Phenolic Diterpenoid Derivatives as Anti-Influenza A Virus Agents

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EXPERIMENTAL SECTION

Material.

All chemicals including compounds and synthetic reagents were purchased from Sigma-Aldrich if unspecified. Oseltamivir and amantadine were purchased from Tszchem and LKT laboratories and Sigma-Aldrich, respectively. The IAV VR1679 and MDCK cells were obtained from ATCC and the PR8 (A/Puerto Rico/8/34) virus was kindly provided by Dr. Elizabeth Ramsburg at Duke University. Chicken RBCs were purchased from Rockland Immunochemicals Inc.

Methods.

Anti-influenza virus assay. MDCK cells at 5,000 cells/well were cultured in 96-well plates for a day before IAV infection. The infection medium used was MEM containing 1% FBS, 1 μg/mL trypsin (Sigma-Aldrich), 1 mM sodium pyruvate, 0.1 mM non-essential amino acid, and 100 U/mL penicillin/streptomycin. Various concentrations of compounds were added to the cell culture. The cells were then infected with IAV at a multiplicity of infection (MOI) of 1, unless otherwise indicated. After 48 h of incubation, Promega CellTiter-Glo® reagent was added to each well following the protocol provided by the supplier. The luminescence (RLU) emitted from each well was quantified with a Promega Victor III plate reader. The % protection of MDCK cells from the cytocidal effect of the flu virus was calculated by the following formula: 100 x [(RLUvs - RLUv) / (RLUctr - RLUv)], where RLUvs is the relative luminescence unit (RLU) from the cells cultured with virus and compounds, RLUv is the RLU from the flu virus infected cells only, and RLUctr is the cells cultured in the absence of virus and compounds. EC_{50}, the concentration required to protect 50% of MDCK cells from cytocidal effect of flu viruses, was calculated using the software CalcuSyn (Biosoft, Cambridge, UK).

Hemolysis inhibition assay. Fresh chicken RBCs were washed twice with PBS and re-suspend in PBS to make a 2% (V/V) suspension to be stored at 4 °C until use. Compounds in 50 μL of PBS (or DMSO in PBS for no-compound control) and equal volume of virus in culture medium were incubated at room temperature for 30 min. Chicken RBCs (2%, 100 μL) pre-warmed to 37 °C was added to each compound/virus mixture and incubated at 37 °C for another 30 min. To trigger hemolysis, 50 μL of sodium acetate buffer (0.15 M, pH 5.0) was added to each mixture and incubated at 37 °C for 45 min. The samples were centrifuged on an Eppendorf 5415C centrifuge at 3000 rpm for 2 min. The supernatants were collected, and formaldehyde was added to a final concentration of 0.5%. The absorbance (OD540) of the supernatants was recorded using a PerkinElmer microplate reader. Percentage of protection of hemolysis by a compound was calculated as $[1- (OD540_{compound} - OD540_{background\ control})/(OD540_{no\text{-}compound\ control} - OD540_{background\ control})] \times 100\%$, where background control is the sample without virus and compound, and no-compound control is the virus-induced hemolysis without compounds.

Immunostaining and confocal microscopy. MDCK cells cultured in 96-well glass-bottom plates were treated with compounds and infected with IAV (MOI = 1) for 6 h. The cells were fixed with 4% formaldehyde in PBS for 15 min. The cells were then treated with a blocking buffer containing 5% FBS and 0.3% Triton X-100 in PBS for 60 min. Immunostaining was carried out by incubating FITC-conjugated anti-influenza A NP antibody (Thermo Fisher Scientific) with the cells at 4 °C overnight. The samples were washed three times in PBS before treated with Prolong® Gold Anti-Fade Reagent with DAPI (Cell Signaling Technology) for

nuclear staining. Confocal images were acquired using a Nikon A1R confocal microscope. Confocal image analysis was performed with NIS-Elements AR 3.0 software.

***In vitro* stability in human plasma.** Plasma stability of compounds **2** and **19** were evaluated in a duplicated experiment using Benfluorex (Toronto Research Chemical Inc.) as a control compound. The stability of the compounds was analyzed by HPLC with UV absorption set at 210 nm or 220/270 nm. Podocarpic acid (**1**) was used as the internal standard for HPLC analysis. HPLC instruments and conditions were the same as described in Chemistry section below.

To carry out the experiment, 1170 μL of human plasma (Innovative Research, Inc.) diluted to 80% with PBS (pH 7.4) was placed in each well of a 24-well plate before adding 30 μL of a compound in DMSO. The final concentration of each compound was 1 μM containing 2.5% of DMSO. The plate was then sealed and incubated at 37 °C on a shaker for various durations. The incubation mixture (200 μl) was collected from each well and added to 4 °C acetonitrile (400 μl) spiked with the internal standard. After vortexing for 1 min, the sample was centrifugation at 14,000 rpm at 4 °C for 15 min. The clear supernatant was filtered with a 0.2 μm syringe filter before HPLC analysis. The relative quantity of parent compound (Q) was normalized using the internal standard. The % of remaining parent compound in each sample was calculated as Q_t/Q_0 x 100%, where Q_0 is the quantity of parent compound obtained at 0 incubation time and Q_t is the quantity of the parent compound found in the samples extracted at different incubation time points.

Chemistry.

General. Podocarpic acid (**1**), totarol (**24**), and derivatives **2**, **19** - **21** were known compounds. Compounds **2**, **20** and **21** were synthesized by previously reported methods.[15,16] Compounds (**3** - **19**, **22**, and **23**) were synthesized and analyzed with positive or negative HR-FABMS on a Shimadzu LCMS-IT-TOF or a Joel SX-102 mass spectrometer. ^{1}H NMR spectra were measured on a Varian 400 or 500 MHz spectrometer as indicated. Samples were dissolved in $CDCl_3$ with TMS as an internal standard unless specified. Biotage Initiator (Biotage) was used for microwave heating in synthesis. Silica gel chromatography was carried out on an ISCO CombiFlash Rf flash chromatograph system with a pre-packed Redi Sep Rf Si gel column (Teledyne ISCO) and mobile phase of Hexane/EtOAc or DCM/MeOH in a gradient of increasing polarity. Compounds were purified on HPLC using a Varian ProStar HPLC system with a PDA detector and Agilent Zorbax C18 columns (5 μm particle size, 4.6 × 250 mm or 9.4 × 250 mm). The mobile phase used for HPLC was the combination of solution A (5% ACN in H_2O with 0.045%TFA) and B (ACN/MeOH/H_2O = 85:10:5 and with 0.045% TFA) in a gradient of decreasing polarity. All synthesized compounds were confirmed having a purity of over 95% by HPLC.

Benzyl podocarpate (3): To a mixture of **1** (116 mg, 0.6 mmol) and potassium carbonate (124 mg, 0.9 mmol) in 10 mL acetone was added benzyl bromide (89 μL, 0.75 mmol). The mixture was heated to 90 °C by microwave for 45 min. The reaction mixture was filtered to separate the salt (which was washed two times by acetone). Further purification was performed by Si-gel column chromatography with Hexane : EtOAc (100:0 to 80:20) as the eluent to yield **14 (**210 mg, 96%). ^{1}H NMR (400 MHz) δ 7.35 (m, 5H), 6.90 (d, 1H, *J* = 8.4 Hz), 6.73 (d, 1H, *J* = 2.8 Hz), 6.58 (dd, 1H, *J* = 8.0 Hz, *J* = 2.4 Hz), 5.11 (dd, 2H, *J* = 15.6 Hz, *J* = 12.4 Hz), 2.65-2.83 (m,

2H), 2.31 (m, 1H), 2.18 (m, 2H), 1.98 (m, 2H), 1.65-1.52 (m, 2H), 1.41 (m, 1H), 1.30 (s, 3H), 1.22 (m, 1H), 0.98 (s, 3H). Calcd for $C_{24}H_{29}O_3$ $(M+H)^+$: 365.2111. Found: 365.2116.

12-Hydroxy-15-methylamino-podocarpa-8,11,13-triene-15-one (22): To **1** (54 mg, 0.2 mmol) in 2 mL THF at 0 °C was added BOP reagent (86 mg, 0.2 mmol). After 5 min, methylamine hydrochloride (20 mg, 0.3 mmol) and TEA (52 µL, 0.4 mmol) were added. The reaction mixture was stirred overnight under N_2 at room temperature. After the solvent was removed under vacuum, the residue was chromatographed with Hexane : EtOAc (100:0 to 50:50) as the eluent to yield **22 (**15 mg, 25%). ^{1}H NMR (400 MHz) ($CDCl_3$/CD_3OD = 1:9) δ 6.79 (d, 1H, *J* = 8.0 Hz), 6.65 (s, 1H), 6.51 (dd, 1H, *J* = 8.4 Hz, *J* = 2.4 Hz), 6.06 (m, 1H), 2.68 (m, 5H), 2.12 (m, 2H), 2.04 (m, 1H), 1.95-1.87 (m, 2H), 1.56 (m, 1H), 1.40 (m, 1H), 1.31 (m, 1H), 1.16 (s, 3H), 1.11 (m, 1H), 0.98 (s, 3H). Calcd for $C_{18}H_{26}NO_2$ $(M+H)^+$: 288.1958. Found: 288.1958.

Synthesis of compounds 4, 5, and 23: To a mixture of **2** (15 mg, 0.05 mmol) and acrylic acid (11 µL, 0.15 mmol) in 2 mL DCM was added DCC (31 mg, 0.15 mmol) and DMAP (2 mg, 0.015 mmol). The mixture was heated to 90 °C by microwave for 15 min. After the solvent was removed under vacuum, the residue was chromatographed with Si-gel chromatography (Hexane : EtOAc = 100:0 to 90:10) to give **4** (13 mg, 19%). Compounds **5** (91%) and **23 (**23%) were synthesized by the same method with starting materials **3** and **22**, respectively.

Methyl 12-*O*-acryloyl-podocarpate (4): ^{1}H NMR (400 MHz) δ 7.05 (d, 1H, J = 8.0 Hz), 6.99 (s, 1H), 6.85 (d, 1H, *J* = 8.0 Hz), 6.58 (d, 1H, *J* = 18.8 Hz), 6.31 (dd, 1H, *J* = 10.8 Hz, *J* = 17.2 Hz), 5.98 (d, 1H, *J* = 10.8 Hz), 3.66 (s, 3H), 2.91 (m, 1H), 2.78 (m, 1H), 2.20 (m, 3H), 1.99 (m, 2H),

1.55 (m, 2H), 1.42 (m, 1H), 1.27 (s, 3H), 1.09 (m, 1H), 1.02 (s, 3H). Calcd for $C_{21}H_{27}O_4$ $(M+H)^+$: 343.1904. Found: 343.1907.

Benzyl 12-*O*-acryloyl-podocarpate (5): ^{1}H NMR (500 MHz) δ 7.35 (m, 5H), 7.04 (d, 1H, *J* = 8.0 Hz), 6.98 (d, 1H, *J* = 2.5 Hz), 6.85 (dd, 1H, *J* = 2.5 Hz, *J* = 8.5 Hz), 6.58 (m, 1H), 6.30 (m, 1H), 5.99 (m, 1H), 5.11 (m, 2H), 2.89 (m, 1H), 2.75 (m, 1H), 2.25 (m, 1H), 2.19 (m, 2H), 1.98 (m, 2H), 1.62 (m, 2H), 1.45 (m, 1H), 1.31 (s, 3H), 1.12 (m, 1H), 0.99 (s, 3H). Calcd for $C_{27}H_{31}O_4$ $(M+H)^+$: 419.2217. Found: 419.2214.

12-*O*-Acryloyl-15-methylamino-podocarpa-8,11,13-triene-15-one (23): ^{1}H NMR (500 MHz) (CD_3OD) δ 7.05 (d, 1H, J = 8.5 Hz), 7.00 (d, 1H, *J* = 2.5 Hz), 6.81 (dd, 1H, *J* = 2.5 Hz, *J* = 8.5 Hz), 6.54 (dd, 1H, *J* = 1.5 Hz, *J* = 18.5 Hz), 6.35 (m, 1H), 6.03 (dd, 1H, *J* = 1.5 Hz, *J* = 11.5 Hz), 2.90 (m, 1H), 2.77 (m, 1H), 2.71 (s, 3H), 2.22 (m, 3H), 2.05 (m, 2H), 1.64 (m, 1H), 1.53 (m, 1H), 1.40 (m, 1H), 1.27 (m, 1H), 1.25 (s, 3H), 1.08 (s, 3H). Calcd for $C_{21}H_{28}NO_3$ $(M+H)^+$: 342.2064. Found: 342.2068.

Synthesis of compounds 6 - 9: To a mixture of **2** (29 mg, 0.1 mmol) in 0.5 mL dry DMF at 0 °C was added sodium hydride (4.4 mg, 0.11 mmol). The mixture was stirred at 0 °C for 20 min under N_2. Succinic anhydride (10 mg, 0.1 mmol) was added and the mixture was stirred at 0 °C for 30 min under N_2. The mixture was poured over 0.1 N HCl and extracted with DCM. The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was chromatographed with Si-gel chromatography (DCM : MeOH = 100:0 to 95:5) to give **7** (30 mg, 77%). Compounds **6**, **8**, and **9** were synthesized from **1**, **2** or **3** by the same method with different anhydrides in 72%, 47%, and 66% yields, respectively.

12-*O*-Succinyl-podocarpic acid (6): ^{1}H NMR (400 MHz) δ 7.02 (d, 1H, *J* = 8.0 Hz), 6.90 (d, 1H, *J* = 2.4 Hz), 6.78 (dd, 1H, *J* = 2.4 Hz, *J* = 8.0 Hz), 2.82 (m, 6H), 2.21 (m, 3H), 2.05 (m, 2H), 1.65 (m, 1H), 1.62 (m, 1H), 1.46 (m, 1H), 1.34 (s, 3H), 1.10 (m, 1H), 1.07 (s, 3H). Calcd for $C_{21}H_{26}O_6 \cdot NH_4$ $(M+NH_4)^+$: 392.2068. Found: 392.2068.

Methyl 12-*O*-succinyl-podocarpate (7): ^{1}H NMR (500 MHz) δ 7.02 (d, 1H, *J* = 8.0 Hz), 6.95 (d, 1H, *J* = 1.5 Hz), 6.80 (dd, 1H, *J* = 2.5 Hz, *J* = 8.5 Hz), 3.66 (s, 3H), 2.79 (m, 6H), 2.20 (m, 3H), 1.96 (m, 2H), 1.62 (m, 1H), 1.52 (m, 1H), 1.40 (m, 1H), 1.27 (s, 3H), 1.11 (m, 1H), 1.02 (s, 3H). Calcd for $C_{22}H_{28}O_6 \cdot NH_4$ $(M+NH_4)^+$: 389.1959. Found: 389.1963.

Methyl 12-*O*-glutaryl-podocarpate (8): ^{1}H NMR (400 MHz) δ 7.03 (d, 1H, *J* = 8.4 Hz), 6.94 (d, 1H, *J* = 2.4 Hz), 6.80 (dd, 1H, *J* = 2.4 Hz, *J* = 8.4 Hz), 3.66 (s, 3H), 2.89 (m, 1H), 2.78 (m, 1H), 2.60 (m, 2H), 2.51 (m, 2H), 2.29-1.93 (m, 7H), 1.62 (m, 1H), 1.52 (m, 1H), 1.40 (m, 1H), 1.27 (s, 3H), 1.11 (m, 1H), 1.02 (s, 3H). Calcd for $C_{23}H_{30}O_6 \cdot Cl$ $(M+Cl)^-$: 437.1736. Found: 437.1733.

Benzyl 12-*O*-succinyl-podocarpate (9): ^{1}H NMR (400 MHz) δ 7.33 (m, 5H), 7.01 (d, 1H, *J* = 8.0 Hz), 6.93 (d, 1H, *J* = 2.4 Hz), 6.80 (dd, 1H, *J* = 2.4 Hz, *J* = 8.4 Hz), 5.10 (m, 2H), 2.85 (m, 6H), 2.30 (m, 1H), 2.17 (m, 2H), 1.98 (m, 2H), 1.61 (m, 1H), 1.54 (m, 1H), 1.40 (m, 1H), 1.30 (s, 3H), 1.09 (m, 1H), 0.97 (s, 3H). Calcd for $C_{28}H_{32}O_6 \cdot Cl$ $(M+Cl)^-$: 499.1893. Found: 499.1891.

Synthesis of compounds 10 and 11: To a mixture of **2** (51 mg, 0.18 mmol) in 1 mL dry DMF at 0 °C was added sodium hydride (19 mg, 0.47 mmol). The mixture was stirred at 0 °C for 30 min under N_2. 3-bromopropionic acid (33 mg, 0.22 mmol) was added and the mixture was stirred at room temperature overnight. The reaction mixture was poured over dilute HCl solution (0.1 N) and extracted with DCM. The organic layer was dried over Na_2SO_4, filtered, and concentrated

under reduced pressure. The residue was chromatographed with Si-gel chromatography (DCM : MeOH = 100:0 to 95:5). Further purification was performed by HPLC to yield **10 (**11 mg, 17%). Compound **11** was synthesized from **3** by the same method with 31% yield.

Methyl 12-*O*-(3'-propionic acid)podocarpate (10): ^{1}H NMR (400 MHz) δ 6.96 (d, 1H, J = 8.0 Hz), 6.81 (d, 1H, J = 2.4 Hz), 6.67 (dd, 1H, *J* = 2.8 Hz, *J* = 8.5 Hz), 4.21 (t, 2H, *J* = 6.4 Hz), 3.66 (s, 3H), 2.82 (m, 3H), 2.73 (m, 1H), 2.22 (m, 3H), 1.96 (m, 2H), 1.62 (m, 1H), 1.51 (m, 1H), 1.39 (m, 1H), 1.27 (s, 3H), 1.11 (m, 1H), 1.02 (s, 3H). Calcd for $C_{21}H_{27}O_5$ $(M-H)^-$: 359.1864. Found: 359.1867.

Benzyl 12-*O*-(3'-propionic acid)podocarpate (11): ^{1}H NMR (400 MHz) δ 7.30 (m, 5H), 6.94 (d, 1H, *J* = 8.4 Hz), 6.90 (d, 1H, J = 2.8 Hz), 6.66 (dd, 1H, *J* = 2.4 Hz, *J* = 8.4 Hz), 5.10 (m, 2H), 4.20 (t, 2H, *J* = 6.4 Hz), 2.82 (m, 3H), 2.69 (m, 1H), 2.26 (m, 1H), 2.19 (m, 2H), 1.94 (m, 2H), 1.64 (m, 1H), 1.53 (m, 1H), 1.41 (m, 1H), 1.30 (s, 3H), 1.13 (m, 1H), 0.98 (s, 3H). Calcd for $C_{27}H_{31}O_5$ $(M-H)^-$: 435.2177. Found: 435.2176.

Synthesis of compound 12 - 18: To a mixture of 3-bromopropionic acid (153 mg, 1.0 mmol) in 1.5 mL DCM was added DCC (134 mg, 0.65 mmol) at room temperature. The mixture was stirred for 20 min. A solution of **2** (38 mg, 0.13 mmol) and DMAP (7.6 mg, 0.06 mmol) in 1 mL DCM was added to above mixture. The resulting reaction mixture was heated to 110 °C by microwave for 45 min. After cooling, the reaction mixture was filtered to remove the solid. Further purification was performed by Si-gel column chromatography with Hexane : EtOAc (100:0 to 85:15) as the eluent to yield **12 (**12 mg, 22%). Compounds **13 - 18** were synthesized with corresponding carboxylic acids by the same method with 69%, 40%, 65%, 72%, 54%, and 75% yields, respectively.

Methyl 12-*O*-(3'-bromopropionyl)podocarpate (12): ^{1}H NMR (400 MHz) δ 7.04 (d, 1H, *J* = 9.6 Hz), 6.97 (s, 1H), 6.83 (d, 1H, *J* = 7.6 Hz), 3.68 (m, 5H), 3.15 (m, 2H), 2.86 (m, 2H), 2.28 (m, 1H), 2.18 (m, 2H), 1.98 (m, 2H), 1.62 (m, 1H), 1.44 (m, 1H), 1.40 (m, 1H), 1.28 (s, 3H), 1.08 (m, 1H), 1.03 (s, 3H). Calcd for $C_{21}H_{26}BrO_4 \cdot NH_4$ $(M+NH_4)^+$: 440.1431. Found: 440.1436.

Methyl 12-*O*-(4'-bromobutanoyl)podocarpate (13): ^{1}H NMR (400 MHz) δ 7.04 (d, 1H, *J* = 9.2 Hz), 6.94 (s, 1H), 6.81 (d, 1H, *J* = 8.0 Hz), 3.66 (s, 3H), 3.54 (m, 2H), 2.89 (m, 1H), 2.78 (m, 2H), 2.28 (m, 3H), 2.18 (m, 2H), 2.00 (m, 2H), 1.57 (m, 3H), 1.41 (m, 1H), 1.28 (s, 3H), 1.09 (m, 1H), 1.02 (s, 3H). Calcd for $C_{22}H_{29}BrO_4$ $(M+H)^+$: 437.1332. Found: 437.1335.

Methyl 12-*O*-(5'-bromopentanoyl)podocarpate (14): ^{1}H NMR (400 MHz) δ 7.04 (d, 1H, *J* = 7.6 Hz), 6.94 (s, 1H), 6.80 (d, 1H, *J* = 9.6 Hz), 3.66 (s, 3H), 3.45 (m, 2H), 2.89 (m, 1H), 2.78 (m, 1H), 2.28 (m, 1H), 2.18 (m, 2H), 2.02-1.89 (m, 7H), 1.62 (m, 3H), 1.43 (m, 1H), 1.27 (s, 3H), 1.08 (m, 1H), 1.02 (s, 3H). Calcd for $C_{23}H_{30}BrO_4 \cdot NH_4$ $(M+NH_4)^+$: 468.1744. Found: 468.1745.

Methyl 12-*O*-(6'-bromohexanoyl)podocarpate (15): ^{1}H NMR (400 MHz) δ 7.03 (d, 1H, *J* = 7.6 Hz), 6.94 (s, 1H), 6.80 (d, 1H, *J* = 9.6 Hz), 3.66 (s, 3H), 3.42 (m, 2H), 2.89 (m, 1H), 2.78 (m, 1H), 2.28 (m, 1H), 2.18 (m, 2H), 2.02-1.89 (m, 4H), 1.78 (m, 2H), 1.59 (m, 6H), 1.44 (m, 1H), 1.27 (s, 3H), 1.11 (m, 1H), 1.02 (s, 3H). Calcd for $C_{24}H_{32}BrO_4 \cdot NH_4$ $(M+NH_4)^+$: 482.1900. Found: 482.1910.

Methyl 12-*O*-isobutyryl-podocarpate (16): ^{1}H NMR (500 MHz) δ 7.04 (d, 1H, *J* = 8.0 Hz), 6.94 (s, 1H), 6.80 (d, 1H, *J* = 8.0 Hz), 3.67 (s, 3H), 2.91 (m, 1H), 2.78 (m, 2H), 2.29 (m, 1H), 2.19 (m, 2H), 1.97 (m, 2H), 1.63 (m, 1H), 1.56 (m, 1H), 1.43 (m, 1H), 1.31 (m, 6H), 1.27 (s, 3H), 1.09 (m, 1H), 1.03 (s, 3H). Calcd for $C_{22}H_{30}O_4 \cdot NH_4$ $(M+NH_4)^+$: 376.2482. Found: 376.2472.

Methyl 12-*O*-cyclopropanoyl-podocarpate (17): ^{1}H NMR (500 MHz) δ 7.04 (d, 1H, *J* = 8.5 Hz), 6.97 (s, 1H), 6.82 (d, 1H, *J* = 8.5 Hz), 3.67 (s, 3H), 2.91 (m, 1H), 2.77 (m, 1H), 2.28 (m, 1H), 2.20 (m, 2H), 1.98 (m, 2H), 1.82 (m, 1H), 1.63 (m, 1H), 1.57 (m, 1H), 1.42 (m, 1H), 1.27 (s, 3H), 1.13 (m, 2H), 1.09 (m, 1H), 1.03 (s, 3H), 1.01 (m, 2H). Calcd for $C_{22}H_{29}O_4$ $(M+H)^+$: 357.2060. Found: 357.2053.

Methyl 12-*O*-(2'-fluoropropanoyl)podocarpate (18): ^{1}H NMR (500 MHz) δ 7.06 (d, 1H, *J* = 8.4 Hz), 6.98 (s, 1H), 6.84 (d, 1H, *J* = 8.4 Hz), 5.23 (dq, 1H, *J* = 6.8 Hz, 48.4 Hz), 3.66 (s, 3H), 2.91 (m, 1H), 2.79 (m, 1H), 2.28 (m, 1H), 2.20 (m, 2H), 2.00 (m, 2H), 1.74 (dd, 3H, *J* = 6.8 Hz, 23.2 Hz), 1.64 (m, 1H), 1.52 (m, 1H), 1.41 (m, 1H), 1.28 (s, 3H), 1.09 (m, 1H), 1.03 (s, 3H). Calcd for $C_{21}H_{27}FO_4 \cdot NH_4$ $(M+NH_4)^+$: 380.2232. Found: 380.2228.

Synthesis of compound 19: To a mixture of **2** (20 mg, 0.07 mmol) in 1 mL dry pyridine was added acetic anhydride (17 µL, 0.17 mmol) at room temperature. The mixture was stirred for 17 h, and then poured into a saturated NH_4Cl solution. The mixture was extracted three times with ethyl acetate. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was chromatographed with Si-gel chromatography with Hexane : EtOAc (100:0 to 85:15) to yield **19** (17 mg, 74%).

Methyl 12-*O*-acetyl-podocarpate (19): ^{1}H NMR (500 MHz) δ 7.04 (d, 1H, *J* = 8.0 Hz), 6.94 (d, 1H, *J* = 2.4 Hz), 6.80 (dd, 1H, *J* = 8.0 Hz, 2.4 Hz), 3.66 (s, 3H), 2.89 (m, 1H), 2.77 (m, 1H), 2.27 (s, 3H), 2.26 (m, 1H), 2.17 (m, 2H), 1.98 (m, 2H), 1.63 (m, 1H), 1.56 (m, 1H), 1.42 (m, 1H), 1.27 (s, 3H), 1.08 (m, 1H), 1.03 (s, 3H). Calcd for $C_{20}H_{27}O_4$ $(M+H)^+$: 331.1904. Found: 331.1897.