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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and cytotoxic activity of 3-amino substituted fusidane triterpenoids

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Abstract

New 3-amino substituted derivatives, containing linear, aromatic, and heterocyclic fragments, as well as conjugates with biogenic amines—spermine and spermidine, were synthesized from 3,11-dioxo analogs of fusidic acid and its methyl ester. Antitumor activity of the compounds was studied in vitro towards the 60 cell lines of nine different types of human tumors of the NCI collection. Introduction of pyrrolidine, *n*-butylamine, benzylamine, and ethylenediamine substituents into the molecules were found to provide a pronounced selective effect on five cell lines of leukemia: HL-60, K-562, MOLT-4, RPMI-8226, and SR, inhibiting their growth from 70% to complete death of cancer cells. Methylfusidate derivative with a spermine fragment was shown to exhibit the widest spectrum of antiproliferative action among the obtained compounds, inhibiting the growth of leukemia, NSC lung cancer, colon cancer, and melanoma cell lines of 68–92%.

Keywords Triterpenoids · Fusidic acid · Fusidane amines · Antitumor activity

Introduction

Cancer is one of the most devastating and rapidly developing diseases affecting the lives of millions of people around the world (Markman et al. 2013). While the development of new pharmaceutical products requires significant time and material costs, the situation is complicated by tumor resistance to anticancer drugs and side effects associated with its high toxicity (Speck-Planche 2019). Thus, the search and development of new agents that possess the high antiproliferative activity and do not cause serious side effects is an important task of modern medicinal chemistry.

The perspective objects of the search are the natural compounds that could show a new mechanism of action via specific targets in the tumor and normal tissues together with lesser toxicity, pharmacokinetic properties and better selectivity (Buyel 2018; Tripathi et al. 2018; Mazumder et al. 2018).

Among the natural compounds a tetracyclic triterpenoid fusidic acid (FA), which is produced by *Fusidium coccineum* fungi and belongs to a small family of fusidane antibiotics (Reynolds 1996), is used in clinical practice. It is characterized by low toxicity and allergic reactions, as well as the lack of cross-resistance with other clinically used antibiotics. FA exhibits a bacteriostatic effect and applied for the treatment of severe staphylococcal infections, including methicillin-resistant strains (Kucers and Bennett 1997).

Since the discovery of this antibiotic, many works have been published on the study of various biological effects of FA. For example, antiparasitic (Salama et al. 2013), antituberculosis (Cicek-Saydam et al. 2001), antiinflammatory (Kilic et al. 2002), antiviral (Tyrrell 1969), and anti-HIV (Faber et al. 1987) activities of FA are known. The application of this antibiotic for the treatment of a wide range of less common infections (Golledge 1999) shows the prospect of FA potential use in neurosurgical practice as adjunctive therapy for bacterial endophthalmitis and legionella pneumonia, as well as for leprosy. Based on nanofibers enriched with an encapsulated mixture of FA and rifampicin, a biodegradable drug delivery system was developed to prevent infections accompanied the installation of orthopedic implants (Gilchrist et al. 2013). Moreover, the activity of

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ester and amide FA derivatives was detected towards the *Plasmodium falciparum* malaria parasite strains (Singh et al. 2017). However, despite extensive studies of FA, its cytotoxic effect practically has not been studied. In 2019, the first evidence emerged that FA derivatives may have potential antitumor activity. The authors (Ni et al. 2019) synthesized a number of FA esters containing a benzyl substituent at 21-COOH group and fragments with terminal amino or carboxyl groups in the C3 position of the molecule and studied their antiproliferative activity against cancer cell lines HeLa, U87, KBV, and MKN45. This study demonstrated that the functionalization of the 3-OH group of FA molecule using amino acids with terminal amino substituents provides antitumor activity, while the introduction of carboxyl or heterocyclic substituents does not have an obvious antitumor effect.

Thus, in continuation of our search for promising biologically active substances in the series of fusidane triterpenoids, we synthesized new analogs of FA containing linear, aromatic, heterocyclic amino groups, and polyamine fragments at the C3 position of the molecule, and investigated antitumor activity of the obtained derivatives for in vitro.

Material and methods

General

One-dimensional (^{1}H and ^{13}C) and two-dimensional (COSY, NOESY, HSQC, and HMBC) NMR spectra of compounds were recorded on *Bruker Avance 400* spectrometer (400.13 MHz for ^{1}H and 100.62 MHz for ^{13}C) and *Bruker Avance II 500 HD Ascend* spectrometer (500.17 MHz for ^{1}H and 125.78 MHz for ^{13}C). All the experiments were set up with standard Bruker pulse sequences. Chemical shifts are given in ppm relative to TMS as the internal standard. Mass spectra were measured by MALDI TOF/TOF method on *Bruker Autoflex III* spectrometer with the registration of positive ions; 3-(4-hydroxy-3,5-dimethoxyphenyl)prop-2-enoic acid was used as a matrix. The elemental analyses were carried out on a Carlo Erba 1106 analyzer. The melting points were determined on a PHMK 80/2617 apparatus. The reaction progress was monitored by TLC using Sorbfil plates (PTSHAF-V, Sorbopolymer, Russia), visualization by 10% solution of sulfuric acid with subsequent heating at 100–120 °C during 2–3 min. Column chromatography was performed on KSK silica gel (100–200 µm, Sorbopolymer, Russia). FA was purchased in the company "Hangzhou Hyper Chemicals Limited", purity 99.3%. 3,11-dioxo derivatives of FAs **1** and **2** were synthesized according to the known procedure (Salimova et al. 2018a). Synthesis of amines **3**, **4**, **6**, and **9** –**10** are described in Salimova et al. (2018b).

Chemistry

Procedure for the preparation of amino derivatives (5), (7–8)

Diketone (**1** or **2**) (0.5 mmol), heterocyclic or aromatic amines (pyrrolidine or benzylamine) (1.5 mmol), and AcOH (1.0 mmol, 0.06 g) were mixed in dry chloroform (5 mL) and the mixture was stirred under argon at room temperature for 12 h. Then mixture treated with sodium triacetoxyborohydride (1.0 mmol, 0.21 g). The reaction mass was quenched by adding 10% $NaHCO_3$, and the product was extracted with chloroform. The organic extract was washed with brine and dried over $MgSO_4$. The solvent was evaporated to give the crude amine, which was purified by column chromatography on silica gel using as eluents chloroform-methanol, 40:1 for compounds (**5**), (**7**); chloroform, for compound (**8**).

(2Z)-2-[(3β,4α,8α,14β,16β)-16-(Acetyloxy)-3-(benzylamino)-4,8,10,14-tetramethyl-11-oxogonan-17-ylidene]-6-methylhept-5-enoic acid (5) Yellow powder; yield 90%; mp 140–142 °C; $[\alpha]_D^{20}$ +66.5° (*c* 0.724, MeOH); ^{1}H NMR (CD_3OD, 500.17 MHz,): $\delta = 7.54$ (2H, d, $J = 7.5$ Hz, H-3′, 7′), 7.40–7.50 (3H, m, H-4′, 5′, 6′), 5.86 (1H, d, $J = 7.5$ Hz, H-16), 5.16 (1H, t, $J = 7.2$ Hz, H-24), 4.30 (1H, t, $J =$ 13.2 Hz, H-1′a), 4.13 (1H, t, $J = 13.2$ Hz, H-1′b), 3.19 (1H, d, $J = 2.5$ Hz, H-3), 2.91 (1H, t, $J = 9.2$ Hz, H-13), 2.88–2.94 (1H, m, H-12a), 2.81 (1H, s, H-9), 2.70–2.85 (1H, m, H-12b), 2.35 (1H, t, $J = 8.0$ Hz, H-22a), 2.21–2.29 (1H, m, H-22b), 2.10 (2H, m, H-2), 2.07–2.19 (1H, m, H-15a), 2.05–2.21 (2H, m, H-23), 2.03 (3H, s, O-C(O)C$\underline{H}_3$), 1.99–2.09 (1H, m, H-7a), 1.90–2.00 (2H, m, H-1a, H-4), 1.81-1.73-1.88 (1H, m, H-1b), 1.71–1.81 (1H, m, H-5), 1.68 (3H, s, H-26), 1.63 (3H, s, H-27), 1.51–1.66 (1H, m, H-6a), 1.35 (1H, d, $J = 14.0$ Hz, H-15b), 1.25 (3H, s, H-18), 1.20 (3H, s, H-30), 1.18–1.32 (1H, m, H-7b), 1.05 (3H, s, H-19), 1.03–1.21 (1H, m, H-6b), 0.85 (3H, d, $J = 7.1$ Hz, H-28); ^{13}C NMR (CD_3OD, 125.78 MHz,): $\delta = 211.3$ (C, C-11), 176.7 (C, C-21), 171.4 (C, O-$\underline{C}$(O)CH_3), 138.1 (C, C-20), 135.9 (C, C-17), 132.4 (C, C-2′), 131.4 (C, C-25), 129.9 (CH, C-6′), 129.5 (CH, C-4′), 128.9 (CH, C-7′), 128.9 (CH, C-3′), 128.8 (CH, C-5′), 123.6 (CH, C-24), 74.3 (CH, C-16), 58.3 (CH, C-9), 57.9 (CH, C-3), 50.4 (CH_2, C-1′), 48.6 (C, C-14), 46.7 (CH, C-13), 44.9 (CH_2, C-12), 41.3 (C, C-8), 40.1 (CH, C-5), 38.1 (CH_2, C-15), 36.7 (C, C-10), 32.5 (CH, C-4), 31.6 (CH_2, C-7), 29.4 (CH_2, C-22), 28.9 (CH_2, C-1), 27.4 (CH_2, C-23), 24.6 (CH_3, C-26), 23.3 (CH_2, C-2), 21.8 (CH_3, C-30), 21.2 (CH_3, C-19), 20.3 (CH_2, C-6), 19.8 (CH_3, O-C(O)$\underline{C}H_3$), 16.6 (CH_3, C-27), 16.0 (CH_3, C-18), 14.0 (CH_3, C-28); MALDI TOF/TOF: m/z (I_{rel}, %):

626.332 [+Na] (29), 642.296 [+K] (100); Anal. Calcd. for $C_{38}H_{53}NO_5$: C, 75.59; H, 8.85; N, 2.32%. Found: C, 76.00; H, 9.01; N, 2.26%.

Methyl (2Z)-2-[(3β,4α,8α,14β,16β)-16-(acetyloxy)-4,8,10,14-tetramethyl-11-oxo-3-pyrrolidin-1-ylgonan-17-ylidene]-6-methylhept-5-enoate (7) Yellow powder; yield 80%; mp 228–230 °C; $[\alpha]_D^{20}$ +14.3° (*c* 1.227, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500.17 MHz,): $\delta = 5.77$ (1H, t, $J = 7.0$ Hz, H-16), 4.96 (1H, t, $J = 7.0$ Hz, H-24), 3.56 (3H, s, C(O)OC$\underline{H}_3$), 3.41–3.50 (1H, m, H-3), 3.20–3.40 (4H, m, H-1′, 4′), 2.69–2.86 (1H, m, H-13), 2.68–2.81 (1H, m, H-12a), 2.61–2.73 (1H, m, H-1a), 2.48–2.63 (1H, m, H-12b), 2.47 (1H, s, H-9), 2.19–2.35 (2H, m, H-22), 2.17–2.29 (1H, m, H-4), 2.08–2.20 (1H, m, H-2a), 1.95–2.14 (1H, m, H-15a), 1.92 (3H, s, O-C(O)C$\underline{H}_3$), 1.91–2.44 (4H, m, H-2′, 3′), 1.86–1.98 (1H, m, H-7a), 1.80–2.02 (2H, m, H-23), 1.56 (3H, s, H-26), 1.54–1.63 (1H, m, H-1b), 1.49–1.63 (1H, m, H-2b), 1.49 (3H, s, H-27), 1.43–1.82 (1H, m, H-6a), 1.28–1.38 (1H, m, H-5), 1.16–1.18 (3H, m, H-28), 1.23–1.36 (1H, m, H-15b), 1.09–1.25 (1H, m, H-7b), 1.09 (3H, s, H-30), 1.08 (3H, s, H-18), 1.01 (3H, s, H-19), 0.95–1.06 (1H, m, H-6b); ^{13}C NMR ($CDCl_3$, 125.78 MHz,): $\delta = 209.6$ (C, C-11), 170.0 (C, C-21), 169.8 (C, O-$\underline{C}$(O)CH_3), 145.2 (C, C-17), 132.9 (C, C-25), 131.4 (C, C-20), 122.4 (CH, C-24), 73.8 (CH, C-16), 65.9 (CH, C-3), 60.1 (CH, C-9), 51.4 (CH_3, C(O)O$\underline{C}H_3$) 48.4 (C, C-14), 47.2 (CH, C-13), 46.5 (CH, C-5), 44.8 (CH_2, C-12), 44.0 (CH_2, C-1′), 43.5 (CH_2, C-4′), 40.6 (C, C-8), 37.9 (CH_2, C-15), 37.0 (CH, C-4), 35.2 (C, C-10), 33.2 (CH_2, C-1), 33.1 (CH_2, C-7), 32.0 (CH_2, C-2), 28.7 (CH_2, C-22), 27.7 (CH_2, C-23), 25.6 (CH_3, C-26), 23.8 (CH_2, C-2′, C-3′), 23.4 (CH_3, C-30), 23.2 (CH_3, C-19), 21.2 (CH_2, C-6), 20.8 (CH_3, O-C(O)$\underline{C}H_3$), 17.6 (CH_3, C-27), 16.8 (CH_3, C-18), 15.2 (CH_3, C-28); MALDI TOF/TOF: m/z (I_{rel}, %): 580.428 [−H] (100), 582.442 [+H] (41); Anal. Calcd. for $C_{36}H_{55}NO_5$: C, 74.32; H, 9.53; N, 2.41%. Found: C, 74.11; H, 9.21; N, 2.36%.

Methyl (2Z)-2-[(3β,4α,8α,14β,16β)-16-(acetyloxy)-3-(benzylamino)-4,8,10,14-tetramethyl-11-oxogonan-17-ylidene]-6-methylhept-5-enoate (8) Yellow powder; yield 92%; mp 124–126 °C; $[\alpha]_D^{20}$ +61.4° (*c* 0.711, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500.17 MHz,): $\delta = 7.55$–7.64 (2H, m, H-3′, 7′), 7.27–7.31 (2H, m, H-4′, 6′), 7.17 (1H, dd, $J = 7.5$, 17.0 Hz, H-5′), 5.74 (1H, dd, $J = 8.0$, 17.2 Hz, H-16), 4.98 (1H, d, $J = 5.5$ Hz, H-24), 4.37 (1H, d, $J = 13.5$ Hz, H-1′a), 4.01 (1H, d, $J = 13.5$ Hz, H-1′b), 3.55 (3H, s, C(O)OC$\underline{H}_3$), 2.93–3.03 (1H, m, H-3), 2.76–2.94 (1H, m, H-13), 2.72–2.91 (1H, m, H-12a), 2.60 (1H, t, $J = 8.1$ Hz, H-12b), 2.55–2.71 (1H, m, H-2a), 2.45 (1H, s, H-9), 2.27–2.38 (1H, m, H-1a), 2.25–2.43 (2H, m, H-22), 1.96–2.19 (1H, m, H-15a), 1.94–2.23 (2H, m, H-23), 1.88 (3H, s, O-C(O)C$\underline{H}_3$), 1.84 (1H, d, $J = 13.5$ Hz, H-4), 1.82–1.96 (1H, m, H-7a), 1.77–1.96 (1H, m, H-2b), 1.58 (3H, s, H-26), 1.52–1.74 (1H, m, H-1b), 1.50–1.66 (1H, m, H-6a), 1.50 (3H, s, H-27), 1.27 (1H, d, $J = 13.5$Hz, H-5), 1.23–1.39 (1H, m, H-15b), 1.22 (3H, s, H-30), 1.11–1.35 (1H, m, H-7b), 1.08 (3H, s, H-18), 0.93–1.06 (1H, m, H-6b), 0.92 (3H, s, H-19), 0.86 (3H, br.s, H-28); ^{13}C NMR ($CDCl_3$, 125.78 MHz,): $\delta = 209.7$ (C, C-11), 170.5 (C, O-$\underline{C}$(O)CH_3), 170.0 (C, C-21), 145.5 (C, C-17), 138.2 (C, C-2′), 132.9 (C, C-25), 130.6 (C, C-20), 128.5 (CH, C-4′, 6′), 127.7 (CH, C-3′, 7′), 127.3 (CH, C-5′), 122.5 (CH, C-24), 74.1 (CH, C-16), 58.5 (CH, C-9), 57.0 (CH, C-3), 51.4 (CH_3, C(O)O$\underline{C}H_3$), 50.1 (CH_2, C-1′), 48.6 (C, C-14), 47.5 (CH, C-13), 46.8 (CH, C-5), 43.6 (CH_2, C-12), 41.2 (C, C-8), 40.6 (CH, C-4), 38.1 (CH_2, C-15), 37.0 (C, C-10), 32.2 (CH_2, C-7), 31.5 (CH_2, C-1), 28.8 (CH_2, C-22), 27.8 (CH_2, C-23), 25.7 (CH_3, C-26), 23.4 (CH_2, C-2), 23.0 (CH_3, C-30), 22.3 (CH_3, O-C(O)$\underline{C}H_3$), 21.6 (CH_3, C-19), 20.8 (CH_2, C-6), 17.7 (CH_3, C-27), 16.7 (CH_3, C-18), 15.5 (CH_3, C-28); MALDI TOF/TOF: m/z (I_{rel}, %): 733.466 [+3 K, −H] (100); Anal. Calcd. for $C_{39}H_{55}NO_5$: C, 75.81; H, 8.97; N, 2.27%. Found: C, 75.20; H, 9.01; N, 2.31%.

Procedure for the preparation of amino derivatives (11–20)

A mixture of the diketone (**1** or **2**) (0.5 mmol), titanium (IV) isopropoxide (0.17 mmol, 0.06 g) and primary or secondary amine (pyrrolidine, *n*-butylamine, benzylamine, spermine, or spermidine) (1.5 mmol) in absolute methanol (5 mL) was stirred under argon at room temperature for 3 h. Sodium borohydride (1.0 mmol, 0.04 g) was then added at −78 °C and the resulting mixture was stirred for an additional 2 h raising the temperature to 20 °C. The reaction was then quenched by adding water (5 mL). Stirring was maintained at room temperature for 20 min. After the filtration, washing with methanol, the organic layer was separated and concentrated in vacuo to afford the expected crude amine, which was purified by column chromatography on silica gel using as eluents chloroform-methanol (4:1) for compounds (**11**), (**13**); chloroform-methanol (20:1) for compounds (**12**), (**14**), (**15**); chloroform-methanol (40:1) for compound (**16**); and methanol for compounds (**17**–**20**).

(2Z)-2-[(3β,4α,8α,11α,14β,16β)-16-(Acetyloxy)-3-(butylamino)-11-hydroxy-4,8,10,14-tetramethylgonan-17-ylidene]-6-methylhept-5-enoic acid (11) Yellow powder; yield 82%; mp 182–184 °C; $[\alpha]_D^{20}$ +20.0° (*c* 0.421, MeOH); ^{1}H NMR ($CDCl_3$, 500.17 MHz,): $\delta = 5.82$ (1H, t, $J = 7.5$ Hz, H-16), 5.05–5.09 (1H, m, H-24), 4.22–4.26 (1H, m, H-11), 3.60 (1H, d, $J = 10.2$ Hz, H-3), 2.93 (1H, d, $J = 11.2$ Hz, H-13), 2.63–2.84 (2H, m, H-1′), 2.29–2.45 (2H, m, H-22), 2.18–2.30 (1H, m, H-12a), 1.97–2.16 (3H, m, H-15a, H-23), 1.95 (3H, s, O-C(O)C$\underline{H}_3$), 1.72–1.83 (1H, m, H-12b), 1.68–1.93 (2H, m, H-2), 1.64–1.73 (1H, m, H-7a), 1.62

(3H, s, H-26), 1.56 (3H, s, H-27), 1.55–1.65 (1H, m, H-4), 1.54–1.72 (1H, m, H-6a), 1.53–1.63 (1H, m, H-5), 1.52–1.70 (3H, m, H-1, H-2′a), 1.50–1.53 (1H, m, H-9), 1.25 (3H, s, H-30), 1.20–1.39 (2H, m, H-3′), 1.14–1.28 (1H, m, H-15b), 1.13–1.28 (1H, m, H-2′b), 1.02–1.16 (2H, m, H-6b, H-7b), 0.94 (6H, br.s, H-19, H-28), 0.90 (3H, t, J = 7.0 Hz, H-4′), 0.88 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 125.78 MHz,): δ = 177.6 (C, C-21), 171.5 (C, O-C(O)CH_3), 138.1 (C, C-17), 137.8 (C, C-20), 131.3 (C, C-25), 124.2 (CH, C-24), 74.6 (CH, C-16), 67.9 (CH, C-11), 48.6 (CH, C-3), 48.5 (CH, C-9), 47.1 (C, C-14), 43.5 (CH_2, C-1′), 43.4 (CH, C-5), 42.6 (CH, C-13), 39.2 (CH_2, C-15), 39.0 (C, C-8), 36.2 (CH_2, C-12), 36.1 (C, C-10), 34.6 (CH, C-4), 32.2 (CH_2, C-7), 29.7 (CH_2, C-1), 29.6 (CH_2, C-23), 28.8 (CH_2, C-2′), 28.1 (CH_2, C-22), 25.6 (CH_3, C-26), 24.9 (CH_2, C-2), 23.7 (CH_3, C-30), 23.5 (CH_3, C-19), 21.0 (CH_2, C-6), 20.1 (CH_3, O-C(O)CH_3), 20.0 (CH_2, C-3′), 17.7 (CH_3, C-18), 17.5 (CH_3, C-27), 15.4 (CH_3, C-28), 13.6 (CH_3, C-4′); MALDI TOF/TOF: m/z (I_{rel}, %): 572.442 [+H] (100), 610.432 [+K] (59); Anal. Calcd. for $C_{35}H_{57}NO_5$: C, 73.51; H, 10.05; N, 2.45%. Found: C, 73.20; H, 9.93; N, 2.37%.

Methyl (2Z)-2-[(3β,4α,8α,11α,14β,16β)-16-(acetyloxy)-3-(butylamino)-11-hydroxy-4,8,10,14-tetramethylgonan-17-ylidene]-6-methylhept-5-enoate (12) Yellow powder; yield 89%; mp 155–157 °C; $[\alpha]_D^{20}$ +10.3° (c 0.671, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500.17 MHz,): δ = 5.79 (1H, d, J = 7.2 Hz, H-16), 4.95–5.05 (1H, m, H-24), 4.20–4.30 (1H, m, H-11), 3.59 (3H, s, C(O)OCH_3), 2.98–3.07 (1H, m, H-3), 2.97 (1H, d, J = 10.5 Hz, H-13), 2.87–3.26 (2H, m, H-1′), 2.26–2.52 (2H, m, H-22), 2.16–2.31 (1H, m, H-12a), 2.02–2.16 (1H, m, H-15a), 1.92 (3H, s, O-C(O)CH_3), 1.92–2.19 (2H, m, H-23), 1.84–1.95 (1H, m, H-1a), 1.73–1.85 (1H, m, H-7a), 1.69–1.82 (1H, m, H-12b), 1.63–1.73 (1H, m, H-1b), 1.61 (3H, s, H-26), 1.56–1.62 (2H, m, H-2), 1.54 (3H, s, H-27), 1.52–1.62 (1H, m, H-6a), 1.51–1.21 (1H, m, H-2′b), 1.43–1.51(1H, m, H-4), 1.38–1.48 (1H, m, H-9), 1.32–1.62 (1H, m, H-2′a), 1.28–1.34 (1H, m, H-5), 1.26 (3H, s, H-30), 1.22–1.47 (3H, m, H-6b, H-3′), 1.13–1.28 (1H, m, H-15b), 1.08 (3H, s, H-19), 1.00–1.16 (1H, m, H-7b), 0.89 (3H, d, J = 8.5 Hz, H-28), 0.85 (3H, s, H-18), 0.82–0.96 (3H, m, H-4′); ^{13}C NMR ($CDCl_3$, 125.78 MHz,): δ = 170.6 (C, C-21), 170.4 (C, O-C(O)CH_3), 148.2 (C, C-17), 132.4 (C, C-25), 130.3 (C, C-20), 122.9 (CH, C-24), 76.3 (CH, C-3), 74.3 (CH, C-16), 68.0 (CH, C-11), 51.4 (CH_3, C(O)OCH_3), 51.3 (CH, C-9), 48.6 (C, C-14), 43.8 (CH, C-13), 42.8 (CH, C-4), 39.4 (CH, C-5), 39.3 (CH_2, C-1′), 38.9 (CH_2, C-15), 38.6 (C, C-8), 36.6 (C, C-10), 35.8 (CH_2, C-12), 34.1 (CH_2, C-1), 32.6 (CH_2, C-7), 31.4 (CH_2, C-2), 29.6 (CH_2, C-2′), 28.8 (CH_2, C-22), 28.2 (CH_2, C-23), 25.7 (CH_3, C-26), 24.0 (CH_3, C-30), 23.7 (CH_3, C-19), 20.9 (CH_3, O-C(O)CH_3), 20.8 (CH_2, C-6), 20.0 (CH_2, C-3′), 17.8 (CH_3, C-27), 17.7 (CH_3, C-18), 15.4 (CH_3, C-28), 13.7 (CH_3, C-4′); MALDI TOF/TOF: m/z (I_{rel}, %): 665.438 [+2K, +2H] (47), 705.460 [+3K, +3H] (100); Anal. Calcd. for $C_{36}H_{59}NO_5$: C, 73.80; H, 10.15; N, 2.39%. Found: C, 73.35; H, 9.98; N, 2.36%.

(2Z)-2-[(3β,4α,8α,11α,14β,16β)-16-(Acetyloxy)-11-hydroxy-4,8,10,14-tetramethyl-3-pyrrolidin-1-ylgonan-17-ylidene]-6-methylhept-5-enoic acid (13) Yellow powder; yield 75%; mp 210–212 °C; $[\alpha]_D^{20}$ +19.6° (c 0.374, MeOH); ^{1}H NMR ($CDCl_3$, 500.17 MHz,): δ = 5.85 (1H, d, J = 7.5 Hz, H-16), 5.10 (1H, t, J = 7.0 Hz, H-24), 4.26–4.30 (1H, m, H-11), 3.31–3.44 (2H, m, H-1′), 3.27–3.38 (1H, m, H-4′a), 3.13–3.21 (1H, m, H-4′b), 3.09–3.17 (1H, m, H-3), 2.97 (1H, d, J = 11.5 Hz, H-13), 2.43–2.59 (1H, m, H-22a), 2.28–2.40 (1H, m, H-12a), 2.21–2.40 (1H, m, H-22b), 2.14–2.25 (1H, m, H-1a), 2.02–2.20 (2H, m, H-23), 2.00–2.19 (1H, m, H-15a), 1.97 (3H, s, O-C(O)CH_3), 1.94–2.13 (2H, m, H-3′), 1.89–2.02 (1H, m, H-1b), 1.79–1.92 (3H, m, H-2, H-12b), 1.77–1.87 (1H, m, H-6a), 1.76–1.93 (2H, m, H-2′), 1.74–1.87 (1H, m, H-7a), 1.73–1.85 (1H, m, H-5), 1.68–1.82 (1H, m, H-4), 1.65 (3H, s, H-26), 1.58 (3H, s, H-27), 1.54–1.56 (1H, m, H-9), 1.27 (3H, s, H-30), 1.18–1.30 (1H, m, H-6b), 1.12–1.29 (2H, m, H-7b, H-15b), 1.09 (3H, d, J = 5.5 Hz, H-28), 1.08 (3H, s, H-19), 0.96 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 125.78 MHz,): δ = 175.4 (C, C-21), 171.3 (C, O-C(O)CH_3), 141.7 (C, C-17), 135.4 (C, C-20), 131.7 (C, C-25), 123.9 (CH, C-24), 74.6 (CH, C-16), 67.9 (CH, C-3), 66.2 (CH, C-11), 53.4 (CH_3, C-4′) 49.8 (CH, C-9), 49.5 (C, C-14), 48.6 (CH_2, C-1′), 43.3 (CH, C-5), 43.0 (CH, C-13), 39.1 (C, C-8), 39.0 (CH_2, C-15), 36.1 (CH_2, C-12), 34.9 (C, C-10), 33.8 (CH, C-4), 32.1 (CH_2, C-1), 31.1 (CH_2, C-7), 29.5 (CH_2, C-22), 28.2 (CH_2, C-23), 25.6 (CH_3, C-26), 24.2 (CH_2, C-2′, C-3′), 23.7 (CH_3, C-19), 23.5 (CH_2, C-2), 22.9 (CH_3, C-30), 20.9 (CH_2, C-6), 20.3 (CH_3, O-C(O)CH_3), 17.7 (CH_3, C-27), 17.5 (CH_3, C-18), 15.1 (CH_3, C-28); MALDI TOF/TOF: m/z (I_{rel}, %): 570.284 [+H] (100); Anal. Calcd. for $C_{35}H_{55}NO_5$: C, 73.77; H, 9.73; N, 2.46%. Found: C, 73.28; H, 9.89; N, 2.50%.

Methyl (2Z)-2-[(3b,4a,8a,11a,14b,16b)-16-(acetyloxy)-11-hydroxy-4,8,10,14-tetramethyl-3-pyrrolidin-1-ylgonan-17-ylidene]-6-methylhept-5-enoate (14) Yellow powder; yield 85%; mp 195–197 °C; $[\alpha]_D^{20}$ +22.3° (c 0.890, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500.17 MHz,): δ = 5.84 (3H, d, J = 8.3 Hz, H-16), 5.08 (1H, t, J = 6.5 Hz, H-24), 4.31–4.40 (1H, m, H-11), 3.64 (3H, s, C(O)OCH_3), 3.62–3.68 (2H, m, H-1′), 3.37–3.57 (2H, m, H-4′), 3.09–3.17 (1H, m, H-3), 2.97–3.08 (1H, m, H-13), 2.32–2.53 (2H, m, H-22), 2.25–2.34 (1H, m, H-12a), 2.11–2.23 (1H, m, H-15a), 2.00–2.26 (2H, m, H-23), 1.98 (3H, s, O-C(O)CH_3), 1.97–2.01 (1H, m, H-2′a), 1.93–2.00 (1H, m, H-3′a), 1.91–1.99 (1H, m, H-1a), 1.86–1.90 (1H, m, H-3′b),

1.82–1.94 (2H, m, H-2a, H-12b), 1.81–1.86 (1H, m, H-2′b), 1.72–1.78 (1H, m, H-1b), 1.71–1.81 (1H, m, H-7a), 1.67 (3H, s, H-26), 1.59–1.72 (1H, m, H-2b), 1.59 (3H, s, H-27), 1.52–1.57 (1H, m, H-9), 1.51–1.71 (1H, m, H-6a), 1.50–1.57 (1H, m, H-5), 1.36–1.42 (1H, m, H-4), 1.32 (3H, s, H-30), 1.21–1.34 (1H, m, H-15b), 1.09–1.16 (1H, m, H-6b), 1.08–1.16 (1H, m, H-7b), 1.00 (3H, s, H-19), 0.96 (3H, d, $J = 6.0$ Hz, H-28), 0.91 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 125.78 MHz,): $\delta = 170.7$ (C, C-21), 170.4 (C, O-C(O)CH_3), 148.1 (C, C-17), 132.5 (C, C-25), 130.4 (C, C-20), 123.0 (CH, C-24), 76.5 (CH, C-3), 74.3 (CH, C-16), 68.2 (CH, C-11), 51.4 (CH_3, C(O)OCH_3), 48.9 (CH, C-9), 48.6 (C, C-14), 47.6 (CH_3, C-4′), 45.1 (CH_2, C-1′), 43.8 (CH, C-13), 42.8 (CH, C-5), 39.5 (CH, C-4), 39.3 (C, C-8), 39.0 (CH_2, C-15), 36.6 (C, C-10), 35.8 (CH_2, C-12), 34.2 (CH_2, C-1), 32.7 (CH_2, C-7), 31.5 (CH_2, C-2), 28.8 (CH_2, C-22), 28.2 (CH_2, C-23), 28.0 (CH_2, C-3′), 25.7 (CH_3, C-26, CH_2, C-2′), 24.1 (CH_3, C-30), 23.7 (CH_3, C-19), 20.9 (CH_3, O-C(O)CH_3, CH_2, C-6), 17.8 (CH_3, C-18), 17.7 (CH_3, C-27), 15.3 (CH_3, C-28); MALDI TOF/TOF: m/z (I_{rel}, %): 645.702 [+K, +Na] (100); Anal. Calcd. for $C_{36}H_{57}NO_5$: C, 74.06; H, 9.84; N, 2.40%. Found: C, 73.87; H, 9.93; N, 2.37%.

(2Z)-2-[(3β,4α,8α,11α,14β,16β)-16-(Acetyloxy)-3-(benzylamino)-11-hydroxy-4,8,10,14-tetramethylgonan-17-ylidene]-6-methylhept-5-enoic acid (15) Yellow powder; yield 90%; mp 155–157 °C; $[\alpha]_D^{20}$ +21.7° (*c* 0.358, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500.17 MHz,): $\delta = 7.35$ (2H, d, $J = 6.5$ Hz, H-3′, 7′), 7.30–7.37 (2H, m, H-4′, 6′), 7.24–7.29 (1H, m, H-5′), 5.86 (1H, d, $J = 8.0$ Hz, H-16), 5.11 (1H, t, $J = 7.0$ Hz, H-24), 4.71 (1H, dd, $J = 2.5$, 15.5 Hz, H-1′a), 4.60 (1H, dd, $J = 2.5$, 15.5 Hz, H-1′b), 4.32–4.37 (1H, m, H-11), 3.69–3.75 (1H, m, H-3), 3.06 (1H, d, $J = 11.5$ Hz, H-13), 2.40–2.65 (2H, m, H-22), 2.23–2.35 (1H, m, H-12a), 2.12–2.26 (1H, m, H-23a), 2.08–2.22 (1H, m, H-15a), 2.03–2.17 (1H, m, H-4), 2.02–2.11 (1H, m, H-23b), 1.98 (3H, s, O-C(O)CH_3), 1.80–1.87 (1H, m, H-1a), 1.77–1.86 (1H, m, H-12b), 1.69–1.76 (1H, m, H-7a), 1.68 (3H, s, H-26), 1.66–1.78 (2H, m, H-2a, H-1b), 1.61 (3H, s, H-27), 1.56–1.67 (1H, m, H-6a), 1.51–1.62 (2H, m, H-5, H-9), 1.46–1.54 (1H, m, H-2b), 1.38 (3H, s, H-30), 1.30 (1H, d, $J = 14.5$ Hz, H-15b), 1.08–1.17 (1H, m, H-7b), 1.05–1.16 (1H, m, H-6b), 0.98 (3H, s, H-19), 0.92 (3H, d, $J = 6.5$ Hz, H-28), 0.91 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 125.78 MHz,): $\delta = 170.3$ (C, O-C(O)CH_3), 169.1 (C, C-21), 149.8 (C, C-17), 140.1 (C, C-2′), 132.6 (C, C-25), 129.5 (C, C-20), 128.4 (CH, C-4′, 6′), 128.2 (CH, C-3′, 7′), 126.9 (CH, C-5′), 123.0 (CH, C-24), 74.4 (CH, C-16), 71.4 (CH, C-3), 68.1 (C, C-11), 51.7 (CH_2, C-1′), 49.3 (CH, C-9), 48.6 (C, C-14), 44.2 (CH, C-13), 39.4 (C, C-8), 38.9 (CH_2, C-15), 36.8 (C, C-10), 36.5 (CH, C-5), 35.7 (CH, C-4), 35.5 (CH_2, C-12), 31.9 (CH_2, C-7), 30.0 (CH_2, C-1), 29.8 (CH_2, C-2), 28.9 (CH_2, C-22), 28.4 (CH_2, C-23), 25.7 (CH_3, C-26), 23.7 (CH_3, C-30), 23.1 (CH_3, C-19), 21.0 (CH_3, O-C(O)CH_3), 20.9 (CH_2, C-6), 17.7 (CH_3, C-27, C-18), 15.9 (CH_3, C-28); MALDI TOF/TOF: m/z (I_{rel}, %): 606.244 [+H] (100), 644.573 [+K] (55); Anal. Calcd. for $C_{38}H_{55}NO_5$: C, 75.33; H, 9.15; N, 2.31%. Found: C, 75.74; H, 9.21; N, 2.29%.

Methyl (2Z)-2-[(3β,4α,8α,11α,14β,16β)-16-(acetyloxy)-3-(benzylamino)-11-hydroxy-4,8,10,14-tetramethylgonan-17-ylidene]-6-methylhept-5-enoate (16) Yellow powder; yield 92%; mp 133–135 °C; $[\alpha]_D^{20}$ +25.8° (*c* 0.420, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500.17 MHz,): $\delta = 7.34$ (2H, d, $J = 3.0$ Hz, H-3′, 7′), 7.33 (2H, t, $J = 3.0$ Hz, H-4′, 6′), 7.28–7.32 (1H, m, H-5′), 5.85 (1H, d, $J = 8.5$ Hz, H-16), 5.10 (1H, t, $J = 7.5$ Hz, H-24), 4.33–4.41 (1H, m, H-11), 3.90 (1H, d, $J = 13.0$ Hz, H-1′a), 3.69 (1H, d, $J = 13.0$ Hz, H-1′b), 3.65 (3H, s, C(O)OCH_3), 3.02 (1H, d, $J = 11.5$ Hz, H-13), 2.30 (1H, dt, $J = 3.0$, 13.0 Hz, H-12a), 2.29–2.54 (2H, m, H-22), 2.10–2.22 (1H, m, H-15b), 2.05–2.14 (1H, m, H-3), 1.99 (3H, s, O-C(O)CH_3), 1.97–2.27 (2H, m, H-23), 1.96–2.10 (1H, m, H-2a), 1.87 (1H, dd, $J = 2.5$, 12.5 Hz, H-12b), 1.73–1.92 (2H, m, H-1), 1.70–1.82 (1H, m, H-7a), 1.69 (3H, s, H-26), 1.66–1.75 (1H, m, H-6a), 1.61 (3H, s, H-27), 1.52–1.58 (1H, m, H-9), 1.49–1.60 (1H, m, H-5), 1.39–1.59 (1H, m, H-2b), 1.35–1.46 (1H, m, H-4), 1.33 (3H, s, H-30), 1.21–1.31 (1H, m, H-15a), 1.08–1.16 (1H, m, H-7b), 1.04–1.15 (1H, m, H-6b), 1.00 (3H, s, H-19), 0.95 (3H, d, $J = 6.0$ Hz, H-28), 0.92 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 125.78 MHz,): $\delta = 170.7$ (C, O-C(O)CH_3), 170.4 (C, C-21), 148.1 (C, C-17), 138.2 (C, C-2′), 132.5 (C, C-25), 130.4 (C, C-20), 128.7 (CH, C-6′), 128.4 (CH, C-4′), 128.2 (CH, C-7′), 127.8 (CH, C-3′), 127.5 (CH, C-5′), 123.1 (CH, C-24), 74.3 (CH, C-16), 68.3 (C, C-11), 62.2 (CH, C-3), 51.4 (CH_3, C(O)OCH_3), 51.2 (CH_2, C-1′), 49.0 (CH, C-9), 48.6 (C, C-14), 43.9 (CH, C-13), 43.7 (CH, C-5), 39.3 (C, C-8), 39.0 (CH_2, C-15), 37.5 (CH, C-4), 36.8 (C, C-10), 35.7 (CH_2, C-12), 34.8 (CH_2, C-1), 32.8 (CH_2, C-7), 28.9 (CH_2, C-22), 28.6 (CH_2, C-2), 28.3 (CH_2, C-23), 25.7 (CH_3, C-26), 24.1 (CH_3, C-30), 23.7 (CH_3, C-19), 21.4 (CH_2, C-6), 20.9 (CH_3, O-C(O)CH_3), 17.8 (CH_3, C-27), 17.7 (CH_3, C-18), 15.9 (CH_3, C-28); MALDI TOF/TOF: m/z (I_{rel}, %): 658.437 [+K] (100); Anal. Calcd. for $C_{39}H_{57}NO_5$: C, 75.57; H, 9.27; N, 2.26%. Found: C, 75.33; H, 9.31; N, 2.23%.

(2Z)-2-[(3β,4α,8α,11α,14β,16β)-16-(Acetyloxy)-3-({3-[(4-aminobutyl)amino]propyl}amino)-11-hydroxy-4,8,10,14-tetramethylgonan-17-ylidene]-6-methylhept-5-enoic acid (17) Yellow powder; yield 78%; mp 248–250 °C; $[\alpha]_D^{20}$ +10.0° (*c* 1.126, MeOH); ^{1}H NMR (CD_3OD, 500.17 MHz,): $\delta =$ 5.84 (1H, d, $J = 8.2$ Hz, H-16), 5.16 (1H, t, $J = 7.5$ Hz, H-24), 4.28–4.34 (1H, m, H-11), 3.11–3.23 (1H, m, H-4′a), 3.06–3.14 (2H, m, H-7′), 3.01–3.15 (2H, m, H-3′), 3.01–3.08 (1H, m, H-13), 2.96–3.06 (2H, m, H-1′),

2.77–2.82 (1H, m, H-4′b), 2.51–2.61 (1H, m, H-22a), 2.33–2.43 (1H, m, H-22b), 2.29–2.36 (1H, m, H-12a), 2.21–2.34 (2H, m, H-5′), 2.13–2.22 (1H, m, H-1a), 2.10–2.20 (3H, m, H-15a, H-6′), 2.06–2.19 (2H, m, H-23), 2.04–2.11 (1H, m, H-2a), 2.00 (3H, s, O-C(O)C$\underline{H}_3$), 1.92–1.99 (1H, m, H-1b), 1.83–1.93 (1H, m, H-12b), 1.76–1.87 (1H, m, H-7a), 1.75–1.84 (1H, m, H-2b), 1.74–1.86 (3H, m, H-2′, H-5), 1.72–1.84 (1H, m, H-4), 1.69 (3H, s, H-26), 1.63 (3H, s, H-27), 1.60–1.67 (1H, m, H-9), 1.35 (3H, s, H-30), 1.21–1.30 (1H, m, H-15b), 1.19–1.28 (1H, m, H-6a), 1.14–1.27 (1H, m, H-7b), 1.09 (3H, d, $J = 6.4$ Hz, H-28), 1.07 (3H, s, H-19), 0.96 (3H, s, H-18), 0.94–0.98 (1H, m, H-6b); ^{13}C NMR (CD_3OD, 125.78 MHz,): $\delta = 175.7$ (C, C-21), 171.5 (C, O-$\underline{C}$(O)CH_3), 133.6 (C, C-17), 131.8 (C, C-25), 131.6 (C, C-20), 123.3 (CH, C-24), 74.3 (CH, C-16), 66.8 (CH, C-11), 63.1 (CH, C-3), 48.8 (CH, C-9), 48.4 (C, C-14), 47.0 (CH_2, C-3′), 44.8 (CH_2, C-4′), 43.1 (CH, C-13), 42.9 (CH, C-5), 39.1 (C, C-8), 38.7 (CH_2, C-1′), 38.6 (CH_2, C-15), 36.6 (CH_2, C-7′), 36.0 (C, C-10), 35.8 (CH_2, C-12), 34.9 (CH, C-4), 33.3 (CH_2, C-1), 31.6 (CH_2, C-7), 28.8 (CH_2, C-22), 27.8 (CH_2, C-23), 24.5 (CH_3, C-26), 24.0 (CH_2, C-2, C2′), 23.0 (CH_2, C-6′), 22.9 (CH_2, C-5′), 22.8 (CH_3, C-19), 22.5 (CH_3, C-30), 21.0 (CH_2, C-6), 19.5 (CH_3, O-C(O)$\underline{C}H_3$), 16.5 (CH_3, C-27), 15.9 (CH_3, C-18), 14.3 (CH_3, C-28); MALDI TOF/TOF: m/z (I_{rel}, %): 644.628 [+H] (100), 682.631 [+K] (64); Anal. Calcd. for $C_{38}H_{65}N_3O_5$: C, 70.88; H, 10.17; N, 6.53%. Found: C, 70.58; H, 10.09; N, 6.48%.

Methyl (2Z)-2-[(3β,4α,8α,11α,14β,16β)-16-(acetyloxy)-3-({3-[(4-aminobutyl)amino]propyl} amino)-11-hydroxy-4,8,10,14-tetramethylgonan-17-ylidene]-6-methylhept-5-enoate (18) Yellow powder; yield 82%; mp 230–232 °C; $[\alpha]_D^{20}$ +7.4° (*c* 0.921, MeOH); ^{1}H NMR (CD_3OD, 500.17 MHz,): $\delta =$ 5.83 (1H, d, $J = 8.5$ Hz, H-16), 5.12 (1H, t, $J = 7.5$ Hz, H-24), 4.26–4.38 (1H, m, H-11), 3.65 (3H, s, C(O)OC$\underline{H}_3$), 3.12–3.20 (2H, m, H-4′), 3.06–3.11 (3H, m, H-13, H-7′), 3.03–3.12 (2H, m, H-3′), 2.79–2.87 (2H, m, H-1′), 2.77–2.90 (1H, m, H-3), 2.52–2.63 (1H, m, H-22a), 2.37–2.47 (1H, m, H-22b), 2.33 (1H, dt, $J = 3.0$, 13.0 Hz, H-12a), 2.16–2.22 (2H, m, H-5′), 2.11–2.23 (1H, m, H-1a), 2.05–2.21 (1H, m, H-15a), 2.01–2.20 (2H, m, H-23), 1.97–2.04 (2H, m, H-6′), 1.96 (3H, s, O-C(O)C$\underline{H}_3$), 1.96–2.10 (1H, m, H-2a), 1.91–1.98 (1H, m, H-1b), 1.78–1.92 (1H, m, H-12b), 1.77–1.89 (1H, m, H-2b), 1.73–1.82 (1H, m, H-5), 1.71–1.77 (1H, m, H-4), 1.70–1.83 (1H, m, H-7a), 1.69–1.92 (2H, m, H-2′), 1.68 (3H, s, H-26), 1.62–1.70 (1H, m, H-6a), 1.61 (3H, s, H-27), 1.58–1.64 (1H, m, H-9), 1.36 (3H, s, H-30), 1.20–1.28 (1H, m, H-15b), 1.17–1.28 (1H, m, H-6b), 1.11–1.23 (1H, m, H-7b), 1.09 (3H, s, H-19), 1.07 (3H, d, $J = 6.5$ Hz, H-28), 0.94 (3H, s, H-18); ^{13}C NMR (CD_3OD, 125.78 MHz,): $\delta = 170.9$ (C, C-21), 170.8 (C, O-$\underline{C}$(O)CH_3), 132.2 (C, C-17), 131.0 (C, C-25), 130.2 (C, C-20), 122.7 (CH, C-24), 74.3 (CH, C-16), 66.7 (C, C-11), 63.1 (CH, C-3), 50.6 (CH_3, C(O)O$\underline{C}H_3$), 48.8 (CH, C-9), 48.4 (C, C-14), 46.9 (CH_2, C-3′), 45.4 (CH_2, C-1′), 44.9 (CH_2, C-4′), 43.8 (CH, C-13), 42.9 (CH, C-5), 41.6 (CH_2, C-7′), 39.1 (C, C-8), 38.69 (CH_2, C-15), 36.03 (CH_2, C-12), 35.80 (C, C-10), 35.00 (CH, C-4), 33.34 (CH_2, C-1), 31.6 (CH_2, C-7), 28.3 (CH_2, C-22), 27.9 (CH_2, C-23), 24.5 (CH_3, C-26), 23.9 (CH_2, C-2), 23.6 (CH_2, C-2′), 23.5 (CH_2, C-6′), 23.0 (CH_2, C-5′), 22.8 (CH_3, C-19), 22.5 (CH_3, C-30), 21.1 (CH_2, C-6), 19.6 (CH_3, O-C(O)$\underline{C}H_3$), 16.5 (CH_3, C-27), 16.4 (CH_3, C-18), 14.3 (CH_3, C-28); MALDI TOF/TOF: m/z (I_{rel}, %): 656.404 [−H] (100); Anal. Calcd. for $C_{39}H_{67}N_3O_5$: C, 71.19; H, 10.26; N, 6.39%. Found: C, 71.11; H, 10.29; N, 6.37%.

(2Z)-2-((3β,4α,8α,11α,14β,16β)-16-(Acetyloxy)-3-{[3-({4-[(3-aminopropyl)amino]butyl}amino) propyl]amino}-11-hydroxy-4,8,10,14-tetramethylgonan-17-ylidene)-6-methylhept-5-enoic acid (19) Yellow powder; yield 75%; mp 253–255 °C; $[\alpha]_D^{20}$ +9.2° (*c* 1.110, MeOH); ^{1}H NMR (CD_3OD, 500.17 MHz,): δ = 5.91 (1H, d, $J = 7.5$ Hz, H-16), 5.16 (1H, d, $J = 6.0$ Hz, H-24), 4.24–4.36 (1H, m, H-11), 3.26–3.33 (2H, m, H-3′), 3.23–3.34 (2H, m, H-8′), 3.15 (2H, t, $J = 7.0$ Hz, H-7′), 3.09 (2H, d, $J = 10.0$ Hz, H-10′), 3.02–3.13 (3H, m, H-4′, H-13), 2.95–3.03 (2H, m, H-1′), 2.78–2.90 (1H, m, H-3), 2.47–2.59 (1H, m, H-9′a), 2.35–2.44 (1H, m, H-9′b), 2.28–2.37 (1H, m, H-12a), 2.11–2.23 (1H, m, H-1a), 2.07–2.23 (1H, m, H-2a), 2.05–2.15 (3H, m, H-15a, H-2′), 2.02–2.35 (2H, m, H-22), 1.97 (3H, s, O-C(O)C$\underline{H}_3$), 1.92–2.02 (1H, m, H-1b), 1.81–1.92 (4H, m, H-5′, H-6′), 1.80–1.89 (1H, m, H-12b), 1.74–1.81 (1H, m, H-5), 1.73–2.22 (2H, m, H-23), 1.73–1.87 (1H, m, H-7a), 1.71–1.79 (1H, m, H-4), 1.69 (3H, s, H-26), 1.65–1.72 (1H, m, H-2b), 1.64 (3H, s, H-27), 1.55–1.69 (1H, m, H-9), 1.35 (3H, s, H-30), 1.25–1.31 (1H, m, H-6a), 1.23–1.31 (1H, m, H-15b), 1.14–1.23 (1H, m, H-6b), 1.13–1.24 (1H, m, H-7b), 1.07 (6H, br.s, H-19, H-28), 0.97 (3H, s, H-18); ^{13}C NMR (CD_3OD, 125.78 MHz,): $\delta = 172.3$ (C, C-21, O-$\underline{C}$(O)CH_3), 136.8 (C, C-17), 132.1 (C, C-20), 131.4 (C, C-25), 123.8 (CH, C-24), 74.6 (CH, C-16), 66.9 (CH, C-11), 63.1 (CH, C-3), 53.8 (CH_2, C-3′), 48.8 (CH, C-9), 48.5 (C, C-14), 47.1 (CH_2, C-4′), 45.1 (CH_2, C-7′), 44.7 (CH_2, C-1′), 43.1 (CH, C-13), 42.8 (CH, C-5), 41.8 (CH_2, C-8′), 39.1 (C, C-8), 38.86 (CH_2, C-15), 36.76 (CH_2, C-10′), 36.13 (CH_2, C-12), 35.86 (C, C-10), 34.97 (CH, C-4), 33.4 (CH_2, C-1), 31.7 (CH_2, C-7), 29.3 (CH_2, C-22), 27.8 (CH_2, C-23), 24.5 (CH_3, C-26), 24.2 (CH_2, C-2′), 24.0 (CH_2, C-9′), 23.5 (CH_2, C-2), 23.4 (CH_2, C-5′), 23.1 (CH_2, C-6′), 22.5 (CH_3, C-30), 22.2 (CH_3, C-19), 21.1 (CH_2, C-6), 19.9 (CH_3, O-C(O)$\underline{C}H_3$), 16.6 (CH_3, C-27, C-18), 14.4 (CH_3, C-28); MALDI TOF/TOF: m/z (I_{rel}, %): 701.405 (100); Anal. Calcd. for $C_{41}H_{72}N_4O_5$: C, 70.24; H, 10.35; N, 7.99%. Found: C, 70.29; H, 10.31; N, 8.01%.

Methyl (2Z)-2-((3β,4α,8α,11α,14β,16β)-16-(acetyloxy)-3-{[3-({4-[(3-aminopropyl)amino]butyl} amino)propyl]amino}-11-hydroxy-4,8,10,14-tetramethylgonan-17-ylidene)-6-methylhept-5-enoate (20) Yellow powder; yield 80%; mp 245–247 °C; $[\alpha]_D^{20}$ +8.9° (*c* 1.137, MeOH); ^{1}H NMR (CD_3OD, 500.17 MHz,): $\delta = 5.83$ (1H, d, $J = 3.2$ Hz, H-16), 5.12 (1H, t, $J = 7.0$ Hz, H-24), 4.24–4.37 (1H, m, H-11), 3.64 (3H, s, C(O)OC$\underline{H}_3$), 3.20–3.31 (2H, m, H-8′), 3.08–3.13 (2H, m, H-3′), 3.01–3.11 (2H, m, H-10′), 3.01–3.07 (1H, m, H-13), 3.00–3.16 (2H, m, H-7′), 2.90–3.01 (2H, m, H-4′), 2.73–2.83 (1H, m, H-3), 2.58–2.64 (1H, m, H-1′a), 2.52–2.62 (1H, m, H-22a), 2.43–2.46 (1H, m, H-1′b), 2.37–2.47 (1H, m, H-22b), 2.33 (1H, dt, $J = 3.5$, 13.5 Hz, H-12a), 2.12–2.20 (1H, m, H-1a), 2.11–2.45 (1H, m, H-15a), 2.01–2.16 (2H, m, H-23), 2.00–2.35 (2H, m, H-2′), 1.99–2.18 (2H, m, H-9′), 1.96 (3H, s, O-C(O)C$\underline{H}_3$), 1.90–1.96 (1H, m, H-1b), 1.80 (1H, t, $J = 3.2$ Hz, H-5), 1.77–1.91 (1H, m, H-12b), 1.76–2.02 (2H, m, H-2),1.74–1.91 (4H, m, H-5′, 6′), 1.73–1.83 (1H, m, H-7a), 1.68–1.75 (1H, m, H-4), 1.68 (3H, s, H-26), 1.61 (3H, s, H-27), 1.56–1.78 (1H, m, H-6a), 1.35 (3H, s, H-30), 1.25 (1H, d, $J = 7.3$ Hz, H-15b), 1.10–1.20 (1H, m, H-7b), 1.06 (3H, s, H-19), 1.04 (3H, d, $J = 9.5$ Hz, H-28), 0.93 (3H, s, H-18), 0.91–0.97 (1H, m, H-6b); ^{13}C NMR (CD_3OD, 125.78 MHz,): $\delta = 171.0$ (C, C-21), 170.8 (C, O-$\underline{C}$(O)CH_3), 148.8 (C, C-17), 132.2 (C, C-25), 130.2 (C, C-20), 122.8 (CH, C-24), 74.4 (CH, C-16), 66.8 (CH, C-11), 63.1 (CH, C-3), 50.7 (CH_3, C(O)O$\underline{C}H_3$), 48.8 (CH, C-9), 48.5 (C, C-14), 47.2 (CH_2, C-4′), 45.9 (CH_2, C-7′), 45.0 (CH_2, C-1′), 43.9 (CH, C-13), 42.9 (CH, C-5), 42.8 (CH_2, C-8′), 42.6 (CH_2, C-3′), 39.1 (C, C-8), 38.7 (CH_2, C-15), 37.2 (CH_2, C-10′), 36.0 (CH_2, C-12), 35.8 (C, C-10), 35.2 (CH, C-4), 33.4 (CH_2, C-1), 31.7 (CH_2, C-7), 28.4 (CH_2, C-22), 27.9 (CH_2, C-23), 24.9 (CH_2, C-9′), 24.6 (CH_3, C-26), 24.4 (CH_2, C-2′), 24.2 (CH_2, C-2), 24.1 (CH_2, C-5′), 23.7 (CH_2, C-6′), 23.0 (CH_3, C-30), 22.3 (CH_3, C-19), 21.1 (CH_2, C-6), 19.6 (CH_3, O-C(O)$\underline{C}H_3$), 16.6 (CH_3,C-18), 16.5 (CH_3,C-27), 14.6 (CH_3, C-28); MALDI TOF/TOF: m/z (I_{rel}, %): 738.646 [+Na] (100); Anal. Calcd. for $C_{42}H_{74}N_4O_5$: C, 70.55; H, 10.43; N, 7.84%. Found: C, 70.47; H, 10.39; N, 7.81%.

Pharmacological studies

The newly synthesized FA derivatives were exposed to comprehensive in vitro screening at the National Cancer Institute (NCI) 60 cancer cell lines panel at Bethesda, MD, USA, representing different types cancer, including leukemia, nonsmall cell lung cancer, colon cancer, melanoma, ovarian cancer, renal carcinoma, prostate cancer, and breast cancer (https://dtp.cancer.gov/compsub/). A single dose (10 μM) of the tested analogs was employed in the full NCI 60 cell lines panel assay (Grever et al. 1992; Monks et al. 1991; Boyd and Paul 1995; Skehan et al. 1990). Each cell line was inoculated and preincubated on a microtiter plate after that test compound was added and the culture was incubated for 48 h. The data reported as mean-graph of the percent growth of the treated cells and presented as percentage growth inhibition (GI%) for the tested analogs.

Results and discussion

Chemistry

New nitrogen-containing FA derivatives were synthesized by the reductive amination reaction (Abdel-Magid et al. 1996; Loncle et al. 2007), using the corresponding 3,11-dioxo analogs as starting compounds. *N*-butylamine, pyrrolidine, and benzylamine were chosen as the amine component, as well as 1,2-diaminoethane, spermine, and spermidine polyamines, since polyamines are biologically active compounds with a wide spectrum of action (Bachrach and Heimer 1989; Woster and Casero 2011; Gerner and Meyskens 2004) and an important component of the life cycle of prokaryotic and eukaryotic cells (Agostinelli et al. 2004; Yatin 2002; Thomas and Thomas 2001).

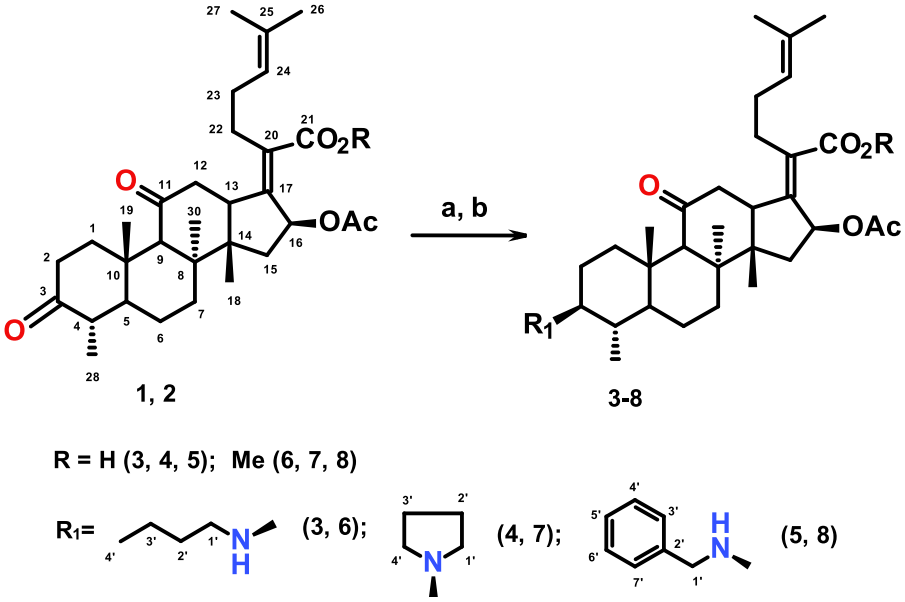
Compounds **3**–**8** were obtained by the reaction of diketones **1** or **2** with 3 eq. of *n*-butylamine, pyrrolidine, or benzylamine in chloroform in the presence of acetic acid, followed by the reaction mass treating with 2 eq. of $NaBH(OAc)_3$ (Scheme 1). Reductive amination of compounds **1** and **2** proceeded with high chemo- and stereoselectivity at the C3 atom with the formation of compounds **3**–**8** with yields of 75–92%, while other functional groups of triterpenoid were not affected.

Derivatives **9**–**20** were synthesized by the reaction of diketones **1** or **2** with 3 eq. of the corresponding amines in dry methanol in the presence of Ti(O*i*-Pr)$_4$ as a catalyst followed by reduction with 2 eq. of $NaBH_4$ (Scheme 2). As a result, monoamino-substituted analogs **9**–**20** were obtained. The keto group at the C11 atom was not involved into amination, however, it was reduced to the hydroxyl group.

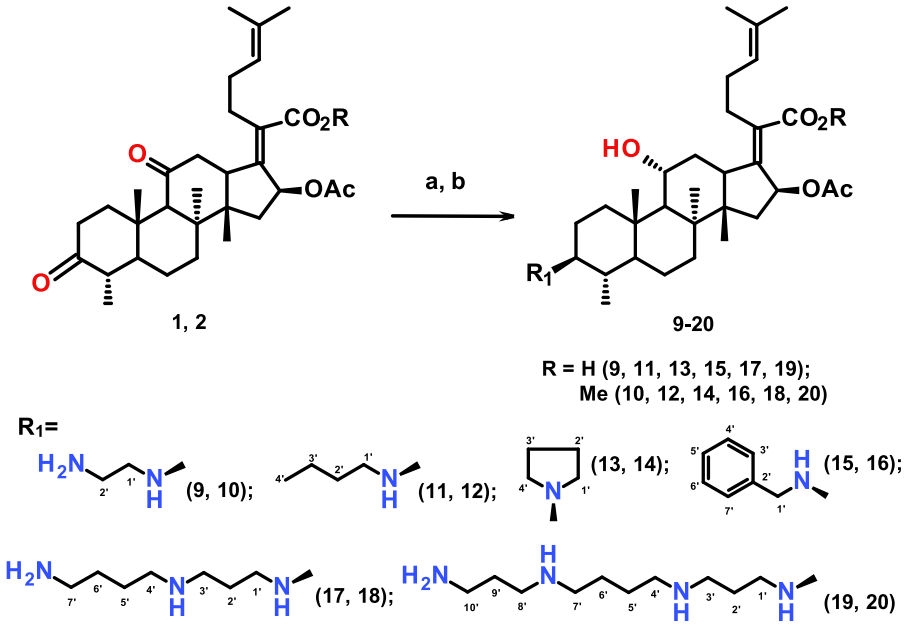
The structure of the synthesized amines **3**–**20**, as well as the configuration of the newly formed stereocenters at C3 and C11 (for derivatives **9**–**20**), were confirmed by a complex of spectral studies, including the data of one-dimensional and two-dimensional NMR spectroscopy. In all cases, molecular ion peaks registered by the means of MALDI TOF/TOF spectrometry corresponded to molecular masses of the synthesized compounds.

Biological activity

Eighteen synthesized amino derivatives of FA were evaluated for their in vitro antitumor activity (cytotoxicity) towards 60 cell lines of nine different types of human



Scheme 1 Synthesis of 11-oxo-3β-amino-derivatives of fusidane triterpenoids: **a** Pyrrolidine, *n*-butylamine or benzylamine, AcOH, $CHCl_3$, 22 °C, 12 h; **b** $NaBH(OAc)_3$, 22 °C, 15 min (85% for **3**, 75% for **4**, 90% for **5**; 88% for **6**, 80% for **7**, 92% for **8**)



Scheme 2 Synthesis of 11α-hydroxy-3β-amino-derivatives of fusidane triterpenoids: **a** Pyrrolidine, *n*-butylamine, benzylamine, ethylenediamine, spermine or spermidine, MeOH, $Ti(i\text{-}PrO)_4$, 22 °C, 3 h; **b** $NaBH_4$, −78→22 °C, 2 h (78% for **9**, 85% for **10**, 82% for **11**; 89% for **12**, 75% for **13**, 85% for **14**, 90% for **15**, 92% for **16**, 78% for **17**, 82% for **18**, 75% for **19**, 80% for **20**)

cancers (lung, colon, central nervous system, ovary, renal, prostate, breast tumors, leukemia, and melanoma) according to the protocols available at the National Cancer Institute (NCI, Bethesda, USA). Results were reported as the percentage of growth of the treated cells compared with the untreated control cells (negative numbers indicate cell kill (Table 1). According to the NCI criteria (reduction of the growth of anyone of the cancer cell lines to ca. 32% or less), amino derivatives **7**, **8**, **10**, **11**, **13**, **16**, and **20** showed a pronounced selective effect in relation to the entire subpanel of the leukemia cell line. Amines with pyrrolidine (**7**, **13**) and ethylenediamine (**10**) substituents had high antiproliferative activity, completely suppressing the growth of leukemia HL-60 cells (negative values in Table 1). 3-[(2-aminoethyl) amino] -fusidate **10** also caused complete death of cell lines K-562, MOLT-4 и SR. Pyrrolidine substituted derivative of FA **13** promoted the death of cancer cells MOLT-4, K-562, and SR showing a high cytotoxic effect, leaving no more than 24% of tumor cells viable. Pyrrolidine 11-oxo-fusidate **7**, as well as butylamino- substituted derivative **11** exhibited high antileukemia activity against HL-60, K-562, and MOLT-4 cell lines (cell viability was ≤30.5%). Benzylamino- 11-oxo-fusidate **8** exhibited good growth inhibition effects against K-562 and

Table 1 Percentage cell growth of 60 human tumor cell line anticancer screening data of the tested compounds at single dose assay (10 μM concentration)

Subpanel tumor cell lines	Percentage cell growth for compounds								
	3	4	5	6	7	8	9	10	11
Leukemia									
CCRF-CEM	97.95	99.50	98.76	82.82	78.19	76.41	101.91	79.40	**41.31**
HL-60(TB)	99.42	98.52	101.09	94.16	**−51.41**	84.70	97.68	**−47.45**	**0.47**
K-562	96.92	98.93	88.58	60.24	**12.46**	**28.79**	98.34	**−7.67**	**30.54**
MOLT-4	99.74	92.39	90.61	52.88	**9.72**	61.73	99.56	**−13.18**	**10.58**
RPMI-8226	100.09	102.76	96.93	59.99	85.47	**49.47**	97.41	70.01	95.91
SR	93.87	98.01	93.26	52.03	56.27	**1.93**	96.30	**−17.79**	74.54
NSC lung cancer									
A549/ATCC	101.24	100.32	91.67	89.17	104.11	81.72	108.47	88.93	103.12
EKVX	101.96	96.14	90.85	83.10	91.91	87.87	97.33	82.19	96.91
HOP-62	101.13	99.41	99.22	99.37	101.63	87.28	99.44	93.03	103.19
HOP-92	88.67	100.85	88.24	71.29	77.20	**49.72**	98.73	53.08	86.54
NCI-H226	96.57	98.96	–	99.52	112.67	–	–	83.47	97.99
NCI-H23	103.64	96.16	97.48	100.30	91.54	93.42	98.09	88.69	90.49
NCI-H322M	99.52	97.78	86.56	97.89	99.58	103.21	99.24	83.53	97.20
NCI-H460	109.91	111.65	106.42	97.42	100.65	88.84	106.48	99.61	100.18
NCI-H522	96.82	92.31	95.32	35.11	101.83	108.45	96.26	86.91	93.05
Colon cancer									
COLO 205	112.20	107.63	103.41	92.93	106.31	59.98	101.79	80.26	104.35
HCC-2998	106.23	105.30	100.93	108.73	114.31	66.65	107.05	91.31	99.34
HCT-116	101.68	102.62	92.23	98.05	84.86	73.00	95.12	**36.77**	101.52
HCT-15	102.67	103.30	96.05	81.89	73.40	72.14	102.93	54.97	98.77
HT29	104.43	111.12	98.33	79.01	68.22	**37.84**	96.33	**33.52**	95.66
KM12	110.32	101.94	103.68	99.60	105.22	100.91	106.06	88.94	104.47
SW-620	104.95	101.37	101.69	96.03	102.94	81.45	96.99	85.42	95.85
CNS Cancer									
SF-268	97.45	98.39	96.20	81.72	97.58	79.56	97.54	76.58	100.14
SF-295	103.17	99.84	98.07	103.44	98.33	98.17	100.54	98.18	98.98
SF-539	97.35	96.40	97.79	82.18	101.83	82.86	104.51	90.20	99.32
SNB-19	95.26	91.75	95.99	89.53	106.76	95.68	99.74	95.07	104.12
SNB-75	92.88	91.66	86.34	78.31	99.23	83.24	97.08	72.96	82.88
U-251	97.45	100.07	98.32	84.44	101.13	74.02	105.75	69.12	98.17
Melanoma									
LOX IMVI	99.81	91.84	99.54	98.37	101.28	81.75	97.26	84.55	97.94
MALME-3M	101.06	104.97	92.58	99.72	100.02	92.54	93.53	89.39	96.93
M14	100.80	102.19	95.98	96.53	93.06	89.74	97.14	86.99	96.25
MDA-MB-435	102.65	99.39	97.23	96.90	97.48	91.25	101.98	93.63	97.99
SK-MEL-2	104.82	106.23	107.53	102.12	105.50	105.80	110.23	92.64	105.81
SK-MEL-28	108.37	98.83	100.55	100.54	93.58	96.65	106.07	89.22	93.37
SK-MEL-5	99.96	105.16	101.50	98.71	99.15	89.59	98.65	88.35	93.67
UACC-257	101.42	102.68	102.79	98.92	109.78	99.71	103.60	99.67	98.08
UACC-62	92.99	91.75	91.98	89.18	97.57	86.70	98.86	89.51	95.53
Ovarian cancer									
IGROV1	93.68	102.04	91.62	72.78	102.10	73.36	103.37	86.58	99.15
OVCAR-3	101.70	103.81	94.05	92.06	99.14	89.19	104.04	83.79	106.09
OVCAR-4	100.59	98.23	93.72	81.64	97.53	80.73	99.31	90.19	102.24
OVCAR-5	105.83	98.75	102.00	103.01	96.88	95.04	108.39	104.28	91.36
OVCAR-8	97.49	99.00	94.68	90.12	95.74	92.37	95.57	86.30	101.59
NC/ADR-RES	106.23	102.86	96.78	96.00	99.30	88.31	103.32	93.36	100.06
SK-OV-3	106.17	103.07	100.20	98.11	108.47	97.45	103.21	93.56	104.28
Renal cancer									
786-0	102.13	99.59	96.35	78.65	93.07	65.93	100.81	73.45	99.20
A498	88.02	98.79	87.51	73.94	105.47	91.88	108.04	94.35	97.65
ACHN	103.64	101.10	91.06	104.01	103.62	99.33	95.56	99.56	91.72
CAKI-1	94.93	90.83	91.13	97.60	106.07	86.81	90.67	88.27	90.58
RXF 393	111.70	108.81	106.34	88.51	98.30	69.43	105.86	57.77	102.96
SN12C	103.84	98.66	96.46	87.08	89.00	87.00	98.51	81.20	94.55

Table 1 (continued)

Subpanel tumor cell lines	Percentage cell growth for compounds								
	3	4	5	6	7	8	9	10	11
TK-10	104.95	101.96	103.64	104.11	102.09	111.21	115.54	101.45	97.42
UO-31	79.89	78.88	75.36	62.96	70.17	69.88	79.20	69.41	81.47
Prostate cancer									
PC-3	89.94	91.84	90.34	69.57	89.12	70.39	96.92	59.60	95.19
DU-145	107.86	107.09	100.65	95.42	100.96	96.49	107.77	65.36	112.02
Breast cancer									
MCF7	94.92	100.82	98.29	69.13	74.63	82.18	99.16	72.26	90.77
MDA-MB-231/ATCC	107.04	92.79	97.14	99.37	130.20	90.54	96.92	93.04	113.83
HS 578T	94.51	97.80	89.97	80.77	89.11	73.66	96.11	77.93	87.25
BT-549	104.89	99.83	109.09	92.27	92.51	89.74	104.55	84.80	93.51
T-47D	100.75	106.51	93.91	77.59	94.93	92.26	95.37	83.15	104.85
MDA-MB-468	95.51	106.10	97.40	73.70	99.71	87.19	100.17	58.61	88.94
	12	13	14	15	16	17	18	19	20
Leukemia									
CCRF-CEM	93.08	42.82	98.20	82.40	50.91	106.84	67.13	94.47	70.62
HL-60(TB)	96.60	**−74.30**	102.48	89.79	**42.95**	95.97	92.99	79.15	**37.18**
K-562	78.93	**14.77**	91.33	65.89	**11.02**	95.71	59.00	67.86	**33.37**
MOLT-4	79.34	**−57.86**	85.18	67.72	**28.06**	98.94	59.10	94.57	65.36
RPMI-8226	66.67	88.06	86.74	59.08	**16.92**	99.89	65.44	60.54	**20.16**
SR	83.85	**24.01**	89.05	**44.88**	**0.25**	99.45	**46.87**	89.28	**42.30**
NSC lung cancer									
A549/ATCC	92.86	101.97	102.35	68.13	59.03	105.81	86.32	87.43	62.13
EKVX	98.39	94.01	100.72	65.69	62.95	98.87	97.17	78.23	66.24
HOP-62	112.08	100.48	107.15	81.91	83.97	94.93	104.31	68.25	52.10
HOP-92	86.17	83.72	94.56	**49.59**	**48.80**	94.46	81.22	83.20	52.63
NCI-H226	–	94.56	–	–	–	–	–	–	–
NCI-H23	104.60	95.70	104.48	78.05	81.41	98.76	96.07	79.41	61.40
NCI-H322M	108.74	100.59	106.13	89.92	91.54	100.01	100.12	80.86	67.89
NCI-H460	104.56	100.91	109.23	86.41	**45.10**	87.19	92.19	**37.81**	**19.36**
NCI-H522	102.90	91.16	95.64	76.08	80.03	101.99	98.53	100.08	93.73
Colon cancer									
COLO 205	104.89	108.15	111.33	101.95	**50.00**	103.56	94.78	**38.94**	**21.56**
HCC-2998	106.89	104.07	99.28	88.00	72.80	101.56	105.21	70.59	52.15
HCT-116	101.95	99.87	104.25	75.64	**20.21**	94.07	79.27	81.89	**47.14**
HCT-15	89.51	94.53	101.95	82.71	**31.02**	104.12	84.37	83.45	76.83
HT29	92.85	103.34	105.03	81.94	**14.84**	100.10	59.69	**48.97**	**31.10**
KM12	101.62	102.16	108.34	78.76	72.91	90.88	88.25	**39.03**	**21.66**
SW-620	101.64	96.20	104.33	100.09	63.49	100.23	93.88	74.77	55.83
CNS cancer									
SF-268	94.56	98.93	98.97	84.33	64.08	101.23	87.03	94.13	81.29
SF-295	100.40	96.29	104.28	72.64	85.68	100.11	100.97	69.09	**41.95**
SF-539	99.50	96.10	104.45	71.78	69.96	107.61	99.85	88.34	65.96
SNB-19	98.67	99.61	98.17	82.20	81.86	99.07	96.09	79.75	61.76
SNB-75	87.91	97.71	80.65	63.75	65.11	108.54	90.81	101.82	93.42
U-251	89.67	99.46	99.98	76.80	**49.17**	105.95	82.08	88.25	71.51
Melanoma									
LOX IMVI	100.15	97.94	97.36	77.62	**47.29**	90.77	85.58	83.81	52.94
MALME-3M	99.41	95.56	102.26	93.60	67.98	94.71	95.29	72.05	**47.24**
M14	99.85	92.21	98.88	78.26	**49.75**	95.09	91.31	73.80	**39.98**
MDA-MB-435	98.21	99.69	103.96	88.04	75.71	102.51	91.64	62.01	**8.03**
SK-MEL-2	122.53	100.36	105.08	97.22	94.42	105.77	113.82	105.96	98.11
SK-MEL-28	107.39	92.92	110.44	94.36	74.56	104.06	103.96	61.12	**19.88**
SK-MEL-5	86.95	96.17	90.98	74.76	59.44	100.88	95.82	57.39	**12.55**

Table 1 (continued)

	12	13	14	15	16	17	18	19	20
UACC-257	103.95	98.07	111.24	93.14	75.28	103.52	99.32	102.13	91.88
UACC-62	92.58	94.90	90.00	70.98	69.28	95.70	98.08	63.02	**31.92**
Ovarian cancer									
IGROV1	102.70	105.16	105.89	62.90	70.28	97.14	89.80	51.43	**41.91**
OVCAR-3	102.20	100.57	119.02	75.64	69.56	107.71	94.18	101.91	82.96
OVCAR-4	100.74	101.33	105.34	66.79	61.24	100.46	96.70	91.85	85.09
OVCAR-5	103.71	102.14	112.60	104.81	88.71	101.19	101.71	100.24	88.10
OVCAR-8	96.14	102.25	99.29	70.02	76.06	93.72	91.02	85.11	54.55
NC/ADR-RES	95.43	95.11	97.65	76.88	64.61	102.59	98.38	88.20	65.85
SK-OV-3	112.77	100.21	106.60	107.91	76.69	100.55	104.35	86.36	91.31
Renal cancer									
786-0	90.88	105.49	95.75	84.04	55.39	93.86	97.10	64.79	**44.78**
A498	112.22	104.47	102.63	83.94	98.11	92.35	100.58	76.76	69.09
ACHN	104.30	102.47	102.95	76.09	79.21	96.50	95.44	70.78	**42.39**
CAKI-1	89.20	91.59	94.86	77.96	72.45	96.34	95.36	81.10	61.89
RXF 393	103.20	98.92	107.33	71.69	**49.56**	103.32	90.48	88.83	75.83
SN12C	100.39	98.37	99.09	76.02	75.63	100.78	90.18	63.49	**38.18**
TK-10	109.72	95.07	111.55	98.73	110.77	102.26	96.28	84.43	69.36
UO-31	79.91	81.22	94.31	58.56	**47.51**	83.35	78.27	61.63	51.69
Prostate cancer									
PC-3	83.54	92.76	91.22	60.43	**31.32**	96.65	84.29	91.51	77.25
DU-145	102.12	105.48	102.31	98.78	74.76	107.04	98.08	91.01	70.53
Breast cancer									
MCF7	98.03	90.73	102.32	84.42	**49.52**	102.77	84.25	95.31	95.61
MDA-MB-231/ATCC	109.68	109.04	104.36	72.44	63.62	98.20	102.34	88.23	55.96
HS 578T	92.75	95.27	98.05	74.97	67.28	94.43	89.95	96.03	82.69
BT-549	113.65	100.69	106.21	94.96	76.20	104.27	114.05	93.74	80.38
T-47D	98.90	102.81	98.75	67.66	62.34	95.75	88.97	65.14	56.97
MDA-MB-468	93.40	94.57	109.79	81.94	76.78	100.99	89.62	92.76	78.52

Survival of cells cultivated in the presence of 10 μM of a compound under examination (in percent) compared with control cells (without the addition of a compound to the culture medium) is given. Negative values correspond to cell death. The symbol "–" designates the absence of data

Most active compounds are shown in bold

SR cell lines (cell viability <29%). 3-(Benzylamino)-11-hydroxy-fusidate **16** showed high antiproliferative effect against leukemia cells: K-562, MOLT-4, RPMI-8226, and SR (cell viability 0.25–28%), colon cancer: HCT-116, HCT-15, and HT-29 (cell viability were 20, 31, and 15%, respectively) and prostate cancer PC-3 (cell viability 31%). Methylfusidate derivative containing spermine substitute (**20**) demonstrated the widest range of cytotoxic action among the synthesized compounds. It turned out to be highly active against cell lines: leukemia (RPMI-8226), NSC lung cancer (NCI-H460), colon cancer (COLO 205, HT29, KM12), and melanoma (MDA-MB-435, SK-MEL-28, SK-MEL-5, UACC-62), leaving no more than 32% of cancer cells viable. As well, amines **15**, **16**, **18**–**20** exhibited moderate growth inhibition effects against leukemia (SR), NSC lung cancer (HOP-92, NCI-H460), colon cancer (COLO-205, HCT-116, HT-29, KM12), CNS cancer (SF-295, U-251), melanoma (LOX IMVI, MALME-3M, M14), ovarian cancer (IGROVI), renal cancer (786-0, ACHN, RXF 393, SN12C, UO-31), and breast cancer (MCF7) (cell viability 38–50%) (Table 1).

Based on the results of the antitumor activity study, the following conclusions on the structure-activity relationships should be formulated:

(1) Comparison of the antitumor activity of 3-amino-substituted compounds **3**–**8** containing keto group at C11 showed that the transformation of the carboxyl group at C21 into ester leads to an increase in the cytotoxic effect on the leukemia cell line; methylfusidate with pyrrolidine fragment at the C3 position (**7**) was the most active;
(2) The antiproliferative action of compounds **11**–**14** shows that in presence of 11-OH group the greatest cytotoxic effect was found for the derivatives with a free 21-carboxyl function of the molecule; pyrrolidine substituted derivative **13** showed the better activity;
(3) In the series of compounds **9**, **10**, and **17**–**20** with polyamine fragments, the introduction of short-chain diamine group into the FA structure provides an increasing of the selectivity towards the leukemia cells (HL-60 (TB), K-562, MOLT-4, and SR), while the

functionalization of the molecule by long-chain amines extends the spectrum of antitumor action (leukemia, NSC lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, and renal cancer); thus, 3-[(2-aminoethyl) amino] -fusidate **10** had the highest selectivity, the widest spectrum of antiproliferative activity was shown by 3-{[3-({4-[(3-aminopropyl) amino]butyl} amino)propyl]amino}-fusidate **20**;

(4) Comparison of FA analogs activity with simple amine substituents-pyrrolidine or *n*-butylamine indicates the fact that the introduction of a pyrrolidine fragment into the molecule leads to an increase in antitumor activity toward the leukemia cell line;

(5) In most cases, the presence of an ester group at C21 turned out to be an important factor for the manifestation of a cytotoxic effect: the amino derivatives of methylfusidate exhibited a greater antiproliferative effect as compared with similar analogs of FA.

Conclusion

Thus, a number of new amino derivatives of fusidic triterpenoids were synthesized, containing linear, aromatic, heterocyclic, and polyamine substituents. The results of biological in vitro tests showed that the introduction of pyrrolidine, *n*-butylamine, benzylamine, and ethylenediamine fragments into C3 position of FA molecule contributes to the appearance of a pronounced selective action in relation to the entire subpanel of the leukemia cell line. Functionalization of the molecule with long-chain amines—spermine and spermidine—leads to the expansion of the spectrum of antitumor action. The results of our work highlight the potential of 3-amino derivatives of FA as a starting point for more in-depth research and development of new cytotoxic agents with antitumor action.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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