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Original article

Antitumor agents 292. Design, synthesis and pharmacological study of *S*- and *O*-substituted 7-mercapto- or hydroxy-coumarins and chromones as potent cytotoxic agents

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ABSTRACT

Thirty-five *S*- and *O*-substituted 7-mercaptocoumarin (**9**–**23**) and 7-hydroxy- or 7-mercapto-chromone (**24**–**43**) analogs were designed, synthesized and evaluated *in vitro* against four human tumor cell lines [KB (nasopharyngeal), KB-vin (vincristine-resistant subline), A549 (lung) and DU145 (prostate)] with paclitaxel as the positive control. Many of the synthesized compounds exhibited potent cytotoxic activity. Among them, compounds **10** and **18** showed broad spectrum activity with GI_{50} values ranging from 0.92 to 2.11 μM and 2.06–14.07 μM, respectively. However, **33**, a 3-brominated compound, displayed significant and selective inhibition against MDR KB-vin with a GI_{50} of 5.84 μM. Regardless of the size of the 7-alkoxy group, 2-α-bromoethyl-8-bromomethyl compounds (**40**–**43**) exhibited increased cytotoxicity compared with 2-ethyl-8-bromomethyl compounds (**36**–**39**). Moreover, in a preliminary pharmacological study, **10** not only remarkably increased cellular apoptosis in a concentration-dependent manner, but also clearly induced A549 cell cycle arrest at the G2/M phase. Thus, these coumarin derivatives merit investigation as novel potential antitumor agents with further structural modification to produce an optimal lead compound and elucidate the detailed pharmacological mechanism(s).

1. Introduction

Coumarins and their derivatives play an important role in the agricultural and pharmaceutical industries. They are widely present in higher plants such as Rutaceae, Apiaceae, Asteraceae, Leguminosae, Thymelaeaceae, as well as occur as animal and microbial metabolites [1]. Most of them show a wide spectrum of pharmacological effects, including antimicrobial, anti-arrhythmic, anti-osteoporosis, anti-HIV, and antitumor activities [2–5]. Accordingly, many reports have described various structures and biological evaluations of numerous coumarin analogs newly synthesized or isolated from plants. For example, in 2005, Andre reported that coumarin (**1**, Fig. 1) and its analogs interact directly with cytochrome P450 (CYP) to inhibit the mutagenicity of 2-amino-3-methylimidazo[4,5-*f*]quinoline in *Salmonella typhimurium* TA98 [6], and libanoridin (**2**, Fig. 1), isolated from *Corydalis heterocarpa* in 2009, was found to have significant anti-inflammatory potency against HT-29 human colon carcinoma cells [7]. Since the 1950s, warfarin (**3**, Fig. 1) has been successfully used in the clinic to prevent thromboembolic disease, and its analog tecarfarin (**4**, Fig. 1) was also found to be a novel orally active vitamin K epoxide reductase inhibitor that can reduce the levels of vitamin K-dependent coagulation factors (factors II, VII, IX, and X) and prolong prothrombin time in canine and rabbit thrombosis models [8].

Multidrug resistance (MDR) always presents a major obstacle in the treatment of human cancers with chemotherapeutic agents. Recently, some natural products, including

Abbreviations: HIV, Human immunodeficiency virus; CYP, Cytochrome P450; MDR, Multidrug resistance; DMDCK, (±)-3′-*O*-4′-*O*-bis(3,4-dimethoxycinnamoyl)-*cis*-khellactone; DSP, 3′*R*,4′*R*-disubstituted-2′,2′-dimethyldihydropyrano [2,3-*f*] chromone; DCK, Dicamphanoyl-khellactone; DCP, Dicamphanoyl-dihydropyranochromone; GI, Growth inhibition.

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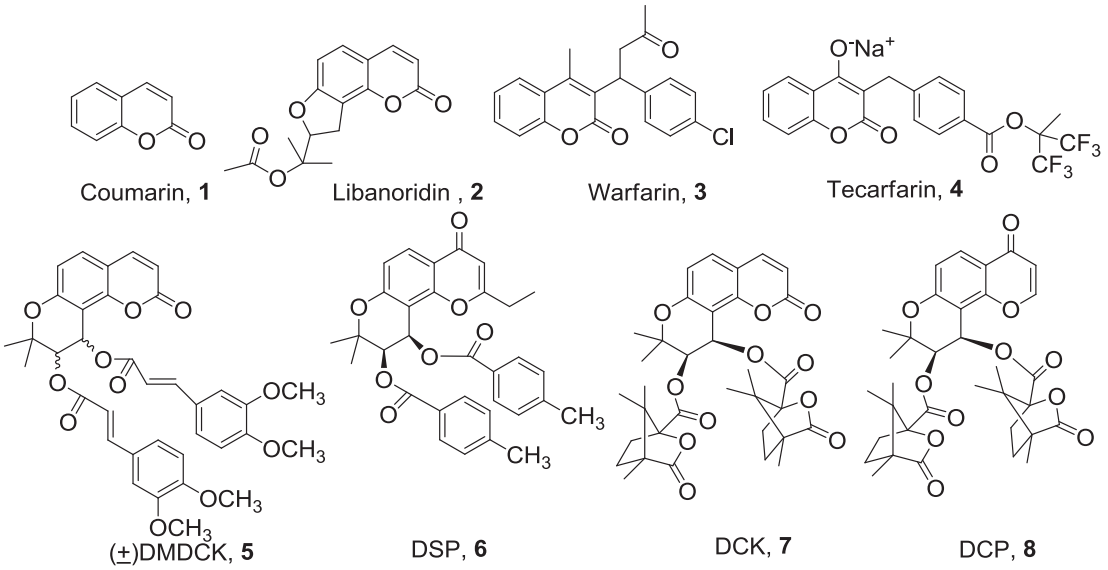
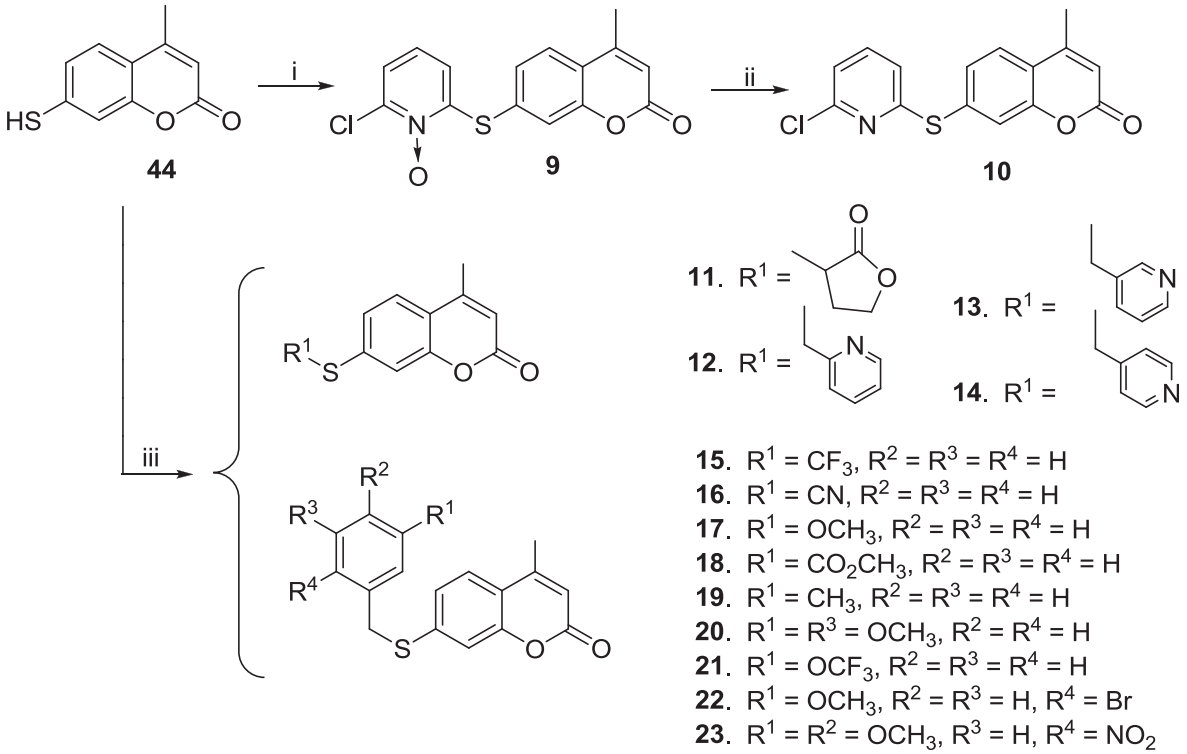
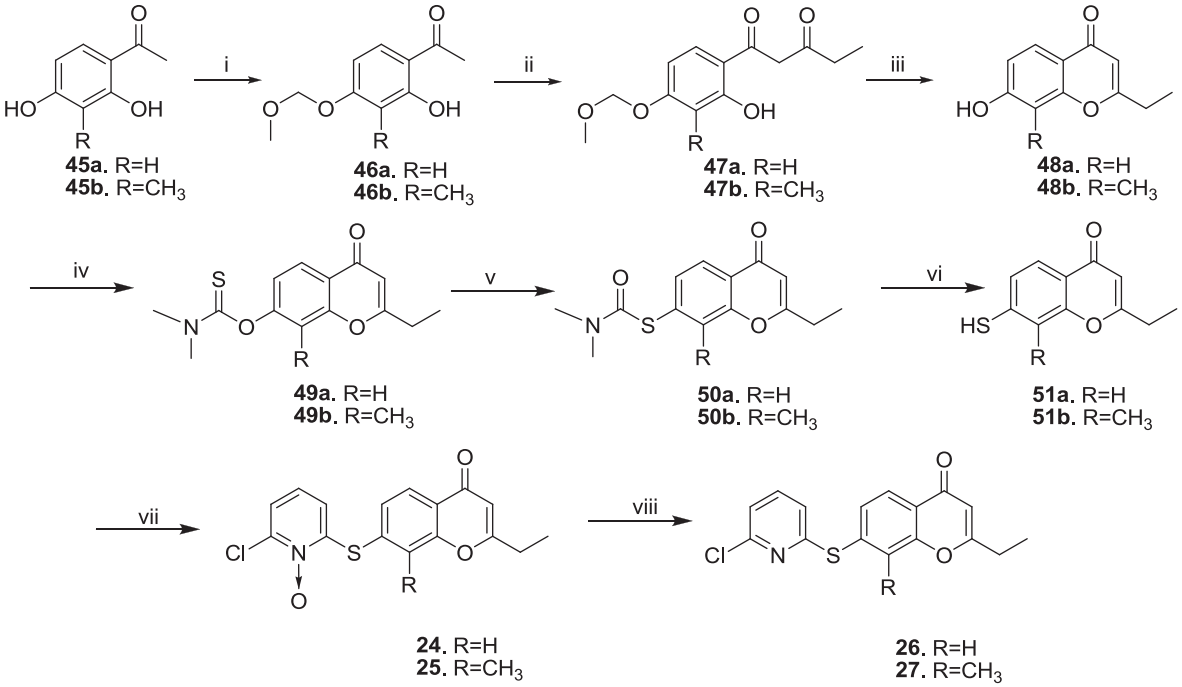


Fig. 1. Structures of bioactive natural and synthetic coumarin derivatives.

(±)-3′-*O*-4′-*O*-bis(3,4-dimethoxycinnamoyl)-*cis*-khellactone [(±) DMDCK, **5**, Fig. 1] and certain related synthetic compounds such as 3′*R*,4′*R*-disubstituted-2′,2′-dimethydihydropyrano[2,3-*f*]chromone (DSP, **6**, Fig. 1), exhibited significant potency in reversing P-gp-mediated MDR [9,10]. In our previous research, hundreds of dicamphanoyl-khellactone (DCK, **7**, Fig. 1) and dicamphanoyl-dihydropyranochromone (DCP, **8**, Fig. 1) analogs containing a coumarin or chromone moiety were designed and synthesized as anti-HIV agents [5,11–13]. In these studies, we also found that some 7-mercapto- and 7-hydroxy-coumarin or -chromone intermediates exhibited considerable cytotoxicity. These findings prompted us to design and synthesize additional new *S*- and *O*-substituted 7-mercapo or 7-hydroxy-coumarin and chromone analogs and screen them for cytotoxicity using paclitaxel as a potent antitumor reference. The most potent cytotoxic analog **10** was selected for further pharmacological studies in the A549 human lung cancer cell line to more fully elucidate the compound's mechanism of action and corresponding target. Herein the synthesis, cytotoxicity against four human tumor cell lines, and preliminary pharmacological mechanism of the new compounds are reported.



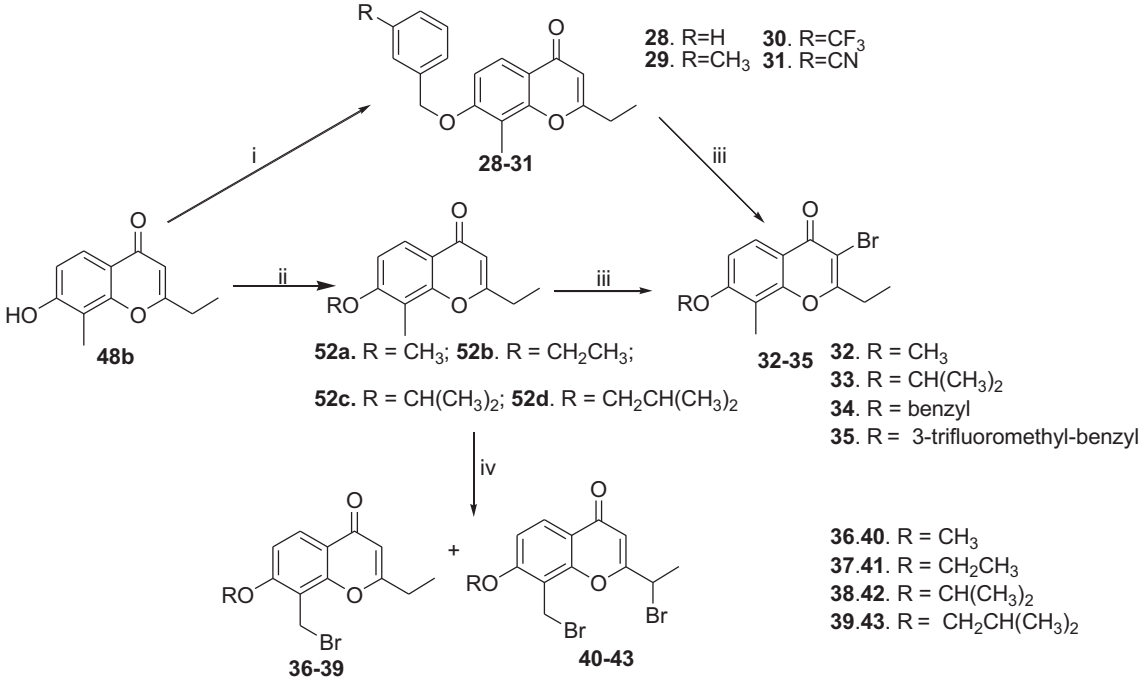
Scheme 1. The synthesis of 7-*S*-heterocyclic and aryl substituted coumarins (**9**–**23**). Reaction conditions: (i) 2,6-dichloropyridine *N*-oxide/pyridine/rt; (ii) $PCl_3/CHCl_3$/reflux; (iii) variable bromides/K_2CO_3/acetone/rt.



Scheme 2. The synthesis of 2-ethyl-7-*S*-heterocyclic substituted chromen-4-one analogs (**24**–**27**). Reaction conditions: (i) chloromethyl methyl ether/K_2CO_3/acetone/rt; (ii) ethyl propionate/NaH/THF/reflux; (iii) conc. HCl/EtOH/reflux; (iv) dimethylthiocarbamoyl chloride/DMF/K_2CO_3; (v) 220–230 °C; (vi) KOH/MeOH/N_2/reflux; (vii) 2,6-dichloropyridine *N*-oxide/pyridine/rt; (viii) PCl_3/$CHCl_3$/reflux.

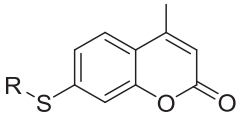
2. Design

Bioisosterism is a commonly used technique in drug research, although bioactive sulfur-containing coumarins have rarely been reported. Therefore, fifteen *S*-substituted 4-methyl-7-mercaptocoumarins (**9**–**23**), four *S*-substituted 2-ethyl-7-mercapto-chromones and four *O*-substituted analogs (**28**–**31**) were designed, synthesized, screened for cytotoxic activity and evaluated for the effect of diverse substituents on the potency. Based on the promising degree of cytotoxicity shown by certain brominated chromone intermediates obtained in our prior anti-HIV agents research, twelve new 2-ethyl-7-alkyloxy- and -aryloxy-chromen-4-one bromides (**32**–**43**) were also synthesized in this study. All synthesized compounds were screened *in vitro*

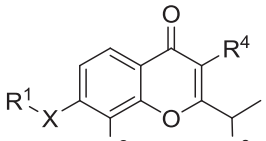


Scheme 3. The synthesis of 2-ethyl-7-*O*-alkyl and aryl substituted chromen-4-one analogs and bromides (**28**–**43**). Reaction conditions: (i) variable halides/K_2CO_3/DMF/50–60 °C; (ii) variable halides/K_2CO_3/acetone/rt; (iii) *N*-bromosuccinimide/CH_3CN/rt; (iv) *N*-bromosuccinimide/CCl_4/reflux.

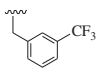
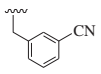
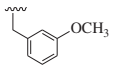
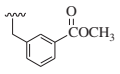
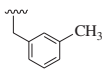
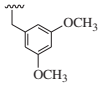
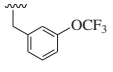
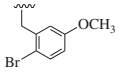
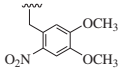
Table 1
In vitro anticancer activity of **9**–**43** against KB, KB-vin, A549 and DU145 cell lines.



9-23



24-43

Compd	R	X	R^1	R^2	R^3	R^4	GI_{50}(μM)			
							KB	KB-vin	A549	DU145
9							>31.27	24.52	>31.27	>31.27
10							0.92	0.92	2.11	1.15
11							>36.19	>36.19	>36.19	>36.19
12							>35.29	>35.29	>35.29	>35.29
13							>35.29	>35.29	>35.29	>35.29
14							>35.29	>35.29	>35.29	>35.29
15							>28.54	>28.54	>28.54	>28.54
16							>32.53	>32.53	>32.53	>32.53
17							>32.01	>32.01	>32.01	>32.01
18							2.06	5.41	7.70	14.07
19							>33.74	>33.74	>33.74	>33.74
20							>29.20	>29.20	>29.20	>29.20
21							14.77	15.28	17.36	15.48
22							15.95	>25.56	15.28	>25.56
23							>25.81	>25.81	>25.81	>25.81
24		S		H	H	H	>29.96	>29.96	>29.96	>29.96
25		S		Me	H	H	>28.75	>28.75	>28.75	>28.75

(*continued on next page*)

Table 1 (*continued*)

Compd	R	X	R^1	R^2	R^3	R^4	GI_{50}(μM)			
							KB	KB-vin	A549	DU145
26		S		H	H	H	>31.47	>31.47	>31.47	>31.47
27		S		Me	H	H	>30.14	23.93	>30.14	>30.14
28		O		Me	H	H	>33.97	24.80	>33.97	27.69
29		O		Me	H	H	28.60	24.91	24.94	24.09
30		O		Me	H	H	>27.60	>27.60	>27.60	>27.60
31		O		Me	H	H	>31.31	>31.31	>31.31	>31.31
32		O	Me	Me	H	Br	>33.65	23.19	>33.65	>33.65
33		O		Me	H	Br	22.60	5.84	>30.75	>30.75
34		O		Me	H	Br	>26.79	>26.79	>26.79	>26.79
35		O		Me	H	Br	>22.66	>22.66	>22.66	>22.66
36		O	Me	CH_2Br	H	H	>33.65	>33.65	>33.65	>33.65
37		O	Ethyl	CH_2Br	H	H	>32.14	>32.14	>32.14	>32.14
38		O		CH_2Br	H	H	>30.75	>30.75	>30.75	>30.75
39		O		CH_2Br	H	H	>29.48	>29.48	>29.48	>29.48
40		O	Me	CH_2Br	Br	H	15.08	16.99	>26.59	>26.59
41		O	Ethyl	CH_2Br	Br	H	15.59	12.77	19.84	20.92
42		O		CH_2Br	Br	H	12.77	11.41	12.79	15.47
43		O		CH_2Br	Br	H	10.93	9.45	8.71	10.52
Paclitaxel							4.12 nM	>1	5.46 nM	3.86 nM

against four different human cancer cell lines, KB (nasopharnygeal), KB-vin (its vincristine-resistant subline), A549 (lung) and DU145 (prostate). Compound **10** with the best activity was tested independently in cell proliferation, apoptosis and cell cycle arrest assays in the A549 human lung cancer cell line.

3. Chemistry

As shown in Scheme 1, commercially available 7-mercapto-4-methyl-2*H*-chromen-2-one (**44**) was reacted with 2,6-dichloropyridine *N*-oxide in pyridine at room temperature to form compound **9**, which was subsequently converted to 7-(6-chloropyridin-2-ylthio)-4-methyl-2*H*-chromen-2-one (**10**) in the presence of PCl_3. Target compounds (**11**–**23**) were synthesized *via* treatment of **44** with various heterocyclic and aryl bromides in the presence of potassium carbonate at room temperature.

The synthesis of 2-ethyl-7-*S*-heterocyclic substituted chromen-4-ones is described in Scheme 2. The commercially available compounds **45a**,**b** were selectively protected as the 4-methoxymethyl (MOM) ethers (**46a**,**b**), followed by condensation with ethyl propionate to afford **47a** and **47b**, which were further treated with concentrated HCl in EtOH to provide the bicyclic compounds (**48a**,**b**). The intermediate 7-mercapto compounds (**51a**,**b**) were prepared from **48a** and **48b** in three steps, *a*-acylation with dimethylthiocarbamoyl chloride to give **49a** and **49b**, followed by Newman–Kwart rearrangement to give **50a** and **50b**,

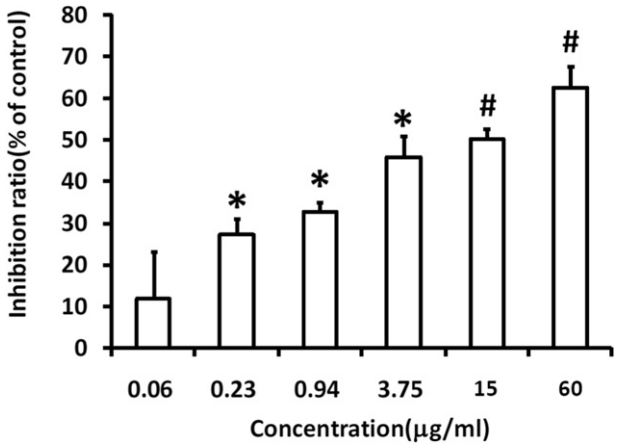


Fig. 2. Anti-proliferative effect of **10** in A549 human lung cancer cells. Each value represents means ± SD in three independent experiments. $^{*}p < 0.05$ *vs.* control group, $^{\#}p < 0.01$ *vs.* control group.

and finally hydrolysis in the presence of methanolic potassium hydroxide. Subsequently, the nucleophilic alkylation of **51a** and **51b** with 2,6-dichloropyridine *N*-oxide yielded the desired compounds (**24**–**27**) using the same procedures as for the synthesis of **10**.

The synthesis of four 7-benzyl ethers (**28**–**31**) and twelve bromides (**32**–**43**) is depicted in Scheme 3. Compounds **28**–**31** were obtained by the etherification of 2-ethyl-7-hydroxy-8-methyl-4*H*-chromen-4-one (**48b**) with four different benzyl bromides in the presence of K_2CO_3 in DMF at 50–60 °C. 7-Alkyl ethers (**52a**–**d**) were synthesized by the alkylation of **48b** with aliphatic halides in acetone at room temperature. Bromides **32**–**43** were prepared by reaction **28**, **29**, and **52a**–**d** with *N*-bromosuccinimide in CH_3CN at room temperature or in CCl_4 under reflux, respectively.

4. Results and discussion

Thirty-five newly synthesized compounds (**9**–**43**) were screened for *in vitro* cytotoxic activity against a panel of human tumor cell lines, including KB (nasopharyngeal), KB-vin (its vincristine-resistant subline), A549 (lung) and DU145 (prostate), with paclitaxel as a reference. The screening results are shown in Table 1.

Overall, compound **10** [7-(6-chloropyridin-2-ylthio)-4-methyl-2*H*-chromen-2-one] was the most potent compound with GI_{50} values ranging from 0.92 to 2.11 μM, while its *N*-oxide (**9**) exhibited only weak cytotoxicity. Interestingly, the four chromones **24**–**27**, which have an isomeric 2-ethyl-4*H*-chromen-4-one coumarin core, with or without an 8-methyl group, showed much lower cytotoxicity than **10**. This result implied that the 4-methyl-2*H*-chromen-2-one skeleton may fit more favorably into the target binding site compared with the 2-ethyl-4*H*-chromen-4-one structure. Most of the remaining coumarin (**11**–**17**) and chromone (**19**–**31**) derivatives, which were modified at the 7-*S* or -*O* position with heterocyclic and aryl rings substituted with electron-donating or -withdrawing groups, showed, at most, weak cytotoxic activity; thus a direct relationship was not found between electronic factors and activity. However, compound **18**, the 1′-methoxy-formyl-benzyl substituted coumarin analog, exhibited promising cancer cell growth inhibitory activity with GI_{50} values ranging from 2.06 to 14.07 μM.

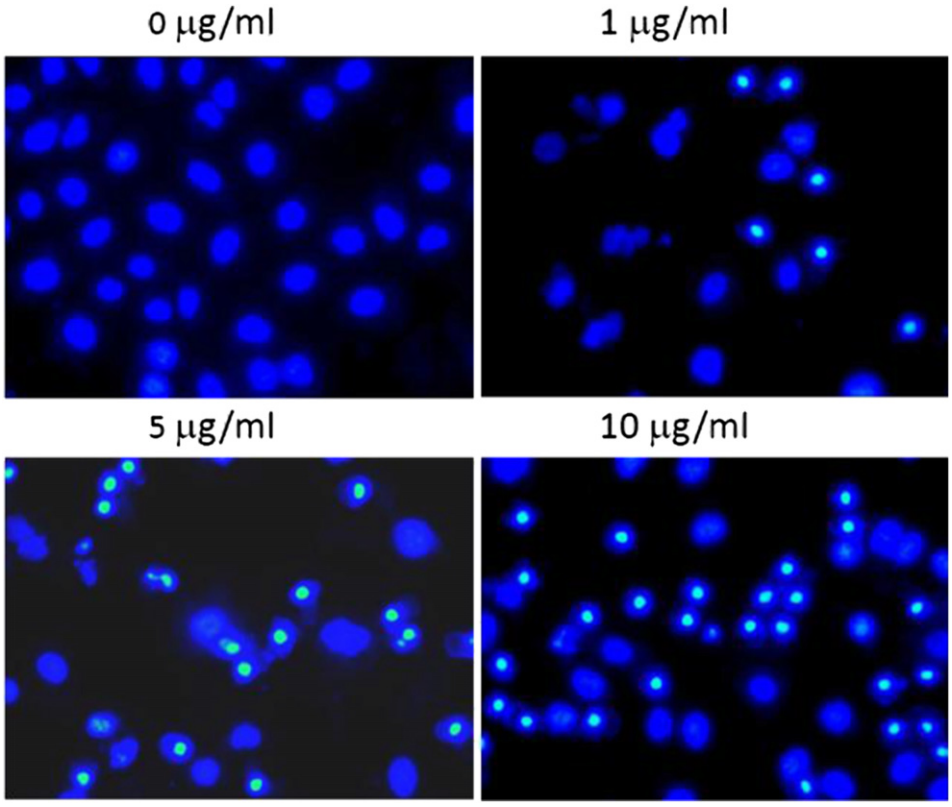


Fig. 3. Compound **10** induced apoptosis in A549 human lung cancer cells. Condensed and fragmented nuclei with bright staining were considered to be apoptotic cells. The results represent three independent experiments.

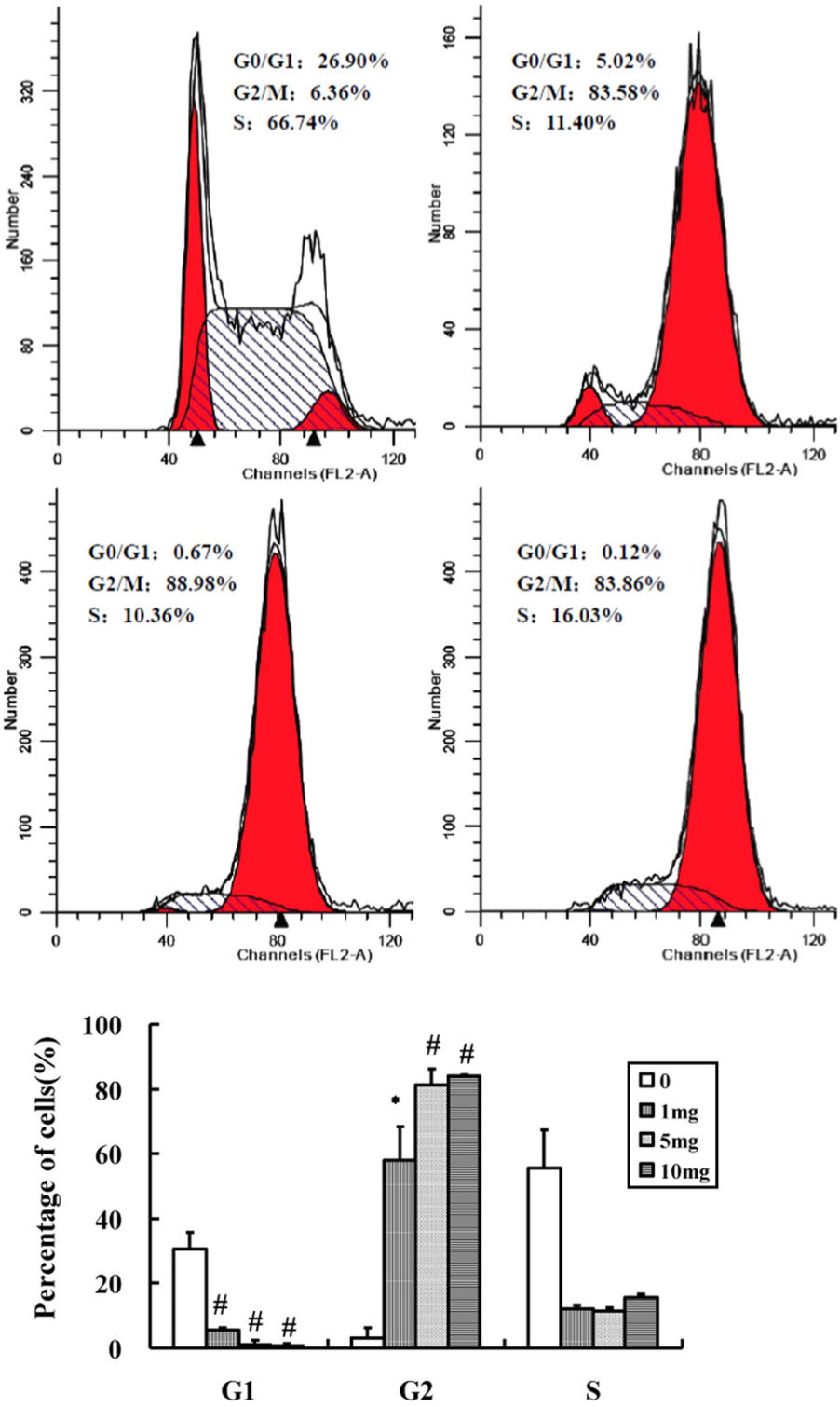


Fig. 4. G2/M cell cycle arrest in A549 cells by **10**. Each value represents mean ± SD from three independent experiments. $^{*}p < 0.05$ *vs.* control group, $^{\#}p < 0.01$ *vs.* control group.

Twelve compounds (**32**–**43**) contained either one or two brominated substituents. While the four 7-*O*-alkyl-8-bromomethyl-2-ethyl analogs (**36**–**39**) were essentially inactive against the four tested cancer cell lines, adding a second bromine at the α-position of the 2-ethyl group increased the inhibitory activity of the dibromo analogs (**40**–**43**). The size of the 7-*O*-alkyl group also affected the activity, with following rank order of potency: methyl < ethyl < isopropyl < isobutyl. Thus, both the size of the 7-alkoxy group and presence of a 2-α-bromine were important for optimal cytotoxicity of the brominated compounds. Interestingly, these compounds showed similar potency against the KB and KB-vin cell lines. Moreover, 3-bromo-2-ethyl-7-isopropoxy-8-methyl-4*H*-chromen-4-one (**33**) selectively inhibited the KB-vin MDR cancer cell line with a GI_{50} value of 5.84 μM, compared with the 1 μM GI_{50} value of the control paclitaxel in the same assay. Analogs with 7-methoxy (**32**) and 7-benzyloxy (**34**)

substitutions did not show the same potency or selectivity against this cell line.

Because compound **10** had a significantly broader spectrum of activity against the four tested human cancer cell lines, it was selected for further pharmacological screening including cell proliferation, apoptosis, and cell cycle arrest assays in the A549 human lung cancer cell line with docetaxel as a control. The data (Figs. 2 and 3) showed that **10** markedly inhibited the proliferation rate of A549 cells and increased the cellular apoptosis, respectively, in a concentration-dependent manner.

Furthermore, the results of **10** on A549 cell cycle progression are shown in Fig. 4. As compared to the control, G2/M phases of A549 cells treated with **10** (0, 1, 5 and 10 μg/mL) for 48 h were 6.36%, 83.58%, 88.98%, and 83.86%, while G0/G1 phases were 26.90%, 5.02%, 0.67%, and 0.12%. These results showed that compound **10** could induce A549 cell cycle arrest at the G2/M phase ($p < 0.01$).

5. Conclusion

Twenty-three novel coumarin and chromone analogs with heterocyclic- and aryl-substituents at the 7-*S* and *O*-positions, and twelve brominated coumarin derivatives were designed and synthesized. Among them, compound **10** not only showed a broader spectrum of activity against four tumor cells, but also remarkably increased cellular apoptosis in a concentration-dependent manner and induced A549 cell cycle arrest at the G2/M phase. Moreover, 3-bromo-2-ethyl-7-isopropoxy-8-methyl-4*H*-chromen-4-one (**33**) showed high selective suppression of MDR KB-vin cell growth, suggesting that groups at the 7-position might interact selectively with different binding pockets of the molecular target. 8-Bromomethyl-2-ethyl compounds (**36**–**39**) were inactive, while the cytotoxic activity of 2-α-bromoethyl-8-bromomethyl compounds (**40**–**43**) increased according to the size of 7-*O*-alkyl group. These findings show that potency is affected by structural change and also indicate that the binding site of the target cancer cell may have a strict structural requirement. Based on these preliminary results, further modification and optimization of the most potent compound, as well as detailed study of the pharmacological target, are warranted.

6. Experimental

6.1. General

Melting points were measured with a Fisher Johns melting apparatus without correction. The proton nuclear magnetic resonance (^{1}H NMR) spectra were measured on 300 MHz Varian Gemini 2000 spectrometer using TMS as internal standard. The solvent used was $CDCl_3$ unless indicated. Mass spectra were measured on HP5973N analytical mass spectrometers. High resolution mass spectra (HRMS) were measured on a Shimadzu LCMSIT-TOF with ESI interface. HPLC purity determinations were conducted using a Shimadzu LCMS-2010 with a Grace Alltima 2.1 × 150 mm HP C18 3 μM column and Shimadzu SPD-M20A detector at 254 nm wavelength in MeCN/H_2O and MeOH/H_2O two different solvent conditions. All target compounds had purity greater than 95%. Commercially available silica gel H was used for column chromatography. Thin-layer chromatography (TLC) was performed on PLC silica gel 60 F254 plates.

6.2. N-Oxide-7-(6-chloropyridin-2-ylthio)-4-methyl-2H-chromen-2-one (**9**)

A mixture of **44** (300 mg, 1.56 mmol), 2,6-dichloropyridine *N*-oxide (256 mg, 1.56 mmol) in pyridine (6 mL) was stirred for 2 h at rt. Then, 2 N HCl (50 mL) was added to the reaction, followed by filtration, and removal of the solvent *in vacuo*. The residue was purified by column chromatography (eluent: CH_2Cl_2/EtOAc 99:1) to afford the desired compound (**9**) as white crystals in 48% yield. Mp 236–237 °C. ^{1}H NMR δ 2.50 (3H, s, 4-CH_3), 6.41 (1H, s, 3-H), 6.55 (1H, dd, *J1* = 8.1 Hz, *J2* = 1.8 Hz, 6-H), 7.01 (1H, t, *J* = 8.4 Hz, H of pyridine), 7.28, 7.54 (each 1H, dd, *J1* = 8.4 Hz, *J2* = 1.8 Hz, 2H of pyridine), 7.62 (1H, d, *J* = 1.8 Hz, 8-H), 7.73 (1H, d, *J* = 8.1 Hz, 5-H). ^{13}C NMR δ 18.83, 58.42, 116.76, 120.27, 122.30, 124.00, 125.77, 126.45, 131.11, 133.28, 141.80, 151.75, 154.05, 154.91, 159.97. ESI MS *m*/*z* 320 ($M^+ + 1$).

6.3. 7-(6-Chloropyridin-2-ylthio)-4-methyl-2H-chromen-2-one (**10**)

A solution of compound **9** (500 mg, 1.56 mmol) in PCl_3 (2 mL) and $CHCl_3$ (20 mL) was heated at reflux temperature for 1.5 h. The mixture was diluted with ice-water (200 mL) and extracted with CH_2Cl_2 (30 mL × 4). After drying the organic phase over anhydrous Na_2SO_4, the mixture was filtered and the solvent was removed under reduced pressure. Then, the residue was purified by column chromatography (eluent: CH_2Cl_2/MeOH 20:1) to give **10** as a white crystalline solid in 48% yield. Mp 138–140 °C. ^{1}H NMR δ 2.46 (3H, s, 4-CH_3), 6.33 (1H, s, 3-H), 7.01, 7.13, 7.52 (each 1H, d, *J* = 7.8 Hz, 3H of pyridine), 7.45 (1H, d, *J* = 8.4 Hz, 6-H), 7.48 (1H, s, 8-H), 7.62 (1H, d, *J* = 8.4 Hz, 5-H). ^{13}C NMR δ 18.94, 116.55, 121.00, 122.05, 122.26, 122.31, 126.24, 129.81, 136.78, 140.25, 152.41, 152.80, 154.75, 160.35, 161.25. ESI MS *m*/*z* 304 ($M^+ + 1$).

6.4. General procedure for synthesis of 4-methyl-7-heterocyclic and aryl-substituted 7-thio-chromen-2H-2-ones (**11**–**23**)

A mixture of **44** (300 mg, 1.56 mmol), K_2CO_3 (372 mg, 2.34 mmol), and halogenated compound (1 equiv) in acetone (10 mL) was stirred for 0.5–3.5 h at rt. After filtering the mixture and removing the solvent *in vacuo*, the residue was purified by column chromatography (eluent: hexane/EtOAc 85:15) to obtain the target compounds (**11**–**23**) as white solids.

6.4.1. 4-Methyl-7-((2-oxo-tetrahydrofuran-3-yl)methylthio)-2H-chromen-2-one (**11**)

Yield 81%, mp 137–138 °C. ^{1}H NMR δ 2.34, 2.80 (2H, m, *J* = 6.3 Hz, furanone 3-H_2), 2.43 (3H, s, 4-CH_3), 4.06 (1H, t, *J* = 6.3 Hz, furanone 2-H), 4.40 (2H, m, *J* = 6.3 Hz, furanone 4-H_2), 6.29 (1H, s, 3-H), 7.42 (1H, dd, *J1* = 8.4 Hz, *J2* = 1.5 Hz, 6-H), 7.44 (1H, d, *J* = 1.5 Hz, 8-H), 7.55 (1H, d, *J* = 8.4 Hz, 5-H). ESI MS *m*/*z* 299 ($M^+ + Na$).

6.4.2. 4-Methyl-7-(pyridin-2-ylmethylthio)-2H-chromen-2-one (**12**)

Yield 37%, mp 92–93 °C. ^{1}H NMR δ 2.39 (3H, s, 4-CH_3), 4.36 (2H, s, 7-SCH_2–), 6.21 (1H, s, 3-H), 7.17–7.27 (3H, m, *J1* = 8.4 Hz, *J2* = 7.8 Hz, 6-H & 8-H & 1H of pyridine), 7.41–7.66 (3H, m, *J1* = 8.4 Hz, *J2* = 7.8 Hz, 2H of pyridine & 5-H), 8.57 (1H, d, *J* = 7.8 Hz, 1H of pyridine). ESI MS *m*/*z* 284 ($M^+ + H$).

6.4.3. 4-Methyl-7-(pyridin-3-ylmethylthio)-2H-chromen-2-one (**13**)

Yield 57%, mp 128–129 °C. ^{1}H NMR δ 2.40 (3H, s, 4-CH_3), 4.20 (2H, s, 7-SCH_2–), 6.24 (1H, s, 3-H), 7.15 (1H, dd, *J1* = 8.4 Hz, *J2* = 1.5 Hz, 6-H), 7.20 (1H, d, *J* = 1.5 Hz, 8-H), 7.26 (1H, t, *J* = 7.8 Hz, 1H of pyridine), 7.46 (1H, d, *J* = 8.4 Hz, 5-H), 7.71 (1H, dt, *J1* = 7.8 Hz, *J2* = 1.8 Hz, 1H of pyridine), 8.50, 8.57 (2H, m, *J1* = 7.8 Hz, *J2* = 1.8 Hz, 2H of pyridine). ESI MS *m*/*z* 282 ($M^+ - H$).

6.4.4. 4-Methyl-7-(pyridin-4-ylmethylthio)-2H-chromen-2-one (**14**)

Yield 67.39%, mp 155–156 °C. ^{1}H NMR δ 2.40 (3H, s, 4-CH_3), 4.18 (2H, s, 7-SCH_2–), 6.23 (1H, s, 3-H), 7.13 (1H, dd, *J1* = 8.7 Hz,

J2 = 1.8 Hz, 6-H), 7.17 (1H, d, *J* = 1.8 Hz, 8-H), 7.31, 8.55 (4H, dd, *J1* = 6.0 Hz, *J2* = 1.8 Hz, H of pyridine), 7.45 (1H, d, *J* = 8.7 Hz, 5-H). ESI MS *m/z* 282 ($M^+ - H$).

*6.4.5. 4-Methyl-7-(3-trifluoromethyl)benzylthio-2H-chromen-2-one (**15**)*

Yield 85.96%, mp 139–140 °C. ^{1}H NMR δ 2.40 (3H, s, 4-CH_3), 4.25 (2H, s, 7-SCH_2–), 6.23 (1H, s, 3-H), 7.13–7.18 (2H, m, *J* = 8.7 Hz, 6-H & 8-H), 7.45–7.61 (5H, m, *J1* = 8.7 Hz, *J2* = 6.6 Hz, 4H of aromatic & 5-H). ESI MS *m/z* 349 ($M^+ - 1$).

*6.4.6. 3-((4-Methyl-2-oxo-2H-chromen-7-ylthio)methyl) benzonitrile (**16**)*

Yield 10% (starting from 200 mg of **44**), mp 172–173 °C. ^{1}H NMR δ 2.41 (3H, s, 4-CH_3), 4.22 (2H, s, 7-SCH_2–), 6.23 (1H, s, 3-H), 7.13–7.17 (2H, m, *J1* = 9.0 Hz, *J2* = 2.1 Hz, 6-H & 8-H), 7.41–7.66 (5H, m, *J1* = 9.0 Hz, *J2* = 7.5 Hz, 4H of aromatic & 5-H). ESI MS *m/z* 330 ($M^+ + Na$).

*6.4.7. 7-(3-Methoxybenzylthio)-4-methyl-2H-chromen-2-one (**17**)*

Yield 17% (starting from 200 mg of **44**), mp 137–138 °C. ^{1}H NMR δ 2.39 (3H, s, 4-CH_3), 3.79 (3H, s, OCH_3 of aromatic), 4.19 (2H, s, 7-SCH_2–), 6.21 (1H, s, 3-H), 6.81, 6.68 (3H, dd, *J1* = 8.7 Hz, *J2* = 2.7 Hz, H of aromatic), 7.14–7.26 (3H, m, *J1* = 8.4 Hz, *J2* = 8.7 Hz, 1H of aromatic and 6-H & 8-H), 7.44 (1H, d, *J* = 8.4 Hz, 5-H). ESI MS *m/z* 311 ($M^+ - H$).

*6.4.8. Methyl 3-((4-methyl-2-oxo-2H-chromen-7-ylthio)methyl) benzoate (**18**)*

Yield 12% (starting from 200 mg of **44**), mp 121–123 °C. ^{1}H NMR δ 2.40 (3H, s, 4-CH_3), 3.92 (3H, s, –OCH_3), 4.25 (2H, s, 7-SCH_2–), 6.22 (1H, s, 3-H), 7.14–7.18 (2H, m, *J1* = 8.1 Hz, *J2* = 2.1 Hz, 6-H & 8-H), 7.38, 7.57, 7.94 (3H, d, *J* = 7.5 Hz, H of aromatic), 7.45 (1H, d, *J* = 8.1 Hz, 5-H), 8.06 (1H, s, H of aromatic). ESI MS *m/z* 339 ($M^+ - H$).

*6.4.9. 7-(3-Methylbenzylthio)-4-methyl-2H-chromen-2-one (**19**)*

Yield 84.32%, mp 100–101 °C. ^{1}H NMR δ 2.35 (3H, s, CH_3 of aromatic), 2.40 (3H, s, 4-CH_3), 4.19 (2H, s, 7-SCH_2–), 6.21 (1H, s, 3-H), 7.08 (1H, d, *J* = 8.1 Hz, 6-H), 7.14–7.22 (5H, m, *J* = 7.2 Hz, 4H of aromatic & 8-H); 7.45 (1H, d, *J* = 8.1 Hz, 5-H). ESI MS *m/z* 295 ($M^+ - 1$).

*6.4.10. 7-(3,5-Dimethoxybenzylthio)-4-methyl-2H-chromen-2-one (**20**)*

Yield 14%, mp 146–148 °C. ^{1}H NMR δ 2.39 (3H, s, 4-CH_3), 3.77 (6H, s, 2× OCH_3 of aromatic), 4.16 (2H, s, 7-SCH_2–), 6.21 (1H, s, 3-H), 6.37, 6.55, 6.81 (3H, s, H of aromatic), 7.16 (1H, dd, *J1* = 8.1 Hz, *J2* = 1.5 Hz, 6-H), 7.18 (1H, d, *J* = 1.5 Hz, 8-H), 7.45 (1H, d, *J* = 8.1 Hz, 5-H). ESI MS *m/z* 341 ($M^+ - H$).

*6.4.11. 7-(3-(Trifluoromethoxy)benzylthio)-4-methyl-2H-chromen-2-one (**21**)*

Yield 89%, mp 106–107 °C. ^{1}H NMR δ 2.40 (3H, s, 4-CH_3), 4.21 (2H, s, 7-SCH_2–), 6.22 (1H, s, 3-H), 7.13–7.18 (3H, m, *J* = 6.5 Hz, H of aromatic), 7.22 (1H, s, H of aromatic), 7.33 (1H, dd, *J1* = 8.4 Hz, *J2* = 1.5 Hz, 6-H), 7.35 (1H, d, *J* = 1.5 Hz, 8-H), 7.45 (1H, d, *J* = 8.4 Hz, 5-H). ESI MS *m/z* 365 ($M^+ - H$).

*6.4.12. 7-(2-Bromo-5-methoxybenzylthio)-4-methyl-2H-chromen-2-one (**22**)*

Yield 84%, mp 136–137 °C. ^{1}H NMR δ 2.40 (3H, s, 4-CH_3), 3.74 (3H, s, OCH_3 of aromatic), 4.28 (2H, s, 7-SCH_2–), 6.23 (1H, s, 3-H), 6.71 (1H, d, *J1* = 9.0 Hz, *J2* = 3.0 Hz, H of aromatic), 6.99 (1H, d, *J* = 3.0 Hz, H of aromatic), 7.17–7.20 (2H, m, *J* = 9.0 Hz, 6-H & 8-H), 7.26 (1H, s, H of aromatic), 7.47 (1H, d, *J* = 9.0 Hz, 5-H). ESI MS *m/z* 391 ($M^+ + 1$).

*6.4.13. 7-(4,5-Dimethoxy-2-nitrobenzylthio)-4-methyl-2H-chromen-2-one (**23**)*

Yellow solid, yield 21% (starting from 100 mg of **44**), mp 173–175 °C. ^{1}H NMR δ 2.40 (3H, s, 4-CH_3), 3.88, 3.96 (each 3H, s, 2× OCH_3 of aromatic), 4.62 (2H, s, 7-SCH_2–), 6.24 (1H, s, 3-H), 6.94, 7.69 (each 1H, s, 2H of aromatic), 7.18 (1H, dd, *J1* = 8.4 Hz, *J2* = 1.8 Hz, 6-H), 7.23 (1H, d, *J* = 1.8 Hz, 8-H), 7.47 (1H, d, *J* = 8.4 Hz, 5-H). ESI MS *m/z* 389 ($M^+ + 2$).

*6.5. 1-(2-Hydroxy-4-(methoxymethoxy)-3-methylphenyl)ethanone (**46b**)*

Chloromethyl methyl ether (9.14 mL, 120 mmol) was added dropwise into a mixture of **45b** (10.0 g, 60.2 mmol) and potassium carbonate (20.8 g, 150 mmol) in anhydrous acetone (60 mL) in an ice-bath. The reaction mixture was allowed to warm to rt and stirred for 1.5 h. The mixture was then filtered, and the filtrate was dissolved in brine and extracted with EtOAc (35 mL). The organic layer was dried *in vacuo* to provide **46b** (13.9 g) as brown oil, 91% yield. ^{1}H NMR δ 2.14 (3H, s, 3-CH_3), 2.56 (3H, s, 1-$COCH_3$), 3.50 (3H, s, 4-OCH_2OCH_3), 5.27 (2H, s, 4-OCH_2OCH_3), 6.65 (1H, d, *J* = 8.7 Hz, 6-H), 7.57 (1H, d, *J* = 8.7 Hz, 5-H), 12.80 (1H, s, 2-OH). ESI MS *m/z* 209 ($M^+ - 1$).

*6.6. Preparation of **48a** and **48b***

*6.6.1. 2-Ethyl-7-hydroxy-4H-chromen-4-one (**48a**)*

The synthesis of the intermediate **48a** from **45a** was reported previously in Ref. [9].

*6.6.2. 2-Ethyl-7-hydroxy-8-methyl-4H-chromen-4-one (**48b**)*

Sodium hydride (60% in mineral oil, 9.52 g/15.9 g, 397 mmol) was added slowly to a mixture of **46b** (13.9 mg, 66.1 mmol) and ethyl propionate (19.0 mL, 165 mmol) in absolute THF under nitrogen. The mixture was warmed to reflux temperature for 2 h, cooled, and neutralized to pH 8 with 37% HCl (25 mL). Water (60 mL) was added and the mixture was extracted with EtOAc (4 × 30 mL). The organic layer was collected and evaporated *in vacuo* to yield **47b** as dark oil. This crude product and 37% HCl (3 mL) were dissolved in EtOH (60 mL) and refluxed for 45 min to give **48b**, which was used in the next reaction without further purification. Mp 223–224 °C. ^{1}H NMR (DMSO, δ) 1.23 (3H, t, *J* = 7.2 Hz, 2-CH_2CH_3), 2.20 (3H, s, 8-CH_3), 2.65 (2H, q, *J* = 7.2 Hz, 2-CH_2CH_3), 6.06 (1H, s, 3-H), 6.98 (1H, d, *J* = 8.7 Hz, 6-H), 7.68 (1H, d, *J* = 8.7 Hz, 5-H). ESI MS *m/z* 203 ($M^+ - 1$).

*6.7. Preparation of **49a** and **49b***

*6.7.1. O-2-Ethyl-4-oxo-4H-chromen-7-yl dimethylcarbamothioate (**49a**)*

Compound **48a** (1. 0 g, 5.26 mmol) and K_2CO_3 (1.82 g, 13.15 mmol) were dissolved in DMF (5 mL) and reacted with dimethylthiocarbamoyl chloride (1.3 g, 10.52 mmol) for 1.5 h at rt. The mixture was poured into ice-water (200 mL) and filtered to afford crude product, which was purified by column chromatography (eluent: petroleum ether/EtOAc 4:1) to give **49a** as a white solid (1.37 mg). Yield 94%, mp 137–139 °C. ^{1}H NMR δ 1.31 (3H, d, *J* = 7.5 Hz, 2-CH_2CH_3), 2.65 (2H, q, *J* = 7.5 Hz, 2-CH_2CH_3), 3.38, 3.48 (each 3H, s, N–$(CH_3)_2$), 6.17 (1H, s, 3-H), 7.10 (1H, dd, *J1* = 8.7 Hz, *J2* = 2.1 Hz, 6-H), 7.18 (1H, d, *J* = 2.1 Hz, 8-H), 8.20 (1H, d, *J* = 8.7 Hz, 5-H). ESI MS *m/z* 278 ($M^+ + H$).

*6.7.2. O-2-Ethyl-8-methyl-4-oxo-4H-chromen-7-yl dimethylcarbamothioate (**49b**)*

The procedure was the same as that used for the preparation of **49a**. Yield 76% (starting from 500 mg of **48b**), mp 164–166 °C. ^{1}H

NMR δ 1.33 (3H, d, J = 7.5 Hz, 2-CH_2CH_3), 2.30 (3H, s, 8-CH_3), 2.70 (2H, q, J = 7.5 Hz, 2-CH_2CH_3), 3.41, 3.49 (each 3H, s, N–$(CH_3)_2$), 6.19 (1H, s, 3-H), 7.05 (1H, d, J = 8.7 Hz, 6-H), 8.05 (1H, d, J = 8.7 Hz, 5-H). ESI MS m/z 292 ($M^+ + H$).

6.8. Preparation of **50a** and **50b**

6.8.1. S-2-Ethyl-4-oxo-4H-chromen-7-yl dimethylcarbamothioate (**50a**)

Compound **49a** (300 mg, 1.08 mmol) was heated to 220–230 °C with stirring for 1 h. The crude product was purified by column chromatography (eluent: CH_2Cl_2/EtOAc 99:1) to give **50a** as light yellow crystals (170 mg). Yield 57%, mp 121–123 °C. ^{1}H NMR δ 1.31 (3H, d, J = 7.5 Hz, 2-CH_2CH_3), 2.65 (2H, q, J = 7.5 Hz, 2-CH_2CH_3), 3.06, 3.12 (each 3H, s, N–$(CH_3)_2$), 6.19 (1H, s, 3-H), 7.46 (1H, dd, $J1$ = 8.4 Hz, $J2$ = 1.5 Hz, 6-H), 7.67 (1H, d, J = 1.5 Hz, 8-H), 8.16 (1H, d, J = 8.4 Hz, 5-H). ESI MS m/z 278 ($M^+ + H$).

6.8.2. S-2-Ethyl-8-methyl-4-oxo-4H-chromen-7-yl dimethylcarbamothioate (**50b**)

The procedure was the same as that used for the preparation of **50a**. Yield 34% (starting from 450 mg of **49b**), mp 202–204 °C. ^{1}H NMR δ 1.33 (3H, d, J = 7.5 Hz, 2-CH_2CH_3), 2.57 (3H, s, 8-CH_3), 2.70 (2H, q, J = 7.5 Hz, 2-CH_2CH_3), 3.04, 3.16 (each 3H, s, N–$(CH_3)_2$), 6.20 (1H, s, 3-H), 7.50 (1H, d, J = 8.1 Hz, 6-H), 8.01 (1H, d, J = 8.1 Hz, 5-H). ESI MS m/z 292 ($M^+ + H$).

6.9. Preparation of **51a** and **51b**

6.9.1. 2-Ethyl-7-mercapto-4H-chromen-4-one (**51a**)

Under nitrogen and in the dark, compound **50a** (170 mg, 0.61 mmol) was hydrolyzed in the presence of KOH (103 mg, 1.84 mmol) in MeOH (10 mL) for 1.5 h at reflux temperature. After cooling to rt, conc. HCl was added to pH 1–2 and ice-water (50 mL) was added. The mixture was extracted with EtOAc (20 mL × 3), and the combined organic layer was dried over anhydrous Na_2SO_4. Product **51a** (120 mg) was obtained after filtration and removal of solvent under reduced pressure. Yield 95%, mp 73–75 °C. ^{1}H NMR δ 1.30 (3H, d, J = 7.8 Hz, 2-CH_2CH_3), 2.64 (2H, q, J = 7.8 Hz, 2-CH_2CH_3), 3.73 (1H, s, 7-SH), 6.15 (1H, s, 3-H), 7.20 (1H, dd, $J1$ = 8.4 Hz, $J2$ = 1.8 Hz, 6-H), 7.30 (1H, d, J = 1.8 Hz, 8-H), 8.02 (1H, d, J = 8.4 Hz, 5-H). ESI MS m/z 205 ($M^+ - H$).

6.9.2. 2-Ethyl-7-mercapto-8-methyl-4H-chromen-4-one (**51b**)

The procedure was the same as that used for the preparation of **51a**. Yield 88% (starting from 150 mg of **50b**), mp 107–109 °C. ^{1}H NMR δ 1.33 (3H, d, J = 7.8 Hz, 2-CH_2CH_3), 2.43 (3H, s, 8-CH_3), 2.69 (2H, q, J = 7.8 Hz, 2-CH_2CH_3), 3.62 (1H, s, 7-SH), 6.16 (1H, s, 3-H), 7.23 (1H, d, J = 8.4 Hz, 6-H), 7.88 (1H, d, J = 8.4 Hz, 5-H). ESI MS m/z 219 ($M^+ - H$).

6.10. Preparation of **24** and **25**

6.10.1. N-Oxide-7-(6-chloropyridin-2-ylthio)-2-ethyl-4H-chromen-4-one (**24**)

The procedure was the same as that used for the preparation of **9**. Yield 51% (starting from 130 mg of **51a**), mp 163–164 °C. ^{1}H NMR δ 1.34 (3H, d, J = 7.5 Hz, 2-CH_2CH_3), 2.70 (2H, q, J = 7.5 Hz, 2-CH_2CH_3), 6.26 (1H, s, 3-H), 6.55, 7.29 (each 1H, dd, $J1$ = 8.1 Hz, $J2$ = 1.8 Hz, 2H of pyridine), 7.01 (1H, t, J = 8.1 Hz, H of pyridine), 7.58 (1H, dd, $J1$ = 8.1 Hz, $J2$ = 1.8 Hz, 6-H), 7.76 (1H, d, J = 1.8 Hz, 8-H), 8.29 (1H, d, J = 8.1 Hz, 5-H). ^{13}C NMR δ 11.07, 18.58, 27.68, 58.59, 109.66, 120.32, 122.31, 125.28, 127.79, 131.58, 135.47, 141.76, 154.72, 156.83, 171.61, 177.67. ESI MS m/z 256 ($M^+ + Na$).

6.10.2. N-Oxide-7-(6-chloropyridin-2-ylthio)-2-ethyl-8-methyl-4H-chromen-4-one (**25**)

The procedure was the same as that used for the preparation of **9**. Yield 53% (starting from 100 mg of **51b**), mp 190–192 °C. ^{1}H NMR δ 1.37 (3H, d, J = 7.8 Hz, 2-CH_2CH_3), 2.60 (3H, s, 8-CH_3), 2.74 (2H, q, J = 7.8 Hz, 2-CH_2CH_3), 6.27 (1H, s, 3-H), 6.34, 7.27 (each 1H, dd, $J1$ = 8.1 Hz, $J2$ = 1.8 Hz, 2H of pyridine), 6.98 (1H, t, J = 8.1 Hz, H of pyridine), 7.62 (1H, d, J = 8.4 Hz, 6-H), 8.12 (1H, d, J = 8.4 Hz, 5-H). ^{13}C NMR δ 11.17, 13.71, 18.57, 27.78, 58.60, 109.37, 119.73, 122.07, 125.14, 132.47, 133.65, 134.32, 141.93, 154.00, 155.34, 171.25, 178.30. ESI MS m/z 270 ($M^+ + Na$).

6.11. Preparation of **26** and **27**

6.11.1. 7-(6-Chloropyridin-2-ylthio)-2-ethyl-4H-chromen-4-one (**26**)

The procedure was the same as that used for the preparation of **10**. Yield 12% (starting from 60 mg of **24**), mp 106–107 °C. ^{1}H NMR δ 1.32 (3H, d, J = 7.5 Hz, 2-CH_2CH_3), 2.67 (2H, q, J = 7.5 Hz, 2-CH_2CH_3), 6.21 (1H, s, 3-H), 7.02, 7.14 (each 1H, d, J = 7.8 Hz, 2H of pyridine), 7.48 (1H, dd, $J1$ = 8.1 Hz, $J2$ = 1.8 Hz, 6-H), 7.51 (1H, t, J = 7.8 Hz, H of pyridine), 7.66 (1H, d, J = 1.8 Hz, 8-H), 8.18 (1H, d, J = 8.1 Hz, 5-H). ESI MS m/z 318 ($M^+ + H$).

6.11.2. 7-(6-Chloropyridin-2-ylthio)-2-ethyl-8-methyl-4H-chromen-4-one (**27**)

The procedure was the same as that used for the preparation of **10**. Yield 6% (starting from 50 mg of **25**), mp 117–118 °C. ^{1}H NMR δ 1.36 (3H, d, J = 7.5 Hz, 2-CH_2CH_3), 2.57 (3H s, 8-CH_3), 2.73 (2H, q, J = 7.8 Hz, 2-CH_2CH_3), 6.24 (1H, s, 3-H), 6.73, 7.08 (each 1H, d, J = 7.8 Hz, 2H of pyridine), 7.44 (1H, t, J = 7.8 Hz, H of pyridine), 7.55 (1H, d, J = 8.1 Hz, 6-H), 8.06 (1H, d, J = 8.1 Hz, 5-H). ESI MS m/z 332 ($M^+ + H$).

6.12. Synthesis of 7-aryloxy-substituted-8-methyl-4H-chromen-4-one (**28**–**31**)

The procedure was similar to that used in the preparation of **11**–**23** with DMF/50–60 °C instead of reaction in acetone at room temperature.

6.12.1. 7-(Benzyloxy)-2-ethyl-8-methyl-4H-chromen-4-one (**28**)

Yield 98% (starting from 340 mg of **48b**), mp 98–99 °C. ^{1}H NMR δ 1.33 (3H, t, J = 7.2 Hz, 2-CH_2CH_3), 2.36 (3H, s, 8-CH_3), 2.68 (2H, q, J = 7.2 Hz, 2-CH_2CH_3), 5.21 (2H, s, 7-OCH_2–), 6.12 (1H, s, 3-H), 7.01 (1H, d, J = 9.0 Hz, 6-H), 7.37–7.45 (5H, m, H of aromatic), 8.03 (1H, d, J = 9.0 Hz, 5-H). ESI MS m/z 293 ($M^+ - 1$).

6.12.2. 2-Ethyl-8-methyl-7-(3-methylbenzyloxy)-4H-chromen-4-one (**29**)

Yield 62% (starting from 322 mg of **48b**), mp 93–94 °C. ^{1}H NMR δ 1.33 (3H, t, J = 7.8 Hz, 2-CH_2CH_3), 2.34 (3H, s, 3-CH_3 of aromatic), 2.38 (3H, s, 8-CH_3), 2.69 (2H, q, J = 7.8 Hz, 2-CH_2CH_3), 5.22 (2H, s, 7-OCH_2–), 6.13 (1H, s, 3-H), 7.04 (1H, d, J = 9.0 Hz, 6-H), 7.15 (1H, d, J = 7.8 Hz, H of aromatic), 7.25 (3H, m, J = 7.8 Hz, H of aromatic), 8.13 (1H, d, J = 9.0 Hz, 5-H). ESI MS m/z 307 ($M^+ - 1$).

6.12.3. 2-Ethyl-8-methyl-7-(3-(trifluoromethyl)benzyloxy)-4H-chromen-4-one (**30**)

Yield 47% (starting from 500 mg of **48b**), mp 122–123 °C. ^{1}H NMR δ 1.34 (3H, t, J = 7.5 Hz, 2-CH_2CH_3), 2.38 (3H, s, 8-CH_3), 2.69 (2H, q, J = 7.5 Hz, 2-CH_2CH_3), 5.25 (2H, s, 7-OCH_2–), 6.13 (1H, s, 3-H), 6.99 (1H, d, J = 9.0 Hz, 6-H), 7.55 (1H, t, J = 7.2 Hz, H of aromatic), 7.64 (2H, t, J = 7.2 Hz, H of aromatic), 7.73 (1H, s, H of aromatic), 8.04 (1H, d, J = 9.0 Hz, 5-H). ESI MS m/z 363 ($M^+ + 1$).

*6.12.4. 3-((2-Ethyl-8-methyl-4-oxo-4H-chromen-7-yloxy)methyl) benzonitrile (**31**)*

Yield 52% (starting from 500 mg of **48b**), mp 176–177 °C. ^{1}H NMR δ 1.34 (3H, t, J = 7.8 Hz, 2-CH_2CH_3), 2.38 (3H, s, 8-CH_3), 2.69 (2H, q, J = 7.8 Hz, 2-CH_2CH_3), 5.23 (2H, s, 7-OCH_2–), 6.13 (1H, s, 3-H), 6.97 (1H, d, J = 9.0 Hz, 6-H), 7.55–7.67 (3H, t, J = 8.1 Hz, H of aromatic), 7.77 (1H, s, H of aromatic), 8.03 (1H, d, J = 9.0 Hz, 5-H). ESI MS m/z 320 ($M^+ + 1$).

*6.13. Synthesis of 2-ethyl-7-alkoxy-8-methyl-4H-chromen-4-one (**52a–d**)*

The procedure was the same as that used for the preparation of **11–23**.

*6.13.1. 2-Ethyl-7-methoxy-8-methyl-4H-chromen-4-one (**52a**)*

Yield 75% (starting from 2.0 g of **48b**), mp 104–106 °C. ^{1}H NMR δ 1.32 (3H, t, J = 7.5 Hz, 2-CH_2CH_3), 2.30 (3H, s, 8-CH_3), 2.68 (2H, q, J = 7.5 Hz, 2-CH_2CH_3), 3.95 (3H, s, 7-OCH_3), 6.11 (1H, s, 3-H), 6.96 (1H, d, J = 9.0 Hz, 6-H), 8.10 (1H, d, J = 9.0 Hz, 5-H). ESI MS m/z 219 ($M^+ + 1$).

*6.13.2. 7-Ethoxy-2-ethyl-8-methyl-4H-chromen-4-one (**52b**)*

Yield 73%, mp 85–87 °C. ^{1}H NMR δ 1.33 (3H, t, J = 7.5 Hz, 2-CH_2CH_3), 1.48 (3H, t, J = 7.2 Hz, 7-OCH_2CH_3), 2.31 (3H, s, 8-CH_3), 2.68 (2H, q, J = 7.5 Hz, 2-CH_2CH_3), 4.17 (2H, q, J = 7.2 Hz, 7-OCH_2CH_3), 6.11 (1H, s, 3-H), 6.93 (1H, d, J = 9.0 Hz, 6-H), 8.01 (1H, d, J = 9.0 Hz, 5-H). ESI MS m/z 233 ($M^+ + 1$).

*6.13.3. 2-Ethyl-7-isopropoxy-8-methyl-4H-chromen-4-one (**52c**)*

Yield 79% (starting from 2.0 g of **48b**), mp 99–101 °C. ^{1}H NMR δ 1.33 (3H, t, J = 6.9 Hz, 2-CH_2CH_3), 1.38 (6H, d, J = 6.0 Hz, 7-$OCH(CH_3)_2$), 2.29 (3H, s, 8-CH_3), 2.68 (2H, q, J = 6.9 Hz, 2-CH_2CH_3), 4. 70 (2H, m, J = 6.0 Hz, 7-$OCH(CH_3)_2$), 6.11 (1H, s, 3-H), 6.95 (1H, d, J = 9.0 Hz, 6-H), 8.01 (1H, d, J = 9.0 Hz, 5-H). ESI MS m/z 247 ($M^+ + 1$).

*6.13.4. 2-Ethyl-7-isobutoxy-8-methyl-4H-chromen-4-one (**52d**)*

Yield 82%, mp 61–62 °C. ^{1}H NMR δ 1.07 (6H, d, J = 6.6 Hz, 7-$OCH_2CH(CH_3)_2$), 1.33 (3H, t, J = 7.2 Hz, 2-CH_2CH_3), 2.16 (1H, m, J = 6.6 Hz, 7-$OCH_2CH(CH_3)_2$), 2.32 (3H, s, 8-CH_3), 2.68 (2H, J = 7.2 Hz, 2-CH_2CH_3), 3.86 (2H, d, J = 6.6 Hz, 7-$OCH_2CH(CH_3)_2$), 6.11 (1H, s, 3-H), 6.93 (1H, d, J = 9.0 Hz, 6-H), 8.01 (1H, d, J = 9.0 Hz, 5-H). ESI MS m/z 261 ($M^+ + 1$).

*6.14. Synthesis of 3-bromo-2-ethyl-7-alkoxy or 7-aryloxy-8-methyl-4H-chromen-4-one (**32–35**)*

A mixture of **28**, **30**, **52a** or **52c** (1.0 equiv) and *N*-bromosuccinimide (1.2 equiv) in CH_3CN (5 mL) was stirred for 1 day at rt. After filtration and removal of the solvent, the residue was purified by column chromatography (eluent: CH_2Cl_2/MeOH 99:1) to obtain 3-brominated compounds (**32–35**) as white solids.

*6.14.1. 3-Bromo-2-ethyl-7-methoxy-8-methyl-4H-chromen-4-one (**32**)*

Yield 44% (starting from 50 mg of **52a**), mp 115–117 °C. ^{1}H NMR δ 1.38 (3H, t, J = 7.5 Hz, 2-CH_2CH_3), 2.31 (3H, s, 8-CH_3), 3.01 (2H, q, J = 7.5 Hz, 2-CH_2CH_3), 3.96 (3H, s, 7-OCH_3), 6.99 (1H, d, J = 8.7 Hz, 6-H), 8.08 (1H, d, J = 8.7 Hz, 5-H). ESI MS m/z 297 ($M^+ + 1$).

*6.14.2. 3-Bromo-2-ethyl-7-isopropoxy-8-methyl-4H-chromen-4-one (**33**)*

Yield 41% (starting from 50 mg of **52c**), mp 96–98 °C. ^{1}H NMR δ 1.35–1.42 (9H, m, *J1* = 6.0 Hz, *J2* = 7.5 Hz, 2-CH_2CH_3 & 7-$OCH(CH_3)_2$), 2.30 (3H, s, 8-CH_3), 3.01 (2H, q, J = 7.5 Hz, 2-CH_2CH_3), 4.70 (2H, m, J = 6.0 Hz, 7-$OCH(CH_3)_2$), 6.98 (1H, d, J = 9.0 Hz, 6-H), 8.04 (1H, d, J = 9.0 Hz, 5-H). ESI MS m/z 325 ($M^+ + 1$).

*6.14.3. 7-(Benzyloxy)-3-bromo-2-ethyl-8-methyl-4H-chromen-4-one (**34**)*

Yield 34% (starting from 2.23 g of **28**), mp 155–157 °C. ^{1}H NMR δ 1.39 (3H, t, J = 7.5 Hz, 2-CH_2CH_3), 2.36 (3H, s, 8-CH_3), 3.00 (2H, q, J = 7.5 Hz, 2-CH_2CH_3), 5.19 (2H, s, 7-OCH_2–), 7.04 (1H, d, J = 9.0 Hz, 6-H), 7.35–7.46 (5H, m, H of aromatic), 8.06 (1H, d, J = 9.0 Hz, 5-H). ESI MS m/z 373 ($M^+ + 1$).

*6.14.4. 3-Bromo-2-ethyl-8-methyl-7-(3-(trifluoromethyl) benzyloxy)-4H-chromen-4-one (**35**)*

Yield 41% (starting from 200 mg of **30**), mp 159–161 °C. ^{1}H NMR δ 1.39 (3H, t, J = 7.2 Hz, 2-CH_2CH_3), 2.39 (3H, s, 8-CH_3), 3.02 (2H, q, J = 7.2 Hz, 2-CH_2CH_3), 5.26 (2H, s, 7-OCH_2–), 7.03 (1H, d, J = 9.0 Hz, 6-H), 7.55, 7.63 (3H, t, J = 7.2 Hz, H of aromatic), 7.72 (1H, s, H of aromatic), 8.08 (1H, d, J = 9.0 Hz, 5-H). ESI MS m/z 441 ($M^+ + 1$).

*6.15. Synthesis of bromides (**36–43**)*

A mixture of **52a–d** (1 equiv) and NBS (1.2 equiv) in CCl_4 was refluxed for 16 h. After filtration and removal of the solvent, the residue was purified by column chromatography with a gradient eluent of CH_2Cl_2/MeOH (40:1 to 30:1) to afford pure mono- (**36–39**) and di-brominated (**40–43**) products in yields of 40–78% and 11–20%, respectively.

*6.15.1. 8-(Bromomethyl)-2-ethyl-7-methoxy-4H-chromen-4-one (**36**)*

Yield 40% (starting with 300 mg of **52a**), mp 162–164 °C. ^{1}H NMR δ 1.36 (3H, t, J = 7.5 Hz, 2-CH_2CH_3), 2.72 (2H, q, J = 7.5 Hz, 2-CH_2CH_3), 4.03 (3H, s, 7-OCH_3), 4.78 (2H, s, 8-CH_2Br), 6.15 (1H, s, 3-H), 6.99 (1H, d, J = 9.0 Hz, 6-H), 8.16 (1H, d, J = 9.0 Hz, 5-H). ESI MS m/z 297 ($M^+ + 1$).

*6.15.2. 8-(Bromomethyl)-7-ethoxy-2-ethyl-4H-chromen-4-one (**37**)*

Yield 58% (starting with 880 mg of **52b**), mp 114–116 °C. ^{1}H NMR δ 1.36 (3H, t, J = 7.8 Hz, 2-CH_2CH_3), 1.52 (3H, t, J = 6.9 Hz, 7-OCH_2CH_3), 2.72 (2H, q, J = 7.8 Hz, 2-CH_2CH_3), 4.25 (2H, q, J = 6.9 Hz, 7-OCH_2CH_3), 4.79 (2H, s, 8-CH_2Br), 6.14 (1H, s, 3-H), 6.96 (1H, d, J = 9.0 Hz, 6-H), 8.13 (1H, d, J = 9.0 Hz, 5-H). ESI MS m/z 311 ($M^+ + 1$).

*6.15.3. 8-(Bromomethyl)-2-ethyl-7-isopropoxy-4H-chromen-4-one (**38**)*

Yield 45% (starting with 1.2 g of **52c**), mp 66–68 °C. ^{1}H NMR δ 1.36 (3H, t, J = 7.8 Hz, 2-CH_2CH_3), 1.44 (6H, d, J = 6.0 Hz, 7-$OCH(CH_3)_2$), 2.71 (2H, q, J = 7.8 Hz, 2-CH_2CH_3), 4.72–4.81 (3H, m, J = 6.0 Hz, 7-$OCH(CH_3)_2$ & 8-CH_2Br), 6.14 (1H, s, 3-H), 6.96 (1H, d, J = 9.0 Hz, 6-H), 8.12 (1H, d, J = 9.0 Hz, 5-H). ESI MS m/z 325 ($M^+ + 1$).

*6.15.4. 8-(Bromomethyl)-2-ethyl-7-isobutoxy-4H-chromen-4-one (**39**)*

Yield 78% (starting with 200 mg of **52d**), mp 91–93 °C. ^{1}H NMR δ 1.25 (6H, d, J = 6.6 Hz, 7-$OCH_2CH(CH_3)_2$), 1.50 (3H, t, J = 7.5 Hz, 2-CH_2CH_3), 2.35 (1H, m, J = 6.6 Hz, 7-$OCH_2CH(CH_3)_2$), 2.85 (2H, J = 7.5 Hz, 2-CH_2CH_3), 4.07 (2H, d, J = 6.6 Hz, 7-$OCH_2CH(CH_3)_2$), 4.93 (2H, s, 8-CH_2Br), 6.28 (1H, s, 3-H), 7.09 (1H, d, J = 9.0 Hz, 6-H), 8.27 (1H, d, J = 9.0 Hz, 5-H). ESI MS m/z 339 ($M^+ + 1$).

*6.15.5. 2-(1-Bromoethyl)-8-(bromomethyl)-7-methoxy-4H-chromen-4-one (**40**)*

Yield 14% (starting with 300 mg of **52a**), mp 153–156 °C. ^{1}H NMR δ 2.09 (3H, d, J = 7.2 Hz, 2-$CHBrCH_3$), 4.04 (3H, s, 7-OCH_3), 4.81 (2H, s, 8-CH_2Br), 4.94 (1H, q, J = 7.2 Hz, 2-$CHBrCH_3$), 6.33 (1H, s,

3-H), 7.02 (1H, d, $J = 9.0$ Hz, 6-H), 8.16 (1H, d, $J = 9.0$ Hz, 5-H). ESI MS m/z 377 ($M^+ + 1$).

*6.15.6. 2-(1-Bromoethyl)-8-(bromomethyl)-7-ethoxy-4H-chromen-4-one (**41**)*

Yield 20% (starting with 880 mg of **52b**), mp 138–140 °C. ^{1}H NMR δ 1.53 (3H, t, $J = 6.9$ Hz, 7-OCH$_2$*CH*$_3$), 2.09 (3H, d, $J = 7.2$ Hz, 2-CHBr*CH*$_3$), 4.26 (2H, q, $J = 6.9$ Hz, 7-O*CH*$_2$CH$_3$), 4.82 (2H, s, 8-CH$_2$Br), 4.95 (1H, q, $J = 7.2$ Hz, 2-C*H*BrCH$_3$), 6.33 (1H, s, 3-H), 6.99 (1H, d, $J = 9.0$ Hz, 6-H), 8.12 (1H, d, $J = 9.0$ Hz, 5-H). ESI MS m/z 391 ($M^+ + 1$).

*6.15.7. 2-(1-Bromoethyl)-8-(bromomethyl)-7-isopropoxy-4H-chromen-4-one (**42**)*

Yield 15% (starting with 1.2 g of **52c**), mp 95–97 °C. ^{1}H NMR δ 1.45 (6H, d, $J = 6.0$ Hz, 7-OCH(*CH*$_3$)$_2$), 2.85 (3H, d, $J = 6.9$ Hz, 2-CHBr*CH*$_3$), 4.80 (3H, m, $J = 6.0$ Hz, 7-O*CH*(CH$_3$)$_2$ & 8-CH$_2$Br), 4.94 (1H, q, $J = 6.9$ Hz, 2-*CH*BrCH$_3$), 6.32 (1H, s, 3-H), 7.00 (1H, d, $J = 9.0$ Hz, 6-H), 8.12 (1H, d, $J = 9.0$ Hz, 5-H). ESI MS m/z 405 ($M^+ + 1$).

*6.15.8. 2-(1-Bromoethyl)-8-(bromomethyl)-7-isobutoxy-4H-chromen-4-one (**43**)*

Yield 11% (starting with 200 mg of **52d**), mp 108–109 °C. ^{1}H NMR δ 1.11 (6H, d, $J = 6.6$ Hz, 7-OCH$_2$CH(*CH*$_3$)$_2$), 2.09 (3H, d, $J = 6.9$ Hz, 2-CHBr*CH*$_3$), 2.22 (1H, m, $J = 6.6$ Hz, 7-OCH$_2$*CH*(CH$_3$)$_2$), 3.95 (2H, d, $J = 6.6$ Hz, 7-O*CH*$_2$CH(CH$_3$)$_2$), 4.83 (2H, s, 8-CH$_2$Br), 4.94 (1H, $J = 6.9$ Hz, 2-*CH*BrCH$_3$), 6.33 (1H, s, 3-H), 7.0 (1H, d, $J = 9.0$ Hz, 6-H), 8.12 (1H, d, $J = 9.0$ Hz, 5-H). ESI MS m/z 419 ($M^+ + 1$).

6.16. Sulforhodamine B (SRB) assay

For the anti-proliferation assay in tumor cells, KB (nasopharyngeal), KB-vin (its vincristine-resistant subline), DU145 (prostate) and A549 (lung) cells were fixed *in situ* with 10% trichloroacetic acid (TCA) to represent a measurement of the cell population at the time of drug addition. Briefly, the plates were incubated for an additional 72 h after attachment and drug addition, and the assay was terminated by 10% TCA. Then, 0.4% SRB dye in 1% HOAc was added to stain the cells for 10 min. Unbound dye was removed by repeated washing with 1% HOAc, and the plates were air dried. Bound stain was subsequently solved with 10 mM trizma base, and the absorbance is read under 515 nm. Growth inhibition of 50% (GI_{50}) was calculated as the drug concentration that caused a 50% reduction in the net protein increase in control cells during the drug incubation.

6.17. A549 human lung cancer cell proliferation assay

Cell proliferation was measured by the MTT method. Briefly, A549 cells were collected and seeded in 96-well plates at a density of 5×10^3 cells/well. After incubation with **10** (0.06, 0.23, 0.94, 3.75, 15, 60 μg/mL) for 48 h, the medium was removed and replaced with fresh medium (180 μL/well). Twenty μL of MTT solution (5 mg/mL) were added to each well and the plates were incubated for an additional 4 h at 37 °C. The medium was aspirated off, and DMSO (150 μL) was added to each well. The absorbance was read at 570 nm using a microplate reader (TECAN Infinite 200). Cell viability was expressed as a percentage of the untreated cells.

6.18. A549 cellular apoptosis assay

A549 cells were seeded in 24-well plates at a density of 5×10^3 cells/well, and incubated with **10** (0, 1, 5 and 10 μg/mL) for 48 h. The cellular monolayer was fixed and stained with DNA fluorochrome Hoechst 33258 for 20 min. After washing with phosphate buffered saline (PBS), the morphological features of apoptosis (including cellular nucleus shrinkage, chromatin condensation, intense fluorescence and nuclear fragmentation) were monitored by fluorescence microscopy (Zeiss, German).

6.19. A549 human lung cancer cell cycle arrested assay

A549 cells (3×10^5/well) were seeded into 6-well plates and exposed to **10** at various concentrations (0, 1, 5 and 10 μg/mL) for 48 h, and then harvested and washed with PBS, and fixed in 70% EtOH at 4 °C. Staining was performed in PBS containing 40 μg/mL RNaseA and 10 μg/mL PI in the dark at rt for 30 min. The cell cycle was measured using FACScan Flow Cytometry (BD FACSCalibur).

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Appendix. Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejmech.2011.12.025.

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