**Supplementary material**

**Materials and methods**

***Synthesis of artelinic acid-choline derivative (AD)***

N-dodecyl-N-(2-hydroxyethyl)-N, N-dimethyl ammonium bromide (choline derivative, CD) was synthesized by reaction between the N, N-dimethyl ethanolamine and 1-bromododecane. The DHA benzyl ethers were obtained by reaction of DHA with the substituted phenols in the presence of Et2O·BF3 at room temperature. Benzyl ethers were hydrolyzed at 0.5% KOH in ethanol to give the free acids, which were further transformed into aryl esters by coupling with CD. Detailed synthesis routes and chemical structure were obtained from Figure S1. The reaction process was monitored by TLC.

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Figure S1. Chemical synthetic routes of artelinic acid-choline derivative (AD) and the preparations process of its liposome (ADLs).

DHA, dihydroartemisinin; AA, artelinic acid; CD, choline derivative; AD, artelinic acid-choline derivative.

***Preparation and characterization of AD liposomes (ADLs)***

***Preparation of ADLs***

Soybean lecithin (48 mg), cholesterol (4.8 mg) and AD (8.0 mg) were added in a flask and dissolved with 10 mL chloroform. Then, it was evaporated to remove the organic solvent by rotary evaporation at 30 ℃ to obtain a lipid film. The lipid film was hydrated with 4 mL distilled water for 10 minutes at 30 ℃. The suspensions were sonicated using the probe sonicator for 5 minutes and filtered through a 0.22 μm filter to obtain ADLs. The samples were stored at 4 ℃ before use.

***Characterization of ADLs***

Particle size (size) and polydispersity index (PDI) were used as the indexes to evaluate the stability of ADLs at 4 ℃ within one month. The changes on size and PDI were recorded every ten days. The surface morphology of ADLs was observed by transmission electron microscopy (TEM), and the release behavior of liposomes was determined by *in vitro* dialysis.

***Determination of the encapsulation efficiency (EE) and drug loading efficiency (DL) of ADLs***

The performed liposomes were centrifuged at 13000 r /min (11525×*g*) for 10 min, and the encapsulated drug content was analyzed by HPLC.

M1 refers to the weight of drug encapsulated in the liposome, M2 refers to the total weight of drug, M3 refers to the total weight of liposome in the preparation.

***In vivo* Antimalarial efficacy study**

***Dose setting of in vivo Antimalarial*** ***efficacy study***

Our dose setting was based on the clinical oral dose of DHA. The human oral dose of DHA was 1.7 mg/kg/day, and the first dose has been doubled here. According to the equivalent dose ratio between human and animal based on body surface area, the oral dose of mice was set at 15.6 mg/kg/day (54.9 μmol/kg/day). Considering the bioavailability for oral administration was 0.25~0.33 times of intravenous injection, so the intravenous dose of mice was set at (13.7 ~ 18.1) μmol/kg/day. Accordingly, we designed five dose groups of 4.4, 8.8, 17.6, 35.2 and 70.4 μmol/kg/day, respectively.

**Results**

***Synthesis of AD***

The structure of AD was confirmed by Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS, Figure S2 A) and 1H-NMR (Figure S2 B). The accurate ions of m/z 658.4680 were detected as cationic compound ([M-Br] +, C39H64NO, -0.46 ppm) for AD, which was also in agreement with the theoretical m/z values ([M-Br]+, C39H64NO). Chemical shifts value of AD 1H-NMR and 13C-NMR was shown below.

AD, 1H-NMR (400 MHz, DMSO-d6, ppm): δ 7.95 (d, J = 8.4 Hz, 2 H), 7.46 (d, J = 8.3 Hz, 2 H), 5.36 (d, J = 4.1 Hz, 1 H), 4.83 – 4.77 (m, 1 H), 4.70 – 4.62 (m, 2 H), 4.51 (d, J = 13.5 Hz, 1 H), 3.81 – 3.74 (m, 2 H), 3.41 – 3.32 (m, 3 H), 3.11 (s, 6 H), 2.15 (tt, J = 16.4, 8.1 Hz, 1 H), 1.97 (dd, J = 10.4, 2.8 Hz, 1 H), 1.77 (dd, J = 9.3, 6.4 Hz, 2 H), 1.67 (dd, J = 8.8, 4.1 Hz, 3 H), 1.55 – 1.47 (m, 1 H), 1.39 (dd, J = 12.4, 5.0 Hz, 1 H), 1.35 (d, J = 5.1 Hz, 1 H), 1.33 – 1.30 (m, 1 H), 1.26 (s, 4 H), 1.19 (s, 16 H), 0.88-0.79 (m, 11 H).

AD, 13C NMR (101 MHz, DMSO-d6, ppm) δ 129.95, 127.64, 103.97, 87.62, 68.98, 64.39, 62.24, 59.02, 55.46, 52.55, 51.20, 44.26, 40.69, 40.49, 40.28, 40.07, 39.86, 39.65, 39.46, 39.17, 37.16, 36.54, 34.61, 31.83, 31.02, 29.55, 29.24, 29.04, 26.34, 26.15, 24.55, 22.61, 22.36, 20.61, 14.45, 13.33.

After four steps of synthesis and purification, the white powder of CD was obtained by adding with cold acetone (yield 60% to 70%). AA and AD were purified by column chromatography (yield 30% to 35%, 60% to 65%, respectively). The unsatisfactory yields of the AA and AD might due to the adsorption of column chromatography and formation of byproducts from esterification and etherification with catalyst in synthesis routes. And how to increase the yields also needs to further improve.

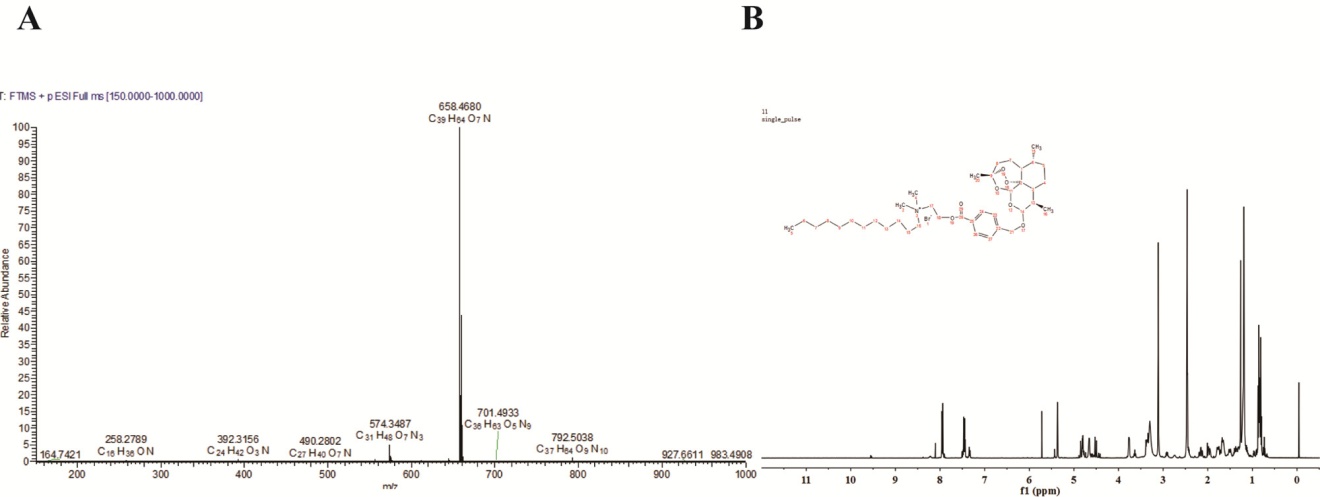


Figure S2. FT-ICR MS (A) and 1H-NMR of AD (B).

***Preparation and characterization of ADLs***

The thin-film hydration method was used to prepare liposomes. The encapsulation efficiency (EE) and drug loading efficiency (DL) of ADLs were (95.74 ± 0.10) %, (12.60 ± 0.05) %, respectively. The size, zeta potential and PDI of ADLs were (146.4 ± 1.8) nm, + (40.87 ± 0.86) mV and 0.35 ± 0.03, respectively.

The morphology of ADLs observed by transmission electron microscopy (TEM) was presented in Figure S3. The preparations exhibited spherical nano-liposome structure with unilamellar vesicles (ULV) and multilamellar vesicles (MLV). Larger PDI values indicated the presence of ULV and MLV or particle aggregates caused by defective particles extrusion (Gorshkova et al. 2017), which may be responsible for the relatively larger variation in particle size and PDI of ADLs within one month (Figure 1A). In order to avoid the effect of aggregation, ADLs should be prepared instantly when it is used.

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Figure S3. The TEM image of ADLs

***Statistic analysis of in vivo*** ***antimalarial activity results***

***Tests of between doses and groups effects.***

**Table S1 Tests of between-doses and groups effects**

Dependent Variable: suppression percentage

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Source | Type III Sum of Squares | df | Mean Square | *F* | *P* |
| Corrected Model | 14084.930a | 24 | 586.872 | 44.699 | .000 |
| Intercept | 737776.206 | 1 | 737776.206 | 56192.595 | .000 |
| Doses | 9686.217 | 4 | 2421.554 | 184.437 | .000 |
| Groups | 3765.305 | 4 | 941.326 | 71.696 | .000 |
| Doses \* Groups | 633.408 | 16 | 39.588 | 3.015 | .001 |
| Error | 984.707 | 75 | 13.129 |  |  |
| Total | 752845.843 | 100 |  |  |  |
| Corrected Total | 15069.637 | 99 |  |  |  |

a. R Squared = .935 (Adjusted R Squared = .914)

***The differences analysis between different treatment groups at same dose***

**Table S2 Pairwise Comparisons between different groups at same dose**

Dependent Variable: suppression percentage

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Doses | (I) Groups | (J) Groups | Mean Difference (I-J) | Std. Error | *P*b |
|
| 4.40 | ADLs | DHALs | 15.190\* | 2.562 | .000 |
| AALs | 15.782\* | 2.562 | .000 |
| AALs+CDLs | 18.192\* | 2.562 | .000 |
| CDLs | 30.215\* | 2.562 | .000 |
|  |  |  |  |  |
| DHALs | ADLs | -15.190\* | 2.562 | .000 |
| AALs | .592 | 2.562 | .818 |
| AALs+CDLs | 3.002 | 2.562 | .245 |
| CDLs | 15.025\* | 2.562 | .000 |
|  |  |  |  |  |
| AALs | ADLs | -15.782\* | 2.562 | .000 |
| DHALs | -.592 | 2.562 | .818 |
| AALs+CDLs | 2.410 | 2.562 | .350 |
| CDLs | 14.433\* | 2.562 | .000 |
|  |  |  |  |  |
| AALs+CDLs | ADLs | -18.192\* | 2.562 | .000 |
| DHALs | -3.002 | 2.562 | .245 |
| AALs | -2.410 | 2.562 | .350 |
| CDLs | 12.023\* | 2.562 | .000 |
|  |  |  |  |  |
| CDLs | ADLs | -30.215\* | 2.562 | .000 |
| DHALs | -15.025\* | 2.562 | .000 |
| AALs | -14.433\* | 2.562 | .000 |
| AALs+CDLs | -12.023\* | 2.562 | .000 |
|  |  |  |  |  |  |
| 8.80 | ADLs | DHALs | 8.495\* | 2.562 | .001 |
| AALs | 10.125\* | 2.562 | .000 |
| AALs+CDLs | 13.733\* | 2.562 | .000 |
| CDLs | 22.068\* | 2.562 | .000 |
|  |  |  |  |  |
| DHALs | ADLs | -8.495\* | 2.562 | .001 |
| AALs | 1.630 | 2.562 | .527 |
| AALs+CDLs | 5.237\* | 2.562 | .044 |
| CDLs | 13.572\* | 2.562 | .000 |
|  |  |  |  |  |
| AALs | ADLs | -10.125\* | 2.562 | .000 |
| DHALs | -1.630 | 2.562 | .527 |
| AALs+CDLs | 3.608 | 2.562 | .163 |
| CDLs | 11.942\* | 2.562 | .000 |
|  |  |  |  |  |
| AALs+CDLs | ADLs | -13.733\* | 2.562 | .000 |
| DHALs | -5.237\* | 2.562 | .044 |
| AALs | -3.608 | 2.562 | .163 |
| CDLs | 8.335\* | 2.562 | .002 |
|  |  |  |  |  |
| CDLs | ADLs | -22.068\* | 2.562 | .000 |
| DHALs | -13.572\* | 2.562 | .000 |
| AALs | -11.942\* | 2.562 | .000 |
| AALs+CDLs | -8.335\* | 2.562 | .002 |
|  |  |  |  |  |  |
| 17.60 | ADLs | DHALs | 4.788 | 2.562 | .046 |
| AALs | 4.987 | 2.562 | .050 |
| AALs+CDLs | 6.035\* | 2.562 | .021 |
| CDLs | 18.052\* | 2.562 | .000 |
|  |  |  |  |  |
| DHALs | ADLs | -4.788 | 2.562 | .046 |
| AALs | .200 | 2.562 | .938 |
| AALs+CDLs | 1.247 | 2.562 | .628 |
| CDLs | 13.265\* | 2.562 | .000 |
|  |  |  |  |  |
| AALs | ADLs | -4.987 | 2.562 | .050 |
| DHALs | -.200 | 2.562 | .938 |
| AALs+CDLs | 1.048 | 2.562 | .684 |
| CDLs | 13.065\* | 2.562 | .000 |
|  |  |  |  |  |
| AALs+CDLs | ADLs | -4.987 | 2.562 | .055 |
| DHALs | -1.247 | 2.562 | .628 |
| AALs | -1.048 | 2.562 | .684 |
| CDLs | 12.017\* | 2.562 | .000 |
|  |  |  |  |  |
| CDLs | ADLs | -18.052\* | 2.562 | .000 |
| DHALs | -13.265\* | 2.562 | .000 |
| AALs | -13.065\* | 2.562 | .000 |
| AALs+CDLs | -12.017\* | 2.562 | .000 |
|  |  |  |  |  |  |
| 35.20 | ADLs | DHALs | 1.438 | 2.562 | .576 |
| AALs | 2.280 | 2.562 | .376 |
| AALs+CDLs | 3.522 | 2.562 | .173 |
| CDLs | 13.727\* | 2.562 | .000 |
|  |  |  |  |  |
| DHALs | ADLs | -1.438 | 2.562 | .576 |
| AALs | .842 | 2.562 | .743 |
| AALs+CDLs | 2.085 | 2.562 | .418 |
| CDLs | 12.290\* | 2.562 | .000 |
|  |  |  |  |  |
| AALs | ADLs | -2.280 | 2.562 | .376 |
| DHALs | -.842 | 2.562 | .743 |
| AALs+CDLs | 1.243 | 2.562 | .629 |
| CDLs | 11.448\* | 2.562 | .000 |
|  |  |  |  |  |
| AALs+CDLs | ADLs | -3.522 | 2.562 | .173 |
| DHALs | -2.085 | 2.562 | .418 |
| AALs | -1.243 | 2.562 | .629 |
| CDLs | 10.205\* | 2.562 | .000 |
|  |  |  |  |  |
| CDLs | ADLs | -13.727\* | 2.562 | .000 |
| DHALs | -12.290\* | 2.562 | .000 |
| AALs | -11.448\* | 2.562 | .000 |
| AALs+CDLs | -10.205\* | 2.562 | .000 |
|  |  |  |  |  |  |
| 70.40 | ADLs | DHALs | .907 | 2.562 | .724 |
| AALs | .978 | 2.562 | .704 |
| AALs+CDLs | 1.193 | 2.562 | .643 |
| CDLs | 10.545\* | 2.562 | .000 |
|  |  |  |  |  |
| DHALs | ADLs | -.907 | 2.562 | .724 |
| AALs | .070 | 2.562 | .978 |
| AALs+CDLs | .285 | 2.562 | .912 |
| CDLs | 9.638\* | 2.562 | .000 |
|  |  |  |  |  |
| AALs | ADLs | -.978 | 2.562 | .704 |
| DHALs | -.070 | 2.562 | .978 |
| AALs+CDLs | .215 | 2.562 | .933 |
| CDLs | 9.567\* | 2.562 | .000 |
|  |  |  |  |  |
| AALs+CDLs | ADLs | -1.193 | 2.562 | .643 |
| DHALs | -.285 | 2.562 | .912 |
| AALs | -.215 | 2.562 | .933 |
| CDLs | 9.352\* | 2.562 | .000 |
|  |  |  |  |  |
| CDLs | ADLs | -10.545\* | 2.562 | .000 |
| DHALs | -9.638\* | 2.562 | .000 |
| AALs | -9.567\* | 2.562 | .000 |
| AALs+CDLs | -9.352\* | 2.562 | .000 |

Based on estimated marginal means

\*. The mean difference is significant at the 0.05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments)

**References**

**Gorshkova, Y. E., Kuklin A. I., Gordeliy, V. I.** (2017) Structure and phase transitions of DMPC multilamellar vesicles in the presence of Ca2+ ions. *Journal of Surface Investigation: X-ray, Synchrotron and Neutron Techniques* **11**, 27-37.