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Research paper

Phosphoramidate protides of five flavones and their antiproliferative activity against HepG2 and L-O2 cell lines

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ABSTRACT

A series of flavone-7-phosphoramidate derivatives were synthesized and tested for their antiproliferative activity in vitro against human hepatoma cell line HepG2 and human normal hepatic cell line L-O2. Compound **8d**, **16d** and **17d**, incorporating the amino acid alanine, exhibited high inhibitory activity on HepG2 cell line with IC_{50} values of 9.0 μmol/L, 5.5 μmol/L and 6.6 μmol/L. The introduction of acyl groups played a pivotal role in the selective inhibition toward human hepatoma HepG2 cells, except for compound **8a**, **9a** and **16b**. Compound **8d**, **16d** and **17d** could significantly induce G2/M arrest in HepG2 cells. Specially, Compound **16d** could lead early apoptosis in HepG2 cells.

1. Introduction

The study of the flavonoids dated back to the 17th century and since then, various discoveries have been made regarding their biological functions [1]. Chrysin, apigenin, luteolin, daidzein and genistein (Fig. 1), polyphenolic compounds available in foods and traditional Chinese medicines of plant origin, belong to the flavone subclass of flavonoids usually occurring as glycosylated forms. These five flavones demonstrate versatile biological activities, such as antibacterial activities [2], antioxidant [3], anti-inflammatory [4], antiviral [5], and antitumor [6–11]. Some reports have been dedicated to the improvement of the anticancer activities and the structure-activity relationships of these flavones' derivatives [12,13].

A strategy known as 'phosphoramidate protide' was introduced by McGuiganet al. as a means of improving the therapeutic potential of a prototype drug for HIV-1 [14,15]. Traditionally, phosphoramidate groups are covalently linked to nucleosides in 'phosphoramidate protide'. Phosphoramidate chemistry has also been applied to nucleoside analogues to generate a new class of anti-cancer agents, which overcome diverse resistance mechanisms [16]. The potency of the compounds varies with the individual components (aryl, ester, and amino acid) of the phosphoramidate moiety. Our interest is focused on the application of phosphoramidate chemistry to the five flavones and the change in the antiproliferation in vitro. In this regard, herein we report the synthesis of a series of amino acid-based flavone phosphoramidates. Subsequently, structurally related flavone phosphoramidates were evaluated in vitro against human hepatoma cell line HepG2 and human normal hepatic cell line L-O2.

2. Results and discussion

2.1. Chemistry

To expand the structural diversity of title compounds, we prepared a series of flavone-7-phosphoramidate derivatives by altering the amino acid and flavone moieties. The structures of target compounds were confirmed on the basis of NMR and mass spectrum. The target compounds were prepared using phosphorochloridate chemistry [17,18]. The key reagent to prepare flavone derivatives is the phenyl aminoacyl phosphorochloridate **2a** ~ **2e** (Scheme 1). It was prepared using two reagents: an amino acid ester and a phenyloxy phosphorodichloridate. The first reagent, an amino acid ester, can be prepared by esterification of the appropriate amino acid via standard esterification methods [19]. Phenyl

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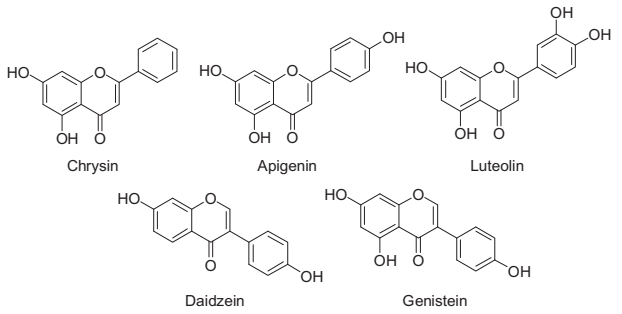
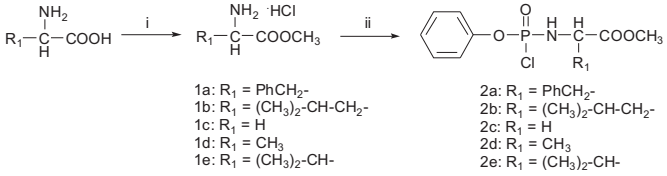


Fig. 1. Structures of five flavones.



Scheme 1. Synthesis of phenyl aminoacyl phosphorochloridates (**2a**–**e**). Reagents and conditions: (i) $SOCl_2$, CH_3OH, −10 °C; (ii) phenyl phosphorodichloridate, TEA, anhydrous CH_2Cl_2, −70 °C.

phosphorodichloridate is commercially available. The phenyl aminoacyl phosphorochloridates were formed by reaction of phenyl phosphorodichloridates with appropriate amino acid esters in the presence of triethylamine [17]. As noted in Table 1, we varied the amino acid from phenylalanine to leucine, glycine, L-alanine and valine. Because of their limited stability, the crude product of compounds **2a** ~ **2e** was directly used as materials in the ProTide syntheses.

Flavone-7-phosphoramidate derivatives were synthesized from phenyl aminoacyl phosphorochloridate and different kinds of flavone. The preparation of flavone-7-phosphoramidate derivatives is illustrated in Scheme 2. The synthetic route for isoflavone-7-phosphoramidate derivatives is outlined in Scheme 3. In order to favor the 7-regioselectivity of the phosphorylation with the phenyl aminoacyl phosphorochloridates, apigenin, luteolin, daidzein and genistein were acetylated with Ac_2O/pyridine and then selectively deprotected on 7-OH group with PhSH/imidazole [20]. Selectively deacylation on 7-OH group was optimized in mixture solvents of N-methyl pyrrolidone/THF (1:3) with short reaction time and good yield (74.8%–85.0%).

Each aminoacyl phosphorochloridate (**2a** ~ **2e**) was reacted with the 7-OH group of compounds chrysin, **6**, **7**, **14** and **15**. The phosphorochloridates **2a** ~ **2e** were allowed to react with chrysin or compound **7** in the presence of TEA to generate the desired compounds **3a** ~ **3e** and **9a** ~ **9e** in THF. But to compounds **6**, **14** and **15**, the reaction lasted more than 4 days when catalyzed by TEA in THF. When the reaction system of K_2CO_3/acetone was used, the phosphoramidate derivatives (**8a** ~ **8e**) syntheses were completed within six hours [21]. The reaction between phosphorochloridates **2a** ~ **2e** and isoflavone derivatives **14** or **15** was very slow using K_2CO_3/acetone. So microwave reaction was used to shorten the reaction time. The synthetic conditions of the acetoxyflavones-7-phosphoramidate derivatives were summarized (Table S1). Finally, the deacylation conditions were screened [22–26] and pyrrolidine was used to deacetylate [27] (Table S2). While deacylating compounds **10a** ~ **10e** and **11a** ~ **11e** using pyrrolidine, the product became complex and difficult to be purified. Therefore, daidzein and genistein were tried to react with phosphorochloridates **2a** ~ **2e** under microwave-assistant condition and turned to be successful (Scheme 3).

2.2. Biological studies

Among the screened compounds, compound **8d**, **16d** and **17d** from acetoxyflavone-7-phosphoramidate series were found to be active in cell based cytotoxicity screening at less than 10 μM concentration. These three compounds were selected for detailed mechanistic investigations.

2.2.1. Compounds inhibit cell proliferation in HepG2 and L-O2 cell lines

Cytotoxicity effects of compounds in HepG2 (hepatocellular carcinoma) cell lines and L-O2 (human liver) cell lines were analyzed by using MTT assay. Most of the compounds inhibited cellular proliferation in HepG2 and L-O2 cell lines. The IC_{50} values (μM) for 48 h incubation were reported (Table 1). Among the 39 tested derivatives, 28 flavone-7-phosphoramidates showed inhibition of HepG2 cells with IC_{50} values less than 100 μmol/L. The results revealed that most of the synthetic compounds exhibited moderate inhibition of cell proliferation. Compound **8d** (IC_{50} 9.0 ± 1.7 μM)、**16d** (IC_{50} 5.5 ± 1.3 μM) and **17d** (IC_{50} 6.6 ± 1.3 μM) displayed higher potency. Interestingly, these three compounds were all L-alanine phosphoramidates. This is consistent with the most potent gemcitabine Protides [16]. In addition, the acetyl group plays an important role to increase the cytotoxicity toward tumor cells HepG2 (Table 1), except for compounds **8a**, **9a** and **16b**. Especially, acetylation for isoflavone-7-phosphoramidates improved the selectivity between HepG2 and L-O2 more than six fold.

2.2.2. Compound *8d*, *16d* and *17d* induces G2/M phase cell cycle arrest in HepG2 cell line

Apigenin, daidzein and genistein were previously shown to induce G2/M cell cycle arrest in human oesophageal adenocarcinoma cells [7], breast cancer cells [28–30], colon cancer cells [31,32], T24 human bladder cancer cells [33], and human malignant glioma cells [34]. In view of the above-mentioned effects on cell growth, it was needed to examine whether the flavone-7-phosphoramidate derivatives **8d**, **16d** and **17d** were able to induce G2/M phase arrest in HepG2. Treatment of HepG2 cell line with compound **8d**, **16d** and **17d** at IC_{50} concentration induced cell cycle arrest in G2/M phase (Fig. 2). Control cells showed 16.4% cells in G2/M phase which was increased to 72.6%, 98.1% and 96.6% by the treatment with compound **8d**, **16d** and **17d**, respectively. Apigenin could increase T24 human bladder cancer cells in G2/M phase to 37.94% at the concentration of 160 μM and incubated for 24 h, in contrast with control (14.45%) [33]. Daidzein could cause cell cycle arrest at G2/M phase (21.8%–38.9%, 22.7% for control) in human breast cancer MCF-7 at various concentration of 1–100 μM and incubated for 72 h [30]. Genistein could also induce cell cycle arrest at G2/M phase (58%, 34% for control) in human malignant glioma LNT-229 cell line at the concentration of 100 μM and incubated for 24 h [34]. Compared with the literature, daidzein derivative **16d** and genistein derivative **17d** almost completely arrested the cell cycle at G2/M phase. We could infer confidently that the action of apigenin, daidzein and genistein on cell cycle was increased by the introduction of phosphoramidate groups dramatically.

2.2.3. Compound 16d leads early apoptosis in HepG2 cell line

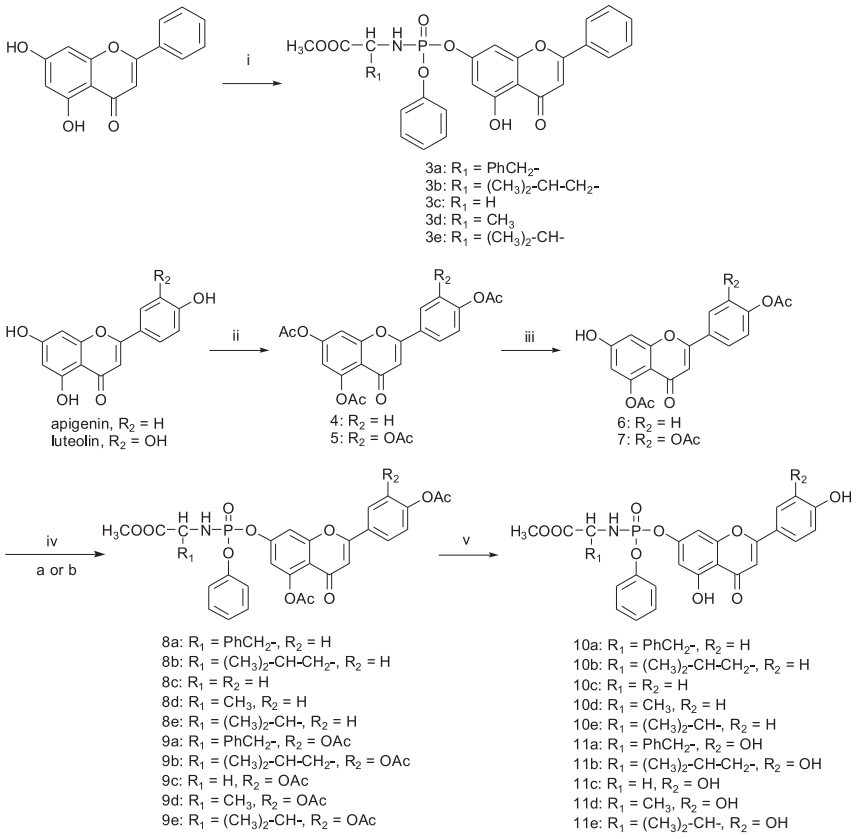
The effects on cell growth and cell cycle progression of the flavone-7-phosphoramidate derivatives **8d**, **16d** and **17d** were better than that of flavones. So the apoptosis was further measured by double staining of cells with Annexin-V/PI and quantified by

Table 1
IC_{50}(μM) Values of 39 flavones derivatives against HepG2 and L-O2 cell lines and impact of acetoxy group on selective antiproliferation.

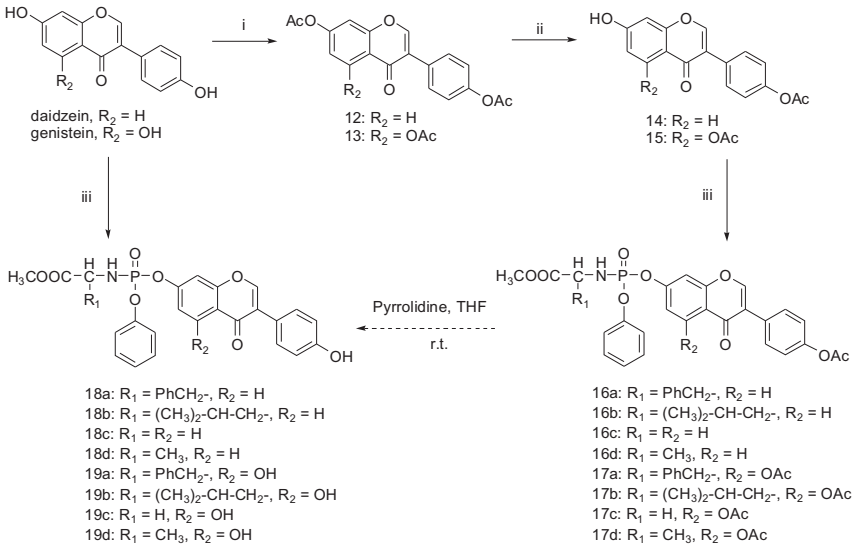
Compd.	Substituents		IC_{50} ± SD(μmol/L)[a]		L-O2/HepG2
	AA	Flavone	HepG2	L-O2	
chrysin	–	chrysin	NA[b]	NA[b]	–
3a	Phe	chrysin	NA[b]	NA[b]	–
3b	Leu	chrysin	NA[b]	NA[b]	–
3c	Gly	chrysin	42.6 ± 3.7	96.5 ± 4.8	2.265
3d	Ala	chrysin	35.3 ± 3.6	62.3 ± 4.3	1.765
3e	Val	chrysin	21.1 ± 2.6	NA[b]	>5
apigenin	–	apigenin	70.18 ± 2.1	NA[b]	>1.425
8a	Phe	4′, 5-diacetoxyapigenin	38.5 ± 0.8	45.9 ± 2.9	1.192
10a	Phe	apigenin	36.6 ± 3.8	NA[b]	>2.732
8a/10a	Phe	–	1.052	<0.459	**<0.436**
8b	Leu	4′, 5-diacetoxyapigenin	22.1 ± 0.5	59.8 ± 1.4	2.706
10b	Leu	apigenin	37.2 ± 1.0	52.8 ± 1.5	1.419
8b/10b	Leu	–	0.594	1.133	**1.907**
8c	Gly	4′, 5-diacetoxyapigenin	47.8 ± 1.4	NA[b]	>2.092
10c	Gly	apigenin	59.7 ± 0.7	71.5 ± 1.0	1.198
8c/10c	Gly	–	0.801	>1.399	**>1.746**
8d	Ala	4′, 5-diacetoxyapigenin	9.0 ± 1.7	22.2 ± 1.4	2.467
10d	Ala	apigenin	34.7 ± 3.2	35.3 ± 1.0	1.017
8d/10d	Ala	–	0.259	0.629	**2.429**
8e	Val	4′, 5-diacetoxyapigenin	18.4 ± 0.8	29.4 ± 0.9	1.590
10e	Val	apigenin	27.3 ± 3.2	26.0 ± 0.7	0.952
8e/10e	Val	–	0.674	1.131	**1.678**
luteolin	–	luteolin	34.7 ± 5.9	NA[b]	>2.882
9a	Phe	3′, 4′, 5-triacetoxyluteolin	27.1 ± 0.6	30.3 ± 2.5	1.118
11a	Phe	luteolin	15.7 ± 3.3	39.1 ± 1.4	2.490
9a/11a	Phe	–	1.726	0.775	**0.449**
9b	Leu	3′, 4′, 5-triacetoxyluteolin	21.0 ± 0.3	38.2 ± 1.0	1.819
11b	Leu	luteolin	22.4 ± 2.4	34.0 ± 0.1	1.581
9b/11b	Leu	–	0.938	1.124	**1.198**
9c	Gly	3′, 4′, 5-triacetoxyluteolin	26.4 ± 2.9	57.1 ± 2.6	2.163
11c	Gly	luteolin	47.7 ± 5.4	47.7 ± 1.7	1
9c/11c	Gly	–	0.553	1.197	**2.163**
9d	Ala	3′, 4′, 5-triacetoxyluteolin	27.3 ± 2.9	51.2 ± 0.6	1.875
11e	Val	luteolin	16.6 ± 1.5	8.2 ± 0.2	0.494
daidzein	–	daidzein	NA[b]	NA[b]	–
16a	Phe	4′-acetoxydaidzein	60.2 ± 3.8	NA[b]	>1.660
18a	Phe	daidzein	52.1 ± 3.9	39.3 ± 2.5	0.754
16a/18a	Phe	–	1.155	>2.545	>**2.203**
16b	Leu	4′-acetoxydaidzein	60.0 ± 1.5	44.8 ± 0.7	0.747
18b	Leu	daidzein	66.0 ± 3.4	47.7 ± 1.6	0.723
16b/18b	Leu	–	0.909	0.939	**1.033**
16c	Gly	4′-acetoxydaidzein	NA[b]	NA[b]	–
18c	Gly	daidzein	48.8 ± 2.0	NA[b]	2.049
16c/18c	Gly	–	>2.049	–	–
16d	Ala	4′-acetoxydaidzein	5.5 ± 1.3	24.3 ± 3.0	4.418
18d	Ala	daidzein	28.8 ± 2.4	20.5 ± 3.0	0.712
16d/18d	Ala	–	0.191	1.185	**6.204**
genistein	–	genistein	NA[b]	NA[b]	–
17a	Phe	4′, 5-diacetoxygenistein	63.2 ± 2.5	NA[b]	1.582
19a	Phe	genistein	NA[b]	NA[b]	–
17a/19a	Phe	–	<0.632	–	–
17b	Leu	4′, 5-diacetoxygenistein	54.0 ± 0.4	60.1 ± 2.0	1.113
19b	Leu	genistein	72.3 ± 1.6	48.2 ± 0.6	0.667
17b/19b	Leu	–	0.747	1.247	**1.669**
17c	Gly	4′, 5-diacetoxygenistein	39.7 ± 1.1	55.2 ± 1.0	1.390
19c	Gly	genistein	NA[b]	44.5 ± 7.5	<0.445
17c/19c	Gly	–	<0.397	1.240	>**3.123**
17d	Ala	4′, 5-diacetoxygenistein	6.6 ± 1.3	11.9 ± 1.3	1.803
19d	Ala	genistein	57.2 ± 4.8	15.7 ± 0.4	0.274
17d/19d	Ala	–	0.115	0.758	**6.591**
erlotinib	–	–	5.8 ± 0.6	18.5 ± 1.1	**3.190**

The shade indicates the impact of acetoxy group on the selective antiproliferation.
[a] The IC_{50} value represents the concentration of each compound resulting in 50% inhibition in cell growth after 48 h incubation, and was the mean values of three repeated experiments.
[b] NA: Compounds having IC50 value > 100 μM.

flow cytometry to simultaneously differentiate viable, early apoptotic, late apoptotic and necrotic cells. HepG2 cells were treated with compound **8d**, **16d** and **17d** at IC_{50} values and analyzed by flow cytometry (Fig. 3 and Table S3). Compared with untreated control, compound **8d** and **17d** mainly resulted in necrosis. Control cells showed 6.7% necrosis cells which were increased to 9.88% and 18.62% by the treatment with compound **8d** and **17d** for 24 h incubation, respectively. But 5.5 μM compound **16d** resulted in 44.16% early apoptosis cells, compared with the 0.41% early apoptosis cells for control. Daidzein induced cancer cells apoptosis [30,35]. 80 μM



Scheme 2. Synthetic method to obtain compounds **3a**–**e**, **8a**–**e**, **9a**–**e**, **10a**–**e** and **11a**–**e**. Reagents and conditions: (i) phosphorochloridates **2a**–**e**, TEA, anhydrous THF, r.t.; (ii) Ac_2O, Pyridine, reflux; (iii) PhSH, imidazole, NMP/THF, −10 °C; (iv) a: phosphorochloridates **2a**–**e**, TEA, anhydrous THF, r.t.; b: phosphorochloridates **2a**–**e**, K_2CO_3, anhydrous acetone, r.t.; (v) pyrrolidine, THF, r.t.



Scheme 3. Synthetic method to obtain compounds **16a**–**e**, **17a**–**e**, **18a**–**e** and **19a**–**e**. Reagents and conditions: (i) Ac_2O, Pyridine, reflux; (ii) PhSH, imidazole, NMP/THF, −10 °C; (iii) phosphorochloridates **2a**–**e**, K_2CO_3, anhydrous acetone, r.t., MW 600w.

daidzein leaded 32.5% BGC-823 cells in apoptosis (1.5% apoptosis cells for control) [35]. We could conclude that the introduction of phosphoramidate groups significantly improve the apoptosis induction of compound **16d**.

3. Conclusion

We herein report the successful application of ProTide technology to five flavones. Most of the series of flavone-7-phosphoramidate derivatives were proved to be more potential than original flavones in vitro. The studies revealed that derivatives

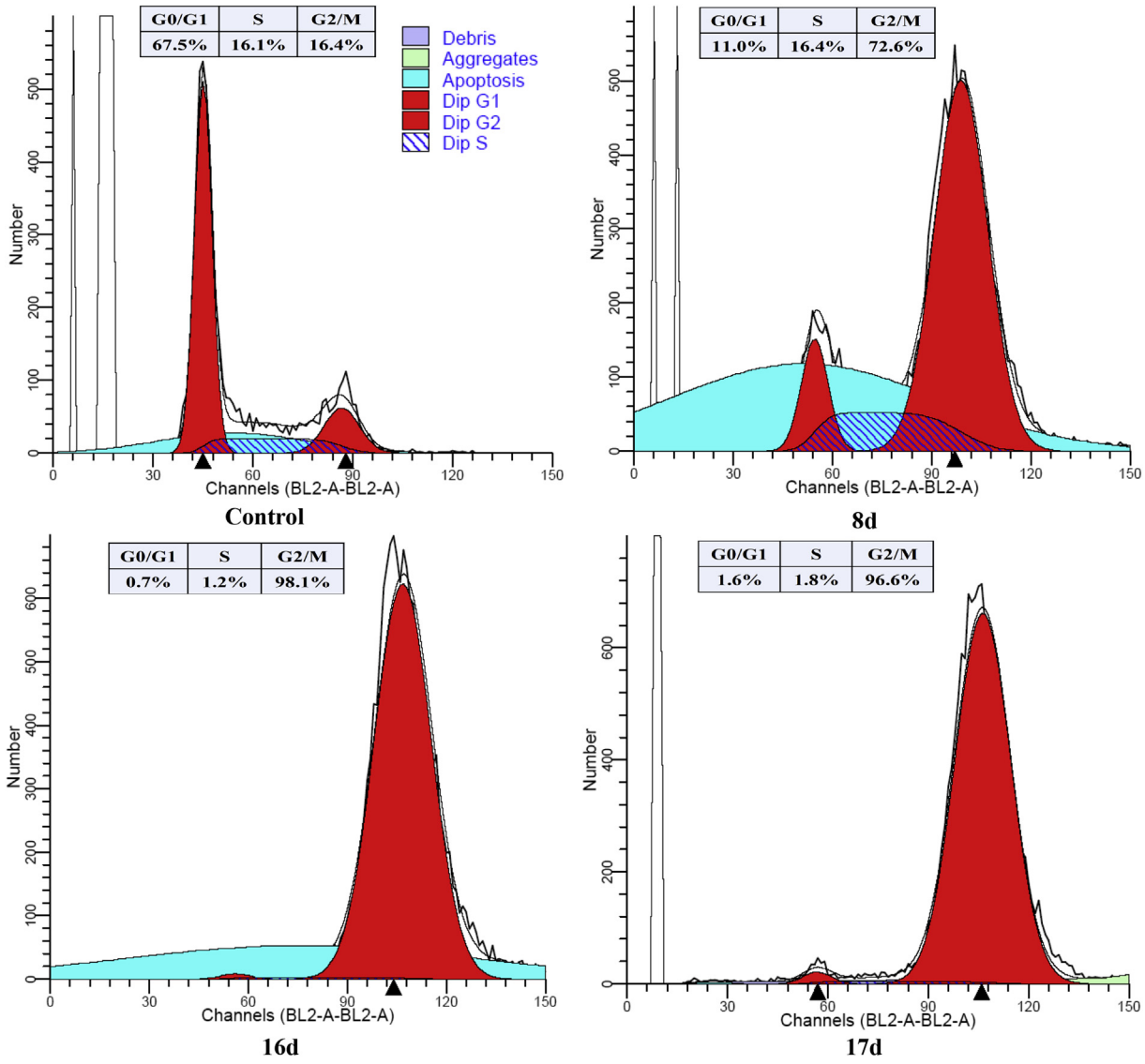


Fig. 2. G2/M phase arrest in HepG2 cells after 24 h incubation with compound **8d**, **16d** and **17d**.

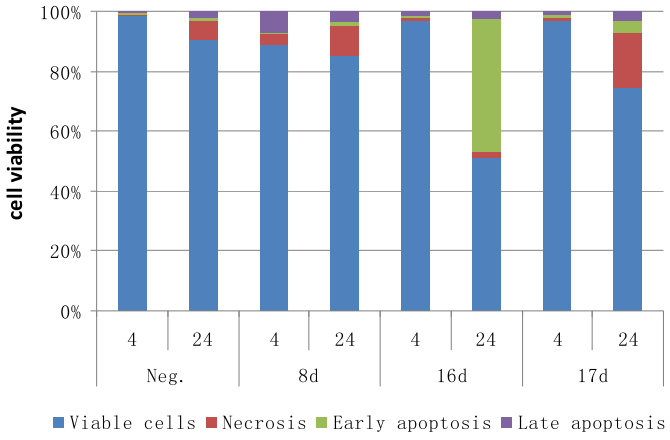


Fig. 3. Induction of apoptosis/necrosis in HepG2 cells after 4 h and 24 h incubation with compound **8d**, **16d** and **17d** at IC_{50} value.

with acetyl group had lower IC_{50} concentration than corresponding derivatives without acetyl group, except for **8a**, **9a** and **16b**. So the protecting group on hydroxyl of flavones may be another key point for structure modification and deserves more attention. Specifically, compound **8d**, **16d** and **17d** could almost thoroughly induce G2/M phase arrest in HepG2 cells. One daidzein phosphoramidate derivative in particular, **16d**, showed significant induction of early apoptosis in HepG2 cells. Further, studies related to the mechanisms by which **16d** induces early apoptosis in HepG2 cells can be planned.

4. Experimental section

4.1. Chemistry

All chemicals and reagents used in current study were of analytical grade. The reactions were monitored by thin layer chromatography (TLC) on Merck pre-coated silica GF254 plates. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AvanceII400M spectrometer (^{1}H, 400 MHz; ^{13}C, 100 MHz). All NMR spectra were run at ambient temperature. Chemical shifts of ^{1}H NMR spectra are given in d values relative to tetramethylsilane (TMS) peak used as

the internal reference. High-resolution mass spectra were obtained using TOF-ESI-MS spectrometer. Microwave reactions were conducted using a XH-100B Initiator reactor (Xiang Hao Corp., Beijing, China). Separation of the compounds by column chromatography was carried out with silica gel 60.

*4.1.1. General procedure for synthesis of the amino acid methyl ester hydrochlorides **1a–e***

A suspension of L-amino acid (50 mmol) in methanol (50 mL) was stirred under ice cold conditions. Thionyl chloride (5 mL) was slowly dropped to the solution at −5 °C. Then, the mixture was allowed to slowly warm to room temperature while being stirred. The reaction was monitored by TLC (N-butanol: water: acetic acid = 4:1:1). When the reaction was completed, the solvent was evaporated under reduced pressure to afford the crude product, which was recrystallized from methanol/ether to give a white solid.

L-Phenylalanine methyl ester hydrochloride (**1a**) Yield 98.1% from L-Phenylalanine.

L-Leucine methyl ester hydrochloride (**1b**) Yield 95% from L-Leucine.

L-Glycine methyl ester hydrochloride (**1c**) Yield 98% from L-Glycine.

L-Alanine methyl ester hydrochloride (**1d**) Yield 96.4% from L-Alanine.

L-Valine methyl ester hydrochloride (**1e**) Yield 70.2% from L-Valine.

*4.1.2. General procedure for synthesis of phosphochloridate **2a–e***

Amino acid methyl ester hydrochloride (3 mmol) and dichlorophenyl phosphate (0.45 mL, 3 mmol) were dissolved in dichloromethane (24 mL) and cooled at −70 °C in a dry ice-acetone bath. Then, triethylamine (0.9 mL) was slowly dropped to the solution. After one hour, the mixture was stirred at room temperature until the reaction was deemed complete by TLC (chloroform: methanol = 30: 1). The solvent was evaporated under reduced pressure. The crude product was filtered and washed with ether for three times. Finally, the filtrate was concentrated to give clear oily liquid **2a–e**. The clear oily liquid was used for the next reaction without separation.

Phenylalanine phosphochloridate (**2a**) was purified by column chromatography in the yield of 96.7%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38–7.10 (m, 10H, Ph-H), 4.49–4.37 (m, 1H, −NH), 4.24–4.10 (m, 1H, −CH), 3.75 (s, 3H, $-OCH_3$), 3.19–3.10 (m, 2H, $-CH_2$).

4.1.3. Acylation of apigenin, luteolin, daidzein and genistein

To solution of apigenin (3 mmol) in acetic anhydride (6 mL) was added pyridine (0.6 mL). Then, the mixture was heated to reflux until apigenin consumption and was poured into ice-cold water (70 mL) to afford **4** as a white solid. The crude product was recrystallized to get pure compound. Compounds **5**, **12** and **13** were synthesized according to the method for **4**.

*4.1.3.1. 4′, 5, 7- O-triacetylapigenin (**4**).* Compound **4** was recrystallized from ethyl acetate. Yield 87.2%; White solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (d, 2H, J = 8.8 Hz, H-2′, H-6′), 7.35 (d, 1H, J = 2.0 Hz, H-8), 7.27 (d, 2H, J = 8.8 Hz, H-3′, H-5′), 6.85 (d, 1H, J = 2.0 Hz, H-6), 6.62 (s, 1H, H-3), 2.44 (s, 3H, $-OCOCH_3$), 2.35 (s, 3H, $-OCOCH_3$), 2.34 (s, 3H, $-OCOCH_3$).

*4.1.3.2. 3′, 4′, 5, 7- O-tetraacetylluteolin (**5**).* Compound **5** was recrystallized from chloroform/methanol. Yield 92.0%; White solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (dd, 1H, J = 8.8, 2.0 Hz, H-6′), 7.71 (d, 1H, J = 2.0 Hz, H-2′), 7.37 (d, 1H, J = 8.8 Hz, H-5′), 7.36 (d, 1H, J = 2.0 Hz, H-8), 6.85 (d, 1H, J = 2.0 Hz, H-6), 6.61 (s, 1H, H-3), 2.44 (s, 3H, $-OCOCH_3$), 2.35 (s, 3H, $-OCOCH_3$), 2.35 (s, 3H, $-OCOCH_3$), 2.33 (s, 3H, $-OCOCH_3$).

*4.1.3.3. 4′, 7- O-diacetyldaidzein (**12**).* Compound **12** was recrystallized from ethyl acetate. Yield 74.9%; White solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (d, 1H, J = 8.0 Hz, H-5), 8.01 (s, 1H, H-2), 7.58 (d, 2H, J = 8.0 Hz, H-2′, H-6′), 7.31 (d, 1H, J = 2.0 Hz, H-8), 7.19–7.16 (m, 3H, H-6, H-3′, H-5′), 2.36 (s, 3H, $-OCOCH_3$), 2.32 (s, 3H, $-OCOCH_3$).

*4.1.3.4. 4′, 5, 7-O-triacetylgenistein (**13**).* Compound **13** was recrystallized from ethyl acetate. Yield 98.0%; White solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (s, 1H, H-2), 7.49 (d, 2H, J = 8.0 Hz, H-2′, H-6′), 7.25 (d, 1H, J = 2.0 Hz, H-8), 7.15 (d, 2H, J = 8.0 Hz, H-3′, H-5′), 6.86 (d, 1H, J = 2.0 Hz, H-6), 2.41 (s, 3H, $-OCOCH_3$), 2.34 (s, 3H, $-OCOCH_3$), 2.31 (s, 3H, $-OCOCH_3$).

4.1.4. Deprotection of 7-actyl group

Compound **4**, or **5**, or **12**, or **13** (2.5 mmol) was dissolved in N-methylpyrrolidone (12.5 mL) and tetrahydrofuran (37.5 mL) at −10 °C, followed by the addition of imidazole (0.06 g) and thiophenol (0.3 mL). After that, the mixture was allowed to slowly warm to room temperature, and monitored by TLC. The reaction solution was evaporated under reduced pressure distillation. Then, the residue was diluted with ethyl acetate (100 mL) and washed with 1 M HCl aq (30 mL × 5). The organic phase was dried over anhydrous Na_2SO_4overnight. After filtration and concentration, the crude was washed with ethanol to afford product **6**, **7**, **14** and **15**.

*4.1.4.1. 4′, 5-diacetylapigenin (**6**).* Yield 74.8%; White solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.13 (s, 1H, 7-OH), 8.09 (d, 2H, J = 8.8 Hz, H-2′, H-6′), 7.33 (d, 2H, J = 8.8 Hz, H-3′, H-5′), 6.94 (d, 1H, J = 2.2 Hz, H-8), 6.75 (s, 1H, H-3), 6.57 (d, 1H, J = 2.2 Hz, H-6), 2.31 (s, 3H, $-OCOCH_3$), 2.30 (s, 3H, $-OCOCH_3$).

*4.1.4.2. 3′, 4′, 5-triacetylluteolin (**7**).* Yield 85.0%; White solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.22 (s, 1H, 7-OH), 8.01–7.99 (m, 2H, H-2′, H-6′), 7.49–7.47 (m, 1H, H-5′), 6.96 (d, 1H, J = 1.6 Hz, H-8), 6.78 (s, 1H, H-3), 6.58 (d, 1H, J = 1.6 Hz, H-6), 2.33 (s, 3H, $-OCOCH_3$), 2.32 (s, 3H, $-OCOCH_3$), 2.30 (s, 3H, $-OCOCH_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 175.7, 169.3, 168.6, 168.5, 162.8, 159.9, 158.7, 150.5, 145.0, 142.9, 129.9, 125.2, 124.9, 122.1, 109.9, 109.3, 108.4, 101.5, 21.4, 20.8, 20.8. HRMS (ESI, m/z) for $C_{21}H_{15}O_9$ ($[M-H]^-$) Calcd: 411.0716; Found: 411.0720.

*4.1.4.3. 4′-Acetyldaidzein (**14**).* Yield 80.4%; White solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.87 (s, 1H, 7-OH), 8.43 (s, 1H, H-2), 8.00 (d, 1H, J = 8.8 Hz, H-5), 7.60 (d, 2H, J = 8.8 Hz, H-2′, H-6′), 7.18 (d, 2H, J = 8.8 Hz, H-3′, H-5′), 6.95 (dd, 1H, J = 8.8, 2.4 Hz, H-6), 6.89 (d, 1H, J = 2.4 Hz, H-8), 2.32 (s, 3H, $-OCOCH_3$).

*4.1.4.4. 4′, 5-diacetylgenistein (**15**).* Yield 77.0%; White solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.17 (s, 1H, 7-OH), 8.35 (s, 1H, H-2), 7.50 (d, 2H, J = 8.4 Hz, H-2′, H-6′), 7.17 (d, 2H, J = 8.4 Hz, H-3′, H-5′), 6.81 (d, 1H, J = 2.4 Hz, H-8), 6.59 (d, 1H, J = 2.4 Hz, H-6), 2.29 (s, 3H, $-OCOCH_3$), 2.28 (s, 3H, $-OCOCH_3$).

*4.1.5. Synthesis of chrysin-7-yl phosphoramidate derivatives **3a–e***

To a solution of chrysin (0.381 g, 1.5 mmol) in THF (60 mL) was added phosphochloridate **2a-e** (2 mmol) and stirred at room temperature. Then, the mixed solution of TEA (0.5 mL) and THF (5 mL) was added dropwise to the mixture. The resulting mixture was stirred at room temperature until complete by TLC. The solvent was removed under reduced pressure and the crude product was purified by column chromatography or crystallized from the appropriate solvent.

*4.1.5.1. ((Phenoxy)(5-hydroxy-2-phenyl-4-oxo-4H-chromene-7-yl) phosphoryl) phenylalanine methyl ester (**3a**).* The residue was recrystallized from ethyl acetate/diethyl ether. Yield 51.9%; Yellow solid; mp: 152–153 °C. ^{1}H NMR (400 MHz, Acetone-d_6) δ 12.90 (s, 1H, 5-OH), 8.14–8.11 (m, 2H, H-2′, H-6′), 7.66–7.62 (m, 3H, H-3′, H-4′, H-5′), 7.40–7.35 (m, 2H, H-3″, H-5″), 7.23–7.16 (m, 7H, Ph-H), 7.13–7.08 (m, 1H, H-4‴), 6.94(2dd, 1H, J = 2.0, 1.0 Hz, H-8), 6.92 (s, 1H, H-3), 6.59 (dd, 1H, J = 2.0, 1.0 Hz, H-6), 5.59–5.50 (m, 1H, −NH), 4.31–4.27 (m, 1H, −CH), 3.61 (s, 3H, $-OCH_3$), 3.12–2.90 (m, 2H, $-CH_2$). ^{13}C NMR (100 MHz, Acetone-d_6) δ 182.8, 172.3, 164.7, 162.0, 157.0, 156.4, 150.8, 136.7, 132.2, 131.1, 129.7, 129.7, 129.4, 129.4, 129.2, 129.2, 128.2, 128.2, 126.6, 126.6, 126.6, 125.0, 120.3, 120.2, 107.9, 105.7, 103.5, 99.2, 56.6, 51.5, 39.7. HRMS (EI, m/z) for $C_{31}H_{26}NO_8P$([M+H]$^+$) Calcd: 571.1396; Found: 571.1384.

*4.1.5.2. ((Phenoxy)(5-hydroxy-2-phenyl-4-oxo-4H-chromene-7-yl) phosphoryl)) leucine methyl ester (**3b**).* The residue was recrystallized from ethanol. Yield 41.7%; Yellow solid; mp: 146–147 °C. ^{1}H NMR (400 MHz, Acetone-d_6) δ 12.96 (s, 1H, 5-OH), 8.16–8.13 (m, 2H, H-2′, H-6′), 7.67–7.62 (m, 3H, H-3′, H-4′, H-5′), 7.46–7.42 (m, 2H, H-3″, H-5″), 7.38–7.34 (m, 2H, H-2″, H-6″), 7.26 (t, 1H, J = 7.2 Hz, H-4″), 7.15 (dd, 1H, J = 2.0, 1.0 Hz, H-8), 6.95 (s, 1H, H-3), 6.75 (dd, 1H, J = 2.0, 1.0 Hz, H-6), 5.53–5.45 (m, 1H, −NH), 4.14–4.05 (m, 1H, −C<u>H</u>−NH), 3.63 (s, 3H, $-OCH_3$), 1.74–1.62 (m, 1H, −CH), 1.60–1.52 (m, <u>2</u>H, $-CH_2$), 0.89–0.85 (m, 6H, $-CH(C\underline{H}_3)_2$).

*4.1.5.3. ((Phenoxy)(5-hydroxy-2-phenyl-4-oxo-4H-chromene-7-yl) phosphoryl)) glycine methyl ester (**3c**).* The residue was recrystallized from ethanol. Yield 61.9%; Yellow solid; mp: 160–161 °C. ^{1}H NMR (400 MHz, Acetone-d_6) δ 12.93 (s, 1H, 5-OH), 8.13–8.10 (m, 2H, H-2′, H-6′), 7.67–7.59 (m, 3H, H-3′, H-4′, H-5′), 7.42 (t, 2H, J = 8.0 Hz, H-3″, H-5″), 7.37–7.34 (m, 2H, H-2″, H-6″), 7.22 (t, 1H, J = 8.0 Hz, H-4″), 7.15–7.14 (m, 1H, H-8), 6.92 (s, 1H, H-3), 6.75–6.74 (m, 1H, H-6), 5.47–5.40 (m, 1H, −NH), 3.96 (dd, 2H, J = 13.2, 7.0 Hz, $-CH_2$), 3.66 (s, 3H, $-OCH_3$).

*4.1.5.4. ((Phenoxy)(5-hydroxy-2-phenyl-4-oxo-4H-chromene-7-yl) phosphoryl)) alanine methyl ester (**3d**).* The residue was purified by column chromatography using chloroform/methanol (130: 1) as an eluent. Yield 41.8%; Pale yellow solid; mp: 134–135 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.89 (s, 1H, 5-OH), 8.13 (d, 2H, J = 8.4 Hz, H-2′, H-6′), 7.67–7.58 (m, 3H, Ph-H), 7.46–7.41 (m, 2H, Ph-H), 7.32–7.22 (m, 3H, Ph-H), 7.16 (s, 1H, H-3), 7.11–7.10 (m, 1H, H-8), 6.77–6.75 (m, 1H, H-6), 5.59–5.37 (m, 1H, −NH), 4.07–3.98 (m, 1H, −CH), 3.57 (s, 3H, $-OCH_3$), 1.26–1.23 (m, 3H, $-CH_3$). ^{13}C NMR (100 MHz, Acetone-d_6) δ 182.7, 173.1, 164.7, 162.1, 161.8, 157.1, 156.6, 150.9, 132.2, 131.1, 129.7, 129.7, 129.2, 129.2, 126.6, 126.6, 125.1, 120.3, 107.4, 105.7, 103.6, 99.4, 51.5, 50.5, 23.7. HRMS (ESI, m/z) for $C_{25}H_{22}NO_8NaP$ ([M+Na]$^+$) Calcd: 518.4076; Found: 518.1467.

*4.1.5.5. ((Phenoxy)(5-hydroxy-2-phenyl-4-oxo-4H-chromene-7-yl) phosphoryl)) valine methyl ester (**3e**).* The residue was purified by column chromatography using chloroform/methanol (130: 1) as an eluent. Yield 41.8%; Pale yellow solid; mp: 136–137 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.86 (s, 1H, 5-OH), 8.12 (d, 2H, J = 8.0 Hz, H-2′, H-6′), 7.62–7.57 (m, 3H, Ph-H), 7.43–7.39 (m, 2H, Ph-H), 7.29–7.20 (m, 3H, Ph-H), 7.14 (s, 1H, H-3), 7.12 (dd, 1H, J = 8.0, 0.8 Hz, H-8), 6.71 (d, 1H, J = 8.0 Hz, H-6), 6.71–6.57 (m, 1H, −NH), 3.68–3.63 (m, 1H, -C<u>H</u>-NH), 3.54 (s, 3H, $-OCH_3$), 1.96–1.89 (m, 1H, −CH), 0.82–0.76 (m, <u>3</u>H, $-CH(C\underline{H}_3)_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.9, 172.9, 164.6, 161.6, 157.0, 156.4, 150.7, 132.9, 130.8, 130.3, 130.3, 129.7, 129.7, 127.1, 127.1, 125.6, 120.7, 120.6, 107.9, 106.2, 103.9, 99.9, 60.9, 52.1, 31.7, 19.3, 18.4. HRMS (ESI, m/z) for $C_{27}H_{26}NO_8NaP$ ([M+Na]$^+$) Calcd:546.1294; Found:546.1287.

*4.1.6. Synthesis of 4′,5-diacetylapigenin-7-yl phosphoramidate derivatives **8a**–**e***

Method A: A solution of a compound **6** (0.354 g, 1 mmol) dissolved in acetone (60 mL) was added phosphoramidate **2a-d** (2 mmol), anhydrous potassium carbonate (0.27 g, 2 mmol) and stirred at room temperature. The reaction was monitored by TLC. After filtration and concentration, the crude product was washed with ethanol to afford product **8a**–**d**. *Method B:* A solution of a compound **6** (0.354 g, 1 mmol) dissolved in acetone (60 mL) was added phosphoramidate **2e** (2 mmol), anhydrous potassium carbonate (0.27 g, 2 mmol). The mixture was stirred at 25 °C (600 W) under microwave irradiation for 40 min. After filtration and concentration, the crude product was washed with ethanol to afford product **8e**.

*4.1.6.1. ((Phenoxy)(5-acetoxy-2-(4-acetoxyphenyl)-4-oxo-4H-chromene-7-yl)phosphoryl) phenylalanine methyl ester (**8a**).* Yield 73.2%; White solid; mp: 139–140 °C. ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.13 (d, 2H, J = 8.6 Hz, H-2′, H-6′), 7.40–7.35 (m, 5H, Ph-H), 7.23–7.16 (m, 7H, Ph-H), 7.12–7.08 (m, 1H, H-4″), 6.91 (dd, 1H, J = 2.8, 0.6 Hz, H-8), 6.74 (d, 1H, J = 2.8 Hz, H-6), 5.65–5.57 (m, 1H, −NH), 4.37–4.27 (m, 1H, −CH), 3.62 (s, 3H, $-OCH_3$), 3.13–3.07 (m, 2H, $-CH_2$), 2.34 (s, 3H, $-OCOCH_3$), 2.31 (s, 3H, $-OCOCH_3$). ^{13}C NMR (100 MHz, Acetone-d_6) δ 175.3, 172.2, 168.5, 161.5, 161.4, 157.7, 154.3, 153.7, 150.8, 150.5, 136.6, 129.7, 129.4, 129.4, 128.6, 128.2, 128.2, 127.7, 127.7, 126.6, 125.1, 122.6, 122.6, 120.3, 120.2, 114.3, 112.3, 107.9, 106.9, 78.3, 56.6, 51.5, 39.6, 20.2, 20.1.HRMS (EI, m/z) for $C_{35}H_{30}NO_{11}P$ ([M+H]$^+$) Calcd: 671.1556; Found: 671.1605.

*4.1.6.2. ((Phenoxy)(5-acetoxy-2-(4-acetoxyphenyl)-4-oxo-4H-chromene-7-yl)phosphoryl) leucine methyl ester (**8b**).* Yield 62.9%; White solid; mp: 149–150 °C. ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.14–8.11 (m, 2H, H-2′, H-6′), 7.60 (dd, 1H, J = 2.4, 1.0 Hz, H-8), 7.43–7.40 (m, 2H, H-3″, H-5″), 7.38–7.34 (m, 2H, H-3′, H-5′), 7.34–7.31 (m, 2H, H-2″, H-6″), 7.26–7.22 (m, 1H, H-4″), 7.06 (dd, 1H, J = 2.4, 1.0 Hz, H-6), 6.74 (s, 1H, H-3), 5.52–5.46 (m, 1H, −NH), 4.13–4.05 (m, 1H, −CH−NH), 3.61 (s, 3H, $-OCH_3$), 2.34 (s, 3H, $-OCOCH_3$), 2.31 (s, 3H, $-OCOCH_3$), 1.71–1.61 (m, 1H, $(CH_3)_2$−CH−CH_2), 1.57–1.52 (m, 2H, $-CH_2$), 0.88–0.83 (m, 6H, $(CH_3)_2$−CH). ^{13}C NMR (100 MHz, Acetone-d6) δ 176.2, 174.3, 169.4, 169.4, 162.4, 158.8, 155.5, 154.7, 151.7, 151.6, 130.6, 130.6, 129.5, 128.6, 128.6, 126.1, 123.5, 123.5, 121.3, 121.3, 115.0, 113.4, 108.9, 108.1, 54.3, 52.4, 43.6, 24.9, 23.1, 21.6, 21.1, 21.0. HRMS (EI, m/z) for $C_{32}H_{32}NO_{11}P$ ([M+H]$^+$) Calcd:637.1713; Found:637.1741.

*4.1.6.3. ((Phenoxy)(5-acetoxy-2-(4-acetoxyphenyl)-4-oxo-4H-chromene-7-yl)phosphoryl) glycine methyl ester (**8c**).* Yield 60.6%; White solid; mp: 143–144 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.15 (d, 2H, J = 8.0 Hz, H-2′, H-6′), 7.64 (dd, 1H, J = 2.2, 0.8 Hz, H-8), 7.42 (t, 2H, J = 8.0 Hz, H-3″, H-5″), 7.37 (d, 2H, J = 8.0 Hz, H-3′, H-5′), 7.27 (d, 2H, J = 8.0 Hz, H-2″, H-6″), 7.24 (t, 1H, J = 8.0 Hz, H-4″), 7.10 (d, 1H, J = 2.0 Hz, H-6), 6.92 (s, 1H, H-3), 6.70–6.65 (m, 1H, −NH), 3.85 (dd, 2H, J = 13.2, 7.0 Hz, -−CH_2), 3.60 (s, 3H, $-OCH_3$), 2.33 (s, 3H, $-OCOCH_3$), 2.31 (s, 3H, $-OCOCH_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 175.8, 171.3, 169.4, 169.2, 161.5, 157.8, 154.4, 153.7, 150.6, 150.2, 130.4, 130.4, 128.5, 128.4, 128.4, 125.7, 123.2, 123.2, 120.8, 120.7, 114.1, 112.9, 108.4, 107.9, 52.3, 42.9, 21.4, 21.3. HRMS (EI, m/z) for $C_{28}H_{24}NO_{11}P$([M+H]$^+$) Calcd:581.1087; Found:581.1071.

*4.1.6.4. ((Phenoxy)(5-acetoxy-2-(4-acetoxyphenyl)-4-oxo-4H-chromene-7-yl)phosphoryl) alanine methyl ester (**8d**).* Yield 54.4%; White solid; mp: 144–145 °C. ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.14 (d, 2H, J = 8.0 Hz, H-2′, H-6′), 7.62–7.56 (m, 1H, H-8), 7.42 (t, 2H, J = 8.0 Hz, H-3″, H-5″), 7.33 (d, 2H, J = 8.0 Hz, H-3′, H-5′), 7.32 (d, 2H, J = 8.0 Hz, H-2″, H-6″), 7.23 (t, 1H, J = 8.0 Hz, H-4″), 7.09–7.04

(m, 1H, H-6), 6.75 (s, 1H, H-3), 5.62–5.56 (m, 1H, –NH), 4.22–4.16 (m, 1H, –CH), 3.63 (s, 3H, $-OCH_3$), 2.34 (s, 3H, $-OCOCH_3$), 2.32 (s, 3H, $-OCOCH_3$), 1.36 (d, 3H, J = 7.0 Hz, $-CH_3$). 13C NMR (100 MHz, Acetone-d_6) δ 175.3, 173.1, 168.5, 168.5, 161.5, 157.9, 154.5, 153.7, 150.7, 150.6, 129.7, 129.7, 128.6, 127.7, 127.7, 125.2, 122.6, 122.6, 120.4, 120.3, 114.4, 112.5, 107.9, 107.3, 51.6, 50.4, 50.3, 20.2, 19.6. HRMS (EI, m/z) for $C_{29}H_{26}NO_{11}P([M+H]^+)$ Calcd:595.1243; Found:595.1262.

*4.1.6.5. ((Phenoxy)(5-acetoxy-2-(4-acetoxyphenyl)-4-oxo-4H-chromene-7-yl)phosphoryl) valine methyl ester (**8e**).* Yield 63.2%; White solid; mp: 157–158 °C. ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.13 (dd, 2H, J = 8.0, 1.6 Hz, H-2′, H-6′), 7.59 (dd, 1H, J = 2.2, 0.9 Hz, H-8), 7.44–7.40 (m, 6H, Ph-H), 7.24 (t, 1H, J = 8.0 Hz, H-4″), 7.06 (d, 1H, J = 2.2 Hz, H-6), 6.75 (s, 1H, H-3), 5.53–5.45 (m, 1H, –NH), 3.93–3.84 (m, 1H, –CH–NH), 3.62 (s, 3H, $-OCH_3$), 2.34 (s, 3H, $-OCOCH_3$), 2.32 (s, 3H, $-OCOCH_3$), 2.09–2.04 (m, 1H, –CH), 0.92–0.88 (m, 6H, $(CH_3)_2$–CH). ^{13}C NMR (100 MHz, Acetone-d_6) δ 175.3, 171.1, 168.5, 168.5, 161.5, 157.8, 154.6, 153.7, 153.1, 150.6, 129.7, 128.6, 127.7, 127.7, 125.2, 122.6, 122.6, 120.4, 120.3, 112.5, 112.4, 108.0, 107.2, 107.1, 60.6, 51.3, 31.8, 20.2, 18.4, 17.3, 17.1. HRMS (ESI, m/z) for $C_{31}H_{30}NO_{11}NaP$ ($[M+Na]^+$) Calcd: 646.5335; Found: 646.2009.

*4.1.7. Synthesis of 3′, 4′, 5-triacetylluteolin -7-yl phosphoramidate derivatives **9a**–**e***

Compound **9a**–**e** was prepared from **7** according to the same procedure described for **3a**.

*4.1.7.1. ((Phenoxy)(5-acetoxy-2-(3,4-diacetoxyphenyl)-4-oxo-4H-chromene-7-yl)phosphoryl) phenylalanine methyl ester (**9a**).* Yield 88.0%; White solid; mp:145–146 °C. ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.02 (dd, 1H, J = 8.4, 2.2 Hz, H-6′), 7.98 (d, 1H, J = 2.2 Hz, H-2′), 7.49 (d, 1H, J = 8.4 Hz, H-5′), 7.39–7.35 (m, 3H, Ph-H), 7.22–7.16 (m, 7H, Ph-H), 7.11–7.07 (m, 1H, H-4″), 6.90 (d, 1H, J = 2.4 Hz, H-6), 6.79–6.77 (m, 1H, H-3), 5.65–5.57 (m, 1H, –NH), 4.34–4.25 (m, 1H, –CH), 3.62 (s, 3H, $-OCH_3$), 3.12–2.90 (m, 2H, $-CH_2$), 2.33 (s, 3H, $-OCOCH_3$), 2.32 (s, 3H, $-OCOCH_3$), 2.30 (s, 3H, $-OCOCH_3$). ^{13}C NMR (100 MHz, Acetone-d_6) δ 175.7, 172.9, 169.2, 168.7, 168.5, 160.5, 157.6, 150.6, 150.5, 145.3, 143.0, 137.2, 130.3, 130.3, 129.7, 129.7, 129.5, 129.4, 128.5, 128.5, 127.7, 125.6, 125.4, 125.1, 122.3, 120.5, 120.4, 114.0, 113.5, 108.8, 107.5, 56.8, 56.5, 52.4, 21.3, 20.9, 20.8. HRMS (ESI, m/z) for $C_{37}H_{32}NO_{13}NaP$ ($[M+Na]^+$) Calcd: 752.1509; Found: 752.0060.

*4.1.8. ((Phenoxy)(5-acetoxy-2-(3,4-diacetoxyphenyl)-4-oxo-4H-chromene-7-yl)phosphoryl) leucine methyl ester (**9b**)*

Yield 86.0%; White solid; mp: 121–122 °C. ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.02 (dd, 1H, J = 8.4, 1.8 Hz, H-6′), 7.98 (d, 1H, J = 1.8 Hz, H-2′), 7.60 (d, 1H, J = 1.8 Hz, H-8), 7.48 (d, 1H, J = 8.4 Hz, H-5′), 7.41 (t, 2H, J = 8.0 Hz, H-3″, H-5″), 7.33 (t, 2H, J = 8.0 Hz, H-2″, H-6″), 7.24 (t, 1H, J = 8.0 Hz, H-4″), 7.06 (d, 1H, J = 1.8 Hz, H-6), 6.77 (s, 1H, H-3), 5.59–5.50 (m, 1H, -NH), 4.13–4.05 (m, 1H, –CH–NH), 3.60 (s, 3H, $-OCH_3$), 2.34 (s, 3H,$-OCOCH_3$), 2.33 (s, 3H, $-OCOCH_3$), 2.32 (s, 3H, $-OCOCH_3$), 1.72–1.60 (m, 1H, –CH), 1.58–1.53 (m, 2H, $-CH_2$), 0.87–0.83 (m, 6H, $-CH(CH_3)_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.5, 175.7, 173.8, 169.2, 168.7, 164.7, 161.9, 157.8, 154.4, 150.7, 150.2, 146.3, 145.3, 130.3, 125.7, 122.3, 120.7, 119.8, 116.5, 115.7, 113.8, 108.8, 103.8, 99.3, 94.3, 53.4, 52.3, 42.3, 24.1, 23.1, 22.7, 22.4, 21.3, 20.8. HRMS (ESI, m/z) for $C_{34}H_{34}NO_{13}NaP$ ($[M+Na]^+$) Calcd:718.1665; Found:718.1659.

*4.1.9. ((Phenoxy)(5-acetoxy-2-(3,4-diacetoxyphenyl)-4-oxo-4H-chromene-7-yl)phosphoryl) glycine methyl ester (**9c**)*

Yield 68.5%; White solid; mp: 133–134 °C. ^{1}H NMR (400 MHz, Acetone-d6) δ 8.02 (dd, 1H, J = 8.4, 2.2 Hz, H-6′), 7.99 (d, 1H, J = 2.2 Hz, H-2′), 7.62 (dd, 1H, J = 2.2, 1.0 Hz, H-8), 7.48 (d, 1H, J = 8.4 Hz, H-5′), 7.41 (t, 2H, J = 8.0 Hz, H-3″, H-5″), 7.34 (d, 2H, J = 8.0 Hz, H-2″, H-6″), 7.24 (t, 1H, J = 8.0 Hz, H-4″), 7.07 (d, 1H, J = 2.2 Hz, H-6), 6.77 (s, 1H, H-3), 5.51–5.48 (m, 1H, –NH), 3.95 (dd, 2H, J = 13.4, 7.0 Hz, $-CH_2$), 3.65 (s, 3H, $-OCH_3$), 2.34 (s, 3H, $-OCOCH_3$), 2.33 (s, 3H, $-OCOCH_3$), 2.32 (s, 3H, $-OCOCH_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.1, 175.8, 171.3, 169.2, 168.7, 164.7, 160.6, 157.8, 154.5, 150.2, 145.3, 143.0, 130.4, 129.7, 125.7, 121.9, 120.7, 120.5, 119.4, 115.7, 114.2, 113.0, 108.8, 104.1, 94.3, 52.3, 43.0, 22.7, 21.3, 20.8. HRMS (ESI, m/z) for $C_{30}H_{26}NO_{13}NaP$ ([M+Na]+) Calcd:662.1039; Found:662.1027.

*4.1.9.1. (((Phenoxy)(5-acetoxy-2-(3,4-diacetoxyphenyl)-4-oxo-4H-chromene-7-yl)phosphoryl) alanine methyl ester (**9d**).* Yield 44.6%; White solid; mp: 109–110 °C. ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.02 (dd, 1H, J = 8.4, 2.0 Hz, H-6′), 7.98 (d, 1H, J = 2.0 Hz, H-2′), 7.62 (d, 1H, J = 2.0 Hz, H-8), 7.48 (d, 1H, J = 8.0 Hz, H-5′), 7.41 (t, 2H, J = 8.0 Hz, H-3″, H-5″), 7.34 (d, 2H, J = 8.0 Hz, H-2″, H-6″), 7.24 (t, 1H, J = 8.0 Hz, H-4″), 7.08 (d, 1H, J = 2.0 Hz, H-6), 6.77 (s, 1H, H-3), 5.60–5.55 (m, 1H, –NH), 4.24–4.13 (m, 1H, -–CH), 3.63 (s, 3H, $-OCH_3$), 2.34 (s, 3H, $-OCOCH_3$), 2.33 (s, 3H, $-OCOCH_3$), 2.32 (s, 3H, $-OCOCH_3$), 1.37 (d, 3H, J = 7.0 Hz, $-CH_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.1, 174.8, 172.5, 169.5, 168.7, 164.9, 164.3, 161.5, 157.7, 150.3, 146.3, 130.4, 129.8, 129.4, 129.2, 121.9, 120.7, 119.3, 116.5, 115.7, 113.9, 104.0, 103.2, 99.3, 94.4, 53.2, 50.3, 47.9, 45.8, 22.8, 17.7. HRMS (ESI, m/z) for $C_{31}H_{28}NO_{13}NaP$ ($[M+Na]^+$) Calcd: 676.1196; Found: 676.1207.

*4.1.10. ((Phenoxy)(5-acetoxy-2-(3,4-diacetoxyphenyl)-4-oxo-4H-chromene-7-yl)phosphoryl) valine methyl ester (**9e**)*

Yield 65.9%; White solid; ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.01 (dd, 1H, J = 8.6, 1.8 Hz, H-6′), 7.98 (d, 1H, J = 1.8 Hz, H-2′), 7.60 (d, 1H, J = 2.2 Hz, H-8), 7.48 (d, 1H, J = 8.6 Hz, H-5′), 7.41 (t, 2H, J = 8.0 Hz, H-3″, H-5″), 7.33 (d, 2H, J = 8.0 Hz, H-2″, H-6″), 7.23 (t, 1H, J = 8.0 Hz, H-4″), 7.06 (d, 1H, J = 2.2 Hz, H-6), 6.77 (s, 1H, H-3), 5.54–5.46 (m, 1H, –NH), 3.93–3.83 (m, 1H, –CH-–NH), 3.62 (s, 3H, -–OCH_3), 2.34 (s, 3H, $-OCOCH_3$), 2.33 (s, 3H, $-OCOCH_3$), 2.32 (s, 3H, $-OCOCH_3$), 2.10–2.06 (m, 1H, –CH), 0.92–0.88 (m, 6H, $(CH_3)_2$–CH). ^{13}C NMR (100 MHz, Acetone-d_6) δ 175.2, 172.4, 168.4, 167.8, 167.6, 160.6, 157.8, 154.6, 154.6, 150.6, 145.3, 143.2, 129.7, 129.7, 129.6, 125.2, 124.6, 124.4, 121.8, 120.4, 120.4, 114.1, 112.6, 108.4, 107.2, 60.6, 51.3, 31.9, 20.2, 19.6, 19.6, 18.4, 17.1. HRMS (ESI, m/z) for $C_{33}H_{32}NO_{13}NaP$ ($[M+Na]^+$) Calcd: 704.1509; Found: 704.1519.

*4.1.11. Synthesis of apigenin-7-yl phosphoramidate derivatives **10a**–**e***

To a solution of **8a**–**e** (0.05 mmol) in tetrahydrofuran (30 mL) was added dropwise the mixed solution of pyrrolidine (0.4 mL) and tetrahydrofuran (3 mL). Then, the mixture was stirred at room temperature until the reaction was deemed complete by TLC. The solution was neutralized with 0.5 M HCl and the solvent was removed under reduced pressure. The residue was diluted in ethyl acetate (120 mL) and washed with water (40 mL × 4). The organic layer was dried over anhydrous Na_2SO_4 and concentrated to give a crude product which was purified by column chromatography or crystallized from the appropriate solvent to give **10a**–**e**.

*4.1.11.1. ((Phenoxy)(5-hydroxy-2-(4-hydroxyphenyl)-4-oxo-4H-chromene-7-yl)phosphoryl) phenylalanine methyl ester (**10a**).* Compound **10a** was purified by column chromatography using chloroform/methanol (40:1) as an eluent. Yield 80.5%; Yellow solid; mp: 104–105 °C· ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.02 (s, 1H, 5-OH), 10.46 (s, 1H, 4′-OH), 8.00 (dd, 2H, J = 8.0, 1.7 Hz, H-2′, H-6′), 7.36 (t, 2H, J = 8.0 Hz, H-3″, H-5″), 7.22–7.13 (m, 5H, Ph-H), 7.09 (d,

2H, J = 8.0 Hz, H-2″, H-6″), 7.08 (t, 1H, J = 8.0 Hz, H-4″), 6.96 (s, 1H, H-3), 6.95 (d, 2H, J = 8.0 Hz, H-3′, H-5′), 6.85–6.84 (m, 1H, H-8), 6.48 (d, 1H, J = 2.0 Hz, H-6), 4.10–4.04 (m, 1H, −CH), 3.54 (s, 3H, $-OCH_3$), 2.98–2.75 (m, 2H, $-CH_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.6, 172.9, 165.2, 162.0, 161.4, 156.7, 155.9, 150.6, 137.2, 130.3, 130.3, 129.7, 129.7, 129.3, 128.5, 128.5, 126.8, 125.5, 121.2, 120.5, 120.4, 116.5, 116.5, 107.7, 103.8, 103.5, 99.4, 79.6, 56.8, 52.3, 31.2. HRMS (EI, m/z) for $C_{31}H_{26}NO_9P([M+H]^+)$ Calcd: 587.1345; Found:587.1353.

4.1.12. ((Phenoxy)(5-hydroxy-2-(4-hydroxyphenyl)-4-oxo-4H-chromene-7-yl)phosphoryl) leucine methyl ester (**10b**)

Compound **10b** was purified by column chromatography using chloroform/methanol (40:1) as an eluent. Yield 85.5%; Yellow solid; mp: 130–131 °C·^{1}H NMR (400 MHz, DMSO-d_6) δ 13.05 (s, 1H, 5-OH), 10.44 (s, 1H, 4′-OH), 8.00 (dd, 2H, J = 8.8, 2.0 Hz, H-2′, H-6′), 7.45–7.40 (m, 2H, H-3″, H-5″), 7.29–7.22 (m, 3H, H-2″, H-4″, H-6″), 7.07 (d, 1H, J = 1.6 Hz, H-8), 6.96 (s, 1H, H-3), 6.95 (d, 2H, J = 8.8 Hz, H-3′, H-5′), 6.68 (d, 1H, J = 1.6 Hz, H-6), 6.65–6.62 (m, 1H, −NH), 3.91–3.84 (m, 1H, -–CH), 3.53 (s, 3H, $-OCH_3$), 1.89–1.74 (m, 1H, $(CH_3)_2$–CH–CH_2), 1.53–1.38 (m, 2H, $-CH_2$), 0.80–0.72 (m, 6H, $(CH_3)_2$–CH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.6, 173.7, 165.2, 162.0, 161.6, 156.9, 156.1, 150.7, 130.4, 130.3, 129.3, 129.3, 125.6, 121.2, 120.8, 120.5, 116.5, 116.5, 107.7, 103.8, 103.7, 99.7, 53.4, 52.3, 42.3, 29.0, 18.9, 18.9. HRMS (ESI, m/z) for $C_{28}H_{27}NO_9P$ ($[M-H]^-$) Calcd:552.1423; Found:552.1432.

4.1.13. ((Phenoxy)(5-hydroxy-2-(4-hydroxyphenyl)-4-oxo-4H-chromene-7-yl)phosphoryl) glycine methyl ester (**10c**)

Compound **10c** was recrystallized from ethyl acetate/diethyl ether. Yield 84.0%; Yellow solid; mp: 77–78 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.05 (s, 1H, 5-OH), 10.44 (s, 1H, 4′-OH), 8.01 (d, 2H, J = 8.8 Hz, H-2′, H-6′), 7.43 (t, 2H, J = 8.0 Hz, H-3″, H-5″), 7.28 (d, 2H, J = 8.0 Hz, H-2″, H-6″), 7.24 (t, 1H, J = 8.0 Hz, H-4″), 7.10 (d, 1H, J = 1.6 Hz, H-8), 6.96 (s, 1H, H-3), 6.94 (d, 2H, J = 8.8 Hz, H-3′, H-5′), 6.69 (d, 1H, J = 1.6 Hz, H-6), 6.61–6.54 (m, 1H, −NH), 3.80 (dd, 2H, J = 16.0, 7.6 Hz, $-CH_2$), 3.60 (s, 3H, $-OCH_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.7, 171.2, 165.2, 162.0, 161.5, 156.9, 156.1, 150.7, 130.4, 130.4, 129.3, 129.3, 125.6, 121.2, 120.7, 120.7, 116.5, 116.5, 107.8, 103.8, 99.8, 79.6, 52.2, 42.9. HRMS (ESI, m/z) for $C_{24}H_{19}NO_9P$ ($[M-H]^-$) Calcd:496.0797; Found:496.0785.

4.1.14. ((Phenoxy)(5-hydroxy-2-(4-hydroxyphenyl)-4-oxo-4H-chromene-7-yl)phosphoryl) alanine methyl ester (**10d**)

Compound **10d** was purified by column chromatography using chloroform/methanol (40:1) as an eluent. Yield 58.0%; Yellow solid; mp: 81–82 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.06 (s, 1H, 5-OH), 10.46 (s, 1H, 4′-OH), 8.00 (d, 2H, J = 8.0 Hz, H-2′, H-6′), 7.46–7.41 (m, 2H, H-3″, H-5″), 7.31–7.22 (m, 1H, H-4″), 7.26 (d, 2H, J = 8.0 Hz, H-2″, H-6″), 7.11–7.05 (m, 1H, H-8), 6.96 (s, 1H, H-3), 6.94 (d, 2H, J = 8.0 Hz, H-3′, H-5′), 6.75–6.71 (m, 1H, H-6), 6.70–6.65 (m, 1H, −NH), 4.04–3.97 (m, 1H, -–CH), 3.56 (s, 3H, $-OCH_3$), 1.25–1.22 (m, 3H, $-CH_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.7, 173.7, 165.2, 162.0, 161.6, 156.9, 156.1, 150.7, 130.4, 130.4, 129.3, 129.3, 125.6, 121.2, 120.7, 120.7, 116.5, 116.5, 107.8, 103.8, 103.7, 99.8, 52.36, 50.3, 50.3. HRMS (EI, m/z) for $C_{25}H_{22}NO_9P([M+H]^+)$ Calcd:511.1032; Found:511.1031.

4.1.15. ((Phenoxy)(5-hydroxy-2-(4-hydroxyphenyl)-4-oxo-4H-chromene-7-yl)phosphoryl) valine methyl ester (**10e**)

Compound **10e** was recrystallized from ethyl acetate/diethyl ether. Yield 53.4%; Yellow solid; mp: 87–88 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.05 (s, 1H, 5-OH), 10.47 (s, 1H, 4′-OH), 8.00 (d, 2H, J = 8.0 Hz, H-2′, H-6′), 7.42 (t, 2H, J = 8.0 Hz, H-3″, H-5″), 7.26 (t, 2H, J = 8.0 Hz, H-2″, H-6″), 7.23 (t, 1H, J = 8.0 Hz, H-4″), 7.08 (d, 1H, J = 2.0 Hz, H-8), 6.97 (s, 1H, H-3), 6.94 (d, 2H, J = 8.0 Hz, H-3′, H-5′), 6.69 (d, 1H, J = 2.0 Hz, H-6), 6.69–6.60 (m, 1H, −NH), 3.70–3.61 (m, 1H, −CH–NH), 3.55 (s, 3H, $-OCH_3$), 1.96–1.89 (m, 1H, −CH), 0.82–0.76 (m, 6H, $(CH_3)_2$–CH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.6, 175.9, 172.9, 165.2, 162.0, 161.5, 156.8, 156.1, 130.3, 130.3, 129.3, 129.3, 125.5, 121.2, 120.7, 120.6, 116.5, 116.5, 107.7, 103.8, 103.7, 99.8, 60.9, 52.1, 49.1, 18.4, 18.3. HRMS (ESI, m/z) for $C_{25}H_{21}NO_9P$ ($[M-H]^-$) Calcd:538.1267; Found:538.1273.

4.1.16. Synthesis of luteolin-7-yl phosphoramidate derivatives **11a–e**

Compound **11a–e** was prepared from **9a–e** according to the same procedure described for **10a–11e**.

4.1.16.1. ((Phenoxy)(5-hydroxy-2-(3,4-dihydroxyphenyl)-4-oxo-4H-chromene-7-yl)phosphoryl) phenylalanine methyl ester (**11a**).

Compound **11a** was purified by preparative TLC. Yield 16.0%; Pale yellow solid; mp: 181–182 °C·^{1}H NMR (400 MHz, DMSO-d_6) δ 13.01 (s, 1H, 5-OH), 10.01 (s, 1H, 4′-OH), 9.45 (s, 1H, 3′-OH), 7.48 (dd, 1H, J = 8.4, 1.6 Hz, H-6′), 7.45 (d, 1H, J = 1.6 Hz, H-2′), 7.37 (t, 2H, J = 8.0 Hz, H-3″, H-5″), 7.22–7.06 (m, 8H, Ph-H), 6.90 (d, 1H, J = 8.0 Hz, H-5′), 6.84–6.80 (m, 2H, Ph-H), 6.49 (d, 1H, J = 1.6 Hz, H-6), 4.10–4.04 (m, 1H, −CH), 3.54 (s, 3H, $-OCH_3$), 2.97–2.74 (m, 2H, $-CH_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.5, 172.9, 165.2, 161.5, 156.5, 155.9, 150.7, 150.6, 146.3, 137.2, 130.3, 130.3, 129.7, 129.7, 128.6, 128.5, 126.8, 125.5, 121.6, 120.5, 120.4, 120.3, 119.9, 116.6, 114.0, 107.7, 103.8, 99.2, 56.8, 52.3, 45.6. HRMS (ESI, m/z) for $C_{31}H_{25}NO_{10}P$ ($[M-H]^-$) Calcd:602.1216; Found:602.1102.

4.1.17. ((Phenoxy)(5-hydroxy-2-(3,4-dihydroxyphenyl)-4-oxo-4H-chromene-7-yl)phosphoryl) leucine methyl ester (**11b**)

Compound **11b** was purified by column chromatography using chloroform/methanol (40:1) as eluent. Yield 20.0%; Pale yellow solid; mp: 193–194 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.07 (s, 1H, 5-OH), 10.03 (s, 1H, 4′-OH), 9.45 (s, 1H, 3′-OH), 7.49 (dd, 1H, J = 8.0, 2.0 Hz, H-6′), 7.47 (d, 1H, J = 2.0 Hz, H-2′), 7.45–7.41 (m, 2H, H-3″, H-5″), 7.30–7.23 (m, 3H, H-2″, H-6″, H-4″), 7.05 (d, 1H, J = 1.8 Hz, H-8), 6.92 (d, 1H, J = 8.0 Hz, H-5′), 6.85 (s, 1H, H-3), 6.67 (d, 1H, J = 1.8 Hz, H-6), 6.68–6.67 (m, 1H, −NH), 3.91–3.84 (m, 1H, −CH), 3.54 (s, 3H, $-OCH_3$), 1.50–1.42 (m, 3H, $-(CH_3)_2\underline{CH}\underline{CH_2}$), 0.80 (d, 6H, J = 6.4 Hz, $-CH(\underline{CH_3})_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.5, 173.8, 165.3, 161.6, 156.8, 156.1, 156.1, 150.7, 146.3, 130.4, 130.3, 125.7, 121.5, 120.8, 120.5, 119.8, 116.5, 114.0, 107.7, 103.8, 99.5, 99.5, 53.3, 52.3, 41.8, 24.1, 23.2, 21.4. HRMS (ESI, m/z) for $C_{28}H_{27}NO_{10}P$ ($[M-H]^-$) Calcd: 568.1378; Found:568.1108.

4.1.18. ((Phenoxy)(5-hydroxy-2-(3,4-dihydroxyphenyl)-4-oxo-4H-chromene-7-yl)phosphoryl) glycine methyl ester (**11c**)

Compound **11c** was purified by column chromatography using chloroform/methanol (30:1) as eluent. Yield 52.0%; Pale yellow solid; mp: 156–157 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.96 (s, 1H, 5-OH), 9.66 (s, 1H, -OH), 7.49 (dd, 1H, J = 8.0, 2.0 Hz, H-6′), 7.45 (d, 1H, J = 2.0 Hz, H-2′), 7.42 (t, 2H, J = 8.0 Hz, H-3″, H-5″), 7.28 (d, 2H, J = 8.0 Hz, H-2″, H-6″), 7.23 (t, 1H, J = 8.0 Hz, H-4″), 7.06 (s, 1H, H-8), 6.91 (d, 2H, J = 8.0 Hz, H-5′), 6.84 (s, 1H, H-3), 6.68 (s, 1H, H-6), 6.59–6.55 (m, 1H, −NH), 3.80 (dd, 2H, J = 4.5, 14.7 Hz, $-CH_2$), 3.61 (s, 3H, $-OCH_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.6, 171.3, 165.3, 161.6, 156.8, 156.1, 150.8, 150.7, 146.3, 130.4, 130.4, 125.6, 121.5, 120.7, 120.7, 119.9, 116.5, 114.0, 107.8, 103.8, 99.7, 99.6, 52.3, 42.9. ^{31}P NMR (162 MHz, DMSO-d_6): δ −0.39. HRMS (ESI, m/z) for $C_{24}H_{19}NO_{10}P$ ($[M-H]^-$) Calcd:512.0747; Found:512.0432.

4.1.19. ((Phenoxy)(5-hydroxy-2-(3,4-dihydroxyphenyl)-4-oxo-4H-chromene-7-yl)phosphoryl) alanine methyl ester (**11d**)

Compound **11d** was purified by column chromatography using

chloroform/methanol (40:1) as eluent. Yield 42.4%; Pale yellow solid; mp: 165–166 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.07 (s, 1H, 5-OH), 10.05 (s, 1H, 4′-OH), 9.45 (s, 1H, 3′-OH), 7.45 (s, 4H, Ph-H), 7.25 (s, 3H, Ph-H), 7.07 (s, 1H, Ph-H), 6.90–6.69 (m, 3H, Ph-H), 6.69(1H, −NH), 4.02 (s, 1H, −CH), 3.56 (s, 3H, −OCH_3), 1.23 (s, 3H, −CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.6, 173.7, 165.3, 161.6, 156.8, 156.1, 150.8, 150.7, 146.3, 130.3, 130.3, 125.6, 121.5, 120.7, 120.7, 119.8, 116.5, 114.0, 107.7, 103.8, 103.7, 99.6, 52.4, 50.3, 20.1. ^{31}P NMR (162 MHz, DMSO-d_6): δ −1.53, −1.77. HRMS (ESI, *m*/*z*) for $C_{25}H_{21}NO_{10}P$ ([M − H]$^-$) Calcd:526.0903; Found:526.0549.

*4.1.20. ((Phenoxy)(5-hydroxy-2-(3,4-dihydroxyphenyl)-4-oxo-4H-chromene-7-yl)phosphoryl) valine methyl ester (**11e**)*

Compound **11e** was purified by column chromatography using chloroform/methanol (40:1) as eluent. Yield 62.4%; Pale yellow solid; mp: 178–179 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.05 (s, 1H, 5-OH), 10.02 (s, 1H, −OH), 9.43 (s, 1H, −OH), 7.48 (dd, 1H, *J* = 8.4, 2.0 Hz, H-6′), 7.45 (d, 1H, *J* = 2.0 Hz, H-2′), 7.50–7.40 (m, 2H, H-3″, H-5″), 7.28 (d, 2H, *J* = 8.0 Hz, H-2″, H-6″), 7.22 (t, 1H, *J* = 8.0 Hz, H-4″), 7.04 (d, 1H, *J* = 1.6 Hz, H-8), 6.91 (d, 1H, *J* = 8.4 Hz, H-5′), 6.84 (s, 1H, H-2), 6.67 (d, 1H, *J* = 1.6 Hz, H-6), 3.70–3.61 (m, 1H, −C<u>H</u>−NH), 3.54 (s, 3H, −OCH_3), 1.96–1.88 (m, 1H, −CH), 0.83–0.77 (m, 6H, −CH(C<u>H</u>$_3$)$_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.5, 172.9, 165.3, 161.6, 156.8, 156.1, 150.7, 150.6, 146.3, 130.3, 130.3, 125.6, 121.6, 120.8, 120.5, 119.8, 116.5, 114.0, 107.7, 103.9, 103.7, 99.6, 60.9, 52.1, 31.7, 19.3, 18.4. HRMS (ESI, *m*/*z*) for $C_{27}H_{25}NO_{10}P$ ([M − H]$^-$) Calcd:554.1216; Found:554.1215.

*4.1.21. Synthesis of 4′-acetyldaidzein-7-yl phosphoramidate derivatives **16a–d***

A 250 mL, three-necked flask was changed with compound **14** (0.296 g, 1 mmol), a solution of phosphoramidate **2a–d** (2–5 mmol) in acetone (60 mL), anhydrous potassium carbonate (2–5 mmol). The mixture was stirred at 25 °C (600 W) under microwave irradiation for 4–5 h (TLC). The solvent was removed under reduced pressure and the filtrate was concentrated to give a crude residue as an oil, which was purified by column chromatography on silica gel to afford the product **16a–e**.

*4.1.22. ((Phenoxy)(2-(4-acetoxyphenyl)-4-oxo-4H-chromene-7-yl) phosphoryl) phenylalanine methyl ester (**16a**)*

Yield 69.6%; White solid; mp: 142–143 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.59 (s, 1H, H-2), 8.09 (d, 1H, *J* = 8.8 Hz, H-5), 7.65 (d, 2H, *J* = 8.0 Hz, H-2′, H-6′), 7.37 (t, 2H, *J* = 8.0 Hz, H-3″, H-5″), 7.30 (d, 1H, *J* = 2.2 Hz, H-8), 7.23–7.16 (m, 8H, Ph-H), 7.15–7.09 (m, 3H, Ph-H), 6.94–6.85 (m, 1H, −NH), 4.13–4.07 (m, 1H, −CH), 3.55 (s, 3H, −OCH_3), 3.02–2.75 (m, 2H, −CH_2), 2.30 (s, 3H, −$OCOCH_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.9, 172.8, 169.7, 156.6, 155.3, 154.8, 150.8, 150.6, 137.2, 130.5, 130.5, 130.3, 130.3, 129.7, 129.7, 129.6, 128.6, 128.6, 127.9, 126.9, 125.5, 123.7, 122.1, 122.1, 121.2, 120.5, 120.4, 118.7, 109.1, 57.0, 56.7, 52.4, 21.3. HRMS (ESI, *m*/*z*) for $C_{33}H_{28}NO_9NaP$ ([M+Na]$^+$) Calcd: 636.1399; Found: 636.0933.

*4.1.23. ((Phenoxy)(2-(4-acetoxyphenyl)-4-oxo-4H-chromene-7-yl) phosphoryl) leucine methyl ester (**16b**)*

Yield 65.9%; White solid; mp: 37–38 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (d, 1H, *J* = 8.8 Hz, H-5), 7.99 (s, 1H, H-2), 7.58 (d, 2H, *J* = 8.6 Hz, H-2′, H-6′), 7.45 (dd, 1H, *J* = 2.2, 1.0 Hz, H-8), 7.38–7.33 (m, 2H, Ph-H), 7.30–7.20 (m, 4H, Ph-H), 7.17 (d, 2H, *J* = 8.6 Hz, H-3′, H-5′), 4.13-–4.08 (m, 1H, −NH), 3.89–3.79 (m, 1H, −NH−C<u>H</u>), 3.66 (s, 3H, −OCH_3), 2.32 (s, 3H, −$OCOCH_3$), 1.68–1.48 (m, 3H, −C<u>H</u>$_2$−C<u>H</u>−$(CH_3)_2$), 0.91–0.87 (m, 6H, −CH−(C<u>H</u>$_3$)$_2$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.4, 173.7, 169.5, 156.9, 154.7, 153.2, 150.7, 150.4, 130.0, 130.0, 129.8, 129.2, 128.2, 128.2, 125.4, 124.7, 121.7, 121.7, 121.6, 120.2, 120.1, 118.4, 109.1, 53.4, 52.4, 43.7, 30.9, 24.4, 22.7, 21.8. HRMS (ESI, *m*/*z*) for $C_{30}H_{30}NO_9NaP$ ([M+Na]$^+$) Calcd: 602.1556; Found: 602.0697.

*4.1.24. ((Phenoxy)(2-(4-acetoxyphenyl)-4-oxo-4H-chromene-7-yl) phosphoryl) glycine methyl ester (**16c**)*

Yield 53.5%; Colorless oil. ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.34 (s, 1H, H-2), 8.20 (d, 1H, *J* = 8.0 Hz, H-5), 7.66 (d, 2H, *J* = 8.6 Hz, H-2′, H-6′), 7.56–7.55 (m, 1H, H-8), 7.43–7.32 (m, 5H, Ph-H), 7.27–7.22 (m, 1H, Ph-H), 7.18 (d, 2H, *J* = 8.6 Hz, H-3′, H-5′), 5.59–5.52 (m, 1H, −NH), 3.97–3.92 (m, 2H, −CH_2), 3.65 (s, 3H, −OCH_3), 2.28 (s, 3H, −$OCOCH_3$). ^{13}C NMR (100 MHz, Acetone-d_6) δ 174.6, 170.7, 168.9, 156.8, 155.0, 154.1, 150.9, 150.8, 130.0, 130.0, 129.8, 129.8, 129.5, 127.7, 125.2, 124.4, 124.0, 121.6, 121.6, 121.4, 120.4, 118.6, 109.3, 51.5, 42.7, 20.2. HRMS (ESI, *m*/*z*) for $C_{26}H_{22}NO_9NaP$ ([M+Na]$^+$) Calcd: 546.0930; Found: 546.0288.

*4.1.25. ((Phenoxy)(2-(4-acetoxyphenyl)-4-oxo-4H-chromene-7-yl) phosphoryl) alanine methyl ester (**16d**)*

Yield 84.3%; Colorless oil. ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.36 (s, 1H, H-2), 8.22 (d, 1H, *J* = 8.0 Hz, H-5), 7.67 (d, 2H, *J* = 8.6 Hz, H-2′, H-6′), 7.57 (dd, 1H, *J* = 2.0, 1.2 Hz, H-8), 7.44–7.32 (m, 5H, Ph-H), 7.27–7.23 (m, 1H, Ph-H), 7.18 (d, 2H, *J* = 8.6 Hz, H-3′, H-5′), 5.67–5.60 (m, 1H, −H), 4.22–4.15 (m, 1H, −CH), 3.62 (s, 3H, −OCH_3), 2.28 (s, 3H, −$OCOCH_3$), 1.37–1.34 (m, 3H, CH_3). ^{13}C NMR (100 MHz, Acetone-d_6) δ 174.5, 173.2, 168.8, 156.8, 155.1, 154.1, 150.9, 150.8, 130.0, 130.0, 129.8, 129.8, 129.5, 127.7, 125.2, 124.4, 124.0, 121.6, 121.6, 121.4, 120.4, 118.6, 109.3, 51.5, 51.4, 20.1, 19.8.HRMS (ESI, *m*/*z*) for $C_{27}H_{24}NO_9NaP$ ([M+Na]$^+$) Calcd: 560.1086; Found: 560.0339.

*4.1.26. Synthesis of 4′, 5-diacetylgenistein-7-yl phosphoramidate derivatives **17a–d***

Compound **17a–d** was prepared from **15** according to the same procedure described for **16a–e**.

*4.1.26.1. ((Phenoxy)(5-acetoxy-2-(4-acetoxyphenyl)-4-oxo-4H-chromene-7-yl)phosphoryl) phenylalanine methyl ester (**17a**).* Yield 69.3%; White solid; mp: 144–145 °C.^{1}H NMR (400 MHz, DMSO-d_6) δ 8.51 (s, 1H, H-2), 7.55 (d, 2H, *J* = 8.0 Hz, H-2′, H-6′), 7.37 (t, 2H, *J* = 8.0 Hz, H-3″, H-5″), 7.22–7.19 (m, 8H, Ph-H), 7.14–7.08 (m, 3H, Ph-H), 7.01–6.95 (m, 1H, Ph-H), 6.93-–6.91 (m, 1H, −NH), 4.13–4.08 (m, 1H, −CH), 3.53 (s, 3H, −OCH_3), 3.04–2.75 (m, 2H, −CH_2), 2.32 (s, 3H, −$OCOCH_3$), 2.30 (s, 3H, −$OCOCH_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.9, 172.8, 169.7, 169.2, 157.7, 154.3, 154.2, 150.8, 150.7, 150.7, 137.2, 130.8, 130.8, 130.3, 130.3, 129.7, 129.6, 129.2, 128.6, 126.9, 125.6, 124.6, 122.1, 122.1, 122.0, 120.5, 120.4, 114.6, 112.8, 107.1, 56.8, 56.7, 52.4, 21.3, 21.3. HRMS (ESI, *m*/*z*) for $C_{35}H_{30}NO_{11}NaP$ ([M+Na]$^+$) Calcd: 694.1454; Found: 694.0739.

*4.1.26.2. ((Phenoxy)(5-acetoxy-2-(4-acetoxyphenyl)-4-oxo-4H-chromene-7-yl)phosphoryl) leucine methyl ester (**17b**).* Yield 80.3%; White solid; mp: 150–151 °C. ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.31 (s, 1H, H-2), 7.58 (d, 2H, *J* = 8.0 Hz, H-2′, H-6′), 7.47 (s, 1H, H-8), 7.42 (t, 2H, *J* = 8.0 Hz, H-3″, H-5″), 7.32 (d, 2H, *J* = 8.0 Hz, H-2″, H-6″), 7.24 (t, 1H, *J* = 8.0 Hz, H-4″), 7.18 (d, 2H, *J* = 8.0 Hz, H-3′, H-5′), 7.08 (s, 1H, H-6), 4.11–4.05 (m, 1H, −NH−C<u>H</u>), 3.62 (s, 3H, −OCH_3), 2.32 (s, 3H, −$OCOCH_3$), 2.28 (s, 3H, −$OCOCH_3$), 1.67–1.62 (m, 1H, −CH_2−C<u>H</u>), 1.59–1.53 (m, 2H, −C<u>H</u>$_2$−C<u>H</u>), 0.89–0.83 (m, 6H, −CH−(C<u>H</u>$_3$)$_2$). ^{13}C NMR (100 MHz, Acetone-d_6) δ 173.6, 173.3, 168.7, 168.5, 157.9, 154.5, 153.1, 151.2, 151.0, 150.8, 130.2, 130.2, 129.7, 129.1, 125.2, 125.0, 121.5, 121.5, 120.4, 120.4, 114.7. HRMS (ESI, *m*/*z*) for $C_{32}H_{32}NO_{11}NaP$([M+Na]$^+$) Calcd: 660.1611; Found: 660.1012.

*4.1.26.3. ((Phenoxy)(5-acetoxy-2-(4-acetoxyphenyl)-4-oxo-4H-chromene-7-yl)phosphoryl) glycine methyl ester (**17c**).* Yield 50.2%;

White solid; mp: 152–153 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.53 (s, 1H, H-2), 7.53 (d, 2H, J = 8.0 Hz, H-2′, H-6′), 7.53 (d, 1H, J = 1.4 Hz, H-8), 7.43 (t, 2H, J = 8.0 Hz, H-3″, H-5″), 7.29 (d, 2H, J = 8.0 Hz, H-2″, H-6″), 7.25 (t, 1H, J = 8.0 Hz, H-4″), 7.20 (d, 2H, J = 8.0 Hz, H-3′, H-5′), 7.13 (d, 1H, J = 1.4 Hz, H-6), 6.73–6.69 (m, 1H, −NH), 3.83 (dd, 2H, J = 15.0, 7.0 Hz, $-CH_2$), 3.60 (s, 3H, $-OCH_3$), 2.32 (s, 3H, $-OCOCH_3$), 2.30 (s, 3H, $-OCOCH_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.9, 171.2, 169.7, 169.2, 157.8, 154.4, 154.3, 154.3, 150.8, 150.6, 130.8, 130.8, 130.4, 130.4, 129.2, 125.7, 124.6, 122.1, 122.1, 120.7, 120.7, 114.7, 113.2, 107.7, 52.3, 42.9, 21.3, 21.3. ^{31}P NMR (162 MHz, DMSO-d_6): δ −0.26. HRMS (ESI, m/z) for $C_{28}H_{24}NO_{11}NaP$ ([M+Na]$^+$) Calcd: 604.0985; Found: 604.0985.

*4.1.26.4. ((Phenoxy)(5-acetoxy-2-(4-acetoxyphenyl)-4-oxo-4H-chromene-7-yl)phosphoryl) alanine methyl ester (**17d**).* Yield 47.5%; White solid; mp: 142–143 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.53 (s, 1H, H-2), 7.54 (d, 2H, J = 8.0 Hz, H-2′, H-6′), 7.44 (d, 1H, 2.4 Hz, H-8), 7.46–7.41 (m, 2H, H-3″, H-5″), 7.32 (d, 1H, J = 8.0 Hz, H-4″), 7.25 (d, 2H, J = 8.0 Hz, H-2″, H-6″), 7.20 (d, 2H, J = 8.0 Hz, H-3′, H-5′), 7.16 (d, 1H, 2.4 Hz, H-6), 6.84–6.78 (m, 1H, −NH), 4.06–4.04 (m, 1H, −CH), 3.57 (s, 3H, $-OCH_3$), 2.32 (s, 3H, $-OCOCH_3$), 2.30 (s, 3H, $-OCOCH_3$), 1.25–1.22 (m, 3H, $-CH_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.9, 173.6, 169.7, 169.2, 157.8, 154.4, 154.3, 150.8, 150.6, 150.5, 130.8, 130.8, 130.4, 130.4, 129.2, 125.7, 124.6, 122.1, 122.1, 120.7, 120.7, 114.7, 113.1, 107.5, 52.4, 50.3, 21.3, 21.3, 20.0. ^{31}P NMR (162 MHz, DMSO-d_6): δ −1.41, -1.63. HRMS (ESI, m/z) for $C_{29}H_{26}NO_{11}NaP$ ([M+Na]$^+$) Calcd: 618.1141; Found: 618.0414.

4.1.27. Synthesis of daidzein-7-yl phosphoramidate derivatives

Compound **18a**–**e** was prepared from daidzein according to the same procedure described for **16a-16e**.

*4.1.28. ((Phenoxy)(2-(4-hydroxyphenyl)-4-oxo-4H-chromene-7-yl) phosphoryl) phenylalanine methyl ester (**18a**)*

Yield 84.9%; Colorless oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.61 (s, 1H, 4′-OH), 8.45 (s, 1H, H-2), 8.07 (d, 1H, J = 8.0 Hz, H-5), 7.43 (d, 2H, J = 8.0 Hz, H-2′, H-6′), 7.37 (t, 2H, J = 8.0 Hz, H-3″, H-5″), 7.27 (s, 1H, Ph-H), 7.22–7.08 (m, 9H, Ph-H), 6.93–6.90 (m, 1H, Ph-H), 6.84 (d, 2H, J = 8.0 Hz, H-3′, H-5′), 4.11–4.07 (m, 1H, −CH), 3.55 (s, 3H, $-OCH_3$), 3.03–2.75 (m, 2H, $-CH_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 175.2, 172.9, 157.8, 156.6, 154.6, 154.2, 150.6, 137.2, 130.6, 130.6, 130.3, 130.3, 129.7, 129.7, 128.6, 128.6, 127.9, 126.9, 125.5, 124.4, 122.5, 121.2, 120.5, 120.4, 118.5, 115.5, 115.4, 109.0, 56.8, 52.36, 31.15. HRMS (ESI, m/z) for $C_{31}H_{25}NO_8P^-$ ([M − H]$^-$) Calcd: 570.1318; Found: 570.1325.

*4.1.29. ((Phenoxy)(2-(4-hydroxyphenyl)-4-oxo-4H-chromene-7-yl) phosphoryl) leucine methyl ester (**18b**)*

Yield 46.8%; Colorless oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.59 (s, 1H, 4′-OH), 8.45 (s, 1H, H-2), 8.17 (d, 1H, J = 8.0 Hz, H-5), 7.52 (s, 1H, H-8), 7.42 (d, 2H, J = 8.0 Hz, H-2′, H-6′), 7.41 (t, 2H, J = 8.0 Hz, H-3″, H-5″), 7.36 (dd, 1H, J = 8.8, 1.2 Hz, H-6), 7.29–7.21 (m, 3H, H-2″, H-4″, H-6″), 6.83 (d, 2H, J = 8.0 Hz, H-3′, H-5′), 6.69–6.63 (m, 1H, −NH), 3.90–3.86(m, −CH−NH), 3.54 (s, 3H, $-OCH_3$), 1.49–1.36 (m, 3H, $-CH_2-CH-(CH_3)_2$), 0.79 (d, 6H, J = 6.2 Hz, $-CH-(CH_3)_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 175.2, 173.8, 157.9, 156.7, 154.9, 154.2, 150.7, 130.6, 130.6, 130.3, 130.3, 128.0, 125.7, 124.5, 122.5, 121.3, 120.8, 120.8, 118.8, 115.5, 115.5, 109.3, 53.4, 52.3, 42.3, 24.1, 23.1, 21.4. HRMS (ESI, m/z) for $C_{28}H_{27}NO_8P^-$ ([M − H]$^-$) Calcd: 536.1474; Found: 536.1479.

*4.1.30. ((Phenoxy)(2-(4-hydroxyphenyl)-4-oxo-4H-chromene-7-yl) phosphoryl) glycine methyl ester (**18c**)*

Yield 68.2%; Colorless oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.63 (s, 1H, 4′-OH), 8.46 (s, 1H, H-2), 8.17 (d, 1H, J = 8.0 Hz, H-5), 7.56 (d, 1H, J = 2.0 Hz, H-8), 7.43 (t, 2H, J = 8.0 Hz, H-3″, H-5″), 7.42 (d, 2H, J = 8.0 Hz, H-2′, H-6′), 7.38 (dd, 1H, J = 8.0, 2.0 Hz, H-6), 7.30 (d, 2H, J = 8.0 Hz, H-2″, H-6″), 7.25 (t, 1H, J = 8.0 Hz, H-4″), 6.84 (d, 2H, J = 8.0 Hz, H-3′, H-5′), 6.66–6.55 (m, 1H, −NH), 3.86–3.80 (m, 2H, $-CH_2$), 3.61 (s, 3H, $-OCH_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 175.2, 171.3, 157.8, 156.7, 154.8, 154.2, 150.7, 130.6, 130.6, 130.4, 130.4, 128.0, 125.6, 124.4, 122.5, 121.3, 120.7, 120.7, 118.9, 115.5, 115.5, 109.5, 52.3, 42.9. HRMS (ESI, m/z) for $C_{24}H_{19}NO_8P^-$ ([M − H]$^-$) Calcd: 480.0848; Found: 480.1865.

*4.1.31. ((Phenoxy)(2-(4-hydroxyphenyl)-4-oxo-4H-chromene-7-yl) phosphoryl) alanine methyl ester (**18d**)*

Yield 44.6%; Colorless oil. ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.56 (s, 1H, 4′-OH), 8.24 (s, 1H, H-2), 8.21 (d, 1H, J = 8.0 Hz, H-5), 7.55 (s, 1H, H-8), 7.47 (d, 2H, J = 8.0 Hz, H-2′, H-6′), 7.44–7.33 (m, 5H, Ph-H), 7.23 (t, 1H, J = 8.0 Hz, H-4″), 6.88 (d, 2H, J = 8.0 Hz, H-3′, H-5′), 5.66–5.63 (m, 1H, -–NH), 4.24–4.17 (m, 1H, −CH), 3.63 (s, 3H, $-OCH_3$), 1.37 (t, 3H, J = 6.4 Hz, $-CH_3$). ^{13}C NMR (100 MHz, Acetone-d_6) δ 174.9, 173.1, 157.5, 156.7, 154.8, 153.2, 150.8, 130.2, 130.2, 129.8, 129.8, 129.7, 127.7, 125.2, 124.7, 122.9, 121.5, 120.4, 118.3, 115.1, 115.1, 109.2, 51.6, 50.4, 19.7. HRMS (ESI, m/z) for $C_{25}H_{21}NO_8P^-$ ([M − H]$^-$) Calcd: 494.1005; Found: 494.2001.

4.1.32. Synthesis of genistein-7-yl phosphoramidate derivatives

Compound **19a**–**e** was prepared from genistein according to the same procedure described for **16a-16e**.

*4.1.32.1. ((Phenoxy)(5-hydroxy-2-(4-hydroxyphenyl)-4-oxo-4H-chromene-7-yl)phosphoryl) phenylalanine methyl ester (**19a**).* Yield 68.0%; Colorless oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.91 (s, 1H, 5-OH), 9.66 (s, 1H, 4′-OH), 8.49 (s, 1H, H-2), 7.42 (d, 2H, J = 8.0 Hz, H-2′, H-6′), 7.37 (t, 2H, J = 8.0 Hz, H-3″, H-5″), 7.30–7.26 (m, 4H, Ph-H), 7.13–7.05 (m, 4H, Ph-H), 6.85 (d, 2H, J = 8.0 Hz, H-3′, H-5′), 6.75–6.73 (m, 1H, Ph-H), 6.55 (d, 1H, J = 2.0 Hz, H-6), 4.10–3.99 (m, 1H, −CH), 3.55 (s, 3H, $-OCH_3$), 3.01–2.74 (m, 2H, $-CH_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.6, 171.3, 165.4, 162.4, 158.0, 154.6, 137.8, 137.1, 136.0, 130.6, 129.9, 129.8, 129.6, 129.2, 128.9, 128.8, 128.5, 127.5, 127.1, 126.7, 122.7, 121.6, 122.0, 120.6, 115.6, 104.7, 99.6, 94.3, 57.0, 52.6, 37.6. HRMS (ESI, m/z) for $C_{31}H_{25}NO_9P^-$ ([M − H]$^-$) Calcd:586.1267; Found: 586.1258.

*4.1.33. ((Phenoxy)(5-hydroxy-2-(4-hydroxyphenyl)-4-oxo-4H-chromene-7-yl)phosphoryl) leucine methyl ester (**19b**)*

Yield 46.8%; Colorless oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.04 (s, 1H, 5-OH), 9.71 (s, 1H, 4′-OH), 8.56 (s, 1H, H-2), 7.49–7.44 (m, 4H, Ph-H), 7.33–7.26 (m, 3H, Ph-H), 7.02 (d, 1H, J = 1.6 Hz, H-8), 6.88 (d, 2H, J = 8.4 Hz, H-3′, H-5′), 6.80–6.75 (m, 1H, H-6), 6.73–6.70 (m, 1H, −NH), 3.95–3.85 (m, 1H, −CH−NH), 3.59 (s, 3H, $-OCH_3$), 1.53–1.47 (m, 3H, $-CH_2-CH-(CH_3)_2$), 0.89–0.75 (m, 6H, $-CH-(CH_3)_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 181.2, 173.8, 162.1, 158.1, 157.1, 156.3, 155.6, 150.6, 130.6, 130.6, 130.4, 130.3, 125.7, 123.4, 121.2, 120.7, 120.4, 115.6, 115.6, 108.4, 103.6, 99.4, 53.3, 52.3, 42.3, 24.1, 23.1, 21.4. HRMS (ESI, m/z) for $C_{28}H_{28}NO_9NaP$ ([M+Na]$^+$) Calcd: 576.1399; Found: 576.1766.

*4.1.33.1. ((Phenoxy)(5-hydroxy-2-(4-hydroxyphenyl)-4-oxo-4H-chromene-7-yl)phosphoryl) glycine methyl ester (**19c**).* Yield 68.3%; Colorless oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.00 (s, 1H, 5-OH), 9.67 (s, 1H, 4′-OH), 8.50 (s, 1H, H-2), 7.45–7.37 (m, 4H, Ph-H), 7.29 (d, 2H, J = 8.0 Hz, H-2″, H-6″), 7.24 (t, 1H, J = 8.0 Hz, H-4″), 7.00 (d, 1H, J = 2.0 Hz, H-8), 6.85 (d, 2H, J = 8.0 Hz, H-3′, H-5′), 6.73 (d, 1H, J = 2.0 Hz, H-6), 6.66–6.59 (m, 1H, −NH), 3.83 (dd, 2H, J = 15.0, 7.0 Hz, $-CH_2$), 3.61 (s, 3H, $-OCH_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 181.2, 171.3, 162.1, 158.1, 157.1, 156.2, 155.6, 150.6, 130.7, 130.7, 130.4, 130.4, 125.7, 123.4, 121.2, 120.7, 120.7, 115.6, 115.6, 108.5,

103.8, 99.6, 52.3, 42.9. HRMS (ESI, *m/z*) for $C_{24}H_{20}NO_9NaP$ ($[M+Na]^+$) Calcd: 520.0773; Found: 520.0952.

*4.1.33.2. ((Phenoxy)(5-hydroxy-2-(4-hydroxyphenyl)-4-oxo-4H-chromene-7-yl)phosphoryl) alanine methyl ester (**19d**).* Yield 78.3%; Colorless oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.00 (s, 1H, 5-OH), 9.65 (s, 1H, 4′-OH), 8.50 (s, 1H, H-2), 7.46–7.41 (m, 4H, Ph-H), 7.36–7.22 (m, 3H, Ph-H), 7.01 (d, 1H, J = 2.0 Hz, H-8), 6.85 (d, 2H, J = 8.0 Hz, H-3′, H-5′), 6.75 (d, 1H, J = 2.0 Hz, H-6), 6.75–6.69 (m, 1H, −NH), 4.04–4.02 (m, 1H, −CH), 3.58 (s, 3H, $-OCH_3$), 1.27–1.23 (m, 3H, $-CH_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 181.3, 173.6, 162.1, 158.1, 157.1, 156.2, 155.5, 150.6, 130.6, 130.6, 130.3, 130.3, 129.8, 125.6, 121.2, 120.8, 120.7, 115.6, 115.6, 108.5, 103.7, 99.5, 52.4, 50.3, 20.1. HRMS (ESI, *m/z*) for $C_{25}H_{21}O_9P$ ($[M-H]^-$) Calcd:510.0954; Found: 510.0943.

4.2. MTT assay for cell growth inhibition

The inhibition of the title compounds against HepG2 human liver cancer cells and L-O2 human liver cells were evaluated using a standard MTT-based colorimetric assay. Five thousand corresponding cells per well were seeded into 96-well plates and incubated at 37 °C, 5% CO_2 for 24 h. And then 100 μL a series of concentration of drug-containing medium were dispensed into wells to maintain the final concentration as 100, 80, 60, 40, 20 and 1 μmol/L. Each concentration was in triplicate. After 48 h incubation, cell survival was determined by the addition of 20 μL MTT (Sigma–Aldrich, St. Louis, USA) work solution (5 mg/mL MTT dissolved in Phosphate Buffer Solution (PBS)). After post-incubation at 37 °C for 4 h, the medium was discarded following by adding 100 μL DMSO (Sigma–Aldrich, St. Louis, USA). The plates were then votexed for 10 min for complete dissolution. The optical absorbance was measured at 570 nm. The data represented the mean of three independent experiments in triplicate and were expressed as mean ± SD. The IC_{50} value was defined as the concentration at which 50% of the cells could survive.

4.3. Cell cycle analysis

HepG2 (1.5×10^5 cells/well in 6-well plates) cells were cultured for 24 h followed by compounds treatment for 24 h. Cells were harvested and fixed in 70% ice-cold ethanol overnight. Subsequently, the cells were centrifuged, the supernatant was discarded and the pellet was treated with RNase A (50 μg/mL) for 5 min at room temperature. The treated cells were stained with propidium iodide (50 μg/mL) for 15 min at room temperature in dark. The cells were then analyzed for cell cycle distribution by flow cytometry (Attune NxT, Thermo Fisher, USA).

4.4. Assessment of apoptosis

Apoptosis was quantified by flow cytometry and double staining of cells with Annexin-V/PI (Annexin V-FITC Kit, Beckman Coulter Inc., USA) to simultaneously differentiate viable, early apoptotic, late apoptotic and necrotic cells. HepG2 cells (1.5×10^5) in 6-well plates were cultured for 24 h followed by compounds treatment at IC_{50} values for 4 h and 24 h. Cells were harvested, centrifuged, washed out with cold phosphate-buffered saline (PBS) and stained with the Annexin V/PI according to the manufacturer's instructions. The data were processed using flow cytometry (Attune NxT, Thermo Fisher, USA).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2016.02.012

References and notes

[1] J.B. Harborne, H. Baxter, The Handbook of Natural Flavonoids vol. 1–2, John Wiley & Sons, New York, 1999.
[2] L. Feng, M.M. Maddox, A. Md Zahidul, L.S. Tsutsumi, N. Gagandeep, D.F. Bruhn, X. Wu, S. Shayna, R.B. Lee, C.J. Simmons, J. Med. Chem. 57 (2014) 8398–8420, http://dx.doi.org/10.1021/jm500312x.
[3] G. Cao, E. Sofic, R.L. Prior, Free Radic. Biol. Med. 22 (1997) 749–760, http://dx.doi.org/10.1016/S0891-5849(96)00351-6.
[4] S. Prasad, K. Phromnoi, V.R. Yadav, M.M. Chaturvedi, B.B. Aggarwal, Planta Medica 76 (2010) 1044–1063, http://dx.doi.org/10.1055/s-0030-1250111.
[5] M.M. Liu, Z. Lu, P.L. He, Y.N. Zhang, J.Y. Zhou, Q. Shen, X.W. Chen, J.P. Zuo, L. Wei, D.Y. Ye, Eur. J. Med. Chem. 52 (2012) 33–43, http://dx.doi.org/10.1016/j.ejmech.2012.03.002.
[6] C. Kanadaswami, L.T. Lee, P.P.H. Lee, J.J. Hwang, K.E. Ferng-Chun, Y.T. Huang, M.T. Lee, Vivo 19 (2005) 895–909.
[7] Q. Zhang, X.H. Zhao, Z.J. Wang, Food Chem. Toxicol. 46 (2008) 2042–2053, http://dx.doi.org/10.1016/j.fct.2008.01.049.
[8] G.H. An, M.E. Morris, Pharm. Res. 27 (2010) 1296–1308, http://dx.doi.org/10.1007/s11095-010-0108-8.
[9] C.M. Lin, K.G. Shyu, B.W. Wang, H. Chang, Y.H. Chen, J.H. Chiu, J. Agric. Food Chem. 58 (2010) 7082–7087, http://dx.doi.org/10.1021/jf100421w.
[10] A. Pick, H. Müller, R. Mayer, B. Haenisch, I.K. Pajeva, M. Weigt, H. Bönisch, C.E. Müller, M. Wiese, Bioorg. Med. Chem. 19 (2011) 2090–2102, http://dx.doi.org/10.1016/j.bmc.2010.12.043.
[11] Y.W. Chin, J.Y. Kong, S.Y. Han, Bioorg. Med. Chem. Lett. 23 (2013) 1768–1770, http://dx.doi.org/10.1016/j.bmcl.2013.01.049.
[12] P. Basabe, M.D. Román, I.S. Marcos, D. Diez, A. Blanco, O. Bodero, F. Mollinedo, B.G. Sierra, J.G. Urones, Eur. J. Med. Chem. 45 (2010) 4258–4269, http://dx.doi.org/10.1016/j.ejmech.2010.06.025.
[13] K. Hu, W. Wang, H. Cheng, S.S. Pan, J. Ren, Med. Chem. Res. 20 (2011) 838–846, http://dx.doi.org/10.1007/s00044-010-9395-1.
[14] C. McGuigan, R.N. Pathirana, N. Mahmood, A.J. Hay, Bioorg. Med. Chem. Lett. 2 (1992) 701–704, http://dx.doi.org/10.1016/s0960-894x(00)80395-9.
[15] C. Mcguigan, R.N. Pathirana, N. Mahmood, K.G. Devine, A.J. Hay, Antivir. Res. 17 (1992) 311–321, http://dx.doi.org/10.1016/0166-3542(92)90026-2.
[16] S. Magdalena, L. Monica Huerta, B. Jan, M. Malcolm, W.G. Jiang, B. Sarah, T. Emely, G. Essam, M.G. Christopher, J. Med. Chem. 57 (2014) 1531–1542, http://dx.doi.org/10.1021/jm401853a.
[17] C. McGuigan, R.N. Pathirana, J. Balzarini, E. De Clercq, J. Med. Chem. 36 (1993) 1048–1052, http://dx.doi.org/10.1021/jm00060a013.
[18] X. Chen, J. Yuan, S. Zhang, L. Qu, Y. Zhao, Phosphorus Sulfur. 185 (2010) 274–278, http://dx.doi.org/10.1080/10426500902772858.
[19] T.W. Greene, P.G.M. Wuts, Protection for the Carbonyl Group, John Wiley & Sons, Inc., 2002.
[20] M. Li, X. Han, B. Yu, J. Org. Chem. 68 (2003) 6842–6845, http://dx.doi.org/10.1021/jo034553e.
[21] M.K. Kim, K.-S. Park, C. Lee, H.R. Park, H. Choo, Y. Chong, J. Med. Chem. 53 (2010) 8597–8607, http://dx.doi.org/10.1021/jm101252m.
[22] C. McGuigan, M.R. Kelleher, P. Perrone, S. Mulready, G. Luoni, F. Daverio, S. Rajyaguru, S.L. Pogam, I. Najera, J.A. Martin, K. Klumpp, D.B. Smith, Bioorg. Med. Chem. Lett. 19 (2009) 4250–4254, http://dx.doi.org/10.1016/j.bmcl.2009.05.099.
[23] Y.P. Huo, S.Z. Zhu, S. Hu, Tetrahedron 66 (2010) 8635–8640, http://dx.doi.org/10.1016/j.tet.2010.09.039.
[24] E. Alvarez-Manzaneda, R. Chahboun, E. Alvarez, J.M. Ramos, J.J. Guardia, I. Messouri, I. Chayboun, A.I. Mansour, A. Dahdouh, Synthesis 2010 (3493) (2010) 3503, http://dx.doi.org/10.1055/s-0030-1258226.
[25] G. Buechi, S.M. Weinreb, J. Am. Chem. Soc. 93 (1971) 746–752, http://dx.doi.org/10.1021/ja00732a032.
[26] S.K. Singh, V.K. Sharma, C.E. Olsen, J. Wengel, V.S. Parmar, A.K. Prasad, J. Org. Chem. 75 (2010) 7932–7935, http://dx.doi.org/10.1021/jo101565e.
[27] F. Lu, J. Ralph, J. Agric. Food Chem. 46 (1998) 2911–2913, http://dx.doi.org/10.1021/jf980440y.
[28] M.C. Pagliacci, M. Smacchia, G. Migliorati, F. Grignani, C. Riccardi, I. Nicoletti, Eur. J. Cancer 30 (1994) 1675–1682, http://dx.doi.org/10.1016/0959-8049(94)00262-4.
[29] A.I. Constantinou, N. Kamath, J.S. Murley, Eur. J. Cancer 34 (1998) 1927–1934, http://dx.doi.org/10.1016/0959-8049(94)00262-4.
[30] E.J. Choi, G.H. Kim, Phytomedicine 15 (2008) 683–690, http://dx.doi.org/

10.1016/j.phymed.2008.04.006.
[31] W. Wang, L. Heideman, C.S. Chung, J.C. Pelling, K.J. Koehler, D.F. Birt, Mol. Carcinog. 28 (2000) 102–110, http://dx.doi.org/10.1002/1098-2744(200006)28:2<102::AID-MC6>3.0.CO;2–2.
[32] G. Salti, S. Grewal, R.R. Mehta, G.T. Das, A.J. Boddie, A. Constantinou, Eur. J. Cancer 36 (2000) 796–802, http://dx.doi.org/10.1016/S0959-8049(00)00017-4.
[33] Z. Yi, Y. Mao, C. Hong, Y. Lin, Z. Hu, W. Jian, X. Xin, X. Xu, Q. Jie, L. Xie, Cancer Cell Int. 13 (2013) 370–373, http://dx.doi.org/10.1186/1475-2867-13-54.
[34] F. Schmidt, C.B. Knobbe, B. Frank, H. Wolburg, M. Weller, Oncol. Rep. 19 (2008) 1061–1066, http://dx.doi.org/10.3892/or.19.4.1061.
[35] S. Tang, J. Hu, Q. Meng, X. Dong, K. Wang, Y. Qi, C. Chu, X. Zhang, L. Hou, Cell Biochem. Biophys. 65 (2013) 197–202, http://dx.doi.org/10.1007/s12013-012-9418-2.