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Original article

Semisynthesis and *in vitro* cytotoxic evaluation of new analogues of 1-*O*-acetylbritannilactone, a sesquiterpene from *Inula britannica*

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ABSTRACT

Semisynthetic analogues of the natural product 1-*O*-acetylbritannilactone (ABL), a sesquiterpene isolated from the medicinal plant *Inula britannica*, have been prepared and exhibited significant *in vitro* cytotoxic activities against four cell lines including three human cancer cell lines (HCT116, HEp-2 and HeLa) and one normal hamster cell line (CHO). Structure–activity relationships indicate that esterification of 6-OH (enhanced lipophilicity) and α-methylene-γ-lactone functionalities play important roles in conferring cytotoxicity. Among the tested compounds, **14** bearing a lauroyl group (12C) at the 6-OH position displayed most potent *in vitro* cytotoxic activity, with IC_{50} values between 2.91 and 6.78 μM, comparable to the positive control etoposide (VP-16, IC_{50} values between 2.13 and 4.79 μM). Moreover, the compound **14** triggered remarkable apoptosis at a low concentration, and induced cell cycle arrest in G2/M phase in HCT116 cells. The biological assays conducted with normal cells (CHO) revealed that all the synthetic compounds are no selective against cancer cell lines tested.

1. Introduction

Inula britannica L. (Xuan fu hua in Chinese) from Compositae family is one of the most popular and multi-purpose traditional Chinese medicinal herbs, and has high sesquiterpenes content [1,2]. 1-*O*-acetylbritannilactone (ABL, Fig. 1), a 1,10-*seco*-eudesmanolide sesquiterpene extracted from *I. Britannica*, has several biological effects including anti-inflammatory, antibacterial, antihepatitic, antidiabetes, and antitumour activities [3–8]. It has been shown to possess anticancer effects in various cancer cells [4,5,9–12], including anti-proliferation, cell cycle arrest, induction of apoptosis and increased sensitivity to apoptosis. ABL induced cell cycle arrest in G0/G1 phase of human colon cancer HT-29 cells accompanied by a strong decrease of cyclin E and CDK4 protein levels, and an increase in p21 protein expression [4]. ABL-induced growth inhibition is also associated with the upregulation of KLF4 expression [4] and ABL induced phosphorylation of Bcl-2 in breast, ovary and prostate cancer cell lines [9]. Moreover, ABL and celecoxib (Fig. 1) could interact synergistically to suppress breast cancer cell growth via COX-2-dependent and -independent mechanisms [13].

An analogue of ABL, 1,6-*O*,*O*-diacetylbritannilactone (OABL, Fig. 1) isolated from *I. britannica*, was ten times more potent than ABL in HL-60 and MCF-7 cells [3,5,14]. OABL also showed strong induced-apoptosis associated with activation of caspase-8, -9, and -3, phosphorylation of Bcl-2 and Bid, and increased release of cytochrome *c* from mitochondria in promyelocytic leukemia HL-60 [11]. The more potent activity of OABL has been considered due to the acetyl group at 6-hydroxy (6-OH) position enhancing the molecular lipophilicity [15]. Another important structural factor related to their bioactivity may be the presence of an electrophilic α-methylene-γ-lactone motif, which can bind thiols of proteins or residues as alkylating reagent to induce the DNA-fragmentation and apoptosis mediated by glutathione depletion of the cells [16–18]. This was also confirmed in many studies of other sesquiterpene lactones [18], such as parthenolide [19,20], helenalin [21] (Fig. 1). Besides, potent bioactivity of OABL may correlate other factors, such as molecular geometry and chemical environment.

The high content (0.2%–0.5% weight of dry flowers and stems) [22] of ABL and potent anticancer activity associated with ABL and OABL in *I. britannica* has prompted interest in designing novel ABL analogues with anticancer activity superior to that of the parent compound [14,15,23,24]. So far, structural modifications of ABL focused mainly on alterations at 6-OH moiety, and some of these analogues have turned out to be more cytotoxic against various human cancer cells than ABL and OABL [14,15,23,24]. For example,

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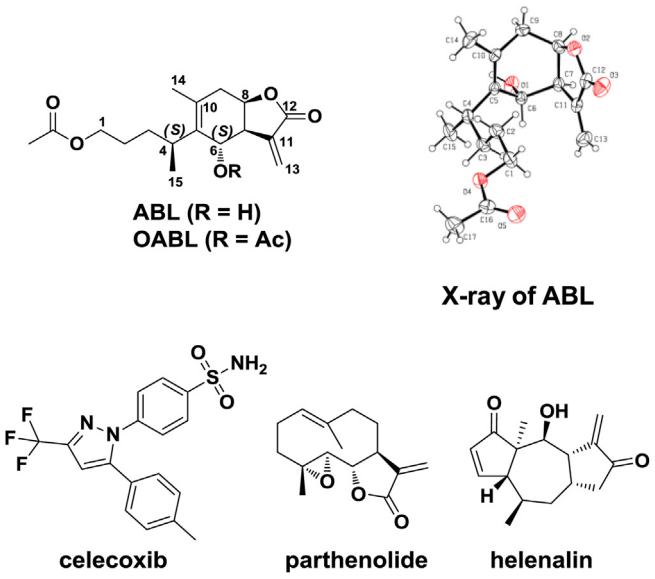


Fig. 1. The structures of 1-*O*-acetylbritannilactone (ABL), 1,6-*O*,*O*-diacetylbritannilactone (OABL), X-ray of ABL, Celecoxib and two sesquiterpene lactones.

Ho and co-workers have synthesized some 1-*O*-acetylbritannilactone oxime esters, of which four esters show better cytotoxic activity on HL-60 and human hepatoma Bel-7402 cell lines [14,15,23].

Our ongoing efforts focused on the introduction of chemical diversity in the molecular framework in order to prepare plant-derived pharmacologically interesting compounds [25–27]. This prompted us to synthesize a series of new ABL analogues and evaluate their structure–activity relationships. We firstly performed the isolation of ABL from *I. britannica*, and the structure of ABL was elucidated by NMR, ESI-MS spectroscopy and X-ray crystallographic data (Fig. 1). Then, using ABL as the starting material, we semisynthesized a series of 6-OH modified and C13-modified arylation analogues. For 6-OH modified analogues, we tried out several standard esterification methods to convert ABL into a series of new esters of various aliphatic and cinnamic carboxylic acids (Schemes 1 and 2). Furthermore, to examine effect of the oxidation of 6-OH on cytotoxic properties, we oxidized 6-OH into 6-ketone using Dess–Martin oxidation (Scheme 3). For C13-modified arylation analogues, we conducted a series of reactions with readily available aryl iodides using the previously reported Heck reaction conditions [28,29] (Scheme 4). Herein, we report the semisynthesis, the structure–activity relationship of ABL analogues based on *in vitro* cytotoxic activities and preliminary cytotoxic mechanism of the most active compound.

2. Results and discussion

2.1. Chemistry

2.1.1. Isolation and characterization of ABL

The AcOEt-soluble fraction of the ethanolic extract of the dried flowers of *I. britannica* was repeatedly subjected to column chromatography over silica gel, followed by recrystallization from EtOH to give the pure compound (+)-ABL ($[\alpha]_D^{25}$ = +103.5 in $CHCl_3$), which was found to be identical to the natural product [2,3,7] by comparison of the spectral and optical rotation data. However, three relative configurations of ABL present in the same plant have been reported, *i.e.*, (4*S*,6*R*) – [9,14,15], (4*R*,6*S*) – [7,30], and (4*S*,6*S*)-ABL [2,31], but the three stereoisomers had similar NMR spectral and optical rotation data. So to determine which the relative configuration of ABL was correctly depicted in the literatures, we obtained X-ray diffraction data of ABL from a SuperNova, Dual, Eos diffractometer with Cu-Kα radiation [32] (Fig. 1) (see Table S1 in Supplementary data). The X-ray diffraction analysis of ABL unambiguously verified the out-of-plane of 4-methyl and the 6α-orientation of the secondary hydroxyl group. As a result, the relative configuration of (+)-ABL should be revised as 4*S**, 6*S**, which is in accord with that of (+)-britannilactone ([α] = +86.0 (*c* = 0.57 in $CHCl_3$)) obtained by total synthesis [33]. It can be concluded that in the crystal structure (Fig. 1) a six-membered ring adopted a slightly twisted boat conformation and was fused by a planar five-membered ring; and 6-OH of ABL located in the backside of the two rings with small steric hindrance could be a modifiable site.

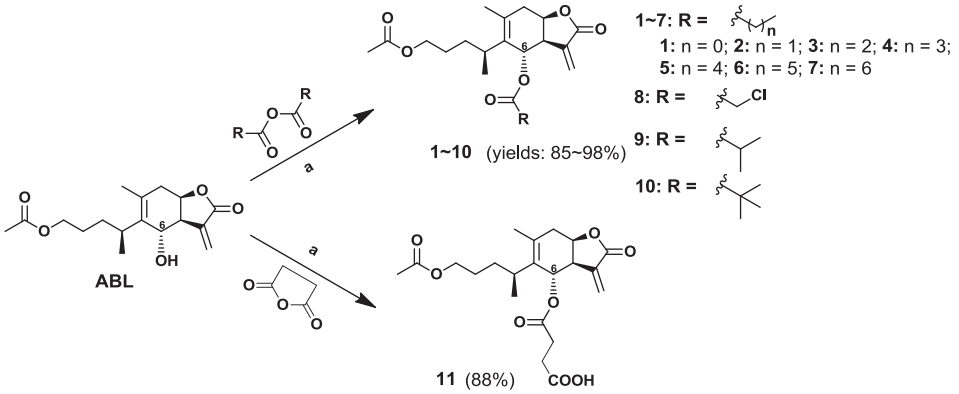
2.1.2. Synthesis of ABL 6-OH modified analogues (**1**–**19**)

The synthesis of ABL 6-OH modified analogues **1**–**19** commenced from starting material ABL. In a conventional procedure, ABL was converted to the corresponding ester analogues with aliphatic side chains and aromatic moieties using different acid anhydrides (Scheme 1) or long chain acids (Scheme 2). This upon treatment with various acid anhydrides in the presence of triethylamine (Et_3N) and catalytic dimethylaminopyridine (DMAP) in dried dichloromethane (DCM) afforded ester analogues **1**–**11** in excellent yields (85%–98%) and in short time (10 min–4 h). Treatment of ABL with various long chain fatty acids in anhydrous DCM solution using dicyclohexylcarbodiimide (DCC) as activating reagent of carboxylic acid gave the desired compounds **12**–**16** in 70%–75% yields. Furthermore, reaction of ABL with *p*-Br- or *p*-CF_3-substituted cinnamic acids provided the expected compounds **17** and **18** in 65% and 83% yields, respectively. Additionally, mild oxidation of ABL with Dess–Martin periodinane (DMP) afforded ketone **19** in 43% yield.

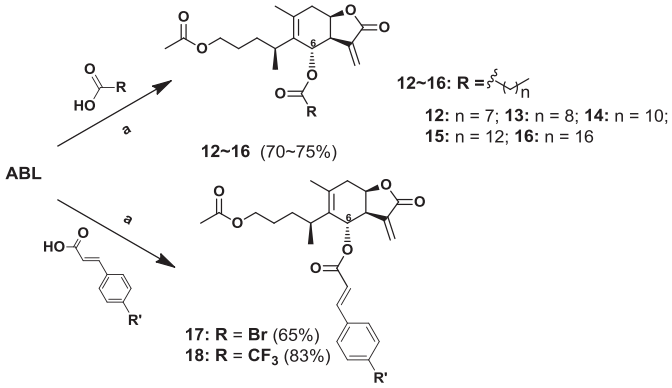
2.1.3. Synthesis of OABL C13-modified arylation analogues (**20**–**24**)

The synthetic route of target compounds was obtained in Scheme 4. Five OABL arylation analogues **20**–**24** were synthesized by Heck coupling reaction of more active OABL with readily available aryl iodides. Indeed, when 5 mol% of $Pd(OAc)_2$ with Et_3N in anhydrous DMF at 80 °C was used, a main product with an exocyclic olefin was isolated in each case after purification in moderate to good yields (41%–82%). The substitution pattern on the aromatic ring and the presence of electron-donating or electron-withdrawing substituents did not affect the yields or the preference for a main olefin geometry in the isolated product. The assignment of the C11–C13 olefin geometry of **20** was determined using NOESY NMR experiments to be the *E*-olefin. Specifically, NOESY signals were readily apparent between the two protons of phenyl ring and the protons attached to C6 and C7 on the six-membered ring (See Fig. S1 in Supplementary data). Also, the C13 benzylic proton for **20** had a chemical shift of 7.60 ppm that supports the assignment as an *E*-olefin. The assignment of the *E*-geometry for the compound **23** was further verified following determination of an X-ray crystal structure (Fig. 2) [32]. For **21**, **22** and **24**, the assignment of the *E*-olefin geometry was accomplished by the diagnostic chemical shift of the C13 proton. These data support that the preferential selectivity for the *E*-isomer (over the *Z*-isomer) from the Heck reaction [34], which is in accord with prior work results of Colby and co-workers [28], whom semisynthetized sesquiterpene lactone derivatives by the palladium-catalyzed arylation for parthenolide containing a α-methylene-γ-lactone motif.

The structures of all 24 synthetic compounds were confirmed by 1D-NMR, 2D-NMR and HR-ESI-MS. Among them, 23 compounds except **1** (OABL) were new. The purity of all compounds was confirmed to be greater than 95% by HPLC with UV and evaporative



Scheme 1. Conditions and reagents: (a) DMAP, Et_3N, CH_2Cl_2, 0 °C to rt.



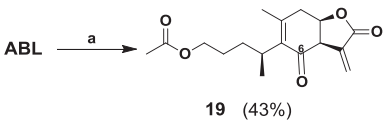
Scheme 2. Conditions and reagents: (a) DCC, DMAP, Et_3N, CH_2Cl_2, rt.

light scattering detection (ELSD) (Supplementary data) prior to their use in the evaluation of their biological efficacies. It should be noted that the synthetic approach for this family of 6-OH modified compounds used in the current study is a simple, effective method, especially reaction with cheap acid anhydrides, short time and high yields. For C13-modified compounds, the arylation of OABL via cross-coupling reaction catalyzed by palladium is achievable. Besides, all these compounds showed ester stability at the 10 mM of concentration stayed for at least 3 days in PBS (pH = 7.4) or culture medium with HPLC analysis (data not shown).

2.2. Cytotoxicity

ABL and its analogues **1**–**24** were evaluated for *in vitro* cytotoxic activities against three cancer cell lines HCT116 (human colorectal cancer), HEp-2 (human larynx epidermal cancer) and HeLa (human cervix cancer) using the SRB assay [35]. Etoposide was used as positive control in this experiment. The IC_{50} values (the concentration to cause 50% inhibition of cell viability) of these compounds are summarized in Tables 1 and 2.

For the ABL 6-OH modified analogues **1**–**19**, most compounds generally showed good cytotoxicity against the three cancer cell lines tested. Notably, acetylation of 6-OH of ABL gave OABL **1**, which showed high cytotoxicity toward HCT116, HEp-2 and HeLa cells, with IC_{50} values of 10.1, 7.60 and 11.2 μM, respectively, with being approximately 3-fold more efficacious than the parent compound ABL. In addition, Ho et al. claimed that **1** has more anticancer effects than ABL on four human cancer cell lines [36], promyelocytic leukemia HL-60, colon adenocarcinoma HT-29, colon adenocarcinoma COLO 205 and gastric carcinoma AGS. These results suggest that **1** would exhibit different sensitivity to various cancer cell lines. Compounds **2**–**10** (except **7** with IC_{50} 25.0 μM to HEp-2) and **12**–**15**, possessing different aliphatic chains at 6-OH position, exhibited higher cytotoxic activity than ABL against HCT116, HEp-2 and HeLa cells (IC_{50} data ranging from 3 to 28 μM). However, in contrast, **11** bearing a succinyl chain (−$COCH_2CH_2COOH$) did not show any activity with IC_{50} values all more than 70 μM. These data indicate that introduction of the appropriate lipophilic aliphatic chains improved potency in the activity.



Scheme 3. Conditions and reagents: (a) DMP, CH_2Cl_2, rt.

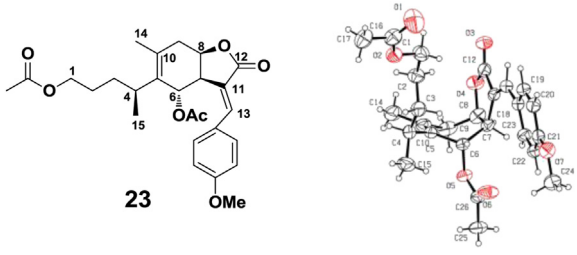
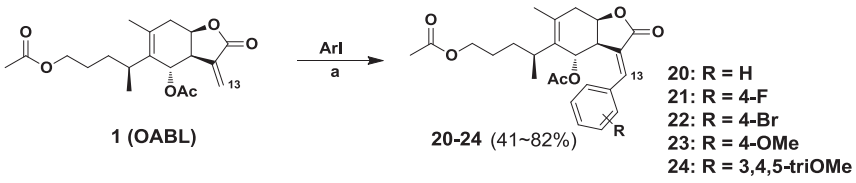
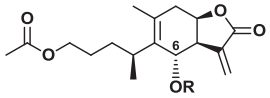


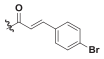
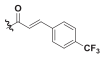
Fig. 2. The structure and X-ray of compound **23**.



Scheme 4. Conditions and reagents: (a) $Pd(OAc)_2$ (5 mol%), Et_3N, DMF, 80 °C.

Table 1
Cytotoxic activities (IC_{50}) of ABL and its 6-OH modified analogues (**1**–**19**).



No.	R	IC_{50}[a] (μM)			
		HCT116	HEp-2	HeLa	CHO
ABL	H	36.1 ± 3.1	19.3 ± 1.5	32.6 ± 2.5	41.3 ± 4.5
1 (OABL)	Ac	10.1 ± 1.1	7.60 ± 0.51	11.2 ± 0.8	8.57 ± 1.25
2		7.78 ± 1.51	9.19 ± 1.78	7.78 ± 0.34	4.97 ± 0.43
3		8.39 ± 0.81	9.40 ± 1.20	7.98 ± 1.13	5.20 ± 0.50
4		12.1 ± 0.6	15.2 ± 1.6	18.2 ± 2.1	6.35 ± 0.51
5		12.7 ± 1.5	18.2 ± 2.1	19.2 ± 2.3	12.2 ± 1.1
6		10.2 ± 1.3	14.9 ± 1.6	17.5 ± 0.5	16.5 ± 2.1
7		12.4 ± 1.6	25.0 ± 3.5	28.2 ± 1.5	19.4 ± 2.5
8		18.8 ± 2.3	18.6 ± 4.2	28.5 ± 3.1	10.5 ± 1.2
9		6.54 ± 1.55	10.5 ± 1.9	14.1 ± 1.6	8.44 ± 1.56
10		10.3 ± 0.55	10.8 ± 0.59	18.2 ± 1.9	16.7 ± 2.4
11		>100	71.3 ± 8.9	>100	91.8 ± 10.5
12		8.97 ± 2.64	16.4 ± 1.8	21.3 ± 2.5	15.2 ± 3.5
13		8.40 ± 1.54	15.0 ± 1.64	16.9 ± 0.7	11.9 ± 2.5
14		2.91 ± 0.61	5.85 ± 0.45	6.78 ± 0.23	5.97 ± 0.12
15		5.54 ± 2.51	14.0 ± 1.5	15.6 ± 1.2	14.9 ± 2.5
16		21.9 ± 3.5	>100	>100	>100
17		7.69 ± 0.51	13.1 ± 0.5	18.2 ± 2.1	24.1 ± 2.5
18		10.2 ± 0.8	9.80 ± 0.51	18.3 ± 0.8	9.54 ± 1.57
19	O	32.6 ± 4.5	91.2 ± 11.5	>100	91.0 ± 4.5
Etoposide	–	2.13 ± 0.23	4.79 ± 0.54	2.97 ± 0.25	2.60 ± 0.15

[a] The IC_{50} values represent the concentration to cause 50% inhibition of cell viability. Cancer cell lines HCT116 (human colorectal cancer), HEp-2 (human larynx epidermal cancer) and HeLa (human cervix cancer) and normal cell line CHO (Chinese hamster ovary) were treated with ABL and its 6-OH modified analogues for 72 h. All data (mean ± SD) are the average of three or four determinations.

Interestingly, of the test synthesized esters, compound **14**, bearing a lauroyl group (12C) at the 6-OH position, displayed the highest potency with IC_{50} values of 2.91, 5.85 and 6.78 μM toward HCT116, HEp-2 and HeLa cells, respectively. This compound was about 10, 4 and 5 times, respectively, more active than ABL, which is comparable to etoposide (IC_{50} data of 2.13, 4.79 and 2.97 μM, respectively), the commonly used chemotherapeutic agent. In contrast to **14**, compounds **15** with a myristoyl group (14C) and **16** with a stearoyl group (18C) had low activity, especially **16** led to a marked reduction in the cytotoxicity against HCT116 cells (IC_{50} = 21.9 μM) and to a loss in activity against HEp-2 and HeLa cells (IC_{50} > 100 μM). This suggests that the aliphatic chain length at 6-OH is decisive for the activity, and 12C aliphatic side chain may be optimal length for the cytotoxic activity. The improvement of bioactivity is a comprehensive effect of many factors, but in the case perhaps the most important reason is the introduction of 12 carbons aliphatic side chain ester which perhaps fit for good binding at the molecular target's pocket. While the succinate **11** with terminal COOH moiety loss the activity may not fit for good binding at the molecular target.

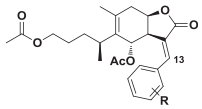
On the other hand, it can also be seen from Table 1 that introduction of substituted cinnamic acids to ABL could improve cytotoxicity towards HCT116, HEp-2 and HeLa. For example, **17** and **18**, with IC_{50} data of 7–18 μM, had similar activity but had both stronger activity than ABL. This indicates that the presence of a *p*-bromo or *p*-CF_3 (trifluoromethyl) group in the benzene ring appeared to have no significant on the activity.

When 6-OH of ABL was oxidated to a ketone group providing analogue **19** (IC_{50} = 32.6 μM), which had a similar cytotoxic effect to that of the parent (IC_{50} = 36.1 μM) against HCT116 cells, whereas it had no effects on other two cancer cell lines tested (IC_{50} > 90 μM), implying that conversion of 6-OH of ABL to a ketone group resulted in loss of activity. In addition, it seems to be concluded from Table 1 that HCT116 cells, compared with other two cells, were higher sensitivity to most ABL analogues.

In order to enrich the chemical diversity of ABL molecular framework, aryl groups were introduced into the α-methylene-γ-lactone motif of **1** (OABL) to decrease α-methylene nucleophilic activity giving **20**–**24**. Although the electrophilic α-methylene-γ-lactone is known to trap nucleophilic intracellular thiols, such as cysteine residues [16–18], the biological effect of arylation of this functional group for OABL is not known. From Table 2, it could be seen that arylation analogues **20**–**24** resulted in decreased potency (IC_{50} > 50 μM and >40 μM on HCT116 and HeLa cells, respectively) compared with that of the parent ABL and OABL, which hinted at the importance of the unscreened α-methylene functionality.

Moreover, we assessed whether ABL and analogues had any differential sensitivity to normal versus cancer cells. Recently, the enone-containing natural product piperlongumine has been found to possess remarkable selectivity for promoting the death of cancer cells versus normal cells [37]. Similarly, to determine if ABL and its analogues are selective, we measured their cytotoxicity against normal cell lines CHO (Chinese hamster ovary). As shown in Tables 1 and 2, the sensitivity of all compounds was low with approximate IC_{50} data, suggesting that ABL analogues may have no selectivity toward cancer cells.

Table 2
Cytotoxic activities (IC_{50}) of OABL arylation analogues (**20**–**24**).



No.	R	IC_{50}[a] (μM)			
		HCT116	HEp-2	HeLa	CHO
20	H	59.1 ± 2.3	–	92.1 ± 2.5	42.4 ± 5.5
21	4-F	>100	–	73.5 ± 6.1	23.6 ± 3.8
22	4-Br	70.2 ± 5.3	–	45.5 ± 4.9	10.5 ± 1.4
23	4-OMe	>100	–	77.1 ± 7.0	–
24	3,4,5-triOMe	>100	–	90.9 ± 1.6	–

[a] The IC_{50} values represent the concentration to cause 50% inhibition of cell viability. Cancer cell lines HCT116, HEp-2 and HeLa and normal cell line CHO were treated with OABL arylation analogues for 72 h. All data (mean ± SD) are the average of three or four determinations.

2.3. Apoptosis-inducing effects of ABL, **1** (OABL) and **14** in HCT116 cells

It is well recognized that apoptosis is a very important mechanism involved in the anti-cancer effect and can be characterized by changes in nuclear morphology [38]. In order to determine the mechanism involved in ABL and its active analogues-induced cytotoxicity, nuclear morphology in HCT116 cells was evaluated by fluorescent microscopy. Staining with Hoechst 33258 showed fragmentation and condensation of chromatin in HCT116 cells treated with 50 μM ABL and 20 μM **1** (OABL) for 48 h, compared with the untreated control (Fig. 3), demonstrating a proapoptotic activity of ABL and OABL, which is similar with reported results in HL-60, COLO 205, HT-29 and AGS cells [4,11,36]. This apoptotic tendency was more apparent in **14** (lauroyl) at relatively low concentration (5 μM).

2.4. Effects of ABL, **1** (OABL) and **14** on cell cycle of HCT116 cells

To further explore the mechanisms by which ABL, **1** (OABL) and **14** exert their cytotoxic potencies, their effects on the cell cycle distribution of HCT116 cells were evaluated by flow cytometry after cells were stained with propidium iodide [39]. As shown in Fig. 4, treatment with 50 μM ABL resulted in accumulation of 46.4% cells at G0/G1 phase, as compared to 39.1% in untreated cells at 24 h. Similar accumulation of cells in G0/G1 phase has been reported previously in another human colon cancer HT-29 cells exposed to ABL [4]. However, treatment of the compounds **1** (OABL, 20 μM) resulted in a clear block of cells in G2/M phase, accumulation of 25.4% compared with 17.9% in untreated cells, which is similar to previous reports at colorectal cancer cells and breast cancer cells [5]. When HCT116 cells were in treatment of **14** (5 μM) for 24 h, a clear block of cells in G2/M phase was also observed with 26.9% cells accumulation, revealing that the superior cytotoxicity of **14** over ABL and **1** (OABL) was associated with a mechanism different from that of ABL and similar with that of OABL in cell cycle progression.

3. Conclusion

In summary, we have described determination of the absolute configuration of ABL, the semisynthesis of a series of new ABL analogues by modifying the 6-OH and C13 methylene groups, and evaluated their *in vitro* cytotoxicity to three human cancer cell lines (HCT116, HEp-2 and HeLa) and one normal hamster cell line (CHO) *in vitro*. Compounds **2**–**6**, **8**–**10**, **12**–**15**, **17** and **18** were found to have significant cytotoxic effects on these cancer cell lines, while exhibited no selectivity toward normal cells. The present study indicated that introduction of appropriate enhanced lipophilic aliphatic chains at 6-OH of ABL leads to an increase in the activity, while arylation (compounds **20**–**24**) of α-methylene-γ-lactone resulted in decreased potency. Impressively, compound **14**, bearing a lauroyl group (12C), demonstrated most potent *in vitro* cytotoxic effects, comparable to the positive drug etoposide, and cytotoxicity induced by the compound against HCT116 cells was mediated by apoptosis and cell cycle arrest at G2/M phase. These results indicate that **14** could act as a potential hit for the development of newer anticancer agents and would be further worth clarifying molecular protein target of the compound.

4. Materials and methods

4.1. Chemistry

4.1.1. General

Optical rotations were measured on a Rudolph Autopol III automatic polarimeter (Rudolph Research Analytical). All NMR spectra were recorded on a 400 MHz AMX Bruker NMR spectrometer in $CDCl_3$ with TMS as internal standard for protons and solvent signals as internal standard for carbon spectra. Chemical shift values are mentioned in δ (ppm) and coupling constants (J) are

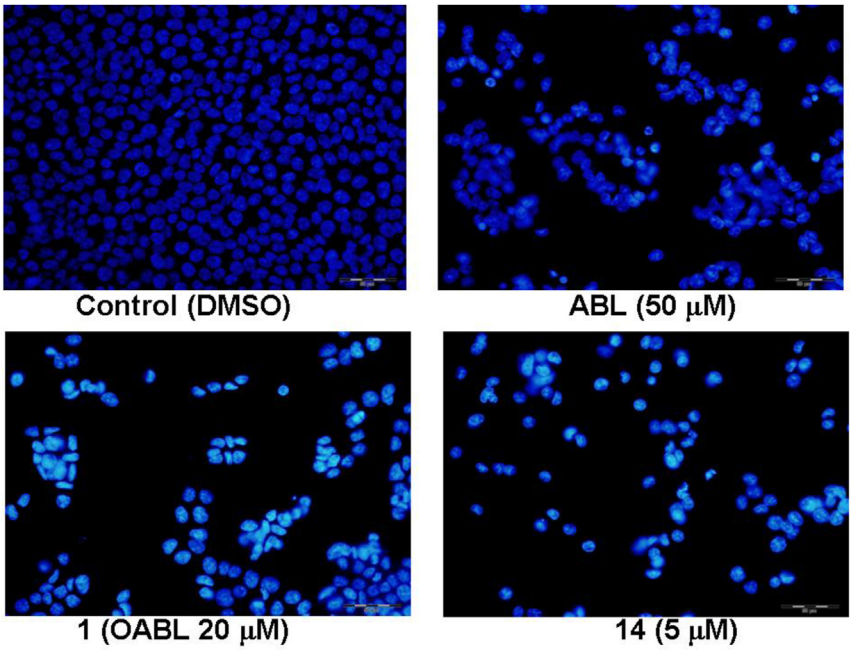


Fig. 3. Induction of apoptosis by ABL, **1** (OABL) and **14** at the indicated concentrations in HCT116 cells. 48 h after the treatment of these compounds at the indicated concentrations, cells were fixed, washed with PBS, stained with Hoechst 33258, and analyzed for morphological characteristics associated with apoptosis by fluorescence microscopic analysis (40×).

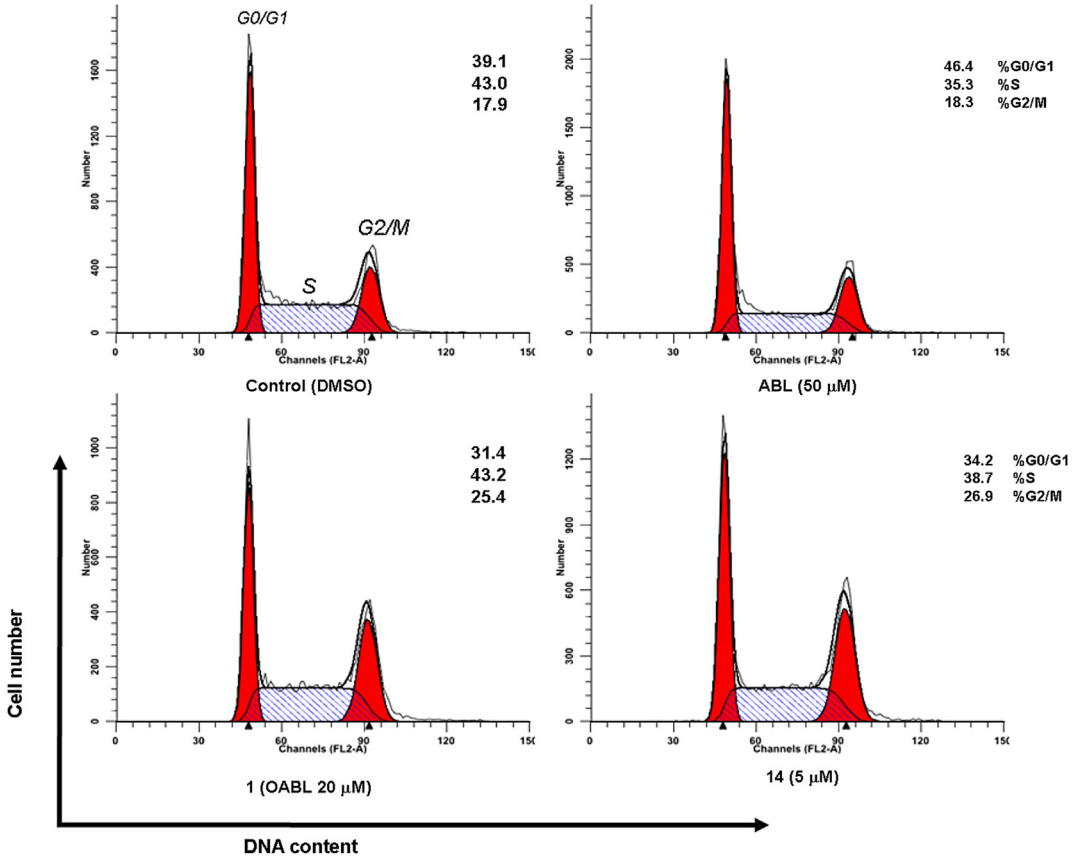


Fig. 4. Effects of ABL, **1** (OABL) and **14** at the indicated concentrations on the cell cycle of HCT116 cells. The cultured cells were treated with these compounds at the indicated concentrations for 24 h, then harvested, and analyzed by flow cytometry. Each experiment was performed in triplicate.

given in Hz. Mass spectra were recorded on an API2000 mass spectrometer (AB Sciex) or ESI-Thermo Fisher LTQ Fleet instrument spectrometer (Thermo Scientific). HR-ESI-MS spectra were obtained on a Bruker Daltonics APEx II FT-ICR mass spectrometer. Analytical HPLC was performed on a Waters 1525 series with UV detection at 215 or 254 nm along with evaporative light scattering detection (ELSD), Method = Agilent TC-C18, 5 μm, 4.6 × 250 mm, 10 min gradient, 80%MeOH:20%H_2O to 100% MeOH. Column chromatography (CC) was performed over silica gel (200–300 mesh, Qingdao Marine Chemical Ltd.). The progress of all reactions was monitored by TLC on 2 cm × 5 cm precoated silica gel 60 F_{254} plates of thickness of 0.25 mm (Qingdao Marine Chemical Group, Co.). Spots were visualized UV light (254, 365 nm) and/or by staining with 5% phosphomolybdic acid followed by heating. All commercially available solvents and reagents were freshly purified and dried by standard techniques prior to use.

4.1.2. Extraction and isolation

ABL was obtained from the flowers of *I. britannica* according to a reported procedure [3]. The dried flowers (50 kg) were extracted with 95% EtOH (3 × 200 L) for 12 h under refluxing. Evaporation of the EtOH solution under reduced pressure gave a crude extract, to which water (20 L) was added. The aqueous extract was successively partitioned with petroleum ether (PE, 3 × 20 L), EtOAc (3 × 20 L), and *n*-BuOH (3 × 20 L). The EtOAc-soluble part (2 kg) was subjected to silica gel column chromatography eluted with a gradient solvent system of PE/EtOAc (100:0–1:1) and then $CHCl_3$–MeOH (1:1) afforded 15 fractions. PE/EtOAc (1:1) fraction gave crude ABL as crystals, and the pure ABL (ca. 25 g) was obtained by recrystallization from anhydrous EtOH.

4.1.2.1. 1-O-Acetylbritannilactone (ABL). Cubic crystals, $[\alpha]_D^{25} = +103.5$ ($c = 0.52$ in $CHCl_3$; see Ref. [3] $[\alpha]_D = +101.6$ ($c = 0.25$ in $CHCl_3$)); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.95–1.04 (m, 1H, H-3b), 1.07 (d, $J = 6.9$ Hz, 3H, H-15), 1.19–1.31 (m, 2H, H-2b, H-3a), 1.34–1.47 (m, 1H, H-2a), 1.76 (s, 3H, H-14), 2.04 (s, 3H, AcO-1), 2.46 (dd, $J = 16.2$, 2.0 Hz, 1H, H-9b), 2.69 (ddd, $J = 9.5$, 6.9, 4.4 Hz, 1H, H-4), 2.77–2.90 (m, 1H, H-9a), 3.50–3.59 (m, 1H, H-7), 3.86–4.02 (m, 2H, H-1), 4.18 (s, 1H, H-6), 4.97–5.05 (m, 1H, H-8), 5.72 (d, $J = 2.3$ Hz, 1H, H-13b), 6.31 (d, $J = 2.7$ Hz, 1H, H-13a); ^{13}C NMR (100 MHz, $CDCl_3$): δ 19.3 (C-15), 20.6 (C-14), 20.9 (CH_3CO-1), 26.5 (C-2), 31.0 (C-3), 32.9 (C-4), 34.4 (C-9), 45.0 (C-7), 64.2 (C-1), 68.3 (C-6), 75.9 (C-8), 123.8 (C-13), 131.1 (C-10), 136.5 (C-5), 136.9 (C-11), 169.9 (C-12), 171.2 (CH_3CO-1); ESI-MS: 331.4 $[M+Na]^+$; HPLC: t_R = 3.184 min, purity > 99% at ELSD.

4.1.3. X-ray experimental

Single crystals of ABL and compound **23** were obtained by recrystallization in ethanol. A suitable crystal was selected and analysed on a SuperNova, Dual, Cu at zero, Eos diffractometer. The crystal was kept at 293(2) K during data collection. Using Olex2 [40], the structure was solved with the Superflip [41] structure solution program using Charge Flipping and refined with the ShelXL [42] refinement package using Least Squares minimisation. The X-ray data is available in the reference [32].

4.1.4. Chemical synthesis

*4.1.4.1. Procedure for the synthesis of ABL analogues (**1**–**11**).* To a suspension of acid anhydride (0.15 mmol) and DMAP in anhydrous CH_2Cl_2 (1 mL) in an ice-bath was added ABL (0.1 mmol) in anhydrous CH_2Cl_2 (1 mL) solution. After completion of the reaction from

10 min to 4 h at room temperature, ice water (2 mL) was added to the solvent and stirred for 20 min, then extracted with CH_2Cl_2, dried and filtered. After removal of the solvent, the crude product was purified by silica gel chromatography (EtOAc/PE) to afford compounds **1**–**11** in 85%–98% yields.

*4.1.4.1.1. 1,6-O,O-Diacetylbritannilactone (**1**).* White solid. Yield: 87%; $[\alpha]_D^{25} = -39.5$ ($c = 0.57$ in $CHCl_3$; see Ref. [33] $[\alpha]_D = -38.4$ ($c = 0.5$ in $CHCl_3$)); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (d, $J = 6.9$ Hz, 3H, H-15), 0.97–1.09 (m, 1H, H-3b), 1.21–1.32 (m, 2H, H-2b, H-3a), 1.35–1.46 (m, 1H, H-2a), 1.81 (s, 3H, H-14), 2.05 (s, 3H, AcO-6), 2.06 (s, 3H, AcO-1), 2.50 (dd, $J = 16.2$, 2.1 Hz, 1H, H-9b), 2.63–2.76 (m, 2H, H-4, H-9a), 3.46–3.51 (m, 1H, H-7), 3.86–4.01 (m, 2H, H-1), 4.90–5.00 (m, 1H, H-8), 5.22 (d, $J = 1.70$ Hz, 1H, H-6), 5.95 (d, $J = 2.3$ Hz, 1H, H-13b), 6.38 (d, $J = 2.7$ Hz, 1H, H-13a); ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.4 (C-15), 20.5 (C-14), 21.0 (*C*H_3CO-6), 21.3 (*C*H_3CO-1), 26.5 (C-2), 31.1 (C-3), 33.0 (C-4), 34.5 (C-9), 42.9 (C-7), 64.2 (C-1), 69.2 (C-6), 74.9 (C-8), 125.0 (C-13), 132.0 (C-5), 133.8 (C-10), 136.2 (C-11), 169.5 (C-12), 170.9 (CH_3*C*O-6), 171.2 (CH_3*C*O-1); ESI-MS: 389.0 $[M+K]^+$; HPLC: $t_R = 4.164$ min, purity = 98% at ELSD, 97% at 215 nm.

*4.1.4.1.2. 1-O-Acetyl-6-O-propionylbritannilactone (**2**).* Yellow solid. Yield: 98%; $[\alpha]_D^{25} = -32.3$ ($c = 0.16$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.87 (d, $J = 6.9$ Hz, 3H, H-15), 0.98–1.07 (m, 1H, H-3b), 1.10–1.21 (m, 3H, C*H*$_3$CH$_2$COO-6), 1.23–1.30 (m, 2H, H-2b, H-3a), 1.34–1.46 (m, 1H, H-2a), 1.80 (s, 3H, H-14), 2.04 (s, 3H, AcO-1), 2.26–2.34 (m, 2H, CH$_3$C*H*$_2$COO-6), 2.45–2.52 (m, 1H, H-9b), 2.62–2.75 (m, 2H, H-4, H-9a), 3.42–3.52 (m, 1H, H-7), 3.86–4.00 (m, 2H, H-1), 4.90–4.98 (m, 1H, H-8), 5.21 (d, $J = 1.70$ Hz, 1H, H-6), 5.94 (d, $J = 2.31$ Hz, 1H, H-13b), 6.37 (d, $J = 2.68$ Hz, 1H, H-13a); ^{13}C NMR (100 MHz, $CDCl_3$): δ 9.1 (*C*H$_3$CH$_2$COO-6), 18.5 (C-15), 20.5 (C-14), 21.0 (*C*H$_3$COO-1), 26.5 (C-2), 27.8 (CH$_3$*C*H$_2$COO-6), 31.0 (C-3), 33.0 (C-4), 34.5 (C-9), 42.9 (C-7), 64.2 (C-1), 69.1 (C-6), 74.9 (C-8), 124.9 (C-13), 132.0 (C-5), 133.7 (C-10), 136.3 (C-11), 169.5 (C-12), 171.2 (CH_3*C*OO-1), 174.3 (CH$_3$CH$_2$*C*OO-6); ESI-MS: 403.0 $[M+K]^+$; HR-ESI-MS: calcd for $C_{20}H_{28}NaO_6$ $[M+Na]^+$: 387.1778; found: 387.1782, error = 1 ppm; HPLC: $t_R = 4.758$ min, purity = 99% at ELSD, 98% at 215 nm.

*4.1.4.1.3. 1-O-Acetyl-6-O-butyrylbritannilactone (**3**).* White oil. Yield: 89%; $[\alpha]_D^{25} = -47.5$ ($c = 0.14$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.87 (d, $J = 6.9$ Hz, 3H, H-15), 0.94 (t, $J = 7.4$ Hz, 3H, C*H*$_3$(CH$_2$)$_2$COO-6), 0.98–1.08 (m, 1H, H-3b), 1.20–1.32 (m, 2H, H-2b, H-3a), 1.33–1.50 (m, 1H, H-2a), 1.55–1.71 (m, 2H, CH$_3$C*H*$_2$CH$_2$COO-6), 1.80 (s, 3H, H-14), 2.04 (s, 3H, AcO-1), 2.22–2.32 (m, 2H, CH$_3$CH$_2$C*H*$_2$COO-6), 2.49 (dd, $J = 16.2$, 2.1 Hz, 1H, H-9b), 2.61–2.79 (m, 2H, H-4, H-9a), 3.43–3.54 (m, 1H, H-7), 3.82–4.02 (m, 2H, 1-H), 4.88–5.00 (m, 1H, H-8), 5.22 (d, $J = 1.8$ Hz, 1H, H-6), 5.94 (d, $J = 2.4$ Hz, 1H, H-13b), 6.37 (d, $J = 2.7$ Hz, 1H, H-13a); ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.6 (*C*H$_3$(CH$_2$)$_2$COO-6), 18.4 (CH$_3$*C*H$_2$CH$_2$COO-6), 18.5 (C-15), 20.5 (C-14), 21.0 (*C*H$_3$COO-1), 26.5 (C-2), 31.0 (C-3), 33.0 (C-4), 34.5 (C-9), 36.4 (CH$_3$CH$_2$*C*H$_2$COO-6), 42.9 (C-7), 63.9 (C-1), 69.0 (C-6), 74.9 (C-8), 124.9 (C-13), 132.1 (C-5), 133.7 (C-10), 136.3 (C-11), 169.5 (C-12), 171.2 (CH$_3$*C*OO-1), 173.5 (CH$_3$(CH$_2$)$_2$*C*OO-6); ESI-MS: 401.0 $[M+Na]^+$; HR-ESI-MS: calcd for $C_{21}H_{30}NaO_6$ $[M+Na]^+$: 401.1935; found: 401.1943, error = 2 ppm; HPLC: $t_R = 5.217$ min, purity > 99% at ELSD, >99% at 215 nm.

*4.1.4.1.4. 1-O-Acetyl-6-O-valerylbritannilactone (**4**).* White solid. Yield: 94%; $[\alpha]_D^{25} = -49.0$ ($c = 0.12$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.87 (d, $J = 6.9$ Hz, 3H, H-15), 0.92 (t, $J = 7.4$ Hz, 3H, C*H*$_3$(CH$_2$)$_3$COO-6), 0.95–1.09 (m, 1H, H-3b), 1.19–1.48 (m, 5H, H-2, H-3a, CH$_3$C*H*$_2$(CH$_2$)$_2$COO-6), 1.50–1.66 (m, 2H, CH$_3$CH$_2$C*H*$_2$CH$_2$COO-6), 1.80 (s, 3H, H-14), 2.04 (s, 3H, AcO-1), 2.28 (t, $J = 7.6$ Hz, 2H, CH$_3$(CH$_2$)$_2$C*H*$_2$COO-6), 2.49 (dd, $J = 16.1$, 2.0 Hz, 1H, H-9b), 2.60–2.78 (m, 2H, H-4, H-9a), 3.33–3.54 (m, 1H, H-7), 3.83–4.04 (m, 2H, H-1), 4.81–5.01 (m, 1H, H-8), 5.21 (d, $J = 1.6$ Hz, 1H, H-6), 5.94 (d, $J = 2.3$ Hz, 1H, H-13b), 6.37 (d, $J = 2.7$ Hz, 1H, H-13a); ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.7 (*C*H$_3$(CH$_2$)$_3$COO-6), 18.5 (C-15), 20.5 (C-14), 21.0 (*C*H$_3$COO-1), 22.2 (CH$_3$CH$_2$C*H*$_2$CH$_2$COO-6), 26.5 (C-2), 27.0 (CH$_3$C*H*$_2$(CH$_2$)$_2$COO-6), 31.0 (C-3), 33.0 (C-4), 34.3 (CH$_3$(CH$_2$)$_2$C*H*$_2$COO-6), 34.5 (C-9), 42.9 (C-7), 64.2 (C-1), 69.0 (C-6), 74.9 (C-8), 124.9 (C-13), 132.1 (C-5), 133.7 (C-10), 136.3 (C-11), 169.5 (C-12), 171.2 (CH$_3$*C*OO-1), 173.7 (CH$_3$(CH$_2$)$_3$*C*OO-6); HR-ESI-MS: calcd for $C_{22}H_{32}NaO_6$ $[M+Na]^+$: 415.2091; found: 415.2100, error = 2.2 ppm; HPLC: $t_R = 5.997$ min, purity = 99% at ELSD, 98% at 215 nm.

*4.1.4.1.5. 1-O-Acetyl-6-O-hexanoylbritannilactone (**5**).* White oil. Yield: 86%; $[\alpha]_D^{25} = -45.5$ ($c = 0.24$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.83–0.92 (m, 6H, H-15, C*H*$_3$(CH$_2$)$_4$COO-6), 0.97–1.08 (m, 1H, H-3b), 1.21–1.35 (m, 6H, H-2b, H-3a, CH$_3$C*H*$_2$C*H*$_2$(CH$_2$)$_2$COO-6), 1.36–1.45 (m, 1H, H-2a), 1.60 (q, $J = 7.1$ Hz, 2H, CH$_3$CH$_2$CH$_2$C*H*$_2$CH$_2$COO-6), 1.79 (s, 3H, H-14), 2.03 (s, 3H, AcO-1), 2.27 (t, $J = 7.6$ Hz, 2H, CH$_3$(CH$_2$)$_3$C*H*$_2$COO-6), 2.48 (dd, $J = 16.1$, 1.7 Hz, 1H, H-9b), 2.61–2.77 (m, 2H, H-4, H-9a), 3.43–3.50 (m, 1H, H-7), 3.85–4.00 (m, 2H, H-1), 4.93 (dt, $J = 4.0$, 1.9 Hz, 1H, H-8), 5.20 (d, $J = 1.2$ Hz, 1H, H-6), 5.93 (d, $J = 2.2$ Hz, 1H, H-13b), 6.36 (d, $J = 2.6$ Hz, 1H, H-13a); ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.8 (*C*H$_3$(CH$_2$)$_4$COO-6), 18.5 (C-15), 20.4 (C-14), 20.9 (*C*H$_3$COO-1), 22.2 (CH$_3$CH$_2$C*H*$_2$(CH$_2$)$_2$COO-6), 24.6 (CH$_3$CH$_2$C*H*$_2$(CH$_2$)$_2$COO-6), 26.4 (C-2), 31.0 (C-3), 31.2 (CH$_3$CH$_2$CH$_2$C*H*$_2$CH$_2$COO-6), 33.0 (C-4), 34.5 (C-9), 34.5 (CH$_3$(CH$_2$)$_3$CH$_2$*C*OO-6), 42.8 (C-7), 64.2 (C-1), 69.0 (C-6), 74.9 (C-8), 124.9 (C-13), 132.0 (C-5), 133.7 (C-10), 136.2 (C-11), 169.5 (C-12), 171.2 (CH$_3$*C*OO-1), 173.7 (CH$_3$(CH$_2$)$_4$*C*OO-6); ESI-MS: 429.2 $[M+Na]^+$; HR-ESI-MS: calcd for $C_{23}H_{34}NaO_6$ $[M+Na]^+$: 429.2248; found: 429.2244, error = 0.9 ppm; HPLC: $t_R = 7.352$ min, purity > 99% at ELSD, 98% at 215 nm.

*4.1.4.1.6. 1-O-Acetyl-6-O-heptanoylbritannilactone (**6**).* Colourless oil. Yield: 85%; $[\alpha]_D^{25} = -58.6$ ($c = 0.11$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.83–0.92 (m, 6H, H-15, C*H*$_3$(CH$_2$)$_5$COO-6), 0.97–1.08 (m, 1H, H-3b), 1.22–1.34 (m, 8H, H-2b, H-3a, CH$_3$(C*H*$_2$)$_3$(CH$_2$)$_2$COO-6), 1.35–1.46 (m, 1H, H-2a), 1.54–1.68 (m, 2H, CH$_3$(CH$_2$)$_3$C*H*$_2$CH$_2$COO-6), 1.80 (s, 3H, H-14), 2.04 (s, 3H, AcO-1), 2.28 (t, $J = 7.5$ Hz, 2H, CH$_3$(CH$_2$)$_4$C*H*$_2$COO-6), 2.49 (dd, $J = 16.1$, 2.0 Hz, 1H, H-9b), 2.59–2.79 (m, 2H, H-4, H-9a), 3.43–3.52 (m, 1H, H-7), 3.83–4.03 (m, 2H, H-1), 4.86–4.99 (m, 1H, H-8), 5.21 (d, $J = 1.7$ Hz, 1H, H-6), 5.94 (d, $J = 2.3$ Hz, 1H, H-13b), 6.37 (d, $J = 2.7$ Hz, 1H, H-13a); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.0 (*C*H$_3$(CH$_2$)$_5$COO-6), 18.5 (C-15), 20.5 (C-14), 21.0 (*C*H$_3$COO-1), 22.4 (CH$_3$C*H*$_2$CH$_2$(CH$_2$)$_3$COO-6), 24.9 (CH$_3$CH$_2$C*H*$_2$(CH$_2$)$_3$COO-6), 26.5 (C-2), 28.8 (CH$_3$(CH$_2$)$_2$CH$_2$(CH$_2$)$_2$COO-6), 31.0 (C-3), 31.4 (CH$_3$(CH$_2$)$_3$CH$_2$CH$_2$COO-6), 33.0 (C-4), 34.5 (C-9), 34.5 (CH$_3$(CH$_2$)$_4$CH$_2$*C*OO-6), 42.9 (C-7), 64.2 (C-1), 69.0 (C-6), 75.1 (C-8), 124.9 (C-13), 132.1 (C-5), 133.7 (C-10), 136.3 (C-11), 169.5 (C-12), 171.2 (CH$_3$*C*OO-1), 173.7 (CH$_3$(CH$_2$)$_5$*C*OO-6); ESI-MS: 459.0 $[M+K]^+$; HR-ESI-MS: calcd for $C_{24}H_{36}NaO_6$ $[M+Na]^+$: 443.2404; found: 443.2409, error = 1.1 ppm; HPLC: $t_R = 9.587$ min, purity > 99% at ELSD.

*4.1.4.1.7. 1-O-Acetyl-6-O-decoylbritannilactone (**7**).* Colourless oil. Yield: 85%; $[\alpha]_D^{25} = -38.8$ ($c = 0.07$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.83–0.91 (m, 6H, H-15, C*H*$_3$(CH$_2$)$_6$COO-6), 1.01 (d, $J = 9.5$ Hz, 1H, H-3b), 1.19–1.34 (m, 10H, H-2b, H-3a, CH$_3$(C*H*$_2$)$_4$CH$_2$CH$_2$COO-6), 1.35–1.43 (m, 1H, H-2a), 1.54–1.66 (m, 2H, CH$_3$(CH$_2$)$_4$C*H*$_2$CH$_2$COO-6), 1.79 (s, 3H, H-14), 2.03 (s, 3H, AcO-1), 2.27 (t, $J = 7.4$ Hz, 2H, CH$_3$(CH$_2$)$_5$ C*H*$_2$COO-6), 2.48 (dd, $J = 16.1$, 2.0 Hz, 1H, H-9b), 2.62–2.74 (m, 2H, H-4, H-9a), 3.42–3.50 (m, 1H, H-7), 3.85–3.99 (m, 2H, H-1), 4.90–4.95 (m, 1H, H-8), 5.20 (d, $J = 1.8$ Hz, 1H, H-6), 5.93 (d, $J = 2.3$ Hz, 1H, H-13b), 6.36 (d, $J = 2.9$ Hz, 1H, H-13a); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.0 (*C*H$_3$(CH$_2$)$_6$COO-6), 18.5 (C-15), 20.4 (C-14), 20.9 (*C*H$_3$COO-1), 22.5 (CH$_3$CH$_2$(CH$_2$)$_5$COO-6), 24.9 (CH$_3$CH$_2$CH$_2$(CH$_2$)$_4$COO-6), 26.4 (C-2), 28.8 (CH$_3$(CH$_2$)$_2$CH$_2$(CH$_2$)$_3$COO-6), 29.0 (CH$_3$(CH$_2$)$_3$CH$_2$(CH$_2$)$_2$COO-6), 31.0 (C-3), 31.6 (CH$_3$(CH$_2$)$_4$CH$_2$CH$_2$COO-6), 33.0 (C-4), 34.5 (C-9),

42.8 (C-7), 53.4 ($CH_3(CH_2)_5CH_2COO$-6), 64.2 (C-1), 69.0 (C-6), 74.9 (C-8), 124.9 (C-13), 132.0 (C-5), 133.7 (C-10), 136.2 (C-11), 169.5 (C-12), 171.2 (CH_3COO-1), 173.7 ($CH_3(CH_2)_6COO$-6); ESI-MS: 457.3 $[M+Na]^+$; HR-ESI-MS: calcd for $C_{25}H_{37}NaO_6$ $[M+Na]^+$: 457.2561; found: 457.2554, error = 1.5 ppm; HPLC: t_R = 9.502 min, purity > 99% at ELSD, >99% at 215 nm.

*4.1.4.1.8. 1-O-Acetyl-6-O-chloracetylbritannilactone (**8**).* White solid. Yield: 99%; $[\alpha]_D^{25} = -54.0$ (c = 0.13 in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 0.87 (d, J = 6.8 Hz, 3H, H-15), 0.93–1.06 (m, 1H, H-3b), 1.17–1.30 (m, 2H, H-2b, H-3a), 1.32–1.43 (m, 1H, H-2a), 1.80 (s, 3H, H-14), 2.02 (s, 3H, AcO-1), 2.50 (d, J = 16.2 Hz, 1H, H-9b), 2.61–2.75 (m, 2H, H-4, H-9a), 3.45–3.55 (m, 1H, H-7), 3.85–3.98 (m, 2H, H-1), 3.99–4.05 (m, 2H, $ClCH_2COO$-6), 4.91–4.99 (m, 1H, H-8), 5.25 (s, 1H, H-6), 5.93 (s, 1H, H-13b), 6.38 (s, 1H, H-13a); ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.5 (C-15), 20.5 (C-14), 20.9 (CH_3COO-1), 26.4 (C-2), 31.0 (C-3), 32.9 (C-4), 34.5 (C-9), 40.9 ($ClCH_2COO$-6), 42.7 (C-7), 64.1 (C-1), 70.9 (C-6), 74.5 (C-8), 125.2 (C-13), 131.2 (C-5), 134.8 (C-10), 135.8 (C-11), 167.1 (CH_2ClCOO-6), 169.2 (C-12), 171.1 (CH_3COO-1); ESI-MS: 407.2 $[M+Na]^+$; HR-ESI-MS: calcd for $C_{19}H_{26}ClO_6$ $[M+H]^+$: 385.1412; found: 385.1428, error = 4.2 ppm; HPLC: t_R = 4.338 min, purity > 99% at ELSD, 99% at 215 nm.

*4.1.4.1.9. 1-O-Acetyl-6-O-isobutyrylbritannilactone (**9**).* White solid. Yield: 99%; $[\alpha]_D^{25} = -49.0$ (c = 0.09 in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 0.86 (d, J = 6.9 Hz, 3H, H-15), 0.97–1.08 (m, 1H, H-3b), 1.15 (d, J = 6.9 Hz, 6H, $(CH_3)_2CHCOO$-6), 1.21–1.31 (m, 2H, H-2b, H-3a), 1.34–1.45 (m, 1H, H-2a), 1.80 (s, 3H, H-14), 2.04 (s, 3H, AcO-1), 2.45–2.56 (m, 2H, H-9b, $(CH_3)_2CHCOO$-6), 2.60–2.76 (m, 2H, H-4, H-9a), 3.41–3.49 (m, 1H, H-7), 3.85–4.00 (m, 2H, H-1), 4.90–4.98 (m, 1H, H-8), 5.19 (d, J = 1.7 Hz, 1H, H-6), 5.94 (d, J = 2.3 Hz, 1H, H-13b), 6.37 (d, J = 2.9 Hz, 1H, H-13a); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.6 (C-15), 18.7 (CH_3CH_3CHCOO-6), 18.8 (CH_3CH_3CHCOO-6), 20.5 (C-14), 21.0 (CH_3COO-1), 26.5 (C-2), 31.0 (C-3), 33.0 (C-4), 34.1, 34.6 (C-9), 42.9 (C-7), 64.2 (C-1), 69.0 (C-6), 74.9 (C-8), 124.9 (C-13), 132.1 (C-5), 133.6 (C-10), 136.3 (C-11), 169.5 (C-12), 171.2 (CH_3COO-1), 176.9 ($(CH_3)_2CHCOO$-6); ESI-MS: 401.2 $[M+Na]^+$; HR-ESI-MS: calcd for $C_{21}H_{30}NaO_6$ $[M+Na]^+$: 401.1935; found: 401.1946, error = 2.7 ppm; HPLC: t_R = 5.394 min, purity > 99% at ELSD, >99% at 215 nm.

*4.1.4.1.10. 1-O-Acetyl-6-O-pivaloylbritannilactone (**10**).* Pale yellow oil. Yield: 86%; $[\alpha]_D^{25} = -31.0$ (c = 0.10 in $CHCl_3$); ^1H NMR (100 MHz, $CDCl_3$): δ 0.83–0.90 (m, 3H, H-15), 1.00–1.09 (m, 1H, H-3b), 1.15–1.19 (m, 9H, $(CH_3)_3CCOO$-6), 1.21–1.31 (m, 3H, H-2, H-3a), 1.81 (d, J = 0.9 Hz, 3H, H-14), 2.04 (s, 3H, AcO-1), 2.49 (dd, J = 16.1, 2.1 Hz, 1H, H-9b), 2.67 (dd, J = 3.6, 1.0 Hz, 2H, H-4, H-9a), 3.42–3.46 (m, 1H, H-7), 3.93 (m, 2H, H-1), 4.90–4.98 (m, 1H, H-8), 5.17 (d, J = 1.8 Hz, 1H, H-6), 5.94 (d, J = 2.4 Hz, 1H, H-13b), 6.37 (d, J = 2.8 Hz, 1H, H-13a); ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.7 (C-15), 20.5 (C-14), 20.9 (CH_3COO-1), 26.5 (C-2), 26.9 (3C, $(CH_3)_3CCOO$-6), 31.0 (C-3), 33.0 (C-4), 34.6 (C-9), 42.8 (C-7), 64.2 (C-1), 69.1 (C-6), 74.9 (C-8), 124.9 (C-13), 132.3 (C-5), 133.6 (C-10), 136.2 (C-11), 169.5 (C-12), 171.2 (CH_3COO-1), 178.2 ($(CH_3)_3CCOO$-6); ESI-MS: 415.1 $[M+Na]^+$; HR-ESI-MS: calcd for $C_{22}H_{32}NaO_6$ $[M+Na]^+$: 415.2091; found: 415.2099, error = 1.9 ppm; HPLC: t_R = 11.152 min, purity > 99% at ELSD, 98% at 215 nm.

*4.1.4.1.11. 1-O-Acetyl-6-O-carboxypropionylbritannilactone (**11**).* White oil. Yield: 88%; $[\alpha]_D^{25} = -112.0$ (c = 0.13 in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 0.86 (d, J = 6.8 Hz, 3H, H-15), 0.95–1.06 (m, 1H, H-3b), 1.19–1.31 (m, 2H, H-2b, H-3a), 1.39 (m, 1H, H-2a), 1.79 (s, 3H, H-14), 2.02 (s, 3H, AcO-1), 2.47 (dd, J = 16.2, 2.0 Hz, 1H, H-9b), 2.54–2.60 (m, 2H, H-4, H-9a), 2.61–2.73 (m, 4H, $HOOC(CH_2)_2COO$-6), 3.45–3.51 (m, 1H, H-7), 3.85–3.99 (m, 2H, H-1), 4.87–4.97 (m, 1H, H-8), 5.22 (d, J = 1.7 Hz, 1H, H-6), 5.92 (d, J = 2.3 Hz, 1H, H-13b), 6.36 (d, J = 2.6 Hz, 1H, H-13a); ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.4 (C-15), 20.4 (C-14), 20.9 (CH_3COO-1), 26.4 (C-2), 28.8 ($HOOCCH_2CH_2COO$-6), 29.1 ($HOOCCH_2CH_2COO$-6), 31.0 (C-3), 33.0 (C-4), 34.5 (C-9), 42.6 (C-7), 64.2 (C-1), 69.6 (C-6), 74.9 (C-8), 125.1 (C-13), 131.7 (C-5), 134.0 (C-10), 136.1 (C-11), 169.6 (C-12), 171.3 (CH_3COO-1), 172.0 ($HOOC(CH_2)_2COO$-6), 177.7 ($HOOC(CH_2)_2COO$-6); ESI-MS: 431.1 $[M+Na]^+$; HR-ESI-MS: calcd for $C_{21}H_{29}O_8$ $[M+H]^+$: 409.1857; found: 409.1865, error = 2 ppm; HPLC: t_R = 0.410 min, purity > 99% at ELSD, >99% at 215 nm.

*4.1.4.2. Procedure for the synthesis of ABL analogues (**12**–**18**).* To a suspension of a corresponding acid (pelargonic acid for **12**, capric acid for **13**, lauric acid for **14**, myristic acid for **15**, stearic acid for **16**, *p*-bromocinnamic acid for **17**, *p*-trifluoromethylcinnamic acid for **18**) (0.2 mmol), DMAP and DCC in anhydrous CH_2Cl_2 (1 mL) was added ABL (0.1 mmol) in anhydrous CH_2Cl_2 (1 mL) solution. After completion of the reaction from 5 to 20 h at room temperature, water (2 mL) was added to the mixture and stirred for 20 min, then extracted with CH_2Cl_2, dried and filtered. After removal of the solvent, the crude product was purified by silica gel chromatography (EtOAc/PE) to afford analogues **12**–**18** in the yield from 65% to 83%.

*4.1.4.2.1. 1-O-Acetyl-6-O-nonanoylbritannilactone (**12**).* Colourless oil. Yield: 71%; $[\alpha]_D^{25} = -50.7$ (c = 0.17 in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 0.77–0.89 (m, 6H, H-15, $CH_3(CH_2)_7COO$-6), 0.94–1.08 (m, 1H, H-3b), 1.19–1.31 (m, 13H, H-2, H-3a, $CH_3(CH_2)_5CH_2CH_2COO$-6), 1.37 (ddd, J = 18.08, 7.12, 3.53 Hz, 1H), 1.51–1.67 (m, 2H, $CH_3(CH_2)_5CH_2CH_2COO$-6), 1.78 (s, 3H, H-14), 2.02 (s, 3H, AcO-1), 2.21–2.32 (m, 2H, H-4, H-9a), 2.47 (dd, J = 16.1, 2.0 Hz, 1H, H-9b), 2.59–2.74 (m, 2H, $CH_3(CH_2)_5CH_2CH_2COO$-6), 3.40–3.50 (m, 1H, H-7), 3.83–4.02 (m, 2H, H-1), 4.86–4.96 (m, 1H, H-8), 5.19 (d, J = 1.7 Hz, 1H, H-6), 5.93 (d, J = 2.2 Hz, 1H, H-13b), 6.35 (d, J = 2.6 Hz, 1H, H-13a); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.3 ($CH_3(CH_2)_7COO$-6), 18.8 (C-15), 20.7 (C-14), 21.2 (CH_3COO-1), 22.9 ($CH_3CH_2(CH_2)_6COO$-6), 25.2 ($CH_3(CH_2)_5CH_2CH_2COO$-6), 26.7 (C-2), 29.4 ($CH_3(CH_2)_2(CH_2)_3(CH_2)_2COO$-6), 31.3 (C-3), 32.0 ($CH_3CH_2CH_2(CH_2)_5COO$-6), 33.3 (C-4), 34.8 (C-9), 34.8 ($CH_3(CH_2)_6CH_2COO$-6), 43.1 (C-7), 64.5 (C-1), 69.3 (C-6), 75.2 (C-8), 125.2 (C-13), 132.3 (C-5), 134.0 (C-10), 136.5 (C-11), 169.8 (C-12), 171.5 (CH_3COO-1), 174.0 ($CH_3(CH_2)_7COO$-6); ESI-MS: 487.2 $[M+K]^+$; HR-ESI-MS: calcd for $C_{26}H_{40}NaO_6$ $[M+Na]^+$: 471.2717; found: 471.2710, error = 1.5 ppm; HPLC: t_R = 11.531 min, purity > 99% at ELSD, >99% at 215 nm.

*4.1.4.2.2. 1-O-Acetyl-6-O-decanoylbritannilactone (**13**).* Colourless oil. Yield: 74%; $[\alpha]_D^{25} = -40.4$ (c = 0.20 in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 0.84–0.91 (m, 6H, H-15, $CH_3(CH_2)_8COO$-6), 0.98–1.08 (m, 1H, H-3b), 1.20–1.34 (m, 14H, H-2b, H-3a, $CH_3(CH_2)_6CH_2CH_2COO$-6), 1.35–1.45 (m, 1H, m, 1H, H-2a), 1.54–1.70 (m, 2H, $CH_3(CH_2)_6CH_2CH_2COO$-6), 1.80 (s, 3H, H-14), 2.03 (s, 3H, AcO-1), 2.27 (t, J = 7.5 Hz, 2H, $CH_3(CH_2)_6CH_2CH_2COO$-6), 2.48 (dd, J = 16.1, 2.0 Hz, 1H, H-9b), 2.63–2.75 (m, 2H, H-4, H-9a), 3.43–3.52 (m, 1H, H-7), 3.86–4.01 (m, 2H, H-1), 4.88–4.98 (m, 1H, H-8), 5.21 (d, J = 1.8 Hz, 1H, H-6), 5.94 (d, J = 2.3 Hz, 1H, H-13b), 6.36 (d, J = 2.6 Hz, 1H, H-13a); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.0 ($CH_3(CH_2)_8COO$-6), 18.5 (C-15), 20.5 (C-14), 20.9 (CH_3COO-1), 22.6 ($CH_3CH_2(CH_2)_7COO$-6), 24.9 ($CH_3(CH_2)_6CH_2CH_2COO$-6), 26.5 (C-2), 29.1–29.4 (4C, $CH_3(CH_2)_2(CH_2)_4(CH_2)_2COO$-6), 31.1 (C-3), 31.8 ($CH_3CH_2CH_2(CH_2)_6COO$-6), 33.1 (C-4), 34.5 (C-9), 34.6 ($CH_3(CH_2)_7CH_2COO$-6), 42.9 (C-7), 64.2 (C-1), 69.0 (C-6), 74.9 (C-8), 124.9 (C-13), 132.1 (C-5), 133.7 (C-10), 136.3 (C-11), 169.5 (C-12), 171.1 (CH_3COO-1), 173.7 ($CH_3(CH_2)_8COO$-6); ESI-MS: 485.1 $[M+Na]^+$; HR-ESI-MS: calcd for $C_{27}H_{42}NaO_6$ $[M+Na]^+$: 485.2874; found: 485.2886, error = 2.5 ppm; HPLC: t_R = 12.862 min, purity = 95% at ELSD.

*4.1.4.2.3. 1-O-Acetyl-6-O-lauroylbritannilactone (**14**).* White oil. Yield: 73%; $[\alpha]_D^{25} = -37.7$ (c = 0.23 in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 0.83–0.94 (m, 6H, H-15, $CH_3(CH_2)_{10}COO$-6), 0.97–1.11 (m, 1H, H-3b), 1.19–1.47 (m, 19H, H-2, H-3a, $CH_3(CH_2)_8CH_2CH_2COO$-6),

1.54–1.69 (m, 2H, $CH_3(CH_2)_8CH_2CH_2COO$-6), 1.80 (s, 3H, H-14), 2.02–2.08 (m, 3H, AcO-1), 2.27 (t, J = 7.5 Hz, 2H, $CH_3(CH_2)_8CH_2CH_2COO$-6), 2.44–2.53 (m, 1H, H-9b), 2.61–2.78 (m, 2H, H-4, H-9a), 3.43–3.52 (m, 1H, H-7), 3.85–4.03 (m, 2H, H-1), 4.93 (ddd, J = 5.8, 3.8, 1.8 Hz, H-8), 5.21 (d, J = 1.6 Hz, 1H, H-6), 5.94 (d, J = 2.3 Hz, 1H, H-13b), 6.37 (d, J = 2.8 Hz, 1H, H-13a); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.3 ($CH_3(CH_2)_{10}COO$-6), 18.7 (C-15), 20.6 (C-14), 21.1 (CH_3COO-1), 22.8 ($CH_3CH_2(CH_2)_9COO$-6), 25.1 ($CH_3(CH_2)_8CH_2CH_2COO$-6), 26.7 (C-2), 29.2–29.7 (6C, $CH_3(CH_2)_2(CH_2)_6(CH_2)_2COO$-6), 31.2 (C-3), 32.0 ($CH_3CH_2CH_2(CH_2)_8COO$-6), 33.2 (C-4), 34.7 (C-9), 34.74 ($CH_3(CH_2)_9CH_2COO$-6), 43.1 (C-7), 64.4 (C-1), 69.2 (C-6), 75.1 (C-8), 125.1 (C-13), 132.3 (C-5), 133.8 (C-10), 136.5 (C-11), 169.7 (C-12), 171.3 (CH_3COO-1), 178.9 ($CH_3(CH_2)_{10}COO$-6); ESI-MS: 513.0 $[M+Na]^+$; HR-ESI-MS: calcd for $C_{29}H_{46}NaO_6$ $[M+Na]^+$: 513.3187; found: 513.3180, error = 1.4 ppm; HPLC: t_R = 12.873 min, purity = 99% at ELSD, 98% at 215 nm.

*4.1.4.2.4. 1-O-Acetyl-6-O-myristoylbritannilactone (**15**).* White solid. Yield: 70%; $[\alpha]_D^{25}$ = −37.4 (c = 0.10 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.83–0.92 (m, 6H, H-15, $CH_3(CH_2)_{12}COO$-6), 0.97–1.09 (m, 1H, H-3b), 1.19–1.45 (m, 23H, H-2, H-3a, $CH_3(CH_2)_{10}CH_2CH_2COO$-6), 1.54–1.71 (m, 2H, $CH_3(CH_2)_{10}CH_2CH_2COO$-6), 1.80 (s, 3H, H-14), 2.04 (d, J = 1.3 Hz, 3H, AcO-1), 2.24–2.32 (m, 2H, $CH_3(CH_2)_{10}CH_2CH_2COO$-6), 2.49 (d, J = 16.2 Hz, 1H, H-9b), 2.62–2.76 (m, 2H, H-4, H-9a), 3.47 (d, J = 7.8 Hz, 1H, H-7), 3.85–4.01 (m, 2H, H-1), 4.89–4.97 (m, 1H, H-8), 5.21 (s, 1H, H-6), 5.94 (s, 1H, H-13b), 6.37 (d, J = 2.3 Hz, 1H, H-13a); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1 ($CH_3(CH_2)_{12}COO$-6), 18.5 (C-15), 20.5 (C-14), 20.9 (CH_3COO-1), 22.6 ($CH_3CH_2(CH_2)_{11}COO$-6), 24.9 ($CH_3(CH_2)_{10}CH_2CH_2COO$-6), 26.5 (C-2), 29.1–29.7 (8C, $CH_3(CH_2)_2(CH_2)_8(CH_2)_2COO$-6), 31.1 (C-3), 31.9 ($CH_3CH_2CH_2(CH_2)_{10}COO$-6), 33.1 (C-4), 34.5 ($CH_3(CH_2)_{11}CH_2COO$-6), 34.6 (C-9), 42.9 (C-7), 64.2 (C-1), 69.0 (C-6), 74.9 (C-8), 124.9 (C-13), 132.1 (C-5), 133.7 (C-10), 136.3 (C-11), 169.5 (C-12), 171.1 (CH_3COO-1), 173.7 ($CH_3(CH_2)_{12}COO$-6); ESI-MS: 541.2 $[M+Na]^+$; HR-ESI-MS: calcd for $C_{31}H_{50}NaO_6$ $[M+Na]^+$: 541.3500; found: 541.3506, error = 1.1 ppm; HPLC: t_R = 14.696 min, purity = 99% at ELSD, 96% at 215 nm.

*4.1.4.2.5. 1-O-Acetyl-6-O-stearoylbritannilactone (**16**).* White waxy solid. Yield: 75%; $[\alpha]_D^{25}$ = −30.1 (c = 0.112 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.84–0.92 (m, 6H, H-15, $CH_3(CH_2)_{16}COO$-6), 0.99–1.08 (m, 1H, H-3b), 1.20–1.35 (m, 30H, H-2b, H-3a, $CH_3(CH_2)_{14}CH_2CH_2COO$-6), 1.35–1.45 (m, 1H, H-2a), 1.55–1.65 (m, 2H, $CH_3(CH_2)_{14}CH_2CH_2COO$-6), 1.81 (s, 3H, H-14), 2.03–2.07 (m, 3H, AcO-1), 2.28 (t, J = 7.5 Hz, 2H, $CH_3(CH_2)_{14}CH_2CH_2COO$-6), 2.49 (dd, J = 16.1, 2.0 Hz, 1H, H-9b), 2.62–2.76 (m, 2H, H-4, H-9a), 3.44–3.52 (m, 1H, H-7), 3.85–4.01 (m, 2H, H-1), 4.89–4.98 (m, 1H, H-8), 5.21 (d, J = 1.8 Hz, 1H, H-6), 5.95 (d, J = 2.3 Hz, 1H, H-13b), 6.38 (d, J = 2.8 Hz, 1H, H-13a); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1 ($CH_3(CH_2)_{16}COO$-6), 18.5 (C-15), 20.5 (C-14), 21.0 (CH_3COO-1), 22.7 ($CH_3CH_2(CH_2)_{15}COO$-6), 25.0 ($CH_3(CH_2)_{14}CH_2CH_2COO$-6), 26.5 (C-2), 29.1–29.7 (12C, $CH_3(CH_2)_2(CH_2)_{12}(CH_2)_2COO$-6), 31.0 (C-3), 31.9 ($CH_3CH_2CH_2(CH_2)_{14}COO$-6), 33.1 (C-4), 34.6 (C-9), 34.6 ($CH_3(CH_2)_{15}CH_2COO$-6), 42.9 (C-7), 64.2 (C-1), 69.0 (C-6), 75.0 (C-8), 125.0 (C-13), 132.1 (C-5), 133.7 (C-10), 136.3 (C-11), 169.5 (C-12), 171.2 (CH_3COO-1), 173.7 ($CH_3(CH_2)_{16}COO$-6); ESI-MS: 597.2 $[M+Na]^+$; HR-ESI-MS: calcd for $C_{35}H_{58}NaO_6$ $[M+Na]^+$: 597.4126; found: 597.4109, error = 2.2 ppm; HPLC: t_R = 6.846 min, purity = 97% at ELSD, 95% at 215 nm.

*4.1.4.2.6. 1-O-Acetyl-6-O-(p-bromocinnamoyl)britannilactone (**17**).* Colourless oil. Yield: 65%; $[\alpha]_D^{25}$ = −133.8 (c = 0.50 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.89 (d, J = 6.9 Hz, 3H, H-15), 1.00–1.11 (m, 1H, H-3b), 1.21–1.34 (m, 2H, H-2b, H-3a), 1.35–1.46 (m, 1H, H-2a), 1.83 (s, 3H, H-14), 2.04 (s, 3H, AcO-1), 2.53 (dd, J = 16.2, 2.1 Hz, 1H, H-9b), 2.66–2.74 (m, 1H, H-4), 2.78 (dd, J = 16.2, 2.7 Hz, 1H, H-9a), 3.53–3.59 (m, 1H, H-7), 3.87–4.01 (m, 2H, H-1), 4.94–5.00 (m, 1H, H-8), 5.34 (d, J = 1.7 Hz, 1H, H-6), 5.99 (d, J = 2.2 Hz, 1H, H-13b), 6.39 (d, J = 2.7 Hz, 1H, H-13a), 6.40 (d, J = 16.0 Hz, 1H, p-BrPhCH = C*H*COO-6), 7.38 (d, J = 8.5 Hz, 2H, p-Br*Ph*CH=CHCOO-6), 7.52 (d, J = 8.5 Hz, 2H, p-Br*Ph*CH=CHCOO-6), 7.59 (d, J = 16.0 Hz, 1H, p-BrPhC*H*=CHCOO-6); ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.5 (C-15), 20.5 (C-14), 20.9 (CH_3COO-1), 26.4 (C-2), 31.1 (C-3), 33.1 (C-4), 34.6 (C-9), 42.5 (C-7), 64.2 (C-1), 69.4 (C-6), 74.9 (C-8), 118.3 (p-BrPhCH=*C*HCOO-6), 124.8 (C-13), 125.0 (p-Br*Ph*CH=CHCOO-6), 129.5 (2C, p-Br*Ph*CH=CHCOO-6), 132.0 (p-Br*Ph*CH=CHCOO-6), 132.1 (2C, p-Br*Ph*CH=CHCOO-6), 132.9 (C-5), 133.9 (C-10), 136.2 (C-11), 144.0 (p-BrPhCH=CHCOO-6), 166.4 (p-BrPhCH=CHCOO-6), 169.5 (C-12), 171.1 (CH_3COO-1); ESI-MS: 539.0 $[M+Na]^+$; HR-ESI-MS: calcd for $C_{26}H_{29}BrNaO_6$ $[M+Na]^+$: 539.1040; found: 539.1022, error = 3.3 ppm; HPLC: t_R = 9.184 min, purity = 98% at ELSD, 96% at 215 nm.

*4.1.4.2.7. 1-O-Acetyl-6-O-(p-trifluoromethylcinnamoyl)britannilactone (**18**).* Yellow oil. Yield: 83%; $[\alpha]_D^{25}$ = −111.8 (c = 0.07 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.91 (d, J = 6.9 Hz, 3H, H-15), 1.02–1.13 (m, 1H, H-3b), 1.23–1.35 (m, 2H, H-2b, H-3a), 1.38–1.47 (m, 1H, H-2a), 1.85 (s, 3H, H-14), 2.05 (s, 3H, AcO-1), 2.55 (dd, J = 16.2, 2.0 Hz, 1H, H-9b), 2.72 (ddd, J = 9.4, 6.9, 4.6 Hz, 1H, H-4), 2.80 (dd, J = 16.2, 2.6 Hz, 1H, H-9a), 3.55–3.61 (m, 1H, H-7), 3.88–4.03 (m, 2H, H-1), 4.95–5.01 (m, 1H, H-8), 5.37 (d, J = 1.8 Hz, 1H, H-6), 6.00 (d, J = 2.3 Hz, 1H, H-13b), 6.41 (d, J = 2.6 Hz, 1H, H-13a), 6.48 (d, J = 16.1 Hz, 1H, p-F_3CPhCH=C*H*COO-6), 7.61–7.71 (m, 5H, p-F_3CPhC*H*=CHCOO-6); ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.5 (C-15), 20.6 (C-14), 20.9 (CH_3COO-1), 26.5 (C-2), 31.1 (C-3), 33.1 (C-4), 34.7 (C-9), 42.9 (C-7), 64.2 (C-1), 69.7 (C-6), 74.9 (C-8), 120.3 (p-F_3CPhCH=*C*HCOO-6), 122.4 (p-F_3*C*PhCH=CHCOO-6), 125.1 (C-13), 125.9 (2C, p-F_3C*Ph*CH=CHCOO-6), 128.3 (3C, p-F_3C*Ph*CH=CHCOO-6), 132.1 (C-5), 134.1 (C-10), 136.2 (C-11), 137.4 (p-F_3C*Ph*CH=CHCOO-6), 143.6 (p-F_3CPh*C*H=CHCOO-6), 166.1 (p-F_3CPhCH=*C*HCOO-6), 169.4 (C-12), 171.1 (CH_3COO-1); ESI-MS: 529.0 $[M+Na]^+$; HR-ESI-MS: calcd for $C_{27}H_{29}F_3NaO_6$ $[M+Na]^+$: 529.1808; found: 529.1800, error = 1.5 ppm; HPLC: t_R = 7.019 min, purity > 99% at ELSD, 98% at 215 nm.

*4.1.4.3. Procedure for the synthesis of ABL analogue (**19**).* ABL (0.1 mmol) in CH_2Cl_2 (1 mL) was added to a suspension of DMP (42.4 mg, 0.1 mmol) in CH_2Cl_2 (1 mL). After stirring at 20 °C for 3 h, the complete solution was subjected to silica gel chromatography (PE:EtOAc = 1:1) to give compound **19**.

*4.1.4.3.1. 1-O-Acetyl-6-oxobritannilactone (**19**).* Yellow oil. Yield: 43%; $[\alpha]_D^{25}$ = +54.6 (c = 0.17 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.19 (d, 3H, H-15), 1.23–1.35 (m, 1H, H-3b), 1.41–1.53 (m, 3H, H-2, H-3a), 2.00 (s, 3H, H-14), 2.02 (s, 3H, AcO-1), 2.64–2.84 (m, 3H, H-9, H-4), 3.74 (dt, J = 7.1, 2.3 Hz, 1H, H-7), 3.94 (t, J = 6.5 Hz, 2H, H-1), 5.00 (ddd, J = 7.2, 4.7, 3.4 Hz, 1H, H-8), 5.98 (d, J = 2.3 Hz, 1H, H-13b), 6.32 (d, J = 2.4 Hz, 1H, H-13a); ^{13}C NMR (100 MHz, $CDCl_3$): δ 19.3 (C-15), 20.9 (C-14), 21.6 (CH_3CO-1), 27.3 (C-2), 29.9 (C-3), 33.3 (C-4), 34.6 (C-9), 50.0 (C-7), 64.3 (C-1), 73.2 (C-8), 125.5 (C-13), 133.3 (C-11), 139.0 (C-5), 149.6 (C-10), 168.7 (C-12), 171.1 (CH_3CO-1), 191.8 (C-12); ESI-MS: 329.0 $[M+Na]^+$; HR-ESI-MS: calcd for $C_{17}H_{23}O_5$ $[M+H]^+$: 307.1540; found: 307.1554, error = 4.6 ppm; HPLC: t_R = 3.694 min, purity > 99% at 254 nm.

*4.1.4.4. Procedure for the synthesis of OABL C13-modified arylation analogues (**20**–**24**).* A mixture of OABL (35 mg, 0.1 mmol), triethylamine (30 mg, 0.3 mmol), and Iodobenzene (40.8 mg, 0.2 mmol) in DMF (750 μL) was treated with palladium(II) acetate (1.12 mg, 0.005 mmol) and then heated at 80 °C under Ar atmosphere. After stirring for 24–40 h, the reaction mixture was allowed to cool to rt, water (5 mL) was added, and the resultant mixture was extracted with dichloromethane (5 mL × 3). The organic extract was evaporated to give an oily residue which was further purified by silica gel chromatography (PE: EtOAc = 5:1 to 1:1).

*4.1.4.4.1. 1,6-O,O-Diacetyl-(E)-13-phenylbritannilactone (**20**).* Yellow oil. Yield: 41%; $[\alpha]_D^{25} = +10.3$ ($c = 0.35$ in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 0.67–0.79 (m, 4H, H-15, H-3b), 0.88–1.01 (m, 1H, H-3a), 1.22–1.44 (m, 2H, H-2), 1.80 (s, 3H, H-14), 2.03 (s, 3H, AcO-6), 2.06 (s, 3H, AcO-1), 2.52 (dd, $J = 15.8$, 2.7 Hz, 1H, H-9b), 2.55–2.64 (m, 1H, H-4), 2.86 (dd, $J = 15.8$, 2.7 Hz, 1H, H-9a), 3.80–3.88 (m, 1H, H-1a), 3.89–3.98 (m, 1H, H-1b), 4.08 (dt, $J = 7.8$, 2.3 Hz, 1H, H-7), 5.02–5.11 (m, 1H, H-8), 5.57 (d, $J = 2.1$ Hz, 1H, H-6), 7.40–7.55 (m, 3H, Ar-13), 7.60 (d, $J = 2.6$ Hz, 1H, H-13), 7.78 (d, $J = 7.2$ Hz, 2H, Ar-13); ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.3 (C-15), 20.2 (C-14), 21.0 (CH_3CO-6), 21.3 (CH_3CO-1), 26.6 (C-2), 30.6 (C-3), 33.2 (C-4), 35.2 (C-9), 42.7 (C-7), 64.2 (C-1), 64.7 (C-6), 74.7 (C-8), 124.0 (C-11), 129.0 (2C, Ar-13), 130.6 (Ar-13), 130.9 (2C, Ar-13), 132.7 (C-5), 133.2 (C-10), 133.9 (Ar-13), 139.9 (C-13), 170.2 (C-12), 171.2 (CH_3CO-6), 171.5 (CH_3CO-1); ESI-MS: 426.8 $[M+H]^+$; HR-ESI-MS: calcd for $C_{25}H_{30}NaO_6$ $[M+Na]^+$: 449.1935; found: 449.1942, error = 1.6 ppm; HPLC: $t_R = 6.480$ min, purity = 98% at ELSD.

*4.1.4.4.2. 1,6-O,O-Diacetyl-(E)-13-(4-fluorophenyl)britannilactone (**21**).* Yellow oil. Yield: 52%; $[\alpha]_D^{25} = +16.7$ ($c = 0.21$ in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 0.63–0.76 (m, 4H, H-15, H-3b), 0.86–1.02 (m, 1H, H-3a), 1.19–1.30 (m, 1H, H-2b), 1.35 (dt, $J = 10.7$, 6.6 Hz, 1H, H-2a), 1.80 (s, 3H, H-14), 2.03 (s, 3H, AcO-6), 2.06 (s, 3H, AcO-1), 2.52 (dd, $J = 16.0$, 2.0 Hz, 1H, H-9b), 2.55–2.64 (m, 1H, H-4), 2.86 (dd, $J = 16.0$, 2.0 Hz, 1H, H-9a), 3.82 (dt, $J = 10.9$, 7.1 Hz, 1H, H-1b), 3.93 (dt, $J = 10.9$, 7.1 Hz, 1H, H-1a), 4.03 (d, $J = 7.8$ Hz, 1H, H-7), 5.02–5.12 (m, 1H, H-8), 5.51 (d, $J = 2.0$ Hz, 1H, H-6), 7.19 (t, $J = 8.7$ Hz, 2H, Ar-13), 7.55 (d, $J = 2.6$ Hz, 1H, H-13), 7.81 (dd, $J = 8.7$, 5.4 Hz, 2H, Ar-13); ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.3 (C-15), 20.2 (C-14), 21.0 (CH_3CO-6), 21.2 (CH_3CO-1), 26.6 (C-2), 30.7 (C-3), 33.2 (C-4), 35.2 (C-9), 42.5 (C-7), 64.2 (C-1), 64.6 (C-6), 74.6 (C-8), 116.3 (2C, $J_{C\text{-}F} = 22.0$ Hz, Ar-13), 123.4 (C-11), 129.0 ($J_{C\text{-}F} = 2.9$ Hz, Ar-13), 133.1 (2C, $J_{C\text{-}F} = 8.8$ Hz, Ar-13), 133.4 (C-5), 133.6 (C-10), 138.6 (C-13), 163.8 ($J_{C\text{-}F} = 253.1$ Hz, Ar-13), 170.4 (C-12), 171.2 (CH_3CO-6), 171.4 (CH_3CO-1); ESI-MS: 467.0 $[M+Na]^+$; HPLC: $t_R = 16.5$ min, purity > 99% at ELSD.

*4.1.4.4.3. 1,6-O,O-Diacetyl-(E)-13-(4-bromophenyl)britannilactone (**22**).* Yellow oil. Yield: 67%; $[\alpha]_D^{25} = +9.5$ ($c = 0.38$ in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 0.69 (dd, $J = 10.3$, 4.9 Hz, 1H, H-3b), 0.73 (d, $J = 6.9$ Hz, 3H, H-15), 0.88 (dd, $J = 6.8$, 4.9 Hz, 1H, H-3a), 0.92–1.04 (m, 1H, H-2b), 1.12–1.28 (m, 1H, H-2a), 1.80 (s, 3H, H-14), 2.03 (s, 3H, AcO-6), 2.06 (s, 3H, AcO-1), 2.49–2.55 (m, 1H, H-9b), 2.55–2.62 (m, 1H, H-4), 2.86 (dd, $J = 16.0$, 2.9 Hz, 1H, H-9a), 3.82 (dt, $J = 10.9$, 7.0 Hz, 1H, H-1b), 3.94 (dt, $J = 10.9$, 6.5 Hz, 1H, H-1a), 4.02 (dt, $J = 7.8$, 2.3 Hz, 1H, H-7), 5.00–5.10 (m, 1H, H-8), 5.49 (d, $J = 2.0$ Hz, 1H, H-6), 7.51 (d, $J = 2.6$ Hz, 1H, H-13), 7.64 (d, $J = 8.6$ Hz, 2H, Ar-13), 7.69 (d, $J = 8.6$ Hz, 2H, Ar-13); ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.3 (C-15), 20.2 (C-14), 21.0 (CH_3CO-6), 21.2 (CH_3CO-1), 26.6 (C-2), 30.7 (C-3), 33.2 (C-4), 35.1 (C-9), 42.6 (C-7), 64.1 (C-1), 64.6 (C-6), 74.7 (C-8), 124.6 (Ar-13), 125.2 (C-11), 131.5 (Ar-13), 132.3 (4C, Ar-13), 132.4 (C-5), 133.4 (C-10), 133.6 (C-13), 138.5 (Ar-13), 170.4 (C-12), 171.2 (CH_3CO-6), 171.3 (CH_3CO-1); ESI-MS: 505.0 $[M+H]^+$; HR-ESI-MS: calcd for $C_{25}H_{30}BrO_6$ $[M+H]^+$: 505.1220; found: 505.1227, error = 1.4 ppm; HPLC: $t_R = 7.99$ min, purity = 97% at ELSD.

*4.1.4.4.4. 1,6-O,O-Diacetyl-(E)-13-(4-methoxyphenyl)britannilactone (**23**).* White solid. Yield: 43%; $[\alpha]_D^{25} = +34.9$ ($c = 0.19$ in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 0.67–0.78 (4H, H-15, H-3b), 0.90–1.03 (m, 1H, H-3a), 1.19–1.40 (2H, H-2), 1.79 (s, 3H, C-14), 2.02 (s, 3H, AcO-6), 2.07 (s, H, AcO-1), 2.51 (dd, $J = 15.9$, 2.0 Hz, 1H, H-9b), 2.54–2.65 (m, 1H, H-4), 2.86 (dd, $J = 15.6$, 2.4 Hz, 1H, H-9a), 3.78–3.85 (m, 1H, H-1b), 3.87 (s, 3H, CH_3O-Ar-13), 3.88–3.96 (m, 1H, H-1a), 4.01 (dt, $J = 7.8$, 2.2 Hz, 1H, H-7), 4.99–5.07 (m, 1H, H-8), 5.61 (d, $J = 2.1$ Hz, 1H, H-6), 7.01 (d, $J = 8.9$ Hz, 1H, Ar-13), 7.53 (d, $J = 2.6$ Hz, 1H, H-13), 7.78 (d, $J = 8.9$ Hz, 1H, Ar-13); ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.4 (C-15), 20.2 (C-14), 21.0 (CH_3CO-6), 21.3 (CH_3CO-1), 26.6 (C-2), 30.6 (C-3), 33.2 (C-4), 35.3 (C-9), 42.8 (C-7), 55.4 (CH_3O-Ar-13), 64.3 (C-1), 64.7 (C-6), 74.5 (C-8), 114.5 (2C, Ar-13), 120.7 (C-11), 125.4 (Ar-13), 133.0 (2C, Ar-13), 133.2 (C-5), 133.8 (C-10), 139.6 (C-13), 161.5 (Ar-13), 170.4 (C-12), 171.2 (CH_3CO-6), 172.0 (CH_3CO-1); ESI-MS: 456.9 $[M+H]^+$; HR-ESI-MS: calcd for $C_{26}H_{32}NaO_7$ $[M+Na]^+$: 479.2040; found: 479.2054, error = 2.9 ppm; HPLC: $t_R = 6.66$ min, purity > 99% at ELSD.

*4.1.4.4.5. 1,6-O,O-Diacetyl-(E)-13-(3,4,5-trimethoxyphenyl)britannilactone (**24**).* White solid. Yield: 82%; $[\alpha]_D^{25} = +30.9$ ($c = 0.24$ in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 0.75 (d, $J = 6.9$ Hz, 3H, H-15), 0.70–0.90 (m, 1H, H-3b), 0.95–1.04 (m, 1H, H-3a), 1.23–1.34 (m, 1H, H-2b), 1.35–1.45 (m, 1H, H-2a), 1.79 (s, 3H, C-14), 2.01 (s, 3H, AcO-6), 2.03 (s, 3H, AcO-1), 2.51 (dd, $J = 15.9$, 2.0 Hz, 1H, H-9b), 2.54–2.62 (m, 1H, H-4), 2.83 (dd, $J = 15.6$, 2.4 Hz, 1H, H-9a), 3.78–3.87 (m, 1H, H-1b), 3.93 (s, 9H, CH_3O-Ar-13), 3.88–3.96 (m, 1H, H-1a), 4.03–4.10 (m, 1H, H-7), 4.99–5.07 (m, 1H, H-8), 5.50 (d, $J = 2.2$ Hz, 1H, H-6), 6.92 (s, 2H, Ar-13), 7.49 (d, $J = 2.3$ Hz, 1H, H-13); ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.4 (C-15), 20.3 (C-14), 20.9 (CH_3CO-6), 21.2 (CH_3CO-1), 26.7 (C-2), 30.7 (C-3), 33.4 (C-4), 35.3 (C-9), 42.4 (C-7), 56.4 (2C, CH_3O-Ar-13), 61.0 (CH_3O-Ar-13), 64.3 (C-1), 65.2 (C-6), 74.6 (C-8), 108.4 (2C, Ar-13), 122.9 (C-11), 128.3 (Ar-13), 133.3 (C-5), 134.0 (C-10), 140.2 (Ar-13), 153.5 (2C, Ar-13), 169.7 (C-12), 171.2 (CH_3CO-6), 171.6 (CH_3CO-1); ESI-MS: 533.8 $[M+NH_4]^+$; HPLC: $t_R = 15.2$ min, purity = 97.5% at ELSD.

4.2. Assay for cytotoxicity

4.2.1. Cell culture

The HCT116 cell line was originally obtained from Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. The HEp-2, HeLa and CHO cells were granted by Prof. Lei group of college of life sciences, Northwest A&F university. The HCT116 cells were grown in RPMI-1640 (Gibco), and HEp-2, HeLa and CHO cells were grown in high glucose-DMEM (Gibco) medium containing 10% (v/v) thermally inactivated foetal bovine serum (FBS), penicillin (100 KU/L) and streptomycin (100 KU/L) at 37 °C in a 5% CO_2 humidified incubator. Cells were always used at <90% of confluence. All compounds were dissolved in DMSO and diluted in cell culture media to a final concentration of less than or equal to 0.1% (v/v), which did not interfere with the cell growth. The 6-OH ester analogues **6**–**8**, **12**–**18** and C13 arylation analogues **20**–**24** although soluble in DMSO, started slightly precipitating (visible only under a microscope) in the cell culture medium, and hence were added into 96-well plates through violently vortex and taken forward for the estimation of IC_{50} values.

4.2.2. Cytotoxicity assays

Cytotoxicity *in vitro* was assessed by the SRB colorimetric assay, which estimates cell number indirectly by measuring total basic amino acids of cultured cells [35]. Briefly 100 μL aliquots of the exponentially growing cells containing 2.5×10^4 cells/mL were added to each well of a 96-well flat-microtiter plate and let cells attach for 24 h. Then the medium was replaced by fresh medium and cells were incubated with various amounts of the test compound for an additional 72 h. Four replicate wells were used in each point in the experiments. After incubation at 37 °C, culture medium was moved and cells were fixed *in situ* with 100 μL aliquots of cold 10% trichloroacetic acid (TCA), and plates were incubated for 1 h at 4 °C. Thereafter, supernatant was discarded and plates were washed 5 times with distilled water and air dried. Sulforhodamine B solution at 0.4% (w/v) in 1% acetic acid was added to each well and plates were incubated for 20–30 min at room temperature. The unbound dye is removed by washing 5 times with 1% acetic acid and plates were air dried. Bound sulforhodamine B was subsequently solubilized with 10 mM Tris base, and the absorbance was

read at 560 nm using an Epoch (Bio-Tek) microplate reader. The percentage of cell viability was calculated relative to control wells designated as 100% viable cells.

4.2.3. Analysis of chromatin condensation

Condensation of chromatin is usually the late event in apoptosis and is detected by nuclear staining with Hoechst 33258 as described previously [38]. 2.5 mL aliquots of the exponentially growing HCT116 cells containing 1.0×10^5 cells/mL were cultured on coverslips, which were kept in six-well plates for 24 h before treatment. To observe cells undergoing apoptosis after 48 h treatment with ABL, **1** (OABL) and **14**, Hoechst 33258 staining was performed according to the kit's instructions (Beyotime Institute Biotechnology, China). Untreated cells (control, 0.1% DMSO) or cells treated with the solvent (≤0.1% DMSO) of the compound were included. The cells were observed using a fluorescence microscopy with a ×40 objective lens (Olympus BX53 + DP72; Japan).

4.2.4. Cell cycle analysis using flow cytometry

2.5 mL aliquots of HCT116 cells containing 1.0×10^5 cells/mL were plated in 6-well plates and incubated at 37 °C for 24 h. Cells were then incubated with tested compound ABL, **1** (OABL) and **14**. Untreated cells (control, 0.1% DMSO) or cells treated with the solvent (≤0.1% DMSO) of the compound were included. After 24 h treatment, cells were centrifuged and fixed in 70% ethanol at 4 °C overnight and subsequently resuspended in PBS containing 100 μL RNase A and 400 μL propidium iodide (PI). Cellular DNA content, for cell cycle distribution analysis, was measured using a FACSCalibur flow cytometer (Becton–Dickinson, San Jose, CA, USA) and analyzed using Modfit LT 3.0 software as described previously [39]. Twenty thousand events were collected per sample. Mean values from three independent experiments were presented.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.04.028.

References

[1] F. Bohlmann, P.K. Mahanta, J. Jakupovic, R.C. Rastogi, A.A. Natu, New sesquiterpene lactones from *Inula* species, Phytochemistry 17 (1978) 1165–1172.
[2] F. Jeske, S. Huneck, J. Jakupovic, Secoeudesmanolides from *Inula japonica*, Phytochemistry 34 (1993) 1647–1649.
[3] B.-N. Zhou, N.-S. Bai, L.-Z. Lin, G. Cordell, Sesquiterpene lactones from *Inula britannica*, Phytochemistry 34 (1993) 249–252.
[4] X.M. Fang, B. Liu, Y.B. Liu, J.J. Wang, J.K. Wen, B.H. Li, M. Han, Acetylbritannilactone suppresses growth via upregulation of kruppel-like transcription factor 4 expression in HT-29 colorectal cancer cells, Oncology Reports 26 (2011) 1181–1187.
[5] C.-T. Ho, M. Rafi, R.S. Dipaola, G. Ghai, R.T. Rosen, N. Bai, Inducing Cell Apoptosis and Treating Cancer Using 1-*O*-Acetylbritannilactone or 1, 6-*O*, *O*-Diacetylbritannilactone, US 6627623, 2003.
[6] J.-L. Qi, Y. Fu, X.-W. Shi, Y.-B. Wu, Y.-Z. Wang, D.-Q. Zhang, Q.-W. Shi, Sesquiterpene lactones and their anti-tumor activity from the flowers of *Inula britannica*, Letters in Drug Design & Discovery 5 (2008) 433–436.
[7] K.-H. Je, A.-R. Han, H.-T. Lee, W. Mar, E.-K. Seo, The inhibitory principle of lipopolysaccharide-induced nitric oxide production from *Inula britannica* var. *chinensis*, Archives of Pharmacal Research 27 (2004) 83–85.
[8] B. Liu, M. Han, J.K. Wen, Acetylbritannilactone inhibits neointimal hyperplasia after balloon injury of rat artery by suppressing nuclear factor-kappa B activation, The Journal of Pharmacology and Experimental Therapeutics 324 (2008) 292–298.
[9] M.M. Rafi, N.-S. Bai, C.-T. Ho, R.T. Rosen, E. White, D. Perez, R.S. DiPaola, A sesquiterpenelactone from *Inula britannica* induces anti-tumor effects dependent on Bcl-2 phosphorylation, Anticancer Research 25 (2005) 313–318.
[10] M. Han, J.K. Wen, B. Zheng, D.Q. Zhang, Acetylbritannilatone suppresses NO and PGE2 synthesis in RAW 264.7 macrophages through the inhibition of iNOS and COX-2 gene expression, Life Sciences 75 (2004) 675–684.
[11] M.H. Pan, Y.S. Chiou, A.C. Cheng, N. Bai, C.Y. Lo, D. Tan, C.T. Ho, Involvement of MAPK, Bcl-2 family, cytochrome c, and caspases in induction of apoptosis by 1,6-*O*,*O*-diacetylbritannilactone in human leukemia cells, Molecular Nutrition & Food Research 51 (2007) 229–238.
[12] Y.P. Liu, J.K. Wen, Y.B. Wu, J. Zhang, B. Zheng, D.Q. Zhang, M. Han, 1,6-*O*,*O*-diacetylbritannilactones inhibits IkappaB kinase beta-dependent NF-kappaB activation, Phytomedicine 16 (2009) 156–160.
[13] B. Liu, J.K. Wen, B.H. Li, X.M. Fang, J.J. Wang, Y.P. Zhang, C.J. Shi, D.Q. Zhang, M. Han, Celecoxib and acetylbritannilactone interact synergistically to suppress breast cancer cell growth via COX-2-dependent and -independent mechanisms, Cell Death & Disease 2 (2011) e185–e193.
[14] S. Liu, H. Liu, W. Yan, L. Zhang, N. Bai, C.T. Ho, Studies on 1-*O*-acetylbritannilactone and its derivative, (2-*O*-butyloxime-3-phenyl)-propionyl-1-*O*-acetylbritannilactone ester, Bioorganic & Medicinal Chemistry Letters 14 (2004) 1101–1104.
[15] S. Liu, H. Liu, W. Yan, L. Zhang, N. Bai, C.T. Ho, Design, synthesis, and antitumor activity of (2-*O*-alkyloxime-3-phenyl)-propionyl-1-*O*-acetylbritannilactone esters, Bioorganic & Medicinal Chemistry 13 (2005) 2783–2789.
[16] S. Amslinger, The tunable functionality of α,β-unsaturated carbonyl compounds enables their differential application in biological systems, ChemMedChem 5 (2010) 351–356.
[17] S.M. Kupchan, D.C. Fessler, M.A. Eakin, T.J. Giacobbe, Reactions of alpha methylene lactone tumor inhibitors with model biological nucleophiles, Science 168 (1970) 376–378.
[18] A. Janecka, A. Wyrebska, K. Gach, J. Fichna, T. Janecki, Natural and synthetic alpha-methylenelactones and alpha-methylenelactams with anticancer potential, Drug Discovery Today 17 (2012) 561–572.
[19] M.L. Guzman, R.M. Rossi, L. Karnischky, X. Li, D.R. Peterson, D.S. Howard, C.T. Jordan, The sesquiterpene lactone parthenolide induces apoptosis of human acute myelogenous leukemia stem and progenitor cells, Blood 105 (2005) 4163–4169.
[20] A. Wyrebska, J. Szymanski, K. Gach, J. Piekielna, J. Koszuk, T. Janecki, A. Janecka, Apoptosis-mediated cytotoxic effects of parthenolide and the new synthetic analog MZ-6 on two breast cancer cell lines, Molecular Biology Reports 40 (2013) 1655–1663.
[21] T.J. Schmidt, Helenanolide-type sesquiterpene lactones-III. Rates and stereochemistry in the reaction of helenalin and related helenanolides with sulfhydryl containing biomolecules, Bioorganic & Medicinal Chemistry 5 (1997) 645–653.
[22] W.-N. Wang, Y.-Z. Wang, D.-Q. Zhang, Study on RP-HPLC determination of 1-*O*-acetylbritannilactone in *Inula britannica* L, Chinese Journal of Pharmaceutical Analysis 2 (2005) 205–207.
[23] D. Zhang, J. Wen, Y. Wu, Y. Fu, W. Yunzhi, X. Shi, J. Qi, 1-*O*-Acetylbritannilactone Derivative, its Preparation Method and Application as Antitumor Agent, CN101186598, 2008.
[24] B. Liu, M. Han, R.H. Sun, J.J. Wang, Y.P. Zhang, D.Q. Zhang, J.K. Wen, ABL-N-induced apoptosis in human breast cancer cells is partially mediated by c-Jun NH_2-terminal kinase activation, Breast Cancer Research: BCR 12 (2010) R9.
[25] J.M. Gao, J.W. Shen, J.Y. Wang, Z. Yang, A.L. Zhang, Microbial transformation of 3beta-acetoxypregna-5,16-diene-20-one by *Penicillium citrinum*, Steroids 76 (2011) 43–47.
[26] N.J. Fan, J.J. Tang, H. Li, X.J. Li, B. Luo, J.M. Gao, Synthesis and cytotoxic activity of some novel steroidal C-17 pyrazolinyl derivatives, European Journal of Medicinal Chemistry 69 (2013) 182–190.
[27] J.J. Tang, F.Y. Zhang, D.M. Wang, J.M. Tian, S. Dong, J.M. Gao, Semisynthesis and antifeedant activity of new derivatives of a dihydro-beta-agarofuran from *Parnassia wightiana*, International Journal of Molecular Sciences 14 (2013) 19484–19493.
[28] C. Han, F.J. Barrios, M.V. Riofski, D.A. Colby, Semisynthetic derivatives of sesquiterpene lactones by palladium-catalyzed arylation of the alpha-methylene-gamma-lactone substructure, The Journal of Organic Chemistry 74 (2009) 7176–7179.
[29] A. Arcadi, M. Chiarini, F. Marinelli, Z. Berente, L. Kollar, Palladium-catalyzed arylation of alpha-methylene-gamma-butyrolactone: 3-benzylfuran-2(5H)-ones vs (Z)-benzylidene-gamma-butyrolactones and their reduction to 3-benzyl-gamma butyrolactones, Organic Letters 2 (2000) 69–72.
[30] A.R. Han, W. Mar, E.K. Seo, X-ray crystallography of a new sesquiterpene lactone isolated from *Inula britannica* var. *chinensis*, Natural Product Sciences 9 (2003) 28–30.
[31] H.Q. Gong, Q.X. Wu, L.L. Liu, J.L. Yang, R. Wang, Y.P. Shi, Sesquiterpenoids from the aerial parts of *Inula japonica*, Helvetica Chimica Acta 94 (2011) 1269–1276.
[32] CCDC-940163 (natural ABL) and CCDC-987147 (compound **23**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif

[33] J. Merten, Y. Wang, T. Krause, O. Kataeva, P. Metz, Total synthesis of the cytotoxic 1,10-seco-eudesmanolides britannilactone and 1,6-*O*,*O*-diacetylbritannilactone, Chemistry – A European Journal 17 (2011) 3332–3334.
[34] I.P. Beletskaya, A.V. Cheprakov, The Heck reaction as a sharpening stone of palladium catalysis, Chemical Reviews 100 (2000) 3009–3066.
[35] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J.T. Warren, H. Bokesch, S. Kenney, M.R. Boyd, New colorimetric cytotoxicity assay for anticancer-drug screening, The Journal of the National Cancer Institute 82 (1990) 1107–1112.
[36] N. Bai, C.-S. Lai, K. He, Z. Zhou, L. Zhang, Z. Quan, N. Zhu, Q.Y. Zheng, M.-H. Pan, C.-T. Ho, Sesquiterpene lactones from *Inula britannica* and their cytotoxic and apoptotic effects on human cancer cell lines, Journal of Natural Products 69 (2006) 531–535.
[37] L. Raj, T. Ide, A.U. Gurkar, M. Foley, M. Schenone, X. Li, N.J. Tolliday, T.R. Golub, S.A. Carr, A.F. Shamji, A.M. Stern, A. Mandinova, S.L. Schreiber, S.W. Lee, Selective killing of cancer cells by a small molecule targeting the stress response to ROS, Nature 475 (2011) 231–234.
[38] F.A. Oberhammer, M. Pavelka, S. Sharma, R. Tiefenbacher, A.F. Purchio, W. Bursch, R. Schultehermann, Induction of apoptosis in cultured hepatocytes and in regressing liver by transforming growth factor beta 1, Proceedings of the National Academy of Sciences of the United States of America 89 (1992) 5408–5412.
[39] J.J. Tang, G.J. Fan, F. Dai, D.J. Ding, Q. Wang, D.L. Lu, R.R. Li, X.Z. Li, L.M. Hu, X.L. Jin, B. Zhou, Finding more active antioxidants and cancer chemoprevention agents by elongating the conjugated links of resveratrol, Free Radical Biology & Medicine 50 (2011) 1447–1457.
[40] O.V. Dolomanov, L.J. Bourhis, R.J. Gildea, J.A. Howard, H. Puschmann, OLEX2: a complete structure solution, refinement and analysis program, Journal of Applied Crystallography 42 (2009) 339–341.
[41] L. Palatinus, G. Chapuis, SUPERFLIP-a computer program for the solution of crystal structures by charge flipping in arbitrary dimensions, Journal of Applied Crystallography 40 (2007) 786–790.
[42] G.M. Sheldrick, A short history of SHELX, Acta Crystallographica Section A 64 (2007) 112–122.