DOI 10.1007/s10600-018-2565-y
Chemistry of Natural Compounds, Vol. 54, No. 6, November, 2018

SYNTHESIS AND CYTOTOXICITY OF CONJUGATES OF BETULINIC ACID AND F-CONTAINING 2-ACYLCYCLOALKANE-1,3-DIONES

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Conjugates of betulinic acid with 2-(fluoroacyl)cycloalkane-1,3-dione motifs were synthesized by reacting 3-chloro-2-(fluoroacyl)-2-cycloalkene-1-ones with an equivalent amount of betulinic acid aminoalkylamide hydrochlorides in the presence of Et_3N in $CHCl_3$. Reduction of betulonic acid conjugates with 2-trifluoroacetylcyclopentane-1,3-dione by $NaBH_4$ in THF produced betulinic acid derivatives containing 2-(1-hydroxy-2,2,2-trifluoroethyl)cyclopentane-1,3-dione. The cytotoxicity of the synthesized compounds was assessed against glioblastoma multiforme (U-87 MG) and breast cancer cell lines (MCF7).

Keywords: betulinic acid, conjugates, 2-(fluoroacyl)cycloalkane-1,3-diones, diamines, cytotoxicity.

Chemical compounds produced by living organisms such as bacteria, fungi, higher plants, and animals play important roles in drug discovery [1]. Conjugation of natural and synthetic compounds is one of the most fruitful approaches to designing new biologically active structures [2]. Pentacyclic triterpenoids are a promising class of natural products for this strategy [3, 4]. Their potential for generating antitumor, anti-inflammatory, hepatoprotective, anti-HIV, and other drugs stems from broad profiles of biological activity, availability, and renewable raw-material sources [5–7]. Lupane-type triterpenoids are highly interesting because of the high cytotoxicity of betulinic acid and its anti-HIV activity [8]. Cyclic β-triketones are common in nature; exhibit various types of biological activity [9], e.g., antitumor [10]; and form an interesting class of polyfunctional compounds. 2-Acylcycloalkane-1,3-diones are widely used to synthesize various classes of bioactive compounds [11, 12]. Currently, methods for synthesizing F-containing compounds of interest as potential drugs and new plant-protection agents are rapidly developing [13, 14]. Previously, efficient synthetic methods for 2-(fluorobenzoyl)cyclohexane-1,3-diones and 2-perfluoroalkanoylcycloalkane-1,3-diones were developed by us and made such compounds accessible for further research on their reactivity [15]. The goals of the present work were to synthesize new betulinic-acid conjugates with F-containing 2-acylcycloalkane-1,3-diones using a diamine linker and to study their cytotoxicity.

Conjugates of betulinic acid (**1**) were synthesized using betulonic acid and 2-(fluoroacyl)cycloalkane-1,3-diones as starting materials. Treatment of **1** with an excess of $(COCl)_2$ synthesized its acid chloride (**2**) [16] that was reacted with polyamines, e.g., putrescine or 2,2′-(ethylenedioxy)bis(ethylamine). Hydrochlorides of betulonic-acid aminoalkylamide derivatives **3a** and **3b** were prepared by our previously developed method [17] in yields up to 84% by treating acid chloride **2** with a four-fold excess of diamine in $CHCl_3$ in the presence of Et_3N followed by work up with HCl solution (5%).

Resulting betulonic-acid derivatives **3a** and **3b** were reduced by $NaBH_4$ in THF to give **4a** and **4b** in 90–92% yield. 3-Chloro-2-(fluoroacyl)cycloalken-1-ones **6a–d** were prepared by treating the corresponding F-containing cyclic β-triketones **5a**–**d** with an excess of $(COCl)_2$ and were used to acylate the hydrochlorides of **4a** and **4b** in $CHCl_3$ in the presence of Et_3N to synthesize betulinic-acid conjugates **7a**–**f** in 50–82% yield. Reaction of the hydrochlorides of the betulonic-acid aminoalkylamide derivatives (**3a** and **3b**) with 2-trifluoroacetyl-3-chloro-2-cyclopenten-1-one (**6c**) and reduction by $NaBH_4$ of the obtained betulonic-acid conjugates **8a** and **8b** synthesized betulinic-acid conjugates **9a** and **9b** in 94–95% yield. Both the 3-carbonyl of the lupane skeleton and the 2-trifluoroacetyl carbonyl were reduced to hydroxyls.

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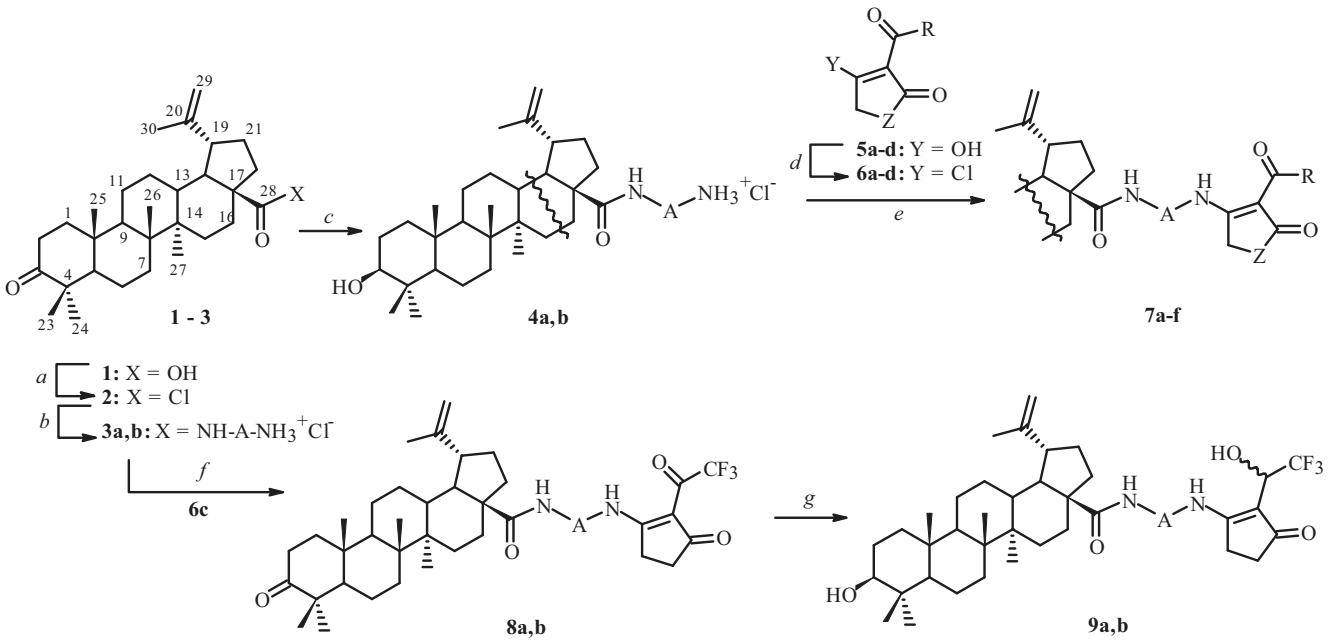
 0009-3130/18/5406-1100

TABLE 1. Cytotoxic Activity of **4a**, **4b**, **7e**, **9a**, and **9b** (IC_{50} ± SEM, μM)

Compound	hTERT	MCF7	U-87 MG	Compound	hTERT	MCF7	U-87 MG
4a	< 10	< 10	< 10	**7e**	35.64 ± 5.01	50.51 ± 7.62	24.47 ± 8.84
4b	< 10	< 10	< 10	**9a**	25.06 ± 1.18	81.93 ± 9.37	19.6 ± 1.87
Doxorubicin	1.82 ± 0.28	2.82 ± 0.28	2.04 ± 0.14	**9b**	81.01 ± 8.33	37.87 ± 6.33	79.43 ± 2.72



3, 4, 8, 9: A = $(CH_2)_4$ (**a**); $(CH_2)_2O(CH_2)_2O(CH_2)_2$ (**b**)
5, 6: R = $3FC_6H_4$, Z = $(CH_2)_2$ (**a**); R = $4FC_6H_4$, Z = $(CH_2)_2$ (**b**); R = CF_3; Z = CH_2 (**c**); R = CF_3; Z = $CH_2C(CH_3)_2$ (**d**)
7: R = $3FC_6H_4$, Z = $(CH_2)_2$, A = $(CH_2)_4$ (**a**); R = $3FC_6H_4$, Z = $(CH_2)_2$, A = $(CH_2)_2O(CH_2)_2O(CH_2)_2$ (**b**)
R = $4FC_6H_4$, Z = $(CH_2)_2$, A = $(CH_2)_4$ (**c**); R = CF_3, Z = CH_2, A = $(CH_2)_4$ (**d**)
R = CF_3; Z = CH_2; A = $(CH_2)_2O(CH_2)_2O(CH_2)_2$ (**e**); R = CF_3, Z = $CH_2C(CH_3)_2$, A = $(CH_2)_4$ (**f**)

a. $(COCl)_2$, CH_2Cl_2; *b*. NH_2-A-NH_2 (4 eq), $CHCl_3$, Et_3N, reflux, 6 h, then 5% HCl; *c*. $NaBH_4$, THF, 4 h; *d*. $(COCl)_2$; *e*. 2-(fluoroacyl)-3-chloro-2-cycloalken-1-one (**6a**–**d**) (1 eq), Et_3N, $CHCl_3$, 0.5 h; *f*. 2-(trifluoroacetyl)-3-chloro-2-cyclopenten-1-one (**6c**) (1 eq), Et_3N, $CHCl_3$, 0.5 h; *g*. $NaBH_4$, THF, 16 h

The structures of **4a**, **4b**, **7a**–**f**, **9a**, and **9b** were established and confirmed using IR, PMR, ^{13}C NMR, and ^{19}F NMR spectroscopy and elemental analysis. PMR spectra of the hydrochlorides of betulinic-acid aminoalkylamide derivatives **4a** and **4b** showed resonances for NH_3^+ at δ 8.08 and 8.24 ppm, respectively, and for the amide proton as a broad singlet at δ 7.68 (**4a**) or a triplet with J = 5.8 Hz at δ 7.70 (**4b**). PMR spectra of betulinic-acid conjugates **7a**–**f** were characterized by amide proton resonances as triplets at δ 5.74–6.28 ppm (J = 5.9–6.2 Hz) (**7b**, **c**, **e**, **f**) or in several instances as broad singlets at δ 5.77–5.79 ppm (**7a** and **7d**) and resonances for NH protons in H-bonds with the carbonyl of the side fluoroacyl group as broad singlets at δ 10.09–11.66 ppm (**7a** and **7c**–**f**) or a triplet at δ 11.56 ppm with J = 5.2 Hz (**7b**). PMR spectra of conjugates **9a** and **9b** were characterized by amide proton resonances as broad singlets at δ 5.84–6.12 ppm and resonances of NH protons in H-bonds with hydroxyls as broad singlets at 6.89–7.08 ppm. Thus, reduction of the trifluoroacetyl carbonyl to an alcohol produced a strong-field shift of the resonance for the NH bonded to the cyclopentane ring as compared with the analogous NH proton in conjugates **7a**–**f**.

Cytotoxicity *in vitro* of the hydrochlorides of betulinic-acid aminoalkylamide derivatives **4a** and **4b**, betulinic-acid conjugates **7a**–**f**, and **9a** and **9b** was assayed using U-87 MG glioblastoma multiforme, MCF7 breast cancer, and hTERT immortalized human fibroblast cell lines and the MTT assay [18]. Table 1 shows that synthesized derivatives **7e** and **9a** exhibited moderate cytotoxicity against U-87 MG cell line; conjugate **9b**, against MCF7 cell line. Compounds **7a**–**d** and **7f** had no cytotoxic activity. In general, the cytotoxicity of the synthesized compounds (**7a**–**f** and **9a** and **9b**) was less than the starting hydrochlorides of aminoalkyl derivatives **4a** and **4b**.

EXPERIMENTAL

PMR, ^{19}F NMR, and ^{13}C NMR spectra were taken in $CDCl_3$ or DMSO-d_6 solutions at 293 K on a Bruker-Biospin Avance 500 spectrometer at operating frequencies 500, 470, and 125 MHz, respectively, using a 5-mm probe (QNP) with a *Z*-gradient. Residual solvent resonances were used as internal standards for PMR [δ_H 7.26 ppm, $CD(H)Cl_3$; 2.50 ppm, DMSO-d_6] and ^{13}C NMR spectra (δ_C 77.16 ppm, $CDCl_3$; 39.52 ppm, DMSO-d_6). An external standard of α,α,α-trifluorotoluene (δ 63 ppm, ^{19}F) was used. IR spectra were taken from KBr pellets on a PerkinElmer Spectrum 100 FT-IR spectrometer. Melting points were determined on a Boetius apparatus. Elemental analysis was performed on a Eurovector EA 3000 CHNS-O analyzer. The course of reactions and purity of products were monitored by TLC on Silufol UV-254 plates (EtOAc–petroleum ether). Column chromatography used silica gel (70–230 mesh) with elution by EtOAc–petroleum ether. Elemental analyses of all compounds agreed with those calculated.

Hydrochlorides of betulonic-acid aminoalkylamide derivatives **3a** and **3b** [17], 2-(fluorobenzoyl)cyclohexane-1,3-diones **5a** and **5b** [19], 2-trifluoroacetylcycloalkane-1,3-diones **5c** and **5d** [20, 21], and 2-(fluoroacyl)-3-chloro-2-cycloalken-1-ones **6a**–**d** [21–23] were prepared using published methods. The physicochemical characteristics of **3a**, **5a**–**d**, and **6a**–**d** corresponded to those in the literature.

2-{2-[2-(3-Oxolup-20(29)-en-28-amido)ethoxy]ethoxy}ethane-1-ammonium Chloride (3b). $C_{36}H_{61}ClN_2O_4$, yield 62%, mp 127–132°C. IR spectrum (KBr, ν, cm^{-1}): 1640, 1705. ^1H NMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.80–2.16 (21H, m, CH, CH_2), 0.90 (3H, s, CH_3), 0.95 (3H, s, CH_3), 0.96 (3H, s, CH_3), 1.00 (3H, s, CH_3), 1.05 (3H, s, CH_3), 1.66 (3H, s, CH_3), 2.35–2.55 (3H, m, CH, CH_2), 2.94–3.89 (13H, m, H-19, $6CH_2$), 4.57 (1H, br.s, H-29), 4.71 (1H, br.s, H-29), 6.62 (1H, t, J = 5.8, NH), 8.25 (3H, s, NH_3^+). ^{13}C NMR spectrum (125 MHz, DMSO-d_6, δ, ppm): 14.7, 16.1, 16.2, 19.6, 19.8, 21.2, 21.6, 25.7, 26.8, 29.5, 31.0, 33.7, 33.9, 34.3, 37.1, 37.8, 38.5, 39.3, 39.8, 40.8, 42.6, 46.8, 47.4, 50.1, 50.2, 55.1, 55.8, 67.1, 70.3, 70.4, 70.5, 77.4, 109.6, 151.0, 176.9, 218.2.

Preparation of Betulinic-acid Aminoalkylamide Hydrochlorides (4a and 4b). A solution of **3a** or **3b** (1 mmol) in anhydrous THF (10 mL) was treated with $NaBH_4$ (4 mmol), stirred for 4 h, diluted with HCl solution (30 mL, 10%) cooled to 0°C, and evaporated (THF) at reduced pressure. Hydrochlorides of **4a** and **4b** were isolated as colorless powders by filtration and drying at reduced pressure.

4-{2-[3β-Hydroxylup-20(29)-en-28-amido]}butane-1-ammonium Chloride (4a). $C_{34}H_{59}ClN_2O_2$, yield 90%, mp 253–257°C (dec.). IR spectrum (KBr, ν, cm^{-1}): 1635. ^1H NMR spectrum (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 0.58–1.80 (27H, m, CH, CH_2), 0.63 (3H, s, CH_3), 0.75 (3H, s, CH_3), 0.83 (3H, s, CH_3), 0.85 (3H, s, CH_3), 0.89 (3H, s, CH_3), 1.61 (3H, s, CH_3), 2.14 (1H, d, J = 12.1), 2.52–2.58 (1H, m), 2.63–3.10 (5H, m), 4.52 (1H, br.s, H-29), 4.63 (1H, br.s, H-29), 7.68 (1H, br.s, NH), 8.08 (3H, br.s, NH_3^+). ^{13}C NMR spectrum (125 MHz, DMSO-d_6, δ, ppm): 14.3, 15.8, 15.9, 17.9, 19.0, 20.5, 24.3, 25.2, 26.2, 27.1, 28.1, 28.9, 30.3, 32.4, 33.9, 36.6, 36.7, 37.6, 37.7, 38.3, 38.4, 38.4, 40.2, 41.8, 46.1, 49.6, 50.1, 54.8, 54.9, 76.7, 109.1, 150.9, 175.5.

2-{2-[2-(3β-Hydroxylup-20(29)-en-28-amido)ethoxy]ethoxy}ethane-1-ammonium Chloride (4b). $C_{36}H_{63}ClN_2O_4$, yield 92%, mp 144–148°C. IR spectrum (KBr, ν, cm^{-1}): 1635. ^1H NMR spectrum (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 0.57–1.81 (23H, m, CH, CH_2), 0.64 (3H, s, CH_3), 0.75 (3H, s, CH_3), 0.83 (3H, s, CH_3), 0.85 (3H, s, CH_3), 0.89 (3H, s, CH_3), 1.61 (3H, s, CH_3), 2.13 (1H, d, J = 12.1), 2.50–2.56 (1H, m), 2.82–3.65 (13H, m), 4.52 (1H, br.s, H-29), 4.64 (1H, br.s, H-29), 7.70 (1H, t, J = 5.8, NH), 8.24 (3H, br.s, NH_3^+). ^{13}C NMR spectrum (125 MHz, DMSO-d_6, δ, ppm): 14.3, 15.8, 16.0, 18.0, 19.1, 20.6, 25.2, 27.2, 28.1, 28.9, 30.3, 32.4, 34.0, 36.7, 36.7, 37.6, 38.2, 38.3, 38.4, 38.5, 40.3, 41.9, 46.2, 49.6, 50.1, 54.9, 55.0, 66.6, 69.1, 69.4, 69.6, 76.8, 109.2, 150.9, 175.7.

Preparation of Betulinic-acid Conjugates 7a–f. A solution of 3-chloro-2-(fluoroacyl)-2-cycloalken-1-ones (**6a**–**d**, 1 mmol) in $CHCl_3$ (10 mL) was treated with a solution of the hydrochloride of betulinic-acid aminoalkylamide derivative **4a** or **4b** in $CHCl_3$ (10 mL) and dropwise with Et_3N (2 mmol), stirred for 30 min, and evaporated at reduced pressure. Conjugates **7a**–**f** were isolated as colorless powders in 50–82% yield by column chromatography over silica gel.

Betulinic Acid *N*-{4-[3-Oxo-2-(3-fluorobenzoyl)cyclohex-1-enylamino]butyl}amide (7a). $C_{47}H_{67}FN_2O_4$, yield 66%, mp 135–139°C. IR spectrum (KBr, ν, cm^{-1}): 1580, 1640. ^1H NMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.64–1.96 (27H, m, CH, CH_2), 0.72 (3H, s, CH_3), 0.78 (3H, s, CH_3), 0.90 (3H, s, CH_3), 0.94 (3H, s, CH_3), 0.95 (3H, s, CH_3), 1.66 (3H, s, CH_3), 2.04 (2H, qt, J = 6.2, CH_2), 2.36 (2H, t, J = 6.2, CH_2), 2.42 (1H, td, J = 12.6, 3.8), 2.67 (2H, t, J = 6.2, CH_2), 3.05–3.50 (6H, m, H-3, 19, $2CH_2$), 4.58 (1H, br.s, H-29), 4.72 (1H, br.s, H-29), 5.79 (1H, br.s, NH), 7.02–7.13 (2H, m, H_{arom}), 7.15–7.22 (1H, m, H_{arom}), 7.24–7.30 (1H, m, H_{arom}), 11.54 (1H, br.s, NH). ^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm, J/Hz): 14.8, 15.5, 16.3, 18.4, 19.6, 20.0, 21.0, 25.7, 26.6, 27.1, 27.4, 27.5, 28.1, 29.6, 29.8, 31.0, 33.8, 34.5, 37.3, 37.4, 37.9, 38.3,

38.6, 38.8, 38.9, 40.9, 42.6, 43.3, 46.9, 50.2, 50.7, 55.5, 55.8, 79.1, 108.2, 109.5, 114.4 (d, J = 22), 116.8 (d, J = 21), 123.2, 129.3 (d, J = 8), 145.2 (d, J = 7), 151.0, 162.4 (d, J = 245), 172.6. ^{19}F NMR spectrum (470 MHz, $CDCl_3$, δ, ppm): –113.75 – (–113.87) (m, F).

Betulinic Acid *N*-[2-(2-{2-[3-Oxo-2-(3-fluorobenzoyl)cyclohex-1-enylamino]ethoxy}ethoxy)ethyl]amide (7b). $C_{49}H_{71}FN_2O_6$, yield 60%, mp 110–115°C. IR spectrum (KBr, ν, cm^{-1}): 1580, 1640. ^{1}H NMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.65–1.96 (23H, m, CH, CH_2), 0.74 (3H, s, CH_3), 0.80 (3H, s, CH_3), 0.92 (3H, s, CH_3), 0.93 (3H, s, CH_3), 0.95 (3H, s, CH_3), 1.66 (3H, s, CH_3), 2.04 (2H, qt, J = 6.2, CH_2), 2.36 (2H, t, J = 6.4, CH_2), 2.38–2.52 (1H, m), 2.69 (2H, t, J = 6.2, CH_2), 3.10 (1H, td, J = 11.1, 3.7, H-19), 3.16 (1H, dd, J = 11.4, 4.8), 3.29–3.77 (12H, m, $6CH_2$), 4.57 (1H, br.s, H-29), 4.71 (1H, br.s, H-29), 6.28 (1H, t, J = 5.8, 1H, NH), 6.98–7.14 (2H, m H_{arom}), 7.14–7.20 (1H, m, H_{arom}), 7.25–7.31 (1H, m, H_{arom}), 11.56 (1H, t, J = 5.2, NH). ^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm, J/Hz): 14.8, 15.5, 16.2, 16.3, 18.5, 19.6, 20.0, 21.1, 25.7, 27.3, 27.5, 28.1, 29.5, 31.0, 33.6, 34.6, 37.3, 37.4, 37.8, 38.4, 38.8, 39.0, 39.1, 40.9, 42.6, 43.6, 46.9, 50.3, 50.7, 55.5, 55.7, 69.0, 70.2, 70.3, 70.9, 79.1, 108.5, 109.4, 114.4 (d, J = 22), 116.8 (d, J = 21), 123.1, 129.3 (d, J = 8.0), 145.3, 151.2, 162.4 (d, J = 245), 172.5, 176.4, 194.2, 196.7. ^{19}F NMR spectrum (470 MHz, $CDCl_3$, δ, ppm): –113.64 – (–113.82) (m, F).

Betulinic Acid *N*-{4-[3-Oxo-2-(4-fluorobenzoyl)cyclohex-1-enylamino]butyl}amide (7c). $C_{47}H_{67}FN_2O_4$, yield 82%, mp 151–155°C. IR spectrum (KBr, ν, cm^{-1}): 1580, 1640. ^{1}H NMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.65–1.96 (27H, m, CH, CH_2), 0.73 (3H, s, CH_3), 0.78 (3H, s, CH_3), 0.91 (3H, s, CH_3), 0.94 (3H, s, CH_3), 0.95 (3H, s, CH_3), 2.04 (2H, qt, J = 6.2, CH_2), 2.36 (2H, t, J = 6.4, CH_2), 2.42 (1H, td, J = 12.4, 3.5), 2.68 (2H, t, J = 6.3, CH_2), 3.07–3.50 (6H, m, H-3, 19, $2CH_2$), 4.58 (1H, br.s, H-29), 4.72 (1H, br.s, H-29), 5.74 (1H, t, J = 6.2, NH), 7.00 (2H, t, J = 8.6, H_{arom}), 7.46 (2H, t, J = 6.8, H_{arom}), 11.46 (1H, br.s, NH). ^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm, J/Hz): 14.8, 15.5, 16.3, 18.4, 19.6, 20.0, 21.1, 25.7, 26.6, 27.1, 27.5, 27.5, 28.1, 29.6, 31.0, 33.9, 34.5, 37.3, 37.5, 37.9, 38.3, 38.6, 38.8, 39.0, 40.9, 42.6, 43.2, 46.9, 50.3, 50.7, 55.5, 55.8, 79.1, 108.2, 109.6, 114.8 (d, J = 22), 130.1 (d, J = 9), 138.9, 151.0, 164.1 (d, J = 250), 172.3, 176.6, 194.3, 196.7. ^{19}F NMR spectrum (470 MHz, $CDCl_3$, δ, ppm): –110.16 – (–110.28) (m, F).

Betulinic Acid *N*-{4-[3-Oxo-2-(trifluoroacetyl)cyclopent-1-enylamino]butyl}amide (7d). $C_{41}H_{61}F_3N_2O_4$, yield 58%, mp 146–150°C. IR spectrum (KBr, ν, cm^{-1}): 1595, 1635. ^{1}H NMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.64–2.00 (27H, m, CH, CH_2), 0.73 (3H, s, CH_3), 0.78 (3H, s, CH_3), 0.88 (3H, s, CH_3), 0.94 (3H, s, CH_3), 0.95 (3H, s, CH_3), 1.66 (3H, s, CH_3), 2.40 (1H, td, J = 12.3, 3.6), 2.50–2.53 (2H, m, CH_2), 2.78–2.89 (2H, m, CH_2), 3.07–3.57 (6H, m, H-3, 19, $2CH_2$), 4.58 (1H, br.s, H-29), 4.71 (1H, br.s, H-29), 5.77 (1H, br.s, NH), 10.09 (1H, br.s, NH). ^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm, J/Hz): 14.8, 15.4, 16.2, 16.3, 18.4, 19.6, 21.1, 24.9, 25.7, 26.7, 27.4, 27.5, 28.1, 29.6, 31.0, 33.5, 33.9, 34.5, 37.3, 37.9, 38.1, 38.6, 38.8, 39.0, 40.9, 42.6, 44.3, 46.9, 50.2, 50.7, 55.5, 55.8, 79.1, 106.4, 109.6, 116.6 (d, J = 287), 150.9, 176.3 (d, J = 38), 176.8, 184.1, 196.2. ^{19}F NMR spectrum (470 MHz, $CDCl_3$, δ, ppm): –75.71 (s, CF_3).

Betulinic Acid *N*-[2-(2-{2-[3-Oxo-2-(trifluoroacetyl)cyclopent-1-enylamino]ethoxy}ethoxy)ethyl]amide (7e). $C_{43}H_{65}F_3N_2O_6$, yield 60%, mp 82–86°C. IR spectrum (KBr, ν, cm^{-1}): 1600, 1635. ^{1}H NMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.65–2.00 (23H, m, CH, CH_2) 0.74 (3H, s, CH_3), 0.80 (3H, s, CH_3), 0.92 (3H, s, CH_3), 0.95 (6H, s, $2CH_3$), 1.66 (3H, s, CH_3), 2.43 (1H, td, J = 12.2, 3.5), 2.50–2.53 (2H, m, CH_2), 2.80–2.83 (2H, m, CH_2), 3.11 (1H, td, J = 11.1, 4.0, H-19), 3.17 (1H, dd, J = 11.4, 4.8), 3.34–3.76 (12H, m), 4.57 (1H, br.s, H-29), 4.72 (1H, br.s, H-29), 6.17 (1H, t, J = 5.9, NH), 10.25 (1H, br.s, NH). ^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm, J/Hz): 14.8, 15.5, 16.2, 16.3, 18.4, 19.6, 21.1, 25.1, 25.7, 27.5, 28.1, 29.5, 31.0, 33.5, 33.7, 34.6, 37.3, 37.9, 38.4, 38.8, 39.0, 39.1, 40.9, 42.6, 44.6, 46.9, 50.2, 50.7, 55.5, 55.8, 68.8, 70.2, 70.3, 70.9, 79.1, 106.6, 109.4, 116.6 (d, J = 288), 151.2, 176.0 (d, J = 39), 176.5, 184.2, 196.3. ^{19}F NMR spectrum (470 MHz, $CDCl_3$, δ, ppm): –75.59 (s, CF_3).

Betulinic Acid *N*-{4-[5,5-Dimethyl-3-oxo-2-(trifluoroacetyl)-cyclohex-1-enylamino]butyl}amide (7f). $C_{44}H_{67}F_3N_2O_4$, yield 50%, mp 139–143°C. IR spectrum (KBr, ν, cm^{-1}): 1590, 1650. ^{1}H NMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.65–1.96 (27H, m, CH, CH_2), 0.74 (3H, s, CH_3), 0.79 (3H, s, CH_3), 0.89 (3H, s, CH_3), 0.95 (3H, s, CH_3), 0.96 (3H, s, CH_3), 1.10 (3H, s, CH_3), 1.10 (3H, s, CH_3), 1.67 (3H, s, CH_3), 2.33 (2H, s, CH_2), 2.35–2.46 (1H, m), 2.53 (2H, s, CH_2), 3.07–3.52 (6H, m, H-3, 19, $2CH_2$), 4.58 (1H, br.s, H-29), 4.72 (1H, s, H-29), 5.78 (1H, t, J = 6.1, NH), 11.66 (1H, br.s, NH). ^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm, J/Hz): 14.8, 15.5, 16.2, 16.3, 18.4, 19.6, 21.1, 25.7, 26.4, 27.4, 27.5, 28.1, 28.4, 28.5, 29.6, 31.0, 31.1, 33.9, 34.5, 37.3, 37.9, 38.2, 38.6, 38.8, 39.0, 40.8, 40.9, 42.6, 43.8, 46.9, 50.2, 50.7, 51.3, 55.5, 55.8, 79.1, 105.1, 109.6, 117.5 (d, J = 287), 150.9, 173.7, 176.8, 179.8 (d, J = 36), 192.6. ^{19}F NMR spectrum (470 MHz, $CDCl_3$, δ, ppm): –72.41 (s, CF_3).

Derivatives **8a** and **8b** were prepared as before [17]. The physicochemical characteristics of **8a** agreed with those in the literature.

Betulonic Acid *N*-{2-[2-(2-(3-Oxo-2-(trifluoroacetyl)cyclopent-1-enylamino)ethoxy)ethoxy]ethyl}amide (8b). $C_{43}H_{63}F_3N_2O_6$, yield 85%, mp 86–90°C. IR spectrum (KBr, ν, cm^{-1}): 1595, 1635, 1705. ^{1}H NMR spectrum (500 MHz,

$CDCl_3$, δ, ppm, J/Hz): 0.81–1.99 (21H, m, CH, CH_2), 0.90 (3H, s, CH_3), 0.95 (3H, s, CH_3), 0.96 (3H, s, CH_3), 1.00 (3H, s, CH_3), 1.04 (3H, s, CH_3), 1.66 (3H, s, CH_3), 2.35–2.52 (5H, m), 2.72–2.89 (2H, m, CH_2), 3.11 (1H, td, J = 11.0, 4.1, H-19), 3.32–3.80 (12H, m, $6CH_2$), 4.57 (1H, br.s, H-29), 4.71 (1H, br.s, H-29), 6.17 (1H, t, J = 5.7, NH), 10.25 (1H, br.s, NH). ^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm, J/Hz): 14.7, 16.0, 16.1, 19.6, 21.1, 21.6, 25.0, 25.7, 26.7, 29.5, 31.0, 33.5, 33.6, 33.8, 34.3, 37.0, 37.9, 38.4, 39.1, 39.7, 40.8, 42.6, 44.6, 46.9, 47.4, 50.1, 50.1, 55.1, 55.7, 68.8, 70.2, 70.3, 70.9, 77.4, 106.6, 109.5, 116.6 (q, J = 288), 151.1, 176.0 (q, J = 37), 176.4, 184.2, 196.3, 218.3. ^{19}F NMR spectrum (470 MHz, $CDCl_3$, δ, ppm): –75.69 (s, CF_3).

Preparation of Derivatives 9a and 9b. A solution of **8a** or **8b** (1 mmol) in THF (15 mL) was treated with $NaBH_4$ (4 mmol), stirred for 16 h at room temperature, and evaporated at reduced pressure. The residue was diluted with HCl solution (5%, 60 mL) cooled to 0°C. The aqueous fraction was extracted with $CHCl_3$ (3 × 20 mL). The combined organic fractions were dried over anhydrous Na_2SO_4 and filtered. The solvent was evaporated at reduced pressure to afford **9a** and **9b** as colorless powders.

Betulinic Acid *N*-{4-[3-Oxo-2-(1-hydroxy-2,2,2-trifluoroethyl)cyclopent-1-enylamino]butyl}amide (9a). $C_{41}H_{63}F_3N_2O_4$, yield 94%, mp 171–175°C. IR spectrum (KBr, ν, cm^{-1}): 1590, 1635. ^1H NMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.65–2.00 (27H, m, CH, CH_2), 0.74 (3H, s, CH_3), 0.80 (3H, s, CH_3), 0.90 (3H, s, CH_3), 0.95 (3H, s, CH_3), 0.96 (3H, s, CH_3), 1.67 (3H, s, CH_3), 2.35–2.45 (3H, m), 2.60–2.62 (2H, m, CH_2), 3.08 (1H, td, J = 11.6, 5.8, H-19), 3.13–3.41 (5H, m, H-3, $2CH_2$), 4.58 (1H, br.s, H-29), 4.72 (1H, br.s, H-29), 4.84–5.05 (1H, m, $CF_3C\underline{H}OH$), 5.84 (1H, br.s, NH), 6.89 (1H, br.s, NH). ^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm, J/Hz): 14.8, 15.5, 16.2, 16.3, 18.4, 19.6, 21.0, 25.0, 25.7, 26.8, 27.5, 27.8, 28.1, 29.6, 31.0, 32.9, 33.9, 34.5, 37.3, 37.9, 38.5, 38.6, 38.9, 39.0, 40.9, 42.6, 44.2, 46.8, 50.2, 50.7, 55.5, 55.8, 66.5 (q, J = 34), 79.1, 104.0, 109.6, 125.7 (d, J = 283), 150.9, 176.3, 177.1, 201.8. ^{19}F NMR spectrum (470 MHz, $CDCl_3$, δ, ppm): –75.02 – (–75.20) (m, CF_3).

Betulinic Acid *N*-[2-(2-{2-[3-Oxo-2-(1-hydroxy-2,2,2-trifluoroethyl)cyclopent-1-enylamino]ethoxy} ethoxy)ethyl]amide (9b). $C_{43}H_{67}F_3N_2O_6$, yield 95%, mp 118–121°C. IR spectrum (KBr, ν, cm^{-1}): 1590, 1635. ^1H NMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.65–2.00 (23H, m, CH, CH_2), 0.74 (3H, s, CH_3), 0.80 (3H, s, CH_3), 0.90 (3H, s, CH_3), 0.95 (6H, s, $2CH_3$), 2.38 (1H, td, J = 13.3, 3.3), 2.47 (2H, br.s, CH_2), 2.65 (2H, br.s, CH_2), 3.07 (1H, td, J = 11.7, 4.3, H-19), 3.17 (1H, dd, J = 11.2, 4.8, H-3), 3.29–3.70 (12H, m), 4.58 (1H, br.s, H-29), 4.72 (1H, br.s, H-29), 4.87–5.14 (1H, m, $CF_3C\underline{H}OH$), 6.12 (1H, br.s, NH), 7.08 (1H, br.s, NH). ^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm, J/Hz): 14.8, 15.4, 15.5, 16.2, 16.3, 18.4, 19.6, 21.0, 25.3, 25.7, 27.5, 28.1, 29.5, 31.0, 32.7, 33.7, 34.5, 37.3, 37.9, 38.5, 38.8, 39.0, 39.2, 40.9, 42.6, 44.2, 46.9, 50.2, 50.7, 55.5, 55.9, 66.0, 66.2 (d, J = 33), 69.8, 70.3, 70.3, 71.1, 71.1, 79.1, 104.9, 109.6, 125.6 (d, J = 283), 151.0, 177.0, 177.3, 200.7. ^{19}F NMR spectrum (470 MHz, $CDCl_3$, δ, ppm): –78.41 – (–78.61) (m, CF_3).

Cytotoxicity of 4a, 4b, 7a–f, 9a, and 9b. Cultures of breast cancer MCF7 (ATCC HTB-22) and U-87 MG glioblastoma multiforme cells (ATCC HTB-14) were obtained commercially (ATCC, USA). The healthy control was immortalized human fibroblasts (hTERT) from A. G. Shilov (FRC ICG, SB, RAS). Cytotoxicity of the tested compounds was assayed using the MTT assay and the standard procedure [18]. Cells were inoculated into 96-well plates (5,000 cells per well) and incubated at 37°C in 5% CO_2 for attachment. Medium in wells was replaced after 24 h with fresh medium containing the tested compounds in DMSO (1 vol%) and incubated for 72 h. Optical density was measured in a plate spectrophotometer as usual. All compounds were tested at concentrations of 10, 25, 50, and 100 μM using the required controls, i.e., negative, DMSO (solvent), and positive, doxorubicin (standard cytostatic). Each experiment was performed independently in triplicate with three tests in each. Results were reported as mean inhibitory concentration IC_{50} ± SEM.

ACKNOWLEDGMENT

The work was financially supported by the National Academy of Sciences of Belarus and the Siberian Branch, Russian Academy of Sciences (Grant X15CO-001, Project 0302-2015-0003).

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