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Synthesis and Biological Evaluation of Novel 7-*O*-Lipophilic Substituted Baicalein Derivatives as Potential Anticancer Agents

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Running title: Anticancer activity of 7-*O*-substituted baicalein derivatives

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Abstract

We synthesized derivatives of baicalein, wogonin, and chrysin through alkylation at the 7-*O*-position of the A ring with lipophilic terpenyl or long chain *n*-alkyl groups, and studied the *in vitro* anticancer activity of the derivatives through the growth inhibition MTT assay. We discovered that baicalein and two of its derivatives were good free radical scavengers. Among 20 synthesized derivatives, 7-*O*-farnesylbaicalein (**5d**) and 7-*O*-dodecylbaicalein (**5i**) demonstrated stronger growth inhibition against human colon cancer SW480 cells compared with baicalein, with half maximal inhibitory concentration (IC_{50}) values of 1.15 and 1.57 μM, respectively. Furthermore, **5d** and **5i** dose- and time-dependently inhibited the growth of SW480 cells. A cell cycle distribution analysis showed that **5d** and **5i** induced SW480 cell arrest at the S phase through an apoptotic mechanism, which was associated with an increase in the generation of reactive oxygen species. In conclusion, the potent anticancer activity of the baicalein derivatives (**5d** and **5i**) suggested that the derivatives are potential anticancer agents for human colon cancer.

Keywords: Baicalein, Lipophilic substituent, Colon cancer, Cytotoxic activity, Flow cytometry

1. Introduction

Colorectal cancer (CRC) is the most commonly diagnosed cancer and is a leading cause of cancer mortality. It remains a considerable major heath concern both globally[1] and in Taiwan, where the number of patients diagnosed with CRC has increased; such patients generally have poor prognosis. Therapeutic strategies to treat CRC include surgery, chemotherapy, and radiation therapy;[2] however, the side effects associated with chemotherapy, and radiation therapy, the high mortality rates and local tumour recurrence associated with surgical procedures[3,4] necessitate alternative therapeutic options for CRC patients.

Flavonoids are a group of compounds found in several plant sources, such as citrus fruits, seeds, olive oil, and cocoa, as well as in tea and red wine.[5] They are low-molecular-weight compounds containing a three-ring (polyphenolic) structure with various substituents.[6] For decades, flavonoids have been utilized for their significant pharmacological activities, including anticancer activity.[7] Studies have shown that flavonoids reduce the risk of cancer, inflammation, and heart diseases.[8] Additional studies have demonstrated that flavonoids possess an antioxidant, anti-inflammatory, anti-allergic, antiviral, and hepatoprotective activity.[9] Both *in vitro* and *in vivo* xenograft models have shown that flavonoids are cytotoxic to various human cancer cell lines, suggesting their potential as anticancer agents.[10–12]

Flavonoids are present in abundant quantities in traditional Chinese medicinal herbs, such as Huang-Qin (*Scutellaria baicalensis* Georgi). Four major flavonoids present in Huang-Qin are baicalein, baicalin, oroxylin A, and wogonin (Fig. 1). Baicalein is widely used as an antioxidant, anti-inflammatory, and anticancer agent.[13,14] A hepatic metabolic study demonstrated that the bioavailability and effectiveness of baicalein decreased rapidly in the intestinal tract upon glucuronidation or sulfation of the hydroxyl group at the 7-*O*-position. More than 90% of baicalein is converted to baicalein-7-*O*-glucuronide (baicalin) in the intestines.[15–17] Baicalein is a selective inhibitor of 12-lipooxygenase, which is responsible for the production of reactive oxygen species (ROS) during arachidonic acid metabolism.[18] Shieh *et al.* demonstrated that baicalein acts as a strong scavenger of the superoxide radicals in a cell-free system through rapid donation of hydrogen ions.[19] Thus, these polyphenols may be vital in preventing human oxidative stress by scavenging hydroxyl, DPPH, and alkyl free radicals.[20,21] Various methods, such as

DPPH and $ABTS^{\bullet+}$ radical scavenging, have been used to estimate the *in vitro* antioxidant activity of baicalein derivatives. The other two flavonoid components isolated from *S. baicalensis* Georgi, oroxylin A and wogonin, have also been reported to possess anticancer activity.[18–23]

The current study was conducted to determine whether baicalein derivatives with lipophilic moieties exhibit increased cell permeability and thus higher intracellular oxidative stress and cytotoxicity. We synthesized and evaluated a series of lipophilic substituted baicalein derivatives, with a focus on substitution at the 7-*O*-position of the A-ring with terpenyl or long chain *n*-alkyl groups. Moreover, we examined the effects of the synthesized derivatives on cell proliferation, cell cycle progress, and apoptosis against the three human colon cancer cell lines, one human liver cancer cell line, and one mouse normal cell line.

2. Chemistry

Derivatives of baicalein (**1**), wogonin (**3**), and chrysin (**4**) were synthesized through 7-*O*-alkylation with a long chain *n*-alkyl or terpenyl bromide. As shown in Scheme 1 and Scheme 2, **5a-i**, **6a-d**, and **7a-d** were synthesized according to a procedure reported in the literature by reacting baicalein (**1**), wogonin (**3**), and chrysin (**4**) with selected *n*-alkyl or terpenyl bromides in anhydrous acetone using anhydrous potassium carbonate (K_2CO_3) as a base under N_2 for 8–24 h.[14]

In brief, the reaction mixture was refluxed for 8–24 h, and the progress of the reaction was monitored using TLC. Subsequently, the reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The crude product was chromatographed on a silica gel column and eluted with EtOAc/*n*-Hex (1/3) to afford the desired baicalein, wogonin, and chrysin derivatives (**5a-i**, **6a-d**, and **7a-d**, respectively) in 40.0–72.3% yield (Scheme 1 and 2). The 7-*O*-substituted derivatives were the major products and the 6,7-*O*-disubstituted derivatives were only minor products in yields less than 10%. The structures of the flavonoid derivatives were determined through ^{1}H and ^{13}C NMR spectra, and liquid chromatography–mass spectrometry.

3. Results and discussion

3.1. Analysis of antioxidant activity of baicalein derivatives

The hydroxyl group of the A ring of baicalein was first alkylated at the 7-*O*-position with terpenyl or long chain *n*-alkyl groups, and free-radical scavenging activity examined using both the DPPH and $ABTS^{\bullet+}$ scavenging methods.[20,21] For comparison, the antioxidant activities of baicalein, oroxylin A, ascorbic acid and quercetin were analyzed. As shown in Table 1, a minor difference in DPPH and $ABTS^{\bullet+}$ free radical activities was observed among analyzed derivatives.

3.2. Analysis of *in vitro* anti-proliferative activity using MTT assay

The cytotoxic activity of the synthesized derivatives was evaluated using *in vitro* growth inhibition assays using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT).[22] The derivatives were rated according to their half maximal inhibitory concentration values (IC_{50}), a measure of the effectiveness of a compound in inhibiting biological and biochemical function; the lower the IC_{50} value, the more effective the compound at inhibiting cancer cell growth. The cytotoxic activity of 20 flavonoid derivatives, along with those of 5-fluorouracil (5-Fu), cisplatin, and doxorubicin as positive controls, were examined against four human cancer cell lines, including SW480 (colon carcinoma), HT29 (colon carcinoma), DLD-1 (colon carcinoma), and HepG2 (liver carcinoma), and one normal murine embryonic liver BNL CL.2 cell line. The cLog*P* and IC_{50} values of tested compounds are listed in Table 2. The anticancer activity of the derivatives increased with the chain length, or lipophilic characteristics, of the substitutes. The lipophilic activity was evident in the two striking bioactive derivatives, namely 7-*O*-farnesylbaicalein (**5d**) and 7-*O*-dodecylbaicalein (**5i**). Both showed significant cell growth inhibition in all four human cancer cell lines with IC_{50} values of 1.15 ± 0.15 and 1.57 ± 0.20 μM, respectively, against the SW480 cell line. These values were 16- to 11-fold more active compared with baicalein (**1**), which exhibited an IC_{50} value of 18.18 ± 0.89 μM. When treated with baicalein (**1**), **5d**, or **5i**, no significant cell death was detected in the normal murine embryonic liver BNL CL.2 cell line. Furthermore, only a marked effect on cell death (< 20%) was observed at the highest concentration tested (20 μM) for **5d** and **5i** after 48 h treatment. Baicalein (**1**) exhibited a slight cytotoxic effect after 48 h, suggesting that both **5d** and **5i** were cytotoxic to human colon cancer cells with no significant adverse effects on normal murine embryonic liver cells.

In the light of the cytotoxicity-related findings described previously, we examined the cytotoxicity of **5d** and **5i** against SW480 cells as a function of time. As shown in Table 3, the *in vitro* cytotoxicity of **5d** and **5i** demonstrated a 12- and 8-fold increase on SW480 cells for 48 h treatment, respectively, compared with baicalein (**1**).

We hypothesized that the increased activity of synthesized derivatives attributable to their enhanced bioavailability and cell membrane permeability that resulted from the increased lipophilicity. To investigate this hypothesis, we calculated cLog*P* values of the 7-*O*-substituted baicalein derivatives and compared their anticancer activity. The cLog*P* values correlated satisfactorily with IC_{50} values (Table 3). Hence, these results support our hypothesis that lipophilicity, or the chain length, of *n*-dodecyl and farnesyl moieties of 7-*O*-substituted baicalein derivatives played a vital role in the anticancer activity of those derivatives.

3.3. Cell morphological assessment

Cell apoptosis was observed by examining Hoechst 33258 stained cell nuclei through fluorescent microscopy. Apoptosis was determined as changes in cell morphology, such as chromatin condensation, nuclear shrinking, and DNA fragmentation.[23] To further investigate the role of apoptosis in the cytotoxicity of **5d** and **5i**, SW480 cells were incubated with 20 μM of baicalein (**1**), **5d**, or **5i**, and in 0.1% DMSO as control, for 48 h. The cells were stained with Hoechst 33258 and examined using fluorescence microscopy for topical morphological changes. The nuclei of the cells in the control sample were round and stained homogenously, whereas those treated with baicalein (**1**), **5d**, and **5i** exhibited typical morphological features of apoptosis such as nuclear shrinkage, chromatin condensation and DNA fragmentation (Fig. 3).[24] These results demonstrated that **5d** and **5i** induced apoptosis in SW480 cells.

3.4. Cell cycle distribution analysis through flow cytometry

To analyze the apoptotic effects of baicalein (**1**), **5d**, and **5i** on cell cycle progression, SW480 cells were treated with the synthesized derivatives at their different concentrations for 48 h. The cell cycle distribution and the subG1 phase were analyzed through flow cytometry after propidium iodide (PI) staining.[25] Untreated cells were used as the control. The percentage of cells at the S phase increased by 33.33%

and 50.00% when treated with 10 and 20 μM of **5d**, respectively, and by 32.12% and 51.02% when treated with 10 and 20 μM of **5i**, respectively, compared with a 26.19% increase in the control (Fig. 4). The increased percentage of cells at the S phase and the cytotoxic activity of **5d** and **5i** suggested that the synthesized derivatives induced SW480 cells arrest in the S phase. In addition, baicalein was also found to increase the percentage of cells at the S phase following the 24 h exposure at the concentration of 50 μM for the test compounds. Analysis of the S-phase arrest, however, showed that the concentration of all cell cycle regulatory molecules cyclin-dependent kinase 4 (CDK4), cyclin B1, and cyclin D1 showed a decreased level.[26] Our results indicated that baicalein did not affect cell cycle in the SW480 cells. A study[26] showed that baicalein dose-dependently inhibited the growth of human lung squamous carcinoma CH27 cells. The results of the current study may suggest that the apoptotic effect of baicalein depends on the type of the cancer cells under treatment.

3.5. Annexin V-FITC/PI staining

To study the bioactivity of baicalein (**1**), **5d**, and **5i** against SW480 cells, the cancer cells were treated with vehicle alone as control, or with one of the three test compounds at their different concentrations (5, 10, and 20 μM). After 48 h, the samples were double-stained with annexin V-FITC and PI.[27] The percentages of cells at various stages of apoptosis are shown in Fig. 5. The data indicated that apoptotic cell death resulting from treatment with **5d** or **5i** was dose-dependent; however, this was not observed in cells treated with baicalein (**1**). Starting from a dose of 10 μM, both **5d** and **5i** induced a higher degree of apoptosis in SW480 cells compared with baicalein (**1**) and cytotoxic effects at both the early and late stages, as determined through annexin V-FITC/PI staining. For baicalein (**1**), the effect was observed only at a higher concentration (20 μM). The analysis confirmed that the superior efficiency of both **5d** and **5i**, in inducing cytotoxicity and inhibiting the proliferation of human colorectal cancer cells.

3.6. Measurement of intracellular ROS production

Several flavonoids induce apoptosis by generating reactive oxygen species (ROS) in mitochondria.[28] Baicalein and its derivatives are hypothesized to induce apoptosis by increasing the concentration of intracellular ROS. Therefore, we

investigated whether baicalein (**1**), **5d**, or **5i** could stimulate generation of ROS in SW480 cells. The fluorescence intensity of dihydroethidium (DHE) in the cells was right-shifted after the cells were treated with all three compounds in a concentration-dependent manner (Fig. 6), indicating that both **5d** and **5i** could stimulate the release of intracellular $O_2^{\bullet-}$ from SW480 cells. As expected, **5d** and **5i**, at the concentrations of 5, 10, and 20 μM, exhibited a more profound effect (mean values = 102.13, 169.24, and 192.86, respectively, for **5d**) and (mean values = 117.62, 165.78, and 215.12, respectively, for **5i**), compared with baicalein (mean values = 104.84, 99.49, and 98.79, respectively) on $O_2^{\bullet-}$ generation in SW480 cells after 48 h of treatment ($P < 0.05$, Fig. 6B). Thus, the results showed that **5d** and **5i** induced apoptosis by increasing the intracellular oxidative stress of SW480 cells, and exhibited a strong capacity to induce apoptosis in SW480 cells in a ROS-dependent manner.

4. Conclusions

Among 20 analyzed derivatives, the baicalein derivatives **5d** and **5i**, which have a farnesyl group and a dodecyl group at the 7-*O*-position, respectively, showed a superior level of cytotoxicity in all human cancer cell lines studied. Furthermore, we discovered that **5d** and **5i** were the most cytotoxic *in vitro*, against human colon adenocarcinoma SW480 cells. In the cell cycle distribution and apoptotic analysis, **5d** and **5i** induced SW480 cells arrest at the S phase. In the Hoechst 33258 staining analysis, **5d** and **5i** markedly induced apoptosis, which was confirmed by the positive rate of annexin V-FITC/PI double staining. Both derivatives induced apoptosis in SW480 cells, by the inducing ROS generation. The results indicate that **5d** and **5i** have an enhanced anticancer activity against human colon cancer cells compared with standard treatments.

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Figures and Tables captions

Scheme 1 Synthesis of 7-*O*-substituted baicalein derivatives.

Scheme 2 Synthesis of 7-*O*-substituted wogonin and chrysin derivatives.

Fig. 1 Chemical structures of baicalein (**1**), baicalin (**2**), wogonin (**3**), and chrysin (**4**).

Fig. 2 *In vitro* cytotoxicity of (A) baicalein (**1**), (B) **5d**, and (C) **5i** in SW480 cells after drug exposure over various time periods.

Fig. 3 Morphological changes in SW480 cells treated with baicalein (**1**), **5d**, or **5i** at 20 μM for 48 h (magnification, 200×). Upper panels show the cell morphology under phase–contrast microscopy, and the lower panels display the Hoechst 33258-stained nuclear patterns detected though fluorescence microscopy (magnification, 200×). Red arrows indicate the apoptotic cells.

Fig. 4 Effect of baicalein (**1**) and its derivatives on SW480 cell cycle distribution. (A) Cell cycle distribution after treatment with baicalein (**1**), **5d**, or **5i** at 5–20 μM in SW480 cells for 48 h. (B) Quantitative difference of cell cycle distribution changed after treatment with baicalein (**1**), **5d**, and **5i** at 5–20 μM in SW480 for 48 h. Data are shown as the mean ± SD of three independent experiments, and $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with control.

Fig. 5 Effect of baicalein (**1**), **5d**, or **5i** on cell apoptosis and necrosis of SW480 cells assessed through flow cytometry. (A) Analysis of cell death pathway after the treatment with baicalein (**1**), **5d**, or **5i** at 5–20 μM in SW480 cells for 48 h. (B) Quantitative analysis of cell death pathway after the treatment with baicalein (**1**), **5d**, and **5i** at 5–20 μM in SW480 cells for 48 h. Plates were examined for apoptotic cells using an Annexin V-FITC apoptosis detection kit. Annexin V–positive/PI–negative cells are in the early stages of apoptosis and double positive cells are in late apoptosis, whereas Annexin V–negative/PI–positive cells are necrotic. Each value represents the mean ± SD of three independent experiments. $^{*}P < 0.05$, compared with control.

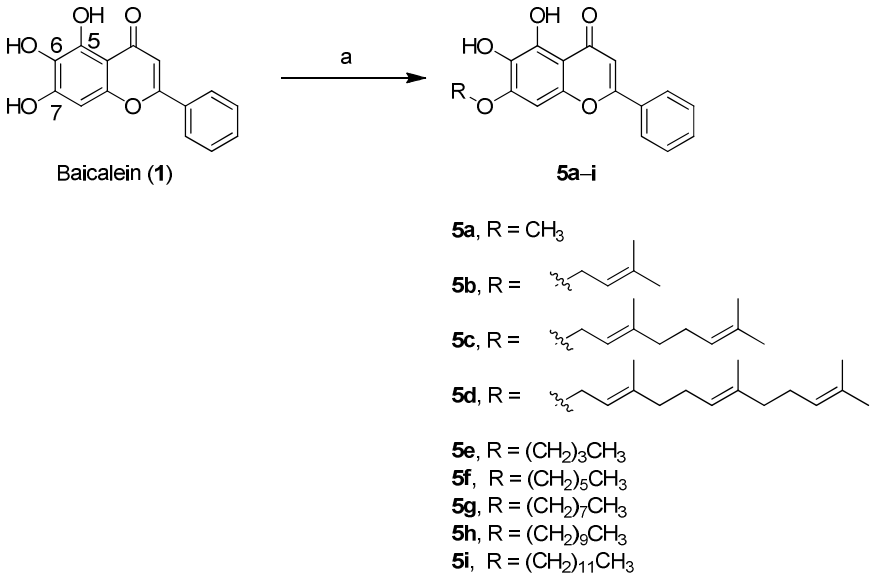
Fig. 6 Effect of baicalein (**1**), **5d**, or **5i** on ROS ($O_2^{\bullet-}$) generation in SW480 cells. (A) Analysis after treatment with baicalein (**1**), **5d**, or **5i** at 5–20 μM in SW480 cells for 48 h to detect $O_2^{\bullet-}$ content. (B) Quantitative analysis after treatment with baicalein (**1**), **5d**, or **5i** at 5–20 μM in SW480 cells for 48 h to detect $O_2^{\bullet-}$ content. Data are shown as the mean ± SD of three independent experiments, and $^*P < 0.05$, compared with control.

Table 1 Antioxidant activity of baicalein (**1**) and its derivatives (**5a-i**), trolox, ascorbic acid, and quercetin

Table 2 cLog*P* values and cytotoxic activities (IC_{50}, μM) of baicalein, wogonin, chrysin and their derivatives against four human cancer cell lines and one normal cell line after drug exposure for 48 h

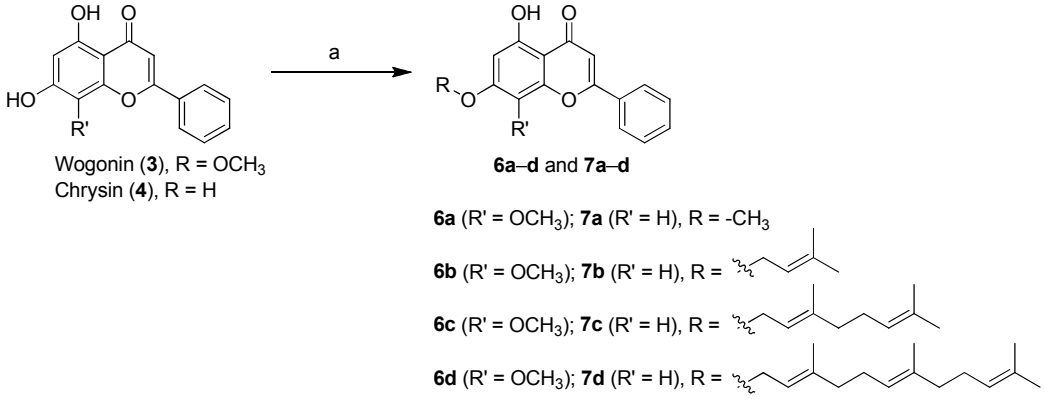
Table 3 *In vitro* cytotoxicity of baicalein (**1**), **5d**, and **5i** against a panel of human colon cancer cell lines over various time periods

Scheme 1 Synthesis of 7-*O*-substituted baicalein derivatives.

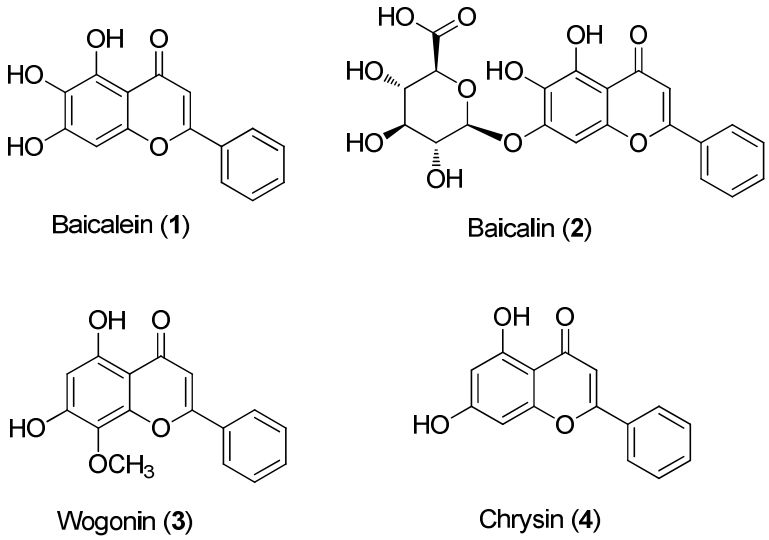


[a]*Reagents and conditions*: (a) *n*-alkyl or terpenyl bromide, anhydrous K_2CO_3, anhydrous acetone, reflux, 8–24 h.

Scheme 2 Synthesis of 7-*O*-substituted wogonin and chrysin derivatives



[a]*Reagents and conditions:* (a) methyl iodide or terpenyl bromide, anhydrous K_2CO_3, anhydrous acetone, reflux, 8–24 h.



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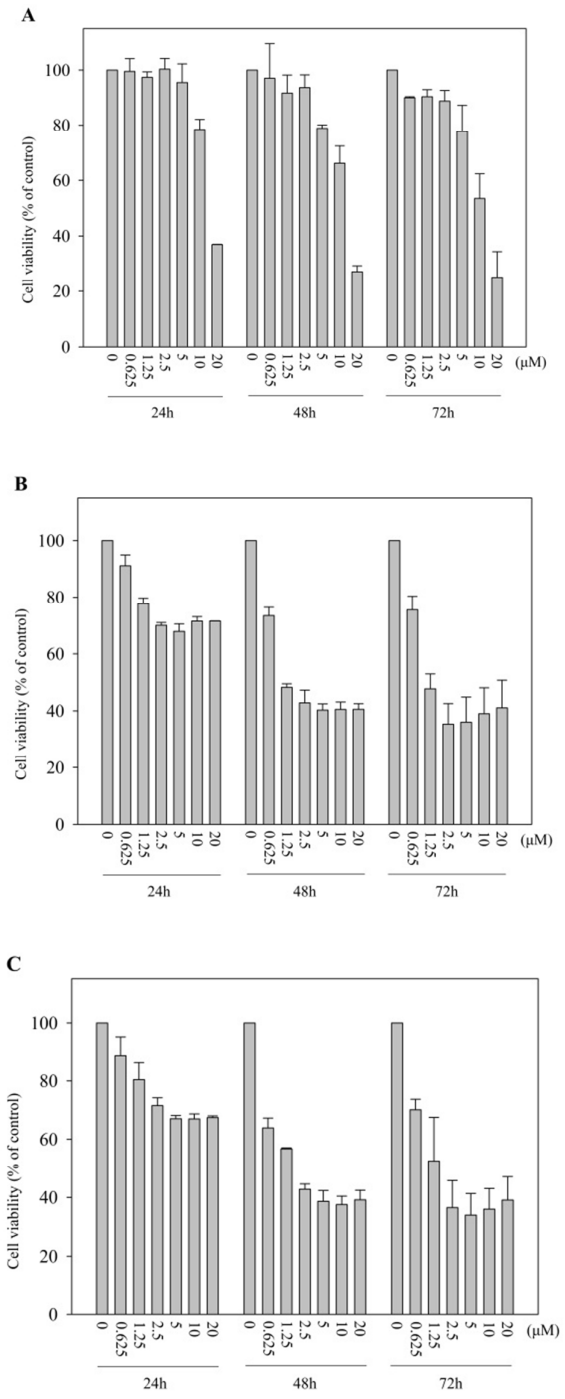

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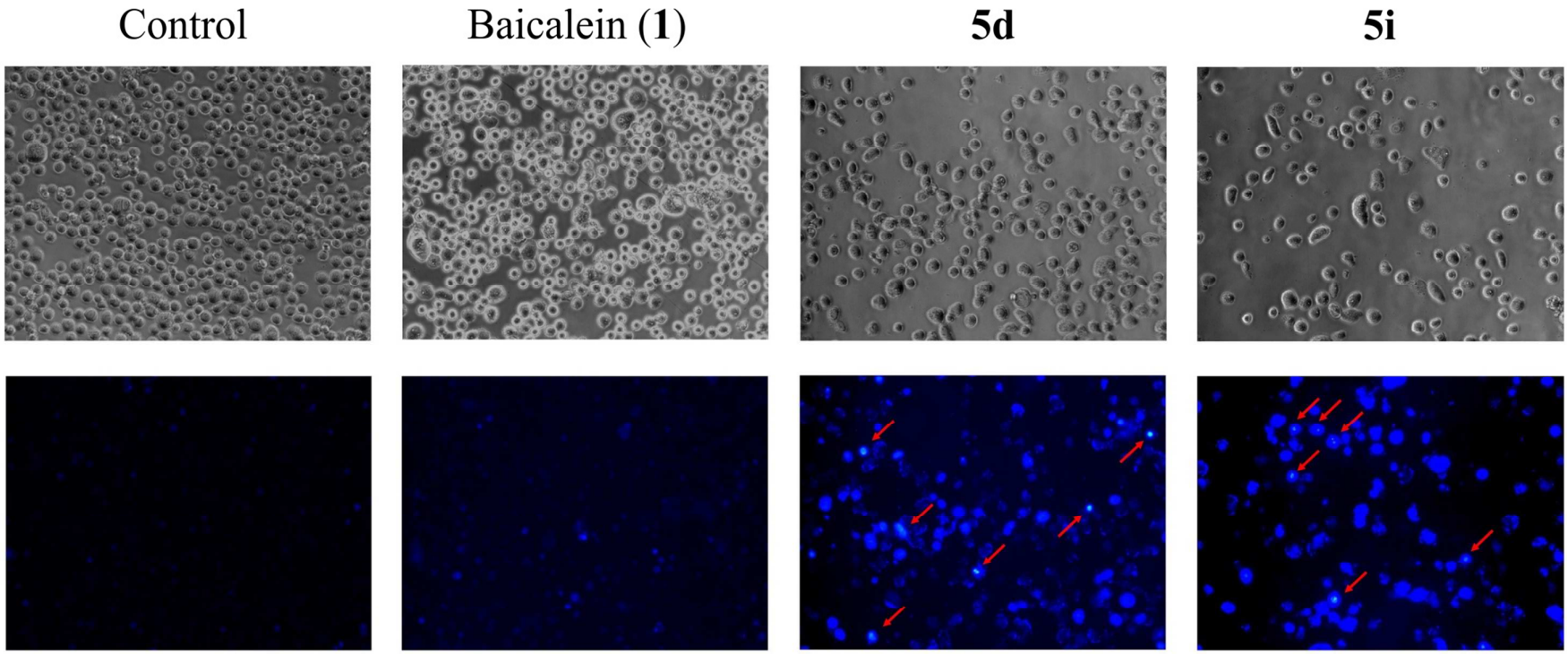

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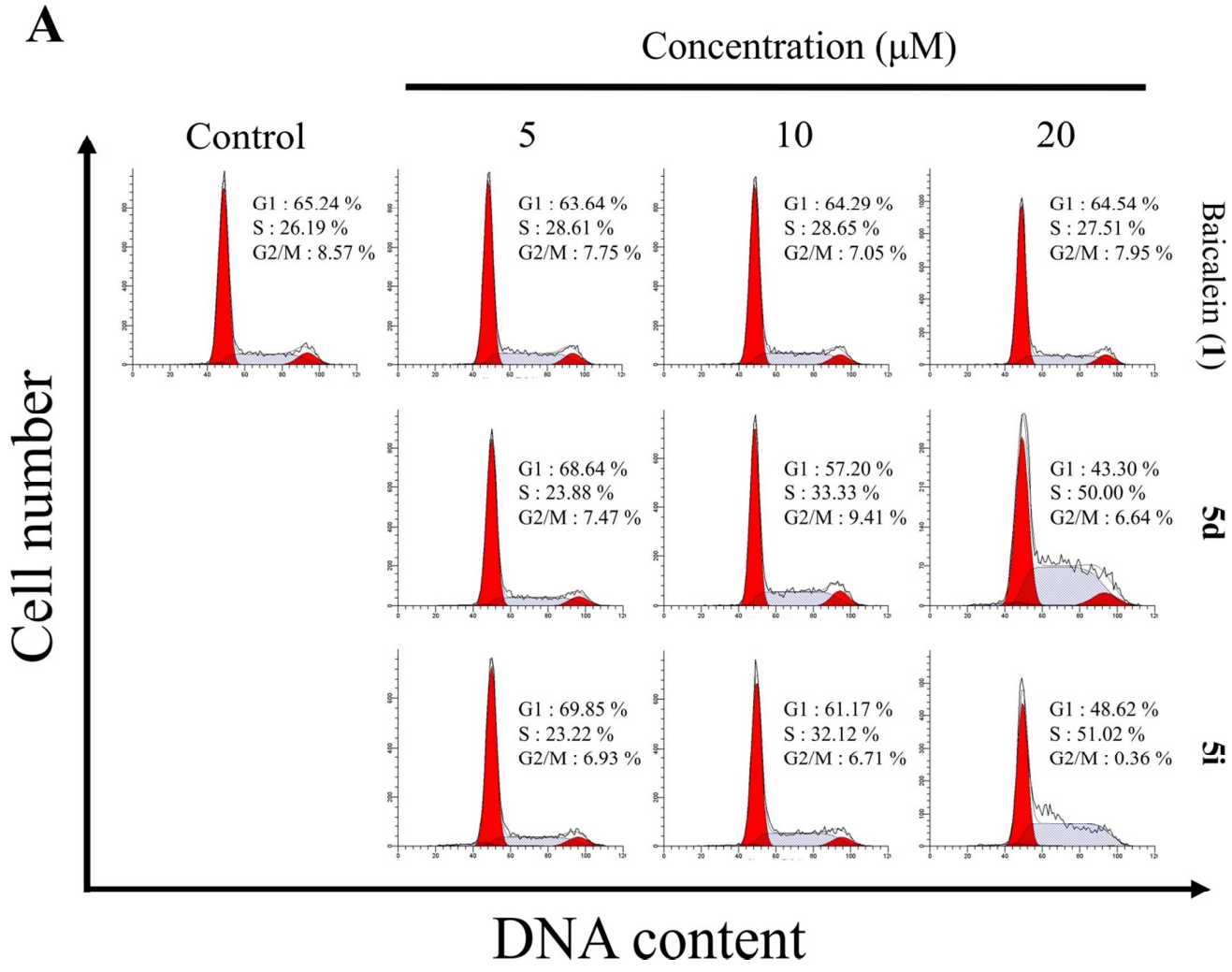

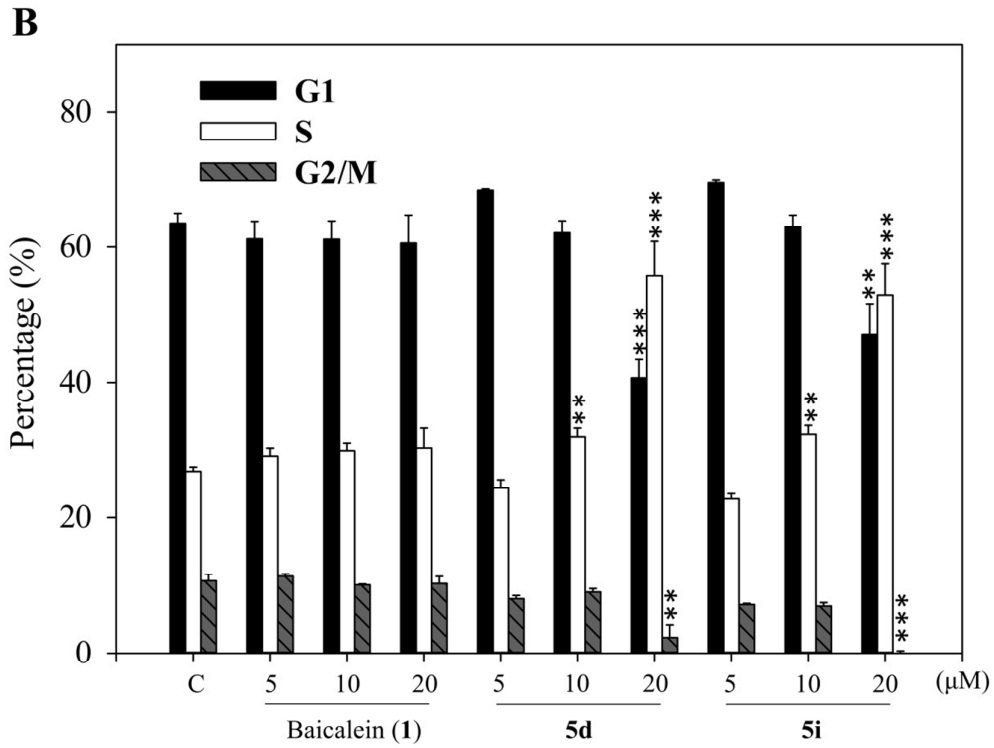

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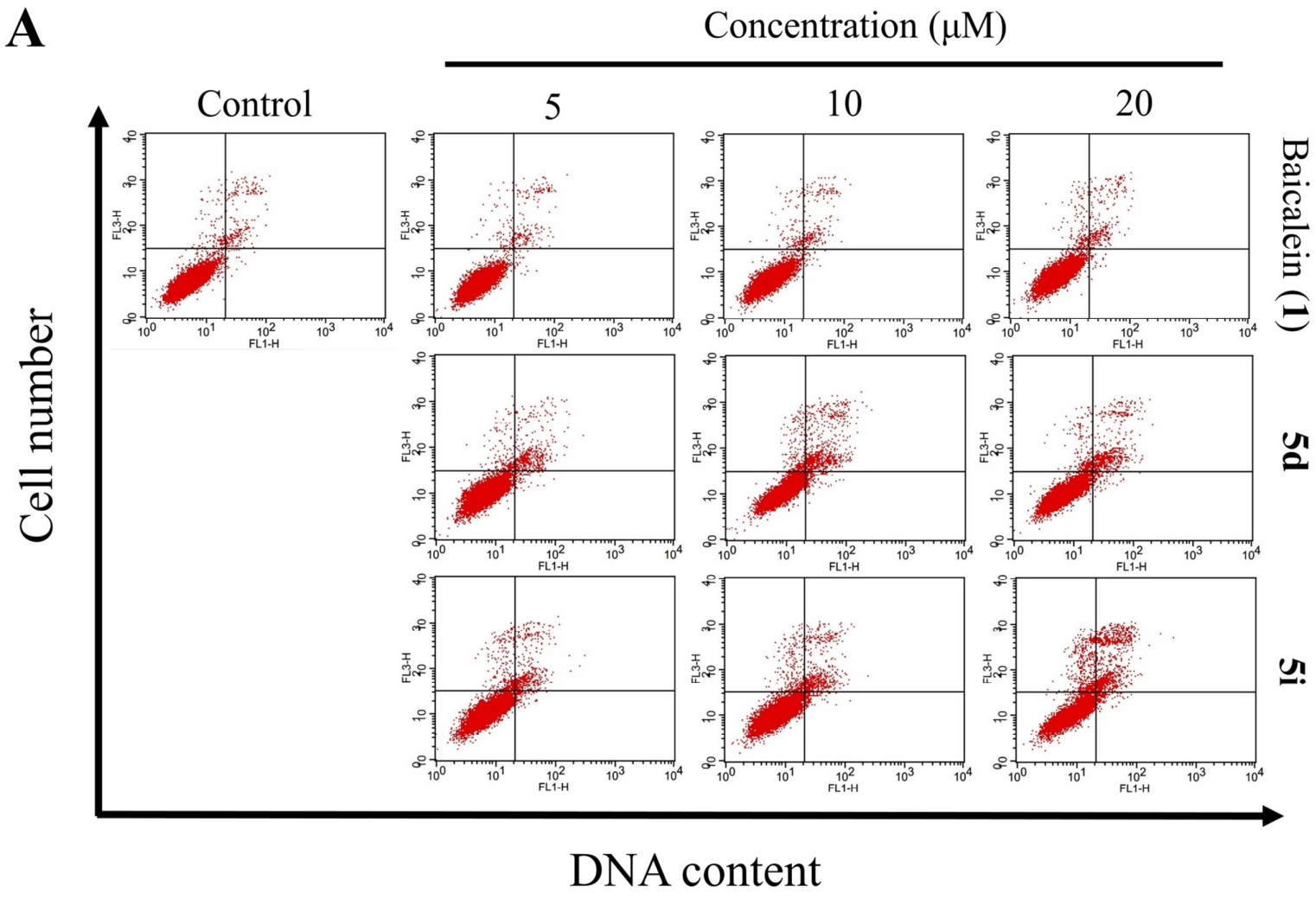


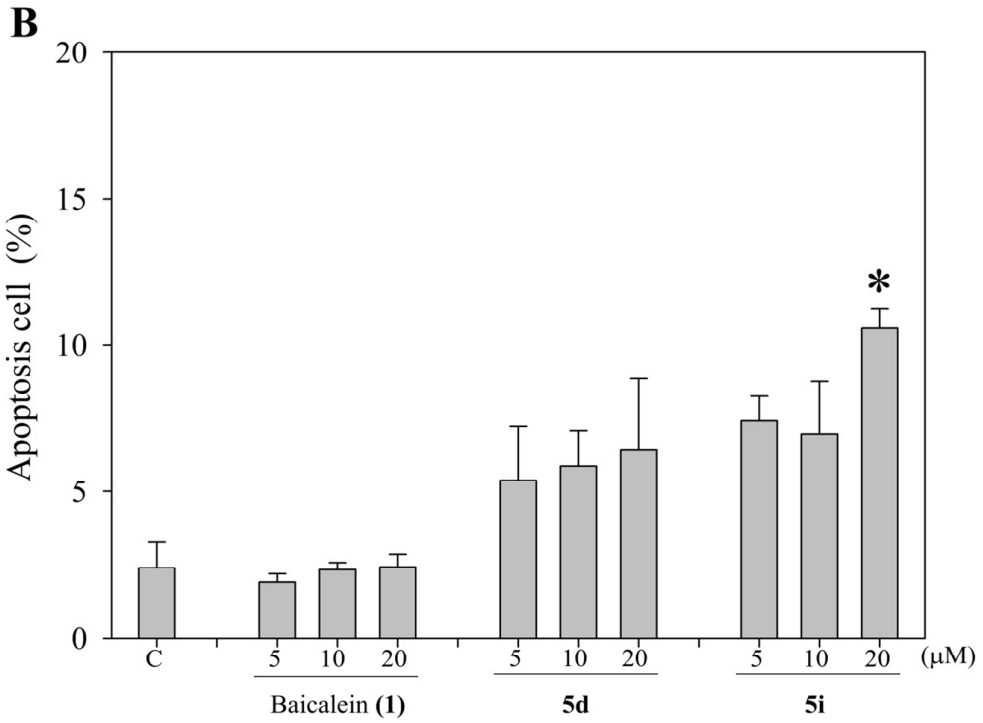
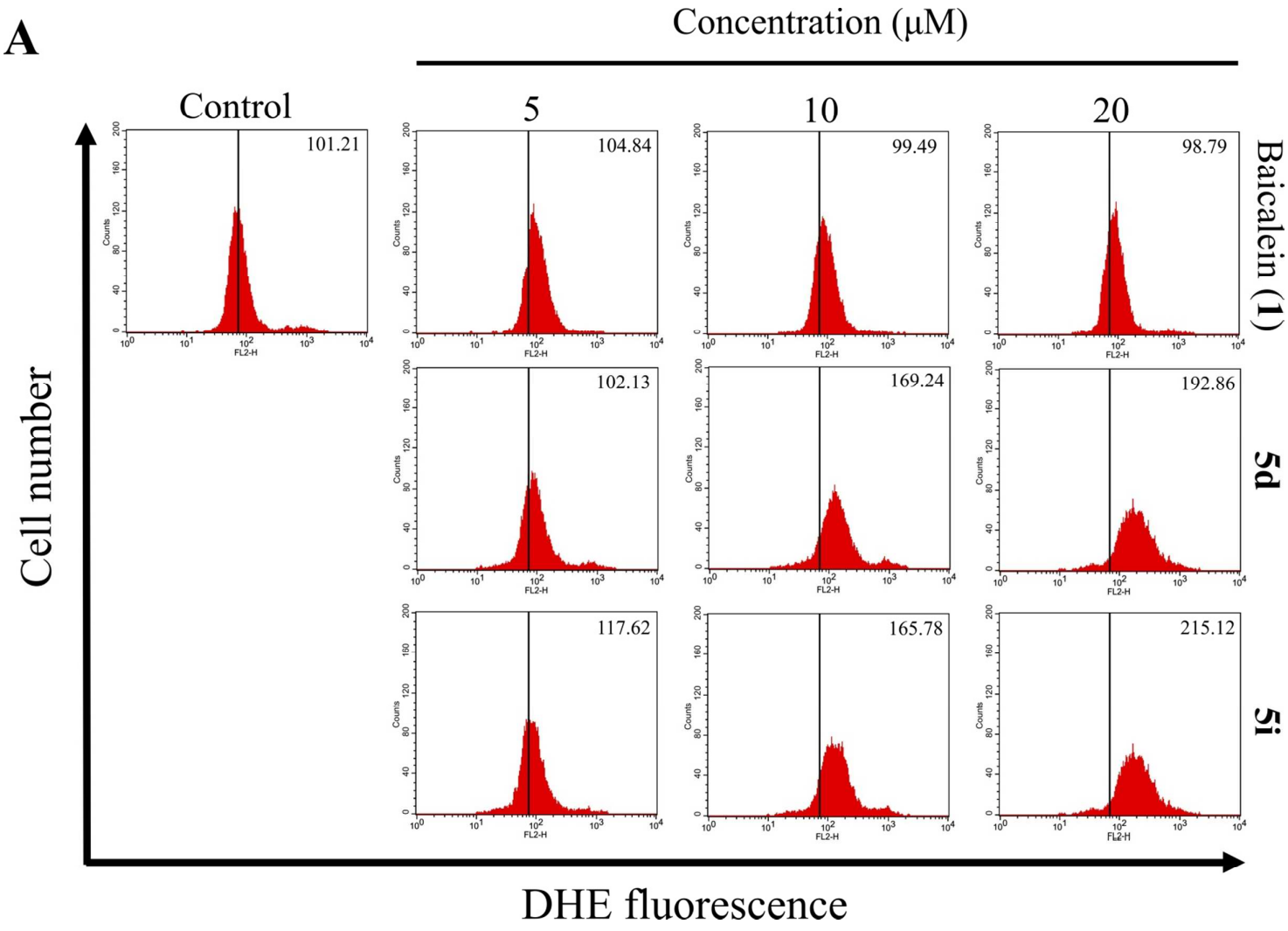


Fig. 5 Effect of baicalein (**1**), **5d**, or **5i** on cell apoptosis and necrosis of SW480 cells assessed through flow cytometry. (A) Analysis of cell death pathway after the treatment with baicalein (**1**), **5d**, or **5i** at 5–20 μM in SW480 cells for 48 h. (B) Quantitative analysis of cell death pathway after the treatment with baicalein (**1**), **5d**, and **5i** at 5–20 μM in SW480 cells for 48 h. Plates were examined for apoptotic cells using an Annexin V-FITC apoptosis detection kit. Annexin V–positive/PI–negative cells are in the early stages of apoptosis and double positive cells are in late apoptosis, whereas Annexin V–negative/PI–positive cells are necrotic. Each value represents the mean ± SD of three independent experiments, and $*P < 0.05$, compared with control.



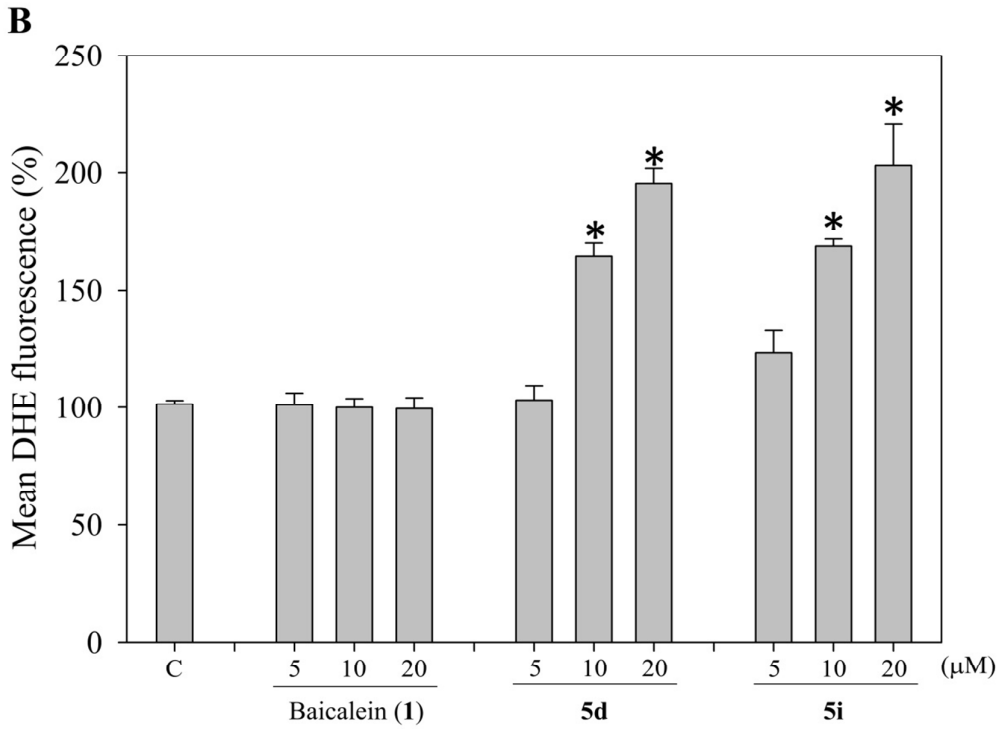


Fig. 6 Effect of baicalein (**1**), **5d**, or **5i** on ROS ($O_2^{\bullet-}$) generation in SW480 cells. (A) Analysis after treatment with baicalein (**1**), **5d**, or **5i** at 5–20 μM in SW480 cells for 48 h to detect $O_2^{\bullet-}$ content. (B) Quantitative analysis after treatment with baicalein (**1**), **5d**, or **5i** at 5–20 μM in SW480 cells for 48 h to detect $O_2^{\bullet-}$ content. Data are shown as the mean ± SD of three independent experiments, and $^*P < 0.05$, compared with control.

Table 1 Antioxidant effect of baicalein (**1**) and its derivatives (**5a-i**), trolox, ascorbic acid, and quercetin

Compound	IC_{50} (μM)[a] (mean ± SD)	
	DPPH	$ABTS^{\bullet+}$
Baicalein (**1**)	17.18 ± 1.26	15.54 ± 1.26
5a	28.40 ± 2.06	20.22 ± 1.36
5b	23.97 ± 1.13	18.83 ± 0.47
5c	24.34 ± 1.56	20.43 ± 1.66
5d	24.59 ± 2.30	23.99 ± 1.64
5e	12.91 ± 0.57	13.98 ± 0.45
5f	23.59 ± 0.30	20.37 ± 0.79
5g	21.53 ± 0.29	20.27 ± 1.50
5h	28.58 ± 0.52	23.40 ± 0.59
5i	23.51 ± 0.60	26.27 ± 1.85
Trolox	22.70 ± 0.03	16.80 ± 0.02
Ascorbic acid	27.30 ± 0.05	18.30 ± 0.55
Quercetin	8.41 ± 0.64	7.26 ± 0.01

[a] Compound concentration required to eliminate rate by 50 %. Data are expressed as the mean ± SD from the dose response curves of at 3–5 independent experiment.

Table 2 cLog*P* values and cytotoxic activities (IC_{50}, μM) of baicalein, wogonin, chrysin and their derivatives against four human cancer cell lines and one normal cell line after drug exposure for 48 h

Compound	clog*P*[a]	IC_{50} (μM)[b] (mean ± SD)				
		SW480[c]	HT-29[c]	DLD-1[c]	HepG2[d]	BNL CL.2[e]
Baicalein (**1**)	3.00	18.18 ± 0.89	30.61 ± 0.46	27.88 ± 0.31	28.09 ± 0.51	> 40
5a	3.33	29.41 ± 0.46	> 40	30.93 ± 0.65	> 40	> 40
5b	5.03	8.60 ± 0.37	> 20	> 20	> 20	> 20
5c	7.06	2.84 ± 0.43	17.02 ± 0.25	9.77 ± 0.93	> 20	> 20
5d	9.09	1.15 ± 0.15	14.95 ± 0.63	6.97 ± 0.15	> 20	> 20
5e	4.92	> 20	19.48 ± 0.35	> 20	> 20	> 20
5f	5.98	9.48 ± 0.47	17.65 ± 0.16	19.01 ± 0.52	> 20	> 20
5g	7.03	3.03 ± 0.46	16.25 ± 0.62	15.41 ± 0.76	> 20	> 20
5h	8.09	1.99 ± 0.38	> 20	15.52 ± 0.47	> 20	> 20
5i	9.15	1.57 ± 0.20	> 20	9.26 ± 0.10	> 20	> 20
Wogonin (**3**)	3.33	35.06 ± 3.84	39.55 ± 0.23	36.87 ± 0.65	37.89 ± 2.26	> 40
6a	3.77	> 20	> 20	> 20	2.72 ± 0.84	> 20
6b	5.48	> 20	> 20	> 20	> 20	> 20
6c	7.51	> 20	> 20	> 20	> 20	> 20
6d	9.54	> 20	> 20	> 20	> 20	> 20
Chrysin (**4**)	3.56	31.08 ± 2.96	19.49 ± 0.38	18.62 ± 0.81	16.50 ± 0.36	> 40
7a	4.15	> 20	> 20	> 20	> 20	> 20
7b	5.85	> 20	> 20	> 20	> 20	> 20
7c	7.88	> 20	> 20	> 20	> 20	> 20
7d	9.91	> 20	> 20	> 20	> 20	> 20
Cisplatin		40.72 ± 1.18	24.07 ± 0.03	-[f]	36.07 ± 3.11	-
5-Fu		32.72 ± 8.32	> 100	-	40.18 ± 7.63	-
Doxorubicin		0.53 ± 0.07	1.70 ± 0.20	-	0.30 ± 0.02	-

[a] Calculated log value of partition coefficient by ChemDraw Ultra 11.0.

[b] Compound concentration (μM) required to inhibit tumor cell proliferation rate by 50 %. Data are expressed as the mean ± SD from the dose response curves of at 3–5 independent experiments.

[c] Human colon adenocarcinoma cell lines

[d] Human hepatocarcinoma cell lines

[e] Normal murine embryonic liver cell lines

[f] Not tested

Table 3 *In vitro* cytotoxicity of baicalein (**1**), **5d**, and **5i** against a panel of human colon cancer cell lines over various time periods

Compound	IC_{50} (μM)[a] (mean ± SD)								
	SW480[b]			HT-29[b]			DLD-1[b]		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
Baicalein (**1**)	16.95 ± 0.47	14.05 ± 1.09	11.55 ± 2.63	> 20	18.05 ± 1.68	18.36 ± 2.98	> 20	> 20	> 20
5d	> 20	1.21 ± 0.03	1.26 ± 0.13	> 20	16.80 ± 0.68	15.40 ± 0.46	16.15 ± 1.02	4.88 ± 0.58	6.86 ± 2.59
5i	> 20	1.84 ± 0.08	1.64 ± 0.58	> 20	> 20	> 20	> 20	7.23 ± 1.42	6.19 ± 1.71

[a] Compound concentration required to inhibit tumor cell proliferation rate by 50 %. Data are expressed as the mean ± SD from the dose response curves of at 3–5 independent experiment.

[b] Human colon adenocarcinoma cell lines

Synthesis and Biological Evaluation of Novel 7-*O*-Lipophilic Substituted Baicalein Derivatives as Potential Anticancer Agents

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Graphical abstract

A series of lipophilic 7-*O*-substituted baicalein derivatives were synthesized and evaluate for their anticancer activity against four human cancer cell lines.