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Design, syntheses, and antitumor activity of novel chromone and aurone derivatives

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Abstract—A series of new chromone analogues bearing heterocyclic thioether moiety and aurone analogues bearing cyclic tertiary amine moiety were designed and synthesized under microwave irradiation. The synthetic protocol was found to present many advantages, such as higher yields, shorter reaction time (10–20 min), mild condition, and readily isolation of the products. The synthesized compounds were assayed for their antitumor activity against four kinds of human solid tumor cell lines including HCCLM-7, Hep-2, MDA-MB-435S, and SW-480. Two compounds, (*Z*)-2-((4-benzyl-piperazin-1-yl)methylene)benzofuran-3(2*H*)-one **5e** and (*Z*)-2-((4-(bis(4-fluorophenyl)methyl)piperazin-1-yl)methylene)benzofuran-3(2*H*)-one **5f**, were identified as the most promising candidates with the IC_{50} values in the range of 4.1–13.1 μM. Further cell cycle studies revealed that compounds **5e** and **5f** arrest the cell cycle in G_0/G_1 phase and displayed apoptosis-inducing effect on Hep-2 cells.

1. Introduction

Flavonoids, occurring widely throughout the plant kingdom, are one of the most representative families of plant secondary metabolites and display a remarkable spectrum of biological activities.[1] Thousands of flavonoids were screened for the purpose of drug discovery or target identification during last decades. It should be especially noted that developing flavonoids as anticancer agents has interested medicinal chemists for many years.[2] Some kinds of molecular mechanisms of flavonoids were identified as carcinogen inactivation, antiproliferation, cell cycle arrest, induction of apoptosis and differentiation, inhibition of angiogenesis, antioxidation and reversal of multidrug resistance.[3] To date some flavonoids have entered clinical trials. For example, Flavopiridol (**1**) was identified as the first cyclin-dependent kinase inhibitor and entered phase II clinical trials.[4]

Most of existing results indicate that the presence of heterocyclic thioether or cyclic tertiary amine feature will benefit the antitumor activities of flavonoids.[5,6] For example, the piperidinyl moieties in Flavopiridol (**1**) and its analogues (**2**) were found to play important roles in their binding with the cyclin-dependent kinase 2 (CDK2).[7] Due to the existence of the thioether moiety at position-2, thioflavopiridol (**2**) not only displayed selectivity within the CDK family but also discriminated between unrelated serine/threonine and tyrosine protein kinases. In addition, piperidinyl-containing benzofuran-3(2*H*)-one derivatives (**3**) were also identified as cyclin-dependent kinase inhibitor. Recently, Qian's group designed and synthesized a series of novel naphthalimide analogues as anticancer agents, DNA intercalators, and DNA photocleavers. Their results demonstrated that introducing sulfur atom to the naphthalimide skeleton would result in higher antitumor activity and/or DNA photocleaving activities.[8]

On the basis of the above observations, we reasoned that compound **4** bearing heterocyclic thioether moiety at position-3 might display anticancer activity with different feature from that of compound **2**. Furthermore, if the piperidinyl moiety at position-7 in compound **3** was moved to the position of 2-benzylidene, the anticancer activity of the resulted compound **5** should be an

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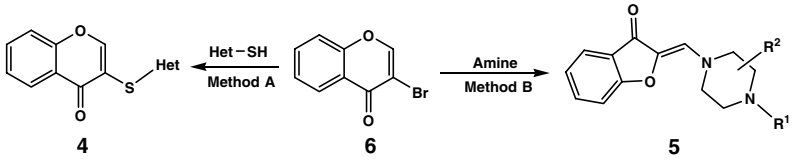
interesting topic. Therefore, we described herein the syntheses and in vitro antitumor activities of compounds **4** and **5** against various human tumor cell lines.

2. Results and discussion

Our recent success in application of microwave irradiation[9] prompted us to carry out microwave-assisted synthesis of the target compounds. By optimizing the temperature, reaction time, solvent, base, and molar ratios of base, the optimized conditions were obtained. Various heterocyclic thiol or 2° amine[10] reacted with potassium *t*-butoxide (1.1 equiv) and 3-bromo-chromone **6** (1.0 equiv) at 90 or 70 °C to afford the desired title compounds **4** and **5** in moderate to good yields. Compared to the reaction time of 12–24 h under conventional heating, it only took 10–20 min to finish the reaction under microwave irradiation. Additionally, a moderate improvement in yields from 2% to 13% was observed by carrying out the reaction under microwave irradiation (Scheme 1 and Table 1).

Gammill and his coworkers described first the addition reaction of amines to 3-bromochromone in 1983 (Scheme 2).[10] Under the reaction conditions (2 equiv amine, 1.5 equiv K_2CO_3, CH_3CN, rt, 18 h), it was found that the primary and secondary amines reacted with 3-bromochromone to afford the ring contraction products **7** and 3-aminochromones **8**, respectively. However, we found the secondary amines reacted with 3-bromochromone under our new conditions (1 equiv amine, 1.1 equiv KOBu-*t*, DMF, 70 °C) to afford the ring contraction products **7**. We put forward a plausible mechanism as shown in Scheme 3 to explain the ring contraction process. Michael addition of the amine to the pyrone ring of **6** yields the α-bromo-β-aminochromone **9**. Elimination of α-H in the presence of potassium *t*-butoxide results in the ring opening, followed by an intramolecular O-alkylation via nucleophilic substitution process to afford the benzofurannone **5**.

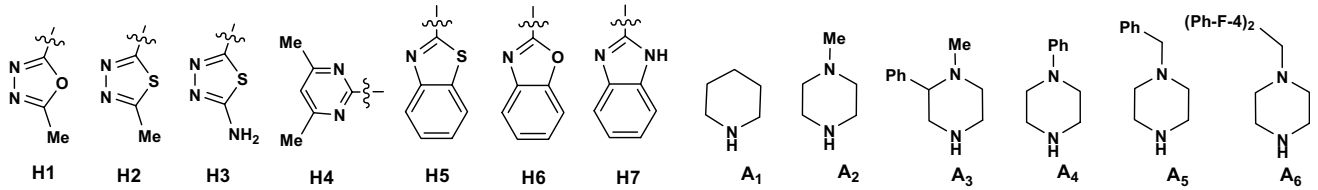
In order to provide much more evidence for the plausible mechanism (Scheme 3) for the formation of the ring contraction product **5**, X-ray crystallography analysis of compound **5e** was performed and the results showed that the product is desirable aurone rather than 3-pirperazinyl chromone. In the crystal structure of compound **5e** as shown in Figure 1,[11] all atoms of benzofuran moiety are nearly coplanar and the piperazine ring adopts a boat conformation. Two intramolecular hydrogen bonds were observed. One is between the



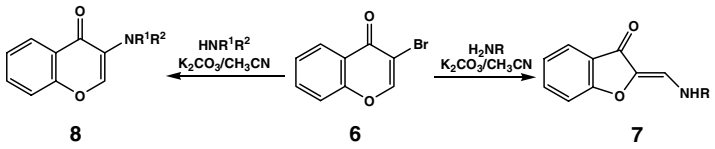
Scheme 1. Synthesis of the title compounds **4** and **5**. Reagents and conditions: (Method A) potassium *t*-butoxide, DMF, 90 °C, MW 200 W; (Method B) potassium *t*-butoxide, DMF, 70 °C, MW 120 W.

Table 1. Results of the syntheses of compounds **4** and **5**

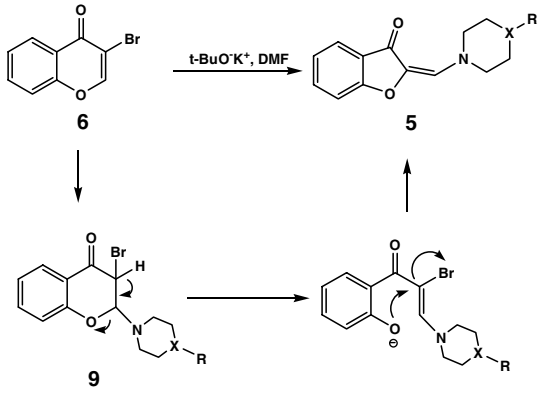
Compound	Het/amine	Traditional heating		Microwave irradiation	
		T (h)[a]	Yield (%)[b]	*T* (min)[a]	Yield (%)[b]
4a	H1	16	77	15	85
4b	H2	16	73	15	75
4c	H3	16	61	15	63
4d	H4	12	84	10	87
4e	H5	24	59	20	65
4f	H6	16	62	20	70
4g	H7	16	60	20	67
5a	A1	12	56	20	62
5b	A2	12	60	20	71
5c	A3	12	52	20	60
5d	A4	12	55	20	68
5e	A5	12	62	20	72
5f	A6	12	65	20	75



[a] Time to finish the reaction monitored by TLC.
[b] Isolated yields.



Scheme 2.



Scheme 3. A plausible mechanism for the formation of the ring contraction product.

oxygen atom $O_{(1)}$ in the benzofuran moiety and the α-C–H atom of the piperazine ring, the other is between the $N_{(4)}$ atom of the piperazine ring and the hydrogen atom at position-2 of the benzyl moiety.

Table 2. Antiproliferative activity of the compounds **4a–g** and **5a–f**

Compound	Cytotoxicity IC_{50}[a] (μM)			
	HCCLM-7	Hep-2	MDA-MB-435S	SW-480
4a	>50	>50	>50	>50
4b	>50	40.2	>50	>50
4c	>50	47.7	>50	>50
4d	>50	>50	>50	>50
4e	>50	31.3	17.1	45.2
4f	>50	29.6	26.1	36.4
4g	>50	42.6	30.3	>50
5a	>50	>50	47.8	>50
5b	>50	>50	40.3	>50
5c	>50	>50	>50	>50
5d	45.1	27.7	25.1	36.4
5e	9.6	5.7	7.6	6.6
5f	12.1	4.7	4.1	13.1
5-FU	18.6	128.7	14.5	8.1

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

The in vitro antitumor activities of the synthesized compounds against four cancer cell lines, including HCCLM-7 (hepatoma carcinoma cell),[12] Hep-2 (laryngocarcinoma cell), MDA-MB-435S (mammary adenocarcinoma cell), and SW-480 (colon carcinoma cell), were assayed by MTT method[13] and the results expressed as IC_{50} are summarized in Table 2. 5-Fluorouricial (5-FU) was used as control. Among chromone derivatives **4a–g** bearing heterocyclic thio-ether moiety, the IC_{50} of **4e** against MDA-MB-435S is 17.2 μM, which is comparable to that of 5-FU (14.5 μM). Most of compounds **4** show moderate activity (30–50 μM) against Hep-2, which is superior to 5-FU (128.7 μM). Within the series bearing the piperazinyl moiety, compounds **5e** and **5f** displayed the most promising antitumor activities against all four tested tumor cell lines. Most interestingly, their activities against Hep-2 (5.7 and 4.7 μM) are 27- and 22-fold higher than that of 5-FU (128.7 μM), respectively. Additionally, **5e** and **5f** displayed higher activities against HCCLM-7 and MDA-MB-435S than 5-FU. For the cancer cell line of SW-480, compound **5e** displayed higher activity than 5-FU, while **5f** showed lower activity than 5-FU. The present study indicated that aurones bearing 4-benzyl piperazinyl displayed higher activities than 4-phenyl piperazine or 4-alkyl piperazine derivatives.

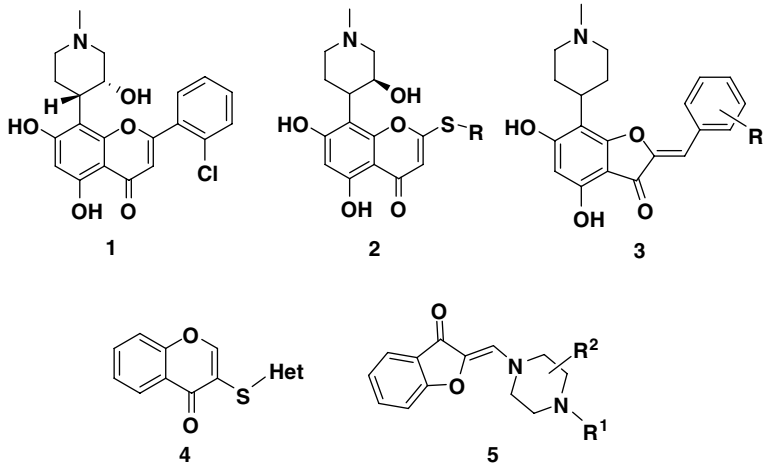


Figure 1.

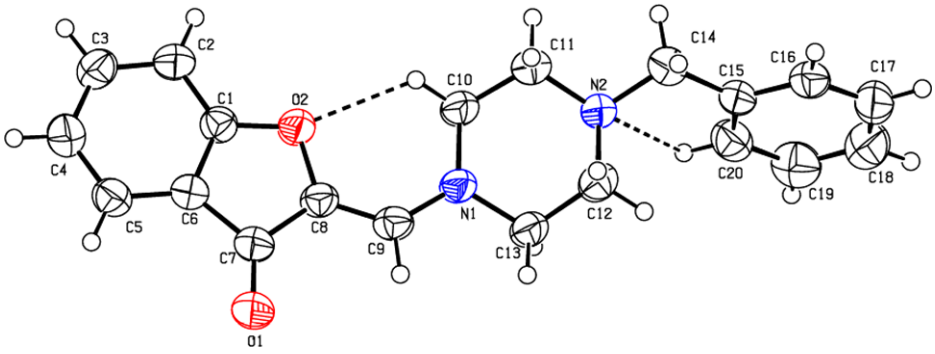


Figure 2. X-ray crystal structure of **5e**.

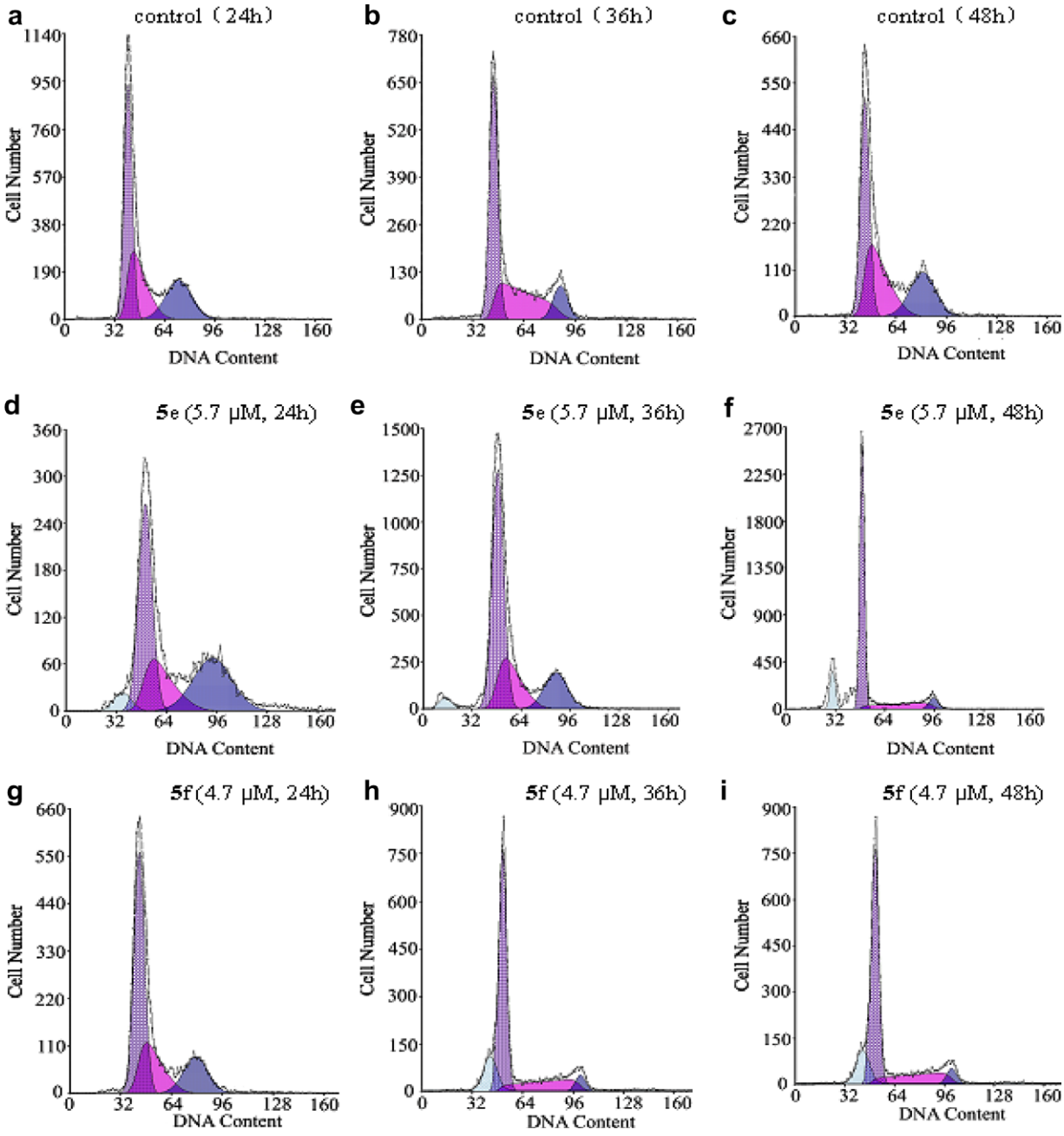


Figure 3. Compounds **5e** and **5f** induced apoptosis in Hep-2 cells.

Table 3. Effects of **5e** and **5f** on cell cycle progression in Hep-2 cells

Compound:	Control				**5e** (5.7 μM)				**5f** (4.7 μM)			
	Sub-G_0	G_0/G_1	S	G_2/M	Sub-G_0	G_0/G_1	S	G_2/M	Sub-G_0	G_0/G_1	S	G_2/M
24 h	0	46.18	29.39	24.42	4.69	44.87	22.98	32.14	0	55.90	25.01	19.09
36 h	0	46.70	39.18	14.32	4.930	58.70	23.84	17.46	5.55	63.03	18.45	18.52
48 h	0	41.96	33.61	24.43	18.20	75.85	17.83	6.32	16.46	73.71	20.89	5.41

To study the effect of the synthesized compounds on cell cycle progression, flow-activated cell sorting analysis was performed.[14] The most promising compounds **5e** and **5f** were tested against Hep-2 cell lines at the 50%-inhibiting concentration according to the MTT assay. Figure 2 and Table 3 show the results after 24-, 36-, and 48-h treatment for **5e** and **5f**. As shown in Figure 2 and Table 3, compounds **5e** and **5f** arrest the cell cycle in G_0/G_1 phase, raising the G_0/G_1 peak from 41.96% to 75.85% (**5e**) and 73.71% (**5f**) after 48-h treatment. Subsequently, cells accumulated in sub-G_0 phase at 18.20% (**5e**) and 16.46% (**5f**). Because the increase of cells in sub-G_0 phase generally informed the increase of apoptotic cell death, **5e** and **5f** might display apoptosis-inducing effect on Hep-2 cells (Fig. 3).

3. Conclusion

In summary, the present work described the molecular design and microwave-assisted syntheses of a series of new chromone analogues with heterocyclic thioether moiety and aurone analogues with piperazine moiety. The synthetic protocol of the title compounds presented many advantages, such as higher yields, shorter reaction time (10–20 min), mild condition, and readily isolation of the products. MTT assay indicated these compounds displayed antitumor activities against HCCLM-7, Hep-2, MDA-MB-435S and SW-480. Two compounds, (*Z*)-2-((4-benzyl-piperazin-1-yl)methylene)benzofuran-3(2*H*)- one **5e** and (*Z*)-2-((4-(bis(4-fluorophenyl)-methyl)piperazin-1-yl)methylene)benzofuran-3(2*H*)-one **5f**, were identified as the most promising candidates. Further flow-activated cell sorting analysis revealed that compounds **5e** and **5f** arrest the cell cycle in G_0/G_1 phase and displayed apoptosis-inducing effect on Hep-2 cells.

4. Experimental

4.1. Chemistry

4.1.1. General methods. ^{1}H NMR spectra were recorded at 400 M Hz in $CDCl_3$ solution on a Varian VNMR 400 MHz spectrometer. MS spectra were determined using a TraceMS 2000 organic mass spectrometry, and the signals are given in *m*/*z*. Melting points were taken on a Buchi B-545 melting point apparatus. Elemental analysis (EA) was carried out on a Vario EL III CHNSO elemental analyzer. X-ray crystallography study was measured on a Bruker APEX CCD area detector diffractometer. Conventional heating was carried out on corning stirrer/hotplates in oil baths. Microwave syntheses were carried out on a Smith synthesizer™.

4.1.2. General method for the preparation of 4. 3-Bromochromone (1 mmol) was added into a solution of heterocyclic thiol (1 mmol) and potassium *t*-butoxide (1.1 mmol) in 2 mL DMF. The resulted mixture was irradiated by 200 W MW at 90 °C for 15–20 min or heated by oil bath at 90 °C for 12–24 h. Completion of the reaction was checked by TLC. The resulting mixture was cooled and diluted with 10 mL of ice water. The obtained solid product was filtered and recrystallized from ethanol to afford the product **4**.

4.1.2.1. 3-(5-Methyl-[1,3,4]oxadiazol-2-ylsulfanyl)-chromen-4-one (4a). Mp 130–132 °C. ^{1}H NMR ($CDCl_3$): 8.51 (s, 1H), 8.26 (d, *J* = 8.0, 1H), 7.75 (t, *J* = 8.0, 1H), 7.49–7.53 (m, 2H), 2.51 (s, 3H). EIMS (probe) 70 eV, *m*/*z* (rel int.): 260 $[M]^+$ (100), 219 (11.2), 178 (10.6), 133 (12.5), 108 (23.5), 83 (58.1); Anal. Calcd for $C_{12}H_8N_2O_3S$: C, 55.38; H, 3.10; N, 10.76. Found: C, 55.21; H, 3.30; N, 10.59.

4.1.2.2. 3-(5-Methyl-[1,3,4]thiadiazol-2-ylsulfanyl)-chromen-4-one (4b). Mp 75–76 °C. ^{1}H NMR ($CDCl_3$): 8.51 (s, 1H), 8.25 (d, *J* = 8.0, 1H), 7.73 (t, *J* = 8.0, 1H), 7.46–7.52 (m, 2H), 2.71 (s, 3H). EIMS (probe) 70 eV, *m*/*z* (rel int.): 276 $[M]^+$ (100), 235 (41.2), 178 (20.1), 149 (9.56), 124 (23.5), 91 (58.1); Anal. Calcd for $C_{12}H_8N_2O_2S_2$: C, 52.16; H, 2.92; N, 10.14. Found: C, 51.95; H, 3.19; N, 10.01.

4.1.2.3. 3-(5-Amino-[1,3,4]thiadiazol-2-ylsulfanyl)-chromen-4-one (4c). Mp 233–234 °C. ^{1}H NMR ($CDCl_3$): 8.46 (s, 1H), 8.24 (d, *J* = 8.0, 1H), 7.74 (t, *J* = 8.0, 1H), 7.456–7.51 (m, 2H), 2.71 (br, 1H). EIMS (probe) 70 eV, *m*/*z* (rel int.): 277 $[M]^+$ (100), 207 (82.2), 178 (20.1), 149 (25.2), 103 (48.8), 88 (68.1); Anal. Calcd for $C_{11}H_7N_3O_2S_2$: C, 47.64; H, 2.54; N, 15.15. Found: C, 47.46; H, 2.704; N, 14.98.

4.1.2.4. 3-(4,6-Dimethyl-pyrimidin-2-ylsulfanyl)-chromen-4-one (4d). Mp 199–201 °C. ^{1}H NMR ($CDCl_3$): 8.35 (s, 1H), 8.27 (d, *J* = 8.0, 1H), 7.73 (t, *J* = 8.0, 1H), 7.53 (d, *J* = 8.0, 1H), 7.46 (t, *J* = 8.0, 1H), 6.721 (s, 3H), 2.33 (s, 6H). EIMS (probe) 70 eV, *m*/*z* (rel int.): 284 $[M]^+$ (100), 255 (30.82), 239 (11.1), 148 (7.112), 106 (16.8), 91 (11.3), 66 (33.6); Anal. Calcd for $C_{15}H_{12}N_2O_2S$: C, 63.36; H, 4.25; N, 9.85. Found: C, 63.20; H, 4.38; N, 9.72.

4.1.2.5. 3-(Benzothiazol-2-ylsulfanyl)-chromen-4-one (4e). Mp 165–168 °C. ^{1}H NMR ($CDCl_3$): δ 8.58 (s, 1H), 8.30 (d, *J* = 8.0, 1H), 7.92 (d, *J* = 8.0, 1H), 7.72 (t, *J* = 8.0, 1H), 7.57 (d, *J* = 8.0, 1H), 7.50 (d, *J* = 8.0, 1H), 7.44 (t, *J* = 4.8, 1H), 7.33 (t, *J* = 8.0, 1H). EIMS (probe) 70 eV, *m*/*z* (rel int.): 311 $[M]^+$ (79.5), 282

(27.0), 223 (100), 195 (20.3), 144 (10.9), 91 (50.3); Anal. Calcd for $C_{16}H_9NO_2S_2$: C, 61.72; H, 2.91; N, 4.50. Found: C, 61.542; H, 3.14; N, 4.30.

4.1.2.6. 3-(Benzooxazol-2-ylsulfanyl)-chromen-4-one (4f). Mp 160–162 °C. ^{1}H NMR ($CDCl_3$): δ 8.61 (s, 1H), 8.27 (d, J = 8.0, 1H), 7.79 (t, J = 8.0, 1H), 7.69 (t, J = 4.8, 1H), 7.59 (d, J = 8.0, 1H), 7.49–7.53 (m, 2H), 7.31–7.385 (m, 2H), 6.64 (br, 1H). EIMS (probe) 70 eV, *m*/*z* (rel int.): 295 $[M]^+$ (19.2), 266 (8.11), 206 (6.14), 174 (72.1), 133 (75.5), 120 (100), 91 (94.4); Anal. Calcd for $C_{16}H_9NO_3S$: C, 65.07; H, 3.07; N, 4.74. Found: C, 64.82; H, 3.30; N, 4.55.

4.1.2.7. 3-(1*H*-Benzoimidazol-2-ylsulfanyl)-chromen-4-one (4g). Mp 182–184 °C. ^{1}H NMR ($CDCl_3$): δ 11.10 (s, 1H), 8.41 (s, 1H), 7.96 (d, J = 8.0, 1H), 7.85 (d, J = 8.0, 1H), 7.77 (d, J = 8.0, 1H), 7.62 (t, J = 8.0, 1H), 7.48 (t, J = 8.0, 1H), 7.37 (t, J = 8.0, 1H), 7.15 (d, J = 8.0, 1H), 7.08 (t, J = 8.0, 1H). EIMS (probe) 70 eV, *m*/*z* (rel int.): 294 $[M]^+$ (100), 266 (17.4), 206 (16.1), 120 (80.2), 92 (66.5); Anal. Calcd for $C_{16}H_{10}N_2O_2S$: C, 65.29; H, 3.42; N, 9.52. Found: C, 65.11; H, 3.58; N, 9.35.

4.1.3. General method for the preparation of 5. 3-Bromochromone (1 mmol) was added into a solution of 1-benzylpiperazine (1 mmol) and potassium *t*-butoxide (1.1 mmol) in 2 mL of DMF. The resulted mixture was irradiated by MW (120 W) at 70 °C for 20 min or heated by oil bath at 70 °C for 12 h. Completion of the reaction was checked by TLC. The resulting mixture was cooled and diluted with 10 mL of ice water. The obtained solid product was filtered and recrystallized from ethanol to afford the product **5**.

4.1.3.1. (*Z*)-2-(Piperidin-1-ylmethylene)benzofuran-3(2*H*)-one (5a). Mp 102–104 °C. ^{1}H NMR ($CDCl_3$): δ 8.26 (d, J = 8.0, 1H), 7.62 (t, J = 8.0, 1H), 7.60 (s, 1H), 7.41 (d, J = 8.0, 1H), 7.35 (t, J = 8.0, 1H), 2.98 (br, 4H), 1.78 (br, 4H), 1.59 (br, 2H). EIMS (probe) 70 eV, *m*/*z* (rel int.): 229 $[M]^+$ (87.8), 209 (8.53), 199 (78.8), 144 (80.6), 120 (15.6), 102 (78.5), 90 (98.6), 67 (100); Anal. Calcd for $C_{14}H_{15}NO_2$: C, 73.34; H, 6.59; N, 6.11. Found: C, 73.16; H, 6.75; N, 5.97.

4.1.3.2. (*Z*)-2-((4-Methylpiperazin-1-yl)methylene)benzofuran-3(2*H*)-one (5b). Mp 105–107 °C. ^{1}H NMR ($CDCl_3$): δ 7.80 (d, J = 8.0, 1H), 7.52 (t, J = 8.0, 1H), 7.21 (d, J = 8.0, 1H), 7.16 (t, J = 8.0, 1H), 7.00 (s, 1H), 3.78 (br, 4H), 2.61 (br, 4H), 2.40 (s, 31H). EIMS (probe) 70 eV, *m*/*z* (rel int.): 244 $[M]^+$ (22.5), 186 (3.68), 146 (6.44), 92 (7.78), 69 (100); Anal. Calcd for $C_{14}H_{16}N_2O_2$: C, 68.83; H, 6.60; N, 11.47. Found: C, 68.60; H, 6.79; N, 11.35.

4.1.3.3. (*Z*)-2-((4-Methyl-2-phenylpiperazin-1-yl)methylene)benzofuran-3(2*H*)-one (5c). Mp 151–153 °C. ^{1}H NMR ($CDCl_3$): δ 7.79 (d, J = 8.0, 1H), 7.48–7.52 (m, 3H), 7.38 (t, J = 8.0, 2H), 7.33 (d, J = 8.0, 1H), 7.19 (d, J = 8.0, 1H), 7.15 (t, J = 8.0, 1H), 7.07 (s, 1H), 5.11 (br, 1H), 3.87 (br, 1H), 3.22 (br, 2H), 2.37–2.48 (m, 3H). EIMS (probe) 70 eV, *m*/*z* (rel int.): 320 $[M]^+$ (6.98), 292 (59.8), 232 (62.6), 206 (36.4), 133 (74.5), 116 (100), 89 (51.6); Anal. Calcd for $C_{20}H_{20}N_2O_{22}$: C, 74.98; H, 6.29; N, 8.74. Found: C, 74.79; H, 6.45; N, 8.56.

4.1.3.4. (*Z*)-2-((4-Phenylpiperazin-1-yl)methylene)benzofuran-3(2*H*)-one (5d). Mp 145–146 °C. ^{1}H NMR ($CDCl_3$): δ 7.82 (d, J = 8.0, 1H), 7.53 (t, J = 8.0, 1H), 7.23–7.38 (m, 4H), 7.18 (t, J = 8.0, 1H), 6.96–7.02 (m, 3H), 3.98 (br, 4H), 3.36 (br, 4H). EIMS (probe) 70 eV, *m*/*z* (rel int.): 306 $[M]^+$ (100), 201 (5.03), 160 (5.79), 132 (14.9), 104 (14.0), 77 (6.17); Anal. Calcd for $C_{19}H_{18}N_2O_2$: C, 74.49; H, 5.92; N, 9.14. Found: C, 74.26; H, 6.12; N, 9.01.

4.1.3.5. (*Z*)-2-((4-Benzylpiperazin-1-yl)methylene)benzofuran-3(2*H*)-one (5e). Mp 168–169 °C. ^{1}H NMR ($CDCl_3$): δ 7.79 (d, J = 8.0, 1H), 7.50 (t, J = 8.0, 2H), 7.32–7.37 (m, 5H), 7.11–7.19 (m, 2H), 6.98 (s. 1H), 3.60 (br, 6H), 2.61 (br, 4H). EIMS (probe) 70 eV, *m*/*z* (rel int.): 320 $[M]^+$ (7.21), 292 (15.9), 232 (10.7), 220 (32.2), 189 (69.0), 100 (100); Anal. Calcd for $C_{20}H_{20}N_2O_2$: C, 74.98; H, 6.29; N, 8.74. Found: C, 75.12; H, 6.49; N, 8.48.

4.1.3.6. (*Z*)-2-((4-(Bis(4-fluorophenyl)methyl)piperazin-1-yl)methylene)benzofuran-3(2*H*)-one (5f). Mp 204–206 °C. ^{1}H NMR ($CDCl_3$): δ 8.13 (d, J = 8.0, 1H), 7.77 (d, J = 8.0, 1H), 7.52 (t, J = 8.0, 1H), 7.32–7.40 (m, 4H), 7.13 (t, J = 8.0, 1H), 6.98–7.06 (m, 4H), 5.49 (s. 1H), 4.29 (s. 1H), 3.65 (br, 4H), 2.50 (br, 4H). EIMS (probe) 70 eV, *m*/*z* (rel int.): 432 $[M]^+$ (76.3), 404 (8.69), 337 (6.16), 229 (37.8), 203 (100), 183 (88.7), 146 (21.3), 92 (31.4); Anal. Calcd for $C_{26}H_{22}F_2N_2O_2$: C, 72.21; H, 5.13; F, 8.79; N, 6.48. Found: C, 72.03; H, 5.31; F, 8.62; N, 6.35.

4.2. Biological assays

4.2.1. Cell culture. Human-derived cell lines (MDA-MB-435S breast carcinoma; HCC LM 7 hepatoma; SW480 colon carcinoma; Hep-2 laryngocarcinoma) were cultured in DMEM (Gibico) containing 10% FBS (Invitrogen), 4.5 g/L glucose, 10,000 U/mL of penicillin, and 10 mg/mL of streptomycin at 37 °C and 5% CO_2. During the experiment time, the medium was replaced by medium containing different concentrations of the synthesized compounds, which were dissolved in DMSO vehicle. The vehicle control only received DMSO (0.5% v/v).

4.2.2. Cell proliferation by MTT assay.[13] 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.

4.2.3. Flow activating cell sorting analysis (FACS).[14] The effect of **5e** and **5f** on Hep-2 laryngocarcinoma cell cycle phase distribution was assessed using flow cytometry. When the cells were grown to about 70% confluence in 6-well microplates and treated with **5e** and **5f** at given concentrations (IC_{50} concentration). After 24, 36, and 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).

4.2.4. Statistical analysis. For each assay, three different experiments were performed in triplicate. The results were statistically evaluated by Student's *t*-test.[15] The IC_{50}, 95% confidence limits.

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11. Crystal data of **5e**. $C_{20}H_{20}N_2O_2$, $M = 320.38$, monoclinic, $a = 9.519$ (1), $b = 19.799$ (2), $c = 9.716$ (1) Å, $\beta = 114.8°$, $V = 1663$ (1) Å^3, $T = 292$ (2) K, space group $P21/c$, $Z = 4$, $Dc = 1.280$ g/cm^3, μ (Mo-Kα) = 0.083 mm^{-1}, $F(000) = 680$. 9756 reflections measured, 3629 unique ($R_{int} = 0.0409$), which were used in all calculation. Fine $R_1 = 0.0490$, $wR(F^2) = 0.1094$ (all data). Full crystallographic details of **5e** have been deposited at the Cambridge Crystallographic Data Center and allocated the deposition number CCDC 294565.
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