Anticancer effect of three topotecan derivatives on NCI-H460 human non-small cell lung cancer growth in nude mice xenograft model

Abstract In this study the NCI-H460 human non-small cell lung cancer nude mice xenograft model was established and applied for in vivo anti-cancer activity evaluation of three topotecan derivatives. The result showed that topotecan derivatives I, II and III could inhibit nude mice xenograft NCI-H460 human non-small cell lung cancer growth. The inhibitory rate of compound III given i.v. at the dosage of 1.5625mg/kg and 0.78125 mg/kg was 55.8％ and 46.4％，respectively. The inhibitory rate of compound II given i.v. at the dosage of 5mg/kg and 2.5 mg/kg was 49.7％ and 43.2％，respectively. The inhibitory rate of compound I given i.p. at the dosage of 0.625mg/kg and 0.3125 mg/kg was 42.5％ and 39.9％，respectively. The inhibitory rate of Topotecan given i.v. at the dosage of 2mg/kg was 48.7％. In conclusion, the anticancer activity of three topotecan derivatives on NCI-H460 human non-small cell lung cancer was Compound III> Compound II> Compound I.

Objective To evaluate the anticancer activity of topotecan derivatives I, II and III on nude mice xenografted NCI-H460 human non-small cell lung cancer according to the SFDA Anticancer New Drug Preclinical R&D Guidelines.

Drugs to be tested

Name: Compound I, II and III

Provider: China Pharmaceutical University

Drug preparations: Compound I 1mg/ml；Compound II 2mg/ml；Compound III 1mg/ml. The drugs were diluted to certain concentrations by saline before use.

Control Drug's Name：Topotecan，5.7mg/ml. The drug was diluted to certain concentrations by saline before use.

Dosage design

Dosage of Compound I: 0.625mg/kg and 0.3125mg/kg.

Dosage of Compound II: 5mg/kg and 2.5mg/kg.

Dosage of Compound III: 1.5625mg/kg and 0.78125mg/kg.

Dosage of Topotecan: 2mg/kg.

The dosage of the three compounds was designed according to the result of the mouse acute toxicity performance (Tab 1.). The dosage in nude mice of the three compounds was the highest safety dosage in acute toxicity at which no obvious side effects were observed.

Tab 1. Side effects of three topotecan derivatives on mice

Compound	Concentration (mg/ml)	Route of administration	ICR mice number	Volume of administration (ml/20g)	Dosage (mg/Kg)	Mice performance
Compound I	1	i.v.	5	0.1	5	Dead
	1	i.p.	5	0.25	12.5	Dead
	1	i.p.	5	0.1	5	Movement difficulty. Decrease in body weight gain.
	0.5	i.p.	5	0.1	2.5	Shock after administration. Recover 5 min later but showed movement difficulty.
	0.25	i.p.	5	0.1	1.25	4 mice normal but one mice showed abnormal of behavior.
	0.125	i.p.	5	0.1	0.625	Normal
Compound II	2	i.v.	5	0.2	20	1 mice normal but four mice showed abnormal of behavior.

	2	i.v.	5	0.1	10	4 mice normal but one mice showed abnormal of behavior.
	1	i.v.	5	0.1	5	Normal
Compound III	1	i.v.	5	0.25	12.5	3 normal. 2 dead.
	1	i.v.	5	0.125	6.25	4 normal. 1 dead.
	0.5	i.v.	5	0.125	3.125	4 normal. one mice showed abnormal of behavior.
	0.25	i.v.	5	0.125	1.5625	normal
Topotecan	0.1425	i.v.	5	0.28	2	normal

Animal

BALB/c nude mice, provided by Shanghai SLAC Experimental Animal Co., Ltd（Experimental animal production license：SCXK（Shanghai）2003-0003；Experimental animal application license：SYXK（Jiangsu）2002-0053）。

Age：35-40 days

Body weight：18-22 g

Sex：Half male and half female

Animal numbers per group：8 nude mice per group

Human cancer nude mice xenograft model

NCI-H460 human non-small cell lung cancer nude mice xenograft model was established by s.c. injection of 1×10^6 NCI-H460 cells into nude mice axilla. After formation of tumor the xenograft was passaged for three generations in nude mice.

Methods

The tumor growing in Log phase was isolated from nude mice and sliced into 1.5 mm^3 particles. Under SPF conditions, the tumor particles were inoculated s.c. of nude mice right axilla. The xenografted tumor was measured the length and width by vernier caliper. Nude mice were randomly divided into eight groups when the bearing tumor reached 100—300mm^3 volume. Tumor length and width were measured twice every week to dynamic evaluate the anticancer activity of the tested compounds. Compound I was given by i.p. twice every week, compound II was given by i.v. twice every week, compound III was given by i.v. twice every week, Topotecan was given by i.v. twice every week. In model group saline was give i.v. twice every week. After four weeks drug administration, nude mice were sacrificed and tumor was isolated by surgery for weight detection.

Tumor volume (TV) was calculated by the following formula:

$TV = 1/2\times a\times b^2$

a and b represents length and width, respectively.

Results and Discussion

The anticancer effect of the three topotecan derivatives on NCI-H460 human lung cancer xenograft model was shown in Tab 2, 3 and Fig 1-9. The result showed that topotecan derivatives I, II and III could inhibit nude mice xenograft NCI-H460 human non-small cell lung cancer growth. The inhibitory rate of compound III given i.v. at the dosage of 1.5625mg/kg and 0.78125 mg/kg was 55.8％ and 46.4％，respectively. The inhibitory rate of compound II given i.v. at the dosage of 5mg/kg and 2.5 mg/kg was 49.7％ and 43.2％，respectively. The inhibitory rate of compound I given i.p. at the dosage of 0.625mg/kg and 0.3125 mg/kg was 42.5％ and 39.9％，respectively. The inhibitory rate of Topotecan given i.v. at the dosage of 2mg/kg was 48.7％. In conclusion, the anticancer activity of three topotecan derivatives on NCI-H460 human non-small cell lung cancer was Compound III> Compound II> Compound I. The compound I, II and III showed dose-dependent anticancer effect. All the three compounds showed inhibitory effect on mice body weight gain.

Conclusion

The anticancer activity of three topotecan derivatives on NCI-H460 human non-small cell lung cancer was Compound III> Compound II> Compound I.

References

1. New Drug Preclinical R&D Guidelines. China SFDA.

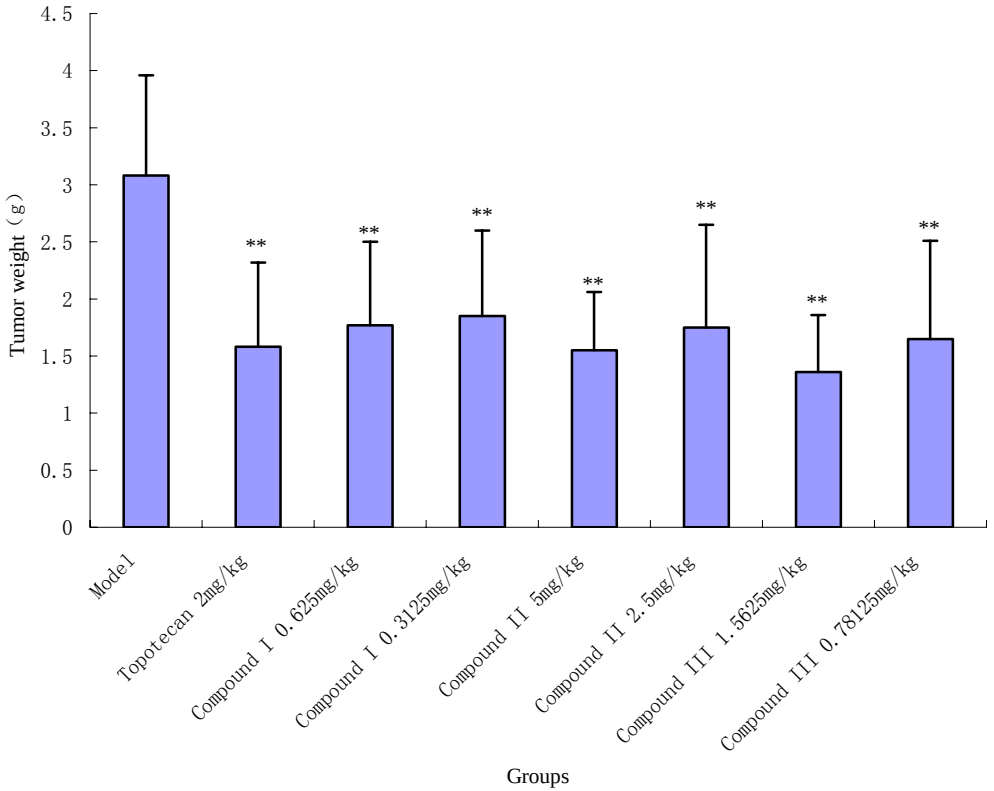
2.Geiger T, et al. Antitumor activity of a PKC-alpha antisense oligonucleotide in combination with standard chemotherapeutic agents against various human tumors transplanted into nude mice. Anticancer Drug Des. 13: 35-45, 1998.

Tab 2. Growth inhibitory effect of topotecan derivatives on NCI－H460 human non-small cell lung cancer nude mice xenograft model (X±SD, n=8)

Groups	Dosage （mg/kg）	Body weight at beginning（g）	Body weight in the end（g）	Tumor weight（g）	Tumor growth inhibitory rate (%)
Model	—	18.34±1.78	22.07±1.68	3.08±0.88	—
Topotecan	2	17.91±1.34	17.32±1.18**	1.58±0.74**	48.7
Compound I	0.625	18.14±1.83	18.86±1.60**	1.77±0.73**	42.5
Compound I	0.3125	18.54±1.24	18.59±1.46**	1.85±0.75**	39.9
Compound II	5	17.74±1.56	18.93±1.49**	1.55±0.51**	49.7
Compound II	2.5	18.11±1.47	17.82±1.06**	1.75±0.90**	43.2
Compound III	1.5625	17.96±1.71	19.63±0.77**	1.36±0.50**	55.8
Compound III	0.78125	18.18±1.34	18.42±1.04**	1.65±0.86**	46.4

Compared with model group，*P<0.05,**P<0.01

Fig 1. Growth inhibitory effect of topotecan derivatives on NCI-H460 human non-small lung cancer nude mice xenograft model



Tab 3. Effect of topotecan derivatives on tumor volume change of NCI－H460 human lung cancer xenografted in nude mice (X±SD, n=8，mm^3)

Group	Dosage （mg/kg）	0.5 week	1 week	1.5 weeks	2 weeks	2.5 weeks	3 weeks	3.5 weeks	4 weeks
Model	—	223.0±117.2	635.9±205.5	1269.8±524.3	1525.3±592.3	1606.2±712.5	1994.7±795.8	2222.7±1109.2	2440.6±1023.3
Topotecan	2	209.6±65.9	574.0±387.8	759.8±419.9*	933.1±496.8*	1220.7±611.4	1273.3±685.1	1413.0±717.0	1639.9±888.2
Compound I	0.625	220.9 ± 178.6	504.2±338.2	706.7±450.3*	1104.7±759.6	1367.1±880.2	1566.7±867.8	1798.2±767.2	2088.1±950.3
Compound I	0.3125	194.9±84.7	560.8±383.8	766.0±480.3	1245.7±585.6	1459.9±793.3	1638.9±827.1	1909.7±464.4	2209.8±760.6
Compound II	5	205.2±115.8	413.8±296.6	602.1±339.3**	733.7±497.0*	950.0±578.0	1147.2±598.0*	1632.9±752.6	1639.4±687.3
Compound II	2.5	182.6±74.4	472.2±327.9	706.8±360.0*	861.1±523.9*	1084.9±536.3	1269.1±644.1	1841.8±859.5	2029.4±766.3
Compound III	1.5625	237.9 ± 185.2	392.9±287.8	547.2±390.0**	719.6±315.8**	958.4±486.2	1093.2±692.2*	1400.4±670.6	1588.3±867.9
Compound III	0.78125	211.8±155.2	455.2±259.8	624.6±371.5*	795.4±389.0*	999.8±562.9	1291.5±564.4	1553.2±706.3	1908.5±639.5

Compared with model group，*P<0.05,**P<0.01

Fig 2. Anticancer effect of three topotecan derivatives on NCI-H460 human lung cancer nude mice xenograft model

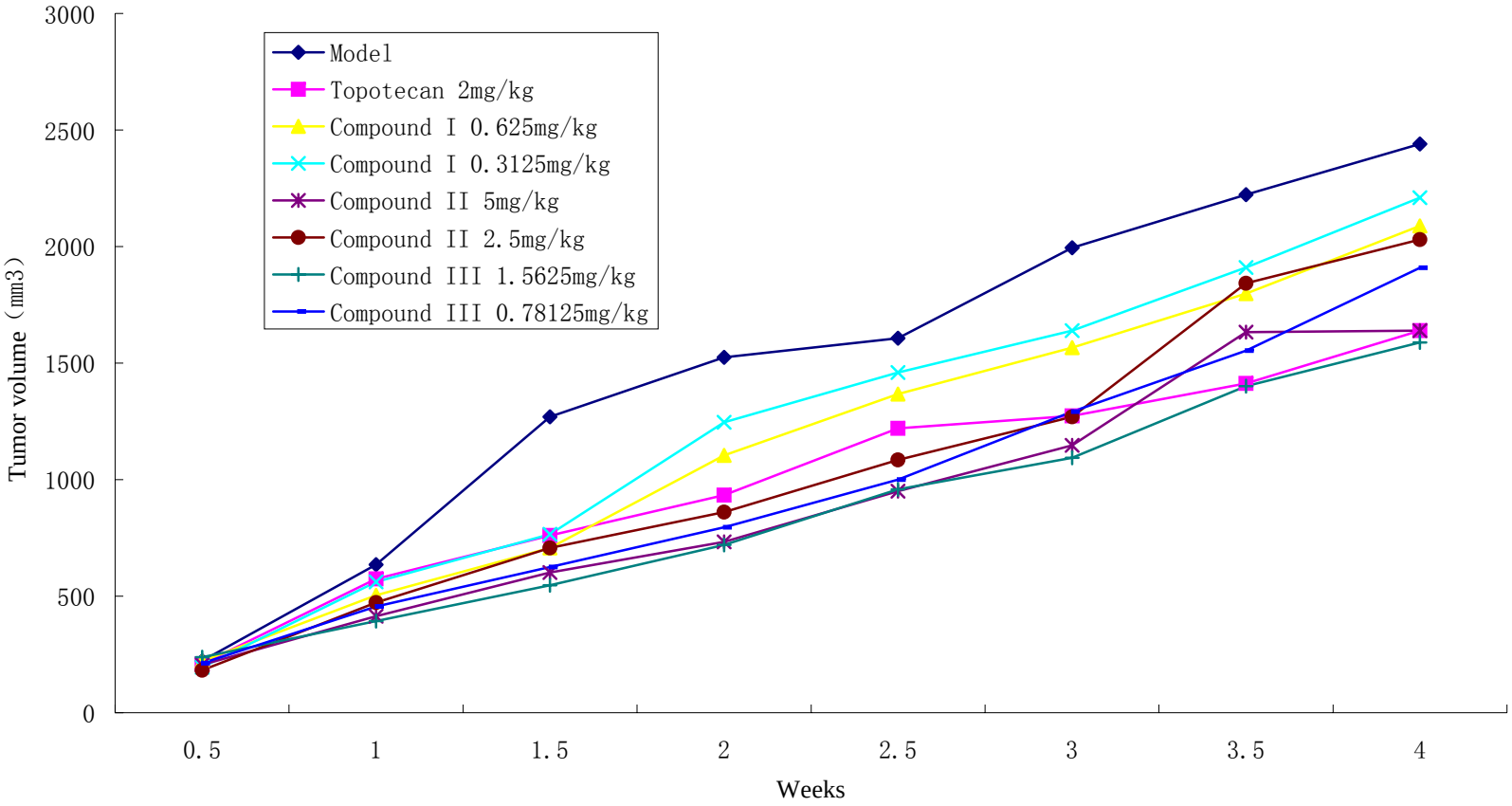


Fig 3. Growth inhibitory effect of topotecan derivatives on NCI-H460 human lung cancer nude mice xenograft model

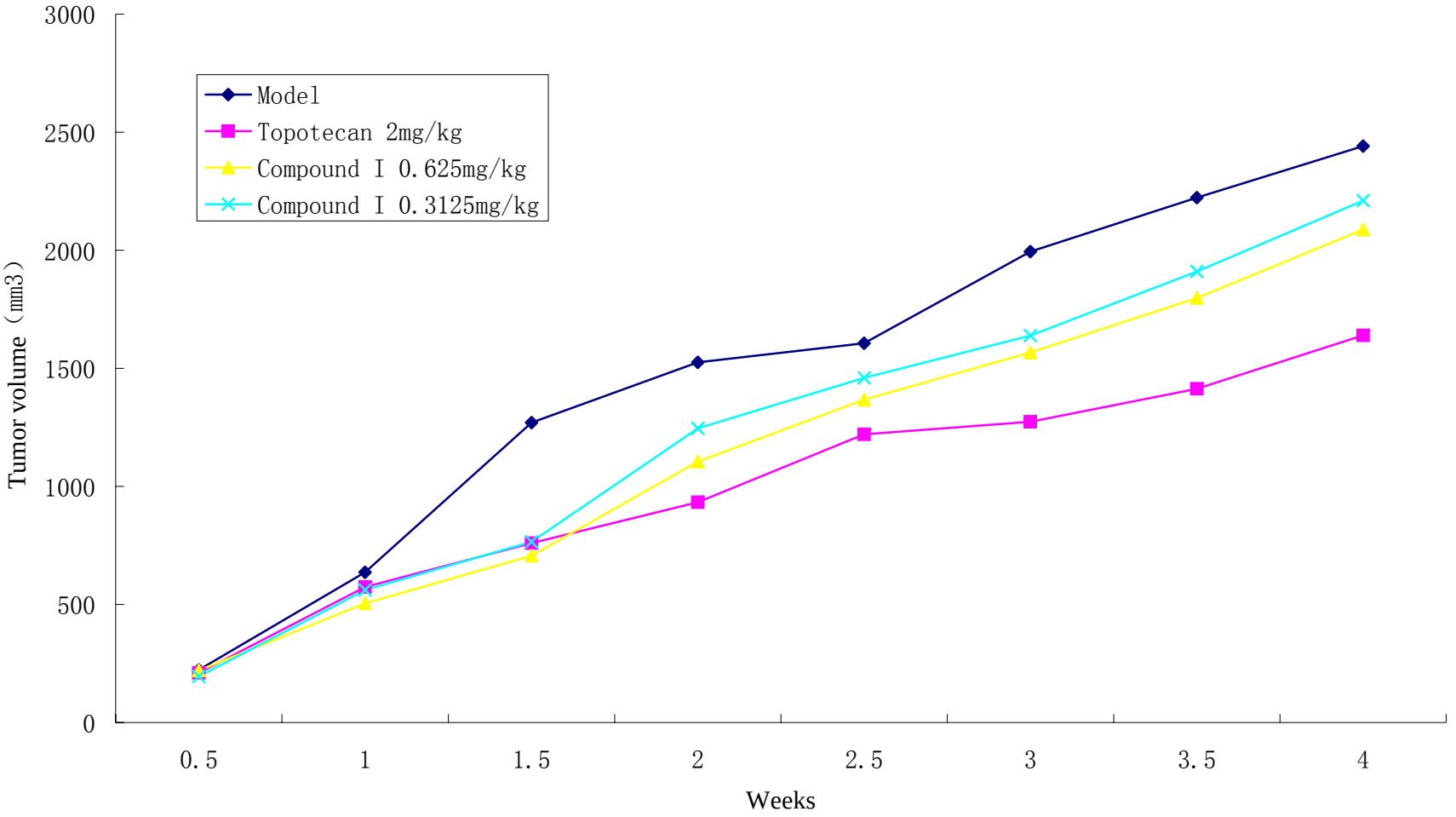


Fig 4. Growth inhibitory effect of topotecan derivatives on NCI-H460 human lung cancer nude mice xenograft model

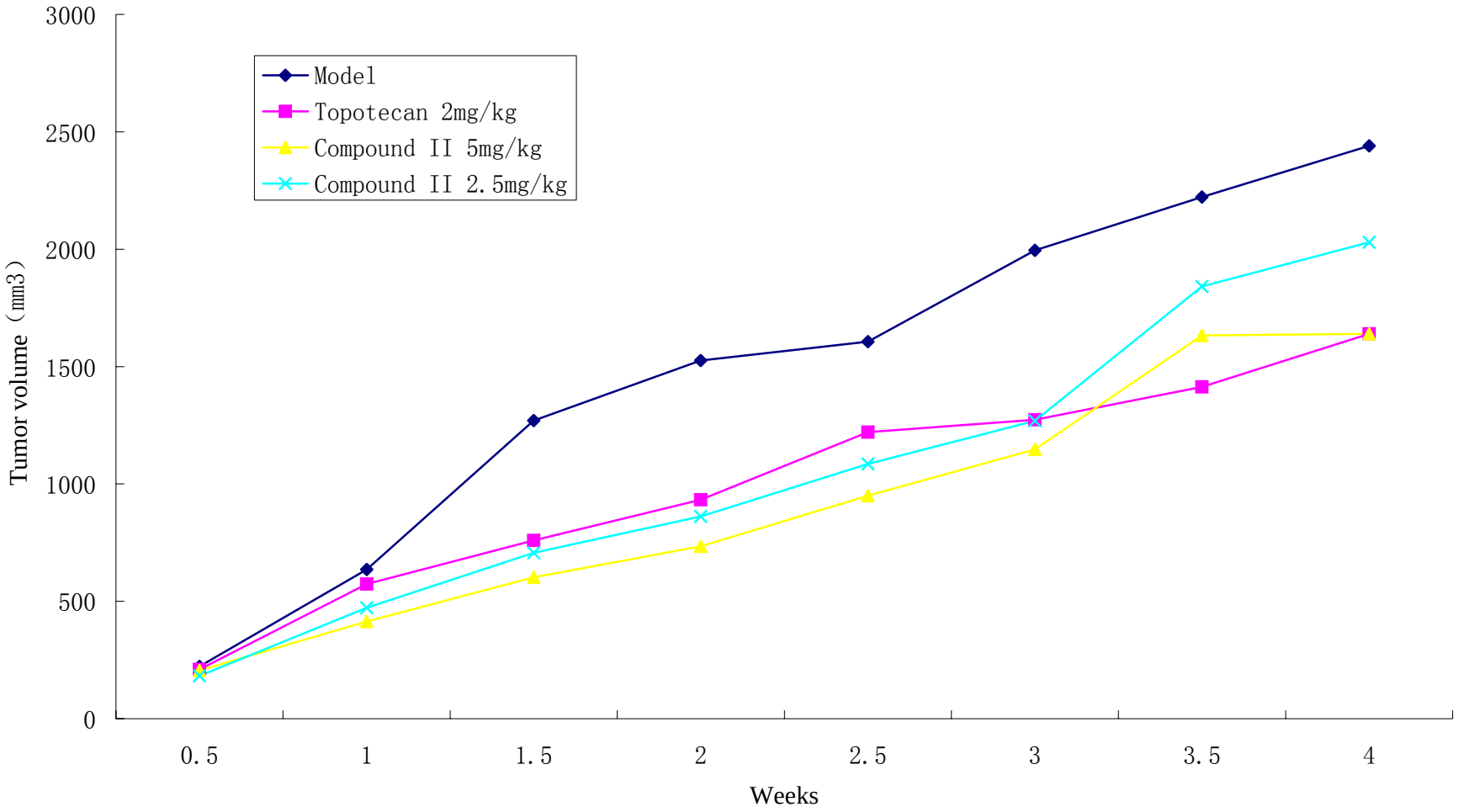


Fig 5. Growth inhibitory effect of topotecan derivatives on NCI-H460 human lung cancer nude mice xenograft model

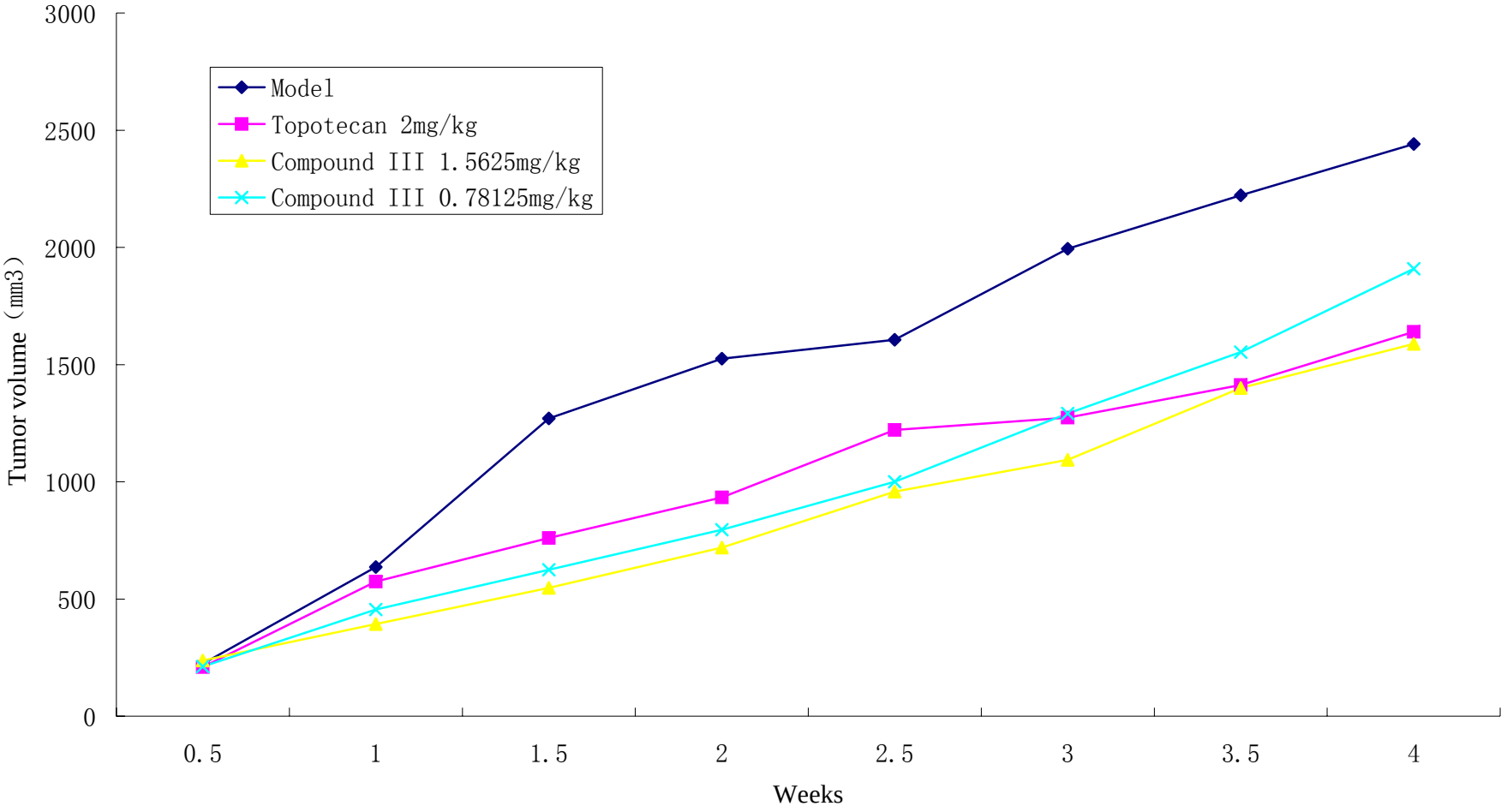


Fig 6. Growth inhibitory effect of topotecan derivatives on NCI-H460 human lung cancer nude mice xenograft model

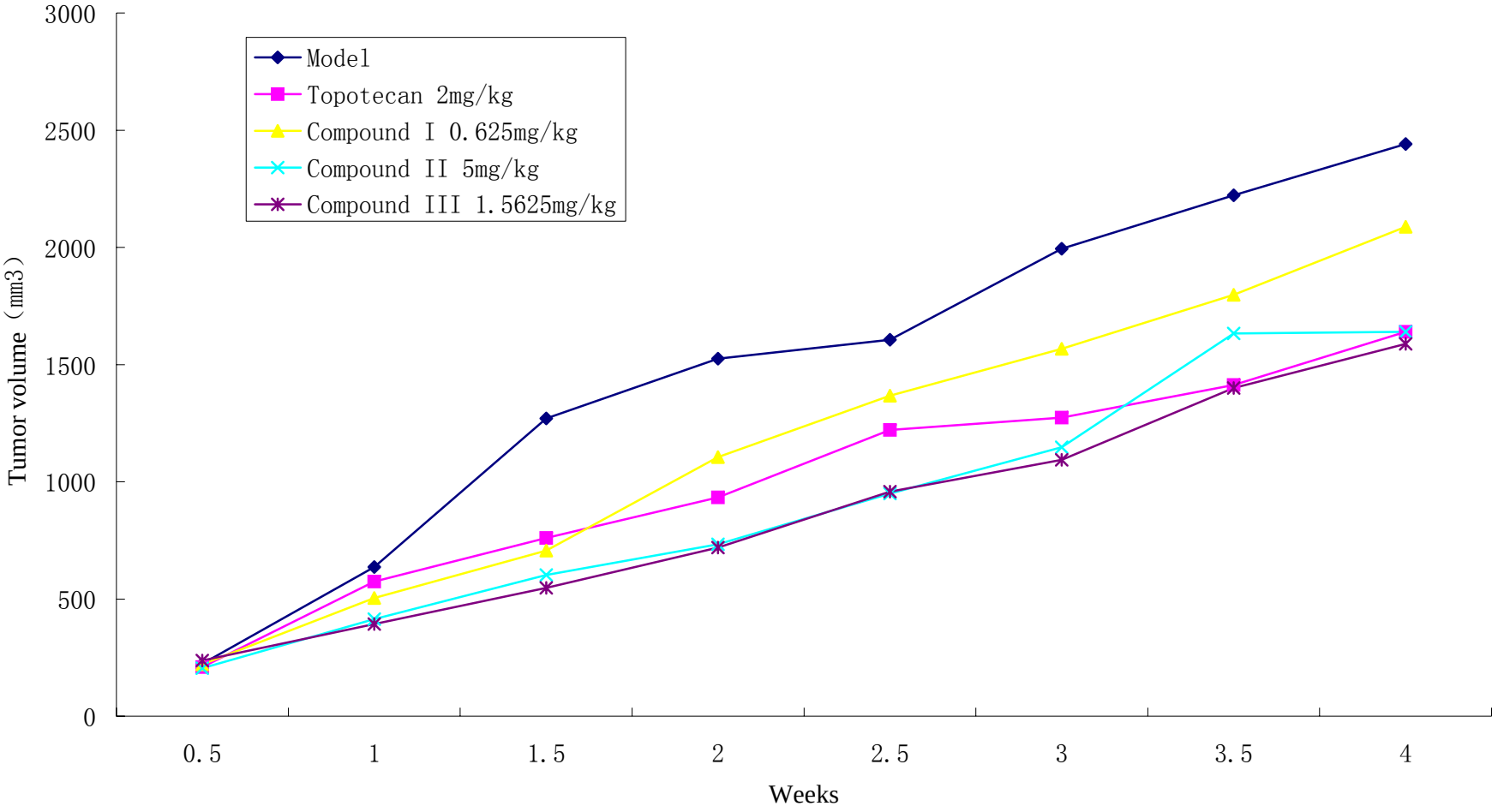
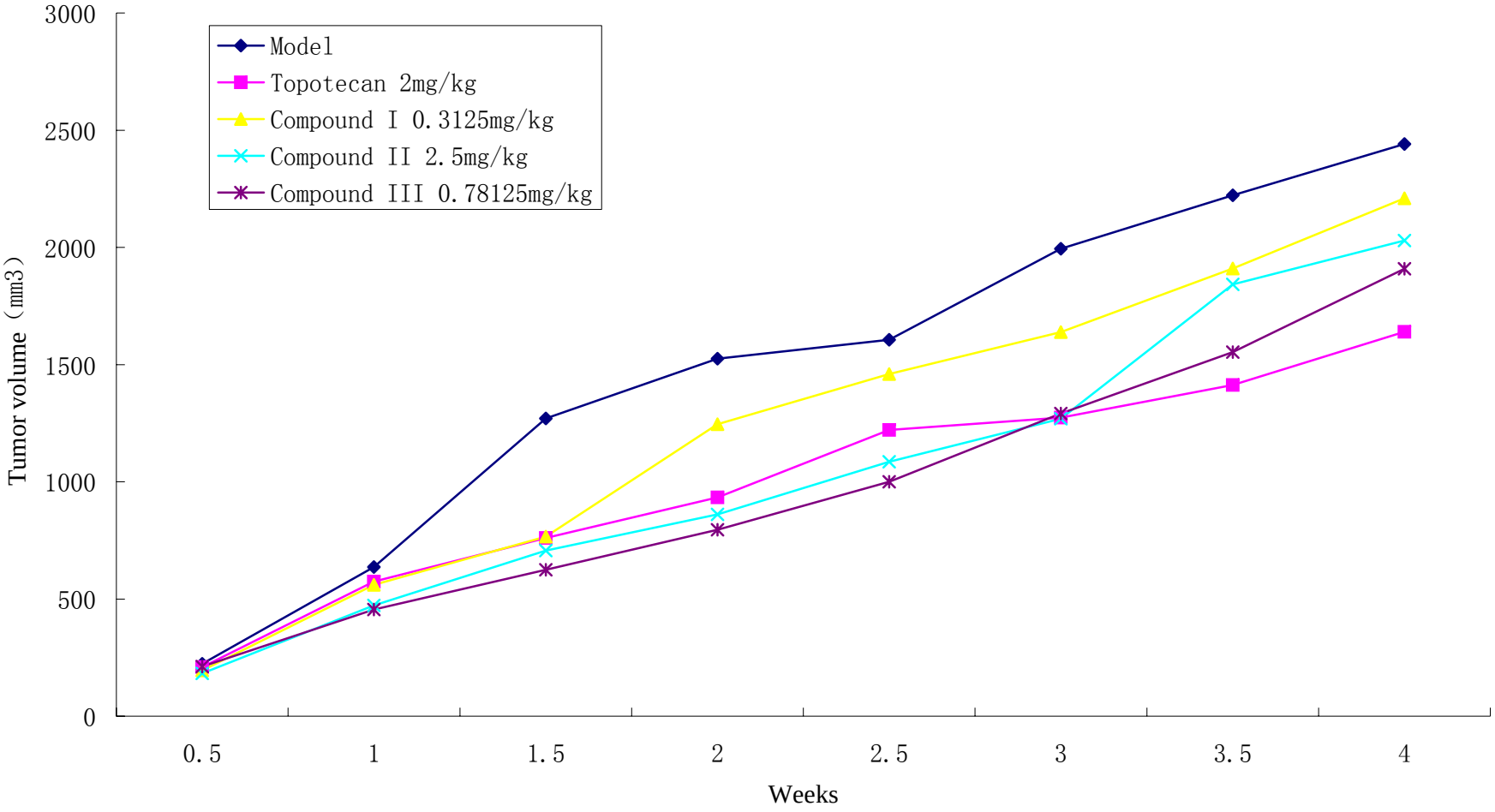
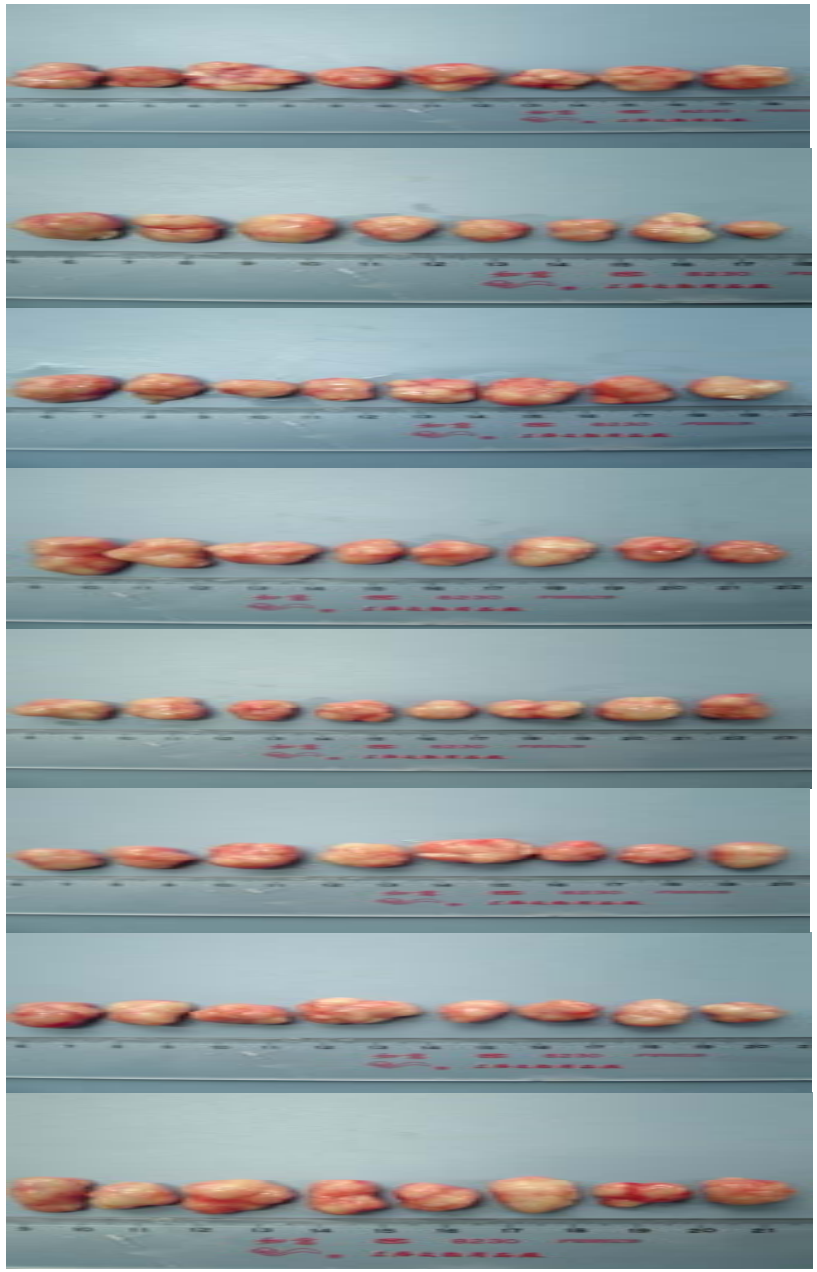


Fig 7. Growth inhibitory effect of topotecan derivatives on NCI-H460 human lung cancer nude mice xenograft model





Model

Topotecan group

Compound I high dose

Compound I low dose

Compound II high dose

Compound II low dose

Compound III high dose

Compound III low dose

Fig 8. Tumors isolated from each mice

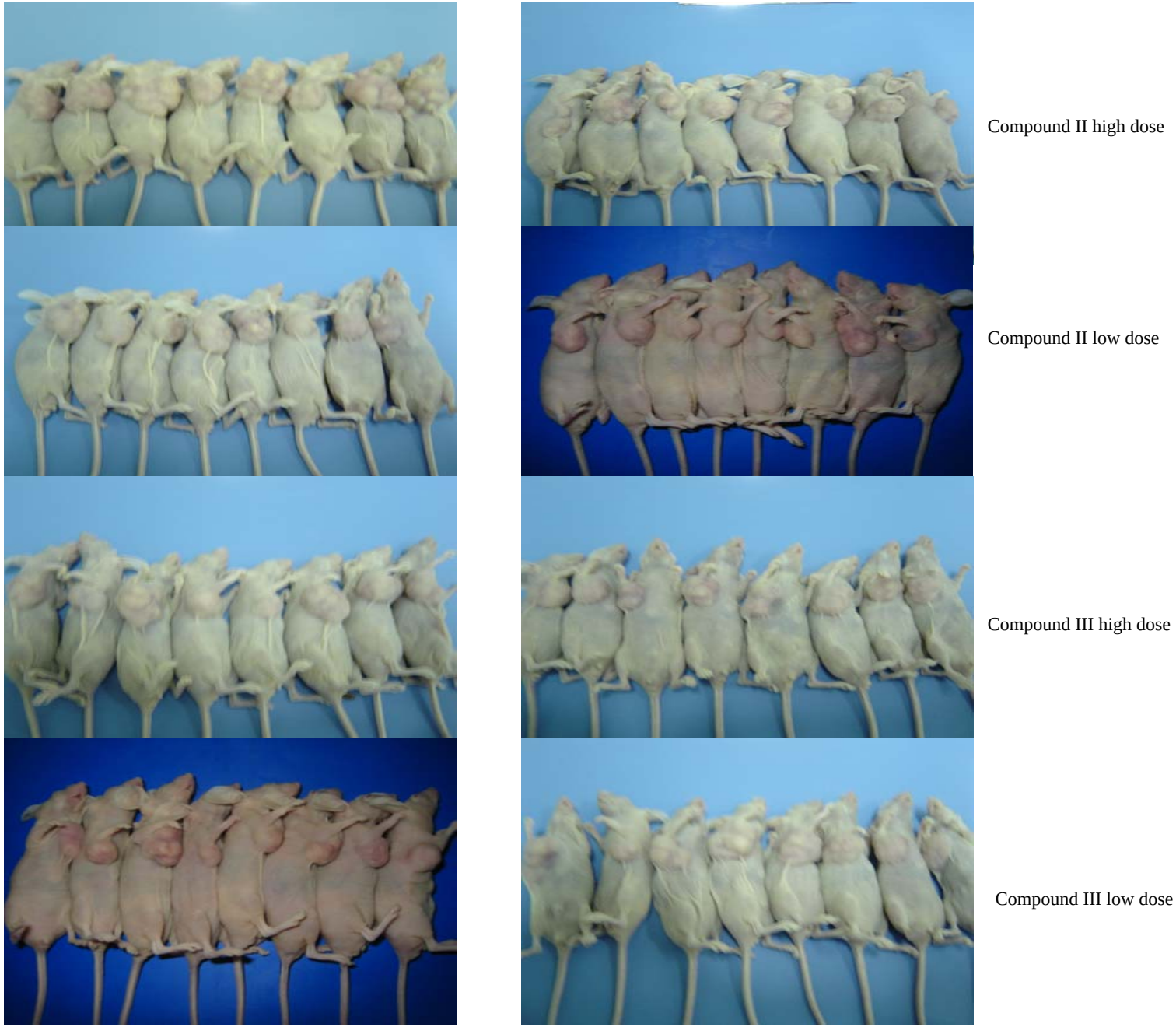


Fig 9. Nude mice bearing NCI-H460 human lung cancer.

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