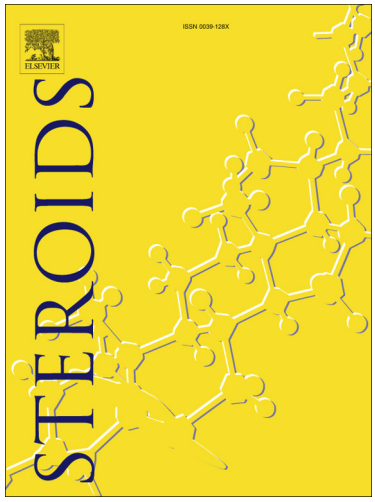

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Enhancing effect of cystamine in its amides with betulinic acid as antimicrobial and antitumor agent *in vitro*

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Abstract

Amides of betulinic acid with cystamine were synthesized to investigate their antimicrobial and antitumor activity, and their influence on the cell cycle and cell apoptosis. The former target amide (**6**) displayed cytotoxicity in CEM cell line after 72 h of treatment (IC_{50} = 3.0 ± 0.7 μM; TI = 20), and induced apoptosis by caspase-3/7 activation in CEM cells. The latter target amide (**9**) displayed antimicrobial activity against *Streptococcus mutans* (MIC 3.125 μM; MBC 3.125 μM) and *Bacillus cereus* (MIC 25 μM; MBC 25 μM). The achieved results demonstrate enhancing of their biological activity over that of the parent compounds. However, two intermediate compounds (**2** and **7**) displayed either considerable cytotoxicity (**2**; 7.5 ± 0.8 μM; TI = 10, against G361) or antimicrobial activity (**7**; both against *Actinomyces odontolycus* and *Clostridium perfrigens* with MIC 12.5 μM and MBC 12.5 μM). The experimental data were compared with the *in silico* calculated physico-chemical and ADME parameters of the target compounds, including successful intermediates.

Keywords: Betulinic acid; cystamine; cytotoxicity; apoptosis; antimicrobial activity.

1. Introduction

Chemical biology of natural products is a challenging area of investigation. Increasing importance has been paid to the investigation of different conjugates formed on the basis of plant products for the possibility to study enhancement of biological effects of the target conjugates in comparison with the biological effects of their parent components [1]. Betulinic acid is a natural product, found in a number of plants [2]. Even if it is practically insoluble in water [3], it displays activity against HIV [4], cancer [5,6] and diabetes [7,8]. To improve the pharmacological characteristics of betulinic acid, especially bioavailability, its novel derivatives with polyamines, simple and complex aromatic amines or amino acids and

peptides have always been a challenge for researchers [9-11]. The anti-tumor effect of betulinic acid and its derivatives was found against a variety of tumor cell lines, such as malignant brain tumor, primitive neuroectodermal tumor [12], human chronic myelogenous leukemia, and against most of prevalent human cancer types, such as cervical, prostate, breast, lung or colorectal cancer [9-11].

The radioprotective activity of cysteamine was described as early as in 1953, and it relied on its effect against the formation of OH radicals [13]. Both, cysteamine and its oxidized form, cystamine, were used in clinical trials to treat radiation sickness in patients receiving radiotherapy [14]. Cystamine also displays potent effect against HIV [14]. Cystamine and cysteamine show anti-cancer effects, and they have been subjects of clinical trials for neurodegenerative diseases [15]. Both compounds have been selected as potential practical candidate drugs for treating these diseases on the basis of experimental animal work [16,17]. Cystamine gives sulfane sulfur (S^0) when metabolized by diamine oxidase. Its beneficial effects, including prevention of carcinogen-induced cancer, dementia, diabetes, lowering of blood cholesterol, decreasing plasma homocysteine levels or preventing atherosclerosis and heart disease have already been reported [15]. The secondary main reason for choosing cystamine for this investigation consisted in its structure that mimics the structure of di- and polyamines used for the structural modification of betulinic acid earlier [10].

The objective of the current investigation was (a) to study aspects of chemical biology with amide conjugates of betulinic acid with cystamine, (b) to find out if the target compounds are cytotoxic and cause cell apoptosis, (c) to find out if they display antimicrobial activity, and (d) to find out if they enhance the effects of betulinic acid. Amide bond formation is easily possible to form either at C(28) carboxyl group or at the short chained diacid (e.g., succinic) hemiester formed at the C(3)-OH in advance. To support the obtained experimental data, *in*

silico calculation of physico-chemical and ADME parameters has been done to find potential coincidence between the experimental and calculated data.

2. Material and Methods

2.1. General

The ^{1}H NMR and the ^{13}C NMR spectra were recorded on a Bruker AVANCE 600 MHz spectrometer at 600.13 MHz and 150.90 MHz in deuterochloroform, using tetramethylsilane ($\delta = 0.0$) as internal reference. ^{1}H NMR data are presented in the following order: chemical shift (δ) expressed in ppm, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), coupling constants in Hertz, number of protons. For unambiguous assignment of both ^{1}H and ^{13}C signals 2D NMR ^{1}H,^{13}C gHSQC and gHMBC spectra were measured using standard parameters sets and pulse programs delivered by producer of the spectrometer. Infrared spectra (IR) were measured with a Nicolet 205 FT-IR spectrometer. Mass spectra (MS) were measured with a Waters ZMD mass spectrometer in a positive ESI mode. Optical rotations were measured on an Autopol IV instrument (Rudolph Research Analytical, USA) at 589 nm wavelength, and the values were corrected to 20 ^{o}C. A PE 2400 Series II CHNS/O Analyzer (Perkin Elmer, USA) was used for simultaneous determination of C, H, and N (accuracy of CHN determination better than 0.30 % abs.). TLC was carried out on silica gel plates (Merck $60F_{254}$) and the visualization was performed by the UV detection and by spraying with the methanolic solution of phosphomolybdic acid (5%) followed by heating. For column chromatography, silica gel 60 (0.063-0.200 mm) from Merck was used. All chemicals and solvents were purchased from regular commercial sources in analytical grade and the solvents were purified by general methods before use. Betulinic acid was purchased from Dr. Jan Šarek – Betulinines (www.betulinines.com).

2.2. (3β)-3-(Acetyloxy)lup-20(29)-en-28-oic acid (**2**)

Acetic anhydride (0.45 mL; 4.7 mmol; 1.44 eq), DMAP (52 mg; 0.43 mmol, 0.13 eq) and EDIPA (1 mL) was added to a solution of betulinic acid (1.5 g; 3.3 mmol) in dry THF (10 ml). The reaction mixture was heated under reflux over 3 h. After stopping the reaction, water (10 mL) was added, and the mixture was stirred for an additional 1 h. The resulting mixture was extracted with chloroform, and dried over sodium sulfate. Evaporation of the solvent afforded a solid and its purification by a column chromatography yielded 1.55 g (95 %) of **2**. ^{1}H NMR: δ 0.83 (3H, s, H23), 0.84 (3H, s, H25), 0.85 (3H, bs, H24), 0.93 (3H, s, H26), 0.97 (3H, bs, H27), 1.05 (2H, dq, J_1 = 4.9 Hz, J_2 = 13.0 Hz, J_3 = 13.0 Hz, J_4 = 13.0 Hz, H12), 1.19 (2H, dt, J_1 = 3.2 Hz, J_2 = 3.2 Hz, J_3 = 13.6 Hz, H21), 1.70 (3H, dd, J_1 = 0.7 Hz, J_2 = 1.4 Hz, H29), 2.04 (3H, s, OAc), 2.13 (2H, ddd, J_1 = 3.7 Hz, J_2 = 11.6 Hz, J_3 = 12.8 Hz, H1), 2.27 (2H, dt, J_1 = 3.8 Hz, J_2 = 3.8 Hz, J_3 = 13.1 Hz, H16), 3.01 (1H, dt, J_1 = 5.0 Hz, J_2 = 10.9 Hz, J_3 = 10.9 Hz, H19), 4.47 (1H, dd, J_1 = 5.5 Hz, J_2 = 10.9 Hz, H3), 4.61 (1H, dq, J_1 = 1.4 Hz, J_2 = 1.4 Hz, J_3 = 1.4 Hz, J_4 = 2.3 Hz, H30), 4.74 (1H, dq, J_1 = 0.7 Hz, J_2 = 0.7 Hz, J_3 = 0.7 Hz, J_4 = 2.3 Hz, H30). ^{13}C NMR: δ 14.64 (q, C27), 16.02 (q, C24), 16.17 (q, C25), 16.46 (q, C26), 18.15 (t, C6), 19.32 (q, OAc), 19.33 (q, C29), 20.84 (t, C11), 23.68 (t, C2), 25.43 (t, C12), 27.94 (q, C23), 29.68 (t, C21), 30.54 (t, C15), 32.13 (t, C16), 34.22 (t, C22), 37.03 (d, C13), 37.11 (s, C10), 37.79 (t, C7), 38.37 (s, C4), 38.39 (t, C1), 40.68 (s, C8), 42.41 (s, C14), 46.92 (d, C19), 49.25 (d, C18), 50.38 (d, C9), 55.40 (d, C5), 56.35 (s, C17), 80.94 (d, C3), 109.74 (t, C30), 150.36 (s, C20), 171.06 (s, OAc), 181.47 (s, C28). MS (ES$^-$): *m/z* 497.1 [M-H]$^-$. IR (KBr; cm^{-1}): 2945 (C–H), 2871 (–CH_3), 1735 (–C=O), 1694 (–C=O). For $C_{32}H_{50}O_4$ (498.74) calculated C (77.06), H (10.10), found C (77.09), H (10.08). M.p. 263-266 ^{o}C.

2.3. (3β)-28-{[2-({2-[(*tert*-Butoxycarbonyl)amino]ethyl}disulfanediyl)ethyl]-amino}-28-oxolup-20(29)-en-3-yl acetate (**3**)

A solution of oxalyl chloride in dry dichloromethane (2M; 1.6 mL; 3.2 mmol; 8 eq) was added to a solution of **2** (200 mg, 0.4 mmol) in dry dichloromethane (4 mL), and the reaction mixture was stirred overnight. The reaction mixture was evaporated, and the residue was dissolved in dry dichloromethane (3 mL). EDIPA (0.182 mL; 1 mmol; 2.6 eq) and Boc protected cystamine hydrochloride (174 mg, 0.6 mmol; 1.5 eq) were added. The reaction mixture was stirred at r.t. overnight. After stopping the reaction, evaporation of the solvent afforded a solid. Its purification by column chromatography yielded 273 mg (93 %) of **3**. ^{1}H NMR: δ 0.82 (3H, s, H23), 0.84 (3H, d, J = 0.6 Hz, H24), 0.84 (3H, s, H25), 0.93 (3H, s, H26), 0.96 (3H, s, H27), 1.45 (9H, s, Boc), 1.68 (3H, dd, J_1 = 0.7 Hz, J_2 = 1.4 Hz, H29), 1.80 (2H, bdd, J_1 = 7.7 Hz, J_2 = 12.0 Hz, H1), 1.94 (2H, ddt, J_1 = 8.2 Hz, J_2 = 10.7 Hz, J_3 = 10.7 Hz, J_4 = 13.2 Hz, H21), 2.04 (3H, s, OAc), 2.46 (1H, bdt, J_1 = 3.5 Hz, J_2 = 12.2 Hz, J_3 = 12.2 Hz, H13), 2.74-2.79 (2H, m, H3'), 2.79-2.86 (2H, m, H2'), 3.11 (1H, dt, J_1 = 4.5 Hz, J_2 = 11.1 Hz, J_3 = 11.1 Hz, H19), 3.38-3.52 (2H, m, H4'), 3.38-3.52 (1H, m, H1'), 3.65 (1H, dq, J_1 = 5.9 Hz, J_2 = 5.9 Hz, J_3 = 5.9 Hz, J_4 = 14.0 Hz, H1'), 4.47 (1H, dd, J_1 = 6.2 Hz, J_2 = 10.3 Hz, H3), 4.59 (1H, dq, J_1 = 1.4 Hz, J_2 = 1.4 Hz, J_3 = 1.4 Hz, J_4 = 2.4 Hz, H30), 4.73 (1H, dq, J_1 = 0.7 Hz, J_2 = 0.7 Hz, J_3 = 0.7 Hz, J_4 = 2.4 Hz, H30), 5.02 (1H, bt, J = 5.0 Hz, NH), 6.31 (1H, bt, J = 5.0 Hz, NH). ^{13}C NMR: δ 14.60 (q, C27), 16.18 (q, C24), 16.21 (q, C25), 16.47 (q, C26), 18.18 (t, C6), 19.46 (q, C29), 20.96 (t, C11), 21.32 (q, OAc), 23.69 (t, C2), 25.58 (t, C12), 27.93 (q, C23), 28.37 (q, C7'), 29.48 (t, C21), 30.88 (t, C15), 33.62 (t, C16), 34.32 (t, C22), 37.11 (s, C10), 37.67 (d, C13), 37.79 (t, C7), 37.92 (t, C3'), 38.03 (t, C4'), 38.33 (s, C4), 38.39 (t, C1), 38.45 (t, C2'), 39.41 (t, C1'), 40.79 (s, C8), 42.45 (s, C14), 46.74 (d, C19), 50.13 (d, C18), 50.55 (d, C9), 55.46 (d, C5), 55.70 (s, C17), 79.65 (s, C6'), 80.95 (d, C3), 109.36 (t, C30), 150.95 (s, C20), 155.81 (s, C5'), 171.03 (s, OAc), 176.56 (s, C28). MS (ES^{+}): *m/z* 733.5 [M+H]$^{+}$, 755.4 [M+Na]$^{+}$; (ES^{-}): 731.4 [M-H]$^{-}$. IR (KBr; cm^{-1}): 3447 (N–H), 2926 (C–H), 2870 (–CH_3), 1718 (–C=O), 1701 (–C=O), 1642 (–C=O).

2.4. *tert*-Butyl {2-[(2-{[(3β)-3-hydroxy-28-oxolup-20(29)-en-28-yl]amino}ethyl)-disulfanediyl]ethyl}carbamate (**4**) and (3β,3'β)-*N*,*N*'-(disulfanediyldiethane-2,1-diyl)bis[3-hydroxylup-20(29)-en-28-amide] (**5**)

$LiOH \cdot H_2O$ (0.36 g; 8.6 mmol; 21 eq) was added to a solution of **3** (294 mg; 0.4 mmol) in methanol (25 mL). The reaction mixture was stirred for 1 day. After stopping the reaction, the mixture was diluted by water, extracted by chloroform (10 times 10-15 mL each) and dried over sodium sulfate. Evaporation of the solvent afforded a solid that was purified by column chromatography, yielding 155 mg (56 %) of **4** and 80 mg (39 %) of **5**.

4: ^{1}H NMR: δ 0.75 (3H, s, H23), 0.81 (3H, d, J = 0.6 Hz, H24), 0.92 (3H, s, H25), 0.96 (3H, s, H26), 0.96 (3H, s, H27), 1.44 (9H, s, Boc), 1.66 (2H, dt, J_1 = 3.5 Hz, J_2 = 3.5 Hz, J_3 = 13.0 Hz, H16), 1.67 (3H, dd, J_1 = 0.7 Hz, J_2 = 1.4 Hz, H29), 2.78 (2H, t, J = 5.8 Hz, H3'), 2.82 (2H, t, J = 5.8 Hz, H2'), 3.11 (1H, dt, J_1 = 4.4 Hz, J_2 = 11.1 Hz, J_3 = 11.1 Hz, H19), 3.18 (1H, dd, J_1 = 4.7 Hz, J_2 = 11.5 Hz, H3), 3.39-3.52 (1H, m, H4'), 3.39-3.52 (2H, m, H1'), 3.64 (1H, dt, J_1 = 5.8 Hz, J_2 = 5.8 Hz, J_3 = 5.8 Hz, J_4 = 13.9 Hz, H4'), 4.58 (1H, dq, J_1 = 1.4 Hz, J_2 = 1.4 Hz, J_3 = 1.4 Hz, J_4 = 2.4 Hz, H30), 4.73 (1H, bd, J_1 = 0.1 Hz, J_2 = 0.1 Hz, J_3 = 0.1 Hz, J_4 = 2.4 Hz, H30), 4.97 (1H, bt, J = 4.6 Hz, NH), 6.32 (1H, bt, J = 4.6 Hz, NH). ^{13}C NMR: δ 14.64 (q, C27), 15.34 (q, C24), 16.14 (q, C25), 16.17 (q, C26), 18.29 (t, C6), 19.49 (q, C29), 20.94 (t, C11), 25.62 (t, C12), 27.41 (t, C2), 27.97 (q, C23), 28.37 (q, C7'), 29.49 (t, C21), 30.89 (t, C15), 33.62 (t, C16), 34.38 (t, C22), 37.19 (s, C10), 37.69 (d, C13), 37.91 (t, C3'), 38.02 (t, C4'), 38.33 (t, C7), 38.43 (t, C2'), 38.71 (s, C4), 38.84 (t, C1), 39.41 (t, C1'), 40.77 (s, C8), 42.45 (s, C14), 46.71 (d, C19), 50.13 (d, C18), 50.64 (d, C9), 55.37 (d, C5), 55.70 (s, C17), 78.97 (d, C3), 79.63 (s, C6'), 109.30 (t, C30), 150.97 (s, C20), 155.81 (s, C5'), 176.54 (s, C28). MS (ES^+): *m/z* 691.3 $[M+H]^+$, 713.3 $[M+Na]^+$; (ES^-): 689.0 $[M-H]^-$. IR (KBr; cm^{-1}): 2957 (C–H), 2870 (–CH_3), 1695 (–C=O), 1641 (–C=O).

5: ^{1}H NMR: δ 0.76 (3H, s, H23), 0.82 (3H, bs, H24), 0.93 (3H, s, H25), 0.96 (3H, s, H26), 0.96 (3H, s, H27), 1.36-1.39 (1H, m, H21), 1.42-1.45 (1H, m, H7), 1.54-1.57 (1H, m, H16), 1.68 (3H, dd, J_1 = 0.6 Hz, J_2 = 1.4 Hz, H29), 1.83 (1H, dd, J_1 = 7.5 Hz, J_2 = 12.2 Hz, H7), 1.93 (1H, ddd, J_1 = 8.0 Hz, J_2 = 11.2 Hz, J_3 = 13.2 Hz, H21), 2.07 (1H, dt, J_1 = 3.4 Hz, J_2 = 3.4 Hz, J_3 = 13.4 Hz, H16), 2.49 (1H, ddd, J_1 = 3.7 Hz, J_2 = 11.5 Hz, J_3 = 13.0 Hz, H13), 2.81-2.88 (2H, m, H2'), 3.12 (1H, dt, J_1 = 4.4 Hz, J_2 = 11.1 Hz, J_3 = 11.1 Hz, H19), 3.18 (1H, dd, J_1 = 4.8 Hz, J_2 = 11.6 Hz, H3), 3.50 (1H, dq, J_1 = 6.6 Hz, J_2 = 6.6 Hz, J_3 = 6.6 Hz, J_4 = 14.0 Hz, H1'), 3.61 (1H, dq, J_1 = 7.1 Hz, J_2 = 7.1 Hz, J_3 = 7.1 Hz, J_4 = 14.0 Hz, H1'), 4.59 (1H, dq, J_1 = 1.4 Hz, J_2 = 1.4 Hz, J_3 = 1.4 Hz, J_4 = 2.4 Hz, H30), 4.72 (1H, bd, J = 2.4 Hz, H30), 6.48 (1H, t, J = 6.9 Hz, NH). ^{13}C NMR: δ 14.62 (q, C27), 15.34 (q, C24), 16.12 (q, C25), 16.17 (q, C26), 18.29 (t, C6), 19.51 (q, C29), 20.96 (t, C11), 25.64 (t, C12), 27.41 (t, C2), 27.97 (q, C23), 29.51 (t, C15), 30.91 (t, C21), 33.75 (t, C16), 34.39 (t, C22), 37.20 (s, C10), 37.61 (d, C13), 38.17 (t, C2'), 38.45 (t, C1'), 38.45 (t, C7), 38.72 (t, C1), 38.85 (s, C4), 40.79 (s, C8), 42.45 (s, C14), 46.61 (d, C19), 50.15 (d, C18), 50.62 (d, C9), 55.38 (d, C5), 55.69 (s, C17), 79.00 (d, C3), 109.29 (t, C30), 150.96 (s, C20), 176.72 (s, C28). MS (ES^+): *m/z* 1029.8 $[M+H]^+$, 1051.8 $[M+Na]^+$; (ES^-): 1027.8 $[M-H]^-$. IR (KBr; cm^{-1}): 2930 (C–H), 2870 (–CH_3), 1640 (–C=O). $[\alpha]_D^{20}$ = –6.8 (*c* 0.191; CH_3OH).

2.5. (3β)-*N*-{2-[(2-Aminoethyl)disulfanediyl]ethyl}-3-hydroxylup-20(29)-en-28-amide (**6**)

A solution of HCl in 1,4-dioxane (4 M; 0.2 mL; 0.7 mmol; 20 eq) was added to a solution of **4** (50 mg; 0.072 mmol) in dry 1,4-dioxane (3 mL). The reaction mixture was stirred for 7 h at 30 °C. After stopping the reaction, the mixture was filtered, washed with a large amount of diethyl ether and dried over sodium sulfate, yielding 29 mg (68 %) of **6**.

^{1}H NMR: δ 0.68 (1H, dd, J_1 = 2.0 Hz, J_2 = 11.5 Hz, H5), 0.75 (3H, s, H23), 0.81 (3H, d, J = 0.6 Hz, H25), 0.92 (3H, s, H26), 0.95 (3H, s, H24), 0.96 (3H, d, J = 0.7 Hz, H27), 1.63 (2H,

dt, J_1 = 3.5 Hz, J_2 = 3.5 Hz, J_3 = 11.0 Hz, H16), 1.67 (3H, dd, J_1 = 0.7 Hz, J_2 = 1.4 Hz, H29), 2.42 (1H, ddd, J_1 = 3.7 Hz, J_2 = 11.5 Hz, J_3 = 13.0 Hz, H13), 2.78-2.82 (2H, m, H3'), 2.79 (2H, t, J = 6.0 Hz, H1'), 3.04 (2H, t, J = 6.0 Hz, H2'), 3.11 (1H, dt, J_1 = 4.5 Hz, J_2 = 11.1 Hz, J_3 = 11.1 Hz, H19), 3.14 (1H, dd, J_1 = 4.7 Hz, J_2 = 11.5 Hz, H3), 3.48-3.54 (1H, m, H4'), 3.58-3.66 (1H, m, H4'), 4.59 (1H, dq, J_1 = 1.4 Hz, J_2 = 1.4 Hz, J_3 = 1.4 Hz, J_4 = 2.3 Hz, H30), 4.73 (1H, dq, J_1 = 0.7 Hz, J_2 = 0.7 Hz, J_3 = 0.7 Hz, J_4 = 2.3 Hz, H30), 6.03 (2H, t, J = 5.9 Hz, NH). ^{13}C NMR: δ 14.64 (q, C27), 15.34 (q, C24), 16.14 (q, C25), 16.18 (q, C26), 18.29 (t, C6), 19.46 (q, C29), 20.92 (t, C11), 25.60 (t, C12), 27.40 (t, C2), 27.97 (q, C23), 29.48 (t, C15), 30.86 (t, C21), 33.73 (t, C16), 34.38 (t, C22), 37.19 (s, C10), 37.75 (d, C13), 37.75 (t, C4'), 37.94 (t, C2'), 38.33 (t, C7), 38.70 (t, C1), 38.86 (s, C4), 40.42 (t, C1'), 40.77 (s, C8), 41.94 (t, C3'), 42.47 (s, C14), 46.78 (d, C19), 50.08 (d, C18), 50.61 (d, C9), 55.36 (d, C5), 55.73 (s, C17), 78.97 (d, C3), 109.38 (t, C30), 150.87 (s, C20), 176.37 (s, C28). MS (APCI): *m/z* 591.4 $[M+H]^+$, 589.3 $[M-H]^-$, 635.4 $[M+HCOO]^-$. IR (KBr; cm^{-1}): 2945 (C–H), 2870 ($-CH_3$), 1640 (–C=O). $[\alpha]_D^{20}$ = –9.1 (*c* 0.407; CH_3OH).

2.6. (3β)-3-[(3-Carboxypropanoyl)oxy]lup-20(29)-en-28-oic acid (**7**)

Succinic anhydride (1.62 g; 16.2 mmol; 3.5 eq) and DMAP (170 mg; 1.39 mmol, 0.30 eq) were added to a solution of betulinic acid (2.1 g; 4.6 mmol) in dry pyridine (20 mL). The reaction mixture was stirred at r.t. for 5 days. After stopping the reaction, the resulting mixture was poured onto ice, and hydrochloric acid was added to adjust pH = 7, extracted with chloroform, and dried over sodium sulfate. Evaporation of the solvent afforded a solid that was purified by a column chromatography, yielding 1.93 g (75 %) of **7**.

^{1}H NMR: δ 0.79 (3H, s, H23), 0.79 (3H, s, H24), 0.80 (3H, s, H25), 0.87 (3H, s, H26), 0.94 (3H, s, H27), 1.64 (3H, dd, J_1 = 0.7 Hz, J_2 = 1.4 Hz, H29), 2.09-2.13 (2H, m, H16), 2.22 (1H, ddd, J_1 = 3.6 Hz, J_2 = 11.6 Hz, J_3 = 12.9 Hz, H13), 2.44-2.52 (4H, m, H2'-H3'), 2.95 (1H, dt,

J_1 = 4.8 Hz, J_2 = 11.0 Hz, J_3 = 11.0 Hz, H19), 4.38 (1H, dd, J_1 = 4.7 Hz, J_2 = 11.6 Hz, H3), 4.56 (1H, dq, J_1 = 1.4 Hz, J_2 = 1.4 Hz, J_3 = 1.4 Hz, J_4 = 2.4 Hz, H30), 4.69 (1H, dq, J_1 = 0.7 Hz, J_2 = 0.7 Hz, J_3 = 0.7 Hz, J_4 = 2.4 Hz, H30). ^{13}C NMR: δ 14.31 (q, C27), 15.65 (q, C24), 15.81 (q, C25), 16.35 (q, C26), 17.67 (t, C6), 18.90 (q, C29), 20.42 (t, C11), 23.27 (t, C2), 25.00 (t, C12), 27.56 (q, C23), 28.77 (t, C2'), 29.15 (t, C21), 29.15 (t, C3'), 30.06 (t, C15), 31.64 (t, C16), 33.68 (t, C22), 36.28 (t, C1), 36.60 (s, C10), 37.38 (s, C4), 37.54 (d, C13), 37.67 (t, C7), 40.21 (s, C8), 41.99 (s, C14), 46.57 (d, C19), 48.50 (d, C18), 49.61 (d, C9), 54.62 (d, C5), 55.36 (s, C17), 79.98 (d, C3), 109.58 (t, C30), 150.26 (s, C20), 171.56 (s, C1'), 173.32 (s, C4'), 177.17 (s, C28). MS (ES$^-$): *m/z* 555.2 [M-H]$^-$. IR (KBr; cm^{-1}): 2946 (C–H), 2872 (–CH_3), 1734 (–C=O). For $C_{34}H_{52}O_6$ (556.77) calculated C (73.34), H (9.41), found C (73.37), H (9.40). M.p. 244-246 ^{o}C.

2.7. (3β)-3-[(2,2-dimethyl-4,13,16-trioxo-3-oxa-8,9-dithia-5,12-diazahexadecan--16-yl)oxy]lup-20(29)-en-28-oic acid (**8**)

Boc-protected cystamine hydrochloride (118 mg; 0.4 mmol; 1.1 eq) and T3P (0.64 mL; 1 mmol; 3 eq) were added to a solution of **7** (200 mg; 0.36 mmol) in dry pyridine (10 mL), and the reaction mixture was stirred at r.t. for 1 day. The resulting mixture was washed with water, extracted with chloroform, and dried over sodium sulfate. Evaporation of the solvent afforded a solid that was purified by a column chromatography, yielding 215 mg (76 %) of **8**. ^{1}H NMR: δ 0.82 (1H, dd, J_1 = 1.8 Hz, J_2 = 11.2 Hz, H5), 0.86 (3H, s, H23), 0.87 (3H, s, H26), 0.89 (3H, d, J = 0.4 Hz, H25), 0.98 (3H, s, H24), 1.02 (3H, s, H27), 1.07 (2H, dq, J_1 = 4.6 Hz, J_2 = 13.1 Hz, J_3 = 13.1 Hz, J_4 = 13.1 Hz, H2), 1.18 (2H, dt, J_1 = 3.2 Hz, J_2 = 3.2 Hz, J_3 = 13.5 Hz, H21), 1.27 (2H, dq, J_1 = 4.8 Hz, J_2 =13.0 Hz, J_3 = 13.0 Hz, J_4 = 13.0 Hz, H11), 1.43 (9H, s, Boc), 1.70 (3H, dd, J_1 = 0.7 Hz, J_2 = 1.4 Hz, H29), 2.24 (2H, dt, J_1 = 3.4 Hz, J_2 = 3.4 Hz, J_3 = 12.6 Hz, H16), 2.31 (1H, ddd, J_1 = 3.6 Hz, J_2 = 11.7 Hz, J_3 = 12.9 Hz, H13), 2.50 (2H, t, J =

6.6 Hz, H2'), 2.56-2.65 (2H, m, H3'), 2.78 (2H, t, J = 6.7 Hz, H6'), 2.81 (2H, t, J = 6.6 Hz, H7'), 3.02 (1H, dt, J_1 = 4.8 Hz, J_2 = 10.9 Hz, J_3 = 10.9 Hz, H19), 3.32-3.37 (2H, m, H8'), 3.43-3.51 (2H, m, H5'), 4.46 (1H, dd, J_1 = 5.1 Hz, J_2 = 11.3 Hz, H3), 4.59 (1H, dq, J_1 = 1.4 Hz, J_2 = 1.4 Hz, J_3 = 1.4 Hz, J_4 = 2.4 Hz, H30), 4.71 (1H, dq, J_1 = 0.7 Hz, J_2 = 0.7 Hz, J_3 = 0.7 Hz, J_4 = 2.4 Hz, H30). ^{13}C NMR: δ 15.14 (q, C27), 16.64 (q, C24), 16.77 (q, C25), 17.09 (q, C26), 19.27 (t, C6), 19.55 (q, C29), 22.12 (t, C11), 24.68 (t, C12), 26.83 (t, C2), 28.54 (q, C23), 28.79 (q, Boc), 30.75 (t, C2'), 30.83 (t, C21), 31.40 (t, C3'), 31.70 (t, C15), 33.34 (t, C16), 35.48 (t, C22), 38.14 (t, C1), 38.30 (d, C13), 38.62 (t, C6'), 38.94 (s, C10), 39.07 (t, C7'), 39.57 (t, C7), 39.62 (s, C4), 40.78 (t, C5'), 40.90 (t, C8'), 41.94 (s, C8), 43.61 (s, C14), 48.50 (d, C19), 50.42 (d, C18), 51.87 (d, C9), 56.88 (d, C5), 57.48 (s, C17), 80.18 (s, Boc), 82.62 (d, C3), 110.20 (t, C30), 151.97 (s, C20), 158.41 (s, Boc), 174.09 (s, C1'), 174.39 (s, C4'), 180.07 (s, C28). MS (ES$^+$): *m/z* 791.5 $[M+H]^+$, 813.3 $[M+Na]^+$; (ES$^-$): 789.4 $[M-H]^-$. IR (KBr; cm^{-1}): 2938 (C–H), 2874 (–CH_3), 1732 (–C=O), 1695 (–C=O), 1662 (–C=O).

2.8. (3β)-3-{[4-({2-[(2-aminoethyl)disulfanediyl]ethyl}amino)-4-oxobutanoyl]-oxy}lup-20(29)-en-28-oic acid (**9**)

A solution of HCl in 1,4-dioxane (4 M; 0.6 mL; 2.4 mmol; 13 eq) was added to a solution of **8** (142 mg; 0.179 mmol) in dry 1,4-dioxane (9 mL). Reaction mixture was stirred at 30 °C for 20 h. Then the reaction mixture was put to the freezer, next day it was melted, filtered, washed with diethyl ether and chloroform and dried over sodium sulfate, yielding 101 mg (82 %) of **9**.

^{1}H NMR: δ 0.83 (1H, dd, J_1 = 2.3 Hz, J_2 = 3 x 11.8 Hz, H5), 0.86 (3H, s, H23), 0.87 (2H, d, J = 0.7 Hz, H26), 0.89 (3H, s, H25), 0.97 (3H, s, H24), 1.02 (2H, d, J = 0.5 Hz, H27), 1.27 (2H, dq, J_1 = 4.7 Hz, J_2 = 3 x 13.1 Hz, H2), 1.27 (2H, dq, J_1 = 4.6 Hz, J_2 = 3 x 12.8 Hz, H11), 1.70 (3H, dd, J_1 = 0.7 Hz, J_2 = 1.4 Hz, H29), 2.23 (2H, dt, J_1 = 3.4 Hz, J_2 = 3.4 Hz, J_3 = 12.8 Hz,

H16), 2.43 (1H, ddd, J_1 = 3.6 Hz, J_2 = 11.2 Hz, J_3 = 13.1 Hz, H13), 2.49 (2H, m, H2'), 2.59 (2H, m, H3'), 2.85 (2H, dt, J_1 = 0.8 Hz, J_2 = 6.8 Hz, H6'), 2.98 (2H, dt, J_1 = 0.8 Hz, J_2 = 6.8 Hz, H7'), 3.02 (1H, dt, J_1 = 4.9 Hz, J_2 = 11.0 Hz, J_3 = 11.0 Hz, H19), 3.28 (2H, bt, J = 6.8 Hz, H5'), 3.50 (2H, bt, J = 6.8 Hz, H8'), 4.47 (1H, dd, J_1 = 5.1 Hz, J_2 = 11.2 Hz, H3), 4.59 (1H, dq, J_1 = 3 x 1.4 Hz, J_2 = 2.4 Hz, H30), 4.71 (1H, dq, J_1 = 3 x 0.7 Hz, J_2 = 2.4 Hz, H30). ^{13}C NMR: δ 15.11 (q, C27), 16.62 (q, C24), 16.75 (q, C25), 17.06 (q, C26), 19.26 (t, C6), 19.54 (q, C29), 22.11 (t, C11), 24.68 (t, C12), 26.82 (t, C2), 28.53 (q, C23), 30.69 (t, C21), 30.82 (t, C3'), 31.37 (t, C2'), 31.68 (t, C15), 33.30 (t, C16), 35.46 (t, C22), 35.51 (t, C6'), 38.11 (t, C1), 38.22 (d, C13), 38.29 (s, C10), 38.94 (t, C7'), 39.38 (t, C5'), 39.55 (s, C4), 39.57 (t, C7), 39.61 (t, C8'), 41.93 (s, C8), 43.59 (s, C14), 48.49 (d, C19), 50.40 (d, C18), 51.87 (d, C9), 56.89 (d, C5), 57.45 (s, C17), 82.63 (d, C3), 110.19 (t, C30), 151.96 (s, C20), 174.12 (s, C1'), 174.53 (s, C4'), 179.96 (s, C28). MS (ES^+): *m/z* 691.3 $[M+H]^+$; (ES^-): 689.3 $[M-H]^-$. IR (KBr; cm^{-1}): 2941 (C–H), 1728 (–C=O), 1695 (–C=O), 1662 (–C=O), 1642 (N–H). $[\alpha]_D^{20}$ = +13.6 (*c* 0.381; CH_3OH).

2.9. Cell cultures

The screening cell lines, i.e., T-lymphoblastic leukemia, CEM; cervical carcinoma, HeLa; breast carcinoma, MCF7; human malignant melanoma, G-361; and human foreskin fibroblasts, BJ, were obtained from the American Type Culture Collection (Manassas, VA, USA). Cells were cultured in DMEM (Dulbecco's Modified Eagle Medium, Sigma Aldrich, MO, USA). Media used were supplemented with 10% of fetal bovine serum, 2 mM of L-glutamine, and 1% of penicillin-streptomycin (all from Sigma Aldrich, MO, USA). The cell lines were maintained under standard cell culture conditions at 37 °C and 5% CO_2 in humid environment. Cells were subcultured twice or three times a week using the standard trypsinization procedure.

3.10. Cytotoxicity screening tests

Description of the experimental procedure used in cytotoxicity assay was already published [10]. IC_{50} values in CEM, MCF7, HeLa, G-361 and BJ measured with the compounds **2**, **5**, **6**, **7** and **9** after 72 h of treatment are shown in Table 1.

3.11. Flow cytometry analysis

CEM cancer cells were treated with **6** and **9** for 24 h, stained with propidium iodide and their DNA contents were measured by a flow cytometer (FACS Verse^{TM}, Becton Dickinson, NJ, USA). The distribution of cells in subG_1 ("apoptotic cells"), G_0/G_1, S and G_2/M peaks were quantified by histogram analysis using BD FACSuite software (Becton Dickinson, NJ, USA). The experiments were repeated three times. The differences between control and treated cells were analyzed by one-way analysis of variance (ANOVA) using Microsoft Excel 2010. Signification was marked with asterisk ($p < 0.05$).

2.12. SDS–polyacrylamide gel electrophoresis and immunoblotting

CEM cells were treated for 24 h with tested compounds. The procedure was done as described earlier [18]. The antibody against caspase-7 recognized the zymogen and fragment. The experiments were repeated three times. The protein expression in treated cells was compared with the untreated controls.

2.13. Activities of caspase 3/7

After 24 h, treated CEM cells were analyzed for activity of caspases 3/7 using a procedure published earlier [18]. The experiments were repeated three times in triplicates. The

differences between control and treated cells were analyzed by one-way analysis of variance (ANOVA) using Microsoft Excel 2010. Signification was marked with asterisk ($p < 0.05$).

3. Results and Discussion

3.1. Chemistry

The amide **6** (Scheme 1) was prepared by a series of reactions, starting from betulinic acid (**1**), protected at the C(3)-OH by acetate formation, yielding **2** [11]. Subsequently, the C(17)-COOH group of **2** was converted to the corresponding acyl chloride [11], which was used directly in the synthesis of **3**, using mono-Boc-protected cystamine under the presence of EDIPA as a base in dichloromethane. When the acetyl protecting group was removed from **3** to prepare the required amide **4**, a side reaction was observed, resulting in obtaining a mixture of the products **4** and **5** (Scheme 1), which were separated by column chromatography, finally yielding 56 % of **4** and 39 % of **5**. Partial substitution of the Boc-protecting group in **4** by another molecule appeared, resulting in the dimeric by-product **5**. Protecting Boc group was removed from the purified **4** by a solution of hydrogen chloride in 1,4-dioxane [11], yielding the amide **6**.

The amide **9** (Scheme 2) was also prepared from betulinic acid (**1**), which was converted to its hemisuccinate at the C(3)-OH center (**7**), using succinic anhydride as a reagent and DMAP as a reaction promotor, in dry pyridine [10]. Because the C(17)-COOH in **7** is much less reactive than the free carboxyl group in hemisuccinate group, no protection of the C(17)-COOH group was necessary before the subsequent amide bond formation at the hemisuccinate carboxyl group to get **8**. This amide bond was made by reacting **7** with mono-Boc-protected cystamine in dry pyridine, using T3P as reaction promotor. Finally, the Boc-protecting group in **8** was removed by a solution of hydrogen chloride in 1,4-dioxane [11], yielding the required amide **9**.

3.2. Biological activity

The amides **6** and **9**, the dimeric product **5** and two intermediates (**2** and **7**) were subjected to the cytotoxicity screening tests *in vitro* for 72 h. The compounds were tested in human T-lymphoblastic leukemia cell line (CEM), breast carcinoma cell line (MCF7), cervical carcinoma cell line (HeLa) and malignant melanoma cell line (G-361). Normal human fibroblasts (BJ) were used for comparison of the toxicity. The dimeric product **5** displayed cytotoxicity towards BJ but did no activity in either of the cancer cell lines. A difference was found between treating the cells with **6** and **9** for 72 h: while the amide **6** was not toxic towards normal human fibroblasts BJ, the amide **9** showed comparable toxicity in MCF7 and in BJ. Neither **6** nor **9** were cytotoxic in HeLa and G-361. The amide **6** showed selectivity against CEM cancer line (IC_{50} = 3.0 ± 0.7 μM; TI = 20; 72 h). In turn, only the amide **9** showed antimicrobial activity against *Streptococcus mutans* (MIC 3.125 μM; MBC 3.125 μM), and *Bacillus cereus* (MIC 25 μM; MBC 25 μM). The intermediate compound **2** was found to be cytotoxic on G361 cancer cell line (IC_{50} = 7.5 ± 0.8 μM; TI = 10; 72 h). The intermediate compound **7** displayed no cytotoxicity at all, however, its antimicrobial activity against *Actinomyces odontolycus* and *Clostridium perfrigens* (MIC 12.5 μM; MBC 12.5 μM) demonstrated its potential and selectivity. The cytotoxicity data of **2**, **5**, **6**, **7** and **9** and the antimicrobial activity of **7** and **9** are summarized in Table 1. The cytotoxicity and antimicrobial screening methods were described earlier [10].

We have investigated whether **6** and **9** influence the cell cycle and cause apoptosis in human acute lymphoblastic leukemia cells CEM. Cells were treated with 1, 5 and 15 μM of **6** and **9** for 24 h. Then the cells were harvested, stained and analyzed by flow cytometry, western blotting or caspase activation assay. Flow cytometric analysis showed an increase in the $subG_1$ phase of the cell cycle (apoptotic cells) in CEM cell line after treatment with the amide

9. In contrast, the amide **6** did not enhance the number of subG$_1$ cells, but slightly induced the proportion of cells in G$_0$/G$_1$ and G$_2$/M phases with decrease of S-phase cells. In these cells, treatment with the amide **9** resulted in increasing the proportion of cells in the G$_0$/G$_1$ phase and decreased their proportion in the concomitant phases, S and G$_2$/M (Fig. 1A). Histograms with the distributions of CEM cells in the G$_0$/G$_1$, S, and G$_2$/M cell cycle phases (Fig. 1A), and the subG$_1$ fraction of cells are shown in Fig. 1B, and were measured by flow cytometric analysis, after 24 h treatment with the amides **6** and **9** relative to untreated control.

Apoptotic markers were detected by western blotting to examine the antiproliferative activities of tested amides **6** and **9**. Caspase cascade activation initiates apoptosis [19]. Caspase-3 and caspase-7 as executioner proteases cleave poly-(ADP-ribose) polymerase (PARP) and cause subsequent DNA degradation and apoptotic death [20]. The western blots showed degradation of caspase-7 into cleaved fragments after treatments with the amides **6** and **9** in CEM cells in a dose-dependent manner (Fig. 2). A final step of caspase activation, PARP cleavage, was also observed in human lymphoblastic leukemia cells treated by the amides **6** and **9** (Fig. 2). No changes in the expressions of anti-apoptotic proteins (Bcl-2 and Mcl-1) and p53 were observed.

These data were supplemented by estimating the caspase-3/7 activity in CEM treated cells using the fluorogenic substrate Ac-DEVD-AMC and/or caspase-3/7 inhibitor Ac-DEVD-CHO. Compound **6** increased activity of caspase-3/7 after the treatment with 5 μM up to eleven fold; compound **9** induced increase in the effector caspases up to eight fold compared to the untreated control (Fig. 3). These results show that the amides **6** and **9** can induce apoptosis by caspase-3/7 activation in CEM cells.

3.3. *In silico* calculation of physico-chemical and ADME parameters

To support experimental data determined for the target compounds **5**, **6** and **9**, and for the intermediates **2** and **7**, their physico-chemical and ADME parameters have been *in silico* calculated using ACD/iLabs software and databases [21]. The calculated physico-chemical parameters were compared with the Lipinski [22] rule of five and with the Ghose [23] rule. The rules describe molecular properties important for a small molecule drug pharmacokinetics in the human body, including their absorption, distribution, metabolism and excretion (known as ADME parameters). However, the rules do not predict displaying of the pharmacological activity. We described in details those rules and their importance in designing pharmacologically prospective structures recently [18]. The target compounds **5**, **6** and **9** and the intermediates **2** and **7** do not correspond to the whole range given for any of the Lipinski [22] and Ghose [23] rules, except of several parameters (Table 2). Despite those facts, the compounds **2**, **6** and **9** display cytotoxicity (Table 1). Exceptions are already known, when pharmacologically active compounds do not correspond to all rules, and we have observed such result as well [10,18,24]. More details on this topic can be found in the Supplementary material.

4. Conclusion

To conclude, we can state that the planned synthetic procedure corresponded well with the technical procedure of the synthesis realized. The only unexpected product was the dimer **5**, which appeared due to a partial substitution of the Boc-protecting group in **4** by another molecule, and which structure was proven by the analytical data. However, this compound (**5**) was cytotoxic in the normal fibroblasts, but displayed no effect in the tumor cell lines. The compound **6** showed selective cytotoxicity against CEM cell line (IC_{50} 3.0 ± 0.7 μM, TI = 20), while the compound **9** was less selective. The intermediate compound **2** was successfully cytotoxic on the G361 cancer cell line (IC_{50} 7.5 ± 0.8 μM, TI = 10). Both compounds (**6** and

9) can induce apoptosis by caspase-3/7 activation in CEM cells, and they enhance the effect of betulinic acid (**1**) due to their better bioavailability in comparison with it. In addition, the amide **9** showed antimicrobial activity against *Streptococcus mutans* (MIC 3.125 μM; MBC 3.125 μM), and it is also active against *Bacillus cereus* (MIC 25 μM; MBC 25 μM). Therefore, a synthesis of the target molecules **6** and **9** resulted in compounds displaying either cytotoxicity or antimicrobial activity with a clear selectivity of their effects, and with enhancing the corresponding effects of their parent components. Finally, the intermediate compound **7** displayed antimicrobial activity against *Actinomyces odontolycus* and *Clostridium perfrigens* (MIC 12.5 μM; MBC 12.5 μM).

Supporting Information Summary

More details on physico-chemical and ADME parameters are given, including *in silico* calculated activity values of the compounds **6** and **9** on central nervous system that are shown in Figs. S1 and S2.

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[Scheme and Figure Captions]

Scheme 1

Scheme 1. Synthetic procedure for **6**: i: Acetic anhydride, EDIPA, DMAP, THF; ii: oxalyl chloride, dichloromethane, then cystamine, EDIPA, dichloromethane; iii: $LiOH•H_2O$, methanol; iv: HCl (g) in 1,4-dioxane, additional 1,4-dioxane.

Scheme 2

Scheme 2. Synthetic procedure for **9**: i: Succinic anhydride, DMAP, pyridine; (ii) cystamine, T3P, pyridine; (iii) HCl in 1,4-dioxane, additional 1,4-dioxane.

Figure 1

Figure 1. Histograms with the distributions of CEM cells.

Legend: Histograms with the distributions of CEM cells in the G_0/G_1, S, and G_2/M cell cycle phases (**A**), and the $subG_1$ fraction of cells (**B**) measured by flow cytometric analysis, after 24 h treatment with **6** and **9** relative to untreated control. Data indicate the percentages (%) of the number of cells in respective phases. Experiments were repeated three times in triplicates. Error bars are omitted for clarity. (**A**) Analysis of variance (one-way ANOVA) between control and treated cells failed due to high biological variability in replicates. (**B**) Differences between control and treated cells with **9** among $subG_1$ phase were significant ($p < 0.05$) *.

Figure 2

Figure 2. Western blot analysis.

Legend: Western blot analysis of apoptotic proteins (PARP, zymogen and fragment of caspase-7) in CEM cells treated with amides. The expression of proteins in cells treated with

1, 5 and 15 μM by compounds **6** and **9** for 24 h were compared with the protein expression of untreated control cells (0+ means control with DMSO). The expression of β-actin was used as a protein loading control.

Figure 3. Activity of caspase-3/7 in CEM cells.

Legend: Activity of caspase-3/7 in CEM cells treated with 1, 5 and 15 μM of amides **6** and **9** compared with untreated control cells for 24 h. The data indicate the relative increase in caspase-3/7 activity. Experiments were repeated three times in triplicates. * Differences between control and treated cells were significant ($p < 0.05$).

Table 1. Cytotoxicity and antimicrobial activity of **2**, **5**, **6**, **7** and **9**

Compound	MW	Cytotoxicity (IC_{50} [μM]), 72 h				
		CEM	MCF7	HeLa	G-361	BJ
2	498.75	18.9 ± 0.8	16.9 ± 1.3	14.6 ± 1.8	7.5 ± 0.8 [a]	> 50
5	1029.67	> 50	> 50	> 50	> 50	13.4 ± 4.0
6	590.97	3.0 ± 0.7 [b]	46.0 ± 5.2	> 50	> 50	> 50
7 [c]	556.78	> 50	> 50	>50	>50	> 50
9 [d]	691.04	4.0 ± 1.9	16.8 ± 0.3	> 50	> 50	28.0 ± 4.5

[a] therapeutic index TI = 10; [b] therapeutic index TI = 20; [c] antimicrobial activity of **7**: *Actinomyces odontolycus* (MIC 12.5 μM; MBC 12.5 μM), *Clostridium perfrigens* (MIC 12.5 μM; MBC 12.5 μM); [d] antimicrobial activity of **9**: *Streptococcus mutans* (MIC 3.125 μM; MBC 3.125 μM), *Bacillus cereus* (MIC 25 μM; MBC 25 μM).

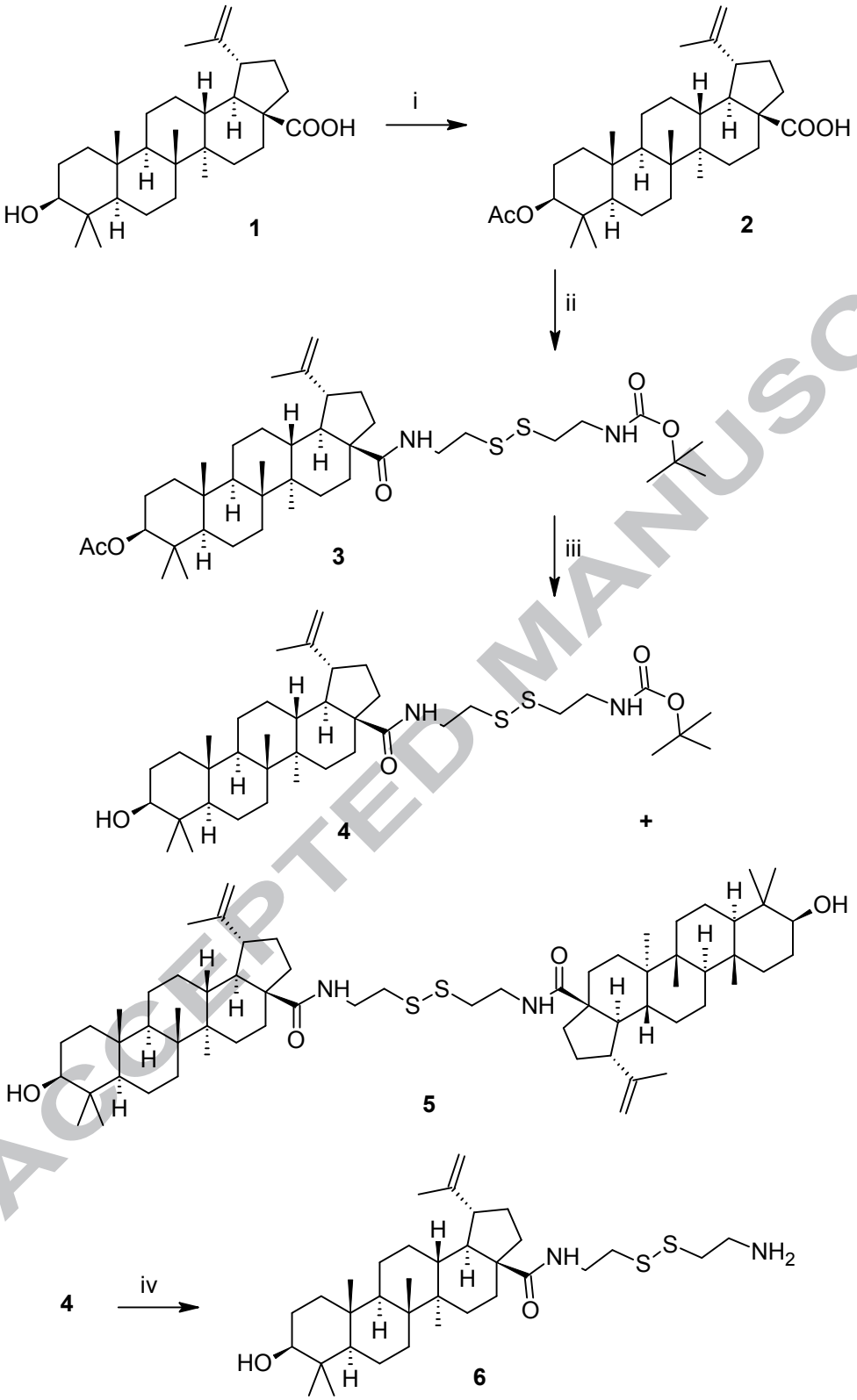
Table 2. Physico-chemical and ADME parameters of the compounds **2**, **5**, **6**, **7** and **9** calculated *in silico* using the ACD/iLabs software [21]

Compd. or ref. No.	MW	Physico-chemical and ADME parameters [a]									
		log *P*	log *D* (pH 7.4)	log *S* (pH 7.4)	bioav. [%]	log *PS* * $f_{u,\ brain}$	log *PS*	log *PB*	log *BB*	PPB [%]	H_{acc} / H_{don} / n.m.b.
2	498.74	7.45	4.96	-4.31	< 30	-5.2	-3.4	-0.55	-0.0	99.56	4/1/4
5	1029.65	14.32	14.32	-10.1	30-70	-10.2	-7.9	-2.00	-2.0	100.00	6/4/11
6	590.97	6.66	4.80	-4.83	30-70	-5.0	-2.8	-0.00	-0.0	99.74	4/4/8
7	556.77	6.67	1.80	-2.52	< 30	-5.0	-3.2	-0.59	-0.0	99.59	6/2/7
9	691.04	7.10	4.60	-8.26	< 30	-5.5	-3.3	-0.60	-0.0	99.84	7/4/13

[a] log *P* – partition coefficient; log *D* – distribution coefficient; log *S* – predicted aqueous solubility; bioav. = bioavailability – the degree of availability of a chemical by the target tissue; log *PS* * $f_{u,\ brain}$ – the brain/plasma equilibration rate, the parameter that is a mathematical modeling parameter based on time required for reaching brain equilibrium; log *PS* – logarithm of the permeability-surface area coefficient; log *PB* – the extent of brain penetration parameter; log *BB* – a hybrid parameter determined by permeability, plasma and brain tissue binding, and active transport mechanism; PPB – plasma protein binding; H_{acc} / H_{don} / n.m.b. = number of H-bond acceptors / number of H-bond donors / number of

movable bonds; limits given in literature [22,23] for several physico-chemical parameters: MW max. 500 [22,23]; log *P* max. 5.0 [22]; max. 5.6 [23]; n.m.b. max. 10 / max. 5 / - [22].

Scheme 1



Scheme 2

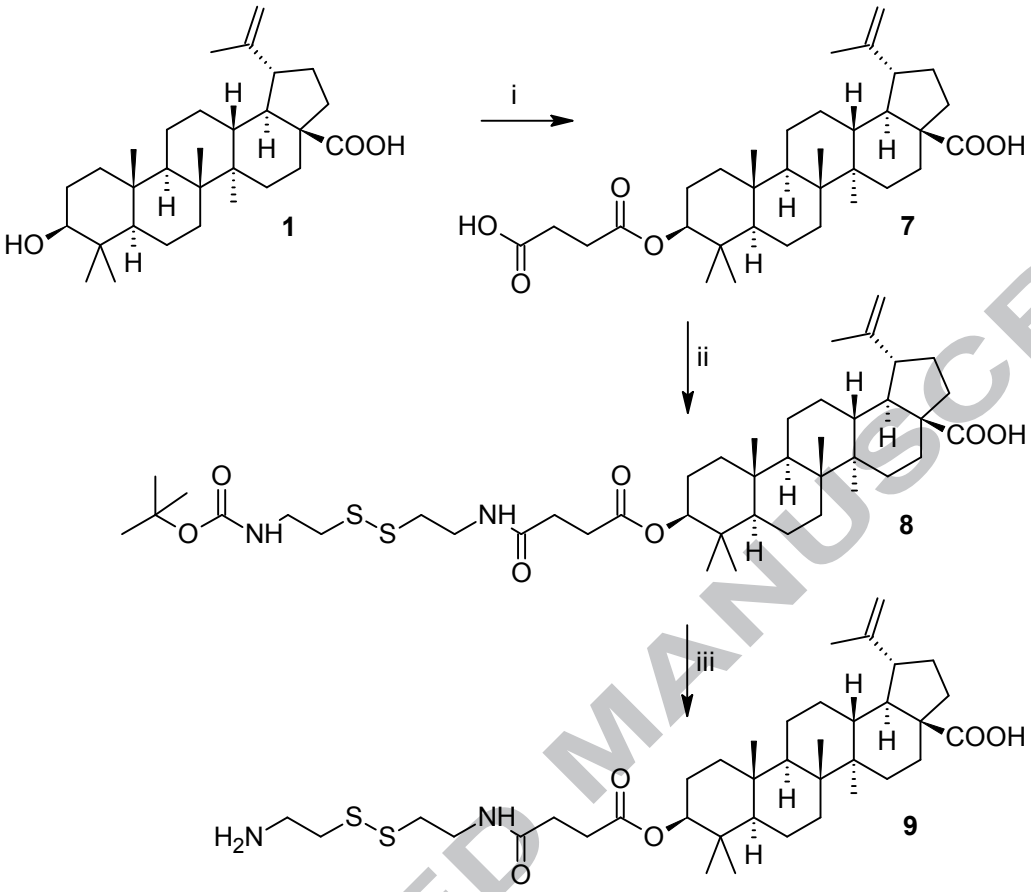
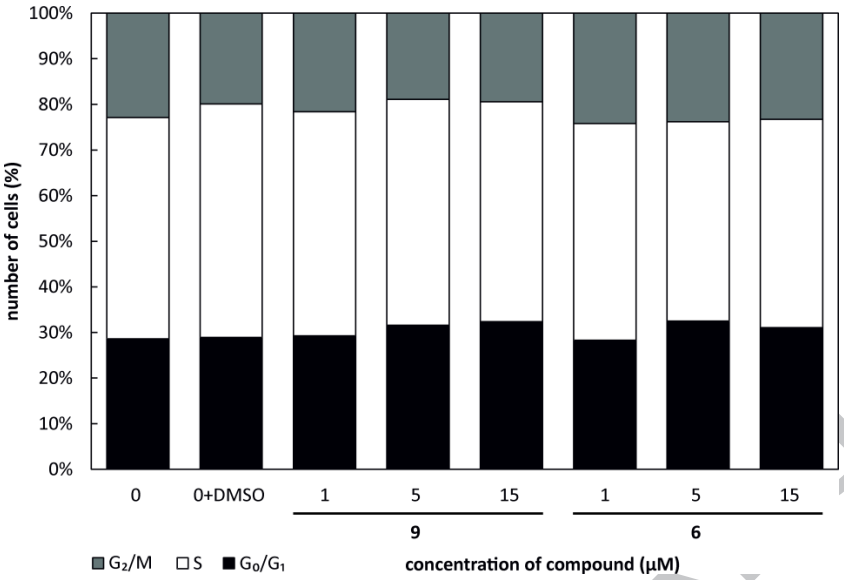


Figure 1

A



B

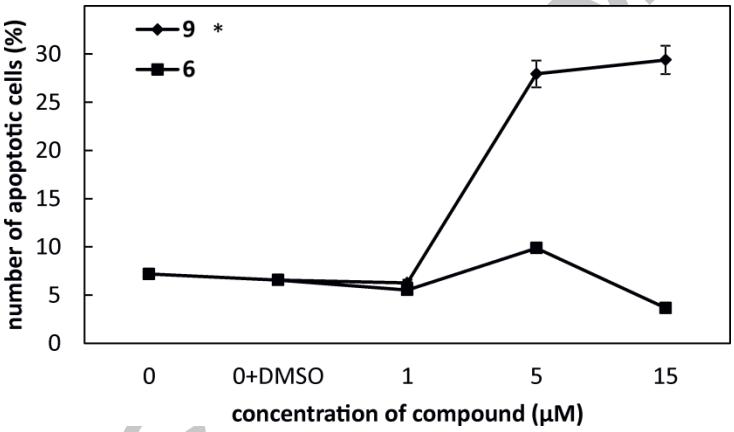


Figure 2

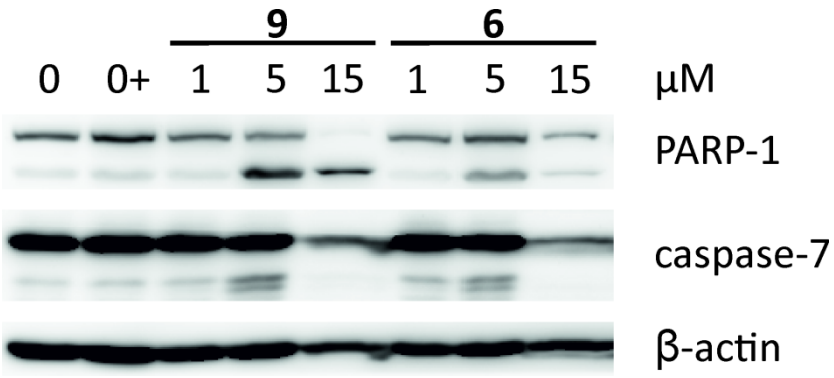
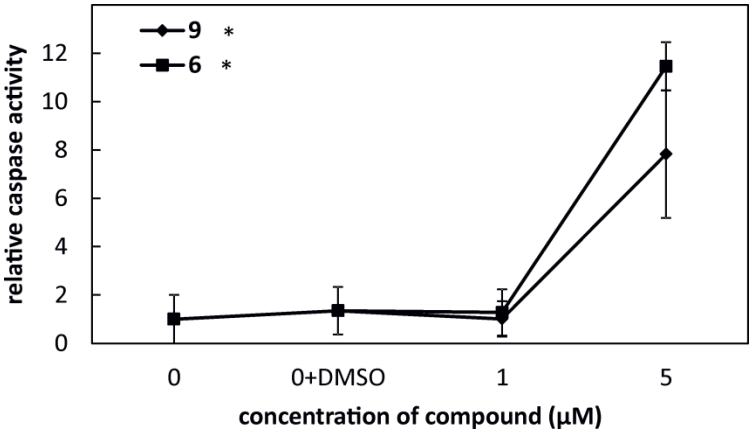
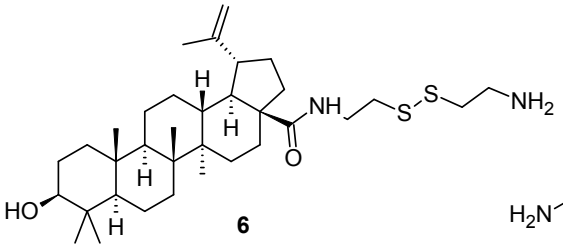


Figure 3

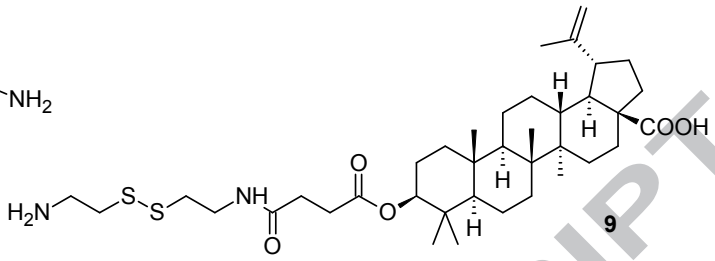


Graphical abstract



6

CEM: IC_{50} 3.0 ± 0.7 μM; TI = 20



9

CEM: IC_{50} 4.0 ± 1.9 μM

Highlights

Enhancing effect of cystamine in its amides with betulinic acid as antimicrobial and antitumor agent *in vitro*

Uladzimir Bildziukevich, Lucie Rárová, Lucie Janovská, David Šaman and Zdeněk Wimmer

- Amides of cystamine with betulinic acid synthesized for biological investigation;
- Antimicrobial activity, cytotoxicity and cell apoptosis were investigated;
- Comparison of substitution at the C(3)-OH and C(17)-COOH on cytotoxicity studied;
- Cytotoxicity of the target compounds on CEM found with TI = 20, and cell apoptosis documented;
- Antimicrobial activity found high on *Streptococcus mutans*