Xerophilusin B Induces Cell Cycle Arrest and Apoptosis in Esophageal Squamous Cell Carcinoma Cells and Does Not Cause Toxicity in Nude Mice

Ran Yao,[†] Zhaoli Chen,[†] Chengcheng Zhou,[†] Mei Luo,[†] Xuejiao Shi,[†] Jiagen Li,[†] Yibo Gao,[†] Fang Zhou,[†] Jianxin Pu,[,‡] Handong Sun,[‡] and Jie He[*,†]*

[†]Department of Thoracic Surgery, Cancer Institute and Hospital, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing 100021, People's Republic of China

[‡]State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, Yunnan, People's Republic of China

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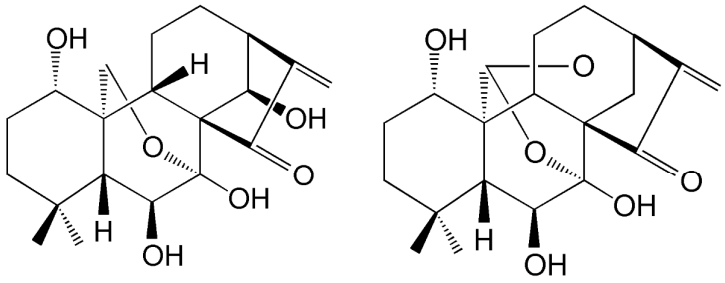
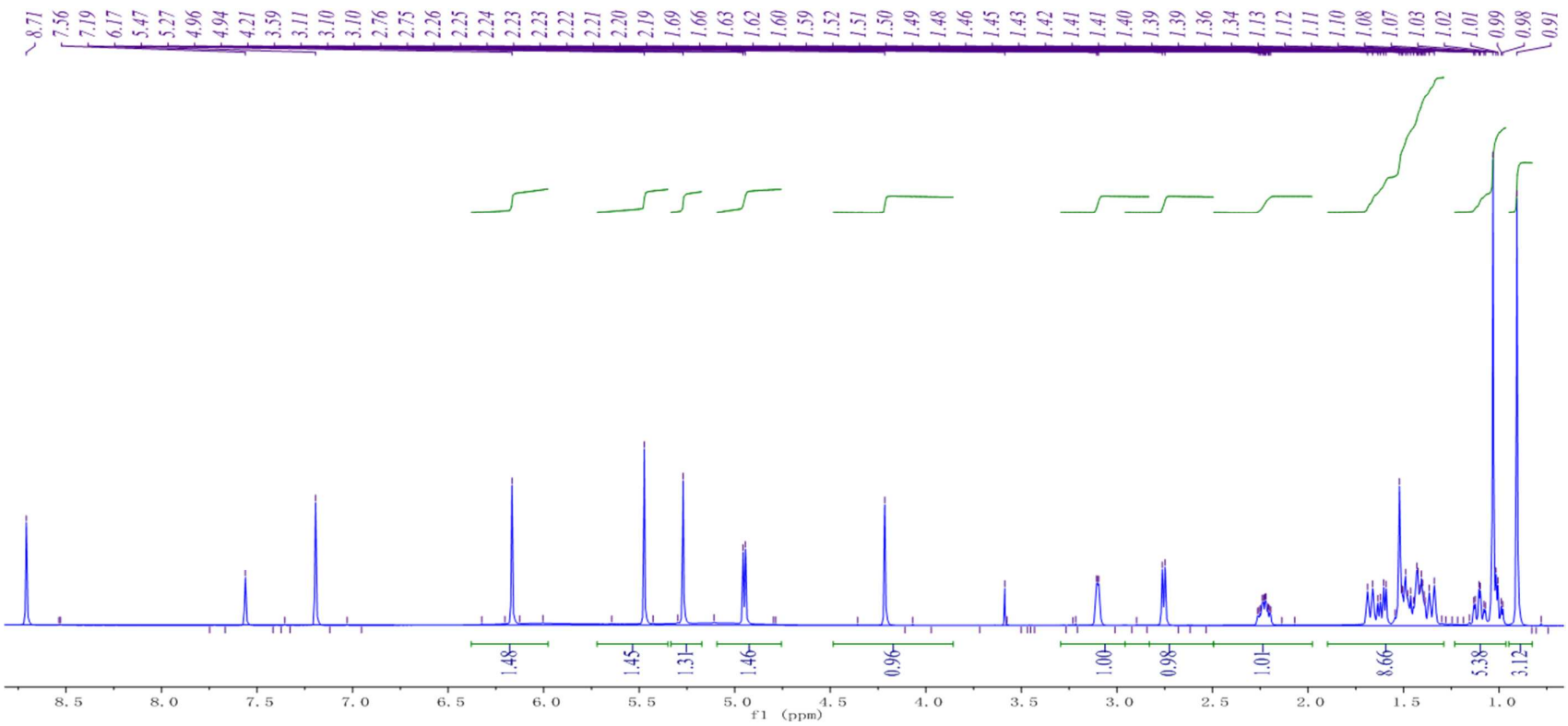
S1. **Figure S1.** Chemical structures of oridonin and ponicidin.

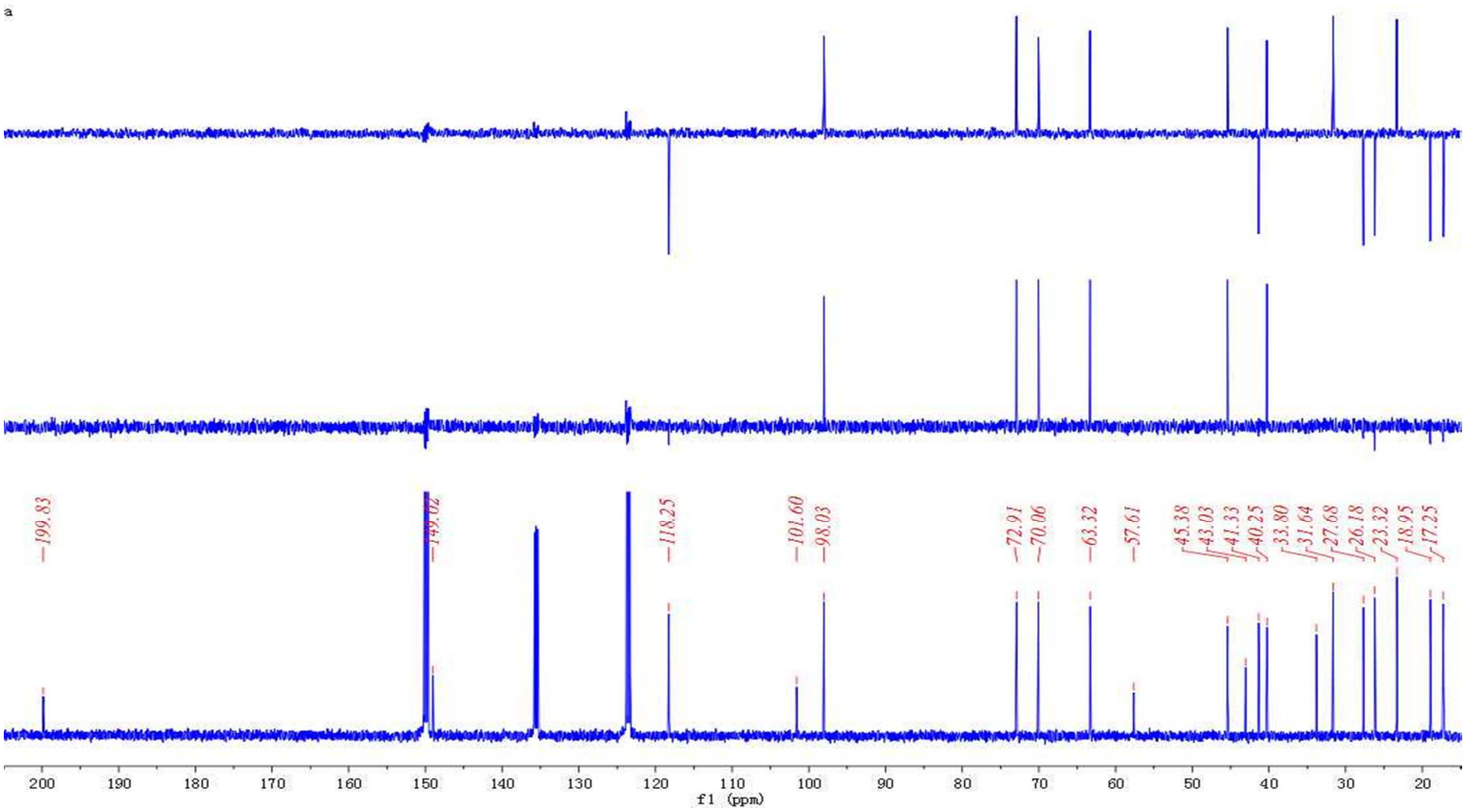


Oridonin Ponicidin

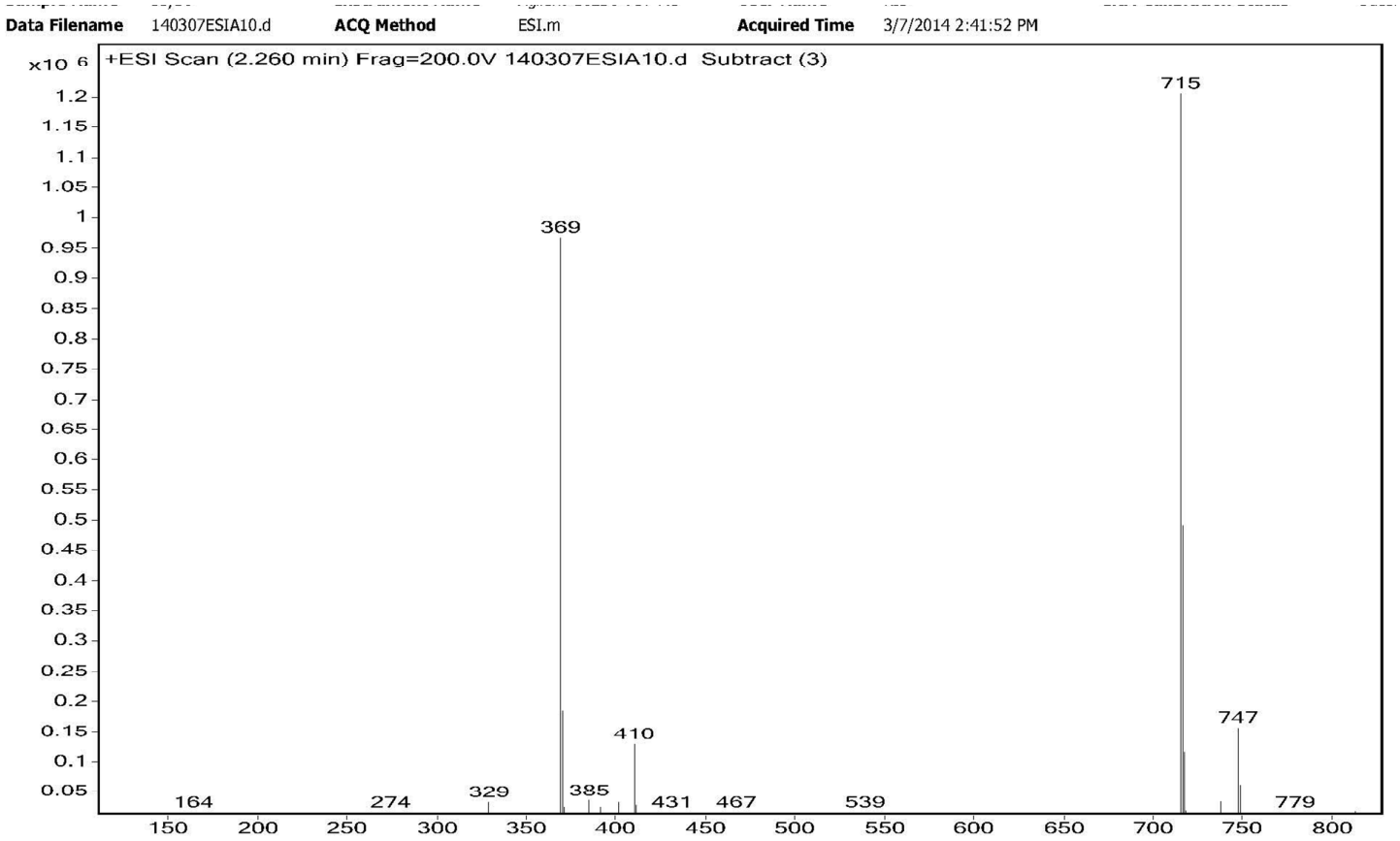
S2. **Figure S2.** ^{1}H-NMR spectrum (400 MHz, $CDCl_3$) of xerophilusin B (**1**).



S3. **Figure S3.** ^{13}C-NMR spectrum (100 MHz, $CDCl_3$) of xerophilusin B (**1**).



S4. **Figure S4.** ESI spectra xerophilusin B (**1**)



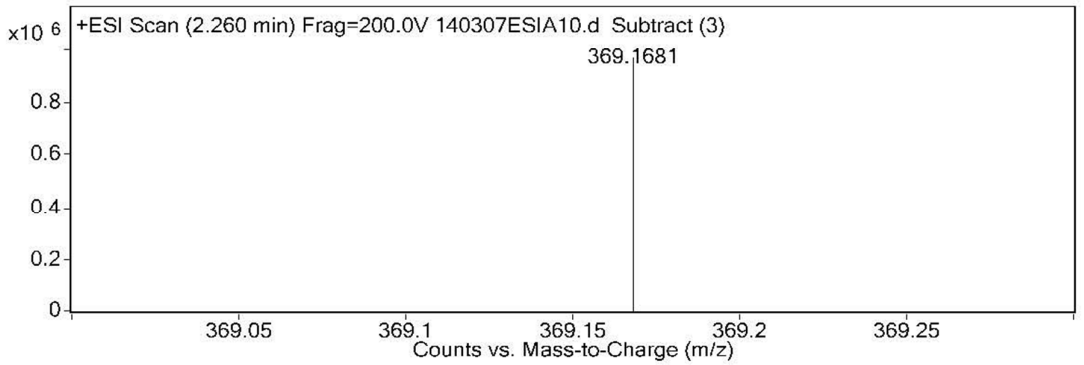
S5. **Figure S5.** HREI spectra of xerophilusin B (**1**).

Data Filename	140307ESIA10.d	**Sample Name**	scy36
Sample Type	Sample	**Position**	
Instrument Name	Agilent G6230 TOF MS	**User Name**	KIB
Acq Method	ESI.m	**Acquired Time**	3/7/2014 2:41:52 PM
IRM Calibration Status	Success	**DA Method**	ESIN.m
Comment			

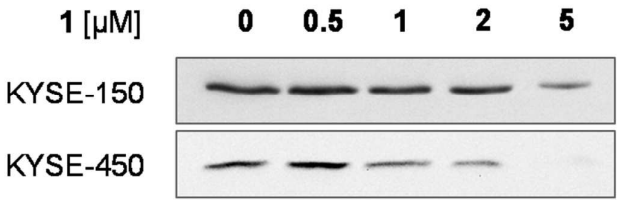
Sample Group	**Info.**
Acquisition SW Version	6200 series TOF/6500 series Q-TOF B.05.01 (B5125.1)

User Spectra

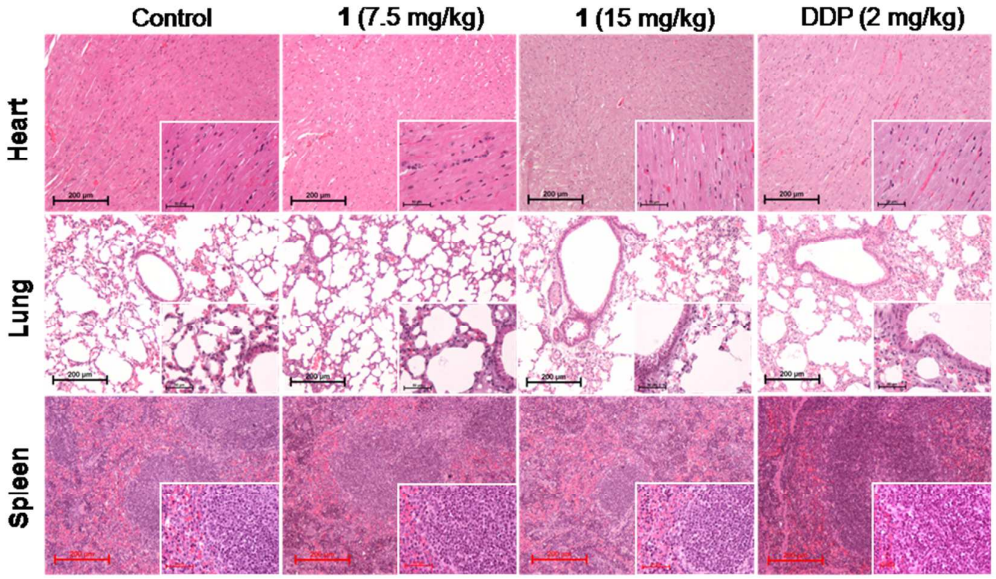
Fragmentor Voltage	**Collision Energy**	**Ionization Mode**
200	0	ESI



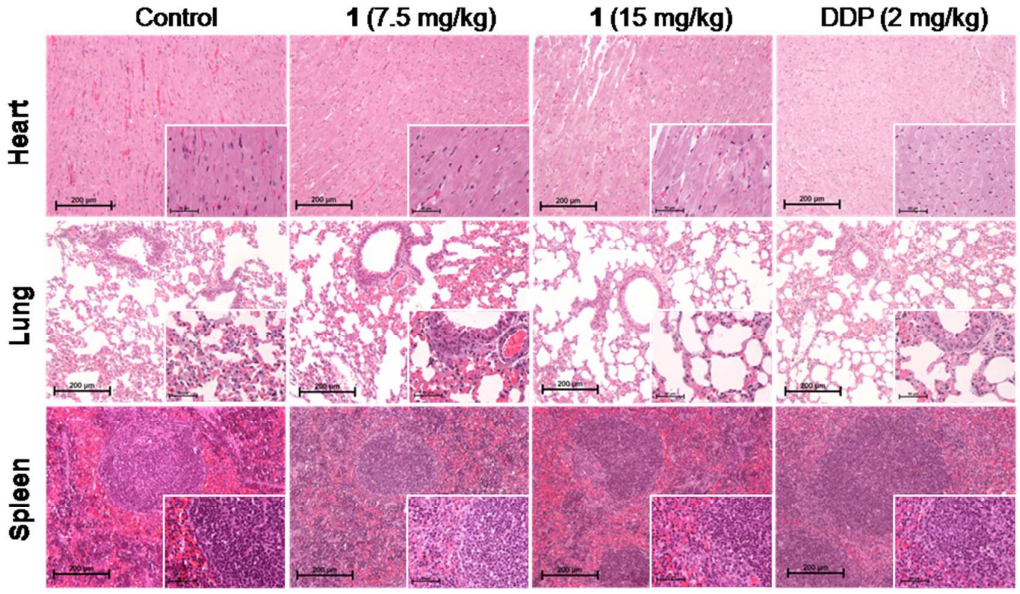
S6. **Figure S6.** Western blot results for β-actin of KYSE-150 and KYSE-450 cells treated with xerophilusin B (**1**) at various concentrations. KYSE-150 and KYSE-450 cells were treated with **1** at various concentrations (0-5 μM) for 24 h. Then expression of β-actin was assessed by western blot. The bands of β-actin from both **1**-treated KYSE-150 and KYSE-450 cells at the concentration of 5 μM were too dim to be identified by the imaging system for western blot detection.



S7. **Figure S7.** Histopathological analysis of organ tissue structure in mice bearing KYSE-150 xenografts. Tissue sections of heart (1_{st} panel), lung (2_{nd} panel), and spleen (3_{rd} panel) were stained with H&E. No tumor metastasis, significant architectural or pathologic changes in organs were observed by microscopic examination. The images of each group were depicted at 100× and 400× magnifications.



S8. **Figure S8.** Histopathological analysis of organ tissue structure in bearing KYSE-450 xenografts. Tissue sections of heart (1_{st} panel), lung (2_{nd} panel), and spleen (3_{rd} panel) were stained with H&E. No tumor metastasis, significant architectural or pathologic changes in organs above were found by microscopic examination. The images of each group were depicted at 100× and 400× magnifications.



Supplemental Table 1. Blood biochemical parameters data for xerophilusin B (**1**) in KYSE-150 and KYSE-450 xenograft mice

Variable	KYSE-150				KYSE-450			
	Control (N=5)	**1** 7.5mg/kg (N=5)	**1** 15mg/kg (N=5)	DDP 2mg/kg (N=5)	Control (N=5)	**1** 7.5mg/kg (N=5)	**1** 15mg/kg (N=5)	DDP 2mg/kg (N=5)
ALT	38.20±1.9	36.6±3.37	56.8±7.6	95.4±4.09***	50.4±7.79	43.4±3.33	53.2±5.6	50.4±3.59
AST	188.6±16.22	145±6.01	160.2±14.69	361±23.51***	158±10.22	143±3.27	302±81.53	212±71.68
TP	50.72±0.68	50.85±1.5	47.79±0.68	51.31±1.29	47.14±0.81	50.06±0.54	45.5±0.66	50.23±0.58
ALB	26.7±0.85	27.68±0.84	29.13±0.85	33.17±0.64***	31.62±1.67	32.34±0.58	30.63±0.57	34.5±0.27
GLOB	24.02±1.05	23.17±1.41	18.67±0.74	18.14±0.78***	15.52±1.1	17.72±0.75	14.87±0.27	15.72±0.64
TBIL	0.18±0.08	0.4±0.19	0.58±0.15	2.52±0.18***	2.78±0.31	2.27±0.07	2.27±0.06	2.92±0.47
ALP	77.8±16.37	49.6±6.1	77.80±8.76	92.4±10.33	71.6±5.42	72.8±3.83	93.4±24.03	97±9.27
GGT	0.2±0.2	1.2±0.73	0	0	0	0.2±0.2	0	0
GLU	4.42±0.48	3.88±0.56	4.52±0.61	3.95±0.49	5.40±0.23	5.18±0.35	6.09±0.4	5.44±0.5
UN	10.35±0.44	8.31±0.95	7.49±0.39	9.46±0.6	12.49±0.86	13.48±0.71	10.98±0.26	13.29±0.91
CREA	11.74±0.6	11.11±0.91	9.44±0.54	13.38±0.59	10.74±0.35	11.98±0.79	9.92±0.61	11.54±0.74
UA	119.31±12.53	122.65±7.22	129.45±16.35	147.82±16.9	132.41±4.94	141.65±11.13	123.89±3.49	163.46±14.93
CHO	2.19±0.18	2.85±0.25	2.55±0.12	2.93±0.21	2.36±0.06	2.83±0.24	2.52±0.19	2.82±0.17
TG	1.25±0.22	1.69±0.41	1.62±0.19	0.74±0.06	1.87±0.15	2.57±0.39	2.27±0.24	2.4±0.29
LDH	2251±179.7	1669.75±247.25	1305.8±163.85	2192.4±170.78	1627.8±93.39	1726.6±121.59	1677±105.3	1684.6±115.71
A/G	1.12±0.08	1.22±0.09	1.58±0.1	1.83±0.06***	2.1±0.24	1.84±0.1	2.06±0.05	2.21±0.09

S9. **Supplemental Table 1.** Blood biochemical parameters data for xrophilusin B (**1**) in KYSE-150 and KYSE-450 xenograft mice. The full names of sixteen biochemical parameters were described in the experimental section. The units of the parameters are: ALT (U/L), AST (U/L), TP (g/L), ALB (g/L), GLOB (g/L), TBIL (μmol/L), ALP (U/L), GGT (U/L), GLU (mmol/L), UN (mmol/L), CREA (μmol/L), UA (μmol/L),

CHO (mmol/L), TG (mmol/L), LDH (U/L). All data are presented as mean ± SEM (n=5). The effect of the **1** or cisplatin (DDP) treatment was determined by comparing with the vehicle control group using one-way ANOVA test (***$p < 0.001$).