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Original article

Design and synthesis of novel 1,2,3-triazole-dithiocarbamate hybrids as potential anticancer agents

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ABSTRACT

A series of novel 1,2,3-triazole-dithiocarbamate hybrids were designed, synthesized and evaluated for anticancer activity against four selected human tumor cell lines (MGC-803, MCF-7, PC-3, EC-109). Majority of the synthesized compounds exhibited moderate to potent activity against MGC-803 and MCF-7. Among them, compounds **3a** and **3c** showed excellent broad spectrum anticancer activity with IC_{50} values ranging from 0.73 to 11.61 μM and 0.49–12.45 μM, respectively. Particularly, compound **3a** was more potent than 5-fluorouracil against all tested human cancer cell lines. Flow cytometry analysis demonstrated that treatment of MGC-803 with **3c** led to cell cycle arrest at G2/M phase accompanied by an increase in apoptotic cell death after 12 h.

1. Introduction

1,2,3-Triazoles have occupied an important role not only in organic chemistry but also in medicinal chemistry due to their easy synthesis by click chemistry and attractive features as well as numerous biological activities [1–3]. 1,2,3-Triazoles are highly stable under basic and acid hydrolysis and reductive and oxidative conditions, indicative of a high aromatic stabilization [4,5]. Moreover, this heterocycle has a high dipole moment and is capable of hydrogen bonding, which could be favorable in the binding of biomolecular targets [6]. 1,2,3-Triazole is one of the key structural units found in a large variety of bioactive molecules as anti-fungal [7], anti-bacterial [8,9], anti-allergic [10], anti-HIV [11,12], anti-tubercular [13,14] and anti-inflammatory agents [15]. Several 1,2,3-triazole-containing drug molecules including tazobactam [16], cefatrizine [4], carboxyamidotriazole [17] are now available in the market. In recent years, people are increasingly focused on their anticancer activity [18–26]. By combining 1,2,3-triazole with other pharmacophores via click chemistry, a number of compounds with potent antitumor activity were synthesized. A series of 1,2,3-triazole-bearing podophyllotoxins were synthesized by H.M.S. Kumar, majority of the compounds proved to be more potent than etoposide in selected human cancer cell lines [27]. A library of 1,2,3-triazole analogs of combretastatin A-4 were prepared by Odlo, and one of the triazole analogs displayed potent cytotoxic activity against several cancer cell lines with IC_{50} values in the nanoMolar range [28]. A family of 1,2,3-triazole-tethered β-lactam-chalcones bifunctional hybrids were designed and synthesized by V. Kumar, preliminary studies showed that several compounds exhibited moderate to good cytotoxic activity [29]. By incorporating the 1,2,3-triazole with arylamides, M.J. Miller identified N-((1-benzyl-1H-1,2,3-triazol-4-yl) methyl)arylamide as a novel and proprietary small molecule scaffold for potential antitumor agents, and one of the compounds exhibited an IC_{50} of 46 nM against MCF-7 cancer cell line [30].

On the other hand, dithiocarbamates have been attracting considerable interest because of their diverse activities. In the literature, dithiocarbamate derivatives have been described as anti-fungal [31], anti-bacterial [32] and carbonic anhydrases inhibitor [33,34]. In particular, their applications in the treatment of cancer have been exploring [35–43]. Our group recently reported the synthesis of novel butenolide-containing dithiocarbamates, and several compounds exhibited good anticancer activity [44,45].

While the pharmacological fight against cancer has made significant progress in the last twenty years, novel molecules to fight this disease are still urgently needed. Inspired by the biological importance of 1,2,3-triazoles and dithiocarbamates as anticancer agents, we

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herein reported the synthesis of novel 1,2,3-triazole-dithiocarbamates hybrids and their anticancer activity. The anticancer activity evaluation results revealed that the 1,2,3-triazole-dithiocarbamate hybrids exhibited potent anticancer activity.

2. Results and discussion

2.1. Chemistry

The synthetic route for 1,2,3-triazole-dithiocarbamate hybrids **3**–**6** is outlined in Scheme 1. Commercially available compound **1** reacting with CS_2 and propargyl bromide in the presence of $Na_3PO_4{\cdot}12H_2O$ in one pot gave compound **2**, which was further reacted with appropriately substituted benzyl azides or aromatic azides by click reaction to afford compounds **3a**–**h** and **6a**–**d** with good yields. The benzyl azides and aromatic azides were previously obtained according to references [46,47]. Compounds **4a**–**h** were synthesized by removing the tertiary butyloxycarbonyl group of the **3a**–**h** in a TFA/CH_2Cl_2 solution. Without further purification, coupling **4a**–**h** to carbobenzoxy chloride yielded compounds **5a**–**h**.

The 1,2,3-triazole-dithiocarbamate hybrids bearing a coumarin ring (**9** and **13**) were synthesized according to Scheme 2 and Scheme 3. Starting from m-hydroxy phenol (Scheme 2), compound 7 could be obtained directly following literature procedures [48,49]. 7 was converted to the azide derivative **8** via nucleophilic substitution by using sodium azide in acetonitrile. Compound **9** was prepared by Cu(I)-mediated Huisgen cycloaddition reaction of compound **2** with the azide derivative **8**. Compounds **13a**–**b** were obtained from 4-methylumbelliferone and 7-hydroxy coumarin, respectively (Scheme 3). In the first step, **11a**–**b** were prepared by alkylation of phenolic group with 1,2-dibromoethane. Then, compounds **11a**–**b** reacted with sodium azide in acetone-water at reflux temperature to form compounds **12a**–**b,** which were subjected to click reaction with **2** to yield compounds **13a**–**b**. All the synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR, HRMS and IR.

2.2. Evaluation of biological activity

2.2.1. Anticancer activity

The IC_{50} values (concentration required to inhibit tumor cell proliferation by 50%) for the synthesized compounds against four human cancer cell lines including MGC-803 (human gastric cancer cell line), MCF-7 (human breast cancer cell line), PC-3 (human prostate cancer cell line), and EC-109 (human esophageal cancer cell line) were determined using MTT assay method. The IC_{50} values were listed in Table 1 and the well-known anticancer drug 5-fluorouracil was used as positive control.

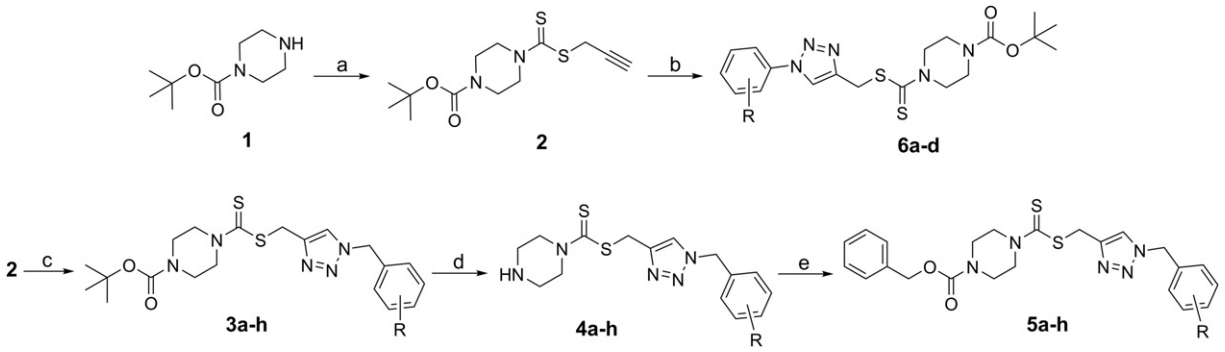
From the screening results in Table 1, it was observed that compounds **3a**–**h** exhibited moderate to good anticancer activity against MGC-803 and MCF-7. Two of the most active compounds are **3a** and **3c**, with IC_{50} values against the four tested human cancer cell lines ranging from 0.73 to 11.61 μM and 0.49–12.45 μM, respectively. Compound **3a** was more cytotoxic than 5-fluorouracil against all tested four human cancer cell lines, while **3c** was less active than 5-fluorouracil only against the EC-109 cells. Compound **3c** proved to be 14-fold more potent than 5-fluorouracil in the case of MGC-803. Starting from compound **3a**–**h**, removing the tertiary butyloxycarbonyl group resulted in a dramatic drop of potency (**4a**–**h**). Replacing the tertiary butyloxycarbonyl group with a carbobenzoxy group caused a slight loss of the IC_{50} values (**5a**–**h**). The substituents on benzyl azides had a profound influence on anticancer activity, such as **3c** (0.49 μM) as compared to **3f** (22.83 μM) against MGC-803, **3a** (2.44 μM) as compared to **3b** (58.9 μM) against EC-109. Compounds **6a**–**d** showed weak or no cytotoxicity against all tested cell lines, suggesting that the length of azides may play an important role in determining activity. The 1,2,3-triazole-dithiocarbamate hybrids bearing a coumarin ring (**9**) also displayed good anticancer activity against MGC-803 and MCF-7 but less than **3a** and **3c** bearing a benzene ring, while **13a-b** showed weak anticancer activity against all tested cell lines.

2.2.2. Apoptosis assay

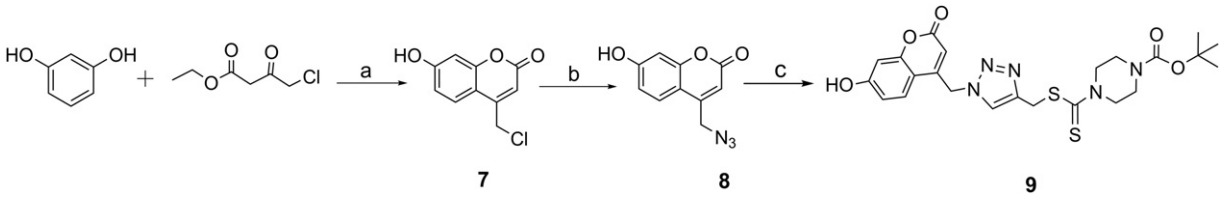
Because compound **3c** had a remarkable broad spectrum activity against all tested human cancer cell lines and the best activity against MGC-803 cell line, it was chosen to be further investigated regarding its mechanism of action. In order to better characterize the mode of cell death induced by compound **3c**, we performed a biparametric cytofluorimetric analysis using propidium iodide (PI) and annexin-V-FITC in MGC-803 cells. After treatment with compound **3c** for 12 h at different concentrations (0, 0.25, 0.5, 1.0 μmol/L), MGC-803 cells were labeled with the two dyes, and the resulting red (PI) and green (FITC) fluorescence was monitored by flow cytometry. It can be observed from Fig. 1 that the apoptosis rates were significantly increased from 3.7% (DMSO control) to 35.1%. The results showed that **3c** markedly increased the cellular apoptosis in a concentration-dependent manner.

2.2.3. Cell cycle analysis

Many anticancer drugs interact with cells leading to cell growth arrest. To determine whether the high anticancer effects of the hybrids were caused by cell cycle accumulated at a certain phase, the effects of different concentrations of compound **3c** on cell cycle progression were examined with MGC-803 cell line. After treatment with compound **3c** at various concentrations (0, 0.25, 0.5, 1.0 μmol/L) for 12 h, it was observed that the percentage of cells in



Scheme 1. Synthesis of the 1,2,3-triazole-dithiocarbamate hybrids (**3-6**). Reagent and reaction conditions: (a) CS_2, $Na_3PO_4{\cdot}12H_2O$, propargyl bromide, acetone, rt; (b) ArN_3, $CuSO_4{\cdot}5H_2O$, Sodium ascorbate, THF-H_2O (1:1), rt; (c) BnN_3, $CuSO_4{\cdot}5H_2O$, Sodium ascorbate, THF-H_2O (1:1), rt; (d) CF_3COOH, CH_2Cl_2, rt; (e) CbzCl, K_2CO_3, CH_2Cl_2, rt.



Scheme 2. Synthesis of the 1,2,3-triazole-dithiocarbamate hybrid (**9**). Reagent and conditions: (a) Con H_2SO_4, 0 °C; (b) NaN_3, CH_3CN, reflux; (c) **2**, $CuSO_4 \cdot 5H_2O$, Sodium ascorbate, THF-H_2O (1:1), rt.

G2/M phase were 17.59%, 22.20%, 34.29%, and 64.10%, respectively (Fig. 2 A), whereas after treatment of compound **3c** (0, 0.25, 0.5, 1.0 μmol/L) for 24 h, the percentage of cells in G2/M phase were 15.12%, 38.75%, 68.10%, and 80.05%, respectively (Fig. 2 B). The results suggested that **3c** caused a clear G2/M arrest pattern in a concentration and time-dependent manner, with a concomitant decrease of cells in other phases of the cell cycle.

3. Conclusions

In conclusion, a new class of 1,2,3-triazole-dithiocarbamate hybrids were synthesized and screened for anticancer activity against four human cancer cell lines. Compounds **3a** and **3c** exhibited excellent broad spectrum anticancer activity in vitro, especially compound **3a**, it was more potent than 5-fluorouracil against all tested human cancer cell lines. The results of apoptosis assay and cell cycle analysis demonstrated that **3c** could obviously inhibit the proliferation of MGC-803 cancer cells by inducing apoptosis and arresting the cell cycle at G2/M phase. These compounds are currently being evaluated for their in vivo efficacy in animal models. The 1,2,3-triazole-dithiocarbamate hybrids have simple structures and are easy to synthesize. These findings have encouraged us to continue the development and testing of novel 1,2,3-triazole-dithiocarbamate hybrids to conduct further studies to investigate the structure–activity relationship and elucidate the detailed pharmacological mechanism(s).

4. Experimental section

4.1. General

Reagents and solvents were purchased from commercial sources and were used without further purification. Melting points were determined on a X-5 micromelting apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 400 MHz and 100 MHz spectrometer respectively. IR spectra were recorded on a Nicolet iS10 infrared spectrometer. High resolution mass spectra (HRMS) were recorded on a Waters Micromass Q-T of Micromass spectrometer.

4.2. Procedure for the synthesis of tert butyl 4-((prop-2-ynylthio) carbonothioyl)piperazine-1-carboxylate (**2**)

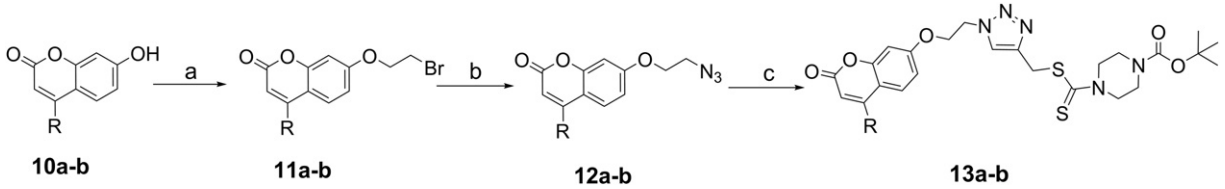
CS_2 (2.28 g, 30 mmol) was added drop wise to the solution of 1-Boc-piperazine (1.86 g, 10 mmol) and $Na_3PO_4 \cdot 12H_2O$ (2.28 g, 6 mmol) in acetone (40 mL). The reaction mixture was stirred at room temperature for 0.5 h. Then propargyl bromide (1.31 g, 11 mmol) was added to the mixture, the reaction mixture was stirred at room temperature for another 0.5 h. Upon completion, the reaction mixture was filtered and the filtrate was concentrated under reduced pressure, the residue was dissolved in EtOAc (50 mL), washed with water, brine, dried over anhydrous Na_2SO_4 and concentrated under vacuum to afford compound **2** (2.78 g, yield 92.2%). white solid. Mp: 87–88 °C. IR (KBr, cm^{-1}) ν: 3215, 2980, 1670, 1420, 1124, 931, 845, 767, 657, 544; ^{1}H NMR (400 MHz, Acetone-d_6, δ, ppm): 4.28 (br, 2H), 4.14 (d, 2H, $J = 2.7$ Hz), 4.00 (br, 2H), 3.58 (br, 4H), 2.78 (t, 1H, $J = 2.7$ Hz), 1.46 (s, 9H); HRMS (ESI) calcd for $C_{13}H_{21}N_2O_2S_2$ $[M + H]^+$: 301.1044, found: 301.1046.

4.3. General procedure for the synthesis of compounds **3a–h**, **6a–d**, **9** and **13a–b**

In a round-bottom flask equipped with a magnetic stirred bar, **2** (1.51 g, 5 mmol), azide derivatives (5.5 mmol), $CuSO_4 \cdot 5H_2O$ (62 mg, 0.25 mmol), sodium ascorbate (100 mg, 0.5 mmol), THF (20 mL) and H_2O (20 mL) were added. The resulting mixture was stirred at room temperature. The disappearance of compound **2** was monitored by TLC. Upon completion, water (40 mL) was added and the reaction mixture was extracted with EtOAc (3 × 40 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under vacuum to afford the crude product. The crude product was recrystallized from acetone to yield the pure product.

4.3.1. tert Butyl 4-(((1-(2-fluorobenzyl)-1H-1,2,3-triazol-4-yl) methylthio)carbonothioyl)-piperazine-1-carboxylate (**3a**)

yield 79.0%. white solid. Mp: 109–110 °C; IR (KBr, cm^{-1}) ν: 3447, 2979, 1693, 1494, 1478, 1424, 1279, 1167, 1012, 986, 932, 791, 757, 695; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.66 (s, 1H), 7.09–7.38 (m, 4H), 5.55 (s, 2H), 4.69 (s, 2H), 4.29 (br, 2H), 3.91 (br, 3H), 3.54 (t, 4H,



Scheme 3. Synthesis of the 1,2,3-triazole-dithiocarbamate hybrids (**13**). Reagent and conditions: (a) 1,2-dibromoethane, K_2CO_3, CH_3CN, reflux; (b) NaN_3, Acetone-H_2O (4:1), reflux; (c) **2**, $CuSO_4 \cdot 5H_2O$, Sodium ascorbate, THF-H_2O (1:1), rt.

Table 1
Inhibitory results of 1,2,3-triazole-dithiocarbamate hybrids against four human cancer cell lines.

Com.	R	IC_{50} (μM)[a]			
		MGC-803	MCF-7	PC-3	EC-109
3a	*o*-F	0.73 ± 0.11	5.67 ± 0.91	11.61 ± 1.59	2.44 ± 0.10
3b	*p*-F	1.93 ± 0.13	3.34 ± 0.40	>128	58.9 ± 3.15
3c	*o*-Cl	0.49 ± 0.07	6.09 ± 0.97	12.45 ± 1.63	11.93 ± 1.60
3d	*p*-Cl	9.79 ± 1.41	2.96 ± 0.30	>128	>128
3e	*p*-CH_3	3.49 ± 0.43	19.65 ± 2.11	17.25 ± 1.98	47.86 ± 2.97
3f	*p*-OCH_3	22.83 ± 2.21	4.96 ± 0.78	>128	36.84 ± 2.66
3g	*p*, *m*-diCl	33.66 ± 2.38	19.09 ± 1.91	>128	>128
3h	*m*, *p*, *m*-triOCH_3	27.34 ± 1.97	22.65 ± 1.63	>128	44.39 ± 3.82
4a	*o*-F	19.35 ± 2.45	33.16 ± 2.08	56.16 ± 2.87	>128
4b	*p*-F	32.46 ± 1.39	49.89 ± 2.17	>128	>128
4c	*o*-Cl	45.19 ± 4.47	38.86 ± 3.77	nt[b]	>128
4d	*p*-Cl	nt[b]	nt[b]	>128	>128
4e	*p*-CH_3	64.27 ± 3.42	53.16 ± 2.84	19.76 ± 2.41	>128
4f	*p*-OCH_3	nt[b]	nt[b]	>128	>128
4g	*p*, *m*-diCl	nt[b]	nt[b]	nt[b]	>128
4h	*m*, *p*, *m*-triOCH_3	nt[b]	nt[b]	>128	>128
5a	*o*-F	5.06 ± 0.80	7.95 ± 0.33	68.29 ± 3.13	26.96 ± 2.30
5b	*p*-F	17.61 ± 1.92	10.32 ± 1.44	>128	32.03 ± 2.45
5c	*o*-Cl	15.50 ± 1.04	21.37 ± 2.08	>128	56.79 + 2.88
5d	*p*-Cl	31.30 ± 2.38	27.50 ± 2.27	>128	>128
5e	*p*-CH_3	9.37 ± 1.36	37.78 ± 2.65	>128	32.56 ± 5.40
5f	*p*-OCH_3	23.80 ± 2.16	30.47 ± 2.37	>128	58.27 ± 2.94
5g	*p*, *m*-diCl	7.14 ± 1.08	10.63 ± 1.39	>128	29.41 ± 4.28
5h	*m*, *p*, *m*-triOCH_3	10.86 + 1.40	>128	>128	>128
6a	*o*-F	119.40 ± 3.93	89.59 ± 4.91	>128	>128
6b	*p*-CH_3	48.30 ± 2.04	74.16 ± 4.45	>128	113.60 ± 3.80
6c	*m*-CF_3	66.84 ± 3.10	80.38 ± 3.11	>128	69.32 ± 2.62
6d	*p*-OCH_3	87.33 ± 3.97	93.05 ± 4.77	>128	>128
9		4.96 ± 0.78	10.44 ± 2.34	36.84 ± 2.66	>128
13a	CH_3	76.90 ± 3.56	90.38 ± 4.05	>128	>128
13b	H	67.05 ± 2.98	79.80 ± 3.09	>128	>128
5-Fu		7.01 ± 1.34	7.54 ± 0.7	27.07 ± 4.21	3.34 ± 0.86

[a] Inhibitory activity was assayed by exposure for 72 h to substances and expressed as concentration required to inhibit tumor cell proliferation by 50% (IC_{50}). Data are presented as the means ± SDs of three independent experiments.
[b] Not tested.

J = 5.1 Hz), 1.47 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 196.42, 161.72, 159.26, 154.41, 144.00, 130.89, 130.81, 130.51, 130.48, 124.82, 124.78, 122.96, 121.98, 121.84, 115.92, 115.71, 80.63, 47.66, 47.61, 31.84, 28.34; HRMS (ESI) calcd for $C_{20}H_{27}FN_5O_2S_2$ $[M + H]^+$: 452.1590, found: 452.1598.

*4.3.2. tert Butyl 4-(((1-(4-fluorobenzyl)-1H-1,2,3-triazol-4-yl) methylthio)carbonothioyl)-piperazine-1-carboxylate (**3b**)*

yield 81.5%. white solid. Mp: 171–172 °C; IR (KBr, cm^{-1}) *ν*: 3482, 3139, 2974, 1690, 1470, 1420, 1281, 1167, 1051, 994, 824, 781, 541; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.59 (s, 1H), 7.04–7.27 (m, 4H), 5.46 (s, 2H), 4.68 (s, 2H), 4.31 (br, 2H), 3.90 (br, 3H), 3.53 (s, 4H), 1.47 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 196.40, 164.07, 161.60, 154.40, 130.49, 130.45, 129.96, 129.87, 116.20, 115.98, 80.66, 53.42, 31.78, 28.34; HRMS (ESI) calcd for $C_{20}H_{27}FN_5O_2S_2$ $[M + H]^+$: 452.1590, found: 452.1588.

*4.3.3. tert Butyl 4-(((1-(2-chlorobenzyl)-1H-1,2,3-triazol-4-yl) methylthio)carbonothioyl)-piperazine-1-carboxylate (**3c**)*

yield 78.7%. white solid. Mp: 105–106 °C; IR (KBr, cm^{-1}) *ν*: 3447, 2975, 1691, 1460, 1423, 1219, 1167, 1039, 935, 788, 746; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.70 (s, 1H), 7.46 (dd, 1H, J_1 = 1.4 Hz, J_2 = 7.8 Hz), 7.23–7.33 (m, 2H), 7.18 (dd, 1H, J_1 = 1.6 Hz, J_2 = 7.4 Hz), 5.62 (s, 2H), 4.70 (s, 2H), 4.28 (br, 2H), 3.93 (br, 2H), 3.54 (t, 4H, *J* = 5.2 Hz), 1.47 (s, 9H); ^{13}C NMR (100 MHz, Acetone-d_6, δ, ppm): 195.68, 154.33, 143.01, 133.30, 133.16, 130.60, 130.31, 129.74, 127.71, 123.79, 79.78, 50.99, 31.63, 27.63; HRMS (ESI) calcd for $C_{20}H_{27}ClN_5O_2S_2$ $[M + H]^+$: 468.1295, found: 468.1292.

*4.3.4. tert Butyl 4-(((1-(4-chlorobenzyl)-1H-1,2,3-triazol-4-yl) methylthio)carbonothioyl)-piperazine-1-carboxylate (**3d**)*

yield 85.5%. white solid. Mp: 177–178 °C; IR (KBr, cm^{-1}) *ν*: 3130, 2979, 2912, 1678, 1491, 1422, 1224, 1161, 1024, 994, 932, 777, 543, 499; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.59 (s, 1H), 7.35 (d, 2H, *J* = 8.4 Hz), 7.20 (d, 2H, *J* = 8.4 Hz), 5.45 (s, 2H), 4.68 (s, 2H), 4.29 (br, 2H), 3.90 (br, 2H), 3.54 (t, 4H, *J* = 5.2 Hz), 1.47(s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm):196.39, 154.42, 144.35, 134.78, 133.11, 129.36, 129.31, 122.79, 80.69, 53.40, 31.72, 28.35; HRMS (ESI) calcd for $C_{20}H_{27}ClN_5O_2S_2$ $[M + H]^+$: 468.1295, found: 468.1291.

*4.3.5. tert Butyl 4-(((1-(4-methylbenzyl)-1H-1,2,3-triazol-4-yl) methylthio)carbonothioyl)-piperazine-1-arboxylate (**3e**)*

yield 81.9%. white solid. Mp: 182–183 °C; IR (KBr, cm^{-1}) *ν*: 3454, 2975, 1682, 1457, 1422, 1224, 1078, 994, 933, 867, 772, 524; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.55 (s, 1H), 7.16–7.21 (m, 4H), 5.43 (s, 2H), 4.67 (s, 2H), 4.29 (br, 2H), 3.91 (br, 2H), 3.54 (t, 4H, *J* = 5.2 Hz), 2.35 (s, 3H), 1.47 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 196.49, 154.41, 138.67, 131.57, 129.75, 128.09, 80.64, 54.01, 31.91, 28.35, 21.15; HRMS (ESI) calcd for $C_{21}H_{30}N_5O_2S_2$ $[M + H]^+$:448.1841, found: 448.1840.

*4.3.6. tert Butyl 4-(((1-(4-methoxybenzyl)-1H-1,2,3-triazol-4-yl) methylthio)carbonothioyl)-piperazine-1-carboxylate (**3f**)*

yield 85.8%. white solid. Mp: 129–130 °C; IR (KBr, cm^{-1}) *ν*: 3502, 3125, 2975, 1686, 1542, 1453, 1281, 1173, 1016, 982, 932, 775, 698; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.54 (s, 1H), 7.21(d, 2H, *J* = 8.7 Hz), 6.88 (d, 2H, *J* = 8.7 Hz), 5.41 (s, 2H), 4.67 (s, 2H), 4.29 (br,

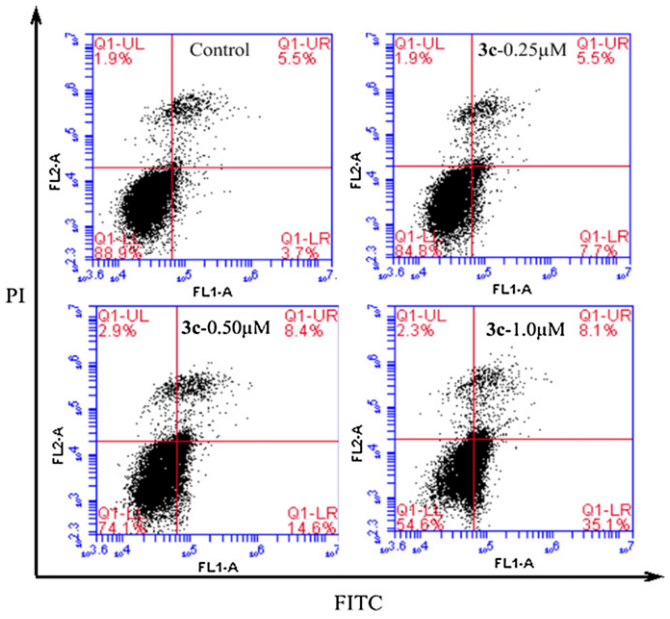


Fig. 1. Apoptosis effect on human MGC-803 cell line induced by compound **3c**. Apoptotic cells were detected with Annexin V/PI double staining after incubation with compounds **3c** (0, 0.25, 0.5, 1.0 μmol/L) for 12 h. The lower left quadrants represent live cells, the lower right quadrants are for early/primary apoptotic cells, upper right quadrants are for late/secondary apoptotic cells, while the upper left quadrants represent cells damaged during the procedure. The experiments were performed three times, and a representative experiment is shown.

2H), 3.90 (br, 2H), 3.80 (s, 3H), 3.51 (t, 4H, J = 5.2 Hz), 1.47 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 196.47, 159.89, 154.40, 143.86, 129.62, 126.59, 122.52, 114.44, 80.61, 55.34, 53.70, 31.90, 28.35; HRMS (ESI) calcd for $C_{21}H_{30}N_5O_3S_2$ $[M + H]^+$: 464.1790, found: 464.1794.

*4.3.7. tert Butyl 4-(((1-(3,4-dichlorobenzyl)-1H-1,2,3-triazol-4-yl) methylthio)carbonothioyl)-piperazine-1-carboxylate (**3g**)*

yield 90.2%. white solid. Mp: 153–154 °C; IR (KBr, cm^{-1}) ν: 3129, 2984, 1693, 1470, 1347, 1159, 1131, 990, 963, 798, 740, 698, 542; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.64 (s, 1H), 7.43 (d, 1H, J = 8.2 Hz), 7.35 (d, 1H, J = 2.0 Hz), 7.08 (dd, 1H, J_1 = 2.0 Hz, J_2 = 8.2 Hz), 5.44 (s, 2H), 4.70 (s, 2H), 4.30 (br, 2H), 3.91 (br, 2H), 3.52 (t, 4H, J = 5.1 Hz), 1.47 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 196.29, 154.39, 144.59, 134.77, 133.26, 133.08, 131.08, 129.86, 127.18, 122.94, 80.65, 52.82, 31.65, 28.34; HRMS (ESI) calcd for $C_{20}H_{26}Cl_2N_5O_2S_2$ $[M + H]^+$: 502.0905, found: 502.0900.

*4.3.8. tert Butyl 4-(((1-(3,4,5-trimethoxybenzyl)-1H-1,2,3-triazol-4-yl)methylthio)carbonothioyl)-piperazine-1-carboxylate (**3h**)*

yield 83.4%. white solid. Mp: 134–135 °C; IR (KBr, cm^{-1}) ν: 3148, 2970, 1688, 1464, 1424, 1384, 1242, 1127, 1011, 933, 843, 774, 738, 615; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.63 (s, 1H), 6.48 (s, 2H), 5.41 (s, 2H), 4.70 (s, 2H), 4.30 (br, 2H), 3.91 (br, 2H), 3.85 (s, 3H), 3.84 (s, 6H), 3.55 (t, 4H, J = 5.2 Hz), 1.48 (s, 9H); HRMS (ESI) calcd for $C_{23}H_{34}N_5O_2S_2$ $[M + H]^+$: 524.2001, found: 524.2005.

*4.3.9. tert Butyl 4-(((1-(4-fluorophenyl)-1H-1,2,3-triazol-4-yl) methylthio)carbonothioyl)-piperazine-1-carboxylate (**6a**)*

yield 77.5%. white solid. Mp: 178–179 °C; IR (KBr, cm^{-1}) ν: 3447, 3086, 2983, 1694, 1514, 1456, 1419, 1224, 1165, 1012, 992, 939, 838, 746, 553; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 8.09 (s, 1H), 7.18–7.71 (m, 4H), 4.79 (s, 2H), 4.32 (br, 2H), 3.93 (br, 2H), 3.56 (t, 4H, J = 5.2 Hz), 1.47 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 196.30, 163.64, 161.17, 154.40, 133.32, 122.56, 122.48, 116.78, 116.55, 80.67, 31.56, 28.35; HRMS (ESI) calcd for $C_{19}H_{25}FN_5O_2S_2$ $[M + H]^+$: 438.1434, found: 438.1437.

*4.3.10. tert Butyl 4-(((1-p-tolyl-1H-1,2,3-triazol-4-yl)methylthio) carbonothioyl)piperazine-1-carboxylate (**6b**)*

yield 78.4%. white solid. Mp: 130–131 °C; IR (KBr, cm^{-1}) ν: 3140, 2975, 1686, 1598, 1497, 1366, 1223, 1124, 1019, 995, 934, 836, 770, 519; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 8.08 (s, 1H), 7.59 (d, 2H, J = 8.4 Hz), 7.30 (d, 2H, J = 8.4 Hz), 4.79 (s, 2H), 4.32 (br, 2H), 3.93 (br, 2H), 3.56 (t, 4H, J = 5.2 Hz), 2.41 (s, 3H), 1.47 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 196.39, 154.40, 138.81, 134.73, 130.17, 121.09, 120.43, 80.62, 31.71, 28.35, 21.09; HRMS (ESI) calcd for $C_{20}H_{28}N_5O_2S_2$ $[M + H]^+$: 434.1684, found: 434.1681.

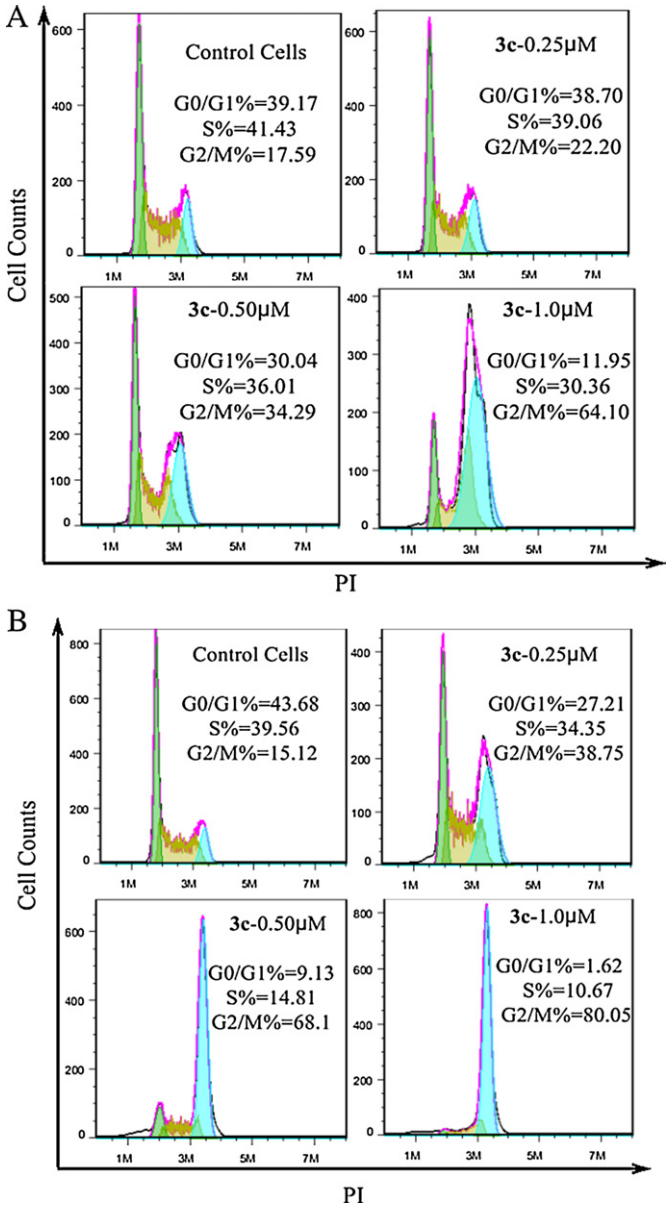


Fig. 2. Effect of compound **3c** on the cell cycle distribution of MGC-803 cells. Cells were treated with different concentrations (0, 0.25, 0.5, 1.0 μmol/L) for 12 h or 24 h. Then the cells were fixed and stained with PI to analyze DNA content by flow cytometry. (A) incubated for 12 h; (B) incubated for 24 h. The experiments were performed three times, and a representative experiment is shown.

*4.3.11. tert Butyl 4-(((1-(3-(trifluoromethyl)phenyl)-1H-1,2,3-triazol-4-yl)methylthio)-carbonothioyl) piperazine-1-carboxylate (**6c**)*

yield 87.6%. white solid. Mp: 109–110 °C; IR (KBr, cm^{-1}) ν: 3142, 2983, 1687, 1598, 1483, 1417, 1325, 1283, 1224, 1166, 1071, 1037, 993, 937, 807, 772, 698, 540; ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 8.21 (s, 1H), 8.01 (s, 1H), 7.96 (d, 1H, $J = 7.6$ Hz), 7.66–7.73 (m, 2H), 4.82 (s, 2H), 4.34 (br, 2H), 3.95 (br, 2H), 3.57 (t, 4H, $J = 5.2$ Hz), 1.48 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 196.13, 154.39, 145.29, 137.34, 132.87, 132.54, 132.21, 131.88, 130.51, 127.38, 125.37, 125.34, 125.30, 125.26, 124.67, 123.56, 121.96, 121.10, 119.25, 117.49, 117.46, 117.42, 117.38, 80.67, 31.45, 28.33; HRMS (ESI) calcd for HRMS (ESI) calcd for $C_{20}H_{25}F_3N_5O2S_2$ $[M + H]^+$: 488.1402, found: 488.1398.

*4.3.12. tert Butyl 4-(((1-(4-methoxyphenyl)-1H-1,2,3-triazol-4-yl) methylthio)carbonothioyl)-piperazine-1-carboxylate (**6d**)*

yield 83.7%. white solid. Mp: 133–134 °C; IR (KBr, cm^{-1}) ν: 3146, 2976, 1686, 1518, 1421, 1254, 1161, 1125, 1041, 995, 935, 810, 770, 694, 532; ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 8.04 (s, 1H), 7.61 (d, 2H, $J = 9.0$ Hz), 7.01 (d, 2H, $J = 9.0$ Hz), 4.79 (s, 2H), 4.32 (br, 2H), 3.94 (br, 2H), 3.86 (s, 3H), 3.56 (t, 4H, $J = 5.3$ Hz), 1.47 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 196.43, 159.80, 154.41, 144.33, 130.48, 122.17, 121.24, 114.72, 80.64, 55.63, 31.72, 28.35; HRMS (ESI) calcd for $C_{20}H_{28}N_5O_3S_2$ $[M + H]^+$: 450.1634, found: 450.1638.

*4.3.13. tert Butyl 4-(((1-((7-hydroxy-2-oxo-2H-chromen-4-yl) methyl)-1H-1,2,3-triazol-4-yl)-methylthio)carbonothioyl) piperazine-1-carboxylate (**9**)*

yield 91.3%. Yellow white solid. Mp: 219–220 °C; IR (KBr, cm^{-1}) ν: 3161, 2972, 2852, 1693, 1562, 1423, 1280, 1152, 1007, 932, 797, 689, 504; ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 10.73 (s, 1H), 8.24 (s, 1H), 7.68 (d, 1H, $J = 9.0$ Hz), 6.82 (d, 1H, $J = 9.0$ Hz), 6.77 (s, 1H), 5.88 (s, 2H), 5.54 (s, 1H), 4.63 (s, 2H), 4.23 (br, 2H), 3.92 (br, 2H), 3.45 (t, 4H, $J = 5.2$ Hz), 1.42 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 194.86, 162.14, 160.39, 155.57, 154.15, 150.99, 143.22, 126.54, 125.42, 113.65, 109.84, 109.66, 103.01, 79.87, 67.48, 49.64, 31.77, 28.49, 25.59; HRMS (ESI) calcd for $C_{23}H_{27}N_5NaO_2S_2$ $[M + Na]^+$: 540.1351, found: 540.1353.

*4.3.14. tert Butyl 4-(((1-(2-(4-methyl-2-oxo-2H-chromen-7-yloxy) ethyl)-1H-1,2,3-triazol-4-yl)-methylthio)carbonothioyl)piperazine-1-carboxylate (**13a**)*

yield 89.6%. white solid. Mp: 137–138 °C; IR (KBr, cm^{-1}) ν: 2954, 2104, 1708, 1613, 1508, 1392, 1273, 1071, 916, 847, 535; ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.16 (s, 1H), 7.68 (d, 1H, $J = 8.8$ Hz), 6.99 (d, 1H, $J = 2.4$ Hz), 6.95 (dd, 1H, $J_1 = 2.4$ Hz, $J_2 = 8.8$ Hz), 6.22 (s, 1H), 4.80 (t, 2H, $J = 4.8$ Hz), 4.58 (s, 2H), 4.52 (t, 2H, $J = 4.8$ Hz), 4.23 (br, 2H), 3.90 (br, 2H), 3.44 (s, 4H), 2.40 (s, 3H), 1.41 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 195.02, 170.79, 161.33, 160.65, 155.74, 154.14, 144.70, 142.49, 130.02, 124.84, 124.50, 113.29, 101.97, 79.86, 67.25, 60.22, 49.26, 31.93, 31.15, 28.48, 21.23; HRMS (ESI) calcd for $C_{25}H_{32}N_5O_5S_2$ $[M + H]^+$: 546.1845, found: 546.1846.

*4.3.15. tert Butyl 4-(((1-(2-(2-oxo-2H-chromen-7-yloxy)ethyl)-1H-1,2,3-triazol-4-yl)methylthio)-carbonothioyl)piperazine-1-carboxylate (**13b**)*

yield 94.1%. white solid. Mp: 135–136 °C; IR (KBr, cm^{-1}) ν: 2977, 1735, 1686, 1612, 1460, 1420, 1365, 1226, 1126, 994, 937, 832, 758; ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.88 (s, 1H), 7.64 (d, 1H, $J = 9.5$ Hz), 7.39 (d, 1H, $J = 8.5$ Hz), 6.84 (dd, 1H, $J_1 = 2.0$ Hz, $J_2 = 8.5$ Hz), 6.79 (d, 1H, $J = 2.0$ Hz), 6.29 (d, 1H, $J = 9.5$ Hz), 4.78 (t, 2H, $J = 5.0$ Hz), 4.70 (s, 2H), 4.43 (t, 2H, $J = 5.0$ Hz), 4.32 (br, 2H), 3.91 (br, 2H), 3.53 (s, 4H), 1.47 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 195.01, 170.80, 161.24, 160.52, 155.09, 154.14, 153.79, 126.99, 124.85, 114.00, 112.90, 111.89, 101.96, 79.86, 67.23, 60.22, 49.27, 31.93, 31.15, 28.48; HRMS (ESI) calcd for $C_{24}H_{30}N_5O_5S_2$ $[M + H]^+$: 532.1688, found: 532.1686.

*4.4. General procedure for the synthesis of compounds **4a-h***

CF_3COOH (4.56 g, 40 mmol) was added to a solution of **3** (2 mmol) in CH_2Cl_2 (20 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred at the same temperature. Upon completion, the reaction mixture was concentrated under vacuum, the residue was dissolved in CH_2Cl_2, washed with saturated $NaHCO_3$, brine, dried over anhydrous Na_2SO_4 and concentrated under vacuum to afford compounds **4**, which were used in the next reaction without further purification.

*4.4.1. (1-(2-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl piperazine-1-carbodithioate (**4a**)*

yield 96.7%. white solid. Mp: 93–94 °C; IR (KBr, cm^{-1}) ν: 3211, 2908, 1492, 1469, 1387, 1261, 1222, 1121, 1021, 983, 852, 753, 735; ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.67 (s, 1H), 7.09–7.36 (m, 4H), 5.55 (s, 2H), 4.69 (s, 2H), 4.30 (br, 2H), 3.93 (br, 2H), 2.96 (t, 4H, $J = 4.8$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 195.81, 161.72, 159.26, 144.27, 130.88, 130.80, 130.50, 130.47, 124.82, 124.79, 122.98, 122.00, 121.86, 115.93, 115.72, 47.66, 47.61, 45.58, 31.76, 30.93; HRMS (ESI) calcd for $C_{15}H_{19}FN_5S_2$ $[M + H]^+$: 352.1066, found: 352.1064.

*4.4.2. (1-(4-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl piperazine-1-carbodithioate (**4b**)*

yield 93.9%. white solid. Mp: 138–139 °C; IR (KBr, cm^{-1}) ν: 3152, 2912, 1601, 1508, 1425, 1243, 1215, 1141, 1136, 1031, 987, 843, 775, 729, 528; ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.60 (s, 1H), 7.04–7.27 (m, 4H), 5.45 (s, 2H), 4.69 (s, 2H), 4.31 (br, 2H), 3.89 (br, 2H), 2.93 (s, 4H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 196.03, 164.07, 161.60, 144.33, 130.47, 130.44, 129.97, 129.88, 122.74, 116.21, 116.00, 53.41, 45.15, 31.76; HRMS (ESI) calcd for $C_{15}H_{19}FN_5S_2$ $[M + H]^+$: 352.1066, found: 352.1063.

*4.4.3. (1-(2-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl piperazine-1-carbodithioate (**4c**)*

yield 94.8%. white solid. Mp: 98–99 °C; IR (KBr, cm^{-1}) ν: 3143, 2927, 1468, 1407, 1388, 1250, 1211, 1139, 1105, 1014, 953, 806, 774, 531; ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.68 (s, 1H), 7.43 (dd, 1H, $J_1 = 1.4$ Hz, $J_2 = 7.8$ Hz), 7.24–7.33 (m, 2H), 7.18 (dd, 1H, $J_1 = 1.6$ Hz, $J_2 = 7.4$ Hz), 5.61 (s, 2H), 4.64 (s, 2H), 4.45 (br, 4H), 3.34 (s, 4H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 197.25, 143.28, 133.50, 132.25, 130.34, 130.19, 129.97, 127.65, 123.43, 51.57, 43.19, 32.18; HRMS (ESI) calcd for $C_{15}H_{19}ClN_5S_2$ $[M + H]^+$: 368.0770, found: 368.0772.

*4.4.4. (1-(4-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl piperazine-1-carbodithioate (**4d**)*

yield 95.1%. white solid. Mp: 79–80 °C; IR (KBr, cm^{-1}) ν: 3138, 2921, 1472, 1407, 1386, 1258, 1226, 1139, 1118, 1014, 980, 806, 774, 501; ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.61 (s, 1H), 7.35 (d, 2H, $J = 8.1$ Hz), 7.20 (d, 2H, $J = 8.1$ Hz), 5.45 (s, 2H), 4.69 (s, 2H), 4.31 (br, 2H), 3.90 (br, 2H), 2.94 (s, 4H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 195.63, 144.54, 134.71, 133.17, 129.35, 129.27, 122.81, 53.36, 45.61, 31.65; HRMS (ESI) calcd for $C_{15}H_{19}ClN_5S_2$ $[M + H]^+$: 368.0770, found: 368.0767.

*4.4.5. 1-(4-methylbenzyl)-1H-1,2,3-triazol-4-yl)methyl piperazine-1-carbodithioate (**4e**)*

yield 94.0%. white solid. Mp: 74–75 °C; IR (KBr, cm^{-1}) ν: 3267, 2919, 1514, 1474, 1420, 1385, 1258, 1137, 1021, 979, 803, 772, 525; ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.56 (s, 1H), 7.14–7.27 (m, 4H), 5.43 (s, 2H), 4.67 (s, 2H), 4.31 (br, 2H), 3.92 (br, 2H), 2.96 (br, 4H), 2.35 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 195.82, 138.61, 131.59, 129.74, 128.08, 122.72, 53.96, 31.80, 29.68, 21.17; HRMS (ESI) calcd for $C_{16}H_{22}N_5S_2$ $[M + H]^+$:348.1317, found: 348.1319.

*4.4.6. (1-(4-methoxybenzyl)-1H-1,2,3-triazol-4-yl)methyl piperazine-1-carbodithioate (**4f**)*

yield 92.7%. white solid. Mp: 95–96 °C; IR (KBr, cm^{-1}) ν: 3279, 2916, 1610, 1514, 1420, 1230, 1178, 1125, 1025, 992, 901, 838, 784, 694, 554; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.55 (s, 1H), 7.24 (d, 2H, J = 8.7 Hz), 6.91 (d, 2H, J = 8.7 Hz), 5.42 (s, 2H), 4.68 (s, 2H), 4.31 (br, 2H), 3.95 (br, 2H), 3.80 (s, 3H), 2.96 (t, 4H, J = 4.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 195.75, 159.89, 144.12, 129.61, 126.61, 122.53, 114.45, 55.34, 53.69, 45.63, 31.79; HRMS (ESI) calcd for $C_{16}H_{22}N_5OS_2$ $[M + H]^+$:364.1266, found: 364.1263.

*4.4.7. (1-(3,4-dichlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl piperazine-1-carbodithioate (**4g**)*

yield 95.5%. white solid. Mp: 122–123 °C; IR (KBr, cm^{-1}) ν: 3238, 2914, 1472, 1418, 1256, 1224, 1047, 978, 776, 739, 537; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.65 (s, 1H), 7.45 (d, 1H, J = 8.3 Hz), 7.35 (d, 1H, J = 2.0 Hz), 7.10 (dd, 1H, J_1 = 2.0 Hz, J_2 = 8.3 Hz), 5.44 (s, 2H), 4.70 (s, 2H), 4.31 (br, 2H), 3.92 (br, 2H), 2.95 (s, 4H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 195.62, 144.87, 134.79, 133.27, 133.07, 131.09, 129.86, 127.17, 122.92, 52.81, 45.63, 31.56; HRMS (ESI) calcd for $C_{15}H_{18}Cl_2N_5S_2$ $[M + H]^+$:402.0381, found: 402.0379.

*4.4.8. (1-(3,4,5-trimethoxybenzyl)-1H-1,2,3-triazol-4-yl)methyl piperazine-1-carbodithioate (**4h**)*

yield 96.0%. white solid. Mp: 120–121 °C; IR (KBr, cm^{-1}) ν: 3331, 2945, 1509, 1425, 1418, 1361, 1328, 1119, 1044, 979, 838, 784, 726, 536; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.64 (s, 1H), 6.47 (s, 2H), 5.40 (s, 2H), 4.69 (s, 2H), 4.30 (br, 2H), 3.91 (br, 2H), 3.83 (s, 3H), 3.82 (s, 6H), 2.93 (s, 4H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 195.63, 153.66, 144.42, 138.23, 130.15, 122.83, 105.16, 60.85, 56.23, 54.35, 45.72, 31.68; HRMS (ESI) calcd for $C_{18}H_{26}N_5O_3S_2$ $[M + H]^+$: 424.1477, found: 424.1472.

*4.5. General procedure for the synthesis of compounds **5a-h***

A mixture of **4** (2 mmol), K_2CO_3 (0.28 g, 2 mmol) and CbzCl (0.38 g, 2.2 mol) in CH_2Cl_2 (20 mL) was stirred at room temperature. Upon completion, K_2CO_3 was removed by filtration and the solvent was diluted with CH_2Cl_2, washed with water, brine, dried over anhydrous Na_2SO_4 and concentrated under vacuum to give the crude product, which were recrystallized from acetone to provide compound **5** as white solids.

*4.5.1. Benzyl 4-(((1-(2-fluorobenzyl)-1H-1,2,3-triazol-4-yl) methylthio)carbonothioyl)piperazine-1-carboxylate (**5a**)*

yield 74.6%. white solid. Mp: 138–139 °C; IR (KBr, cm^{-1}) ν: 3446, 3131, 1686, 1492, 1465, 1422, 1360, 1211, 1095, 791, 761, 693, 584; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.66 (s, 1H), 7.08–7.37 (m, 9H), 5.54 (s, 2H), 5.15 (s, 2H), 4.69 (s, 2H), 4.30 (br, 2H), 3.95 (br, 2H), 3.61 (t, 4H, J = 5.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 196.61, 161.73, 159.27, 155.04, 143.97, 136.21, 130.94, 130.85, 130.54, 130.51, 128.61, 128.30, 128.09, 124.84, 124.80, 123.04, 121.93, 121.79, 115.94, 115.73, 67.64, 47.72, 47.68, 43.02, 31.85; HRMS (ESI) calcd for $C_{23}H_{25}FN_5O_2S_2$ $[M + H]^+$: 486.1434, found: 486.1432.

*4.5.2. Benzyl 4-(((1-(4-fluorobenzyl)-1H-1,2,3-triazol-4-yl) methylthio)carbonothioyl)piperazine-1-carboxylate (**5b**)*

yield 90.2%. white solid. Mp: 114–115 °C; IR (KBr, cm^{-1}) ν: 3446, 3056, 1697, 1511, 1474, 1425, 1360, 1130, 1094, 976, 785, 727, 692, 523; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.58 (s, 1H), 7.03–7.36 (m, 9H), 5.45 (s, 2H), 5.15 (s, 2H), 4.67 (s, 2H), 4.29 (br, 2H), 3.94 (br, 2H), 3.62 (t, 4H, J = 5.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 196.53, 164.04, 161.57, 155.02, 136.21, 130.54, 130.51, 129.98, 129.90, 128.60, 128.30, 128.08, 122.84, 116.19, 115.97, 67.62, 53.37, 43.01, 31.85; HRMS (ESI) calcd for $C_{23}H_{24}FN_5NaO_2S_2$ $[M + Na]^+$: 508.1253, found: 508.1250.

*4.5.3. Benzyl 4-(((1-(2-chlorobenzyl)-1H-1,2,3-triazol-4-yl) methylthio)carbonothioyl)piperazine-1-carboxylate (**5c**)*

yield 71.7%. white solid. Mp: 91–92 °C; IR (KBr, cm^{-1}) ν:3457, 2974, 1692, 1511, 1474, 1425, 1360, 1130, 1094, 976, 785, 727, 692, 523; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.67 (s, 1H), 7.13–7.43 (m, 9H), 5.61 (s, 2H), 5.15 (s, 2H), 4.69 (s, 2H), 4.28 (br, 2H), 3.95 (br, 2H), 3.61 (t, 4H, J = 5.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 196.60, 155.05, 143.86, 136.20, 133.42, 132.46, 130.20, 129.90, 128.61, 128.31, 128.10, 127.58, 126.98, 123.20, 67.65, 51.40, 43.04, 31.89; HRMS (ESI) calcd for $C_{23}H_{25}ClN_5O_2S_2$ $[M + H]^+$: 502.1138, found: 502.1137.

*4.5.4. Benzyl 4-(((1-(4-methylbenzyl)-1H-1,2,3-triazol-4-yl) methylthio)carbonothioyl)piperazine-1-carboxylate (**5e**)*

yield 81.8%. white solid. Mp: 99–100 °C; IR (KBr, cm^{-1}) ν: 3141, 2983, 1686, 1598, 1417, 1325, 1224, 1165, 1037, 993, 936, 858, 806, 771, 698, 540; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.55 (s, 1H), 7.14–7.37 (m, 9H), 5.44 (s, 2H), 5.16 (s, 2H), 4.67 (s, 2H), 4.29 (br, 2H), 3.96 (br, 2H), 3.59 (t, 4H, J = 5.1 Hz), 2.35 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 196.67, 155.04, 138.68, 136.21, 131.56, 129.76, 128.61, 128.31, 128.10, 122.73, 67.64, 54.02, 43.03, 31.95, 21.17; HRMS (ESI) calcd for $C_{24}H_{28}N_5O_2S_2$ $[M + H]^+$: 482.1684, found: 482.1683.

*4.5.5. Benzyl 4-(((1-(4-methoxybenzyl)-1H-1,2,3-triazol-4-yl) methylthio)carbonothioyl)piperazine-1-carboxylate (**5f**)*

yield 75.5%. white solid. Mp: 113–114 °C; IR (KBr, cm^{-1}) ν: 3351, 3143, 2952, 1678, 1513, 1425, 1360, 1130, 1095, 986, 780, 727, 692, 542; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.53 (s, 1H), 7.31–7.39 (m, 5H), 7.22 (d, 2H, J = 8.6 Hz), 6.89 (d, 2H, J = 8.6 Hz), 5.40 (s, 2H), 5.15 (s, 2H), 4.66 (s, 2H), 4.28 (br, 2H), 3.89 (br, 2H), 3.80 (s, 3H), 3.61 (t, 4H, J = 5.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 196.63, 159.90, 155.03, 143.75, 136.22, 129.64, 128.60, 128.29, 128.08, 126.61, 122.54, 114.45, 67.61, 55.35, 53.69, 43.02, 31.97; HRMS (ESI) calcd for $C_{24}H_{28}N_5O_3S_2$ $[M + H]^+$: 498.1634, found: 498.1639.

*4.5.6. Benzyl 4-(((1-(3,4-dichlorobenzyl)-1H-1,2,3-triazol-4-yl) methylthio)carbonothioyl)-piperazine-1-carboxylate (**5g**)*

yield 86.5%. white solid. Mp: 106–107 °C; IR (KBr, cm^{-1}) ν: 3138, 2910, 1693, 1474, 1425, 1365, 1219, 1132, 1031, 996, 933, 785, 731, 694, 548; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.63 (s, 1H), 7.45 (d, 1H, J = 8.3 Hz), 7.33–7.37 (m, 6H), 7.09 (dd, 1H, J_1 = 2.0 Hz, J_2 = 8.3 Hz), 5.43 (s, 2H), 5.15 (s, 2H), 4.69 (s, 2H), 4.29 (br, 2H), 3.95 (br, 2H), 3.62 (t, 4H, J = 5.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 196.45, 155.02, 144.45, 136.20, 134.82, 133.22, 133.04, 131.08, 129.88, 128.60, 128.30, 128.08, 127.21, 122.98, 67.63, 52.79, 43.01, 31.74; HRMS (ESI) calcd for $C_{23}H_{24}Cl_2N_5O_2S_2$ $[M + H]^+$: 536.0748, found: 536.0749.

*4.5.7. Benzyl 4-(((1-(3,4,5-trimethoxybenzyl)-1H-1,2,3-triazol-4-yl) methylthio)carbonothioyl)-piperazine-1-carboxylate (**5h**)*

yield 82.0%. white solid. Mp: 134–135 °C. IR (KBr, cm^{-1}) ν: 3141, 2911, 1713, 1594, 1506, 1459, 1431, 1385, 1218, 1094, 951, 695, 575; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.63 (s, 1H), 7.35–7.37 (m, 5H), 6.47 (s, 2H), 5.40 (s, 2H), 5.15 (s, 2H), 4.68 (s, 2H), 4.28 (br, 2H), 3.96 (br, 2H), 3.83 (s, 3H), 3 0.82 (s, 6H), 3.62 (t, 4H, J = 5.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 196.60, 155.02, 153.69, 144.02, 138.30, 136.19, 130.08, 128.60, 128.30, 128.09, 122.82, 105.23, 67.64, 60.85, 56.25, 54.38, 43.01, 31.86; HRMS (ESI) calcd for $C_{26}H_{32}N_5O_5S_2$ $[M + H]^+$: 558.1845, found: 558.1841.

*4.6. Procedure for the synthesis of 4-(azidomethyl)-7-hydroxy-2H-chromen-2-one compound **8***

To a magnetically stirred solution of compound **7** (0.63 g, 3 mmol) in CH_3CN (10 mL), sodium azide (0.59 g, 9 mmol) was added carefully and the reaction mixture was refluxed for 10 h. Upon completion, the reaction mixture was concentrated under

vacuum, the residue was dissolved in EtOAc (30 mL) and washed with water, brine, dried over anhydrous Na_2SO_4 and concentrated under vacuum to give compound **8** (0.60 g, yield 92.0%), which was used in the next reaction without further purification. yellow solid. Mp: 139–140 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.79 (s, 2H), 6.39 (s, 1H), 6.82 (d, 1H, J = 8.8 Hz), 6.75 (s, 1H), 7.57 (d, 1H, J = 8.8 Hz), 10.69 (s, 1H); HRMS (ESI) calcd for $C_{10}H_8N_3O_3$ $[M + H]^+$: 218.0566, found: 218.0566.

4.7. General procedure for the synthesis of compounds *11a-b*

K_2CO_3 (1.66 g, 12 mmol) and 1,2-dibromoethane (1.12 g, 6 mmol) were added to a solution of **10a** or **10b** (2 mmol) in CH_3CN (20 mL). The reaction mixture was refluxed for 2 h. Upon completion, K_2CO_3 was removed by filtration and the solvent was concentrated under vacuum, the residue was dissolved in CH_2Cl_2, washed with water, brine, dried over anhydrous Na_2SO_4 and concentrated under vacuum to give compounds **11a-b**, which were used in the next reaction without further purification.

4.7.1. 7-(2-bromoethoxy)-4-methyl-2H-chromen-2-one (*11a*)

yield 94.5%. white solid. Mp: 104–105 °C; IR(KBr, cm^{-1}) ν: 3073, 2956, 1713, 1619, 1511, 1460, 1288, 1265, 1174, 1012, 986, 887, 845, 572, 520; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.51 (d, 1H, J = 8.8 Hz), 6.89 (dd, 1H, J_1 = 2.5 Hz, J_2 = 8.8 Hz), 6.79 (d, 1H, J = 2.5 Hz), 6.14 (s, 1H), 4.36 (t, 2H, J = 6.0 Hz), 3.69 (t, 2H, J = 6.0 Hz), 2.40 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 161.07, 161.01, 155.12, 152.48, 125.77, 114.11, 112.48, 112.29, 101.69, 68.18, 28.59, 18.67; HRMS (ESI) calcd for $C_{12}H_{12}BrO_3$ $[M + H]^+$: 282.9970, found: 282.9973.

4.7.2. 7-(2-bromoethoxy)-2H-chromen-2-one (*11b*)

yield 93.7%. white solid. Mp: 176–177 °C; IR (KBr, cm^{-1}) ν: 3056, 1697, 1511, 1474, 1425, 1360, 1130, 1094, 976, 785, 727, 692, 523; ^{1}H NMR (400 MHz, actone-d_6, δ, ppm): 7.91 (d, 1H, J = 9.5 Hz), 7.61 (d, 1H, J = 8.6 Hz), 6.97 (dd, 1H, J_1 = 2.4 Hz, J_2 = 8.6 Hz), 6.93 (d, 1H, J = 2.4 Hz), 6.24 (d, 1H, J = 9.5 Hz), 4.51 (t, 2H, J = 5.4 Hz), 3.84 (t, 2H, J = 5.4 Hz); HRMS (ESI) calcd for $C_{11}H_{10}BrO_3$ $[M + H]^+$: 268.9813, found: 268.9814.

4.8. General procedure for the synthesis of compounds *12a-b*

To a magnetically stirred solution of compound **11a** or **11b** (3 mmol) in acetone (32 mL), a solution of sodium azide (0.39 g, 6 mmol) in water (8 mL) was added drop wise and the reaction mixture was refluxed for 8 h. Upon completion, the reaction mixture was concentrated under vacuum to remove acetone, the residue was extracted with EtOAc (3 × 30 mL), the combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under vacuum to get **12a-b,** which were used in the next reaction without further purification.

4.8.1. 7-(2-azidoethoxy)-4-methyl-2H-chromen-2-one (*12a*)

yield 77.9%. white solid. Mp: 101–102 °C; IR (KBr, cm^{-1}) ν: 2954, 2875, 2104, 1708, 1613, 1508, 1424, 1369, 1273, 1154, 1071, 988, 916, 847, 796, 535; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.52 (d, 1H, J = 8.8 Hz), 6.90 (dd, 1H, J_1 = 2.5 Hz, J_2 = 8.8 Hz), 6.82 (d, 1H, J = 2.5 Hz), 6.16 (s, 1H), 4.21 (t, 2H, J = 4.8 Hz), 3.66 (t, 2H, J = 4.8 Hz), 2.40(s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 161.13, 161.10, 155.18, 152.45, 125.74, 114.11, 112.64, 112.34, 101.46, 67.40, 49.94, 18.68; HRMS (ESI) calcd for $C_{12}H_{12}N_3O_3$ $[M + H]^+$: 246.0879, found: 246.0875.

4.8.2. 7-(2-azidoethoxy)-2H-chromen-2-one (*12b*)

yield 76.5%. white solid. Mp: 154–155 °C; IR (KBr, cm^{-1}) ν: 3075, 2124, 1732, 1608, 1507, 1405, 1390, 1125, 1053, 995, 914, 892, 834, 749, 616, 522; NMR (400 MHz, actone-d_6, δ, ppm): 7.66 (d, 1H, J = 9.5 Hz), 7.41 (d, 1H, J = 8.6 Hz), 6.90 (dd, 1H, J_1 = 2.4 Hz, J_2 = 8.6 Hz), 6.83 (d, 1H, J = 2.4 Hz), 6.29 (d, 1H, J = 9.5 Hz), 4.22 (t, 2H, J = 4.9 Hz), 3.67 (t, 2H, J = 4.9 Hz).^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 161.31, 161.00, 155.79, 143.29, 128.95, 113.57, 113.05, 112.95, 101.47, 67.45, 49.92; HRMS (ESI) calcd for $C_{11}H_{10}N_3O_3$ $[M + H]^+$: 232.0722, found:232.0720.

4.9. Anticancer activity assays

Exponentially growing cells were seeded into 96-well plates at a concentration of 5 × 10^3 cells per well. After 24 h incubation at 37 °C, the culture medium was removed and replaced with fresh medium containing the candidate compounds in different concentrations. The cells were incubated for another 72 h. Afterward, 20 μL of MTT solution (5 mg/mL) was added to all wells and incubated for 4 h at 37 °C. Discarded the suspension and added 150 μL of dimethyl sulfoxide (DMSO) to each well and shook the plates to dissolve the dark blue crystals (formazan); the absorbance was measured using a microplate reader at a wavelength of 570 nm. Each concentration was analyzed in triplicate and the experiment was repeated three times. The average 50% inhibitory concentration (IC_{50}) was determined from the dose–response curves according to the inhibition ratio for each concentration.

4.10. Flow cytometric analysis of cell cycle distribution

For flow cytometric analysis of DNA content, 5 × 10^5 MGC-803 cells in exponential growth were treated with different concentrations of the test compounds for 12 or 24 h. After an incubation period, the cells were collected, centrifuged and fixed with icecold ethanol (70%). The cells were then treated with buffer containing RNAse A and 0.1% Triton X-100 and then stained with PI. Samples were analyzed on Accuri C6 flow cytometer (Becton, Dickinson). Data obtained from the flow cytometer was analyzed using the FlowJo software (Tree Star, Inc., Ashland, OR, USA).

4.11. Analysis of cellular apoptosis

MGC-803 cells were plated in 6-well plates (5.0 × 10^4 cells/mL) and incubated at 37 °C for 12 h. Exponentially growing cells were then incubated for 12 h with complete medium (blank) or with the compound **3c**. Cells were then harvested and the Annexin-V-FITC/PI apoptosis kit (Biovision) was used according to the manufacturer's instructions to detect apoptotic cells. Ten thousand events were collected for each sample and analyzed by Accuri C6 flow cytometer.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2012.12.046.

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