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Synthesis and biological evaluation of cytotoxic activity of novel anthracene L-rhamnopyranosides

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ABSTRACT

A series of anthracene L-rhamnopyranosides were designed and synthesized in a practical way and their cytotoxic activity was examined in vitro. Most compounds exhibited both potent cytotoxicity against several tumor cell lines and high DNA binding capacity. The preliminary results showed that subtle modifications of rhamnosyl moiety in anthracene rhamnosides with acetyl group had a selective toxicity for different tumor cells and the displacement of C-10 carbonyl group in emodin by acetylmethylene group was helpful to improve the inhibitory activity. Lipophilicity of the anthracene glycosides was not a crucial factor for cytotoxicity and most molecules with good cytotoxicity could inhibit the catalytic activity of Top2α.

1. Introduction

As is well known, proliferative disorders are expected to be one of the major causes of death in the 21st century. Although many strategies can be followed by drug designers in order to identify new chemical entities for a more effective treatment of cancer, the crucial decision is always the selection of a suitable starting point from the vast chemical space.[1] It is the chemical modification of bioactive components from medicinal herbs that is one of the most common approaches in drug discovery and improvement of therapeutic properties.[2] Many natural and synthetic compounds based on tricyclic planar chromophore framework, fully or partially consisting of anthraquinone, anthrapyrazole, or acridine, show interesting cytostatic and antitumor properties.[3,4]

Emodin, as an important component of a Chinese herb, has received considerable attention by many synthetic chemists and biochemists. Structurally it belongs to anthraquinones as do daunorubicin and mitoxantrone which are some of the most powerful cytostatics. Recently it was reported that emodin could induce the apoptosis of certain cancer cells.[5,6] However, emodin showed low DNA binding affinity, and low or insignificant cytotoxicity against various cancer cells.[2] It was proved that the addition of side chains such as polymethyleneamine, sugar or heterocycle to emodin, was usually effective to gain higher antitumor activity.[7,8] We are more attracted by two natural emodin glycosides 2′,3′-di-*O*-acetylfrangulin A (**1**) and frangulin B (**2**) (Fig. 1), which bear L-rhamnosyl moieties at C_3–OH of emodin and showed much more cytotoxic activity against KB cells than emodin,[9,10] indicating that such sugar appendages lead to conspicuous changes of their antitumor activity.[9] Another two natural products **3** and **4** (Fig. 1), analogs of glycoside **1** with two acetyl groups on the different hydroxyls of rhamnosyl moiety, were isolated by Peter and Abegaz,[11] while their antitumor activity has not been reported up to now.

With the continuous interest in the effect of sugar attachments to a planar aromatic molecule on the biological activity and in order to search for potential new antitumor agents, we decided to investigate the effect of subtle modifications of rhamnosyl moiety of emodin rhamnosides on the tumor cell growth inhibitory activity. Here, we would like to report the synthesis of three natural emodin glycosides **2**, **3** and **4** along with their analogs **5**, **6** and **7** (Fig. 1), which bear the L-rhamnosyl moieties with acyl modification. Another three emodin derivatives **8**, **9** and **10** (Fig. 1) were also provided by the installation of rhamnosyl residue onto 1,8-di-*O*-methoxy-emodin and 10-acetyl-emodin in order to study the influence of subtle modifications of emodin skeleton on the tumor cell growth inhibitory activity. Both cytotoxic activity and DNA binding capacity of all natural and designed compounds were evaluated in vitro. The results obtained have rendered important clues to the understanding of the cytotoxic profile for these types of compounds.

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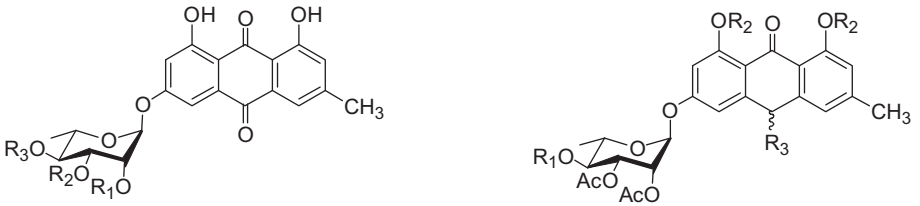


Figure 1. Chemical structures of anthracene glycosides **1**–**10**.

2. Results and discussion

2.1. Chemistry

We have reported the successful synthesis of compound **1** previously by glycosylation of 1,8-*O*-di-*n*-hexanoyl emodin[12] with L-rhamnopyranosyl trichloroacetimidate under the promotion of $BF_3{\cdot}Et_2O$, followed by deprotection of Hex groups in the presence of PhSH and DBU.[12] In the present study, the synthesis of target compounds **2**–**6** was done by the similar route as that for compound **1** described previously by us.[12]

First four trichloroacetimidate donors **11**–**14** were prepared as shown in Scheme 1, respectively. Treatment of the known compound **15**[13]or **16**[13] with levulinic acid in the presence of EDC·HCl and DMAP afforded compound **17** or **18**, respectively. Selective cleavage of PMB at C_3–OH of known compound **19**[13] was performed smoothly with DDQ, and then the subsequent acylation gave compound **20**. The known compound **21**[13] was subjected to removal of the 2,3-*O*-isopropylidene group and then butyrylation of the corresponding OH to provide compound **22**. Hydrolysis of **17**, **18**, **20** or **22** with *N*-bromosuccinimide (NBS) in acetone–H_2O, followed by treatment with CCl_3CN and DBU gave trichloroacetimidate **11**–**14**, respectively.

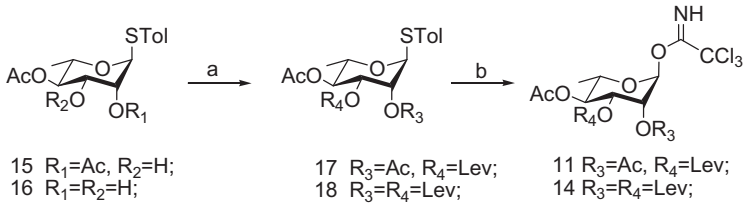
The preparation of the target compounds **2**–**6** was performed as shown in Scheme 2. With the known acceptor **23**[12] and required donors **11**–**14** in hand, the coupling reactions were performed under the promotion of $BF_3{\cdot}Et_2O$ to provide the desired rhamnosylanthraquinones **24**–**27**. The subsequent deprotection of the *n*-hexanoyl group and the Lev group with hydrazine acetate was carried out simultaneously to afford compounds **3**–**6**. To the best of our knowledge, this was the first report that phenol ester was removed successfully with hydrazine acetate, which greatly facilitated the synthesis of emodin glycosides.[12] Cleavage of all phenol and alkyl esters of the intermediate **24** was performed smoothly with MeONa to provide **2** in 85.7% yield. Thus, the total synthesis of three natural occurring anthraquinone L-rhamnopyranosides **2**, **3** and **4** was achieved for the first time.

The synthesis of the target compound **7** was depicted in Scheme 3. With the acceptor **1** and donor **28**[12] in hand, their coupling reaction was performed under the promotion of TMSOTf to provide **29**, which was subjected to the Lev cleavage with hydrazine acetate to obtain the target compound **7** smoothly.

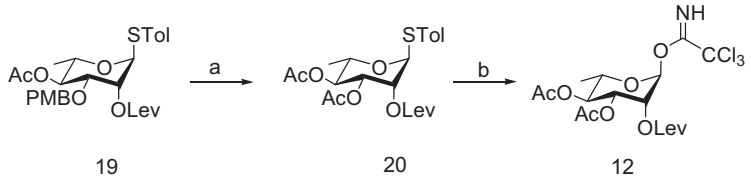
Rhamnosides of emodin derivatives **8**, **9**, **10** were prepared as outlined in Scheme 4. Treatment of the known compound **30**[12] with MeI in the presence of K_2CO_3 afforded intermediate **31**, which was subjected to hydrazine acetate to cleave the Lev group, furnishing the target compound **8**. Acetylation of compound **32**[12] led to the formation of the target compound **9** as a diastereoisomer mixture along with 1,8-*O*-diacetate byproduct **33**. The ratio of each diastereoisomer for compounds **9** and **33** was about 3:1 and 4:1, respectively, which could be determined by NMR. However, Sévenet and co-workers[9] reported only one pure diastereoisomer of **9** was afforded under the similar conditions. The preparation of the target compound **10** was first tried by the similar route as that for **9**. Therefore, intermediate **34** was first synthesized from compound **35**, which was afforded by reducing the known compound **30** with Zn–AcOH. During a trial of the preparation of **10** by treating **34** with hydrazine acetate, we found that two pairs of the *meso* and *racemic* bianthrones **36**[9] were obtained without the target compound **10** observed. Then we replaced the Lev group in **34** with benzyl group. Thus treatment compound **1** with benzyl trichloroacetimidate **37**[14] was performed under the promotion of TfOH to provide compound **38**, which was then reduced by Zn–AcOH and followed by acetylation to furnish **40**. Finally debenzylation was carried out by hydrogenolysis using Pd/C to provide the target compound **10** as a diastereoisomer mixture, of which the ratio of each diastereoisomer was about 100:1, which was determined by NMR.

2.2. Cytotoxicity against tumor cells

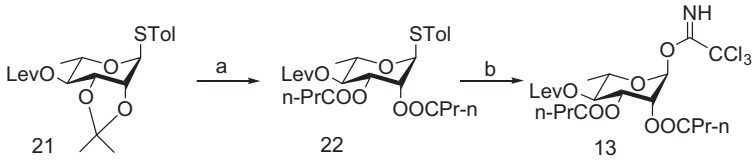
To examine the potential ability of the natural and artificially designed anthracene L-rhamnopyranosides **1**–**10**, both their cytotoxicity and DNA binding capacity were tested in vitro, respectively. Table 1 shows the IC_{50} values of compounds **1**–**10** and **33** against three types of tumor cells in culture. The results indicate that (a) in the case of the emodin rhamnopyranosides **1**–**7**, most compounds except **3** and **5** exhibited better cytotoxicity against the three tumor cells than emodin, which proved that the addition of L-rhamnosyl residue was critical to improve cytotoxicity. (b)



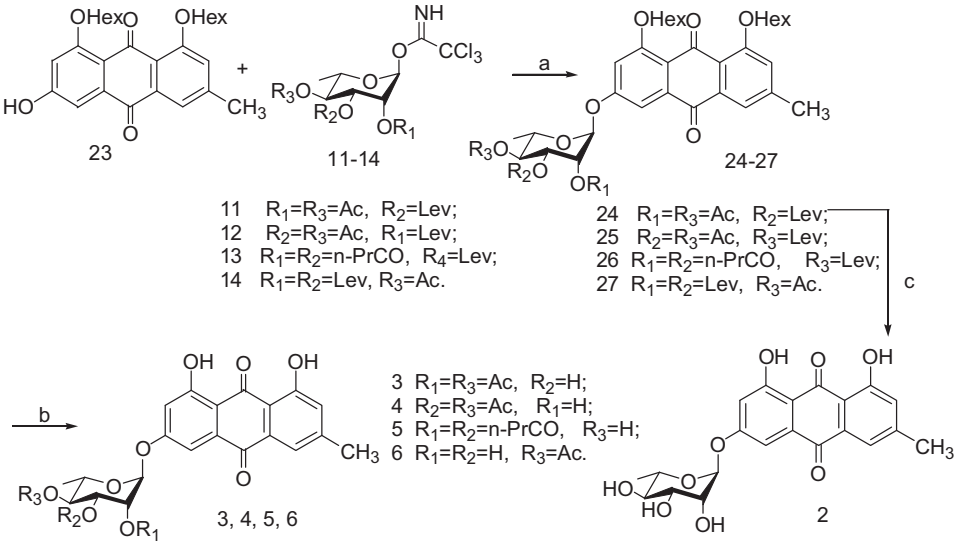
Scheme 1.1. Reagents and conditions: (a) EDC·HCl, DMAP, levulinic acid, CH_2Cl_2, 83.5% for **17**, 97.5% for **18**; (b) (i) NBS, acetone–H_2O; (ii) DBU, $CNCCl_3$, CH_2Cl_2.



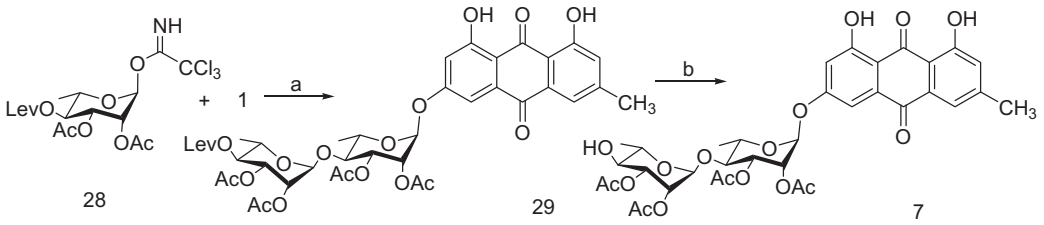
Scheme 1.2. Reagents and conditions: (a) (i) DDQ; (ii) Ac_2O, DMAP, pyridine, 90.0% for two steps; (b) (i) NBS, acetone–H_2O; (ii) DBU, $CNCCl_3$, CH_2Cl_2.



Scheme 1.3. Reagents and conditions: (a) (i) 80% AcOH; (ii) *n*-PrCOCl, DMAP, pyridine, 72.4% over two steps; (b) (i) NBS, acetone–H_2O; (ii) DBU, $CNCCl_3$, CH_2Cl_2.



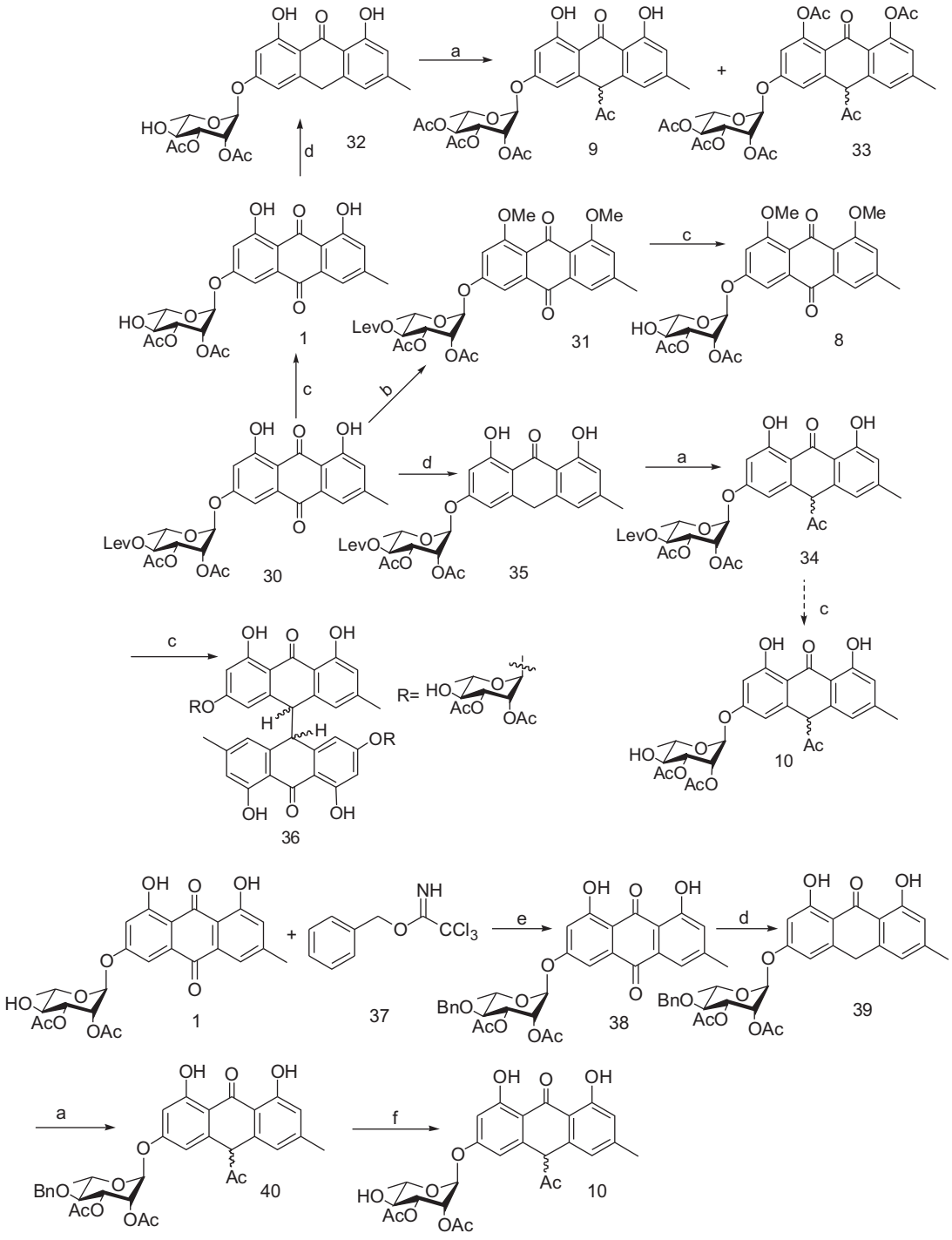
Scheme 2. Reagents and conditions: (a) $BF_3{\cdot}Et_2O$, CH_2Cl_2, 98.0% for **24**, 98.2% for **25**, 96.9% for **26**, 93.8% for **27**; (b) AcOH–NH_2NH_2, 77.8% for **3**, 78.2% for **4**, 75.6% for **5**, 75.2% for **6**; (c) MeONa, 85.7%.



Scheme 3. Reagents and conditions: (a) TMSOTf, CH_2Cl_2, 94.1%; (b) AcOH–NH_2NH_2, 76.2%.

Among compounds **1**–**7**, compound **1** showed the strongest cytotoxicity against KB and HL-60 cells, while compound **4** exhibited stronger cytotoxicity against MDA-MB-231 than the other emodin rhamnopyranosides, indicating subtle modifications of rhamnosyl moiety with acetyl group had a selective toxicity for different tumor cells. (c) The influence of the length of acyl chain on cytotoxicity was clear when compared compound **1** and **5**. (d) The decreased inhibition of compound **7** against KB and HL-60 cells (about eightfold and fourfold) comparing with **1** suggested introducing more sugar residue did not favor the activity. (e) Blocking both C_1–OH and C_8–OH of compound **1** with methyl group (**8**) resulted in a loss of antitumor activity, which proved that both C_1–OH and C_8–OH of emodin were essential for the activity. (f) Much stronger inhibition of **10** than **1** (about 10-fold increase) suggested replacing C-10 carbonyl group of emodin with acetylmethylene group favored the activity.

Compound **9** displayed the good cytotoxicity among the compounds above. In order to investigate its cell selectivity, cytotoxic activity against a normal cell line, human microvascular endothelial cell (HMEC), was tested. The IC_{50} value of compound



Scheme 4. Reagents and conditions: (a) Ac_2O, pyridine, 42.6% for **9**, 38.7% for **34**, 40.6% for **40**; (b) K_2CO_3, MeI, acetone, 95.3%; (c) AcOH–NH_2NH_2, 91.3%; (d) Zn/AcOH, 79.4%; (e) **37**, TfOH, CH_2Cl_2, 67.8%; (f) Pd/C, H_2, 90.4%.

9 against HMEC was 16.2 μM, which was reduced almost 20 times as much as against tumor cells. The result suggested that compound **9** had cell selectivity to a certain extent.

2.3. Prediction of lipophilicity

The partition coefficient log *P* is a parameter which describes the manner in which a drug partitions between polar and non-polar phases, and it has been demonstrated to be an indispensable tool in predicting the transport and activity of drugs. Table 2 gathered the values of log *P*, determined by using ACD lab program for each compound **1**–**10** and **33**. All the compounds **1**–**10** and **33** showed similar lipophilicity with log *P* values in the range of −1.18 to −1.68 (Table 2). Lipophilicity did not exert a significant effect on cytotoxicity for compounds, pointing out that lipophilicity of the molecules was not a crucial factor for cytotoxicity.

2.4. DNA binding properties

Competitive displacement (C_{50}) fluorometric assays with DNA-bound ethidium can be used[15] to determine 'apparent' equilibrium constants (K_{app}) for drug binding, as the C_{50} value is approximately

Table 1
IC_{50} cytotoxicity values (μM) of anthracene L-rhamnopyranosides against tumor cells (data derived from the mean of three independent assays)

Compound	KB	HL-60	MDA-MB-231
1	1.1	3.6	>20
2	2.1	15.7	>20
4	>20	>20	1.2
6	>20	>20	7.9
7	8.3	11.8	>20
9	0.7	1.1	0.8
10	0.3	0.8	0.4
33	1.1	1.5	1.7
3, **5**, **8**	>20	>20	>20
Emodin	>20	>20	>20

Table 2
Lipophilicity (log *P*) of the target compounds **1**–**10** and **33**

Compound	log *P*
1	−1.18
2	−1.44
3	−1.18
4	−1.18
5	0.87
6	−1.31
7	−1.63
8	−1.11
9	−1.25
10	−1.38
33	−1.68

inversely proportional to the binding constant.[16] The K_{app} values of anthracene L-rhamnopyranosides **1**–**10** and **33** calculated for calf thymus DNA, are reported in Table 3. All the glycosides had higher DNA binding constants than emodin, indicating that the introduction of rhamnosyl residue dramatically increased the DNA binding capacity of emodin or its derivatives. The relative binding affinities as indicated by the binding constants K_{app} are in the order of **1** > **8** and **9** > **33**, which indicates that blocking the C_1–OH and C_8–OH in emodin resulted in the reduction of the DNA binding capacity.

There is not quantitative correspondence between binding with calf thymus DNA and in vitro potency. It seems that the best results in cytotoxicity are obtained where high values of K_{app} with calf thymus DNA are combined with good cytotoxicity in vitro.

2.5. Inhibitions of DNA topoisomerase IIα

DNA topoisomerase II (Top2) is a well-known anticancer target. Anthracyclines antibiotics, especially adriamycin (ADM), are the typical representatives of DNA topoisomerase II inhibitors. In order to investigate whether anthracene L-rhamnopyranosides shared the similar mechanism as adriamycin, inhibition of TopoII α activity was measured by the ATP-dependent decatenation of kDNA in a cell-free system. As shown in Figure 2, in the absence of compounds, kDNA was decatenated to minicircles which disappeared in the presence of adriamycin and compound **1**, **2**, **6**, **7**, **9**, **33**, indicating that compound **1**, **2**, **6**, **7**, **9**, **33** could inhibit the catalytic activity of Top2α.

Table 3
DNA binding ability of the target compounds **1**–**10** and **33**

Compound	K^a_{app} (10^{-7} M^{-1})	Compound	K_{app} (10^{-7} M^{-1})
Emodin	0.0075	**6**	1.52
1	1.43	**7**	1.88
2	1.19	**8**	0.28
3	0.81	**9**	1.36
4	1.58	**10**	1.20
5	2.86	**33**	0.33

$K^a_{app} = (1.26/C_{50}) \times 10^{-7}$ in which 1.26 is the concentration (μM) of ethidium in ethidium–DNA complex, C_{50} is drug concentration (μM) to effect 50% drop in fluorescence of bound ethidium, and 10^7 is the value of K_{app} assumed for ethidium in the complex.

3. Conclusions

A series of novel anthracene rhamnopyranosides based on emodin and its derivatives were synthesized in a practical way and their cytotoxic activity was tested in vitro. The preliminary results showed that the introduction of some L-rhamnosyl moieties to emodin or its derivatives could significantly improve cytotoxicity, notably subtle modifications of rhamnosyl moiety with acetyl group had a selective toxicity for different tumor cells, both C_1–OH and C_8–OH of emodin L-rhamnopyranosides were essential for antitumor activity, and displacement of C-10 carbonyl group of emodin by acetylmethylene group was helpful to improve the inhibitory activity. All anthracene glycosides had higher DNA binding constants but there was not quantitative correspondence between binding with calf thymus DNA and in vitro potency. Lipophilicity of the molecules was not a crucial factor for cytotoxicity and most anthracene glycosides with good cytotoxicity could inhibit the catalytic activity of Top2α.

4. General methods

Solvents were purified in a conventional manner. Thin layer chromatography (TLC) was performed on precoated E. Merck Silica Gel 60 F254 plates. Flash column chromatography was performed on silica gel (200–300 mesh, Qingdao, China). Optical rotations were determined with a Perkin–Elmer Model 241 MC polarimeter. ^{1}H NMR and ^{13}C NMR spectra were taken on a JEOL JNM-ECP 600 spectrometer with tetramethylsilane as an internal standard, and chemical shifts are recorded in ppm values. Mass spectra were recorded on a Q-TOF Global mass spectrometer.

4.1. *p*-Tolyl 3-*O*-levulinyl-2,4-di-*O*-acetyl-1-thio-α-L-rhamnopyranoside (17)

To a solution of compound **15** (2.0 g, 5.6 mmol) in dry CH_2Cl_2 (50 mL), levulinic acid (786.1 mg, 6.8 mmol), EDC (1.4 g, 6.8 mmol), and DMAP (68.9 mg, 0.6 mmol) were added under argon. The mixture was stirred for 12 h, diluted with CH_2Cl_2 (150 mL), washed with H_2O (100 mL), 1 M HCl (2 × 100 mL), saturated aqueous $NaHCO_3$ (2 × 100 mL), and brine (2 × 100 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (10:1, v/v, petroleum ether/EtOAc) to give **17** (2.13 g, 83.5%) as a white solid; R_f 0.44 (2:1, petroleum ether/EtOAc); $[\alpha]_D^{25}$ −90.4 (*c* 0.10, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 7.35 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.12 (d, 2H, *J* = 8.0 Hz, Ar-H), 5.47 (dd, 1H, *J* = 3.2, 1.8 Hz, H-2), 5.33 (d, 1H, *J* = 1.4 Hz, H-1), 5.29 (dd, 1H, *J* = 10.1, 3.2 Hz, H-3), 5.15 (t, 1H, *J* = 9.9 Hz, H-4), 4.34–4.39 (m, 1H, H-5), 2.77–2.82, 2.55–2.66, 2.41–2.46 (m, 4H, $COCH_2$), 2.32 (s, 3H, $PhCH_3$), 2.18, 2.14, 2.13 (each s, each 3H, 3 × $COCH_3$), 1.24 (d, 3H, *J* = 6.0 Hz, 6-CH_3); ^{13}C NMR ($CDCl_3$): δ 206.2, 171.6, 170.2, 170.0, 138.2, 132.4, 129.9, 129.4, 86.0, 71.2, 70.8, 69.6, 67.6, 37.6, 29.7, 27.8, 21.1, 20.9, 20.8, 17.3; ESI MS: calcd for $[M+H]^+$ *m/z* 453.2; found, 453.2.

4.2. *p*-Tolyl 2,3-di-*O*-levulinyl-4-*O*-acetyl-1-thio-α-L-rhamnopyranoside (18)

To a solution of compound **16** (1.0 g, 3.2 mmol) in dry THF (50 mL), levulinic acid (0.95 g, 8.01 mmol), EDC (1.84 g,

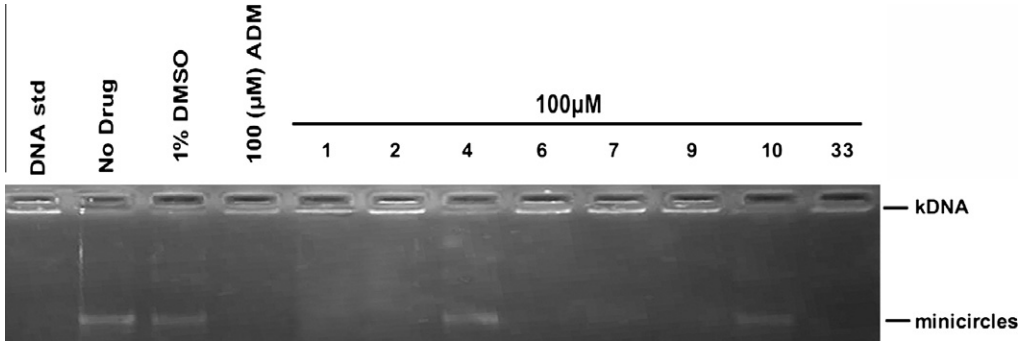


Figure 2. Anthracene L-rhamnopyranosides or adriamycin inhibited topo II-mediated kDNA decatenation in the cell-free system. The positions of kDNA and minicircles were indicated.

9.60 mmol) and DMAP (0.16 g, 1.28 mmol) were added under argon. The mixture was stirred for 12 h and then concentrated. The residue was dissolved with CH_2Cl_2 (150 mL), washed with H_2O (100 mL), 1 M HCl (2 × 100 mL), saturated aqueous $NaHCO_3$ (2 × 100 mL), and brine (2 × 100 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (3:1, v/v, petroleum ether/EtOAc) to give **18** (1.58 g, 97.5%) as a white solid; R_f 0.44 (1:1, petroleum ether/EtOAc); $[\alpha]_D^{25}$ −50.2 (*c* 0.11, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.40 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.17 (d, 2H, *J* = 8.0 Hz, Ar-H), 5.55 (dd, 1H, *J* = 3.6, 1.2 Hz, H-2), 5.36 (d, 1H, *J* = 1.4 Hz, H-1), 5.32 (dd, 1H, *J* = 10.2, 3.6 Hz, H-3), 5.17 (t-like, 1H, *J* = 10.2, 9.6 Hz, H-4), 4.40–4.43 (m, 1H, H-5), 2.49–2.87 (m, 8H, 4 × CH_2), 2.38 (s, 3H, PhCH_3), 2.25, 2.23, 2.18 (each s, each 3H, 3 × COCH_3), 1.29 (d, 3H, *J* = 6.0 Hz, 6-CH_3); ^{13}C NMR ($CDCl_3$): δ 207.4, 206.3, 171.7, 170.8, 170.1, 138.6, 132.6 (two), 130.0 (two), 129.2, 86.0, 71.4, 70.9, 69.6, 67.7, 37.8, 37.7, 29.9, 29.8, 28.0, 27.9, 21.2, 20.9, 17.4; ESIMS: calcd for $[M+H]^+$ *m/z* 509.2; found, 509.2.

4.3. *p*-Tolyl 2-*O*-levulinyl-3,4-di-*O*-acetyl-thio-1-α-L-rhamnopyranoside (20)

To a solution of compound **19** (1.24 g, 2.31 mmol) in CH_2Cl_2 (15 mL) and H_2O (1 mL), DDQ (0.67 g, 2.96 mmol) was added under argon. The mixture was stirred for 2 h, diluted with CH_2Cl_2 (100 mL), washed with saturated aqueous $NaHCO_3$ (2 × 50 mL), and brine (2 × 50 mL), dried over Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography (2:1, v/v, petroleum ether/EtOAc) to afford a white solid (0.98 g, 90.7%); to a stirred solution of the white solid (0.68 g, 1.66 mmol) in dry CH_2Cl_2 (30 mL) at 0 °C were added Ac_2O (0.24 mL, 2.50 mmol), Et_3N (0.69 mL, 4.98 mmol) and DMAP (40.5 mg, 0.33 mmol). The mixture was stirred for 12 h, diluted with CH_2Cl_2 (100 mL), washed with 1 M HCl (2 × 50 mL), saturated aqueous $NaHCO_3$ (2 × 50 mL), and brine (2 × 50 mL), dried over Na_2SO_4, and concentrated to dryness. The residue was purified by silica gel column chromatography (3:1, v/v, petroleum ether/EtOAc) to give compound **20** (0.70 g, 99.2%); R_f 0.47 (1:1, petroleum ether/EtOAc); $[\alpha]_D^{25}$ −60.4 (*c* 0.17, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.42 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.20 (d, 2H, *J* = 8.0 Hz, Ar-H), 5.57 (dd, 1H, *J* = 3.0, 1.2 Hz, H-2), 5.39 (d, 1H, *J* = 1.4 Hz, H-1), 5.35 (dd, 1H, *J* = 10.1, 3.0 Hz, H-3), 5.19 (t, 1H, *J* = 10.2 Hz, H-4), 4.42–4.47 (m, 1H, H-5), 2.40 (s, 3H, Ar-CH_3), 2.20, 2.15, 2.09 (each s, each 3H, 3 × COCH_3), 1.31 (d, 3H, *J* = 6.0 Hz, 6-CH_3); ^{13}C NMR ($CDCl_3$): δ 206.1, 171.7, 170.1, 170.0, 138.3, 132.6, 130.1, 129.4, 86.0, 715, 71.3, 69.4, 67.7, 37.9, 29.9, 28.1, 21.2, 20.9, 20.7, 17.4; ESIMS: calcd for $[M+H]^+$ *m/z* 453.2; found, 453.2.

4.4. *p*-Tolyl 2,3-di-*O*-butyryl-4-*O*-levulinyl-1-thio-α-L-rhamnopyranoside (22)

The solution of compound **21** (1.51 g, 3.80 mmol) in 80% AcOH (30 mL) was stirred for 1.5 h at 80 °C, then concentrated. To a solution of the residue in dry pyridine (30 mL) were added BtCl (1.18 mL, 11.40 mmol) and DMAP (0.26 g, 1.52 mmol) at 0 °C. The reaction mixture was stirred for an additional 8 h while warming to room temperature. When TLC (petroleum ether/EtOAc, 3:1) showed complete conversion, MeOH (2 mL) was carefully added to destroy excess Bt_2O, and the mixture was diluted with CH_2Cl_2 (200 mL) The organic layer was shaken with 1 M HCl (3 × 100 mL), followed by washing with saturated aqueous $NaHCO_3$ (3 × 100 mL) and water (3 × 100 mL). Finally the organic layer was separated, dried over Na_2SO_4, and evaporated to syrup. The crude product was purified by silica gel column chromatography (5:1, v/v, petroleum ether/EtOAc) to give **22** (1.40 g, 72.4%); R_f 0.83 (1:1, petroleum ether/EtOAc); $[\alpha]_D^{25}$ −93.7 (*c* 0.11, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.37 (d, 2H, *J* = 8.3 Hz, Ar-H), 7.13 (d, 2H, *J* = 8.2 Hz, Ar-H), 5.52 (dd, 1H, *J* = 3.0, 1.2 Hz, H-2), 5.33 (d, 1H, *J* = 1.8 Hz, H-1), 5.31 (dd, 1H, *J* = 10.1, 3.0 Hz, H-3), 5.16 (t-like, 1H, *J* = 10.2, 9.6 Hz, H-4), 4.34–4.40 (m, 1H, H-5), 2.74–2.78 (m, 2H, COCH_2), 2.52–2.62 (m, 2H, COCH_2), 2.37–2.38 (m, 2H, COCH_2), 2.33 (s, 3H, PhCH_3), 2.23–2.28 (m, 2H, COCH_2), 2.20 (s, 3H, CH_3CO), 1.60–1.69 (m, 4H, 2 × CH_2), 1.26 (d, 3H, *J* = 6.6 Hz, CH_3), 0.97 (t, 3H, *J* = 7.8 Hz, CH_3), 0.93 (t, 3H, *J* = 7.8 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 206.2, 173.3, 172.7, 171.9, 138.3, 132.6 (two), 130.2 (two), 129.6, 86.2, 71.5, 71.2, 69.1, 67.8, 37.8, 36.1, 35.9, 29.9, 28.0, 21.2, 18.5, 18.2, 17.4, 13.7; ESIMS: calcd for $[M+Na]^+$ *m/z* 531.2; found, 531.2.

4.5. General procedure for the preparation of different suitable protected rhamnopyranosyl trichloroacetimidate (11–14)

To a stirred solution of **17**, **18**, **20**, **22** (1.0 equiv) in acetone and H_2O (v/v = 9:1) at −20 °C was added NBS (2.0 equiv). After 20 min, the reaction was quenched with saturated aqueous $NaHCO_3$ and concentrated. The residue was diluted with $CHCl_3$ and washed with saturated aqueous $NaHCO_3$, brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (petroleum ether/EtOAc) to afford each product. To a stirred solution of above product (1 equiv) and CCl_3CN (6.0 equiv) in dry CH_2Cl_2 at 0 °C was added DBU (0.5 equiv). After 2 h, the solution was concentrated. The residue was purified fast by silica gel column chromatography (3:1, v/v, petroleum ether/EtOAc) to afford crude **11**–**14**, which could be used directly in the next step.

4.6. General procedure for the preparation of 1,8-di-*O*-*n*-hexanoyl-3-(2′,3′,4′-substituted-α-L-rhamnopyranosyl)-emodin (24–27)

To a solution of compound **23** (1.0 equiv), appropriate rhamnopyranosyl trichloroacetimidate **11**–**14** (1.2 equiv) and 4 Å molecular sieves in dry CH_2Cl_2 (20 mL) was added $BF_3{\cdot}Et_2O$ (1 equiv) at 0 °C under argon. The reaction mixture was allowed to stir for 2 h under this condition, while warmed to room temperature until TLC indicated that the reaction was complete. The reaction was quenched by Et_3N (five drops) and concentrated. The residue was purified by silica gel column chromatography (5:1→2:1, v/v, petroleum ether/EtOAc) to give a appropriate compound **24**–**27**.

4.6.1. 1,8-Di-*O*-*n*-hexanoyl-3-(2′,4′-di-*O*-acetyl-3′-*O*-levulinyl-α-L-rhamnopyranosyl)-emodin (24)

An orange solid, yield 98.0%; R_f 0.18 (2:1, petroleum ether/EtOAc); $[\alpha]_D^{25}$ −78.7 (*c* 0.11, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.97 (s-like, 1H, Ar-H), 7.83 (t, 1H, *J* = 2.4 Hz, Ar-H), 7.16 (s-like, 1H, Ar-H), 7.02 (t, 1H, *J* = 2.4 Hz, Ar-H), 5.63 (s-like, 1H, H-1′), 5.46 (td, 1H, *J* = 10.3, 3.6 Hz, H-3′), 5.40 (dd, 1H, *J* = 3.2, 1.8 Hz, H-2′), 5.19 (td, 1H, *J* = 10.2, 2.4 Hz, H-4′), 3.85–3.89 (m, 1H, H-5′), 2.54–2.79 (m, 8H, 4 × CH_2CO), 2.46 (s, 3H, Ar-CH_3), 2.19 (s, 3H, CH_3CO), 2.16 (s, 3H, CH_3CO), 2.09 (s, 3H, CH_3CO), 1.78–1.82 (m, 4H, 2 × CH_2), 1.38–1.42 (m, 8H, 4 × CH_2), 1.23 (dd, 3H, *J* = 6.0, 1.8 Hz, CH_3), 0.92–0.94 (m, 6H, 2 × CH_3); ^{13}C NMR ($CDCl_3$): δ 206.2, 182.0, 179.6, 172.3, 172.0, 171.8, 170.2, 170.1, 159.6, 152.4, 150.4, 146.0, 136.2, 134.2, 131.0, 126.0, 123.4, 121.1, 118.0, 111.9, 95.5, 70.3, 69.2, 68.9, 68.1, 37.8, 34.4 (two), 31.6, 29.8, 27.9, 24.3, 22.5, 21.7, 20.9, 20.7, 17.5, 14.1; ESIMS: calcd for $[M+Na]^+$ *m/z* 817.3; found, 817.3.

4.6.2. 1,8-Di-*O*-*n*-hexanoyl-3-(3′,4′-di-*O*-acetyl-2′-*O*-levulinyl-α-L-rhamnopyranosyl)-emodin (25)

A yellow solid, yield 98.2%; R_f 0.18 (2:1, petroleum ether/EtOAc); $[\alpha]_D^{25}$ −63.4 (*c* 0.10, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.01 (d, 1H, *J* = 1.2 Hz, Ar-H), 7.87 (d, 1H, *J* = 2.6 Hz, Ar-H), 7.19 (d, 1H, *J* = 1.2 Hz, Ar-H), 7.08 (d, 1H, *J* = 2.4 Hz, Ar-H), 5.65 (d, 1H, *J* = 1.2 Hz, H-1′), 5.45–5.47 (m, 1H, H-2′, H-3′), 5.17 (t-like, 1H, *J* = 10.3, 9.7 Hz, H-4′), 3.90–3.92 (m, 1H, H-5′), 2.70–2.83 (m, 6H, 3 × CH_2CO), 2.50 (s, 3H, Ar-CH_3), 2.27–2.30 (m, 2H, CH_2CO), 2.24 (s, 3H, CH_3CO), 2.07 (s, 3H, CH_3CO), 2.06 (s, 3H, CH_3CO), 1.83–1.86 (m, 4H, 2 × CH_2), 1.42–1.46 (m, 8H, 4 × CH_2), 1.22 (d, 3H, *J* = 6.6 Hz, CH_3), 0.97 (t, 3H, *J* = 7.1 Hz, CH_3), 0.96 (t, 3H, *J* = 7.1 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 206.0, 182.0, 179.6, 172.3, 172.0, 171.7, 170.1, 170.0, 159.6, 152.4, 150.4, 146.0, 136.2, 134.2, 131.0, 126.0, 123.4, 121.1, 118.0, 112.0, 95.5, 70.6, 69.3, 68.6, 68.0, 38.0, 34.4 (two), 31.6, 29.9, 28.0, 24.3 (two), 22.5, 21.7, 20.8, 20.7, 17.5, 14.1; ESIMS: calcd for $[M+Na]^+$ *m/z* 817.3; found, 817.3.

4.6.3. 1,8-Di-*O*-*n*-hexanoyl-3-(2′,3′-di-*O*-butyryl-4′-*O*-levulinyl-α-L-rhamnopyranosyl)-emodin (26)

A yellow solid, yield 96.9%; R_f 0.55 (2:1, petroleum ether/EtOAc); $[\alpha]_D^{25}$ −70.2 (*c* 0.11, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.93 (s-like, 1H, Ar-H), 7.80 (d, 1H, *J* = 2.4 Hz, Ar-H), 7.11 (s-like, 1H, Ar-H), 7.00 (d, 1H, *J* = 2.4 Hz, Ar-H), 5.57 (d, 1H, *J* = 1.2 Hz, H-1′), 5.43 (dd, 1H, *J* = 10.2, 3.6 Hz, H-3′), 5.40 (dd, 1H, *J* = 3.6, 1.8 Hz, H-2′), 5.12 (t-like, 1H, *J* = 10.2, 9.6 Hz, H-4′), 3.84–3.86 (m, 1H, H-5′), 2.62–2.68 (m, 6H, 3 × CH_2CO), 2.45–2.48 (m, 2H, CH_2CO), 2.42 (s, 3H, Ar-CH_3), 2.35–2.38 (m, 2H, CH_2CO), 2.19–2.26 (m, 2H, CH_2CO), 2.10 (s, 3H, CH_3CO), 1.74–1.79 (m, 4H, 2 × CH_2), 1.62–1.67 (m, 4H, 2 × CH_2), 1.54–1.58 (m, 2H, CH_2), 1.16 (d, 3H, *J* = 6.0 Hz, CH_3), 0.86–0.95 (m, 12H, 4 × CH_3); ^{13}C NMR ($CDCl_3$): δ 206.1, 182.0, 179.6, 172.7, 172.6, 172.3, 172.0, 171.8, 163.6, 159.7, 152.3, 150.4, 146.0, 136.2, 134.2, 131.0, 126.1, 123.4, 121.0, 118.0, 112.0, 95.7, 70.8, 69.0, 68.3, 68.1, 37.8, 36.1, 35.9, 34.4 (two), 31.6, 29.8, 28.0, 24.3 (two), 22.5, 21.7, 18.5, 18.3, 17.5, 14.3, 13.7; ESIMS: calcd for $[M+Na]^+$ *m/z* 851.4; found, 851.4.

4.6.4. 1,8-Di-*O*-*n*-hexanoyl-3-(2′,3′-di-*O*-levulinyl-4′-*O*-acetyl-α-L-rhamnopyranosyl)-emodin (27)

A yellow solid, yield 93.8%; R_f 0.10 (2:1, petroleum ether/EtOAc); $[\alpha]_D^{25}$ −50.7 (*c* 0.10, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.98 (d, 1H, *J* = 1.2 Hz, Ar-H), 7.83 (d, 1H, *J* = 2.6 Hz, Ar-H), 7.16 (d, 1H, *J* = 1.3 Hz, Ar-H), 7.03 (d, 1H, *J* = 2.4 Hz, Ar-H), 5.61 (d, 1H, *J* = 1.8 Hz, H-1′), 5.41–5.44 (m, 1H, H-2′, H-3′), 5.14 (t, 1H, *J* = 10.3 Hz, H-4′), 3.86–3.89 (m, 1H, H-5′), 2.24–2.80 (m, 12H, 6 × CH_2CO), 2.46 (s, 3H, Ar-CH_3), 2.20 (s, 3H, CH_3CO), 2.18 (s, 3H, CH_3CO), 2.09 (s, 3H, CH_3CO), 1.80–1.82 (m, 4H, 2 × CH_2), 1.40–1.44 (m, 8H, 4 × CH_2), 1.19 (d, 3H, *J* = 6.0 Hz, CH_3), 0.94 (t, 3H, *J* = 7.1 Hz, CH_3), 0.93 (t, 3H, *J* = 7.1 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 206.4, 206.1, 182.0, 179.6, 172.3, 172.0, 171.8, 171.1, 170.0, 159.6, 150.4, 146.0, 136.2, 134.2, 131.0, 126.0, 123.4, 121.1, 118.0, 112.0, 95.5, 70.2, 69.2, 68.9, 68.0, 37.9, 37.8, 34.4 (two), 31.6, 29.9, 29.8, 28.0 (two), 24.3 (two), 22.5, 21.7, 20.9, 17.5, 14.1; ESIMS: calcd for $[M+Na]^+$ *m/z* 873.3; found, *m/z* 873.3.

4.7. General procedure for the preparation of 3-(2′,3′,4′-substituted-α-L-rhamnopyranosyl)-emodin (3–6)

To a stirred solution of **24**–**27** (1 equiv) in CH_2Cl_2 and CH_3OH (v/v, 1:1) was added NH_2NH_2–HOAc (10 or 20 equiv). After 5 h, the solution was concentrated. Then the residue was purified by silica gel column chromatography (petroleum ether/acetone) to afford compound **3**–**6**, respectively.

4.7.1. 3-(2′,4′-Di-*O*-acetyl-α-L-rhamnopyranosyl)-emodin (3)

A red solid, yield 77.8%; R_f 0.41 (2:1, petroleum ether/acetone); $[\alpha]_D^{25}$ −82.8 (*c* 0.11, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 12.20 (s, 1H, O*H*), 11.98 (s, 1H, O*H*), 7.58 (s-like, 1H, Ar-H), 7.40 (d, 1H, *J* = 2.4 Hz, Ar-H), 7.04 (s, 1H, Ar-H), 6.84 (d, 1H, *J* = 2.4 Hz, Ar-H), 5.65 (s, 1H, H-1′), 5.21–5.22 (m, 1H, H-2′), 4.91 (t-like, 1H, *J* = 10.3, 9.6 Hz, H-4′), 4.18–4.19 (m, 1H, H-3′), 3.78–3.83 (m, 1H, H-5′), 2.40 (s, 3H, Ar-CH_3), 2.19 (s, 3H, CH_3CO), 2.10 (s, 3H, CH_3CO), 1.17 (d, 3H, *J* = 6.6 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 191.1, 181.7, 171.5, 170.6, 165.0, 162.7, 162.4, 148.9, 135.5, 133.2, 124.7, 121.6, 113.7, 111.6, 109.5, 95.1, 74.2, 72.0, 68.3, 67.7, 22.3, 21.1, 17.5; HRESIMS: calcd for $C_{25}H_{23}O_{11}$ 499.1240; found, 499.1237.

4.7.2. 3-(3′,4′-Di-*O*-acetyl-α-L-rhamnopyranosyl)-emodin (4)

A red solid, yield 78.2%; R_f 0.40 (2:1, petroleum ether/acetone); $[\alpha]_D^{25}$ −145.1 (*c* 0.09, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 12.27 (s, 1H, O*H*), 12.05 (s, 1H, O*H*), 7.64 (d, 1H, *J* = 1.2 Hz, Ar-H), 7.51 (d, 1H, *J* = 2.4 Hz, Ar-H), 7.10 (s-like, 1H, Ar-H), 6.95 (d, 1H, *J* = 2.4 Hz, Ar-H), 5.71 (d, 1H, *J* = 1.8 Hz, H-1′), 5.42 (dd, 1H, *J* = 10.2, 2.4 Hz, H-3′), 4.91 (t-like, 1H, *J* = 10.3, 9.6 Hz, H-4′), 4.31 (br s, 1H, H-2′), 3.90–3.93 (m, 1H, H-5′), 2.47 (s, 3H, Ar-CH_3), 2.16 (s, 3H, CH_3CO), 2.07 (s, 3H, CH_3CO), 1.22 (d, 3H, *J* = 6.0 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 191.1, 181.6, 171.2 170.1 165.0, 162.7, 162.5, 148.9, 135.5, 133.1, 124.6, 121.5, 113.6, 111.5, 109.5 (two), 97.2, 71.3, 70.9, 69.1, 67.9, 29.8, 22,3, 21.0, 20.9, 17.5; HRESIMS: calcd for $C_{25}H_{23}O_{11}$ 499.1240; found, 499.1243.

4.7.3. 3-(2′,3′-Di-*O*-butyryl-α-L-rhamnopyranosyl)-emodin (5)

A red solid, yield 75.6%; R_f 0.51(2:1, petroleum ether/acetone); $[\alpha]_D^{25}$ −119.8 (*c* 0.10, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 12.20 (s, 1H, O*H*), 12.00 (s, 1H, O*H*), 7.59 (s-like, 1H, Ar-H), 7.45 (d, 1H, *J* = 1.8 Hz, Ar-H), 7.04 (s, 1H, Ar-H), 6.88 (d, 1H, *J* = 2.4 Hz, Ar-H), 5.57 (d, 1H, *J* = 1.2 Hz, H-1′), 5.42 (dd, 1H, *J* = 3.6, 1.8 Hz, H-2′), 5.31 (dd, 1H, *J* = 9.6, 3.6 Hz, H-3′), 3.76–3.79 (m, 1H, H-5′), 3.70 (td, 1H, *J* = 10.2, 4.8 Hz, H-4′), 2.42 (s, 3H, Ar-CH_3), 2.24–2.32 (m, 4H, 2 × CH_2CO), 1.64–1.70 (m, 4H, 2 × CH_2), 1.32

(d, 3H, J = 6.0 Hz, CH_3), 0.98 (t, 3H, J = 7.2 Hz, CH_3), 0.94 (t, 3H, J = 7.2 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 191.1, 181.6, 173.9, 172.6, 164.9, 162.7, 162.5, 148.9, 135.5, 133.2, 124.6, 121.5, 113.6, 111.5, 109.6, 109.4, 95.7, 71.6, 71.2, 70.3, 69.3, 36.1, 22.3, 18.6, 18.3, 17.7, 13.7 (two); HRESIMS: calcd for $C_{29}H_{31}O_{11}$ 555.1866; found, 555.1859.

4.7.4. 3-(4′-*O*-Acetyl-α-L-rhamnopyranosyl)-emodin (6)

A red solid, yield 75.2%; R_f 0.12 (2:1, petroleum ether/acetone); $[\alpha]_D^{25}$ −146.1 (c 0.10, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 12.26 (s, 1H, OH), 12.04 (s, 1H, OH), 7.62 (d, 1H, J = 1.2 Hz, Ar-H), 7.46 (d, 1H, J = 2.4 Hz, Ar-H), 7.09 (s-like, 1H, Ar-H), 6.93 (d, 1H, J = 2.4 Hz, Ar-H), 5.72 (d, 1H, J = 1.2 Hz, H-1′), 4.89 (t, 1H, J = 9.6 Hz, H-4′), 4.19 (dd, 1H, J = 3.6, 1.2 Hz, H-2′), 4.10 (dd, 1H, J = 9.6, 3.6 Hz, H-3′), 3.81–3.83 (m, 1H, H-5′), 2.45 (s, 3H, Ar-CH_3), 2.19 (s, 3H, CH_3CO), 2.07 (s, 3H, CH_3CO), 1.20 (d, 3H, J = 6.0 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 191.1, 181.8, 172.3, 165.0, 162.7, 162.6, 148.9, 135.5, 133.2, 124.7. 121.5, 113.7, 111.4, 109.5, 109.4, 97.3, 75.1, 70.4, 70.1, 67.2, 22.3, 21.1, 17.6; HRESIMS: calcd for $C_{23}H_{21}O_{10}$ 457.1135; found, 457.1135.

4.8. 3-(α-L-Rhamnopyranosyl)-emodin (2)

To a stirred solution of **24** (0.40 g, 0.50 mmol) in CH_2Cl_2 (5 mL) and CH_3OH (5 mL) was added MeONa (20 mg). The reaction mixture was stirred at room temperature for 30 min, after which the reaction mixture was neutralized with Dowex 50 × 8(H^+) resin until pH 7, filtered and concentrated. The residue was recrystallized from absolute ethanol to give compound **2** (0.18 g, 85.7%); $[\alpha]_D^{25}$ −104.6 (c 0.12, DMSO); ^{1}H NMR (DMSO-d_6): δ 11.98 (s, 1H, OH), 11.86 (s, 1H, OH), 7.41 (br s, 1H, Ar-H), 7.19 (d, 1H, J = 1.8 Hz, Ar-H), 7.11 (br s, 1H, Ar-H), 6.87 (d, 1H, J = 1.8 Hz, Ar-H), 5.59 (s, 1H, H-1′), 5.23 (d, 1H, J = 4.4 Hz, OH), 4.96 (d, 1H, J = 5.8 Hz, OH), 4.88 (d, 1H, J = 5.9 Hz, OH), 3.87–3.89 (m, 1H, H-3′), 3.65–3.3.69 (m, 1H, H-5′), 3.41–3.45 (m, 1H, H-2′), 3.31–3.34 (m, 1H, H-4′), 2.39 (s, 3H, Ar-CH_3), 1.14 (d, 3H, J = 6.2 Hz, CH_3); ^{13}C NMR (DMSO-d_6): δ 190.5, 181.4, 164.3, 163.2, 162.0, 149.1, 135.2, 133.2, 124.7, 121.1, 113.8, 111.2, 109.6, 109.0, 99.0, 72.1, 70.8, 70.7, 70.3, 22.1, 18.3; HRESIMS: calcd for $C_{21}H_{19}O_9$ 415.1029; found, 415.1042.

4.9. 3-(2″,3″-Di-*O*-acetyl-4″-*O*-levulinyl-α-L-rhamnopyranosyl-(1→4)-2′,3′-di-*O*-acetyl-α-L-rhamnopyranosyl)-emodin (29)

To a mixture of compound **1** (200 mg, 0.41 mmol), **28** (260 mg, 0.53 mmol) and 4 Å molecular sieves in dry CH_2Cl_2 (20 mL) was added TMSOTf (15 μL, 0.082 mmol) at −30 °C under argon. The reaction mixture was allowed to stir for 1 h under this condition, while warmed to room temperature until TLC indicated that the reaction was complete. After the reaction was quenched by Et_3N (five drops), the mixture was concentrated. The obtained residue was purified by silica gel column chromatography (5:1, v/v, petroleum ether/acetone) to give a yellow solid **29** (0.32 g, 94.1%); R_f 0.40 (2:1, petroleum ether/acetone); $[\alpha]_D^{25}$ −126.0 (c 0.10, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 12.25 (s, 1H, OH), 12.05 (s, 1H, OH), 7.65 (d, 1H, J = 1.5 Hz, Ar-H), 7.53 (d, 1H, J = 2.6 Hz, Ar-H), 7.09 (d, 1H, J = 1.5 Hz, Ar-H), 6.93 (d, 1H, J = 2.5 Hz, Ar-H), 5.59 (d, 1H, J = 1.4 Hz, H-1′), 5.48 (dd, 1H, J = 3.3, 1.9 Hz, H-2′), 5.43 (dd, 1H, J = 9.5, 3.3 Hz, H-3″), 5.24 (dd, 1H, J = 9.5, 3.3 Hz, H-3′), 5.13 (dd, 1H, J = 3.3, 1.8 Hz, H-2″), 5.09 (t-like, 1H, J = 10.3, 9.9 Hz, H-4″), 5.00 (d, 1H, J = 1.8 Hz, H-1″), 3.87–3.97 (m, 2H, H-5′, H-5″), 3.78 (t, 1H, J = 9.5 Hz, H-4′), 2.73 (t, 2H, J = 6.2 Hz, CH_2CO), 2.49–2.52 (m, 2H, CH_2CO), 2.46 (s, 3H, Ar-CH_3), 2.18, 2.17, 2.14, 2.10, 2.03 (each s, each 3H, CH_3CO), 1.37 (d, 3H, J = 6.2 Hz, CH_3), 1.22 (d, 3H, J = 6.4 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 206.0, 191.1, 181.5, 171.8, 170.0, 169.9, 169.8, 164.8, 162.6, 162.3, 148.8, 135.5, 133.1, 124.5, 121.4, 113.6, 111.6, 109.7, 109.1, 99.4, 95.2, 37.6, 29.7, 27.9, 22.2, 20.9, 20.8, 20.7, 18.1, 17.2; ESIMS: calcd for $[M+H]^+$ m/z 827.2; found: m/z 827.1.

4.10. 3-(2″,3″-Di-*O*-acetyl-α-L-rhamnopyranosyl-(1→4)-2′,3′-di-*O*-acetyl-α-L-rhamnopyranosyl)-emodin (7)

Compound **7** was prepared in a similar way as **3** in 76.2% yield; R_f 0.43 (3:1, petroleum ether/acetone); $[\alpha]_D^{25}$ −163.1 (c 0.13, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 12.24 (s, 1H, OH), 12.05 (s, 1H, OH), 7.64 (d, 1H, J = 1.1 Hz, Ar-H), 7.52 (d, 1H, J = 2.6 Hz, Ar-H), 7.09 (d, 1H, J = 0.8 Hz, Ar-H), 6.93 (d, 1H, J = 2.5 Hz, Ar-H), 5.59 (d, 1H, J = 1.9 Hz, H-1′), 5.47 (dd, 1H, J = 3.3, 1.8 Hz, H-2′), 5.43 (dd, 1H, J = 9.5, 3.3 Hz, H-3′), 5.11 (dd, 1H, J = 3.3, 1.8 Hz, H-2″), 5.03 (dd, 1H, J = 9.5, 3.3 Hz, H-3″), 4.99 (d, 1H, J = 1.8 Hz, H-1″), 3.77–3.91 (m, 3H, H-4″, H-5′, H-5″), 3.64 (t, 1 H, J = 9.5 Hz, H-4′), 2.46 (s, 3H, Ar-CH_3), 2.18, 2.13, 2.11, 2.09 (each s, each 3H, CH_3CO), 1.36 (d, 3H, J = 5.9 Hz, CH_3), 1.34 (d, 3H, J = 6.2 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 191.1, 181.5, 171.2, 170.1, 169.8, 164.8, 162.6, 162.3, 148.8, 135.5, 133.1, 124.5, 121.5, 113.6, 111.6, 109.7, 109.2, 99.4, 95.2, 78.4, 71.8, 71.2, 71.1, 70.4, 69.8, 69.3, 68.5, 22.2, 20.9, 20.8, 20.7, 18.1, 17.3; HRESIMS: calcd for $C_{35}H_{37}O_{17}$ 729.2031; found, 729.2018.

4.11. 1,8-Di-*O*-methxyl-3-(2′,3′-di-*O*-acetyl-4′-*O*-levulinyl-α-L-rhamnopyranosyl)-emodin (31)

To a stirred solution of **30** (0.10 g, 0.17 mmol) in dry acetone (10 mL) were added K_2CO_3 (1.16 g, 8.36 mmol) and MeI (0.32 mL, 5.1 mmol). The reaction mixture was refluxed at 60 °C under argon for 24 h, then filtrated and concentrated in vacuo. The residue was purified by silica gel column chromatography (3:1, v/v, petroleum ether/acetone) to give a yellow solid **31** (100.5 mg, 95.3%) with R_f 0.17 (2:1, petroleum ether/acetone); $[\alpha]_D^{25}$ −101.3 (c 0.12, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.65 (s-like, 1H, Ar-H), 7.52 (d, 1H, J = 2.6 Hz, Ar-H), 7.10 (s-like, 1H, Ar-H), 6.95 (d, 1H, J = 2.6 Hz, Ar-H), 5.66 (d, 1H, J = 1.9 Hz, H-1′), 5.54 (dd, 1H, J = 9.9, 3.3 Hz, H-3′), 5.44 (dd, 1H, J = 3.3, 1.8 Hz, H-2′), 5.19 (t, 1H, J = 9.9 Hz, H-4′), 3.99, 3.98 (each s, each 3H, each OCH_3), 3.93–3.95 (m, 1H, H-5′), 2.74–2.77 (m, 2H, CH_2CO), 2.52–2.55 (m, 2H, CH_2CO), (m, 2H, CH_2CO), 2.47 (s, 3H, Ar-CH_3), 2.21, 2.18, 2.10 (each s, each 3H, CH_3CO), 1.22 (d, 3H, J = 6.2 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 206.1, 183.9, 181.7, 171.8, 170.3, 170.1, 161.7, 159.8, 159.5, 144.9, 137.9, 136.5, 134.4, 129.0, 128.2, 125.3, 119.7, 119.5, 118.9, 106.3, 105.0, 95.2, 70.8, 69.4, 68.6, 67.8, 56.7, 56.5, 37.7, 29.7, 29.4, 27.9, 22.7, 22.1, 21.5, 20.9, 20.8, 17.4; ESIMS: calcd for $[M+H]^+$ m/z 627.2; found: m/z 627.2.

4.12. 1,8-Di-*O*-methxyl-3-(2′,3′-di-*O*-acetyl-α-L-rhamnopyranosyl)-emodin (8)

8 was prepared in a similar way as **3** in 91.3% yield; R_f 0.15 (2:1, petroleum ether/acetone); $[\alpha]_D^{25}$ −117.3 (c 0.10, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.63 (s-like, 1H, Ar-H), 7.52 (d, 1H, J = 2.4 Hz, Ar-H), 7.10 (s-like, 1H, Ar-H), 6.96 (d, 1H, J = 2.4 Hz, Ar-H), 5.66 (d, 1H, J = 1.8 Hz, H-1′), 5.45 (dd, 1H, J = 3.0, 1.8 Hz, H-2′), 5.39 (dd, 1H, J = 9.6, 3.3 Hz, H-3′), 3.99, 3.98 (each s, each 3H, each OCH_3), 3.85–3.87 (m, 1H, H-5′), 3.77 (t, 1H, J = 9.6 Hz, H-4′), 2.47 (s, 3H, Ar-CH_3), 2.20, 2.12 (each s, each 3H, CH_3CO), 1.35 (d, 3H, J = 6.6 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 183.9, 181.8, 171.0, 170.1, 167.4, 161.7, 159.8, 153.2, 144.9, 136.4, 134.5, 121.5, 119.7, 119.4, 119.0, 106.4, 105.3, 95.5, 71.6, 70.7, 70.1, 69.7, 56.7, 56.5, 26.0, 22.9, 22.7, 22.1, 21.0, 20.9, 17.7; HRESIMS: calcd for $C_{27}H_{29}O_{11}$ 529.1710; found, 529.1721.

4.13. 3-(2′,3′,4′-Tri-*O*-acetyl-α-L-rhamnopyranosyl)-10-acetyl-emodin (9) and 1,8-di-*O*-acetyl-3-(2′,3′,4′-tri-*O*-acetyl-α-L-rhamnopyranosyl)-10-acetyl-emodin (33)

To a stirred solution of **32** (0.10 g, 0.21 mmol) in Ac_2O (10 mL) was added dry pyridine (125 μL, 1.55 mmol). The reaction mixture was stirred at room temperature for 6 h, then concentrated and purified by silica gel column chromatography (5:1, v/v, petroleum ether/acetone) to give a yellow solid **9** (50 mg, 42.6%); ^{1}H NMR ($CDCl_3$): δ 12.57 (s, 1H, O*H*), *12.55 (s, 1H, O*H*), 12.25 (s, 1H, O*H*), *12.24 (s, 1H, O*H*), 6.82 (s-like, 1H, Ar-H), 6.71 (d, 2H, *J* = 1.4 Hz, Ar-H), *6.70 (d, 2H, *J* = 2.9 Hz, Ar-H), 6.61 (d, 1H, *J* = 2.2 Hz, Ar-H), *6.60 (d, 1H, *J* = 2.5 Hz, Ar-H), 5.56 (d, 1H, *J* = 1.5 Hz, H-1′), *5.53 (d, 1H, *J* = 1.5 Hz, H-1′), *5.46 (dd, 1H, *J* = 9.9, 3.7 Hz, H-3′), 5.45 (dd, 1H, *J* = 9.9, 3.7 Hz, H-3′), 5.42 (dd, 1H, *J* = 3.7, 1.8 Hz, H-2′), 5.17 (t, 1H, *J* = 9.9 Hz, H-4′), *5.16 (t, 1H, *J* = 9.9 Hz, H-4′), *5.06 (s, 1H, H-10), 5.03 (s, 1H, H-10), 3.87–3.93 (m, 1H, H-5′), 2.38 (s, 3H, Ar-C*H*$_3$), 2.22, 2.07, 2.06, 2.04 (each s, each 3H, C*H*$_3$CO), 1.21 (d, 3H, *J* = 6.2 Hz, C*H*$_3$), *1.20 (d, 3H, *J* = 6.2 Hz, C*H*$_3$); ^{13}C NMR ($CDCl_3$): δ 202.0, 191.6 (two), 170.0 (two), 169.9, 165.7, 165.6, 163.3, 161.9, 161.8, 148.6, 140.1, 137.4, 120.0, 117.8, 112.4, 110.4, 108.0, 107.6, 103.8, 103.7, 95.3, 95.2, 70.6, 69.2, 69.1, 68.7, 68.6, 67.8 (two), 59.0, 58.9, 29.7, 24.7, 24.6, 22.1, 20.9, 20.8, 20.7, 17.5, 17.4; HRESIMS: calcd for $C_{29}H_{29}O_{12}$ 569.1659; found, 569.1674. The other yellow solid **33** (55 mg, 40.9%); ^{1}H NMR ($CDCl_3$): δ 7.13 (br s, 2H, Ar-H), *7.04 (d, 1H, *J* = 2.6 Hz, Ar-H), *7.02 (d, 1H, *J* = 2.6 Hz, Ar-H), 6.96 (br s, 2H, Ar-H), *6.88 (d, 1H, *J* = 2.2 Hz, Ar-H), *6.87 (d, 1H, *J* = 2.6 Hz, Ar-H), 5.55 (s-like, 1H, H-1′), *5.53 (s-like, 1H, H-1′), 5.44 (dd, 1H, *J* = 9.9, 3.3 Hz, H-3′), 5.41–5.42 (m, 1H, H-2′), 5.16 (t, 1H, *J* = 9.9 Hz, H-4′), 5.09 (s, 1H, H-10), *5.07 (s, 1H, H-10), 3.88–3.91 (m, 1H, H-5′), 2.42 (s, 3H, Ar-C*H*$_3$), 2.21, 2.20, 2.06, 2.04 (each s, each 3H, C*H*$_3$CO), 1.21 (d, 3H, *J* = 6.2 Hz, C*H*$_3$), *1.20 (d, 3H, *J* = 6.2 Hz, C*H*$_3$); ^{13}C NMR ($CDCl_3$): δ 202.7, 202.5, 180.5, 180.3, 169.9, 169.8, 169.4 (two), 159.1, 159.0, 152.7, 150.8, 150.7, 145.3, 140.0, 137.7, 129.6, 129.0, 128.2, 126.3, 124.7, 121.9, 119.7, 115.2, 112.8, 112.4, 112.2, 112.1, 95.5, 70.5, 69.1 (two), 68.5, 67.8 (two), 60.1, 59.9, 53.4, 24.5, 24.4, 21.5, 21.1 (two), 20.8, 20.7 (two), 17.4 (two); HRESIMS: calcd for $C_{33}H_{35}O_{14}$ 655.2027; found, 655.2011.

4.14. 3-(2′,3′-Di-*O*-acetyl-4′-*O*-levulinyl-α-L-rhamnopyranosyl)-10-acetyl-emodin (34)

In a similar way as **9** with **35** instead of **32**, **34** was afforded in 38.7% yield. ^{1}H NMR ($CDCl_3$): δ 12.79 (s, 1H, O*H*), *12.76 (s, 1H, O*H*), 12.06 (s, 1H, O*H*), *12.05 (s, 1H, O*H*), 6.97 (s-like, 2H, Ar-H), 6.96 (d, 2H, *J* = 0.7 Hz, Ar-H), *6.84 (d, 2H, *J* = 2.9 Hz, Ar-H), *6.81 (s-like, 1H, Ar-H), 6.80 (s-like, 1H, Ar-H), 5.63 (d, 1H, *J* = 1.5 Hz, H-1′), *5.60 (d, 1H, *J* = 1.5 Hz, H-1′), 5.58 (dd, 1H, *J* = 3.7, 1.8 Hz, H-2′), 5.47–5.51 (m, 1H, H-3′), 5.18–5.23 (m, 1H, H-4′), *5.05 (s, 1H, H-10), 5.03 (s, 1H, H-10), 3.87–3.96 (m, 1H, H-5′), 2.74–2.79 (m, 2H, C*H*$_2$CO), 2.52–2.56 (m, 2H, C*H*$_2$CO), 2.40 (s, 3H, Ar-C*H*$_3$), *2.39 (s, 3H, Ar-C*H*$_3$), 2.21, 2.20, 2.19, 2.18, 2.17, 2.10, 2.09 (each s, each 3H, C*H*$_3$CO), 1.25 (d, 3H, *J* = 6.2 Hz, C*H*$_3$), *1.23 (d, 3H, *J* = 6.2 Hz, C*H*$_3$); ^{13}C NMR ($CDCl_3$): δ 206.1, 200.9, 200.8, 191.6, 191.5, 171.8 (two), 170.2, 170.1 (two), 170.0, 164.5, 164.4, 163.1 (two), 157.9, 157.8, 148.8, 148.7, 140.1, 137.0 (two), 120.5, 120.4, 117.8 (two), 104.5, 104.2, 103.6, 103.5, 103.4, 96.1, 95.9, 70.7, 70.5, 69.2, 68.4, 68.3, 67.9, 59.5, 59.3, 37.7, 29.7, 27.9, 27.4, 26.8, 24.7, 22.3, 20.9, 20.7, 17.4, 17.3; HRESIMS: calcd for $C_{32}H_{33}O_{13}$ 625.1921; found, 625.1949.

4.15. 3-(2′,3′-Di-*O*-acetyl-4′-*O*-benzyl-α-L-rhamnopyranosyl)-10-acetyl-emodin (40)

By use of the same procedure as **9** starting from **39**, **40** was synthesized in 40.6% yield; R_f 0.56 (3:1, petroleum ether/acetone); $[\alpha]_D^{25}$ −57.3 (*c* 0.16, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 12.55 (s, 1H, O*H*), *12.53 (s, 1H, O*H*), 12.25 (s, 1H, O*H*), *12.24 (s, 1H, O*H*), 7.35 (t-like, 3H, *J* = 8.0, 6.6 Hz, Ph-H), 7.28–7.31 (m, 2H, Ph-H), 6.80 (s-like, 1H, Ar-H), *6.71 (s-like, 1H, Ar-H), 6.69 (d, 1H, *J* = 1.4 Hz, Ar-H), 6.58 (dd, 1H, *J* = 4.4, 2.2 Hz, Ar-H), 5.52 (d, 1H, *J* = 1.5 Hz, H-1′), *5.49 (d, 1H, *J* = 1.1 Hz, H-1′), 5.58 (dd, 1H, *J* = 3.7, 1.8 Hz, H-2′), 5.47 (dd, 1H, *J* = 9.9, 3.3 Hz, H-3′), *5.46 (dd, 1H, *J* = 9.9, 3.3 Hz, H-3′), 5.42 (dd, 1H, *J* = 3.7, 1.8 Hz, H-2′), 5.04 (s, 1H, H-10), *5.02 (s, 1H, H-10), 4.72 (d, 1H, *J* = 11.3 Hz, Ph-C*H*$_2$-1), *4.71 (d, 1H, *J* = 11.3 Hz, Ph-C*H*$_2$-1), 4.66 (d, 1H, *J* = 11.3 Hz, Ph-C*H*$_2$-2), *4.65 (d, 1H, *J* = 11.3 Hz, Ph-C*H*$_2$-2), 3.81–3.88 (m, 1H, H-5′), 3.59 (t, 1H, *J* = 9.5 Hz, H-4′), 2.37 (s, 3H, Ar-C*H*$_3$), 2.20, 2.17, 2.02, (each s, each 3H, C*H*$_3$CO), 1.33 (d, 3H, *J* = 6.2 Hz, C*H*$_3$), *1.29 (d, 3H, *J* = 6.2 Hz, C*H*$_3$); ^{13}C NMR ($CDCl_3$): δ 201.9, 201.8, 191.6, 191.5, 169.9, 169.8, 165.6, 165.5, 163.2, 162.1, 162.0, 148.4, 140.0 (two), 137.8, 137.5, 137.4, 128.4, 127.9, 127.6, 120.0, 117.7, 112.4, 110.3, 110.2, 108.1, 107.6, 103.8, 103.5, 103.6, 95.3, 95.2, 78.4, 75.2, 71.0, 69.7, 69.6, 69.1 (two), 59.0, 58.9, 24.7, 24.6, 22.1, 20.9, 17.9 (two); ESIMS: calcd for $[M-H]^-$ *m*/*z* 617.2; found, 617.1.

4.16. 3-(2′,3′-Di-*O*-acetyl-4′-*O*-levulinyl-α-L-rhamnopyranosyl)-10-methylene-emodin (35)

To a solution of compound **30** (0.35 g, 0.59 mmol) in AcOH (20 mL) was added Zn (1.34 g, 20.48 mmol) at 80 °C under argon. After stirred for 8 h, the mixture was filtrated and concentrated in vacuo. The residue was purified by silica gel column chromatography (3:1, v/v, petroleum ether/acetone) to give a yellow solid **35** (0.27 g, 79.4%) with R_f 0.48 (2:1, petroleum ether/acetone); $[\alpha]_D^{25}$ −94.4 (*c* 0.11, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 12.58 (s, 1H, O*H*), 12.25 (s, 1H, O*H*), 6.61 (d, 2H, *J* = 2.5 Hz, Ar-H), 6.59 (d, 2H, *J* = 1.1 Hz, Ar-H), 5.58 (d, 1H, *J* = 1.4 Hz, H-1″), 5.51 (dd, 1H, *J* = 10.3, 3.7 Hz, H-3′), 5.42 (dd, 1H, *J* = 3.7, 1.9 Hz, H-2′), 5.18 (t, 1H, *J* = 9.8 Hz, H-4′), 4.24 (s, 2H, Ar-C*H*$_2$), 3.93–3.94 (m, 1H, H-5′), 2.74–2.77 (m, 2H, C*H*$_2$CO), 2.52–2.55 (m, 2H, C*H*$_2$CO), 2.36 (s, 3H, Ar-C*H*$_3$), 2.20, 2.18, 2.09 (each s, each 3H, C*H*$_3$CO), 1.22 (d, 3H, *J* = 7.3 Hz, C*H*$_3$); ^{13}C NMR ($CDCl_3$): δ 206.1, 192.1, 171.8, 170.2, 170.0, 165.1, 162.8, 161.4, 147.6, 143.8, 141.1, 119.7, 115.9, 113.4, 111.4, 107.2, 102.2, 95.2, 70.8, 69.4, 68.3, 67.7, 37.7, 32.9, 29.7, 27.8, 22.1, 20.9, 20.7, 17.4; HRESIMS: calcd for $C_{30}H_{31}O_{12}$ 583.1816; found, 583.1821.

4.17. 3-(2′,3′-Di-*O*-acetyl-4′-*O*-benzyl-α-L-rhamnopyranosyl)-10-methylene-emodin (39)

Compound **39** was prepared in a similar way as **35** in 78.4% yield; R_f 0.27 (3:1, petroleum ether/EtOAc); $[\alpha]_D^{25}$ −130.2 (*c* 0.10, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 12.57 (s, 1H, O*H*), 12.26 (s, 1H, O*H*), 7.26–7.35 (m, 5H, Ph-H), 6.69 (s, 2H, Ar-H), 6.60 (s, 1H, Ar-H), 6.56 (s, 1H, Ar-H), 5.49–5.52 (m, 2H, H-2′, H-3′), 5.43 (s, 1H, H-1′), 4.72 (d, 1H, *J* = 11.0 Hz, Ph-C*H*$_2$-1), 4.67 (d, 1H, *J* = 11.0 Hz, Ph-C*H*$_2$-2), 4.22 (s, 2H, Ar-C*H*$_2$), 3.87–3.90 (m, 1H, H-5′), 3.59 (t, 1H, *J* = 9.7 Hz, H-4′), 2.35 (s, 3H, Ar-C*H*$_3$), 2.20 (s, 3H, C*H*$_3$CO), 2.02 (s, 3H, C*H*$_3$CO), 1.32 (d, 3H, *J* = 6.4 Hz, C*H*$_3$); ^{13}C NMR ($CDCl_3$): δ 192.1, 170.0, 169.9, 165.1, 162.7, 161.6, 147.5, 143.7, 141.1, 137.8, 128.5, 127.9, 127.6, 119.7, 115.9, 113.4, 111.2, 107.5, 102.0, 95.1, 78.5, 75.2, 71.2, 69.8, 69.0, 32.8, 22.1, 20.9 (two), 17.9; HRESIMS: calcd for $C_{32}H_{31}O_{10}$ 575.1917; found, 575.1920.

4.18. 3-(2′,3′-Di-*O*-acetyl-4′-*O*-benzyl-α-L-rhamnopyranosyl)-emodin (38)

To a solution of compound **1** (200 mg, 0.41 mmol), **37** (260 mg, 0.82 mmol) and 4 Å molecular sieves in dry CH_2Cl_2 (20 mL) was added TfOH (185 μL, 0.21 mmol) at 0 °C under argon. The reaction mixture was allowed to stir for 12 h under this condition, while

warmed to room temperature until TLC indicated that the reaction was complete. The reaction was quenched by Et_3N (five drops) and concentrated. The residue was purified by silica gel column chromatography (5:1, v/v, petroleum ether/acetone) to give a yellow solid **38** (0.16 g, 67.8%); $[\alpha]_D^{25}$ −98.2 (*c* 0.12, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 12.25 (s, 1H, O*H*), 12.06 (s, 1H, O*H*), 7.64 (d, 1H, *J* = 1.3 Hz, Ar-H), 7.50 (d, 1H, *J* = 2.3 Hz, Ar-H), 7.34–7.36 (m, 2H, Ph-H), 7.29–7.31 (m, 3H, Ph-H), 7.09 (br s, 1H, Ar-H), 6.91 (d, 1H, *J* = 2.7 Hz, Ar-H), 5.60 (d, 1H, *J* = 1.8 Hz, H-1′), 5.49 (dd, 1H, *J* = 9.2, 3.2 Hz, H-3′), 5.46 (dd, 1H, *J* = 3.2, 1.8 Hz, H-2′), 4.73 (d, 1H, *J* = 11.0 Hz, Ph-C*H*$_2$-1), 4.67 (d, 1H, *J* = 11.5 Hz, Ph-C*H*$_2$-2), 3.84–3.88 (m, 1H, H-5′), 3.60 (t-like, 1H, *J* = 9.6, 9.2 Hz, H-4′), 2.45 (s, 3H, Ar-C*H*$_3$), 2.21 (s, 3H, C*H*$_3$CO), 2.03 (s, 3H, C*H*$_3$CO), 1.32 (d, 3H, *J* = 6.4 Hz, C*H*$_3$); ^{13}C NMR ($CDCl_3$): δ 191.0, 181.5, 170.0, 169.8, 164.8, 162.5, 162.3, 148.7, 137.8, 135.3, 133.1, 129.1, 128.4, 127.9, 127.6, 124.5, 121.4, 113.5, 111.4, 109.5, 109.2, 95.3, 78.3, 75.1, 71.1, 69.6, 69.2, 22.2, 20.9, 17.9; HRESIMS: calcd for $C_{32}H_{29}O_{11}$ 589.1710; found, 589.1701.

4.19. Dimerization of prinoidin (36)

To a stirred solution of **35** (0.10 g, 0.17 mmol) in CH_2Cl_2 (10 mL) and CH_3OH (10 mL) was added NH_2NH_2–HOAc (0.16 g, 1.7 mmol). After 3 h, the solution was concentrated. Then the residue was purified by silica gel column chromatography (3:1, v/v, petroleum ether/acetone) to afford a yellow solid **36** (0.12 g, 73.5%); ^1H NMR ($CDCl_3$): δ 12.46 (s, 1H, O*H*), 12.19 (s, 1H, O*H*), 12.14 (s, 1H, O*H*), 12.04 (s, 1H, O*H*), 11.92 (s, 1H, O*H*), 11.84 (s, 1H, O*H*), 11.67 (s, 1H, O*H*), 6.97, 6.95 (each br s, each 1H, Ar-H), 6.93 (s-like, 1H, Ar-H), 6.86 (br s, 1H, Ar-H), 6.81 (s-like, 1H, Ar-H), 6.73 (d, 1H, *J* = 2.6 Hz, Ar-H), 6.69 (d, 1H, *J* = 2.2 Hz, Ar-H), 6.64, 6.63 (each br s, each 1H, Ar-H), 6.50 (d, 1H, *J* = 2.5 Hz, Ar-H), 6.49 (d, 1H, *J* = 2.2 Hz, Ar-H), 5.81 (d, 1H, *J* = 1.4 Hz, H-1′), *5.61 (d, 1H, *J* = 1.8 Hz, H-1′), 5.52 (dd, 1H, *J* = 3.3, 1.4 Hz, H-2′), *5.49 (dd, 1H, *J* = 3.7, 1.9 Hz, H-2′), 5.44 (dd, 1H, *J* = 10.2, 3.7 Hz, H-3′), *5.39 (dd, 1H, *J* = 3.7, 1.9 Hz, H-2′), *5.36 (dd, 1H, *J* = 9.9, 3.7 Hz, H-3′), *5.29 (dd, 1H, *J* = 3.0, 2.2 Hz, H-2′), *5.19 (dd, 1H, *J* = 9.9, 3.7 Hz, H-3′), 5.17 (br s, 1H, Ar-H), 4.53 (s, 1H, H-10), *4.49 (d, 1H, *J* = 3.7 Hz, H-10), 4.46 (d, 1H, *J* = 4.0 Hz, H-10), *4.37 (s, 1H, H-10), 3.92–3.97 (m, 1H, H-4′), 3.65–3.79 (m, 1H, H-5′), 2.52, 2.50 (each s, each 3H, Ar-C*H*$_3$), 2.24, 2.23, 2.22, 2.19, 2.17, 2.16, 2.15, 2.14, 2.13, 2.05 (each s, each 3H, C*H*$_3$CO), 1.44 (d, 3H, *J* = 6.2 Hz, C*H*$_3$), 1.42 (d, 3H, *J* = 6.2 Hz, C*H*$_3$), *1.36 (d, 3H, *J* = 5.9 Hz, C*H*$_3$), *1.35 (d, 3H, *J* = 6.2 Hz, C*H*$_3$); ^{13}C NMR ($CDCl_3$): δ 190.6, 190.2, 171.9, 170.9 (two), 170.0 (two), 164.8, 164.4, 163.3, 162.3, 161.9, 161.2, 161.1, 159.8, 159.5, 129.7, 121.8, 121.1, 120.7, 120.6, 117.5, 117.2, 117.1, 113.3, 113.0, 111.1, 110.6, 11.0, 109.5, 108.6, 104.0, 101.8, 101.6, 95.6, 94.9, 94.5 (two), 72.1, 71.4, 71.3, 71.1, 70.8, 70.1, 70.0, 69.8, 69.7 (two), 69.6, 69.4, 56.6, 56.0, 55.7, 22.7, 22.3, 22.2, 21.9, 21.8, 21.1, 21.0, 20.9 (two), 20.8, 17.7, 17.6, 17.5 (two); ESIMS: calcd for $[M-H]^-$ *m/z* 969.2; found: *m/z* 969.2.

4.20. 3-(2′,3′-Di-*O*-acetyl-α-L-rhamnopyranosyl)-10-acetyl-emodin (10)

A suspension of **40** (0.11 g, 0.18 mmol) and Pd/C (80 mg, 10%) in CH_2Cl_2–EtOH (1:1, 10 mL) was stirred under H_2 for 12 h and then filtered and concentrated. The residue was purified by silica gel column chromatography (3:1, v/v, petroleum ether/acetone) give **10** (85 mg, 90.4%) with R_f 0.76 (2:1, petroleum ether/acetone); $[\alpha]_D^{25}$ −94.5 (*c* 0.11, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 12.55 (s, 1H, O*H*), *12.53 (s, 1H, O*H*), 12.24 (s, 1H, O*H*), *12.23 (s, 1H, O*H*), 6.80 (s, 1H, Ar-H), *6.70 (s-like, 2H, Ar-H), 6.59 (s, 1H, Ar-H), 5.55 (d, 1H, *J* = 1.4 Hz, H-1′), *5.51 (d, 1H, *J* = 1.4 Hz, H-1′), 5.40 (dd, 1H, *J* = 2.9, 1.4 Hz, H-2′), 5.30 (dd, 1H, *J* = 9.9, 3.7 Hz, H-3′), 5.04 (s, 1H, H-10), *5.01 (s, 1H, H-10), 3.76–3.82 (m, 1H, H-5′), 3.73 (t-like, 1H, *J* = 9.5, 9.2 Hz, H-4′), 2.39 (s, 3H, Ar-C*H*$_3$), 2.20 (s, 3H, C*H*$_3$CO), 2.19 (s, 3H, C*H*$_3$CO), 2.18 (s, 3H, C*H*$_3$CO), 2.17 (s, 3H, C*H*$_3$CO), 2.12 (s, 3H, C*H*$_3$CO), *1.36 (d, 3H, *J* = 6.4 Hz, C*H*$_3$), 1.34 (d, 3H, *J* = 6.4 Hz, C*H*$_3$); ^{13}C NMR ($CDCl_3$): δ 202.1, 201.8, 191.6, 191.5, 171.0, 169.9, 165.6, 165.5, 163.2, 162.1, 161.9, 148.5, 140.0 (two), 137.5, 137.4, 120.0, 117.7, 112.4, 110.3, 110.2, 108.1, 107.6, 103.8, 103.6, 95.5, 95.3, 71.6, 70.8, 70.0, 69.9, 69.4 (two), 59.0, 58.8, 29.7, 29.2, 24.6 (two), 22.1, 20.9, 20.8, 17.6, 17.5; HRESIMS: calcd for $C_{27}H_{27}O_{11}$ 527.1553; found, 527.1566.

4.21. Cytotoxic assay

The cytotoxicity of compounds was examined using a normal cell line, human microvascular endothelial cell line(HMEC) and a panel of human tumor cell lines, including one human promyelocytic leukemia cell line (HL-60), one human oral epidermoid carcinoma cell line (KB), and one human breast carcinoma cell lines (MDA-MB-231). Cells were seeded into 96-well plates and treated in triplicate with gradient concentrations of compounds at 37 °C for 72 h. Cytotoxicity to leukemia cells was assessed by MTT assay as previously described.[17,18] SRB was applied to adherent tumor cells.[18–20] The cytotoxicity of compounds was expressed as an IC_{50}, determined by the Logit method from at least three independent experiments.

4.22. Fluorescence binding studies

The fluorometric assays was described like that reported by McConnaughie.[15] The C_{50} values for ethidium displacement from calf thymus DNA was determined using aqueous buffer (10 mM Na_2HPO_4, 10 mM NaH_2PO_4, 1 mM EDTA, pH 7.0) containing 1.26 μM ethidium bromide and 1 μM calf thymus DNA, respectively.

All measurements were made in 10 mm quartz cuvettes at 25 °C using a Perkin–Elmer LS5 instrument (excitation at 522 nm; emission at 584 nm) following serial addition of aliquots of a stock drug solution (~5 mM in DMSO). The C_{50} values are defined as the drug concentrations which reduce the fluorescence of the DNA-bound ethidium by 50% and are reported as the mean from three determinations. Apparent equilibrium binding constants were calculated from the C_{50} values (in μM) using the following equation: $K_{app} = (1.26/C_{50}) \times 10^{-7} K_{ethidium}$, and with a value of $K_{ethidium} = 10^7\ M^{-1}$ for ethidium bromide.[21]

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