Supporting information

Influence of a Basic Side Chain on the Properties of Hypoxia-Selective Nitro Analogues of the Duocarmycins – Demonstration of Substantial Anticancer Activity in Combination with Irradiation or Chemotherapy

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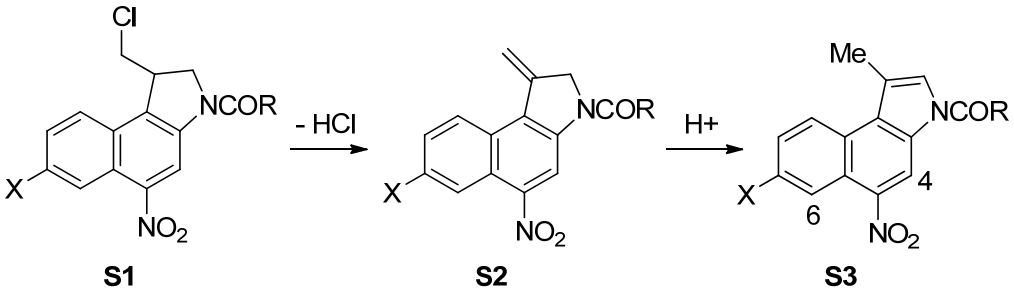
Contents

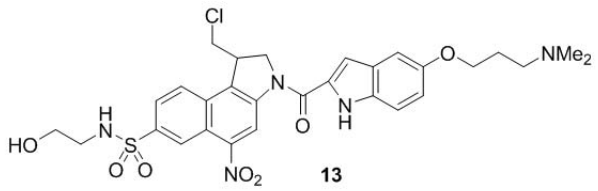
Purity of final products

NitroCBIs (**S1** in Scheme S1), particularly those bearing strong electron-withdrawing substituents X, are susceptible to base-induced elimination of HCl to give the corresponding exomethylene compound (**S2**). This is a common impurity found after introduction of the side chain R, even if very weakly basic conditions are used to wash away excess side chain acid RCO_2H. The exomethylene byproduct exhibits diagnostic signals in the ^{1}H NMR spectrum (in d_6-DMSO signals centred at δ 6.3, 5.8, and 5.5 in a 1:1:2 integral ratio) and is generally very slightly faster running than the desired chloromethyl compound under the HPLC conditions used. Subsequent acid treatment (e.g. in the formation of an HCl salt or the deprotection of TBDMS or t-Bu protecting groups) causes isomerization to the corresponding 1-methylindole (**S3**), which shows down-field shifts of some aromatic protons (H-4 and H-6 are the most easily detected) and is generally very slightly slower running than the chloromethylindoline compound under the HPLC conditions used. Since these byproducts are incapable of forming a spirocyclic intermediate their presence at low levels was considered of little consequence to the measured biological properties of the final products. In support of this interpretation the largest single impurity of 6.2% (tentatively identified as the corresponding 1-methylindole) was found for **13** yet the cytotoxicity of this nitroCBI under hypoxia was not compromised and in fact **13** generated very similar IC_{50}s to the more pure close analogue **12**.

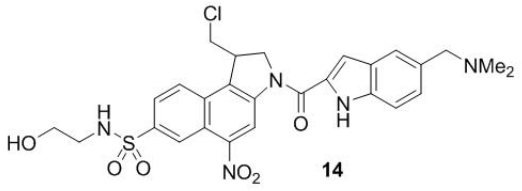


Scheme S1. Formation of exomethylene and 1-methylindole nitroCBI byproducts.

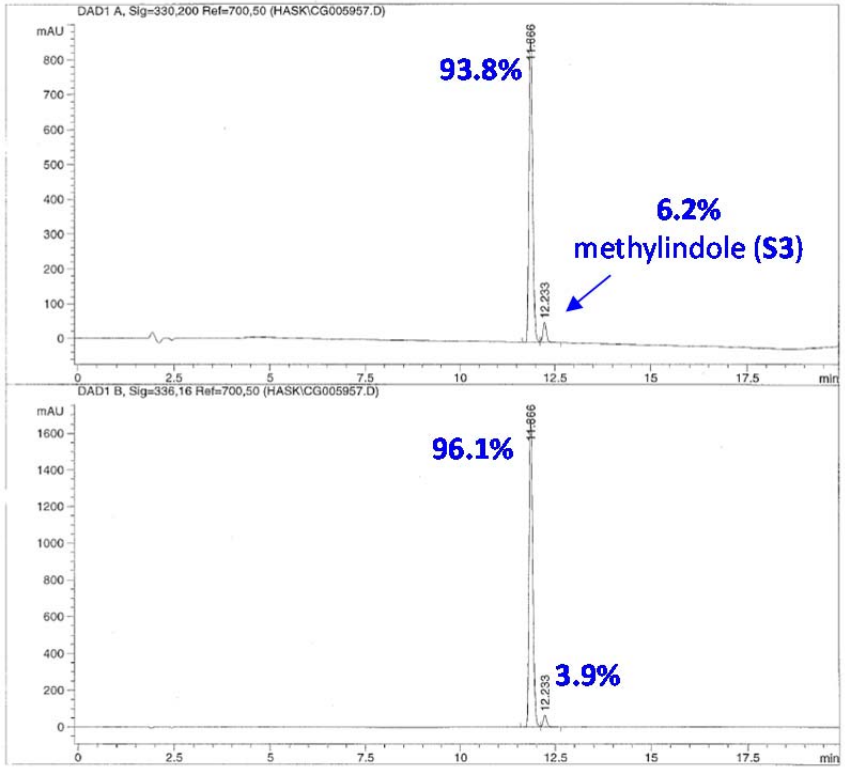
Figure S1. HPLC chromatograms of all final products with a purity of <95%. Chromatograms are reproduced on the following 8 pages. For each compound the upper panel shows chromatograms at 330 ± 200 nm (used for determining the reported purity) and the lower panel at λ_{max} ± 16 nm. For impurities >2% tentative identities are indicated.

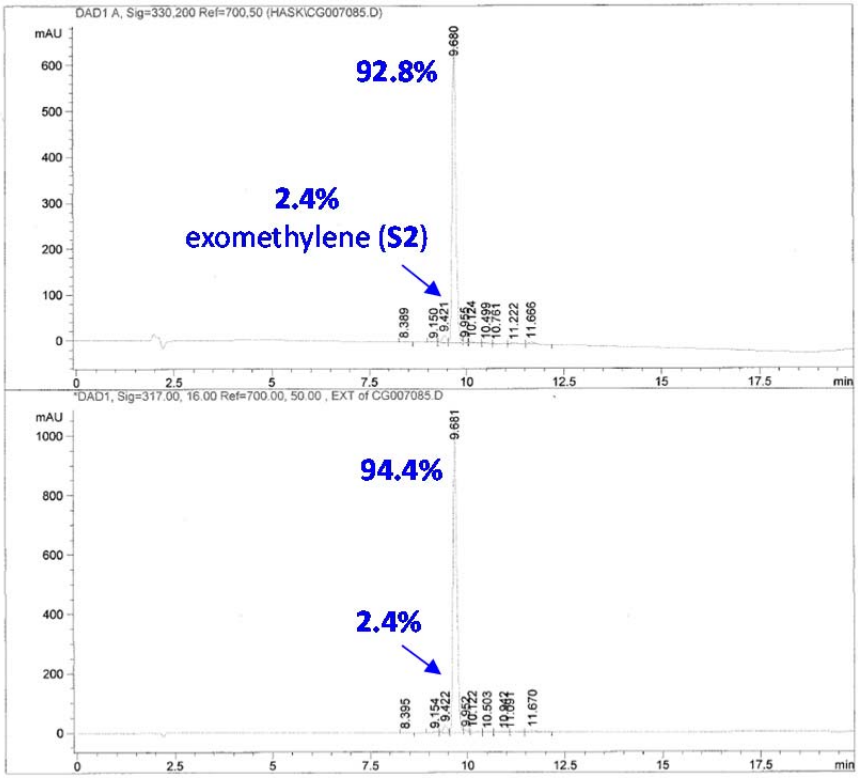

Cl
NMe2
HO
NO2

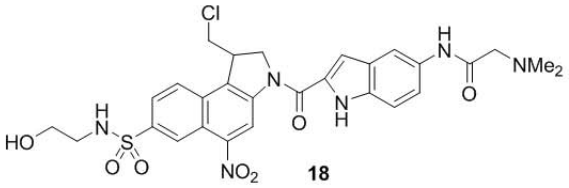
13


Cl
NMe2
HO
NO2

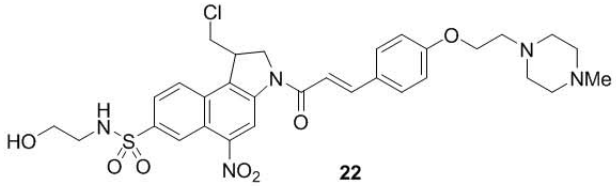
14


DAD1 A, Sig=330,200 Ref=700,50 (HASK\CG005957.D)
93.8%
6.2%
methylindole (S3)
DAD1 B, Sig=336,16 Ref=700,50 (HASK\CG005957.D)
96.1%
3.9%

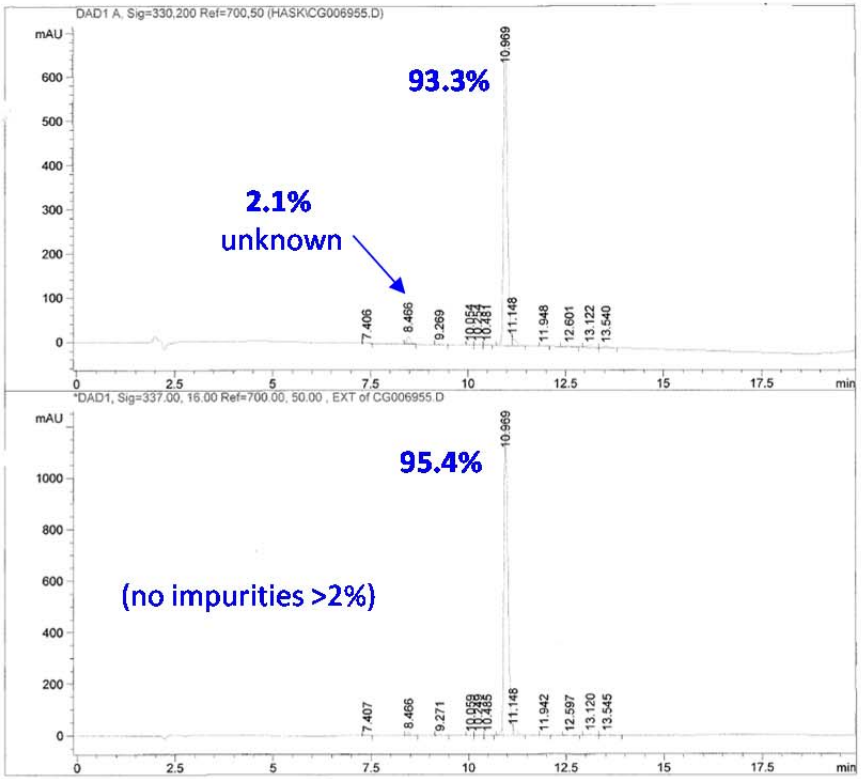

DAD1 A, Sig=330,200 Ref=700,50 (HASK\CG007085.D)
92.8%
2.4%
exomethylene (S2)
DAD1, Sig=317,00, 16,00 Ref=700,00, 50,00 , EXT of CG007085.D
94.4%
2.4%


Cl
HO
NO2
NMe2

18


Cl
HO
NO2
NMe

22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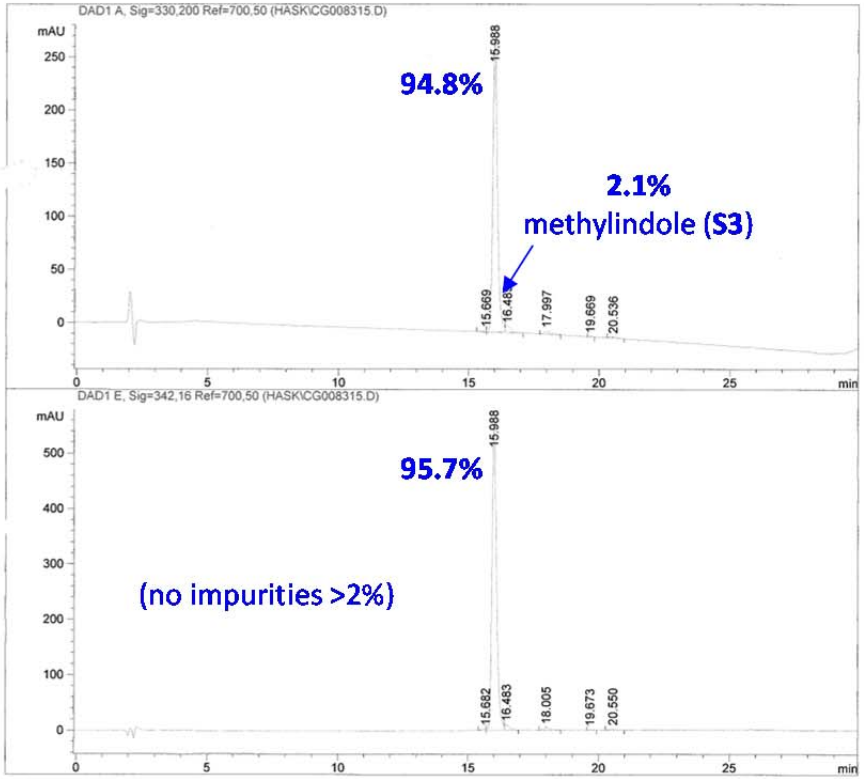
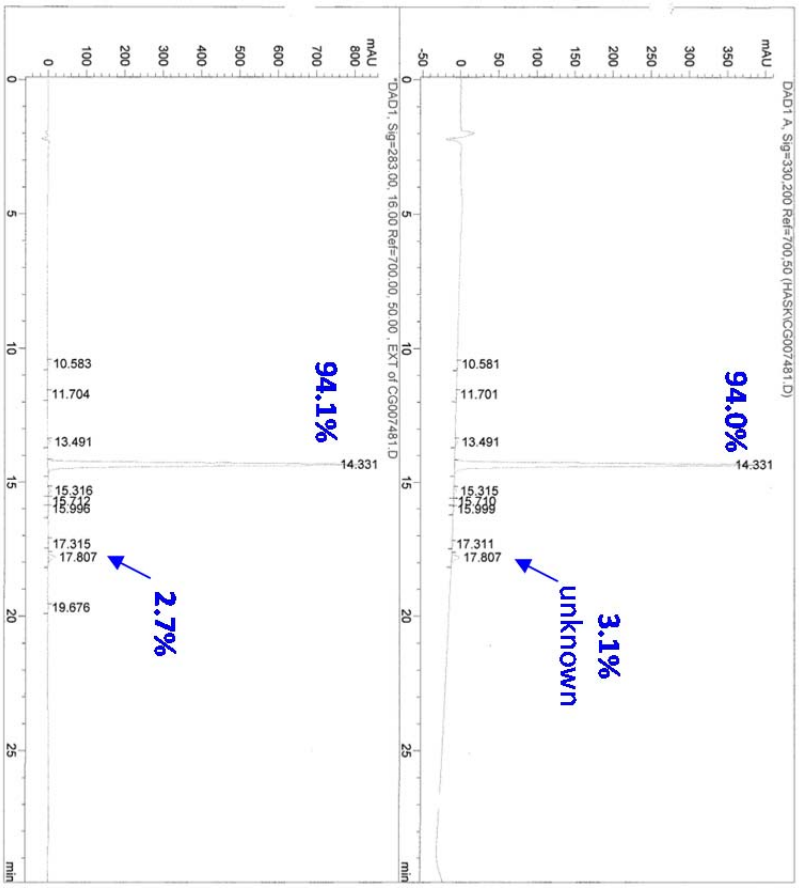
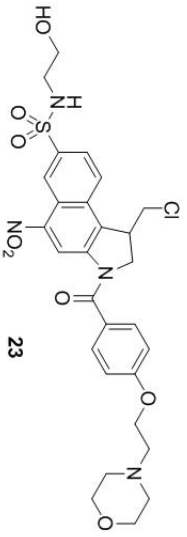
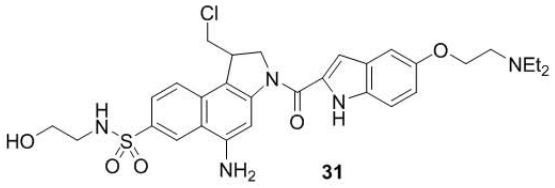
93.3%
2.1%
unknown
*DAD1, Sig=337.00, 16.00 Ref=700.00, 50.00 , EXT of CG006955.D
95.4%
(no impurities >2%)


DAD1 A, Sig=330,200 Ref=700,50 (HASK\CG008315.D)
94.8%
2.1%
methylindole (S3)
DAD1 E, Sig=342,16 Ref=700,50 (HASK\CG008315.D)
95.7%
(no impurities >2%)

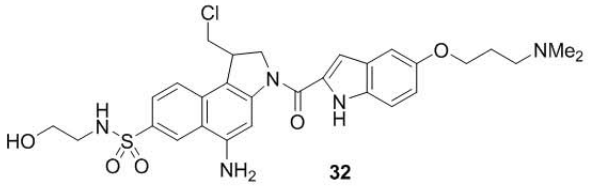

DAD1 A, Sig=330,200 Ref=700,50 (HASKICG007481.D)
mAU
94.0%
10.581
11.701
13.491
14.331
15.315
15.710
15.999
17.311
17.807
3.1%
unknown
DAD1 Sig=283,00, 16,00 Ref=700,00, 50,00 , EXT of CG007481.D
mAU
94.1%
10.583
11.704
13.491
14.331
15.316
15.712
15.996
17.315
17.807
19.676
2.7%
min



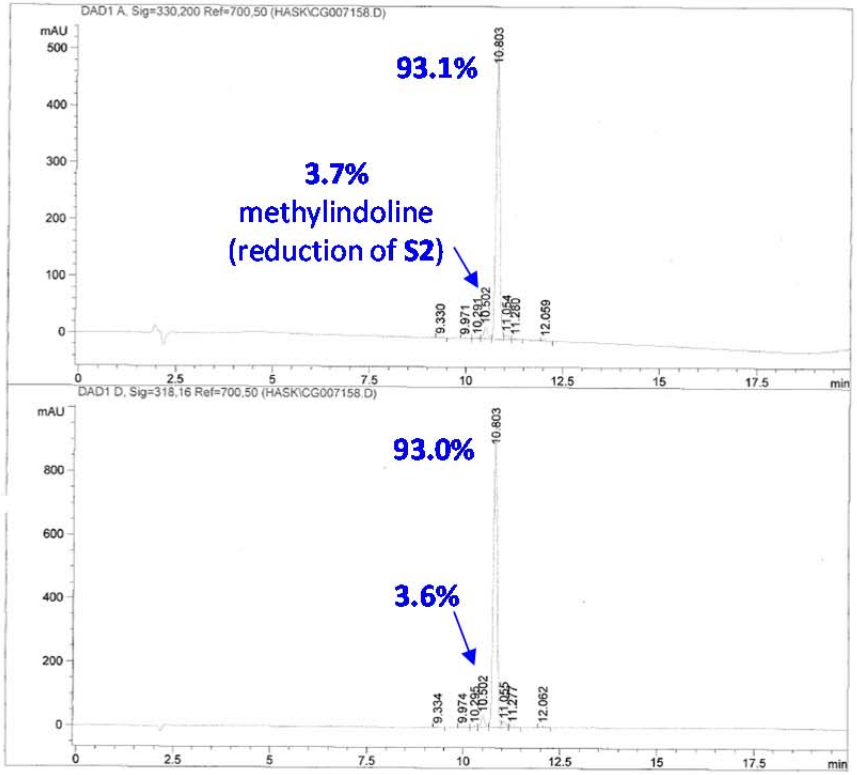
23

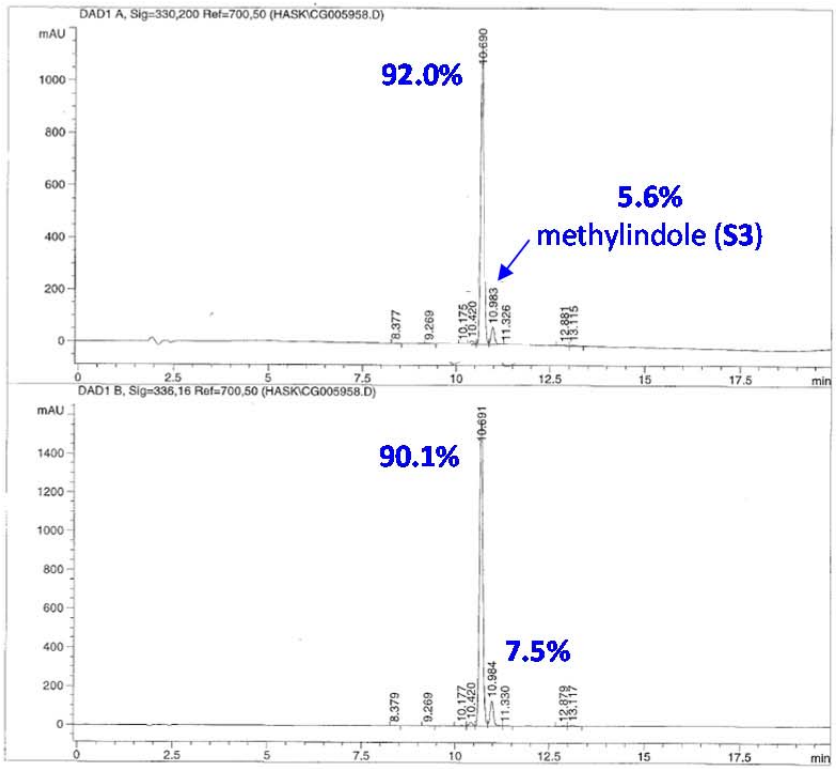

Cl
NEt2
O
N
H
HO
S
O O
NH2

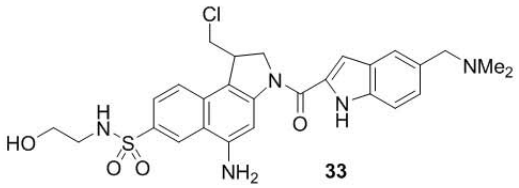
31


Cl
NMe2
O
N
H
HO
S
O O
NH2

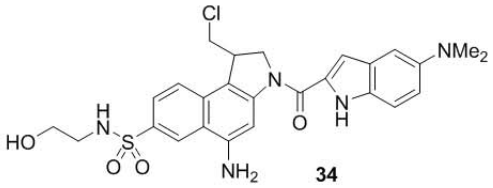
32


DAD1 A, Sig=330,200 Ref=700,50 (HASK\CG007158.D)
mAU
93.1%
10.803
3.7%
methylindoline
(reduction of S2)
9.330
9.971
10.502
11.054
11.280
12.059
min
DAD1 D, Sig=318,16 Ref=700,50 (HASK\CG007158.D)
93.0%
10.803
3.6%
9.334
9.974
10.502
11.055
11.277
12.062
min

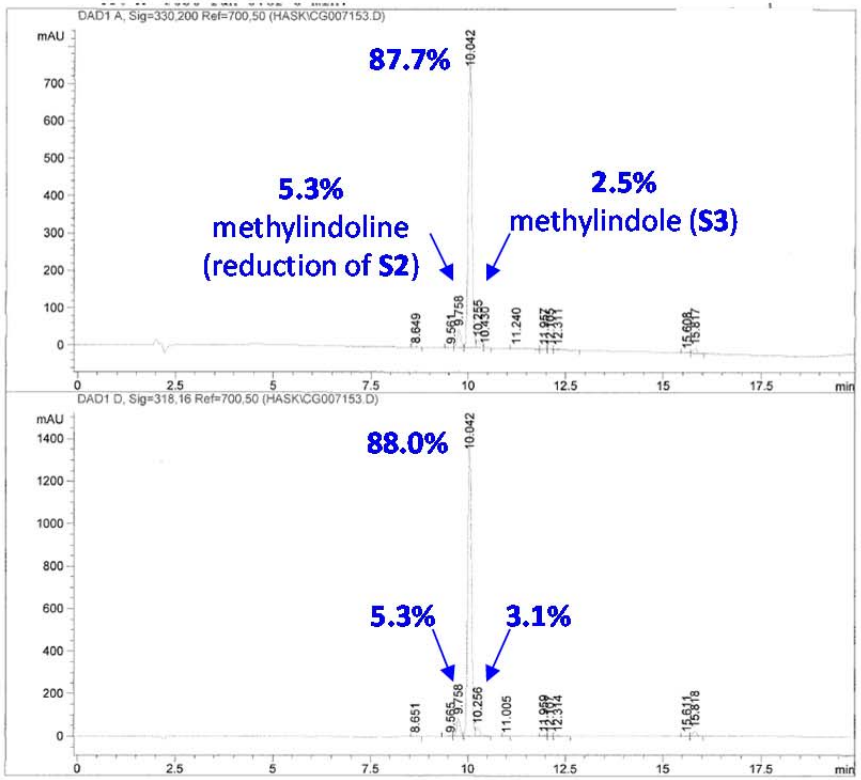

DAD1 A, Sig=330,200 Ref=700,50 (HASK\CG005958.D)
mAU
92.0%
10.690
5.6%
methylindole (S3)
8.377
9.269
10.175
10.420
10.983
11.326
13.881
13.115
min
DAD1 B, Sig=336,16 Ref=700,50 (HASK\CG005958.D)
90.1%
10.691
7.5%
8.379
9.269
10.177
10.420
10.984
11.330
13.878
13.117
min

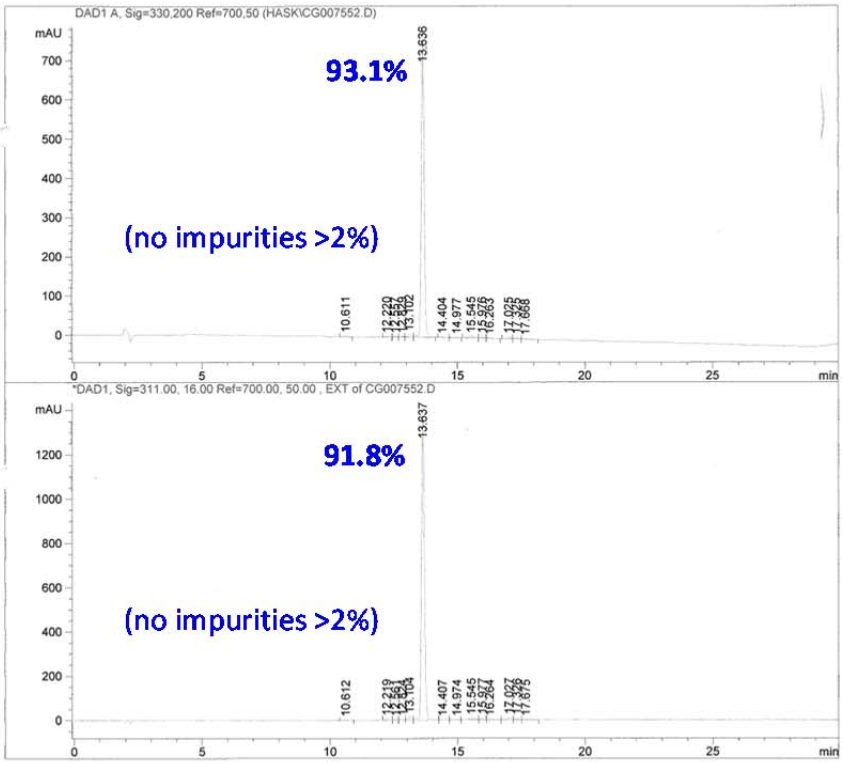

Cl
NMe2
HO
H
N
S
O O
NH2
O
N
H

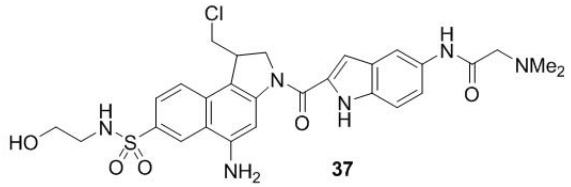
33


Cl
NMe2
HO
H
N
S
O O
NH2
O
N
H

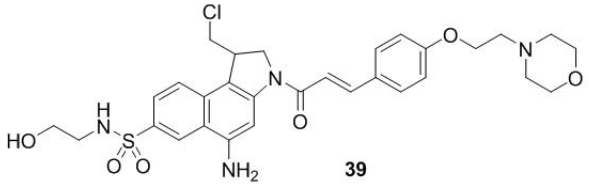
34


DAD1 A, Sig=330,200 Ref=700,50 (HASK\CG007153.D)
mAU
87.7%
10.042
5.3%
methylindoline
(reduction of S2)
2.5%
methylindole (S3)
min
DAD1 D, Sig=318,16 Ref=700,50 (HASK\CG007153.D)
mAU
88.0%
10.042
5.3%
3.1%
min

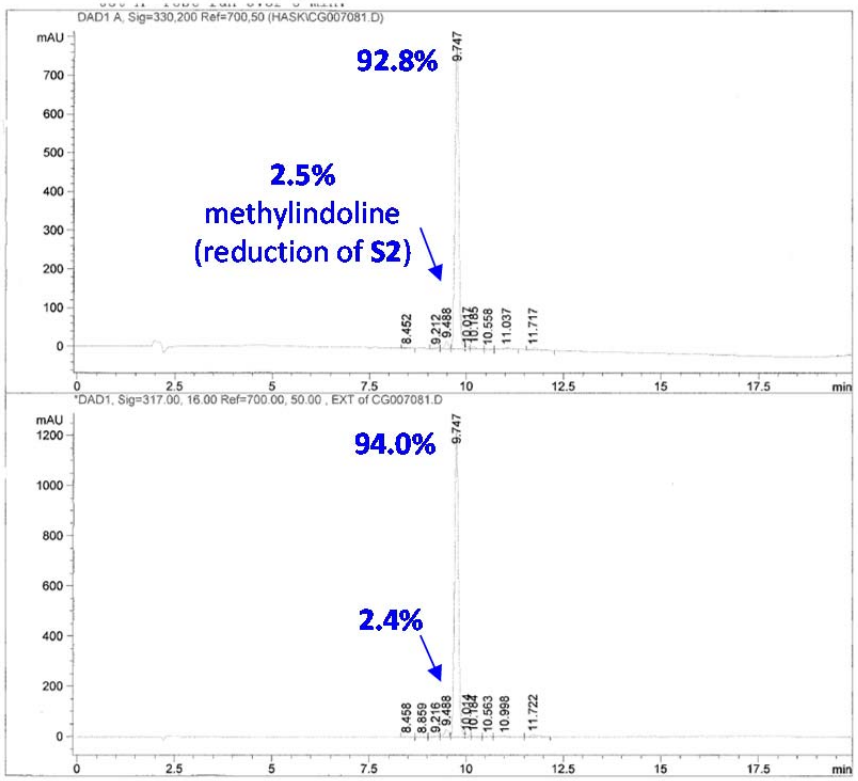

DAD1 A, Sig=330,200 Ref=700,50 (HASK\CG007552.D)
mAU
93.1%
13.636
(no impurities >2%)
min
*DAD1, Sig=311.00, 16.00 Ref=700.00, 50.00 , EXT of CG007552.D
mAU
91.8%
13.637
(no impurities >2%)
min


Cl
H
N
NMe2
O
N
H
HO
H
N
S
O O
NH2

37


Cl
O
N
O
HO
H
N
S
O O
NH2

39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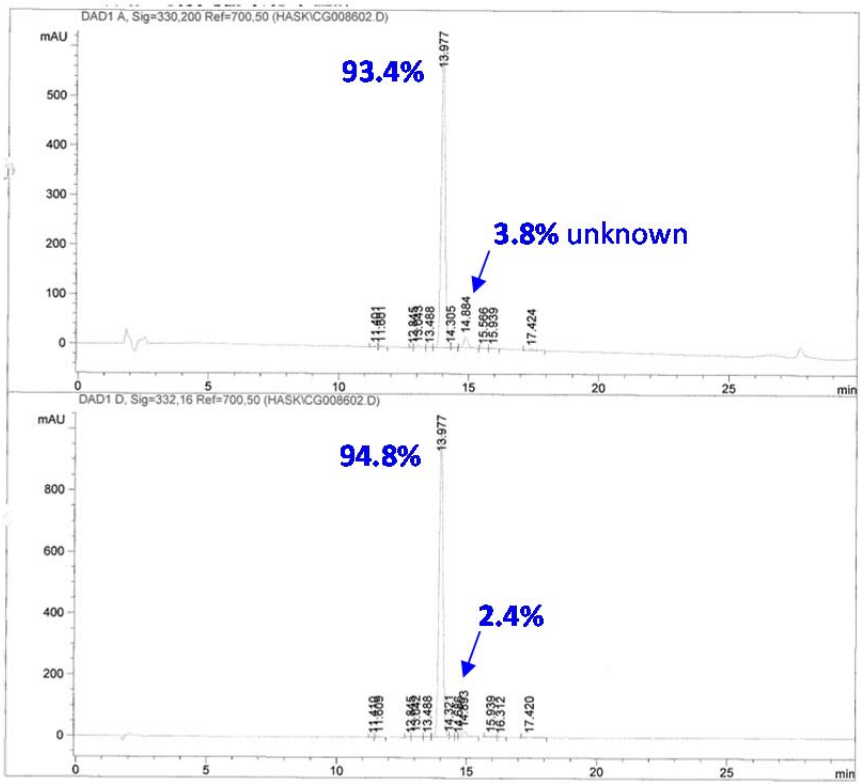
92.8%
2.5%
methylindoline
(reduction of S2)
*DAD1, Sig=317.00, 16.00 Ref=700.00, 50.00 , EXT of CG007081.D
94.0%
2.4%
mAU
min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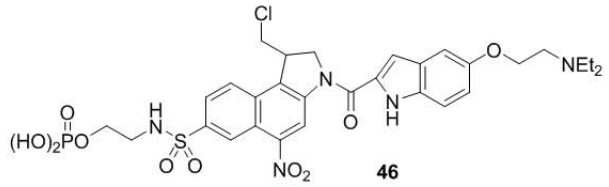
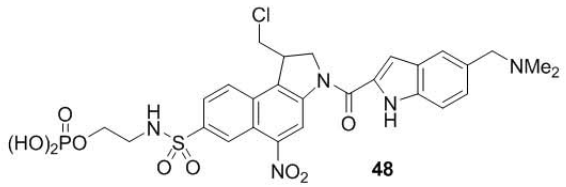
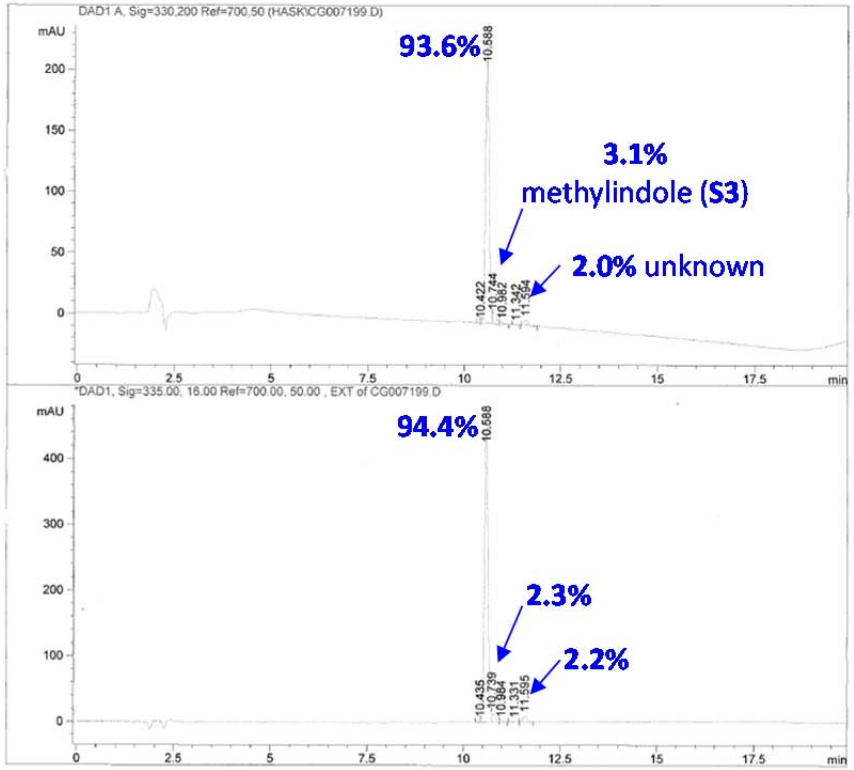
3.8% unknown
DAD1 D, Sig=332,16 Ref=700,50 (HASK\CG008602.D)
94.8%
2.4%
mAU
min


Cl
NEt2
(HO)2PO
NO2

46


Cl
NMe2
(HO)2PO
NO2

48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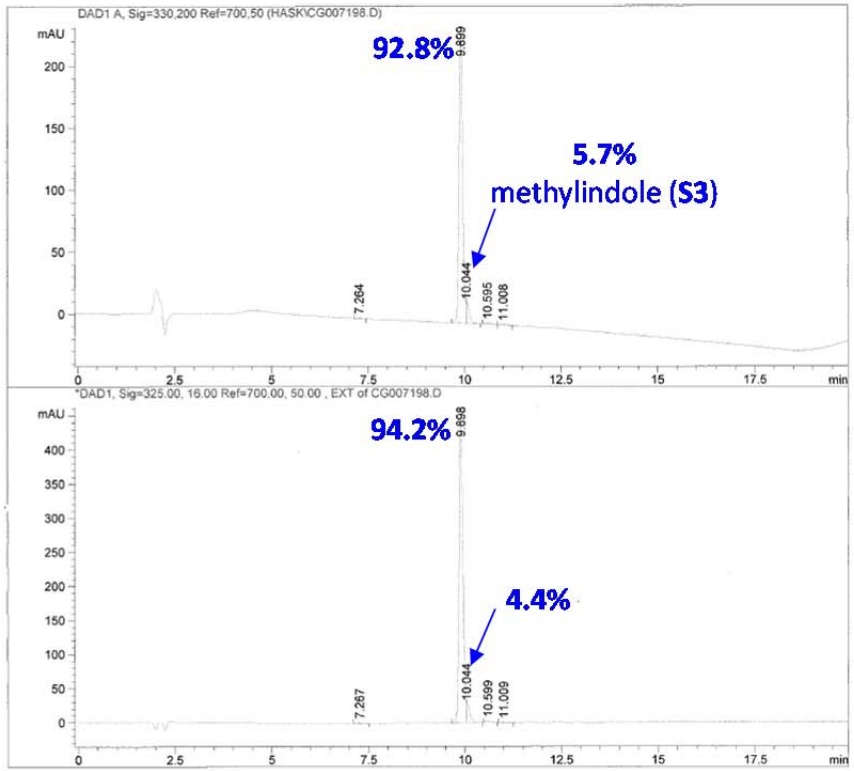
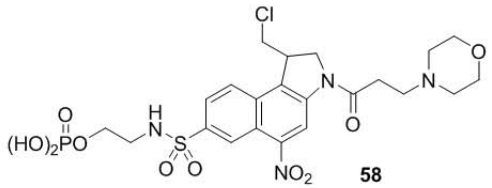
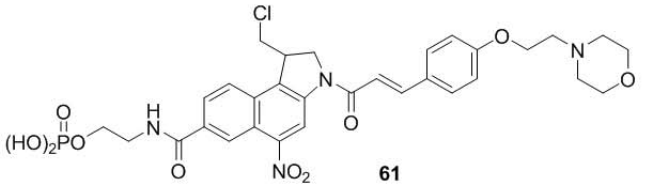
93.6%
3.1%
methylindole (S3)
2.0% unknown
*DAD1, Sig=335.00, 16.00 Ref=700.00, 50.00 , EXT of CG007199.D
94.4%
2.3%
2.2%

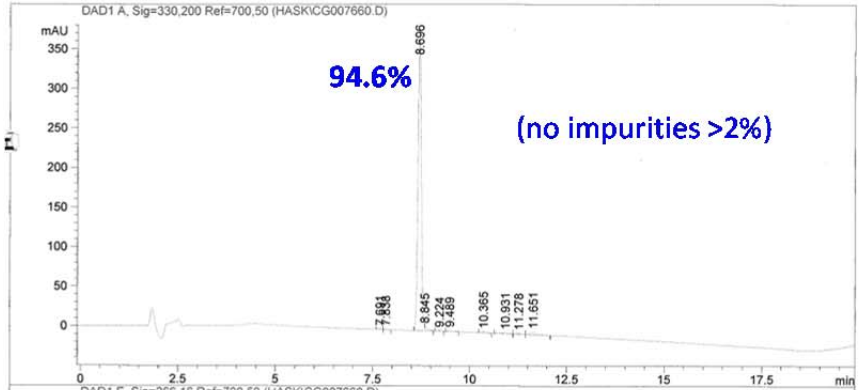

DAD1 A, Sig=330,200 Ref=700,50 (HASKI\CG007198.D)
92.8%
5.7%
methylindole (S3)
*DAD1, Sig=325.00, 16.00 Ref=700.00, 50.00 , EXT of CG007198.D
94.2%
4.4%



58



61


DAD1 A, Sig=330,200 Ref=700,50 (HASK\CG007660.D)
94.6%
(no impurities >2%)
DAD1 E, Sig=266,16 Ref=700,50 (HASK\CG007660.D)
95.6%
(no impurities >2%)

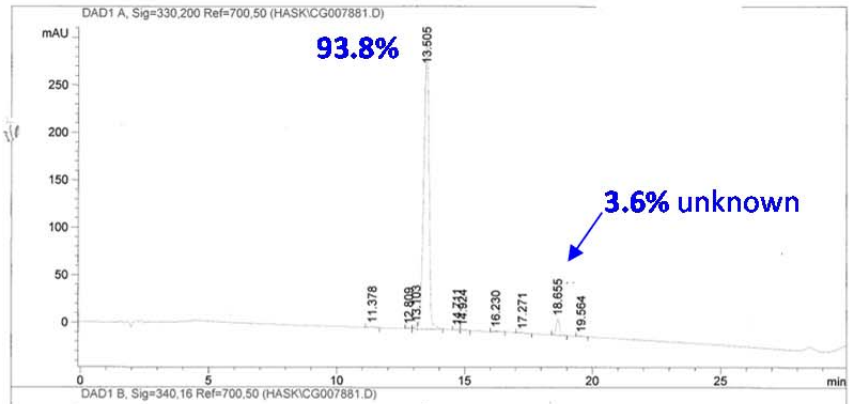

DAD1 A, Sig=330,200 Ref=700,50 (HASK\CG007881.D)
93.8%
3.6% unknown
DAD1 B, Sig=340,16 Ref=700,50 (HASK\CG007881.D)
94.7%
3.7%

Table S1. In Vitro Cytotoxicity Data for NitroCBIs.

	HT29			SiHa		
	IC_{50} (μM)[a]		HCR[b]	IC_{50} (μM)[a]		HCR[b]
	oxic	hypoxic		oxic	Hypoxic	
10[c]	28 ± 2	11 ± 2	2.1 ± 0.1	13 ± 1	3.5 ± 2.5	16 ± 4
11[c]	9.3 ± 1.4	0.090 ± 0.020	160 ± 40	2.0 ± 0.2	0.065 ± 0.013	46 ± 10
12	4.2 ± 1.2	0.073 ± 0.019	59 ± 5	1.3 ± 0.6	0.044 ± 0.005	35 ± 19
13	3.1 ± 0.3	0.064 ± 0.009	50 ± 7	1.1 ± 0.1	0.043 ± 0.011	27 ± 5
14	20 ± 2	0.63 ± 0.25	42 ± 14	14 ± 4	0.21 ± 0.03	68 ± 13
15	24 ± 3	10 ± 2	2.6 ± 0.1	22 ± 3	1.8 ± 0.7	20 ± 8
16	21 ± 1	2.5 ± 0.1	8.9 ± 0.1	11 ± 1	0.53 ± 0.12	27 ± 5
17	4.3 ± 0.5	0.96 ± 0.06	4.4 ± 0.5	1.2 ± 0.2	0.39 ± 0.14	4.1 ± 1.6
18	5.8 ± 1.9	0.75 ± 0.12	7.6 ± 1.7	6.8 ± 1.1	0.45 ± 0.06	16 ± 3
19	25 ± 4	1.3 ± 0.2	21 ± 4	6.1 ± 1.2	0.29 ± 0.05	22 ± 5
20	9.1 ± 0.9	3.2 ± 0.5	2.9 ± 0.3	6.0 ± 1.8	1.2 ± 0.4	5.5 ± 0.8
21	3.3 ± 0.1	2.3 ± 0.2	1.4 ± 0.1	1.6 ± 0.3	0.74 ± 0.16	2.2 ± 0.1
22	3.1 ± 0.3	0.71 ± 0.17	4.9 ± 1.1	3.1 ± 0.7	0.29 ± 0.04	10 ± 1
23	16 ± 2	14 ± 4	1.1 ± 0.2	17 ± 1	5.7 ± 1.4	3.3 ± 0.8
24	43 ± 2	27 ± 5	1.9 ± 0.5	47 ± 3	21 ± 2	2.3 ± 0.3
25[c]	5.7 ± 1.8	3.2 ± 0.2	1.7 ± 0.5	ND	ND	ND
26[c]	12 ± 2	0.11 ± 0.07	490 ± 200	4.2 ± 1.1	0.24 ± 0.14	20 ± 4
27	8.3 ± 1.2	6.4 ± 2.6	0.96 ± 0.22	6.8 ± 0.7	0.36 ± 0.07	23 ± 7
28	4.1 ± 0.3	6.0 ± 0.2	0.68 ± 0.04	3.5 ± 0.3	1.6 ± 0.8	2.2 ± 0.7

[a]Drug concentration to reduce cell density to 50% of that of controls following 4 h of exposure. Values are the means ± SEM for two to fourteen experiments. [b]Hypoxic cytotoxicity ratio = IC_{50}(oxic)/IC_{50}(hypoxic). Values are the mean of intra-experiment ratios ± SEM for two to fourteen experiments. [c]Data previously reported.[1] ND = not determined.

Table S2. In Vitro Cytotoxicity Data for AminoCBIs.

	HT29			SiHa		
	IC_{50} (μM)[a]		HCR[b]	IC_{50} (μM)[a]		HCR[b]
	oxic	hypoxic		oxic	Hypoxic	
29[c]	0.18 ± 0.01	0.27 ± 0.03	0.68 ± 0.03	0.053 ± 0.009	0.041 ± 0.009	1.3 ± 0.2
30[c]	0.086 ± 0.006	0.072 ± 0.008	1.3 ± 0.2	0.017 ± 0.002	0.013 ± 0.001	1.3 ± 0.1
31	0.13 ± 0.03	0.090 ± 0.026	1.7 ± 0.4	0.036 ± 0.014	0.014 ± 0.001	2.9 ± 1.3
32	0.042 ± 0.025	0.049 ± 0.016	0.87 ± 0.52	0.014 ± 0.005	0.0091 ± 0.0026	1.5 ± 0.1
33	0.36 ± 0.07	0.19 ± 0.08	2.6 ± 1.0	0.078 ± 0.024	0.024 ± 0.001	3.3 ± 1.1
34	0.17 ± 0.01	0.18 ± 0.05	1.0 ± 0.2	0.057 ± 0.013	0.043 ± 0.006	1.3 ± 0.2
35	0.11 ± 0.01	0.12 ± 0.04	0.90 ± 0.08	0.024 ± 0.004	0.024 ± 0.005	1.1 ± 0.2
36	0.070 ± 0.003	0.064 ± 0.023	1.1 ± 0.1	0.016 ± 0.001	0.017 ± 0.002	1.0 ± 0.1
37	0.51 ± 0.09	0.48 ± 0.02	1.1 ± 0.2	0.11 ± 0.02	0.078 ± 0.015	1.5 ± 0.4
38	0.50 ± 0.03	0.47 ± 0.08	1.1 ± 0.3	0.16 ± 0.01	0.073 ± 0.015	2.3 ± 0.5
39	0.13 ± 0.02	0.12 ± 0.02	1.1 ± 0.1	0.034 ± 0.004	0.041 ± 0.004	0.85 ± 0.17
40	0.092 ± 0.031	0.081 ± 0.009	1.1 ± 0.4	0.029 ± 0.006	0.031 ± 0.002	0.94 ± 0.17
41	4.1 ± 0.5	4.8 ± 1.3	0.90 ± 0.14	1.6 ± 0.1	1.3 ± 0.1	1.2 ± 0.2
42	12 ± 2	14 ± 3	0.88 ± 0.01	4.4 ± 0.4	3.5 ± 0.1	1.3 ± 0.1
43[c]	0.23 ± 0.04	0.21 ± 0.03	1.2 ± 0.2	0.031 ± 0.006	0.048 ± 0.008	0.64 ± 0.06
44	0.25 ± 0.03	0.28 ± 0.03	0.91 ± 0.15	0.035 ± 0.007	0.040 ± 0.008	0.79 ± 0.08

[a]Drug concentration to reduce cell density to 50% of that of controls following 4 h of exposure. Values are the means ± SEM for two to twelve experiments. [b]Hypoxic cytotoxicity ratio = IC_{50}(oxic)/IC_{50}(hypoxic). Values are the mean of intra-experiment ratios ± SEM for two to twelve experiments. [c]Data previously reported.[1]

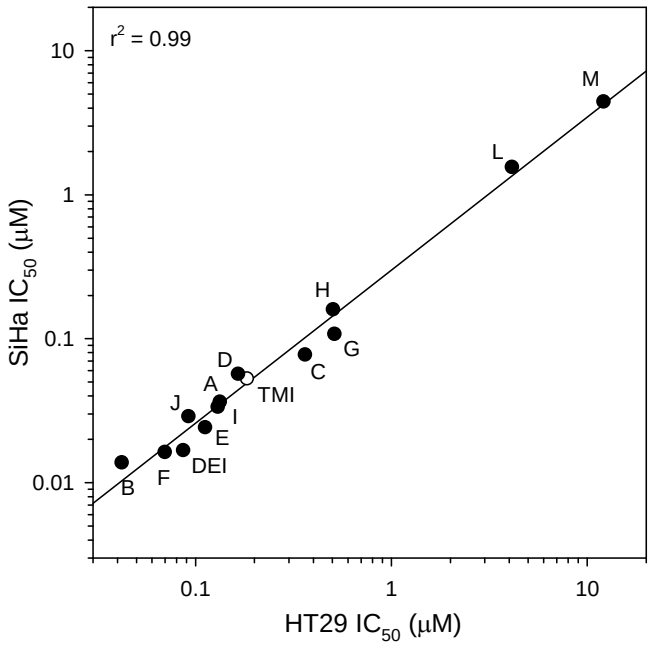


Figure S2. Correlation of cytotoxicity of aminoCBIs in two human carcinoma cell lines. IC_{50}s for all sulfonamide aminoCBIs (**29**-**42**) determined under oxic conditions. The letters refer to the side chain structures illustrated in Table 1. A similar analysis of IC_{50}s for the same compounds under hypoxic conditions gives an equally high correlation (r^2 = 0.99).

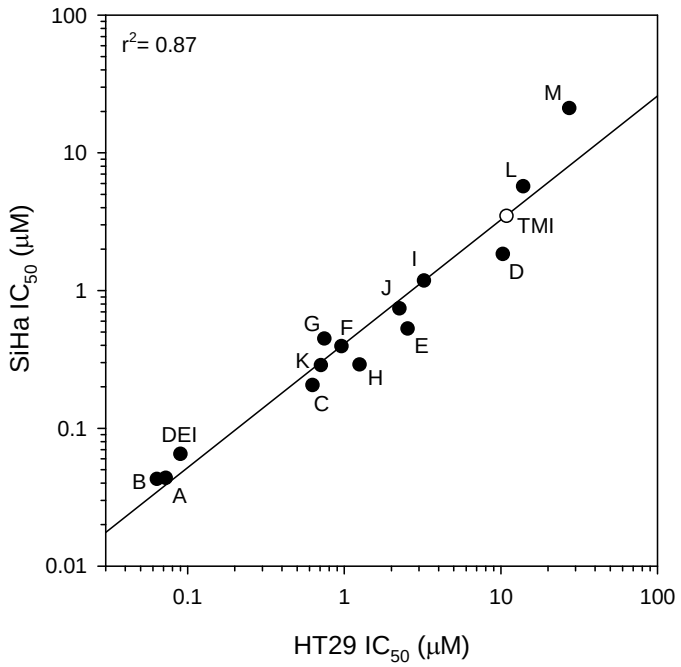


Figure S3. Correlation of hypoxic cytotoxicity of nitroCBIs in two human carcinoma cell lines. IC_{50}s for all sulfonamide nitroCBIs (**10**-**24**) determined under hypoxic conditions. The letters refer to the side chain structures illustrated in Table 1.

Table S3. Activity of nitroCBI phosphates against SiHa xenografts.

Compound[a]	Log_{10} Cell Kill[b]		
	Compound Alone (vs Control)	Radiation Alone (vs Control)	Radiation + Compound (vs Radiation Alone)
9[c,d]	0.63 ± 0.20* 1.05 ± 0.04** 0.63 ± 0.26* 0.65 ± 0.21*	2.01** 2.03** 1.79** 1.91**	1.36 ± 0.09** 1.63 ± 0.12** 1.83 ± 0.18** 1.56 ± 0.17**
45[c]	0.34 ± 0.31	2.11**	0.55 ± 0.14*
46	0.31 ± 0.14	1.79**	0.57 ± 0.08**
47	0.92 ± 0.05**	2.03**	1.77 ± 0.29**
48	0.26 ± 0.09	1.79**	0.86 ± 0.12**
49	0.21 ± 0.04	1.91**	1.46 ± 0.31**
50[e]	1.16 ± 0.05** 1.34 ± 0.37* 0.68 ± 0.40	2.03** 1.78** 2.33**	2.41 ± 0.12** 1.73 ± 0.21** 2.19 ± 0.15[f]**
51	1.26 ± 0.06**	2.03**	2.26 ± 0.10**
52	0.27 ± 0.15	1.79**	1.36 ± 0.13**
54	1.05 ± 0.09**	1.91**	2.58 ± 0.13[f]**
55	0.15 ± 0.05	1.69**	0.33 ± 0.24
56	-0.01 ± 0.01	1.69**	1.54 ± 0.35**
57	0.08 ± 0.04	1.91**	0.32 ± 0.11
58	0.17 ± 0.03	1.91**	0.30 ± 0.09
59[c,g]	ND 0.79 ± 0.20**	2.01** 2.03**	2.04 ± 0.11** 2.21 ± 0.22[h]**
60	1.49 ± 0.32**	2.37**	1.47 ± 0.20**
61	0.36 ± 0.23	2.37**	1.72 ± 0.22**

[a]All compounds were dosed at 42 μmolkg^{-1} except for **46** (23.7 μmol/kg). [b][Log_{10} clonogens(g tumor tissue)$^{-1}$ for specified comparator] minus [log_{10} clonogens(g tumor tissue)$^{-1}$ for specified treatment]. [c]This data previously reported.[1] [d]Tested in 4 separate experiments. [e]Tested in 3 separate experiments. [f]No clonogens detected in 3/5 treated tumors (excluded from calculation of mean). [g]Tested in 2 separate experiments. [h]No clonogens detected in 1/5 treated tumors (excluded from calculation of mean). ND = Not determined. All groups within a given experiment

were pairwise compared for statistical differences against the appropriate control group using one way ANOVA with the Holm-Sidak method for post hoc testing with SigmaPlot v13 (Systat Software, Inc.). *, p<0.05; **, p<0.01.

Table S4. Activity of nitroCBI phosphates against H460 xenografts.

Compound[a]	**Log_{10} Cell Kill**[b]		
	Compound Alone (vs Control)	**Radiation Alone (vs Control)**	**Radiation + Compound (vs Radiation Alone)**
9[c]	-0.07 ± 0.01 0.24 ± 0.05 0.47 ± 0.02* 0.27 ± 0.05	1.03** 1.43** 1.45** 1.26**	0.41 ± 0.10* 0.27 ± 0.14 0.17 ± 0.15 0.43 ± 0.16
46	0.03 ± 0.09	1.45**	-0.03 ± 0.10
47	0.14 ± 0.04	1.43**	0.12 ± 0.06
48	-0.03 ± 0.05	1.45**	0.07 ± 0.07
49	0.12 ± 0.05	1.26**	0.27 ± 0.08
50	0.13 ± 0.02	1.43**	0.76 ± 0.15**
51	0.61 ± 0.01**	1.43**	0.79 ± 0.12**
52	0.24 ± 0.08	1.45**	0.32 ± 0.04
54	0.48 ± 0.14**	1.26**	1.11 ± 0.22**
55	-0.18 ± 0.22	0.97**	0.25 ± 0.11
56	0.09 ± 0.05	0.97**	0.44 ± 0.13
57	0.12 ± 0.01	1.26**	0.05 ± 0.06
58	-0.13 ± 0.13	1.26**	0.09 ± 0.06
59	0.11 ± 0.08	1.43**	0.38 ± 0.21
60	0.25 ± 0.04**	1.16**	0.12 ± 0.08
61	ND	1.16**	0.54 ± 0.12**

[a]All compounds were dosed at 75 μmolkg^{-1} except for **46** (23.7 μmol/kg), **49** (42.2 μmol/kg), **54** (56.3 μmol/kg), and **57** (13.3 μmol/kg). [b][Log_{10} clonogens(g tumor tissue)$^{-1}$ for specified comparator] minus [log_{10} clonogens(g tumor tissue)$^{-1}$ for specified treatment]. [c]Tested in 4 separate experiments, one of which previously reported.[1] ND = Not determined. All groups within a given experiment were pairwise compared for statistical differences against the appropriate control group

using one way ANOVA with the Holm-Sidak method for post hoc testing with SigmaPlot v13 (Systat Software, Inc.). *, p<0.05; **, p<0.01.

Table S5. Activity of nitroCBI phosphates against H1299 xenografts.

Compound[a]	**Log_{10} Cell Kill**[b]		
	Compound Alone (vs Control)	**Radiation Alone (vs Control)**	**Radiation + Compound (vs Radiation Alone)**
9[c]	0.85 ± 0.16**	1.26**	1.82 ± 0.21**
47	0.62 ± 0.03**	1.26**	1.40 ± 0.37**
50	1.06 ± 0.01**	1.26**	2.89 ± 0.17[d]**
51	1.39 ± 0.31**	1.26**	2.24 ± 0.30**
59	1.05 ± 0.05**	1.26**	2.16 ± 0.27**

[a]All compounds were dosed at 75 μmolkg^{-1}. [b][Log_{10} clonogens(g tumor tissue)$^{-1}$ for specified comparator] minus [log_{10} clonogens(g tumor tissue)$^{-1}$ for specified treatment]. [c]This data previously reported.[1] [d]No clonogens detected in 1/5 treated tumors (excluded from calculation of mean). All groups within a given experiment were pairwise compared for statistical differences against the appropriate control group using one way ANOVA with the Holm-Sidak method for post hoc testing with SigmaPlot v13 (Systat Software, Inc.). *, p<0.05; **, p<0.01.

Table S6. Activity of nitroCBI phosphates against HCT116 xenografts.

Compound[a]	**Log_{10} Cell Kill**[b]		
	Compound Alone (vs Control)	**Radiation Alone (vs Control)**	**Radiation + Compound (vs Radiation Alone)**
9[c]	0.92 ± 0.08*	2.21**	0.97 ± 0.18**
47	ND	2.21**	1.34 ± 0.19**
50	ND	2.21**	2.60 ± 0.06[d]**
51	1.79 ± 0.21**	2.21**	2.40 ± 0.34[e]**
59	0.72 ± 0.07*	2.21**	1.36 ± 0.20**

[a]All compounds were dosed at 56.3 μmolkg^{-1}. [b][Log_{10} clonogens(g tumor tissue)$^{-1}$ for specified comparator] minus [log_{10} clonogens(g tumor tissue)$^{-1}$ for specified treatment]. [c]This data previously reported.[1] [d]No clonogens detected in 3/5 treated tumors (excluded from calculation of mean). [e]No clonogens detected in 4/5 treated tumors (excluded from calculation of mean). ND = Not

determined. All groups within a given experiment were pairwise compared for statistical differences against the appropriate control group using one way ANOVA with the Holm-Sidak method for post hoc testing with SigmaPlot v13 (Systat Software, Inc.). *, p<0.05; **, p<0.01.

Table S7. Activity of **50** against 22Rv1 xenografts.

Compound[a]	**Log_{10} Cell Kill**[b]		
	Compound Alone (vs Control)	**Radiation Alone (vs Control)**	**Radiation + Compound (vs Radiation Alone)**
50	ND	2.30**	1.18 ± 0.23**

[a]**50** was dosed at 30 μmolkg^{-1}. [b][Log_{10} clonogens(g tumor tissue)$^{-1}$ for specified comparator] minus [log_{10} clonogens(g tumor tissue)$^{-1}$ for specified treatment]. ND = Not determined. All groups within a given experiment were pairwise compared for statistical differences against the appropriate control group using one way ANOVA with the Holm-Sidak method for post hoc testing with SigmaPlot v13 (Systat Software, Inc.). *, p<0.05; **, p<0.01.

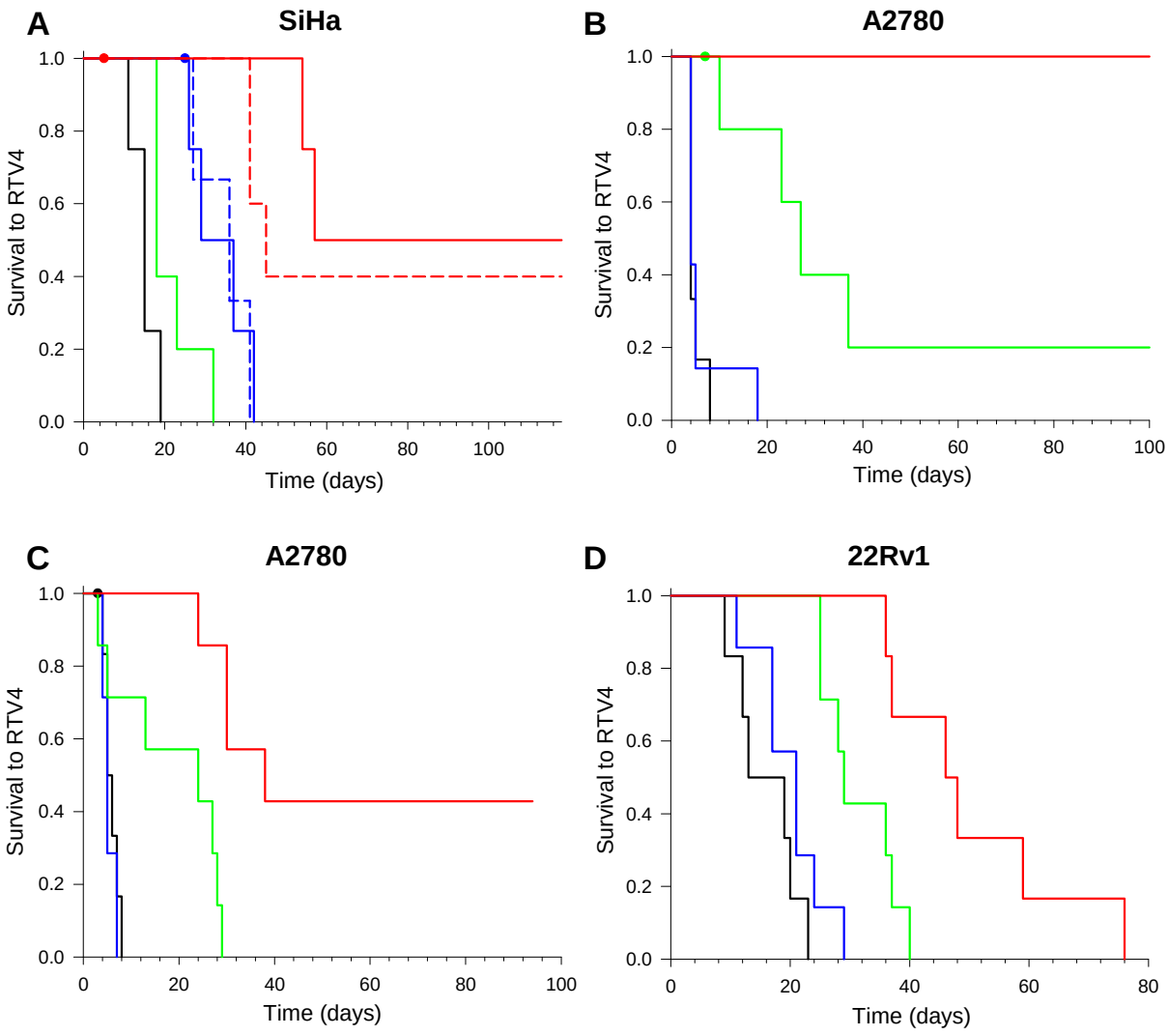


Figure S4. Kaplan-Meier survival curves for growth delay experiments. Conditions and groups are as described in Figure 5. Survival is measured as time to quadrupling of the initial tumor volume. Censored events marked with circles are as follows: A, one in **9** only group (42 μmol/kg) with average tumor diameter >15 mm, and one in combination group (56 μmol/kg) with body weight loss >15%; B, one in gemcitabine only group with body weight loss >15%; C, one in control group with tumor ulceration.

Table S8. Statistical analysis of Kaplan-Meier survival curves.

Xenograft	Dose (μmol/kg)		Median RTV4 Growth Delay in Days (p[a])		
	NitroCBI	Chemotherapy	NitroCBI[b]	Chemotherapy[c]	Combination[d]
SiHa	**9** (42)	gemcitabine (237)	21 (0.007)	3 (ns)	27 (0.002)
	9 (56)	gemcitabine (237)	14 (0.007)		39 (0.004)
A2780	**9** (56)	gemcitabine (133)	0 (ns)	23 (<0.001)	>73 (0.007)
A2780	**50** (15)	gemcitabine (100)	0 (ns)	19 (0.03)	14 (0.001)
22Rv1	**50** (14)	docetaxel (32)	8 (ns)	16 (<0.001)	17 (0.007)

[a]Groups were pairwise compared for statistical significance using Log-Rank with SigmaPlot v13 (Systat Software, Inc.); ns, $p \geq 0.05$. [b]NitroCBI alone compared to control. [c]Chemotherapy alone compared to control. [d]NitroCBI plus chemotherapy *compared to chemotherapy alone*.

Table S9. Body weight nadirs for growth delay experiments.

Xenograft	Schedule	Dose (μmol/kg)		Maximum body weight loss	
		NitroCBI	Chemotherapy	Mean ± SE (%)	p vs control[a]
SiHa	q3d×4	-	-	-2.5 ± 1.4	-
		-	gemcitabine (237)	-2.3 ± 0.8	ns
		9 (42)	-	-3.3 ± 1.4	ns
		9 (56)	-	0.0 ± 0.4	ns
		9 (42)	gemcitabine (237)	-5.7 ± 1.2	ns
		9 (56)	gemcitabine (237)	-7.9 ± 2.1	ns
A2780	q3d×4	-	-	-2.3 ± 0.3	-
		-	gemcitabine (133)	-5.0 ± 2.3	ns
		9 (56)	-	-3.3 ± 0.8	ns
		9 (56)	gemcitabine (133)	-5.6 ± 1.6	ns
A2780	q3d×4	-	-	-0.3 ± 0.1	-
		-	gemcitabine (100)	-1.8 ± 0.7	ns
		50 (15)	-	-0.6 ± 0.4	ns
		50 (15)	gemcitabine (100)	-1.4 ± 0.5	ns
22Rv1	qw×2	-	-	-0.8 ± 0.3	-
		-	docetaxel (32)	-8.3 ± 1.1	<0.001
		50 (14)	-	-1.3 ± 0.3	ns
		50 (14)	docetaxel (32)	-4.8 ± 1.2	0.005

[a]All treatment groups were pairwise compared with control for statistical significance using one way ANOVA with the Holm-Sidak method for post hoc testing with SigmaPlot v13 (Systat Software, Inc.); ns, $p \geq 0.05$.

Reference

1. Tercel, M.; Atwell, G. J.; Yang, S.; Ashoorzadeh, A.; Stevenson, R. J.; Botting, K. J.; Gu, Y.; Mehta, S. Y.; Denny, W. A.; Wilson, W. R.; Pruijn, F. B. Selective treatment of hypoxic tumor cells in vivo: phosphate pre-prodrugs of nitro analogues of the duocarmycins. *Angew. Chem. Int. Ed.* **2011**, *50*, 2606–2609.