Synthesis of hybrid compounds of 24-nor-lupane triterpene and amino acids via C-28 amide linkage

Nguyen Hoang Sa1,2*, Nguyen Ha Thanh1,3, Tran Van Loc1, Le Nhat Thuy Giang1,3, Le Cong Hoan2, Ngo Thi Uyen Tuyen2, Dang Thi Tuyet Anh1,3

1 Institute of Chemistry, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Nghia Do, Cau Giay, Hanoi, Vietnam
2 University of Khanh Hoa, 01 Nguyen Chanh, Nha Trang, Khanh Hoa, Vietnam
3 Graduate University of Science and Technology, VAST, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam

* Correspondence to Nguyen Hoang Sa <nhoangsa@gmail.com>
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Abstract. The betulinic acid derivatives exhibited different biological activity against different targets, such as anticarcinogenic activity in experimental animals, or anti-HIV, antihyperglycemia, anti-inflammatory in human. In this work, the synthetic procedure of 24-nor-3-oxo-20(29)-lupen-28-oic acid (1) derivatives was investigated. Starting from compound 1 and L-leucine methyl ester, the synthesis proceeded through amidification, reduction, and hydrolysis under basic condition. Finally, three hybrid compounds (2b, 3b, and 4b) were successfully synthesized and elucidated by spectral analysis.

Keywords: 24-nor-3-oxo-20(29)-lupen-28-oic acid, L-leucine, amide, hybrid compounds

1 Introduction

The hybrid compounds containing two or more biologically active structures, could generate candidates with extraordinary bioactivity and potential synergic effects. In recent times, numerous hybrid molecules have been synthesized to discover their biological activities. For instance, a series of hybrids containing aromatic amides and lupane triterpenoid acids exhibited significant anti-inflammatory and antioxidant effects [1]. In our recent study, a series of quinazoline–triazole hybrid compounds were designed and synthesized. Molecular docking study indicated that some hybrids displayed AChEI activity as dual binding site (anionic and peripheral anionic) inhibitors [2].

Actually, natural products have been used to generate preliminary therapeutic agents and continuously provide a significant guide to the discovery of new lead compounds. The pentacyclic triterpene is a terpenoid class of natural products bearing several biological activities because of their pharmacological effects while being devoid of prominent toxicity [3]. Among these compounds, betulinic acid and its derivatives have possessed interesting biological properties, such as anticancer, antitumor, anti-inflammatory, antibacterial, antimalarial, antiviral, and anti-HIV effects [3-5]. Previously, 3α-hydroxy-lup-20(29)-ene-23,28-dioic acid (1a), isolated from Schefflera octophylla (Araliaceae), was used as a reactant to produce 24-nor-3-oxo-20(29)-lupen-28-oic acid (1) through the oxidation – decarboxylation reaction [6]. Besides that, compound 24-nor-11α-hydroxy-3-oxo-lup-20(29)-en-28-oic acid (1b) was isolated in large quantity (0.26%) from Acanthopanax trifoliatus (Araliaceae). Simultaneous modifications of compound 1b at C-28 led to four amide derivatives with moderate...
cytotoxic activity in four different human tumor cell models (KB, HepG2, MCF-7, and LU) [7].

Continuing our research project on the development of hybrid compounds containing two biologically active moieties, herein, the synthesis of hybrid compounds consisting lupan triterpene derivatives, amino acid, and a C-28 amide linkage, was carried out. This study indicated an expecting starting point for drug discovery projects.

2 Methodology

2.1 General procedure for preparation of compound 2b

A mixture of compound 1 (198 mg, 0.45 mmol) and oxalyl chloride (254 mg, 2 mmol) in CH2Cl2 (10 mL) was stirred at r.t. for 8 h and concentrated under reduced pressure. The residue was then added into a mixture of L-Leucine methyl ester hydrochloride (109 mg, 0.6 mmol) and triethylamine (121 mg, 1.2 mmol) in CH2Cl2 (15 mL). After stirring at r.t. for 12 hours, the reaction mixture was washed with water, dried with Na2SO4 and solvent was removed in vacuum. The residue was purified by flash chromatography (n-hexane/EtOAc (5:1) to afford compound 2b (72%).

Methyl 24-nor-3β-hydroxy -20(29)-lupen-28-carbonyl-L-leucinate (2b). Yield 184 mg (72%), m.p. 136 °C. 1H-NMR (Chloroform-d, 500 MHz) δH 5.86 (1H, d, J = 8.0 Hz, -OH), 4.74 (1H, br s, H-29a), 4.59 (1H, br s, H-29b), 4.56 (1H, dt, J = 8.0, 4.5 Hz, H-2', overlap), 3.70 (3H, s, -OCH3), 3.12 (1H, td, J = 11.0, 4.5 Hz, H-19), 1.70 (3H, brs), 1.00 (3H, s), 0.98 (3H, s), 0.97 (3H, d, J = 7.0 Hz), 0.96 (3H, s), 0.94 (3H, s), 0.93 (6H, d, J = 7.0 Hz). 13C NMR (Chloroform-d, 125 MHz) δC 212.84 (C-3), 175.87 (C-28), 173.22 (C-1'), 149.24 (C-20), 110.13 (C-29), 53.49 (C-17), 52.49 (C-2'), 52.31 (OCH3), 50.29 (C-5), 49.60 (C-19), 49.31 (C-9), 48.13 (C-18), 45.47 (C-4), 42.91 (C-18), 41.16 (C-8), 40.51 (C-3'), 39.91 (C-10), 37.98 (C-1), 36.75 (C-13), 36.20 (C-2), 35.06 (C-21), 33.04 (C-7), 31.36 (C-16), 30.14 (C-15), 29.86 (C-22), 26.33 (C-12), 24.46 (C-4'), 22.84 (C-11), 22.42 (C-5', C-6'), 21.77 (C-6), 19.80 (C-30), 18.61 (C-26), 15.86 (C-25), 15.02 (C-27), 12.97 (C-23). ESI-MS m/z 568.5 [M+H]+ found for CsH25NO13.

2.2 General procedure for preparation of compound 3b

A mixture of compound 2b (134 mg, 0.236 mmol) in MeOH (15 mL) and THF (10 mL) was added NaBH4 (38 mg, 1 mmol) The reaction mixture was stirred at rt for 2 hours. Reaction was observed by TLC until 2b completely converted. The excess of NaBH4 was decomposed with 5 % HCl and product was extracted by EtOAc, the organic phase was washed with water, dried with Na2SO4 and concentrated. The residue was purified by flash chromatography (n-hexane/EtOAc = 4:1) to afford 3b (50 %).

Methyl 24-nor-3β-hydroxy -20(29)-lupen-28-carbonyl-L-leucinate (3b). Yield 70 mg (30 %), m.p 136 °C. 1H-NMR (Chloroform-d, 500 MHz) δH 5.86 (1H, d, J = 8.0 Hz, -OH), 4.73 (1H, br s, H-29a), 4.59 (1H, br s, H-29b), 4.57 (1H, dt, J = 8.0, 4.5 Hz, H-2', overlap), 3.72 (3H, s, -OCH3), 3.12 (1H, td, J = 11.0, 4.5 Hz, H-19), 3.05 (1H, td, J = 10.5, 5.0 Hz, H-3), 2.43 (1H, td, J = 12.5, 3.5 Hz, H-13), 1.71 (3H, brs), 1.00 (3H, s), 0.98 (3H, d, J = 7.0 Hz), 0.97 (3H, s), 0.96 (3H, s), 0.93 (6H, d, J = 7.0 Hz), 0.77 (3H, s). 13C NMR (Chloroform-d, 125 MHz) δC 175.87 (C-28), 173.22 (C-1'), 149.24 (C-20), 110.13 (C-29), 75.26 (C-3), 53.49 (C-17), 52.49 (C-2'), 52.31 (OCH3), 49.60 (C-19), 49.30 (C-9), 48.40 (C-18), 48.13 (C-5), 42.91 (C-14), 41.18 (C-8), 40.51 (C-4), 39.91 (C-10), 39.51 (C-1), 37.28 (C-13), 36.33 (C-21), 35.06 (C-12), 33.33 (C-7), 31.36 (C-3'), 30.14 (C-15), 29.86 (C-22), 26.31 (C-2'), 24.46 (C-4'), 22.42 (C-5', C-6'), 21.75 (C-11), 21.72 (C-6), 19.80 (C-30), 18.61 (C-26), 16.14 (C-25), 15.68 (C-23), 15.02 (C-27). ESI-MS m/z 568.5 [M−H]+ found for CsH25NO13.
2.3 General procedure for preparation of compound 4b

Compound 3b (85 mg, 0.15 mmol) was hydrolyzed with KOH (168 mg, 3.0 mmol) in MeOH (5 mL) by stirring at r.t for 5h. H2O (20 mL) 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 Na2SO4 and concentrated. The residue was purified by flash chromatography (n-hexane/EtOAc (2:1) to afford 4b (89%).

24-Nor-3β-hydroxy-20(29)-lupen-28-carbonyl-L-leucine (4b). Yield 74 mg (89%), m.p 137 °C. 1H-NMR (Methanol-d4, 500 MHz) δ 4.73 (1H, d, J = 2.0 Hz, H-29β), 4.60 (1H, br s, H-29α), 4.47 (1H, dt, J = 10.0, 4.5 Hz, H-2′), 3.06 (1H, td, J = 11.0, 5.0 Hz, H-3α), 2.99 (1H, td, J = 11.5, 5.0 Hz, H-19), 2.63 (1H, td, J = 13.0, 4.0 Hz, H-13), 1.71 (3H, brs, H-30), 1.00 (3H, s, H-27), 0.98 (3H, s, H-25), 0.97 (6H, d, J = 7.0 Hz, H-5′, H-6′), 0.95 (3H, d, J = 6.5 Hz, H-23), 0.83 (3H, s, H-26). 13C NMR (Methanol-d4, 125 MHz) δc 177.89 (C-28), 175.76 (C-1′), 151.33 (C-20), 108.97 (C-29), 76.26 (C-3), 56.01 (C-17), 52.05 (C-2′), 50.06 (C-19), 50.04 (C-9), 47.52 (C-18), 47.01 (C-5), 42.63 (C-14), 40.69 (C-8), 40.38 (C-4), 38.53 (C-10), 38.40 (C-1), 38.36 (C-13), 37.75 (C-21), 36.65 (C-3′), 33.71 (C-7), 33.00 (C-16), 30.99 (C-2), 30.59 (C-15), 29.40 (C-22), 26.11 (C-12), 25.29 (C-4′), 22.55 (C-5′, C-6′), 21.51 (C-11), 21.03 (C-6), 19.78 (C-30), 18.63 (C-26), 16.10 (C-25), 15.84 (C-23), 14.99 (C-27). ESI-MS m/z 556.4 [M+H]+ found for C38H60NO5.

3 Results and Discussion

Synthesis of hybrid compounds with the modification at the C-28 position was described in Scheme 1-2. 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-L-leucinate to afford 2b (Scheme 1) in good yields (72%). The chemical structure of the synthesized compound 2b was confirmed by 1D NMR and MS spectroscopy.

Scheme 1. Synthesis of amide derivatives 2b

The 1H-NMR spectrum showed the signals of olefinic methylene protons of isoprenyl in 24-nor-lupane triterpene skeleton at δH 4.74 (1H, br s, 29α), 4.59 (1H, br s, 29β). The different signal at δc 52.49 (−NH−CH−COOR) corresponding to δH 4.56 (1H, dt, 8.0, 4.5 Hz). Other carbon signals of leucinate unit resonated at δc 40.51 (C-3′), 24.46 (C-4′), 22.42 (C-5′, C-6′) and 52.31 (−OCH3).

Subsequently, compounds 3b and 4b were synthesized following the synthetic procedure as depicted in Scheme 2. The selective reduction of the ketone group in 2b with sodium borohydride reagent gave 3β-OH isomer (3b) as the main products.[8] After the purification, 3β-OH isomer (3b) were obtained 50% yield.

Then, the hydrolysis of esters at new side chain for 3b with dilute base condition furnished compounds 4b in good yields (89%).

Similar to compounds 2b and 3b, both 1H-NMR and 13C-NMR spectra of 4b contained the
signals of 24-nor-lupane triterpene skeleton, amino acid unit and amide linkage. The $^{13}$C NMR data of 4b showed the signals of 35 carbons. The signals of two carbonyls at $\delta$ 177.89 (C-28) and 175.76 (C-1’) indicated that this compound contained amide and carboxyl, respectively. Two olefinic methylene carbons had chemical shift at $\delta$: 151.33 (C-20) and 108.97 (C-29). One hydroxyl methine group at 76.26 (C-3). Moreover, the $\beta$-configuration of C-3 hydroxyl and $\alpha$-configuration of C-4 methyl were confirmed by the coupling constant of 4b H-3 (H-3: 3.06, td, $J = 11.0, 5.0$ Hz). The signals of leucine moiety resonated at 52.05 (C-2’), 36.51 (C-3’), 25.29 (C-4’), 22.55 (C-5’, C-6’) and 52.31 (-OCH$_3$).

**Scheme 2. Synthesis of derivatives 3b and 4b**

### 4 Conclusion

In summary, the synthesis of new 24-nor-lupane triterpenoid hybrids containing amide linkage were designed and synthesized. We developed a simple synthesis of hybrid compounds containing amide linkage. The synthetic pathway included the transformation of carboxylic acid into acid chloride, then coupled with amino acids to form C-28 amide (2b). Stereoselective reduction of C-3 ketones using NaNH$_4$ in dry methanol led to $\beta$-configuration of the hydroxyl group at C-3 (3b). Hydrolysis of esters under alkaline condition afforded the corresponding acid (4b) in excellent yields. All compounds have 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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