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Synthesis and Cytotoxicity of Dihydroartemisinin Ethers Containing Cyanoarylmethyl Group

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Abstract—A new type of ether of dihydroartemisinin containing cyano and aryl groups was prepared and tested for cytotoxicity to A549, P388, L1210 and HT29 cells using the MTT assay. **12k** and **12l** were the most cytotoxic compounds. **13** lacking the peroxy group showed a 1000-fold less potency than **12l**. Similarly, the inactive compound **14** indicated that the position of cyano groups was also important. Flow cytometry data showed that the compounds caused an accumulation of P388 cells in the G_1-phase of the cell cycle.

Introduction

Traditional Chinese medicine Qinghao (*Artemisia annua* L.) was recorded as a versatile remedy for malaria, dermatitis and other diseases in many Chinese ancient medical books. Based on these clinical experiences, Chinese scientists have developed artemisinin and its derivatives (artemisinin **1**, dihydroartemisinin **2**, artemether **3a** and artesunate **3b**) as a totally new type of antimalarial drugs since 1970s. Up to now, these drugs have been used in the clinic all over the world. Their quick onset, powerful effect and good toleration may be attributed to the 1,2,4-trioxane moieties in their molecules. A number of researchers have been focusing their attention on other components of the plant, artemisinin derivatives/analogues and their bioactivities.[1] More than 20 research papers reporting the cytotoxicity of artemisinin family were recently published.[2–21]

Some natural components of *A. annua* (such as artemisinin, arteannuin-B **4**, artemisinic acid **5,** artemisitene **6**, flavones and other terpenoids) showed cytotoxic properties at varying concentrations against L 1210, P 388, A 549, HT 29, MCF 7 and KB in vitro.[2,4,9]

Artemisinin and related compounds were tested against Ehrlich Ascites tumour cells, on which artemisinin, artemether, arteether and artesunate exhibited moderate cytotoxicity (IC_{50} 12.2–29.8 μM), artemisitene **6** was slightly more active (IC_{50} 6.8 μM), and a dimer of dihydroartemisinin **7a** was the most potent (IC_{50} 1.4μM). It was found the nonsymmetric dimer **7a** was more cytotoxic than the symmetric dimer **7b** due to their slight different stereochemistry.[5–7] Other artemisinin dimers, trimers and tetramers and their cytotoxicities was reported recently.[8]

The antitumor effect of artesunate was tested in vitro and in vivo in China.[10–12] It was shown to be cytotoxic for six cell lines (IC_{50} 1∼100 μg/mL) and displayed antitumor effects on human nasopharyngeal cancer (CNE2, SUNE-1) and human liver cancer (BEL-7402) grafted in nude mice. Recently artesunate has been analyzed for its antitumor activity against 55 cell lines.[13] Leukemia and colon cancer cell lines were the most sensitive lines, and no cross resistance against CEM leukemia sub-lines

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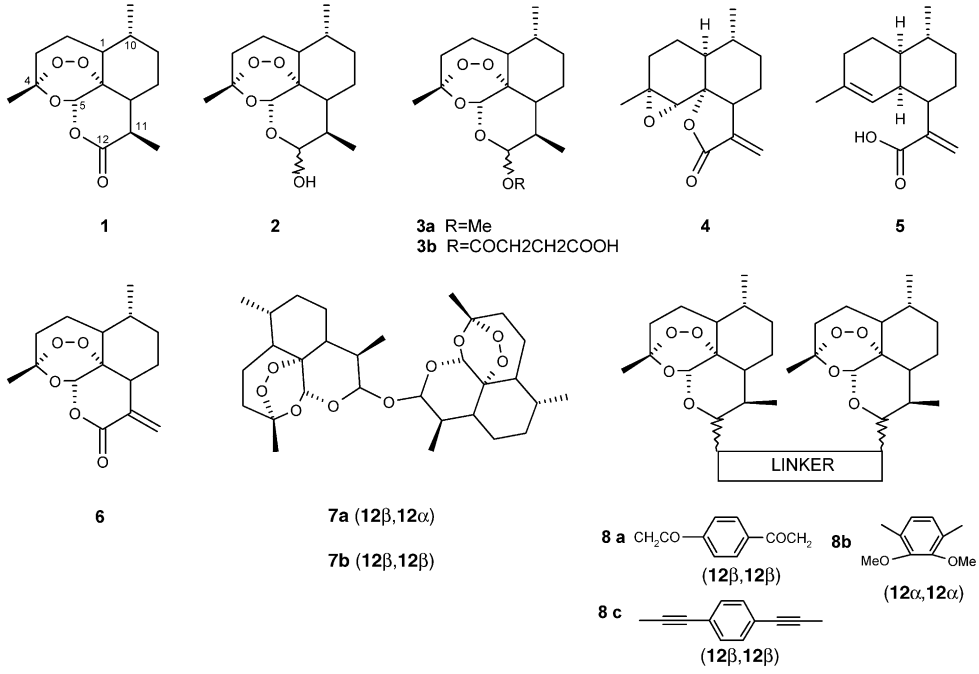


Chart 1.

which are resistant to either doxorubicin, vincristine, methotrexate or hydroxyurea were observed.

Lai's group found that dihydroartemisinin can selectively kill cancer cells in presence of holotransferrin which can increase intracellular iron concentrations, whereas normal breast cells (HTB 125) and lymphocytes were not affected under the same condition. It seems that the mechanisms underlying both antitumor and antimalarial properties are similar.[14–16]

Posner's group found some new artemisinin derivatives and trioxane dimers which had antimalarial, antiproliferative and antitumor activities. Dimer **8a**, **8b**, **8c** were especially potent and selective at inhibiting growth of some human cancer cell lines, and **8a** was evaluated in vivo assay (Chart 1).[17,18]

In order to search for new artemisinin derivatives with more potent antimalarial and antitumor activity, we synthesized compounds **9**, **10** and investigated their pharmacological properties. They showed lower antimalarial activity than artemether but stronger antitumor activity in vitro. The most active compound was **10** ($R = p\text{-}COOCH_3$, $IC_{50} = 1.91$, 0.33, 0.02 μM against KB, HCT-8, A 2780 cell lines, respectively).[19,20] Thereafter, we noted that artemisinin derivatives **11** and **12a** were so quite different in their antitumor activity. **12a** with a phenyl group was much more active than **11**. This finding led us to explore the structure–activity relationship of this kind of derivatives. Herein we report synthesis and preliminary biological evaluation of **12** and related compounds (Charts 2 and 3). The brief report has been published in 2001.

Results and Discussion

Chemistry

In the presence of an acidic catalyst, dihydroartemisinin reacted with corresponding cyanohydrin to

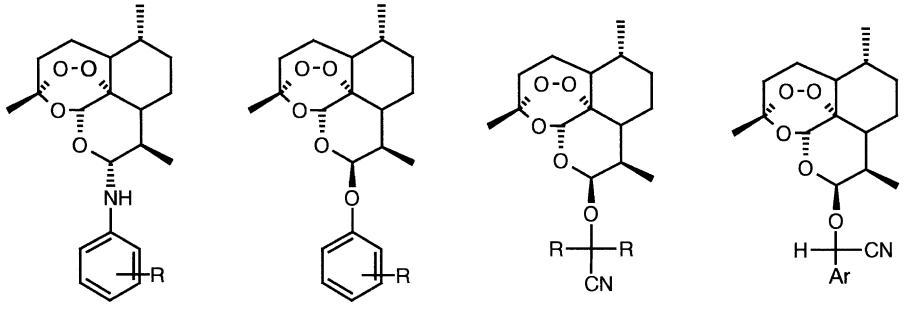


Chart 2.

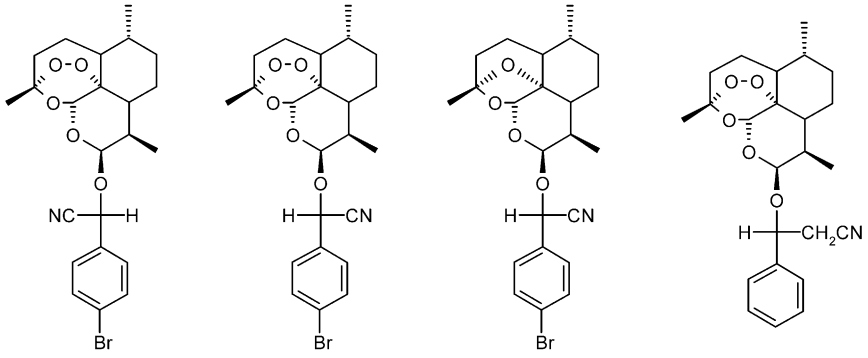


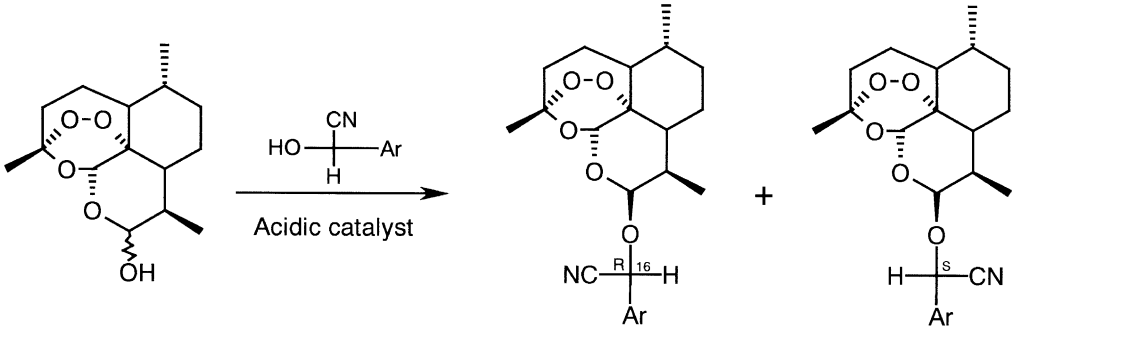
Chart 3.

give **12**(16-*R* and 16-*S*) in moderate yield. Generally, the pair of isomers could be separated by careful column chromatography. They are 12-β ethers as indicated by the small coupling constants ($J \sim 3.5$ Hz) between 11-H and 12-H in their ^{1}H NMR.[22] The R configuration of C-16 of **12k** was established by X-ray diffraction.[21] In the ^{1}H NMR spectra of **12k** and **12l**, the values of chemical shifts of C-12 and C-16 were distinguishable (for **12k** 5.22, 5.25; for **12l**, 4.79, 5.46 respectively). Taking these data as a criterion, the stereochemistry of other isomers could be deduced. When dihydroartemisinin reacted with α-hydroxy-2-naphthylacetonitrile, three compound were yielded, normal products **12s**, **12t** and byproduct **12u**, in which the signal due to 11α-methyl group appeared downfield (δ 1.30) as compared with that of the 11β-methyl group in **12s** (δ 1.04) and in **12t** (δ 0.96) in their ^{1}H NMR spectra (Scheme 1).[23]

In order to examine the role of peroxy group and cyano group, we synthesized compound **13** and **14**. **13** was derived from **12l** by reduction with zinc powder in acetic acid[21] and **14** was prepared by condensation of dihydroartemisinin and 3-hydroxy-3-phenyl-propionitrile (Scheme 2).

Biological results

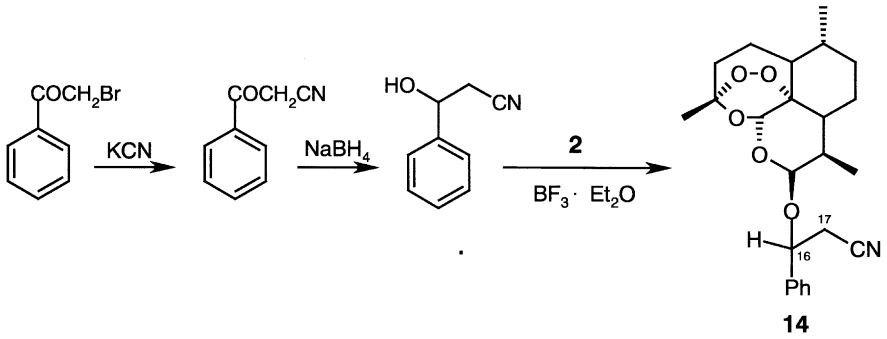
Cytotoxicity and effect on the cell cycle distribution were measured according to the procedures described



12a Ar = C_6H_5
12h Ar = 2-Br-C_6H_4
12j Ar = 3-Br-C_6H_4
12k Ar = 4-Br-C_6H_4
12n Ar = 2,4-$(OMe)_2$-C_6H_3
12o Ar = 3,4-$(OMe)_2$-C_6H_3
12q Ar = 1-naphthyl
12s Ar = 2-naphthyl
12u Ar = 2-naphthyl (11S)
12v Ar = 3-C_6H_4O- C_6H_4-CF_3
12x Ar = 9-anthryl (R / S)

12b Ar = C_6H_5
12c Ar = 2-F-C_6H_4
12d Ar = 4-F-C_6H_4
12e Ar = 2-Cl-C_6H_4
12f Ar = 4-Cl-C_6H_4
12g Ar = 2-Br-C_6H_4
12i Ar = 3-Br-C_6H_4
12l Ar = 4-Br-C_6H_4
12m Ar = 2,4-$(OMe)_2$-C_6H_3
12p Ar = 2,4-Me_2-C_6H_3
12r Ar = 1-naphthyl
12t Ar = 2-naphthyl
12w Ar = 3-C_6H_4O- C_6H_4-CF_3

Scheme 1.



Scheme 2.

previously.[21] VCR was used as a reference compound with IC_{50} values of 18 and 2 nM for A549 and P388 cells, respectively. Table 1 showed compound **12** to be cytotoxic against L1210, A549, P388 and HT29 cells. At the concentration of 25- 500 nM, they induced a G_1 accumulation in L1210 cells and apoptosis in P388 cells (see Table 1).

Conclusion

From these data, the following conclusion in terms of structure–activity relationship can be drawn:

1. Compound **12** was more toxic against A549, P388, L1210 cells than HT29 cells.
2. Among the compound **12** series, **12g**, **12h**, **12i**, **12j**, **12k**, and **12l** bearing Br substituent on the phenyl ring were more active than others. **12k** and **12l** were the most active and their IC_{50} could be comparable to that of VCR, a very potent cytotoxic agent.
3. From Table 1 (**12k** vs **12l**, **12m** vs **12n**, **12s** vs **12t**, **12v** vs **12w**), it can be seen that the configuration of C-16 had non-significant effect on the cytotoxicity, cell cycle and apoptosis.
4. Compound **12l** was 1000-fold more cytotoxic than **13**. The peroxy group is clearly essential for cytotoxicity.[4,6]
5. Compound **14** was devoid of effect on cell proliferation. This lack of cytotoxicity suggested that the cyanohydrin moiety must be present in the molecule. The activity of compound **12** may be due partly to its degradation products arylcyanohydrin. So far as we know, mandelonitrile glucosides having some similarity with the chemical structure of compound **12** were studied in cancer research and cancer theraphy.[24]

Experimental

Melting points were taken in open capillary on BUCHI-510 melting point apparatus and were uncorrected. The

Table 1. Inhibition of cell proliferation, perturbation of the cell cycle and induction of apoptosis by compounds **12, 13**

No.	IC_{50} (nM)				Cell-cycle effect L1210	Cell-cycle apoptosis P388		
	L1210	A549	P388	HT29	% G1 (nM)	% G1	% apoptose	nM
11		79,432	1855			65	7	10,000
12a	113	1227	238	4095	69 (400)	67	53	500
12b	34	662	48	2213		61	45	100
12c	38	364	43	447		72	43	200
12d	41	306	77	1471		71	51	250
12e	470	10,600	290	16,200	68 (1000)			
12f	620	8800	440	7400	67 (2500)			
12g	27	193	28	9638		70	46	100
12h	150	741	92	809	71 (500)			
12i	19	85	20	7788		72	58	100
12j	151	437	108	748	68 (500)			
12k	15	47	12	179		62	49	25
12l	10	39	11	490		63	51	25
12m			105			68	20	500
12n			152			68	16	500
12o	17	221	13	1923		64	49	50
12p			84			71	28	500
12s			70			70	24	250
12t			76			71	26	250
12v	76	172	61	441	68 (250)	70	ND	250
12w	67	140	45	414	67 (250)	69	ND	250
12x			71			70	28	250
13		41,990	24,200					
14		Inactive	Inactive					
Control						42	4	

IR spectra were run on a Perkin-Elmer 599B spectrophotometer. ^{1}H NMR spectra were determined in $CDCl_3$ solution on a NMR Brucker AM-400. Elemental analyses were performed on a CE 1106 elemental analyser and all the results were within 0.4% of the theoretical values.

General procedure: synthesis of cyano-aryl-methyl 12β-deoxoartemisinyl ether (12)

To a dichloromethane solution of dihydroartemisinin (1.42 g, 5 mmol) and a 1.5 to 2-fold excess of a cyanohydrin fresh prepared from arylaldehyde at 0 to −20 °C was added trimethylsilyl trifluoromethanesulfonate or boron trifluoride etherate (0.05–0.1 mL). The resulting mixture was stirred at room temperature for 24 h, and then washed with aqueous sodium bicarbonate and brine. The organic layer was dried ($MgSO_4$) and concentrated in vacuo to give the crude products. Pure **12**(16-*R*) and **12**(16-*S*) were obtained by column chromatography (silica gel) using petroleum ether–ethyl acetate (100: 3∼5 v/v) as eluent.

(16-*R*) Cyano-phenyl-methyl 12β-deoxoartemisinyl ether (12a) and (16-*S*) cyano- phenyl-methyl 12β-deoxoartemisinyl ether (12b). 12a: white crystal, mp 135–137 °C (from ethyl ether), yield 22%, ^{1}H NMR (400 MHz, δ, ppm, $CDCl_3$): 7.44 (5H, m, aromatic–H); 5.68 (1H, s, 5-H); 5.28 (1H, s, 16-H); 5.24 (1H, d, J = 3.6 Hz, 12-H); 1.43 (3H, s, 4-CH_3); 1.01 (3H, d, J = 7.3 Hz, 11CH_3), 0.87 (3H, d, J = 6.0 Hz, 10-CH_3), IR (KBr, cm^{-1}): 1458, 1375, 1103, 1011, 875, 700. Anal. calcd for $C_{23}H_{29}NO_5$: C 69.15, H 7.32, N 3.51; found: C 68.86, H 7.53, N 3.33.

12b: white crystal, mp 98–100 °C (from ethyl ether), yield 15%, ^{1}H NMR (400 MHz, δ, ppm, $CDCl_3$): 7.42 (5H, m, aromatic–H); 5.60 (1H, s, 5-H); 5.50 (1H, s, 16-H); 4.81 (1H, d, J = 3.5 Hz, 12-H); 1.44 (3H, s, 4-CH_3); 0.95 (3H, d, J = 6.6 Hz, 11-CH_3), 0.84 (3H, d, J = 7.5 Hz, 10-CH_3), IR (KBr, cm^{-1}): 1452, 1377, 1192, 1101, 878, 743. Anal. calcd for $C_{23}H_{29}NO_5$: C 69.15, H 7.32, N 3.51; found: C 68.85, H 7.42, N 3.18.

(16-S) Cyano-(*o*-fluoro-phenyl)-methyl 12β-deoxoartemisinyl ether (12c). 12c: white crystal, mp 147–148 °C (from ethyl acetate–petroleum ether), yield 29%, ^{1}H NMR (400 MHz, δ, ppm, $CDCl_3$): 7.53 (1H, t, J = 7.6 Hz, aromatic–H); 7.38 (1H, m, aromatic–H); 7.19 (1H, t, J = 7.3 Hz, aromatic–H); 7.09 (1H, t, J = 9.5 Hz, aromatic–H); 5.70 (1H, s, 5-H); 5.61 (1H, s, 16-H); 4.90 (1H, d, J = 3.5 Hz, 12-H); 1.38 (3H, s, 4-CH_3); 0.85 (3H, d, J = 6.4 Hz, 11-CH_3); 0.81 (3H, d, J = 7.5 Hz, 10-CH_3). Anal. calcd for $C_{23}H_{28}FNO_5$: C 66.17, H 6.76, N 3.36; found: C 66.07, H 6.78, N 3.35.

(16-*S*) Cyano-(*p*-fluoro-phenyl)-methyl 12β-deoxoartemisinyl ether (12d). 12d: white crystal, mp 146–147 °C (from ethyl acetate–petroleum ether), yield 24%, ^{1}H NMR (400 MHz, δ, ppm, $CDCl_3$): 7.43 (1H, d, J = 8.6 Hz, aromatic-H); 7.42 (1H, d, J = 8.6 Hz, aromatic-H); 7.09 (1H, t, J = 8.6 Hz, aromatic–H); 7.08 (1H, t, J = 8.5 Hz, aromatic–H); 5.57 (1H, s, 5-H); 5.47 (1H, s, 16-H); 4.78 (1H, d, J = 3.5 Hz, 12-H); 1.41 (3H, s, 4-CH_3); 0.88 (3H, d, J = 6.7 Hz, 11-CH_3); 0.82 (3H, d, J = 7.5 Hz, 10-CH_3). Anal. calcd for $C_{23}H_{28}FNO_5$: C 66.17, H 6.76, N 3.36, found: C 66.16, H 6.74, N 3.33.

(16-*S*) Cyano-(*o*-chloro-phenyl)-methyl 12β-deoxoartemisinyl ether (12e). 12e: white crystal, mp 118–120 °C (from ethyl acetate–petroleum ether), yield 27%, ^{1}H NMR (400 MHz, δ, ppm, $CDCl_3$): 7.63 (1H, m, aromatic–H); 7.42 (1H, m, aromatic–H); 7.36 (2H, m, aromatic–H); 5.76 (1H, s, 5-H); 5.66 (1H, s, 16-H); 5.01 (1H, d, J = 3.5 Hz, 12-H); 1.45 (3H, s, 4-CH_3); 0.96 (3H, d, J = 6.4 Hz, 11-CH_3); 0.88 (3H, d, J = 7.2 Hz, 10-CH_3). Anal. calcd for $C_{23}H_{28}ClNO_5$: C 63.66, H 6.50, N 3.23; found: C 63.42, H 6.29, N 3.08.

(16-*S*) Cyano-(*p*-chloro-phenyl)-methyl 12β-deoxoartemisinyl ether (12f). 12f: white crystal, mp 126–128 °C (from ethyl acetate–petroleum ether), yield 39%, ^{1}H NMR (400 MHz, δ, ppm, $CDCl_3$): 7.51 (2H, d, J = 8.5 Hz, aromatic–H); 7.24 (2H, d, J = 8.5 Hz, aromatic-H); 5.57 (1H, s, 5-H); 5.46 (1H, s, 16-H); 4.78 (1H, d, J = 3.5 Hz, 12-H); 1.42 (3H, s, 4-CH_3); 0.98 (3H, d, J = 6.5 Hz, 11-CH_3); 0.81 (3H, d, J = 7.4 Hz, 10-CH_3). Anal. calcd for $C_{23}H_{28}ClNO_5$: C 63.66, H 6.50, N 3.23; found: C 63.47, H 6.37, N 3.11.

(16-*S*) Cyano-(*o*-bromo-phenyl)-methyl 12β-deoxoartemisinyl ether (12g). 12g: white needle crystal, mp 128–129 °C (from ethyl acetate–petroleum ether), yield 18%, ^{1}H NMR (400 MHz, δ, ppm, $CDCl_3$): 7.65 (1H, d, J = 7.5 Hz, aromatic–H); 7.63 (1H, d, J = 7.8 Hz, aromatic–H); 7.39 (1H, t, J = 7.5 Hz, aromatic–H); 7.25 (1H, t, J = 7.8 Hz, aromatic–H); 5.73 (1H, s, 5-H); 5.66 (1H, s, 16-H); 5.04 (1H, d, J = 3.5 Hz, 12-H); 1.45 (3H, s, 4-CH_3); 0.96 (3H, d, J = 6.7 Hz, 11-CH_3); 0.89 (3H, d, J = 7.5 Hz, 10-CH_3). IR (KBr, cm^{-1}): 1571, 1470, 1446, 1377, 1194, 1101, 1032, 982, 931, 878. Anal. calcd for $C_{23}H_{28}BrNO_5$: C 57.75, H 5.90, N 2.93; found: C 57.89, H 5.92, N 2.99.

(16-*R*) Cyano-(*o*-bromo-phenyl)-methyl 12β-deoxoartemisinyl ether (12h). 12h: amorphous, yield 20%, ^{1}H NMR (400 MHz, δ, ppm, $CDCl_3$): 7.66 (1H, d, J = 7.8 Hz, aromatic–H); 7.65 (1H, d, J = 7.5 Hz, aromatic–H); 7.41 (1H, d, J = 8.0 Hz, aromatic–H); 7.25 (1H, d, J = 8.0 Hz, aromatic–H); 5.99 (1H, s, 5-H); 5.43 (1H, s, 16-H); 5.30 (1H, d, J = 3.7 Hz, 12-H); 1.43 (3H, s, 4-CH_3); 1.01 (3H, d, J = 5.6 Hz, 11-CH_3); 0.89 (3H, d, J = 5.8 Hz, 10-CH_3). IR (KBr, cm^{-1}): 1471, 1448, 1375, 1446, 1194, 1101, 1009, 875. Anal. calcd for $C_{23}H_{28}BrNO_5$: C 57.75, H 5.90, N 2.93; found: C 57.84, H 5.83, N 2.82.

(16-*S*) Cyano-(*m*-bromo-phenyl)-methyl 12β-deoxoartemisinyl ether (12i). 12i: white crystal, mp 145–146 °C (from ethyl acetate–petroleum ether), yield 19%, ^{1}H NMR (400 MHz, δ, ppm, $CDCl_3$): 7.59 (1H, s, aromatic–H); 7.54 (1H, d, J = 7.9 Hz, aromatic–H); 7.38 (1H, d, J = 7.8 Hz, aromatic–H); 7.28 (1H, t, J = 7.8 Hz, aromatic–H); 5.58 (1H, s, 5-H); 5.47 (1H, s, 16-H); 4.81 (1H, d, J = 3.5 Hz, 12-H); 1.44 (3H, s, 4-CH_3); 0.96 (3H, d, J = 6.4 Hz, 11-CH_3); 0.87 (3H, d, J = 7.2 Hz, 10-CH_3). IR (KBr, cm^{-1}): 1576, 1473, 1433, 1379, 1194, 1101, 1036, 984, 957, 874. Anal. calcd for $C_{23}H_{28}BrNO_5$: C 57.75, H 5.90, N 2.93; found: C 57.79, H 5.90, N 2.99.

(16-*R*) Cyano-(*o*-bromo-phenyl)-methyl 12β-deoxoartemisinyl ether (12j). 12j: amorphous, yield 22%, ^{1}H NMR (400 MHz, δ, ppm, $CDCl_3$): 7.61 (1H, s, aromatic–H); 7.55 (1H, d, $J=7.9$ Hz, aromatic–H); 7.39 (1H, d, $J=7.8$ Hz, aromatic–H); 7.31 (1H, t, $J=7.8$ Hz, aromatic–H); 5.64 (1H, s, 5-H); 5.26 (1H, s, 16-H); 5.23 (1H, d, $J=3.7$ Hz, 12-H); 1.43 (3H, s, 4-CH_3); 1.01 (3H, d, $J=7.5$ Hz, 11-CH_3); 0.90 (3H, d, $J=6.0$ Hz, 10-CH_3). IR (KBr, cm^{-1}): 1574, 1475, 1377, 1446, 1194, 1103, 1013, 874. Anal. calcd for $C_{23}H_{28}BrNO_5$: C 57.75, H 5.90, N 2.93; found: C 57.94, H 5.92, N 2.92.

(16-*R*) Cyano-(*p*-bromo-phenyl)-methyl 12β-deoxoartemisinyl ether (12k). 12k: white crystal, mp 128–129 °C (from ethyl acetate–petroleum ether), yield 23%, ^{1}H NMR (400 MHz, δ, ppm, $CDCl_3$): 7.57 (2H, d, $J=8.5$ Hz, aromatic–H); 7.33 (2H, d, $J=8.5$ Hz, aromatic–H); 5.63 (1H, s, 5-H); 5.25 (1H, s, 16-H); 5.22 (1H, d, $J=3.7$ Hz, 12-H); 1.43 (3H, s, 4-CH_3); 1.00 (3H, d, $J=7.4$ Hz, 11-CH_3); 0.90 (3H, d, $J=6.1$ Hz, 10-CH_3). IR (KBr, cm^{-1}): 1593, 1489, 1375, 1101, 1032, 1011, 955, 876. Anal. calcd for $C_{23}H_{28}BrNO_5$: C 57.75, H 5.90, N 2.93; found: C 57.80, H 6.07, N 2.85.

(16-*S*) Cyano-(*p*-bromo-phenyl)-methyl 12β-deoxoartemisinyl ether (12l). 12l: white needle crystal, mp 144–145 °C (from petroleum ether), yield 25%, ^{1}H NMR (400 MHz, δ, ppm, $CDCl_3$): 7.55 (2H, d, $J=8.3$ Hz, aromatic–H); 7.32 (2H, d, $J=8.4$ Hz, aromatic–H); 5.58 (1H, s, 5-H); 5.46 (1H, s, 16-H); 4.79 (1H, d, $J=3.4$ Hz, 12-H); 1.44 (3H, s, 4-CH_3); 0.96 (3H, d, $J=6.3$ Hz, 11-CH_3); 0.85 (3H, d, $J=7.3$ Hz, 10-CH_3). IR (KBr, cm^{-1}): 1593, 1487, 1394, 1144, 1103, 1036, 955, 879. Anal. calcd for $C_{23}H_{28}BrNO_5$: C 57.75, H 5.90, N 2.93; found: C 57.80, H 5.89, N 2.96.

(16-*S*) Cyano-(o, *p*-dimethoxy-phenyl)-methyl 12β-deoxoartemisinyl ether (12m). 12m: white crystal, mp 149–150 °C (from ethyl acetate–petroleum ether), yield 20%, ^{1}H NMR (400 MHz, δ, ppm, $CDCl_3$): 7.40 (1H, d, $J=8.5$ Hz, aromatic–H); 6.49 (1H, d, $J=8.4$ Hz, aromatic–H); 6.43 (1H, s, aromatic–H); 5.71 (1H, s, 5-H); 5.63 (1H, s, 16-H); 4.91 (1H, d, $J=3.5$ Hz, 12-H); 3.81 (3H, s, OCH_3); 3.80 (3H, s, OCH_3); 1.45 (3H, s, 4-CH_3); 0.95 (3H, d, $J=6.4$ Hz, 11-CH_3); 0.81 (3H, d, $J=7.5$ Hz, 10-CH_3). IR (KBr, cm^{-1}): 1616, 1585, 1508, 1464, 1265, 1209, 1103, 1099, 987, 955, 937, 874. Anal. calcd for $C_{25}H_{33}NO_7$: C 65.34, H 7.24, N 3.05; found: C 65.48, H 7.29, N 3.11.

(16-*R*) Cyano-(o, *p*-dimethoxy-phenyl)-methyl 12β-deoxoartemisinyl ether (12n). 12n: white crystal, mp 149–152 °C (from ethyl acetate–petroleum ether), yield 22%, ^{1}H NMR (400 MHz, δ, ppm, $CDCl_3$): 7.46 (1H, d, $J=8.4$ Hz, aromatic–H); 6.51 (1H, d, $J=8.4$ Hz, aromatic–H); 6.45 (1H, s, aromatic–H); 5.98 (1H, s, 5-H); 5.40 (1H, s, 16-H); 5.21 (1H, d, $J=3.4$ Hz, 12-H); 3.83 (3H, s, OCH_3); 3.81 (3H, s, OCH_3); 1.44 (3H, s, 4-CH_3); 0.97 (3H, d, $J=7.4$ Hz, 11-CH_3); 0.90 (3H, d, $J=6.0$ Hz, 10-CH_3). IR (KBr, cm^{-1}): 1616, 1589, 1510, 1464, 1284, 1211, 1126, 1097, 1040, 995, 957, 870. Anal. calcd for $C_{25}H_{33}NO_7$: C 65.34, H 7.24, N 3.05; found: C 65.41, H 7.15, N 3.05.

(16-*R*) Cyano-(m, *p*-dimethoxy-phenyl)-methyl 12β-deoxoartemisinyl ether (12o). 12o oil, yield 25%, ^{1}H NMR (400 MHz, δ, ppm, $CDCl_3$): 7.02 (1H, d, $J=8.4$ Hz, aromatic–H); 6.95 (1H, s, aromatic–H); 6.88 (1H, d, $J=8.1$ Hz, aromatic–H); 5.60 (1H, s, 5-H); 5.26 (1H, s, 16-H); 5.20 (1H, d, $J=3.6$ Hz, 12-H); 3.83 (3H, s, OMe); 3.81 (3H, s, OMe); 1.43 (3H, s, 4-CH_3); 1.00 (3H, d, $J=7.4$ Hz, 11-CH_3); 0.88 (3H, d, $J=6.2$ Hz, 10-CH_3). IR (KBr, cm^{-1}): 1684, 1597, 1518, 1456, 1100, 1040, 950, 875. Anal. calcd for $C_{25}H_{33}NO_7$: C 65.34, H 7.24, N 3.05; found: C 65.45, H 7.22, N 2.89.

(16-*S*) Cyano-(*o*, *p*-dimethyl-phenyl)-methyl 12β-deoxoartemisinyl ether (12p). 12p: oil, yield 64%, ^{1}H NMR (400 MHz,δ, ppm, $CDCl_3$): 7.41 (1H, d, $J=7.7$ Hz, aromatic–H); 7.04 (1H, d, $J=7.7$ Hz, aromatic–H); 7.03 (1H, s, aromatic–H); 5.77 (1H, s, 5-H); 5.46 (1H, d, $J=5.4$ Hz, 12-H); 5.38 (1H, s, 16-H); 2.41 (3H, s, Me); 2.31 (3H, s, Me); 1.45 (3H, s, 4-CH_3); 1.23 (3H, d, $J=7.2$ Hz, 11-CH_3); 0.91 (3H, d, $J=6.0$ Hz, 10-CH_3). IR (KBr, cm^{-1}): 1616, 1502, 1452, 1377, 1194, 1101, 1010, 956, 875. Anal. calcd for $C_{25}H_{33}NO_5$: C 70.23, H 7.78, N 3.28; found: C 70.37, H 7.58, N 3.19.

(16-*R*) Cyano-(1′-naphthyl)-methyl 12β-deoxoartemisinyl ether (12q) and (16-*S*) cyano- (1′-naphthyl)-methyl 12β-deoxoartemisinyl ether (12r). 12q: amorphous, yield 24%, ^{1}H NMR (400 MHz,δ, ppm, $CDCl_3$): 8.17 (1H, d, $J=8.6$ Hz aromatic–H); 7.92 (1H, t, $J=7.1$ Hz, aromatic–H); 7.75 (1H, d, $J=7.0$ Hz, aromatic–H); 7.59 (3H, m, aromatic–H); 7.51 (1H, t, $J=7.7$ Hz, aromatic–H); 6.26 (1H, s, 5-H); 5.39 (1H, d, $J=3.7$ Hz, 12-H); 5.30 (1H, s, 16-H); 1.47 (3H, s, 4-CH_3); 1.09 (3H, d, $J=7.3$ Hz, 11-CH_3), 0.82 (3H, d, $J=6.2$ Hz, 10-CH_3). IR (KBr, cm^{-1}): 1512, 1450, 1377, 1194, 1101, 1009, 955, 876. Anal. calcd for $C_{27}H_{31}NO_5$: C 72.14, H 6.95, N 3.12; found: C 72.47, H 7.33, N 2.99.

12r: amorphous, yield 15%, ^{1}H NMR (400 MHz, δ, ppm, $CDCl_3$): 8.19 (1H, d, $J=8.2$ Hz, aromatic–H); 7.92 (1H, t, $J=6.0$ Hz, aromatic–H); 7.60 (4H, m, aromatic–H); 7.48 (1H, t, $J=4.1$ Hz, aromatic–H); 6.03 (1H, s, 5-H); 5.70 (1H, s, 16-H); 4.86 (1H, d, $J=3.6$ Hz, 12-H); 1.48 (3H, s, 4- CH_3); 0.99 (3H, d, $J=6.3$ Hz, 11-CH_3), 0.71 (3H, d, $J=7.4$ Hz, 10-CH_3). IR (KBr, cm^{-1}): 1512, 1452, 1373, 1194, 1101, 1011, 986, 876. Anal. calcd for $C_{27}H_{31}NO_5$: C 72.14, H 6.95, N 3.12; found: C 72.27, H 6.92, N 3.08.

(16-*R*) Cyano-(2′-naphthyl)-methyl 12β-deoxoartemisinyl ether (12s), (16-*S*) cyano- (2′-naphthyl)-methyl 12β-deoxoartemisinyl ether (12t) and (16-*R*) cyano- (2′-naphthyl)-methyl 12β-(11*S*) deoxoartemisinyl ether (12u). 12s: amorphous, yield 25%, ^{1}H NMR (400 MHz, δ, ppm, $CDCl_3$): 7.97 (1H, s, aromatic–H); 7.90 (3H, m, aromatic–H); 7.54 (3H, m, aromatic–H); 5.84 (1H, s, 5-H); 5.34 (1H, s, 16-H); 5.30 (1H, d, $J=3.6$ Hz, 12-H); 1.45 (3H, s, 4-CH_3); 1.04 (3H, d, $J=7.3$ Hz, 11-CH_3), 0.86 (3H, d, $J=6.2$ Hz, 10-CH_3). IR (KBr, cm^{-1}): 1602, 1510, 1450, 1377, 1194, 1101, 1011, 955, 939, 876, 825. Anal. calcd for $C_{27}H_{31}NO_5$: C 72.14, H 6.95, N 3.12, found: C 72.20, H 6.74, N 3.19.

12t: amorphous, yield 26%, ^{1}H NMR (400 MHz, δ, ppm, $CDCl_3$): 7.91 (1H, s, aromatic–H); 7.86 (3H, m, aromatic–H); 7.54 (3H, m, aromatic–H); 5.67 (1H, s, 5-H); 5.64 (1H, s, 16-H); 4.86 (1H, d, $J=3.5$ Hz, 12-H); 1.46 (3H, s, 4-CH_3); 0.96 (3H, d, $J=6.3$ Hz, 11-CH_3), 0.84 (3H, d, $J=7.2$ Hz, 10-CH_3). IR (KBr, cm^{-1}): 1602, 1510, 1450, 1377, 1280, 1226, 1194, 1101, 1032, 955, 937, 876, 825. Anal. calcd for $C_{27}H_{31}NO_5$: C 72.14, H 6.95, N 3.12; found: C 71.96, H 6.90, N 3.28.

12u: white crystal, mp 144–146 °C, yield 5%, ^{1}H NMR (400 MHz, δ, ppm, $CDCl_3$): 8.01 (1H, s, aromatic–H); 7.91–7.84 (3H, m, aromatic–H); 7.58 (1H, d, $J=8.5$ Hz aromatic–H); 7.52 (2H, m aromatic–H); 5.88 (1H, s, 5-H); 5.50 (1H, d, $J=4.3$ Hz, 12-H); 5.45 (1H, s, 16-H); 1.47 (3H, s, 4-CH_3); 1.30 (3H, d, $J=6.2$ Hz, 11-CH_3), 0.91 (3H, d, $J=6.1$ Hz, 10-CH_3). IR (KBr, cm^{-1}): 1604, 1510, 1458, 1377, 1203, 1126, 1090, 1003, 885. Anal. calcd for $C_{27}H_{31}NO_5$: C 72.14, H 6.95, N 3.12; found: C 72.01, H 6.78, N 3.12.

(16-*R*) Cyano-[*m*-(3′-trifluorophenoxy)-phenyl]-methyl 12β-deoxoartemisiny ether (12v) and (16-*S*) cyano-[*m*-(3′-trifluorophenoxy)-phenyl]-methyl 12β-deoxo-artemisiny ether (12w). 12v: amorphous, yield 23%, ^{1}H NMR (400 MHz, δ, ppm, $CDCl_3$): 7.46–7.37 (4H, m, aromatic–H); 7.21 (1H, s, aromatic–H); 7.17 (1H, d, $J=7.8$ Hz, aromatic–H); 7.08 (1H, s, aromatic–H); 7.05 (1H, d, $J=8.1$ Hz, aromatic–H); 5.57 (1H, s, 5-H); 5.48 (1H, s, 16-H); 4.82 (1H, d, $J=3.2$ Hz, 12-H) ;1.43 (3H, s, 4-CH_3); 0.95 (3H, d, $J=6.2$ Hz, 11-CH_3); 0.80 (3H, d, $J=7.3$ Hz, 10-CH_3). IR (KBr, cm^{-1}): 1589, 1489, 1450, 1329, 1130, 876. Anal. calcd for $C_{30}H_{32}F_3NO_6$: C 64.39, H 5.76, N 2.50; found: C 64.32, H 5.77, N 2.50.

12w: amorphous, yield 22%, ^{1}H NMR (400 MHz, δ, ppm, $CDCl_3$): 7.47–7.37 (4H, m, aromatic–H); 7.25 (1H, s, aromatic–H); 7.16 (1H, d, $J=7.9$ Hz, aromatic–H); 7.12 (1H, s, aromatic–H); 7.06 (1H, d, $J=7.7$ Hz, aromatic–H); 5.67 (1H, s, 5-H); 5.24 (1H, s, 16-H); 5.23 (1H, d, $J=3.0$ Hz, 12-H); 1.42 (3H, s, 4-CH_3); 0.98 (3H, d, $J=7.3$ Hz, 11-CH_3); 0.89 (3H, d, $J=6.2$ Hz, 10-CH_3). IR (KBr, cm^{-1}): 1589, 1489, 1450, 1329, 1130, 876. Anal. calcd for $C_{30}H_{32}F_3NO_6$: C 64.39, H 5.76, N 2.50; found: C 64.54, H 5.81, N 2.38.

(16-*R*/*S*) Cyano-(9-anthryl)-methyl 12β-deoxoartemisinyl ether (12x). 12x: white crystal, mp 184–186 °C (from dichloromethane–petroleum ether), yield 15%, ^{1}H NMR (400 MHz, δ, ppm, $CDCl_3$): 8.56 (1H, s, aromatic–H); 8.51 (2H, d, $J=8.0$ Hz, aromatic–H); 8.04 (2H, d, $J=8.4$ Hz, aromatic–H); 7.61 (2H, t, $J=7.2$ Hz, aromatic–H); 7.50 (2H, t, $J=7.3$ Hz, aromatic–H); 7.14, 7.07 (1H, s, s, 5-H); 5.78, 5.65 (1H, s, s, 16-H); 4.78, 4.63 (1H, d, d, $J=4.9$ Hz, $J=3.5$ Hz, 12-H); 1.49 (3H, s, 4-CH_3); 0.94 (3H, d, $J=6.6$ Hz, 11-CH_3); 0.46 (3H, d, $J=7.4$ Hz, 10-CH_3). IR (KBr, cm^{-1}): 1626, 1448, 1373, 1192, 1099, 1007, 980, 878. Anal. calcd for $C_{31}H_{33}NO_5$: C 74.53, H 6.66, N 2.80; found: C 74.42, H 6.48, N 2.79.

(16-*R*) Cyano-(*p*-bromo-phenyl)-methyl 12β-deoxo-deoxy-artemisinyl ether (13). To a stirring solution of **12l** (100 mg, 0.21 mmole) in 5 mL of glacial acetic acid at 0 °C was added Zn powder (100 mg). Then the mixture was stirred at room temperature for 2 h. Iced water was added and the mixture was extracted with chloroform. The organic layer was washed with aqueous sodium bicarbonate and brine. The organic layer was dried ($MgSO_4$) and concentrated in vacuo. The crude product was recrystallized to give pure **13** (58 mg, yield 60%).

13: white crystal, mp 122–122.5 °C (from ethyl acetate–petroleum ether), ^{1}H NMR (400 MHz, δ, ppm, $CDCl_3$): 7.54 (2H, d, $J=8.5$ Hz, aromatic–H); 7.31 (2H, d, $J=8.5$ Hz, aromatic–H); 5.47 (1H, s, 5-H); 5.34 (1H, s, 16-H); 4.78 (1H, d, $J=4.1$ Hz, 12-H); 1.47 (3H, s, 4-CH_3); 0.91 (3H, d, $J=7.3$ Hz, 11-CH_3); 0.88 (3H, d, $J=6.4$ Hz, 10-CH_3). IR (KBr, cm^{-1}): 1593, 1487, 1394, 1211, 1103, 930, 868. Anal. calcd for $C_{23}H_{28}BrNO_4$: C 59.74, H 6.10, N 3.03; found: C 59.85; H 5.78, N 3.31.

(Cyanomethyl)-benzyl 12β-deoxoartemisinyl ether (14). Dihydroartemisinin (200 mg, 0.7 mmol) reacted with 3-hydroxy-3-phenyl-propionitrile using boron trifluoride etherate as a catalyst, according to the general procedure. The product was a mixture of 16*R* and 16*S* isomers (amorphous, 84 mg, yield 29%).

^{1}H NMR (400 MHz, δ, ppm, $CDCl_3$): 7.38 (5H, m, aromatic–H); 5.74, 5.63 (1H, s, s, 5-H); 5.19, 5.17 (1H, m, 16-H); 4.79, 4.65 (1H, d, d, $J=3.7$ Hz, 12-H); 2.68 (2H, m, 17-H); 1.39 (3H, s, 4-CH_3); 0.94 (3H, d, $J=6.2$ Hz, 11-CH_3), 0.87 (3H, d, $J=7.3$ Hz, 10-CH_3). IR (KBr, cm^{-1}): 1456, 1375, 1194, 1101, 1013, 981, 875 Anal. calcd for $C_{24}H_{31}NO_5$: C 69.71, H 7.56 , N 3.39; found: C 69.52H 7.56 N 2.99.

References and Notes

1. Li, Y. *Med. Trop.* **1998**, *58*, 9.
2. Jung, M.; ElSohly, H. N.; McChesney, J. D. *Planta Med.* **1990**, *56*, 624.
3. Sun, W. S.; Han, J. X.; Yang, W. Y.; Deng, D. A.; Yue, X. F. *Acta Pharmacol. Sin.* **1992**, *13*, 541.
4. Zheng, G. Q. *Planta Med.* **1994**, *60*, 54.
5. Woerdenbag, H. J.; Moskai, T. A.; Pras, N.; Malingre, T. M.; El-Feraly, F. S.; Kampinga, H. H.; Konings, A. W. T. *J. Nat. Prod.* **1993**, *56*, 849.
6. Beekman, A. C.; Barentsen, A. R. W.; Woerdenbag, H. J.; Uden, W. V.; Pras, N.; Konings, A. W. T.; El-Feraly, F. S.; Galal, A. M.; Wikström, H. *J. Nat. Prod.* **1997**, *60*, 325.
7. Beekman, A. C.; Wierenga, P. K.; Woerdenbag, H. J.; Uden, W. V.; Pras, N.; Konings, A. W. T.; El-Feraly, F. S.; Galal, A. M.; Wikström, H. *Planta Med.* **1998**, *64*, 615.
8. Ekthawatchai, S.; Kamchonwongpaisan, S.; Kongsaeree, P.; Tarnchompoo, B.; Thebtaranonth, Y.; Yuthavong, Y. *J. Med. Chem.* **2001**, *44*, 4688.
9. Jung, M. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1091.
10. Yang, X. P.; Pan, Q. C.; Liang, Y. G.; Zhang, Y. L. *Cancer* **1997**, *16*, 186 (in Chinese).
11. Zhang, X.; Yang, X. P.; Pan, Q. C. *Chin. Tradit. Herb. Drugs* **1998**, *29*, 467.
12. Zhang, J. X.; Wang, S. X.; Zhang, F. G.; Zhang, Y. H.; Liu, A. H. *Chin. Tradit. Herb. Drugs* **2001**, *32*, 345.
13. Efferth, T.; Dunstan, H.; Sauerbrey, A.; Miyachi, H.; Chitambar, C. R. *Int. J. Oncol.* **2001**, *18*, 767.

14. Lai, H.; Singh, N. P. *Cancer Lett*. **1995**, *91*, 41.
15. Moore, J. C.; Lai, H.; Li, J. R.; Ren, R. L.; McDougall, J. A.; Singh, N. P.; Chou, C. K. *Cancer Lett*. **1995**, *98*, 83.
16. Singh, N. P.; Lai, H. *Life Sci*. **2001**, *70*, 49.
17. Posner, G. H.; Ploypradith, P.; Hapangama, W.; Wang, D.; Cumming, J. N.; Kensler, T. W.; Klinedinst, D.; Shapiro, T. A.; Zheng, Q. Y.; Murray, C. K.; Pilkington, L. G.; Jayasinghe, L. R.; Bray, J. F.; Daughenbaugh, R. *Bioorg. Med. Chem*. **1997**, *5*, 1257.
18. Posner, G. H.; Ploypradith, P.; Parker, M. H.; O'Dowd, H.; Woo, S. H.; Northrop, J.; Krasavin, M.; Dolan, P.; Kensler, T. W.; Xie, S. J.; Shapiro, T. A. *J. Med. Chem*. **1999**, *42*, 4275.
19. Yang, Y. H.; Li, Y.; Yang, J. D.; Wu, B. A. *Bioorg. Med. Chem. Lett*. **1995**, *5*, 1791.
20. Liang, J.; Li, Y. *Chin. J. Med. Chem*. **1996**, *6*, 22.
21. Li, Y.; Shan, F.; Wu, J. M.; Wu, G. S.; Ding, J.; Xiao, D.; Yang, W. Y.; Atassi, G.; Leonce, S.; Caignard, D. H.; Renard, P. *Bioorg. Med. Chem. Lett*. **2001**, *11*, 5.
22. Li, Y.; Yu, P. L.; Chen, Y. X.; Li, L. Q.; Gai, Y. Z.; Wang, D. S.; Zhang, Y. P. *Acta Pharma. Sin.;* **1981**, *16*, 429.
23. Wang, D. Y.; Wu, Y. K.; Wu, Y. L.; Li, Y.; Shan, F. *J. Chem. Soc., Perkin Trans. 1* **1999**, 1827.
24. Reitnauer, P. G. *Arzneim.-Forsch*. **1972**, *22*, 1347.