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Development of New Amide Derivatives of Betulinic Acid: Synthetic Approaches and Structural Characterization

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Abstract

In this study, we report the synthesis of three new derivatives of betulinic acid, a pentacyclic triterpenoid known for its antitumor activity. These derivatives were synthesized via amide bond formation at the C-28 position using 3-[(Ethylimino)methylidene]amino-*N*,*N*-dimethylpropan-1-amine (EDC)/Hydroxybenzotriazole (HOBt) activation and various amines as nucleophiles. The synthesized compounds were characterized by nuclear magnetic resonance (NMR) techniques, including proton (^{1}H), carbon-13 (^{13}C), COSY, HSQC, and DEPT, as well as ultraviolet–visible (UV-VIS) spectroscopy, Fourier-transform infrared (IR) and elemental analysis. This work highlights the potential of semi-synthetic modification of betulinic acid to enhance anticancer properties while addressing challenges in solubility and bioavailability. Further structural optimization and formulation studies are warranted to improve drug-like properties and therapeutic applicability.

Keywords: betulinic acid; antitumor; drug discovery; spectroscopy

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1. Introduction

Pentacyclic triterpenoids are a class of secondary metabolites widely found in plants; their unique chemical structures and diverse biological activities have made them hotspots in natural medicine research. Among them, betulinic acid (BA), as a representative molecule of lupane-type triterpenoids, has attracted much attention due to its remarkable antitumor [1], antiviral [2], anti-inflammatory [3] and other pharmacological activities [2].

BA was initially isolated from the bark of *Betula alba*, and its structure is characterized by a rigid pentacyclic skeleton (lupane skeleton) [4] containing two key active sites, the hydroxyl group at C-3 position and the carboxyl group at C-28 position, which provide an important basis for structural modification [5]. The biosynthesis of BA occurs via the mevalonate pathway, with lupeol serving as its immediate precursor. Owing to its rigid five-ring backbone and functional group arrangement, BA displays remarkable biological activity with relatively low toxicity to normal cells, making it an attractive lead compound for drug development.

Cancer is a group of diseases characterized by uncontrolled cell division, the ability to invade surrounding tissues, and potential metastasis to distant organs. It results from genetic and epigenetic changes that disrupt normal cell regulation mechanisms. Globally,

cancer is a leading cause of morbidity and mortality, with the most common types including lung [6], breast [7], colorectal [8], liver [9], and prostate cancers [10]. Despite significant advances in diagnosis and treatment, including surgery, chemotherapy, radiotherapy, targeted therapy, and immunotherapy, cancer remains a major clinical challenge due to issues such as drug resistance, recurrence, and treatment-associated toxicity [11]. In the field of oncology, betulinic acid has attracted extensive attention due to its ability to selectively induce apoptosis in cancer cells by regulating multiple signalling pathways. Multiple studies have explored its anticancer mechanism and revealed that it primarily acts by disrupting mitochondrial membrane potential in cancer cells. The compound directly targets mitochondria, leading to excessive oxygen species (ROS), which contribute to mitochondrial dysfunction and subsequent caspase activation. This mitochondrial-mediated apoptosis is significant not only for its generation of reactive oxygen species but also because betulinic acid does not appear to induce drug resistance in cancer cells, a common limitation of conventional chemotherapeutics [12].

Despite the broad-spectrum bioactivity of betulinic acid itself, its low solubility, poor bioavailability, and lack of selectivity limit its clinical application, so optimizing its physico-chemical and pharmacological properties through semi-synthetic modifications has become an important direction of current research [1].

To address these limitations, researchers in medicinal chemistry have turned to organic synthesis and semi-synthetic strategies to develop new biologically active derivatives of betulinic acid (Figure 1i). Structural modifications on ring A and the C20-C29 double bond have the potential to markedly enhance the therapeutic efficacy of betulinic acid, and linking the C-28 carboxyl group with ester or amide bonds could enhance the cytotoxicity of betulinic acid [1,13] (Figure 1ii). A wide range of betulinic acid derivatives have demonstrated superior antitumor activity compared to both the parent compound and several clinically approved reference drugs. In this context, this study synthesizes three new betulinic acid derivatives by attaching three different amines to the carboxyl group of the polar group C-28 (Figure 1iii) [14]. These modifications aim to improve solubility, enhance cytotoxic potency, and overcome pharmacokinetic limitations associated with the parent compound. These derivatives were evaluated through UV-VIS, IR and NMR techniques.

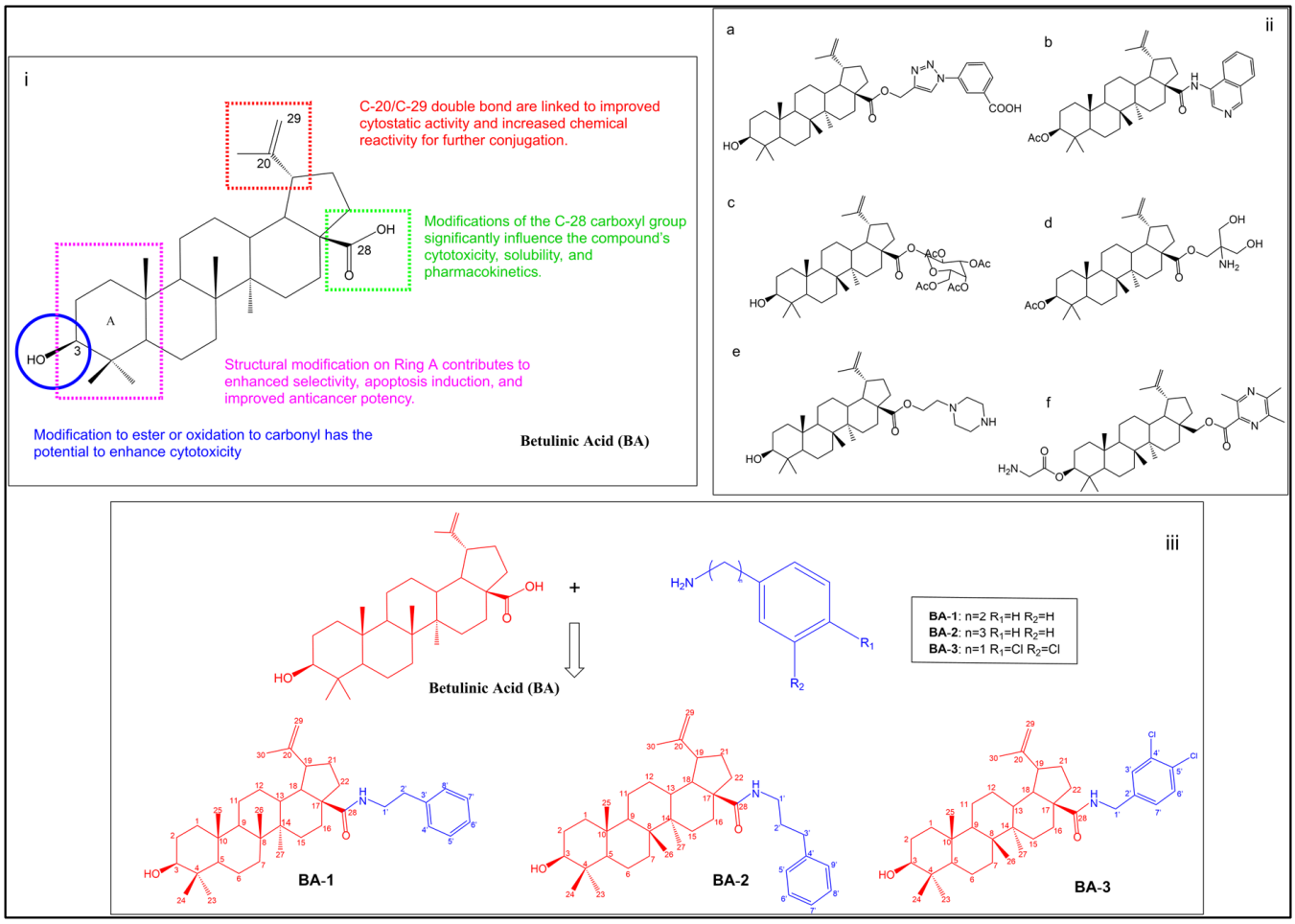


Figure 1. (**i**) Functional groups of betulinic acid that are important for its biological activity. (**ii**) Examples of betulinic acid derivatives reported in the literature: (a) [15], (b) [16], (c) [17], (d) [18], (e) [19] and (f) [20]. (**iii**) Synthesis of **BA-1**, **BA-2**, **BA-3** by using betulinic acid (BA) and different amines.

2. Results and Discussion

The synthetic route for **BA-1**, **BA-2** and **BA-3** started from betulinic acid (Figure 2).

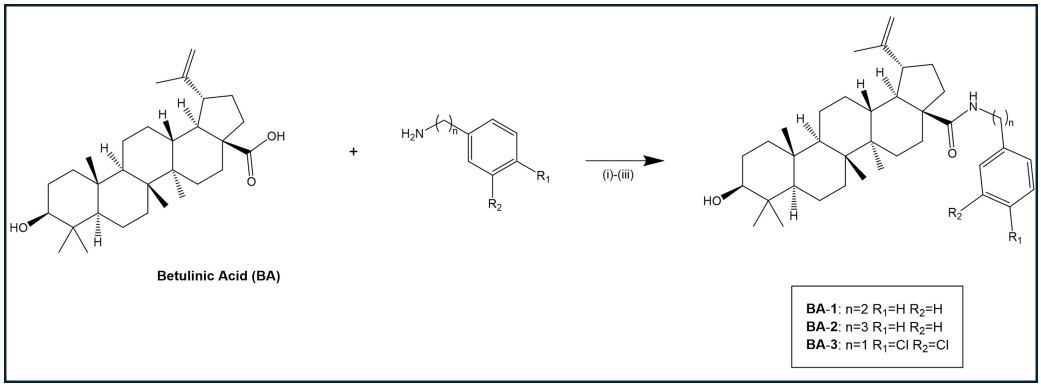


Figure 2. (i) Synthesis of **BA-1**: (R)-Phenylethylamine (1.5 equivalent), HOBt (1.5 equivalents), EDC hydrochloride (1.5 equivalents), DIPEA (1.5 equivalents). (ii) Synthesis of **BA-2**: (*R*)-3-phenylpropylamine (1.5 equivalent), HOBt (1.5 equivalents), EDC hydrochloride (1.5 equivalents), DIPEA (1.5 equivalents). (iii) Synthesis of **BA-3**: (*R*) 3,4-Dichlorobenzylamine (3 equivalents), HOBt (3 equivalents), EDC hydrochloride (3 equivalents), DIPEA (5 equivalents).

The design concept of this study is based on the conclusion in existing literature that modifications at the C-3 and C-28 positions can significantly alter cell membrane permeability, targeting, and metabolic stability [21]. An EDC/HOBt condensation system was used to introduce different amine substituents to improve pharmacological properties.

A substantial amount of research has demonstrated that amide or ester modifications at the C-28 position can significantly enhance the anticancer potency of betulinic acid. Hoenke and his research group [16] reported that betulinic acid amides displayed highly selective cytotoxic properties and pro-apoptotic characteristics. In contrast, Yang et al. [19] have designed betulinic acid–nitrogen heterocyclic derivatives, which have been shown to enhance water solubility and exhibit potent antiproliferative effects. A comparison of the betulinic acid derivatives examined in this study with their analogues reveals that they are similarly modified at the C-28 position. However, they incorporate a diverse array of aromatic and aliphatic amino substituents, a strategy employed to optimize lipophilicity and pharmacokinetic properties.

The amide bond formation in this study was achieved using the EDC/HOBt coupling system in the presence of DIPEA or Et3N, a strategy that has been widely adopted in peptide synthesis and carboxylic acid derivatization. A comparison of EDC/HOBt with traditional coupling agents such as DCC reveals several advantages. These include milder conditions, higher solubility in polar solvents, and reduced side-product formation due to the stabilization provided by HOBt. An extant study reveals a variety of methods for modifying betulinic acid [14]. These methods include the synthesis of ester derivatives using alkyl halides (e.g., BnCl), as well as the conversion of the carboxylic acid into isocyanate via DPPA prior to its reaction with amines to form urea derivatives. Furthermore, a preliminary investigation was conducted prior to the implementation of the EDC/HOBt coupling system, which involved the assessment of EDC/DMAP. However, this approach yielded low product purity and was prone to byproduct formation. Conversely, the EDC/HOBt approach demonstrated a higher cost-effectiveness, yielding favourable outcomes (70–87%) under relatively mild conditions without the need for extensive purification steps. Consequently, the method adopted in this study offers competitive advantages over existing protocols, striking a balance between practicality, scalability, and efficiency. The selected amines are all short-chain compounds, facilitating easier interaction formation. Halogenated benzylamines were chosen for their greater hydrophobicity and the potential for halogen-mediated metabolic shielding. We prioritized candidate amines based on three criteria: chain length, electronic effects, and operability [22]. Ultimately, three amine functional groups were adopted that represent key variables and permit direct comparison under uniform conditions. These modifications not only expanded the chemical diversity of betulinic acid derivatives but also provided novel candidates for SAR studies.

Following the protocol reported in the literature [23,24], **BA-1** and its homologue **BA-2** were prepared by the reaction of betulinic acid (0.219 mmol) and two different amines (phenylethylamine and 3-phenylpropylamine) (1.5 eq) stirred at room temperature in DMF (3 mL) for 20 h and 39 h, respectively. **BA-3** was prepared by the reaction of betulinic acid (0.33 mmol) and 3,4-Dichlorobenzylamine (3 eq) stirred at room temperature in DCM (20 mL) for 17 h. The target compounds were purified on a silica gel column with DCM/ MeOH (98:2, *v*/*v*) to yield **BA-1** 73.4 mg (73.4%), **BA-2** 87.2 mg (87.2%) and **BA-3** 105.9 mg (70.6%)

The chemical structure of **BA-1**, **BA-2** and **BA-3** was confirmed by NMR, IR, UV, and elemental analysis.

The ^{1}H-NMR spectrum of **BA-1** displayed a single peak at 5.56 ppm, assigned to the amine N–H proton (Supplementary Figure S3). In the ^{13}C-NMR spectrum of **BA-1**, two peaks at 40.41 ppm and 36.00 ppm correspond to the methylene groups between the

benzene ring and the amino group, and multiple signals in the aromatic region (139.27 ppm, 128.87 ppm, 128.80 ppm, and 126.64 ppm) constitute the most relevant features for validating the reaction with the aromatic amine (Supplementary Figures S4 and S5). A total of thirty-eight carbon signals were observed, corresponding to six methyl groups, thirteen methylene carbons, eleven methines, and eight quaternary carbons.

Further structural assignments were supported by 2D NMR experiments, including Heteronuclear Single Quantum Coherence (HSQC) and Correlation Spectroscopy (COZY). In the ^{1}H–^{1}H COSY spectrum of compound **BA-1**, the N-H proton (δ5.56 ppm) exhibited clear cross-peaks with the methylene protons at C-1′ (δ3.55/3.50 ppm) and C-2′ (δ2.83/2.80 ppm), confirming the spin–spin coupling networks. These correlations firmly establish the connectivity between the amide N-H and the benzylic side chain, in agreement with the proposed structure. In the HSQC spectrum of **BA-1**, the methylene protons at C-1′ (δH 3.55/3.50 ppm) correlated with the carbon resonance at δC 40.41 ppm, confirming their direct attachment. Similarly, the protons at C-2′ (δH 2.83/2.80 ppm) showed correlations with the carbon at δC 36.00 ppm. As expected, the amide NH proton did not exhibit a direct HSQC correlation, consistent with its attachment to nitrogen. In addition, the DEPT-135 and DEPT-90 spectra further confirmed the expected CH_2 and CH signals at C-1′ and C-2′, consistent with the proposed assignments (Supplementary Figures S6–S9, Table S1).

The IR spectrum of **BA-1** revealed O-H stretching at 3446 cm^{-1}, N–H stretching at 3387 cm^{-1}, C=C stretching at 3068 cm^{-1}, C=O amide stretching peaks at 1506 cm^{-1} and aromatic stretching at 1641 cm^{-1} (Supplementary Figure S24), while its UV spectrum exhibited an absorbance value of 0.757 at 226.50 nm—an absorption attributable to the benzene ring (Supplementary Figure S27).

The ^{1}H-NMR spectrum of **BA-2** displayed a single peak at 5.56 ppm, assigned to the amine N–H proton (Supplementary Figure S10). In the ^{13}C-NMR spectrum, multiple signals in the aromatic region (141.76 ppm, 128.64 ppm, 128.52 ppm, and 126.13 ppm) constitute the most relevant features for validating the reaction with the aromatic amine (Supplementary Figures S11 and S12). In the spectrum of **BA-2**, there are three peaks that differ in the 10–60 ppm region, at 39.05 ppm, 36.64 ppm, and 33.68 ppm, corresponding to signals of methylene groups between the benzene ring and the amino group. A total of thirty-nine carbon signals were observed, corresponding to six methyl groups, fourteen methylene carbons, eleven methines, and eight quaternary carbons.

Further structural assignments were supported by 2D NMR experiments, including Heteronuclear Single Quantum Coherence (HSQC) and Correlation Spectroscopy (COZY). In the ^{1}H–^{1}H COSY spectrum of compound **BA-2**, the N-H proton (δ5.56 ppm) exhibited clear cross-peaks with the methylene protons at C-1′ (δ3.33/3.23 ppm), C-2′ (δ2.88 ppm) and C-3′ (δ2.65 ppm), confirming the spin–spin coupling networks. These correlations firmly establish the connectivity between the amide N-H and the benzylic side chain, in agreement with the proposed structure.

In the HSQC spectrum of **BA-2**, the methylene protons at C-1′ (δH 3.55/3.50 ppm) correlated with the carbon resonance at δC 39.05 ppm, confirming their direct attachment. Similarly, the protons at C-2′ (δH 2.88 ppm) showed correlations with the carbon at δC 36.64 ppm, and C-3′ (δH 2.65 ppm) correlated with the carbon resonance at δC 33.68 ppm. As expected, the amide NH proton did not exhibit a direct HSQC correlation, consistent with its attachment to nitrogen. Distortionless enhancement by polarization transfer (DEPT-135, DEPT-90 and DEPT-45) enabled the classification of carbon environments in compound **BA-2**. In addition, the DEPT-135 and DEPT-90 spectra further confirmed the expected CH_2 signals at C-1′, C-2′ and C-3′, consistent with the proposed assignments (Supplementary Figures S13–S16, Table S2).

The IR spectrum of **BA-2** revealed N–H stretching at 3381 cm^{-1} and C=O amide stretching peaks at 1516 cm^{-1} (Supplementary Figure S25), while its UV spectrum exhibited an absorbance value of 0.744 at 226.50 nm, attributable to the benzene ring (Supplementary Figure S28).

The ^{1}H-NMR spectrum of **BA-3** displayed a single peak at 6.03 ppm, assigned to the amine N–H proton (Supplementary Figure S17). The ^{13}C-NMR spectrum shows multiple signals in the aromatic region (139.77 ppm, 132.74 ppm, 131.38 ppm, 130.67 ppm, 129.68 ppm and 127.19 ppm) (Supplementary Figure S11). In the spectrum of **BA-3**, there is only one peak that differs from betulinic acid, at 42.30 ppm, corresponding to the signal of the methylene group between the benzene ring and the amino group. A total of thirty-seven carbon signals were observed, corresponding to six methyl groups, twelve methylene carbons, nine methines, and ten quaternary carbons.

Further structural assignments were supported by 2D NMR experiments, including Heteronuclear Single Quantum Coherence (HSQC) and Correlation Spectroscopy (COZY). In the ^{1}H–^{1}H COSY spectrum of compound **BA-3**, the N-H proton (δ6.03 ppm) exhibited clear cross-peaks with the methylene protons at C-1′ (δ4.27 ppm), confirming the spin–spin coupling networks. These correlations firmly establish the connectivity between the amide N-H and the benzylic side chain, in agreement with the proposed structure.

In the HSQC spectrum of **BA-3**, the methylene protons at C-1′ (δH 4.27 ppm) correlated with the carbon resonance at δC42.30 ppm, confirming their direct attachment. As expected, the amide NH proton did not exhibit a direct HSQC correlation, consistent with its attachment to nitrogen and distortionless enhancement by polarization transfer (DEPT-135, DEPT-90 and DEPT-45), which enabled the classification of carbon environments in compound **BA-3**. In addition, the DEPT-135 and DEPT-90 spectra further confirmed the expected CH_2 signals at C-1′, consistent with the proposed assignments (Supplementary Figures S13–S16, Table S2, Supplementary Figures S18 and S19, Table S3).

The IR spectrum of **BA-3** revealed N–H stretching at 3371 cm^{-1}, C=C stretching at 3068 cm^{-1}, aromatic stretching at 1450–1600 cm^{-1}, C=O amide stretching peaks at 1668 cm^{-1} and C-Cl stretching at 740 cm^{-1} (Supplementary Figure S26), while its UV spectrum exhibited an absorbance value of 0.409 at 264.00 nm (Supplementary Figure S29).

3. Materials and Methods

Chemistry

Silica gel (FCP 230–400 mesh) was used for column chromatography. Thin-layer chromatography was carried out on Merck precoated silica gel 60 F254 plates (Merck, Darmstadt, Germany) and visualization was performed with phosphomolybdic acid, iodine, or a UV–visible lamp. All chemicals were purchased from Bide Pharmatech., Ltd. (Shanghai, China). ^{1}H NMR and ^{13}C-NMR spectra were collected in $CDCl_3$ at 25 °C on a Bruker Ascend®-600 (Magnet System 600′54 Ascend LH, San Jose, CA, USA) NMR spectrometer (600 MHz for ^{1}H and 150 MHz for ^{13}C). All chemical shifts were reported in the standard δ notation of parts per million using the peak of the residual proton signals of $CDCl_3$ as an internal reference ($CDCl_3$, δC 77.2 ppm, δH 7.26 ppm). UV analysis was performed by a Shimadzu UV–2600 (Osaka, Japan) with a 1 cm quartz cell and a slit width of 2.0 nm. The analysis was carried out using wavelengths in the range of 200–700 nm. IR analysis (KBr) was performed on a Shimadzu IRAffinity-1S (Kyoto, Japan) with a frequency range of 4000–500 cm^{-1}.

(1R,3aS,5aR,5bR,7aR,9S,11aR,11bR,13aR,13bR)-N-butyl-9-hydroxy-5a,5b,8,8,11a-pentamethyl-1-(prop-1-en-2-yl)icosahydro-3aH cyclopenta[a]chrysene-3a-carboxamide (**BA-1**)

In a round-bottom flask, betulinic acid (100 mg, 0.219 mmol), HOBt (44 mg, 0.328 mmol), EDC (63 mg, 0.328 mmol), DIPEA (57 μL, 0.328 mmol) and 3 mL of

DMF as a solvent were sequentially added. The mixture was stirred at rt for 30 min. Phenylethylamine (39 μL, 0.328 mmol) was then added and stirred at rt for 20 h. After the reaction was complete, 200 mL saturated brine was added for extraction, and then partitioned with ethyl acetate (20 mL × 3). Then, the organic phases were consolidated, dried over anhydrous Na_2SO_4 then evaporated, obtaining the crude product. The crude product was purified by silica gel column chromatography with dichloromethane/methanol (98:2, *v*/*v*) as the eluent. The target compound was concentrated and yielded a white solid compound 73.4 mg, a yield of 73.4%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.31 (t, *J* = 7.5 Hz, 2H), 7.25–7.18 (m, 3H), 4.72 (dd, *J* = 13.1, 2.3 Hz, 1H), 4.62–4.56 (m, 1H), 3.60–3.45 (m, 2H), 3.17 (dd, *J* = 11.4, 4.8 Hz, 1H), 3.04 (td, *J* = 11.1, 3.8 Hz, 1H), 2.82 (ddt, *J* = 27.9, 13.8, 6.9 Hz, 2H), 2.39 (td, *J* = 12.4, 3.6 Hz, 1H), 1.99–1.85 (m, 1H), 1.81 (dt, *J* = 13.5, 3.4 Hz, 1H), 1.72–1.43 (m, 6H), 1.35 (tddd, *J* = 16.8, 14.2, 7.8, 4.6 Hz, 6H), 1.28–1.16 (m, 2H), 1.06 (dt, *J* = 13.5, 3.2 Hz, 1H), 1.01–0.92 (m, 7H), 0.90 (s, 3H), 0.87 (dd, *J* = 13.2, 4.5 Hz, 1H), 0.81 (s, 3H), 0.75 (s, 3H), 0.67 (tq, *J* = 6.2, 3.0 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$)δ 176.19, 151.07, 139.27, 128.87, 128.80, 126.64, 109.49, 79.15, 55.83, 55.53, 50.76, 50.16, 46.92, 42.61, 40.88, 40.41, 39.00, 38.87, 38.49, 37.87, 37.35, 36.00, 34.52, 33.91, 31.07, 30.98, 29.53, 28.13, 27.57, 25.73, 21.04, 19.60, 18.45, 16.32, 16.27, 15.50, 14.75. UV-Vis (CH_2Cl_2) peaks 259.00, 253.50 and 226.50 nm. IR (FTIR) 3446, 3387, 3068, 1641, 1379cm^{-1}. Elemental Analysis: C, 81.22; H, 10; N, 2.4; O, 5.70. HRMS (ESI$^+$) *m/z*: [M + H]$^+$ calcd for $C_{38}H_{58}NO_2^+$ 560.4462; found 560.4467 (Δ = 0.9 ppm).

(1R,3aS,5aR,5bS,7aR,9S,11aR,11bS,13aS,13bS)-9-hydroxy- 5b,8,8,11a-tetramethyl-N-(3-phenylpropyl)-1-(prop-1-en-2-yl)icosahydro-3aHcyclopenta[a]chrysene-3a-carboxamide (**BA-2**)

In a round-bottom flask, betulinic acid (100 mg, 0.219 mmol), HOBt (44 mg, 0.328 mmol), EDC (63 mg, 0.328 mmol) and DIPEA (57 μL, 0.328 mmol) were added in 3 mL of DMF and stirred at rt for 30 min, then 3-phenylpropylamine (47 μL, 0.328 mmol) was added at room temperature and reacted continuously for 39 h. Following the completion of the reaction, 200 mL of brine was added to the mixture for extraction and partitioned with ethyl acetate (20 mL), and the organic layer was collected, dried with anhydrous Na_2SO_4, then evaporated to collect the crude product. The target compound was purified by silica gel column chromatography using DCM/MeOH (98:2, *v*/*v*) as the eluent. In this step of purification, 87.2 mg of the target compound was obtained with a yield of 87.2%. The synthesis method of the second product has significant differences from the other two products in key operations such as solvent polarity, base selection and addition method, and temperature.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.296 (t, *J*= 7.6 Hz, 2H), 7.18 (m, 2H),7.20 (m, 1H), 5.56 (t, *J* = 5.9 Hz, 1H), 4.73 (d, *J* = 2.5 Hz, 1H), 4.58 (m, 1H), 3.33 (dq, *J* = 13.2, 6.7 Hz, 1H), 3.23 (dt, *J* = 13.0, 6.4 Hz, 1H), 3.17 (dt, *J* = 9.2, 3.8 Hz, 1H), 2.65 (td, *J* = 7.6, 3.0 Hz, 2H), 2.46 (ddd, *J* = 12.9, 11.4, 3.6 Hz, 1H), 1.99–1.85 (m, 1H), 1.81 (dt, *J* = 13.5, 3.4 Hz, 2H), 1.69 (m, 1H), 1.67 (s, 4H), 1.62 (m, 1H), 1.60 (m, 1H), 1.56 (m, 1H), 1.54 (m, 1H), 1.52 (m, 1H), 1.50 (m, 1H), 1.47 (m, 1H), 1.45 (m, 1H), 1.41 (m, 1H), 1.38 (m, 1H), 1.37 (m, 1H), 1.34 (tddd, *J* = 16.8, 14.2, 7.8, 4.6 Hz, 6H), 1.28–1.16 (m, 2H), 1.13–1.10 (m, 1H), 0.96 (s, 3H), 0.93 (dd, *J* = 13.2, 4.5 Hz, 3H), 0.81 (s, 3H), 0.75 (s, 3H), 0.67 (tq, *J* = 6.2, 3.0 Hz, 1H). ^{13}C NMR (151 MHz, $CDCl_3$)δ176.18, 162.72, 151.15, 141.76, 128.52, 126.13, 109.46, 55.74, 55.54, 50.80, 50.28, 49.40, 46.90, 42.64, 40.91, 39.05, 39.00, 38.87, 38.60, 37.86, 37.35, 34.57, 33.97, 33.68, 31.66, 31.04, 29.63, 28.12, 27.56, 25.78, 21.08, 19.64, 18.44, 16.28, 15.50, 14.78. UV-Vis (CH_2Cl_2) peaks 260.00, 254.00, 248.00 nm, 226.50 nm. IR (FTIR) 3442, 3381, 1641, 1379 cm^{-1}. Elemental Analysis: C, 81.12; H, 10.3; N, 2.12; O, 5.20. HRMS (ESI$^+$) *m/z*: [M + H]$^+$ calcd for $C_{39}H_{60}NO_2^+$ 574.4619; found 574.5088 (Δ0.05 ppm).

(1R,3aS,5aR,5bS,7aR,9S,11aR,11bS,13aS,13bS)-N-(3,4-dichlorobenzyl)-9-hydroxy-5b,8,8,11a-tetramethyl-1-(prop-1-en-2-yl)icosahydro-3aH-cyclopenta[a]chrysene-3a-carboxamide (**BA-3**)

In a round-bottom flask, 150 mg (0.33 mmol) of betulinic acid, 136.7 mg (0.99 mmol) of HOBt and 190 mg (0.99 mmol) of EDC were placed into 20 mL of DCM. The mixture was stirred for 20 min at 0–5 °C and 230 µL (1.65 mmol) of Et_3N was added. The mixture was stirred for 1 h at 0–5 °C, then 3,4-dichlorobenzylamine (140 µL, 0.99 mmol) was added into the mixture. Then the mixture was stirred at room temperature for 17 h. After the reaction, 50 mL of distilled water was added to the mixture for extraction, followed by partitioning with ethyl acetate (20 mL); the organic layer was obtained, dried over anhydrous Na_2SO_4 and evaporated to yield the crude product. Purification of the target compound was performed by silica gel column chromatography with DCM/MeOH (98:2, *v*/*v*) as the eluent. The purification gave 105.9 mg of the target product with a yield of 70.6%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.41–7.33 (m, 2H), 7.13 (dd, *J* = 8.2, 2.1 Hz, 1H), 6.03 (t, *J* = 6.1 Hz, 1H), 4.74 (d, *J* = 2.4 Hz, 1H), 4.64–4.56 (m, 1H), 4.45 (dd, *J* = 15.1, 6.1 Hz, 1H), 4.27 (dd, *J* = 15.1, 5.9 Hz, 1H), 3.21–3.10 (m, 2H), 2.44 (ddd, *J* = 13.0, 11.5, 3.7 Hz, 1H), 1.94 (ddq, *J* = 13.2, 9.7, 3.4 Hz, 2H), 1.75 (dd, *J* = 12.2, 7.6 Hz, 1H), 1.62–1.58 (m, 2H), 1.56–1.52 (m, 1H), 1.51–1.43 (m, 2H), 1.41 (dd, *J* = 15.0, 5.8 Hz, 3H), 1.38–1.33 (m, 3H), 1.32–1.26 (m, 1H), 1.26–1.20 (m, 2H), 1.14 (dt, *J* = 13.5, 3.3 Hz, 1H), 0.96 (s, 6H), 0.87 (s, 3H), 0.82 (s, 3H), 0.76 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ176.34, 150.90, 139.77, 132.74, 131.38, 130.67, 129.68, 127.19, 109.61, 79.13, 55.85, 55.53, 50.78, 50.27, 46.78, 42.63, 42.30, 40.88, 38.99, 38.87, 38.52, 37.86, 37.34, 34.55, 33.88, 30.97, 29.59, 28.12, 27.55, 25.77, 21.06, 19.63, 18.44, 16.29, 15.49, 14.77. UV-Vis (CH_2Cl_2) peaks 282.20, 273.80, 264.00 nm. IR (FTIR) 3441, 3371, 3068, 1668, 1029 and 740 cm^{-1}. Elemental Analysis: C, 72.0; H, 8.64; Cl, 11.33; N, 2.1; O, 5.02. HRMS (ESI$^+$) *m/z*: $[M + H]^+$ calcd for $C_{37}H_{54}Cl_2NO_2^+$ 613.3527; found 614.3523 (Δ1.0 ppm).

4. Conclusions

This study focused on betulinic acid (BA), a pentacyclic triterpenoid natural product with broad-spectrum pharmacological activity. By introducing different amine fragments at the C-28 positions, three semi-synthetic derivatives were synthesized and characterized by UV-VIS, IR, NMR spectroscopy and elemental analysis. This study provides an experimental foundation and theoretical reference for the design of BA derivatives and the development of novel anticancer candidate drugs.

Supplementary Materials: The following supporting information can be downloaded at: Figure S1: 1H NMR spectrum (CDCl3, 600 MHz) of betulinic acid (BA); Figure S2: 13C NMR spectrum (CDCl3, 150 MHz) of betulinic acid (BA); Figure S3: 1H NMR spectrum (CDCl3, 600 MHz) of BA-1; Figure S4: 13C NMR spectrum (CDCl3, 150 MHz) of BA-1; Figure S5: 13C NMR spectrum (CDCl3, 150 MHz) of BA-1 (10–60ppm); Figure S6: DEPT-135 NMR spectrum (CDCl3, 600 MHz) of BA-1; Figure S7: DEPT-90 NMR spectrum (CDCl3, 600 MHz) of BA-1; Figure S8: COSY NMR spectrum (CDCl3, 600 MHz) of BA-1; Figure S9: HSQC NMR spectrum (CDCl3, 600 MHz) of BA-1; Figure S10: 1H NMR spectrum (CDCl3, 600 MHz) of BA-2; Figure S11: 13C NMR spectrum (CDCl3, 150 MHz) of BA-2; Figure S12: 13C NMR spectrum (CDCl3, 150 MHz) of BA-2 (10–60 ppm); Figure S13: DEPT-135 spectrum (CDCl3, 600 MHz) of BA-2; Figure S14: DEPT-90 NMR spectrum (CDCl3, 600 MHz) of BA-2; Figure S15: COSY NMR spectrum (CDCl3, 600 MHz) of BA-2; Figure S16: HSQC NMR spectrum (CDCl3, 600 MHz) of BA-2; Figure S17: 1H NMR spectrum (CDCl3, 600 MHz) of BA-3; Figure S18: 13C NMR spectrum (CDCl3, 600 MHz) of BA-3; Figure S19: 13C NMR spectrum (CDCl3, 600 MHz) of BA-3 (10–60 ppm); Figure S20: DEPT-135 NMR spectrum (CDCl3, 600 MHz) of BA-3; Figure S21: DEPT-90 NMR spectrum (CDCl3, 600 MHz) of BA-3; Figure S22: COSY NMR spectrum (CDCl3, 600 MHz) of BA-3; Figure S23: HSQC NMR spectrum (CDCl3, 600 MHz) of BA-3; Table S1: 1H and 13C- (NMR) chemical shifts and the structure of BA-1; Table S2: 1H and 13C- (NMR) chemical shifts and the structure of BA-2; Table S3: 1H and 13C- (NMR) chemical shifts and the structure of BA-3; Figure S24: IR spectrum of BA-1; Figure S25: IR spectrum of BA-2; Figure S26: IR spectrum of BA-3;

Figure S27: UV spectrum of BA-1; Figure S28: UV spectrum of BA-2; Figure S29: UV spectrum of BA-3. Figure S30: Mass spectrometry of BA-1; Figure S31: Mass spectrometry of BA-2; Figure S32: Mass spectrometry of BA-3.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

EDC	3-[(Ethylimino)methylidene]amino-*N*,*N*-dimethylpropan-1-amine
HOBT	Hydroxybenzotriazole
DIPEA	*N*,*N*-Diisopropylethylamine
DCM	Dichloromethane
DMF	Dimethylformamide
MeOH	Methanol
ROS	Reactive oxygen species
BBB	Blood–brain barrier

References

1. Zhong, Y.; Liang, N.; Liu, Y.; Cheng, M.S. Recent progress on betulinic acid and its derivatives as antitumor agents: A mini review. *Chin. J. Nat. Med.* **2021**, *19*, 641–647. [CrossRef]
2. Lou, H.; Li, H.; Zhang, S.; Lu, H.; Chen, Q. A Review on Preparation of Betulinic Acid and Its Biological Activities. *Molecules* **2021**, *26*, 5583. [CrossRef]
3. Oliveira-Costa, J.F.; Meira, C.S.; Neves, M.V.G.D.; Dos Reis, B.P.Z.C.; Soares, M.B.P. Anti–Inflammatory Activities of Betulinic Acid: A Review. *Front. Pharmacol.* **2022**, *13*, 883857. [CrossRef]
4. Özdemir, Z.; Wimmer, Z. Selected plant triterpenoids and their amide derivatives in cancer treatment: A review. *Phytochemistry* **2022**, *203*, 113340. [CrossRef]
5. Yang, S.J.; Liu, M.C.; Xiang, H.M.; Zhao, Q.; Xue, W.; Yang, S. Synthesis and in vitro antitumor evaluation of betulin acid ester derivatives as novel apoptosis inducers. *Eur. J. Med. Chem.* **2015**, *102*, 249–255. [CrossRef] [PubMed]
6. Arbour, K.C.; Riely, G.J. Systemic Therapy for Locally Advanced and Metastatic Non—Small Cell Lung Cancer: A Review. *JAMA J. Am. Med. Assoc.* **2019**, *322*, 764–774. [CrossRef] [PubMed]
7. Wendt, C.; Margolin, S. Identifying breast cancer susceptibility genes—A review of the genetic background in familial breast cancer. *Acta Oncol.* **2019**, *58*, 135–146. [PubMed]
8. Manatakis, D.K.; Tasis, N.; Antonopoulou, M.I.; Kordelas, A.; Balalis, D.; Korkolis, D.P. Tseleni-Balafouta S, Colorectal cancer metastases to the thyroid gland—A systematic review: Colorectal cancer thyroid metastases. *Hormones* **2021**, *20*, 85–91.
9. Sergi, C.M. *Liver Cancer*; Exon Publications: Brisbane, Australia, 2021.
10. Dall'Era, M.A.; Cooperberg, M.R.; Chan, J.M.; Davies, B.J.; Albertsen, P.C.; Klotz, L.H.; Warlick, C.A.; Holmberg, L.; Bailey, D.E., Jr.; Wallace, M.E.; et al. Active surveillance for early-stage prostate cancer: Review of the current literature. *Cancer* **2008**, *112*, 1650–1659.
11. Niculescu, A.G.; Grumezescu, A.M. Novel Tumor–Targeting Nanoparticles for Cancer Treatment—A Review. *Int. J. Mol. Sci.* **2022**, *23*, 5253.

12. Bildziukevich, U.; Rárová, L.; Šaman, D.; Wimmer, Z. Picolyl amides of betulinic acid as antitumor agents causing tumor cell apoptosis. *Eur. J. Med. Chem.* **2018**, *145*, 41–50. [CrossRef] [PubMed]
13. Jiang, A.; Anyanwu, M.; Leong, K.; Li, J.; Gianoncelli, A.; Coghi, P.; Ribaudo, G. 3-[(1*H*-Benzo[d][1,2,3]triazol-1-yl)oxy]propyl 9-hydroxy-5a,5b,8,8,11a-pentamethyl-1-(prop-1-en-2-yl)icosahydro-3a*H*-cyclopenta[a]chrysene-3a-carboxylate. *Molbank* **2022**, *2022*, M1419. [CrossRef]
14. Genet, C.; Strehle, A.; Schmidt, C.; Boudjelal, G.; Lobstein, A.; Schoonjans, K.; Souchet, M.; Auwerx, J.; Saladin, R.; Wagner, A. Structure—Activity Relationship Study of Betulinic Acid, A Novel and Selective TGR5 Agonist, and Its Synthetic Derivatives: Potential Impact in Diabetes. *J. Med. Chem.* **2010**, *53*, 178–190. [CrossRef]
15. Dangroo, N.A.; Singh, J.; Rath, S.K.; Gupta, N.; Qayum, A.; Singh, S.; Sangwan, P.L. A convergent synthesis of novel alkyne—Azide cycloaddition congeners of betulinic acid as potent cytotoxic agent. *Steroids* **2017**, *123*, 1–12. [CrossRef]
16. Hoenke, S.; Heise, N.V.; Kahnt, M.; Deigner, H.P.; Csuk, R. Betulinic acid derived amides are highly cytotoxic, apoptotic and selective. *Eur. J. Med. Chem.* **2020**, *207*, 112815. [CrossRef] [PubMed]
17. Eignerova, B.; Tichy, M.; Krasulova, J.; Kvasnica, M.; Rarova, L.; Christova, R.; Urban, M.; Bednarczyk-Cwynar, B.; Hajduch, M.; Sarek, J. Synthesis and antiproliferative properties of new hydrophilic esters of triterpenic acids. *Eur. J. Med. Chem.* **2017**, *140*, 403–420. [CrossRef] [PubMed]
18. Spivak, A.; Khalitova, R.; Nedopekina, D.; Dzhemileva, L.; Yunusbaeva, M.; Odinokov, V.; D'yakonov, V.; Dzhemilev, U. Synthesis and Evaluation of Anticancer Activities of Novel C-28 Guanidine-Functionalized Triterpene Acid Derivatives. *Molecules* **2018**, *23*, 3000. [CrossRef]
19. Yang, Y.; Xie, T.; Tian, X.; Han, N.; Liu, X.; Chen, H.; Qi, J.; Gao, F.; Li, W.; Wu, Q.; et al. Betulinic Acid-Nitrogen Heterocyclic Derivatives: Design, Synthesis, and Antitumor Evaluation In Vitro. *Molecules* **2020**, *25*, 948. [CrossRef]
20. Guo, W.B.; Zhang, H.; Yan, W.Q.; Liu, Y.M.; Zhou, F.; Cai, D.S.; Zhang, W.X.; Huang, X.M.; Jia, X.H.; Chen, H.S.; et al. Design, synthesis, and biological evaluation of ligustrazine—Betulin amino-acid/dipeptide derivatives as anti-tumor agents. *Eur. J. Med. Chem.* **2020**, *185*, 111839. [CrossRef]
21. Jiang, W.; Li, X.; Dong, S.; Zhou, W. Betulinic acid in the treatment of tumour diseases: Application and research progress. *Biomed. Pharmacother.* **2021**, *142*, 111990. [CrossRef]
22. Bildziukevich, U.; Vida, N.; Rárová, L.; Kolář, M.; Šaman, D.; Havlíček, L.; Drašar, P.; Wimmer, Z. Polyamine derivatives of betulinic acid and β-sitosterol: A comparative investigation. *Steroids* **2015**, *100*, 27–35. [CrossRef] [PubMed]
23. Kodr, D.; Stanková, J.; Rumlová, M.; Džubák, P.; Řehulka, J.; Zimmermann, T.; Křížová, I.; Gurská, S.; Hajdúch, M.; Drašar, P.B.; et al. Betulinic Acid Decorated with Polar Groups and Blue Emitting BODIPY Dye: Synthesis, Cytotoxicity, Cell-Cycle Analysis and Anti-HIV Profiling. *Biomedicines* **2021**, *9*, 1104. [CrossRef] [PubMed]
24. Guo, Y.; Xiao, Y.; Coluccini, C.; Coghi, P. ((1*R*,3a*S*,5a*R*,5b*R*,7a*R*,9*S*,11a*R*,11b*R*,13a*R*,13b*R*)-9-Acetoxy-5a,5b,8,8,11a- pentamethyl-1-(prop-1-en-2-yl)icosahydro-3a*H*-cyclopenta[a]chrysen-3a-yl)methyl 2-Bromo-3-methylbenzoate. *Molbank* **2025**, *2025*, M1971. [CrossRef]

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