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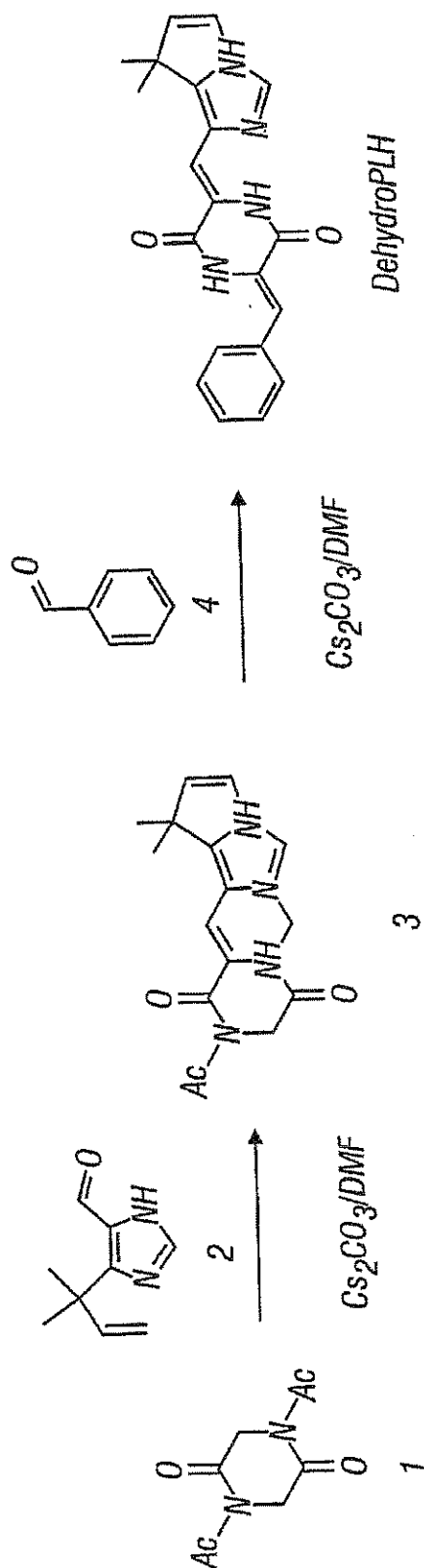


FIG. 1

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HPLC conditions:

Column: YMC-Pack ODS-AM
(20 X 250 mm)

Gradient: 50% to 100% MeOH
in H₂O for 30 min

Flow rate: 12mL/min

O.D.: 230 nm

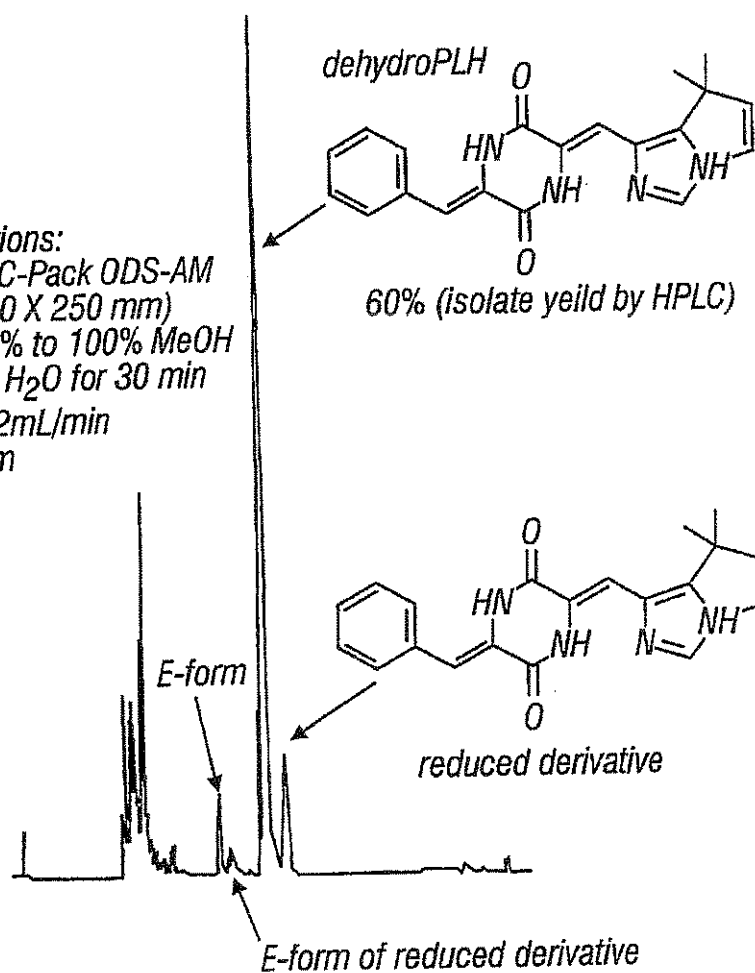


FIG. 2

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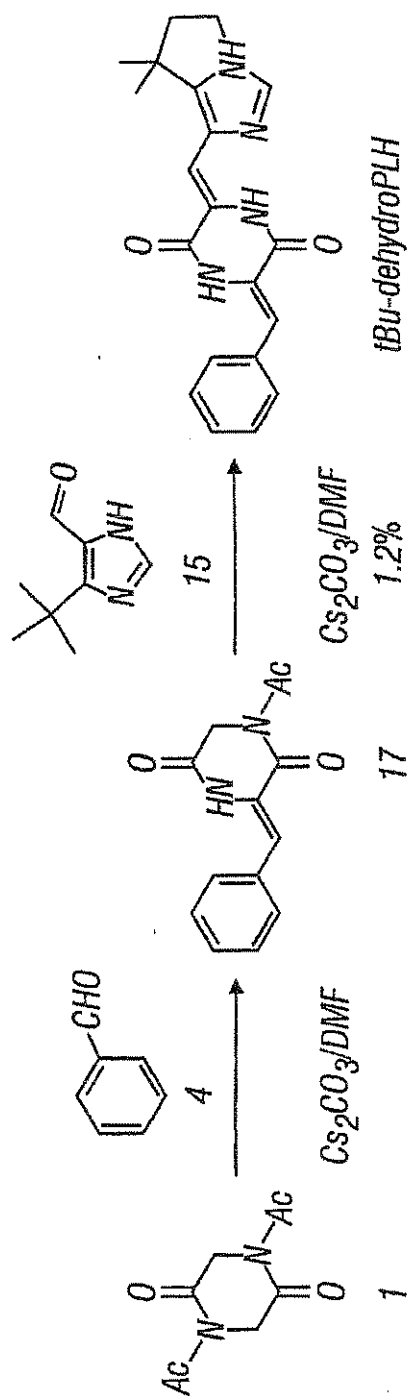


FIG. 3

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HPLC conditions:
Column: YMC-Pack ODS-AM
(20 X 250 mm)
Gradient: 50% to 100% MeOH
in H₂O for 30 min
Flow rate: 12mL/min
O.D.: 230 nm

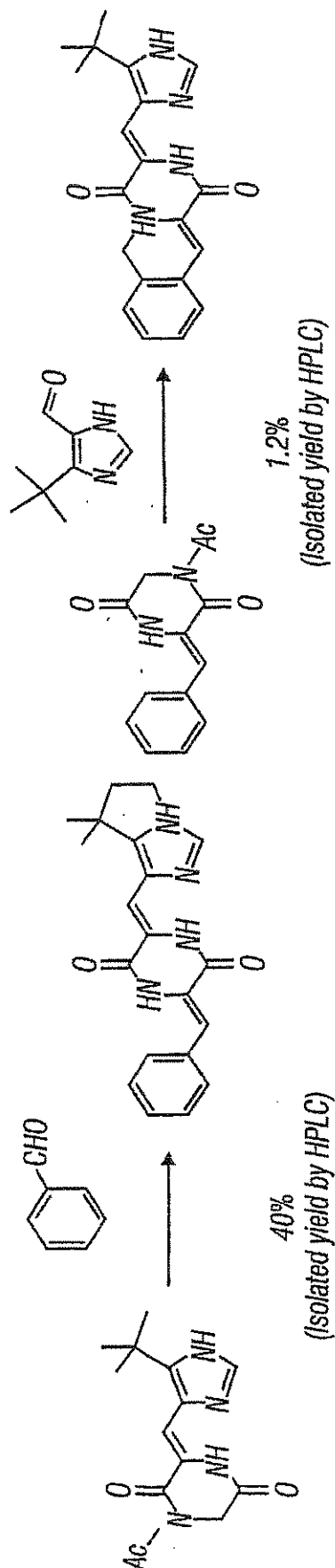
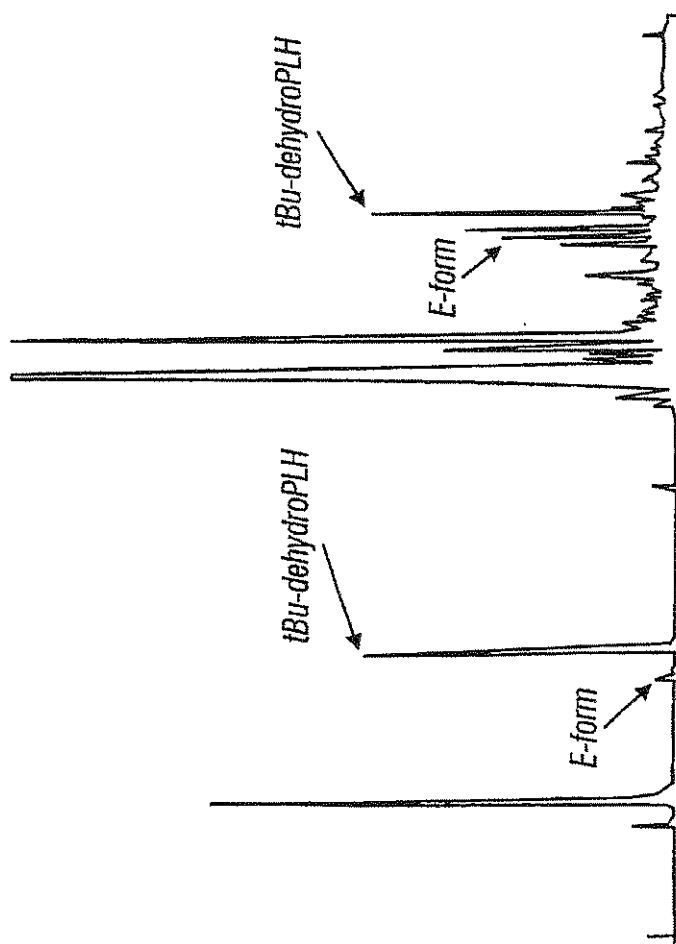


FIG. 4

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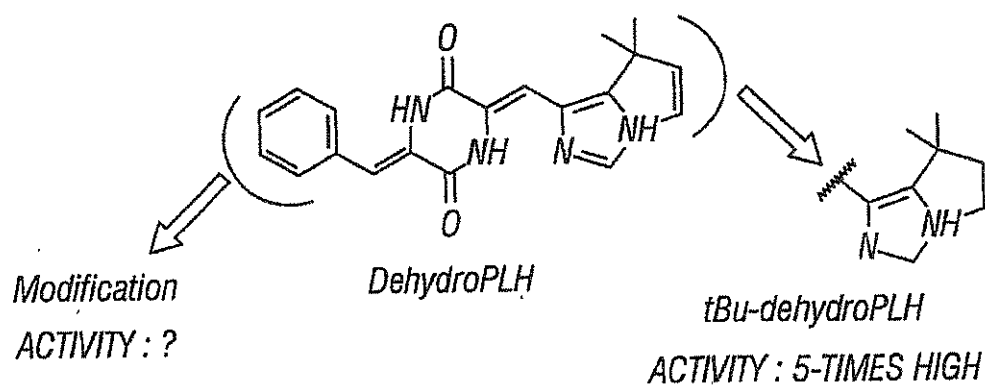


FIG. 5

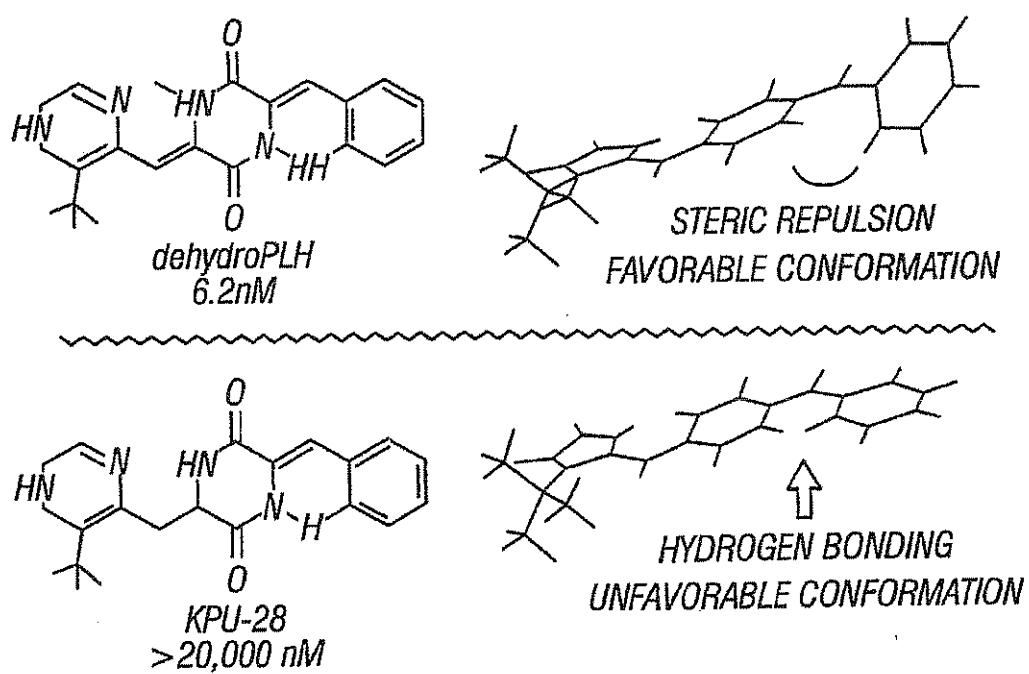


FIG. 6

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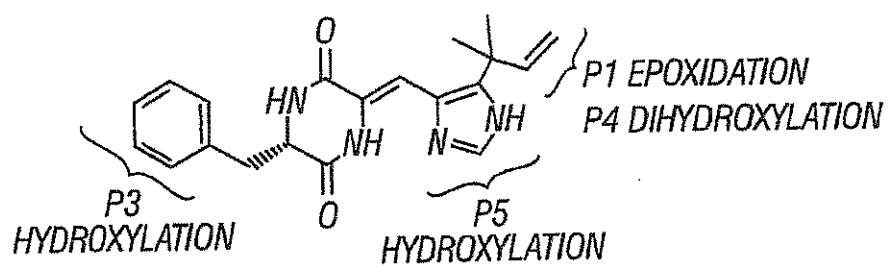


FIG. 7

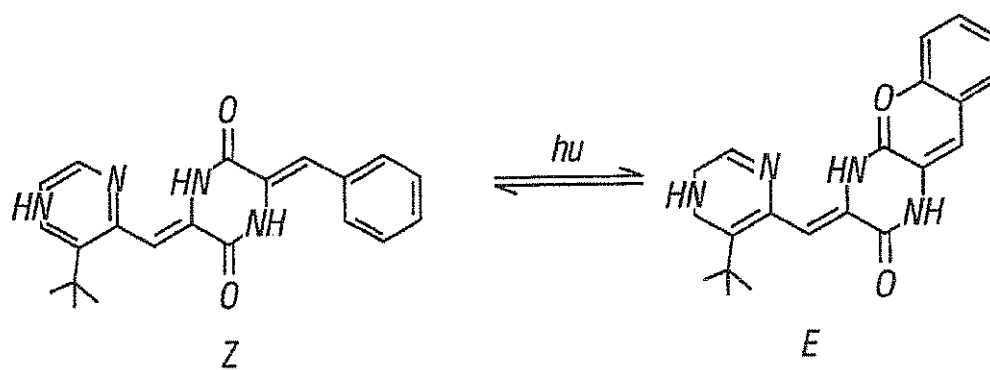


FIG. 8

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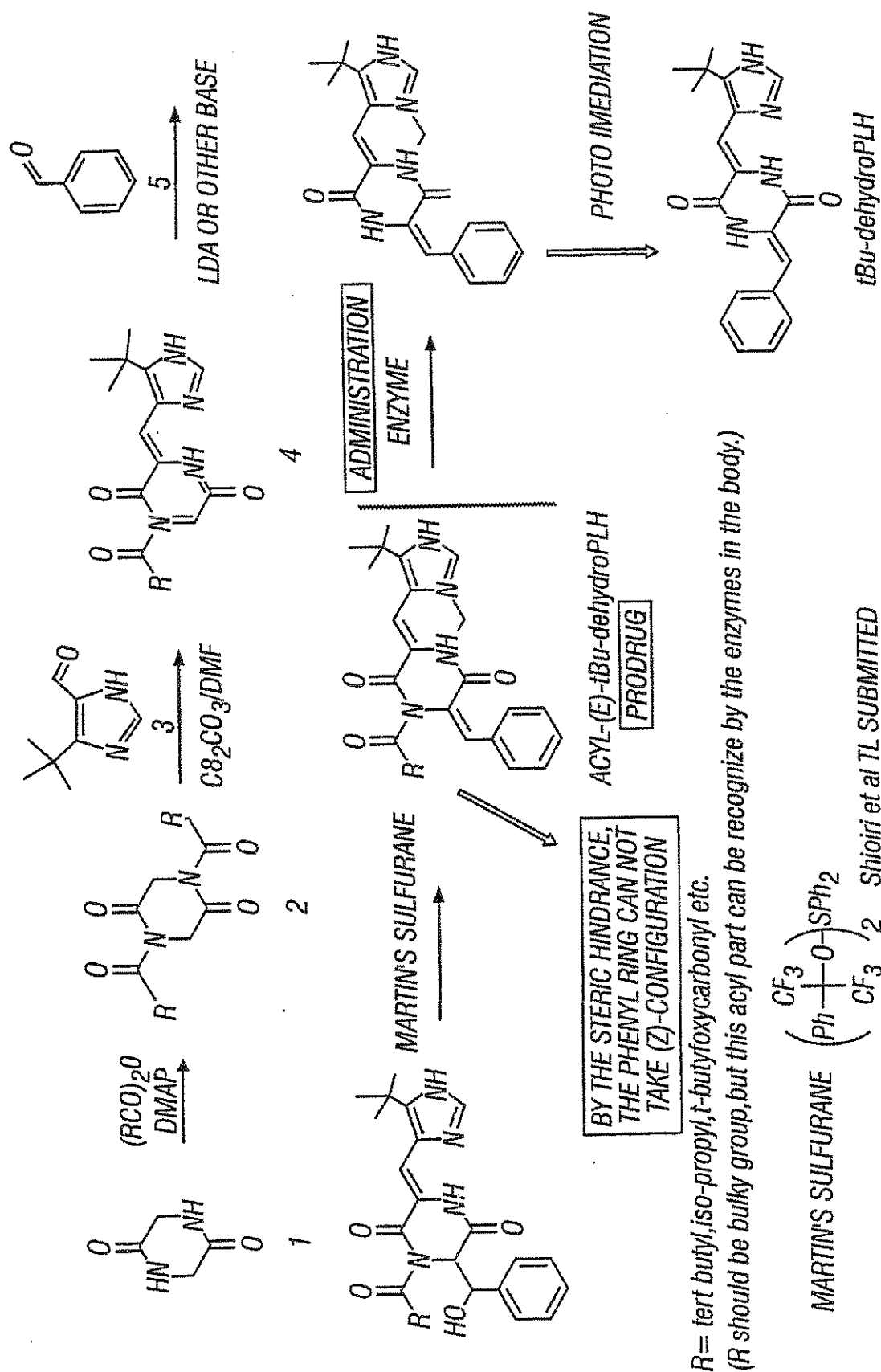
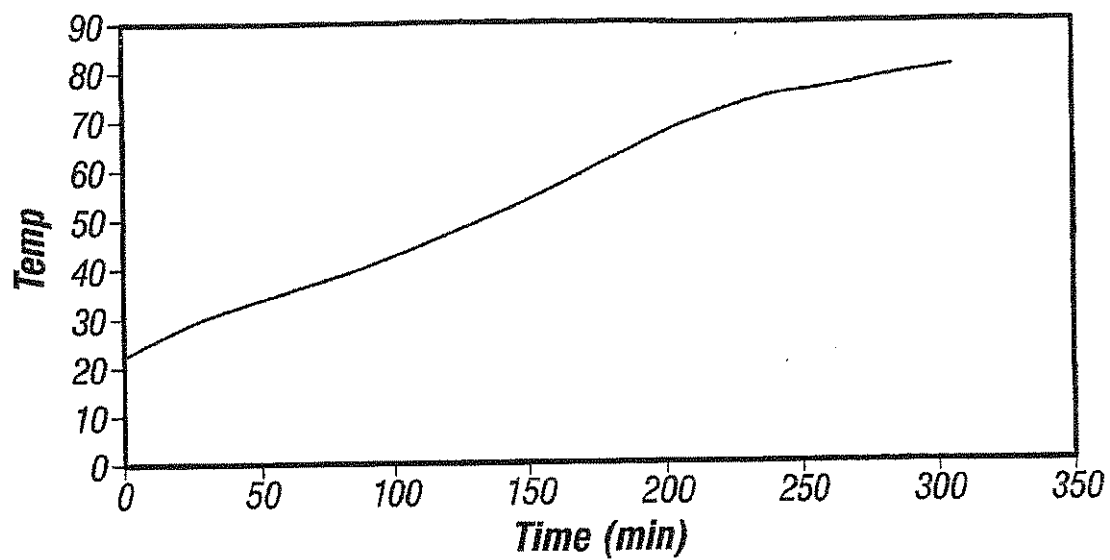
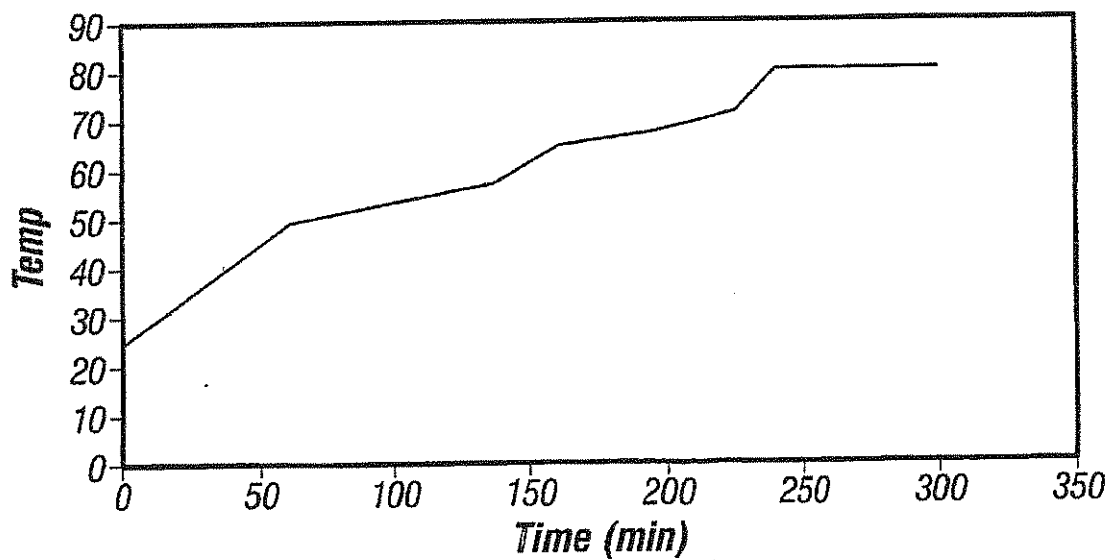
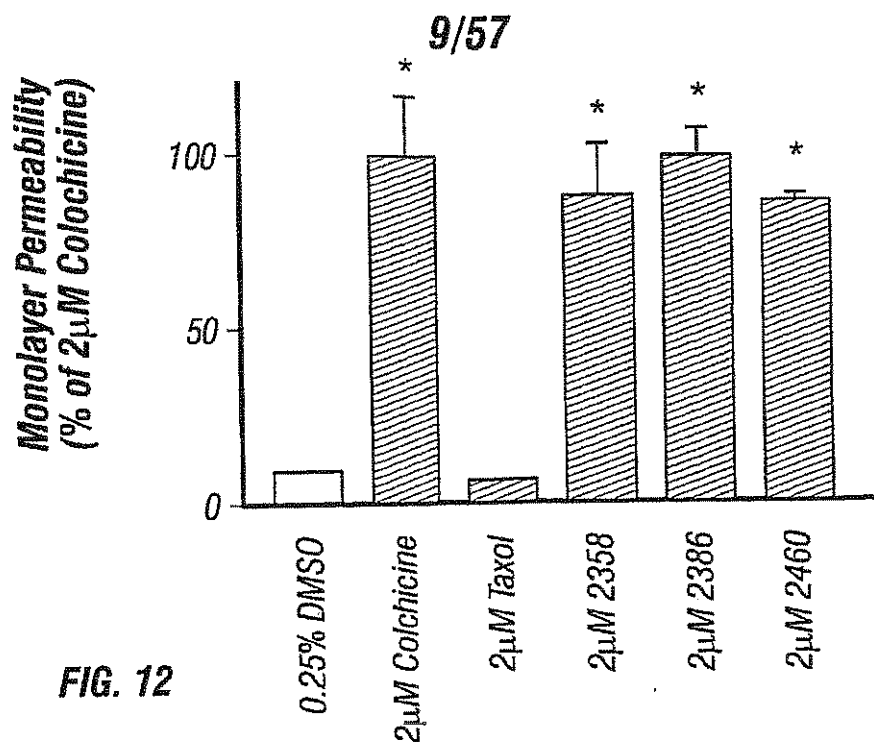
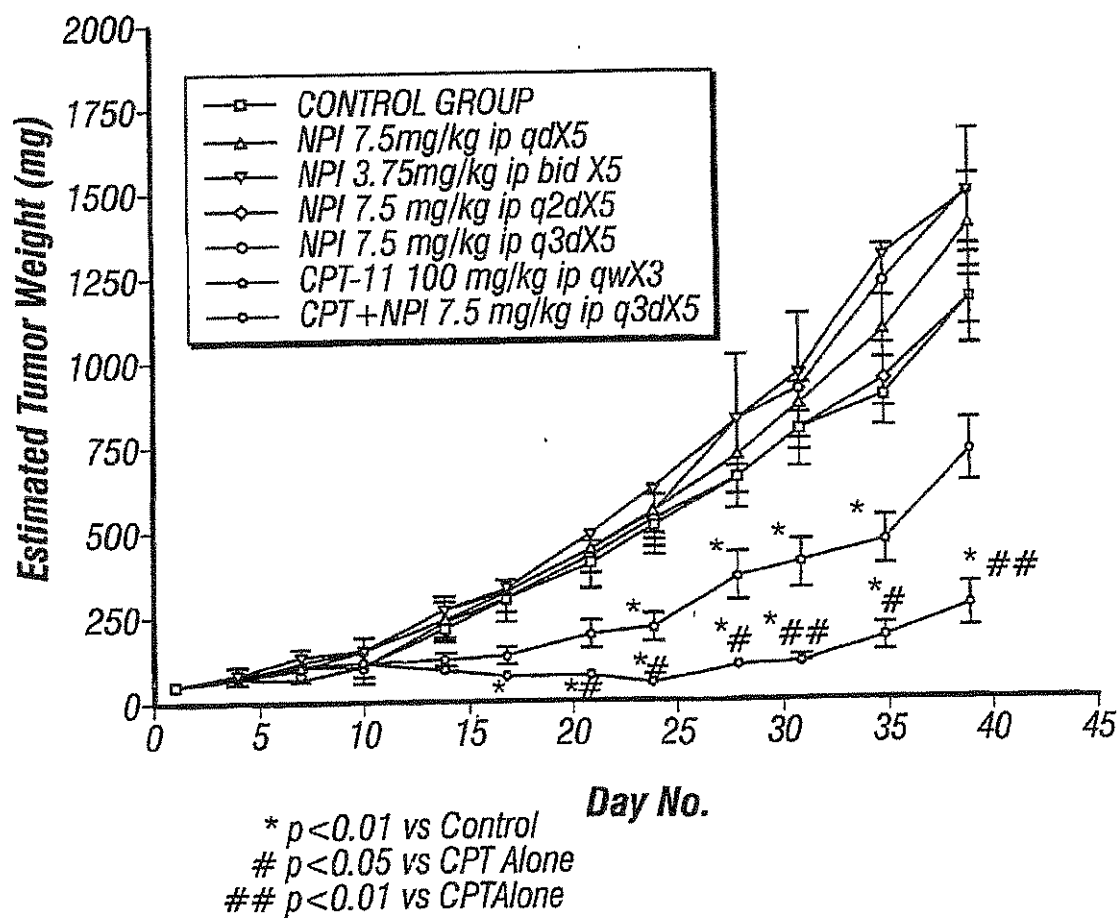


FIG. 9

8/57**FIG. 10****FIG. 11**

**FIG. 12****FIG. 13**

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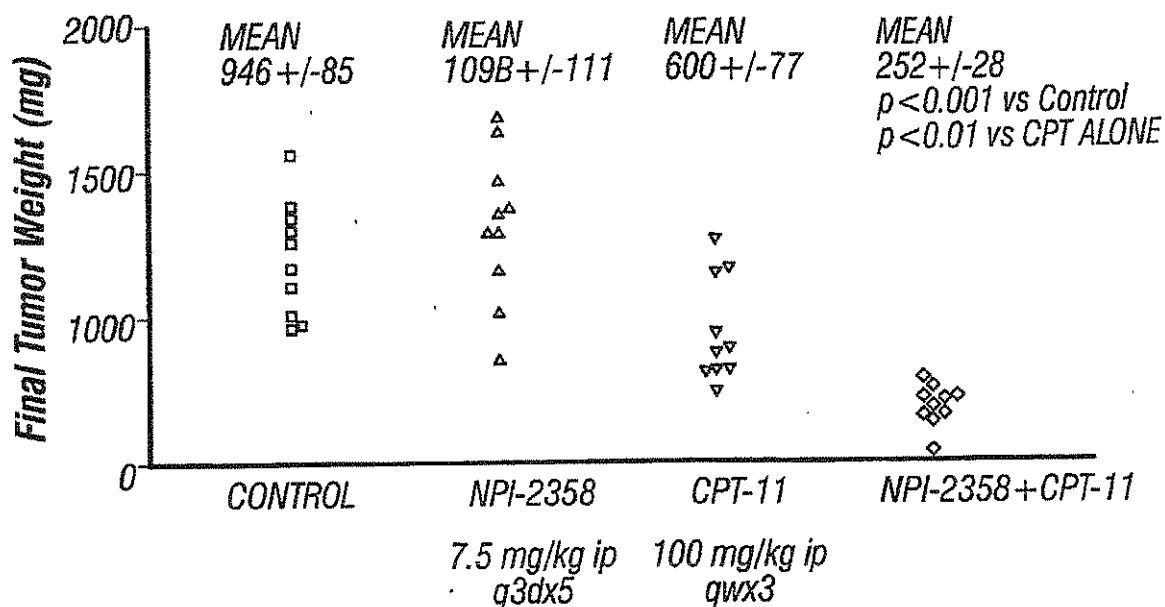


FIG. 14

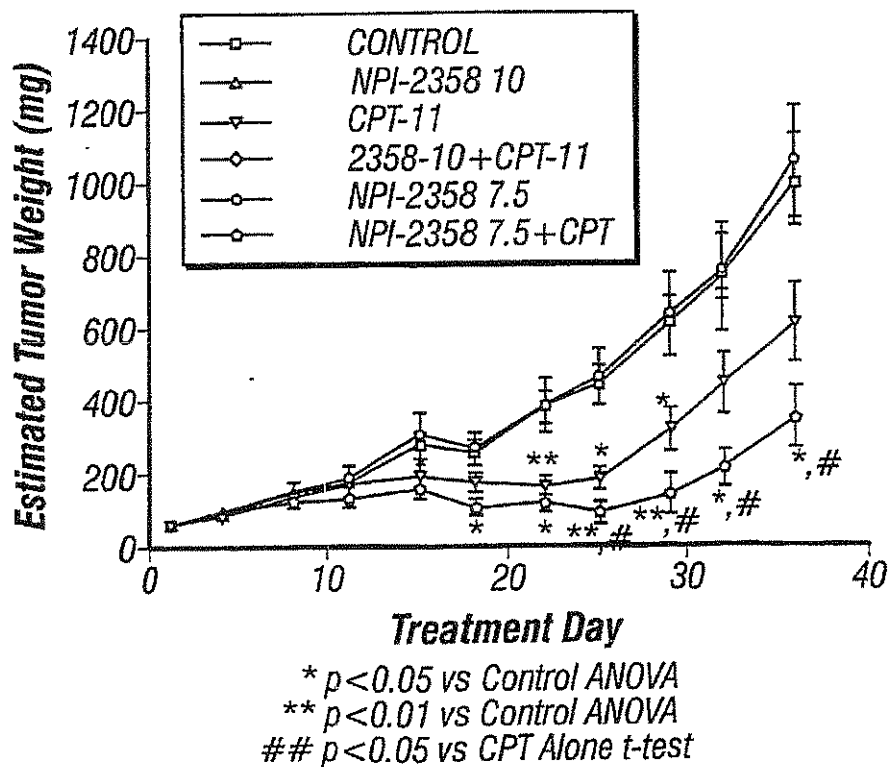


FIG. 15

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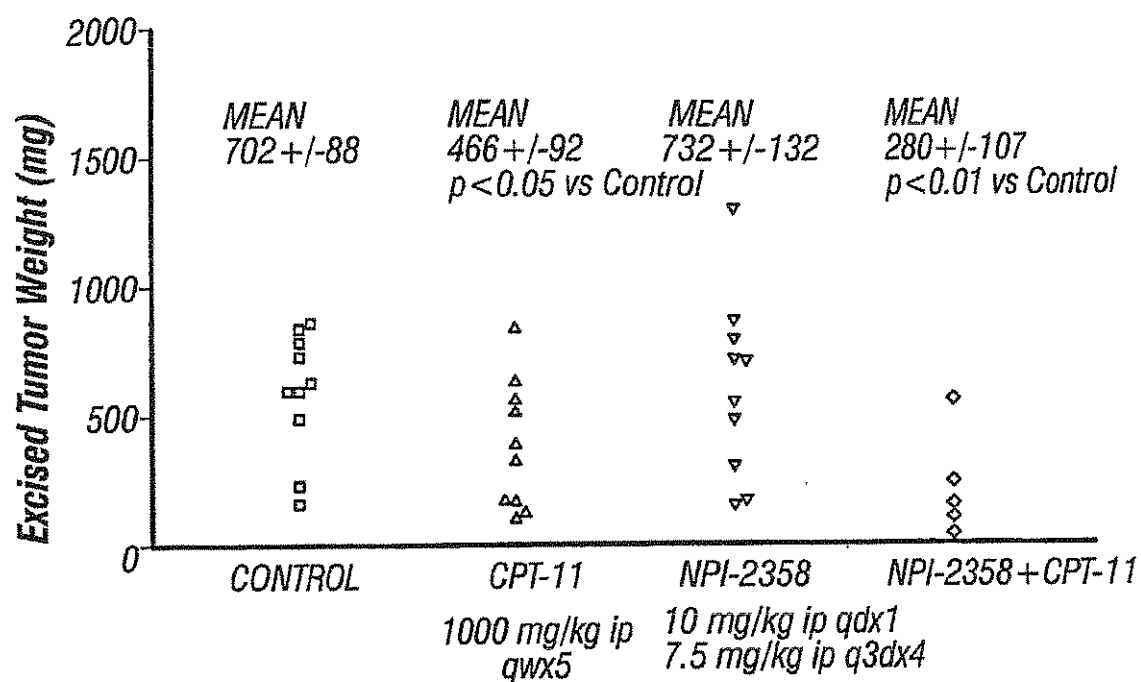


FIG. 16

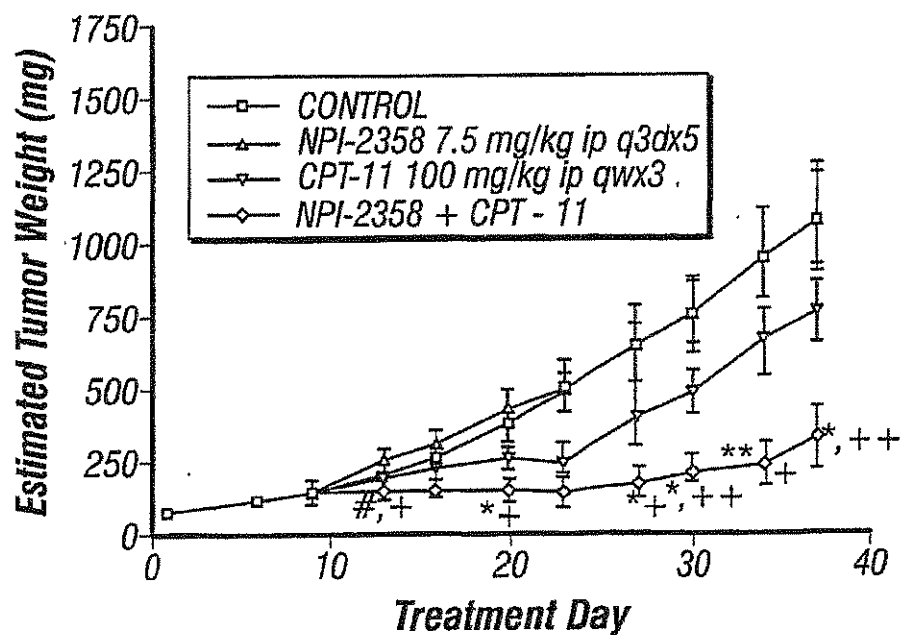


FIG. 17A

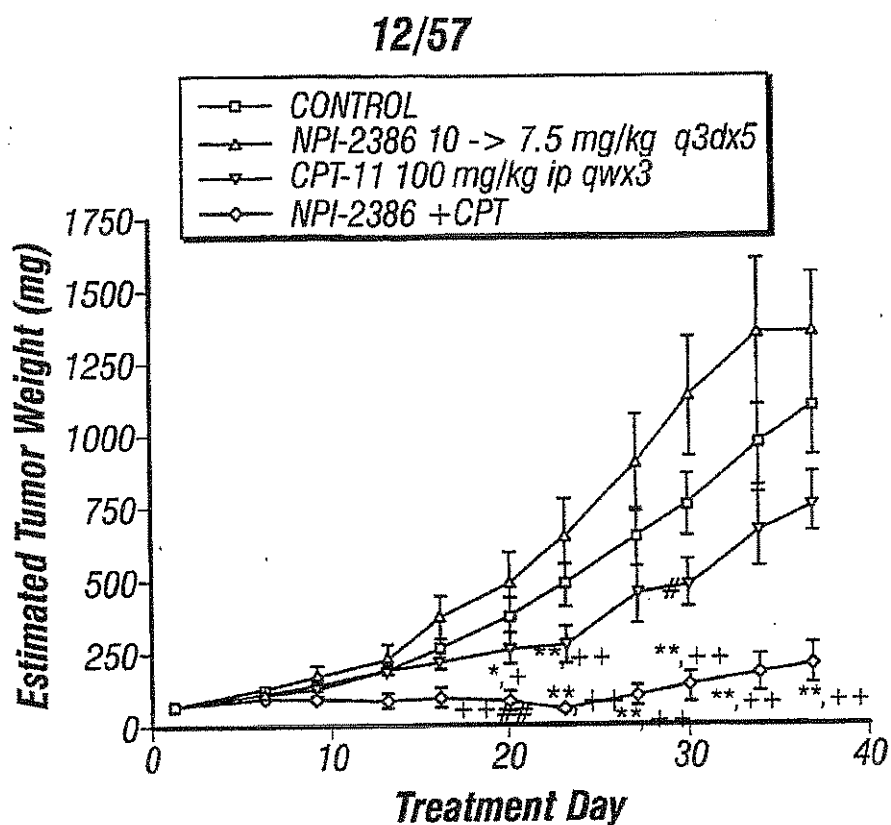


FIG. 17B

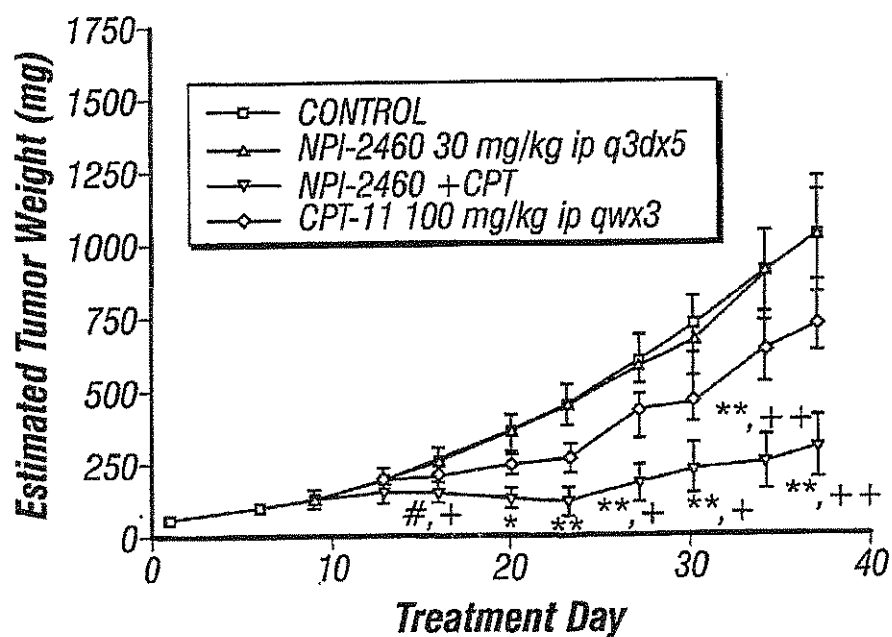


FIG. 17C

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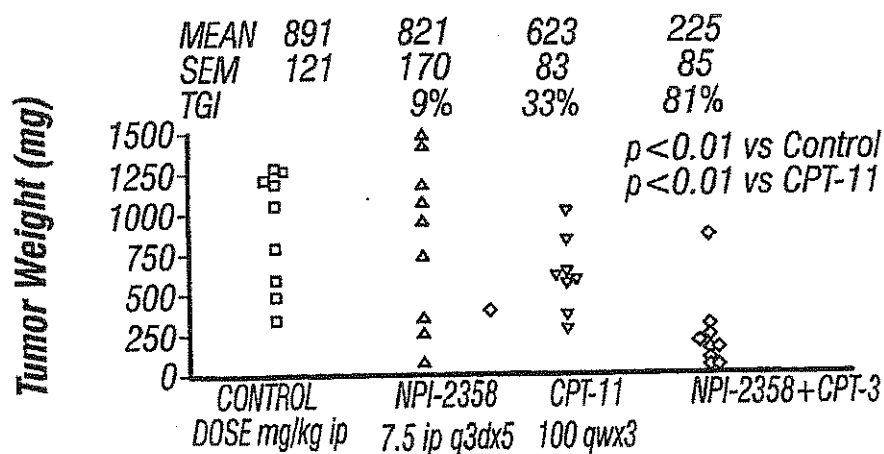


FIG. 18A

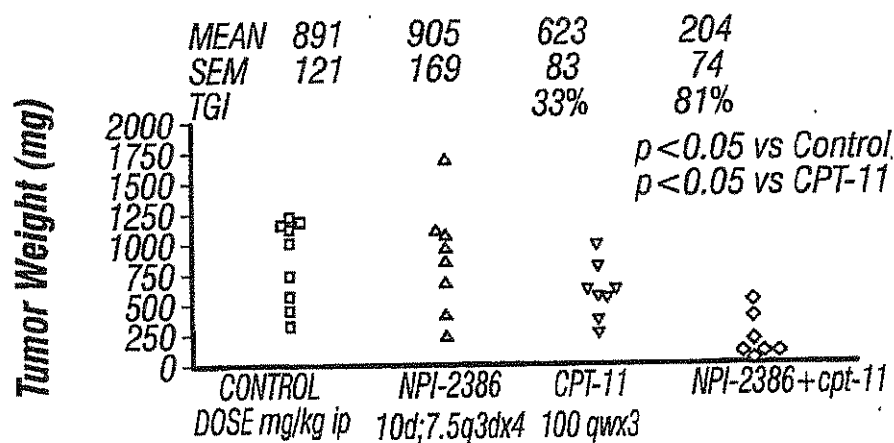


FIG. 18B

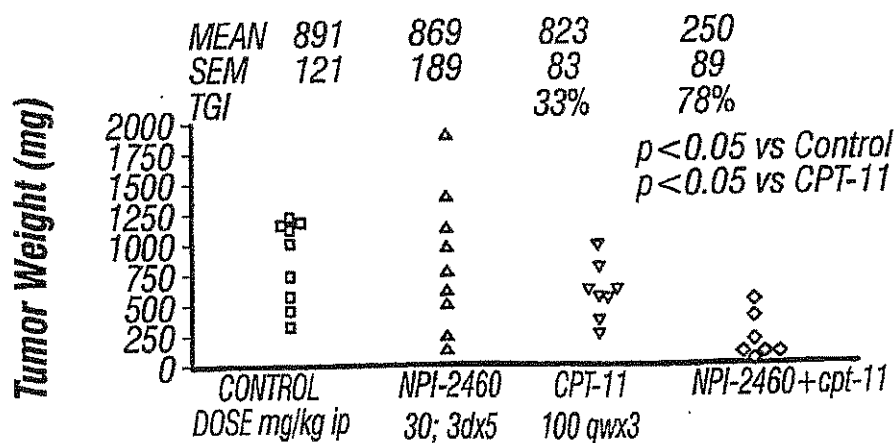
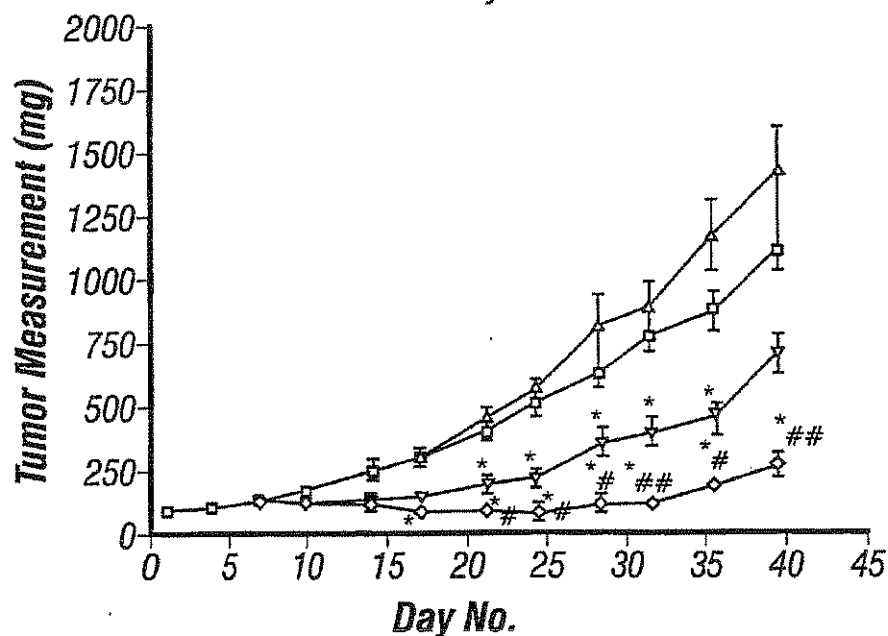
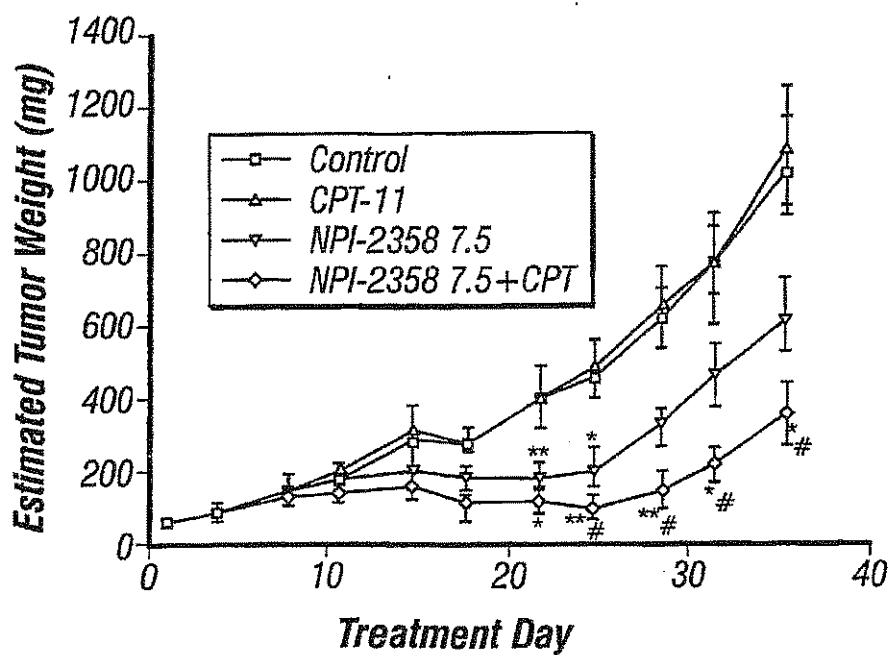


FIG. 18C

14/57**Study 2164****FIG. 19A****Study 2288****FIG. 19B**

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Study 2139

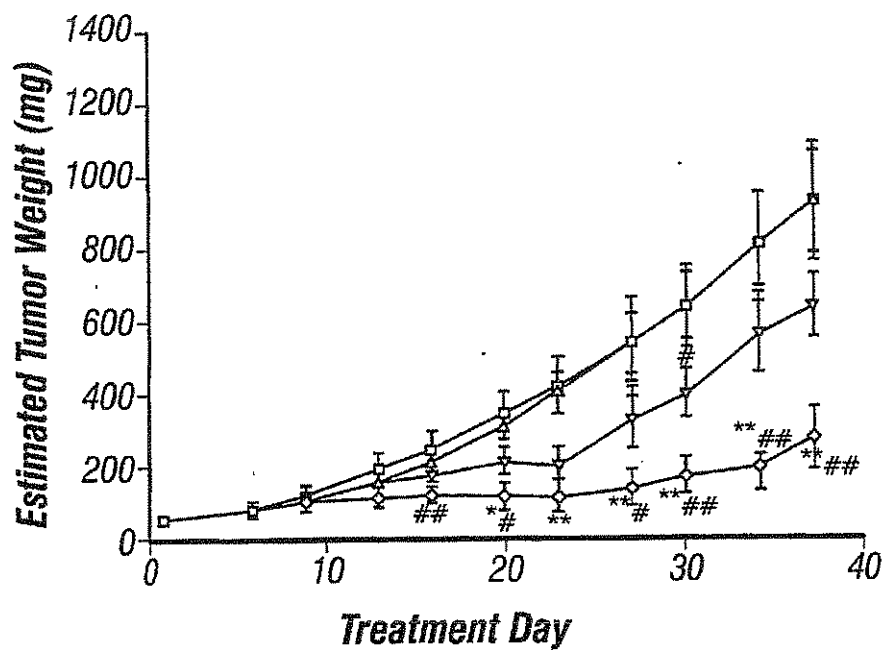


FIG. 19C

Study 2164

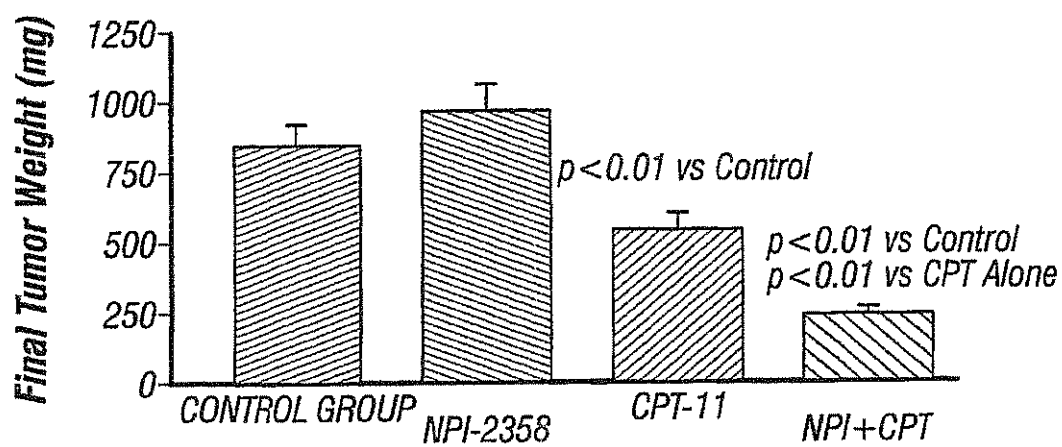


FIG. 20A

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Study 2288

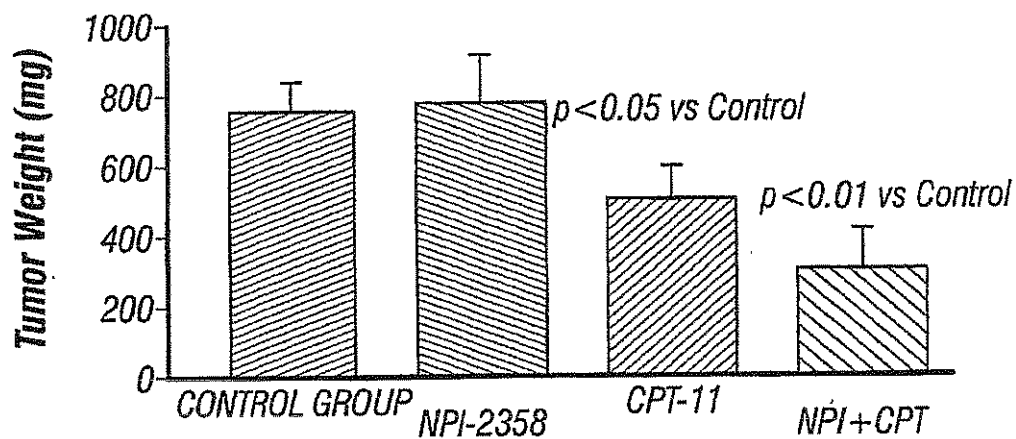


FIG. 20B

Study 2139

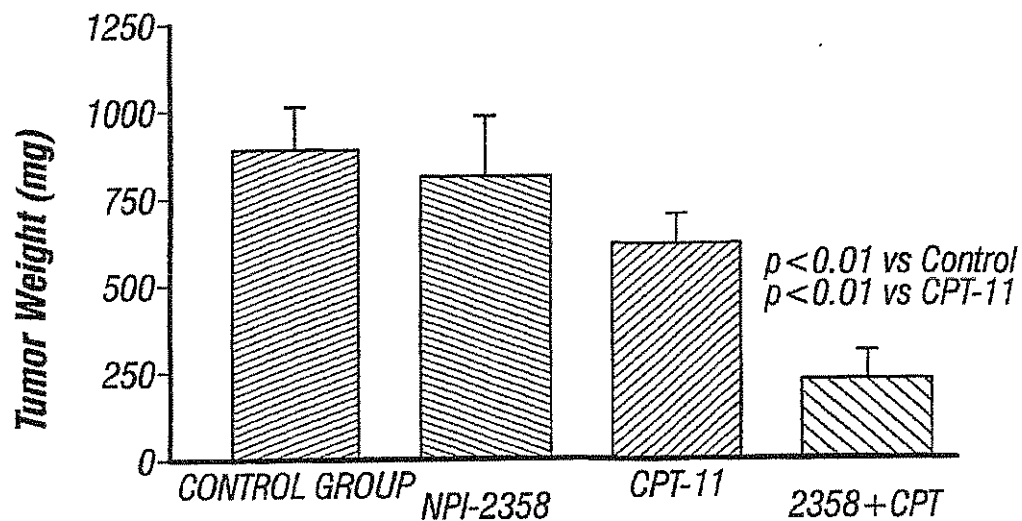
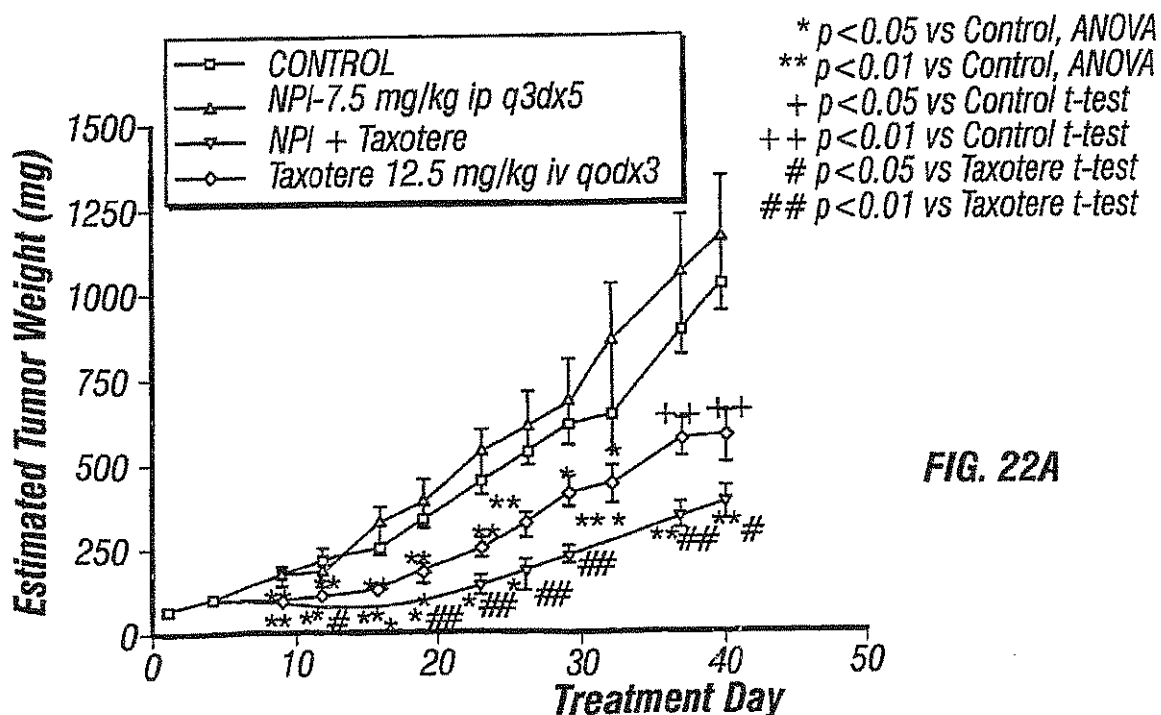
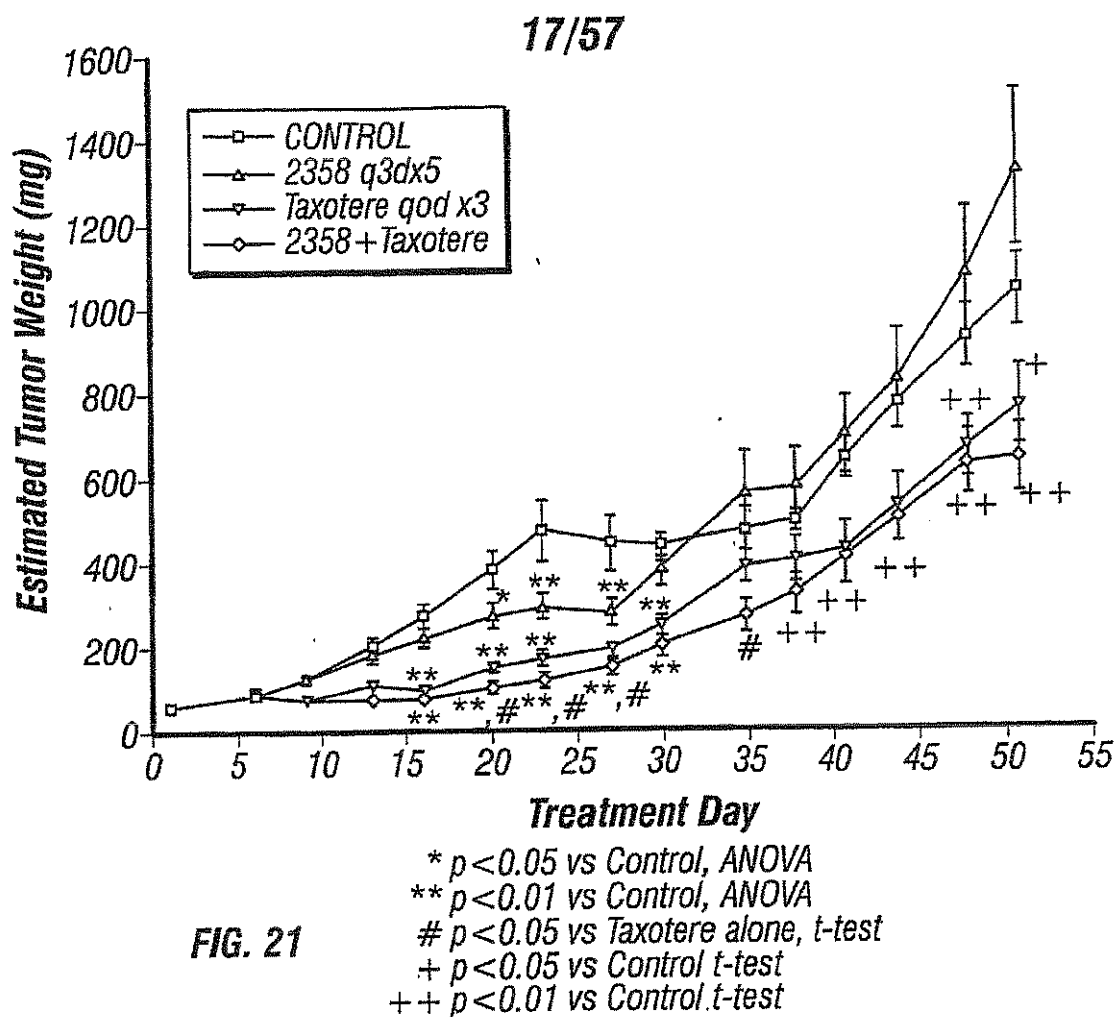


FIG. 20C



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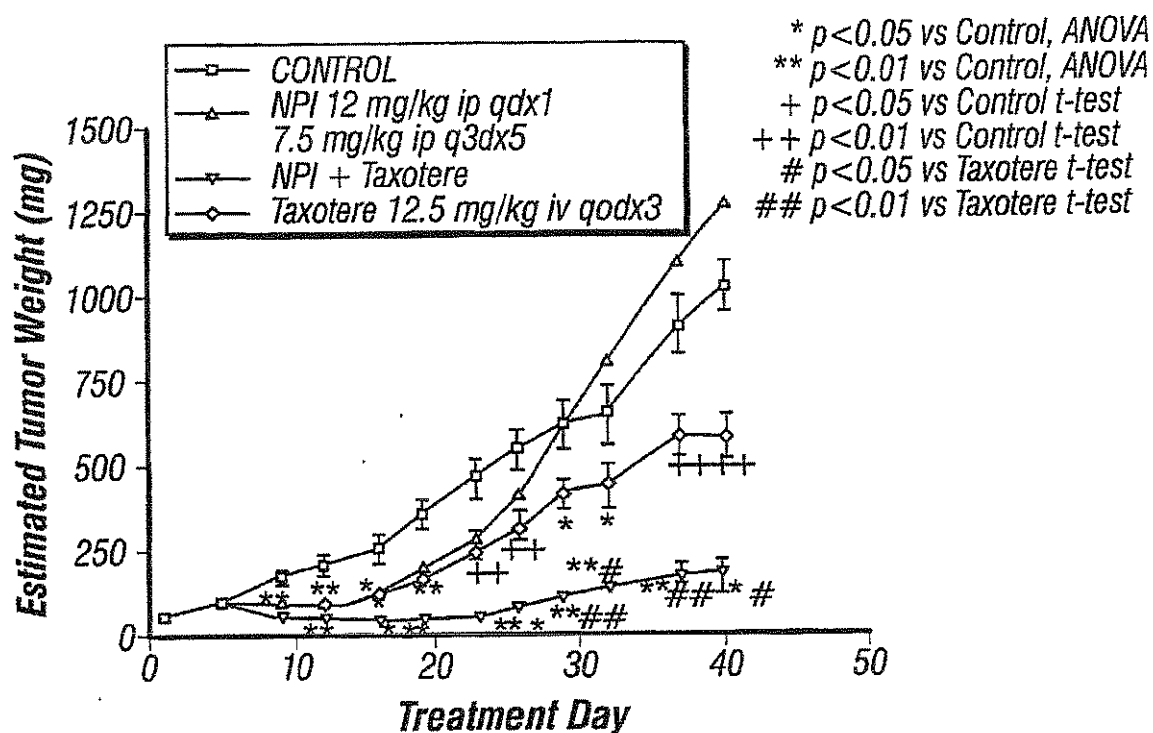


FIG. 22B

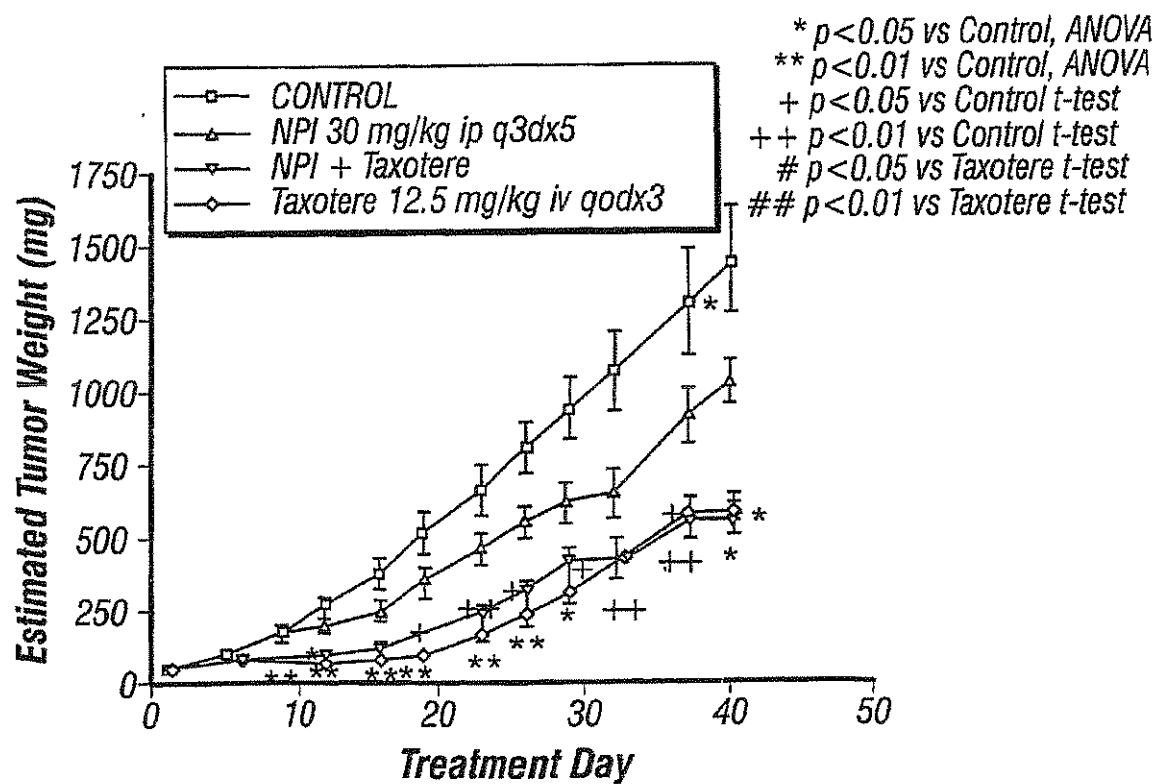


FIG. 22C

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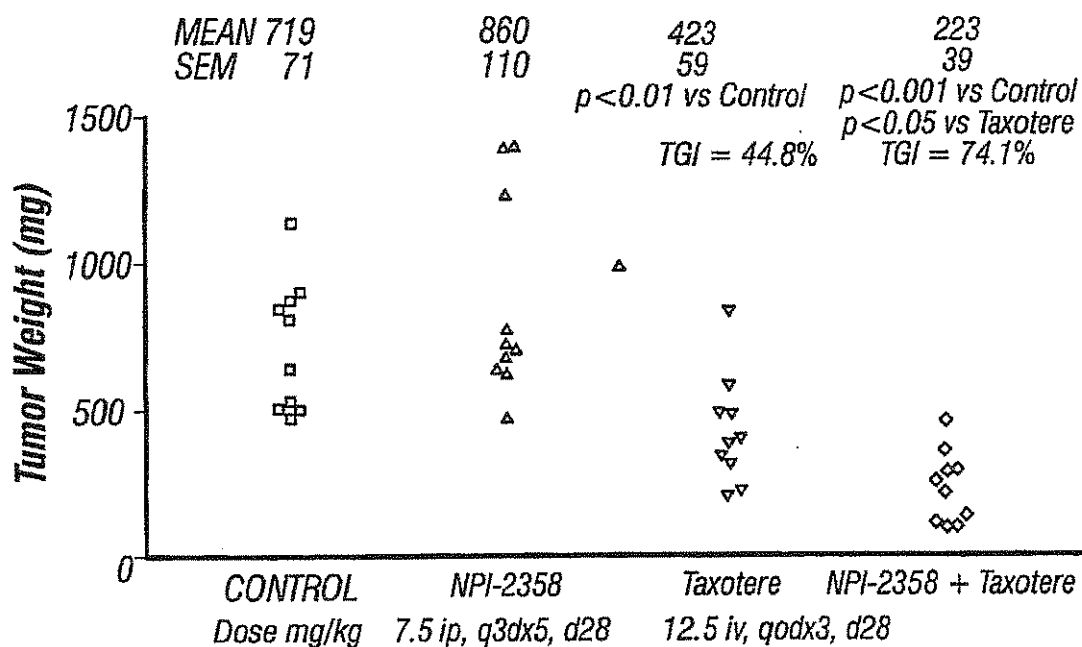


FIG. 23

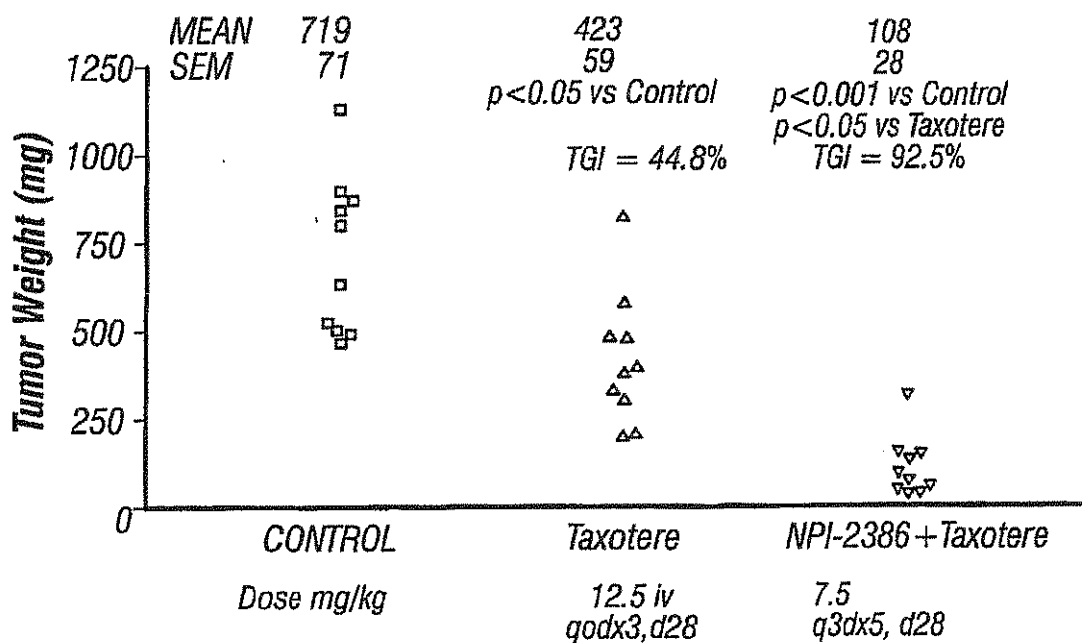


FIG. 24

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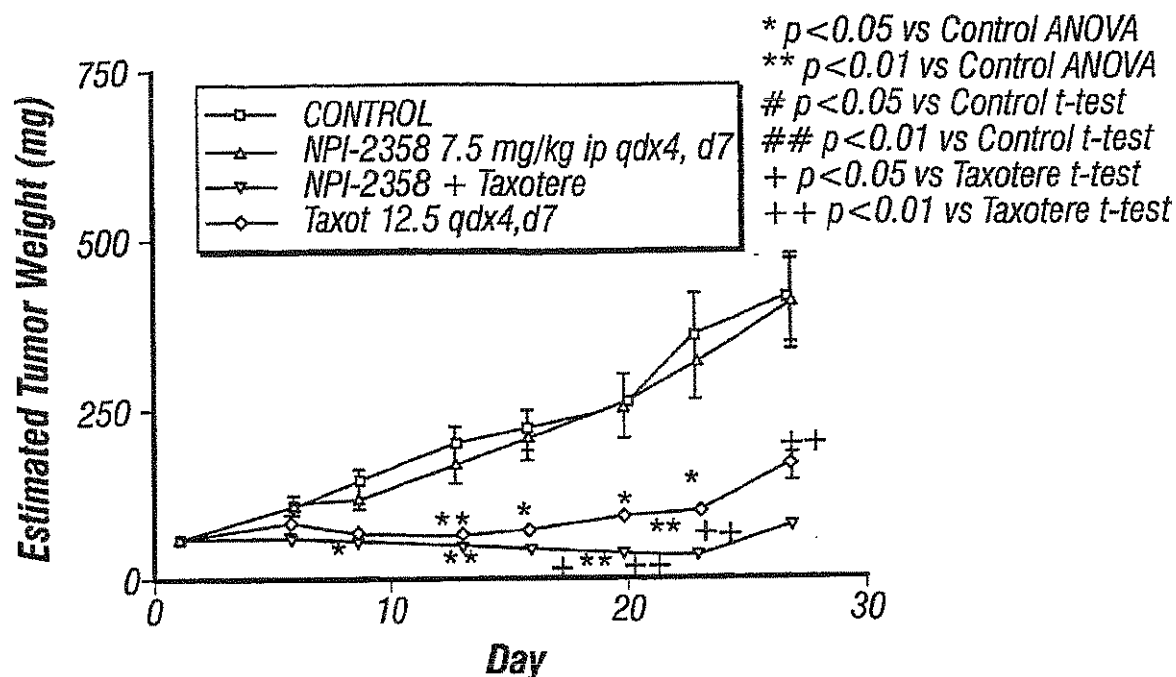


FIG. 25A

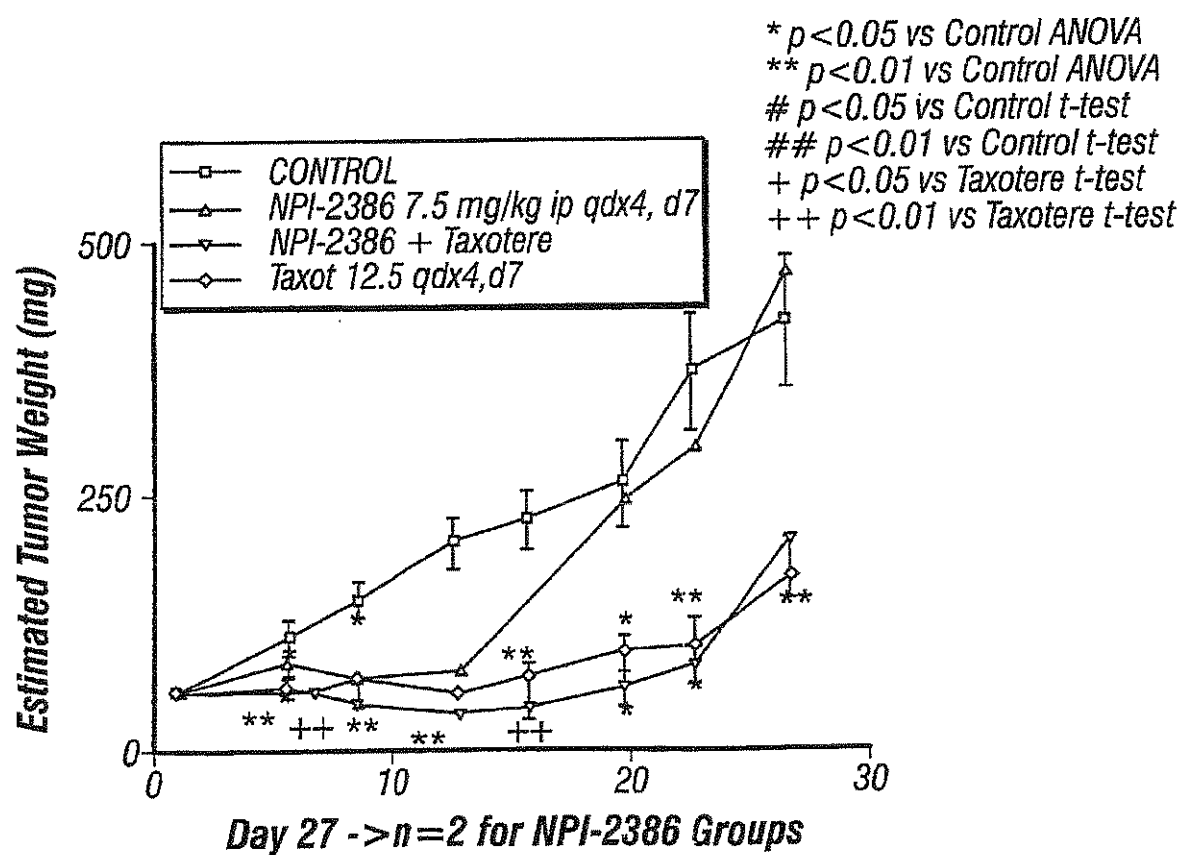


FIG. 25B

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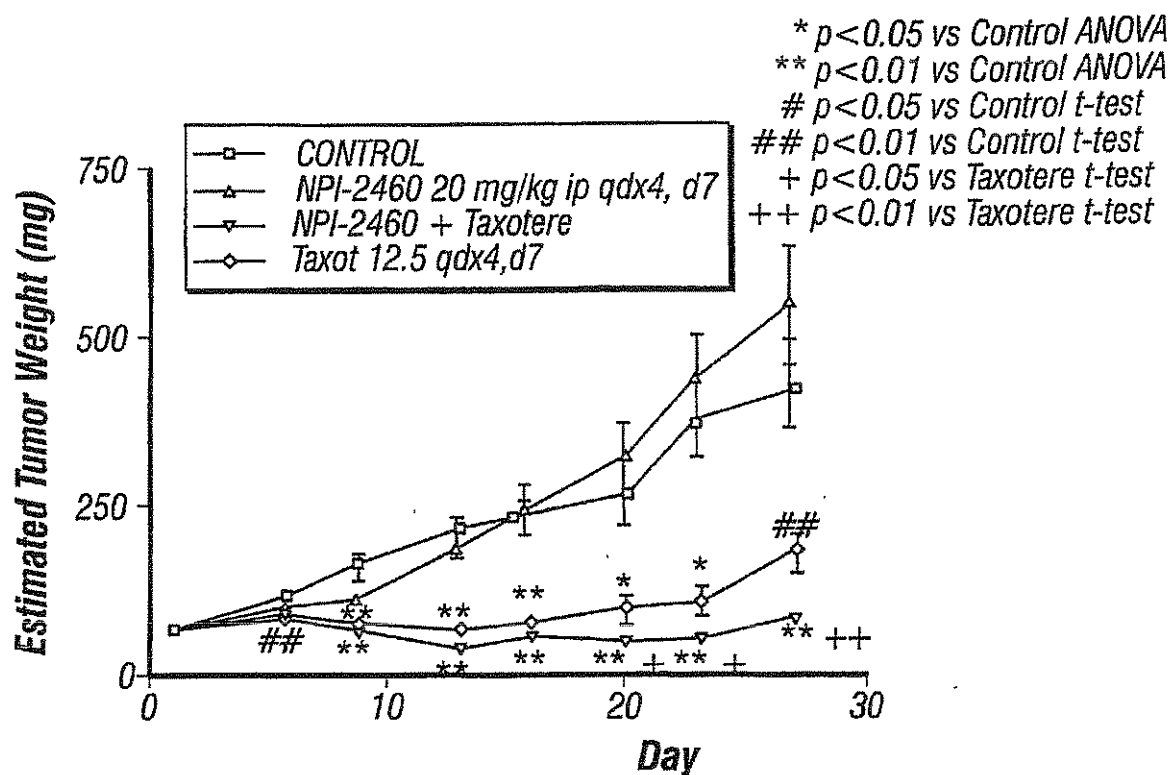


FIG. 25C

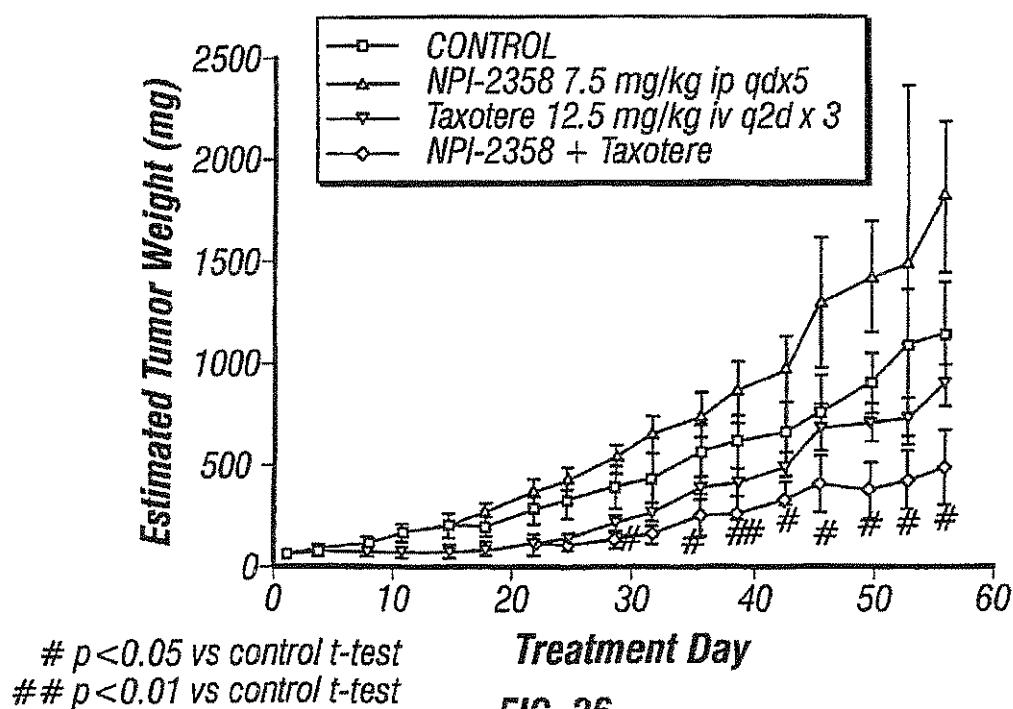
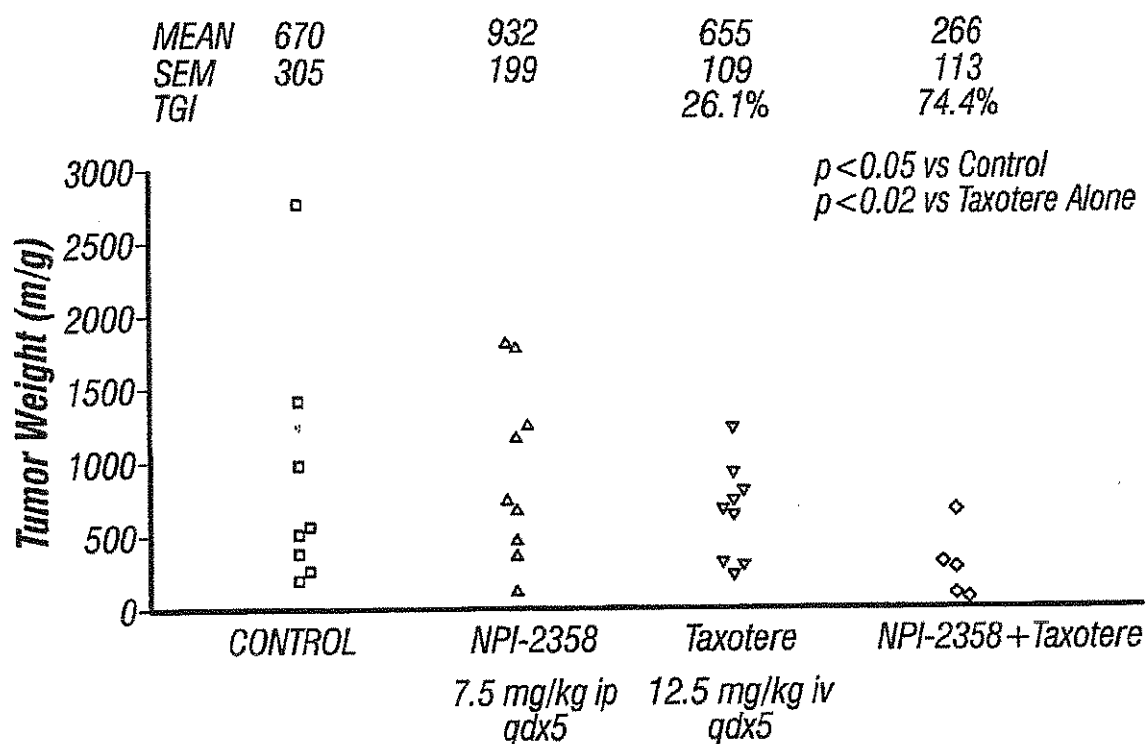
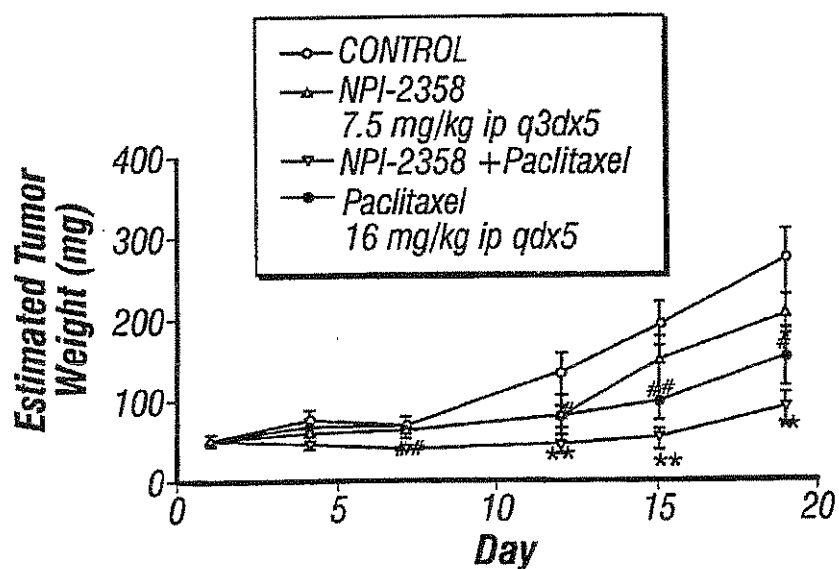


FIG. 26

22/57**FIG. 27**

** $p < 0.01$ vs Control ANOVA
$p < 0.05$ vs Control t-test
$p < 0.01$ vs Control t-test
+ $p < 0.05$ vs Paclitaxel, t-test

FIG. 28

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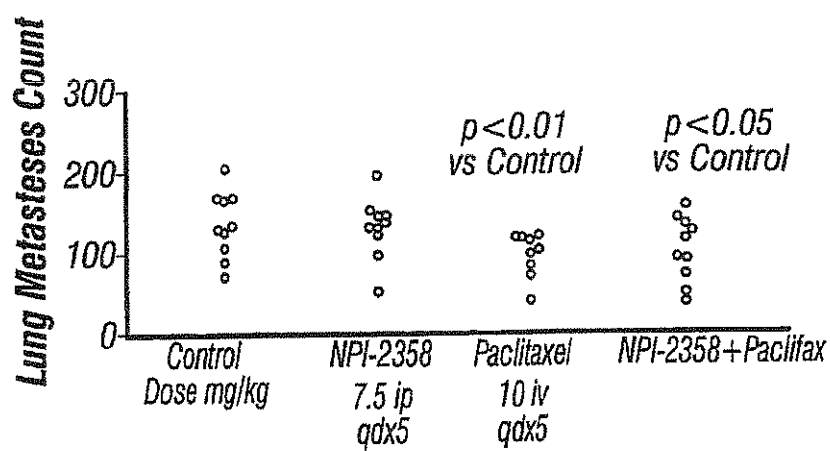


FIG. 29A

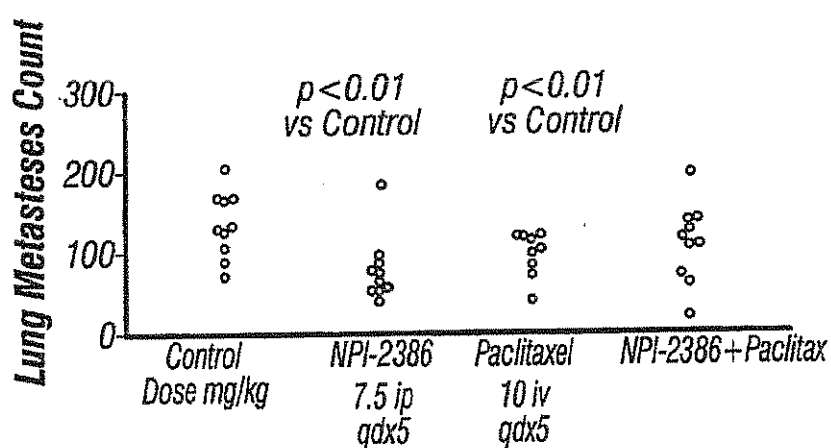


FIG. 29B

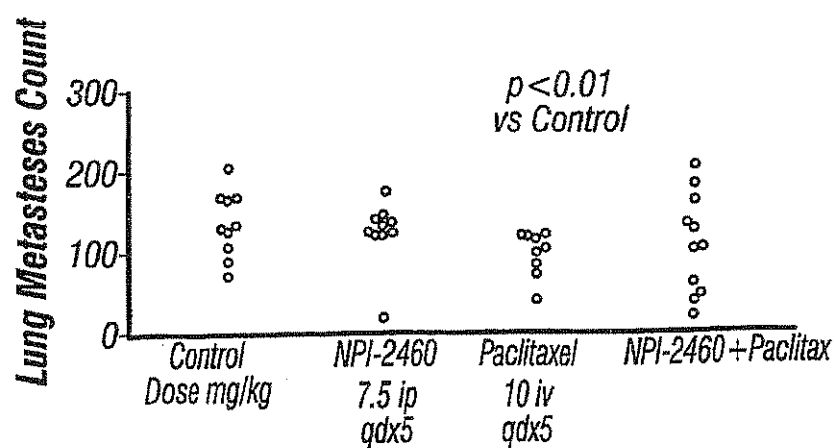


FIG. 29C

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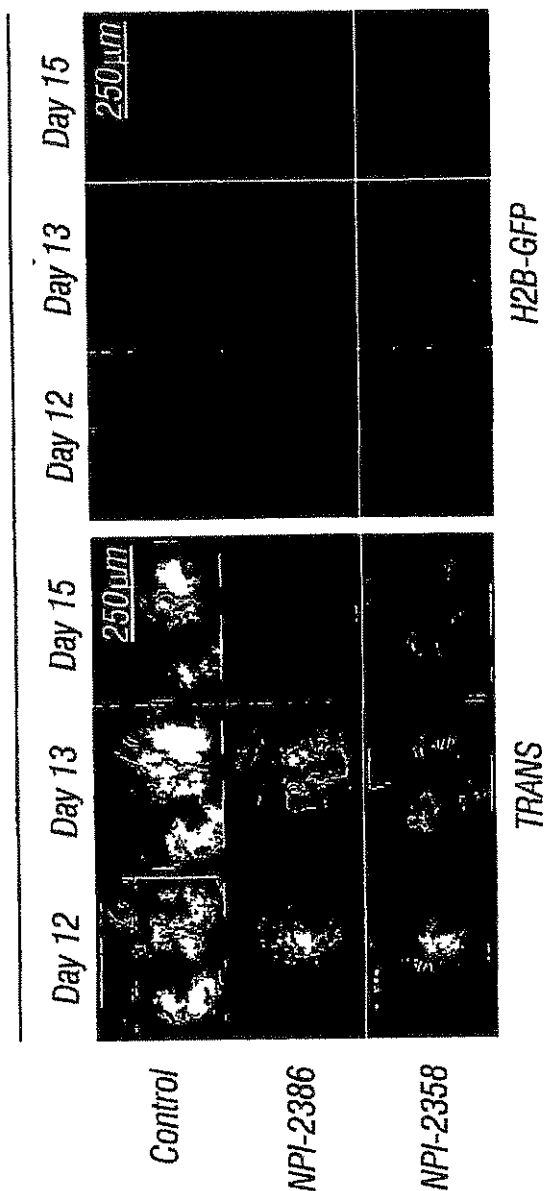


FIG. 30

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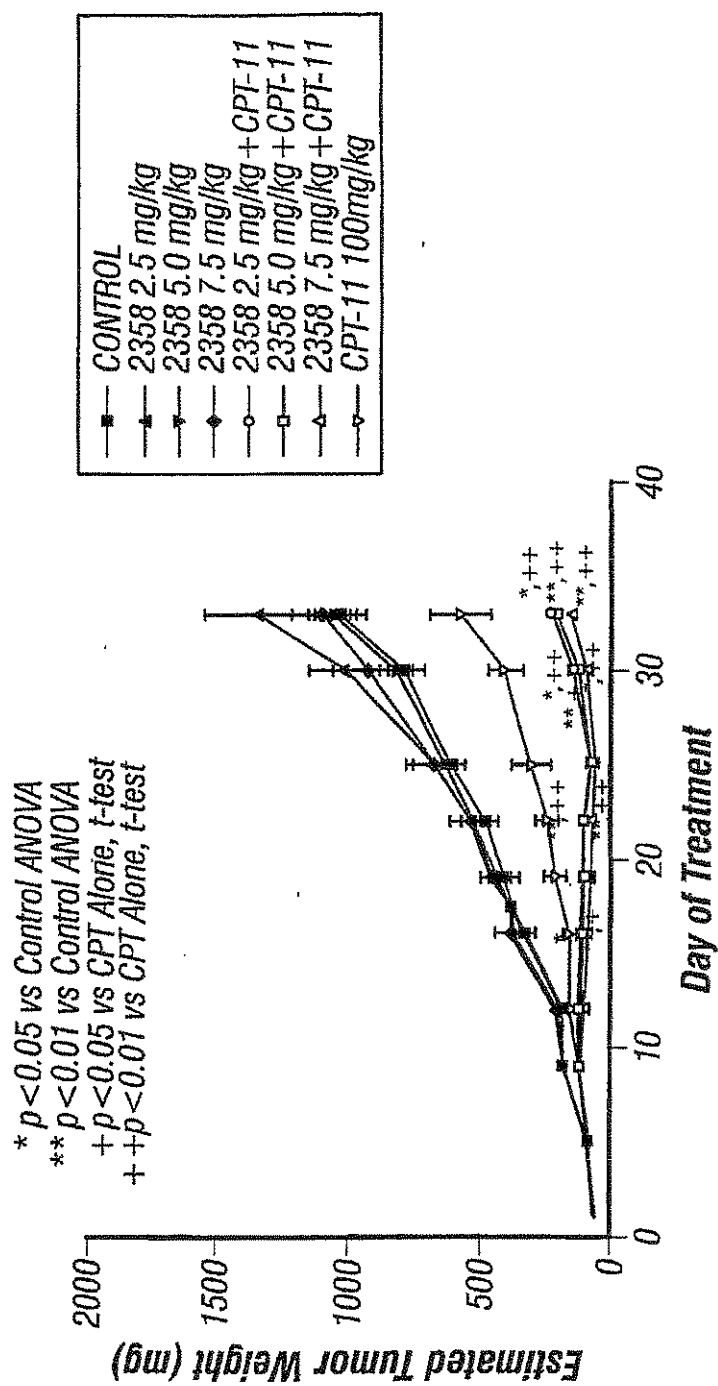
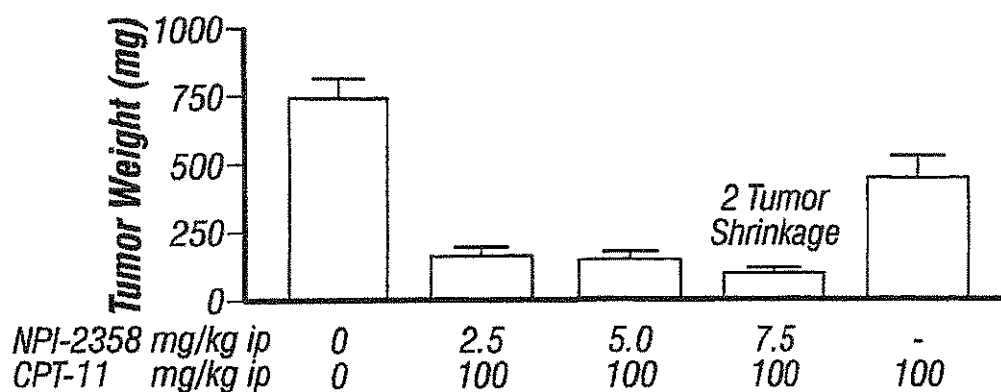
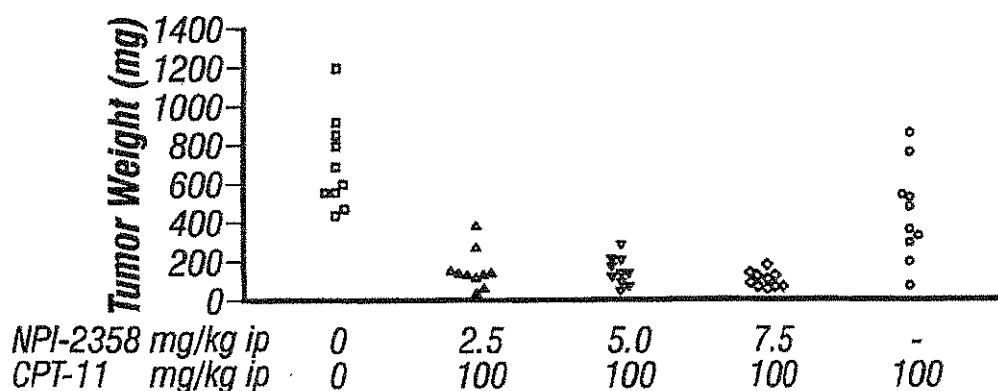


FIG. 31

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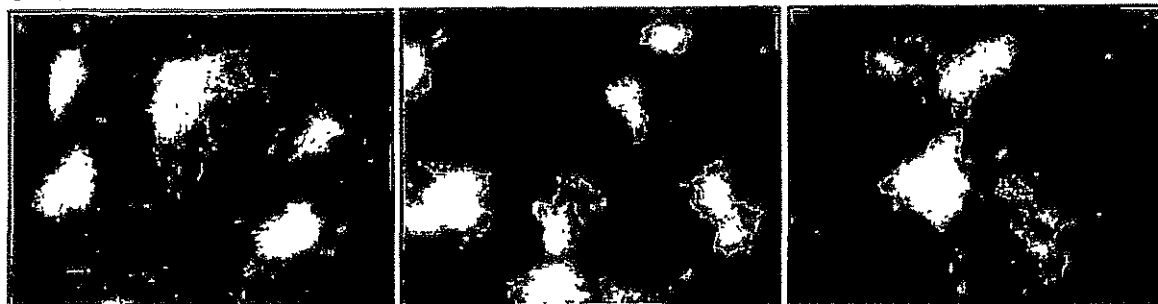
Mean (mg)	736.9	164.3	151.9	101.6	452.3
SEM	76.7	33.2	23.7	12.9	78.7
ANOVA vs Control		<0.01	<0.01	<0.001	NS
TGI (%)		85.3	85.7	2.5 mg/kg	42.3
T-Test				<0.05 vs 2.5 mg/kg	

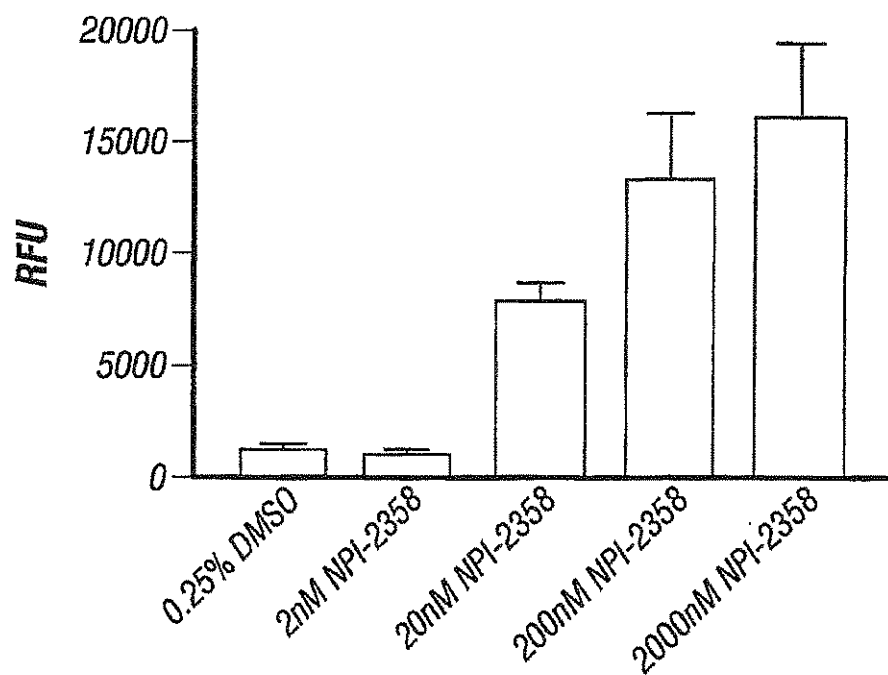
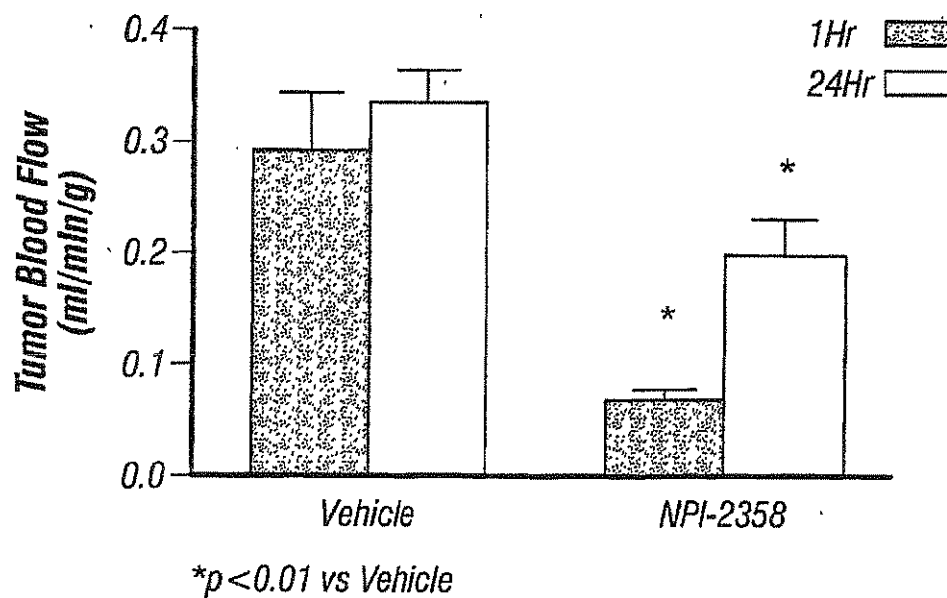
**FIG. 32A****FIG. 32B**

0.25% DMSO

200nM NPI-2358

200nM NPI-2386

**FIG. 33**

27/57**FIG. 34****FIG. 35A**

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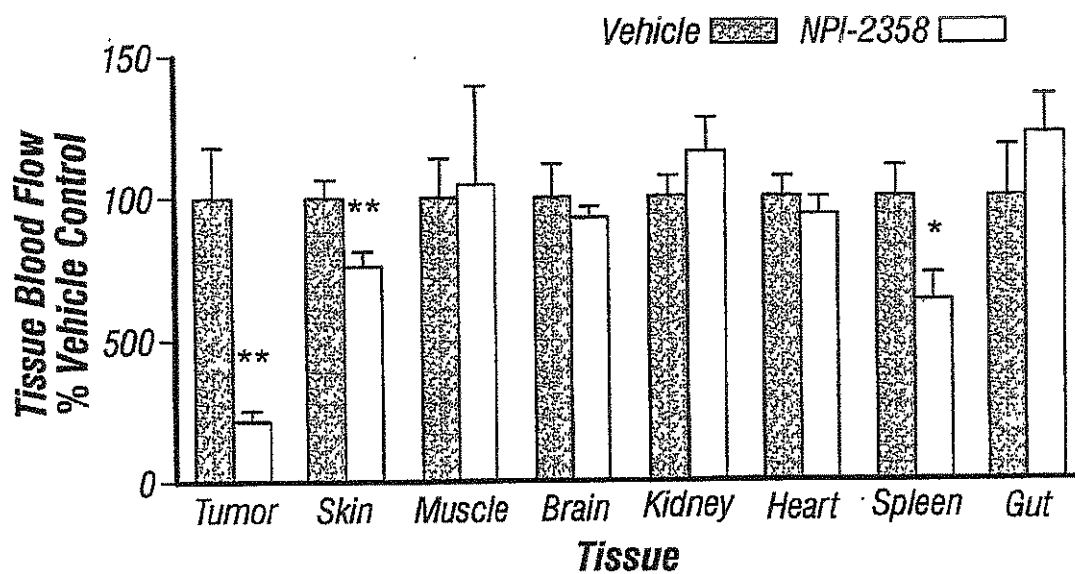
* $p < 0.05$; ** $p < 0.01$ vs Vehicle Control

FIG. 35B

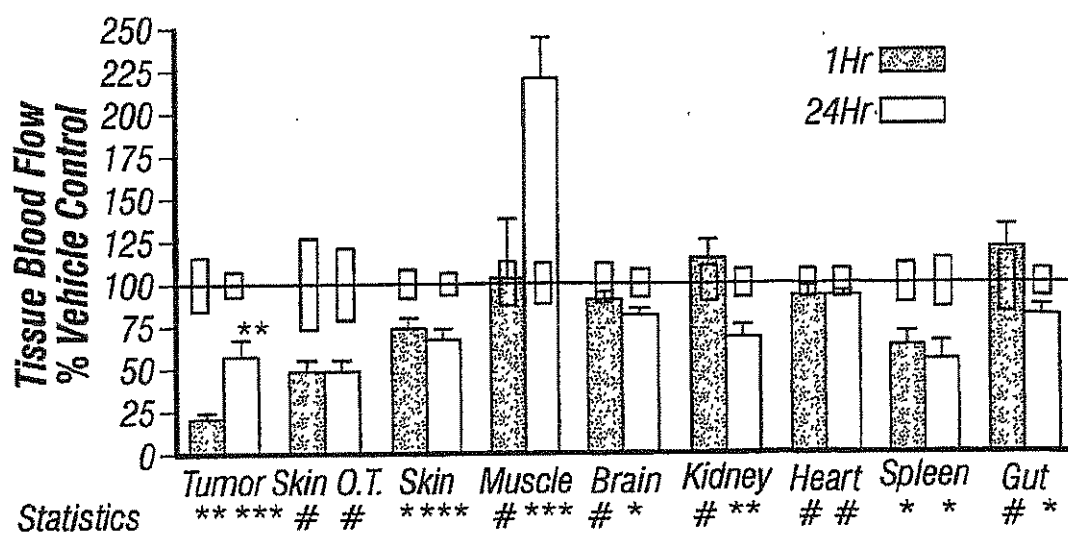
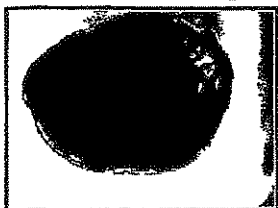
* $p < 0.05$; ** $p < 0.01$; ***= $p < 0.001$; # = NS vs Vehicle
Open Rectangles = 1 SEM of Vehicle

FIG. 36

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1. KPU-02 7.5 mg/kg



2. KPU-02 15.0 mg/kg

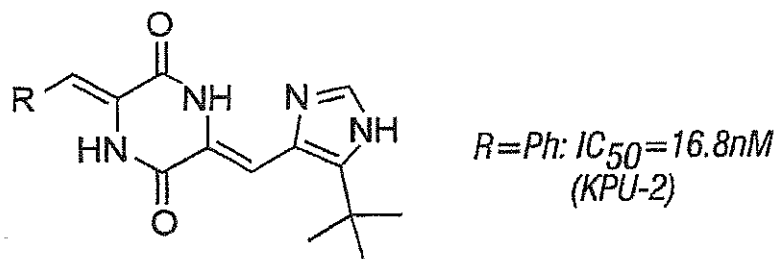


3. Vehicle Control



FIG. 37

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compd.	R	$IC_{50}/HT-29$ cells (nM)
--------	-----	----------------------------

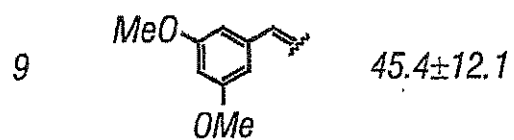
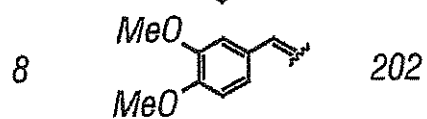
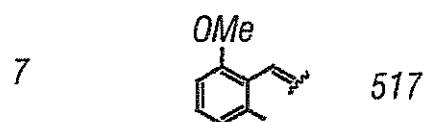
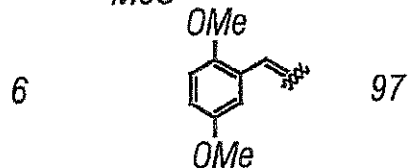
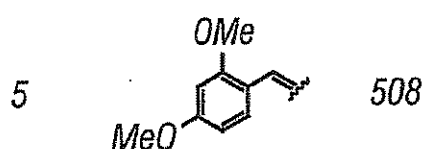
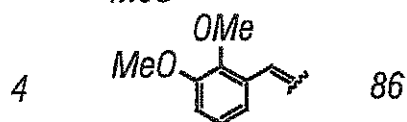
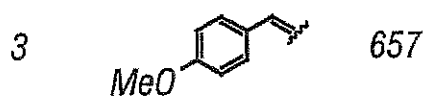
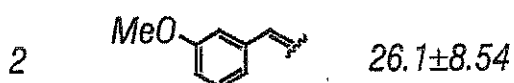
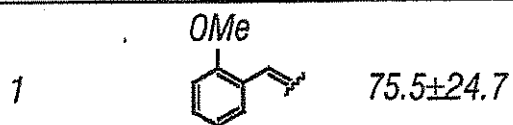


FIG. 38

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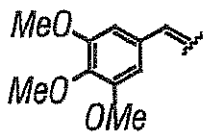
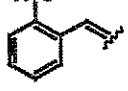
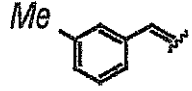
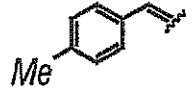
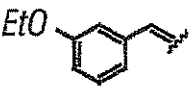
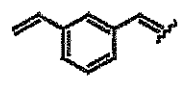
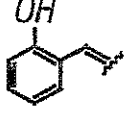
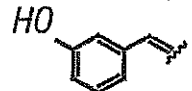
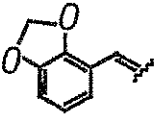
compd.	R 	IC_{50} /HT-29 cells (nM)
1		819
1		45.8 ± 11.5
1		46.7 ± 9.64
1		483 ± 61.5
1		45.9 ± 10.2
1		17.0 ± 1.52
1		6160 ± 1230
1		364 ± 187
1		52

FIG. 38
(Continued)

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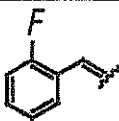
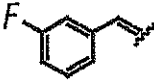
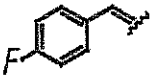
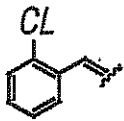
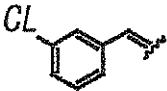
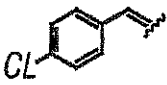
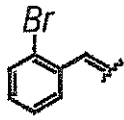
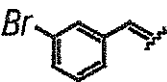
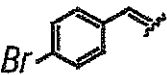
compd.	R 	$IC_{50}/HT-29$ cells (nM)
1		30 ± 16.5
2		13.1 ± 5.32
2		501 ± 84.8
2		46.3 ± 13.5
2		24.5 ± 8.73
2		70
2		40.5 ± 10.9
2		31.1 ± 7.29
2		71

FIG. 38
(Continued)

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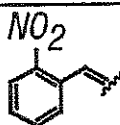
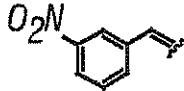
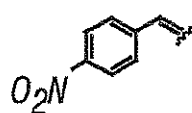
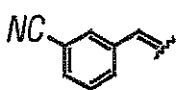
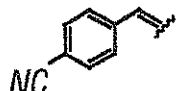
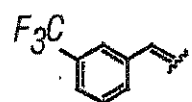
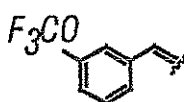
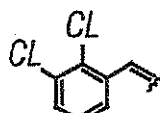
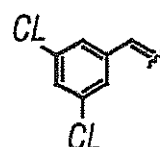
compd.	R 	IC_{50} /HT-29 cells (nM)
2		50.3 ± 12.4
2		44.7 ± 11.9
3		>20000
3		35.8 ± 14.0
3		>20000
3		44.0 ± 7.4
3		14.3 ± 4.93
3		29.9 ± 4.46
3		94.9 ± 5.05

FIG. 38
(Continued)

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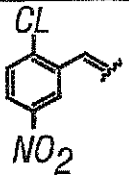
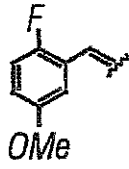
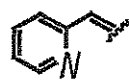
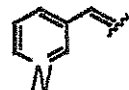
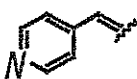
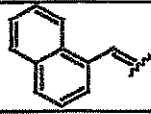
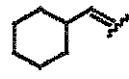
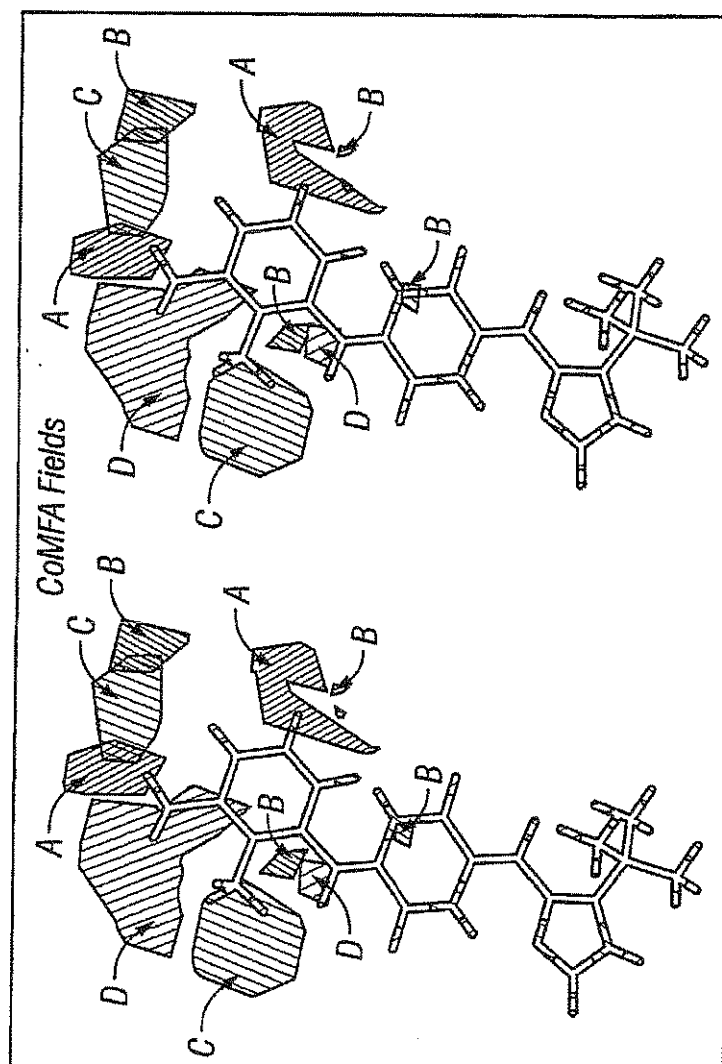
compd.	R	IC ₅₀ /HT-29 cells (nM)
3		53.4±4.85
3		20.3± 2.12
3		>20000
4		103±11.6
4		54
4		14.9± 2.12
4		173
4		78.9±14.1
paclitaxel		16.2±3.04

FIG. 38
(Continued)

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3D-QSAR (CoMFA)



- A: Sterically favorable.
 B: Sterically unfavorable.
 C: Positive electrostatic potentials are favorable.
 D: Negative electrostatic potentials are favorable.

$$pl_{50}=5.62+$$

[CoMFA steric & electrostatic fields]

Component Number=9

$$n=64 \quad s=0.21 \quad r^2=0.93$$

$$s_{CV}=0.62 \quad q^2=0.37$$

Relative Contribution: St=58%

El=42%

Molecular Models: Structures of compounds were optimized by the molecular orbital method, PM3.
 Atomic charges: Calculated by MNDO-ESP.

CoMFA: Carried out with the Advanced CoMFA module of SYBYL 6.8.

Probe: C(sp³) for the calculation of the steric interaction potential.
 +1.0 charge for the calculation of the electrostatic potential.

FIG. 39

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Proposed active conformation of KPU-2
from X-ray crystallographic analysis

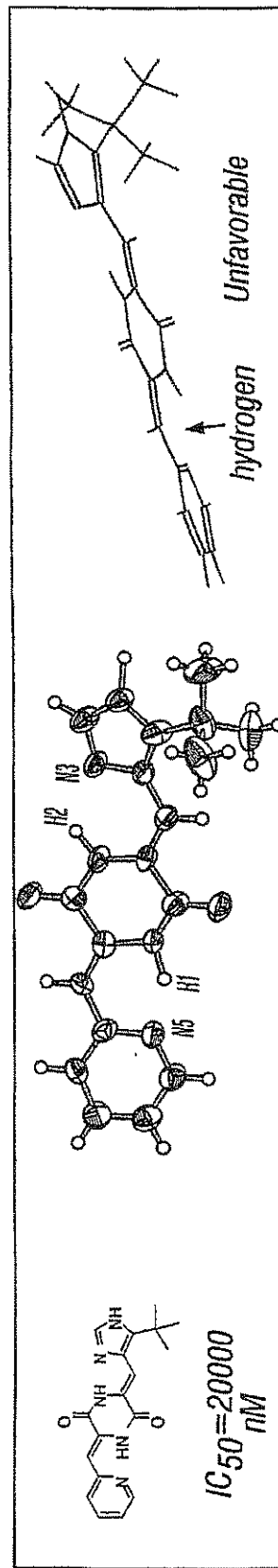
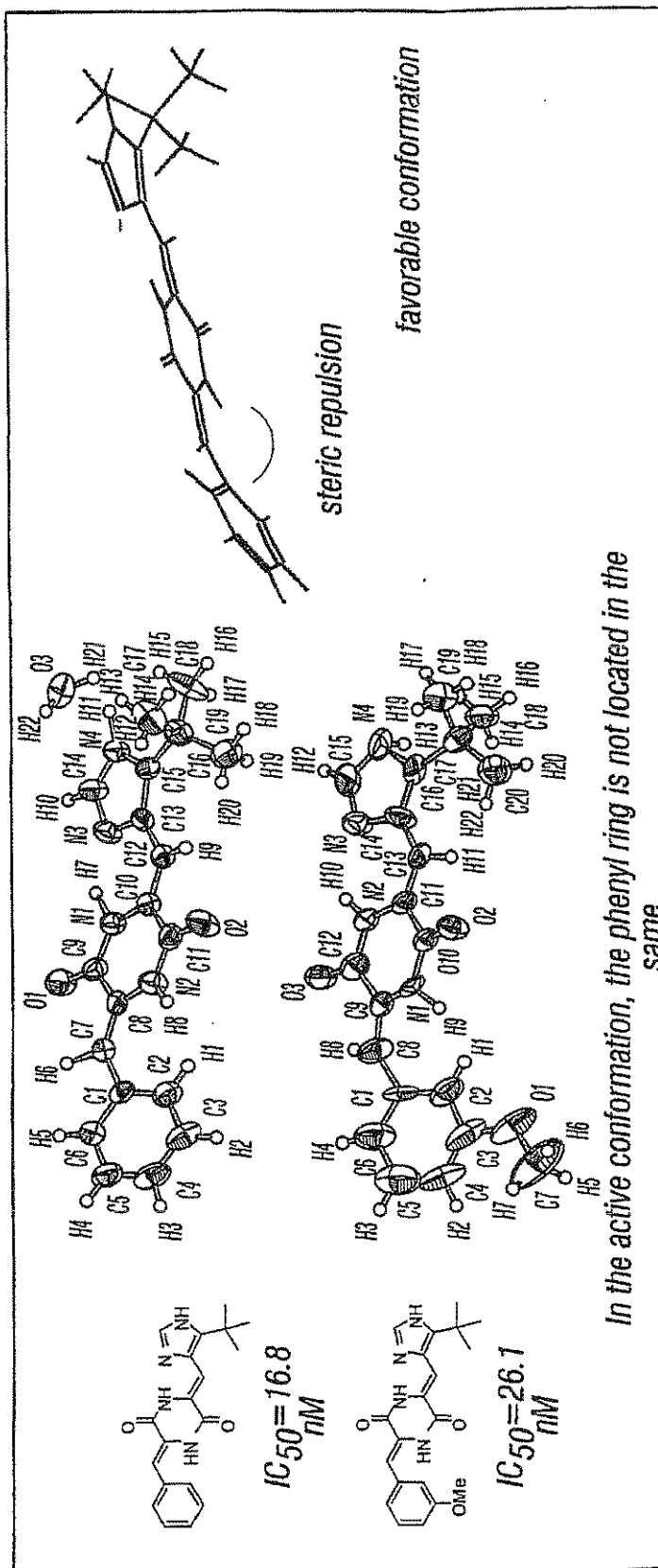


FIG. 40

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compound	structure	IC ₅₀ (μM)		IC ₅₀ (nM, cytotoxicity)		
		tubulin polymerization		HT-29	PC-3	P-
(-)-PLH		31. 3		394	n.t.	388 180
ΔPLH		20. 9		43	4.7	36
KPU-2 (NPI-2358)		16. 8		14.9	1.0	2.8
colchicine		96. 7		n.t.	n.t.	210

Cytoskeleton Immunofluorescence Staining og PC-3 Cells

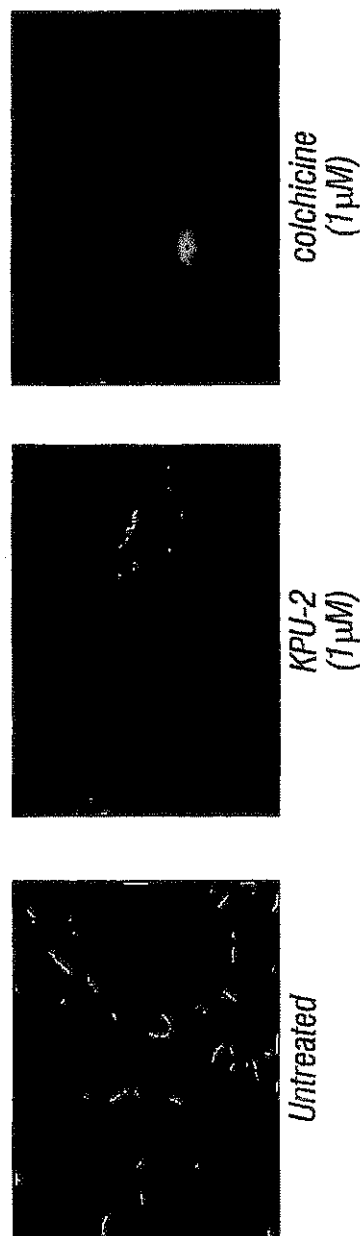


FIG. 41

KPU-2 showed more potent activity that ΔPLH.

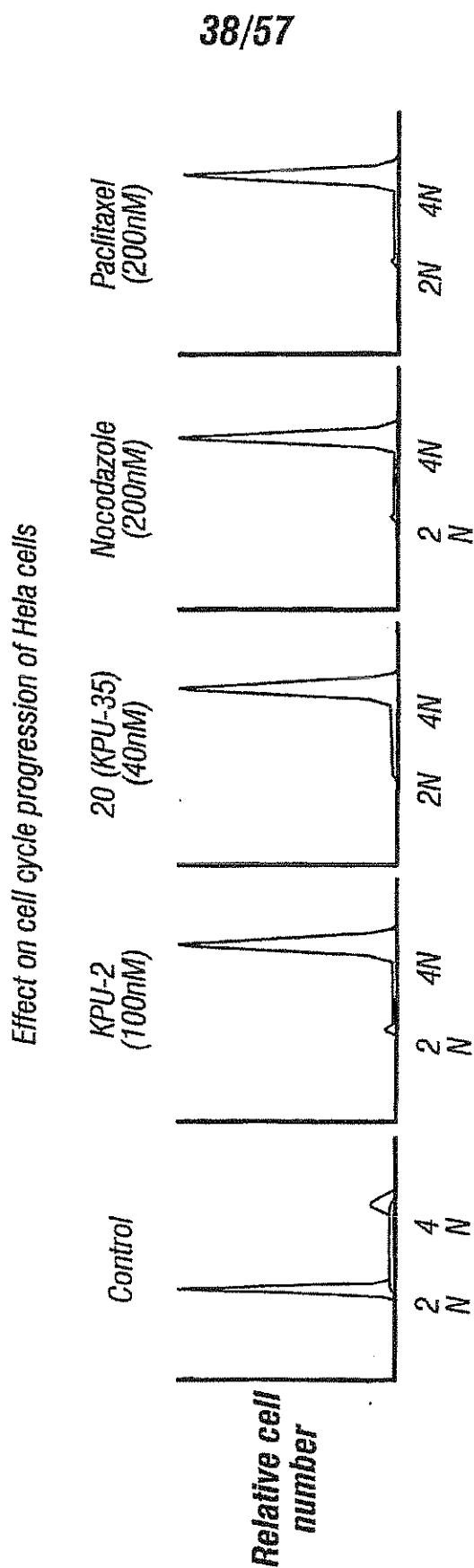


FIG. 42

39/57*Effect of PLH derivatives on drug-sensitive and
-resistance tumor cell lines*

<i>compound</i>	<i>MES-SA uterine sarcoma cyclosporin pretreatment</i>		<i>MES-SA/DX-5 (resistant) cyclosporin pretreatment</i>	
	<i>(-)</i>	<i>(+)</i>	<i>(-)</i>	<i>(+)</i>
<i>ΔPLH</i>	116 $\pm$ 15	114 $\pm$ 32	98 $\pm$ 24	88 $\pm$ 15
<i>KLU-2</i>	34 $\pm$ 10	31 $\pm$ 5	23 $\pm$ 7	38 $\pm$ 2
<i>paclitaxel</i>	13 $\pm$ 4	18 $\pm$ 1	>100	27 $\pm$ 2

FIG. 43

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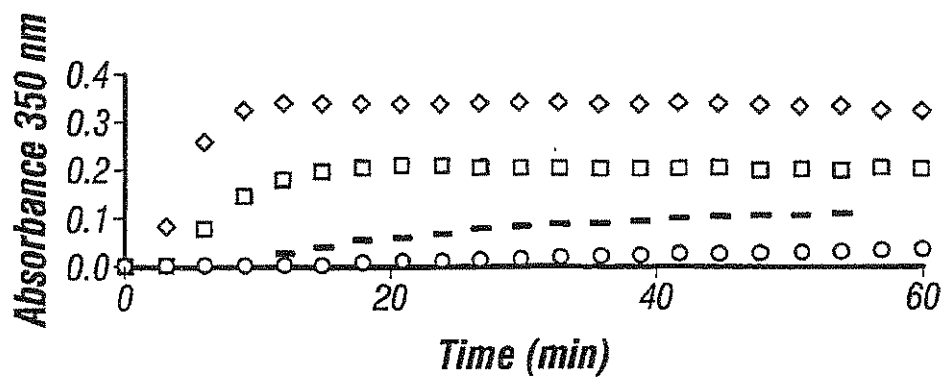


FIG. 44A

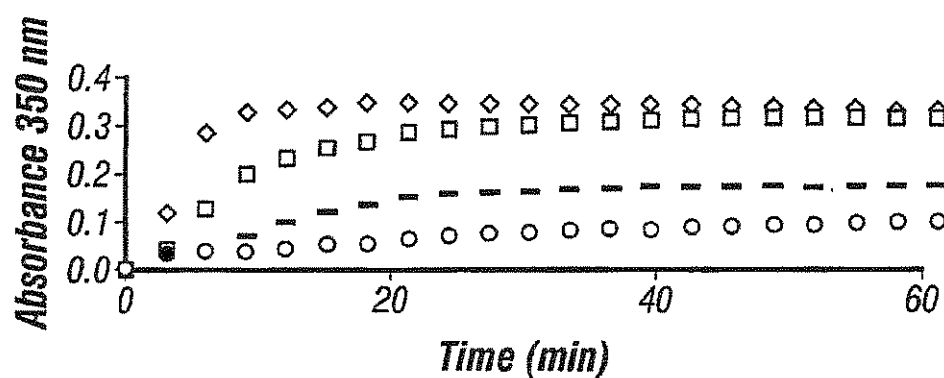


FIG. 44B

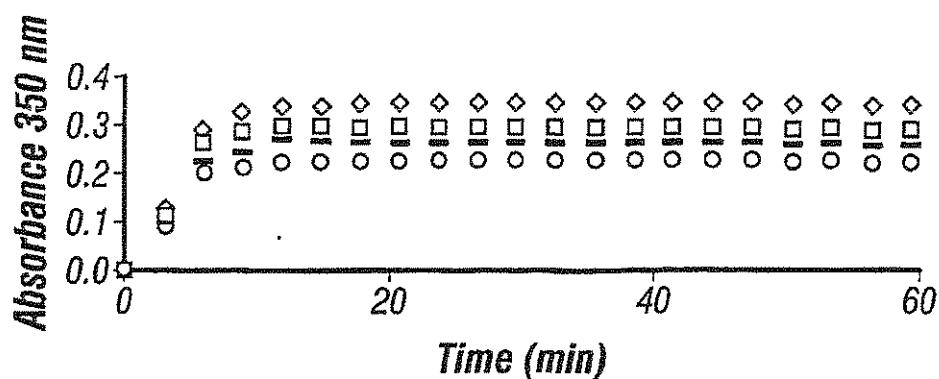


FIG. 44C

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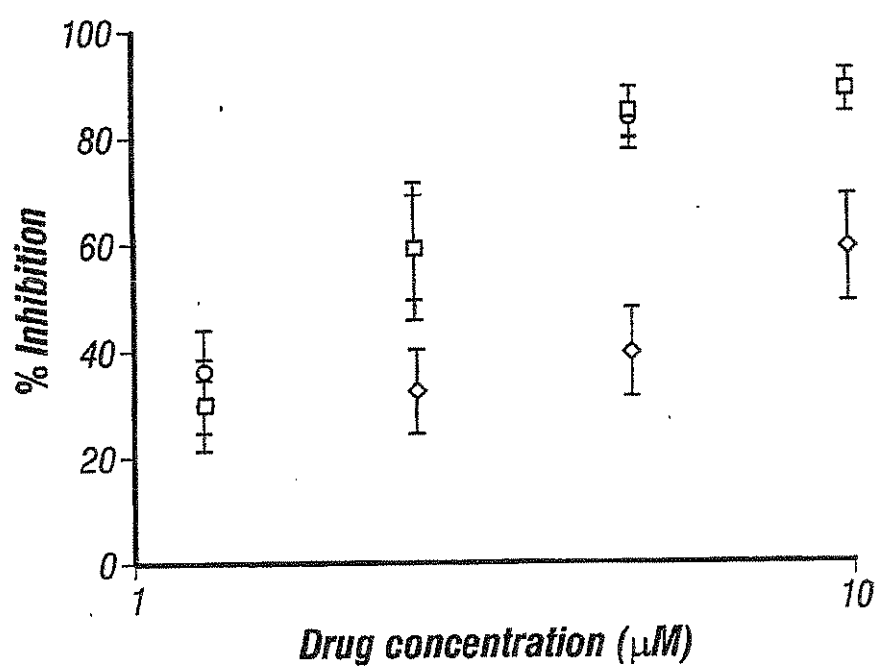


FIG. 45

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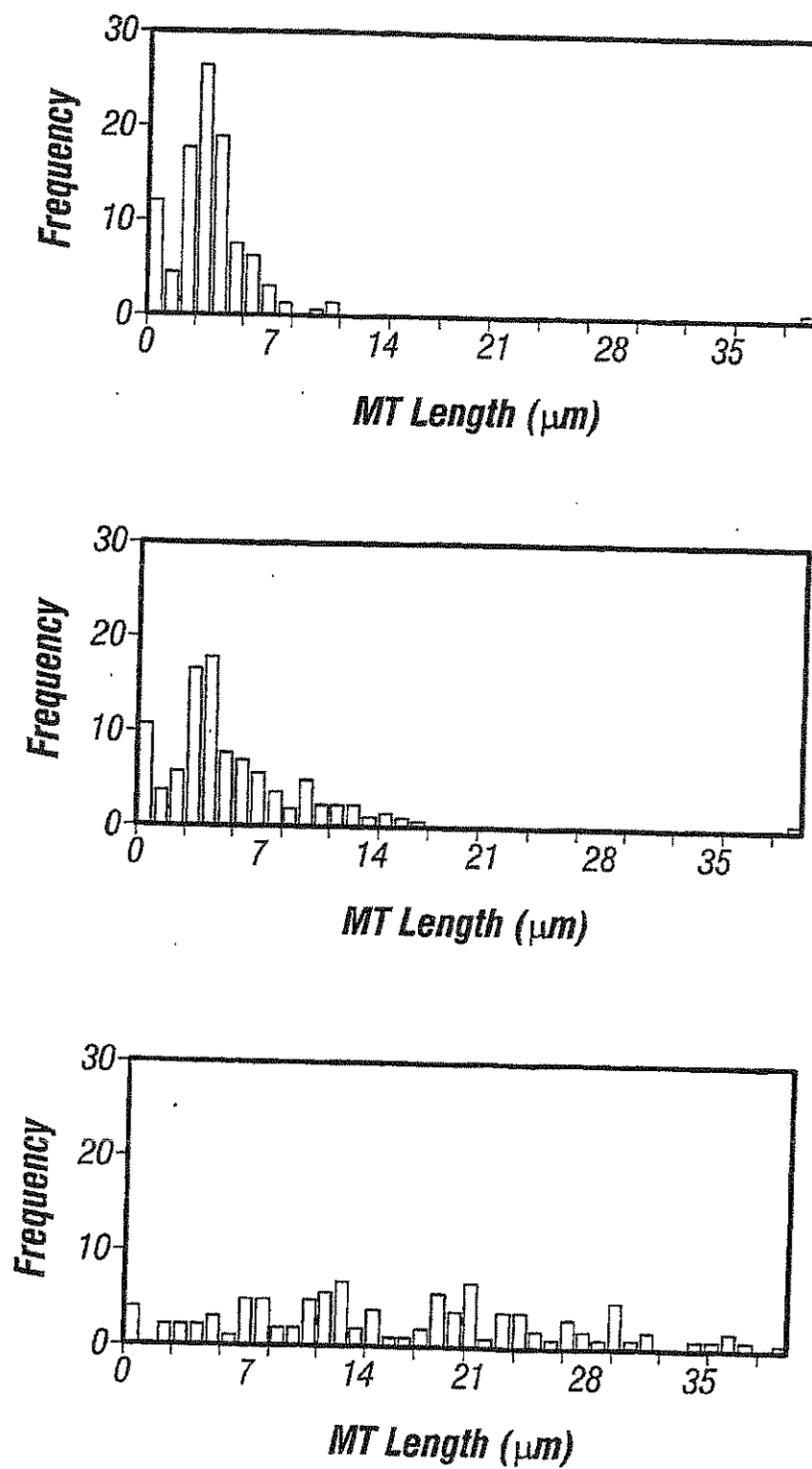


FIG. 46A

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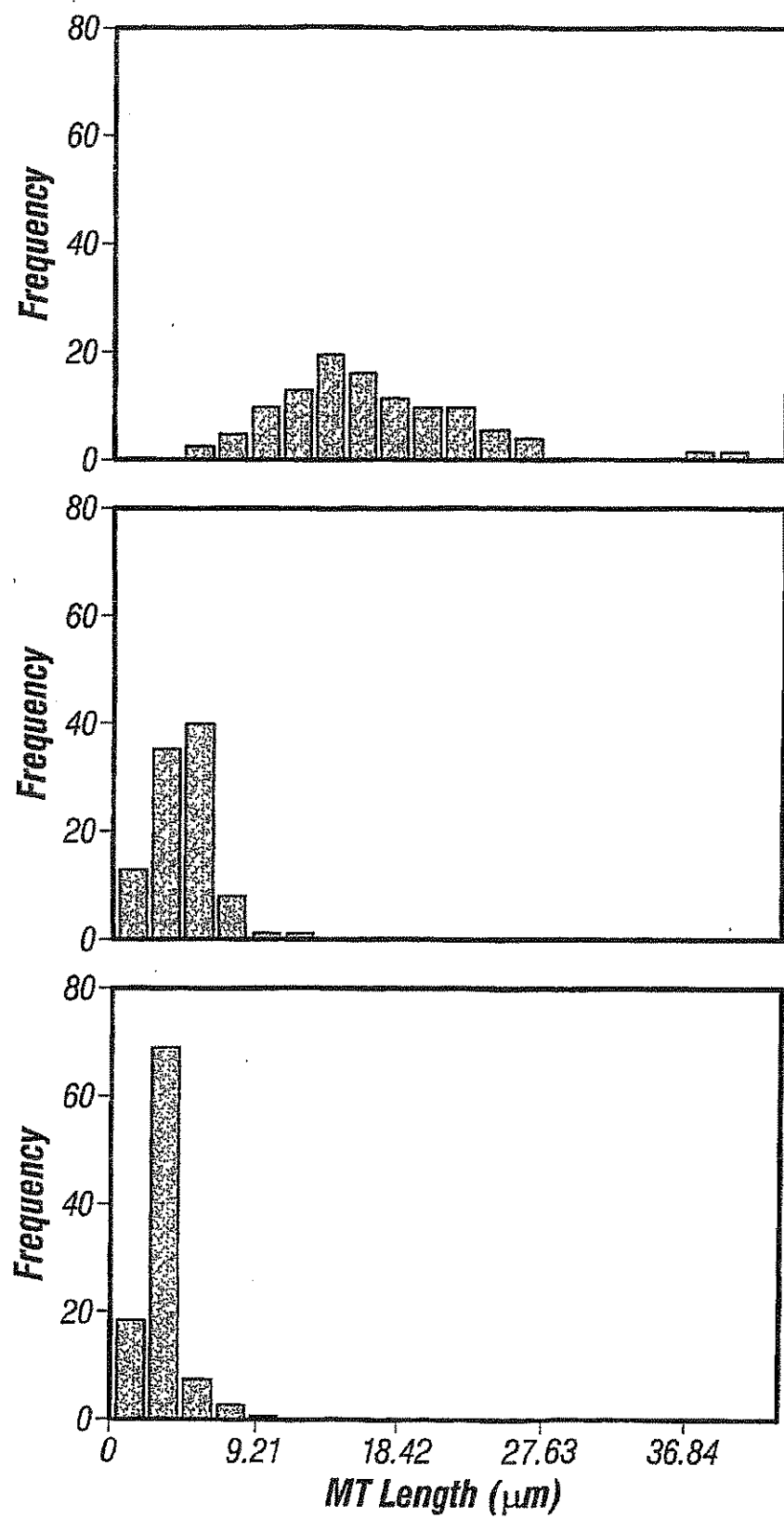


FIG. 46B

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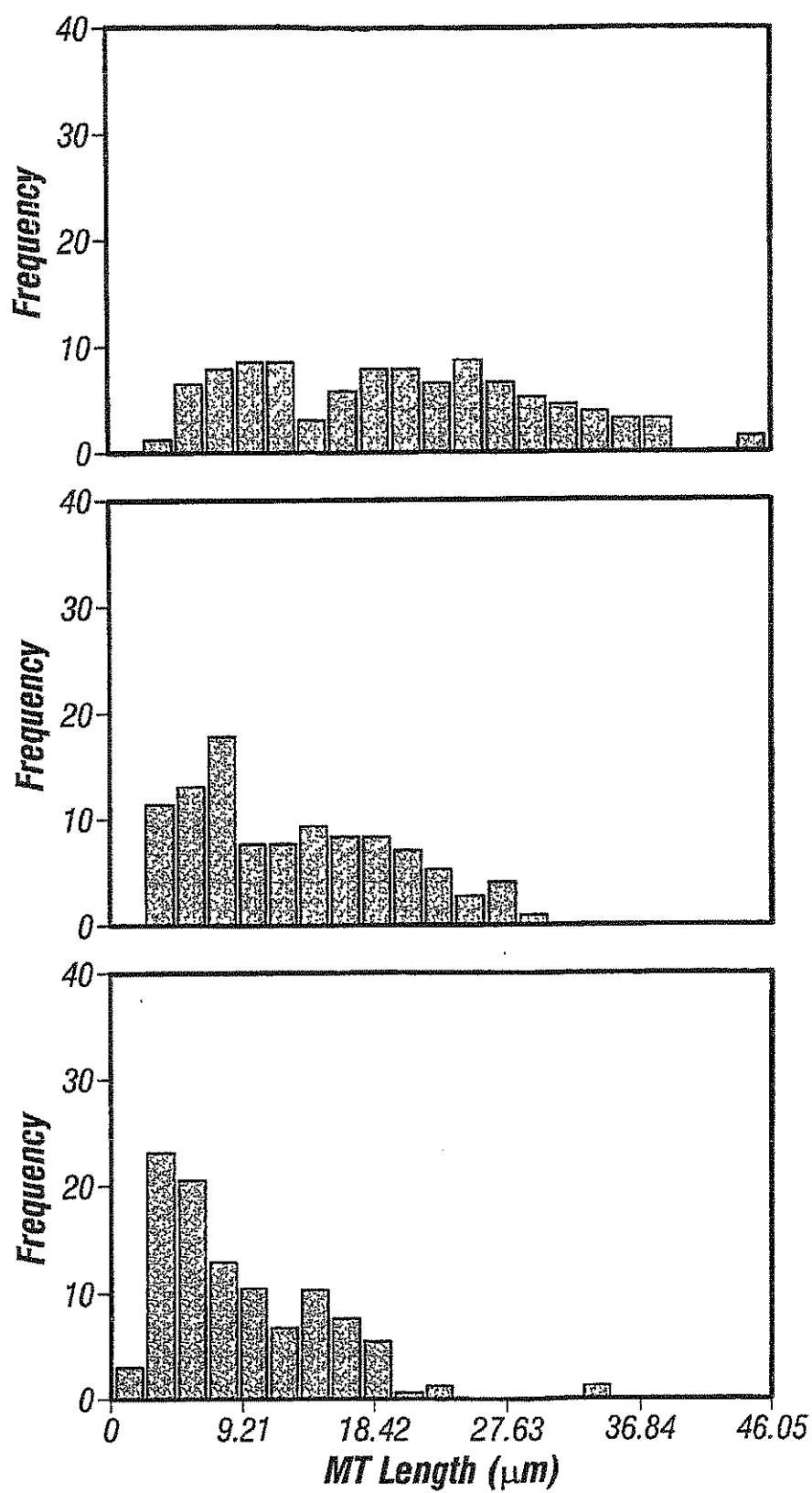


FIG. 46C

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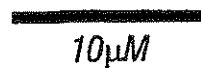
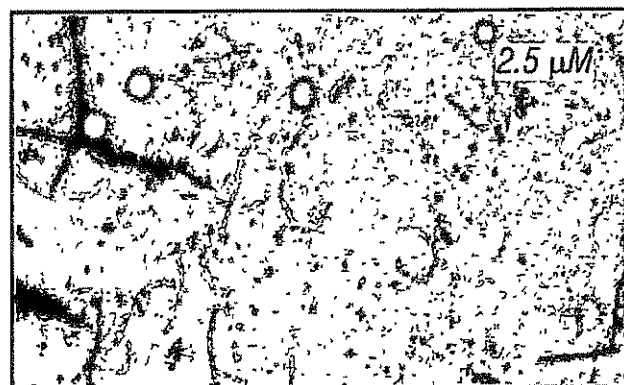
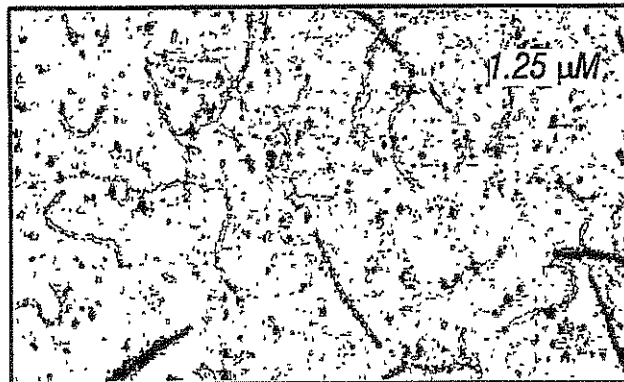
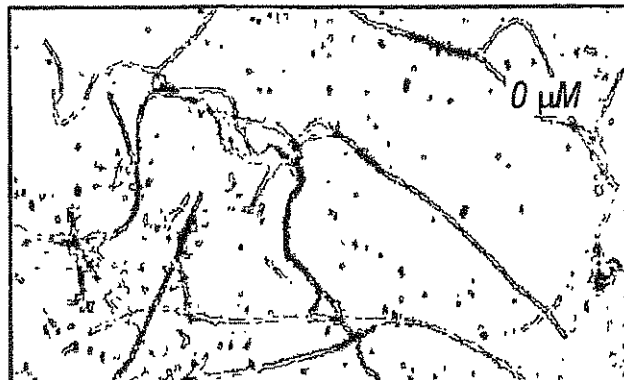
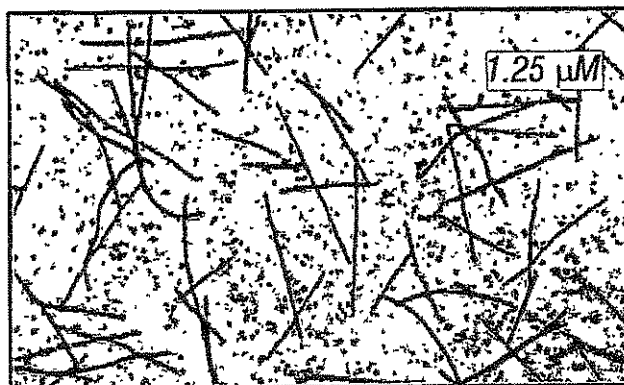


FIG. 47A

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10μm

FIG. 47B

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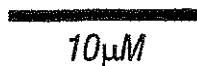
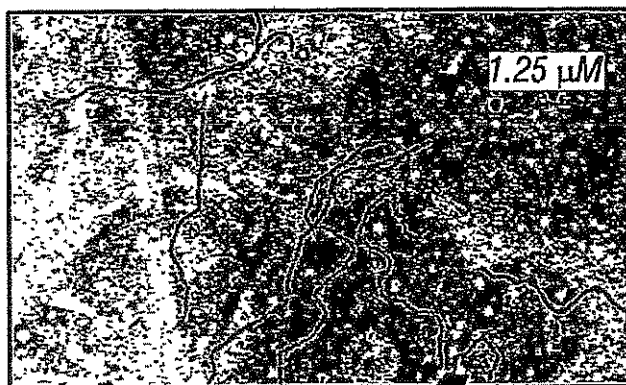
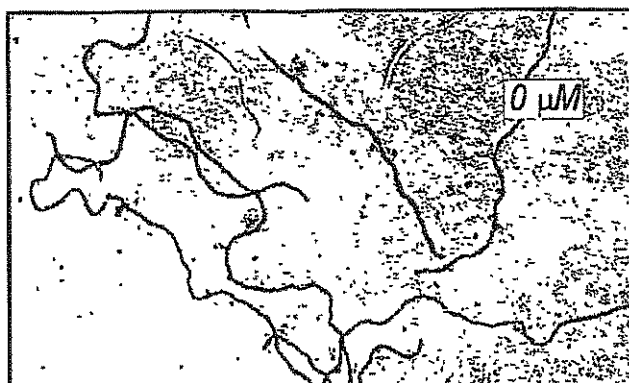


FIG. 47C

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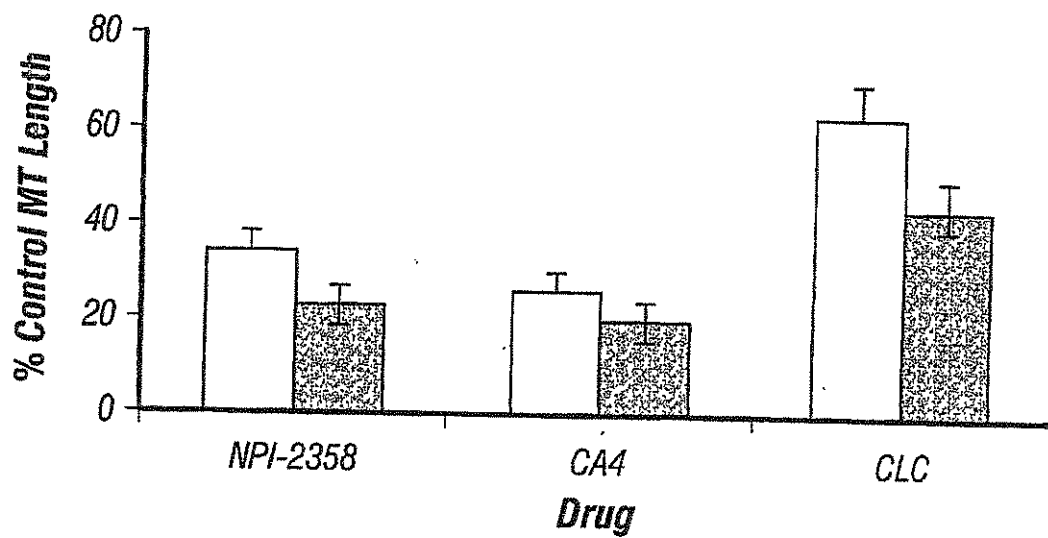


FIG. 48

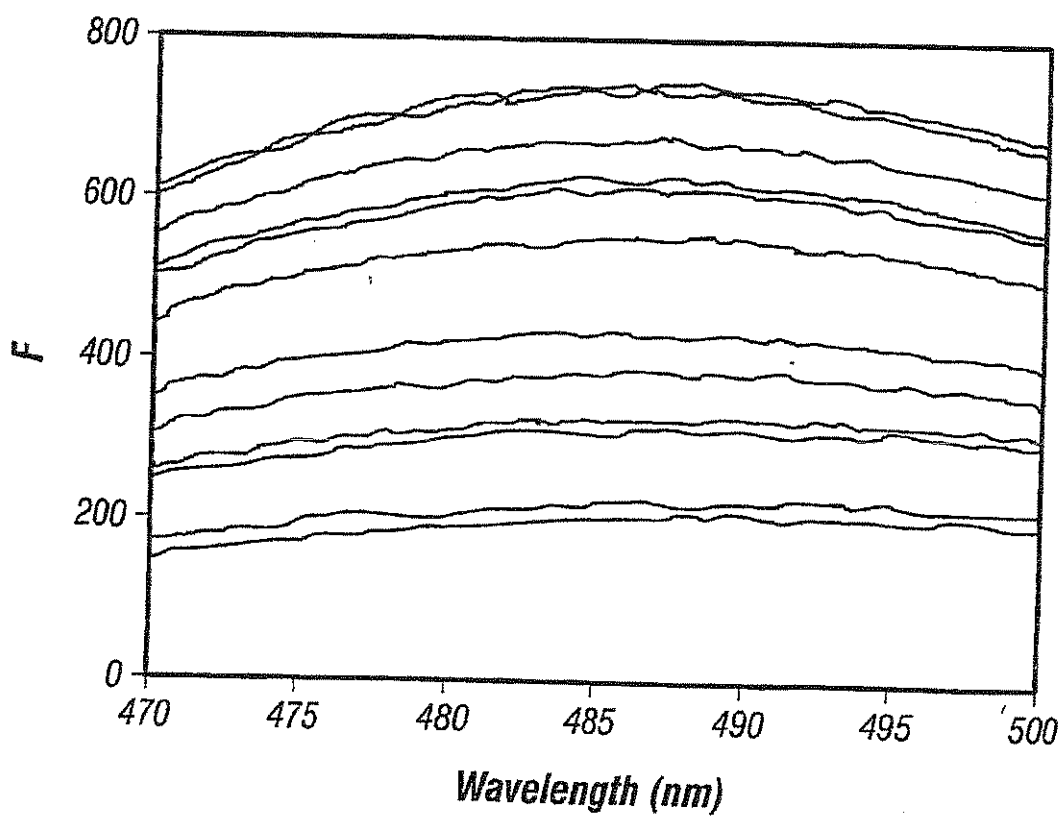


FIG. 49A

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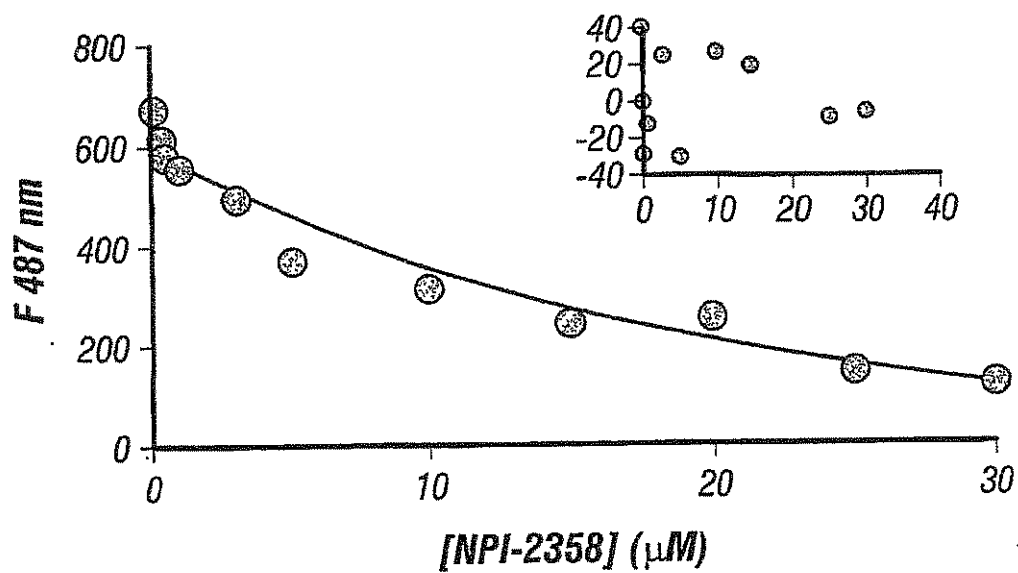


FIG. 49B

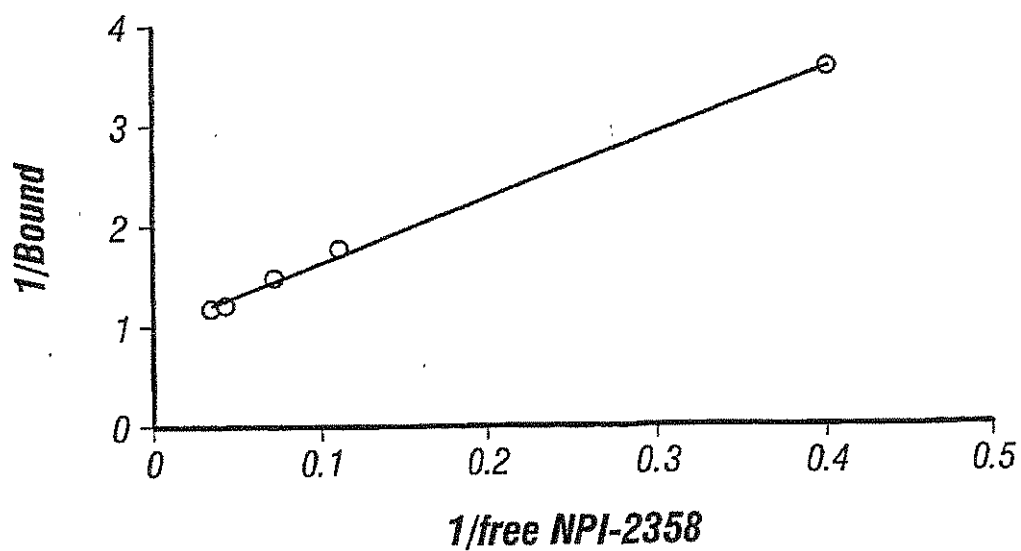


FIG. 49C

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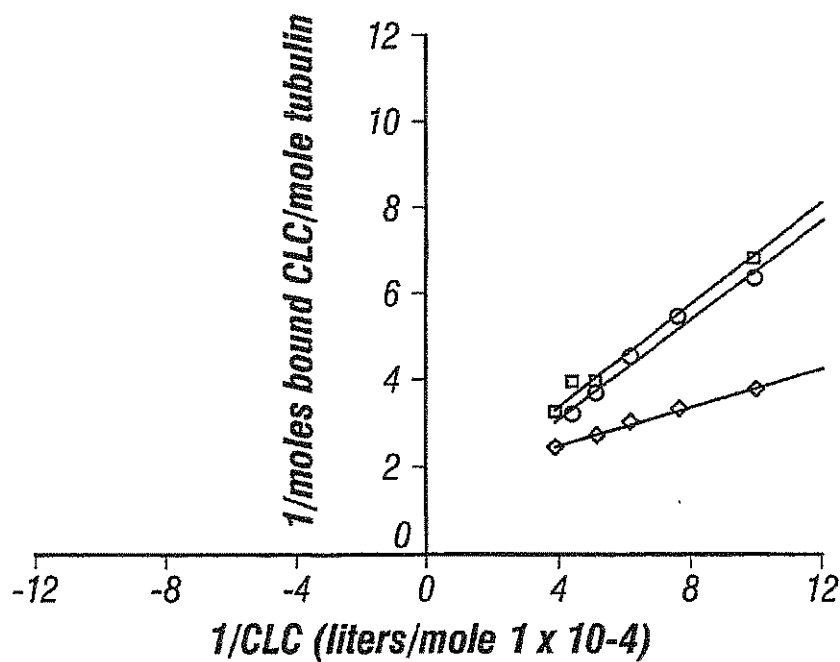


FIG. 50

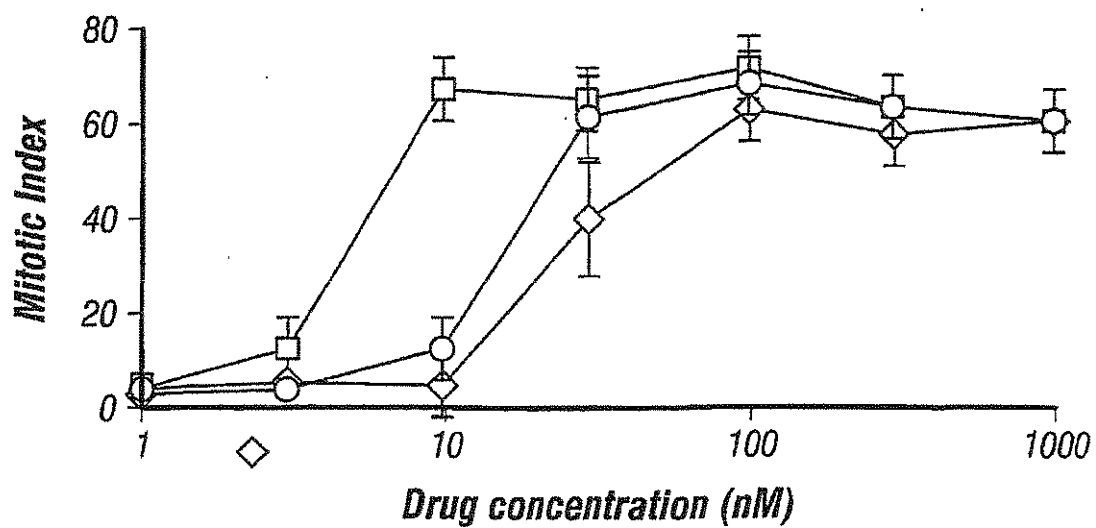


FIG. 51

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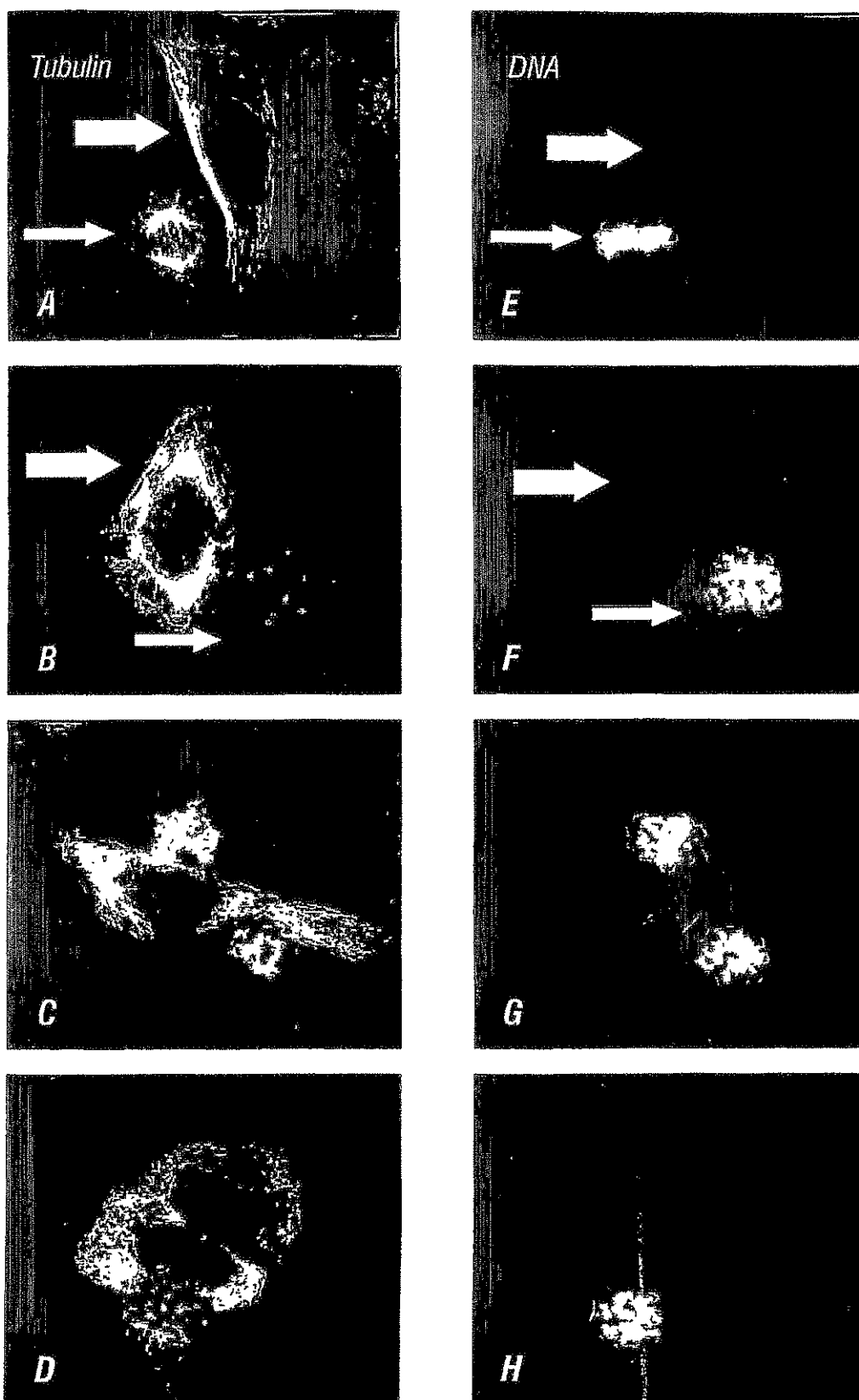


FIG. 52

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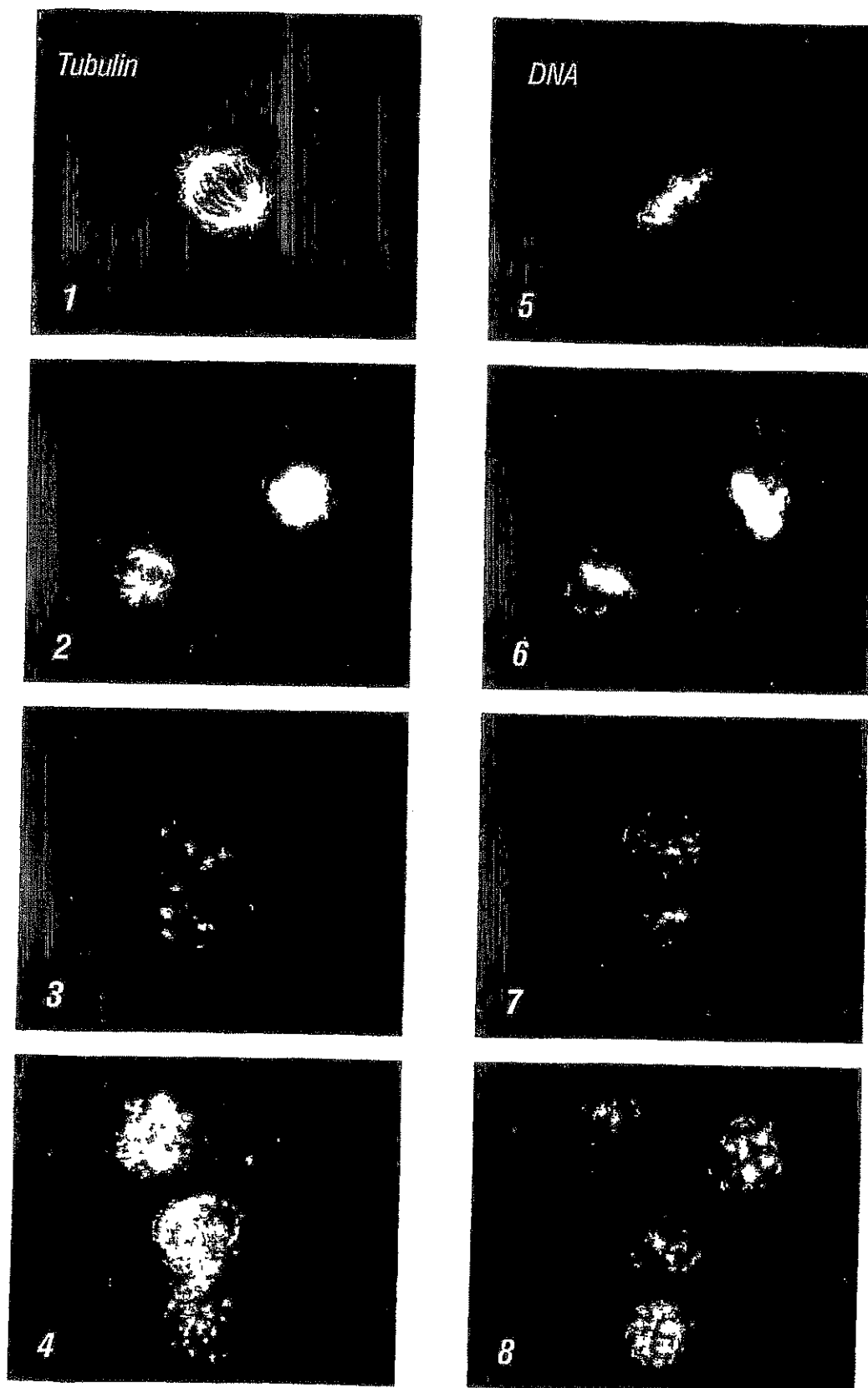


FIG. 53A

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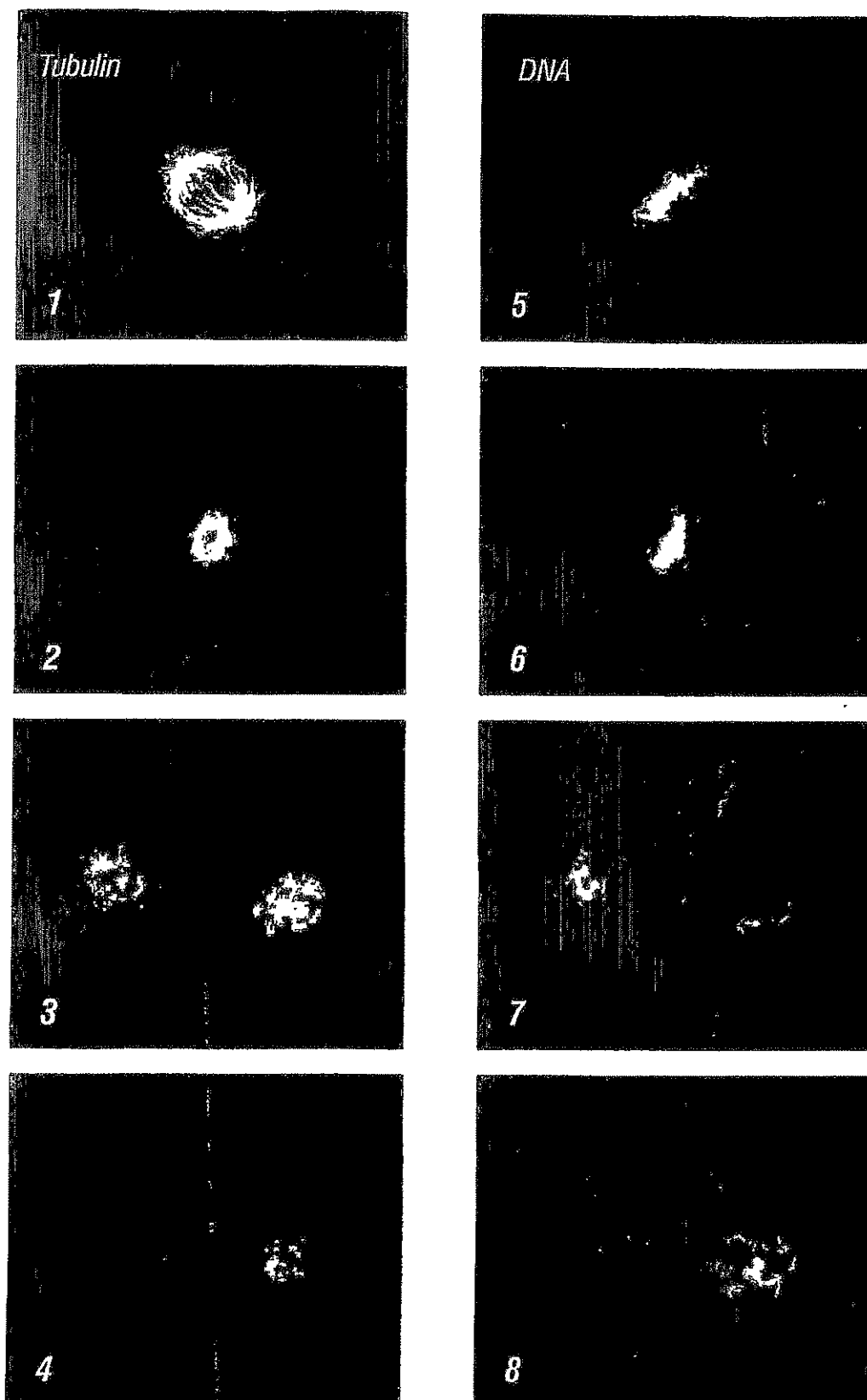


FIG. 53B

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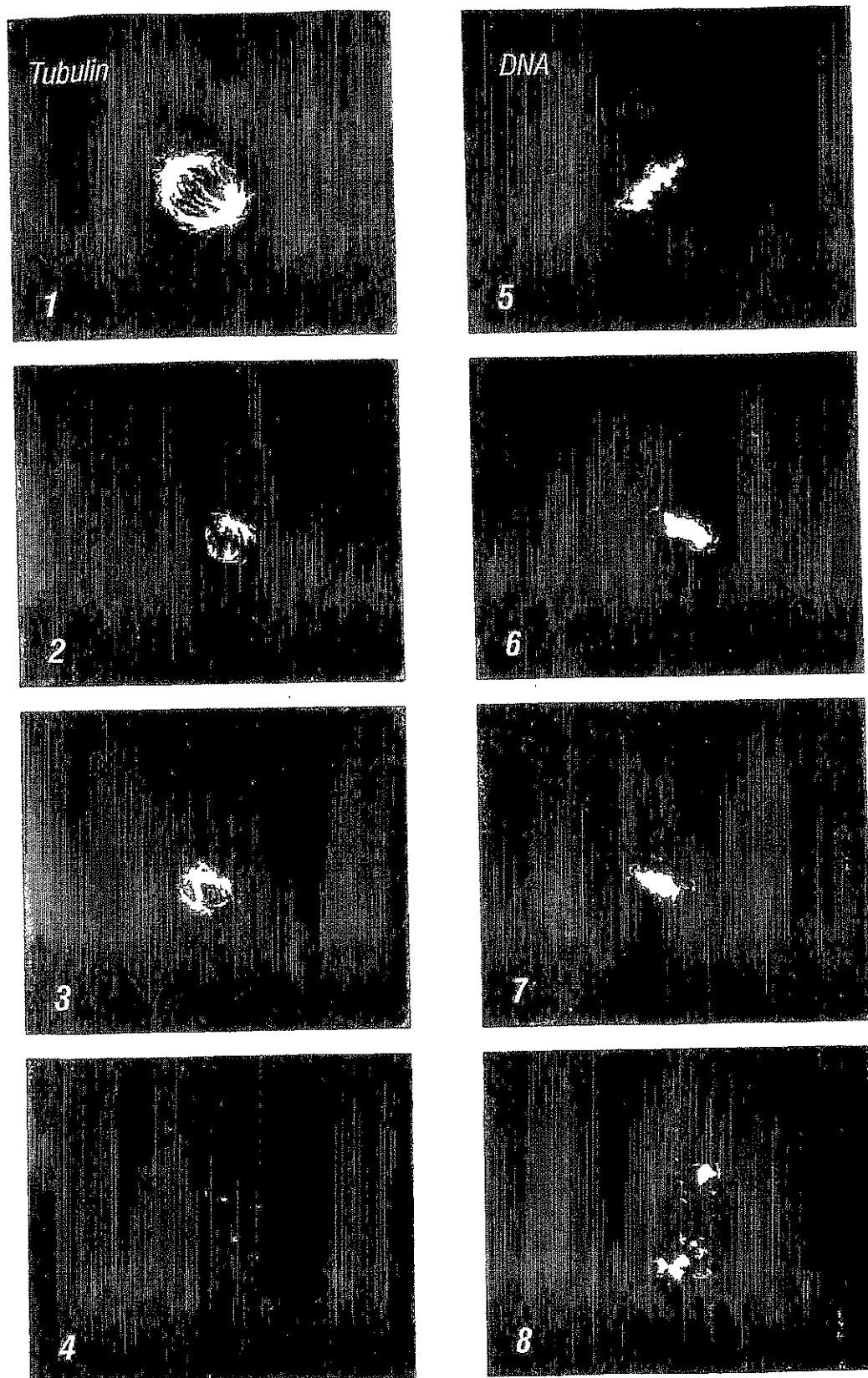


FIG. 53C

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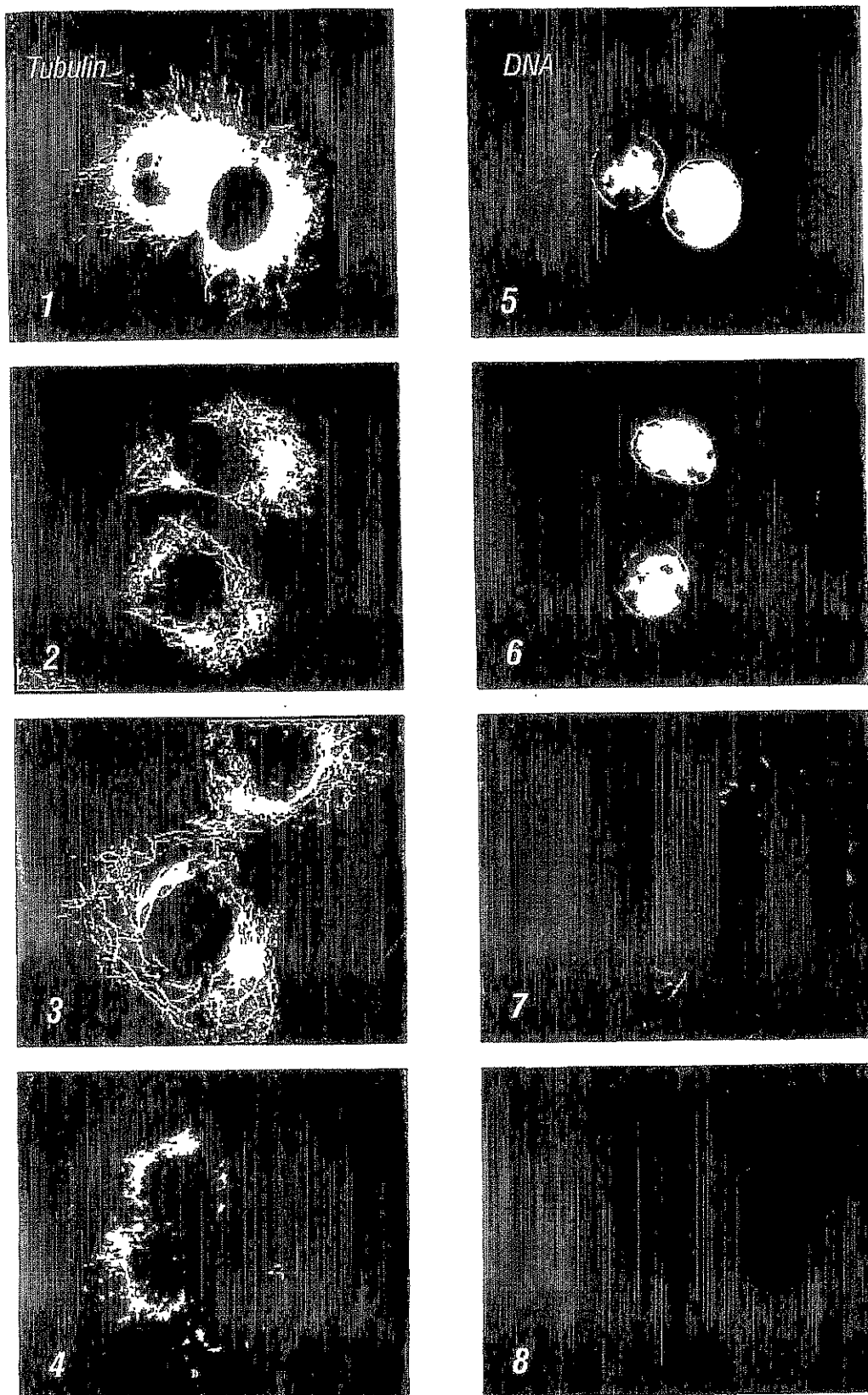


FIG. 54A

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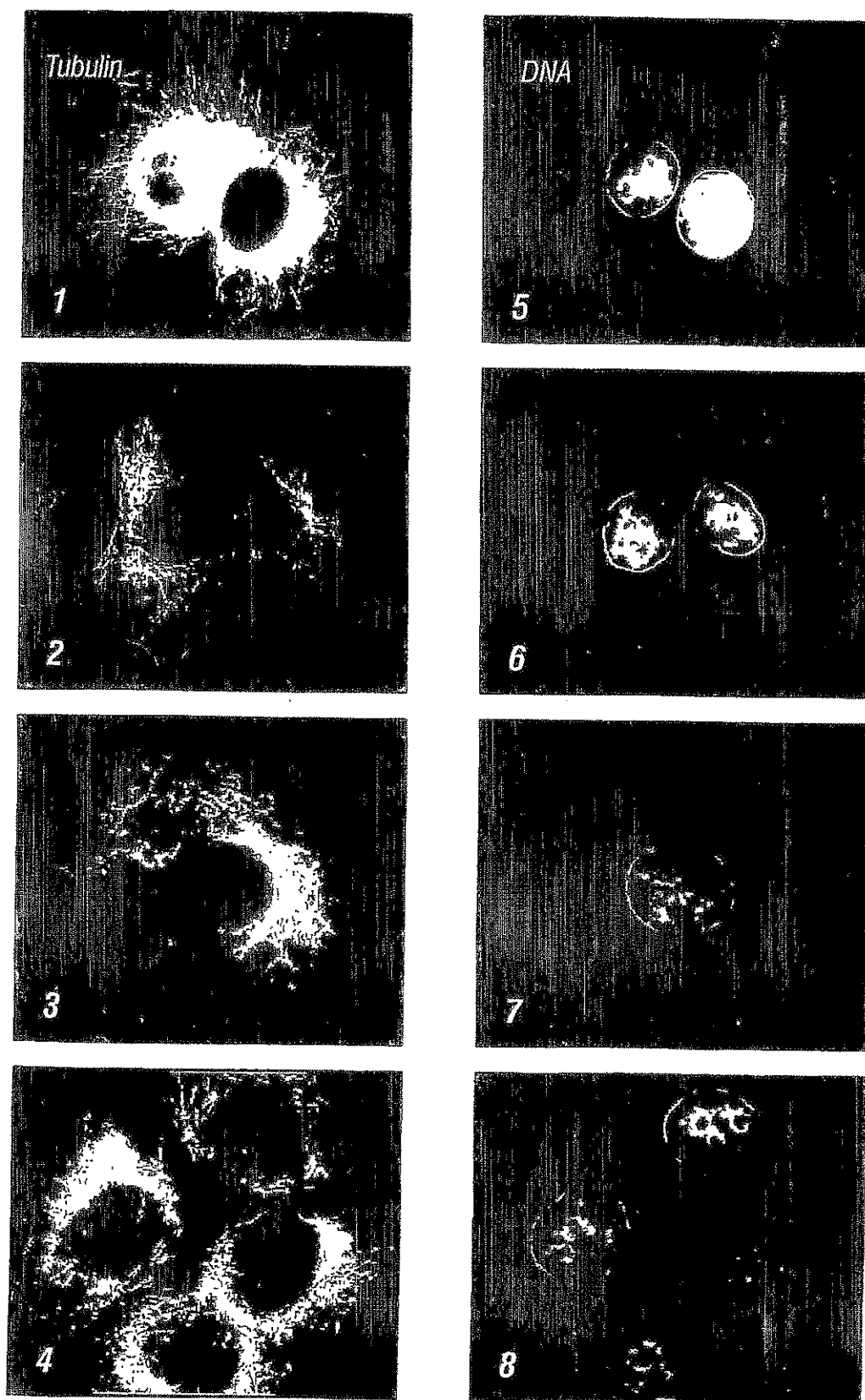


FIG. 54B

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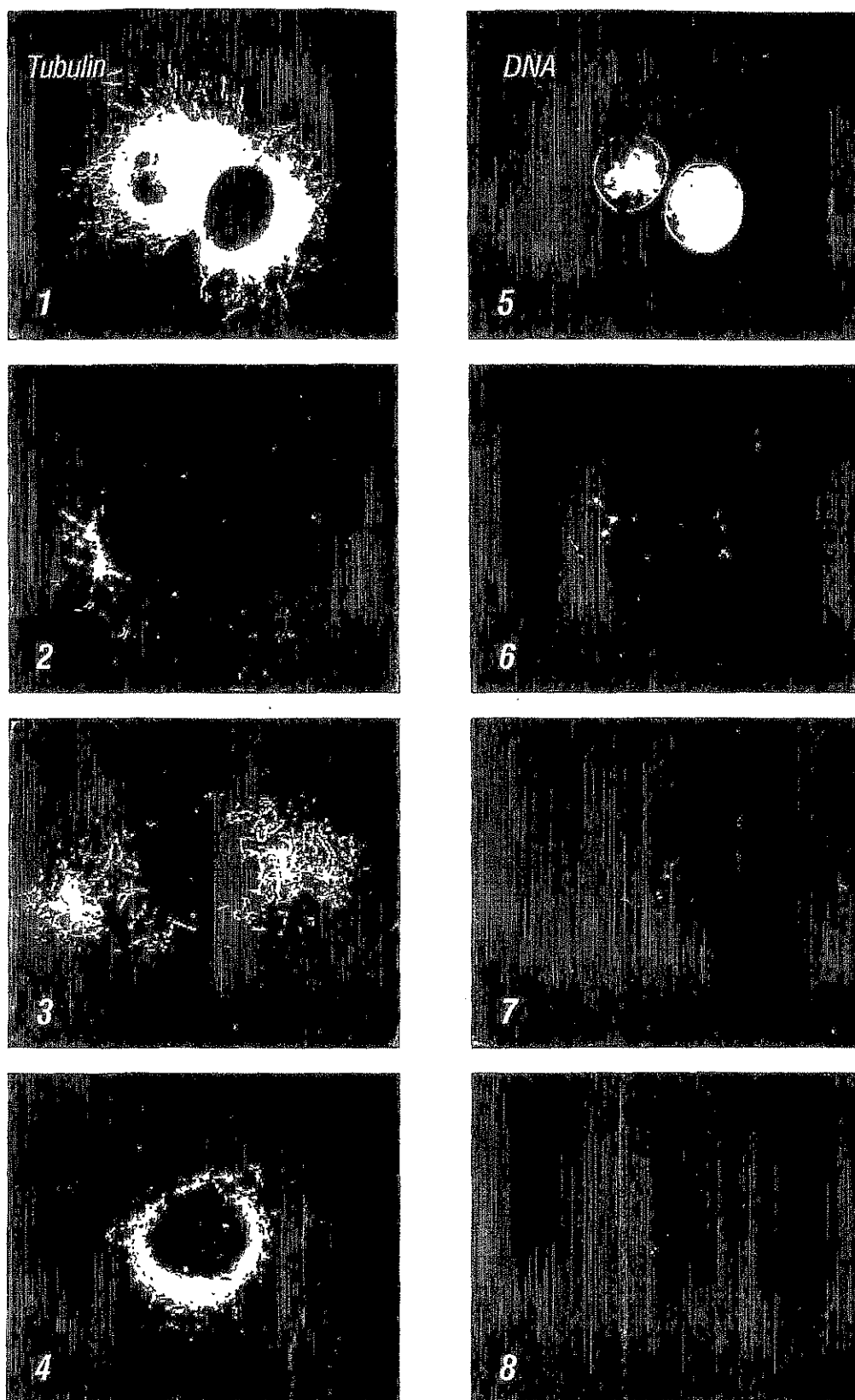


FIG. 54C