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Synthesis and Immunosuppressive Activity of New Artemisinin Derivatives. 1. [12(β or □)-Dihydroartemisininoxy]phen(ox)yl Aliphatic Acids and Esters

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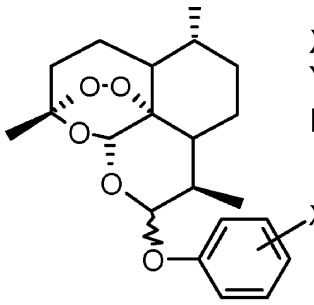

X=none, -O-, -OCO-, -CONH;
Y=-CH_2, -CH_2CH_2-, -CHMe;
R= H, Me, Et, -$CH_2CH_2NMe_2$,
2-Methoxyphenyl

X—Y—COOR

11b, 12-β, p-, X = O, Y = -CH_2-

	[SI]	
Inhibition on T cell		Inhibition on B cell
11b	848	28473
CsA	963	7
Artemether	3	48

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Synthesis and Immunosuppressive Activity of New Artemisinin Derivatives. 1. [12(β or α)-Dihydroartemisininoxy]phen(ox)yl Aliphatic Acids and Esters

Zhong-Shun Yang,[§] Wen-Liang Zhou,[‡] Yi Sui,[§] Jun-Xia Wang,[‡] Jin-Ming Wu,[§] Yu Zhou,[‡] Yu Zhang,[§] Pei-Lan He,[‡] Ji-Ye Han,[§] Wei Tang,[‡] Ying Li,*[,§] and Jian-Ping Zuo*[,‡]

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A series of novel dihydroartemisinin derivatives were synthesized and evaluated on their immunosuppressive activity in the search for potential immunosuppressive agents with high efficacy and low toxicity. These compounds were assayed in their cytotoxicity of lymphocyte, inhibition activity on concanavalin A (ConA) induced T cell proliferation and lipopolysaccharide (LPS) induced B cell proliferation. Among them, **11b**, **13b**, **14d**, **15b**, **16**, and **17** remarkably exhibited lower cytotoxicity and higher inhibition activity on the mitogen-induced T cell and B cell proliferation in comparison with artemisinin, artesunate, and artemether in vitro. More significantly, compound **11b** displayed reduced cytotoxicity by over 100-fold compared with cyclosporin A (CsA) and comparable inhibition activity (SI = 848) on ConA-induced T cell proliferation to CsA (SI = 963) and more than 4000 times the inhibitory effect (SI = 28473) on LPS-induced B cell proliferation compared with CsA (SI = 7) in vitro. The in vivo experimental results showed that compound **16** could inhibit 2,4-dinitrofluorobenzene (DNFB)-induced delayed-type hypersensitivity (DTH) reaction and sheep red blood cell (SRBC) induced antibody production, respectively. The structure and activity relationships (SAR) of these compounds were also discussed.

Introduction

Since Murry succeeded in carrying out the first kidney transplant operation in 1954,[1] organ transplantation has grown tremendously during the last part of the 20th century.[2] The knowledge that the T-lymphocyte played an integral role in transplant rejection brought cyclosporine A (CsA) and tacrolimus (FK506) to the fore as therapeutic immunosuppressants.[3] However, the currently clinically used immunosuppressive drugs such as glycocorticoids (e.g., cortisol), cyclosporin A (CsA), tacrolimus (FK506), and sirolimus (rapamycin), etc., despite their undeniable clinical advantages, were proved to possess rather serious side effects including renal toxicity, liver toxicity, infection, malignancy, cosmetic effects, and other bad responses.[4−8]

In the search for new potential immunosuppressive agents with high efficacy and low toxicity, we turned our attention to the traditional Chinese medicine artemisinin (qinghaosu, **1**). It is well-known that artemisinin opened a new era in malaria chemotherapy.[9] In fact, artemisinin and its derivatives have a variety of bioactivities besides their outstanding antimalarial activity. In general, antimalarial drugs possess immunosuppressive action and have been used for the treatment of dermatosis.[10] Since the 1980s, the immunosuppressive action of artemisinin and its derivatives has been studied in China. The experimental results suggested that qinghaosu **1**, dihydroartemisinin **2**, artemether **3**, arteether **4**, and artesunic acid **5** (Figure 1) had definite immunosuppressive activity.[11−18] Qinghao extracts and qinghaosu were tried for treatment of the systemic lupus erythematosus (SLE) patients.[19] Besides, high immunosuppressive action of sodium artesunate had been demonstrated on SLE mice model and patients with lupus erythematosus.[20] Recently, both in vivo (delayed-type hypersensitivity (DTH) response) and in vitro (spectrofluorometry) studies indicated the activity of artemisinin as an immunosuppressive agent was more than that of cyclosporin A (CsA).[21]

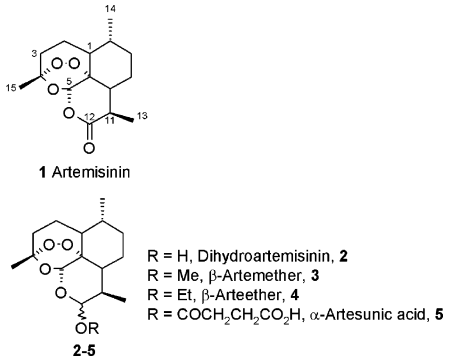

Figure 1. Artemisnin and its semisynthetic derivatives.

Currently, nonsteroidal antiinflammatory drugs (NSAIDs) and some immunosuppressive agents remain the first-line therapy for most people with arthritis. In addition, lessons from allograft tolerance could be extended to the treatment of autoimmune diseases[22] such as rheumatoid arthritis (RA)[23−29] and SLE.[30]

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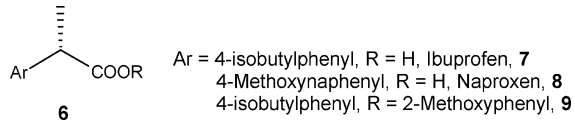


Figure 2. Several important nonsteroidal anti-inflammatory drugs (NSAIDs).

Considering the certain relevance of NSAIDs with the immunosuppressive activity, we are interested in combining artemisinin and the NSAIDs to develop a new class of immunosuppressive agents. 2-Arylpropanoic acids are a type of widely used nonsteroidal antiinflammatory drugs with excellent antipyretic, antiinflammatory and analgesic effects. Among them, ibuprofen **7**, naproxen **8**, and guaiacol ester of ibuprofen **9** were the typical representatives[31] with a general formula, **6** (Figure 2). We observed that each of them consists of both a hydrophilic part (carboxylic group) and a lipophilic part (aromatic ring); thus, we supposed that amphiphilicity may be one of the most important features of these compounds.

In view of the aforementioned observations, we attempted to link the artemisinin nucleus with various arylaliphatic acids and esters, which are similar to compound **6** in structure, to improve their bioactivity and bioavailability. Thus, as part of an ongoing research project in the search for highly potent, low toxicity, specific-selective, and tolerance-inducing immunosuppressive agents, a series of [12(β or α)-dihydroartemisininoxy]phen(ox)yl aliphatic acids and esters **10**−**17** were synthesized and tested in cytotoxicity T cell and B cell functional assays for evaluating their immunosuppressive activity in vitro. Furthermore, some of them have been tested in vivo for their immunosuppressive effects. Compound **16**, the representative of these new artemisinin derivatives, was investigated in the animal models of 2,4-dinitrofluorobenzene (DNFB) induced delayed-type hypersensitivity (DTH) reaction and sheep red blood cell (SRBC) induced antibody production.

Chemistry

Dihydroartemisinin **2** was prepared by sodium borohydride reduction of artemisinin **1** as previously reported.[32] Dihydroartemisinin acetate, **25**, and **26** were prepared according to the literature procedure.[33] To probe their structure−activity relationships (SAR), we synthesized eight classes of novel structure-related dihydroartemisinin derivatives **10**−**17** (Figure 3).

The new aryl ethers (**10b**, **11b**, **12b**, **12d**, **13b**, **14b**, **14d**, **15b**) of dihydroartemisinin were prepared by treatment of dihydroartemisinin acetate **18** with appropriately substituted phenols **19**−**24** in the presence of trifluoacetic acid (TFA) or boron trifluoride etherate (BF_3·Et_2O) at room temperature (Schemes 1−4). Similar to previous work,[33,34] these aryl ether derivatives are almost all 12-β epimers under the catalysis of TFA, with the exception of compound **12b** wherein the 12-α epimer is predominant. In our former work, the reaction of **18** with resorcinol afforded the 12-C glycoside compound **12h**.[35] However, a similar reaction of **18** with **21**, the monoether of resorcinol, gave not the 12-C glycoside isomer **12g** but only the 12-O glycoside isomer **12d** (Scheme 2). This result may be caused by the steric hindrance effect of substrate **21** with a bulky substituent. These aryl ethers, after purification by column chromatography, were hydrolyzed with 0.5% KOH/EtOH solution followed by acidification and then recrystallization or column chromatography of the crude products to furnish the corresponding free acids (**10a**, **11a**, **12a**, **12c**, **13a**, **14a**, **14c**, **15a**), whose composition (α/β) was the same as that of precursory esters. The free acids then were converted into the corresponding target esters (**11c**, **12e**, **12f**, **13c**, **15c**) in moderate to good yields by condensation with the appropriate alcohol (for **11c**) or phenol. It is worth noting that an additional chiral center present in compounds **12a**−**f**, **13a**−**c**, and **15a**−**c** was due to the employment of material (*dl*)-ethyl

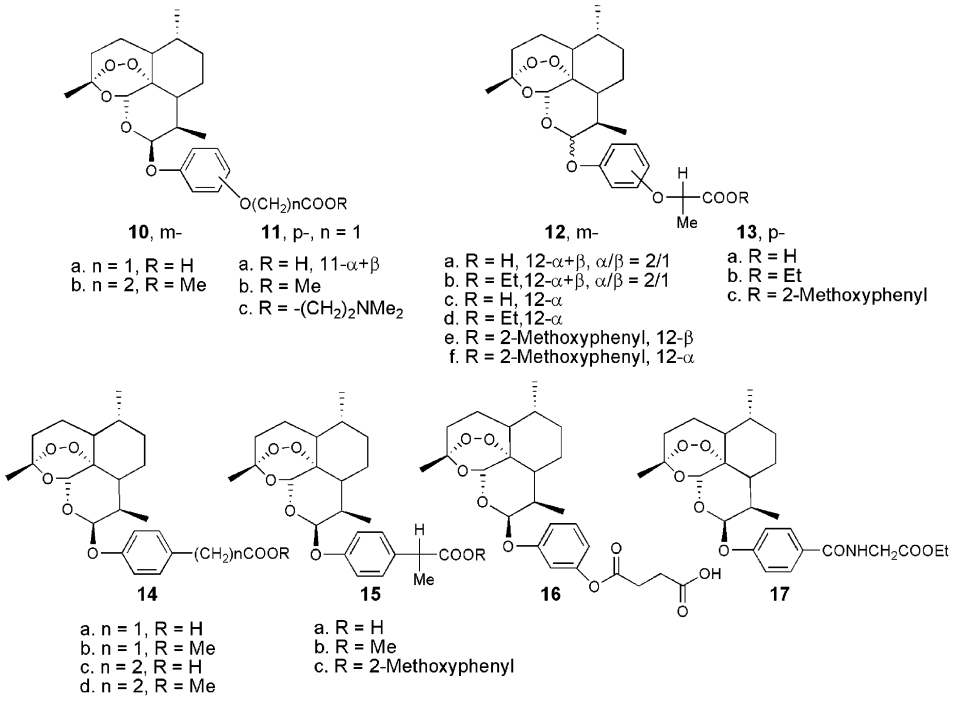
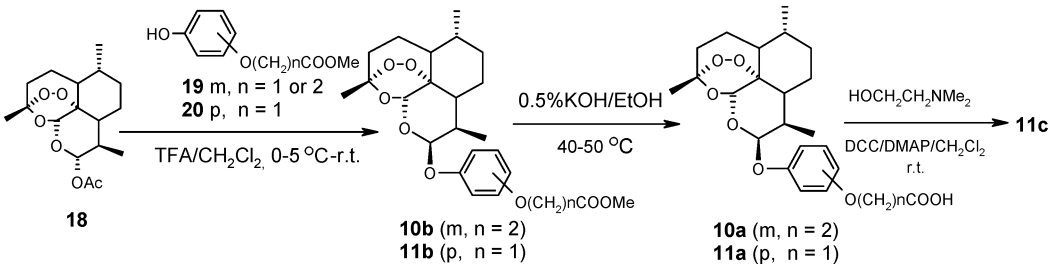
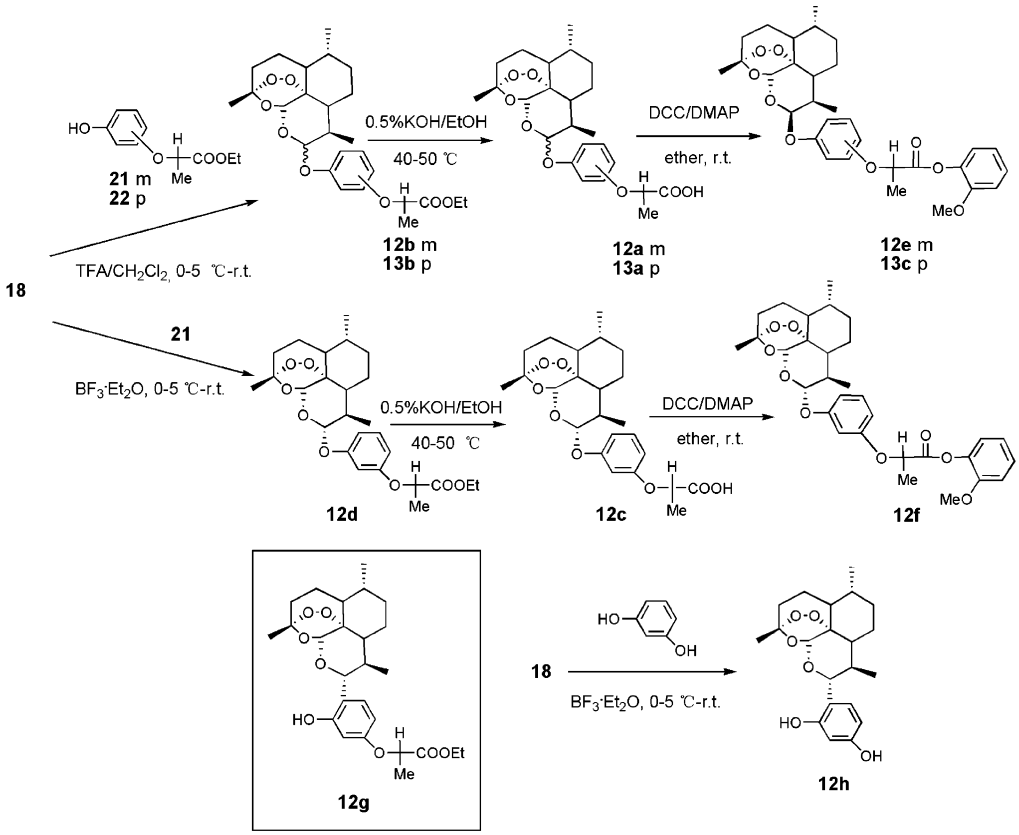


Figure 3. Eight classes of novel dihydroartemisinin derivatives.

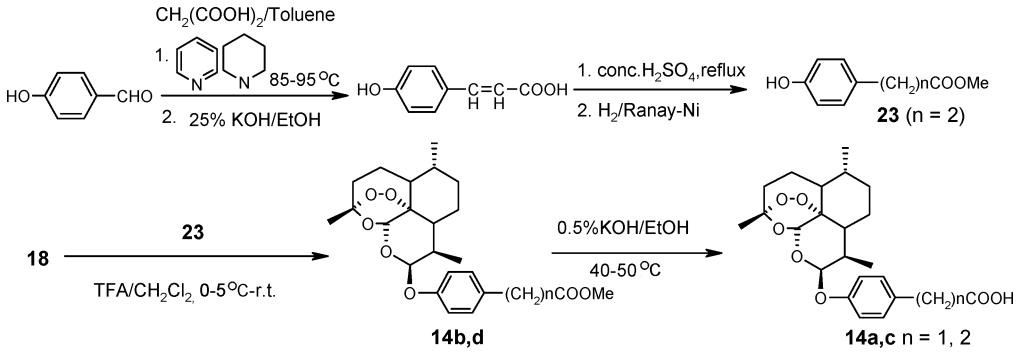
Scheme 1. Synthetic Routes to **10a**,**b** and **11a**−**c**



Scheme 2. Synthetic Routes to **12a**−**h**, **13a**−**c**



Scheme 3. Synthetic Routes to **14a**−**d**



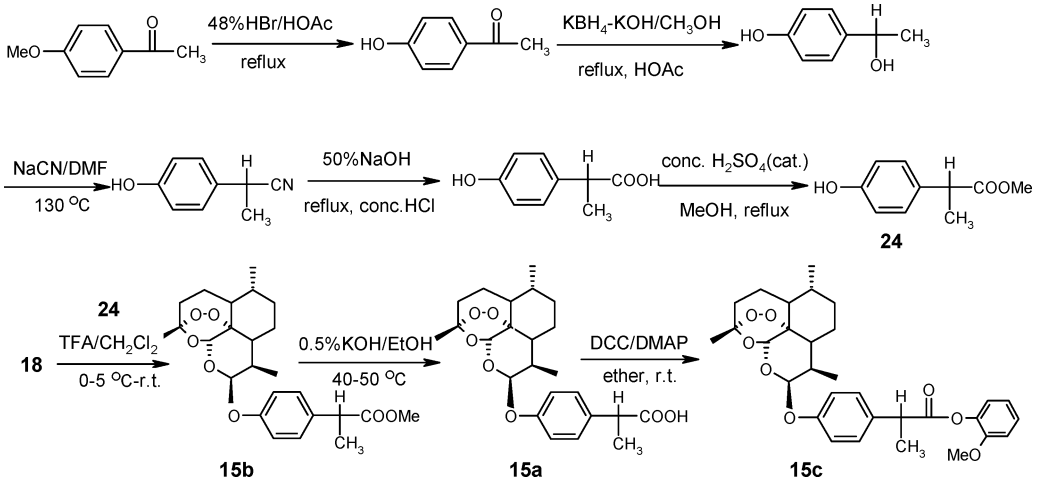
2-bromopropionate or nonstereoselective preparation of intermediate **24**.

It was interesting that the racemization of the 11-methyl group in the artemisinin nucleus from compound **11a** arose during the course of hydrolysis, which was embodied in its proton NMR spectra.

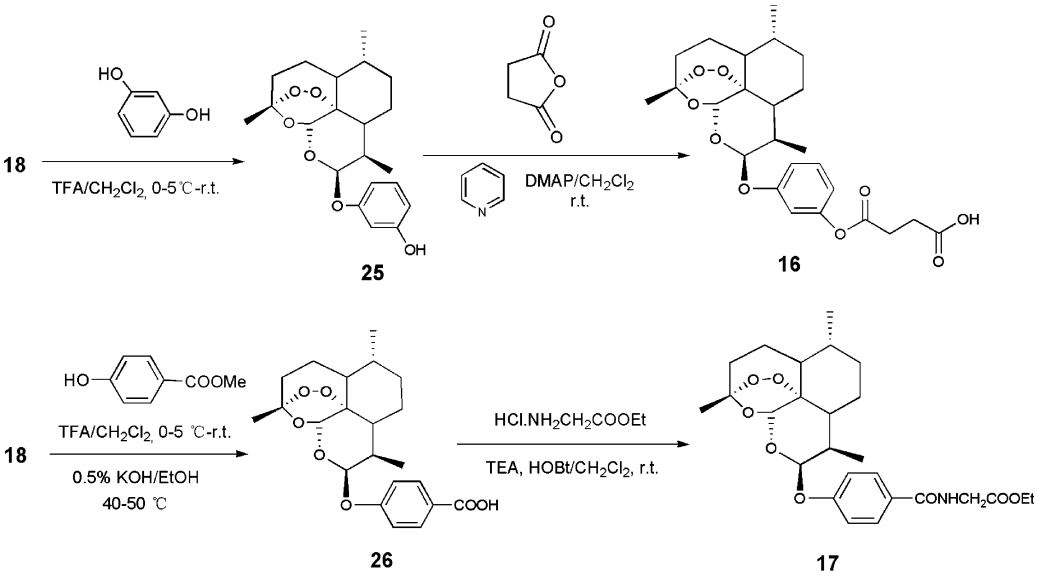
[12-β-Dihydroartemisininoxy]phenoxylsuccinic acid **16**, structurally close to artesunate, was synthesized by treatment of **25** and succinic anhydride in the presence of pyridine and DMAP at room temperature. Compound **17** was prepared by condensation between **26** and H-Gly-OEt·HCl in the presence of HOBt at room temperature (Scheme 5).

The starting substituted phenols **19**, **20**, and **23** were prepared by methyl esterification of commercially available acids catalyzed with concentrated sulfuric acid. Synthetic intermediates **21**, **22**, and **24** were prepared according to the literature procedure.[36,37]

Scheme 4. Synthetic Routes to **15a**−**c**



Scheme 5. Synthetic Routes to **16** and **17**



Results and Discussion

The new dihydroartemisinin derivatives were tested in vitro for their cytotoxicity on murine spleen cells, inhibition activity on concanavalin A (ConA) induced T cell proliferation, and lipopolysaccharide (LPS) induced B cell proliferation, with cyclosporin A (CsA), artemisinin, artesunate, and artemether 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. In the experiments for testing bioactivity of each compound, results were expressed as the mean ± standard error. Student's *t*-test was used to determine variances between groups where appropriate, although these data are not shown in Table 1. On the basis of the inhibition of ConA-induced T lymphocyte proliferation, artemether (SI = 23) was more potent than artemisinin (SI = 6) and artesunate (SI = 7) but far less potent than cyclosporin A (SI = 963). However, the cytotoxicity of compounds **10a**, **11a**, **11b**, **13a**, **13b**, **14a**, and **17** was about 10- to 100-fold less than that ofartemether and cyclosporin A. The SI values of inhibition on ConA-induced T cell proliferation showed that compounds **11b**, **13b**, **14d**, **15b**, **16**, and **17** were much more effective than artemether, among which compound **11b** (SI = 848) is the most active one and comparable to cyclosporin A (SI = 963). The SI values of inhibition on LPS-induced B cell proliferation indicated that the derivatives of **11b**, **13a**, **13b**, **13c**, **14c**, **14d**, **16**, and **17**, especially **11b** (SI = 28473), had much higher inhibition activity than artemether (SI = 49), even cyclosporin A (SI = 7). Compound **16**, as a representative of these derivatives, has been under systematic investigations both in vitro and in vivo experiments. The immunosuppressive activity of compound **16** was tested in BALB/c mice by DNFB-induced DTH response in comparison to CsA (Figure 4). The DTH reaction is a cell-mediated pathologic response involved with T cell activation and the production of many cytokines.[38,39] Compound **16** could effectively reduce the ear swelling in DTH mice at a dose of 20 mg/kg. Compound **16** was also assessed on its immunosuppressive activity by

Table 1. Inhibitory Effects of Cyclosporin A, Artemisinin, and Its Derivatives on Spleen Lymphocyte Proliferation Induced by Mitogens in Vitro

		IC_{50} (M) [SI[b]]	
compd[a]	cytotoxicity CC_{50} (M)	inhibition of ConA-induced T lymphocyte proliferation	inhibition of LPS-induced B lymphocyte proliferation
10a	3.25×10^{-4}	5.25×10^{-6} [62]	1.70×10^{-5} [19]
11a	3.54×10^{-4}	5.34×10^{-6} [66]	5.98×10^{-6} [59]
11b	2.07×10^{-4}	2.44×10^{-7} [848]	7.27×10^{-9} [28473]
11c	2.08×10^{-6}	1.96×10^{-6} [1]	4.96×10^{-8} [42]
12a	3.51×10^{-5}	8.60×10^{-6} [4]	7.40×10^{-7} [47]
12b	1.36×10^{-6}	1.73×10^{-6} [1]	3.50×10^{-7} [4]
12c	5.54×10^{-6}	7.64×10^{-6} [1]	1.05×10^{-6} [5]
12d	2.99×10^{-6}	3.59×10^{-6} [1]	5.60×10^{-7} [5]
12e	2.99×10^{-6}	4.12×10^{-6} [1]	5.70×10^{-7} [5]
12f	8.23×10^{-6}	5.73×10^{-6} [1]	8.20×10^{-7} [10]
13a	$>1.00 \times 10^{-4}$	7.27×10^{-6} [>14]	8.60×10^{-7} [>116]
13b	$>1.00 \times 10^{-4}$	2.60×10^{-7} [>385]	8.50×10^{-7} [>118]
13c	3.91×10^{-5}	2.33×10^{-6} [17]	2.70×10^{-7} [145]
14a	$>1.00 \times 10^{-4}$	1.18×10^{-5} [>9]	1.02×10^{-5} [>10]
14b	8.00×10^{-6}	7.13×10^{-7} [11]	1.79×10^{-7} [45]
14c	1.04×10^{-5}	4.00×10^{-6} [3]	1.07×10^{-7} [97]
14d	4.17×10^{-5}	1.38×10^{-7} [302]	1.78×10^{-8} [2343]
15a	5.96×10^{-5}	3.60×10^{-6} [17]	8.60×10^{-6} [7]
15b	6.53×10^{-6}	1.37×10^{-8} [477]	7.80×10^{-7} [8]
15c	5.33×10^{-6}	4.80×10^{-7} [11]	9.47×10^{-8} [56]
16	5.27×10^{-5}	3.27×10^{-7} [161]	2.65×10^{-7} [199]
17	1.30×10^{-4}	2.53×10^{-6} [51]	5.37×10^{-8} [2421]
CsA	1.29×10^{-6}	1.34×10^{-9} [963]	1.84×10^{-7} [7]
artemisinin	2.83×10^{-5}	4.43×10^{-6} [6]	8.96×10^{-6} [3]
artemether	8.60×10^{-5}	3.82×10^{-6} [23]	1.78×10^{-6} [48]
artesunate	3.29×10^{-5}	4.58×10^{-6} [7]	9.89×10^{-7} [33]

[a] The compounds tested for immunosuppressive activity were consistent with the description in the Experimental Section; [b] 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.

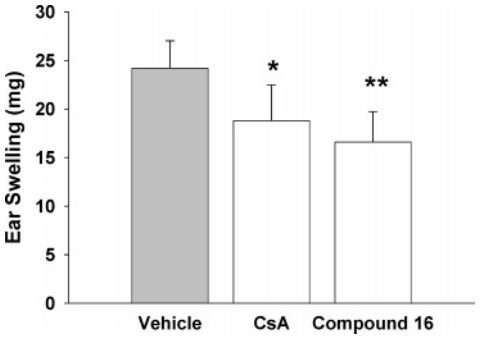


Figure 4. Inhibitory effects of compound **16** on DNFB-induced ear swelling in BALB/c mice. BALB/c mice were initially sensitized with DNFB on days 0 and 1 and then challenged with DNFB on day 9. Vehicle, compound **16** (20 mg/kg), and CsA (20 mg/kg) were administered on days 1−3 before and 0.5 h after challenge. Ear swelling was calculated as the difference between the weights of left (DNFB treated) and right (untreated) ear punches 30 h after challenge. Values represent the mean ± SEM ((*) $P < 0.05$, (**) $P < 0.01$) compared with vehicle. $n = 12$ mice/group. Three individual experiments were performed with similar results.

quantitative hemolysis of SRBC (QHS) assay, which is based on the principle that B lymphocytes are activated in vivo by SRBC and could produce anti-SRBC specific antibody ex vivo. Administration of compound **16** at a dose of 20 mg/kg for 4 days effectively reduced the hemolysis of SRBC more than dexamethasone (DEX) (Figure 5).

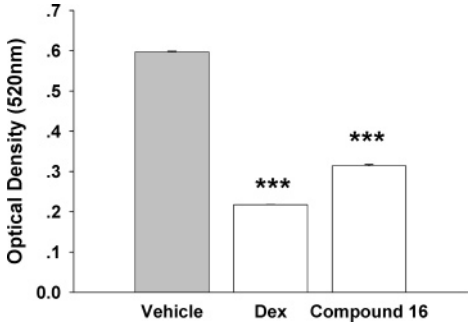


Figure 5. Suppressive effects of compound **16** on anti-SRBC antibody production in vivo. BALB/c mice were given with vehicle, compound **16** (20 mg/kg), or DEX (10 mg/kg) for 4 days. Quantitative hemolysis of SRBC was determined as previously described. Values represent the mean ± SEM ((***) $p < 0.001$)) compared with vehicle. $n = 12$ mice/group. Three individual experiments were performed with similar results.

Structure−Activity Relationships (SAR)

From the preliminary in vitro immunosuppressive evaluation in Table 1, we could found the following. (i) All the methyl or ethyl esters (except **14b**) in the new dihydroartemisinin derivatives showed a much more potent inhibitory effect on T cell proliferation than their corresponding acids, with their SI ratios of **11b** to **11a** (848/66), **13b** to **13a** (385/14), **14d** to **14c** (302/3), and **15b** to **15a** (477/17) ranging from 13 to 101. (ii) All the free acids (except **16**) and their other esters exhibited activity similar to that of artemether, even if their structures differ from each other. (iii) As a group, dihydroartemisinin derivatives **10a**, **12a**−**f**, and **14a**,**b** are almost equipotent but less potent than artemether and CsA. Compounds **13a**−**c** are more potent than their homologous region isomers **12a**−**f**. (iv) In comparison with **14b**, compounds **11b**, **14d**, and **15b** structurally has one more O atom between the benzene ring and methoxycarbonylmethyl group, one more methano ($-CH_2-$) group in the aliphatic side chain, and another methyl group at the vicinal position of carbonyl group. However, the last three displayed similar immunosuppressive activity to each other but much more efficacy than **14b**. (v) Compound **13b** was no more active than **15b**, although holding another O atom between the benzene ring and the 2-substituted propionate moiety. (vi) Compound **16** represented a more powerful inhibitory effect on T cell proliferation than its structure-related prototype artesunate. Apparently, free carboxylic acids, the hydrophilic part of some dihydroartemisinin derivatives, could not increase the immunosuppressive activity in vitro as we anticipated. Esterification of the free acids instead with proper small aliphatic groups such as methyl and ethyl groups did improve greatly their immunosuppressive activity.

Conclusions

In summary, a series of novel dihydroartemisinin derivatives, potential immunosuppressants, have been synthesized and assessed for their cytotoxicity of lymphocyte, inhibition activity on ConA-induced T cell proliferation, and LPS-induced B cell proliferation in comparison with artemisinin, artesunate, artemether, and cyclosporin A (CsA) in vitro. Among them, **11b**, **13b**,

14d, **15b**, **16**, and **17** obviously exhibited their lower cytotoxicity and higher inhibition activity on the mitogen-induced T cell and B cell proliferation compared with the controls. It is worth noting that compound **11b** displayed the most outstanding immunosuppressive effects in vitro, and it will be tested in additional in vitro studies and in vivo animal model experiments. From the preliminary in vivo tests of compound **16**, the results outlined the great potential of these compounds for further exploitation as immunosuppressants.

In view of the above observations and analyses, we presumed that one important factor contributing to high immunosuppressive potency is the presence of a lipophilic artemisinin nucleus and an appropriate aliphatic ester side chain. Further modification of analogue and optimization of their synthetic strategy are now in progress. The qualitative structure−activity relationship was expected to be useful in guiding the design of new immunosuppressive agents with high efficacy and low toxicity.

Experimental Section

Chemistry. All commercially available reagents were used without further purification unless otherwise stated. The solvents used were all 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 (4/1 v/v).

All melting points were taken in open capillary tubes on a Buchi-510 melting point apparatus and are uncorrected. The IR spectra through the range from 4000 to 600 cm^{-1} 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. The chemical shifts are expressed as δ values in parts per million (ppm) downfield from TMS as the internal standard, and the coupling constants (J) are given in hertz (Hz). The peak patterns are indicated by the following abbreviations: bs = broad singlet, d = doublet, m = multiple, p = penta, q = quarter, s = singlet, and t = triplet. 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.

Preparation of Synthetic Intermediates 21, 22, 24. Ethyl 2-(*m* or *p*-hydroxyphenoxy)propionate **21** and **22** were prepared on the basis of the modified literature procedure,[36] which is detailed below.

To a solution of sodium ethoxide, prepared by dissolving sodium (2.30 g, 100 mmol) in absolute EtOH (50 mL), there was added consecutively resorcinol or hydroquinone (5.50 g, 50 mmol). Ethyl 2-bromopropionate (3.8 mL, 5.7 g, 33 mmol) was added over 0.5 h at reflux. The mixture was refluxed overnight, and the solvent was then removed by distillation. After drying, EtOAc (50 mL) was added, and the organic extract was washed with water and brine, dried over anhydrous $MgSO_4$, and concentrated before purifying by chromatography (20:1, petroleum ether/EtOAc) to give **21** (5.2 g, 50%) or **22** (5.2 g, 50%) as a colorless liquid.

2-(4-Hydroxyphenyl)propanoic acid **24** was synthesized from *p*-methoxyacetophenone according to the modified U.S. patent method,[37] and the detailed procedure modified is as follows.

DMF (50 mL) and sodium cyanide (2.7 g, 20.4 mmol) were added to a solution of 1-(4-hydroxyphenyl)ethanol in DMF (10 mL). The mixture was stirred for 6 h at 130 °C, which was then allowed to stand overnight, and the solvent was removed under vacuum. To the residue was added 50% sodium hydroxide solution (10 mL) and water (10 mL). The aqueous mixture was then heated under reflux for 5 h and was then cooled and made acidic to pH 2.0 with hydrochloride. To it was added ethyl acetate (50 mL), and the two-phase mixture was stirred for 5 min. It was then filtered through a filter aid pad, and the pad was rinsed with EtOAc (20 mL). The organic layer of the filtrate was separated, dried over anhydrous $MgSO_4$, and evaporated under vacuum to dryness. The residue was taken up in ethyl acetate (30 mL) and petroleum ether (30 mL) and stirred in an ice bath. The product crystallized within a few minutes, and the suspension was stirred for 1 h before being filtered. The solids were dried under vacuum at 40 °C to obtain the desired product **24** (2.6 g, 80%). Its melting point was 128−130 °C.

General Method for Preparation of New Dihydroartemisinin Derivatives. 1. Condensation of Dihydroartemisinin Acetate (18) with Substituted Phenols (19−24) To Prepare New Aryl Ether Derivatives of Dihydroartemisnin (10b, 11b, 12b, 12d, 13b, 14b, 14d, 15b). To a solution of dihydroartemisinin acetate (**18**, 3.26 g, 10 mmol) and appropriate ethyl (or methyl) esters of the corresponding substituted phenol (**21**−**25**, 12 mmol) in CH_2Cl_2 (60 mL) was added 1.2 equiv of trifluoroacetic acid (TFA, 0.9 mL, 12 mmol) or several drops $BF_3{\cdot}Et_2O$ (for **21**) 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 (1:20, v/v) as the eluent to give new ether derivatives (**10b**, **11b**, **12b**, **12d**, **13b**, **14b**, **14d**, **15b**), whose yields ranged from 30% to 50%. The Lewis acid TFA was employed for the syntheses of all the new aryl ethers, with the exception of compound **12d** under the catalysis of $BF_3{\cdot}Et_2O$.

Methyl [3-(12-*β*-Artemisininoxy)]phenoxylpropionate (10b). White crystals. Mp: 115−117 °C (from ethyl acetate−petroleum ether). Yield: 36%. ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 6.83−7.26 (4H, m, Ar−H), 5.49 (1H, s, H-5), 5.48 (1H, d, J = 3.66 Hz, H-12), 4.33 (2H, t, J = 7.69 Hz, Ar−OCH_2−), 3.65 (3H, s, −OMe), 2.78 (1H, m, H-11), 2.62 (2H, d, J = 7.13 Hz, −CH_2CO−), 2.36 (1H, m, H-3β), 1.44 (3H, s, Me-15), 0.98 (3H, d, J = 7.33 Hz, Me-13), 0.97 (3H, d, J = 4.76 Hz, Me-14). IR (KBr, cm^{-1}): 1740 (C=O, ester), 1591 (C=C, benzene ring). Anal. ($C_{25}H_{34}O_7$) C, H.

Methyl [4-(12-*β*-Artemisininoxy)]phenoxylacetate (11b). White catkin/flossy crystals. Mp: 97−99 °C (from ethyl acetate−petroleum ether). Yield: 30%. ^{1} H NMR (400 MHz, $CDCl_3$, δ ppm): 7.02 (2H, d, J = 9.15 Hz, Ar−H), 6.82 (2H, d, J = 9.16 Hz, Ar−H), 5.49 (1H, s, H-5), 5.36 (1H, d, J = 3.29 Hz, H-12), 4.57 (2H, s, Ar−O−CH_2−), 3.78 (3H, s, −OMe), 2.75 (1H, m, H-11), 2.36 (1H, m, H-3β), 1.42 (3H, s, Me-15), 0.99 (3H, d, J = 7.32 Hz, Me-13), 0.94 (3H, d, J = 6.22 Hz, Me-14). IR (KBr, cm^{-1}): 1770 (C=O, ester), 1508 (C=C, benzene ring). Anal. ($C_{24}H_{32}O_8$) C, H.

Ethyl 2-[3-(12-Artemisininoxy)phenoxylpropionate (12b). 12-α + 12-β mixture, α/β = 2/1. Colorless oil. Yield: 45%. ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 7.16 (1H, m, Ar−H), 6.78 (1H, m, Ar−H), 6.73 (1H, m, Ar−H), 6.49 (1H, m, Ar−H), 5.54, 5.47 (1H, d × 2, J = 2.20, 10.71 Hz, H-12), 5.46 (1H, s × 2, H-5), 4.72 (1H, m, Ar−O−C**H**−Me), 3.93 (2H, m, −O−C**H**$_2$−Me), 2.78 (1H, m, H-11), 2.37 (1H, m, H-3β), 1.60 (3H, m, Ar−O−CH−**Me**), 1.43 (3H, s × 2, Me-15), 1.26 (3H, m, −OCH_2**Me**), 1.00 (3H, d × 2, J = 7.41, 7.42 Hz, Me-13), 0.96 (3H, d × 2, J = 7.96, 8.98 Hz, Me-14). IR (film, cm^{-1}): 1755, 1732 (C=O, ester), 1593, 1489 (C=C, benzene ring). Anal. ($C_{26}H_{36}O_8$) C, H.

Ethyl 2-[3-(12-α-Artemisininoxy)]phenoxylpropionate (12d). Colorless oil. Yield: 40%. ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 7.15 (1H, td, J = 8.16, 1.65 Hz, Ar−H), 6.76 (1H, m, Ar−H), 6.65 (1H, t + d, J = 5.05, 5.50 Hz, Ar−H), 6.49 (1H, dt, J = 8.25, 2.66 Hz, Ar−H), 5.47 (2H, s + d, J = 8.25 Hz,

H-5, H-12), 4.73 (1H, q × 2, *J* = 6.78, 6.78 Hz, Ar−O−C**H**−Me), 4.22 (2H, m, −O−CH_2−), 2.78 (1H, m, H-11), 2.37 (1H, m, H-3*β*), 1.60 (3H, d, *J* = 6.78 Hz, Ar−O−CH−**Me**), 1.44 (3H, s, Me-15), 1.26 (3H, m, −O−CH_2−**Me**), 1.01 (3H, d × 2, *J* = 7.33, 7.31 Hz, Me-13), 0.97 (3H, d, *J* = 6.05 Hz, Me-14). IR (film, cm^{-1}): 1755, 1734 (C=O, ester), 1593, 1489 (C=C, benzene ring). Anal. ($C_{26}H_{36}O_8$) C, H.

Ethyl 2-[4-(12-*β*-Artemisininoxy)]phenoxylpropionate (13b). Colorless oil. Yield: 45%. ^{1}H NMR (400 MHz, $CDCl_3$, *δ* ppm): 6.92 (2H, d, *J* = 9.14 Hz, Ar−H), 6.72 (2H, dd, *J* = 9.07, 2.27 Hz, Ar−H), 5.42 (1H, s, H-5), 5.27 (1H, d, *J* = 3.43 Hz, H-12), 4.56 (1H, q, *J* = 6.82 Hz, Ar−O−C**H**−Me), 4.12 (2H, q × 2, *J* = 7.14, 7.00 Hz, −O−C**H**$_2$−Me), 2.68 (1H, m, H-11), 2.29 (1H, m, H-3*β*), 1.50 (3H, d, *J* = 6.73 Hz, Ar−O−CH−**Me**), 1.35 (3H, s, Me-15), 1.18 (3H, m, −O−CH_2−**Me**), 0.92 (3H, d, *J* = 7.42 Hz, Me-13), 0.87 (3H, d, *J* = 7.00 Hz, Me-14). IR (film, cm^{-1}): 1755, 1738 (C=O, ester), 1593, 1506 (C=C, benzene ring). Anal. ($C_{26}H_{36}O_8$) C, H.

Methyl [4-(12-*β*-Artemisininoxy)]phenylacetate (14b). Colorless crystals. Mp: 108−112 °C (from ethyl acetate−petroleum ether). Yield: 30%. ^{1} H NMR (400 MHz, $CDCl_3$, *δ* ppm): 7.19 (2H, d, *J* = 8.43 Hz, Ar−H), 7.06 (2H, d, *J* = 8.61 Hz, Ar−H), 5.48 (1H, d, *J* = 4.09 Hz, H-12), 5.43 (1H, s, H-5), 3.64 (3H, s, −OMe), 3.52 (2H, s, Ar−CH_2−), 2.79 (1H, m, H-11), 2.37 (1H, ddd, *J* = 4.03, 3.85, 3.85 Hz, H-3*β*), 1.43 (3H, s, Me-15), 1.00 (3H, d, *J* = 7.33 Hz, Me-13), 0.96 (3H, d, *J* = 6.05 Hz, Me-14). IR (KBr, cm^{-1}): 1734 (C=O, ester), 1512 (C=C, benzene ring). Anal. ($C_{24}H_{32}O_7$) C, H.

Methyl 3-[4-(12-*β*-Artemisininoxy)]phenylpropionate (14d). White needle crystals. Mp: 82−84 °C (from ethyl acetate−petroleum ether). Yield: 35%. ^{1} H NMR (400 MHz, $CDCl_3$, *δ* ppm): 7.11 (2H, d, *J* = 8.52 Hz, Ar−H), 7.03 (2H, d, *J* = 8.67 Hz, Ar−H), 5.49 (1H, s, H-5), 5.46 (1H, d, *J* = 2.88 Hz, H-12), 3.67 (3H, s, −OMe), 2.89 (2H, t, *J* = 7.84 Hz, Ar−CH_2−), 2.77 (1H, m, H-11), 2.59 (2H, t, *J* = 7.77 Hz, −CH_2−CO−), 2.36 (1H, m, H-3*β*), 1.43 (3H, s, Me-15), 1.01 (3H, d, *J* = 7.29 Hz, Me-13), 0.96 (3H, d, *J* = 5.78 Hz, Me-14). IR (KBr, cm^{-1}): 1736 (C=O, ester), 1608, 1512 (C=C, benzene ring). Anal. ($C_{25}H_{34}O_7$) C, H.

Methyl 2-[4-(12-*β*-Artemisininoxy)]phenylpropionate (15b). Colorless oil. Yield: 40%. ^{1}H NMR (400 MHz, $CDCl_3$, *δ* ppm): 7.20 (2H, dd, *J* = 8.69, 1.56 Hz, Ar−H), 7.06 (2H, dd, *J* = 6.73, 2.06 Hz, Ar−H), 5.49 (1H, d, *J* = 3.30 Hz, H-12), 5.47 (1H, s, H-5), 3.67 (1H, q, *J* = 6.50 Hz, Ar−C**H**−Me), 3.64 (3H, s, −OMe), 2.78 (1H, m, H-11), 2.36 (1H, m, H-3*β*), 1.46 (3H, d, *J* = 7.14 Hz, Ar−CH−**Me**), 1.43 (3H, s, Me-15), 1.00 (3H, d, *J* = 7.42 Hz, Me-13), 0.95 (3H, d, *J* = 9.62 Hz, Me-14). IR (film, cm^{-1}): 1738 (C=O, ester), 1610, 1510 (C=C, benzene ring). Anal. (C_{25} $H_{34}O_7$) C, H.

2. Hydrolysis of Ethers To Prepare Corresponding Free Acids 10a, 11a, 12a, 12c, 13a, 14a, 14c, 15a. The ether (**10b**−**15b**, **12d**−**14d**; 10 mmol) was dissolved in 0.5% KOH/EtOH solution (150 mL), and the mixture was stirred at about 40−50 °C overnight. After neutralization with acetic acid, the solution was evaporated in vaccum. 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 acids (**10a**, **11a**, **12a**, **12c**, **13a**, **14a**, **14c**, **15a**).

[3-(12-*β*-Artemisininoxy)]phenoxylacetic Acid (10a). White amorphous solid. Yield: 90%. ^{1} H NMR (400 MHz, $CDCl_3$, *δ* ppm): 7.18 (1H, m, Ar−H), 6.77 (1H, d, *J* = 8.05 Hz, Ar−H), 6.70 (1H, s, Ar−H), 6.54 (1H, d, *J* = 8.34 Hz, Ar−H), 5.48 (1H, d, *J* = 3.35 Hz, H-12), 5.46 (1H, s, H-5), 4.64 (2H, s, −OCH_2−), 3.98 (1H, bs, −OH), 2.76 (1H, m, H-11), 2.36 (1H, m, H-3*β*), 1.42 (3H, s, Me-15), 0.99 (3H, d, *J* = 7.26 Hz, Me-13), 0.94 (3H, d, *J* = 5.98 Hz, Me-14). IR (KBr, cm^{-1}): 3435 (OH), 1740 (C=O, carboxylic acid), 1595, 1491 (C=C, benzene ring) Anal. ($C_{23}H_{30}O_8$) C, H.

[4-(12-*β*-Artemisininoxy)]phenoxylacetic Acid (11a). White needle crystals. Mp: 132−132.5 °C (from ethyl acetate−petroleum ether). Yield: 90%. ^{1} H NMR (400 MHz, $CDCl_3$, *δ* ppm): 7.04 (2H, d, *J* = 9.22 Hz, Ar−H), 6.84 (2H, d, *J* = 9.20 Hz, Ar−H), 5.53, 5.49 (1H, s × 2, H-5), 5.68, 5.38 (1H, d × 2, *J* = 6.29, 3.35 Hz, H-12), 4.60 (2H, s, −OCH_2−), 3.85 (1H, bs, −OH), 2.76 (1H, m, H-11), 2.36 (1H, m, H-3*β*), 1.42 (3H, s, Me-15), 1.00 (3H, d, *J* = 7.17 Hz, Me-13), 0.94 (3H, d, *J* = 5.93 Hz, Me-14). IR (KBr, cm^{-1}): 3429 (OH), 1724 (C=O, carboxylic acid), 1662, 1506 (C=C, benzene ring). Anal. ($C_{23}H_{30}O_8$) C, H.

2-[3-(12-Artemisininoxy)]phenoxylpropionic Acid (12a). 12-α + 12-*β* mixture, α/*β* = 2/1. White amorphous solid. Yield: 95%. ^{1}H NMR (400 MHz, $CDCl_3$, *δ* ppm): 7.14 (1H, m, Ar−H), 6.78−6.70 (1H, m, Ar−H), 6.53−6.42 (2H, m, Ar−H), 5.54 (1H, s, H-5), 5.48 (1H, d × 2, *J* = 4.36, 10.70 Hz, H-12), 4.78 (1H, m, Ar−O−C**H**−Me), 2.80 (1H, m, H-11), 2.40 (1H, m, H-3*β*), 1.65 (3H, d × 2, *J* = 6.38, 7.38 Hz, Ar−O−CH−**Me**), 1.43 (3H, s × 2, Me-15), 0.99 (3H, d × 2, *J* = 7.39, 6.05 Hz, Me-13), 0.96 (3H, d × 2, *J* = 6.05, 7.05 Hz, Me-14). IR (KBr, cm^{-1}): 3429 (OH), 1732 (C=O, carboxylic acid), 1603, 1491 (C=C, benzene ring). Anal. ($C_{24}H_{32}O_8$) C, H.

2-[3-(12-α-Artemisininoxy)]phenoxylpropionic Acid (12c). White amorphous solid. Yield: 95%. ^{1}H NMR (400 MHz, $CDCl_3$, *δ* ppm): 7.19 (1H, t, *J* = 8.25 Hz, Ar−H), 6.80 (1H, m, Ar−H), 6.71 (1H, q, *J* = 2.20 Hz, Ar−H), 6.54 (1H, d, *J* = 8.11 Hz, Ar−H), 5.48 (2H, s + d, *J* = 9.49 Hz, H-5, H-12), 4.81 (1H, q × 2, *J* = 6.97, 6.73 Hz, Ar−O−C**H**−Me), 2.79 (1H, m, H-11), 2.38 (1H, m, H-3*β*), 1.65 (3H, d, *J* = 6.87 Hz, Ar−O−CH−**Me**), 1.44 (3H, s, Me-15), 1.01 (3H, d, *J* = 7.25 Hz, Me-13), 0.97 (3H, d, *J* = 7.14 Hz, Me-14). IR (KBr, cm^{-1}): 3417 (OH), 1732 (C=O, carboxylic acid), 1603, 1593, 1491(C=C, benzene ring). Anal. ($C_{24}H_{32}O_8$) C, H.

2-[4-(12-*β*-Artemisininoxy)]phenoxylpropionic Acid (13a). White amorphous solid. Yield: 95%. ^{1}H NMR (400 MHz, $CDCl_3$, *δ* ppm): 7.05 (2H, m, Ar−H), 6.85 (2H, d, *J* = 9.07 Hz, Ar−H), 5.50 (1H, s, H-5), 5.39 (1H, d, *J* = 3.69 Hz, H-12), 4.72 (1H, q, *J* = 6.72 Hz, Ar−O−C**H**−Me), 2.78 (1H, m, H-11), 2.36 (1H, m, H-3*β*), 1.62 (3H, d × 2, *J* = 7.05, 6.71 Hz, Ar−O−CH−**Me**), 1.43 (3H, s, Me-15), 1.01 (3H, d, *J* = 7.38 Hz, Me-13), 0.96 (3H, d, *J* = 6.84 Hz, Me-14). IR (KBr, cm^{-1}): 3435 (OH), 1732 (C=O, carboxylic acid), 1506 (C=C, benzene ring). Anal. ($C_{24}H_{32}O_8$) C, H.

[4-(12-*β*-Artemisininoxy)]phenylacetic Acid (14a). White crystals. Mp:154−156 °C (from ethyl acetate−petroleum ether). Yield: 90%. ^{1} H NMR (400 MHz, $CDCl_3$, *δ* ppm): 7.20 (2H, d, *J* = 8.79 Hz, Ar−H), 7.08 (2H, d, *J* = 8.66 Hz, Ar−H), 5.49 (1H, d, *J* = 3.57 Hz, H-12), 5.47 (1H, s, H-5), 3.59 (2H, s, Ar−CH_2−), 2.79 (1H, m, H-11), 2.38 (1H, m, H-3*β*), 1.43 (3H, s, Me-15), 1.00 (3H, d, *J* = 7.42 Hz, Me-13), 0.96 (3H, d, *J* = 6.04 Hz, Me-14). IR (KBr, cm^{-1}): 3267 (OH), 1730 (C=O, carboxylic acid), 1610, 1512 (C=C, benzene ring). Anal. ($C_{23}H_{30}O_7$) C, H.

3-[4-(12-*β*-Artemisininoxy)]phenylpropionic Acid (14c). White amorphous solid. Yield: 90%. ^{1}H NMR (400 MHz, $CDCl_3$, *δ* ppm): 7.07 (2H, d × 2, *J* = 8.42, 8.42 Hz, Ar−H), 7.01, 6.75 (2H, d ×2, *J* = 8.79, 8.43 Hz, Ar−H), 5.49 (1H, s, H-5), 5.45 (1H, d, *J* = 3.30 Hz, H-12), 2.87 (2H, td, *J* = 7.51, 3.66 Hz, Ar−CH_2−), 2.77 (1H, m, H-11), 2.62 (2H, t, *J* = 7.69 Hz, −CH_2−CO−), 2.36 (1H, m, H-3*β*), 1.42 (3H, s, Me-15), 0.99 (3H, d, *J* = 7.32 Hz, Me-13), 0.94 (3H, d, *J* = 5.86 Hz, Me-14). IR (KBr, cm^{-1}): 3400 (chel OH), 1709 (C=O, carboxylic acid), 1612, 1512 (C=C, benzene ring). Anal. ($C_{24}H_{32}O_7$) C, H.

2-[4-(12-*β*-Artemisininoxy)]phenylpropionic Acid (15a). White particle crystals. Mp: 152−154 °C (from ethyl acetate−petroleum ether). Yield: 95%. ^{1}H NMR (300 MHz, $CDCl_3$, *δ* ppm): 7.24 (2H, m, Ar−H), 7.08 (2H, d, *J* = 8.52 Hz, Ar−H), 5.49 (1H, d, *J* = 3.30 Hz, H-12), 5.47 (1H, s, H-5), 3.68 (1H, q, *J* = 7.23 Hz, Ar−C**H**−Me), 2.79 (1H, m, H-11), 2.37 (1H, m, H-3*β*), 1.48 (3H, d, *J* = 7.14 Hz, Ar−CH−**Me**), 1.44 (3H, s, Me-15), 1.00 (3H, d, *J* = 7.42 Hz, Me-13), 0.96 (3H, d, *J* = 6.04 Hz, Me-14). IR (KBr, cm^{-1}): 3400 (chel OH), 1725, 1705 (C=O, carboxylic acid), 1608, 1510 (C=C, benzene ring). Anal. ($C_{24}H_{32}O_7$) C, H.

3. Condensation of Acid with *N*,*N*-Dimethylethanol or *o*-Methoxyphenol To Prepare Corresponding Esters 11c, 12e, 12f, 13c, 15c. To a solution of free acid (**11a**, **12a**, **12c**,

13a, **15a**; 2.0 mmol) and *o*-methoxyphenol (0.24 mL, 2.2 mmol) or *N*,*N*-dimethylethanol (2.2 mmol, for **11a**) in dry ether (50 mL), DCC (453.2 g, 2.2 mmol) and DMAP (cat. 24.4 mg, 0.1 mmol) were added, and the solution was stirred for 2−5 h at room temperature. After the condensation 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 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 1:20 v/v as the eleunt) to give the target ester (**11c**, **12e**, **12f**, **13c**, **15c**).

Dimethylaminoethyl [4-(12-*β*-Artemisininoxy)]phenoxylacetate (11c). White amorphous solid. Yield: 45%. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.04 (2H, d, J = 9.14 Hz, Ar−H), 6.83 (2H, d, J = 9.16 Hz, Ar−H), 5.51 (1H, s, H-5), 5.37 (1H, d, J = 3.30 Hz, H-12), 4.68 (2H, s, Ar−OCH_2−), 3.93, 3.66 (2H, m, −COO−CH_2−), 2.77 (1H, m, H-11), 2.38 (1H, m, H-3β), 1.69 (2H, m, −CH_2N−), 1.62 (6H, s, −NMe_2), 1.43 (3H, s, Me-15), 1.01 (3H, d, J = 7.34 Hz, Me-13), 0.96 (3H, d, J = 6.24 Hz, Me-14). IR (KBr, cm^{-1}): 1700, 1660 (C=O, ester), 1545, 1510 (C=C, benzene ring). Anal. ($C_{27}H_{39}O_8N$) C, H, N.

2-Methoxyphenyl 2-[3-(12-*β*-Artemisininoxy)]phenoxylpropionate (12e). White amorphous solid. Yield: 40%. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.20 (2H, td, J = 8.24, 2.74 Hz, Ar−H), 7.00 (1H, d, J = 7.83 Hz, Ar−H), 6.93 (2H, q, J = 7.96 Hz, Ar−H), 6.86−6.78 (1H, m + brd, J = 8.52 Hz, Ar−H), 6.76 (1H, dt, J = 6.96, 2.27 Hz, Ar−H), 6.65 (1H, dt, J = 8.24, 2.74 Hz, Ar−H), 5.49 (1H, s, H-5), 5.51 (1H, d, J = 3.43 Hz, H-12), 4.99 (1H, q, J = 6.73 Hz, Ar−O−C**H**−Me), 3.78 (3H, s, Ar−OMe), 2.79 (1H, m, H-11), 2.37 (1H, m, H-3β), 1.79 (3H, d, J = 6.73 Hz, Ar−O−CH−**Me**), 1.43 (3H, s, Me-15), 1.01 (3H, d, J = 7.42 Hz, Me-13), 0.92 (3H, d, J = 6.05 Hz, Me-14). IR (KBr, cm^{-1}): 1782 (C=O, ester), 1606, 1502 (C=C, benzene ring). Anal. ($C_{31}H_{38}O_9$) C, H.

2-Methoxyphenyl 2-[3-(12-α-Artemisininoxy)]phenoxylpropionate (12f). White amorphous solid. Yield: 30%. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.18 (2H, m, Ar−H), 6.94 (3H, m, Ar−H), 6.77 (2H, m, Ar−H), 6.65 (1H, m, Ar−H), 5.48 (1H, s, H-5), 5.06 (1H, d, J = 9.34 Hz, H-12), 4.99 (1H, q, J = 6.86 Hz, Ar−OC**H**−Me), 3.77 (3H, s, Ar−OMe), 2.71 (1H, m, H-11), 1.80 (3H, d × 2, J = 6.87, 6.73 Hz, Ar−OCH−**Me**), 1.43 (3H, s, Me-15), 0.99 (3H, d, J = 7.00 Hz, Me-13), 0.97 (3H, d, J = 7.14 Hz, Me-14). IR (KBr, cm^{-1}): 1782 (C=O, ester), 1606, 1502 (C=C, benzene ring). Anal. ($C_{31}H_{38}O_9$) C, H.

2-Methoxyphenyl 2-[4-(12-*β*-Artemisininoxy)]phenoxylpropionate (13c). White particle crystal (from ethyl acetate−petroleum ether). Mp: 142−144 °C. Yield: 45%. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.20 (1H, td, J = 7.65, 1.97 Hz, Ar−H), 7.14 (2H, d, J = 10.18 Hz, Ar−H), 6.98 (1H, m, Ar−H), 6.94 (4H, m, Ar−H), 5.52 (1H, s, H-5), 5.39 (1H, d, J = 3.44 Hz, H-12), 4.92 (1H, q × 2, J = 6.73, 6.86 Hz, Ar−O−C**H**−Me), 3.79 (3H, s, Ar−OMe), 2.78 (1H, m, H-11), 2.38 (1H, m, H-3β), 1.78 (3H, d, J = 6.87 Hz, Ar−O−CH−**Me**), 1.03 (3H, d, J = 7.28 Hz, Me-13), 0.97 (3H, d, J = 6.18 Hz, Me-14). IR (KBr, cm^{-1}): 1774 (C=O, ester), 1610, 1504 (C=C, benzene ring). Anal. ($C_{31}H_{38}O_9$) C, H.

2-Methoxylphenol 2-[4-(12-*β*-Artemisininoxy)]phenylpropionate (15c). White amorphous solid. Yield: 75%. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.34 (2H, d, J = 8.40 Hz, Ar−H), 7.16 (1H, m, Ar−H), 7.11 (2H, dd, J = 8.80, 0.80 Hz, Ar−H), 6.96−6.87 (3H, m, Ar−H), 5.52 (1H, d, J = 2.40 Hz, H-12), 4.49 (1H, s, H-5), 3.96 (1H, q, J = 7.20 Hz, Ar−C**H**−Me), 3.74 (3H, s, Ar−OMe), 2.80 (1H, m, H-11), 2.38 (1H, m, H-3β), 1.59 (3H, d, J = 7.20 Hz, Ar−CH−**Me**), 1.44 (3H, s, Me-15), 1.02 (3H, d, J = 7.68 Hz, Me-13), 0.96 (3H, d, J = 6.00 Hz, Me-14). IR (KBr, cm^{-1}): 1759 (C=O, ester), 1608, 1600, 1582 (C=C, benzene ring). Anal. ($C_{31}H_{38}O_8$) C, H.

Synthesis of [3-(12-*β*-Artemisininoxy)]phenoxylsuccinic Acid (16). To a solution of **25** (3.6 g, 10 mmol) in dry CH_2Cl_2 (60 mL) was added succinic anhydride (SAA, 1.2 g, 12 mmol), pyridine (1.0 mL, 12 mmol), and catalytic DMAP (1.2 mg, 1 mmol) at room temperature. The mixture was stirred for 2 h at the same conditions until the reaction was complete, then washed with 10% HCl, H_2O, and brine and dried over anhydrous $MgSO_4$. After evaporation of the solvent under reduced pressure, the crude products were purified with a silica gel chromatography using ethyl acetate−petroleum ether (1:5, v/v) as the eluent to give the target acid **16** (3.1 g, 63%) as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.25 (1H, s, Ar−H), 7.00 (1H, dd, J = 8.51, 2.23 Hz, Ar−H), 6.84 (1H, t, J = 2.23 Hz, Ar−H), 6.72 (1H, dd, J = 7.80, 2.01 Hz, Ar−H), 5.46 (1H, d, J = 3.55 Hz, H-12), 5.45 (1H, s, H-5), 2.98 (1H, bs, −OH), 2.85 (2H, t, J = 6.87 Hz, −CH_2COOH), 2.77 (2H, t, J = 6.67, 7.12 Hz, Ar−$OCOCH_2$−), 2.36 (1H, m, H-3β), 1.42 (3H, s, Me-15), 0.98 (3H, d, J = 7.50 Hz, Me-13), 0.94 (3H, d, J = 6.10 Hz, Me-14). IR (KBr, cm^{-1}): 3300 (chel OH), 1786, 1765 (C=O, ester), 1740, 1713 (C=O, carboxylic acid), 1608, 1600, 1487 (C=C, benzene ring). Anal. ($C_{25}H_{32}O_9 \cdot {}^3/_4H_2O$) C, H.

Synthesis of Ethyl [4-(12-*β*-Artemisininoxy)]benzoylaminoacetate (17). A solution of **26** (4.0 g, 10 mmol), H-Gly-OEt·HCl (2.8 g, 20 mmol), triethylamine (TEA, 2.8 mL, 20 mmol), and 1-hydroxybenzotriazole (HOBt, 2.0 g, 15 mmol) in dry dichloromethane (DCM, 80 mL) was stirred at room temperature for 5 h. After completion, the reaction mixture was washed with water and brine and and dried over anhydrous $MgSO_4$. Concentration and purification with silica gel chromatography using ethyl acetate−petroleum ether (1:20, v/v) as the eluent afforded the target compound **17** (3.9 g, 80%) as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.78 (2H, d, J = 8.79 Hz, Ar−H), 7.15 (2H, d, J = 8.80 Hz, Ar−H), 6.59 (1H, t, J = 4.88 Hz, −NH−), 5.55 (1H, d, J = 3.29 Hz, H-12), 5.45 (1H, s, H-5), 4.26 (2H, q, J = 7.15 Hz, −OCH_2−), 4.22 (2H, d, J = 5.08 Hz, −NCH_2−), 2.83 (1H, m, H-11), 2.37 (1H, m, H-3β), 1.44 (3H, s, Me-15), 1.31 (3H, t, J = 7.08 Hz, −OCH_2**Me**), 1.03 (3H, d, J = 7.42 Hz, Me-13), 0.96 (3H, d, J = 6.18 Hz, Me-14). IR (KBr, cm^{-1}): 3371 (NH), 1755 (C=O, ester), 1645 (C=O, acylamide), 1608, 1504 (C=C, benzene ring). Anal. ($C_{26}H_{35}O_8N$) C, H, N.

Biological Assay. Materials. Stock solutions of compounds were 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-dimethylthylthiazol-2-yl)-2,5-diphenyltetrazolium 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.[40]

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 of light/12 h of dark photoperiod, 22 ± 1 °C, 55% ± 5% relative humidity). All husbandry and experimental contacts made with the mice were conducted under 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.

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 three times with PBS containing 2% FBS and were resuspended in RPMI 1640 medium at the indicated concentration.

Cytotoxicity Assay. Fresh spleen cells were obtained from BALB/C mice (male, 7−9 weeks old). Spleen cells (5×10^5 cells) were cultured in 96-well flat plates with 200 μL of 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. An amount of 18 μL of MTT (5 mg/mL) was added to each well at the final 5 h of 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 using the following formula:

$$\text{cytotoxicity (\%)} = \frac{\text{compounds } (OD_{570}) - \text{background } (OD_{570})}{\text{control } (OD_{570}) - \text{background } (OD_{570})} \times 100$$

T Cell and B Cell Function Assay. Fresh spleen cells were obtained from BALB/C mice (male, 7−9 weeks old). The 5×10^5 spleen cells were cultured at the same conditions as those 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 cells or B cells proliferative responses, 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 of 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, PerkinElmer Life Sciences).

DNFB-Induced Delayed-Type Hypersensitivity (DTH) Response. The DTH assay was carried out as previously described with some modifications.[40] Briefly, mice were initially sensitized with 0.5% DNFB (2,4-dinitrofluorobenzene, Pharmigen) dissolved in acetone−olive oil (4:1) on each hind foot on days 0 and 1. On day 9, mice were challenged with 0.4% DNFB on both sides of their left ear. Vehicle, compound **16** (20 mg/kg), and cyclosporine A (CsA, 20 mg/kg) were administered to each group intraperitoneally (ip) on days 1−3, before and 0.5 h after challenge. At least 12 BALB/c mice were prepared for each group. Ear swelling was expressed as the difference between the weight of the left and right ear patches obtained from 8 mm punches 30 h after challenge. The punches were obtained in a blinded manner.

Quantitative Hemolysis of Sheep Red Blood Cells (QHS). Twelve female BALB/c mice were prepared for each group. We performed the QHS assay as previously described with slight modifications.[41] Briefly, on day 0, mice were immunized ip with 0.2 mL of diluted fresh SRBC, which had been washed 3 times with saline (1500−2000 rpm, 10−15 min) and diluted 1:5 (v/v) with saline. Vehicle (saline), compound **16** (20 mg/kg), and dexamethasone (DEX, 10 mg/kg) were administered ip to each group on days 1−4 once a day. At day 5, the spleen cells of mice were tested for anti-SRBC antibody production using quantitative hemolysis of the SRBC method. Briefly, diluted 1:20 of fresh SRBC (1 mL), diluted 1:10 of fresh guinea pig serum (1 mL), and 2×10^7/mL spleen cell suspensions (1 mL) from drug-treated or untreated mice were incubated for 1 h at 37 °C. Hemolysis was determined by reading the optical density (OD) at 520 nm in a blinded manner.

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Supporting Information Available: Elemental analysis data for the target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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