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Synthesis of Water Soluble C-10-Phenoxy Artemisinin-Chitosan Conjugate

DAN XIAO[1], BO YANG[1,*], YUN-JIAN CHEN[2], XIA-LI LIAO[1], XUE-MIN YANG[1], QI-XUE QIN[1] and DONG YI[1]

[1]Faculty of Life Science and Technology, Kunming University of Science and Technology, Kunming 650500, P.R. China
[2]Kunming Pharmaceutical Group Corporation, Kunming 650100, P.R. China

*Corresponding author: Tel: +86 13064281879; E-mail: yangbo6910@sina.com

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A sort of C-10-phenoxy artemisinin-chitosan conjugate, in which C-10-phenoxy artemisinin was covalently bound to chitosan, was prepared and its aqueous solubility was evaluated. The results indicated that the conjugate (1.013 mg/mL) had much higher aqueous solubility than artemisinin (0.0084 mg/mL) and C-10-phenoxy artemisinin (0.0245 mg/mL). The conjugate will be potentially useful for their application as the prodrug of artemisinin.

Key Words: C-10-Phenoxy artemisinin, Chitosan, Conjugate, Aqueous solubility.

INTRODUCTION

Malaria has a devastating effect throughout tropical regions. There are *ca.* 300-500 million clinical cases each year resulting in 1.5-2.7 million deaths. Nearly all fatal cases are caused by *Plasmodium falciparum*[1]. Artemisinin (Fig. 1) is the active principle of the Chinese traditional antimalarial drug *Artemisia annua* L[2]. Its semisynthetic derivatives, such as dihydroartemisinin, artemether, arteether and artesunate, are effective against both chloroquine-sensitive and chloroquine-resistant *Plasmodium falciparum* and are clinically used for the treatment of cerebral malaria[3]. The hydrolytic instability of these semisynthetic derivatives has still led scientists to prepare some new derivatives of artemisinin[4].

In previous reports, a series of new C-10 phenoxy derivatives of artemisinin were synthesized and studied with biological activity *in vitro*. The result showed that these derivatives were relatively stable in aqueous solution at neutral aqueous solution and had high *in vivo* activity in murine screens when administered orally[5-7]. However, C-10-phenoxy derivatives of artemisinin are a poorly aqueous soluble drug and have low bioavailability by oral administration due to slow drug dissolution and decomposition in stomach and intestine[8,9].

Chitosan (Fig. 2) which is a natural cationic polysaccharide composed by β-(1-4)-linked glucosamine units together with some N-acetyl-dglucosamine units, is obtained by exhaustive deacetylation of chitin[10]. Owing to the favorable biodegradable, nontoxic and antimicrobial properties, chitosan has been used in different biomedical and drug delivery applications[11,12].

In our previous study, we have prepared an inclusion complex of the drug artemether in hydroxypropyl-β-cyclodextrin, which showed an increase in aqueous solubility and bioavailability of drug[13]. In this paper, C-10-phenoxy artemisinin-chitosan conjugate was prepared and the aqueous solubility of conjugate was evaluated. These may provide a useful approach to develop a highly effective prodrug candidate of artemisinin.

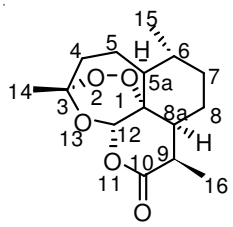


Fig. 1. Structure of the artemisinin

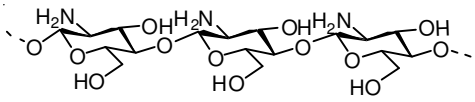


Fig. 2. Structure of the chitosan

EXPERIMENTAL

Artemisinin was obtained from Kunming Pharmaceutical Corporation (PC > 99 %) in Yunnan Province, P.R. China. Chitosan was commercially available ($\overline{Mn}$ = 1800, $\overline{Mw}$ / $\overline{Mn}$ = 1.31) (Nanjing Reagent Factory). N,N-Dimethylformamide (DMF) was predried over calcium hydride for 2 days and then distilled under a reduced pressure prior to use. 4-Dimethylamio-

pyridine (DMAP) was commercially available (Chengdu Reagent Factory). Trimethylsilyl trifluoromethane sulfonate was commercially available (Aladdin Reagent Factory). Dicyclohexylcarbodiimide (DCC) was commercially available (Shanghai Reagent Factory) and used without further purification. Other chemicals and solvents were of analytical-reagent grade and deionized double-distilled water was used throughout the study.

All reactions were monitored by TLC, melting points were determined by the capillary method without correction. ^{1}H NMR spectra and MS data were recorded on a Bruker DRX 500 NMR spectrometer and a ZAB-2F mass spectrometer, respectively.

Synthesis of dihydroartemisinin (2): To a stirred solution of artemisinin 1.500 g (5 mmol) in MeOH (130 mL) and maintained at 0-5 ºC, the mixture was added $NaBH_4$ 1.300 g (24 mmol), it was stirred at 0-5 º for 1 h. Acetic acid was added to adjust the pH = 7, Then it was added to cold water (100 mL) and stirred for 15 min at room temperature. The white precipitate was collected and washed with H_2O-MeOH (2:1, 2 mL × 200 mL). The wet crops were pooled and dissolved in CH_2Cl_2 (4 mL × 30 mL). After drying (40 g $MgSO_4$) and evaporation of the solvent, the solution were dried over anhydrous sodium sulphate and solvent was removed *in vacuo* to afford white solid (1.260 g) 84 % yield. m.p. 147-150 ºC (lit[14] 149-153 ºC). ^{1}H NMR (500 MHz, $CDCl_3$): 5.61-5.39 (m, 1H, O-CH-O), 5.30-4.76 (d, 1H, CHOH), 2.63(m, 0.5H, $CHCH_3$), 0.91-1.02 (m, 6H, $CHCH_3$), MS (m/z): 284 (M^+).

Synthesis of acetylated artemisinin (3): To a solution of dihydroartemisinin (**2**) (568 mg, 2 mmol) in dry pyridine (5 mL) at 0 ºC was slowly added acetic anhydride (6 mL). The reaction mixture was stirred at 0 ºC for 1 h and then a catalytic amount of 4-(dimethylamino)-pyridine (DMAP, 10 mg) was added. The reaction mixture was then allowed to warm to room temperature. The solution was allowed to stir for 2 h. The clear mixture was slowly poured into 10 mL of fast stirring ice-water. The solution was extracted with ethyl acetate (30 mL × 3 mL) and washed with 1 mol/L HCl, water and brine. After the solution was dried over Na_2SO_4 and filtered, the solvent was removed to give a crude product. Quick purification of the residues through a short silica gel pad with hexanes/EtOAc (10:1) gave the pure product (350 mg) in 95 % yield lit[15]. ^{1}H NMR (500 MHz, $CDCl_3$): 5.61-5.39 (m, 1H, O-CH-O), 5.30-4.76 (d, 1H, CHOH), 2.63 (m, 0.5H, $CHCH_3$), 0.91-1.02 (m, 6H, $CHCH_3$), MS (m/z): calcd. (%) for $C_{17}H_{26}O_6Na$ 349, found (%) 349.

Synthesis of methyl 4-[(10-dihydroartemisininoxy) methyl]benzoate of artemisinin (5): To a solution of the acetylated artemisinin (**3**) (0.100 mg, 0.306 mmol) and methyl *p*-hydroxymethylbenzoate (0.056 mg, 0.336 mmol) in anhydrous $CHCl_3$ (1 mL) was added TMSOTf (0.013 mL, 0.06 mmol) with stirring at room temperature. After 40 min, the reaction mixture was quenched with saturated $NaHCO_3$ solution (0.5 mL) and extracted with $CHCl_3$. The organic layer was washed with water and brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue obtained was purified by flash column, chromatography using 20 % EtOAc/hexane to give artelinic acid methyl ester (0.105 g, 80 %) lit[16]. ^{1}H NMR (500 MHz, $CDCl_3$): 0.97 (m, 7H), 1.28 (m, 2H), 1.47 (brs, 4H), 1.64 (m, 1H), 1.83 (m, 2H), 1.89 (m, 1H), 2.07 (m, 1H), 2.39 (dt, 1H), 2.71 (brs, 1H), 4.57 (d, *J* = 12.9 Hz, 1H), 4.94 (m, 2H), 5.46 (s, 1H), 7.39 (d, *J* = 9.9 Hz, 2H), 7.75 (d, *J* = 9.9Hz, 2H); 0.91-1.02 (m, 6H, $CHCH_3$), MS (m/z): calcd. (%) for $C_{17}H_{26}O_6Na$ 441, found (%) 441.

Synthesis of C-10-phenoxy artemisinin-chitosan conjugate (6): To a solution of (**5**) (418 mg, 1.0 mmol) in dry DMF (50 mL), chitosan (180 mg, 0.1 mmol) and DCC (226 mg, 1.1 mmol) was added. The reaction mixture was stirred for 2 days in an ice bath and another 2 days at room temperature and then allowed to stand for 1 h. The precipitate was removed by filtration and the filtrate was poured into 300 mL of acetone. The precipitate was collected and subsequently purified on a Sephadex G-25 column with water as eluent. After the residue was dried *in vacuo*, we got the yellow solid (185 mg) in 32 % yield. ^{1}H NMR (500 MHz, D_2O + HCl): 7.39 (d, *J* = 9.9 Hz, 2H), 7.75 (d, *J* = 9.9 Hz, 2H); 0.91-1.02 (m, 6H, $CHCH_3$), 5.80-5.44 (m, O-CH-O), 2.64-2.78 (m, O=CCH_2), 3.649-3.874 (m, H-3, H-4, H-5, H-6 of chitosan), 3.280-3.312 (H-2 of chitosan), 0.97 (d, $CHCH_3$), 0.87 (m, $CHCH_3$).

Measurement of water solubility: The water solubility of C-10-phenoxy derivatives of artemisinin-chitosan conjugate was assessed by preparation of its saturated aqueous solution. An excess amount of complex was put in 5 mL of water (pH *ca*. 7) and the mixture was stirred for 1 h. After removing the insoluble substance by filtration, the filtrate was evaporated under reduced pressure to dryness and the residue was dosed by weighing method.

RESULTS AND DISCUSSION

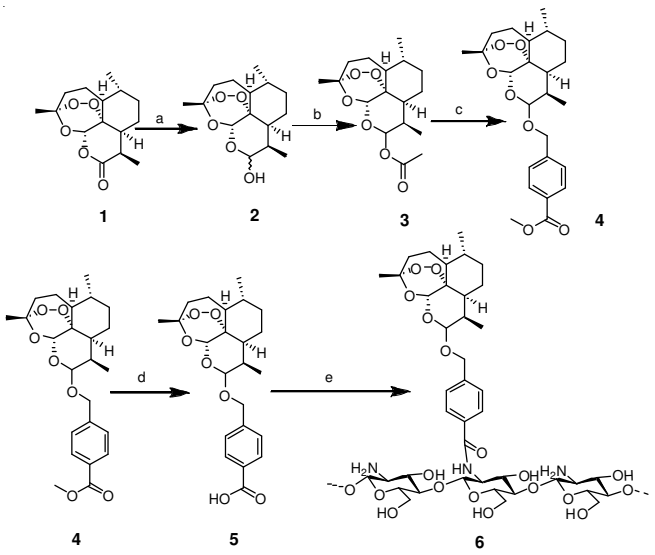
Two kinds of C-10-phenoxy artemisinin-chitosan conjugate that contain different amounts of artemisinin were synthesized according to the synthetic route shown in Fig. 3. The results are summarized in Table-1. The yields of C-10-phenoxy chitosan-artemisinin conjugates were 0.32 and 0.21 mol %, respectively and could be controlled by varying the feed ratio between chitosan and artesunate (**3**). All the polymers obtained showed high water solubility .The chemical composition of the polymers was confirmed by means of ^{1}H NMR. Fig. 4 showed a representative ^{1}H NMR spectrum of the chitosan-artemisinin conjugate and demonstrated the presence of covalently bound chitosan amide in the polymers. We could calculate the produced rate according to ^{1}H NMR spectrum.

TABLE-1
SYNTHETIC RESULTS DERIVED FROM CHITOSAN-ARTEMISININ CONJUGATE

Molar feed ratio conjugate (6)	$\overline{Mn}$ (g/mol)*	$\overline{Mn}$ (g/mol)**	Yield (mol %)
1:8	5000	1050	21
1:10	5800	1865	32

*The theoretical value. **Calculated from the peak integration of ^{1}H NMR spectra.

Table-2 showed that the conjugate (1.013 mg/mL) had much higher solubility than artemisinin (0.0084 mg/mL) and C-10-phenoxy artemisinin (0.0245 mg/mL). As the shortcoming of poorly aqueous soluble C-10-phenoxy artemisinin, we can anticipate that these may provide a useful approach to develop a highly effective prodrug candidate of artemisinin.



Reagents and conditins: (a) $NaBH_4$ (b) acetic anhydride (c) methyl *p*-hydroxymethylbenzoate/TMSOTF (d) $NaHCO_3$ (3) DCC

Fig. 3. Synthesis of C-10-phenoxy derivatives of artemisinin-chitosan conjugate

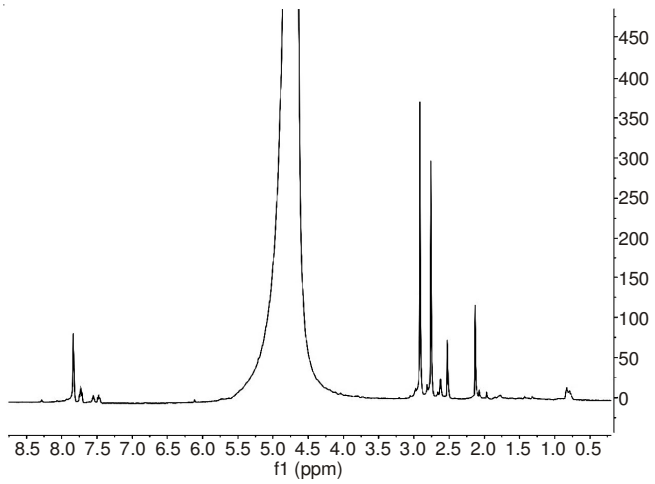


Fig. 4. ^1H NMR spectrum of C-10-phenoxy derivatives of artemisinin-chitosan conjugate in D_2O

TABLE-2
SOME PHYSICOCHEMICAL PROPERTIES OF ARTEMISININ AND CHITOSAN-ARTEMISININ CONJUGATE

Compound	Solubility (g/dL)[a]
Artemisinin	0.0084
Derivatives (5)	0.0245
Conjugate (6)	1.0130

[a]In water at 25 ºC.

Conclusion

We successfully prepared a new C-10-phenoxy artemisinin-chitosan conjugate and the results showed that the conjugate had much higher aqueous solubility compared to artemisinin. This conjugate will be potentially useful for their application as the prodrug of artemisinin.

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