

Synthesis and Cytotoxic Activity of Ursolic Acid Derivatives

Dao Duc Thien^a, Nguyen Thanh Tam^a, Dinh Gia Thien^b, Nguyen Thi Hoang Anh^a, and Tran Van Sung^a

^a Institute of Chemistry, Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet road, Cau Giay, Hanoi, Vietnam

^b Le Quy Don High School, Tam Ky, Quang Nam, Vietnam

Reprint requests to Prof. Dr. Tran Van Sung. Fax: 0084 4 38361283.

E-mail: tranvansungvhh@gmail.com

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Fourteen ursolic acid derivatives, among them four novel compounds, were synthesized by modification either at the C-3, C-28 or both positions. The cytotoxic activity of the obtained derivatives was evaluated against the four human cancer cell lines KB (human mouth epidermal carcinoma), HepG2 (human hepatocellular carcinoma), MCF7 (human breast carcinoma) and Lu (human lung carcinoma). As the result, compounds **7** and **8** were from two to three times more active than ursolic acid on all four tested cell lines. This is the first report on cytotoxic effects of the synthesized ursolic acid derivatives **4**, **8**, and **10–15**.

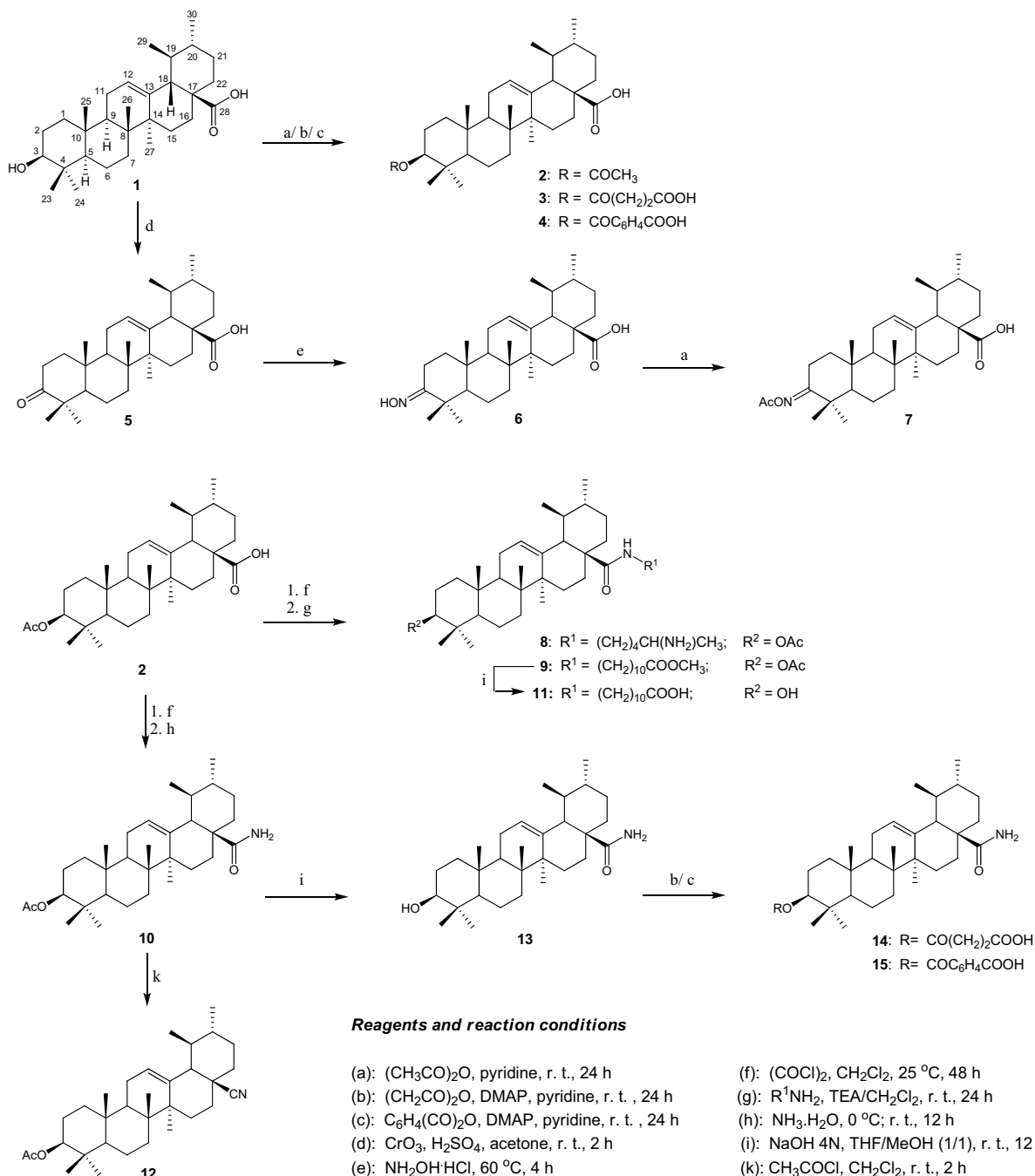
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Introduction

The genus *Eriobotrya* (Rosaceae) contains about 26 species. Only one of these species, *Eriobotrya japonica* (Thunb.) Lindl. (loquat) was hitherto intensively studied and contains many interesting chemical constituents with biological activities. Our phytochemical investigation of leaves of *Eriobotrya poilanei* J. E. Vid. growing in Vietnam showed the presence of ursolic acid in large amount (0.32 % of dry leaves weight). Ursolic acid has been reported to possess a series of biological activities such as antitumor, antimicrobial, anti-inflammatory, hepatoprotective, and cardioprotective properties [1]. A series of ursolic acid derivatives and their biological activities have been previously published [2, 3]. This article describes the isolation of ursolic acid from *Eriobotrya poilanei*'s leaves, the synthesis of its derivatives, among them four are new compounds (**8**, **10**, **14**, **15**), and their cytotoxic activity against the human cancer cell lines KB, HepG2, MCF7, and LU. The cytotoxic activity test results show that ten derivatives exhibited activity on all four tested cancer cell lines, two of them, compounds **7** and **8**, are two to three times more active than ursolic acid itself.

Results and Discussion

Scheme 1 outlines the synthesis of ursolic acid derivatives **2–15**. The hydroxyl group at C-3 of ursolic acid (**1**) was acylated with acetic, succinic and phthalic anhydrides to afford esters **2** (90 %), **3** (60 %) and **4** (60 %), respectively. Jones oxidation of ursolic acid yielded ketone **5** as the main product with a yield of 65 %. With the aim to introduce a nitrogen function to the ursolic acid skeleton, ketone **5** was transformed into ketoxime **6** and then to its acetyl product **7** with good yield (62 % for **6**; 87 % for **7**). For the synthesis of derivatives with a nitrogen function at C-18 of ursolic acid, the 3-hydroxy group was acetylated and the acetyl product **2** reacted with oxalyl chloride, then with the corresponding amines giving the amides **8**, **9** and **11** (after hydrolysis of **9**). The correlations between the amide proton at $\delta_{\text{H}} = 6.00$ ppm and the carbonyl carbon ($\delta_{\text{C}} = 178.5$ ppm) and the methylene carbon ($\delta_{\text{C}} = 39.71$ ppm) in the HMBC spectrum as well as the correlation between this proton and two methylene protons at $\delta_{\text{H}} = 2.99$ and 3.31 ppm (each 1 H, m) in the ^1H - ^1H COSY spectrum of compound **8** confirmed that the amidation took place at the primary amine group. If an aqueous ammonia solution



Scheme 1. Synthesis of ursolic acid derivatives.

was used instead of the amines, the amide **10** was obtained (85 % yield) which was hydrolyzed to amide **13** and then to the acid amides **14** and **15** with the corresponding diacid anhydrides. Interestingly, in the re-

action of amide **10** with acetyl chloride after 2 hours at room temperature the dehydration product **12** was obtained (76 %) instead of an expected diacetyl product.

Table 1. The cytotoxic activity of ursolic acid (**1**) and the synthesized derivatives.

Compound	IC ₅₀ (μg mL ⁻¹)			
	KB	HepG2	MCF7	LU
1 (Ursolic acid)	10.23	11.75	8.0	12.23
2	8.00	4.73	27.50	34.79
3	19.60	6.17	21.07	24.35
4	17.05	8.00	19.73	40.72
5	5.31	4.36	25.36	19.79
7	4.32	4.31	3.43	5.44
8	4.90	5.03	4.78	4.96
10	> 128	> 128	> 128	> 128
11	11.60	17.90	14.44	17.56
12	> 128	> 128	> 128	> 128
13	62.92	53.46	43.44	71.23
14	26.0	9.14	44.58	33.57
15	6.39	8.00	44.62	49.31
Ellipticin	0.51	0.79	0.72	0.68

Ursolic acid (**1**) and its twelve synthesized derivatives have been tested against four human cancer cell lines: human mouth epidermal carcinoma (KB), human hepatocellular carcinoma (Hep-G2), human breast carcinoma (MCF-7), and human lung carcinoma (LU). The results show that, except compounds **10** and **12**, all other compounds were active against all 4 tested cancer cell lines with different IC₅₀ values (Table 1). Especially compounds **7** and **8**, where the nitrogen function has been introduced at the position C-3, the cytotoxicity was from 2 to 3 times higher than that of ursolic acid itself against all tested cell lines. Besides these two derivatives (**7** and **8**) there are some other good active ones, for example compound **2** against Hep-G2 (IC₅₀ = 4.73 μg mL⁻¹) and **5** against KB and Hep-G2 (IC₅₀ = 5.31 and 4.36 μg mL⁻¹, respectively).

It has been reported that 3-oxo-ursolic acid (**5**) and 3β-O-acetylursolic acid (**2**) possess cytotoxic activity against HONE-1 (human nasopharyngeal carcinoma), KB, and HT29 (colorectal carcinoma) cancer cell lines [4]. Among the components isolated from the dichloromethane extract of the dried fruits of *Chaenomeles speciosa* (Sweet) Nakai (Rosaceae), 3β-O-acetyl ursolic acid showed the highest activity against both prostaglandin-H-synthase isoenzymes [5]. 3β-Succinoyl-urs-12-en-28-oic acid (**3**) exhibited cytotoxicity against NTUB1 (human bladder cancer cell line) with IC₅₀ = 8.65 μM [6]. This is the first report about the cytotoxicity against MCF7 and LU cancer cell lines of 3β-O-acetylursolic acid (**2**) and 3-acetoxymino-urs-12-en-28-oic acid (**7**). Until now there were no investigations on the cytotoxicity of the

remaining synthesized derivatives. Our results indicate again a potential for the study of the structure-activity relationship of ursolic acid derivatives.

Experimental Section

General

FT-IR: Nicolet IMPACT 410. ESI-MS: AGILENT 1100 LC-MSD Trap spectrometer. HR-ESI-MS: Qstar pulsar (Applied Biosystems). NMR: Bruker Avance 500 MHz. Column chromatography (CC): silica gel (70–230 and 230–400 mesh, Merck). Thin layer chromatography (TLC): DC-Alufolien 60 F₂₅₄ (Merck).

Isolation of ursolic acid (**1**)

The leaves of *Eriobotrya poilanei* were collected in March 2009 in Bi Dup National Park, Nui Ba, Lam Dong province of Vietnam, and identified by Dr. Nguyen Tien Hiep, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology. A voucher specimen (VH4212) is deposited at the Herbarium of this Institute.

Dried leaves (1800 g) were extracted exhaustively with methanol-water = 85 : 15 at room temperature. The organic solvent was evaporated under reduced pressure, and the aqueous solution was successively extracted with *n*-hexane, ethyl acetate and *n*-butanol. Evaporation of these extracts yielded 9.4, 50 and 130 g of a residue, respectively. The ethyl acetate extract (50 g) was purified on a silica gel column eluting with increasing polarity of *n*-hexane/ethyl acetate and then ethyl acetate/methanol to furnish 5.8 g of **1** (0.32 % of the dried plant material). The spectral data of **1** are in good agreement with those reported for ursolic acid [7].

Bioactivity assays

Bioactivity assays were carried out in the Institute of Chemistry, Vietnam Academy of Science and Technology (VAST), Hanoi, Vietnam. Cytotoxic assays were performed according to Likhiwitayawuid *et al.* [8] and Skehan *et al.* [9] at different concentrations in 96-well plates. The Hep-G2, KB and MCF-7 cell lines were maintained in the RPMI-1640 culture medium with 10 % fetal bovine serum (FBS). The LU cell line was maintained in DMEM culture medium with 10 % fetal bovine serum (FBS).

3β-Acetoxy-urs-12-ene-28-oic acid (**2**)

Ursolic acid (**1**) was treated with acetic anhydride and pyridine at r.t. overnight and worked up as usual to give acetyl-ursolic acid (**2**). The NMR spectroscopic data of **2** are in good agreement with those of acetyl-ursolic acid [10].

*3 β -Succinoyl-urs-12-en-28-oic acid (3) and
3 β -phthaloyl-urs-12-en-28-oic acid (4)*

A mixture of ursolic acid (**1**) (0.1 mmol), succinic (0.5 mmol) or phthalic anhydrides (0.5 mmol) and 4-dimethylaminopyridine (DMAP, 0.3 mmol) in pyridine (5 mL) was stirred at 60 °C. After stirring for 8 h, water was added, and the mixture was extracted with EtOAc. The organic phase was neutralized with 1N HCl solution, washed with water, dried, evaporated and chromatographed on a silica gel column (CH₂Cl₂-MeOH = 95 : 5) to furnish 32 mg of **3** or 35 mg of **4** as colorless solids (60%).

Compound 3: colorless powder. – MS ((+)-ESI): m/z = 555 [M-H]⁻, 579 [M+Na]⁺ (C₃₄H₅₂O₆). – ¹H NMR (500 MHz, CDCl₃ + CD₃OD): δ = 0.80 (3H, s, CH₃), 0.82 (3H, d, 6.2 Hz, CH₃), 0.86 (3H, s, CH₃), 0.87 (3H, s, CH₃), 0.94 (3H, d, 6.0 Hz, CH₃), 0.95 (3H, s, CH₃), 1.09 (3H, s, CH₃), 4.52 (1H, dd, 7.0, 9.0 Hz, 3-H), 5.24 ppm (1H, t-like, 12-H). – ¹³C NMR (125 MHz, CDCl₃ + CD₃OD): δ g 15.35q, 16.59q, 16.77q, 16.89q, 18.07t, 21.04q, 23.17t, 23.41q, 24.04t, 27.88q, 28.74t, 28.82t, 28.91t, 29.52t, 32.80t, 36.68t, 36.75s, 37.61t, 38.12s, 38.77t, 38.95d, 39.37d, 41.92s, 47.34s, 47.73d, 51.76s, 52.61d, 55.19d, 81.44d, 125.35d, 138.04s, 172.33s, 173.12s, 181.31s ppm.

Compound 4: colorless powder. – MS ((+)-ESI): m/z = 603 [M-H]⁻, 627 [M+Na]⁺ (C₃₈H₅₂O₆). – ¹H NMR (500 MHz, CDCl₃ + CD₃OD): δ = 0.82 (3H, s, CH₃), 0.87 (3H, d, 6.0 Hz, CH₃), 0.92 (3H, s, CH₃), 0.94 (3H, d, 8.0 Hz, CH₃), 0.97 (3H, s, CH₃), 1.11 (3H, s, CH₃), 1.26 (3H, s, CH₃), 4.72 (1H, dd, 3.7, 11.3 Hz, 3-H), 5.25 (1H, t-like, 12-H), 7.50–7.72 ppm (4H, m). – ¹³C NMR (125 MHz, CDCl₃ + CD₃OD): δ = 15.32q, 16.70q, 16.76q, 16.90q, 18.09t, 21.03q, 23.19t, 23.40q, 24.08t, 27.90t, 28.02q, 29.56t, 30.56t, 32.84t, 36.70s, 36.77t, 37.82s, 38.20t, 38.79d, 38.97d, 39.38s, 41.96s, 47.37d, 47.70s, 52.69d, 55.35d, 82.61d, 125.26d, 128.45d, 128.62d, 130.05d ($\times$ 2), 130.83s, 131.92s, 138.13s, 168.21s ($\times$ 2), 180.66s ppm.

3-Oxo-urs-12-en-28-oic acid (5)

To a solution of ursolic acid (**1**) (46 mg, 0.1 mmol) in acetone (10 mL) were added CrO₃ (100 mg, 1.0 mmol) and H₂SO₄ (20%, 10 mL). The reaction mixture was stirred at r. t. for 4 h, then concentrated *in vacuo* and extracted with ethyl acetate (50 mL). The organic phase was washed with water (2 $\times$ 20 mL), dried over Na₂SO₄, filtered and concentrated *in vacuo*, then purified by silica gel column chromatography (*n*-hexane-ethyl acetate = 7 : 1) to give 30 mg (65%) of **5** as a colorless solid. – IR (KBr): ν = 3423, 3174, 2932, 1697, 1460, 1387, 1275, 1030 cm⁻¹.

3-Hydroxyimino-urs-12-en-28-oic acid (6)

A mixture of **5** (46 mg, 0.1 mmol) and NH₂OH·HCl (40% in water, 1 mL) in pyridine/ethanol (1/1.2 mL) was heated at

60 °C for 4 h. After cooling to r. t., the reaction mixture was evaporated and extracted with ethyl acetate (3 times). The organic phase was washed with water, dried over Na₂SO₄, filtered, concentrated and chromatographed on a silica gel column (dichloromethane-methanol = 98 : 2) to furnish 29 mg (62%) of **6** as a colorless powder. The NMR spectroscopic data of compounds **5** and **6** were identical with published data [3].

3-Acetoxyimino-urs-12-en-28-oic acid (7)

A mixture of **6** (30 mg, 0.064 mmol) and acetic anhydride (0.1 mL) in pyridine (1 mL) was stirred at r. t. After 2 h, the reaction mixture was concentrated *in vacuo* to dryness and then purified using a silica gel column, eluted with *n*-hexane-ethyl acetate = 4 : 1 to furnish 28 mg (87%) of **7** as a colorless amorphous powder. – IR (KBr): ν = 2947, 2869, 1774, 1692, 1623, 1459, 1371, 1211 cm⁻¹. – ¹H NMR (500 MHz, CDCl₃): δ = 0.80 (3H, s, CH₃), 0.86 (3H, d, 6.3 Hz, CH₃), 0.94 (3H, d, 6.3 Hz, CH₃), 1.02 (3H, s, CH₃), 1.07 (3H, s, CH₃), 1.12 (3H, s, CH₃), 2.18 (3H, s, -COCH₃), 2.19 (1H, d, 11.5 Hz, H-18), 2.30 (1H, m, H-2a), 2.93 (1H, m, H-2b), 5.25 ppm (1H, m, 12-H).

Compounds **8**, **9** and **10** were synthesized as following: To a solution of **2** (49 mg, 0.1 mmol) in CH₂Cl₂ (3 mL) was added oxalyl chloride (0.1 mL) at r. t. After 48 h, the mixture was evaporated *in vacuo* to yield **2a**.

N-(3 β -Acetoxy-urs-12-en-28-oyl)-2,6-diaminohexane (8)

A solution of **2a** in CH₂Cl₂ (5 mL) was added to a mixture of NH₂(CH₂)₄CH(CH₃)NH₂ (58 mg, 0.5 mmol), triethylamine (15 mg) and CH₂Cl₂ (10 mL) at r. t. After stirring overnight, the reaction mixture was diluted with CH₂Cl₂ (30 mL) and washed with water. The organic layer was dried over Na₂SO₄, evaporated, purified by silicagel column chromatography using dichloromethane-methanol = 100 : 5 as eluent to give 42 mg of **8** as a colorless powder, 70%. – MS ((+)-ESI): m/z = 595 [M-H]⁻. – HRMS ((+)-ESI): m/z = 597.49961 (calcd. 597.49952 for C₃₈H₆₅N₂O₃, [M+H]⁺). – ¹H NMR (500 MHz, CDCl₃): δ = 0.77 (3H, s, CH₃), 0.86 (3H, d, 5.0 Hz, CH₃), 0.87 (3H, s, CH₃), 0.88 (3H, s, CH₃), 0.95 (3H, s, CH₃), 0.96 (3H, d, 6.2 Hz, CH₃), 0.96 (3H, s, CH₃), 1.09 (3H, s, CH₃), 2.05 (3H, s, OCOCH₃), 2.71 [1H, m, -CH(CH₃)-NH₂], 2.99 (1H, m, -NH-CH₂-), 3.31 (1H, m, -NH-CH₂-), 4.50 (1H, dd, 5.0, 10.0 Hz, 3-H), 5.31 (1H, t-like, 12-H), 6.00 ppm (1H, br s, NH). – ¹³C NMR (125 MHz, CDCl₃): δ = 15.58q, 16.74q, 16.98q, 17.30q, 17.51q, 18.17t, 21.25q, 21.32q, 23.23q, 23.43t, 23.55t, 24.89t, 26.70t, 27.85t, 28.08q, 30.91t, 31.48t, 32.70t, 36.86s, 37.26t, 37.70s, 38.33t, 39.09d, 39.41d, 39.58s, 39.71t, 39.79d, 42.52s, 47.09s, 47.48t, 47.68d, 53.87d, 55.24d, 80.85d (C-3), 125.49d (C-12), 140.05s (C-13), 171.02s (OCOCH₃), 178.5s ppm (NH-C=O).

N-(3 β -Acetoxy-urs-12-en-28-oyl)-11-aminoundecanoic acid methyl ester (**9**)

Compound **2a** (51 mg) in CH₂Cl₂ (5 mL) and 50 L of triethylamine were dropped slowly into a solution of 22 mg (0.1 mmol) of NH₂(CH₂)₁₀COOCH₃ in CH₂Cl₂ (3 mL) and stirred at r.t. for 12 h. The mixture was then diluted with CH₂Cl₂ (30 mL), washed with water (20 mL), dried over Na₂SO₄, evaporated *in vacuo* and chromatographed over a silica gel column (*n*-hexane-ethyl acetate = 6 : 1) to give 60 mg of **9** as a colorless powder, 87%. – ¹H NMR (500 MHz, CDCl₃): δ = 0.84 (3H, s, CH₃), 0.88 (3H, d, 6.4 Hz, CH₃), 0.95 (3H, s, CH₃), 1.05 (3H, d, 5.6 Hz, CH₃), 1.06 (3H, s, CH₃), 1.09 (3H, s, CH₃), 1.11 (3H, s, CH₃), 2.30 (2H, t, 7.5 Hz, -CH₂-COOCH₃), 2.56, 3.03 (each 1H, m, -CONH-CH₂), 3.28 (1H, m, 3-H), 3.66 (3H, s, -COOCH₃) 5.33 (1H, br s, 12-H), 5.88 ppm (1H, t, *J* = 5.5 Hz, -CONH-).

3 β -Acetoxy-urs-12-en-28-carboxamide (**10**)

A solution of **2a** (94 mg) in THF (10 mL) was slowly added to aqueous NH₃ (25%, 10 mL) at 0 °C. The reaction mixture was stirred at r.t. overnight and then concentrated. After addition of ethyl acetate, the solution was washed with 1 N HCl, neutralized with NaHCO₃, washed with water, dried, and evaporated *in vacuo*. The residue was chromatographed on a silica gel column (*n*-hexane-ethyl acetate = 3 : 1) to afford 77 mg (85%) of compound **10** as a colorless powder. – IR (KBr): ν = 3477, 3173, 2947, 2869, 1730, 1674, 1607, 1458, 1377, 1251, 1038 cm⁻¹. – HRMS ((+)-ESI): *m/z* = 498.39461 (calcd. 498.39472 for C₃₂H₅₂NO₃, [M+H]⁺). – ¹H NMR (500 MHz, CDCl₃): δ = 0.85 (3H, s, CH₃), 0.86 (3H, d, 5.5 Hz, CH₃), 0.87 (3H, s, CH₃), 0.88 (3H, d, 6.8 Hz, CH₃), 0.95 (3H, s, CH₃), 0.96 (3H, s, CH₃), 1.10 (3H, s, CH₃), 2.05 (3H, s, OCOCH₃), 4.50 (1H, dd, 5.5, 10.0 Hz, 3-H), 5.30 (1H, t-like, 12-H), 5.90 ppm (2H, s, NH₂). – ¹³C NMR (125 MHz, CDCl₃): δ = 15.53q, 16.68q, 17.11q, 17.23q, 18.12t, 21.18q, 21.26q, 23.18q, 23.35t, 23.50t, 24.81t, 27.84t, 28.03q, 30.84t, 32.70t, 36.83s, 37.11t, 37.65s, 38.28t, 39.04d, 39.38s, 39.71d, 42.43s, 47.44d, 47.91s, 54.21d, 55.22d, 80.81d (C-3), 125.62d (C-12), 139.74s (C-13), 170.95s (OCOCH₃), 181.36s ppm (C-28).

N-(3 β -Hydroxy-urs-12-ene-28-oyl)-11-amino undecanoic acid (**11**) and 3 β -hydroxy-urs-12-ene-28-carboxamide (**13**)

Compounds **11** and **13** were obtained through the hydrolysis of **9** and **10**, respectively. A 4 N NaOH solution (0.5 mL) was added to a mixture of **9** or **10** (0.05 mmol in 5 mL of THF/CH₃OH = 1/1) at r.t. After stirring for 15 h, the reaction mixture was evaporated *in vacuo*. The residue was dissolved in water, neutralized with 2 N HCl to pH = 7 and extracted with CH₂Cl₂ (50 mL). The organic layer was washed

with water, dried over Na₂SO₄, filtered and evaporated *in vacuo*. Purification of the crude product on a silica gel column (*n*-hexane-ethyl acetate = 2 : 1) furnished 30 mg of **11**, while chloroform-methanol = 4 : 1 yielded 35 mg of **13**.

Compound 11: colorless powder, yield 95%. – IR (KBr): ν = 3405, 2923, 2874, 1717, 1632, 1530, 1459, 1383, 1263, 1034 cm⁻¹. – ¹H NMR (500 MHz, CDCl₃): δ = 0.78 (s, 3H, CH₃), 0.87 (3H, d, 6.5 Hz, CH₃), 0.93 (3H, d, 7.0 Hz, CH₃), 0.99 (6H, s, CH₃), 1.07 (3H, s, CH₃), 1.26 (3H, s, CH₃), 2.32 (2H, t, 7.5 Hz, -CH₂-COOH), 3.02 (1H, br s), 3.22 (1H, m), 3.28 (1H, m, 3-H), 5.31 (1H, br s, 12-H), 5.93 ppm (1H, br s, -CONH-).

Compound 13: colorless powder, yield 76%. – MS ((+)-ESI): *m/z* = 454 [M-H]⁻ (C₃₀H₄₉O₂N). – IR (KBr): ν = 3498, 3413, 3194, 2933, 2876, 1671, 1601, 1460, 1376, 1200, 1036 cm⁻¹. – ¹H NMR (500 MHz, CDCl₃): δ = 0.78 (3H, s, CH₃), 0.85 (3H, s, CH₃), 0.87 (3H, d, 6.5 Hz, CH₃), 0.92 (3H, d, 7.4 Hz, CH₃), 0.96 (3H, s, CH₃), 0.99 (3H, s, CH₃), 1.11 (3H, s, CH₃), 3.22 (1H, dd, 4.6, 11.1 Hz, 3-H), 5.31 (1H, t-like, 12-H), 5.78, 5.89 ppm (each 1H, br s, NH₂).

3 β -Acetoxy-urs-12-en-17-nitrile (**12**)

A solution of acetyl chloride (0.3 mL) in CH₂Cl₂ (5 mL) was dropped into a solution of **10** (0.2 mmol) in CH₂Cl₂ (10 mL) and DMAP (0.5 mmol) at r.t. After stirring for 2 h at r.t., 10 mL of 1 N HCl was added. The organic layer was washed with saturated aqueous NaHCO₃, dried and chromatographed on a silica gel column (CH₂Cl₂-MeOH = 100 : 3) to furnish 75 mg (76%) of **12** as a colorless solid. – MS ((+)-ESI): *m/z* = 502 [M+Na]⁺. – HRMS ((+)-ESI): *m/z* = 502.36555 (calcd. 502.36610 for C₃₂H₄₉NO₂Na, [M+Na]⁺). – ¹H NMR (500 MHz, CDCl₃): δ = 0.84 (3H, d, 6.5 Hz, CH₃), 0.87 (3H, s, CH₃), 0.88 (3H, s, CH₃), 0.94 (3H, d, 7.5 Hz, CH₃), 0.99 (3H, s, CH₃), 1.08 (6H, s, 2 $\times$ CH₃), 2.05 (3H, s, OCOCH₃), 4.50 (1H, dd, 7.0, 9.3 Hz), 5.36 ppm (1H, t-like, 12-H). – ¹³C NMR (125 MHz, CDCl₃): δ = 15.64q, 16.74q, 16.93q, 17.40q, 18.19t, 20.85q, 21.28q, 23.03q, 23.34t, 23.55t, 25.42t, 28.09q, 28.54t, 29.99t, 33.31t, 36.45s, 36.88s, 37.69s, 38.42t, 38.68t, 39.68d, 39.78d, 42.30s, 47.52s, 47.52d, 55.32d, 55.36d, 80.85d (C-3), 125.00s (CN), 127.32d (C-12), 136.75s (C-13), 170.99s ppm (OCOCH₃).

3 β -Succinoyl-urs-12-en-28-carboxamide (**14**) and 3 β -phthaloyl-urs-12-en-28-carboxamide (**15**)

Compound **13** was converted into **14** and **15** by the same manner and with the same reagents as for **3** and **4**, respectively.

Compound 14: colorless powder, yield 60%. – IR (KBr): ν = 3458, 3201, 2922, 2865, 1735, 1713, 1641, 1577, 1454, 1385, 1267, 1213, 1000 cm⁻¹. – MS ((+)-ESI): *m/z* = 556 [M+H]⁺; 554 [M-H]⁻. – HRMS ((+)-

ESI): $m/z = 556.40019$ (calcd. 556.40020 for $C_{34}H_{54}NO_5$, $[M+H]^+$). – 1H NMR (300 MHz, $CDCl_3$): $\delta = 0.81$ (3H, s, CH_3), 0.85 (3H, d, 8.5 Hz, CH_3), 0.89 (3H, s, CH_3), 0.92 (3H, d, 6.2 Hz, CH_3), 0.95 (3H, s, CH_3), 0.96 (3H, s, CH_3), 1.10 (3H, s, CH_3), 2.64 (4H, br s, H-2' and H-3'), 4.52 (1H, dd, 6.0, 10.0 Hz, 3-H), 5.30 (1H, br s, 12-H), 5.93, 6.78 ppm (each 1H, br s, NH_2). – ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 15.71q$, 16.90q, 17.16q, 17.23q, 18.19t, 21.19q, 23.24q, 23.35t ($\times 2$), 24.61t, 27.81t, 28.11q, 29.24t, 29.67t, 30.74t, 32.52t, 36.83s, 37.11t, 37.72s, 38.04t, 39.00d, 39.33s, 39.66d, 42.28s, 47.20d, 47.79s, 53.94d, 55.09d, 81.25d, 125.73d, 139.50s, 171.84s, 176.32s, 182.81s ppm.

Compound 15: colorless powder, yield 60%. – IR (KBr): $\nu = 3519$, 3392, 2968, 2919, 1717, 1627, 1570, 1454, 1280, 1134 cm^{-1} . – MS ((+)-ESI): $m/z = 602$ $[M-H]^-$. – HRMS ((+)-ESI): $m/z = 604.40031$ (calcd. 604.40020 for $C_{38}H_{54}O_5N$, $[M+H]^+$). – 1H NMR (500 MHz, $[D_6]DMSO$): $\delta = 0.77$ (3H, s, CH_3), 0.82 (3H, d, 5.0 Hz, CH_3), 0.82 (3H, s,

CH_3), 0.91 (3H, d, 6.1 Hz, CH_3), 0.91 (3H, s, CH_3), 0.92 (3H, s, CH_3), 1.06 (3H, s, CH_3), 4.61 (1H, dd, 4.1, 11.0 Hz, 3-H), 5.22 (1H, t-like, 12-H), 6.60, 6.67 (each 1H, s, NH_2), 7.54 (3H, m), 7.70 ppm (1H, d, 2.6 Hz). – ^{13}C NMR (125 MHz, $[D_6]DMSO$): $\delta = 15.08q$, 16.62q, 16.82q, 16.97q, 17.71t, 21.02q, 22.60t, 22.80t, 23.14q, 23.59t, 27.36t, 27.86q, 30.32s, 30.44t, 32.48t, 36.40s, 36.89t, 37.40s, 37.73t, 38.41d, 38.86d, 41.61s, 46.60d, 46.84s, 52.15d, 54.80d, 81.38d, 124.37d ($\times 2$), 127.75d, 128.57d, 130.36d, 132.75s, 138.50s, 138.50s, 167.21s, 178.98s ($\times 2$) ppm.

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- [1] J. Liu, *J. Ethnopharmacol.* **1995**, *49*, 57–68.
- [2] Y.-Q. Meng, D. Liu, L.-Li Cai, H. Chen, B. Cao, Y.-Z. Wang, *Bioorg. Med. Chem.* **2009**, *17*, 848–854.
- [3] C.-M. Ma, S.-Q. Cai, J.-R. Cui, R.-Q. Wang, P.-F. Tu, M. Hattori, M. Daneshmandi, *Eur. J. Med. Chem.* **2005**, *40*, 582–589.
- [4] Y.-M. Chiang, J.-Y. Chang, C.-C. Kuo, C.-Y. Chang, Y.-H. Kuo, *Phytochemistry* **2005**, *66*, 495–501.
- [5] E. Reininger, R. Bauer, *Nat. Prod. Commun* **2008**, *3*, 1991–1994.
- [6] H.-Y. Tu, A.-M. Huang, B.-L. Wei, K.-H. Gan, T.-C. Hour, S.-C. Yang, Y.-S. Pu, C.-N. Lin, *Bioorg. Med. Chem.* **2009**, *17*, 7265–7272.
- [7] M. Goretii, V. Silva, *Molecules* **2008**, *13*, 2482–2487.
- [8] K. Likhitwitayawuid, C. K. Angerhofer, G. A. Cordell, J. M. Pezzuto, *J. Nat. Prod.* **1993**, *56*, 30–33.
- [9] P. Skehan, R. Storeng, D. Scudiero, A. Monks, M. J. Mahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney, M. R. Boyd, *J. Natl. Cancer Inst.* **1990**, *82*, 1107–1112.
- [10] A. V. Tkachev, A. Yu. Denisov, *Tetrahedron* **1994**, *50*, 2591–2598.