TẠP CHÍ KHOA HỌC ĐẠI HỌC KHÁNH HÒA
tapchikhoahoc@ukh.edu.vn

SYNTHESIS OF NEW C-3 ESTER-LINKED BETULINIC ACID CONJUGATES WITH LAMIVUDINE

Le Cong Hoan[1], Ngo Thi Uyen Tuyen[1], Dang Thi Tuyet Anh[2], Nguyen Hoang Sa[1]

[1]University of Khanh Hoa,
[2]Institute of Chemistry, Vietnam Academy of Science and Technology

Abstract: *Betulinic acid and its derivatives display diverse bioactivities, such as anti-inflammatory, antitumor, anti-inflammatory, antibacterial, antimalarial, anticancer, and anti-HIV activities. Betulinic acid possesses three sites that are the most flexible toderivatization, including the C-3 hydroxyl, C-20 alkene, and C-28 carboxylic acid positions. This study aims to design and synthesis of a C-3 betulinic acid derivatives.*

Betulinic derivatives were synthesized by using classical esterification or ester - ester exchange reaction. The target product was isolated by using column chromatography. Its structure was determined by combining spectral analysis and comparison with related data.

A new hybrid compound (compound 1c) was successfully synthesized from betulinic acid (BA) and lamivudine (3TC).

Keywords: *betulinic acid, ester, hybrid compounds, lamivudine.*

1. Introduction

Triterpenoids represent a varied class of natural products. Among these triterpenoids, the lupane-type triterpenoids are characterized by a diverse accumulation of bioactive compounds. One of the typical lupane triterpenes is 3*β*-hydroxy-lup-20(29)-en-28-oic acid (betulinic acid, BA), which is widely distributed throughout various plants. BA possesses a number of interesting pharmacological effects, including antitumor, anti-inflammatory, antibacterial, antimalarial, and antiviral activities [1, 2]. Recently, a lot of new articles have been published on transforming functional groups, incuding 3-OH, 28-COOH and C-19 isoprenyl (or C-20 alkene) in betulinic acid skeleton. It has the high synthetic potential and is dynamically used in transformations with the aim to design new drugs. For example, a series of new imidazole carboxylic esters (carbamates) and N-acylimidazole derivatives of betulinic acid shown cytotoxic activity against human cancer cell lines as HepG2, Jurkat and HeLa [3]. A number of C-3 and C-20 derivatives have shown cytotoxic activity on MOLT-4, JurkatE6.1, CEM.CM3, BRISTOL8, U937, DU145, PA-1, A549, and L132 cell lines [4]. A lot of BA-AZT hybrids with interesting anticancer potential on KB and Hep-G2 cell lines [5, 6]. In this paper, we performed synthesis of a new hybrid compound between BA and 3TC (an anti-HIV drug), particularly that derivatized on C-3 hydroxy group.

2. Experimental

Betulinic acid, lamivudine (3TC) and 3-methylglutaric anhydride were purchased from Sigma–Aldrich corporation. All reactions were carried out in the suitable oven-dried glassware and under atmospheric pressure. Column chromatography was performed using Silica gel 60 F-254 (0.25 mm, Merck). ESI-MS spectrum was obtained on an Agilent 1100 LC-MSD Trap spectrometer. ^{1}H-NMR (500MHz) and ^{13}C-NMR (125MHz) spectral data were measured on a Bruker Avance 500 NMR spectrometer at 25^{0}C.

2.1. Preparation of compound 1c

The mixture of BA (*1 equiv.*), 3-methylglutaric anhydride (*2equiv.*) and DMAP (*1 equiv.*) in anhydrous pyridine (5 mL) was refluxed overnight (10-15 hours). The product was

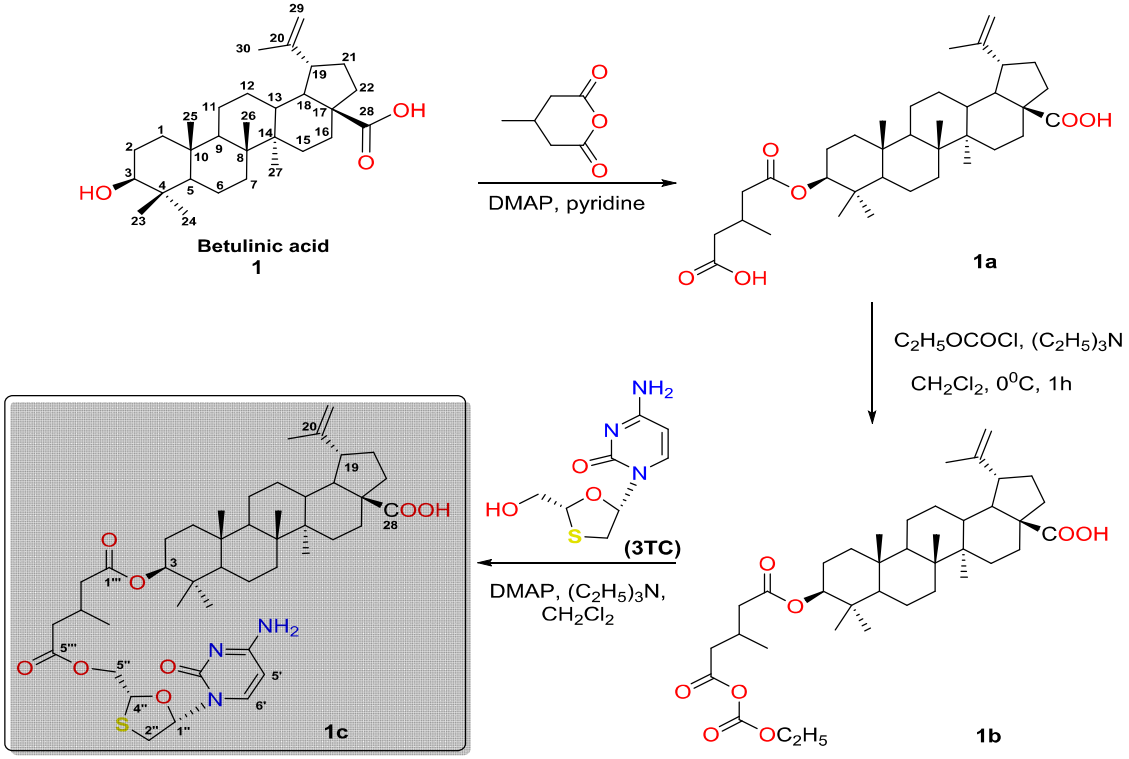
concentrated under reduced pressure, and mixture products were fractioned by CC eluting with *n*-hexane:CH_2Cl_2 (1:1) to afford colorless solid. The crude product (**1a)** was used into the next reaction.The solution of **1a** (*1 equiv.*), ethyl chloroformate (*1 equiv.*) and triethyl amine (*1 equiv.*) in CH_2Cl_2 (10 mL) mixture was stirred at 0^0C for 1 hour. The reaction was completed as verified by TLC control. The resulting solution (**1b**) was dispensed into triethyl amine (*1 equiv.*), DMAP (*1 equiv.*) and 3TC (*1 equiv.*). After maintaining at 0^0C for 1 hour, the reaction condition was warmed to room temperature for 15 hours till the completion of reaction to give the crude product as white powder. The crude product was purified on a silica gel column using *n*-hexane:CH_2Cl_2 (9:1 to 1:1) to provide **1c**.

2.2. Spectral data of compound 1c

$C_{44}H_{65}N_3O_8S$, yield: 57%, mp. 187-188 ^{0}C, **ESI-MS** (*m/z*): $[M+H]^+$=796.1, $[M+Na]^+$=818.4, **^{1}H-NMR (CDCl$_3$, 500 MHz)** δ_H ppm 7.50 (1H, d, *J*=7.5 Hz, H-6′), 6.31 (1H, t, *J*=5.0 Hz, H-1″), 5.89 (1H, d, *J*=7.5 Hz, H-5′), 5.34 (1H, q, H-4″), 4.72 (1H, s, H-29a), 4.59 (1H, s, H-29b), 4.58-4.55 (1H, m, H-5″a), 4.48 (1H, m, H-5b″), 4.38 (1H, dd, *J*=3.5, 12.0 Hz, H-3*α*), 3.54 (1H, dd, *J*=5.0, 12.0 Hz, H-2″a), 3.11 (1H, dd, *J*=5.0, 12.0 Hz, H-2″b), 2.99 (1H, q, H-19), 2.41-2.36 (1H, m, H-3‴), 2.31-2.22 (4H, overlap, H-2‴, H-4‴), 1.68 (3H, s, H-30), 1.04 (3H, s, H-26), 0.96 (3H, s, H-27), 0.92 (3H, s, H-23), 0.82 (6H, s, H-25, H-24), 0.81 (3H, d, *J*=7.0 Hz, H-6‴). **APT (CDCl$_3$, 125 MHz),** δ_C ppm: 181.1 (C-28), 172.1 (C-5‴), 171.7 (C-1‴), 154.5 (C-2′), 150.0 (C-20), 141.2 (C-6′), 109.6 (C-29), 94.8 (C-5′), 87.6 (C-1″), 83.4 (C-3), 83.3 (C-4″), 81.21 (C-5), 50.4 (C-3‴), 42.4 (C-19), 40.7 (C-4‴), 28.0 (C-2‴), 38.4 (C-2″), 27.5 (C-23), 19.9 (C-30), 19.8 (C-25), 19.3 (C-26), 16.6 (C-24), 16.2 (C-27), 14.7 (C-6‴).

3. Results and discussion

Synthesis of betulinic acid-3TC hybrid (**1c**) with modification at the C-3 position is described in **Scheme 1**. Initially, BA (**1**) was treated with 3-methylglutaric anhydride on refluxing in pyridine for 10-15 hours and the presence of DMAP as catalyst resulted in C-3 ester derivatives **1a**. Esterification of **1a** using ethyl chloroformate in the presence of catalyst triethyl amine in CH_2Cl_2 give ester derivatives **1b**. Our continuous attempts to carry out the formation of hybrid compound of **1b** with 3TC at new side chain. The transesterification had been induced by treatment with triethyl amine and DMAP while being cooled down to 0^0C for 1 hour. Then, the reaction mixture was warmed to room temperature for 15 hours, effecting conversion to the target molecule **1c** in 57% overall yield, after column chromatography. Structure of the new hybrid compound (**1c**) was elucidated by using NMR and MS spectra, and assignment was performed based on our analysis.



Scheme 1. Synthesis of derivatives 1c

Compounds 1c: its molecular formula, $C_{44}H_{65}N_3O_8S$, was suggested from combined analysis of the positive ESI-MS at *m/z* 796 $[M+H]^+$ and NMR spectra. The 3TC moiety was easily

identified from its characteristic signals in the ^{1}H-NMR and APT spectra. Specifically, two aromatic C–H of the 3TC resonated as doublets with typical coupling constant (7.5 Hz) in the less shielding region at δ_H 7.50 (H-6′), 7.30 5.89 (H-5′) and δ_C 141.2 (C-6′), 94.8 (C-5′). The signal of four oxathiolane hydrogens showed at δ_H 6.31 (H-1″), 5.34 (H-4″), 3.54 (H-2″a) and 3.11 (H-2″b). The oxymethylene (H-5″) appeared as two multiplets at δ_H 4.58-4.55 and 4.48. Besides, two multiplets of aliphatic hydrogens on 3-methylglutaroyl side chain with typical deshielding chemical shift resonated at δ_H 2.41-2.36 (1H, H-3‴), 2.31-2.22 (4H, overlap, H-2‴, H-4‴). The methyl group of 3-methylglutaroyl unit resonated at 0.81 (3H, d, 7.0 Hz, H-6‴). The BA moiety was easily identified from its characteristic signals and considering published data [3]. Moreover, the signal at δ_H 4.38 (1H, dd, 3.5 and 12.0 Hz) of H-3α was shifted less shielding against other H-3 of BA (δ_H ~ 3.20-3.50 ppm), which also proved that C-3 attached to an oxygen of ester group.

4. Conclusion

In summary, a new C-3 betulinic acid-3TC hybrid was designed and synthesized by employing analogue methods of Steglich esterification and characterized by basic spectroscopic techniques. The new compound **1c** contained two biologically active moieties, so it is able to be an interesting molecule of potential in therapeutic use after biological tests.

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TỔNG HỢP MỘT C-3 ESTER MỚI, HỢP CHẤT LAI GIỮA BETULINIC ACID VỚI LAMIVUDINE

Lê Công Hoan[1], Ngô Thị Uyên Tuyền[1], Đặng Thị Tuyết Anh[2], Nguyễn Hoàng Sa[1]

[1]Trường Đại học Khánh Hoà,
[2]Viện Hoá học, Viện Hàn lâm Khoa học và Công Nghệ Việt Nam

Tóm tắt: *Betulinic acid và các dẫn xuất của nó có chứa hoạt tính sinh học rất đa dạng, bao gồm kháng viêm, kháng khối u, kháng khuẩn, chống sốt rét, chống ung thư và kháng HIV. Người ta dựa vào 3 vị trí nhóm chức của betulinic acid để tiến hành bán tổng hợp các dẫn xuất, đó là C-3 hydroxyl, C-20 alkene và C-28 carboxylic acid.*

Các dẫn xuất của betulinic acid đã được tổng hợp dựa trên phản ứng ester hóa kinh điển hoặc phản ứng trao đổi ester. Phân tử đích đã được phân lập bằng phương pháp sắc ký cột thường. Cấu trúc của chất này đã được xác định dựa trên sự kết hợp phân tích phổ (MS, NMR) và so sánh đối chiếu với tài liệu tham khảo.

Một dẫn xuất mới là hợp chất lai giữa betulinic acid và lamivudine (chất 1c) đã được tổng hợp thành công.

Từ khóa: *Betulinic acid, Ester, hợp chất lai, lamivudine.*