Supporting Information to:

Identification of a New Natural Camptothecin Analogue in Targeted Screening for HIF-1α Inhibitors

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General Experimental Procedures

NMR data were recorded at 25 °C in acetone-$d_6$ with TMS as internal standard on a Varian 400 MHz INOVA spectrometer. Positive ion FAB-MS was conducted using a VG7070-EHF high resolution mass spectrometer. HR-ESI-MS spectra were obtained on a JEOL SX102 high resolution magnetic sector instrument. HPLC-MS hardware consisted of a Waters 600 pump and 996 photodiodearray spectrometer, a Micromass ZMD electrospray mass spectrometer (cone voltage = 30V) and a Sedex 75 evaporative light scattering detector (ELSD). During dereplication, chromatography was performed by C$_{18}$ HPLC. Scale-up purification was accomplished on Sephadex LH-20 (Pharmacia; Uppsala, Sweden) and phenyl bonded phase HPLC (Varian; Walnut Creek, CA, USA). Optical rotation was determined with a Perkin Elmer 241 polarimeter (Perkin-Elmer, Wellesley, MA, USA). UV spectra were acquired on a Lambda 20 UV/vis spectrometer (PerkinElmer). All solvents were analytical reagent grade.

Extraction and isolation

The dried, ground plant (115 g) was extracted with CH$_2$Cl$_2$-MeOH (1:1) for 16 h by percolation, the solvent removed by rotary evaporation and high vacuum dried, yielding 7.91 g of crude extract. The HPLC method previously published [1], [2], [3] was employed in dereplication. A 100 mg aliquot of the extract was triturated in hexane, with hexane insoluble material then adsorbed onto 0.5 g of C$_{18}$-bonded silica and dried under N$_2$. This was loaded into a syringe barrel fitted with a frit and washed sequentially by vacuum filtration with 1) 4 mL MeCN-H$_2$O (2:8); 2) 2 mL MeCN-MeOH-H$_2$O (85:5:10) + 2 mL MeOH; and 3) CH$_2$Cl$_2$. Upon test, bioactivity was found in the MeCN-MeOH-H$_2$O (85:5:10) fraction. This was filtered through a 0.45 µ membrane, then injected onto a C$_{18}$ Varian Dynamax HPLC column (2.1 × 25 cm, 8 µ, 60 A). The mobile phase was MeCN-20 mM pH 4 NH$_4$OAc run at 15 mL/min under a gradient: 0 – 5 min at (30:70), 5 – 25 min gradient to (50:50), 25 – 45 min gradient to (100:0), 45 – 60 min at (100:0). Using a Gilson fraction collector under MassLynx control, eighty-four 9 mL fractions were collected, which were robotically reformatted to give four ½ log dilutions in a 384 well microtiter plate.
To obtain sufficient mass of \(2 - 4\) for bioassay and structure elucidation, 2 g of extract were partitioned between hexane and MeOH-H\(_2\)O (9:1); the MeOH-H\(_2\)O solubles were adsorbed to C\(_{18}\) bonded phase silica and eluted with 1) MeCN-H\(_2\)O (2:8); 2) MeCN-MeOH-H\(_2\)O (85:5:10); and 3) CH\(_2\)Cl\(_2\). Fraction 2 (260 mg) was passed through a Sephadex LH-20 column, eluting with 620 mL MeOH-CH\(_2\)Cl\(_2\) (9:1); those fractions showing HIF-1\(\alpha\) activity were pooled to give 105 mg. The HIF-1\(\alpha\) active pool was permeated through LH-20 with 840 mL MeOH-CH\(_2\)Cl\(_2\) (1:1). The resulting mixture (39 mg) was highly enriched in camptothecins, which were separated by HPLC on a phenyl bonded phase column at a flow rate of 15 mL/min, eluted with MeOH-H\(_2\)O: 0 – 45 min at (2:1), 45 – 75 min gradient to (1:0). Compounds 1 – 4 were subjected to a final polishing chromatography on Sephadex LH-20 eluted with 230 mL methanol, yielding 1 (0.5 mg, 0.025%), 2 (0.5 mg, 0.025%), 3 (0.5 mg, 0.025%), and 4 (0.4 mg, 0.020%).

The structures of 1 – 3 were confirmed by direct comparison of \(^1\)H-NMR spectra obtained from standards from the NCI repository, as well as with published values [4]. Important \(^1\)H-NMR signals in the confirmation of 3 included a broad doublet attributed to H-9 (\(\delta = 7.49, J = 2.8\) Hz) and the observation of vicinal coupling between H-11 (dd, \(J = 8.8, 2.8\) Hz) and H-12 (br d, \(J = 8.8\) Hz), both consistent with the placement of the methoxy group at C-10. The \(^1\)H-NMR spectrum of 2 exhibited a coupling pattern indicative of a 1,2,3 relationship among the hydrogens of the A-ring. Based on these measurements, the methoxy was assigned to C-9 in 2.

The \(^1\)H-NMR spectrum of 4 contained a singlet at \(\delta = 6.35\) that integrated for two protons, indicative of a methylenedioxy moiety. These methylene protons exhibited HMBC correlations with C-9 (\(\delta = 142\)) and C-10 (\(\delta = 145\)). An HMBC correlation between C-9 (\(\delta = 142\)) and H-7 (\(\delta = 8.51\)) provided further evidence for assigning the methylenedioxy functionality to the 9,10 position. The structure was confirmed by comparison of the \(^1\)H-NMR spectrum with that of a reference sample of synthetic 9,10-methylenedioxy-(20\(R/S\))-camptothecin.

\textbf{20S-Camptothecin (1):} pale yellow powder, \(^1\)H-NMR (acetone-\(d_6\), 400 MHz): \(\delta = 8.69\) (1H, br s, H-7), 8.21 (1H, br d, \(J = 8.4\) Hz, H-9), 8.12 (1H, dd, \(J = 8.4, 1.6\) Hz, H-12), 7.88 (1H, ddd, \(J = 8.4, 6.8, 1.6\) Hz, H-10), 7.72 (1H, ddd, \(J = 8.4, 6.8, 1.2\) Hz, H-11), 7.51 (1H, s, H-14), 5.54 (1H,
4.00 (3H, s, 9-OCH₃), 1.95 (2H, m, H-19), 1.02 (3H, t, J = 7.2 Hz, H-18); ESI-MS: m/z = 349.1 [M + H]⁺.

9-Methoxy-(20S)-camptothecin (2): pale yellow powder; ¹H-NMR (acetone-d₆, 400 MHz): δ = 8.93 (1H, br s, H-7), 7.78 (1H, dd, J = 8.4, 6.0 Hz, H-11), 7.76 (1H, dd, J = 8.4, 2.8 Hz, H-12), 7.49 (1H, s, H-14), 7.15 (1H, dd, J = 6.0, 2.8 Hz, H-10), 5.54 (1H, d, J = 16.4 Hz, H-17a), 5.39 (1H, d, J = 16.4 Hz, H-17b), 5.33 (2H, d, J = 1.2 Hz, H-5), 4.11 (3H, s, 9-OCH₃), 1.95 (2H, m, H-19), 1.01 (3H, t, J = 7.2 Hz, H-18); ESI-MS: m/z = 379.1 [M + H]⁺.

10-Methoxy-(20S)-camptothecin (3): pale yellow powder; ¹H-NMR (acetone-d₆, 400 MHz): δ = 8.55 (1H, br d, J = 8.8 Hz, H-12), 7.51 (1H, dd, J = 8.8, 2.8 Hz, H-11), 7.49 (1H, br d, J = 2.8 Hz, H-9), 7.44 (1H, s, H-14), 5.53 (1H, d, J = 16.0 Hz, H-17a), 5.38 (1H, d, J = 16.4 Hz, H-17b), 5.28 (2H, s, H-5), 4.00 (3H, s, 10-OCH₃), 1.98 (2H, m, H-19), 1.01 (3H, t, J = 7.2 Hz, H-18); ESI-MS: m/z = 379.0 [M + H]⁺.

9,10-(Methylenedioxy)-(20S)-camptothecin (4): Bright yellow powder; [α]D₂⁵: +28.8° (c 0.20, MeOH); UV (MeOH): λmax (log ε) = 224 (4.36), 274 (4.30), 338 (4.24), 374 (4.00) nm; ¹H-NMR (acetone-d₆, 400 MHz): δ = 8.51 (1H, d, J = 0.8 Hz, H-7), 7.82 (1H, dd, J = 8.8, 0.8, H-12), 7.59 (1H, d, J = 8.8 Hz, H-11), 7.43 (1H, s, H-14), 6.35 (2H, s, OCH₂O), 5.53 (1H, d, J = 16.4, H-17a), 5.38 (1H, d, J = 16.4, H-17b), 5.29 (2H, s, H-5), 5.19 (1H, s, 20-OH), 1.98 (2H, m, H-19), 1.01 (3H, t, J = 7.2, H-18); ¹³C-NMR (acetone-d₆, 400 MHz): δ = 173.5 (C, C-20a), 157.8 (C, C-16a), 152.1 (C, C-2), 150.9 (C, C-13), 150.6 (C, C-15), 147.0 (C, C-3), 145.3 (C, C-10), 142.1 (C, C-9), 130.2 (C, C-6), 124.5 (CH, C-12), 123.7 (CH, C-7), 119.6 (C, C-16), 115.3 (C, C-8), 114.6 (CH, C-11), 103.7 (CH₂, O-CH₂-O), 96.9 (CH, C-14), 73.4 (C, C-20), 66.2 (CH₂, C-17), 50.7 (CH₂, C-5), 31.6 (CH₂, C-19), 7.8 (CH₃, C-18); ESI-MS: m/z = 393.1 [M + H]⁺; HR-ESI-MS: m/z = 393.1096 [M + H]⁺ (calcd. for C₂₁H₁₆N₂O₆ + H⁺: 393.1086).

References

