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Synthesis and cytotoxic activity of 17-carboxylic acid modified 23-hydroxy betulinic acid ester derivatives

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Abstract—New 17-carboxylic acid modified 23-hydroxy betulinic acid ester derivatives were prepared and tested for cytotoxic activity on five cancer cell lines in vitro: all tested compounds showed stronger cytotoxic activity than 23-hydroxy betulinic acid and betulinic acid. In addition, compound **5a** was tested for anti-tumor activity in vivo: it had much better anti-tumor activity than 23-OH betulinic acid and had similar anti-tumor activity with cyclophosphamide and 5-fluorouracil.

Lupane derivatives are prevalent in natural sources and have various biological activities. 23-Hydroxy betulinic acid was isolated from the root of *Pulsatilla chinensis* which is a Chinese medicinal herb for 'blood-cooling' and detoxification in traditional Chinese medicine, and as such has been used for the treatment of amoebic dysentery and malaria. 23-Hydroxy betulinic acid was recently turned out to have good anti-tumor and anti-HIV selectivity, such as the cytotoxicity and apoptotic response induced by 23-hydroxy betulinic acid in human leukemia HL-60 cells. The telomerase activity during apoptosis was examined by telomeric repeat amplification protocol (TRAP) assay, and B cell leukemia/lymphoma 2 gene (*bcl-2*) was analyzed to determine whether their expressions correlated with telomerase activity. 23-Hydroxy betulinic acid inhibited telomerase activity and suppressed the expression of *bcl-2* in HL-60 cells. The inhibition of telomerase activity of HL-60 cells was closely associated with the apoptotic events induced by 23-hydroxy betulinic acid.[1–4] Betulinic acid, one of well-known lupines, showed the selective cytotoxic activity toward many cell lines.[5–10]

The research of derivatives of 23-hydroxy betulinic acid about their cytotoxicity has not been reported up to now. In this manuscript, we report a series of derivatives of 23-hydroxy betulinic acid with modification of 17-carboxylic acid. The study results revealed that all derivatives have better cytotoxicity than 23-hydroxy betulinic acid in vitro and many of them have better cytotoxicity than betulinic acid. Compound **5a** shows more potent anti-tumor activity than 23-hydroxy betulinic acid and has slightly less anti-tumor activity than cyclophosphamide and 5-fluorouracil.

Synthesis of 17-carboxylic acid ester derivatives of 23-hydroxy betulinic acid has been described in Scheme 1. 23-Hydroxy betulinic acid (**1**) was provided by the laboratory of Wencai Ye. It was treated with acetic anhydride in pyridine to afford 3,23-*O*-diacetyl derivative **2**. Compound **2** upon reaction with $(COCl)_2$ in CH_2Cl_2 yielded 3,23-*O*-diacetyl-17-acyl chloride derivative **3**. Upon reaction with ethylene glycol, 1,4-butanediol, 1,6-hexanediol, butyne-1,4-diol, allyl alcohol, propargyl alcohol, respectively, yielded corresponding 3,23-*O*-diacetyl-17-carboxylate derivatives **4a–f**, and **4a–f** were then hydrolyzed to give derivatives **5a–f**. In the process of hydrolysis reaction, the esters of 17-position remained the esters but not acids. We think the steric hindrance of this position is the reason. We found the esters were not hydrolyzed in the condition of NaOH/THF/CH_3OH, but they could be transmitted to the acids in the condition of LiI/DMF.[12] All the prepared compounds were characterized by spectroscopic tools.[5–8]

Keywords: 23-Hydroxy betulinic acid; 17-Carboxylic acid modified ester derivatives; Anti-tumor activity.
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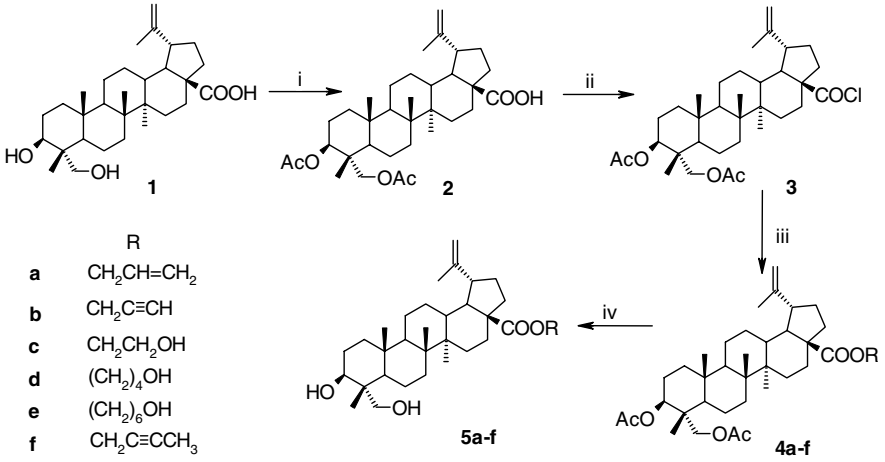

Scheme 1. Reagents and conditions: (i) Ac_2O/Pyr (yield 85%); (ii) $(COCl)_2/CH_2Cl_2$ (yield 90%); (iii) ROH/CH_2Cl_2 (yields 45–70%); (iv) NaOH/THF/CH_3OH (yields 80–90%).

The cytotoxic activity of betulinic acid, 23-hydroxy betulinic acid and its derivatives (**5a–f**) in vitro was determined by performing the MTT cytotoxicity assay, and the result is summarized in Table 1. Many different cell lines were used: A549 (human lung carcinoma), B16 (mice melanoma), SF-763 (human cerebroma), BEL-7402 (human hepatoma), and C6 (mice neuroglioma). The activity of derivatives was compared with that of 23-hydroxy betulinic acid and betulinic acid in each panel.[9,10] It is the first time to report the cytotoxicity of the derivatives of the 23-hydroxy betulinic acid. In human lung carcinoma cell line (A549), compounds **5a–f** have better cytotoxicity than betulinic acid and have similar cytotoxicity as 23-OH betulinic acid. Compound **5a** has the best cytotoxicity in A549. In human hepatoma cell line (BEL-7402), compound **5f** is the best compound which has similar cytotoxicity as betulinic acid. Compounds **5a–f** have better cytotoxicity than 23-OH betulinic acid but none of them have better than betulinic acid. In human cerebroma cell line (SF-763), compound **5a** is the most potent which is almost twofold more potent than betulinic acid and 23-OH betulinic acid. Compounds **5b**, **5e**, and **5f** are almost 1.5-fold more potent than betulinic acid and 23-OH betulinic acid. Compound **5d** is slightly better than betulinic acid and 23-OH betulinic acid. Compound **5c** is a little better than 23-OH betulinic acid but worse than betulinic acid. In mice melanoma cell line (B16), compound **5e** is the best compound which has twofold more potency than 23-OH betulinic acid and is little better than betulinic acid. Compounds **5a–d** and **5f** have better cytotoxicity than 23-betulinic acid but are worse than betulinic acid. In mice neuroglioma cell line (C6), compound **5e** has twofold better cytotoxicity than betulinic acid and 23-OH betulinic acid. Compounds **5c** and **5d** are 1.5-fold more potent than betulinic acid and 23-OH betulinic acid. Compounds **5a**, **5b**, and **5f** are slightly better than betulinic acid and 23-OH betulinic acid.

In conclusion, compounds **5a–f** are more potent than 23-betulinic acid in all cell lines and more potent than betulinic acid in A549 and C6. In SF-763 all compounds have better cytotoxicity than betulinic acid but compound **5c**. In B16 none of compounds are more potent than betulinic acid but compound **5e**. In BEL-7402, none of compounds have better cytotoxicity than betulinic acid. Compounds **5a** and **5f** were the best compounds. So compound **5a** was chosen to test in vivo.

In vivo cytotoxic activity of 23-hydroxy betulinic acid and its derivative **5a** was determined by performing the cytotoxicity assay in murine having H22 liver tumor

Table 1. The cytotoxicity data of 23-hydroxy betulinic acid and its derivatives (**5a–f**) [IC_{50} (μmol/L) ± SD]

Compound	Cell line				
	A549	BEL-7402	SF-763	B16	C6
5a	74.22 ± 17.61*	63.86 ± 2.51	54.72 ± 16.38*	80.54 ± 2.99	82.05 ± 5.44
5b	87.82 ± 7.24	67.01 ± 14.65	63.41 ± 25.19	72.93 ± 7.52	82.73 ± 8.22
5c	83.78 ± 13.70	78.66 ± 21.30	101.15 ± 1.756	80.87 ± 1.56	65.00 ± 24.12
5d	84.145 ± 11.03	74.47 ± 9.21	78.60 ± 9.06	66.03 ± 7.03	69.30 ± 9.11
5e	78.28 ± 18.50	69.17 ± 15.67	64.31 ± 5.80	41.06 ± 3.62*	49.68 ± 30.01*
5f	81.06 ± 77.74	52.96 ± 13.36*	64.31 ± 7.16	63.15 ± 7.52	75.88 ± 14.89
HBA	87.60 ± 8.67	97.32 ± 10.94	104.54 ± 1.04	83.04 ± 4.06	99.48 ± 21.44
BA	97.51 ± 11.78	43.39 ± 9.22	92.07 ± 5.06	53.46 ± 9.47	90.69 ± 21.09

Concentration (μmol/L): 26.20, 32.80, 41.00, 51.20, 64.00, 80.00, and 100.00. Data are means of three experiments. BA, betulinic acid; HBA, 23-hydroxy betulic acid.

* The significant differences among IC_{50} values of these compounds in a cell line.

Table 2. The cytotoxicity data of 23-hydroxy betulinic acid and its derivatives **5a** in mice H22 in vivo

Drugs	Dose	Injection	Number of mice		Weight of mice (g)		Weight of tumor $X \pm SD$ (g)	Ratio of inhibition (%)	*P* value
			Start	End	Start	End			
Normal saline	0.4 ml/mouse	iv	10	10	18.1 ± 0.6	27.3 ± 2.8	1.58 ± 0.49		
Cyclophosphamide	30 mg/kg	iv	10	10	18.1 ± 0.5	26.2 ± 2.3	0.69 ± 0.30	56.3	<0.01
5a	20 mg/kg	ip	10	10	18.5 ± 0.9	25.0 ± 2.7	0.90 ± 0.53	43.0	<0.05
23-OH betulinic acid	20 mg/kg	ip	10	10	18.3 ± 0.9	26.9 ± 1.3	1.42 ± 0.36	10.1	>0.05

Table 3. The cytotoxicity data of 23-hydroxy betulinic acid and its derivatives **5a** in mice B16 in vivo

Drugs	Dose	Injection	Number of mice		Weight of mice (g)		Weight of tumor $X \pm SD$ (g)	Ratio of inhibition (%)	*P* value
			Start	End	Start	End			
Normal saline	0.4 ml/mouse	iv	9	9	18.9 ± 2.3	21.3 ± 3	2.03 ± 0.67		
5-Fluorouracil	25 mg/kg	iv	9	9	19.0 ± 1.4	18.8 ± 1.7	0.56 ± 0.25	72.4	<0.01
5a	20 mg/kg	ip	9	9	19.0 ± 1.4	17.9 ± 2	1.18 ± 0.99	41.9	<0.05
23-OH betulinic acid	20 mg/kg	ip	9	9	18.8 ± 2.4	20.5 ± 2.9	1.76 ± 0.8	13.3	>0.05

and B16 melanoma and the cytotoxicity data are summarized in Tables 2 and 3. ICR female murine with body weight of 18–22 g were transplanted with H22 and B16 subcutaneously into the right oxter according to protocols of transplant tumor research. After 24 h of tumor transplantation, murine were weighed, and each model group was at random divided into 4 groups, each of which had 10 murine in H22 group and 9 murine in B16 group. The groups with 23-OH betulinic acid and **5a** were administered intraperitoneously 20 mg/kg in a vehicle of 20% DMSO/80% saline. respectively. The positive control group was treated with cyclophosphamide (30 mg/kg) in H22 group and 5-fluorouracil (25 mg/kg) in B16 group through intravenous injection in a vehicle of 20% DMSO/ 80% saline. The negative control group received 0.9% normal saline through intravenous injection. All test compounds were given through injections 24 h after tumor transplantation (or inoculation). Treatments were done at a frequency of intravenous or intraperitoneal injection one dose per day for a total of four consecutive days in H22 group and for a total of 11 consecutive days in B16 group. After the treatments, all murine were killed and weighed simultaneously, and then tumor segregated and weighed. Tumor inhibitory ratio was calculated by the following formula and perform *T* test:[11]

$$\text{Tumor inhibitory ratio (\%)} = (1 - \text{average tumor weight of treated group}/\text{average tumor weight of control group}) \times 100\%$$

Compound **5a** has better anti-tumor activity than 23-OH betulinic acid in mice with H22 liver tumor and with B16 melanoma which has slight potent anti-tumor activity than cyclophosphamide in H22 group and 5-fluorouracil in B16 group. Cyclophosphamide and 5-fluorouracil are the normal anti-tumor drugs in clinic.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.09.096.

References and notes

1. Pisha, E.; Chai, H.; Lee, I.-S.; Chagwedera, T. E.; Farnsworth, N. R.; Cordell, G. A.; Beecher, C. W. W.; Fong, H. H. S.; Dogglas Kinghorn, A.; Brown, D. M.; Wani, M. C.; Wall, M. E.; Hieken, T. J.; Das Gupta, T. K.; Pezzut, J. M. *Nat. Med.* **1995**, *1*, 1046.
2. Ye, W.-C.; Ji, N.-N.; Zhao, S.-X.; Liu, J.-H.; Ye, T.; Mckervey, M. A.; Stevensin, P. *Phytochemistry* **1996**, *42*, 799.
3. Ji, Z.-N.; Ye, W.-C.; Liu, G.-G.; Wendy Hsiao, W. L. *Life Sci.* **2002**, *72*, 1.
4. Shi-Jun, Y.; Hong, G. *J. Liaoning Normal Univ. (Nat. Sci. Ed.)* **2002**, *26*, 56.
5. Sun, I.-C.; Wang, H.-K.; Shen, Y. K. J.-K.; Cosentino, L. M.; Chen, C.-H.; Yang, L.-M.; Lee, K.-H. *J. Med. Chem.* **1998**, *41*, 4648.
6. Sun, I.-C.; Chen, C.-H.; Wu, Y. K. J.-H.; Wang, H.-K.; Lee, K.-H. *J. Med. Chem.* **2002**, *45*, 4271.
7. Evers, M.; Poujade, C.; Ribeill, F. S. Y.; James, C.; Lelievre, Y.; Gueguen, J.-C.; Reisdorf, D.; Morize, I.; Pauwels, R.; De Clercq, E.; Henin, Y.; Bousseau, A.; Mayaux, J.-F.; Le Pecq, J.-B.; Dereu, N. *J. Med. Chem.* **1996**, *39*, 1056.

8. Soler, F.; Poujade, C.; Evers, M.; Carry, J.-C.; Henin, Y.; Bousseau, A.; Huet, T.; Pauweis, R.; De Clercq, E.; Mayaux, J.-F.; Le Pecq, J.-B.; Dereu, N.. *J. Med. Chem.* **1996**, *39*, 1069.
9. Mukherjee, R.; Jaggi, M.; Rajendran, P.; Srivastava, S. K.; Siddiqui, M. J. A.; Vardhan, A.; Burman, A. C. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3169.
10. Mukherjee, R.; Jaggi, M.; Siddiqui, M. J. A.; Srivastava, S. K.; Rajendran, P.; Vardhan, A.; Burman, A. C. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4087.
11. Xu, S. Y.; Bian, R. L.; Chen, X. *Pharmacological Experimental Method*; People's Medical Publishing House: Beijing, 1991, 1424.
12. Finlay, H. J.; Honda, T.; Gribble, G. W. *ARKIVOC* **2002**, *xii*, 38.