

Supplementary Information

Seriniquinone, a selective anticancer agent, induces cell death by autophagocytosis, targeting the cancer protective protein dermcidin

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A. Chemical General Methods. Unless otherwise noted, all reagents and chemical compounds were purchased from Alfa Aesar, GFS Chemicals, Strem Chemicals, Sigma-Aldrich or TCI America and used without further purification. Samples of seriniquinone (**1**) were obtained naturally or prepared in two steps using published methods as outlined in Fig. 1c [Matsuoka, M., Iwamoto, A. & Kitao, T. Reaction of 2,3-dichloro-1,4-naphthoquinone with dithiooxamide. Synthesis of dibenzo[b,i]thianthrene-5,7,12,14-tetrone. *J. Heterocyclic Chem.* **28**, 1445-1447 (1991)]. Tetraacetate **2**, probe **15** and probe **16** were prepared via chemical synthesis (Fig. 1d) with a purity >98% by NMR and HPLC analyses and stored as 1 mg/mL stocks in DMSO. While **2** and **16** proved stable as solid materials or DMSO stock solutions, probe **15** proved to be unstable and was prepared fresh and stored in 1 mg/mL stocks in DMSO at -80°C for up to 1 month. NMR spectra were recorded on a Mercury Plus 400 (Varian), ECA500 (Jeol), DMX500 (Bruker) or VX500 equipped with XSens cold probe (Varian) spectrometer. FID files were processed using MestRenova version 8.1 (MestreLab Research) and were referenced residual solvent peaks [S. Budavari, M.J. O'Neil, A. Smith, P.E. Heckelman, The Merck Index, an Encyclopedia of Chemicals, Drugs, and Biologicals, Eleventh Edition, Merck Co., Inc. Rahway, NJ, 1989]. High-resolution mass spectral (HRMS) data were obtained at the mass spectral facilities at the Scripps Research Institute, La Jolla, CA or at the University of California, San Diego Molecular Mass Spectroscopy Facility led by Dr. Yongxuan Su. Electrospray (ESI) and atmospheric pressure chemical ionization (APCI) analysis was performed using a LCQ Deca mass spectrometer (ThermoFinnigan), and fast atom bombardment (FAB) analysis was carried out using a MAT 900 XL mass spectrometer (ThermoFinnigan). UV spectra were measured on a DU800 spectrophotometer (Beckman) with a 1 cm cell. IR spectra were obtained with a Nicolet IR100 FT-IR (ThermoFinnigan). Reversed-phase HPLC separation was performed using a semi-preparative C18 Luna column (250 × 10 mm) at a flow rate of 2.5 mL/min using a 600E pump (Waters) and Lambda-Max model 480 UV detector (Waters).

A1. Selection and characterization of *Serinicoccus sp.* Strain CNJ927 was isolated from a sediment sample collected at a depth of 50 m near Palau in 2004. By analysis of the 16S rDNA gene sequence, CNJ927 was identified as *Serinicoccus sp.* A picture of this strain was provided in Fig. 1b. Strain CNJ927 was cultured at 27°C with shaking at 215 rpm in the medium A1BFe+C (10 g of starch, 4 g of yeast extract, 2 g of peptone, 1 g of CaCO₃, 40 mg of Fe₂(SO₄)₃ • 4 H₂O, 100 mg of KBr per 1 L of seawater) in a 2.8 L Fernbach flask. After 7 d, the 36 L broth (36 flasks) was extracted using with EtOAc (36 L). The EtOAc layer was dried over Na₂SO₄ and concentrated by rotary evaporation to yield 2.0 g of crude extract.

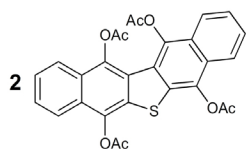
A2. Isolation of seriniquinone (1**).** The crude extract was dissolved in a small volume of CHCl₃, applied on a silica gel column (50 g, 3.2 × 20 cm, 200-450 mesh), and eluted stepwise with 100:0, 50:1, 25:1, 10:1, 5:1, 1:1 and 0:100 (v/v) of CHCl₃:MeOH (150 mL/fraction). Seriniquinone was observed in the first fraction eluted with CHCl₃. This fraction was purified by HPLC; 250 × 10 mm ODS column (Phenomenex), eluting with 70% aq. CH₃CN at a flow rate 2.5 mL/min with detection at 210 nm. Under this condition, seriniquinone (**1**) was eluted as peak with retention time of 28.0 min. This peak was collected and concentrated to afford 2.4 mg of seriniquinone (**1**), as orange crystals (inset of Fig. 1b).

Seriniquinone (1**).** IR (plate) ν_{\max} 1664, 1588, 1500, 1258 cm⁻¹; UV (CHCl₃) λ_{\max} (ϵ) 342 (6900), 289 (24,000), 250 (20,700); ¹H NMR and ¹³C NMR data are summarized in Table S1; HR-ESI-TOFMS [M+H]⁺ m/z 345.0210 (C₂₀H₉O₄S, calcd. 345.0222). The structure and key COSY and HMBC correlations from **1** are provided in Fig. 1a.

#	δ H, mult (<i>J</i> in Hz)	δ C, type	COSY	HMBC
1		177.3, C		
2		152.6, C		
3		141.0, C		
4		178.6, C		
4a		131.9, C		
5	8.25, d (6.6)	127.0, CH	6	C4,C7,C8a
6	7.81, t (6.6)	133.9, CH	5,7	C4a,C8
7	7.87, t (6.6)	135.3, CH	6,8	C5,C8a
8	8.35, d (6.6)	128.6, CH	7	C1,C4a,C6
8a		134.8, C		

Table S1. NMR spectroscopic data for serinoquinone (**1**) in CDCl₃. Assignments are based on COSY, HSQC and HMBC analyses. Carbons C1 and C4, C4a and C8a, C5 and C8 or C6 and C7 could not be distinguished. ¹H NMR and ¹³C NMR data was collected at 500 MHz and 125 MHz, respectively.

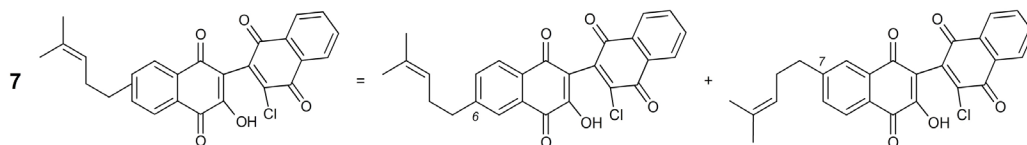
A3. Chemical synthesis. Synthetic efforts were conducted as noted in Fig. 1c-1d of the manuscript. High purity anhydrous solvents were used at all steps. Dichloromethane (CH₂Cl₂), tetrahydrofuran (THF) and *N,N*-dimethylformamide (DMF) were obtained by passing through a solvent column composed of dry activated A1 alumina. DMF was stored on oven dried 4Å molecular sieves for 24 h prior to use. EtN^{*i*}Pr₂ was distilled from ninhydrin, dried (Na₂SO₄), and then redistilled from sodium. Water was obtained after purification via a Milli-Q water purifier (Millipore). All reactions were performed under a positive pressure of dry Ar in oven-dried thick walled round bottom flasks (ChemGlass) or glass vial (VWR Scientific) stirred with a Teflon coated stirbar. Flash chromatography was carried out on 40–63 mesh Geduran Silica Gel 60 (EM Biosciences). Thin layer chromatography (TLC) was conducted on 250 μm Silica Gel 60 F254 glass plates (EMD Chemicals). Visualization was achieved with UV light and stained with ceric ammonium molybdate. Yields and characterization data correspond to isolated, homogeneous materials. Unless otherwise noted, all solvent mixtures are given in v:v ratios. Several reactions were conducted on materials directly prepared without chromatographic purification. In these cases, flash chromatography was used to provide analytical samples for spectroscopic analyses.



Dinaphtho[2,3-*b*:2',3'-*d*]thiophene-5,7,12,13-tetraol tetraacetate (2**).** A mixture of serinoquinone (**1**) (50.0 mg, 0.15 mmol), Zn dust (81.0 mg, 1.23 mmol) and Ac₂O (2 mL) was brought to reflux. After 2 h, the reaction mixture was cooled to rt and the unreacted Zn dust was filtered off and the filtrate was concentrated by rotary evaporation. The crude product was

purified by flash chromatography (1:1 hexanes:CH₂Cl₂ to CH₂Cl₂) to provide peracetoxyhydroquinone **2** (51.2 mg, 67%).

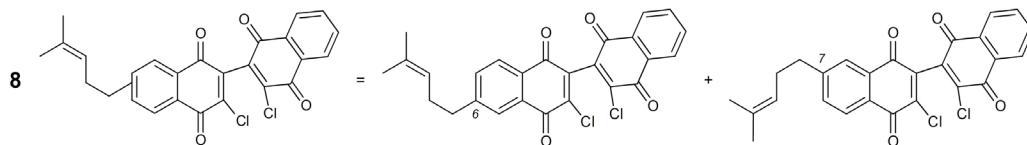
Peracetoxyhydroquinone 2: ¹H NMR (CDCl₃, 500 MHz) δ 8.12 (td, *J* = 1.0, 8.3 Hz, 2H), 7.80 (td, *J* = 0.9, 8.2 Hz, 2H), 7.64 (ddd, *J* = 1.2, 6.8, 8.3 Hz, 2H), 7.59 (ddd, *J* = 1.3, 6.8, 8.1 Hz, 2H), 2.61 (s, 6H), 2.59 (s, 6H); ¹³C NMR (CDCl₃, 125MHz) δ 168.8, 168.7, 140.2, 137.7, 128.7, 127.2, 126.6, 126.2, 125.6, 122.9, 120.6, 20.8, 20.5; HR-ESI-MS *m/z* calcd. for C₂₈H₂₀O₈SNa [M+Na]⁺:539.0879, found 539.0775.



3'-chloro-3-hydroxy-6(7)-(4-methylpent-3-en-1-yl)-[2,2'-binaphthalene]-1,1',4,4'-tetraone

(7). Hydroxynaphthylquinone **6** (2.59 g, 10.11 mmol) prepared according to the methods of [José M. M. del Corral, M. A. Castroa, M. Gordaliza, M. L. Martin, A. B. Oliveira, S. A. Gualberto, M. D. García-Grávalos, A. San Feliciano, Synthesis and biological evaluation of cytotoxic 6(7)-alkyl-2-hydroxy-1,4-naphthoquinones. *Arch. Pharm. Pharm. Med. Chem.* **2002**, *9*, 427–437] and 2,3-dichloro-1,4-naphthoquinone (**3**) (2.30 g, 10.12 mmol) were dissolved in anhydrous CH₃CN (200 mL). The flask was charged with an Ar atmosphere by repeated degassing. Anhydrous CsCO₃ (6.60 g, 20.21 mmol) was added and the contents were recharged with an Ar atmosphere by repeated degassing. The flask was wrapped in foil, to exclude light, and the slurry was stirred at rt. After 72 h, 2 N HCl was added until the pH was 2. The mixture was diluted with H₂O (200 mL) and extracted with EtOAc (3 × 100 mL). The combined organic layers were washed with H₂O (100 mL) then brine (100 mL), dried over Na₂SO₄, and concentrated by rotary evaporation. The crude product (4.55 g) was dried by azeotropic removal of toluene (3 × 100 mL), and used directly in the next step.

Adduct 7: ¹H NMR (CDCl₃, 400 MHz) δ 8.24 (m, 1H), 8.15 (m, 1H), 8.08 (d, *J* = 7.9 Hz, 1H), 8.00 (d, *J* = 1.8 Hz, 1H), 7.80 (m, 2H), 7.66 (bs, 1H), 7.63 (dd, *J* = 1.8, 7.9 Hz, 1H), 5.13 (dddd, *J* = 1.4, 2.8, 5.8, 7.1 Hz, 1H), 2.80 (t, *J* = 7.6 Hz, 2H), 2.37 (q, *J* = 7.1 Hz, 2H), 1.69 (d, *J* = 1.3 Hz, 3H), 1.56 (d, *J* = 1.4 Hz, 3H); ¹³C NMR (CDCl₃, 125 MHz) δ 181.6, 181.1, 180.5, 177.5, 153.2, 149.2, 146.1, 139.0, 136.0, 134.6, 134.3, 133.5, 132.0, 131.5, 130.8, 129.3, 127.6, 127.6, 127.5, 126.8, 122.5, 115.3, 36.2, 29.4, 25.8, 17.9; HR-ESI-MS *m/z* calcd. for C₂₆H₂₀ClO₅ [M+H]⁺: 447.0994, found 447.0996.

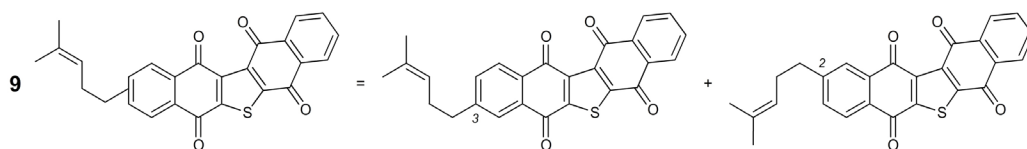


3,3'-Dichloro-6(7)-(4-methylpent-3-en-1-yl)-[2,2'-binaphthalene]-1,1',4,4'-tetraone **(8).**

Dried adduct **7** (4.55 g, 10.18 mmol) was dissolved in dry CH₂Cl₂ (200 mL). After cooling to 0°C, oxalyl chloride (1.78 mL, 20.36 mmol) was added slowly followed by the addition of anhydrous DMF (catalytic, 20 drops). The mixture was warmed to rt. After stirring at rt for an additional 2 h, the mixture was poured onto ice H₂O (100 mL) and stirred for 20 min at rt. The organic layer was then separated and the aqueous layer was extracted with CH₂Cl₂ (3 × 60 mL). The combined organic layers were washed with brine (100 mL), dried over Na₂SO₄, and concentrated on a rotary evaporator. The crude product was purified by flash chromatography

(hexanes to 1:1 hexanes:EtOAc) to afford dichloride **8**, as yellow-orange solid (2.30 g, 49% in 3 steps from **5**).

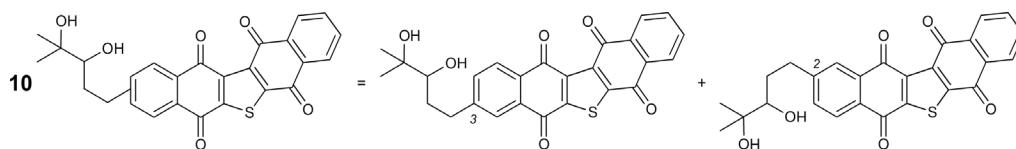
Dichloride 8: ^1H NMR (CDCl_3 , 500 MHz) δ 8.27 (m, 1H), 8.16 (m, 1H), 8.06 (d, $J = 8.3$ Hz, 1H, major isomer), 8.06 (d, $J = 10.7$ Hz, 1H, minor isomer), 7.96 (qd, $J = 0.6, 1.8$ Hz, 1H), 7.85 (m, 2H), 7.63 (m, 1H), 5.14 (ttdd, $J = 1.5, 3.0, 5.6, 7.2$ Hz, 1H), 2.83 (t, $J = 7.5$ Hz, 2H, major isomer), 2.81 (t, $J = 7.3$ Hz, 2H, minor isomer), 2.39 (m, 2H), 1.70 (d, $J = 1.3$ Hz, 3H, major isomer), 1.69 (d, $J = 1.3$ Hz, 3H, minor isomer), 1.57 (dd, $J = 0.6, 1.4$ Hz, 3H, major isomer), 1.56 (dd, $J = 0.6, 1.4$ Hz, 3H, minor isomer); ^{13}C NMR (CDCl_3 , 125 MHz) δ 180.2, 179.9, 179.9, 179.7, 177.3, 177.1, 177.1, 176.9, 150.8, 150.5, 145.3, 145.2, 145.1, 145.0, 140.1, 140.0, 139.9, 139.7, 135.1, 134.9, 134.9, 134.9, 134.7, 133.6, 133.6, 131.6, 131.6, 131.4, 131.4, 131.3, 129.6, 129.4, 128.0, 127.8, 127.7, 127.7, 127.6, 127.4, 122.5, 122.5, 36.4, 36.4, 36.4, 29.4, 29.4, 25.8, 17.9; HR-ESI-MS m/z calcd. for $\text{C}_{26}\text{H}_{18}\text{Cl}_2\text{O}_4\text{Na}$ [$\text{M}+\text{Na}$] $^+$: 487.0474, found 487.0477.



2(3)-(4-Methylpent-3-en-1-yl)dinaphtho[2,3-*b*:2',3'-*d*]thiophene-5,7,12,13-tetraone (9).

Dichloride **8** (3.47 g, 7.46 mmol) was dissolved in THF (75 mL). Na_2S (1.16 g, 14.91 mmol) dissolved in H_2O (75 mL) was added slowly at rt. After stirring at rt for an additional 1 h, the reaction was terminated by the addition of 1 N HCl (100 mL). The organic layer was collected and aqueous layer was further extracted with EtOAc (3×50 mL). The combined organic layers were washed with H_2O (2×50 mL) brine (2×50 mL), dried over Na_2SO_4 and concentrated by rotary evaporation. The residue was passed through a short pad of silica gel (10 g) eluting with EtOAc (2 L). The resulting solution was concentrated on a rotary evaporator to obtain alkene **9**. This compound was rather instable and was best processed immediately after preparation. For characterization purposes, a small sample of **9** was purified by flash chromatography (hexanes to 1:1 hexanes:EtOAc).

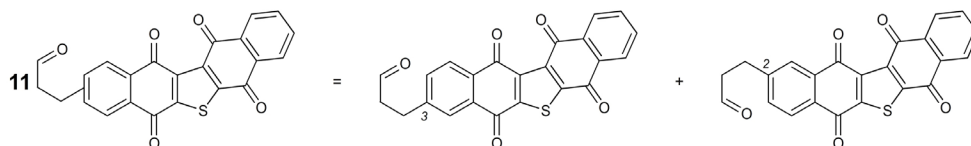
Alkene 9: ^1H NMR (CDCl_3 , 500 MHz) δ 8.33 (dd, $J = 7.6, 1.4$ Hz, 1H), 8.23 (dd, $J = 1.4, 7.6$ Hz, 1H), 8.22 (d, $J = 7.9$ Hz, 1H, major isomer), 8.13 (m, 1H, minor isomer), 8.03 (d, $J = 1.8$ Hz, 1H, major isomer), 7.91 (d, $J = 1.7$ Hz, 1H, minor isomer), 7.85 (dt, $J = 1.5, 7.6$ Hz, 1H, major isomer), 7.79 (dt, $J = 1.4, 7.5$ Hz, 1H, major isomer), 7.75 (m, 1H, minor isomer), 7.69 (m, 1H, minor isomer), 7.64 (dd, $J = 1.8, 8.0$ Hz, 1H, major isomer), 7.59 (dd, $J = 1.7, 8.0$ Hz, 1H, minor isomer), 5.14 (dddd, $J = 1.5, 3.0, 5.7, 7.2$ Hz, 1H, major isomer), 5.09 (m, 1H, minor isomer), 2.81 (t, $J = 7.8$ Hz, 2H, major isomer), 2.75 (t, $J = 7.5$ Hz, 2H, minor isomer), 2.38 (m, 2H), 1.68 (d, $J = 1.5$ Hz, 3H, major isomer), 1.57 (s, 3H, minor isomer), 1.54 (d, $J = 1.5$ Hz, 3H, major isomer); ^{13}C NMR (CDCl_3 , 125 MHz) δ 178.9, 178.6, 177.4, 177.3, 152.6, 152.4, 149.6, 140.9, 140.8, 135.5, 135.2, 135.2, 134.9, 134.9, 134.2, 133.9, 133.5, 132.8, 132.0, 132.0, 131.9, 131.0, 129.9, 128.7, 128.6, 128.6, 128.5, 127.3, 127.2, 127.0, 126.9, 126.8, 122.6, 36.6, 36.3, 29.4, 29.4, 25.8, 17.9; HR-ESI-MS m/z calcd. for $\text{C}_{26}\text{H}_{18}\text{O}_4\text{SNa}$ [$\text{M}+\text{Na}$] $^+$: 449.0818, found 449.0824.



2(3)-(3,4-Dihydroxy-4-methylpentyl)dinaphtho[2,3-*b*:2',3'-*d*]thiophene-5,7,12,13-tetraone

(10). Alkene **9** (2.20 g, 5.16 mmol) was dissolved in a mixture of acetone (75 mL) and H₂O (25 mL). *N*-Methylmorpholine-*N*-oxide (1.81 g, 15.50 mmol) and K₂OsO₄ • 2 H₂O (95.0 mg, 0.26 mmol) were added sequentially as solids. After stirring for 16 h at rt, H₂O (100 mL) was added and acetone was removed by rotary evaporation. The mixture was extracted with EtOAc (3 × 60 mL). The organic layers were combined, washed with brine (50 mL), dried over Na₂SO₄, and concentrated by rotary evaporation. The crude product was purified by flash chromatography (1000:1 CH₂Cl₂:MeOH to 30:1 CH₂Cl₂:MeOH) to provide diol **10** (1.59 g, 67% over 2 steps from dichloride **8**).

Diol 10: ¹H NMR (CDCl₃, 500 MHz) δ 8.31 (ddd, *J* = 0.6, 1.4, 7.7 Hz, 1H), 8.22 (m, 2H), 8.12 (d, *J* = 11.7 Hz, 1H, major isomer), 8.11 (d, *J* = 5.6 Hz, 1H, minor isomer), 8.04 (dd, *J* = 0.5, 2.0 Hz, 1H, minor isomer), 7.85 (dt, *J* = 1.4, 7.5 Hz, 1H), 7.79 (ddt, *J* = 0.8, 1.4, 7.4 Hz, 1H), 7.67 (dd, *J* = 1.9, 8.0 Hz, 1H), 7.63 (dd, *J* = 1.8, 7.9 Hz, 1H, minor isomer), 3.63 (m, 1H), 3.12 (m, 2H), 3.09 (m, 2H), 1.36 (s, 3H), 1.36 (s, 3H); ¹³C NMR (CDCl₃, 125 MHz) δ 178.6, 178.5, 178.5, 178.2, 177.4, 177.3, 177.3, 177.0, 152.7, 152.5, 152.4, 149.3, 147.8, 140.8, 140.7, 140.7, 140.6, 135.4, 135.2, 135.2, 135.0, 134.8, 134.8, 134.3, 133.9, 133.9, 133.1, 132.1, 132.0, 131.9, 130.6, 129.0, 128.5, 128.5, 128.0, 127.4, 127.0, 127.0, 126.5, 36.6, 36.4, 29.9, 29.7, 26.7, 26.7; HR-ESI-MS *m/z* calcd. for C₂₆H₂₁O₆S [M+H]⁺: 461.1014, found 461.1060.

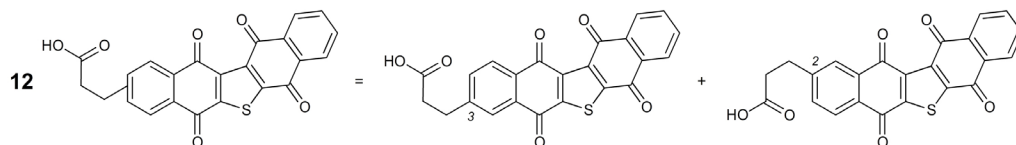


3-(5,7,12,13-Tetraoxo-5,7,12,13-tetrahydrodinaphtho[2,3-*b*:2',3'-*d*]thiophen-2(3)-yl)-

propanal (11). Silica gel supported NaIO₄ was prepared by dissolving NaIO₄ (1.16 g, 5.42 mmol) in H₂O (4 mL) heated to 70°C. Silica gel (230-400 mesh, 8.0 g) was added to this hot solution with vigorous swirling and shaking. The resulting powder was added to a solution of diol **10** (1.25 g, 2.71 mmol) in CH₂Cl₂ (65 mL). After stirring at rt for 2 h, the reaction mixture was filtered through Celite washing thoroughly with CH₂Cl₂ (3 × 50 mL). The solvent was then removed by rotary evaporation to yield aldehyde **11** (1.06 g, 98%) as a yellow solid. Aldehyde **11** was used directly without further purification.

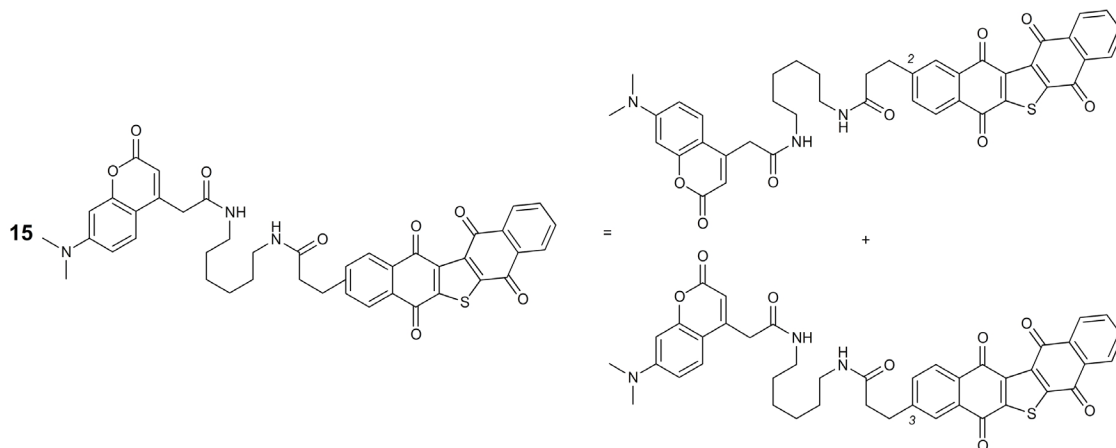
Aldehyde 11: ¹H NMR (CDCl₃, 500 MHz) δ 9.87 (t, *J* = 0.9 Hz, 1H, minor isomer), 9.86 (t, *J* = 0.9 Hz, 1H, major isomer), 8.32 (dd, *J* = 1.2, 7.9 Hz, 1H), 8.24 (m, 2H), 8.15 (t, *J* = 7.9 Hz, 1H, minor isomer), 8.12 (d, *J* = 1.8 Hz, 1H, minor isomer), 8.04 (d, *J* = 1.8 Hz, 1H, major isomer), 7.85 (dt, *J* = 1.4, 7.5 Hz, 1H), 7.80 (tt, *J* = 1.0, 7.5 Hz, 1H), 7.69 (dd, *J* = 1.9, 8.0 Hz, 1H, major isomer), 7.64 (dd, *J* = 1.8, 7.9 Hz, 1H, minor isomer), 3.14 (m, 2H), 2.93 (m, 2H); ¹³C NMR (CDCl₃, 125 MHz) δ 200.2, 200.1, 178.6, 178.6, 178.5, 178.2, 177.4, 177.3, 177.3, 177.1, 152.7, 152.5, 152.4, 152.4, 148.9, 147.5, 140.8, 140.7, 140.7, 140.7, 135.4, 135.2, 135.2, 135.1, 134.8, 134.8, 134.2, 133.9, 133.2, 132.1, 132.0, 131.9, 130.3, 129.0, 128.6, 128.5, 128.1, 127.5, 127.0,

127.0, 126.5, 44.5, 44.4, 28.3, 28.1; HR-ESI-MS m/z calcd. for $C_{23}H_{12}O_5SNa$ $[M+Na]^+$: 423.0298, found 423.0305.



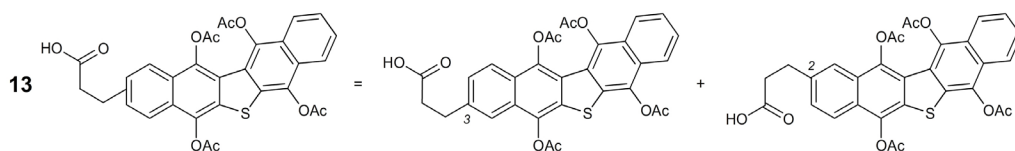
3-(5,7,12,13-Tetraoxo-5,7,12,13-tetrahydrodinaphtho[2,3-*b*:2',3'-*d*]-thiophen-2(3)-yl)propanoic acid (12). Oxone[®] (1.86 g, 3.03 mmol) was added to aldehyde **11** (1.21 g, 3.03 mmol) dissolved in DMF (20 mL). The mixture for was stirred for 2 h at rt. EtOAc (100 mL) and 1 N HCl (30 mL) were added to the reaction mixture. The organic layer was separated and washed with H₂O (2 × 50 mL) and then brine (50 mL), dried over Na₂SO₄, and concentrated on a rotary evaporator to afford acid **12** (1.23 g, 98%). This material was used without further purification. Analytical samples were purified by flash chromatography (1:1 hexanes:CH₂Cl₂ to 5:1 CH₂Cl₂:MeOH).

Acid 12: ¹H NMR (DMSO-*d*₆, 500 MHz) δ 12.19 (bs, 1H), 8.19 (dd, $J = 1.3, 7.7$ Hz, 1H, minor isomer), 8.18 (dd, $J = 1.4, 7.8$ Hz, 1H, major isomer), 8.14 (dd, $J = 1.4, 7.6$ Hz, 1H), 8.10 (d, $J = 8.0$ Hz, 1H, major isomer), 8.06 (d, $J = 7.9$ Hz, 1H, minor isomer), 8.04 (d, $J = 1.8$ Hz, 1H, minor isomer), 8.00 (d, $J = 1.8$ Hz, 1H, major isomer), 7.97 (dt, $J = 1.4, 7.5$ Hz, 1H), 7.92 (dt, $J = 1.4, 7.5$ Hz, 1H), 7.84 (dd, $J = 1.9, 7.9$ Hz, 1H, major isomer), 7.78 (dd, $J = 1.8, 7.9$ Hz, 1H, minor isomer), 3.03 (t, $J = 7.4$ Hz, 2H), 2.66 (t, $J = 7.4$ Hz, 2H); ¹³C NMR (DMSO-*d*₆, 125 MHz) δ 178.3, 178.2, 176.7, 176.5, 173.4, 151.7, 151.7, 147.9, 140.2, 140.1, 135.2, 134.4, 134.0, 132.6, 131.6, 131.6, 127.8, 127.5, 126.3, 126.0, 34.4, 30.2; HR-ESI-MS m/z calcd. for $C_{23}H_{12}O_6SNa$ $[M+Na]^+$: 439.0355, found 439.0217.



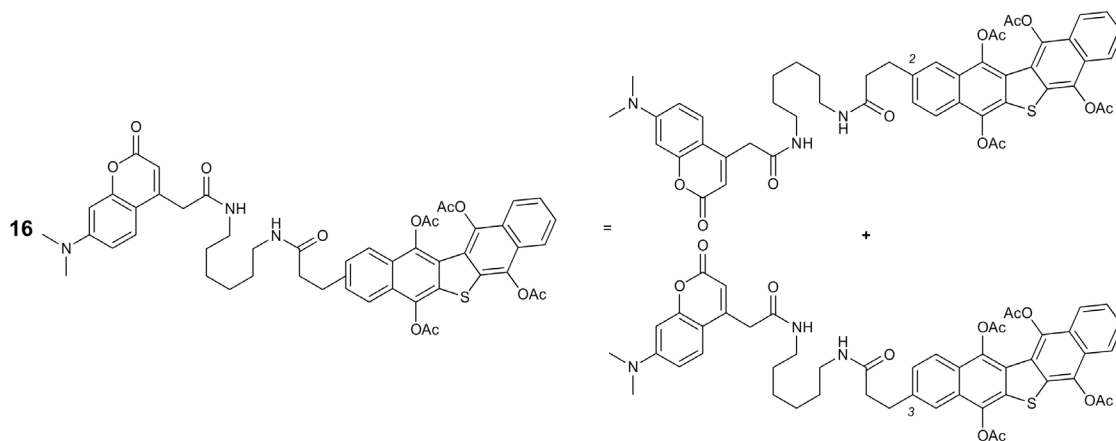
***N*-(6-(2-(7-(Dimethylamino)-2-oxo-2H-chromen-4-yl)acetamido)hexyl)-3-(5,7,12,13-tetraoxo-5,7,12,13-tetrahydrodinaphtho[2,3-*b*:2',3'-*d*]thiophen-2(3)-yl)propanamide (15).** Acid **12** (21.0 mg, 0.05 mmol) was dissolved in dry DMF (0.5 mL) and TSTU (21.1 mg, 0.07 mmol) was added. After 3 h, a solution of *N*-(6-aminohexyl)-2-(7-(dimethylamino)-2-oxo-2H-chromen-4-yl)acetamide (**14a**) (19.2 mg, 0.05 mmol) in DMF (0.5 mL) was added and the mixture was stirred at rt. After 6 h, MeOH (15 mL) was added. The preparation of IAF tag **14a** was described previously [Yu, W. L., et. Al. Spirohexenolide A targets human macrophage migration inhibitory factor (hMIF). *J. Nat. Prod.* **76**, 817-823 (2013) also see Yu, W. L., Guizzunti, G., Foley, T. L.,

Burkart, M. D. & La Clair, J. J. An optimized immunoaffinity fluorescent method for natural product target elucidation. *J. Nat. Prod.* **73**, 1659-1666 (2010)]. The mixture was filtered through a pad of Celite® (1-2 g). The supernatant was collected and dried by rotary evaporation and purified crystallization from 10:1 CH₂Cl₂:MeOH to afford 2.2 mg (8 %) of probe **15**. This material proved instable to flash or preparative HPLC and was not sufficiently stable to collect adequate ¹³C NMR data. Samples of probe **15** were dissolved at 1 mg/mL in DMSO, aliquotted, and stored at -80°C until usage for up to 24 days. Copies of ¹H NMR and ¹H,¹H gCOSY NMR spectra from probe **15** have been provided within the Supplemental Information. Due to stability issues, this material was not fully characterized. HR-ESI-MS *m/z* calcd. for C₄₂H₃₈N₃O₈S [M+H]⁺: 744.2380 found 744.2401.



3-(5,7,12,13-Tetraacetyldinaphtho[2,3-*b*:2',3'-*d*]thiophen-2/3-yl)propanoic acid (13). A mixture of acid **12** (80.0 mg, 0.19 mmol), Zn dust (97.0 mg, 1.48 mmol) and Ac₂O (2 mL) was brought to reflux for 2 h. Upon completion, the reaction mixture was cooled to rt. The unreacted Zn dust was filtered off, and the filtrate was concentrated by rotary evaporation. The crude product was purified by flash chromatography (1:1 hexanes:CH₂Cl₂ to 100:1 CH₂Cl₂:MeOH) to provide acid **13** (70.6 mg, 65%).

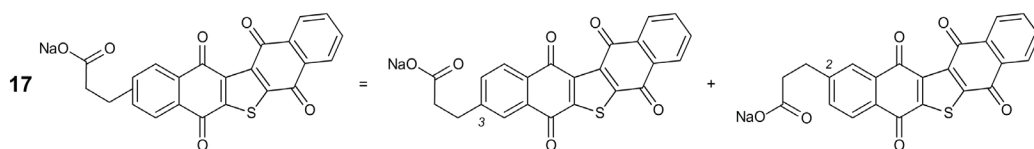
Acid 13: ¹H NMR (CDCl₃, 500 MHz) δ 8.12 (m, 1H), 8.05 (d, *J* = 8.7 Hz, 1H), 7.91 (m, 1H), 7.80 (d, *J* = 8.4 Hz, 1H, major isomer), 7.74 (d, *J* = 8.6 Hz, 1H, minor isomer), 7.63 (m, 1H), 7.60 (m, 1H), 7.49 (dd, *J* = 1.7, 8.7 Hz, 1H, minor isomer), 7.44 (dd, *J* = 1.7, 8.8 Hz, 1H), 3.17 (m, 2H), 2.78 (m, 2H), 2.60-2.57 (multiple singlets, 12H); ¹³C NMR (CDCl₃, 125 MHz) 170.8, 177.9, 168.5, 168.4, 168.4, 168.3, 168.2, 168.2, 152.7, 140.7, 140.7, 140.6, 140.4, 138.6, 138.2, 138.2, 137.9, 130.6, 130.3, 130.2, 129.7, 129.2, 128.1, 128.0, 127.6, 127.4, 127.2, 127.1, 127.0, 126.8, 126.8, 126.6, 126.5, 126.4, 126.4, 126.0, 125.9, 125.6, 123.2, 122.8, 122.7, 121.3, 121.0, 120.5, 120.5, 119.1, 35.2, 35.3, 31.0, 31.0, 21.1, 21.1, 21.1, 20.8; HR-ESI-MS *m/z* calcd. for C₃₁H₂₅O₁₀S [M+H]⁺: 611.1021, found 611.1003.



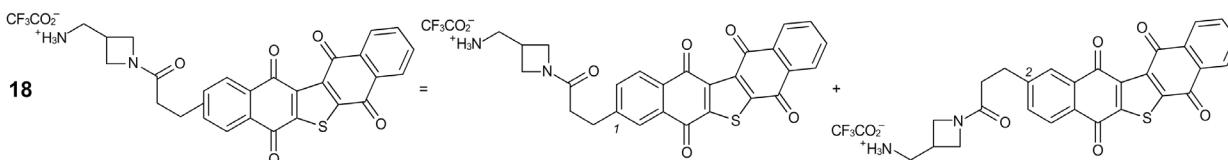
2(3)-(3-((6-(2-(7-(Dimethylamino)-2-oxo-2H-chromen-4-yl)acetamido)hexyl)amino)-3-oxopropyl)dinaphtho[2,3-*b*:2',3'-*d*]thiophene-5,7,12,13-tetrayl tetraacetate (16). Acid **13** (74.0 mg, 0.12 mmol) was dissolved in anhydrous DMF (12 mL). *N*-(6-aminohexyl)-2-(7-

(dimethylamino)-2-oxo-2*H*-chromen-4-yl)acetamide (**14a**) (65.0 mg, 0.15 mmol) was added followed by EtN^{*i*}Pr₂ (0.064 mL, 0.37 mmol) and HATU (61.0 mg, 0.16 mmol) at 0°C. The preparation of IAF tag **14a** was described previously [Yu, W. L., et. Al. Spirohexenolide A targets human macrophage migration inhibitory factor (hMIF). *J. Nat. Prod.* **76**, 817-823 (2013) also see Yu, W. L., Guizzunti, G., Foley, T. L., Burkart, M. D. & La Clair, J. J. An optimized immunoaffinity fluorescent method for natural product target elucidation. *J. Nat. Prod.* **73**, 1659-1666 (2010)]. The reaction was then warmed to rt. After 3 h at rt, the DMF was removed by rotary evaporation. The crude product was purified by flash chromatography (100:1 CH₂Cl₂:MeOH to 20:1 CH₂Cl₂:MeOH) to provide probe **16** (32.0 mg, 29%).

Probe 16: ¹H NMR (CDCl₃, 500 MHz) δ 8.10 (d, *J* = 9.1 Hz, 1H), 8.03 (d, *J* = 8.7 Hz, 1H), 7.78 (d, *J* = 10.1 Hz, 1H), 7.60 (m, 3H), 7.56 (d, *J* = 7.0 Hz, 1H), 7.33 (d, *J* = 8.9 Hz, 1H), 6.51 (dd, *J* = 9.0, 2.6 Hz, 1H), 6.44 (d, *J* = 2.4 Hz, 1H), 5.83 (m, 1H), 5.79 (s, 1H), 5.45 (m, 1H), 3.21 (s, 2H), 3.16 (m, 2H), 3.07 (m, 2H), 2.98 (m, 6H), 2.93 (m, 2H), 2.59 (m, 12H), 1.06 (m, 10H); ¹³C NMR (DMSO-*d*₆, 125MHz) δ 171.5, 169.4, 169.3, 169.2, 168.2, 161.4, 156.0, 153.5, 152.1, 143.3, 140.8, 140.6, 138.3, 137.9, 129.4, 129.3, 127.7, 127.5, 127.1, 126.9, 126.6, 126.3, 125.5, 125.4, 123.5, 121.2, 119.2, 110.0, 109.6, 108.8, 98.1, 49.2, 39.3, 39.0, 29.7, 29.5, 26.7, 26.6, 21.3, 21.2, 21.1; HR-ESI-MS *m/z* calcd. for C₅₀H₅₀N₃O₁₂S [M+H]⁺: 916.3070, found 916.3146.



2(3)-(3-((6-(2-(7-(Dimethylamino)-2-oxo-2*H*-chromen-4-yl)acetamido)hexyl)amino)-3-oxopropyl)dinaphtho[2,3-*b*:2',3'-*d*]thiophene-5,7,12,13-tetrayl tetraacetate (17). A solution of NaOMe in MeOH (0.05 M, 0.72 mL, 0.036 mmol) was added to acid **13** (15.0 mg, 0.365 mmol) dissolved in MeOH (20 mL). The reaction mixture was stirred at rt for 2 h under Ar atmosphere until all solids dissolved. Upon completion, the solvent was reduced to a final volume of 2 mL by rotary evaporation. Ether (20 mL) was added to this solution with vigorous stirring. The orange precipitate was collected by filtration, washed excessively with ether (5 × 2 mL) then CH₂Cl₂ (5 × 2 mL) and dried under high vacuum to afford sodium salt **17** (4.0 mg, 24%), as an orange solid. Spectral properties corresponded to those reported for acid **12**.



(1(2)-(3-(5,7,12,13-tetraoxo-5,7,12,13-tetrahydrodinaphtho[2,3-*b*:2',3'-*d*]thiophen-3-yl)propanoyl)azetid-3-yl)methanaminium 2,2,2-trifluoroacetate (18). Acid **13** (45.0 mg, 0.11 mmol), *tert*-butyl acetidine-3-ylmethylcarbamate (53.0 mg, 0.14 mmol) and EtN^{*i*}Pr₂ (0.1 mL, 0.54 mmol) were dissolved in anhydrous DMF (2 mL) under an Ar atmosphere. The mixture was cooled to 0°C and HATU (48.0 mg, 0.22 mmol) was added at 0°C. The mixture was warmed to rt and stirred for an additional 2 h at rt. The reaction was terminated by the addition of 1 M sodium phosphate buffer pH 7 (10 mL) and extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with H₂O (15 mL) then brine (15 mL), dried with Na₂SO₄ and concentrated by rotary evaporation. The mixture was purified by flash chromatography (1000:1

CH₂Cl₂:MeOH to 100:1 CH₂Cl₂:MeOH) affording the Boc-protected amide (45.2 mg, 71%). A sample of this material (20 mg) was dissolved in 3:1 CH₂Cl₂:TFA (0.9 mL) and stirred at rt. After 1 h, Et₂O (10 mL) was added under vigorous stirring. The yellow precipitate was collected by filtration, washed excessively with ether (5 × 2 mL) and CH₂Cl₂ ether (5 × 2 mL) and dried under high vacuum to afford amide **18** (7.0 mg, 42%), as a yellow solid.

Amide 18: ¹H NMR (CD₃OD, 500 MHz) δ 8.22-8.08 (m, 3H), 8.01-7.91 (m, 2H), 7.82 (d, *J* = 8.1 Hz, 1H), 7.74 (bs, 2H), 4.15 (t, *J* = 8.5 Hz, 1H), 3.89 (t, *J* = 9.1 Hz, 1H), 3.82 (dd, *J* = 5.3, 8.8 Hz, 1H), 3.62 (dd, *J* = 5.3, 9.8 Hz, 1H), 3.01 (td, *J* = 7.3, 15.3 Hz, 4H), 2.80-2.69 (m, 1H), 2.45 (dd, *J* = 7.4, 13.8 Hz, 2H); ¹³C NMR (DMSO-*d*₆, 125MHz) δ 178.9, 178.8, 177.3, 177.1, 171.7, 153.4, 152.3, 149.0, 140.8, 140.7, 135.9, 135.9, 135.0, 134.7, 132.2, 132.2, 128.4, 128.2, 127.0, 126.7, 53.4, 51.4, 42.5, 32.0, 27.0; HR-ESI-MS *m/z* calcd. for C₂₇H₂₁N₂O₅S [M+H]⁺: 485.1126, found 485.1211.

B. General biological protocols. All reagents and media were used at molecular biological grades. The following sections provide experimental procedures for the biological studies described in Figs. 2-4, as denoted below.

B1. Tissue culture. The bulk of this program was conducted using the HCT-116 (ATCC# CCL-247). However, additional cell lines including Malme-3M (ATCC# HTB-64), PC-3M (DCTD tumor repository, NCI), SF-295 (DCTD tumor repository, NCI), SW-620 (DCTD tumor repository, NCI), Colo 205 (DCTD tumor repository, NCI), WIDR (DCTD tumor repository, NCI), OVCAR-8 (DCTD tumor repository, NCI) and HL-60 (DCTD tumor repository, NCI) were evaluated to validate the activity observed in the HCT-116 cell lines. Often, we conducted confocal or activity assays in multiple cell lines as a mean to carefully validate the observed effects. The following procedures were used throughout this program for growth of the respective cell lines. The cell lines listed above, with the exception of Malme-3M, were cultured in Roswell Park Memorial Institute (RPMI) medium (GIBCO-BRL), supplemented with 10% inactivated fetal calf serum (FCS) and 1% penicillin/streptomycin (GIBCO-BRL). Malme-3M cells were cultured in Iscove's modified Dulbecco's medium (GIBCO-BRL) supplemented with 20% inactivated FCS and 1% penicillin/streptomycin. All cells were manipulated under sterile conditions provided by a class II biohazard safety flow hood and incubated at 37°C in a 5% CO₂ atmosphere. Every 2 - 4 d, cells were detached from flask using a 0.05% trypsin-EDTA solution (GIBCO-BRL) and split 1:4 for routine passage.

B2. Cell lysate preparation. All samples of cell lysate were prepared fresh, stored on ice and used within 4 h of production. Cells were cultured in 75 cm² culture flask to a density of 5 × 10⁴ cells/cm² to 1 × 10⁵ cells/cm². The cells were washed with phosphate buffered saline (PBS) (2 × 5 mL), harvested by gentle removal using a tissue scraper, and suspended in either PBS (5 mL) containing 25-50 μL of a protease inhibitor cocktail (104 mM 4-(2-aminoethyl)-benzenesulfonylfluoride hydrochloride, 80 μM aprotinin, 4 mM bestatin, 1.4 mM E-64, 2 mM, Leupeptin, 1.5 mM pepstatin A in DMSO) or radioimmunoprecipitation assay (RIPA) buffer (5 mL) (25 mM Tris-HCl pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate and 0.1 % sodium dodecylsulfate) containing 25-50 μL of a protease inhibitor cocktail. The resulting cell suspension was then passed through 27.5 gauge needle multiple times using syringe. The crude cell lysate centrifuged at 2,500 × g, concentrated to the desired concentration using a 3 kDa spin concentrator (Millipore), and stored at 4°C until used. When required, the concentration of

protein in the lysates was checked via Bradford analysis or Lowry's analysis using the DC Protein Assay kit (Pierce).

B3. SDS PAGE analysis. Samples were diluted in 1:1 ratio with 2 × Laemmli sample buffer (65.8 mM Tris-HCl, pH 6.8, 2.1% SDS, 26.3% w/v glycerol, 0.01% w/v bromophenol blue, 0.5% v/v β-mercaptoethanol) and run on a 4-12% Bis-Tris NuPAGE gel (Life Technologies) with NuPAGE MES SDS buffer (Life Technologies). Gels were stained using SilverQuest kit (Life Technologies) using the fast staining protocol as described by the manufacturer. Gels were imaged on a conventional flatbed scanner (1260, Epson)

B4. Western blot analysis. Protein extracts were quantified the Lowry assay using the DC Protein Assay kit (Bio-Rad Laboratories). Equal amounts of protein (typically 30 μg) were used for analytical studies. Each sample was diluted 1:1 ratio with 2 × Laemmli sample buffer (Bio-Rad), boiled for 5 min, cooled and then submitted to SDS-PAGE (see Section B3). The resulting gel was transferred to a polyvinylidene difluoride (PVDF) membrane (GE Healthcare or Bio-Rad Laboratories) using a Novex X-cell system (Life Technologies) using Novex Tris-glycine transfer buffer (Life Technologies) containing 20% MeOH. After transfer, the blot was blocked by shaking with 5% non-fat dry milk in TBS (137 mM NaCl, 20 mM Tris) (10 mL) for 1 h at rt. The blots were washed with (3 × 10 mL) TBST (137 mM NaCl, 20 mM Tris, 0.1% Tween-20) and lightly shaken with primary antibody in 5% bovine serum albumin in TBS (10 mL) for 12 h at 4°C. The following primary antibodies were used at the ascribed dilution: caspase-3 at 1:1000 dilution (9662, Cell Signaling Technology Inc.), cleaved caspase-3 at 1:1000 dilution (9664, Cell Signaling Technology Inc.), caspase 7 at 1:1000 dilution (9492, Cell Signaling Technology Inc.), cleaved caspase-7 at 1:1000 dilution (8438, Cell Signaling Technology Inc.), caspase-9 at 1:1000 dilution (9502, Cell Signaling Technology Inc.), cleaved caspase-9 at 1:1000 dilution (7237, Cell Signaling Technology Inc.), PARP at 1:1000 dilution (9542, Cell Signaling Technology Inc.), cleaved PARP at 1:1000 dilution (5625, Cell Signaling Technology Inc.), cyclin A2 at 1:1000 dilution (4656, Cell Signaling Technology Inc.), cyclin B1 at 1:1000 dilution (4138, Cell Signaling Technology Inc.), cyclin D1 at 1:1000 dilution (2978, Cell Signaling Technology Inc.), cyclin D2 at 1:1000 dilution (3741, Cell Signaling Technology Inc.), cyclin D3 at 1:1000 dilution (2936, Cell Signaling Technology Inc.), cyclin E1 at 1:1000 dilution (4129, Cell Signaling Technology Inc.), cyclin E2 at 1:1000 dilution (4132, Cell Signaling Technology Inc.), cyclin H at 1:1000 dilution (2927, Cell Signaling Technology Inc.), LC3A at 1:2000 dilution (4599, Cell Signaling Technology Inc.), LC3B at 1:2000 dilution (3868, Cell Signaling Technology Inc.), anti-IAF XRI-TF35 at 1:1000 to 1:2000 dilution (Xenobe Research Institute), DCD at 1:100 dilution (PIPA513677, ThermoScientific Pierce), or GAPDH at 1:2000 dilution (ABP-MAB-GL001, Allele Biotechnologies). As required, β-Actin at 1:10,000 dilution (ab8227, Abcam) was used to normalize protein loading. After treatment with the primary antibodies, the blots were washed with (3 × 10 mL) TBST (137 mM NaCl, 20 mM Tris, 0.1% Tween-20) and lightly shaken secondary antibody in 2.5% bovine serum albumin in TBS (10 mL) for 2 h at rt. The following secondary alkaline phosphatase conjugated antibodies were used: anti-mouse at 1:500 to 1:2000 dilution, (7056, Cell Signaling Technology Inc.) and anti-rabbit at 1:500 to 1:2000 dilution (7054, Cell Signaling Technology Inc. or 69266, Novagen). Blots were developed by treatment with BCIP/NBT color development substrate (34042, Pierce or S3771, Promega) according to the manufacturer's protocols. Blots were imaged on a conventional flatbed scanner (1260, Epson).

B5. Confocal microscopy. Confocal studies were conducted on a LSM 710 inverted confocal microscope (Zeiss) containing a scanning module with three detection channels, a Plan-Apochromat 63 × 1.4 na objective, and multiple lasers including a diode lasers at CW pulsed (405 nm, 30 mW), an Ar-laser (458 nm, 488 nm, 514 nm, each at 25 mW), and a HeNe-laser (543 nm, 1 mW) and HeNe-laser (633 nm, 5 mW).

B6. Immunoprecipitation with the anti-IAF XRI-TF35 mAb. Cell lysate (1 mL) containing an ascribed concentration of **15** or **16** was added to 100 µL of Affi-Gel 10 resin (Bio-Rad) bearing 1.5 mg/mL of covalently attached anti-IAF XRI-TF35 mAb (Xenobe Research Institute). The resulting slurry were shaken on a Labquake rotator (Thermo Scientific) for 12 h at 4°C. The resin was then collected and washed with ice-cold RIPA buffer (3 × 1 mL). After the wash was complete, all of the remaining aqueous solution was removed by aspiration. The resin was treated 1 mM 7-dimethylaminocoumarin-4-acetic acid in RIPA buffer (50 µL) and centrifuged for 5 min at 2,500 × g. The supernatant was collected as the immunoprecipitated fraction. Samples of the immunoprecipitated fraction were diluted in 1:1 ratio with 2 × Laemmli sample buffer (Bio-Rad Laboratories) and evaluated by SDS-PAGE (see Section B3) or Western blot analysis (see Section B4).

C. Experimental procedures. The following sections provide detailed experimental procedures for the data presented within Figs. 2-4 of the manuscript.

C1. Subcellular localization studies. Five different cell lines (HCT-116, PC-3M, SF-295, HCT-8 or Malme-3M) were evaluated to confirm the uptake and subcellular localization. A common procedure was used for all imaging studies. Cells were seeded 24 h prior to treatment in 35 mm glass-bottom dishes (MatTek Corporation) and cultured until a density of 5×10^3 cells/cm² to 1×10^4 cells/cm². All compounds were added to cells under 1 mL of media from DMSO stocks such that the DMSO content remained under 0.1%. Cells were cultured for the ascribed periods at 37°C in a 5% CO₂ atmosphere and imaged as described in Section B5. Images from these studies were presented in Fig. 2.

C2. Time-course imaging studies. Compound **1** was added at time 0 h and cells were cultured for an ascribed period at 37°C in a 5% CO₂ atmosphere. After incubation, the cells were fixed by treatment with 4% formaldehyde in PBS followed by washing twice with PBS (2 × 1 mL). Images were collected 2 h to 6 h after fixation as described in Section B5. Images from these studies were presented in Fig. 2c.

C3. Colocalization studies. Counterstaining was used to verify the subcellular localization in the endoplasmic reticulum (ER) and autophagosomes. For the ER co-staining, cells were treated with **1** for the ascribed period, and then stained live with 0.5 µM ER-tracker™ Blue-White DPX (Life Technologies) at 37°C. After 30 min, cells were washed PBS (4 × 1 mL) and analyzed immediately by confocal microscopy. The fluorescence from **1** was acquired by red emission at 550-631 nm, while the ER-tracker was acquired by blue emission at 406-450 nm. For autophagosomal co-staining, cells were treated with **1** for the ascribed period, and then stained with 50 µM dansylcadaverine at 37°C. After 15 min, cells were washed PBS (4 × 1 mL) and analyzed immediately by confocal microscopy. Live cells were used for confocal analysis. The fluorescence from **1** was acquired by red emission at 550-631 nm, while the ER-tracker was acquired by blue emission at 415-530 nm (see Section B5.) Images from these studies were presented in Fig. 2d-2j.

C4. Trypan blue assays. Cell viability was determined using the Trypan blue dye exclusion test after incubation of HCT-116 cells. Cells were cultured to 5×10^4 cells/cm² in the presence of either 0.3 μ M **1**, 3 μ M **1**, or 30 μ M **1**. Aliquots were removed from cultures after 24 h or 48 h. The cells that excluded Trypan blue (viable) or did not exclude Trypan blue (non-viable) were differentially counted using a Neubauer chamber (New Optik). Data from these studies was presented in Fig. 3a.

C5. Cytotoxicity analyses. Cytotoxicity analyses were conducted in multiple cell lines (HCT-116, Malme-3M, SW-620, Colo 205, WIDR, OVCAR-8, SF-295 or HL-60). Briefly, cells were cultured (see Section B1) in 96-well plates to a density of 1×10^4 cells/well. Each well was treated with a single concentration of analyte displayed over 5-fold dilutions from 0.0001 μ M to 50.0 μ M. For each analyses, 5-fold dilutions of etoposide (0.05 μ M - 50 μ M) and 1% DMSO were used as positive and negative controls, respectively. The cells were incubated for 24 h, 48 h or 72 h. At the end of the incubation period, cultures were treated with 1 mM 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) for 3 h, wherein viable cells being able to reduce the yellow MTT dye to a purple formazan product. Colorimetric analyses were conducted on a microplate reader (Beckman Coulter Inc.), using absorption readings at 570 nm. All experiments were conducted in triplicate and the deviation over these analyses has been reported. Data from these studies was presented in Table S2.

C6. Cytotoxicity time-course studies. Time course experiments were also conducted in HCT-116 cells to determine the window for optimal efficacy of **1**. Cells were exposed to **1** over for either 1 h, 3 h, 6 h, or 9 h followed by incubation in drug-free media for the remainder of a 24 h period. At the end of the incubation period, cell viability was quantified using the CellTiter96 One Solution Cell Proliferation Assay (Promega), following manufacture's instruction. Colorimetric analyses were conducted on a microplate reader (Molecular Devices), using absorption readings at 490 nm. All experiments were conducted in triplicate and the deviation over these analyses has been reported. Data from these studies was presented in Fig. 3b.

C7. Validation of entry into autophagy. Lysate was prepared in RIPA buffer (see Section B2) from HCT-116 cells that were cultured to 1×10^6 cells/cm² in 75 cm² cell culture flasks over 24 h and then incubated with either 3 μ M **1**, 30 μ M **1**, 17 μ M etoposide (positive control), or 0.3% DMSO (negative control) for 24 h. Equal amounts of protein (30 μ g) as determined by the Lowry method were diluted in a 1:1 ratio with 2 \times Laemmli sample buffer (BioRad Laboratories) and subjected to SDS PAGE (see Section B3) and western blot analysis using LC3A, LC3B and β -actin as the primary antibodies (see Section B4). Blots from this study are presented in Fig. 3c.

C8. Cell cycle and DNA fragmentation analyses. HCT-116 cells were seeded in 24-well plates and grown to 5×10^4 cells/cm², typically over a 24 h period. The cells were then treated with either 0.3 μ M **1**, 3 μ M **1**, 30 μ M **1**, 17 μ M etoposide (positive control) or 1% DMSO (negative control). Cells were then incubated for either 18 h or 24 h. After this period, the cells were recovered by treatment with 0.05% trypsin-EDTA solution (GIBCO-BRL). An 100 μ L aliquot of these cells was incubated for 30 min in the dark with a hypotonic solution containing 50 μ g/mL propidium iodide, 0.1% sodium citrate, and 0.1% Triton X-100. Cell cycle progression and DNA fragmentation were analyzed on an ImageStream system (Amnis). Five thousand events were acquired and data analyses were conducted using ModFit LT for Win32 version 3.1. Data from these studies were presented in Fig. 3d (DNA fragmentation, obtained from the subdiploid population) and Fig. 3e (cell cycle progression, obtained from the G0/G1, S and G2/M populations).

C9. Western blot analysis of cell cycle progression. HCT-116 cells were grown 5×10^4 cells/cm² in a 75 cm² cell culture flasks typically over a 24 h period at which point they were treated with media containing either 3 μ M **1**, 30 μ M **1**, 17 μ M etoposide (positive control) or 0.3% DMSO (negative control). After incubation for 24 h, cell lysates were prepared in RIPA buffer (see Section B2). Samples of lysates containing 30 μ g of protein were subjected western blot analysis (see Section B4) using cyclin A2, cyclin B1, cyclin D1, cyclin D2, cyclin D3, cyclin E1, cyclin E2, or cyclin H as the primary antibodies. The experiment remained comparable over three repetitions and exemplary blots are presented in Fig. 3f.

C10. Western blot analysis of apoptotic markers. HCT-116 cells were grown to 5×10^4 cells/cm² in a 75 cm² cell culture flasks typically over a 24 h period at which point they were treated with media containing either 3 μ M **1**, 30 μ M **1**, 17 μ M etoposide (positive control) or 0.3% DMSO (negative control). After incubation for 24 h, cell lysates were prepared in RIPA buffer (see Section B2). Samples of lysates containing 30 μ g of protein were subjected western blot analysis (see Section B4) using caspase-3, cleaved caspase-3, caspase 7, cleaved caspase-7, caspase-9, cleaved caspase-9, PARP, cleaved PARP, as the primary antibodies. The experiment remained comparable over three repetitions and exemplary blots are presented in Fig. 3g (caspase activity) and Fig. 3g (cyclin activity).

C11. Immunoprecipitation with probe 15. An aliquot of 1.3 mM stock of probe **15** in DMSO was added to 1 mL of cell lysate prepared in PBS (see Section B2) containing 1 mg/mL of net protein to provide a solution of cell lysate containing either 3 μ M **15** or 30 μ M **15**. An aliquot of these solutions (1 mL) was subjected to IP analysis using 100 μ L the resin bearing 1.5 mg/mL of covalently attached anti-IAF XRI-TF35 mAb (see Section B6). This study was repeated on three different cell lysates and representative images of silver stained SDS PAGE gels are provided in Fig. 4a.

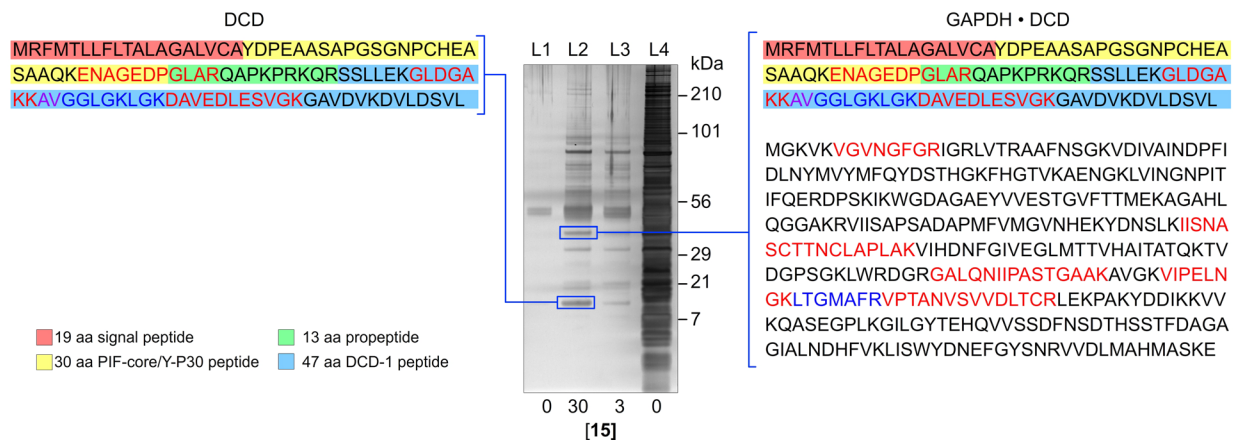


Figure S1 | Protein ID via LC-MS/MS analyses. (left) Data depicting the LC-MS/MS analysis of a band at 12 kDa returned peptides observed in DCD. (right) Data depicting the LC-MS/MS analysis of a band at 50 kDa returned peptides observed in DCD and glyceraldehyde 3-phosphate dehydrogenase (GAPDH). This data serves to further support that provided in Fig. 4a. Peptides identified are colored in either red or blue. Regions of the DCD protein are shaded in either red, yellow, green or blue as indicated within the figure.

C12. Target identification. Silver stained bands were excised from the corresponding SDS-PAGE gel and destained using the SilverQuest kit (Life Technologies) according to the

manufacturers protocols. The destained bands were submitted to LC-MS/MS Protein-ID analysis conducted by the Biomolecular and Proteomics Mass Spectrometry Facility at UC San Diego. Each band was subjected to an in gel trypsin-digestion and peptides were analyzed by LC-MS/MS using a QSTAR-Elite hybrid mass spectrometer (AB/MDS Sciex) interfaced to a nanoscale reversed-phase high-pressure liquid chromatograph (Tempo) using a 10 cm 180 ID glass capillary packed with 5 μm C₁₈ Zorbax beads (Agilent). The buffer compositions were as follows: buffer A was composed of 98% H₂O, 2% CH₃CN, 0.2% formic acid, and 0.005% trifluoroacetic acid (TFA) and buffer B was composed of 100% CH₃CN, 0.2% formic acid, and 0.005% TFA. Peptides were eluted from the C₁₈ column into the mass spectrometer using a linear gradient of 5-60% buffer B over 60 min at 400 $\mu\text{l}/\text{min}$. LC-MS/MS data were acquired in a data-dependent fashion by selecting the 4 most intense peaks with charge state of 2 to 4 that exceeds 20 counts, with exclusion of former target ions set to "360 seconds" and the mass tolerance for exclusion set to 100 ppm. Time-of-flight MS were acquired at m/z 400 Da to m/z 1600 Da for 1 s with 12 time bins to sum. MS/MS data were acquired from ions m/z of 50 Da to m/z of 2,000 Da by using enhance all and 24 time bins to sum, dynamic background subtract, automatic collision energy, and automatic MS/MS accumulation with the fragment intensity multiplier set to 6 and maximum accumulation set to 2 s before returning to the survey scan. Peptide identifications were made using paragon algorithm executed in Protein Pilot 2.0 software (Life Technologies). Representative peptide maps are provided in Fig. 4a and Fig. S1.

C13. Metabolic conversion studies (Fig. S2). A 1.1 mM solution of **16** in DMSO (80 μL) or 1.9 mM solution of **2** in DMSO (80 μL) was added to 1 mL of HCT-116 cell lysate containing 1 mg/mL of total protein (see Section B2). The samples were incubated at either 0°C, rt, or 37°C for 96 h. The samples were extracted with CH₂Cl₂ (3 \times 1 mL), washed with water (1 \times 1 mL) then brine (1 \times 1 mL). Alternatively, cells were treated with **2** or **16** for an ascribed period. After incubation, cell lysates were prepared according standard protocols (see Section B2). The lysate samples were then extracted with CH₂Cl₂ (3 \times 1 mL), washed with water (1 \times 1 mL) then brine (1 \times 1 mL). For both approaches, the conversion of **2** and **16** to **1** and **15** were then determined using LC-HRMS analysis as shown in Fig. S2.

C14. Immunoprecipitation with probe 16. An aliquot of a 1.1 mM stock of probe **16** in DMSO was added to 1 mL of cell lysate prepared in PBS (see Section B2) containing 1 mg/mL in net protein to provide a solution of cell lysate containing either 20 μM **16**, 30 μM **16**, or 40 μM **16**. Aliquot of these solution (1 mL) were subjected to IP analysis (see Section B6). This study was repeated on three different cell lysates and representative images of silver stained gels are provided in L4-L5, Fig. 4b.

C15. Live cell immunoprecipitation with probe 16. A 1.1 mM solution of **16** in DMSO (30 μL) was added to 25 mL of media covering HCT-116 cells cultivated to 1×10^6 cells/cm² on a 75 cm² cell culture flask to afford a final concentration of 30 μM **16**. The dish was incubated for 24 h at 37°C. The cells were washed with PBS (3 \times 5 mL), suspended in 5 mL of PBS, and frozen at -80°C. The flask was removed from the freezer and thawed on ice. Cells were collected and lysate was prepared in PBS (see Section B2). An aliquot of this solution (1 mL) was subjected to IP analysis (see Section B6) and evaluated by SDS PAGE analysis (see Section B3). This study was repeated on three different cell lysates and representative images of silver stained gels are provided in L7-L8 in Fig. 4b.

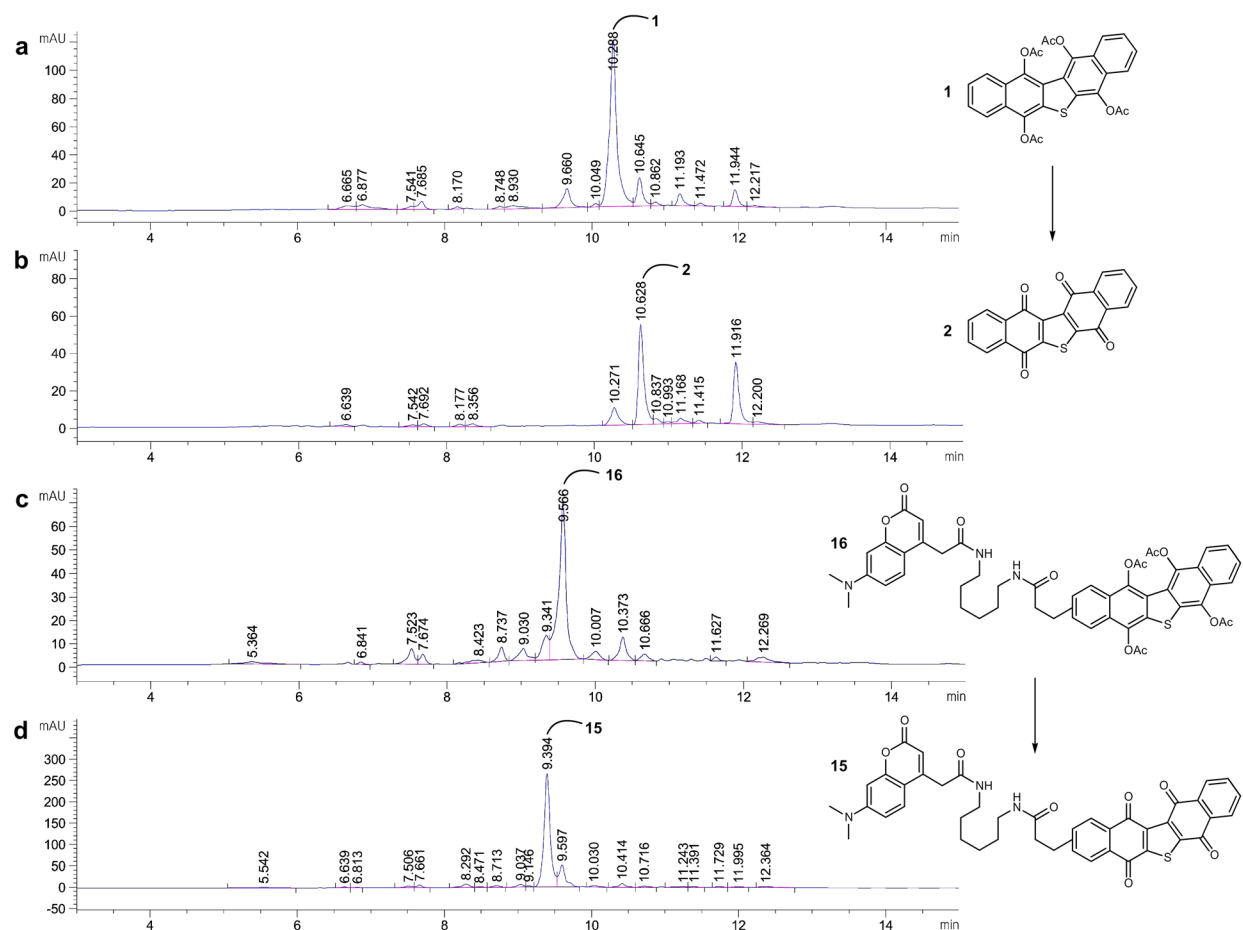


Figure S2 | Peracetate **2** and probe **16** are converted to **1** and **15**, respectively, in HCT-116 cell lysate. **a**, LC/MS trace depicting extracts from HCT-116 cell lysate that were treated with 150 μM **2** for 24 h. **b**, LC/MS trace depicting extracts from HCT-116 cell lysate that were treated with 150 μM **2** for 96 h. **c**, LC/MS trace depicting extracts from HCT-116 cell lysate that were treated with 90 μM **16** for 24 h. **d**, LC/MS trace depicting extracts from HCT-116 cell lysate that were treated with 90 μM **16** for 96 h. Unless otherwise stated, cell lysates contained containing ~ 1 mg/mL in total protein.

C16. Iodoacetamide treatment after immunoprecipitation. A 20 μL aliquot of immunoprecipitated fraction from Section C11 was treated with 5 mM iodoacetamide (freshly prepared). After 1 h at 23 $^{\circ}\text{C}$, the resulting fraction was diluted in a 1:1 ratio with 2 \times Laemmli sample buffer (Bio-Rad Laboratories) and evaluated by SDS PAGE analysis (Section B3). This study was repeated on three different cell lysates and representative images of silver stained gels were provided in L10, Fig. 4c.

C17. Iodoacetamide treatment before immunoprecipitation. Cell lysate (5 mL) containing 1 mg/mL in net protein prepared in PBS (see Section B2) was treated with 0.5 mM iodoacetamide for 1 h at 4 $^{\circ}\text{C}$. The remaining iodoacetamide was removed by spin dialysis with PBS (5 \times 10 mL). Probe **15** was added from a 1.3 mM DMSO stock to provide to provide a final concentration of 30 μM **15** in the cell lysate. An aliquot of this solution (1 mL) was subjected to IP analysis (see Section B6) and evaluated by SDS PAGE analysis (see Section B3). This study

was repeated on three different cell lysates and representative images of silver stained SDS PAGE gels were provided in L11, Fig. 4c.

C18. Western blot validation with probe 15. A 20 μ L aliquot of the IP fraction from Section C11 from lysate treated with 30 μ M **15** was diluted in a 1:1 ratio with 2 \times Laemmli sample buffer (Bio-Rad Laboratories) and subjected to western blot analysis (see Section B4) using the anti-IAF XRI-TF35 (L12-L13), DCD (L14) and GAPDH (L15) as the primary antibodies. Blots from these analyses were provided in L12-L15, Fig. 4d.

C19. Western blot validation with probe 16. A 20 μ L aliquot of the IP fraction from Section C14 or Section C15 was diluted in a 1:1 ratio with 2 \times Laemmli sample buffer (Bio-Rad Laboratories) and subjected to western blot analysis (see Section B4) using the DCD GAPDH as the primary antibodies. Blots from these analyses were provided in L17-L18, Fig. 4e.

C20. qPCR analysis (Fig. 4g). HCT-116 (5×10^4 cells/cm²), Malme-3M (2.5×10^4 cells/cm²) and PC3M (5×10^4 cells/cm²) cells were seeded in 5 mL six-welled dishes. After growth for 24 h, the cells were treated with 0.3 % DMSO (negative control), 15 μ M etoposide (positive control), 3 μ M **1** or 30 μ M **1** in HCT-116 and PC-3M cells, and 30 and 100 nM in Malme-3M cells, and incubated for an additional 24 h. Total RNA was extracted for all samples using the RNeasy Mini Kit (Qiagen). RNA samples were quantified by absorbance measures at 260 nm on microvolume spectrophotometer (Thermo Scientific). Purity of samples was determined by the ratio between measurements at 260 nm and 280 nm. After isolation and quantification, RNA was converted into cDNA by reaction with reverse transcriptase (RT) using an iScript cDNA Synthesis Kit (BioRad) with 4 μ L reaction buffer, 1 μ L RT enzyme stock, 500 ng total RNA, diluted with ultrapure H₂O to a final volume of 20 μ L. The newly synthesized cDNA was then subjected to a q-PCR reaction conducted on an iQ5 thermocycler (BioRad) using the reagent SYBR Green PCR Master Mix (Applied Biosystems) with 10 μ L SYBR, 0.4 μ L Primer F, 0.4 μ L Primer R, 1 μ L cDNA, diluted with ultrapure H₂O to a final volume of 20 μ L. PCR reactions were conducted using the following conditions: initial denaturation at 95 °C for 10 min, followed by 50 cycles at 95°C for 15 s and 58°C for 1 min. The dissociation curve that followed amplification consisted of 75 cycles from 58 °C to 95 °C for 15 s, varying the temperature by 0.5 °C per cycle. Primers (Invitrogen) for DCD (DCD F: AAGCCAAGGAAGCAGAGATCC and DCD R: GCTCCTTTACCCACGCTTTCT) were designed using the OligoPerfect Designer software (Invitrogen), which generated an amplicon with 125 bp. The gene RPLPO (RPLPO F: GCAATGTTGCCAGTGTCTG and RPLPO R: GCCTTGACCTTTTCAGCAA; Invitrogen), which generated an amplicon with 142 bp, was used as internal control, for normalization. Quantitative parameters were obtained by values of the C_T (threshold cycle), where the signal associated to amplification of the PCR product during the exponential phase is first detected. Calculation to measure relative expression of analyzed genes was based on the 2^{- $\Delta\Delta$ CT} method [Kenneth J. Livak, Thomas D. Schmittgen, Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2^{- $\Delta\Delta$ CT} Method, Methods, Volume 25, Issue 4, December 2001, Pages 402-408]. Data from these analyses were provided in Fig. 4g.

C21. Confocal image analysis of probe 15. HCT-116 cells were treated with 5 μ M **15** to 30 μ M probe **15** over 8 h and imaged was imaged under conventional conditions (see Section B5). An exemplary image is provided in Fig. S3.

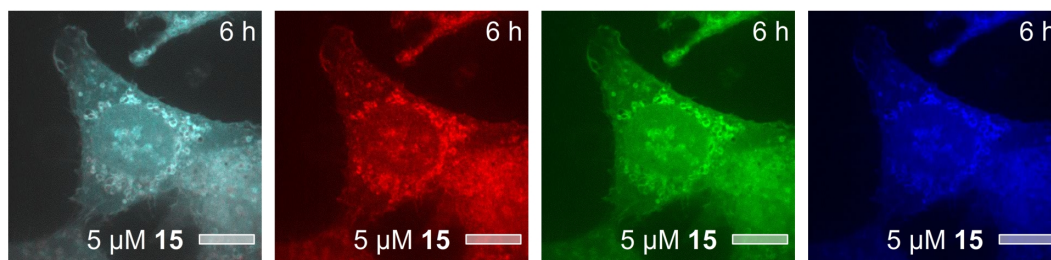


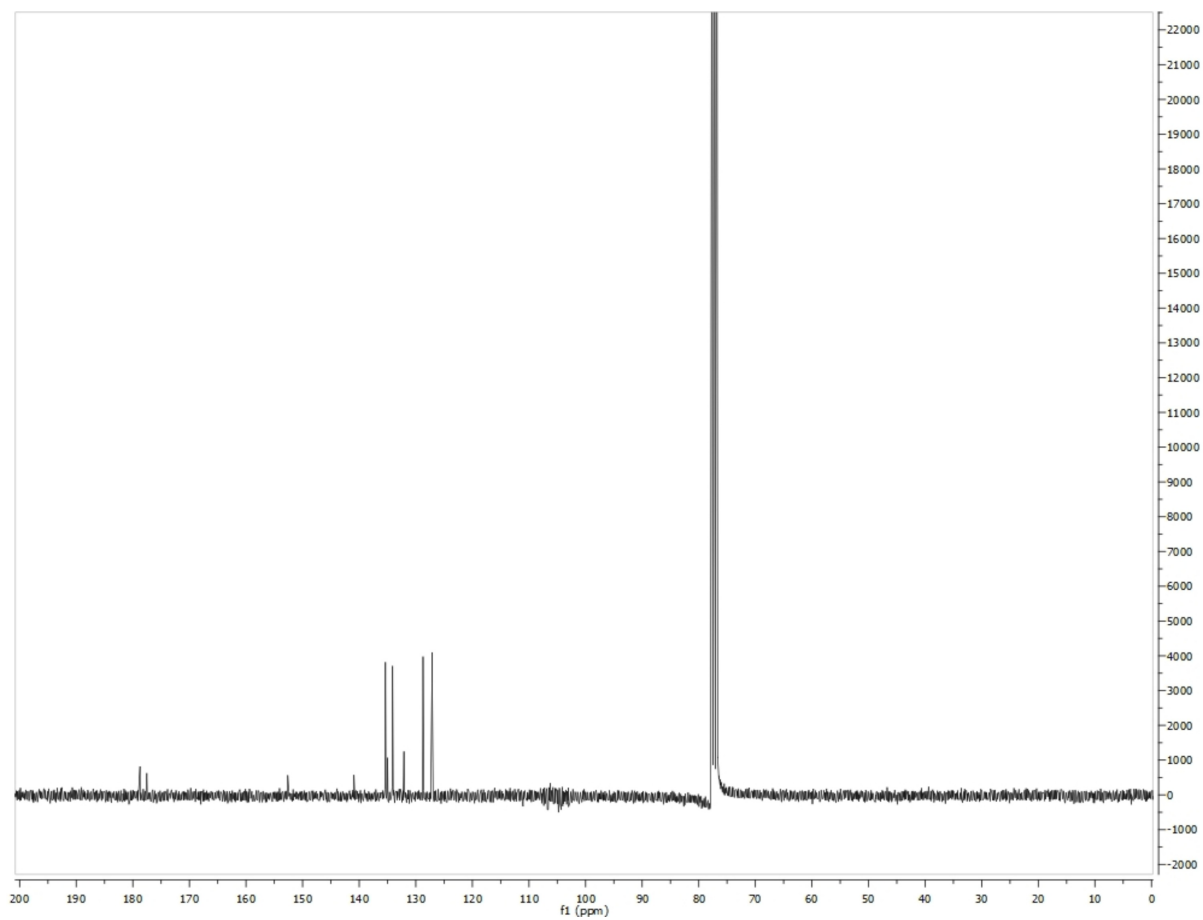
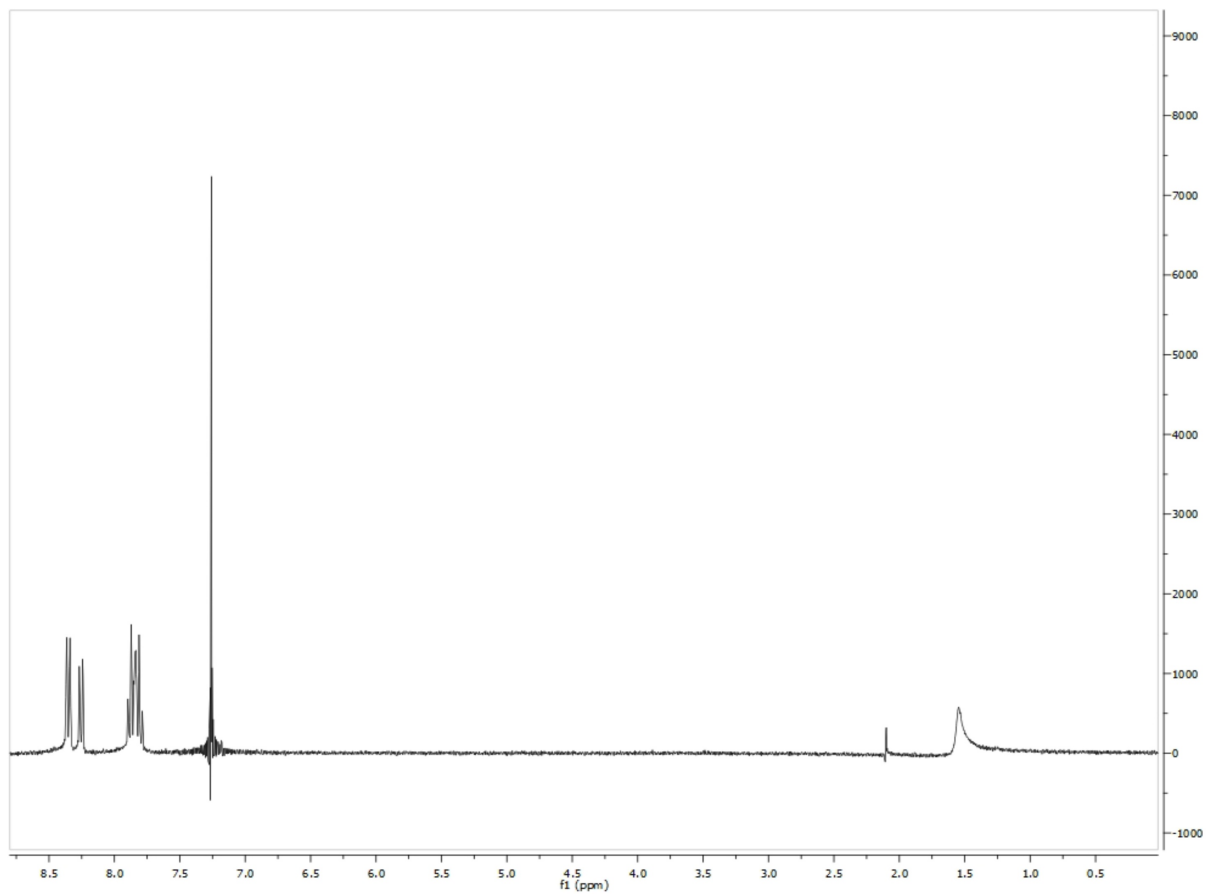
Figure S3 | The IAF probe **15** undergoes comparable localization in the ER and transitioning into the autophagosomes. The image depicts HCT-116 cells that were treated with 5 μM **15** for 6 h. The presence of blue fluorescence from the IAF tag in **15** overlapped with the red and green fluorescence from the seriniquinone core, indicating that probe **15** provides an accurate mimic of **1**.

C22. Cell selectivity and bioactivity data (Table S2).

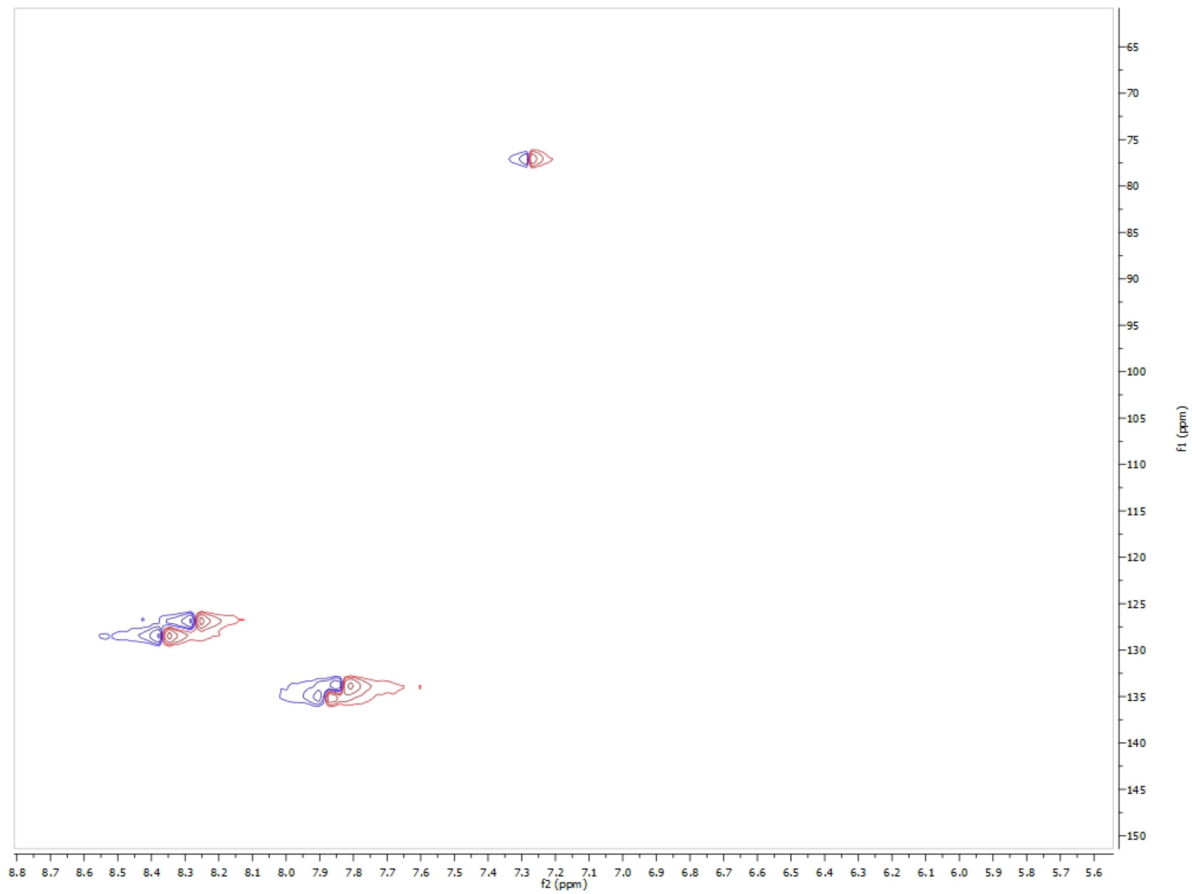
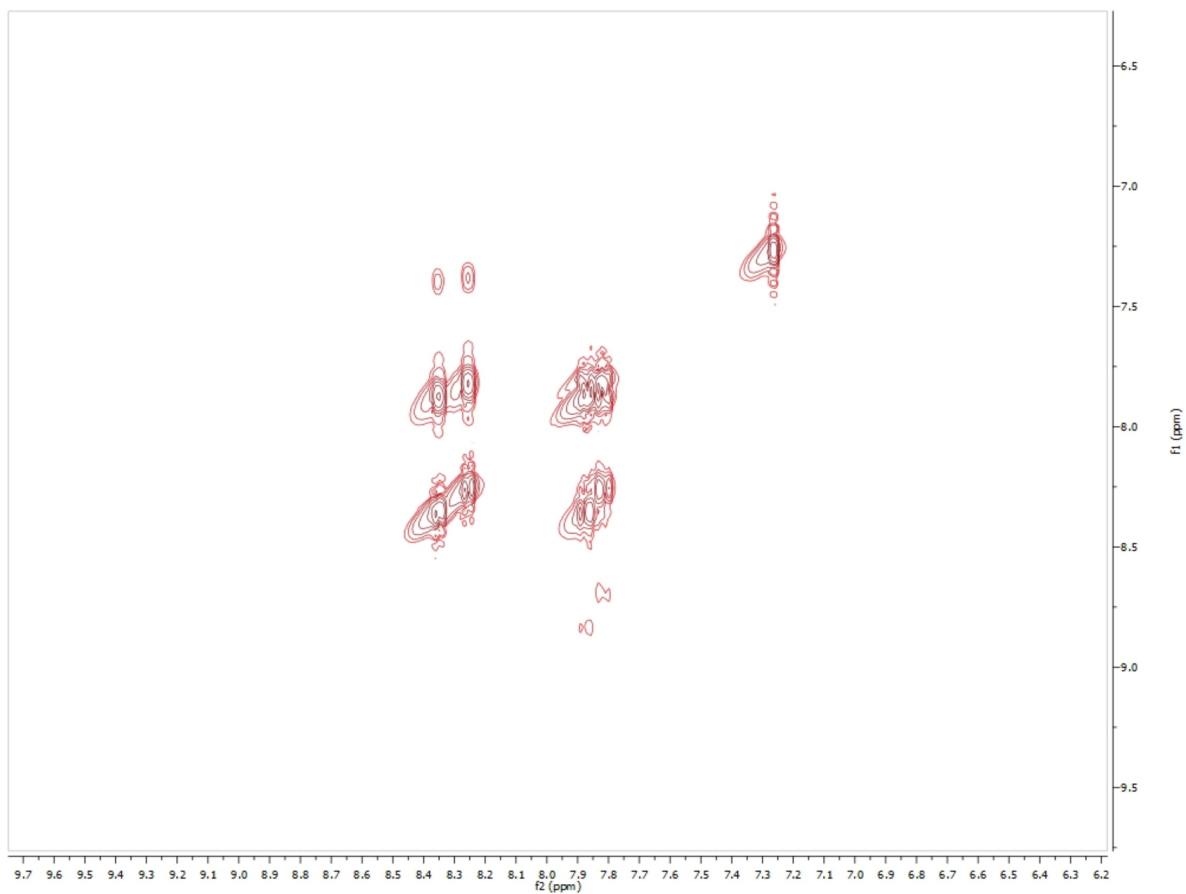
cell line	IC ₅₀ values (μM) after treatment for 72 h				
	1	2	10	17	18
Malme-3M	0.03 \pm 0.01	0.02 \pm 0.01	0.02 \pm 0.01	0.18 \pm 0.09	0.03 \pm 0.01
SW-620	0.23 \pm 0.23	–	0.36 \pm 0.04	1.68 \pm 0.24	0.05 \pm 0.01
Colo 205	1.03 \pm 0.14	–	0.63 \pm 0.07	2.42 \pm 0.64	0.04 \pm 0.01
WIDR	0.72 \pm 0.46	–	–	1.85 \pm 1.12	0.04 \pm 0.01
HCT-116	0.99 \pm 0.19	0.36 \pm 0.03	0.37 \pm 0.12	2.90 \pm 0.54	0.84 \pm 0.08
OVCAR-8	0.58 \pm 0.12	1.21 \pm 0.30	0.36 \pm 0.22	3.34 \pm 0.51	0.68 \pm 0.18
SF-295	0.28 \pm 0.08	1.01 \pm 0.25	0.11 \pm 0.05	0.74 \pm 0.12	0.21 \pm 0.03
HL-60	0.94 \pm 0.04	0.71 \pm 0.16	0.41 \pm 0.12	2.79 \pm 0.32	0.46 \pm 0.16
PC-3M	2.07 \pm 1.04	2.12 \pm 0.33	–	–	–

Table S2 | Cell selectivity and bioactivity remains consistent over a panel of derivatives. Structures appear in Fig. 1. Malme-3M, HCT-116 and PC-3M cell lines were used for the biological studies. These data along with the NCI screening data (see pages S34-S51) define the melanoma selectivity described herein.

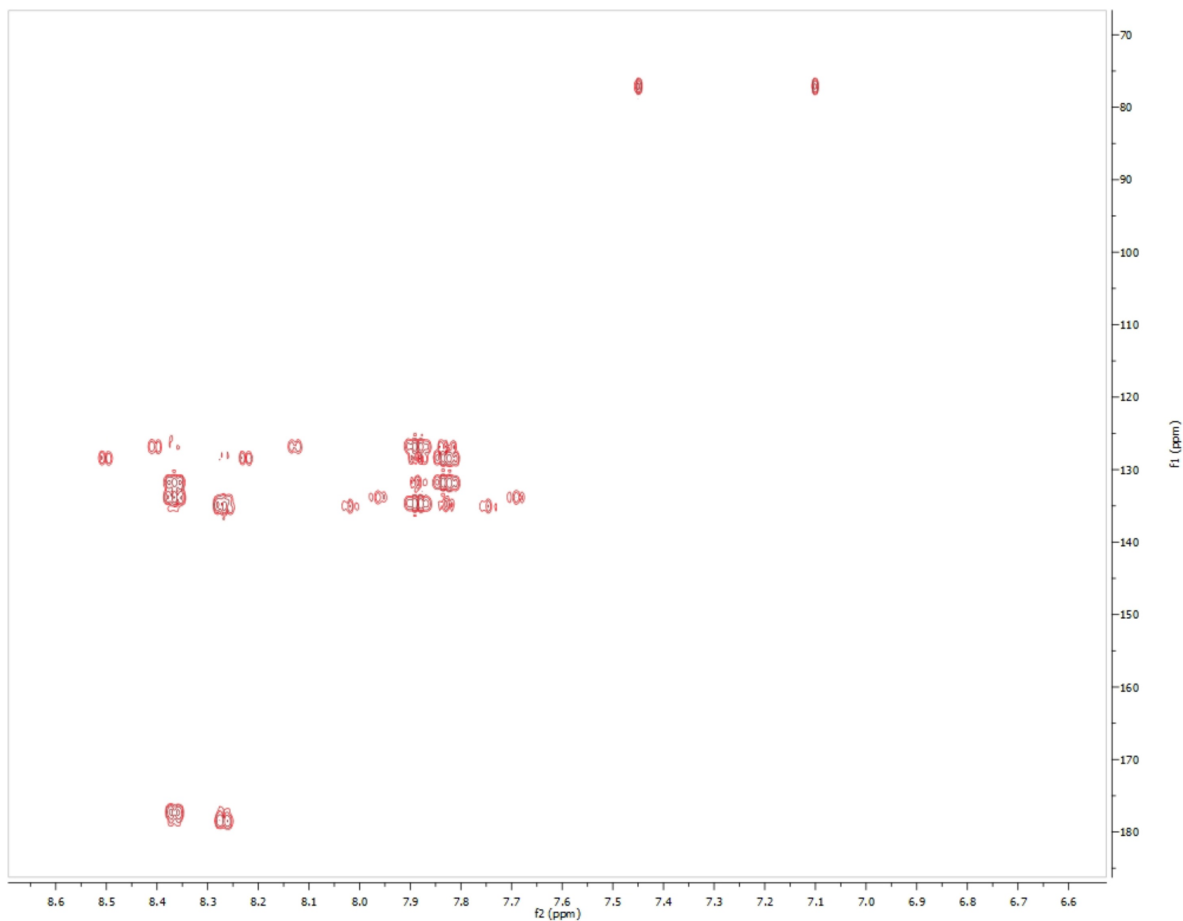
^1H -NMR (500 MHz) and ^{13}C -NMR (125 MHz) spectra of seriniquinone **1** in CDCl_3



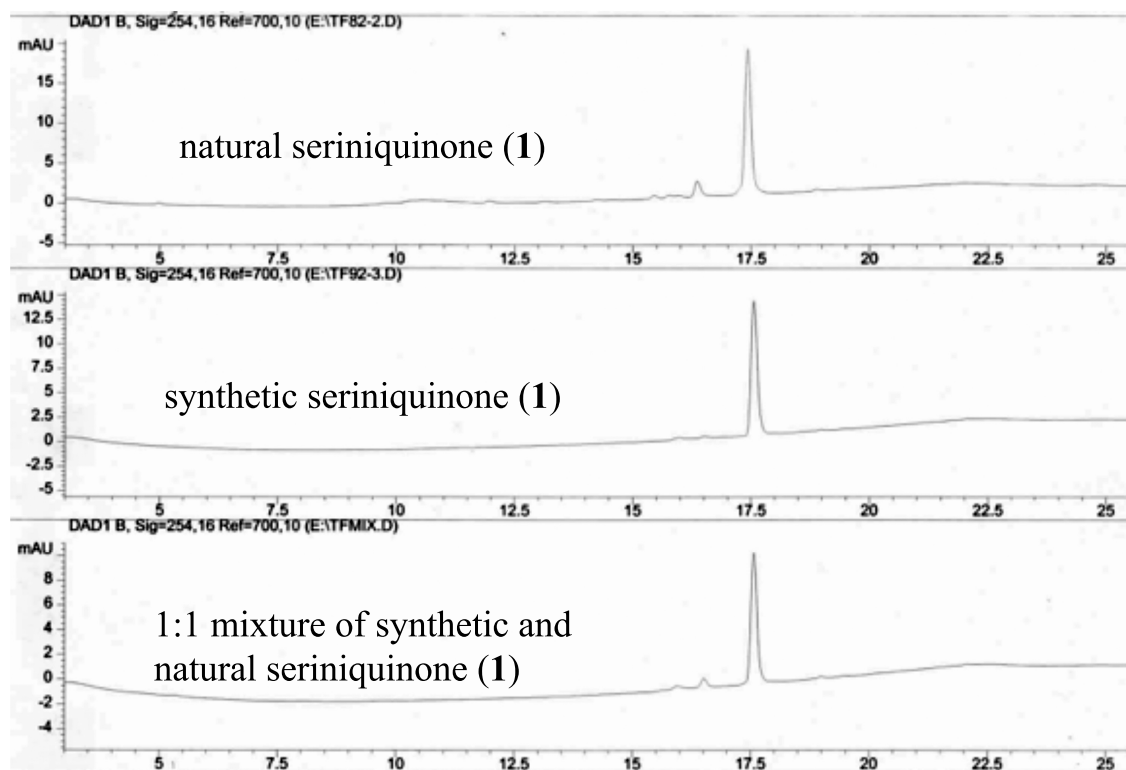
^1H , ^1H -gCOSY (500 MHz) and ^1H , ^{13}C -HSQC (125 MHz) spectra of seriniquinone **1** in CDCl_3



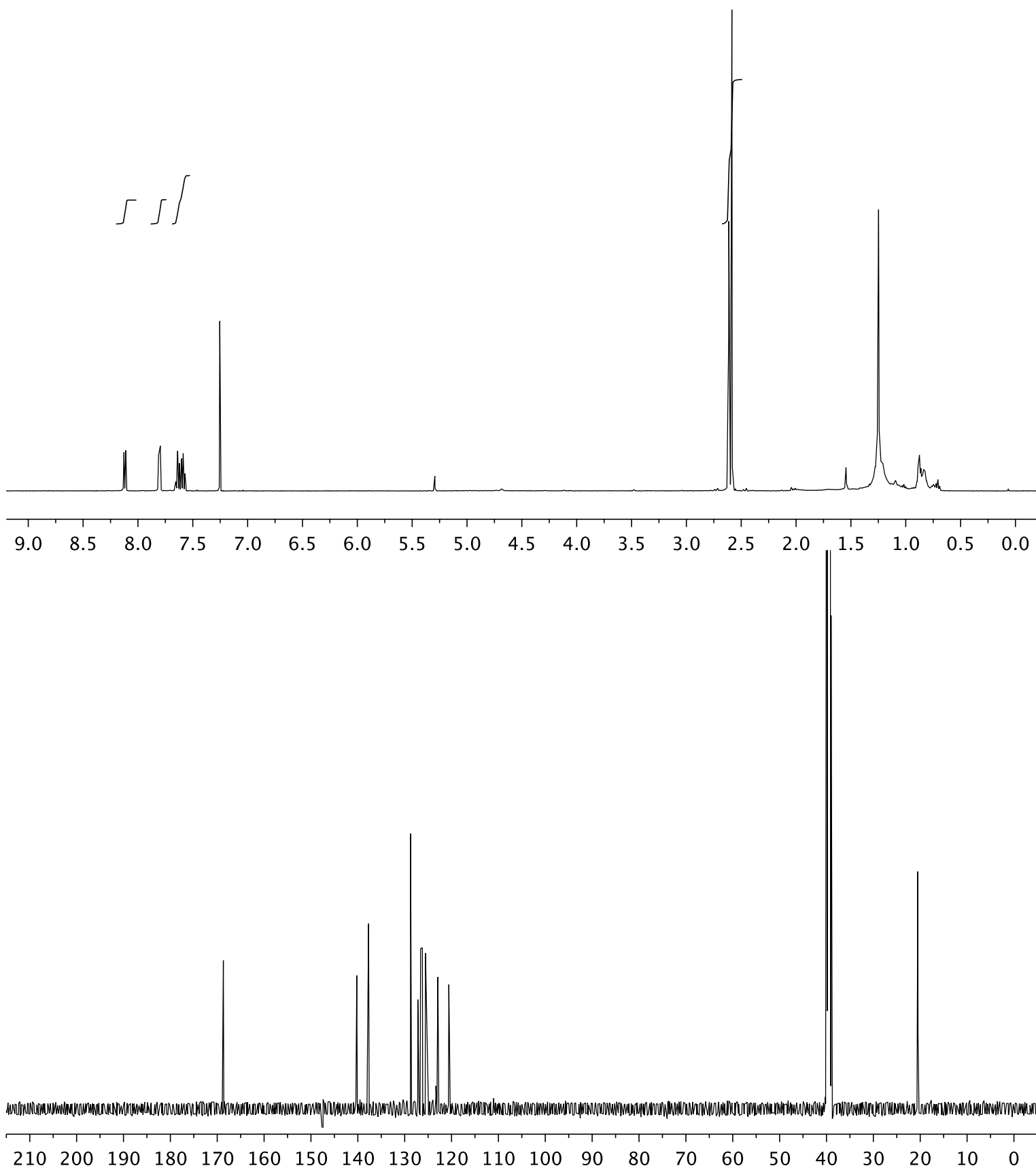
^1H , ^{13}C -HSQC (400 MHz) spectra of seriniquinone **1** in CDCl_3



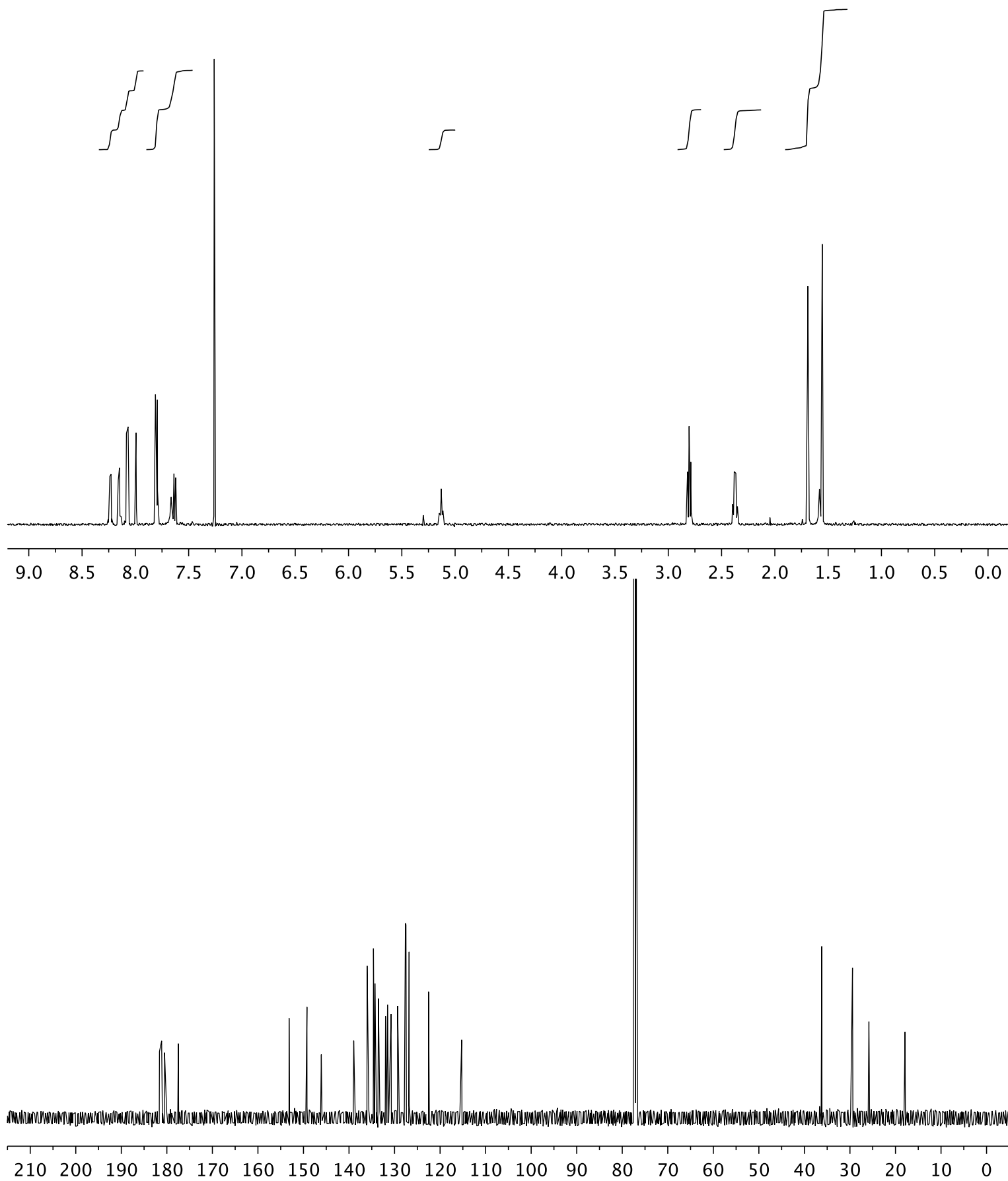
HPLC evaluation of synthetic and natural seriniquinone (**1**)



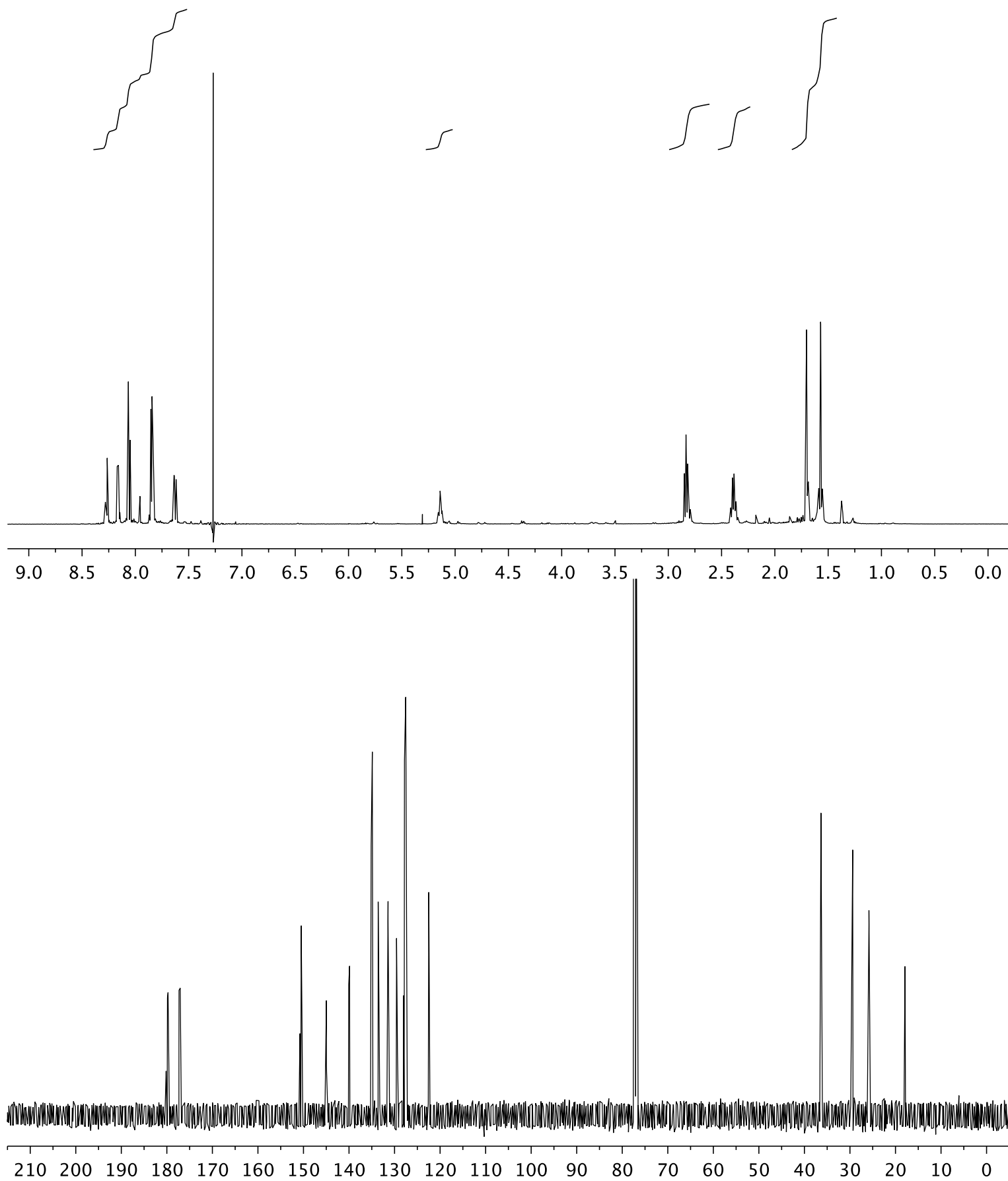
^1H -NMR (500 MHz) and ^{13}C -NMR (125 MHz) spectra of tetracetate **2** in CDCl_3



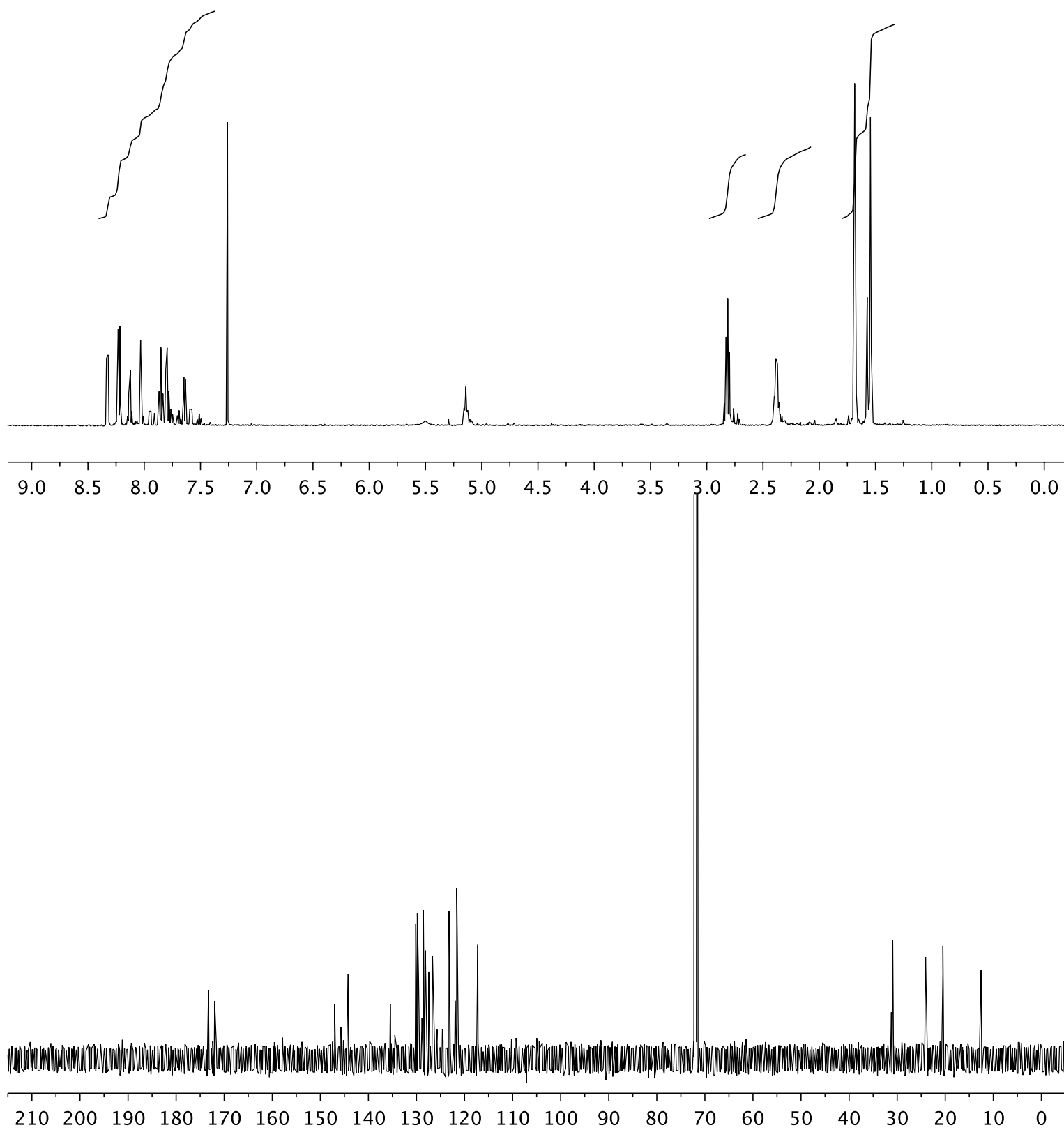
^1H -NMR (500 MHz) and ^{13}C -NMR (125 MHz) spectra of adduct **7** in CDCl_3



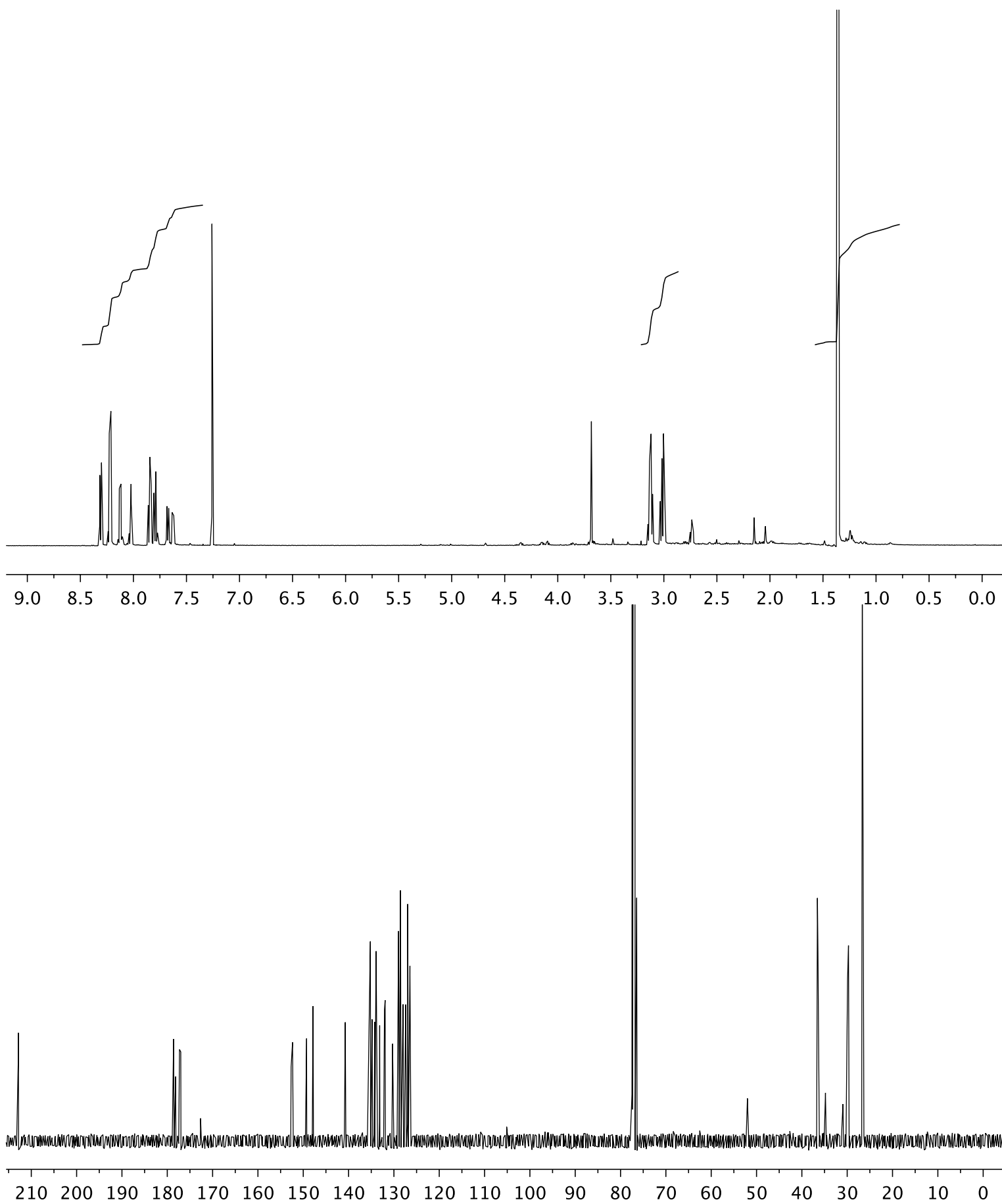
^1H -NMR (500 MHz) and ^{13}C -NMR (125 MHz) spectra of dichloride **8** in CDCl_3



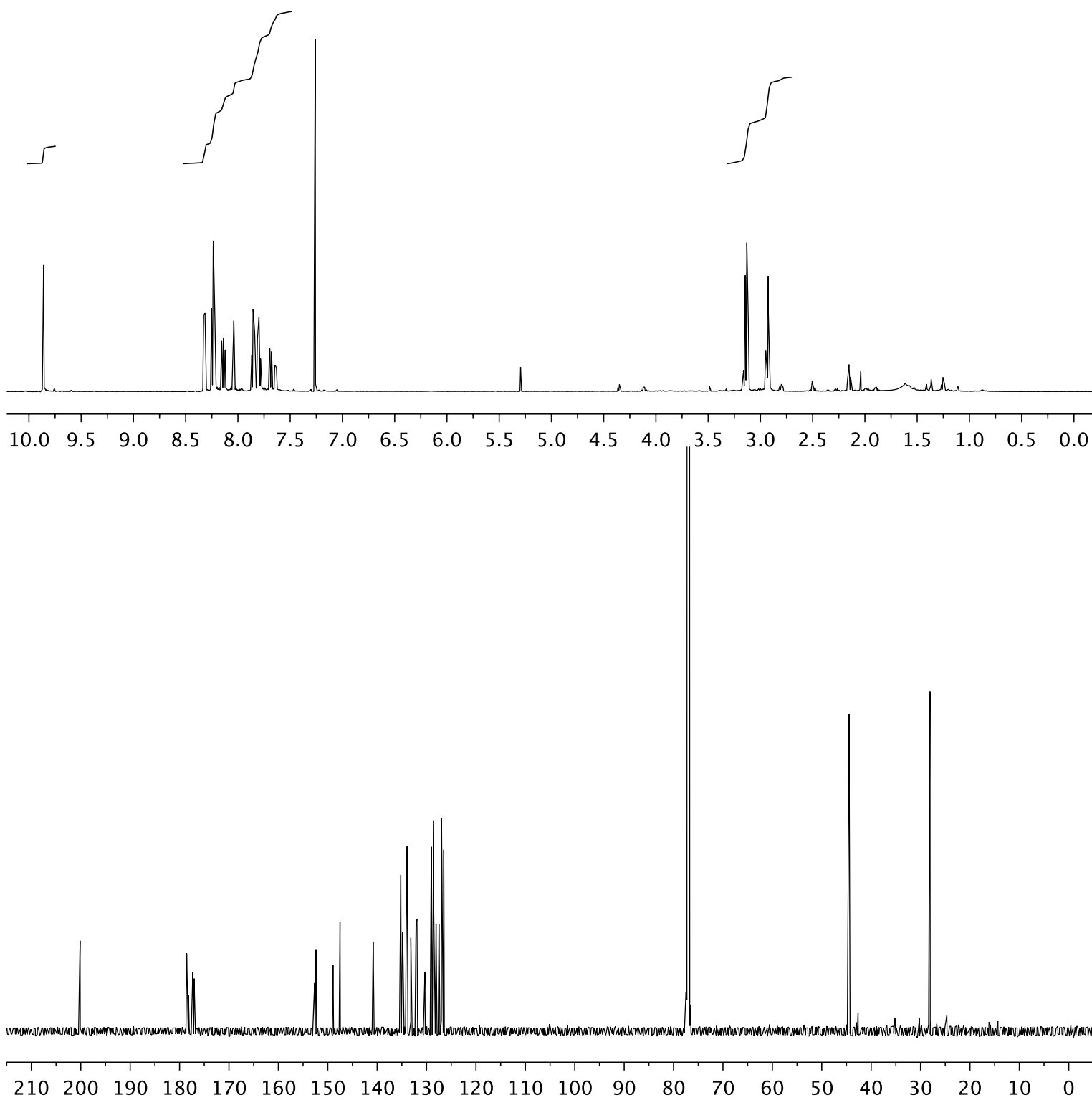
^1H -NMR (500 MHz) and ^{13}C -NMR (125 MHz) spectra of alkene **9** in CDCl_3



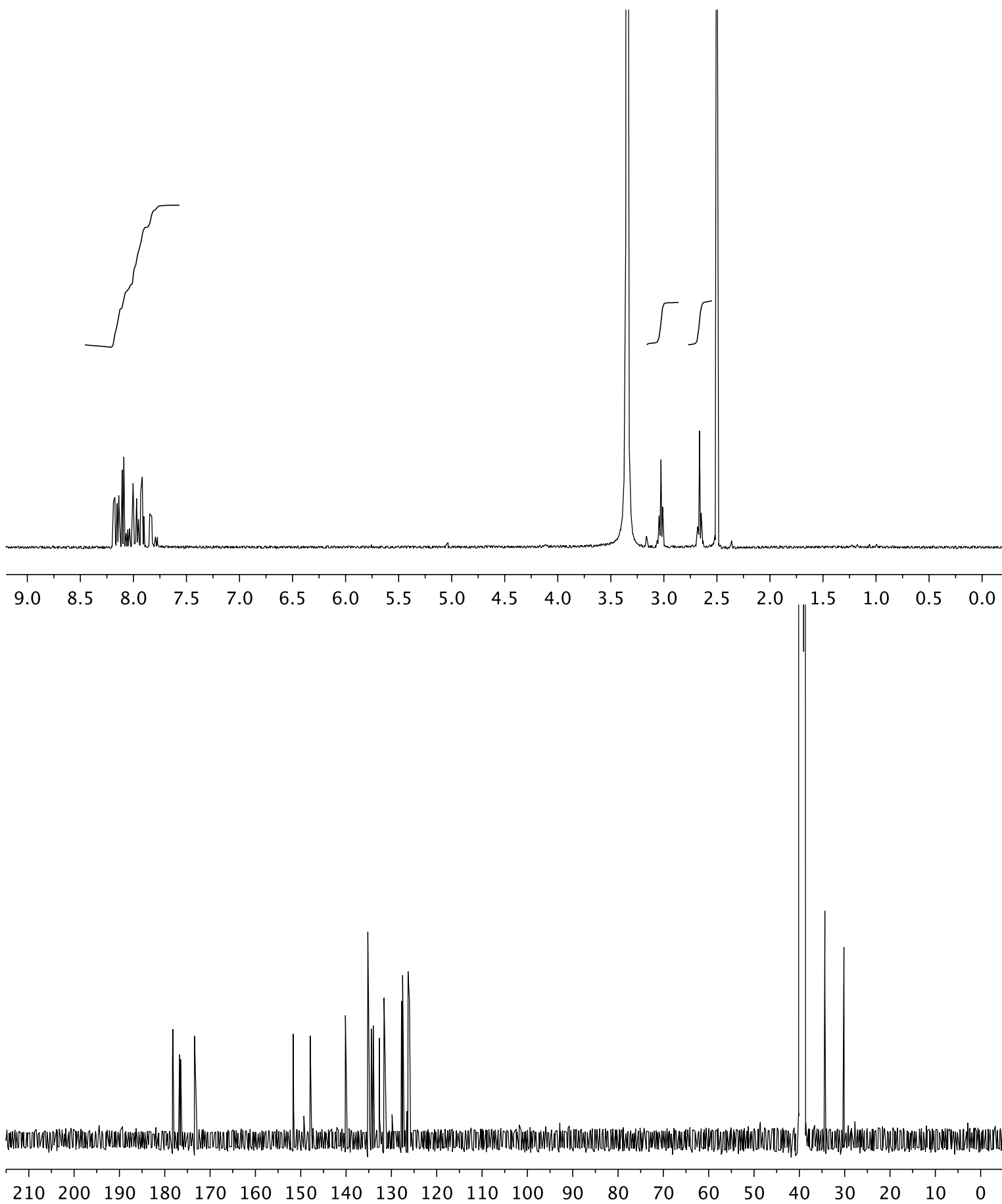
^1H -NMR (500 MHz) and ^{13}C -NMR (125 MHz) spectra of diol **10** in CDCl_3



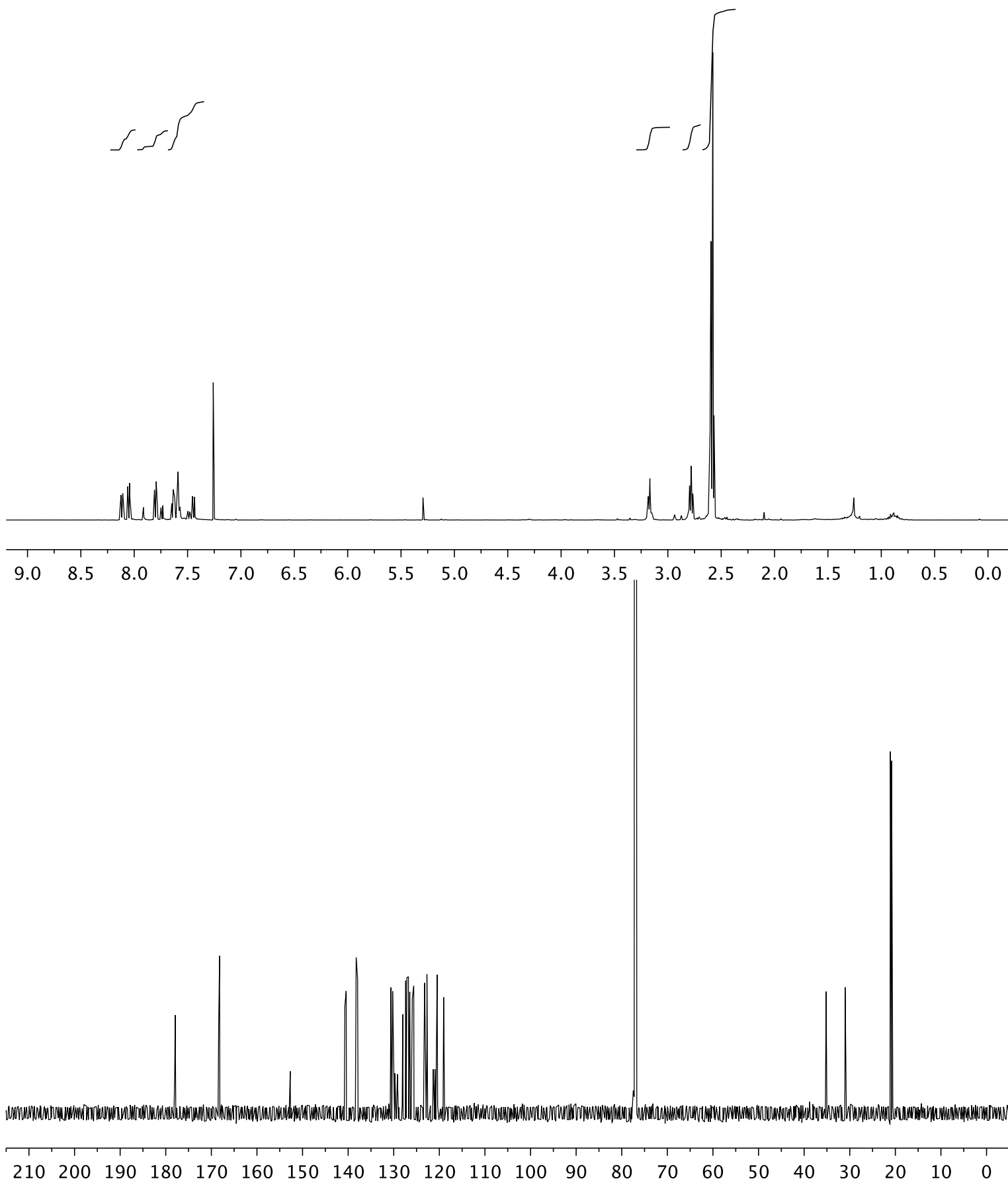
^1H -NMR (500 MHz) and ^{13}C -NMR (125 MHz) spectra of aldehyde **11** in CDCl_3



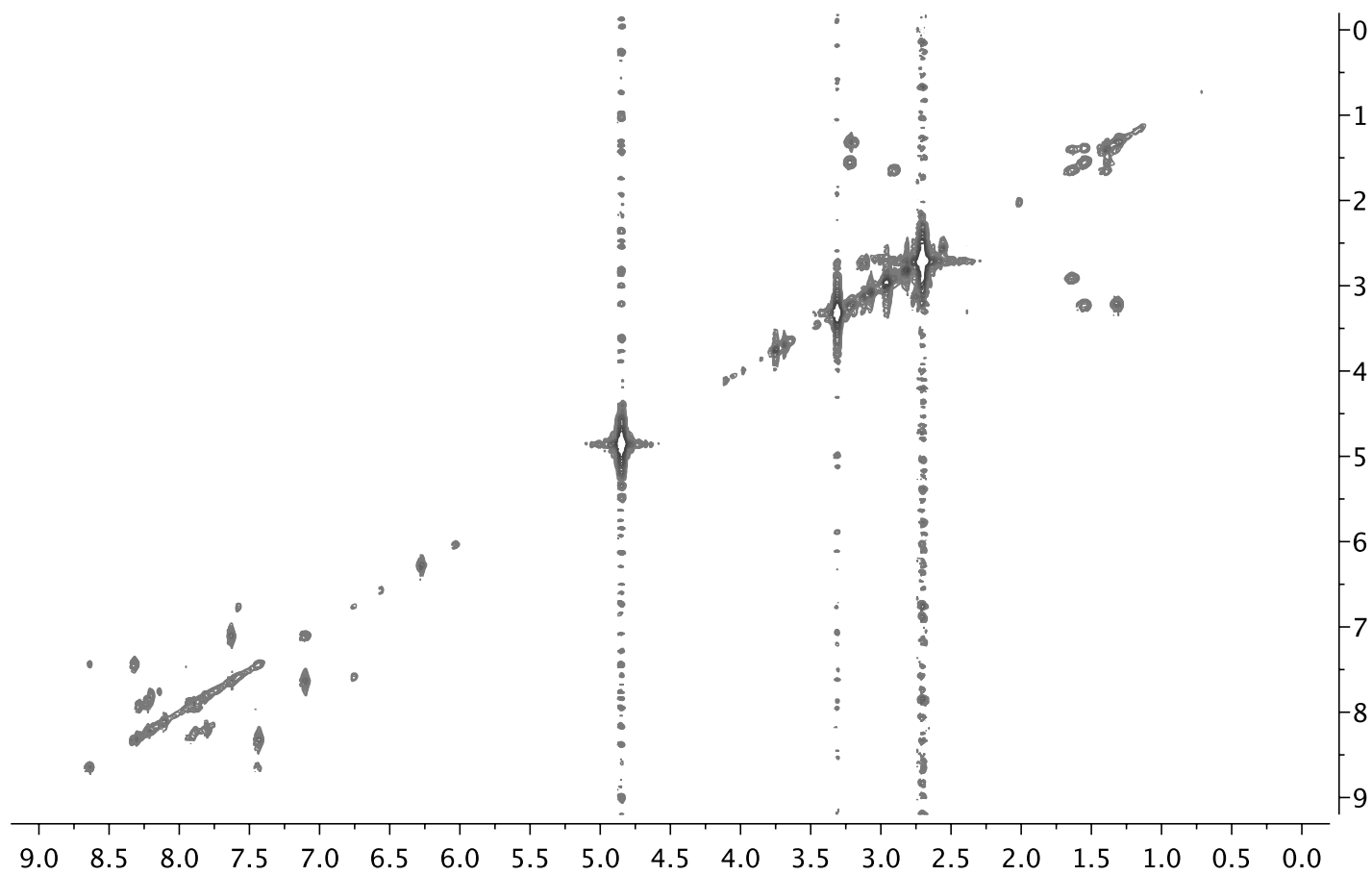
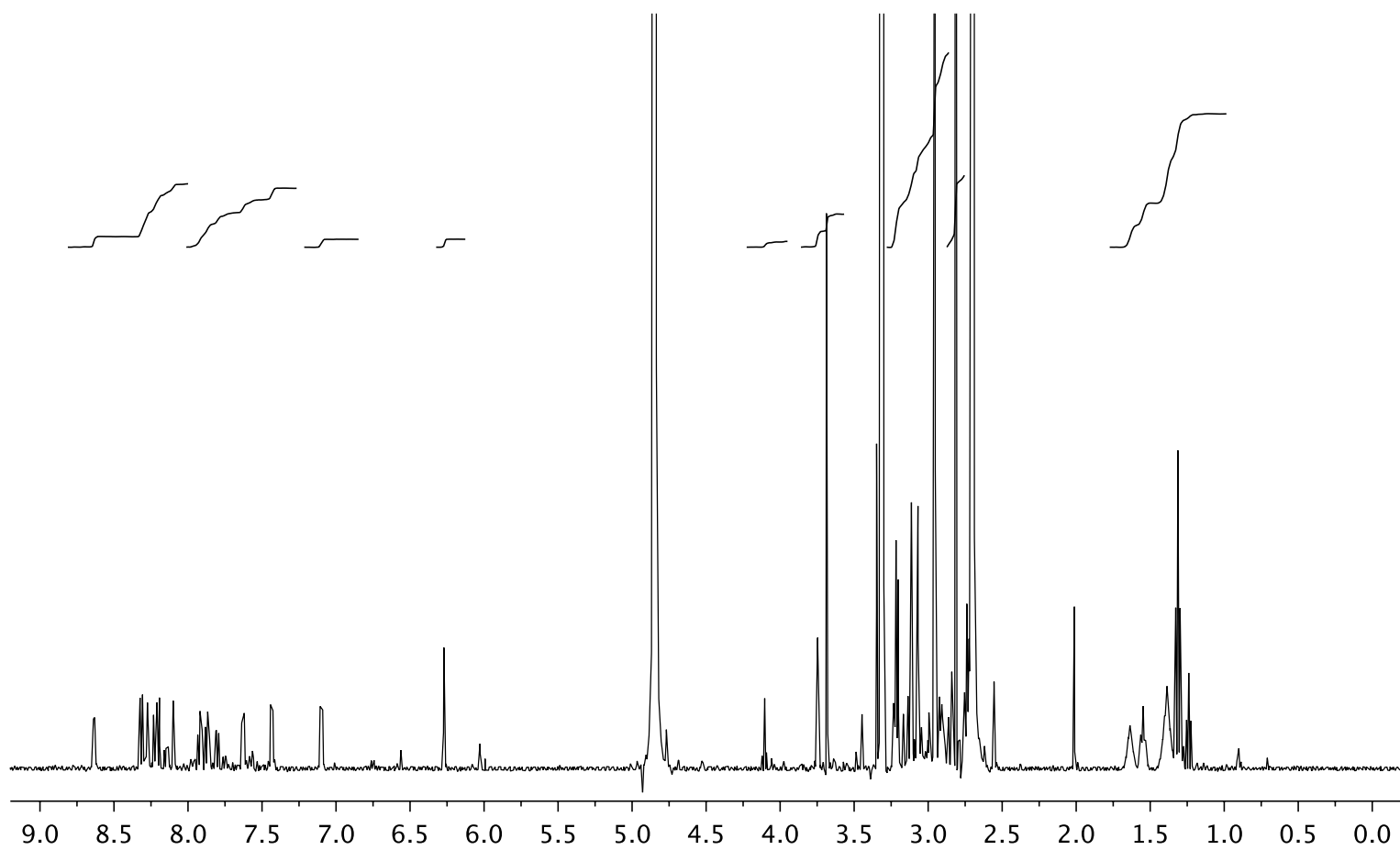
^1H -NMR (500 MHz) and ^{13}C -NMR (125 MHz) spectra of acid **12** in DMSO-d_6



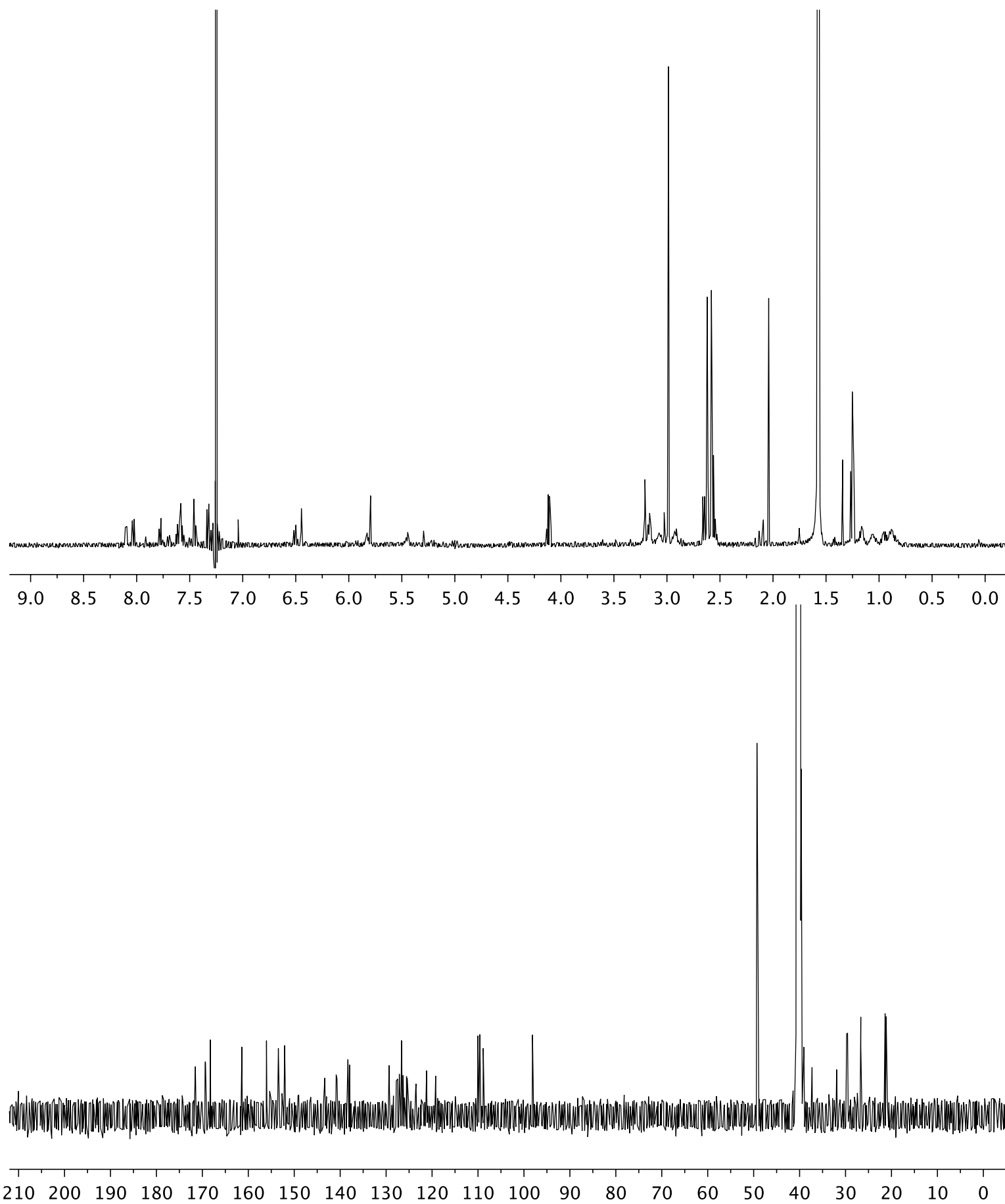
$^1\text{H-NMR}$ (500 MHz) and $^{13}\text{C-NMR}$ (125 MHz) spectra of tetraacetateacid **13** in CDCl_3



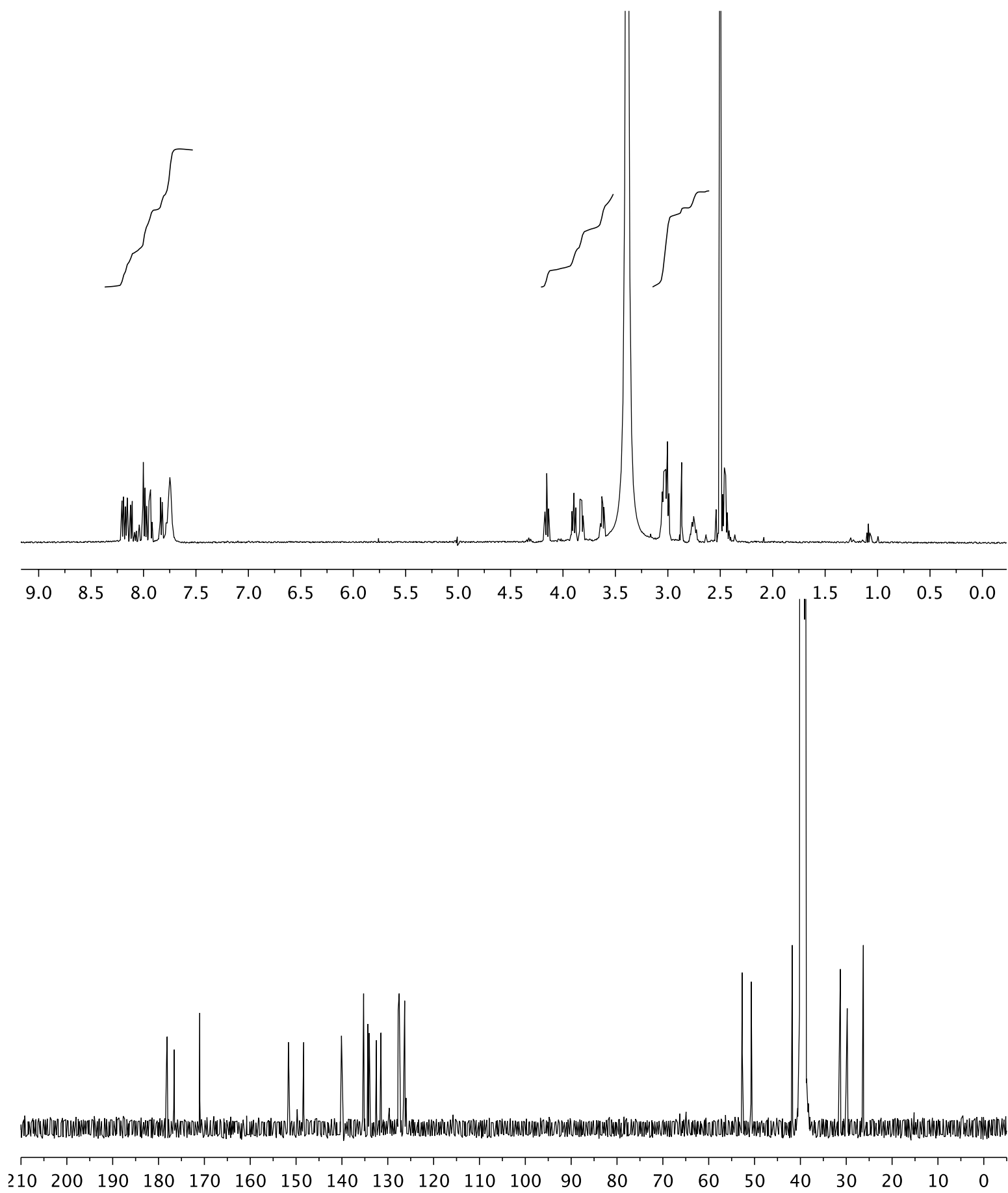
$^1\text{H-NMR}$ (500 MHz) and $^1\text{H-gCOSY}$ (500 MHz) spectra of probe **15** in CD_3OD



^1H -NMR (500 MHz) and ^{13}C -NMR (125 MHz) spectra of probe **16** in CDCl_3

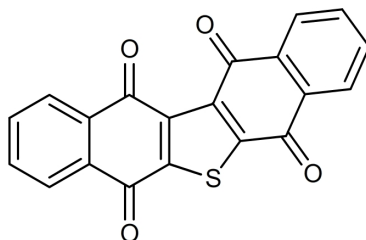


^1H -NMR (500 MHz) and ^{13}C -NMR (125 MHz) spectra of amide **17** in $\text{DMSO-}d_6$

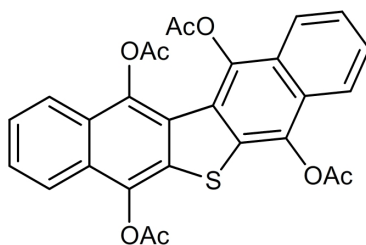


Copies of selected NCI60 analyses.

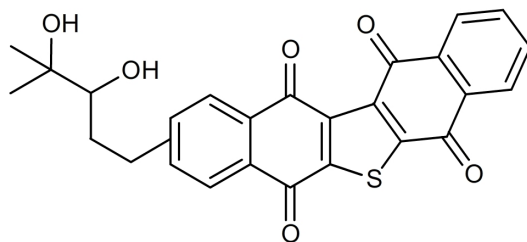
NCI code numbers are provided for data sets collected from compounds **1, 2, 10, 17, 18**.



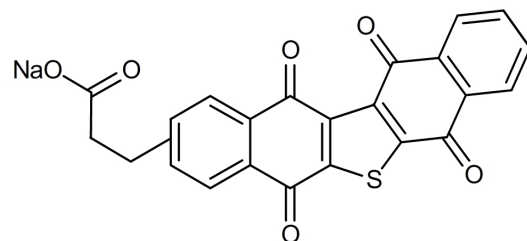
1 S747855



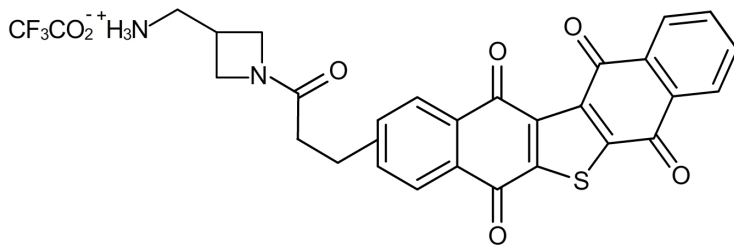
2 S774837



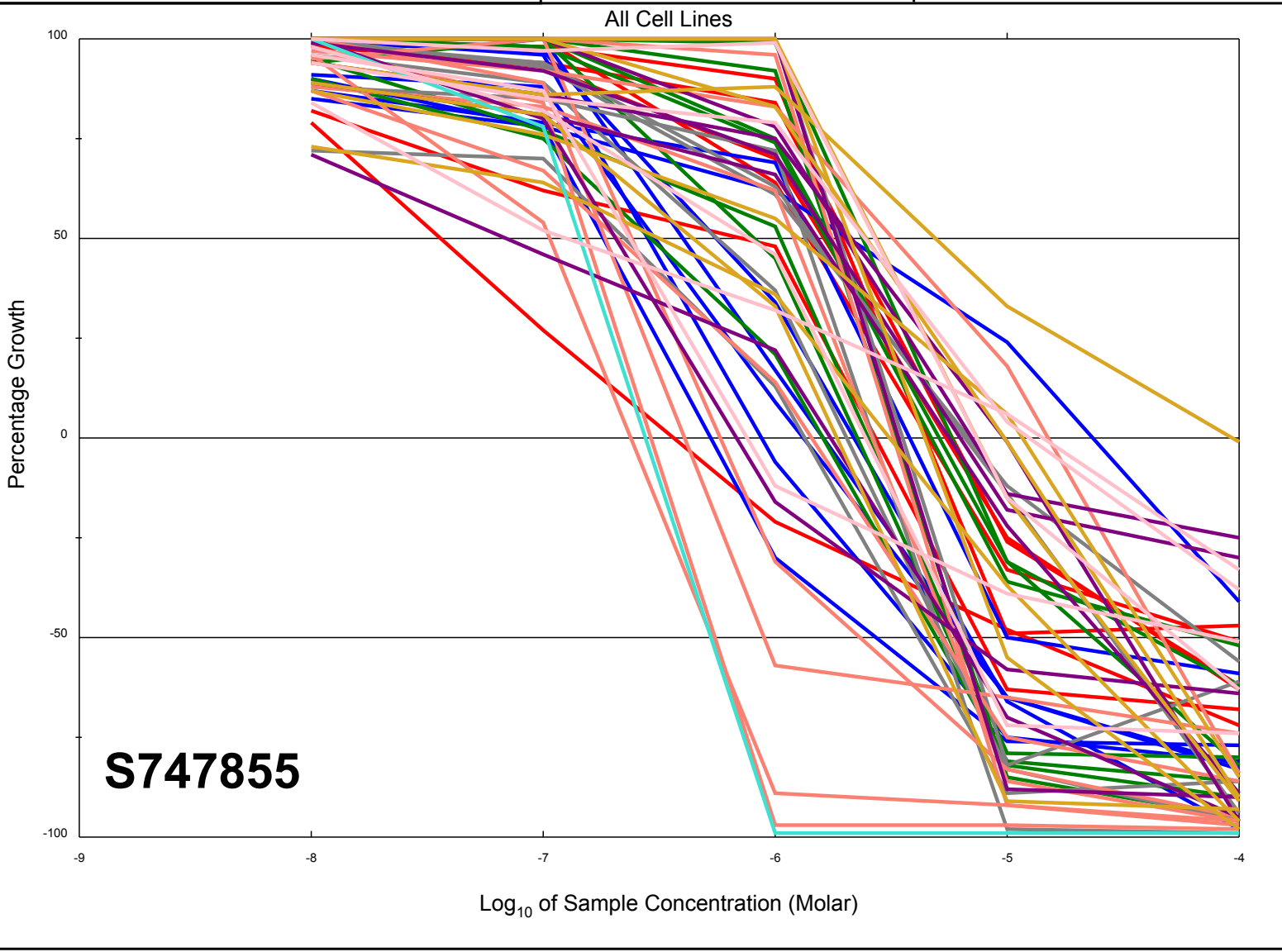
10 D-778345

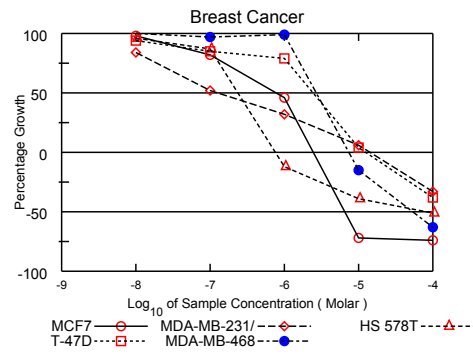
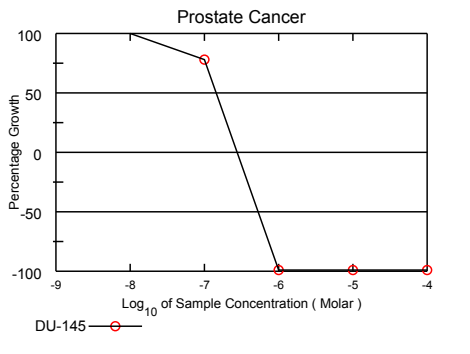
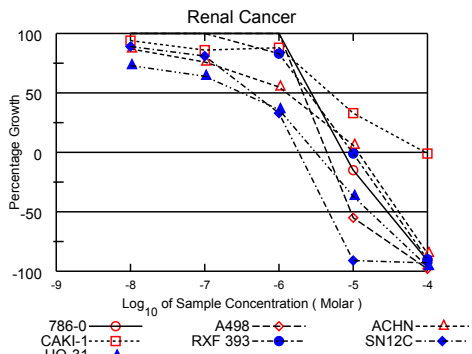
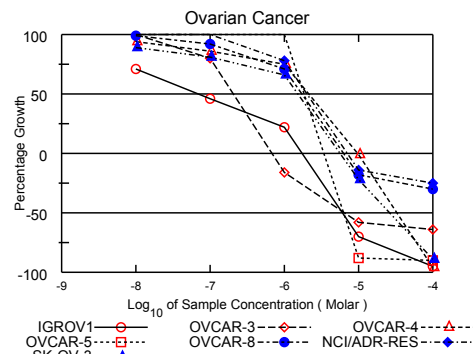
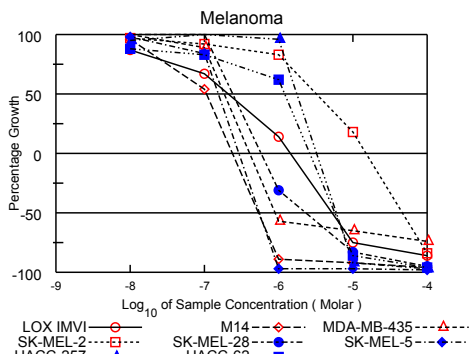
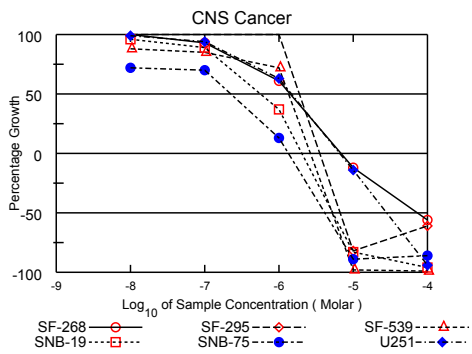
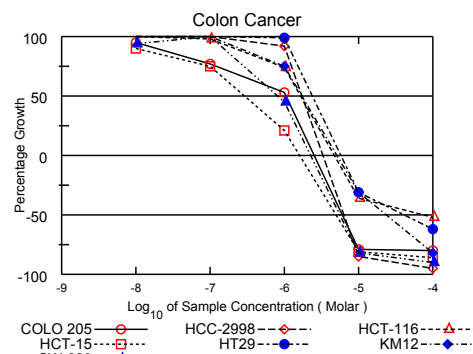
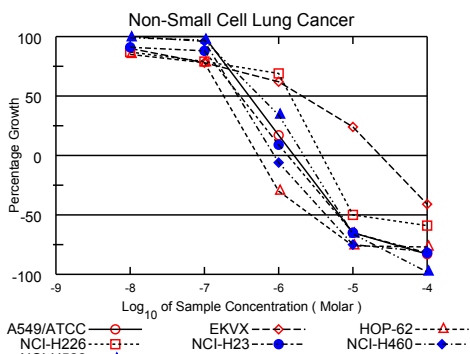
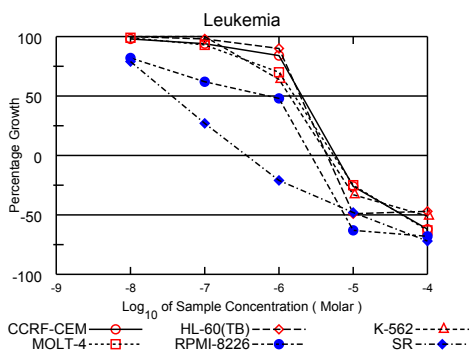


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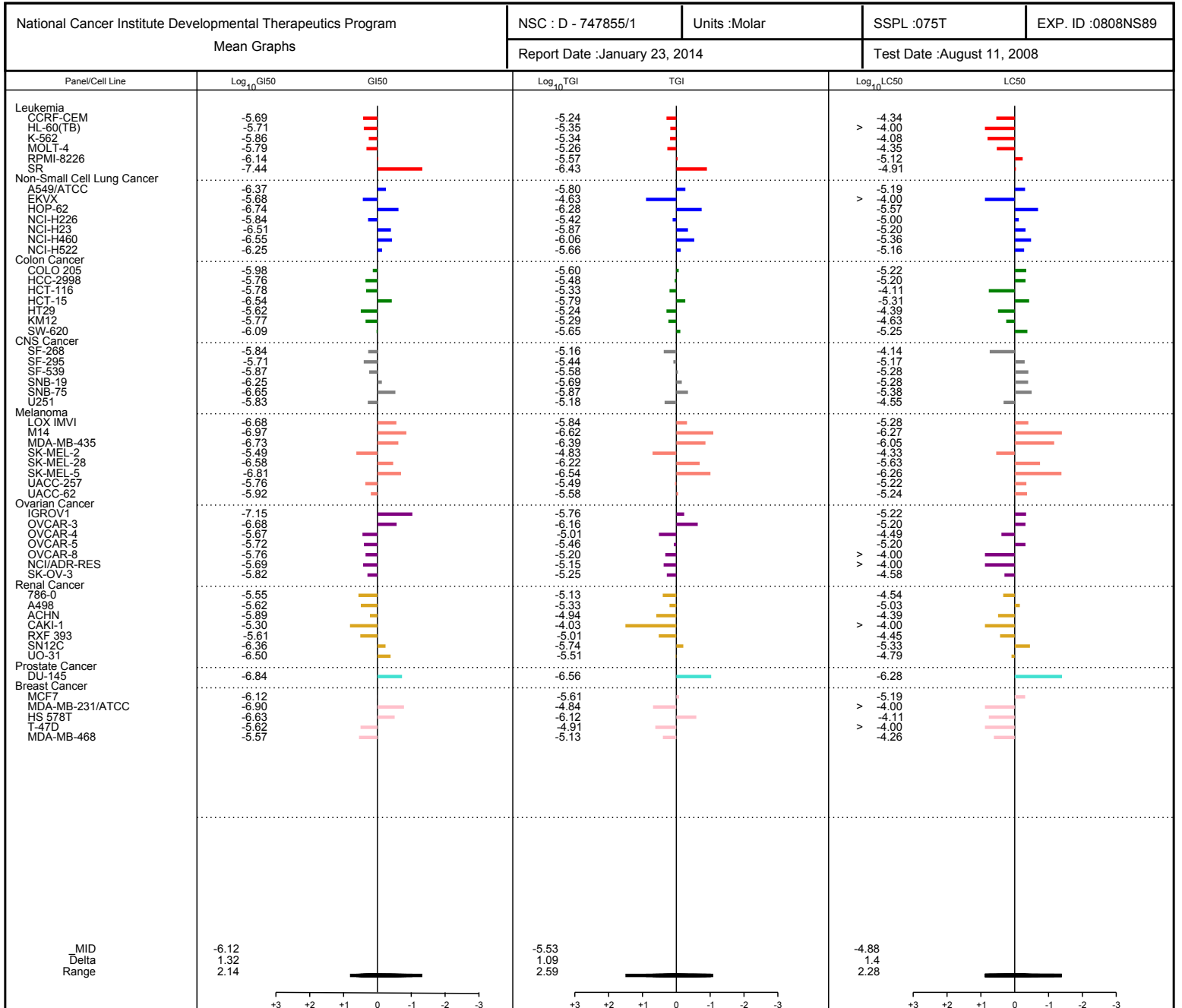


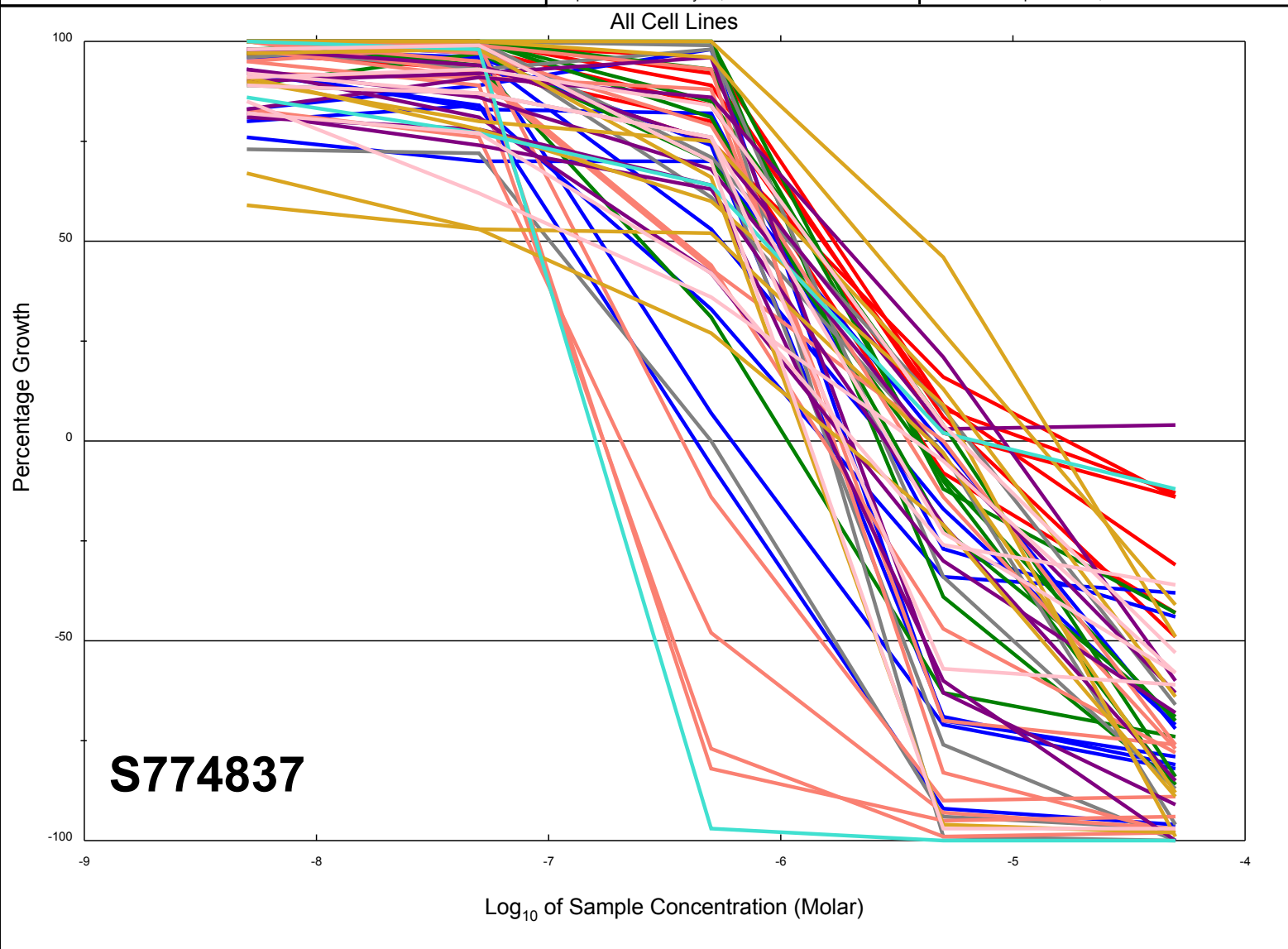


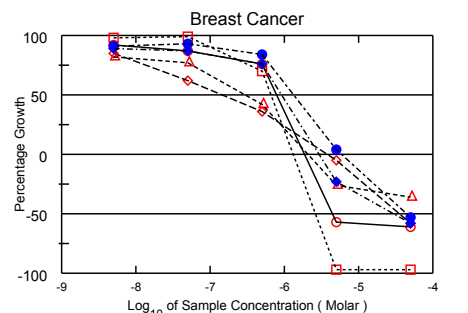
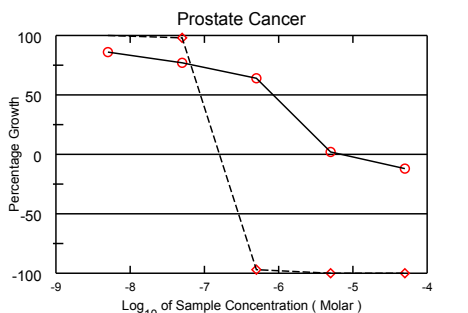
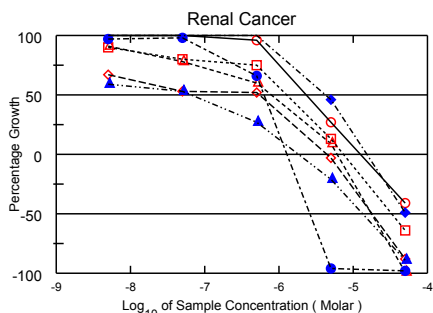
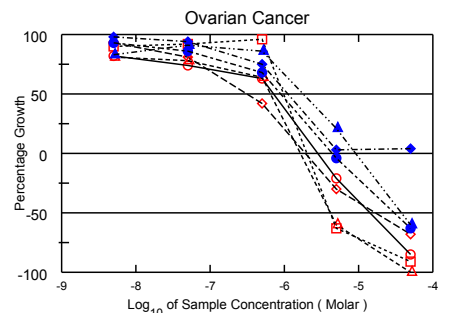
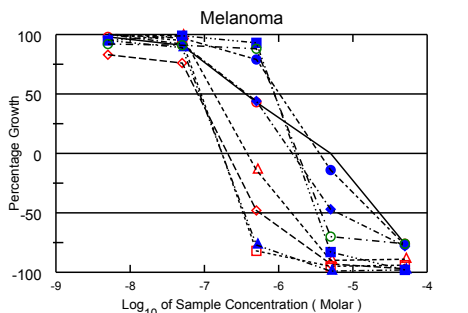
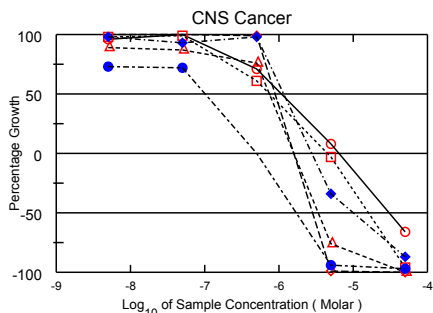
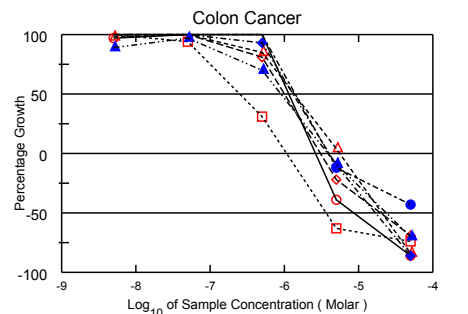
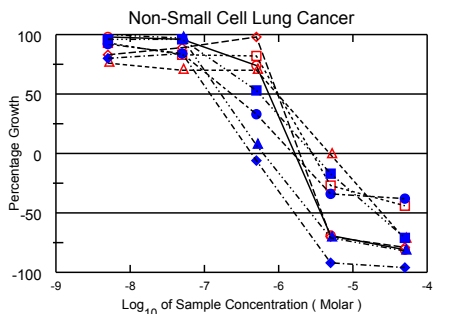
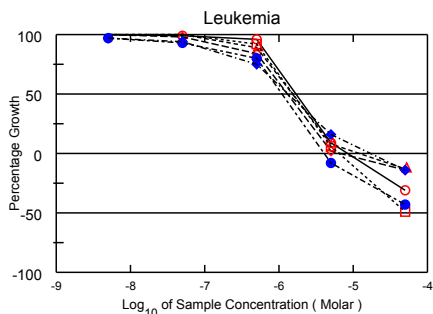
National Cancer Institute Developmental Therapeutics Program In-Vitro Testing Results

NSC : D - 747855 / 1	Experiment ID : 0808NS89	Test Type : 08	Units : Molar
Report Date : January 23, 2014	Test Date : August 11, 2008	QNS :	MC :
COMI : CNJ927.344 (73141)	Stain Reagent : SRB Dual-Pass Related	SSPL : 075T	

Panel/Cell Line	Log10 Concentration																
	Time		Mean Optical Densities						Percent Growth						GI50	TGI	LC50
	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0					
Leukemia																	
CCRF-CEM	0.341	1.192	1.178	1.139	1.053	0.252	0.130	98	94	84	-26	-62	2.03E-6	5.77E-6	4.61E-5		
HL-60(TB)	0.908	2.546	2.546	2.521	2.376	0.467	0.480	100	98	90	-49	-47	1.93E-6	4.45E-6	> 1.00E-4		
K-562	0.330	1.904	1.940	1.904	1.333	0.220	0.161	102	100	64	-33	-51	1.38E-6	4.52E-6	8.39E-5		
MOLT-4	0.615	1.972	1.964	1.873	1.562	0.462	0.225	99	93	70	-25	-63	1.62E-6	5.45E-6	4.46E-5		
RPMI-8226	0.553	1.335	1.197	1.041	0.928	0.206	0.177	82	62	48	-63	-68	7.22E-7	2.71E-6	7.67E-6		
SR	0.528	0.779	0.726	0.597	0.418	0.275	0.147	79	27	-21	-48	-72	3.62E-8	3.69E-7	1.22E-5		
Non-Small Cell Lung Cancer																	
A549/ATCC	0.190	0.916	0.925	0.971	0.311	0.066	0.032	101	108	17	-65	-83	4.29E-7	1.60E-6	6.51E-6		
EKVX	1.107	2.497	2.362	2.189	1.972	1.435	0.655	90	78	62	24	-41	2.07E-6	2.32E-5	> 1.00E-4		
HOP-62	0.757	1.484	1.375	1.328	0.529	0.180	0.176	85	78	-30	-76	-77	1.83E-7	5.28E-7	2.70E-6		
NCI-H226	0.785	1.627	1.519	1.449	1.368	0.392	0.323	87	79	69	-50	-59	1.45E-6	3.80E-6	9.98E-6		
NCI-H23	0.585	2.019	1.889	1.851	0.720	0.206	0.107	91	88	9	-65	-82	3.06E-7	1.34E-6	6.31E-6		
NCI-H460	0.283	1.899	1.897	1.839	0.266	0.071	0.053	100	96	-6	-75	-81	2.84E-7	8.74E-7	4.33E-6		
NCI-H522	1.232	2.327	2.315	2.299	1.603	0.423	0.020	99	97	34	-66	-98	5.57E-7	2.19E-6	6.95E-6		
Colon Cancer																	
COLO 205	0.277	0.770	0.743	0.655	0.538	0.058	0.056	95	77	53	-79	-80	1.05E-6	2.51E-6	6.01E-6		
HCC-2998	0.787	2.082	2.141	2.129	1.979	0.115	0.040	105	104	92	-85	-95	1.72E-6	3.30E-6	6.31E-6		
HCT-116	0.255	1.626	1.652	1.599	1.272	0.163	0.123	102	98	74	-36	-52	1.66E-6	4.70E-6	7.69E-5		
HCT-15	0.193	1.248	1.138	0.981	0.416	0.036	0.028	90	75	21	-81	-86	2.88E-7	1.61E-6	4.94E-6		
HT29	0.162	1.035	1.060	1.031	1.024	0.112	0.062	103	100	99	-31	-62	2.38E-6	5.78E-6	4.11E-5		
KM12	0.251	1.042	1.074	1.045	0.844	0.173	0.046	104	100	75	-31	-82	1.72E-6	5.08E-6	2.35E-5		
SW-620	0.199	1.134	1.082	1.156	0.619	0.035	0.021	94	102	45	-82	-90	8.14E-7	2.25E-6	5.56E-6		
CNS Cancer																	
SF-268	0.300	0.906	0.925	0.866	0.673	0.266	0.131	103	93	61	-12	-56	1.44E-6	6.96E-6	7.22E-5		
SF-295	1.032	2.686	2.796	2.714	2.749	0.181	0.403	107	102	104	-82	-61	1.95E-6	3.61E-6	6.70E-6		
SF-539	0.752	1.903	1.763	1.730	1.580	0.015	0.008	88	85	72	-98	-99	1.35E-6	2.65E-6	5.22E-6		
SNB-19	0.524	1.385	1.354	1.288	0.845	0.087	0.021	96	89	37	-83	-96	5.67E-7	2.04E-6	5.29E-6		
SNB-75	0.605	1.105	0.966	0.957	0.669	0.070	0.083	72	70	13	-89	-86	2.26E-7	1.34E-6	4.17E-6		
U251	0.248	1.235	1.226	1.180	0.871	0.213	0.015	99	94	63	-14	-94	1.48E-6	6.56E-6	2.82E-5		
Melanoma																	
LOX IMVI	0.296	1.831	1.630	1.330	0.509	0.074	0.042	87	67	14	-75	-86	2.11E-7	1.43E-6	5.22E-6		
M14	0.331	1.074	1.041	0.735	0.036	0.025	0.012	96	54	-89	-92	-96	1.07E-7	2.39E-7	5.33E-7		
MDA-MB-435	0.523	2.014	2.103	1.844	0.227	0.183	0.139	106	89	-57	-65	-74	1.84E-7	4.07E-7	8.99E-7		
SK-MEL-2	1.221	2.307	2.270	2.224	2.126	1.413	0.198	97	92	83	18	-84	3.22E-6	1.49E-5	4.64E-5		
SK-MEL-28	0.345	0.857	0.922	0.899	0.240	0.060	0.017	113	108	-31	-83	-95	2.63E-7	6.02E-7	2.36E-6		
SK-MEL-5	0.622	2.309	2.276	2.035	0.017	0.018	0.011	98	84	-97	-97	-98	1.54E-7	2.90E-7	5.48E-7		
UACC-257	0.958	1.614	1.581	1.632	1.585	0.076	0.028	95	103	96	-92	-97	1.75E-6	3.23E-6	5.97E-6		
UACC-62	0.418	1.446	1.320	1.275	1.052	0.059	0.017	88	83	62	-86	-96	1.20E-6	2.62E-6	5.71E-6		
Ovarian Cancer																	
IGROV1	0.326	0.912	0.742	0.598	0.454	0.099	0.016	71	46	22	-70	-95	7.13E-8	1.73E-6	6.08E-6		
OVCAR-3	0.359	0.878	0.890	0.777	0.303	0.149	0.131	102	80	-16	-58	-64	2.07E-7	6.88E-7	6.34E-6		
OVCAR-4	0.491	1.373	1.321	1.252	1.151	0.486	0.018	94	86	75	-1	-96	2.12E-6	9.67E-6	3.26E-5		
OVCAR-5	0.492	1.145	1.160	1.162	1.175	0.059	0.047	102	103	105	-88	-90	1.92E-6	3.49E-6	6.34E-6		
OVCAR-8	0.509	1.808	1.799	1.702	1.431	0.417	0.357	99	92	71	-18	-30	1.72E-6	6.27E-6	> 1.00E-4		
NCI/ADR-RES	0.588	1.645	1.663	1.643	1.418	0.504	0.441	102	100	78	-14	-25	2.03E-6	7.01E-6	> 1.00E-4		
SK-OV-3	0.563	1.064	1.007	0.969	0.892	0.441	0.064	89	81	66	-22	-89	1.51E-6	5.65E-6	2.65E-5		
Renal Cancer																	
786-0	0.908	2.207	2.289	2.345	2.235	0.772	0.082	106	111	102	-15	-91	2.79E-6	7.44E-6	2.89E-5		
A498	0.948	1.471	1.506	1.519	1.540	0.425	0.017	107	109	113	-55	-98	2.37E-6	4.70E-6	9.31E-6		
ACHN	0.377	1.688	1.512	1.370	1.102	0.451	0.056	87	76	55	6	-85	1.28E-6	1.15E-5	4.09E-5		
CAKI-1	0.991	2.535	2.449	2.313	2.347	1.507	0.983	94	86	88	33	-1	4.96E-6	9.44E-5	> 1.00E-4		
RXF 393	0.488	0.680	0.746	0.698	0.647	0.484	0.051	134	109	83	-1	-90	2.46E-6	9.78E-6	3.58E-5		
SN12C	0.397	1.358	1.257	1.180	0.711	0.037	0.026	89	81	33	-91	-93	4.41E-7	1.84E-6	4.67E-6		
UO-31	0.411	1.053	0.879	0.821	0.641	0.258	0.016	73	64	36	-37	-96	3.13E-7	3.09E-6	1.64E-5		
Prostate Cancer																	
DU-145	0.243	0.799	0.843	0.676	0.003	0.003	0.003	108	78	-99	-99	-99	1.44E-7	2.76E-7	5.29E-7		
Breast Cancer																	
MCF7	0.216	1.001	0.988	0.857	0.576	0.060	0.056	98	82	46	-72	-74	7.63E-7	2.44E-6	6.48E-6		
MDA-MB-231/ATCC	0.493	1.124	1.025	0.821	0.695	0.533	0.330	84	52	32	6	-33	1.26E-7	1.44E-5	> 1.00E-4		
HS 578T	0.571	0.944	0.929	0.896	0.503	0.351	0.277	96	87	-12	-39	-51	2.37E-7	7.58E-7	7.68E-5		
T-47D	0.512	1.039	1.007	0.959	0.927	0.533	0.316	94	85	79	4	-38	2.42E-6	1.24E-5	> 1.00E-4		
MDA-MB-468	0.446	0.922	0.949	0.910	0.916	0.379	0.167	106	97	99	-15	-63	2.68E-6	7.38E-6	5.44E-5		



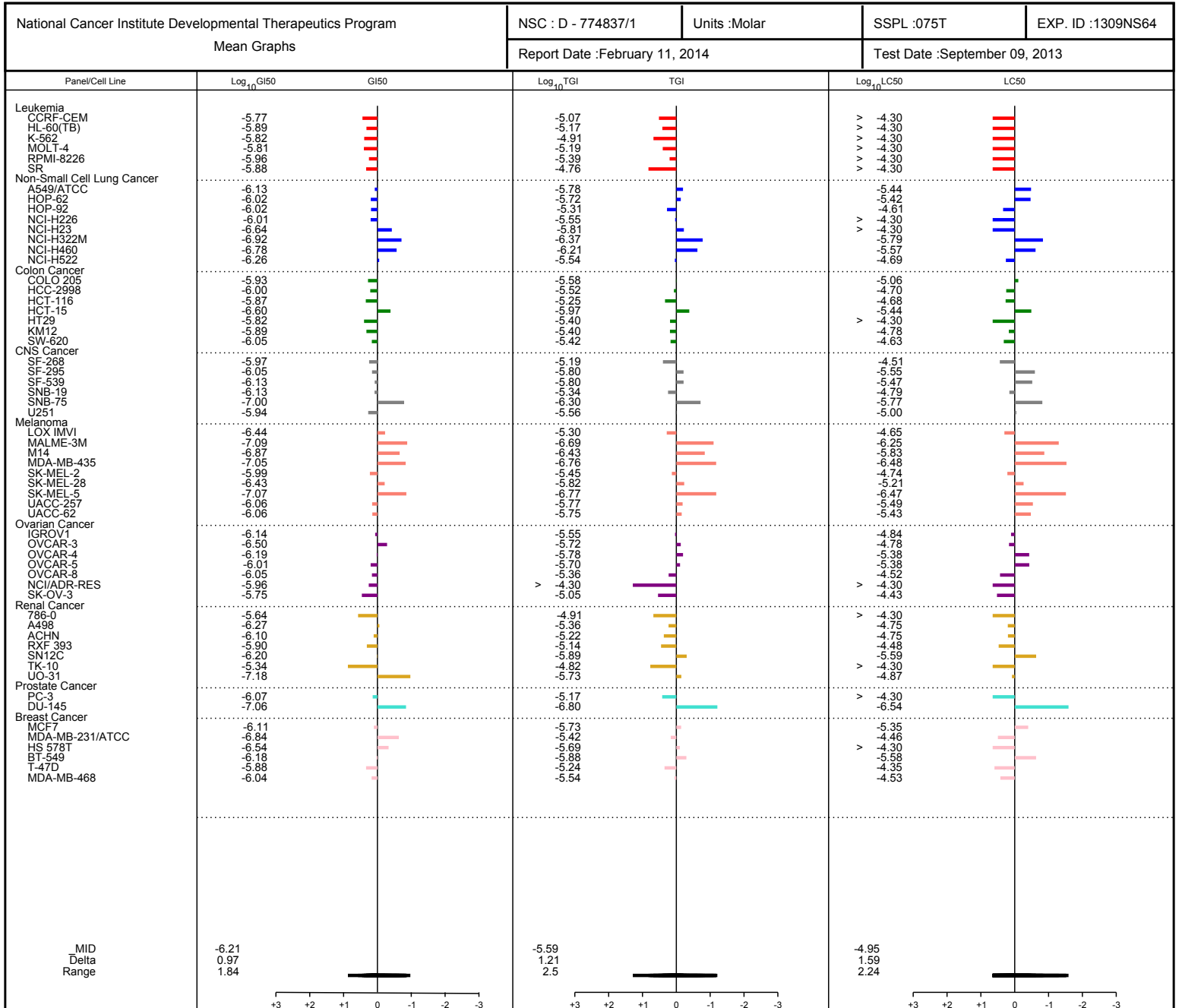




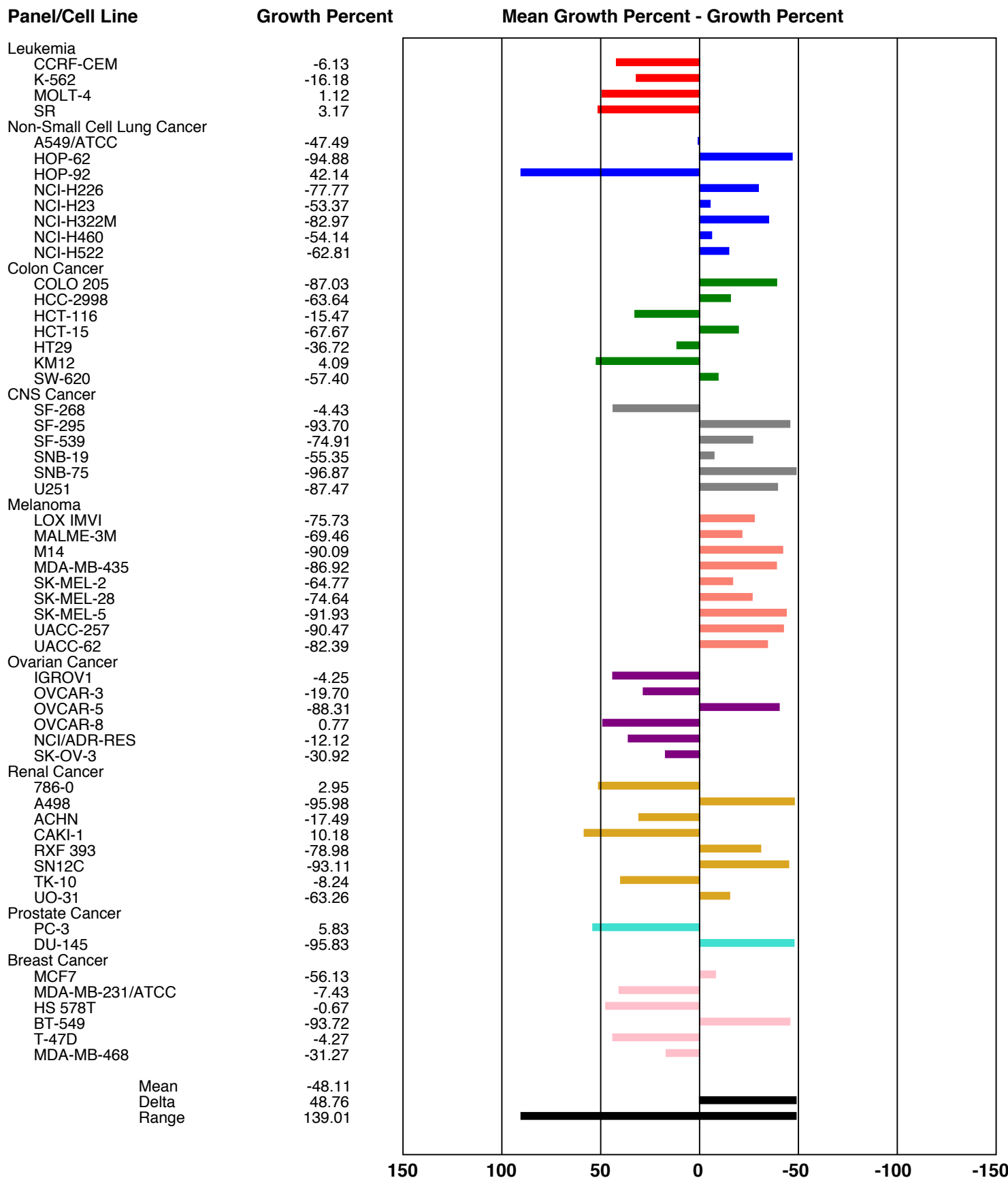
National Cancer Institute Developmental Therapeutics Program In-Vitro Testing Results

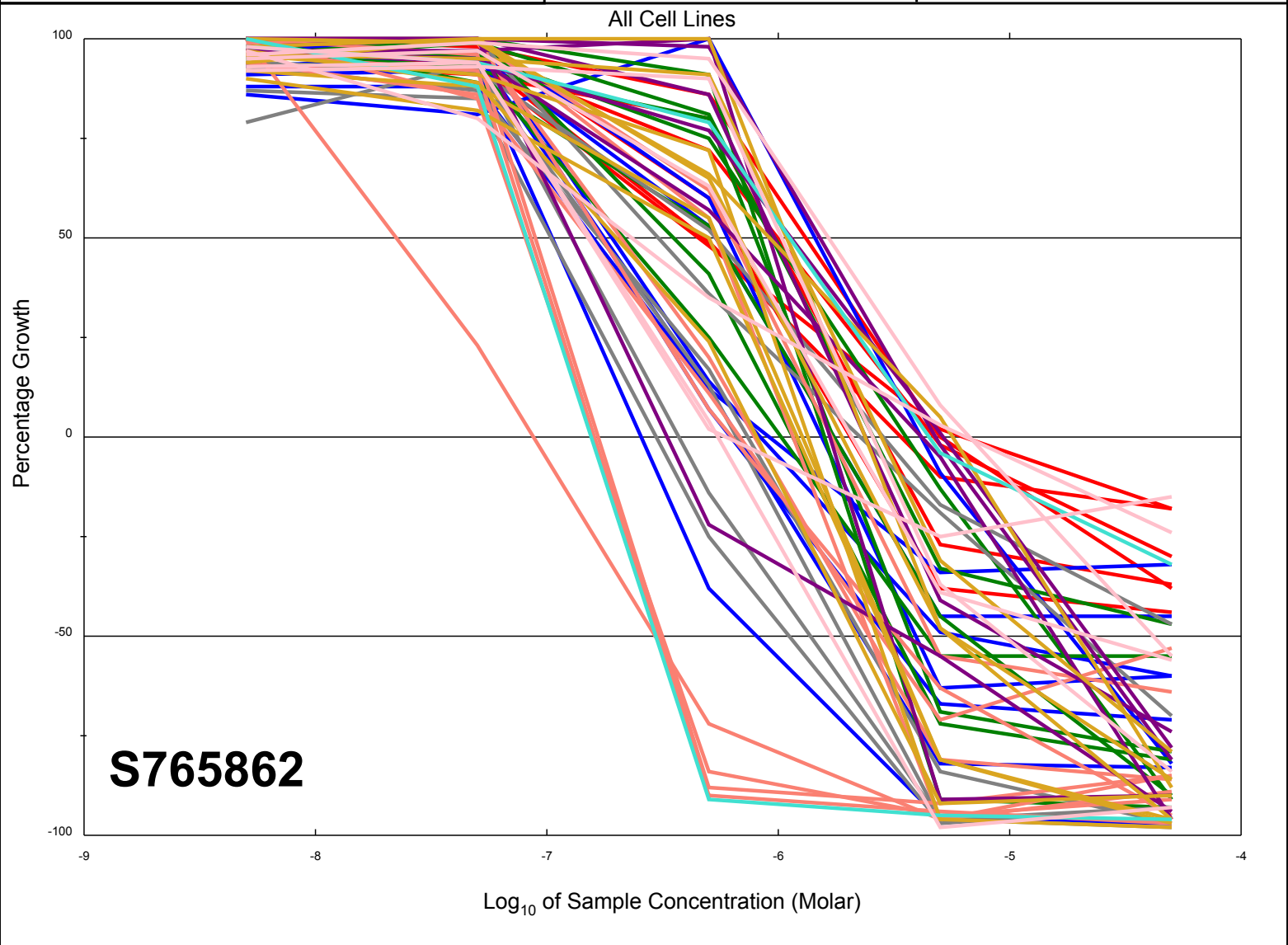
NSC : D - 774837 / 1	Experiment ID : 1309NS64	Test Type : 08	Units : Molar
Report Date : February 11, 2014	Test Date : September 09, 2013	QNS :	MC :
COMI : LT471 (130234)	Stain Reagent : SRB Dual-Pass Related	SSPL : 075T	

Panel/Cell Line	Time Zero	Log10 Concentration											GI50	TGI	LC50		
		Ctrl		Mean Optical Densities						Percent Growth							
		-8.3	-7.3	-6.3	-5.3	-4.3	-8.3	-7.3	-6.3	-5.3	-4.3						
Leukemia																	
CCRF-CEM	0.719	3.153	3.205	3.137	3.066	0.942	0.496	102	99	96	9	-31	1.70E-6	8.45E-6	> 5.00E-5		
HL-60(TB)	0.687	2.910	3.041	2.873	2.551	0.733	0.591	106	98	84	2	-14	1.30E-6	6.70E-6	> 5.00E-5		
K-562	0.270	2.140	2.424	2.310	1.929	0.423	0.236	115	109	89	8	-13	1.51E-6	1.23E-5	> 5.00E-5		
MOLT-4	0.800	2.994	3.093	3.098	2.812	0.941	0.409	105	105	92	6	-49	1.54E-6	6.53E-6	> 5.00E-5		
RPMI-8226	0.897	2.599	2.547	2.476	2.254	0.829	0.510	97	93	80	-8	-43	1.09E-6	4.09E-6	> 5.00E-5		
SR	0.363	1.609	1.567	1.532	1.294	0.565	0.313	97	94	75	16	-14	1.32E-6	1.72E-5	> 5.00E-5		
Non-Small Cell Lung Cancer																	
A549/ATCC	0.429	2.220	2.186	2.155	1.757	0.132	0.080	98	96	74	-69	-81	7.37E-7	1.64E-6	3.67E-6		
HOP-62	0.530	1.214	1.099	1.138	1.201	0.158	0.110	83	89	98	-70	-79	9.65E-7	1.91E-6	3.79E-6		
HOP-92	1.034	1.578	1.446	1.415	1.413	1.027	0.290	76	70	70	-1	-72	9.50E-7	4.88E-6	2.46E-5		
NCI-H226	1.344	2.942	2.826	2.669	2.647	0.977	0.750	93	83	82	-27	-44	9.74E-7	2.81E-6	> 5.00E-5		
NCI-H23	0.703	2.103	1.989	1.884	1.160	0.466	0.437	92	84	33	-34	-38	2.31E-7	1.55E-6	> 5.00E-5		
NCI-H322M	0.872	1.710	1.545	1.576	0.822	0.073	0.031	80	84	-6	-92	-96	1.20E-7	4.31E-7	1.64E-6		
NCI-H460	0.156	1.761	1.764	1.711	0.270	0.046	0.029	100	97	7	-71	-82	1.66E-7	6.17E-7	2.70E-6		
NCI-H522	1.015	2.312	2.265	2.257	1.697	0.845	0.292	96	96	53	-17	-71	5.44E-7	2.86E-6	2.04E-5		
Colon Cancer																	
COLO 205	0.422	1.814	1.778	1.844	1.837	0.259	0.061	97	102	102	-39	-86	1.17E-6	2.65E-6	8.73E-6		
HCC-2998	0.380	1.407	1.445	1.416	1.214	0.296	0.120	104	101	81	-22	-69	1.00E-6	3.05E-6	1.99E-5		
HCT-116	0.223	2.253	2.218	2.285	1.948	0.309	0.037	98	102	85	4	-84	1.36E-6	5.59E-6	2.07E-5		
HCT-15	0.252	1.361	1.398	1.294	0.601	0.092	0.066	103	94	31	-63	-74	2.52E-7	1.07E-6	3.60E-6		
HT29	0.298	1.631	1.668	1.764	1.743	0.262	0.170	103	110	108	-12	-43	1.53E-6	3.97E-6	> 5.00E-5		
KM12	0.339	1.602	1.639	1.672	1.508	0.305	0.046	103	106	93	-10	-86	1.30E-6	3.99E-6	1.67E-5		
SW-620	0.208	1.418	1.290	1.378	1.058	0.189	0.063	89	97	70	-9	-70	9.00E-7	3.84E-6	2.35E-5		
CNS Cancer																	
SF-268	0.630	1.729	1.685	1.725	1.412	0.719	0.217	96	100	71	8	-66	1.08E-6	6.44E-6	3.07E-5		
SF-295	1.089	2.798	2.867	2.875	2.788	0.010	-0.003	104	104	99	-99	-100	8.87E-7	1.58E-6	2.83E-6		
SF-539	0.874	2.572	2.392	2.356	2.159	0.213	0.003	89	87	76	-76	-100	7.39E-7	1.58E-6	3.38E-6		
SNB-19	0.803	2.143	2.119	2.129	1.622	0.782	0.031	98	99	61	-3	-96	7.47E-7	4.55E-6	1.61E-5		
SNB-75	0.673	1.360	1.173	1.165	0.672	0.042	0.017	73	72	.	-94	-97	9.99E-8	4.98E-7	1.70E-6		
U251	0.766	2.604	2.565	2.469	2.563	0.505	0.099	98	93	98	-34	-87	1.15E-6	2.75E-6	9.96E-6		
Melanoma																	
LOX IMVI	0.427	2.835	2.778	2.621	1.475	0.427	0.101	98	91	43	.	-76	3.65E-7	4.97E-6	2.25E-5		
MALME-3M	0.698	1.105	1.035	1.007	0.364	0.047	0.020	83	76	-48	-93	-97	8.10E-8	2.05E-7	5.58E-7		
M14	0.524	2.290	2.285	2.273	0.449	0.055	0.058	100	99	-14	-90	-89	1.35E-7	3.74E-7	1.49E-6		
MDA-MB-435	0.503	2.183	2.227	2.104	0.090	0.025	0.029	103	95	-82	-95	-94	9.00E-8	1.72E-7	3.30E-7		
SK-MEL-2	1.302	2.465	2.475	2.426	2.223	1.115	0.295	101	97	79	-14	-77	1.03E-6	3.51E-6	1.84E-5		
SK-MEL-28	0.643	1.867	1.884	1.773	1.181	0.339	0.142	101	92	44	-47	-78	3.75E-7	1.51E-6	6.10E-6		
SK-MEL-5	1.012	3.163	3.062	2.931	0.228	0.009	0.020	95	89	-77	-99	-98	8.59E-8	1.71E-7	3.42E-7		
UACC-257	0.873	1.956	1.906	1.950	1.885	0.146	0.016	95	99	93	-83	-98	8.81E-7	1.69E-6	3.24E-6		
UACC-62	0.789	2.315	2.197	2.185	2.132	0.234	0.190	92	91	88	-70	-76	8.69E-7	1.80E-6	3.72E-6		
Ovarian Cancer																	
IGROV1	0.738	1.983	1.763	1.653	1.524	0.585	0.113	82	74	63	-21	-85	7.16E-7	2.83E-6	1.43E-5		
OVCAR-3	0.547	1.546	1.477	1.360	0.971	0.381	0.173	93	81	42	-30	-68	3.19E-7	1.91E-6	1.64E-5		
OVCAR-4	0.704	1.368	1.239	1.223	1.128	0.283	-0.001	81	78	64	-60	-100	6.47E-7	1.64E-6	4.17E-6		
OVCAR-5	0.585	1.723	1.609	1.632	1.679	0.217	0.054	90	92	96	-63	-91	9.75E-7	2.01E-6	4.14E-6		
OVCAR-8	0.562	2.275	2.159	2.042	1.731	0.538	0.210	93	86	68	-4	-63	8.93E-7	4.37E-6	3.03E-5		
NCI/ADR-RES	0.595	1.999	1.973	1.911	1.645	0.640	0.649	98	94	75	3	4	1.11E-6	> 5.00E-5	> 5.00E-5		
SK-OV-3	0.550	1.468	1.312	1.386	1.339	0.740	0.219	83	91	86	21	-60	1.78E-6	9.00E-6	3.73E-5		
Renal Cancer																	
786-0	0.453	2.015	2.100	2.094	1.947	0.869	0.267	105	105	96	27	-41	2.29E-6	1.23E-5	> 5.00E-5		
A498	1.817	2.467	2.252	2.161	2.154	1.761	0.219	67	53	52	-3	-88	5.39E-7	4.39E-6	1.79E-5		
ACHN	0.389	1.943	1.795	1.603	1.326	0.529	0.006	91	78	60	9	-99	7.94E-7	6.06E-6	1.77E-5		
RXF 393	0.937	1.977	1.870	1.770	1.719	1.068	0.342	90	80	75	13	-64	1.26E-6	7.32E-6	3.32E-5		
SN12C	0.639	2.031	1.985	1.998	1.564	0.024	0.011	97	98	66	-96	-98	6.31E-7	1.28E-6	2.60E-6		
TK-10	0.884	1.663	1.962	2.016	2.073	1.242	0.450	138	145	153	46	-49	4.58E-6	1.52E-5	> 5.00E-5		
UO-31	0.668	1.777	1.321	1.256	0.967	0.531	0.073	59	53	27	-21	-89	6.54E-8	1.84E-6	1.34E-5		
Prostate Cancer																	
PC-3	0.538	2.040	1.832	1.694	1.506	0.565	0.476	86	77	64	2	-12	8.49E-7	6.77E-6	> 5.00E-5		
DU-145	0.350	1.182	1.250	1.162	0.011	-0.008	-0.006	108	98	-97	-100	-100	8.78E-8	1.59E-7	2.87E-7		
Breast Cancer																	
MCF7	0.578	2.213	2.088	2.005	1.816	0.251	0.227	92	87	76	-57	-61	7.82E-7	1.87E-6	4.45E-6		
MDA-MB-231/ATCC	0.584	1.350	1.234	1.057	0.861	0.555	0.244	85	62	36	-5	-58	1.43E-7	3.78E-6	3.50E-5		
HS 578T	0.614	1.382	1.242	1.204	0.933	0.452	0.396	82	77	42	-26	-36	2.88E-7	2.04E-6	> 5.00E-5		
BT-549	1.051	1.963	1.941	1.955	1.686	0.029	0.032	98	99	70	-97	-97	6.55E-7	1.31E-6	2.60E-6		
T-47D	0.480	1.043	0.991	1.003	0.955	0.500	0.226	91	93	84	4	-53	1.33E-6	5.78E-6	4.42E-5		
MDA-MB-468	0.805	1.796	1.689	1.664	1.557	0.618	0.340	89	87	76	-23	-58	9.12E-7	2.91E-6	2.97E-5		



One Dose Mean Graph



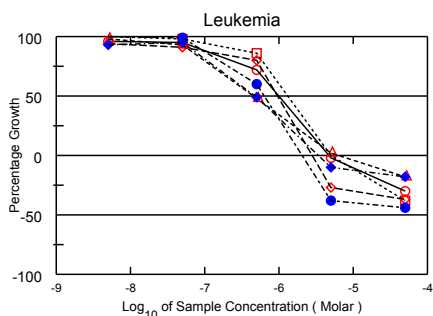


National Cancer Institute Developmental Therapeutics Program
Dose Response Curves

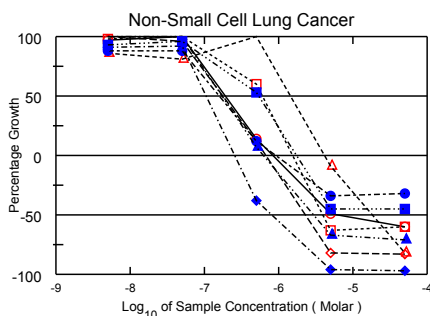
NSC: D - 765862 / 1
Report Date: January 23, 2014

SSPL: 075T
Test Date: August 06, 2012

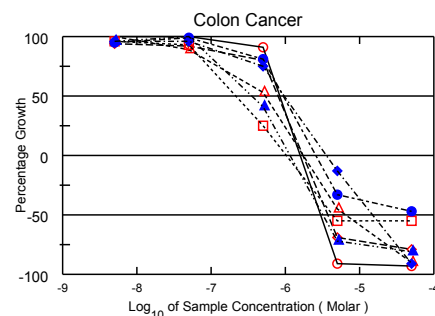
EXP. ID: 1208NS22



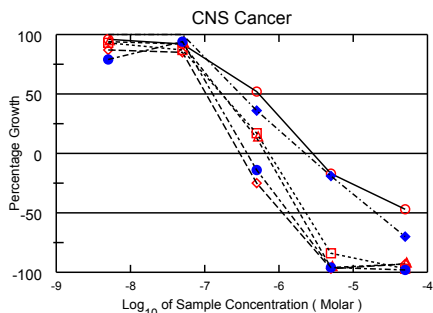
CCRF-CEM —○—
MOLT-4 —□—
HL-60(TB) —◇—
RPMI-8226 —●—
K-562 —△—
SR —◆—



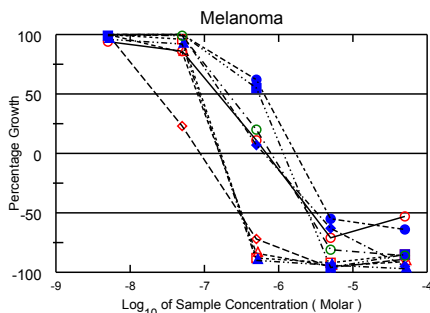
A549/ATCC —○—
NCI-H226 —□—
NCI-H460 —△—
HOP-62 —◇—
NCI-H23 —●—
NCI-H522 —◆—
HOP-92 —△—
NCI-H322M —◆—



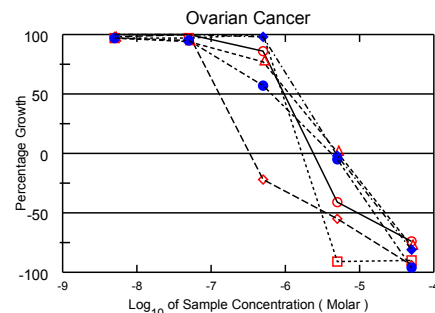
COLO 205 —○—
HCT-15 —□—
SW-620 —△—
HCC-2998 —◇—
HT29 —●—
HCT-116 —△—
KM12 —◆—



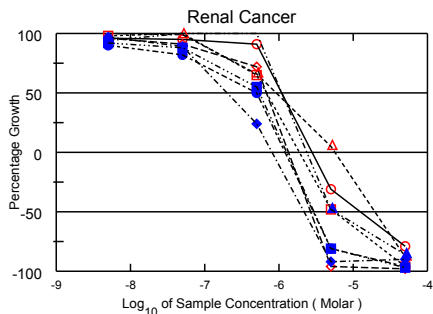
SF-268 —○—
SNB-19 —□—
SF-295 —◇—
SNB-75 —●—
SF-539 —△—
U251 —◆—



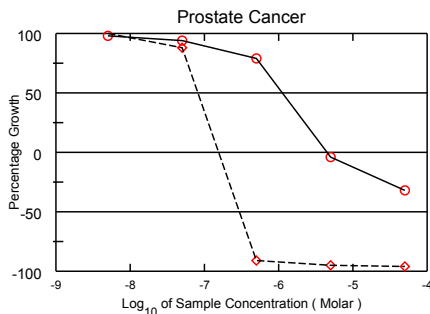
LOX IMVI —○—
MDA-MB-435 —□—
SK-MEL-5 —△—
MALME-3M —◇—
SK-MEL-2 —●—
UACC-257 —◆—
M14 —△—
SK-MEL-28 —◆—
UACC-62 —○—



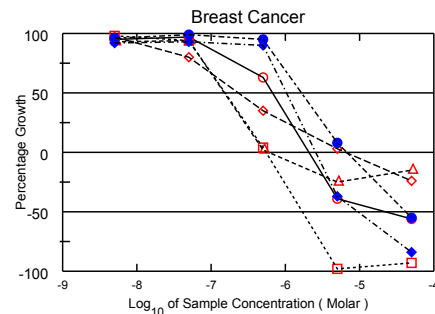
IGROV1 —○—
OV5 —□—
OV5 —◇—
OV3 —●—
OV8 —◆—
OV4 —△—
SK-OV-3 —◆—



786-0 —○—
CAKI-1 —□—
TK-10 —△—
A498 —◇—
RXF 393 —●—
UO-31 —◆—
ACHN —△—
SN12C —◆—



PC-3 —○—
DU-145 —◇—



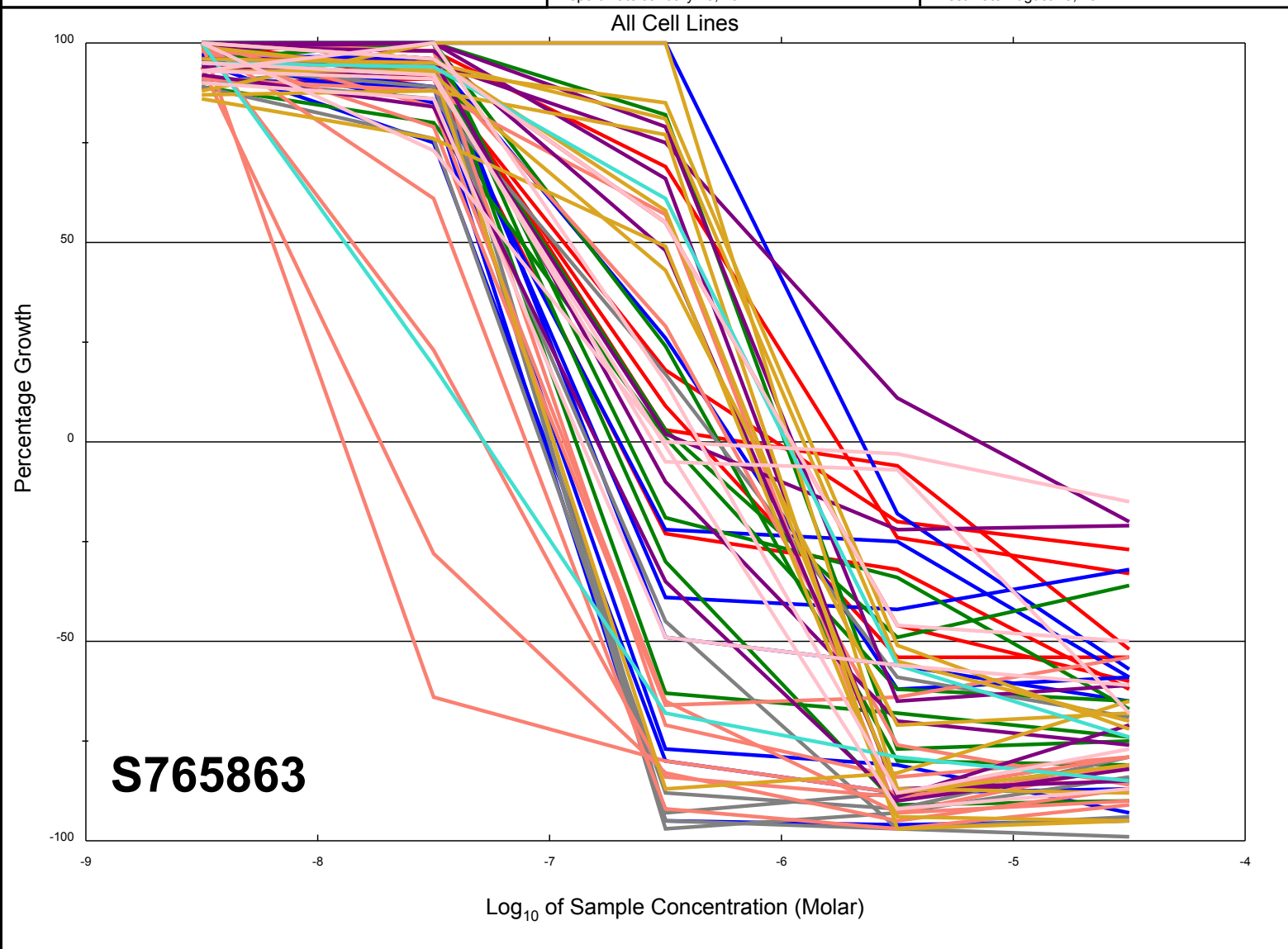
MCF7 —○—
BT-549 —□—
MDA-MB-231/ —◇—
T-47D —●—
MDA-MB-468 —◆—
HS 578T —△—

National Cancer Institute Developmental Therapeutics Program In-Vitro Testing Results

NSC : D - 765862 / 1	Experiment ID : 1208NS22	Test Type : 08	Units : Molar
Report Date : January 23, 2014	Test Date : August 06, 2012	QNS :	MC :
COMI : LT415 (119558)	Stain Reagent : SRB Dual-Pass Related	SSPL : 075T	

Panel/Cell Line	Time Zero	Log10 Concentration											GI50	TGI	LC50	
		Ctrl		Mean Optical Densities					Percent Growth							
		-8.3	-7.3	-6.3	-5.3	-4.3	-8.3	-7.3	-6.3	-5.3	-4.3					
Leukemia																
CCRF-CEM	0.433	2.334	2.257	2.244	1.805	0.425	0.302	96	95	72	-2	-30	9.96E-7	4.70E-6	> 5.00E-5	
HL-60(TB)	0.656	2.554	2.441	2.380	2.178	0.480	0.414	94	91	80	-27	-37	9.57E-7	2.81E-6	> 5.00E-5	
K-562	0.193	1.820	1.783	1.700	0.980	0.226	0.158	98	93	48	2	-18	4.59E-7	6.28E-6	> 5.00E-5	
MOLT-4	0.504	2.124	2.130	2.091	1.900	0.503	0.312	100	98	86	.	-38	1.31E-6	4.96E-6	> 5.00E-5	
RPMI-8226	0.821	2.477	2.538	2.453	1.819	0.513	0.462	104	99	60	-38	-44	6.37E-7	2.07E-6	> 5.00E-5	
SR	0.542	2.606	2.453	2.508	1.549	0.487	0.445	93	95	49	-10	-18	4.71E-7	3.36E-6	> 5.00E-5	
Non-Small Cell Lung Cancer																
A549/ATCC	0.324	1.856	1.817	1.952	0.541	0.167	0.130	97	106	14	-49	-60	2.04E-7	8.40E-7	6.64E-6	
HOP-62	0.404	1.313	1.358	1.276	0.511	0.073	0.068	105	96	12	-82	-83	1.75E-7	6.67E-7	2.28E-6	
HOP-92	1.325	1.681	1.631	1.614	1.721	1.203	0.244	86	81	111	-9	-82	1.61E-6	4.19E-6	1.83E-5	
NCI-H226	0.687	1.154	1.144	1.160	0.968	0.251	0.273	98	101	60	-63	-60	6.05E-7	1.53E-6	3.89E-6	
NCI-H23	1.103	3.185	2.943	2.934	1.344	0.726	0.750	88	88	12	-34	-32	1.57E-7	8.95E-7	> 5.00E-5	
NCI-H322M	0.838	1.634	1.561	1.573	0.521	0.035	0.025	91	92	-38	-96	-97	1.06E-7	2.56E-7	8.10E-7	
NCI-H460	0.324	3.036	3.090	3.088	0.507	0.108	0.094	102	102	7	-67	-71	1.76E-7	6.18E-7	2.96E-6	
NCI-H522	0.930	2.333	2.233	2.272	1.679	0.514	0.511	93	96	53	-45	-45	5.41E-7	1.75E-6	> 5.00E-5	
Colon Cancer																
COLO 205	0.345	1.441	1.446	1.443	1.338	0.030	0.023	100	100	91	-91	-93	8.36E-7	1.57E-6	2.96E-6	
HCC-2998	0.722	2.379	2.274	2.251	2.045	0.226	0.150	94	92	80	-69	-79	7.94E-7	1.72E-6	3.74E-6	
HCT-116	0.111	1.520	1.514	1.370	0.862	0.061	0.012	100	89	53	-45	-90	5.40E-7	1.74E-6	6.46E-6	
HCT-15	0.152	2.054	1.978	1.916	0.633	0.068	0.069	96	93	25	-55	-55	2.15E-7	1.03E-6	4.30E-6	
HT29	0.243	1.603	1.538	1.595	1.343	0.162	0.130	95	99	81	-33	-47	9.31E-7	2.55E-6	> 5.00E-5	
KM12	0.557	2.932	2.845	2.836	2.344	0.485	0.053	96	96	75	-13	-91	9.66E-7	3.56E-6	1.50E-5	
SW-620	0.278	2.263	2.192	2.266	1.094	0.079	0.054	96	100	41	-72	-81	3.53E-7	1.16E-6	3.21E-6	
CNS Cancer																
SF-268	0.730	2.208	2.142	2.085	1.504	0.603	0.384	96	92	52	-17	-47	5.40E-7	2.81E-6	> 5.00E-5	
SF-295	1.109	2.216	2.073	2.051	0.828	0.034	0.076	87	85	-25	-97	-93	1.04E-7	2.95E-7	1.10E-6	
SF-539	0.534	2.548	2.425	2.379	0.806	0.023	0.037	94	92	13	-96	-93	1.70E-7	6.64E-7	1.91E-6	
SNB-19	0.851	2.302	2.198	2.107	1.095	0.133	0.025	93	87	17	-84	-97	1.67E-7	7.32E-7	2.28E-6	
SNB-75	0.807	1.465	1.330	1.427	0.692	0.030	0.016	79	94	-14	-96	-98	1.28E-7	3.69E-7	1.36E-6	
U251	0.360	1.980	2.002	2.133	0.945	0.291	0.109	101	109	36	-19	-70	3.23E-7	2.25E-6	2.04E-5	
Melanoma																
LOX IMVI	0.395	2.343	2.222	2.062	0.612	0.117	0.185	94	86	11	-71	-53	1.50E-7	6.85E-7	2.80E-6	
MALME-3M	0.636	1.136	1.149	0.749	0.180	0.025	0.073	103	23	-72	-96	-89	2.27E-8	8.67E-8	2.94E-7	
M14	0.438	1.778	1.862	1.582	0.069	0.024	0.039	106	85	-84	-95	-91	8.08E-8	1.59E-7	3.14E-7	
MDA-MB-435	0.462	1.840	1.847	1.791	0.057	0.039	0.069	100	96	-88	-92	-85	8.94E-8	1.67E-7	3.12E-7	
SK-MEL-2	1.158	2.455	2.470	2.449	1.959	0.526	0.414	101	100	62	-55	-64	6.31E-7	1.70E-6	4.56E-6	
SK-MEL-28	0.530	1.600	1.685	1.777	0.605	0.197	0.025	108	117	7	-63	-95	2.03E-7	6.30E-7	3.27E-6	
SK-MEL-5	0.564	2.858	2.762	2.670	0.058	0.034	0.020	96	92	-90	-94	-97	8.49E-8	1.60E-7	3.02E-7	
UACC-257	0.755	1.683	1.673	1.686	1.270	0.032	0.112	99	100	55	-96	-85	5.43E-7	1.16E-6	2.49E-6	
UACC-62	0.927	3.011	3.024	2.997	1.348	0.177	0.132	101	99	20	-81	-86	2.10E-7	7.92E-7	2.47E-6	
Ovarian Cancer																
IGROV1	0.700	1.685	1.710	1.727	1.550	0.415	0.185	102	104	86	-41	-74	9.65E-7	2.39E-6	9.54E-6	
OVCAR-3	0.684	2.037	2.009	2.070	0.536	0.310	0.039	98	102	-22	-55	-94	1.32E-7	3.34E-7	3.61E-6	
OVCAR-4	0.481	1.120	1.101	1.079	0.971	0.488	0.106	97	94	77	1	-78	1.13E-6	5.16E-6	2.21E-5	
OVCAR-5	0.410	1.356	1.327	1.327	1.384	0.035	0.041	97	97	103	-91	-90	9.36E-7	1.69E-6	3.06E-6	
OVCAR-8	0.415	2.045	1.990	1.962	1.347	0.394	0.018	97	95	57	-5	-96	6.51E-7	4.13E-6	1.56E-5	
SK-OV-3	0.694	1.654	1.682	1.777	1.630	0.679	0.134	103	113	98	-2	-81	1.50E-6	4.76E-6	2.03E-5	
Renal Cancer																
786-0	0.680	2.552	2.481	2.454	2.385	0.470	0.143	96	95	91	-31	-79	1.09E-6	2.79E-6	1.25E-5	
A498	1.422	2.354	2.315	2.266	2.089	0.063	0.026	96	91	72	-96	-98	6.73E-7	1.34E-6	2.67E-6	
ACHN	0.242	1.822	1.864	1.806	1.287	0.318	0.030	103	99	66	5	-88	9.16E-7	5.63E-6	1.95E-5	
CAKI-1	0.449	1.535	1.510	1.585	1.150	0.234	0.020	98	105	65	-48	-96	6.74E-7	1.88E-6	5.54E-6	
RXF 393	0.783	1.216	1.173	1.139	1.000	0.151	0.031	90	82	50	-81	-96	5.00E-7	1.21E-6	2.91E-6	
SN12C	0.724	2.713	2.662	2.492	1.194	0.061	0.072	97	89	24	-92	-90	1.97E-7	8.01E-7	2.18E-6	
TK-10	0.721	1.542	1.492	1.585	1.656	0.374	0.098	94	105	114	-48	-86	1.24E-6	2.52E-6	5.60E-6	
UO-31	0.842	2.114	2.009	1.966	1.540	0.161	0.025	92	88	55	-81	-97	5.43E-7	1.27E-6	2.96E-6	
Prostate Cancer																
PC-3	0.431	1.236	1.219	1.188	1.063	0.414	0.293	98	94	79	-4	-32	1.11E-6	4.48E-6	> 5.00E-5	
DU-145	0.327	1.611	1.614	1.454	0.028	0.015	0.013	100	88	-91	-95	-96	8.12E-8	1.54E-7	2.94E-7	
Breast Cancer																
MCF7	0.625	2.807	2.695	2.736	1.995	0.380	0.274	95	97	63	-39	-56	6.67E-7	2.06E-6	2.15E-5	
MDA-MB-231/ATCC	0.670	1.512	1.488	1.341	0.964	0.693	0.509	97	80	35	3	-24	2.30E-7	6.29E-6	> 5.00E-5	
HS 578T	1.036	2.074	2.006	2.013	1.060	0.777	0.886	93	94	2	-25	-15	1.51E-7	6.05E-7	> 5.00E-5	
BT-549	0.920	1.957	1.937	1.892	0.961	0.019	0.069	98	94	4	-98	-93	1.53E-7	5.47E-7	1.69E-6	
T-47D	0.709	1.739	1.698	1.728	1.692	0.787	0.316	96	99	95	8	-55	1.64E-6	6.59E-6	4.10E-5	
MDA-MB-468	0.713	1.511	1.446	1.457	1.432	0.446	0.114	92	93	90	-37	-84	1.03E-6	2.54E-6	9.30E-6	

National Cancer Institute Developmental Therapeutics Program		NSC : D - 765862/1		Units :Molar		SSPL :075T		EXP. ID :1208NS22	
Mean Graphs		Report Date :January 23, 2014				Test Date :August 06, 2012			
Panel/Cell Line	Log ₁₀ G150	G150	Log ₁₀ TGI	TGI	Log ₁₀ LC50	LC50			
Leukemia									
CCRF-CEM	-6.00		-5.33		>>> -4.30				
HL-60(TB)	-6.02		-5.55		>>> -4.30				
K-562	-6.34		-5.20		>>> -4.30				
MOLT-4	-5.88		-5.30		>>> -4.30				
RPMI-8226	-6.20		-5.68		>>> -4.30				
SR	-6.33		-5.47		>>> -4.30				
Non-Small Cell Lung Cancer									
A549/ATCC	-6.69		-6.08		>>> -5.18				
HOP-62	-6.76		-6.18		>>> -5.64				
HOP-92	-5.79		-5.38		>>> -4.74				
NCI-H226	-6.22		-5.81		>>> -5.41				
NCI-H23	-6.80		-6.05		>>> -4.30				
NCI-H322M	-6.98		-6.59		>>> -6.09				
NCI-H460	-6.76		-6.21		>>> -5.53				
NCI-H522	-6.27		-5.76		>>> -4.30				
Colon Cancer									
COLO 205	-6.08		-5.80		>>> -5.53				
HCC-2998	-6.10		-5.76		>>> -5.43				
HCT-116	-6.27		-5.76		>>> -5.19				
HCT-15	-6.67		-5.99		>>> -5.37				
HT29	-6.03		-5.59		>>> -4.30				
KM12	-6.01		-5.45		>>> -4.82				
SW-620	-6.45		-5.94		>>> -5.49				
CNS Cancer									
SF-268	-6.27		-5.55		>>> -4.30				
SF-295	-6.98		-6.53		>>> -5.96				
SF-539	-6.77		-6.18		>>> -5.72				
SNB-19	-6.78		-6.14		>>> -5.84				
SNB-75	-6.89		-6.43		>>> -5.87				
U251	-6.49		-5.65		>>> -4.69				
Melanoma									
LOX IMVI	-6.82		-6.16		>>> -5.55				
MALME-3M	-7.64		-7.06		>>> -6.53				
M14	-7.09		-6.80		>>> -6.50				
MDA-MB-435	-7.05		-6.78		>>> -6.51				
SK-MEL-2	-6.20		-5.77		>>> -5.34				
SK-MEL-28	-6.69		-6.20		>>> -5.49				
SK-MEL-5	-7.07		-6.80		>>> -6.52				
UACC-267	-6.26		-5.93		>>> -5.60				
UACC-62	-6.68		-6.10		>>> -5.81				
Ovarian Cancer									
IGROV1	-6.02		-5.62		>>> -5.02				
OVCAR-3	-6.88		-6.48		>>> -5.44				
OVCAR-4	-5.95		-5.29		>>> -4.65				
OVCAR-5	-6.03		-5.77		>>> -5.51				
OVCAR-8	-6.19		-5.38		>>> -4.81				
SK-OV-3	-5.82		-5.32		>>> -4.69				
Renal Cancer									
786-0	-5.96		-5.55		>>> -4.90				
A498	-6.17		-5.87		>>> -5.57				
ACHN	-6.04		-5.25		>>> -4.71				
CAKI-1	-6.17		-5.73		>>> -5.26				
RXF 393	-6.30		-5.92		>>> -5.54				
SN12C	-6.71		-6.10		>>> -5.66				
TK-10	-5.91		-5.60		>>> -5.25				
UO-31	-6.27		-5.90		>>> -5.53				
Prostate Cancer									
PC-3	-5.96		-5.35		>>> -4.30				
DU-145	-7.09		-6.81		>>> -6.53				
Breast Cancer									
MCF7	-6.18		-5.69		>>> -4.67				
MDA-MB-231/ATCC	-6.64		-5.20		>>> -4.30				
HS 578T	-6.82		-6.22		>>> -4.30				
BT-549	-6.81		-6.26		>>> -5.77				
T-47D	-5.78		-5.18		>>> -4.39				
MDA-MB-468	-5.99		-5.59		>>> -5.03				
MID Delta Range									
MID	-6.41		-5.88		-5.19				
Delta	1.23		1.18		1.34				
Range	1.86		1.88		2.23				

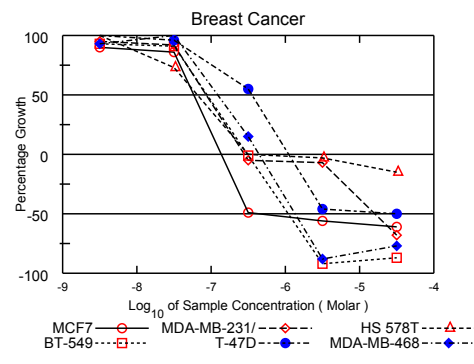
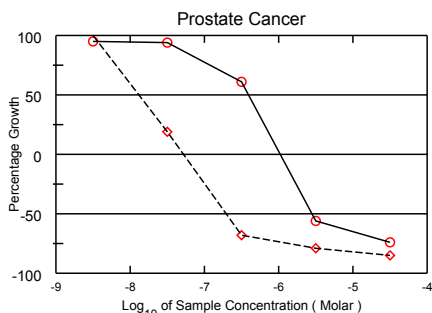
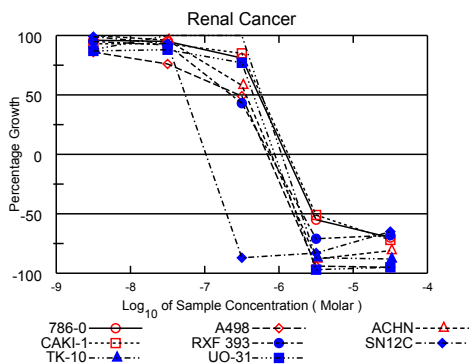
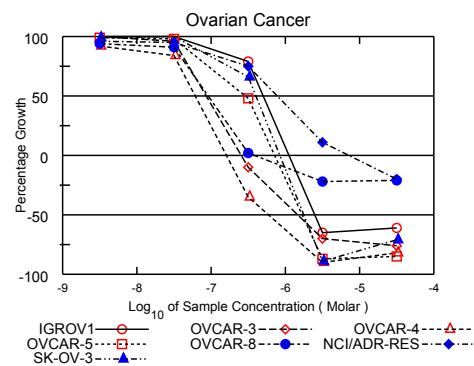
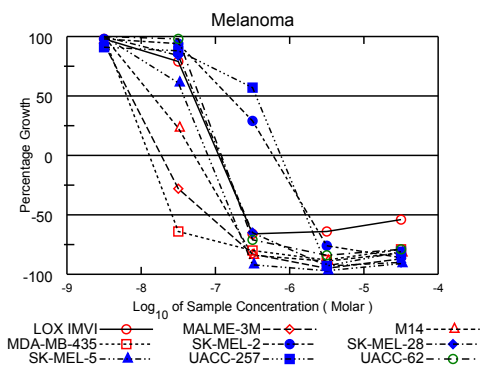
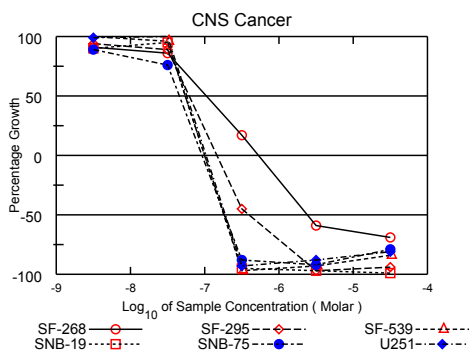
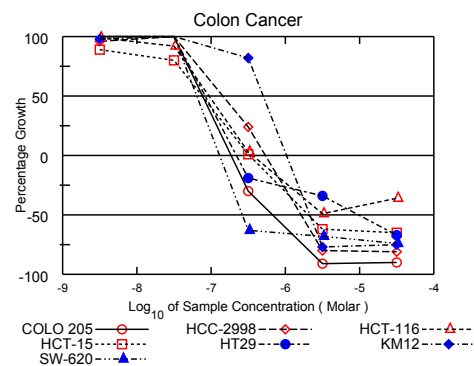
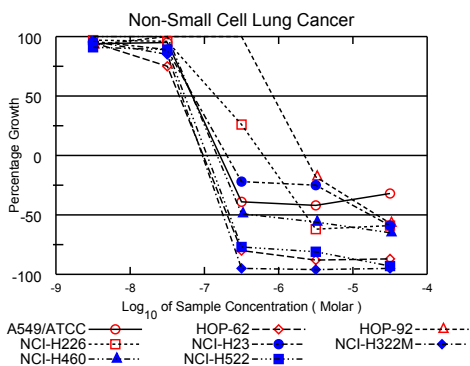
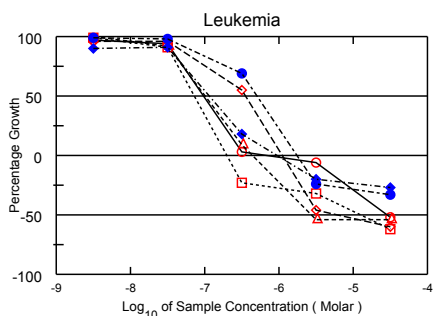


National Cancer Institute Developmental Therapeutics Program
Dose Response Curves

NSC: D - 765863 / 1
Report Date: January 23, 2014

SSPL: 075T
Test Date: August 13, 2012

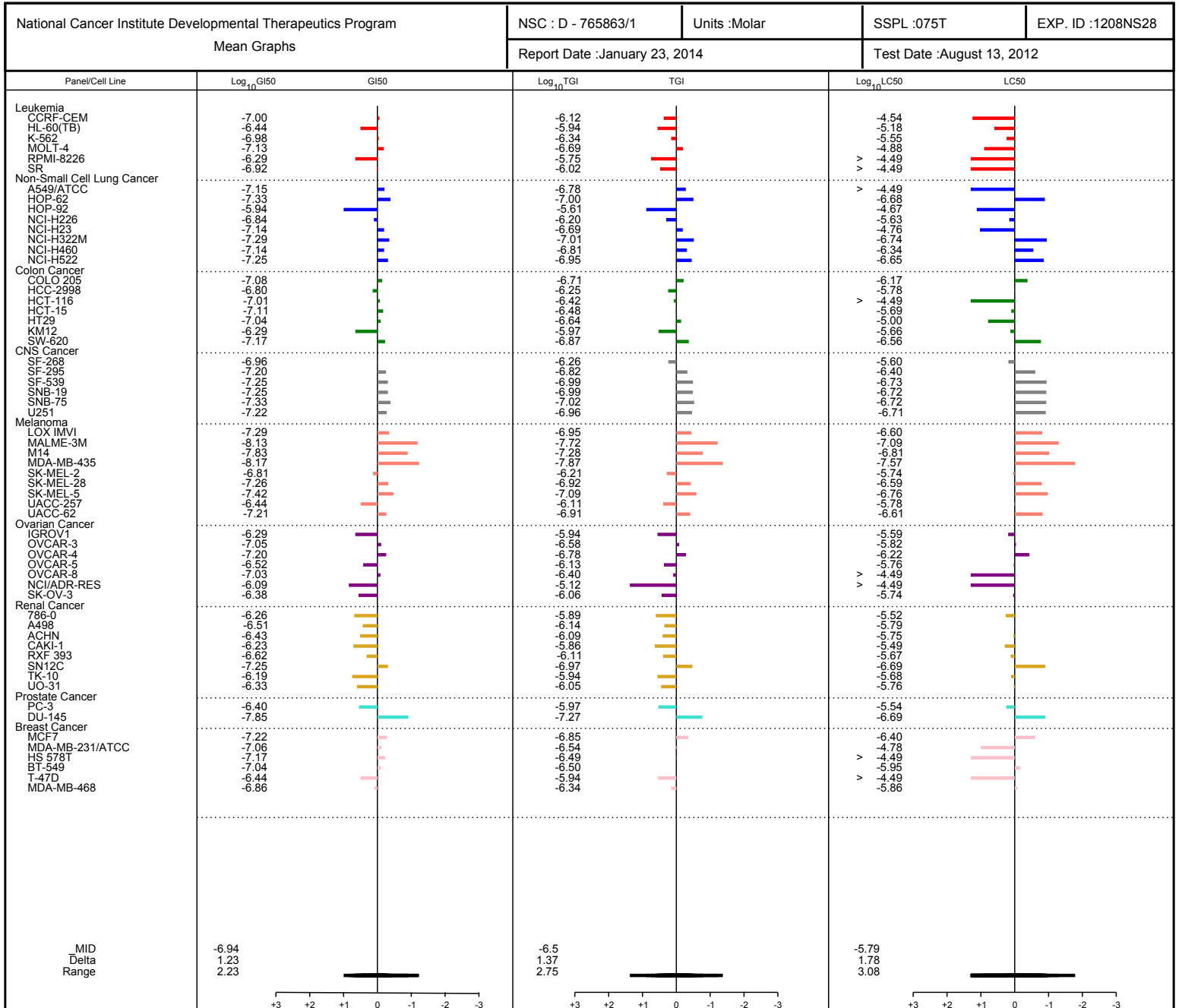
EXP. ID: 1208NS28

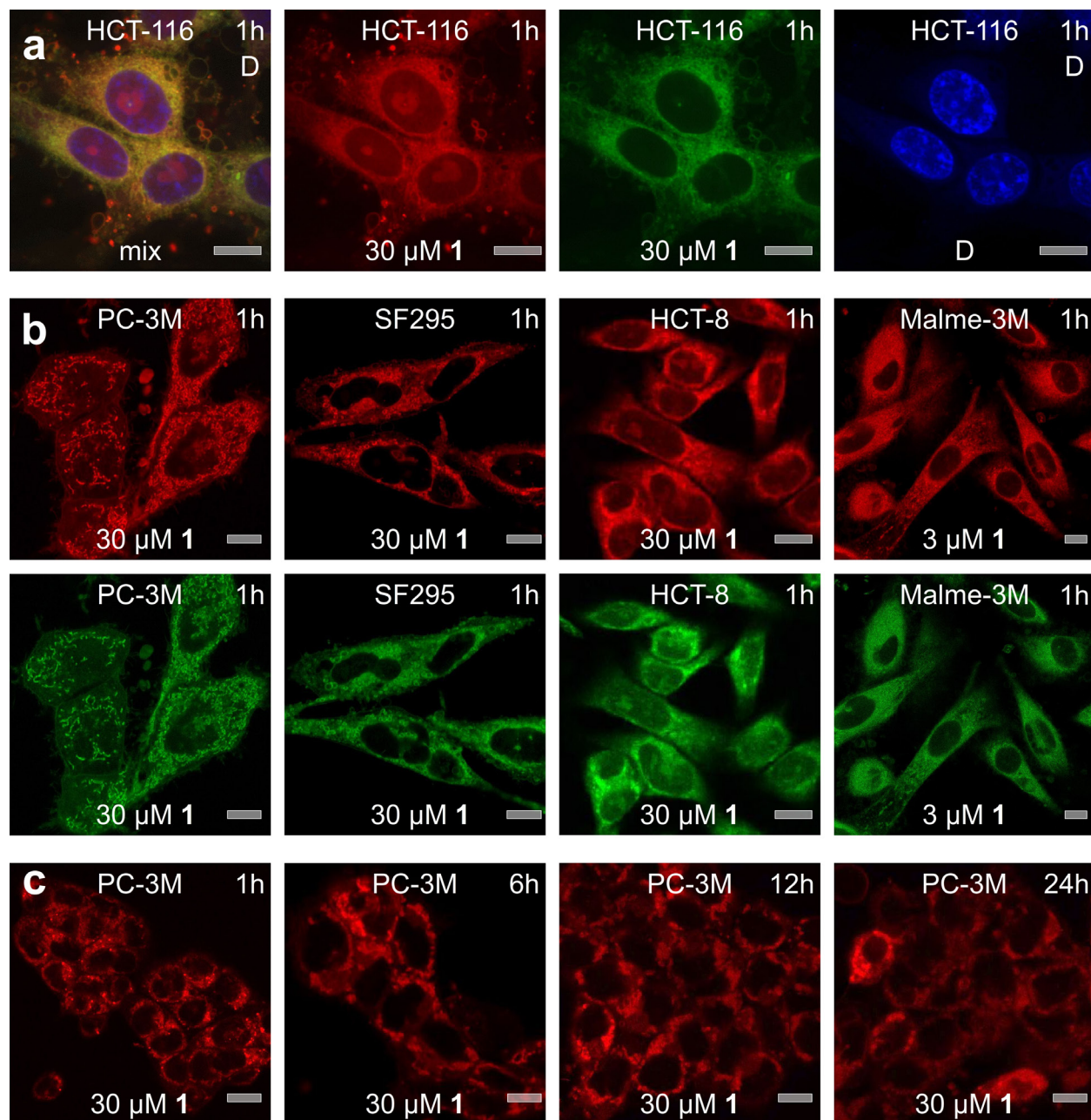


National Cancer Institute Developmental Therapeutics Program In-Vitro Testing Results

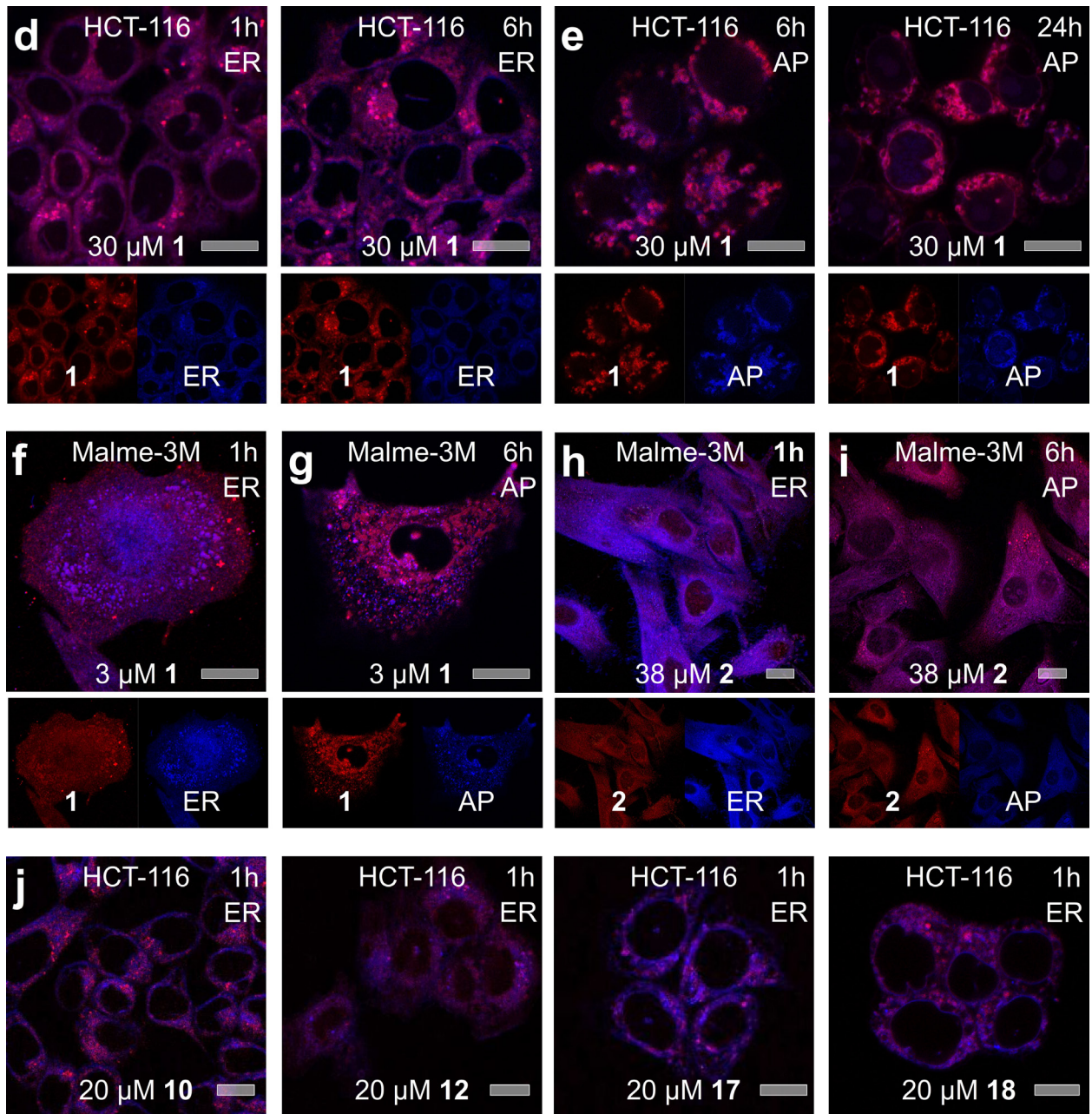
NSC : D - 765863 / 1	Experiment ID : 1208NS28	Test Type : 08	Units : Molar
Report Date : January 23, 2014	Test Date : August 13, 2012	QNS :	MC :
COMI : LT417 (119559)	Stain Reagent : SRB Dual-Pass Related	SSPL : 075T	

Panel/Cell Line	Time	Log10 Concentration											GI50	TGI	LC50		
		Zero		Mean Optical Densities						Percent Growth							
		Ctrl	-8.5	-7.5	-6.5	-5.5	-4.5	-8.5	-7.5	-6.5	-5.5	-4.5					
Leukemia																	
CCRF-CEM	0.332	2.015	1.963	1.920	0.389	0.313	0.158	97	94	3	-6	-52	9.98E-8	7.51E-7	2.88E-5		
HL-60(TB)	0.659	2.606	2.527	2.533	1.730	0.358	0.266	96	96	55	-46	-60	3.64E-7	1.14E-6	6.55E-6		
K-562	0.247	2.089	2.103	1.941	0.413	0.115	0.113	101	92	9	-54	-54	1.04E-7	4.53E-7	2.84E-6		
MOLT-4	0.544	2.308	2.283	2.151	0.417	0.369	0.209	99	91	-23	-32	-62	7.42E-8	2.03E-7	1.31E-5		
RPMI-8226	0.901	2.755	2.745	2.724	2.178	0.681	0.604	99	98	69	-24	-33	5.18E-7	1.78E-6	> 3.25E-5		
SR	0.440	2.175	2.006	2.026	0.751	0.352	0.320	90	91	18	-20	-27	1.19E-7	9.65E-7	> 3.25E-5		
Non-Small Cell Lung Cancer																	
A549/ATCC	0.365	2.023	1.923	1.942	0.224	0.213	0.248	94	95	-39	-42	-32	7.06E-8	1.67E-7	> 3.25E-5		
HOP-62	0.342	1.059	1.022	0.878	0.070	0.042	0.045	95	75	-80	-88	-87	4.70E-8	9.92E-8	2.09E-7		
HOP-92	1.641	2.073	2.041	2.139	2.210	1.343	0.708	93	115	132	-18	-57	1.14E-6	2.46E-6	2.16E-5		
NCI-H226	0.670	1.227	1.209	1.202	0.813	0.254	0.277	97	96	26	-62	-59	1.46E-7	6.36E-7	2.36E-6		
NCI-H23	1.152	3.173	3.084	2.943	0.898	0.863	0.469	96	89	-22	-25	-59	7.26E-8	2.05E-7	1.74E-5		
NCI-H322M	0.838	2.028	1.976	1.854	0.046	0.035	0.046	96	85	-95	-96	-95	5.11E-8	9.69E-8	1.84E-7		
NCI-H460	0.375	3.146	3.213	3.232	0.192	0.165	0.131	102	103	-49	-56	-65	7.26E-8	1.55E-7	4.57E-7		
NCI-H522	0.936	2.277	2.153	2.127	0.220	0.178	0.061	91	89	-77	-81	-93	5.58E-8	1.12E-7	2.25E-7		
Colon Cancer																	
COLO 205	0.473	1.918	1.967	1.995	0.329	0.041	0.048	103	105	-30	-91	-90	8.30E-8	1.94E-7	6.81E-7		
HCC-2998	0.929	2.718	2.639	2.832	1.366	0.184	0.178	96	106	24	-80	-81	1.58E-7	5.56E-7	1.67E-6		
HCT-116	0.193	2.068	2.054	1.926	0.255	0.099	0.123	99	92	3	-49	-36	9.73E-8	3.76E-7	> 3.25E-5		
HCT-15	0.173	1.547	1.391	1.274	0.181	0.065	0.061	89	80	1	-62	-65	7.77E-8	3.32E-7	2.06E-6		
HT29	0.237	1.492	1.530	1.579	0.192	0.157	0.079	103	107	-19	-34	-67	9.20E-8	2.30E-7	9.98E-6		
KM12	0.462	2.321	2.275	2.349	1.987	0.104	0.114	98	101	82	-77	-75	5.16E-7	1.06E-6	2.19E-6		
SW-620	0.281	2.316	2.365	2.387	0.105	0.090	0.074	102	103	-63	-68	-74	6.82E-8	1.36E-7	2.73E-7		
CNS Cancer																	
SF-268	0.667	2.312	2.171	2.085	0.954	0.277	0.206	91	86	17	-59	-69	1.09E-7	5.51E-7	2.51E-6		
SF-295	0.988	3.074	2.944	2.853	0.540	0.032	0.057	94	89	-45	-97	-94	6.37E-8	1.50E-7	4.00E-7		
SF-539	0.547	1.907	1.939	1.851	0.019	0.040	0.085	102	96	-97	-93	-84	5.63E-8	1.02E-7	1.86E-7		
SNB-19	0.894	2.421	2.276	2.350	0.049	0.025	0.005	90	95	-95	-97	-99	5.63E-8	1.03E-7	1.89E-7		
SNB-75	0.738	1.574	1.486	1.377	0.087	0.062	0.157	89	76	-88	-92	-79	4.71E-8	9.46E-8	1.90E-7		
U251	0.530	2.399	2.386	2.463	0.037	0.063	0.101	99	103	-93	-88	-81	6.08E-8	1.09E-7	1.96E-7		
Melanoma																	
LOX IMVI	0.629	3.078	3.017	2.558	0.212	0.226	0.288	98	79	-66	-64	-54	5.13E-8	1.13E-7	2.51E-7		
MALME-3M	0.684	1.567	1.513	0.493	0.117	0.032	0.092	94	-28	-83	-95	-87	7.45E-9	1.92E-8	8.18E-8		
M14	0.534	2.124	2.152	0.893	0.084	0.057	0.094	102	23	-84	-89	-82	1.46E-8	5.28E-8	1.55E-7		
MDA-MB-435	0.421	2.002	2.048	0.150	0.086	0.050	0.088	103	-64	-80	-88	-79	6.73E-9	1.34E-8	2.66E-8		
SK-MEL-2	1.186	2.307	2.289	2.245	1.513	0.279	0.169	98	94	29	-76	-86	1.56E-7	6.13E-7	1.82E-6		
SK-MEL-28	0.443	1.282	1.345	1.148	0.156	0.029	0.045	107	84	-65	-93	-90	5.50E-8	1.19E-7	2.58E-7		
SK-MEL-5	0.574	2.612	2.634	1.819	0.044	0.019	0.051	101	61	-92	-97	-91	3.84E-8	8.13E-8	1.72E-7		
UACC-257	0.809	1.852	1.760	1.726	1.399	0.058	0.157	91	88	57	-93	-81	3.59E-7	7.77E-7	1.68E-6		
UACC-62	0.678	2.653	2.655	2.607	0.198	0.109	0.141	100	98	-71	-84	-79	6.24E-8	1.24E-7	2.45E-7		
Ovarian Cancer																	
IGROV1	0.570	1.943	1.951	1.937	1.656	0.198	0.225	101	100	79	-65	-61	5.17E-7	1.15E-6	2.54E-6		
OVCAR-3	0.593	1.842	1.883	1.795	0.535	0.176	0.141	103	96	-10	-70	-76	8.86E-8	2.62E-7	1.50E-6		
OVCAR-4	0.822	1.698	1.632	1.562	0.531	0.085	0.149	92	84	-35	-90	-82	6.30E-8	1.65E-7	6.04E-7		
OVCAR-5	0.545	1.562	1.552	1.538	1.037	0.073	0.083	99	98	48	-87	-85	3.01E-7	7.42E-7	1.74E-6		
OVCAR-8	0.467	2.146	2.046	1.988	0.501	0.366	0.368	94	91	2	-22	-21	9.33E-8	3.94E-7	> 3.25E-5		
NCI/ADR-RES	0.625	2.313	2.252	2.236	1.893	0.816	0.502	96	95	75	11	-20	8.04E-7	7.52E-6	> 3.25E-5		
SK-OV-3	0.407	1.056	1.048	1.096	0.836	0.044	0.118	99	106	66	-89	-71	4.13E-7	8.66E-7	1.82E-6		
Renal Cancer																	
786-0	0.678	2.481	2.401	2.400	2.147	0.305	0.202	96	95	81	-55	-70	5.53E-7	1.28E-6	2.99E-6		
A498	1.458	2.326	2.205	2.114	1.888	0.093	0.077	86	76	49	-94	-95	3.10E-7	7.21E-7	1.61E-6		
ACHN	0.290	1.528	1.540	1.484	1.012	0.035	0.055	101	96	58	-88	-81	3.71E-7	8.13E-7	1.79E-6		
CAKI-1	0.912	3.064	2.922	2.912	2.736	0.449	0.255	93	93	85	-51	-72	5.87E-7	1.37E-6	3.21E-6		
RXF 393	0.614	1.130	1.104	1.087	0.838	0.181	0.197	95	92	43	-71	-68	2.38E-7	7.81E-7	2.14E-6		
SN12C	0.908	3.035	3.022	2.884	0.120	0.158	0.315	99	93	-87	-83	-65	5.63E-8	1.07E-7	2.03E-7		
TK-10	0.831	1.691	1.586	1.688	1.751	0.106	0.099	88	100	107	-87	-88	6.39E-7	1.15E-6	2.09E-6		
UO-31	0.820	2.396	2.189	2.212	2.032	0.025	0.040	87	88	77	-97	-95	4.64E-7	9.00E-7	1.74E-6		
Prostate Cancer																	
PC-3	0.582	1.840	1.774	1.768	1.347	0.255	0.151	95	94	61	-56	-74	4.02E-7	1.08E-6	2.88E-6		
DU-145	0.350	1.620	1.673	0.591	0.114	0.074	0.054	104	19	-68	-79	-85	1.40E-8	5.38E-8	2.04E-7		
Breast Cancer																	
MCF7	0.395	2.250	2.065	1.984	0.200	0.173	0.154	90	86	-49	-56	-61	5.97E-8	1.40E-7	4.01E-7		
MDA-MB-231/ATCC	0.595	1.470	1.430	1.399	0.564	0.554	0.190	95	92	-5	-7	-68	8.77E-8	2.87E-7	1.64E-5		
HS 578T	1.007	2.087	2.114	1.799	1.004	0.975	0.854	103	73	.	-3	-15	6.74E-8	3.21E-7	> 3.25E-5		
BT-549	0.935	1.859	1.793	1.777	0.927	0.074	0.119	93	91	-1	-92	-87	9.09E-8	3.18E-7	1.12E-6		
T-47D	0.592	1.360	1.385	1.331	1.015	0.319	0.297	103	96	55	-46	-50	3.65E-7	1.14E-6	> 3.25E-5		
MDA-MB-468	0.637	1.406	1.351	1.486	0.752	0.076	0.149	93	110	15	-88	-77	1.39E-7	4.54E-7	1.39E-6		





An expansion of panels a-c from Figure 2



An expansion of the panel d-j from Figure 2