Notes

Synthesis and Cytotoxicity of Water-Soluble Ambrosin Prodrug Candidates

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The potential therapeutic application of the naturally occurring, cytotoxic pseudoguaianolide sesquiterpene lactone ambrosin is limited by its aqueous insolubility. A number of water-soluble ambrosin derivatives have therefore been prepared for potential use as prodrugs. Michael addition of several secondary amines to both the α,β-unsaturated ketone and α-methylene lactone moieties of ambrosin afforded tertiary amine diadducts that were converted to water-soluble hydrochloride salts. The salt of the bis-piperidine adduct proved to be the most potent, producing cytotoxic activity only slightly less potent than that of ambrosin itself in a variety of human cancer cell cultures. The sodium salt of the bis-sulfonic acid derivative of ambrosin was inactive, while the sodium salt of the bis-sulfinic acid analog had low activity. Biological evaluation of several ambrosin analogs with reduced and/or isomerized α,β-unsaturated ketone and α-methylene lactone moieties demonstrated the importance of both of these functional groups for biological activity.

Ambrosin (**1**) is a naturally occurring pseudoguaianolide sesquiterpene lactone first isolated from *Ambrosia maritima* L.[1] Extensive chemical and spectroscopic studies led to the correct two-dimensional structure of ambrosin,[2–4] and X-ray analysis of bromoambrosin established the absolute configuration displayed in structure **1**.[5] Demonstration of the antileukemic activity of ambrosin (**1**)[6] was followed rapidly by its total synthesis.[7] Subsequently, the cytotoxicity of ambrosin (**1**) was shown in a number of cancer cell cultures.[8,9]

Further development of ambrosin (**1**) as a clinically useful anticancer agent has been limited by its aqueous insolubility. As an approach to circumventing this problem, we have considered the synthesis of soluble ambrosin derivatives that might be converted back to ambrosin after administration, either spontaneously or after metabolic activation. The present communication details the synthesis of water-soluble ambrosin derivatives, as well as their cytotoxicities in a variety of cancer cell cultures.

Chemistry

The initial approach involved Michael addition of secondary amines to the two α,β-unsaturated carbonyl systems in ambrosin (**1**). The Michael addition of secondary amines to the α-methylene lactone system of the related sesquiterpene helenalin has been investigated in some detail, and the retro-Michael reaction of methiodides of the resulting Michael adducts is also established.[10] In addition, related monoaddition and diaddition products of secondary amines to the sesquiterpene pseudoguaianolide parthenin have been reported.[11] The Michael addition products of secondary amines to ambrosin (**1**) would be expected to form water-soluble hydrochlorides and quaternary ammonium salts which might be converted back to ambrosin (**1**) after administration by a retro-Michael mechanism. The conversion of the tertiary amine Michael adducts back to ambrosin (**1**) could also conceivably be activated through metabolic *N*-oxidation of the amines to the corresponding *N*-oxides,[12] followed by a Cope elimination reaction.

The diadditions of five secondary amines to ambrosin (**1**) and conversion of the products to their hydrochloride salts resulted in derivatives **2**-**6**. The secondary amine additions resulting in the products **2** and **3** were carried out at 5 °C in THF. The Michael additions resulting in the products **4**–**6** were performed using phase transfer catalysis.[13] These reactions were performed by heating stirred mixtures of ambrosin, the secondary amine, hexadecyltributylammonium bromide, and anhydrous potassium carbonate in refluxing acetone. The ^{1}H NMR spectra of the products showed the absence of the four olefinic proton signals which appear in the range of 6–8 ppm in the spectrum of ambrosin.

Besides ambrosin derivatives containing basic functional groups, analogs having acidic functional groups that could form water-soluble salts were also considered. Michael additions of bisulfite anion (HSO_3^-) to a variety of α,β-unsaturated carbonyl systems have been reported, including α,β-unsaturated aldehydes,[14] ketones,[14,15] amides,[16] and esters.[17] The resulting sulfonates have greatly enhanced water solubilities.[17] Accordingly, ambrosin (**1**) was reacted with sodium bisulfite in aqueous ethanol to obtain the disodium disulfonate derivative **7**. In addition, disodium disulfinate derivatives of ambrosin were also contemplated. Michael addition products derived from reaction of sulfoxylate anion (HSO_2^-) with α,β-unsaturated carbonyl systems have been proposed as intermediates in the dithionite ($S_2O_4^{2-}$) reduction of sulfonyl acrylates to α,β-unsaturated esters,[18] as well as in the phase transfer-catalyzed reduc-

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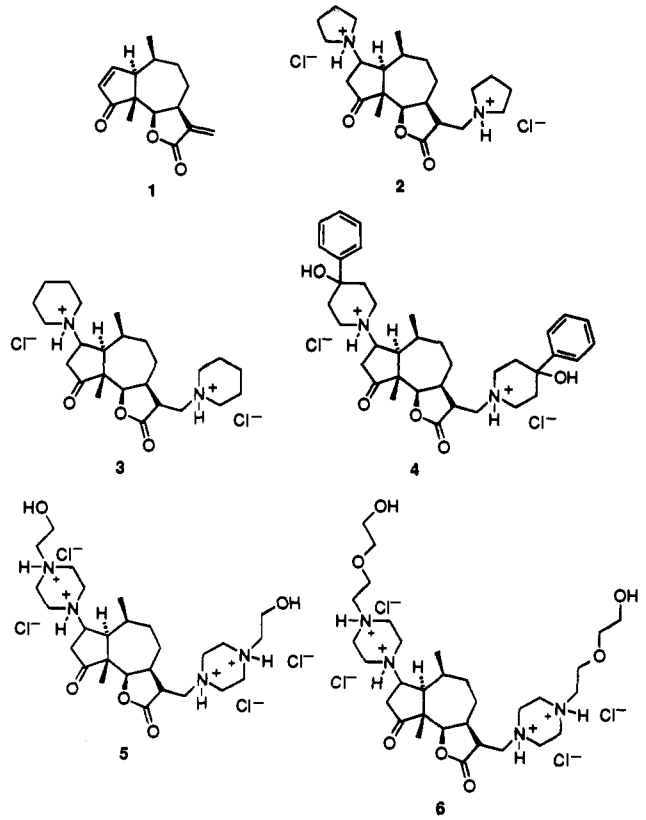
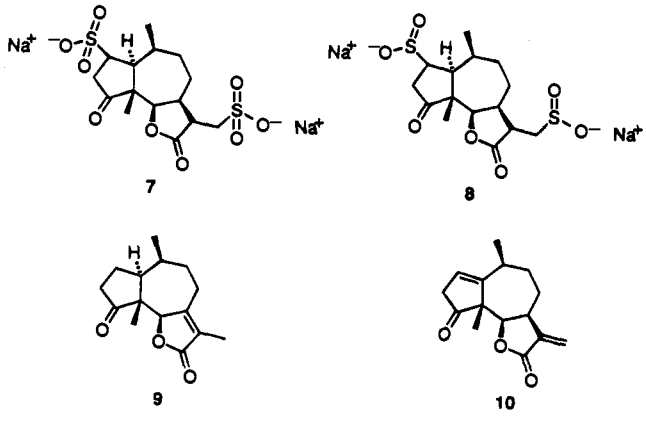


tion of α,β-unsaturated ketones.[19] Ambrosin (**1**) was therefore reacted with sodium dithionite under phase transfer catalysis conditions, and the diadduct **8** was obtained as a solid. As expected, both **7** and **8** were very soluble in water.

Several derivatives were prepared in order to gain evidence about the contribution of the α,β-unsaturated carbonyl moieties in the ambrosin (**1**) system to its cytotoxicity in cancer cell cultures. Prior studies have indicated that the α,β-unsaturated carbonyl groups in certain cytotoxic sesquiterpenes, including ambrosin and helenalin, contribute significantly to their biological activities.[8-10] Catalytic hydrogenation of ambrosin (**1**) over palladium on charcoal yielded the dihydroambrosin derivative **9**.[4,20-22] Subjection of ambrosin (**1**) to 2 equiv of diisopropylamine in refluxing tetrahydrofuran (THF) afforded neoambrosin (**10**), a metabolite of *Hymenoclea salsola*.[21] The acid-catalyzed isomerization of ambrosin (**1**) to neoambrosin (**10**) has been reported previously.[23]



All of the new ambrosin derivatives, **2-8**, prepared in this study were very hygroscopic solids. Elemental analyses indicated that they all remained hydrated and/or solvated even after prolonged heating under vacuum.

Biological Results and Discussion

The new ambrosin derivatives were submitted for cytotoxicity testing in the National Cancer Institute's *in vitro* drug discovery screen.[24-26] The screen has been designed to identify compounds with selective cytotoxicities for various types of human cancer cells. The compounds were tested in 51 different cell lines, representing human leukemia, non-small-cell lung cancer, colon cancer, CNS (central nervous system) cancer, melanoma, ovarian cancer, renal cancer, prostate cancer, and breast cancer cell lines. Results from representative cell lines for each of these eight different types of cancer for ambrosin (**1**) and prodrug candidates **2** and **3** are listed in Table 1, along with mean graph midpoint (MGM) values for all 51 cell lines. Similar results for compounds **3-10** are provided in Table 2 (supporting information).

The ambrosin cytotoxicity results listed in Table 1, as well as the more extensive testing in all 51 cell lines, indicate quite clearly that ambrosin does not possess a significant degree of selectivity for any particular cell line or type of tumor. Similarly, the selectivities of all of the new ambrosin derivatives reported here were low.

The cytotoxicity testing results for compounds **9** and **10** demonstrate the importance of both the α,β-unsaturated ketone and the α-methylene lactone for cytotoxicity. In neoambrosin (**10**), the conjugated double bond in the cyclopentenone ring of ambrosin (**1**) has been isomerized from an α,β-position to a β,γ-position relative to the ketone. This change was reflected by a 2.4-fold decrease in overall cytotoxicity, as shown by the MGM values for all 51 cell lines. Reduction of the cyclopentenone double bond of ambrosin and isomerization of the α-methylene lactone double bond to afford the α,β-unsaturated lactone **9** resulted in a 24-fold decrease in cytotoxicity, as evidenced by a corresponding increase in the MGM value. These results are consistent with previous studies in the helenalin and ambrosin series.[8-10]

As stated previously, one of the main goals of this study was to obtain water-soluble ambrosin derivatives that might retain cytotoxicity through a prodrug mechanism. As expected, the amine hydrochloride derivatives **2-6**, as well as the sulfonic acid **7** and the sulfinic acid **8**, had greatly enhanced aqueous solubilities. The most cytotoxic of the new water-soluble compounds was **3** followed by **2**, **5**, **4**, **6**, **8**, and **7**. It is likely that the cytotoxicities of the more active compounds in this series result from their conversion to ambrosin (**1**) or related α,β-unsaturated derivatives, since the low activity of **9** indicates the necessity of the α,β-unsaturated cyclopentenone and α-methylene lactone moieties for biological activity. The dipiperidinyl dihydrochloride derivative **3** appears to be a promising candidate for further preclinical development as a water-soluble ambrosin prodrug, since the cytotoxicity observed after its administration to human cancer cell cultures is only slightly less potent than that of ambrosin itself.

Experimental Section

General. Melting points were determined in capillary tubes on a Thomas-Hoover Unimelt apparatus and are uncorrected. ^{1}H NMR spectra were recorded at 300 MHz on a

Table 1. Cytotoxicities of Ambrosin and Ambrosin Derivatives

	cytotoxicity (GI_{50} in μM)[a]								
no.	lung NCI-H522	colon HCT-116	CNS SF-539	melanoma LOX-IMVI	ovarian OVCAR-5	renal SN12C	prostate PC-3	breast MCF7	MGM[b]
1	1.0	1.8	2.9	2.2	4.9	3.4	4.5	3.9	3.4
2	1.8	3.8	9.1	3.3	6.3	11	20	4.2	9.1
3	1.1	1.8	5.4	3.6	22	8.1	8.5	4.7	5.2

[a] The cytotoxicity GI_{50} values are the concentrations corresponding to 50% growth inhibition. [b] Mean graph midpoint for all human cancer cell lines (ca. 51) tested. The panels and cell lines employed included the following: leukemia CCRF-CEM, HL-60(TB), K-562, MOLT-4, RPMI-8226, and SR; non-small-cell lung cancer A549/ATCC, EKVX, HOP-62, NCI-H226, NCI-H23, NCI-H322M, NCI-H460, NCI-H522; colon cancer COLO 205, HCC-2998, HCT-116, HCT-15, HT29, KM12, SW-620; CNS cancer SF-268, SF-295, SF-539, SNB-19, SNB-75, U251; ovarian cancer IGROV1, OVCAR-5, OVCAR-8, SK-OV-8, SK-OV-3; renal cancer 786-0, A498, SCHN, SN12C, TK-10; prostate cancer PC-3, DU-145; and breast cancer MCF7, MCF/ADR-RES, MDA-MB-231/ATCC, HS 578T, MDA-MB-435, MDA-N, BT-549, and T-47D.

Bruker ARX 300 spectrometer or at 500 MHz on a Varian VXR-500S spectrometer as noted. IR spectra were obtained on a Perkin-Elmer 1600 FT-IR spectrophotometer. Low-resolution chemical ionization mass spectra (CIMS) were determined on a Finnigan 4000 spectrometer using 2-methylpropane as the reagent gas. Low-resolution fast atom bombardment mass spectra (FABMS) were obtained on a Kratos MS50 spectrometer. Plasma desorption mass spectra (PDMS) were run on a Bioion 20R mass spectrometer.

Ambrosin Pyrrolidine Diadduct Dihydrochloride (2). Ambrosin (37.2 mg, 0.15 mmol) was dissolved in THF (0.5 mL). The solution was filtered through glass wool, the glass wool was washed with THF (3 × 0.1 mL), and the solution was cooled in the freezer for 15 min. Pyrrolidine (21 mg, 0.3 mmol) in THF (0.5 mL) was added to the solution of ambrosin. The mixture was kept in the refrigerator at 5 °C for 20 h. The solvent was evaporated. The residue (57.4 mg) was dissolved in ethyl ether and filtered through silica gel (0.5 g) deactivated with triethylamine. The solvent was evaporated and the residue kept *in vacuo* for 24 h to remove traces of pyrrolidine. The remaining oil was dissolved in dry Et_2O (5 mL), and a solution of HCl in dioxane (4.0 M, 0.3 mL) was added by syringe. The precipitate was filtered off and dried *in vacuo* over anhydrous $CaSO_4$ in a vacuum desiccator for 12 h and at 56 °C over P_2O_5 in an Abderhalden apparatus for 24 h to afford **2** as a solid (43.2 mg, 55.7%): mp 138–143 °C; ^{1}H NMR (CD_3OD, 300 MHz) δ 4.50 (t, 1 H), 3.74 (m, 8 H), 3.25–2.55 (m, 7 H), 2.12–1.4 (m, 14 H), 1.26–1.23 (m, 6 H); IR (KBr) 3424, 2959, 2881, 2604, 2483, 1751, 1630, 1455, 1393, 1201, 1179, 1141, 1120, 1083, 1018, 992, 872, 781, 742 cm^{-1}; FABMS *m/z* (rel intensity) 389 (100). Anal. ($C_{23}H_{38}Cl_2N_2O_3 \cdot H_2O \cdot 0.5Et_2O$) C, H, N.

Ambrosin Piperidine Diadduct Dihydrochloride (3). The procedure for **3** was identical with that used for **2** except that piperidine was used instead of pyrrolidine. The product **3** was obtained as a solid (53.1 mg, 68.5%): mp 134–137 °C; ^{1}H NMR (CD_3OD, 300 MHz) δ 4.30 (m, 1 H), 3.65–3.30 (m, 8 H), 3.10 (m, 5 H), 2.81 (m, 2 H), 2.4–1.4 (m, 18 H), 1.27–1.14 (m, 6 H); IR (KBr) 3422, 2946, 2655, 2537, 1751, 1654, 1457, 1395, 1200, 1120, 1081, 1020, 989, 962 cm^{-1}; FABMS *m/z* (rel intensity) 417.0 (100). Anal. ($C_{25}H_{42}Cl_2N_2O_3 \cdot 1.5H_2O$) C, H, N.

Ambrosin 4-Hydroxy-4-phenylpiperidine Diadduct Dihydrochloride (4). A mixture of ambrosin (37.2 mg, 0.15 mmol), 4-hydroxy-4-phenylpiperidine (104 mg, 0.6 mmol), hexadecyltributylphosphonium bromide (15.1 mg, 0.03 mmol), and powdered anhydrous potassium carbonate (52 mg, 0.3 mmol) in acetone (1.5 mL) was heated at reflux with magnetic stirring for 5 h. The inorganic salts were filtered off, the solvent was evaporated, and the remaining residue was purified by flash chromatography on silica gel (6 g) deactivated with triethylamine (eluent: chloroform–acetone, 1:1, acetone). The acetone fraction was evaporated and the residue (23 mg) dissolved in absolute ethanol (2 mL) and treated with a 1 M solution of HCl in Et_2O (0.1 mL). The solvent was evaporated and the yellow residue dried *in vacuo* to give compound **4** as a yellow solid (23 mg, 19%): ^{1}H NMR (CD_3OD, 300 MHz) δ 7.49 (m, 4 H), 7.33 (m, 4 H), 7.21 (m, 2 H), 3.10 (m, 3 H), 2.85 (m, 3 H), 2.70 (m, 4 H), 2.23–1.95 (m, 7 H), 1.68 (m, 6 H), 1.52 (m, 3 H), 1.39 (m, 10 H); IR (neat) 3380, 2930, 2656, 1764, 1704, 1629, 1448, 1367, 1186, 994, 763, 702 cm^{-1}; PDMS (positive ions) 601 (100). Anal ($C_{37}H_{50}Cl_2N_2O_5 \cdot 1.4\ C_2H_5OC_2H_5 \cdot 1.2C_2H_5OH$) C, H. N.

Ambrosin 1-(2-Hydroxyethyl)piperazine Diadduct Tetrahydrochloride (5). The procedure for **5** was similar to that for **4**, except 1-(2-hydroxyethyl)piperazine was used in place of 4-hydroxy-4-phenylpiperidine and the reaction was carried out for 4.5 h instead of 5 h. The free base was obtained as a yellow oil in 35% yield: ^{1}H NMR (CD_3OD, 500 MHz) δ 3.65 (m, 3 H), 3.40 (m, 4 H), 3.15 (m, 2 H) 2.80–2.50 (m, 19 H), 1.65–0.95 (m, 14 H); IR (neat) 3369, 2925, 2815, 1763, 1578, 1158 cm^{-1}.

The hydrochloride salt obtained after evaporation of diethyl ether was dissolved in 2-propanol, the insoluble impurities were filtered off, and the residue was dried *in vacuo* for 24 h to yield the tetrahydrochloride **5** as a hygroscopic, yellow solid in 15% overall yield: IR (KBr) 3420, 2928, 2683, 1751, 1636, 1458, 1184, 1078, 1012, 668 cm^{-1}; PDMS (positive ions) *m/z* (rel intensity) 461.4 (30), 427.3 (100), 377.3 (38). Anal. ($C_{27}H_{50}Cl_4N_4O_5 \cdot 2HCl \cdot 1.8H_2O \cdot 0.4i\text{-}C_3H_7OH$) C, H, N.

Ambrosin 1-[2-(2-Hydroxyethoxy)ethyl]piperazine Diadduct Tetrahydrochloride (6). The procedure for **6** was similar to that for **4**, except 1-[2-(2-hydroxyethoxy)ethyl]-piperazine (34.8 mg, 0.2 mmol) was used instead of 4-hydroxy-4-phenylpiperidine and the reaction was carried out for 3 h instead of 5 h. The free base was obtained as a yellow oil in 33% yield: ^{1}H NMR (CD_3OD, 500 MHz) δ 3.56 (m, 11 H), 3.44 (m, 6 H), 2.78 (m, 6 H), 2.60–2.40 (m, 21 H), 2.17–0.95 (m, 12 H); IR (neat) 3380, 2928–2820 (multiple bands), 1764, 1459, 1352, 1308, 1122, 1067, 1012 cm^{-1}.

The tetrahydrochloride **6** was isolated as a hygroscopic, yellow solid in 16% overall yield: IR (KBr) 3776, 2924, 2856, 1763, 1652, 1459, 1376, 1069, 1011 cm^{-1}; PDMS (positive ions) *m/z* (rel intensity) 595.9 (100). Anal. ($C_{31}H_{58}Cl_4N_4O_7 \cdot 2.2\ HCl \cdot 4H_2O$) C, H. N.

Ambrosin Sodium Bisulfite Diadduct (7). Ambrosin (37 mg, 0.15 mmol) was dissolved in absolute ethanol (2 mL). A solution of sodium bisulfite (0.4 mmol, 43 mg) in distilled water (0.2 mL) was added, and the mixture was left at ambient temperature overnight. The solvent was evaporated, and the solid residue was extracted twice with boiling 95% ethanol (5 mL). The solvent was evaporated and the solid residue dried *in vacuo* overnight to give the product **7** (62 mg, 78%) as a solid: mp 220 °C dec; ^{1}H NMR (CD_3OD, 300 MHz) δ 3.60 (m, 1 H), 3.15–2.42 (m, 6 H), 1.96–1.60 (m, 4 H), 1.12 (m, 9 H); IR (KBr) 3450, 2929, 1763, 1638, 1422, 1206, 1048, 872, 628 cm^{-1}. Anal. ($C_{15}H_{20}Na_2S_2O_9 \cdot 2H_2O$) C, H.

Ambrosin Sodium Dithionite Diadduct (8). Ambrosin (49 mg, 0.2 mmol), Adogen 464 [methyltrialkyl(C_8–C_{10})-ammonium chloride; 50 mg], sodium bicarbonate (176 mg, 2 mmol), and 85% sodium dithionite (206 mg, 1 mmol) were dissolved in a mixture of benzene (1.5 mL) and water (1.5 mL). The mixture was stirred and heated at reflux under argon for 2.5 h. Ethyl ether (1 mL) was added to the cooled mixture. The organic layer was separated and washed with water (1 mL). The combined water layers were extracted with ethyl ether (1 mL). Water was evaporated, and the residue was dried *in vacuo* overnight. The dry solid was extracted three times with 95% ethanol. The solvent was removed and the residue dried *in vacuo* to give the product (26 mg, 27%): mp

220 °C dec; IR (KBr) 3410, 2927, 1755, 1606, 1368, 1195, 1048 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) δ 4.50 (m, 1 H), 4.10–3.85 (m, 1 H), 3.08 (m, 2 H), 2.74 (m, 1 H), 2.42 (m, 2 H), 2.20 (m, 1 H), 2.10 (m, 1 H), 2.0–1.5 (m, 8 H), 1.2–1.0 (m, 6 H); FABMS *m/z* (rel intensity) 424.2 (M^+ + 2, 100). Anal ($C_{15}H_{20}Na_2S_2O_7 \cdot 3.5H_2O$) C, H.

2,3-Dihydroisoambrosin (9). Ambrosin (37 mg, 0.15 mmol) was dissolved in methanol (15 mL) and stirred at ambient temperature with palladium on charcoal (5%, 25 mg). The catalyst was filtered off. A second portion of palladium on charcoal (5%, 38 mg) was added, and the mixture was hydrogenated at atmospheric pressure and room temperature. The hydrogen uptake corresponded to 0.20 mmol. The catalyst was filtered off, and the solvent was removed. The solid residue was crystallized from ethyl acetate–hexane (1 mL) to give 2,3-dihydroisoambrosin (**9**) as a solid (20 mg, 54%): mp 163–164 °C (lit.[21] mp 164–165 °C); IR (KBr) 2966, 1748, 1730, 1665, 1463, 1457 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 4.62 (brs, 1 H) 2.77 (ddd, 1 H), 2.36–2.23 (m, 4 H), 2.13–1.95 (m, 3 H), 1.82 (s, 3 H), 1.34 (ddd, 1 H), 1.01 (d, J = 7.2 Hz, 3 H), 0.84 (s, 3 H); CIMS *m/z* (rel intensity) 249 (MH^+, 100).

Neoambrosin (10). Ambrosin (50 mg, 0.2 mmol) was added to a solution of diisopropylamine (40 mg, 0.4 mmol) in THF (1 mL). The mixture was heated at reflux for 30 h. The solvent and the excess diisopropylamine were evaporated, and the remaining residue was purified by flash chromatography on silica gel (230–400 mesh, eluent: benzene–ethyl acetate, 4:1) to afford **10** as a solid (28 mg, 56%): mp 123–124 °C (lit.[21] mp 126–127 °C); ^1H NMR ($CDCl_3$, 300 MHz) δ 6.27 (d, J = 3.6 Hz, 1 H), 5.95 (t, J = 4.2 Hz, 1 H), 5.51 (d, J = 3.3 Hz, 1 H), 4.41 (d, J = 8.8 Hz, 1 H), 3.35 (m, 1 H), 3.13 (dd, J = 22.7, 1.9 Hz, 1 H), 2.89 (m, 1 H), 2.81 (dd, J = 22.8, 2.5 Hz, 1 H), 2.09 (m, 2 H), 1.76 (m, 2 H), 1.20 (s, 3 H), 1.17 (d, J = 7.4 Hz, 3 H); IR (KBr) 2930, 2360, 1763, 1662, 1458, 1407, 1380, 1331, 1299, 1276, 1241, 1172, 1146, 1108, 1051, 1009, 977, 943, 815 cm^{-1}; CIMS *m/z* (rel intensity) 247 (MH^+, 100), 229 (14). Anal. ($C_{15}H_{18}O_3 \cdot 0.25H_2O$) C, H.

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Supporting Information Available: Table of cytotoxicity data for compounds **4**–**10** (1 page). Ordering information is given on any current masthead page.

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