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Taccalonolide Microtubule Stabilizers Generated Using Semisynthesis Define the Effects of Mono Acyloxy Moieties at C-7 or C-15 and Disubstitutions at C-7 and C-25

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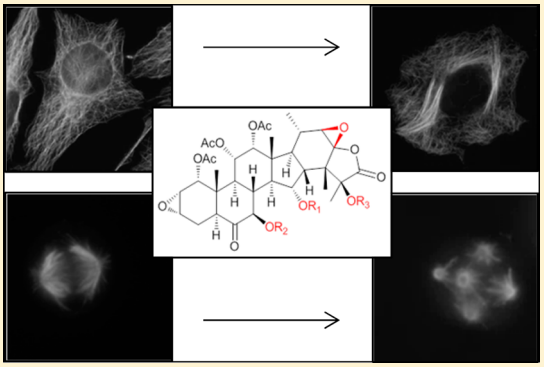
Supporting Information

ABSTRACT: The taccalonolides are a unique class of microtubule stabilizers isolated from *Tacca* spp. that have efficacy against drug-resistant tumors. Our previous studies have demonstrated that a C-15 acetoxy taccalonolide, AF, has superior in vivo antitumor efficacy compared to AJ, which bears a C-15 hydroxy group. With the goal of further improving the in vivo efficacy of this class of compounds, we semisynthesized and tested the biological activities of 28 new taccalonolides with monosubstitutions at C-7 or C-15 or disubstitutions at C-7 and C-25, covering a comprehensive range of substituents from formic acid to anthraquinone-2-carbonyl chloride. The resulting taccalonolide analogues with diverse C-7/C-15/C-25 modifications exhibited IC_{50} values from 2.4 nM to >20 μM, allowing for extensive in vitro structure−activity evaluations. This semisynthetic strategy was unable to provide a taccalonolide with improved therapeutic window due to hydrolysis of substituents at C-7 or C-15 regardless of size or steric bulk. However, two of the most potent new taccalonolides, bearing isovalerate modifications at C-7 or C-15, demonstrated potent and highly persistent antitumor activity in a drug-resistant xenograft model when administered intratumorally. This study demonstrates that targeted delivery of the taccalonolides to the tumor could be an effective, long-lasting approach to treat drug-resistant tumors.



Microtubule-stabilizing drugs have played an important role in cancer therapeutics for more than 20 years.[1] Taxol (paclitaxel), the first microtubule stabilizer identified, was initially isolated from the Pacific yew and its novel mechanism of action elucidated by Dr. Horwitz's laboratory in 1979.[2] Although paclitaxel and other taxanes have achieved significant clinical successes and remain a mainstay in cancer therapy, toxicities and drug resistance have spurred the search for new agents that have a similar mechanism of action.[3] These efforts have been successful, and many chemically diverse classes of microtubule stabilizers have been identified.[4] A screen of tropical plant extracts yielded the taccalonolides, isolated from *Tacca chantrieri* André (Dioscoreaceae), as a new class of microtubule stabilizers.[5] The taccalonolides have potent in vivo antitumor actions,[6−10] and they possess several advantages over many other microtubule stabilizers, including the ability to overcome multiple forms of drug resistance that limit the utility of the taxanes such as the expression of P-glycoprotein, the βIII isotype of tubulin, and mutations in the taxane binding site.[5,8] The mechanism of microtubule stabilization caused by the taccalonolides is unique in that they bind covalently to microtubules to initiate extensive and irreversible inter- and intraprotofilament stabilization.[7,11]

Since the initial discovery of **1** (taccalonolide A) and **2** (taccalonolide B),[12] numerous members of this class have been isolated from several *Tacca* spp., including taccalonolides C−M and W−Y from *T. plantagiana*,[12−17] taccalonolide N from an unidentified *Tacca* sp., taccalonolides R−V from *T. paxiana*,[18] taccalonolides O−Q from *T. subflabellata*,[19] taccalonolides Z, AA, and AB from *T. integrifolia* and *T. chantrieri*,[6] and taccalonolides AC, AD, AE, AF, H2, AI, and AN from *T. chantrieri*.[9,20] In addition to these natural taccalonolides, semisynthesis has been used to further examine the structure−activity relationships (SAR) of this class and identify the moieties optimal for potent biological activity.[6,9,20,21] The

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J. Nat. Prod. XXXX, XXX, XXX−XXX

array of natural and semisynthetic taccalonolides identified and characterized by our laboratory demonstrates the complex SAR of these compounds with contributions from multiple sites throughout the hexacyclic steroidal scaffold.[22] The key moieties that contribute to taccalonolide potency include an epoxide group bridging C-22, C-23 and the presence of a bulky group at C-1.[9] The importance of the C-22, C-23 epoxide group was revealed initially with the identification of **3** (taccalonolide AF), a rare, potent taccalonolide isolated from *T. plantaginea*.[20] Compound **3** is the C-22, C-23 epoxidized analogue of **1**, the major metabolite from *Tacca* spp., which can be produced semisynthetically by a single-step reaction in quantitative yield.[20] Compound **3** is over 200-fold more potent than **1** and directly stimulates the polymerization of purified tubulin.[20] The same single-step epoxidation reaction was also applied to **2**, producing the even more potent semisynthetic taccalonolide **4** (taccalonolide AJ).[20] While both **3** and **4** are microtubule stabilizers with potent antiproliferative and cytotoxic effects in vitro, **3** exhibited antitumor activity at tolerable doses when administered systemically, while **4** demonstrated antitumor effects only when injected directly into tumors.[7,10] Pharmacokinetic analyses suggest that the C-15 hydroxy group of **4** is subject to oxidation by liver microsomes, leading to an increased rate of microsomal clearance as compared to **3** and a shorter plasma half-life that prevents **4** from eliciting antitumor activity when administered systemically.[10]

Taccalonolides **3** and **4** differ only at the C-15 position, with an acetoxy group in **3** and a hydroxy group in **4** (Figure 1).

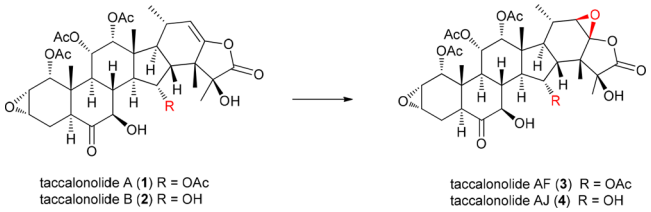


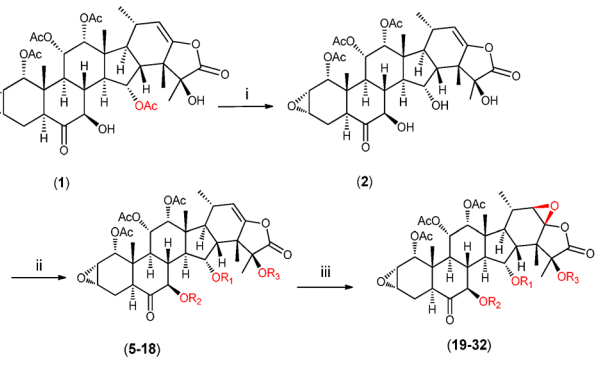
Figure 1. Structures of taccalonolides **1**−**4**.

These compounds have significant differences in antitumor efficacy, which implies that modifications at C-15 are important to understanding taccalonolide SAR, especially as it relates to in vivo efficacy. Since the C-15 acetoxy group on **3** can be readily hydrolyzed to generate **4** under aqueous conditions,[7] we hypothesized that taccalonolides bearing C-15 modifications could improve potency and/or in vivo efficacy. In the present study, semisynthetic reactions were designed and conducted to determine systematically whether moieties of different sizes at the C-15 of **2** could improve the efficacy of the taccalonolides. These reactions yielded taccalonolides with monosubstitutions at C-15 or C-7 as well as compounds with disubstitutions at C-7, C-25. Altogether, 14 new taccalonolides were initially generated: six containing monosubstitutions at C-15, four monosubstituted at C-7, and four with disubstitutions at C-7 and C-25. These new taccalonolides were then epoxidized at C-22, C-23 to generate an additional 14 new C-22,C-23-epoxy-taccalonolides. The new taccalonolides were evaluated in vitro for their antiproliferative and microtubule-stabilizing activities, and the most potent analogues were evaluated in vivo.

RESULTS AND DISCUSSION

Chemistry. The aim of this study was to semisynthesize derivatives of **2** by incorporating substituents of varying size and complexity at C-15, followed by epoxidation at C-22, C-23. The C-15 acetyl group of **1** was first hydrolyzed to produce **2** using 0.05 M $NaHCO_3$,[21] which in turn was used as the precursor to add substituents at C-15. Compound **2** was esterified with isobutyric, isovaleric, or pivalic anhydride; cyclopropane chloride; anthraquinone-2-carbonyl chloride; or formic acid using 4-(dimethylamino)pyridine (DMAP) as the catalyst in the presence of Hünig's base or pyridine (Scheme 1). These esterification reactions resulted in taccalonolides with six different monoester modifications at C-15 (**5**−**10**), four monoester modifications at C-7 (**11**−**14**), and four dual substitutions at C-7 and C-25 (**15**−**18**). Each of the products was further epoxidized at C-22, C-23 with dimethyldioxirane

Scheme 1. Synthesis of Target Compounds 5−32[a]



	R_1	R_2	R_3
5	$(CH_2)_2CHCO$	H	H
6	$(CH_3)_2CHCO$	H	H
7	$(CH_3)_2CHCH_2CO$	H	H
8	$(CH_3)_3CO$	H	H
9	HCO	H	H
10	$C_{14}H_8O_2CO$	H	H
11	H	$(CH_3)_2CHCO$	H
12	H	$(CH_3)_2CHCH_2CO$	H
13	H	$(CH_3)_3CO$	H
14	H	$C_{14}H_8O_2CO$	H
15	H	$(CH_2)_2CHCO$	$(CH_2)_2CHCO$
16	H	$(CH_3)_2CHCO$	$(CH_3)_2CHCO$
17	H	$(CH_3)_2CHCH_2CO$	$(CH_3)_2CHCH_2CO$
18	H	$C_{14}H_8O_2CO$	$C_{14}H_8O_2CO$
19	$(CH_2)_2CHCO$	H	H
20	$(CH_3)_2CHCO$	H	H
21	$(CH_3)_2CHCH_2CO$	H	H
22	$(CH_3)_3CO$	H	H
23	HCO	H	H
24	$C_{14}H_8O_2CO$	H	H
25	H	$(CH_3)_2CHCO$	H
26	H	$(CH_3)_2CHCH_2CO$	H
27	H	$(CH_3)_3CO$	H
28	H	$C_{14}H_8O_2CO$	H
29	H	$(CH_2)_2CHCO$	$(CH_2)_2CHCO$
30	H	$(CH_3)_2CHCO$	$(CH_3)_2CHCO$
31	H	$(CH_3)_2CHCH_2CO$	$(CH_3)_2CHCH_2CO$
32	H	$C_{14}H_8O_2CO$	$C_{14}H_8O_2CO$

[a]Reagents and conditions: (i) $NaHCO_3$/MeOH, (ii) DMAP/*i*-Pr_2Net/CH_2Cl_2, (iii) DMDO/CH_2Cl_2.

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Table 1. Structures and Antiproliferative Potencies of C-7, C-15, and C-7, C-25 Acyloxy Taccalonolides and their Corresponding C-22, C-23 Epoxides

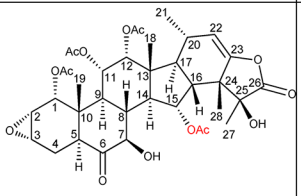
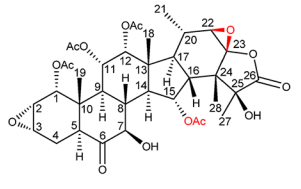
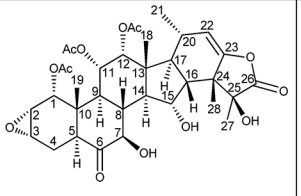
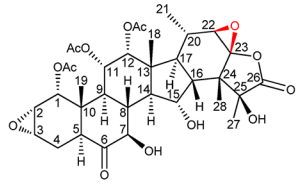
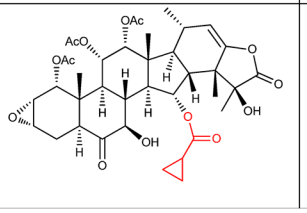
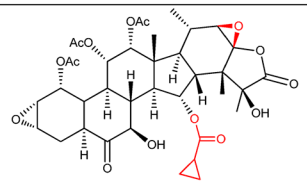
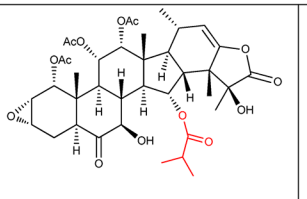
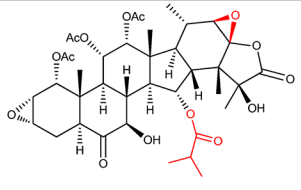
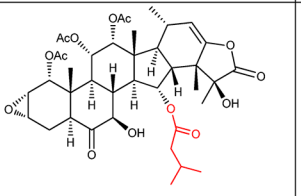
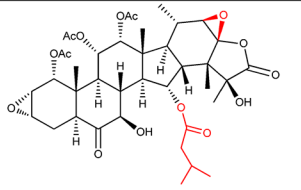
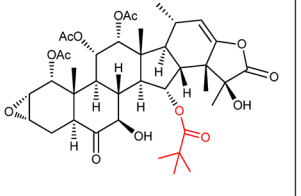
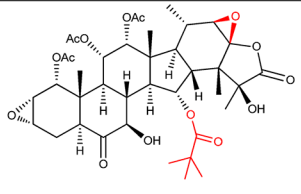
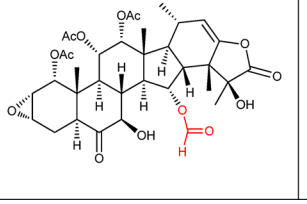
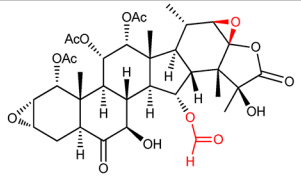
taccalonolide	structure	IC_{50} (nM)	taccalonolide epoxide	structure	IC_{50} (nM)
1		5,400 ± 200[5]	**3**		23 ± 3[5] [235][a]
2		3,100 ± 200[5]	**4**		4.2 ± 0.3[5] [738][a]
5		> 20,000	**19**		27.5 ± 0.9 [> 727][a]
6		> 20,000	**20**		10.6 ± 0.3 [> 1,887][a]
7		1,500 ± 300	**21**		2.7 ± 0.2 [556][a]
8		2,400 ± 500	**22**		4.2 ± 0.8 [571][a]
9		> 20,000	**23**		8.4 ± 0.3 [> 2,381][a]

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Table 1. continued

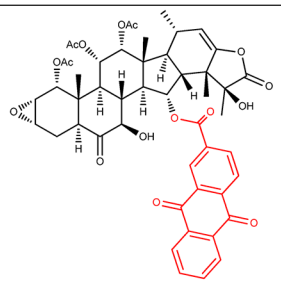
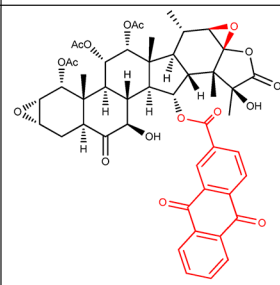
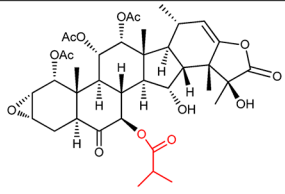
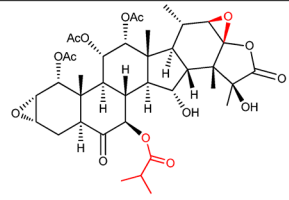
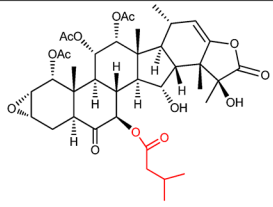
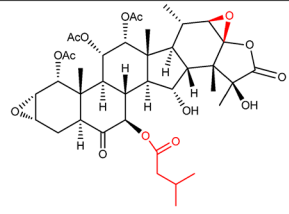
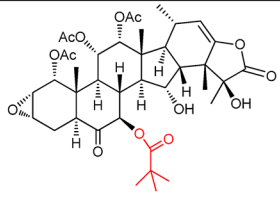
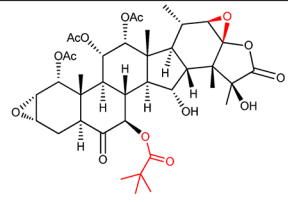
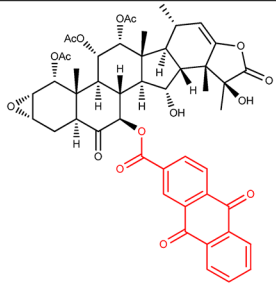
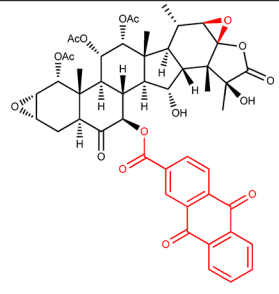
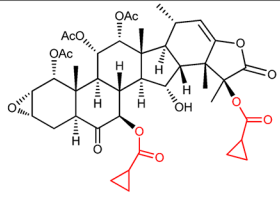
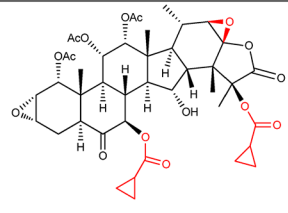
taccalonolide	structure	IC_{50} (nM)	taccalonolide epoxide	structure	IC_{50} (nM)
10		7,100 ± 500	**24**		38 ± 4 [186][a]
11		4,700 ± 600	**25**		19 ± 3 [247][a]
12		> 20,000	**26**		2.4 ± 0.7 [> 8,333][a]
13		10,000 ± 1,000	**27**		9 ± 1 [1,111][a]
14		3,400 ± 400	**28**		22 ± 3 [155][a]
15		> 20,000	**29**		130 ± 20 [> 154][a]

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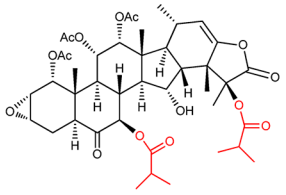
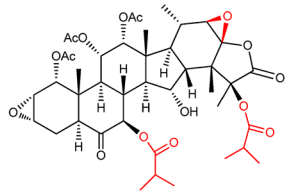
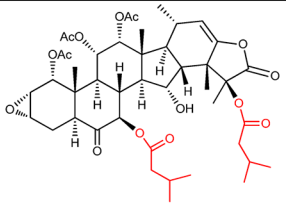
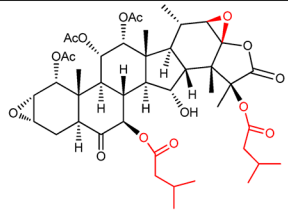
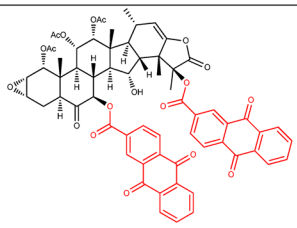
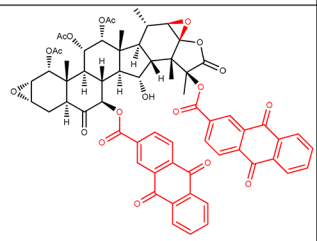
Table 1. continued

taccalonolide	structure	IC_{50} (nM)	taccalonolide epoxide	structure	IC_{50} (nM)
16		> 20,000	**30**		790 ± 40 [> 25][a]
17		> 20,000	**31**		250 ± 30 [> 80][a]
18		> 20,000	**32**		3,000 ± 800 [> 7][a]

[a]The fold increase in potency achieved by C-22, C-23 epoxidation is shown in brackets in the last column.

(DMDO) to produce 14 additional epoxy-taccalonolides (**19**–**32**).

Analysis of the ^{1}H NMR data for each of the monoesters revealed the signals of five methyls (0.68–1.76 ppm), five oxygenated methines (3.97–5.61 ppm), two epoxide methines (3.30–3.50 ppm), and one olefinic methine (4.90–5.09 ppm), which are characteristic of the taccalonolides. Moreover, the downfield chemical shifts of the H-15 protons at 5.45 to 5.61 ppm and additional methyl groups observed in the ^{1}H NMR spectra indicated the presence of a substitution at C-15 in compounds **5**–**10**. The downfield chemical shifts of H-15 have been well documented for all the taccalonolides possessing a bulky substituent at C-15, including taccalonolides A, E, F, R–V, Z, AA, AE, AF, and AI.[6,9,12,14,18,20] Compounds with substitutions at C-7 (**11**–**18**) were recognized readily by the downfield shifts of H-7 from 4.00 to 4.50 ppm to 4.95–5.10 ppm, while substitutions at C-25 (**15**–**18**) were characterized by the downfield shifts of Me-27 from 1.75 to 2.15–2.18 ppm. Each of the structures was confirmed through full analysis of its 1D and 2D NMR data together with LC-MS and HRESIMS (Supporting Information).

SAR of Antiproliferative Effects. Each of the semisynthetic esters of **2** and their corresponding C-22, C-23 epoxides was evaluated to determine their in vitro antiproliferative activities against the HeLa cervical cancer cell line using the sulforhodamine B assay. The new taccalonolides with substituents at C-15 (**5**–**10**) showed a greater than 13-fold difference in potency, with IC_{50} values ranging from 1.5 μM for the C-15 isovalerate (**7**) to greater than 20 μM for the cyclopropyl (**5**), isobutyrate (**6**), and formic acid (**9**) substituents (Table 1). The C-15 pivalate (**8**) and anthraquinone (**10**) substituents were intermediate in potency, with IC_{50} values of 2.4 and 7.1 μM, respectively. Thus, evaluating the different substituents at C-15, the potency ranking was isovalerate (**7**) > pivalate (**8**) > hydroxy (**2**) > acetate (**1**) > anthraquinone (**10**), and each of these was superior to the cyclopropyl (**5**), isobutyrate (**6**), and formic acid (**9**) substituents.

In addition to the desired C-15 monosubstitued products, these reactions also yielded taccalonolides with monosubstitutions at C-7 (**11**–**14**) and disubstitutions at C-7 and C-25 (**15**–**18**). Among the C-7 monosubstituted taccalonolides obtained, the addition of an anthraquinone group (**14**) yielded the most potent of this series with an IC_{50} of 3.4 μM, and the addition of an isobutyric group yielded a compound (**11**) with an IC_{50} of 4.7 μM. The addition of a pivalate group at C-7 resulted in a compound (**13**) with an IC_{50} of 10 μM. In contrast to the 1.5 μM potency obtained with an isovalerate group at C-15 (**7**), a C-7 isovalerate group (**12**) resulted in a taccalonolide with an IC_{50} greater than 20 μM (Table 1). Thus, for C-7 substituents, the ranking of the most potent compounds was hydroxy (**2**) > anthraquinone (**14**) > isobutyrate (**11**) > pivalate (**13**) > isovalerate (**12**). The dual C-7, C-25 cyclopropyl (**15**), isobutyrate (**16**), isovalerate (**17**), or anthraquinone (**18**) substitutions each resulted in taccalonolides with IC_{50} values greater than 20 μM.

Consistent with previous reports demonstrating the role of C-22, C-23 epoxidation in the covalent binding of taccalonolides to β-tubulin,[9,11] C-22, C-23 epoxidation of these semisynthetic taccalonolide esters yielded compounds with highly improved potencies (**19**–**32**). The increases in potency for the monosubstituted esters ranged from 247-fold to greater than 8000-fold (Table 1). Each of the C-15-modified C-22, C-23 epoxy-taccalonolides (**19**–**24**) demonstrated antiproliferative activities in the low nanomolar range, 2.7–38 nM. The most potent C-15 epoxy-taccalonolide (**21**) had an IC_{50} value

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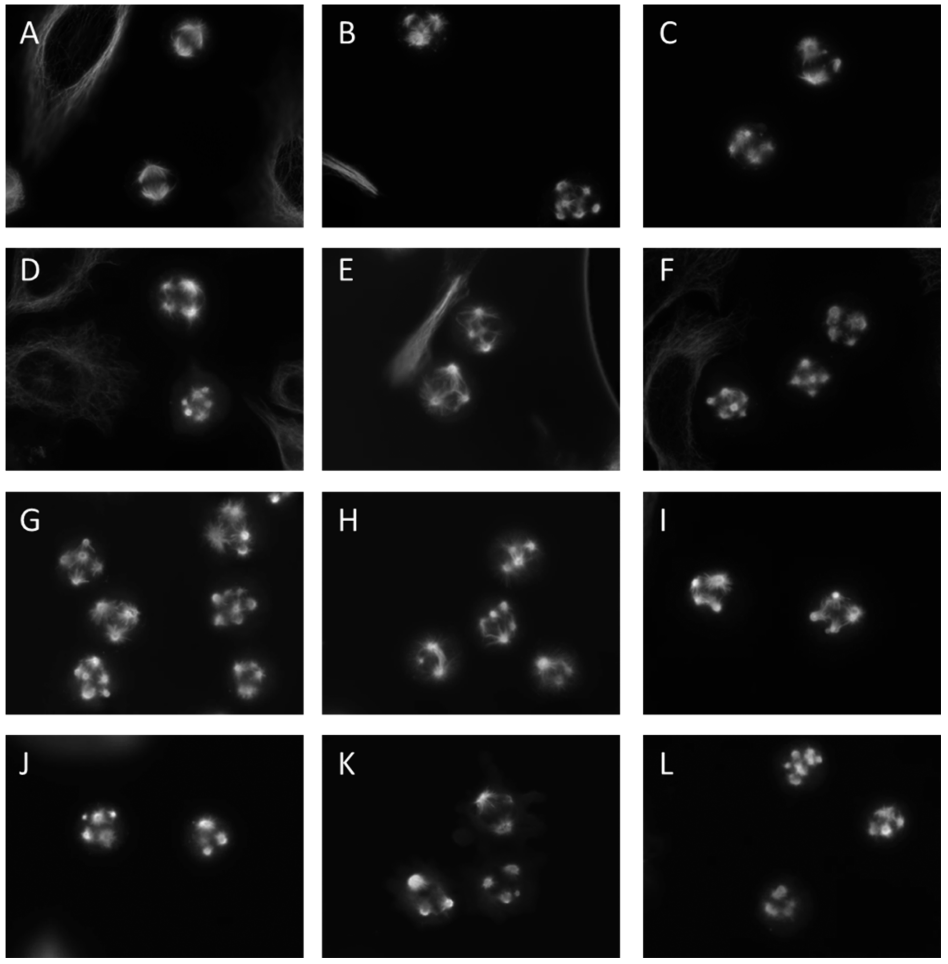

Figure 2. Effect of new acyloxy taccalonolides on mitotic spindles. HeLa cells were treated with (A) vehicle control (EtOH) or 5-fold the IC_{50} value of (B) **19**, (C) **20**, (D) **21**, (E) **22**, (F) **23**, (G) **25**, (H) **26**, (I) **27**, (J) **29**, (K) **30**, and (L) **31** for 18 h. Microtubules were visualized by indirect immunofluorescence using a β-tubulin antibody.

of 2.7 nM and was generated from the most potent parent C-15 compound (**7**) that contained an isovalerate substitution. The second most potent C-22, C-23 epoxidized C-15-modified compound (**22**) was derived from the second most potent nonepoxidized C-15-modified compound (**8**), demonstrating a similar trend in potency in these compounds with and without C-22, C-23-epoxidation. The C-15 cyclopropane (**19**) and C-15 anthraquinone (**24**) C-22, C-23-epoxy derivatives had the lowest potencies of this series, suggesting that cyclic ring substituents at C-15 are not optimal for potency. The most potent C-7-modified C-22, C-23-epoxy-taccalonolide contained an isovalerate group (**26**) and had an IC_{50} value of 2.4 nM, followed by pivalate (**27**), isobutyrate (**25**), and anthraquinone (**28**) modifications with IC_{50} values of 9, 19, and 22 nM, respectively.

The finding that the two most potent taccalonolides generated in this study both contained isovalerate modifications at either C-7 (**26**) or C-15 (**21**) indicated that this longer, less branched chain leads to increased potency regardless of its exact position on the taccalonolide backbone. While the C-22, C-23 epoxidation of the dual-substituted C-7, C-25 taccalonolides (**29**−**32**) increased their potencies to 130−3000 nM, the dual modification consistently resulted in lower-potency compounds as compared to single modification at either C-7 or C-15. It is interesting to note that the dual C-7, C-25 cyclopropyl substitution compound (**29**) was more potent than the other dual substitutions of longer and branched chains (**30**, **31**). Together, these data indicate that a range of monosubstitutions at either C-7 or C-15 can modestly alter potency with no clear distinction between the effects of modification at either site, suggesting the possibility that this area may not be critical for taccalonolide binding or that these modifications are hydrolyzed before binding occurs. The addition of substituents at C-25 was detrimental to antiproliferative potency, suggesting the possibility of steric limitations near the site of the covalent interaction between the C-22, C-23 epoxide of the taccalonolides and β-tubulin.

Evaluation of Microtubule-Stabilizing Effects. The ability of the semisynthetic taccalonolides to elicit microtubule disruption was evaluated. At concentrations 5-fold greater than their IC_{50} values (Table 1), each of the compounds caused the formation of multiple aberrant mitotic spindles (Figure 2) and bundling of interphase microtubules (Figure 3), which are hallmarks of microtubule stabilizers.[4] These cellular findings provide support that the modified taccalonolides retained the microtubule-stabilizing activities typical for this family of natural products. Furthermore, a subset of the most potent new taccalonolides were selected for evaluation for their effects on tubulin polymerization to determine if their cellular potencies were related to the abilities of these compounds to directly interact with and polymerize tubulin. Compounds **19**, **20**, and **27** each caused comparable levels of tubulin

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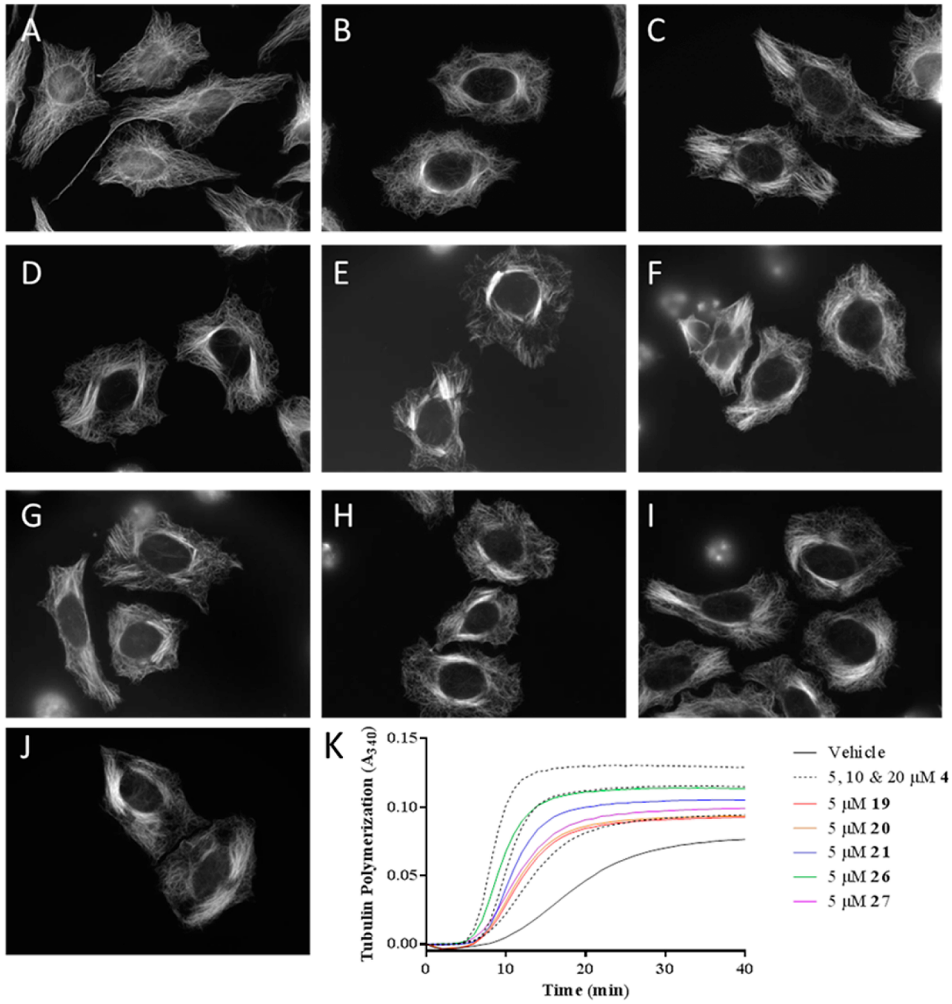

Figure 3. Effect of new acyloxy taccalonolides on microtubules and tubulin polymerization. HeLa cells were treated with (A) vehicle control (EtOH), (B) **19**, (C) **20**, (D) **21**, (E) **22**, (F) **25**, (G) **26**, (H) **27**, (I) **29**, and (J) **31**, for 18 h. Microtubules were visualized by indirect immunofluorescence using a β-tubulin antibody. (K) Tubulin polymerization in the presence of vehicle or taccalonolies. Porcine brain tubulin was allowed to polymerize in the presence of vehicle (solid black line), 5−20 μM **4** (dashed lines), or 5 μM of the new taccalonolides (colored lines). The rate and extent of polymerization was monitored turbidimetrically at 340 nm.

polymerization compared to an equivalent concentration of **4**, suggesting similar efficiencies of tubulin-stabilizing activity (Figure 3K). In contrast, **21** and **26** elicited a greater extent of tubulin polymerization than equivalent concentrations of **4**, with effects similar to 1.5−2-fold higher concentrations of **4**. These findings indicate that compounds **21** and **26**, the C-7 and C-15 isovalerate-modified taccalonolides, are the two most potent compounds in cellular assays as well as the two most potent stimulators of tubulin polymerization.

In Vivo Antitumor Evaluations. The potent C-7- and C-15-modified taccalonolides **19**, **20**, **21**, **22**, and **26** were also evaluated for antitumor efficacy. Initial dose tolerance testing in tumored mice indicated that these taccalonolides are highly potent in vivo, with each compound having a cumulative maximal tolerated dose (MTD) of less than 10 mg/kg when administered by ip injection. However, the lack of observable antitumor effects for any of these compounds in drug-sensitive or -resistant xenograft models during MTD evaluations suggested that these compounds were not able to elicit antitumor activity at systemically tolerable doses; this is similar to what was observed with **4**.[7,10] The antitumor activities of **21** and **26**, the two most potent compounds with isovaleric modifications on C-15 and C-7, respectively, were evaluated in the NCI/ADR-RES drug-resistant xenograft model. While compound **3** has efficacy in this taxane-resistant model with systemic administration, the lower therapeutic windows of **21** and **26** necessitated intratumoral injection, which was used to observe antitumor efficacy of **4** in other models.[10] The tumors of mice in each of the treatment groups grew at a similar rate in the week prior to the initiation of drug treatment, indicating a similar baseline growth rate (Figure 4A). The growth of tumors treated with either **21** (20 μg) or **26** (20 μg) intratumorally abruptly slowed following the first drug administration on day 0, while the growth of vehicle- and paclitaxel (40 μg)-treated tumors was unaffected. This is expected, because the NCI/ADR-RES xenograft is resistant to taxanes.[23] Tumors continued to be treated with the same doses of vehicle, paclitaxel, **21**, or **26** on days 3, 6, 10, and 14.

A two-way ANOVA with Tukey's post hoc test was used to compare the size of tumors in each treatment group to one another throughout the treatment period (individual p-values for each comparison are provided in the Supporting Information). Throughout this treatment schedule, the vehicle- and paclitaxel-injected tumors grew at similar rates that were

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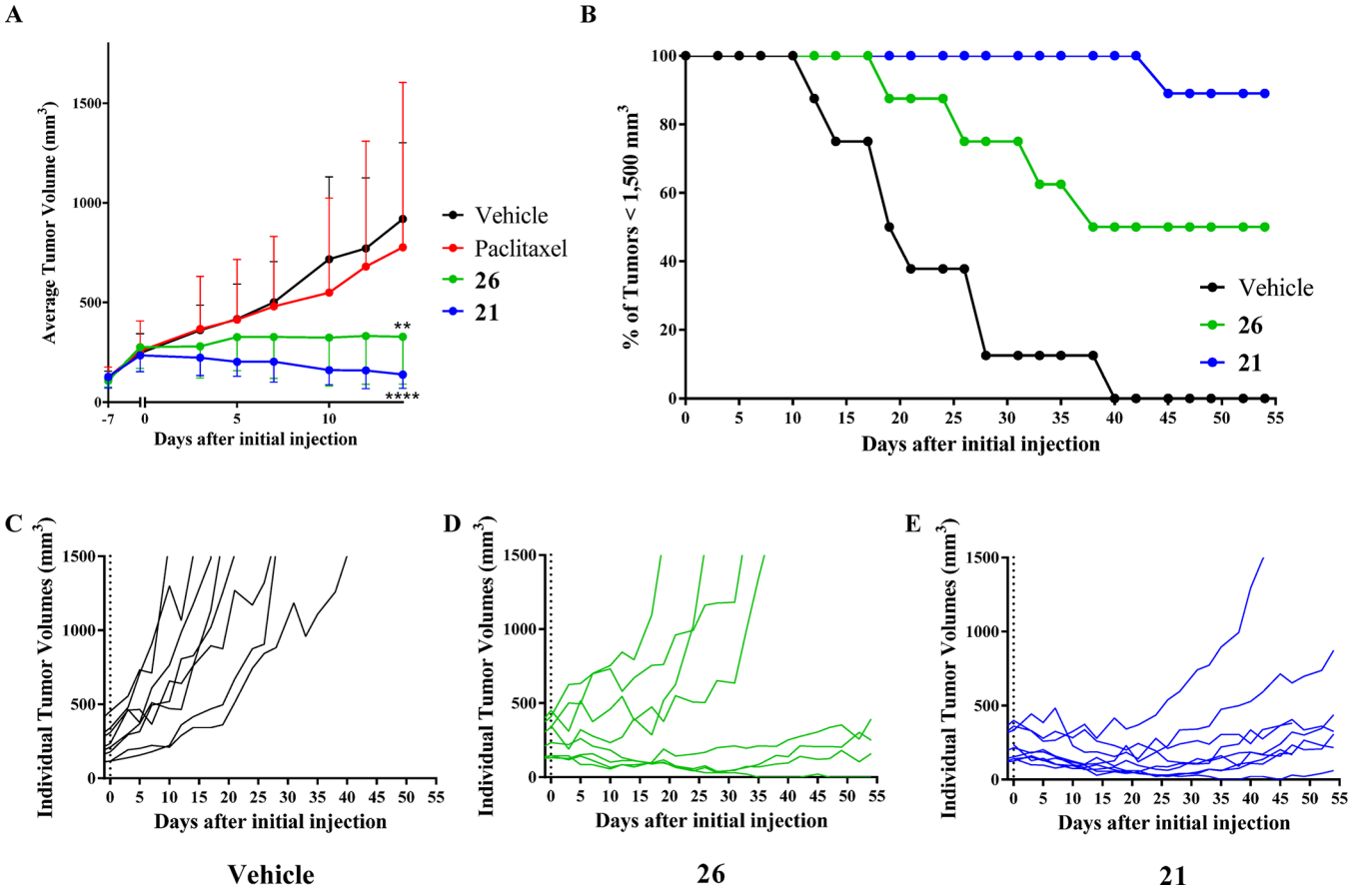

Figure 4. In vivo antitumor activity of **21** and **26**. (A) Effect of intratumoral injections of vehicle, paclitaxel, **21**, or **26** on the growth of drug-resistant NCI/ADR-RES xenograft tumors. A 20 μg amount of **21** or **26**, 40 μg of paclitaxel, or vehicle was administered on days 0, 3, 6, 10, and 14 by intratumoral injection in a volume of 200 μL. Average tumor volumes ±95% CI are graphed over time with day 0 representing the initial dose. A two-way ANOVA with Tukey's post hoc test for multiple comparisons over the duration of the initial 14-day period was performed, and statistical significance between vehicle and drug-treated tumor groups was noted on day 14; $^{**}p = 0.002$; $^{****}p < 0.0001$. (B) Percentage of tumors less than 1,500 mm^3 and (C–E) individual tumor volumes for each of the vehicle-, **26**-, or **21**-treated tumors over time.

not different statistically from one another at any point up to day 14, when mice in these treatment groups were euthanized due to tumor size. In contrast, a statistically significant difference was detected between tumors in vehicle-treated mice versus those in mice administered **21** as early as day 10 with no associated weight loss or other signs of toxicity. The growth of tumors in the mice treated with compound **26** showed statistically significant differences as compared to vehicle-treated animals starting on day 12 with no adverse effects noted. Statistically significant effects were also observed between tumors in animals treated with paclitaxel compared to either **21** or **26** on days 12–14. After dosing was halted on day 14, the tumors of mice in the vehicle and taccalonolide-treated groups continued to be monitored, and mice were euthanized when their tumor volumes reached 1500 mm^3. The percentages of mice in each treatment group with tumors less than 1500 mm^3 over time were graphed (Figure 4B). Fifty percent of mice in the vehicle-treated group reached this threshold 19 days after the initial injection. For tumors treated with **26**, it took twice as long, 38 days, for half of the tumors to reach this volume. Even more strikingly, only one tumor in the group treated with **21** reached a volume of 1500 mm^3 by day 54, which was 40 days after the final dose was administered (Figure 4B).

When individual tumor volumes in each treatment group were graphed, it was evident that the four mice in the group treated with **26** that had the largest initial tumor burdens (338–450 mm^3) were the ones that reached 1500 mm^3 between days 19 and 38, while the other four tumors in this treatment group that had smaller initial volumes (130–233 mm^3) did not increase in size even by day 54 (Figure 4D). Even more strikingly, all the tumors treated with **21**, even some that initially started with volumes greater than 300 mm^3, showed profound delays in tumor growth (Figure 4E). None of the tumors treated with **21** reached a volume of 1500 mm^3 until day 45, which was 31 days after the final dose of **21** and 5 days after the final vehicle-treated tumor reached this size (Figure 4B). Together, these results indicate that **21** and **26** have potent and persistent antitumor effects with no associated toxicities when administered directly to paclitaxel-resistant tumors. This suggests that **21** and **26** may be excellent semisynthetic taccalonolides for targeting drug-resistant tumors.

While these data demonstrate that C-7- and C-15-modified taccalonolides have excellent and highly persistent antitumor efficacy when administered directly into the tumor, the lack of antitumor effects at systemically tolerable doses prompted further analysis. Our original rationale for making C-15-modified taccalonolides was to generate compounds that were less susceptible to hydrolysis at this site, which is likely a limiting factor for the in vivo efficacy of **3**.[10] We hypothesized

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that the similar profiles of in vivo activity observed in this study to those observed for **4** could be the result of hydrolysis of the C-7 and C-15 substituents. Detailed evaluations were carried out using **24**, the most abundant semisynthetic compound bearing a C-15 anthraquinone substituent. Incubation of 10 mg of **24** in 1:1 EtOH/phosphate-buffered saline (PBS) for 36 h resulted in hydrolysis to **4** with a 67% yield. Therefore, taccalonolides with C-15 ester modifications ranging from an acetoxy group (**3**)[7] to an anthraquinone moiety (**24**) are readily hydrolyzed even in an aqueous solution. Importantly, hydrolysis of the C-15 esters generates **4**, which has a higher microsomal clearance, a lower in vivo half-life, and no antitumor efficacy with systemic administration.[10] Therefore, limiting C-15 hydrolysis is a promising strategy to improve the therapeutic window of these compounds. In addition to semisynthetic strategies employed here, approaches to alter drug formulation to limit hydrolysis are also ongoing.

In summary, 28 new C-7/C-15/C-25 taccalonolide derivatives (**5**–**32**) were produced semisynthetically from naturally occurring taccalonolides via DMAP-catalyzed esterification and DMDO epoxidation. The results show that the new taccalonolides have IC_{50} values ranging from 2.4 nM to greater than 20 μM, providing new in vitro SAR for this class of microtubule stabilizers. Compounds **21** and **26** were the most potent in cellular and biochemical experiments and demonstrated in vivo antitumor efficacy in a paclitaxel-resistant tumor that persisted for at least 30 days after the final dose when administered by intratumoral injection. However, none of the C-7/C-15/C-25-modified taccalonolides had a sufficient therapeutic window for evaluating antitumor activity using systemic administration. These experiments demonstrate the strengths and limitations of these substituted analogues, which suggest the potential for targeted delivery of the taccalonolides as a viable method of achieving antitumor efficacy with an acceptable therapeutic window.

EXPERIMENTAL SECTION

General Experimental Procedures. 1D and 2D NMR spectra were acquired using a 500 or 600 MHz Bruker AVANCE spectrometer (Billerica, MA, USA) using a cryoprobe and $CDCl_3$ as solvent. Mass spectra were measured with a LC/MS Waters Alliance mass spectrometer (Malford, MA, USA) equipped with a 2695 HPLC module, 996 photodiode array detector, and Micromass Quattro triple quadrupole mass spectrometer with ESI under positive mode. High-resolution electrospray ionization mass spectroscopy (HRESIMS) was recorded on an Agilent Technologies (Santa Clara, CA, USA) 6224 TOFLC/MS mass spectrometer. TLC was carried out on precoated silica gel plates (silica gel 60 F-254, Merck KGaA, Darmstadt, Germany). Spots were visualized by spraying with 20% sulfuric acid in ethanol followed by heating. Preparative HPLC was performed on a Waters 1528 binary pump and 2487 diode array detector system using a Phenomenex (Torrance, CA, USA) Kinetex 5 μm C_{18} 250 × 21 mm and a Phenomenex Luna 5 μm C_{18} 250 × 21 mm column. Isobutyric anhydride, pivalic anhydride, isovaleric anhydride, cyclopropane chloride, formic acid, DMAP, and *N*,*N*-diisopropylethylamine (Hünig's base) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Anthraquinone-2-carbonyl chloride was purchased from TCI America (Portland, OR, USA).

*Hydrolysis of Taccalonolide A (**1**).* Compound **1** (50 mg, 0.071 mmol) was isolated from the root and rhizomes of *Tacca chantrieri* and hydrolyzed with 0.05 M $NaHCO_3$ as previously described[21] to produce **2** (40 mg, 80%).

*Esterification of Taccalonolide B (**2**) and Isobutyric Anhydrides.* Aliquots of 11.26 μL (0.068 mmol) of isobutyric anhydride and 11.8 μL (0.068 mmol) of Hünig's base were added to a mixture of **2** (15 mg, 0.02 mmol) and DMAP (4.5 mg, 0.03 mmol) in 1 mL of anhydrous CH_2Cl_2. The solution was stirred overnight at room temperature, and the resultant product was then concentrated under a vacuum. The residue was purified by preparative HPLC using a Phenomenex Kinetex 5 μm C_{18} 250 × 21 mm column eluted with 45% CH_3CN/H_2O to afford compounds **6** (5.0 mg, 33%), **11** (0.6 mg, 4%), and **16** (4.8 mg, 32%).

*Esterification of **2** with Isovaleric Anhydride.* Aliquots of 14 μL (0.07 mmol) of isovaleric anhydride were added to a mixture of **2** (15 mg, 0.02 mmol) and DMAP (4.5 mg, 0.03 mmol) in 1 mL of anhydrous CH_2Cl_2. The mixture was stirred at room temperature for 30 min, and the resultant product was then concentrated under a vacuum. The residue was purified by preparative HPLC using a Phenomenex Kinetex 5 μm C_{18} 250 × 21 mm column eluted with 50% CH_3CN/H_2O to afford compounds **7** (3.1 mg, 20.6%), **12** (5.2 mg, 34.6%), and **17** (6.0 mg, 40%).

*Esterification of **2** with Pivalic Anhydride.* Aliquots of 5.47 μL (0.27 mmol) of pivalic anhydride and 14.1 μL (0.08 mmol) of Hünig's base were added to a mixture of **2** (18 mg, 0.027 mmol) and DMAP (3.2 mg, 0.026 mmol) in 1 mL of anhydrous CH_2Cl_2. The mixture was stirred overnight at room temperature, and the resultant product was then concentrated under a vacuum. The residue was purified by preparative HPLC using a Phenomenex Luna 5 μm C_{18} 250 × 21 mm column eluted with 50% CH_3CN/H_2O to afford compounds **8** (2.5 mg, 13.8%) and **13** (2.6 mg, 14.4%).

*Esterification of **2** with Cyclopropane Chloride.* Aliquots of 100 μL of pyridine anhydrous and 6.1 μL (0.068 mmol) of cyclopropane chloride were added to a mixture of **2** (15 mg, 0.02 mmol) and DMAP (4.5 mg, 0.03 mmol) in 400 μL of anhydrous CH_2Cl_2. The mixture was stirred overnight at room temperature, and the resultant product was then concentrated under a vacuum. The residue was purified by preparative HPLC using a Phenomenex Luna 5 μm C_{18} 250 × 21 mm column eluted with 50% CH_3CN/H_2O to afford compounds **5** (1.15 mg, 7.6%) and **15** (0.7 mg, 4.7%).

*Esterification of **2** with Formic Acid.* Aliquots of 20 μL (0.5 mmol) of formic acid and 20 μL (0.11 mmol) of isobutyric anhydride were added to a mixture of **2** (11 mg, 0.017 mmol) and DMAP (1.5 mg, 0.012 mmol). The mixture was stirred at room temperature overnight, and the resultant product was concentrated under a vacuum. The residue was purified by preparative HPLC using a Phenomenex Luna 5 μm C_{18} 250 × 21 mm column eluted with 50% CH_3CN/H_2O to afford compound **9** (5.15 mg, 51.5%).

*Esterification of **2** with Anthraquinone-2-carbonyl Chloride.* Aliquots of 50 μL of anhydrous Et_3N and 40 mg (0.16 mmol) of anthraquinone-2-carbonyl chloride were added to a mixture of **2** (60 mg, 0.08 mmol) and DMAP (9.0 mg, 0.06 mmol) in 4 mL of anhydrous CH_2Cl_2. The mixture was stirred overnight at room temperature, and the resultant product was then concentrated under a vacuum. The residue was purified by semipreparative HPLC using a Phenomenex Gemini 5 μm C_{18} 250 × 10 mm column eluted with 70% CH_3CN/H_2O (with 0.1% formic acid) to afford compounds **10** (12.5 mg, 15.3%), **14** (3.6 mg, 4.4%), and **18** (15.5 mg, 15.1%).

Epoxidation of the Bulky Acyloxy Taccalonolides. Compounds **5**–**18** (0.9–10 mg) were epoxidized using the protocol as previously described[9] to produce compounds **19**–**32** with 100% yield after removal of the solvent.

Statement of Purity. HPLC was performed on a Waters system, equipped with a 600 controller and a 996 photodiode array detector. Different isocratic or gradient solvent systems composed of MeOH/H_2O or MeCN/H_2O were used to generate the best resolution of each compound. The purity of all compounds was judged by the percentage of the integrated signal at UV 205 nm. All final compounds submitted for bioassay were at least 95% pure as judged by this method.

*Compound **5**:* white powder; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.61 (1H, t, J = 9.6 Hz, H-15), 5.33 (1H, dd, J = 10.1, 2.5 Hz, H-11), 5.28 (1H, d, J = 2.7, H-12), 5.09 (1H, s, H-22), 4.76 (1H, d, J = 5.2 Hz, H-1), 4.07 (1H, d, J = 11.3 Hz, H-7), 3.76 (1H, brs, OH-7), 3.48 (1H, t, J = 3.8 Hz, H-2), 3.41 (1H, m, H-3), 2.78 (1H, m, H-5), 2.76 (1H, m, H-9), 2.50 (1H, m, H-14), 2.44 (1H, m, H-16), 2.22 (2H, m, H-4), 2.22 (1H, m, H-20), 2.20 (3H, s, $OCOCH_3$-1), 2.16 (3H, s, $OCOCH_3$-12), 2.01 (3H, s, $OCOCH_3$-11), 1.83 (1H, dd, J = 13.8,

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J. Nat. Prod. XXXX, XXX, XXX−XXX

10.5, H-17), 1.76 (1H, m, H-8), 1.71 (3H, s, H-27), 1.59 (1H, m, H-2′), 1.35 (3H, s, H-28), 1.01 (3H, s, H-18), 0.99 (2H, m, H-3′and H-4′), 0.93 (3H, d, J = 6.95 Hz, H-21), 0.78 (3H, s, H-19), 0.76 (2H, m, H-3′ and H-4′); ^{13}C NMR (125 MHz, $CDCl_3$) 209.8 (C-6), 178.1 (C-26), 175.4 (C-1′), 170.8 (11−$OCOCH_3$), 169.6 (1−$OCOCH_3$), 169.3 (12-$OCOCH_3$), 154.2 (C-23), 111.1 (C-22), 79.2 (C-25), 75.1 (C-7), 74.3 (C-12), 72.9 (C-1), 71.3 (C-15), 70.9 (C-11), 54.5 (C-14), 52.1 (C-3), 51.4 (C-16), 50.1 (C-24), 49.5 (C-2), 48.9 (C-17), 43.8 (C-8), 43.6 (C-13), 43.1 (C-10), 42.6 (C-5), 40.1 (C-9), 30.8 (C-20), 25.1 (C-28), 21.5 ($OCOCH_3$-11), 21.4 (C-4), 20.9 ($OCOCH_3$-1 and $OCOCH_3$-12), 20.6 (C-27), 19.9 (C-21), 14.1 (C-2′), 13.6 (C-18), 12.74 (C-19), 8.2 (C-3′ and C-4′); HRMS m/z 729.3129 $[M + H]^+$ (calcd for $C_{38}H_{49}O_{14}$, 729.3122).

Compound ***6****:* white powder; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.60 (1H, t, J = 9.45 Hz, H-15), 5.34 (1H, dd, J = 11.9, 1.75 Hz, H-11), 5.27 (1H, brd, H-12), 5.07 (1H, s, H-22),), 4.73 (1H, d, J = 5.4 Hz, H-1), 3.97 (1H, dd, J = 10.2, 3.5 Hz, H-7), 3.76 (1H, d, J = 3.5 Hz, OH-7), 3.49 (1H, t, J = 5.0, H-2), 3.39 (1H, m, H-3), 2.79 (1H, m, H-5), 2.69 (1H, m, H-2′), 2.65 (1H, m, H-9), 2.46 (1H, m, H-14), 2.44 (1H, m, H-16), 2.22 (2H, m, H-4), 2.20 (3H, s, $OCOCH_3$-1), 2.18 (1H, m, H-20), 2.15 (3H, s, $OCOCH_3$-12), 2.0 (3H, s, $OCOCH_3$-11), 1.79 (1H, m, H-17), 1.75 (1H, m, H-8), 1.64 (3H, s, H-27), 1.35 (3H, s, H-28), 1.14 (3H, d, J = 7.0 Hz, H-3′), 1.12 (3H, d, J = 7.0 Hz, H-4′), 1.00 (3H, s, H-18), 0.93 (3H, d, J = 6.7 Hz, H-21), 0.78 (3H, s, H-19); ^{13}C NMR (125 MHz, $CDCl_3$) δ 210.1 (C-6), 178.7 (C-26), 176.9 (C-1′), 171.0 (11−$OCOCH_3$), 169.7 (1-$OCOCH_3$), 169.3 (12-$OCOCH_3$), 154.4 (C-23), 111.3 (C-22), 79.0 (C-25), 75.0 (C-7), 74.4 (C-12), 72.8 (C-1), 71.4 (C-15), 70.8 (C-11), 54.7 (C-14), 51.9 (C-3), 50.6 (C-16), 50.5 (C-24), 49.7 (C-2), 48.7 (C-17), 43.7 (C-8), 43.8 (C-13), 42.8 (C-10), 42.2 (C-5), 40.1 (C-9), 34.4 (C-2′), 30.9 (C-20), 25.3 (C-28), 21.3 ($OCOCH_3$-11), 20.9 ($OCOCH_3$-1 and $OCOCH_3$-12), 20.8 (C-4), 20.1 (C-27), 19.5 (C-3′ and C-4′), 19.4 (C-21), 12.8 (C-18), 12.8 (C-19); HRMS m/z 731.3284 $[M + H]^+$ (calcd for $C_{38}H_{51}O_{14}$, 731.3278).

Compound ***7****:* white powder; ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.55 (1H, t, J = 9.6 Hz, H-15), 5.31 (1H, dd, J = 11.5, 2.6 Hz, H-11), 5.27 (1H, d, J = 2.6 Hz, H-12), 5.07 (1H, d, J = 1.3 Hz, H-22), 4.74 (1H, d, J = 5.5 Hz, H-1), 3.97 (1H, dd, J = 10.1, 3.5 Hz, H-7), 3.76 (1H, d, J = 3.5 Hz, OH-7), 3.47 (1H, dd, J = 5.5, 3.7, H-2), 3.38 (1H, m, H-3), 2.77 (1H, dd, J = 11.0, 6.1, H-5), 2.71 (1H, m, H-9), 2.44 (1H, m, H-14), 2.41 (1H, m, H-16), 2.26 (2H, m, H-2′), 2.21 (1H, m, H-4a), 2.18 (1H, m, H-20), 2.17 (3H, s, $OCOCH_3$-1), 2.12 (3H, s, $OCOCH_3$-12), 2.03 (1H, m, H-4b), 2.01 (1H, m, H-3′), 1.99 (3H, s, $OCOCH_3$-11), 1.79 (1H, m, H-17), 1.72 (1H, m, H-8), 1.63 (3H, s, H-27), 1.34 (3H, s, H-28), 0.98 (3H, s, H-18), 0.93 (6H, d, J = 7.0 Hz, H-4′ and H-5′), 0.92 (3H, d, J = 6.7 Hz, H-21), 0.78 (3H, s, H-19); ^{13}C NMR (150 MHz, $CDCl_3$) δ 209.7 (C-6), 178.7 (C-26), 173.5 (C-1′), 170.8 (11-$OCOCH_3$), 169.4 (1-$OCOCH_3$), 169.2 (12-$OCOCH_3$), 154.2 (C-23), 111.1 (C-22), 79.4 (C-25), 75.1 (C-7), 73.9 (C-12), 72.6 (C-1), 71.1 (C-15), 70.8 (C-11), 54.4 (C-14), 52.1(C-3), 51.3 (C-16), 50.1 (C-24), 49.4 (C-2), 48.6 (C-17), 43.7 (C-13), 43.4 (C-3′), 43.2 (C-8), 42.7 (C-10), 42.4 (C-5), 40.4 (C-9), 30.8 (C-20), 25.3 (C-28), 24.1 (C-2′), 22.8 (C-4′ and C-5′), 21.5 (C-4), 21.2 ($OCOCH_3$-11), 20.8 ($OCOCH_3$-12), 20.5 (C-27), 20.3 ($OCOCH_3$-1), 20.2 (C-21), 13.6 (C-18), 12.9 (C-19); HRMS m/z 745.3445 $[M + H]^+$ (calcd for $C_{39}H_{53}O_{14}$, 745.3435).

Compound ***8****:* white powder; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.55 (1H, t, J = 9.7 Hz, H-15), 5.30 (1H, dd, J = 11.7, 2.7 Hz, H-11), 5.26 (1H, d, J = 2.7 Hz, H-12), 5.07 (1H, s, H-22), 4.74 (1H, d, J = 5.5 Hz, H-1), 3.97 (1H, d, J = 10.5 Hz, H-7), 3.76 (1H, brd, OH-7), 3.49 (1H, t, J = 5.3, H-2), 3.38 (1H, m, H-3), 2.78 (1H, m, H-5), 2.65 (1H, m, H-9), 2.45 (1H, dd, J = 9.0, 9.0 Hz, H-14), 2.41 (1H, m, H-16), 2.27 (2H, m, H-4), 2.18 (3H, s, $OCOCH_3$-1), 2.06 (1H, m, H-20), 2.15 (3H, s, $OCOCH_3$-12), 1.99 (3H, s, $OCOCH_3$-11), 1.79 (1H, m, H-17), 1.71 (1H, m, H-8), 1.63 (3H, s, H-27), 1.34 (3H, s, H-28), 1.00 (3H, d, J = 6.5 Hz, H-21), 0.98 (3H, s, H-18), 0.90−0.92 (9H, s, H-3′, H-4′, and H-5′), 0.77 (3H, s, H-19); ^{13}C NMR (125 MHz, $CDCl_3$) δ 209.8 (C-6), 178.2 (C-26), 173.3 (C-1′), 170.8 (11-$OCOCH_3$), 169.5 (1-$OCOCH_3$), 169.2 (12-$OCOCH_3$), 154.5 (C-23), 111.6 (C-22), 79.3 (C-25), 75.2 (C-7), 73.6 (C-12), 72.5 (C-1), 71.1 (C-15), 70.8 (C-11), 54.3 (C-14), 52.3 (C-3), 51.2 (C-16), 50.8 (C-24), 49.8 (C-2), 48.4 (C-17), 43.5 (C-13), 43.4 (C-2′), 43.1 (C-8), 42.5 (C-10), 42.2 (C-5), 40.5 (C-9), 30.7 (C-20), 25.1 (C-28), 23.1 (C-3′ and C-5′), 21.3 ($OCOCH_3$-11), 20.9 ($OCOCH_3$-1 and $OCOCH_3$-12), 20.7 (C-4), 20.4 (C-27), 20.1 (C-21), 13.2 (C-18), 13.0 (C-19); HRMS m/z 745.3422 $[M + H]^+$ (calcd for $C_{39}H_{52}O_{14}$, 745.3435).

Compound ***9****:* white powder; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.95 (1H, s, H-1′), 5.69 (1H, t, J = 9.4 Hz, H-15), 5.32 (1H, dd, J = 11.5, 2.5 Hz, H-11), 5.28 (1H, d, J = 2.5, Hz, H-12), 5.08 (1H, d, J = 1.4, Hz, H-22), 4.73 (1H, d, J = 5.5 Hz, H-1), 4.02 (1H, d, J = 10.3, Hz, H-7), 3.83 (1H, s, OH-7), 3.47 (1H, t, J = 5.2, H-2), 3.38 (1H, m, H-3), 2.74 (1H, m, H-9), 2.72 (1H, m, H-5), 2.52 (1H, t, J = 10.3 Hz, H-14), 2.44 (1H, dd, J = 13.3, 10.0 Hz, H-16), 2.20 (2H, m, H-4), 2.18 (1H, m, H-20), 2.17 (3H, s, $OCOCH_3$-1), 2.12 (3H, s, $OCOCH_3$-12), 1.99 (3H, s, $OCOCH_3$-11), 1.82 (1H, dd, J = 13.4, 9.8 Hz, H-17), 1.74 (1H, m, H-8), 1.65 (3H, s, H-27), 1.32 (3H, s, H-28), 0.99 (3H, s, H-18), 0.93 (3H, d, J = 7.0 Hz, H-21), 0.78 (3H, s, H-19); ^{13}C NMR (150 MHz, $CDCl_3$) δ 209.3 (C-6), 177.8 (C-26), 170.8 (11-$OCOCH_3$), 169.5 (1-$OCOCH_3$), 169.1 (12-$OCOCH_3$), 154.2 (C-23), 111.2 (C-22), 79.6 (C-25), 74.9 (C-7), 73.8 (C-12), 72.3 (C-1), 70.7 (C-11), 70.4 (C-15), 54.1 (C-14), 52.2 (C-3), 51.3 (C-16), 50.1 (C-24), 49.6 (C-2), 48.8 (C-17), 43.2 (C-10), 43.0 (C-13), 42.7 (C-8), 42.2 (C-9), 40.2 (C-5), 30.8 (C-20), 25.4 (C-28), 21.4 (C-4), 21.2 ($OCOCH_3$-11), 20.8 (C-27), 20.5 ($OCOCH_3$-12), 20.3 (C-21), 20.2 ($OCOCH_3$-1), 13.6 (C-18), 12.6 (C-19); HRMS m/z 689.2824 $[M + H]^+$ (calcd for $C_{35}H_{45}O_{14}$, 689.2809).

Compound ***10****:* pale yellow powder; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.81 (1H, d, J = 1.6 Hz, H-3′), 8.43 (1H, dd, J = 8.2, 1.5 Hz, H-15′), 8.35 (1H, d, J = 8.2 Hz, H-14′), 8.33 (2H, m, H-7′ and H-10′), 7.85 (2H, m, H-8′ and H-9′), 5.85 (1H, t, J = 9.7 Hz, H-15), 5.35 (1H, dd, J = 11.6, 2.9 Hz, H-11), 5.35 (1H, s, H-12), 5.08 (1H, d, J = 1.6 Hz, H-22), 4.73 (1H, d, J = 5.5 Hz, H-1), 3.89 (1H, d, J = 10.4 Hz, H-7), 3.47 (1H, dd, J = 5.5, 3.6 Hz, H-2), 3.32 (1H, m, H-3), 2.80 (1H, t, J = 11.2 Hz, H-9), 2.73 (1H, dd, J = 11.0, 5.1 Hz, H-5), 2.64 (1H, t, J = 9.3 Hz, H-14), 2.60 (1H, dd, J = 13.5, 10.2 Hz, H-16), 2.31 (3H, s, $OCOCH_3$-1), 2.27 (3H, s, $OCOCH_3$-12), 2.22 (1H, m, H-20), 2.10 (2H, m, H-4), 2.02 (3H, s, $OCOCH_3$-11), 1.92 (1H, dd, J = 13.5, 9.6 Hz, H-17), 1.92 (1H, m, H-8), 1.71 (3H, s, H-27), 1.39 (3H, s, H-28), 1.07 (3H, s, H-18), 0.93 (3H, d, J = 7.3 Hz, H-21), 0.76 (3H, s, H-19); ^{13}C NMR (100 MHz, $CDCl_3$) δ 209.5 (C-6), 183.4 (C-5′), 182.6 (C-12′), 177.8 (C-26), 170.9 (11-$OCOCH_3$), 169.9 (1-$OCOCH_3$), 169.6 (12-$OCOCH_3$), 165.7 (C-1′), 154.5 (C-23), 137.5 (C-4′), 135.7 (C-13′), 135.1 (C-15′), 134.8 (C-8′), 134.6 (C-9′), 133.6 (C-2′), 133.5 (C-6′ and C-11′), 127.6 (C-3′), 127.6 (C-14′), 127.6 (C-7′), 127.4 (C-10′), 111.3 (C-22), 79.2 (C-25), 75.4 (C-7), 73.9 (C-12), 73.2 (C-15), 72.8 (C-1), 71.0 (C-11), 54.4 (C-14), 52.2 (C-3), 51.4 (C-16), 50.2 (C-24), 49.8 (C-2), 49.1 (C-17), 43.5 (C-13), 43.3 (C-8), 43.1 (C-10), 42.3 (C-5), 40.1 (C-9), 30.8 (C-20), 25.7 (C-28), 21.4 ($OCOCH_3$-11), 21.4 (C-4), 21.3 ($OCOCH_3$-12), 21.0 (C-27), 21.0 ($OCOCH_3$-1), 20.3 (C-21), 13.6 (C-18), 13.1 (C-19); HRMS m/z 917.2980 $[M + Na]^+$ (calcd for $C_{49}H_{50}O_{16}Na$, 917.2991).

Compound ***11****:* white powder; NMR (500 MHz, $CDCl_3$) δ 5.35 (1H, dd, J = 11.5, 2.2 Hz, H-11), 5.25 (1H, d, J = 2.2 Hz, H-12), 5.19 (1H, brd, H-7), 5.06 (1H, s, H-22), 4.74 (1H, d, J = 5.7 Hz, H-1), 4.46 (1H, t, J = 8.0, H-15), 3.44 (1H, t, J = 4.5, H-2), 3.35 (1H, m, H-3), 2.82 (1H, m, H-9), 2.78 (1H, m, H-5), 2.69 (1H, m, H-2′), 2.29 (1H, m, H-14), 2.27 (1H, m, H-16), 2.20 (1H, m, H-8), 2.18 (1H, m, H-20), 2.17 (3H, s, $OCOCH_3$-1), 2.16 (2H, m, H-4), 2.10 (3H, s, $OCOCH_3$-12), 1.99 (3H, s, $OCOCH_3$-11), 1.83 (1H, dd, J = 13.5, 9.7 Hz, H-17), 1.67 (3H, s, H-27), 1.30 (3H, s, H-28), 1.27 (3H, d, J = 7.0 Hz, H-3′), 1.23 (3H, d, J = 7.0 Hz, H-4′), 0.98 (3H, s, H-18), 0.91 (3H, d, J = 7.3 Hz, H-21), 0.80 (3H, s, H-19). ^{13}C NMR (125 MHz, $CDCl_3$) δ 202.5 (C-6), 176.8 (C-26), 176.5 (C-1′), 170.8 (11-$OCOCH_3$), 169.8 (1−$OCOCH_3$), 169.5 (12-$OCOCH_3$), 153.7 (C-23), 111.6 (C-22), 79.2 (C-25), 77.3 (C-7), 74.0 (C-12), 72.7 (C-1), 70.7 (C-11), 70.1 (C-15), 57.6 (C-14), 52.3 (C-3), 51.4 (C-16), 49.7 (C-2), 49.5 (C-24), 47.5 (C-17), 43.8 (C-13), 42.8 (C-5), 42.1 (C-10), 41.1 (C-9), 38.5 (C-8), 34.2 (C-2′), 30.9 (C-20), 25.1 (C-28), 20.9 (C-27), 21.4 (C-4), 21.1 ($OCOCH_3$-12), 20.8 ($OCOCH_3$-1), 20.6 ($OCOCH_3$-11), 20.1 (C-21), 19.1 (C-3′), 18.9 (C-4′), 13.3 (C-

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J. Nat. Prod. XXXX, XXX, XXX−XXX

18), 13.1 (C-19); HRMS m/z 731.3272 $[M + H]^+$ (calcd for $C_{38}H_{51}O_{14}$, 731.3278).

Compound ***12:*** white powder; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.34 (1H, dd, J = 11.5, 2.7 Hz, H-11), 5.24 (1H, d, J = 2.7 Hz, H-12), 5.19 (1H, brd, H-7), 5.07 (1H, d, J = 1.5 Hz, H-22), 4.74 (1H, d, J = 5.5 Hz, H-1), 4.47 (1H, t, J = 7.6, H-15), 3.44 (1H, dd, J = 5.5, 3.7 Hz, H-2), 3.35 (1H, m, H-3), 2.81 (1H, m, H-9), 2.79 (1H, m, H-5), 2.30 (1H, m, H-14), 2.29 (1H, m, H-16), 2.27 (2H, m, H-2′), 2.22 (1H, m, H-8), 2.21 (1H, m, H-20), 2.19 (2H, m, H-4), 2.18 (1H, m, H-3′), 2.17 (3H, s, $OCOCH_3$-1), 2.09 (3H, s, $OCOCH_3$-12), 1.99 (3H, s, $OCOCH_3$-11), 1.80 (1H, dd, J = 13.7, 9.5 Hz, H-17), 1.68 (3H, s, H-27), 1.32 (3H, s, H-28), 1.04 (3H, d, J = 6.6 Hz, H-4′/H-5′), 1.01 (3H, d, J = 6.6 Hz, H-4′/H-5′), 0.98 (3H, s, H-18), 0.91 (3H, d, J = 7.0 Hz, H-21), 0.80 (3H, s, H-19); ^{13}C NMR (125 MHz, $CDCl_3$) δ 202.6 (C-6), 175.6 (C-26), 172.3 (C-1′), 170.6 (11−$OCOCH_3$), 169.8 (1-$OCOCH_3$), 169.2 (12-$OCOCH_3$), 153.4 (C-23), 111.4 (C-22), 79.0 (C-25), 77.1 (C-7), 73.8 (C-12), 72.6 (C-1), 70.9 (C-11), 70.1 (C-15), 57.8 (C-14), 52.2 (C-3), 51.6 (C-16), 49.7 (C-2), 49.5 (C-24), 47.5 (C-17), 43.6 (C-13), 43.1 (C-3′), 42.7 (C-5), 41.8 (C-10), 41.2 (C-9), 38.4 (C-8), 30.6 (C-20), 25.6 (C-2′), 25.1 (C-28), 22.4 (C-4′ and C-5′), 20.9 (C-27), 21.4 (C-4), 21.1 ($OCOCH_3$-12), 20.8 ($OCOCH_3$-1), 20.6 ($OCOCH_3$-11), 20.1 (C-21), 13.4 (C-18), 12.7 (C-19); HRMS m/z 745.3434 $[M + H]^+$ (calcd for $C_{39}H_{53}O_{14}$, 745.3436).

Compound ***13:*** white powder; ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.36 (1H, dd, J = 11.3, 2.7 Hz, H-11), 5.25 (1H, d, J = 2.6, Hz, H-12), 5.22 (1H, brd, H-7), 5.06 (1H, d, J = 1.4, Hz, H-22), 4.76 (1H, d, J = 5.5 Hz, H-1), 4.48 (1H, brt, H-15), 3.45 (1H, dd, J = 5.5, 3.5, H-2), 3.35 (1H, m, H-3), 2.83 (1H, m, H-9), 2.78 (1H, m, H-5), 2.29 (1H, m, H-14), 2.27 (1H, m, H-16), 2.21 (1H, m, H-20), 2.18 (3H, s, $OCOCH_3$-1), 2.17 (2H, m, H-4), 2.15 (1H, m, H-8), 2.10 (3H, s, $OCOCH_3$-12), 2.02 (3H, s, $OCOCH_3$-11), 1.82 (1H, dd, J = 13.6, 9.5 Hz, H-17), 1.68 (3H, s, H-27), 1.30 (9H, s, H-3′, H-4′, andH-5′), 1.29 (3H, s, H-28), 0.99 (3H, s, H-18), 0.92 (3H, d, J = 7.3 Hz, H-21), 0.81 (3H, s, H-19); ^{13}C NMR (125 MHz, $CDCl_3$) δ 201.2 (C-6), 177.9 (C-1′), 176.3 (C-26), 170.8 (11-$OCOCH_3$), 169.8 (1-$OCOCH_3$), 169.5 (12-$OCOCH_3$), 153.3 (C-23), 111.3 (C-22), 79.2 (C-25), 77.5 (C-7), 73.9 (C-12), 72.8 (C-1), 70.8 (C-11), 70.3 (C-15), 57.7 (C-14), 52.2 (C-3), 51.4 (C-16), 49.6 (C-2), 49.4 (C-24), 47.5 (C-17), 44.2 (C-13), 42.8 (C-5), 42.6 (C-10), 41.7 (C-9), 39.7 (C-2′), 38.5 (C-8), 30.7 (C-20), 27.3 (C-3′-5′), 25.30 (C-28), 21.1 (C-27), 21.5 (C-4), 21.0 ($OCOCH_3$-12), 20.6 ($OCOCH_3$-1), 20.5 ($OCOCH_3$-11), 20.1 (C-21), 13.4 (C-18), 13.1(C-19); HRMS m/z 745.3433 $[M + H]^+$ (calcd for $C_{39}H_{53}O_{14}$, 745.3436).

Compound ***14:*** pale yellow powder; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.90 (1H, s, H-3′), 8.42 (1H, d, J = 7.7 Hz, H-15′), 8.41 (1H, d, J = 7.7 Hz, H-14′), 8.31 (2H, dd, J = 7.7, 3.2 Hz, H-7′ and H-10′), 7.84 (2H, td, J = 7.7, 7.7, 3.2 Hz, H-8′ and H-9′), 5.53 (1H, d, J = 8.0 Hz, H-7), 5.42 (1H, dd, J = 11.6, 2.8 Hz, H-11), 5.28 (1H, d, J = 2.7 Hz, H-12), 5.03 (1H, d, J = 1.6 Hz, H-22), 4.78 (1H, d, J = 5.5 Hz, H-1), 4.56 (1H, t, J = 7.9 Hz, H-15), 3.48 (1H, dd, J = 5.5, 3.6 Hz, H-2), 3.36 (1H, m, H-3), 2.93 (1H, m, H-9), 2.89 (1H, m, H-5), 2.38 (2H, m, H-8 and H-14), 2.27 (1H, m, H-16), 2.21 (1H, m, H-20), 2.20 (3H, s, $OCOCH_3$-1), 2.19 (2H, m, H-4), 2.12 (3H, s, $OCOCH_3$-12), 2.01 (3H, s, $OCOCH_3$-11), 1.80 (1H, dd, J = 13.8, 9.7 Hz, H-17), 1.63 (3H, s, H-27), 1.08 (3H, s, H-28), 1.07 (3H, s, H-18), 0.92 (3H, d, J = 7.3 Hz, H-21), 0.86 (3H, s, H-19); ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.3 (C-6), 182.5 (C-12′), 182.4 (C-5′), 176.5 (C-26), 170.8 (11-$OCOCH_3$), 169.7 (1-$OCOCH_3$), 169.5 (12-$OCOCH_3$), 164.2 (C-1′), 153.5 (C-23), 136.5 (C-13′), 134.8 (C-4′), 134.7 (C-8′, C-9′, and C-15′), 133.8 (C-2′), 133.5 (C-6′ and C-11′), 128.3 (C-3′), 127.9 (C-14′), 127.6 (C-7′ and C-10′), 111.6 (C-22), 79.2 (C-25), 78.7 (C-7), 73.9 (C-12), 72.8 (C-1), 71.0 (C-11), 70.3 (C-15), 57.6 (C-14), 52.4 (C-3), 52.0 (C-16), 49.7 (C-2), 49.5 (C-24), 47.6 (C-17), 44.1 (C-13), 43.0 (C-5), 42.6 (C-10), 41.4 (C-9), 38.9 (C-8), 30.6 (C-20), 24.9 (C-28), 21.4 (C-4), 21.4 ($OCOCH_3$-11), 20.9 (C-27), 20.9 ($OCOCH_3$-12), 20.7 ($OCOCH_3$-1), 20.3 (C-21), 13.9 (C-18), 13.0 (C-19); HRMS m/z 917.2993 $[M + Na]^+$ (calcd for $C_{49}H_{50}O_{16}Na$, 917.2991).

Compound ***15:*** white powder; ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.35 (1H, dd, J = 11.7, 2.6 Hz, H-11), 5.24 (1H, d, J = 2.6 Hz, H-12), 5.21 (1H, d, J = 11.3 Hz, H-7), 5.12 (1H, s, H-22), 4.75 (1H, d, J = 5.5 Hz, H-1), 4.62 (1H, t, J = 8.2, H-15), 3.45 (1H, t, J = 4.5, H-2), 3.36 (1H, m, H-3), 2.83 (1H, m, H-9), 2.80 (1H, m, H-5), 2.28 (1H, m, H-14), 2.24 (1H, m, H-16), 2.20 (2H, m, H-4), 2.18 (1H, m, H-20), 2.17 (1H, m, H-8), 2.16 (3H, s, $OCOCH_3$-1), 2.14 (3H, s, H-27), 2.10 (3H, s, $OCOCH_3$-12), 1.99 (3H, s, $OCOCH_3$-11), 1.88 (1H, dd, J = 13.7, 10.0 Hz, H-17), 1.75 (1H, m, H-2″), 1.49 (1H, m, H-2′) 1.42 (3H, s, H-28), 1.10 (2H, m, H-3′/H-4′), 0.98 (3H, s, H-18), 0.96 (2H, m, H-3″/H-4″), 0.92 (2H, m, H-3′/H-4′), 0.93 (3H, d, J = 7.0 Hz, H-21), 0.86 (2H, m, H-3″/H-4″), 0.80 (3H, s, H-19); ^{13}C NMR (150 MHz, $CDCl_3$) δ 203.6 (C-6), 173.3 (C-1′/C-1″), 171.2 (C-26), 170.5 (11-$OCOCH_3$), 169.9 (1-$OCOCH_3$), 169.6 (12-$OCOCH_3$), 151.8 (C-23), 112.0 (C-22), 86.7 (C-25), 77.0 (C-7), 73.7 (C-12), 72.6 (C-1), 70.9 (C-11), 70.0 (C-15), 57.0 (C-14), 52.7 (C-16), 52.2 (C-3), 51.5 (C-24), 49.7 (C-2), 47.3 (C-17), 44.1 (C-13), 42.8 (C-5), 42.4 (C-10), 41.3 (C-9), 38.6 (C-8), 30.6 (C-20), 25.4 (C-28), 22.4 (C-4′/5′ and C-4″/5″), 22.4 (C-4), 21.1 ($OCOCH_3$-12), 20.9 ($OCOCH_3$-1), 20.6 ($OCOCH_3$-11), 20.1 (C-21), 16.8 (C-27), 13.7 (C-18), 13.4 (C-2′), 12.6 (C-19), 12.3 (C-2″), 9.3 (C-3′/C-3″), 8.3 (C-4′/C-4″); HRMS m/z 797.3386 $[M + H]^+$ (calcd for $C_{42}H_{53}O_{15}$, 797.3384).

Compound ***16:*** white powder; ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.33 (1H, dd, J = 11.5, 2.8 Hz, H-11), 5.24 (1H, d, J = 2.8 Hz, H-12), 5.22 (1H, brd, H-7), 5.12 (1H, s, H-22), 4.77 (1H, d, J = 5.5 Hz, H-1), 4.51 (1H, t, J = 9 Hz, H-15), 3.44 (1H, t, J = 4.6, H-2), 3.34 (1H, m, H-3), 2.83 (1H, t, J = 11.4, H-9), 2.80 (1H, dd, J = 10.6, 5.7 Hz, H-5), 2.63 (1H, m, H-2″), 2.49 (1H, m, H-2′), 2.30 (1H, m, H-14), 2.21 (1H, m, H-16), 2.19 (1H, m, H-20), 2.18 (2H, m, H-4), 2.17 (3H, s, $OCOCH_3$-1), 2.16 (3H, s, H-27), 2.14 (1H, m, H-8), 2.10 (3H, s, $OCOCH_3$-12), 1.99 (3H, s, $OCOCH_3$-11), 1.92 (1H, dd, J = 13.6, 10.4 Hz, H-17), 1.39 (3H, s, H-28), 1.29 (3H, d, J = 7.0 Hz, H-3′/H-4′), 1.21 (3H, d, J = 7.0 Hz, H-3′/H-4′), 1.17 (3H, d, J = 7.0 Hz, H-3″/H-4″), 1.16 (3H, d, J = 7.0 Hz, H-3″/H-4″), 0.98 (3H, s, H-18), 0.93 (3H, d, J = 7.3 Hz, H-21), 0.79 (3H, s, H-19); ^{13}C NMR (150 MHz, $CDCl_3$) δ 202.7 (C-6), 175.1 (C-1′), 172.4 (C-1″), 170.8 (C-26), 170.6 (11-$OCOCH_3$), 169.8 (1-$OCOCH_3$), 169.2 (12-$OCOCH_3$), 152.1 (C-23), 111.8 (C-22), 86.6 (C-25), 76.4 (C-7), 73.2 (C-12), 72.4 (C-1), 70.7 (C-11), 69.6 (C-15), 57.4 (C-14), 52.6 (C-16), 51.7 (C-24), 51.5 (C-3), 49.1 (C-2), 47.2 (C-17), 43.4 (C-13), 42.8 (C-5), 41.9 (C-10), 41.2 (C-9), 38.6 (C-8), 35.2 (C-2″), 33.9 (C-2′), 30.6 (C-20), 25.2 (C-28), 21.7 (C-4), 21.4 ($OCOCH_3$-11), 20.6 ($OCOCH_3$-12), 20.5 (C-21), 19.7 (C-3″), 19.3 ($OCOCH_3$-1), 19.2 (C-3′ and C-4′), 18.3 (C-4″), 16.3 (C-27), 14.5 (C-18), 12.2 (C-19); HRMS m/z 801.3704 $[M + H]^+$ (calcd for $C_{42}H_{57}O_{15}$, 801.3697).

Compound ***17:*** white powder; ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.32 (1H, dd, J = 11.5, 2.5 Hz, H-11), 5.23 (1H, d, J = 2.5 Hz, H-12), 5.20 (1H, d, J = 11.3 Hz, H-7), 5.12 (1H, s, H-22), 4.76 (1H, d, J = 5.5 Hz, H-1), 4.51 (1H, t, J = 8.4, H-15), 3.43 (1H, t, J = 4.5, H-2), 3.34 (1H, m, H-3), 2.82 (1H, m, H-9), 2.80 (1H, m, H-5), 2.35 (2H, m, H-3′ and H-3″), 2.26 (1H, m, H-14), 2.23 (1H, m, H-16), 2.17 (1H, m, H-20), 2.16 (3H, s, $OCOCH_3$-1), 2.15 (3H, s, H-27), 2.14 (2H, m, H-4), 2.13 (1H, m, H-8), 2.12 (2H, m, H-2′a and H-2″a), 2.09 (3H, s, $OCOCH_3$-12), 2.01 (1H, m, H-2′b and H- H-2″b), 1.99 (3H, s, $OCOCH_3$-11), 1.89 (1H, dd, J = 13.6, 9.9 Hz, H-17), 1.38 (3H, s, H-28), 1.05 (3H, d, J = 6.4 Hz, H-4′/H-5′), 1.03 (3H, d, J = 6.4 Hz, H-4′/H-5′), 0.97 (3H, s, H-18), 0.96 (6H, brd, H-4″ and H-5″), 0.93 (3H, d, J = 7.0 Hz, H-21), 0.78 (3H, s, H-19); ^{13}C NMR (150 MHz, $CDCl_3$) δ 202.6 (C-6), 171.3 (C-1′/C-1″), 170.8 (C-26), 170.6 (11-$OCOCH_3$), 169.8 (1-$OCOCH_3$), 169.2 (12-$OCOCH_3$), 152.2 (C-23), 112.6 (C-22), 87.9 (C-25), 76.6 (C-7), 73.7 (C-12), 72.7 (C-1), 70.9 (C-11), 69.8 (C-15), 57.3 (C-14), 52.9 (C-16), 51.9 (C-3), 49.3 (C-2), 49.5 (C-24), 47.1 (C-17), 43.9 (C-13), 43.3 (C-3′/C-3″), 42.8 (C-5), 42.4 (C-10), 41.3 (C-9), 38.6 (C-8), 30.9 (C-20), 25.7 (C-2′/C-2″), 20.1 (C-28), 22.4 (C-4′/5′and C-4″/5″), 22.4 (C-4), 21.1 ($OCOCH_3$-12), 20.4 ($OCOCH_3$-1), 20.1 ($OCOCH_3$-11), 19.9 (C-21), 16.5 (C-27), 13.3 (C-18), 12.4 (C-19); HRMS m/z 829.4012 $[M + H]^+$ (calcd for $C_{44}H_{61}O_{15}$, 829.4010).

Compound ***18:*** pale yellow powder; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.12−7.35 (14H, broad, H-3′, H-3″, H-15′, H-15″, H-14′, H-14″, H-7′, H-7″, H-10′, H-10″, H-8′, H-8″, H-9′, and H-9″), 5.64 (1H, brd, J = 11.1 Hz, H-7), 5.44 (1H, dd, J = 11.5, 2.8 Hz, H-11), 5.36 (1H, d, J

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J. Nat. Prod. XXXX, XXX, XXX−XXX

= 2.8 Hz, H-12), 5.26 (1H, s, H-22), 4.90 (1H, brt, H-15), 4.86 (1H, d, *J* = 5.6 Hz, H-1), 3.48 (1H, dd, *J* = 5.6, 3.7 Hz, H-2), 3.36 (1H, m, H-3), 3.02 (1H, t, *J* = 11.3 Hz, H-9), 2.91 (1H, dd, *J* = 11.2, 4.9 Hz, H-5), 2.55 (1H, m, H-14), 2.53 (1H, m, H-16), 2.40 (1H, m, H-8), 2.31 (1H, m, H-20), 2.25 (3H, s, $OCOCH_3$-1), 2.20 (3H, s, $OCOCH_3$-12), 2.12 (2H, m, H-4), 2.11 (1H, m, H-17), 2.05 (3H, s, $OCOCH_3$-11), 2.00 (3H, s, H-27), 1.74 (3H, s, H-28), 1.18 (3H, s, H-18), 1.02 (3H, d, *J* = 6.9 Hz, H-21), 0.84 (3H, s, H-19); ^{13}C NMR (125 MHz, $CDCl_3$) δ 202.0 (C-6), 182.1−181.1 (4C, C-12′, C-5′, C-12″, and C-5″), C-26 (n.d.), 170.7 (11-$OCOCH_3$), 169.6 (1-$OCOCH_3$), 169.4 (12−$OCOCH_3$), 162.9 (2C, C-1′ and C-1″), 153.1 (C-23), 135.7−132.0 (16C, C-13′, C-13″, C-15′, C-15″, C-4′, C-4″, C-8′, C-8″, C-9′, C-9″, C-2′, C-2″, C-6′, C-6″, C-11′, and C-11″), 128.0−126.9 (8C, C-3′, C-3″, C-7′, C-7″, C-10′, C-10″, C-14′, and C-14″), 116.5 (C-25), 113.0 (C-22), 79.0 (C-7), 73.8 (C-12), 72.8 (C-1), 71.4 (C-11), 70.5 (C-15), 57.1 (C-14), 53.4 (C-16), 52.3 (C-3), 52.3 (C-24), 49.6 (C-2), 48.0 (C-17), 43.6 (C-13), 42.7 (C-5), 42.7 (C-10), 41.6 (C-9), 39.3 (C-8), 31.2 (C-20), 25.9 (C-28), 21.3 (C-4), 21.3 ($OCOCH_3$-12), 21.0 ($OCOCH_3$-11), 20.8 ($OCOCH_3$-1), 20.2 (C-21), 13.9 (C-18), 13.0 (C-19), 2.0 (C-27); HRMS *m*/*z* 1151.3316 $[M + Na]^+$ (calcd for $C_{64}H_{56}O_{19}Na$, 1151.3308).

Compound ***19:*** white powder; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.60 (1H, t, *J* = 9.0 Hz, H-15), 5.30 (1H, dd, *J* = 10.1, 2.5 Hz, H-11), 5.28 (1H, d, *J* = 2.7, H-12), 4.76 (1H, d, *J* = 5.0 Hz, H-1), 4.02 (1H, dd, *J* = 10.2, 3.2 Hz, H-7), 3.75 (1H, d, *J* = 3.2, OH-7), 3.49 (1H, t, *J* = 3.8 Hz, H-2), 3.40 (1H, m, H-3), 3.28 (1H, s, H-22), 2.79 (1H, m, H-5), 2.76 (1H, m, H-9), 2.48 (1H, m, H-14), 2.09 (1H, m, H-16), 2.25 (2H, m, H-4), 2.20 (3H, s, $OCOCH_3$-1), 2.16 (3H, s, $OCOCH_3$-12), 2.01 (3H, s, $OCOCH_3$-11), 2.12 (1H, dd, *J* = 13.8, 10.5, H-17), 1.81 (3H, s, H-27), 1.75 (1H, m, H-8), 1.62 (1H, m, H-20), 1.58 (1H, m, H-2′), 1.34 (3H, s, H-28), 1.05 (3H, d, *J* = 6.95 Hz, H-21), 0.96 (2H, m, H-3′/H-4′), 0.90 (3H, s, H-18), 0.79 (3H, s, H-19), 0.76 (2H, m, H-3′/H-4′); ^{13}C NMR (125 MHz, $CDCl_3$) δ 210.2 (C-6), 177.6 (C-26), 174.9 (C-1′), 171.9 (11-$OCOCH_3$), 169.6 (1-$OCOCH_3$), 169.3 (12-$OCOCH_3$), 91.9 (C-23), 78.7 (C-25), 75.3 (C-7), 73.9 (C-12), 72.8 (C-1), 71.5 (C-15), 71.2 (C-11), 65.9 (C-22), 54.2 (C-14), 52.3 (C-3), 49.9 (C-2), 48.0 (C-16), 46.1 (C-24), 45.0 (C-17), 43.8 (C-13), 43.1 (C-8), 42.9 (C-10), 42.2 (C-5), 40.5 (C-9), 32.1 (C-20), 24.0 (C-27), 21.5 (C-4), 21.1 ($OCOCH_3$-1 and $OCOCH_3$-11), 20.4 ($OCOCH_3$-12), 19.9 (C-28), 18.6 (C-21), 14.3 (C-2′), 13.5 (C-18), 13.1 (C-19), 8.6 (C-3′ and C-4′); HRMS *m*/*z* 745.3063 $[M + H]^+$ (calcd for $C_{38}H_{49}O_{15}$, 745.3071).

Compound ***20:*** white powder; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.58 (1H t, *J* = 9.1 Hz,, H-15), 5.29 (1H, dd, *J* = 11.0, 1.7 Hz, H-11), 5.20 (1H, d, *J* = 1.7, H-12), 4.71 (1H, d, *J* = 5.5 Hz, H-1), 3.91 (1H, dd, *J* = 10.3, 3.6 Hz, H-7), 3.75 (1H, d, *J* = 3.6 Hz, OH-7), 3.46 (1H, t, *J* = 5.0, H-2), 3.36 (1H, m, H-3), 3.25 (1H, s, H-22), 2.76 (1H, m, H-5), 2.67 (1H, m, H-9), 2.60 (1H, m, H-2′), 2.40 (1H, t, *J* = 9.1 Hz, H-14), 2.23 (2H, m, H-4), 2.18 (3H, s, $OCOCH_3$-1), 2.15 (3H, s, $OCOCH_3$-12), 2.08 (1H, m, H-16), 2.02 (1H, m, H-17), 1.99 (3H, s, $OCOCH_3$-11), 1.69 (1H, m, H-8), 1.64 (1H, m, H-20), 1.73 (3H, s, H-27), 1.32 (3H, s, H-28), 1.14 (3H, d, *J* = 7.0 Hz, H-3′), 1.12 (3H, d, *J* = 7.0 Hz, H-4′), 1.01 (3H, d, *J* = 7.7 Hz, H-21), 0.88 (3H, s, H-18), 0.76 (3H, s, H-19); ^{13}C NMR (125 MHz, $CDCl_3$) δ 209.4 (C-6), 177.7 (C-26), 176.8 (C-1′), 170.0 (12−OCOCH3), 169.9 (11−OCOCH3), 169.5 (1−OCOCH3), 92.1 (C-23), 78.6 (C-25), 75.1 (C-7), 73.8 (C-12), 72.8 (C-1), 71.4 (C-15), 70.8 (C-11), 65.6 (C-22), 54.2 (C-14), 52.0 (C-3), 49.8 (C-2), 47.8 (C-16), 46.9 (C-24), 44.7 (C-17), 44.3 (C-13), 43.1 (C-10), 42.7 (C-8), 42.5 (C-5), 40.2 (C-9), 34.7 (C-2′), 31.7 (C-20), 23.7 (C-27), 21.4 (C-4), 21.2 ($OCOCH_3$-11), 20.9 ($OCOCH_3$-1), 20.7 ($OCOCH_3$-12), 19.8 (C-28), 18.9 (C-3′ and C-4′), 18.5 (C-21), 13.4 (C-18), 12.9 (C-19); HRMS *m*/*z* 747.3197 $[M + H]^+$ (calcd for $C_{38}H_{51}O_{15}$, 747.3227).

Compound ***21:*** white powder; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.55 (1H, t, *J* = 9.0 Hz, H-15), 5.27 (1H, dd, *J* = 11.8, 2.6 Hz, H-11), 5.17 (1H, d, *J* = 2.6 Hz, H-12), 4.73 (1H, d, *J* = 5.5 Hz, H-1), 3.97 (1H, dd, *J* = 10.2, 3.3 Hz, H-7), 3.77 (1H, d, *J* = 3.3 Hz, OH-7), 3.47 (1H, t, *J* = 5.0, H-2), 3.37 (1H, m, H-3), 3.25 (1H, s, H-22), 2.76 (1H, m, H-5), 2.70 (1H, m, H-9), 2.44 (1H, t, *J* = 9.8 Hz, H-14), 2.32 (2H, m, H-2′), 2.29 (1H, m, H-17), 2.20 (1H, m, H-4a), 2.02 (1H, m, H-20), 2.17 (3H, s, $OCOCH_3$-1), 2.14 (3H, s, $OCOCH_3$-12), 2.09 (1H, m, H-3′), 2.02 (1H, m, H-16), 2.01 (1H, m, H-4b), 1.99 (3H, s, $OCOCH_3$-11), 1.74 (3H, s, H-27), 1.71 (1H, m, H-8), 1.33 (3H, s, H-28), 1.02 (3H, d, *J* = 6.9 Hz, H-21), 0.93 (6H, d, *J* = 7.0 Hz, H-4′ and H-5′), 0.88 (3H, s, H-18), 0.76 (3H, s, H-19); ^{13}C NMR (125 MHz, $CDCl_3$) δ 209.5 (C-6), 176.7 (C-26), 173.2 (C-1′), 170.6 (11-$OCOCH_3$), 169.2 (1-$OCOCH_3$), 169.0 (12-$OCOCH_3$), 92.2 (C-23), 79.1 (C-25), 74.9 (C-7), 73.6 (C-12), 72.6 (C-1), 71.0 (C-15), 70.8 (C-11), 65.6 (C-22), 54.3 (C-14), 52.2 (C-3), 49.7 (C-2), 47.8 (C-16), 47.2 (C-24), 44.0 (C-10), 43.6 (C-17), 43.4 (C-3′), 43.2 (C-8), 43.1 (C-13), 42.2 (C-5), 40.4 (C-9), 31.7 (C-20), 23.7 (C-27), 24.3 (C-2′), 22.6 (C-4′ and C-5′), 21.3 (C-4), 21.2 ($OCOCH_3$-11), 20.8 ($OCOCH_3$-12), 20.3 ($OCOCH_3$-1), 18.7 (C-21), 19.4 (C-28), 13.1 (C-19), 13.4 (C-19); HRMS *m*/*z* 761.3416 $[M + H]^+$ (calcd for $C_{39}H_{53}O_{15}$, 761.3384).

Compound ***22:*** white powder; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.52 (1H, t, *J* = 9.1 Hz, H-15), 5.26 (1H, dd, *J* = 11.8, 2.8 Hz, H-11), 5.18 (1H, d, *J* = 2.8 Hz, H-12), 4.72 (1H, d, *J* = 5.5 Hz, H-1), 3.94 (1H, dd, *J* = 10.2 Hz, H-7), 3.78 (1H, brd, OH-7), 3.46 (1H, t, *J* = 5.4, H-2), 3.37 (1H, m, H-3), 3.26 (1H, s, H-22), 2.76 (1H, m, H-5), 2.67 (1H, m, H-9), 2.40 (1H, dd, *J* = 9.0, 9.0 Hz, H-14), 2.25 (2H, m, H-4), 2.17 (3H, s, $OCOCH_3$-1), 2.14 (3H, s, $OCOCH_3$-12), 2.07 (1H, m, H-16), 2.04 (1H, m, H-17), 1.98 (3H, s, $OCOCH_3$-11), 1.73 (3H, s, H-27), 1.68 (1H, m, H-8), 1.60 (1H, m, H-20), 1.32 (3H, s, H-28), 1.01 (3H, s, H-3′), 0.99 (3H, d, *J* = 7.7 Hz, H-21), 0.92 (3H, s, H-4′), 0.91 (3H, s, H-5′), 0.87 (3H, s, H-18), 0.76 (3H, s, H-19); ^{13}C NMR (125 MHz, $CDCl_3$) δ 209.5 (C-6), 177.6 (C-26), 173.1 (C-1′), 170.8 (12-$OCOCH_3$), 169.8 (11-$OCOCH_3$), 169.1 (1-$OCOCH_3$), 91.5 (C-23), 79.1 (C-25), 74.9 (C-7), 73.8 (C-12), 72.8 (C-1), 70.9 (C-15), 70.7 (C-11), 65.7 (C-22), 54.1 (C-14), 52.0 (C-3), 49.8 (C-2), 47.7 (C-16), 46.8 (C-24), 44.7 (C-17), 44.3 (C-13), 43.9 (C-2′), 43.3 (C-10), 42.4 (C-8), 40.2 (C-9), 40.1 (C-5), 31.7 (C-20), 23.7 (C-27), 22.8 (C-3′−C-5′), 21.4 (C-4), 21.2 ($OCOCH_3$-11), 20.9 ($OCOCH_3$-1/12), 20.7 ($OCOCH_3$-1/12), 19.7 (C-28), 18.7 (C-21), 13.4 (C-18), 12.6 (C-19); HRMS *m*/*z* 761.3371 $[M + H]^+$ (calcd for $C_{39}H_{53}O_{15}$, 761.3384).

Compound ***23:*** white powder; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.95 (1H, s, H-1′), 5.60 (1H, brt, H-11), 5.32 (1H, dd, *J* = 11.0, 2.3 Hz, H-11), 5.20 (1H, d, *J* = 2.3, Hz, H-12), 4.71 (1H, d, *J* = 5.5 Hz, H-1), 4.02 (1H, dd, *J* = 10.4, 3.8 Hz, H-7), 3.89 (1H, s, OH-7), 3.46 (1H, t, *J* = 4.8, H-2), 3.37 (1H, m, H-3), 3.27 (1H, s, H-22), 2.73 (1H, m, H-5), 2.70 (1H, m, H-9), 2.49 (1H, t, *J* = 10.1 Hz, H-14), 2.20 (2H, m, H-4), 2.16 (3H, s, $OCOCH_3$-1), 2.13 (3H, s, $OCOCH_3$-12), 2.11 (1H, dd, *J* = 13.3, 10.0 Hz, H-16), 2.08 (1H, dd, *J* = 13.1, 8.8 Hz, H-17), 1.98 (3H, s, $OCOCH_3$-11), 1.73 (1H, m, H-8), 1.76 (3H, s, H-27), 1.65 (1H, m, H-20), 1.30 (3H, s, H-28), 1.03 (3H, d, *J* = 7.4 Hz, H-21), 0.88 (3H, s, H-18), 0.77 (3H, s, H-19); ^{13}C NMR (150 MHz, $CDCl_3$) δ 209.3 (C-6), 176.9 (C-26), 170.5 (11−$OCOCH_3$), 169.4 (1−$OCOCH_3$), 169.1 (12−$OCOCH_3$), 92.4 (C-23), 79.1 (C-25), 74.7 (C-7), 73.8 (C-12), 72.6 (C-1), 70.8 (C-11), 70.4 (C-15), 65.8 (C-22), 53.8 (C-14), 52.2 (C-3), 49.7 (C-2), 47.9 (C-16), 46.8 (C-24), 45.3 (C-17), 44.0 (C-13), 43.3 (C-10), 42.9 (C-8), 42.2 (C-9), 40.1 (C-5), 31.8 (C-20), 23.9 (C-27), 21.4 (C-4), 21.2 ($OCOCH_3$-11), 20.5 ($OCOCH_3$-12), 20.2 ($OCOCH_3$-1), 19.5 (C-28), 18.8 (C-21), 13.2 (C-18), 13.0 (C-19); HRMS *m*/*z* 705.2750 $[M + H]^+$ (calcd for $C_{35}H_{45}O_{15}$, 705.2758).

Compound ***24:*** pale yellow powder; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.84 (1H, d, *J* = 1.6 Hz, H-3′), 8.44 (1H, dd, *J* = 8.2, 1.6 Hz, H-15′), 8.36 (1H, d, *J* = 8.1 Hz, H-14′), 8.33 (2H, m, H-7′ and H-10′), 7.85 (2H, m, H-8′ and H-9′), 5.83 (1H, t, *J* = 9.3 Hz, H-15), 5.32 (1H, dd, *J* = 11.5, 2.8 Hz, H-11), 5.26 (1H, d, *J* = 2.7 Hz, H-12), 4.71 (1H, d, *J* = 5.5 Hz, H-1), 3.82 (1H, d, *J* = 10.6 Hz, H-7), 3.46 (1H, dd, *J* = 5.5, 3.7 Hz, H-2), 3.32 (1H, m, H-3), 3.30 (1H, s, H-22), 2.77 (1H, t, *J* = 11.4 Hz, H-9), 2.70 (1H, dd, *J* = 10.8, 5.2 Hz, H-5), 2.60 (1H, t, *J* = 10.2 Hz, H-14), 2.30 (3H, s, $OCOCH_3$-1), 2.30 (3H, s, $OCOCH_3$-12), 2.26 (1H, dd, *J* = 13.4, 9.8 Hz, H-16), 2.15 (1H, m, H-17), 2.10 (2H, m, H-4), 2.01 (3H, s, $OCOCH_3$-11), 1.84 (3H, s, H-27), 1.73 (1H, m, H-8), 1.63 1H, (m, H-20), 1.38 (3H, s, H-28), 1.07 (3H, d, *J* = 7.4 Hz, H-21), 0.96 (3H, s, H-18), 0.75 (3H, s, H-19); ^{13}C NMR (100 MHz, $CDCl_3$) δ 209.2 (C-6), 183.4 (C-5′), 182.6 (C-12′), 177.1 (C-26), 170.9 (11-$OCOCH_3$), 169.9 (1-$OCOCH_3$), 169.5 (12-$OCOCH_3$),

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165.5 (C-1′), 137.2 (C-4′), 135.7 (C-13′), 135.2 (C-15′), 134.8 (C-8′), 134.6 (C-9′), 133.6 (C-2′), 133.5 (C-6′, 11′), 127.8 (C-3′), 127.6 (C-14′), 127.6 (C-7′), 127.4 (C-10′), 92.2 (C-23), 79.2 (C-25), 75.4 (C-7), 73.8 (C-12), 73.1 (C-15), 72.8 (C-1), 70.8 (C-11), 66.1 (C-11), 54.0 (C-14), 52.2 (C-3), 49.8 (C-2), 48.1 (C-16), 46.7 (C-24), 45.6 (C-17), 43.7 (C-13), 43.2 (C-8), 43.1 (C-10), 42.3 (C-5), 39.9 (C-9), 32.1 (C-20), 24.1 (C-27), 21.4 (C-4), 21.4 ($OCOCH_3$-11), 21.4 ($OCOCH_3$-12), 21.0 ($OCOCH_3$-1), 20.1 (C-28), 18.8 (C-21), 13.3 (C-18), 13.1 (C-19); HRMS *m/z* 933.2949 $[M + Na]^+$ (calcd for $C_{49}H_{50}O_{17}Na$, 933.2940).

Compound ***25:*** white powder; ^1H NMR (600 MHz, $CDCl_3$) δ 5.31 (1H, dd, *J* = 11.6, 2.8 Hz, H-11), 5.19 (1H, brd, H-7), 5.16 (1H, d, *J* = 2.7, Hz, H-12), 4.73 (1H, d, *J* = 5.5 Hz, H-1), 4.37 (1H, t, *J* = 8.4 Hz, H-15), 4.05 (1H, s, OH-15), 3.45 (1H, dd, *J* = 5.5, 3.7, H-2), 3.36 (1H, m, H-3), 3.26 (1H, s, H-22), 2.80 (1H, m, H-9), 2.78 (1H, m, H-5), 2.67 (1H, m, H-2′), 2.28 (1H, m, H-14), 2.19 (2H, m, H-4), 2.17 (3H, s, $OCOCH_3$-1), 2.16 (1H, m, H-8), 2.12 (3H, s, $OCOCH_3$-12), 2.04 (1H, m, H-17), 1.99 (3H, s, $OCOCH_3$-11), 1.96 (1H, m, H-16), 1.76 (3H, s, H-27), 1.64 (1H, m, H-20), 1.29 (3H, s, H-28), 1.27 (3H, d, *J* = 7.0 Hz, H-3′/H-4′), 1.23 (3H, d, *J* = 7.0 Hz, H-3′/H-4′), 1.01 (3H, d, *J* = 7.3 Hz, H-21), 0.88 (3H, s, H-18), 0.80 (3H, s, H-19); ^{13}C NMR (125 MHz, $CDCl_3$) δ 202.2 (C-6), 177.5 (C-1′), 174.9 (C-26), 170.7 (11−$OCOCH_3$), 169.4 (1−$OCOCH_3$), 169.1 (12−$OCOCH_3$), 91.3 (C-23), 78.9 (C-25), 77.5 (C-7), 73.8 (C-12), 72.6 (C-1), 70.8 (C-11), 70.5 (C-15), 65.7 (C-22), 57.1 (C-14), 52.1 (C-3), 49.8 (C-2), 47.8 (C-16), 46.5 (C-24), 44.2 (C-17), 44.0 (C-13), 42.6 (C-5), 41.7 (C-10), 41.3 (C-9), 38.5 (C-8), 34.8 (C-2′), 31.5 (C-20), 23.9 (C-27), 21.4 (C-4), 21.1 ($OCOCH_3$-12), 20.8 ($OCOCH_3$-1), 20.6 ($OCOCH_3$-11), 19.6 (C-28), 19.1 (C-3′/5′), 18.9 (C-3′/5′), 18.7 (C-21), 13.3 (C-18), 13.0 (C-19); HRMS *m/z* 747.2915 $[M + H]^+$ (calcd for $C_{38}H_{51}O_{15}$, 747.3227).

Compound ***26:*** white powder; ^1H NMR (600 MHz, $CDCl_3$) δ 5.30 (1H, dd, *J* = 11.6, 2.6 Hz, H-11), 5.20 (1H, brd, H-7), 5.16 (1H, d, *J* = 2.6, Hz, H-12), 4.73 (1H, d, *J* = 5.5 Hz, H-1), 4.37 (1H, t, *J* = 8.3 Hz, H-15), 3.45 (1H, dd, *J* = 5.5, 3.7, H-2), 3.36 (1H, m, H-3), 3.26 (1H, s, H-22), 2.80 (1H, m, H-9), 2.78 (1H, m, H-5), 2.34 (2H, m, H-2′), 2.26 (1H, m, H-14), 2.20 (1H, m, H-3′), 2.19 (2H, m, H-4), 2.18 (1H, m, H-8), 2.17 (3H, s, $OCOCH_3$-1), 2.12 (3H, s, $OCOCH_3$-12), 2.02 (1H, m, H-17), 1.99 (3H, s, $OCOCH_3$-11), 1.96 (1H, m, H-16), 1.76 (3H, s, H-27), 1.64 (1H, m, H-20), 1.30 (3H, s, H-28), 1.03 (3H, d, *J* = 6.6 Hz, H-4′/H-5′), 1.02 (3H, d, *J* = 6.6 Hz, H-4′/H-5′), 1.01 (3H, d, *J* = 6.5 Hz, H-21), 0.87 (3H, s, H-18), 0.80 (3H, s, H-19); ^{13}C NMR (125 MHz, $CDCl_3$) δ 202.3 (C-6), 174.8 (C-26), 172.1 (C-1′), 170.6 (11-$OCOCH_3$), 169.3 (1-$OCOCH_3$), 169.1 (12-$OCOCH_3$), 91.4 (C-23), 79.1 (C-25), 77.4 (C-7), 73.6 (C-12), 72.6 (C-1), 70.8 (C-11), 70.4 (C-15), 65.5 (C-22), 57.1 (C-14), 52.1 (C-3), 49.7 (C-2), 47.9 (C-16), 46.5 (C-24), 44.0 (C-17), 44.2 (C-13), 42.7 (C-5), 43.1 (C-3′), 42.7 (C-5), 42.2 (C-10), 41.2 (C-9), 38.3 (C-8), 31.4 (C-20), 25.3 (C-2′), 23.9 (C-27), 22.5 (C-4′ and C-5′), 21.4 (C-4), 21.1 ($OCOCH_3$-12), 20.8 ($OCOCH_3$-1), 20.6 ($OCOCH_3$-11), 19.7 (C-28), 18.7 (C-21), 13.3 (C-18), 13.0 (C-19); HRMS *m/z* 761.3379 $[M + H]^+$ (calcd for $C_{39}H_{53}O_{15}$, 761.3384).

Compound ***27:*** white powder; ^1H NMR (600 MHz, $CDCl_3$) δ 5.30 (1H, dd, *J* = 11.2, 2.7 Hz, H-11), 5.25 (1H, d, *J* = 11.5 Hz, H-7), 5.15 (1H, d, *J* = 2.7 Hz, H-12), 4.73 (1H, d, *J* = 5.6 Hz, H-1), 4.38 (1H, brt, H-15), 4.05 (1H, s, OH-15), 3.45 (1H, t, *J* = 5.4 Hz, H-2), 3.35 (1H, m, H-3), 3.26 (1H, d, *J* = 1.4, Hz, H-22), 2.83 (1H, m, H-9), 2.79 (1H, m, H-5), 2.29 (1H, m, H-14), 2.18 (3H, s, $OCOCH_3$-1), 2.17 (2H, m, H-4), 2.14 (1H, m, H-8), 2.13 (3H, s, $OCOCH_3$-12), 2.06 (1H, dd, *J* = 13.8, 10.1 Hz, H-17), 1.99 (3H, s, $OCOCH_3$-11), 1.96 (1H, m, H-16), 1.75 (3H, s, H-27), 1.64 (1H, m, H-20), 1.29 (9H, s, H-3′, H-4′, and H-5′), 1.28 (3H, s, H-28), 1.00 (3H, s, H-18), 0.89 (3H, d, *J* = 7.3 Hz, H-21), 0.79 (3H, s, H-19); ^{13}C NMR (125 MHz, $CDCl_3$) δ 202.1 (C-6), 177.5 (C-1′), 175.3 (C-26), 170.8 (11-$OCOCH_3$), 169.8 (1-$OCOCH_3$), 169.5 (12−$OCOCH_3$), 90.8 (C-23), 79.6 (C-25), 77.6 (C-7), 73.8 (C-12), 72.8 (C-1), 70.8 (C-11), 70.5 (C-15), 65.7 (C-22), 57.4 (C-14), 52.1 (C-3), 49.8 (C-2), 47.9 (C-16), 47.9 (C-24), 44.2 (C-17), 44.2 (C-13), 42.9 (C-5), 41.8 (C-10), 41.3 (C-9), 39.3 (C-2′), 38.4 (C-8), 31.1 (C-20), 27.3 (C-3′, C-4′, and C-5′), 24.2 (C-27), 22.5 (C-4), 21.1 ($OCOCH_3$-12), 20.6 ($OCOCH_3$-1), 20.5 ($OCOCH_3$-11), 19.5 (C-28), 18.5 (C-21), 13.1 (C-18), 12.9 (C-19); HRMS *m/z* 761.3378 $[M + H]^+$ (calcd for $C_{39}H_{53}O_{15}$, 761.3384).

Compound ***28:*** pale yellow powder; ^1H NMR (400 MHz, $CDCl_3$) δ 8.88 (1H, s, H-3′), 8.42 (1H, d, *J* = 7.7 Hz, H-15′), 8.41 (1H, d, *J* = 7.7 Hz, H-14′), 8.33 (2H, dd, *J* = 7.7, 3.2 Hz, H-7′ and H-10′), 7.85 (2H, td, *J* = 7.7, 7.7, 3.2 Hz, H-8′ and H-9′), 5.53 (1H, d, *J* = 9.9 Hz, H-7), 5.37 (1H, dd, *J* = 11.0, 2.8 Hz, H-11), 5.20 (1H, d, *J* = 2.8 Hz, H-12), 4.76 (1H, d, *J* = 5.5 Hz, H-1), 4.47 (1H, t, *J* = 7.3 Hz, H-15), 3.46 (1H, dd, *J* = 5.6, 3.8 Hz, H-2), 3.35 (1H, m, H-3), 3.23 (1H, s, H-22), 2.92 (1H, m, H-9), 2.87 (1H, m, H-5), 2.35 (2H, m, H-8 and H-14), 2.19 (3H, s, $OCOCH_3$-1), 2.14 (3H, s, $OCOCH_3$-12), 2.15 (2H, m, H-4), 2.03 (1H, m, H-17), 2.00 (3H, s, $OCOCH_3$-11), 1.96 (1H, m, H-16), 1.72 (3H, s, H-27), 1.63 (1H, m, H-20), 1.04 (3H, s, H-28), 1.04 (3H, d, *J* = 7.3 Hz, H-21), 0.97 (3H, s, H-18), 0.85 (3H, s, H-19); ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.9 (C-6), 182.5 (C-12′), 182.3 (C-5′), 175.4 (C-26), 170.8 (11-$OCOCH_3$), 169.6 (1-$OCOCH_3$), 169.4 (12-$OCOCH_3$), 164.3 (C-1′), 153.5 (C-23), 136.6 (C-13′), 134.5 (C-4′), 134.7 (C-8′, C-9′, and C-15′), 133.8 (C-2′), 133.5 (C-6′ and C-11′), 128.3 (C-3′), 127.9 (C-14′), 127.6 (C-7′, 10′), 91.6 (C-23), 79.1 (C-25), 78.9 (C-7), 73.7 (C-12), 72.8 (C-1), 71.0 (C-11), 70.5 (C-15), 65.5 (C-22), 57.0 (C-14), 52.3 (C-3), 49.7 (C-2), 48.2 (C-16), 46.4 (C-24), 44.4 (C-13), 44.3 (C-17), 43.0 (C-5), 42.7 (C-10), 41.3 (C-9), 38.8 (C-8), 31.6 (C-20), 23.9 (C-27), 21.4 (C-4), 21.3 ($OCOCH_3$-11), 21.0 ($OCOCH_3$-12), 20.7 ($OCOCH_3$-1), 19.4 (C-28), 18.8 (C-21), 13.7 (C-18), 13.0 (C-19); HRMS *m/z* 933.2942 $[M + Na]^+$ (calcd for $C_{49}H_{50}O_{17}Na$, 933.2940).

Compound ***29:*** white powder; ^1H NMR (500 MHz, $CDCl_3$) δ 5.33 (1H, dd, *J* = 11.6, 2.1 Hz, H-11), 5.24 (1H, d, *J* = 10.7 Hz, H-7), 5.17 (1H, d, *J* = 2.1, Hz, H-12), 4.75 (1H, d, *J* = 5.3 Hz, H-1), 4.52 (1H, t, *J* = 8.1 Hz, H-15), 3.46 (1H, t, *J* = 5.2, H-2), 3.37 (1H, m, H-3), 3.29 (1H, s, H-22), 2.84 (1H, m, H-9), 2.82 (1H, m, H-5), 2.25 (1H, m, H-14), 2.20 (2H, m, H-4), 2.19 (2H, m, H-8), 2.18 (3H, s, $OCOCH_3$-1), 2.17 (3H, s, H-27), 2.13 (3H, s, $OCOCH_3$-12), 2.08 (1H, m, H-17), 2.01 (3H, s, $OCOCH_3$-11), 1.96 (1H, m, H-16), 1.75 (1H, m, H-2″), 1.68 (1H, m, H-20), 1.54 (1H, m, H-2′), 1.41 (3H, s, H-28), 1.12 (2H, m, H-3″/H-4″), 1.07 (2H, m, H-3′/H-4′), 1.03 (3H, d, *J* = 7.3 Hz, H-21), 0.98 (2H, m, H-3″/H-4″), 0.90 (2H, m, H-3′/H-4′), 0.89 (3H, s, H-18), 0.81 (3H, s, H-19); ^{13}C NMR (125 MHz, $CDCl_3$) δ 202.9 (C-6), 173.3 (C-1′), 171.3 (C-1″), 170.9 (C-26), 170.9 (11-$OCOCH_3$), 169.9 (1-$OCOCH_3$), 169.1 (12-$OCOCH_3$), 91.5 (C-23), 86.8 (C-25), 77.1 (C-7), 73.8 (C-12), 72.5 (C-1), 70.7 (C-11), 69.4 (C-15), 65.5 (C-22), 56.5 (C-14), 52.1 (C-3), 49.7 (C-2), 49.3 (C-16), 47.8 (C-24), 44.2 (C-17), 43.9 (C-13), 42.9 (C-5), 42.6 (C-10), 41.1 (C-9), 38.8 (C-8), 30.9 (C-20), 21.8 (C-4), 21.5 (C-27), 21.1 ($OCOCH_3$-11), 20.8 ($OCOCH_3$-12), 20.4 ($OCOCH_3$-1), 19.8 (C-28), 18.7 (C-21), 13.4 (C-18), 12.9 (C-2′), 12.7 (C-19), 12.1 (C-2″), 9.6 (C-3′/C-3″), 8.23 (C-4′/C-4″); HRMS *m/z* 813.3318 $[M + H]^+$ (calcd for $C_{42}H_{53}O_{16}$, 813.3333).

Compound ***30:*** white powder; ^1H NMR (500 MHz, $CDCl_3$) δ 5.31 (1H, dd, *J* = 11.6, 2.7 Hz, H-11), 5.22 (1H, d, *J* = 11.3 Hz, H-7), 5.15 (1H, d, *J* = 2.7, Hz, H-12), 4.74 (1H, d, *J* = 5.5 Hz, H-1), 4.38 (1H, t, *J* = 8.9 Hz, H-15), 3.44 (1H, dd, *J* = 5.4, 3.7, H-2), 3.34 (1H, m, H-3), 3.27 (1H, s, H-22), 2.82 (1H, dd, *J* = 11.3, 9.5 Hz, H-9), 2.80 (1H, m, H-5), 2.61 (1H, m, H-2′), 2.52 (1H, m, H-2″), 2.23 (1H, dd, *J* = 10.9, 7.9 Hz, H-14), 2.18 (3H, s, H-27), 2.17 (3H, s, $OCOCH_3$-1), 2.15 (2H, m, H-4), 2.12 (1H, m, H-8), 2.11 (3H, s, $OCOCH_3$-12), 2.10 (1H, m, H-17), 1.99 (3H, s, $OCOCH_3$-11), 1.96 (1H, dd, *J* = 13.8, 9.0 Hz, H-16), 1.66 (1H, m, H-20), 1.36 (3H, s, H-28), 1.27 (3H, d, *J* = 7.0 Hz, H-3′/H-4′), 1.21 (3H, d, *J* = 7.0 Hz, H-3′/H-4′), 1.19 (3H, d, *J* = 7.0 Hz, H-3″/H-4″), 1.17 (3H, d, *J* = 7.0 Hz, H-3″/H-4″), 1.02 (3H, d, *J* = 7.3 Hz, H-21), 0.88 (3H, s, H-18), 0.78 (3H, s, H-19); ^{13}C NMR (150 MHz, $CDCl_3$) δ 202.6 (C-6), 175.2 (C-1′), 173.3 (C-1″), 170.6 (11-$OCOCH_3$), 170.1 (C-26), 169.8 (1-$OCOCH_3$), 169.2 (12-$OCOCH_3$), 91.2 (C-23), 85.8 (C-25), 76.8 (C-7), 73.6 (C-12), 72.6 (C-1), 70.9 (C-11), 69.7 (C-15), 65.5 (C-22), 56.9 (C-14), 52.1 (C-3), 49.4 (C-2), 49.1 (C-16), 47.7 (C-24), 44.3 (C-17), 43.8 (C-13), 42.4 (C-5), 42.1 (C-10), 41.2 (C-9), 38.3 (C-8), 35.2 (C-2″), 33.9 (C-2′), 31.3 (C-20), 21.5 (C-4), 21.3 ($OCOCH_3$-11), 20.6 ($OCOCH_3$-12), 20.2 (C-28), 20.1 (C-27), 19.7 (C-3′), 19.3 ($OCOCH_3$-1), 19.2 (C-3″ and C-4″), 18.5 (C-21), 18.3 (C-4′), 16.3 (C-27), 13.8 (C-18),

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12.7 (C-19); HRMS m/z 817.3690 $[M + H]^+$ (calcd for $C_{42}H_{57}O_{16}$, 817.3646).

Compound ***31****:* white powder; ^1H NMR ($CDCl_3$, 500 MHz) 5.28 (1H, dd, J = 11.7, 2.5 Hz, H-11), 5.19 (1H, d, J = 11.3 Hz, H-7), 5.14 (1H, d, J = 2.5, Hz, H-12), 4.74 (1H, d, J = 5.5 Hz, H-1), 4.38 (1H, t, J = 8.4 Hz, H-15), 3.45 (1H, t, J = 4.5, H-2), 3.35 (1H, m, H-3), 3.27 (1H, s, H-22), 2.82 (1H, m, H-9), 2.80 (1H, m, H-5), 2.35 (2H, m, H-3′ and H-3″), 2.26 (1H, m, H-14), 2.20 (2H, m, H-4), 2.18 (2H, m, H-2′a and H-2″a), 2.17 (3H, s, $OCOCH_3$-1), 2.17 (3H, s, H-27), 2.12 (1H, m, H-8), 2.11 (3H, s, $OCOCH_3$-12), 2.04 (1H, m, H-17), 2.08 (1H, m, H-2′b and H-2″b), 1.99 (3H, s, $OCOCH_3$-11), 1.94 (1H, m, H-16), 1.66 (1H, m, H-20), 1.36 (3H, s, H-28), 1.04 (3H, d, J = 7.3 Hz, H-21), 1.01 (6H, d, J = 6.7 Hz, H-4′ and H-4″), 0.97 (6H, d, J = 6.7 Hz, H-5′ and H-5″), 0.86 (3H, s, H-18), 0.77 (3H, s, H-19); ^{13}C NMR (125 MHz, $CDCl_3$) δ 202.6 (C-6), 171.5 (C-1′/C-1″), 170.9 (C-26), 170.6 (11-$OCOCH_3$), 169.8 (1-$OCOCH_3$), 169.2 (12-$OCOCH_3$), 91.0 (C-23), 85.8 (C-25), 76.7 (C-7), 73.8 (C-12), 72.6 (C-1), 71.1 (C-11), 69.7 (C-15), 65.6 (C-22), 56.8 (C-14), 52.3 (C-3), 49.6 (C-2), 49.1 (C-16), 48.5 (C-24), 44.1 (C-13), 43.9 (C-17), 43.1 (C-3′/C-3″), 42.6 (C-5), 42.4 (C-10), 41.0 (C-9), 38.2 (C-8), 30.9 (C-20), 25.3 (C-2′/C-2″), 25.4 (C-28), 22.9 (C-4′/5′and C-4″/5″), 22.3 (C-4), 21.1 ($OCOCH_3$-12), 20.8 (C-27), 20.6 ($OCOCH_3$-1), 20.5 ($OCOCH_3$-11), 18.7 (C-21), 13.3 (C-18), 12.8 (C-19); HRMS m/z 845.3960 $[M + H]^+$ (calcd for $C_{44}H_{61}O_{16}$, 845.3959).

Compound ***32****:* pale yellow powder; ^1H NMR (400 MHz, $CDCl_3$) δ 8.19−7.60 (14H, broad, H-3′, H-3″, H-15′, H-15″, H-14′, H-14″, H-7′, H-7″, H-10′, H-10″, H-8′, H-8″, H-9′, and H-9″), 5.63 (1H, brd, J = 11.2 Hz, H-7), 5.41 (1H, dd, J = 11.5, 2.8 Hz, H-11), 5.28 (1H, d, J = 2.7 Hz, H-12), 4.83 (1H, d, J = 5.5 Hz, H-1), 4.76 (1H, brt, J = 9.0 Hz, H-15), 3.48 (1H, dd, J = 5.6, 3.7 Hz, H-2), 3.36 (1H, m, H-3), 3.36 (1H, s, H-22), 3.01 (1H, t, J = 11.3 Hz, H-9), 2.93 (1H, dd, J = 11.0, 5.3 Hz, H-5), 2.53 (1H, m, H-14), 2.38 (1H, m, H-8), 2.28 (1H, m, H-17), 2.26 (3H, s, $OCOCH_3$-1), 2.21 (3H, s, $OCOCH_3$-12), 2.17 (1H, m, H-16), 2.17 (2H, m, H-4), 2.05 (3H, s, $OCOCH_3$-11), 2.00 (3H, s, H-27), 1.77 (1H, m, H-20), 1.66 (3H, s, H-28), 1.11 (3H, d, J = 7.3 Hz, H-21), 1.07 (3H, s, H-18), 0.85 (3H, s, H-19); ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.9 (C-6), 182.0−180.7 (4C, C-12′, C-5′, C-12″, and C-5″), C-26 (n.d.), 171.0 (11-$OCOCH_3$), 169.6 (1-$OCOCH_3$), 169.3 (12-$OCOCH_3$), 163.2 and 162.4 (2C, C-1′ and C-1″), 135.7−132.0 (16C, C-13′, C-13″, C-15′, C-15″, C-4′, C-4″, C-8′, C-8″, C-9′, C-9″, C-2′, C-2″, C-6′, C-6″, C-11′, and C-11″), 128.1−126.9 (8C, C-3′, C-3″, C-7′, C-7″, C-10′, C-10″, C-14′, and C-14″), 116.4 (C-25), 113.0 (C-22), 91.7 (C-23), 79.0 (C-7), 73.8 (C-12), 72.8 (C-1), 71.2 (C-11), 70.2 (C-15), 66.0 (C-22), 56.5 (C-14), 52.3 (C-3), 50.0 (C-16), 49.7 (C-2), 48.5 (C-24), 44.6 (C-17), 44.0 (C-13), 42.9 (C-5), 42.7 (C-10), 41.5 (C-9), 39.1 (C-8), 31.9 (C-20), 25.9 (C-28), 21.4 (C-4), 21.3 ($OCOCH_3$-12), 21.1 ($OCOCH_3$-11), 20.6 ($OCOCH_3$-1), 18.9 (C-21), 13.6 (C-18), 13.0 (C-19), 2.0 (C-27); HRMS m/z 1167.3274 $[M + Na]^+$ (calcd for $C_{64}H_{56}O_{20}Na$, 1167.3257).

Biological Assays. The antiproliferative potencies of the taccalonolides were evaluated using the sulforhodamine B assay.[24] Briefly, HeLa cells (purchased from ATCC, Manassas, VA, USA, and validated by STR Profiling at Genetica DNA Laboratories, Burlington, NC, USA) were cultured in BME (Sigma) containing 10% fetal bovine serum (Hyclone, GE Healthcare, Pittsburgh, PA, USA) and 2.5% gentamycin (Life Technologies, Carlsbad, CA, USA). Cells were plated at a density of 2500 cells per well in 96-well plates and allowed to adhere overnight prior to compound addition. Cells were exposed to the compounds for 48 h, and then IC_{50} values—the concentration that causes a 50% inhibition of cell density as compared to vehicle control—were calculated using nonlinear regression with Prism 6 (Graphpad, La Jolla, CA, USA). IC_{50} values are the average of 3 or 4 experiments, each performed in triplicate, ±SD. The mitotic and interphase microtubules of HeLa cells treated for 18 h with 5-fold the respective IC_{50} of each compound were visualized by indirect immunofluorescence techniques using a β-tubulin antibody (Sigma #T-4026) and a Nikon Eclipse 80i fluorescence microscope with NIS Elements software (Shinagawa, Tokyo, Japan). Cell-based and biochemical assays that demonstrate the same mechanism of microtubule stabilization and extensive SAR in this and previous articles strongly indicate that the taccalonolides are not assay interference compounds.

Tubulin Polymerization. The effect of the taccalonolides on the rate and extent of tubulin polymerization was evaluated turbidimetrically in a Spectramax plate reader (Molecular Devices, Sunnyvale, CA, USA) using purified porcine brain tubulin (Cytoskeleton, Denver, CO, USA). The indicated concentrations of each taccalonolide or vehicle (1% EtOH) were incubated with tubulin (2 mg/mL) in GPEM buffer (80 mM Pipes pH 6.8, 1 mM $MgCl_2$, 1 mM EGTA) containing 10% glycerol and 1 mM GTP in a final volume of 100 μL. Samples were prepared on ice and warmed to 37 °C to initiate tubulin polymerization.

In Vivo Antitumor Evaluations. NCI/ADR-RES tumor fragments were transplanted s.c. into the flanks of female athymic nude mice (Envigo/Harlan, Huntingdon, Cambridgeshire, UK). When tumors reached an average of 250 mm^3 (100−465 mm^3 range), the mice were placed into one of four treatment groups: vehicle (n = 8), paclitaxel (n = 7), **21** (n = 9), or **26** (n = 9). Tumors were injected directly with 200 μL of either vehicle (10% EtOH in PBS), paclitaxel (40 μg in 1% Cremophor/EtOH in PBS), **21** (20 μg in 10% EtOH in PBS), or **26** (20 μg in 10% EtOH in PBS) on days 0, 3, 6, 10, and 14. Tumor volume was calculated using calipers and the following formula: tumor volume (mm^3) = length (mm) × width (mm) × height (mm). Tumor volumes over time were graphed in Prism 6 (Graphpad), and statistical significance between treatment groups was calculated using a two-way ANOVA with a Tukey's post hoc test to compare among treatments over the time course of the treatment period. Mice were monitored for weight loss or other signs of toxicity throughout the trial, maintained in an AAALAC approved facility, provided food and water ad libitum, and euthanized when tumors approached 2000 mm^3. Animal experiments were performed at UT Health under IACUC protocol 12018x.

Hydrolytic Stability of 24. Compound **24** (10 mg) was stirred in a mixture of EtOH and 1× PBS (1:1) for 36 h at room temperature. The reaction mixture was dried down in a vacuum, extracted with 1 mL of CH_3CN and subjected to semipreparative HPLC using a Phenomenex Gemini 5 μm C_{18} 250 × 10 mm column eluted with 60−100% CH_3CN/H_2O (with 0.1% formic acid) to yield compound **4** (5.1 mg, 67.4% yield).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.7b00967.

Spectra (PDF)

Molecular formula strings spreadsheet (XLS)

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Author Contributions

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Notes

The authors declare the following competing financial interest(s): A.R.B.O., A.L.R., J.P., L.D., R.H.C., and S.L.M. are listed as authors on a pending patent application on the taccalonolides.

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■ DEDICATION

Dedicated to Dr. Susan Band Horwitz, of Albert Einstein College of Medicine, Bronx, NY, for her pioneering work on bioactive natural products.

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