Bioorganic & Medicinal Chemistry Letters 18 (2008) 4741–4744

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and biological evaluation of novel 1-*O*- and 14-*O*-derivatives of oridonin as potential anticancer drug candidates

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ARTICLE INFO

Article history:
Received 13 February 2008
Revised 8 June 2008
Accepted 11 June 2008
Available online 3 July 2008

Keywords:
Oridonin
Derivatives
Cytotoxicity
Anticancer
Structure–activity relationships

ABSTRACT

Novel 1-*O*- and 14-*O*-derivatives of oridonin were synthesized and biologically evaluated. All of the derivatives exhibited stronger cytotoxicity against six cancer cell lines (BGC-7901, SW-480, HL-60, BEL-7402, A549, and B16) than oridonin in vitro, and some of them were more potent than oridonin and cyclophosphamide in vivo. Compounds **Ib** and **IIg** were the most potent with the IC_{50} values of 0.84 μM for **Ib** in HL-60 cell and 1.00 μM for **IIg** in BEL-7402 cell.

Malignant neoplasm seriously threatens human health and takes the second place among all the causes of morality, just inferior to the cardio- and cerebro-vascular diseases. The anticancer drugs usually used in current clinical treatment are cytotoxic agents, which have many adverse effects such as the harm that it causes to human immune system and leading to gastrointestinal disorders. In recent years, a lot of natural compounds, which exhibited low toxicity and high therapeutic efficacy, have been reported.[1]

Oridonin (1) is an *ent*-kaurene diterpenoid compound isolated from the leaves of *Rabdosia rubescens* (Hemsl.) Hara, *Isodon japonicus* (Burm.f.) Hara, and *Isodon trichocarpus* Kudo. It has attracted considerable attention in recent years because of its extensive activities, such as anti-tumor, antibacterial, antiviral, and anti-inflammatory effects.[2,3] Oridonin has been safely used for the treatment of hepatoma and promyelocytic leukemia, but its poor solubility limited its extensive use in clinic,[4,5] and only a few of structure modifications of oridonin derivatives have been described by literatures.[6–8]

Recently, we have noticed that there has been a large body of investigations on the action mechanisms of oridonin, in particular, the action connected with the apoptotic mechanisms and regulation of telomerase, which is a crucial step in the tumorigenesis, seems to be very promising in anticancer therapy.[9–15] Therefore, we assume that the structural modification of oridonin would produce novel drug candidates with improved solubility and higher potency than oridonin. In this letter, our efforts have been focused on synthesis and biological evaluation of 1-*O*- and 14-*O*-derivatives of oridonin. It was found that water solubility of most target compounds has been improved (>50 mg/ml) as compared to that of oridonin. The biological study results revealed that all derivatives exhibited stronger cytotoxicity than oridonin in vitro, and some of them were more potent than oridonin and cyclophosphamide in vivo.

The synthetic routes are shown in Schemes 1 and 2.

The synthetic routes are outlined in Scheme 1. Treatment of oridonin with 2,2-dimethoxypropane in presence of TsOH in acetone afforded 7,14-(1-methylethylene)-dioxy-oridonin derivative **2** in 85% yield. Compound **2** upon reaction with Ac_2O/C_5H_5N or $RSO_2Cl/Et_3N/DMAP/CH_2Cl_2$, respectively, yielded corresponding 1-*O*-acetyl or 1-*O*-sulfonyl derivatives **3a** and **3b** in 95% and 92% yields. Deprotection of **3a** and **3b** with AcOH gave the corresponding compounds **4a** and **4b** in quantitative yields, respectively. Compounds **5a** and **5b** were prepared in high yields 97.5% and 95%, respectively, by reaction of **4a** and **4b** with *N*-Boc-Gly-OH in the presence of DMAP/DCC in CH_2Cl_2. Treatment of **5a** and **5b** with

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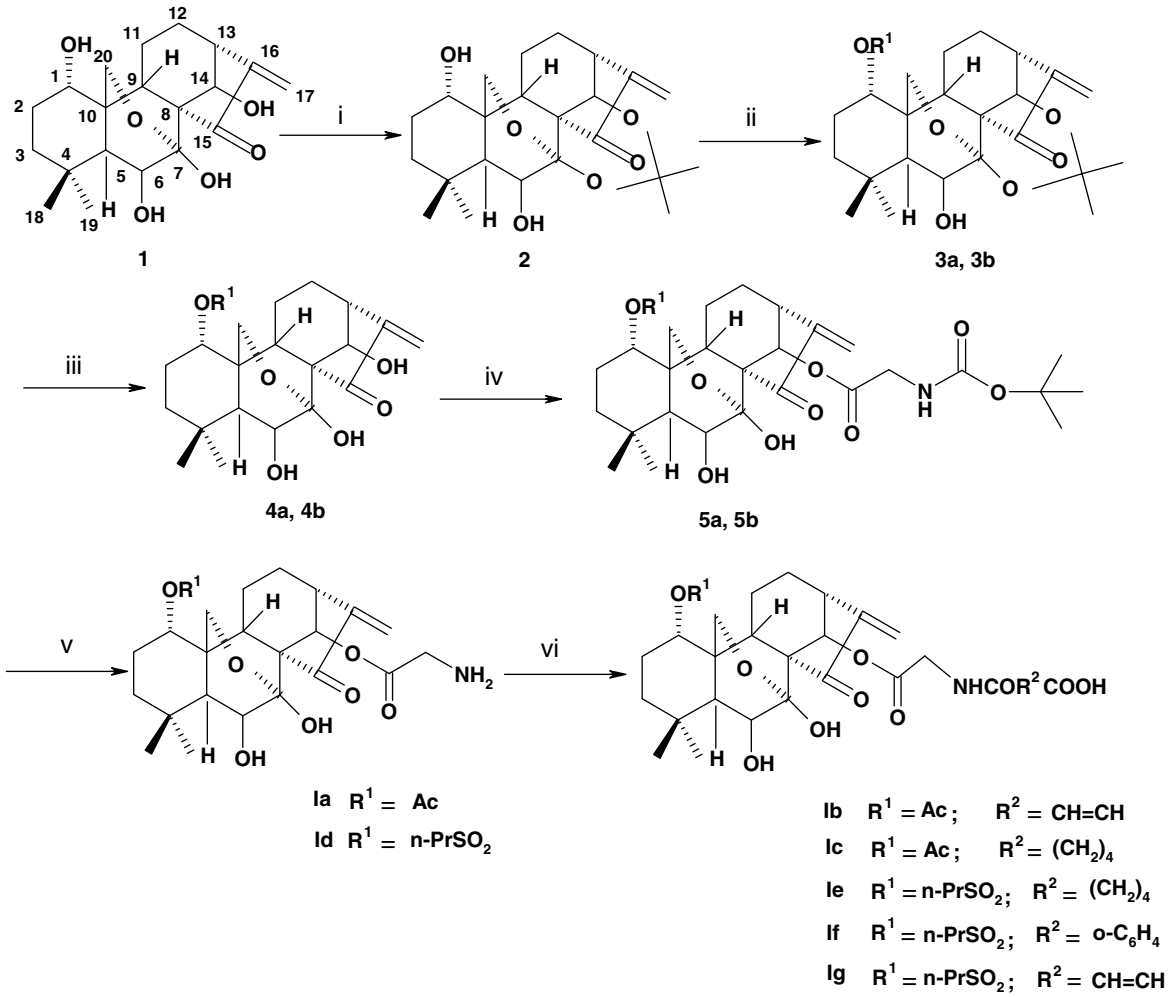
doi:10.1016/j.bmcl.2008.06.097



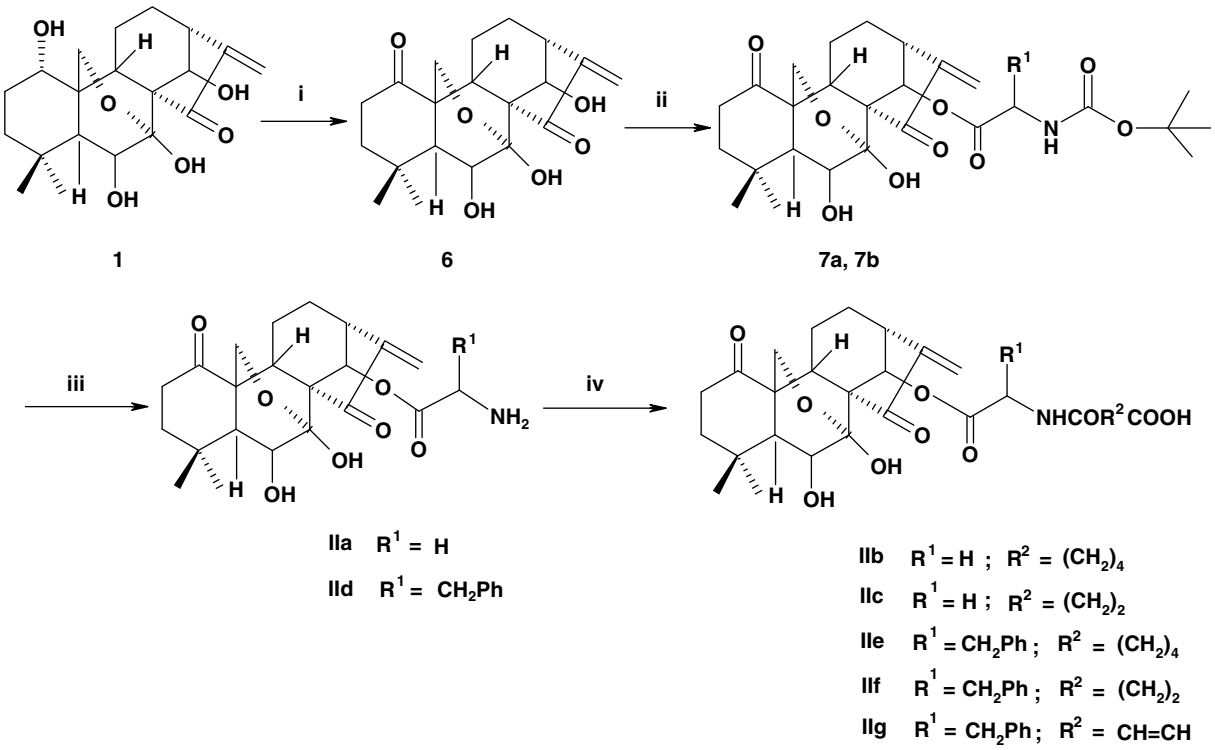
Scheme 1. Reagents and conditions: (i) $(CH_3)_2C(OCH_3)_2$, TsOH, acetone; (ii) Ac_2O, C_5H_5N (R = Ac) or RSO_2Cl (R = *n*-$PrSO_2$), Et_3N/DMAP, CH_2Cl_2; (iii) 80% AcOH; (iv) DMAP/DCC, *N*-Boc-Gly-OH, CH_2Cl_2; (v) CF_3COOH, CH_2Cl_2; (vi) $R^2(CO)_2O$, picoline, CH_2Cl_2.

CF_3COOH obtained target compounds **Ia** and **Id** in yields 96.6% and 98.2%, respectively. In an attempt to improve the solubility of the oridonin derivatives, the terminal carboxylic acid moiety to the compounds **Ia** and **Id** was introduced, and then target compounds **Ib**–**Ic** and **Ie**–**Ig** were prepared by reaction of **Ia** and **Id** with $R^2(CO)_2O$ in the presence of picoline in CH_2Cl_2 in yields (55.3–78.5%), respectively.[16]

As shown in Scheme 2, oxidation of oridonin with Jones reagents in acetone afforded 1-oxo-oridonin derivative **6** in high yield (98.5%). Treatment of **6** with *N*-Boc-R^1-COOH in the presence of DMAP/DCC in CH_2Cl_2 gave compounds **7a** and **7b** (98.8% and 97.6%). Target compounds **IIa** and **IId** were obtained by reaction of **7a** and **7d** with CF_3COOH in yields 98.2% and 98.7%, respectively. Target compounds **IIb**–**IIc** and **IIe**–**IIg** were prepared by reaction of **IIa** and **IId** with $R^2(CO)_2O$ in the presence of picoline in CH_2Cl_2 in yields (57.0–83.6%), respectively.[16]

The cytotoxicity of the target compounds was determined by MTT assay in vitro. Six different cell lines were used: BGC-7901 (human gastric carcinoma), SW-480 (human colon carcinoma), HL-60 (human promyelocytic leukemia), BEL-7402 (human hepatoma), A549 (human lung carcinoma), and B16 (mice melanoma). The activity of derivatives was compared with the parent compound oridonin and positive drug 5-fluorouracil in each panel. As shown in Table 1, the results have revealed that all of the 1-*O*- and 14-*O*-derivatives of oridonin are more cytotoxic than oridonin in vitro, and compounds **Ib** and **IIg** have stronger anti-tumor activity than 5-fluorouracil in all tested cell lines, which were the most potent with the IC_{50} values of 0.84 μM (human promyelocytic leukemia cell line HL-60) and 1.00 μM (human hepatoma cell line BEL-7402), respectively. Compound **Ib** is almost 38-fold more potent than oridonin and 25-fold more potent than 5-fluorouracil in HL-60 cell. Compound **IIg** is almost 40-fold more potent than oridonin and 14-fold more potent than 5-fluorouracil in BEL-7402 cell. Compound **IIf** is almost 14-fold more potent than oridonin and 9-fold more potent than 5-fluorouracil in HL-60 cell.

Based on the in vitro results, we further tested cytotoxicity of compounds **Ib**, **IIf**, and **IIg** in vivo by performing the cytotoxicity assay in mice having H22 liver tumor and B16 melanoma. Institute of Cancer Research (ICR) female mice with body weight of 18–22 g were transplanted with H22 and B16 subcutaneously into the right oxter according to protocols of tumor transplant research,[17] respectively. After 24 h of tumor transplantation, mice in H22 group and in B16 group were weighed and divided into six groups at random, respectively. The groups with oridonin, **Ib**, **IIf**, and **IIg** were administered intraperitoneously 20 mg/kg in a vehicle of 20% DMSO/80% saline, respectively. The positive control group was treated with cyclophosphamide (20 mg/kg) in H22 group and 5-fluorouracil (20 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 of the test compounds were given through injections after 24 h of tumor transplantation (or inoculation). Treatments were



Scheme 2. Reagents and conditions: (i) Jones reagents, acetone; (ii) *N*-Boc-R^1-COOH, DMAP/DCC, CH_2Cl_2; (iii) CF_3COOH, CH_2Cl_2; (iv) $R^2(CO)_2O$, picoline, CH_2Cl_2.

Table 1
The cytotoxicity data of oridonin derivatives (**Ia**–**Ig** and **IIa**–**IIg**) in six cancer cell lines

Compound	Cytotoxicity (IC_{50}, μM)					
	BGC-7901	SW-480	HL-60	BEL-7402	A549	B16
5-Fu[a]	16.35 ± 1.96	13.16 ± 1.78	21.38 ± 1.56	14.41 ± 2.99	10.85 ± 7.02	8.73 ± 0.75
Oridonin	28.30 ± 1.57	31.42 ± 3.22	32.76 ± 1.97	39.80 ± 2.87	27.24 ± 1.13	26.15 ± 2.35
Ia	18.20 ± 1.14	22.31 ± 2.26	12.34 ± 2.51	17.85 ± 3.01	11.03 ± 0.59	12.40 ± 1.01
Ib	2.78 ± 0.53	2.60 ± 0.31	0.84 ± 0.23	1.00 ± 0.54	2.60 ± 0.41	0.93 ± 0.22
Ic	13.16 ± 1.80	10.70 ± 0.81	5.30 ± 0.38	8.26 ± 0.56	10.52 ± 1.03	12.49 ± 0.76
Id	15.91 ± 1.65	24.73 ± 1.02	19.80 ± 2.75	20.18 ± 2.11	16.29 ± 2.38	21.70 ± 3.30
Ie	15.26 ± 2.46	11.21 ± 0.25	9.50 ± 0.16	24.77 ± 1.76	14.02 ± 0.87	13.02 ± 1.73
If	20.85 ± 3.55	25.36 ± 2.43	13.82 ± 2.42	39.05 ± 2.85	22.67 ± 3.02	17.55 ± 0.89
Ig	5.52 ± 0.78	14.65 ± 1.54	1.97 ± 0.30	19.14 ± 0.19	1.33 ± 0.31	5.66 ± 0.43
IIa	20.94 ± 3.26	28.40 ± 2.23	24.78 ± 2.45	30.34 ± 1.33	20.61 ± 1.55	19.14 ± 2.47
IIb	15.13 ± 1.14	10.23 ± 0.14	8.65 ± 0.98	13.79 ± 0.46	7.47 ± 0.62	16.40 ± 2.51
IIc	15.60 ± 1.46	10.17 ± 0.76	7.76 ± 0.69	2.09 ± 0.24	11.45 ± 0.37	8.57 ± 0.83
IId	10.48 ± 1.05	15.04 ± 1.97	27.51 ± 2.57	29.60 ± 1.38	22.17 ± 2.32	10.22 ± 0.42
IIe	7.05 ± 0.46	7.30 ± 0.30	6.24 ± 0.83	12.85 ± 2.01	8.06 ± 0.55	13.91 ± 1.73
IIf	5.96 ± 0.68	12.94 ± 0.89	2.31 ± 1.06	6.50 ± 0.60	17.05 ± 1.80	14.81 ± 1.49
IIg	3.02 ± 0.54	2.89 ± 0.65	2.07 ± 0.20	1.00 ± 0.13	3.46 ± 0.52	8.22 ± 0.78

The data were the mean ± SD obtained from three independent experiments.
[a] 5-Fluorouracil was used as a positive control.

Table 2
The anti-tumor effect of oridonin derivatives **Ib**, **IIf**, and **IIg** in mice with H22

Compound	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	iv	10	10	19.2 ± 1.5	26.1 ± 1.2	1.71 ± 0.64		
Cyclophosphamide[a]	iv	10	10	18.1 ± 1.1	25.8 ± 2.1	0.78 ± 0.43	54.3	<0.01
Oridonin	ip	10	10	18.2 ± 1.4	26.2 ± 1.9	0.98 ± 0.67	42.7	<0.05
Ib	ip	10	10	18.5 ± 0.8	25.5 ± 1.4	0.60 ± 0.91	64.9	<0.01
IIf	ip	10	10	18.1 ± 0.4	25.3 ± 1.8	0.68 ± 1.69	59.1	<0.01
IIg	ip	10	10	18.6 ± 1.5	26.2 ± 1.3	0.64 ± 1.24	62.5	<0.01

[a] Cyclophosphamide was used as a positive control.

Table 3
The anti-tumor effect of oridonin derivatives **Ib**, **IIf**, and **IIg** in mice with B16

Compound	Injection	Number of mice		Weight of mice (g)		Weight of tumor X ± SD (g)	Ratio of inhibition (%)	*P*-Value
		Start	End	Start	End			
Normal saline	iv	10	10	19.0 ± 0.8	25.7 ± 1.5	1.96 ± 0.52		
5-Fluorouracil[a]	iv	10	10	18.9 ± 0.6	20.2 ± 2.1	0.52 ± 0.17	73.5	<0.01
Oridonin	ip	10	10	18.5 ± 1.9	26.6 ± 1.8	1.06 ± 0.84	45.9	<0.05
Ib	ip	10	10	18.3 ± 0.9	26.1 ± 1.7	0.59 ± 0.36	69.9	<0.01
IIf	ip	10	10	18.8 ± 1.3	25.8 ± 2.6	0.85 ± 1.40	56.6	<0.01
IIg	ip	10	10	19.1 ± 1.7	24.2 ± 1.2	0.76 ± 0.82	61.2	<0.01

[a] 5-Fluorouracil was used as a positive control.

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 eleven consecutive days in B16 group. After the treatments, all of the mice were killed and weighed simultaneously, and then tumors were segregated and weighed. The ratio of inhibition of tumor was calculated by the following formula and the cytotoxicity data are summarized in Tables 2 and 3.

Ratio of inhibition of tumor (%) = (1 − average tumor weight of treated group/average tumor weight of control group) × 100%.

As shown in Tables 2 and 3, compounds **Ib**, **IIf**, and **IIg** have stronger anti-tumor activity than oridonin and cyclophosphamide in mice with H22 liver tumor. Compounds **Ib**, **IIf**, and **IIg** have more potent anti-tumor activity than oridonin and slightly less potent anti-tumor activity than 5-fluorouracil in mice with B16 melanoma.

The preliminary analysis of structure–activity relationships (SAR) suggested that the introduction of both ester side chain of lipophilicity and terminal carboxylic acid moiety to the 14-*O* position of oridonin appeared crucial for an increase of the cytotoxicity of target compounds, such as compounds **Ib–Ic**, **Ie–Ig**, **IIb–IIc**, and **IIe–IIg**. Furthermore, the substituents of 1-*O* position of oridonin with acetyl functional group displayed more potent anti-tumor activity than those with propylsulfonyl group, such as compounds **Ia**, **Ib**, and **Ic** showed higher anti-tumor activity than the corresponding compounds **Id**, **Ig**, and **Ie**. However, the derivatives of oxidation of 1-hydroxyl of oridonin, **IIa** and **IIb**, showed slightly less potent anti-tumor activity than the substituents of 1-hydroxyl with acetyl group, **Ia** and **Ic**. So, it suggests that 1-hydroxyl of oridonin has obvious effects on its activity. Secondly, in two series of derivatives, compounds with substituent R^2 containing vinyl moiety, such as **Ib**, **Ig**, and **IIg**, exhibited stronger cytotoxicity. However, whether the high affinities of these derivatives leading to enhanced activity are induced by the conjugation effect of the vinyl group remains unknown and is needed to be further investigated.

In conclusion, a series of novel 1-*O*- and 14-*O*-derivatives of oridonin were synthesized and tested for cytotoxicity against six cancer cell lines (SW-480, BGC-7901, HL-60, BEL-7402, A549, and B16) by MTT assay in vitro. Within this series of compounds, **Ib** and **IIg** are the most potent with the IC_{50} values of 0.84 μM in HL-60 cell and 1.00 μM in BEL-7402 cell, respectively. Compounds **Ib** and **IIg** have stronger anti-tumor activity than oridonin and cyclophosphamide and slightly less potent anti-tumor activity than 5-fluorouracil in mice with H22 liver tumor and with B16 melanoma in vivo. The preliminary SAR of the target compounds was discussed based on the experimental data obtained. As a possible result of present findings, the 1-*O*- and 14-*O*-derivatives of oridonin as potential anticancer drug candidates may be used in clinic.[18] Further studies on the structure modification of oridonin and the mechanism of the derivatives are currently in progress and will be reported in due course.

Acknowledgments

This study was supported by grant from National Natural Science Fund (No. 30472083) and Key fund of Ministry of Education of China (No. 108069) for financial assistance.

References

1. Yang, J. Y.; Xu, J. Y.; Wu, X. M.; Hua, W. Y.. *Chin. J. Mode. Appl. Pharm.* **2007**, *24*, 277.
2. Fujita, E.; Fujita, T.; Katayama, H.; Shibuya, M. *J. Chem. Soc.* **1970**, *C*, 1674.
3. Yin, F.; Liang, J. Y.; Liu, J. *J. Chin. Pharm. Univ.* **2003**, *34*, 302.
4. Zhang, D. R.; Ren, T. C. *Chin. Pharm. J.* **2003**, *38*, 817.
5. Ran, Q.; Xu, J. Y.; Wu, X. M.; Hua, W. Y. *Pharm. Clin. Res.* **2007**, *15*, 91.
6. Nagao, Y.; Fujita, E.; Kohno, T.; Yagi, M. *Chem. Pharm. Bull.* **1981**, *29*, 3202.
7. Fujita, E.; Nagao, Y.; Kohno, T.; Matsuda, M.; Ozaki, M. *Chem. Pharm. Bull.* **1981**, *29*, 3208.
8. Yan, X. B.; Lei, M.; Zhang, J. Y.; Liu, H. M. *Chin. J. Org. Chem.* **2005**, *25*, 222.
9. Ikezoe, T.; Chen, S. S.; Tong, X. J. *Int. J. Oncol.* **2003**, *23*, 1187.
10. Zhang, C. L.; Wu, L. J.; Zuo, H. J.; Onodera, S.; Ikejima, T. *Chin. Pharm. Bull.* **2004**, *20*, 669.
11. Zhang, C. L.; Wu, L. J.; Tashiro, S. I.; Onodera, S.; Ikajima, T. *J. Asian Nat. Prod. Res.* **2004**, *6*, 127.
12. Liu, Y. Q.; Mu, Z. Q.; You, S.; Tashiro, S. I.; Onodera, S.; Ikajima, T. *Biol. Pharm. Bull.* **2006**, *29*, 1873.
13. Jin, S.; She, J. N.; Wang, J.; Huang, G.; Zhou, J. G. *Cancer Biol. Ther.* **2007**, *6*, e1.
14. Han, J.; Ye, M.; Qiao, X.; Wu, W. Y.; Qu, G. Q.; Guo, D. A. *J. Pharm. Sci.* **2007**, *16*, 307.
15. Li, D.; Wu, L. J.; Tashiro, S. I.; Onodera, S.; Ikejima, T. *J. Asian Nat. Prod. Res.* **2008**, *10*, 77.
16. *Analytical data for compound* ***Ib****: mp132–134* °C. IR (KBr, cm^{-1}): 3370, 3093, 2958, 2877, 1714, 1634, 1573, 1242. ^1H MNR ($CDCl_3$, 500 Hz): δ (ppm) 8.58 (1H, t), 6.50 (1H, d, *J* = 12.8 Hz), 6.35 (1H, d, *J* = 12.8 Hz), 6.17 (1H, s, H-17), 6.01 (1H, s, C-6-OH), 5.60 (1H, s, H-17), 4.62 (1H, d, *J* = 5.3 Hz, H-14), 4.26, 3.97 (each 1H, d, *J* = 10.3 Hz, H_2-20), 4.16 (2H, m), 3.78 (1H, d, *J* = 6.5 Hz, H-6), 3.42 (1H, m, H-1), 3.15 (1H, d, *J* = 9.8 Hz, H-13), 2.52 (1H, m, H-12), 2.00 (3H, s), 1.88 (1H, m, H-9), 1.14, 1.12 (each 3H, s, 2× CH_3). ESI-MS: 584 $[M+Na]^+$, 560 $([M-H]^-$. Anal. Calcd for $C_{28}H_{35}O_{11}N$: C, 59.89; H, 6.24; N, 2.50. Found: C, 59.78; H, 6.02; N, 2.63.
Analytical data for compound ***IIg****: mp148–150* °C. IR (KBr, cm^{-1}) 3387, 2961, 2878, 1712, 1637, 1560, 1213. ^1H MNR ($CDCl_3$, 500 Hz): δ (ppm) 7.73 (1H, d, *J* = 7.3 Hz), 7.22 (3H, m), 7.13 (2H, m), 6.29 (2H, m), 6.20 (1H, s, H-17), 5.97 (1H, s, C-6-OH), 5.59 (1H, s, H-17), 4.76 (1H, d, *J* = 6.0 Hz,), 4.28, 4.00 (each 1H, d, *J* = 10.6 Hz, H_2-20), 3.76 (1H, d, *J* = 6.8 Hz, H-6), 3.20, 3.08 (each 1H, dd), 2.94 (1H, d, *J* = 9.3 Hz, H-13), 2.43 (1H, m, H-12), 2.18 (1H, dd, *J* = 5.6 Hz, 12.8 Hz, H-9), 1.83 (4H, m, H-2, H-11, 12) 1.48 (2H, m, H-3), 1.17, 0.98 (each 3H, s, 2× CH_3). ESI-MS: 606 $[M-H]^-$. Anal. Calcd for $C_{33}H_{37}O_{10}N$: C, 65.24; H, 6.10; N, 2.31. Found: C, 65.46; H, 5.97; N 2.52.
17. Xu, S. Y.; Bian, R. L.; Chen, X. *Pharmacological Experimental Method*; People's Medical Publishing House: Beijing, 1991. pp 1424.
18. Xu, J. Y.; Wu, X. M.; Yang, J. Y.; Ran, Q.; Wang, L.; Wang, Z. X.; Yuan, S. T.; Zhang, L. Y.; Shen, M. Q. Chinese Patent Application: CN 10133915.X, 2007.