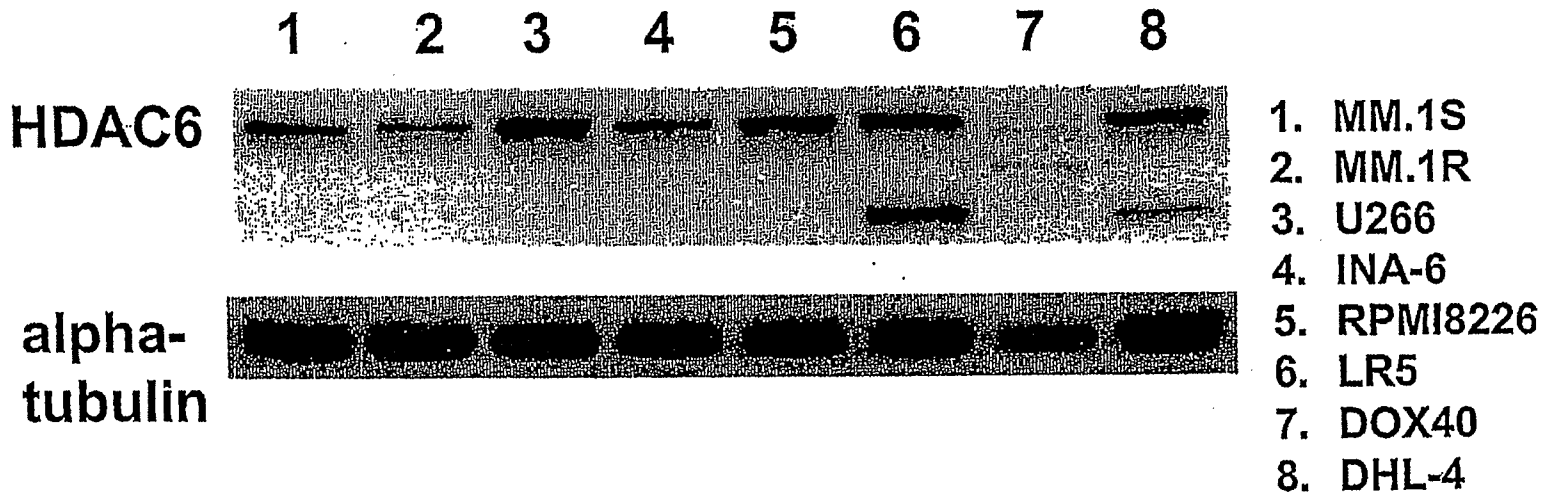

1 2 3 4 5 6 7 8
HDAC6
alpha-tubulin
1. MM.1S
2. MM.1R
3. U266
4. INA-6
5. RPMI8226
6. LR5
7. DOX40
8. DHL-4

Figure 1

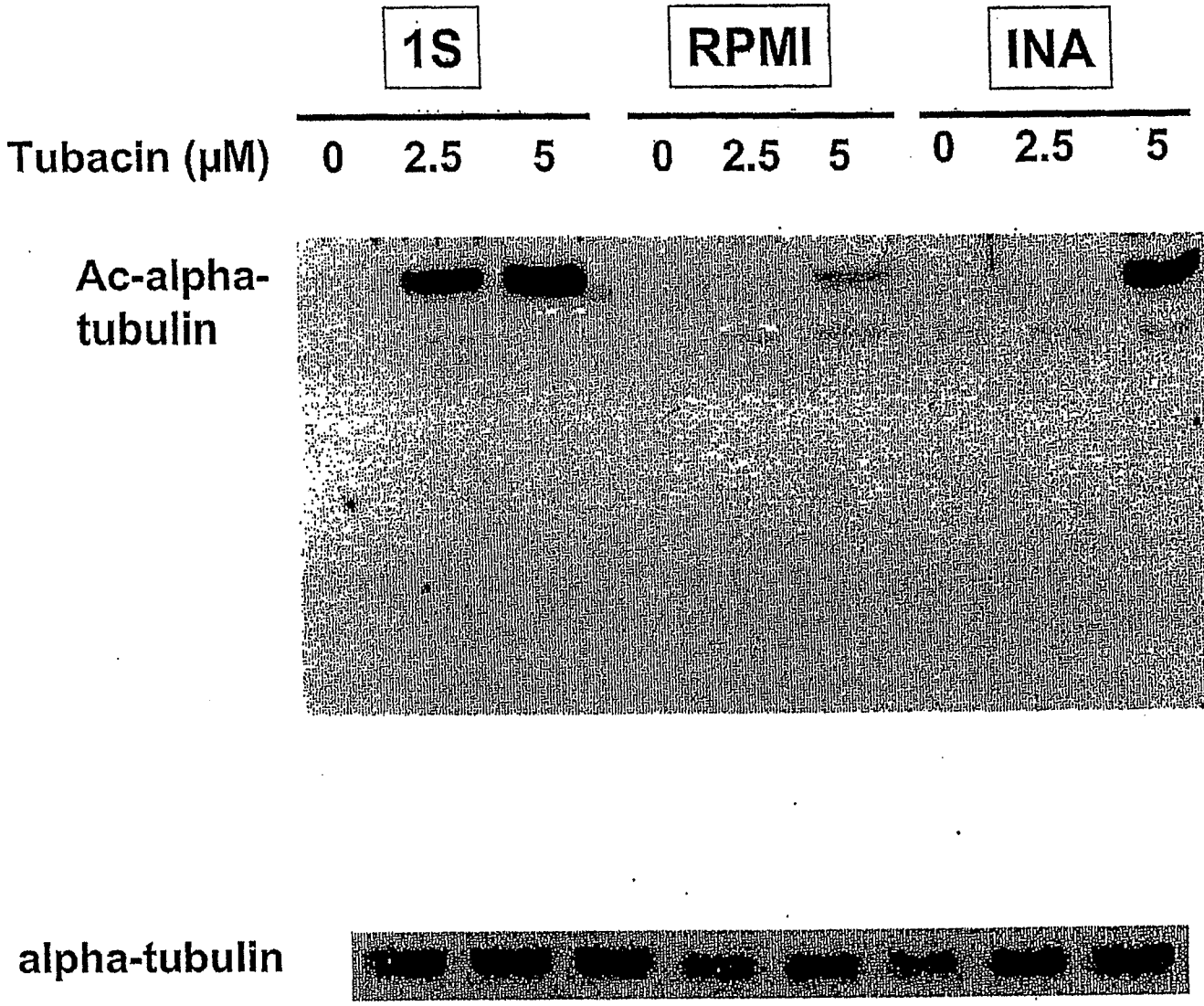

1S
RPMI
INA
Tubacin (μM)
0
2.5
5
0
2.5
5
0
2.5
5
Ac-alpha-tubulin
alpha-tubulin

Figure 2

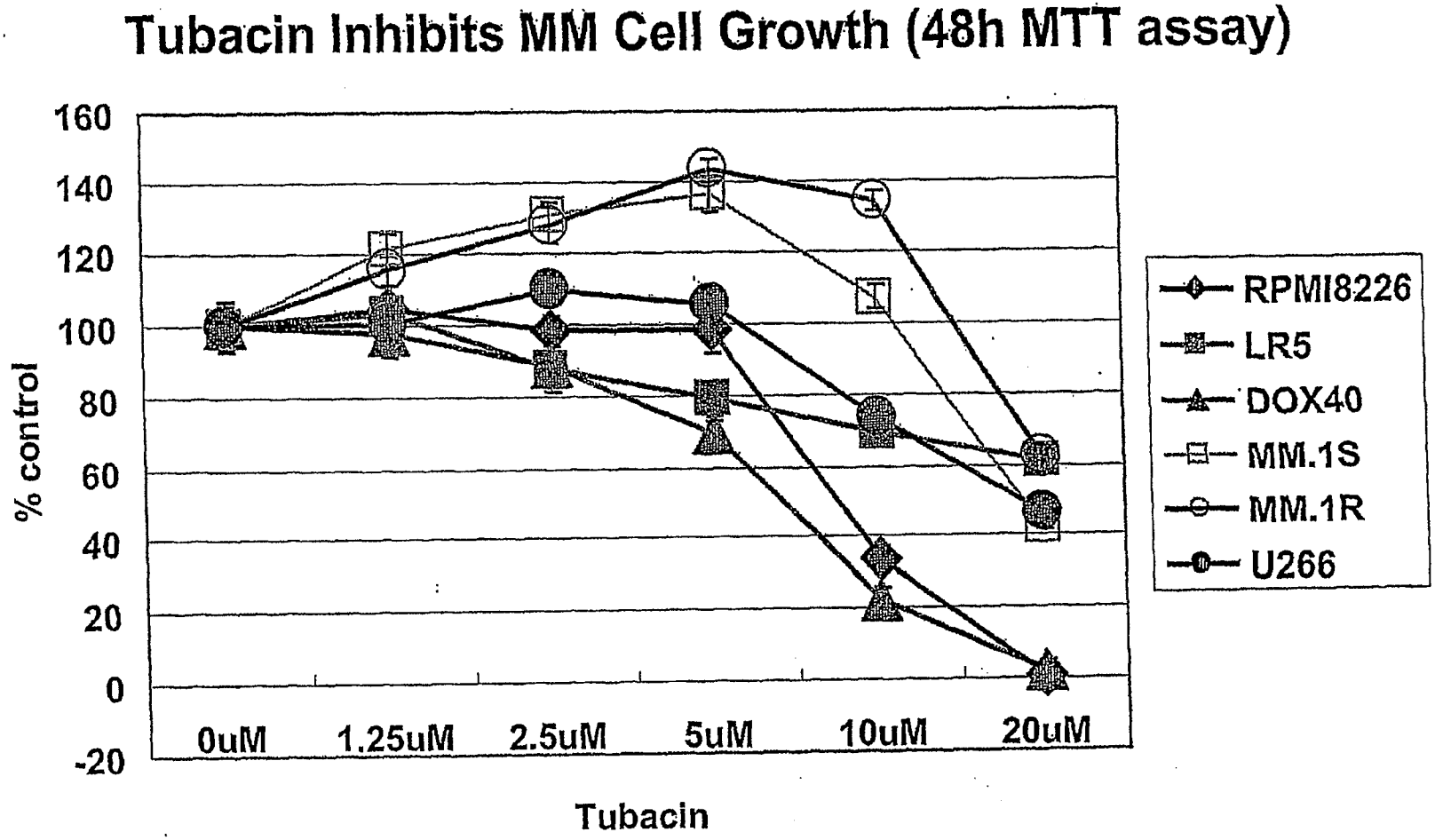

Tubacin Inhibits MM Cell Growth (48h MTT assay)
160
140
120
100
80
60
40
20
0
-20
% control
0uM
1.25uM
2.5uM
5uM
10uM
20uM
Tubacin
RPMI8226
LR5
DOX40
MM.1S
MM.1R
U266

Figure 3A

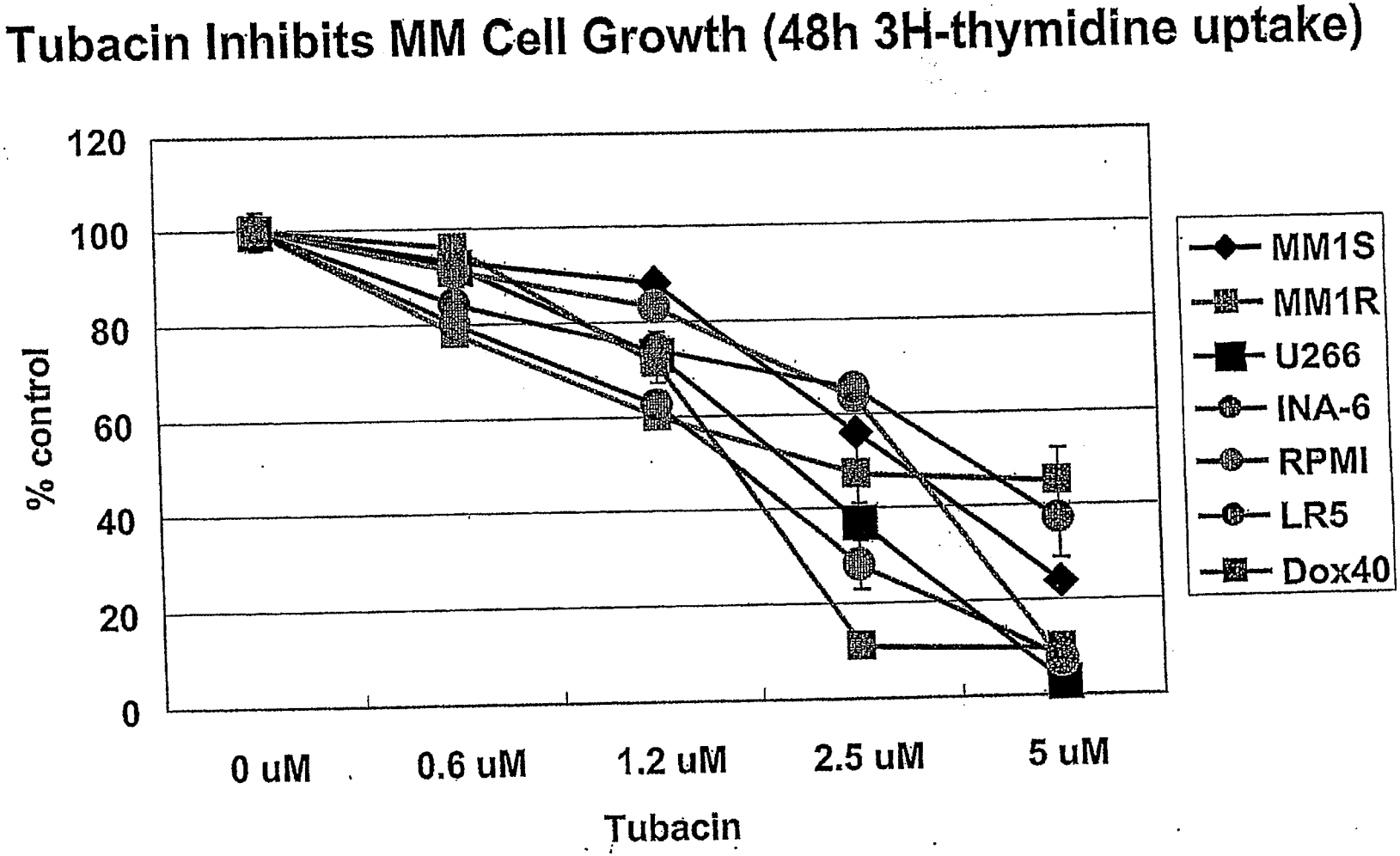

Tubacin Inhibits MM Cell Growth (48h 3H-thymidine uptake)
120
100
80
60
40
20
0
% control
0 uM
0.6 uM
1.2 uM
2.5 uM
5 uM
Tubacin
MM1S
MM1R
U266
INA-6
RPMI
LR5
Dox40

Figure 3B

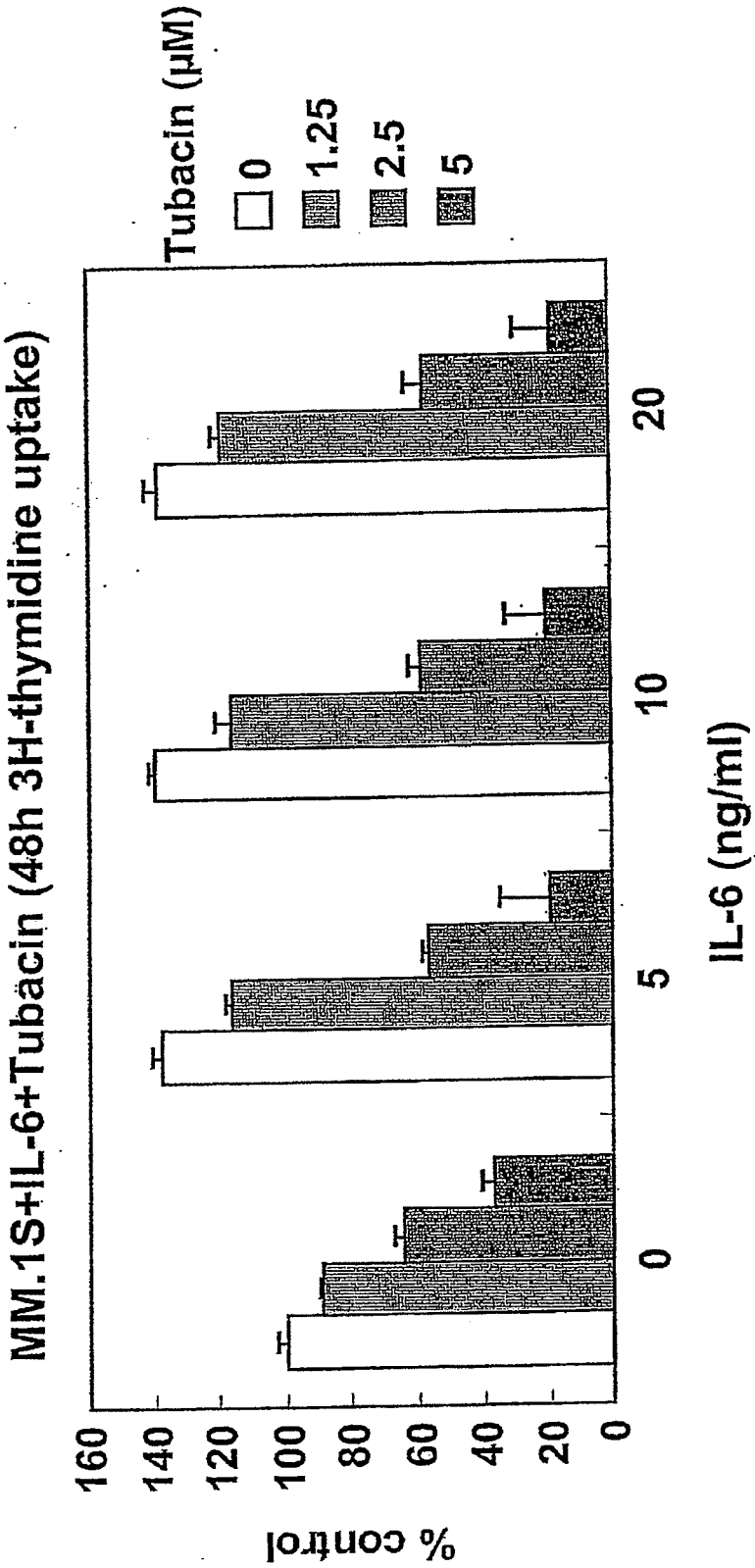

MM.1S+IL-6+Tubacin (48h 3H-thymidine uptake)
Tubacin (μM)
0
1.25
2.5
5
% control
160
140
120
100
80
60
40
20
0
0
5
10
20
IL-6 (ng/ml)

Figure 4

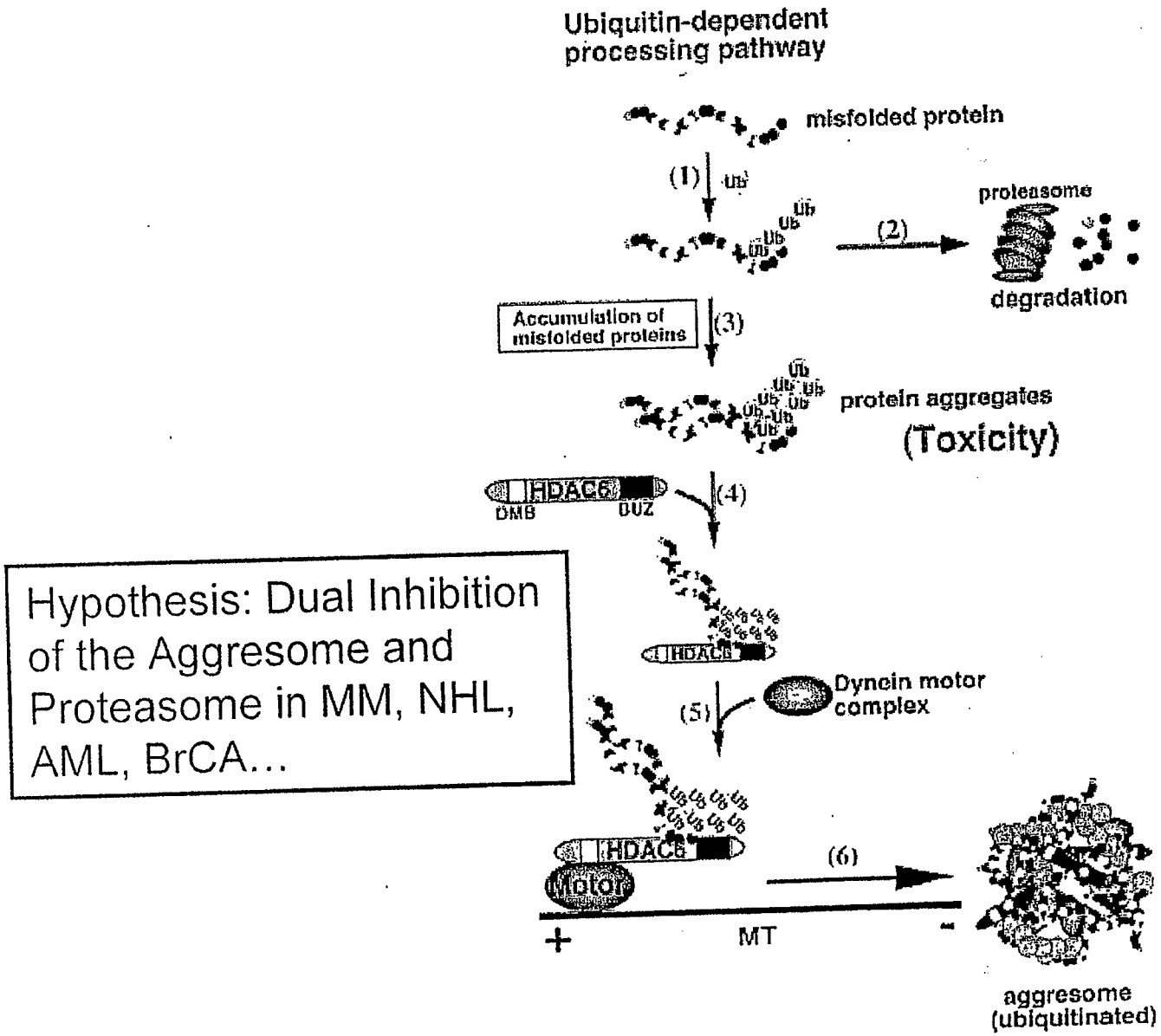

Ubiquitin-dependent processing pathway
misfolded protein
(1)
Ub
proteasome
(2)
degradation
Accumulation of misfolded proteins
(3)
protein aggregates
(Toxicity)
HDAC6
DMB
DUZ
(4)
HDAC6
Dynein motor complex
(5)
HDAC6
Motor
(6)
+
MT
-
aggresome
(ubiquitinated)
Hypothesis: Dual Inhibition of the Aggresome and Proteasome in MM, NHL, AML, BrCA...

Fig. 5

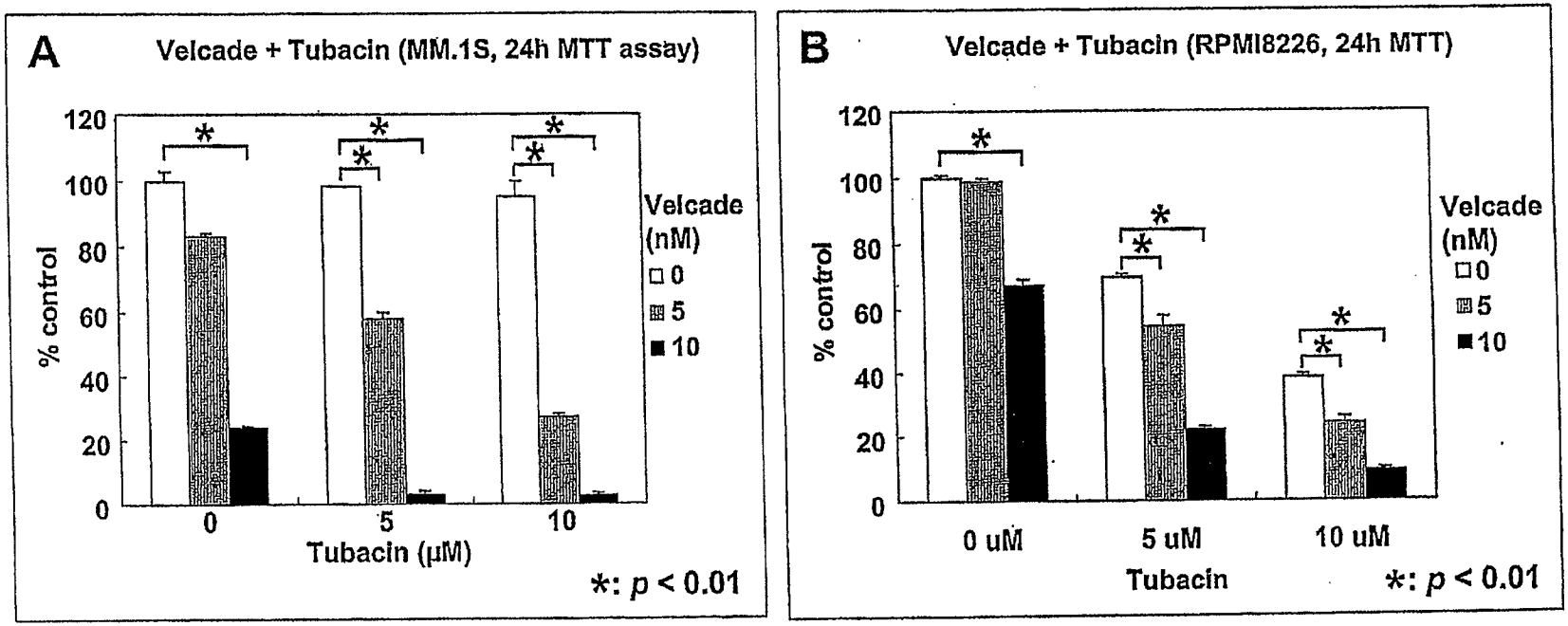

A
Velcade + Tubacin (MM.1S, 24h MTT assay)
% control
120
100
80
60
40
20
0
0
5
10
Tubacin (μM)
Velcade (nM)
0
5
10
*: p < 0.01
B
Velcade + Tubacin (RPMI8226, 24h MTT)
% control
120
100
80
60
40
20
0
0 uM
5 uM
10 uM
Tubacin
Velcade (nM)
0
5
10
*: p < 0.01

Figure 6

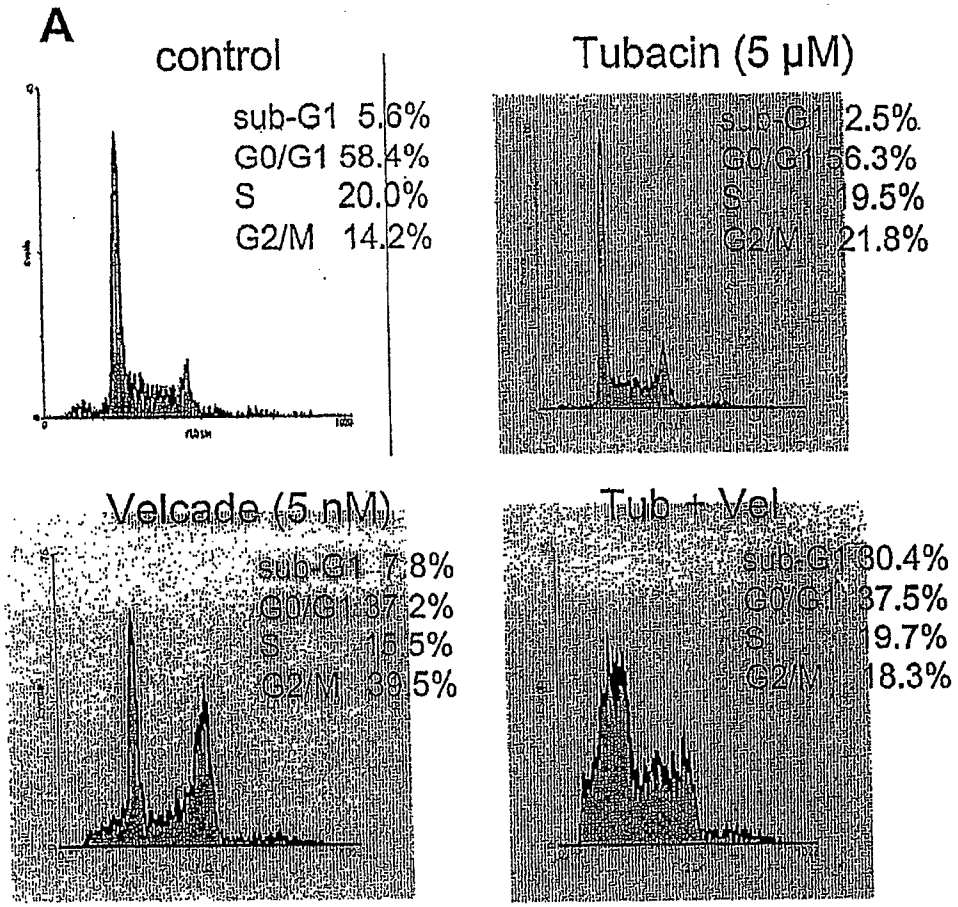

A
control
sub-G1 5.6%
G0/G1 58.4%
S 20.0%
G2/M 14.2%
Tubacin (5 µM)
sub-G1 2.5%
G0/G1 36.3%
S 19.5%
G2/M 21.8%
Velcade (5 nM)
sub-G1 7.8%
G0/G1 37.2%
S 15.5%
G2/M 39.5%
Tub + Vel
sub-G1 30.4%
G0/G1 37.5%
S 9.7%
G2/M 18.3%
B
MM.1S
control
Tub. (5 µM)
PS-341 (5 nM)
Tub.+PS-341
p21Clp1

Figure 7

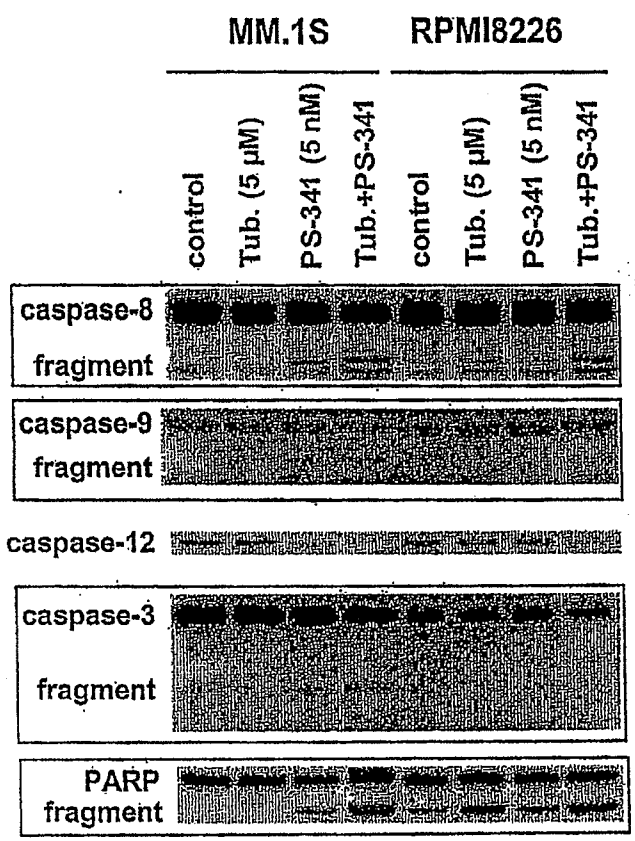

MM.1S
RPMI8226
control
Tub. (5 μM)
PS-341 (5 nM)
Tub.+PS-341
control
Tub. (5 μM)
PS-341 (5 nM)
Tub.+PS-341
caspase-8
fragment
caspase-9
fragment
caspase-12
caspase-3
fragment
PARP
fragment

Figure 8

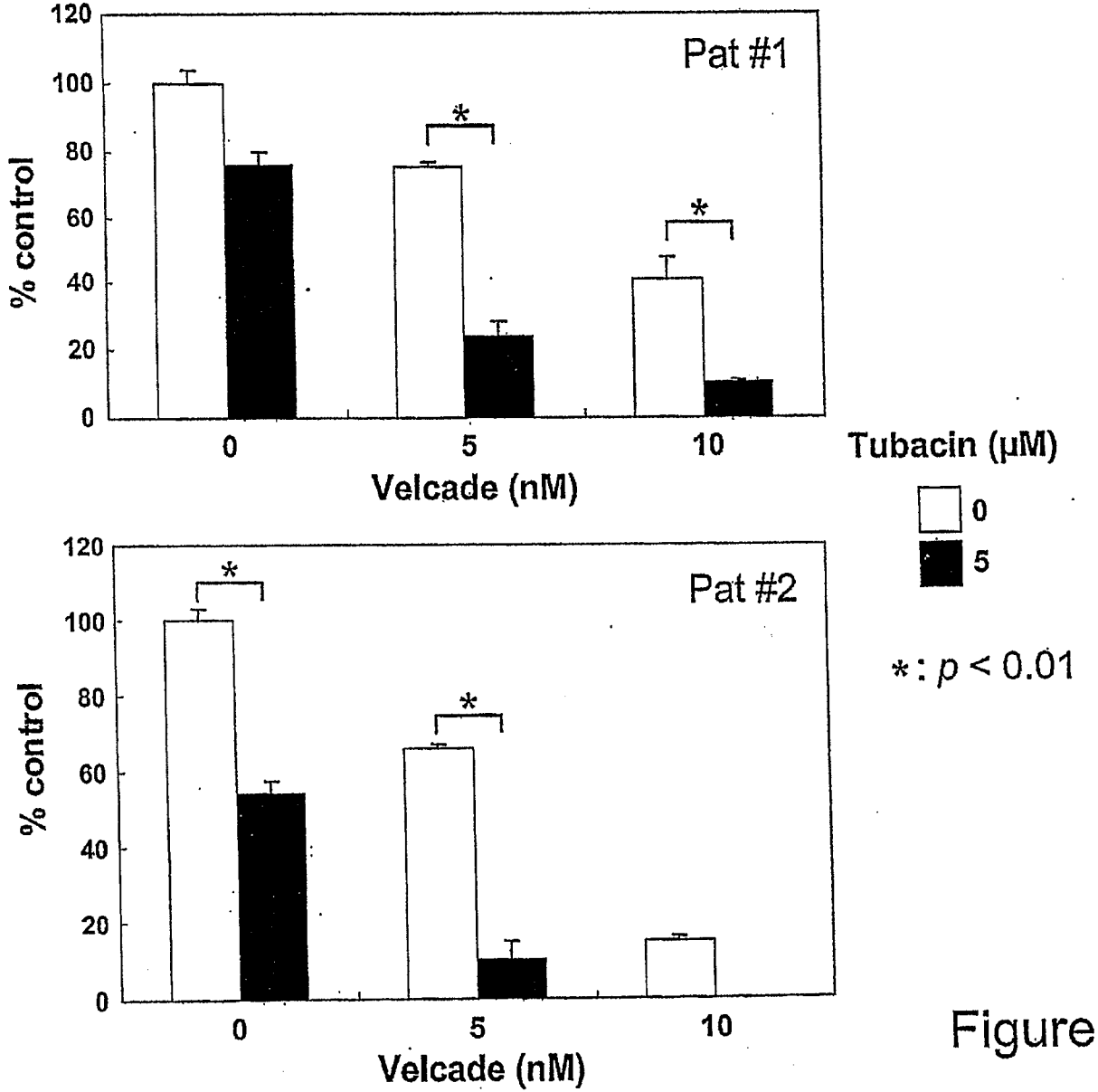
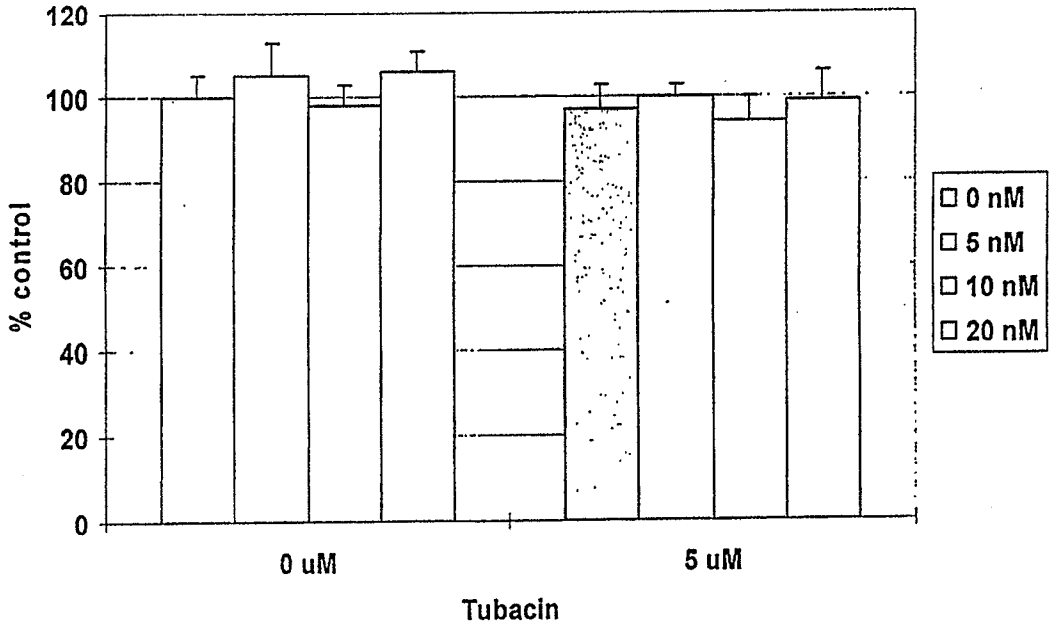

Pat #1
% control
120
100
80
60
40
20
0
*
*
0
5
10
Velcade (nM)
Tubacin (μM)
0
5
Pat #2
% control
120
100
80
60
40
20
0
*
*
0
5
10
Velcade (nM)
*: p < 0.01

Figure 9

Tubacin and bortezomib are not toxic to PBMCs



- Combination is cytotoxic in all myeloma cell lines studied (Universal)
- No demonstrable effect on PBMC (Broad therapeutic window)
- Overcomes cell adhesion-mediated drug resistance (Predicts clinical response)
- Sensitizes patient-derived myeloma cells to 5-10nM bortezomib (Toxicity)

Fig. 10

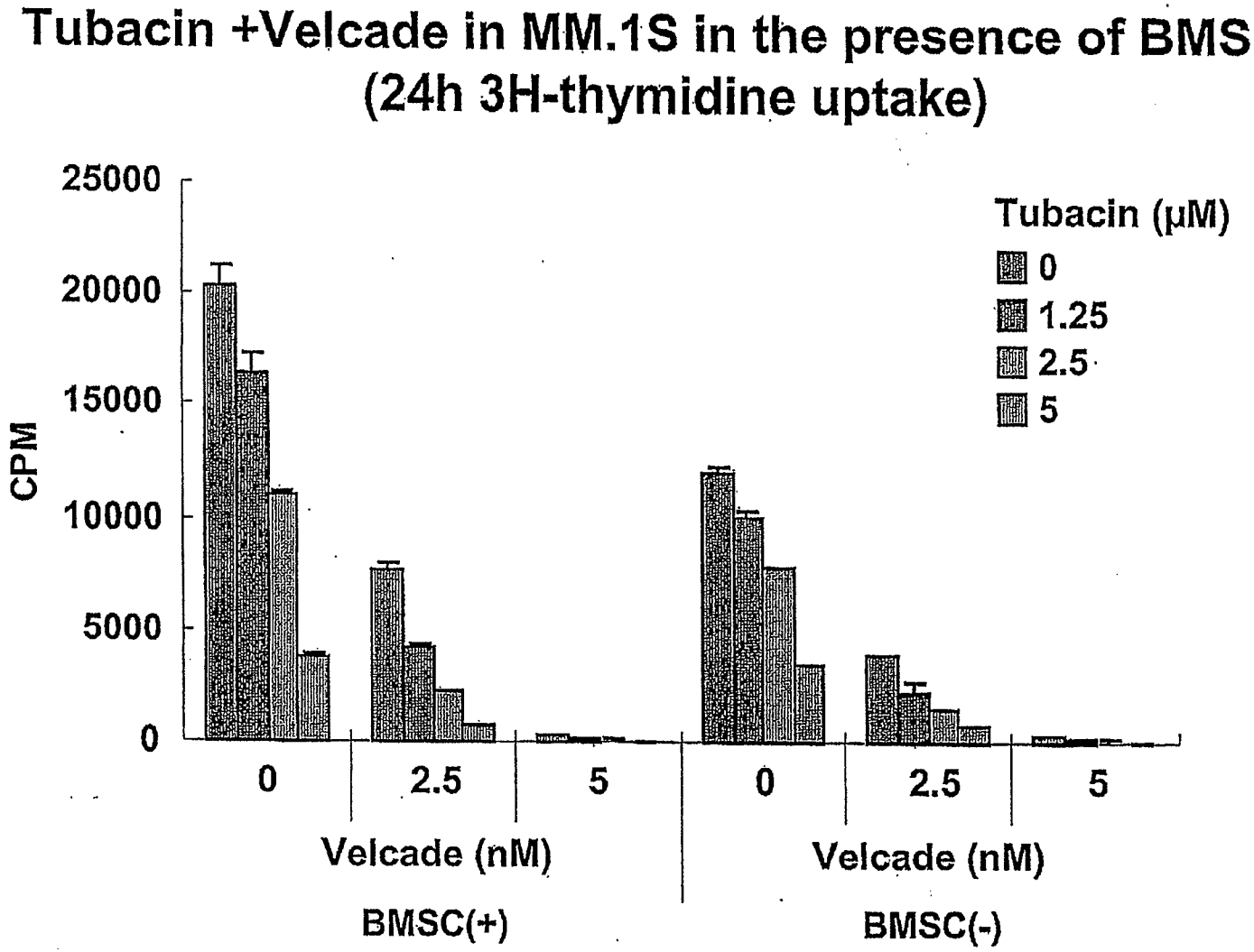

Tubacin +Velcade in MM.1S in the presence of BMSCs
(24h 3H-thymidine uptake)
25000
20000
15000
10000
5000
0
CPM
Tubacin (μM)
0
1.25
2.5
5
0
2.5
5
0
2.5
5
Velcade (nM)
Velcade (nM)
BMSC(+)
BMSC(-)

Figure 11

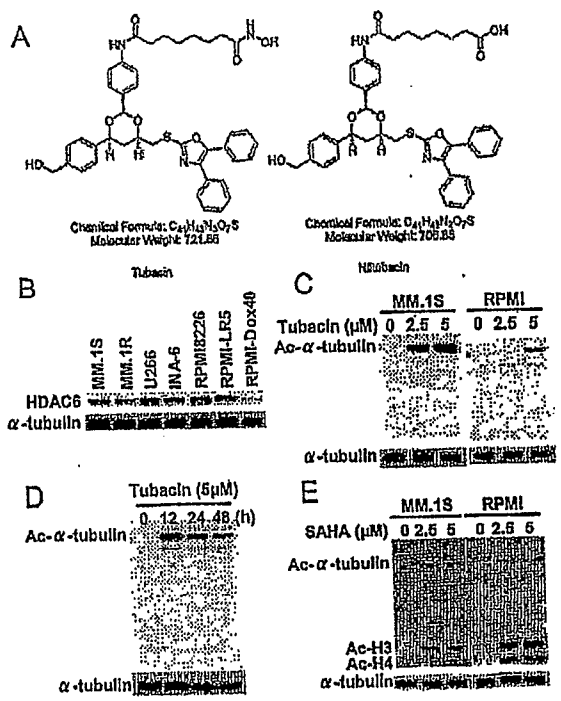

A
Chemical Formula: C41H43N3O7S
Molecular Weight: 721.86
Chemical Formula: C41H41N3O7S
Molecular Weight: 705.85
B
Tubacin
MM.1S
MM.1R
U266
INA-6
RPMI8226
RPMI-LR5
RPMI-Dox40
HDAC6
α-tubulin
C
Nilotinib
MM.1S
RPMI
Tubacin (μM) 0 2.5 5 0 2.5 5
Ac-α-tubulin
α-tubulin
D
Tubacin (5μM)
0 12 24 48 (h)
Ac-α-tubulin
α-tubulin
E
MM.1S
RPMI
SAHA (μM) 0 2.5 5 0 2.5 5
Ac-α-tubulin
Ac-H3
Ac-H4
α-tubulin

Figure 12

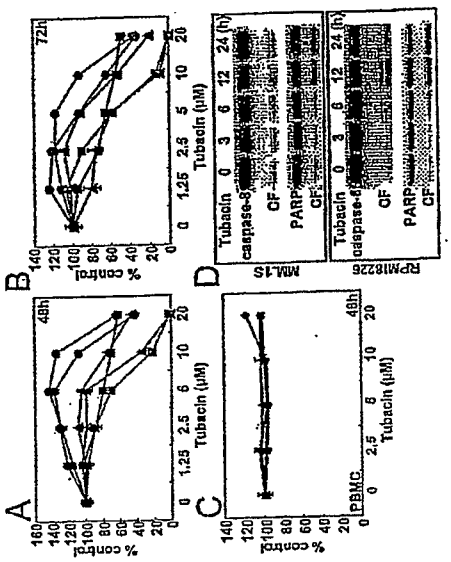

A
B
C
D
48h
72h
% control
Tubacin (μM)
PBMC
MM.1S
RPMI8226
Tubacin
caspase-8
CF
PARP
24 (h)

Figure 3

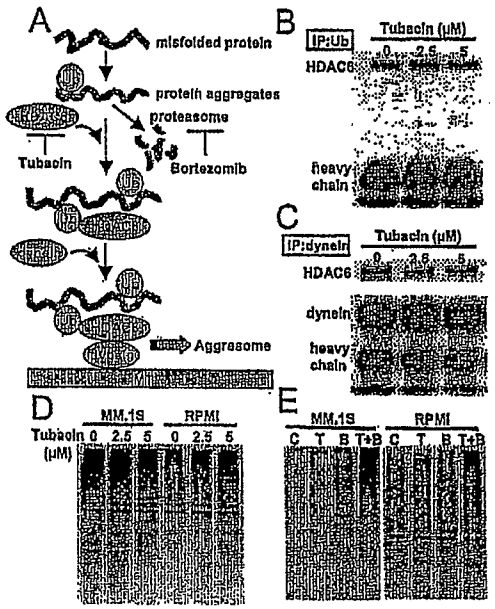

A
misfolded protein
protein aggregates
proteasome
Tubacin
Bortezomib
Aggresome
B
IP:Ub
Tubacin (μM)
0 2.5 5
HDAC6
heavy chain
C
IP:dynein
Tubacin (μM)
0 2.5 5
HDAC6
dynein
heavy chain
D
MM.1S
RPMI
Tubacin (μM)
0 2.5 5 0 2.5 5
E
MM.1S
RPMI
C T B T+B C T B T+B

Figure 14

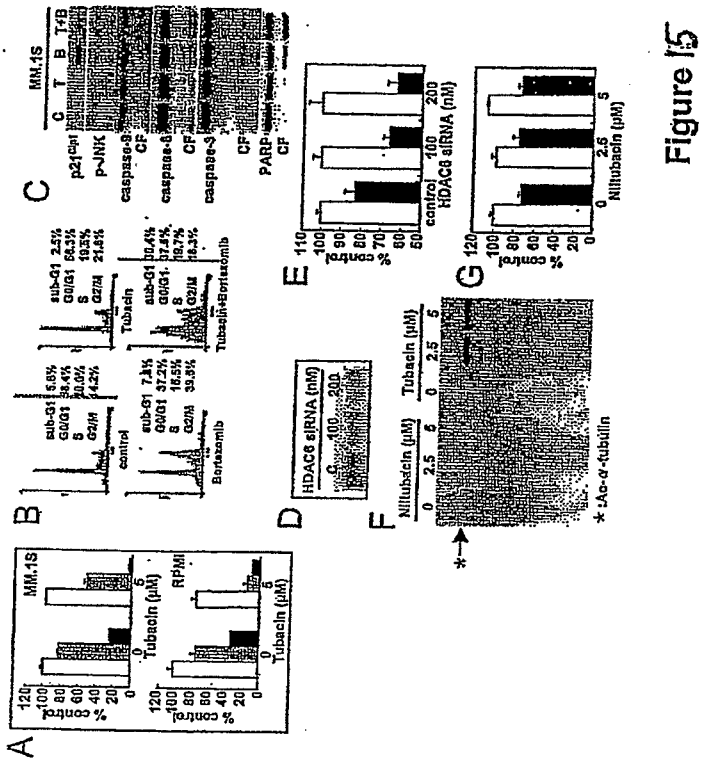

A
MM.1S
RPMI
% control
Tubacin (μM)
B
control
Bortezomib
Tubacin
Tubacin+Bortezomib
sub-G1
G0/G1
S
G2/M
C
MM.1S
C
T
B
T+B
p21Cip1
p-JNK
caspase-8
caspase-3
PARP
CF
D
HDAC6 siRNA (nM)
E
% control
control
HDAC6 siRNA (nM)
F
Nilotubacin (μM)
Tubacin (μM)
* Ac-α-tubulin
G
% control
Nilotubacin (μM)

Figure 15

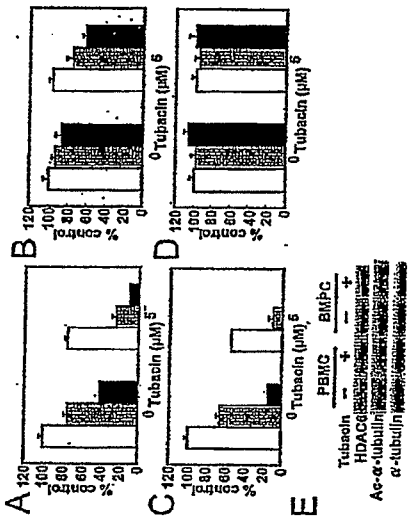

A
B
C
D
E
% control
Tubacin (μM)
Tubacin (μM)
Tubacin (μM)
Tubacin (μM)
PBMC
BMPC
Tubacin
HDAC6
Ac-α-tubulin
α-tubulin

Figure 16

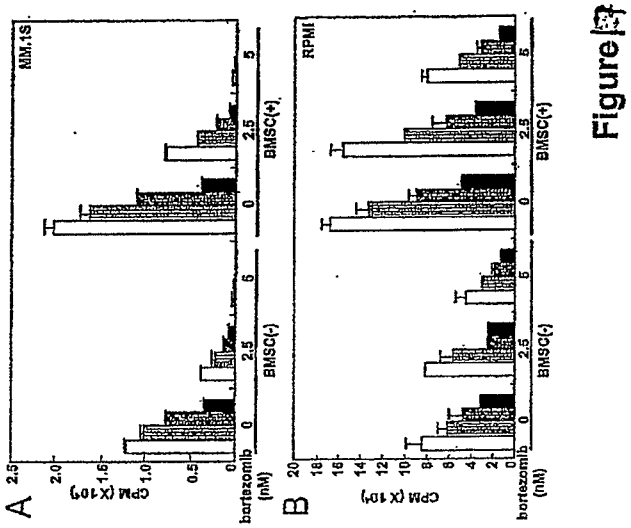

A
MM.1S
CPM (X 10³)
2.5
2.0
1.5
1.0
0.5
0
bortezomib (nM)
0
2.5
5
0
2.5
5
BMSC(-)
BMSC(+)
B
RPMI
CPM (X 10³)
20
18
16
14
12
10
8
6
4
2
0
bortezomib (nM)
0
2.5
5
0
2.5
5
BMSC(-)
BMSC(+)

Figure 1B

Tubacin and bortezomib: potent inhibition of protein catabolism

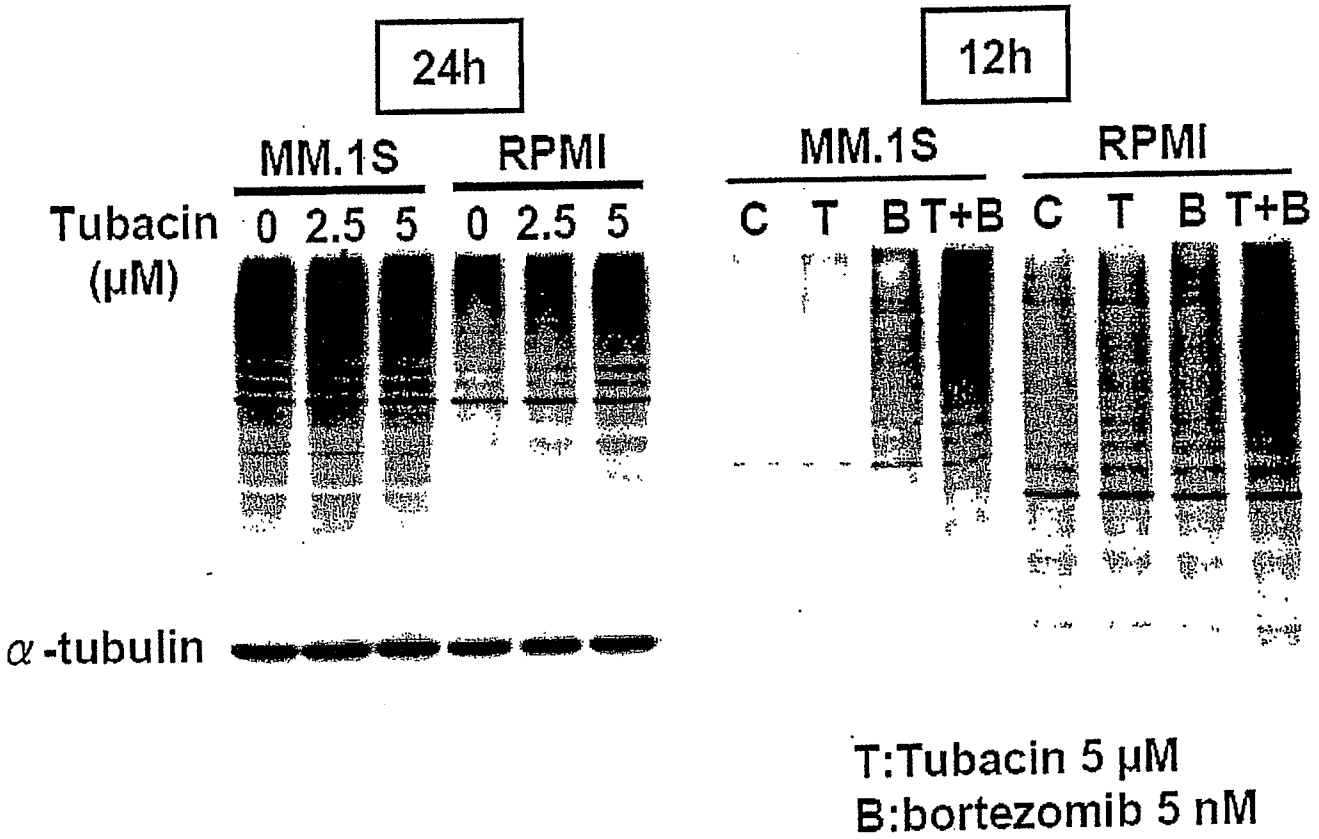
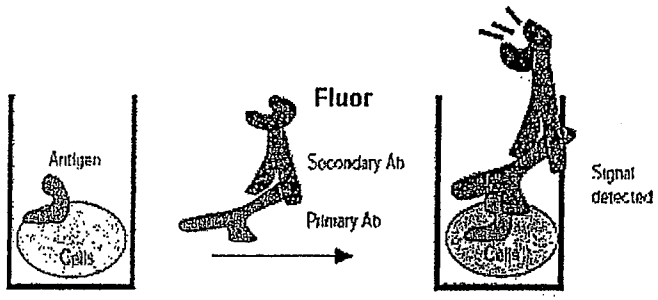
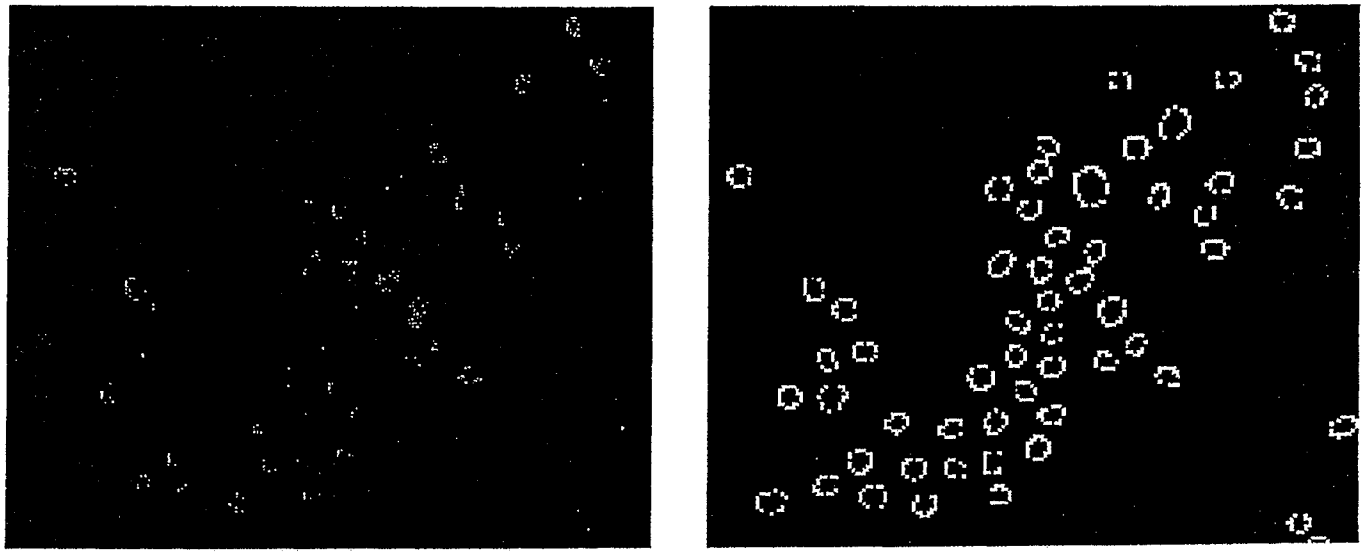


Fig. 18





Medicinal Chemistry: Development of HT/IF Quantitative Assay

Fig. 19

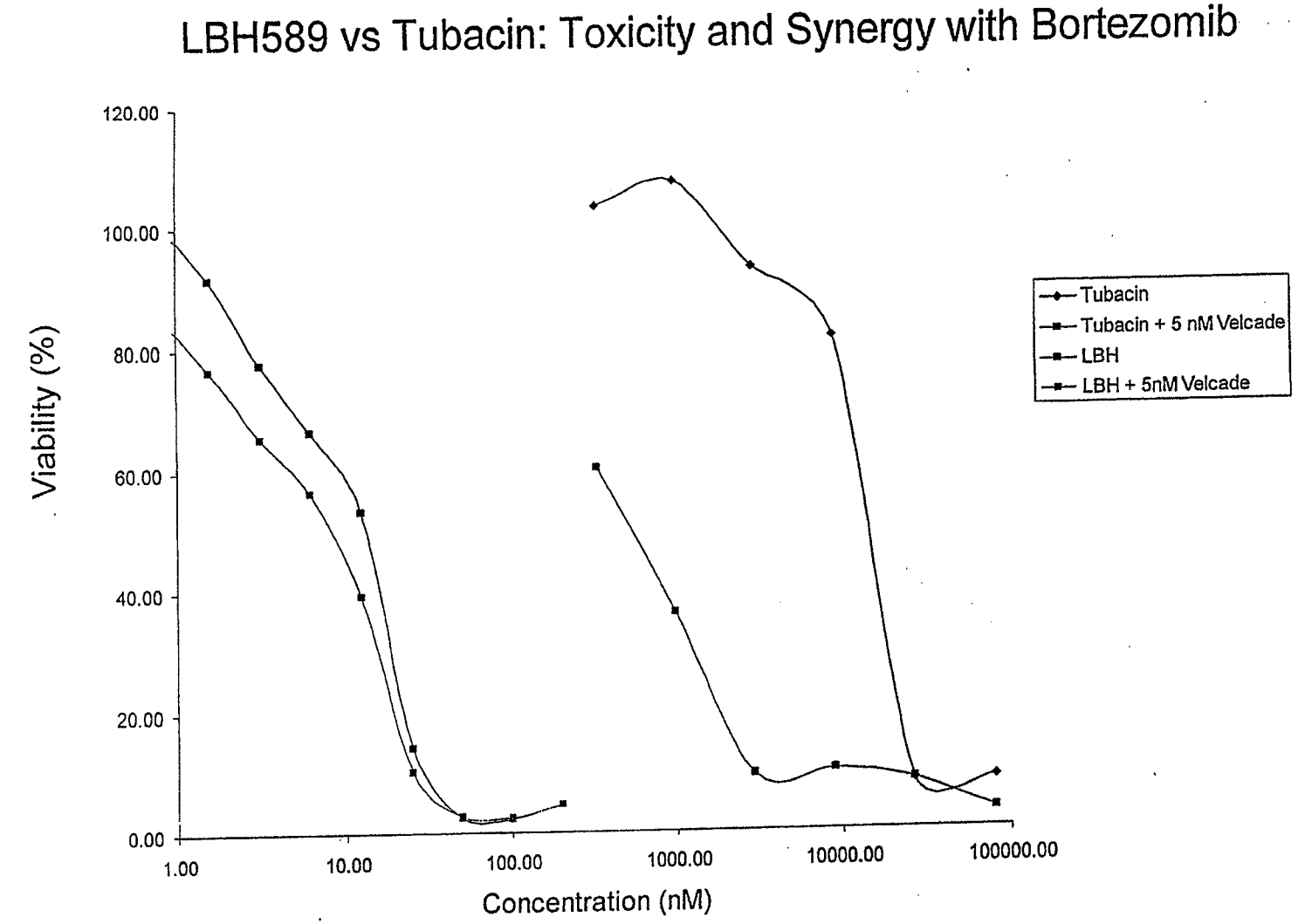

LBH589 vs Tubacin: Toxicity and Synergy with Bortezomib
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Viability (%)
1.00
10.00
100.00
1000.00
10000.00
100000.00
Concentration (nM)
Tubacin
Tubacin + 5 nM Velcade
LBH
LBH + 5nM Velcade
MM.1S (48h)

Fig. 20

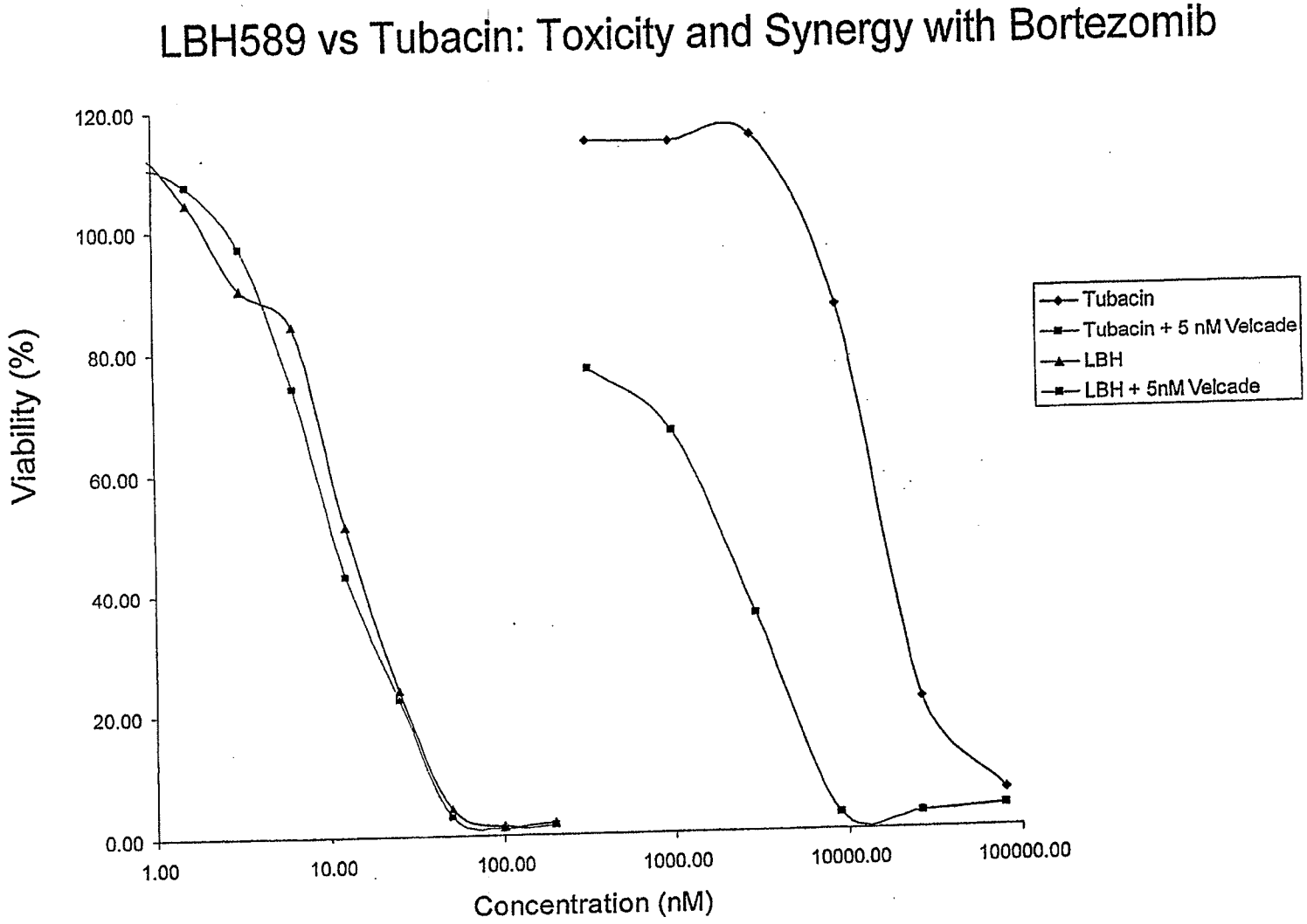

LBH589 vs Tubacin: Toxicity and Synergy with Bortezomib
Viability (%)
120.00
100.00
80.00
60.00
40.00
20.00
0.00
1.00
10.00
100.00
1000.00
10000.00
100000.00
Concentration (nM)
Tubacin
Tubacin + 5 nM Velcade
LBH
LBH + 5nM Velcade
RPMI-8226 (48h)

Fig. 21

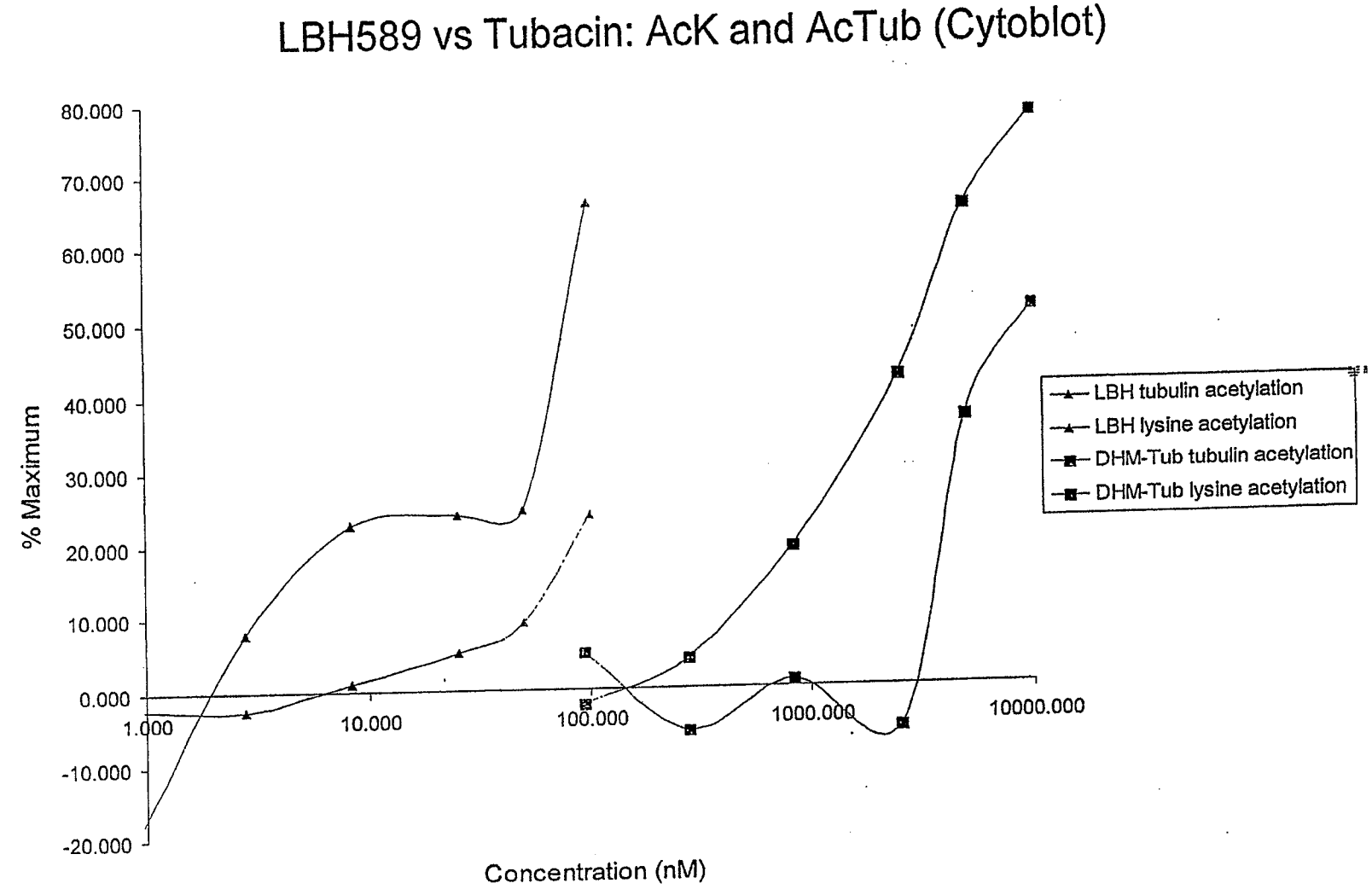

LBH589 vs Tubacin: AcK and AcTub (Cytoblot)
80.000
70.000
60.000
50.000
40.000
30.000
20.000
10.000
0.000
-10.000
-20.000
% Maximum
1.000
10.000
100.000
1000.000
10000.000
Concentration (nM)
LBH tubulin acetylation
LBH lysine acetylation
DHM-Tub tubulin acetylation
DHM-Tub lysine acetylation
293T (12h)

Fig. 22

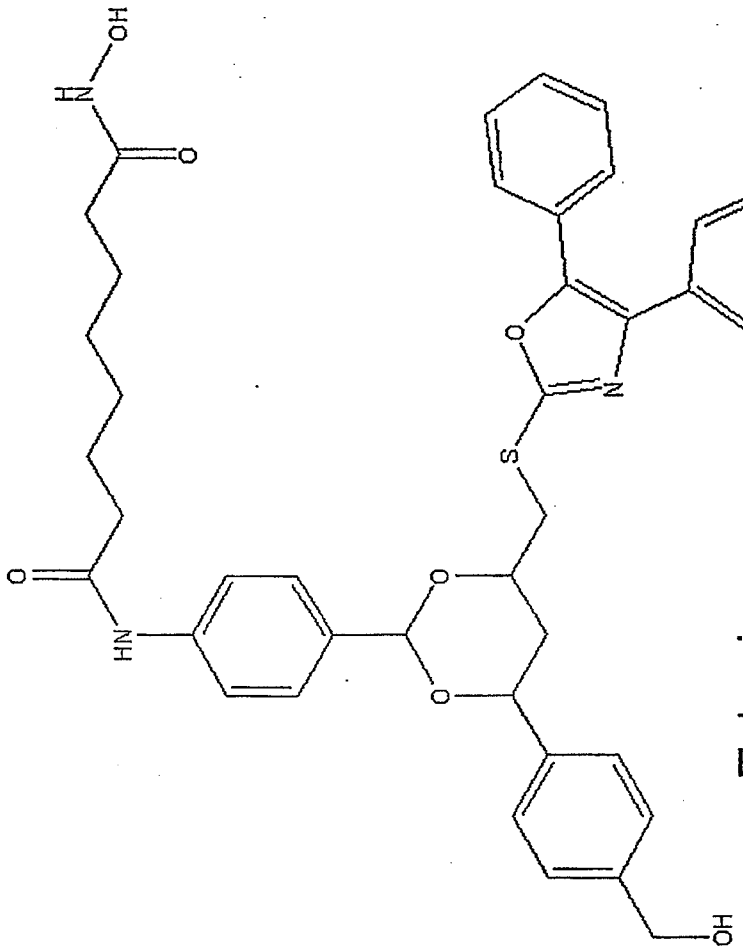


Fig. 23

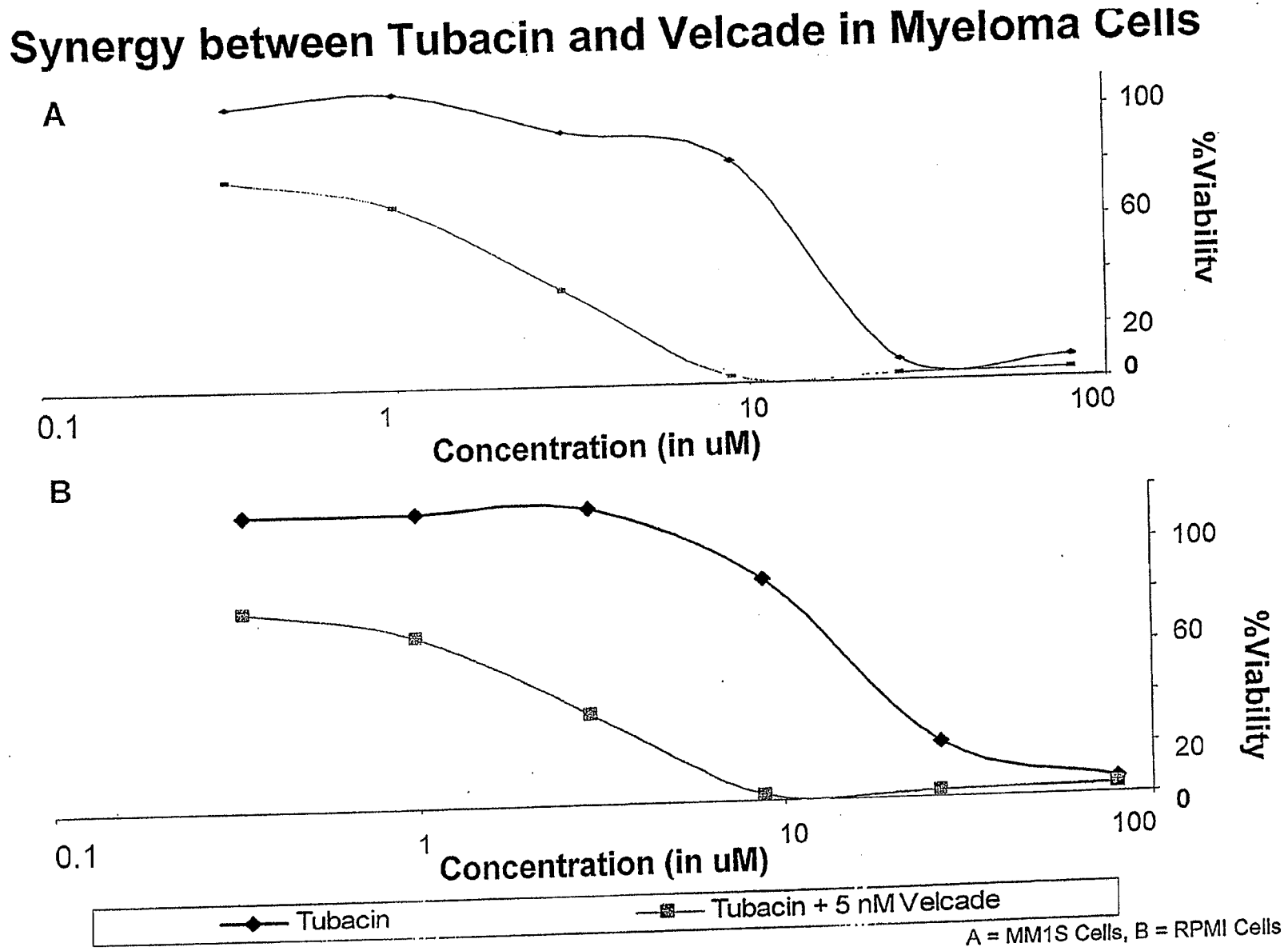

Synergy between Tubacin and Velcade in Myeloma Cells
A
100
60
20
0
%Viability
0.1
1
10
100
Concentration (in uM)
B
100
60
20
0
%Viability
0.1
1
10
100
Concentration (in uM)
Tubacin
Tubacin + 5 nM Velcade
A = MM1S Cells, B = RPMI Cells

Fig. 24

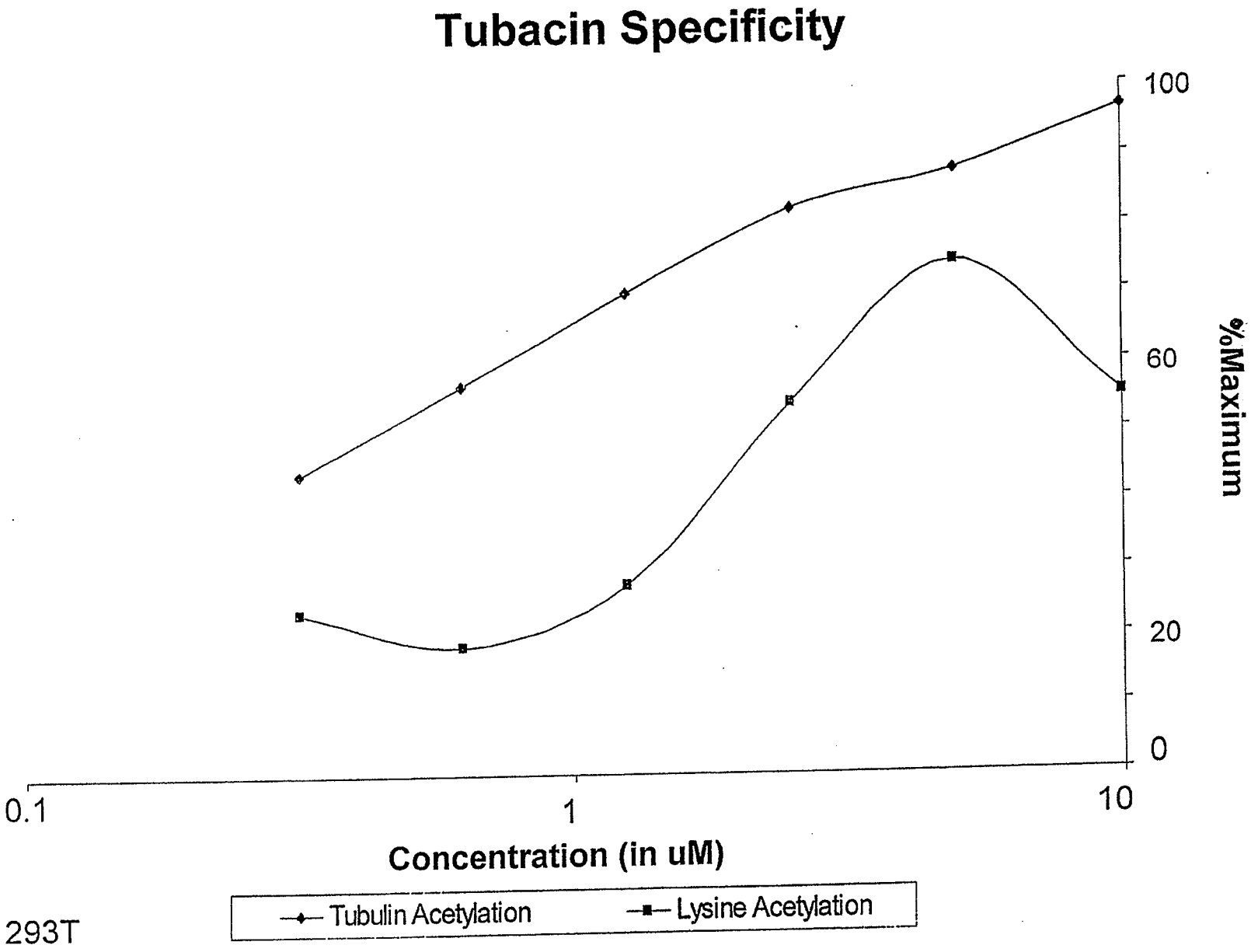
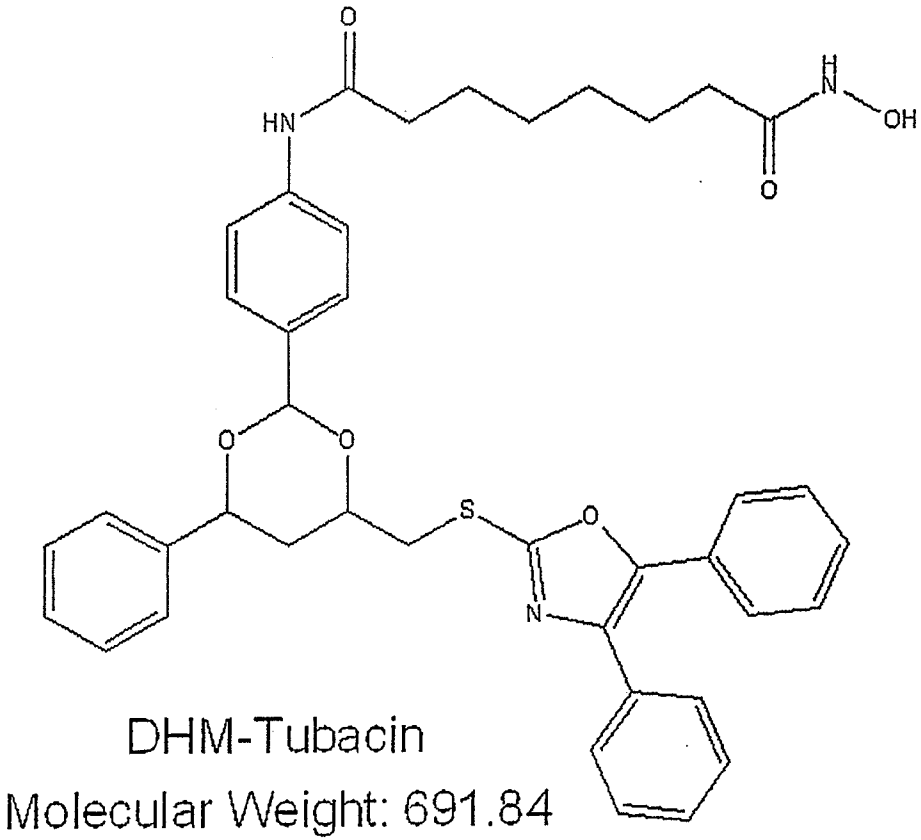

Tubacin Specificity
100
60
20
0
%Maximum
0.1
1
10
Concentration (in uM)
293T
Tubulin Acetylation
Lysine Acetylation

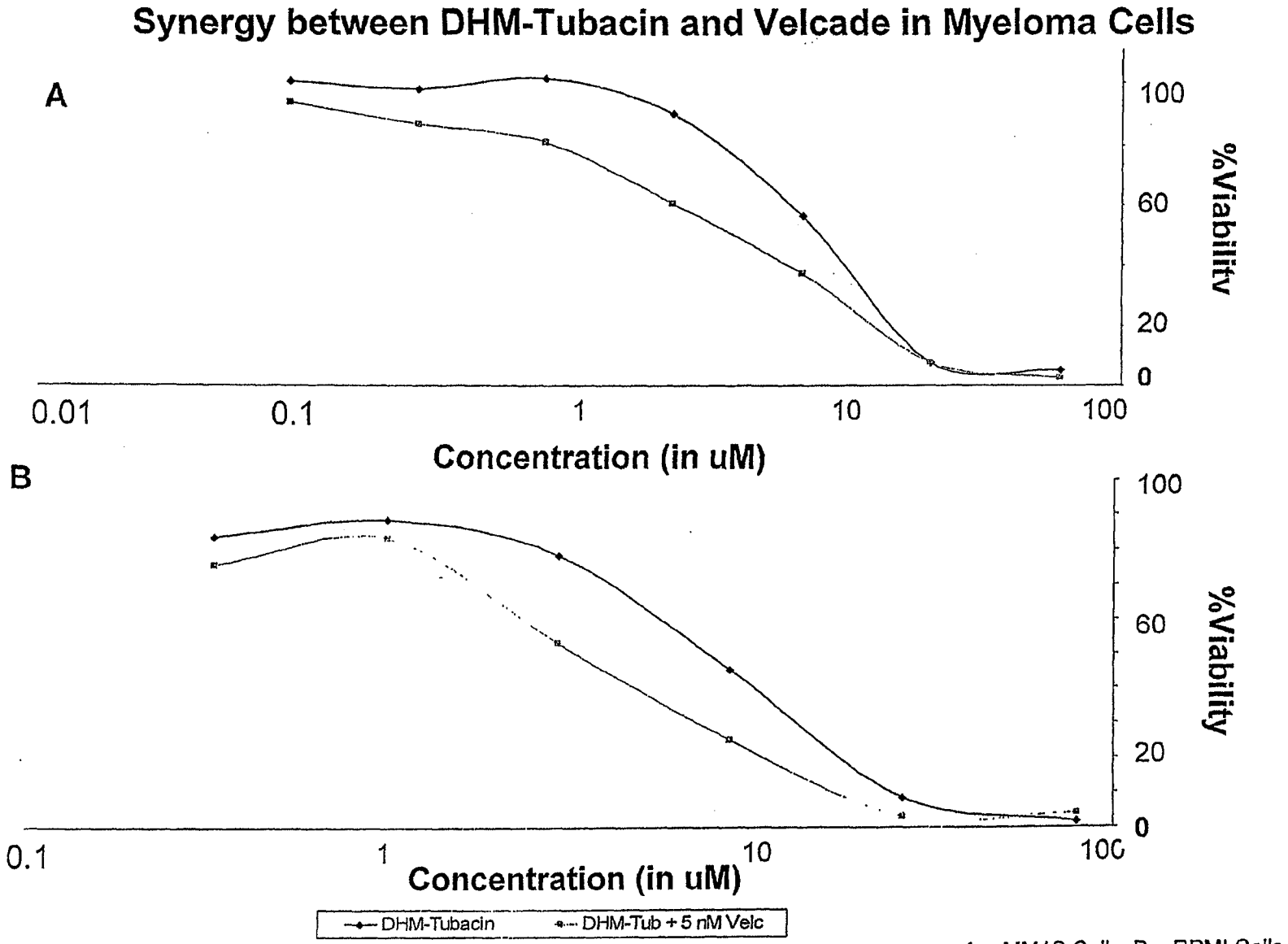
Fig. 25



DHM-Tubacin

Molecular Weight: 691.84

Fig. 26



A = MM1S Cells, B = RPMI Cells

Fig. 27

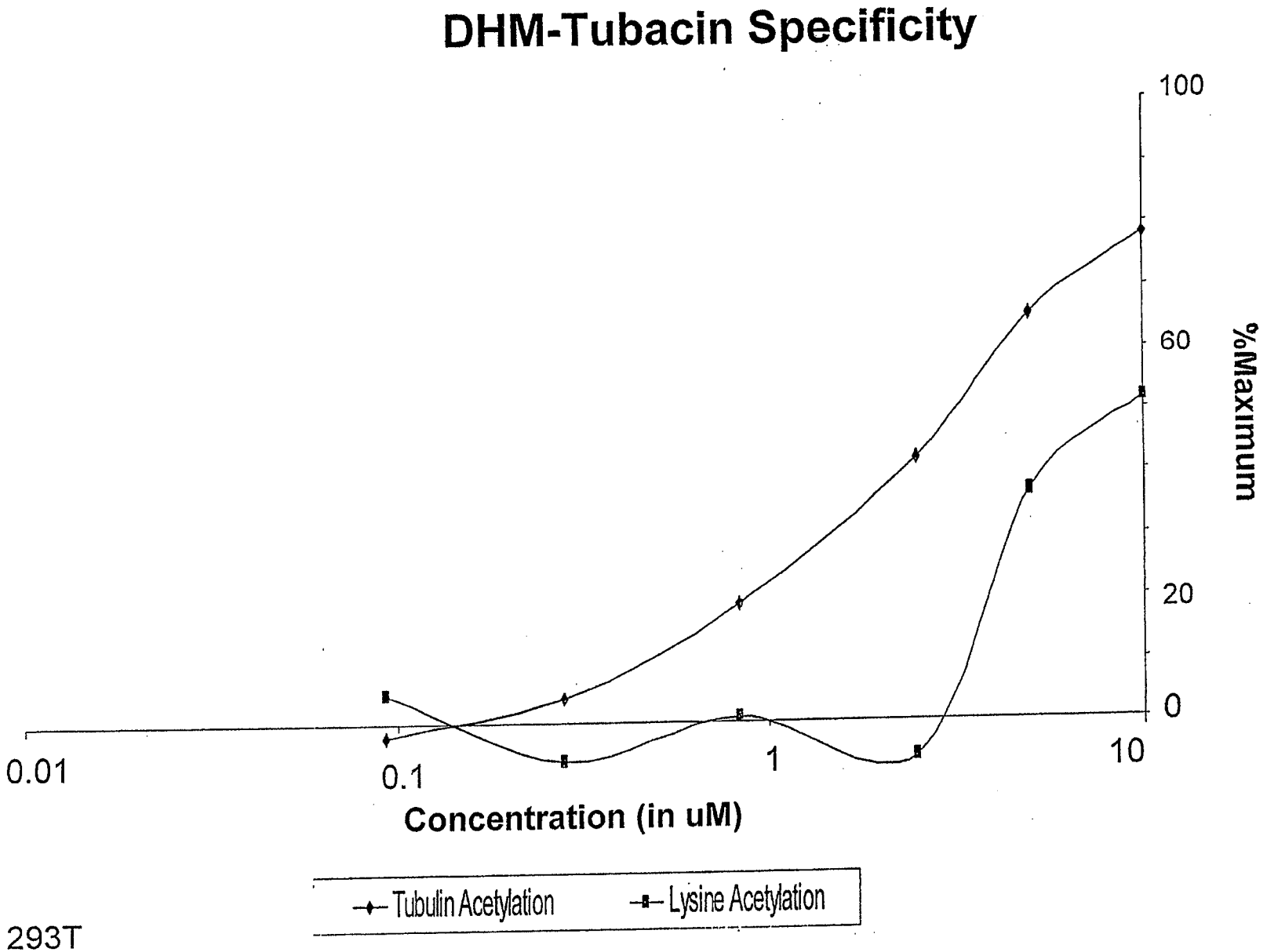

DHM-Tubacin Specificity
100
60
20
0
%Maximum
0.01
0.1
1
10
Concentration (in uM)
Tubulin Acetylation
Lysine Acetylation
293T

Fig. 28

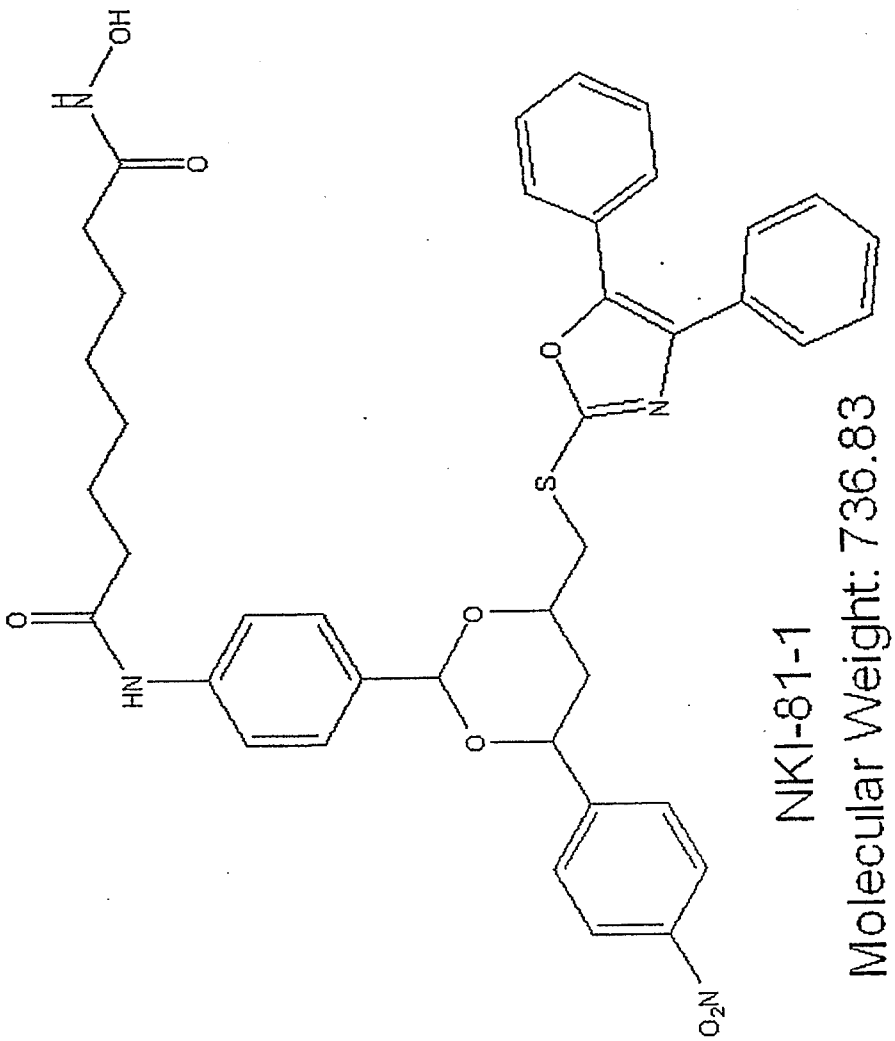


Fig. 29

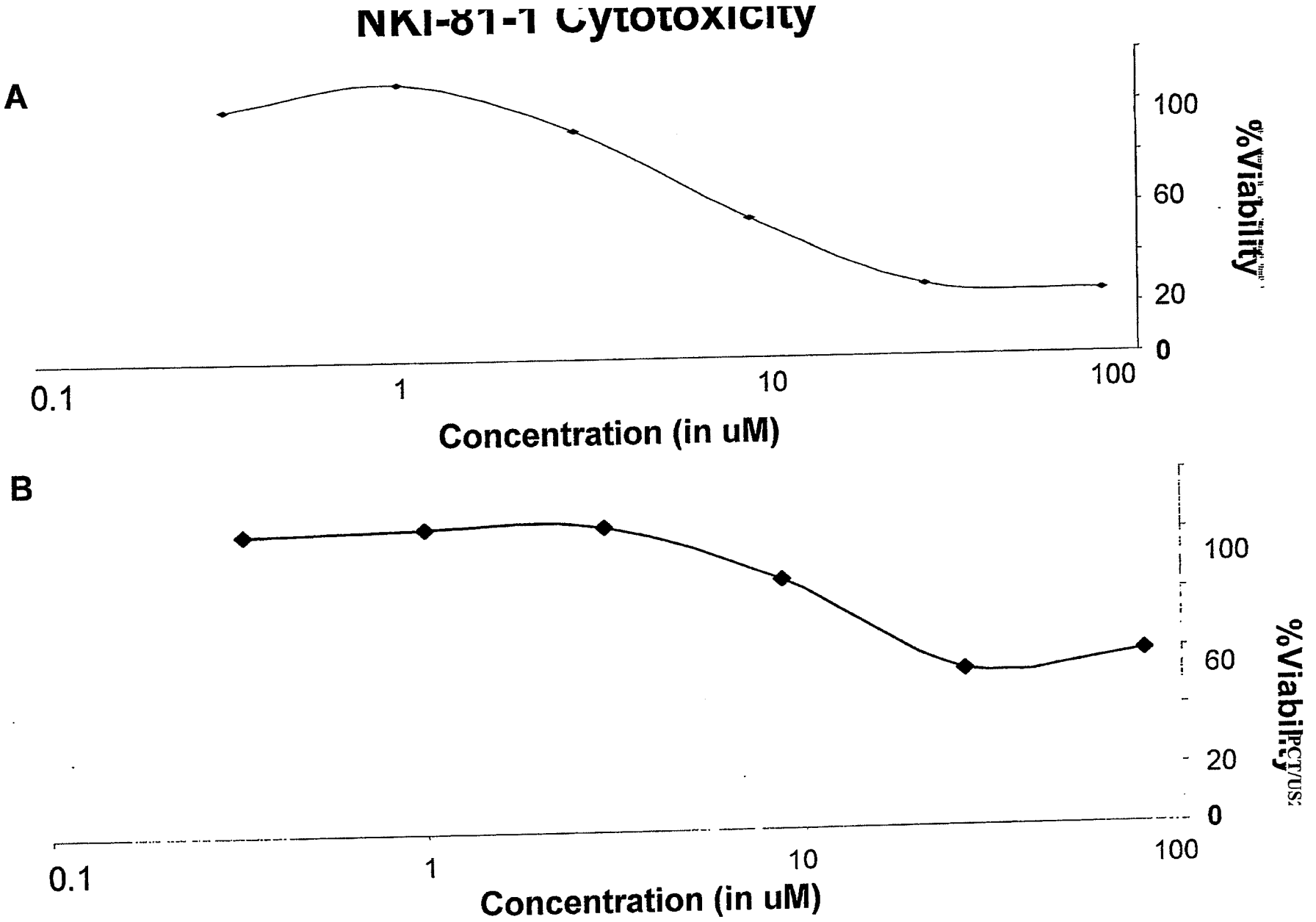

NK1-81-1 Cytotoxicity
A
100
60
20
0
%Viability
0.1
1
10
100
Concentration (in uM)
B
100
60
20
0
%Viability
0.1
1
10
100
Concentration (in uM)
A = MM1S Cells, B = RPMI Cells

Fig. 30

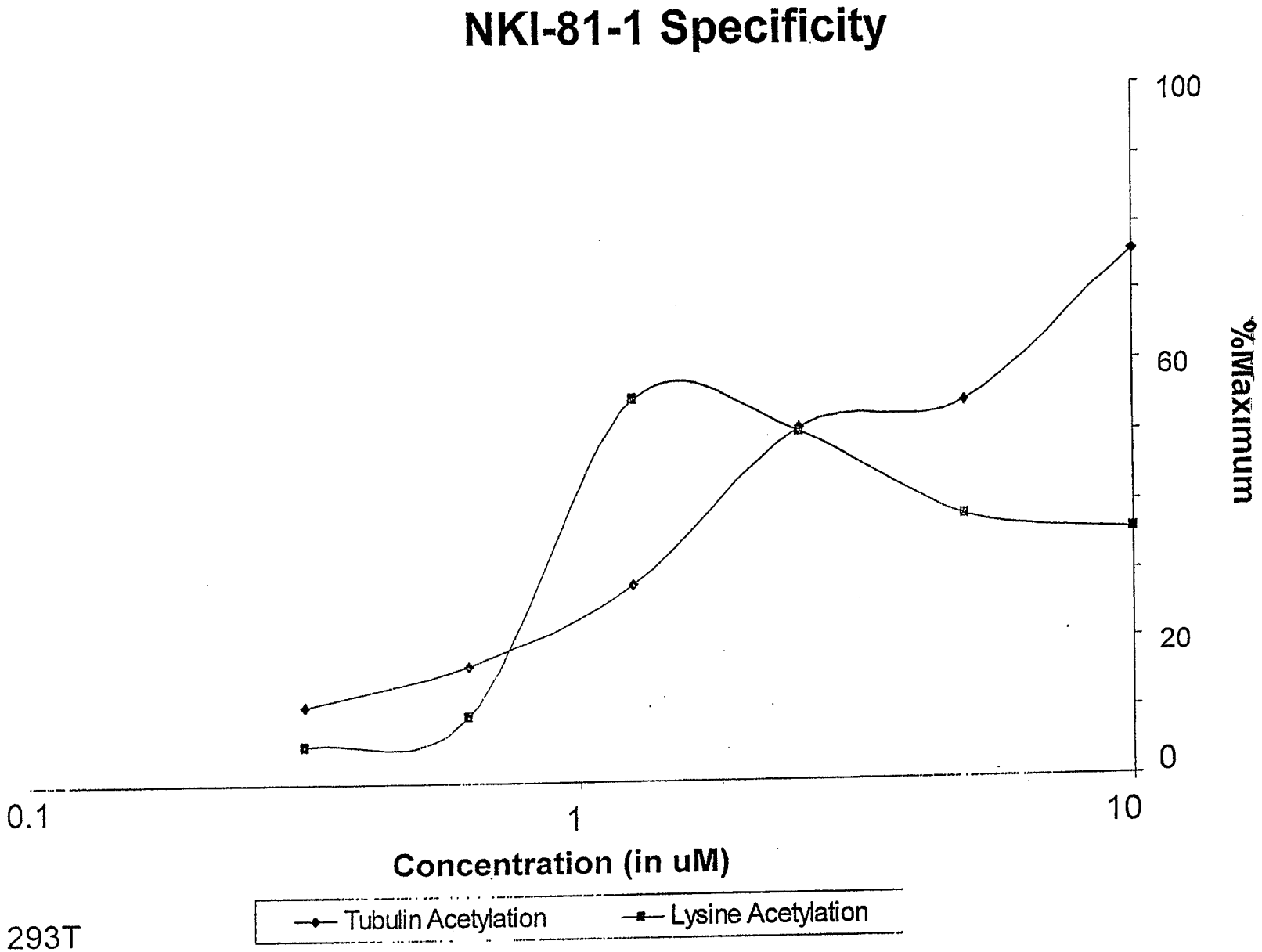
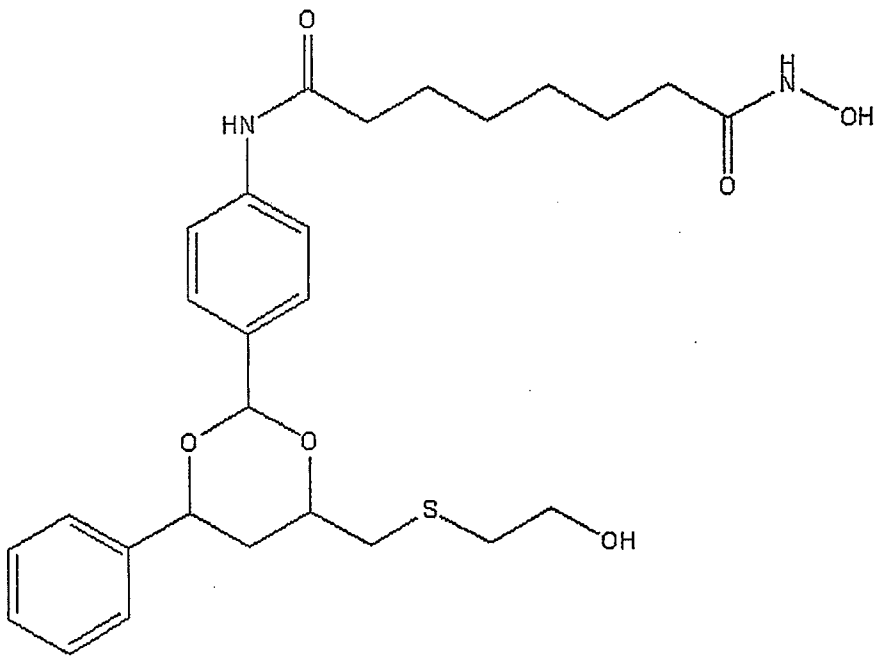

NKI-81-1 Specificity
100
60
20
0
%Maximum
0.1
1
10
Concentration (in uM)
Tubulin Acetylation
Lysine Acetylation
293T

Fig. 31



NKI-94-1

Molecular Weight: 516.65

Fig. 32

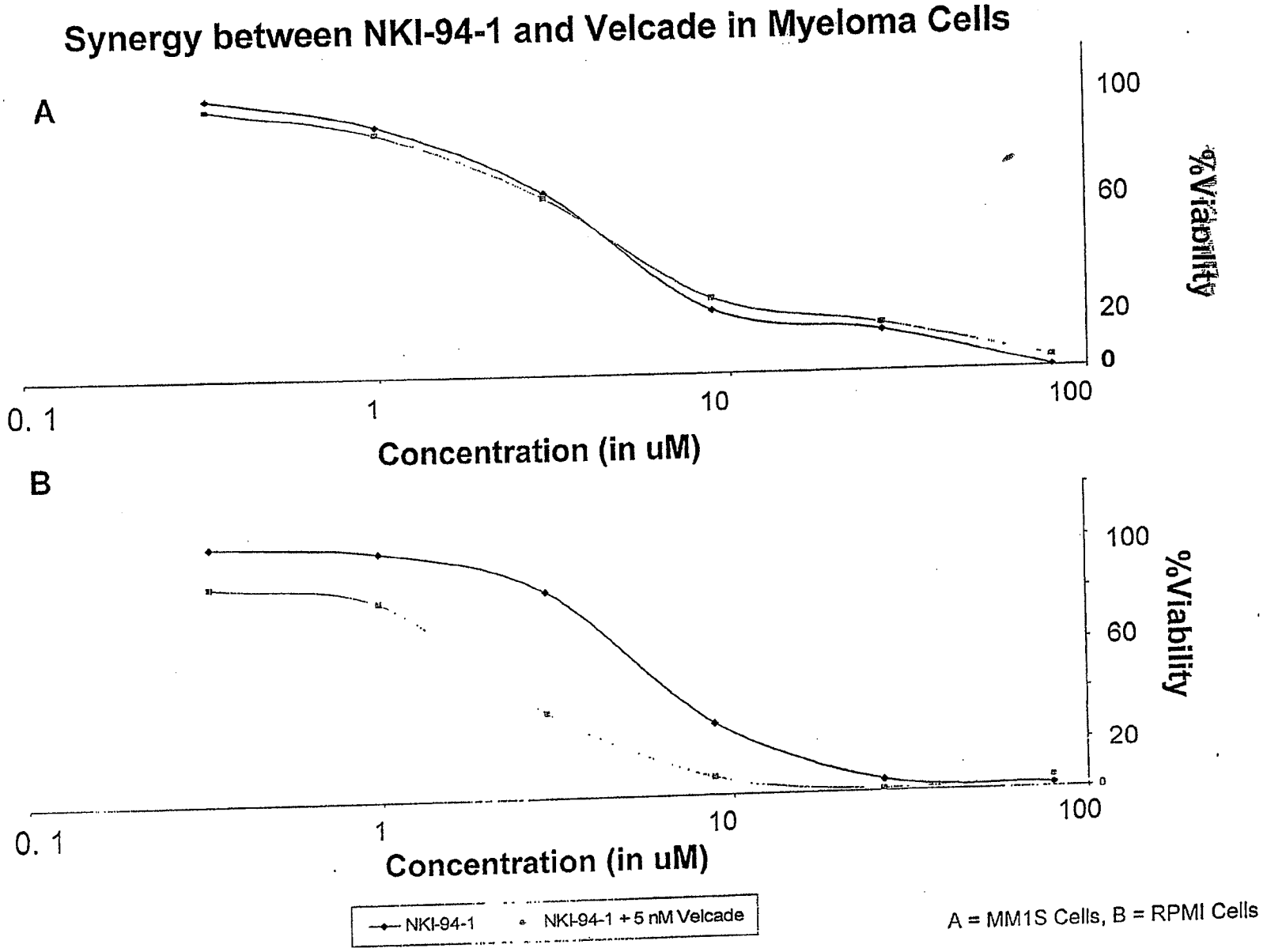

Synergy between NKI-94-1 and Velcade in Myeloma Cells
A
100
60
20
0
%Viability
0. 1
1
10
100
Concentration (in uM)
B
100
60
20
0
%Viability
0. 1
1
10
100
Concentration (in uM)
NKI-94-1
NKI-94-1 + 5 nM Velcade
A = MM1S Cells, B = RPMI Cells

Fig. 33

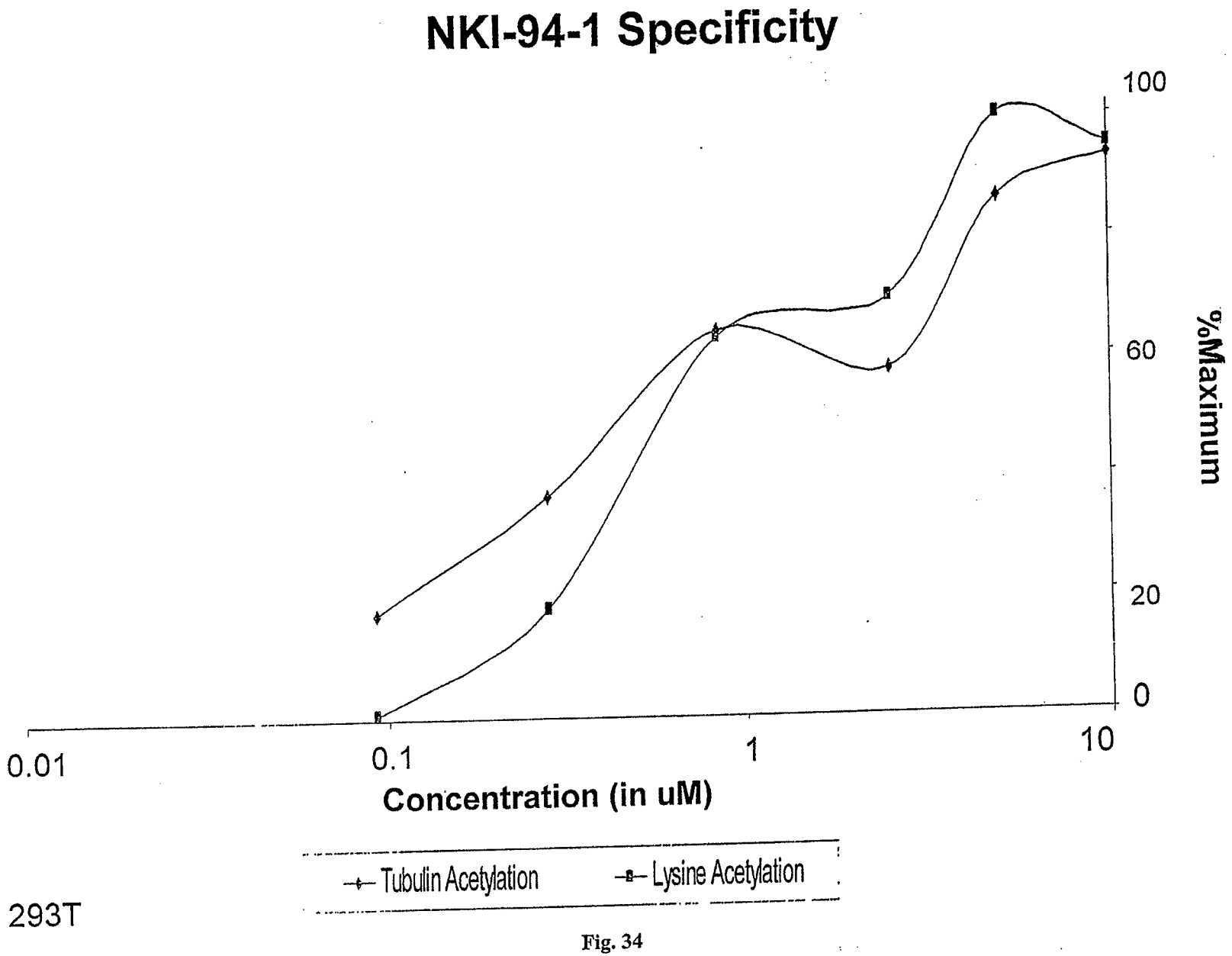

NKI-94-1 Specificity
100
60
20
0
%Maximum
0.01
0.1
1
10
Concentration (in uM)
Tubulin Acetylation
Lysine Acetylation
293T

Fig. 34

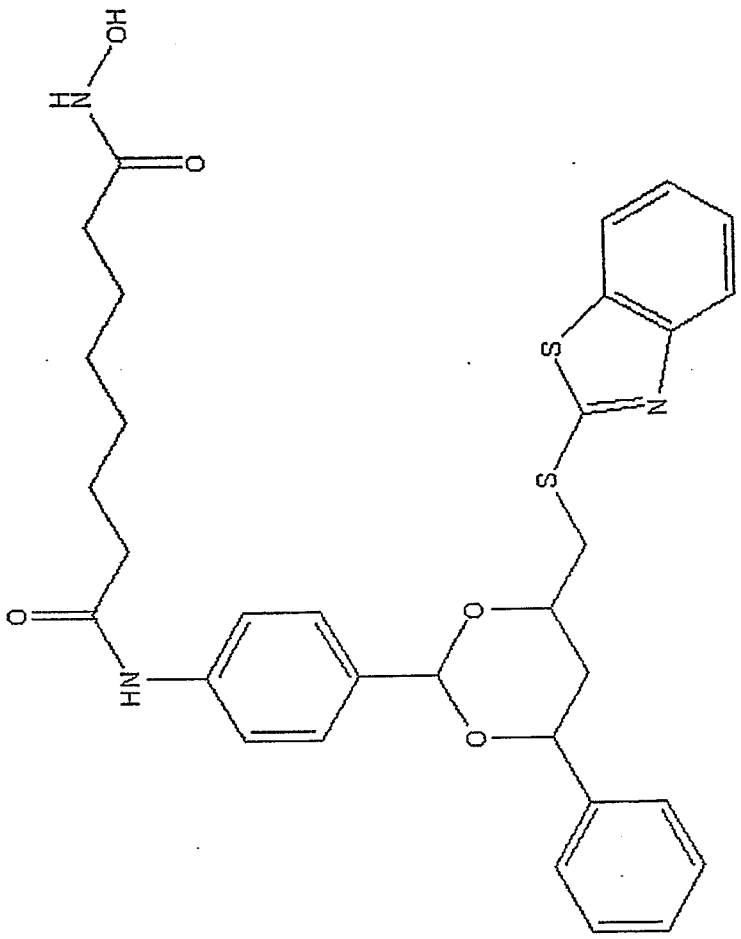


Fig. 35

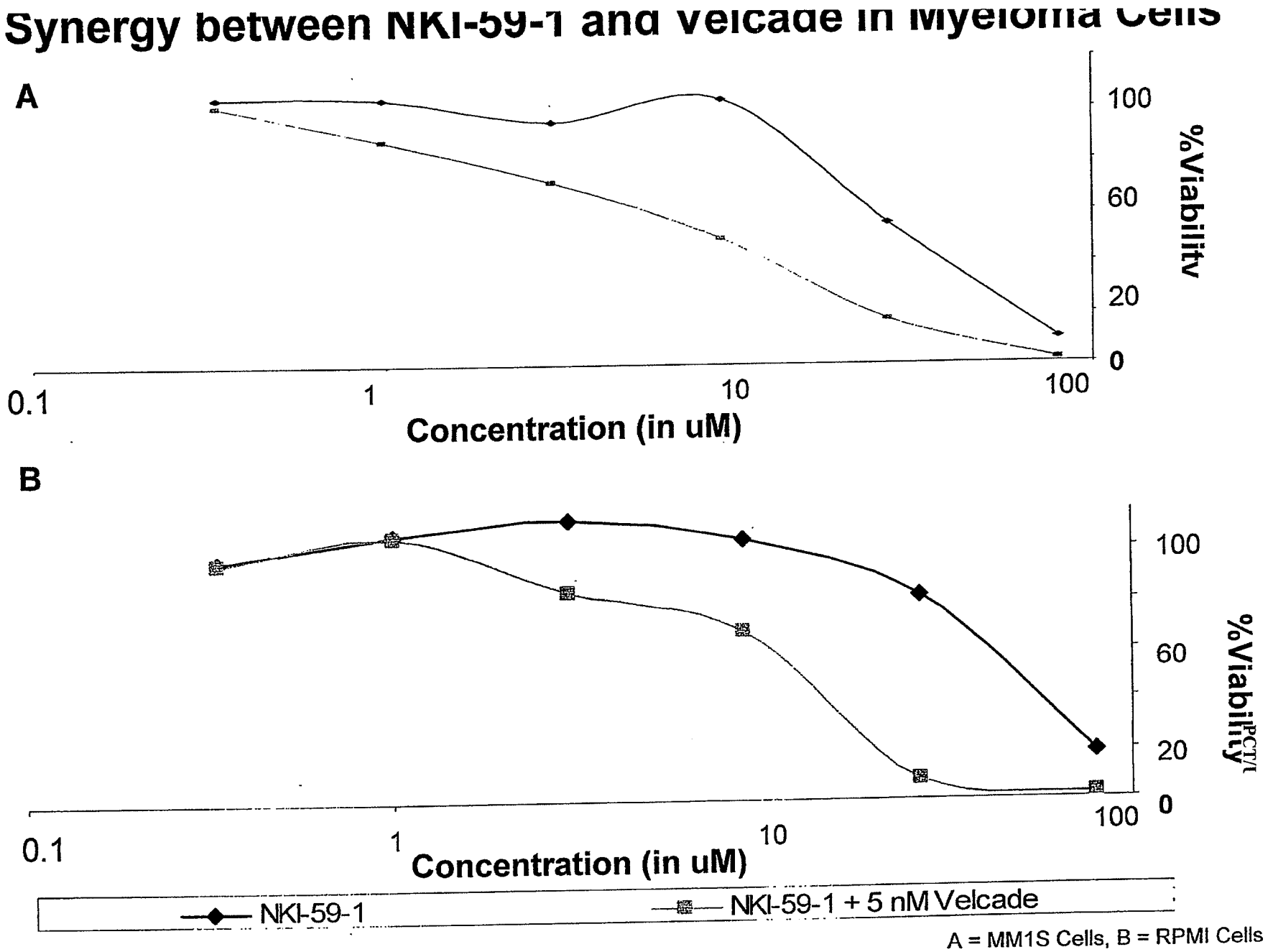

Synergy between NKI-59-1 and Velcade in Myeloma Cells
A
100
60
20
0
%Viability
0.1
1
10
100
Concentration (in uM)
B
100
60
20
0
%Viability
0.1
1
10
100
Concentration (in uM)
NKI-59-1
NKI-59-1 + 5 nM Velcade
A = MM1S Cells, B = RPMI Cells

Fig. 36

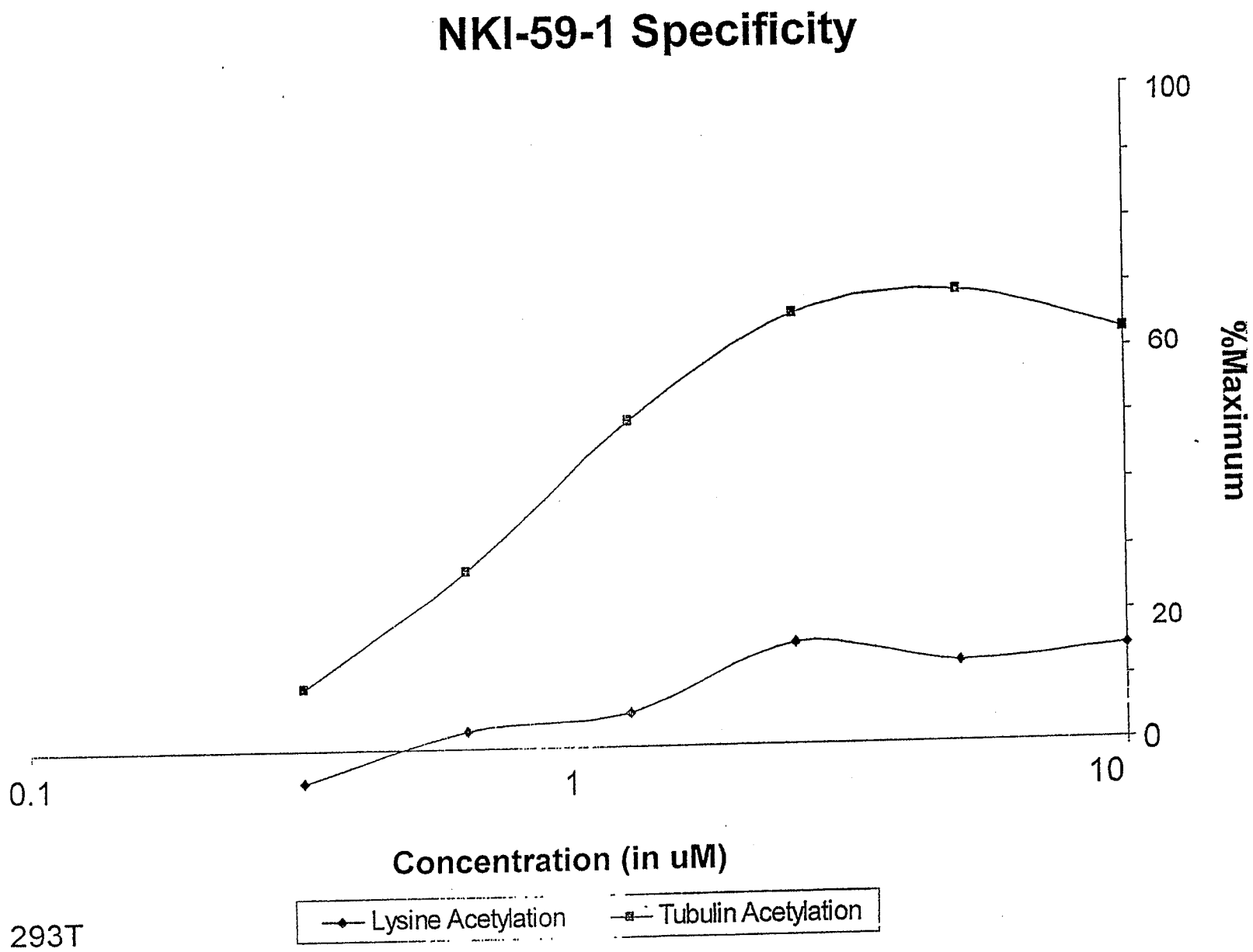
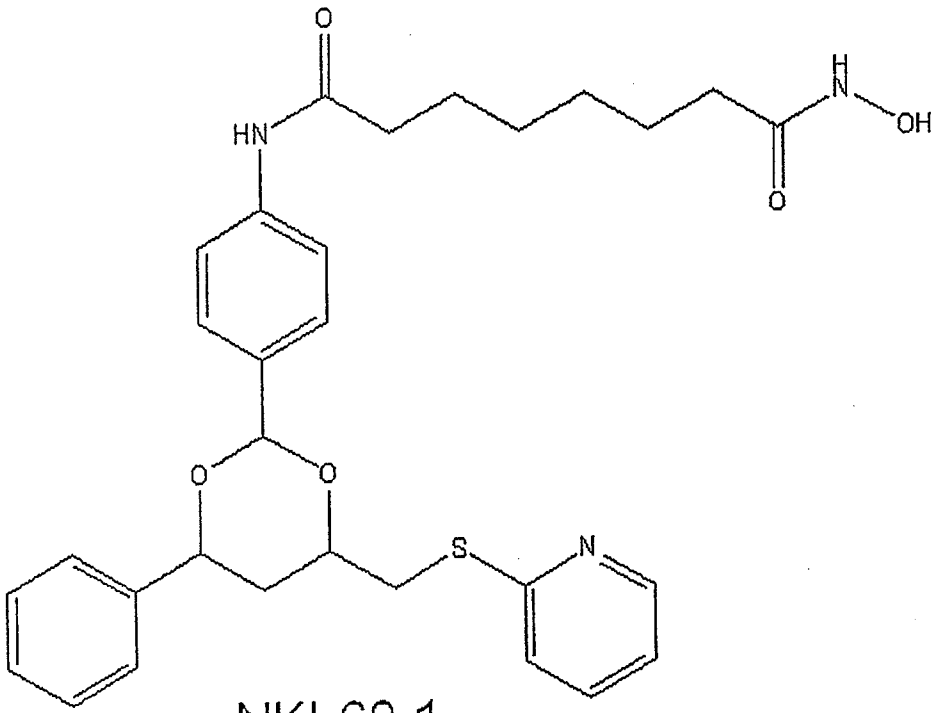

NKI-59-1 Specificity
100
60
20
0
%Maximum
0.1
1
10
Concentration (in uM)
293T
Lysine Acetylation
Tubulin Acetylation

Fig. 37



NKI-60-1

Molecular Weight: 549.68

Fig. 38

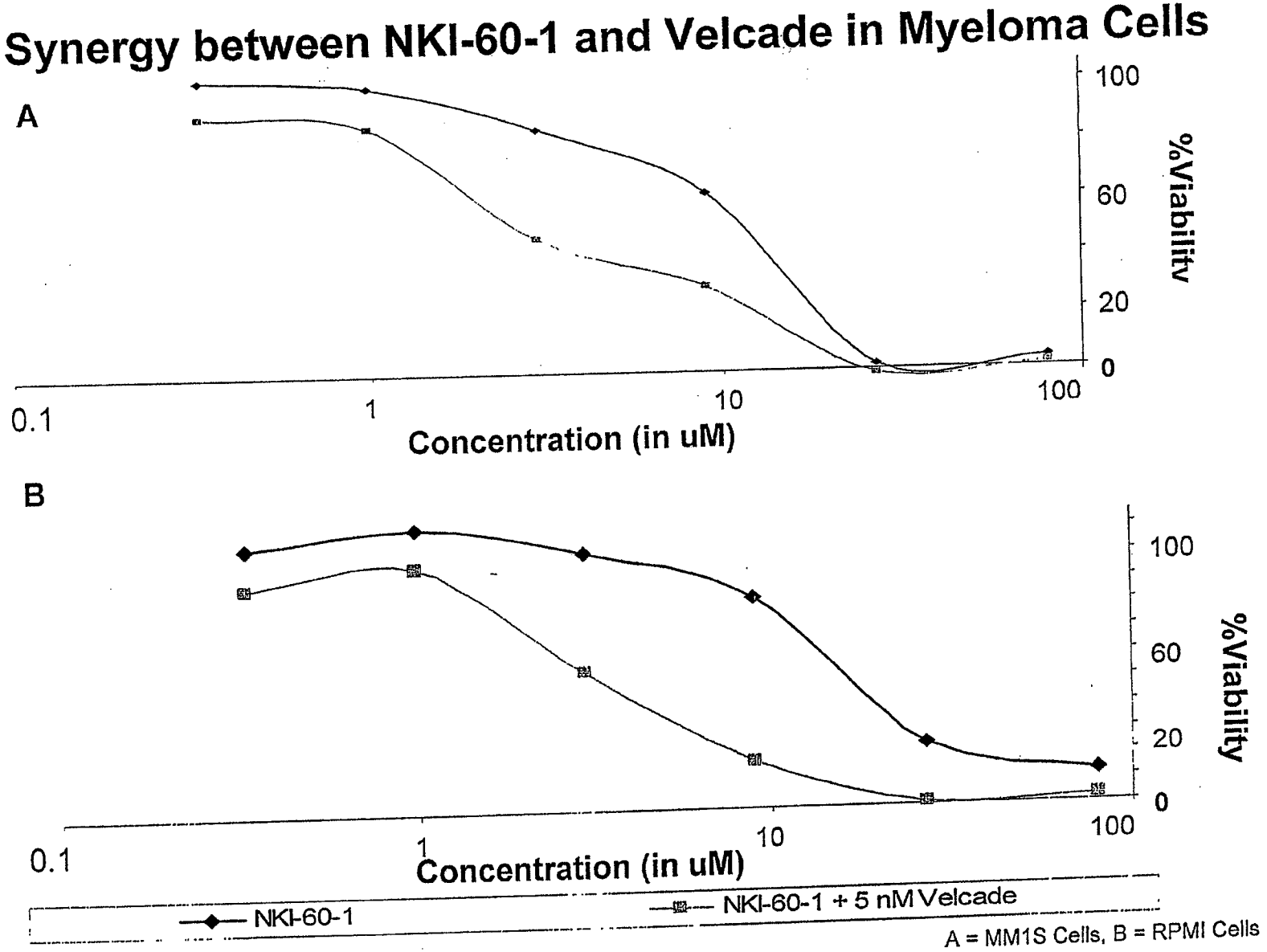

Synergy between NKI-60-1 and Velcade in Myeloma Cells
A
100
60
20
0
%Viability
0.1
1
10
100
Concentration (in uM)
B
100
60
20
0
%Viability
0.1
1
10
100
Concentration (in uM)
NKI-60-1
NKI-60-1 + 5 nM Velcade
A = MM1S Cells, B = RPMI Cells

Fig. 39

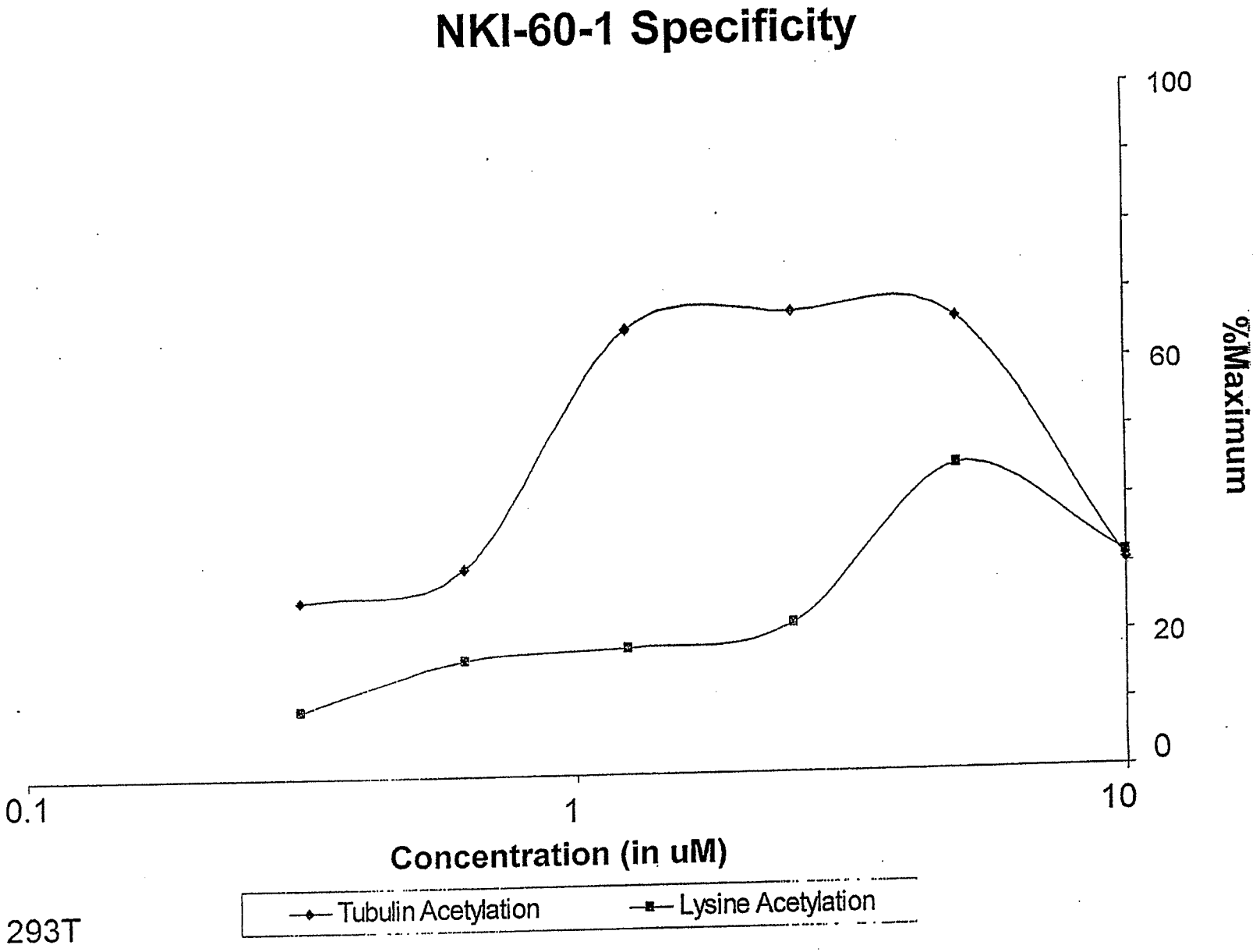
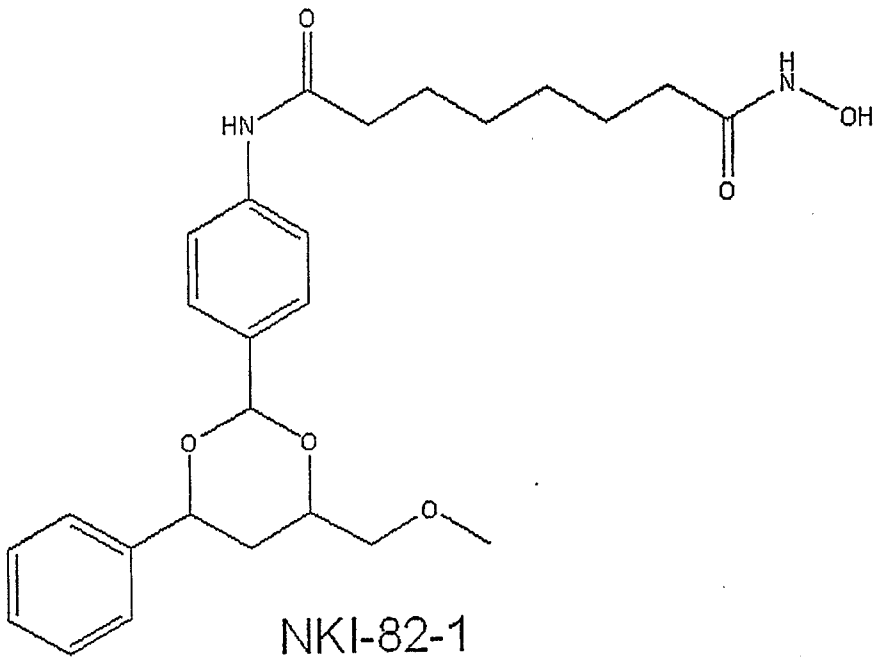

NKI-60-1 Specificity
100
60
20
0
%Maximum
0.1
1
10
Concentration (in uM)
Tubulin Acetylation
Lysine Acetylation
293T

Fig. 40



NKI-82-1

Molecular Weight: 470.56

Fig. 41

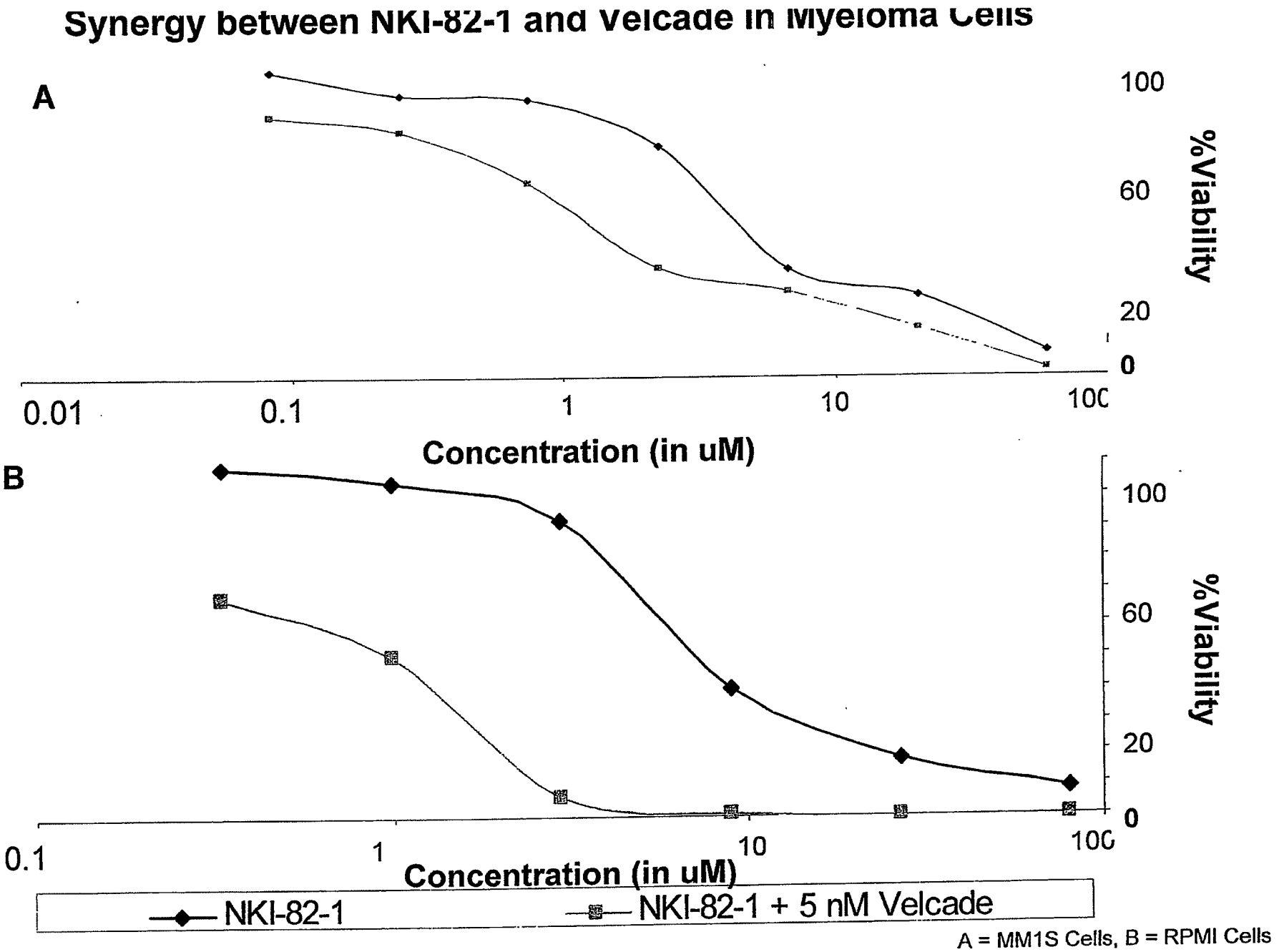

Synergy between NKI-82-1 and Velcade in Myeloma Cells
A
100
60
20
0
%Viability
0.01
0.1
1
10
100
Concentration (in uM)
B
100
60
20
0
%Viability
0.1
1
10
100
Concentration (in uM)
NKI-82-1
NKI-82-1 + 5 nM Velcade
A = MM1S Cells, B = RPMI Cells

Fig. 42

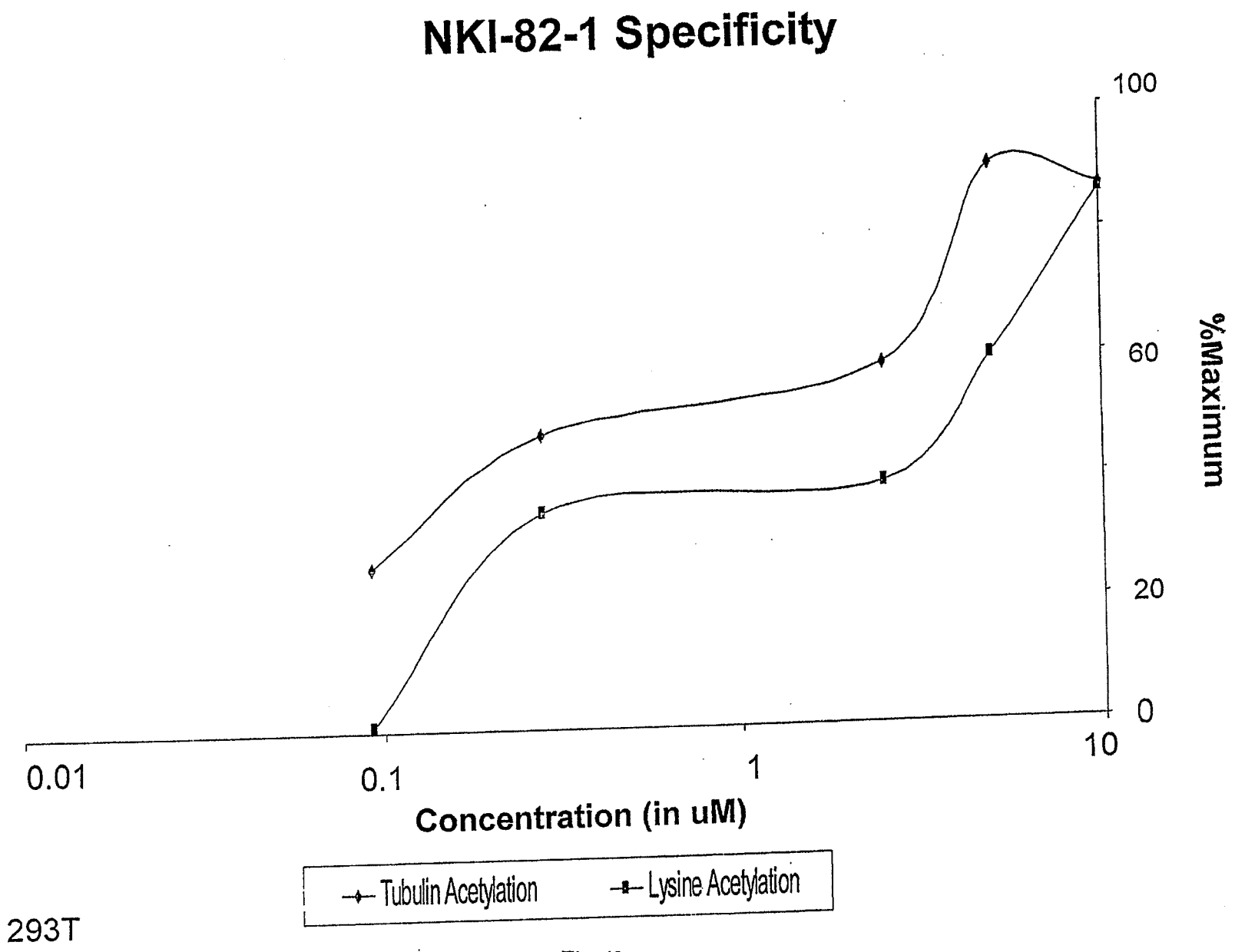
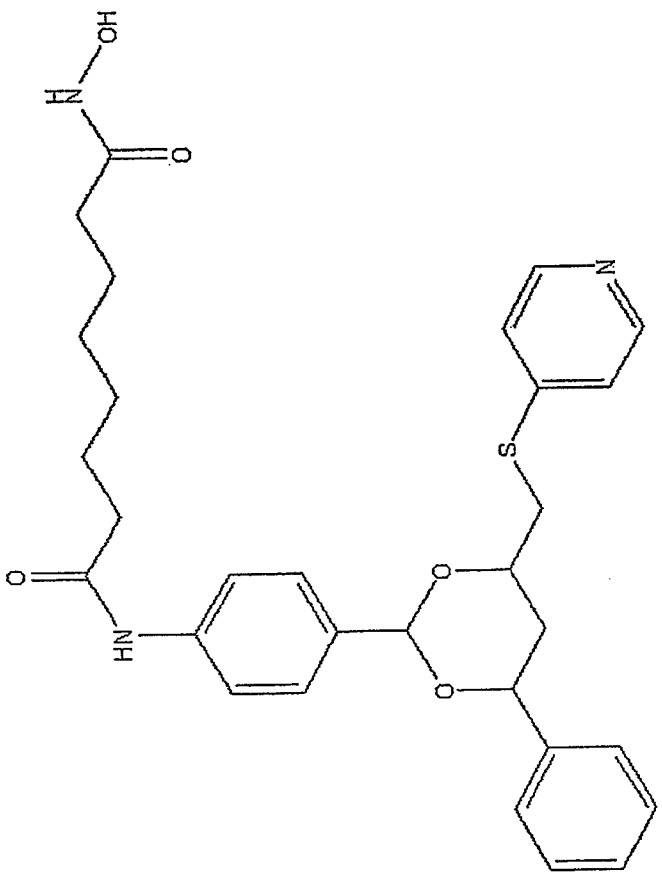

NKI-82-1 Specificity
100
60
20
0
%Maximum
0.01
0.1
1
10
Concentration (in uM)
Tubulin Acetylation
Lysine Acetylation
293T

Fig. 43



NKI-84-1

Molecular Weight: 549.68

Fig. 44

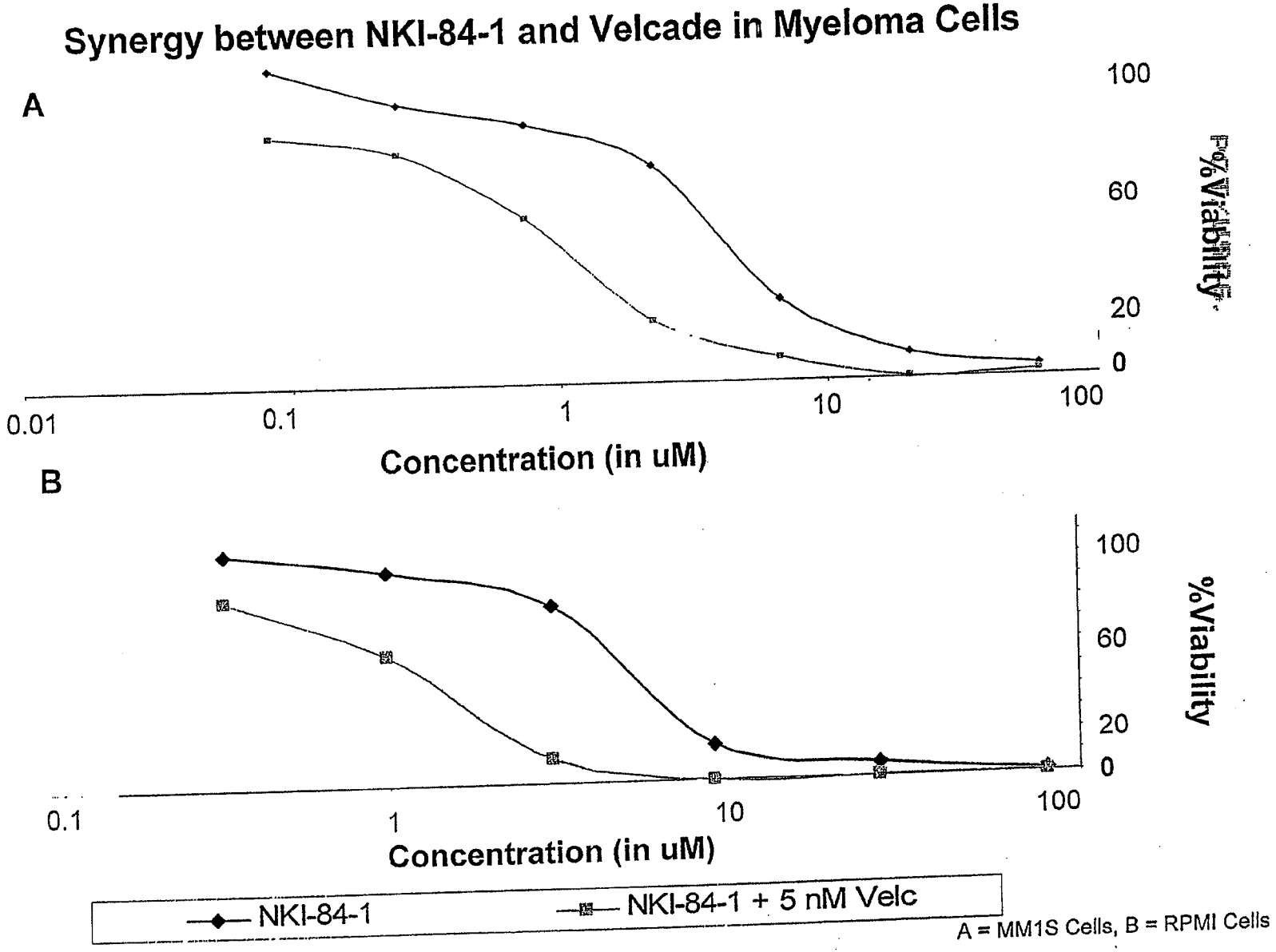

Synergy between NKI-84-1 and Velcade in Myeloma Cells
A
100
60
20
0
%Viability
0.01
0.1
1
10
100
Concentration (in uM)
B
100
60
20
0
%Viability
0.1
1
10
100
Concentration (in uM)
NKI-84-1
NKI-84-1 + 5 nM Velc
A = MM1S Cells, B = RPMI Cells

Fig. 45

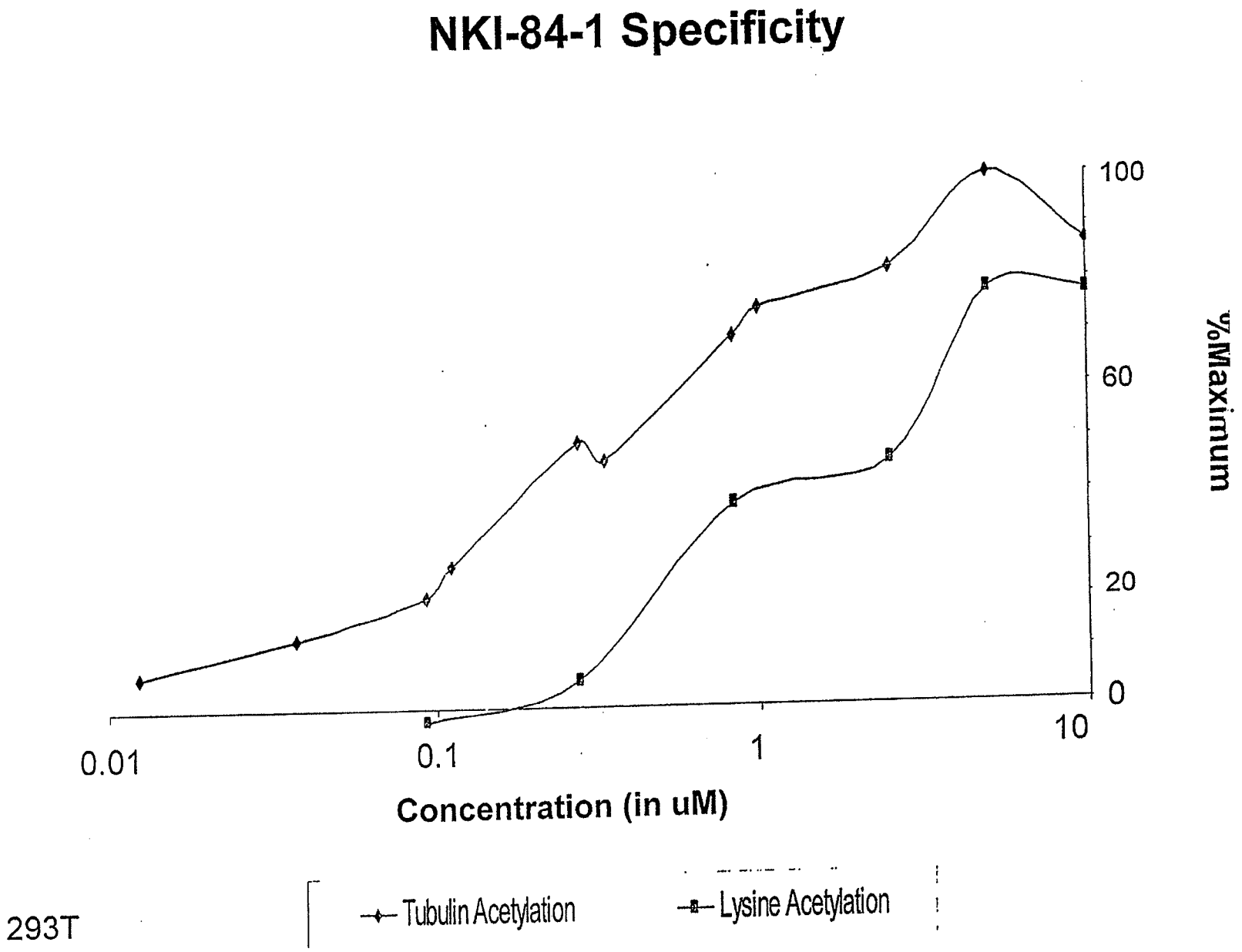
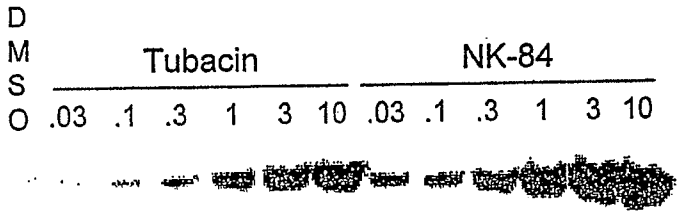

NKI-84-1 Specificity
100
60
20
0
%Maximum
0.01
0.1
1
10
Concentration (in uM)
293T
Tubulin Acetylation
Lysine Acetylation

Fig. 46

Effect of Tubacin and NK-84 on Tubulin Acetylation



RPMI-8226

Fig. 47

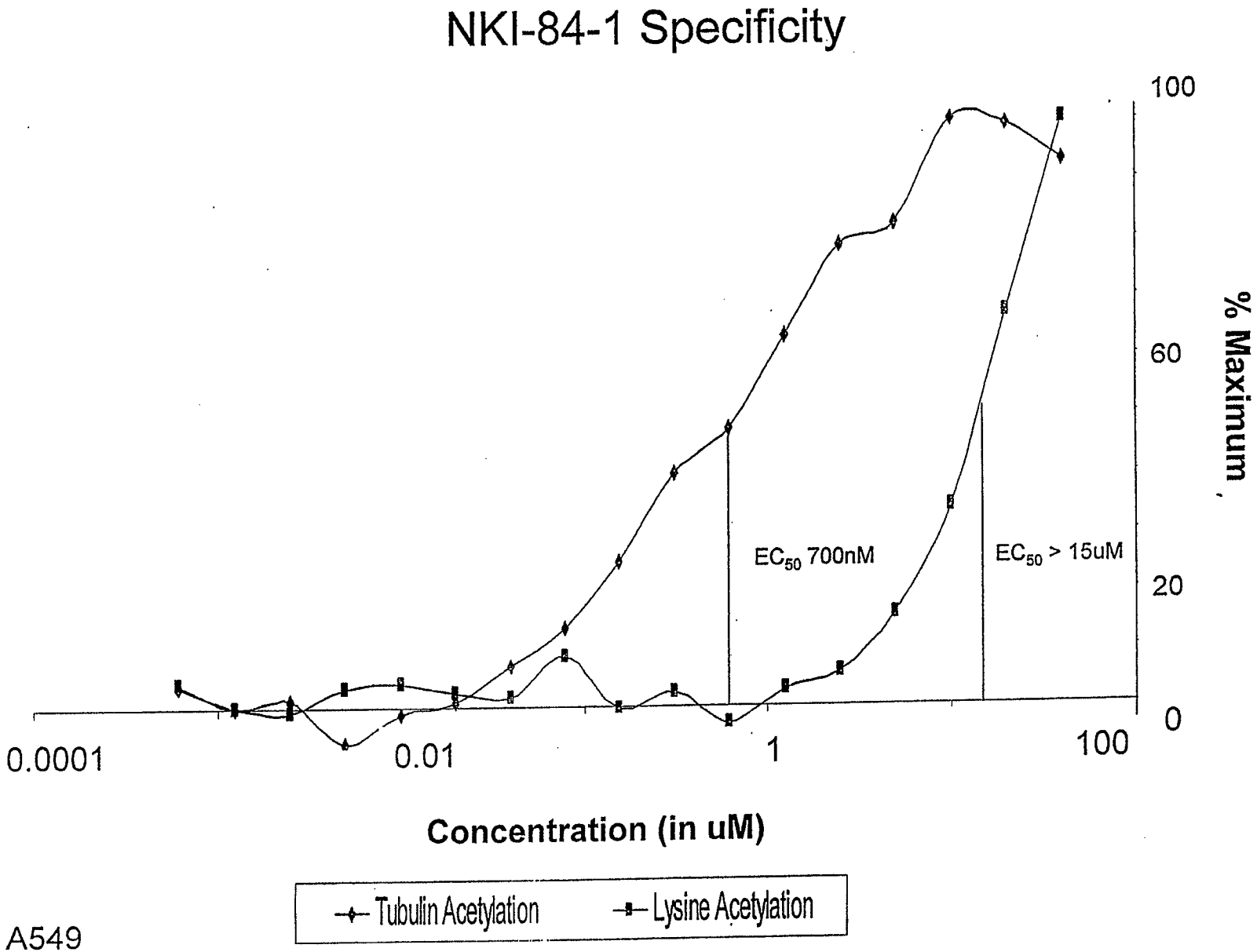

NKI-84-1 Specificity
100
60
20
0
% Maximum
EC50 700nM
EC50 > 15uM
0.0001
0.01
1
100
Concentration (in uM)
Tubulin Acetylation
Lysine Acetylation
A549

Fig. 48

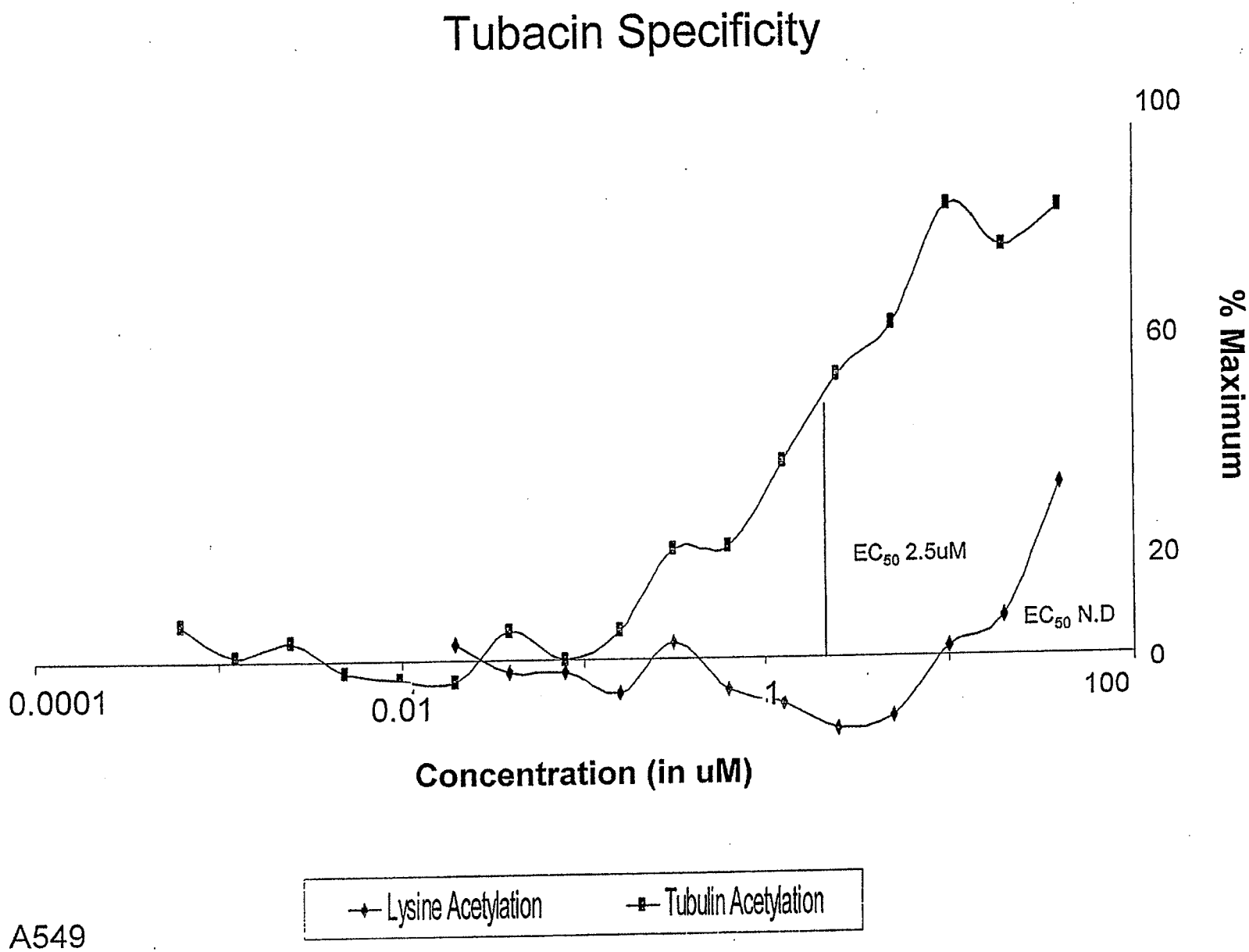
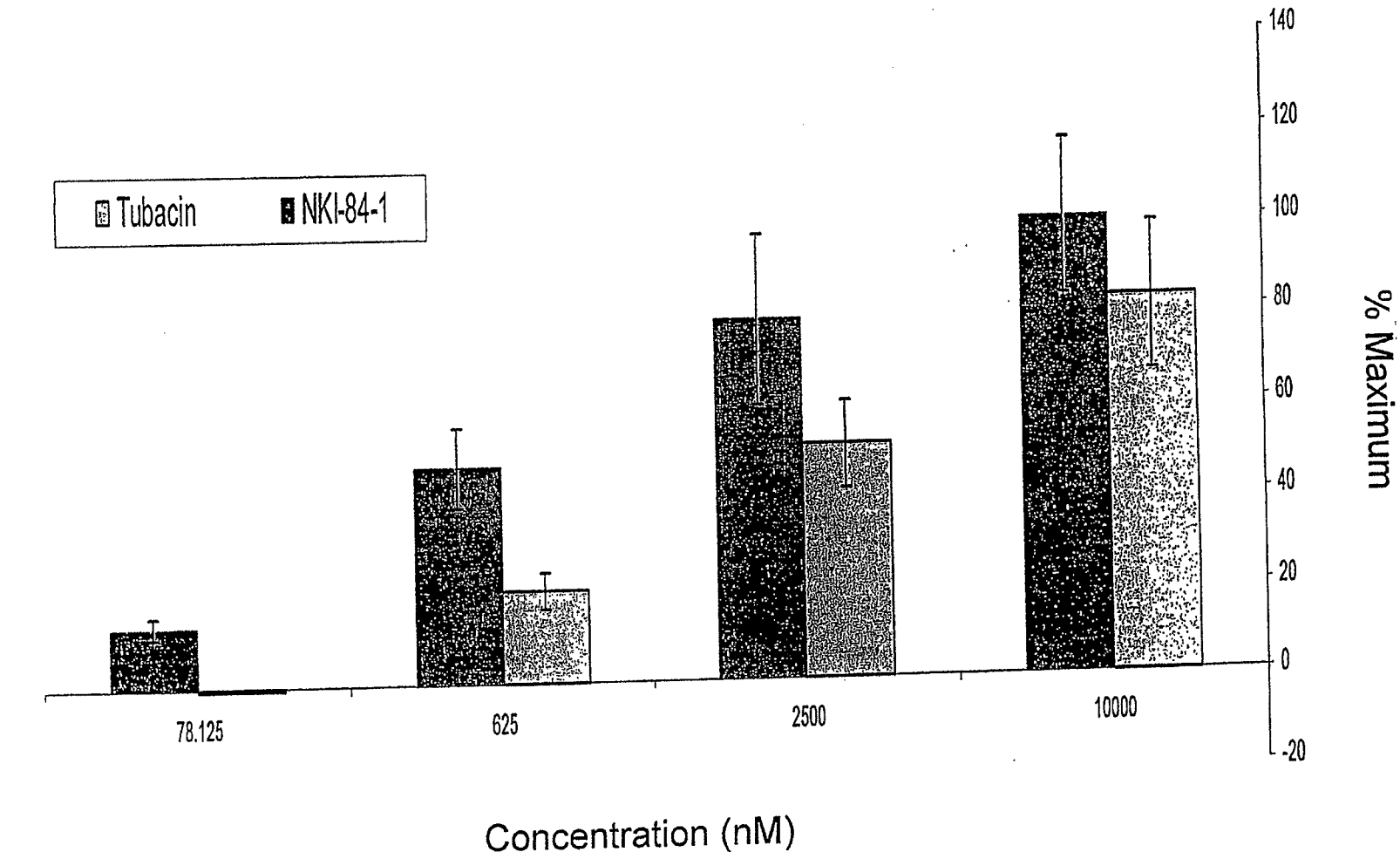
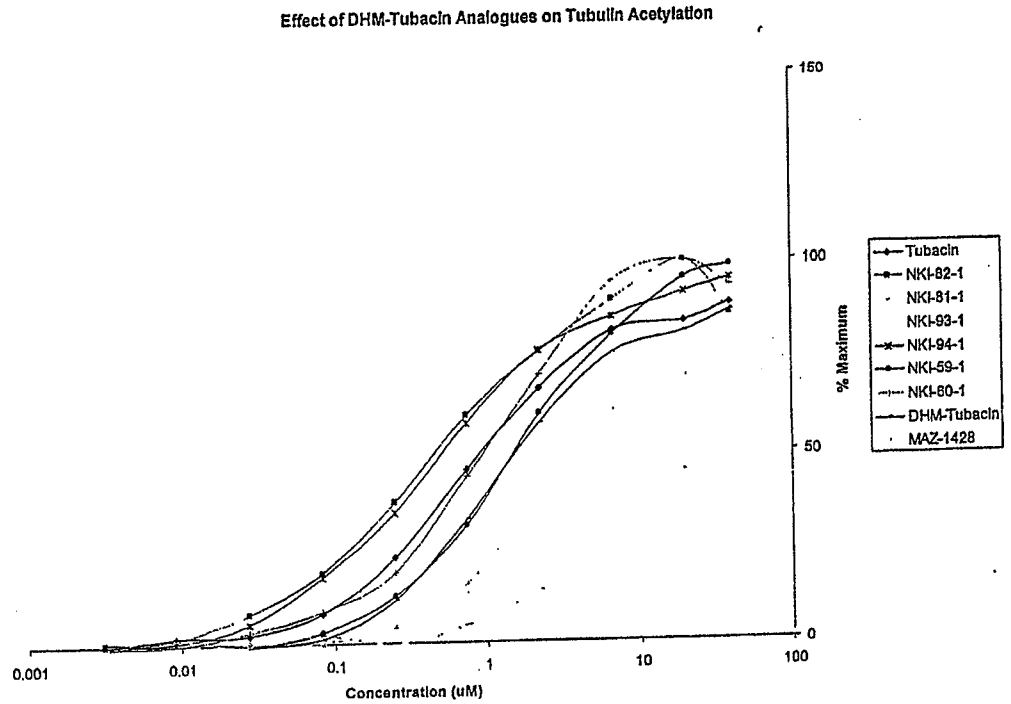

Tubacin Specificity
100
60
20
0
% Maximum
EC50 2.5uM
EC50 N.D
0.0001
0.01
1
100
Concentration (in uM)
Lysine Acetylation
Tubulin Acetylation
A549

Fig. 49


Effect of Tubacin and NKI-84-1 on Tubulin Acetylation
Tubacin
NKI-84-1
140
120
100
80
60
40
20
0
-20
% Maximum
78.125
625
2500
10000
Concentration (nM)

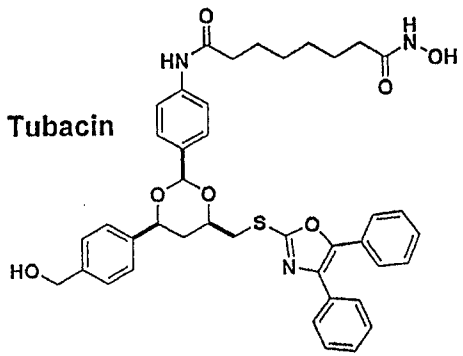
A549

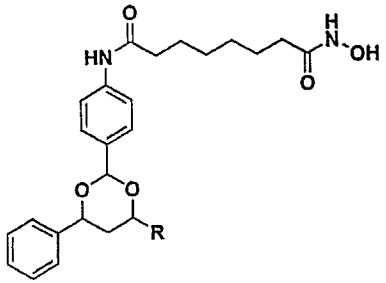
Fig. 50

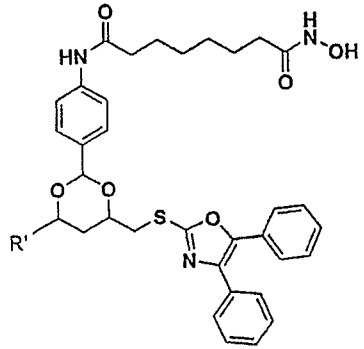


Series 1 Analogs: TDAC Inhibitory Activity

Fig. 51







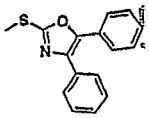
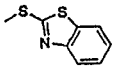
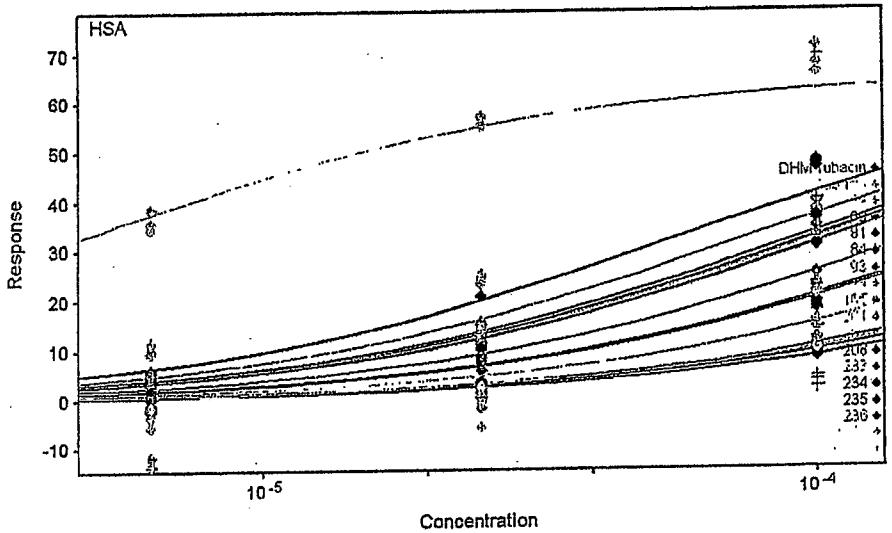
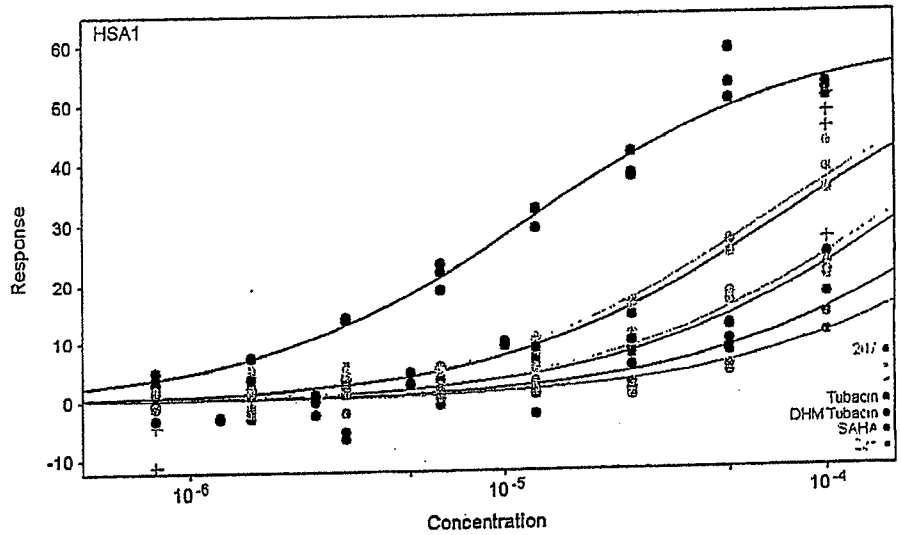
	HDAC Inhibition	TDAC Inhibition	
Tubacin	7.2 uM	0.9 uM	
DHM-Tubacin	25 uM	1.5 uM	
NKI-59-1	11 uM	1.5 uM	
NKI-60-1	3 uM	0.9 uM	
NKI-82-1	1.5 uM	0.45 uM	
NKI-84-1	**1.5 uM**	**160 nM**	
NKI-94-1	1.6 uM	0.5 uM	
NKI-81-1	13 uM	3.8 uM	

Fig. 52

	Average KD (uM)	Average KD (M)	Percent Bound
DHM Tubacin	96.85	9.69E-05	87.5%
Tubacin	5.53	5.53E-06	99.2%
59	100.8	1.01E-04	87.1%
60	67.3	6.73E-05	91.0%
81	244.25	2.44E-04	73.6%
84	80.85	8.09E-05	89.4%
94	95.8	9.58E-05	87.7%
201	73.7	7.37E-05	90.2%
207	202.75	2.03E-04	77.0%
208	184.75	1.85E-04	78.6%
233	99.8	9.98E-05	87.2%
234	563.5	5.64E-04	54.7%
235	676	6.76E-04	50.1%
236	108	1.08E-04	86.3%





ADME-Tox: HSA Binding by SPR

Fig. 53A

PCT/US06/10670

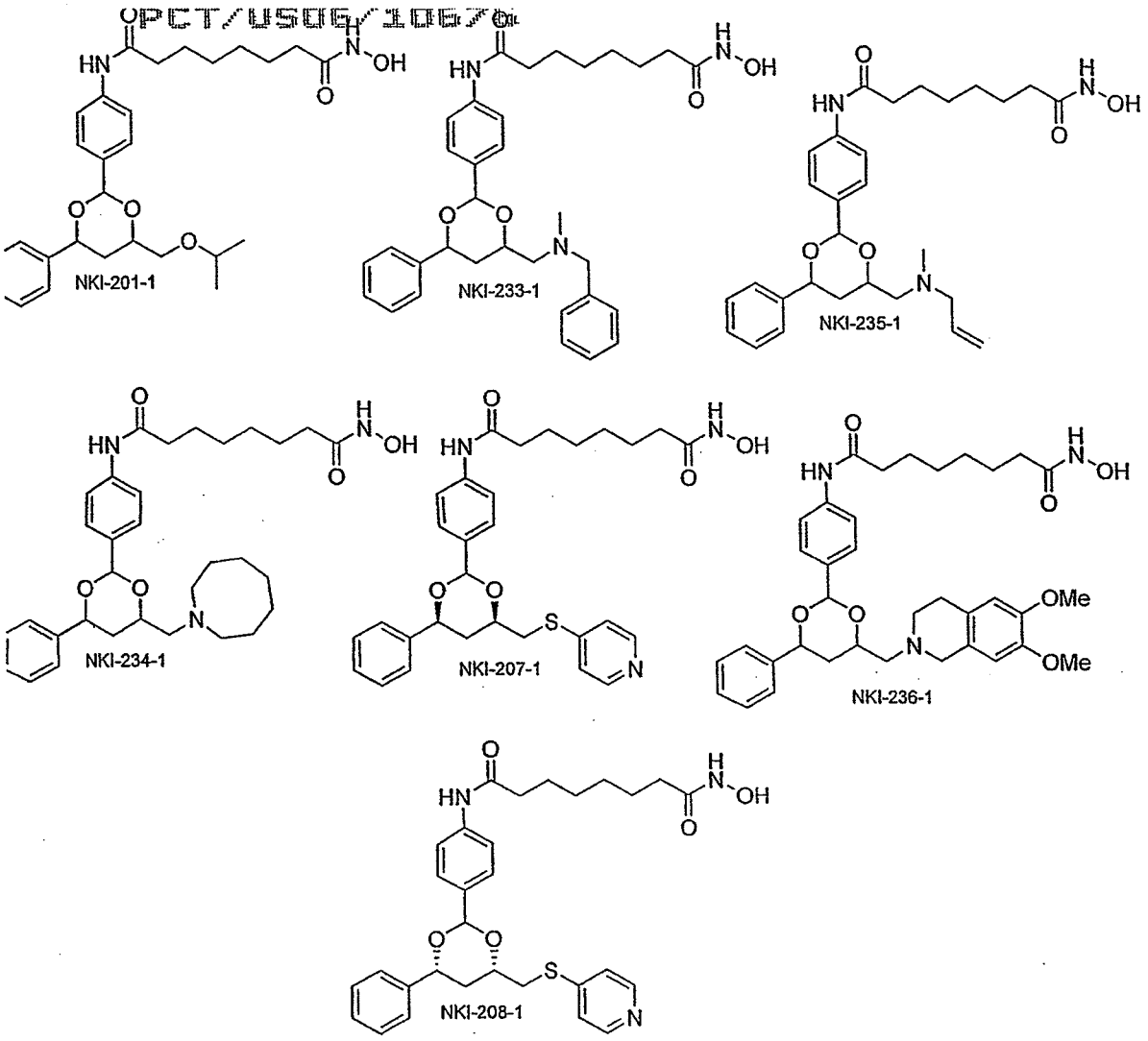
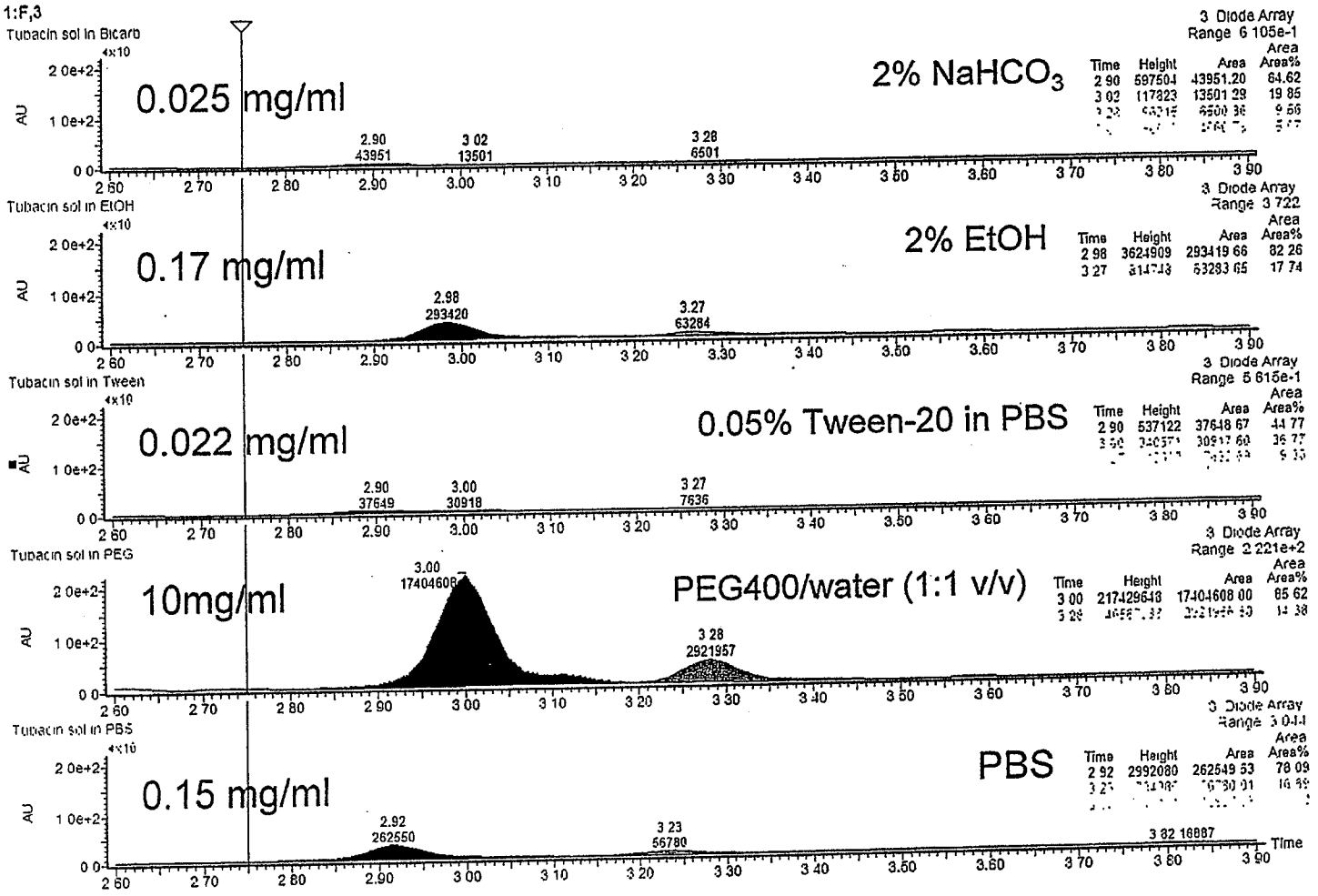


Fig. 53B



ADME: Solubility Testing for Mouse MM Model and PK

Fig. 54

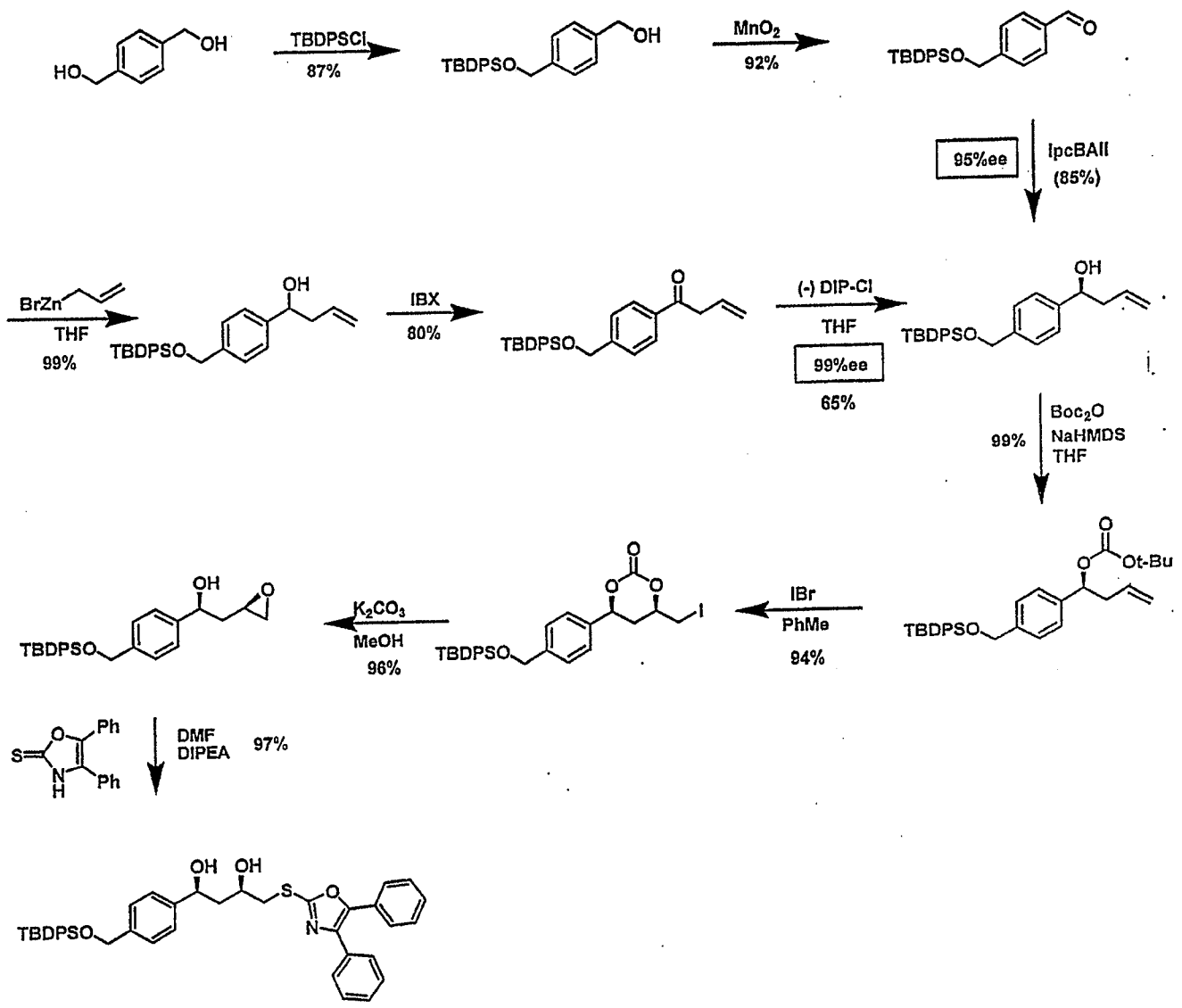

TBDPSCl
87%
MnO2
92%
95%ee
IpcBAll
(85%)
BrZn
THF
99%
IBX
80%
(-) DIP-Cl
THF
99%ee
65%
99%
Boc2O
NaHMDS
THF
IBr
PhMe
94%
K2CO3
MeOH
96%
DMF
DIPEA
97%
TBDPSO
Ot-Bu
Ph

Fig. 55A

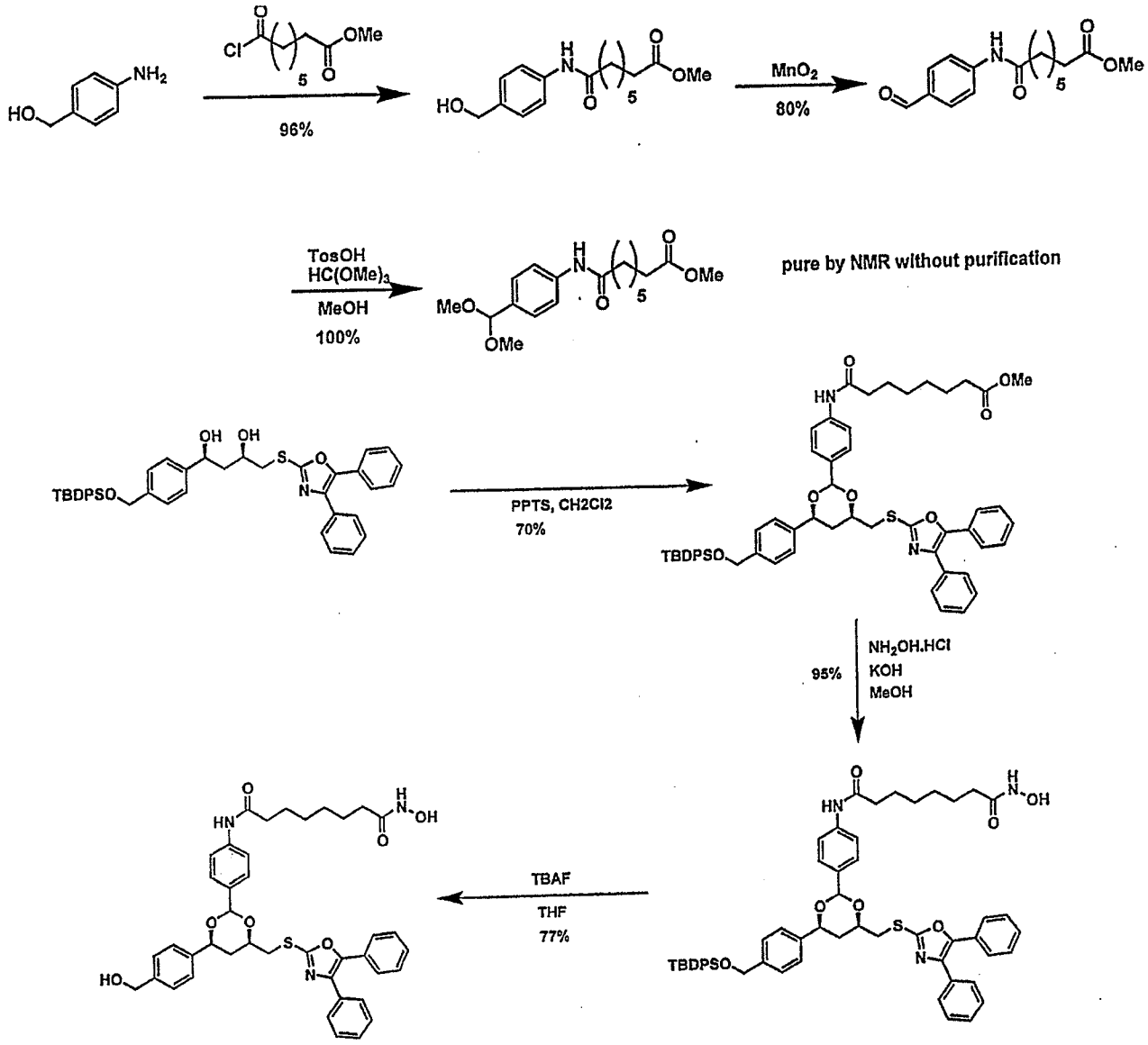

96%
MnO2
80%
TosOH
HC(OMe)3
MeOH
100%
pure by NMR without purification
PPTS, CH2Cl2
70%
NH2OH.HCl
KOH
MeOH
95%
TBAF
THF
77%

Fig. 55B

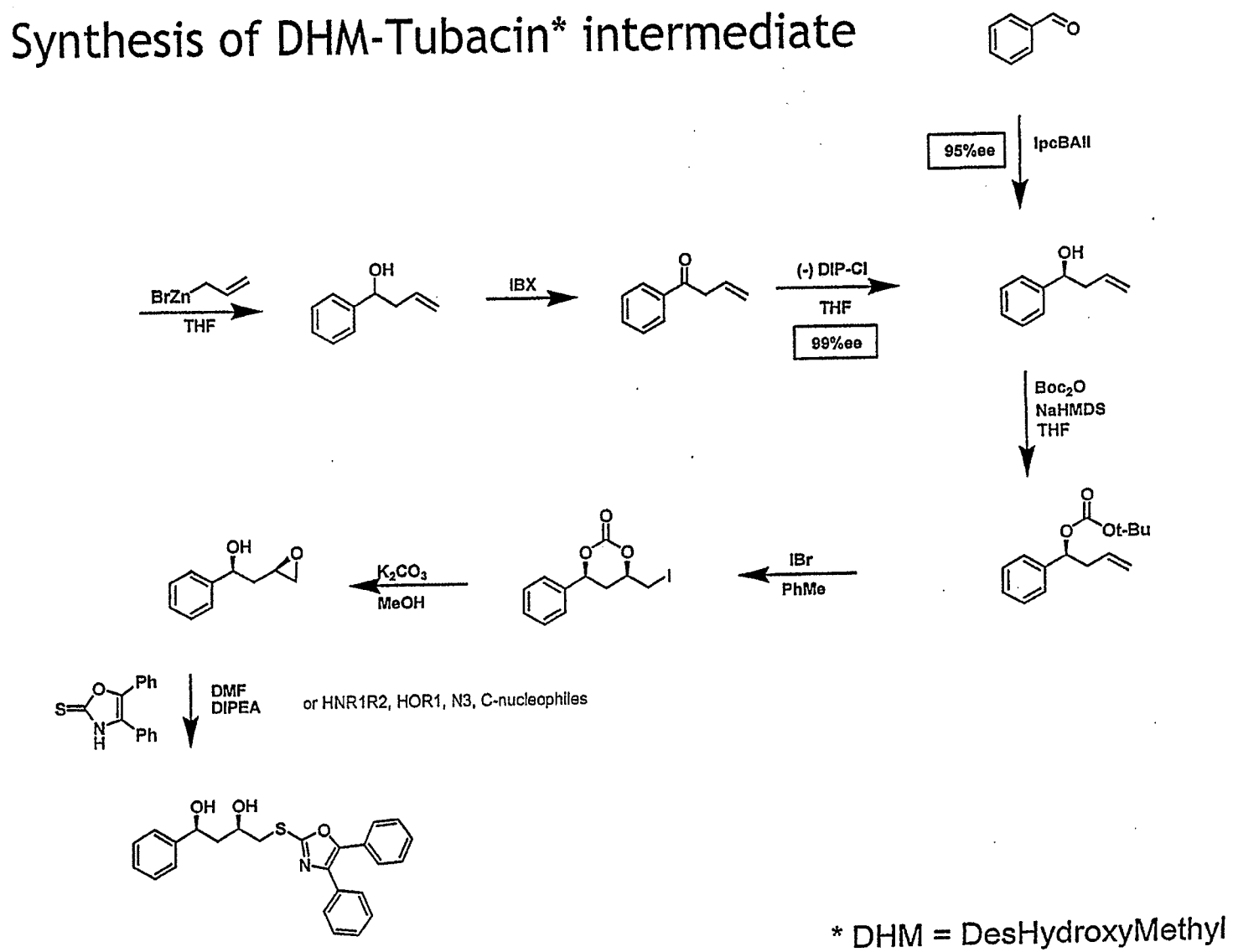

Synthesis of DHM-Tubacin* intermediate
95%ee
IpcBAll
BrZn
THF
OH
IBX
(-) DIP-Cl
THF
99%ee
Boc2O
NaHMDS
THF
Ot-Bu
IBr
PhMe
K2CO3
MeOH
DMF
DIPEA
or HNR1R2, HOR1, N3, C-nucleophiles
Ph
Ph
* DHM = DesHydroxyMethyl

Fig. 56

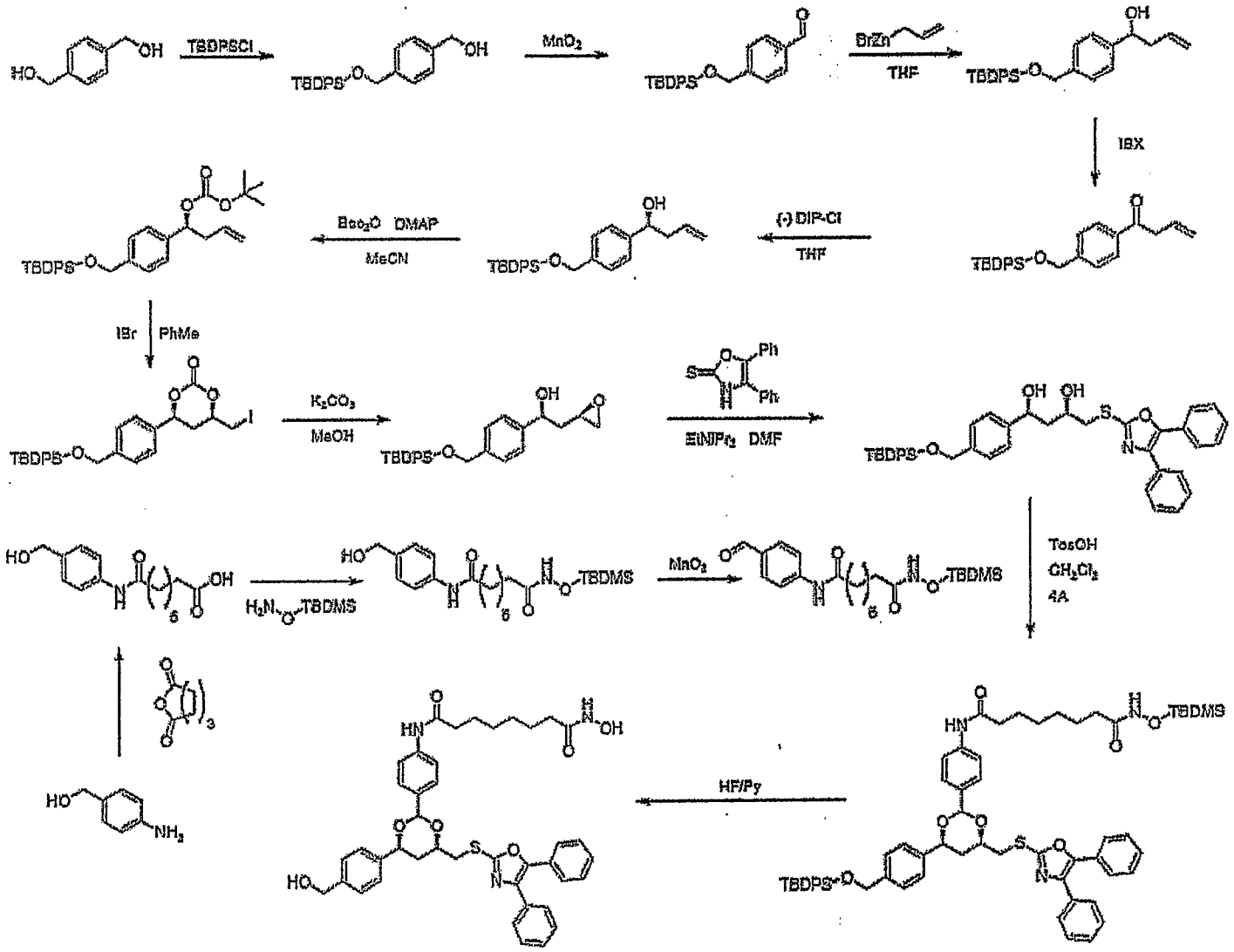

TBDPSCl
MnO2
BrZn
THF
IBX
(-)-DIP-Cl
THF
Boc2O DMAP
MeCN
IBr
PhMe
K2CO3
MeOH
Et(iPr)2 DMF
TosOH
CH2Cl2
4A
MnO2
HF/Py
TBDPS
TBDMS

Fig. 57

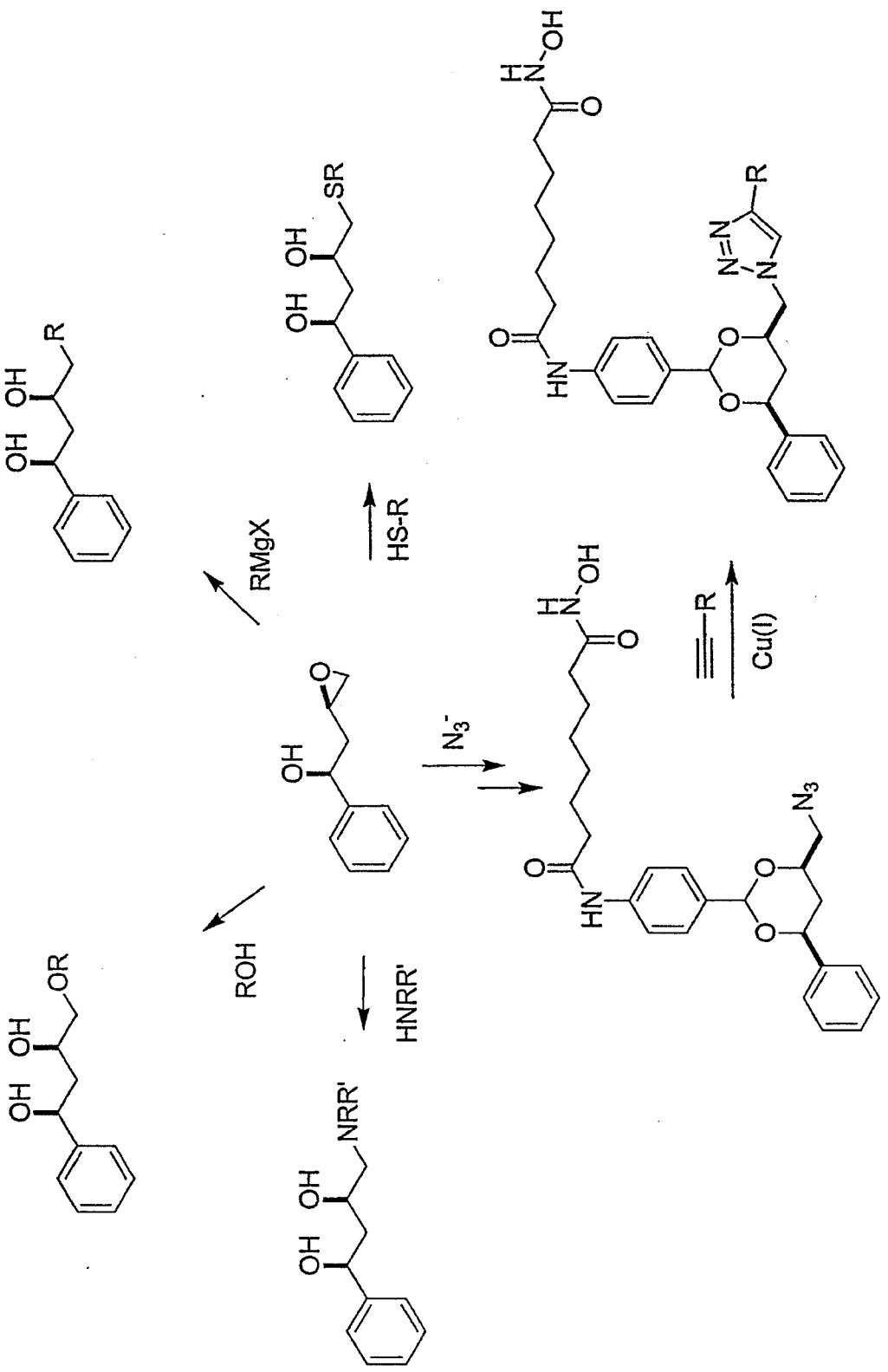
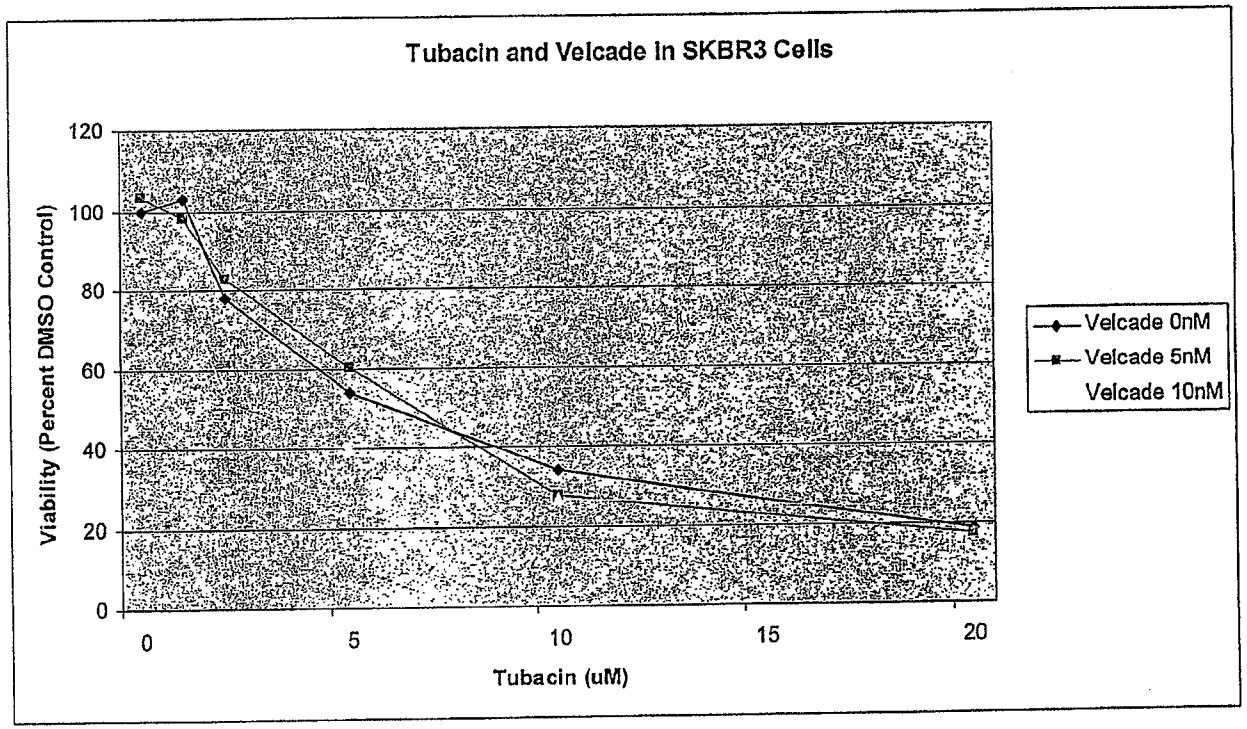


Fig. 58



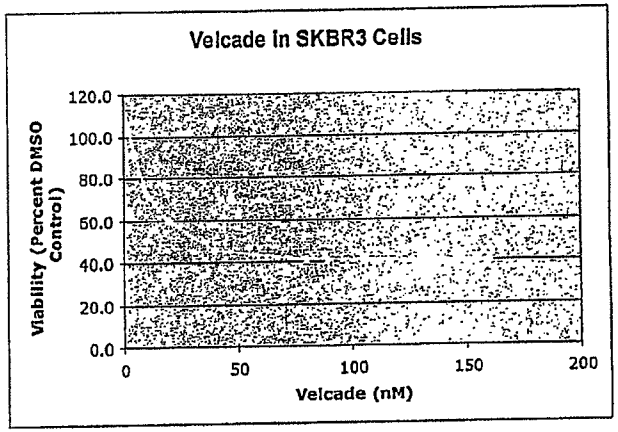


Fig. 59

HDAC6 in Breast Cancer: Tubacin Confers Bortezomib Sensitivity