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Alvaradoins E-N, Antitumor and Cytotoxic Anthracenone C-glycosides from the Leaves of *Alvaradoa haitiensis*

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Abstract

Bioactivity-directed fractionation of an extract of the leaves of *Alvaradoa haitiensis*, using the KB (human oral epidermoid carcinoma) cell line, led to the isolation and identification of 10 new anthracenone C-glycosides, alvaradoins E-N (**1–10**), along with the known compound, chrysophanol (**11**). The cytotoxicity of all compounds was evaluated, and preliminary structure-activity relationships are suggested. The most potent compounds in the in vitro assays (**1** and **2**) were evaluated in vivo vs. the P388 (murine lymphocytic leukemia) model, and alvaradoin E (**1**) showed antileukemic activity (125% T/C) at a dose of 0.2 mg kg⁻¹ per injection when administered intraperitoneally.

The plant genus *Alvaradoa*, formerly classified in the family Simaroubaceae, was recently established in the family Picramniaceae and constitutes the subfamily Alvaradoideae.^{1–3} Members of this genus can be found as shrubs or small-to medium-sized trees up to 15 m tall, and *Alvaradoa* is composed of seven species distributed throughout tropical America from southern Florida and Mexico through Central America and the West Indies to South America.^{1,4} These species thrive in a variety of climates, being found at elevations ranging from sea level to above 1500 m.⁴ The literature reports the isolation of bioactive compounds from only two species of *Alvaradoa*. Thus, an extract from the leaves of *A. amorphides*, which is used traditionally to treat skin rashes and head fever, yielded the compounds chaparrin, chrysophanol, and chrysophanein.^{5,6} From the aerial parts of *A. jamaicensis*, the anthracenone C-glycosides, alvaradoins A-D, were isolated and tested for activity against *Mycobacterium*

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tuberculosis, but were inactive.⁷ Anthracenone C-glycosides of a similar structural type have been isolated from the taxonomically related genus, *Picramnia*, also in the Picramniaceae.^{8–13} However, this class of bioactive plant secondary metabolites has not been explored extensively for potential anticancer activity.

As part of a collaborative research program to explore the plant kingdom for novel anticancer agents,¹⁴ the chloroform-soluble extract of the leaves of *Alvaradoa haitiensis* Urb. (Picramniaceae) exhibited promising cytotoxicity against the KB (human oral epidermoid carcinoma) cell line. Using this assay to monitor subsequent bioactivity-directed fractionation studies, 10 new anthracenone C-glycosides were obtained (**1–10**), all of which displayed potent cytotoxic activities. The known compound, chrysophanol (**11**), was also isolated. The structures of the new compounds **1–10** were elucidated via spectrometric and spectroscopic studies, including the determination of their absolute configurations by circular dichroism (CD). All compounds were evaluated in the KB assay, and preliminary structure-activity relationships (SAR) were developed from these data. The most potent compounds (**1** and **2**) were evaluated further in vivo in the P388 (murine lymphocytic leukemia) model.

Results and Discussion

Compounds **1–8** were isolated in the form of four pairs of diastereoisomers. These structurally related pairs (e.g., compounds **1** and **2**) were separated using normal-phase HPLC over a diol column (see Experimental Section). Hence, to simplify the discussion of the structure elucidation, the data for one compound, usually the *S* isomer, from each pair is described in detail. Then, the differences between it and its epimer are explained in the subsequent paragraphs.

The HRFABMS of the potassium adduct of compound **1** yielded a molecular ion peak $[M + K]^+$ at m/z 469.0916, corresponding to the molecular formula, $C_{22}H_{22}O_9$, and indicating an index of hydrogen deficiency of twelve. Fundamental absorptions in the IR spectrum were observed at 3428, 1740, and 1719 cm^{-1} , suggestive of hydroxy and carbonyl moieties, and four bands in the UV spectrum (λ_{max} 202, 268, 297, and 358 nm) showed the presence of a highly conjugated system.

The ^1H and ^{13}C NMR data for compound **1** are given in Table 1. A ketone carbonyl was noted at δ_{C} 195.0 (C-9) in the ^{13}C NMR spectrum, and it was hydrogen-bonded to two non-equivalent phenolic hydroxy groups at C-1 and C-8, as evidenced by ^1H NMR resonances at δ_{H} 12.01 (OH-1) and 11.92 (OH-8), respectively. Based on HSQC data, five protonated aromatic carbons were determined (Table 1): two *meta*-coupled aromatic resonances at $\delta_{\text{H}}/\delta_{\text{C}}$ 6.73/117.1 and 7.04/122.4 were assigned to H-2/C-2 and H-4/C-4, respectively, while three *ortho*-coupled aromatic resonances at $\delta_{\text{H}}/\delta_{\text{C}}$ 6.85/116.4, 7.04/119.1, and 7.56/136.7 were assigned to H-7/C-7, H-5/C-5, and H-6/C-6. A shielded resonance at $\delta_{\text{H}}/\delta_{\text{C}}$ 2.38/22.2 (H_3 -11/C-11) indicated a methyl group, which was attached to δ_{C} 148.0 (C-3) as a result of an HMBC correlation between H_3 -11 and C-3. Resonances at $\delta_{\text{H}}/\delta_{\text{C}}$ 4.66/44.0 (H-10) displayed HMBC correlations with carbons at δ 118.3 (C-1a), 116.1 (C-8a), 142.3 (C-4a), and 146.7 (C-5a), as well as with C-4 and C-5. From these data, two benzene rings, a non-aromatic ring, and a ketone carbonyl were characterized, all of which could be ascribed to an anthracenone skeleton, similar to alvaradoins A-D⁷ and chrysophanol (**11**).^{15,16}

The remaining resonances observed in the ^1H and ^{13}C NMR spectra of **1** were attributable to a glycosyl unit. The ^1H , ^{13}C , HSQC, and HMBC NMR spectroscopic data (Table 1 and Figure 1) displayed resonances characteristic for a glycosyl moiety containing five methine groups at $\delta_{\text{H}}/\delta_{\text{C}}$ 3.84/81.3 (H-5'/C-5'), 3.59–3.62/68.2 (H-4'/C-4'), 3.65–3.69/73.1 (H-3'/C-3'), 3.65–3.69/70.6 (H-2'/C-2'), and 5.63/94.4 (H-1'/C-1'). This glycone was attached to the anthracenone

core at C-10, as determined by HMBC correlations from H-10 to C-5' and H-5' to C-5a, as shown. H-1' showed HMBC correlations to C-5', C-3', and to a carbonyl at δ_C 168.3 (C-1'') of an acetoxy group, the methyl group of which (δ_H/δ_C 1.76/20.4; C-2'') showed HMBC correlations from H-2'' to C-1''. H-1' was positioned equatorially and the acetoxy group axially based upon the extremely small coupling between H-2' and H-1' ($J < 1$ Hz), and this finding was supported by a strong ROESY correlation between these two hydrogens. H-5' displayed a doublet of doublets splitting pattern in the 1H NMR spectrum, and the J values indicated axial coupling to H-4' (9.8 Hz), thereby placing the anthracenone core in the equatorial position ($J=2.4$ Hz for coupling to H-10). Although the J values for the coupling of H-4' to H-3' could not be interpreted in the crowded, upfield region of the 1H NMR spectrum of **1**, both were presumed to be in axial positions, the former due to the aforementioned diaxial coupling to H-5' and the latter due to ROESY correlations between H-5' and H-3'. In summation, due to the axial protons at C-4' and C-3' and equatorial protons at C-2' and C-1', the glycone moiety was determined to be a lyxopyranose unit containing an O-acetyl group at C-1'.

The absolute configuration of **1** at C-10 was determined using ROESY NMR and circular dichroism (CD) spectroscopic data. Key ROESY correlations were observed between H-10 with H-5', H-4, and H-5, further supporting the connectivity of the glycone unit at C-10 (Table 1). Also, cross-peak correlations between H-5' with H-5, and H-4' with H-4, indicated the *S* configuration for C-10 (Figure 2). This was supported by the CD spectrum, which showed a positive Cotton effect at 297 nm and negative Cotton effects at 323 and 268 nm, as reported for (10*S*)-aloin.^{17,18} These results are analogous to those reported for sarcoside¹² and picramnioside B.⁹

The second $C_{22}H_{22}O_9$ isomer, compound **2**, displayed splitting patterns, chemical shifts, and coupling constants in the NMR spectra that were nearly identical to those of **1** (Table 1). Differences, however, were observed between these two compounds in their ROESY NMR spectra, specific rotation data, CD spectra, and melting points. The relative configuration was determined from observations in the ROESY spectrum from cross-peak correlations between H-5' with H-4, and H-4' with H-5, indicating the *R* configuration at C-10, analogous to mayoside¹¹ and picramnioside C.⁹ Furthermore, **2** showed a negative Cotton effect at 299 nm and positive Cotton effects at 353 and 257 nm, as reported for (10*R*)-aloin,^{17,18} supporting the opposite configuration of C-10 in compounds **1** and **2**. These results are analogous to those reported for similar pairs of diastereoisomers, such as aloins A and B,^{18,19} 10-hydroxyaloin A and B,²⁰ and cascarosides A, B, C, and D.¹⁷ Following the nomenclature first established with a related species of *Alvaradoa* by Harding et al.,⁷ compounds **1** and **2** were ascribed the trivial names alvaradoin E and alvaradoin F, respectively.

The HRFABMS of the potassium adduct of compound **4** yielded a molecular ion peak $[M + K]^+$ at m/z 551.1324 by HRFABMS, corresponding to a molecular formula of $C_{27}H_{28}O_{10}$ and indicating an index of hydrogen deficiency of 14. As with **1** and **2**, absorptions in the IR spectrum were observed at 3414, 1741, 1725, and 1638 cm^{-1} , suggestive of hydroxy and carbonyl moieties, and four bands in the UV spectrum (λ_{max} 201, 268, 296, and 359 nm) indicated a highly conjugated system.

The 1H and ^{13}C NMR data for **4** (Tables 2 and 3) displayed chemical shifts and splitting patterns that were similar to those observed with **1** and **2**. The hydrogen deficiency increased by two and the molecular formula by C_5H_6O over the aforementioned compounds, and this could be rationalized via the addition of a 3-methylbut-2-enoyl (seneciyl) moiety to the glycone. This side chain was characterized via a series of resonances (H-2'', H-4'', and H-5'' in Table 2 and C-1'' to C-5'' in Table 3), and this included discerning which methyl group was *cis* (H-5''/C-5'') vs. *trans* (H-4''/C-4'') to proton H-2'', based on chemical shift differences²¹ and a strong ROESY correlation between H-2'' and H-5''; these findings are opposite of those reported for

a similar series of compounds.⁷ The point of attachment for the glycone to the anthracenone core was analogous to what was observed with **1** and **2**, via resonances at δ_H/δ_C 3.99/81.6 (H-5'/C-5') that displayed axial coupling to H-4' (J value of 9.9 Hz), and was supported by an HMBC correlation of H-5' to C-4a. Similarly, the acetoxy group (H-1'''/C-1''' and H-2'''/C-2''') was connected to position C-1', as in both **1** and **2**, via an HMBC correlation from H-1' to C-1'''. Likewise, hydroxy groups were observed at C-4' and C-2', again based on HMBC correlations. Finally, the chemical shifts of H-3'/C-3' shifted downfield as compared to **1** and **2**, especially apparent in the ¹H NMR data (Table 2), due to attachment of the 3-methylbut-2-enoyl moiety, and this was substantiated by an HMBC correlation from H-3' to C-1''. Analogous to **1**, the absolute configuration of **4** was determined to be 10*S* using ROESY and CD data. Key ROESY signals included correlations between H-5' with H-5, and H-4' with H-4, and the CD spectrum revealed a positive Cotton effect at 296 nm and negative Cotton effects at 322 and 268 nm.

For the second C₂₇H₂₈O₁₀ isomer, **3**, the chemical shifts, splitting patterns and coupling constants were similar to those observed in **4**, but the compounds differed in their specific rotations, CD spectra, and melting points. The absolute configuration at C-10 was determined to be 10*R* from the CD spectrum, in which a negative Cotton effect at 296 nm and positive Cotton effects at 351 and 258 nm were observed, indicating a configuration that was analogous to **2**. This pair was ascribed the trivial names alvaradoin G (**3**) and alvaradoin H (**4**).²²

The HRMS of the sodium adduct of compound **5** displayed a molecular ion peak [M + Na]⁺ at m/z 551.1525, corresponding to the molecular formula C₂₇H₂₈O₁₁. Relative to compound **4**, these data indicated the addition of a single oxygen atom. The NMR data of **4** and **5** were also quite similar (Tables 2 and 3), although the latter displayed an oxygenated quaternary carbon at δ_C 76.3 (C-10). The amalgamation of these data demonstrated that compound **5** is representative of the C-10 hydroxylated analog of compound **4**, and HMBC correlations of δ_H 6.41 (OH-10) with both C-10 and C-5' (δ_C 79.8) supported this structural assignment. The relative configuration of **5** at C-10 was determined by ROESY experiments, which displayed correlations between H-5' and H-5 and between H-4' and H-4, indicating a configuration similar to **4**, although this was now designated as *R* due to the priority of the hydroxy moiety. Similarly, the CD spectrum of **5** was nearly identical to that of **4**, with a positive Cotton effect at 301 nm and negative Cotton effects at 332 and 268 nm.

The other C₂₇H₂₈O₁₁ isomer (compound **6**) had NMR data that were similar to compound **5** (Tables 2 and 3), although clear differences were noted in their specific rotation, CD, and melting point data. Akin to the discussion above, these data suggested that **6** is the hydroxylated analog of **3**, and this finding was supported by HMBC correlations that demonstrated OH-10 is attached to C-10. In the ROESY spectrum, cross-peak correlations were observed between H-5' and H-4 and between H-4' and H-5, which indicated a configuration similar to compound **3** that is indicated as 10*S*, again due to the priority of the hydroxy moiety. The CD spectrum revealed a negative Cotton effect at 299 nm and positive Cotton effects at 353 and 260 nm, supporting the opposite configuration at C-10 for these anthracenone C-glycosides, and accordingly, this pair of compounds were ascribed the trivial names alvaradoin I (**5**) and alvaradoin J (**6**).

The HRMS of the sodium adducts of compounds **7–9** were similar, having peaks for [M + Na]⁺ at m/z 493.1475, 493.1452, and 493.1461, respectively, which corresponded to a molecular formula of C₂₅H₂₆O₉ for each compound. The spectroscopic data were consistent with those observed for the aforementioned compounds (Tables 2 and 3), indicating a very similar ring system and substitution patterns. Compared to compounds **5** and **6**, these compounds did not appear to have a C-10 hydroxy moiety, as evidenced by the ¹³C NMR data for C-10, which was δ_C 44.6 for both **7** and **8** and δ_C 44.9 for **9**. Also in relation to compounds **5** and **6**, the 3-methylbut-2-enoyl moiety was located at C-2' of the glycone in compounds **7**

and **8**, as supported by HMBC correlations between H-2' and C-1". For compound **9** the 3-methylbut-2-enoyl moiety was placed at C-3' of the glycone, as corroborated by the HMBC correlation between δ_{H} 5.07 (H-3') and δ_{C} 166.5 (C-1"). Resonances for an acetate moiety in the glycone were not apparent in any of these three compounds, and instead a hydroxy group was observed at position C-1', as noted by HSQC data for compounds **7–9** via resonances at $\delta_{\text{H}}/\delta_{\text{C}}$ 4.81/92.9, 4.81/93.0, and 4.81/95.7, respectively. Compounds **7** and **8** were nearly identical, based on their 1-D NMR data (Tables 2 and 3), but differed in their relative configuration. As indicated by the ROESY data, cross-peak correlations between H-5' and H-5 and between H-4' and H-4 indicated the *S* configuration at C-10 for **8**, while cross-peak correlations between H-5' and H-4 and between H-4' and H-5 indicated the *R* configuration at C-10 for **7**. Although the 1-D NMR data between compounds **8** and **9** were somewhat different, they displayed similar ROESY correlations, indicating also an *S* configuration at C-10 in compound **9**. The CD data exhibited Cotton effects similar to **1** for both compounds **8** and **9**, and Cotton effects similar to **2** for compound **7** (see Experimental Section). Although the 10*R* analog of compound **9** appeared to be present, not enough material was isolated for thorough structure elucidation studies. This trio of isomers were ascribed the trivial names alvaradoin K (**7**), alvaradoin L (**8**), and alvaradoin M (**9**).

The HRMS of the sodium adduct of compound **10** revealed a peak corresponding to $[\text{M} + \text{Na}]^+$ at m/z 469.1097, suggesting a molecular formula of $\text{C}_{22}\text{H}_{22}\text{O}_{10}$. Based on spectroscopic properties, especially in the ^1H and ^{13}C NMR spectra (Tables 2 and 3), this compound was similar to compound **6**, including the presence of a hydroxy moiety at C-10 (δ_{C} 76.3). The major difference between these compounds was the replacement of the 3-methylbut-2-enoyl moiety at position C-3' in **6** with a hydroxy group in **10**, as supported by HMBC data from δ_{H} 4.05 (OH-3') to δ_{C} 72.7 (C-3'). Using CD data, the absolute configuration of **10** was found to be 10*S*, again analogous to compound **6**. This compound was ascribed the trivial name alvaradoin N, and, as with compound **9**, despite considerable effort, it was not feasible to isolate the 10*R* analog of **10**.

Compounds **1–11** were tested for activity in the KB cell line (human oral epidermoid carcinoma; Table 4). Compounds **1** and **2** were the most cytotoxic, having EC_{50} values that were only an order of magnitude less active than the positive control, camptothecin. In compounds **3** and **4**, where the hydroxy at R₂ of the glycoside of **1** and **2** has been esterified as a 3-methylbut-2-enoyl moiety, the EC_{50} values dropped by another order of magnitude. Analogous results were observed in compounds **7–9**, where an identical conversion was placed at R₃ (**7** and **8**) or R₂ (**9**) of the glycoside and the R₄ acetate was converted to a hydroxy moiety. In compounds **5**, **6**, and **10**, where a hydroxy was inserted at C-10 of the anthracenone, cytotoxicity was reduced substantially. Chrysophanol (**11**), which represents the core anthracenone aglycone of **1–10**, was completely inactive, suggesting that a combination of the anthracenone and glycoside units is essential for cytotoxic activity. Four structurally related compounds (alvaradoins A–D) have been described from a related species of *Alvaradoa*, and although the authors did not report cytotoxicity test results, these compounds were reported as inactive against *Mycobacterium tuberculosis* when tested at 12.5 $\mu\text{g/mL}$.⁷

Compounds **1** and **2** exhibited sufficient cytotoxicity to justify further evaluation using the in vivo P388 murine lymphocytic leukemia model.²³ Modest in vivo activity against intraperitoneal (i.p.) implanted P388 xenografts (T/C of 125%) was observed with compound **1** when mice were injected via the i.p. route daily for 5 days per week at an optimal dose of 0.2 mg kg^{-1} body weight per injection. Compound **2** was evaluated in the same model, but displayed less activity (115% T/C), even when delivered at its maximally tolerated dose of 0.4 mg kg^{-1} per injection using the same model and dosing schedule. This suggests that compound **1** possesses the preferred stereochemistry, and as such, **1** is under consideration for further evaluation, possibly via derivatization and/or analog development of the lead pharmacophore.

Moreover, **1** and **2** were evaluated in a similar P388 model, where both the cancer cells and the treatment were delivered intravenously. In this more rigorous model of chemotherapy, both compounds were found to be inactive, suggesting metabolic degradation or other issues that influence drug distribution or accessibility of leukemia cells in bone marrow. These latter findings are consistent with those reported by our group earlier,²⁴ whereby *in vivo* activity for **1** and **2** was noted in the hollow fiber assay only when the cancer cells were implanted *i.p.*

Experimental Section

General Experimental Procedures

Melting points were measured either on a Kofler hot-stage apparatus or a Mel-Temp II digital thermometer melting point apparatus and are uncorrected. Optical rotations were measured on a Rudolph Research Autopol III automatic polarimeter at 25 °C and the $[\alpha]_D$ values are given in $10^{-1} \text{ deg cm}^2 \text{ g}^{-1}$. CD spectra were obtained from an Aviv Stopped Flow Model 202 spectrometer. UV spectra were recorded on a Varian Cary 3G UV-Visible spectrophotometer, and IR (NaCl or KBr pellet) spectra were recorded on a Nicolet Avatar 360 FT-IR spectrometer. All NMR experiments were performed using a Bruker AMX-500 spectrometer with TMS as an internal standard in acetone- d_6 , except for compound **11**, for which CDCl_3 was utilized. EIMS and ESIMS were recorded on HP5989A and Finnigan LCQ instruments, respectively. HRMS measurements were obtained via direct-insertion probe EI or fast-atom bombardment on a VG70S magnetic sector instrument (Micromass, Beverly, MA) or by an Applied Biosystems (Framingham, MA) TOF/TOF mass spectrometer, equipped with a Nd:YAG laser operating at 355 nm and 200 Hz. This latter instrument was operated in the reflectron mode, and the matrix employed was 2,5-dihydroxybenzoic acid prepared at a concentration of 9 mg/mL in 70:30 (v:v) acetonitrile-0.1% trifluoroacetic acid. Column chromatography was carried out on Si gel 60 (230–400 mesh, Merck, Darmstadt, Germany). Fractions were monitored by TLC (silica gel 60 F254 plates, 0.25 mm thickness) visualized with UV light (254 and 365 nm) and with 1% H_2SO_4 in EtOH. Preparative HPLC was carried out via a Varian Prostar 210 pump system using either a Diol NP or ODS-A (both $250 \times 25 \text{ mm}$, i.d., $5 \mu\text{m}$; YMC, Wilmington, NC) columns. The peaks were detected using a Prostar 330 PDA detector and data were recorded by Star Chromatography Workstation version 5.51 software system. Empirically, flow rates of either 7 or 10 mL/min were utilized on a case-by-case basis. The percent yields of isolated compounds were calculated based on the dry weight of the plant material (w/w).

Plant Material

Leaves of *Alvaradoa haitiensis* Urb. (Picramniaceae) were collected under a Consultant Agreement in February 1996 by F. J. and R. G. at Cordillera Central, San Cristobal Province, Dominican Republic and dried. Voucher specimens (#2047 and 7357) have been deposited at the Field Museum of Natural History, Chicago, IL.

Extraction and Isolation

The dried and ground leaves (5408 g) of *A. haitiensis* were extracted with MeOH ($6 \text{ L} \times 2$) for 24 h at rt and concentrated *in vacuo*. The concentrated MeOH solution was diluted with H_2O to give a MeOH- H_2O (9:1) solution (2 L), defatted with hexane ($2 \text{ L} \times 2$), and then concentrated *in vacuo*. The aqueous MeOH fraction was dissolved in CHCl_3 -MeOH (4:1, 1 L) and partitioned further with H_2O ($1 \text{ L} \times 2$). The organic fraction was washed using 1% saline solution until there was no evidence of tannins,²⁵ and concentrated *in vacuo* to afford 169 g of a crude extract with a high degree of cytotoxicity (1% survival of KB cells tested at a concentration of $20 \mu\text{g/mL}$). This extract was purified by low-pressure column chromatography with Si gel (1300 g) using gradient mixtures of 50→100% CHCl_3 in hexane then 0→10% MeOH in CHCl_3 , resulting in 17 pooled fractions (F01-F17). Of these, F07-F14 showed significant inhibition of KB cancer cells (<10% survival at $2 \mu\text{g/mL}$). A precipitate from F07

and F08 [eluted with CHCl₃-MeOH (99:1); <10% survival at 2 µg/mL] was recrystallized from CHCl₃-MeOH (4:1) to yield 1.6 g of solid, which contained an approximate 3:2 mixture of compounds **3** and **4**, according to the ¹H NMR spectrum. These compounds were separated by gradient normal-phase HPLC with a YMC-Diol NP column under the following conditions: A:B (A = 19:1, CHCl₃-isopropanol; B = hexane) (33:67→40:60 for 28 min) then isocratic (40:60 for 17 min) to yield pure compounds **3** and **4**.

F09 [eluted with CHCl₃-MeOH (98.5:1.5); KB, 5% survival at 2 µg/mL] was subjected to Si gel chromatography using a gradient of hexane-acetone (100:0→0:100) to afford 14 fractions (F15-F28). F26 [eluted with hexane-acetone (3:2); 3% survival at 0.2 µg/mL] was purified by reversed-phase preparative HPLC using a YMC ODS-A C₁₈ column under the following conditions: MeCN-H₂O (45:55→60:40 for 45 min) then (60:40→100:0 for 5 min) resulting in four impure fractions (F29-F32). Further purification of F29 was achieved using normal-phase preparative HPLC using a YMC-Diol column with a solvent system A:B (A = 19:1, CHCl₃-isopropanol; B = hexane) (65:35→90:10 for 50 min) to yield pure compounds **7** and **8**. In turn, F30 was purified using the same HPLC column with solvent system of A:B (A = 9:1, CHCl₃-isopropanol; B = hexane) (30:70→40:60 for 60 min) to yield pure compounds **5**, **6**, and **9**.

F13 [eluted with CHCl₃-MeOH (19:1); KB, <5% survival at 2 µg/mL] was subjected to Si gel chromatography using hexane-acetone (90:10→0:100, gradient mixtures), resulting in 11 fractions (F33-F43). A precipitate formed from F38 [eluted with hexane-acetone (55:45); >5% survival at 2 µg/mL] to give a yellow solid (1.2 g). The solid was purified via preparative HPLC using a YMC-Diol NP under the following conditions: MeOH-CHCl₃ (2:98→6:94 for 40 min), affording pure compounds **1**, **2**, and **10**.

Alvaradoin E [(10S)-C-(1-O-acetyl)-β-L-lyxopyranosyl-1,8-dihydroxy-3-methylanthracen-9(10H)-one, 1]: yellow solid (219.5 mg, yield 0.0041% w/w); *t*_R 14.12 min in 50:50→100:0 MeOH-H₂O over 25 min and *t*_R 13.32 min in 2:98→4:96 MeOH-CHCl₃ over 20 min, with the ODS A and Diol NP columns, respectively; mp 194–196 °C; [α]_D –16.8 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log Σ) 358 (3.97), 297 (3.85), 268 (3.79), 202 (4.43) nm; CD (MeOH) [θ] (nm) –5.87 × 10^{–6} (323), +8.66 × 10^{–6} (297), –5.40 × 10^{–6} (268); IR (KBr) ν_{max} 3428, 2921, 1740, 1636, 1618, 1457, 1294, 1234, 1139, 1021, 959, 806, 761 cm^{–1}; ¹H and ¹³C NMR data, see Table 1; HMBC data, see Table 1; ESIMS *m/z* 453 [M + Na]⁺; HRFABMS *m/z* 469.0916 [M + K]⁺ (calcd for C₂₂H₂₂O₉K, 469.0901).

Alvaradoin F [(10R)-C-(1-O-acetyl)-β-L-lyxopyranosyl-1,8-dihydroxy-3-methylanthracen-9(10H)-one, 2]: yellow solid (93.5 mg, yield 0.0017% w/w); *t*_R 13.95 min in 50:50→100:0 MeOH-H₂O over 25 min and *t*_R 14.39 min in 2:98→4:96 MeOH-CHCl₃ over 20 min, with the ODS A and Diol NP columns, respectively; mp 210–213 °C; [α]_D –107.7 (*c* 0.05, methanol); UV (MeOH) λ_{max} (log Σ) 357 (4.03), 296 (3.94), 268 (3.89), 203 (4.46) nm; CD (MeOH) [θ] (nm) +4.73 × 10^{–6} (353), –2.44 × 10^{–7} (299), +2.63 × 10^{–6} (257); IR (KBr) ν_{max} 3423, 2924, 1750, 1635, 1617, 1457, 1293, 1230, 1139, 1021, 961, 800, 770 cm^{–1}; ¹H and ¹³C NMR data, see Table 1; HMBC data, see Table 1; ESIMS *m/z* 453 [M + Na]⁺; HRFABMS *m/z* 469.0916 [M + K]⁺ (calcd for C₂₂H₂₂O₉K, 469.0901).

Alvaradoin G [(10R)-C-(1-O-acetyl-3-O-senecioid)-β-L-lyxopyranosyl-1,8-dihydroxy-3-methylanthracen-9(10H)-one, 3]: brown solid (6.9 mg, yield 0.00013% w/w); *t*_R 16.64 min in 50:50→100:0 MeOH-H₂O over 25 min and *t*_R 17.11 min in 38:62 A:B (A = 19:1 CHCl₃-isopropanol, B = hexane) over 30 min, with the ODS A and Diol NP columns, respectively; mp 197–200 °C; [α]_D –85.0 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log Σ) 360 (3.99), 295 (3.92), 268 (3.94), 205 (4.67) nm; CD (MeOH) [θ] (nm) +3.11 × 10^{–6} (351), –2.03 × 10^{–7} (296), +1.62 × 10^{–6} (258); IR (NaCl) ν_{max} 3446, 2990, 1759, 1693, 1616, 1596, 1286, 1220, 1127, 1018,

756 cm⁻¹; ¹H and ¹³C NMR data, see Tables 2 and 3; HMBC H-2/C-1, C-3, CH₃-11, C-4; H-4/C-2, C-10, CH₃-11; H-5/C-6, C-10, C-7; H-6/C-5a, C-8; H-7/C-5; H-10/C-4a, C-5a, C-4, C-5, C-1a, C-8a, C-1'; CH₃-11/C-2, C-3, C-4; H-5'/C-4', C-4a, C-5a; H-4'/C-3'; H-3'/C-4', C-1''; H-2'/C-3'; H-1'/C-5', C-3', C-2', C-1'''; H-2''/C-1'', C-4'', C-5''; CH₃-4''/C-2'', C-3'', C-5''; CH₃-5''/C-2'', C-3'', C-4''; CH₃-2'''/C-1'''; OH-1/C-1, C-1a; OH-8/C-8, C-8a; OH-4'/C-5', C-4'; OH-2'/C-3', C-2', C-1'; EIMS *m/z* 512 [M]⁺ (2), 452 (1), 352 (5), 280 (5), 265 (6), 240 (100), 165 (9); HRFABMS *m/z* 551.1336 [M + K]⁺ (calcd for C₂₇H₂₈O₁₀K, 551.1319).

Alvaradoin H [(10S)-C-(1-O-acetyl-3-O-senecioidyl)-β-L-lyxopyranosyl-1,8-dihydroxy-3-methylanthracen-9(10H)-one, 4]: yellow solid (12 mg, yield 0.00022% w/w); *t_R* 16.72 min in 50:50→100:0 MeOH-H₂O over 25 min and *t_R* 15.45 min in 38:62 A:B (A = 19:1 CHCl₃-isopropanol, B = hexane) over 30 min, with the ODS A and Diol NP columns, respectively; mp 240–243 °C; [α]_D –26.0 (c 0.05, MeOH); UV (MeOH) λ_{max} (log Σ) 358 (4.06), 297 (3.98), 268 (3.98), 207 (4.77) nm; CD (MeOH) [θ] (nm) –5.18 × 10⁻⁶ (322), +3.46 × 10⁻⁶ (296), –6.00 × 10⁻⁶ (268); IR (NaCl) ν_{max} 3460, 3026, 2977, 1747, 1693, 1617, 1598, 1294, 1228, 1156, 1022, 752 cm⁻¹; ¹H and ¹³C NMR data, see Tables 2 and 3; HMBC H-2/C-1, C-1a, CH₃-11, C-4; H-4/C-2, C-10, CH₃-11; H-5/C-10; H-6/C-5a, C-8; H-7/C-5, C-8; H-10/C-4a, C-5a, C-4, C-5, C-1a, C-8a, C-5'; CH₃-11/C-2, C-3, C-4; H-5'/C-4', C-4a; H-4'/C-3'; H-3'/C-4'; H-2'/C-3'; H-1'/C-5', C-3', C-2', C-1'''; H-2''/C-1'', C-4'', C-5''; CH₃-4''/C-2'', C-3'', C-5''; CH₃-5''/C-2'', C-3'', C-4''; CH₃-2'''/C-1'''; OH-1/C-1, C-1a, C-2; OH-8/C-8, C-8a; OH-4'/C-5', C-4'; OH-2'/C-2', C-1'; EIMS *m/z* 512 [M]⁺ (1), 452 (2), 352 (5), 280 (34), 265 (45), 240 (100), 165 (18); HRFABMS *m/z* 551.1324 [M + K]⁺ (calcd for C₂₇H₂₈O₁₀K, 551.1319).

Alvaradoin I [(10R)-C-(1-O-acetyl-3-O-senecioidyl)-β-L-lyxopyranosyl-1,8,10-trihydroxy-3-methylanthracen-9-one, 5]: yellow solid (6.0 mg, yield 0.00011% w/w); *t_R* 13.24 min in 50:50→100:0 MeOH-H₂O over 25 min and *t_R* 9.92 min in 20:80→60:40 A:B (A = 9:1 CHCl₃-isopropanol, B = hexane) over 10 min, with the ODS A and Diol NP columns, respectively; mp 148–149 °C; [α]_D –11.7 (c 0.06, MeOH); UV (MeOH) λ_{max} (log Σ) 369 (3.89), 300 (3.71), 213 (4.50) nm; CD (MeOH) [θ] (nm) –1.73 × 10⁶ (332), +7.61 × 10⁶ (301), –8.98 × 10⁶ (268); IR (NaCl) ν_{max} 3394, 3016, 2980, 2917, 1719, 1642, 1220, 1142, 757 cm⁻¹; ¹H and ¹³C NMR data, see Tables 2 and 3; HMBC H-2/C-1, C-1a, CH₃-11, C-4; H-4/C-1a, C-2, C-10, CH₃-11; H-5/C-7, C-8a, C-10; H-6/C-5a, C-8; H-7/C-5, C-8a; CH₃-11/C-2, C-3, C-4; H-1'/C-4a, C-5a, C-10, C-4', C-3', C-1'; H-4'/C-3'; H-3'/C-4', C-2', C-1''; H-1'/C-5', C-3', C-5'; H-2''/C-4'', C-5''; CH₃-4''/C-2'', C-3'', C-5''; CH₃-5''/C-2'', C-3'', C-4''; CH₃-2'''/C-1'''; OH-1/C-1, C-1a; OH-8/C-7, C-8, C-8a; OH-2'/C-2', C-1'; EIMS *m/z* 528 [M]⁺ (3), 468 (1), 315 (3), 298 (8), 273 (14), 256 (100), 240 (2), 213 (24), 83 (53); HRMALDITOFMS *m/z* 551.1518 [M + Na]⁺ (calcd for C₂₇H₂₈O₁₁Na, 551.1529).

Alvaradoin J [(10S)-C-(1-O-acetyl-3-O-senecioidyl)-β-L-lyxopyranosyl-1,8,10-trihydroxy-3-methylanthracen-9-one, 6]: yellow solid (10.4 mg, yield 0.00019% w/w); *t_R* 13.27 min in 50:50→100:0 MeOH-H₂O over 25 min and *t_R* 10.39 min in 20:80→60:40 A:B (A = 9:1 CHCl₃-isopropanol, B = hexane) over 10 min, with the ODS A and Diol NP columns, respectively; mp 138–139 °C; [α]_D –56.7 (c 0.05, MeOH); UV (MeOH) λ_{max} (log Σ) 369 (3.88), 300 (3.68), 213 (4.48) nm; CD (MeOH) [θ] (nm) +2.10 × 10⁶ (353), –1.40 × 10⁷ (299), +4.29 × 10⁶ (260); IR (NaCl) ν_{max} 3417, 3021, 2980, 2923, 1723, 1604, 1287, 1217, 1143, 754; ¹H and ¹³C NMR data, see Tables 2 and 3; HMBC H-2/C-1, C-1a, CH₃-11, C-4; H-4/C-1a, C-2, C-3, C-10, CH₃-11; H-5/C-6, C-7, C-8a, C-10; H-6/C-5a, C-8; H-7/C-5, C-8, C-8a; CH₃-11/C-2, C-3, C-4; H-5'/C-4a, C-5a, C-10, C-4', C-3', C-1'; H-4'/C-3'; H-3'/C-4', C-1''; H-1'/C-5', C-3', C-2', C-1'''; H-2''/C-4'', C-5''; CH₃-4''/C-2'', C-3'', C-5''; CH₃-5''/C-2'', C-3'', C-4''; CH₃-2'''/C-1'''; OH-1/C-1, C-1a, C-2; OH-8/C-7, C-8; OH-10/C-4a, C-10, C-5'; OH-2'/C-2', C-1'; EIMS *m/z* 528 [M]⁺ (2), 468 (1), 315 (2), 298 (2), 285 (3), 273 (15), 256 (100), 240 (3), 213 (23), 83 (48); HRMALDITOFMS *m/z* 551.1525 [M + Na]⁺ (calcd for C₂₇H₂₈O₁₁Na, 551.1529).

Alvaradoin K [(10*R*)-*C*-(2-*O*-senecioidyl)- β -*L*-lyxopyranosyl-1,8-dihydroxy-3-methylanthracen-9(10*H*)-one, 7]: yellow solid (14 mg, yield 0.00026% w/w); t_R 17.56 min in 50:50→100:0 MeOH-H₂O over 25 min and t_R 10.95 min in 80:20→95:5 A:B (A = 19:1 CHCl₃-isopropanol, B = hexane) over 15 min, with the ODS A and Diol NP columns, respectively; mp 143–144 °C; $[\alpha]_D$ –65.0 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log Σ) 359 (3.87), 297 (3.77), 210 (4.42) nm; CD (MeOH) $[\theta]$ (nm) $+1.47 \times 10^6$ (351), -8.51×10^6 (298); IR (NaCl) ν_{max} 3451, 3020, 2975, 2936, 1690, 1603, 1291, 1229, 1074, 754 cm⁻¹; ¹H and ¹³C NMR data, see Tables 2 and 3; HMBC H-2/C-1, CH₃-11, C-4; H-4/C-10, CH₃-11; H-5/C-6, C-10; H-6/C-5a, C-8; H-7/C-5; H-10/C-4a, C-5a, C-1a, C-8a, C-5', C-4'; CH₃-11/C-2, C-3, C-4; H-5'/C-10, C-4', C-3', C-1'; H-4'/C-10, C-5', C-3'; H-2'/C-4', C-3', C-1"; H-1'/C-5', C-3', C-2'; H-2"/C-4", C-5"; CH₃-4"/C-2", C-3", C-5"; CH₃-5"/C-2", C-3", C-4"; OH-1/C-1, C-1a, C-2; OH-8/C-8, C-8a; OH-1'/C-2', C-1'; ESIMS m/z 493 [M + Na]⁺; HRMALDITOFMS m/z 493.1475 [M + Na]⁺ (calcd for C₂₅H₂₆O₉Na, 493.1474).

Alvaradoin L [(10*S*)-*C*-(2-*O*-senecioidyl)- β -*L*-lyxopyranosyl-1,8-dihydroxy-3-methylanthracen-9(10*H*)-one, 8]: yellow solid (14 mg, yield 0.00026% w/w); t_R 15.91 min in 50:50→100:0 MeOH-H₂O over 25 min and t_R 11.63 min in 80:20→95:5 A:B (A = 19:1 CHCl₃-isopropanol, B = hexane) over 10 min, with the ODS A and Diol NP columns, respectively; mp 141–142 °C; $[\alpha]_D$ –26.7 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log Σ) 360 (3.92), 298 (3.82), 210 (4.47) nm; CD (MeOH) $[\theta]$ (nm) -3.19×10^6 (327), $+3.64 \times 10^6$ (298), -4.90×10^6 (268); IR (NaCl) ν_{max} 3436, 3020, 2975, 2924, 1691, 1603, 1291, 1231, 1079, 755 cm⁻¹; ¹H and ¹³C NMR data, see Tables 2 and 3; HMBC H-2/C-1, C-1a, CH₃-11, C-4; H-4/C-1a, C-10, CH₃-11; H-5/C-7, C-8a; H-6/C-5a, C-8; H-7/C-5, C-8; H-10/C-4a, C-5a, C-4, C-1a, C-8a, C-5', C-4'; CH₃-11/C-2, C-3, C-4; H-5'/C-4a, C-5a, C-10, C-4'; H-2'/C-4', C-1"; H-1'/C-5', C-3', C-2'; H-2"/C-1", C-4", C-5"; CH₃-4"/C-2", C-3", C-5"; CH₃-5"/C-2", C-3", C-4"; OH-1/C-1, C-1a; OH-8/C-7, C-8, C-8a; OH-4'/C-5'; OH-3'/C-4', C-3'; OH-1'/C-2', C-1'; ESIMS m/z 493 [M + Na]⁺; HRMALDITOFMS m/z 493.1452 [M + Na]⁺ (calcd for C₂₅H₂₆O₉Na, 493.1474).

Alvaradoin M [(10*S*)-*C*-(3-*O*-senecioidyl)- β -*L*-lyxopyranosyl-1,8-dihydroxy-3-methylanthracen-9(10*H*)-one, 9]: yellow solid (6.8 mg, yield 0.00013% w/w); t_R 15.91 min in 50:50→100:0 MeOH-H₂O over 25 min and t_R 11.91 min in 20:80→60:40 A:B (A = 9:1 CHCl₃-isopropanol, B = hexane) over 15 min, with the ODS A and Diol NP columns, respectively; mp 153–154 °C; $[\alpha]_D$ –32.0 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log Σ) 360 (3.86), 297 (3.74), 212 (4.42) nm; CD (MeOH) $[\theta]$ (nm) -1.58×10^6 (322), $+4.97 \times 10^5$ (301), -2.65×10^6 (268); IR (NaCl) ν_{max} 3452, 3019, 2974, 2926, 1699, 1603, 1292, 1230, 1079, 756 cm⁻¹; ¹H and ¹³C NMR data, see Tables 2 and 3; HMBC H-2/C-1, CH₃-11, C-4; H-4/C-1a, C-10, CH₃-11; H-5/C-7, C-8a, C-10; H-6/C-5a, C-8; H-7/C-5; H-10/C-4a, C-5a, C-4, C-5, C-1a, C-5'; CH₃-11/C-2, C-3, C-4; H-5'/C-4a; H-3'/C-4'; H-2"/C-4", C-5"; CH₃-4"/C-2", C-3", C-5"; CH₃-5"/C-2", C-3", C-4"; OH-1/C-1a; OH-8/C-7, C-8a; OH-4'/C-5'; OH-1'/C-2', C-1'; ESIMS m/z 493 [M + Na]⁺; HRMALDITOFMS m/z 493.1461 [M + Na]⁺ (calcd for C₂₅H₂₆O₉Na, 493.1474).

Alvaradoin N [(10*S*)-*C*-(1-*O*-acetyl)- β -*L*-lyxopyranosyl-1,8,10-trihydroxy-3-methylanthracen-9-one, 10]: yellow solid; (6.1 mg, yield 0.00011% w/w); t_R 9.64 min in 50:50→100:0 MeOH-H₂O over 25 min and t_R 17.58 min in 2:98→4:96 MeOH-CHCl₃ over 20 min, with the ODS A and Diol NP columns, respectively; mp 139–140 °C; $[\alpha]_D$ –56.0 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log Σ) 368 (3.70), 301 (3.54), 211 (4.13) nm; CD (MeOH) $[\theta]$ (nm) $+4.61 \times 10^6$ (351), -4.72×10^8 (299), $+1.51 \times 10^8$ (259); IR (NaCl) ν_{max} 3400, 2925, 2854, 1743, 1628, 1607, 1285, 1219, 1071, 756 cm⁻¹; ¹H and ¹³C NMR data, see Tables 2 and 3; HMBC H-2/CH₃-11, C-4; H-4/C-10, CH₃-11; H-5/C-10; H-6/C-5a, C-8; H-7/C-5; CH₃-11/C-2, C-3, C-4; H-5'/C-5a, C-10, C-4', C-3'; H-4'/C-5', C-3'; H-3'/C-4'; H-2'/C-3'; H-1'/C-5', C-3', C-1"; H-2"/C-1"; OH-1/C-1, C-2; OH-8/C-7, C-8; OH-10/C-4a, C-10, C-5'; OH-3'/C-3',

C-2'; OH-2'/C-3', C-2', C-1'; ESIMS m/z 445 $[M - 1]^+$; HRMALDITOFMS m/z 469.1097 $[M + Na]^+$ (calcd for $C_{22}H_{22}O_{10}Na$, 469.1111).

Chrysophanol (11): physical data were comparable to literature values.^{15,16}

KB Cytotoxicity Assay. The extract, fractions, and pure compounds were tested in a human oral epidermoid carcinoma (KB) cell line using established protocols as described previously.²⁶

In Vivo Evaluation of Compounds 1 and 2. Compounds 1 and 2 were evaluated in an in vivo test system using the murine P-388 lymphocytic leukemia model as described previously.²³

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Figure 1.
Key HMBC correlations for compound **1**

Figure 2.
Key ROESY ($\leftrightarrow$) correlations of compounds **1** and **2**

Table 1
¹H and ¹³C NMR Data for Compounds 1 and 2 (acetone-*d*₆)

position	1					2				
	δ_H	δ_C	HMBC	ROESY	δ_H	δ_C	HMBC	ROESY		
1	-	162.9	-	-	-	162.9	-	-		
1a	-	118.3	-	-	-	116.1	-	-		
2	6.73 (1H, s)	117.1	1, 4, CH ₃ -11	CH ₃ -11	6.70 (1H, s)	116.6	1, 1a, 4, CH ₃ -11	CH ₃ -11		
3	-	148.0	-	-	-	148.7	-	-		
4	7.04 (1H, s overlap)	122.4	1a, 2, 10, CH ₃ -11	H-4', OH-4', H-10, CH ₃ -11	6.90 (1H, s)	120.2	1a, 10, CH ₃ -11	H-5', H-10, CH ₃ -11		
4a	-	142.3	-	-	-	146.7	-	-		
5	7.04 (1H, d overlap, 7.5)	119.1	7, 8a, 10	H-6, H-10, H-5'	7.18 (1H, d, 7.5)	121.2	8a, 10	H-4', OH-4'		
5a	-	146.7	-	-	-	142.4	-	-		
6	7.56 (1H, dd overlap, 8.0, 7.9)	136.7	5a, 8	H-5, H-7	7.53 (1H, dd overlap, 8.0, 7.9)	136.1	5a, 8	H-5, H-7		
7	6.85 (1H, d, 8.4)	116.4	5, 8	H-6	6.88 (1H, d, 7.9)	116.8	5	H-6		
8	-	163.2	-	-	-	163.1	-	-		
8a	-	116.1	-	-	-	118.3	-	-		
9	-	195.0	-	-	-	195.0	-	-		
10	4.66 (1H, d, 2.3)	44.0	4, 5, 5', 4a, 5a, 1a, 8a	H-4, H-5, H-4', H-5'	4.66 (1H, d, 2.3)	43.9	4, 5, 5', 4a, 5a, 1a, 8a	H-5'		
CH ₃ -11	2.38 (3H, s)	22.2	2, 3, 4	H-2, H-4	2.40 (3H, s)	22.0	2, 3, 4	H-2, H-4		
1'	5.63 (1H, s)	94.4	3', 5', 1''	H-2', OH-2'	5.62, s	94.4	3', 5', 1''	H-2', OH-2'		
2'	3.65-3.69 (2H, m)	70.6	4'	H-1', OH-2'	3.66-3.70 (2H, m)	70.5	4'	H-1'		
3'	3.65-3.69 (2H, m)	73.1	4', 5'	OH-2', OH-4', H-5'	3.66-3.70 (2H, m)	73.1	4'	-		
4'	3.59-3.62 (1H, m)	68.2	3'	H-4, OH-4', H-5', H-10	3.59-3.61 (1H, m)	68.3	3'	OH-4'		
5'	3.84 (1H, dd, 9.8, 2.4)	81.3	4a, 5a	H-3', H-4', H-5, H-10	3.86 (1H, dd, 9.9, 2.5)	81.4	5a	H-4		
1''	-	168.3	-	-	-	168.3	-	-		
CH ₃ -2''	1.76 (1H, s)	20.4	1''	-	1.78 (1H, s)	20.4	1''	-		
OH-1	12.01 (1H, s)	-	1, 1a, 2	-	11.92 (1H, s)	-	1, 1a, 2,	-		
OH-8	11.92 (1H, s)	-	7, 8, 8a	-	12.04 (1H, s)	-	7, 8, 8a	-		
OH-2'	4.09 (1H, d, 3.7)	-	1', 2'	H-1', H-2'	4.08 (1H, d, 3.6)	-	1', 2'	H-1'		
OH-3'	3.97 (1H, d, 6.8)	-	3'	-	3.96 (1H, d, 6.9)	-	2', 3'	-		
OH-4'	4.50 (1H, d, 4.8)	-	4', 5'	H-4, H-3', H-4'	4.43 (1H, d, 4.7)	-	4', 5'	H-4'		

Table 2

¹H NMR Data of Compounds 3–10 (acetone-*d*₆)

position	3	4	5	6	7	8	9	10
H-2	6.72 (s)	6.73 (s)	6.80 (s)	6.75 (s)	6.70 (s)	6.76 (s)	6.71 (s)	6.73, s
H-3	-	-	-	-	-	-	-	-
H-4	6.96 (s)	7.01 (s)	7.28 (s)	7.36 (s)	6.91 (s overlap)	7.03 (s)	6.99 (s)	7.38, s
H-5	7.16 (d, 7.5)	7.07 (d, 7.5)	7.50 (d, 7.6)	7.41 (dd, 7.6, 1.0)	7.15 (d, 7.4)	7.04 (d, 7.6)	7.06 (d, 7.4)	7.41, (dd, 7.8, 0.9)
H-6	7.58 (d, 7.7)	7.56 (dd, 8.1, 7.2)	7.62 (brt, 8.1, 7.8)	7.63 (t, 8.0)	7.57 (t, 7.9)	7.55 (t, 8.1, 7.8)	7.53 (t, 7.9)	7.62, (t, 7.8, 8.1)
H-7	6.89 (d, 8.3)	6.85 (d, 8.1)	6.90 (d, 8.3)	6.95 (dd, 8.3, 0.8)	6.91 (d overlap, 7.9)	6.84 (d, 8.4)	6.83 (d, 7.6)	6.93, (dd, 8.4, 0.9)
H-10	4.71 (brs)	4.69 (s)	-	-	4.68 (brs)	4.68 (brs)	4.70 (d, 2.1)	-
CH ₃ -11	2.41 (s)	2.38 (s)	2.43 (s)	2.43 (s)	2.39 (s)	2.43 (s)	2.38 (s)	2.41, s
H-1'	5.62 (dd, 5.5, 1.6)	5.63 (d, 4.8)	5.57 (brs)	5.56 (d, 1.8)	4.81 (brd, 6.8)	4.81 (brd, 4.2)	4.81 (m)	5.55 (d, 1.5)
H-2'	3.87 (m)	3.84-3.92 (m)	3.84 (m)	3.84 (m)	4.88 (m)	4.89 (m)	3.81 (m)	3.63 (overlap)
H-3'	4.98 (dd, 8.8, 3.6)	4.96 (dd, 9.6, 3.3)	4.96 (dd, 9.1, 3.2)	4.97 (dd, 9.1, 3.3)	3.96 (m)	3.97 (m)	5.07 (dd, 9.7, 3.1)	3.69 (overlap)
H-4'	3.87 (m)	3.84-3.92 (m)	3.95 (brt, 9.3, 9.2)	3.93 (m)	3.65 (brt, 9.7, 9.5)	3.70 (brt, 9.6)	3.89 (m)	3.63 (overlap)
H-5'	4.01 (dd,)	3.99 (dd, 9.9, 2.1)	3.72 (d, 9.7)	3.74 (d, 9.6)	4.05 (dd, 9.9, 2.3)	4.05 (dd, 9.8, 2.1)	4.15 (dd, 9.9, 2.3)	3.57 (overlap)
H-2''	5.67 (dd, 2.8, 1.4)	5.66 (dd, 2.4, 1.3)	5.62 (s)	5.62 (m)	5.32 (brs)	5.33 (brs)	5.66 (m)	-
CH ₃ -4''	2.14 (s)	2.12 (s)	2.12 (s)	2.11 (d, 1.1)	1.90 (s)	1.89 (s)	2.13 (d, 1.2)	-
CH ₃ -5''	1.89 (s)	1.88 (s)	1.89 (s)	1.88 (d, 1.2)	1.84 (s)	1.85 (s)	1.88 (d, 1.2)	-
CH ₃ -2'''	1.83 (s)	1.80 (s)	1.79 (s)	1.81 (s)	-	-	-	1.75, s
OH-1	11.95 (s)	12.00 (s)	11.93 (s overlap)	11.85 (s)	11.85 (s)	11.86 (s)	11.95 (s)	11.84, s
OH-8	12.06 (s)	11.91 (s)	11.92 (s overlap)	12.04 (s)	11.95 (s)	11.95 (s)	11.85 (s)	12.03, s
OH-10	-	-	6.41 (s)	6.40 (brs)	-	-	-	6.54, s
OH-1'	-	-	-	-	5.74 (d, 4.4)	5.70 (d, 4.5)	5.44 (d, 4.3)	-
OH-2'	4.47 (d, 4.0)	4.42 (d, 4.5)	4.46 (brs)	4.40 (d, 4.5)	-	-	3.85 (d, 4.4)	4.08 (d, 3.6)
OH-3'	-	-	-	-	4.14 (brs)	4.12 (brs)	-	4.05 (d, 6.6)
OH-4'	4.83 (d, 6.0)	4.81 (d, 5.7)	5.73 (brs)	5.63 (brs)	4.40 (brs)	4.44 (brs)	4.54 (d, 6.1)	5.65 (d, 2.1)

Table 3

¹³C NMR Data of Compounds 3–10 (acetone-*d*₆)^a

carbon	3	4	5	6	7	8	9	10
C-1	163.4	162.8	163.2	162.9	162.7	163.2	162.5	162.8
C-1a	116.3	116.4	114.9	114.9	116.2	116.4	116.5	114.9
C-2	117.1	117.2	118.1	117.7	116.5	116.9	116.9	117.6
C-3	148.2	148.0	148.1	148.8b	148.7	147.8	147.8	148.8
C-4	120.5	122.2	120.0	118.5	120.7	122.5	122.4	118.4
C-4a	146.5	142.2	145.8	148.9b	146.9	143.2	143.2	149.1
C-5	121.2	119.2	117.7	118.8	121.2	119.6	119.6	118.8
C-5a	142.4	146.3	149.0	145.8	143.3	147.1	147.1	146.1
C-6	136.3	136.7	136.9	136.3	135.9	136.9	136.9	136.2
C-7	116.7	116.5	117.2	118.0	116.6	116.3	116.2	117.8
C-8	163.3	163.2	162.6	162.9	162.9	162.6	163.1	162.9
C-8a	118.4	117.1	117.1	116.9	118.5	118.3	118.3	116.9
C-9	193.1	194.9	194.0	193.9	195.1	195.2	195.2	193.9
C-10	44.2	44.0	76.3	76.2	44.6	44.6	44.4	76.3
CH ₃ -11	22.3	22.1	22.4	22.2	22.1	22.2	22.2	22.2
C-1'	94.6	94.4	94.1	94.0	92.9	93.0	95.7	93.9
C-2'	68.7	68.5	68.2	68.1	73.3	73.4	70.5	70.2
C-3'	74.9	74.8	74.5	74.4	70.5	70.6	75.0	72.7
C-4'	65.9	65.6	67.3	67.3	69.1	69.1	66.2	70.0
C-5'	81.9	81.6	79.8	79.9	79.2	79.2	79.4	79.5
C-1''	166.5	166.0	166.2	166.2	166.0	166.1	166.5	-
C-2''	116.9	116.7	116.6	116.6	116.8	116.9	117.1	-
C-3''	157.7	157.8	158.0	157.9	157.0	156.9	157.2	-
CH ₃ -4''	20.2	20.1	20.1	20.1	20.3	20.3	20.0	-
CH ₃ -5''	27.3	27.2	27.2	27.2	27.3	27.4	27.2	-
C-1'''	168.4	168.0	168.2	168.1	-	-	-	168.1
CH ₃ -2'''	20.6	20.4	20.4	20.3	-	-	-	20.3

^aData are based on DEPT, HSQC, and HMBC experiments.

^bSignals may be interchangeable.

Table 4Cytotoxicity of Compounds Isolated from *A. haitiensis* against the KB Cell Line.

compound	KB ^a
1	0.050 ± 0.019
2	0.065 ± 0.026
3	0.65 ± 0.15
4	1.07 ± 0.43
5	12.5 ± 3.03
6	15.9 ± 3.41
7	0.27 ± 0.072
8	0.59 ± 0.10
9	0.38 ± 0.043
10	2.94 ± 1.30
11	>20
camptothecin ^b	0.0036 ± 0.0029

^a Human oral epidermoid carcinoma. Results are expressed as EC₅₀ values in μ M (see Experimental Section). Mean $\pm$ SEM determined from three separate experiments.

^b Positive control as typical average value.