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Synthetic Reactions with Rare Taccalonolides Reveal the Value of C-22,23 Epoxidation for Microtubule Stabilizing Potency

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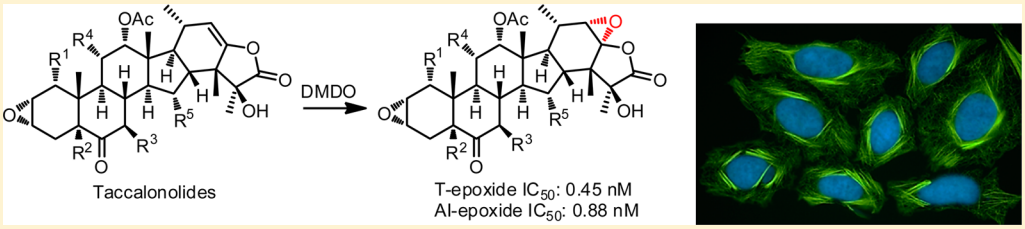
Supporting Information



ABSTRACT: The taccalonolides are microtubule stabilizers isolated from plants of the genus *Tacca*. Taccalonolide AF is 231 times more potent than the major metabolite taccalonolide A and differs only by the oxidation of the C-22,23 double bond in A to an epoxy group in AF. In the current study, 10 other rare natural taccalonolides were epoxidized and in each case epoxidation improved potency. The epoxidation products of taccalonolide T and AI were the most potent, with IC_{50} values of 0.43 and 0.88 nM, respectively. These potent taccalonolides retained microtubule stabilizing effects, and T-epoxide demonstrated antitumor effects in a xenograft model of breast cancer. Additional reactions demonstrated that reduction of the C-6 ketone resulted in an inactive taccalonolide and that C-22,23 epoxidation restored its activity. These studies confirm the value of C-22,23 epoxidation as an effective strategy for increasing the potency of a wide range of structurally diverse taccalonolide microtubule stabilizers.

INTRODUCTION

Microtubules remain an important target for anticancer drug discovery.[1] Paclitaxel, the first microtubule stabilizer identified from the *Taxus brevifolia*, is one of the most successful anticancer drugs currently used in the clinic. Members of a second class of plant-derived microtubule stabilizers, the taccalonolides, have been isolated from a variety of *Tacca* species.[2−11] The taccalonolides are highly acetylated hexacyclic steroid lactones that exhibit effects similar to other microtubule stabilizers in that they increase the density of cellular microtubules, interrupt mitotic progression, and consequentially lead to the apoptosis of cancer cells.[12] Despite their low antiproliferative potencies *in vitro*, taccalonolides A and E were found to be potent and effective antitumor agents *in vivo*[13,14] with the ability to circumvent multiple mechanisms of drug resistance, including mutations in the taxane binding site and the expression of MRP7, βIII-tubulin, and P-glycoprotein (Pgp).[14,15] The recent isolation of taccalonolides with low nanomolar potency facilitated biochemical and structural studies demonstrating that the taccalonolides bind to tubulin covalently and impart unique interprotofilament stability to microtubules.[16] The most potent of these rare natural taccalonolides, AF (**1**), contains an epoxy group at C-22,23 that results in a 231-fold increase in potency compared with taccalonolide A (**2**), which contains a double bond at this site.[17] Taccalonolide AF is an effective antitumor agent that causes tumor regression in the MDA-MB-231 breast cancer xenograft model, albeit with a narrow therapeutic window.[16] A simple and efficient method was developed to semisynthetically epoxidize the C-22,23 double bond in taccalonolides A and B (**3**) to generate taccalonolides AF and AJ (**4**), respectively.[17] Taccalonolide AJ has an IC_{50} value of 4.2 nM, which is 734-fold more potent than the parent molecule, taccalonolide B, suggesting that epoxidation of the C-22,23 double bond is an effective way to increase the potency of this class of molecules. In the current study, the C-22,23 double bond in an additional 10 rare natural microtubule stabilizing taccalonolides was epoxidized, including the newly isolated taccalonolide AI (**5**). Our results demonstrate that this modification increases the potency of each taccalonolide, in some cases leading to subnanomolar potency. These new epoxidized taccalonolides retain microtubule stabilizing activity and some have antitumor efficacy.

RESULTS AND DISCUSSION

A number of taccalonolides, designated A−Y, were previously isolated from various *Tacca sp.* by multiple investigators.[12] We have continued to search for additional rare natural taccalonolides and conduct semisynthetic reactions to fully understand the SAR of this class of compounds with the goal of identifying taccalonolides with optimal properties for consideration for clinical development.

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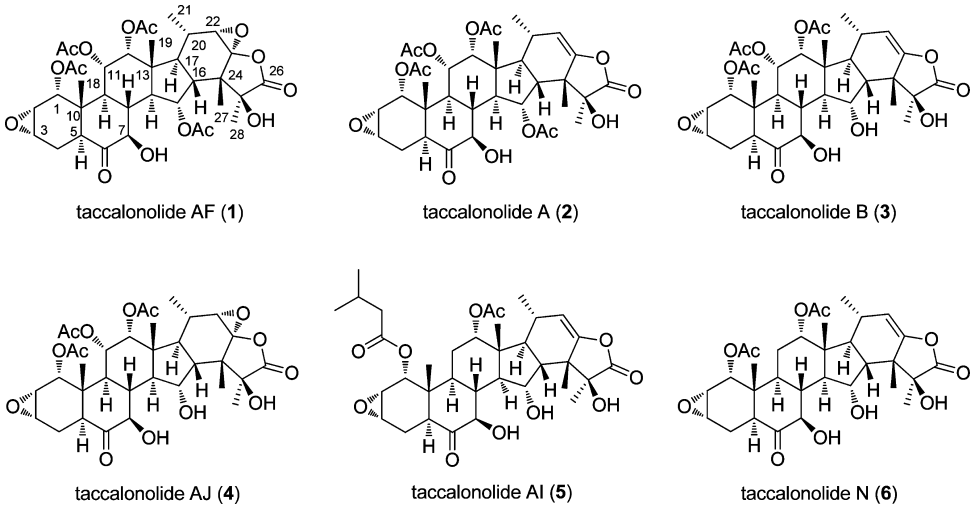


Figure 1. Structures of taccalonolides AF, A, B, AJ, AI, and N.

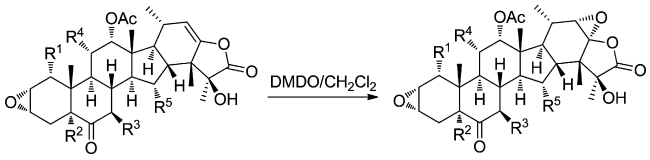
Isolation and Structure Elucidation of 5. The potent semisynthetic **4** is the C-22,23 epoxidation product of the naturally occurring **3**.[17] In the process of isolating highly purified **3** from the roots and rhizomes of *T. chantrieri*, we found a minor product with potent microtubule stabilizing activity. This minor taccalonolide was purified and designated taccalonolide AI (**5**).

Compound **5** was obtained as a white powder. Its molecular formula ($C_{35}H_{48}O_{11}$) was determined by high resolution electrospray ionization mass spectroscopy (HRESIMS), 645.3268 $[M + H]^+$ (calcd for $C_{35}H_{49}O_{11}$ 645.3269), and NMR data. The ^{1}H NMR spectrum of **5** showed characteristics of the taccalonolide backbone, including four methyl singlets at δ 1.67 (s, 3H), 1.36 (s, 3H), 0.82 (s, 3H), and 0.76 (s, 3H), one methyl doublet at δ 0.95 (d, J = 7.0 Hz, 3H, 21-CH_3), one acetyl at δ 2.08 (s, 3H), epoxyl signals at δ 3.55 (t, J = 4.0 Hz, 1H, H-2) and 3.40 (br, 1H, H-3), and an olefin singlet at δ 5.02 (br, 1H, H-22). The upfield shift of H-15 at δ 4.38 (ddd, J = 9.5, 8.3, 2.5 Hz, 1H) indicated a 15-OH similar to **3** and **6**. The only acetoxy group was assigned to C-12 due to the chemical shift of H-12 at δ 4.99 (t, J = 2.8 Hz, 1H) and the HMBC correlation between H-12 and the acetoxy carbon at δ 169.3. The downfield chemical shift of H-1 at δ 4.59 (d, J = 5.2 Hz, 1H) required an acyloxy substitution at C-1. This acyloxy group was determined to be an isovaleryloxy by the ^{1}H NMR signals at δ 2.18 (m, 2H, H-2′), 2.14 (m, 1H, H-3′), 1.01 (d, J = 7.0 Hz, 3H, H-4′), 1.00 (d, J = 6.0 Hz, 3H, H-5′), and 2D NMR correlations. The HMBC correlations between H-1 and the carbonyl carbon of the isovaleryloxy at δ 171.8 confirmed this substitution. Thus, the structure of **5** was determined as shown in Figure 1.

Compound **5** retains the microtubule stabilizing activity of other taccalonolides and has antiproliferative activity with an IC_{50} of 47 nM in HeLa cells, which makes it one of the most potent natural taccalonolides isolated to date (Table 1). The potency of this taccalonolide is consistent with previously determined SAR, which indicates that a large bulky group at C-1 is optimal for activity.[13,16] The bulky isovaleryloxy group at C-1 is the only difference between **5** and **6**, which has an acetoxy at this site and is approximately 200-fold less potent (Table 1). Additionally, the only difference between **5** and taccalonolide AM[18] is the absence of the C-5 hydroxyl in **5**, which confers a 42-fold increase in potency (Table 1). Therefore, the combination of modifications at C-1 and C-5 appear to be important for the potency of **5**.

Epoxidation of the Taccalonolides. There are many methods for epoxidation of alkenes. Considering the multiple fragile functional groups present in the taccalonolides and small quantity of natural taccalonolides available (less than 1 mg in some cases), a mild and efficient epoxidation method was needed. Dimethyldioxirane (DMDO) can rapidly epoxidize alkenes under neutral and mild conditions, and it is also well suited for the synthesis of sensitive epoxides of enol esters and enol lactones, as are present in the taccalonolides.[19] The reaction is highly efficient, generally furnishing the desired epoxides in almost quantitative yield. It is also very convenient, and in most cases pure products are obtained after evaporation of the solvent. Due to the dramatic increases in potency conferred by the epoxidation of the C-22,23 double bond in **2** and **3** to generate **1** and **4**, we applied this method to epoxidize a wide variety of other natural taccalonolides: E (**7**, 1.15 mg), N (**6**, 1.75 mg), R (**8**, 1.44 mg), T (**9**, 2.1 mg), Z (**10**, 0.74 mg), AA (**11**, 1.0 mg), AB (**12**, 0.94 mg), AD (**13**, 1.28 mg), AI (**5**, 0.62 mg), and AN (**14**, 0.65 mg) (see Scheme 1). Most of these

Scheme 1. Epoxidation of Taccalonolides



natural taccalonolides are very rare with estimated content in the plant at ppm levels or less. Only the α epoxy was obtained as determined by the small coupling constant between H20/H22. We hypothesize that this is a result of spatial strains of the β, axial orientation of the 27-CH_3.

The antiproliferative potency of each C-22,C23 epoxy-taccalonolide was evaluated in HeLa cells and compared with the IC_{50} of the parent compound (Table 1). In each case, C-22,23 epoxidation resulted in an increase in potency with an over 200-fold improvement in potency observed for 5 of the 10 new epoxy-taccalonolides (Table 1). Remarkably, the C-22,23 epoxidation of **9** and **5** resulted in the generation of the first ever compounds of this class with subnanomolar potencies of

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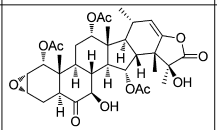
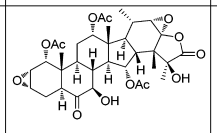
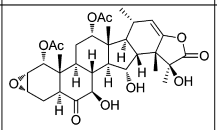
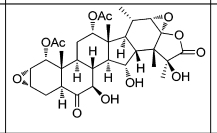
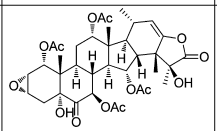
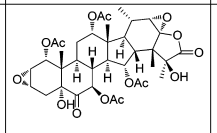
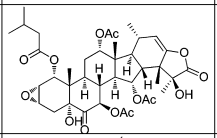
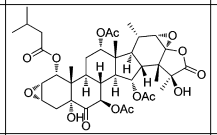
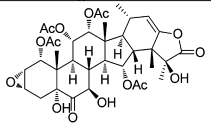
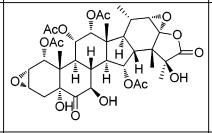
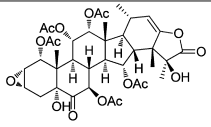
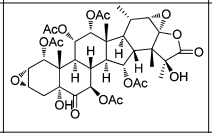
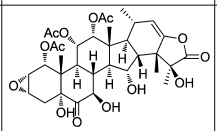
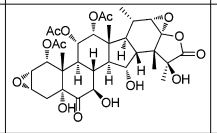
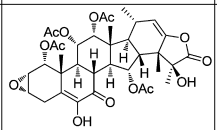
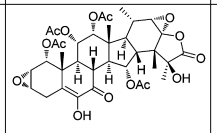
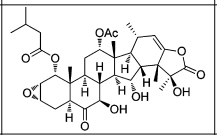
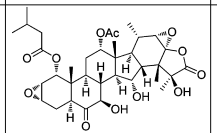
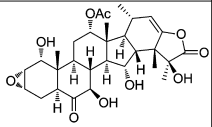
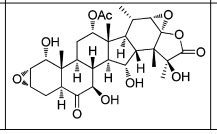
Table 1. Antiproliferative Potencies of Natural Taccalonolides and Their Corresponding C-22,23 Epoxides

Taccalonolide	Structure	IC_{50} Value (nM)[a]	Taccalonolide Epoxide	Structure	IC_{50} Value (nM)[a]
E (**7**)		39,500 ± 4,700	E-epoxide (**15**)		188 ± 28 [210][b]
N (**6**)		8,500 ± 400	N-epoxide (**16**)		14.5 ± 1.2 [567][b]
R (**8**)		13,144 ± 1,390	R-epoxide (**17**)		29.6 ± 2.0 [438][b]
T (**9**)		335 ± 24	T-epoxide (**18**)		0.45 ± 0.04 [744][b]
Z (**10**)		120 ± 8	Z-epoxide (**19**)		17.2 ± 0.3 [7][b]
AA (**11**)		32 ± 2	AA-epoxide (**20**)		15.6 ± 0.2 [2][b]
AB (**12**)		2,767 ± 107	AB-epoxide (**21**)		8.4 ± 0.7 [329][b]
AD (**13**)		3,480 ± 230	AD-epoxide (**22**)		820 ± 7 [4][b]
AI (**5**)		47 ± 3	AI-epoxide (**23**)		0.88 ± 0.01 [53][b]
AN (**14**)		1,510 ± 78	AN-epoxide (**24**)		685 ± 19 [2][b]

[a] IC_{50} values were determined in HeLa cells. [b] The fold increase in potency achieved by C-22,23 expoxidation is shown in brackets in the last column.

0.45 and 0.88 nM, respectively. This makes **18** and **23** more potent than paclitaxel, which had an IC_{50} of 1.2 nM in this assay.

Microtubule Stabilizing Effects of C-22,23 Epoxidized Taccalonolides. In addition to their potent antiproliferative effects, each epoxidized taccalonolide caused interphase microtubule bundling in HeLa cells. These effects are depicted in Figure 2 for the two most potent taccalonolides, **18** and **23**, and representative images of the cellular microtubule stabilizing effects of other, less potent epoxy taccalonolides are shown in the Supporting Information. In addition to their microtubule stabilizing effects in cells, both **18** and **23** enhanced the polymerization of purified porcine brain tubulin in turbidimetric assays (Figure 3). Similarly to other potent taccalonolides, including **1** and **4**,[17] they enhanced the extent of tubulin polymerization compared with vehicle controls without affecting the time required to initiate tubulin polymerization, a feature that makes this class of microtubule stabilizers distinct from other microtubule stabilizers that bind to the paclitaxel or laulimalide binding sites.[16]

***In Vivo* Efficacy of Potent Epoxidized Taccalonolides.** Despite the fact that **9** and **5** are rare natural products, both semisynthetic C-22,23 epoxidation products **23** and **18** were generated in sufficient quantities for *in vivo* antitumor analyses.

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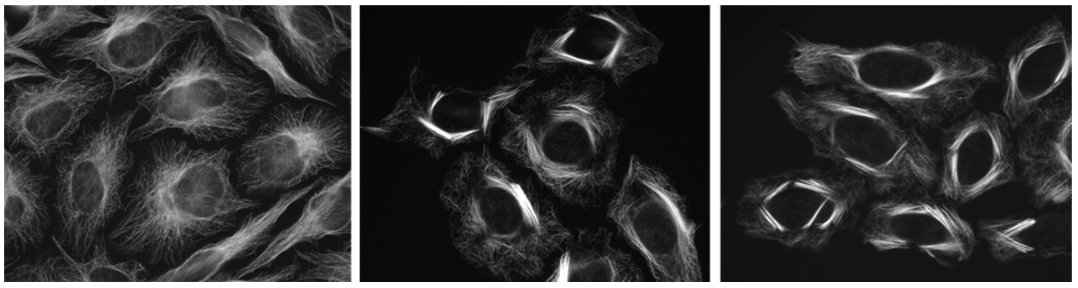

Figure 2. Effect of potent epoxidized taccalonolides on cellular microtubules. Microtubules were visualized by immunofluorescence using a β-tubulin antibody after treatment of HeLa cells for 18 h with (A) vehicle (EtOH), (B) 4 nM **18**, or (C) 10 nM **23**.

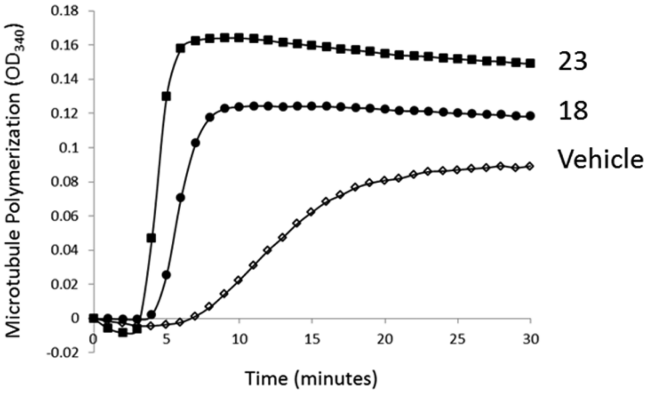

Figure 3. Effect of potent epoxidized taccalonolides on purified tubulin polymerization. Purified porcine brain tubulin was incubated with 6 μM **23**, 0.7 μM **18**, or EtOH vehicle, and microtubule polymerization was monitored turbidimetrically after shift to 37 °C.

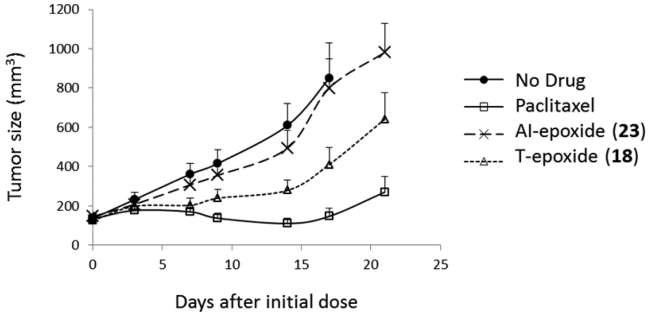

Figure 4. *In vivo* efficacy of a cumulative total dose of 2.25 mg/kg **23** or 0.5 mg/kg **18** in an MDA-MB-231 xenograft model of triple negative breast cancer compared with a cumulative total dose of 45 mg/kg paclitaxel. Measurements are an average of 10 tumors with standard error.

Antitumor studies could be performed with small quantities of material due to the exquisite potency of the taccalonolides *in vivo*.[13,14,16] The ability of taccalonolides to covalently bind to microtubules likely contributes to their *in vivo* potency.[16] The low doses of taccalonolides needed to observe effects *in vivo* allow them to be diluted in aqueous solvents, in this case less than 10% EtOH in phosphate buffered saline (PBS). This is in contrast to paclitaxel, which requires administration in Cremophor. The antitumor efficacies of **18** and **23** were compared with nontreated tumors as a negative control and three doses of 15 mg/kg paclitaxel administered on days 0, 3, and 7 as a positive control for antitumor activity.

Individual doses of 0.25 mg/kg **18** were administered twice in the first week (days 0, 3) based on preliminary dose tolerance studies that showed a total dose of 0.5 mg/kg was acceptable. No additional drug was administered due to an average 10% body weight loss observed on day 7. Despite the low total dose administered (0.5 mg/kg), tumor growth was completely inhibited through day 7 (Figure 4). During days 7–14, the mice gradually recovered to 4% body weight loss while significant antitumor effects were sustained for over a week after the final dose was administered (Figure 4). By day 17, the mice had fully recovered from drug-induced weight loss and some antitumor effects persisted. One lethality was encountered on day 21, which was 18 days after the final dose and after a full recovery of body weight loss; it is therefore unclear whether this was a drug-related toxicity.

As has been noted for other taccalonolides, **18** has a narrow therapeutic window based on a concurrent study where two doses of 0.375 mg/kg administered on days 0 and 3 (0.75 total dose) resulted in an LC_{40} with 2 of the 5 mice succumbing 8–11 days after the final dose. Despite this narrow therapeutic window, the ability of a total dose of 0.5 mg/kg **18** to produce antitumor effects highlights the exceptional *in vivo* potency of the taccalonolides.

Taccalonolide **23** was administered at 0.75 mg/kg on days 0, 3, and 7 for a total dose of 2.25 mg/kg. Only slight antitumor effects were observed, but dosing could not be increased due to a limited amount of material, which was fully expended on day 7. Although **23** was only 2-fold less potent than **18** *in vitro*, no significant antitumor effects or weight loss were observed with **23** at a dose 3-fold higher than the dose of **18** that produced antitumor effects. Although we cannot rule out the possibility that **23** may have antitumor efficacy at higher concentrations, these results highlight that *in vitro* potency cannot be used as a sole predictor of *in vivo* efficacy, even when drugs of the same class are being compared. These results are consistent with studies showing that **1** had excellent antitumor effects while **4** did not.[16]

Reduction of the Carbonyl Group in Taccalonolide A.

Almost all taccalonolides possess a carbonyl group at C-6. The role of this carbonyl group in the microtubule stabilizing activity of the taccalonolides is unknown. Reduction of this carbonyl group with $NaBH_4$ resulted in two new semisynthetic products, **25** and **26**, that were isolated with respective 2% and 9% yields after HPLC purification (Scheme 2). Due to the spatial strain of the β orientation of both 18-CH_3 and 7-OH, we hypothesize that the hydride attached to the carbonyl from the α face resulting in the 6β reduction products of **25** and **26**.

Compound **25** was obtained as white powder, and a molecular formula of $C_{34}H_{46}O_{13}$ was deduced from the HRMS, 663.3021 (calcd for $C_{34}H_{47}O_{13}$ 663.3011). The ^1H NMR showed signals only for three acetyl methyl groups, suggesting the loss of one acetyl group. The chemical shift of

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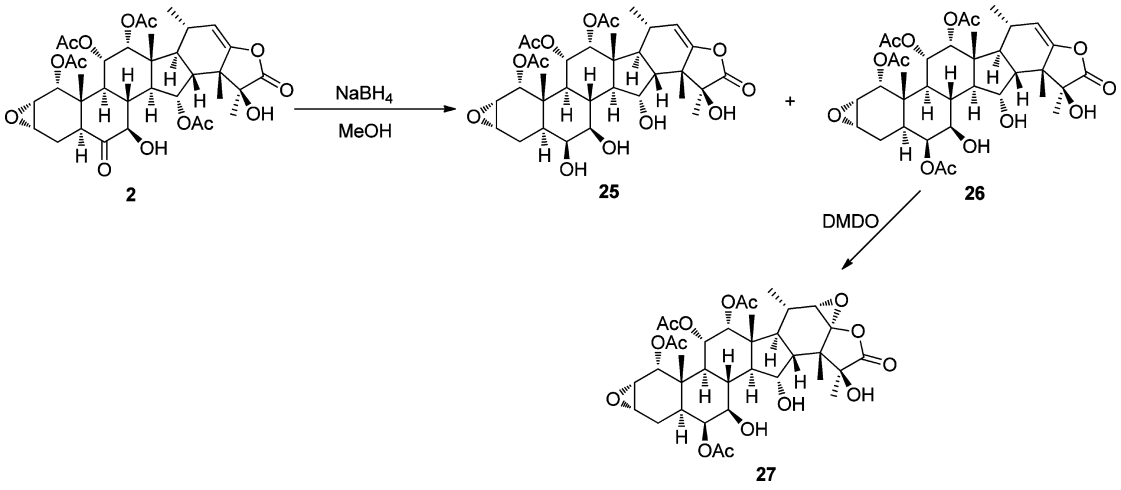
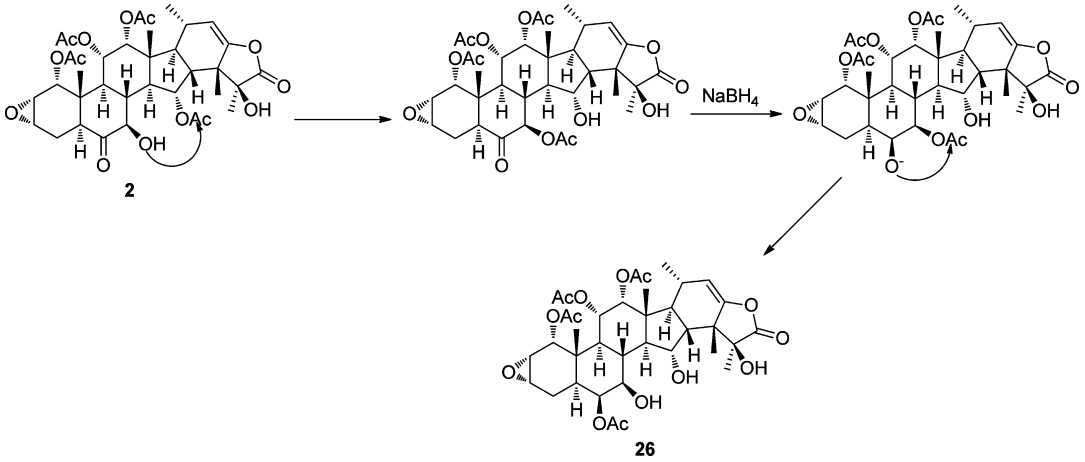
Scheme 2. Reduction of the Carbonyl of 2



Scheme 3. Proposed Mechanism of the Formation of 26 from 2



H-15 at δ 4.39 (t, J = 8.7 Hz), ca. 1.1 ppm higher than that of taccalonolide A, indicated that the acetyl group at 15-OH was lost. This is consistent with previous studies showing that the C-15 acetoxy can easily be hydrolyzed.[14,18] The C-6 carbonyl signal was also lost, and instead, signals for a hydroxymethine at δ_H 3.80 (br, H-6) and δ_C 73.5 (C-6) were observed, indicating the successful reduction of the C-6 carbonyl group. The orientation of the 6-OH was determined to be β (equatorial) due to the small coupling constant of H-6. The rest of the molecule was found to be unchanged by 2D NMR. Thus, **25** is a product of the reduction of the C-6 carbonyl and the hydrolysis of the C-15 acetoxyl group.

Compound **26** was obtained as white powder. The molecular formula of $C_{36}H_{48}O_{14}$ was determined by HRMS, 705.3137 (calcd for $C_{36}H_{49}O_{14}$ 705.3168), corresponding to the reduction of a carbonyl group. The ^{1}H NMR showed signals for four acetyl methyl groups. H-15 resonance at δ 4.37, suggested the hydrolysis of this acetyl group to give 15-OH. Interestingly, this acetyl group appears to shift to the newly generated C-6 hydroxyl group based on the δ_H 5.07 (br, H-6), δ_C 76.7 (C-6), and COSY correlations between H-6/H-5 (δ 2.05, m), H-6/H-7 (δ 3.79, dd, J = 9.5, 2.5 Hz), and H-7/H-8 (δ 1.94, m). The configuration of 6-OAc was determined to be β (equatorial) due to the small coupling constant of H-6. We hypothesize that the migration of the acetyl group from 15-OH to 6-OH might have occurred through two steps (See Scheme 3). The 15-acetyl group could have first migrated to 7-OH since they are very close and the 15-acetoxy is prone to loss of the acetyl group as demonstrated previously. Then the newly formed alkoxide from the reduction of the 6-ketone could have attached to the carbonyl carbon of the 7-OAc group to yield **26**, the 6-OAc product.

Both **25** and **26** were found to have no antiproliferative effects at concentrations up to 50 μM, suggesting the importance of the C-6 ketone for activity. However, **26** was generated in sufficient quantity to epoxidize at C-22,23. Similarly to the results observed in Table 1, C-22,23 epoxidation of **26** resulted in an over 300-fold improvement of potency to generate an active taccalonolide, **27**, with an IC_{50} of 163 $\pm$ 10 nM.

Structure–Activity Relationships. The newly isolated taccalonolide **5** and the known taccalonolide **9** showed relatively potent antiproliferative activities with IC_{50} values of 47 and 335 nM, respectively, which are 181- and 39-fold more potent than **6** and **8**. The only difference between taccalonolides **5**/**6** and **9**/**8** is that both **5** and **9** have an isovaleryloxy group at C-1, while **6** and **8** have an acetyloxy group at C-1. This result supports previous assertions that a bulky substitution at C-1 is preferred for the antiproliferative activity of the taccalonolides.[13]

Epoxidation of the C-22,23 double bond significantly increased the potency of every taccalonolide analyzed. Taking

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into account **1** and **4** (the epoxy products of **2** and **3**, respectively), epoxidation of the C-22,23 double bond resulted in an over 200-fold increase in potency for 7 of the 12 natural taccalonolides tested (Table 1).[17] Although these data show that a C-22,23 epoxide is optimal for the potency of a wide range of taccalonolides, further information can be gleaned from the relative effect that this epoxidation has on activity. Two taccalonolides, **10** and **11**, showed relatively modest 7- and 2-fold increases in potency, respectively, after epoxidation. However, it is important to note that these two taccalonolides were relatively potent before epoxidation (120 and 32 nM, respectively) and that their epoxidized forms are some of the most potent taccalonolides identified to date (Table 1). Therefore, it appears that there may be a maximum potency that can be conferred to some taccalonolides by epoxidation. The notable outliers to this are the epoxidized forms of **9** and **5**, which each contain a bulky isovaleryloxy group at C-1 and are the first taccalonolides to show subnanomolar potency. Together, these results suggest that the combination of a C-22,23 epoxide with a bulky C-1 modification is optimal for taccalonolide potency.

Taccalonolides **13** and **14** also showed only modest increases of 2−4-fold in potency after C-22,23 epoxidation. These compounds differ from those previously mentioned in that even the epoxidized forms, **22** and **24**, have only moderate potencies, between 685−820 nM. Therefore, although epoxidation increased the potency of these taccalonolides, the resulting activities remained modest. Taccalonolide **13** contains a C-6,7 keto−enol tautomerization on the bottom of the molecule. We hypothesize that these nonoptimal substituents on C-6 and C-7 limit the potency of **13** even when epoxidized at C-22,23. A similar limit in potency was conferred by the hydrolysis of the C-1 group in **14**.

Both C-6 reductive products of the major metabolite taccalonolide A, **25** and **26**, exhibited decreased potency, further demonstrating the importance of the C-6 ketone for activity. However, the finding that C-22,23 epoxidation of **26** leads to an over 300-fold improvement in potency demonstrates that this epoxidation can dramatically improve the activity even of taccalonolides that otherwise have no detectable antiproliferative effects. Together, these data provide more extensive SAR for the taccalonolides, including the critical importance of C-22,23, C-1, and C-6 modifications as well as the interplay between these substituents.

CONCLUSIONS

Each of the 11 taccalonolides epoxidized at C-22,23 in this study exhibited increased potency compared with their precursors, the majority showing over 200-fold improvement in activity. Two of these products, **18** and **23**, are the first taccalonolides identified with subnanomolar potency. These two epoxidized taccalonolides have potent microtubule stabilizing activities in cells and with purified tubulin, but only **18** demonstrated antitumor efficacy at the dose and schedule tested. These results demonstrate that a C-22,23 epoxy combined with a bulky C-1 isovaleryloxy group facilitates optimal potency for the taccalonolide class of microtubule stabilizers and further enhances our understanding of the structure−activity relationship and antitumor efficacy for this group of microtubule stabilizers. While both C-22,23 epoxidated and nonepoxidated taccalonolides cause microtubule stabilization in cells, the recent finding that some epoxy taccalonolides can covalently bind to microtubules leads to a hypothesis that the C-22,23 epoxide may facilitate their irreversible binding, which would be consistent with the increased potency afforded by this modification. Additional studies to directly test this hypothesis are ongoing.

EXPERIMENTAL SECTION

General Experimental Procedures. NMR spectra were acquired on Bruker Avance 500, 600, or 700 MHz instruments equipped with CryoProbes using $CDCl_3$ as solvent. All spectra were measured and reported in ppm using trimethylsilane as an internal standard. ^{13}C NMR data were obtained from HMQC and HMBC spectra. The HRMS data were obtained on an Aglient Technologies 6224 TOFLC/MS mass spectrometer. LC/MS was performed with a Waters Alliance 2695 HPLC module, 996 photodiode array detector, and Micromass Quattro triple quadrupole mass spectrometer equipped with ESI under the positive mode. TLC was performed on aluminum sheets (silica gel 60 F254, Merck KGaA, Germany). Spots were visualized by spraying with 20% sulfuric acid in ethanol followed by heating. Final compounds were tested to be >95% pure by LC/MS.

Isolation of Taccalonolides. Taccalonolides E (**7**), N (**6**), R (**8**), T (**9**), Z (**10**), AA (**11**), AB (**12**), AD (**13**), and AN (**14**) were obtained previously.[13−15,17,18] A taccalonolide B (**3**) enriched fraction[14] was further purified by reversed phase HPLC (Phenomenex Luna, 5 μm C_{18} 250 × 21.2 mm column) eluting with a gradient of 40−100% acetonitrile in H_2O in 50 min to yield highly pure **3** and the minor product, taccalonolide AI (**5**).

*Taccalonolide AI (**5**).* White powder. HRMS (m/z) calcd for $C_{35}H_{49}O_{11}$ $[M + H]^+$ 645.3269, found 645.3268; ^1H NMR (500 MHz, $CDCl_3$) δ 5.23 (d, J = 2.6 Hz, 1H, OH-15), 5.02 (br, 1H, H-22), 4.99 (t, J = 2.8 Hz, 1H, H-12), 4.72 (s, 1H, OH-25), 4.59 (d, J = 5.2 Hz, 1H, H-1), 4.45 (br, 1H, OH-7), 4.38 (ddd, J = 9.5, 8.3, 2.5 Hz, 1H, H-15), 4.01 (d, J = 10.3 Hz, 1H, H-7), 3.55 (t, J = 4.0 Hz, 1H, H-2), 3.40 (br, 1H, H-3), 2.70 (dd, J = 11.3, 4.5 Hz, 1H, H-5), 2.39 (dd, J = 13.1, 10.9 Hz, 1H, H-16), 2.26 (dd, J = 16.1, 4.5 Hz, 1H, H-4a), 2.19 (m, 1H, H-20), 2.18 (m, 2H, H-2′), 2.15 (m, 1H, H-9), 2.14 (m, 1H, H-3′), 2.12 (m, 1H, H-4b), 2.08 (s, 3H, 12−OCOCH_3), 2.07 (m, 1H, H-14), 1.98 (dd, J = 13.2, 10.1 Hz, 1H, H-17), 1.71 (m, 1H, H-8), 1.69 (m, 2H, H-11), 1.67 (s, 3H, H-28), 1.36 (s, 3H, H-27), 1.01 (d, J = 7.0 Hz, 3H, H-4′), 1.00 (d, J = 6.0 Hz, 3H, H-5′), 0.95 (d, J = 7.0 Hz, 3H, H-21), 0.82 (s, 3H, H-19), 0.76 (s, 3H, H-18). ^{13}C NMR (125 MHz, $CDCl_3$) δ 209.1 (C-6), 175.4 (C-26), 171.8 (C-1′), 169.3 (12-OCOCH$_3$), 154.9 (C-23), 110.4 (C-22), 78.9 (C-25), 77.5 (C-7), 73.5 (C-12), 71.8 (C-15), 70.4 (C-1), 57.3 (C-14), 52.5 (C-3), 50.8 (C-24), 49.9 (C-16), 49.3 (C-2), 47.7 (C-17), 43.8 (C-13), 43.4 (C-5), 42.6 (C-2′), 42.2 (C-8), 41.2 (C-10), 36.1 (C-9), 30.8 (C-20), 25.1 (C-27), 25.4 (C-3′), 24.7 (C-11), 21.9 (C-4′,5′), 21.7 (C-28), 20.9 (C-4), 20.7 (12-OCOCH$_3$), 19.3 (C-21), 12.9 (C-18), 12.3 (C-19).

Epoxidation of Taccalonolides. Dimethyldioxirane was prepared by reaction of oxone with acetone, and the concentration of dimethyldioxirane was determined by UV.[20] Each taccalonolide (ca. 1−3 μmol) was dissolved in 500 μL of CH_2Cl_2 and cooled to −20 °C. DMDO (2−3 equiv) was added, and the mixture was stirred at room temperature until the taccalonolide was completely epoxidized (1−4 h). The epoxides were obtained after removal of the solvents and reagent with no further purification required.

*Taccalonolide E-Epoxide (**15**).* White powder. HRMS (m/z) calcd for $C_{34}H_{45}O_{13}$ $[M + H]^+$ 661.2854, found 661.2851; ^1H NMR (500 MHz, $CDCl_3$) δ 5.47 (t, J = 9.1 Hz, 1H, H-15), 4.91 (d, J = 3.0 Hz, 1H, H-12), 4.60 (d, J = 5.3 Hz, 1H, H-1), 3.86 (dd, J = 10.1, 3.7 Hz, 1H, H-7), 3.83 (br, 7-OH), 3.51 (t, J = 4.7 Hz, 1H, H-2), 3.39 (br, 1H, H-3), 3.30 (s, 1H, H-22), 2.67 (m, 2H, H-5, 25-OH), 2.32 (dd, J = 10.9, 8.6 Hz, 1H, H-14), 2.26−2.03 (m, 6H), 2.12 (s, 3H, 1-OCOCH_3), 2.10 (s, 3H, 12-OCOCH_3), 2.01 (s, 3H, 15-OCOCH_3), 1.76 (s, 3H, H-28), 1.75−1.60 (m, 4H), 1.35 (s, 3H, H-27), 1.08 (d, J = 7.4 Hz, 3H, H-21), 0.75 (s, 3H, H-19), 0.70 (s, 3H, H-18).

*Taccalonolide N-Epoxide (**16**).* White powder. HRMS (m/z) calcd for $C_{32}H_{43}O_{12}$ $[M + H]^+$ 619.2749, found 619.2757; ^1H NMR (500 MHz, $CDCl_3$) δ 5.25 (d, J = 3.1 Hz, 1H, H-12), 5.08 (br, 1H, OH), 4.88 (d, J = 3.0 Hz, 1H, OH), 4.59 (d, J = 5.4 Hz, 1H, H-1), 4.44 (br,

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1H, OH), 4.30 (t, J = 10.0 Hz, 1H, H-15), 4.01 (d, J = 10.4 Hz, 1H, H-7), 3.53 (t, J = 4.3 Hz, 1H, H-2), 3.41 (br, 1H, H-3), 3.28 (s, 1H, H-22), 2.71 (dd, J = 11.7, 4.7 Hz, 1H, H-5), 2.31−2.03 (m, 6H), 2.10 (s, 3H, 1-OCOCH_3), 2.09 (s, 3H, 12-OCOCH_3), 1.76 (s, 3H, H-28), 1.75−1.67 (m, 4H), 1.37 (s, 3H, H-27), 1.07 (d, J = 7.3 Hz, 3H, H-21), 0.74 (s, 3H, H-19), 0.71 (s, 3H, H-18).

*Taccalonolide R-Epoxide (**17**).* White powder. HRMS (m/z) calcd for $C_{36}H_{47}O_{15}$ $[M + H]^+$ 719.2910, found 719.2907; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.55 (d, J = 10.8 Hz, 1H, H-7), 5.50 (t, J = 8.6 Hz, 1H, H-15), 4.88 (br, 1H, H-12), 4.81 (d, J = 5.1 Hz, 1H, H-1), 3.70 (t, J = 4.5 Hz, 1H, H-2), 3.58 (d, J = 3.8 Hz, 1H, H-3), 3.31 (s, 1H, 5-OH), 3.29 (s, 1H, H-22), 2.82 (td, J = 10.8, 7.1 Hz, 1H, H-9), 2.70 (s, 1H, 25-OH), 2.54−2.47 (m, 2H, H-4a,14), 2.26−1.91 (m, 6H), 2.16 (s, 3H, 1-OCOCH_3), 2.14 (s, 3H, 7-OCOCH_3), 2.12 (s, 3H, 12-OCOCH_3), 1.97 (s, 3H, 15-OCOCH_3), 1.73 (s, 3H, H-28), 1.67 (m, 3H, H_2-11, H-20), 1.33 (s, 3H, H-27), 0.80 (d, J = 7.4 Hz, 3H, H-21), 0.78 (s, 3H, H-19), 0.70 (s, 3H, H-18).

*Taccalonolide T-Epoxide (**18**).* White powder. HRMS (m/z) calcd for $C_{39}H_{53}O_{15}$ $[M + H]^+$ 761.3379, found 761.3362; ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.55 (d, J = 10.7 Hz, 1H, H-7), 5.50 (t, J = 8.7 Hz, 1H, H-15), 4.88 (t, J = 3.2 Hz, 1H, H-12), 4.79 (d, J = 5.1 Hz, 1H, H-1), 3.72 (t, J = 4.4 Hz, 1H, H-2), 3.57 (br, 1H, H-3), 3.30 (br, 1H, 5-OH), 3.29 (br, 1H, H-22), 2.82 (dt, J = 11.6, 8.5 Hz, 1H, H-9), 2.72 (s, 1H, 25-OH), 2.50 (m, 2H, H-4a,14), 2.25−2.16 (m, 5H, H4b, H-17, H_2-2′, H-3′), 2.16 (s, 3H, 7-OCOCH_3), 2.13 (s, 3H, 12-OCOCH_3), 1.97 (s, 3H, 15-OCOCH_3), 1.95 (m, 1H, H-8), 2.02 (dd, J = 13.9, 9.2 Hz, 1H, H-16), 1.73 (s, 3H, H-28), 1.67 (m, 3H, H_2-11, H-20), 1.32 (s, 3H, H-27), 1.07 (d, J = 7.3 Hz, 3H, H-21), 1.01 (t, J = 6.6 Hz, 6H, H-4′,5′), 0.77 (s, 3H, H-19), 0.70 (s, 3H, H-18). ^{13}C NMR (150 MHz, $CDCl_3$) δ 201.5 (C-6), 177.9 (C-26), 172.1 (1-OCOCH$_3$), 172.3 (15-OCOCH$_3$), 171.4 (7-OCOCH$_3$), 169.7 (12-OCOCH$_3$), 92.8 (C-23), 79.9 (C-5), 79.6 (C-25), 76.4 (C-7), 74.5 (C-12), 72.5 (C-1), 72.0 (C-15), 66.2 (C-22), 55.0 (C-3), 54.4 (C-14), 50.8 (C-2), 48.5 (C-16), 46.9 (C-24), 45.1 (C-17), 44.9 (C-13), 44.7 (C-10), 43.4 (C-2′), 34.4 (C-9), 32.0 (C-20), 27.3 (C-4), 26.6 (C-3′), 26.4 (C-11), 23.9 (C-28), 23.4 (15-OCOCH$_3$), 22.9 (C-4′,5′), 21.9 (7,12-OCOCH$_3$), 20.0 (C-27), 18.9 (C-21), 15.1 (C-18), 13.9 (C-19).

*Taccalonolide Z-Epoxide (**19**).* White powder. HRMS (m/z) calcd for $C_{36}H_{47}O_{16}$ $[M + H]^+$ 735.2859, found 735.2867; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.53 (t, J = 8.9 Hz, 1H, H-15), 5.19 (brd, J = 12.2 Hz, 1H, H-11), 5.16 (br, 1H, H-12), 4.85 (d, J = 5.3 Hz, 1H, H-1), 4.71 (dd, J = 10.3, 5.2 Hz, 1H, H-7), 3.74 (t, J = 4.7 Hz, 1H, H-2), 3.64 (br, 1H, 5-OH), 3.61 (br, 1H, H-3), 3.47 (d, J = 4.9 Hz, 1H, 7-OH), 3.27 (s, 1H, H-22), 3.16 (t, J = 11.5 Hz, 1H, H-9), 2.66 (s, 1H, 25-OH), 2.57 (d, J = 16.5 Hz, 1H, H-4a), 2.50 (t, J = 10.0 Hz, 1H, H-14), 2.23 (d, J = 16.5 Hz, 1H, H-4b), 2.17 (s, 3H, 1-OCOCH_3), 2.16 (s, 3H, 12-OCOCH_3), 2.15−2.03 (m, 2H, H-16,17), 2.09 (s, 3H,11-OCOCH_3), 1.98 (s, 3H, 15-OCOCH_3), 1.76 (s, 3H, H-28), 1.58 (m, 1H, H-20), 1.34 (s, 3H, H-27), 1.01 (d, J = 7.4 Hz, 3H, H-21), 0.88 (s, 3H, H-19), 0.72 (s, 3H, H-18).

*Taccalonolide AA-Epoxide (**20**).* White powder. HRMS (m/z) calcd for $C_{38}H_{49}O_{17}$ $[M + H]^+$ 777.2964, found 777.2947; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.70 (d, J = 10.9 Hz, 1H, H-7), 5.53 (t, J = 8.3 Hz, 1H, H-15), 5.31 (br, 1H, 5-OH), 5.19 (d, J = 12.6 Hz, 1H, H-11), 5.18 (br, 1H, H-12), 4.90 (d, J = 5.2 Hz, 1H, H-1), 3.72 (t, J = 4.8 Hz, 1H, H-2), 3.59 (br, 1H, H-3), 3.28 (m, 2H, H-9,22), 2.69 (s, 1H, 25-OH), 2.60 (t, J = 10.2 Hz, 1H, H-14), 2.56 (d, J = 17.1 Hz, 1H, H-4a), 2.15−2.00 (m, 3H, H-4b, 8,16), 2.20 (s, 3H, 1-OCOCH_3), 2.17 (s, 3H, 7-OCOCH_3), 2.16 (s, 3H, 12-OCOCH_3), 1.99 (s, 6H, 11,15-OCOCH_3), 1.75 (s, 3H, H-28), 1.62 (m, 2H, H-17,20), 1.32 (s, 3H, H-27), 1.09 (d, J = 7.3 Hz, 1H), 1.02 (d, J = 7.3 Hz, 3H, H-21), 0.93 (s, 3H, H-19), 0.71 (s, 3H, H-18).

*Taccalonolide AB-Epoxide (**21**).* White powder. HRMS (m/z) calcd for $C_{34}H_{45}O_{15}$ $[M + H]^+$ 693.2753, found 693.2771; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.30 (s, 1H, 25-OH), 5.22 (dd, J = 12.0, 2.5 Hz, 1H, H-11), 5.13 (d, J = 2.4 Hz, 1H, H-12), 4.96 (br, 2H, 15,25-OH), 4.91 (dd, J = 10.6, 4.5 Hz, 1H, H-7), 4.81 (d, J = 5.4 Hz, 1H, H-1), 4.38 (td, J = 9.1, 3.7 Hz, 1H, H-15), 4.03 (d, J = 4.5 Hz, 1H, 7-OH), 3.75 (t, J = 4.5 Hz, 1H, H-2), 3.68 (s, 1H, 5-OH), 3.62 (br, 1H, H-3), 3.25 (s, 1H, H-22), 3.15 (t, J = 11.6 Hz, 1H, H-9), 2.54 (dd, J = 16.6, 2.1 Hz, 1H, H-4a), 2.27 (d, J = 16.6, Hz, 1H, H-4b), 2.21 (m, 1H, H-14), 2.17 (s, 3H, 1-OCOCH_3), 2.15 (s, 3H, 12-OCOCH_3), 2.09 (m, 2H, H-16,17), 1.98 (s, 3H, 11-OCOCH_3), 1.77 (s, 3H, H-28), 1.61 (m, 2H, H-8,20), 1.36 (s, 3H, H-27), 0.99 (d, J = 7.4 Hz, 3H, H-21), 0.85 (s, 3H, H-19), 0.76 (s, 3H, H-18).

*Taccalonolide AD-Epoxide (**22**).* White powder. HRMS (m/z) calcd for $C_{36}H_{45}O_{15}$ $[M + H]^+$ 717.2753, found 717.2738; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.19 (s, 1H, 6-OH), 5.64 (t, J = 8.5 Hz, 1H, H-15), 5.43 (dd, J = 11.3, 2.3 Hz, 1H, H-11), 5.26 (d, J = 2.3 Hz, 1H, H-12), 4.90 (d, J = 5.5 Hz, 1H, H-1), 3.55 (t, J = 4.2 Hz, 1H, H-2), 3.41 (t, J = 3.7 Hz, 1H, H-3), 3.38 (d, J = 19.7 Hz, 1H, H-4a), 3.28 (br, 1H, H-22), 2.83 (t, J = 12.3 Hz, 1H, H-11), 2.74 (s, 1H, 25-OH), 2.62 (m, 3H, H-4b,8,14), 2.44 (dd, J = 11.4, 8.0 Hz, 1H, H-16), 2.18 (s, 3H, 15-OCOCH_3), 2.11 (s, 3H, 1-OCOCH_3), 2.10 (s, 3H, 11-OCOCH_3), 2.00 (s, 3H, 12-OCOCH_3), 1.75 (m, 2H, H-17,20), 1.71 (s, 3H, H-28), 1.31 (s, 3H, H-27), 1.21 (s, 3H, H-18), 1.07 (d, J = 6.8 Hz, 3H, H-21), 0.92 (s, 3H, H-19).

*Taccalonolide AI-Epoxide (**23**).* White powder. HRMS (m/z) calcd for $C_{35}H_{49}O_{12}$ $[M + H]^+$ 661.3219, found 661.3211; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.26 (d, J = 3.0 Hz, 1H, 15-OH), 5.08 (s, 1H, 25-OH), 4.90 (t, J = 2.7 Hz, 1H, H-12), 4.57 (d, J = 5.3 Hz, 1H, H-1), 4.44 (d, J = 3.1 Hz, 1H, 7-OH), 4.29 (t, J = 9.5 Hz, 1H, H-15), 4.01 (d, J = 10.3 Hz, 1H, H-7), 3.55 (t, J = 4.4 Hz, 1H, H-2), 3.40 (d, J = 3.2 Hz, 1H, H-3), 3.27 (s, 1H, H-22), 2.69 (dd, J = 11.4, 4.8 Hz, 1H, H-5), 2.27 (d, J = 16.7, 5.0 Hz, 1H, H-4a), 2.24−2.02 (m, 7H, H-4b,9,14,16,3′, H_2−2′), 2.10 (s, 3H, 12-OCOCH_3), 1.67 (s, 3H, H_2-11, H-20), 1.76 (s, 3H, H-28), 1.37 (s, 3H, H-27), 1.06 (d, J = 7.4 Hz, 3H, H-21), 1.02 (dd, J = 6.2, 3.6 Hz, 6H, H-4′,5′), 0.75 (s, 3H, H-19), 0.71 (s, 3H, H-18).

*Taccalonolide AN-Epoxide (**24**).* White powder. HRMS (m/z) calcd for $C_{30}H_{41}O_{11}$ $[M + H]^+$ 577.2643, found 577.2657; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.18 (d, J = 3.1 Hz, 1H, 15-OH), 5.08 (s, 1H, H-12), 4.94 (br, 1H, 25-OH), 4.30 (t, J = 9.9 Hz, 1H, H-15), 4.35 (br, 1H, 7-OH), 3.98 (d, 1H, J = 10.3 Hz, 1H, H-7), 3.71 (dd, J = 8.4, 5.7 Hz, 1H, H-1), 3.55 (br, 1H, H-3), 3.42 (t, J = 5.1 Hz, 1H, H-2), 3.28 (br, 1H, H-22), 2.51 (dd, J = 11.1, 5.1 Hz, 1H, H-5), 2.28−2.11 (m, 4H, H_2-4, H-9,16), 2.10 (s, 3H, 12-OCOCH_3), 2.08−2.02 (m, 2H, H-14,17), 1.76 (s, 3H, H-28), 1.70−1.58 (m, 2H, H-8,11), 1.37 (s, 3H, H-17), 1.08 (d, J = 7.2 Hz, 1H, H-21), 0.72 (s, 3H, H-19), 0.63 (s, 3H, H-18).

Reduction of Taccalonolide A (2). Compound **2** (10.2 mg) was dissolved in 2 mL of MeOH and cooled in a sodium chloride ice bath. Excess $NaBH_4$ was added and reacted for 10 min. After removal of MeOH, the residue was dissolved in CH_2Cl_2 at room temperature. The CH_2Cl_2 soluble material was subjected to HPLC separation to yield compounds **25** (0.15 mg) and **26** (0.93 mg).

*TA-NaBH$_4$-10 (**25**).* White powder. HRMS (m/z) calcd for $C_{34}H_{47}O_{13}$ $[M + H]^+$ 663.3011, found 663.3021; ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.40 (d, J = 2.9 Hz, 1H, 15-OH), 5.35 (dd, J = 11.7, 2.8 Hz, 1H, H-11), 5.23 (d, J = 2.8 Hz, 1H, H-12), 5.01 (s, 1H, H-22), 4.64 (s, 25-OH), 4.56 (d, J = 5.4 Hz, 1H, H-1), 4.38 (t, J = 8.9 Hz, 1H, H-15), 3.80 (s, 1H, H-6), 3.51 (m, 1H, H-7), 3.47 (m, 1H, H-2), 3.39 (s, 1H, H-3), 2.39 (dd, J = 13.4, 10.6 Hz, 1H, H-16), 2.25 (t, J = 13.8 Hz, 1H, H-4a), 2.15 (m, 2H, H-9,20), 2.11 (s, 3H, 1-OCOCH_3), 2.07 (s, 3H, 12-OCOCH_3), 1.95 (s, 3H, 11-OCOCH_3), 2.00 (m, 1H, H-8), 1.97 (m, 1H, H-14), 1.94 (m, 1H, H-4b), 1.70 (m, 2H, H-5,17), 1.66 (s, 3H, H-28), 1.34 (s, 3H, H-27), 1.24 (s, 1H), 1.02 (s, 3H, H-18), 0.99 (s, 3H, H-19), 0.91 (d, J = 7.1 Hz, 3H, H-21). ^{13}C NMR (150 MHz, $CDCl_3$) δ 176.2 (C-26), 171.3 (11-OCOCH$_3$), 170.2 (1-OCOCH$_3$), 169.8 (12-OCOCH$_3$), 154.8 (C-23), 111.2 (C-22), 78.9 (C-25), 74.2 (H-11), 74.1 (2xC, C-1,7), 73.5 (C-6), 72.0 (C-15), 71.0 (C-12), 56.3 (C-14), 50.9 (C-24), 48.2 (C-17), 44.6 (C-13), 40.5 (C-10), 37.5 (C-9), 35.3 (C-8), 33.4 (C-5), 31.4 (C-20), 26.0 (C-4), 25.6 (C-27), 22.0 (C-28), 21.7 (11-OCOCH$_3$), 21.3 (12-OCOCH$_3$), 21.0 (1-OCOCH$_3$), 20.5 (C-21), 14.6 (C-18), 13.7 (C-19).

*TA-NaBH$_4$-12 (**26**).* White powder. HRMS (m/z) calcd for $C_{36}H_{49}O_{14}$ $[M + H]^+$ 705.3168, found 705.3137; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.47 (s, 1H, 15-OH), 5.36 (dd, J = 11.7, 2.6 Hz, 1H, H-11), 5.23 (d, J = 2.6 Hz, 1H, H-12), 5.06 (t, J = 2.7 Hz, 1H, H-6), 4.98 (d, J = 1.1 Hz, 1H, H-22), 4.74 (br, 1H, OH), 4.64 (d, J = 5.5 Hz,

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1H, H-1), 4.36 (t, J = 9.2 Hz, 1H, H-15), 3.78 (d, J = 7.0 Hz, 1H, H-7), 3.59 (s, 1H, OH), 3.46 (dd, J = 5.1, 3.9 Hz, 1H, H-2), 3.34 (d, J = 3.3 Hz, 1H, H-3), 2.37 (dd, J = 13.3, 10.6 Hz, 1H, H-16), 2.22 (m, 1H, H-9), 2.21 (s, 3H, 6-OCOCH_3), 2.17 (m, 1H, H-20), 2.12 (s, 3H, 1-OCOCH_3), 2.06 (s, 3H, 12-OCOCH_3), 2.05 (m, 1H, H-5), 1.97 (s, 3H, 11-OCOCH_3), 1.98−1.92 (m, 4H, H-8,14, H_2-4), 1.80 (dd, J = 13.4, 9.8 Hz, 1H, H-17), 1.65 (s, 3H, H-28), 1.32 (s, 1H, H-27), 1.03 (s, 1H, H-18), 1.01 (s, 1H, H-19), 0.90 (d, J = 7.0 Hz, 3H, H-21). ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.5 (C-26), 173.7 (6-OCOCH$_3$), 170.8 (11-OCOCH$_3$), 169.6 (1-OCOCH$_3$), 169.4 (12-OCOCH$_3$), 154.5 (C-23), 110.4 (C-22), 78.9 (C-25), 76.2 (C-6), 74.0 (C-7), 73.6 (C-12), 73.4 (C-1), 71.2 (C-15), 70.5 (C-11), 56.1 (C-14), 52.0 (C-3), 51.2 (C-16), 50.7 (C-24), 49.9 (C-2), 47.6 (C-17), 44.2 (C-13), 39.9 (C-9), 37.0 (C-10), 34.8 (C-8), 31.7 (C-5), 30.8 (C-20), 25.2 (2xC, C-4,27), 21.6 (C-28), 21.0 (11-OCOCH$_3$), 20.7 (1,6-OCOCH$_3$), 20.4 (12-OCOCH$_3$), 20.1 (C-21), 13.9 (C-18), 13.3 (C-19).

TA-NaBH$_4$-12 Epoxide (27). White powder. HRMS (m/z) calcd for $C_{36}H_{49}O_{15}$ $[M + H]^+$ 721.3066, found 721.3043; ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.53 (d, J = 2.8 Hz, 1H, 15-OH), 5.32 (dd, J = 11.8, 2.9 Hz, 1H, H-11), 5.15 (d, J = 2.9 Hz, 1H, H-12), 5.11 (s, 1H, OH), 5.05 (t, J = 2.8 Hz, 1H, H-6), 4.63 (d, J = 5.6 Hz, 1H, H-1), 4.27 (brt, J = 7.2 Hz, 1H, H-15), 3.79 (brd, J = 9.5 Hz, 1H, H-7), 3.53 (s, 1H, OH), 3.46 (m, J = 4.7 Hz, 1H, H-2), 3.33 (br, 1H, H-3), 3.23 (s, 1H, H-22), 2.21 (s, 3H, 6-OCOCH_3), 2.26−1.87 (m, 9H, H_2-4, H-5,8,9,14,16,17,20), 2.12 (s, 3H, 1-OCOCH_3), 2.08 (s, 3H, 12-OCOCH_3), 1.96 (s, 3H, 11-OCOCH_3), 1.73 (s, 3H, H-28), 1.32 (s, 3H, H-27), 1.02 (s, 3H, H-18), 1.01 (d, J = 7.4 Hz, 3H, H-21), 0.90 (s, 3H, H-19).

Inhibition of Cellular Proliferation. The antiproliferative activities and IC_{50} values of the taccalonolides were determined using the sulforhodamine B assay in HeLa cells as previously described.[14] The data are from an average of three experiments, each performed in triplicate, with standard deviation.

Immunofluorescence. Microtubules in interphase HeLa cells were visualized by immunofluorescence using a β-tubulin antibody (Sigma no. 4026). Images were acquired 18 h after indicated drug or vehicle treatment using a Nikon Eclipse 80i fluorescence microscope and NIS Elements AR 3.0 software. Paclitaxel was used as a positive control for microtubule stabilization.

Tubulin Polymerization. The ability of the taccalonolides **23** and **18** to enhance tubulin polymerization was determined as previously described.[17] Briefly, 1 μL of a 100× stock solution of each compound in EtOH was added to a final volume of 100 μL containing 2 mg/mL purified porcine brain tubulin (Cytoskeleton) in GPEM buffer (80 mM Na-Pipes, pH 6.9, 1 mM EGTA, 1 mM $MgCl_2$, 1 mM GTP, and 10% glycerol), and microtubule formation was monitored turbidimetrically at OD_{340} on a Spectramax 96-well plate reader.

***In Vivo* Antitumor Testing.** The antitumor efficacy of the two most potent taccalonolides, **23** and **18**, was evaluated in a MDA-MB-231 triple negative breast cancer xenograft model. Tumor fragments were bilaterally implanted in female nude (nu/nu) mice. Mice were randomly placed into separate treatment groups (n = 5), and dosing was initiated when the average tumor size was 134 mm^3. Taccalonolides **23** and **18** were solubilized in 100% EtOH at 1 and 1.25 mg/mL, respectively, while paclitaxel stocks were solubilized in 50% Cremophor/50% EtOH at 15 mg/mL. Drug stocks were diluted a minimum of 1:10 in 200 μL of phosphate buffered saline immediately prior to intraperitoneal injection. Dose and schedule were determined from prior dose tolerance testing. Taccalonolide **23** was administered at a concentration of 0.7 mg/kg on days 0, 3, and 7 after which dosing was halted due to expenditure of all available material. Taccalonolide **18** was administered at a concentration of 0.25 mg/kg on days 0 and 3, after which dosing was halted due to an average 10% weight loss. Paclitaxel was administered at 15 mg/kg on days 0, 3, and 7. During the period of drug administration, mice were monitored daily, and weight and tumor measurements were taken 2−3 times weekly. Control treated mice were sacrificed on day 17 due to large tumor size; all other mice were sacrificed on day 21. The mice were purchased from Harlan Laboratories, housed in an AALAC-approved facility under fully licensed veterinary care, and provided water and food *ad libitum*.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

NMR spectra of new isolated and synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

‖All authors have given approval to the final version of the manuscript. Jiangnan Peng and April L. Risinger contributed equally to this manuscript.

Notes

Patents protecting the class of compounds disclosed in this paper were filed by the University of Texas Health Science Center at San Antonio.

The authors declare the following competing financial interest(s): All authors are inventors on a patent application for the taccalonolides that is assigned to the University of Texas System.

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■ ABBREVIATIONS USED

DMDO dimethyldioxirane; PBS phosphate-buffered saline

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