Accepted Manuscript

Novel 3′,5′-diprenylated chalcones inhibited the proliferation of cancer cells *in vitro* by inducing cell apoptosis and arresting cell cycle phase

Zhonghang Wen, Yongqiang Zhang, Xinghui Wang, Xiaoping Zeng, Zhanxing Hu, Yi Liu, Yuxin Xie, Guangyi Liang, Jianguo Zhu, Heng Luo, Bixue Xu

PII: S0223-5234(17)30245-3

DOI: 10.1016/j.ejmech.2017.03.077

Reference: EJMECH 9337

To appear in: *European Journal of Medicinal Chemistry*

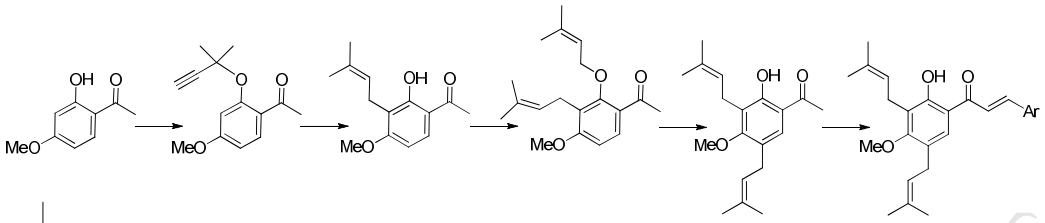
Received Date: 31 December 2016

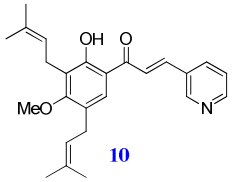
Revised Date: 3 March 2017

Accepted Date: 30 March 2017

Please cite this article as: Z. Wen, Y. Zhang, X. Wang, X. Zeng, Z. Hu, Y. Liu, Y. Xie, G. Liang, J. Zhu, H. Luo, B. Xu, Novel 3′,5′-diprenylated chalcones inhibited the proliferation of cancer cells *in vitro* by inducing cell apoptosis and arresting cell cycle phase, *European Journal of Medicinal Chemistry* (2017), doi: 10.1016/j.ejmech.2017.03.077.

A double Claisen rearrangements synthetic strategy was established for the total synthesis of a series of 3',5'-diprenylated chalcones.





10

selceted compound

IC_{50} = 2.92 μM PC3
3.14 μM MDA
1.85 μM HEL
2.64 μM K562

Compound 10 inhibited the proliferation of cancer cells *in vitro* by inducing cell apoptosis and arresting cell cycle phase.

Novel 3',5'-diprenylated chalcones inhibited the proliferation of cancer cells *in vitro* by inducing cell apoptosis and arresting cell cycle phase

Zhonghang Wen [a,b,c#], Yongqiang Zhang [a,b,c#], Xinghui Wang [a,b], Xiaoping Zeng [a,b], Zhanxing Hu [a,b], Yi Liu [a,b,c], Yuxin Xie [d], Guangyi Liang [a,b], Jianguo Zhu [e*], Heng Luo [a,b*], Bixue Xu [a,b*]

[a] *State Key Laboratory of Functions and Applications of Medicinal Plants, Guizhou Medical University, Guiyang 550014, P.R. China;*
[b] *Key Laboratory of Chemistry for Natural Products of Guizhou Province and Chinese Academy of Sciences, Guiyang 550014, P.R. China;*
[c] *School of Pharmaceutical Sciences, Guizhou University, Guiyang 550025, P.R. China;*
[d] *School of Pharmaceutical Sciences, Guizhou Medical University, Guiyang 550025, P.R. China;*
[e] *Guizhou Provincial People's Hospital, Guiyang 550002, P.R. China.*

Abstract

A double Claisen rearrangements synthetic strategy was established for the total synthesis of 4,4'-dimethyl medicagenin (compound **6c**). A series of its analogs also were prepared, including two novel 3',5'-diprenylated chalcones, in which ring B was replaced by azaheterocycle. The structures of the twenty-two newly synthesized compounds were confirmed by ^{1}H NMR, ^{13}C NMR and ESI-MS. *In vitro*, the cytotoxicity of the target compounds was evaluated using cancer cells. Noticeably, compound **10** exhibited broad-spectrum cytotoxicity on PC3 prostate cancer cells, MDA-MB-231 breast cancer cells (MDA), HEL and K562 erythroleukemia cells with IC_{50} values of 2.92, 3.14, 1.85 and 2.64 μM, respectively. Further studies indicated that compound **10** induced apoptosis and arrested the cell cycle phase of the above mentioned four cancer cell lines. By contrast, compound **6g** selectively displayed potent inhibitory activity against the proliferation of HEL cells with an IC_{50} value of 4.35 μM. Compound **6g** slightly induced apoptosis and arrested cell cycle phase of HEL cells. Preliminary structure-activity relationship studies indicated that, in all cancer cell lines evaluated, the 3-pyridinyl group was essential for cytotoxicity.

1 Introduction

Cancer is the second leading cause of morbidity and mortality worldwide [1]. The use of conventional chemotherapy is the primary treatment approach for curing cancer. Despite enormous efforts in developing better leads and novel chemotherapeutic strategies, the lack of selectivity and development of drug-resistance diminishes the efficacy of cancer chemotherapy [2]. Therefore, the search and development for improved treatment strategies and more effective anti-cancer agents

Corresponding authors. Tel.: +86 851-83807713; E-mail: bixue_xu@126.com (B.X. Xu); Tel.: +86 851-83876210; E-mail: luohengzz@sina.com (H. Luo); Tel.: +86 851-83876458; E-mail: doctorzhujianguo@163.com (J.G. Zhu)
[#] Both authors contributed equally to this work.

remains an essential task.

Prenylated chalcones have drawn a great deal of attention owing to their unique structure and extensive bioactivities. Various prenylated chalcones have been investigated as anti-malarial [3], anti-inflammatory [4], anti-bacterial [5], anti-diabetic [6] and anti-cancer [7] agents. Natural prenylated chalcones under the current study have xanthohumol [8], broussochalcone A [9], spinochalcone A [10], 4,4'-dimethyl medicagenin [11], etc. Xanthohumol and its analogs have been hot topics in the field of anti-cancer research [12-20], broussochalcone A enhanced cytotoxicity of mitomycin C in treatment of MCF-7 cells may be associated with the quinone reductase-inducing potential of broussochalcone A [21], spinochalcone A showed good anti-proliferative activity against DU-145 cells, and high selectivity compared with normal cells [22]. However, few reports have been published on the biological activities of 4,4'-dimethyl medicagenin (**6c**), and there is a lack of diversity about the structural modification of its ring B. Therefore it seems to be important to develop synthetic routes of 4,4'-dimethyl medicagenin and its analogs for elucidation of the relation between structures and promising activities. In this paper, we report on the design and synthesis of a series of prenylated chalcones as analogs of 4,4'-dimethyl medicagenin, comprising of two novel 3',5'-diprenylated chalcones, in which ring B was replaced by azaheterocycle. The inhibitory activity was assessed *in vitro* using four cancer cell lines, including PC3, MDA-MB-231 (MDA), HEL and K562 cells.

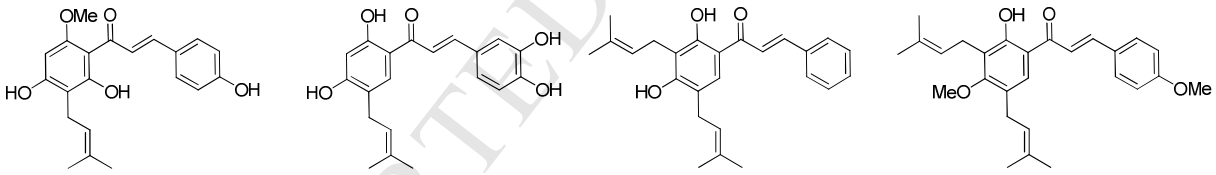


Figure 1. Chemical structure of four natural prenylated chalcones. Xanthohumol was isolated from *Humulus lupulus* L., broussochalcone A was isolated from *Broussonetia papyrifera*, spinochalcone A was isolated from *Tephrosia spinosa*, and 4,4'-dimethyl medicagenin was isolated from *sophora subprostrata* Chun et T. Chen.

2 Results and discussion

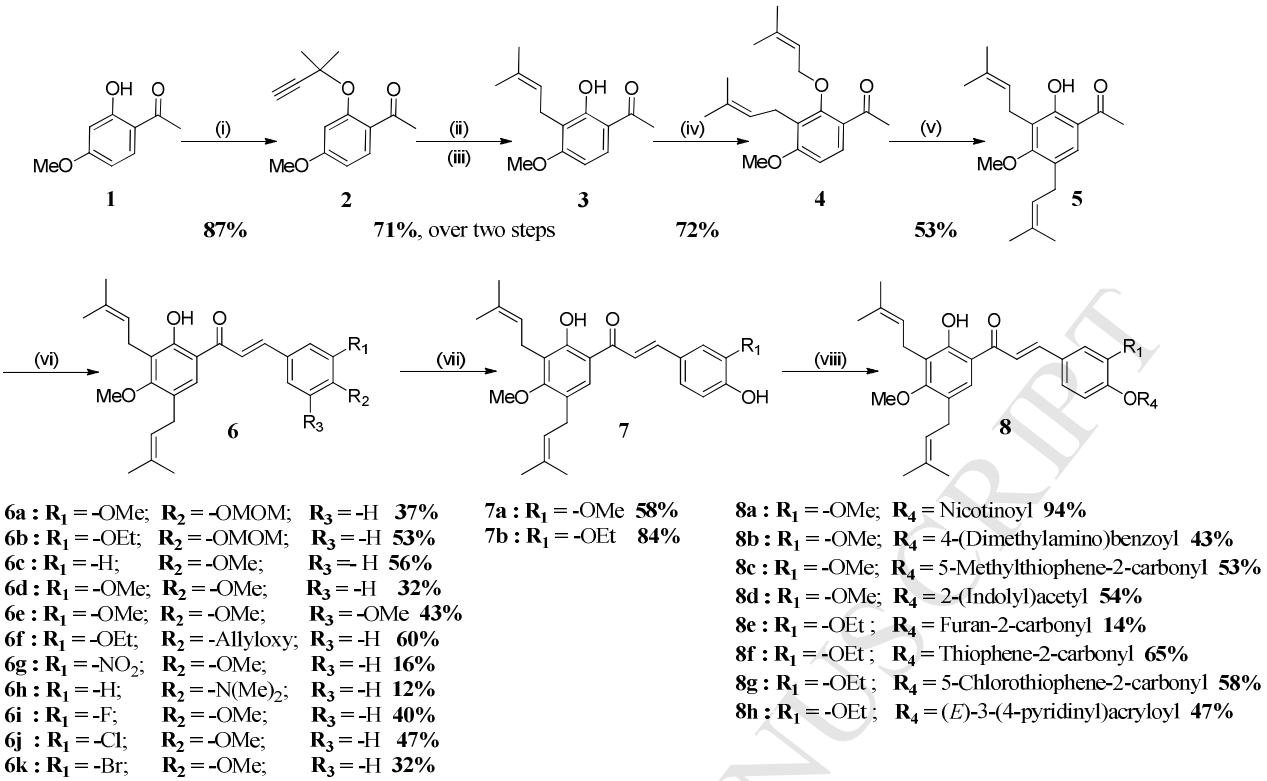
2.1 Chemistry

Several methods have been reported for the preparation of 3',5'-diprenylated chalcones, all showing as a synthetic challenge for the formation of the double C-prenyl side-chains. Sugamoto et al [23]. carried out the double prenylation of the C-3 and C-5 of 2'-hydroxy-4'-methoxyacetophenone by using Montmorillonite K10 catalyzed 4'-methoxy-2'-prenoxyacetophenone via [1,3]-sigmatropic rearrangement, only two steps it taken, however, the yield was only 7%. Narender et al [3]. accomplished the total synthesis of Medicagenin, 2',4'-

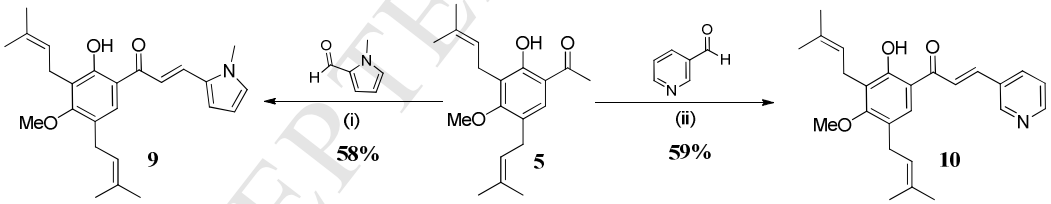
dihydroxy-3',5'-C-diprenylacetophenone as the key intermediate was obtained by reacting 2',4'-dihydroxyacetophenone with 2-methyl-but-3-en-2-ol in the presence of $BF_3 \cdot OEt_2$ in low yield (21%), and this synthetic condition was not easy to control. Wang et al [7]. also finished the total synthesis of Medicagenin in 8 steps and 8.5% overall yield, the formation of the double C-prenyl side-chains of chalcone by using Florisil® catalyzed prenyl phenyl ethers, however, the yield was very low (2%). Therefore, the challenging synthetic route and accompanying poor yield limited this approach for further use.

To optimize the synthetic route as well as to improve the yield of the total synthesis of 3',5'-diprenylated chalcones, we developed an efficient method as showed in Scheme 1, Lindlar catalytic hydrogenation [24], double Claisen rearrangements and Aldol condensation are the key steps in this route, the most important intermediate 2'-hydroxy-4'-methoxy-3',5'-diprenylacetophenone (**5**) was achieved in 5 steps and 24% overall yield. Currently, this approach has not been described in other studies.

Twenty-one 3',5'-diprenylated chalcones were synthesized through the route described in Scheme 1. Treating the starting material **1** with 3-chloro-3-methylbutyne at -5 ºC in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and $CuCl_2$ provided compound **2** in 87% yield [25]. Reduction of **2** by Lindlar catalytic hydrogenation and subsequently Claisen rearrangement at 120 ºC in *N*,*N*-diethylaniline afforded compound **3** in 71% yield over two steps [24]. The compound **5** was synthesized by prenylation of **3** and followed by Claisen rearrangement at 170 ºC in *N*,*N*-diethylaniline. Aldol coupling [26] compound **5** with different derivatives of benzaldehyde afforded the corresponding 3',5'-diprenylated chalcones (**6a**-**6k**) in low to good yields (12-60%). The deprotection of compounds **6a** and **6b** were performed by using two methods [27,28], treatment of **6a** with diluted hydrochloric acid (3 M) in methanol at 60 ºC to give **7a** in 58% yield, the reaction of **6b** with *p*-toluene sulfonic acid monohydrate (*p*-$TsOH.H_2O$) in methanol at room temperature provided **7b** in better yield (84%) than former. Different substituents were introduced to the 4-hydroxyl of **7a** or **7b** to afford the corresponding compounds (**8a**-**8h**) in low to good yields (14-94%) by using Kim's method [29]. As shown in Scheme 2, two novel 3',5'-diprenylated chalcones (**9**, **10**), in which ring B was replaced by azaheterocycle were synthesized by Aldol coupling between compound **5** and the *N*-methylpyrrole-2-carboxaldehyde or 3-pyridinecarboxaldehyde, respectively. In addition, we have tried to replace ring B of 4,4'-dimethyl medicagenin with a variety of nitrogen heterocycles by treating compound **5** with a variety of aromatic aldehydes, which contain nitrogen atoms, such as, 2-pyrrolecarbaldehyde, imidazole-4-carboxaldehyde, 5-methoxyindole-3-carboxaldehyde, 2-pyridinecarboxaldehyde, 4-pyridinecarboxaldehyde, 4-quinolinecarboxaldehyde, etc. Unfortunately, we failed to get the desired compounds.



Scheme 1. Synthetic route of 3',5'-diprenylated chalcones. Reagents and conditions: (i) $CuCl_2$, DBU, acetonitrile, 3-chloro-3-methylbutyne, -5 ºC, 11 h; (ii) Lindlar catalyst($Pd/CaCO_3$), H_2, ethyl acetate, RT, 12 h; (iii) *N*,*N*-diethylaniline, 120 ºC, 45 h; (iv) K_2CO_3, DMF, prenyl bromine, 50 ºC, 30 h; (v) *N*,*N*-diethylaniline, 170 ºC, 24 h; (vi) benzaldehyde derivatives, NaOH, ethanol, RT; (vii) HCl (3M), methanol, 60 ºC, 1 h (**7a**); $TsOH.H_2O$, methanol, RT, 15 h (**7b**); (viii) R_4OH, DMAP, EDCI, CH_2Cl_2, 0 ºC-RT (**8a**-**8h**).



Scheme 2. Synthesis of target compound **9** and **10**. Reagents and conditions: (i) NaOH, ethanol, RT, 37 h; (ii) NaOH, ethanol, RT, 35 h.

2.2 In vitro cytotoxicity assay

The cytotoxic activities of the synthesized 3', 5'-diprenylated chalcones were evaluated using four cell lines, including PC3, MDA, HEL and K562 cells. We used the MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide) assay to determine the cell viability after treatment with 5 μmol/L of compound (Figure 2 and Table 1). The result indicated that compound **6g** only displayed a potent inhibitory effect on the proliferation of HEL cells at a relatively low concentration. By contrast, compound **10** exhibited broad-spectrum cytotoxic effects on all cancer cells evaluated.

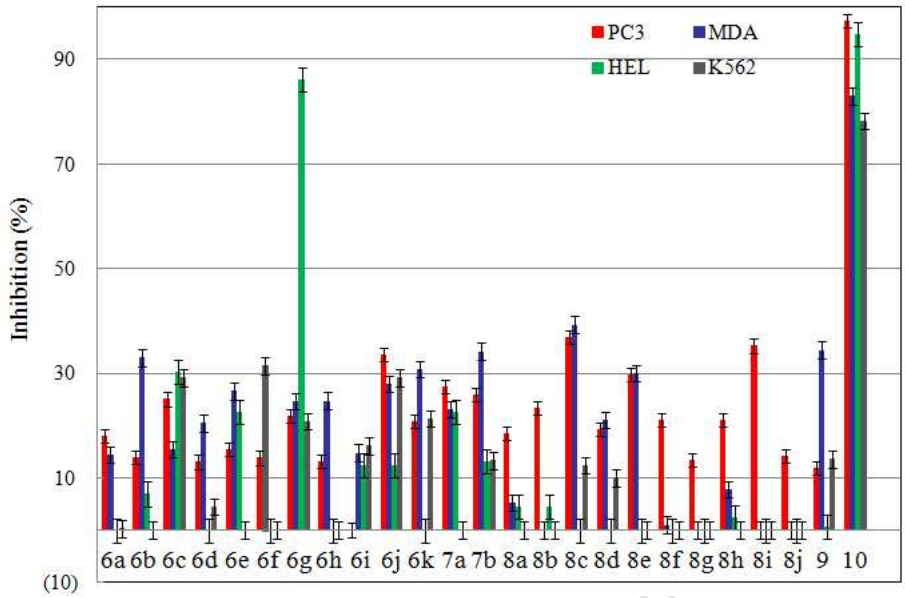
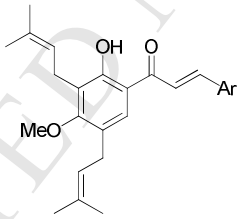
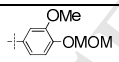
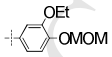


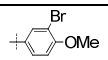
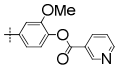
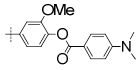
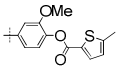
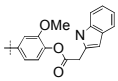
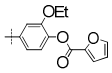
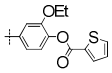
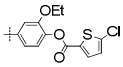
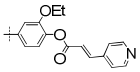
Figure 2. Inhibition of 3',5'-diprenylated chalcones on the proliferation of cancer cells *in vitro*. Cancer cells lines PC3, MDA, HEL and K562 were incubated with chalcones at a concentration of 5 μmol/L and the effect on cell proliferation was investigated. Data are presented as mean ± SD of three independent experiments.

Table 1

The structures and inhibition after treating cancer cells with 5 μmol/L of target compounds respectively. Data are presented as the mean ± SD of three independent experiments.

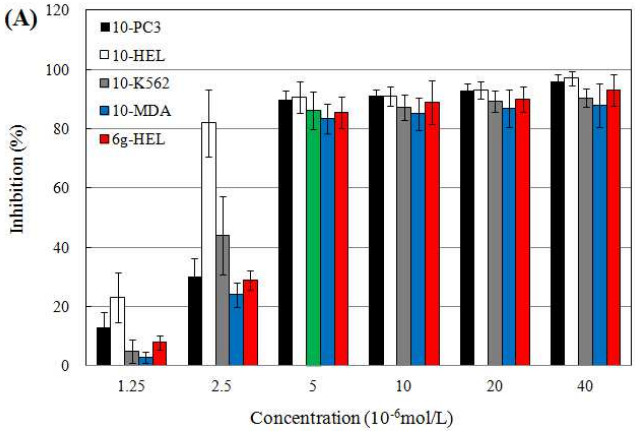


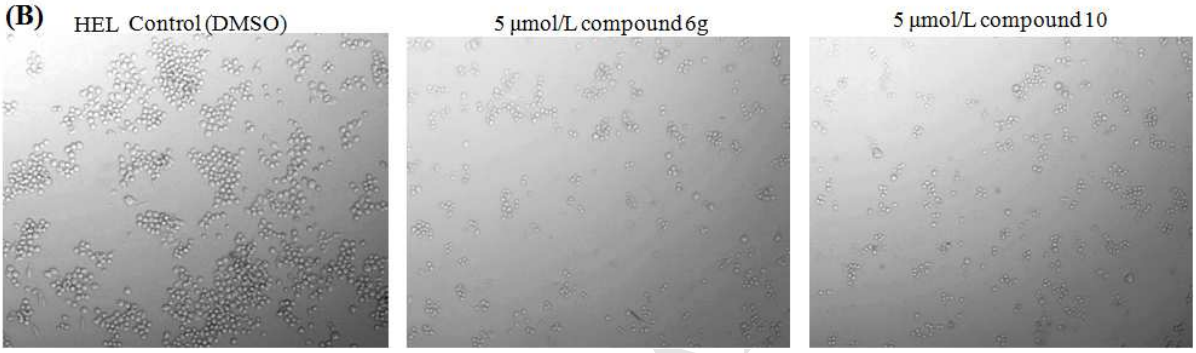
Compd	Ar	Inhibition (%)			
		PC3	MDA	HEL	PC3
6a		17.3 ± 1.7	14.0 ± 0.4	NA[a]	3.9 ± 0.3
6b		13.1 ± 2.1	29.9 ± 4.1	6.3 ± 0.7	NA
6c		24.6 ± 1.3	14.5 ± 1.1	29.5 ± 3.2	32.0 ± 0.7
6d		10.6 ± 0.9	19.0 ± 1.7	NA	7.5 ± 0.6
6e		15.0 ± 1.8	26.2 ± 8.3	23.6 ± 5.7	NA
6f		13.4 ± 2.2	27.7 ± 1.6	NA	NA
6g		21.2 ± 3.6	23.4 ± 2.1	85.4 ± 4.4	23.0 ± 5.8
6h		11.6 ± 2.8	21.5 ± 5.3	NA	NA
6i		NA	12.1 ± 2.9	12.3 ± 1.7	19.3 ± 3.6
6j		31.8 ± 2.0	26.5 ± 1.9	11.7 ± 4.4	31.9 ± 2.0

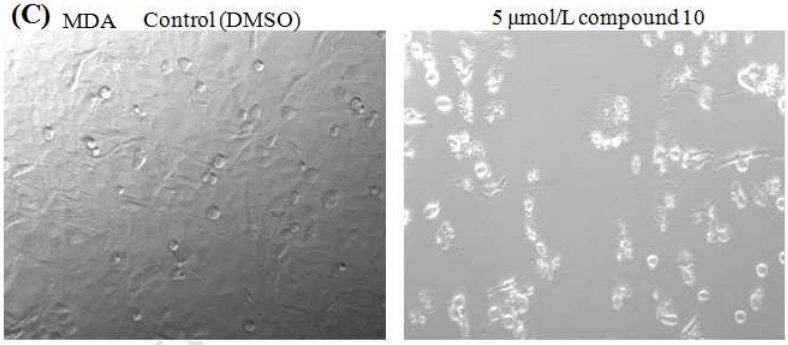
Compd	Ar	Inhibition (%)			
		PC3	MDA	HEL	PC3
6k		20.9 ± 0.4	30.2 ± 1.4	NA	18.9 ± 5.4
7a		26.0 ± 2.2	19.3 ± 1.6	22.2 ± 3.9	NA
7b		25.6 ± 1.9	33.5 ± 5.7	12.7 ± 5.4	10.2 ± 3.6
8a		23.0 ± 1.2	NA	5.7 ± 2.0	NA
8b		36.5 ± 1.0	38.5 ± 2.4	NA	8.1 ± 1.0
8c		19.2 ± 6.9	19.6 ± 1.8	NA	6.3 ± 1.4
8d		29.9 ± 4.1	28.3 ± 7.5	NA	NA
8e		12.7 ± 2.3	NA	NA	NA
8f		21.4 ± 5.3	7.4 ± 2.7	4.0 ± 1.2	NA
8g		34.3 ± 1.0	NA	NA	NA
8h		13.5 ± 1.8	NA	NA	NA
9		11.7 ± 1.3	33.8 ± 1.3	NA	9.9 ± 1.8
10		90.4 ± 3.2	83.4 ± 5.4	91.7 ± 2.7	86.2 ± 5.6
doxorubicin[b]		89.7 ± 5.4	87.1 ± 4.2	89.2 ± 4.7	90.6 ± 3.8

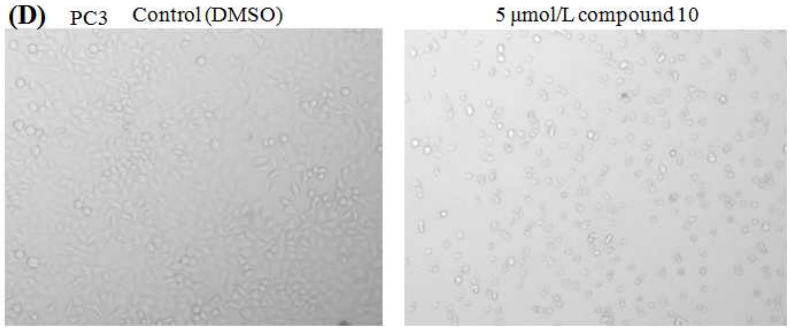
[a] No activity; [b] Used as a positive contro1.

The relation between concentration and inhibition was analyzed to calculate the IC_{50} values of selected compounds **6g** and **10** (Figure 3A). The results indicated a dose-dependent trend of the inhibitory response of compound **6g** on HEL cells and compound **10** on PC3, MDA, HEL and K562 cells. The IC_{50} values are summarized in Table 2 and showed that the cytotoxicity of compound **10** on all cancer cells was close to that of the positive control (doxorubicin). In contrast, the cytotoxicity of compound **6g** on HEL cells was lower than that of doxorubicin ($P < 0.05$). Microscopical observation indicated morphological changes of the cancer cells (Figure 3B-E), we found the numbers of HEL cells **(**Figure 3B) were significantly reduced after the treatment of 5 μmol/L of compounds **6g** or **10**. In addition, a reduced number of apoptotic bodies were found when compared to the control. Compared to the control, an increase in apoptotic bodies, rounder cells and an increased intercellular space were found in MDA cells (Figure 3C), PC3 cells (Figure 3D) and K562 cells (Figure 3E) after treatment with compound **10.** In addition, spindle-like untreated MDA cells and PC3 cells easily adhered, and demonstrated a clear cell membrane, which indicated that MDA cells and PC3 cells underwent apoptosis after treatment with compound **10**.


(A)
10-PC3
10-HEL
10-K562
10-MDA
6g-HEL
Inhibition (%)
120
100
80
60
40
20
0
1.25
2.5
5
10
20
40
Concentration (10-6mol/L)


(B)
HEL Control (DMSO)
5 μmol/L compound 6g
5 μmol/L compound 10


(C) MDA Control (DMSO)
5 μmol/L compound 10


(D) PC3 Control (DMSO)
5 μmol/L compound 10

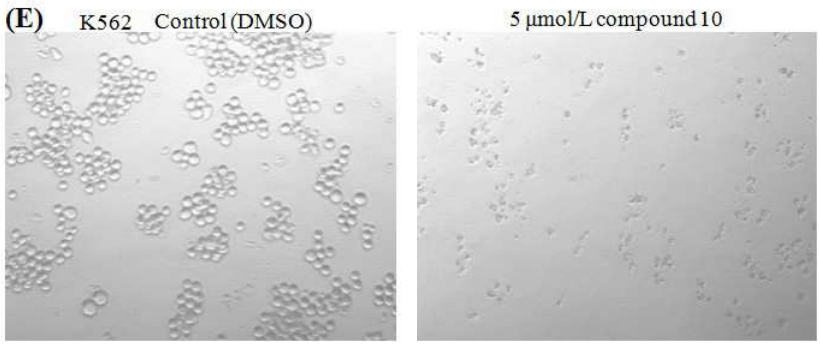


Figure 3. (A) Inhibition on growth of cancer cells *in vitro* after treatment with compounds. (B-E) Morphological changes of cancer cell lines HEL (B), MDA (C), PC3 (D) and K562 (E) Induced by treatment with 5 μmol/L of tested compound for 48 h, morphological changes were observed by an inverted microscope.

Table 2

Cytotoxicity screening of selected compounds *in vitro*.

Compd	IC_{50}/ μM			
	PC3	**MDA**	**HEL**	**PC3**
6g	ND[a]	ND	4.35 ± 0.84*	ND
10	2.92 ± 0.47	3.14 ± 0.35	1.85 ± 0.28	2.64 ± 0.18
doxorubicin[b]	2.63 ± 0.38	2.25 ± 0.42	2.13 ± 0.18	2.41 ± 0.39

[a] Not determined; [b] Used as a positive contro1; *$P < 0.05$, versus the control group.

Cells were treated with 5 μmol/L of compound **10** for 48 h at 37 ºC and then cells were harvested and stained with annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (PI). Cells were analyzed by flow cytometry to confirm that cancer cells underwent apoptosis. Four cancer cell lines that were treated with compound **10** showed a significant increase in apoptosis when compared to untreated cells ($P < 0.01$, Figure 4A and B). This was especially true for PC3 cells and MDA cells. At 5 μmol/L, compound **6g** displayed a lower apoptosis rate, whereas at 10 μmol/L, a higher apoptosis rate was observed in HEL cells ($P < 0.01$, Figure 5A and B).

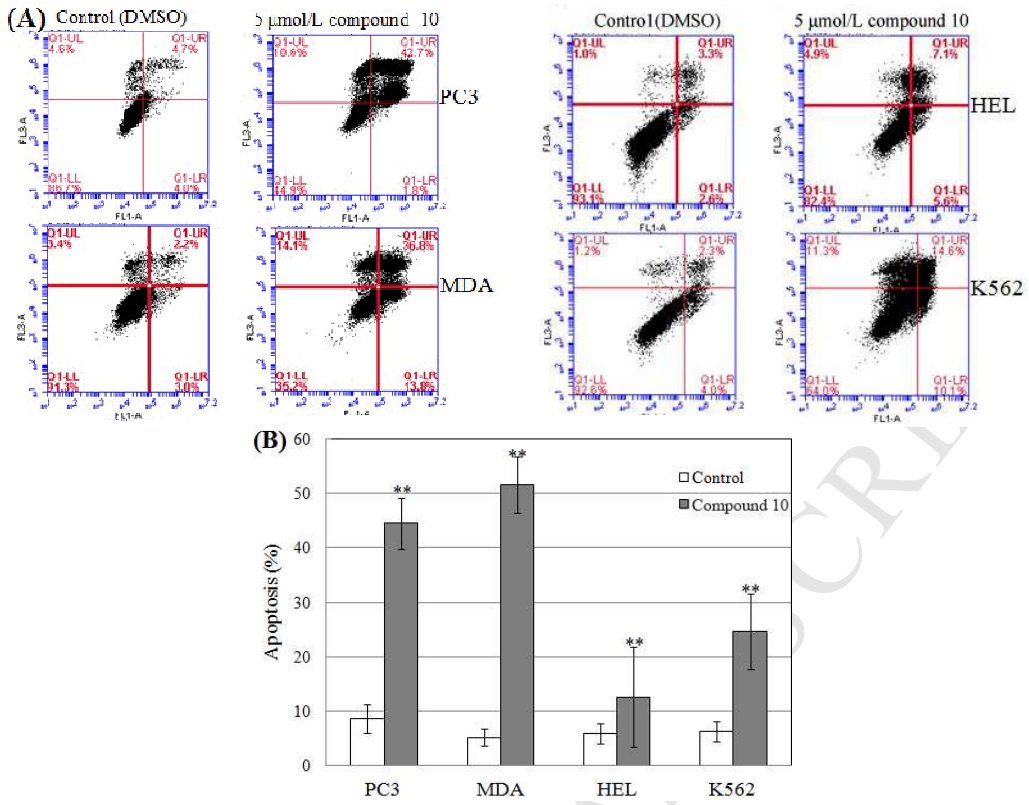
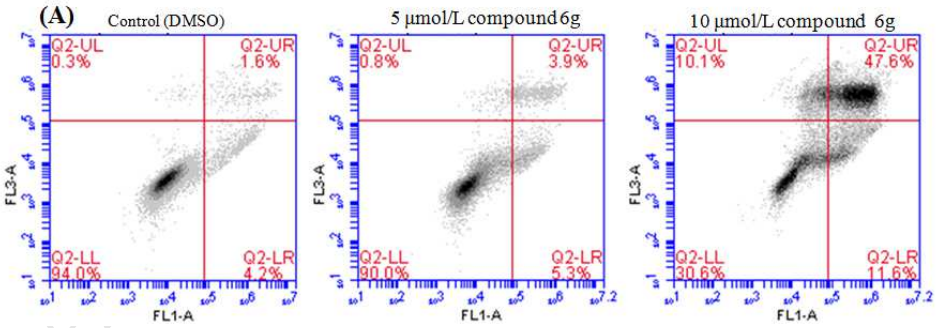


Figure 4. (A) Flow cytometric histograms of apoptosis induced by compound **10** in four cancer cell lines**.** After treatment for 48 h with 5 μmol/L of compound **10**, cells were harvested, stained with annexin-V-FITC and PI and analyzed by flow cytometry. (B) Compound **10**-induced apoptosis in the investigated cell lines compared to DMSO-treated control cells. Data show the percentages of early and late apoptotic cells (right quadrants) and present the mean ± SD of three independent experiments. $^{*}P < 0.05$; $^{**}P < 0.01$, versus the control group.



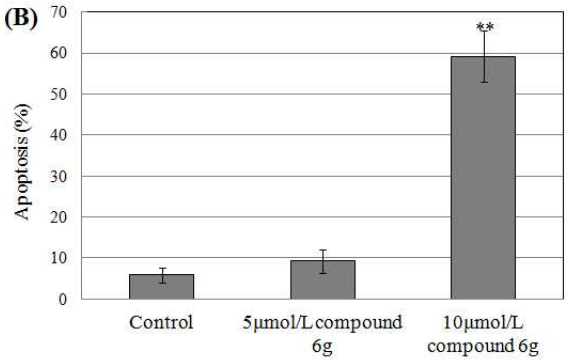
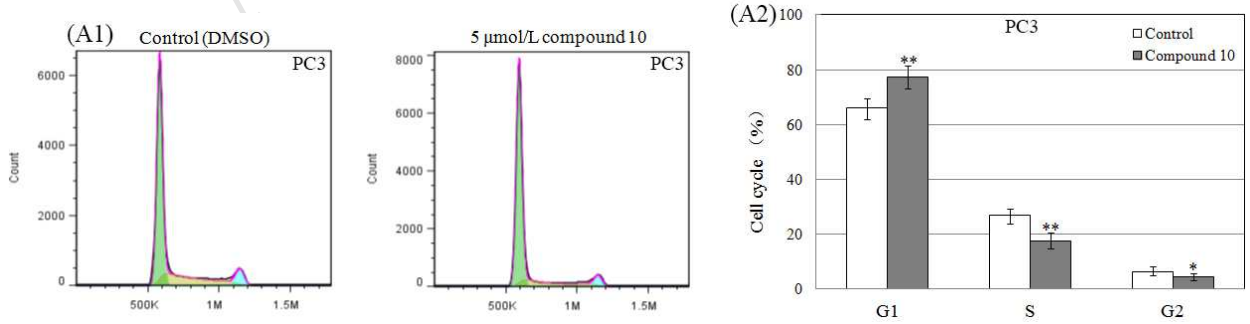


Figure 5. (A) Flow cytometric histograms of apoptotic HEL cells, 48 h after treatment with 5 μmol/L or 10 μmol/L of compound **6g**. Cells were harvested, stained with annexin-V-FITC and PI and analyzed by flow cytometry. (B) Compound **6g**-induced apoptosis in HEL cells compared to DMSO-treated control cells. Data show the percentages of early and late apoptotic cells (right quadrants) and present the mean ± SD of three independent experiments. $^{*}P < 0.05$; $^{**}P < 0.01$, versus the control group.

2.3 Effects of active compounds on cell cycle phase arrest of cancer cells

The effects of two selected compounds **6g** and **10** on cell cycle phase arrest in cancer cells were evaluated by flow cytometry. After treatment for 48 h with 5 μmol/L of compound **6g** or **10**, cells were harvested and analyzed by flow cytometry. Results showed that in PC3 cells, compound **10** can prolong the G1 phase ($P < 0.01$), shorten the S phase ($P < 0.01$) and G2 phase ($P < 0.05$) compared to the DMSO-treated control cells (Figure 6A1 and A2). Cell cycle phase arrest of compound **10** in MDA cells differed from that in PC3 cells, compound **10** only shortened the S phase ($P < 0.01$) and prolonged the G2 phase ($P < 0.01$) of MDA cells (Figure 6B1 and B2). By contrast, compound **10** can significantly reduce G1 ($P < 0.01$) and slightly shorten G2 phases ($P < 0.05$) of K562 cells (Figure 6C1 and C2), and noticeably prolong the G1 phase ($P < 0.01$) and considerably shorten the S phase ($P < 0.01$) of HEL cells (Figure 6D1 and D2). Compound **6g** slightly prolonged the G1 phase ($P < 0.05$) and shortened the G2 phase ($P < 0.05$) of HEL cells (Figure 7A1 and A2). The above results showed that compound **10** caused cell cycle phase arrest in several cancer cells, indicating that the anti-cancer activity of compound **10** may be involved in the regulation of cell cycle-associated genes.



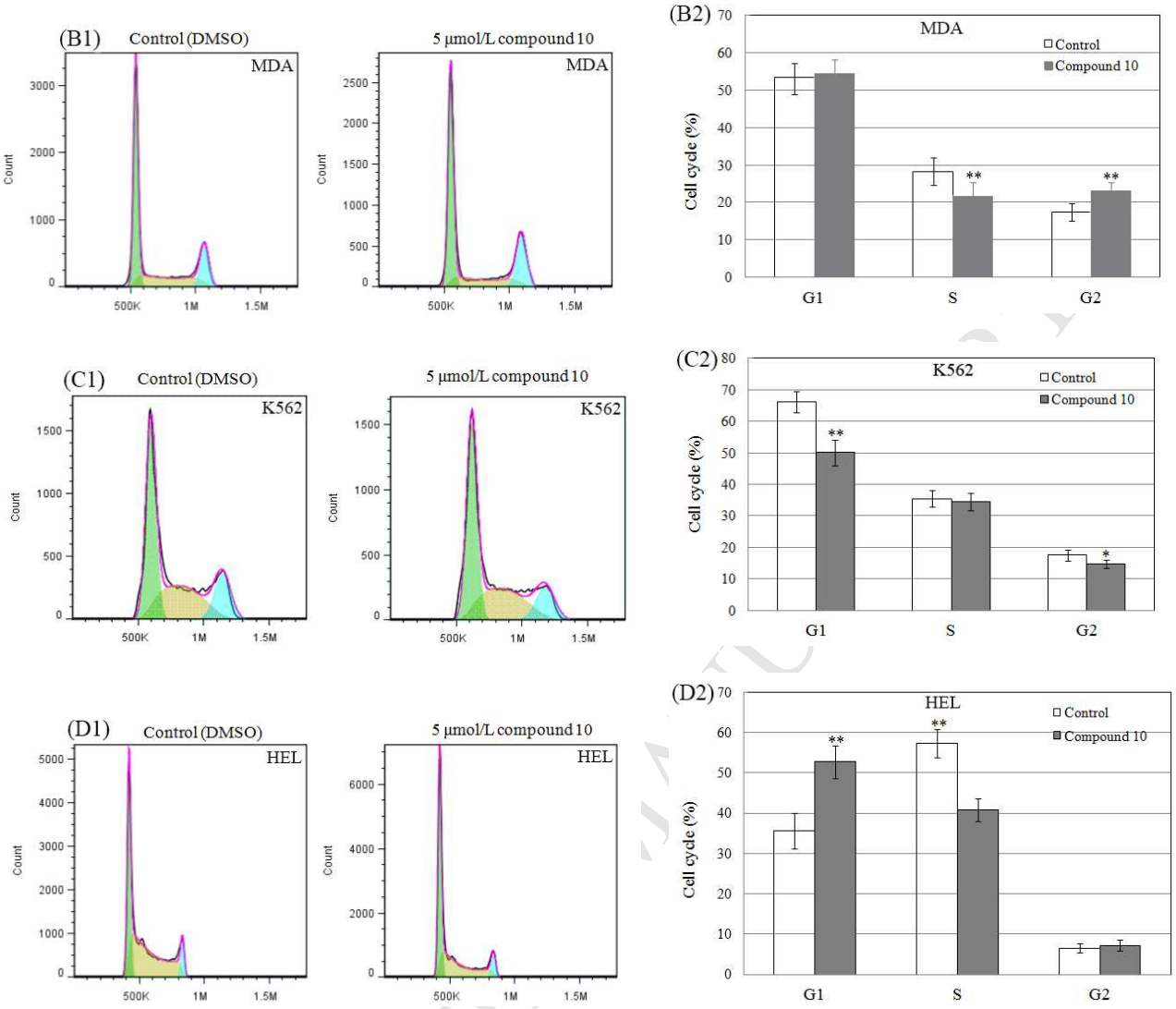


Figure 6. Effects of compound **10** on cell cycle phase arrest in PC3 (A1), MDA (B1), K562 (C1) and HEL (D1) cells. Cells were treated for 48 h with 5 μmol/L of compound **10**. Cells were then fixed and stained with PI to analyze the DNA content by flow cytometry. Percentages of PC3 (A2), MDA (B2), K562 (C2) and HEL (D2) cells in different phases of the cell cycle. Data are presented as the mean ± SD from three independent experiments. *$P < 0.05$; **$P < 0.01$, versus the control group.

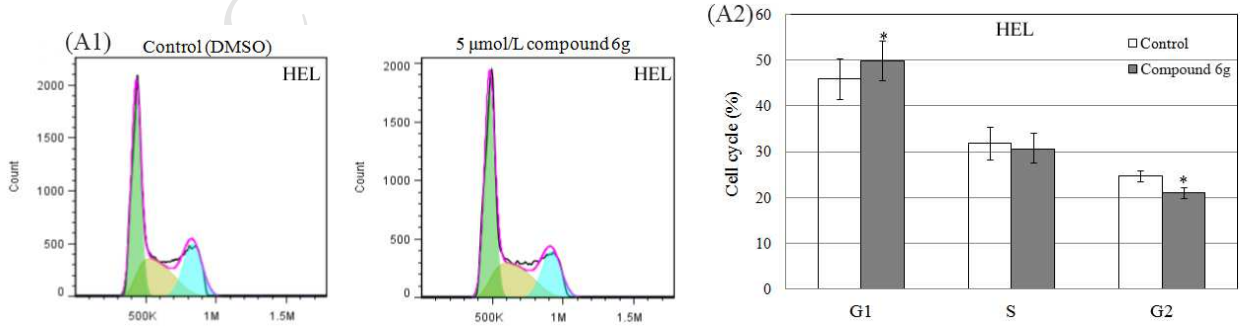


Figure 7. (A1) Effects of compound **6g** on cell cycle phase arrest in HEL cells. Cells were treated for 48 h with 5 μmol/L of compound **6g**. Cells were fixed and stained with PI to analyze the DNA content by flow cytometry. (A2) Percentages of cells in the different phases of the cell cycle. Data are presented as the mean ± SD from three

independent experiments. *$P < 0.05$; **$P < 0.01$, versus the control group.

2.4 Structure-activity relationship studies

To obtain a series of analogs, the aromatic ring B of 4,4'-dimethyl medicagenin (**6c**) was modified. Based on the cytotoxicity results (Table 1-2), preliminary structure-activity relationship could be drawn. Replacement of the ring B of compound **6c** with 3-pyridinyl resulted in greatly improved cytotoxicity in all cancer cell lines evaluated, the improvement was approximately three-four fold compared to the standard compound **6c**. While the replacement of ring B of compound **6c** with N-methyl-2-pyrrolyl only slightly improved cytotoxicity in MDA cells. Changing the substituents on ring B of compound **6c** was detrimental to activity versus erythroleukemia cells (HEL and K562), only addition of a nitro group to position 3 of compound **6c** could increase cytotoxic activity in HEL cells. Molecules bearing electron-donating groups, such as in compounds **6b**, **6f** and **7b**, show a better inhibitory effect in MDA cells, whereas the introduction of electron-withdrawing groups to position 4 of compound **7b** (compounds **8e**-**8h,** respectively) resulted in decreased cytotoxicity in MDA cells. Addition of a group to position 3 of compound **6c** slightly improved cytotoxicity in MDA cells, such as in compounds **6d**, **6g**, **6j** and **6k**. However, this structural change was not associated with improved cytotoxic activity in PC3 cells. Cumulatively, the evaluation of the compounds mentioned above is indicative of a greater preference for the structural modification of 4,4'-dimethyl medicagenin (**6c**) in relation to cytotoxicity. Therefore, the best-performing new compound **10** may be selected as a lead compound that further explored in future.

3 Conclusion

In this study, a library of novel 3′,5′-diprenylated chalcones as analogs of 4,4'-dimethyl medicagenin (**6c**) were synthesized. Cytotoxicity of these target compounds was evaluated by MTT assays, which indicated that compound **10** possessed broad-spectrum cytotoxicity in PC3, MDA, HEL and K562 cells with IC_{50} values of 2.92, 3.14, 1.85 and 2.64 μM, respectively. Further studies indicated that compound **10** induced apoptosis and arrested cell cycle phase of PC3, MDA, HEL and K562 cells. By contrast, compound **6g** selectively displayed potent inhibitory activity against proliferation of HEL cells with an IC_{50} value of 4.35 μM. Compound **6g** slightly induced apoptosis and arrested cell cycle phase of HEL cells. Preliminary structure-activity relationship studies indicated that, in all cancer cell lines evaluated, the 3-pyridinyl group was essential for cytotoxicity.

4 Experimental section

4.1 General

Mass spectra (MS) were obtained on an electrospray ionization (ESI) mode on HP-5793 mass

spectrometer (Hewlett-Packard, USA). Nuclear magnetic resonance (NMR) spectra were recorded on INOVA-400 (^{1}H NMR, 400 MHz; ^{13}C NMR, 100 MHz, Varian Unit, USA) or WNMR-I (^{1}H NMR, 500 MHz; ^{13}C NMR, 125 MHz, WIPM, China) with TMS as an internal standard. Melting points (mp) were determined on an X-4 microscope melting point apparatus. Column chromatography was performed on silica gel (300-400 mesh) and thin-layer chromatography (TLC) analysis was carried out on silica gel plates GF_{254} purchased from Qingdao Ocean Chemical Reagent Co. (Qingdao, China). Other reagents were analytical grade or guaranteed reagent commercial product and used without further purification, unless otherwise noted.

4.2 Chemical synthesis

*4.2.1 Synthesis of 4'-methoxy-2'-((2-methylbut-3-yn-2-yl)oxy)acetophenone (**2**)*

To a solution of 2-hydroxy-4-methoxyacetylbenzene **1** (10.11 g, 59.62 mmol) in 65 mL CH_3CN, $CuCl_2$ (43.0 mg, 0.06 mmol) was added, after stirring at -5 ºC for 20 min, the 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 13.65 mL, 89.43 mmol) followed by 3-chloro-3-methylbutyne (10.60 mL, 89.43 mmol) were added dropwise. Then the mixture was stirred further 11 h at -5 ºC, ice concentrated hydrochloric acid was added to adjust pH = 2. After removal of solvent, the residue was poured into 100 mL water, and the light gray precipitate was collected by suction filtration, washed with petroleum ether and dried to obtain **2** (12.05 g, 87%). ESI-MS *m/z*: 255.1 $[M + Na]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.75 (d, *J* = 8.8 Hz, 1H), 7.18 (d, *J* = 2.4 Hz, 1H), 6.59 (dd, *J* = 8.8, 2.4 Hz, 1H), 3.83 (s, 3H), 2.68 (s, 1H), 2.57 (s, 3H), 1.74 (s, 6H).

*4.2.2 Synthesis of 2'-hydroxy-4'-methoxy-3'-prenylacetophenone (**3**)*

To a solution of **2** (11.59 g, 49.90 mmol) in 20 mL ethyl acetate was added 5% $Pd/CaCO_3$ (1.16 g, 10% wt. of **2**). Three purges of vacuum/argon followed by three purges of vacuum/H_2 were performed. After stirring for 12 h at room temperature under hydrogen, the reaction mixture was filtered through diatomite and the filtrate was concentrated, the obtained product was used in the next reaction without further purification. The previous crude product (6.19 g) was dissolved in 30 mL *N*, *N*-diethylaniline and heated at 120 ºC for 45 h under argon. The reaction mixture was cooled to room temperature, 100 mL 1 M dilute hydrochloric acid was added and the product was extracted with ethyl acetate, and the organic phase was washed with brine, dried over $MgSO_4$. Then the solvent was evaporated under reduced pressure, the obtained residue was purified by column chromatography on silica gel (1:35 ethyl acetate–petroleum ether) to afford **3** (4.8 g, 71% yield, over two steps) as yellow oil. ESI-MS *m/z*: 257.0 $[M + Na]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 12.72 (s, 1H), 7.60 (d, *J* = 8.9 Hz, 1H), 6.46 (d, *J* = 8.9 Hz, 1H), 5.22 – 5.17 (m, 1H), 3.90 (s, 3H), 3.36 (s, 2H), 2.58 (s, 3H), 1.79 (s, 3H), 1.68 (s, 3H).

*4.2.3 Synthesis of 4'-methoxy-2'-prenoxy-3'-prenylacetophenone (**4**)*

To a mixture of **3** (5.74 g, 24.51 mmol) and anhydrous K_2CO_3 (7.10 g，51.0 mmol) in 18 mL of anhydrous DMF, prenyl bromide (6.5 mL, 50.25 mmol) was added under argon. The contents were stirred at 50 ºC for 30 h, after cooling to room temperature, the reaction mixture was poured into water and extracted with ethyl acetate, and the organic phase was washed with brine, dried over $MgSO_4$. After removal of solvent, the obtained residue was purified by column chromatography on silica gel (1:60 ethyl acetate–petroleum ether) to afford **4** (5.3 g, 72% yield) as light yellow oil. ESI-MS *m/z*: 325.0 $[M + Na]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.55 (d, *J* = 8.7 Hz, 1H), 6.68 (d, *J* = 8.7 Hz, 1H), 5.54 – 5.47 (m, 1H), 5.19 – 5.12 (m, 1H), 4.30 (d, *J* = 7.1 Hz, 2H), 3.86 (s, 3H), 3.38 (d, *J* = 6.7 Hz, 2H), 2.59 (s, 3H), 1.76 (s, 6H), 1.67 (d, *J* = 1.1 Hz, 3H), 1.59 (s, 3H).

*4.2.4 Synthesis of 2'-hydroxy-4'-methoxy-3',5'-diprenylacetophenone (**5**)*

A solution of **4** (5.30 g, 17.53 mmol) in 30 mL *N*,*N*-diethylaniline was heated at 170 ºC for 24 h under argon. The reaction mixture was cooled to room temperature, 100 mL 1 M dilute hydrochloric acid was added and the product was extracted with ethyl acetate, and the organic phase was washed with brine, dried over $MgSO_4$. After removal of solvent, the obtained residue was purified by column chromatography on silica gel (1:60 ethyl acetate–petroleum ether) to give **5** (2.83 g, 53% yield) as yellow oil. ESI-MS *m/z*: 325.1 $[M + Na]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.40 (s, 1H), 5.29 – 5.19 (m, 2H), 3.76 (s, 3H), 3.38 (d, *J* = 6.7 Hz, 2H), 3.30 (d, *J* = 7.2 Hz, 2H), 2.57 (s, 3H), 1.79 (s, 3H), 1.76 (s, 3H), 1.73 (s, 3H), 1.68 (d, *J* = 1.1 Hz, 3H).

*4.2.5 Synthesis of compounds **6a-6k**, **9** and **10***

*4.2.5.1 (E)-2'-Hydroxy-3,4'-dimethoxy-4-methoxymethoxy-3',5'-diprenylchalcone (**6a**)*

To solution of **5** (1.20 g, 3.97 mmol) and 3-methoxy-4-(methoxymethoxy) benzaldehyde (1.17 g, 5.96 mmol) in 14 mL ethanol, NaOH (2.48 g, 37.57 mmol) was added and the reaction mixture was stirred at room temperature for 41 h. After neutralized the excessive NaOH with 1 M dilute hydrochloric acid, the resulting mixture was extracted with ethyl acetate, and the organic phase was washed with brine, dried over $MgSO_4$. After removal of solvent under reduced pressure, the obtained residue was purified by column chromatography on silica gel (2:3 chloroform–petroleum ether) to give **6a** (714.7 mg, 37% yield) as yellow oil. ESI-MS *m/z*: 503.0 $[M + Na]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 13.27 (s, 1H), 7.84 (d, *J* = 15.4 Hz, 1H), 7.58 (s, 1H), 7.46 (d, *J* = 15.4 Hz, 1H), 7.24 (d, *J* = 8.7 Hz, 1H), 7.20 (d, *J* = 8.3 Hz, 1H), 7.17 (s, 1H), 5.33 – 5.23 (m, 4H), 3.97 (s, 3H), 3.78 (s, 3H), 3.53 (s, 3H), 3.42 (d, *J* = 6.5 Hz, 2H), 3.34 (d, *J* = 7.0 Hz, 2H), 1.81 (s, 3H), 1.79 (s, 3H), 1.76 (s, 3H), 1.70 (s, 3H).

*4.2.5.2 (E)-3-Ethoxy-2'-hydroxy-4-methoxymethoxy-4'-methoxy-3',5'-diprenylchalcone (**6b**)*

6b was obtained from **5** and 3-ethoxy-4-(methoxymethoxy) benzaldehyde as described for the

synthesis of **6a**: 53% yield as yellow oil. ESI-MS *m/z*: 517.3 $[M + Na]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 13.27 (s, 1H), 7.82 (d, *J* = 15.4 Hz, 1H), 7.58 (s, 1H), 7.45 (d, *J* = 15.4 Hz, 1H), 7.22 (dd, *J* = 8.4, 1.6 Hz, 1H), 7.19 (d, *J* = 8.3 Hz, 1H), 7.17 (d, *J* = 1.5 Hz, 1H), 5.33 – 5.23 (m, 4H), 4.18 (q, *J* = 7.0 Hz, 2H), 3.78 (s, 3H), 3.54 (s, 3H), 3.42 (d, *J* = 6.5 Hz, 2H), 3.35 (d, *J* = 7.1 Hz, 2H), 1.81 (s, 3H), 1.80 (s, 3H), 1.77 (s, 3H), 1.70 (s, 3H), 1.51 (t, *J* = 7.0 Hz, 3H).

*4.2.5.3 (E)-2'-Hydroxy-4,4'-dimethoxy-3',5'-diprenylchalcone (**6c**)*

6c was obtained from **5** and 4-methoxybenzaldehyde as described for the synthesis of **6a**: 56% yield as yellow oil. ESI-MS *m/z*: 443.2 $[M + Na]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 13.32 (s, 1H), 7.86 (d, *J* = 15.4 Hz, 1H), 7.62 (s, 1H), 7.60 (s, 1H), 7.59 (s, 1H), 7.47 (d, *J* = 15.4 Hz, 1H), 6.97 (s, 1H), 6.95 (d, *J* = 1.9 Hz, 1H), 5.34 – 5.23 (m, 2H), 3.87 (s, 3H), 3.78 (s, 3H), 3.42 (d, *J* = 6.6 Hz, 2H), 3.34 (d, *J* = 7.0 Hz, 2H), 1.81 (s, 3H), 1.79 (s, 3H), 1.77 (s, 3H), 1.70 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 192.94, 166.34, 162.96, 162.36, 161.93, 144.56, 133.00, 132.03, 130.50, 128.42, 127.65, 125.56, 123.77, 123.17, 122.63, 118.24, 116.74, 114.60, 61.41, 55.58, 28.36, 25.92, 25.88, 23.10, 18.13, 18.10.

*4.2.5.4 (E)-2'-Hydroxy-3,4,4'-trimethoxy-3',5'-diprenylchalcone (**6d**)*

6d was obtained from **5** and 3,4-dimethoxybenzaldehyde as described for the synthesis of **6a**: 32% yield as yellow oil. ESI-MS *m/z*: 473.2 $[M + Na]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 13.30 (s, 1H), 7.84 (d, *J* = 15.3 Hz, 1H), 7.59 (s, 1H), 7.45 (d, *J* = 15.4 Hz, 1H), 7.26 – 7.24 (m, 1H), 7.16 (d, *J* = 1.7 Hz, 1H), 6.92 (d, *J* = 8.3 Hz, 1H), 5.31 (t, *J* = 7.1 Hz, 1H), 5.26 (t, *J* = 6.6 Hz, 1H), 3.97 (s, 3H), 3.95 (s, 3H), 3.78 (s, 3H), 3.42 (d, *J* = 6.5 Hz, 2H), 3.35 (d, *J* = 7.1 Hz, 2H), 1.81 (s, 3H), 1.79 (s, 3H), 1.76 (s, 3H), 1.70 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 192.81, 162.99, 162.39, 151.68, 149.35, 144.81, 133.10, 132.08, 128.32, 127.90, 125.47, 123.80, 123.50, 123.10, 122.59, 118.43, 116.70, 111.25, 110.20, 61.40, 56.18, 56.03, 28.23, 25.92, 25.90, 23.10, 18.12.

*4.2.5.5 (E)-2'-Hydroxy-3,4,4',5-tetramethoxy-3',5'-diprenylchalcone (**6e**)*

6e was obtained from **5** and 3,4,5-trimethoxybenzaldehyde as described for the synthesis of **6a**: 43% yield as yellow oil. ESI-MS *m/z*: 503.3 $[M + Na]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 13.23 (s, 1H), 7.80 (d, *J* = 15.4 Hz, 1H), 7.58 (s, 1H), 7.46 (d, *J* = 15.4 Hz, 1H), 6.87 (s, 2H), 5.33 – 5.28 (m, 1H), 5.28 – 5.24 (m, 1H), 3.94 (s, 6H), 3.91 (s, 3H), 3.79 (s, 3H), 3.42 (d, *J* = 6.6 Hz, 2H), 3.35 (d, *J* = 7.1 Hz, 2H), 1.81 (s, 3H), 1.79 (s, 3H), 1.76 (s, 3H), 1.70 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 192.66, 163.14, 162.44, 153.59, 144.74, 140.63, 133.17, 132.10, 130.39, 128.30, 125.51, 123.84, 123.01, 122.52, 119.90, 116.60, 105.79, 61.37, 61.16, 56.28, 28.14, 25.88, 23.06, 18.08.

*4.2.5.6 (E)-4-Allyloxy-3-ethoxy-2'-hydroxy-4'-methoxy-3',5'-diprenylchalcone (**6f**)*

6f was obtained from **5** and 4-allyloxy-3-ethoxybenzaldehyde as described for the synthesis of

6a: 60% yield as yellow powder, m.p. 43-45 ºC. ESI-MS *m/z*: 513.2 $[M + Na]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 13.30 (s, 1H), 7.82 (d, *J* = 15.3 Hz, 1H), 7.58 (s, 1H), 7.43 (d, *J* = 15.4 Hz, 1H), 7.21 (dd, *J* = 8.3, 1.8 Hz, 1H), 7.18 (d, *J* = 1.8 Hz, 1H), 6.92 (d, *J* = 8.3 Hz, 1H), 6.09 (ddt, *J* = 17.1, 10.5, 5.2 Hz, 1H), 5.44 (dd, *J* = 17.3, 1.4 Hz, 1H), 5.34 – 5.28 (m, 2H), 5.28 – 5.24 (m, 1H), 4.68 (dd, *J* = 3.9, 1.4 Hz, 2H), 4.17 (q, *J* = 7.0 Hz, 2H), 3.78 (s, 3H), 3.42 (d, *J* = 6.6 Hz, 2H), 3.35 (d, *J* = 7.1 Hz, 2H), 1.81 (s, 3H), 1.80 (s, 3H), 1.77 (s, 3H), 1.71 (s, 3H), 1.51 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 192.83, 162.97, 162.38, 151.09, 149.06, 144.85, 132.98, 132.05, 128.33, 128.10, 125.46, 123.78, 123.23, 123.10, 122.60, 118.42, 118.18, 116.71, 113.49, 112.52, 69.87, 64.78, 61.38, 28.22, 25.88, 23.08, 18.10, 14.94.

*4.2.5.7 (E)-2'-Hydroxy-4,4'-dimethoxy-3-nitro-3',5'-diprenylchalcone (**6g**)*

6g was obtained from **5** and 4-methoxy-3-nitrobenzaldehyde as described for the synthesis of **6a**: 16% yield as yellow oil. ESI-MS *m/z*: 464.2 $[M - H]^-$; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 13.10 (s, 1H), 8.18 (d, J = 2.1 Hz, 1H), 7.80 (d, J = 15.7 Hz, 1H), 7.77 (dd, J = 9.2, 2.6 Hz, 1H), 7.57 (s, 1H), 7.53 (d, J = 15.5 Hz, 1H), 7.16 (d, J = 8.7 Hz, 1H), 5.33 – 5.22 (m, 2H), 4.03 (s, 3H), 3.79 (s, 3H), 3.42 (d, J = 6.7 Hz, 2H), 3.35 (d, J = 7.0 Hz, 2H), 1.81 (s, 3H), 1.79 (s, 3H), 1.77 (s, 3H), 1.70 (s, 3H). ^{13}C NMR (100 MHz, CDCl3) δ 192.21, 163.44, 162.54, 154.43, 141.54, 134.90, 133.25, 132.21, 128.47, 127.60, 125.99, 124.96, 123.95, 122.98, 122.43, 121.09, 116.47, 114.01, 61.46, 56.95, 32.07, 29.85, 29.52, 28.38, 25.91, 23.08, 22.85, 18.16, 18.12, 14.30.

*4.2.5.8 (E)-2'-Hydroxy-4-dimethylamino-4'-methoxy-3',5'-diprenylchalcone (**6h**)*

6h was obtained from **5** and 4-dimethylaminobenzaldehyde as described for the synthesis of **6a**: 12% yield as yellow powder, m.p. 118-120 ºC. ESI-MS *m/z*: 456.1 $[M + Na]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 13.52 (s, 1H), 7.87 (d, *J* = 15.2 Hz, 1H), 7.59 (s, 1H), 7.56 (d, *J* = 8.8 Hz, 2H), 7.38 (d, *J* = 15.2 Hz, 1H), 6.72 (d, *J* = 8.8 Hz, 2H), 5.34 – 5.25 (m, 2H), 3.78 (s, 3H), 3.42 (d, *J* = 6.5 Hz, 2H), 3.35 (d, *J* = 7.0 Hz, 2H), 3.07 (s, 6H), 1.81 (s, 3H), 1.80 (s, 3H), 1.78 (s, 3H), 1.71 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 192.89, 162.54, 162.29, 162.23, 152.26, 145.70, 132.90, 131.93, 130.76, 128.27, 125.24, 123.62, 123.30, 122.79, 116.98, 115.07, 111.97, 61.39, 40.31, 28.35, 25.94, 25.89, 23.11, 18.12.

*4.2.5.9 (E)-3-Fluoro-2'-hydroxy-4,4'-dimethoxy-3',5'-diprenylchalcone (**6i**)*

6i was obtained from **5** and 3-fluoro-4-methoxybenzaldehyde as described for the synthesis of **6a**: 40% yield as yellow powder, m.p. 62-64 ºC. ESI-MS *m/z*: 461.2 $[M + Na]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.79 (d, *J* = 15.4 Hz, 1H), 7.57 (s, 1H), 7.47 – 7.39 (m, 2H), 7.36 (d, *J* = 8.6 Hz, 1H), 7.00 (t, *J* = 8.5 Hz, 1H), 5.28 (mf, *J* = 13.4, 4.7 Hz, 2H), 3.95 (s, 3H), 3.78 (s, 3H), 3.41 (d, *J* = 6.5 Hz, 2H), 3.34 (d, *J* = 6.9 Hz, 2H), 1.80 (s, 3H), 1.79 (s, 3H), 1.77 (s, 3H), 1.70 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 192.63, 163.16, 162.43, 153.51, 151.54, 149.95, 143.38, 133.09,

132.12, 128.43, 128.13, 126.53, 125.76, 123.85, 123.09, 122.54, 119.52, 116.62, 114.97, 114.82, 113.26, 61.43, 56.39, 28.37, 25.91, 25.88, 23.08, 18.14, 18.10.

*4.2.5.10 (E)-3-Chloro-2'-hydroxy-4,4'-dimethoxy-3',5'-diprenylchalcone (**6j**)*

6j was obtained from **5** and 3-chloro-4-methoxybenzaldehyde as described for the synthesis of **6a**: 47% yield as yellow powder, m.p. 101-103 ºC. ESI-MS *m/z*: 477.0 $[M + Na]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 13.20 (s, 1H), 7.77 (d, *J* = 15.4 Hz, 1H), 7.71 (d, *J* = 1.8 Hz, 1H), 7.58 (s, 1H), 7.49 (dd, *J* = 8.5, 1.7 Hz, 1H), 7.46 (d, *J* = 15.4 Hz, 1H), 6.97 (d, *J* = 8.5 Hz, 1H), 5.32 – 5.28 (m, 1H), 5.28 – 5.23 (m, 1H), 3.97 (s, 3H), 3.79 (s, 3H), 3.42 (d, *J* = 6.6 Hz, 2H), 3.35 (d, *J* = 6.9 Hz, 2H), 1.81 (s, 3H), 1.80 (s, 3H), 1.78 (s, 3H), 1.70 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 192.61, 163.20, 162.44, 156.99, 142.98, 133.11, 132.09, 129.63, 129.44, 128.49, 128.47, 125.73, 123.85, 123.42, 123.11, 122.56, 119.55, 116.62, 112.16, 61.41, 56.45, 29.83, 28.38, 25.88, 23.08, 18.14, 18.09, 18.04.

*4.2.5.11 (E)-3-Bromo-2'-hydroxy-4,4'-dimethoxy-3',5'-diprenylchalcone (**6k**)*

6k was obtained from **5** and 3-bromo-4-methoxybenzaldehyde as described for the synthesis of **6a**: 32% yield as yellow powder, m.p. 120-122 ºC. ESI-MS *m/z*: 522.0 $[M + Na]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.91 – 7.88 (m, 1H), 7.77 (d, *J* = 15.4 Hz, 1H), 7.58 (s, 1H), 7.54 (dd, *J* = 8.5, 1.8 Hz, 1H), 7.46 (d, *J* = 15.4 Hz, 1H), 6.94 (d, *J* = 8.5 Hz, 1H), 5.33 – 5.21 (m, 1H), 3.96 (s, 3H), 3.78 (s, 3H), 3.41 (d, *J* = 6.5 Hz, 2H), 3.35 (d, *J* = 6.9 Hz, 2H), 1.80 (s, 6H), 1.77 (s, 3H), 1.70 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 192.59, 163.18, 162.44, 157.84, 142.84, 133.14, 132.77, 132.12, 130.20, 128.97, 128.47, 125.73, 123.84, 123.10, 122.54, 119.50, 116.61, 112.57, 111.97, 61.42, 56.56, 28.37, 25.91, 25.89, 23.08, 18.15, 18.11.

*4.2.5.12 (E)-1-(2-Hydroxy-4-methoxy-3,5-diprenyl)phenyl-3-(N-methyl-2-pyrrolyl)-pro- pene-1-one (**9**)*

9 was obtained from **5** and *N*-methyl-pyrrole-2-carbaldehyde as described for the synthesis of **6a**: 58% yield as yellow oil. ESI-MS *m/z*: 416.1 $[M + Na]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 13.49 (s, 1H), 7.86 (d, *J* = 15.0 Hz, 1H), 7.55 (s, 1H), 7.32 (d, *J* = 15.0 Hz, 1H), 6.87 (dd, *J* = 4.0, 1.2 Hz, 1H), 6.85 (d, *J* = 2.2 Hz, 1H), 6.25 (dd, *J* = 3.5, 2.7 Hz, 1H), 5.33 – 5.24 (m, 2H), 3.79 (s, 3H), 3.78 (s, 3H), 3.41 (d, *J* = 6.6 Hz, 2H), 3.34 (d, *J* = 7.1 Hz, 2H), 1.81 (s, 3H), 1.79 (s, 3H), 1.76 (s, 3H), 1.70 (d, *J* = 0.8 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 192.43, 162.67, 162.21, 133.05, 132.09, 131.95, 130.44, 128.35, 128.09, 125.34, 123.65, 123.11, 122.72, 116.79, 115.31, 112.91, 110.12, 61.37, 34.56, 28.23, 25.91, 25.88, 23.08, 18.10.

*4.2.5.13 (E)-1-(2-Hydroxy-4-methoxy-3,5-diprenyl)phenyl-3-(3-pyridinyl)-propene-1-one (**10**)*

10 was obtained from **5** and nicotinaldehyde as described for the synthesis of **6a**: 59% yield as yellow oil. ESI-MS *m/z*: 414.0 $[M + Na]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 12.77 (s, 1H), 8.56

(d, *J* = 1.3 Hz, 1H), 8.35 – 8.31 (m, 1H), 7.63 (d, *J* = 7.9 Hz, 1H), 7.54 (d, *J* = 15.6 Hz, 1H), 7.34 (d, *J* = 15.6 Hz, 1H), 7.07 (dd, *J* = 7.8, 4.8 Hz, 1H), 5.00 – 4.92 (m, 2H), 3.48 (s, 3H), 3.11 (d, *J* = 6.6 Hz, 2H), 3.04 (d, *J* = 7.0 Hz, 2H), 1.51 (s, 3H), 1.48 (s, 3H), 1.46 (s, 3H), 1.40 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm):192.19, 163.48, 162.54, 151.24, 150.01, 140.74, 134.86, 133.16, 132.11, 130.67, 128.44, 125.94, 123.91, 122.89, 122.62, 122.40, 116.39, 61.38, 28.28, 25.87, 25.83, 23.03, 18.08, 18.04.

*4.2.6 Synthesis of (E)-2',4-Dihydroxy-3,4'-dimethoxy-3',5'-diprenylchalcone (**7a**)*

6a (714.7 mg, 1.49 mmol) was dissolved in 25 mL methanol. To this solution, 7.2 mL of 3 M diluted hydrochloric acid was added. After the contents were stirred at 60 ºC for 1 h, the reaction mixture was poured into water and extracted with ethyl acetate, and the organic phase was washed with brine, dried over $MgSO_4$. After removal of solvent under reduced pressure, the obtained residue was purified by column chromatography on silica gel (2:1 chloroform–petroleum ether) to give **7a** (373.4.0 mg, 57.5% yield) as yellow oil. ESI-MS *m/z*: 459.1 $[M + Na]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 13.31 (s, 1H), 7.83 (d, *J* = 15.3 Hz, 1H), 7.58 (s, 1H), 7.43 (d, *J* = 15.3 Hz, 1H), 7.24 (dd, *J* = 8.2, 1.6 Hz, 1H), 7.13 (d, *J* = 1.6 Hz, 1H), 6.98 (d, *J* = 8.2 Hz, 1H), 5.97 (s, 1H), 5.33 – 5.24 (m, 2H), 3.98 (s, 3H), 3.79 (s, 3H), 3.42 (d, *J* = 6.6 Hz, 2H), 3.35 (d, *J* = 7.0 Hz, 2H), 1.82 (s, 3H), 1.79 (s, 3H), 1.77 (s, 3H), 1.71 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 192.83, 162.97, 162.37, 148.55, 146.91, 145.00, 133.04, 132.06, 128.34, 127.53, 125.48, 123.79, 123.56, 123.15, 122.60, 118.17, 116.70, 115.07, 110.30, 61.40, 56.08, 28.28, 25.91, 25.89, 23.09, 18.12, 18.11.

*4.2.7 Synthesis of (E)-3-Ethoxy-2',4-dihydroxy-4'-methoxy-3',5'-diprenylchalcone (**7b**)*

6b (1.07 g, 2.16 mmol) was dissolved in 20 mL methanol. To this solution, *p*-toluenesulfonic acid monohydrate (2.39 g, 12.56 mmol) was added. After the contents were stirred at room temperature for 15 h, the reaction mixture was poured into water and extracted with ethyl acetate, and the organic phase was washed with brine, dried over $MgSO_4$. After removal of solvent under reduced pressure, the obtained residue was purified by column chromatography on silica gel (2:1 chloroform–petroleum ether) to give **7b** (800.6 mg, 83.7% yield) as yellow powder, m.p. 86-88 ºC. ESI-MS *m/z*: 473.1 $[M + Na]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.82 (d, *J* = 15.4 Hz, 1H), 7.58 (s, 1H), 7.42 (d, *J* = 15.4 Hz, 1H), 7.23 (dd, *J* = 8.3, 1.2 Hz, 1H), 7.11 (d, *J* = 1.3 Hz, 1H), 6.97 (d, *J* = 8.2 Hz, 1H), 6.03 (s, 1H), 5.34 – 5.22 (m, 2H), 4.19 (q, *J* = 7.0 Hz, 2H), 3.78 (s, 3H), 3.41 (d, *J* = 6.5 Hz, 2H), 3.34 (d, *J* = 7.0 Hz, 2H), 1.81 (s, 3H), 1.79 (s, 3H), 1.76 (s, 3H), 1.70 (s, 3H), 1.50 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm): 192.86, 162.98, 162.38, 148.68, 146.22, 145.07, 133.03, 132.03, 128.34, 127.49, 125.47, 123.79, 123.39, 123.18, 122.63, 118.13, 116.72, 115.03, 111.24, 64.78, 61.38, 28.28, 25.88, 23.09, 18.10, 14.95.

*4.2.8 Synthesis of compounds **8a-8h***

*4.2.8.1 (E)-2'-Hydroxy-3,4'-dimethoxy-4-nicotinoyloxy-3',5'-diprenylchalcone (**8a**)*

To an ice solution of **7a** (50.0 mg, 0.115 mmol) and nicotinic acid (14.3 mg, 0.116 mmol) in 2 mL dry CH_2Cl_2, 4-dimethylaminopyridine (DMAP, 21.1 mg, 0.174 mmol) followed by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI, 54.9 mg, 0.288 mmol) was added under argon. After the contents were stirred at 0 ºC-RT for 16 h, the reaction mixture was poured into saturated ammonium chloride solution and extracted with ethyl acetate, and the organic phase was washed with brine, dried over $MgSO_4$. After removal of solvent, the obtained residue was purified by preparation plate on silica gel (1:1 ethyl acetate–petroleum ether) to give **8a** (58.5 mg, 94% yield) as yellow oil. ESI-MS *m/z*: 564.3 $[M + Na]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 13.19 (s, 1H), 9.42 (d, *J* = 1.6 Hz, 1H), 8.87 (dd, *J* = 4.8, 1.6 Hz, 1H), 8.48 (dt, *J* = 8.0, 1.9 Hz, 1H), 7.87 (d, *J* = 15.4 Hz, 1H), 7.59 (s, 1H), 7.55 (d, *J* = 15.5 Hz, 1H), 7.49 (dd, *J* = 7.9, 4.9 Hz, 1H), 7.33 (dd, *J* = 8.3, 1.7 Hz, 1H), 7.26 (d, *J* = 3.8 Hz, 1H), 7.25 (d, *J* = 8.2 Hz, 2H), 5.33 – 5.24 (m, 2H), 3.90 (s, 3H), 3.79 (s, 3H), 3.43 (d, *J* = 6.6 Hz, 2H), 3.35 (d, *J* = 7.0 Hz, 2H), 1.81 (s, 3H), 1.78 (s, 3H), 1.76 (s, 3H), 1.71 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 192.67, 163.34, 163.31, 162.50, 154.19, 151.66, 151.63, 143.81, 141.54, 137.89, 134.33, 133.17, 132.12, 128.44, 125.73, 125.32, 123.91, 123.62, 123.52, 123.01, 122.52, 121.53, 121.25, 116.60, 112.20, 61.41, 56.11, 28.26, 25.90, 25.88, 23.09, 18.11, 18.10.

*4.2.8.2 (E)-2'-Hydroxy-3,4'-dimethoxy-4-(4-dimethylaminobenzoyloxy)-3',5'-diprenyl chalcone (**8b**)*

8b was obtained from **7a** and 4-(dimethylamino)benzoic acid as described for the synthesis of **8a**: 43% yield as yellow oil. ESI-MS *m/z*: 606.2 $[M + Na]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 13.22 (s, 1H), 8.09 (s, 1H), 8.07 (s, 1H), 7.87 (d, *J* = 15.4 Hz, 1H), 7.59 (s, 1H), 7.53 (d, *J* = 15.4 Hz, 1H), 7.30 (dd, *J* = 8.2, 1.7 Hz, 1H), 7.23 (s, 1H), 7.22 (d, *J* = 8.1 Hz, 2H), 6.72 (s, 1H), 6.70 (s, 1H), 5.33 – 5.29 (m, 1H), 5.28 – 5.24 (m, 1H), 3.89 (s, 3H), 3.79 (s, 3H), 3.42 (d, *J* = 6.6 Hz, 2H), 3.35 (d, *J* = 7.0 Hz, 2H), 3.09 (s, 6H), 1.81 (s, 3H), 1.78 (s, 3H), 1.76 (s, 3H), 1.71 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 192.80, 164.97, 163.17, 162.44, 153.87, 152.10, 144.31, 142.74, 133.45, 133.20, 132.33, 132.11, 128.46, 125.65, 123.99, 123.83, 122.99, 122.55, 121.66, 120.61, 116.64, 115.46, 112.14, 110.90, 61.41, 56.13, 40.24, 28.22, 25.91, 23.08, 18.11.

*4.2.8.3 (E)-2'-Hydroxy-3,4'-dimethoxy-4-(5-methylthiophene-2-carbonyloxy)-3',5'-diprenylchalcone (**8c**)*

8c was obtained from **7a** and 5-methylthiophene-2-carboxylic acid as described for the synthesis of **8a**: 53% yield as yellow oil. ESI-MS *m/z*: 583.3 $[M + Na]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 13.19 (s, 1H), 7.86 (d, *J* = 15.4 Hz, 1H), 7.82 (d, *J* = 3.7 Hz, 1H), 7.58 (s, 1H), 7.53 (d, *J* = 15.5 Hz, 1H), 7.30 (dd, *J* = 8.2, 1.7 Hz, 1H), 7.23 – 7.21 (m, 2H), 6.86 (d, *J* = 3.7 Hz,

1H), 5.33 – 5.23 (m, 2H), 3.90 (s, 3H), 3.79 (s, 3H), 3.42 (d, *J* = 6.6 Hz, 2H), 3.35 (d, *J* = 7.1 Hz, 2H), 2.58 (s, 3H), 1.81 (s, 3H), 1.78 (s, 3H), 1.76 (s, 3H), 1.71 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 192.72, 163.22, 162.46, 159.97, 151.89, 149.72, 144.03, 141.76, 135.67, 133.94, 133.19, 132.14, 129.54, 128.45, 126.89, 125.69, 123.86, 123.75, 122.98, 122.51, 121.53, 120.92, 116.60, 112.18, 61.41, 56.14, 28.23, 25.90, 23.07, 18.11, 16.06.

*4.2.8.4 (E)-2'-Hydroxy-3,4'-dimethoxy-4-(2-indolylacetyloxy)-3',5'-diprenylchalcone (**8d**)*

8d was obtained from **7a** and 2-indolylacetic acid as described for the synthesis of **8a**: 54% yield as yellow powder, m.p. 143-145 ºC. ESI-MS *m/z*: 616.2 $[M + Na]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 13.11 (s, 1H), 8.15 (s, 1H), 7.81 (d, *J* = 15.2 Hz, 1H), 7.72 (d, *J* = 7.8 Hz, 1H), 7.56 (s, 1H), 7.49 (d, *J* = 15.3 Hz, 1H), 7.39 (d, *J* = 8.0 Hz, 1H), 7.29 – 7.27 (m, 1H), 7.25 – 7.21 (m, 2H), 7.20 – 7.15 (m, 2H), 7.08 (d, *J* = 8.1 Hz, 1H), 5.34 – 5.30 (m, 1H), 5.30 – 5.26 (m, 1H), 4.12 (s, 2H), 3.85 (s, 3H), 3.83 (s, 3H), 3.47 (d, *J* = 6.6 Hz, 2H), 3.39 (d, *J* = 7.0 Hz, 2H), 1.88 (s, 3H), 1.85 (s, 3H), 1.82 (s, 3H), 1.77 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 192.73, 169.94, 163.24, 162.46, 151.62, 144.00, 142.07, 136.24, 133.85, 133.19, 132.14, 128.44, 127.32, 125.68, 123.87, 123.49, 123.46, 122.98, 122.52, 122.43, 121.48, 120.90, 119.84, 119.08, 116.60, 112.08, 111.35, 107.96, 61.40, 55.93, 31.24, 28.23, 25.90, 25.89, 23.08, 18.10.

*4.2.8.5 (E)-3-Ethoxy-4-(furan-2-carbonyloxy)-2'-hydroxy-4'-methoxy-3',5'-diprenylchalcone (**8e**)*

8e was obtained from **7b** and furan-2-carboxylic acid as described for the synthesis of **8a**: 14% yield as yellow oil. ESI-MS *m/z*: 567.0 $[M + Na]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 13.18 (s, 1H), 7.84 (d, *J* = 15.4 Hz, 1H), 7.69 (d, *J* = 0.8 Hz, 1H), 7.57 (s, 1H), 7.52 (d, *J* = 15.5 Hz, 1H), 7.42 (d, *J* = 3.1 Hz, 1H), 7.29 (dd, *J* = 8.3, 1.6 Hz, 1H), 7.24 – 7.21 (m, 2H), 6.61 (dd, *J* = 3.5, 1.7 Hz, 1H), 5.33 – 5.28 (m, 1H), 5.28 – 5.24 (m, 1H), 4.14 (q, *J* = 7.0 Hz, 2H), 3.79 (s, 3H), 3.42 (d, *J* = 6.6 Hz, 2H), 3.34 (d, *J* = 7.0 Hz, 2H), 1.81 (s, 3H), 1.78 (s, 3H), 1.76 (s, 3H), 1.70 (s, 3H), 1.38 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 192.73, 163.26, 162.49, 156.30, 151.12, 147.39, 143.99, 143.80, 141.54, 134.10, 133.21, 132.14, 128.44, 125.70, 123.90, 123.61, 123.00, 122.53, 121.46, 121.03, 119.80, 116.62, 113.40, 112.36, 64.81, 61.42, 28.25, 25.90, 23.09, 18.12, 14.78.

*4.2.8.6 (E)-3-Ethoxy-2'-hydroxy-4'-methoxy-3',5'-diprenyl-4-(thiophene-2-carbonyloxy)chalcone (**8f**)*

8f was obtained from **7b** and thiophene-2-carboxylic acid as described for the synthesis of **8a**: 65% yield as yellow oil. ESI-MS *m/z*: 583.0 $[M + Na]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 12.87 (s, 1H), 7.68 (dd, *J* = 3.7, 1.2 Hz, 1H), 7.52 (d, *J* = 15.4 Hz, 1H), 7.36 (dd, *J* = 5.0, 1.2 Hz, 1H), 7.20 (d, *J* = 15.4 Hz, 1H), 6.97 (dd, *J* = 8.2, 1.6 Hz, 1H), 6.93 (s, 1H), 6.92 – 6.90 (m, 2H), 6.88 – 6.85 (m, 1H), 5.01 – 4.96 (m, 1H), 4.96 – 4.91 (m, 1H), 3.82 (q, *J* = 7.0 Hz, 2H), 3.47 (s,

3H), 3.11 (d, *J* = 6.6 Hz, 2H), 3.03 (d, *J* = 7.0 Hz, 2H), 1.50 (s, 3H), 1.47 (s, 3H), 1.45 (s, 3H), 1.39 (s, 3H), 1.05 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 192.72, 163.23, 162.46, 160.01, 151.18, 144.02, 142.05, 134.96, 133.95, 133.70, 133.16, 132.46, 132.09, 128.45, 128.16, 125.67, 123.85, 123.60, 123.00, 122.53, 121.47, 120.92, 116.60, 113.46, 64.84, 61.39, 28.23, 25.87, 23.07, 18.09, 14.79.

*4.2.8.7 (E)-4-(5-Chloro-thiophene-2-carbonyloxy)-3-ethoxy-2'-hydroxy-4'-methoxy-3',5'-diprenyl-chalone (**8g**)*

8g was obtained from **7b** and 5-chloro-thiophene-2-carboxylic acid as described for the synthesis of **8a**: 58% yield as yellow oil. ESI-MS *m/z*: 617.0 $[M + Na]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 13.19 (s, 1H), 7.84 (d, *J* = 15.4 Hz, 1H), 7.79 (d, *J* = 4.0 Hz, 1H), 7.58 (s, 1H), 7.52 (d, *J* = 15.5 Hz, 1H), 7.28 (dd, *J* = 8.3, 1.6 Hz, 1H), 7.23 – 7.20 (m, 2H), 7.02 (d, *J* = 4.0 Hz, 1H), 5.33 – 5.28 (m, 1H), 5.28 – 5.24 (m, 1H), 4.13 (q, *J* = 7.0 Hz, 2H), 3.79 (s, 3H), 3.42 (d, *J* = 6.6 Hz, 2H), 3.35 (d, *J* = 7.1 Hz, 2H), 1.81 (s, 3H), 1.79 (s, 3H), 1.76 (s, 3H), 1.71 (s, 3H), 1.37 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 192.69, 163.26, 162.48, 159.01, 151.06, 143.92, 141.76, 138.78, 134.58, 134.12, 133.17, 132.11, 130.56, 128.44, 127.72, 125.69, 123.88, 123.49, 123.01, 122.53, 121.42, 121.05, 116.60, 113.39, 64.83, 61.40, 28.24, 25.88, 23.08, 18.10, 14.80.

*4.2.8.8 (E)-4-((E)-3-(4-Pyridinyl)acryloyloxy)-3-ethoxy-2'-hydroxy-4'-methoxy-3',5'-diprenyl-chalcone (**8h**)*

8h was obtained from **7b** and (*E*)-3-(pyridin-4-yl) acrylic acid as described for the synthesis of **8a**: 47% yield as yellow oil. ESI-MS *m/z*: 604.2 $[M + Na]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 13.18 (s, 1H), 8.82 (d, *J* = 1.3 Hz, 1H), 8.65 (d, *J* = 3.6 Hz, 1H), 7.93 (d, *J* = 8.0 Hz, 1H), 7.88 (d, *J* = 16.1 Hz, 1H), 7.84 (d, *J* = 15.4 Hz, 1H), 7.58 (s, 1H), 7.52 (d, *J* = 15.4 Hz, 1H), 7.39 (dd, *J* = 7.9, 4.9 Hz, 1H), 7.28 (dd, *J* = 8.2, 1.6 Hz, 1H), 7.23 (d, *J* = 1.6 Hz, 1H), 7.18 (d, *J* = 8.1 Hz, 1H), 6.75 (d, *J* = 16.1 Hz, 1H), 5.33 – 5.28 (m, 1H), 5.28 – 5.23 (m, 1H), 4.15 (q, *J* = 7.0 Hz, 2H), 3.79 (s, 3H), 3.42 (d, *J* = 6.6 Hz, 2H), 3.35 (d, *J* = 7.1 Hz, 2H), 1.81 (s, 3H), 1.79 (s, 3H), 1.77 (s, 3H), 1.70 (s, 3H), 1.42 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 192.69, 164.13, 163.25, 162.47, 151.45, 151.00, 150.01, 143.98, 143.13, 141.98, 134.61, 133.96, 133.16, 132.10, 130.08, 128.42, 125.67, 124.01, 123.87, 123.46, 123.00, 122.51, 121.46, 120.95, 119.08, 116.59, 113.25, 64.72, 61.39, 28.23, 25.87, 23.07, 18.09, 14.83.

4.3 Cytotoxicity assay

4.3.1 Cell culture

Cancer cell lines were obtained from the biology laboratory in the Key Laboratory of

Chemistry for Natural Products of Guizhou Province and Chinese Academy of Sciences (Guiyang, China). Cells were maintained in DMEM (supplemented with 10% FBS, 100 μg/ mL penicillin, and 100 μg/mL streptomycin) and cultured at 37 ºC in a CO_2 incubator (5% CO_2 and 95% air, 95% humidity). Freshly thawed cells were passaged at least 3 times before they were used in experiments.

4.3.2 MTT assay

Cytotoxicity was evaluated by MTT assay. Briefly, PC3, MDA, K562 and HEL cells were seeded in 96-well plates at density of 4×10^3 to 8×10^3 cells/well and allowed to adhere for 24 h. After cells were exposed for 48 h to different concentrations of compounds, cells were evaluated under an inverted microscope equipped with a CCD camera (Nikon, Japan). 20 μL of MTT solution (5 mg/mL) was then added to each well and incubated at 37ºC for 4 h. The supernatant was removed, and 150 μL DMSO solution was added. Plates were gently shaken to dissolve blue formazan crystals and the absorbance was read at 490 nm using a microplate reader (Thermo Scientific, Vario Skan Flash, USA).

4.4 Cell apoptosis assay

Apoptosis was evaluated by a microscope and flow cytometry based on the annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (PI) staining kit (BD Pharmingen, San Diego, USA). Apoptotic cells were defined as Annexin-V-positive. Compound-treated cells were trypsinized, washed twice with PBS and transferred into microcentrifuge tubes for centrifugation at 1,000 rpm for 5 min at room temperature and resuspended in binding buffer. PI (Sigma, St. Louis, USA) was then added to a final concentration of 20 μg/mL. The cells was transferred to a round-bottom 96-well plate and analyzed by flow cytometry (Becton Dickinson, Franklin Lakes, USA).

4.5 Cell cycle analysis

After treatment of the cells with compounds, cells were trypsinized, washed twice with PBS, and transferred to microcentrifuge tubes for centrifugation at 1,000 rpm for 5 min at room temperature. To avoid cell clumping, cells were fixed by addition of ice-cold ethanol and blocked for 1 h at 4 °C in 5% BSA/PBS. After washing twice with PBS, the cells were then resuspended in PBS containing 1 μg/mL RNase and incubated at 37ºC for 30 min. PI was added to a final concentration of 20 μg/mL. Cells was transferred to round-bottom 96-well plates and analyzed by flow cytometry using FACS Array (Becton Dickinson, Franklin Lakes, USA). Histograms of PI signal intensity were generated and the percentages of cells in G_0, G_1, S and G_2/M phases were determined.

4.6 Statistical analysis

Data were analyzed by SPSS 18.0 software and presented as mean ± SD of three independent experiments. For all measurements, one-way ANOVA followed by Student's test was used to assess the statistical significance between groups. The LSD method was used to assess the statistical significance between every two groups. A statistically significant difference was considered at the level of $P < 0.05$.

Declaration of ethics

The authors declare that there is no ethical issue to declare.

Declaration of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgment

The authors are deeply thankful to the staff members of the analytical center for spectral measurement and activity test centre for anti-cancer activity of the Key Laboratory of Chemistry for Natural Products of Guizhou Province and Chinese Academy of Sciences (Guiyang, China). The work was supported by the Specific Project of China Postdoctoral Science Foundation (No. 2015T80106), P.R. China, the Special Fund for Outstanding Youth in Science and Technology, Guizhou Province (No. QKHRZ [2015]24), P.R. China, the Main Science Project of Guizhou province (No. [2013]6012), P.R. China, the postdoctoral Science Foundation of Beijing (No. 2015-ZZ-45), P.R. China and the Natural Science Foundation of Guizhou province (No. [2014]2099), P.R. China.

References

[1] W.Q. Chen, R.S. Zheng, P.D. Baade, S.W. Zhang, H.M. Zeng, F. Bray, A. Jemal, X.Q. Yu, J. He, Cancer statistics in China, 2015, CA Cancer J. Clin. 66 (2) (2016) 115-132.

[2] Q. Wu, Z.P. Yang, Y.Z. Nie, Y.Q. Shi, D.M. Fan, Multi-drug resistance in cancer chemotherapeutics: Mechanisms and lab approaches, Cancer Lett. 347 (2) (2014), 159-166.

[3] N. Tadigoppula, V. Korthikunta, S. Gupta, P. Kancharla, T. Khaliq, A. Soni, R.K. Srivastava, K. Srivastava, S.K. Puri, K.S. Raju, Wahajuddin, P.S. Sijwali, V. Kumar, I.S. Mohammad, Synthesis and insight into the structure-activity relationships of chalcones as antimalarial agents, J. Med. Chem. 56 (1) (2013) 31-45.

[4] K. Damodar, J.K. Kim, J.G. Jun, Synthesis and pharmacological properties of naturally occurring prenylated and pyranochalcones as potent anti-inflammatory agents, Chin. Chem. Lett. 27 (5) (2016) 698-702.

[5] O.A. Battenberg, Y.L. Yang, S.H. Verhelst, S.A. Sieber, Target profiling of 4-hydroxyderricin in S. aureus reveals seryl-tRNA synthetase binding and inhibition by covalent modification, Mol. Bio. Syst. 9 (3) (2013) 343-351.

[6] S.A. Ma, Y.W. Huang, Y.Y. Zhao, G.X. Du, L. Feng, C. Huang, Y.M. Li, F.J. Guo, Prenylflavone derivatives from the seeds of *Psoralea corylifolia* exhibited PPAR-γ agonist activity, Phytochem. Lett. 16 (2016) 213-218.

[7] H.M. Wang, L. Zhang, J. Liu, Z.L. Yang, H.Y. Zhao, Y. Yang, D. Shen, K. Lu, Z.C. Fan, Q.W. Yao, Y.M. Zhang, Y.O. Teng, Y. Peng, Synthesis and anti-cancer activity evaluation of novel prenylated and geranylated chalcone natural products and their analogs, Eur. J. Med. Chem. 92 (2015) 439-448.

[8] J.F. Stevens, M. Ivancic, V.L. Hsu, M.L. Deinzer, Prenylflavonoids from Humulus lupulus, Phytochemistry 44 (8) (1997) 1575-1585.

[9] C.N. Lin, C.M. Lu, Novel antiplatelet constituents from formosan moraceous plants, J. Nat. Prod. 59 (9) (1996) 834-838.

[10] E.V. Ran, Y.R. Prasad, Two chalcones from tephrosia spinosa, Phytochemistry 31(6) (1992) 2121-2122.

[11] K. Kyogoku, K. Hatayama, K. Suzuki, S. Yokomori, K. Maejima, M. Komatsu, Studies on constituents of Guang-Dou-Gen (root of *sophora subprostrata* Chun et T. Chen), isolation of 2 new flavanones, Chem. Pharm. Bull. 21 (8) (1973)1777-1780.

[12] S. Vogel, S. Ohmayer, G. Brunner, J. Heilmann, Natural and non-natural prenylated chalcones: Synthesis, cytotoxicity and anti-oxidative activity, Bioorg. Med. Chem. 16 (8) (2008) 4286-4293.

[13] B.X. Zhang, D.Z. Duan, C.P. Ge, J. Yao, Y.P. Liu, X.M. Li, J.G. Fang, Synthesis of xanthohumol analogues and discovery of potent thioredoxin reductase inhibitor as potential anticancer agent, J. Med. Chem. 58 (4) (2015) 1795-1805.

[14] C.L. Miranda, G.L. Aponso, J.F Stevens, M.L. Deinzer, D.R. Buhler, Prenylated chalcones and avanones as inducers of quinone reductase in mouse Hepa 1c1c7 cells, Cancer Lett. 149 (1) (2000) 21-29.

[15] T. Tronina, A. Bartmańska, B. F. Psurska, J. Wietrzyk, J. Popłoński, E. Huszcza, Bioorg. Med. Chem. 21 (7) (2013) 2001-2006.

[16] R. Weiskirchen, A. Mahli, S. Weiskirchen, C. Hellerbrand, The hop constituen txanthohumol exhibits hepatoprotective effects and inhibits the activation of hepatic stellate cells at different levels, Front. Physiol. 6 (2015) 140-150.

[17] M. Kłósek, A. Mertas, W. Król, D. Jaworska, J. Szymszal, E. Szliszka, Tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis in prostate cancer cells after treatment with xanthohumol—a natural compound present in *Humulus lupulus L.*, Int. J. Mol. Sci. 17 (6) (2016) 837-853.

[18] Y.B. Liu, X.H. Gao, D. Deeb, A.S. Arbab, S.A. Dulchavsky, S.C. Gautam, Anticancer agent xanthohumol inhibits IL-2 induced signaling pathways involved in T cell proliferation, J. Exp. Ther. Oncol. 10 (1) (2012) 1-8.

[19] X.Q. Zhao, K. Jiang, B. Liang, X.Q. Huang, Anticancer effect of xanthohumol induces growth inhibition and apoptosis of human liver cancer through NF-κB/p53-apoptosis signaling pathway, Oncol. Rep. 35 (2) (2016) 669-675.

[20] M.R. Cook, J. Luo, M. Ndiaye, H. Chen, M. Kunnimalaiyaan, Xanthohumol inhibits the neuroendocrine transcription factor achaete-scute complex-like 1, suppresses proliferation, and induces phosphorylated ERK1/2 in medullary thyroid cancer, Am. J. Surg. 199 (3) (2010) 315-318.

[21] C.S. Kwon, J. Lee, J.H. Kim, J.S. Kim, Increased sensitivity to mitomycin C by pretreatment with broussochalcone A in MCF-7 cells, J. Food Sci. Biotechnol. 13 (6) (2004) 777-780.

[22] E.E. Caamal-Fuentes, S.R. Peraza-Sánchez, L.W. Torres-Tapia, R.E. Moo-Puc-, Isolation and identification of cytotoxic compounds from Aeschynomene fascicularis, a mayan medicinal plant, Molecules 20 (8) (2015) 13563-13574.

[23] K. Sugamoto, Y.I. Matsusita, K. Matsui, C. Kurogi, T. Matsui, Synthesis and antibacterial activity of chalcones bearing prenyl or geranyl groups from Angelica keiskei, Tetrahedron 67 (29) (2011) 5346-5359.

[24] Z.L. Liu, X.J. Wang, N.G. Li, H.P. Sun, J.X. Wang, Q.D. You, Total synthesis of aldehyde-containing garcinia natural products isomorellin and gaudichaudione A, Tetrahedron 67 (26) (2011) 4774-4779.

[25] D. Cao, X.L. Han, G.C. Wang, Z. Yang, F. Peng, L. Ma, R.H. Zhang, H.Y. Ye, M.H. Tang, W.S. Wu, K. Lei, J.L. Wen, J.Y. Chen, J.X. Qiu, X.L. Liang, Y. Ran, Y. Sang, M.L. Xiang, A.H. Peng, L.J. Chen, Synthesis and biological evaluation of novel pyranochalcone derivatives as a new class of microtubule stabilizing agents, Eur. J. Med. Chem. 62 (2013) 579-589.

[26] K. Dahlén, E.A. Wallén, M. Grøtli, K. Luthman, Synthesis of 2,3,6,8-tetrasubstituted chromone scaffolds, J. Org. Chem. 71 (18) (2006) 6863-6871.

[27] J.B. Daskiewicz, F. Depeint, L. Viornery, C. Bayet, G Comte-Sarrazin, Effects of flavonoids on cell proliferation and caspase activation in a human colonic cell line HT29: An SAR study, J. Med. Chem. 48 (8) (2005) 2790-2804.

[28] K. Sugamoto, C. Kurogi, Y.I. Matsushita, T. Matsui, Synthesis of 4-hydroxyderricin and related derivatives, Tetrahedron Lett. 49 (47) (2008) 6639-6641.

[29] Y.S. Kim, S.G.L. Keyser, J.S. Schneekloth, Synthesis of 2', 3', 4'-trihydroxyflavone (2-D08), an inhibitor of protein sumoylation, Bioorg. Med. Chem. Lett. 24 (4) (2014) 1094-1097.

Highlights

1. A double Claisen rearrangements synthetic strategy was established for synthesis of 3',5'-diprenylated chalcones.
2. Twenty-three novel 3',5'-diprenylated chalcones were prepared.
3. Preliminary SARs of these compounds against four cancer cell lines were analyzed.
4. Compound **10** showed potent cytotoxicity versus all cancer cells tested.
5. Compound **10** inhibited the proliferation of cancer cells *in vitro* by inducing cell apoptosis and arresting cell cycle phase.