# Acylphloroglucinol Derivatives from *Hypericum prolificum*

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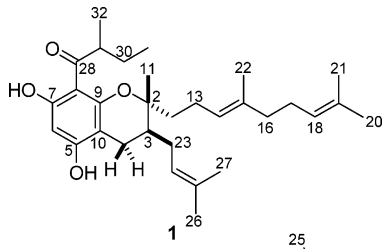
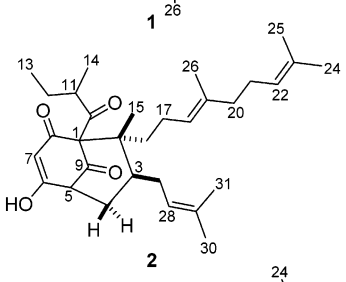
Three new acylphloroglucinol derivatives have been isolated from the hexane extract of the aerial parts of *Hypericum prolificum* L.: prolificin A (**1**), prolifenone A (**2**), and prolifenone B (**3**). The structures were elucidated on the basis of extensive 2D NMR and MS data. All three compounds were evaluated for in vitro cell proliferation inhibitory activity against human breast (MCF-7), lung (NCI-H460), CNS (SF-268), stomach (AGS), and colon (HCT-116) tumor cell lines. Prolificin A showed growth inhibition of all cell lines with $IC_{50}$ values ranging from 23 to 36 $\mu$M. Prolifenones A and B were inactive at the concentrations tested.

In recent years, the genus *Hypericum* (Clusiaceae) has received widespread interest owing to the antidepressant properties of *H. perforatum* (St. John's wort).[1] Several other *Hypericum* species have been studied, resulting in the isolation of natural products possessing anti-HIV, antitumor, anti-inflammatory, antibacterial, and antioxidant properties.[2−6]

As part of a study to characterize the bioactive constituents of *Hypericum* species growing in Pennsylvania, we have examined the hexane extract of the aerial parts of *H. prolificum* and report



**1**



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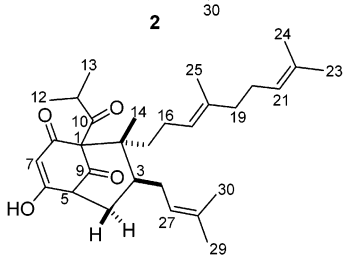

**3**

herein the isolation and structure elucidation of three new acylphloroglucinol derivatives: prolificin A (**1**), prolifenone A (**2**), and prolifenone B (**3**). The compounds were evaluated for lipid peroxidation and COX-1 and -2 inhibitory activities, as well as tumor cell growth inhibition toward human breast (MCF-7), lung (NCI-H460), CNS (SF-268), stomach (AGS), and colon (HCT-116) tumor cell lines.

Prolificin A (**1**) was purified by repeated column chromatography and obtained as a yellow oil. The IR spectrum showed absorption bands at 3351 and 1622 $cm^{-1}$, providing evidence for the presence of hydroxyl and carbonyl groups, respectively. The HR-MALDIMS gave a molecular formula of $C_{31}H_{46}O_4$, indicating nine degrees of unsaturation. The DEPT spectrum of compound **1** provided evidence for 10 quaternary, six methine, seven methylene, and eight methyl carbons. The resonances for a carbonyl carbon ($\delta$ 210), a trioxygenated aromatic ring ($\delta$ 95.9, 100.3, 106.2, 156.8, 160.2, 166.2), and three trisubstituted carbon−carbon double bonds ($\delta$ 122.3, 124.1, 124.5, 131.2, 131.5, 136.5) were observed in its $^{13}C$ NMR spectrum (Table 1). The presence of the trisubstituted double bonds was confirmed by the $^1H$ NMR data, which showed resonances at $\delta$ 5.13, 5.17, and 5.24. These data accounted for eight of the nine required degrees of unsaturation, indicating the presence of an additional ring. Detailed analysis of the $^1H-^1H$ COSY and HMBC data revealed the presence of a chroman ring system appended by methyl, (*E*)-4,8-dimethyl-3,7-nonadienyl (C-12 to C-22), 3-methylbut-2-enyl (C-23 to C-27), and 2-methylbutanoyl (C-28 to C-32) groups, which are commonly found in compounds isolated from the *Hypericum* genus. In the $^1H-^1H$ COSY spectrum, H-3 ($\delta$ 1.75) showed correlations to $H_2$-4 ($\delta$ 2.15, 2.81) and $H_2$-23 ($\delta$ 2.10, 1.68), placing the 3-methylbut-2-enyl group at position 3 on the chroman ring. This connectivity was further confirmed by the HMBC correlations between C-23 ($\delta$ 29.1) and the C-4 methylene hydrogens. HMBC correlations between $H_3$-11 ($\delta$ 0.98) and C-2 ($\delta$ 81.0), C-3 ($\delta$ 36.0), and C-12 ($\delta$ 39.4) established the placement of the C-11 methyl and the (*E*)-4,8-dimethyl-3,7-nonadienyl groups at C-2 (Figure 1). The singlet at $\delta$ 5.82 showed HMBC cross-peaks to the aromatic carbons C-5, C-7, C-8, and C-10, confirming its location at position 6 on the ring. The sharp deshielded signal at $\delta$ 14.7 in the $^1H$ NMR spectrum suggested that the carbonyl carbon was hydrogen-bonded to one of the phenolic hydrogens. The hydrogen-bonded hydroxyl showed strong HMBC correlations to aromatic carbons at $\delta$ 95.9 (C-6), 106.2 (C-8), and 166.2 (C-7), placing the 2-methylbutanoyl group at position 8 on the chroman ring.

On the basis of the spectroscopic data, two regioisomers are possible for prolificin A: one having the ether linkage *ortho* (C-9) to the 2-methylbutanoyl group and the other with the linkage at the *para* (C-5) position. The placement of the ether linkage at the

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**Table 1.** NMR Data for Prolificin A (**1**) in Benzene-$d_6$[a]

| pos. | $\delta_C$, mult. | $\delta_H$ ($J$ in Hz) | HMBC[b] | COSY |
|---|---|---|---|---|
| 2 | 81.0, qC | | 3, 4, 11 | |
| 3 | 36.0, CH | 1.75, m | 4, 11 | 4, 23 |
| 4 | 22.2, $CH_2$ | $\beta$ 2.15, dd (16.5, 9.9) | | 3 |
| | | $\alpha$ 2.81, dd (16.5, 5.1) | | |
| 5 | 160.2, qC | | 4,6, C-5 OH | |
| 6 | 95.9, CH | 5.82, s | C-7 OH | |
| 7 | 166.2, qC | | 6, C-7 OH | |
| 8 | 106.2, qC | | 6, C-7 OH | |
| 9 | 156.8, qC | | 4 | |
| 10 | 100.3, qC | | 4, 6, C-5 OH | |
| 11 | 19.8, $CH_3$ | 0.98, s | | |
| 12 | 39.4, $CH_2$ | 1.68, m | 11 | 13 |
| 13 | 21.9, $CH_2$ | 2.06, m | 12, 14 | 12, 14 |
| 14 | 122.3, CH | 5.17, t (7.0) | 13, 16, 22 | 13, 16, 22 |
| 15 | 136.5, qC | | 13, 16, 17, 22 | |
| 16 | 40.0, $CH_2$ | 2.06, m | 14, 17, 22 | 14, 17 |
| 17 | 26.8, $CH_2$ | 2.05, m | 16 | 16, 18 |
| | | 2.18, m | | |
| 18 | 124.5, CH | 5.24, t (6.6) | 17, 20, 21 | 17, 20, 21 |
| 19 | 131.2, qC | | 17, 20, 21 | |
| 20 | 25.63, $CH_3$ | 1.71, s | 18, 21 | 18 |
| 21 | 17.54, $CH_3$ | 1.59, s | 18, 20 | 18 |
| 22 | 16.0, $CH_3$ | 1.55, s | 14, 16 | 14 |
| 23 | 29.1, $CH_2$ | 1.68, m | 4 | 3, 24 |
| | | 2.10, m | | |
| 24 | 124.1, CH | 5.13, t (6.5) | 23, 26, 27 | 23, 26, 27 |
| 25 | 131.5, qC | | 26, 27 | |
| 26 | 25.57, $CH_3$ | 1.66, s | 24, 27 | 24 |
| 27 | 17.45 $CH_3$ | 1.56, s | 24, 26 | 24 |
| 28 | 210.0, qC | | 29, 30, 32 | |
| 29 | 45.9, CH | 3.94, m | 30, 31, 32 | 30, 32 |
| 30 | 27.2, $CH_2$ | 1.49, m | 29, 31, 32 | 29, 31 |
| | | 1.98, m | | |
| 31 | 11.8, $CH_3$ | 0.93, t (7.7) | 29, 30 | 30 |
| 32 | 16.8, $CH_3$ | 1.27, d (6.6) | 29, 30 | 29 |
| C-5 OH | | 5.08 br, s | | |
| C-7 OH | | 14.7, s | | |

[a] $^1$H and $^{13}$C NMR assignments were guided by DEPT and HMQC correlations. [b] Proton correlating with $^{13}$C resonance.

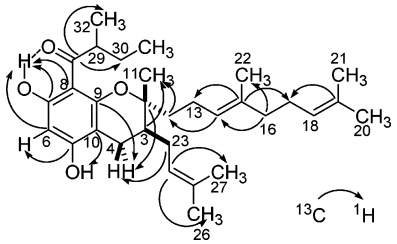


**Figure 1.** Selected HMBC correlations of prolificin A (**1**).

*ortho* position was based on HMBC correlations between the phenolic hydrogen ($\delta$ 5.1) and the carbons at $\delta$ 100.3 (C-10) and 160.2 (C-5). Furthermore, irradiation of H-29 ($\delta$ 3.94) of compound **1** in an NOE experiment enhanced the signal intensity of the C-2 methyl group, which confirmed their spatial proximity.

The relative configuration at C-2 and C-3 was assigned on the basis of coupling constants, NOE correlations, and comparison with data reported for hypercalyxone B isolated from *Hypericum amblycalyx*.[7] A coupling constant of 9.9 Hz for H-4$\beta$ ($\delta$ 2.15) suggested that it was in a diaxial relationship with H-3 ($\delta$ 1.75). Irradiation of $H_3$-11 gave enhanced signal intensities for H-4$\beta$ and the $H_2$-23 methylene hydrogens, suggesting that the methyl group and the 3-methylbut-2-enyl side chain were co-facial, and confirmed the proposed structure for compound **1**.

Prolifenone A (**2**) was isolated as a yellow oil, and its molecular formula was determined to be $C_{31}H_{46}O_4$ by HR-MALDIMS, indicating nine degrees of unsaturation. The IR spectrum provided evidence for hydroxyl (3411 cm$^{-1}$), two isolated carbonyl (1726, 1718 cm$^{-1}$), and conjugated carbonyl (1685 cm$^{-1}$) groups. These data were corroborated by the $^{13}$C NMR data, which showed signals for three carbonyls at $\delta$ 208.4, 205.5, and 191.4 (Table 2). The NMR data suggested the presence of 2-methylbutanoyl, 3-methylbut-2-enyl, and (*E*)-4,8-dimethyl-3,7-nonadienyl groups identical to those found in prolificin A. The isolated carbonyls, the conjugated carbonyl system, and the carbon−carbon double bonds of the side chains accounted for seven of the nine observed degrees of unsaturation, indicating that the compound contained two rings. The absence of resonances associated with an aromatic ring in the $^{13}$C NMR spectrum suggested the absence of a chroman moiety. Analysis of the NMR data of **2** provided evidence for a bicyclo-[3.3.1]nonane core, which is a common feature among the acylphloroglucinols.[2] The structure of the bicyclic core and the location of the attached groups in **2** were determined on the basis of $^1$H−$^1$H COSY and HMBC correlations (Table 2, Figure 2).

The $^1$H−$^1$H COSY spectrum provided evidence for the following proton sequence: H-5−$H_2$-4−H-3−$H_2$-27. The quaternary carbon C-2 ($\delta$ 48.5) displayed HMBC correlations to H-3, $H_2$-4, $H_3$-15, and $H_2$-16. The deshielded chemical shift value for C-1 ($\delta$ 82.5) suggested that it was flanked by the three carbonyl groups (C-8, C-9, C-10).[8] HMBC correlations between C-1 and H-5, H-7, $H_3$-15, and $H_2$-16 necessitated three-bond relationships to all these positions. The C-6 enolic carbon ($\delta$ 179.8) showed correlations to $H_2$-4, H-5, and H-7. Further correlations were observed between C-9 ($\delta$ 205.5) and $H_2$-4 and H-5. The lower chemical shift value observed for the C-8 carbonyl ($\delta$ 191.4) suggested that it was in conjugation with the enol moiety, completing the assignment of the bicyclic core and the attached groups in compound **2**.

The relative configuration at C-2 and C-3 was determined by NOE correlations. Irradiation of $H_3$-15 ($\delta$ 1.18) showed enhancement of the $H_2$-27 ($\delta$ 2.25, 2.42) signals, indicating that the methyl group and the 3-methylbut-2-enyl groups were co-facial. The orientation of the diastereotopic $H_2$-4 hydrogens was based on HMBC and NOE data. The C-9 carbonyl group ($\delta$ 205.5) showed a strong correlation to H-4$\alpha$ ($\delta$ 2.0), suggesting that the carbonyl and H-4$\alpha$ were in an *anti* relationship. Furthermore, NOE correlations were observed between $H_3$-15 and H-4$\beta$ ($\delta$ 1.48), indicating their *syn* relationship.

Prolifenone B (**3**) was also obtained as a yellow oil. The IR spectrum displayed peaks corresponding to a hydroxyl (3394 cm$^{-1}$) and three carbonyl (1732, 1717, 1683 cm$^{-1}$) groups. A detailed analysis of its NMR spectroscopic data (Table 2) revealed that the structure was similar to prolifenone A (**2**). The data indicated the presence of bicyclo[3.3.1]nonane, 3-methylbut-2-enyl, and (*E*)-4,8-dimethyl-3,7-nonadienyl moieties, identical to those in prolifenone A. The $^{13}$C spectrum of **3** showed one fewer carbon, which was corroborated by the mass spectrometric data with a molecular formula of $C_{30}H_{44}O_4$. The absence of a methyl triplet and the presence of two methyl doublets at $\delta$ 1.28 and 1.41 in the $^1$H NMR spectrum, which showed HMBC correlations to C-10 ($\delta$ 208.9), suggested the presence of an isobutanoyl group instead of a 2-methylbutanoyl group. The rest of the spectroscopic data of prolifenone B were almost identical to those of prolifenone A, and hence confirmed its structure as **3**. The relative configuration at C-2 and C-3 was determined in a manner similar to **2**. NOE correlations were observed between the $H_3$-14 methyl ($\delta$ 1.19) and the methylene protons $H_2$-26 ($\delta$ 2.45), indicating a *syn* relationship between the methyl and the 3-methylbut-2-enyl groups.

Lipid peroxidation and COX-1 and -2 enzyme inhibitory assays revealed that all three compounds were inactive at 103.7 $\mu$M. These compounds showed poor solubility in the assay buffer, and therefore we were unable to test them at higher concentrations. In vitro human tumor cell proliferation studies were carried out on prolificin A, prolifenone A, and prolifenone B, using the MTT cell viability assay.[9] Prolificin A showed moderate growth inhibition of human breast (MCF-7), lung (NCI-H460), CNS (SF-268), stomach (AGS), and colon (HCT-116) tumor cell lines, with $IC_{50}$ values of 23.7, 29.9, 30.9, 32.4, and 35.1 $\mu$M, respectively. Prolifenones A and B

**Table 2.** NMR Data for Prolifenone A (**2**) and Prolifenone B (**3**) in Benzene-$d_6$[a]

| | prolifenone A (**2**) | | | | prolifenone B (**3**) | |
|---|---|---|---|---|---|---|
| pos. | $\delta_C$, mult. | $\delta_H$ ($J$ in Hz) | HMBC[b] | COSY | $\delta_C$, mult. | $\delta_H$ ($J$ in Hz) |
| 1 | 82.5, qC | | 5, 7, 15, 16 | | 82.5, qC | |
| 2 | 48.5, qC | | 3, 4, 15, 16 | | 48.4, qC | |
| 3 | 42.3, CH | 1.85, m | 5,15 | 4, 27 | 42.2, CH | 1.85, m |
| 4 | 31.8, $CH_2$ | $\beta$ 1.48, m | 5 | 3, 5 | 31.9, $CH_2$ | 1.48, m |
| | | $\alpha$ 2.00, m | | | | 2.03, m |
| 5 | 54.9, CH | 3.25, dd (4.4, 2.2) | 7 | 4 | 55.0, CH | 3.27, dd (4.8, 2.2) |
| 6 | 179.8, qC | | 4, 5, 7 | | 180.5, qC | |
| 7 | 108.7, CH | 6.04, s | | | 108.6, CH | 6.11, s |
| 8 | 191.4, qC | | | | 191.2, qC | |
| 9 | 205.5, qC | | 4, 5 | | 205.5, qC | |
| 10 | 208.4, qC | | 11, 12, 14 | | 208.9, qC | |
| 11 | 49.0, CH | 2.15, m | 12, 13, 14 | 12, 14 | 42.3, CH | 2.33, m |
| 12 | 27.8, $CH_2$ | 1.60, m | 13, 14 | 11, 13 | 21.8, $CH_3$ | 1.28, d (6.4) |
| | | 2.02, m | | | | |
| 13 | 11.5, $CH_3$ | 0.84, t (7.7) | 11, 12 | 12 | 20.8, $CH_3$ | 1.41, d (6.4) |
| 14 | 16.9, $CH_3$ | 1.40, d (6.2) | 11 | 11 | 14.1, $CH_3$ | 1.19, s |
| 15 | 14.0, $CH_3$ | 1.18, s | | | 37.2, $CH_2$ | 1.83, m |
| | | | | | | 2.25, m |
| 16 | 37.3, $CH_2$ | 1.78, m | 15, 17 | 17 | 27.5, $CH_2$ | 1.67, m |
| | | 2.20, m | | | | 2.15, m |
| 17 | 27.5, $CH_2$ | 1.68, m | 18 | 17, 18 | 122.7, CH | 4.98, t (6.4) |
| | | 2.15, m | | | | |
| 18 | 122.7, CH | 4.97, t (7.3) | 17, 20, 26 | 17, 20, 26 | 136.8, qC | |
| 19 | 136.8, qC | | 17, 20, 21, 26 | | 39.9, $CH_2$ | 1.98, m |
| 20 | 39.9, $CH_2$ | 1.97, m | 18, 21, 26 | 18, 21 | 26.7, $CH_2$ | 2.11, m |
| 21 | 26.7, $CH_2$ | 2.09, m | 20 | 20, 22 | 124.4, CH | 5.16, t (7.0) |
| 22 | 124.3, CH | 5.15, t (7.0) | 21, 24, 25 | 21, 24, 25 | 131.0, qC | |
| 23 | 131.2, qC | | 24, 25 | | 25.67, $CH_3$ | 1.71, s |
| 24 | 25.62, $CH_3$ | 1.70, s | 22, 25 | 22 | 17.55, $CH_3$ | 1.56, s |
| 25 | 17.58, $CH_3$ | 1.56, s | 24 | 22 | 16.0, $CH_3$ | 1.46, s |
| 26 | 16.0, $CH_3$ | 1.46, s | 18, 20 | 18 | 25.3, $CH_2$ | 2.45, m |
| 27 | 25.4, $CH_2$ | 2.25, m | 3, 4 | 3, 28 | 125.0, CH | 5.21, t (7.0) |
| | | 2.42, m | | | | |
| 28 | 125.0, CH | 5.20, t (7.0) | 27, 30 | 27, 30, 31 | 131.2, qC | |
| 29 | 131.0, qC | | 27, 30 | | 25.63, $CH_3$ | 1.63, s |
| 30 | 25.66, $CH_3$ | 1.63, s | 28, 31 | 28 | 17.58, $CH_3$ | 1.66, s |
| 31 | 17.54, $CH_3$ | 1.66, s | 28, 30 | 28 | | |

[a] $^1$H and $^{13}$C NMR assignments were guided by DEPT and HMQC correlations. [b] Proton correlating with $^{13}$C resonances.

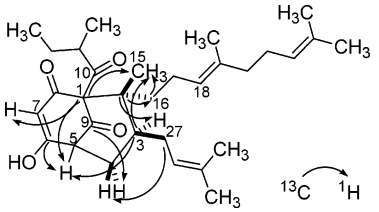


**Figure 2.** Selected HMBC correlations of prolifenone A (**2**).

did not inhibit the proliferation of these tumor cell lines at the concentrations tested. Adriamycin (doxorubicin) was used as positive control and showed potent cytotoxicity against all cell lines, with $IC_{50}$ values below 1.60 $\mu$M (MCF-7, 0.43 $\mu$M; NCI-H460, 0.66 $\mu$M; SF-268, 1.55 $\mu$M; AGS, 0.31 $\mu$M; HCT-116, 0.41 $\mu$M).

In summary, we have isolated three new acylphloroglucinol derivatives from *H. prolificum*. The chroman moiety in prolificin A (**1**) and the bicyclo[3.3.1]nonane moiety in prolifenones A (**2**) and B (**3**) are present in a number of biologically active molecules, including those isolated from St. John's wort.[2,5−7] Only prolificin A was active against a panel of human tumor cell lines, suggesting the potential for structural modifications to enhance human cancer cell cytotoxicity. Given the widespread research focus on St. John's wort, the current study warrants further investigation of compounds **1**−**3** and also other *Hypericum* species for the identification of novel natural products with broad spectrum biological properties.

## Experimental Section

**General Experimental Procedures.** Optical rotations were determined using a Rudolph Autopol IV polarimeter. UV data were recorded on a Varian Cary 4000 UV−vis spectrophotometer. FTIR data were acquired using a Nicolet Magna IR 560 spectrometer. NMR spectra were recorded on a JEOL ECP 400 MHz spectrometer ($^1$H, 400 MHz; $^{13}$C, 100 MHz) using benzene-$d_6$ as solvent and TMS as internal standard. LR-ESIMS (negative mode) data were obtained on an LCQ Classic Finnigan LC-MS/MS system (ThermoFinnigan, San Jose, CA). HR-MALDIMS data were recorded on an IonSpec 7.0 Ultima FTMS (NSF grant no. CHE 0092036). Column chromatography was performed using Fisher Scientific silica gel (230−400 mesh), and analytical TLC was performed using Sigma-Aldrich polyester-backed plates precoated with silica gel $UV_{254}$. All solvents were ACS grade and were obtained from Fisher Scientific and Aldrich Chemical Co.

**Plant Material.** The aerial parts of *H. prolificum* L. were collected in Lawrence County, PA, in June 2005 and identified by Joseph A. Isaac. A voucher specimen (#18641) has been deposited at the Carnegie Museum Herbarium in Pittsburgh, PA.

**Extraction and Isolation.** The plant material was ground using a coffee grinder to produce a fine powder (496 g). The powdered plant material was extracted successively at room temperature with hexane (2 × 1.6 L), acetone (3 × 1.4 L), and MeOH (3 × 1.4 L). The solvents were allowed to percolate for one night each time. The extracts were concentrated on a rotary evaporator to afford green gums weighing 14.27, 28.99, and 121.66 g for hexane, acetone, and MeOH, respectively. A portion of the hexane extract (13.95 g) was subjected to silica gel column chromatography, eluting with an acetone−hexane solvent gradient with increasing concentrations of acetone (5 to 100%) to afford eight fractions (A1−A8). Fraction A2 (3.93 g), eluted with 5% acetone−hexane, was chromatographed using 5 and 7% EtOAc−hexane sequentially to afford subfractions B1−B7. Subfraction B4 (1.69 g) was purified using 3 and 5% TBME−hexane to afford prolificin A, **1** (1.0 g). Fraction A6 (0.74 g), eluted with 20% acetone−hexane, was purified by column chromatography using 30% TBME−hexane to afford prolifenone A, **2** (29.5 mg), and prolifenone B, **3** (100 mg).

**Prolificin A (1):** yellow oil; $[\alpha]^{25}_{D}$ +8.77 (*c* 0.57, MeOH); UV (EtOH) $\lambda_{max}$ (log $\epsilon$) 294 (4.08) nm; IR (NaCl) $\nu_{max}$ 3351, 1622, 1229, 1138 $cm^{-1}$; $^1H$ NMR ($C_6D_6$, 400 MHz), see Table 1; $^{13}C$ NMR ($C_6D_6$, 100 MHz), see Table 1; LR-ESIMS *m*/*z* 481 $[M - H]^+$ (100); HR-MALDIMS *m*/*z* 505.3277 $[M + Na]^+$ (calcd for $C_{31}H_{46}O_4Na$, 505.3288).

**Prolifenone A (2):** yellow oil; $[\alpha]^{25}_{D}$ +13.3 (*c* 0.145, MeOH); UV (EtOH) $\lambda_{max}$ (log $\epsilon$) 278 (3.96) nm; IR (NaCl) $\nu_{max}$ 3411, 1726, 1718, 1685, 1652, 1635, 1458, 1380, 1229 $cm^{-1}$; $^1H$ NMR ($C_6D_6$, 400 MHz), see Table 2; $^{13}C$ NMR ($C_6D_6$, 100 MHz), see Table 2; LR-ESIMS *m*/*z* 481 $[M - H]^+$ (100); HR-MALDIMS *m*/*z* 505.3304 $[M + Na]^+$ (calcd for $C_{31}H_{46}O_4Na$, 505.3288).

**Prolifenone B (3):** yellow oil; $[\alpha]^{25}_{D}$ −0.58 (*c* 1.30, MeOH); UV (EtOH) $\lambda_{max}$ (log $\epsilon$) 281 (3.84) nm; IR (NaCl) $\nu_{max}$ 3394, 1732, 1717, 1683, 1653, 1635, 1456, 1377, 1230 $cm^{-1}$; $^1H$ NMR ($C_6D_6$, 400 MHz), see Table 2; $^{13}C$ NMR ($C_6D_6$, 100 MHz), see Table 2; LR-ESIMS *m*/*z* 467 $[M - H]^+$ (100); HR-MALDIMS *m*/*z* 491.3139 $[M + Na]^+$ (calcd for $C_{30}H_{44}O_4Na$, 491.3132).

**Tumor Cell Proliferation Assay.** Prolificin A, prolifenone A, and prolifenone B were dissolved in DMSO to obtain the desired concentrations (15 to 130 $\mu$M), and the tumor cell proliferation inhibition assay was conducted according to the established procedure at the Bioactive Natural Products and Phytoceuticals (BNPP) laboratory at MSU.[9] Only prolificin A was found to inhibit the growth of all tumor cell lines studied.

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## References and Notes

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