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Design, synthesis and antiproliferative activity studies of novel dithiocarbamate–chalcone derivates

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ABSTRACT

A series of novel dithiocarbamate–chalcone derivates were designed, synthesized and evaluated for antiproliferative activity against three selected cancer cell lines (EC-109, SK-N-SH and MGC-803). Majority of the synthesized compounds exhibited moderate to potent activity against all the cancer cell lines assayed. Particularly, compounds **II2** and **II5** exhibited the excellent growth inhibition against SK-N-SH with IC_{50} values of 2.03 μM and 2.46 μM, respectively. Further mechanism studies revealed that compound **II2** could obviously inhibit the proliferation of SK-N-SH cells by inducing apoptosis and arresting the cell cycle at G0/G1 phase.

Cancer, being one of the leading causes of death globally, poses a major socioeconomic hazard to humanity at large. Although there have been progresses in the development of treatment and prevention of cancer, the successful treatment of cancer remains a challenge.[1] Therefore, there is still an urgent need to search for novel antiproliferative agents that have broader spectrum of cytotoxicity to tumor cells.[2]

Chalcones are considered to be the precursors of flavonoids and isoflavonoids[3] that have been screened for their wide range of pharmacological activities as antibacterial,[4,5] anti-tumor,[6,7] antiinflammatory,[8,9] antifungal and antioxidant agents.[10,11] Dithiocarbamate is considered privileged scaffold in drug discovery with a wide array of biological activities. In the literature, dithiocarbamate derivatives have been described as anti-fungal,[12] anti-bacterial,[13] and carbonic anhydrase inhibitors.[14] Besides, the dithiocarbamate has always been used as a linkage to combine different biologically active scaffold to design new chemical entities. Our group have reported two series of dithiocarbamates derivates **1** and **2** as antitumor agents, which can inhibit gastric cancer cell growth, invasion and migration (Fig. 1).[15,16]

Molecular hybridization is a strategy of rational design of new ligands or prototypes based on the recognition of pharmacophoric subunits in the molecular structure of two or more known bioactive derivatives.[17] In the course of our search for new anticancer agents, we recently reported the synthesis and antiproliferative activity of a series of novel *N*-Acyl homoserine lactones (AHLs) analogs **3** with the chalcone and homoserine lactone scaffold linked by the dithiocarbamate group through molecular hybridization approach.[18] The preliminary structure–activity relationship (SAR) studies revealed that the chalcone scaffold and dithiocarbamate group were critical for their inhibitory activity. So in this study, these two biologically important groups are retained and we choose derivative **3** (namely **11a** in Ref. 18) as the lead compound due to its most potent inhibitory activity against MGC-803 cells than other AHLs analogs. In continuation with our efforts toward the discovery of novel anticancer agents, we herein design and optimize chalcone–dithiocarbamate hybrids (Fig. 2).

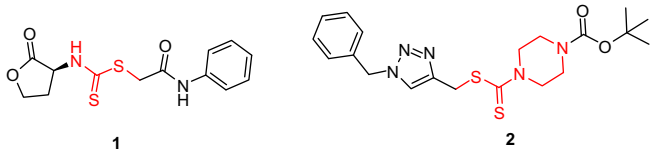

Figure 1. Structures of dithiocarbamates as antitumor agents previously reported.

The synthetic routes towards dithiocarbamate–chalcone analogues (**II1**–**II17**) were shown in Scheme 1. Commercially available substituted benzaldehydes were reacted with substituted acetophenones to form chalcones by Claisen–Schmidt condensation, which was subjected to etherification reaction with 1,3-dibromopropane or 1,2-dibromoethane to afford **5** and **IIb**. The target analogues were easily obtained in high yields with the mature reaction

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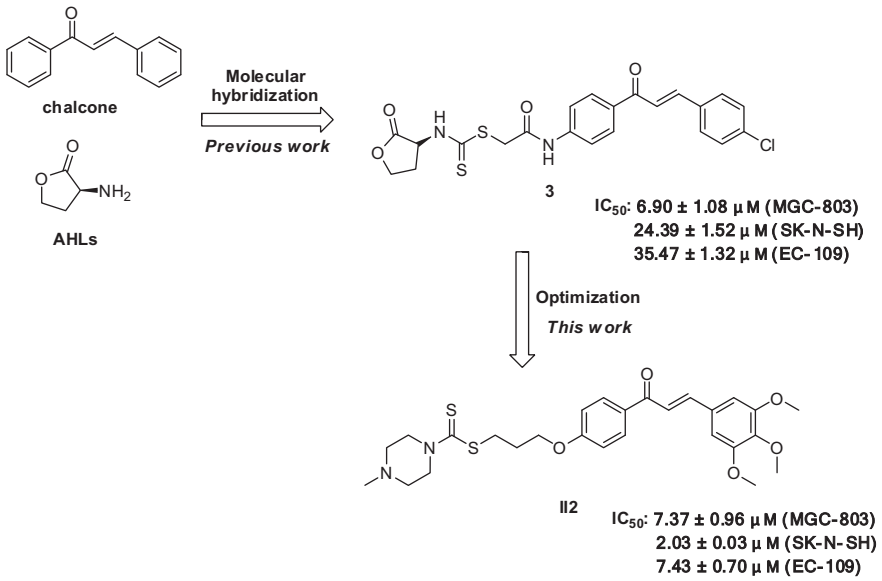
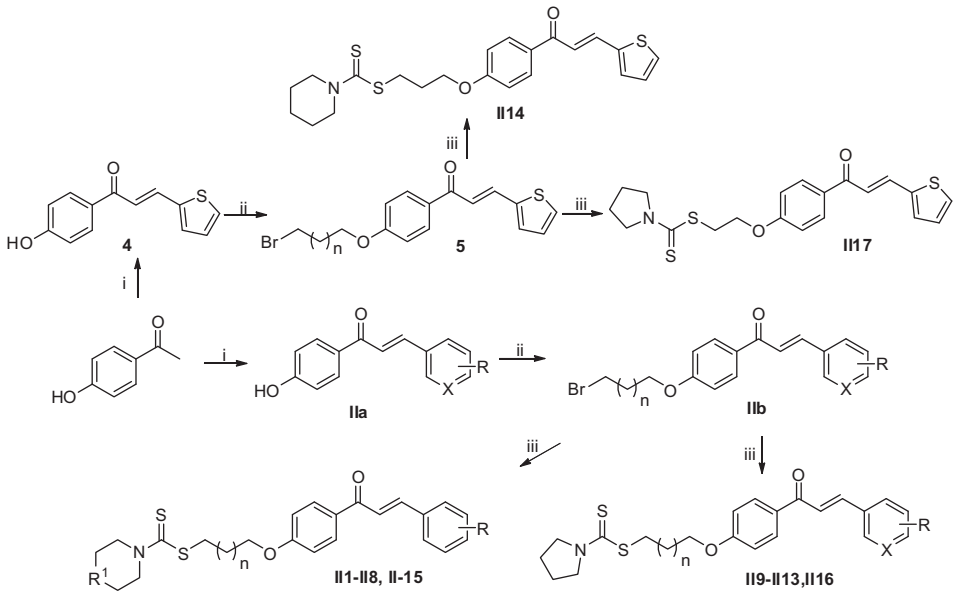


Figure 2. The design and optimization of chalcone–dithiocarbamate derivates.



II1 n = 1, R = 4-Cl, $R^1 = CH_3N$
II2 n = 1, R = 3,4,5-triOMe, $R^1 = CH_3N$
II3 n = 1, R = 3,5-diF, $R^1 = CH_3N$
II4 n = 1, R = 3,5-diCl, $R^1 = CH_3N$
II5 n = 0, R = 3,4,5-triOMe, $R^1 = CH_3N$
II6 n = 1, R = 4-Cl, $R^1 = CH_2$
II7 n = 0, R = 4-Cl, $R^1 = CH_2$
II8 n = 0, R = 3,4,5-triOMe, $R^1 = CH_2$
II15 n = 0, R = 4-Cl, $R^1 = O$
II18 n = 1, R = 4-OCH_3, $R^1 = CH_3N$

II9 n = 1, R = 4-Cl, X = C
II10 n = 1, R = 3,4,5-triOMe, X = C
II11 n = 1, R = 3,5-diF, X = C
II12 n = 0, R = 4-Cl, X = C
II13 n = 1, R = H, X = N
II16 n = 0, R = 3,4,5-triOMe X = C

Scheme 1. Synthesis of analogues **II1**–**II17**. Reagents and conditions: (i) substituted aromatic aldehyde, NaOH, EtOH, rt, 60–83% yield; (ii) 1,3-dibromopropane or 1,2-dibromoethane, K_2CO_3, THF, reflux, 72–79% yield; (iii) CS_2, substituted amine, $Na_3PO_4{\cdot}12H_2O$, acetone, rt, 63–76% yield.

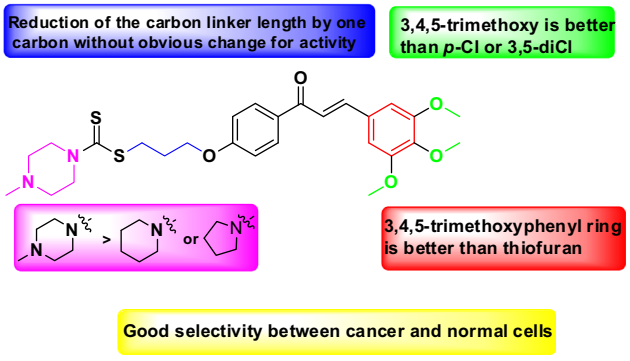
conditions developed by our group.[18] Finally, all the chalcone–dithiocarbamate derivatives were fully characterized by ^{1}H NMR, ^{13}C NMR and HRMS.

All synthesized compounds were evaluated for their antiproliferative activity against three human cancer cell lines (MGC-803, EC-109, SK-N-SH) using MTT assay method and compared with the well-known anticancer drug 5-fluorouracil and lead compound AHLs derivative **3** (Table 1). The majority of the synthesized compounds exhibited moderate to potent activity against all the cancer cell lines. Particularly, compounds **II2** and **II5** exhibited excellent

Table 1
Antiproliferative activity of chalcone–dithiocarbamate derivatives

Compound	IC_{50}[a] (μM)		
	EC-109	SK-N-SH	MGC-803
II1	34.19 ± 0.68	31.88 ± 1.31	29.36 ± 0.72
II2	7.43 ± 0.70	2.03 ± 0.03	7.37 ± 0.96
II3	14.50 ± 0.56	13.20 ± 0.72	16.26 ± 0.78
II4	12.43 ± 1.07	24.62 ± 0.92	19.36 ± 1.27
II5	10.02 ± 0.52	2.46 ± 0.02	8.28 ± 0.36
II6	42.61 ± 1.26	32.23 ± 2.13	56.82 ± 1.02
II7	26.12 ± 1.14	27.38 ± 0.48	12.80 ± 1.69
II8	38.90 ± 1.31	48.36 ± 0.91	41.33 ± 0.78
II9	18.61 ± 0.36	19.05 ± 1.17	34. 54 ± 0.77
II10	11.12 ± 1.27	12.78 ± 0.91	14.45 ± 0.69
II11	28.78 ± 0.30	36.12 ± 0.90	28.37 ± 0.81
II12	27.81 ± 0.98	18.34 ± 1.37	47.93 ± 1.29
II13	29.82 ± 0.28	24.46 ± 0.52	19.48 ± 0.48
II14	36.38 ± 1.22	38.17 ± 1.34	41.32 ± 1.99
II15	15.42 ± 0.96	11.74 ± 0.42	11.46 ± 0.30
II16	10.71 ± 0.81	12.12 ± 1.62	31.66 ± 0.58
II17	18.25 ± 1.08	19.08 ± 0.67	32.36 ± 0.35
II18	>100	29.54 ± 1.53	19.44 ± 1.09
3[18]	35.47 ± 1.32	24.39 ± 1.52	6.90 ± 1.08
5-FU	10.30 ± 0.83	9.84 ± 0.75	7.21 ± 1.04

[a] Antiproliferative 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 from the dose–response curves of three independent experiments.



Scheme 2. Summary of SAR of chalcone–dithiocarbamate derivatives.

growth inhibition activity against SK-N-SH with IC_{50} values of 2.03 μM and 2.46 μM, respectively.

Compounds **II1**–**II4**, **II9**–**II11** and **II18** with different substituents on the aryl ring were synthesized and evaluated for their cytotoxicity. Compound **II2** with a 3,4,5-trimethoxy group showed the most potent activity against SK-N-SH cells, with an IC_{50} of 2.03 μM, which is 15 times higher than compound **II1** with a halogen atom Cl on the phenyl ring. In addition, compound **II10** with a 3,4,5-trimethoxy group was better than compound **II9** with a 4-Cl and compound **II11** with a 3,5-diF for the inhibitory activity against SK-N-SH cells. For SK-N-SH cells and MGC-803 cells, analogs **II3** substituted by 3,5-diF exhibited more potent antiproliferative activity than **II4** substituted by 3,5-diCl. Meanwhile, replacing 3,4,5-trimethoxy group with electron-donating group 4-methoxy led to a complete loss of potency against EC-109 cells (**II2** vs **II18**). From the biological data of compounds **II1**–**II4**, **II9**–**II11**, and **II18**, we can conclude that the substituent on the phenyl group of chalcone had a remarkable effect on their cytotoxic activity.

The importance of the aromatic ring of chalcone scaffold in the anticancer activity was also explored. Replacing the phenyl scaffold of compound **II16** with thiophene ring of **II17** led to a loss of

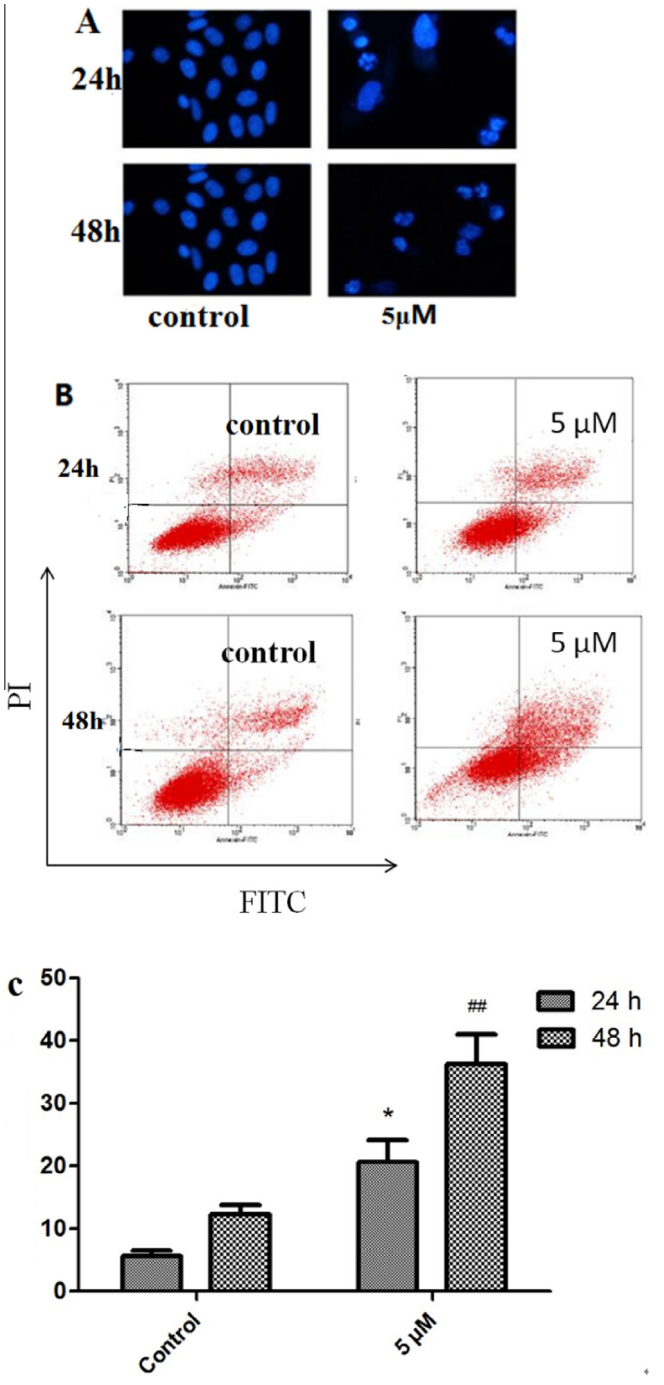


Figure 3. Compound **II2** induced apoptosis in SK-N-SH cells. (A) Apoptosis analysis with Hoechst-33258 staining after 24 h, 48 h treatment of **II2** in SK-N-SH cells. (B and C) Quantitative analysis of apoptotic cells using annexin V-FITC/PI double staining and flow-cytometry calculation. (*) P <0.05 was considered statistically highly significant. (##) P <0.01 was considered statistically highly significant. Data are the mean ± SD. All experiments were carried out at least three times.

activity. However, replacing the phenyl scaffold of compound **II11** with a pyridine ring of **II13** led to an increase of activity. The results revealed that the aromatic rings of chalcones had a profound effect on the activity. Structure activity relationship analysis showed that different dithiocarbamates effected the antitumor activity, for example, dithiocarbamate contained 1-methylpiperazine of compound **II2** was more active than dithiocarbamate contained pyrrolidine of compound **II10** against SK-N-SH cells. Based on the above findings, we further tested whether the length of

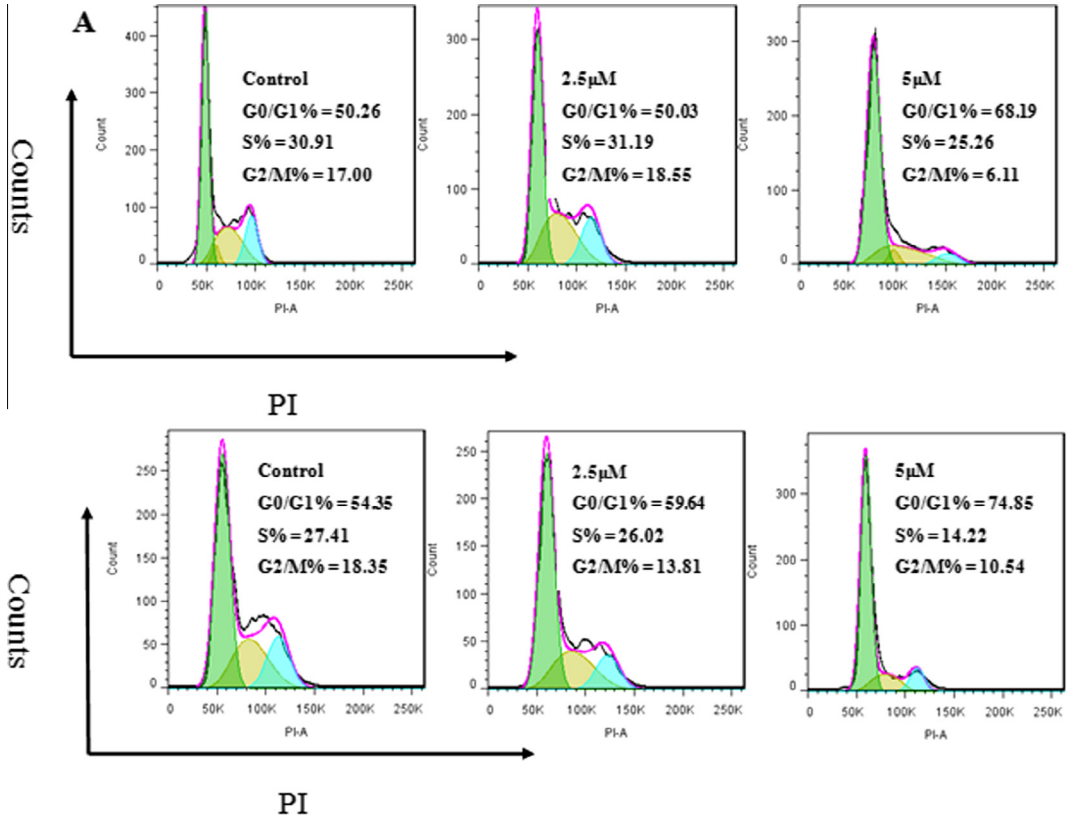


Figure 4. Effect of compound **II2** on the cell cycle distribution of SK-N-SH cells. Cells were treated with different concentrations (2.5 μM, 5 μM) for 24 h or 48 h. Then the cells were fixed and stained with PI to analyze DNA content by flow cytometry. (A) Incubated for 24 h; (B) incubated for 48 h. The experiments were performed three times, and a representative experiment is shown.

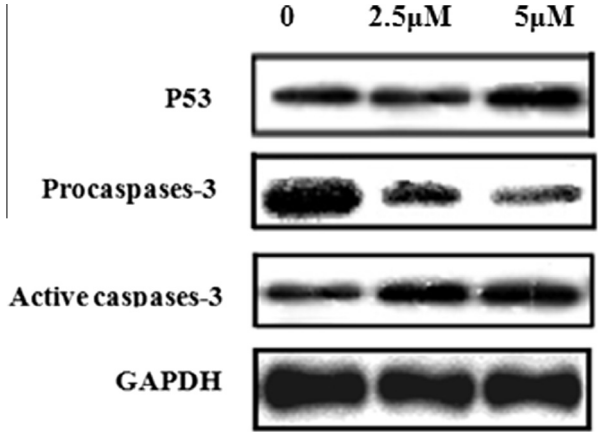


Figure 5. Expression of p53, pro-caspases 3 and Active-caspase 3 in SK-N-SH cells after treatment by compound **II2** for 48 h.

carbon linker between the dithiocarbamates and chalcones affect the anticancer activity. With reduction of the carbon tether length by one carbon (**II2** vs **II5**, **II6** vs **II7**, **II9** vs **II12**, **II10** vs **II16**), an obvious increase of potency was not observed.

Compounds **II2**, **II5** were further examined for possible cytotoxicity against GES-1 (normal human gastric epithelial cell line). We found that compounds **II2**, **II5** exhibited no cytotoxicity against GES-1 (>64 μM, >64 μM, respectively). However, compounds **II2**, **II5** exhibited potent cytotoxicity against the selected MGC-803 cell line (2.03 μM, 2.46 μM, respectively). The results indicated that compounds **II2**, **II5** had good selectivity between the selected cancer cell line (MGC-803) and a normal cell line (GES-1). The detailed illustration for structure activity relationship of target derivatives was showed in Scheme 2.

To explore cytotoxicity of **II2** in SK-N-SH, cell apoptosis was investigated with Hoechst 33258 staining. After 24 h and 48 h incubation with **II2** at indicated concentration, characteristic apoptotic morphological changes were observed by fluorescence microscope, including cell rounding, chromatin shrinkage, and formation of apoptotic bodies (Fig. 3A). The apoptotic analysis was also performed with annexin V-FITC/PI double staining and quantitated by flow cytometry. Compound **II2** treatment of SK-N-SH cells time dependently increased the percentage of the apoptotic population up to 20.6% and 36.8%, respectively, compared to control (Fig. 3B and C).

To have a better understanding of the mechanism of action of cytotoxic activity of compound **II2**, a cell-cycle cytotoxicity assay was performed by treating SK-N-SH cells with different concentrations with compound **II2** (2.5 μM, 5 μM). After treatment SK-N-SH cells for 24 h, it was observed that the percentage of cells in G0/G1 phase at different concentrations were 50.03% and 68.19%, respectively (Fig. 4A), whereas treatment for 48 h, the percentage of cells in G0/G1 phase were 59.64% and 74.85%, respectively (Fig. 4B). The results suggested that **II2** caused an obvious G0/G1 arrest pattern in a concentration- and time-dependent manner with a concomitant decrease in terms of the number of cells in other phases of the cell cycle. Detailed activity evaluation and apoptosis mechanism studies are currently underway in our laboratory.

At the same time, we also evaluated the level of p53, caspases 3 and pro-caspase 3 (inactive form of apoptotic effector caspase 3) in SK-N-SH cells (Fig. 5). Cleavage of pro-caspases 3 results in the production of an active effector (caspase 3), which can cleave essential

structural proteins such as cytokeratins, nuclear lamins, and also an inhibitor of caspase-activated DNase (iCAD), which liberates the DNase (CAD) to digest chromosomal DNA and cause cell death.[19] We can see from the result that compound **II2** dramatically increased the level of p53 and reduced the level of procaspase 3, which was most likely due to the fact that procaspase 3 was cleaved to the active form (caspase 3), which could promote apoptosis of SK-N-SH.

In summary, we designed and synthesized a series of chalcone–dithiocarbamate analogues displaying high activity against the proliferation of different cancer cells in vitro. Particularly, compound **II2** exhibited excellent growth inhibition against SK-N-SH with an IC_{50} value of 2.03 μM. The mechanism may be related with inducing apoptosis and arresting the cell cycle at G0/G1 phase. The further mechanism investigations are under way and will be reported in due course.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.07.012.

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