SYNTHESIS OF HYBRID COMPOUND OF 24-NOR-LUPANE TRITERPENE AND 11-AMINO-UNDECANOIC ACID VIA C-28 AMIDE LINKAGE

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Title:
Tổng hợp một số chất lai của 24-Nor-lupane triterpene với 11-Amino-undecanoic acid qua cầu nối C-28 amide

Từ khóa:
24-nor-3-oxo-20(29)-lupen-28-oic acid, 11-amino-undecanoic acid, hợp chất lai

Keywords:
24-nor-3-oxo-20(29)-lupen-28-oic acid, 11-amino-undecanoic acid, hybrid compounds

ABSTRACT
*Starting from 24-nor-3-oxo-20(29)-lupen-28-oic acid (**1**), a new derivatives **(2)** have been synthesized. The synthetic approach C-28 amide conjugates of **1** with 11-amino-undecanoic acid was described in this report. The new hybrid containing two biologically active moieties via a amide linkage. The chemical structure of **2** was elucidated mass (MS), nuclear magnetic resonance (NMR) spectroscopy.*

TÓM TẮT
*Từ hợp chất ban đầu là 24-nor-3-oxo-20(29)-lupen-28-oic acid (**1**), một dẫn xuất mới (**2**) đã được tổng hợp. Quá trình tổng hợp chất qua cầu nối C-28 amide của chất **1** với 11-amino-undecanoic đã được mô tả trong công bố này. Hợp chất lai mới chứa hai mảnh ghép có hoạt tính qua một cầu nối amide. Cấu trúc của chất **2** đã được làm sáng tỏ bằng sự phân tích kết hợp các phổ khối lượng và phổ cộng hưởng từ hạt nhân.*

1. Introduction

The pentacyclic lupane triterpenoid, which possesses wide range of biological effects, including anticancer, antitumor, anti-inflammatory, antibacterial, antimalarial, and antiviral activities (MH Cháirez-Ramírez *et al*, 2016, Jimin Shin *et al*, 2011, Cristina A Dehelean *et al*, 2011). A lot of novel of lupane triterpenoid derivatives have possesed new or much higher bioactivities than their starting materials. For example, a series of betulinic acid – zudovidine (AZT) hybrids was found as potent cytotoxic agents against KB and Hep-G2 cell lines (Dang Thi Tuyet Anh *et al*, 2014). The modifications of compound 24-nor-11α-hydroxy-3-oxo-lup-20(29)-en-28-oic acid at C-28 led to four amide derivatives with moderate cytotoxic activity in four different human tumor cell models, including KB, HepG2, MCF-7, and LU (Dang Thi Tuyet Anh *et al*, 2015). In recent, several phytochemical and pharmacological studies have been carried out. The result showed that, some 24-nor-lupane triterpenes were isolated, semi-synthesis and elucidated structure. Among these, several compounds showed stronger cytotoxicity on KB, HepG2, MCF7, and Lu cell lines (Phan Van Kiem *et al*, 2003; Nguyen Thanh Tam, 2015). In this communication, we describe the detailed the design and synthesis of hybrid compound consisting triterpene derivatives, amino acid via a C-28 amide linkage.

2. Experimental

2.1. General experimental procedures

NMR spectra were recorded on Bruker Avance 500. Chemical shifts were expressed in δ (ppm) downfield from as methanol-d_4 ($^1H = \delta_H$ 3.31, $^{13}C = \delta_C$ 49.00), and coupling constants (*J*) were reported in Hertz. ESI-MS: Agilent LC-MSD-Trap SL. melting point was measured as celsius degree based on KRUSS M5000. TLC: Silica gel 60 F_{254} (0.25mm, Merck); CC: Silica gel 60 (230-400 mesh, Merck) for the first column, silica gel 60, 40-63 μm (Merck), and Sephadex

LH-20 for the following columns.

2.2. Material

Previously, compound 3-α-hydroxy-lup-20(29)-ene-23,28-dioic acid (**1a**) was isolated from *Schefflera octophylla* (Araliaceae). Then, compound 24-nor-3-oxo-20(29)-lupen-28-oic acid (**1**) was produced by the oxidation – decarboxylation reaction of **1a** (Dang Thi Tuyet Anh *et al*, 2018).

2.3. General procedure for preparation of the hybrid compound

A mixture of compound **1** (198 mg, 0.45 mmol) and oxalyl chloride (254 mg, 2 mmol) in CH_2Cl_2 (10 mL) was stirred at r.t. for 10h and concentrated under reduced pressure. The residue was then added into a mixture of amino undecanoic methylester hydrochloride (158 mg, 0.6 mmol) and triethylamine (121 mg, 1.2 mmol) in CH_2Cl_2 (15 mL). After stirring at r.t. for 24 hours, the reaction mixture was washed with water, dried with Na_2SO_4 and solvent was removed in vacuum to give a while amorphous powder. After that, the solution of MeOH (15mL), THF (10 mL), and $NaBH_4$ (38 mg, 1 mmol) was added. The reaction mixture was stirred at rt for 4 hours. The excess of $NaBH_4$ was decomposed with 5% HCl and product was extracted by EtOAc, the organic phase was washed with water, dried with Na_2SO_4 and concentrated. Lastly, the intermediate product was hydrolyzed with KOH (168 mg, 3.0 mmol) in MeOH (5mL) by stirring at r.t for 5h. H_2O (20ml) was added. The reaction mixture was neutralized by 2N HCl to pH = 4 and then extracted with ethylacetate. The organic phase was washed with water, dried with Na_2SO_4 and concentrated. The residue was applied to silica gel column and eluted with a solvent system of *n*-hexane: EtOAc (from 3:1 to 1:1), and then further purified on Sephadex LH-20 with CH_2Cl_2:MeOH (5:95) to afford **2** (over all 72%).

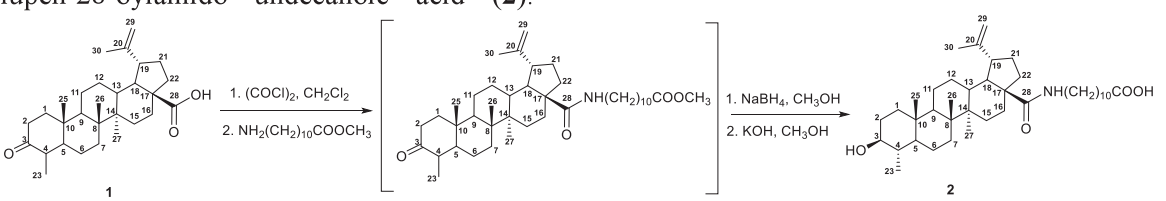
Methyl 24-nor-3-β-hydroxy-20(29)-lupen-28-oylamido undecanoic acid (**2**). Yield 86 mg (72%), m.p 137 ºC. ^{1}H-NMR (Methanol-d_4, 500 MHz) δ_H 7,54 (1H, t, J = 5.5 Hz, N**H**), 4.72 (1H, d, J = 2.0 Hz, H-29a), 4.60 (1H, br s, H-29b), 3.29 (1H, *m*, H-11'a), 3.16 (1H, td, J = 11.0, 4.0 Hz, H-3α), 3.05 (1H, *m*, H-11'b), 2.97 (1H, td, J = 11.0, 5.0 Hz, H-19), 2.62 (1H, td, J = 13.0, 3.5 Hz, H-13), 2.28 (2H, t, J = 7.5 Hz, H-2'), 1.71 (3H, brs, H-30), 1.02 (3H, s, H-27), 1.00 (3H, s, H-25), 0.96 (3H, d, J = 10.0 Hz, H-23), 0.84 (3H, s, H-26).

^{13}C-NMR (Methanol-d_4, 125 MHz) δ_C 178.64 (C-1'), 176.82 (C-28), 149.32 (C-20), 110.19 (C-29), 75.41 (C-3), 56.02 (C-17), 49.65 (C-19), 48.86 (C-9), 48.45 (C-18), 48.22 (C-5), 42.96 (C-14), 41.14 (C-8), 41.03 (C-11'), 39.95 (C-10), 39.57 (C-4), 37.25 (C-1), 36.48 (C-13), 35.21 (C-21), 34.38 (C-2'), 33.52 (C-7), 32.44, (C-16), 30.20 (C-15), 30.11 (C-22) 29.63 (C-2), 29.45, 29.39, 29.33, 29.20, 29.03, 28.73, 26.40 (C-12), 25.32, 24.68, 21.86 (C-11), 20.01 (C-6), 19.80 (C-30), 18.58 (C-26), 16.70 (C-25), 16.03 (C-23), 14.99 (C-27).

ESI-MS *m/z* 626.5 $[M+H]^+$ found for $C_{40}H_{68}NO_4^+$.

3. Results and discussion

Synthesis of hybrid compounds with the modification at the C-28 position was described in Scheme 1. Initially, compound **1** was treated with oxalyl chloride in dichloromethane at reflux to give an acid chloride. Then, the solution was subjected to 1–1.2 equivalent of methyl 11-aminoundecanoate to form C-28 amide bond. The reduction of the ketone group in 1 with sodium borohydride reagent gave 3-OH bond. Then, the hydrolysis of esters at new side chain for intermediate product with dilute base condition furnished compounds **2** in good yields (72%). The chemical structure of the synthesized compound **2** was confirmed by analysed of NMR and MS spectroscopy.



Scheme 1. Synthesis of the hybrid compound 2

Compound 2 was obtained as a amorphous powder (86 mg), melting poit 137 °C, and had a positive reaction in the Liebermann–Burchard test. The molecular formula was obtained as $C_{40}H_{67}NO_4$ with ESI-MS and with the observation of NMR spectra, the substance was suggested to be triterpenoid. NMR spectra of **2** contained the signals of 24-nor-lupane triterpene skeleton, amino-undecanoic acid unit and amide linkage. The ^{13}C NMR and DEPT spectra showed 40 carbon signals of a triterpene skeleton whose most of the carbon signals resonated in the more shielding region from 14.99 to 56.02 ppm. The skeleton of 24-nor-lupane triterpene moiety was recognized to be lupane triterpenoid with the typical alkenyl carbons at δ_C 149.32 (C-20) and 110.19 (C-29), five methyl carbons at δ_C 19.80 (C-30), 18.58 (C-26), 16.70 (C-25), 16.03 (C-23), and 14.99 (C-27). The spectral data also supported the presences of one hydroxyl methine signal at δ_C 79.7 was assigned to C-3 as usual. The signals of one carbonyls at δ_C 176.82 (C-28), and 173.97(C-1′) indicated that this compound contained amide and acid carboxylic, respectively.

The ^1H NMR spectrum of **2** showed the presence of five methyl groups as singlets at δ_H 11.71 (3H, brs, H-30), 1.02 (3H, s, H-27), 1.00 (3H, s, H-25) 0.84 (3H, s, H-26) and doublet at δ_H .96 (3H, d, J = 10.0 Hz, H-23). The *axial* position of the hydroxyl methine proton was confirmed by the signal at δ_H 3.16 (1H, td, J = 11.0, 4.0 Hz, H-3α). Besides that, ^1H-NMR spectrum showed the signals of olefinic methylene protons of isoprenyl in 24-nor-lupane triterpene skeleton at δ_H 4.72 (1H, d, J = 2.0 Hz, 29a), 4.60 (1H, br s, 29''). The signal at δ_H 5.85 7,54 (1H, t, J = 5.5 Hz, N**H**), 3.29 (1H, m, H-11'a) and 3.05 (1H, m, H-11'b) indicated the presence of —$NHCH_2$— group. Other signals at δ_H 2.28 (2H, CH_2COOH), 1.49-1.50 (4H), and 1.25-1.27 (12H) indicated the presence of undecanoic moiety.

4. Conclusion

In summary, a 24-nor-lupane triterpene — 11-amino-undecanoic acid hybrids via amide linkage were designed and synthesized. The chemical structures of hybrid compounds were confirmed by means of NMR and MS spectroscopy.

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