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Cytotoxic Phloroglucinols from the Leaves of *Myrtus communis*

Filippo Cottiglia,*[†] Laura Casu,[†] Marco Leonti,[†] Pierluigi Caboni,[†] Costantino Floris,[‡] Bernardetta Busonera,[§] Pamela Farci,[§] Aicha Ouhtit,[§] and Giuseppina Sanna[§]

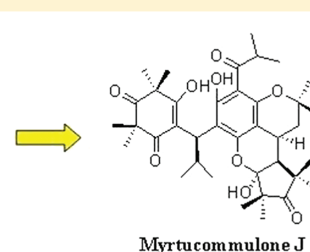
[†]Dipartimento Farmaco Chimico Tecnologico, University of Cagliari, Via Ospedale 72, 09124 Cagliari, Italy

[‡]Dipartimento di Scienze Chimiche, University of Cagliari, Cittadella Universitaria, SS 554 Km 4,500, 09042 Monserrato, Italy

[§]Dipartimento di Scienze e Tecnologie Biomediche, Sez. di Microbiologia e Virologia Generale e Biotecnologie Microbiche, University of Cagliari, Cittadella Universitaria, SS 554 Km 4,500, 09042 Monserrato, Italy

Supporting Information

ABSTRACT: Bioactivity-guided fractionation of a dichloromethane extract of the leaves of *Myrtus communis* led to the isolation of phloroglucinol derivatives. The structures of the new myrtucommulones J, K, and L (**1**–**3**) and the previously known myrtucommulone A (**4**) were elucidated on the basis of extensive 1D and 2D NMR experiments as well as high-resolution mass spectrometry. Myrtucommulone J was obtained as a tautomeric pair (**1**/**1a**). The compounds were tested in vitro for their cytotoxic and antibacterial activities.



Myrtus communis L. (Myrtaceae) is an evergreen shrub widely distributed in the Mediterranean region. In Sardinia, the leaves of *M. communis* are used as a culinary spice, and maceration of the berries and leaves in alcohol is used to prepare red and white "Mirto" liquor.[1] *M. communis* also has a long history in traditional medicine, well known for its antiseptic and anti-inflammatory properties.[2,3] The leaves contain unique non-prenylated alkyl and acylphloroglucinols.[4−7] These *M. communis* acylphloroglucinols (myrtucommulones A−E) are characterized by a phloroglucinol ring connected via an isobutyl bridge to one or two syncarpic acids. These compounds have received attention due to their interesting antibacterial,[5−8] anti-inflammatory,[9−11] and antioxidant properties,[12] as well as for their α-glucosidase inhibitory activity.[7] Despite the structural similarity of myrtucommulones to the antitumoral filixic acids,[13] little attention has been paid to their cytotoxic activity. Mediation of apoptotic cell death has been reported for myrtucommulone A,[14] while antiproliferative activity on HaCat keratinocytes has been reported for myrtucommulones A and B.[15] In our continuous search for plant-derived compounds with cytotoxic activity,[16] the present paper describes the isolation, structure elucidation, and cytotoxic and antibacterial activity of four phloroglucinols, three of which are new, obtained from an extract of leaves of *M. communis*. Bioguided fractionation of the DCM extract was conducted following cytotoxicity against MT-4 cells in vitro. Pure compounds were tested against a panel of cancer cell lines and against a strain of *Staphylococcus aureus*. The DCM fractions were also evaluated, but showed no significant activity (data not shown), against members of the Retroviridae, Flaviviridae, Picornaviride, Poxviridae, and Herpesviridae families.

RESULTS AND DISCUSSION

The DCM extract from the leaves of *M. communis* exhibited cytotoxic activity against MT-4 cells derived from human hematological tumor. Bioguided fractionation was carried out in order to identify the cytotoxic compounds. The DCM extract was subjected to vacuum liquid chromatography (VLC) to yield seven major fractions (F1−F7). Fraction F3 exhibited the highest activity, with an IC_{50} value of 3.7 μg/mL. Subsequently, this fraction was further evaluated versus solid tumor cell lines (HepG2, DU145) and against "normal" human tissue cells (CRL7065). Fraction F3 was then subjected to open column chromatography and semipreparative HPLC to afford three new (**1**−**3**) and one known (**4**) phloroglucinol.

The ^{13}C NMR spectrum of myrtucommulone J (**1**) exhibited 38 carbon signals, which were sorted by DEPT experiments into 14 CH_3, one CH_2, five CH, and 18 quaternary carbons. This corresponded to the molecular formula $C_{38}H_{52}O_9$, in agreement with a molecular peak at m/z 653 $[M + H]^+$ in low-resolution ESIMS and a pseudomolecular ion at m/z 653.3680 $[M + H]^+$ (calcd 653.3684) in the HR-TOF-ESI mass spectrum. The ^{1}H NMR spectrum of **1**, however, revealed doubled signal patterns in a ratio of approximately 9:1 (derived from the ^{1}H NMR signal intensities). Because only one pseudomolecular peak at m/z 653 was detected in the ESIMS, the ^{1}H NMR signal pattern indicated the presence of two isomeric forms. Also, by HPLC analysis, only one peak could be detected for compound **1**, suggesting an equilibrium mixture of two tautomers or rotamers (**1** and **1a**). The NMR signal assignments presented below refer to the most abundant isomer (**1**). The ^{1}H NMR spectrum of compound **1** showed two low-field

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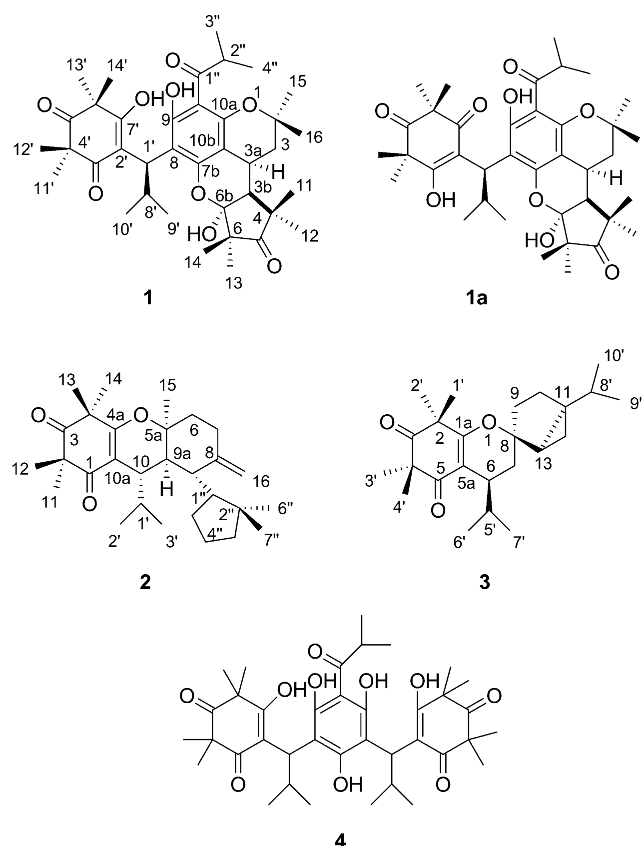

signals (δ_H 14.30, 9.15) suggesting the presence of OH protons that participate in strong hydrogen bonds. Ten tertiary [δ_H 0.91, 1.33 (2CH$_3$), 1.37, 1.38 (2CH$_3$), 1.39, 1.41, 1.42, 1.51] and four secondary (δ_H 0.53, 0.77, 1.14, 1.21) methyl signals were present. A methine proton (δ_H 4.08) in a 2-methylpropionyl side chain, four further methines (δ_H 2.93, 3.43, 3.44, 3.48), and one methylene (δ_H 1.93, 1.56) signal were also detected. HMBC correlations between the methine of the 2-methylpropionyl side chain at δ 4.08 and C-1″ (δ_C 212.4) and C-10 (δ_C 105.5) and between the OH proton at δ_H 14.3 and C-1″, C-10a (δ_C 163.4), C-9 (δ_C 154.8), and C-10 (Figure 1) are characteristics of acylphloroglucinols with an OH at C-9. Four quaternary methyl groups in the HMBC spectrum (δ_H 1.37, 1.38, 1.42, and 1.51) showed correlations with C-6′, C-4′, C-7′, C-3′, and C-5′, indicating the presence of an enolized 1,1,3,3-tetramethylcyclohexatrione (syncarpic acid).[17] The DQF-COSY experiment showed correlations of the following spin system: the methine proton at δ_H 3.43 showed a cross-peak with the methine proton at δ_H 2.93, which in turn correlated with two methyl groups (δ_H 0.53 and 0.77) (Figure 1). This was indicative of an isobutyl group, and cross-peaks in the HMBC spectrum between the methine proton at δ_H 3.43 and C-2′ (δ_C 114.0), C-3′, C-7′, C-8 (δ_C 104.9), C-9, and C-7b (δ_C 159.3) demonstrated that this group was attached to C-2′ of the syncarpic acid and to C-8 of the acylphloroglucinol. HMBC correlations of the OH at δ_H 9.15 with C-7′, C-6′, C-3′, and C-2′ determined that the OH was at C-7′. In the DQF-COSY spectrum, a further spin system was observed, and the partial structure −CH−CH−CH$_2$− was deduced by cross-peaks between H-3a (δ_H 3.48) and the H-3 methylene protons (δ_H 1.93 and 1.59) and H-3b (δ_H 3.34), while H-3b correlated with H-3a. H-3a also showed HMBC correlations with C-10b, C-6b, C-3, and C-3b. This indicated that the −CH−CH−CH$_2$− moiety was linked to C-10b of the central 1,3,5-trioxygenate aromatic group. The methine proton at δ_H 3.34 correlated with C-3 and C-3a (δ_C 35.4) and with the quaternary oxygenated C-6b, suggesting a C-7b/C-10b fusion of the aromatic nucleus with a pyran unit. Further cross-peaks of the proton at δ_H 3.34 with C-4 (δ_C 51.0) and C-5 (δ_C 216.9) and of the *gem*-dimethyl group at δ_H 0.91 and 1.41 with C-3b, C-4, and C-5, and of a further *gem*-dimethyl group at δ_H 1.33 and 1.39 with C-5, C-6 (δ_C 54.0), and C-6b suggested the presence of a tetramethylcyclopentanone fused at C-3b/C-6b of the pyran ring. Finally, a second pyran ring was deduced from HMBC correlations between the methylene protons at C-3 and C-2 (δ_C 86.7), C-15 (δ_C 30.5), and C-16 (δ_C 28.3).

The relative configuration of compound **1** was determined by ROESY experiments. With geminal hydrogens of a conformationally locked six-membered ring the equatorial hydrogen is typically deshielded with respect to the axial hydrogen. Therefore the resonance at δ_H 1.93 of one of the methylene hydrogens at position 3 was assigned to H-3$_{eq}$, while that at δ_H 1.59 was assigned to H-3$_{ax}$. In the ROESY spectrum H-3$_{eq}$ showed correlations with H-3a, which in turn correlated with the CH$_3$-15 at δ_H 1.33 (Figure 2). This observation suggested that H-3a and CH$_3$-15 were both on the α-face of the molecule. Also, H-3b was on the α-face of the benzodipyran system, as a strong cross-peak was evident between H-3b and H-3$_{eq}$. In the same spectrum a correlation between H-13 and H-10′ indicated that the isopropyl group attached to C-1′ was β. The structure

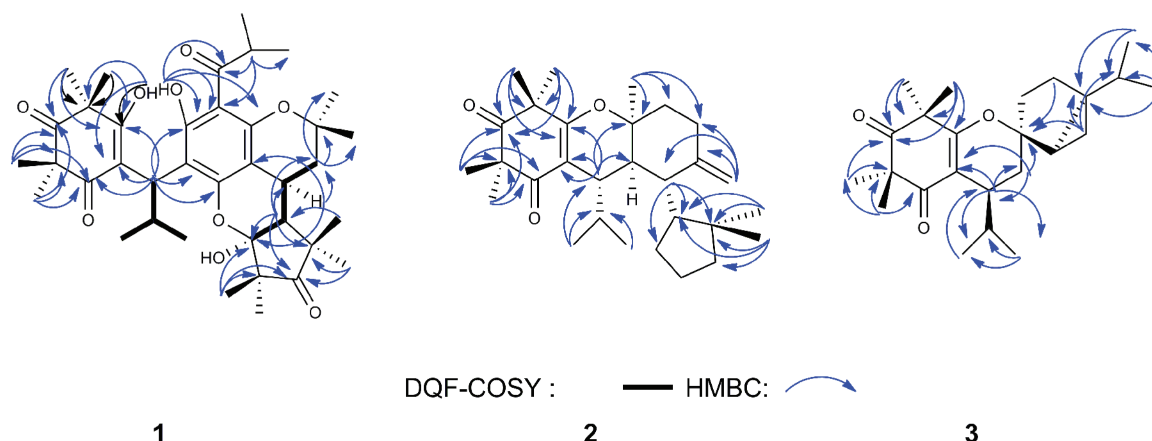

Figure 1. Main HMBC and DQF-COSY correlations of myrtucommulones J (**1**), K (**2**), and L (**3**).

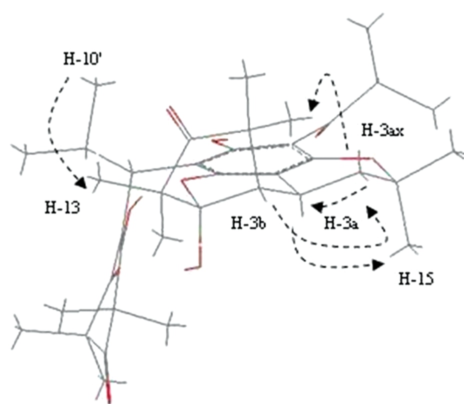

Figure 2. Selected ROE cross-peaks of myrtucommulone J (**1**).

assignment of **1** suggests that the perceived isomeric forms are due to a tautomeric equilibrium of the enolized syncarpic acid.

The ^{13}C NMR spectrum of myrtucommulone K (**2**) exhibited 29 carbon signals, which were sorted by DEPT experiments into nine CH_3, six CH_2, five CH, and nine quaternary carbons. This corresponded to the molecular formula $C_{29}H_{44}O_3$, in agreement with a molecular peak at m/z 441 $[M + H]^+$ in low-resolution ESIMS and a pseudomolecular ion at m/z 441.3353 $[M + H]^+$ (calcd 441.3363) in the HR-TOF-ESI mass spectrum. The ^{1}H NMR spectrum of **2** showed signals at δ_H 4.89 and 4.87, indicating a terminal methylene, one CH (δ_H 2.67), nine CH_3 groups (δ_H 0.66, 0.91, 0.95, 1.13, 1.31 (2CH_3), 1.32, 1.33, and 1.36), and a number of overlapping aliphatic signals in the range between 1.45 and 2.40 ppm. Correlations of the methyl singlets at δ_H 1.32 and 1.36 to C-1 (δ_C 198.1), C-2 (δ_C 55.1), and C-3 (δ_C 214.0) and of the methyl singlets at δ_H 1.31 and 1.33 to C-3, C-4 (δ_C 47.8), and C-4a (δ_C 170.2) in the HMBC spectrum (Figure 1) suggested that an enolized 1,1,3,3-tetramethylcyclohexatrione core was present also in compound **2**. Judging from the HMBC correlations of the methine at δ_H 2.67 with C-10a (δ_C 112.4), C-4a, C-1, C-9a (δ_C 39.3), and C-5a (δ_C 85.5) an isobutyl group was attached to C-10a of the syncarpic acid moiety. While in myrtucommulone J the isobutyl group was attached to an aromatic acylphloroglucinol, in compound **2** it was linked to a methylenecyclohexane ring. This was evidenced by long-range correlations in the HMBC spectrum between the methine group at δ_H 2.67 and C-9a, C-9 (δ_C 41.7), and C-5a, as well as between the two methylene protons at δ_H 4.89 and 4.87 and C-8 (δ_C 151.0), C-9, and C-7 (δ_C 35.5), and finally between the methyl group at δ_H 1.31 and C-9a, C-5a, and C-6 (δ_C 44.0). A *gem*-dimethylcyclopentane ring linked to C-9 of the xanthene nucleus was also present, as judged from HMBC correlations of the methine at δ_H 2.40 with C-1″ (δ_C 56.8), C-2″ (δ_C 34.3), and C-5″ (δ_C 25.1) and from correlations of the *gem*-dimethyl group at δ_H 0.91 and 0.95 with C-1″, C-2″, and C-3″ (δ_C 36.4).

The relative configuration of compound **2** was determined by ROESY experiments. Correlations were observed between the methyl singlet H-15 and H-9a and the isopropylic methyl group H-3′, suggesting that the three groups were on the α-side of the molecule. In the same spectrum, a strong cross-peak between H-9 and H-10 suggested that the dimethylcyclopentane ring was fixed on the α-side of the xanthene nucleus. The structure of myrtucommulone K was therefore assigned as **2**.

The HR-TOF-ESI mass spectrum of myrtucommulone L (**3**) displayed a molecular ion peak at m/z 373.2748 (calcd 373.2737) $[M + H]^+$. This molecular mass in combination with ^{1}H and ^{13}C NMR data established the molecular formula as $C_{24}H_{36}O_3$. The ^{1}H and ^{13}C NMR data for compound **3** showed signals characteristic of a syncarpic acid-derived tetramethylcyclohexenedione system, already observed in compounds **1** and **2**. The ^{1}H NMR spectrum also showed four isopropylic methyl groups at δ 0.60, 0.94, 0.86, and 0.88, four methines at δ 2.91, 2.59, 1.29, and 1.23, and four methylenes (δ_H 1.82, 1.63; δ_H 1.29; δ_H 1.68; and δ_H 0.68, 0.32). One isopropyl group (δ_H 0.60, 0.94 and δ 2.59) was linked to C-6 of a tetramethyltetrahydrochromenedione nucleus, as judged from long-range correlations in the HMBC spectrum between H-6 at δ 2.91 and C-5a (δ 112.5), C-7 (δ 31.4), C-5′ (δ 26.3), C-6′ (δ 15.5), and C-7′ (δ 20.7) and between the two methylene protons at C-7 (δ 1.82, 1.63) and C-6 (δ 33.7), C-5a as well as C-8 (δ 87.8) (Figure 1). Correlations observed in the DQF-COSY and HMBC spectra of compound **3** suggested the presence of a bicyclic monoterpene of the thujane type. HMBC cross-peaks of C-8 with 2H-7, 2H-10 (δ_H 1.68), and 2H-12 (δ_H 0.32; 0.68) established that this ring was linked to the tetramethyltetrahydrochromenedione nucleus via a quaternary spirocenter at C-8.

The relative configuration of compound **3** was determined by ROESY experiments. Correlations were observed between H-7_{ax} and the two methyls H-6′ and H-7′ and between H-7_{eq} and methyl H-7′, suggesting that the isopropyl group attached at C-6 was on the β-face of the tetramethyltetrahydrochromenedione nucleus. Further correlations between H-7_{eq} and H_3-10′ and between H-13 and H_3-9′ and H_3-10′ indicated β-disposition of the cyclopropyl ring with respect to the above-mentioned nucleus. The structure of myrtucommulone L was therefore assigned as **3**.

The spectroscopic data of the known compound myrtucommulone A (**4**) were in agreement with those found in the literature.[4,5]

Compounds **1**/**1a**–**4** were evaluated against the human hematological tumor cell line MT-4. Compounds **1**/**1a** and **4**, which showed significant activity, were also evaluated against solid tumor cell lines (HepG2, DU145) and against "normal" human tissue cells (CRL7065). The most active compound versus cancer cells was **1**/**1a**, with IC_{50} values ranging from 2.1 to 3.0 μM, and **4** had IC_{50} values in the range of 4.7 and 14.0 μM. Compounds **2** and **3** were not cytotoxic against MT-4 cells (IC_{50} values above 41.0 μM). With respect to the active compounds **4** and **1**/**1a**, compounds **2** and **3** both lack an aromatic phloroglucinol core, which seems to be essential for the cytotoxic activity.

Because antibacterial activity was reported for different myrtucommulones,[5−8] we also evaluated the antibacterial potential of **1**/**1a**–**4** against *Staphylococcus aureus*. The similar range of antiproliferative activity indicates that the toxicity of **2**–**4** toward bacteria and eukaryotic cells is due to a nonspecific mode of action. Compound **1**/**1a**, however, showed a certain specificity, being 35-fold more active against *S. aureus* with respect to normal eukaryotic cells with a MIC of 0.38 μM. Compounds **4** and **1**/**1a** show high structural similarity to filixic acid. Remarkably, some filixic acid derivatives isolated from the genus *Dryopteris* inhibited tumorigenesis in an animal model.[13] The mechanism of action involved in the cytotoxicity of *Myrtus* acylphloroglucinols remains to be determined, although it is reported that myrtucommulone A induces apoptosis in cancer cells via the mitochondrial cytochrome *c*/Apaf-1/caspase-9 pathway.[14]

■ EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured in $CHCl_3$ at 25 °C using a Perkin-Elmer 241 polarimeter.

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UV spectra were recorded on a GBC Cintra 5 spectrophotometer. ^{1}H and 2D NMR spectra of **1**/**1a** were recorded on a Bruker AVANCE 600 spectrometer (operating at 600.13 MHz for ^{1}H and 150.92 MHz for ^{13}C). All other spectra were measured on a Varian Unity INOVA 400 MHz spectrometer, operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. All spectra were measured at 25 °C in $CDCl_3$ and referenced against residual C*H*Cl_3 in $CDCl_3$ (^{1}H 7.27 ppm) and $CDCl_3$ (^{13}C 77.0 ppm). HR-ESIMS (positive mode) were measured on a Agilent 6520 time of flight (TOF) MS instrument, while ESIMS experiments were performed on a Varian 1200 L triple quadrupole. Column chromatography (CC) was carried out under TLC (thin-layer chromatography) monitoring using silica gel (40−63 μm, Merck) and Sephadex LH-20 (25−100 μm, Pharmacia). Silica gel (40−63 μm) (Merck) was used for VLC. TLC was performed on silica gel 60 F_{254} or RP-18 F_{254} (Merck). Semipreparative HPLC was conducted by means of a Varian 920 LH instrument fitted with an autosampler module with a 1000 μL loop. The columns were a 250 × 10 mm Spherisorb silica, particle size 5 μm (Waters), and a 250 × 10 mm Polaris C-18-A, particle size 5 μm (Varian). The UV detection wavelength was 254 nm. For molecular modeling a PC Spartan Pro software program (Wavefunction Inc.) was used.

Plant Material. *Myrtus communis* leaves were collected in June 2008 at Capoterra, Sardinia, Italy. A voucher specimen (No. 0322) was deposited in the Herbarium of the Dipartimento Farmaco Chimico Tecnologico, University of Cagliari.

Extraction and Isolation. Air-dried and powdered leaves of *M. communis* (507 g) were ground and extracted with CH_2Cl_2 (4 L) by percolation at room temperature to give 24.4 g of dried extract. An aliquot (10 g) of the CH_2Cl_2 extract was subjected to VLC (silica gel, 90 g) using a step gradient of petroleum ether/CH_2Cl_2/EtOAc (9:1:0 to 0:1:9, 500 mL each) to yield six main fractions (F1−F6). The bioactive fraction (F3) (1.1 g) eluted with petroleum ether/CH_2Cl_2 (2.5:7.5) was separated by CC over silica gel using hexane/EtOAc (9:1) as eluent to obtain six subfractions (F3.1−F3.6). Fraction F3.1 (0.5 g) was subjected to VLC over silica using a step gradient of hexane/EtOAc (9.5:0.5 to 9:1, 50 mL each), yielding three subfractions (F3.1.1−F3.1.3). Fraction F3.1.2 (120 mg) was first purified by RP-18 HPLC using acetonitrile/trifluoroacetic acid (TFA) (99.95:0.05) and then rechromatographed by NP HPLC using hexane/EtOAc (9.6:0.4) as eluents, yielding compound **3** (2.5 mg). Fraction F3.2 (70 mg) was purified by RP-18 HPLC using acetonitrile/TFA (99.95:0.05) to give compound **2** (4.7 mg). Fractions F3.3 (11.0 mg) and F3.4 (62.8 mg) were combined and purified by solid-phase extraction (SPE) (RP-18) with acetonitrile to give a yellow solid (45 mg). The solid was purified further by RP-18 HPLC using acetonitrile/TFA (99.95:0.05) to give compound **4** (18 mg). Fraction F3.5 (50.6 mg) was purified by SPE (RP-18) using acetonitrile as eluent and then with RP-18 HPLC using acetonitrile/TFA (99.95:0.05) to afford compound **1**/**1a** (11.6 mg).

*Myrtucommulone J (**1**):* yellow, amorphous powder; $[\alpha]^{25}_D$ 0 (*c* 0.05, CH_2Cl_2); UV (CH_2Cl_2) λ_{max} (log ε) 224 (4.8), 289 (3.9); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 14.30 (1H, s, OH-9), 9.15 (1H, s, OH-7′), 4.08 (1H, hept, *J* = 6.6 Hz, H-2″), 3.48 (m, H-3a), 3.43 (1H, d, *J* = 3.0 Hz, H-1′), 3.34 (1H, d, *J* = 4.8 Hz, H-3b), 2.93 (1H, m, H-8′), 1.93 (1H, dd, *J* = 12.6, 12.0 Hz, H-3_{eq}), 1.59 (1H, dd, *J* = 12.6, 12.2 Hz, H-3_{ax}), 1.51 (3H, s, H-14′), 1.42 (3H, s, H-11′), 1.41 (3H, s, H-11), 1.39 (3H, s, H-13), 1.38 (6H, s, H-16, H-13′), 1.37 (3H, s, H-12′), 1.33 (6H, s, H-14, H-15), 1.21 (3H, d, *J* = 6.6 Hz, H-3″), 1.14 (3H, d, *J* = 6.6 Hz, H-4″), 0.91 (3H, s, H-12), 0.77 (3H, d, *J* = 6.6 Hz, H-10′), 0.53 (3H, d, *J* = 6.6 Hz, H-9′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 216.9 (C, C-5), 212.4 (C, C-1″), 212.1 (C, C-5′), 203.1 (C, C-3′), 177.6 (C, C-7′), 163.4 (C, C-10a), 159.3 (C, C-7b), 154.8 (C, C-9), 115.4 (C, C-10b), 114.0 (C, C-2′), 105.5 (C, C-10), 104.9 (C, C-8), 99.9 (C, C-6b), 86.7 (C, C-2), 54.9 (C, C-4′), 54.0 (C, C-6), 51.0 (C, C-4), 48.7 (C, C-6′), 40.5 (CH, C-1′), 39.6 (CH, C-2″), 38.9 (CH_2, C-3), 36.8 (CH, C-3b), 35.4 (CH, C-3a), 30.5 (CH_3, C-15), 28.8 (CH_3, C-11), 28.3 (CH_3, C-16), 26.4 (CH, C-8′), 25.5 (CH_3, C-13′, C-14′), 25.4 (CH_3, C-11′), 24.6 (CH_3, C-12′), 24.5 (CH_3, C-14), 23.6 (CH_3, C-12), 22.1 (CH_3, C-10′), 21.7 (CH_3, C-9′), 19.7 (CH_3, C-3″), 19.4 (CH_3, C-13), 19.2 (CH_3, C-4″); HR-TOF-ESIMS (*m*/*z*) 653.3680 $[M + H]^+$ (calcd for $C_{38}H_{53}O_9$ 653.3684); EIMS (*m*/*z*) 651 $[M - H]^+$.

*Myrtucommulone K (**2**):* white, amorphous powder; $[\alpha]^{25}_D$ +22.2 (*c* 0.07, CH_2Cl_2); UV (CH_2Cl_2) λ_{max} (log ε) 205 (4.7), 220 (4.8), 265 (3.4); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.89 (1H, br s, H-16a), 4.87 (1H, br s, H-16b), 2.67 (1H, dd, *J* = 3.6, 3.2 Hz, H-10), 2.40 (1H, m, H-9), 2.22 (1H, m, H-7a), 2.07 (1H, m, H-7b), 2.04 (1H, m, H-1′), 2.02 (1H, m, H-6a), 1.81 (1H, m, H-9a), 1.80 (1H, m, H-5″a), 1.71 (1H, m, H-5″b), 1.64 (1H, m, H-4″a), 1.63 (1H, m, H-3″a), 1.56 (1H, m, H-1″), 1.51 (1H, m, H-3″b), 1.47 (1H, m, H-6b), 1.45 (1H, m, H-4″b), 1.36 (3H, s, H-11), 1.33 (3H, s, H-14), 1.32 (3H, s, H-12), 1.31 (6H, s, H-13, H-15), 1.13 (3H, d, *J* = 6.8 Hz, H-3′), 0.95 (3H, s, H-6″), 0.91 (3H, s, H-7″), 0.66 (3H, d, *J* = 6.8 Hz, H-2′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 214.0 (C, C-3), 198.1 (C, C-1), 170.2 (C, C-4a), 151.0 (C, C-8), 112.4 (C, C-10a), 110.9 (CH_2, C-16), 85.5 (C, C-5a), 56.8 (CH, C-1″), 55.1 (C, C-2), 47.8 (C, C-4), 44.0 (CH_2, C-6), 41.7 (CH, C-9), 39.3 (CH, C-9a), 36.4 (CH_2, C-3″), 36.2 (CH, C-10), 35.5 (CH_2, C-7), 34.3 (C, C-2″), 29.8 (CH_3, C-7″), 26.6 (CH_3, C-3′), 25.9 (CH, C-1′), 25.8 (CH_3, C-14), 25.6 (CH_3, C-11), 25.1 (CH_2, C-5″), 24.8 (CH_3, C-12), 24.1 (CH_3, C-13), 23.5 (CH_2, C-4″), 22.9 (CH_3, C-15), 21.7 (CH_3, C-6″), 19.7 (CH_3, C-2′); HR-TOF-ESIMS (*m*/*z*) 441.3363 $[M + H]^+$ (calcd for $C_{29}H_{45}O_3$ 441.3353); EIMS (*m*/*z*) 441 $[M + H]^+$, 463 $[M + Na]^+$, 903 $[2 M + Na]^+$.

*Myrtucommulone L (**3**):* colorless oil; $[\alpha]^{25}_D$ 0 (*c* 0.08, CH_2Cl_2); UV (CH_2Cl_2) λ_{max} (log ε) 220 (4.9), 265 (3.9); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.91 (1H, ddd, *J* = 11.6, 7.6, 3.6 Hz, H-6), 2.59 (1H, m, H-5′), 1.82 (1H, dd, *J* = 13.6, 7.6 Hz, H-7_{eq}), 1.68 (2H, m, H-10), 1.63 (1H, dd, *J* = 13.6, 11.6 Hz, H-7_{ax}), 1.35 (3H, s, H-2′), 1.32 (3H, s, H-4′), 1.31 (3H, s, H-1′), 1.29−1.27 (3H, m, H-9, H-8′), 1.28 (3H, s, H-3′), 1.23 (1H, m, H-13), 0.94 (3H, d, *J* = 7.2 Hz, H-7′), 0.88 (3H, d, *J* = 6.8 Hz, H-10′), 0.86 (3H, d, *J* = 6.8 Hz, H-9′), 0.68 (1H, dd, *J* = 5.2, 4.0 Hz, H-12a), 0.60 (3H, d, *J* = 7.2 Hz, H-6′), 0.32 (1H, dd, *J* = 7.2, 4.4 Hz, H-12b); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 213.7 (C, C-3), 198.1 (C, C-5), 172.6 (C, C-1a), 112.5 (C, C-5a), 87.8 (C, C-8), 55.5 (C, C-4), 48.6 (C, C-2), 34.1 (CH, C-8′), 33.7 (CH, C-6), 32.5 (C, C-11), 32.5 (CH_2, C-9), 31.4 (CH_2, C-7), 29.7 (CH, C-13), 26.3 (CH, C-5′), 25.8 (CH_3, C-3′), 24.1 (CH_2, C-10), 24.0 (CH_3, C-2′), 23.3 (CH_3, C-4′), 23.2 (CH_3, C-1′), 20.7 (CH_3, C-7′), 19.6 (CH_3, C-9′, C-10′), 15.5 (CH_3, C-6′), 11.9 (CH_2, C-12); HR-TOF-ESIMS (*m*/*z*) 373.2748 $[M + H]^+$ (calcd for $C_{24}H_{37}O_3$ 373.2737).

Cytotoxicity Assays. Cell lines were purchased from American Type Culture Collection (ATCC). The absence of mycoplasma contamination was checked periodically by the Hoechst staining method. Exponentially growing MT-4 cells, hepatocellular carcinoma cells (HepG2), prostate carcinoma cells (DU145), and normal tissues, foreskin fibroblasts (CRL7065), were seeded at an initial density of 1×10^5 cells/mL in 96-well plates in RPMI-1640 medium supplemented with 10% fetal bovine serum, 100 units/mL penicillin G, and 100 μg/mL streptomycin. Cell cultures were then incubated at 37 °C in a humidified, 5% CO_2 atmosphere in the absence or presence of serial dilutions of test compounds. Cell viability was determined after 96 h at 37 °C by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide method.[18] Camptothecin and etoposide were used as reference drugs in the cytotoxic assays. DCM fractions (F1−F7) were dissolved in DMSO at 100 μg/mL. Compounds **1**−**4** were dissolved at 100 mM and subsequently diluted in culture medium. Cell growth at each test concentration was expressed as percentage of untreated controls, and concentrations resulting in 50% (IC_{50}) growth inhibition were determined by linear regression analysis.

Antibacterial Activity. Minimum inhibitory concentration (MIC) against *Staphylococcus aureus* ATCC 25923 was determined with the doubling dilution method using 96-well plates as described in Liu et al.[19] Overnight cultures of bacteria at 37 °C in nutrient broth (Liofilchem s.r.l.) were adjusted to a concentration of approximately 10^5 cells/mL. To 25 μL of compound stock solution (4.1 mg/mL and 16 μg/mL) in EtOH brought into the first well of the first column were added 75 μL of sterile broth and 100 μL of culture suspension. Subsequently, 2-fold dilutions in 100 μL of culture suspensions were made, reaching 1 and 0.03 μg/μL as the final concentration. After 20 h incubation at 37 °C and moist atmosphere, 20 μL of aqueous thiazolyl blue tetrazolium bromide (Sigma-Aldrich) was added to each well and the plates were reincubated for another 4 h. Living bacteria were

revealed as violet, turbid solutions. Chloramphenicol was used as a positive control, showing a MIC of 4 μM.

■ ASSOCIATED CONTENT

Supporting Information

NMR and HR-ESIMS spectra of compounds **1**/**1a**–**3** and biological test data of **1**/**1a**–**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: + 39-706758979. Fax: + 39-706758553. E-mail: cottiglf@unica.it.

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