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Novel Antitumor Artemisinin Derivatives Targeting G1 Phase of the Cell Cycle

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Abstract—Modification of artemisinin structure led us to the discovery of a novel class of antitumor compounds. These artemisinin derivatives containing cyano and aryl groups showed potent antiproliferative effect in vitro against P388 and A549 cells. This activity was reflected in P388 murine leukemia by an accumulation of cells in G1 phase, and induction of apoptosis.

Artemisinin (qinghaosu, **1**) and its derivatives artemether (**3**), artesunate (**5**), dihydroartemisinin (**2**) and arteether (**4**) have been developed as new kinds of antimalarial drugs active against multidrug-resistant *Plasmodium falciparum* strains.[1] Their rapid action, powerful effect and good tolerance may be attributed to the 1,2,4-trioxane moiety in their molecules. Such a novel chemical structure from rich natural resources encourages chemists and pharmacologists to attempt further exploration. It was reported that artemisinin and many of its analogues possessed not only antiparasitic effect against *P. falciparum, Schistosoma japonicum and Clonorchis sinensi* but also immuno-modulation effects.[2,3] Moreover, the cytotoxic effect of some artemisinin derivatives attracted many investigators.[4–16]

In the early nineties, it was found in our laboratory that cyano artemether (**6a**, which was first synthesized by researchers in Hoechest Company[17]) possessed inhibitory effect against *P. falciparum* and no cytotoxic effect against P388 cells in vitro. However, a pair of isomers (**6b** and **6c**: R = H, R′ = phenyl) in test of antiproliferative potential displayed in vitro cytotoxicity against P388 and L1210 murine leukemia cell lines. Hence, a research program on this series of compounds (**6**: R = H, CH_3, R′ = substituted or unsubstituted aryl) was launched. In this paper, the synthesis and the antiproliferative effect of these compounds against P388 and A549 tumor cell lines are briefly reported.

Chemistry

These compounds were synthesized in moderate yield by acid catalyzed condensation of dihydroartemisinin and corresponding cyanohydrin. Generally, a pair of isomers were produced, but individual pure isomer could be separated by careful column chromatography. Herewith, the preparation of **6d** and **6e** (Fig. 1) as a typical example and their antitumor activity is described. In order to test whether the peroxy group is essential for antitumor activity, **6f** was prepared too.

Under stirring, boron trifluoride diethyl etherate (0.1 mL, 0.8 mmol) was added at −20 °C to a dichloromethane solution (20 mL) of dihydroartemisinin (1.5 g, 5.3 mmol) and 2-(4-bromophenyl)-2-hydroxyacetonitrile newly prepared from 4-bromobenzaldehyde (1.9 g, 10 mmol) with potassium cyanide. The reaction temperature was then allowed to rise to room temperature.

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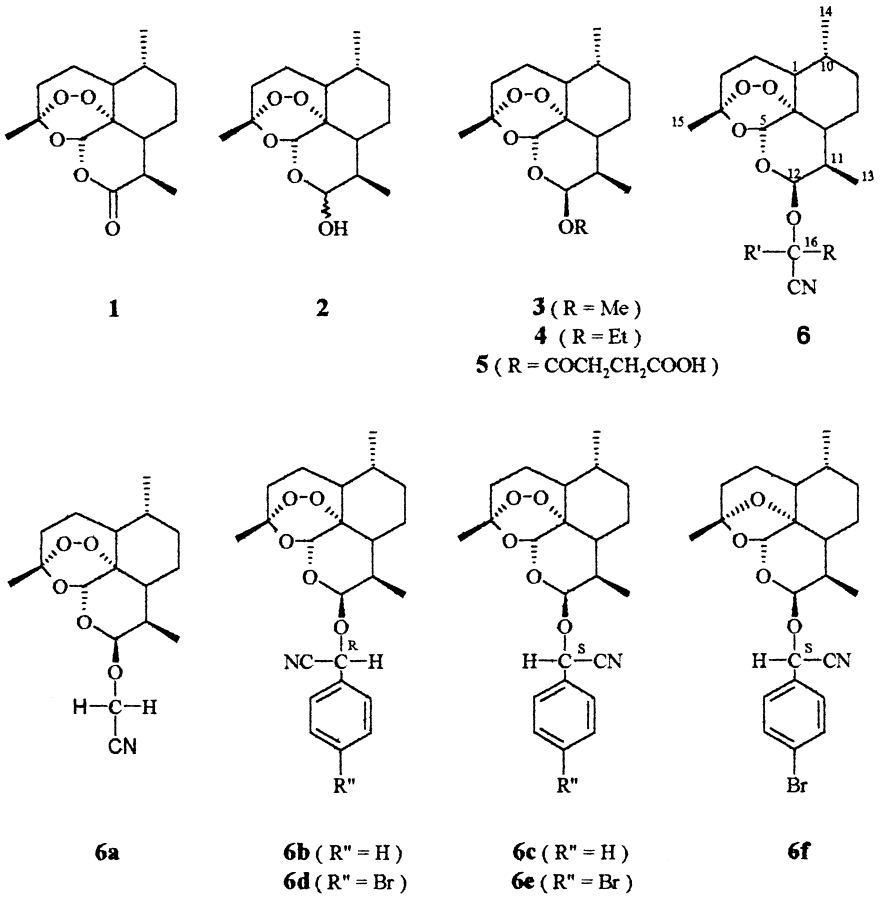

Figure 1. Chemical structures of artemisinin derivatives.

After 24 h, the solution was washed with aqueous sodium bicarbonate, water and brine. The organic layer was dried ($MgSO_4$), filtered and the solvent evaporated. The residue was chromatographed on silica gel to yield 0.57 g of **6d** (22%) and 0.63 g of **6e** (25%).[18] From their ^1H NMR spectra, both isomers were shown to be 12-β derivatives of artemisinin, their sole difference was the configuration of C-16. The *R* configuration of C-16 in **6d** was determined by X-ray diffraction of **6d** (Fig. 2).[19] **6f** was prepared from **6e** by reduction with zinc in acetic acid according to the general procedure in 60% yield.[20]

Pharmacological Studies

Cell culture and cytotoxicity

The murine P388 leukemia and human lung A549 carcinoma cell lines were cultivated in RPMI 1640 medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 units/mL penicillin, 100 μg/mL streptomycin, and 10 mM HEPES buffer (pH: 7.4). Cytotoxicity was measured by the microculture tetrazolium assay as described.[21] Briefly, P388 and A549 cells were exposed to graded concentrations of drug for 48 and 96 h, respectively (four doubling times). Results are expressed as IC_{50}, the concentration which reduced by 50% the optical density of treated cells with respect to the density of untreated cells.

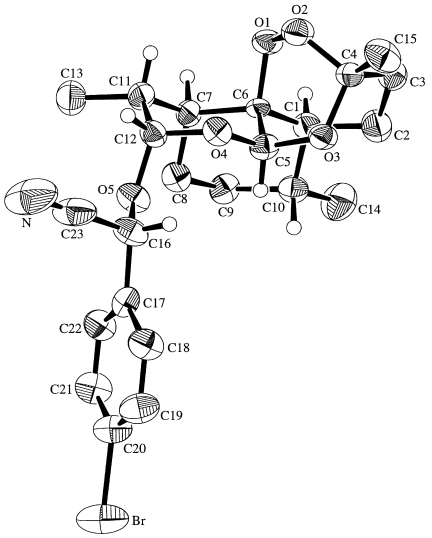

Figure 2. X-ray crystal structure of **6d**.

Cell cycle and apoptosis

P388 cells (2.5×10^5/mL) were incubated for 21 h with various concentrations of drugs then cells were fixed by 70% ethanol and incubated for 30 min in PBS containing 100 μg/mL RNAse and 50 μg/mL propidium iodide (PI, Sigma). For each sample, 10^4 cells were analyzed on an Epics XL/MCL flow cytometer (Beckman counter,

Table 1. Inhibition of cell proliferation, cell cycle effect and induction of apoptosis

Compound	IC_{50} (nM)			Cell cycle and apoptosis (P388)		
	P388	A549	NIH-3T3	% G1	% apoptose	nM
6a	1855	79,432	[a]NT	65	7	10,000
6b	238	1227	NT	67	53	500
6c	48	662	NT	61	45	100
6d	12	47	3,050,000	62	49	25
6e	11	39	5,120,000	63	51	25
6f	24,200	41,990	NT	NT		
VCR	2	18				
Control				42	4	

[a]NT, not tested.

France). Results are expressed as the percentage of cells in G1 phase of the cell cycle and in the sub-G1 phase (apoptotic cells). Apoptotic cells were also quantified by flow cytometry using the annexin-V-FITC labeling.

The results are shown in Table 1.

Results and Discussion

As shown in Table 1 and other data to be published later, **6d** and **6e** were the most potent to inhibit the proliferation of P388 and A549 cells as compared to other compounds. Furthermore, **6d** and **6e** induced an accumulation of P388 cells in the G1 phase of the cell cycle and approximately 50% of apoptotic cells.

The structure–activity relationships can be drawn from these results: (i) The aryl group seems to be necessary for the potent antiproliferation. **6a** without a phenyl group was much less potent when compared to **6b** and **6c**. (ii) The peroxy group appears to be essential for cytotoxicity as in the case of antimalarial activity. **6f** without a peroxy group showed poor antiproliferation. (iii) The configuration of C-16 has insignificant influence on the activity, **6d** and **6e** were equipotent for the inhibition of the proliferation and the cell cycle progression, and for the apoptosis.

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18. Data for **6b**: mp 135–137 °C. ^{1}H NMR (400 MHz), δ: 0.87 (3H, d, J = 6.02 Hz, 10-CH_3), 1.01 (3H, d, J = 7.27 Hz, 11-CH_3), 1.43 (3H, s, 4-CH_3), 5.24 (1H, d, J = 3.62 Hz, 12-H), 5.28 (1H, s, 16-H), 5.68 (1H, s, 5-H), 7.44 (5H, m, aromatic H). IR (KBr): 1457.9, 1103.1, 875.5 cm^{-1}. Anal calcd for ($C_{23}H_{29}NO_5$): C 69.15, H 7.32, N 3.51. Found: C 68.86, H 7.35, N 3.33. **6c**: mp 98–100 °C. ^{1}H NMR (400 MHz), δ: 0.84 (3H, d, J = 7.48 Hz, 10-CH_3), 0.95 (3H, d, J = 6.59 Hz, 11-CH_3), 1.44 (3H, s, 4-CH_3), 4.81 (1H, d, J = 3.43 Hz, 12-H), 5.50 (1H, s, 16-H), 5.60 (1H, s, 5-H), 7.42 (5H, m, aromatic H). IR (KBr): 1452.2, 1101.2, 877.5 cm^{-1}. 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. **6d**: mp 128–129 °C. ^{1}H NMR (400 MHz), δ: 0.90 (3H, d, J = 6.10 Hz, 10-CH_3), 1.00 (3H, d, J = 7.36 Hz, 11-CH_3), 1.43 (3H, s, 4-CH_3), 5.22 (1H, d, J = 3.66 Hz, 12-H), 5.25 (1H, s, 16-H), 5.63 (1H, s, 5-H), 7.33 (2H, d, J = 8.48 Hz, aromatic H), 7.57 (2H, d, J = 8.48 Hz, aromatic H). IR (KBr): 1592.9, 1488.8, 1375.0, 1101.2, 1031.7, 1010.5, 954.6, 875.5 cm^{-1}. 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. **6e**: mp 144–145 °C. ^{1}H NMR (400 MHz), δ: 0.85 (3H, d, J = 7.32 Hz, 10-CH_3), 0.96 (3H, d, J = 6.32 Hz, 11-CH_3), 1.44 (3H, s, 4-CH_3), 4.79 (1H, d, J = 3.40 Hz, 12-H), 5.46 (1H, s, 16-H), 5.58 (1H, s, 5-H), 7.32 (2H, d, J = 8.40 Hz, aromatic H), 7.55 (2H, d, J = 8.30 Hz, aromatic H). IR (KBr): 1592.9, 1486.9, 1103.1, 1035.6, 954.6, 879.4 cm^{-1}. 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.

19. The X-ray crystallographic data have been deposited at the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK.
20. Data for **6f**: mp 122–122.5 °C. ^{1}H NMR (400 MHz), δ: 0.88 (3H, d, J = 6.37 Hz, 10-CH_3), 0.91 (3H, d, J = 7.25 Hz, 11-CH_3), 1.47 (3H, s, 4-CH_3), 4.78 (1H, d, J = 4.06 Hz, 12-H), 5.34 (1H, s, 16-H), 5.47 (1H, s, 5-H), 7.31 (2H, d, J = 8.46 Hz, aromatic H), 7.54 (2H, d, J = 8.49 Hz, aromatic H). IR (KBr): 1592.9, 1486.9, 1394.3, 1211.1, 1103.0, 929.8, 867.8 cm^{-1}. 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.
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