MS (*m/z*): Calcd for $C_{12}H_{16}N_4S_2$: 281.0895 $[M + H]^+$, Found 281.0898.

3-Phenyl-4-amino-5-(n-pentyldisulfanyl)-1,2,4-triazole (**8f**) White solid, Yield 46%, m.p.: 99.9–101.7 °C. IR (KBr, cm^{-1}): 3474, 3413, 3134, 1638, 1453, 1401, 1136, 975, 769, 690. ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 8.03–8.05 (m, 2H, Ar–H), 7.53–7.56 (m, 3H, Ar–H), 6.25 (s, 2H, NH_2), 2.99 (t, $J = 7.2$ Hz, 2 H, SCH_2), 1.70–1.77 (m, 2H, CH_2), 1.28–1.36 (m, 4H, CH_2), 0.87 (t, $J = 7.2$ Hz, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm): 154.85, 153.01, 130.39, 128.97, 128.46, 127.23, 38.69, 30.36, 28.23, 21.14, 14.29. HR-ESI-MS (*m/z*): Calcd for $C_{13}H_{18}N_4S_2$: 295.1051 $[M + H]^+$, Found 295.1053.

3-Phenyl-4-amino-5-(n-hexyldisulfanyl)-1,2,4-triazole (**8g**) White solid, Yield 51%, m.p.: 103.8–104.5 °C. IR (KBr, cm^{-1}): 3474, 3413, 3134, 2362, 1638, 1618, 1453, 1401, 975, 769, 690. ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 8.03–8.05 (m, 2H, Ar–H), 7.53–7.55 (m, 3H, Ar–H), 6.25 (s, 2H, NH_2), 2.99 (d, $J = 7.2$ Hz, 2H, SCH_2), 1.69–1.76 (m, 2H, CH_2), 1.25–1.37 (m, 6H, CH_2), 0.86 (t, $J = 6.8$ Hz, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm): 154.56, 153.57, 129.40, 128.95, 128.45, 127.36, 38.65, 31.26, 28.47, 27.80, 22.44, 14.32. HR-ESI-MS (*m/z*): Calcd for $C_{14}H_{20}N_4S_2$: 309.1208 $[M + H]^+$, Found 309.1210.

3-Phenyl-4-amino-5-benzyldisulfanyl-1,2,4-triazole (**8h**) White solid, Yield 51%, m.p.: 129.3–130.6 °C. IR (KBr, cm^{-1}): 3475, 3413, 3144, 2360, 1638, 1618, 1469, 1453, 1401, 1238, 1137, 970, 863, 773, 764, 715, 695. ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 8.04–8.07 (m, 2H, Ar–H), 7.54–7.57 (m, 3 H, Ar–H), 7.28–7.43 (m, 5H, Ar–H), 6.25 (s, 2H, NH_2), 4.27 (s, 2H, SCH_2). ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm): 154.88, 153.05, 137.07, 130.45, 129.99, 129.01, 128.97, 128.50, 128.02, 127.19, 42.55. HR-ESI-MS (*m/z*): Calcd for $C_{15}H_{14}N_4S_2$: 315.0738 $[M + H]^+$, Found 315.0742.

3-Phenyl-4-amino-5-(4-chlorobenzyldisulfanyl)-1,2,4-triazole (**8i**) White solid, Yield 48%, m.p.: 148.4–149.8 °C. IR (KBr, cm^{-1}): 3475, 3413, 3147, 1656, 1595, 1489, 1465, 1402, 991, 837, 825, 804, 772, 739, 718, 693. ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 8.04–8.06 (m, 2H, Ar–H), 7.54–7.56 (m, 3H, Ar–H), 7.41–7.47 (AB × 2, $J_{AB} = 8.8$ Hz, 4H, Ar–H), 6.26 (s, 2H, NH_2), 4.25 (s, 2H, SCH_2). ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm): 155.04, 153.93, 136.18, 134.55, 131.71, 130.89, 129.40, 128.96, 128.49, 126.26, 41.38. HR-ESI-MS (*m/z*): Calcd for $C_{15}H_{13}ClN_4S_2$: 349.0349 $[M + H]^+$, Found 349.0352.

3-(4-Chlorophenyl)-4-amino-5-ethyldisulfanyl-1,2,4-triazole (**8j**) Pale yellow solid; Yield 48%, m.p.: 107.6–109.2 °C. IR (KBr, cm^{-1}): 3474, 3414, 3146, 2361, 1638, 1618, 1474, 1448, 1401, 1276, 1244, 1012, 974, 925, 833, 782, 768, 751, 724, 681. ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 8.09 (d, $J = 8.4$ Hz, 2H, Ar–H), 7.63 (d, $J = 8.8$ Hz, 2H, Ar–H), 6.28 (s, 2H, NH_2), 2.96–3.02 (q, $J = 7.2$ Hz, 2H, SCH_2), 1.35 (t, $J = 7.2$ Hz, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm): 153.89, 153.39, 135.28, 130.13, 129.13, 126.05, 32.74, 14.43. HR-ESI-MS (*m/z*): Calcd for $C_{10}H_{11}ClN_4S_2$: 287.0192 $[M + H]^+$, Found 287.0195.

3-(4-Chlorophenyl)-4-amino-5-(n-propyldisulfanyl)-1,2,4-triazole (**8k**) White solid, Yield 48%, m.p.: 110.4–112.3 °C. IR (KBr, cm^{-1}): 3473, 3413, 3133, 2360, 1638, 1618, 1401, 1275, 1243, 1232, 1014, 974, 904, 828, 767, 683. ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 8.09 (d, $J = 8.4$ Hz, 2H, Ar–H), 7.63 (d, $J = 8.4$ Hz, 2H, Ar–H), 6.28 (s, 2H, NH_2), 2.96 (t, $J = 7.2$ Hz, 2H, SCH_2), 1.71–1.80 (m, 2H, CH_2), 0.96 (t, $J = 7.2$ Hz, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm): 153.88, 153.34, 135.29, 130.13, 129.13, 126.04, 41.11, 21.94, 13.19. HR-ESI-MS (*m/z*): Calcd for $C_{11}H_{13}ClN_4S_2$: 301.0349 $[M + H]^+$, Found 301.0348.

3-(4-Chlorophenyl)-4-amino-5-(i-propyldisulfanyl)-1,2,4-triazole (**8l**) Pale yellow solid, Yield 51%, m.p.: 115.6–116.9 °C. IR (KBr, cm^{-1}): 3474, 3413, 3134, 2863, 2360, 2027, 1638, 1618, 1474, 1447, 1401, 1154, 1014, 975, 950, 931, 832, 768, 749, 725, 695, 682. ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 8.09 (d, $J = 8.8$ Hz, 2H, Ar–H), 7.63 (d, $J = 8.4$ Hz, 2H, Ar–H), 6.27 (s, 2H, NH_2), 3.38 (m, 1H, SCH), 1.34 (d, $J = 6.8$ Hz, 6H, CH_3). ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm): 153.82, 153.51, 135.26, 130.12, 129.13, 126.05, 41.68, 22.46. HR-ESI-MS (*m/z*): Calcd for $C_{11}H_{13}ClN_4S_2$: 301.0349 $[M + H]^+$, Found 301.0350.

3-(4-Chlorophenyl)-4-amino-5-(n-butyldisulfanyl)-1,2,4-triazole (**8m**) White solid, Yield 53%, m.p.: 104.5–105.9 °C. IR (KBr, cm^{-1}): 3475, 3414, 3126, 2956, 2929, 2360, 2027, 1638, 1618, 1452, 1401, 1273, 1243, 1220, 1114, 996, 834, 747, 730, 681. ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 8.09 (d, $J = 8.8$ Hz, 2H, Ar–H), 7.63 (d, $J = 8.4$ Hz, 2H, Ar–H), 6.27 (s, 2H, NH_2), 2.99 (t, $J = 7.2$ Hz, 2H, SCH_2), 1.68–1.75 (m, 2H, CH_2), 1.34–1.43 (m, 2H, CH_2), 0.89 (t, $J = 7.2$ Hz, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm): 153.88, 153.34, 135.29, 130.12, 129.13, 126.05, 38.40, 30.63, 21.36, 13.95. HR-ESI-MS (*m/z*): Calcd for $C_{12}H_{15}ClN_4S_2$: 315.0505 $[M + H]^+$, Found 315.0504.

3-(4-Chlorophenyl)-4-amino-5-(i-butyldisulfanyl)-1,2,4-triazole (**8n**) Pale yellow solid, Yield 48%, m.p.: 101.2–103.1 °C. IR (KBr, cm^{-1}): 3473, 3413, 3137, 2966, 2870, 2360, 1638, 1618, 1472, 1449, 1401, 1242, 1014, 946, 829, 767, 750, 725, 695, 682. ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 8.09 (d, $J = 8.4$ Hz, 2H, Ar–H), 7.63 (d, $J = 8.4$ Hz, 2H, Ar–H), 6.28 (s, 2H, NH_2), 2.90 (d, $J = 6.8$ Hz, 2H, SCH_2), 1.98–2.10 (m, 1H, CH), 0.97 (d, $J = 6.8$ Hz, 6H, CH_3). ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm): 153.89, 153.30, 135.28, 130.12, 129.14, 126.06, 47.81, 27.78, 21.83. HR-ESI-MS (*m/z*): Calcd for $C_{12}H_{15}ClN_4S_2$: 315.0505 $[M + H]^+$, Found 315.0502.

3-(4-Chlorophenyl)-4-amino-5-(n-pentyldisulfanyl)-1,2,4-triazole (**8o**) White solid, Yield 50%, m.p.: 107.7–109.3 °C. IR (KBr, cm^{-1}): 3473, 3414, 3128, 2926, 2857, 2361, 2342, 2027, 1638, 1618, 1452, 1401, 1273, 1244, 1093, 995, 952, 833, 747, 730, 690. ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm):

8.09 (d, $J = 8.8$ Hz, 2H, Ar–H), 7.63 (d, $J = 8.4$ Hz, 2H, Ar–H), 6.27 (s, 2 H, NH_2), 2.98 (t, $J = 6.8$ Hz, 2H, SCH_2), 1.71–1.75 (m, 2H, CH_2), 1.24–1.37 (m, 4H, CH_2), 0.88 (t, $J = 6.8$ Hz, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm): 153.87, 153.35, 135.27, 130.12, 129.15, 126.04, 38.65, 30.35, 28.21, 22.15, 14.30. HR-ESI-MS (*m/z*): Calcd for $C_{13}H_{17}ClN_4S_2$: 329.0661 $[M + H]^+$, Found 329.0667.

3-(4-Chlorophenyl)-4-amino-5-(n-hexyldisulfanyl)-1,2,4-triazole (**8p**) White solid; Yield 55%, m.p.: 110.4–112.1 °C. IR (KBr, cm^{-1}): 3474, 3414, 3126, 2957, 2926, 2855, 2360, 2342, 2027, 1638, 1618, 1451, 1401, 1243, 1095, 998, 836, 749, 731, 679. ^1H NMR (DMSO-d_6, 400 MHz) δ (ppm): 8.09 (d, $J = 8.8$ Hz, 2H, Ar–H), 7.63 (d, $J = 8.4$ Hz, 2H, Ar–H), 6.27 (s, 2H, NH_2), 2.98 (t, $J = 7.2$ Hz, 2H, SCH_2), 1.67–1.76 (m, 2H, CH_2), 1.26–1.37 (m, 6H, CH_2), 0.87 (t, $J = 6.4$ Hz, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm): 153.86, 153.32, 135.27, 130.11, 129.13, 126.07, 38.76, 31.24, 28.49, 27.82, 22.44, 14.33. HR-ESI-MS (*m/z*): Calcd for $C_{14}H_{19}ClN_4S_2$: 343.0818 $[M + H]^+$, Found 343.0817.

3-(4-Chlorophenyl)-4-amino-5-benzyldisulfanyl-1,2,4-triazole (**8q**) White solid, Yield 52%, m.p.: 152.9–154.2 °C. IR (KBr, cm^{-1}): 3474, 3413, 3125, 2260, 2341, 1638, 1618, 1599, 1399, 1013, 971, 914, 862, 834, 766, 750, 731, 697, ^1H NMR (DMSO-d_6, 400 MHz) δ (ppm): 8.11 (d, $J = 8.4$ Hz, 2H, Ar–H), 7.64 (d, $J = 8.8$ Hz, 2H, Ar–H), 7.28–7.42 (m, 5H, Ar–H), 6.27 (s, 2H, NH_2), 4.27 (s, 2H, SCH_2). ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm): 153.88, 153.33, 137.03, 135.31, 130.16, 129.97, 129.16, 128.96, 128.02, 126.05, 42.63. HR-ESI-MS (*m/z*): Calcd for $C_{15}H_{13}ClN_4S_2$: 349.0349 $[M + H]^+$, Found 349.0341.

3-(4-Chlorophenyl)-4-amino-5-(4-chlorobenzyldisulfanyl)-1,2,4-triazole (**8r**) White solid; Yield 48%, m.p.: 125.3–126.7 °C. IR (KBr, cm^{-1}): 3472, 3415, 3133, 2361, 2342, 1638, 1618, 1490, 1401, 1109, 994, 866, 831, 732, 719. ^1H NMR (DMSO-d_6, 400 MHz) δ (ppm): 8.10 (d, $J = 8.4$ Hz, 2H, Ar–H), 7.64 (d, $J = 8.4$ Hz, 2H, Ar–H), 7.41–7.46 (q, $J = 8.8$ Hz, 4H, Ar–H), 6.28 (s, 2H, NH_2), 4.24 (s, 2H, SCH_2). ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm): 153.88, 153.16, 136.26, 135.32, 132.73, 131.80, 130.16, 129.15, 128.98, 126.02, 41.53. HR-ESI-MS (*m/z*): Calcd for $C_{15}H_{12}Cl_2N_4S_2$: 382.9959 $[M + H]^+$, Found 382.9960.

Biological assay

The following established in vitro cell lines were used: SMMC-7721, Hela, A549, and L929, which were obtained from theTumor Cell Resources Bank, Chinese Academy of MedicalSciences. Cell counting kit-8 was from Dojindo (Japan).

Compounds **8a–r** and 5-fluorouracil were respectively dissolved in DMSO to make stock solutions at a concentration of 1.0×10^{-2} mol/L. During the experiment, cell culture medium RPMI-1640 was used to dilute the stock solution to the desired concentration. Cells in the exponential phase were seeded in 96-well culture plates at the confluence of 1×10^4 cells/ well, kept in 37 °C, 5% CO_2 incubator for 24 h. The medium containing different concentrations of compounds was replaced with fresh medium, at 37 °C, 5% CO_2 incubator for 48 h. Then 90 μL of fresh medium and 10 μL of CCK-8 were added into culture plates and cultivated at the same conditions for 1 h. The sample cell was added into 96-well microplate reader and the plate was read at 450 nm and the absorbance value (OD) was recorded. Cell viability was calculated from the mean values for three wells using the following formula:

$$\text{Relative cell viability} = (\text{OD value for the test group} - \text{blank OD})/(\text{control OD value} - \text{blank OD value}) \times 100\%$$

The IC_{50} value is defined as the concentration that causes a 50% cell proliferation inhibition.

Acknowledgements We are grateful to the National Natural Science Foundation of China (20971097) and Tianjin Municipal Natural Science Foundation (13JCYBJC24500) for financial support.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

References

Abdo NYM, Kamel MM (2015) Synthesis and anticancer evaluation of 1,3,4-oxadiazoles, 1,3,4-thiadiazoles, 1,2,4-triazoles and mannich bases. Chem Pharm Bull 63:369–376

Aktas-Yokus O, Yuksek H, Gursoy-Kol O, Alpay-Karaoglu S (2015) Synthesis and biological evaluation of new 1,2,4-triazole derivatives with their potentiometric titrations. Med Chem Res 24:2813–2824

Anand P, Kunnumakara AB, Sundaram C, Harikumar KB, Tharakan ST, Lai OS, Sung B, Aggarwal BB (2008) Cancer is a preventable disease that requires major lifestyle changes. Pharm Res 25:2097–2116

Ashour HM, Shaaban OG, Rizk OH, El-Ashmawy IM (2013) Synthesis and biological evaluation of thieno[2′,3′:4,5]pyrimido [1,2-b][1,2,4]triazines and thieno[2,3-d][1,2,4]triazolo[1,5-a] pyrimi-dines as anti-inflammatory and analgesic agents. Eur J Med Chem 62:341–351

Aswathanarayanappa C, Bheemappa E, Bodke YD, Krishnegowda PS, Venkata SP, Ningegowda R (2013) Synthesis and evaluation of antioxidant properties of novel 1,2,4-triazole-based schiff base heterocycles. Arch Pharm Chem Life Sci 346:922–930

Bera H, Lee MH, Sun LY, Dolzhenko AV, Chui YK (2013) Synthesis, anti-thymidine phosphorylase activity and molecular docking of 5-thioxo-[1,2,4]triazolo[1,5-a][1,3,5]triazin -7-ones. Bioorg Chem 50:34–40

Botros S, Khalil NA, Naguib BH, El-Dash Y (2013) Synthesis and anticonvulsant activity of new phenytoin derivatives. Eur J Med Chem 60:57–63

Chelamalla R, Akena V, Manda S (2017) Synthesis of N′-arylidene-2-(5-aryl-1H-1, 2, 4-triazol-3-ylthio)acetohydrazides as anti-depressants. Med Chem Res 26:1359–1366

Cesarini S, Spallarossa A, Ranise A, Schenone S, Bruno O, Colla PL, Casula L, Collu G, Sanna G, Loddo R (2008) Parallel one-pot synthesis and structure-activity relationship study of symmetric formimidoester disulfides as a novel class of potent non-nucleoside HIV-1 reverse transcriptase inhibitors. Bioorg Med Chem 16:6353–6363

Diraimondo TR, Plugis NM, Jin X, Khosla C (2013) Selective inhibition of extracellular thioredoxin by asymmetric disulfides. J Med Chem 56:1301–1310

El-Serwy WS, Mohamed NA, Abbas EM, Abdel-Rahman RF (2013) Synthesis and anti-inflammatory properties of novel 1,2,4-triazole derivatives. Res Chem Intermed 39:2543–2554

Hunter R, Kaschula CH, Parker IM, Caira MR, Richards P, Travis S, Taute F, Qwebani T (2008) Substituted ajoenes as novel anti-cancer agents. Bioorg Med Chem Lett 18:5277–5279

Hou YP, Sun J, Pang ZH, Lv PC, Li DD, Yan L, Zhang HJ, Zheng EX, Zhao J, Zhu HL (2011) Synthesis and antitumor activity of 1,2,4-triazoles having 1,4-benzodioxan fragment as a novel class of potent methionine aminopeptidase type II inhibitors. Bioorg Med Chem 19:5948–5954

Kahveci B, Menteşe E, Akkaya E, Yılmaz F, Doğan IS, Özel A (2014) Synthesis of some novel 1,2,4-triazol-3-one derivatives bearing the salicyl moiety and their anticonvulsant activities. Arch Pharm Chem Life Sci 347:449–455

Kamel MM, Abdo NYM (2014) Synthesis of novel 1,2,4-triazoles, triazolothiadiazines and triazolothiadiazoles as potential anticancer agents. Eur J Med Chem 86:75–80

Li BL, Li B, Zhang RL, Zhao JJ, Wang XF, Liu YM, Shi YP, Liu JB, Chen BQ (2016) Synthesis and antiproliferative evaluation of novel 1,2,4-triazole derivatives incorporating benzisoselenazolone scaffold. Bioorg Med Chem Lett 26: 1279–1281

Li C, Liu JC, Li YR, Gou C, Zhang ML, Liu HY, Li XZ, Zheng CJ, Piao HR (2015) Synthesis and antimicrobial evaluation of 5-aryl-1,2,4-triazole-3-thione derivatives containing a rhodanine moiety. Bioorg Med Chem Lett 25:3052–3056

Li LJ, Ding H, Wang BJ, Yu SC, Zou Y, Chai XY, Wu QY (2014) Synthesis and evaluation of novel azoles as potent antifungal agents. Bioorg Med Chem Lett 24:192–194

Li YF, Zhang HJ, Quan ZS (2016) Synthesis and anti-inflammatory activity evaluation of novel 3-alkyl-6-(4H-1,2,4-triazol-4-yl)-3,4-dihydro-2H-benzo[e][1,3]oxazine derivatives. Med Chem Res 25:2280–2288

Nagender P, Kumar RN, Reddy GM, Swaroop DK, Poornachandra Y, Kumar CG, Narsaiah B (2016) Synthesis of novel hydrazone and azole functionalized pyrazolo[3,4-b]pyridine derivatives as promising anticancer agents. Bioorg Med Chem Lett 26:4427–4432

Padmaja A, Pedamalakondaiah D, Sravya G, Reddy GM, Kumar MVJ (2015) Synthesis and antioxidant activity of a new class of sulfone/sulfonamide-linked bis (oxadiazoles), bis (thiadiazoles), and bis (triazoles). Med Chem Res 24:2011–2020

Park HB, Kwon HC, Lee CH, Yang HO (2009) Glionitrin A, an antibiotic-antitumor metabolite derived from competive interaction between abandoned mine microbes. J Nat Prod 72: 248–252

Patel NB, Khan IH, Rajani SD (2010) Pharmacological evaluation and characterizations of newly synthesized 1,2,4-triazoles. Eur J Med Chem 45:4293–4299

Popiołek Ł, Rzymowska J, Kosikowska U, Hordyjewska A, Wujec M, Malm A (2014) Synthesis, antiproliferative and antimicrobial activity of new Mannich bases bearing 1,2,4-triazole moiety. J Enzyme Inhib Med Chem 29:786–795

Qiao RZ, Hui XP, Xu PF, Zhang ZY, Cheng DL (2001) Microwave induced synthesis of 3-aryl-6-(6-/8-substituted 4-chloroquinoline-3-yl)-s-triazolo [3,4-b]-1,3,4-thiadiazoles. Chin J Chem 19:800–806

Rubino S, Busà R, Attanzio A, Alduina R, Stefano VD, Girasolo MA, Orecchio S, Tesoriere L (2017) Synthesis, properties, antitumor and antibacterial activity of new Pt(II) and Pd(II) complexes with 2,20-dithiobis(benzothiazole) ligand. Bioorg Med Chem 25:2378–2386

Ryu EK, Choe YS, Byun SS, Lee KH, Chi DY, Choi Y, Kim BT (2004) Synthesis of radioiodine labeled dibenzyl disulfide for evaluation of tumor cell uptake. Bioorg Med Chem 12:859–864

Sarigol D, Uzgoren-Baran A, Tel BC, Somuncuoglu EI, Kazkayasi I, Ozadali-Sari K, Unsal-Tan O, Okay G, Ertan M, Tozkoparan B (2015) Novel thiazolo[3,2-b]-1,2,4-triazoles derived from naproxen with analgesic/anti-inflammatory properties: Synthesis, biological evaluation and molecular modeling studies. Bioorg Med Chem 23:2518–2528

Shi YJ, Song XJ, Li X, Ye TH, Xiong Y, Yu LT (2013) Synthesis and biological evaluation of 1,2,4-triazole and 1,3,4-thiadiazole derivatives as potential cytotoxic agents. Chem Pharm Bull 61:1099–1104

Sirakawa K, Aki O, Tsujikawa T, Tsuda T (1970) S-Alkylthioisothioureas. I. Chem Pharm Bull 18:235–242

Sokmen BB, Gumrukcuoglu N, Ugras S, Ugras HI, Yanardag R (2013) Synthesis, antibacterial, antielastase, antiurease and anti-oxidant activities of new methoxy substituted bis-1,2,4- triazole derivatives. J Enzyme Inhib Med Chem 28:72–77

Sun XQ, Hong ZX, Liu MY, Guo S, Yang D, Wang Y, Lan T, Gao LY, Qi HX, Gong P, Liu YJ (2017) Design, synthesis, and biological activity of novel tetrahydropyrazolopyridone derivatives as FXa inhibitors with potent anticoagulant activity. Bioorg Med Chem 25:2800–2810

Teha M, Ismail NH, Imran S, Wadood A, Rahim F, Muqarrabin LMRA, Ahmat N (2016) Synthesis of novel disulfide and sulfone hybrid scaffolds as potent β-glucuronidase inhibitor. Bioorg Chem 68:15–22

Turos E, Revell KD, Ramaraju P, Gergeres DA, Greenhalgh K, Young A, Sathyanarayan N, Dickey S, Lim D, Alhamadsheh MM, Reynolds K (2008) Unsymmetric aryl-alkyl disulfide growth inhibitors of methicillin-resistant staphylococcus aureus and bacillus anthracis. Bioorg Med Chem 16:6501–6508

Vijesh AM, Isloor AM, Shetty P, Sundershan S, Fun HK (2013) New pyrazole derivatives containing 1,2,4-triazoles and benzoxazoles as potent antimicrobial and analgesic agents. Eur J Med Chem 62:410–415

Wang F, Yang Z, Liu YB, Ma L, Wu YZ, He L, Shao MF, Yu K, Wu WS, Pu YZ, Nie CL, Chen LJ (2015) Synthesis and biological evaluation of diarylthiazole derivatives as antimitotic and anti-vascular agents with potent antitumor activity. Bioorg Med Chem 23:3337–3350

Wang XM, Mao S, Cao L, Xie XX, Xin MH, Lian JF, Cao YX, Zhang SQ (2015) Modification of N-(6-(2-methoxy-3-(4-fluorophenylsulfonamido)pyridin-5-yl)-[1,2,4]triazolo[1,5-a]pyridin-2-yl)acetamide as PI3Ks inhibitor by replacement of the acetamide group with alkylurea. Bioorg Med Chem 23:5662–5671

Wang Z, Shi XH, Wang J, Zhou T, Xu YZ, Huang TT, Li YF, Zhou YL, Yang L, Yang SY, Yu LT, Wei YQ (2011) Synthesis, structure–activity relationships and preliminary antitumor

evaluation of benzothiazole-2-thiol derivatives as novel apoptosis inducers. Bioorg Med Chem Lett 21:1097–1101

Yadagiri B, Gurrala S, Bantu R, Nagarapu L, Polepalli S, Srujana G, Jain N (2015) Synthesis and evaluation of benzosuberone embedded with 1,3,4-oxadiazole, 1,3,4-thiadiazole and 1,2,4-triazole moieties as new potential antiproliferative agents. Bioorg Med Chem Lett 25:2220–2224

Yu SC, Wang N, Chai XY, Wang BG, Cui H, Zhao QJ, Zou Y, Sun QY, Meng Q, Wu QY (2013) Synthesis and antifungal activity of the novel triazole derivatives containing 1,2,3-triazole fragment. Arch Pharm Res 36:1215–1222

Zhao PL, Duan AN, Zou M, Yang HK, You WW, Wu SG (2012) Synthesis and cytotoxicity of 3,4-disubstituted-5-(3,4,5-trimethoxyphenyl)-4H-1,2,4-triazoles and novel 5,6-dihydro-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole derivatives bearing 3,4,5-trimethoxyphenyl moiety. Bioorg Med Chem Lett 22:4471–4474