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Artemisinin Derivatives Bearing Mannich Base Group: Synthesis and Antimalarial Activity

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Abstract—Novel artemisinin derivatives bearing Mannich base group were prepared and tested for their antimalarial activity. These water-soluble artemisinin derivatives were more stable than sodium artesunate and few compounds were found to be more active against *Plasmodium berghei* in mice than artesunic acid by oral administration. Two most potent derivatives **17b** and **17d** were examined for their antimalarial activity against *Plasmodium knowlesi* in rhesus monkeys.

Introduction

Sodium artesunate (**3**) is the first water-soluble artemisinin derivative, which has been successfully used for treatment of chloroquine-resistant malaria patients by iv or im administration since the 1980's. Due to the instability of its aqueous solution, some research groups attempted to find more stable water-soluble artemisinin derivatives, and their efforts were continuously reported.[1–11] Artelinic acid (**4**), in which an ether bond links artemisinin nucleus with the acidic moiety was once considered as a good substitute for artesunate owing to its better activity and stability.[1] However, further study revealed that sodium artelinate was slightly less active than artemisinin and artesunate both in culture and in vivo.[12] Based on the knowledge of known basic antimalarial drugs, another strategy of introducing basic group into the artemisinin molecule was considered. Thus compounds **5**, **6**, **7** (Fig. 1) were prepared and tested in vivo by our groups.[8] Some of these compounds showed more active against *P. berghei* in mice but less active against *P. knowlesi* in rhesus monkeys than artesunate. Besides, more basic artemisinin derivatives (Fig. 2) were also evaluated in vitro and in vivo.[3–6,11]

In the modification of chemical structure of chloroquine (**8**), a number of Mannich base derivatives (Fig. 3) were developed. Amodiaquine (**9**) and amopyroquine (**11**) had a slight advantage over chloroquine on chloroquine-resistant strains of *Plasmodium falciparum*. Later, it was found that cycloquine (**10**) and tebuquine (**12**) were more active than amodiaquine in vivo and in clinic.[13–17] It seemed that bis-Mannich bases substituted on the amino-phenol was superior to mono-Mannich base for antimalarial activity.

During the 1960's–1970's, Chinese researchers synthesized M 6407 (**13**), changroline (**14**), 6701 (**15**), pyronaridine (**16**) and their analogues (Fig. 4). According to expectation, these compounds had good antimalarial activity in vivo and in clinical trials.[18–23] Among them, pyronaridine was the most promising and marketed in China and until now related studies of pyronaridine and its combinations have been performed by some groups.[24–26] It was interesting that changroline showed obvious antiarrhythmia activity during clinical trials for treatment of malaria patients suffering from arrhythmia, hence it became a lead compound of a new type of antiarrythmia drugs.[27–29]

From these observations, the Mannich base was recognized to be a useful pharmacophoric group for antimalarial activity. Thus, we decided to introduce the

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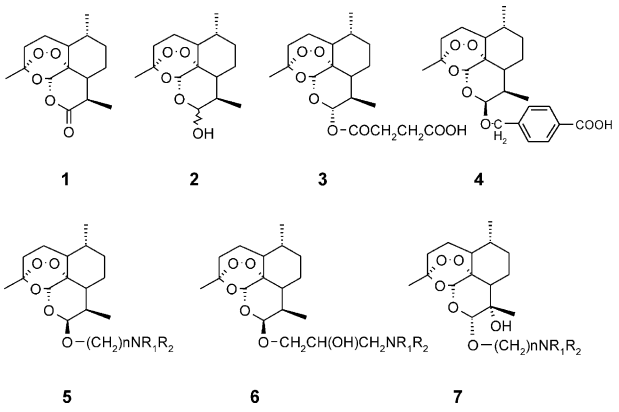


Figure 1.

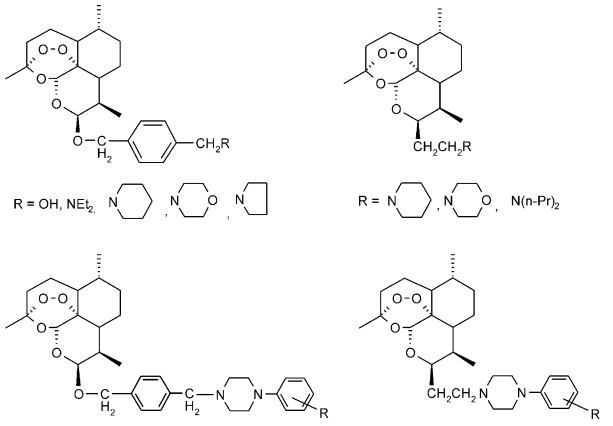


Figure 2.

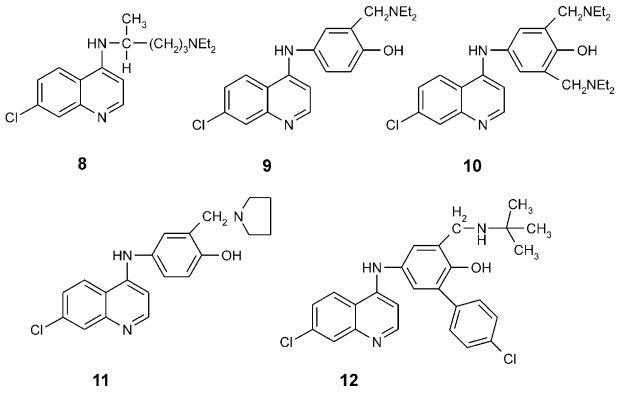


Figure 3.

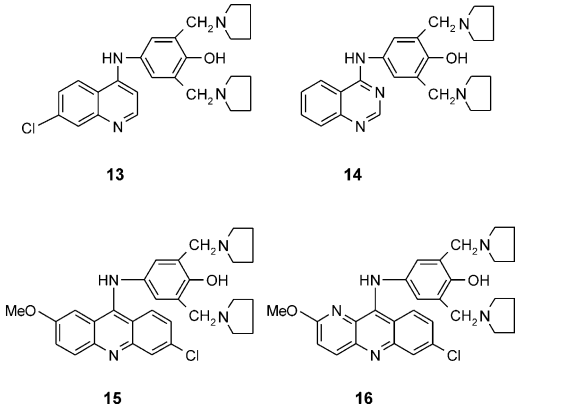


Figure 4.

Mannich base group into artemisinin molecule (Fig. 5) and evaluate the antimalarial activity. The details of their syntheses and bioevaluation as antimalarial agents are presented.

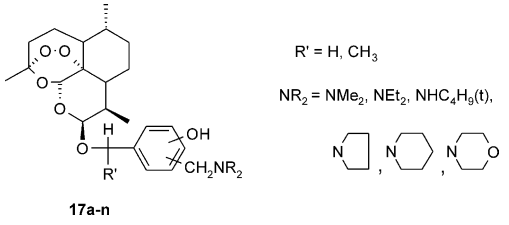


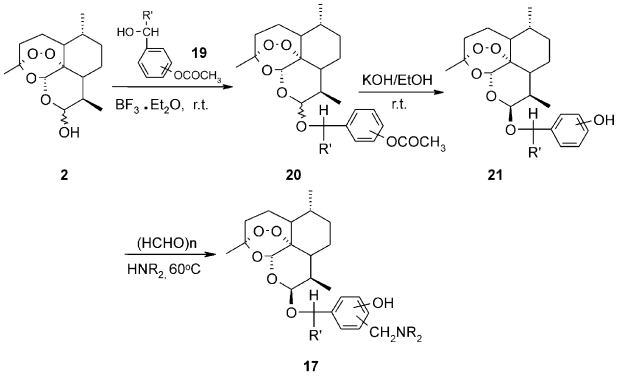
Figure 5.

Results and Discussion

Preparation of 17

The synthesis of compound **17** is described in Scheme 1. In the presence of $BF_3{\cdot}Et_2O$, dihydroartemisinin **2** reacted with corresponding *m*- or *p*-acetoxy benzyl alcohol **19** to afford ether **20** (a pair of 12-epimers). Without purification, the basic hydrolysis of compound **20** was carried out with 0.25% KOH/EtOH followed by the column chromatography of the crude products to furnish **21** (12-βisomer, major product) in good yield. Compound **21** reacted with formalin or paraformaldehyde and various amines to give **17** in moderate yield. It was worth noting that Mannich reaction of **21** was carried out only in dioxane or THF solution, if in alcohol or aqueous solution, the reaction products were always too complex for separation.

Pure free Mannich bases may be obtained by column chromatography. They combined with organic acids (oxalic acid, maleic acid, etc.) to yield the corresponding salts. A few compounds were directly prepared from



Scheme 1.

crude Mannich bases and organic acid, and then purified by recrystallization.

The stability of their aqueous solution was determined by TLC. They could be kept at room temperature for several weeks without any decomposition. Undoubtedly, the stability of this new type of artemisinin derivatives was much greater than that of sodium artesunate.

Biological Results

17b, **17e** and **17g** were tested in vitro against K1 and NF54 strains of *P. falciparum* and exhibited potent activity (IC_{50} 0.18–0.36 ng/mL or 3–6 nM) (Table 1). The most of compound **17** were evaluated on their antimalarial activities in vivo. The in vivo antimalarial activity was assessed in mice infected with 1.5×10^7 *P. berghei* K_{173} and artesunic acid or sodium artesunate was used as a control. The compounds to be tested were dissolved in water and administrated orally or subcutaneously once a day for D_0–D_3. Groups of 10 mice received these compounds at dose levels of 0.615, 1.25, 2.5 or 10 mg/kg. Blood smears were made on D_4, stained and examined under microscope. The results in Table 1 showed that **17b** and **17d** were much more active than artesunic acid by oral administration. In contrast with compound **5**,[8] their antimalarial activities via subcutaneous injection were not drastically decreased and were comparable with sodium artesunate via intravenous injection. Hence **17b** and **17d** were further evaluated in the monkey model. Each monkey was inoculated with 2×10^7 *P. knowlesi* intravenously on D_0. Compound **17b** at 10.0 and 3.16 mg/kg/day and **17d** at 3.16 mg/kg/day were given orally from D_0 to D_6. The experimental results are listed in Table 2. Compound **17b** reduced parasites more rapidly than artesunic acid, but at 3.16 mg/kg/day, **17b** and **17d** could not cleanse all parasites like compound **5** did.[8] Whereas artesunic acid could cleanse all parasites at 3.16 mg/kg/day, and no recrudescence within 105 days was observed.

Conclusion

In summary, artemisinin derivatives bearing Mannich base group, another new type of water-soluble compounds, showed higher stability and good antimalarial activity though they were found to be less potent than artesunic acid in the monkey model.

Experimental

Melting points were taken in open capillary on BUCHI-510 melting point apparatus and were uncorrected. The IR spectra were run on Perkin-Elmer 599B spectrophotometer. ^1H NMR spectra were determined in CD_3OCD_3, D_2O (for salts), or $CDCl_3$ solution on a

Table 1. Antimalarial activity of **17** against *P. berghei* K_{173} and *P. falciparum* K_1 and NF_{54}

Compd	R′	OH	NR_2	ED_{50} (mg/kg/day)	ED_{90} (mg/kg/day)	IC_{50} (ng/mL)	
						K_1	NF_{54}
3				6.33 (po) 0.97 (iv)	23.37 (po) 5.25 (iv)	1.20	1.20
17a	H	3′	4′-CH_2NMe_2	ND	ND	ND	ND
17b	H	3′	4′-CH_2NEt_2	**1.00** (po) **0.96** (sc)	**2.68** (po) **4.11** (sc)	**0.18**	**0.36**
17c	H	3′	4′-CH_2-pyrrolidino	> 5.0	ND	ND	ND
17 d	H	3′	4′-CH_2-morpholino	**0.64** (po) **2.45** (sc)	**2.72** (po) **4.68** (sc)	ND	ND
17e	H	3′	4′-CH_2-*t*-butylamino	> 5.0	ND	**0.25**	**0.17**
17f	H	4′	3′-CH_2NMe_2	ND	ND	ND	ND
17g	H	4′	3′-CH_2NEt_2	2.80	11.52	**0.26**	**0.29**
17h	H	4′	3′,5′-bis-(CH_2-pyrrolidino)	> 2.0	ND	ND	ND
17i	H	4′	3,5-bis-(CH_2-morpholino)	> 10.0	ND	ND	ND
17j	Me	3′	4′-CH_2-pyrrolidino	2.62	9.50	ND	ND

ND, not done.

Table 2. Rapidity of parasite clearance of **17b** and **17d** oral administrated on *P. knowlesi* in Rhesus monkeys (7-day treatment test)

Compd	Dose (mg/kg/day)	Number of monkeys	Parasitemia before treatment (‰)	Mean time for 50% reduction (h)	Mean time for 90% reduction (h)	Time of parasitemia clearance (h)
3	10.0	3	35.0	8.21	13.74	50
	3.16	3	42.7	7.84	13.30	54
17b	10.0	2	38.0	4.70	11.58	44
	3.16	2	34.5	2.00	10.13	NC
17d	3.16	1	112.0	—	—	NC

NC, no clearance.

JNM PS-100 or Brucker AM-400 spectrophotometer. ^{13}C NMR spectra were determined in DMSO-d_6 solution on Brucker AM-400 spectrophotometer. Elemental analyses were performed on CE 1106 elemental analyser and all the results had deviation within 0.4% of the theoretical values.

General method for preparation of compound 21

Method A. To a solution of dihydroartemisinin (**2**, 5.7 g, 0.02 mol) and acetoxybenzyl alcohol (**19**, R′ = H or CH_3, 0.03 mol) in 150 mL of CH_2Cl_2 was added five drops of $BF_3{\cdot}Et_2O$ at 0 °C. The mixture was stirred at room temperature until the reaction completed (monitored by TLC). The solution was evaporated under reduced pressure. The resultant oil was dissolved in 150 mL of 0.25% KOH/EtOH and stirred at room temperature overnight. After neutralizing with dilute hydrochloric acid, the solution was evaporated in vacuum. The residue was dissolved in CH_2Cl_2, washed, dried, concentrated and purified by recrystallization or column chromatography (silica gel, using ethyl acetate–petroleum ether 5:100 v/v as the eluent) to give **21**.

Method B. Compound **20** was first isolated by column chromatography to afford pure 12-β isomer and then saponified as the procedure above mentioned.

12β-Dihydroartemisinyl *m*-hydroxybenzyl ether (21a, R′ = H). White crystal, mp 140–142 °C (from ethyl acetate–petroleum ether), yield: 57% from **2**. ^1H NMR (100 MHz, ppm, CD_3COCD_3):δ 6.60–7.20 (4H, m, aromatic H), 5.42 (1H, s, 5-H), 4.78 (1H, d, J = 4 Hz, 12-H), 4.70, 4.35 (2H, q, J_{AB} = 12 Hz, OCH_2), 3.32 (1H, s, OH), 1.28 (3H, s, 15-CH_3), 0.88–0.80 (6H, m, 13-CH_3, 14-CH_3). Anal. calcd for $C_{22}H_{30}O_6$: C, 67.67, H, 7.74, found: C, 68.04, H 8.04.

12β - Dihydroartemisinyl *p* - hydroxybenzyl ether (21b, R′ = H) and its epimer. White crystal, mp 155–156 °C (from ethyl acetate–petroleum ether), yield: 68% from **2**. ^1H NMR (400 MHz, ppm, $CDCl_3$): δ 7.09, 6.74 (4H, q, J_{AB} = 8.4 Hz, aromatic H), 5.42 (1H, s, 5-H), 4.79 (1H, d, J = 3.4 Hz, 12-H), 4.71, 4.38 (2H, q, J_{AB} = 11.9 Hz, OCH_2), 1.39 (3H, s, 15-CH_3), 0.88 (3H, d, J = 5.9 Hz, 13-CH_3), 0.83 (3H, d, J = 7.3 Hz, 14-CH_3). Anal. calcd for $C_{22}H_{30}O_6$: C, 67.67, H, 7.74, found: C, 68.02, H 7.97.

Its epimer: **12α-dihydroartemisinyl *p*-hydroxybenzyl ether**.

White crystal, mp 146–148 °C (from ethyl acetate–petroleum ether), yield: 4% from **2**. ^1H NMR (400 MHz, ppm, $CDCl_3$): δ 7.18, 6.78 (4H, q, J_{AB} = 8.4 Hz, aromatic H), 5.42 (1H, s, 5-H), 4.85, 4.52 (2H, q, J_{AB} = 11.9 Hz, OCH_2), 4.48 (1H, d, J = 9.2 Hz, 12-H), 1.40 (3H, s, 15-CH_3), 0.88 (3H, d, J = 5.9 Hz, 13-CH_3), 0.80 (3H, d, J = 7.1 Hz, 14-CH_3). Anal. calcd for $C_{22}H_{30}O_6$: C, 67.67, H 7.74, found: C, 67.93, H 7.85.

12β - Dihydroartemisinyl *m* - hydroxy - α - methyl - benzyl ether (21c, R′ = CH_3). White crystal, mp 144–145 °C (from ethyl acetate–petroleum ether), yield: 63% from **2**, ^1H NMR (100 MHz, ppm, $CDCl_3$): δ 7.00 (1H, m, aromatic H), 6.70 (3H, m, aromatic H), 5.48 (1H, s, 5-H), 4.80 (1H, m, OC**H**CH_3), 4.52 (1H, d, J = 4.0 Hz, 12-H), 1.36 (3H, s, 15-CH_3), 1.32 (3H, m, OCHC**H$_3$**), 0.90 (3H, d, J = 4.0 Hz, 13-CH_3), 0.74 (3H, d, J = 7.0 Hz, 14-CH_3). Anal. calcd for $C_{23}H_{32}O_6$: C, 68.29, H 7.97, found: C, 68.44, H 8.20.

12β - Dihydroartemisinyl *p* - hydroxy - α - methyl - benzyl ether (21d, R′ = CH_3). White crystal, mp 157–158 °C (from ethyl acetate–petroleum ether), yield: 63% from **2**. ^1H NMR (100 MHz, ppm, $CDCl_3$): δ 7.10, 6.80 (4H, q, J_{AB} = 8.0 Hz, aromatic H), 6.79 (2H, q, J_{AB} = 8.4 Hz, aromatic H), 5.46 (1H, s, 5-H), 4.80 (1H, m, OC**H**CH_3), 4.14 (1H, d, J = 4.0 Hz, 12-H), 1.44 (3H, s, 15-CH_3), 1.30 (3H, m, OCHC**H$_3$**), 0.88–0.80 (6H, m, 13-CH_3, 14-CH_3). Anal. calcd for $C_{23}H_{32}O_6$: C, 68.29, H 7.97, found: C 68.44, H 8.20.

General method for preparation of compound 17

Method A. To a solution of **21** (5 mmol) in 80 mL of dioxane (or THF) was added excess paraformaldehyde (occasionally, formalin, 15∼20 mmol) and amine (10∼30 mmol). The resulting mixture was heated at 60 °C over 12 h and then evaporated in vacuum. The residue was extracted with CH_2Cl_2 (or EtOAc), the extract was washed successively with water, brine and then dried ($MgSO_4$) and concentrated in vacuum. The crude products were purified by column chromatography (silica gel) using ethyl acetate–petroleum ether (1:4 to 1:0) as the eluent to give **17**.

Method B. A solution of excess paraformaldehyde and amine (as mentioned above) in dioxane was refluxed for 2 h then cooled to 60 °C. After adding **21**, the mixture was stirred for 24 h at the same temperature. The workup was the same as above.

3′-Hydroxy-4′-dimethylaminomethyl-benzyl 12-β-dihydroartemisinyl ether oxalate (17a). White crystal, mp 171–174 °C (from ethyl ether), yield 30% from **21a**. ^1H NMR (free base, 400 MHz, ppm, $CDCl_3$): δ 6.93 (1H, d, J = 7.7 Hz, aromatic H), 6.81 (1H, s, aromatic H), 6.73 (1H, d, J = 6.6 Hz, aromatic H), 5.46 (1H, s, 5-H), 4.91 (1H, d, J = 3.6 Hz, 12-H), 4.87 (1H, d, J = 12.4 Hz, OCH_2), 4.46 (1H, d, J = 12.4 Hz, OCH_2), 3.65 (2H, s, CH_2N), 2.34 (6H, s, NCH_3 × 2), 1.47 (3H, s, 15-CH_3), 0.97 (3H, d, J = 7.7 Hz, 13-CH_3), 0.89 (3H, d, J = 6.9 Hz, 14-CH_3). IR (KBr, cm^{-1}): 1626, 1585, 1466, 1377, 1281, 1101, 1013, 980, 876, 825. Anal. calcd for $C_{25}H_{37}NO_6$: C, 67.07, H, 8.34, N 3.13, found: C, 66.68, H 8.37, N 3.20.

3′-Hydroxy-4′-diethylaminomethyl-benzyl 12-β-dihydroartemisinyl ether oxalate (17b). White crystal, mp 148–150 °C (from acetone–THF), yield 50% from **21a**. ^1H NMR (400 MHz, ppm, D_2O): δ 7.42 (1H, m, aromatic H), 6.98 (2H, d, J = 8.3 Hz, aromatic H), 5.39 (1H, s, 5-H), 4.90 (1H, d, J = 3.7 Hz, 12-H), 4.71 (1H, d, J = 12.5 Hz, OCH_2), 4.58 (1H, d, J = 12.5 Hz, OCH_2), 4.32 (2H, s, $ArCH_2N$), 3.21 (4H, m, N**CH_2**CH_3 × 2), 1.42 (3H, s, 15-CH_3), 1.33 (6H, m, CH_2C**H$_3$** × 2), 0.93

(3H, d, $J=6.2$ Hz, 13-CH_3), 0.90 (3H, d, $J=3.5$ Hz, 14-CH_3). ^{13}C NMR (400 MHz, ppm, DMSO-d_6): δ 9.72 (q), 12.99 (q), 20.26 (q), 24.14 (t), 24.34 (t), 25.72 (q), 30.59 (d), 34.12 (t), 36.07 (t), 36.70 (t), 38.91 (d), 43.81 (d), 46.04 (t), 52.04 (t), 52.05 (d), 68.72 (t), 80.41 (s), 86.95 (d), 100.36 (d), 103.23 (s), 113.81 (d), 117.36 (d), 117.92 (s), 130.59 (d), 140.06 (s), 156.62 (s), 164.23 (s).[30,31] Anal. calcd for $C_{29}H_{43}NO_{10}$: C, 61.58, H, 7.66, N, 2.48, found: C, 61.68, H, 7.64, N, 2.29.

3′-Hydroxy-4′-pyrrolidinomethyl-benzyl 12-β-dihydroartemisinyl ether oxalate (17c). White crystal, mp 165–166 °C (from ethyl acetate–acetone), yield 43% from **21a**. ^{1}H NMR (400 MHz, ppm, D_2O): δ 7.38 (1H, d, $J=6.7$ Hz, aromatic H), 7.04 (2H, s, aromatic H), 5.42 (1H, s, 5-H), 4.90 (1H, d, $J=3.7$ Hz, 12-H), 4.73 (1H, d, $J=12.5$ Hz, OCH_2), 4.64 (1H, d, $J=12.5$ Hz, OCH_2), 4.38 (2H, s, $ArCH_2N$), 3.53, 3.23 (2H, 2H, m, m, CH_2NCH_2), 2.17, 2.02 (2H, 2H, m, m, CH_2CH_2), 1.43 (3H, s, 15-CH_3), 0.97 (6H, m, 13-CH_3, 14-CH_3). Anal. calcd for $C_{29}H_{40}NO_{10}\cdot 3/4H_2O$: C, 60.46, H, 7.26, N, 2.43, found: C, 60.46, H, 7.29, N, 2.34.

3′-Hydroxy-4′-morpholinomethyl-benzyl 12-β-dihydroartemisinyl ether oxalate (17d). White crystal, mp 163–164 °C (from acetone–ether), yield 68% from **21a**. ^{1}H NMR (400 MHz, ppm, D_2O): δ 7.39 (1H, d, $J=8.2$ Hz, aromatic H), 7.05 (2H, m, aromatic H), 5.41 (1H, s, 5-H), 4.95 (1H, d, $J=3.4$ Hz, 12-H), 4.73 (1H, $J=12.6$ Hz, OCH_2), 4.64 (1H, $J=12.6$ Hz, OCH_2), 4.40 (2H, s, $ArCH_2N$), 4.10, 3.82 (2H, 2H, d, d, $J=13.2$ Hz, CH_2OCH_2), 3.49, 3.31 (2H, 2H, d, d, $J=12.9$ Hz, CH_2NCH_2), 1.41 (3H, s, 15-CH_3), 0.97 (3H, d, $J=7.4$ Hz, 13-CH_3), 0.95 (3H, d, $J=5.0$ Hz, 14-CH_3), ^{13}C NMR (400 MHz, ppm, DMSO-d_6)[30,31]: δ 13.00 (q), 20.26 (q), 24.15 (t), 24.33 (t), 25.71 (q), 30.58 (d), 34.11 (t), 36.06 (t), 36.70 (d), 43.80 (d), 51.38 (t), 52.04 (d), 55.38 (t), 64.12 (t), 68.67 (t), 80.41 (s), 86.95 (d), 100.37 (d), 103.24 (s), 113.79 (d), 116.47 (s), 117.42 (d), 131.58 (d), 140.53 (s), 156.33(s), 163.17(s).[30,31] Anal. calcd for $C_{29}H_{41}NO_{11}$: C, 60.09, H, 7.13, N, 2.42; found: C, 59.69, H, 7.09, N, 2.36.

3′-Hydroxy-4′-*t*-butylmethylamino-benzyl 12-β-dihydroartemisinyl ether maleate (17e). White crystal, mp 161–162 °C (from acetone), yield 27% from **21a**. ^{1}H NMR (400 MHz, ppm, CD_3COCD_3): δ 7.37 (1H, d, $J=7.7$ Hz, aromatic H), 7.04 (1H, s, aromatic H), 6.85 (1H, d, $J=7.8$ Hz, aromatic H), 6.16 (2H, s, CH=CH), 5.45 (1H, s, 5-H), 4.81 (1H, d, $J=3.9$ Hz, 12-H), 4.78 (1H, $J=12.5$ Hz, OCH_2), 4.43 (1H, $J=12.6$ Hz, OCH_2), 4.31 (2H, s, $ArCH_2N$), 1.58 (9H, s, C_4H_9), 1.34 (3H, m, 15-CH_3), 0.94 (6H, m, 13-CH_3, 14-CH_3). Anal. calcd for $C_{31}H_{45}NO_{10}$: C, 62.93, H, 7.67, N, 2.37, found: C, 62.66, H, 7.72, N, 2.18.

4′-Hydroxy-3′-dimethylaminomethyl-benzyl 12-β-dihydroartemisinyl ether maleate (17f). White crystal, mp 103–106 °C (from ethyl acetate–petroleum ether), yield 33% from **21b**. ^{1}H NMR (free base, 400 MHz, ppm, $CDCl_3$): δ 7.13 (1H, d, $J=8.1$ Hz, aromatic H), 6.90 (1H, s, aromatic H), 6.80 (1H, d, $J=8.4$ Hz, aromatic H), 5.43 (1H, s, 5-H), 4.87 (1H, d, $J=3.3$ Hz, 12-H), 4.74 (1H, d, $J=11.7$ Hz, OCH_2), 4.42 (1H, d, $J=12.1$ Hz, OCH_2), 3.67 (2H, m, $ArCH_2N$), 2.33 (6H, s, $NCH_3\times 2$), 1.45 (3H, s, 15- CH_3), 0.93 (3H, d, $J=6.2$ Hz, 13-CH_3), 0.88 (3H, d, $J=7.3$ Hz, 14-CH_3). IR (KBr, cm^{-1}): 1614, 1495, 1467, 1375, 1263, 1103, 1030, 937, 881, 825. Anal. calcd for $C_{25}H_{37}NO_6$: C, 67.09, H, 8.33, N, 3.13; found: C, 67.24, H, 8.31, N, 2.84.

4′-Hydroxy-3′-diethylaminomethyl-benzyl 12-β-dihydroartemisinyl ether oxalate (17g). White crystal, mp 152–154 °C (from acetone), yield 41% from **21b**. ^{1}H NMR (400 MHz, ppm, D_2O): δ 7.41 (1H, m, aromatic H), 7.37 (1H, s, aromatic H), 6.98 (1H, d, $J=8.3$ Hz, aromatic H), 5.52 (1H, s, 5-H), 4.90 (1H, d, $J=3.7$ Hz, 12-H), 4.71 (1H, d, $J=11.8$ Hz, OCH_2), 4.52 (1H, d, $J=11.8$ Hz, OCH_2), 4.32 (2H, q, $J=13.3$ Hz, $ArCH_2N$), 3.21 (4H, m, $NC\mathbf{H_2}CH_3\times 2$), 1.42 (3H, s, 15-$CH_3$), 1.33 (6H, m, $CH_2C\mathbf{H_3}\times 2$), 0.93 (3H, d, $J=5.4$ Hz, 13-CH_3), 0.90 (3H, d, $J=7.3$ Hz, 14-CH_3). Anal. calcd for $C_{29}H_{43}NO_{10}$: C, 61.59, H, 7.66, N, 2.48, found: C, 61.96, H, 8.01, N, 2.89.

4′-Hydroxy-3′, 5′-bis(pyrrolidinomethyl)-benzyl 12-β-dihydroartemisinyl ether fumarate (17h). White crystal, mp 152–154 °C (from ethyl alcohol–petroleum ether), yield 57% from **21b**. ^{1}H NMR (free base, 100 MHz, ppm, $CDCl_3$): δ 7.07 (1H, s, aromatic H), 6.80 (1H, s, aromatic H), 5.14 (1H, s, 5-H), 4.67 (1H, d, $J=3.8$ Hz, 12-H), 4.41 (2H, q, $J=12$ Hz, OCH_2), 3.85 (4H, s, $ArCH_2N\times 2$), 2.60 (8H, m, $CH_2NCH_2\times 2$), 1.76 (8H, m, $CH_2CH_2\times 2$), 1.38 (3H, s, 15-CH_3), 0.88 (6H, m, 13-CH_3, 14-CH_3). Anal. calcd for $C_{40}H_{56}N_2O_{14}$: C, 60.90, H, 7.15, N, 3.55, found: C, 60.82, H, 7.34, N, 3.77.

4′-Hydroxy-3′, 5′-bis(morpholinomethyl)-benzyl 12-β-dihydroartemisinyl ether fumarate (17i). White crystal, mp 147–148 °C (from acetone), yield 40% from **21b**. ^{1}H NMR (400 MHz, D_2O): δ 7.42 (2H, s, aromatic H), 6.57 (2H, s, CH=CH), 5.44 (1H, s, 5-H), 4.90 (1H, d, $J=3.7$ Hz, 12-H), 4.62 (2H, q, $J=12$ Hz, OCH_2), 4.32 (4H, s, $ArCH_2N\times 2$), 3.92 (8H, m, $CH_2OCH_2\times 2$), 3.22 (8H, m, $CH_2NCH_2\times 2$), 1.40 (3H, s, 15-CH_3), 0.92 (3H, d, $J=5.7$ Hz, 13-CH_3), 0.90 (3H, d, $J=7.3$ Hz, 14-CH_3). Anal. calcd for $C_{36}H_{54}N_2O_{13}$: C, 59.82, H, 7.53. N, 3.88, found: C, 60.21, H, 7.45, N, 3.73.

3′-Hydroxy- 4′-pyrrolidinomethyl-α-methyl-benzyl 12-β-dihydroartemisinyl ether oxalate (17j). White crystal, mp 157–158 °C (from acetone–water), yield 38% from **21c**. ^{1}H NMR (400 MHz, ppm, D_2O): δ 7.35 (1H, m, aromatic H), 6.95 (2H, m, aromatic H), 5.62 (1H, s, 5-H), 4.62 (1H, m, $OC\mathbf{H}CH_3$), 4.33 (2H, d, $J=9.0$ Hz, $ArCH_2N$), 3.50, 3.17 (2H, 2H, m, m, CH_2NCH_2), 2.14, 2.00 (2H, 2H, m, m, CH_2CH_2), 1.44 (3H, d, $J=7.6$ Hz, $OCHC\mathbf{H_3}$), 1.41 (3H, s, 15-CH_3), 0.95 (3H, d, $J=5.2$ Hz, 13-CH_3), 0.86 (3H, d, $J=6.5$ Hz, 14-CH_3). Anal. calcd for $C_{30}H_{43}NO_{10}$: C, 62.38, H, 7.50. N, 2.42, found: C, 62.33, H, 7.69, N, 2.35.

4′-Hydroxy-3′-dimethylaminomethyl-α-methyl-benzyl 12-β-dihydroartemisinyl ether oxalate (17k). White crystal, mp 128–130 °C (from ethyl acetate–acetone), yield 48% from **21d**. ^{1}H NMR (free base, 400 MHz, ppm, $CDCl_3$): δ 7.10 (1H, m, aromatic H), 6.86 (1H, d,

$J = 2.4$ Hz, aromatic H), 6.75 (1H, m, aromatic H), 5.48 (1H, s, 5-H), 4.81 (1H, m, OC**H**CH_3), 4.61 (1H, d, $J = 3.7$ Hz, 12-H), 3.60 (2H, m, $ArCH_2N$), 2.30 (6H, s, $NCH_3 \times 2$), 1.44 (3H, s, 15-CH_3), 1.39 (3H, d, $J = 6.7$ Hz, OCHC**H$_3$**), 0.97 (3H, d, $J = 6.1$ Hz, 13-CH_3), 0.74 (3H, d, $J = 7.3$ Hz, 14-CH_3). IR (KBr, cm^{-1}): 3200–2500, 1600, 1498, 1466, 1373, 1257, 1099, 987, 875. Anal. calcd for $C_{28}H_{41}NO_{10}$: C, 60.97, H, 7.49, N, 2.54; found: C, 60.88, H, 7.82, N, 2.85.

4′-Hydroxy-3′,5′-bis(diethylaminomethyl)-α-methyl-benzyl-12-β-dihydro artemisinyl ether oxalate (17l). White crystal, mp 136–138 °C (from ethyl alcohol–petroleum ether), yield 56% from **21d**. ^{1}H NMR (free base, 400 MHz, ppm, $CDCl_3$): δ 6.99 (2H, s, aromatic H), 5.49 (1H, s, 5-H), 4.83 (1H, q, $J = 6.4$ Hz, OC**H**CH_3), 4.64 (1H, d, $J = 3.4$ Hz, 12-H), 3.66 (4H, $ArCH_2N \times 2$), 2.58 (8H, m, N**CH_2**$CH_3 \times 4$), 1.44 (3H, s, 15-CH_3), 1.40 (3H, d, $J = 6.7$ Hz, OCHC**H$_3$**), 1.24 (12H, t, $J = 6.5$ Hz, NCH_2C**H$_3$** $\times 4$), 0.96 (3H, d, $J = 6.4$ Hz, 13-CH_3), 0.75 (3H, d, $J = 7.2$ Hz, 14-CH_3). IR (KBr, cm^{-1}): 3300–2500, 1608, 1470, 1375, 1227, 1099, 989, 875, 825. Anal. calcd for $C_{37}H_{58}N_2O_{14}$: C, 58.87, H, 7.74. N, 3.71, found: C, 58.85, H, 7.74, N, 3.70.

4′-Hydroxy-3′-pyrrolidinomethyl-α-methyl-benzyl 12-β-dihydroartemisinyl ether oxalate (17m). White crystal, mp 160–162 °C (from acetone–petroleum ether), yield 44% from **21d**. ^{1}H NMR (free base, 400 MHz, ppm, $CDCl_3$): δ 7.10 (1H, m, aromatic H), 6.90 (1H, m, aromatic H), 6.78 (1H, t, $J = 8.4$ Hz, aromatic H), 5.50 (1H, s, 5-H), 4.80 (1H, m, OC**H**CH_3), 4.63 (1H, d, $J = 3.3$ Hz, 12-H), 3.78 (2H, m, $ArCH_2N$), 2.60 (4H, m, CH_2NCH_2), 1.84 (3H, OCHC**H$_3$**), 1.46 (3H, s, 15-CH_3), 1.39 (4H, m, CH_2CH_2), 0.97 (3H, d, $J = 6.4$ Hz, 13-CH_3), 0.75 (3H, d, $J = 7.3$ Hz, 14-CH_3). IR (KBr, cm^{-1}): 3300–2500, 1600, 1498, 1462, 1257, 1099, 985, 875. Anal. calcd for $C_{30}H_{43}NO_{10}$: C, 62.38, H, 7.50. N, 2.42, found: C, 62.49, H, 7.42, N, 2.52.

4′-Hydroxy-3′, 5′-bis(piperidinomethyl)-α-methyl-benzyl 12-β-dihydro artemisinyl ether oxalate (17n). White crystal, mp 104–106 °C (from ethyl alcohol–petroleum ether), yield 36% from **21d**. ^{1}H NMR (free base, 400 MHz, ppm, $CDCl_3$): δ 6.94 (2H, s, aromatic H), 5.49 (1H, s, 5-H), 4.80 (1H, q, $J = 6.5$ Hz, OC**H**CH_3), 4.62 (1H, d, $J = 3.6$ Hz, 12-H), 3.56 (4H, s, $ArCH_2N \times 2$), 2.40 (8H, m, $CH_2NCH_2 \times 2$), 1.42–1.56 (12H, m, $CH_2CH_2CH_2 \times 2$), 1.38 (3H, s, 15-CH_3), 1.35 (3H, d, $J = 6.0$ Hz, OCHC**H$_3$**), 0.94 (3H, d, $J = 7.8$ Hz, 13-CH_3), 0.75 (3H, d, $J = 7.4$ Hz, 14-CH_3). IR (KBr, cm^{-1}): 3427, 2540, 1612, 1456, 1226, 1099, 989, 721. Anal. calcd for $C_{39}H_{58}N_2O_{14}$: C, 60.14, H, 7.51, N, 3.60, found: C, 60.21, H, 7.41, N, 3.81.

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