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Synthesis and antiproliferative evaluation of amide-containing flavone and isoflavone derivatives

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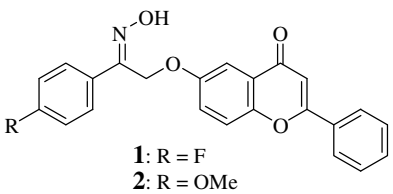
ABSTRACT

Certain amide-containing flavone and isoflavone derivatives were synthesized and evaluated for their antiproliferative activities. These compounds were synthesized via alkylation of hydroxyl precursors followed by the reaction with H_2SO_4 and NaN_3 (Schmidt reaction).

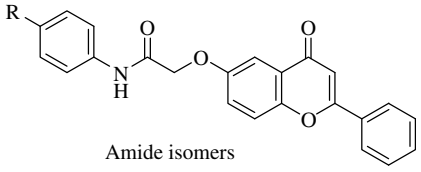
The preliminary assays indicated that the inhibitory activity against the growth of NCI-H661 decreased in an order of linked chromophore flavone-6-yl **16a–d** > flavone-7-yl **17a–d** > flavone-3-yl **15a–d** and isoflavone-7-yl **18a–d**. Among these flavone-6-yl derivatives, *N*-(4-methoxyphenyl)-2-(4-oxo-2-phenyl-4*H*-chromen-6-yloxy)acetamide (**16c**) was the most potent with a GI_{50} value of 0.84 μM. The inhibitory activity against the growth of NPC-TW01 decreased in an order of linked chromophore flavone-6-yl **16a–d** > isoflavone-7-yl **18a–d** > flavone-7-yl **17a–d** > flavone-3-yl **15a–d**. Flavone-6-yl derivatives **16a–d** demonstrated significant inhibitory activities against the growth of NPC-TW01 cell with an average GI_{50} value of 0.84 μM. The oxime derivatives **1** and **2** caused accumulation of NPC-TW01 cell in G_2/M phase which were distinct from that of their amide isomers **16b** and **16c**, respectively, which induced cell-cycle arrest in G_0/G_1 phase followed by apoptosis. Therefore, the antiproliferative mechanism of flavone derivatives was affected not only by the phenyl benzopyran-4-one pharmacophore but also by the peripheral substituents.

1. Introduction

Flavonoids and isoflavonoids are polyphenolic compounds which have been found in plants and dietary components such as fruits, soy beans, vegetables, and red wine. Their extensive biological activities including anticancer,[1–8] antifungal,[9] antiviral,[10] anti-inflammatory,[11] antioxidant,[12,13] cardiovascular,[14–18] and anti-osteoporotic effects[19,20] have attracted substantial attentions. Although the skeleton of phenyl benzopyran-4-one pharmacophore plays an important role in biological effects of flavonoid and isoflavonoid derivatives, the type of peripheral substituents is also crucial. For example, isoflavones containing a sulfur or oxygen hinge with an amine-bearing side chain are potential selective estrogen receptor modulators,[6] while substitution of 2-pyridylmethylthio or 2-thioazole group on isoflavone pharmacophore enhanced aromatase inhibitory activity.[7,8] Recently, we have reported preparation of certain oxime-bearing flavone and isoflavone derivatives and investigated for their antiproliferative activities.[21–24] Among them, (*Z*)-6-[2-hydroxyimino-2-(4-fluorophenyl)ethoxy]-2-phenyl-4*H*-1-benzopyran-4-one (**1**) and (*Z*)-6-[2-hydroxyimino-2-(4-methoxyphenyl)-ethoxy]-2-phenyl-4*H*-1-benzopyran-4-one (**2**) exhibited significant antiproliferative activities.[24] To further explore and optimize antiproliferative effects, the present report describes the preparation of isomeric amide derivatives of **1** and **2** on the ground that a number of biologically active compounds possess the amide functional moiety.[25–27] To establish a detailed structure–activity relationship (SAR), other amide-containing flavone and isoflavone derivatives have also been synthesized for evaluation.



1: R = F
2: R = OMe



Amide isomers

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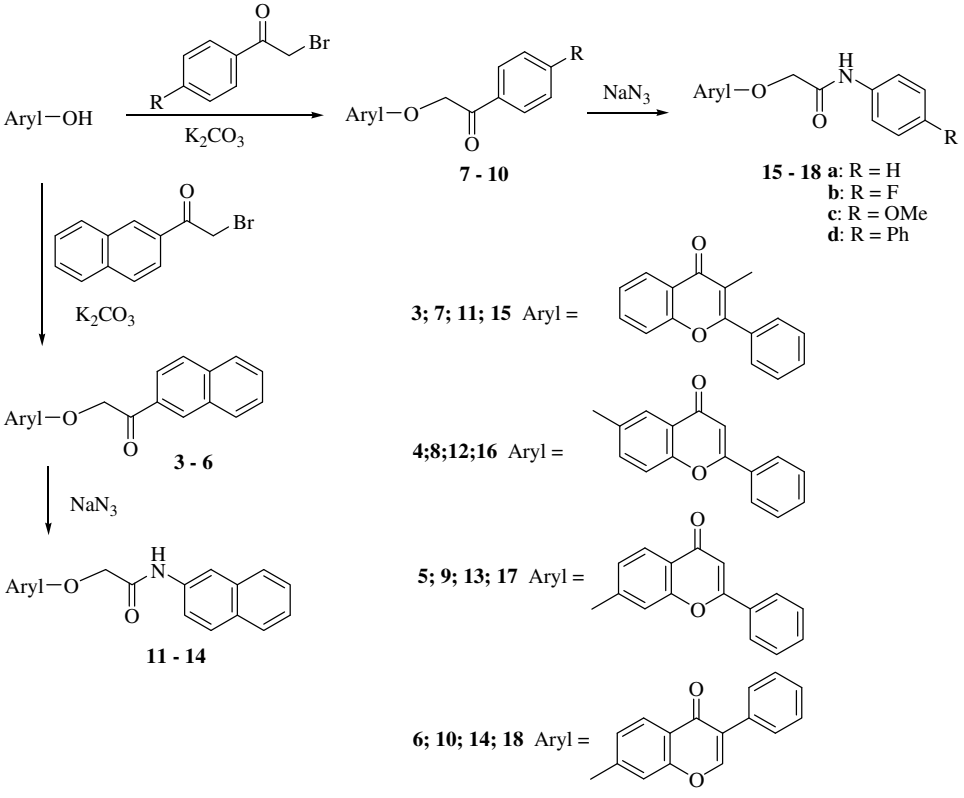
2. Chemistry

The preparation of amide-containing flavone and isoflavone derivatives is illustrated in Scheme 1. Alkylation of 3-hydroxyflavone with 2-bromo-1-(naphthalen-2-yl)ethanone gave 3-(2-(naphthalen-2-yl)-2-oxoethoxy)-2-phenyl-4*H*-chromen-4-one (**3**), which was then treated with H_2SO_4 and NaN_3 to afford *N*-(naphthalen-2-yl)-2-(4-oxo-2-phenyl-4*H*-chromen-3-yloxy)acetamide (**11**) in a good overall yield. The same synthetic procedures were applied for the synthesis of **12–14** from their respective ketone precursor **4**, **5**, and **6**, respectively. Accordingly, **15a–d**, **16a–d**, **17a–d**, and **18a–d** were prepared from **7a–d**, **8a–d**, **9a–d**, and **10a–d**, respectively.[24]

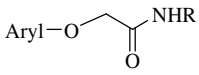
3. Pharmacological results and discussion

Flavonoids are emerging as prospective anticancer drug candidates and some of them have already entered in clinical trials.[28] In view of the therapeutic potential of flavonoids, our laboratories have tried to elucidate possible structure–activity relationships that might lead to new drug discovery. Our current work continues efforts to design and synthesize a series of amide containing flavone and isoflavone derivatives and to evaluate their biological activities. Initially, the growth inhibitory activity of all compounds is evaluated in vitro against a panel of human cancer cell lines, including lung carcinoma (NCI-H661), nasopharyngeal (NPC-TW01), and T-cell leukemia (MT-2) cells. Results from Table 1 indicated that MT-2 is relatively insensitive to the treatment of these amide derivatives with exception of *N*-(4-fluorophenyl)-2-(4-oxo-2-phenyl-4*H*-chromen-6-yloxy)acetamide (**16b**) which exhibited a GI_{50} value of 0.47 μM. The inhibitory activity against the growth of NCI-H661 decreased in an order of linked chromophore flavone-6-yl **16a–d** (GI_{50} values of 5.60, 1.47, 0.84, and 9.71 μM, respectively) > flavone-7-yl **17a–d** > flavone-3-yl **15a–d** and isoflavone-7-yl **18a–d** with an exception of *N*-(biphenyl-4-yl)-2-(4-oxo-2-phenyl-4*H*-chromen-7-yloxy)acetamide (**17d**), which was more active than that of **16d** (GI_{50} values of 1.28 and 9.71 μM, respectively). Among these flavone-6-yl derivatives, *N*-(4-methoxyphenyl)-2-(4-oxo-2-phenyl-4*H*-chromen-6-yloxy)acetamide (**16c**) was the most potent with a GI_{50} value of 0.84 μM. The inhibitory activity against the growth of NPC-TW01 decreased in an order of linked chromophore flavone-6-yl **16a–d** (GI_{50} values of 1.26, 0.74, 0.71, and 0.64 μM, respectively) > isoflavone-7-yl **18a–d** > flavone-7-yl **17a–d** > flavone-3-yl **15a–d** with an exception of *N*-(4-fluorophenyl)-2-(4-oxo-3-phenyl-4*H*-chromen-7-yloxy)acetamide (**18b**), which was more active than that of **16b** (GI_{50} values of 0.55 and 0.74 μM, respectively). All the four flavone-6-yl derivatives demonstrated significant inhibitory activities against the growth of NPC-TW01 cell with an average GI_{50} value of 0.84 μM. Our data have shown that derivatives of flavone-3-yl were inactive against the growth of three cells tested probably due to the steric effect between C-2 phenyl and C-3 substituent, which hindered the coplanar formation of the molecule. In addition, the peripheral substituent of naphthalen-2-yl is not favorable for both flavone and isoflavone chromophores in which compounds **11–14** were inactive. Results from this study have shown that the most preferred pharmacophore is flavone-6-yl and two of the most active substituents (R group) are 4-F-Ph and 4-MeO-Ph. Although a slightly different antiproliferative efficacy of **16b** and **16c** against the growth of NCI-H661 and MT-2 was observed, similar GI_{50} values were identified for both compounds against the growth of NPC-TW01. Thus, **16b** and **16c** along with their respective oxime isomers **1** and **2** were further evaluated on their effects of cell-cycle distribution and the results are summarized in Table 2. The oxime derivatives **1** and **2** caused accumulation of NPC-TW01 cell in G_2/M phase which were similar to that of genistein (an isoflavone derivative)[29] and certain flavonoids such as casticin[30] and apigenin.[31] However, the amide isomers **16b** and **16c** induced cell-cycle arrest in G_0/G_1 phase which were similar to that of quercetin and luteolin



Scheme 1.

Table 1
Antiproliferative activity of flavone and isoflavone derivatives



Compound	Substituents		GI_{50} (μM)[a,b]		
	Aryl	R	NCI-H661	NPC-TW01	MT-2
11	Flavone-3-yl	Naph	6.50 ± 0.52	6.52 ± 0.61	>10
12	Flavone-6-yl	Naph	6.71 ± 0.61	6.75 ± 0.42	>10
13	Flavone-7-yl	Naph	>10	>10	>10
14	Isoflavone-7-yl	Naph	>10	1.37 ± 0.22	7.68 ± 0.92
15a	Flavone-3-yl	Ph	>10	>10	6.72 ± 0.52
15b	Flavone-3-yl	4-F–Ph	>10	9.50 ± 0.82	>10
15c	Flavone-3-yl	4-MeO–Ph	6.74 ± 0.72	5.48 ± 0.62	>10
15d	Flavone-3-yl	4-Ph–Ph	>10	>10	>10
16a	Flavone-6-yl	Ph	5.60 ± 0.52	1.26 ± 0.32	1.43 ± 0.33
16b	Flavone-6-yl	4-F–Ph	1.47 ± 0.15	0.74 ± 0.20	0.47 ± 0.32
16c	Flavone-6-yl	4-MeO–Ph	0.84 ± 0.25	0.71 ± 0.15	3.01 ± 0.52
16d	Flavone-6-yl	4-Ph–Ph	9.71 ± 1.52	0.64 ± 0.23	>10
17a	Flavone-7-yl	Ph	>10	>10	>10
17b	Flavone-7-yl	4-F–Ph	5.77 ± 0.82	>10	6.41 ± 1.21
17c	Flavone-7-yl	4-MeO–Ph	5.50 ± 0.52	5.97 ± 0.92	6.70 ± 1.03
17d	Flavone-7-yl	4-Ph–Ph	1.28 ± 0.58	1.61 ± 0.42	2.48 ± 0.72
18a	Isoflavone-7-yl	Ph	>10	>10	>10
18b	Isoflavone-7-yl	4-F–Ph	>10	0.55 ± 0.20	2.50 ± 0.72
18c	Isoflavone-7-yl	4-MeO–Ph	9.53 ± 1.13	6.70 ± 1.14	4.55 ± 0.82
18d	Isoflavone-7-yl	4-Ph–Ph	>10	1.75 ± 0.42	>10

[a] All tested compounds were dissolved in 100% DMSO at a concentration of 20 mM as the stock solution. Cells were cultured without or in the presence of flavone or isoflavone derivatives at different concentrations for 72 h. Cell survival was determined by MTT assay. Drug molar concentration causing 50% cell growth inhibition (GI_{50}) was calculated. Each value represents the mean ± SD of three independent experiments.
[b] NCI-H661: human lung carcinoma; NPC-TW01: human nasopharyngeal carcinoma; MT-2: human T-cell leukemia.

Table 2
Effect of flavone derivatives on cell-cycle progression of NPC-TW01[a]

Compound	G_0/G_1 (%)	S (%)	G_2/M (%)	Sub-G_1 (%)
Control	48.7 ± 6.4	36.5 ± 3.7	14.2 ± 2.2	0.6 ± 0.2
1	22.3 ± 2.7	33.2 ± 3.1	42.8 ± 4.4	1.7 ± 0.6
2	0.5 ± 0.2	27.5 ± 2.3	70.8 ± 5.7	1.2 ± 0.8
16b	77.2 ± 9.5	13.6 ± 3.0	5.2 ± 0.6	4.0 ± 1.5
16c	77.8 ± 8.2	13.6 ± 1.5	5.3 ± 0.7	3.3 ± 2.0

[a] The human nasopharyngeal carcinoma cells (NPC-TW01) were co-cultured with flavone derivatives at 15 × GI_{50} (6.0 μM, 48.0 μM, 10.5 μM, and 10.5 μM for compound **1**, **2**, **16b**, and **16c**, respectively) for 72 h. Control cells were cultivated with complete medium in the present of 0.5% DMSO for 72 h. The DNA content was evaluated on a Becton Dickinson FACScan flow cytometer and the percentage of cells in various cell-cycle phases was determined by using the ModFit LT software. Data are mean ± SD of three independent experiments.

(flavone derivatives).[32,33] Therefore, the antiproliferative mechanism of flavone derivatives was affected not only by the phenyl benzopyran-4-one pharmacophore but also by the peripheral substituents in which oxime derivatives **1** and **2** were distinct from that of their amide isomers **16b** and **16c**. Figure 1 shows the effect of **16b** on cell-cycle progression in NPC-TW01 cell. The proportion of cells was slightly increased in the sub-G_1 and accumulated in G_0/G_1 phase after 24 h treatment. After 96 h, the accumulation of the cells in G_0/G_1 DNA content was decreased, while the hypodiploid (sub-G_1 phase) cells increased. Compound **16b** inhibited proliferation of NPC-TW01 by the alteration of cell division, accumulation of cells in G_0/G_1 phase at early 72 h which was then decreased followed by the increase of apoptotic cells (sub-G_1 phase) after 96 h treatment. Thus, compound **16b** induces cell-cycle arrest followed by apoptosis.

4. Conclusion

A number of amide containing flavone and isoflavone derivatives were synthesized and evaluated for their antiproliferative activities. The results indicated that the most preferred pharmacophore is flavone-6-yl and two of the most active peripheral substituents are 4-F-Ph and 4-MeO-Ph. All the four flavone-6-yl derivatives demonstrated significant inhibitory activity against the growth of NPC-TW01 cell with an average GI_{50} value of 0.84 μM. Among these flavone-6-yl derivatives, **16b** was the most active against the growth of MT-2 with a GI_{50} value of 0.47 μM, while **16c** was the most active against the growth of NCI-H661 with a GI_{50} value of 0.84 μM. Compounds **16b** and **16c** induced cell-cycle arrest in G_0/G_1 phase followed by apoptosis which was distinct from that of their isomeric oxime derivatives **1** and **2** which induced cell-cycle arrest in G_2/M phase. Further evaluation of **16b** and **16c** as potential anticancer drugs is on-going.

5. Experimental

5.1. General

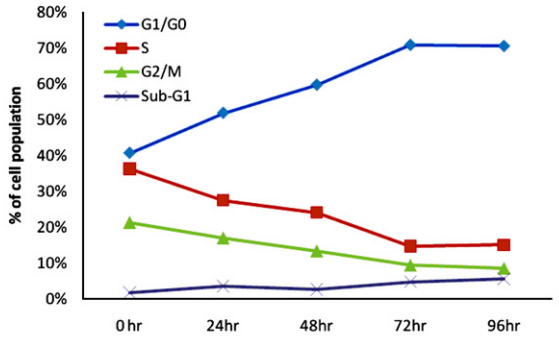
TLC. Precoated (0.2 mm) silica gel 60 F_{254} plates from EM Laboratories, Inc.; detection by UV light (254 nm). Mp: Electrothermal IA9100 digital melting-point apparatus; uncorrected. ^{1}H NMR spectra: Varian-Unity-400 spectrometer at 400, chemical shifts δ in ppm with $SiMe_4$ as an internal standard (=0 ppm), coupling constants J in Hz. Elemental analyses were carried out on a Heraeus CHN-O-Rapid elemental analyzer, and results were within ±0.4% of calculated values.

5.1.1. 3-(2-(Naphthalen-2-yl)-2-oxoethoxy)-2-phenyl-4*H*-chromen-4-one (3)

A solution of 3-hydroxyflavone (0.24 g, 1 mmol) in DMF (20 mL) was added a solution of 2-bromo-1-(naphthalen-2-yl)ethanone (0.25 g, 1 mmol) in DMF (2 mL). The mixture was stirred at room temperature for 48 h (TLC monitoring) and evaporated to give a residual solid. The white solid thus obtained was collected and purified by flash column chromatography (FC; silica gel;

【30 μM】

	Control	24	48	72	96
G1/G0	40.71%	51.95%	59.84%	71.02%	70.71%
S	36.33%	27.57%	24.16%	14.72%	15.07%
G2/M	21.34%	17.04%	13.41%	9.47%	8.60%
Sub-G1	1.62%	3.45%	2.59%	4.79%	5.62%



【60 μM】

	Control	24	48	72	96
G1/G0	40.71%	51.52%	68.32%	73.70%	66.74%
S	36.33%	26.38%	18.35%	15.17%	12.78%
G2/M	21.34%	19.83%	10.46%	6.64%	7.78%
Sub-G1	1.62%	2.27%	2.87%	4.50%	12.70%

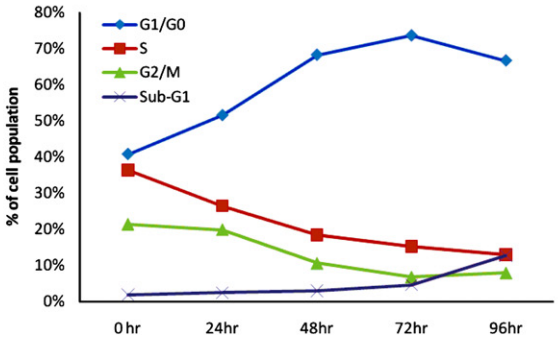


Figure 1. The time- and dose-effect of **16b** on cell-cycle distribution in NPC-TW01. Cells were treated with 30 μM (top panel) or 60 μM (lower panel) of compound **16b** for the time indicated. Control cells were cultivated with complete medium in the presence of 0.3% DMSO without **16b** for the time period indicated. The DNA content of treated cells was analyzed by flow cytometry.

n-hexane/EtOAc 1:1) and recrystallized from CH_2Cl_2 to give **3** (0.38 g, 93%). Mp 170–171 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 5.80 (s, OCH_2), 7.47–7.54 (m, 4H, arom. H), 7.58–7.68 (m, 2H, arom. H), 7.77–7.86 (m, 2H, arom. H), 7.92–8.02 (m, 3H, arom. H), 8.06–8.09 (m, 2H, arom. H), 8.17–8.20 (m, 2H, arom. H), 8.64 (s, 1H, arom. H). ^{13}C NMR (100 MHz, DMSO-d_6): 74.23 (CH_2O), 119.16, 123.89, 123.99, 125.62, 125.88, 127.73, 128.41, 129.15, 129.22, 129.34, 129.53, 130.26, 130.45, 131.18, 131.59, 132.22, 132.77, 134.91, 135.92, 140.08, 154.97, 155.37, 174.54 (C(4)), 194.90 (C=O). Anal. Calcd for $C_{27}H_{18}O_4$: C 79.79, H 4.46. Found: C 79.43, H 4.42.

The same procedure was applied to convert 6-hydroxyflavone to **4**; 7-hydroxyflavone to **5**; and 7-hydroxyisoflavone to **6**, respectively.

5.1.2. 6-(2-(Naphthalen-2-yl)-2-oxoethoxy)-2-phenyl-4*H*-chromen-4-one (4)

Yield: 81%. Mp 178–179 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 5.87 (s, OCH_2), 6.98 (s, 1H–C(3)), 7.47 (d, J = 3.2, 1H–C(5)), 7.53 (dd, J = 9.2, 3.2, 1H–C(7)), 7.55–7.59 (m, 3H, arom. H), 7.62–7.71 (m, 2H, arom. H), 7.77 (d, J = 9.2, 1H–C(8)), 8.00 (s, 1H, arom. H), 8.02 (d, J = 1.6, 1H–C(1′)), 8.05–8.08 (m, 3H, arom. H), 8.14 (d, J = 7.6, 1H, arom. H), 8.80 (s, 1H, arom. H). ^{13}C NMR (100 MHz, DMSO-d_6): 71.37 (CH_2O), 106.84, 120.88, 123.95, 124.38, 124.67, 127.00, 127.84, 128.49, 129.21, 129.67, 129.82, 130.31, 130.71, 131.89, 132.22, 132.46, 132.81, 136.05, 151.28, 156.09, 163.07, 177.54 (C(4)), 194.76 (C=O). Anal. Calcd for $C_{27}H_{18}O_4$: C 79.79, H 4.46. Found: C 79.41, H 4.45.

5.1.3. 7-(2-(Naphthalen-2-yl)-2-oxoethoxy)-2-phenyl-4*H*-chromen-4-one (5)

Yield: 86%. Mp 201–202 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 5.98 (s, OCH_2), 6.99 (s, 1H–C(3)), 7.21 (dd, J = 8.8, 2.4, 1H–C(6)), 7.48 (d, J = 2.4, 1H–C(8)), 7.54–7.60 (m, 3H, arom. H), 7.66–7.75 (m, 2H, arom. H), 7.99 (d, J = 8.8, 1H–C(5)), 8.05–8.12 (m, 5H, arom. H), 8.17 (d, J = 8.0, 1H, arom. H), 8.82 (s, 1H, arom. H). ^{13}C NMR (100 MHz, DMSO-d_6): 71.50 (CH_2O), 102.66, 107.50, 115.82, 118.13, 123.98, 126.89, 126.92, 127.88, 128.51, 129.18, 129.68, 129.77, 130.28, 130.69, 131.85, 132.15, 132.38, 132.79, 136.07, 158.10, 162.91, 163.43, 177.13 (C(4)), 194.09 (C=O). Anal. Calcd for $C_{27}H_{18}O_4$: C 79.79, H 4.46. Found: C 79.69, H 4.43.

5.1.4. 7-(2-(Naphthalen-2-yl)-2-oxoethoxy)-3-phenyl-4*H*-chromen-4-one (6)

Yield: 66%. Mp 192–193 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 5.91 (s, OCH_2), 7.19 (dd, J = 8.8, 2.4, 1H–C(6)), 7.27 (d, J = 2.4, 1H–C(8)), 7.33–7.43 (m, 3H, arom. H), 7.53–7.56 (m, 2H, arom. H), 7.62–7.71 (m, 2H, arom. H), 7.99–8.06 (m, 4H, arom. H), 8.12 (d, J = 8.0, 1H–C(5)), 8.40 (s, 1H, arom. H), 8.77 (s, 1H, arom. H). ^{13}C NMR (100 MHz, DMSO-d_6): 71.43 (CH_2O), 102.41, 115.83, 118.61, 123.90, 124.50, 127.72, 127.84, 128.47, 128.51, 128.83, 129.19, 129.59, 129.68, 130.26, 130.70, 132.10, 132.57, 132.75, 136.06, 154.85, 158.02, 163.24, 175.20 (C(4)), 194.15 (C=O). Anal. Calcd for $C_{27}H_{18}O_4$: C 79.79, H 4.46. Found: C 79.91, H 4.85.

5.1.5. *N*-(Naphthalen-2-yl)-2-(4-oxo-2-phenyl-4*H*-chromen-3-yloxy)acetamide (11)

A solution of **3** (0.41 g, 1 mmol) in H_2SO_4 (3 mL) was stirred at rt for 10 min. To this solution, sodium azide (0.13 g, 2 mmol) was added in one portion. The mixture was stirred at rt for 1 h (TLC monitoring) and then poured into ice-water (100 mL). The white solid thus obtained was collected and purified by flash column chromatography (FC; silica gel; MeOH/EtOAc 1:1) and recrystallized from CH_2Cl_2 to give **11** (0.30 g, 71%). Mp 190–191 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 4.73 (s, OCH_2), 7.38–7.49 (m, 2H, arom. H), 7.51–7.55 (m, 1H, arom. H), 7.58–7.60 (m, 3H, arom. H), 7.66 (dd, J = 8.8, 2.4, 1H, arom. H), 7.79–7.89 (m, 5H, arom. H), 8.14–8.19 (m, 3H, arom. H), 8.30 (d, J = 1.6, 1H–C(1′)), 10.53 (s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 71.91 (CH_2O), 116.27, 119.25, 120.78, 123.92, 125.48, 125.75, 126.05, 127.19, 128.04, 128.17, 129.15, 129.37, 129.44, 130.62, 130.94, 131.86, 134.02, 135.14, 136.59, 140.59, 155.53, 155.93, 167.71 (CONH), 174.99 (C(4)). Anal. Calcd for $C_{27}H_{19}NO_4$: C 76.95, H 4.54, N 3.32. Found: C 76.99, H 4.55, N 3.26.

The same procedure was applied to convert **4** to **12**; **5** to **13**; **6** to **14**; **7a–d** to **15a–d**; **8a–d** to **16a–d; 9a–d** to **17a–d**; and **10a–d** to **18a–d**, respectively.

5.1.6. *N*-(Naphthalen-2-yl)-2-(4-oxo-2-phenyl-4*H*-chromen-6-yloxy)acetamide (12)

Yield: 89%. Mp 253–254 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 4.91 (s, OCH_2), 7.02 (s, 1H–C(3)), 7.38–7.48 (m, 2H, arom. H), 7.51 (d,

J = 3.2, 1H–C(5)), 7.56–7.59 (m, 4H, arom. H), 7.65 (dd, J = 8.8, 2.0, 1H, arom. H), 7.80–7.89 (m, 4H, arom. H), 8.08–8.11 (m, 2H, arom. H), 8.31 (d, J = 1.6, 1H–C(1′)), 10.39 (s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 68.16 (CH_2O), 106.87, 116.57, 120.88, 120.97, 124.41, 124.66, 125.55, 127.03, 127.21, 128.04, 128.17, 129.15, 129.85, 130.65, 131.86, 132.52, 133.99, 136.61, 151.41, 155.93, 163.16, 167.14 (CONH), 177.55 (C(4)). Anal. Calcd for $C_{27}H_{19}NO_4 \cdot 0.1H_2O$: C 76.62, H 4.57, N 3.30. Found: C 76.39, H 4.56, N 3.29.

5.1.7. *N*-(Naphthalen-2-yl)-2-(4-oxo-2-phenyl-4*H*-chromen-7-yloxy)acetamide (13)

Yield: 77%. Mp 202–203 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 4.98 (s, OCH_2), 6.97 (s, 1H–C(3)), 7.21 (dd, J = 8.8, 2.4, 1H–C(6)), 7.38–7.48 (m, 3H, arom. H), 7.54–7.58 (m, 3H, arom. H), 7.65 (dd, J = 8.8, 2.0, 1H, arom. H), 7.80–7.89 (m, 3H, arom, H), 7.99 (d, J = 8.8, 1H–C(5)), 8.06–8.08 (m, 2H, arom. H), 8.31 (s, 1H, arom. H), 10.40 (s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 68.03 (CH_2O), 102.79, 107.53, 115.73, 116.61, 118.29, 120.88, 125.55, 126.92, 127.01, 127.21, 128.04, 128.17, 129.15, 129.81, 130.65, 131.82, 132.42, 133.99, 136.60, 157.98, 162.99, 163.19, 166.72 (CONH), 177.15 (C(4)). Anal. Calcd for $C_{27}H_{19}NO_4$: C 76.95, H 4.54, N 3.32. Found: C 76.67, H 4.55, N 3.30.

5.1.8. *N*-(Naphthalen-2-yl)-2-(4-oxo-3-phenyl-4*H*-chromen-7-yloxy)acetamide (14)

Yield: 88%. Mp 239–240 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 4.97 (s, OCH_2), 7.22–7.24 (m, 2H, arom. H), 7.36–7.48 (m, 5H, arom. H), 7.55–7.57 (m, 2H, arom. H), 7.65 (dd, J = 8.8, 2.0, 1H–C(6)), 7.80–7.85 (m, 2H, arom. H), 7.88 (d, J = 8.8, 1H–(C5)), 8.09 (dd, J = 8.4, 1.2, 1H, arom. H), 8.31 (d, J = 1.2, 1H, arom. H), 8.46 (s, 1H–C(2)), 10.41 (s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 68.12 (CH_2O), 102.38, 115.88, 116.63, 118.76, 120.88, 124.47, 125.56, 127.22, 127.80, 128.06, 128.17, 128.54, 128.85, 129.15, 129.63, 130.67, 132.59, 133.99, 136.56, 154.98, 157.91, 163.00, 166.68 (CONH), 175.13 (C(4)). Anal. Calcd for $C_{27}H_{19}NO_4$: C 76.95, H 4.54, N 3.32. Found: C 76.68, H 4.55, N 3.30.

5.1.9. 2-(4-Oxo-2-phenyl-4*H*-chromen-3-yloxy)-*N*-phenylacetamide (15a)

Yield: 78%. Mp 156–157 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 4.64 (s, OCH_2), 7.05–7.08 (m, 1H, arom. H), 7.29–7.33 (m, 2H, arom. H), 7.50–7.64 (m, 6H, arom. H), 7.78–7.88 (m, 2H, arom. H), 8.13–8.17 (m, 3H, arom. H), 10.31 (s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 71.89 (CH_2O), 119.24, 120.16, 123.92, 124.37, 125.74, 126.05, 129.34, 129.43, 129.47, 130.91, 131.86, 135.14, 138.99, 140.57, 155.52, 155.97, 167.39 (CONH), 174.96 (C(4)). Anal. Calcd for $C_{23}H_{17}NO_4$: C 74.38, H 4.61, N 3.77. Found: C 74.33, H 4.63, N 3.71.

5.1.10. *N*-(4-Fluorophenyl)-2-(4-oxo-2-phenyl-4*H*-chromen-3-yloxy)acetamide (15b)

Yield: 85%. Mp 191–192 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 4.60 (s, OCH_2), 7.12–7.16 (m, 2H, arom. H), 7.52–7.63 (m, 6H, arom. H), 7.76–7.84 (m, 2H, arom. H), 8.11–8.13 (m, 3H, arom. H), 10.35 (s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 71.79 (CH_2O), 115.94, 116.16, 119.22, 122.10, 122.18, 123.84, 125.71, 126.11, 129.31, 129.44, 130.80, 131.91, 135.21, 140.43, 155.50, 156.16, 157.78, 160.17, 167.44 (CONH), 174.98 (C(4)). Anal. Calcd for $C_{23}H_{16}FNO_4$: C 70.95, H 4.14, N 3.60. Found: C 70.99, H 4.27, N 3.66.

5.1.11. *N*-(4-Methoxyphenyl)-2-(4-oxo-2-phenyl-4*H*-chromen-3-yloxy)acetamide (15c)

Yield: 72%. Mp 212–213 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 3.71 (s, OCH_3), 4.60 (s, OCH_2), 6.87–6.89 (m, 2H, arom. H), 7.50–7.59 (m, 6H, arom. H), 7.78–7.88 (m, 2H, arom. H), 8.13–8.16 (m, 3H, arom. H), 10.17 (s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 55.85 (MeO), 71.88 (CH_2O), 114.56, 119.24, 121.75, 123.92, 125.74, 126.04, 129.34, 129.43, 130.91, 131.86, 132.08, 135.13, 140.55, 155.52, 156.02, 156.19, 166.85 (CONH), 174.94 (C(4)). Anal. Calcd for $C_{24}H_{19}NO_5 \cdot 0.2H_2O$: C 71.17, H 4.82, N 3.46. Found: C 71.00, H 4.75, N 3.31.

5.1.12. *N*-(Biphenyl-4-yl)-2-(4-oxo-2-phenyl-4*H*-chromen-3-yloxy)acetamide (15d)

Yield: 65%. Mp 171–172 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 4.67 (s, OCH_2), 7.30–7.34 (m, 1H, arom. H), 7.41–7.45 (m, 2H, arom. H), 7.51–7.65 (m, 8H, arom. H), 7.73–7.88 (m, 4H, arom. H), 8.14–8.18 (m, 3H, arom. H); 10.43 (s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 71.93 (CH_2O), 119.25, 120.53, 123.92, 125.75, 126.05, 126.99, 127.68, 127.79, 129.35, 129.44, 129.61, 130.91, 131.87, 135.15, 136.01, 138.49, 140.32, 140.59, 155.53, 155.99, 167.47 (CONH), 174.99 (C(4)). Anal. Calcd for $C_{29}H_{21}NO_4$: C 77.84, H 4.73, N 3.13. Found: C 77.52, H 4.77, N 3.17.

5.1.13. 2-(4-Oxo-2-phenyl-4*H*-chromen-6-yloxy)-*N*-phenylacetamide (16a)

Yield: 91%. Mp 224–225 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 4.84 (s, OCH_2), 7.01 (s, 1H–C(3)), 7.05–7.08 (m, 1H, arom. H), 7.29–7.33 (m, 2H, arom. H), 7.47 (d, J = 3.2, 1H–C(5)), 7.53 (dd, J = 9.2, 3.2, 1H–C(7)), 7.55–7.63 (m, 5H, arom. H), 7.79 (d, J = 9.2, 1H–C(8)), 8.07–8.10 (m, 2H, arom. H), 10.18 (s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 68.08 (CH_2O), 106.78, 106.85, 120.35, 120.94, 124.38, 124.45, 124.64, 127.02, 129.48, 129.83, 131.85, 132.50, 139.01, 151.38, 155.89, 163.13, 166.83 (CONH), 177.53 (C(4)). Anal. Calcd for $C_{23}H_{17}NO_4$: C 74.38, H 4.61, N 3.77. Found: C 74.28, H 4.65, N 3.76.

5.1.14. *N*-(4-Fluorophenyl)-2-(4-oxo-2-phenyl-4*H*-chromen-6-yloxy)acetamide (16b)

Yield: 96%. Mp 241–242 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 4.83 (s, OCH_2), 7.01 (s, 1H–C(3)), 7.13–7.18 (m, 2H, arom. H), 7.47 (d, J = 3.2, 1H–C(5)), 7.53 (dd, J = 9.2, 3.2, 1H–C(7)), 7.56–7.59 (m, 3H, arom. H), 7.62–7.66 (m, 2H, arom. H), 7.79 (d, J = 9.2, 1H–C(8)), 8.07–8.10 (m, 2H, arom. H), 10.23 (s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 68.07 (CH_2O), 106.86, 115.95, 116.17, 120.94, 122.20, 122.28, 124.35, 124.64, 127.02, 129.83, 131.85, 132.51, 135.36, 135.38, 151.39, 155.84, 157.78, 160.16, 163.15, 166.81 (CONH), 177.52 (C(4)). Anal. Calcd for $C_{23}H_{16}FNO_4$: C 70.95, H 4.14, N 3.60. Found: C 70.88, H 4.23, N 3.57.

5.1.15. *N*-(4-Methoxyphenyl)-2-(4-oxo-2-phenyl-4*H*-chromen-6-yloxy)acetamide (16c)

Yield: 94%. Mp 194–195 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 3.70 (s, OCH_3), 4.79 (s, OCH_2), 6.87–6.90 (m, 2H, arom. H), 7.01 (s, 1H–C(3)), 7.47 (d, J = 3.2, 1H–C(5)), 7.52 (dd, J = 9.2, 3.2, 1H–C(7)), 7.53–7.59 (m, 5H, arom. H), 7.79 (d, J = 9.2, 1H–C(8)), 8.07–8.10 (m, 2H, arom. H), 10.04 (s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 55.84 (MeO), 68.11 (CH_2O), 106.85, 114.56, 120.92, 122.02, 124.37, 124.64, 127.02, 129.83, 131.85, 132.06, 132.50, 151.37, 155.90, 156.25, 163.12, 166.35 (CONH), 177.53 (C(4)). Anal. Calcd for $C_{24}H_{19}NO_5$: C 71.81, H 4.77, N 3.49. Found: C 71.49, H 4.79, N 3.51.

5.1.16. *N*-(Biphenyl-4-yl)-2-(4-oxo-2-phenyl-4*H*-chromen-6-yloxy)acetamide (16d)

Yield: 61%. Mp 254–255 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 4.89 (s, 2H, OCH_2), 7.05 (s, 1H–C(3)), 7.32–7.38 (m, 1H, arom. H), 7.43–7.47 (m, 2H, arom. H), 7.52 (d, J = 3.2, 1H–C(5)), 7.58 (dd, J = 9.2, 3.2, 1H–C(7)), 7.59–7.63 (m, 3H, arom. H), 7.65–7.67 (m, 4H, arom. H), 7.75–7.77 (m, 2H, arom. H), 7.83 (d, J = 9.2, 1H–C(8)), 8.11–8.13 (m, 2H, arom. H), 10.30 (s, NH), ^{13}C NMR (100 MHz, DMSO-d_6): 68.17 (CH_2O), 106.88, 120.69, 120.96, 124.38, 124.67, 126.99, 127.03, 127.68, 127.80, 129.60, 129.80, 129.84, 131.88, 132.50, 136.09, 138.50, 140.30, 151.40, 155.91, 163.13, 166.90 (CONH),

177.51 (C(4)). Anal. Calcd for $C_{29}H_{21}NO_4$: C 77.84, H 4.73, N 3.13. Found: C 77.56, H 4.75, N 3.05.

5.1.17. 2-(4-Oxo-2-phenyl-4*H*-chromen-7-yloxy)-*N*-phenylacetamide (17a)

Yield: 87%. Mp 207–208 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 4.92 (s, OCH_2), 6.98 (s, 1H–C(3)), 7.07–7.11 (m, 1H, arom. H), 7.19 (dd, J = 8.8, 2.4, 1H–C(6)), 7.31–7.35 (m, 2H, arom. H), 7.37 (d, J = 2.4, 1H–C(8)), 7.57–7.66 (m, 5H, arom. H), 7.99 (d, J = 8.8, 1H–C(5)), 8.07–8.09 (m, 2H, arom. H), 10.20 (s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 67.98 (CH_2O), 102.73, 107.52, 115.68, 118.26, 120.37, 124.49, 126.91, 126.99, 129.49, 129.81, 131.81, 132.41, 138.99, 157.96, 162.98, 163.14, 166.42 (CONH), 177.13 (C(4)). Anal. Calcd for $C_{23}H_{17}NO_4$: C 74.38, H 4.61, N 3.77. Found: C 74.00, H 4.60, N 3.75.

5.1.18. N-(4-Fluorophenyl)-2-(4-oxo-2-phenyl-4*H*-chromen-7-yloxy)acetamide (17b)

Yield: 85%. Mp 207–208 °C. ^{1}H-NMR (400 MHz, DMSO-d_6): 4.90 (s, OCH_2), 6.96 (s, 1H–C(3)), 7.15 (dd, J = 9.2, 2.4, 1H–C(6)), 7.16–7.19 (m, 2H, arom. H), 7.36 (d, J = 2.4, 1H–C(8)), 7.55–7.67 (m, 5H, arom. H), 7.97 (d, J = 9.2, 1H–C(5)), 8.05–8.08 (m, 2H, arom. H), 10.24 (s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 67.95 (CH_2O), 102.75, 107.52, 115.70, 115.96, 116.17, 118.30, 122.23, 122.31, 126.91, 126.99, 129.81, 131.81, 132.41, 135.36, 157.79, 157.95, 160.18, 162.97, 163.08, 166.40 (CONH), 177.13 (C(4)). Anal. Calcd for $C_{23}H_{16}FNO_4{\cdot}0.1H_2O$: C 70.62, H 4.17, N 3.58. Found: C 70.48, H 4.18, N 3.59.

5.1.19. *N*-(4-Methoxyphenyl)-2-(4-oxo-2-phenyl-4*H*-chromen-7-yloxy)acetamide (17c)

Yield: 67%. Mp 188–189 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 3.70 (s, OCH_3), 4.86 (s, OCH_2), 6.87–6.90 (m, 2H, arom. H), 6.96 (s, 1H–C(3)), 7.17 (dd, J = 8.8, 2.4, 1H–C(6)), 7.35 (d, J = 2.4, 1H–C(8)), 7.52–7.58 (m, 5H, arom. H), 7.97 (d, J = 8.8, 1H–C(5)), 8.05–8.08 (m, 2H, arom. H), 10.04 (s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 55.84 (MeO), 68.03 (CH_2O), 102.73, 107.52, 114.56, 115.72, 118.26, 122.04, 126.91, 126.98, 129.81, 131.81, 132.03, 132.41, 156.27, 157.95, 162.97, 163.12, 165.93 (CONH), 177.13 (C(4)). Anal. Calcd for $C_{24}H_{19}NO_5$: C 71.81, H 4.77, N 3.49. Found: C 71.42, H 4.88, N 3.41.

5.1.20. *N*-(Biphenyl-4-yl)-2-(4-oxo-2-phenyl-4*H*-chromen-7-yloxy)acetamide (17d)

Yield: 85%. Mp 216–217 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 4.94 (s, OCH_2), 6.97 (s, 1H–C(3)), 7.19 (dd, J = 8.8, 2.4, 1H–C(6)), 7.30–7.33 (m, 1H, arom. H)), 7.38 (d, J = 2.4, 1H–C(8)), 7.40–7.44 (m, 2H, arom. H), 7.56–7.65 (m, 7H, arom. H), 7.72–7.75 (m, 2H, arom. H), 7.99 (d, J = 8.8, 1H–C(5)), 8.06–8.09 (m, 2H, arom. H), 10.30 (s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 68.03 (CH_2O), 102.76, 107.53, 115.71, 118.30, 120.72, 126.92, 126.98, 127.02, 127.68, 127.81, 129.61, 129.82, 131.82, 132.42, 136.11, 138.47, 140.26, 157.97, 162.99, 163.15, 166.49 (CONH), 177.14 (C(4)). Anal. Calcd for $C_{29}H_{21}NO_4$: C 77.84, H 4.73, N 3.13. Found: C 77.89, H 4.74, N 3.07.

5.1.21. 2-(4-Oxo-3-phenyl-4*H*-chromen-7-yloxy)-*N*-phenylacetamide (18a)

Yield: 89%. Mp 233–234 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 4.90 (s, OCH_2), 7.05–7.09 (m, 1H, arom. H), 7.19–7.21 (m, 2H, arom. H), 7.30–7.42 (m, 5H, arom. H), 7.55–7.57 (m, 2H, arom. H), 7.61–7.63 (m, 2H, arom. H), 8.07 (d, J = 9.2, 1H–C(5)), 8.45 (s, 1H–C(2)), 10.20 (s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 68.07 (CH_2O), 102.32, 115.85, 118.75, 120.38, 124.48, 124.51, 127.79, 128.54, 128.85, 129.49, 129.63, 132.58, 138.96, 154.96, 157.89, 162.96, 166.38 (CONH), 175.13 (C(4)). Anal. Calcd for $C_{23}H_{17}NO_4$: C 74.38, H 4.61, N 3.77. Found: C 74.28, H 4.60, N 3.70.

5.1.22. N-(4-Fluorophenyl)-2-(4-oxo-3-phenyl-4*H*-chromen-7-yloxy)acetamide (18b)

Yield: 93%. Mp 269–270 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 4.89 (s, OCH_2), 7.14–7.21 (m, 4H, arom. H), 7.35–7.44 (m, 3H, arom. H), 7.55–7.57 (m, 2H, arom. H), 7.62–7.66 (m, 2H, arom. H), 8.07 (d, J = 8.8, 1H–C(5)), 8.46 (s, 1H–C(2)), 10.25 (s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 68.03 (CH_2O), 102.36, 115.86, 115.97, 116.19, 118.78, 122.25, 122.34, 124.49, 127.80, 128.55, 128.85, 129.63, 132.58, 135.30, 135.33, 154.98, 157.89, 162.91, 166.36 (CONH), 175.13 (C(4)). Anal. Calcd for $C_{23}H_{16}FNO_4$: C 70.95, H 4.14, N 3.60. Found: C 70.92, H 4.16, N 3.59.

5.1.23. *N*-(4-Methoxyphenyl)-2-(4-oxo-3-phenyl-4*H*-chromen-7-yloxy)acetamide (18c)

Yield: 91%. Mp 228–229 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 3.70 (s, OCH_3), 4.85 (s, OCH_2), 6.87–6.91 (m, 2H, arom. H), 7.18–7.21 (m, 2H, arom. H), 7.34–7.44 (m, 3H, arom. H), 7.50–7.55 (m, 3H, arom. H), 7.56 (d, J = 1.6, 1H, arom. H), 8.06 (d, J = 9.2, 1H–C(5)), 8.44 (s, 1H–C(2)), 10.06 (s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 55.85 (MeO), 68.11 (CH_2O), 102.33, 114.56, 115.89, 118.74, 122.06, 124.48, 127.77, 128.54, 128.85, 129.63, 131.99, 132.58, 154.97, 156.29, 157.89, 162.96, 165.89 (CONH), 175.13 (C(4)). Anal. Calcd for $C_{24}H_{19}NO_5$: C 71.81, H 4.77, N 3.49. Found: C 71.79, H 4.78, N 3.47.

5.1.24. *N*-(Biphenyl-4-yl)-2-(4-oxo-3-phenyl-4*H*-chromen-7-yloxy)acetamide (18d)

Yield: 90%. Mp 285–286 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 4.92 (s, OCH_2), 7.20–7.22 (m, 2H, arom. H), 7.30–7.44 (m, 6H, arom. H), 7.55–7.57 (m, 2H, arom. H), 7.62–7.65 (m, 4H, arom. H), 7.72–7.74 (m, 2H, arom. H), 8.08 (d, J = 9.6, 1H–C(5)), 8.46 (s, 1H–C(2)), 10.31 (s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 68.10 (CH_2O), 102.35, 115.87, 118.76, 120.73, 124.48, 126.98, 127.68, 127.81, 128.55, 128.85, 129.63, 132.58, 136.14, 138.43, 140.26, 154.98, 157.90, 162.96, 166.45 (CONH), 175.13 (C(4)). Anal. Calcd for $C_{29}H_{21}NO_4$: C 77.84, H 4.73, N 3.13. Found: C 77.81, H 4.75, N 3.08.

5.2. Antiproliferative activity

5.2.1. Cell lines

Human non-small cell lung carcinoma (NCI-H661) was purchased from American Type Culture Collection (ATCC; Rockville, MD). T-cell leukemia (MT-2) was obtained from Japanese Collection of Research Bioresources (JCRB) and nasopharyngeal carcinoma (NPC-TW01) was purchased from Bioresource Collection and Research Center (BCRC, Taiwan). All the tumor cell lines were maintained in either RPMI-1640 or Modified essential medium (MEM) supplied with 10% fetal bovine serum at 37 °C in a humidified atmosphere of 5% CO_2/95% air in the absence of antibiotics.

5.2.2. Growth inhibition assay

Logarithmic phase cells were seeded in a 96-well plate and incubated overnight prior to addition of the designated compounds. After incubation with different concentrations of the tested compounds for 72 h, cells were incubated with MEM containing 0.4 mg/mL MTT for 2 h. The conversion of MTT to formazan by metabolically viable cells was measured by the absorbance at 570 nm in a 96-well microtiter plate reader. The percentage conversion by mock-treated control cells was used to evaluate the effect of the chemicals on cell growth and to determine the concentration that inhibited 50% of growth (GI_{50}).

5.2.3. Cell-cycle analysis

Exponentially growing cells were incubated with various concentrations of the tested compounds for the indicated times. The cells were then fixed, incubated with RNase, and stained with

50 μg/mL of propidium iodide. DNA content was evaluated on a Becton Dickinson FACScan flow cytometer and the percentage of cells in various cell-cycle phases was determined by using the ModFit LT software (Verity Software House, Inc.). For each analysis, 10,000 events were recorded.

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