Synthesis of Novel Ure Derivatives of Artemisinin

Do Thi Trang, Trinh Thi Nhung, Doan Thi Hien, Vu Dinh Tien, Tran Khac Vu*

Hanoi University of Science and Technology – No. 1, Dai Co Viet Str., Hai Ba Trung, Ha Noi, Viet Nam

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Abstract

The synthesis of novel artemisinin derivatives containing ure linkages 6a-c was described. 10β-azidoartemisinin (4) was first obtained in good yield by the reaction of dihydroartemisinin (2) with NaN_3 in the presence of $(CH_3)_3SiCl$ and a catalytic amount of KI in CH_2Cl_2 at ice water temperature. This compound was then hydrolyzed by Ph_3P in THF/H_2O at 65 °C for 6 h to furnish 10β-aminoartemisinin (5). The reaction of 5 with different isocyanates in CH_2Cl_2 at ambient temperature gave the target compounds 6a-c. The structures of synthesized compounds were confirmed based on spectroscopic methods: ^1H and ^{13}C NMR and comparison with published data.

Keywords. Artemisinin, dihydroartemisinin, artemether, arteether, sodium azide, isocyanate

1. Introduction

Artemisinin (1), a sesquiterpene lactone endoperoxide isolated from *Artemisia annua* L has been widely used as an important starting material in antimalarial drug development and research [1]. Since the discovery of artemisinin, a lot of semi-synthetic artemisinin derivatives have been developed into drug for the treatment of malaria such as artemether (3a), arteether (3b) and artesunate (3c) (Fig 1) [2-5]. Recently, clinical researches revealed that several artemisinin derivatives, besides the antimalarial activity, exerted pharmacological properties including anti-cancer, anti-virus, anti-fungi and immunosuppressive activity [6]. Accordingly, the research on artemisinin derivatives has recieved increasing attention in the drug development especially in the filed of cancer treatment throughout the world. More recently, in this direction, a few artemisinin derivatives have been reported to exhibit *in vitro* potential activities against several human cancer cell lines [7-11]. Therefore, in continuity of our research program toward new anti-cancer agent discovery, we have designed, synthesized a novel series of artemisinin drivatives containing ure linkages which is often contained in the molecule of cancer drugs. The current paper reports the results of this study.

2. Experimental

Dihydroartemisinin (2) was purchased from Duoc Khoa company, Hanoi University of Pharmacy. All products were examined by thin-layer chromatography (TLC), performed on Whatman 250lm Silica Gel GF Uniplates and visualized under UV light at 254 nm. Melting points determined in open capillaries on Electrothermal IA 9200 Shimazu apparatus and uncorrected. Purification was done by crystallization and the open silica gel column chromatography using Merck silica gel 60 (240–400 mesh). Nuclear magnetic resonance spectra (^1H and ^{13}C NMR) were recorded using tetramethylsilane (TMS) as an internal standard on a Bruker 500 MHz spectrometer with $CDCl_3$ as a solvent. Chemical shifts are reported in parts per million (ppm) downfield from TMS as internal standard, and coupling constants (*J*) are expressed in hertz (Hz). Multiplicities are shown as the abbreviations: s (singlet), brs (broad singlet), d (doublet), t (triplet), m (multiplet). Reagents and solvents were purchased from Aldrich or Fluka Chemical Corp. (Milwaukee, WI, USA) or Merck unless noted otherwise. Solvents were distilled and dried before use.

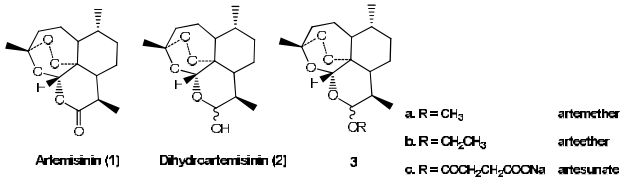


Fig.1. Some antimalarial derivatives

Synthesis of 10β-azidoartemisinin (4)

A mixture of dihydroartemisinin (2) (2,84g, 0,01 mol, 1eq), sodium azide (0.98 g, 0.015 mol, 1.5 eq), KI (83 mg, 0.5 mmol, 0.05 eq) and $(CH_3)_3SiCl$ (d = 0.85, 2.54 ml, 2eq) in CH_2Cl_2 (10 mL) was cooled in a ice-water bath and stirred for 2.5 h. The reaction was monitored by TLC (*n*-hexane: ethyl acetate = 10:1). The reaction mixture was then extracted with water, neutralized by $NaHCO_3$. The organic phase was dried on anhydrous Na_2SO_4, and was evaporated to the residues. Purification was performed by silica gel column chromatography using *n*-hexane: ethyl

* Corresponding author: Tel.: (+84) 904306925
Email: vu.trankhac@hust.edu.vn

acetate = 90:1 as an eluting system to give 10*β*-azidoartemisinin (4) (2.5g, 81 %). White crystal: Mp: 41-43 ^{o}C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.53 (s, 1H, H-12), 5.37 (d, *J* = 4.0 Hz, 1H, H-10), 2.71 (m, 1H), 2.40-2.33 (m, 1H), 2.06-2.03 (m, 1H), 1.91-1.86 (m, 1H), 1.82-1.81 (m, 1H), 1.89-1.87 (m, 1H), 1.82-1.76 (m, 1H), 1.72-1.63 (m, 2H), 1.52-1.47 (m, 2H), 1.44-1.42 (m, 3H), 1.37-1.34 (m, 1H), 1.26-1.22 (m, 1H), 0.96-0.90 (m, 6 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 104.4 (C-12), 91.8 (C-3), 88.6 (C-12a), 80.6 (C-10), 52.5; 44.1, 37.3, 36.2, 34.5, 30.2, 25.9, 24.6, 23.5 (C-14), 20.3 (C-15), 13.1 (C-16).

Synthesis of 10β-aminoartemisinin (5)

A mixture of 10β-azidoartemisinin (4) (1.90 g, 6.15 mmol, 1 eq), Ph_3P (2.417 g, 9.20 mmol, 1.5 eq)

in THF (10 mL) and H_2O (15 mL) was stirred at 65 ^{o}C for 8 h. The reaction was monitored by TLC using CH_2Cl_2 : MeOH = 10 : 1 as a developing solvent system. The reaction mixture was then diluted with CH_2Cl_2 (15mL), and extracted with H_2O (2 × 10 mL). The organic phase was separated, dried on anhydrous Na_2SO_4, and evaporated to the residues. The residues were kept in the fridge and washed several times with a cold mixture of *n*-hexane: ethylacetate (10 : 1) to remove Ph_3PO. Compound 5 was obtained as a yellowish oil (2.108 g, 81%), enough purity for the next step (1.067g, 61 %).

Synthesis of ure artemisinin derivatives 6a-c General procedure

A mixture of 10*β*-aminoartemisinin (5) (1 eq) and isocyanates: (*p*-tolylisocyanate, 3-chloro-4-methylphenyl isocyanate and 4-methoxy-2-methyl phenyl isocyanate) (1.1 eq) in CH_2Cl_2 (10 mL) was stirred at room temperature for 7 h. The reaction was monitored by TLC (CH_2Cl_2 : MeOH = 99 : 1). The reaction mixture was then washed with water. The organic phase was dried on anhydrous Na_2SO_4, and was evaporated to the residues. Purification was performed by silica gel column chromatography using CH_2Cl_2 : MeOH = 99 : 1 as an eluting system to give target compounds 6a-c in moderate yields.

Compound 6a: yield 68 %; light solid; mp: 136-137^{o}C. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.82 (s, 1H, NH), 7.15 (d, *J* = 8.5 Hz, 2H, Ar-H), 6.93(d, *J* = 8.5Hz, 2H, Ar-H), 6.37(d, *J* = 10.0 Hz, NH), 5.60(s, 1H, H-12), 5.41 (t, *J* = 10.0 Hz, 1H, H-10), 2.55-2.52 (m, 1H), 2.42-2.36 (dt, *J* = 4.0 Hz, 14.0 Hz, 1H), 2.23(s, 3H, CH_3, Ar-CH_3), 2.04-2.00 (m, 1H), 1.94-1.90(m, 1H), 1.84-1.81 (m, 1H), 1.76-1.73 (m, 2H), 1.67-1.63 (m, 1H), 1.53-1.46 (m, 3H), 1.41-1.36 (m, 4H), 1.29-1.32 (m, 1H), 1.06-1.00 (m, 1H), 0.99-0.98 (d, *J* = 6.0 Hz, 3H, CH_3, H-15), 0.96-0.94 (d, *J* = 7.0 Hz, 3H, CH_3, H-16).^{13}C NMR (125 MHz, $CDCl_3$) δ 154.93 (-NH-CO-NH-); 136.29, 131.79, 128.94, 119.90, 105.19 (C-3), 91.89 (C-10), 80.94 (C-12), 78.99 (C-12a), 51.64, 45.92, 37.48, 36.49, 34.08, 32.32, 25.47(C-14), 24.67, 21.75, 20.76 (Ar-CH_3); 20.28 (C-15), 13.409 (C-16).

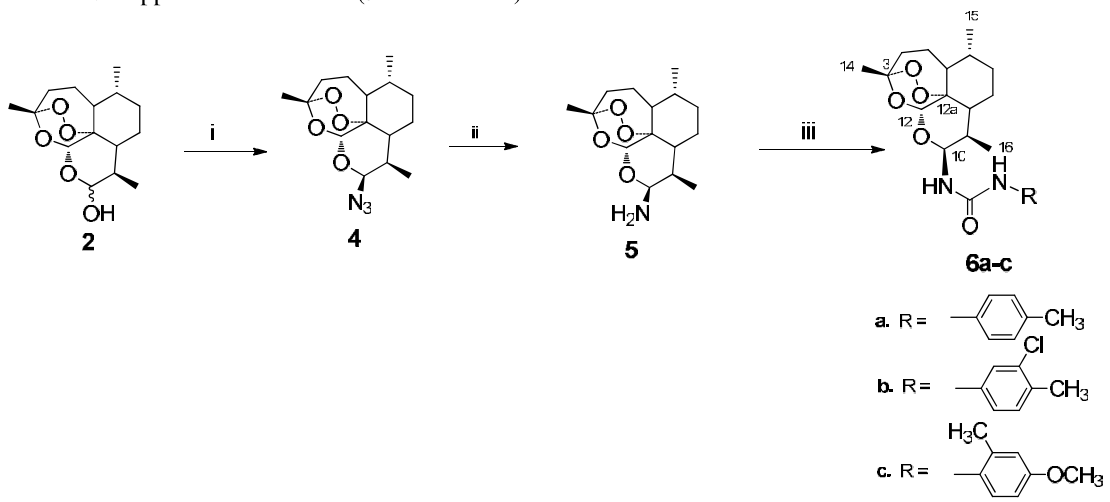
Compound 6b: yield 58%; light solid; mp: 137-139 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.86 (s, 1H, NH), 7.38-7.26 (m, 1H, H-Ar), 6.95 (m, 2H, H-Ar), 6.42 (d, *J* = 10.0 Hz, NH), 5.62 (s, 1H, H-12), 5.38 (t, *J* =10.5 Hz, 1H, H-10), 2.55 (m, 1H), 2.43-2.37 (dt, *J* = 4.0 Hz, 9.5 Hz, 1H), 2.24 (s, 3H, Ar-CH_3), 2.06-2.04 (m, 1H), 1.95-1.91 (m, 1H), 1.85-1.81 (m, 1H), 1.77-1.74 (m, 1H), 1.68-1.64 (m, 3H), 1.55-1.44 (m, 2H), 1.40 (s, 3H, CH_3, H-14), 1.38-1.26 (m, 2H), 1.09-1.04 (m, 1H), 1.0 (d, 6.0 Hz, 3H, CH_3, H-15), 0.96 (d, *J* = 6.5 Hz, 3H, CH_3, H-16). ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.74 (-NH-CO-NH-), 137.59, 133.93, 130.40, 129.74, 119.87, 117.78, 105.27 (C-3), 91.93 (C-10), 80.98 (C-12), 79.17 (C-12a), 51.61, 45.86, 37.49, 36.45, 34.06, 32.22, 25.46 (C-14), 24.65, 21.75, 20.27 (Ar-CH_3), 19.29 (C-15), 13.32 (C-16).

Compound 6c: yield: 47%; white solid; mp: 126-128 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.36 (d, *J* = 9.0 Hz, 1H, H-Ar), 7.12 (s, 1H, NH), 6.63 (d, *J* = 3.0 Hz, 1H, H-Ar), 6.55-6.52 (dd, *J* = 3.0 Hz, 9.0 Hz, 1H, H-Ar), 5.48 (s, 1H, H-12), 5.42 (t, *J* = 10.5 Hz, 1H, H-10), 3.72 (s, Ar-OCH_3), 2.56-2.54 (m, 1H), 2.40-2.35 (m, 1H), 2.16 (s, Ar-CH_3), 2.04-1.98 (m, 1H), 1.91-1.87(m, 1H), 1.84-1.80 (m, 1H), 1.756-1.72 (m, 2H), 1.68-1.62 (m, 1H), 1.52-1.49 (m, 2H), 1.44 (s, 3H, H-14), 1.31-1.25 (m, 1H), 1.08-1.00 (m, 1H), 0.97-0.95 (m, 6H, $2CH_3$, H-15, H-16). ^{13}C NMR (125 MHz, $CDCl_3$) δ 155.58 (-NH-CO-NH-), 115.71, 111.24, 104.99 (C-3), 91.90 (C-10), 80.87 (C-12), 78.88 (C-12a), 55.33 (Ar-OCH_3), 51.74, 45.95, 37.41, 36.50, 34.10, 32.34, 25.82 (C-14), 24.63, 21.77, 20.27 (C-15), 18.23 (Ar-CH_3), 13.58 (C-16).

3. Results and disscustion

The synthesis of artemisinin-based ure derivatives 6a-c is illustrated in Scheme 1. In the first step, dihydroartemisinin (2) reacted with sodium azide in the presence of trimethylsilyl chloride and potassium iodide in dichloromethane at 0–5 ^{o}C to give the 10β-azidoartemisinin intermediate (4) in good yield according to known procedure [12, 13]. This compound was then hydrolyzed by triphenylphosphine (Ph_3P) in mixture of tetrahydrofuran (THF) and water (1:1) at 65 ^{o}C to furnish 10β-aminoartemisinin (5) as yellowish oil in rather good yield by a more facile modified procedure without column chromatography. Next, the reaction of compound 5 with different isocyanates in CH_2Cl_2 afforded a series of novel derivatives 6a-c. The

structures of synthesized compounds were confirmed based on spectroscopic methods including ^{1}H and ^{13}C NMR and comparison with published data. Compound 6a was used as an example for structural elucidation. The ^{1}H NMR showed the presence of all protons in the molecule, in which signals at 7.82 and 6.34 ppm corresponding to protons of the ure group were observed. The aryl moiety could be seen through the presence of two couples of protons at 7.15 and 6.93 ppm as doublets (J = 8.5 Hz). Additionally, a singlet at 5.60 ppm was assigned to H-12 and a characteristic signal of H-10 was observed at 5.4 ppm as a triplet (t, J = 10.0 Hz). The ^{13}C NMR indicated the signals of 23 carbons, in which the signal at the lowest field was assigned to the carbon of ure group at δ: 154.92 ppm. Other characteristic signals at δ: 136.29, 131.78, 128.94 and 119.90 were observed and confirmed the presence of aryl moiety.



Scheme. 1. Synthesis of novel artemisinin derivatives: condition and reagents: i) $(CH_3)_3SiCl$, NaN_3, KI, CH_2Cl_2, 0 ^{o}C, 2.5 h, 81%; ii) Ph_3P, THF/H_2O, 65 ^{o}C, 6 h, 61%; iii) isocyanates, CH_2Cl_2, ambient temperature, 7h, 47-68%.

4. Conclusion

A series of novel artemisinin derivatives containing ure linkages has been sythesized. The structure of these derivatives has been characterized using spectroscopic methods such as ^{1}H and ^{13}C NMR and compared with published data. The bio-assay results of the synthesized derivatives will be addressed in due time.

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