Cytotoxic Ergostane derivatives from *Termitomyces microcarpus* (Lyophyllaceae)

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SUPPLEMENTARY MATERIAL

1. NMR spectra for Compounds **1-6**.
2. Fig. S1: Flow chart showing the isolation of compounds from *Termitomyces microcarpus*.
3. Table S1-S6: NMR data for compounds **1-6**
4. Table S7: Growth percentage of against compounds **1-6** in the NCI *in vitro* 60 cell Drug Screen Program (Single dose of 10⁻⁵M)
5. Figure S2: One Dose Data for Compounds **1-6**
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7. Figure S3: Dose Response Curves for Figure **3**.
8. Table S9: *In vitro* testing results for Compound **3**.

^1H NMR spectrum for compound 1 in CDCl_3

^{13}C NMR spectrum for compound 1 in CDCl_3

DEPT spectrum for Compound 1 in CDCl₃

HSQCDEPT spectrum for compound 1 in CDCl₃

HMBC spectrum for compound 1 in CDCl₃

COSY spectrum for compound 1 in CDCl₃

NOESY spectrum for compound 1 in CDCl_3

^1H NMR spectrum for compound 2 in CDCl_3

^{13}C NMR spectrum for compound Tm 2 in CDCl_3

DEPT spectrum for compound 2 in CDCl_3

HSQCDEPT spectrum for compound 2 in CDCl_3

HMBC spectrum for compound 2 in CDCl_3

COSY spectrum for compound 2 in CDCl₃

NOESY spectrum for compound 2 in CDCl₃

^1H NMR spectrum for compound 3 in CDCl_3

^{13}C NMR spectrum and chemical shift for compound 3 in CDCl_3

DEPT spectrum for compound 3 in CDCl₃

HSQCDEPT spectrum for compound 3 in CDCl₃

HMBC spectrum for compound 3 in CDCl₃

COSY spectrum for compound 3 in CDCl₃

NOESY spectrum for compound 3 in CDCl_3

^1H NMR spectrum for compound 4 in CDCl_3

^{13}C NMR spectrum for compound 4 in CDCl_3

DEPT spectrum for Compound 4 in CDCl_3

HSQCDEPT spectrum for compound 4 in CDCl_3

HMBC spectrum for compound 4 in CDCl_3

COSY spectrum for compound 4 in CDCl₃

NOESY spectrum for compound 4 in CDCl₃

^1H NMR spectrum for compound 5 in CDCl_3

^{13}C NMR spectrum for compound 5 in CDCl_3

HMBC spectrum for compound 5 in CDCl₃

COSY spectrum for compound 5 in CDCl₃

NOESY spectrum for compound 5 in CDCl_3

^1H NMR spectrum for compound 6 in CD_3OD

^{13}C NMR spectrum for compound 6 in CD_3OD

DEPT spectrum for compound 6 in CD_3OD

HSQCDEPT spectrum for compound 6 in CD_3OD

HMBC spectrum for compound 6 in CD₃OD

COSY spectrum for compound 6 in CD₃OD

NOESY spectrum for compound 6 in CDCl_3

Figure S1: Flow chart showing the isolation of compounds from *Termitomyces microcarpus*

Table S1: NMR data for compound 1 in CDCl₃ compared against reference values

Position	¹³ C NMR (125 MHz)	¹³ C NMR (125. MHz) (Mansoor <i>et al.</i> , 2005)	¹ H NMR (500 MHz)	¹ H NMR (500 MHz) (Mansoor <i>et al.</i> , 2005)
1	170.9 C	173.6	-	-
2	112.6 CH	112.6	5.66 d <i>J</i> =1.9 Hz	5.67 d
3	170.6 C	172.9	-	-
4	104.9 C	104.1	-	-
5 α	35.5 CH ₂	36.2	2.27 ddd <i>J</i> =2.2, 3.7, 6.7 Hz	2.23 ddd
5 β			1.07	
6 α	35.3 CH ₂	36.5	1.99 ddd <i>J</i> =2.1, 4.7, 7.0 Hz	1.98 ddd
6 β			1.62	-
7	49.1 C	49.0	-	-
8	50.6 CH	51.7	2.65 m	2.66 ddd
9 α	21.6 CH ₂	22.3	1.72 m	1.73 m
9 β			1.51 m	-
10 α	29.1 CH ₂	30.2	1.40 m	-
10 β			1.90 m	1.92
11	55.6 CH	56.7	1.49 m	1.50 m
12	11.9 CH ₃	12.1	0.61s	0.68 s
13	40.3 CH	41.5	2.06 m	2.08 m
14	21.3 CH ₃	21.4	1.04 d, <i>J</i> =6.20	1.04 d
15	134.8 CH	137.5	5.17 dd <i>J</i> =8.0, 15.5 Hz	5.21 m
16	133.1 CH	133.2	5.26 dd, <i>J</i> =7.8. 15.5 Hz	5.26 m
17	43.1 CH	43.1	1.87 m	1.85 m
18	33.3 CH	29.7	1.49m	1.57 m
19	20.2 CH ₃	22.0	0.84 d, <i>J</i> =7.2 Hz	0.85 d, <i>J</i> =7.0 Hz
20	19.9 CH ₃	21.5	0.84 d, <i>J</i> =7.2 Hz	0.85 d, <i>J</i> =7.0 Hz
21	17.8 CH ₃	20.0	0.92 d <i>J</i> =6.5 Hz	0.94 d <i>J</i> =6.5 Hz

Table S2: NMR data for compound 2 in CDCl₃ compared against reference values

Position	¹³ C NMR (125 MHz)	¹³ C NMR 125. MHz) (Gao <i>et al.</i> , 2007)	¹ H NMR (500 MHz)	¹ H NMR (500 MHz) (Gao <i>et al.</i> , 2007)
1 α	32.6 CH ₂	32.8	1.68 m	1.70
1 β			1.06 m	-
2 α	30.6 CH ₂	30.8	1.50 m	-
2 β			1.25 m	-
3 α	66.3 CH	66.4	4.01 m	3.98
4 α	36.1 C	36.2	2.13 m	-
4 β			1.94 m	-
5	82.7 C	83.0	-	-
6	135.5 CH	135.2	6.28 d, <i>J</i> =8.9 Hz	6.30 d, <i>J</i> =8.5Hz
7	130.8 C	135.8	6.60 d, <i>J</i> =9.0 Hz	6.61 d <i>J</i> =8.5 Hz
8	78.4 C	78.5	-	-
9	142.6 C	142.8	-	-
10	38.0 C	38.0	-	-
11	119.7 CH	119.8	5.43 dd, <i>J</i> =7.0 Hz	5.43 dd, <i>J</i> =6.0 Hz
12 α	41.2 CH ₂	41.3	2.27 m	-
12 β			2.11 m	-
13	43.6 C	43.7	-	-
14	48.2 CH	48.3	1.36 m	-
15 α	22.8CH ₂	22.8	1.09 m	-
15 β			1.53 m	-
16 α	28.6 CH ₂	28.7	1.36 m	-
16 β			1.8 m	-
17	56.6 CH	56.0	1.84 m	1.85 m
18	12.9 CH ₃	12.8	0.76 s	0.75 s
19	25.6 CH ₃	25.5	1.08 s	1.09 s
20	39.9 CH	39.9	2.03	-
21	20.9CH ₃	20.9	1.01 d <i>J</i> =6.5 Hz	1.01 d <i>J</i> =6.5 Hz
22	135.1 CH	135.5	5.24 dd, <i>J</i> =7.8, 15.3 Hz	5.24 dd <i>J</i> =15.1
23	134.4 CH	132.5	5.17 dd, <i>J</i> =7.8, 15.3 Hz	5.15 dd, <i>J</i> =15.1
24	42.8CH	42.8	1.85 m	-
25	33.1.0 CH	33.11	1.47 m	-
26	22.1 CH ₃	21.91	0.84 d, <i>J</i> =6.8 Hz	0.83 d, <i>J</i> = 6.8 Hz
27	20.0 CH ₃	20.0	0.86 d, <i>J</i> =6.9 Hz	0.83 d, <i>J</i> = 6.7 Hz
28	17.6 CH ₃	17.5	0.94 d, <i>J</i> =7.1 Hz	0.92 d <i>J</i> =7.2 s

Table S3: NMR data for compound 3 in CDCl₃ compared against reference values

Position	¹³ C NMR (125 MHz)	¹³ C NMR (100 MHz) (Kwon <i>et al.</i> , 2002)	¹ H NMR (500 MHz)	¹ H NMR (400 MHz) (Kwon <i>et al.</i> , 2002)
1 α	34.9 CH ₂	34.7	1.95 m	1.9
1 β			1.69 m	
2 α	30.3CH ₂	30.1	1.83m	1.5
2 β			1.54m	
3	66.7 CH	66.5	3.96m	3.97 m
4 α	37.1 C	37.0	2.10m	1.91m
4 β			1.91m	
5	82.3 C	82.1	-	-
6	135.6 CH	135.4	6.50 d, <i>J</i> =8.5 Hz	6.50 d, <i>J</i> =8.5 Hz
7	130.7C	130.7	6.22 d, <i>J</i> =8.5 Hz	6.24 d, <i>J</i> =8.5 Hz
8	79.6 C	79.4	-	-
9	51.3 CH	51.1	1.55	1.51 m
10	37.1 CH	36.9	-	-
11 α	23.6CH ₂	23.4	1.21m	1.5 m
11 β			1.50m	-
12 α	39.6CH ₂	39.3	1.94m	1.9 m
12 β			1.23m	-
13	44.7 C	44.6	-	
14	51.9 CH	51.7	1.55m	1.57m
15 α	20.8 CH ₂	20.6	1.59m	1.68 m
15 β			1.40m	-
16 α	28.9 CH ₂	28.6	1.75m	1.96 m
16 β			1.35m	-
17	56.4 CH	56.2	1.22 m	1.85 m
18	13.1 CH ₃	12.9	0.81 s	0.82 s
19	18.4 CH ₃	18.2	0.88s	0.88 s
20	39.9 CH	39.7	2.02m	2.02m
21	21.0CH ₃	20.9	0.99 d <i>J</i> =6.5 Hz	1.00 d <i>J</i> =6.5 Hz
22	135.4 CH	135.2	5.22 dd, <i>J</i> =8.3, 15.4 Hz	5.24 dd <i>J</i> =8.0, 15.1 Hz
23	132.3 CH	132.3	5.15 dd, <i>J</i> =7.5, 15.4 Hz	5.15 dd, <i>J</i> =8.0, 15.1 Hz
24	42.9 CH	42.8	1.85m	1.8
25	33.3 CH	33.1	1.47	-
26	20.2 CH ₃	19.6	0.82 d, <i>J</i> =6.8 Hz	0.82 d, <i>J</i> = 6.8 Hz
27	19.9 CH ₃	19.9	0.81 d, <i>J</i> =6.9 Hz	0.83 d, <i>J</i> = 6.7 Hz
28	17.7 CH ₃	17.5	0.90 d, <i>J</i> =6.8 Hz	0.91 d <i>J</i> =7.2 Hz

Table S4: NMR data for compound 4 in CDCl₃ compared against reference values

No.	¹³ C NMR (125 MHz)	¹³ C NMR (100 MHz) (Kwon <i>et al.</i> , 2002)	¹ H NMR (500 MHz)	¹ H NMR (400 MHz) (Kwon <i>et al.</i> , 2002)
1α	32.4 CH ₂	32.9	1.66 m	-
1β			1.44 m	-
2α	31.3 CH ₂	31.8	1.93m	1.5
2β			1.54m	-
3	68.9 CH	69.4	3.91 m	3.93 m
4α	39.8 CH ₂	39.9	2.20 m	2.20 m
4β			1.40 m	-
5	68.0 C	68.5	-	-
6	61.5 CH	62.0	3.13 d, <i>J</i> =3.2 Hz	3.14 d, <i>J</i> =3.2 Hz
7	65.3 CH	65.7	4.41 d, <i>J</i> =3.5 Hz	4.42 d, <i>J</i> =3.4 Hz
8	125.7 C	125.9	-	-
9	38.9 CH	39.4	2.34	-
10	36.0 C	36.5	-	-
11α	19.2 CH ₂	19.7	1.49 m	-
11β			1.38 m	-
12α	36.6 CH ₂	37.3	1.92 m	-
12β			1.14 m	-
13	44.7 C	42.2	-	-
14	153.0 C	153.3	-	-
15α	25.2 CH ₂	25.7	2.58 m	-
15β			2.24 m	
16α	27.4 CH ₂	27.9	1.73 m	-
16β			1.35-m	-
17	57.1 CH	57.5	1.26 m	1.85 m
18	18.3 CH ₃	18.3	0.92 s	0.86 s
19	16.7 CH ₃	17.2	0.86 s	0.88 s
20	39.4 CH	40.3	2.08 m	-
21	21.5 CH ₃	21.9	0.99 d <i>J</i> =6.8 Hz	1.01 d <i>J</i> =7.0 Hz
22	135.4 CH	135.9	5.19 dd, <i>J</i> =8.0, 15.5 Hz	5.19 dd <i>J</i> =7.5, 16.0 Hz
23	132.5 CH	132.9	5.23 dd, <i>J</i> =7.8, 15.6 Hz	5.21 dd, <i>J</i> =7.5, 16.0 Hz
24	43.0 CH	43.5	1.84 m	-
25	33.3 CH	33.8	1.46	-
26	20.2 CH ₃	20.4	0.83 d, <i>J</i> =6.4 Hz	0.82 d, <i>J</i> = 7.0 Hz
27	19.9 CH ₃	20.7	0.83 d, <i>J</i> =6.5 Hz	0.84 d, <i>J</i> = 7.0 Hz
28	17.8 CH ₃	18.8	0.90 d, <i>J</i> =7.2 Hz	0.90 d <i>J</i> =6.8 Hz

Table S5: NMR data for compound 5 in CDCl₃ compared against reference values

No.	¹³ C NMR (125 MHz)	¹³ C NMR (75 MHz) (Li <i>et al.</i> , 2005)	¹ H NMR (500 MHz)	¹ H NMR (300 MHz) (Li <i>et al.</i> , 2005)
1 α	33.1 CH ₂	33.0	1.46 m	1.53-1.66 m
1 β			2.30 m	
2 α	31.1 CH ₂	30.9	1.41 m	1.40-1.50 m
2 β			1.84 m	1.84-1.92 m
3	67.9 CH	67.7	4.07 m	4.08 t J=11.3,4.9 Hz
4 α	40.6 C	39.5	2.02 m	1.74-1.82 m
4 β			1.74 m	2.11-2.20 m
5	76.1 C	76.0	-	-
6	73.7CH	73.7	3.60 d, J=5.0 Hz	3.62 d, J=5.1 Hz
7	117.5 CH	117.6	5.30 d, J=5.0 Hz	5.33-5.38 m
8	144.0 C	144.0	-	-
9	43.9 CH	43.6	1.94	1.93-2.01 m
10	37.4 CH	37.2	-	-
11 α	22.3 CH	22.1	1.50 m	1.54-1.65m
11 β			1.27 m	
12 α	39.4 CH ₂	39.2	1.31 m	1.29-1.38 m
12 β			2.09 m	2.02-2.11 m
13	43.7 C	43.8	-	
14	54.9 CH	54.8	1.89 m	1.88-1.97 m
15 α	23.1 CH ₂	22.9	1.50 m	1.39-1.50 m
15 β			1.40 m	1.51-1.59 m
16 α	27.9 CH ₂	27.9	1.75 m	1.96 m
16 β			1.35 m	
17	56.0 CH	56.0	1.29 m	1.27-1.34 m
18	12.3 CH ₃	12.3	0.60 s	0.60 s
19	19.1 CH ₃	18.8	1.09 s	1.09 s
20	39.7 CH	40.4	2.04 m	1.99-2.08 m
21	21.3 CH ₃	21.1	1.02 d J=6.5 Hz	1.03 d J=6.5 Hz
22	135.4 CH	135.4	5.16 dd, J=8.0, 15.4 Hz	5.13 dd J=7.8, 15.3Hz
23	132.2 CH	132.20	5.22 dd, J=7.5, 15.4 Hz	5.23 dd, J=7.1,15.2Hz
24	43.0 CH	42.8	1.85 m	1.81-1.90 m
25	33.3 CH	33.1	1.47	1.43-1.52 m
26	20.1 CH ₃	20.0	0.83 d, J=6.8 Hz	0.84 d, J= 6.4 Hz
27	19.9 CH ₃	19.7	0.85 d, J=6.9 Hz	0.82 d, J= 6.5 Hz
28	17.9 CH ₃	17.6	0.90 d, J=6.8 Hz	0.92 d J=6.9 Hz

Table S6: NMR data for compound 6 in CDCl₃ compared against reference values

No.	¹³ C NMR (125 MHz)	¹³ C NMR (75 MHz) (Uddin <i>et al.</i> , 2011)	¹ H NMR (500 MHz)	¹ H NMR (300 MHz) (Uddin <i>et al.</i> , 2011)
1	40.2 CH ₂	40.5	0.99 m	-
2	28.2 CH ₂	29.6	1.90 m	1.82
3	79.8 CH	79.3	4.50 dd, <i>J</i> =11.3, 4.7 Hz	4.47 dd, <i>J</i> =10.0, 5.5 Hz
4	40.0 C	40.8	-	-
5	57.6 CH	57.2	0.75 m	-
6	19.6 CH ₂	20.0	1.53 m	-
7	35.7 CH ₂	36.1	1.62 m	-
8	43.7C	42.3	-	-
9	50.6 CH	52.2	1.58 m	1.60
10	42.1 C	38.8	-	-
11	22.2 CH ₂	22.4	1.93 m	4.56
12	27.0 CH	27.4	5.29 t, <i>J</i> =3.75	4.59
13	39.8 C	39.8	2.71	2.71
14	40.2 C	44.1	-	-
15	30.9 CH ₂	31.5	1.54 m	1.51
16	31.9 CH ₂	34.1	2.0 m	2.02
17	49.7 C	57.9	-	-
18	49.6 CH	51.0	2.22 m	1.51
19	49.7 CH	49.0	3.17 m	3.0 t, <i>J</i> =10.2, 5.5, 1.45 Hz
20	152.1 C	152.6	-	-
21	31.8 CH ₂	32.5	1.49 m	1.37
22	38.2 CH ₂	38.9	1.76 m	-
23	28.1 CH ₃	28.1	0.96 s	0.93 s
24	16.3 CH ₃	16.4	0.77 s	0.78 s
25	16.7 CH ₃	16.4	0.81 s	0.82 s
26	16.9 CH ₃	16.3	0.95 s	0.97 s
27	15.3 CH ₃	14.9	0.93 s	0.96 s
28	180.2 C	180.1	-	-
29	110.3 CH ₂	110.0	4.60 (1H, br, s) 4.72 (1H, br, s)	4.66 (1H, br, s) 4.73 (1H, br, s)
30	19.6 CH ₃	19.5	1.96 s	1.68 s

Table S7: Growth percentage of compounds 1-6 in the NCI *in vitro* 60 cell Drug Screen Program (Single dose of 10⁻⁵M)

	Compounds/growth percentage ^a					
	1	2	3	4	5	6
<u>Panel/cell lines</u>	<u>NSC777920</u>	<u>NSC777922</u>	<u>NSC777923</u>	<u>NSC777926</u>	<u>NSC777924</u>	<u>NSC777925</u>
Leukemia						
CCRF-CEM	22.58	62.47	-6.81	106.71	102.04	67.51
HL-60(TB)	46.13	120.53	36.84	122.43	117.51	116.70
K-562	70.59	95.75	-29.29	116.60	101.85	95.15
MOLT-4	39.78	79.49	5.30	103.23	105.62	84.20
RPMI-8226	24.89	99.64	14.66	108.28	101.57	92.69
SR	35.18	71.90	-2.50	104.68	89.42	70.69
Non-Small Cell Lung Cancer						
A549/ATCC	53.34	91.23	-39.92	105.42	87.87	100.94
HOP-62	87.76	110.81	-37.57	101.51	95.94	114.44
HOP-92	39.14	106.31	5.48	95.71	91.14	91.29
NCI-H226	75.49	107.96	27.59	92.27	93.37	98.72
NCI-H23	56.09	102.12	0.68	102.50	101.18	98.36
NCI-H322M	90.46	102.96	-8.57	117.34	112.11	110.99
NCI-H460	50.11	101.04	-26.89	110.06	106.70	101.32
NCI-H522	69.70	38.09	-74.73	114.17	102.23	83.12
Colon Cancer						
COLO 205	70.82	110.71	-36.93	98.10	105.57	106.12
HCC-2998	66.31	121.29	-46.41	108.11	113.09	112.29
HCT-116	38.17	91.42	-92.71	86.07	103.36	82.94
HCT-15	37.96	80.54	-53.15	107.62	104.33	87.95
HT29	56.90	90.00	-69.31	110.70	115.25	103.80
KM12	75.88	105.91	-44.40	104.79	108.83	106.51
SW-620	70.35	105.93	-35.79	102.31	105.15	108.01
CNS Cancer						
SF-268	81.65	105.61	-46.82	105.11	103.35	108.65
SF-295	56.91	100.37	-40.51	96.66	102.70	106.14
SF-539	82.30	106.11	-16.16	108.80	109.85	100.19
SNB-19	78.14	98.45	-13.31	101.27	98.78	106.38
SNB-75	56.23	102.55	20.89	97.70	99.44	99.44
U251	48.07	89.97	-67.68	103.44	96.51	99.85

Melanoma

LOX IMVI	59.80	76.82	-88.68	103.79	101.31	85.57
MALME-3M	48.90	91.30	56.15	105.86	107.43	107.29
M14	53.94	100.73	-62.82	98.45	94.55	99.35
MDA-MB-435	50.92	99.47	-36.85	107.78	109.72	101.47
SK-MEL-2	85.36	120.49	-7.87	115.80	122.82	109.02
SK-MEL-28	34.22	99.98	9.04	107.22	97.09	104.72
SK-MEL-5	31.60	92.92	34.66	98.31	99.29	85.99
UACC-257	54.64	93.71	62.51	99.84	90.61	98.48
UACC-62	28.35	88.79	-22.12	92.89	100.07	91.73

Ovarian Cancer

IGROV1	86.10	65.57	5.38	113.05	120.10	117.30
OVCAR-3	78.35	112.09	-17.65	106.80	110.33	111.75
OVCAR-4	61.59	97.49	7.77	113.19	107.54	107.21
OVCAR-5	97.31	101.63	-11.18	110.98	100.74	129.12
OVCAR-8	49.07	82.97	-11.44	102.23	100.79	94.38
NCI/ADR-RES	76.95	97.90	-3.56	112.33	102.12	101.65
SK-OV-3	101.80	117.92	-37.69	99.67	95.92	122.14

Renal Cancer

786-0	74.59	107.81	-22.00	101.51	100.52	104.19
A498	51.37	112.87	-0.74	101.21	100.05	93.40
ACHN	71.94	97.07	-52.69	104.03	110.08	102.50
CAKI-1	75.18	100.41	-13.02	104.37	100.74	106.93
XF 393	43.47	105.60	-16.97	95.41	114.71	NT
SN12C	59.94	99.35	-3.80	102.12	97.50	102.55
TK-10	130.47	142.39	-14.05	116.31	110.78	129.20
UO-31	48.89	85.04	-51.34	99.05	95.94	97.96

Prostate Cancer

PC-3	35.69	84.28	-27.63	100.65	92.41	78.18
DU-145	74.84	106.13	-55.08	112.16	115.17	108.39

Breast Cancer

MCF7	19.54	83.95	-37.05	98.63	103.60	79.94
MDA-MB-231/ATCC	71.30	107.99	-59.25	105.51	100.03	91.67
HS 578T	65.05	94.74	10.18	100.04	99.47	96.16
T-47D	18.71	60.80	NT	108.15	NT	NT
MDA-MB-468	90.09	89.14	-12.67	99.23	94.49	100.01
BT-549	NT	89.54	-5.15	95.43	95.40	96.26

^aData obtained from NCI *in vitro* 60-cell drug screen program at 10⁻⁵ M concentration

Figure S2: One Dose Data for Compounds 1-6

Compound **1** one dose data

Compound 2 one dose data

Compound 3 one dose data

Compound 4 one dose curve

Compound 5 one dose curve

Compound 6 one dose curve

Table S8: *In vitro* testing of compound 3

<u>Panel/cell lines</u>	<u>Compound 3^a</u>		
	<u>GI₅₀^b(μM)</u>	<u>TGI₅₀ (μM)</u>	<u>LC₅₀^c (μM)</u>
Leukemia			
CCRF-CEM	1.30	6.90	>10
HL-60(TB)	3.31	>10	>10
K-562	1.40	4.02	>10
MOLT-4	2.03	5.53	>10
RPMI-8226	1.92	9.54	>10
SR	1.04	4.63	>10
Non-Small Cell Lung Cancer			
A549/ATCC	1.65	3.46	7.23
HOP-62	2.44	5.48	>10
HOP-92	2.32	7.11	>10
NCI-H226	4.90	>10	>10
NCI-H23	2.00	5.34	>10
NCI-H322M	3.86	>10	>10
NCI-H460	1.52	3.34	7.36
NCI-H522	1.55	3.25	6.88
Colon Cancer			
COLO 205	2.14	4.91	>10
HCC-2998	1.88	4.27	9.68
HCT-116	1.29	2.75	5.87
HCT-15	1.39	4.88	>10
HT29	1.92	3.73	7.25
KM12	1.57	3.50	7.81
SW-620	1.32	2.73	5.65
CNS Cancer			
SF-268	1.67	3.68	8.10
SF-295	2.04	5.07	>10
SF-539	2.36	6.86	>10
SNB-19	2.16	4.60	9.78
SNB-75	1.69	8.53	>10
U251	1.70	3.47	7.09
Melanoma			
LOX IMVI	1.10	2.62	6.26
MALME-3M	>10	>10	>10
M14	2.25	5.75	>10
MDA-MB-435	1.43	3.20	7.14

SK-MEL-2	2.59	6.04	>10
SK-MEL-28	2.49	6.91	>10
SK-MEL-5	5.75	>10	>10
UACC-257	>10	>10	>10
UACC-62	1.68	5.64	>10
Ovarian Cancer			
IGROV1	2.45	6.82	>10
OVCAR-3	1.86	3.76	7.60
OVCAR-4	1.70	6.03	>10
OVCAR-5	2.32	7.16	>10
OVCAR-8	1.88	5.96	>10
NCI/ADR-RES	1.78	4.35	>10
SK-OV-3	3.58	>10	>10
Renal Cancer			
786-0	2.28	8.66	>10
A498	2.25	7.07	>10
ACHN	1.52	3.83	9.65
CAKI-1	1.31	7.34	>10
SN12C	3.10	>10	>10
TK-10	3.04	6.95	>10
UO-31	1.60	3.54	7.84
Prostate Cancer			
PC-3	1.31	3.83	>10
DU-145	1.85	3.94	8.38
Breast Cancer			
MCF7	1.37	4.07	>10
MDA-MB-231/ATCC	1.71	4.27	>10
HS 578T	2.42	7.92	>10
BT-549	5.07	>10	>10
T-47D	0.651	6.20	>10
MDA-MB-468	1.65	4.30	>10

^aData obtained from NCI's *in vitro* disease-oriented human tumor cell lines screen (Grever *et al.*, 1992)

^bGI₅₀ was the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation, determined at five concentration levels (100, 10, 1.0, 0.1 and 0.001 mM).

^cLC₅₀ is a parameter of cytotoxicity and reflects the molar concentration needed to kill 50% of the cells

Figure S3: Dose Response Curves for Figure 3.

Figure 4: Five dose response curve for compound 3

Compound 3 data for Growth inhibition at five concentration with GI₅₀, TGI and LC₅₀

Table S9: *In vitro* testing results for Compound 3.

National Cancer Institute Developmental Therapeutics Program In-Vitro Testing Results															
NSC : D - 777923 / 1			Experiment ID : 1402NS22						Test Type : 08			Units : Molar			
Report Date : February 12, 2015			Test Date : February 03, 2014						QNS :			MC :			
COMI : NAT3 (134151)			Stain Reagent : SRB Dual-Pass Related						SSPL : 0YHL						
Log10 Concentration															
Panel/Cell Line	Time Zero	Ctrl	-9.0	-8.0	-7.0	-6.0	-5.0	-9.0	-8.0	-7.0	-6.0	-5.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.524	2.291	2.311	2.259	2.120	1.554	0.444	101	98	90	58	-15	1.30E-6	6.19E-6	> 1.00E-5
HL-60(TB)	0.770	2.223	2.140	2.230	2.293	2.131	0.910	94	100	105	94	10	3.31E-6	> 1.00E-5	> 1.00E-5
K-562	0.244	1.343	1.447	1.260	1.423	0.970	0.139	109	92	107	66	-43	1.40E-6	4.02E-6	> 1.00E-5
MOLT-4	0.622	1.966	2.034	2.111	2.068	1.770	0.438	105	111	108	85	-30	2.03E-6	5.53E-6	> 1.00E-5
RPMI-8226	0.780	2.093	2.122	2.039	2.025	1.705	0.769	102	96	95	70	-1	1.92E-6	9.54E-6	> 1.00E-5
SR	0.276	0.714	0.757	0.715	0.706	0.500	0.205	110	100	98	51	-26	1.04E-6	4.63E-6	> 1.00E-5
Non-Small Cell Lung Cancer															
A549/ATCC	0.433	1.671	1.677	1.706	1.656	1.474	0.122	100	103	99	84	-72	1.65E-6	3.46E-6	7.23E-6
HOP-62	0.874	1.767	1.628	1.648	1.733	1.813	0.549	84	87	96	105	-37	2.44E-6	5.48E-6	> 1.00E-5
HOP-92	1.040	1.571	1.515	1.494	1.480	1.506	0.881	90	85	83	88	-15	2.32E-6	7.11E-6	> 1.00E-5
NCI-H226	1.503	3.004	2.991	2.998	2.984	2.953	1.940	99	100	99	97	29	4.90E-6	> 1.00E-5	> 1.00E-5
NCI-H23	0.583	1.501	1.484	1.424	1.470	1.368	0.396	98	92	97	86	-32	2.00E-6	5.34E-6	> 1.00E-5
NCI-H322M	0.892	1.785	1.868	1.824	1.831	1.764	1.040	109	104	105	98	17	3.86E-6	> 1.00E-5	> 1.00E-5
NCI-H460	0.262	1.675	1.754	1.652	1.596	1.343	0.080	106	98	94	76	-69	1.52E-6	3.34E-6	7.36E-6
NCI-H522	0.889	1.691	1.695	1.688	1.663	1.525	0.222	100	100	96	79	-75	1.55E-6	3.26E-6	6.88E-6
Colon Cancer															
COLO 205	0.580	1.637	1.578	1.622	1.574	1.591	0.332	94	99	94	96	-43	2.14E-6	4.91E-6	> 1.00E-5
HCC-2998	0.863	2.199	2.226	2.297	2.344	2.047	0.415	102	107	111	89	-52	1.88E-6	4.27E-6	9.68E-6
HCT-116	0.300	2.133	1.927	1.925	1.945	1.524	0.045	89	89	90	67	-85	1.29E-6	2.75E-6	5.87E-6
HCT-15	0.309	1.650	1.474	1.488	1.458	1.153	0.221	87	88	86	63	-28	1.39E-6	4.88E-6	> 1.00E-5
HT29	0.261	1.189	1.225	1.240	1.263	1.180	0.068	104	105	108	99	-74	1.92E-6	3.73E-6	7.25E-6
KM12	0.563	2.269	2.285	2.344	2.370	1.892	0.195	101	104	106	78	-65	1.57E-6	3.50E-6	7.81E-6
SW-620	0.240	1.166	1.148	1.129	1.134	0.882	0.026	98	96	96	69	-89	1.32E-6	2.73E-6	5.65E-6
CNS Cancer															
SF-268	0.553	1.648	1.641	1.685	1.693	1.455	0.203	99	103	104	82	-63	1.67E-6	3.68E-6	8.10E-6
SF-295	0.941	2.843	2.586	2.701	2.608	2.642	0.589	86	92	88	89	-37	2.04E-6	5.07E-6	> 1.00E-5
SF-539	0.912	2.313	2.186	2.305	2.249	2.175	0.751	91	99	95	90	-18	2.36E-6	6.86E-6	> 1.00E-5
SNB-19	0.652	1.920	1.896	1.872	1.937	1.935	0.317	98	96	101	101	-51	2.16E-6	4.60E-6	9.78E-6
SNB-75	0.876	1.600	1.442	1.507	1.494	1.356	0.833	78	87	85	66	-5	1.69E-6	8.53E-6	> 1.00E-5
U251	0.556	2.060	2.115	2.119	2.055	1.865	0.144	104	104	100	87	-74	1.70E-6	3.47E-6	7.09E-6
Melanoma															
LOX IMVI	0.321	1.744	1.687	1.748	1.701	1.111	0.074	96	100	97	55	-77	1.10E-6	2.62E-6	6.26E-6
MALME-3M	0.834	1.327	1.357	1.340	1.363	1.403	1.182	106	103	107	115	71	> 1.00E-5	> 1.00E-5	> 1.00E-5
M14	0.417	1.379	1.297	1.302	1.328	1.313	0.294	91	92	95	93	-29	2.25E-6	5.75E-6	> 1.00E-5
MDA-MB-435	0.588	2.216	2.078	2.123	2.056	1.764	0.171	92	94	90	72	-71	1.43E-6	3.20E-6	7.14E-6
SK-MEL-2	1.168	2.257	2.313	2.274	2.310	2.323	0.820	105	102	105	106	-30	2.59E-6	6.04E-6	> 1.00E-5
SK-MEL-28	0.727	1.864	1.803	1.845	1.828	1.804	0.595	95	98	97	95	-18	2.49E-6	6.91E-6	> 1.00E-5
SK-MEL-5	0.892	3.138	3.100	3.155	3.111	2.864	1.747	98	101	99	88	38	5.75E-6	> 1.00E-5	> 1.00E-5
UACC-257	0.999	1.897	1.935	1.956	2.008	2.028	1.533	104	107	112	115	59	> 1.00E-5	> 1.00E-5	> 1.00E-5
UACC-62	1.037	2.801	2.697	2.703	2.623	2.296	0.792	94	94	90	71	-24	1.68E-6	5.64E-6	> 1.00E-5
Ovarian Cancer															
IGROV1	0.558	1.698	1.808	1.733	1.840	1.628	0.454	110	103	112	94	-19	2.45E-6	6.82E-6	> 1.00E-5
OVCAR-3	0.411	1.061	1.073	1.103	1.096	1.022	0.126	102	106	105	94	-69	1.86E-6	3.76E-6	7.60E-6
OVCAR-4	0.662	1.242	1.158	1.199	1.200	1.073	0.530	86	93	93	71	-20	1.70E-6	6.03E-6	> 1.00E-5
OVCAR-5	0.725	1.771	1.664	1.705	1.684	1.638	0.618	90	94	92	87	-15	2.32E-6	7.16E-6	> 1.00E-5
OVCAR-8	0.535	1.750	1.844	1.823	1.839	1.473	0.416	108	106	107	77	-22	1.88E-6	5.96E-6	> 1.00E-5
NCI/ADR-RES	0.550	1.507	1.481	1.503	1.504	1.335	0.295	97	100	100	82	-46	1.78E-6	4.35E-6	> 1.00E-5
SK-OV-3	1.162	2.148	2.050	2.132	2.091	2.145	1.260	90	98	94	100	10	3.58E-6	> 1.00E-5	> 1.00E-5
Renal Cancer															
786-0	0.679	2.446	2.352	2.370	2.314	2.109	0.643	95	96	93	81	-5	2.28E-6	8.66E-6	> 1.00E-5
A498	1.329	1.901	1.922	1.772	1.836	1.817	1.128	104	77	89	85	-15	2.25E-6	7.07E-6	> 1.00E-5
ACHN	0.437	1.471	1.437	1.450	1.443	1.190	0.210	97	98	97	73	-52	1.52E-6	3.83E-6	9.65E-6
CAKI-1	0.611	2.291	2.116	2.197	2.130	1.584	0.556	90	94	90	58	-9	1.31E-6	7.34E-6	> 1.00E-5
SN12C	1.400	3.100	2.987	3.133	3.113	2.940	1.535	93	102	101	91	8	3.10E-6	> 1.00E-5	> 1.00E-5
TK-10	0.968	1.890	1.926	1.913	1.934	2.050	0.755	104	103	105	117	-22	3.04E-6	6.95E-6	> 1.00E-5
UO-31	0.694	1.898	1.846	1.837	1.846	1.653	0.241	96	95	96	80	-65	1.60E-6	3.54E-6	7.84E-6
Prostate Cancer															
PC-3	0.583	2.292	2.237	2.222	2.118	1.655	0.322	97	96	90	63	-45	1.31E-6	3.83E-6	> 1.00E-5
DU-145	0.320	1.153	1.206	1.186	1.182	1.076	0.123	106	104	103	91	-62	1.85E-6	3.94E-6	8.38E-6
Breast Cancer															
MCF7	0.443	1.923	1.757	1.717	1.723	1.394	0.261	89	86	86	64	-41	1.37E-6	4.07E-6	> 1.00E-5
MDA-MB-231/ATCC	0.670	1.701	1.738	1.655	1.711	1.487	0.360	104	96	101	79	-46	1.71E-6	4.27E-6	> 1.00E-5
HS 578T	1.217	2.123	2.120	2.041	2.095	2.009	1.097	100	91	97	87	-10	2.42E-6	7.92E-6	> 1.00E-5
BT-549	1.381	2.458	2.335	2.415	2.367	2.306	1.758	89	96	92	86	35	5.07E-6	> 1.00E-5	> 1.00E-5
T-47D	0.810	1.466	1.394	1.400	1.267	1.109	0.714	89	90	70	46	-12	6.51E-7	6.20E-6	> 1.00E-5
MDA-MB-468	0.875	1.638	1.590	1.585	1.544	1.458	0.488	94	93	88	76	-44	1.65E-6	4.30E-6	> 1.00E-5