
BI 2536
Coated
Tube
Add buffer
then bath
sonicate to
dissolve BI
2536
500µM
BI 2536
Add hexanol
then extract
BI 2536 by
rotating vial
for an hour
Hexanol
500µM
BI 2536
Briefly
centrifuge
Hexanol
BI 2536
Measure BI 2536
fluorescence in
hexanol layer
Ex: 330nm
Em: 370nm

**Fig. 1**

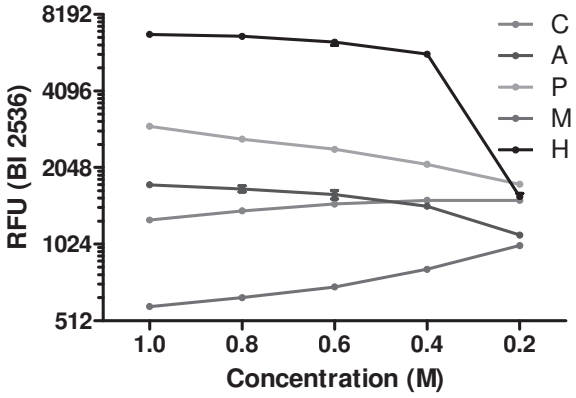

8192
4096
2048
1024
512
RFU (BI 2536)
1.0
0.8
0.6
0.4
0.2
Concentration (M)
C
A
P
M
H

**Fig. 2**

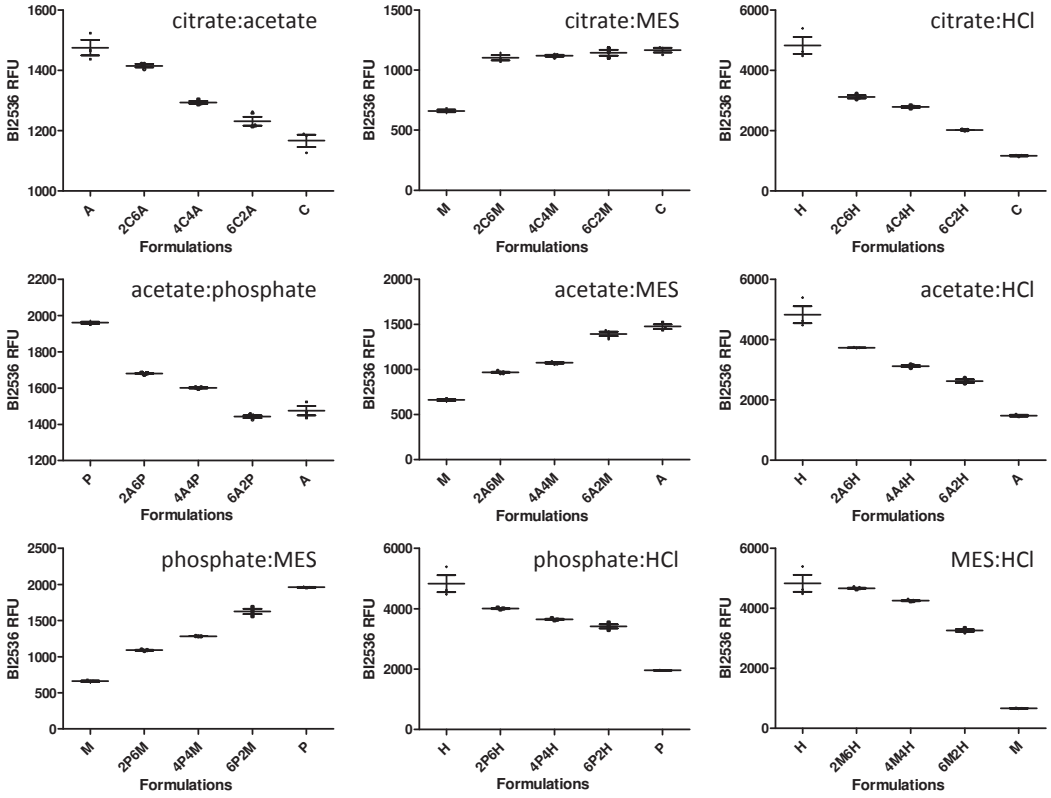

citrate:acetate
BI2536 RFU
1600
1400
1200
1000
A
2C6A
4C4A
6C2A
C
Formulations
citrate:MES
BI2536 RFU
1500
1000
500
0
M
2C6M
4C4M
6C2M
C
Formulations
citrate:HCl
BI2536 RFU
6000
4000
2000
0
H
2C6H
4C4H
6C2H
C
Formulations
acetate:phosphate
BI2536 RFU
2200
2000
1800
1600
1400
1200
P
2A6P
4A4P
6A2P
A
Formulations
acetate:MES
BI2536 RFU
2000
1500
1000
500
0
M
2A6M
4A4M
6A2M
A
Formulations
acetate:HCl
BI2536 RFU
6000
4000
2000
0
H
2A6H
4A4H
6A2H
A
Formulations
phosphate:MES
BI2536 RFU
2500
2000
1500
1000
500
0
M
2P6M
4P4M
6P2M
P
Formulations
phosphate:HCl
BI2536 RFU
6000
4000
2000
0
H
2P6H
4P4H
6P2H
P
Formulations
MES:HCl
BI2536 RFU
6000
4000
2000
0
H
2M6H
4M4H
6M2H
M
Formulations

**Fig. 3**

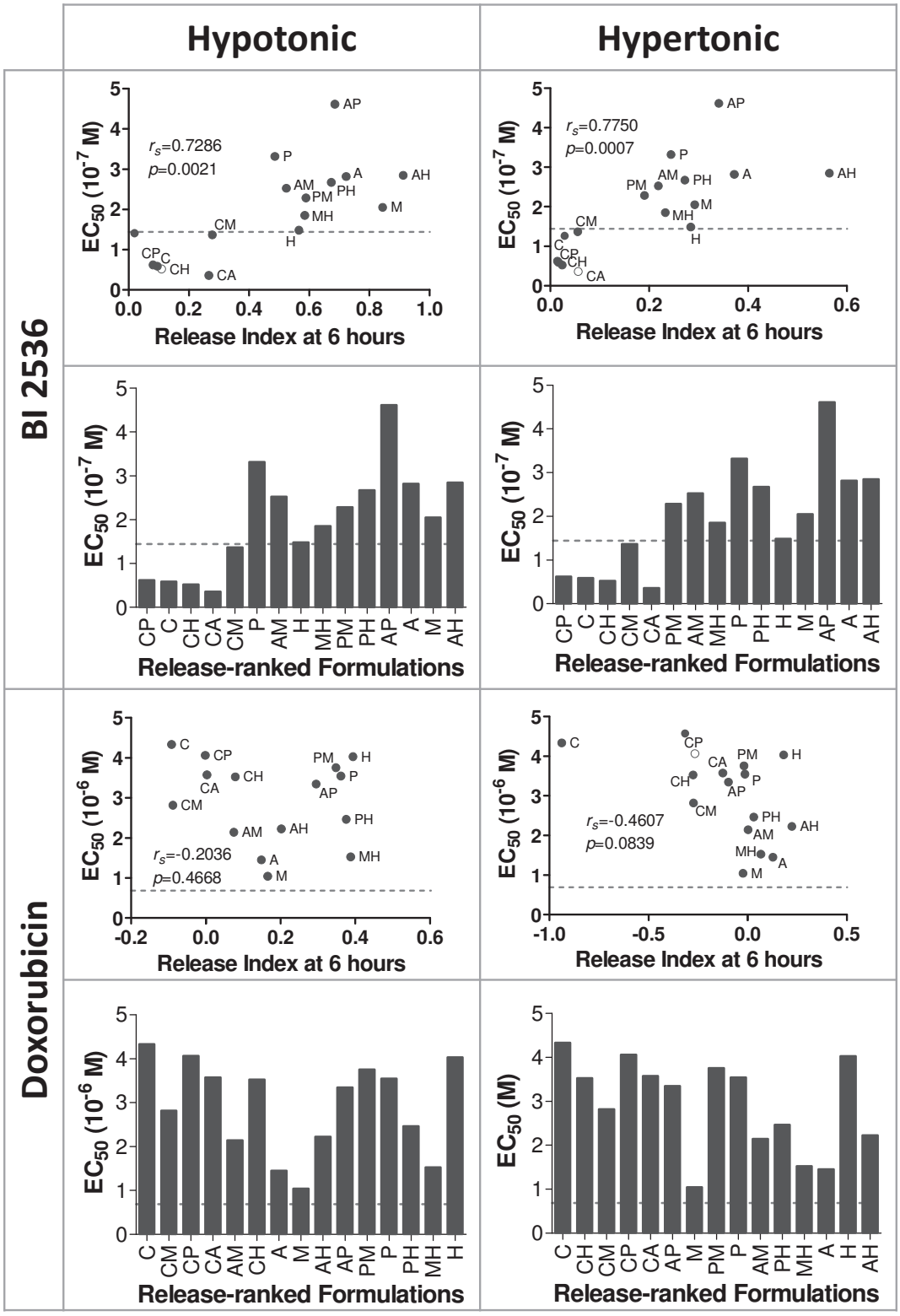

Hypotonic
Hypertonic
BI 2536
Doxorubicin
EC50 (10-7 M)
EC50 (10-6 M)
EC50 (M)
rs=0.7286
p=0.0021
rs=0.7750
p=0.0007
rs=-0.2036
p=0.4668
rs=-0.4607
p=0.0839
Release Index at 6 hours
Release-ranked Formulations

**Fig. 4**

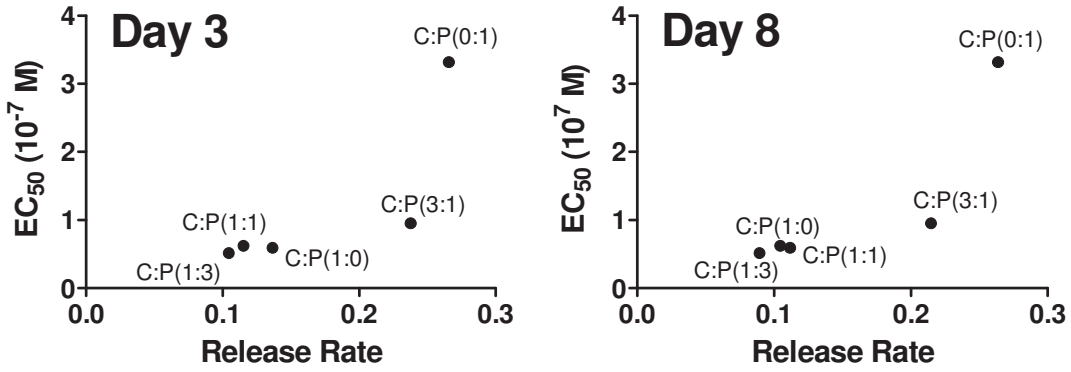

Day 3
C:P(0:1)
C:P(3:1)
C:P(1:1)
C:P(1:0)
C:P(1:3)
EC50 (10-7 M)
Release Rate
Day 8
C:P(0:1)
C:P(3:1)
C:P(1:0)
C:P(1:1)
C:P(1:3)
EC50 (107 M)
Release Rate

**Fig. 5A**

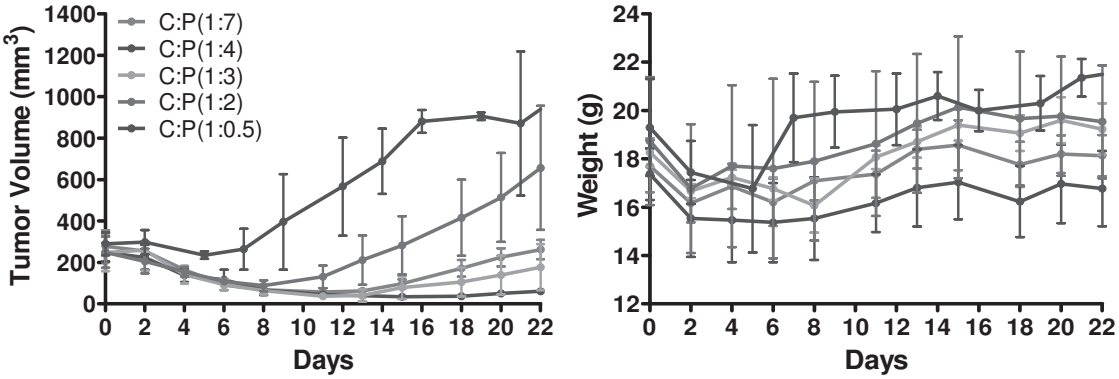

C:P(1:7)
C:P(1:4)
C:P(1:3)
C:P(1:2)
C:P(1:0.5)
Tumor Volume (mm3)
Days
Weight (g)
Days

**Fig. 5B**

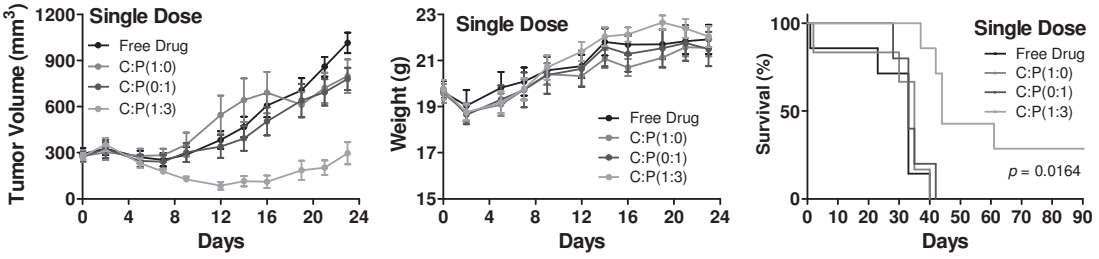

Single Dose
Tumor Volume (mm3)
Free Drug
C:P(1:0)
C:P(0:1)
C:P(1:3)
Days
Single Dose
Weight (g)
Free Drug
C:P(1:0)
C:P(0:1)
C:P(1:3)
Days
Single Dose
Survival (%)
Free Drug
C:P(1:0)
C:P(0:1)
C:P(1:3)
p = 0.0164
Days

**Fig. 6A**

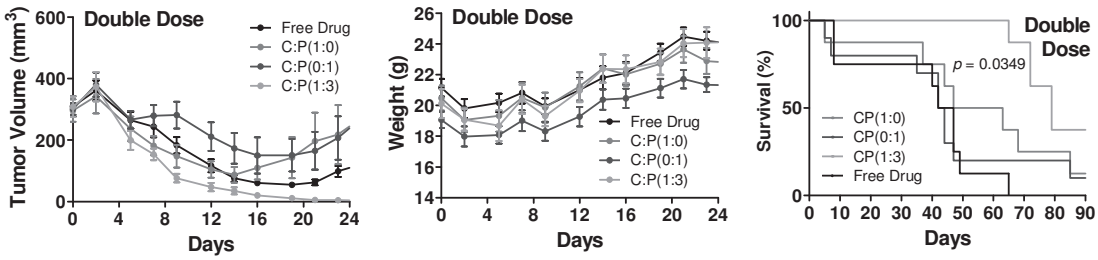

Double Dose
Tumor Volume (mm3)
Free Drug
C:P(1:0)
C:P(0:1)
C:P(1:3)
Days
Double Dose
Weight (g)
Free Drug
C:P(1:0)
C:P(0:1)
C:P(1:3)
Days
Double Dose
Survival (%)
p = 0.0349
CP(1:0)
CP(0:1)
CP(1:3)
Free Drug
Days

**Fig. 6B**

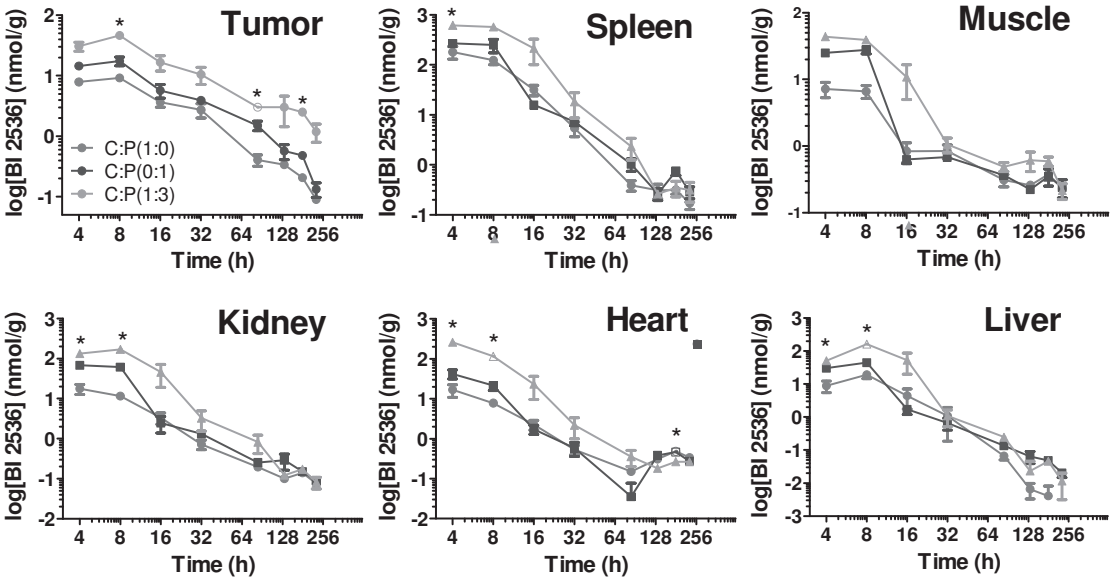



Fig. 7A

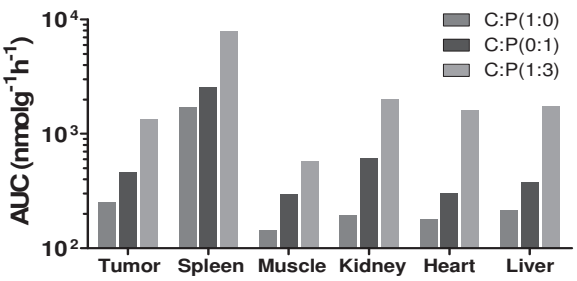



Fig. 7B

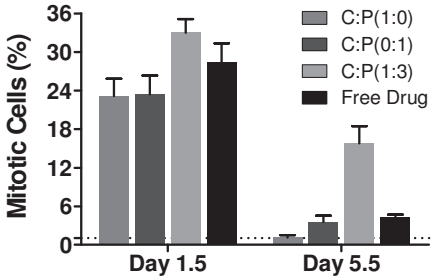



Fig. 7C

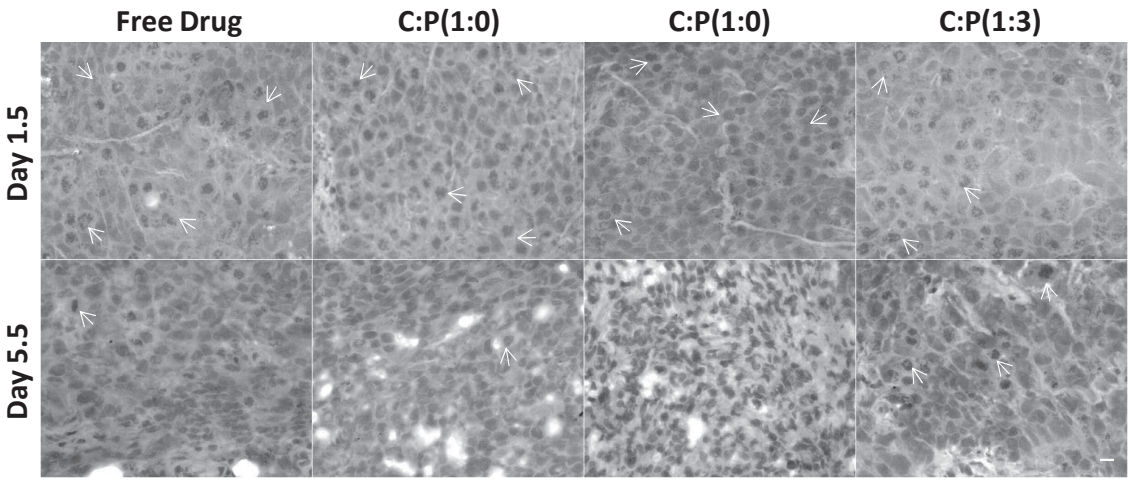



**Fig. 7D**

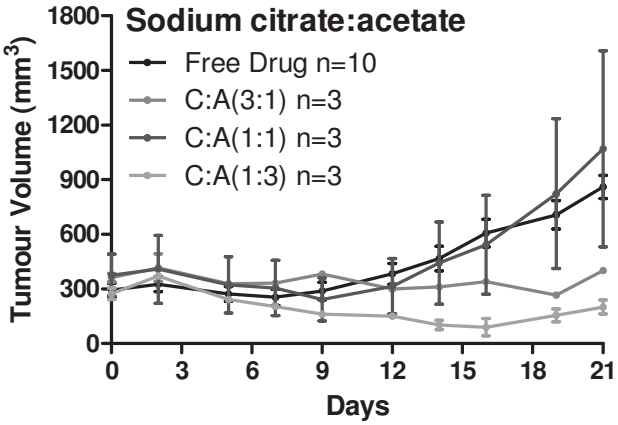

Sodium citrate:acetate
Free Drug n=10
C:A(3:1) n=3
C:A(1:1) n=3
C:A(1:3) n=3
Tumour Volume (mm3)
1800
1500
1200
900
600
300
0
0
3
6
9
12
15
18
21
Days

**Fig. 8A**

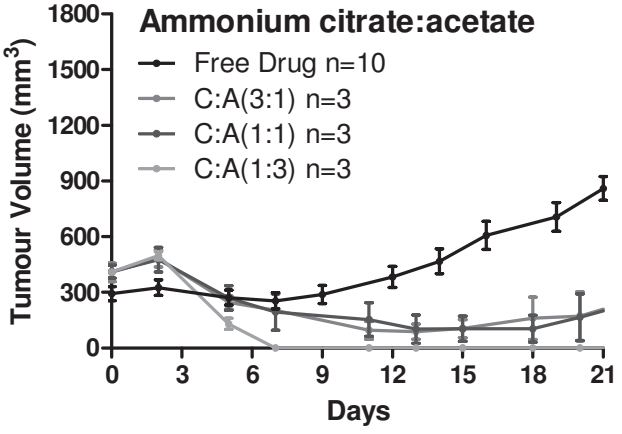

Ammonium citrate:acetate
Free Drug n=10
C:A(3:1) n=3
C:A(1:1) n=3
C:A(1:3) n=3
Tumour Volume (mm3)
1800
1500
1200
900
600
300
0
0
3
6
9
12
15
18
21
Days

**Fig. 8B**