New Candidaspongiolides, Tedanolide Analogs that Selectively Inhibit Melanoma Cell Growth

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Supporting Information

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General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. UV spectra were acquired in spectroscopy grade MeOH using a Varian Cary 50 UV-Vis spectrophotometer. NMR data were collected using an Avance III 600 (^{1}H 600 MHz, ^{13}C 150 MHz) NMR spectrometer (Bruker Biospin) with a 3-mm PATXI probe, referenced to residual solvent. MS spectra were measured with an Agilent Technologies 6510 Q-TOF LC-MS and an Applied Biosystems, Inc. QSTAR XL hybrid triple-quad time-of-flight (QqTOF) mass spectrometer. Initial fractionation was performed on Diol SPE cartridges (Applied Separations) and Sephadex LH-20 resin (Amersham Biosciences). HPLC purification was performed on a Rainin SD-1/UV-1 system.

Biological Material. Two different collections from Papua New Guinea (0CDN1808 and 0CDN5955), used in this investigation, were initially identified as *Euryspongia* sp. They were subsequently compared to Great Barrier Reef specimens of *C. flabellata* (the original source of the candidaspongiolides) and reclassified as *Candidaspongia* sp.[1] The Papua New Guinea specimens are a darker color and have somewhat sharper conules, while the *C. flabellata* specimens from Australia had thicker fibers and a thicker sand coat on the surface. Vouchers for the Papua New Guinea collections are maintained at the Smithsonian Sorting Center, Suitland, Maryland.

Extraction and Isolation. The Papua New Guinea *Candidaspongia sp*. specimens were repeatedly extracted according to the methodology outlined in McCloud[2] to give the aqueous crude extracts. A portion of this extract (520 mg) was subjected to size-exclusion chromatography on Sephadex LH-20 (2.5 × 90 cm) using hexanes/CH_2Cl_2/MeOH (2:5:1) to yield seven fractions (141A-141G). Fraction 141F was chromatographed on C_{18} (2.0 × 17 cm)

[1] Meragelman, T. L.; Willis, R. H.; Woldemichael, G. M.; Heaton, A.; Murphy, P. T.; Snader, K. M.; Newman, D. J.; van Soest, R.; Boyd, M. R.; Cardellina II, J. H.; McKee, T. C. J. Nat. Prod. **2007**, *70*, 1133.
[2] McCloud, T. G. Molecules, **2010**, *15*, 4526.

using 50/50 50% CH_3CN/50% H_2O (+0.1% AcOH) to yield precandidaspongiolides A/B (**1**/**2**, 11.2 mg). Fraction 141C was chromatographed on C_{18} (2.0 × 17 cm) using 55% CH_3CN/45% H_2O (+0.1% AcOH) to yield seven fractions (154A-154G). Fraction 154F was purified by HPLC using a Rainin Dynamax C_{18} column (250 × 10 mm) employing a gradient of 35% CH_3CN/65% H_2O (+0.1% AcOH) to 85% CH_3CN at 4.5 mL/min over 20 min to yield candidaspongiolides A/B (**3**/**4**, 0.9 mg). Fraction 141D was purified by HPLC utilizing the same method to yield tedanolide (**5**, 0.4 mg).

Precandiaspongiolides A and B (1/2): $[\alpha]^{25}_{D}$ + 58.3 (*c* 0.23, MeOH); UV (MeOH) λ_{max} (log ε) 205 (3.69) nm; ^1H NMR and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 665.3146 $[M+Na]^+$ (calcd for $C_{32}H_{50}O_{13}Na$, 665.3144).

Candidaspongiolides A and B (3/4): $[\alpha]^{25}_{D}$ + 20.0 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.48) nm; ^1H NMR and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 707.3235 $[M+Na]^+$ (calcd for $C_{34}H_{52}O_{14}Na$, 707.3249).

(+)-Tedanolide (**5**): $[\alpha]^{25}_{D}$ + 20 (*c* 0.02, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.79) nm; ^1H NMR and ^{13}C NMR data in $CDCl_3$ and CD_3OD, see Tables S2 and S3; HRESIMS *m/z* 633.3232 $[M+Na]^+$ (calcd for $C_{32}H_{50}O_{11}Na$, 633.3245).

Acetylation of Precandidaspongiolides A and B (1/2). A stock solution of AcCl was prepared on ice by dissolving 1 μL of AcCl in 50 μL of anhydrous CH_2Cl_2. 7 μL of the AcCl solution (2 μmol) was added to a stirring solution of **1**/**2** (1.1 mg, 1.7 μmol) in 2,4,6-trimethylpyridine (excess) at –40 °C.[3] The reaction was monitored by LC-MS for the production of mono-acetylated product and allowed to stir at –40 °C for 3h and then 25°C for 19h. After the mono-acetylated product formation was observed, the reaction mixture was diluted with H_2O and

[3] Ishihara, K.; Kurihara, H.; Yamamoto, H. *J. Org. Chem.* 1993, 58, 3791.

dried under N_2. The reaction mixture was then purified by HPLC using a Rainin Dynamax C_{18} column (250 × 10 mm) employing a gradient of 35% CH_3CN/65% H_2O (+0.1% AcOH) to 85% CH_3CN at 4.5 mL/min over 20 min to yield 28-acetyl-precandidaspongiolide A (**6**, 0.48 mg, 41% yield) and unreacted precandidaspongiolides A/B (**1**/**2**, 0.57 mg, 52% yield).

28-acetyl-precandidaspongiolide A (**6**): $[\alpha]^{25}_{D}$ + 28.6 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.38) nm; ^1H NMR and ^{13}C NMR data, see Table S4; HRESIMS *m/z* 707.3245 $[M+Na]^+$ (calcd for $C_{34}H_{52}O_{14}Na$, 707.3249).

Reduction of Precandidaspongiolides A and B (1/2). $NaBH_4$ (~3 mg, excess) was added to a semi-pure (~75%) solution of **1**/**2** (13.0 mg, 20.2 umol) in MeOH (200 μL). The reaction was allowed to stir at rt for 10 min. The reaction mixture was diluted with H_2O and desalted by passing through a C_{18} SPE column. The products were then purified by HPLC using a Rainin Dynamax C_{18} column (250 × 10 mm) employing a gradient of 25% CH_3CN/75% H_2O (+0.1% AcOH) to 75% CH_3CN at 4.5 mL/min over 20 min to yield 11*R*-dihydro-precandidaspongiolide A (**7**, 4.76 mg, 37% yield) and 11*S*-dihydro-precandidaspongiolide A (**8**, 0.49 mg, 4% yield).

11*R*-dihydro precandidaspongiolide A (**7**): $[\alpha]^{25}_{D}$ + 49.7 (*c* 0.18, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.60) nm; ^1H NMR and ^{13}C NMR data, see Table S5; HRESIMS *m/z* 667.3304 $[M+Na]^+$ (calcd for $C_{32}H_{52}O_{13}Na$, 667.3300).

11*S*-dihydroprecandidaspongiolide A (**8**): $[\alpha]^{25}_{D}$ + 43.5 (*c* 0.02, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.41) nm; ^1H NMR and ^{13}C NMR data, see Table S6; HRESIMS *m/z* 667.3290 $[M+Na]^+$ (calcd for $C_{32}H_{52}O_{13}Na$, 667.3300).

MTT Cytotoxicity Assay. Cell survival was measured by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) cytotoxicity assay (Sigma, St Louis, MO).[4] Cells were seeded in 100 μL of medium at a density of 4×10^3 cells/well and allowed to incubate at 37°C in 5% CO_2 for 24 hours. Serially diluted drugs were then added in an additional 100 μL of medium and incubated for 72 hours. After removal of medium containing drug, MTT (0.5mg/mL) in IMDM growth medium was added to each well and incubated for four hours. The media solution was then removed from the wells, and 100 μL acidified 80% ethanol solution was added to lyse cells and dissolve the formazan product. Cell viability was measured spectrophotometrically at 570 nm and background corrected at 690 nm. All MTT assays were performed three times in triplicate. Cytotoxicity (IC_{50}) was defined as the drug concentration that reduced cell viability to 50% of the untreated control. Both KB-3-1 and KB-V1 were also coincubated with **1**/**2** and 100 nM tariquidar (Dr. Susan Bates, NCI).

Cell lines. The cell lines used were: the human epithelial adenocarcinoma cell line KB-3-1 and its P-gp-expressing multidrug resistant sub-line KB-V1; the human breast cancer cell line MCF-7; the human lung carcinoma cell line H460; and the human melanoma cell lines M14, LOX IMVI, and UACC-257. All cell lines were grown at 37°C in 5% CO_2 and cultured as follows. The KB, MCF7, M14, and UACC-257 cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM,) supplemented with 10% fetal bovine serum, 5 mM L-glutamine, 50 units/mL penicillin, and 50 μg/mL streptomycin, all obtained from Life Technologies (Carlsbad, California, USA). LOX IMVI cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium from Life Technologies (Carlsbad, California, USA) and supplemented as

[4] Brimacombe, K. R., Hall, M. D., Auld, D. S., Inglese, J., Austin, C. P., Gottesman, M. M., and Fung, K. L., *Assay Drug Dev. Technol.* **2009**, *7*, 233.

described above. Additionally, the multidrug resistant cell line KB-V1 was cultured in 1 μg/mL vinblastine to maintain P-glycoprotein expression.[5]

NCI-60 cell line screen. Growth inhibiton of 50% (GI_{50}) is defined as the concentration of a compound that causes a 50% reduction in cell growth compared to the untreated control. The GI_{50} is comparable to an IC_{50}, but takes into account the cell count at time zero and in untreated controls at the end of the assay period; the IC_{50} is calculated based on the number of cells in the untreated control when the assay endpoint is read. The GI_{50} is calculated as $100 \times (T - T_0)/(C - T_0) = 50$, where T is the test optical density, T_0 is the optical density at time zero, and C is the control optical density. The total growth inhibition (TGI) signifies a cytostatic effect, and is calculated as $100 \times (T - T_0)/(C - T_0) = 0$. The lethal concentration of 50% (LC_{50}) signifies a cytotoxic effect, and is calculated as $100 \times (T - T_0)/T_0 = -50$. The control optical density is not used in the calculation of LC_{50}.[6]

[5] Shen, D. W., Cardarelli, C., Hwang, J., Cornwell, M., Richert, N., Ishii, S., Pastan, I., and Gottesman, M. M. *J. Biol. Chem*, **1986**, *261*, 7762.
[6] Monks, A., Scudiero, D., Skehan, P., Showmaker, R., Paull, K., Vistica, D., Hose, C., Langley, J., Cronise, P., Vaigro-Wolff, A., Gray-Goodrich, M., Campbell, H., Mayo, J., Boyd, M., *J. Natl. Cancer Inst.*, ***1991***, *83*, 757.

Table S1. NMR Data for Candidaspongiolide A (**3**) in Acetone-d_6 (600 MHz, 500MHz[a])

No.	δ_C	δ_H, mult (J, Hz)
1	172.6[a]	—
2	72.3	3.79, d (1.9)
3	84.7	3.83, dd (9.5, 1.9)
4	48.5	3.19, dq (9.5, 7.0)
5	214.6[a]	—
6	49.0	3.41, dq (10.6, 7.0)
7	80.3	5.40, d (10.6)
8	134.2[a]	—
9	132.5	5.46, br d (9.8)
10	45.7	3.43, dq (9.8, 6.8)
11	210.9[a]	—
12a	43.8	2.72, dd (17.7, 9.7)
12b		2.31, dd (17.7, 1.7)
13	69.2	4.50, dd (9.7, 1.7)
14	85.2[a]	—
15	215.9[a]	—
16	48.0	4.12, ddd (11.0, 10.8, 4.1)
17	77.9	3.22, d (10.8)
18	63.0[a]	—
19	66.2	2.64, d (9.3)
20	31.9	2.47, ddd (10.7, 9.3, 6.6)
21	131.4	5.34 ddq (10.9, 10.7, 1.7)
22	125.3	5.49, dq (10.9, 6.8)
23	13.3	1.64, dd (6.8, 1.7)
24	14.9[b]	1.21, d (7.0)
25	14.6	1.16, d (7.0)
26	10.6	1.67, br s
27	15.2	0.96, d (6.8)
28	65.3[c]	3.77,[c] s
29a	64.4	4.34, dd (10.6, 4.1)
29b		3.90, dd (11.0, 10.6)
30	11.3	1.35, s
31	18.6	1.08, d (6.6)
32	60.9	3.40, s
33	170.0[a]	—
34	20.5	2.01, s

[a] Quaternary carbons obtained from original candidaspongiolide core NMR data (500 MHz).
[b] Originally misassigned. Careful inspection of original HSQC data for candidaspongiolide A (candidaspongiolide core) showed no correlation between δ_C 22.9 and δ_H 1.21.
[c] The multiplicity-edited HSQC used to characterize **3** clearly indicated a CH_2 multiplicity (opposite phase to CH/CH_3) for the correlation between δ_C 65.3 and δ_H 3.77, suggesting it was the C-28 primary alcohol. However, the $^1H/^{13}C$ chemical shifts did not match those in the literature for candidaspongiolide A (candidaspongioilde core). Further evaluation of the original COSY and HMBC spectra for candidapsongiolide A (candidaspongiolide core) indicated that δ_C 65.3 and δ_H 3.77 were the correct assignments for C-28, and H-28, respectively, and a minor contaminant was responsible for the correlation observed between δ_C 70.9 and δ_H 3.61.[7]

[7] See Supporting information for Meragelman, T. L.; Willis, R. H.; Woldemichael, G. M.; Heaton, A.; Murphy, P. T.; Snader, K. M.; Newman, D. J. ; van Soest, R.; Boyd, M. R.; Cardellina II, J. H.; McKee, T. C. *J. Nat. Prod.* **2007**, *70*, 1133.

Table S2. NMR Data for Tedanolide (**5**) (600 MHz, $CDCl_3$)

No.	δ_C	δ_H, mult (*J*, Hz)
1	171.8	—
2	71.7	3.86, br dd (8.7, 1.7)
3	83.5	3.66, dd (8.6, 1.7)
4	48.8	3.02,[a] (8.6, 7.0)
5	215.7	—
6	50.3	3.02,[a] (10.2, 6.9)
7	80.1	4.10, br dd (10.2, 2.5)
8	136.6	—
9	129.8	5.46,[a]
10	45.7	3.40, m (6.8)
11	213.1	—
12a	45.1	2.57, dd (17.0, 9.5)
12b		2.47, dd (17.0, 2.9)
13	68.7	4.29, m (9.5, 2.9)
14	53.6	3.03,[a] (6.9)
15	214.5	—
16	52.4	3.53, ddd (11.6, 9.6, 4.0)
17	77.4	3.23, br d (9.6, 3.2)
18	63.2	—
19	67.0	2.60, d (9.3)
20	31.6	2.42, ddq (10.6, 9.3, 6.5)
21	130.6	5.22, ddq (10.8, 10.6, 1.6)
22	125.5	5.45,[a] (10.8, 6.8)
23	13.6	1.60, dd (6.8, 1.6)
24	14.5	1.21, d (7.0)
25	15.8	1.27, d (6.9)
26	10.7	1.61, s
27	16.9	1.07, d (6.8)
28	10.9	1.10, d (6.9)
29a	64.3	4.25, dd (10.5, 4.0)
29b		4.11, dd (11.6, 10.5)
30	11.8	1.37, s
31	18.8	1.11, d (6.5)
32	60.8	3.29, s
2-OH		2.75, br d (8.7)
7-OH		1.44, br d (2.5)
13-OH		3.32, br d (3.2)
17-OH		2.13, br d (3.2)

[a] Signals overlapped.

Table S3. NMR Data for Tedanolide (**5**) (600 MHz, CD_3OD)

No.	δ_C	δ_H, mult (*J*, Hz)
1	171.5	—
2	72.6	3.76, d (1.7)
3	84.7	3.68, dd (9.6, 1.7)
4	51.0	3.12,[a] (9.6, 7.1)
5	216.4	—
6	49.6	3.11,[a] (10.5, 7.1)
7	80.2	4.00, d (10.5)
8	138.9	—
9	130.1	5.35, br d (10.0)
10	47.0	3.41, dq (10.0, 6.9)
11	211.2	—
12a	47.5	2.60, dd (16.9, 9.6)
12b		2.34, dd (16.9, 1.7)
13	69.3	4.22, ddd (9.6, 7.0, 1.7)
14	55.8	2.91, dq (7.0, 7.0)
15	214.1	—
16	54.5	3.43, ddd (11.2, 10.5, 3.9)
17	78.0	3.13,[a] (10.5)
18	63.9	—
19	67.7	2.58, d (9.3)
20	32.7	2.49, ddq (10.4, 9.3, 6.6)
21	132.1	5.31, ddq (10.8, 10.4, 1.6)
22	126.4	5.48, dq (10.8, 6.9)
23	13.6	1.63, dd (6.9, 1.6)
24	15.2	1.24, d (7.1)
25	16.1	1.27, d (7.1)
26	10.6	1.65, s
27	15.9	1.05, d (6.9)
28	12.0	1.15, d (7.0)
29a	65.5	4.25, dd (10.7, 3.9)
29b		4.00, dd (11.2, 10.7)
30	11.6	1.37, s
31	18.8	1.10, d (6.6)
32	61.1	3.34, s

[a] Signals overlapped.

Table S4. NMR Data for 28-acetyl-precandidaspongiolide A (**6**) (600 MHz, CD_3OD)

No.	δ_C	$\delta_{H,}$ mult (*J*, Hz)
1	172.5	—
2	72.2	3.79, d (2.1)
3	83.7	3.74, dd (9.3, 2.1)
4	49.0	3.10, dq (9.3, 7.1)
5	216.5	—
6	50.0	3.12, dq (10.1, 7.0)
7	79.5	4.00, d (10.1)
8	137.7	—
9	129.0	5.34, br d (9.6)
10	45.9	3.42, dq (9.6, 6.9)
11	212.1	—
12a	43.3	2.75, dd (17.1, 9.3)
12b		2.36, dd (17.1, 2.1)
13	68.7	4.38, dd (9.3, 2.1)
14	82.0	—
15	212.5	—
16	48.0	4.02,[a] (10.3, 3.4)
17	77.4	3.09, d (10.3)
18	63.1	—
19	66.5	2.61, d (9.3)
20	31.6	2.49, ddq (10.5, 9.3, 6.6)
21	130.8	5.31, ddq (10.8, 10.5, 1.6)
22	125.2	5.50, dq (10.8, 6.9)
23	12.7	1.64, dd (6.9, 1.6)
24	14.0	1.22, d (7.1)
25	14.9	1.25, d (7.0)
26	9.8	1.66, br s
27	15.4	1.06, d (6.9)
28a	64.2	4.25, d (11.0)
28b		4.20, d (11.0)
29a	63.2	4.35, dd (11.2, 3.4)
29b		4.01,[a] (11.2)
30	10.6	1.38, s
31	18.0	1.11, d (6.6)
32	60.5	3.36, s
33	171.6	—
34	19.9	2.01, s

[a] Signals overlapped.

Table S5. NMR Data for 11*R*-dihydro precandidaspongiolide A (**7**) (600 MHz, CD_3OD)

No.	δ_C	δ_H, mult (*J*, Hz)
1	172.2	—
2	71.6	3.64, d (2.2)
3	84.8	3.72,[a] dd (10.0, 2.2)
4	47.9	3.26, dq (10.0, 6.9)
5	217.0	—
6	51.1	3.23, dq (9.7, 7.2)
7	78.3	4.17, d (9.7)
8	135.8	—
9	130.0	5.62, br d (9.0)
10	38.1	2.19, dq (9.0, 7.1, 1.7)
11	76.3	3.74,[a] (10.5, 1.7, 1.7)
12a	36.2	1.53, ddd (14.1, 10.5, 10.5)
12b		1.06, ddd (14.1, 1.7, 1.7)
13	73.2	4.28, dd (10.5, 1.7)
14	84.9	—
15	215.6	—
16	47.1	4.03, ddd (11.1, 10.9, 4.1)
17	77.3	3.29,[b] (10.9)
18	62.8	—
19	66.4	2.64, d (9.3)
20	31.6	2.46, ddq (10.5, 9.3, 6.5)
21	130.8	5.37, ddq (10.7, 10.5, 1.6)
22	125.2	5.54, dq (10.7, 6.9)
23	12.5	1.62, dd (6.9, 1.6)
24	14.8	1.20, d (6.9)
25	13.9	1.26, d (7.2)
26	9.3	1.54, s
27	17.1	1.00, d (7.1)
28a	64.8	3.81, d (11.6)
28b		3.77, d (11.6)
29a	64.7	4.26, dd (10.6, 4.1)
29b		3.88, dd (11.1, 10.6)
30	10.6	1.30, s
31	17.7	1.11, d (6.5)
32	60.4	3.43, s

[a] Signals overlapped. [b] Buried under CD_3OD signal.

Table S6. NMR Data for 11*S*-dihydro precandidaspongiolide A (**8**) (600 MHz, CD_3OD)

No.	δ_C	δ_H, mult (*J*, Hz)
1	172.2	—
2	71.5	3.59, d (1.9)
3	84.1	3.84,[a] (10.0, 1.9)
4	48.1	3.25, dq (10.0, 6.9)
5	217.8	—
6	50.8	3.18, dq (10.3, 7.0)
7	78.7	4.07, d (10.3)
8	135.0	—
9	133.2	5.18, br d (8.9)
10	39.5	2.09, ddq (9.9, 8.9, 6.8)
11	73.3	3.40, ddd (11.0, 9.9, 1.5)
12a	38.4	1.48, ddd (14.1, 10.9, 1.5)
12b		0.88, ddd (14.1, 11.0, 1.8)
13	69.5	4.27,[a] (10.9, 1.8)
14	85.2	—
15	216.7	—
16	47.3	4.05, ddd (11.3, 11.1, 4.0)
17	77.2	3.29, d (11.1)
18	62.6	—
19	66.5	2.63, d (9.5)
20	31.5	2.46, ddq (10.6, 9.5, 6.6)
21	130.9	5.36, ddq (10.8, 10.6, 1.3)
22	125.2	5.54, ddq (10.8, 6.8)
23	12.6	1.62, dd (6.8, 1.3)
24	14.6	1.22, d (6.9)
25	14.3	1.26, d (7.0)
26	9.5	1.55, br s
27	17.1	1.01, d (6.8)
28a	66.5	3.85, d (11.7)
28b		3.81, d (11.7)
29a	64.9	4.27,[a] (4.0)
29b		3.82,[a] (11.3)
30	10.4	1.30, s
31	17.6	1.11, d (6.6)
32	60.3	3.42, s

[a] Signals overlapped.

Figure S1. NCI 60-cell line screen, single dose (10^{-5} M) of **1/2**

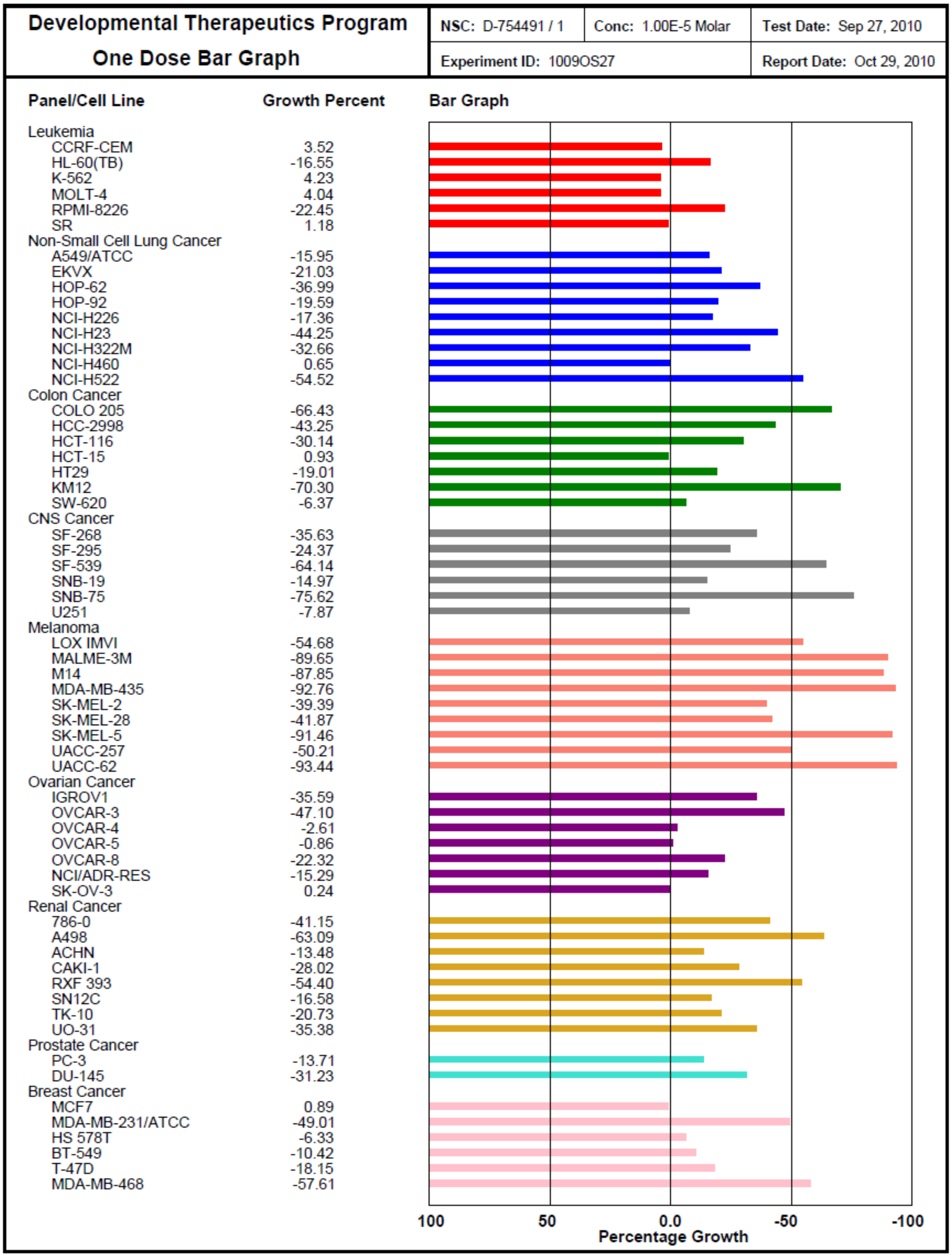


Figure S2. NCI 60-cell line screen, single dose (10^{-5} M) of **1**/**2**

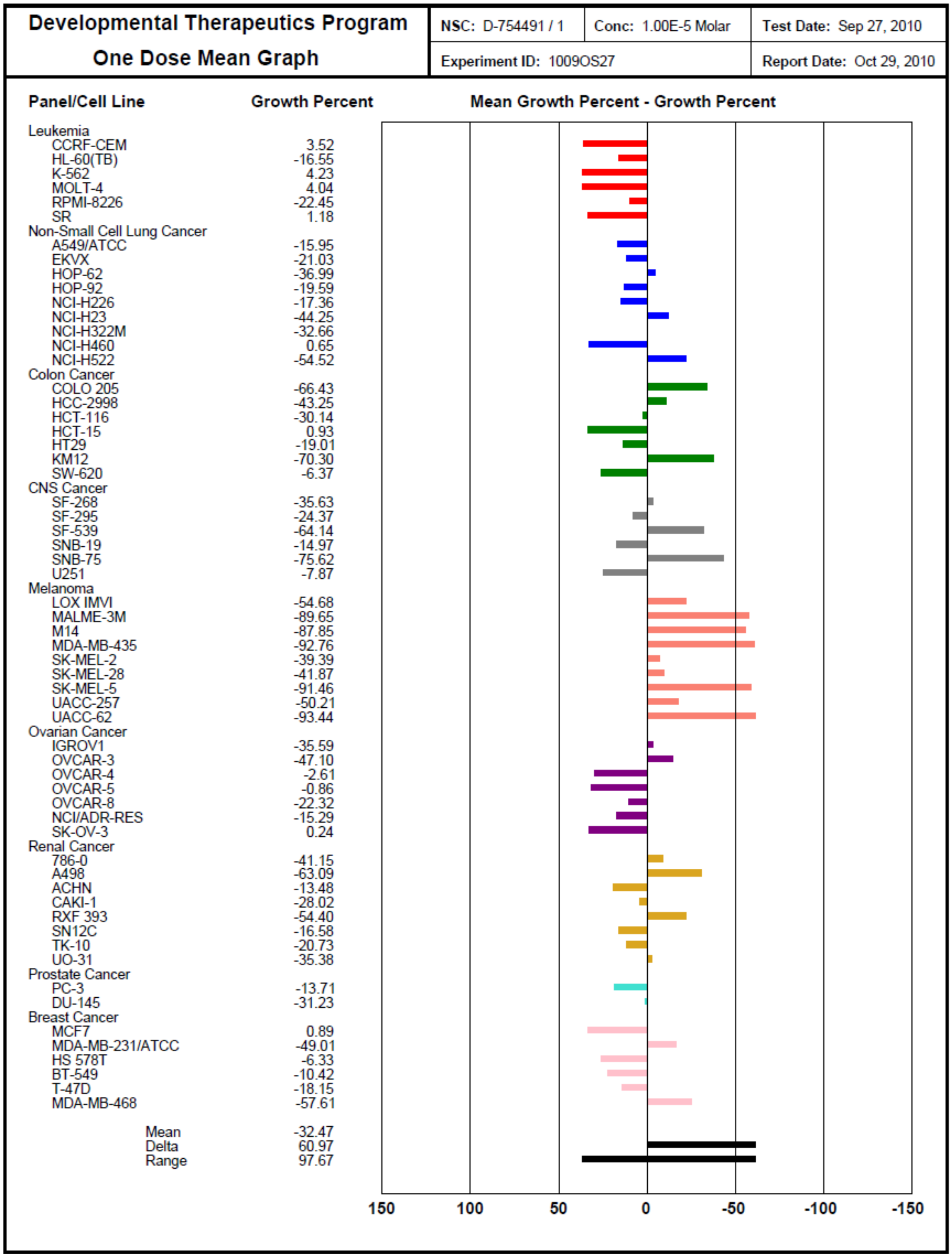


Figure S3. NCI 60-cell line screen, dose response curves for **1**/**2**

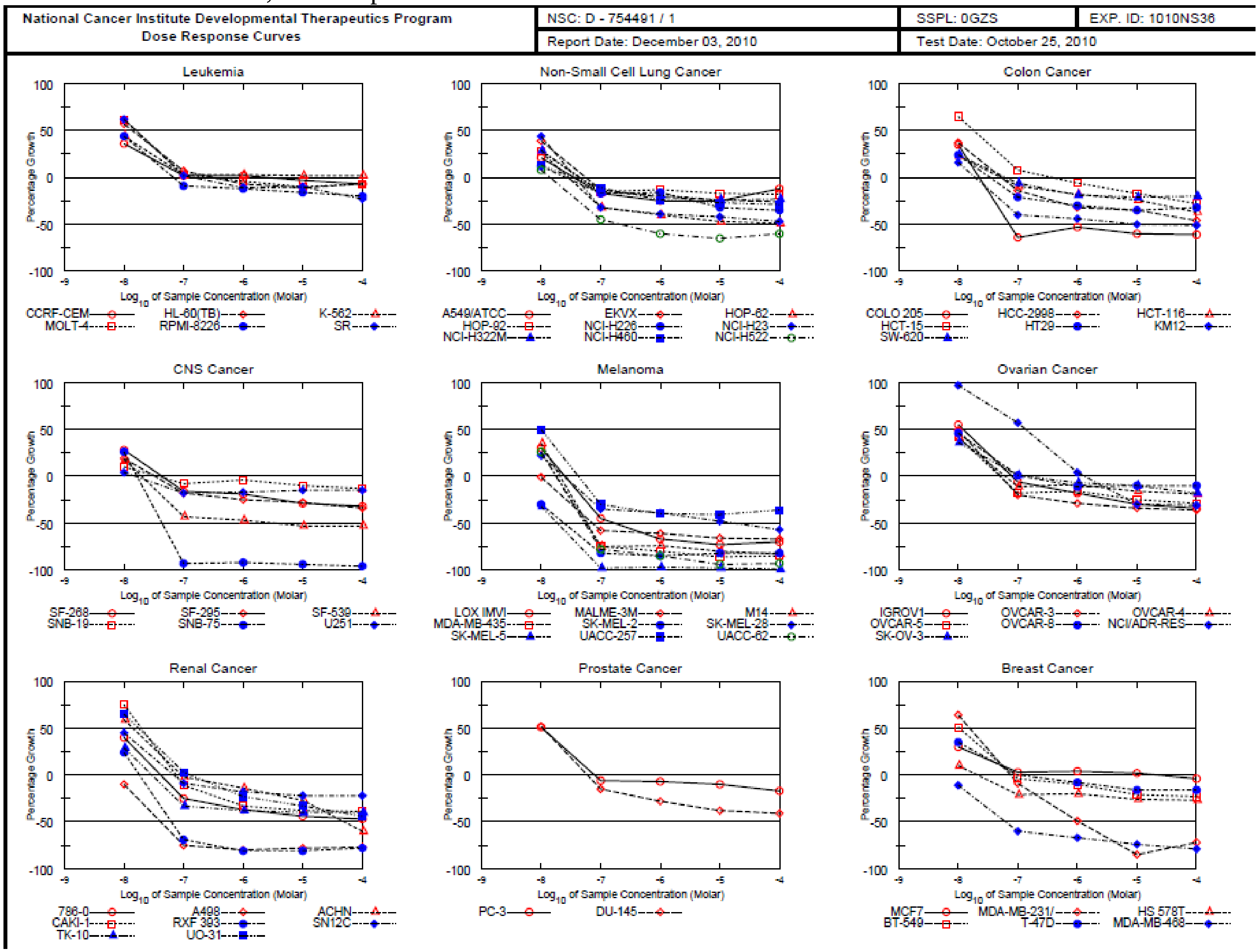


Figure S4. NCI 60-cell line screen, mean bar graphs for **1/2**

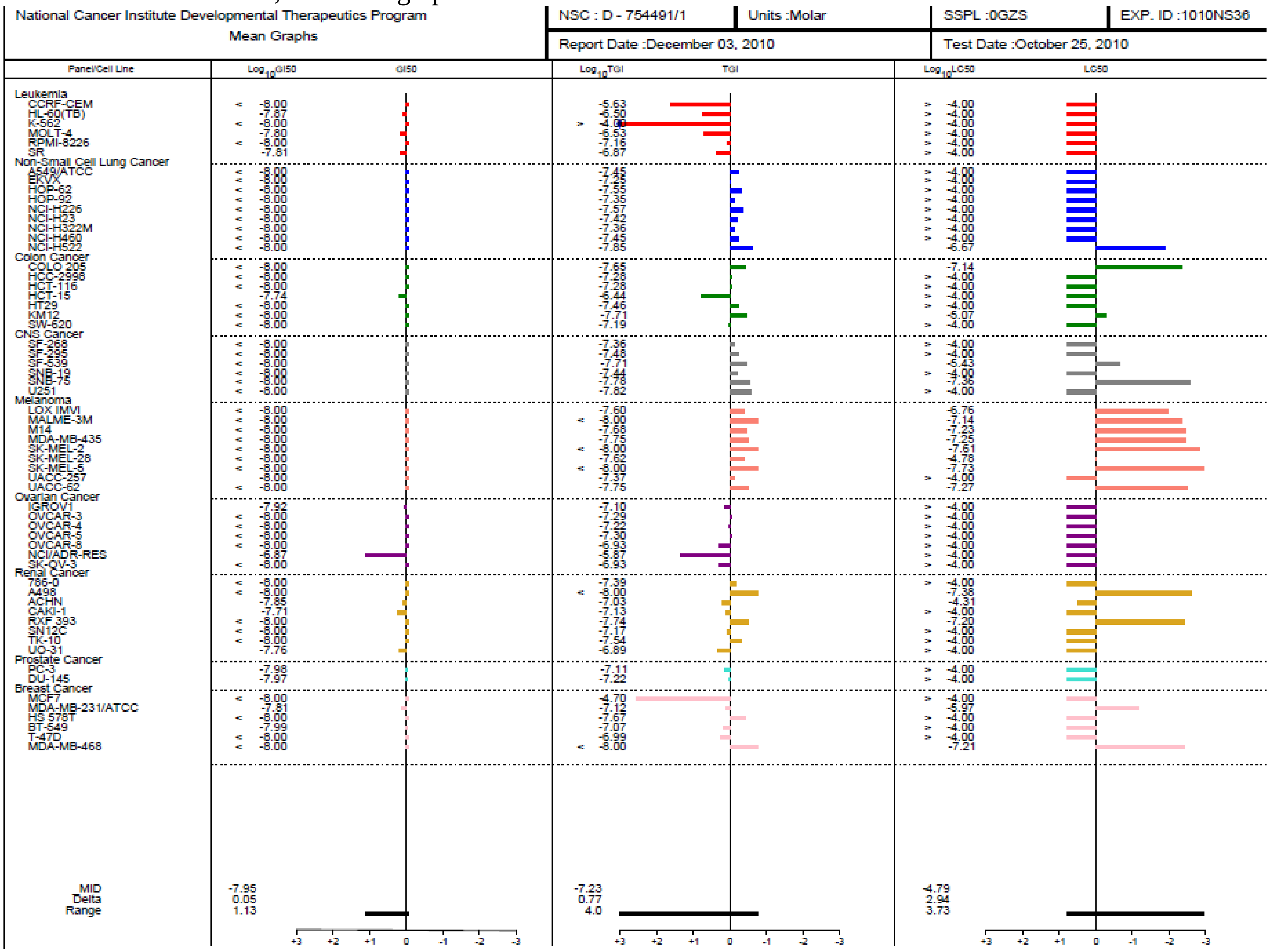


Figure S5. NCI 60-cell line screen, dose response curves for candidaspongiolide acyl ester mixture

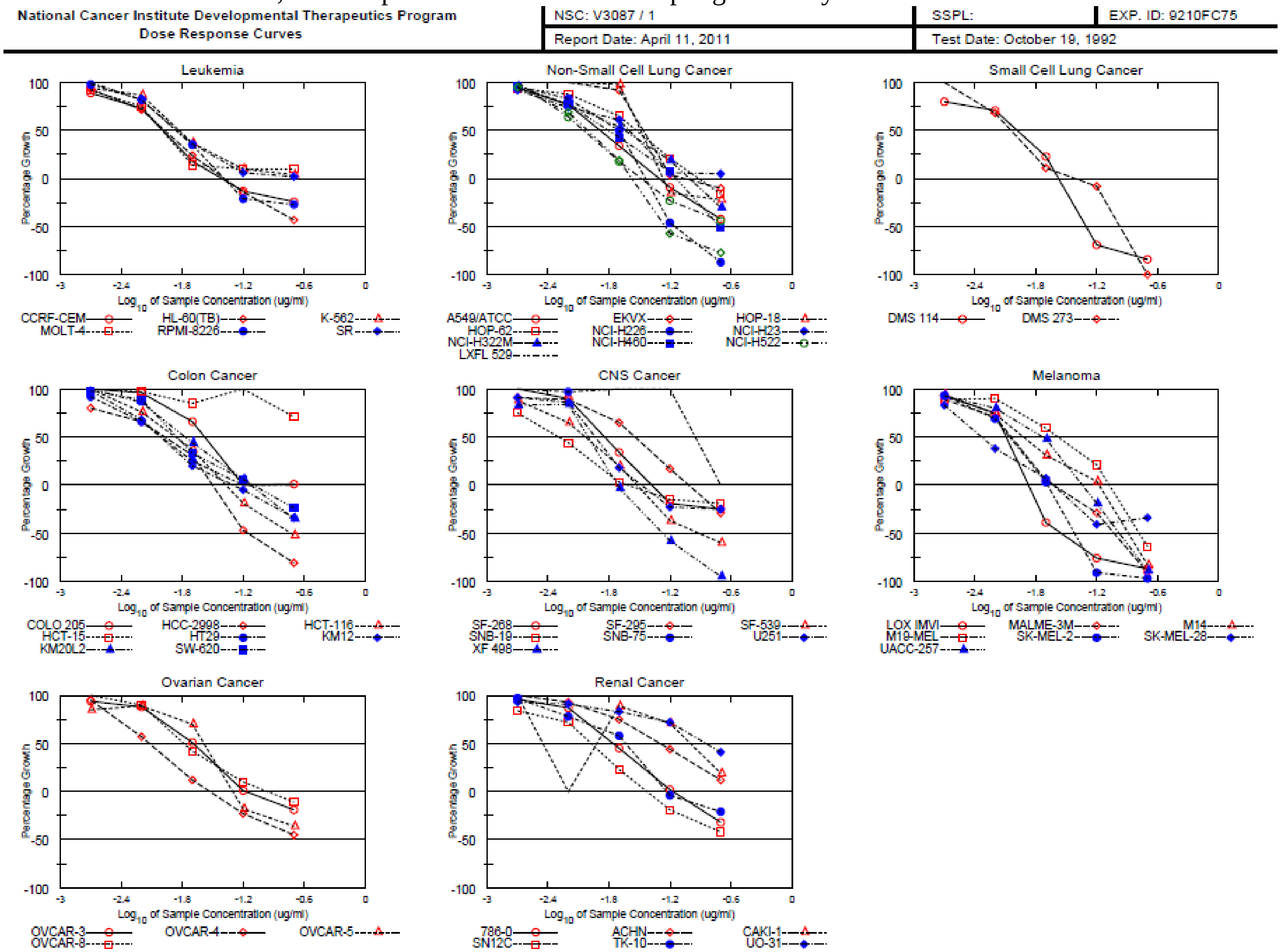


Figure S6. NCI 60-cell line screen, mean bar graphs for candidaspongiolide acyl ester mixture

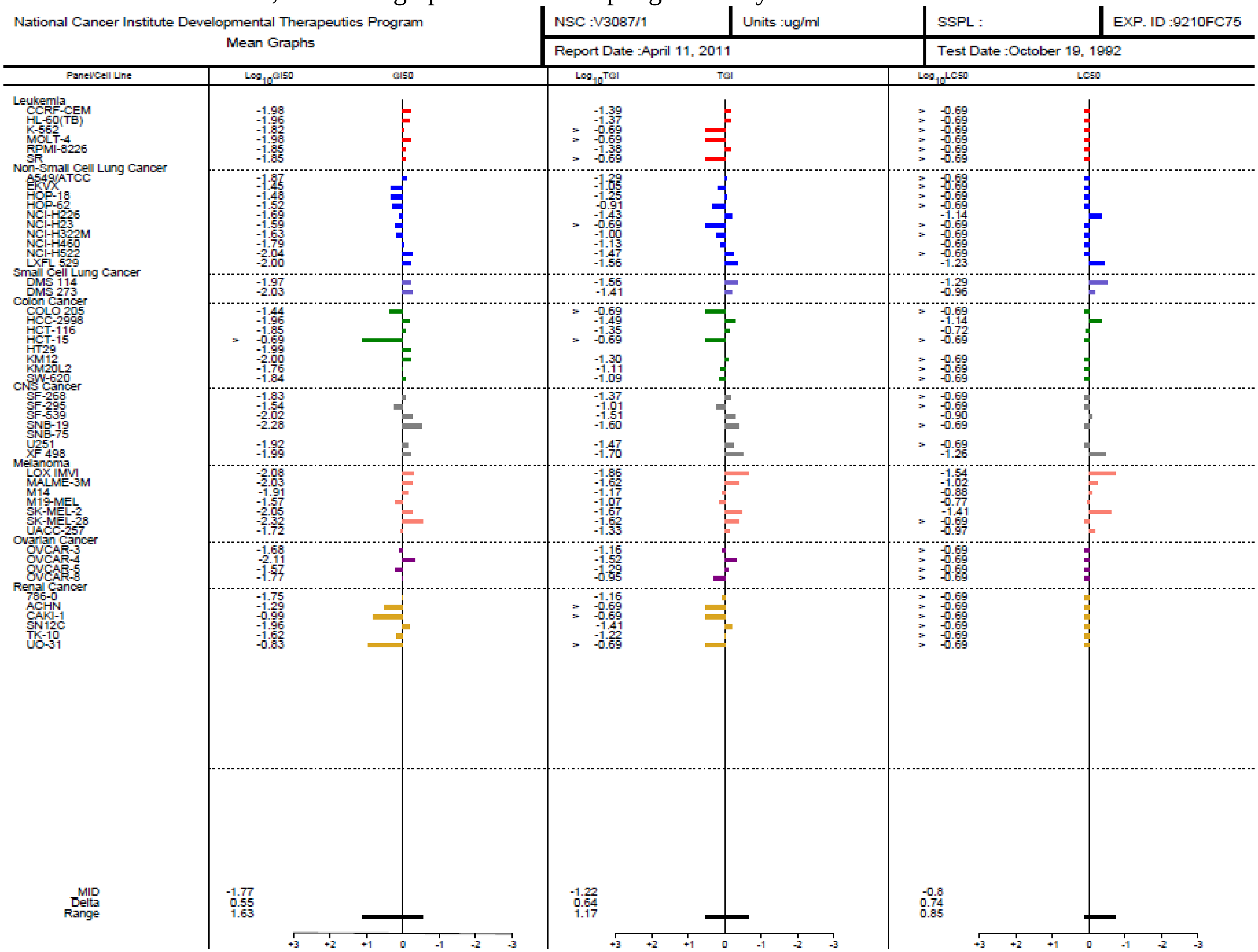


Figure S7. NCI 60-cell line screen, dose response curves for candidaspongiolide A (macrolide core)

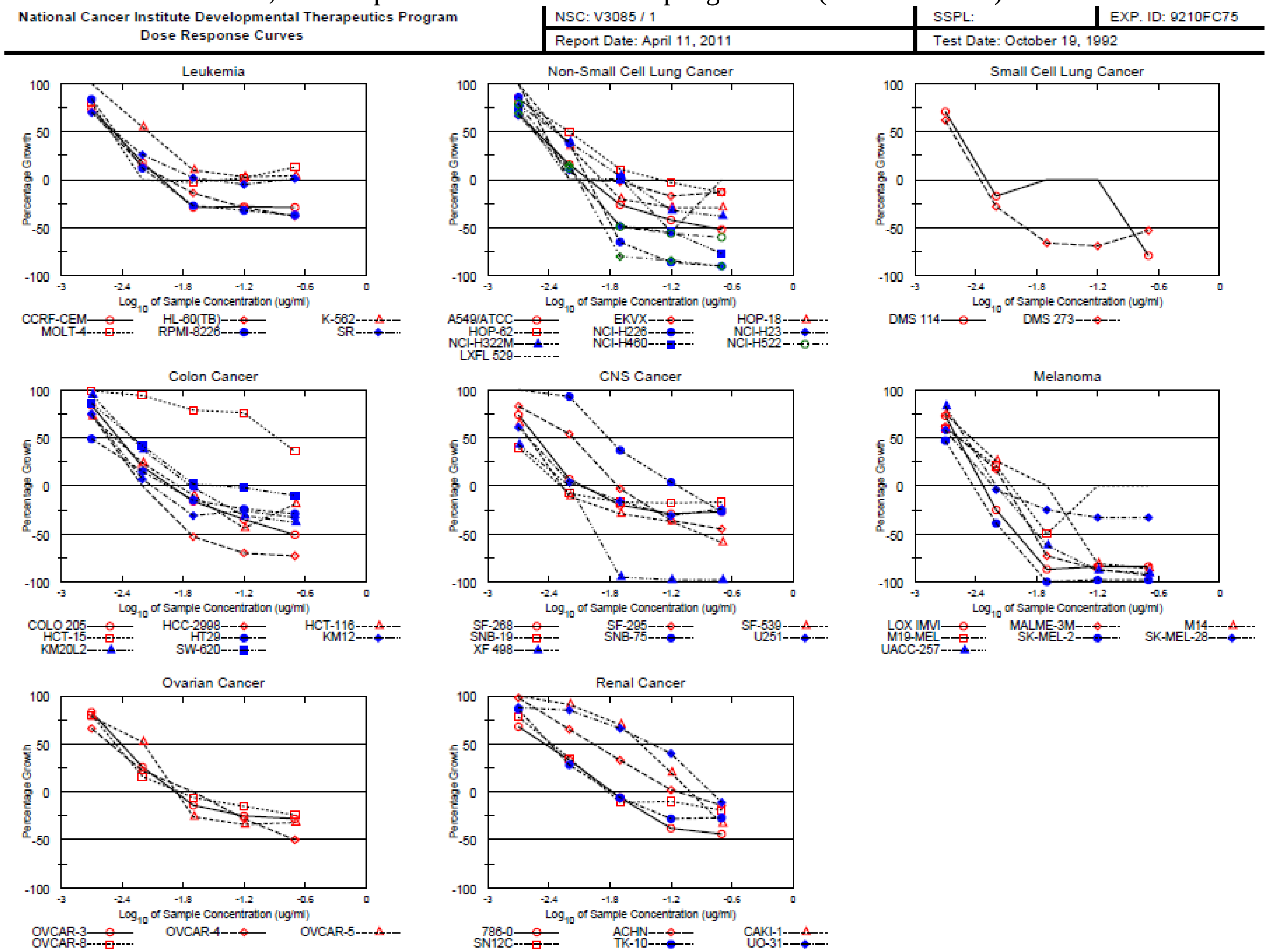


Figure S8. NCI 60-cell line screen, mean bar graphs for candidaspongiolide A (macrolide core)

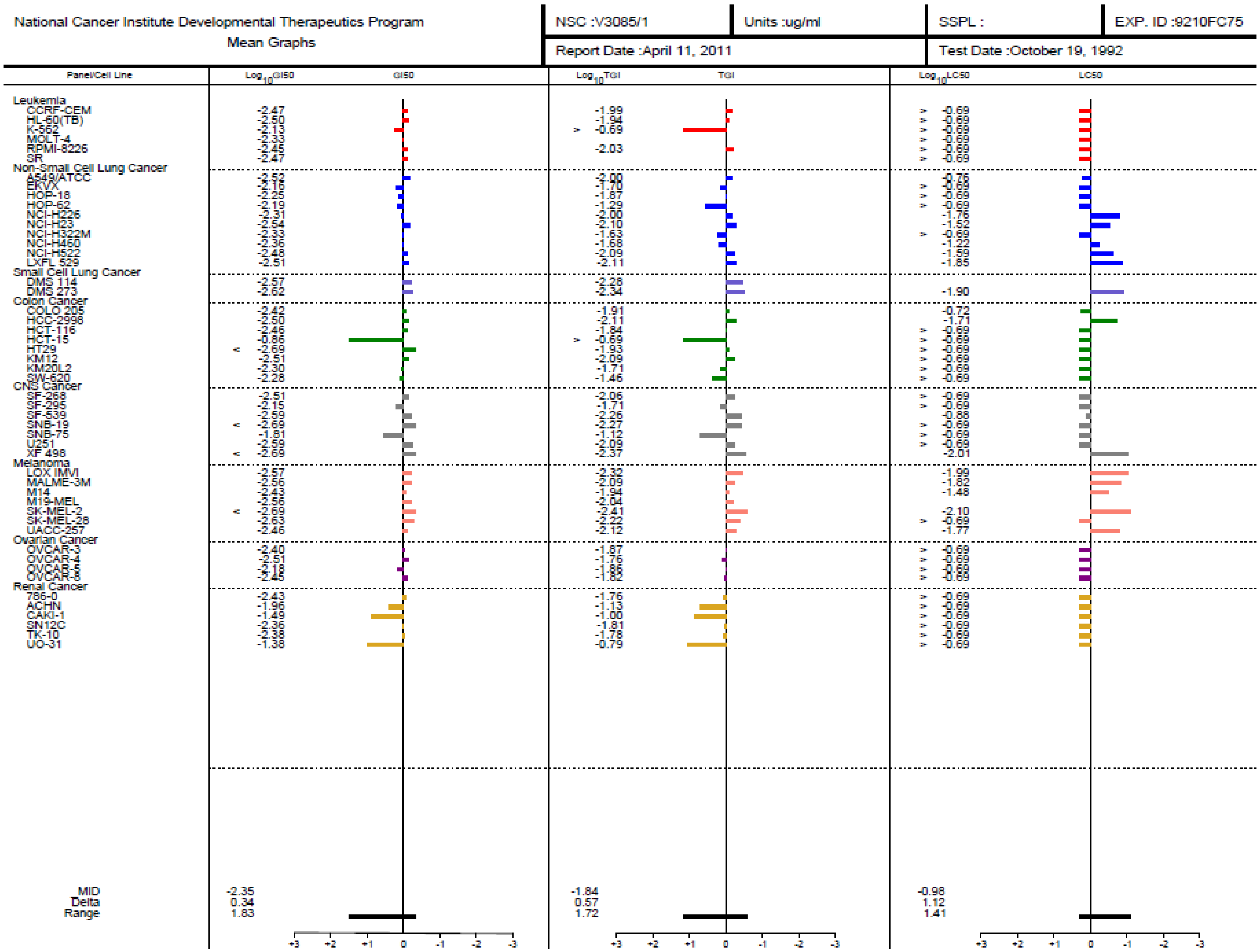


Table S7. Melanoma GI_{50}'s for candidaspongiolide acyl ester mixture

Melanoma Cell Line	GI_{50} (nM)[a]
LOX IMVI	9.0
MALME-3M	10.1
M14	13.3
M19-MEL	29.0
SK-MEL-2	9.6
SK-MEL-28	5.2
UACC-257	20.6
Melanoma Average	**13.8**
Mean GI_{50} All NCI-60 Cell Lines	18.3

[a] Based on an estimated average mass of 927.1, calculated utilizing the reported fatty acid compositions (894.5 = 4.4%; 908.5 = 10.6%; 922.6 = 47.3%; 936.6 = 22.7%; 948.6 = 5.2%; 950.6 = 9.8%). Note: 10% of the fatty acid mixture was unidentified and percentages have been re-calculated to total 100%.[8]

Table S8. Melanoma GI_{50}'s for candidaspongiolide A (macrolide core)

Melanoma Cell Line	GI_{50} (nM)
LOX IMVI	3.9
MALME-3M	4.0
M14	5.4
M19-MEL	4.0
SK-MEL-2	< 3.0
SK-MEL-28	3.4
UACC-257	5.1
Melanoma Average	< **4.1**
Mean GI_{50} All NCI-60 Cell Lines	6.5

[8] Meragelman, T. L.; Willis, R. H.; Woldemichael, G. M.; Heaton, A.; Murphy, P. T.; Snader, K. M.; Newman, D. J. ; van Soest, R.; Boyd, M. R.; Cardellina II, J. H.; McKee, T. C. *J. Nat. Prod.* **2007**, *70*, 1133.

Figure S9. Melanoma IC_{50} curves for **1**/**2**, **3**/**4**, **5-8**

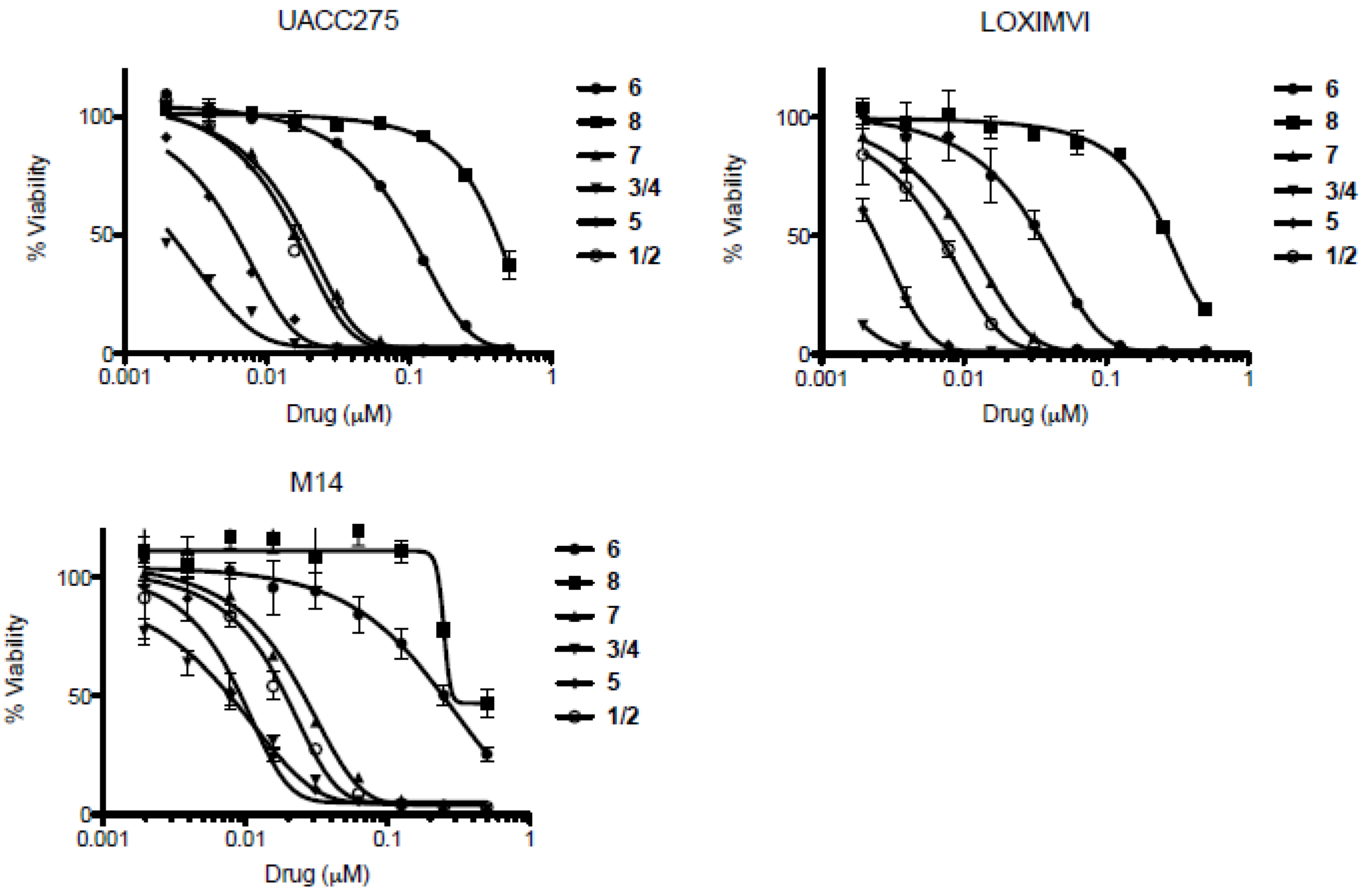


Figure S10. Breast and Lung IC_{50} curves for **1**/**2**, **3**/**4**, **5-8**

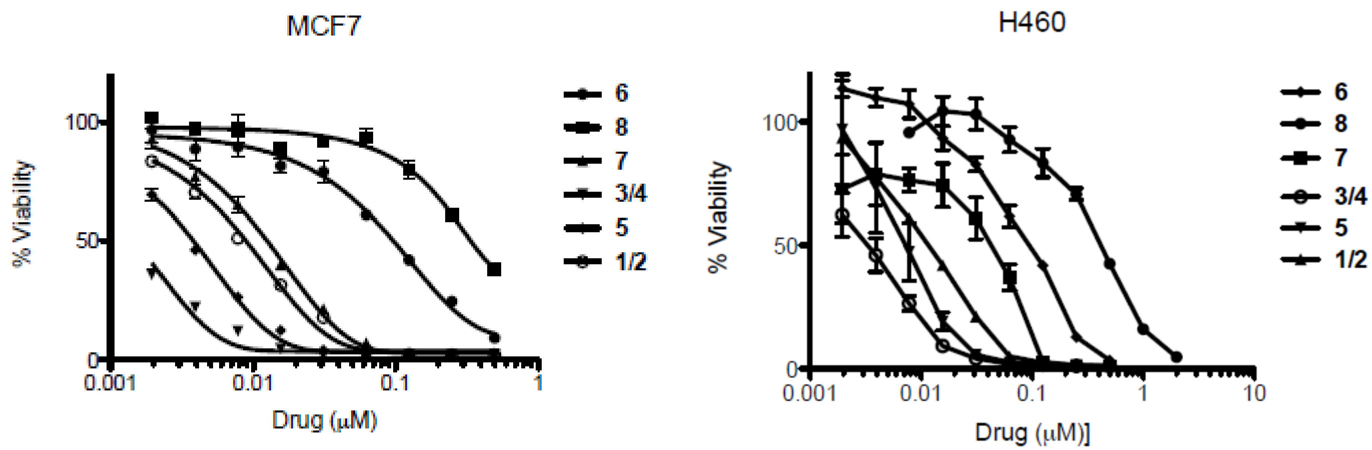
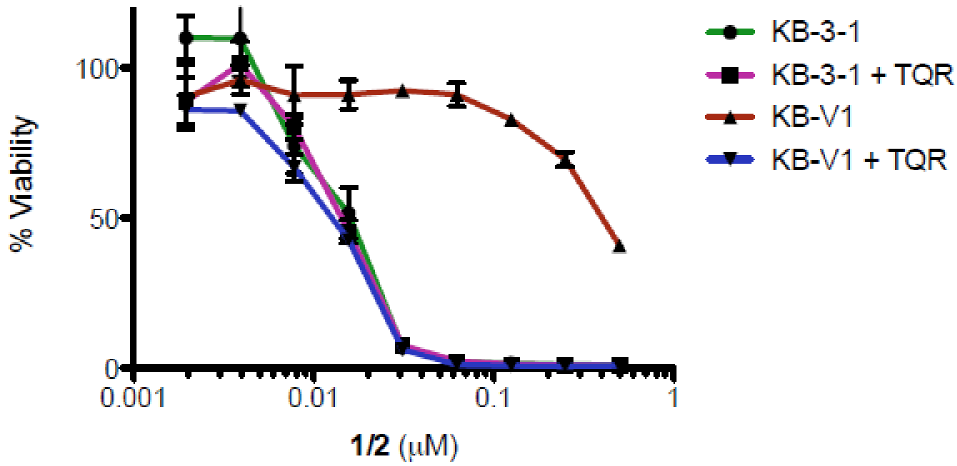


Figure S11. Precandidaspongiolides A (**1**) and B (**2**) are P-gp substrates



	1/2 (nM)	**1/2** + TQR (nM)
KB-3-1	15.7 ± 4.9	14.7 ± 1.5
KB-V1	419.3 ± 15.0	13.1 ± 0.8
RR	26.7	N/A