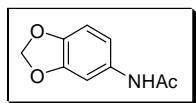


Supporting Information for

Synthesis and Biological Evaluation of 10,11-Methylenedioxy-14-azacamptothecin

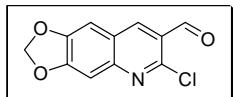
**Mark A. Elban, Wenyue Sun, Brian M. Eisenhauer,
Sidney M. Hecht**

General Methods. Reagents and solvents were reagent grade and used without further purification. Anhydrous grade solvents were purchased from VWR. All reactions involving air or moisture sensitive reagents or intermediates were performed under a nitrogen or argon atmosphere. Flash chromatography was performed using Silicycle 40-60 mesh silica gel. Analytical TLC was performed using 0.25 mm EM silica gel 60 F₂₅₀ plates that were visualized under ultraviolet light (254 nm) or by staining with Hanessian's stain (cerium molybdate). Optical rotations were obtained using a Jasco digital polarimeter. ¹H and ¹³C NMR spectra were recorded using 300 MHz and 500 MHz Varian instruments. Chemical shifts are reported in parts per million (ppm δ) referenced to the residual ¹H resonance of the solvent (CDCl₃, 7.26 ppm; DMSO-*d*₆, 2.49 ppm; CD₃OD, 3.31 ppm). ¹³C spectra were referenced to the residual ¹³C resonance of the solvent (CDCl₃, 77.0 ppm; DMSO-*d*₆, 39.5 ppm; CD₃OD, 49.0 ppm). Splitting patterns are designated as follows: s, singlet; br, broad; d, doublet; dd, doublet of doublets; t, triplet; q, quartet; m, multiplet. High resolution mass spectra were obtained at the Michigan State University-NIH Mass Spectrometry Facility. Melting points were determined for all crystalline compounds and are uncorrected.

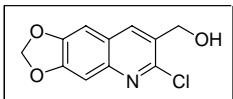


N-Benzo[1,3]dioxol-5-yl-acetamide (6). To a solution containing 10.0 g (73.0 mmol) of 3,4-(methylenedioxy)aniline in 100 mL of CH₂Cl₂ at 0 °C was added 30.4 mL (219 mmol) of NEt₃ and 10.0 mL (109 mmol) of Ac₂O. The reaction mixture was stirred at this temperature for 30 min at which time it was diluted with 100 mL of brine and washed sequentially with 100-mL portions of 1 N HCl, sat. NaHCO₃(aq), and H₂O. The organic layer was dried over anhydrous MgSO₄, filtered, and excess solvent was removed

under diminished pressure to afford **6** as a dark purple solid: yield 12.7 g (97%); mp 132-134 °C, lit.¹ 135-136 °C; silica gel TLC R_f 0.32 (10% MeOH in 1:1 ethyl acetate-hexanes); ¹H NMR (DMSO-*d*₆) δ 2.02 (s, 3H), 5.95 (s, 2H), 6.79 (m, 1H), 6.95 (m, 1H), 7.34 (m, 1H) and 9.85 (m, 1H); ¹³C NMR (DMSO-*d*₆) δ 23.82, 100.94, 101.50, 107.91, 111.89, 133.82, 124.80, 147.07 and 168.05.

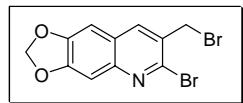


6-Chloro[1,3]dioxolo[4,5-g]quinoline-7-carbaldehyde (7). To a solution containing 18.3 mL (195 mmol) of POCl₃ in 6.51 mL of DMF at 0 °C was added 5.00 g (27.9 mmol) of **6**. The reaction mixture was heated at reflux for 2 h, cooled to room temperature, and then carefully poured into 50 mL of ice water. After the reaction mixture was made basic with sat. NaHCO₃(aq), it was extracted with three 200-mL portions of CHCl₃. The combined organic layer was dried over anhydrous MgSO₄, filtered, and excess solvent was removed under diminished pressure to afford **7** as a light brown solid: yield 3.80 g (58%); mp 211-213 °C; silica gel TLC R_f 0.78 (10% MeOH in 1:1 ethyl acetate-hexanes); ¹H NMR (DMSO-*d*₆) δ 6.30 (s, 2H), 7.40 (s, 1H), 7.59 (s, 1H), 8.69 (s, 1H) and 10.31 (s, 1H); ¹³C NMR (DMSO-*d*₆) δ 103.1, 104.14, 104.21, 123.62, 124.18, 138.71, 147.34, 147.98, 148.73, 154.11 and 189.22; mass spectrum (FAB), *m/z* 236.0115 (M + H)⁺ (C₁₁H₇O₂NCl requires 236.0114).



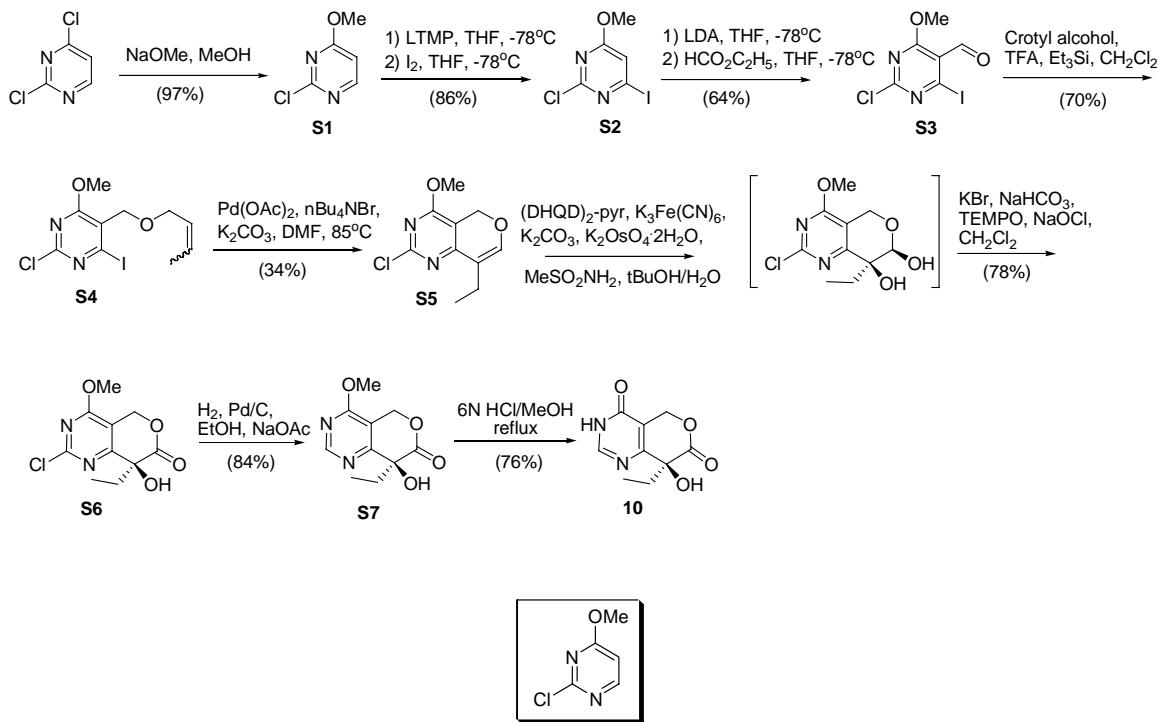
(6-Chloro-[1,3]dioxolo[4,5-g]quinolin-7-yl)methanol (8). To a solution containing 3.10 g (13.2 mmol) of **7** in 50 mL of EtOH at 0 °C was added 4.90 g (132 mmol) of NaBH₄. The reaction mixture was allowed to warm to room temperature and was stirred

for 1.5 h, at which time it was carefully quenched with sat. $\text{NH}_4\text{Cl}_{(\text{aq})}$, diluted with 50 mL of brine, and extracted with three 200-mL portions of CH_2Cl_2 . The combined organic layer was dried over anhydrous MgSO_4 , filtered, and excess solvent was removed under diminished pressure to afford **8** as a light brown solid: yield 3.10 g (99%); mp 198-200 °C; silica gel TLC R_f 0.72 (10% MeOH in 1:1 ethyl acetate–hexanes); ^1H NMR (DMSO- d_6) δ 4.55 (s, 2H), 5.60 (br s, 1H), 6.20 (s, 2H), 7.23 (s, 1H), 7.36 (s, 1H) and 8.19 (s, 1H); ^{13}C NMR (DMSO- d_6) δ 59.76, 102.22, 102.68, 103.82, 124.14, 131.37, 134.83, 144.05, 145.71, 147.84 and 150.77; mass spectrum (FAB), m/z 238.0272 ($\text{M} + \text{H}^+$)⁺ ($\text{C}_{11}\text{H}_9\text{O}_3\text{NCl}$ requires 238.0271).



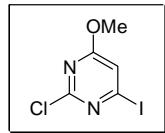
6-Bromo-7-bromomethyl[1,3]dioxolo[4,5-g]quinoline (9). To a solution containing 2.00 g (8.44 mmol) of **8** in 50 mL benzene was added 8.01 mL (84.4 mmol) of PBr_3 . The reaction mixture was heated to reflux for 18 h at which time it was poured into 100 mL of ice/ H_2O , made basic with solid NaHCO_3 , and extracted with three 100-mL portions of CHCl_3 . The combined organic layer was dried over anhydrous MgSO_4 , filtered, and excess solvent was removed under diminished pressure to afford a mixture of monobromide and dibromide. Accordingly, the material was dissolved in 20 mL of toluene and treated again with 4.00 mL (42.2 mmol) of PBr_3 . The reaction mixture was heated to reflux for 18 h at which time it was poured into 100 mL of ice/ H_2O , made basic with solid NaHCO_3 and extracted with three 100-mL portions of CHCl_3 . The combined organic layer was dried over anhydrous MgSO_4 , filtered, and excess solvent was removed under diminished pressure to afford **9** as a light yellow solid: yield 1.96 g (67%); mp 150-152 °C; silica gel TLC R_f 0.87 (10% MeOH in 1:1 ethyl acetate–hexanes); ^1H NMR

(CDCl₃) δ 4.65 (s, 2H), 6.12 (s, 2H), 6.98 (s, 1H), 7.28 (s, 1H) and 7.96 (s, 1H); ¹³C NMR (CDCl₃) δ 32.60, 102.49, 102.74, 105.29, 124.64, 129.64, 137.68, 140.74, 146.52, 148.92 and 152.24; mass spectrum (FAB), *m/z* 343.8920 (M + H)⁺ (C₁₁H₈O₂Br₂N requires 343.8922).

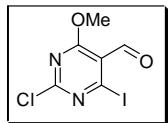


2-Chloro-4-methoxypyrimidine (S1).² To a solution containing 30.0 g (207 mmol) of 2,4-dichloropyrimidine in 70 mL of MeOH was added 140 mL of NaOMe (4.75 g (207 mmol) of Na in 140 mL of MeOH) at 0 °C. The reaction mixture was stirred while warming to room temperature overnight, at which time excess solvent was removed under diminished pressure. Purification was accomplished by dissolving the residue in 200 mL of Et₂O and filtering off the NaCl. The salts were washed with excess Et₂O and the excess solvent was concentrated under diminished pressure followed by co-evaporation with hexanes to give **S1** as a colorless amorphous solid: yield 28.4 g (97%);

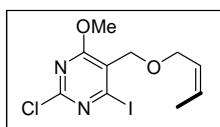
silica gel TLC R_f 0.65 (1:1 ethyl acetate–hexanes); ^1H NMR (CDCl_3) δ 3.89 (s, 3H), 6.56 (d, 1H, J = 5.8 Hz) and 8.17 (d, 1H, J = 5.8 Hz); ^{13}C NMR (CDCl_3) δ 54.29, 106.85, 158.39, 159.81 and 170.41.



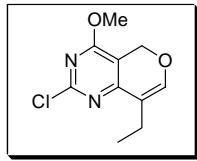
2-Chloro-4-iodo-6-methoxypyrimidine (S2).³ To a solution containing 29.8 mL (74.6 mmol) of *n*-BuLi (2.5 M in hexane) in 200 mL of THF at -30 °C was added 13.3 mL (78.0 mmol) of 2,2,6,6-tetramethylpiperidine over a period of 5 min. The reaction mixture was stirred at -30 °C for 15 min and then cooled to -78 °C for 15 min. A solution containing 4.90 g (33.9 mmol) of **S1** in 25 mL of THF was added dropwise over a period of 20 min and the reaction mixture was stirred for 1 h at -78 °C. To this was added 10.3 g (40.7 mmol) of I₂ in 50 mL of THF and the reaction mixture was stirred at -78 °C for 1.5 h. Hydrolysis was carried out at -78 °C using a mixture of 50 mL of (1:2:2 35% HCl_(aq)–EtOH–THF). The reaction mixture was warmed gently to room temperature and treated with 100 mL of sat. NaHCO_{3(aq)}. The reaction mixture was then concentrated under diminished pressure and then extracted with four 100-mL portions of CH₂Cl₂. The combined organic layer was dried over anhydrous MgSO₄, filtered, and excess solvent was removed under diminished pressure. The residue was purified by flash chromatography on a silica gel column (20 x 5 cm). Elution with 1:10 ethyl acetate–hexanes gave **S2** as a light yellow solid: yield 7.88 g (86%); mp 108–110 °C, lit.³ 109–111 °C; silica gel TLC R_f 0.66 (1:4 ethyl acetate–hexanes); ^1H NMR (CDCl_3) δ 3.94 (s, 3H) and 7.11 (s, 1H); ^{13}C NMR (CDCl_3) δ 54.96, 117.51, 127.03, 158.26 and 169.48.



2-Chloro-4-iodo-6-methoxy-pyrimidine-5-carbaldehyde (S3).⁴ To a solution containing 19.5 mL (48.8 mmol) of *n*-BuLi (2.5 M in hexane) in 200 mL of THF at -30 °C was added 6.62 mL (51.0 mmol) of iPr₂NH over a period of 5 min. The reaction mixture was stirred at -30 °C for 15 min and then cooled to -78 °C for 15 min. A solution containing 6.00 g (22.2 mmol) of **S2** in 25 mL of THF was added dropwise over a period of 20 min and the reaction mixture was stirred for 1 h at -78 °C. To this was added 14.3 mL (117 mmol) of ethyl formate and the reaction mixture was stirred at -78 °C for 2 h. Hydrolysis was carried out at -78 °C using 50 mL of (1:2:2 35% HCl_(aq)–EtOH–THF). The reaction mixture was gently warmed to room temperature and treated with 100 mL of sat. NaHCO_{3(aq)}. The reaction mixture was concentrated under diminished pressure and then extracted with four 100-mL portions of CH₂Cl₂. The combined organic layer was dried over anhydrous MgSO₄, filtered, and the solvent was concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (20 x 5 cm). Elution with 1:9 ethyl acetate–hexanes as eluant gave **S3** as a colorless solid: yield 4.24 g (64%); mp 123–125 °C; silica gel TLC *R_f* 0.40 (1:4 ethyl acetate–hexanes); ¹H NMR (CDCl₃) δ 4.12 (s, 3H) and 10.07 (s, 1H); ¹³C NMR (CDCl₃) δ 56.09, 116.93, 132.42, 161.01, 168.31 and 188.49; mass spectrum (electrospray), *m/z* 298.8 (M + H)⁺ theoretical *m/z* 298.9 (M + H)⁺; mass spectrum (FAB), *m/z* 298.9083 (M + H)⁺ (C₆H₅ClN₂O₂ requires 298.9084).

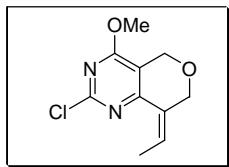


5-But-2-enyloxymethyl-2-chloro-4-iodo-6-methoxypyrimidine (S4).⁴ To a solution containing 4.18 g (14.0 mmol) of aldehyde **S3** in 15 mL of CH₂Cl₂ at 0 °C was added 3.93 mL (46.3 mmol) of crotyl alcohol, 4.48 mL (28.1 mmol) of triethylsilane and 7.13 mL (92.7 mmol) of trifluoroacetic acid. The reaction mixture was stirred at room temperature for 48 h and poured into 300 mL of sat. NaHCO₃(aq). The resulting reaction mixture was extracted with three 100-mL portions of hexanes. The combined organic layer was dried over anhydrous MgSO₄, filtered, and excess solvent removed under diminished pressure. The residue was purified by flash chromatography on a silica gel column (20 x 5 cm). Elution with 1:9 ethyl acetate–hexanes as eluant gave **S4** as a colorless oil: yield 3.46 g (70%); silica gel TLC *R_f* 0.68 (1:4 ethyl acetate–hexanes); ¹H NMR (CDCl₃) δ 1.65 (d, 3H, *J* = 6.0 Hz), 3.92 (m, 2H), 3.96 (s, 3H), 4.40 (s, 2H), 5.52 (m, 1H) and 5.65 (m, 1H); ¹³C NMR (CDCl₃) δ 17.66, 55.40, 67.26, 71.72, 121.87, 126.78, 130.34, 134.90, 157.68, and 167.17; mass spectrum (electrospray), *m/z* 354.9 (M + H)⁺, theoretical *m/z* 355.0 (M + H)⁺; mass spectrum (FAB) *m/z* 354.9712 (M + H)⁺ (C₁₀H₁₃ClIN₂O₂ requires 354.9710).

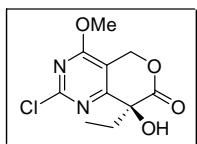


2-Chloro-8-ethyl-4-methoxy-5H-pyrano[4,3-d]pyrimidine S5.⁴ To a solution containing 2.60 g (7.33 mmol) of crotyl ether **S4** in 50 mL of DMF (degassed with N₂ for 30 min) at room temperature was added successively 2.36 g (7.33 mmol) of tetra-*n*-butylammonium bromide, 2.02 g (14.7 mmol) of K₂CO₃, and 0.093 g (0.367 mmol) of Pd(OAc)₂. The reaction mixture was stirred at 85 °C for 13 h. The cooled reaction mixture was filtered through a pad of Celite, and rinsed with 300 mL of ether. The

organic layer was washed with two 30-mL portions of ice water, one 30-mL portion of brine, dried over anhydrous MgSO_4 , filtered, and excess solvent was removed under diminished pressure. The residue was purified by flash chromatography on a silica gel column (20 x 5 cm). Elution with 1:9 ethyl acetate–hexanes as eluant gave **S5** as a colorless solid: yield 0.575 g (34%); mp 109–110 °C; silica gel TLC R_f 0.81 (1:4 ethyl acetate–hexanes); ^1H NMR (CDCl_3) δ 1.04 (t, 3H, J = 7.5 Hz), 2.30 (dq, 2H, J = 6.3 and 1.2 Hz), 3.94 (s, 3H), 5.02 (s, 2H) and 6.65 (s, 1H); ^{13}C NMR (CDCl_3) δ 13.30, 19.12, 54.39, 62.15, 103.11, 116.90, 150.42, 158.86, 159.88 and 164.74; mass spectrum (electrospray), m/z 226.8 ($\text{M} + \text{H}$) $^+$, theoretical m/z 227.1 ($\text{M} + \text{H}$) $^+$; mass spectrum (FAB) m/z 227.0586 ($\text{M} + \text{H}$) $^+$ ($\text{C}_{10}\text{H}_{12}\text{ClN}_2\text{O}_2$ requires 227.0587).



The *exo*-ethylidene side product was isolated as an off-white solid: yield 0.164 g (10%); mp 106–108 °C; silica gel TLC R_f 0.54 (1:4 ethyl acetate–hexanes); ^1H NMR (CDCl_3) δ 1.78 (d, 3H, J = 7.2 Hz), 3.98 (s, 3H), 4.50 (s, 2H), 4.60 (s, 2H) and 7.00 (m, 1H); ^{13}C NMR (CDCl_3): δ 13.27, 54.65, 62.50, 65.04, 110.72, 128.07, 128.33, 157.71, 158.44 and 166.81; mass spectrum (electrospray), m/z 226.8 ($\text{M} + \text{H}$) $^+$, theoretical m/z 227.1 ($\text{M} + \text{H}$) $^+$.

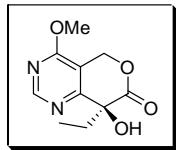


2-Chloro-8-ethyl-8-hydroxy-4-methoxy-5,8-dihydro-pyrano[4,3-d]pyrimidin-7-one (S6).⁴ A solution containing 0.022 g (0.025 mmol) of $(\text{DHQD})_2\text{PYR}$ ligand, 2.51 g

(7.62 mmol) of $K_3Fe(CN)_6$, 1.05 g (7.62 mmol) of K_2CO_3 , 0.0017 g (0.0048 mmol) of $K_2OsO_4 \bullet 2H_2O$, and 0.242 g (2.54 mmol) of $MeSO_2NH_2$ in 30 mL of 1:1 *t*-BuOH– H_2O was stirred at room temperature for 5 min. This mixture was added to 0.575 g (2.54 mmol) of **S5** at 0 °C. The reaction mixture was stirred at 4 °C for 24 h at which time 2.43 g (19.3 mmol) of Na_2SO_4 was added carefully, followed by 10 mL of H_2O . The resulting mixture was stirred for 10 min and extracted with three 100-mL portions of EtOAc. The combined organic phase was dried over anhydrous $MgSO_4$, filtered, and excess was solvent removed under diminished pressure. The residue was purified by flash chromatography on a silica gel column (10 x 5 cm). Elution with 1:1 ethyl acetate–hexanes as eluant gave the diol as a colorless oil. The residue was dissolved in 12 mL of CH_2Cl_2 and 1 mL of H_2O . To this solution at 0 °C was added 0.036 g (0.304 mmol) of KBr, 0.032 g (0.38 mmol) of $NaHCO_3$, and 0.015 g (0.101 mmol) of TEMPO. After stirring for 15 min at 0 °C, 14.1 mL (7.59 mmol) of $NaOCl$ (4% solution in H_2O) was added dropwise over a period of 30 min, and then 20 mL of H_2O was added and the reaction mixture was extracted with three 100-mL portions of CH_2Cl_2 . The combined organic phase dried over anhydrous $MgSO_4$, filtered, and excess solvent was removed under diminished pressure. The residue was purified by flash chromatography on a silica gel column (20 x 5 cm). Elution with 10% MeOH in 1:1 ethyl acetate–hexanes as eluant gave **S6** as an colorless solid: yield 0.510 g (78%); mp 201–202 °C; silica gel TLC R_f 0.60 (10% MeOH in 1:1 ethyl acetate–hexanes); $[\alpha]^{21}_D +39.2$ (*c* 2.7, $CHCl_3$); 1H NMR ($CDCl_3$) δ 0.96 (t, 3H, *J* = 7.5 Hz), 1.83 (q, 2H, *J* = 7.2 Hz), 3.89 (s, 1H), 4.07 (s, 3H), 5.16 (d, 1H, *J* = 15.9 Hz) and 5.39 (d, 1H, *J* = 15.9 Hz); ^{13}C NMR ($CDCl_3$) δ 7.49, 31.68, 5.49, 62.90, 73.75, 107.72, 160.52, 164.45, 165.66 and 171.07; mass spectrum

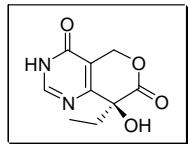
(electrospray), m/z 258.8 ($M + H$)⁺, theoretical m/z 259.0 ($M + H$)⁺; mass spectrum (FAB) m/z 259.0485 ($M + H$)⁺ ($C_{10}H_{12}ClN_2O_4$ requires 259.0486).

The enantiomeric purity of **S6** was determined as 80% ee by chiral HPLC on a Chirobiotic T column (250 x 4.6 mm); the mobile phase was 10% EtOH in hexane at a flow rate of 1.0 mL/min, UV detection was at 300 nm. The retention time of the desired isomer was 43.0 min; that of the optical antipode was 46.6 min. Crystallization from hexane–CH₂Cl₂ (70% yield) gave **S6** having an optical purity >98% ee as determined by chiral HPLC.

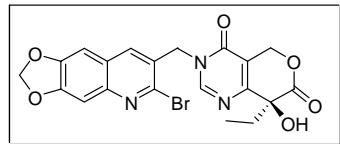


8-Ethyl-8-hydroxy-4-methoxy-5,8-dihydropyrano[4,3-d]pyrimidin-7-one (S7).⁴ A solution containing 0.386 g (1.49 mmol) of lactone **S6**, 0.306 g (3.73 mmol) of NaOAc, and ~0.050 g of 10% Pd/C in 20 mL abs. EtOH was stirred at 25 °C under 45 psi of hydrogen for 2 h. The catalyst was filtered through a pad of Celite and washed with 300 mL of EtOH. The combined organic fraction was concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (20 x 5 cm). Elution with 10% MeOH in 1:1 ethyl acetate–hexanes as eluant gave **S7** as light yellow oil: yield 0.283 g (84%); silica gel TLC R_f 0.36 (10% MeOH in 1:1 ethyl acetate–hexanes); $[\alpha]^{21}_D +59.6$ (*c* 1.5, CHCl₃); ¹H NMR (CDCl₃) δ 0.95 (t, 3H, *J* = 7.2 Hz), 1.83 (q, 2H, *J* = 7.5 Hz), 4.05 (s, 3H), 4.13 (br, 1H), 5.19 (d, 1H, *J* = 15.9 Hz), 5.44 (d, 1H, *J* = 15.9 Hz) and 8.79 (s, 1H); ¹³C NMR (CDCl₃) δ 7.30, 31.33, 54.40, 62.98, 73.65, 109.05, 158.27, 161.96, 164.31 and 171.66; mass spectrum (electrospray), m/z 224.8 ($M + H$)⁺,

theoretical m/z 225.1 ($M + H$)⁺; mass spectrum (FAB), m/z 225.0874 ($M + H$)⁺ ($C_{10}H_{13}N_2O_4$ requires 225.0875).

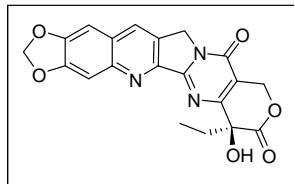


8-Ethyl-8-hydroxy-5,8-dihydro-3H-pyranopyrimidine-4,7-dione (10). To a solution containing 0.404 g (1.80 mmol) of **S7** in 5 mL of 6 N HCl was added 6 mL of MeOH. The reaction mixture was stirred at 75 °C for 3 h at which time the solvent was concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (20 x 5 cm). Elution with 10% MeOH in CH₂Cl₂ gave **10** as a colorless solid: yield 0.293 g (76%); mp 198-199 °C; silica gel TLC R_f 0.36 (10% MeOH in CH₂Cl₂); $[\alpha]^{21}_D +62.6$ (*c* 0.75, MeOH); ¹H NMR (CD₃OD) δ 0.92 (t, 3H, *J* = 7.5 Hz), 1.96 (m, 2H), 5.19 (d, 1H, *J* = 16.0 Hz), 5.32 (d, 1H, *J* = 16.5 Hz) and 8.31 (s, 1H); ¹³C NMR (CD₃OD) δ 8.17, 32.16, 64.88, 74.39, 117.60, 151.31, 159.71, 160.91 and 173.91; mass spectrum (electrospray), m/z 210.8 ($M + H$)⁺, theoretical m/z 211.1 ($M + H$)⁺, mass spectrum (FAB), m/z 211.0718 ($M + H$)⁺ ($C_9H_{11}N_2O_4$ requires 211.0719).



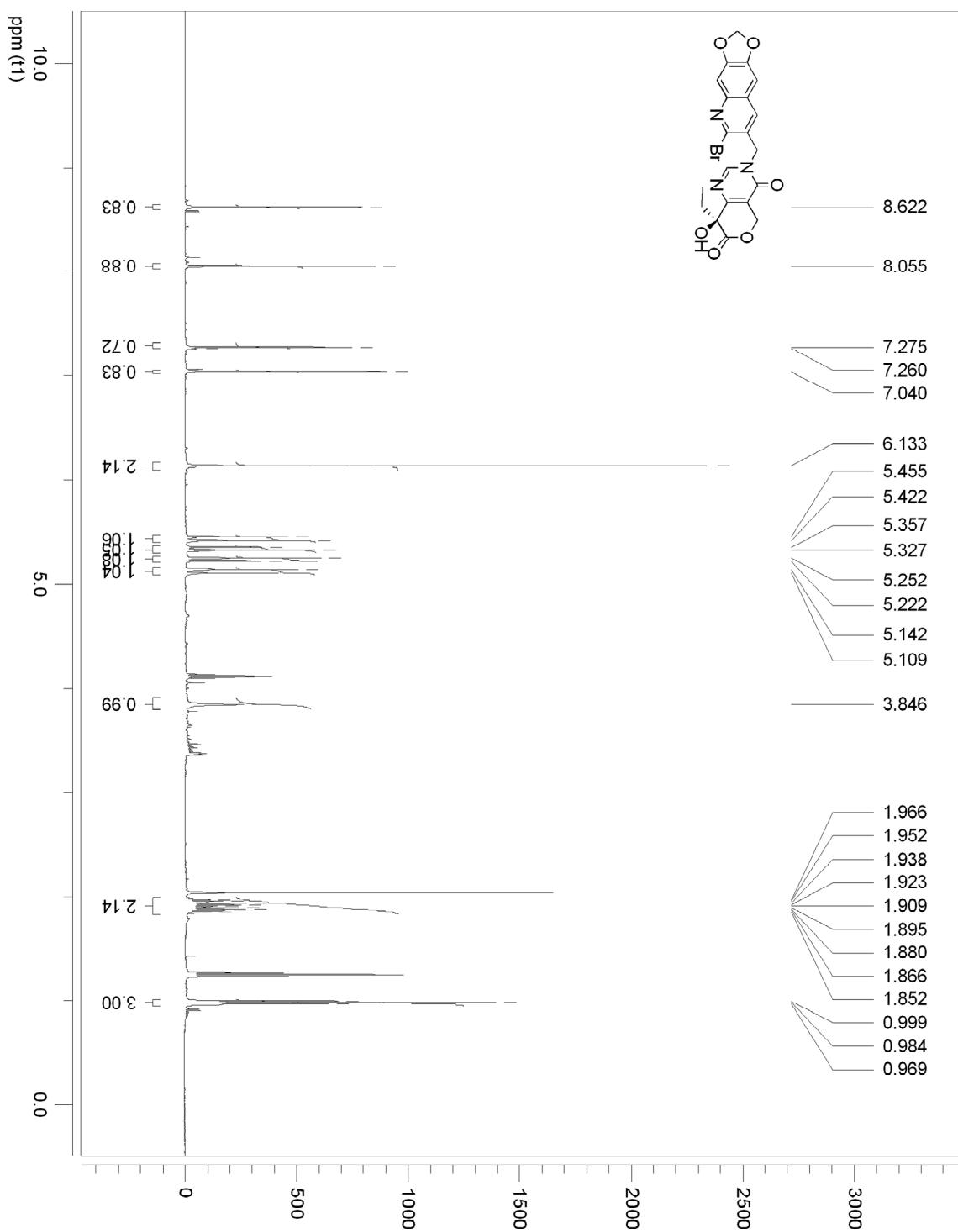
3-(6-Bromo-[1,3]dioxolo[4,5-g]quinolin-7-ylmethyl)-8-ethyl-8-hydroxy-5,8-dihydro-3H-pyranopyrimidine-4,7-dione (11). To a solution containing 0.034 g (0.180 mmol) of **10** in 6 mL of dry dimethoxyethane at 25 °C was added 0.026 g (0.230 mmol) of potassium *tert*-butoxide. The reaction mixture was stirred at room temperature for 30 min. To this was added 0.124 g (0.360 mmol) of **9** in one portion. The reaction mixture

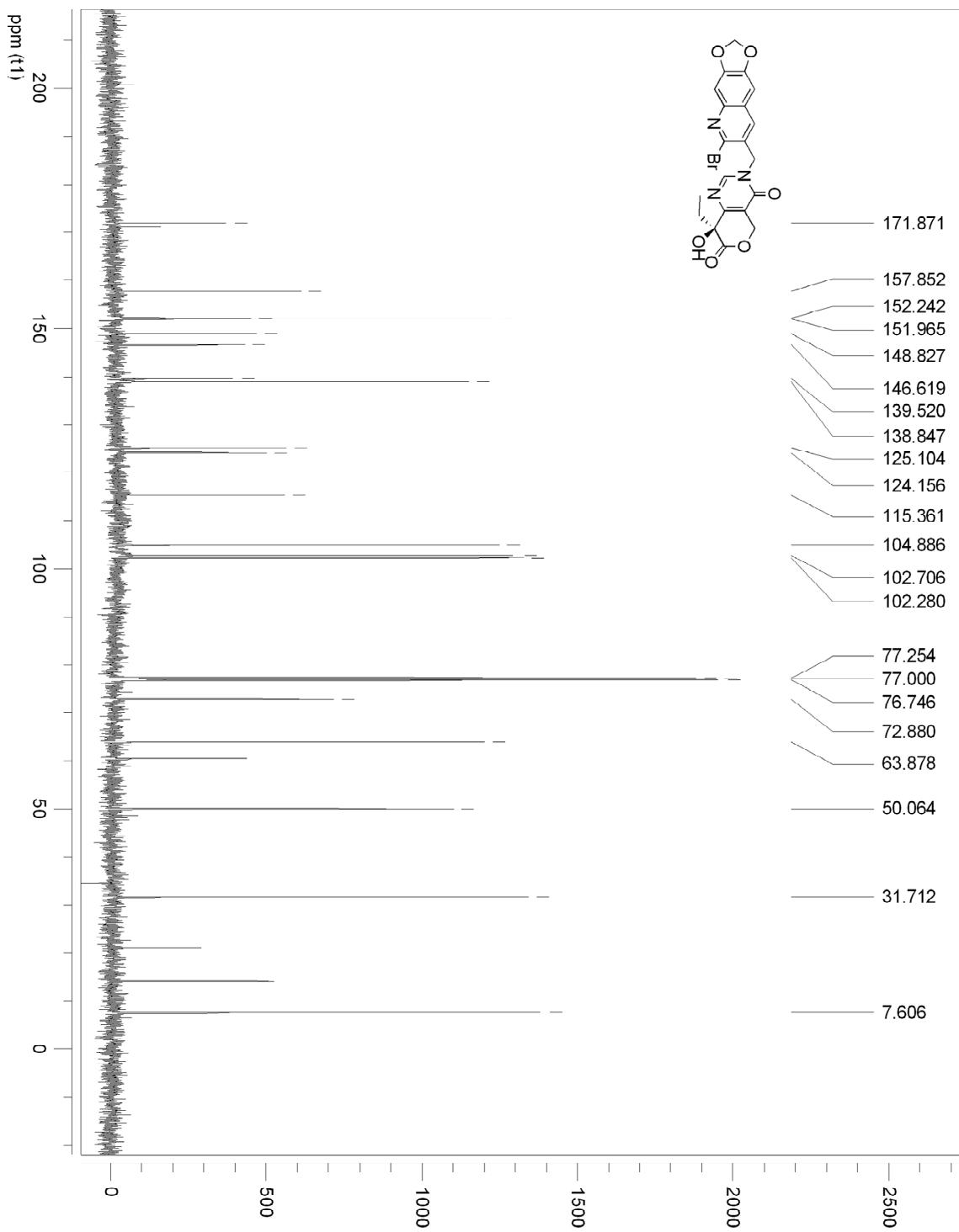
was stirred at reflux for 12 h, allowed to cool, and concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (30 x 2 cm). Elution with 10% MeOH in 1:1 ethyl acetate–hexanes gave **11** as a colorless solid: yield 0.059 g (69%); silica gel TLC R_f 0.40 (10% MeOH in 1:1 ethyl acetate–hexanes); $[\alpha]^{21}_D +13.2$ (*c* 1.7, 1:1 MeOH–CHCl₃); ¹H NMR (CDCl₃) δ 0.98 (t, 3H, *J* = 7.5 Hz), 1.91 (m, 2H), 3.85 (br, 1H), 5.11 (d, 1H, *J* = 16.5 Hz), 5.22 (d, 1H, *J* = 15.0 Hz), 5.33 (d, 1H, *J* = 15.0 Hz), 5.42 (d, 1H, *J* = 16.5 Hz), 6.13 (s, 2H), 7.04 (s, 1H), 7.28 (s, 1H), 8.06 (s, 1H) and 8.62 (s, 1H); ¹³C NMR (CDCl₃) δ 7.61, 31.71, 50.06, 63.88, 72.88, 102.28, 102.71, 104.89, 115.36, 124.16, 125.10, 138.85, 139.52, 146.62, 148.83, 151.97, 152.24, 157.85 and 171.87; mass spectrum (FAB), *m/z* 474.0303 (M + H)⁺ (C₂₀H₁₇O₆N₃Br requires 474.0301).

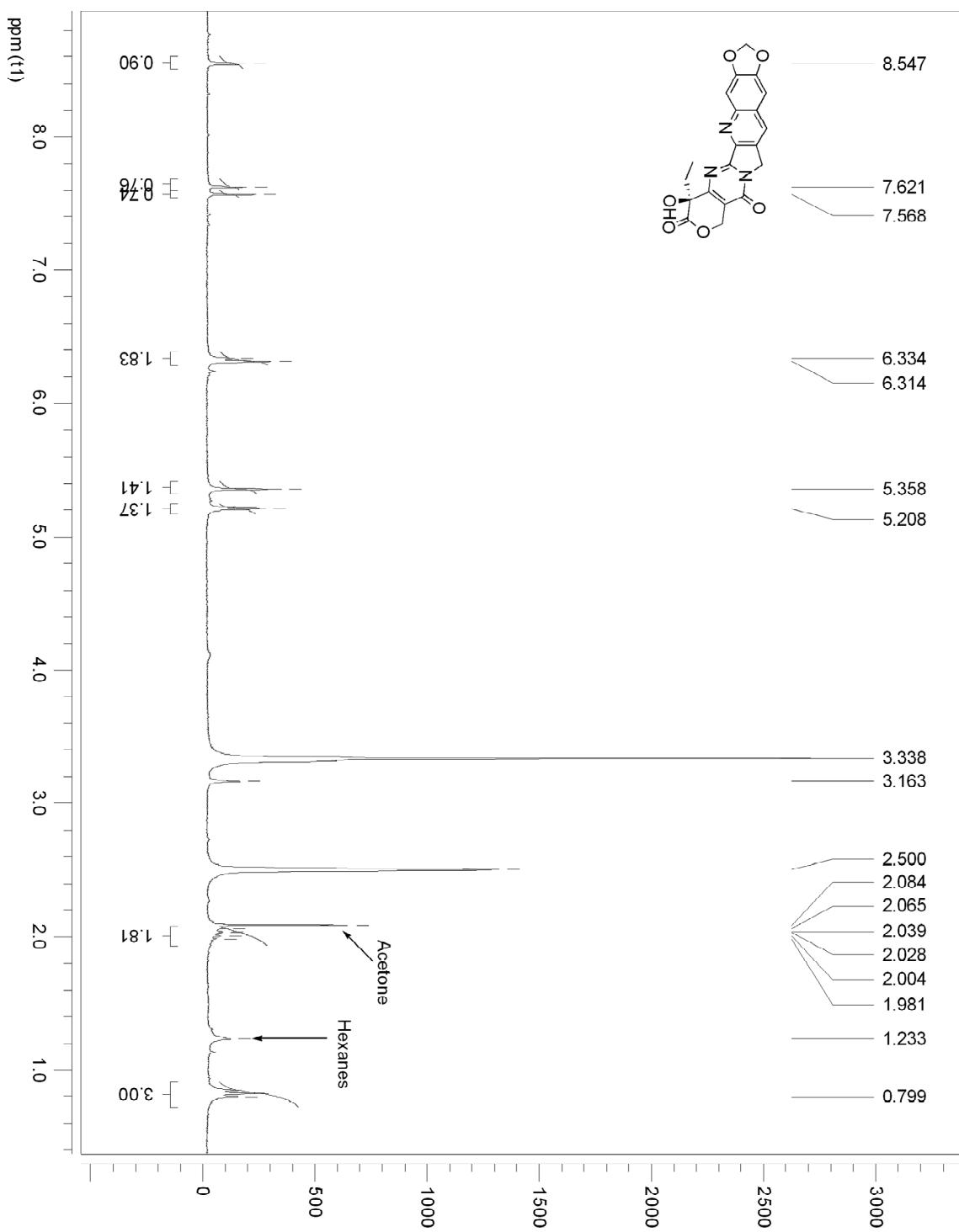


10,11-Methylenedioxy-14-azacamptothecin (5). To a solution containing 0.023 g (0.048 mmol) of **11** in 5 mL of benzene was added a catalytic amount of AIBN and 0.015 mL (0.048 mmol) of tris(trimethylsilyl)silane. The reaction mixture was stirred at 80 °C for 3 h. Analysis by silica gel TLC indicated that the reaction was not complete. Accordingly, a catalytic amount of AIBN and 0.015 mL (0.048 mmol) of tris(trimethylsilyl)silane was added to the solution and the reaction mixture was heated at reflux for an additional 3 h. After removal of the solvent under diminished pressure, the residue was purified by flash chromatography on a silica gel column (25 x 1 cm). Elution with 1% MeOH in 1:1 hexanes–acetone as eluant gave **5** as a colorless solid: yield 1.1 g

(6%); silica gel TLC R_f 0.58 (5% MeOH in ethyl acetate); $[\alpha]^{21}_{D} +41.7$ (c 0.03, MeOH); ^1H NMR (DMSO- d_6) δ 0.80 (t, 3H, J = 7.2 Hz), 1.98 (m, 2H), 5.21 (s, 2H), 5.36 (s, 2H), 6.31 (s, 2H), 7.57 (s, 1H), 7.62 (s, 1H) and 8.55 (s, 1H); mass spectrum (FAB), m/z 394.1041 ($\text{M} + \text{H}$) $^+$ ($\text{C}_{20}\text{H}_{16}\text{O}_6\text{N}_3$ requires 394.1039).

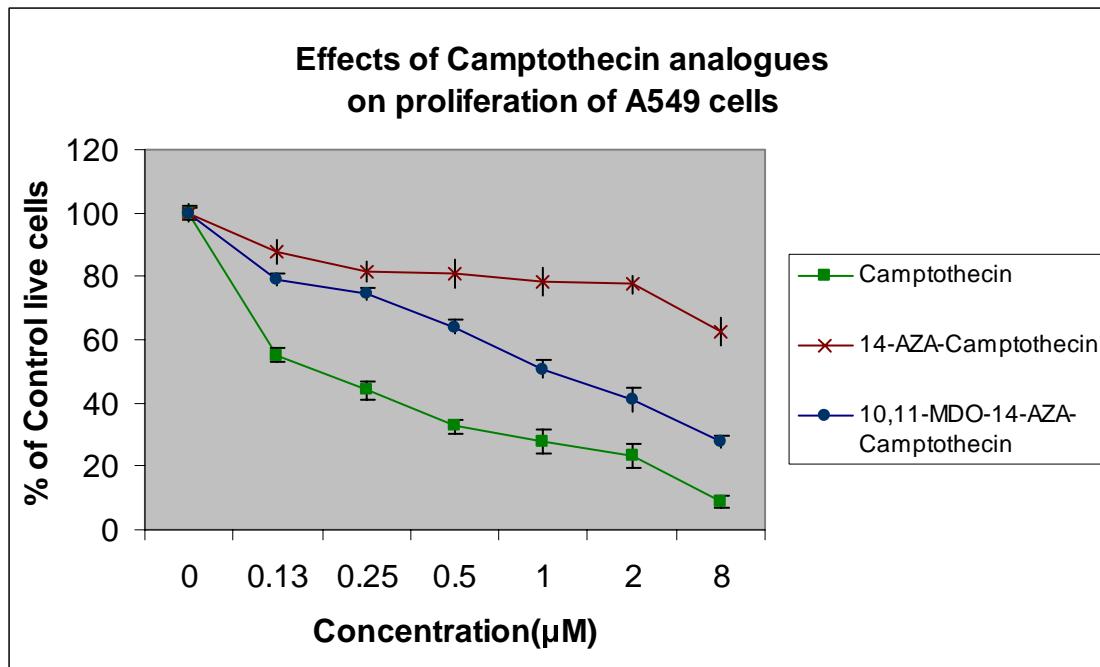






MTT assay

The A549 cells were maintained in Kaighn's modification of Ham's F12 medium (F12K) with 2 mM L-glutamine supplemented with 1.5 g/L sodium bicarbonate and 10% fetal bovine serum at 37 °C in a 5% CO₂ in air atmosphere. Cytotoxicity was determined by MTT assay. Two hundred microliters of culture samples containing approximately 5.0 × 10⁴ of A549 cells were placed in each well of 96-well culture plates and allowed to attach and grow overnight. The medium was then replaced with 200 μL of growth medium containing the appropriate concentration of DMSO (the control) or CPT analogues. The cultures were incubated at 37 °C for 72 h in a 5% CO₂ in air atmosphere. After removal of the culture medium and addition of 15 μL of MTT (5 mg/mL) to each well, the samples were incubated for an additional 4 h at 37 °C in a 5% CO₂ atmosphere. Two hundred microliters of DMSO was then added to each well. The OD₅₇₀ value was obtained from a microplate reader. Five replicate wells were assayed for each condition, and SDs were determined. The percentage of cell survival was determined as follows: $A_{\text{exp group}}/A_{\text{control}} \times 100$. An IC₅₀ (concentration of drug inducing 50% decrease in survival) was determined by extrapolation of results of MTT assays whereby percentage survival was plotted against log₂ concentration.



Exponential growth phase cultures were treated with indicated CPT, medium replaced every 12 hours, and proliferation relative to control assessed by MTT assay after 72 hours. Results were calculated as the percentage of values obtained with untreated cells and represent mean \pm SD.

Camptothecin IC₅₀: 0.147 μM

14-azacamptothecin IC₅₀: >10 μM

10,11-MDO-14-azacamptothecin IC₅₀: 1.28 μM

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