FIGURE 1

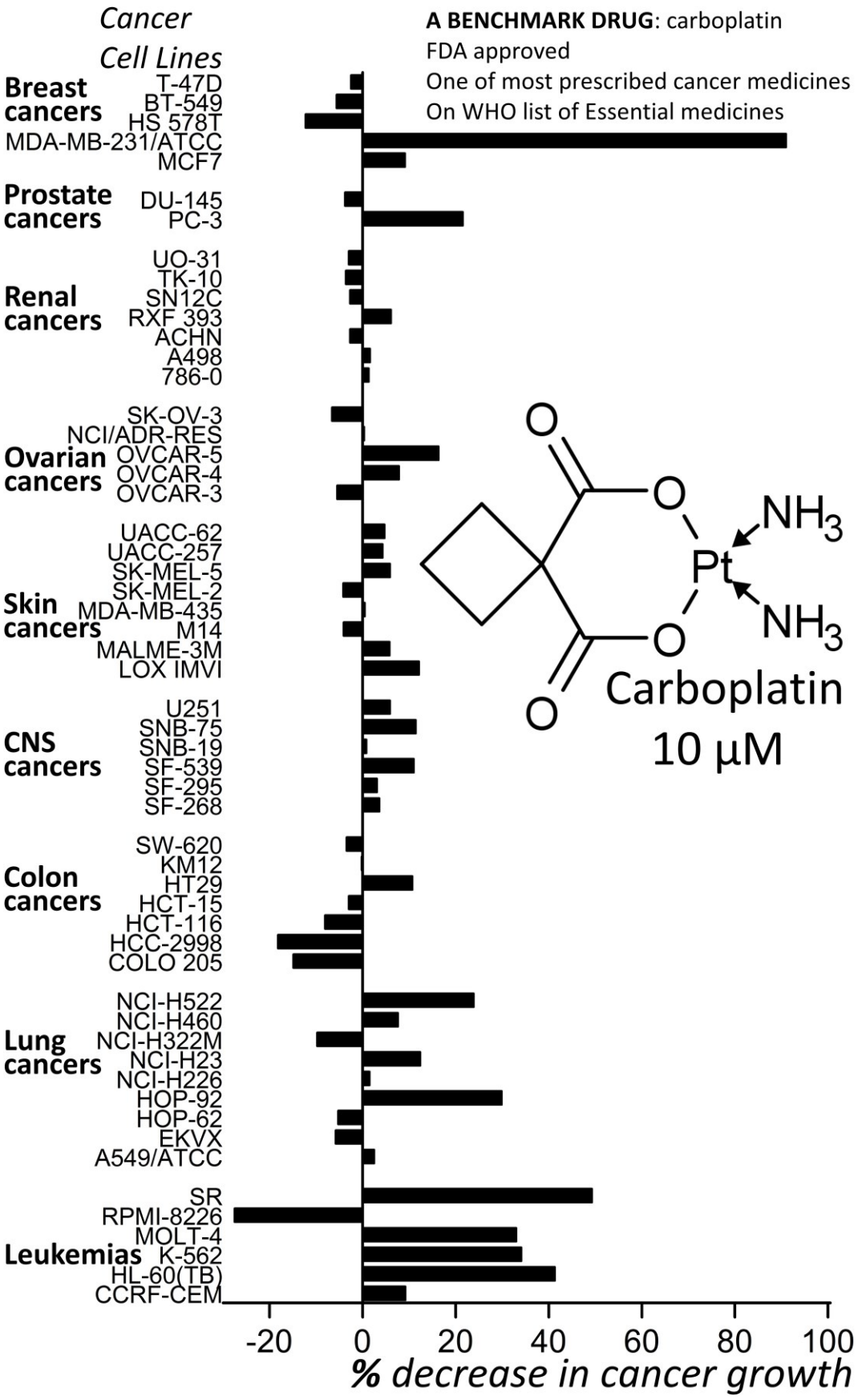

Cancer Cell Lines
Breast cancers
T-47D
BT-549
HS 578T
MDA-MB-231/ATCC
MCF7
Prostate cancers
DU-145
PC-3
Renal cancers
UO-31
TK-10
SN12C
RXF 393
ACHN
A498
786-0
Ovarian cancers
SK-OV-3
NCI/ADR-RES
OVCAR-5
OVCAR-4
OVCAR-3
Skin cancers
UACC-62
UACC-257
SK-MEL-5
SK-MEL-2
MDA-MB-435
M14
MALME-3M
LOX IMVI
CNS cancers
U251
SNB-75
SNB-19
SF-539
SF-295
SF-268
Colon cancers
SW-620
KM12
HT29
HCT-15
HCT-116
HCC-2998
COLO 205
Lung cancers
NCI-H522
NCI-H460
NCI-H322M
NCI-H23
NCI-H226
HOP-92
HOP-62
EKVX
A549/ATCC
Leukemias
SR
RPMI-8226
MOLT-4
K-562
HL-60(TB)
CCRF-CEM
A BENCHMARK DRUG: carboplatin
FDA approved
One of most prescribed cancer medicines
On WHO list of Essential medicines
O
O
O
O
Pt
NH3
NH3
Carboplatin
10 µM
-20
0
20
40
60
80
100
% decrease in cancer growth

FIGURE 2

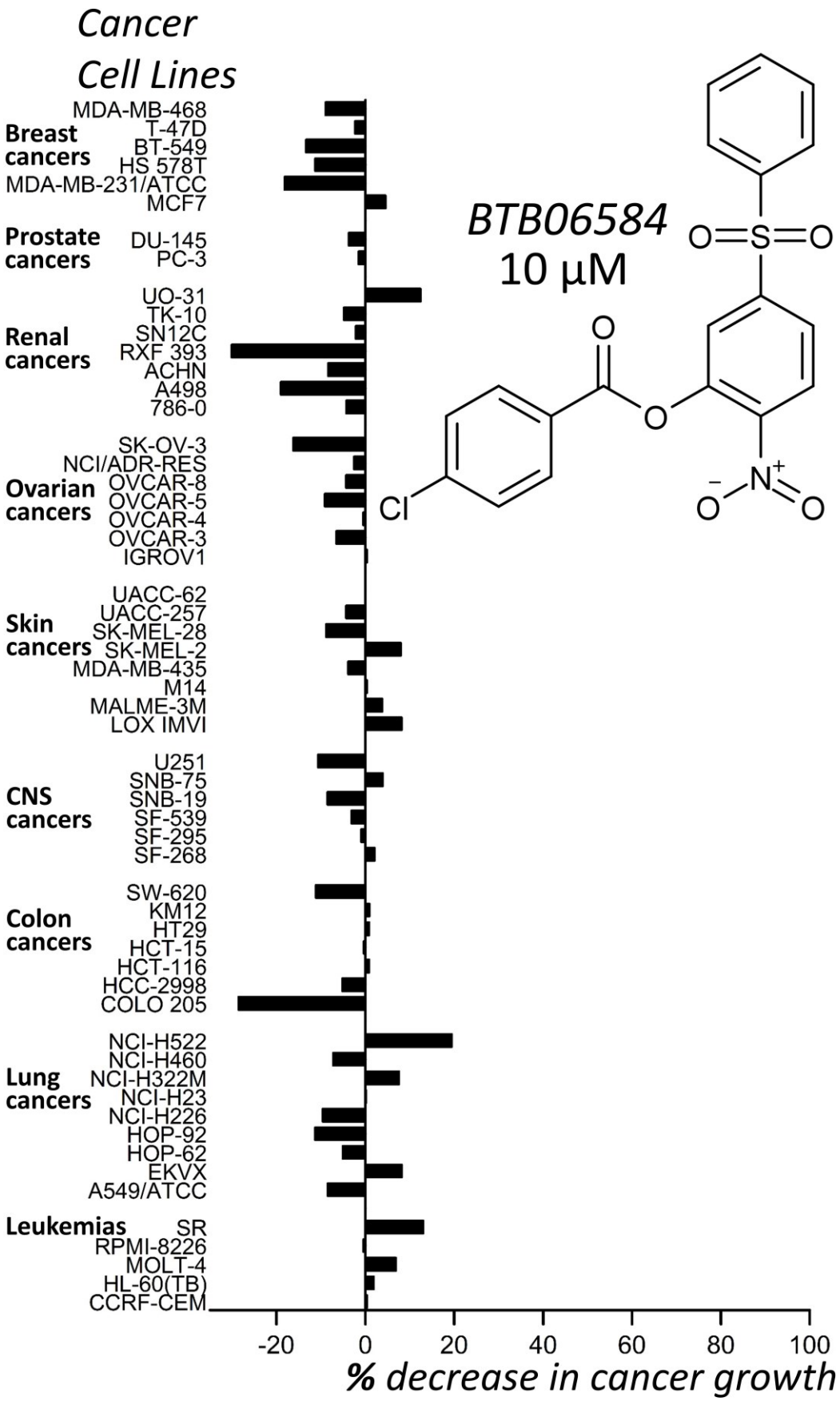

Cancer
Cell Lines
Breast cancers
MDA-MB-468
T-47D
BT-549
HS 578T
MDA-MB-231/ATCC
MCF7
Prostate cancers
DU-145
PC-3
Renal cancers
UO-31
TK-10
SN12C
RXF 393
ACHN
A498
786-0
Ovarian cancers
SK-OV-3
NCI/ADR-RES
OVCAR-8
OVCAR-5
OVCAR-4
OVCAR-3
IGROV1
Skin cancers
UACC-62
UACC-257
SK-MEL-28
SK-MEL-2
MDA-MB-435
M14
MALME-3M
LOX IMVI
CNS cancers
U251
SNB-75
SNB-19
SF-539
SF-295
SF-268
Colon cancers
SW-620
KM12
HT29
HCT-15
HCT-116
HCC-2998
COLO 205
Lung cancers
NCI-H522
NCI-H460
NCI-H322M
NCI-H23
NCI-H226
HOP-92
HOP-62
EKVX
A549/ATCC
Leukemias
SR
RPMI-8226
MOLT-4
HL-60(TB)
CCRF-CEM
BTB06584
10 µM
Cl
O
S
N+
-20
0
20
40
60
80
100
% decrease in cancer growth

FIGURE 3

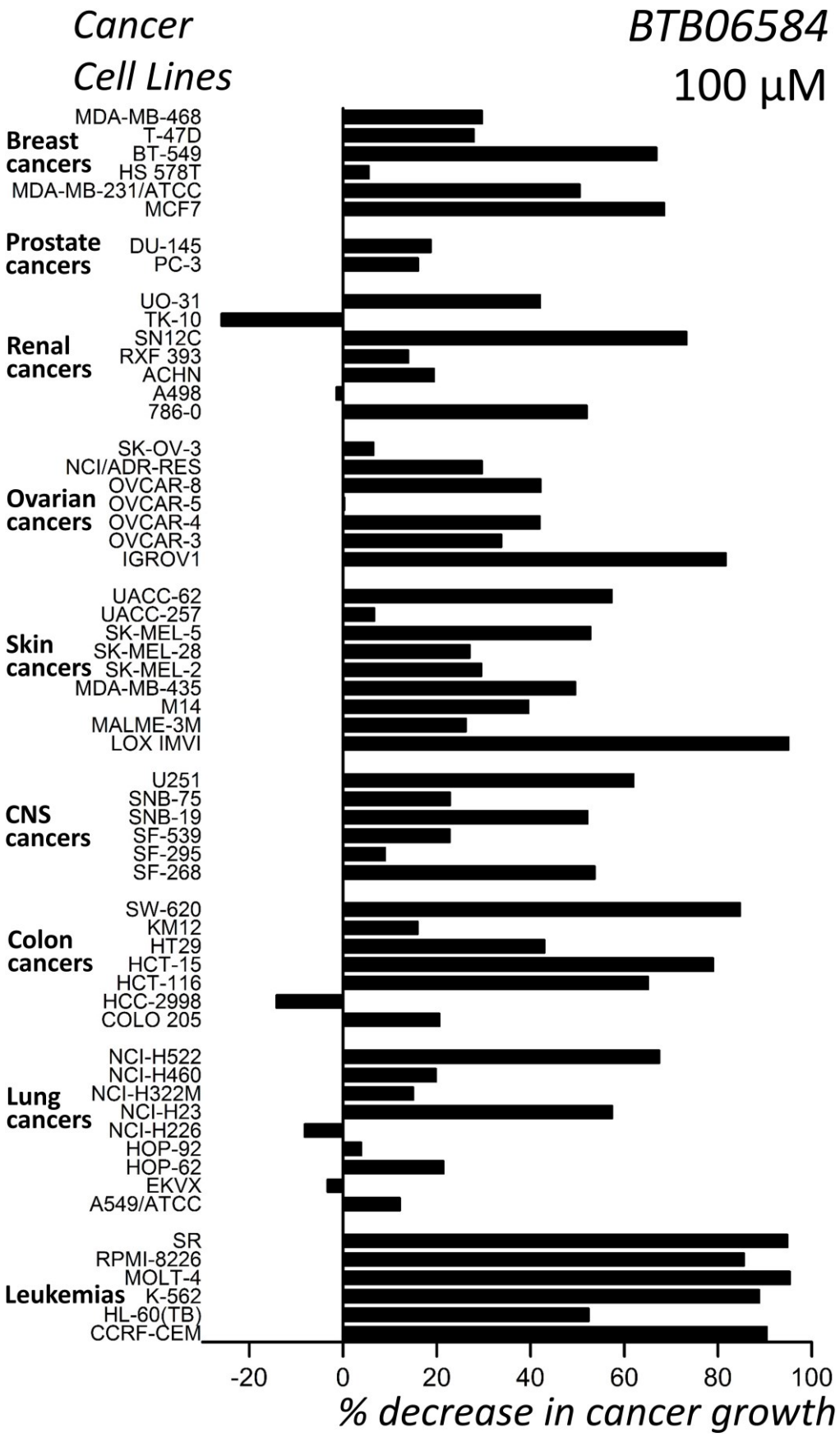

Cancer Cell Lines
BTB06584
100 µM
Breast cancers
MDA-MB-468
T-47D
BT-549
HS 578T
MDA-MB-231/ATCC
MCF7
Prostate cancers
DU-145
PC-3
Renal cancers
UO-31
TK-10
SN12C
RXF 393
ACHN
A498
786-0
Ovarian cancers
SK-OV-3
NCI/ADR-RES
OVCAR-8
OVCAR-5
OVCAR-4
OVCAR-3
IGROV1
Skin cancers
UACC-62
UACC-257
SK-MEL-5
SK-MEL-28
SK-MEL-2
MDA-MB-435
M14
MALME-3M
LOX IMVI
CNS cancers
U251
SNB-75
SNB-19
SF-539
SF-295
SF-268
Colon cancers
SW-620
KM12
HT29
HCT-15
HCT-116
HCC-2998
COLO 205
Lung cancers
NCI-H522
NCI-H460
NCI-H322M
NCI-H23
NCI-H226
HOP-92
HOP-62
EKVX
A549/ATCC
Leukemias
SR
RPMI-8226
MOLT-4
K-562
HL-60(TB)
CCRF-CEM
-20
0
20
40
60
80
100
% decrease in cancer growth

FIGURE 4

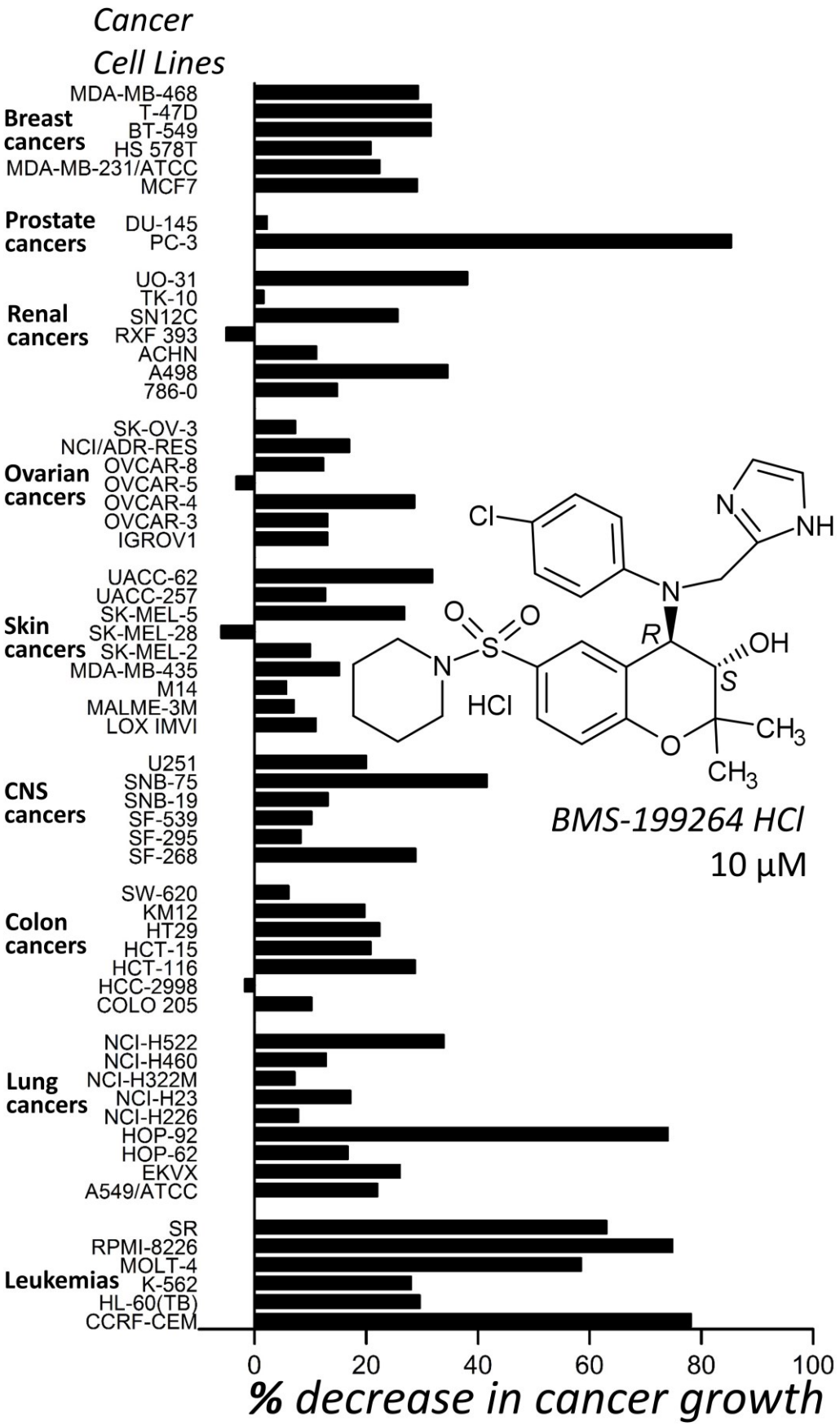

Cancer
Cell Lines
Breast cancers
MDA-MB-468
T-47D
BT-549
HS 578T
MDA-MB-231/ATCC
MCF7
Prostate cancers
DU-145
PC-3
Renal cancers
UO-31
TK-10
SN12C
RXF 393
ACHN
A498
786-0
Ovarian cancers
SK-OV-3
NCI/ADR-RES
OVCAR-8
OVCAR-5
OVCAR-4
OVCAR-3
IGROV1
Skin cancers
UACC-62
UACC-257
SK-MEL-5
SK-MEL-28
SK-MEL-2
MDA-MB-435
M14
MALME-3M
LOX IMVI
CNS cancers
U251
SNB-75
SNB-19
SF-539
SF-295
SF-268
Colon cancers
SW-620
KM12
HT29
HCT-15
HCT-116
HCC-2998
COLO 205
Lung cancers
NCI-H522
NCI-H460
NCI-H322M
NCI-H23
NCI-H226
HOP-92
HOP-62
EKVX
A549/ATCC
Leukemias
SR
RPMI-8226
MOLT-4
K-562
HL-60(TB)
CCRF-CEM
0
20
40
60
80
100
% decrease in cancer growth
Cl
N
NH
N
R
OH
S
O
O
N
HCl
S
O
CH3
CH3
BMS-199264 HCl
10 µM

FIGURE 5

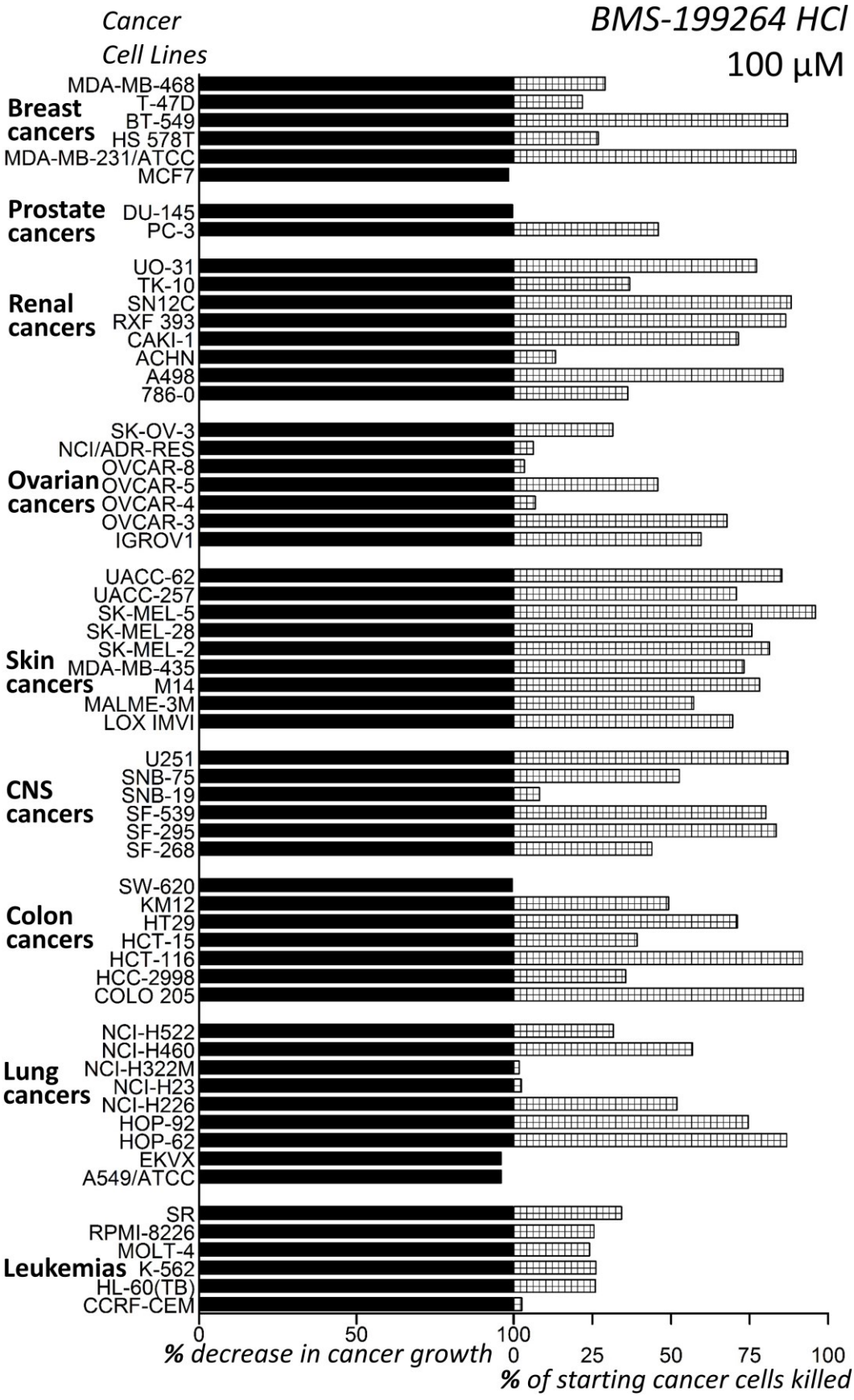

Cancer
Cell Lines
BMS-199264 HCl
100 µM
Breast cancers
MDA-MB-468
T-47D
BT-549
HS 578T
MDA-MB-231/ATCC
MCF7
Prostate cancers
DU-145
PC-3
Renal cancers
UO-31
TK-10
SN12C
RXF 393
CAKI-1
ACHN
A498
786-0
Ovarian cancers
SK-OV-3
NCI/ADR-RES
OVCAR-8
OVCAR-5
OVCAR-4
OVCAR-3
IGROV1
Skin cancers
UACC-62
UACC-257
SK-MEL-5
SK-MEL-28
SK-MEL-2
MDA-MB-435
M14
MALME-3M
LOX IMVI
CNS cancers
U251
SNB-75
SNB-19
SF-539
SF-295
SF-268
Colon cancers
SW-620
KM12
HT29
HCT-15
HCT-116
HCC-2998
COLO 205
Lung cancers
NCI-H522
NCI-H460
NCI-H322M
NCI-H23
NCI-H226
HOP-92
HOP-62
EKVX
A549/ATCC
Leukemias
SR
RPMI-8226
MOLT-4
K-562
HL-60(TB)
CCRF-CEM
0
50
100
% decrease in cancer growth
0
25
50
75
100
% of starting cancer cells killed

FIGURE 6

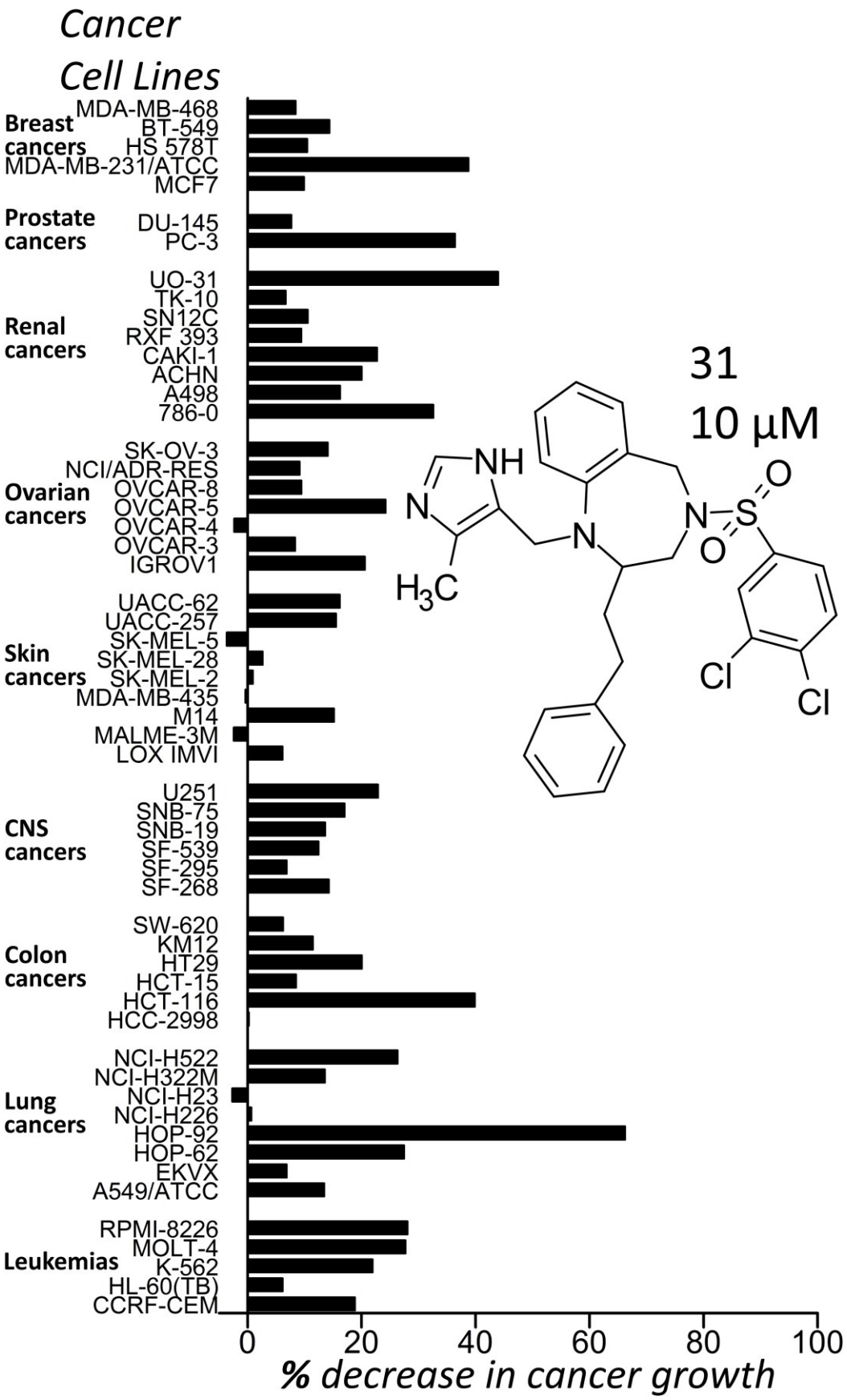

Cancer Cell Lines
Breast cancers
MDA-MB-468
BT-549
HS 578T
MDA-MB-231/ATCC
MCF7
Prostate cancers
DU-145
PC-3
Renal cancers
UO-31
TK-10
SN12C
RXF 393
CAKI-1
ACHN
A498
786-0
Ovarian cancers
SK-OV-3
NCI/ADR-RES
OVCAR-8
OVCAR-5
OVCAR-4
OVCAR-3
IGROV1
Skin cancers
UACC-62
UACC-257
SK-MEL-5
SK-MEL-28
SK-MEL-2
MDA-MB-435
M14
MALME-3M
LOX IMVI
CNS cancers
U251
SNB-75
SNB-19
SF-539
SF-295
SF-268
Colon cancers
SW-620
KM12
HT29
HCT-15
HCT-116
HCC-2998
Lung cancers
NCI-H522
NCI-H322M
NCI-H23
NCI-H226
HOP-92
HOP-62
EKVX
A549/ATCC
Leukemias
RPMI-8226
MOLT-4
K-562
HL-60(TB)
CCRF-CEM
0
20
40
60
80
100
% decrease in cancer growth
31
10 µM
NH
N
N
N
S
O
O
H3C
Cl
Cl

FIGURE 7

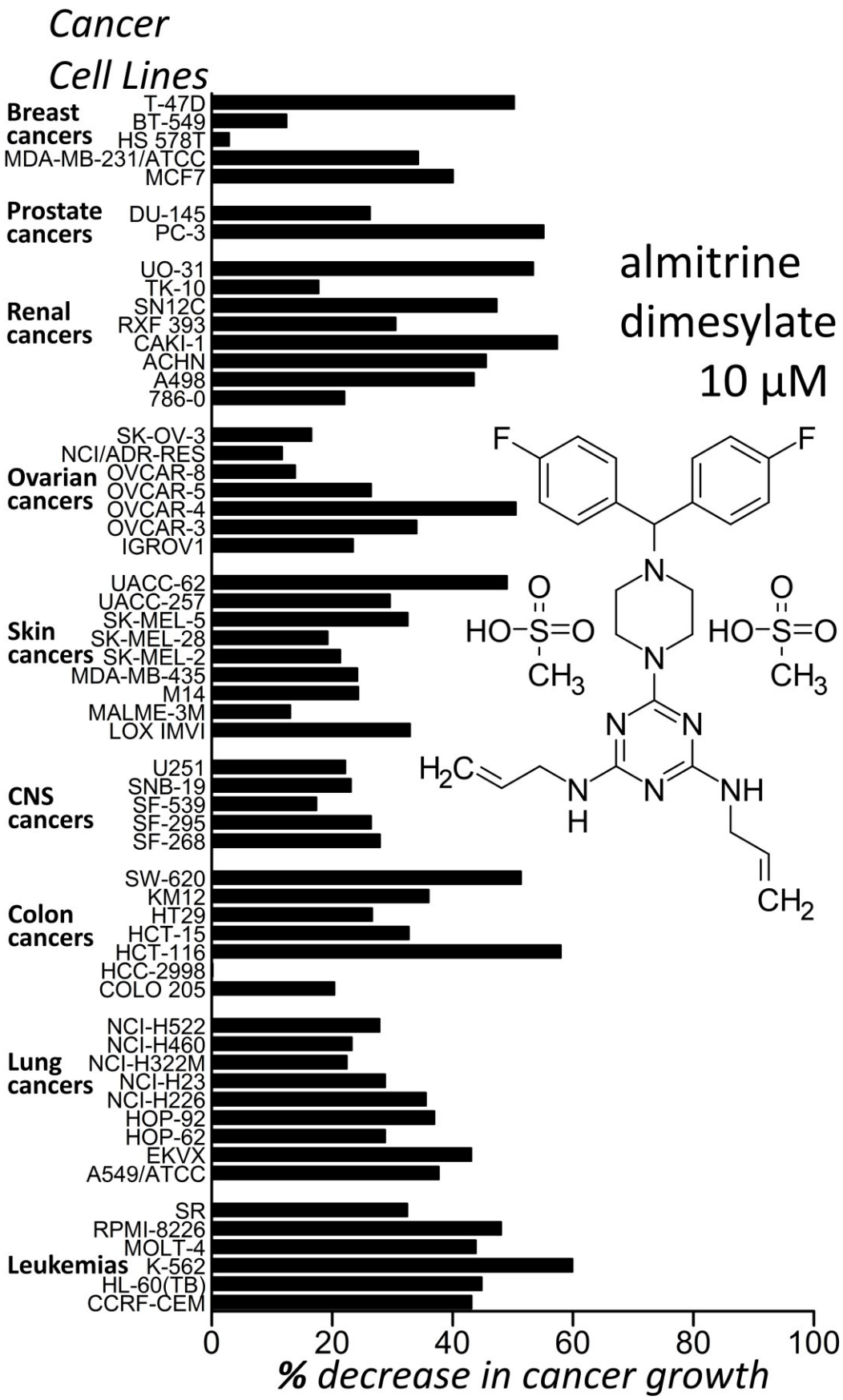

Cancer Cell Lines
Breast cancers
T-47D
BT-549
HS 578T
MDA-MB-231/ATCC
MCF7
Prostate cancers
DU-145
PC-3
Renal cancers
UO-31
TK-10
SN12C
RXF 393
CAKI-1
ACHN
A498
786-0
Ovarian cancers
SK-OV-3
NCI/ADR-RES
OVCAR-8
OVCAR-5
OVCAR-4
OVCAR-3
IGROV1
Skin cancers
UACC-62
UACC-257
SK-MEL-5
SK-MEL-28
SK-MEL-2
MDA-MB-435
M14
MALME-3M
LOX IMVI
CNS cancers
U251
SNB-19
SF-539
SF-295
SF-268
Colon cancers
SW-620
KM12
HT29
HCT-15
HCT-116
HCC-2998
COLO 205
Lung cancers
NCI-H522
NCI-H460
NCI-H322M
NCI-H23
NCI-H226
HOP-92
HOP-62
EKVX
A549/ATCC
Leukemias
SR
RPMI-8226
MOLT-4
K-562
HL-60(TB)
CCRF-CEM
0
20
40
60
80
100
% decrease in cancer growth
almitrine dimesylate 10 µM

FIGURE 8A

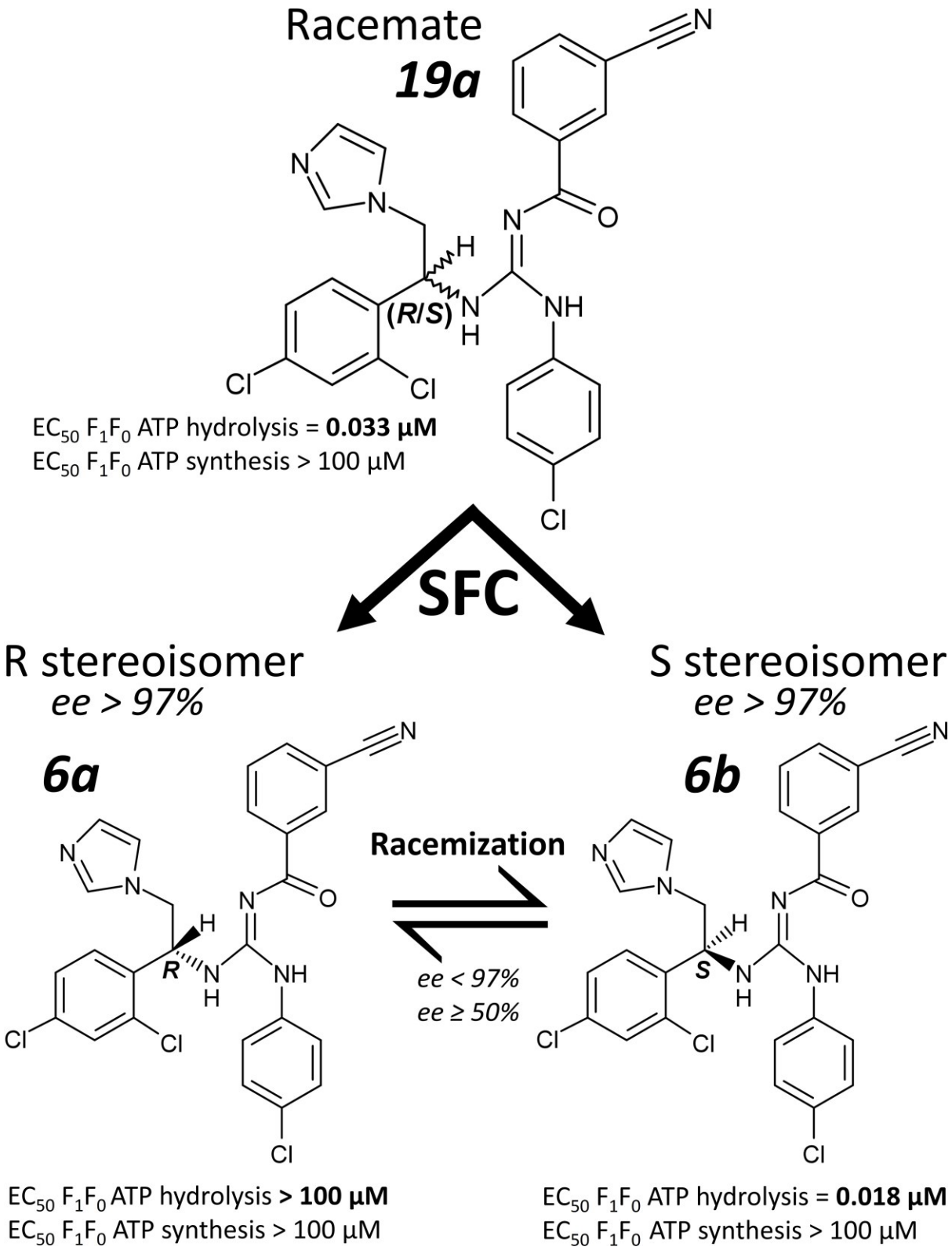

Racemate
19a
(R/S)
EC50 F1F0 ATP hydrolysis = 0.033 µM
EC50 F1F0 ATP synthesis > 100 µM
SFC
R stereoisomer
ee > 97%
6a
EC50 F1F0 ATP hydrolysis > 100 µM
EC50 F1F0 ATP synthesis > 100 µM
Racemization
ee < 97%
ee ≥ 50%
S stereoisomer
ee > 97%
6b
EC50 F1F0 ATP hydrolysis = 0.018 µM
EC50 F1F0 ATP synthesis > 100 µM

FIGURE 8B

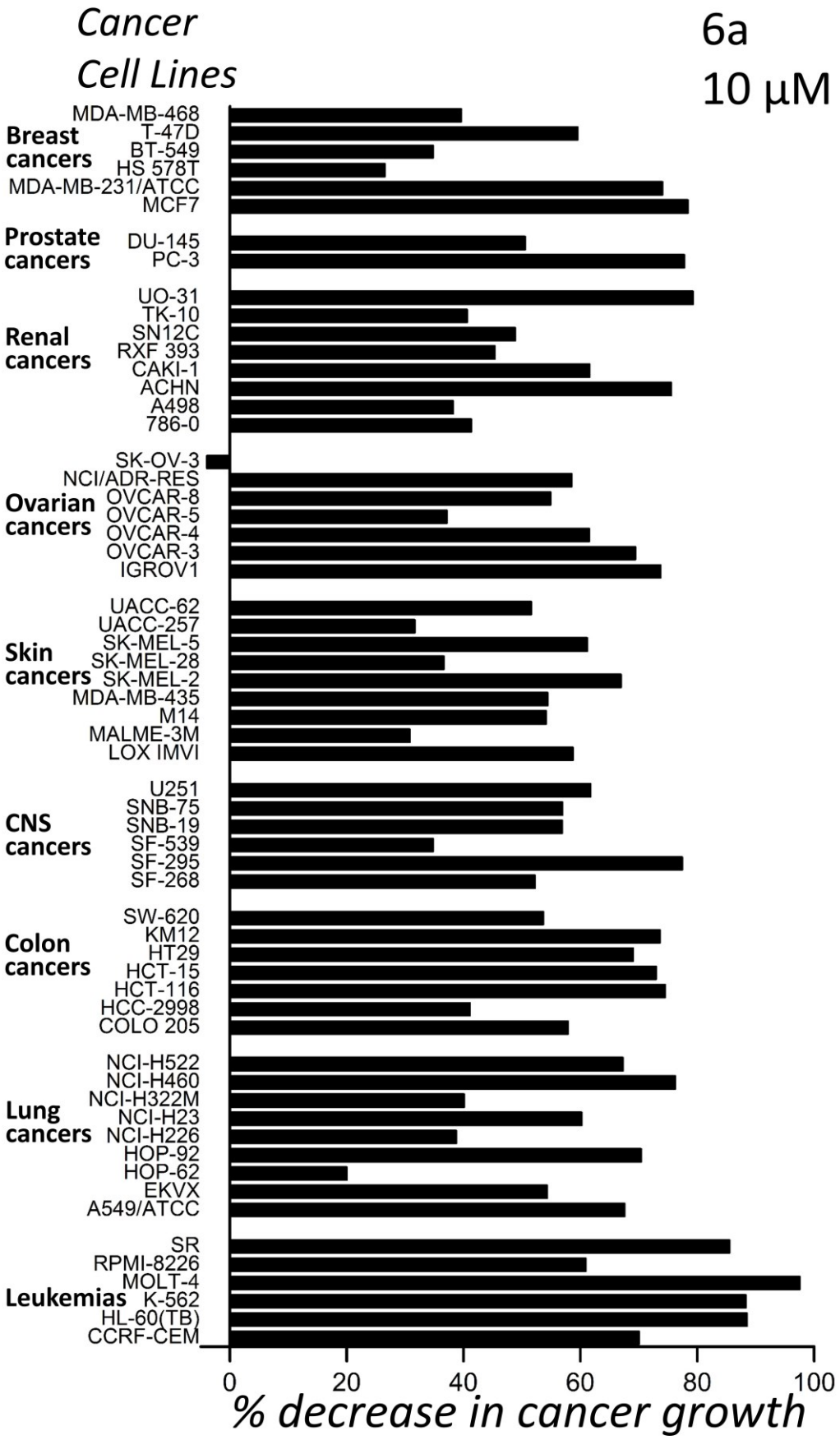

Cancer
Cell Lines
6a
10 µM
Breast cancers
MDA-MB-468
T-47D
BT-549
HS 578T
MDA-MB-231/ATCC
MCF7
Prostate cancers
DU-145
PC-3
Renal cancers
UO-31
TK-10
SN12C
RXF 393
CAKI-1
ACHN
A498
786-0
Ovarian cancers
SK-OV-3
NCI/ADR-RES
OVCAR-8
OVCAR-5
OVCAR-4
OVCAR-3
IGROV1
Skin cancers
UACC-62
UACC-257
SK-MEL-5
SK-MEL-28
SK-MEL-2
MDA-MB-435
M14
MALME-3M
LOX IMVI
CNS cancers
U251
SNB-75
SNB-19
SF-539
SF-295
SF-268
Colon cancers
SW-620
KM12
HT29
HCT-15
HCT-116
HCC-2998
COLO 205
Lung cancers
NCI-H522
NCI-H460
NCI-H322M
NCI-H23
NCI-H226
HOP-92
HOP-62
EKVX
A549/ATCC
Leukemias
SR
RPMI-8226
MOLT-4
K-562
HL-60(TB)
CCRF-CEM
0
20
40
60
80
100
% decrease in cancer growth

FIGURE 8C

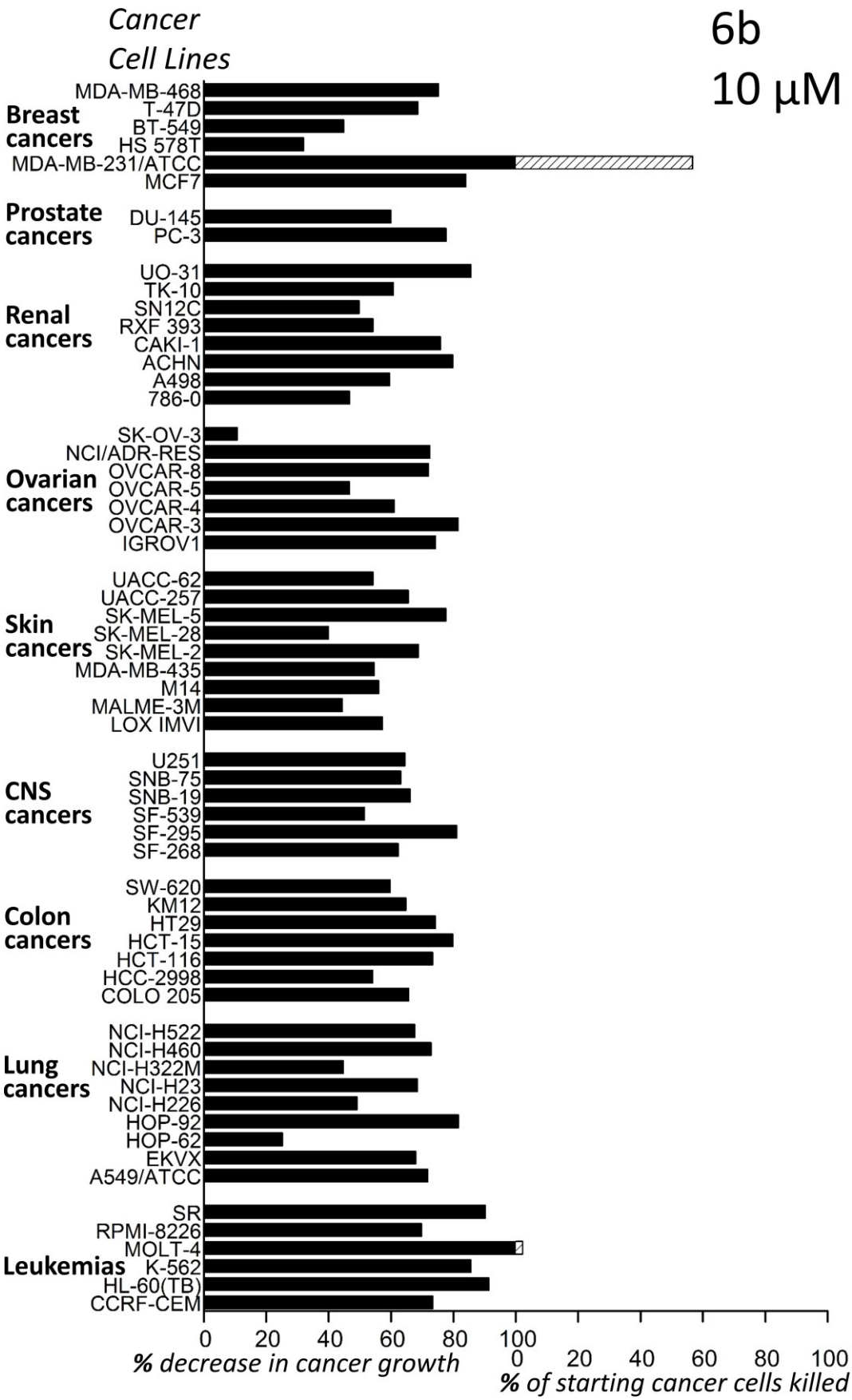

Cancer
Cell Lines
6b
10 μM
Breast cancers
MDA-MB-468
T-47D
BT-549
HS 578T
MDA-MB-231/ATCC
MCF7
Prostate cancers
DU-145
PC-3
Renal cancers
UO-31
TK-10
SN12C
RXF 393
CAKI-1
ACHN
A498
786-0
Ovarian cancers
SK-OV-3
NCI/ADR-RES
OVCAR-8
OVCAR-5
OVCAR-4
OVCAR-3
IGROV1
Skin cancers
UACC-62
UACC-257
SK-MEL-5
SK-MEL-28
SK-MEL-2
MDA-MB-435
M14
MALME-3M
LOX IMVI
CNS cancers
U251
SNB-75
SNB-19
SF-539
SF-295
SF-268
Colon cancers
SW-620
KM12
HT29
HCT-15
HCT-116
HCC-2998
COLO 205
Lung cancers
NCI-H522
NCI-H460
NCI-H322M
NCI-H23
NCI-H226
HOP-92
HOP-62
EKVX
A549/ATCC
Leukemias
SR
RPMI-8226
MOLT-4
K-562
HL-60(TB)
CCRF-CEM
0 20 40 60 80 100
% decrease in cancer growth
0 20 40 60 80 100
% of starting cancer cells killed

FIGURE 8D

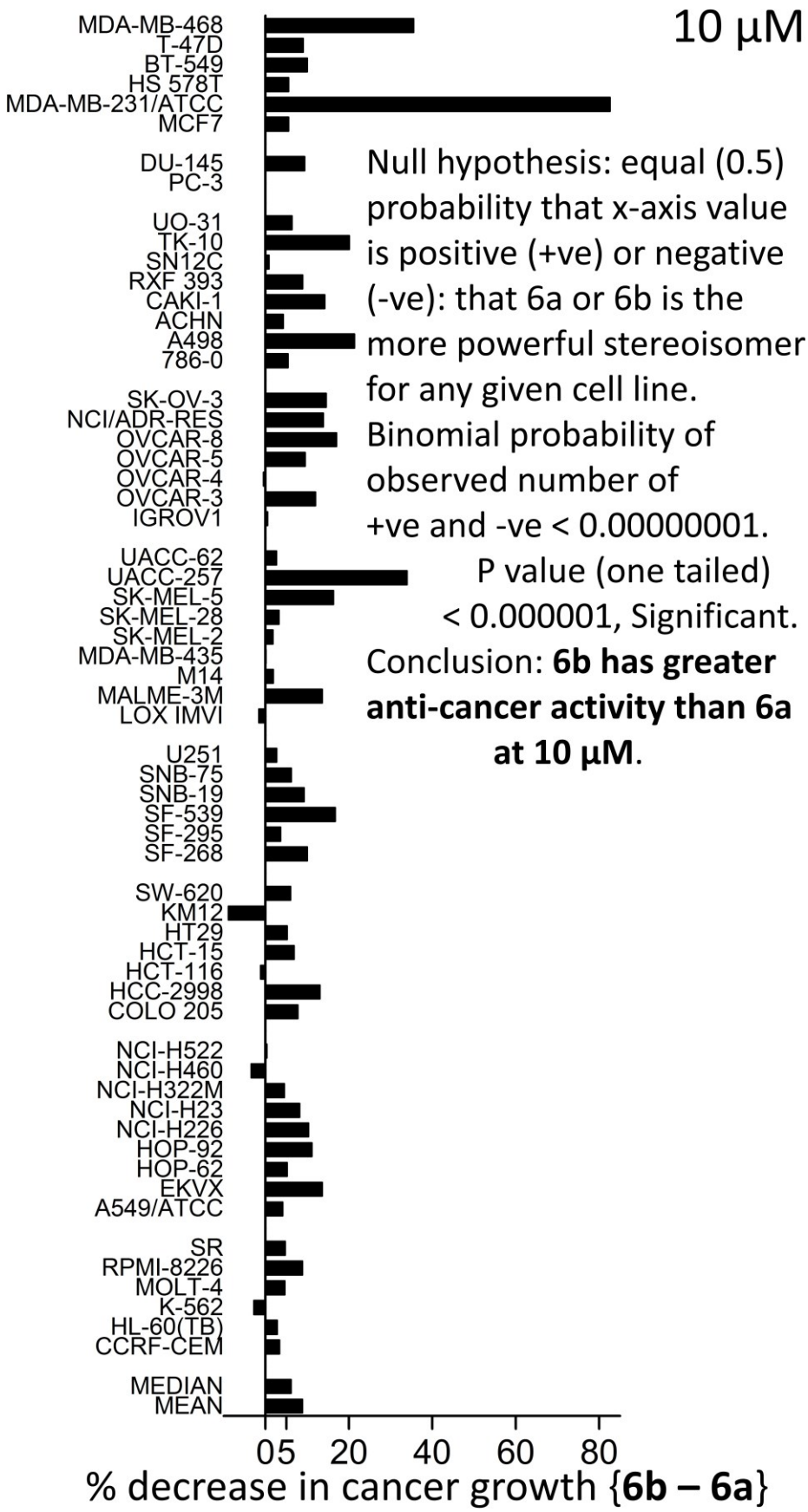

10 µM
MDA-MB-468
T-47D
BT-549
HS 578T
MDA-MB-231/ATCC
MCF7
DU-145
PC-3
UO-31
TK-10
SN12C
RXF 393
CAKI-1
ACHN
A498
786-0
SK-OV-3
NCI/ADR-RES
OVCAR-8
OVCAR-5
OVCAR-4
OVCAR-3
IGROV1
UACC-62
UACC-257
SK-MEL-5
SK-MEL-28
SK-MEL-2
MDA-MB-435
M14
MALME-3M
LOX IMVI
U251
SNB-75
SNB-19
SF-539
SF-295
SF-268
SW-620
KM12
HT29
HCT-15
HCT-116
HCC-2998
COLO 205
NCI-H522
NCI-H460
NCI-H322M
NCI-H23
NCI-H226
HOP-92
HOP-62
EKVX
A549/ATCC
SR
RPMI-8226
MOLT-4
K-562
HL-60(TB)
CCRF-CEM
MEDIAN
MEAN
05
20
40
60
80
% decrease in cancer growth {6b – 6a}
Null hypothesis: equal (0.5) probability that x-axis value is positive (+ve) or negative (-ve): that 6a or 6b is the more powerful stereoisomer for any given cell line.
Binomial probability of observed number of +ve and -ve < 0.00000001.
P value (one tailed) < 0.000001, Significant.
Conclusion: 6b has greater anti-cancer activity than 6a at 10 µM.

FIGURE 8E

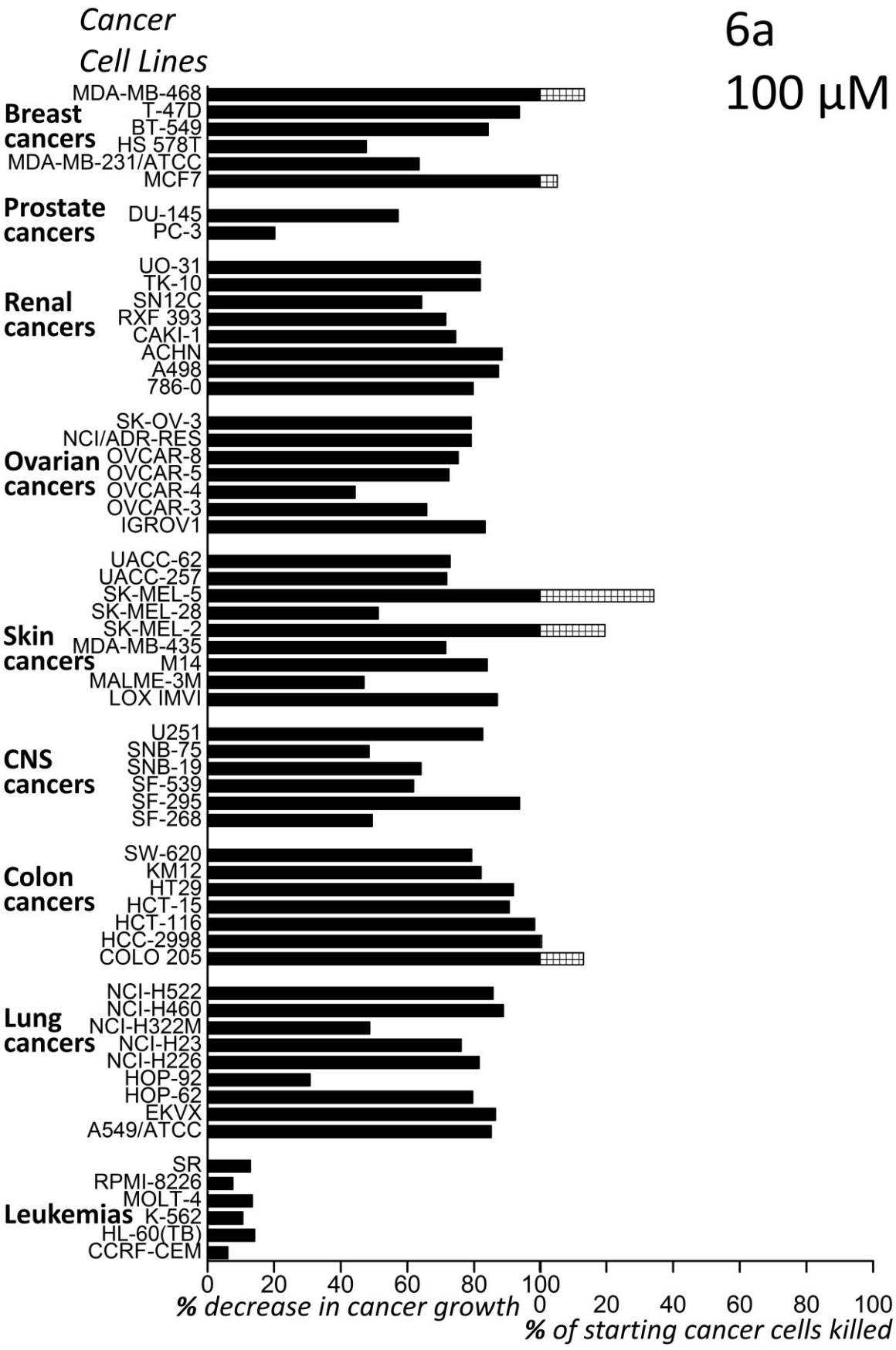

Cancer
Cell Lines
6a
100 µM
Breast cancers
MDA-MB-468
T-47D
BT-549
HS 578T
MDA-MB-231/ATCC
MCF7
Prostate cancers
DU-145
PC-3
Renal cancers
UO-31
TK-10
SN12C
RXF 393
CAKI-1
ACHN
A498
786-0
Ovarian cancers
SK-OV-3
NCI/ADR-RES
OVCAR-8
OVCAR-5
OVCAR-4
OVCAR-3
IGROV1
Skin cancers
UACC-62
UACC-257
SK-MEL-5
SK-MEL-28
SK-MEL-2
MDA-MB-435
M14
MALME-3M
LOX IMVI
CNS cancers
U251
SNB-75
SNB-19
SF-539
SF-295
SF-268
Colon cancers
SW-620
KM12
HT29
HCT-15
HCT-116
HCC-2998
COLO 205
Lung cancers
NCI-H522
NCI-H460
NCI-H322M
NCI-H23
NCI-H226
HOP-92
HOP-62
EKVX
A549/ATCC
Leukemias
SR
RPMI-8226
MOLT-4
K-562
HL-60(TB)
CCRF-CEM
0
20
40
60
80
100
% decrease in cancer growth
% of starting cancer cells killed

FIGURE 8F

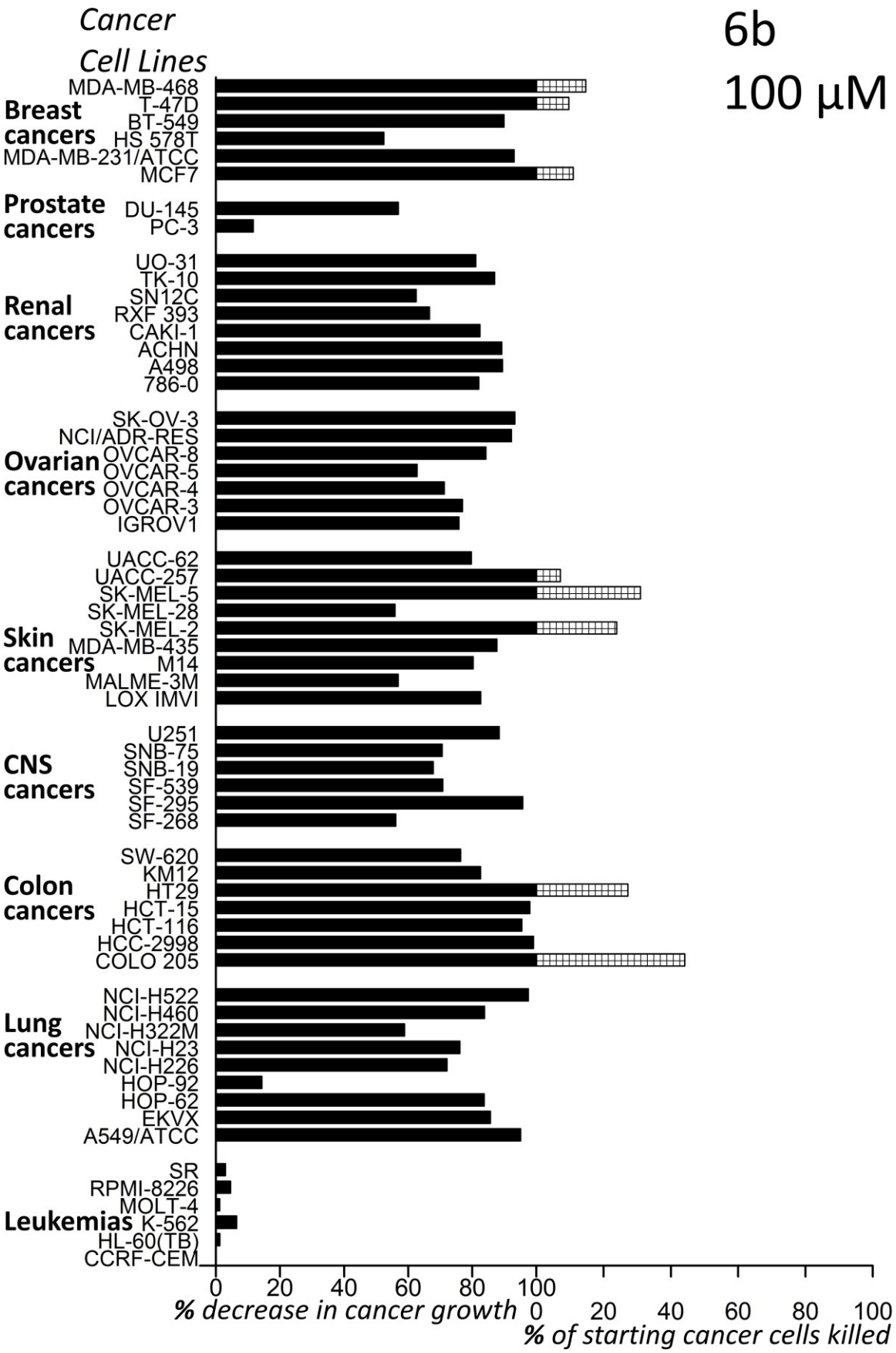

Cancer Cell Lines
6b
100 µM
Breast cancers
MDA-MB-468
T-47D
BT-549
HS 578T
MDA-MB-231/ATCC
MCF7
Prostate cancers
DU-145
PC-3
Renal cancers
UO-31
TK-10
SN12C
RXF 393
CAKI-1
ACHN
A498
786-0
Ovarian cancers
SK-OV-3
NCI/ADR-RES
OVCAR-8
OVCAR-5
OVCAR-4
OVCAR-3
IGROV1
Skin cancers
UACC-62
UACC-257
SK-MEL-5
SK-MEL-28
SK-MEL-2
MDA-MB-435
M14
MALME-3M
LOX IMVI
CNS cancers
U251
SNB-75
SNB-19
SF-539
SF-295
SF-268
Colon cancers
SW-620
KM12
HT29
HCT-15
HCT-116
HCC-2998
COLO 205
Lung cancers
NCI-H522
NCI-H460
NCI-H322M
NCI-H23
NCI-H226
HOP-92
HOP-62
EKVX
A549/ATCC
Leukemias
SR
RPMI-8226
MOLT-4
K-562
HL-60(TB)
CCRF-CEM
0 20 40 60 80 100
% decrease in cancer growth
0 20 40 60 80 100
% of starting cancer cells killed

FIGURE 9

Anti-cancer activity scales with EC_{50} F_1F_0 ATP hydrolase

BTB06584
Inhibits F_1F_0 ATP hydrolase ≥ **100** µM
No inhibition of F_1F_0 ATP synthesis @ 100 µM

BMS-199264 HCl
EC_{50} F_1F_0 ATP hydrolase = **0.48** µM
EC_{50} F_1F_0 ATP synthesis = 18 µM

19a racemate
EC_{50} F_1F_0 ATP hydrolase = **0.033** µM
EC_{50} F_1F_0 ATP synthesis > 100 µM

***6b, S* stereoisomer**
EC_{50} F_1F_0 ATP hydrolase = **0.018** µM
EC_{50} F_1F_0 ATP synthesis > 100 µM

***6a*, *R* stereoisomer**
EC_{50} F_1F_0 ATP hydrolase **>100** µM
EC_{50} F_1F_0 ATP synthesis > 100 µM

31
EC_{50} F_1F_0 ATP hydrolase = **0.022** µM
EC_{50} F_1F_0 ATP synthesis > 30 µM
EC_{50} CYP2C9 inhibition = 2 µM

MEAN % CANCER GROWTH INHIBITION OF NCI-60 CANCER CELL LINES

	BTB06584	BMS-199264 HCl	6b	6a	31
10 µM	-3%[α]	23%	66%	57%	15%
100 µM	39%	148%[β]	75%	71%	Untested

α *Negative % = cancer growth promotion (rather than growth inhibition)*
β *% > 100 = less cancer cells at experiment end than start; if = 200% = all cancer cells dead*

Because of racemization during NCI testing: 6b≈6a≈19a
31 consumed by CYP2C9, so its anti-cancer activity is less than its EC_{50} F_1F_0 ATP hydrolase value would predict

FIGURE 10A

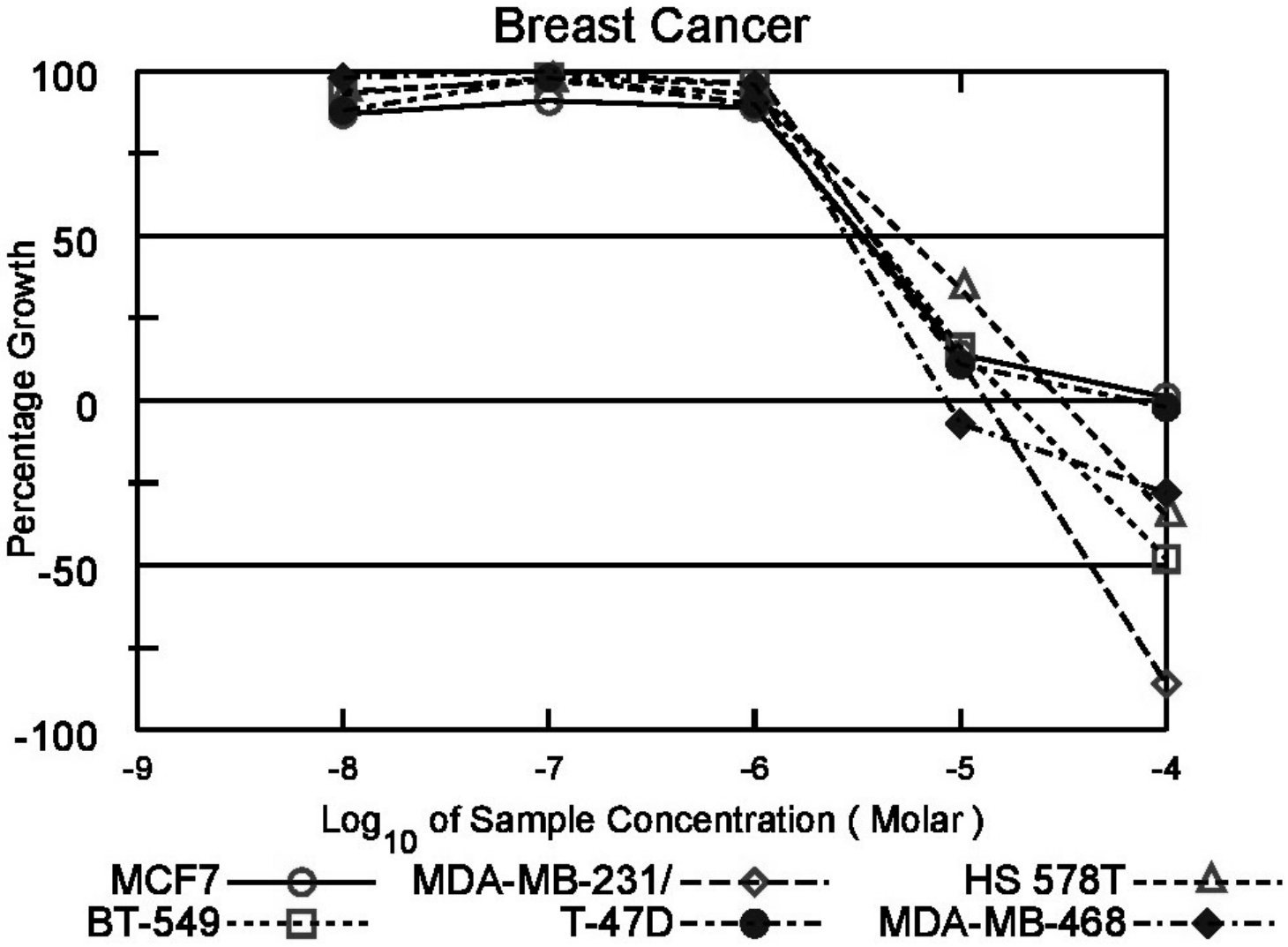

Breast Cancer
100
50
0
-50
-100
Percentage Growth
-9
-8
-7
-6
-5
-4
Log10 of Sample Concentration (Molar)
MCF7
MDA-MB-231/
HS 578T
BT-549
T-47D
MDA-MB-468

FIGURE 10B

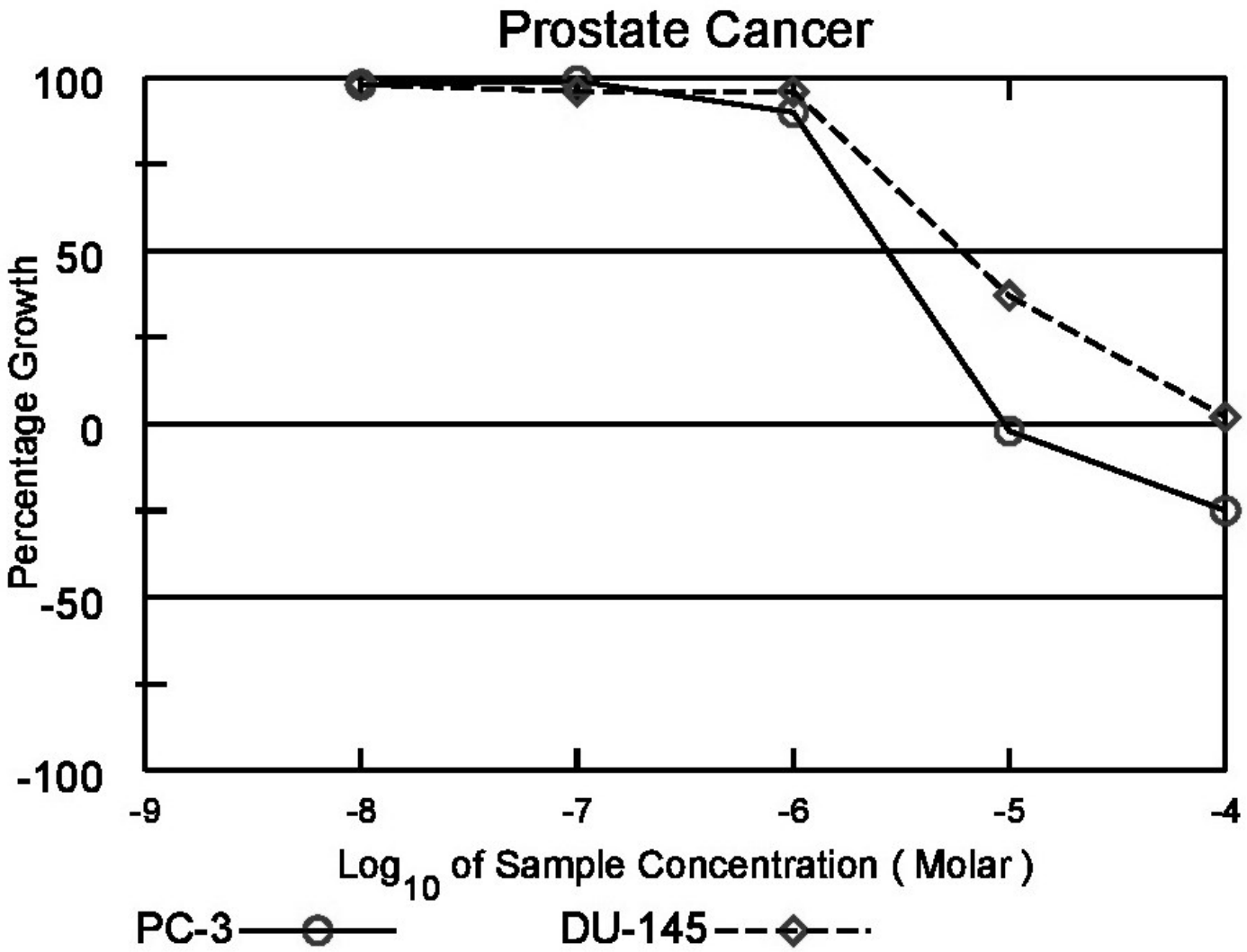

Prostate Cancer
100
50
0
-50
-100
Percentage Growth
-9
-8
-7
-6
-5
-4
Log10 of Sample Concentration (Molar)
PC-3
DU-145

FIGURE 10C

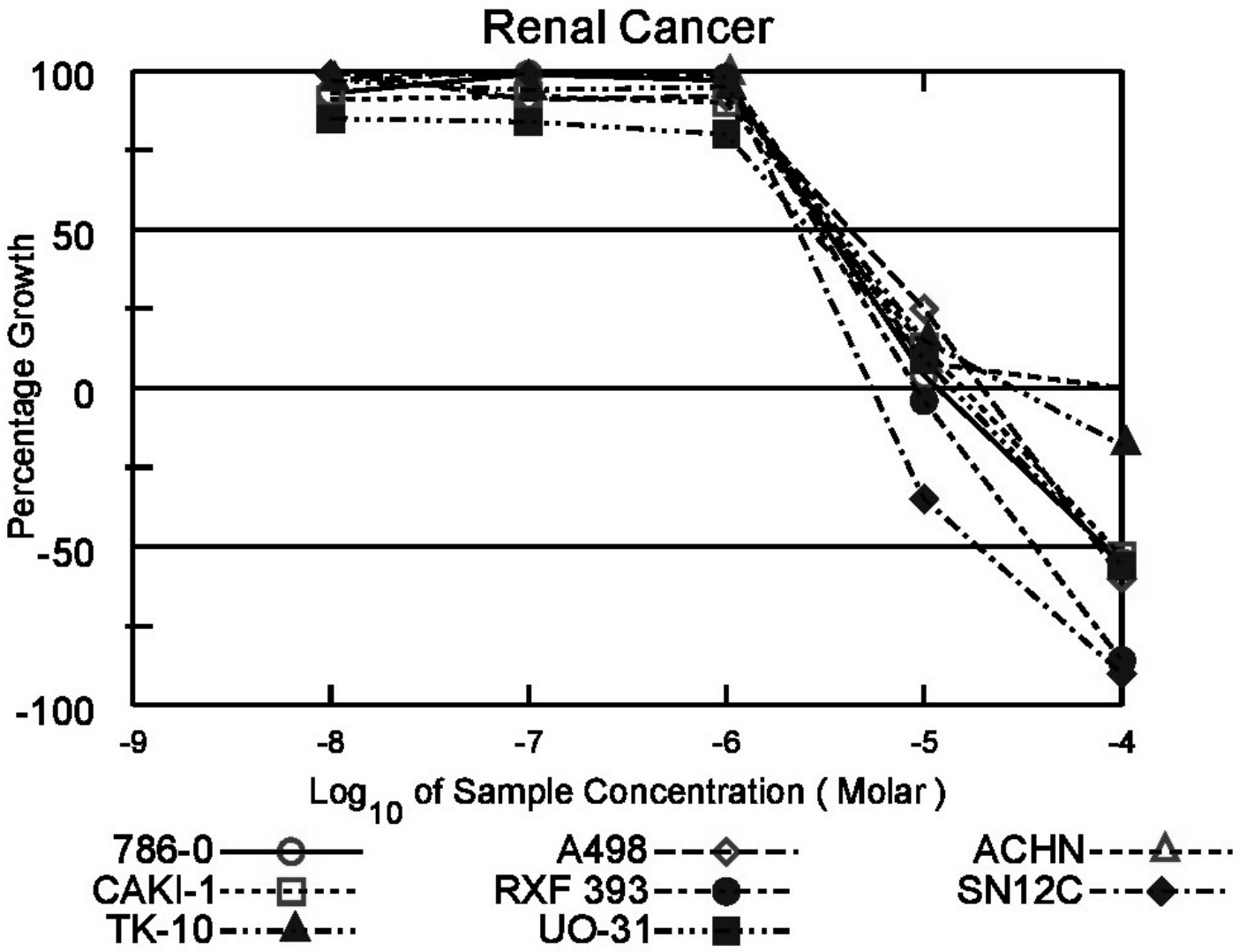

Renal Cancer
100
50
0
-50
-100
Percentage Growth
-9
-8
-7
-6
-5
-4
Log10 of Sample Concentration (Molar)
786-0
A498
ACHN
CAKI-1
RXF 393
SN12C
TK-10
UO-31

FIGURE 10D

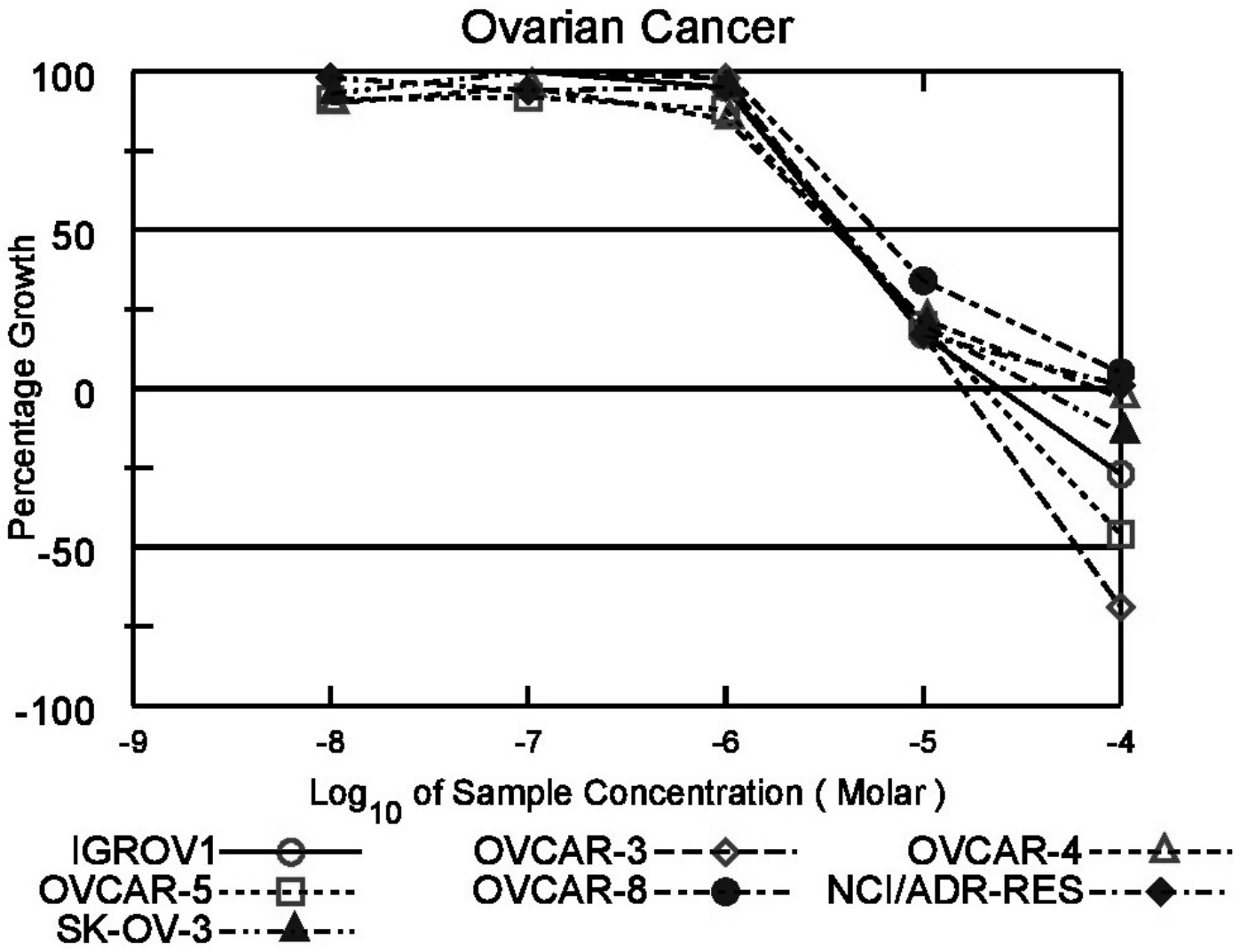

Ovarian Cancer
100
50
0
-50
-100
Percentage Growth
-9
-8
-7
-6
-5
-4
Log10 of Sample Concentration (Molar)
IGROV1
OVCAR-3
OVCAR-4
OVCAR-5
OVCAR-8
NCI/ADR-RES
SK-OV-3

FIGURE 10E

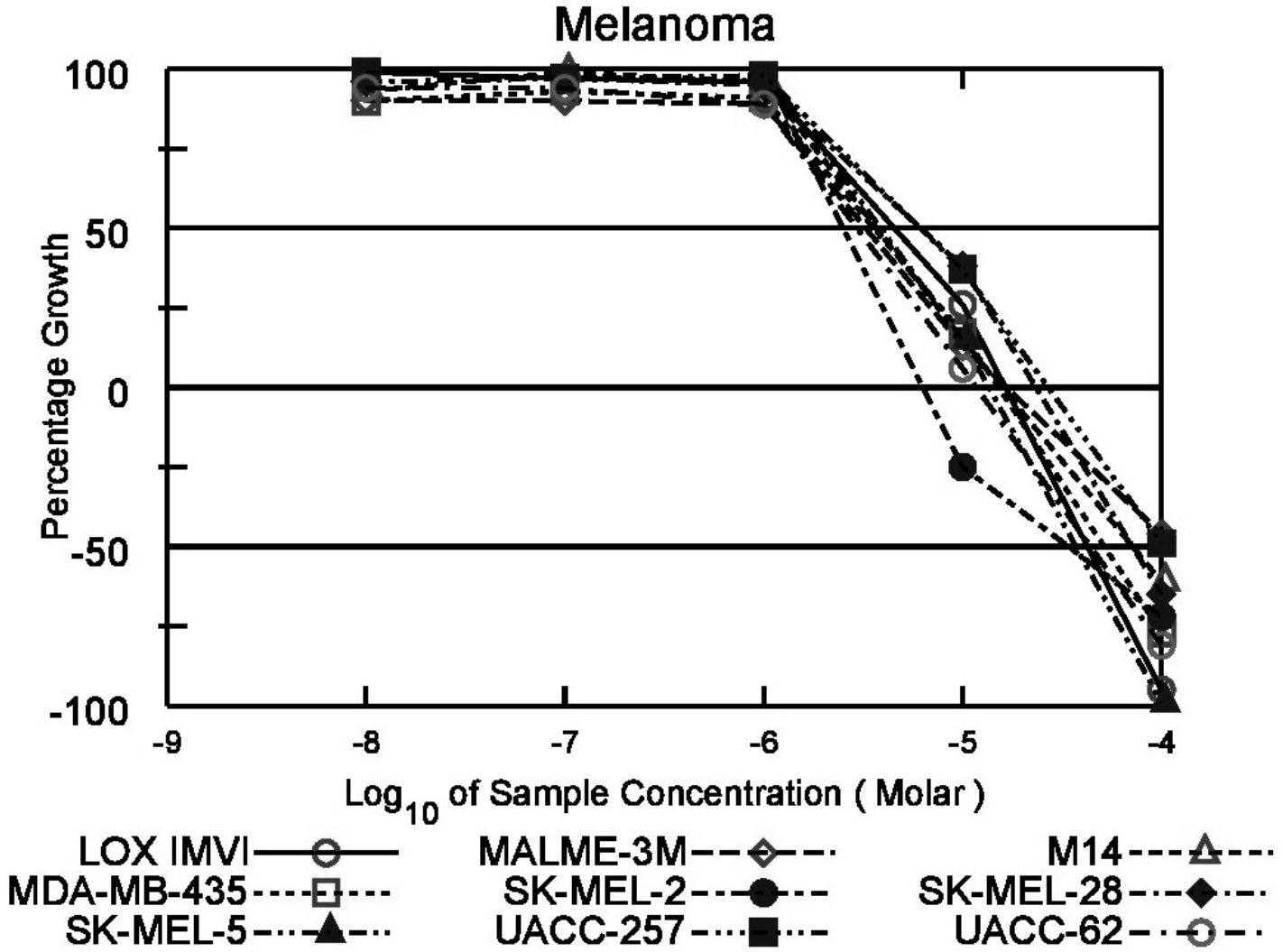

Melanoma
100
50
0
-50
-100
Percentage Growth
-9
-8
-7
-6
-5
-4
Log10 of Sample Concentration (Molar)
LOX IMVI
MALME-3M
M14
MDA-MB-435
SK-MEL-2
SK-MEL-28
SK-MEL-5
UACC-257
UACC-62

FIGURE 10F

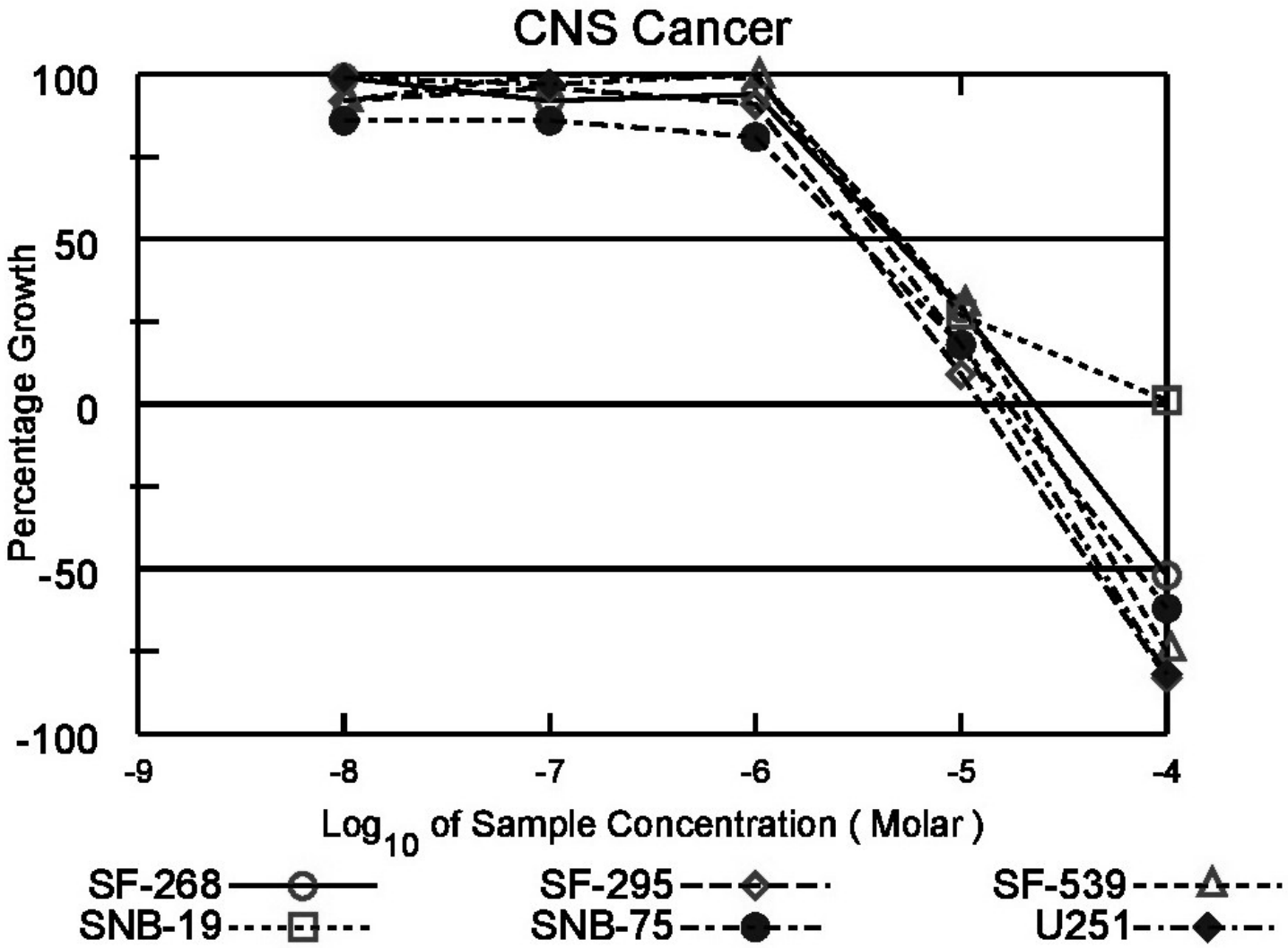

CNS Cancer
100
50
0
-50
-100
Percentage Growth
-9
-8
-7
-6
-5
-4
Log10 of Sample Concentration (Molar)
SF-268
SF-295
SF-539
SNB-19
SNB-75
U251

FIGURE 10G

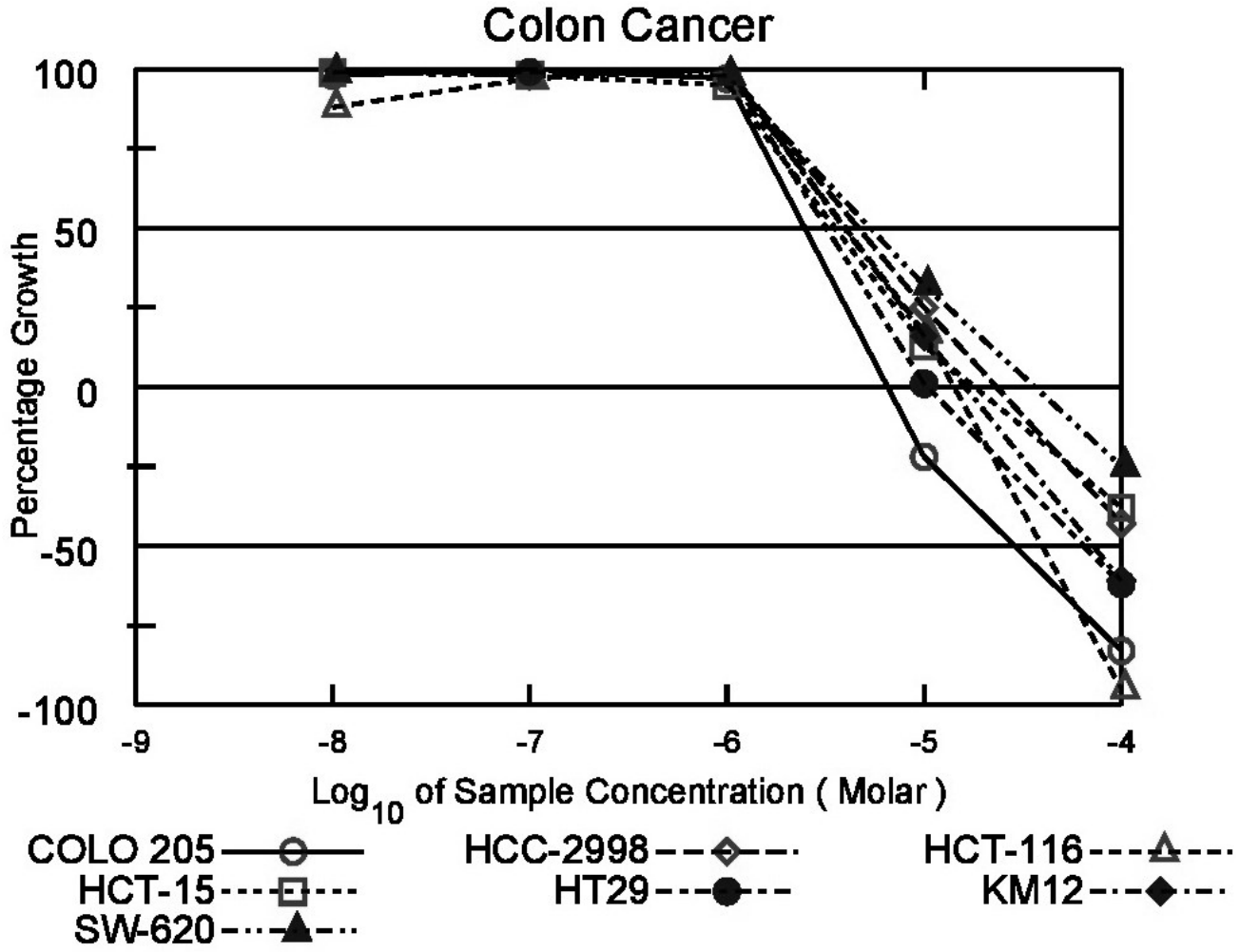

Colon Cancer
100
50
0
-50
-100
Percentage Growth
-9
-8
-7
-6
-5
-4
Log10 of Sample Concentration (Molar)
COLO 205
HCC-2998
HCT-116
HCT-15
HT29
KM12
SW-620

FIGURE 10H

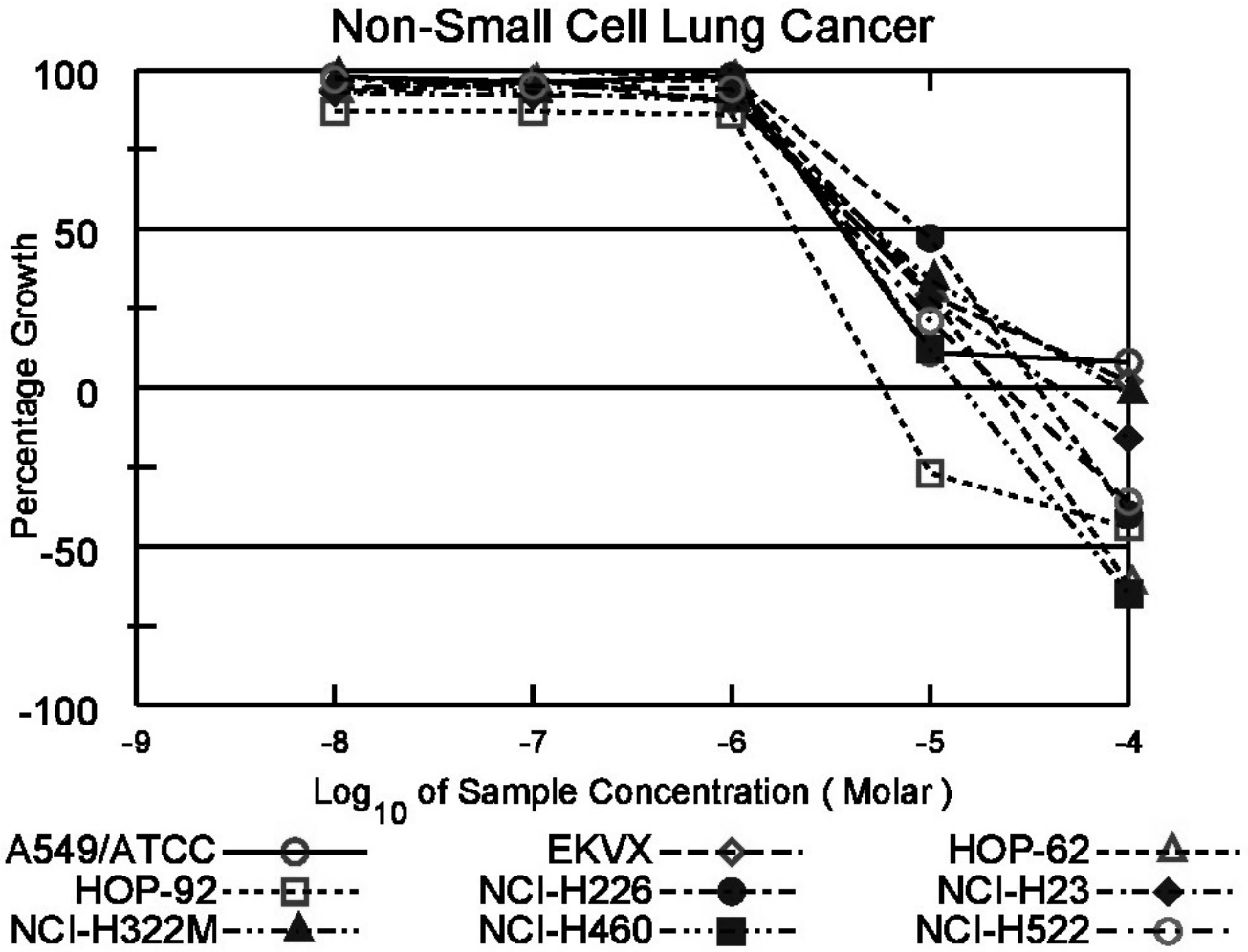

Non-Small Cell Lung Cancer
100
50
0
-50
-100
Percentage Growth
-9
-8
-7
-6
-5
-4
Log10 of Sample Concentration (Molar)
A549/ATCC
EKVX
HOP-62
HOP-92
NCI-H226
NCI-H23
NCI-H322M
NCI-H460
NCI-H522

FIGURE 10I

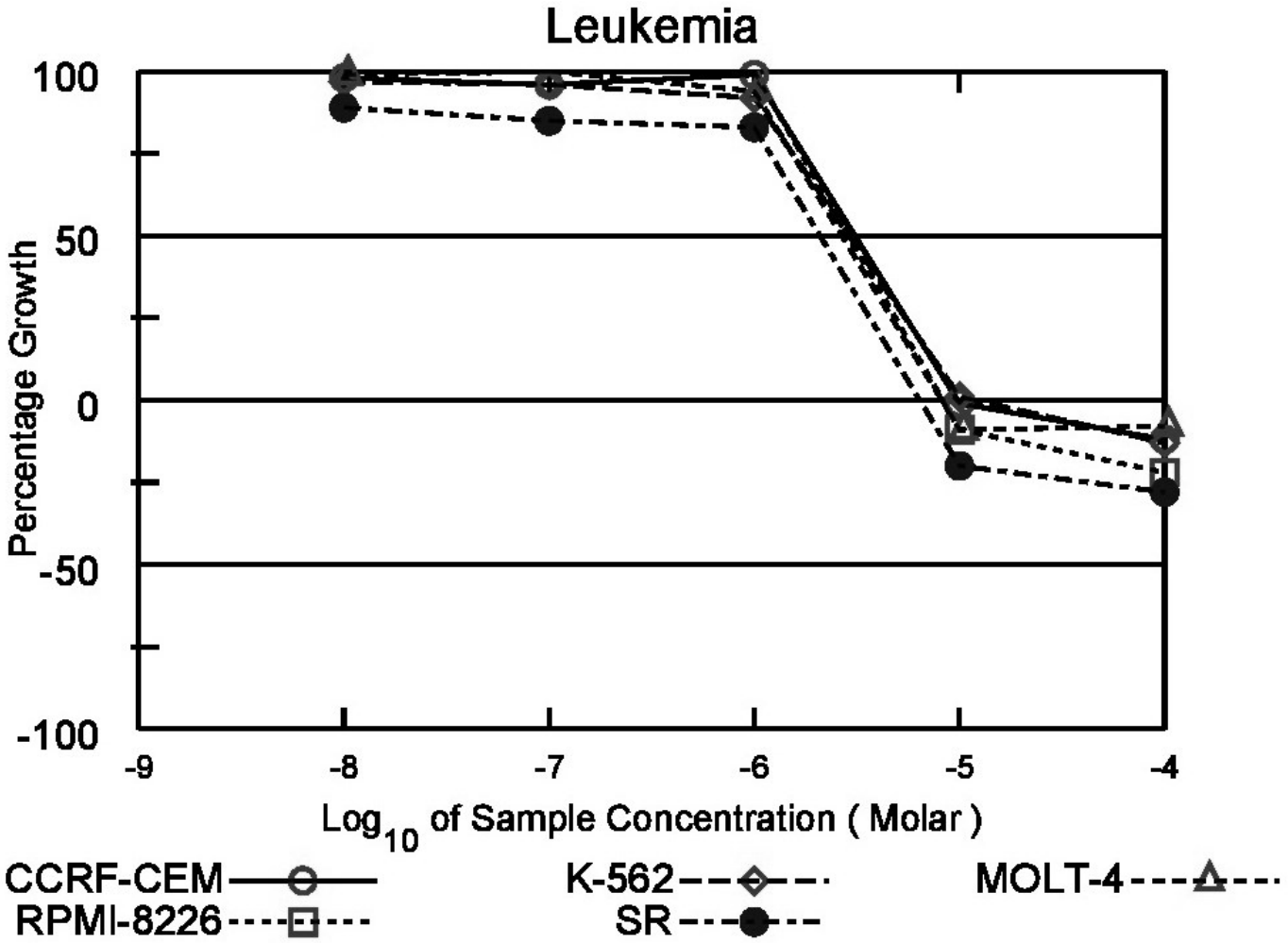

Leukemia
100
50
0
-50
-100
Percentage Growth
-9
-8
-7
-6
-5
-4
Log10 of Sample Concentration (Molar)
CCRF-CEM
K-562
MOLT-4
RPMI-8226
SR

FIGURE 11A

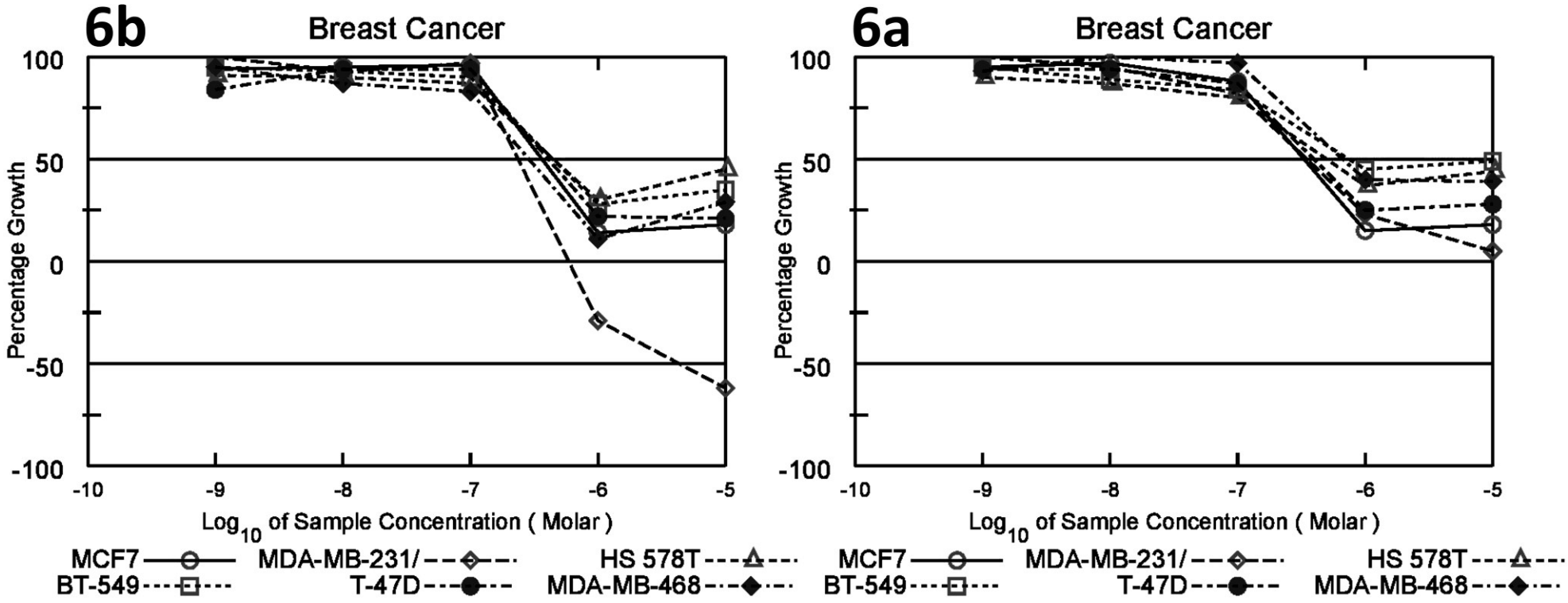

6b
Breast Cancer
100
50
0
-50
-100
Percentage Growth
-10
-9
-8
-7
-6
-5
Log10 of Sample Concentration (Molar)
MCF7
MDA-MB-231/
HS 578T
BT-549
T-47D
MDA-MB-468
6a
Breast Cancer
100
50
0
-50
-100
Percentage Growth
-10
-9
-8
-7
-6
-5
Log10 of Sample Concentration (Molar)
MCF7
MDA-MB-231/
HS 578T
BT-549
T-47D
MDA-MB-468

FIGURE 11B

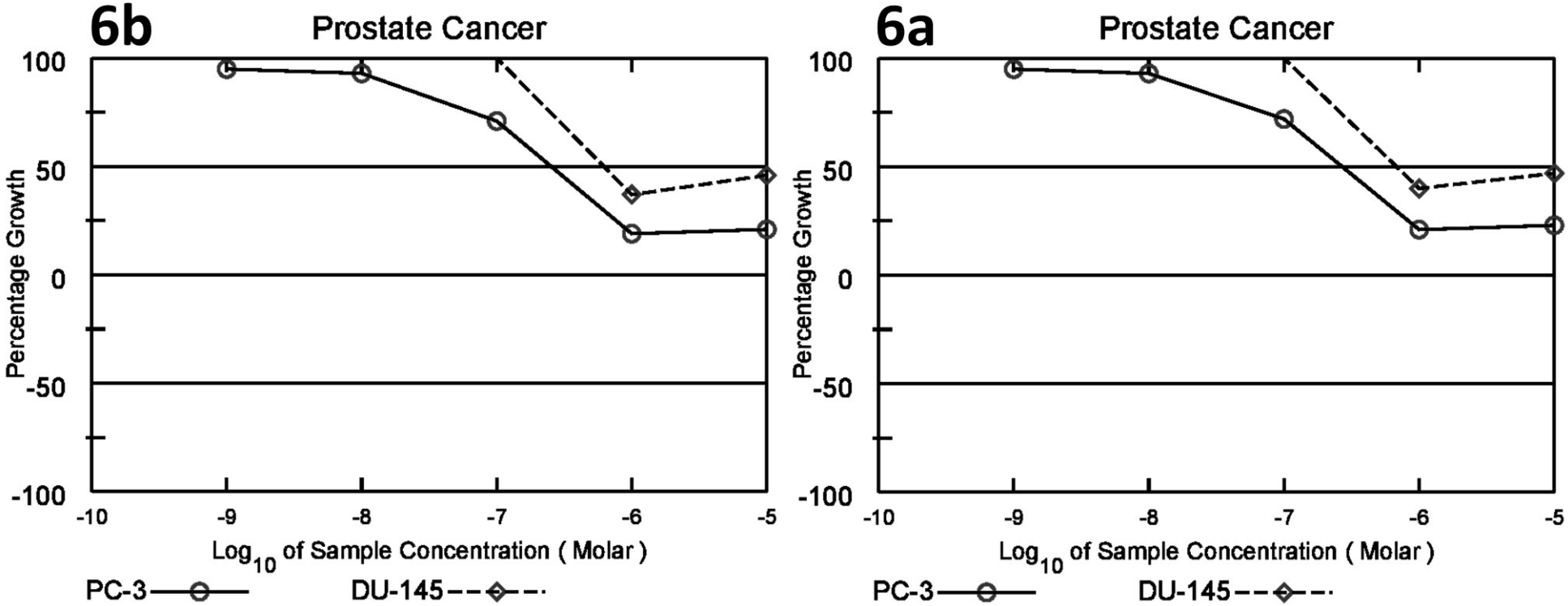

6b
Prostate Cancer
100
50
0
-50
-100
Percentage Growth
-10
-9
-8
-7
-6
-5
Log10 of Sample Concentration (Molar)
PC-3
DU-145
6a
Prostate Cancer
100
50
0
-50
-100
Percentage Growth
-10
-9
-8
-7
-6
-5
Log10 of Sample Concentration (Molar)
PC-3
DU-145

FIGURE 11C

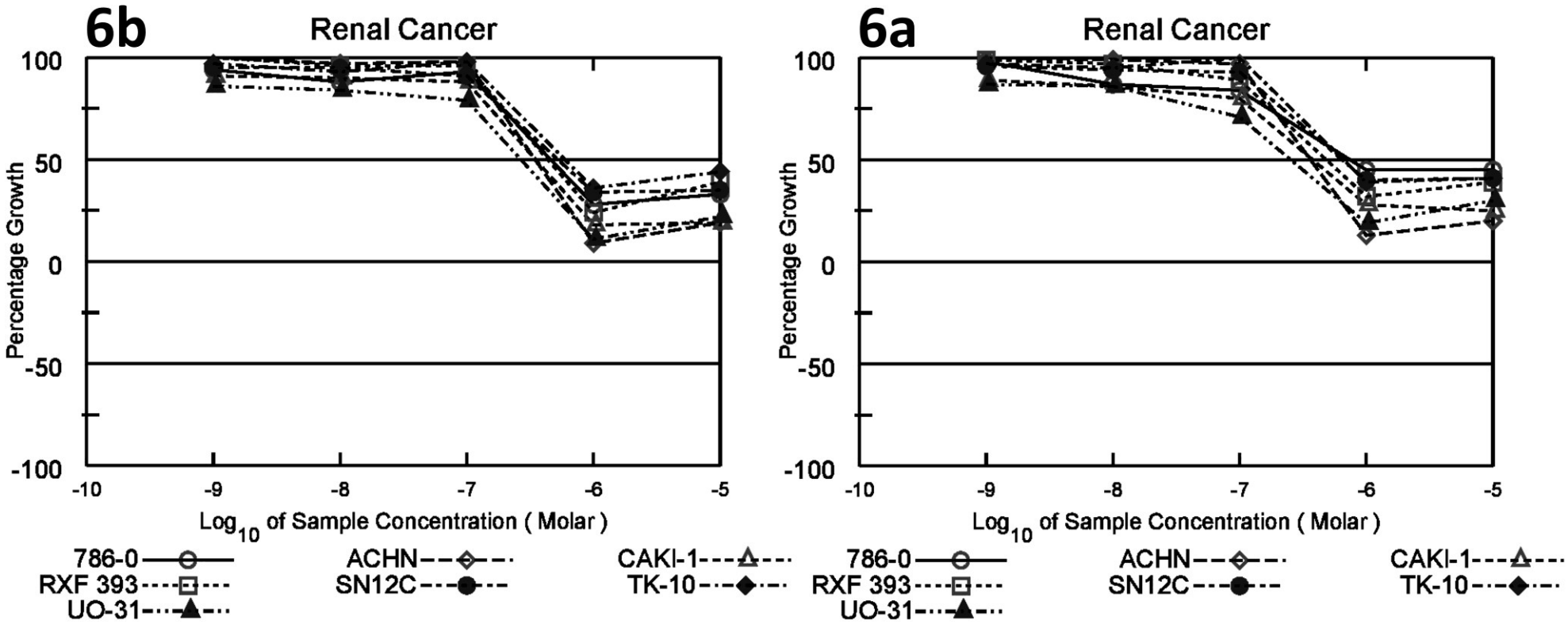

6b
Renal Cancer
100
50
0
-50
-100
Percentage Growth
-10
-9
-8
-7
-6
-5
Log10 of Sample Concentration (Molar)
786-0
ACHN
CAKI-1
RXF 393
SN12C
TK-10
UO-31
6a
Renal Cancer
100
50
0
-50
-100
Percentage Growth
-10
-9
-8
-7
-6
-5
Log10 of Sample Concentration (Molar)
786-0
ACHN
CAKI-1
RXF 393
SN12C
TK-10
UO-31

FIGURE 11D

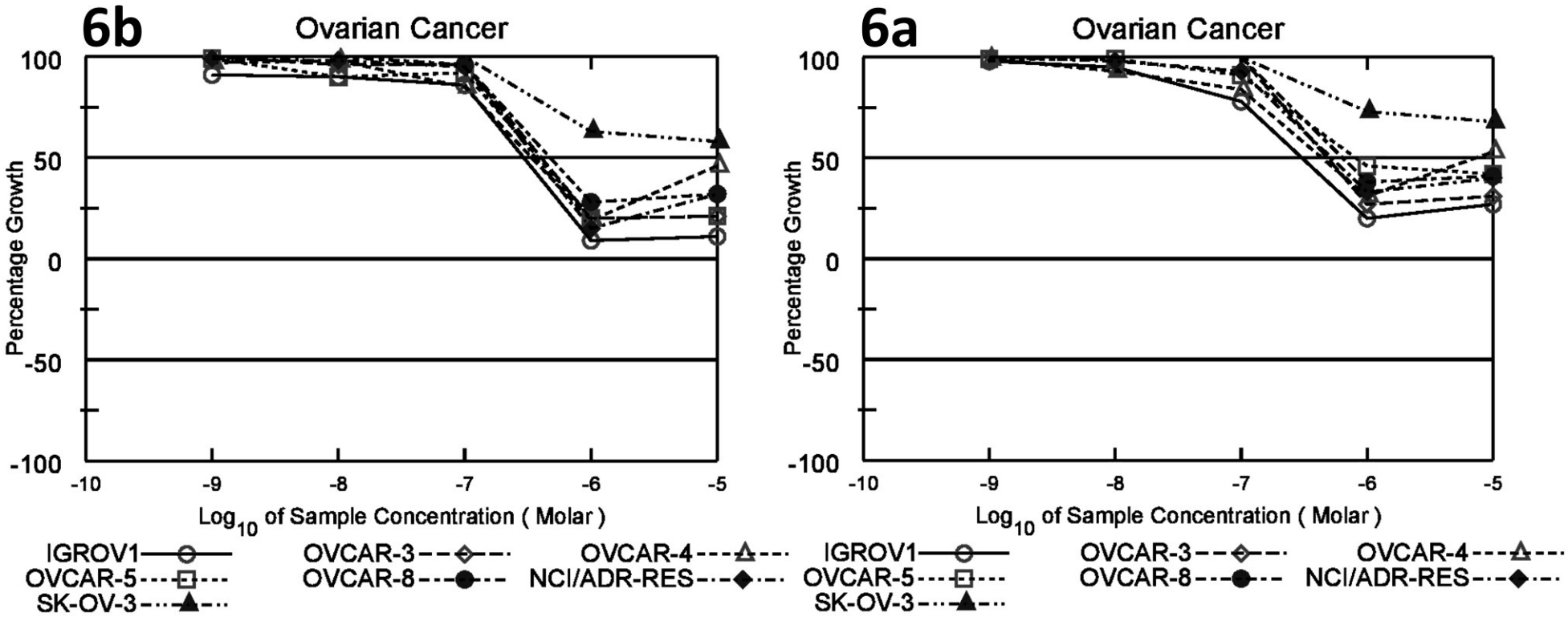

6b
Ovarian Cancer
100
50
0
-50
-100
Percentage Growth
-10
-9
-8
-7
-6
-5
Log10 of Sample Concentration (Molar)
IGROV1
OVCAR-3
OVCAR-4
OVCAR-5
OVCAR-8
NCI/ADR-RES
SK-OV-3
6a
Ovarian Cancer
100
50
0
-50
-100
Percentage Growth
-10
-9
-8
-7
-6
-5
Log10 of Sample Concentration (Molar)
IGROV1
OVCAR-3
OVCAR-4
OVCAR-5
OVCAR-8
NCI/ADR-RES
SK-OV-3

FIGURE 11E

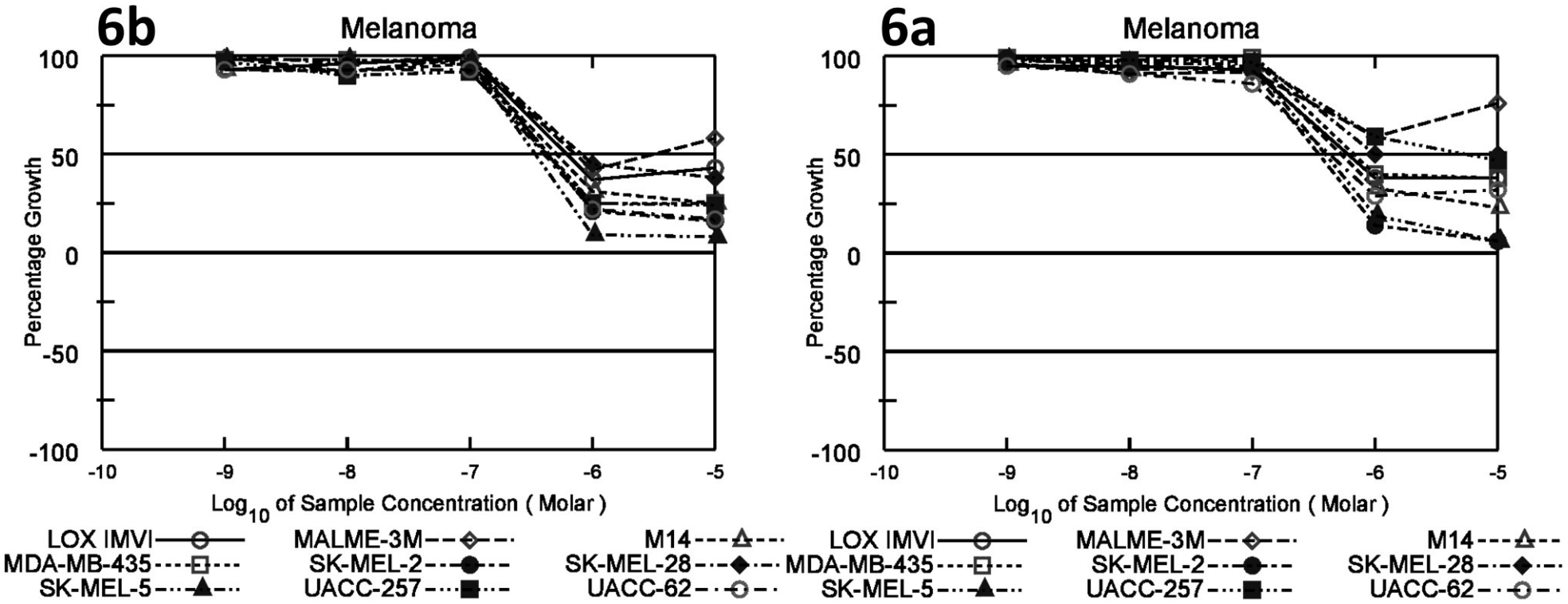

6b
Melanoma
100
50
0
-50
-100
Percentage Growth
-10
-9
-8
-7
-6
-5
Log10 of Sample Concentration (Molar)
LOX IMVI
MALME-3M
M14
MDA-MB-435
SK-MEL-2
SK-MEL-28
SK-MEL-5
UACC-257
UACC-62
6a
Melanoma
100
50
0
-50
-100
Percentage Growth
-10
-9
-8
-7
-6
-5
Log10 of Sample Concentration (Molar)
LOX IMVI
MALME-3M
M14
MDA-MB-435
SK-MEL-2
SK-MEL-28
SK-MEL-5
UACC-257
UACC-62

FIGURE 11F

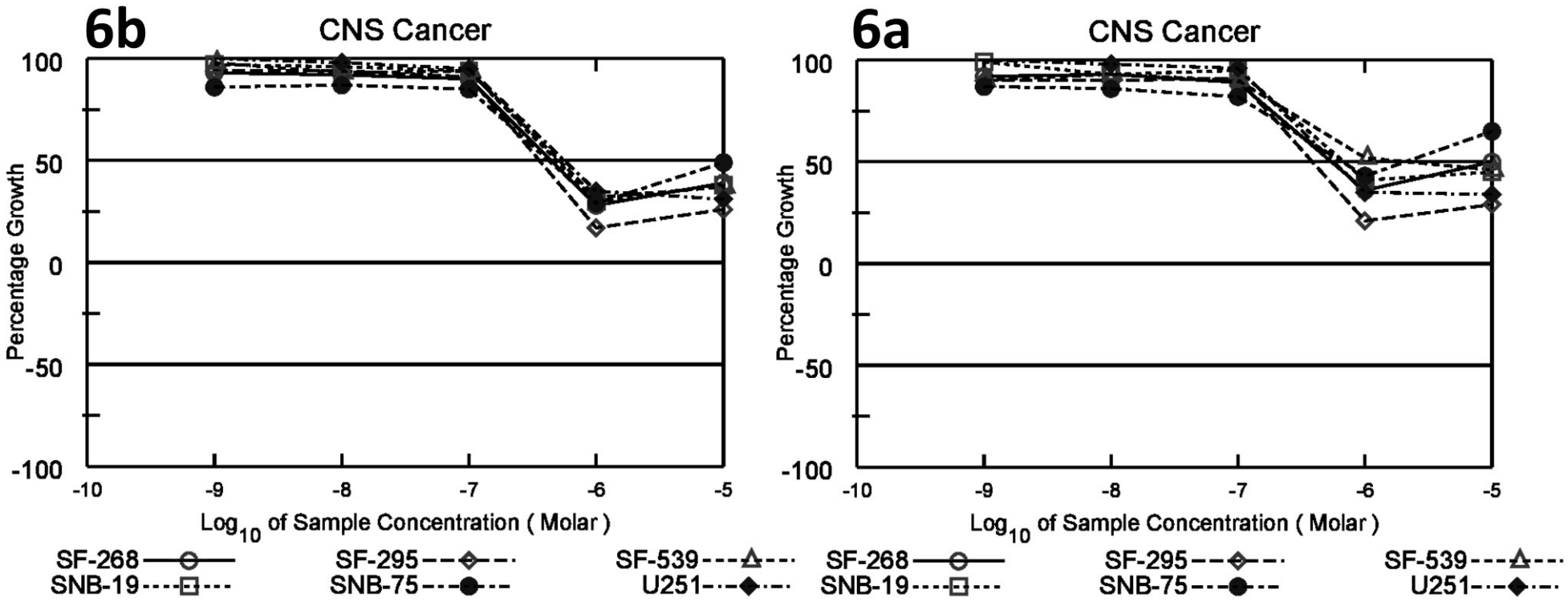

6b
CNS Cancer
100
50
0
-50
-100
Percentage Growth
-10
-9
-8
-7
-6
-5
Log10 of Sample Concentration (Molar)
SF-268
SF-295
SF-539
SNB-19
SNB-75
U251
6a
CNS Cancer
100
50
0
-50
-100
Percentage Growth
-10
-9
-8
-7
-6
-5
Log10 of Sample Concentration (Molar)
SF-268
SF-295
SF-539
SNB-19
SNB-75
U251

FIGURE 11G

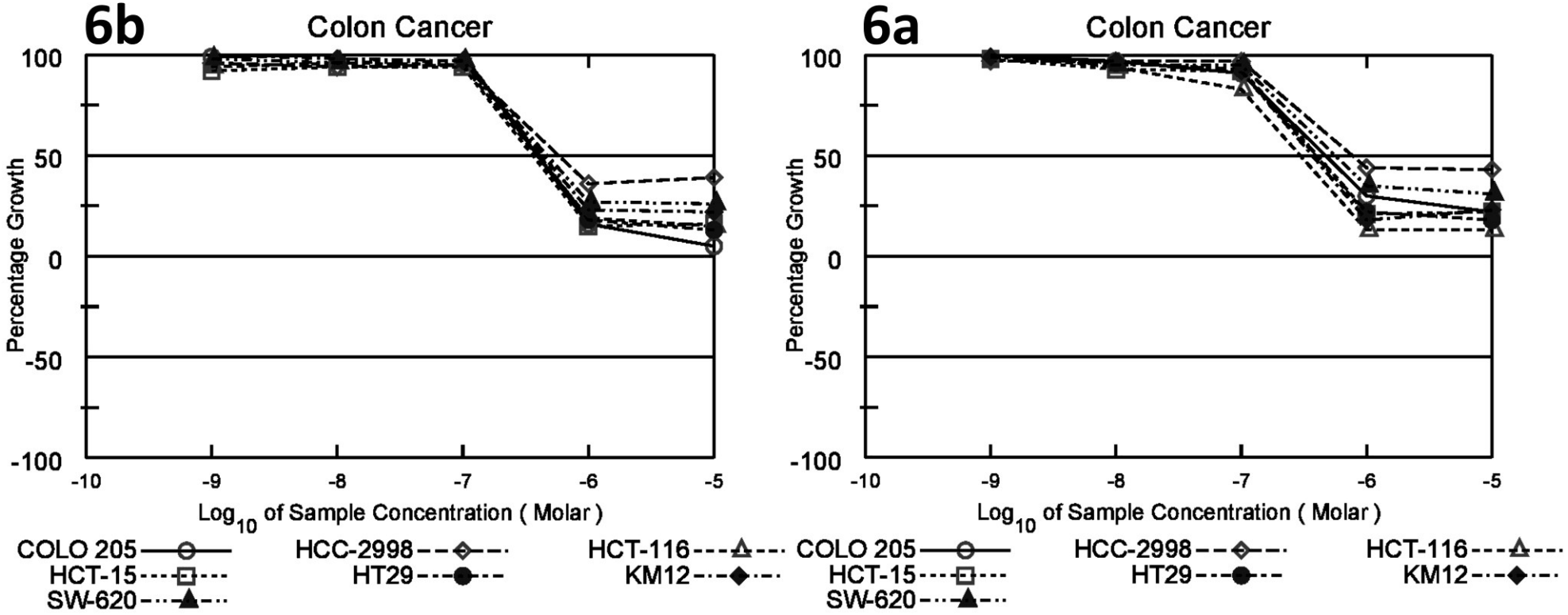

6b
Colon Cancer
100
50
0
-50
-100
Percentage Growth
-10
-9
-8
-7
-6
-5
Log10 of Sample Concentration (Molar)
COLO 205
HCC-2998
HCT-116
HCT-15
HT29
KM12
SW-620
6a
Colon Cancer
100
50
0
-50
-100
Percentage Growth
-10
-9
-8
-7
-6
-5
Log10 of Sample Concentration (Molar)
COLO 205
HCC-2998
HCT-116
HCT-15
HT29
KM12
SW-620

FIGURE 11H

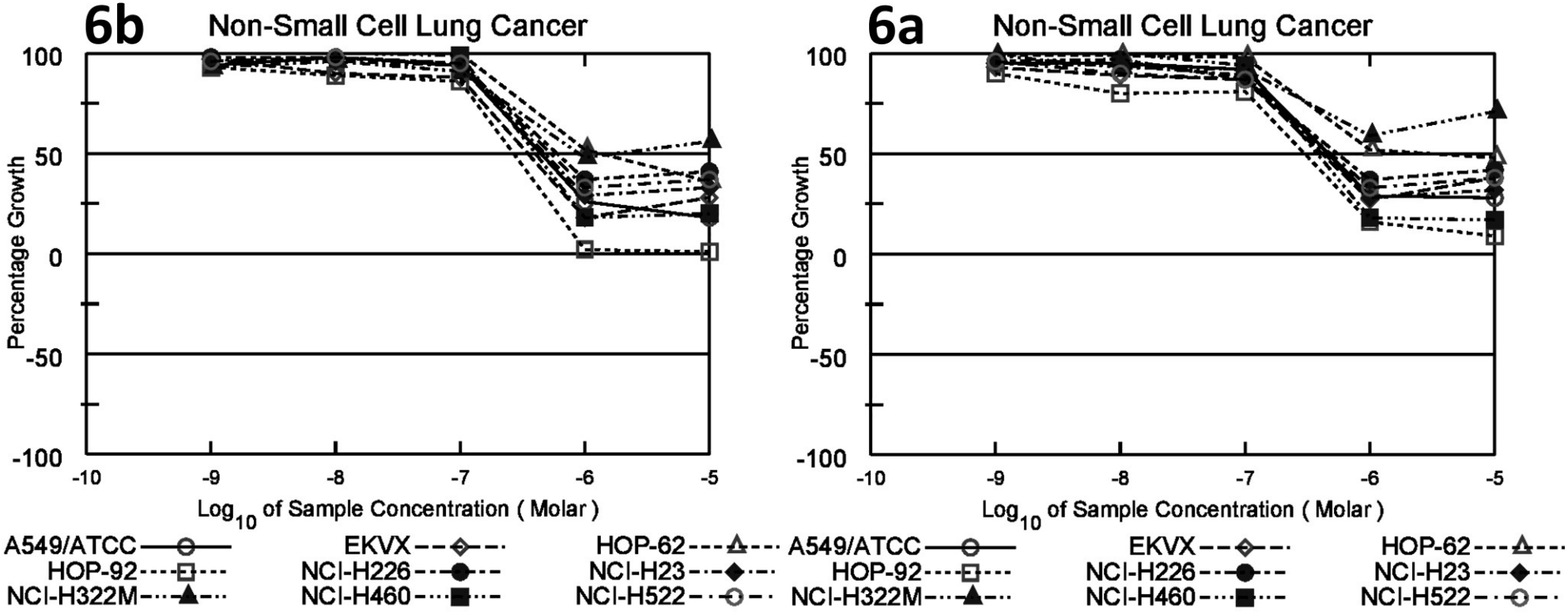

6b
Non-Small Cell Lung Cancer
Percentage Growth
100
50
0
-50
-100
-10
-9
-8
-7
-6
-5
Log10 of Sample Concentration (Molar)
A549/ATCC
EKVX
HOP-62
HOP-92
NCI-H226
NCI-H23
NCI-H322M
NCI-H460
NCI-H522
6a
Non-Small Cell Lung Cancer
Percentage Growth
100
50
0
-50
-100
-10
-9
-8
-7
-6
-5
Log10 of Sample Concentration (Molar)
A549/ATCC
EKVX
HOP-62
HOP-92
NCI-H226
NCI-H23
NCI-H322M
NCI-H460
NCI-H522

FIGURE 11I

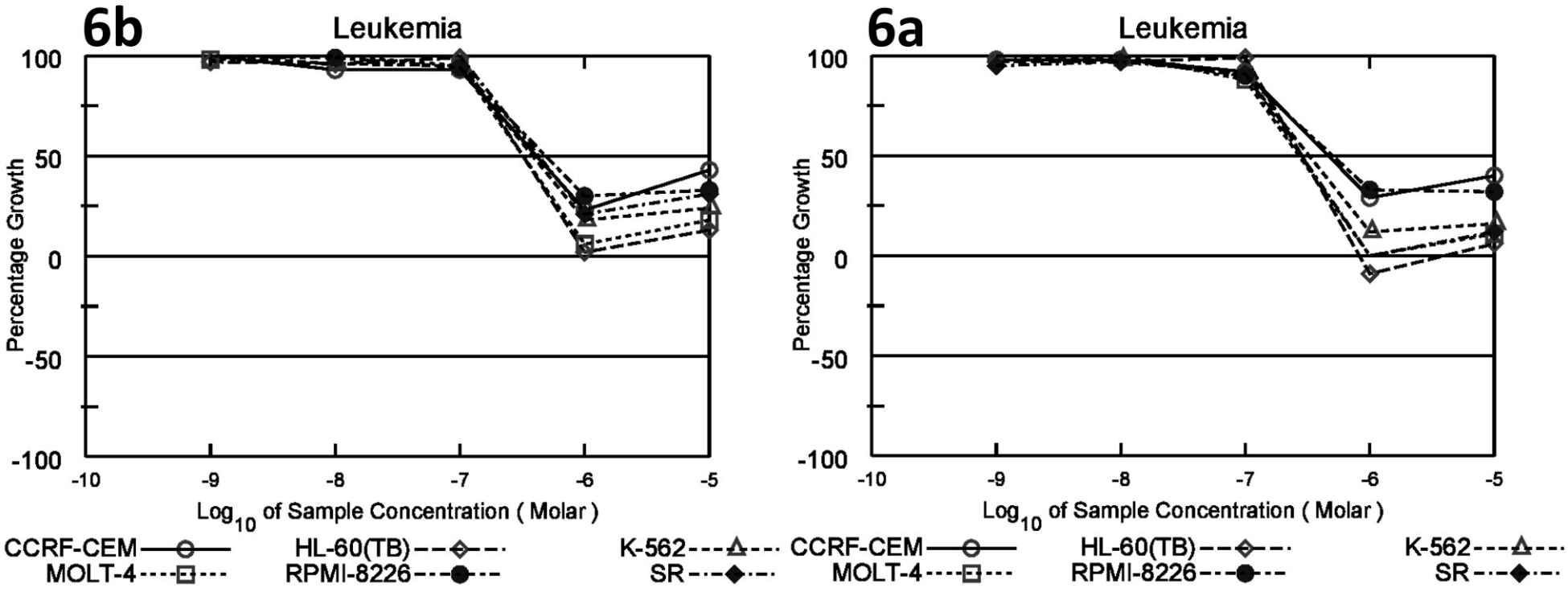
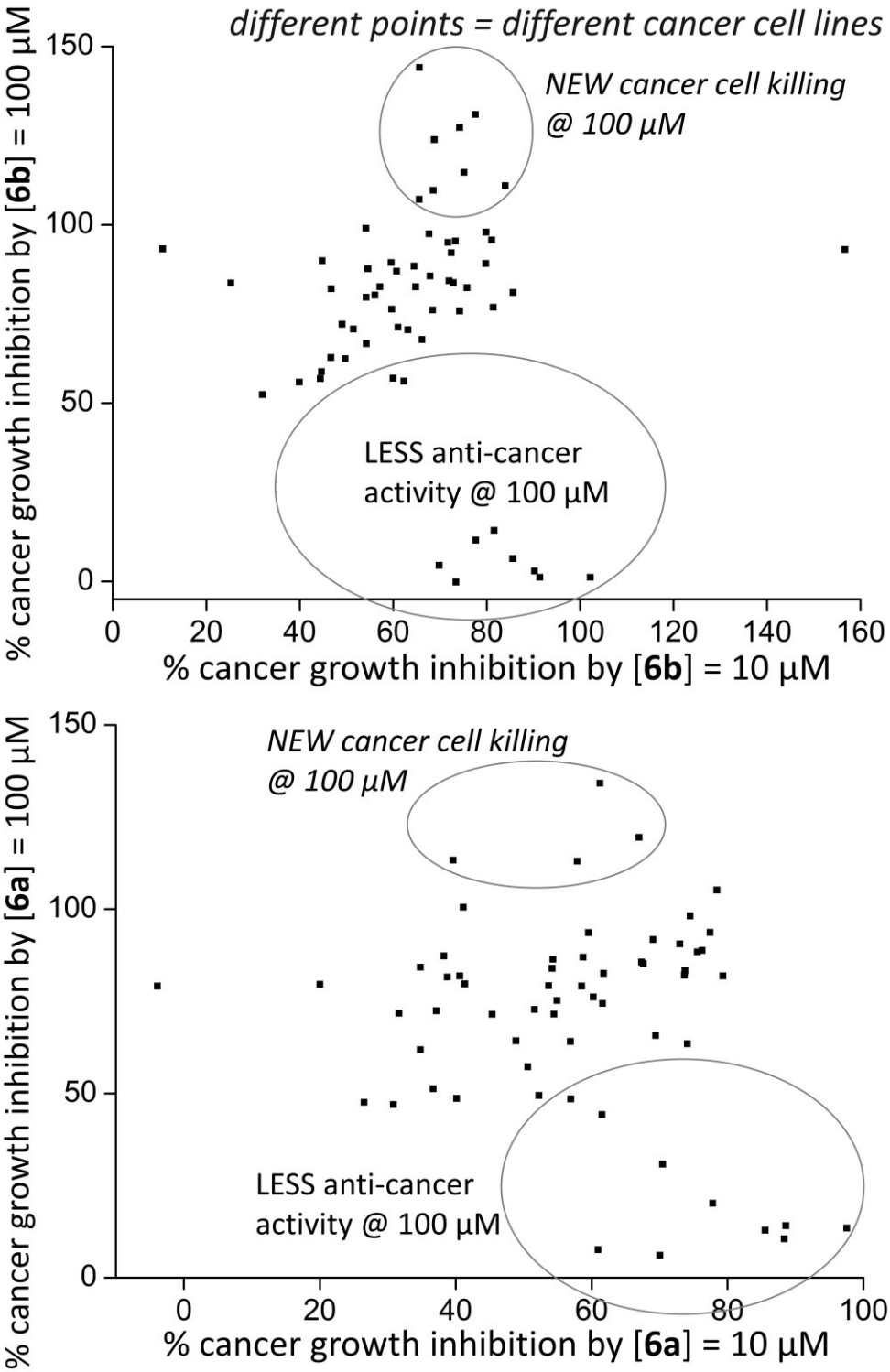

6b
Leukemia
100
50
0
-50
-100
Percentage Growth
-10
-9
-8
-7
-6
-5
Log10 of Sample Concentration (Molar)
CCRF-CEM
HL-60(TB)
K-562
MOLT-4
RPMI-8226
SR
6a
Leukemia
100
50
0
-50
-100
Percentage Growth
-10
-9
-8
-7
-6
-5
Log10 of Sample Concentration (Molar)
CCRF-CEM
HL-60(TB)
K-562
MOLT-4
RPMI-8226
SR

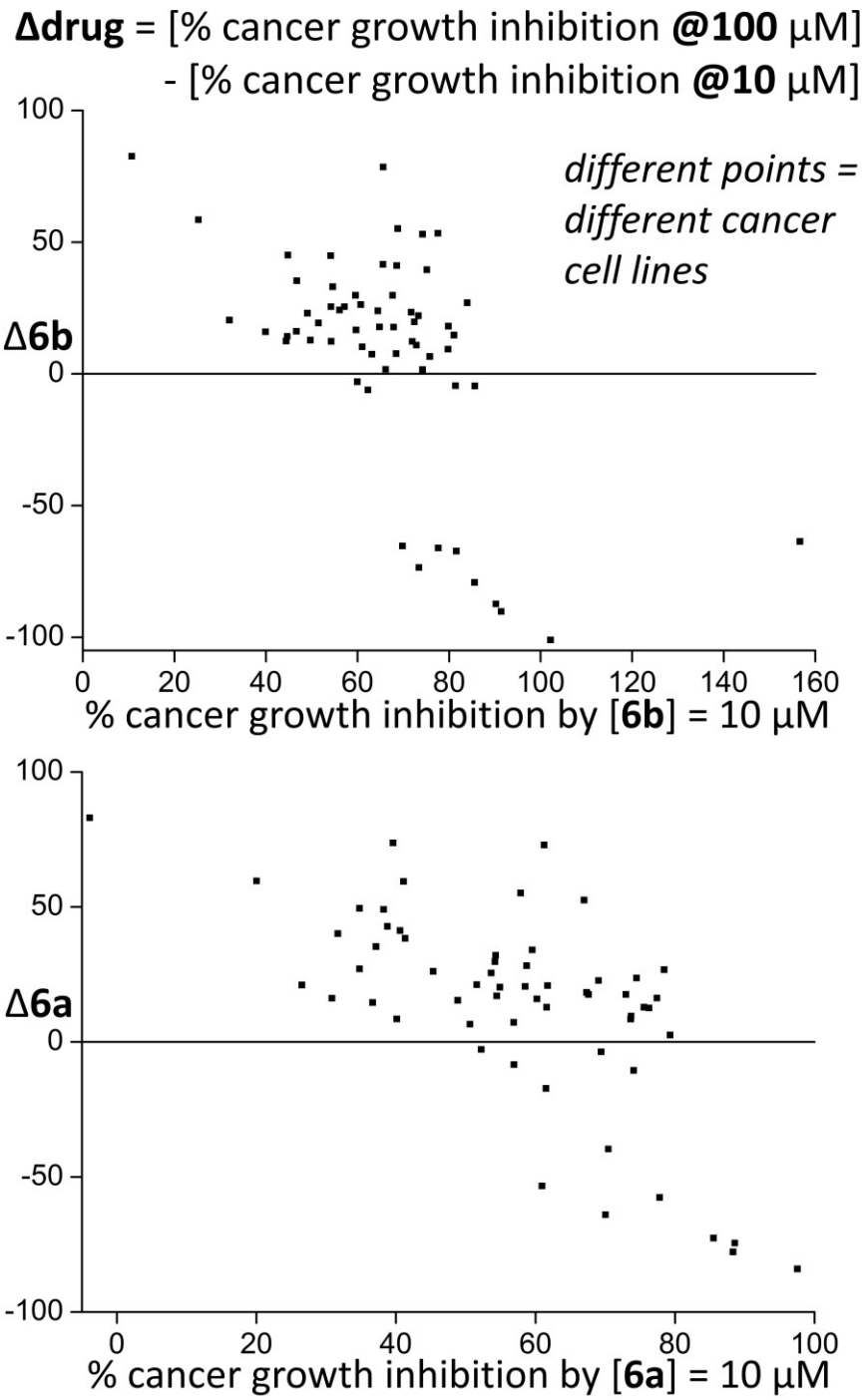
FIGURE 12A



{if % cancer growth inhibition > 100
= less cancer cells at experiment end than start;
if = 200% = all cancer cells dead}

% cancer growth inhibition 10 & 100 µM data pooled:
Pearson R (6a, 6b) = **0.9** {p < 0.00001, Significant}

FIGURE 12B

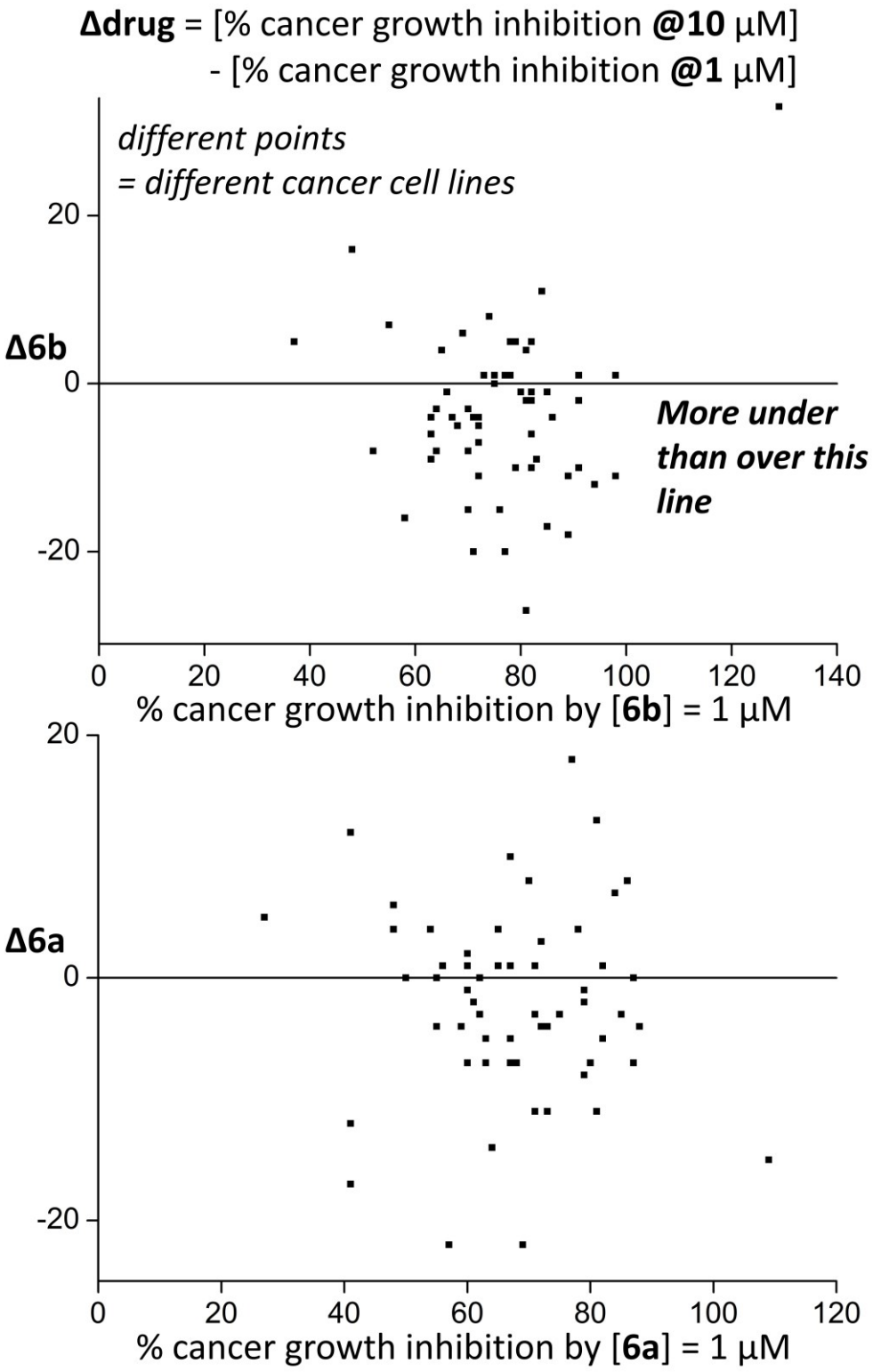


{if % cancer growth inhibition > 100 = less cancer cells at experiment end than start; if = 200% = all cancer cells dead}

CONCLUSION: 6a and 6b can exert LESS anti-cancer activity at 100 μM than 10 μM

Pearson R (Δ6a, Δ6b) = **0.94** {p < 0.00001, Significant}

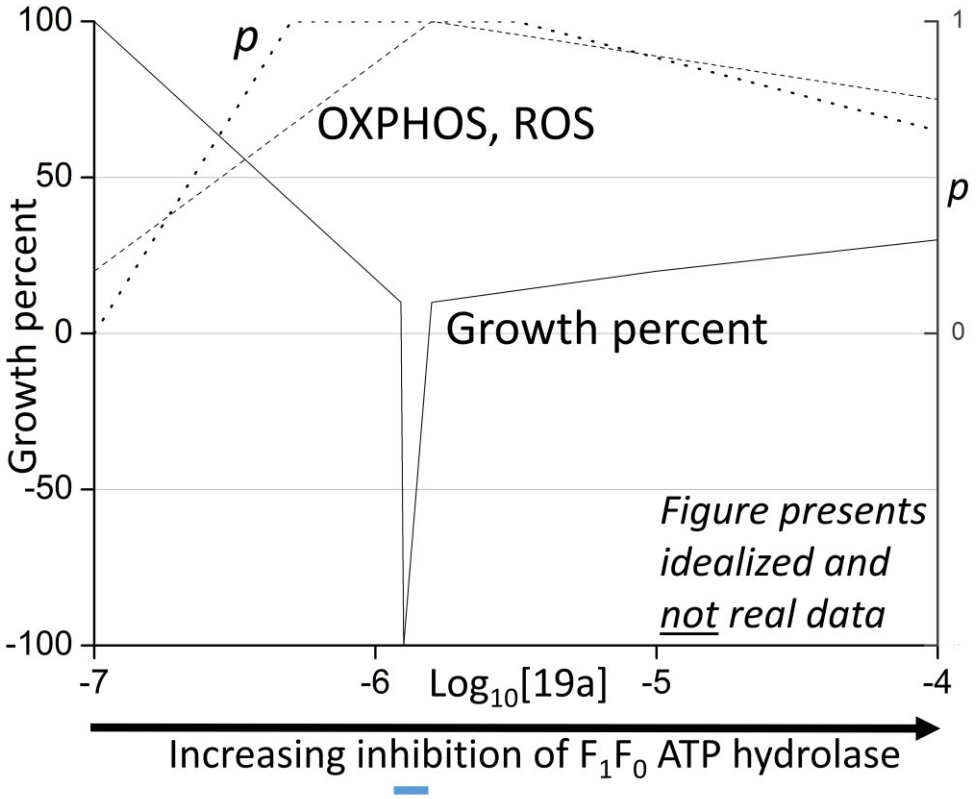
FIGURE 12C



CONCLUSION: 6a and 6b can exert LESS anti-cancer activity at 10 μM than 1 μM

Pearson R (Δ6a, Δ6b) = **0.78** {$p < 0.00001$, Significant}

FIGURE 13A



ROS driven apoptosis

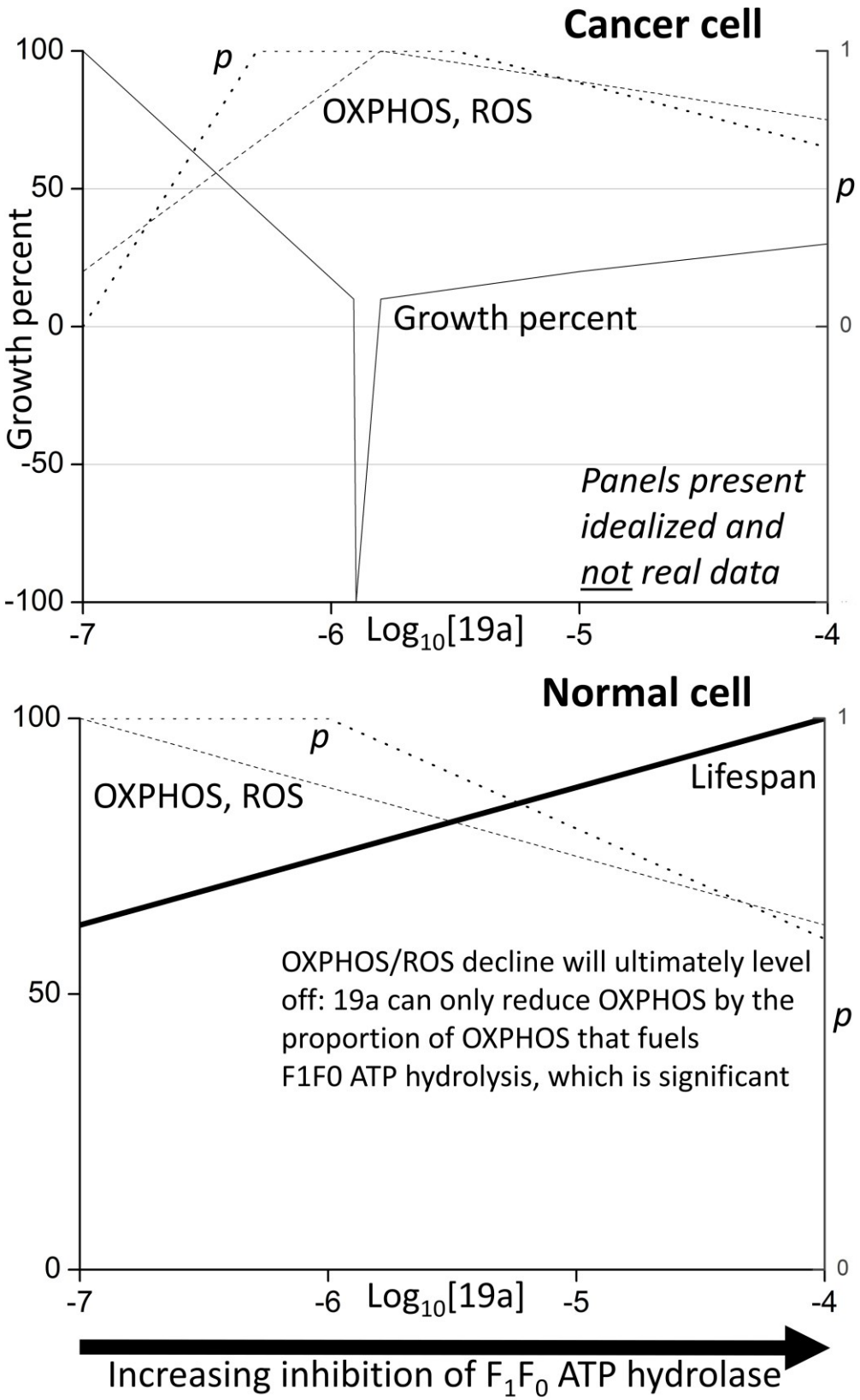
INCREASING	**DECREASING**
ROS driven cell cycle checkpoint blockade + ROS damages information fidelity and reduces number of possible cell divisions	ROS driven cell cycle checkpoint blockade + ROS damages information fidelity and reduces number of possible cell divisions

Number of cell divisions possible, N = Hayflick limit/p
Hayflick limit = 40 to 60, p is between 0 and 1,
when p=0, N=∞ ("limitless replicative potential", a Hallmark of cancer),
when p=1, N= Hayflick limit

Different cancer cell lines present the 3 phases with different concentration ranges

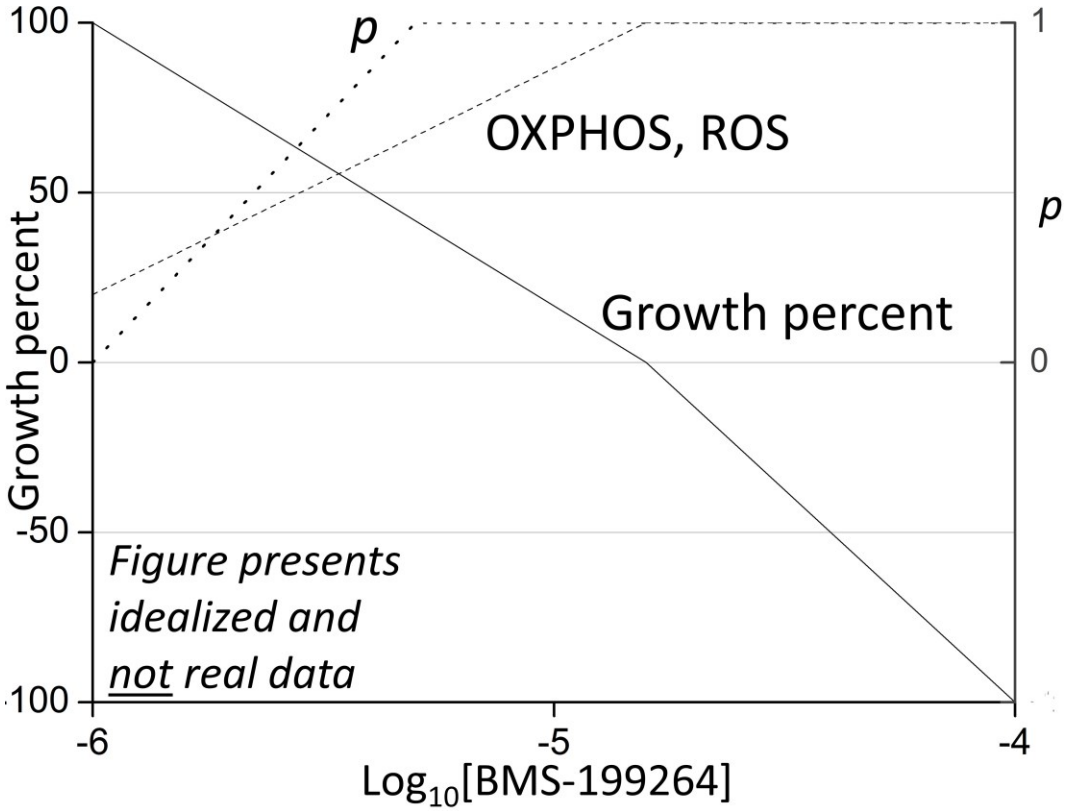
Apoptosis isn't often seen in data because its range is narrow and typically falls inbetween tested concentrations

FIGURE 13B



Cancers use ES cell phenotype and commit apoptosis at lower [ROS] than normal cells

FIGURE 14A



Increasing inhibition of F_1F_0 ATP hydrolase

Increasing uncoupling

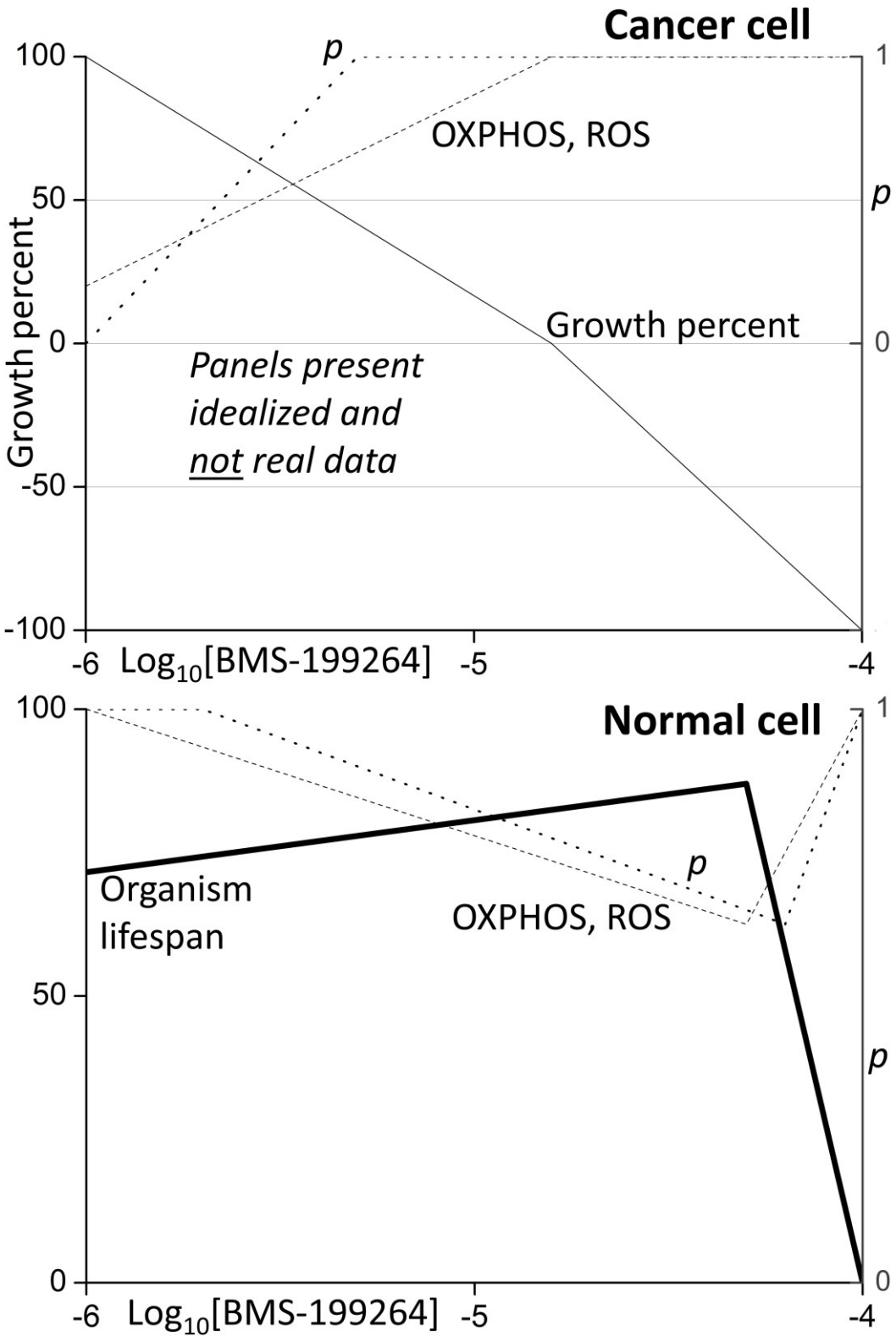
ROS driven cell cycle checkpoint blockade + ROS damages information fidelity and reduces number of possible cell divisions

ROS driven apoptosis

Number of cell divisions possible, N = Hayflick limit/p
Hayflick limit = 40 to 60, p is between 0 and 1,
when p=0, N=∞ ("limitless replicative potential", a Hallmark of cancer),
when p=1, N= Hayflick limit

Different cancer cell lines present the 2 phases with different concentration ranges

FIGURE 14B



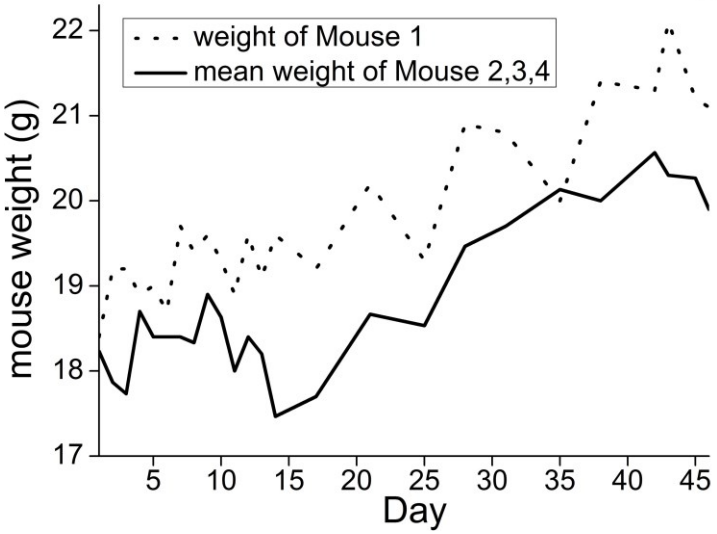
THERAPEUTIC WINDOW

{Cancers use ES cell phenotype and commit apoptosis at lower [ROS] than normal cells}

FIGURE 15A

	Day 1	Day 4	Day 7	Day 10	Day 13	Day 22	Day 39	Day 44	Day 45	Day 98
Mouse 1	vehicle	vehicle	vehicle	vehicle	vehicle	vehicle	vehicle	vehicle		SACRIFICED
Mouse 2	0.2 mg/kg	2 mg/kg	10 mg/kg	20 mg/kg	nothing	0.2 mg/kg	0.2 mg/kg	2 mg/kg		SACRIFICED
Mouse 3	0.2 mg/kg	2 mg/kg	10 mg/kg	20 mg/kg	40 mg/kg	2 mg/kg	2 mg/kg	20 mg/kg, DIED		
Mouse 4	0.2 mg/kg	2 mg/kg	10 mg/kg	20 mg/kg	40 mg/kg	20 mg/kg	vehicle (ethanol component replaced by water)	40 mg/kg	DIED	

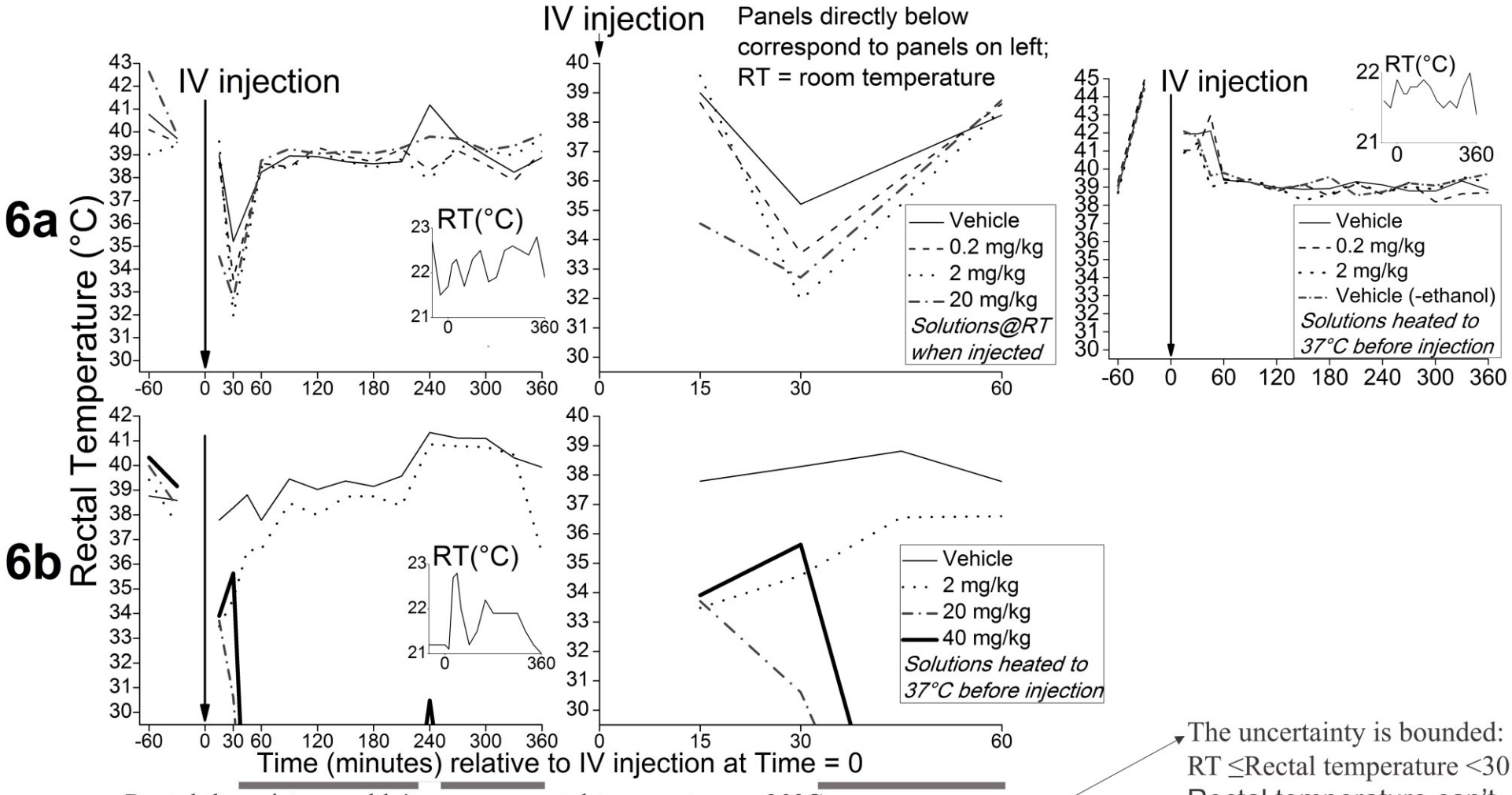
drug=6a drug=6b



Day 22, 39, 44

rectal temperature recorded every 30 minutes for 6 hours after IV injection (extra recording in 1st hour at 15 minutes {on Day 22} and at 15 and 45 minutes {on Day 39 and Day 44}, all rectal recording experiments started at approximately same time of day: ~0900 AM); data presented elsewhere herein

FIGURE 15B

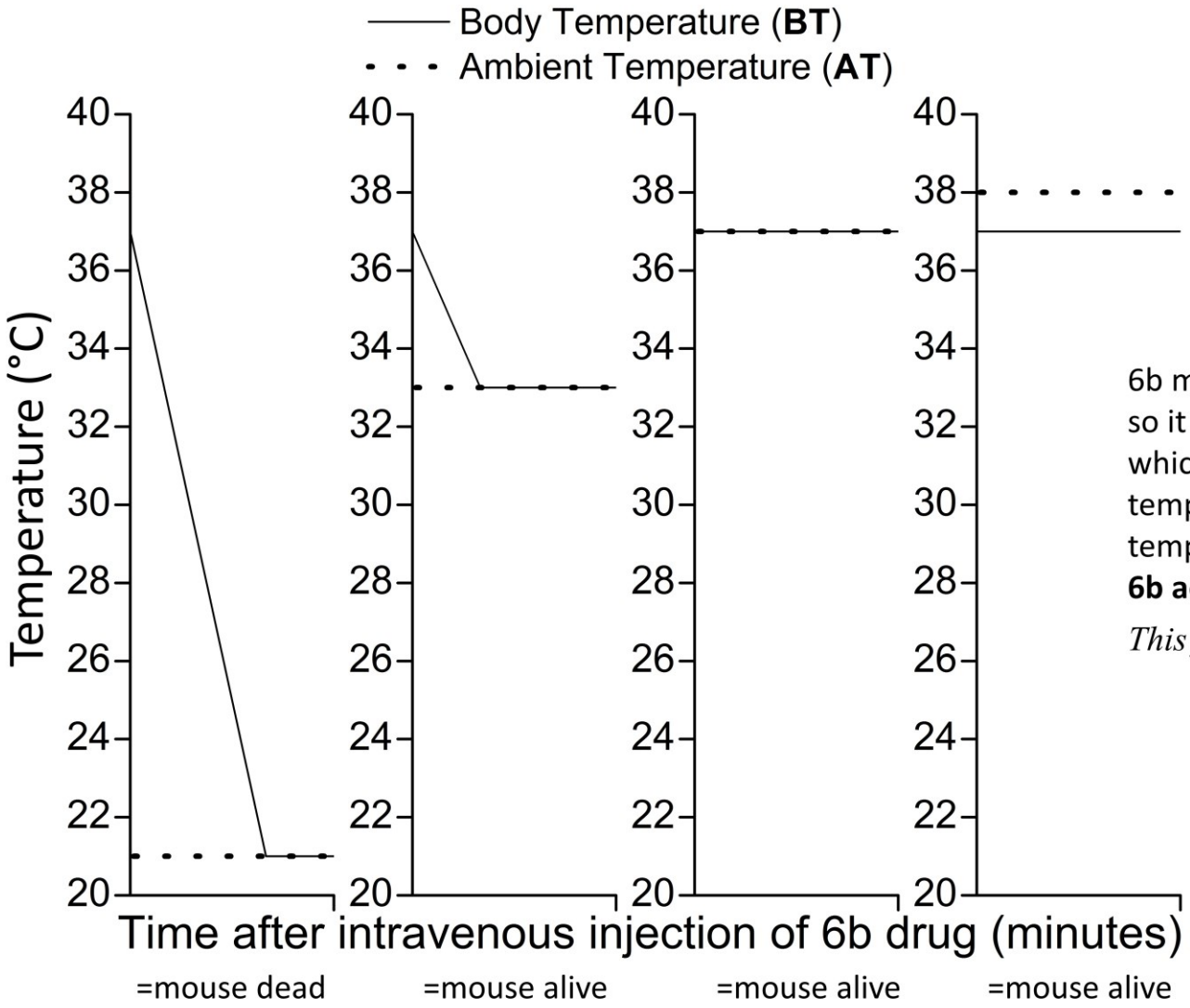


Rectal thermister couldn't measure rectal temperatures <30°C

6b: 20 & 40 mg/kg make rectal temperature fall below 30°C and out of range

The uncertainty is bounded:
RT ≤Rectal temperature <30
Rectal temperature can't fall below RT

FIGURE 15C



Upon 6b administration:
If (BT>AT) {BT=AT@AT}
If (BT=AT) {BT=AT}
If (BT<AT) {BT<AT}

6b makes the body more efficient,
so it produces less heat,
which is not problematic if ambient temperature (AT) equals a life-permissive body temperature (BT) because
6b administration cannot make BT fall below AT

This figure presents illustrative and not real data

6b induced metabolic efficiency gain,
if AT equals a life-permissive BT,
is useful to combat cachexia, the leading cause of death in cancer sufferers

FIGURE 16

6a sample Post-synthesis

	6a	6b
Retention time (minutes)	2.35	0
Area (%)	100	0

Enantiomeric excess (ee) stability: HIGH

Recorded by chiral SFC-MS

6b sample Post-synthesis

	6a	6b	6a	6b	6a	6b	6a	6b	6a	6b
			After 2.5 months stored as powder		After 72 hours in water@RT		After 48 hours in phosphate solution@37°C		After 5 days in phosphate solution@37°C	
Retention time (minutes)	2.34	2.69	1.68	1.91	1.67	1.88	1.83	2.12	1.89	2.14
Area (%)	1.21	98.79	0.66	99.34	0.59	99.41	1.29	98.71	0.34	99.66

Retention times can vary because of different chiral-SFC columns (of different lengths) used

Phosphate buffer solution, 1 M, sourced from Sigma-Aldrich (product code: *P3619*), its recorded pH at the 37°C temperature used = 7.41

RT=room temperature, 6b in water assay: water was in contact with air (and so CO_2), thence water pH ~5.6

FIGURE 17A

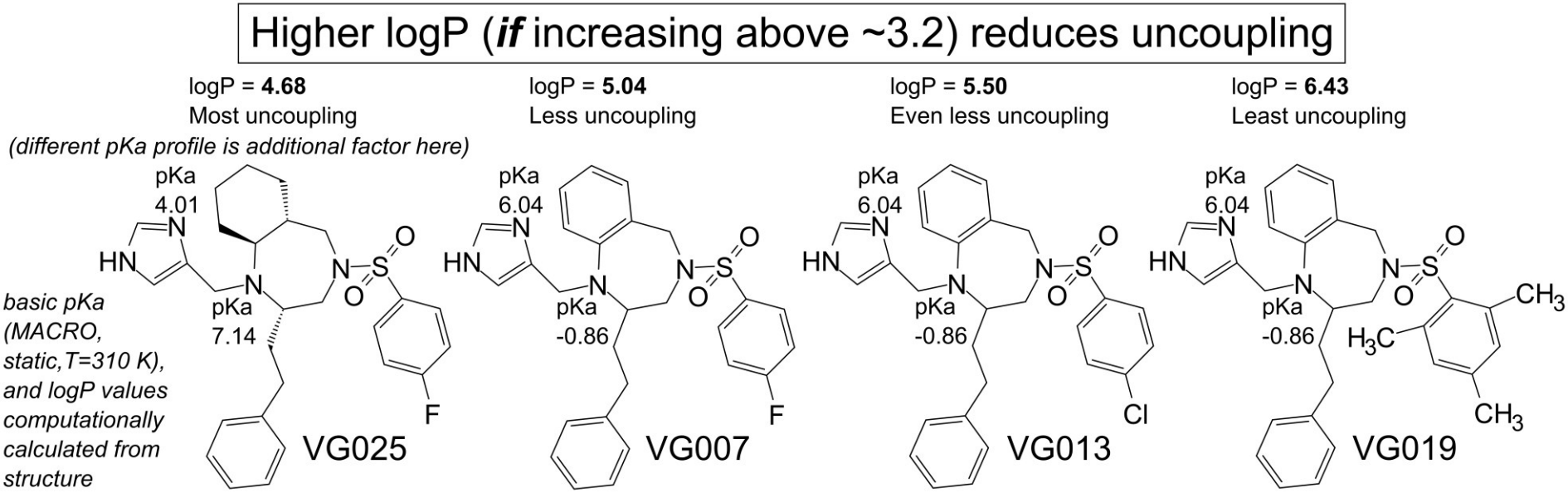

Higher logP (*if* increasing above ~3.2) reduces uncoupling
logP = **4.68**
Most uncoupling
logP = **5.04**
Less uncoupling
logP = **5.50**
Even less uncoupling
logP = **6.43**
Least uncoupling
(different pKa profile is additional factor here)
basic pKa (MACRO, static, T=310 K), and logP values computationally calculated from structure
pKa 4.01
pKa 7.14
VG025
pKa 6.04
pKa -0.86
VG007
pKa 6.04
pKa -0.86
VG013
pKa 6.04
pKa -0.86
VG019

FIGURE 17B

Higher logP (***if*** increasing above ~3.2) reduces uncoupling

	VG025 logP = **4.68** *Most uncoupling* ω	VG007 logP = **5.04** *Less uncoupling*	VG013 logP = **5.50** *Even less uncoupling*	VG019 logP = **6.43** *Least uncoupling*	BENCHMARK DRUGS
ΔO_2 consumption (**without** respiratory chain inhibitor)	-1.19% (10 μM) +75% (100 μM)	+25% (100 μM)	+3.32% (10 μM) +20% (100 μM)	-0.34% (100 μM)	Oligomycin (3 μM) -40% FCCP (500 nM) +50%
$\Delta\psi_{IM}$ (**without** respiratory chain inhibitor)	-7.3% (10 μM) -47.5% (100 μM)	0% (100 μM)	0% (100 μM)	0% (100 μM)	Oligomycin (3 μM) 0% μ FCCP (500 nM) -50%
$\Delta\psi_{IM}$ (**with** respiratory chain inhibitor)	-46% (10 μM) -64% (100 μM)	-42.61% (100 μM)	-30.15% (10 μM) -40.79% (100 μM)	-29.74% (100 μM)	Oligomycin (3 μM) -49.1% FCCP (500 nM) -52.5%

Uncoupling = increased O_2 consumption

$\Delta\psi_{IM}$ *-% = depolarization*

+% = hyperpolarization

ω, different pKa profile is additional factor here

μ, oligomycin should hyperpolarize the membrane slightly: presumably the assay used isn't sensitive enough to detect small hyperpolarizations