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Original article

Synthesis and antitumor activity of novel dithiocarbamate substituted chromones

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ABSTRACT

A series of chromone derivatives bearing diverse dithiocarbamate moieties were designed and synthesized *via* a three-component reaction protocol. Their *in vitro* antitumor activities were evaluated by MTT method against HCCLM-7, Hela, MDA-MB-435S, SW-480, Hep-2 and MCF-7. Two compounds (3-chloro-4-oxo-4*H*-chromen-2-yl)methyl piperidine-1-carbodithioate (**Iq**) and (6-chloro-4-oxo-4*H*-chromen-3-yl)methyl piperidine-1-carbodithioate (**II**u), were identified as the most promising candidate due to their high potency and broad-spectrum. Further flow-activated cell sorting analysis revealed that compounds **Iq** and **II**u arrest the cell cycle of SW-480 and MDA-MB-435s both in G_2/M phase with dose-dependent effect and might display apoptosis-inducing effect on these tumor cell lines.

1. Introduction

As one of the most representative families of plant secondary metabolites, flavonoids have been found to be associated with a remarkable spectrum of biological activities [1–3]. In last decades, medicinal chemists have paid great attentions on the isolation, screening and structural modifications of new flavonoids. Most interestingly, it has been found recently that some flavonoids displayed anticancer activity with novel mechanisms, such as carcinogen inactivation, antiproliferation, cell cycle arrest, induction of apoptosis and differentiation, inhibition of angiogenesis, antioxidation and reversal of multidrug resistance [4]. To date, some flavonoids with novel action mechanism have entered clinical trials. For example, as shown in Fig. 1, flavopiridol was identified as the first cyclin-dependent kinase inhibitor and entered Phase II clinical trials [5].

Dithiocarbamate (DTC) derivatives are well known as organic intermediates, rubber additive, additive of polluted water, vulcanizing agents and fungicides [6]. As shown in Fig. 1, for example, DSF, an irreversible inhibitor of aldehyde dehydrogenase, is one of the two drugs approved by FDA for treatment of alcoholism [7]. Clinical trials have shown the efficacy of DSF without toxicity. In clinical trials DDTC was used in patients with HIV-1 infection and found to delay progression to AIDS. PDTC is a stable pyrrolidine derivative of dithiocarbamates and an antioxidant. Previous studies have shown that PDTC strongly inhibits replication of human rhinoviruses and coxsackievirus myocarditis. PDTC also showed inhibitory ability against murine colon adenocarcinoma bearing mice through the inhibition of nuclear factor κB in the tumor tissue [6,7]. Recently, Dou et al. reported that the DSF–Cu complex showed inhibition of proteasome activity and induction of apoptotic cell death [7].

Based on the above considerations, we proposed that chromones bearing DTC moiety should display some interesting anticancer activity. Therefore, we designed compounds **I**, **II**, **III** and **IV** as shown in Fig. 2 with the aim to discover lead structure with anticancer activity. Herein, we described the detailed synthetic route, screening results and structure–activity relationships of these designed compounds. Fortunately, two compounds with promising broad-spectrum anticancer activity were identified.

2. Results and discussion

2.1. Chemistry

The synthetic route for compound **I** is outlined in Scheme 1. According to the reported procedures [8,9], 2-methyl chromone **1** underwent bromination reaction with NBS to give the desired 2-bromomethyl chromone **2** in yields of 70–82%. Then, at the presence of potassium phosphate as base [10], a subsequent three-component reaction of the corresponding amine, carbon disulfide and the intermediate **2** produced compound **I** in good yields

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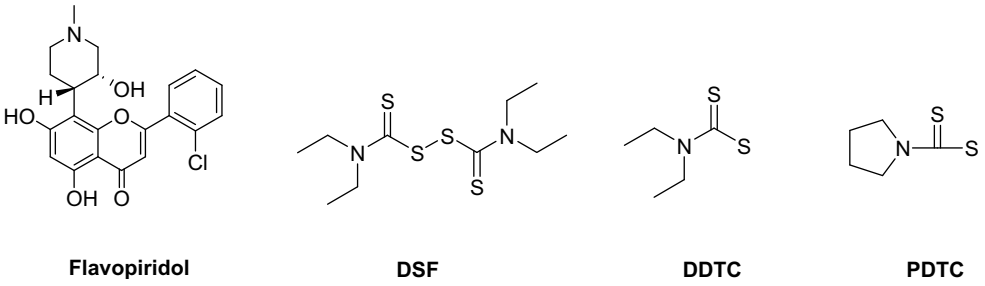


Fig. 1. Structures of flavopiridol and some representative dithiocarbamates.

(72–87%). As shown in Schemes 2 and 3, compounds **II** and **III** could be easily prepared by the similar three-component reaction using 3-cholоromethyl chromone **3** and 3-bromochromone **4** as substrate, respectively [11,12]. It should be noted that sodium methoxide rather than potassium phosphate was used as base for the three-component reaction of 3-bromochromone **4**. Scheme 4 showed the synthetic route of compound **IV**. The bromination of the starting material **5** with NBS afforded 6-bromomethyl chromone **6** smoothly in a yield of 80% [9]. Then, three-component reaction of the corresponding amine, carbon disulfide and intermediate **6** produced the desired compound **IV** in good yields (82–88%) when potassium phosphate was used as base.

The structures of all the target compounds were characterized by elemental analyses, ^{1}H NMR and EI-MS spectrum. In addition, the crystal structures of **I**c, **II**s and **III**b were determined by X-ray diffraction analyses [13]. As shown in Fig. 3, it can be found that the special tropism of DTC side chain shows big difference. The dihedral angles between the pyrone ring and DTC plane are 75.05° (**I**c), 36.61° (**II**s) and 78.67° (**III**b), respectively. The distance between the centroids of pyrone ring and DTC unit are 4.46 Å (**I**c), 4.65 Å (**II**s) and 3.91 Å (**III**b), respectively.

2.2. Pharmacology

The *in vitro* antiproliferative activities of the synthesized compounds **I**, **II**, **III**, and **IV** against six cancer cell lines, including HCCLM-7 (hepatoma carcinoma cell), Hela (cervical carcinoma cell), MDA-MB-435S (mammary adenocarcinoma cell), SW-480 (colon carcinoma cell), Hep-2 (laryngocarcinoma cell) and MCF-7 (mammary adenocarcinoma cell), were assayed by MTT method [14] and the results expressed as IC_{50} were summarized in Table 1.

As shown in Table 1, the substituent on benzene ring of compound **I** displayed remarkable effect on the antitumor activity. For the derivatives bearing the same DTC moiety, introduction of methoxyl group onto the position-7 always improved significantly the antitumor activity, such as compounds **I**a and **I**f, **I**c and **I**g, **I**d and **I**h. However, introduction of Cl atom onto the position-7 did not improve the activity. However, most interestingly, some compounds bearing a chlorine atom at 3-position were found to display good antitumor activities with broad-spectrum. For example, the IC_{50} values of compound **I**p range from 0.6 μM against Hep-2 to 2.3 μM against MCF-7. The IC_{50} values of compound **I**q are 0.77 μM, 0.69 μM, 0.94 μM and 1.0 μM against MDA-MB-435s, SW-480, Hep-2 and MCF-7, respectively.

In most cases, compound **II** except for **II**h and **II**i displayed higher antitumor activity than the corresponding compounds **I**. For example, compound **II**a and **I**a (R = H, D = D_2), **II**b and **I**b (R = H, D = D_3), **II**c and **I**c (R = H, D = D_4), **II**d and **I**d (R = H, D = D_5), **II**j and **I**f (R = 7-OMe, D = D_2), **II**l and **I**g (R = 7-OMe, D = D_4), **II**m and **I**h (R = 7-OMe, D = D_5), **II**t and **I**k (R = 7-OMe, D = D_3), **II**u and **I**l (R = 7-OMe, D = D_4), **II**v and **I**m (R = 7-OMe, D = D_5). Most fortunately, compound **II**u displayed excellent antitumor activity with broad-spectrum. Its IC_{50} values against the tested six cell lines are all under 1.0 μM, identified itself as the most promising candidate. However, after the bridge CH_2 group between the DTC moiety and the chromone ring of compound **II** was removed, the antitumor activity of the resulted compound **III** reduced significantly, indicating that the flexibility of the DTC group might be a vital factor for the activity. In addition, when the DTC moiety was moved onto

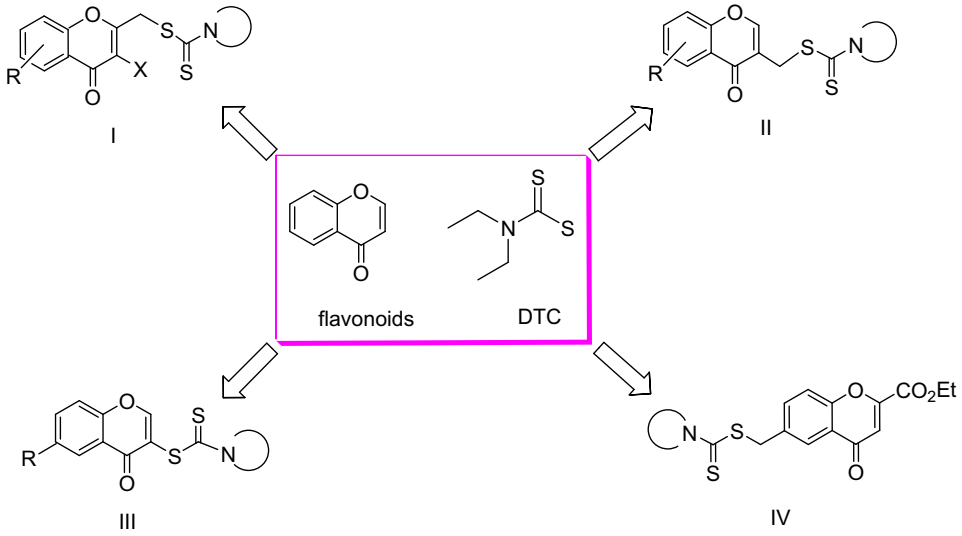
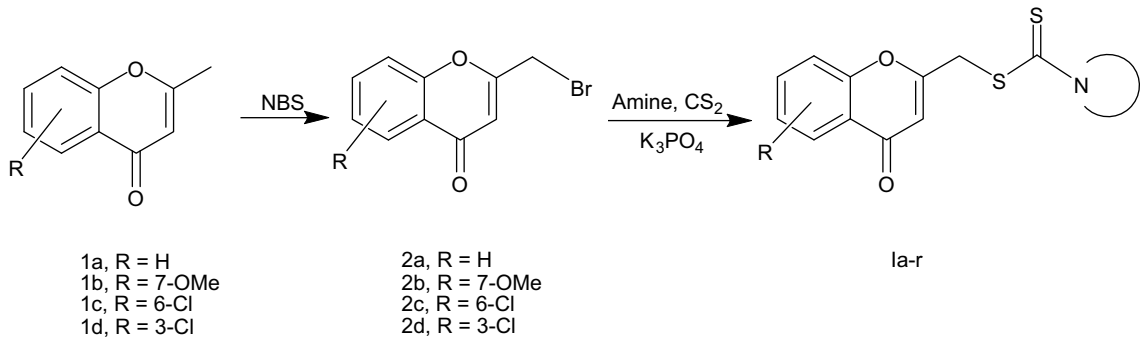
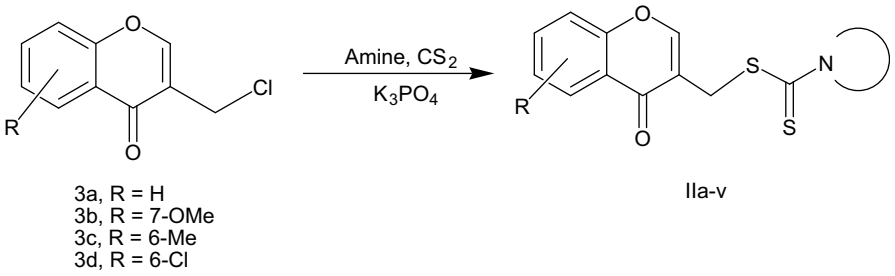


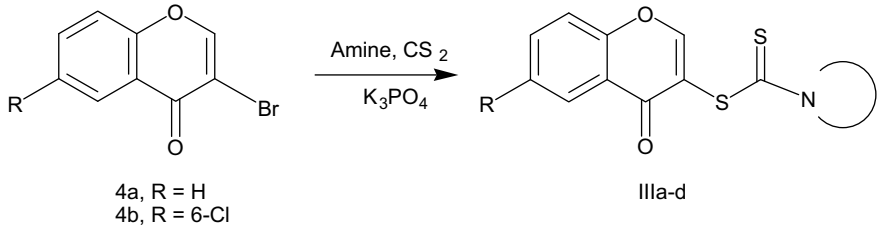
Fig. 2. Design of the title compounds.



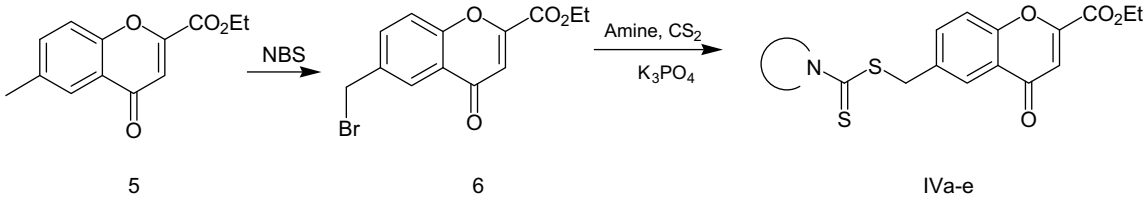
Scheme 1. Synthesis of 2-DTC chromones **I**.



Scheme 2. Synthesis of 3-DTC chromones **II**.



Scheme 3. Synthesis of 3-DTC chromones **III**.



Scheme 4. Synthesis of 6-DTC chromones **IV**.

position-6 of chromone ring, the antitumor activities of the resulted compound **IV** were reduced significantly.

In addition, from the results as shown in Table 1, we can also conclude that the bulky DTC moieties, such as D_7, D_8, and D_9, are not favorable for the antitumor activity. In addition, although its mechanism is not clear, D_{10} and D_{11} also seemed to display negative effect on the antitumor activity. For example, compounds bearing D_{10} and D_{11} moiety always displayed lower activity, such as compounds **I**e, **II**i, **II**r, and **IV**e.

To study the effect of the synthesized compounds on cell cycle progression, flow-activated cell sorting (FACS) analysis was performed [15]. The most promising compounds **I**q and **II**u were tested against SW-480 and MDA-MB-435s cell lines respectively at three different concentrations according the MTT assay. Fig. 4 and Table 2 show the results after 48 h treatment for **I**q and **II**u, respectively. As shown in Fig. 4 and Table 2, compound **I**q arrest the cell cycle of SW-480 in G_2/M phase with dose-dependent effect, raising the G_2/M peak from 15.00% to 27.77% (0.5 μM), 27.96% (0.7 μM) and 39.27% (1.0 μM) after 48 h treatment. Similarly, compound **II**u arrest the cell cycle of MDA-MB-435s in G_2/M phase with dose-dependent effect, raising the G_2/M peak from 8.72% to 13.89% (0.12 μM), 20.62% (0.13 μM) and 24.87% (0.14 μM) after 48 h

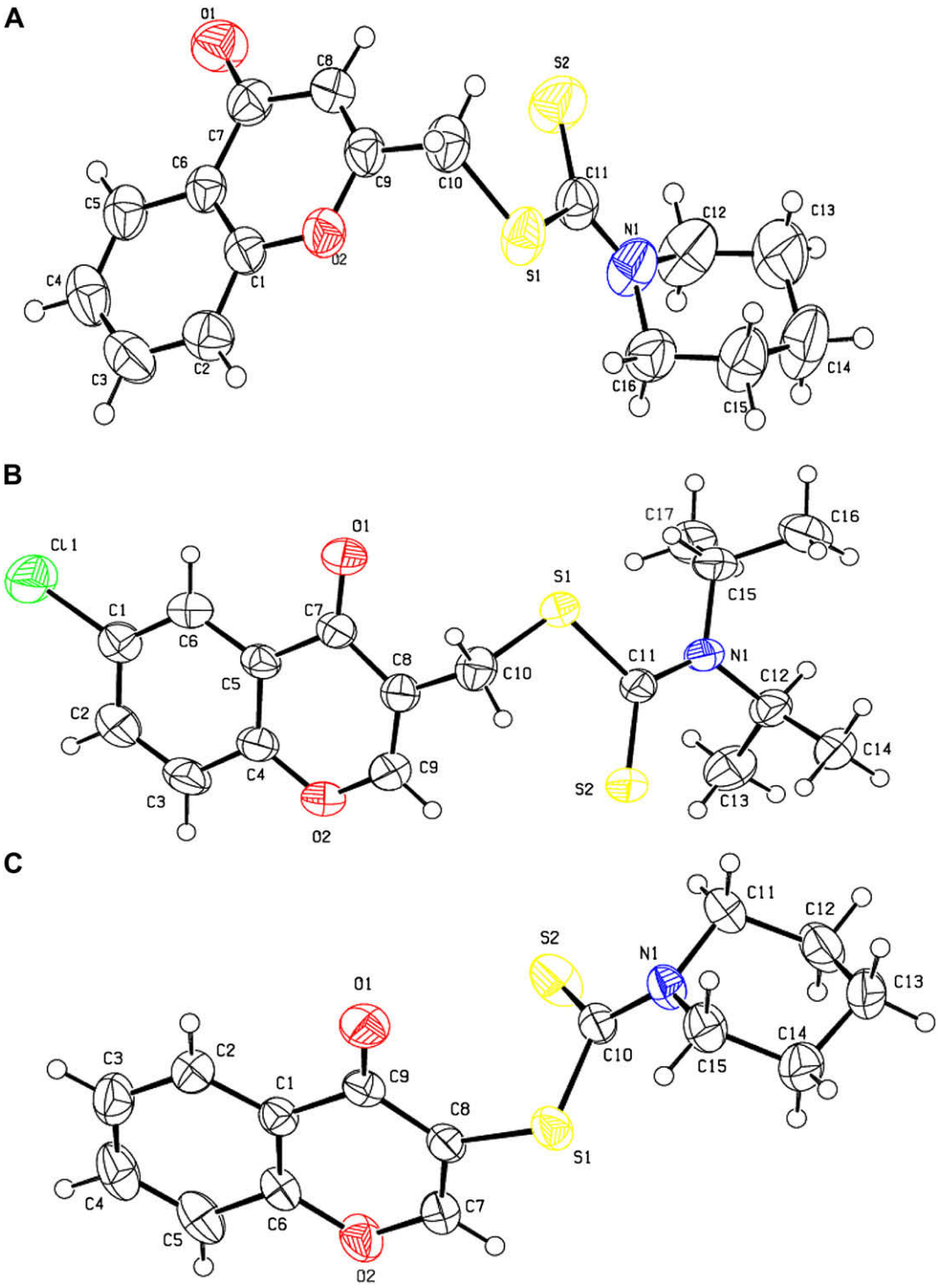


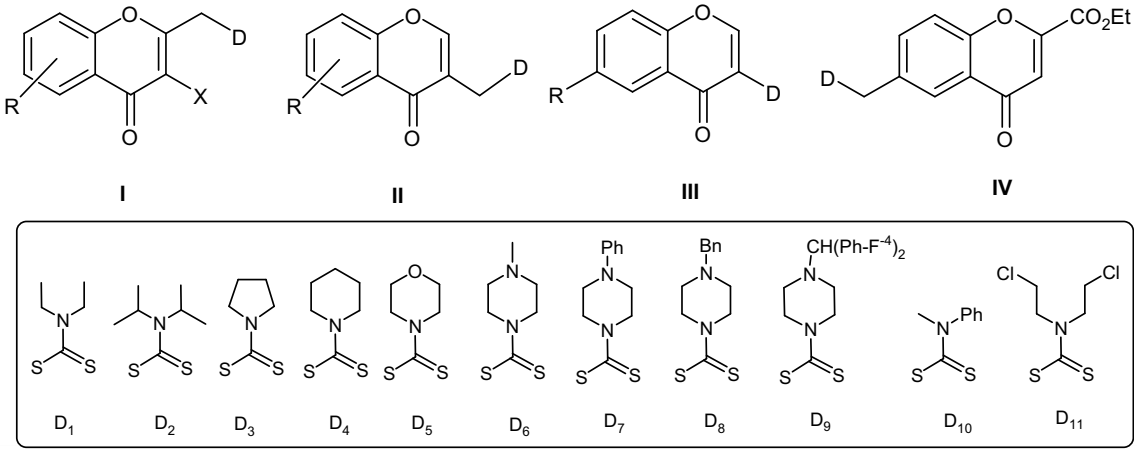
Fig. 3. X-ray crystal structures of **I**c (A), **II**s (B) and **III**b (C).

treatment. Based on the results of FACS analysis, we can conclude compounds **I**q and **II**u might display apoptosis-inducing effect on SW-480 and MDA-MB-435s cell lines, respectively.

3. Conclusion

In conclusion, a series of chromones bearing diverse dithiocarbamate moieties were designed and synthesized using a three-component reaction protocol. Based on the result of MTT assay, two compounds (3-chloro-4-oxo-4*H*-chromen-2-yl)methyl piperidine-1-carbodithioate (**I**q) and (6-chloro-4-oxo-4*H*-chromen-3-yl)-methyl piperidine-1-carbodithioate (**II**u), were identified as the most promising candidate due to their high potency and broad-spectrum. Further flow-activated cell sorting analysis revealed that compounds **I**q and **II**u arrest respectively the cell cycle of SW-480 and MDA-MB-435s in G_2/M phase with dose-dependent effect and might display apoptosis-inducing effect on these tumor cell lines.

Table 1
Antiproliferative activity of the DTC–chromone derivatives.



N.O.	R	X	D	Antiproliferative activity (IC_{50},[a] μM)					
				LM-7	Hela	MDA-MB-435s	SW-480	Hep-2	MCF-7
Ia	H	H	D_2	>25	–	>40	>40	13.6	31.7
Ib	H	H	D_3	40.0	23.1	>30	23.4	9.1	35.8
Ic	H	H	D_4	>25	>25	>25	13.7	12.0	>25
Id	H	H	D_5	25.2	35.6	22.2	24.4	>25	21.0
Ie	H	H	D_{11}	>25	>25	3.7	25.1	15.6	>25
If	7-OMe	H	D_2	19.1	>25	22.1	7.2	7.6	>25
Ig	7-OMe	H	D_4	5.0	11.9	1.5	2.3	1.5	10.0
Ih	7-OMe	H	D_5	11.0	>25	3.4	3.1	3.0	>25
Ii	6-Cl	H	D_1	8.8	8.7	23.5	>25	8.8	>25
Ij	6-Cl	H	D_2	>30	>30	>25	>25	22.7	>25
Ik	6-Cl	H	D_3	>25	>40	>25	>25	>40	>25
Il	6-Cl	H	D_4	13.8	>25	3.0	2.5	1.6	>25
Im	6-Cl	H	D_5	>25	33.3	>25	>25	20.6	>25
In	6-Cl	H	D_7	15.9	25.9	18.6	21.2	15.9	>25
Io	H	Cl	D_2	>40	>40	23.6	18.1	20.0	>40
Ip	H	Cl	D_3	1.9	0.7	1.8	1.4	0.6	2.3
Iq	H	Cl	D_4	3.3	9.8	0.77	0.69	0.94	1.0
Ir	H	Cl	D_5	19.2	4.5	4.1	1.8	9.0	11.1
IIa	H	–	D_2	21.9	>30	7.9	6.8	3.0	>30
IIb	H	–	D_3	6.0	>25	3.8	2.3	3.6	>25
IIc	H	–	D_4	4.6	2.3	0.47	1.2	0.39	1.7
IId	H	–	D_5	12.0	–	3.9	5.9	4.4	4.0
IIe	H	–	D_6	17.6	5.9	2.2	–	2.4	22.9
IIf	H	–	D_7	2.9	7.1	9.7	5.2	16.7	>30
IIg	H	–	D_8	19.1	7.9	4.4	4.5	2.9	9.6
IIh	H	–	D_9	>25	>25	>40	>40	9.6	>30
IIi	H	–	D_{10}	>25	>25	>25	>25	>25	>25
IIj	7-OMe	–	D_2	4.7	5.4	1.8	4.8	8.9	>40
IIk	7-OMe	–	D_3	6.0	12.1	1.8	4.8	3.1	34.1
IIl	7-OMe	–	D_4	4.7	3.5	0.93	6.7	4.3	>40
IIm	7-OMe	–	D_5	>40	9.5	>40	7.1	4.8	11.8
IIn	6-Me	–	D_2	6.3	1.4	0.24	>25	0.70	6.6
IIo	6-Me	–	D_3	3.4	4.4	1.9	5.9	3.6	41.4
IIp	6-Me	–	D_4	5.1	>25	1.7	>25	0.9	0.8
IIq	6-Me	–	D_5	14.3	6.1	5.5	3.8	4.0	20.7
IIr	6-Me	–	D_{11}	14.5	23.3	6.7	18.7	8.0	16.2
IIs	6-Cl	–	D_2	>25	>25	>25	>25	4.2	–
IIt	6-Cl	–	D_3	2.6	1.3	6.4	7.8	2.1	0.60
IIu	6-Cl	–	D_4	0.85	0.44	0.24	0.79	0.28	0.38
IIv	6-Cl	–	D_5	6.2	2.0	0.6	6.2	0.9	8.1
IIIa	H	–	D_3	–	3.8	1.9	>25	7.4	12.2
IIIb	H	–	D_4	7.8	9.0	17.4	16.3	5.8	–
IIIc	Cl	–	D_3	24.6	16.3	24.0	>25	26.0	>25
IIId	Cl	–	D_4	>25	8.3	13.3	>25	12.2	>25
IVa	–	–	D_2	>25	>25	8.1	>25	15.9	>25
IVb	–	–	D_3	18.0	6.6	3.4	>25	8.9	>25
IVc	–	–	D_4	16.9	5.8	2.9	>25	3.4	21.9
IVd	–	–	D_5	>25	>25	>25	>25	>25	>25
IVe	–	–	D_{10}	>25	18.1	13.5	>25	–	>25

[a] The IC_{50} values represent the concentration resulting in a 50% decrease in cell growth after 72 h incubation, which were the mean values of three repeated experiments.

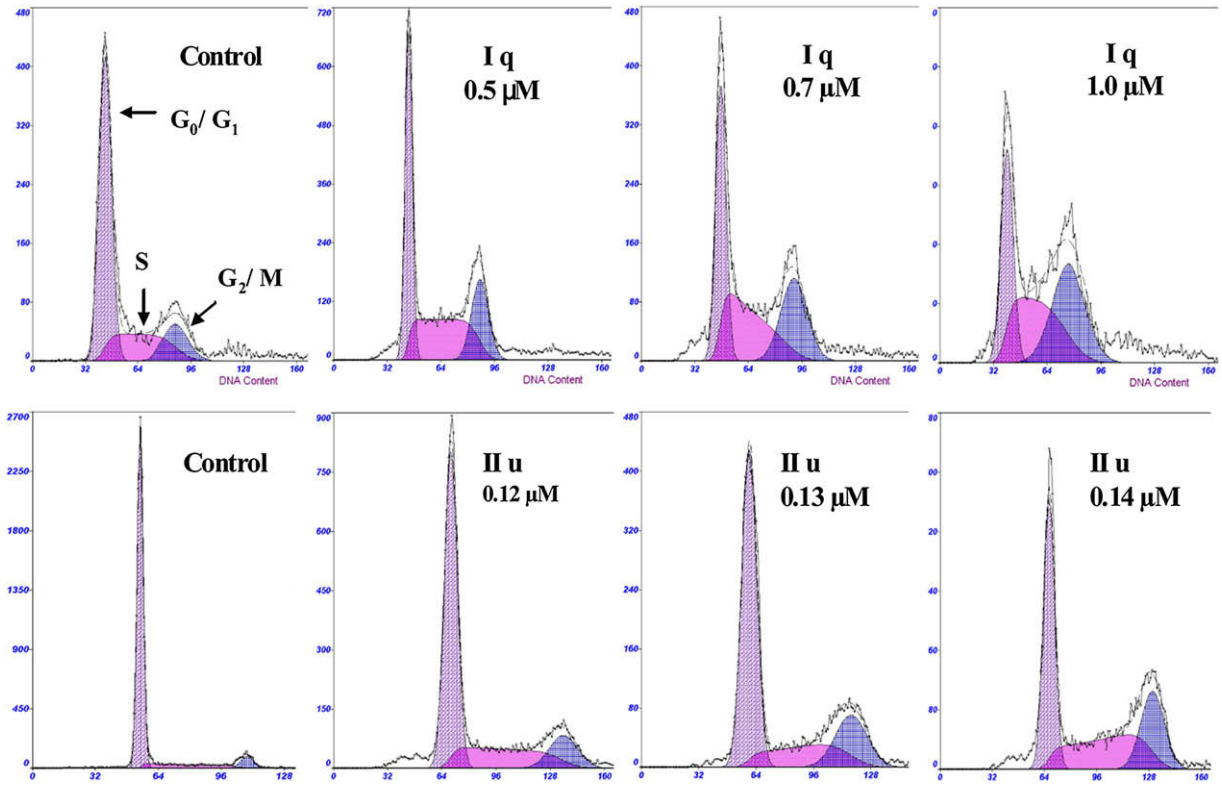


Fig. 4. Compounds **I**q and **II**u induced apoptosis in sw-480 cells and MDA-MS-435s respectively.

4. Experimental protocol

4.1. Chemistry

4.1.1. General methods

^{1}H NMR spectra were recorded at 400 MHz, in $CDCl_3$ solution on a Mercury-Plus400 spectrometer and chemical shifts were recorded in parts per million (ppm) with TMS as the internal reference. MS spectra were determined using a Tracems2000 organic mass spectrometry, and signals were given in *m*/*z*. Melting points were taken on a Buchi B-545 melting point apparatus. Element analysis (EA) was measured on a Vario ELIII CHNSO elemental analyzer. All commercially available solvents and reagents were used as supplied by Acros Organics unless otherwise stated. The silica gel (200–300 meshes) for flash column chromatography was from Qingdao Marine Chemical Factory in China.

4.1.2. General procedure for synthesis of *I*

The intermediate 2-methyl chromone **1** was synthesized according to the reported method [8]. The CCl_4 solution (20 mL) of **1** (5 mmol), NBS (5 mmol) and AIBN (0.1 mmol) was irradiated with 200 W lamp. After the reaction completed, the mixture was cooled to room temperature and filtrated. The filtrate was concentrated in vacuo. The crude product was chromatographed on silica gel with acetone/petroleum (15/85), giving the **2** in 70–82% yield [9].

Table 2
Effects of **I**q and **II**u on cell cycle progression.

N.O.	**I**q to sw-480				**II**u to MDA-MB-435s			
	Control	0.5 μM	0.7 μM	1.0 μM	Control	0.12 μM	0.13 μM	0.14 μM
G_0/G_1	62.28	39.39	36.84	26.51	83.62	60.82	58.68	41.57
S	22.72	37.84	35.20	34.22	7.62	25.29	20.70	33.56
G_2/M	15.00	27.77	27.96	39.27	8.72	13.89	20.62	24.87

2-Methyl-4*H*-chromen-4-one (**1**a): Mp 70–72 °C (Ref. [8d]: 72–73 °C) ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.33$ (s, 3H, CH_3), 6.87 (s, 1H, 3-CH), 7.47 (t, $J = 7.2$ Hz, 1H, ArH), 7.55 (d, $J = 8.4$ Hz, 1H, ArH), 7.71 (t, $J = 8.4$ Hz, 1H, ArH), 7.92 (d, $J = 7.2$ Hz, 1H, ArH); Reported: 2.36 (s, 3H, CH_3), 6.90 (s, 1H, 3-CH), 7.46–7.74 (m, 3H, ArH), 7.75–7.95 (m, 1H, ArH).

7-Methoxy-2-methyl-4*H*-chromen-4-one (**1**b): Mp 95–97 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.97$ (s, 3H, CH_3), 3.91 (s, 3H, OCH_3), 6.19 (s, 1H, 3-CH), 6.74 (d, $J = 8.8$ Hz, 1H, ArH), 7.21 (s, 1H, ArCH), 7.98 (d, $J = 8.8$ Hz, 1H, ArH).

6-Chloro-2-methyl-4*H*-chromen-4-one (**1**c): Mp 116–118 °C (Ref. [8d]: 114–116 °C); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.07$ (s, 3H, CH_3), 5.30 (s, 1H, 3-CH), 7.39 (d, $J = 9.2$ Hz, 1H, ArH), 7.47 (d, $J = 9.2$ Hz, 1H, ArH), 7.89 (s, 1H, ArCH); Reported: 2.10 (s, 3H, CH_3), 5.30 (s, 1H, 3-CH), 7.41 (d, $J = 9.2$ Hz, 1H, ArH), 7.49 (d, $J = 9.2$ Hz, 1H, ArH), 7.91 (s, 1H, ArCH).

3-Chloro-2-methyl-4*H*-chromen-4-one (**1**d): Mp 115–117 °C (Ref. [8e]: 118 °C); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.60$ (s, 3H, CH_3), 7.12 (t, $J = 8.0$ Hz, 1H, ArH), 7.28 (d, $J = 8.4$ Hz, 1H, ArH), 7.43 (t, $J = 8.0$ Hz, 1H, ArH), 8.20 (d, $J = 8.0$ Hz, 1H, ArH); Reported: 2.58 (s, 3H, CH_3), 7.07–7.45 (m, 3H, ArH), 8.21 (m, 1H, ArH).

2-(Bromomethyl)-4*H*-chromen-4-one (**2**a): Mp 98–99 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 4.43$ (s, 2H, CH_2), 6.55 (s, 1H, 3-CH), 7.36 (t, $J = 7.2$ Hz, 1H, ArH), 7.45 (d, $J = 8.4$ Hz, 1H, ArH), 7.66 (t, $J = 8.4$ Hz, 1H, ArH), 8.14 (d, $J = 7.2$ Hz, 1H, ArH); Ms (*m*/*z*) 239 (M^+).

2-(Bromomethyl)-7-methoxy-4*H*-chromen-4-one (**2**b): Mp 102–103 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 3.91$ (s, 3H, CH_3), 4.27 (s, 2H, CH_2), 6.43 (s, 1H, 3-CH), 6.89 (s, 1H, ArH), 6.99 (d, $J = 9.2$ Hz, 1H, ArH), 8.08 (d, $J = 8.8$ Hz, 1H, ArCH); Ms (*m*/*z*) 268 (M^+).

2-(Bromomethyl)-6-chloro-4*H*-chromen-4-one (**2**c): Mp 110–112 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 4.54$ (s, 2H, CH_2), 6.68

(s, 1H, 3-CH), 7.34 (d, $J = 9.2$ Hz, 1H, ArH), 7.58 (d, $J = 9.2$ Hz, 1H, ArH), 7.73 (s, 1H, ArCH); Ms (m/z) 272 (M^+).

2-(Bromomethyl)-3-chloro-4*H*-chromen-4-one (**2**d): Mp 105–107 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 4.82$ (s, 2H, CH_2), 7.41 (t, $J = 8.0$ Hz, 1H, ArH), 7.57 (d, $J = 8.4$ Hz, 1H, ArH), 7.73 (t, $J = 8.0$ Hz, 1H, ArH), 8.20 (d, $J = 8.0$ Hz, 1H, ArH); Ms (m/z) 272 (M^+).

To a solution of amine (1 mmol) in DMF (2 mL) was added dropwise carbon disulfide (2 mmol) and anhydrous potassium phosphate (1 mmol). The resulted mixture was stirred at room temperature for 30 min. Then brominated chromone **2** (1 mmol) was added by one-portion and stirring was continued. After completion of the reaction (monitored by TLC), the mixture was diluted with ice-cold water (20 mL) and the precipitate was filtered, and recrystallized from ethanol to give the target compound **I**.

(4-Oxo-4*H*-chromen-2-yl)methyl diisopropylcarbamodithioate (**I**a): Mp 106–108 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.75$ (b, 12H, CH_3), 3.59 (b, 2H, iPr-CH), 4.64 (s, 2H, CH_2), 6.51 (s, 1H, 3-CH), 7.36 (t, $J = 7.2$ Hz, 1H, ArH), 7.45 (d, $J = 8.4$ Hz, 1H, ArH), 7.66 (t, $J = 8.4$ Hz, 1H, ArH), 8.14 (d, $J = 7.2$ Hz, 1H, ArH); Ms (m/z) 335 (M^+); Anal. calcd for $C_{17}H_{21}NO_2S_2$: C, 60.86; H, 6.31; N, 4.18. Found: C, 60.71; H, 6.50; N, 4.29.

(4-Oxo-4*H*-chromen-2-yl)methyl pyrrolidine-1-carbodithioate (**I**b): Mp 114–116 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.01$ (m, 2H, CH_2), 2.13 (m, 2H, CH_2), 3.71 (t, $J = 7.2$ Hz, 2H, CH_2), 3.96 (t, $J = 7.2$ Hz, 2H, CH_2), 4.64 (s, 2H, CH_2), 6.53 (s, 1H, 3-CH), 7.39 (t, $J = 7.2$ Hz, 1H, ArH), 7.46 (d, $J = 8.4$ Hz, 1H, ArH), 7.66 (t, $J = 8.4$ Hz, 1H, ArH), 8.18 (d, $J = 7.2$ Hz, 1H, ArH); Ms (m/z) 305 (M^+); Anal. calcd for $C_{15}H_{15}NO_2S_2$: C, 58.99; H, 4.95; N, 4.59. Found: C, 58.70; H, 5.05; N, 4.39.

(4-Oxo-4*H*-chromen-2-yl)methyl piperidine-1-carbodithioate (**I**c): Mp 120–122 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.76$ (b, 6H, CH_2), 3.92 (m, 2H, CH_2), 4.33 (m, 2H, CH_2), 4.65 (s, 2H, CH_2), 6.52 (s, 1H, 3-CH), 7.37 (t, $J = 7.2$ Hz, 1H, ArH), 7.45 (d, $J = 8.4$ Hz, 1H, ArH), 7.66 (t, $J = 8.4$ Hz, 1H, ArH), 8.18 (d, $J = 7.2$ Hz, 1H, ArH); Ms (m/z) 319 (M^+); Anal. calcd for $C_{16}H_{17}NO_2S_2$: C, 60.16; H, 5.36; N, 4.38. Found: C, 60.02; H, 5.51; N, 4.43.

(4-Oxo-4*H*-chromen-2-yl)methyl morpholine-4-carbodithioate (**I**d): Mp 131–134 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 3.79$ (b, 4H, CH_2), 3.96 (b, 2H, CH_2), 4.35 (b, 2H, CH_2), 4.64 (s, 2H, CH_2), 6.52 (s, 1H, 3-CH), 7.38 (t, $J = 7.2$ Hz, 1H, ArH), 7.46 (d, $J = 8.4$ Hz, 1H, ArH), 7.66 (t, $J = 8.4$ Hz, 1H, ArH), 8.18 (d, $J = 7.2$ Hz, 1H, ArH); Ms (m/z) 321 (M^+); Anal. calcd for $C_{15}H_{15}NO_3S_2$: C, 56.05; H, 4.70; N, 4.36. Found: C, 56.12; H, 4.81; N, 4.40.

(4-Oxo-4*H*-chromen-2-yl)methyl bis(2-chloroethyl)carbamodithioate (**I**e): Mp 142–144 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 3.26$ (t, $J = 7.2$ Hz, 2H, CH_2), 3.57 (b, 4H, CH_2), 3.73 (t, $J = 7.2$ Hz, 2H, CH_2), 4.49 (s, 2H, CH_2), 7.35 (d, $J = 8.4$ Hz, 1H, ArH), 7.49 (d, $J = 8.4$ Hz, 1H, ArH), 7.78 (t, $J = 8.8$ Hz, 1H, ArH), 7.94 (t, $J = 8.4$ Hz, 1H, ArH), 8.19 (s, 1H, 3-CH); Ms (m/z) 375 (M^+); Anal. calcd for $C_{15}H_{15}Cl_2NO_2S_2$: C, 47.87; H, 4.02; N, 3.72. Found: C, 47.60; H, 4.19; N, 3.84.

(7-Methoxy-4-oxo-4*H*-chromen-2-yl)methyl diisopropylcarbamodithioate (**I**f): Mp 121–123 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.22$–1.66 (b, 14H, i-Pr), 3.91 (s, 3H, CH_3), 4.60 (s, 2H, CH_2), 4.62 (s, 2H, CH_2), 6.43 (s, 1H, 3-CH), 6.86 (s, 1H, ArH), 6.96 (d, $J = 9.2$ Hz, 1H, ArH), 8.08 (d, $J = 8.8$ Hz, 1H, ArCH); Ms (m/z) 365 (M^+); Anal. calcd for $C_{18}H_{23}NO_3S_2$: C, 59.15; H, 6.34; N, 3.83. Found: C, 59.02; H, 6.40; N, 3.76.

(7-Methoxy-4-oxo-4*H*-chromen-2-yl)methyl piperidine-1-carbodithioate (**I**g): Mp 130–132 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.73$ (b, 6H, CH_2), 3.90 (m, 5H, OMe & CH_2), 4.30 (m, 2H, CH_2), 4.64 (s, 2H, CH_2), 6.45 (s, 1H, 3-CH), 6.86 (s, 1H, ArH), 6.96 (d, $J = 8.8$ Hz, 1H, ArH), 8.07 (d, $J = 9.2$ Hz, 1H, ArCH); Ms (m/z) 349 (M^+); Anal. calcd for $C_{17}H_{19}NO_3S_2$: C, 58.43; H, 5.48; N, 4.01. Found: C, 58.32; H, 5.60; N, 4.12.

(7-Methoxy-4-oxo-4*H*-chromen-2-yl)methyl morpholine-4-carbodithioate (**I**h): Mp 141–143 °C; ^{1}H NMR (400 MHz, $CDCl_3$): 3.79 (b, 4H, CH_2), 3.94–3.98 (b, 5H, OMe & CH_2), 4.35 (b, 2H, CH_2), 4.61 (s, 2H, CH_2), 6.46 (s, 1H, 3-CH), 6.86 (s, 1H, ArH), 6.96 (d, $J = 9.2$ Hz, 1H, ArH), 8.08 (d, $J = 8.8$ Hz, 1H, ArCH); Ms (m/z) 351 (M^+); Anal. calcd for $C_{16}H_{17}NO_4S_2$: C, 54.68; H, 4.88; N, 3.99. Found: C, 54.57; H, 4.968; N, 4.12.

(6-Chloro-4-oxo-4*H*-chromen-2-yl)methyl diethylcarbamodi thioate (**I**i): Mp 120–122 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.31$ (m, 6H, CH_3), 3.78 (m, 2H, CH_2), 4.04 (m, 2H, CH_2), 4.62 (s, 2H, CH_2), 6.52 (s, 1H, 3-CH), 7.42 (d, $J = 8.8$ Hz, 1H, ArH), 7.60 (d, $J = 8.8$ Hz, 1H, Het-CH), 8.12–8.15 (m, 2H, ArCH); Ms (m/z) 341 (M^+); Anal. calcd for $C_{15}H_{16}ClNO_2S_2$: C, 52.70; H, 4.72; N, 4.10. Found: C, 52.70; H, 4.72; N, 4.10.

(6-Chloro-4-oxo-4*H*-chromen-2-yl)methyl diisopropylcarbamodithioate (**I**j): Mp 104–106 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.20$–1.83 (b, 14H, i-Pr), 4.62 (s, 2H, CH_2), 6.52 (s, 1H, 3-CH), 7.43 (d, $J = 8.8$ Hz, 1H, ArH), 7.60 (d, $J = 8.8$ Hz, 1H, Het-CH), 8.14 (s, 1H, ArCH); Ms (m/z) 369 (M^+); Anal. calcd for $C_{17}H_{20}ClNO_2S_2$: C, 55.19; H, 5.45; N, 3.79. Found: C, 55.03; H, 5.61; N, 3.84.

(6-Chloro-4-oxo-4*H*-chromen-2-yl)methyl pyrrolidine-1-carbodithioate (**I**k): Mp 120–122 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.01$ (q, 2H, CH_2), 3.73 (q, 2H, CH_2), 3.71 (d, $J = 6.8$ Hz, 1H, CH_2), 3.94 (d, $J = 6.8$ Hz, 2H, CH_2), 4.62 (s, 2H, CH_2), 6.54 (s, 1H, 3-CH), 7.42 (d, $J = 9.2$ Hz, 1H, ArH), 7.60 (d, $J = 9.2$ Hz, 1H, Het-CH), 8.13 (s, 1H, ArCH); Ms (m/z) 339 (M^+); Anal. calcd for $C_{15}H_{14}ClNO_2S_2$: C, 53.01; H, 4.15; N, 4.12. Found: C, 52.89; H, 4.10; N, 4.22.

(6-Chloro-4-oxo-4*H*-chromen-2-yl)methyl piperidine-1-carbodithioate (**I**l): Mp 131–133 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.73$ (b, 6H, CH_2), 3.91 (b, 2H, CH_2), 4.29 (b, 2H, CH_2), 4.60 (s, 2H, CH_2), 6.51 (s, 1H, 3-CH), 7.39 (d, $J = 8.8$ Hz, 1H, ArH), 7.60 (d, $J = 8.8$ Hz, 1H, Het-CH), 8.14 (s, 1H, ArCH); Ms (m/z) 353 (M^+); Anal. calcd for $C_{16}H_{16}ClNO_2S_2$: C, 54.30; H, 4.56; N, 3.96. Found: C, 54.22; H, 4.71; N, 3.87.

(6-Chloro-4-oxo-4*H*-chromen-2-yl)methyl morpholine-4-carbodithioate (**I**m): Mp 135–137 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 3.80$ (b, 4H, CH_2), 3.98 (b, 2H, CH_2), 4.35 (b, 2H, CH_2), 4.64 (s, 2H, CH_2), 6.52 (s, 1H, 3-CH), 7.42 (d, $J = 10.4$ Hz, 1H, ArH), 7.60 (d, $J = 10.4$ Hz, 1H, ArH), 8.14 (s, 1H, ArCH); Ms (m/z) 355 (M^+); Anal. calcd for $C_{15}H_{14}ClNO_3S_2$: C, 50.63; H, 3.97; N, 3.94. Found: C, 50.47; H, 4.12; N, 4.05.

(6-Chloro-4-oxo-4*H*-chromen-2-yl)methyl 4-phenylpiperazine-1-carbodithioate (**I**n): Mp 163–165 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 3.31$ (b, 4H, CH_2), 4.13 (b, 2H, CH_2), 4.51 (b, 2H, CH_2), 4.65 (s, 2H, CH_2), 6.54 (s, 1H, 3-CH), 6.91–6.94 (m, 3H, ArH), 7.26–7.33 (m, 2H, ArH), 7.43 (d, $J = 9.2$ Hz, 1H, ArH), 7.61 (d, $J = 9.2$ Hz, 1H, ArH), 8.14 (s, 1H, ArCH); Ms (m/z) 430 (M^+); Anal. calcd for $C_{21}H_{19}ClN_2O_2S_2$: C, 58.52; H, 4.44; N, 6.50. Found: C, 58.36; H, 4.23; N, 6.41.

(3-Chloro-4-oxo-4*H*-chromen-2-yl)methyl diisopropylcarbamodithioate (**I**o): Mp 112–114 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.77$ (b, 12H, CH_3), 3.648 (b, 2H, iPr-CH), 4.92 (s, 2H, CH_2), 7.37 (t, $J = 8.0$ Hz, 1H, ArH), 7.47 (d, $J = 8.4$ Hz, 1H, ArH), 7.70 (t, $J = 8.0$ Hz, 1H, ArH), 8.25 (d, $J = 8.0$ Hz, 1H, ArH); Ms (m/z) 369 (M^+); Anal. calcd for $C_{17}H_{20}ClNO_2S_2$: C, 55.19; H, 5.45; N, 3.79. Found: C, 55.10; H, 5.54; N, 3.68.

(3-Chloro-4-oxo-4*H*-chromen-2-yl)methyl pyrrolidine-1-carbodithioate (**I**p): Mp 126–128 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.93$ (m, 2H, CH_2), 2.05 (m, 2H, CH_2), 3.62 (t, $J = 7.2$ Hz, 2H, CH_2), 3.92 (t, $J = 7.2$ Hz, 2H, CH_2), 4.92 (s, 2H, CH_2), 7.37 (t, $J = 8.0$ Hz, 1H, ArH), 7.47 (d, $J = 8.4$ Hz, 1H, ArH), 7.70 (t, $J = 8.0$ Hz, 1H, ArH), 8.25 (d, $J = 8.0$ Hz, 1H, ArH); Ms (m/z) 339 (M^+); Anal. calcd for $C_{15}H_{14}ClNO_2S_2$: C, 53.01; H, 4.15; N, 4.12. Found: C, 52.90; H, 4.02; N, 4.09.

(3-Chloro-4-oxo-4*H*-chromen-2-yl)methyl piperidine-1-carbodithioate (**I**q): Mp 140–142 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.74$

(b, 6H, CH_2), 3.91 (b, 2H, CH_2), 4.32 (b, 2H, CH_2), 4.93 (s, 2H, CH_2), 7.39 (t, $J = 8.0$ Hz, 1H, ArH), 7.48 (d, $J = 8.4$ Hz, 1H, ArH), 7.70 (t, $J = 8.0$ Hz, 1H, ArH), 8.24 (d, $J = 8.0$ Hz, 1H, ArH); Ms (m/z) 353 (M^+); Anal. calcd for $C_{16}H_{16}ClNO_2S_2$: C, 54.30; H, 4.56; N, 3.96. Found: C, 54.17; H, 4.60; N, 3.90.

(3-Chloro-4-oxo-4*H*-chromen-2-yl)methyl morpholine-4-carbodithioate (**I**r): Mp 141–143 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 3.75$ (b, 4H, CH_2), 3.94 (b, 2H, CH_2), 4.32 (b, 2H, CH_2), 4.97 (s, 2H, CH_2), 7.39 (t, $J = 8.0$ Hz, 1H, ArH), 7.51 (d, $J = 8.4$ Hz, 1H, ArH), 7.78 (t, $J = 8.0$ Hz, 1H, ArH), 8.32 (d, $J = 8.0$ Hz, 1H, ArH); Ms (m/z) 355 (M^+); Anal. calcd for $C_{15}H_{14}ClNO_3S_2$: C, 50.63; H, 3.97; N, 3.94. Found: C, 50.49; H, 4.08; N, 3.85.

4.1.3. General procedure for synthesis of ***II***

The intermediate 3-cholоromethyl chromone **3** was synthesized according to the reported method [11].

3-(Chloromethyl)-4*H*-chromen-4-one (**3**a): Mp 105–107 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 4.64$ (s, 2H, CH_2), 7.43 (d, $J = 8.0$ Hz, 1H, ArH), 7.57 (t, $J = 8.0$ Hz, 1H, ArH), 7.76 (d, $J = 8.0$ Hz, 1H, ArH), 8.08 (d, $J = 8.0$ Hz, 1H, ArH), 8.14 (s, 1H, 2-CH); Ms (m/z) 194 (M^+).

3-(Chloromethyl)-7-methoxy-4*H*-chromen-4-one (**3**b): Mp 146–148 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 3.87$ (s, 3H, CH_3), 4.43 (s, 2H, CH_2), 6.82 (s, 1H, 2-CH), 7.45 (d, $J = 8.8$ Hz, 1H, ArH), 7.63 (d, $J = 8.8$ Hz, 1H, ArH), 8.10 (s, 1H, ArH); Ms (m/z) 224 (M^+).

3-(Chloromethyl)-6-methyl-4*H*-chromen-4-one (**3**c): Mp 130–132 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.42$ (s, 3H, CH_3), 4.42 (s, 2H, CH_2), 7.50 (d, $J = 8.4$ Hz, 1H, ArH), 7.64 (d, $J = 8.4$ Hz, 1H, ArH), 8.01 (s, 1H, ArH), 8.25 (s, 1H, 2-CH); Ms (m/z) 208 (M^+).

6-Chloro-3-(chloromethyl)-4*H*-chromen-4-one (**3**d): Mp 159–161 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 4.53$ (s, 2H, CH_2), 7.45 (d, $J = 8.8$ Hz, 1H, ArH), 7.63 (d, $J = 8.8$ Hz, 1H, ArH), 8.10 (s, 1H, ArH), 8.21 (s, 1H, 2-CH); Ms (m/z) 228 (M^+).

To a solution of amine (1 mmol) in DMF (2 mL) was added dropwise carbon disulfide (2 mmol) and anhydrous potassium phosphate (1 mmol). The resulted mixture was stirred at room temperature for 30 min. Then chlorinated chromone **3** (1 mmol) was added by one-portion and stirring was continued. After completion of the reaction (monitored by TLC), the mixture was diluted with ice-cold water (20 mL) and the precipitate was filtered, and recrystallized from ethanol to give the target compound **II**.

(4-Oxo-4*H*-chromen-3-yl)methyl diisopropylcarbamodithioate (**II**a): Mp 94–96 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.33$ (b, 14H, CH_2), 4.51 (s, 2H, CH_2), 6.43 (d, $J = 9.6$ Hz, 1H, Het-CH), 7.42 (t, $J = 8.0$ Hz, 1H, ArH), 7.45 (d, $J = 8.4$ Hz, 1H, ArH), 7.65 (t, $J = 8.0$ Hz, 1H, ArH), 8.24 (d, $J = 8.0$ Hz, 1H, ArH), 8.43 (s, 1H, 2-CH); Ms (m/z) 335 (M^+); Anal. calcd for $C_{17}H_{21}NO_2S_2$: C, 60.86; H, 6.31; N, 4.18. Found: C, 60.69; H, 6.13; N, 4.24.

(4-Oxo-4*H*-chromen-3-yl)methyl pyrrolidine-1-carbodithioate (**II**b): Mp 103–105 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.93$ (m, 2H, CH_2), 2.05 (m, 2H, CH_2), 3.62 (t, $J = 7.2$ Hz, 2H, CH_2), 3.92 (t, $J = 7.2$ Hz, 2H, CH_2), 4.48 (s, 2H, CH_2), 7.39–7.45 (m, 2H, ArH), 7.65 (t, $J = 8.0$ Hz, 1H, ArH), 8.24 (d, $J = 8.0$ Hz, 1H, ArH), 8.49 (s, 1H, 2-CH); Ms (m/z) 305 (M^+); Anal. calcd for $C_{15}H_{15}NO_2S_2$: C, 58.99; H, 4.95; N, 4.59. Found: C, 58.80; H, 5.17; N, 4.63.

(4-Oxo-4*H*-chromen-3-yl)methyl piperidine-1-carbodithioate (**II**c): Mp 118–120 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.01$ (b, 6H, CH_2), 2.71 (b, 2H, CH_2), 4.18 (b, 2H, CH_2), 4.64 (s, 2H, CH_2), 7.21–7.25 (m, 2H, ArH), 7.57 (t, $J = 8.0$ Hz, 1H, ArH), 8.20 (d, $J = 8.0$ Hz, 1H, ArH), 8.34 (s, 1H, 2-CH); Ms (m/z) 319 (M^+); Anal. calcd for $C_{16}H_{17}NO_2S_2$: C, 60.16; H, 5.36; N, 4.38. Found: C, 60.32; H, 5.21; N, 4.31.

(4-Oxo-4*H*-chromen-3-yl)methyl morpholine-4-carbodithioate (**II**d): Mp 131–134 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.63$ (b, 4H, CH_2), 2.71 (b, 2H, CH_2), 4.12 (b, 2H, CH_2), 5.01 (s, 2H, CH_2), 7.30–7.35 (m, 2H, ArH), 7.61 (t, $J = 8.0$ Hz, 1H, ArH), 8.26 (d, $J = 8.0$ Hz, 1H, ArH), 8.40 (s, 1H, 2-CH); Ms (m/z) 321 (M^+); Anal. calcd for $C_{15}H_{15}NO_3S_2$: C, 56.05; H, 4.70; N, 4.36. Found: C, 55.95; H, 4.91; N, 4.50.

(4-Oxo-4*H*-chromen-3-yl)methyl 4-methylpiperazine-1-carbodithioate (**II**e): Mp 122–124 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.33$ (s, 3H, CH_3), 2.50 (b, 4H, CH_2), 4.36 (b, 2H, CH_2), 4.48 (b, 2H, CH_2), 4.52 (s, 2H, CH_2), 6.54 (s, 1H, 3-CH), 7.27 (t, $J = 7.2$ Hz, 1H, ArH), 7.39 (d, $J = 9.0$ Hz, 1H, ArH), 7.66 (t, $J = 7.2$ Hz, 1H, ArH), 8.24 (d, $J = 9.0$ Hz, 1H, ArH), 8.44 (s, 1H, ArCH); Ms (m/z) 334 (M^+); Anal. calcd for $C_{16}H_{18}N_2O_2S_2$: C, 57.46; H, 5.42; N, 8.38. Found: C, 57.21; H, 5.60; N, 8.27.

(4-Oxo-4*H*-chromen-3-yl)methyl 4-phenylpiperazine-1-carbodithioate (**II**f): Mp 151–153 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 3.31$ (b, 4H, CH_2), 4.13 (b, 2H, CH_2), 4.51 (b, 2H, CH_2), 4.65 (s, 2H, CH_2), 6.54 (s, 1H, 3-CH), 6.91–6.94 (m, 3H, ArH), 7.26–7.33 (m, 2H, ArH), 7.43 (d, $J = 9.2$ Hz, 1H, ArH), 7.61 (d, $J = 9.2$ Hz, 1H, ArH), 8.14 (s, 1H, ArCH); Ms (m/z) 396 (M^+); Anal. calcd for $C_{21}H_{20}N_2O_2S_2$: C, 63.61; H, 5.08; N, 7.06. Found: C, 63.45; H, 5.31; N, 7.21.

(4-Oxo-4*H*-chromen-3-yl)methyl 4-benzylpiperazine-1-carbodithioate (**II**g): Mp 160–162 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 3.31$ (b, 4H, CH_2), 4.13 (b, 2H, CH_2), 4.51 (b, 2H, CH_2), 4.65 (s, 2H, CH_2), 6.54 (s, 1H, 3-CH), 6.91–6.94 (m, 3H, ArH), 7.26–7.33 (m, 2H, ArH), 7.43 (d, $J = 9.2$ Hz, 1H, ArH), 7.61 (d, $J = 9.2$ Hz, 1H, ArH), 8.14 (s, 1H, ArCH); Ms (m/z) 410 (M^+); Anal. calcd for $C_{22}H_{22}N_2O_2S_2$: C, 64.36; H, 5.40; N, 6.82. Found: C, 64.21; H, 5.52; N, 6.76.

(4-Oxo-4*H*-chromen-3-yl)methyl 4-(bis(4-fluorophenyl) methyl) piperazine-1-carbodithioate (**II**h): Mp 175–177 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 3.31$ (b, 4H, CH_2), 4.13 (b, 2H, CH_2), 4.51 (b, 2H, CH_2), 4.65 (s, 2H, CH_2), 6.54 (s, 1H, 3-CH), 6.91–6.94 (m, 3H, ArH), 7.26–7.33 (m, 2H, ArH), 7.43 (d, $J = 9.2$ Hz, 1H, ArH), 7.61 (d, $J = 9.2$ Hz, 1H, ArH), 8.14 (s, 1H, ArCH); Ms (m/z) 522 (M^+); Anal. calcd for $C_{28}H_{24}F_2N_2O_2S_2$: C, 64.35; H, 4.63; N, 5.36. Found: C, 64.24; H, 4.45; N, 5.30.

(4-Oxo-4*H*-chromen-3-yl)methyl methyl(phenyl)carbamodithioate (**II**i): Mp 138–140 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 3.79$ (s, 3H, CH_3), 4.51 (s, 2H, CH_2), 7.31 (d, $J = 6.8$ Hz, 2H, ArH), 7.45–7.51 (m, 4H, ArH), 7.58 (d, $J = 8.0$ Hz, 1H, ArH), 7.78 (t, $J = 7.2$ Hz, 1H, ArH), 8.17 (d, $J = 8.0$ Hz, 1H, ArH); Ms (m/z) 341 (M^+); Anal. calcd for $C_{18}H_{15}NO_2S_2$: C, 63.32; H, 4.43; N, 4.10. Found: C, 63.24; H, 4.61; N, 4.03.

(7-Methoxy-4-oxo-4*H*-chromen-3-yl)methyl diisopropylcarbamodithioate (**II**j): Mp 96–98 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.73$ (b, 12H, CH_3), 3.58 (b, 2H, iPr-CH), 3.89 (s, 3H, CH_3), 4.47 (s, 2H, CH_2), 6.81 (s, 1H, 2-CH), 6.96 (d, $J = 9.0$ Hz, 1H, ArH), 8.14 (d, $J = 9.6$ Hz, 1H, ArH), 8.37 (s, 1H, ArH); Ms (m/z) 365 (M^+); Anal. calcd for $C_{18}H_{23}NO_3S_2$: C, 59.15; H, 6.34; N, 3.83. Found: C, 59.01; H, 6.40; N, 3.91.

(7-Methoxy-4-oxo-4*H*-chromen-3-yl)methyl pyrrolidine-1-carbodithioate (**II**k): Mp 106–108 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.93$ (m, 2H, CH_2), 2.05 (m, 2H, CH_2), 3.62 (t, $J = 7.2$ Hz, 2H, CH_2), 3.92 (t, $J = 7.2$ Hz, 2H, CH_2), 3.99 (s, 3H, CH_3), 4.43 (s, 2H, CH_2), 6.82 (s, 1H, 2-CH), 6.91 (d, $J = 9.0$ Hz, 1H, ArH), 8.15 (d, $J = 9.6$ Hz, 1H, ArH), 8.35 (s, 1H, ArH); Ms (m/z) 335 (M^+); Anal. calcd for $C_{16}H_{17}NO_3S_2$: C, 57.29; H, 5.11; N, 4.18. Found: C, 57.31; H, 5.20; N, 4.30.

(7-Methoxy-4-oxo-4*H*-chromen-3-yl)methyl piperidine-1-carbodithioate (**II**l): Mp 124–126 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.73$ (b, 6H, CH_2), 3.90 (m, 5H, OMe & CH_2), 4.30 (m, 2H, CH_2), 4.56 (s, 2H, CH_2), 6.89 (s, 1H, 2-CH), 7.03 (d, $J = 9.0$ Hz, 1H, ArH), 8.19 (d, $J = 9.6$ Hz, 1H, ArH), 8.51 (s, 1H, ArH); Ms (m/z) 349 (M^+); Anal. calcd for $C_{17}H_{19}NO_3S_2$: C, 58.43; H, 5.48; N, 4.01. Found: C, 58.54; H, 5.51; N, 4.05.

(7-Methoxy-4-oxo-4*H*-chromen-3-yl)methyl morpholine-4-carbodithioate (**II**m): Mp 136–138 °C; ^{1}H NMR (400 MHz, $CDCl_3$): 3.72 (b, 4H, CH_2), 3.88–3.93 (b, 5H, OMe & CH_2), 4.32 (b, 2H, CH_2),

4.49 (s, 2H, CH_2), 6.82 (s, 1H, 2-CH), 6.94 (d, $J = 9.0$ Hz, 1H, ArH), 8.1 (d, $J = 8.4$ Hz, 1H, ArH), 8.35 (s, 1H, ArH); Ms (m/z) 351 (M^+); Anal. calcd for $C_{16}H_{17}NO_4S_2$: C, 54.68; H, 4.88; N, 3.99. Found: C, 54.46; H, 4.95; N, 4.05.

(6-Methyl-4-oxo-4*H*-chromen-3-yl)methyl diisopropylcarbamodithioate (**II**n): Mp 98–100 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.75$ (b, 12H, CH_3), 2.35 (s, 3H, CH_3), 3.58 (b, 2H, iPr-CH), 4.48 (s, 2H, CH_2), 7.34 (d, $J = 8.4$ Hz, 1H, ArH), 7.46 (d, $J = 8.4$ Hz, 1H, ArH), 8.01 (s, 1H, 2-CH), 8.42 (s, 1H, ArH); Ms (m/z) 349 (M^+); Anal. calcd for $C_{18}H_{23}NO_2S_2$: C, 61.86; H, 6.63; N, 4.01. Found: C, 61.72; H, 6.80; N, 4.10.

(6-Methyl-4-oxo-4*H*-chromen-3-yl)methyl pyrrolidine-1-carbodithioate (**II**o): Mp 110–113 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.93$ (m, 2H, CH_2), 2.04 (m, 2H, CH_2), 2.46 (s, 3H, CH_3), 3.62 (t, $J = 7.2$ Hz, 2H, CH_2), 3.92 (t, $J = 7.2$ Hz, 2H, CH_2), 4.60 (s, 2H, CH_2), 7.33 (d, $J = 8.4$ Hz, 1H, ArH), 7.46 (d, $J = 8.4$ Hz, 1H, ArH), 8.01 (s, 1H, 2-CH), 8.46 (s, 1H, ArH); Ms (m/z) 319 (M^+); Anal. calcd for $C_{16}H_{17}NO_2S_2$: C, 60.16; H, 5.36; N, 4.38. Found: C, 60.08; H, 5.50; N, 4.43.

(6-Methyl-4-oxo-4*H*-chromen-3-yl)methyl piperidine-1-carbodithioate (**II**p): Mp 121–123 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.74$ (b, 6H, CH_2), 2.45 (s, 3H, CH_3), 3.91 (m, 2H, CH_2), 4.31 (m, 2H, CH_2), 4.56 (s, 2H, CH_2), 7.32 (d, $J = 8.4$ Hz, 1H, ArH), 7.45 (d, $J = 8.4$ Hz, 1H, ArH), 8.03 (s, 1H, 2-CH), 8.40 (s, 1H, ArH); Ms (m/z) 333 (M^+); Anal. calcd for $C_{17}H_{19}NO_2S_2$: C, 61.23; H, 5.74; N, 4.20. Found: C, 61.10; H, 5.90; N, 4.31.

(6-Methyl-4-oxo-4*H*-chromen-3-yl)methyl morpholine-4-carbodithioate (**II**q): Mp 131–134 °C; ^{1}H NMR (400 MHz, $CDCl_3$): 2.45 (s, 3H, CH_3), 3.75 (b, 4H, CH_2), 3.94 (b, 2H, CH_2), 4.32 (b, 2H, CH_2), 4.61 (s, 2H, CH_2), 7.36 (d, $J = 8.4$ Hz, 1H, ArH), 7.48 (d, $J = 8.4$ Hz, 1H, ArH), 8.07 (s, 1H, 2-CH), 8.49 (s, 1H, ArH); Ms (m/z) 335 (M^+); Anal. calcd for $C_{16}H_{17}NO_3S_2$: C, 57.29; H, 5.11; N, 4.18. Found: C, 57.13; H, 5.32; N, 4.30.

(6-Methyl-4-oxo-4*H*-chromen-3-yl)methyl bis(2-chloroethyl)-carbamodithioate (**II**r): Mp 146–148 °C; ^{1}H NMR (400 MHz, $CDCl_3$): 3.26 (t, $J = 7.2$ Hz, 2H, CH_2), 3.70 (s, 3H, CH_3), 3.72 (t, $J = 7.2$ Hz, 2H, CH_2), 4.50 (s, 2H, CH_2), 7.41–7.47 (m, 2H, ArH), 7.67 (d, $J = 8.4$ Hz, 1H, ArH), 8.21–8.24 (m, 2H, ArH & 2-CH); Ms (m/z) 390 (M^+); Anal. calcd for $C_{16}H_{17}Cl_2NO_2S_2$: C, 49.23; H, 4.39; N, 3.59. Found: C, 49.36; H, 4.34; N, 3.61.

(6-Chloro-4-oxo-4*H*-chromen-3-yl)methyl diisopropylcarbamodithioate (**II**s): Mp 110–112 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.32$–1.58 (b, 12H, CH_3), 4.08 (b, 1H, i-Pr-CH), 4.49 (s, 2H, CH_2), 5.01 (b, 1H, i-Pr-CH), 7.41 (d, $J = 8.8$ Hz, 1H, ArH), 7.60 (d, $J = 8.8$ Hz, 1H, ArH), 8.20 (s, 1H, ArH), 8.44 (s, 1H, 2-CH); Ms (m/z) 369 (M^+); Anal. calcd for $C_{17}H_{20}ClNO_2S_2$: C, 55.19; H, 5.45; N, 3.79. Found: C, 54.98; H, 5.62; N, 3.65.

(6-Chloro-4-oxo-4*H*-chromen-3-yl)methyl pyrrolidine-1-carbodithioate (**II**t): Mp 125–127 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.94$–1.97 (m, 2H, CH_2), 2.03–2.06 (m, 2H, CH_2), 3.62 (d, $J = 6.8$ Hz, 1H, CH_2), 3.92 (d, $J = 6.8$ Hz, 2H, CH_2), 4.46 (s, 2H, CH_2), 7.40 (d, $J = 8.8$ Hz, 1H, ArH), 7.59 (d, $J = 8.8$ Hz, 1H, ArH), 8.19 (s, 1H, ArH), 8.50 (s, 1H, 2-CH); Ms (m/z) 339 (M^+); Anal. calcd for $C_{15}H_{14}ClNO_2S_2$: C, 53.01; H, 4.15; N, 4.12. Found: C, 52.78; H, 4.25; N, 4.09.

(6-Chloro-4-oxo-4*H*-chromen-3-yl)methyl piperidine-1-carbodithioate (**II**u): Mp 124–127 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.68$ (b, 6H, CH_2), 3.86 (b, 2H, CH_2), 4.27 (b, 2H, CH_2), 4.51 (s, 2H, CH_2), 7.41 (d, $J = 9.2$ Hz, 1H, ArH), 7.60 (d, $J = 9.2$ Hz, 1H, ArH), 8.19 (s, 1H, ArH), 8.47 (s, 1H, 2-CH); Ms (m/z) 353 (M^+); Anal. calcd for $C_{16}H_{16}ClNO_2S_2$: C, 54.30; H, 4.56; N, 3.96. Found: C, 54.42; H, 4.65; N, 3.90.

(6-Chloro-4-oxo-4*H*-chromen-3-yl)methyl morpholine-4-carbodithioate (**II**v): Mp 131–133 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 3.73$ (b, 4H, CH_2), 3.92 (b, 2H, CH_2), 4.31 (b, 2H, CH_2), 4.51 (s, 2H, CH_2), 7.41 (d, $J = 9.2$ Hz, 1H, ArH), 7.61 (d, $J = 8.8$ Hz, 1H, ArH), 8.19 (s, 1H, ArH), 8.44 (s, 1H, 2-CH); Ms (m/z) 355 (M^+); Anal. calcd for $C_{15}H_{14}ClNO_3S_2$: C, 50.63; H, 3.97; N, 3.94. Found: C, 50.50; H, 4.03; N, 4.11.

4.1.4. General procedure for synthesis of *III*

The intermediate 3-bromo-chromones **4** was synthesized according to the reported method [12].

3-Bromo-4*H*-chromen-4-one (**4**a): Mp 120–122 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.39$ (t, $J = 7.2$ Hz, 1H, ArH), 7.52 (d, $J = 8.4$ Hz, 1H, ArH), 7.73 (t, $J = 7.2$ Hz, 1H, ArH), 8.15 (s, 1H, 2-CH), 8.31 (d, $J = 7.6$ Hz, 1H, ArH); Ms (m/z) 224 (M^+).

3-Bromo-6-chloro-4*H*-chromen-4-one (**4**b): Mp 132–134 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.48$ (d, $J = 8.8$ Hz, 1H, ArH), 7.66 (d, $J = 8.8$ Hz, 1H, ArH), 8.13 (s, 1H, ArH), 8.24 (s, 1H, 2-CH); Ms (m/z) 258 (M^+).

To a solution of amine (1 mmol) in DMF (2 mL) was added dropwise carbon disulfide (2 mmol) and anhydrous sodium methoxide (1 mmol). The resulted mixture was stirred at room temperature for 30 min. Then brominated chromone **4** (1 mmol) was added by one-portion and stirring was continued. After completion of the reaction (monitored by TLC), the mixture was diluted with ice-cold water (20 mL) and the precipitate was filtered, and recrystallized from ethanol to give the target compound **III**.

4-Oxo-4*H*-chromen-3-yl pyrrolidine-1-carbodithioate (**III**a): Mp 130–132 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.10$ (m, 2H, CH_2), 2.21 (m, 2H, CH_2), 3.68 (t, $J = 7.2$ Hz, 2H, CH_2), 3.90 (t, $J = 7.2$ Hz, 2H, CH_2), 7.39 (t, $J = 7.2$ Hz, 1H, ArH), 7.52 (d, $J = 8.4$ Hz, 1H, ArH), 7.73 (t, $J = 7.2$ Hz, 1H, ArH), 8.15 (s, 1H, 2-CH), 8.31 (d, $J = 7.6$ Hz, 1H, ArH); Ms (m/z) 291 (M^+); Anal. calcd for $C_{14}H_{13}NO_2S_2$: C, 57.71; H, 4.50; N, 4.81. Found: C, 57.56; H, 4.70; N, 4.67.

4-Oxo-4*H*-chromen-3-yl piperidine-1-carbodithioate (**III**b): Mp 119–121 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.74$ (b, 6H, CH_2), 4.06 (b, 2H, CH_2), 4.27 (b, 2H, CH_2), 7.45 (t, $J = 7.2$ Hz, 1H, ArH), 7.51 (d, $J = 8.4$ Hz, 1H, ArH), 7.71 (t, $J = 7.2$ Hz, 1H, ArH), 8.13 (s, 1H, 2-CH), 8.27 (d, $J = 7.6$ Hz, 1H, ArH); Ms (m/z) 305 (M^+); Anal. calcd for $C_{15}H_{15}NO_2S_2$: C, 58.99; H, 4.95; N, 4.59 6. Found: C, 58.86; H, 5.11; N, 4.47.

6-Chloro-4-oxo-4*H*-chromen-3-yl pyrrolidine-1-carbodithioate (**III**c): Mp 123–125 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.01$ (m, 2H, CH_2), 2.17 (m, 2H, CH_2), 3.90 (m, 4H, CH_2), 7.48 (d, $J = 8.8$ Hz, 1H, ArH), 7.66 (d, $J = 8.8$ Hz, 1H, ArH), 8.13 (s, 1H, ArH), 8.24 (s, 1H, 2-CH); Ms (m/z) 325 (M^+); Anal. calcd for $C_{14}H_{12}ClNO_2S_2$: C, 51.61; H, 3.71; N, 4.30. Found: C, 51.45; H, 3.90; N, 4.42.

6-Chloro-4-oxo-4*H*-chromen-3-yl piperidine-1-carbodithioate (**III**d): Mp 130–133 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.78$ (b, 6H, CH_2), 4.13 (b, 2H, CH_2), 4.29 (b, 2H, CH_2), 7.51 (d, $J = 8.8$ Hz, 1H, ArH), 7.68 (d, $J = 8.8$ Hz, 1H, ArH), 8.13 (s, 1H, ArH), 8.27 (s, 1H, 2-CH); Ms (m/z) 339 (M^+); Anal. calcd for $C_{15}H_{14}ClNO_2S_2$: C, 53.01; H, 4.15; N, 4.12. Found: C, 53.12; H, 4.09; N, 4.23.

4.1.5. General procedure for synthesis of *IV*

The intermediate ethyl 6-methyl-4-oxo-4*H*-chromene-2-carboxylate **5** was synthesized according to the reported method [8,9]. The CCl_4 solution (20 mL) of **5** (5 mmol), NBS (5 mmol) and AIBN (0.1 mmol) was irradiated with 200 W lamp. After the reaction completed, the mixture was cooled to room temperature and filtrated. The filtrate was concentrated in vacuo. The crude product was chromatographed on silica gel with acetone/petroleum (15/85), giving **6** in 80% yield.

Ethyl ethyl 6-methyl-4-oxo-4*H*-chromene-2-carboxylate (**5**): Mp 137–139 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.45$ (t, $J = 7.2$ Hz, 3H, CO_2 $CH2_3\underline{CH_3}$), 2.00 (s, 3H, Ar-CH_3), 4.36 (q, $J = 7.2$ Hz, 2H, CO_2CH_2), 7.16 (s, 1H, 3-CH), 7.53 (d, $J = 9.0$ Hz, 1H, ArH), 7.88 (d, $J = 8.4$ Hz, 1H, ArH), 8.20 (s, 1H, ArH); Ms (m/z) 232 (M^+).

Ethyl 6-(bromomethyl)-4-oxo-4*H*-chromene-2-carboxylate (**6**): Mp 147–149 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.44$ (t, $J = 7.2$ Hz, 3H, CH_3), 4.47 (q, $J = 7.2$ Hz, 2H, CO_2CH_2), 4.72 (s, 2H, CH_2), 7.11 (s, 1H, 3-CH), 7.56 (d, $J = 9.0$ Hz, 1H, ArH), 7.85 (d, $J = 8.4$ Hz, 1H, ArH), 8.17 (s, 1H, ArH); Ms (*m*/*z*) 291 (M^+).

Target compound **IV** can be synthesized from **6**, amine and carbon disulfide under the similar procedure to that of the synthesis of **I**.

Ethyl 6-((diisopropylcarbamothioylthio)methyl)-4-oxo-4*H*-chromene-2-carboxylate (**IV**a): Mp 142–144 °C; 1H NMR (400 MHz, $CDCl_3$): $\delta = 1.34$ (b, 14 H, CH_2), 1.73 (b, 12 H, CH_3), 3.58 (b, 2H, iPr-CH), 4.72 (s, 2H, CH_2), 7.12 (s, 1H, 3-CH), 7.57 (d, $J = 9.0$ Hz, 1H, ArH), 7.86 (d, $J = 8.4$ Hz, 1H, ArH, H), 8.17 (s, 1H, ArH); Ms (*m*/*z*) 407 (M+); Anal. calcd for $C_{20}H_{25}NO_4S_2$: C, 58.94; H, 6.18; N, 3.44. Found: C, 58.79; H, 6.34; N, 3.55.

Ethyl 4-oxo-6-((pyrrolidine-1-carbonothioylthio)methyl)-4*H*-chromene-2-carboxylate (**IV**b): Mp 136–138 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.44$ (t, $J = 7.2$ Hz, 3H, CH_3), 1.98 (m, 2H, CH_2), 2.09 (m, 2H, CH_2), 3.65 (t, $J = 7.2$ Hz, 2H, CH_2), 3.96 (t, $J = 7.2$ Hz, 2H, CH_2), 4.47 (q, $J = 7.2$ Hz, 2H, CO_2CH_2), 4.72 (s, 2H, CH_2), 7.11 (s, 1H, 3-CH), 7.56 (d, $J = 9.0$ Hz, 1H, ArH), 7.85 (d, $J = 8.4$ Hz, 1H, ArH), 8.17 (s, 1H, ArH); Ms (*m*/*z*) 377 (M^+); Anal. calcd for $C_{18}H_{19}NO_4S_2$: C, 57.27; H, 5.07; N, 3.71. Found: C, 57.12; H, 5.00; N, 3.81.

Ethyl 4-oxo-6-((piperidine-1-carbonothioylthio)methyl)-4*H*-chromene-2-carboxylate (**IV**c): Mp 151–153 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.43$ (t, $J = 7.2$ Hz, 3H, CH_3), 2.02 (b, 6H, CH_2), 2.71 (b, 2H, CH_2), 4.16 (b, 2H, CH_2), 4.72 (s, 2H, CH_2), 7.11 (s, 1H, 3-CH), 7.54 (d, $J = 9.0$ Hz, 1H, ArH), 7.87 (d, $J = 8.4$ Hz, 1H, ArH, H), 8.14 (s, 1H, ArH); Ms (*m*/*z*) 391 (M^+); Anal. calcd for $C_{19}H_{21}NO_4S_2$: C, 58.29; H, 5.41; N, 3.58. Found: C, 58.09; H, 5.60; N, 3.63.

Ethyl 6-((morpholine-4-carbonothioylthio)methyl)-4-oxo-4*H*-chromene-2-carboxylate (**IV**d): Mp 156–158 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.44$ (t, $J = 7.2$ Hz, 3H, CH_3), 3.76 (b, 4H, CH_2), 3.95 (b, 2H, CH_2), 4.35 (b, 2H, CH_2), 4.47 (q, $J = 7.2$ Hz, 2H, CO_2CH_2), 4.72 (s, 2H, CH_2), 7.11 (s, 1H, CH_2), 7.57 (d, $J = 9.0$ Hz, 1H, 3-CH), 7.82 (d, $J = 8.4$ Hz, 1H, ArH, H), 8.18 (s, 1H, ArH); Ms (*m*/*z*) 393 (M^+); Anal. calcd for $C_{18}H_{19}ClNO_5S_2$: C, 54.94; H, 4.87; N, 3.56. Found: C, 54.80; H, 4.99; N, 3.60.

Ethyl 6-((methyl(phenyl)carbamothioylthio)methyl)-4-oxo-4*H*-chromene-2-carboxylate (**IV**e): Mp 171–173 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.43$ (t, $J = 7.2$ Hz, 3H, CH_3), 1.56 (s, 3H, CH_3), 2.05 (b, 6H, CH_2), 2.77 (b, 2H, CH_2), 4.19 (b, 2H, CH_2), 4.77 (s, 2H, CH_2), 7.13 (s, 1H, 3-CH), 7.54–7.59 (m, 3H, ArH), 7.64–7.69 (m, 3H, ArH), 7.89 (d, $J = 8.4$ Hz, 1H, ArH, H), 8.18 (s, 1H, ArH); Ms (*m*/*z*) 413 (M^+); Anal. calcd for $C_{21}H_{19}NO_4S_2$: C, 61.00; H, 4.63; N, 3.39. Found: C, 61.08; H, 4.71; N, 3.48.

4.2. Pharmacology

4.2.1. Cell proliferation by MTT assay [14]

Cells seeded in 96-well microplates at 8000 cells/well were incubated with the test compounds for 72 h, respectively. Then, 20 μL (0.5 mg/mL, final concentration) of MTT (Sigma, USA) was added to each well and incubated for 4 h. MTT is converted to a blue formazan product by mitochondrial succinate dehydrogenase. This product was eluted from cells by addition of 150 mL of DMSO and absorbance at 570 nm was determined by an ELISA using a NJ-2300 microplate spectrophotometer.

4.2.2. Flow activating cell sorting analysis (FACS) [15]

The effect of compounds **I**q and **II**u on cancer cell cycle phase distribution was assessed using flow cytometry. The cells were grown to about 70% confluence in 6-well microplates and then treated with **I**q and **II**u at different given concentrations. After 48 h, cells were harvested by trypsinization, washed in PBS and fixed in 70% ice-cold (4 °C) ethanol overnight. They were washed with PBS, incubated with RNase (100 μg/mL final concentration) at 37 °C for 30 min, stained with propidium iodide (50 μg/mL final concentration), and analyzed by flow cytometry (Beckman Coulter). For each assay, three different experiments were performed in triplicate. The results were statistically evaluated by the Student's *t*-test. For all the tests, the level of significance was set at $P < 0.05$.

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