Supplementary Information

Indenoisoquinoline Topoisomerase Inhibitors Strongly Bind and Stabilize the *MYC* Promoter G-Quadruplex and Downregulate *MYC*

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Table S1. The DNA sequences and primers used in this study.

Sequence Name	**DNA Sequence** (5′ to 3′)
MycPu28	TGGGGAGGGTGGGGAGGGTGGGGAAGGT
MycPu22	TGAGGGTGGG TAGGGTGGGTAA
K-RasG4	AGGGCGGTGTGGGAAGAGGGAAGAGGGGGAGG
Telomeric G4	TTAGGGTTAGGGTTAGGGTTAGGGTT
qRT-PCR Primers	
MYC Forward	GCTGCTTAGACGCTGGATT
MYC Reverse	TCCTCCTCGTCGCAGTAGA
GAPDH Forward	CATGAGAAGTATGACAACAGCCT
GAPDH Reverse	AGTCCTTCCACGATACCAAAGT

Table S2. Competitor binding affinities (K_i) of the five indenoisoquinolines determined by competition fluorescence displacement experiments.

	K_i (nM)				
Compound	**MycPu22**	**MycPu28**	**K-RasG4**	**Telomeric G4**	**calf thymus dsDNA[a]**
5	16	18	21	164	~11000
6	40	33	32	286	~12000
9	12	11	11	28	5171
12	7	7	7	14	3624
13	26	19	23	146	2138

[a]The K_i value of the calf thymus ds-DNA refers to the base-pair concentration.

Table S3. MYC inhibition, Top1 inhibition, and GI_{50} values of the 29 indenoisoquinolines.

	1	2	3	4	5	6	7	8	9	10	11	12	13	19	20
MYC Inhibition[a]	+	+++	+++	++	+++	+++	+++	+	+++	+++	+++	+++	+++	+	+
Top1 Inhibiton[b]	+	++	++	+	++	+	++	+	++	+++	+++	++++	++++	+++	+++
MGM[c]	0.58	0.63	0.60	2.90	0.16	0.24	0.22	0.22	0.05	0.07	0.18	0.06	0.40	2.70	1.26
Cancer cell lines	**Antiproliferative activities [GI_{50} (μM)]**[d]														
Leukemia															
CCRF-CEM	0.08	0.03	0.06	0.53	0.11	0.26	0.16	0.04	<0.01	0.04	0.05	<0.01	0.01	1.57	1.37
HL-60 (TB)	0.11	6.23	0.60	18.00	0.16	0.22	0.20	0.09	0.03	0.04	0.10	<0.01	0.21	4.28	1.80
K-562	0.56	0.79	1.14	1.19	0.13	0.17	0.21	0.15	0.02	0.04	0.23	0.11	0.18	5.13	1.42
MOLT-4	0.04	0.02	0.04	0.35	0.04	0.04	0.05	0.03	<0.01	0.03	0.03	<0.01	<0.01	0.87	0.29
RPMI-8226	0.87	5.26	1.47	13.90	0.15	0.20	0.14	0.05	0.04	0.08	0.17	0.03	0.10	1.85	1.84
SR	0.12	0.05	0.14	0.58	0.08	0.12	0.13	0.05	<0.01	0.01	0.02	<0.01	<0.01	0.29	0.31
Lung															
A549	0.06	0.61	0.45	4.17	0.07	0.11	0.06	0.02	0.05	0.07	0.12	0.04	0.06	1.96	0.59
EKVX	1.37	2.18	1.20	7.73	0.30	0.30	0.33	0.44	0.30	0.27	0.45	1.01	0.43	4.91	ND
HOP-62	0.21	0.05	0.22	0.95	0.11	0.14	0.15	0.07	<0.01	0.03	0.15	0.01	0.03	1.41	1.25
HOP-92	1.12	2.20	1.19	4.68	0.28	0.37	0.36	0.14	0.55	0.24	0.87	1.38	0.15	4.47	1.42
NCI-H226	1.04	3.27	1.35	12.30	0.15	0.16	0.14	0.15	0.10	0.09	0.17	0.04	1.42	2.79	1.32
NCI-H23	1.01	0.86	0.92	6.27	0.18	0.31	0.30	0.23	0.03	0.04	0.15	0.02	0.21	3.10	1.86
NCI-H322M	1.26	1.68	1.28	4.34	0.32	0.45	0.44	0.96	0.05	0.16	0.28	0.04	0.34	5.44	2.47
NCI-H460	0.04	0.02	0.05	0.38	0.04	0.05	0.05	0.03	<0.01	0.02	0.04	<0.01	<0.01	0.44	0.41
NCI-H522	0.61	0.27	0.37	6.32	0.04	0.06	0.06	0.12	0.06	0.08	0.08	<0.01	0.02	1.06	1.84
Colon															
COLO 205	0.78	0.16	0.50	1.30	0.09	0.07	0.07	0.15	0.04	0.06	0.30	0.24	0.07	1.74	0.47
HCC-2998	1.29	5.07	1.41	11.30	0.40	1.15	1.06	1.05	0.16	0.20	0.30	1.03	0.19	3.44	2.17
HCT-116	0.30	0.10	0.55	0.98	0.09	0.14	0.14	0.09	<0.01	0.03	ND	ND	0.04	1.27	0.44
HCT-15	0.26	1.73	1.11	4.08	0.16	0.25	0.23	0.13	0.06	0.12	0.30	1.17	0.15	2.90	1.03
HT29	0.66	0.16	1.08	1.37	0.07	0.15	0.15	0.15	0.02	0.06	0.15	0.03	0.12	2.48	1.01
KM12	0.55	2.11	1.10	3.37	0.17	0.24	0.26	0.17	0.14	0.16	0.40	1.11	0.19	3.80	1.61
SW-620	0.09	0.03	0.08	0.30	0.12	0.18	0.17	0.11	<0.01	0.03	0.13	0.01	0.04	1.68	0.46
CNS															
SF-268	1.07	0.17	0.40	4.75	0.21	0.38	0.39	0.28	0.02	0.07	0.08	<0.01	0.07	1.75	2.15
SF-295	0.08	0.03	0.13	0.67	0.09	0.19	0.19	0.13	<0.01	0.03	0.07	0.01	0.08	1.27	0.75
SF-539	1.33	1.98	0.73	2.15	0.25	0.37	0.32	0.53	0.03	0.05	0.31	0.04	0.11	1.96	0.85
SNB-19	1.06	1.03	0.61	13.80	0.18	0.22	0.16	0.13	0.04	0.04	0.11	0.01	0.11	2.13	1.75
SNB-75	0.29	0.35	0.26	0.93	0.22	0.25	0.36	0.37	0.05	0.13	0.27	0.03	0.23	3.63	0.66
U251	0.31	0.04	0.30	7.24	0.08	0.11	0.10	0.08	0.01	0.04	0.08	<0.01	0.10	1.72	1.05
Melanoma															
LOX IMVI	0.17	0.04	0.16	0.78	0.11	0.14	0.14	0.18	<0.01	0.03	0.04	<0.01	0.05	1.34	0.81
MALME-3M	1.54	5.90	1.48	5.77	0.20	0.32	0.33	1.13	0.06	0.18	0.34	0.62	0.21	4.49	ND
M14	1.23	0.87	0.57	1.71	0.23	0.31	0.34	0.37	<0.01	0.04	0.07	<0.01	0.32	2.85	1.57
MDA-MB-435	1.25	5.06	1.07	1.53	0.31	0.47	0.48	0.58	0.06	0.15	0.42	0.48	0.39	4.67	1.93
SK-MEL-2	2.11	7.17	13.20	5.29	0.51	1.60	1.13	1.71	1.01	0.35	1.59	1.56	0.55	19.50	3.92
SK-MEL-28	1.81	8.34	1.62	2.30	0.94	1.44	1.23	1.52	10.60	0.39	1.13	1.18	1.11	8.03	1.56
SK-MEL-5	1.07	2.54	1.12	11.00	0.16	0.31	0.25	0.22	0.07	0.11	0.26	0.03	0.07	1.50	1.00
UACC-257	2.19	7.85	2.24	4.04	0.47	0.49	0.40	1.29	0.18	0.18	0.59	0.58	1.18	6.60	2.02
UACC-62	1.43	2.09	0.57	1.81	0.27	0.61	0.34	1.26	0.02	0.02	0.04	<0.01	0.55	1.58	1.30
Ovarian															
IGROV1	1.13	2.47	1.14	10.40	0.21	0.32	0.33	0.48	0.02	0.21	0.42	0.08	ND	ND	1.91
OVCAR-3	1.43	5.97	1.41	15.10	0.19	0.26	0.28	0.34	0.06	0.17	0.32	0.14	0.41	2.70	2.41
OVCAR-4	1.17	1.43	1.27	2.19	0.30	0.42	0.46	0.89	0.08	0.17	0.33	0.48	0.23	3.13	1.93
OVCAR-5	1.74	5.02	1.69	3.56	0.28	0.35	0.33	0.33	0.16	0.14	0.45	1.04	0.38	6.31	2.44
OVCAR-8	1.10	0.81	1.18	3.09	0.14	0.19	0.18	0.13	0.09	0.09	0.17	0.02	0.07	3.11	0.95
NCI/ADR-RES	1.09	0.22	0.54	0.98	0.23	0.43	0.37	0.86	0.03	0.06	0.13	0.02	1.04	3.14	2.72
SK-OV-3	1.16	1.47	0.66	10.10	0.22	0.23	0.22	0.21	0.03	0.06	0.14	0.01	0.22	2.67	2.22
Renal															
786-0	0.47	1.47	0.32	2.47	0.12	0.20	0.20	0.13	0.03	0.04	0.22	0.02	0.06	1.78	1.16
A498	0.55	0.38	0.53	0.96	0.20	0.37	0.25	0.35	ND	0.03	ND	ND	0.13	1.64	ND
ACHN	0.18	0.08	0.22	0.78	0.06	0.12	0.13	0.06	<0.01	0.04	0.04	<0.01	0.04	1.67	0.29
CAKI-1	0.41	0.03	0.06	2.97	ND	ND	ND	ND	<0.01	0.03	0.04	<0.01	0.24	3.17	0.57
RXF 393	1.04	0.70	1.30	1.90	0.23	0.32	0.26	0.27	0.06	0.07	0.39	0.03	0.96	7.16	2.31
SN12C	0.49	2.05	1.18	10.20	0.16	0.21	0.15	0.08	0.04	0.05	0.08	<0.01	0.16	4.10	1.12
TK-10	ND[e]	ND	ND	ND	0.18	0.33	0.30	0.27	0.37	0.32	0.73	1.34	0.41	4.63	3.02
UO-31	1.03	0.60	1.08	3.74	0.08	0.13	0.12	0.13	<0.01	0.03	0.05	<0.01	0.10	0.42	0.57
Prostate															
PC-3	0.98	0.86	1.02	3.60	0.28	0.37	0.30	0.60	0.07	0.13	0.57	1.28	0.28	19.50	2.05
DU-145	0.24	0.19	0.33	3.18	0.21	0.20	0.22	0.06	0.02	0.05	0.06	<0.01	0.06	2.31	1.89
Breast															
MCF7	0.09	0.02	0.05	0.49	0.03	0.03	0.04	0.03	<0.01	0.01	0.03	<0.01	<0.01	0.20	0.37
MDA-MB-231	1.35	6.15	1.62	10.70	0.40	0.70	0.40	0.48	0.68	0.27	0.63	1.61	0.68	11.20	2.58
HS 578T	1.49	6.87	1.84	12.90	0.75	2.24	1.62	2.08	1.91	1.17	3.85	1.82	0.66	8.95	3.08
BT-549	1.53	3.80	0.65	15.20	0.66	0.58	0.94	1.25	0.35	0.37	0.29	0.05	0.28	4.11	1.67
T-47D	8.98	1.21	1.12	22.80	0.08	0.16	0.15	0.27	0.02	0.03	0.11	<0.01	0.05	1.07	2.04
MDA-MB-468	0.55	0.06	1.28	1.19	0.23	0.22	0.14	0.21	0.03	0.03	0.12	0.03	ND	ND	1.57

Table S3. (continued)

	25	30	36	37	38	42	44	45	46	47	48	49	50	55
MYC Inhibition[a]	0	+	+++	+++	+	++	++	+	+	++	+	+	++	+
Top1 Inhibiton[b]	++	0	++	++	++	+++++	++++	+++	+++	++++	+	+	++++	++
MGM[c]	0.79	0.60	0.20	0.74	0.07	0.08	0.21	0.16	0.88	0.35	0.77	12.00	0.11	0.60
Cancer cell lines	**Antiproliferative activities [GI_{50} (μM)][d]**													
Leukemia														
CCRF-CEM	0.34	0.03	0.05	0.66	0.11	<0.01	<0.01	<0.01	<0.01	<0.01	0.30	2.44	<0.01	<0.01
HL-60 (TB)	1.17	0.03	0.08	0.59	1.48	0.02	<0.01	0.22	0.25	1.60	<0.01	19.50	0.29	0.30
K-562	0.83	0.34	0.09	2.25	0.77	1.49	0.02	0.09	0.28	ND	1.59	6.49	<0.01	0.19
MOLT-4	0.10	0.02	0.03	0.24	0.15	<0.01	<0.01	<0.01	<0.01	<0.01	0.42	<0.01	<0.01	<0.01
RPMI-8226	0.27	0.12	0.12	0.70	1.02	<0.01	0.01	<0.01	0.48	0.30	0.20	2.39	0.02	0.25
SR	0.31	0.02	0.03	0.03	0.04	<0.01	<0.01	<0.01	ND	<0.01	ND	ND	<0.01	<0.01
Lung														
A549	0.22	0.06	0.52	ND	1.31	10.50	0.03	0.11	0.74	0.89	0.65	18.70	<0.01	0.11
EKVX	1.18	2.91	ND	ND	ND	70.70	0.26	ND	2.06	1.46	1.31	17.70	2.57	ND
HOP-62	1.06	0.18	0.12	0.59	0.46	<0.01	0.01	0.06	0.05	ND	0.42	18.00	<0.01	0.19
HOP-92	1.72	0.13	0.28	13.60	1.34	0.19	0.83	1.65	3.27	1.30	ND	19.80	<0.01	1.66
NCI-H226	0.56	0.27	0.45	ND	1.18	0.37	0.21	1.39	0.65	1.40	0.66	13.90	0.05	2.12
NCI-H23	1.05	0.24	0.17	0.68	0.38	0.09	<0.01	0.13	0.05	0.19	0.71	11.90	0.02	0.71
NCI-H322M	0.45	7.05	0.66	72.60	1.39	<0.01	0.05	ND	0.53	1.56	1.05	15.60	3.18	1.00
NCI-H460	0.31	0.04	0.03	0.24	0.37	<0.01	<0.01	0.02	0.43	0.28	0.24	5.81	<0.01	0.03
NCI-H522	0.96	0.84	0.05	0.10	0.78	<0.01	<0.01	0.07	0.40	0.31	13.10	15.40	0.02	0.04
Colon														
COLO 205	0.62	4.92	7.76	7.90	0.69	<0.01	0.38	0.30	0.08	0.94	1.14	10.30	<0.01	0.31
HCC-2998	0.35	10.00	1.42	37.10	0.62	29.30	ND	0.10	ND	0.13	0.14	ND	ND	3.32
HCT-116	ND[e]	0.39	0.22	0.74	0.15	0.17	0.07	0.12	0.13	0.22	0.12	1.49	<0.01	0.16
HCT-15	0.73	0.09	1.53	<0.01	1.49	<0.01	0.55	0.14	0.91	0.20	1.11	16.00	0.07	1.02
HT29	0.35	0.34	1.18	4.08	0.48	<0.01	<0.01	0.09	ND	0.07	1.04	16.90	<0.01	0.32
KM12	1.13	0.53	1.04	9.39	1.34	<0.01	0.26	0.15	1.00	1.29	0.68	12.80	1.62	1.01
SW-620	0.16	0.20	0.08	0.81	0.34	0.06	0.11	0.13	0.25	<0.01	0.57	11.20	<0.01	0.11
CNS														
SF-268	0.95	1.85	0.04	0.27	0.55	ND	0.11	1.20	0.38	1.11	2.41	11.90	<0.01	0.07
SF-295	1.16	0.36	0.18	0.33	0.28	<0.01	<0.01	<0.01	0.87	0.02	0.57	10.70	<0.01	0.03
SF-539	1.61	1.04	0.27	0.37	0.67	<0.01	ND	0.01	0.25	0.16	0.64	17.70	<0.01	0.24
SNB-19	0.54	0.33	0.39	12.80	1.08	<0.01	ND	ND	0.02	0.55	1.39	<0.01	<0.01	0.31
SNB-75	0.95	1.39	0.21	2.97	0.16	0.23	0.01	0.13	ND	0.79	1.30	16.00	<0.01	0.40
U251	0.64	0.18	0.12	0.27	0.33	<0.01	<0.01	0.03	0.01	0.06	0.52	11.40	<0.01	0.05
Melanoma														
LOX IMVI	0.15	0.10	0.09	0.30	0.30	0.06	<0.01	0.11	0.03	0.07	0.46	3.81	<0.01	0.05
MALME-3M	1.42	0.93	1.16	1.79	2.16	1.60	0.15	1.01	0.69	1.07	ND	24.80	<0.01	1.25
M14	1.05	1.65	0.08	ND	0.46	<0.01	0.01	0.32	0.37	1.10	1.34	ND	ND	0.15
MDA-MB-435	1.20	10.30	0.89	2.84	1.30	<0.01	0.53	1.23	0.36	0.65	1.81	<0.01	<0.01	0.95
SK-MEL-2	1.71	18.20	9.44	30.80	2.18	<0.01	1.62	1.93	3.39	4.99	3.01	<0.01	19.60	5.32
SK-MEL-28	1.53	2.01	1.37	16.40	1.88	<0.01	0.17	1.27	0.25	1.48	1.42	16.50	0.68	11.80
SK-MEL-5	0.70	0.48	0.22	1.67	1.26	0.22	0.14	1.33	0.48	0.33	2.26	14.20	0.21	1.15
UACC-257	1.69	12.30	0.48	25.30	1.57	<0.01	0.29	1.54	0.95	1.34	7.72	<0.01	0.12	0.70
UACC-62	1.03	0.72	0.05	0.21	0.95	<0.01	0.03	0.36	0.27	0.54	1.38	15.10	0.01	0.33
Ovarian														
IGROV1	0.84	0.58	1.52	11.30	1.24	6.72	0.27	0.32	2.57	1.04	18.90	7.05	0.02	0.11
OVCAR-3	1.05	6.09	0.57	1.35	1.69	16.40	0.58	0.43	0.10	1.19	0.71	15.30	0.04	1.23
OVCAR-4	0.34	1.45	1.29	1.41	1.11	<0.01	0.12	0.14	0.10	0.33	0.25	4.17	1.48	2.21
OVCAR-5	2.12	1.51	3.60	<0.01	1.67	<0.01	0.15	0.25	0.79	ND	1.53	17.60	0.13	12.80
OVCAR-8	0.40	0.16	0.45	0.89	1.01	1.03	0.11	0.14	0.28	0.48	0.59	18.10	<0.01	0.34
NCI/ADR-RES	1.16	1.48	0.28	<0.01	2.55	0.35	ND	ND	8.70	0.03	2.44	1.55	<0.01	1.50
SK-OV-3	1.05	1.21	0.24	0.56	1.15	<0.01	0.60	1.40	ND	1.29	2.18	12.30	<0.01	0.21
Renal														
786-0	1.34	0.22	0.21	0.33	0.34	<0.01	<0.01	0.11	0.09	0.17	0.76	12.90	<0.01	0.29
A498	ND	10.60	0.07	ND	0.96	7.17	0.38	1.13	ND	20.20	5.94	ND	1.52	ND
ACHN	0.39	0.09	0.05	0.31	0.28	<0.01	<0.01	0.09	0.11	0.15	0.64	10.10	<0.01	0.07
CAKI-1	1.05	ND	0.12	0.22	0.29	0.32	<0.01	0.11	0.29	0.20	0.38	10.30	<0.01	0.10
RXF 393	1.29	1.66	0.46	1.35	1.13	8.00	0.14	0.62	2.97	2.48	1.30	20.50	0.05	0.80
SN12C	0.33	0.25	0.20	0.42	0.45	<0.01	<0.01	0.59	0.26	0.94	0.44	11.40	<0.01	0.24
TK-10	1.33	2.75	2.12	2.54	1.85	73.70	ND	ND	2.95	1.66	0.52	17.10	12.50	ND
UO-31	0.77	0.30	0.07	ND	0.69	0.03	0.02	0.29	16.90	1.05	3.75	20.60	0.15	0.32
Prostate														
PC-3	0.86	1.21	0.67	7.22	0.64	34.90	ND	ND	1.15	ND	1.13	15.80	0.50	0.62
DU-145	0.33	0.20	0.14	0.33	0.43	<0.01	<0.01	0.05	0.25	0.16	0.11	10.60	<0.01	0.04
Breast														
MCF7	0.30	0.04	0.05	0.08	0.16	<0.01	<0.01	<0.01	0.36	0.09	0.38	8.82	<0.01	0.07
MDA-MB-231	0.51	1.27	2.06	18.10	1.57	77.80	0.46	0.25	0.64	0.97	0.68	15.30	0.15	1.59
HS 578T	1.44	4.90	1.97	<0.01	ND	54.20	2.03	2.29	0.55	1.93	2.85	13.90	1.52	4.70
BT-549	1.27	3.10	0.12	0.35	0.36	ND	0.75	1.30	0.88	ND	2.41	80.80	2.40	5.51
T-47D	1.55	0.63	0.56	12.00	0.70	<0.01	0.02	0.12	0.07	0.50	0.73	ND	<0.01	0.55
MDA-MB-468	0.95	7.84	0.14	ND	0.24	ND	ND	ND	ND	ND	ND	ND	ND	ND

[a] The MYC inhibition levels were determined based on the western blotting results as shown in Figures 3A and S2. MYC inhibition levels were classified into four levels: strong inhibition, +++, MYC expression inhibited at 0.5 to 1.0 μM; medium inhibition, ++, MYC expression inhibited at 2.0 μM, or no clear dose-dependent MYC inhibition; weak inhibition, +, MYC expression inhibited at 4.0 μM; no inhibition, 0, no MYC expression inhibition up to 4.0 μM.

[b] The relative topoisomerase I (Top1) inhibition levels of the compounds were previously determined and classified into six levels (0 – 5, +++++ = 5).[1-9]

[c] The MGM values for each compound are the average of GI_{50} values across the entire panel of NCI-60 cancer cell lines, where compounds with GI_{50} values that fall outside the test range of 10^{-4} to 10^{-8} M are assigned values of 10^{-4} or 10^{-8} M.

[d] The antiproliferative activities (GI_{50} values) listed are the concentrations corresponding to 50% growth inhibition which were determined in the NCI-60 cancer cell lines drug screen.

[e] GI_{50} value not determined.

Table S4. Raw log_{10}MGM data of the 29 indenoisoquinolines.

The 29 indenoisoquinolines were grouped by their MYC inhibition levels and topoisomerase I inhibition levels. The overall anticancer activity of each group was determined by the $mean(log_{10}\text{MGM})$ value.

log_{10}MGM (μM)		MYC Inhibition Levels*			
		3	**2**	**1**	**0**
Topoisomerase I Inhibition Levels**	**0**			-0.22	
				Mean: -0.22	
	1	-0.62***	0.46	-0.66, -0.24, -0.12, 1.08	
		Mean: -0.62**	**Mean: 0.46**	**Mean: 0.02**	
	2	-1.35, -0.78, -0.70, -0.66, -0.22, -0.20, -0.13		-1.18, -0.22	-0.10
		Mean: -0.58		**Mean: -0.70**	**Mean: -0.10**
	3	-1.13, -0.75		-0.80, -0.06, 0.10, 0.43	
		Mean: -0.94		**Mean: -0.08**	
	4	-1.26, -0.40	-0.95, -0.68, -0.46		
		Mean: -0.83	**Mean: -0.69**		
	5		-1.10		
			Mean: -1.10		

* The MYC inhibition levels were determined based on the western blotting results as shown in Figures 3A and S2 (3 = strong, 2 = medium, 1 = weak, and 0 = no inhibition).

** The topoisomerase I inhibition levels were previously determined.[1-9]

*** log_{10}MGM value of each individual compound. The MGM values for each compound are the average of GI_{50} values across the entire panel of NCI-60 cancer cell lines, where compounds with GI_{50} values that fall outside the test range of 10^{-4} to 10^{-8} M are assigned values of 10^{-4} or 10^{-8} M. 50% growth inhibition (GI_{50}) values were determined in the NCI-60 cancer cell lines drug screen.

**** The $mean(log_{10}\text{MGM})$ value of all compounds in each group.

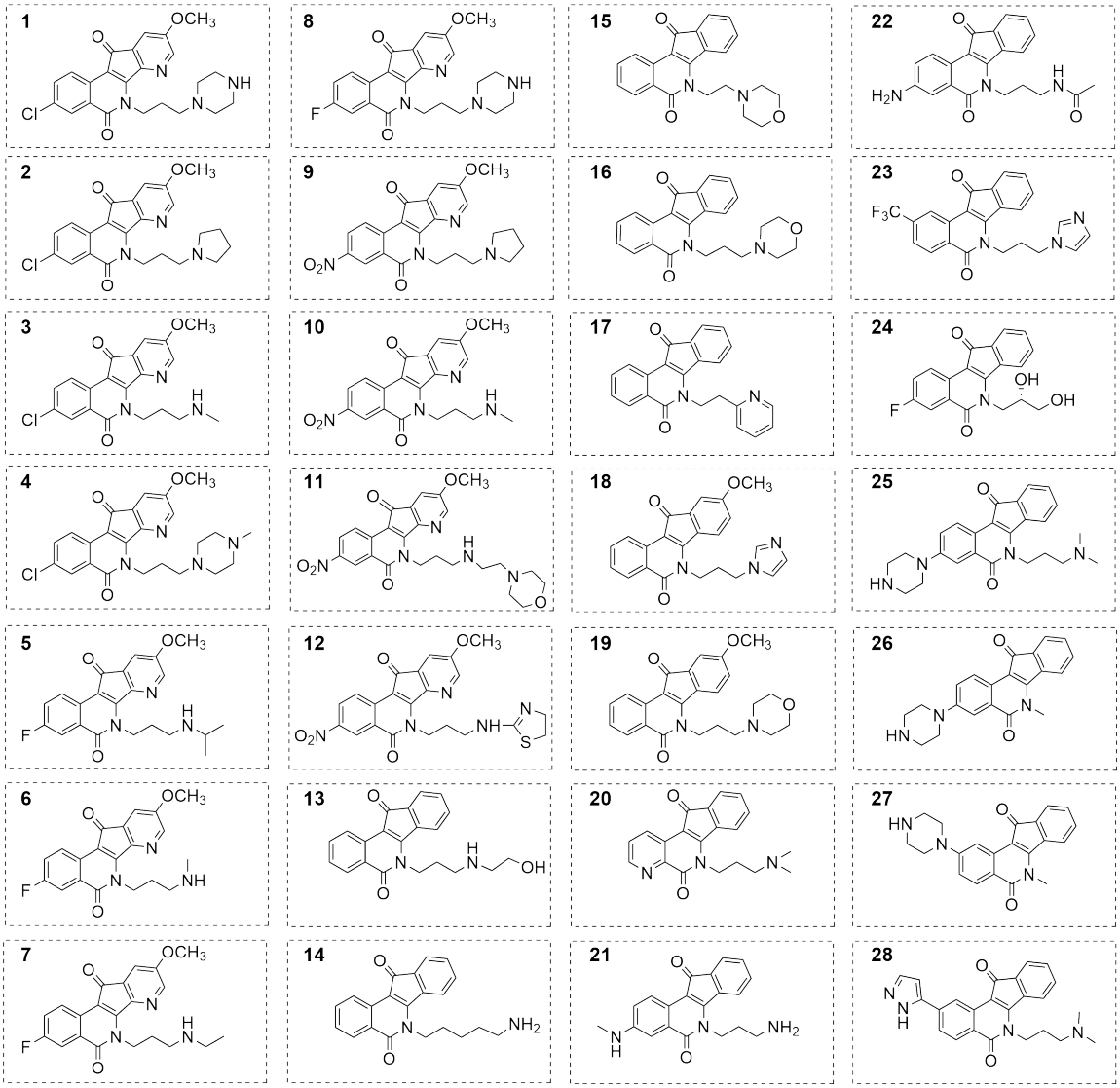


Figure S1. Chemical structures of the 56 indenoisoquinoline analogs.

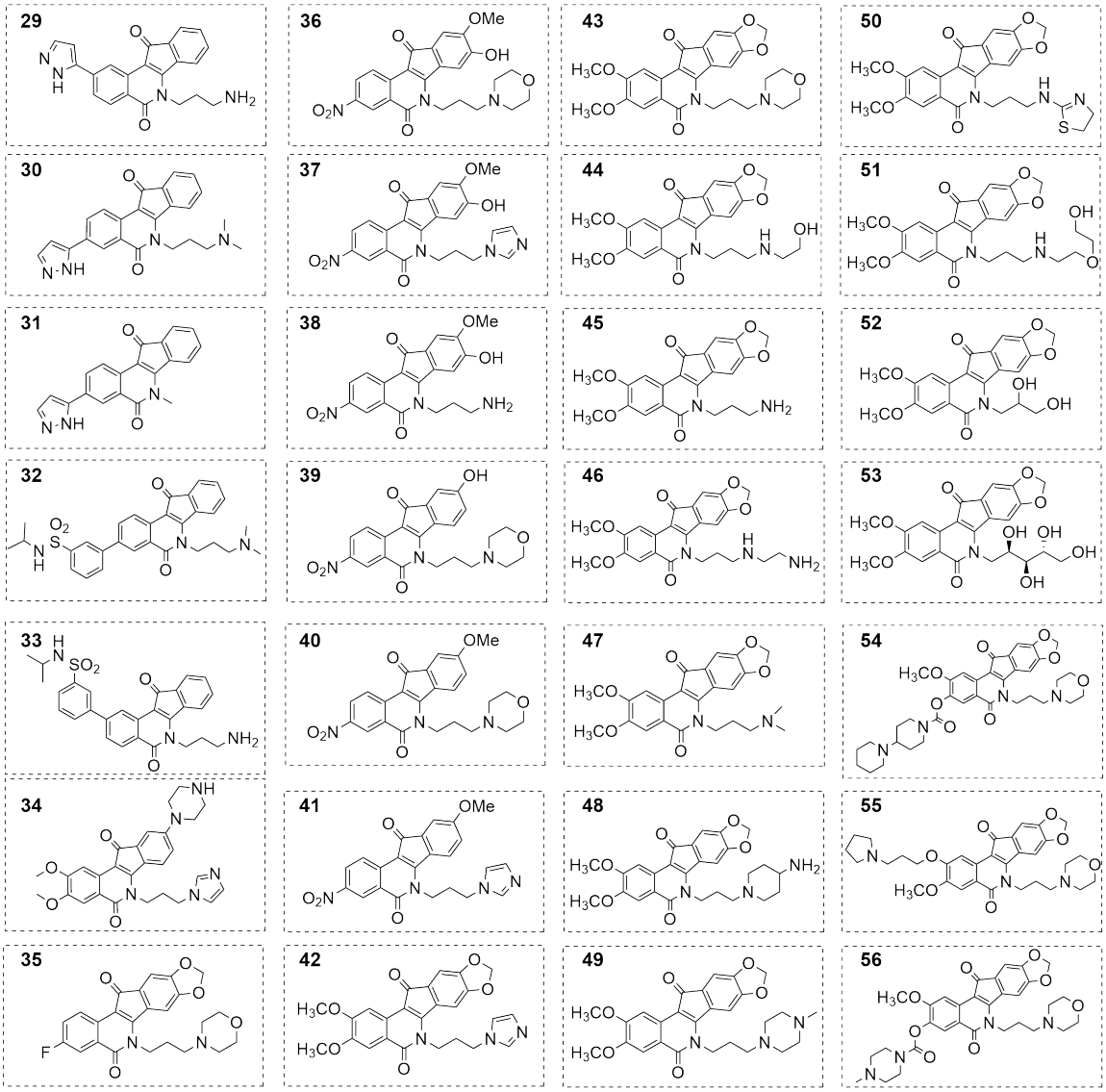


Figure S1. (continued)

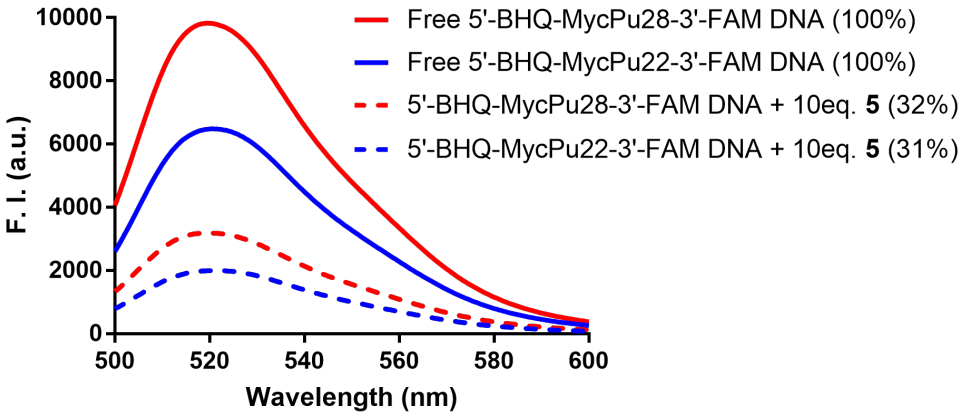


Figure S2. Fluorescence emission spectra of 5′-BHQ-MycPu28-3′-FAM (1 μM) or 5′-BHQ-MycPu22-3′-FAM (1 μM) in the presence or absence of 10 μM indenoisoquinoline **5**. The levels of reduction in the fluorescence induced by indenoisoquinoline **5** are very similar for the MycPu28 and MycPu22, as shown by the numbers in parentheses. Conditions: 25 °C, 50 mM Tris·acetate, pH 7.

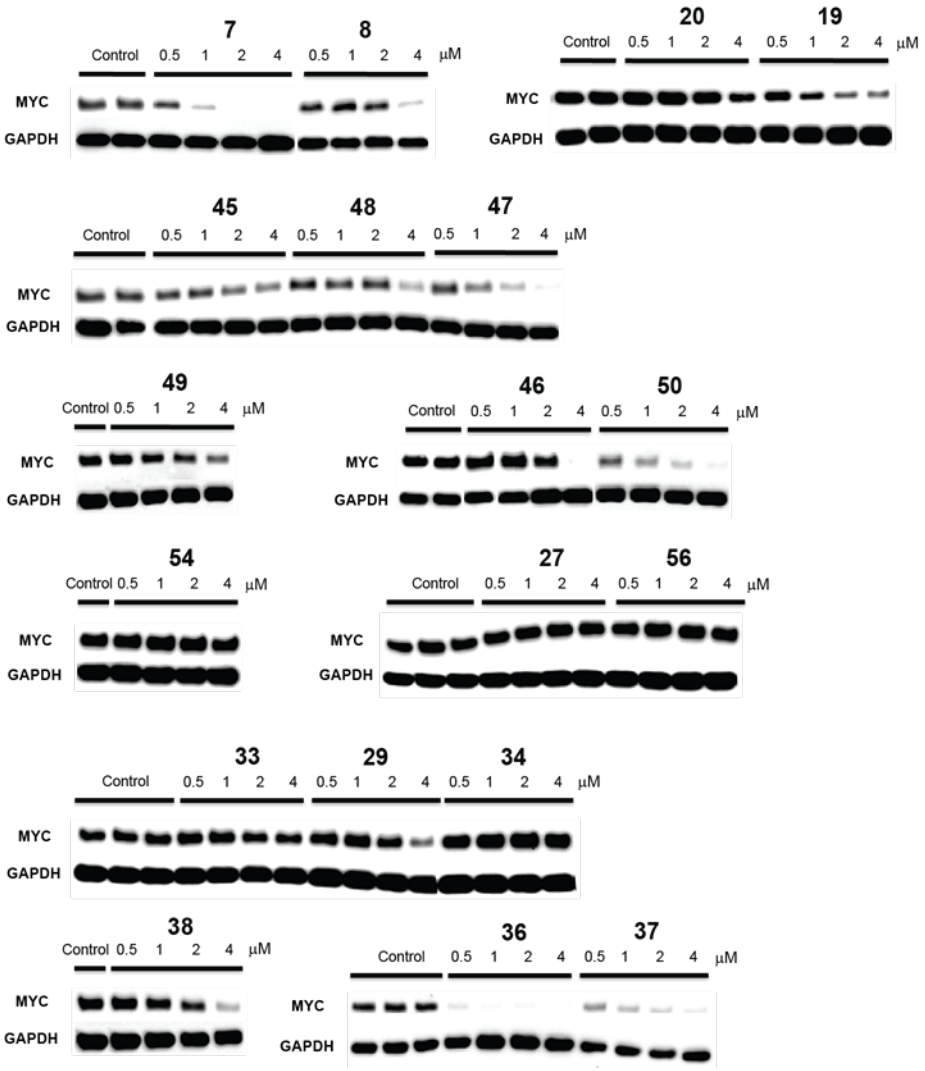


Figure S3. MYC protein expression levels in the absence and presence of various concentrations of indenoisoquinolines (24 h treatment) obtained by western blotting experiments in MCF-7 breast cancer cell lines. GAPDH was used as an internal control.

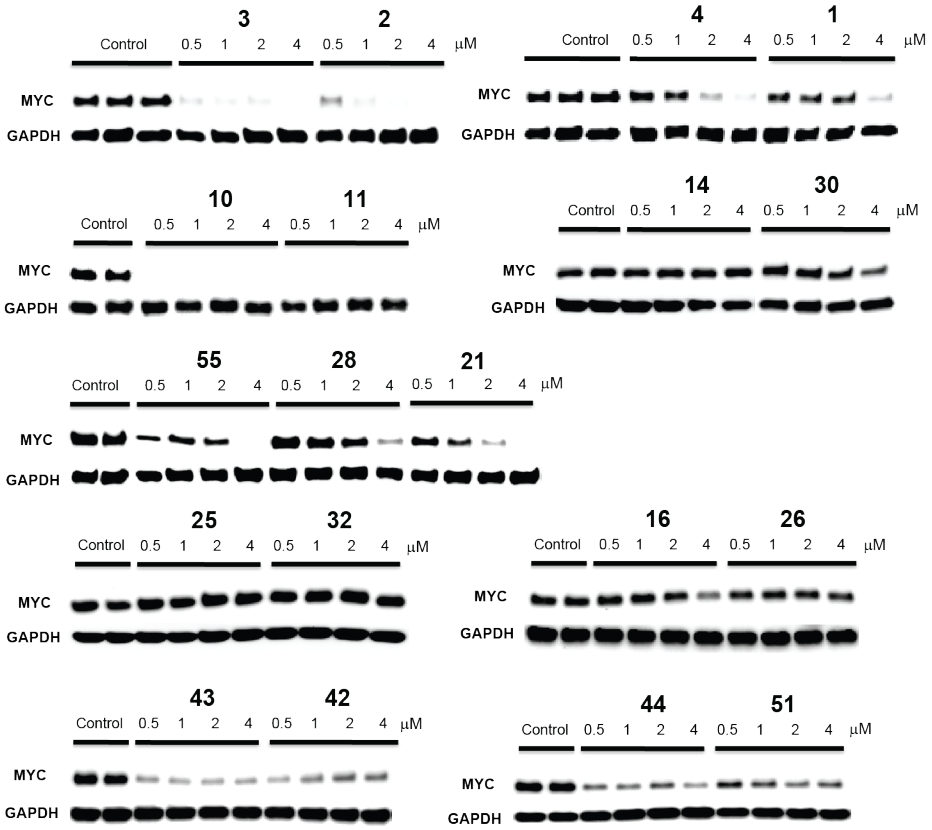


Figure S3. (continued)

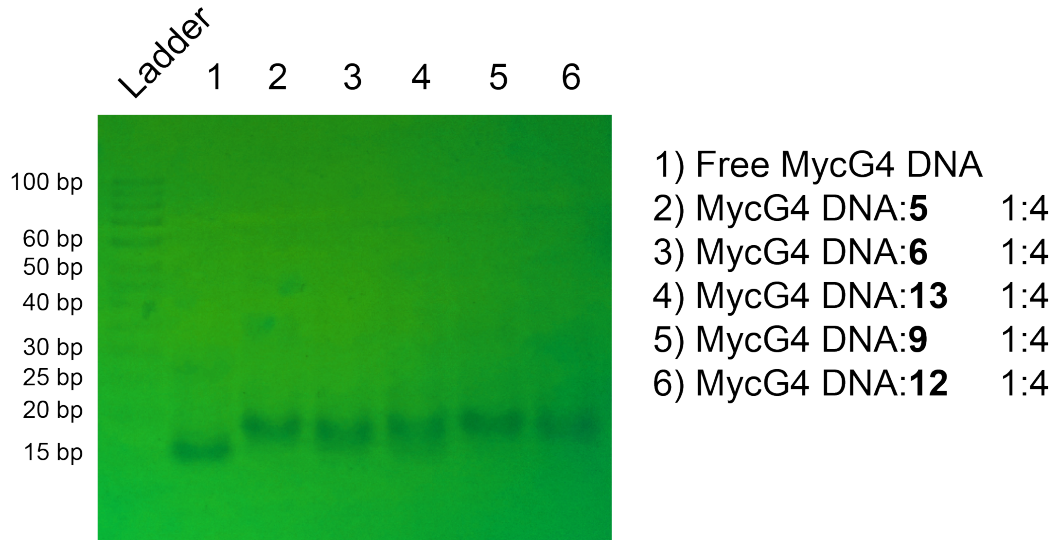


Figure S4. Native PAGE experiments of MycPu22 G-quadruplex DNA in the presence and absence of various indenoisoquinolines. DNA bands were visualized using UV light. Each sample contains 4 μL of 150 μM DNA. Conditions: 25 °C, TBE buffer containing 12.5 mM KCl, pH 8.0.

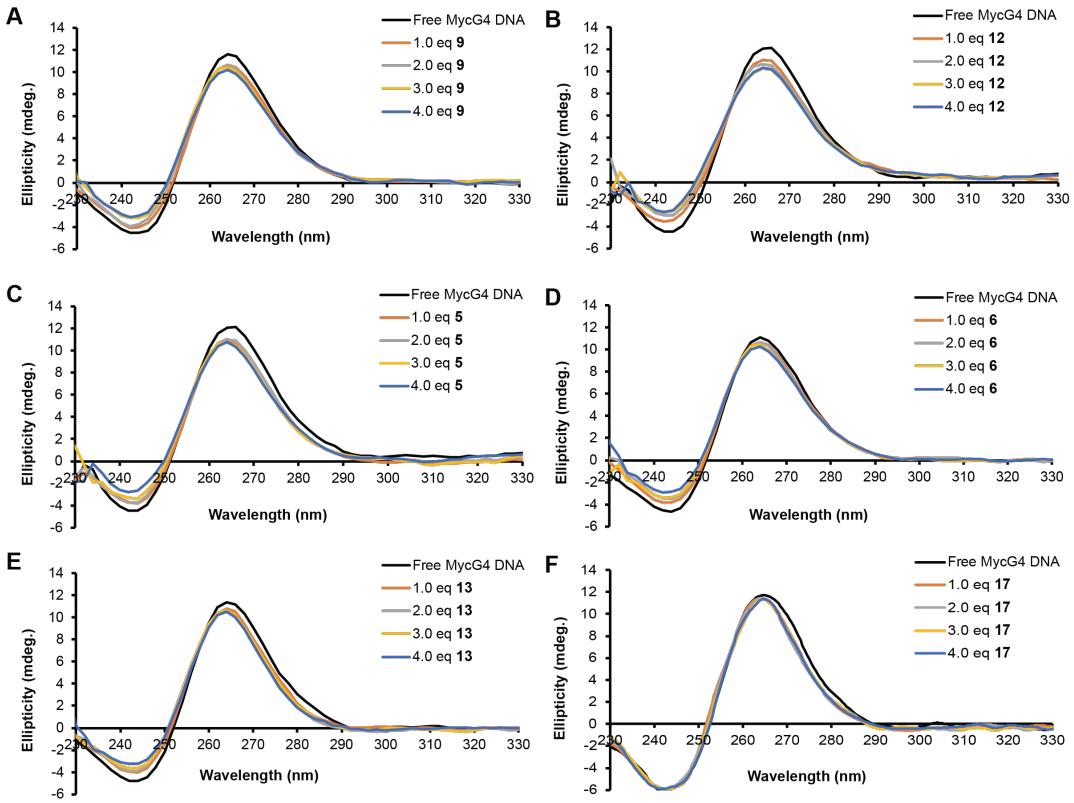
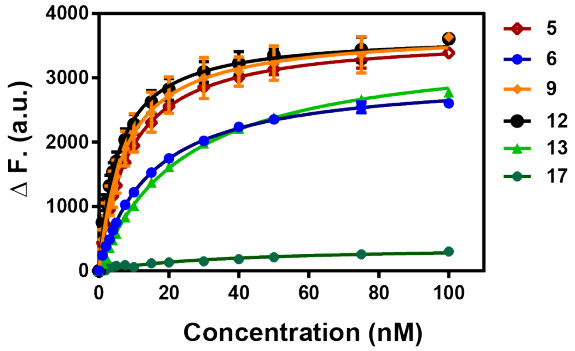


Figure S5. CD spectra of MycPu22 G-quadruplex DNA (15 μM) with addition of 1, 2, 3, and 4 equivalents of indenoisoquinoline compound **9** (A), **12** (B), **5** (C), **6** (D), **13** (E), and **17** (F). Conditions: 25 °C, pH 7, 5 mM K^+.

A



B

Compound	$K_{d,app}$ (nM)
5	8.5 ± 0.5
6	14.8 ± 0.3
9	7.3 ± 0.3
12	5.6 ± 0.2
13	23.9 ± 0.7
17	N.D.

Figure S6. Apparent binding affinities of the five indenoisoquinolines with MycPu22 determined by fluorescence-based binding assay. (A) Fluorescence intensity change of 3′-TAMRA-labeled MycPu22 DNA (0.5 nM) at 580 nm upon respective titration of six indenoisoquinolines. Conditions: 20 °C, pH 7, 100 mM K^+. (B) Apparent K_d values determined for six indenoisoquinolines. N.D. indicates that the value was not determined due to the negligible change of fluorescence signal. The apparent binding affinity K_d values were determined by fitting the data to a one-site specific binding model using GraphPad Prism software, with a simplified equation of $\Delta F_{obs} = \Delta F_{max} \frac{[L]_T}{[L]_T + K_{d,app}}$, where ΔF represents the fluorescence intensity change of the indenoisoquinolines bound to MycPu22 DNA and $[L]_T$ represents the total ligand concentration.

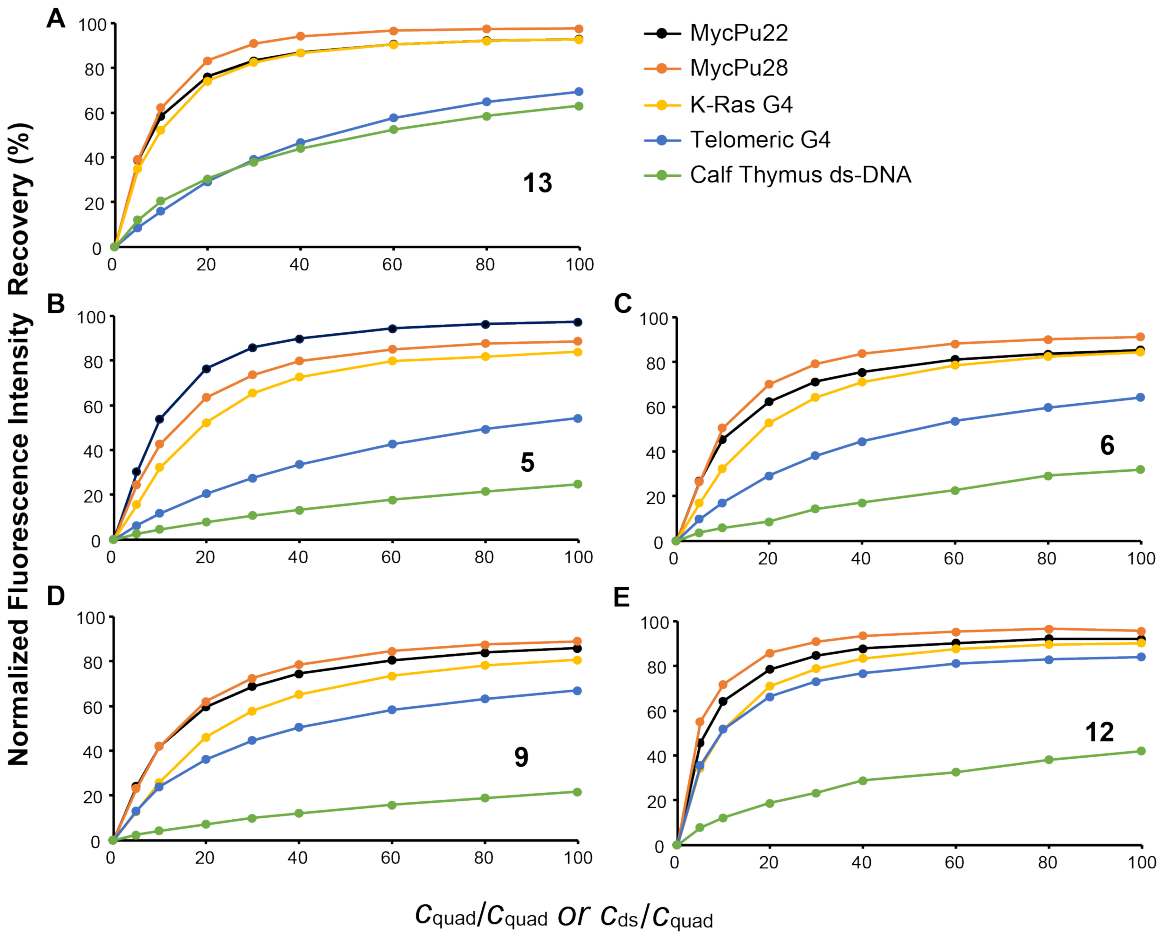


Figure S7. Binding selectivities of MycG4-interactive indenoisoquinolines. Competition fluorescence displacement experiments with increasing concentrations of unlabeled G4s and ds-DNA were added to 3′-TAMRA labeled MycPu22 (20 nM) mixed with 5 equivalents of indenoisoquinoline compound **13** (A), **5** (B), **6** (C), **9** (D), and **12** (E). The normalized TAMRA fluorescence intensities at 580 nm were plotted as a function of molar ratio of G4 (in 3 G-tetrads) or calf thymus ds-DNA (in 11 bp) to labeled MycPu22. The fluorescence intensity of free 3′-TAMRA-labeled MycPu22 was defined as 100%, and the fluorescence intensity of a 1:5 mixture of 3′-TAMRA-labeled MycPu22 and indenoisoquinoline was defined as 0%. Conditions: 20 °C, pH 7, 100 mM K^+.

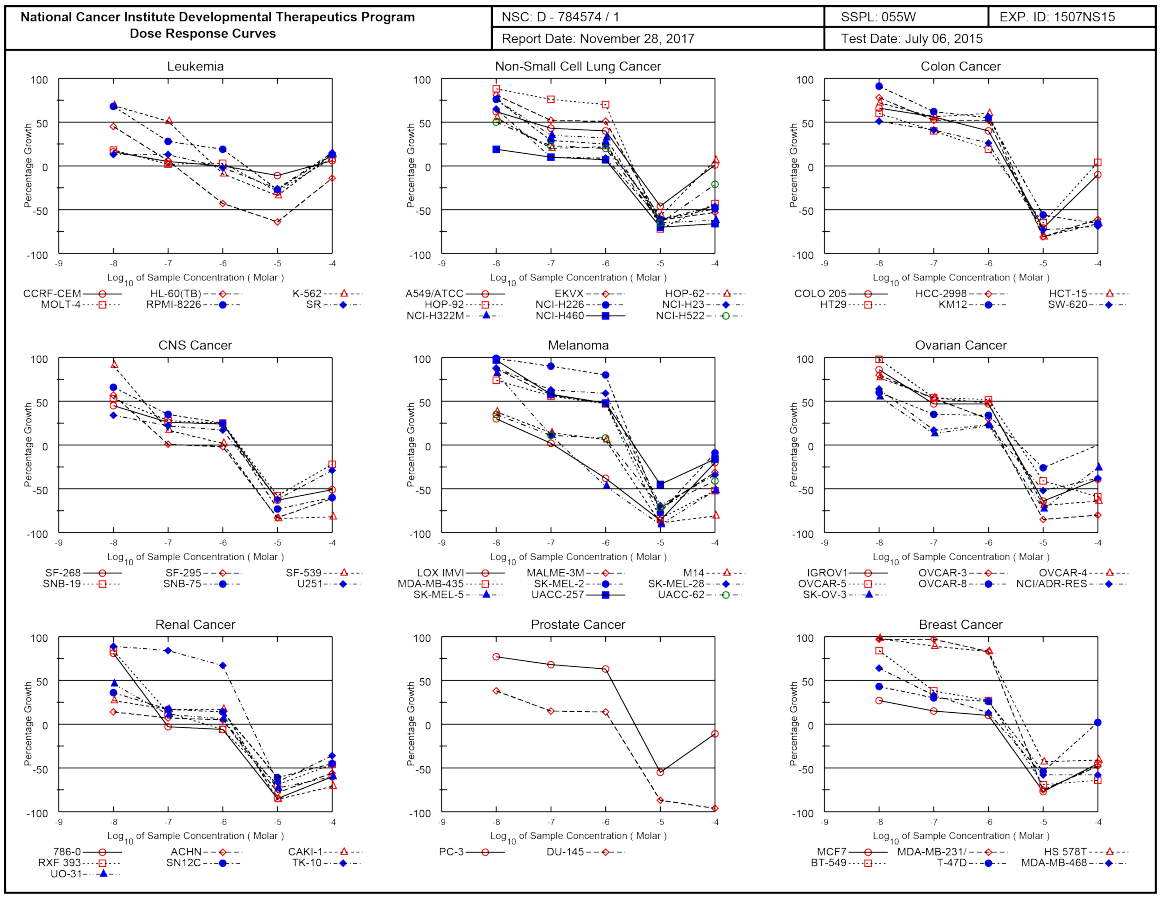


Figure S8. Example dose response curves from the NCI-60 cancer cell line drug screen (compound **12**). The response parameters GI_{50} (50% growth inhibition) and LC_{50} (50% lethal concentration) are extracted from concentration–response curves. TGI (total growth inhibition) is read as the x-axis intercept.

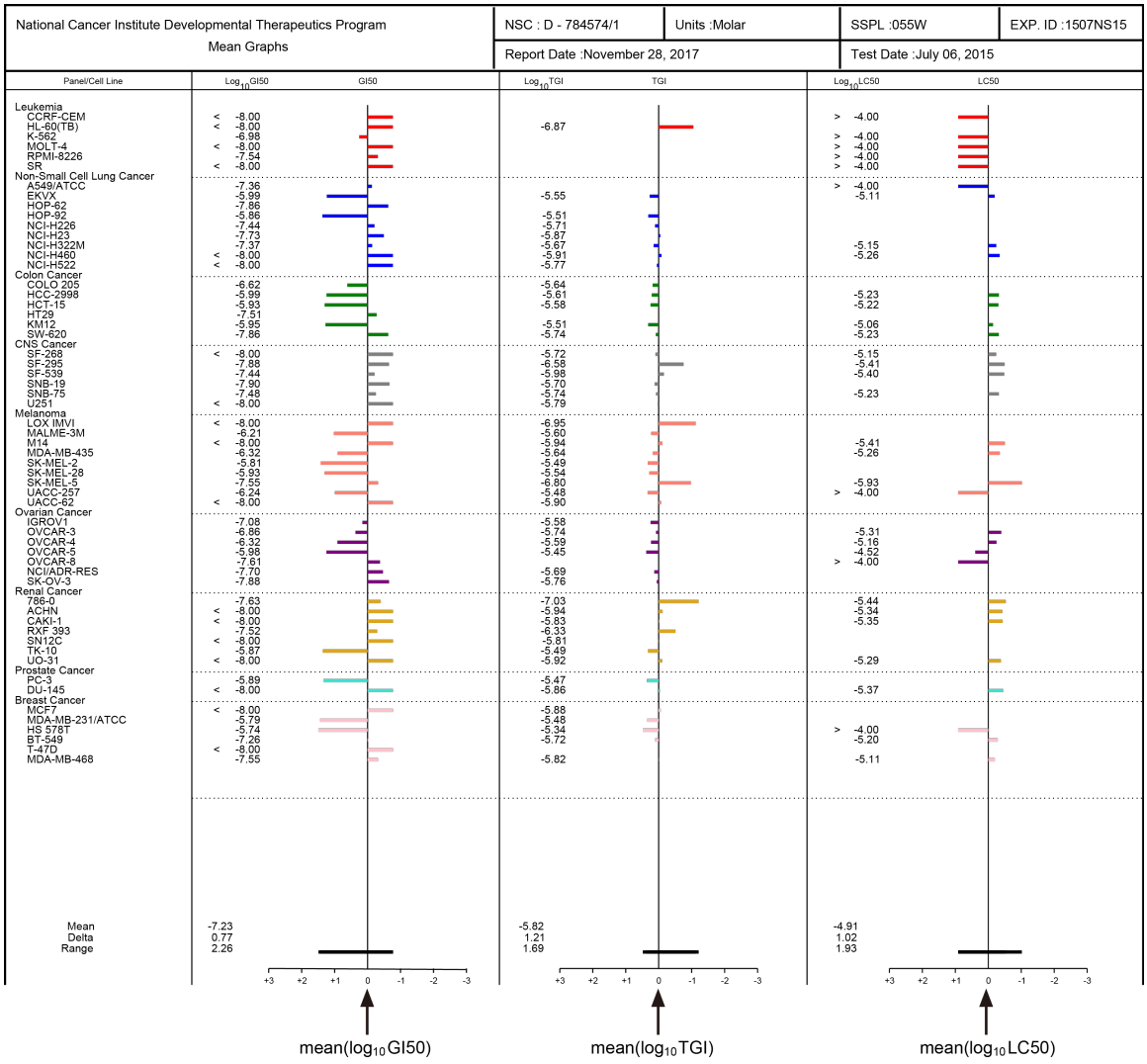


Figure S9. An example of Mean Graphs from the NCI-60 cancer cell line drug screen (compound **12**). Mean Graphs are constructed for GI_{50}, TGI, and LC_{50} values, with bars depicting the deviation of individual cancer cell lines from the overall mean value for all the NCI-60 cancer cell lines tested. GI_{50} Mean Graph: 50% growth inhibition Mean Graph. TGI Mean Graph: total growth inhibition Mean Graph. LC_{50} Mean Graph: 50% lethal concentration Mean Graph. The mean value across the entire panel of NCI-60 cancer cell lines (Mean), the maximum difference from the mean (Delta), and the difference between the highest and lowest values (Range) are indicated below each profile.

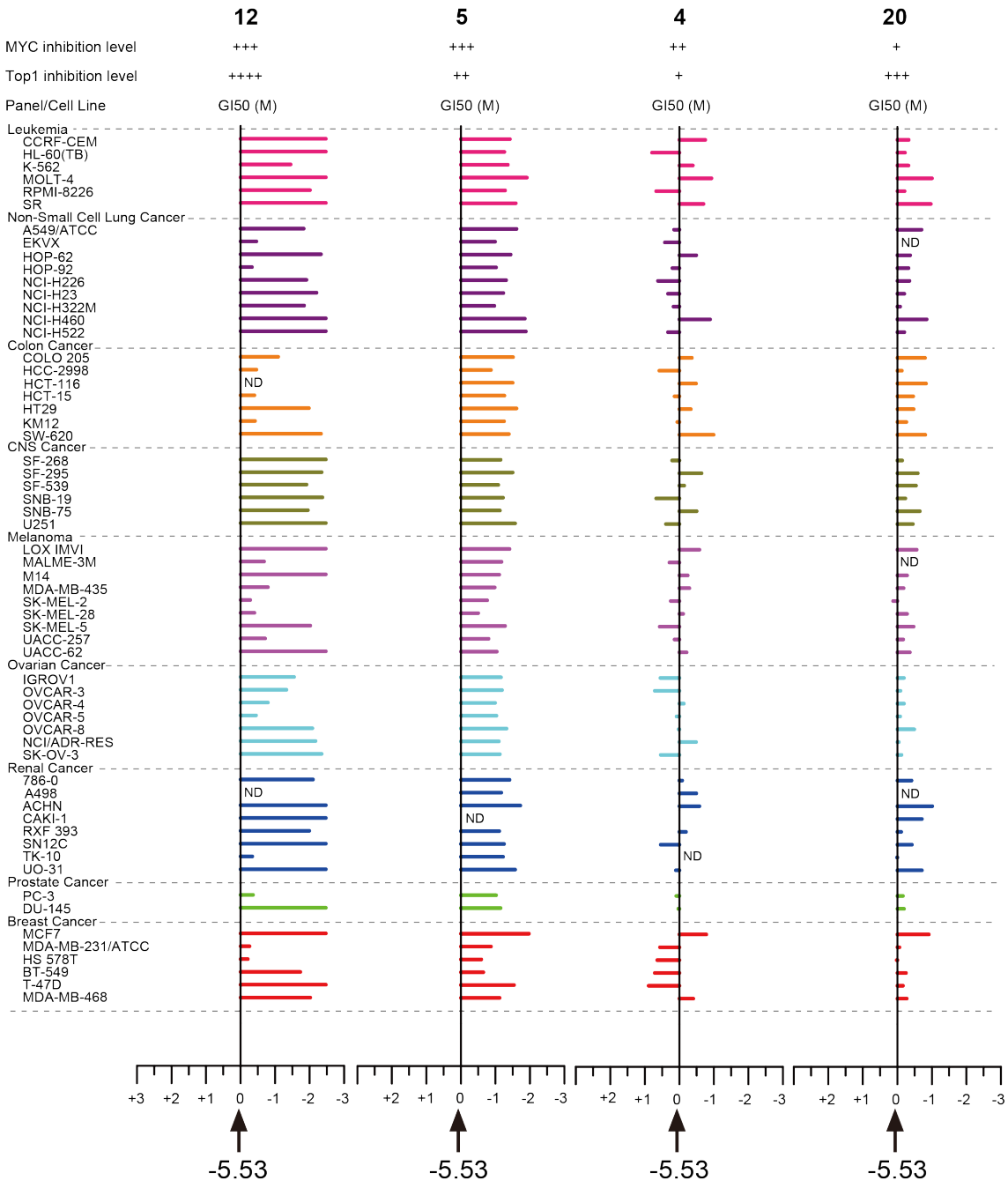


Figure S10. Bar graphs showing the antiproliferation profiles (GI_{50}) of indenoisoquinoline **12**, **5**, **4**, and **20** from the NCI-60 cancer cell line drug screen. MYC inhibition and Topoisomerase I inhibition levels are shown at the top. Bar graphs are constructed for each compound, with bars depicting the deviation of individual cancer cell lines from the compound **4** $mean(log_{10}GI_{50})$ value of -5.53. Compounds **12** and **5** with strong MYC inhibition and topoisomerase I inhibition show more potent anticancer activities compared to compounds **4** and **20**. ND: GI_{50} value not determined.

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