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Synthesis and immunosuppressive activity of new artemisinin derivatives. Part 2: 2-[12(β or α)-Dihydroartemisinoxymethyl-(or 1′-ethyl)]phenoxyl propionic acids and esters

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Abstract—Another series of novel dihydroartemisinin derivatives were synthesized and assessed for their cytotoxicity of lymphocyte, inhibitory activity on mitogen-induced spleen lymphocyte proliferation in vitro. Some of the compounds exhibited inhibitory effects on ConA-induced T cell and LPS-induced B cell proliferation comparable to or more potent than parent artemisinin.

1. Introduction

The immunosuppressive action of artemisinin and its derivatives has been studied in China for many years. Many experimental results in vitro and in vivo suggested that the new type of antimalarial drugs, such as artemisinin (qinghaosu) **1**, dihydroartemisinin **2**, artemether **3**, and artesunic acid **4** (Fig. 1), possessed definite immunosuppressive activity.[1–7]

In search for new potential immunosuppressive agents with much higher efficacy and lower toxicity, we synthesized a class of novel artemisinin derivatives **5**–**8** (Fig. 2) and found that introduction of phen(ox)yl aliphatic acid and ester into artemisinin nucleus did enhance their immunosuppressive activity.[8]

Herein, we will report our continuous work, that is, synthesis and immunosuppressive activity of another series of new artemisinin derivatives **9**. Compared with compound **8**, the only difference in the chemical structure of **9** is the link between artemisinin nucleus and phenoxyl aliphatic acid or ester, that is OCH_2 or OCH(Me) group instead of O atom (Fig. 3). These compounds were tested in MTT assay for their cytotoxicity, T cell and B cell functional assays for evaluating their immunosuppressive activity in vitro.

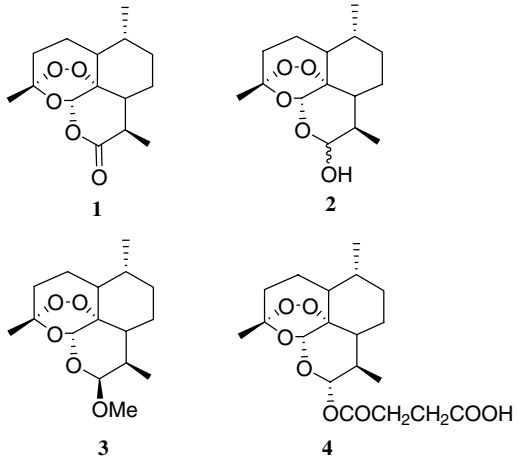

Figure 1. Artemisinin and its first-generation semi-synthetic derivatives.

Keywords: Artemisinin derivatives; Synthesis; Immunosuppressive activity; Inhibitory effect.
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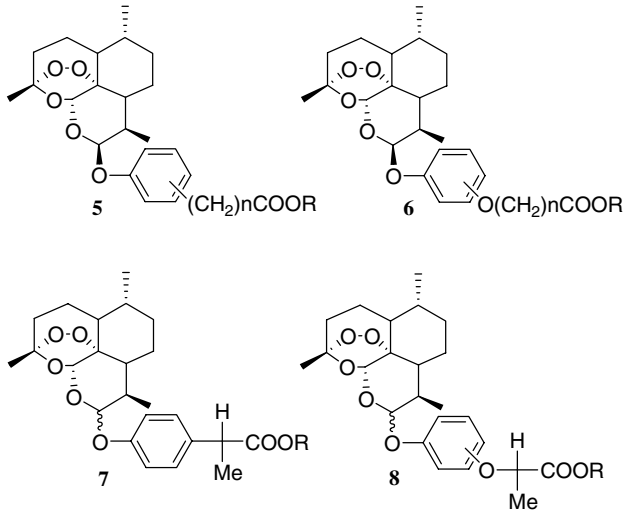


Figure 2. Artemisinin derivatives **5**–**8**.

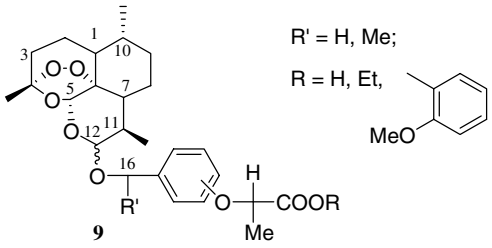


Figure 3. New class of artemisinin derivatives **9**.

2. Results and discussion

2.1. Chemistry

The new dihydroartemisinin benzyl ethers (**9b**, **9d**, **9g**, **9j**, **9l**, and **9n**) were prepared by treatment of dihydroartemisinin with appropriately substituted phenols **10**–**12** in the presence of boron trifluoride etherate ($BF_3{\cdot}Et_2O$) at room temperature (Schemes 1 and 2). Similar to our previous work,[9] these benzyl ether derivatives (except for **9n)** were composed of almost equal 12β and 12α epimers and can be separated by column chromatography. These benzyl ethers then were hydrolyzed with 0.5% KOH/EtOH solution to give the corresponding free acids (**9a**, **9c**, **9f**, **9h**, **9k**, and **9m**). These free acids finally were converted into their corresponding aryl esters (**9e**, **9j**, and **9o**) by coupling with *o*-methoxyphenol in moderate to good yields.

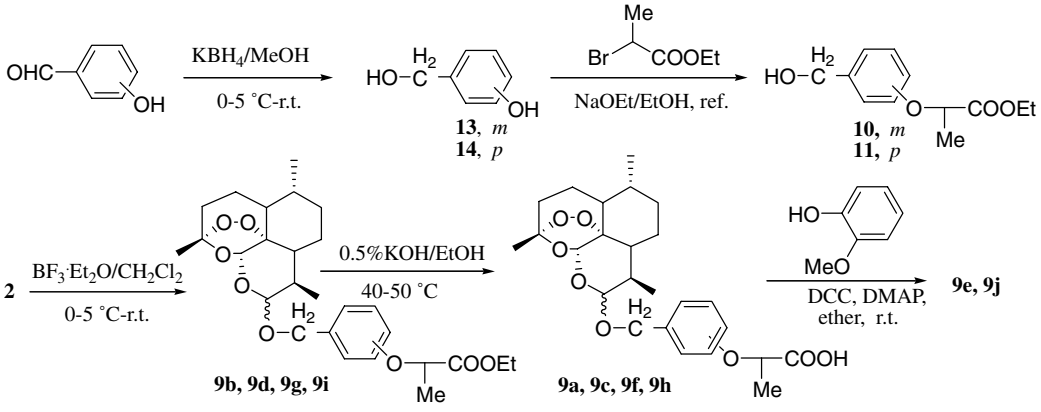
Synthetic intermediates **10**–**12** were prepared according to literature procedure starting with hydroxybenzaldehyde or hydroxyacetophenone.[10]

Because compounds **9** with one or two chiral centers in the side chain are diastereoisomeric mixture, the proton assignment in their ^1H NMR spectra became more difficult. But in the cases of free acids or *para* substituent benzyl derivatives, their ^1H NMR data may be easily confirmed.

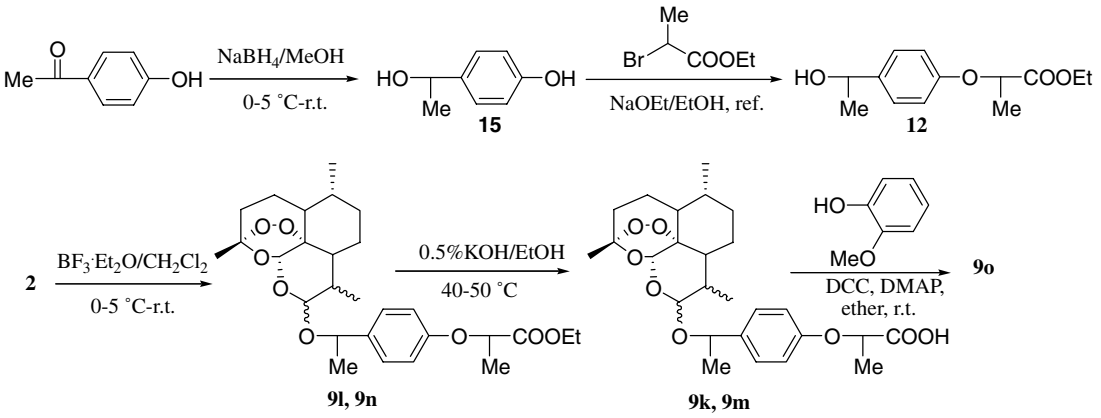
2.2. Biological activity

The new dihydroartemisinin derivatives were tested in vitro for their cytotoxicity on murine spleen cells, and inhibitory activity on concanavalin A (ConA)-induced T cell proliferation or lipopolysaccharide (LPS)-induced B cell proliferation with artemisinin, artemether, and artesunate as the controls. The pharmacological results of these compounds are summarized in Table 1. The cytotoxicity of each compound was expressed as the concentration of compound that reduced cell viability to 50% (CC_{50}). The immunosuppressive activity of each compound was expressed as the concentration of compound that inhibited ConA-induced T cell proliferation and LPS-induced B cell proliferation to 50% (IC_{50}) of the control value. The selective index (SI) value was used to evaluate the bioactivity of compounds.

The results showed that, among them, **9a**, **9e**, **9h**, and **9j** had 5- to 9-fold higher bioactivity than artemisinin in the ConA-induced T cell proliferation. In the inhibition of LPS-induced B cell proliferation, **9e**, **9f**, **9g**, **9j**, **9k**, **9m**, **9n**, and **9o** exhibited 30- to 88-fold higher bioactivity than artemisinin. It was unfortunate that the cytotoxicity of compounds **9** examined in MTT assay relatively increased compared with that of artemisinin. It seemed



Scheme 1. Synthetic routes to **9a**–**j**.



Scheme 2. Synthetic routes to **9k–o**.

Table 1. Inhibitory effects of artemisinin and its derivatives **9** on spleen lymphocyte proliferation induced by T cell or B cell mitogen (ConA or LPS) in vitro

Compound	CC_{50} (M)	IC_{50} (M), [SI][a]	
		T lymphocyte	B lymphocyte
9a	1.2×10^{-5}	6.8×10^{-7} [17.4]	8.0×10^{-7} [14.8]
9b	7.7×10^{-6}	9.1×10^{-7} [8.5]	5.0×10^{-7} [15.4]
9c	1.1×10^{-5}	2.4×10^{-6} [4.4]	4.5×10^{-7} [23.6]
9d	8.2×10^{-6}	4.2×10^{-6} [2.0]	6.7×10^{-7} [12.2]
9e	3.2×10^{-6}	4.6×10^{-7} [6.9]	2.8×10^{-7} [11.5]
9f	2.7×10^{-6}	1.4×10^{-6} [1.9]	1.6×10^{-7} [16.9]
9g	8.8×10^{-6}	1.8×10^{-6} [4.8]	3.0×10^{-7} [29.8]
9h	1.3×10^{-5}	7.0×10^{-7} [19.0]	7.6×10^{-7} [17.5]
9i	2.4×10^{-5}	2.4×10^{-6} [9.8]	4.0×10^{-7} [59.0]
9j	2.7×10^{-6}	8.4×10^{-7} [3.3]	2.2×10^{-7} [12.3]
9k	9.5×10^{-6}	9.8×10^{-7} [9.7]	1.8×10^{-7} [52.9]
9l	6.6×10^{-7}	3.6×10^{-6} [<1]	5.0×10^{-7} [1.3]
9m	1.1×10^{-5}	4.4×10^{-6} [2.4]	2.8×10^{-7} [38.2]
9n	1.3×10^{-5}	1.4×10^{-5} [<1]	1.0×10^{-7} [128.4]
9o	3.2×10^{-6}	1.4×10^{-6} [2.3]	1.7×10^{-7} [18.6]
Artemisinin	2.8×10^{-5}	4.4×10^{-6} [6]	9.0×10^{-6} [3]
Artemether	8.6×10^{-5}	3.8×10^{-6} [23]	1.8×10^{-6} [48]
Artesunate	3.3×10^{-5}	4.6×10^{-6} [7]	9.9×10^{-7} [33]

[a] Selectivity index [SI] is determined as the ratio of the concentration of the compound that reduced cell viability to 50% (CC_{50}) to the concentration of the compound needed to inhibit the proliferation to 50% (IC_{50}) of the control value.

that the inhibitory effect of most artemisinin derivatives **9** on ConA-induced T cell proliferation was comparable to or slightly higher than that of parent artemisinin. While their inhibitory activity (except for **9l**) on LPS-induced B cell proliferation was much more potent than that of artemisinin.

3. Conclusion

In summary, as part of continuous research plan to search for potential immunosuppressive agents with higher efficacy and lower toxicity, another new series of new dihydroartemisinin derivatives **9** were synthesized and assessed for their cytotoxicity of lymphocyte, inhibitory activity on ConA-induced T cell proliferation and LPS-induced B cell proliferation in comparison with artemisinin.

In the current study, it was found that when the linking chain between artemisinin nucleus and phenoxyl aliphatic acid or ester, that is, oxygen atom in compound **8** was replaced with OCH_2 or OCH(Me) group, their immunosuppressive activity greatly reduced and was only comparable to or slightly more potent than that of parent artemisinin.

4. Experimental

4.1. General

All commercially available reagents were used without further purification unless otherwise stated. The solvents used were all of AR grade and were distilled under positive pressure of dry nitrogen atmosphere where necessary. $BF_3{\cdot}Et_2O$ was redistilled by the standard methods.

The progress of the reactions was monitored by analytical thin-layer chromatography (TLC) performed on homemade $HSGF_{254}$ precoated silica gel plates. Visualization was performed by UV or development using vanillin solution in sulfuric acid and ethanol (v/v = 4/1).

All melting points were taken in open capillary tubes on a Buchi-510 melting points apparatus and are uncorrected. The IR spectra through the range from 4000 to 600 were run on a Perkin-Elmer 599B spectrophotometer with KBr pellets or as thin films and are reported in reciprocal centimeters (cm^{-1}). ^1H NMR spectra were determined in $CDCl_3$ solution on a Brucker AM 400 spectrophotometer at ambient temperature. Elemental analyses were performed on a CE 1106 elemental analyzer and all the results had deviation within ±0.4% of the theoretical values. Yields were of purified compounds and were not optimized.

4.2. Preparation of synthetic intermediates 10–12

Ethyl 2-[*m* or *p*-hydroxymethyl (or 1′-ethyl) phenoxy] propionate **10**–**12** were prepared on the basis of the modified literature procedure,[10] which is described below. To a solution of sodium ethoxide, prepared by dissolving sodium (2.50 g, 110 mmol) in absolute EtOH (120 mL), were added consecutively hydroxybenzyl alcohol **13**–**15** (100 mmol) prepared by potassium borohydride reduction of hydroxybenzaldehyde or hydroxyacetophenone and (*dl*)-ethyl 2-bromopropionate (15.6 mL, 120 mmol). The mixture solution was refluxed for 5 h, and the solvent was then removed by distillation. EtOAc (100 mL) was added and the organic extract was washed with water and brine, dried over anhydrous $MgSO_4$, and concentrated. The resultant residue was purified by silica gel chromatography (petroleum ether/EtOAc, 20:1) to give **10**–**12** as colorless oil (yield: 50%).

4.3. Synthesis of benzyl ether artemisinin derivatives (9b, 9d, 9g, 9j, 9l, and 9n)

To a solution of dihydroartemisinin (**2**, 5.7 g, 20.0 mmol) and ethyl 2-[*m* or *p*-hydroxymethyl (or 1′-ethyl) phenoxy] propionate (**10**–**12**, 24.0 mmol) in CH_2Cl_2 (80 mL) was added $BF_3{\cdot}Et_2O$ (0.5 mL) at 0 °C. The mixture was stirred at room temperature until the condensation reaction was complete (monitored by TLC) and then washed by saturated aqueous $NaHCO_3$ solution, H_2O, and brine, dried over anhydrous $MgSO_4$, and evaporated to dryness under reduced pressure. The resultant crude products were purified using silica gel chromatography with ethyl acetate/petroleum ether (1:25, v/v) as the eluent to give new ether derivatives (**9b**, **9d**, **9g**, **9j**, **9l**, and **9n**), whose yields ranged from 25% to 35%.

4.3.1. Ethyl 2-[3-(12-β-artemisinoxymethyl)]phenoxyl propionate (9b). Colorless oil. Yield: 30%. ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 7.22 (1H, t, J = 7.90 Hz, Ar-H); 6.92 (1H, dd, J = 7.55, 0.82 Hz, Ar-H); 6.84 (1H, br s, Ar-H); 6.77 (1H, dd, J = 8.24, 1.65 Hz, Ar-H); 5.44 (1H, s, 5-H); 4.89 (1H, d, J = 3.43 Hz, 12-H); 4.85 (1H, dd, J = 12.57, 5.70 Hz, 16-Ha); 4.73 (1H, q, J = 6.80 Hz, Ar-O–C**H**-Me); 4.47 (1H, d, J = 12.49 Hz, 16-Hb); 4.21 (2H, dq, J = 7.15, 1.37 Hz, –O–C**H**$_2$-Me); 2.66 (1H, m, 11-H); 2.37 (1H, m, 3-Hβ); 1.61 (3H, d, J = 6.73 Hz, Ar-O–CH-**Me**); 1.45 (3H, s, 4-Me); 1.25 (3H, dt, J = 7.14, 0.96 Hz, –O–CH$_2$-**Me**); 0.96 (3H, d, J = 6.73 Hz, 10-Me); 0.94 (3H, d, J = 7.42 Hz, 11-Me).

IR (Film, cm^{-1}): 2939, 2874, 1755; 1736 (C=O), 1587; 1489 (C=C), 1448, 1375, 1263, 1194, 1176, 1101, 1022, 878, 785.

Anal. Calcd for $C_{27}H_{38}O_8$: C, 66.10; H, 7.81. Found: C, 66.31; H, 7.67.

4.3.2. Ethyl 2-[3-(12-α-artemisinoxymethyl)]phenoxyl propionate (9d). Colorless oil. Yield: 20%. ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 7.24 (1H, t, J = 7.88 Hz, Ar-H); 6.96 (1H, d, J = 7.55 Hz, Ar-H); 6.88 (1H, br s, Ar-H); 6.78 (1H, dt, J = 8.23, 1.26 Hz, Ar-H); 5.33 (1H, s, 5-H); 4.93 (1H, d,d, J = 12.59, 1.93 Hz, 16-Ha); 4.74 (1H, dq, J = 6.80,1.50 Hz, Ar-O–C**H**-Me); 4.59 (1H, d, J = 12.58 Hz, 16-Hb); 4.49 (1H, d, J = 9.41 Hz, 12-H); 4.21 (2H, m, –O–C**H**$_2$-Me); 2.49 (1H, m, 11-H); 2.38 (1H, m, 3-Hβ); 1.61 (3H, d, J = 6.71 Hz, Ar-O–CH-**Me**); 1.46 (3H, s, 4-Me); 1.25 (3H, t, J = 7.05, Hz, –O–CH$_2$-**Me**); 0.95 (3H, d, J = 5.87 Hz, 10-Me); 0.94 (3H, d, J = 6.04 Hz, 11-Me).

IR (Film, cm^{-1}): 2937, 2874, 1755; 1736 (C=O), 1610; 1587 (C=C), 1454, 1375, 1263, 1032, 879, 850, 785.

Anal. Calcd for $C_{27}H_{38}O_8$: C, 66.10; H, 7.81. Found: C, 66.15; H, 7.91.

4.3.3. Ethyl 2-[4-(12-β-artemisinoxymethyl)]phenoxyl propionate (9g). Colorless oil. Yield: 35%. ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 7.21 (2H, d, J = 8.79 Hz, Ar-H); 6.84 (2H, d, J = 8.70 Hz, Ar-H); 5.45 (1H, s, 5-H); 4.87 (1H, d, J = 3.52 Hz, 12-H); 4.81 (1H, d, J = 11.92 Hz, 16-Ha); 4.73 (1H, q, J = 6.74 Hz, Ar-O–C**H**-Me); 4.44 (1H, d, J = 12.02 Hz, 16-Hb); 4.21 (2H, dq, J = 7.13, 1.27 Hz, –O–C**H**$_2$-Me); 2.64 (1H, m, 11-H); 2.37 (1H, m, 3-Hβ); 1.61 (3H, d, J = 6.74 Hz, Ar-O–CH-**Me**); 1.45 (3H, s, 4-Me); 1.25 (3H, dt, J = 7.18, 1.56 Hz, –O–CH$_2$-**Me**); 0.96 (3H, d, J = 6.15 Hz, 10-Me); 0.91 (3H, d, J = 7.32 Hz, 11-Me).

IR (Film, cm^{-1}): 2939, 2874, 1751; 1732 (C=O), 1612; 1510 (C=C); 1448, 1375, 1232, 1194, 1136, 1101, 1032, 878, 825.

Anal. Calcd for $C_{27}H_{38}O_8$: C, 66.10; H, 7.81. Found: C, 66.03; H, 7.70.

4.3.4. Ethyl 2-[4-(12-α-artemisinoxymethyl)]phenoxyl propionate (9i). Colorless oil. Yield: 25%. ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 7.26 (1H, d, J = 8.51 Hz, Ar-H); 7.25 (1H, d, J = 8.65 Hz, Ar-H); 6.85 (1H, d, J = 8.65 Hz, Ar-H); 6.83 (1H, d, J = 8.51 Hz, Ar-H); 5.32 (1H, s, 5-H); 4.88 (1H, d, J = 12.09 Hz, 16-Ha); 4.73 (1H, q, J = 6.73 Hz, Ar-O–C**H**-Me); 4.56 (1H, d,

J = 12.09 Hz, 16-Hb); 4.47 (1H, d, J = 9.20 Hz, 12-H); 4.21 (2H, dq, J = 7.14, 1.16 Hz, –O–C**H**$_2$-Me); 2.47 (1H, m, 11-H); 2.38 (1H, m, 3-Hβ); 1.61 (3H, d, J = 7.56 Hz, Ar-O–CH-**Me**); 1.46 (3H, s, 4-Me); 1.26 (3H, dt, J = 7.14, 2.06 Hz, –O–CH$_2$-**Me**); 0.94 (3H, d, J = 5.91 Hz, 10-Me); 0.86 (3H, dd, J = 7.14, 1.65 Hz, 11-Me).

IR (Film, cm^{-1}): 2937, 2874, 1751; 1736 (C=O), 1612; 1510 (C=C); 1448, 1375, 1240, 1200, 1134, 1030, 879, 825.

Anal. Calcd for $C_{27}H_{38}O_8$: C, 66.10; H, 7.81. Found: C, 66.18; H, 7.49.

4.3.5. Ethyl 2-[4-(12-β-artemisinoxy)-1′-ethyl]phenoxyl propionate (9l). Colorless oil. Yield: 30%. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.20 (2H, d, J = 9.13 Hz, Ar-H); 6.80 (2H, d, J = 9.20 Hz, Ar-H); 5.50 (1H, s, 5-H); 4.88 (1H, q, J = 6.45 Hz, Ar-O–C**H**-Me); 4.72 (1H, dq, J = 6.73, 2.20 Hz, Q-O–C**H**-Me); 4.61 (1H, d, J = 3.57 Hz, 12-H); 4.20 (2H, m, –O–C**H**$_2$-Me); 2.50 (1H, m, 11-H); 2.36 (1H, m, 3-Hβ); 1.60 (3H, d, J = 6.18 Hz, Ar-O–CH-**Me**); 1.44 (3H, s, 4-Me); 1.40 (3H, d, J = 6.45 Hz, Q-O–CH-**Me**) 1.24 (3H, m, –O–CH$_2$-**Me**); 0.97 (3H, d, J = 6.32 Hz, 10-Me); 0.77 (3H, dd, J = 7.28, 2.00 Hz, 11-Me).

IR (Film, cm^{-1}): 2924, 2874, 1755; 1736 (C=O), 1610; 1510 (C=C), 1448, 1375, 1240, 1194, 1134, 1099, 1032, 1011, 984, 876, 829.

Anal. Calcd for $C_{28}H_{40}O_8$: C, 66.65; H, 7.99. Found: C, 66.60; H, 7.88.

4.3.6. Ethyl 2-[4-(12-artemisinoxy)-1′-ethyl]phenoxyl propionate (9n). A mixture of 12-α and 12-β isomers (α/β = 1/2). Colorless oil. Yield: 25%. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.22 (1H, d, J = 7.69 Hz, Ar-H); 7.20 (1H, d, J = 8.25 Hz, Ar-H); 6.82 (2H, d, J = 8.51 Hz, Ar-H); 5.19, 5.17, (1H, s, s, 5-H); 5.03, 4.21 (1H, d,d, J = 3.44, 9.47 Hz, 12-H); 4.96, 4.88 (1H, q, q, J = 6.46, 6.32 Hz, Ar-O–C**H**-Me); 4.72 (1H, m, Q-O–C**H**-Me); 4.20 (2H, m, –O–C**H**$_2$-Me); 2.66, 2.42 (1H, m, m, 11-H); 2.36 (1H, m, 3-Hβ); 1.61 (3H, d, J = 6.46 Hz, Ar-O–CH-**Me**); 1.46, 1.35 (3H, s, s, 4-Me); 1.41, (3H, m, Q-O–CH-**Me**) 1.24 (3H, m, –O–CH$_2$-**Me**); 0.95, 0.91 (3H, d, d, J = 7.42, 10.30 Hz, 10-Me); 0.89, 0.78 (3H, d, d, J = 10.44, 7.35 Hz, 11-Me).

IR (Film, cm^{-1}): 2926, 2873, 1755; 1736 (C=O), 1610; 1512 (C=C), 1448, 1375, 1281, 1240, 1195, 1134, 1099, 1016, 984, 876, 829.

Anal. Calcd for $C_{28}H_{40}O_8$: C, 66.65; H, 7.99. Found: C, 66.77; H, 8.11.

4.4. Synthesis of free acid artemisinin derivatives (9a, 9c, 9f, 9h, 9k, and 9m)

The benzyl ether derivative (**9b**, **9d**, **9g**, **9j**, **9l**, **9n**, 4.0 mmol) was dissolved in 0.5% KOH/EtOH solution (60 mL), and the mixture solution was stirred at about 40–50 °C overnight. After neutralization with acetic acid, the solution was evaporated in vacuum. The residue was dissolved in EtOAc, washed with brine, dried over anhydrous $MgSO_4$ and evaporated to dryness under reduced pressure. The resultant solid was purified by recrystallization or column chromatography (silica gel, using ethyl acetate as the eluent) to give the corresponding free acid (**9a**, **9c**, **9f**, **9h**, **9k**, and **9m**).

4.4.1. 2-[3-(12-β-Artemisinoxymethyl)]phenoxyl propionic acid (9a). White amorphous solid. Yield: 95%. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.26 (1H, t, J = 7.90 Hz, Ar-H); 6.95 (1H, d, J = 7.27 Hz, Ar-H); 6.89 (1H, br s, Ar-H); 6.83 (1H, d, J = 8.33 Hz, Ar-H); 5.44 (1H, s, 5-H); 4.90 (1H, d, J = 2.99 Hz, 12- H); 4.85 (1H, d,d, J = 12.39, 3.85 Hz, 16-Ha); 4.79 (1H, q, J = 6.95 Hz, Ar-O–C**H**-Me); 4.50 (1H, d, J = 12.61 Hz, 16-Hb); 2.68 (1H, m, 11- H); 2.38 (1H, m, 3-Hβ); 1.66 (3H, d, J = 6.84 Hz, Ar-O–CH-**Me**); 1.46 (3H, s, 4-Me); 0.94 (6H, d, J = 6.41 Hz, 11-Me, 10-Me).

IR (Film, cm^{-1}): 3435 (–OH), 2941, 2874, 1736 (C=O), 1587; 1490 (C=C); 1450, 1377, 1263, 1101, 1030, 1011,876, 826.

Anal. Calcd for $C_{25}H_{34}O_8$: C, 64.92; H, 7.41. Found: C, 64.64; H, 7.61.

4.4.2. 2-[3-(12-α-Artemisinoxymethyl)]phenoxyl propionic acid (9c). White amorphous solid. Yield: 95%. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.24 (1H, t, J = 7.91, 3.50 Hz, Ar-H); 6.97 (1H, dd, J = 7.48, 4.27 Hz, Ar-H); 6.93 (1H, bs, Ar-H); 6.82 (1H, dt, J = 7.91, 2.59 Hz, Ar-H); 5.34 (1H, s, 5-H); 4.92 (1H, dd, J = 12.61, 7.91 Hz, 16-Ha); 4.79 (1H, q, J = 6.84 Hz, Ar-O–C**H**-Me); 4.60 (1H, d, J = 12.61, 8.33 Hz, 16-Hb); 4.50, 4.45 (1H, d,d, J = 9.40, 9.20 Hz, 12-H); 2.49 (1H, m, H-11); 2.39 (1H, m, 3-Hβ); 1.65 (3H, d, J = 6.84 Hz, Ar-O–CH-**Me**); 1.46, 1.45 (3H, s, s, 4 -Me); 0.95, 0.94 (3H, d, d, J = 5.99, 5.77 Hz, 10-Me); 0.91, 0.89 (3H, d, d, J = 7.05, 7.05 Hz, 11-Me).

IR (Film, cm^{-1}): 3427 (–OH), 2928, 2874, 1736 (C=O), 1587; 1490 (C=C); 1454, 1377, 1263, 1155, 1134, 1032, 878, 825.

Anal. Calcd for $C_{25}H_{34}O_8$: C, 64.92; H, 7.41. Found: C, 64.76; H, 7.66.

4.4.3. 2-[4-(12-β-Artemisinoxymethyl)]phenoxyl propionic acid (9f). White amorphous solid. Yield: 95%. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.22 (2H, d, J = 8.56 Hz, Ar-H); 6.86 (2H, d, J = 8.56 Hz, Ar-H); 5.45 (1H, s, 5-H); 4.88 (1H, d, J = 3.36 Hz, 12-H); 4.80(1H, d, J = 11.24 Hz, 16-Ha); 4.78 (1H, q, J = 7.05 Hz, Ar-O–C**H**-Me); 4.43 (1H, d, J = 11.92 Hz, 16-Hb); 2.64 (1H, m, 11-H); 2.37 (1H, m, 3-Hβ); 1.65 (3H, d, J = 6.88 Hz, Ar-O–CH-**Me**); 1.45 (3H, s, 4-Me); 0.94 (3H, d, J = 6.21 Hz, 10-Me); 0.93 (3H, d, J = 7.39 Hz, 11-Me).

IR (KBr, cm^{-1}): 3425 (–OH); 3100 (Ar-H), 2941, 2873, 1736 (C=O); 1612; 1512 (C=C), 1450, 1377, 1240, 1134, 1099, 1009, 876, 825.

Anal. Calcd for $C_{25}H_{34}O_8$: C, 64.92; H, 7.41. Found: C, 64.72; H, 7.67.

4.4.4. 2-[4-(12-α-Artemisinoxymethyl)]phenoxyl propionic acid (9h). White amorphous solid. Yield: 95%. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.28 (2H, d, J = 8.73 Hz, Ar-H); 6.86 (2H, d, J = 8.56 Hz, Ar-H); 5.33 (1H, s, 5-H); 4.88 (1H, d, J = 11.92 Hz, 16-Ha); 4.78 (1H, q, J = 6.83 Hz, Ar-O–C**H**-Me); 4.56 (1H, d, J = 12.09 Hz, 16-Hb); 4.48 (1H, d, J = 9.23 Hz, 12-H); 2.48 (1H, m, 11-H); 2.38 (1H, m, 3-Hβ); 1.65 (3H, d, J = 6.88 Hz, Ar-O–CH-**Me**); 1.46 (3H, s, 4-Me); 0.95 (3H, d, J = 6.04 Hz, 10-Me); 0.88 (3H, d, d, J = 7.21, 1.51 Hz, 11-Me).

IR (KBr, cm^{-1}): 3425; 3250 (–OH), 2937, 2874, 1736 (C=O); 1614; 1512 (C=C), 1454, 1377, 1240-1132, 1032, 876, 825.

Anal. Calcd for $C_{25}H_{34}O_8$: C, 64.92; H, 7.41. Found: C, 64.56; H, 7.75.

4.4.5. 2-[4-(12-β-Artemisinoxy)-1′-ethyl]phenoxyl propionic acid (9k). White amorphous solid. Yield: 95%. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.21 (2H, dd, J = 8.62, 1.65 Hz, Ar-H); 6.83 (2H, d, J = 8.62 Hz, Ar-H); 5.51 (1H, s, 5-H); 4.88 (1H, p, J = 6.32 Hz, Ar-O–C**H**-Me); 4.77 (1H, dq, J = 6.78, 2.01 Hz, Q-O–C**H**-Me); 4.62, 4.61 (1H,d,d, J = 2.75 Hz, 12-H); 2.52 (1H, m, 11-H); 2.37 (1H, m, 3-Hβ); 1.66 (3H, d, J = 6.59 Hz, Ar-O–CH-**Me**); 1.44 (3H, s, 4-Me); 1.40 (3H, d, J = 8.07 Hz, Q-O–CH-**Me**); 0.98 (1H, J = 6.23, 10-Me); 0.79,0.78 (1H, dd, J = 7.33, 2.57 Hz, 11-Me).

IR (Film, cm^{-1}): 3430 (–OH), 2926, 2860, 1736 (C=O), 1610; 1512 (C=C), 1450, 1377, 1240, 1099, 1032, 984, 876, 827.

Anal. Calcd for $C_{26}H_{36}O_8$: C, 65.53; H, 7.61. Found: C, 65.42; H, 7.75.

4.4.6. 2-[4-(2-Artemisinoxy)-1′-ethyl]phenoxyl propionic acid (9m). A mixture of 12-α and 12-β isomers (α/β = 1/1). White amorphous solid. Yield: 95%. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.24 (2H, dd, J = 8.07, 2.02 Hz, Ar-H); 6.86 (2H, dd, J = 8.70, 2.10 Hz, Ar-H); 5.19 (1H, s, 5-H); 5.05, 4.21 (1H, d,d, J = 3.49, 9.35 Hz, 12-H); 4.98, 4.88 (1H, q, q, J = 6.41, 6.41 Hz, Ar-O–C**H**-Me); 4.79 (1H, m, Q-O–C**H**-Me); 2.68, 2.42 (1H, m, m, 11-H); 2.34 (1H, m, 3-Hβ); 1.66 (3H, d, d, J = 6.96, 1.10 Hz, Ar-O–CH-**Me**); 1.47, 1.42 (3H, s, s, 4-Me); 1.45, 1.37 (3H, m, Q-O–CH-**Me**); 0.97, 0.91 (3H, d, d, J = 7.33, 5.68 Hz, 10-Me); 0.90, 0.80 (3H, d, d, J = 7.15, 7.33 Hz, 11-Me).

IR (KBr, cm^{-1}): 3429 (–OH), 2928, 2873, 1736 (C=O), 1610; 1512 (C=C), 1452, 1377, 1240, 1134, 1099, 1011, 984, 876, 827.

Anal. Calcd for $C_{26}H_{36}O_8$: C, 65.53; H, 7.61. Found: C, 65.47; H, 7.61.

4.5. Synthesis of aryl ester artemisinin derivatives (9e, 9j, and 9o)

To a solution of above free acid (**9a**, **9c**, **9f**, **9h**, **9k**, **9m**, 5.0 mmol) and *o*-methoxyphenol (0.6 mL, 5.5 mmol) in dry ether (50 mL), DCC (1.13 g, 5.5 mmol) and DMAP (cat. 122 mg, 0.5 mmol) were added, and the solution was stirred for 2–5 h at room temperature. After the condensation reaction was complete, the solvent was evaporated and the solid residue was macerated with cold acetone. The acetone-insoluble portion melted at 225–227 °C and was identified as dicyclohexylurea (DCU). The acetone-soluble fraction was evaporated to dryness, and the residue was purified by column chromatography (silica gel, using ethyl acetate/petroleum ether 1:25 v/v as the eluent) to give the target aryl esters (**9e**, **9j**, and **9o**).

4.5.1. 2-Methoxyphenyl 2-[3-(12-β-artemisinoxymethyl)]phenoxyl propionate (9e). White amorphous solid. Yield: 40%. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.27 (1H, t, J = 7.84 Hz, Ar-H); 7.20 (1H, dt, J = 7.42, 1.79 Hz, Ar-H); 6.99–6.85 (6H, m, Ar-H); 5.46 (1H, s, 5-H); 5.00 (1H, dq, J = 6.78, 1.65 Hz, Ar-O–C**H**-Me); 4.92 (1H, d, J = 3.43 Hz, 12-H); 4.89 (1H, dd, J = 12.24, 2.20 Hz, 16-Ha); 4.51 (1H, d, J = 11.41 Hz, 16-Hb); 3.77 (3H, s, Ar-O-Me); 2.67 (1H, m, 11-H); 2.37 (1H, m, 3-Hβ); 1.81 (3H, d, J = 6.74 Hz, Ar-O–CH-**Me**); 1.46 (3H, s, 4-Me); 0.96 (3H, d, J = 7.42 Hz, 10-Me); 0.91 (3H, d, J = 6.05 Hz, 11-Me).

IR (KBr, cm^{-1}): 2941, 2874, 1782 (C=O), 1608; 1502 (C=C), 1456, 1375, 1259-1028, 1109, 1028, 876, 825, 750.

Anal. Calcd for $C_{32}H_{40}O_9$: C, 67.59; H, 7.09. Found: C, 67.54; H, 7.17.

4.5.2. 2-Methoxyphenyl 2-[4-(12-β-artemisinoxymethyl)]phenoxyl propionate (9j). White amorphous solid. Yield: 45%. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.26 (2H, d, J = 8.41 Hz, Ar-H); 7.20 (1H, td, J = 7.73 Hz, J = 1.76 Hz, Ar-H); 7.02–6.96 (4H, m, Ar-H); 6.95–6.84 (1H, m, Ar-H); 5.46 (1H, s, 5-H); 4.99 (1H, q, J = 6.78 Hz, Ar-O–C**H**-Me); 4.90 (1H, d, J = 3.13 Hz, 12-H); 4.84 (1H, d, J = 11.74 Hz, 16-Ha); 4.46 (1H, d, J = 11.93 Hz, 16-Hb); 3.77 (3H, s, Ar-O-Me); 2.66 (1H, m, 11-H); 2.38 (1H, m, 3-Hβ); 1.82 (3H, d, J = 6.85 Hz, Ar-O–CH-**Me**); 1.47 (3H, s, 4-Me); 0.95 (3H, d, J = 6.85 Hz, 10-Me); 0.93 (3H, d, J = 7.63 Hz, 11-Me).

IR (KBr, cm^{-1}): 2939, 2874, 1782 (C=O), 1610; 1502 (C=C), 1456, 1375, 1248-1159, 1109, 1026, 876, 825, 750.

Anal. Calcd for $C_{32}H_{40}O_9$: C, 67.59; H, 7.09. Found: C, 67.55; H, 7.21.

4.5.3. 2-Methoxyphenyl 2-[4-(12-β-Artemisinoxy)-1′-ethyl]phenoxyl propionate (9o). White amorphous solid. Yield: 40%. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.28–7.16 (3H, m, Ar-H); 7.00–6.84 (5H, m, Ar-H);

5.52 (1H, s, 5-H); 4.98 (1H, q, $J = 6.78$ Hz, Ar-O–C**H**-Me); 4.92 (1H, q, $J = 6.55$ Hz, Q-O–C**H**-Me); 4.65 (1H, d, $J = 3.29$ Hz, 12-H); 3.89, 3.77 (3H, s, s, Ar-O-Me); 2.53 (1H, m, 11-H); 2.38 (1H, m, 3-Hβ); 1.81 (3H, d, $J = 6.73$ Hz, Ar-O–CH-**Me**); 1.46 (3H, s, 15-Me); 1.43 (3H, d, $J = 6.59$ Hz, Q-O–CH-**Me**); 0.98 (3H, d, $J = 6.32$ Hz, 10-Me); 0.80 (3H, d, $J = 7.41$ Hz, 11-Me).

IR (Film, cm^{-1}): 2924, 2874, 1782 (C=O), 1610; 1502 (C=C); 1456, 1375, 1259, 1097, 1032, 990, 876, 827.

Anal. Calcd for $C_{33}H_{42}O_9$: C, 68.02; H, 7.27. Found: C, 68.00; H, 7.13.

4.6. Biological assay

4.6.1. Materials. Stock solution of compounds was prepared with 100% dimethylsulfoxide (DMSO, Sigma) and diluted with RPMI 1640 medium containing 10% fetal bovine serum (FBS). RPMI 1640 medium powder was purchased from Gibco-BRL, Life Technologies. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide], concanavalin A (ConA), and lipopolysaccharide (LPS) were purchased from Sigma. [^{3}H]Thymidine (1 mci/mL) was purchased from Shanghai Institute of Atomic Energy (SIAE). The related in vitro experimental procedure was performed according to our previous work.[11]

4.6.2. Animals. BALB/c mice, used at 6–8 weeks of age, were purchased from Shanghai Experimental Animal Center and were housed in a controlled environment (12-h light/12-h dark photoperiod, 22 ± 1 °C, 55% ± 5% relative humidity). All husbandry and experimental contacts made with the mice were maintained at specific pathogen-free conditions. All mice were allowed to acclimatize in our facility for 1 week before any experiment started. All experiments were carried out according to the NIH Guide for Care and Use of Laboratory Animals and were approved by the Bioethics Committee of the Shanghai Institute of Materia Medica.

4.6.3. Preparation of spleen cell from mice. BALB/C mice were sacrificed and spleens were removed aseptically. A single cell suspension was prepared after cell debris and clumps were removed. Erythrocytes were depleted with ammonium chloride buffer solution. Lymphocytes were washed 3 times with PBS containing 2% FBS and were resuspended in RPMI 1640 medium at the indicated concentration.

4.6.4. Cytotoxicity assay. Fresh spleen cells were obtained from BALB/C mice (male, 7–9 weeks old). 5×10^5 of spleen cells were cultured in 96-well flat plates with 200 μL RPMI 1640 media containing 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin in a humidified, 37 °C, 5% CO_2-containing incubator for 48 h, in the presence or absence of various concentrations of compounds. Eighteen microliters of MTT (5 mg/mL) was added to each well at the final 5 h culture. Then 90 μL of lysis buffer (10% SDS, 50% DMF, pH 7.2) was added to each well for 6–7 h and the absorbance values at 570 nm were collected by microplate reader (Bio-Rad, Model 550). The percentage of cell death was determined by using the following calculating formula:

$$\text{Cytotoxicity}(\%) = \frac{\text{Compounds } (OD_{570}) - \text{Background } (OD_{570})}{\text{Control } (OD_{570}) - \text{Background } (OD_{570})} \times 100$$

4.6.5. T cell and B cell function assay. Fresh spleen cells were obtained from BALB/C mice (male, 7–9 weeks old). 5×10^5 of spleen cells were cultured at the same conditions to that mentioned above. The cultures were unstimulated or stimulated with 5 μg/mL of concanavalin A (ConA) or 10 μg/mL of lipopolysaccharide (LPS) to induce T cell or B cell proliferative responses for 48 h, respectively. The compounds were added to cultures with indicated concentrations to test their bioactivities. Proliferation was assessed in terms of uptake of [^{3}H]thymidine during 8 h pulsing with 20 kBq of [^{3}H]thymidine for each well, and then cells will be harvested onto glass fiber filters by a Basic 96 harvester. The incorporated radioactivity was counted by a liquid scintillation counter (1540 MicroBeta Trilux, Perkin-Elmer Life Sciences).

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