## New Candidaspongiolides, Tedanolide Analogs that Selectively Inhibit Melanoma Cell Growth

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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 (<sup>1</sup>H 600 MHz, <sup>13</sup>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.<sup>1</sup> 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<sup>2</sup> to give the aqueous crude extracts. A portion of this extract (520 mg) was subjected to size-exclusion chromatography on Sephadex LH-20 ( $2.5 \times 90$  cm) using hexanes/CH<sub>2</sub>Cl<sub>2</sub>/MeOH (2:5:1) to yield seven fractions (141A-141G). Fraction 141F was chromatographed on C<sub>18</sub> ( $2.0 \times 17$  cm)

<sup>&</sup>lt;sup>1</sup> 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.

<sup>&</sup>lt;sup>2</sup> McCloud, T. G. Molecules, **2010**, *15*, 4526.

using 50/50 50% CH<sub>3</sub>CN/50% H<sub>2</sub>O (+0.1% AcOH) to yield precandidaspongiolides A/B (1/2, 11.2 mg). Fraction 141C was chromatographed on C<sub>18</sub> (2.0 × 17 cm) using 55% CH<sub>3</sub>CN/45% H<sub>2</sub>O (+0.1% AcOH) to yield seven fractions (154A-154G). Fraction 154F was purified by HPLC using a Rainin Dynamax C<sub>18</sub> column (250 × 10 mm) employing a gradient of 35% CH<sub>3</sub>CN/65% H<sub>2</sub>O (+0.1% AcOH) to 85% CH<sub>3</sub>CN 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):**  $[α]^{25}_{D}$  + 58.3 (*c* 0.23, MeOH); UV (MeOH)  $λ_{max}$  (log ε) 205 (3.69) nm; <sup>1</sup>H NMR and <sup>13</sup>C NMR data, see Table 1; HRESIMS *m/z* 665.3146 [M+Na]<sup>+</sup> (calcd for C<sub>32</sub>H<sub>50</sub>O<sub>13</sub>Na, 665.3144).

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

(+)-**Tedanolide** (5):  $[\alpha]_{D}^{25} + 20$  (*c* 0.02, MeOH); UV (MeOH)  $\lambda_{max}$  (log  $\varepsilon$ ) 204 (3.79) nm; <sup>1</sup>H NMR and <sup>13</sup>C NMR data in CDCl<sub>3</sub> and CD<sub>3</sub>OD, see Tables S2 and S3; HRESIMS *m/z* 633.3232 [M+Na]<sup>+</sup> (calcd for C<sub>32</sub>H<sub>50</sub>O<sub>11</sub>Na, 633.3245).

Acetylation of Precandidaspongiolides A and B (1/2). A stock solution of AcCl was prepared on ice by dissolving 1  $\mu$ L of AcCl in 50  $\mu$ L of anhydrous CH<sub>2</sub>Cl<sub>2</sub>. 7  $\mu$ L of the AcCl solution (2  $\mu$ mol) was added to a stirring solution of 1/2 (1.1 mg, 1.7  $\mu$ mol) in 2,4,6trimethylpyridine (excess) at -40 °C.<sup>3</sup> 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<sub>2</sub>O and

<sup>&</sup>lt;sup>3</sup> Ishihara, K.; Kurihara, H.; Yamamoto, H. J. Org. Chem. 1993, 58, 3791.

dried under N<sub>2</sub>. The reaction mixture was then purified by HPLC using a Rainin Dynamax C<sub>18</sub> column ( $250 \times 10$  mm) employing a gradient of 35% CH<sub>3</sub>CN/65% H<sub>2</sub>O (+0.1% AcOH) to 85% CH<sub>3</sub>CN 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)  $\lambda_{max}$  (log  $\epsilon$ ) 204 (3.38) nm; <sup>1</sup>H NMR and <sup>13</sup>C NMR data, see Table S4; HRESIMS *m/z* 707.3245 [M+Na]<sup>+</sup> (calcd for C<sub>34</sub>H<sub>52</sub>O<sub>14</sub>Na, 707.3249).

Reduction of Precandidaspongiolides A and B (1/2). NaBH<sub>4</sub> (~3 mg, excess) was added to a semi-pure (~75%) solution of 1/2 (13.0 mg, 20.2 umol) in MeOH (200  $\mu$ L). The reaction was allowed to stir at rt for 10 min. The reaction mixture was diluted with H<sub>2</sub>O and desalted by passing through a C<sub>18</sub> SPE column. The products were then purified by HPLC using a Rainin Dynamax C<sub>18</sub> column (250 × 10 mm) employing a gradient of 25% CH<sub>3</sub>CN/75% H<sub>2</sub>O (+0.1% AcOH) to 75% CH<sub>3</sub>CN 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**):  $[α]^{25}_{D}$  + 49.7 (*c* 0.18, MeOH); UV (MeOH)  $λ_{max}$ (log ε) 204 (3.60) nm; <sup>1</sup>H NMR and <sup>13</sup>C NMR data, see Table S5; HRESIMS *m/z* 667.3304 [M+Na]<sup>+</sup> (calcd for C<sub>32</sub>H<sub>52</sub>O<sub>13</sub>Na, 667.3300).

**11S-dihydroprecandidaspongiolide A** (8):  $[\alpha]^{25}_{D}$  + 43.5 (*c* 0.02, MeOH); UV (MeOH)  $\lambda_{max}$ (log  $\varepsilon$ ) 204 (3.41) nm; <sup>1</sup>H NMR and <sup>13</sup>C NMR data, see Table S6; HRESIMS *m*/*z* 667.3290 [M+Na]<sup>+</sup> (calcd for C<sub>32</sub>H<sub>52</sub>O<sub>13</sub>Na, 667.3300). MTT Cytotoxicity Assay. Cell survival was measured by the MTT (3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide) cytotoxicity assay (Sigma, St Louis, MO).<sup>4</sup> Cells were seeded in 100  $\mu$ L of medium at a density of 4 × 10<sup>3</sup> cells/well and allowed to incubate at 37°C in 5% CO<sub>2</sub> for 24 hours. Serially diluted drugs were then added in an additional 100  $\mu$ L 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  $\mu$ L 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<sub>50</sub>) 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<sub>2</sub> 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

<sup>&</sup>lt;sup>4</sup> 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  $\mu$ g/mL vinblastine to maintain P-glycoprotein expression.<sup>5</sup>

**NCI-60 cell line screen.** Growth inhibiton of 50% (GI<sub>50</sub>) is defined as the concentration of a compound that causes a 50% reduction in cell growth compared to the untreated control. The GI<sub>50</sub> is comparable to an IC<sub>50</sub>, but takes into account the cell count at time zero and in untreated controls at the end of the assay period; the IC<sub>50</sub> is calculated based on the number of cells in the untreated control when the assay endpoint is read. The GI<sub>50</sub> is calculated as  $100 \times (T - T_0)/(C - T_0) = 50$ , where T is the test optical density, T<sub>0</sub> 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<sub>50</sub>) 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<sub>50</sub>.<sup>6</sup>

<sup>&</sup>lt;sup>5</sup> 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.

<sup>&</sup>lt;sup>6</sup> 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.

No.	$\frac{\delta_{\rm C}}{\delta_{\rm C}}$	$\frac{\text{durifor cundiduspongroude (V(S) m}}{\delta_{\text{H}} \text{ mult } (J, \text{Hz})}$
1	172.6ª	
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 <sup>a</sup>	
6	49.0	3.41, dq (10.6, 7.0)
7	80.3	5.40, d (10.6)
8	134.2 <sup>a</sup>	<u> </u>
9	132.5	5.46, br d (9.8)
10	45.7	3.43, dq (9.8, 6.8)
11	210.9 <sup>a</sup>	_
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	$85.2^{\mathrm{a}}$	_
15	215.9 <sup>a</sup>	_
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 <sup>b</sup>	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 <sup>c</sup>	3.77, <sup>°</sup> 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 <sup>a</sup>	
<u>34</u>	20.5	2.01, s
i matern	ary carnons c	onamed from original candidaspongiolide core NMR data (500

**Table S1**. NMR Data for Candidaspongiolide A (3) in Acetone- $d_6$  (600 MHz, 500MHz<sup>a</sup>)

<sup>a</sup> Quaternary carbons obtained from original candidaspongiolide core NMR data (500 MHz).

<sup>b</sup> Originally misassigned. Careful inspection of original HSQC data for candidaspongiolide A (candidaspongiolide core) showed no correlation between  $\delta_C$  22.9 and  $\delta_H$  1.21.

<sup>c</sup> The multiplicity-edited HSQC used to characterize **3** clearly indicated a CH<sub>2</sub> multiplicity (opposite phase to CH/CH<sub>3</sub>) for the correlation between  $\delta_C$  65.3 and  $\delta_H$  3.77, suggesting it was the C-28 primary alcohol. However, the <sup>1</sup>H/<sup>13</sup>C chemical shifts did not match those in the literature for candidaspongiolide A (candidaspongiolide core). Further evaluation of the original COSY and HMBC spectra for candidapsongiolide A (candidaspongiolide core) indicated that  $\delta_C$  65.3 and  $\delta_H$  3.77 were the correct assignments for C-28, and H-28, respectively, and a minor contaminant was responsible for the correlation observed between  $\delta_C$  70.9 and  $\delta_H$  3.61.<sup>7</sup>

<sup>&</sup>lt;sup>7</sup> 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.

No.	$\delta_{C}$	$\delta_{\rm H}$ mult ( <i>J</i> , 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, <sup>a</sup> (8.6, 7.0)
5	215.7	_
6	50.3	3.02, <sup>a</sup> (10.2, 6.9)
7	80.1	4.10, br dd (10.2, 2.5)
8	136.6	
9	129.8	5.46, <sup>a</sup>
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 <sup>, a</sup> (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, <sup>a</sup> (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	overlanne	2.13, br d (3.2)

 Table S2. NMR Data for Tedanolide (5) (600 MHz, CDCl<sub>3</sub>)

No.	$\delta_{C}$	$\delta_{\rm H} \text{ mult } (J, \text{Hz})$
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, <sup>a</sup> (9.6, 7.1)
5	216.4	_
6	49.6	3.11, <sup>a</sup> (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, <sup>a</sup> (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)
$\frac{32}{a \text{ Signala}}$	61.1	3.34, s

Table S3. NMR Data for Tedanolide (5) (600 MHz, CD<sub>3</sub>OD)

No.	$\delta_{C}$	$\delta_{\rm H,}$ mult ( <i>J</i> , 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, <sup>a</sup> (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, <sup>a</sup> (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
<sup>a</sup> Signals	s overlappe	d

Table S4. NMR Data for 28-acetyl-precandidaspongiolide A (6) (600 MHz, CD<sub>3</sub>OD)

No.	$\delta_{\rm C}$	$\delta_{\rm H}$ mult (J, Hz)
1	172.2	
2	71.6	3.64, d (2.2)
3	84.8	3.72, <sup>a</sup> 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 <sup>, a</sup> (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 <sup>, b</sup> (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	10 -	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 1. <sup>b</sup> Buried under CD <sub>2</sub> OD signal.

Table S5. NMR Data for 11*R*-dihydro precandidaspongiolide A (7) (600 MHz, CD<sub>3</sub>OD)

<sup>a</sup> Signals overlapped. <sup>b</sup> Buried under CD<sub>3</sub>OD signal.

No.	$\delta_{\rm C}$	$\delta_{\rm H,}$ mult (J, Hz)
1	172.2	_
2	71.5	3.59, d (1.9)
3	84.1	3.84, <sup>a</sup> (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, <sup>a</sup> (10.9, 1.8)
14	85.2	<u> </u>
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 <sup>, a</sup> (4.0)
29b		3.82, <sup>a</sup> (11.3)
30	10.4	1.30, s
31	17.6	1.11, d (6.6)
32	60.3	3.42, s
<sup>a</sup> Signa	ls overla	nnad

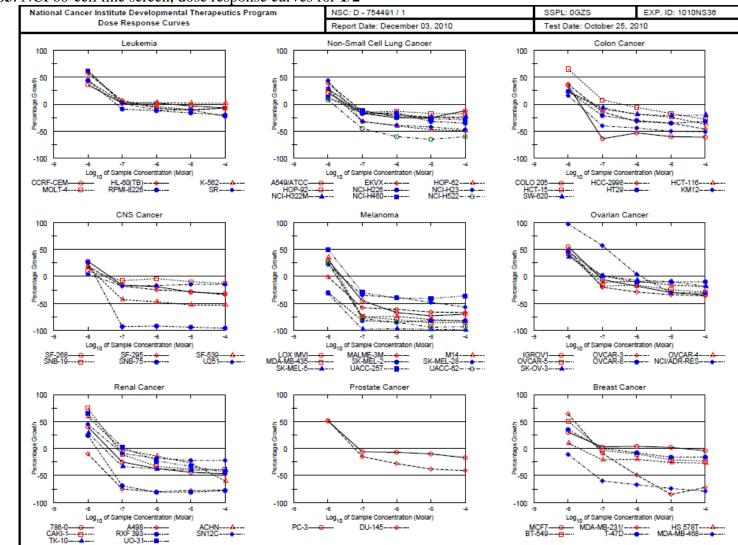
**Table S6**. NMR Data for 11*S*-dihydro precandidaspongiolide A (8) (600 MHz, CD<sub>3</sub>OD)

Developmental Therap	eutics Program	NSC: D-754491/1	Conc: 1.00E-5 Molar	Test Date: Sep 27, 2010
One Dose Bar G	raph	Experiment ID: 1009	Report Date: Oct 29, 2010	
Panel/Cell Line	Growth Percent	Bar Graph		
Leukemia				
CCRF-CEM HL-60(TB)	3.52 -16.55			
K-562	4.23			
MOLT-4	4.04			
RPMI-8226	-22.45			
SR	1.18			
Non-Small Cell Lung Cancer A549/ATCC	-15.95			
EKVX	-21.03			
HOP-62	-36.99			<b>_</b>
HOP-92	-19.59			
NCI-H226	-17.36			
NCI-H23	-44.25			
NCI-H322M NCI-H460	-32.66 0.65			
NCI-H522	-54.52			
Colon Cancer				
COLO 205	-66.43			
HCC-2998	-43.25			
HCT-116	-30.14			
HCT-15 HT29	0.93 -19.01			
KM12	-70.30			
SW-620	-6.37			
CNS Cancer				
SF-268	-35.63			
SF-295 SF-539	-24.37			
SF-009 SNB-19	-64.14 -14.97			
SNB-75	-75.62			
U251	-7.87			
Melanoma				
LOX IMVI	-54.68			
MALME-3M M14	-89.65 -87.85			
MDA-MB-435	-92.76			
SK-MEL-2	-39.39			_
SK-MEL-28	-41.87			_
SK-MEL-5	-91.46			
UACC-257 UACC-62	-50.21 -93.44			
Ovarian Cancer	-30.44			
IGROV1	-35.59			-
OVCAR-3	-47.10			
OVCAR-4	-2.61			
OVCAR-5 OVCAR-8	-0.86 -22.32			
NCI/ADR-RES	-15.29			
SK-OV-3	0.24			
Renal Cancer				
786-0	-41.15			
A498 ACHN	-63.09 -13.48			
CAKI-1	-13.46 -28.02			
RXF 393	-54.40			<b>—</b>
SN12C	-16.58			
TK-10	-20.73			_
UO-31 Prostate Cancer	-35.38			-
PC-3	-13.71			
DU-145	-31.23			
Breast Cancer				
MCF7	0.89			
MDA-MB-231/ATCC	-49.01			
HS 578T BT-549	-6.33 -10.42			
T-47D	-18.15			
MDA-MB-468	-57.61			
		100 50	0.0	-50 -100
			Percentage Growth	

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

Developmental Thera	apeutics <b>P</b> rogram	NSC: D-754491/1	Conc: 1.00E-5 Molar	Test Date: Sep 27, 2010
One Dose Mea	an Graph	Experiment ID: 1009	IOS27	Report Date: Oct 29, 2010
Panel/Cell Line	Growth Percent	Mean Growth	Percent - Growth Perc	cent
Leukemia CCRF-CEM	3.52 -16.55			
HL-60(TB) K-562	-16.55 4.23			
MOLT-4	4.04			
RPMI-8226 SR	-22.45 1.18			
Non-Small Cell Lung Cancer A549/ATCC				
EKVX	-15.95 -21.03			
HOP-62 HOP-92	-36.99 -19.59			
NCI-H226	-17.36			
NCI-H23 NCI-H322M	-44.25 -32.66		-	
NCI-H460	0.65			
NCI-H522 Colon Cancer	-54.52			
COLO 205	-66.43			
HCC-2998 HCT-116	-43.25 -30.14			
HCT-15	0.93			
HT29 KM12	-19.01 -70.30			
SW-620 CNS Cancer	-6.37			
SF-268 SF-295	-35.63 -24.37			
SF-295 SF-539	-24.37 -64.14			
SNB-19	-14.97			
SNB-75 U251	-75.62 -7.87			
Melanoma				
LOX IMVI MALME-3M	-54.68 -89.65			
M14 MDA-MB-435	-87.85 -92.76			
SK-MEL-2	-39.39		-	
SK-MEL-28 SK-MEL-5	-41.87 -91.46		_	
UACC-257 UACC-62	-50.21			
Ovarian Cancer	-93.44			
IGROV1 OVCAR-3	-35.59 -47.10			
OVCAR-4	-2.61			
OVCAR-5 OVCAR-8	-0.86 -22.32		_	
NCI/ADR-RES SK-OV-3	-15.29 0.24			
Renal Cancer				
786-0 A498	-41.15 -63.09			
ACHN	-13.48 -28.02			
CAKI-1 RXF 393	-54.40			
SN12C TK-10	-16.58 -20.73			
UO-31	-35.38		•	
Prostate Cancer PC-3	-13.71			
DU-145 Breast Cancer	-31.23		1	
MCF7	0.89			
MDA-MB-231/ATCC HS 578T	-49.01 -6.33			
BT-549 T-47D	-10.42 -18.15			
MDA-MB-468	-18.15 -57.61			
Mean	-32.47			
Delta Range	60.97 97.67			
rungo	01.01			
	150	100 50	0 -50	-100 -150

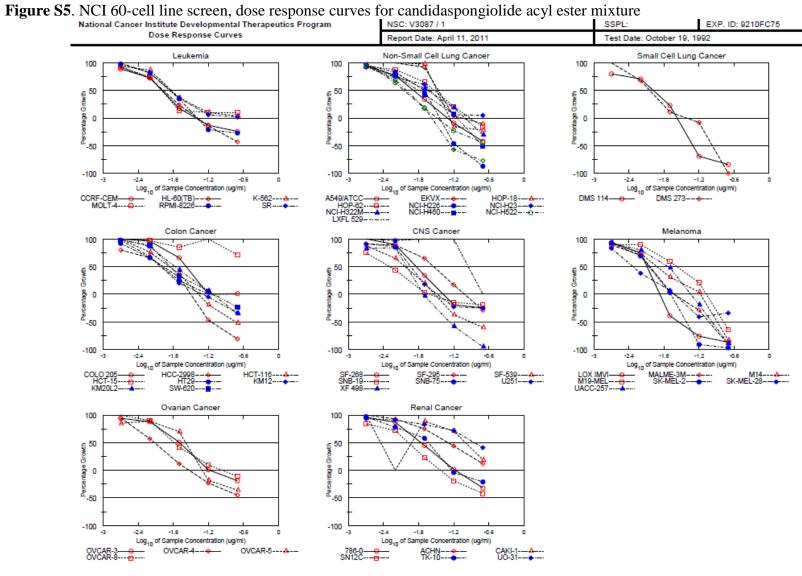
**Figure S2**. NCI 60-cell line screen, single dose  $(10^{-5} \text{ M})$  of 1/2



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

National Cancer Institute Developmental Therapeutics Program		NSC : D - 754491/1	Units :Molar	SSPL :0GZS	EXP. ID :1010NS3		
Mean Graphs			Report Date :Decemb	Report Date :December 03, 2010		Test Date :October 25, 2010	
Pane/Cell Line	Log 10 <sup>GI50</sup>	GI50	Log <sub>10</sub> TGI	TGI	Log <sub>10</sub> LC50	LC50	
ukemia CCRF-CEM HL-60(TB) K-562 MOLT-4 RPMI-8226 SR	< -8.00 -7.87 < -8.00 -7.80 < -8.00 < -8.00 -7.81	4	-5.63 -6.50 6.53 6.53 -7.16	1	* 4400 * 44400 * * * * * 000 * * * * 000		
SR, n-Small Cell Lung Cancer APA/A/TCC HOP-82 HOP-92 NCI-H226 NCI-H226 NCI-H228 NCI-H322M NCI-H460 NCI-H322	V - 8000 V - 8000 V - 8000 V - 8000 V - 8000 V - 8000 V - 6000 V - 6000 V - 6000		745 7755 7755 7755 7757 7742 7745 7745	L	> 4.00 > 4.00 > 4.00 > 4.00 > 4.00 > 4.00 > 4.00 > 4.00 > 4.00 > 4.00		
NCH-H522 COLO 205 HCC-2996 HCT-116 HCT-15 HT29 KM12 SW-620 IS Cancer	< -8.00 < -8.00 < -8.00 -7.74 < -8.00 < -8.00 < -8.00 < -8.00		-7.65 -7.28 -7.28 -6.44 -7.46 -7.71 -7.71	-	-7.14 > -4.00 > -4.00 > -4.00 > -4.00 > -4.00 > -5.07 > -4.00		
SW-520 SF-295 SF-295 SF-539 SFB-19 SNB-75 U251 Hanoma	< -8.00 < -8.00 < -8.00 < -8.00 < -8.00 < -8.00 < -8.00		-7.36 -7.48 -7.71 -7.71 -7.44 -7.78 -7.82		> 4.00 > 4.00 - 5.43 - 4.00 - 7.30 - 7.30		
Vanoma LOX II/W/I MALME-3M MI4 MDA-MB-435 SK-MEL-28 SK-MEL-28 SK-MEL-28 UACC-257 UACC-257	<ul> <li>-8.00</li> <li>&lt; -8.00</li> </ul>				-6.76 -7.12 -7.225 -7.251 -7.618 -7.733 > -4.00 -7.27		
UACC-52 arian Cancer (GROVI) OVCAR-3 OVCAR-3 OVCAR-5 OVCAR-5 OVCAR-5 OVCAR-5 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3 SkOV-3	-7.92 < -8.00 < -8.00 < -8.00 < -8.00 -6.87 < -8.00	_	-7.10 -7.225 -7.225 -7.200 -4.003		> -4.00 > -4.00 > -4.00 > -4.00 > -4.00 > -4.00 > -4.00		
786-0 4498 ACHN 2AK7-1 XKF 393 SM12C FK-10 J0-31	< -8.00 8.00 -7.7.85 -7.71 < -8.00 < -8.00 < -8.00 -7.76	1	-7.39 -8.00 -7.03 -7.13 -7.13 -7.14 -7.17 -7.54 -6.89		> 4.00 -7.38 -4.31 > 4.00 -7.20 > 4.00 > 4.00 > 4.00		
20-3	-7.98 -7.97		-7.11 -7.22		> -4.00 > -4.00		
DU-145 Bast Cancer MCF7 MDA-MB-231/ATCC HS 578T BT-549 T-47D MDA-MB-468	< -8.00 -7.81 < -8.00 -7.99 < -8.00 < -8.00 < -8.00		4,70 -7.12 -7.67 -7.67 -7.07 -8.00 < -8.00	-	> -4.00 -5.97 > 4.00 > 4.00 > -7.21		
MID Delta Range	-7.95 0.05 1.13		-7.23 0.77 4.0		4.79 2.94 3.73		

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



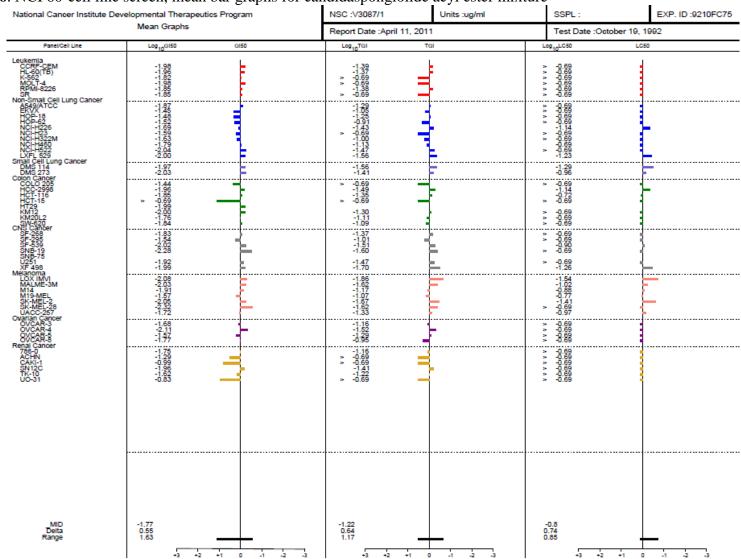


Figure S6. NCI 60-cell line screen, mean bar graphs for candidaspongiolide acyl ester mixture

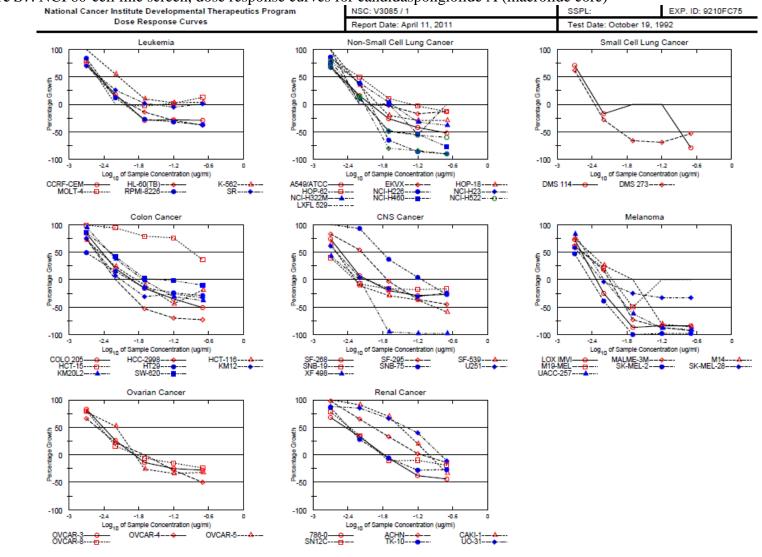
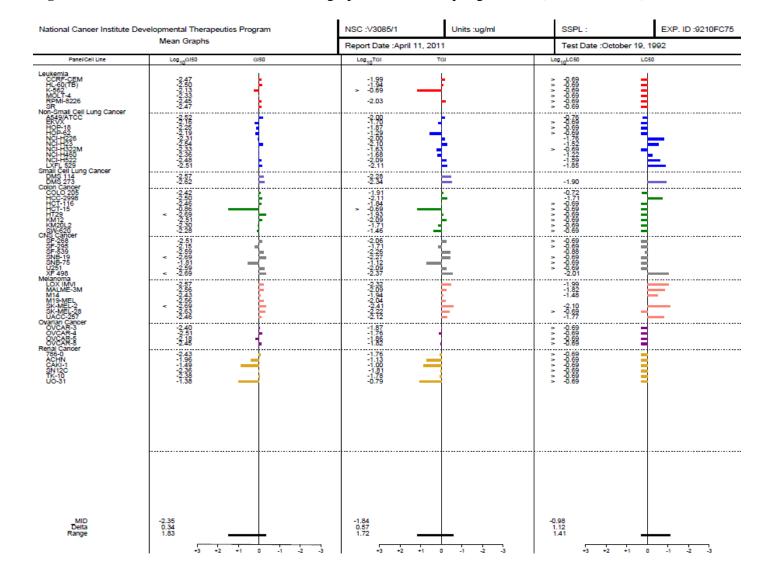


Figure S7. NCI 60-cell line screen, dose response curves for candidaspongiolide A (macrolide core)



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

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 <sub>50</sub> All NCI-60 Cell Lines	18.3

Table S7. Melanoma GI<sub>50</sub>'s for candidaspongiolide acyl ester mixture

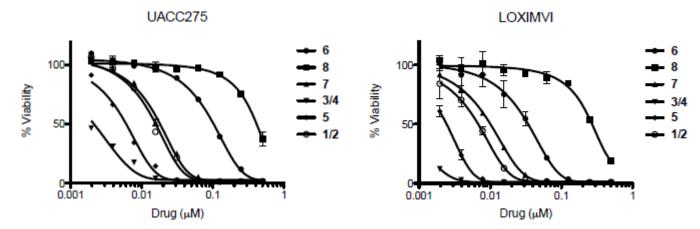
<sup>a</sup> Based on an estimated average mass of 927.1, calculated utilizing the reported fatty acid compositions (894.5 = 4.4%; 908.5 = 10.6%; 922.6 = 47.3%; 936.6 = 22.7%; 948.6 = 5.2%; 950.6 = 9.8%). Note: 10% of the fatty acid mixture was unidentified and percentages have been re-calculated to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

50	
Melanoma Cell Line	GI <sub>50</sub> (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 <sub>50</sub> All NCI-60 Cell Lines	6.5

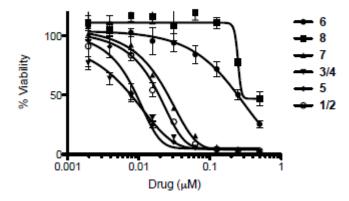
Table S8. Melanoma GI<sub>50</sub>'s for candidaspongiolide A (macrolide core)

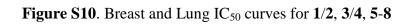
<sup>&</sup>lt;sup>8</sup> Meragelman, T. L.; Willis, R. H.; Woldemichael, G. M.; Heaton, A.; Murphy, P. T.; Snader, K. M.; Newman, D. J. ; van Soest, R.; Boyd, M. R.; Cardellina II, J. H.; McKee, T. C. *J. Nat. Prod.* **2007**, *70*, 1133.

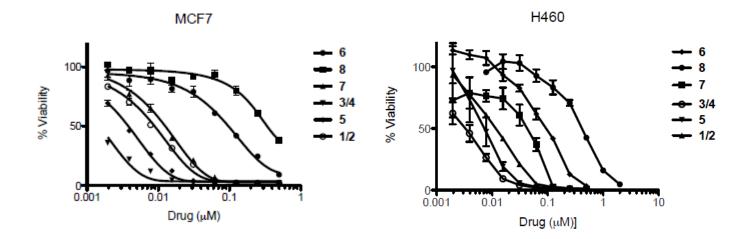


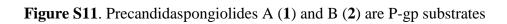


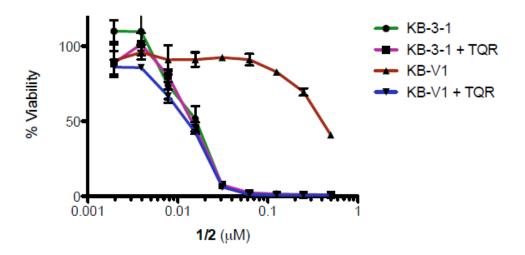












	1/2 (nM)	1/2 + TQR (nM)
KB-3-1	$15.7 \pm 4.9$	$14.7 \pm 1.5$
KB-V1	$419.3 \pm 15.0$	$13.1 \pm 0.8$
RR	26.7	N/A