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SYNTHESIS AND ANTITUMOUR ACTIVITY OF NOVEL DITERPENEQUINONE SALVICINE AND THE ANALOGS¹

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Abstract: A novel diterpenequinone named salvicine (4), structurally modified derivative of a natural product, and a series of the novel analogs have been prepared. Most of the analogs were found to be potently active against tumor cell lines *in vitro*. Further study on 4 *in vivo* demonstrated that it possessed a significant antineoplastic activity against murine S-180 Sarcoma and Lewis lung cancer, and human lung adenocarcinoma xenografts A-549 and LAX-83. The preclinical studies of 4 are now under way. © 1999 Elsevier Science Ltd. All rights reserved.

Compound 1 was isolated from a Chinese medicinal plant *Salvia prionitis* that was used for antibacterial, antitubercular and antiphlogistic drug as a folk medicine. Studies on the chemical constituents of the medicinal plant have resulted in the isolation of more than 40 compounds so far^[1-4]. Compound 1 [4,5-seco-5,10-friedo-abieta-3,5(10),6,8,13-pentaene-11,12-dione] was one of these ingredients and was found to display a cytotoxicity against P388 leukemia cell *in vitro*^[5]. As a part of our screening program for antitumour compounds, we selected compound 1 as a lead to carry out the structural modification and prepared 9 derivatives 2-10. In this paper, we report the synthesis and antitumor activities.

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Chemistry

The compound 1, isolated from Chinese medicinal plant *Salvia prienitis*, was considered as a lead to carry out chemical modification as illustrated in scheme 1. Selective terminal attack on 1 with N-bromosuccinimide in tert-butyl alcohol gave the bromohydrin $2^{[6]}$. The epoxide 3 was prepared by m-chloroperbenzoic acid epoxidation of 1. Hydration of 3 with water and 8% perchloric acid in tetrahydrofuran resulted in a mixture of four compounds: the expected diol 4 (salvicine) and 5, 6, $7^{[7]}$. Compound 5 might be a product by singlet oxygen oxidation mechanism of olefin intermediate. Xiaoyuan Li *et al* reported preparation for this class of compounds ^[8]. Compound 7 was an anhydride-type side product. Kusumi *et al* reported preparation of an anhydride-type compound with a skeleton similar to 7 by photo-oxidation of a diterpenequinone^[9]. So compound 7 might be produced by a same photo-oxidation mechanism. Compound 4 was treated with acetic anhydride and pyridine at room temperature overnight to afford acetyl derivative 8. Further oxidation of 4 by pyridinium chlorochromate (PCC) in dichloromethane gave compound 9 (main product) and 10.



Scheme 1. Synthesis of compounds 2-10

In a typical experiment (All compounds described herein are racemic, the prefix dl is omitted.): To a stirred solution of m-chloroperbenzoic acid (4.3g, 25mmol) in CHCl₃ (100ml) held at 0° C was added a solution of compound 1 (6.0g, 20mmol) dissolved in CHCl₃ (60ml) during a period of 30 minutes. The mixture was stirred overnight at room temperature, washed with 10% NaHCO₃ solution and dried over anhydrous MgSO₄. The solvent was concentrated *in vacuo* and the residue was purified by CC on silica gel eluting with a mixture of

c-C₄H₁-EtOAc (9:1,v/v) to afford the epoxide 3 (5.4g, 85% yield): ¹H NMR (400 MHz, CDCl₃) δ (ppm): 1.10, 1.15 (each 3H, d, J=7.0 Hz, CH₂-16.17), 1.25,1.30 (each 3H, s, CH₂-18, 19), 1.68 (1H, m, H-2), 1.79 (1H, m, H-2), 2.37 (3H, s, C₄-CH₃), 2.92 (1H, m, H-15), 3.11 (1H, m, H-1), 3.28 (1H, m, H-3), 7.05 (1H, d, J=7.8 Hz, H-7), 7.07 (1H, s, H-14), 7.36 (1H, d, J=7.8 Hz, H-6). EIMS m/z: 312 (M⁺), 284 (M-CO), 267, 254, 240, 227, 213. Salvicine(4) [4,5-seco-5,10-friedo-abieta-3,4-dihydroxy-5(10),6,8,13-tetraene-11,12-dione]: To a solution of epoxide 3 (4.0 g, 0.013 mol) in 120 ml of tetrahydrofuran was added 23 ml of water. The solution was stirred and 4 ml of 8% perchloric acid was added. After stirring for 6 hr under N₂ at room temperature, 300 ml of brine was added and the mixture was extracted several times with ether. The organic phase was washed with dilute sodium bicarbonate and brine, dried (MgSO₄), evaporated under reduced pressure and purified by cc on silica gel eluted with cyclohexane – ethyl acetate mixture (4:1, v/v) to afford diol 4 (2.7 g, 65% yield), mp. 104° C. ¹H NMR (400 MHz, CDCl₃) δ (ppm): 1.12, 1.13 (each 3H, d, J=6.0 Hz CH₃-16, 17), 1.13, 1.20 (each 3H, s, CH₄-18, 19), 1.46 (1H, m, H-2), 1.77 (1H, m, H-2), 2.39 (3H, s, C₅-CH₃), 3.02 (1H, m, H-15), 3.08 (1H, m, H-1), 3.25 (1H, m, H-1), 3.54 (1H, dd, J=10.2, 1.2 Hz, H-3), 7.05 (1H, d, J=7.7 Hz, H-7), 7.07 (1H, s, H-14), 7.36 (1H, d, J=7.7 Hz, H-6). ¹³C NMR(25.25MHz, CDCl₃) δ (ppm): 19.9 (C-20), 21.5 (C-16), 21.5 (C-17), 23.4 (C-19), 26.4 (C-15), 26.9 (C-18), 27.6 (C-2), 30.7 (C-1), 73.0 (C-4), 78.6 (C-3), 128.1 (C-8), 128.4 (C-14), 135.1 (C-9), 137.0 (C-7), 140.2 (C-10), 140.2 (C-6), 144.7 (C-13), 148.3 (C-5), 181.3 (C-12), 182.6 (C-11). HRMS: 330.1859 for $C_{20}H_{26}O_4$, calcd 330.1831.

Biology

Study on the cytotoxic activity of the novel diterpenequinone analogs was carried out first *in vitro* against two leukemia cell lines (P388 mouse and HL-60 human leukemia cells) and two solid tumor cell lines (SPC-A4 lung cancer and SGC-7901 stomach cancer cells). The four cell lines were exposed to compounds 1-10 for 48 h. The *in vitro* cytotoxic activity was measured by microculture terazolium colorimetric assay (MTT). Most of the analogs showed cytotoxic activity. The results were reported in Table 1.

Compound 4 was further evaluated *in vivo* against four subcutaneously transplanting tumor animal models including two murine tumor models S-180 sarcoma, Lewis lung cancer, and two human lung adenocarcinoma xenografts A-549 and LAX-83. Etoposide (VP-16) and Miltomycinum C (MMC) were used as positive control drugs, respectively. The results are listed in Tables 2-5. The results showed compound 4 exhibited markedly activity against all four experimental animal models. The potent doses (inhibition > 30%, P < 0.05) for S-180 sarcoma, Lewis lung cancer, A-549 and LAX-83 human lung adenocarcinoma were 7.5, 7.5, 20 and 30 mg / Kg, respectively. Based on above pharmacological test data, salvicine (4) will be a promising compound to be developed as a new anticancer drug. The preclinical studies of salvicine are in progress in our institute.

Compound	P388	HL-60	SPC-A ₄	SGC-7901
1	1.95	2.36	2.75	1.37
2	3.99	4.61	1.45	6.12
3	3.38	4.48	1.88	71.46
4	3.49	3.57	2.46	1.84
5	3.70	3.70	2.90	2.22
6	0.83	0.27	3.38	1.98
7	1.47	1.43	2.76	89.63
8	3.39	5.04	2.61	/
9	1.78	2.39	2.68	52.36
10	1.46	2.84	2.62	66.90

Table 1: In vitro inhibitory effects (IC₅₀, µM) of compounds 1-10 on tumor cell lines.

Table 2: In vivo antitumor activity of salvicine (4) against murine S-180 sarcoma.

Drug	Dose (mg/kg)*day	Route	Mice In."/Fi. ^b	Body WT.(g) In /Fi	Tumor WT.(g) X ± SD	Inhibition (%)	Р
			+ _ +				
NS	0.2°7°	i.p.	20/20	21.1/28.5	1.66±0.66		
Salvicine	3.75*7	i.p.	10/10	21.0/26.9	1.52±0.62	8.4	>0.05
Salvicine	7.5*7	i.p.	10/10	20.9/26.1	1.07±0.61	35.5	<0.05
Salvicine	15'6	i.p.	10/10	20.9/22.8	0.93±0.57	44.0	< 0.01
VP-16	3* 7	i.p.	10/10	20.9/25.7	0.99±0.57	40.0	<0.05
NS	0.2*7	i. p .	20/20	21.6/25.5	1.80±0.53	1	
Salvicine	3.75 * 7	i.p.	10/10	21.4/24.8	1.99±0.66	1	
Salvicine	7.5*7	i.p.	10/10	21.6/22.9	1.22±0.37	32.2	< 0.05
Salvicine	15'6	i.p.	10/10	21.7/21.0	0.92±0.50	48.9	< 0.01
VP-16	3* 7	i.p.	10/10	21.4/23.5	1.09±0.52	39.4	<0.01
NS	0.2*7	i.p.	20/20	21.3/26.1	2.74±0.57	1	
Salvicine	3.75*7	i.p.	10/10	21.4/23.8	1.72±0.50	37.2	<0.01
Salvicine	7.5' 7	i.p.	10/10	21.3/23.1	1.40±0.39	48.9	<0.01
Salvicine	15'6	i.p.	10/10	21.4/20.4	1.06±0.43	61.3	<0.01
VP-16	3*7	i.p.	10/10	21.3/21.4	1.06±0.33	61.3	<0.01

^a: initial stage of experiment; ^b: final stage of experiment; ^c: ml/mouse.

Table 3: In vivo antitumor activity of salvicine (4) against murine Lewis lung cancer.

Drug	Dose (mg/kg) [•] day	Route	Mice In. [®] /Fi. ^b	Body WT.(g)	Tumor WT.(g)	Inhibition (%)	Р
				in./F1.	$X \pm SD$		
NS	0.2*10°	i.p.	20/20	19.1/20.3	1.47±0.63		
Salvicine	3.75*10	i.p.	10/10	19.2/20.5	1.28±0.45	12.9	>0.05
Salvicine	7.5* 10	i.p.	10/10	19.1/19.0	0.97±0.35	34.0	< 0.05
Salvicine	15*9	i.p.	10/10	19.0/16.6	0.75±0.35	49.0	<0.01
VP-16	5* 10	i.p.	10/10	19.3/17.0	0.73±0.2	50.3	<0.01
NS	0.2*10	i.p.	20/20	20.1/22.2	2.00±0.57	1	1
Salvicine	3.75*10	i.p.	10/10	20.1/22.0	1.85±0.71	7.50	>0.05
Salvicine	7.5* 10	i.p.	10/10	19.8/19.5	1.32±0.61	34.0	< 0.05
Salvicine	15 * 9	i.p.	10/10	19.9/18.3	0.91±0.47	54.5	< 0.01
VP-16	5'10	i.p.	10/10	20.2/19.7	1.03 ± 0.46	48.5	<0.01

^a: initial stage of experiment; ^b: final stage of experiment; ^c: ml/mouse.

Drug	Dose	Route	Schedule	Mice	Body WT.(g)	Tumor	Inhibition	P
	(mg/kg)			In."/Fi."	In./Fi.	WT.(g) X + SD	(%)	
						<u></u>		
CONTROL	0.2°	ip	Q2d×10	11/11	22.8/26.4	1.62±0.54	/	/
MMC	2.0	ip	Q2d×10	5/5	22.2/21.25	0.52±0.24	67.90	< 0.05
Salvicine	10	ip	Q2d×10	5/5	22.0/22.6	0.84±0.46	48.19	< 0.05
Salvicine	20	ip	Q2d×10	5/5	22.3/23.5	0.64±0.41	60.55	< 0.05
Salvicine	30	ip	Q2d×10	5/5	21.5/21.2	0.72±0.38	55.60	< 0.05
CONTROL	0.2°	ip	O2d×10	12/12	18.7/20.2	2.64±1.41	/	1
MMC	2.0	ip	Q2d×10	6/6	18.3/16.8	1.25±0.94	52.69	< 0.05
Salvicine	10	ip	Q2d×10	6/6	18.7/16.0	1.85±1.86	30.15	>0.05
Salvicine	20	ip	Q2d×10	6/6	18.5/19.0	1.91±1.17	27.76	>0.05
Salvicine	30	ip	Q2d×10	6/6	18.5/14.7	0.53±0.42	80.14	<0.01
CONTROL	0.2°	ip	Q2d×9	14/14	21.3/23.9	3.43±1.86	/	1
MMC	2.0	ip	O2d×9	6/6	21.3/18.0	0.71±0.47	79.24	< 0.01
Salvicine	10	ip	Q2d×9	6/6	21.1/24.6	2.55±0.79	25.50	>0.05
Salvicine	20	ip	Q2d×9	6/6	21.4/23.0	1.59±1.08	53.61	< 0.05
Salvicine	30	ip	Q2d×9	6/6	21.5/21.9	1.67±0.39	51.25	<0.05

Table 4: Inhibition effects of salvicine (4) on the human lung adenocarcinoma xenograft A-549.

*: initial stage of experiment; b: final stage of experiment; c: ml/mouse.

Table 5: Inhibition effects of salvicine (4) on the human lung adenocarcinoma xenograft LAX-83.

Drug	Dose	Route	Schedule	Mice	Body	Tumor	Inhibition	Р
	(mg/kg)			In."/F1."	w i .(g) In./Fi.	$\frac{WI}{X \pm SD}$	(%)	
<u> </u>								
CONTROL	0.2°	ip	Q2d×10	8/8	25.2/26.1	$1.71 \pm .051$	/	/
MMC	2.0	ip	Q2d×10	4/4	26.0/25.5	0.62±0.13	63.49	< 0.05
Salvicine	10	ip	Q2d×10	4/4	24.8/26.0	1.23±0.45	28.02	>0.05
Salvicine	20	ip	Q2d×10	4/4	24.5/25.0	1.25±0.81	26.73	>0.05
Salvicine	30	ip	Q2d×10	4/4	22.8/23.2	1.02 ± 0.41	40.31	< 0.05
CONTROL	0.2°	ip	Q2d×10	11/11	21.0/22.0	1.91±0.94	/	/
MMC	2.0	ip	Q2d×10	6/6	22.7/21.3	0.57±0.53	70.10	<0.01
Salvicine	10	ip	Q2d×10	6/6	21.8/19.5	1.13±0.92	40.98	>0.05
Salvicine	20	ip	Q2d×10	6/6	22.3/21.2	1.58±1.16	17.53	>0.05
Salvicine	30	ip	Q2d×10	6/6	22.2/19.6	0.93 ± 0.84	51.42	< 0.05
				10/10		1 1 5 . 0 . 10	,	,
CONTROL	0.2 ^e	ip	Q2d×10	12/12	20.1/22.4	1.15 ± 0.48	/	/
MMC	2.0	ip	Q2d×10	6/6	19.9/17.0	0.14±0.07	87.54	< 0.01
Salvicine	10	ip	Q2d×10	6/6	19.6/21.3	0.79±0.60	31.23	>0.05
Salvicine	20	ip	Q2d×10	6/6	19.5/19.9	1.04±0.64	9.39	>0.05
Salvicine	30	ip	Q2d×10	6/6	19.8/19.5	0.44±0.29	61.59	< 0.01

*: initial stage of experiment; b: final stage of experiment; c: ml/mouse.

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