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Molecular diversity of phenothiazines: design and synthesis of phenothiazine–dithiocarbamate hybrids as potential cell cycle blockers

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Abstract

Novel phenothiazine–dithiocarbamate analogues were designed by molecular hybridization strategy and synthesized and evaluated for their anticancer activity in vitro against three selected cancer cell lines (EC-109, MGC-803, and PC-3). The preliminary structure–activity relationship (SAR) for this phenothiazine–dithiocarbamate hybrids is explored. Among all analogues, 2-oxo-2-(10H-phenothiazin-10-yl)ethyl 4-ethylpiperazine-1-carbodithioate (**8a**) showed the most potent inhibitory activity with an IC_{50} value of 11.59 μM against PC-3 cells. In addition, compound **8a** could arrest the cell cycle at the G1 phase and regulate the expression of G1 checkpoint-related proteins, suggesting that phenothiazine–dithiocarbamate hybrids might be useful as cell cycle blockers.

Graphical abstract

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Dong-Jun Fu and Ruo-Han Zhao have contributed equally to this work.

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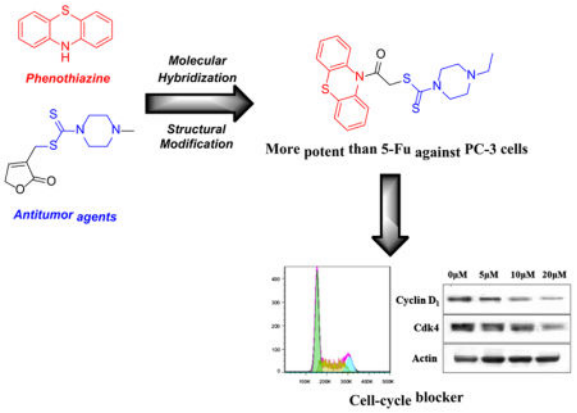
Compliance with ethical standards

Conflicts of interest The authors declare no conflict of interest.



Keywords

Phenothiazine; Dithiocarbamate; Antiproliferative activity; G1 phase; G1 checkpoint-related protein

Introduction

Phenothiazine **1**, a nitrogen- and sulfur-containing tricyclic scaffold, was reported to have many potent pharmacological activities, including anticancer, antibacterial, antiplasmid, and multi-drug resistance (MDR) reversal activities [1–5]. For example, new phenothiazine derivatives **2** and **3** [6] have been synthesized and evaluated in vitro for their ability to inhibit tubulin polymerization and antiproliferative activity against 60 cancer cell lines, including several multi-drug-resistant (MDR) tumor cell lines. Particularly, they exhibited more important cell growth inhibition than phenstatin on human colon Duke's type D, colorectal adenocarcinoma COLO 205, and human kidney adenocarcinoma A498 cell lines. These interesting findings suggested that phenothiazine moiety can be used as a promising anticancer scaffold (Fig. 1).

Dithiocarbamate, as an important scaffold in medicinal chemistry, has displayed various biological activities, including anticancer, antibacterial, antifungal, and inhibition of carbonic anhydrase activities [7–12]. Four series of dithiocarbamates derivatives have been reported as potential antitumor agents by our group: The butenolide-containing dithiocarbamate **4** displayed a potent inhibitory activity with an IC_{50} value of 0.77 μM against HeLa cells [13]; the novel dithiocarbamate–chalcone analogue **5** could induce apoptosis through apoptosis-related proteins and arrest the cell cycle at G0/G1 phase against SK-N-SH cells [14]; triazole–dithiocarbamate hybrid **6** inhibited cell growth, invasion, and migration on MGC-803 cell line [15]; formononetin–dithiocarbamate derivative **7** inhibited migration and growth of PC-3 cells and regulated MAPK/Wnt signaling pathways [16] (Fig. 2).

Molecular hybridization strategy is an useful strategy in drug discovery to design new bioactive ligands as promising lead compound [17,18]. In order to discover more potent anticancer agents [19–22], a molecular hybridization strategy of phenothiazine and

dithiocarbamate to produce a novel hybrid was used in this work. As shown in Fig. 3, a molecular hybridization strategy based on the structures of phenothiazine **1** and a bioactive dithiocarbamate analogue **4** yielded a scaffold which has four parts: (1) a dithiocarbamate group with drug-like properties, (2) a phenothiazine scaffold as an antitumor pharmacophore fragment, (3) a medium-chain alkyl group for lipophilicity, and (4) various aliphatic amine units attached with dithiocarbamate fragment. To the best of our knowledge, to date there have been no literature reports regarding phenothiazine–dithiocarbamate hybrids.

Results and discussion

Synthesis

The synthetic route used is shown in Scheme 1. Commercially available phenothiazine **1** was reacted with 2-chloroacetyl chloride (or 3-chloropropanoyl chloride or 4-chlorobutanoyl chloride), carbon disulfide, and various piperazine analogues to afford **8a–8o** in the presence of sodium phosphate tribasic dodecahydrate.

Structure–activity relationships

To identify novel derivatives with antitumor potential, we evaluated the antiproliferative activity of phenothiazine–dithiocarbamate hybrids **8a–8o** against three cancer cell lines (PC-3, EC-109, and MGC-803) by the MTT assay. Due to the potentially similar mode of action between reported dithiocarbamate derivatives and well-known 5-fluorouracil (5-Fu), 5-Fu was used as the reference drug in the MTT assay [23]. In addition, phenothiazine **1** also was a positive control (Table 1).

The antiproliferative activity results against the cancer cell lines are given in Table 1. Several phenothiazine–dithiocarbamate derivatives (**8b**, **8c**, **8e**, **8f**, **8j**, **8n**, and **8o**) displayed weak inhibitory activity against all three cancer cell lines, indicating that the aromatic group (pyridine ring and phenyl ring) on the piperazine moiety decreased the antiproliferative activity. Interestingly, compound **8o** exhibited more potent antiproliferative activity than 5-Fu against PC-3 cell line with an IC_{50} value of 22.90 μM. However, **8o** exhibited no cytotoxicity against MGC-803 cells and EC-109 cells (>100 μM). These results demonstrate that compound **8o** showed good selectivity between the selected cancer cell lines.

Compared with the phenothiazine **1**, phenothiazine–dithiocarbamate derivatives bearing an alkyl chain on the piperazine moiety (**8a**, **8d**, **8g**, **8h**, **8i**, **8k**, and **8m**) showed good inhibitory activity results with IC_{50} values ranging from 11.59 μM to 90.51 μM. This result suggests that the dithiocarbamate moiety may play a synergistic role in the overall activity. We also found that substituents on the piperazine unit were important for inhibitory activity. Importantly, compound **8a** bearing an ethyl group displayed the best antiproliferative activity with an IC_{50} value of 11.59 μM against PC-3 cells. Replacing the ethyl group (**8a**) by a methyl group (**8g**) or acetyl group (**8h**) caused a decrease in activity. The modifications and SAR studies revealed that the ethyl group on the piperazine moiety is important for inhibitory activity.

In order to expand the SAR study, the length of carbon linker between the phenothiazine scaffold and the dithiocarbamate scaffold was also investigated. By extending the carbon

linker length by one or two carbons, the antiproliferative activity decreased (**8h** vs **8d** or **8m**) indicating the importance of the linker for activity. An illustration for the observed structure–activity relationship is shown in Scheme 2.

Prediction of molecular properties and drug-likeness

Because of the best antiproliferative activity against PC-3 cell line, the drug-likeness of compound **8a** was assessed using free molecular calculation services provided by Molsoft (http://molsoft.com/mprop). As given in Table 2, compound **8a** showed acceptable molecular and drug-likeness properties.

Apoptosis assays

Based on the above antiproliferative results, compound **8a** was chosen for apoptosis assays to investigate whether it could induce apoptosis in PC-3 cells (Fig. 4a, b, c). After treatment with four different concentrations of compound **8a** (0, 5, 10, and 20 μM) for 48 h, PC-3 cells were stained with PI and Annexin V-FITC and then analyzed by flow cytometry. As illustrated in Fig. 4a and b, the percentage of apoptotic cells did not change based on the control group. Then, we performed to examine the expression of the key protein Bax which is involved in driving apoptosis [24]. As shown in Fig. 4c, Bax expression did not increase after treatment of PC-3 cells with compound **8a**. All these results in Fig. 4 illustrate that compound **8a** does not induce apoptosis in PC-3 cells.

Cell cycle assays

Targeting the cell cycle of tumor cells has been recognized as a useful strategy for cancer therapy [25]. In this study, compound **8a** was chosen to investigate the effect of our designed derivatives on the cell cycle of PC-3 cells. After treating PC-3 cells with compound **8a** at different concentrations (0, 5, 10, and 20 μM) for 48 h, PC-3 cells were then fixed and stained with PI for flow cytometry analysis. As shown in Fig. 5, compound **8a** arrested G1 phase (dose-dependent), accompanied with the decrease in cells at G2 and S phase. Specifically, the percentage of cells at G1 phase for the high concentration group (20 μM) was 60.11%, about 25% higher than that of the control group.

The G1-phase arrest induced by **8a** was further confirmed by investigating the changes in G1 checkpoint-related proteins, including cyclin D1 and CDK4 [26]. As shown in Fig. 5c, our results verified that cyclin D-CDK4 complex accumulation promotes G1–S transition in cell cycle [27]. Compound **8a** downregulated cyclin D1 and CDK4 concentration dependently (Fig. 5c), which led to cell arrest in G1 phase.

Conclusions

Following our previous work, we designed a series of new phenothiazine–dithiocarbamate derivatives by a efficient molecular hybridization strategy. All hybrids possessed moderate to good growth inhibition against the tested cancer cells. Particularly, compound **8a** exhibited growth inhibition against PC-3 cells with an IC_{50} value of 11.59 μM. The preliminary SAR indicates that dithiocarbamate, as a reported antitumor scaffold, could play potential synergistic effect with a phenothiazine skeleton.

Mechanistically, it was determined that compound **8a** halts cell cycle progression at the G1 phase. In PC-3 cells, compound **8a** does not induce apoptosis. Overall, compound **8a** could be used as a potential cell cycle blocker.

Experimental section

General

All reagents and solvents were purchased in Zhengzhou Research Biotechnology Co., Ltd. Melting points were determined on an X-5 micromelting apparatus and are uncorrected. ^{1}HNMR and ^{13}CNMR spectra were recorded on a Bruker 400 and 100 MHz spectrometer with TMS as internal standard in DMSO-*d*6. Chemical shifts are given as ppm and Hertz values relative to TMS. High-resolution mass spectra (HRMS) were recorded on a Waters Micromass Q-T of Micromass spectrometer by electrospray ionization (ESI).

Synthesis of target derivatives 8a~8o

To a solution of phenothiazine (0.5 mmol), 2-chloroacetyl chloride (or 3-chloropropanoyl chloride or 4-chlorobutanoyl chloride) (0.5 mmol), carbon disulfide (1.5 mmol), and a piperazine analogue (0.5 mmol) in acetone (10 mL) and sodium phosphate tribasic dodecahydrate (0.75 mmol) were added, and the mixture was stirred at room temperature. Upon completion (monitored by TLC), EtOAc (20 mL) and H_2O (40 mL) were added. The aqueous layer was extracted again with EtOAc (20 mL); the combined organic layers were washed with H_2O for several times to remove the acetone, washed with brine, dried over $MgSO_4$, and evaporated to give crude product. The resulting residue was purified by column chromatography (hexane/EtOAc = 8:1) to obtain desired analogues **8a–8o**.

2-Oxo-2-(10H-phenothiazin-10-yl)ethyl4-ethylpiperazine-1-carbodithioate (8a)—Light yellow solid, yield: 78%, mp: 144–147 °C. ^{1}H NMR (400 MHz, DMSO) δ 7.69 (d, *J* = 7.3 Hz, 2H), 7.57 (d, *J* = 7.7 Hz, 2H), 7.42 (td, *J* = 7.8, 1.1 Hz, 2H), 7.33 (t, *J* = 7.3 Hz, 2H), 4.41 (s, 2H), 4.26 –3.99 (m, 2H), 3.87 (s, 2H), 2.39 (d, *J* = 14.8 Hz, 4H), 2.34 (q, *J* = 7.2 Hz, 2H), 0.99 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO) δ 193.83, 165.78, 137.96, 132.31, 127.87, 127.33, 127.13, 124.50, 51.61, 50.96, 11.78. HRMS (ESI) calcd for $C_{21}H_{24}N_3OS_3$ $[M+H]^+$: 430.1081, found: 430.1083.

2-Oxo-2-(10H-phenothiazin-10-yl)ethyl4-(4-(tert-butyl) benzyl)piperazine-1-carbodithioate (8b)—White solid, yield: 62%, mp: 124–127 °C. ^{1}H NMR (400 MHz, DMSO) δ 7.69 (d, *J* = 7.2 Hz, 2H), 7.56 (d, *J* = 7.5 Hz, 2H), 7.41 (t, *J* = 7.2 Hz, 2H), 7.33 (dd, *J* = 12.0, 5.5 Hz, 4H), 7.22 (d, *J* = 8.1 Hz, 2H), 4.40 (s, 2H), 4.12 (s, 2H), 3.87 (s, 2H), 3.45 (s, 2H), 2.46 –2.31 (m, 4H), 1.27 (s, 9H). ^{13}C NMR (100 MHz, DMSO) δ 193.79, 165.78, 149.39, 137.95, 134.41, 132.30, 128.64, 127.87, 127.32, 127.16, 127.12, 124.93, 60.92, 51.86, 34.14, 31.14. HRMS (ESI) calcd for $C_{30}H_{34}N_3OS_3$ $[M+H]^+$: 548.1864, found: 548.1862.

3-Oxo-3-(10H-phenothiazin-10-yl)propyl4-(4-(tert-butyl) benzyl)piperazine-1-carbodithioate (8c)—White solid, yield: 89%, mp: 142–144 °C. ^{1}H NMR (400 MHz, DMSO) δ 7.60 (d, *J* = 7.8 Hz, 2H), 7.55 (d, *J* = 7.6 Hz, 2H), 7.35 (ddd, *J* = 18.7, 15.2, 7.3

Hz, 6H), 7.22 (d, *J* = 8.0 Hz, 2H), 4.17 (s, 2H), 3.82 (s, 2H), 3.55 – 3.38 (m, 4H), 2.88 (s, 2H), 2.40 (s, 4H), 1.26 (s, 9H). ^{13}C NMR (101 MHz, DMSO) δ 194.54, 169.58, 149.39, 138.11, 134.41, 132.21, 128.66, 127.90, 127.44, 127.27, 127.08, 124.94, 60.96, 51.87, 34.15, 33.47, 31.71, 31.15. HRMS (ESI) calcd for $C_{31}H_{36}N_3OS_3$ $[M + H]^+$: 562.2020, found: 562.2018.

3-Oxo-3-(10H-phenothiazin-10-yl)propyl4-acetylpiperazine-1-carbodithioate (8d)—White solid, yield: 11%, mp: 173–176 °C. ^{1}H NMR (400 MHz, DMSO) δ 7.62 (d, *J* = 7.8 Hz, 2H), 7.57 (d, *J* = 7.5 Hz, 2H), 7.40 (t, *J* = 7.2 Hz, 2H), 7.32 (t, *J* = 7.4 Hz, 2H), 4.18 (d, *J* = 22.6 Hz, 2H), 3.87 (d, *J* = 19.9 Hz, 2H), 3.55 (dd, *J* = 10.0, 5.2 Hz, 4H), 3.46 (t, *J* = 6.4 Hz, 2H), 2.90 (s, 2H), 2.02 (s, 3H). ^{13}C NMR (100 MHz, DMSO) δ 195.68, 170.08, 169.15, 138.59, 132.71, 128.42, 127.95, 127.82, 127.62, 44.85, 33.95, 32.16, 21.70. HRMS (ESI) calcd for $C_{22}H_{23}N_3NaO_2S_3$ $[M + Na]^+$: 480.0850, found: 480.0851.

3-Oxo-3-(10H-phenothiazin-10-yl)propyl4-benzylpiperazine-1-carbodithioate (8e)—Yellow solid, yield: 25%, mp: 161–166 °C. ^{1}H NMR (400 MHz, DMSO) δ 7.61 (d, *J* = 7.8 Hz, 51H), 7.58 (dd, *J* = 22.4, 7.7 Hz, 103H), 7.55 (d, *J* = 7.7 Hz, 51H), 7.39 (dd, *J* = 11.0, 4.3 Hz, 54H), 7.46 – 7.13 (m, 236H), 7.36 – 7.14 (m, 182H), 4.17 (s, 52H), 4.00 (d, *J* = 140.1 Hz, 104H), 3.82 (s, 53H), 3.60 (d, *J* = 49.8 Hz, 9H), 3.50 (s, 54H), 3.54 – 3.28 (m, 171H), 3.44 (t, *J* = 6.5 Hz, 52H), 2.88 (s, 47H), 2.64 (d, *J* = 191.9 Hz, 215H), 2.40 (s, 105H), 2.27 – 2.08 (m, 8H). ^{13}C NMR (100 MHz, DMSO) δ 195.07, 170.08, 138.60, 137.98, 132.69, 132.58, 129.41, 128.72, 128.41, 127.93, 127.78, 127.59, 61.76, 52.36, 34.00, 32.22. HRMS (ESI) calcd for $C_{27}H_{28}N_3OS_3$ $[M + H]^+$: 506.1394, found: 506.1395.

2-Oxo-2-(10H-phenothiazin-10-yl)ethyl4-benzylpiperazine-1-carbodithioate (8f)—White solid, yield: 54%, mp: 167–169 °C. ^{1}H NMR (400 MHz, DMSO) δ 7.69 (d, *J* = 7.2 Hz, 2H), 7.57 (d, *J* = 7.6 Hz, 2H), 7.41 (dd, *J* = 11.0, 4.3 Hz, 2H), 7.36 – 7.20 (m, 7H), 4.40 (s, 2H), 4.12 (s, 2H), 3.87 (s, 2H), 3.50 (s, 2H), 2.47 – 2.31 (m, 4H). ^{13}C NMR (100 MHz, DMSO) δ 193.84, 165.78, 137.95, 137.46, 132.25, 128.90, 128.21, 127.87, 127.32, 127.13, 127.09, 124.50, 61.21, 51.84, 51.13. HRMS (ESI) calcd for $C_{26}H_{26}N_3OS_3$ $[M + H]^+$: 492.1238, found: 492.1237.

2-Oxo-2-(10H-phenothiazin-10-yl)ethyl4-methylpiperazine-1-carbodithioate (8g)—Light yellow solid, yield: 74%, mp: 186–189 °C. ^{1}H NMR (400 MHz, DMSO) δ 7.69 (d, *J* = 7.4 Hz, 2H), 7.57 (dd, *J* = 7.7, 1.1 Hz, 2H), 7.42 (td, *J* = 7.7, 1.4 Hz, 2H), 7.33 (td, *J* = 7.6, 1.0 Hz, 2H), 4.41 (s, 2H), 4.10 (s, 2H), 3.86 (s, 2H), 2.34 (s, 4H), 2.18 (s, 3H). ^{13}C NMR (100 MHz, DMSO) δ 193.97, 165.77, 137.96, 127.88, 127.33, 127.17, 127.14, 124.50, 53.89, 50.97, 45.03. HRMS (ESI) calcd for $C_{20}H_{22}N_3OS_3$ $[M + H]^+$: 416.0925, found: 416.0924.

2-Oxo-2-(10H-phenothiazin-10-yl)ethyl4-acetylpiperazine-1-carbodithioate (8h)—Light yellow solid, yield: 77%, mp: 199–200 °C. ^{1}H NMR (400 MHz, DMSO) δ 7.70 (d, *J* = 6.9 Hz, 2H), 7.58 (d, *J* = 7.6 Hz, 2H), 7.42 (t, *J* = 7.2 Hz, 2H), 7.33 (t, *J* = 7.4 Hz, 2H), 4.44 (s, 2H), 4.12 (d, *J* = 24.2 Hz, 2H), 3.96 (s, 2H), 3.54 (s, 4H), 2.01 (s, 3H). ^{13}C NMR (100 MHz, DMSO) δ 194.43, 168.62, 165.71, 137.94, 132.31, 127.97, 127.89, 127.32,

127.16, 44.38, 21.18. HRMS (ESI) calcd for $C_{21}H_{21}N_3NaO_2S_3$ [M + Na]$^+$: 466.0694, found: 466.0695.

3-Oxo-3-(10H-phenothiazin-10-yl)propyl4-ethylpiperazine-1-carbodithioate (8i)—Yellow solid, yield: 35%, mp: 108–112 °C. ^{1}H NMR (400 MHz, DMSO) δ 7.61 (d, *J* = 7.5 Hz, 2H), 7.56 (dd, *J* = 7.7, 1.0 Hz, 2H), 7.40 (td, *J* = 7.8, 1.3 Hz, 2H), 7.31 (td, *J* = 7.6, 0.9 Hz, 2H), 4.16 (s, 2H), 3.81 (s, 2H), 3.44 (t, *J* = 6.7 Hz, 2H), 2.89 (s, 2H), 2.38 (dd, *J* = 10.0, 4.9 Hz, 4H), 2.33 (t, *J* = 7.2 Hz, 2H), 1.00 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO) δ 194.53, 169.58, 138.12, 132.22, 127.90, 127.44, 127.28, 127.09, 51.68, 50.98, 33.49, 31.68, 11.85. HRMS (ESI) calcd for C22H26N3OS$_3$[M + H]$^+$: 444.1238, found: 444.1236.

3-Oxo-3-(10H-phenothiazin-10-yl)propyl4-(pyridin-2-yl)piperazine-1-carbodithioate (8j)—White solid, yield: 80%, mp: 114–117 °C. ^{1}H NMR (400 MHz, DMSO) δ 8.13 (d, *J* = 3.4 Hz, 1H), 7.71 – 7.47 (m, 5H), 7.41 (dd, *J* = 10.9, 4.3 Hz, 2H), 7.31 (t, *J* = 7.5 Hz, 2H), 6.80 (d, *J* = 8.6 Hz, 1H), 6.68 (dd, *J* = 6.8, 5.1 Hz, 1H), 4.28 (s, 2H), 3.96 (s, 2H), 3.63 (s, 4H), 3.48 (t, *J* = 6.5 Hz, 2H), 2.91 (s, 2H). ^{13}C NMR (100 MHz, DMSO) δ 194.89, 169.60, 158.13, 147.55, 138.11, 137.64, 132.23, 127.90, 127.45, 127.29, 127.10, 113.25, 106.92, 43.53, 33.48, 31.64. HRMS (ESI) calcd for $C_{25}H_{25}N_4OS_3$ [M + H]$^+$: 493.1190, found: 493.1186.

Tert-butyl4-(((2-oxo-2-(10H-phenothiazin-10-yl)ethyl)thio)carbonothioyl)piperazine-1-carboxylate (8k)—Light yellow solid, yield: 63%, mp: 180–183 °C. ^{1}H NMR (400 MHz, DMSO) δ 7.69 (d, *J* = 6.6 Hz, 2H), 7.57 (d, *J* = 7.6 Hz, 2H), 7.42 (t, *J* = 7.5 Hz, 2H), 7.33 (t, *J* = 7.5 Hz, 2H), 4.42 (s, 2H), 4.10 (s, 2H), 3.91 (s, 2H), 3.42 (s, 4H), 1.40 (s, 9H). ^{13}C NMR (100 MHz, DMSO) δ 194.93, 166.22, 154.13, 138.43, 132.81, 132.76, 128.39, 127.82, 127.67, 79.87, 51.08, 49.79, 30.03, 28.48. HRMS (ESI) calcd for $C_{24}H_{27}N_3NaO_3S_3$ [M + Na]$^+$: 524.1112, found: 524.1113.

Tert-butyl4-(((4-oxo-4-(10H-phenothiazin-10-yl)butyl)thio)carbonothioyl)piperazine-1-carboxylate (8l)—White solid, yield: 11%, mp: 173–176 °C. ^{1}H NMR (400 MHz, DMSO) δ 7.63 (d, *J* = 7.8 Hz, 2H), 7.56 (d, *J* = 7.6 Hz, 2H), 7.41 (t, *J* = 7.4 Hz, 2H), 7.32 (t, *J* = 7.4 Hz, 2H), 4.20 (s, 2H), 3.87 (s, 2H), 3.42 (s, 4H), 3.19 (t, *J* = 7.0 Hz, 2H), 2.56 (s, 2H), 1.97 – 1.70 (m, 2H), 1.42 (s, 9H). ^{13}C NMR (100 MHz, DMSO) δ 195.36, 170.43, 153.68, 138.39, 132.31, 127.89, 127.54, 127.25, 126.99, 79.35, 35.28, 32.53, 28.00, 24.05. HRMS (ESI) calcd for $C_{26}H_{31}N_3NaO_3S_3$ [M+ Na]$^+$: 552.1425, found: 552.1428.

4-Oxo-4-(10H-phenothiazin-10-yl)butyl4-acetylpiperazine-1-carbodithioate (8m)—Yellow solid, yield: 14%, mp: 162–164 °C. ^{1}H NMR (400 MHz, DMSO) δ 7.63 (d, *J* = 7.8 Hz, 2H), 7.57 (d, *J* = 7.6 Hz, 2H), 7.41 (t, *J* = 7.3 Hz, 2H), 7.32 (t, *J* = 7.4 Hz, 2H), 4.19 (s, 2H), 3.89 (s, 2H), 3.55 (s, 4H), 3.19 (t, *J* = 7.1 Hz, 2H), 2.56 (s, 2H), 2.03 (s, 3H), 1.92 – 1.75 (m, 2H). ^{13}C NMR (100 MHz, DMSO) δ 195.81, 170.94, 169.16, 138.88, 132.84, 128.41, 128.04, 127.76, 127.51, 44.89, 35.76, 33.02, 24.54, 21.72. HRMS (ESI) calcd for $C_{23}H_{25}N_3NaO_2S_3$ [M + Na]$^+$: 494.1007, found: 494.1008.

4-Oxo-4-(10H-phenothiazin-10-yl)butyl4-phenylpiperazine-1-carbodithioate (8n)—Gray green solid, yield: 27%, mp: 137–140 °C. ^{1}H NMR (400 MHz, DMSO) δ 7.63 (d, *J* = 7.8 Hz, 2H), 7.56 (d, *J* = 7.6 Hz, 2H), 7.40 (t, *J* = 7.6 Hz, 2H), 7.31 (t, *J* = 7.5 Hz, 2H), 7.24 (t, *J* = 7.9 Hz, 2H), 6.94 (d, *J* = 8.1 Hz, 2H), 6.82 (t, *J* = 7.2 Hz, 1H), 4.35 (s, 2H), 3.99 (s, 2H), 3.22 (dd, *J* = 15.5, 8.1 Hz, 6H), 2.57 (s, 2H), 1.87 (p, *J* = 7.0 Hz, 2H). ^{13}C NMR (101 MHz, DMSO) δ 195.46, 170.95, 150.52, 138.89, 132.82, 129.52, 128.41, 128.04, 127.76, 127.51, 119.72, 115.92, 48.02, 35.76, 32.98, 24.56. HRMS (ESI) calcd for $C_{14}H_{11}NNaOS_3$ $[M + Na]^+$: 264.0459, found: 264.0457.

4-Oxo-4-(10H-phenothiazin-10-yl)butyl4-methylpiperazine-1-carbodithioate (8o)—Orange liquid, yield: 12%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (d, *J* = 7.6 Hz, 2H), 7.35 (d, *J* = 7.6 Hz, 2H), 7.24 (t, *J* = 7.4 Hz, 2H), 7.14 (t, *J* = 7.5 Hz, 2H), 4.24 (s, 2H), 3.82 (s, 2H), 3.22 (t, *J* = 7.2 Hz, 2H), 2.51 (s, 2H), 2.38 (s, 4H), 2.23 (s, 3H), 2.04 – 1.87 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.90, 170.33, 137.68, 132.27, 127.00, 126.30, 126.01, 125.84, 53.36, 44.59, 35.05, 32.23, 23.57. HRMS (ESI) calcd for $C_{22}H_{26}N_3OS_3$ $[M + H]^+$: 444.1238, found: 444.1239.

Biological testing

The MTT assay, analysis of cell cycle distribution, detection of apoptosis, western blot analysis, and statistical analysis were carried out as previously reported [16].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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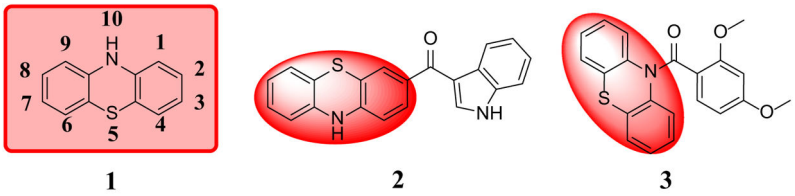


Fig. 1.
Phenothiazine and antiproliferative derivatives

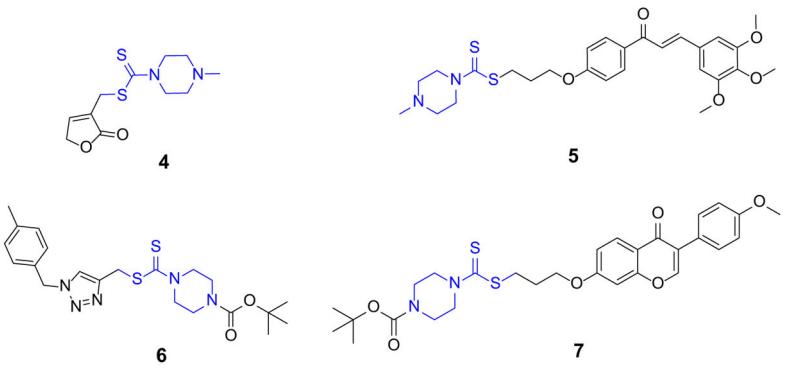


Fig. 2.
Antiproliferative dithiocarbamates reported by our group

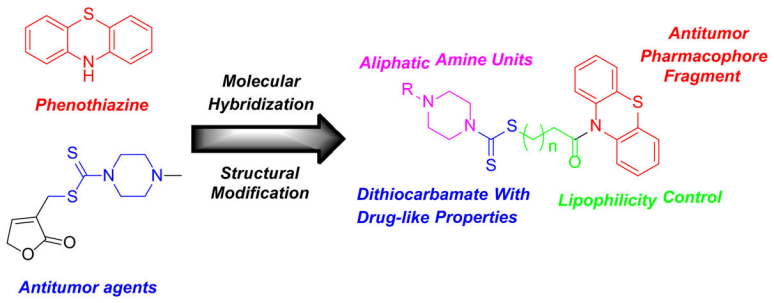


Fig. 3.

Rational molecular hybridization strategy for target antiproliferative derivatives

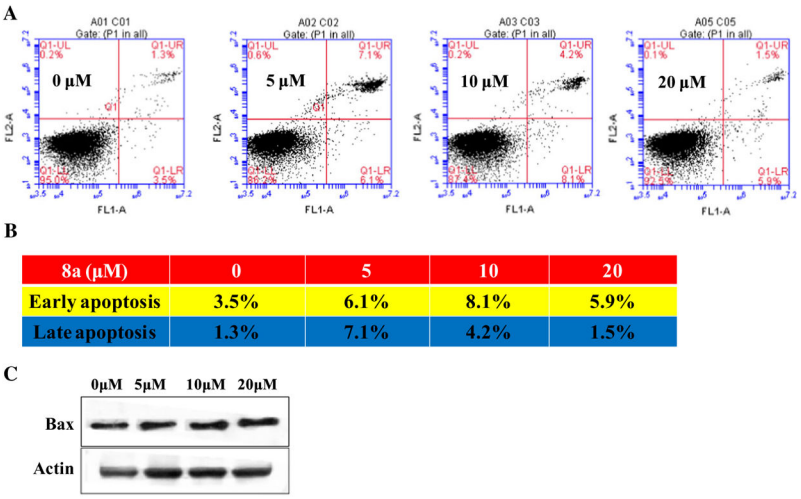


Fig. 4.

Compound **8a** did not induce apoptosis in PC-3 cells. **a** Apoptosis analysis after 48 h in PC-3 cells; **b** quantitative analysis of the percentage of early apoptosis and late apoptosis; **c** western blot analysis of Bax protein

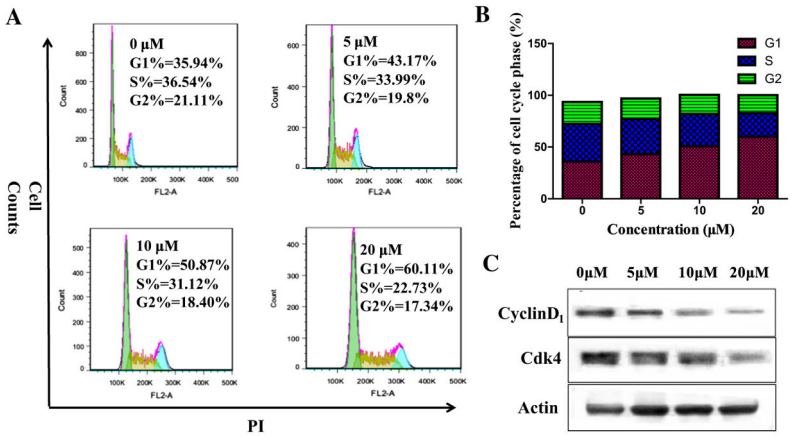
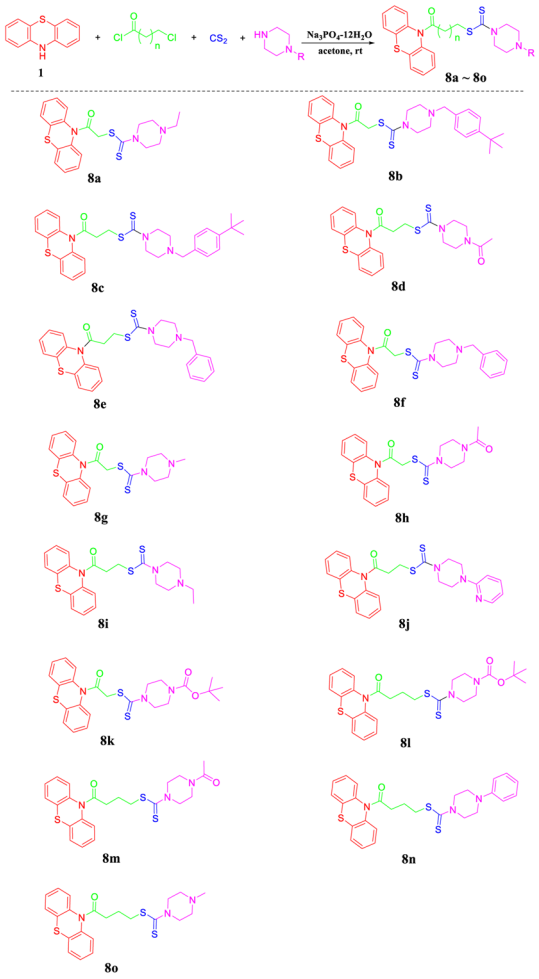


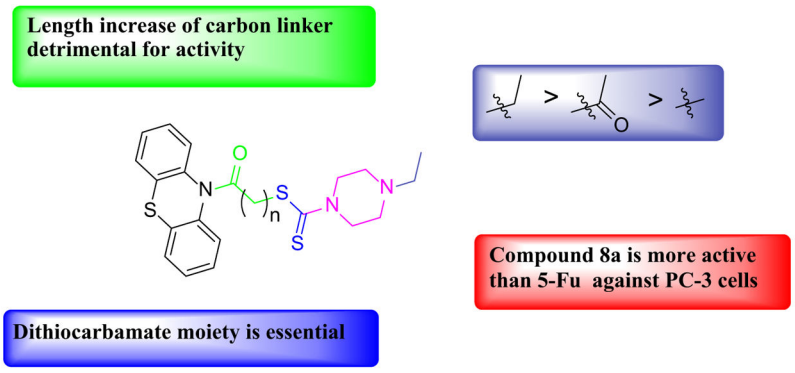
Fig. 5.

a Effects of **8a** on PC-3 cell cycle progress for 48 h; **b** quantitative analysis of the percentage of cell cycle phase; **c** effects of **8a** on G1 regulatory protein. PC-3 cells were treated for 48 h with the indicated concentration of **8a**. The cells were harvested and lysed for the detection of CDK4 and cyclin D1



Scheme 1.

Synthesis of phenothiazine–dithiocarbamate hybrids **8a–8o**



Scheme 2.
Summary of SAR

Table 1

Anticancer activity in vitro of phenothiazine–dithiocarbamate derivatives

Hybrid	MGC-803	IC_{50}(μM)[a]	
		EC-109	PC-3
8a	17.79 ± 0.40	18.96 ± 0.07	11.59 ± 0.64
8b	>100	>100	>100
8c	>100	>100	>100
8d	43.76 ± 3.84	43.89 ± 0.37	55.70 ± 0.05
8e	>100	>100	>100
8f	>100	>100	>100
8g	66.62 ± 2.81	83.51 ± 1.36	35.15 ± 3.40
8h	37.83 ± 1.38	42.99 ± 0.05	19.33 ± 0.58
8i	45.70 ± 0.22	31.52 ± 0.25	31.53 ± 0.25
8j	>100	>100	>100
8k	36.70 ± 4.11	71.94 ± 4.92	39.22 ± 0.38
8l	83.67 ± 2.37	90.51 ± 3.42	79.93 ± 1.08
8m	52.13 ± 1.42	71.36 ± 4.28	58.71 ± 0.04
8n	>100	>100	>100
8o	>100	>100	22.90 ± 1.13
1	>100	>100	>100
5-Fu	12.41 ± 0.94	14.27 ± 1.32	29.31 ± 1.87

[a]Antiproliferative activity was assayed by exposure for 48 h. Data are presented as the means±SDs of three independent experiments

Table 2

Molecular properties and drug-likeness of compound **8a**[a]

Compound	MW	HBA	MolLogP	MolPSA (Å^2)	Drug-likeness score
Desirable value	<500	<10	<5	<140	About 1.0
8a	429.10	5	4.27	19.97	1.18

[a] *MW* molecular weight, *HBA* number of hydrogen bond acceptors, *MolLogP* LogP value predicted by molsoft, *MolPSA* topological polar surface area