Supporting Information

Chou et al. 10.1073/pnas.1111332108

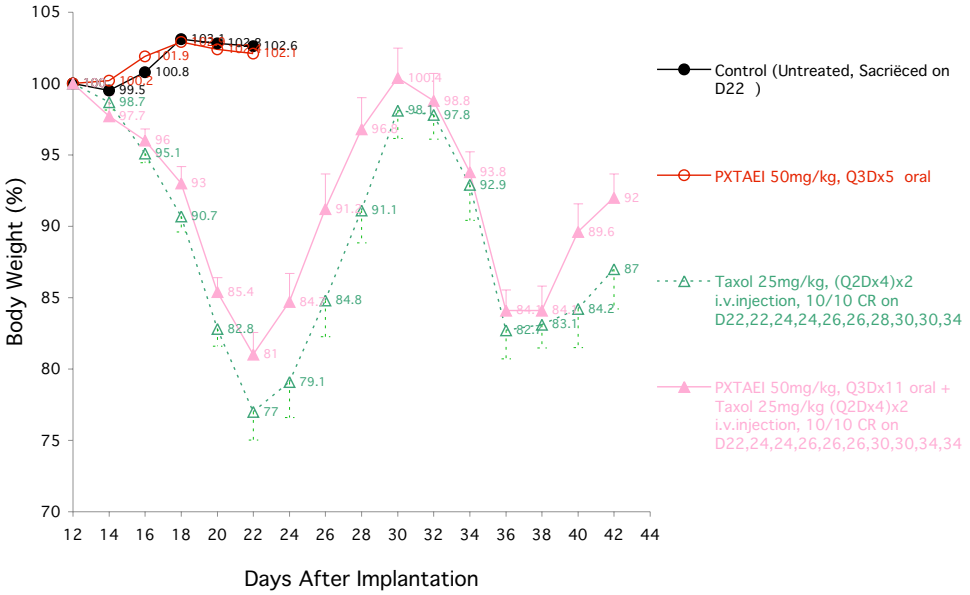


Fig. S1. Reduction of Taxol-induced toxicity by oral administration of PXTAEI in nude mice bearing a human mammary carcinoma MX-1 xenograft. High repeated doses of Taxol (25 mg/kg administered i.v. according to the schedule shown), induced body weight losses (green △). Cotreatment with oral PXTAEI (50 mg/kg administered every 3 d) alleviated body weight losses and improved recovery (pink ▲). The rapid MX-1 tumor growth did not hinder the body weight gain with (red ○) or without (black ●) PXTAEI, and these mice were killed on D22 because of excessive tumor burden (i.e., tumor >10% of body weight).

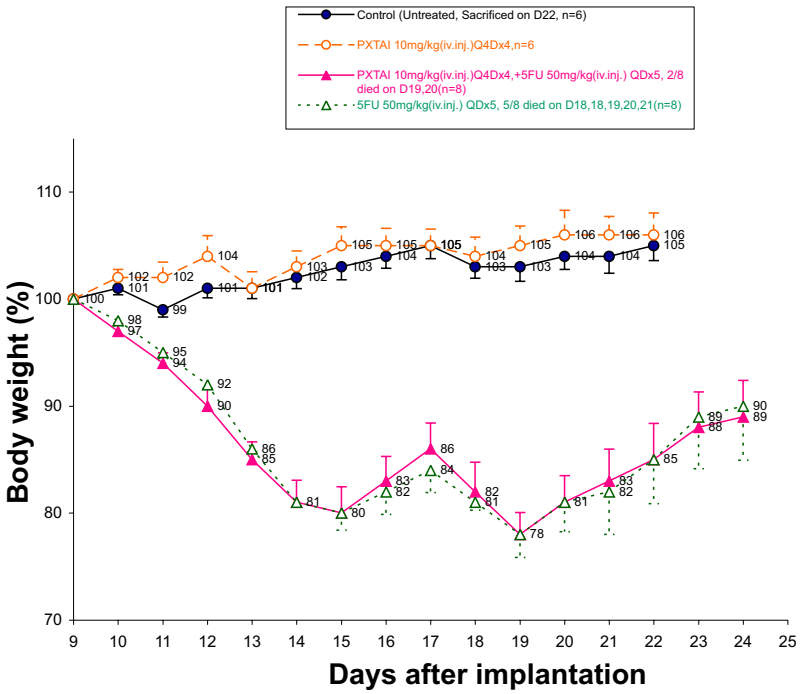
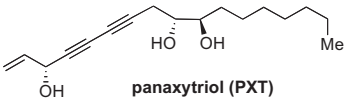
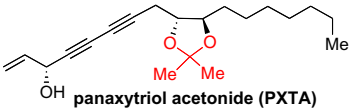
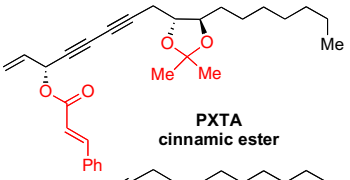
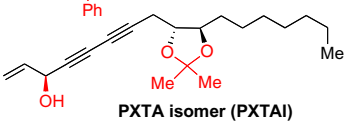
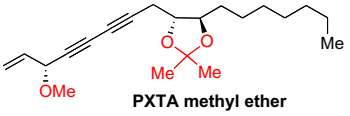
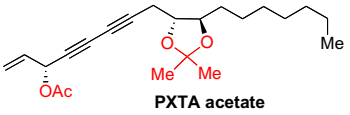
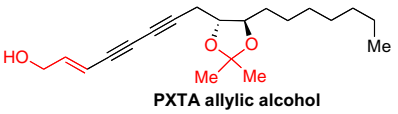
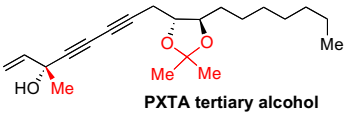
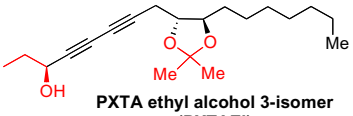
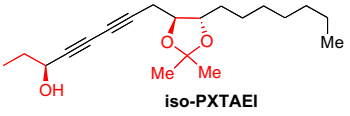
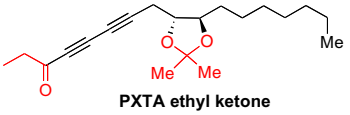


Fig. S2. Reduction of high-dose 5-FU–induced lethality by a low dose of PXTAI (10 mg/kg administered as an i.v. injection every 4 d) in nude mice an bearing MX-1 xenograft tumor. The 5-FU dose was 50 mg/kg administered as an i.v. injection QD for 5 d, which caused the death of five of eight mice (green △). The body weight losses were not alleviated by PXTAI because of drastic 5-FU treatment (pink ▲). The saline-treated control group (●) and the PXTAI-treated group (orange ○) showed continued body weight increases. On D24, 5-FU treatment led to 90.2% tumor suppression, whereas 5-FU plus PXTAI resulted in 93.3% tumor suppression.

Table S1. Comparison of cytotoxicity of PXT and synthetic analogs in vitro: IC50 (μM) against human lymphoblastic leukemia sublines (CCRF-CEM)*,†

Entry	Compounds	CCRF-CEM	CCRF-CEM/ Taxol‡	CCRF-CEM/ vinblastine I‡
1	 panaxytriol (PXT)	5.0 ± 0.9	$5.2_{[1.0\times]}$§	$6.4_{[1.3\times]}$ §
2	 panaxytriol acetonide (PXTA)	2.3 ± 0.01	$2.8_{[1.2\times]}$	$4.4_{[1.9\times]}$
3	 PXTA cinnamic ester	627.1 ± 14.8	$156.3_{[0.2\times]}$	$106.5_{[0.2\times]}$
4	 PXTA isomer (PXTAI)	0.5 ± 0.001	$1.0_{[2.2\times]}$ I‡	$1.0_{[2.0\times]}$ I‡
5	 PXTA methyl ether	21.3 ± 0.3	$15.1_{[0.7\times]}$	$26.6_{[1.3\times]}$
6	 PXTA acetate	3.9 ± 0.9	$7.6_{[2.0\times]}$	$8.6_{[2.2\times]}$
7	 PXTA allylic alcohol	1.9 ± 0.2	$2.9_{[1.5\times]}$	$2.8_{[1.4\times]}$
8	 PXTA tertiary alcohol	3.8 ± 0.5	$6.2_{[1.6\times]}$	$7.3_{[1.9\times]}$
9	 PXTA ethyl alcohol 3-isomer (PXTAEI)	2.0 ± 0.1	$3.6_{[1.8\times]}$	$3.3_{[1.6\times]}$
10	 iso-PXTAEI	6.1 ± 0.2	$6.0_{[1.0\times]}$	$9.0_{[1.5\times]}$
11	 PXTA ethyl ketone	0.8 ± 0.3	$1.7_{[2.1\times]}$	$1.2_{[1.4\times]}$

*All compounds were synthesized according to previously described methods (1, 2).

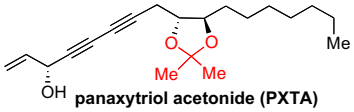
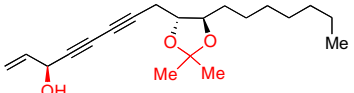
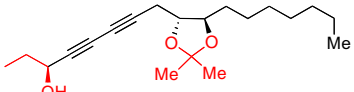
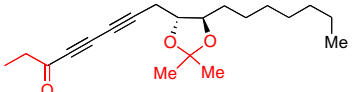
†Cell growth inhibition was measured by cell counting kit 8 (CCK-8) assay for leukemic cells after a 72-h incubation using a Powerwave XS spectrophotometer. IC_{50} values were determined in duplicate or triplicate from the dose–effect relationship at six or seven concentrations of each drug using CompuSyn software by Chou and Martin (3) based on the median-effect principle and plot and serial deletion analysis (4).

‡CCRF-CEM/Taxol and CCRF-CEM/vinblastine are subcell lines of CCRF-CEM cells that are 283-fold resistant to Taxol and 261-fold resistant to vinblastine, respectively, compared with IC_{50} of the parent cell line.

§Numbers in brackets are fold of cross-resistance determined by comparison with IC_{50} of the parent cell line.

1. Yun HD, Danishefsky SJ (2003) Straightforward synthesis of panaxytriol: An active component of Red Ginseng. *J Org Chem* 68:4519–4522.
2. Yun H, et al. (2005) Total synthesis as a resource in drug discovery: The first in vivo evaluation of panaxytriol and its derivatives. *J Org Chem* 70:10375–10380.
3. Chou TC, Martin (2005) CompuSyn for drug combinations. PC software and User's Guide: A Computer program for quantitation of synergism and antagonism in drug combinations and the determination of IC50, ED50, and LD50 values. ComboSyn Inc. (Paramus, NJ).
4. Chou TC (2006) Theoretical basis, experimental design, and computerized simulation of synergism and antagonism in drug combination studies. *Pharmacol Rev* 58:621–681.

Table S2. Cytotoxicity of PXTA derivatives against solid tumor growth: IC50 (μM) against human solid tumor cells*

Compound	MX-1 mammary carcinoma	HCT-116 colon carcinoma	A-549 lung carcinoma	SK-OV-3 ovarian adenocarcinoma
 panaxytriol acetonide (PXTA)	1.9	1.3	4.0	3.0
 PXTA isomer (PXTAI)	5.3	0.6	2.9	5.1
 PXTA ethyl alcohol 3-isomer (PXTAEI)	3.5	3.2	1.0	4.0
 PXTA ethyl ketone	1.5	1.1	12.6	13.0

*Cell counting kit 8 (CCK-8) assay following 72-h drug exposure. The absorbance was measured by a Powerwave XS microplate spectrophotometer.

Table S3. Effect of PXTAEI (i.v. injection) against Taxol-induced neurotoxicity in MX-1/nude mice: A study of reduction of neurotoxicity* by PXTAEI (cycle 1)

		Neurotoxicity (paralysis) on date after implantation (cycle 1)											
Group	No.	09	11	13	15	17	19	21	23	25	27	First cycle total score	
Taxol, 25 mg/kg i.v. injection (D9, D11, D13, D15)	A1	–	–	–	–	+	++	++	++	++	+	10	53
	A2	–	–	–	–	+	++	++	+	+	–	7	
	A3	–	–	–	–	+	++	++	++	+	–	8	
	A4	–	–	–	–	+	++	++	+	+	+	8	
	A5	–	–	–	–	+	++	++	++	+	–	8	
	A6	–	–	–	–	–	+	+	–	–	–	2	
	A7	–	–	–	–	–	–	–	–	–	–	0	
	A8	–	–	–	–	+	++	++	+	–	–	5	
	A9	–	–	–	–	–	–	+	+	+	–	3	
	A10	–	–	–	–	–	+	+	–	–	–	2	
Taxol, 25 mg/kg i.v. injection (D9, D11, D13, D15) + PXTAEI, 30 mg/kg i.v. injection (every 4 d)	B1	–	–	–	–	–	+	+	–	–	–	2	27
	B2	–	–	–	–	+	++	++	++	+	–	8	
	B3	–	–	–	–	–	–	–	–	–	–	0	
	B4	–	–	–	–	–	+	–	–	–	–	1	
	B5	–	–	–	–	–	+	+	–	–	–	2	
	B6	–	–	–	–	–	–	–	–	–	–	0	
	B7	–	–	–	–	+	++	++	+	+	+	8	
	B8	–	–	–	–	–	–	–	–	–	–	0	
	B9	–	–	–	–	–	+	+	+	+	–	4	
	B10	–	–	–	–	–	+	+	–	–	–	2	
	↑: i.v. injection (Taxol)	↑	↑	↑	↑								

*PXTAEI at 30 mg/kg on its own has no toxicity or significant therapeutic effect. Rating: –, normal; +, slightly unable to balance; ++, hind leg paralysis; +++, paralysis of four legs and death.

Table S4. Effect of PXTAEI (i.v. injection) against Taxol-induced neurotoxicity in MX-1/nude mice: A study of reduction of neurotoxicity* by PXTAEI (cycle 2)

		Neurotoxicity (paralysis) on date after implantation (cycle 2)																						
Group	No.	29	31	33	35	37	39	41	43	45	47	49	51	53	55	57	59	61	63	65	Second cycle total score		Overall score	
Taxol, 25 mg/kg i.v. injection (D29, D31, D33, D35)	A1	–	–	–	+	+	++	++	++	++	++	++	++	++	+	+	+	+	+	+	24	181	34	234
	A2	–	–	–	+	++	++	++	++	++	++	+	+	+	+	+	+	+	–	–	20		27	
	A3	–	–	+	++	++	++	++	++	++	++	++	+	+	+	+	+	+	+	+	25		33	
	A4	–	–	–	++	++	++	++	++	++	++	++	++	++	++	+	–	–	–	–	23		31	
	A5	–	–	–	+	+	++	++	++	++	++	++	++	++	++	+	+	+	+	+	25		33	
	A6	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	2		4	
	A7	–	–	–	++	++	++	++	++	++	+	+	+	–	–	–	–	–	–	–	15		15	
	A8	–	–	+	++	++	++	++	++	++	++	++	++	++	++	++	++	++	+	+	30		35	
	A9	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	15		18	
	A10	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	2		4	
Taxol, 25 mg/kg i.v. injection (D29, D31, D33, D35) + PXTAEI 30 mg/kg i.v. injection (every 4 d)	B1	–	–	–	++	++	++	++	++	++	++	++	++	++	++	++	++	++	+	–	29	145	31	172
	B2	–	–	–	++	++	++	++	++	++	+	+	+	+	+	–	–	–	–	–	17		25	
	B3	–	–	–	+	+	+	++	+	+	++	++	+	+	+	+	–	–	–	–	15		15	
	B4	–	–	–	+	++	++	++	+	+	+	+	+	+	+	+	+	+	+	+	19		20	
	B5	–	–	–	–	++	++	++	++	++	++	++	++	++	+	+	–	–	–	–	20		22	
	B6	–	–	–	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	6		6	
	B7	–	–	+	++	++	++	++	++	++	++	++	++	++	++	+	+	+	+	+	28		36	
	B8	–	–	–	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	8		8	
	B9	–	–	–	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	3		7	
	B10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0		2	
	↑: i.v. injection (Taxol)	↑	↑	↑	↑																			

*PXTAEI at 30 mg/kg on its own has no toxicity or significant therapeutic effect. Rating: –, normal; +, slightly unable to balance; ++, hind leg paralysis; +++, paralysis of four legs and death.