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Structure-Activity Relationships of Retro-dihydrochalcones Isolated from *Tacca sp*

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Abstract

Several biologically active compounds have been identified from *Tacca* species, including glycosides, diarylheptanoids, saponins, withanolides, and the taccalonolide class of microtubule stabilizers. More recently, two cytotoxic retro-dihydrochalcones named evelynin A (**7**) and taccabulin A (**6**) were isolated and their biological activities characterized, including the finding that taccabulin has microtubule destabilizing effects. Here we describe the identification and characterization of five new retro-chalcones, named taccabulins B – E (**1–4**) and evelynin B (**5**) from *Tacca sp.* extracts. Their structures were determined using 1D and 2D NMR as well as mass spectroscopic data and modeled into the colchicine binding site of tubulin. The antiproliferative and microtubule effects of each compound were determined experimentally and found to be well correlated with modeling studies. The isolation and biological characterization of several retro-dihydrochalcones facilitated preliminary structure-activity relationships for this compound class concerning its antiproliferative and microtubule depolymerizing activities.

Plants in the genus *Tacca* have yielded an array of biologically active molecules, including the taccalonolide class of microtubule stabilizers.¹ A number of different types of steroids and their glycosides have been reported from *Tacca* spp., including withanolides,² glucosides,^{2–4} and other steroidal glycosides.^{5–9} In addition, diarylheptanoids and diarylheptanoid glycosides were also reported from *T. chantrieri* and *T. plantaginea*.^{10,11} More recently, we have isolated two retro-dihydrochalcones from a *Tacca sp.* named evelynin (**7**)¹² and taccabulin A (**6**).¹³ Evelynin was originally isolated in the process of purification of the taccalonolides and was found to have antiproliferative activity against a

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ASSOCIATED CONTENT

Supporting Information. ¹H, ¹³C, COSY, HSQC, HMBC NMR spectra of compounds **1–5**. This information is available free of charge via the Internet at <http://pubs.acs.org>.

Notes

The authors declare no competing financial interest.

range of cancer cells at low micromolar concentrations.¹² Taccabulin A (**6**) was identified via bioassay-guided fractionation aimed at isolating new microtubule stabilizing taccalonolides from a fraction that had unexpected microtubule destabilizing effects.¹³ Taccabulin A (**6**), which is the first microtubule destabilizer identified from a *Tacca sp.*, was found to have antiproliferative activity against multiple cancer cell lines at nanomolar concentrations and had synergistic effects when combined with the taccalonolides.¹³ The microtubule specific activities of taccabulin A (**6**) were found to be a result of direct binding to the colchicine site on microtubules.

Based on these discoveries, we expanded our investigation of fractions from two *Tacca spp.* for the distinct chemical signatures associated with structurally similar retro-dihydrochalcones. Herein we describe the isolation and characterization of five new retro-dihydrochalcones. Structure-activity relationship (SAR) and modeling studies shed light on the properties of these molecules that are optimal for their biological activities.

RESULTS AND DISCUSSION

Fresh roots and rhizomes of *T. chantrieri* and *T. integrifolia* were extracted using supercritical CO₂. The extracts were fractionated on a silica gel column and fractions screened for antiproliferative activities, their effects on microtubule structure, and chemical signatures associated with retro-dihydrochalcones. The microtubule destabilizing fractions containing taccabulins were further purified by reverse-phase HPLC to yield five new retro-dihydrochalcones named taccabulins B–E (**1–4**) and evelynin B (**5**).

Taccabulin B (**1**) was obtained as a yellow powder. The molecular formula of C₂₁H₂₆O₇ was determined by HRESIMS from an ion with m/z 391.1771 [M + H]⁺ (calcd for C₂₁H₂₇O₇ m/z 391.1757). Its ¹H NMR spectrum was similar to taccabulin A (**6**) but exhibited resonances for an additional methoxy and *O*-methyl group. The *O*-methyl group was determined to be at C-3 based on the up-field shift of H-2, as well as the HMBC correlation between 3-OCH₃ (δ 3.92) and C-3 (δ 149.0). The methoxy group was determined to be on C-3' based on the HMBC correlations from H-5' (δ 6.28) to C-3' (δ 136.5) and C-1' (δ 115.4). Thus, the structure of **1** was determined to be 1-(3,4-dimethoxyphenyl)-3-(2,3,4,6-tetramethoxyphenyl)propan-1-one.

Taccabulin C (**2**) was obtained as a yellow powder. A molecular formula of C₁₈H₂₀O₆ was determined by HRESIMS based on an ion m/z 331.1332 [M + H]⁺ (calcd for m/z C₁₈H₂₁O₆

331.1338). This indicated **2** had one methyl group less than **6**. The ^1H NMR spectrum showed that H-3' (δ 6.18, d, $J = 2.0$ Hz) and H-5' (δ 6.05, d, $J = 2.0$ Hz) were not equivalent, suggesting the loss of the C-2' methyl group in **2**. The chemical shifts and coupling constants of H-2, H-5, and H-6 in **2** suggested a 3, 4-disubstituted A-ring carrying a hydroxy and a methoxy group. The HMBC correlations between $\text{OCH}_3/\text{C-3}$, H-2, H-6/C-4, and H-5/C-3, C-4 indicated a methoxy group at C-3 and hydroxy group at C-4. Thus, the structure of **2** was determined as 1-(4-hydroxy-3-methoxyphenyl)-3-(2-hydroxy-4,6-dimethoxyphenyl)propan-1-one.

Taccabulin D (**3**) was obtained as a yellow powder. A molecular formula of $\text{C}_{19}\text{H}_{22}\text{O}_6$ as **6** was determined by the HRESIMS based on an ion at m/z 347.1487 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{19}\text{H}_{23}\text{O}_6$ m/z 347.1495). The ^1H NMR spectrum of **3** was similar to the spectrum obtained for **6** except for the up-field shift of H-2 and down-field shift of H-5, which suggested that **3** has a 3-methoxy-4-hydroxy substituted A-ring as in **2**. Thus, the structure of **3** was defined as 1-(4-hydroxy-3-methoxyphenyl)-3-(2,4,6-trimethoxyphenyl)propan-1-one and this was supported by full assignment of its 2D NMR data.

Taccabulin E (**4**) was obtained as a pale yellow powder. A molecular formula of $\text{C}_{19}\text{H}_{22}\text{O}_6$ was assigned by HRESIMS based on an ion at m/z 347.1484 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{19}\text{H}_{23}\text{O}_6$ m/z 347.1495). Resonances for three methoxy groups at δ 3.81 (s, 9H) and two aromatic protons at δ 6.16 (s, 2H) were observed in the ^1H NMR spectrum, which suggested that **4** has the same 2,4,6-trimethoxyphenyl unit as **6**. A resonance at δ 5.91 integrating for 2 hydrogens suggested a 3,4-methylenedioxy functionality. An HMBC correlation from the methylenedioxy protons to C-3 and C-4 (δ 148.5 and 148.7) substantiated the illustrated substitution pattern. A resonance at δ 4.13 (dd, $J = 8.5, 5.0$ Hz) correlated with H- α (δ 2.73, m) in the COSY spectrum, indicated the reduction of the carbonyl group to a hydroxy group. Owing to limited quantities of material, the absolute configuration of the C-1 stereogenic center was not determined. Thus, the structure of **4** was determined as 1-(benzo[d][1,3]dioxol-5-yl)-3-(2,4,6-trimethoxyphenyl)propan-1-ol.

Evelynin B (**5**) was obtained as a yellow powder. A molecular formula of $\text{C}_{18}\text{H}_{18}\text{O}_7$ was determined by HRESIMS based on an ion at m/z 345.0965 $[\text{M} + \text{H}]^+$ (calcd for m/z $\text{C}_{18}\text{H}_{19}\text{O}_7$ 345.0974). The ^1H NMR spectrum of **5** differed from that of evelynin A (**7**)¹² by the lack of resonances for two methoxy groups and the presence of a resonance for a methylenedioxy group, suggesting that the 3,4-dimethoxy functionality in **7** was replaced by a methylenedioxy group in **5**. This was supported by the HMBC correlations of the methylenedioxy protons (δ 6.04, 2H) with C-3 and C-4. The other resonances were similar for the two compounds. Thus, the structure of compound **5** was determined as 2-[3-(benzo[d][1,3]dioxol-5-yl)-3-oxopropyl]-3,5-dimethoxycyclohexa-2,5-diene-1,4-dione. Since **5** is similar to the known compound, evelynin (**7**),¹² a trivial name evelynin B was assigned and evelynin will henceforth be referred to as evelynin A (**7**).

The antiproliferative effects of the new taccabulin and evelynin analogues were evaluated against three cancer cell lines: HeLa, A549, and PC-3 (Table 3). The IC_{50} value of evelynin B (**5**) against HeLa cells was found to be 8.8 μM , which is slightly less potent than the 6.3 μM IC_{50} of evelynin A (**7**) in this cell line.¹² This finding indicates that replacement of the

3,4-dimethoxy group in evelynin A (**7**) by a methylenedioxy group in B (**5**) only slightly decreases antiproliferative potency. The IC₅₀ value of evelynin B (**5**) was 8.3 μ M in A549 lung cancer cells, almost identical in HeLa cells, and 5.0 μ M in PC-3 prostate cancer cells. In contrast, each of the new taccabulins was found to be significantly less potent than taccabulin A (**6**), which had an IC₅₀ value of 0.6 μ M in HeLa cells.¹³ Taccabulin D (**3**), which was the most potent of the new taccabulins, was 50-fold less potent than taccabulin A (**6**) with an IC₅₀ value of 28.9 μ M. The only difference between these two compounds is the transposition of hydroxy and methoxy groups at C-3 and C-4, illustrating the importance of these substituents for antiproliferative activity. Taccabulin D (**3**) was even less potent in A549 and PC-3 cells with IC₅₀ values of 40.3 μ M and greater than 50 μ M, respectively. Taccabulins B (**1**), C (**2**), and E (**4**) had IC₅₀ values greater than 50 μ M in all three cell lines. These data indicate that the presence of a hydroxy group at C-2' in taccabulin C (**2**) or reducing the carbonyl group to a hydroxy group in taccabulin E (**4**) is not optimal for activity. Additionally, methylation of the 3-OH or addition of a 3'-methoxy group in taccabulin B (**1**) drastically decreased potency as compared to A (**6**).

The ability of these compounds to directly interact with and inhibit the polymerization of purified tubulin was also determined. Differences in the rate of microtubule formation and total polymer formed (represented by the maximum turbidity at 30 min) are shown in Figure 1. In agreement with previous reports, taccabulin A (**6**) completely inhibited tubulin polymerization at 100 μ M while evelynin A (**7**) had no effect.^{12,13} Evelynin B (**5**) inhibited tubulin polymerization by 40%. The rate of tubulin polymerization was noticeably slowed in the presence of taccabulin D (**3**) along with a 10% decrease in the amount of total polymer formed. Taccabulins E (**4**) and C (**2**) also inhibited total polymer formed to the same degree as taccabulin D (**3**), but unlike **3** they did not alter the rate of polymerization. Interestingly, taccabulin B (**1**) caused a slight but reproducible increase in the rate of microtubule polymerization although total polymer formed was not changed from vehicle treated levels. The extent of inhibition of microtubule polymerization correlated well with antiproliferative potency for all compounds other than evelynin A (**7**), which did not affect microtubule formation in spite of the fact that it was the second most potent antiproliferative compound. This result suggests that the antiproliferative activity of evelynin A (**7**) is unrelated to its ability to bind cellular microtubules, consistent with previous observations.¹² This is not completely unexpected as related structures have been identified that cause cytotoxicity through other mechanisms, including inhibition of topoisomerase I activity or DNA synthesis.^{14,15} The varied effects of the individual compounds on the rate and extent of tubulin polymerization demonstrates that subtle structural differences can have varied and unexpected results on their biological activities.

The microtubule destabilizing activity of each compound is likely directly related to its ability to interact with the colchicine binding site on microtubules. Each of the compounds was modeled into the colchicine binding site on tubulin (Figure 2) and HINT scores for binding affinities were calculated (Table 4). Positive HINT scores, which indicate strong potential binding to the colchicine site, were calculated for taccabulin A (**6**), evelynin B (**5**) and taccabulin E (**4**). In contrast, taccabulins B (**1**), C (**2**), D (**3**) and evelynin A (**7**) showed negative scores, indicative of weak binding. Relatively, taccabulins A (**6**) and B (**1**) are

similar in terms of binding poses, C (2) and D (3) are similar, and E (4) and evelynin B (5) are similar (Figure 2). The 2,4,6-trimethoxyphenyl group of taccabulin A (6) and the 2,3,4,6-tetramethoxyphenyl group of taccabulin B (1) fit into subpocket A of the colchicine binding site (Figure 2A). The second phenyl ring of the two compounds fits into subpocket C and the 1-propanone bridge between the two phenyl rings are located in subpocket B. The 3-hydroxy group of taccabulin A (6) forms hydrogen bonds with both Thr179 α and Val181 α , which is similar to combretastatin A. The 3-methoxy group of taccabulin B (1) only interacts with Val181 α via one hydrogen bond, suggesting taccabulin A (6) binds better than B (1), which is consistent with our biological data (Table 3, Figure 1). The predicted binding poses of taccabulin C (2) and D (3) are slightly different from those of A (6) and B (1) (Figure 2B). However, due to the flexibility of the bridge between the two phenyl rings, the A-ring (in the 2D structure) adopts a similar binding pose. The 3-hydroxy group forms hydrogen bonds with both Thr179 α and Val181 α in a similar manner as taccabulin A (6) does; the 4-methoxy group fits into subpocket A. Within this binding pose, HINT scores predict that taccabulin D (3) binds better than C (2), which is also consistent with our experimental results (Table 3, Figure 1). The binding pose of taccabulin E (4) is similar to the others; the 2,4,6-trimethoxyphenyl ring fits into subpocket A and the A- ring fits into subpocket C (Figure 2C). The difference is that the hydrogen bonding interactions with Thr179 α and Val181 α are not present for this compound and the hydroxy group is located in a hydrophobic area that is not predicted to be favorable for binding. The binding pose of evelynin B (5) is similar to taccabulin E (4) and HINT scores predict similar binding, although the C-3' carbonyl group forms a favorable hydrogen bond with Cys241 β (Figure 2D). Concomitantly, the binding pose of evelynin A (7) is similar to that of taccabulin B (1), with the 3-*O*-methyl clashing with Thr179 α . Although taccabulin E (4) was calculated to have the third highest HINT binding score, its limited ability to inhibit tubulin polymerization suggests that the hydrogen bonding interactions with Thr179 α and Val181 α are more critical for binding than was anticipated by our modeling and that taccabulin E (4) appears to be less acceptable from Cys241 β , which is a critical feature for strongly active compounds that is difficult to score.¹⁶

The finding that the two agents with the highest HINT scores, taccabulin A (6) and evelynin B (5), are the two molecules that inhibit tubulin polymerization further demonstrates the robustness of our modeling analysis even when data are compared across multiple binding poses.

Collectively, the identification, modeling, and experimental determination of antiproliferative and microtubule depolymerizing effects for these seven retro-dihydrochalcones give useful information regarding SARs for this compound class and have facilitated refinement of our model of the colchicine binding site on tubulin.

EXPERIMENTAL SECTION

General Experimental Procedures

NMR spectra were recorded on Bruker Avance 600 MHz and 500 MHz instruments. All NMR data were measured and reported in ppm using TMS as an internal standard. The

HRMS was measured using an Agilent Technologies 6224 TOF LC/MS system. The UV spectrum was obtained online with a Waters 996 PDA Detector. TLC was performed on aluminum sheets (silica gel 60 F254, Merck KGaA, Germany). Preparative HPLC was performed on a Waters Breeze HPLC system using a Phenomenex Luna C₁₈ column (250 × 21.2 mm, 5μm). Gradient elutions were performed using MeCN:H₂O or MeOH:H₂O solvent systems with a flow rate of 9 mL/min. LC/MS was recorded on a Waters Alliance 2695 HPLC equipped with Micromass Quattro triple quadrupole mass spectrometer using the ESI positive mode.

Plant Material

The taccabulins and evelynin B were isolated from pooled minor fractions of the roots and rhizomes of *T. chantrieri* (the black bat flower) and *T. integrifolia* (the white bat flower) obtained from the University of Hawaii at Manoa, Gardino Nurseries in Delray Beach, Florida and the San Antonio Botanical Gardens from 2004–2008. Plants were identified by botanists at each institution: Dr. Will McClatchey, Mr. Sid Gardino, and Mr. Paul Cox, respectively. Voucher specimens of *T. chantrieri* (SLM370, SLM371, SLM373 & SLM374) and *T. integrifolia* (SLM372 & SLM375) are housed in the herbarium of the University of Texas Health Science Center at San Antonio. The roots and rhizomes were collected from living plants and stored at –20 °C until lyophilized.

Extraction and Isolation

The supercritical CO₂ extract (4.21 g) of the roots and rhizomes of *T. chantrieri* and *T. integrifolia* was fractionated on silica gel and eluted with a gradient of CH₂Cl₂:acetone (0~20%). Fraction 11 was separated by C₁₈ HPLC eluted with a gradient MeOH:H₂O (50~100% in 40 min) and was further separated on C₁₈ HPLC using a gradient of MeOH:H₂O (30% to 80% in 40 min) to yield taccabulins A (**6**, 2.0 mg) and B (**1**, 12 mg). Fraction 10, which contained analogues of taccabulin A (**6**) and showed cytotoxicity, was added to a C₁₈ HPLC column, eluted with a MeOH:H₂O gradient, and 10 fractions were collected. Evelynin B (**5**, 4.9 mg) was obtained from fraction 3. Fraction 5 was purified on a C₁₈ HPLC column and eluted with a gradient of MeOH:H₂O to yield taccabulin E (**4**, 0.9 mg). Fraction 2 was further separated on a C₁₈ HPLC column eluted with a gradient MeOH:H₂O solvent system to yield taccabulin C (**2**, 0.8 mg). Fraction 4 was further separated on a C₁₈ HPLC column followed by silica gel PTLC (CH₂Cl₂:acetone 97:3) to obtain taccabulin D (**3**, 0.8 mg).

Taccabulin B (1): yellow powder, UV λ_{max} 230, 276, 306 nm; for ¹H and ¹³C NMR data, see Table 1; HRESIMS *m/z* 391.1771 [M + H]⁺ (calcd for C₂₁H₂₇O₇ 391.1757).

Taccabulin C (2): yellow powder, UV λ_{max} 228, 276, 306 nm; for ¹H and ¹³C NMR data, see Table 1; HRESIMS *m/z* 331.1332 [M + H]⁺ (calcd for C₁₈H₂₁O₆ 331.1338).

Taccabulin D (3): yellow powder, UV λ_{max} 228, 276, 304 nm; for ¹H and ¹³C NMR data, see Table 2; HRESIMS *m/z* 347.1487 [M + H]⁺ (calcd for C₁₉H₂₃O₆ 347.1495).

Taccabulin E (4): yellow powder, UV λ_{max} 231, 282 nm; for ¹H and ¹³C NMR data, see Table 2; HRESIMS *m/z* 347.1484 [M + H]⁺ (calcd for C₁₉H₂₃O₆ 347.1495).

Evelynin B (5): yellow powder, UV λ_{max} 224, 283 nm; for ^1H and ^{13}C NMR data, see Table 2; HRESIMS m/z 345.0965 $[\text{M} + \text{H}]^+$ (calcd $\text{C}_{18}\text{H}_{19}\text{O}_7$ 345.0974).

Biological Assays

HeLa cervical cancer cells, A549 lung cancer cells, and PC-3 prostate cancer cells were purchased from the American Type Culture Collection (ATCC). A549 cells were cultured in Richter's Medium (IMEM) (Life Technologies), PC-3 cells were cultured in RPMI Medium 1640 (Life Technologies) and HeLa cells were cultured in Basal Medium Eagle with Earle's salts (Sigma-Aldrich) each with 10% FBS and 25 – 50 $\mu\text{g/mL}$ gentamicin. The SRB assay was used to determine the antiproliferative potency of the compounds as previously described.¹⁷ The concentration that caused 50% inhibition of proliferation (IC_{50}) was determined from an average of three independent experiments each conducted with triplicate points. Tubulin polymerization experiments were performed with purified porcine tubulin (Cytoskeleton) at a concentration of 3 mg/mL in 10% glycerol GPEM buffer (80 mM Pipes pH 6.8, 1 mM MgCl_2 , 1 mM EGTA, 1mM GTP). Drugs were added to chilled tubulin at a concentration of 100 μM and warmed to 37 $^\circ\text{C}$ to initiate polymerization which was monitored by increased turbidity at A_{340} .

Molecular Modeling

The modeling procedure was generally the same as previously reported.^{16,18} Sybyl 8.1 (Tripos International) was used to prepare the X-ray crystal structures of $\alpha\beta$ -tubulin complexed with different ligands (pdbid: 1SA0, 1SA1, 3HKC, 3HKD and 3HKE) for modeling.^{19,20} The stathmin-like domain and the C and D subunits were deleted. Hydrogen atoms were added and their orientations were optimized by the Tripos force field in Sybyl to a gradient of $0.005 \text{ kcal mol}^{-1} \text{ \AA}^{-1}$. GOLD 5.1²¹ was used for docking. The ligands were docked in the active site, which was defined by the space in a 6 $\text{\AA}$ radius around the complexed small molecule in the crystal. One hundred GOLD Genetic Algorithm runs generated 100 docking conformations for each ligand that were filtered initially by GoldScore. The conformations were further analyzed with HINT.²² The HINT (Hydrophobic INteractions) scoring function evaluates atom-atom interactions using a set of parameters derived from the solvation partition coefficients, LogP, of small molecules measured in the 1-octanol/water system. To select the final "active" conformation, we considered the ligand binding to all five receptor structures and chose the conformation with both a high HINT score and a high degree of similarity to the conformation of the complexed small ligand. The final "active" conformation and the corresponding HINT score were then defined, respectively, as the binding conformation/mode and binding score of the ligand.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Inhibition of tubulin polymerization. Tubulin at a concentration of 30 μM was incubated with vehicle (DMSO) or indicated drug at a final concentration of 100 μM . Tubulin polymerization was monitored by changes in turbidity at A_{340} .

Figure 2.

Modeling of compounds into the colchicine (yellow) binding pocket: **A)** Taccabulin A (red) and taccabulin B (green); **B)** Taccabulin C (orange) and taccabulin D (blue); **C)** Taccabulin E (magenta); **D)** evelynin A (light blue) and evelynin B (maroon).

Table 1¹H and ¹³C NMR Data of Taccabulins B (1) and C (2)

position	1		2	
	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)	δ_{C}
1		130.4		129.3
2	7.55, d (1.9)	110.4	7.54, br	110.0
3		149.0		146.6
4		153.1		151.1
5	6.87, d (8.5)	110.1	6.92, d (8.5)	113.9
6	7.63, dd (8.5, 1.9)	122.9	7.58, brd (8.5)	124.1
1'		115.4		108.8
2'		152.6		156.5
3'		136.5	6.18, d (2.0)	94.7
4'		152.0		160.1
5'	6.29, s	92.6	6.05, d (2.0)	91.3
6'		153.9		159.2
α	3.10, m	38.9	3.34, t (5.5)	38.5
β	2.98, m	19.4	2.93, t (5.5)	16.5
CO		199.1		202.0
3-OCH ₃	3.93, s	56.1	3.93, s	56.1
4-OCH ₃	3.94, s	56.1		
2'-OCH ₃	3.88, s	61.3		
3'-OCH ₃	3.83, s	61.0		
4'-OCH ₃	3.87, s	56.3	3.74, s	55.3
5'-OCH ₃	3.79, s	55.9	3.79, s	55.5
OH			8.87, 6.08, br	

Table 2

¹H and ¹³C NMR Data of Taccabulins D (3), E (4), and Evelynin B (5)

position	3		4		5	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1		130.1		139.0		131.4
2	7.55, d (1.5)	113.7	6.86, d (1.1)	106.7	7.44, br	107.8
3		146.4		147.7		148.2
4		150.0		146.6		151.7
5	6.92, d (8.5)	109.9	6.73, d 8.0	119.3	6.84, d (8.4)	107.9
6	7.60, dd (8.5, 1.5)	123.6	6.77, dd (8.0, 1.1)	108.0	7.56, brd (8.4)	124.4
1'		109.9		110.1		132.0
2'		158.8		158.9		154.7
3'	6.14, s	90.4	6.16, s	90.9		178.0
4'		159.6		159.6		157.3
5'	6.14, s	90.4	6.16, s	90.9	5.85, s	107.0
6'		158.8		158.9		187.2
α	3.03, m	38.3	1.86, m	38.8	3.04, t (7.8)	37.1
β	2.98, m	18.6	2.72, t (6.5)	18.8	2.84, t (7.8)	18.7
CO		199.5		72.8		196.8
3-OCH ₃	3.94, s	56.1				
4'-OCH ₃	3.81, s	55.4	3.81, s	55.5	3.81, s	56.3
6'-OCH ₃	3.78, s	55.6	3.81, s	55.9		
2'-OCH ₃	3.78, s	55.6	3.81, s	55.9	3.98, s	61.0
OCH ₂ O			5.91, s	100.9	6.04 s	101.7

Table 3

Antiproliferative Activities

compounds	IC ₅₀ (μM)		
	<u>HeLa</u>	<u>A549</u>	<u>PC-3</u>
Taccabulin B (1)	> 50	> 50	> 50
Taccabulin C (2)	> 50	> 50	> 50
Taccabulin D (3)	28.9 ± 0.9	40.3 ± 2.9	> 50
Taccabulin E (4)	> 50	> 50	> 50
Evelynin B (5)	8.8 ± 0.5	8.3 ± 0.6	5.0 ± 0.8
Taccabulin A (6)	0.58 ± 0.01	ND	ND
Evelynin A (7)	6.3 ± 0.7	ND	4.7 ± 0.4

The IC₅₀ value of each compound in μM was determined using the SRB assay in HeLa, A549, and PC-3 cancer cells. *n* = 3.

Table 4

HINT Binding Scores

	HINT score
Taccabulin A (6)	261
Taccabulin B (1)	−218
Taccabulin C (2)	−754
Taccabulin D (3)	−546
Taccabulin E (4)	202
Evelynin A (7)	−122
Evelynin B (5)	228