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

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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, 3-α-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 CH_2Cl_2 (10 mL) was stirred at r.t. for 8h 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 CH_2Cl_2 (15 mL). After stirring at r.t. for 12 hours, the reaction mixture was washed with water, dried with Na_2SO_4 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-3-oxo-20(29)-lupen-28-carbonyl-L-leucinate (**2b**). Yield 184 mg (72%), m.p. 136 ^oC. ^1H-NMR (Chloroform-*d*, 500 MHz) δ_H 5.86 (1H, d, *J* = 8.0 Hz, -N**H**), 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, -OCH_3), 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). ^{13}C NMR (Chloroform-*d*, 125 MHz) δ_C 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 (-O**C**H_3), 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 $C_{36}H_{58}NO_4^+$.

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 $NaBH_4$ (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 Na_2SO_4 and concentrated. The residue was purified by flash chromatography (*n*-hexane/EtOAc = 4:1) to afford **3b** (50 %).

Methyl 24-nor-3*β*-hydroxy -20(29)-lupen-28-carbonyl-L-leucinate (**3b**). Yield 70 mg (50 %), m.p 136 ^oC. ^1H-NMR (Chloroform-*d*, 500 MHz) δ_H 5.86 (1H, d, *J* = 8.0 Hz, -O**H**), 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, -OCH_3), 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). ^{13}C NMR (Chloroform-*d*, 125 MHz) δ_C 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 (O**C**H_3), 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 $C_{36}H_{58}NO_4^-$.

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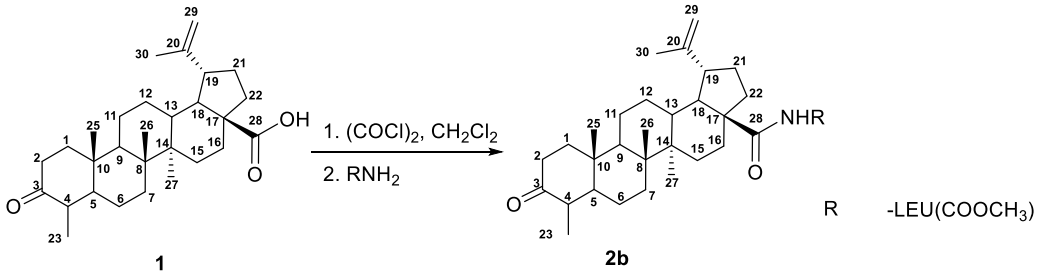
2.3 General procedure for preparation of compound 4b

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 Na_2SO_4 and concentrated. The residue was purified by flash chromatography (*n*-hexane/EtOAc (2:1) to afford **4b** (89 %).

24-Nor-3*β*-hydroxy -20(29)-lupen-28-carbonyl-L-leucine (**4b**). Yield 74 mg (89%), m.p 137 ^oC. ^{1}H-NMR (Methanol-d_4, 500 MHz) δ_H 4.73 (1H, d, *J* = 2.0 Hz, H-29'), 4.60 (1H, br s, H-29''), 4.47 (1H, dt, *J* = 10.0, 4.5 Hz, H-2'), 3.06 (1H, td, *J* = 11.0, 5.0 Hz, H-3*α*), 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). ^{13}C NMR (Methanol-d_4, 125 MHz) δ_C 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 $C_{35}H_{58}NO_4^+$.

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**

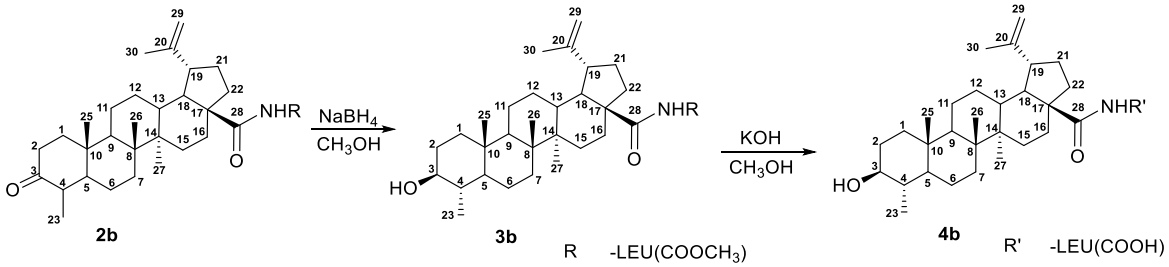
The ^{1}H-NMR spectrum showed the signals of olefinic methylene protons of isoprenyl in 24-nor-lupane triterpene skeleton at δ_H 4.74 (1H, br s, 29a), 4.59 (1H, br s, 29b). The different signal at δ_C 52.49 (—NH—**C**H—COOR) corresponding to δ_H 4.56 (1H, dt, 8.0, 4.5 Hz). Other carbon signals of leucinate unit resonated at δ_C 40.51 (C-3'), 24.46 (C-4'), 22.42 (C-5', C-6') and 52.31 (-OCH_3).

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 3*β*-OH isomer (**3b**) as the main products.[8] After the purification, 3*β*-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 ^{1}H-NMR and ^{13}C-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 δ_C 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 δ_C 151.33 (C-20) and 108.97 (C-29). One hydroxyl methine group at 76.26 (C-3). Moreover, the *β*-configuration of C-3 hydroxyl and *α*-configuration of C-4 methyl were confirmed by the coupling constant of **4b** H-3*α* (δ_H 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 $NaBH_4$ in dry methanol led to *β*-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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