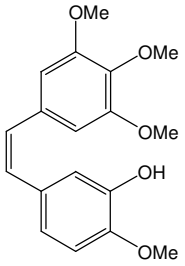
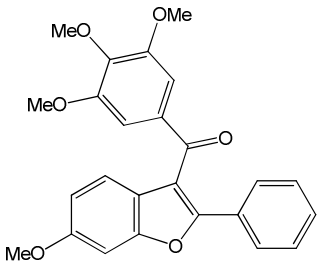
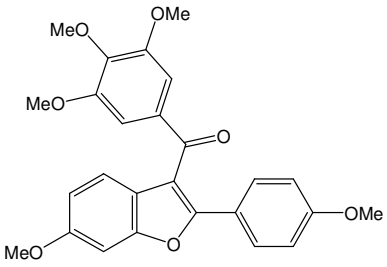
Supplementary Information

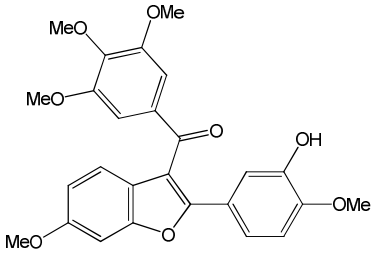
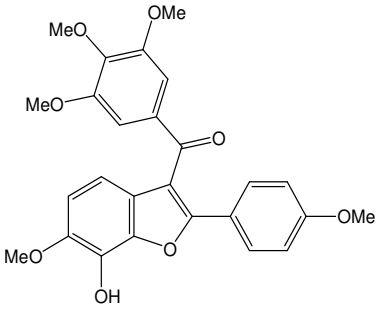
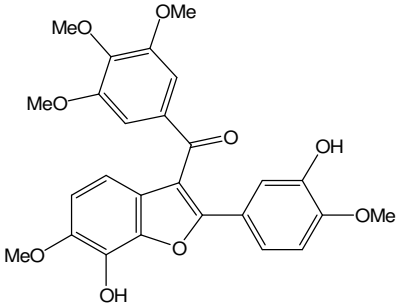
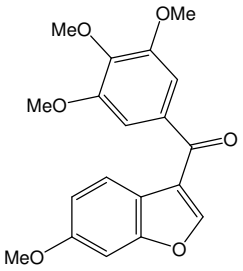
Discovery of 7-Hydroxy-6-methoxy-2-methyl-3-(3,4,5-trimethoxybenzoyl)benzo[*b*]furan (BNC105), a Tubulin Polymerization Inhibitor with Potent Antiproliferative and Tumor Vascular Disrupting Properties.

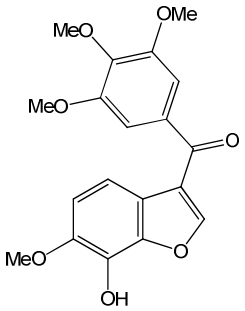
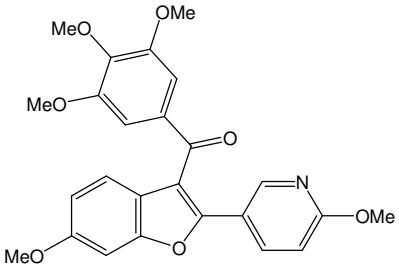
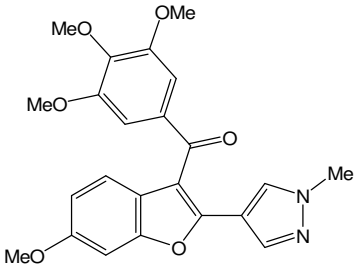
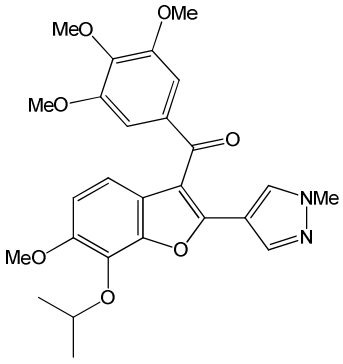
Bernard L. Flynn, Gurmit S. Gill, Damian W. Grobelny, Jason H. Chaplin, Dharam Paul, Annabell F. Leske, Tina C. Lavranos, David K. Chalmers, Susan A. Charman, Edmund Kostewicz, David M. Shackleford, Julia Morizzi, Ernest Hamel, M. Katherine Jung, Gabriel Kremmidiotis.

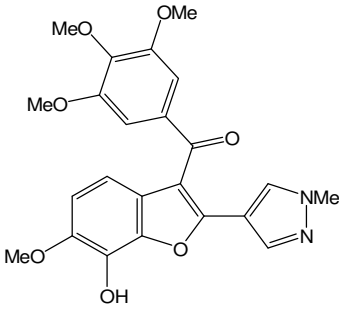
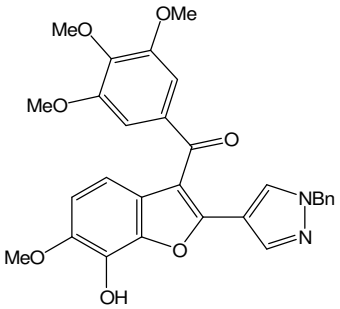
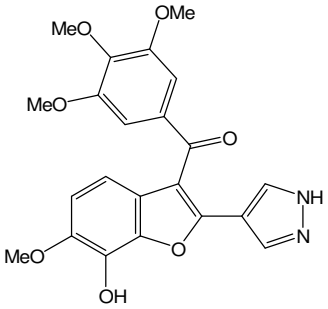
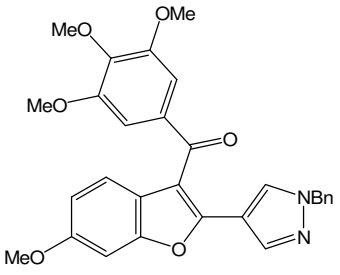
Contents

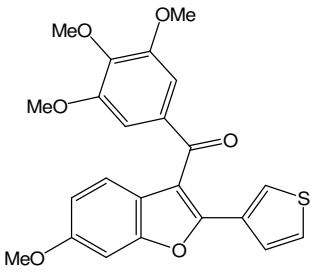
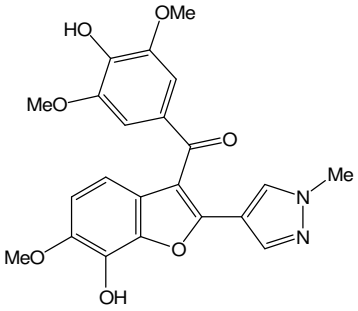
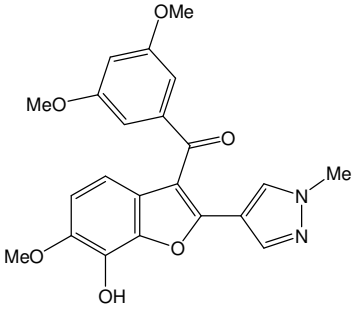
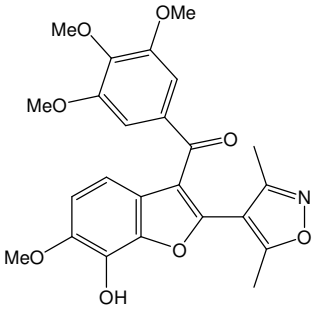
Table SI-1: Complete listing of novel benzo[*b*]furans evaluated

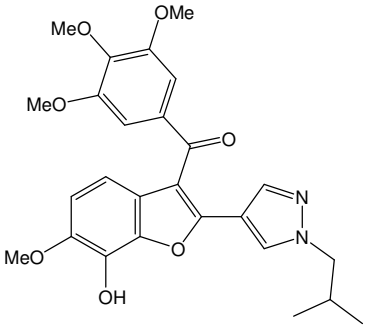
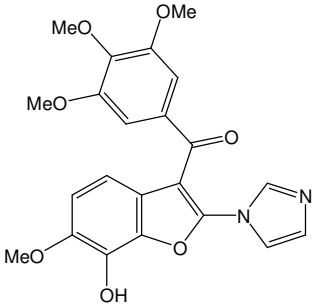
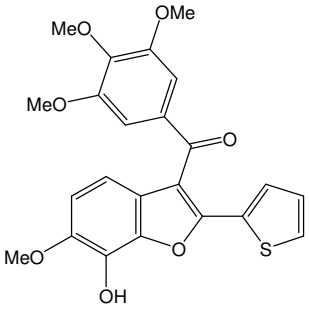
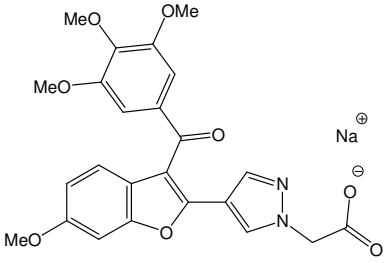
Entry	Structure[a]	Inhib. Tubulin polymer-isation[b] IC_{50} (μM)	Inhibition of cancer cell proliferation[c] IC_{50} (nM)	Inhib HUVECs[e] IC_{50} (nM)
1.		1.8 ± 0.2	<10, 2.9	Activated: 1-10 Quiescent: 1-10
2.		1.5 ± 0.5	88 +/- 10	ND
3.		1.5 ± 0.5	34 +/- 10	ND

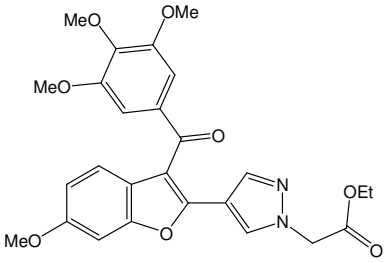
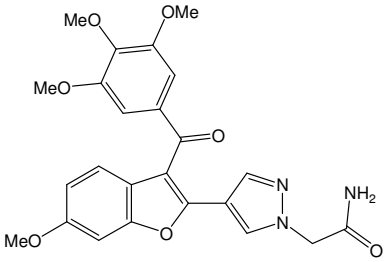
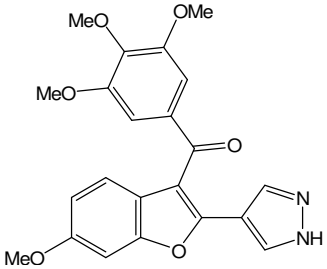
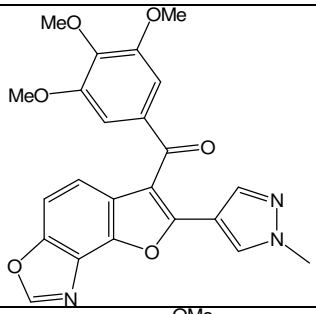
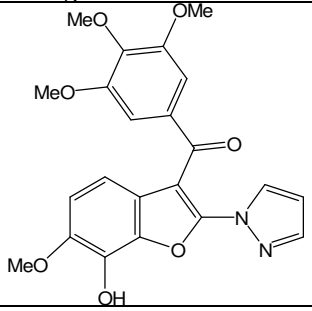
Entry	Structure[a]	Inhib. Tubulin polymer-isation[b] IC_{50} (μM)	Inhibition of cancer cell proliferation[c] IC_{50} (nM)	Inhib HUVECs[e] IC_{50} (nM)
4.		1.3 ± 0.1	57, 21	ND
5.		1.5 ± 0.4	1-2	ND
6.		<4	3.9, 4.1	Activated: 1-10 Quiescent: 1-10
7.		1.6 ± 0.2	60, 50	Activated: 10-100 Quiescent: 10-100

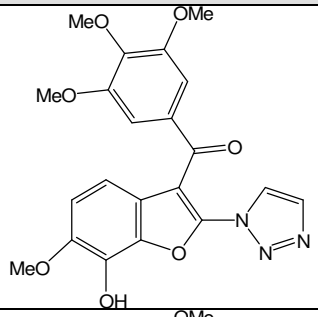
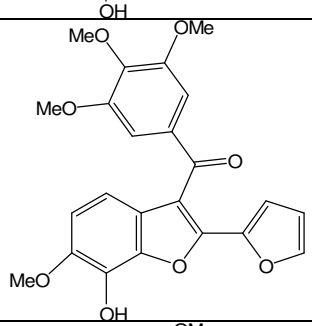
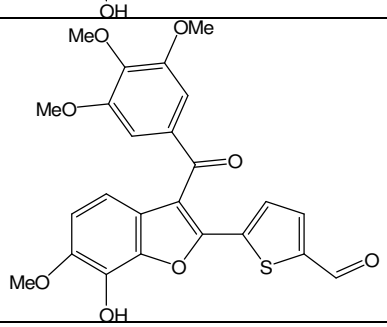
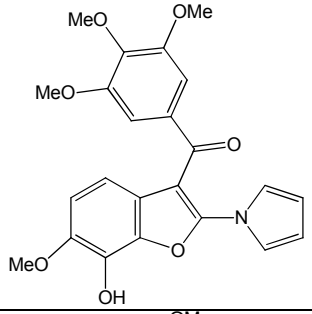
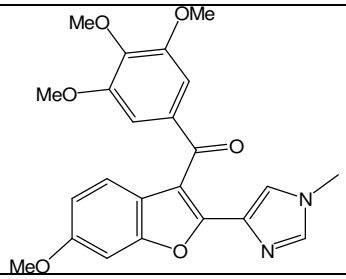
Entry	Structure[a]	Inhib. Tubulin polymer-isation[b] IC_{50} (μM)	Inhibition of cancer cell proliferation[c] IC_{50} (nM)	Inhib HUVECs[e] IC_{50} (nM)
8.			62, 31	Activated: 10-100 Quiescent: 10-100
9.		<4	70, 72	Activated: 10-100 Quiescent: 10-100
10.		1.2 ± 0.04	3.9, 4.0	Activated: 1-10 Quiescent: 10-100
11.		ND	48, 35	Activated: 100-1000 Quiescent: 100-1000

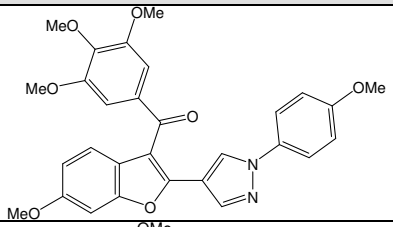
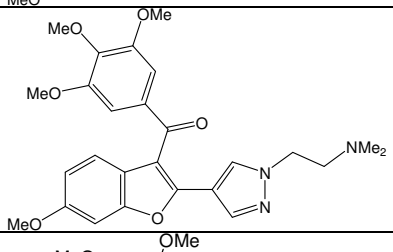
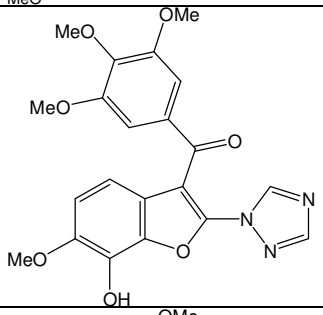
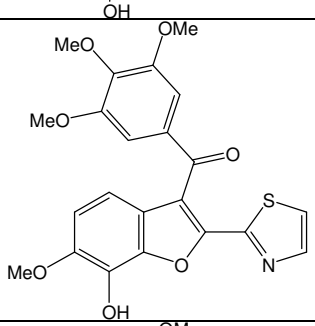
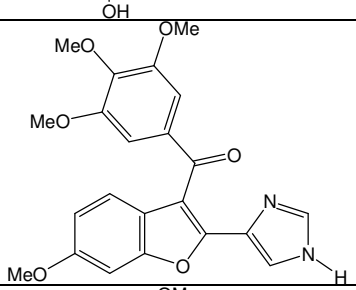
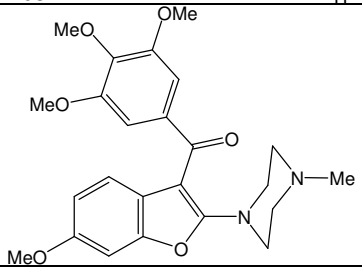
Entry	Structure[a]	Inhib. Tubulin polymer-isation[b] IC_{50} (μM)	Inhibition of cancer cell proliferation[c] IC_{50} (nM)	Inhib HUVECs[e] IC_{50} (nM)
12.		1.3 ± 0.07	4, <1	Activated: 0.1-1.0 Quiescent: 0.1-1.0
13.		ND	4.1, 3.3	ND
14.		ND	3.0, 3.4	Activated: 1-10 Quiescent: 1-10
15.		ND	110, 83	Activated: 100-1000 Quiescent: 100-1000

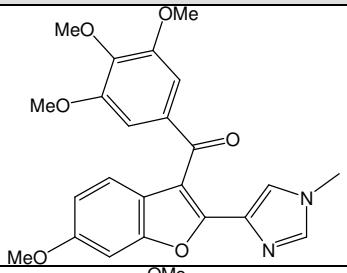
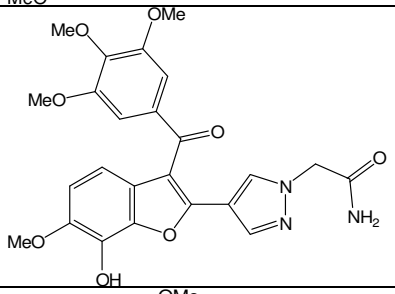
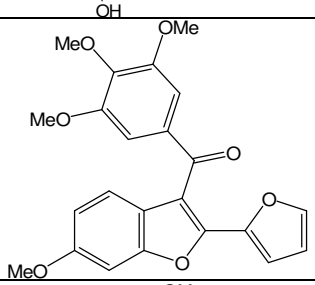
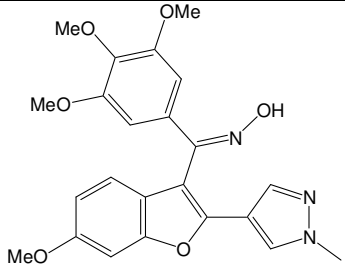
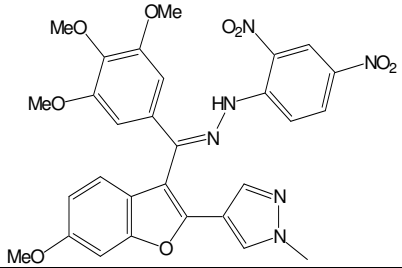
Entry	Structure[a]	Inhib. Tubulin polymer-isation[b] IC_{50} (μM)	Inhibition of cancer cell proliferation[c] IC_{50} (nM)	Inhib HUVECs[e] IC_{50} (nM)
16.		ND	17, 22	Activated: 10-100 Quiescent: 10-100
17.		ND	25, 29	ND
18.		ND	3.3; 4.4	Activated: 1-10 Quiescent: 1-10
19.		ND	68; 57	Activated: 10-100 Quiescent: 10-100

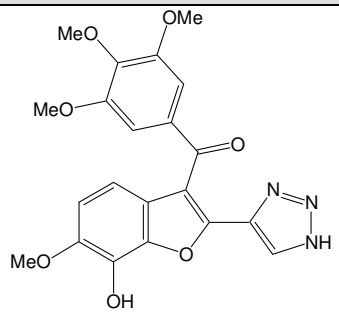
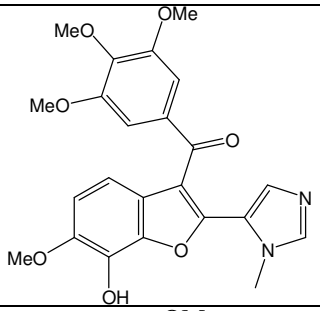
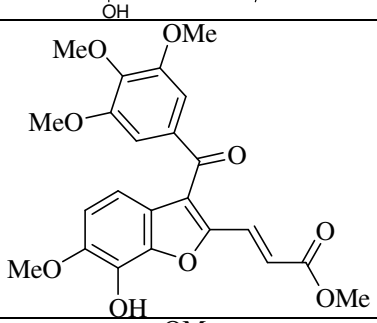
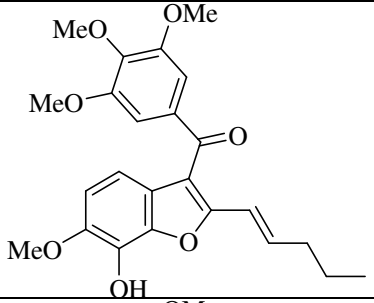
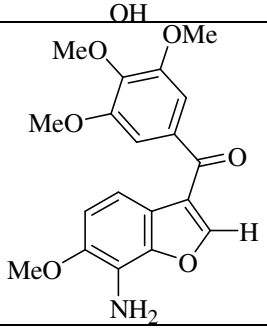
Entry	Structure[a]	Inhib. Tubulin polymer-isation[b] IC_{50} (μM)	Inhibition of cancer cell proliferation[c] IC_{50} (nM)	Inhib HUVECs[e] IC_{50} (nM)
20.		ND	21; 14	Activated: 1-10 Quiescent: 10-100
21.		ND	1.6; <1	Activated: 1-10 Quiescent: 1-10
22.		ND	<1; <1	Activated: 0.1-1.0 Quiescent: 0.1-1.0
23.		ND	900; 540	Activated: 100-1000 Quiescent: 100-1000

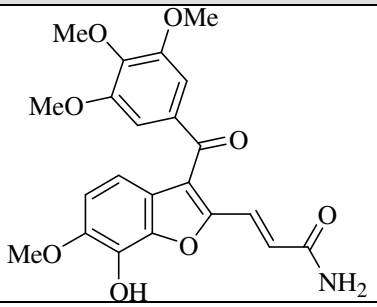
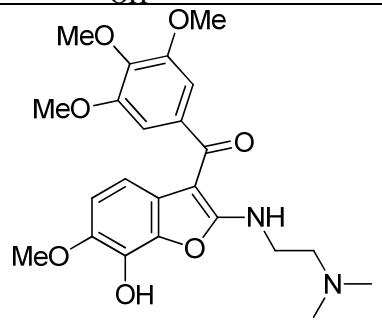
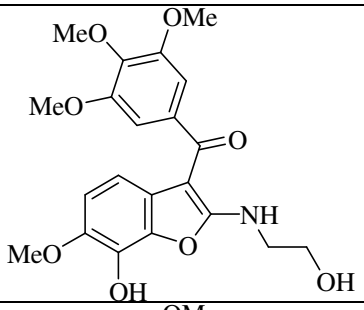
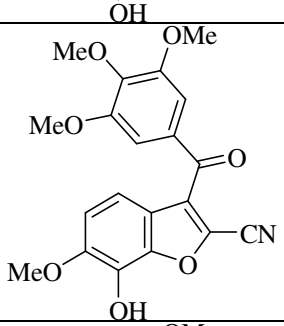
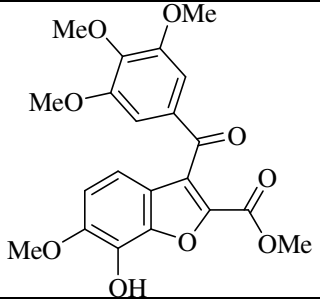
Entry	Structure[a]	Inhib. Tubulin polymer-isation[b] IC_{50} (μM)	Inhibition of cancer cell proliferation[c] IC_{50} (nM)	Inhib HUVECs[e] IC_{50} (nM)
24.		ND	29; 15	Activated: 10-100 Quiescent: 10-100
25.		ND	3.8; 4.0	Activated: 1-10 Quiescent: 1-10
26.		ND	3.6; 4.0	Activated: 1-10 Quiescent: 10-100
27.		ND	540; 600	Activated: 100-1000 Quiescent: 100-1000
28.		ND	3.5; 2.4	Activated: 100-1000 Quiescent: 100-1000

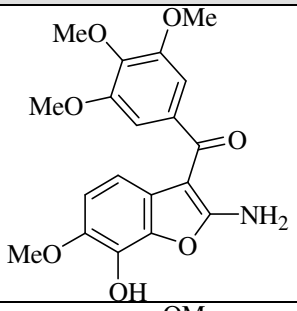
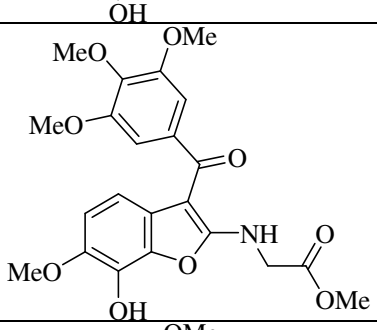
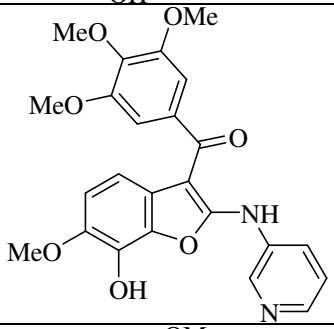
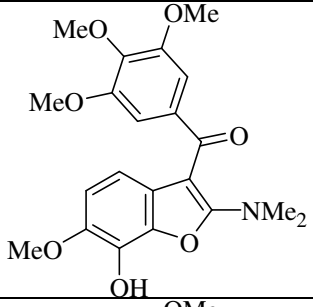
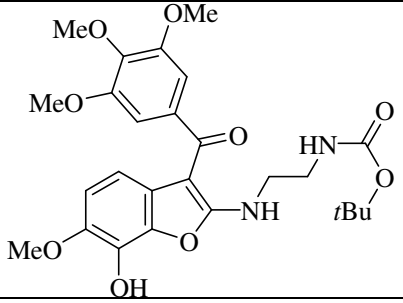
Entry	Structure[a]	Inhib. Tubulin polymer-isation[b] IC_{50} (μM)	Inhibition of cancer cell proliferation[c] IC_{50} (nM)	Inhib HUVECs[e] IC_{50} (nM)
29.		ND	0.42; 1.7	Activated: 1-10 Quiescent: 1-10
30.		8.7 ± 0.8	0.33; 0.75	Activated: 0.1-1.0 Quiescent: 0.1-1.0
31.		ND	1.8; 2.3	Activated: 1-10 Quiescent: 1-10
32.		ND	1.4; 2.1	Activated: 1-10 Quiescent: 1-10
33.		ND	40; 35	Activated: 10-100 Quiescent: 10-100

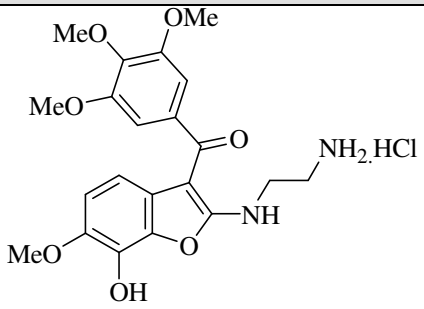
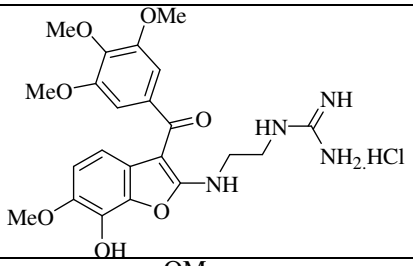
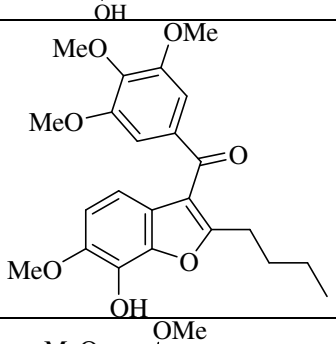
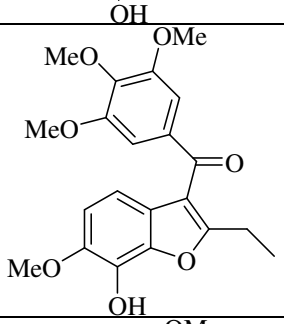
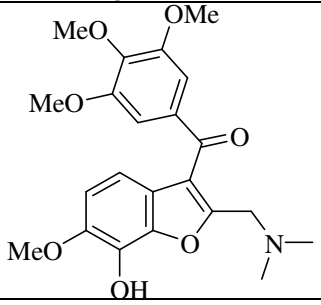
Entry	Structure[a]	Inhib. Tubulin polymer-isation[b] IC_{50} (μM)	Inhibition of cancer cell proliferation[c] IC_{50} (nM)	Inhib HUVECs[e] IC_{50} (nM)
34.		ND	360, 350	Activated: 10-100 Quiescent: 10-100
35.		ND	300; 310	Activated: 100-1000 Quiescent: 100-1000
36.		ND	0.37; 0.32	Activated: 0.1-1.0 Quiescent: 0.1-1.0
37.		2.2 ± 0.4	0.37; 0.32	Activated: 0.1-1.0 Quiescent: 0.1-1.0
38.		ND	4.5; 5.7	Activated: 10-100 Quiescent: 10-100
39.		ND	200, 230	Activated: 100-1000 Quiescent: 100-1000

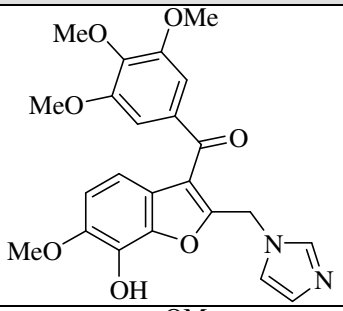
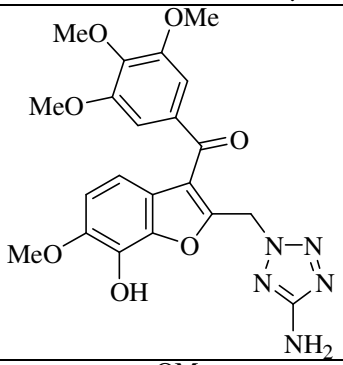
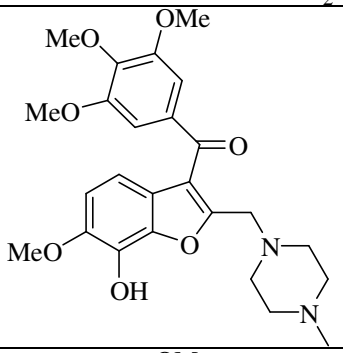
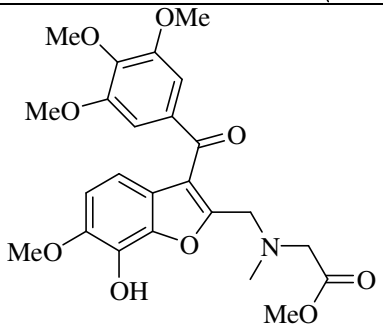
Entry	Structure[a]	Inhib. Tubulin polymer-isation[b] IC_{50} (μM)	Inhibition of cancer cell proliferation[c] IC_{50} (nM)	Inhib HUVECs[e] IC_{50} (nM)
40.		ND	40, 35	Activated: 10-100 Quiescent: 10-100
41.		ND	1.5, 0.62	Activated: 0.1-1.0 Quiescent: 0.1-1.0
42.		ND	6.9, 7.9	Activated: 1-10 Quiescent: 1-10
43.		ND	100-1000[d]	Activated: 100-1000 Quiescent: 100-1000
44.		ND	>1000[d]	Activated: 10-100 Quiescent: >1000

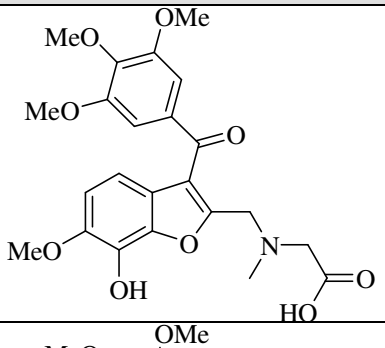
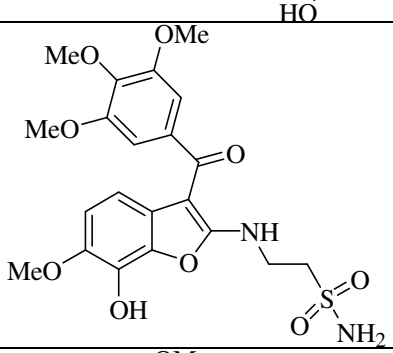
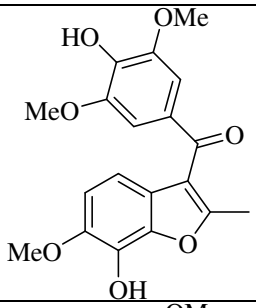
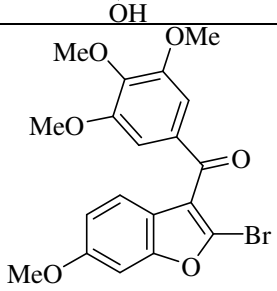
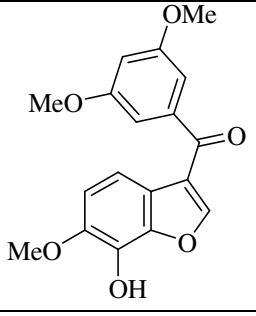
Entry	Structure[a]	Inhib. Tubulin polymer-isation[b] IC_{50} (μM)	Inhibition of cancer cell proliferation[c] IC_{50} (nM)	Inhib HUVECs[e] IC_{50} (nM)
45.		ND	1-10[d]	Activated: 1-10 Quiescent: 1-10
46.		ND	1-10[d]	Activated: 1-10 Quiescent: 1-10
47.		ND	2.0	Activated: 0.1-1 Quiescent: 1-10
48.		ND	8.0	Activated: 1-10 Quiescent: 1-10
49.		ND	1-10[d]	Activated: 1-10 Quiescent: 1-10

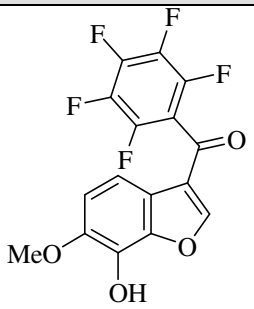
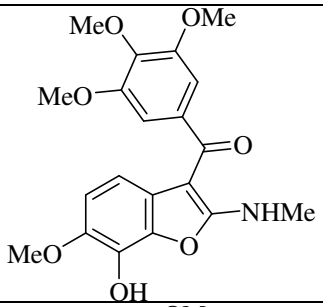
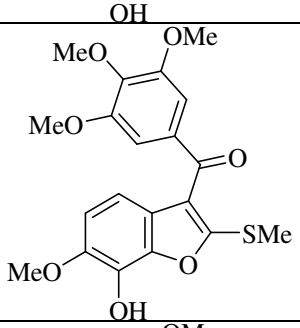
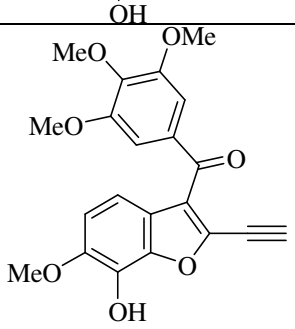
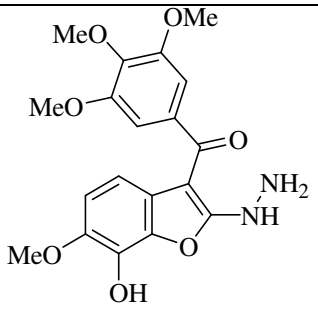
Entry	Structure[a]	Inhib. Tubulin polymer-isation[b] IC_{50} (μM)	Inhibition of cancer cell proliferation[c] IC_{50} (nM)	Inhib HUVECs[e] IC_{50} (nM)
50.		ND	1-10[d]	Activated: 1-10 Quiescent: 1-10
51.		ND	10-100[d]	Activated: 10-100 Quiescent: 10-100
52.		ND	1-10[d]	Activated: 1-10 Quiescent: 1-10
53.		ND	0.1-1[d]	Activated: 1-10 Quiescent: 1-10
54.		ND	1-10[d]	Activated: 1-10 Quiescent: 1-10

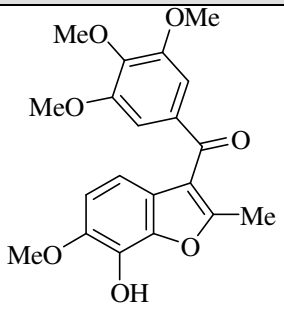
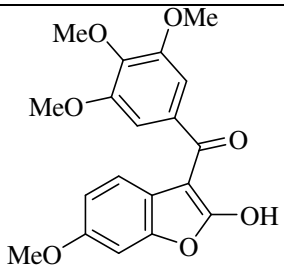
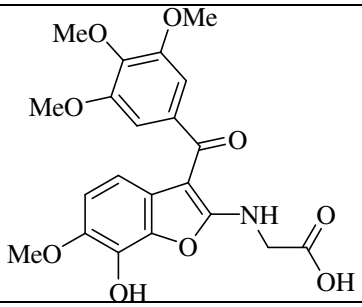
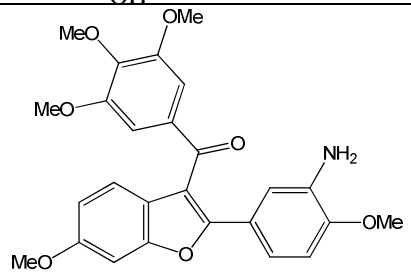
Entry	Structure[a]	Inhib. Tubulin polymer-isation[b] IC_{50} (μM)	Inhibition of cancer cell proliferation[c] IC_{50} (nM)	Inhib HUVECs[e] IC_{50} (nM)
55.		ND	0.1-1[d]	Activated: 1-10 Quiescent: 1-10
56.		ND	1-10[d]	Activated: 1-10 Quiescent: 1-10
57.		ND	1-10[d]	Activated: 1-10 Quiescent: 1-10
58.		ND	1-10[d]	Activated: 1-10 Quiescent: 1-10
59.		ND	ND	Activated: 1-10 Quiescent: 1-10

Entry	Structure[a]	Inhib. Tubulin polymer-isation[b] IC_{50} (μM)	Inhibition of cancer cell proliferation[c] IC_{50} (nM)	Inhib HUVECs[e] IC_{50} (nM)
60.		ND	ND	Activated: 100-1000 Quiescent:100-1000
61.		ND	ND	Activated: 100-1000 Quiescent:10-100
62.		ND	ND	Activated: 1-10 Quiescent:1-10
63.		ND	ND	Activated: 0.1-1.0 Quiescent:0.1-1.0
64.		ND	ND	Activated: 1-10 Quiescent:1-10

Entry	Structure[a]	Inhib. Tubulin polymer-isation[b] IC_{50} (μM)	Inhibition of cancer cell proliferation[c] IC_{50} (nM)	Inhib HUVECs[e] IC_{50} (nM)
65.		ND	ND	Activated: 1-10 Quiescent:1-10
66.		ND	ND	Activated: 1-10 Quiescent:1-10
67.		ND	ND	Activated: 10-100 Quiescent:10-100
68.		ND	ND	Activated: 1-10 Quiescent: 1-10

Entry	Structure[a]	Inhib. Tubulin polymer-isation[b] IC_{50} (μM)	Inhibition of cancer cell proliferation[c] IC_{50} (nM)	Inhib HUVECs[e] IC_{50} (nM)
69.		ND	ND	Activated: 10-100 Quiescent: 10-100
70.		ND	ND	Activated: 1-10 Quiescent: 1-10
71.		ND	ND	Activated: 10-100 Quiescent: 100-1000
72.		ND	500	Tum: 100-1000 Norm: 100-1000
73.		ND	35	Tum: 100-1000 Norm: 100-1000

Entry	Structure[a]	Inhib. Tubulin polymer-isation[b] IC_{50} (μM)	Inhibition of cancer cell proliferation[c] IC_{50} (nM)	Inhib HUVECs[e] IC_{50} (nM)
74.		ND	800	Tum: >1000 Norm: >1000
75.		ND	3.5	Tum: 1-10 Norm: 0.1-1
76.		ND	1.2	Tum: 0.1-1 Norm: 1-10
77.		ND	3.3	Tum: 1-10 Norm: 1-10
78.		ND	35	Tum: 1-10 Norm: 10-100

Entry	Structure[a]	Inhib. Tubulin polymer-isation[b] IC_{50} (μM)	Inhibition of cancer cell proliferation[c] IC_{50} (nM)	Inhib HUVECs[e] IC_{50} (nM)
79.		3.0	2.4	Tum: 0.1-1 Norm: 10-100
80.		ND	575	Tum: 100-1000 Norm: 100-1000
81.		ND	260	Tum: 100-1000 Norm: 100-1000
82.		1.9±0.4	26±5	Tum: 32 Norm: 44

[a] For synthetic methods and spectral data, see: main text experimental and main text refs 25 and 26.

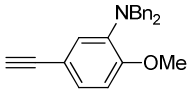
[b] The tubulin concentration was 10 μM. Inhibition of extent of assembly, after a 20 min incubation at 30 °C, was the parameter measured: For a description of the method see Verdier-Pinard, P. *et. al. Mol. Pharmacol.* **1998**, *53*, 62-76

[c] Unless otherwise stated the cell line was MCF-7, using the method described in: Verdier-Pinard, P. *et. al. Mol. Pharmacol.* **1998**, *53*, 62-76.

[d] The cell line used was MDA-MB-231, see main text experimental for method.

[c] The value is given as a range within which the IC_{50} value falls, see main text Experimental for method.

Synthesis of Compounds:



3-*N,N*-Dibenzylamino-4-methoxy-phenylacetylene 17a:

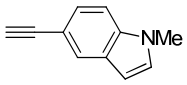
This material was prepared in 4 steps from 2-methoxy-4-iodonitrobenzene:

Step 1, 2-methoxy-4-iodoaniline: A suspension of 2-methoxy-4-iodonitrobenzene (4.00 g, 14.3 mmol) and tin(II) chloride (12.93 g, 57.30 mmol) in 95% ethanol (40 mL) was heated to reflux for 4 h. The solvent was removed under vacuum, and the residue was dissolved in ethyl acetate (100 mL). The organic layer was washed with a 10% sodium bicarbonate solution (100 mL) and brine (100 mL) and dried over $MgSO_4$. The solvent was removed under vacuum to afford the title compound as a dark brown oil (2.73 g, 77%) that was used without further purification in the next step. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.98 (dd, *J* = 8.78, 2.09 Hz, 1H), 6.97 (d, *J* = 1.81 Hz, 1H), 6.50 (d, *J* = 8.90 Hz, 1H), 3.80 (s, 3H).

Step 2, 2-methoxy-4-iodo-*N,N*-dibenzylaniline: To a solution of 2-methoxy-4-iodoaniline (2.73 gm, 11.00 mmol) in dry dimethylformamide (10 mL) under nitrogen was added potassium carbonate (3.35 g, 24.2 mmol), followed by the addition of benzyl bromide (2.9 mL, 24.4 mmol), and the reaction mixture was stirred at 100 ^{o}C for 6.5 h (TLC), then cooled to room temperature, diluted with ethyl acetate (80 mL) and washed with 10% aqueous citric acid (3 x 80 mL) and brine (2 x 80 mL). The organic layer was separated and dried over $MgSO_4$. The solvent was removed under vacuum to afford a viscous brown oil (4.42 gm, 94%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26-7.17 (m, 11H), 7.03 (br s, 1H), 6.60 (d, *J* = 8.50 Hz, 1H), 4.20 (br s, 4H), 3.86 (s, 3H).

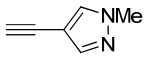
Step 3, 3-*N,N*-dibenzylamino-4-methoxyphenyl-trimethylsilylacetylene: A solution of 2-methoxy-4-iodo-*N,N*-dibenzylaniline (500 mg, 1.16 mmol), $Pd(PPh_3)_2Cl_2$ (25 mg, 0.036 mmol) and triethylamine (320 μL, 2.30 mmol) in dry dichloromethane was deoxygenated by passing nitrogen through the solution for 2 min. To the resulting yellow suspension was added trimethylsilylacetylene (250 μL, 1.77 mmol), followed by the addition of copper (I) iodide (11 mg, 0.058 mmol). The resulting suspension was stirred at room temperature for 1.5 h. The reaction was diluted with dichloromethane (20 mL) and washed with water (20 mL) and brine (20 mL). The organic layer was separated and dried over magnesium sulfate, and the solvent was removed under vacuum to afford the crude product (502 mg) as a brown oil that was used in the next step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25-7.19 (m, 10H), 7.06(dd, *J* = 8.32, 1.94 Hz, 1H), 6.90 (d, *J* = 1.83 Hz, 1H), 6.77 (d, J = 8.35 Hz, 1H), 4.20 (bs, 4H), 3.89 (s, 3H), 0.20 (s, 9H).

Step 4, 3-*N,N*-dibenzylamino-4-methoxy-phenylacetylene **17a**: To a solution of 3-*N,N*-dibenzylamino-4-methoxyphenyl-trimethylsilylacetylene in methanol (5 mL) and tetrahydrofuran (7 mL) was added potassium fluoride (384 mg, 6.6 mmol), and the reaction mixture was stirred at room temperature for 1 h and diluted with ethyl acetate (20 mL). The organic layer was washed with water (2 x 20 mL) and dried over $MgSO_4$. The solvent was removed under vacuum to give an orange oil that was purified by column chromatography to give a slightly tan oil (149 mg, 69%) ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26-7.17(m, 10H), 7.08 (dd, *J* = 8.31, 1.89 Hz, 1H), 6.92 (d, *J* = 1.49 Hz, 1H), 6.79 (d, J = 8.33 Hz, 1H), 4.21 (bs, 4H), 3.90 (s, 3H), 2.98 (s, 1H).



5-Ethynyl-1-methyl-1*H*-indole 17b:

A solution of 1-methyl-5-iodoindole (500 mg, 1.95 mmol) in dry 1,4-dioxane (5 mL) was deoxygenated by passing nitrogen through the solution. Trimethylsilylacetylene (323 μL, 2.33 mmol), $Pd(PPh_3)_2Cl_2$ (68.3 mg, 0.097 mmol) and copper (I) iodide (11.12 mg, 0.058 mmol) were then added sequentially, and the reaction mixture was stirred for 1 h and filtered through celite. The solvent was removed under reduced pressure and the residue dissolved in tetrahydrofuran (6 mL) and treated with tetrabutylammonium fluoride (2.1 mL, 1 M solution in tetrahydrofuran). The reaction was stirred for 15 min and diluted with ethyl acetate (10 mL). The organic layer was separated, washed with 1 M HCl (aq) (5 mL) and dried over $MgSO_4$. The solvent was removed under vacuum, and the crude product was purified by flash chromatography (silica gel, eluent = hexane / diethyl ether 9:1) to afford the title compound as a creamy solid (117 mg, 39%) ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80 (br s, 1H), 7.34 (dd, *J* = 8.48, 1.43 Hz, 1H), 7.24 (d, *J* = 8.48, 1H), 7.05 (d, *J* = 3.11 Hz, 1H), 6.46 (d, *J* = 3.06 Hz, 1H), 3.77 (s, 3H), 2.99 (s, 3H).



4-Ethynyl-1-methyl-1-H-pyrazole 17f:

When 1-methyl-5-iodoindole was replaced by 1-methyl-4-iodopyrazole in example (**17b**), the identical procedure afforded the title compound as a yellow paste that crystallized on standing (890 mg, 58%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57(s, 1H), 7.50 (s, 1H), 3.86 (s, 3H), 2.98 (s, 1H).

Pharmacokinetic Studies of BNC105 / BNC105P

Figure SI-3: Comparison of dose normalized plasma concentrations of CA4 following IV (6 µmol/Kg) administration of CA4 and CA4P to rats

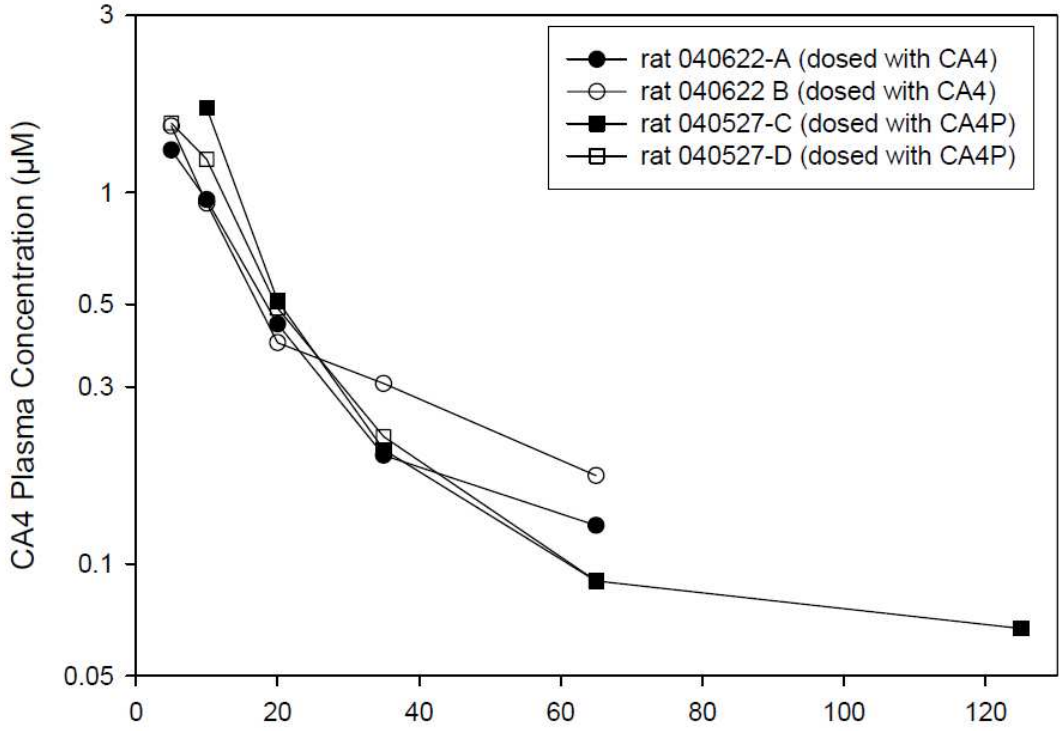


Figure SI-4: Plasma concentrations of BNC105 following IV administration to male Sprague Dawley rats at nominal dose of 6.0 µmol/kg.

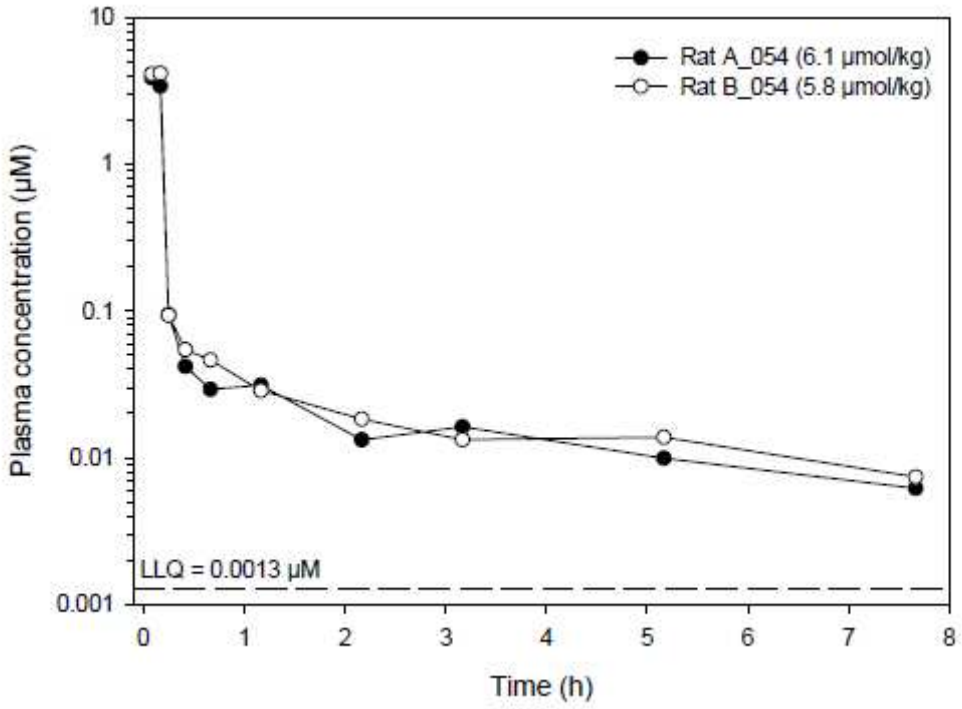


Figure SI-5: Plasma concentrations of BNC105P (closed symbols) and BNC105 (open symbols) following IV administration of BNC105P to male Sprague Dawley rats at a nominal dose of 6.6 µmol/kg.

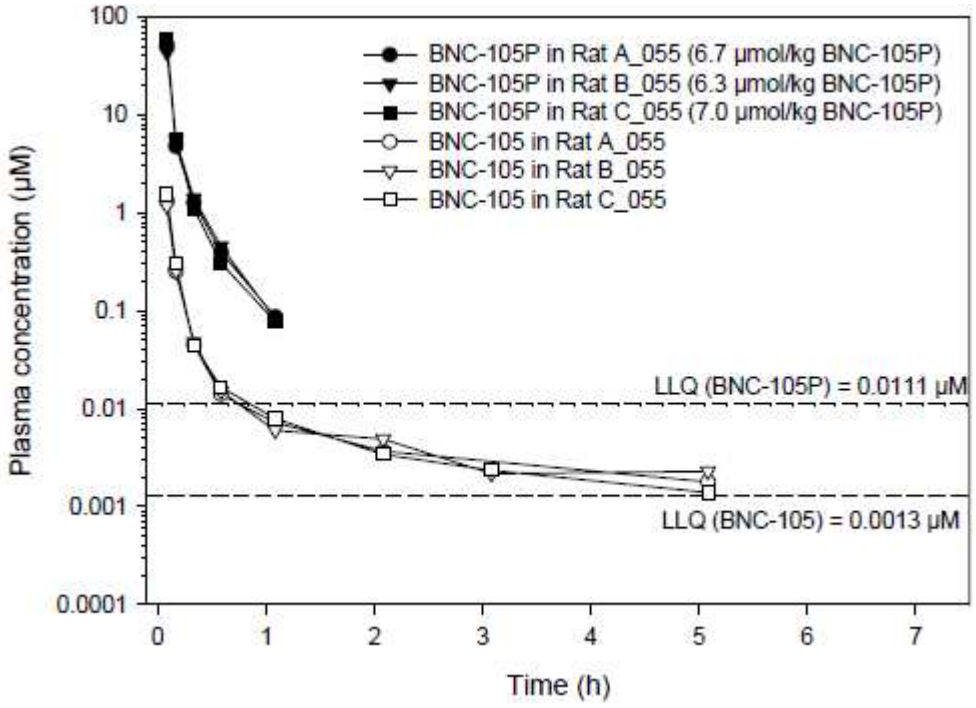


Table SI-2: Tissue distribution of BNC105 in mice after IV administration of BNC105 and BNC105P. Data represent the dose normalized concentrations of BNC105 at T = 15 min and T = 2 h after IV administration of 6.0 µmol/kg of BNC105 and BNC105P.

Sample Time	Compound Dosed	[BNC105] in tissue (ng/g)					
		Tumor	**Liver**	**Heart**	**Spleen**	**Kidney**	**Brain**
T = 15 min	BNC105	936	794	475	2796	1117	5463
	BNC105P	636	527	725	2854	1121	2695
T = 2 h	BNC105	684	188	406	1310	653	3221
	BNC105P	500	206	358	1276	552	1932