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SYNTHESIS AND ANTITUMOUR ACTIVITY OF NOVEL DITERPENEQUINONE SALVICINE AND THE ANALOGS[1]

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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.

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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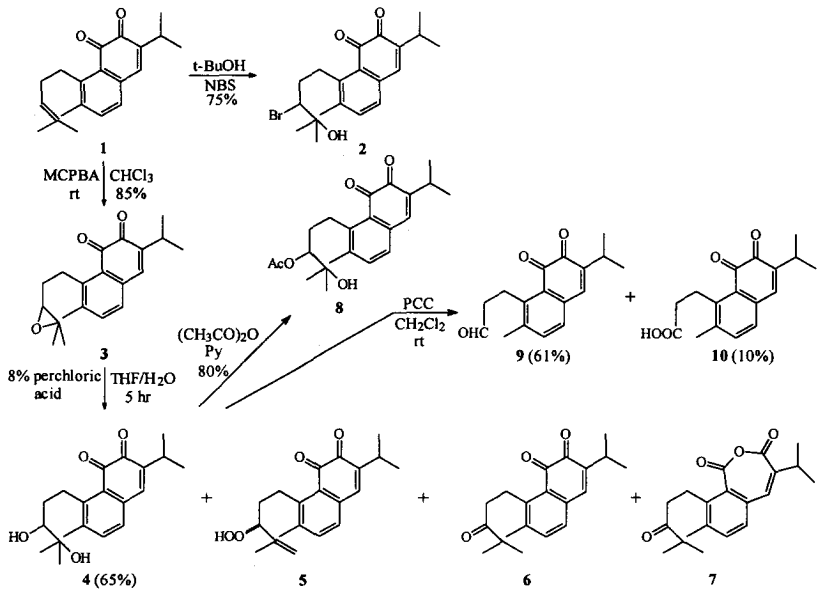
Chemistry

The compound **1**, isolated from Chinese medicinal plant *Salvia prionitis*, 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 $CHCl_3$ (100ml) held at 0^0 C was added a solution of compound **1** (6.0g, 20mmol) dissolved in $CHCl_3$ (60ml) during a period of 30 minutes. The mixture was stirred overnight at room temperature, washed with 10% $NaHCO_3$ solution and dried over anhydrous $MgSO_4$. The solvent was concentrated *in vacuo* and the residue was purified by CC on silica gel eluting with a mixture of

c-C_6H_{12}-EtOAc (9:1,v/v) to afford the epoxide **3** (5.4g, 85% yield): ^{1}H NMR (400 MHz , $CDCl_3$) δ (ppm): 1.10, 1.15 (each 3H, d, J=7.0 Hz, CH_3-16,17), 1.25,1.30 (each 3H, s, CH_3-18, 19), 1.68 (1H, m, H-2), 1.79 (1H, m, H-2), 2.37 (3H, s, C_5-CH_3), 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 solutiion 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 N_2 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 ($MgSO_4$), 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. 104^0 C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.12, 1.13 (each 3H, d, J=6.0 Hz CH_3-16, 17), 1.13, 1.20 (each 3H, s, CH_3-18, 19), 1.46 (1H, m, H-2), 1.77 (1H, m, H-2), 2.39 (3H, s, C_5-CH_3), 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). ^{13}C NMR(25.25MHz, $CDCl_3$) 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.

Table 1: *In vitro* inhibitory effects (IC_{50}, μM) of compounds **1-10** on tumor cell lines.

Compound	P388	HL-60	$SPC\text{-}A_4$	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 2: *In vivo* antitumor activity of salvicine **(4)** against murine S-180 sarcoma.

Drug	Dose (mg/kg)*day	Route	Mice In.[a]/Fi.[b]	Body WT.(g) In./Fi.	Tumor WT.(g) X ± SD	Inhibition (%)	P
NS	0.2*7[c]	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	/	
Salvicine	3.75*7	i.p.	10/10	21.4/24.8	1.99±0.66	/	
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	/	
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.[a]/Fi.[b]	Body WT.(g) In./Fi.	Tumor WT.(g) X ± SD	Inhibition (%)	P
NS	0.2*10[c]	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	/	/
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.

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

Drug	Dose (mg/kg)	Route	Schedule	Mice In.[a]/Fi.[b]	Body WT.(g) In./Fi.	Tumor WT.(g) X ± SD	Inhibition (%)	P
CONTROL	0.2[c]	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[c]	ip	Q2d×10	12/12	18.7/20.2	2.64±1.41	/	/
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[c]	ip	Q2d×9	14/14	21.3/23.9	3.43±1.86	/	/
MMC	2.0	ip	Q2d×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

[a] : 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 (mg/kg)	Route	Schedule	Mice In.[a]/Fi.[b]	Body WT.(g) In./Fi.	Tumor WT.(g) X ± SD	Inhibition (%)	P
CONTROL	0.2[c]	ip	Q2d×10	8/8	25.2/26.1	1.71±.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[c]	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
CONTROL	0.2[c]	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

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

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