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Synthesis of a series of novel dihydroartemisinin derivatives containing a substituted chalcone with greater cytotoxic effects in leukemia cells

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ABSTRACT

Fifteen dihydroartemisinin derivatives containing a substituted chalcone linked by either ether or ester were synthesized and investigated for their cytotoxicity in human leukemia HL-60 and mouse lymphoma P388 cells. These derivatives have greater antiproliferative and cytotoxic effects in both cell lines than dihydroartemisinin. Dihydroartemisinin chalcones linked by ether are more cytotoxic than dihydroartemisinin chalcones linked by ester with apoptosis induction abilities.

Artemisinin (**1**), isolated from *Artemisia annua* L.,[1] contains a 1,2,4-trioxane moiety (Fig. 1). Artemisinin and its derivatives [dihydroartemisinin (DHA, **2**), artemether, arteether, and artesunate] have been developed as a new type of antimalarial drugs.[2] These compounds have been reported to have antitumor activities, but high concentrations are required.[3] Therefore, structure modification might improve their anti-cancer activities. Several groups have performed modifications at the C10 position of artemisinin and reported that the addition of an alkyl carbon chain (C_8H_{17} to $C_{16}H_{33}$) or a cyanoarylmethyl group significantly improved its antitumor activities.[4] In addition, it has been found that the endoperoxide in artemisinin is required for the cytotoxic efficacy. These data provide a rationale for the modification of artemisinin's structure in order to improve its antitumor activity.

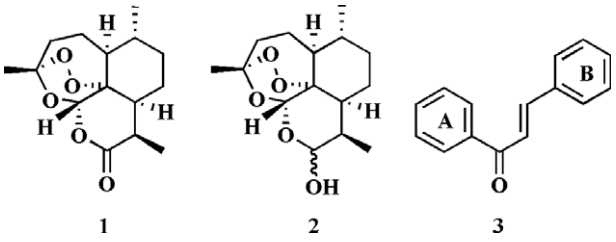

Figure 1. The structures of artemisinin (**1**), dihydroartesiminin (**2**) and chalcone (**3**).

Chalcone (**3**) is an α,β-unsaturated flavonoid identified in *Angelica Keiskei*.[5] Chalcone and related compounds have been found in food products such as tea, fruits and vegetables.[6] Chemically, they are open-chained molecules bearing two aromatic rings linked by a three-carbon enone.[7] Chalcone and its derivatives have been reported to be cytotoxic for cancer cells including leukemia cells.[8] We designed a new group of DHA derivatives containing chalcone with a different substituent linked by ether or ester and examined their vitro cytotoxic activities on human leukemia HL-60 cells and mouse lymphoma P388 cells.

The synthetic pathways are shown in Scheme 1. On the side chain, chalcone analogues **3a**–**3k** were synthesized by Claisen–Schmidt condensation with a better yield based on a method reported previously.[9] In this process, *p*-hydroxyacetophenone was reacted with a corresponding aldehyde and NaOH as base. Compounds **5a**–**5k** were synthesized from the corresponding chalcone analogues and 2-bromoethanol in methyl isobutyl ketone (MIBK). The crude products were recrystallized from ethanol. The reaction of DHA **2** with trifluoroacetic anhydride (TFAA) in the presence of Et_3N gave an active ester at room temperature.[10] The DHA aliphatic ethers were obtained by a reaction of the active ester with compounds **5a**–**5k** in CH_2Cl_2, which were then purified on a silica gel column to produce a minor C-10α form and major C-10β diastereomers **7a**–**7k**, respectively.[11]

On the other hand, the phenolic hydroxyl groups in the compounds **3a**, **3c**, **3d** and **3f** were reduced to aromatic ethers **4a**–**4d**,

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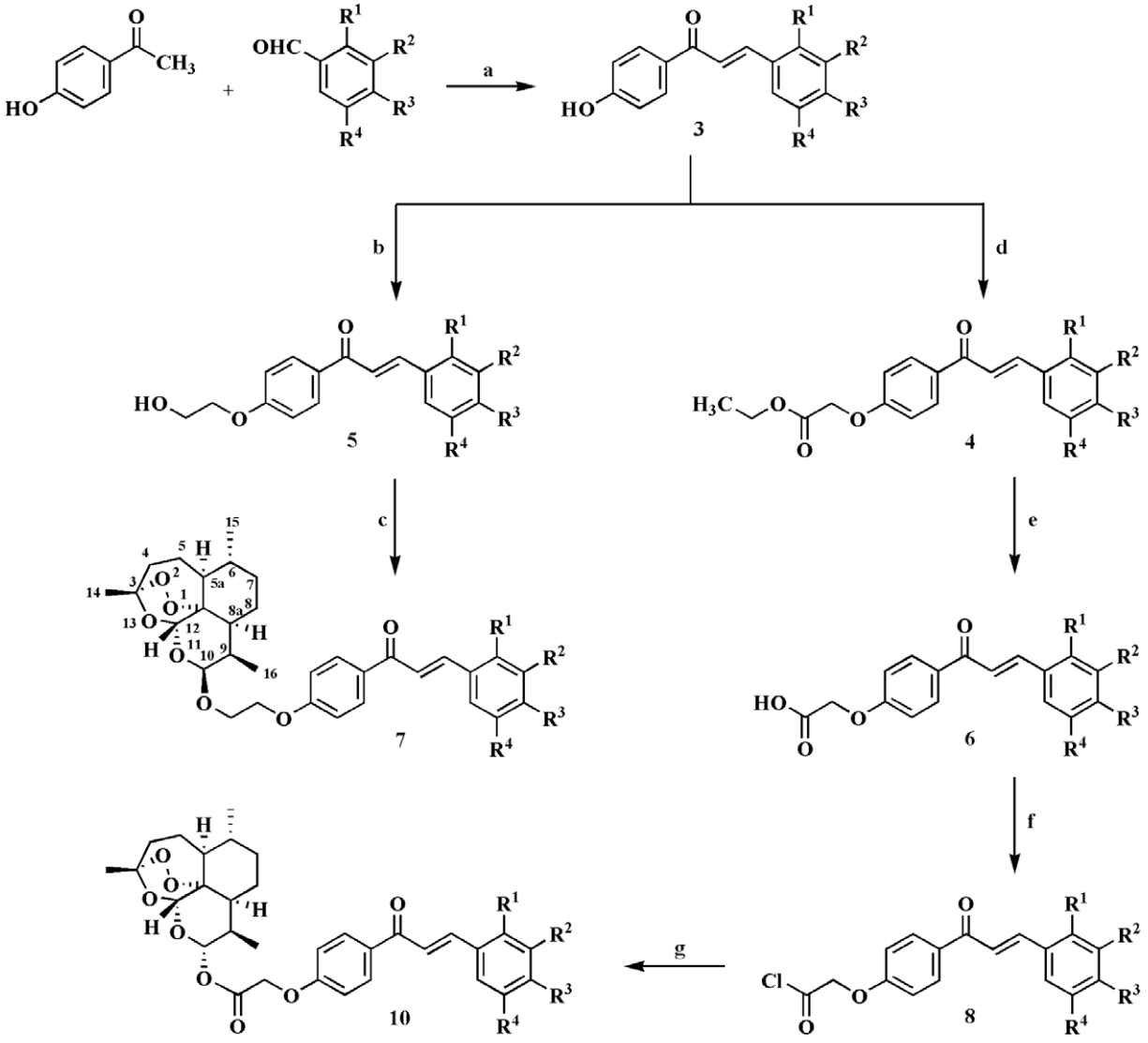

Scheme 1. The synthetic pathways of dihydroartemisinin derivatives. Reagents and conditions: (a) NaOH, MeOH, reflux, 2 h; (b) $HOCH_2CH_2Br$, K_2CO_3, KI, MIBK, reflux, 6 h; (c) DHA, TFAA, Et_3N, CH_2Cl_2, ice-water bath, reflux, 42 h; (d) $ClCH_2COOC_2H_5$, K_2CO_3, KI, MIBK, reflux, 8 h; (e) NaOH, HCl, H_2O, reflux, 4 h; (f) $SOCl_2$, CH_2Cl_2, reflux, 2 h; (g) DHA, Et_3N, CH_2Cl_2, reflux, 24 h.

respectively, by the Williamson reaction with ethyl chloroacetate in MIBK in the presence of sodium bicarbonate. Compounds **4a**–**4d** were reduced by hydrolysis and neutralization in water to yield the acids **6a**–**6d**. The acids **6a**–**6d** reacted with sulfoxide chloride refluxing in CH_2Cl_2 to obtain acyl chlorides **8a**–**8d**, which subsequently reacted with DHA in dry CH_2Cl_2 in the presence of Et_3N to give the DHA esters **10a**–**10d**.[12] Under these conditions, the esterification of DHA was highly stereoselective. The major products **10a**–**10d** are α-isomers, while minor products are β-isomers.[13]

The stereochemistry of these compounds was confirmed by application of ^1H NMR, which analyzing the chemical shift of H-10 and the coupling constant between H-9 and H-10. The DHA aliphatic ethers are β-isomers as indicated by a chemical shift (4.90 ppm) and a small coupling constant (J = 3.3 Hz), while the DHA esters are α-isomers (δ = 5.89 ppm and J = 9.9 Hz).[14] The geometrical configuration of the chalcone side chains was determined to be *trans* by observing that the coupling constant between α-H and β-H was that of α,β-unsaturated ketones (J = 15.6 Hz).

The growth inhibitory effects of these DHA derivatives were determined by counting cells and their cytotoxicities were as-

Table 1
The growth inhibitory effects of dihydroartemisinin derivatives in human leukemia HL-60 cells

Compounds	R^1	R^2	R^3	R^4	IG_{50} (μM)	IC_{50} (μM)
DHA					0.730	2.90
7a	H	H	Cl	H	0.106	0.130
7b	Cl	H	H	H	0.119	0.136
7c	H	H	Br	H	0.040	0.080
7d	H	H	OCH_3	H	0.214	0.147
7e	OCH3	H	H	H	0.165	0.134
7f	H	H	OC_2H_5	H	0.121	0.110
7g	H	H	H	H	0.208	0.193
7h	H	OCH_3	OCH_3	H	0.090	0.300
7i	OCH_3	OCH_3	H	H	0.079	0.194
7j	H	OCH_3	OCH_3	OCH_3	0.070	0.219
7k	H	$-OCH_2O-$		H	0.059	0.089
10a	H	H	Cl	H	0.276	0.537
10b	H	H	Br	H	0.314	0.636
10c	H	H	OCH_3	H	0.174	0.394
10d	H	H	OC_2H_5	H	0.286	0.734

The cells were treated for 72 h. IG_{50}, the concentration that inhibits cell proliferation by 50%; IC_{50}, the concentration that reduces cell viability by 50%.

sessed by Trypan blue exclusion assay we previously reported.[15] The IG_{50} (the concentration that inhibits cell proliferation by 50%) and the IC_{50} (the concentration that reduces cell viability by 50%) values were determined. As shown in Table 1, all of these derivatives have greater growth inhibitory effects in HL-60 cells compared with those of DHA. Since chalcone **3** and its substituted products do not inhibit HL-60 cell growth at a concentration of 1 μM (data not shown), it suggests that the addition of a substituted chalcone enhances the growth inhibitory activity of DHA. By comparing the antiproliferative activities of compounds **7a**–**7g**, it was found that the compound (**7c**) with the addition of Br in the chalcone is more potent than with an introduction of a Cl, OCH_3 or OC_2H_5 group. The compounds with the introduction of two or three OCH_3 groups in the chalcone (**7h**, **7i** and **7j**) are more effective inhibitors than compounds with the introduction of one OCH_3 group (**7d** and **7e**). By comparing the antiproliferative activities of compounds **7a**, **7c**, **7d** and **7f** with those of compounds **10a**, **10b**, **10c** and **10d**, it was found that compounds linked by ether (**7a–7f**) are more potent inhibitors than those compounds linked by ester (**10a–10d**). Although there is approximately fourfold increase in the concentration of DHA required to kill half of the cells as observed by comparing their IG_{50}, there is approximately onefold increase in the concentrations of the derivatives (**7a**–**7k**) required to kill half of cells comparing (Table 1). These data suggest that the mechanism of action of compounds **7a**–**7k** differs from that of DHA. Among these derivatives, compounds **7c** and **7k** are the most potent in inhibiting cell growth and in inducing cytotoxicity (Table 1).

To determine if the cytotoxic effects of these compounds are due to apoptosis induction, HL-60 cells were treated with com-

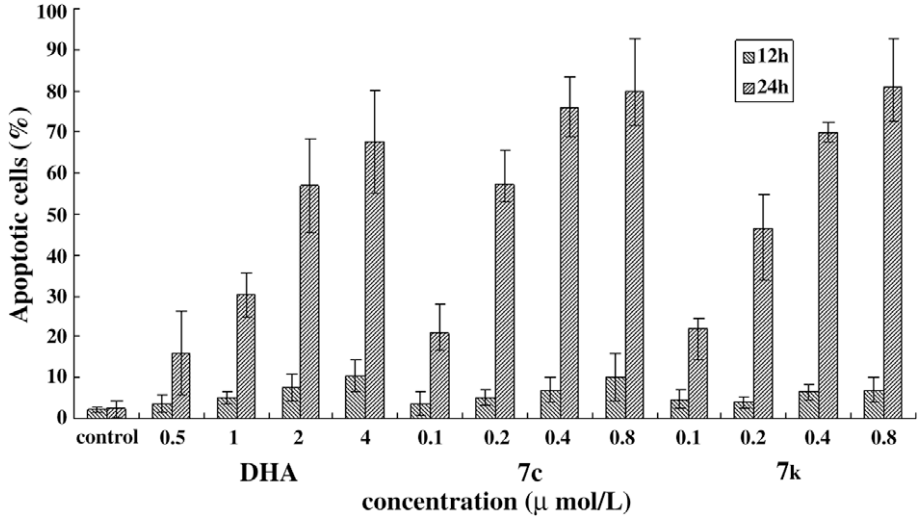

Figure 2. Dose- and time-dependent apoptotic effects of compounds DHA, **7c**, and **7k**. HL-60 cells were treated with the indicated compounds at the indicated concentrations for 12 h and 24 h. The percentages of apoptotic cells were determined by morphologic fluorescent observation after staining with AO and EB. Data shown are means ± SD of three independent experiment.

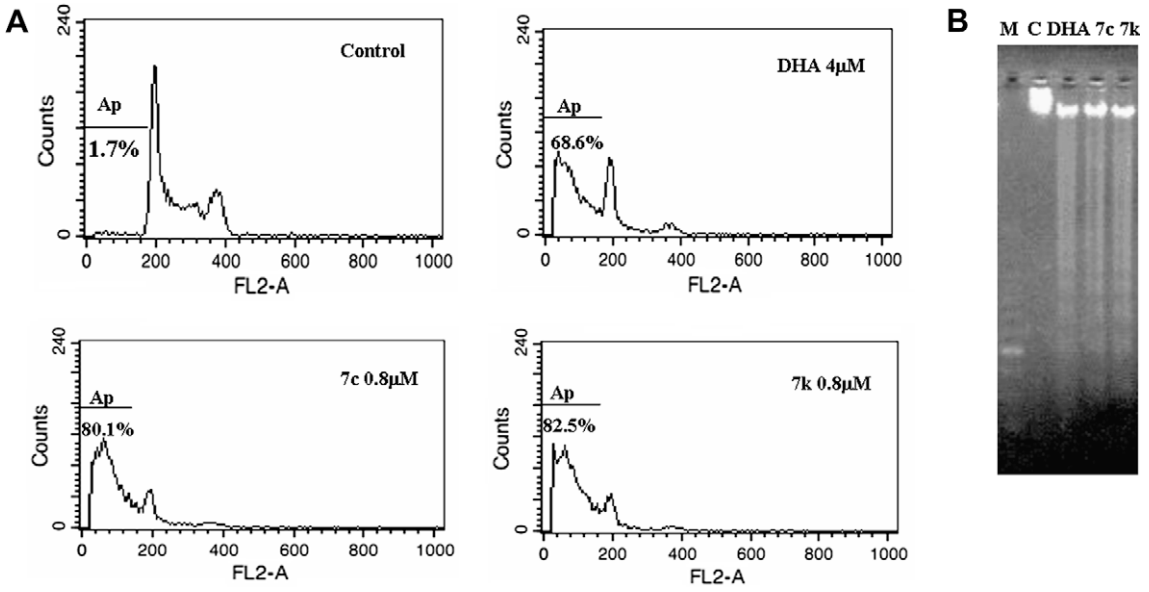

Figure 3. Apoptotics induction determined by FACS and DNA fragmentation. (A) FACS analysis of apoptotic cells. HL-60 cells were treated with DHA, **7c** and **7k** at the indicated concentrations for 24 h. Apoptotic cells were determined after propidium iodide staining. Ap, apoptotic cells. (B) DNA fragmentation. HL-60 cells were treated with the DHA (4 μM), **7c** (0.8 μM) and **7k** (0.8 μM) for 24 h and the fragmentation DNA was visualized after staining with ethidium bromide described in Ref. 15. M, DNA standards; C, control.

Table 2
The antiproliferative effects of **DHA, 7c, 7k** and adriamycin (Adr) in P388 and P388/Adr cells

Compounds	IG_{50} (μM)	
	P388	P388/Adr
DHA	2.30 ± 0.48	1.51 ± 0.32
Adr	0.019 ± 0.002	3.73 ± 0.19
7c	0.098 ± 0.019	0.155 ± 0.001
7k	0.096 ± 0.008	0.178 ± 0.013

Data shown are average ± SD in three independent experiments. The cells were treated for 72 h.

pounds **7c** and **7k**. The percentages of apoptotic cells were determined by morphological observation after staining with acridine orange (AO) and ethidium bromide (EB) as we reported.[15] Cells with nuclear shrinkage, blebbing, and apoptotic bodies were counted as apoptotic cells and percentage was calculated after observing 300 cells. As shown in Figure 2, compounds **7c** and **7k** at a concentration of 0.2 μM induced apoptosis in about 50% of cells after 24 h treatment while DHA induced a similar apoptotic effect only at a concentration of 2 μM (Fig. 2). The apoptotic effects of these compounds were further confirmed by subG1 induction which was determined by flow cytometry and by DNA fragmentation determined by DNA gel electrophoresis as we previously reported.[15] As shown in Figure 3A, compounds **7c** and **7k** induced more than 80% of cells in the subG1 phase (apoptotic cells) at a concentration of 0.8 μM after 24 h of treatment. Both compounds **7c** and **7k** induced DNA fragmentation (Fig. 3B). To obtain the similar subG1 and DNA fragmentation induction ability, 4 μM of DHA was required.

It has been shown that artemisinin and its derivatives are equally active towards drug-sensitive and -resistant cell lines. Using mouse lymphoma P388 and adriamycin-resistant P388/Adr cells, we have compared the cell growth inhibitory and cytotoxic activities of DHA, **7c** and **7k**. As shown in Table 2, compounds **7c** and **7k** were more potent than DHA in inhibiting growth of both P388 and P388/Adr cells.

In conclusion, our data indicate that (1) the DHA aliphatic ethers are in the β-isomer configuration, while the DHA esters are in the α-isomer configuration; (2) DHA derivatives containing a chalcone are more potent in growth inhibition and cytotoxicity than DHA; (3) DHA ethers are more effective than DHA esters in growth inhibition and cytotoxicity.

Acknowledgment

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References and notes

1. Klayman, D. L. *Science* **1985**, *228*, 1049.
2. Efferth, T. *Drug Resist. Update* **2005**, *8*, 85.
3. Li, Y.; Wu, J. M.; Shan, F.; Wu, G. S.; Ding, J.; Xiao, D.; Han, J. X.; Atassi, G.; Leonce, S.; Caignard, D. H.; Renard, P. *Bioorg. Med. Chem.* **2003**, *11*, 977.
4. (a) Liu, Y.; Wong, V. K.; Ko, B. C.; Wong, M. K.; Che, C. M. *Org. Lett.* **2005**, *7*, 1561; (b) Wu, J. M.; Shan, F.; Wu, G. S.; Li, Y.; Ding, J.; Xiao, D.; Han, J. X.; Atassi, G.; Leonce, S.; Caignard, D. H.; Renard, P. *Eur. J. Med. Chem.* **2001**, *36*, 469.
5. Liu, Y. C.; Hsieh, C. W.; Wu, C. C.; Wung, B. S. *Life Sci.* **2007**, *80*, 1420.
6. Aherne, S. A.; O'Brien, N. M. *Nutrition* **2002**, *18*, 75.
7. Nowakowska, Z. *Eur. J. Med. Chem.* **2007**, *42*, 125.
8. (a) Borges-Argáez, R.; Balnbury, L.; Flowers, A.; Giménez-Turba, A.; Ruiz, G.; Waterman, P. G.; Peña-Rodríguez, L. M. *Phytomedicine* **2007**, *14*, 530; (b) Saydam, G.; Aydin, H. H.; Sahin, F.; Kucukoglu, O.; Erciyas, E.; Terzioglu, E.; Buyukkececi, F.; Omay, S. B. *Leukocyte Res.* **2003**, *27*, 57.
9. Song, L. L.; Kosmeder, J. W.; Lee, S. K.; Gerhäuser, C.; Lantvit, D.; Moon, R. C.; Moriarty, R. B.; Pezzuto, J. M. *Cancer Res.* **1999**, *59*, 578.
10. (a) Lin, A. J.; Lee, M.; Klayman, D. L. *J. Med. Chem.* **1989**, *32*, 1249; (b) Lin, A. J.; Zikry, A. B. *J. Med. Chem.* **1997**, *40*, 1396.
11. *General procedure for the preparation of dihydroartemisinin aliphatic ethers* (**7a**–**7k**). A mixture of the *p*-hydroxyacetophenone (10 mmol) and the corresponding aldehyde (11 mmol) in anhydrous MeOH (30 mL) was stirred at room temperature for 5 min. Then, NaOH (40 mmol) was added. The reaction mixture was stirred at room temperature for 2 h. Then water (30 mL) was added and the mixture was acidified with HCl (5 mol/L) until pH 8 was reached. When the chalcones precipitated, they were filtered and recrystallized from EtOH (95%) to yield a yellow solid **3**. K_2CO_3 (0.03 mol) was added to a stirred solution of compound **3** (10 mmol) in MIBK (50 mL) at 50 °C for 30 min followed by adding 2-bromoethanol (9 mmol) and KI as catalysts, and stirring at 120 °C for another 6 h. The reaction mixture was washed with NaOH (5%) and water. The organic layer was dried (Mg_2SO_4) and filtered to yield a crude product **5** which was then purified by crystallization (cyclohexane–acetone). To a CH_2Cl_2 solution of dihydroartemisinin (2 mmol) Et_3N (2.4 mmol) and TFAA (2.4 mmol) were added at 0 °C. The resulting mixture was stirred at room temperature for 18 h, and then compound **5** was added and stired for another 24 h. The reaction was washed with NaOH (5%) and brine. The organic layer was dried ($MgSO_4$) and concentrated in vacuo to give the crude products. Pure products were obtained by column chromatography (silica gel) using petroleum ether–ethyl acetate (8–10:1 v/v) as eluent. Spectral data of the potent compounds: Compound **7h**: yield 16.4%; mp 110–112 °C; IR (KBr): 3439, 2921, 1657, 1602, 1511, 1384, 1261, 1028, 984, 874, 828, 805, 766 cm^{-1}; ESI-MS: 617.7 $(M+Na)^+$; ^1H NMR (600 MHz, $CDCl_3$): δ (ppm) 8.03 (2H, d, J = 8.7 Hz, Ar-H), 7.77 (1H, d, J = 15.6 Hz, –CH=CH–), 7.41 (1H, d, J = 15.6 Hz, –CH=CH–), 7.24 (1H, d, J = 8.3 Hz, Ar-H), 7.17 (1H, s, Ar-H), 6.99 (2H, d, J = 8.7 Hz, Ar-H), 6.91 (1H, d, J = 8.3 Hz, Ar-H), 5.48 (1H, s, H-12), 4.91 (1H, d, J = 3.3 Hz, H-10), 4.22 (3H, m, $–OCH_2CH_2O–$), 3.83 (1H, m, $–OCH_2CH_2O–$), 3.96 (3H, s, $–OCH_3$), 3.94 (3H, s, $–OCH_3$), 2.65 (1H, m, H-9), 2.37 (1H, m, H-4), 2.05 (1H, m, H-4), 1.86 (1H, m, H-5), 1.47 (3H, s, H-14), 0.93 (3H, d, J = 5.7 Hz, H-16), 0.91 (3H, d, J = 7.3 Hz, H-15); ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 188.7, 162.6, 151.3, 149.2, 144.2, 131.4, 130.7, 128.0, 123.0, 119.8, 114.4, 111.1, 110.1, 104.1, 102.2, 87.9, 81.1, 67.5, 66.3, 56.0, 55.9, 52.5, 44.4, 37.5, 36.4, 34.6, 30.9, 26.2, 24.7, 24.4, 20.4, 13.0.
12. (a) Jung, M.; Lee, K.; Jung, H. *Tetrahedron Lett.* **2001**, *42*, 3997; (b) Hindley, S.; Ward, S. A.; Storr, R. C.; Searle, N. L.; Bray, P. G.; Park, B. K.; Davies, J.; O'Neill, P. M. *J. Med. Chem.* **2002**, *45*, 1052.
13. *General procedure for the preparation of dihydroartemisinin esters* (**10a**–**10d**): To a stirred solution of compound **3** (10 mmol) in MIBK (50 mL) K_2CO_3 (0.03 mol) was added at 50 °C for 30 min followed by adding ethyl chloroacetate (9 mmol) and KI as catalysts and stirring at 120 °C for 6 h, and then water (50 mL) was added. The mixture was washed with NaOH (5%) and water. The organic layer was dried (Mg_2SO_4) and filtered to yield crude product **4** which was added to 25% NaOH (30 mL) for 1 h. The mixture was acidified with HCl (3 mol/L) until acid and filtered to yield crude product **6** which was purified by crystallization (ethanol–water). Et_3N (2.4 mmol) was added to a CH_2Cl_2 of DHA (2 mmol), freshly prepared compound **8** from compound **6** and sulfoxide chloride. The resulting mixture was stirred at room temperature for 2 h. The reaction mixture was washed with HCl (1 mol/L) and brine. The organic layer was dried ($MgSO_4$) and concentrated in vacuo to give the crude products. Pure products were obtained by column chromatography (silica gel) using petroleum ether–ethyl acetate (15:1 v/v) as eluent. Spectral data of the potent compounds: Compound **10c**: yield 11.7%; mp 137–139 °C; IR (KBr): 3448, 2928, 1772, 1658, 1600, 1510, 1028, 824 cm^{-1}; ESI-MS: 579.2 $(M+H)^+$, 601.2 $(M+Na)^+$, 617.2 $(M+K)^+$; ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 8.03 (2H, d, J = 8.8 Hz, Ar-H), 7.78 (1H, d, J = 15.6 Hz, –CH=CH–), 7.61 (2H, d, J = 8.7 Hz, Ar-H), 7.42 (1H, d, J = 15.6 Hz, –CH=CH–), 7.00 (2H, d, J = 8.8 Hz, Ar-H), 6.94 (2H, d, J = 8.7 Hz, Ar-H), 5.90 (1H, d, J = 9.9 Hz, H-10), 5.46 (1H, s, H-12), 4.79 (2H, m, $–COCH_2O–$), 3.86(3H, s, $–OCH_3$), 2.62 (1H, m, H-9), 2.38 (1H, m, H-4), 2.05 (1H, m, H-4), 1.45 (3H, s, H-14), 0.97 (3H, d, J = 5.6 Hz, H-16), 0.84 (3H, d, J = 7.1 Hz, H-15); ^{13}C NMR (150 MHz, DMSO): δ (ppm) 188.7, 167.9, 161.7, 161.6, 143.8, 131.8, 131.2, 127.9, 119.9, 115.0, 114.8, 104.1, 93.0, 91.2, 80.3, 65.0, 55.8, 51.5, 45.0, 36.4, 36.3, 34.1, 32.1, 26.0, 24.6, 21.4, 20.5, 12.1.
14. Oh, S.; Jeong, I. H.; Ahn, C. M.; Shin, W. S.; Lee, S. *Bioorg. Med. Chem.* **2004**, *12*, 3783.
15. Liu, D.; Song, D.; Guo, G.; Wang, R.; Lv, J.; Jing, Y.; Zhao, L. *Bioorg. Med. Chem.* **2007**, *15*, 5432.