SYNTHESIS OF SUCCINIC ACID 12α-DEOXOARTEMISINYL ESTER 4′-*O*-DEMETHYL-4β-(4″-NITROANILINO)-4-DESOXYPODOPHYLLOTOXIN

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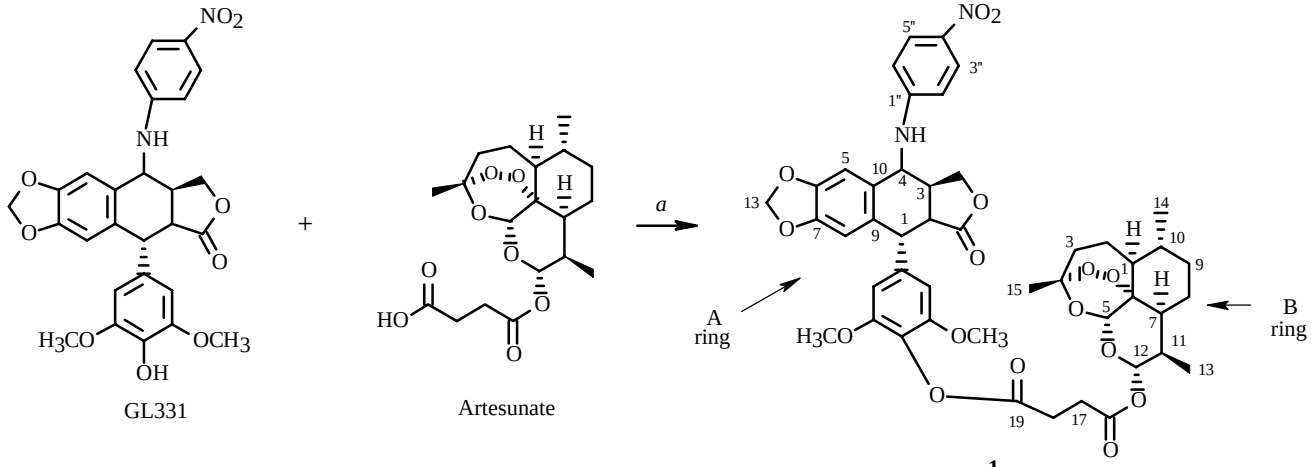
The succinic acid 12α-deoxoartemisinyl ester 4′-O-demethyl-4-β-(4″-nitroanilino)-4-desoxypodophyllotoxin was synthesized for the first time.

Key words: artesunate, Gl331, ester.

4′-*O*-Demethyl-4-β-(4″-nitroanilino)-4-desoxypodophyllotoxin (GL331) is one of the derivatives of podophyllotoxin [1]. Podophyllotoxin and its derivatives exhibit pronounced biological activity as antiviral agents and antineoplastic drugs [2]. The podophyllotoxin derivatives etoposide (VP-16), etopophos (etoposide phosphate), and teniposide (VM-26) are currently used in the chemotherapy of various types of cancer, including small cell lung cancer, lymphoma, testicular carcinoma, and Kaposi's sarcoma [3, 4]. GL331, as well as NK611 and TOP53, are currently under the clinical trial phase [5, 6]. Podophyllotoxin is known as an antimicrotubule agent while GL331 inhibits the catalytic activity of DNA topoisomerase II (topo II) [7, 8].

The antimalarial drug artesunate is a semisynthetic derivative of artemisinin (hemisuccinate of dihydroartemisinin) [9]. Artesunate is active against chloroquine- and mefloquine-resistant strains of *P. falciparum*, with satisfactory tolerability and low side effect [10]. Artemisinin exerts its antimalarial activity by iron-mediated cleavage of the endoperoxide bridge and generation of organic free radicals [11]. Recently, studies have shown that artemisinin and its derivatives, including artesunate, also reveal profound cytotoxic activity against tumor cells and antiviral activity [12]. The iron storage in tumor cells is generally greater than in normal cells; therefore, it may also play a key role in the inhibitory reaction of artesunate against tumor cells [13].

All of the compounds mentioned above share various inhibitory mechanisms, which are understood to some depth at the biochemical level. The present work is the first to prepare a topoisomerase inhibitor possessing multi-target specificity by counjugating the GL331 with artesunate.



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Synthesis of GL331 with artesunate was performed in dry CH_2Cl_2. The yield of ester after recrystallization from methanol was 70%. The structure of compound **1**, including A moiety and B moiety, was confirmed by ^{1}H NMR, ^{13}C NMR, TOF MS, and IR spectra. Signals for the ester C atoms appear at δ 170.66 (19-C of B ring) and 128.73 ppm (4′-C of A ring). In addition to signals for the skeleton of GL331, signals of artesunate (171.47, 105.15, 92.93, 92.13, 80.81, 37.96, 36.92, 34.78, 32.47, 26.60, 30.09, 29.23, 25.26, 22.68 ppm) are found. Further evaluation of the antitumor biological activity of compound **1** will be reported soon.

EXPERIMENTAL

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AM-500 spectrometer in $CDCl_3$ with $SiMe_4$ as internal standard. Melting points were determined on a Boetius X4 microstage. IR spectra were recorded on a Nicolet Magna IR 550 spectrometer. TOF MS spectra were obtained on a Perkin–Elmer SCIEX API 100 spectrometer. Thin layer chromatography (TLC) was carried out on silica gel G eluted by chloroform–acetone (95:5 v/v). Separation and purification were carried out by column chromatography on silica gel (200–300 mesh) (Qingdao Haiyang Chemical Group Co., P.R .China). Optical density was measured on a Perkin–Elmer 241 MC polarimeter with a 1-dm tube. Elemental analyses corresponded with those calculated. GL331 was prepared by the literature method [14].

Succinic Acid 12α-Deoxoartemisinyl Ester 4′-*O*-Demethyl-4β-(4″-nitroanilino)-4-desoxypodophyllotoxin (1). A mixture of 4′-*O*-demethyl-4β-(4″-nitroanilino)-4-desoxypodophyllotoxin (GL331, 0.104 g, 0.2 mmol) artesunate (0.153 g, 0.4 mmol), dimethylaminopyridine (DMAP, 0.03 g, 0.16 mmol), and *N*,*N*-dicyclohexylcarbodiimide (DCC, 0.0 5g, 0.24 mmol) in dried dichloromethane (20 mL) was stirred for 6 h at 0°C. The resulting suspension was filtered, and distilled water (100 mL) was added. After extracting with dichloromethane (100 mL) three times, the organic layer was dried and concentrated to give the crude product, with was then purified by silica gel column diluted with a mixture of chloroform–acetone (97:3 v/v). Solvent was removed in vacuum. The product was recrystallized from methanol.

A yield of 70% (0.073 g) of a light-yellow compound (crystals from methanol) was obtained, R_f 0.40, mp 165–167°C, $[\alpha]_D^{20}$+16° (*c* 0.5, $CHCl_3$), $C_{46}H_{50}O_{16}N_2$.

^{1}H NMR ($CDCl_3$) spectrum: 8.15 (d, J = 8.77 Hz, 2H, H-3″ and H-5″ of A ring), 6.76 (s, 1H, H-5 of A ring), 6.5 (d, 3H, H-8, H-2″ and H-6″ of A ring), 6.32 (s, 2H, H-2′ and H-6′ of A ring), 6.0 (s, 2H, OCH_2O-13 of A ring), 5.8 (d, J = 9.82, 1H, H-12 of B ring), 5.45 (s, 1H, H-5 of B ring), 4.83 (m, 2H, NH and H-1), 4.62 (d, J = 3.96, 1H, H-4), 4.40 (t, 1H, H-11), 3.90 (t, 1H, H-11), 3.72 (s, 6H, -OCH_3), 3.12~3.04 (m, 2H, H-2 and H-3 of A ring), 2.95 (t, 2H, CH_2-18), 2.8 (t, 2H, CH_2-17), 2.55 (m, 1H, H-11 of B ring), 2.36 (m, 1H, H-10 of B ring), 2.03 (m, 2H, CH_2-3 of B ring), 1.90 (m, 1H, CH_2-3 of B ring), 1.80~1.60 (m, 4H, CH_2-9, 8 of B ring), 1.55~1.42 (m, 1H, CH-7 of B ring), 1.39~1.35 (m, 1H, CH-1 of B ring), 1.31 (m, 2H, CH_2-2 of B ring), 0.86 (d, J = 6.95, 3H, CH_3-14 of B ring), 1.00 (d, J = 5.68, 3H, CH_3-13 of B ring), 1.41 (s, 3H, CH_3-15 of B ring).

^{13}C NMR ($CDCl_3$) spectrum: 174.60 (C-12 of A ring), 171.47 (C-16 of B ring), 170.66 (C-19 of B ring), 152.82 (C-3′ and C5′ of A ring), 152.31 (C-1″ of A ring), 149.40 (C-6 of A ring), 148.62 (C-7 of A ring), 139.86 (C-1′ of A ring), 138.14 (C-4″ of A ring), 132.51 (C-1 of A ring), 129.61 (C-9 of A ring), 128.73 (C-4′ of A ring), 127.30 (C-3″ and 5″-C of A ring), 111.79 (C-2″ and C-6″ of A ring), 110.91 (C-8 of A ring), 109.80 (C-5 of A ring), 108.44 (C-2′ and C-6′ of A ring), 105.15 (C-4 of B ring), 102.47 (C-13 of A ring), 92.93 (C-12 of B ring), 92.13 (C-5 of B ring), 80.81 (C-6 of B ring), 68.92 (8-C of A ring), 52.86 (C-7′ of A ring), 52.26 (C-8′ of A ring), 45.94 (C-4 of A ring), 44.29 (C-3 of A ring), 42.50 (C-7 of B ring), 38.97 (C-2 of A ring), 37.96 (C-1 of B ring), 36.92, 34.78 (C-17 and C-18 of B ring), 32.47 (C-2 of B ring), 26.60 (C-10 of B ring), 30.09, 29.23, 25.26, 22.68 (C-2, 3, 8, 9 of B ring), 20.87 (C-14, 15 of B ring), 12.71 (C-13 of B ring).

FTIR (KBr, ν, cm^{-1}) spectrum: 3470 (NH), 2920 (aliphatic C-H), 1775 (lactone), 1115, 881, 831 (-O-C-O-), 1660, 1520, 1490 (aromatic C=O), 1330, 1310 (NO_2).

Mass: (ESI+Na, *m/z*), 909.1

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