Hemisynthesis and antiproliferative properties of mono-[O-(14-benzoylaconine-8-yl)] esters and bis-[O-(14-benzoylaconine-8-yl)]esters. Eur J Med Chem

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Original article

Hemisynthesis and antiproliferative properties of mono-[*O*-(14-benzoylaconine-8-yl)]esters and bis-[*O*-(14-benzoylaconine-8-yl)]esters

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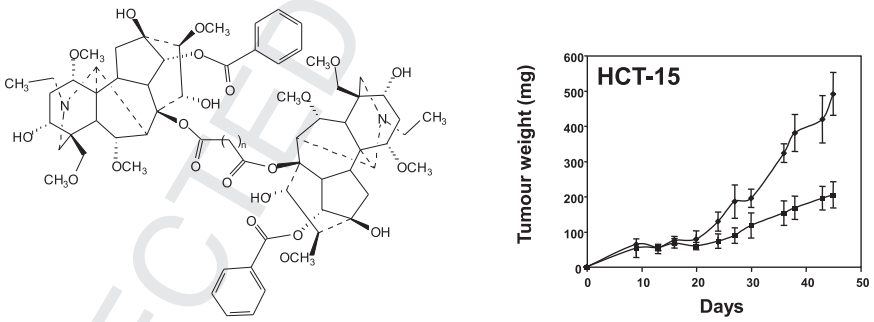
HIGHLIGHTS

- We synthesised a series of compounds derived from *Aconitum* alkaloids.
- Three of them displayed anti-proliferative activity against human tumour cell lines.
- Antitumour activity was detected in human tumour xenografts in immunodeficient mice.

GRAPHICAL ABSTRACT



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ABSTRACT

A series of mono- and bifunctional acyl compounds, build from the 8-*O*-azeloyl-14-benzoylaconine scaffold and differing by the length of the alkyl linker chain, were synthesised and evaluated against a panel of human tumour cell lines, A-549 (lung cancer), MCF-7 (breast cancer) and HCT-15 (colon cancer). None of the mono-[*O*-(14-benzoylaconine-8-yl)]esters displayed *in vitro* activity against tumour cells (IC_{50} > 60 μM). However, three bis-[*O*-(14-benzoylaconine-8-yl)]esters presented a noticeable *in vitro* cytotoxic activity, those bearing 7, 8 and 9 carbon atoms between the two aconitine moieties, with IC_{50}s ranging between ~~3.7 and 22 μM~~. The most active, bis[*O*-(14-benzoylaconine-8-yl)]suberate, was then evaluated *in vivo* in immunodeficient mice bearing human tumour xenografts originating from MCF-7 and HCT-15 cells. For MCF-7 cells, administration of five doses every 4 days, and weekly administration of 4 doses resulted in T/C percent values of 36% ($p = 0.001$) and 56% ($p = 0.02$) on day 45, respectively. For HCT-15 cells, administration of five doses every 3 days resulted in 49% tumour regression on the 25th day ($p = 0.00001$).

1. Introduction

The plant kingdom harbours the largest reservoir of pharmacologically active natural compounds. About 40% of all drugs derive directly or indirectly from plant alkaloids, and only a small fraction of the biosphere has been yet analysed. Several potent anticancer drugs of widespread use were isolated from plants, among which

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vinblastine, paclitaxel (Taxol®) and camptothecin are the most renowned. In an attempt to identify new potential anticancer drugs, we recently discovered that 8-*O*-azeloyl-14-benzoylaconine, an aconitine derivative, extracted from the roots of the Asian plant *Aconitum karakolicum* Rapcs displayed cytotoxic properties against human tumour cells in culture [1].

Plants of the *Aconitum* genus have been used in traditional medicine in many Asian and former USSR countries. In spite of their high toxicity, *Aconitum* plants display analgesic, anti-inflammatory and anti-arrhythmic activities, and their use has been proposed in several diseases and pathological conditions [2]. The principal chemical constituents of the *Aconitum* genus are diterpenoid alkaloids such as aconitine, mesaconitine, lappaconitine and songorine. Other new other new alkaloids have been recently isolated and studied [3–6]. Toxicological and pharmacological properties of purified compounds from *Aconitum* have been studied *in vitro* and *in vivo*: aconitine, 3-acetylaconitine and hypaconitine were found to be the most toxic alkaloids, inhibiting the respiratory and cardiovascular systems [7]. The proposed mechanism of the toxic effect of the principal alkaloid, aconitine, is the block of the voltage-gated Na^+ channels, inducing an influx of Na^+ ions into cells, which finally results in permanent neuron depolarisation and inhibition of neuronal conductivity [8].

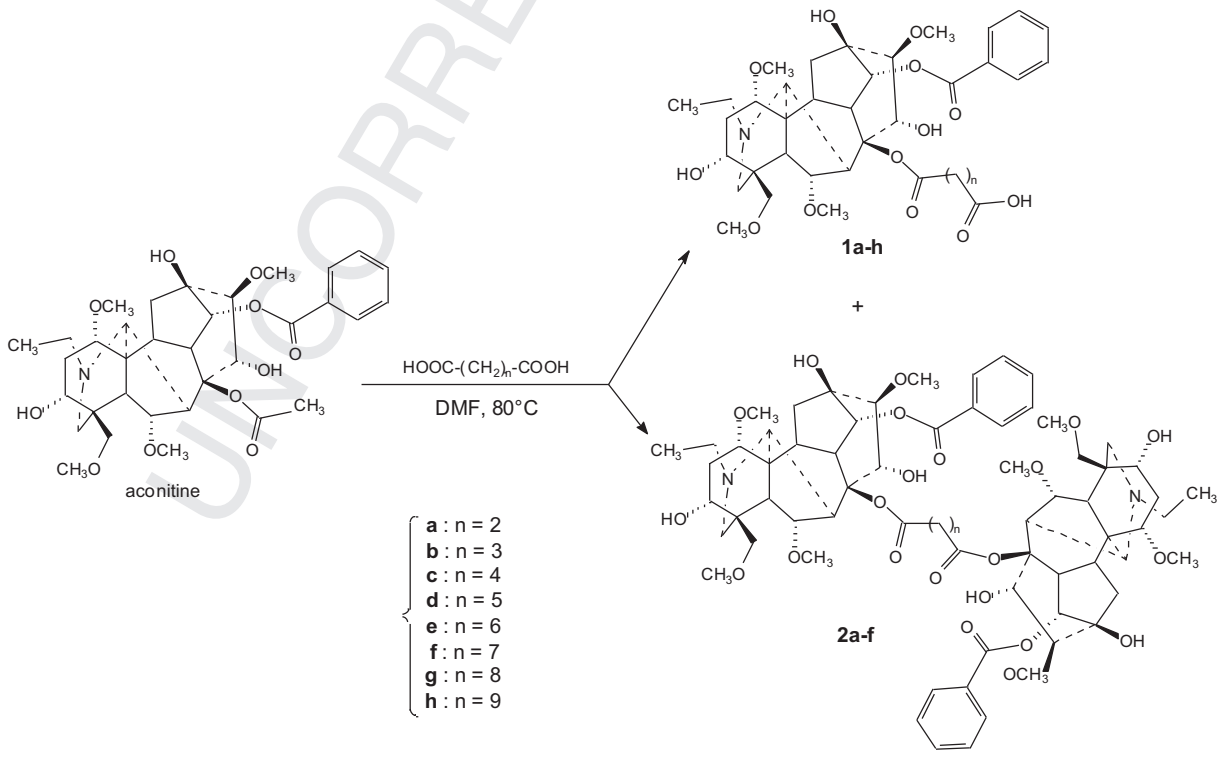
In spite of various and detailed studies of *Aconitum* alkaloids, very little information is available on their antitumour activity. De Ines et al. [9] showed the cytotoxic effect of various C_{19}-norditerpenoid alkaloids against several tumour cell lines. Hazawa et al. [10–12] reported that semi-synthetic derivatives of C_{20}-norditerpenoid alkaloids, *e.g.* 11-veratroylpseudokobustine, 11-(*m*-trifluoromethylbenzoyl)kobustine, 11-(*m*-trifluoromethylbenzoyl) pseudokobustine and 11,15-dianisoylpseudokobusine, exhibited cytotoxic activity against A172, A-549, HeLa and Raji cell lines. Yet, the mechanism of the cytotoxic activity of *Aconitum* derivatives remained unclear.

In the present work, the previously described 8-*O*-azeloyl-14-benzoylaconine [1], was used as a basis for the design and hemisynthesis of new structural analogues. A series of mono- and bifunctional acyl compounds, build from the 8-*O*-azeloyl-14-benzoylaconine scaffold and differing by the length of the alkyl linker, were synthesised and evaluated against a panel of human tumour cell lines. The most active, bis[*O*-(14-benzoylaconine-8-yl)] suberate was then evaluated *in vivo* in appropriate models of immunodeficient mice bearing human tumour xenografts. We report here the hemisynthesis method and preliminary anticancer activity data of new mono-[*O*-(14-benzoylaconine-8-yl)]esters and bis-[*O*-(14-benzoylaconine-8-yl)]esters.

2. Results

2.1. Chemistry

Commercially available reagents were used as received without additional purification. The aliphatic acid mono- and di-esters of aconitine **1a**–**h** and **2a**–**f** were prepared from commercially available aconitine using a modified procedure adapted from Pelletier [13]. The reaction of aconitine with various aliphatic diacids in DMF at 80 °C led to the formation of the mono-[*O*-(14-benzoylaconine-8-yl)]esters **1a**–**h** and bis-[*O*-(14-benzoylaconine-8-yl)]esters **2a**–**f** through a transesterification carried out on the 8-acetyl functionality of the aconitine moiety (Scheme 1). The reaction mixtures were heated during 7 h in an oil bath for which the temperature was regulated at 80 °C, due to the heat-sensitivity of reaction products [14]. Purification by HPLC of the reaction mixture led to the isolation of **1** and **2** with yields ranging from 37 to 44% and from 18 to 25%, respectively. Structural identification was performed using high resolution MS–MS mass spectrometry and ^1H, ^{13}C, 2D NOESY NMR spectroscopy analysis. The isolation of a suitable trifluoroacetate salt of **1d** allowed to establish unambiguously its 3D



Scheme 1. Synthesis of new mono-[*O*-(14-benzoylaconine-8-yl)]esters **1a**–**h** and bis-[*O*-(14-benzoylaconine-8-yl)]esters **2a**–**f**.

spatial structure by single crystal X-ray crystallography, with one alkyl diacid moiety at C-8 of aconitine scaffold (Fig. 1). Moreover, this established the *R*-configuration of carbon C-8 of **1d**. With reference to aconitine [14], no epimerisation occurred during the trans-esterification procedure.

2.2. Biological activity

2.2.1. In vitro antiproliferative activity

The two series of compounds **1a**–**h** and **2a**–**f** were tested against three human tumour cell lines, A-549 (lung cancer), MCF-7 (breast cancer), and HCT-15 (colon cancer). None of the mono-[*O*-(14-benzoylaconine-8-yl)]esters displayed *in vitro* activity against tumour cells ($IC_{50} > 60$ μM) (Fig. 2). Concerning the bis-[*O*-(14-benzoylaconine-8-yl)]esters **2d**–**f**, there was a discrepancy between compounds with a C-4, C-5 or C-6 linker chain and those with a C-7, C-8 or C-9 linker chain. As depicted in Table 1, only the latter group presented a noticeable *in vitro* cytotoxic activity (IC_{50} ranging from ~~3.7 to 22~~ μM) (Figs. 2 and 3, Table 1).

2.2.2. In vivo testing of bis[O-(14-benzoylaconine-8-yl)]suberate (*2e*)

An *in vivo* study was undertaken for the bis[*O*-(14-benzoylaconine-8-yl)]suberate **2e**. It was evaluated for anticancer activity in immunodeficient mice xenografted subcutaneously with human tumour cells, at the dose of 10 mg/kg. The same tumours as those used for the *in vitro* evaluation were chosen for the *in vivo* studies. However, the A-549 cell line grew very slowly *in vivo* and was not considered as suitable for antitumour evaluation.

Compound **2e** was well tolerated and neither mortality nor toxic effects were observed during animal experiments (Table 2). Moreover, concerning weight variation of the treated mice, only one group of treated mice presented a moderate weight loss of 9.34% in reference to the control group.

For MCF-7 cells, administration of five doses every 4 days, and weekly administration of four doses resulted in T/C percent values of 36% ($p = 0.001$) and 56% ($p = 0.02$) on day 45, respectively (Fig. 2). For HCT-15 cells, administration of five doses every 3 days resulted in 49% tumour regression on the 25th day (Fig. 2, $p = 0.00001$). These preliminary *in vivo* results are encouraging and warrant further investigation, which is presently ongoing.

3. Discussion

In a previous study, we reported the preliminary screening cytotoxic activity of the naturally occurring 8-*O*-azeloyl-14-benzoylaconine, isolated from *A. karakolicum*, against three types of *in vitro* human tumour cell lines [1]. We undertook further progress of this work by the design and hemisynthesis of new structural analogues bearing various alkyl chains on the heterocyclic moiety, and the evaluation of their antiproliferative activity.

From the results depicted in Table 1, it appears that the replacement of the azeloyl moiety by C-4 to C-11 analogues (compounds **1a**–**h**) led to compounds devoid of cytotoxic activity ($IC_{50} > 60$ μM). On the other hand, some related di-esterified compounds **2** exhibited notable cytotoxic activities against several tumour cell lines. These findings show that potency is affected by structural change and also indicate that the binding site

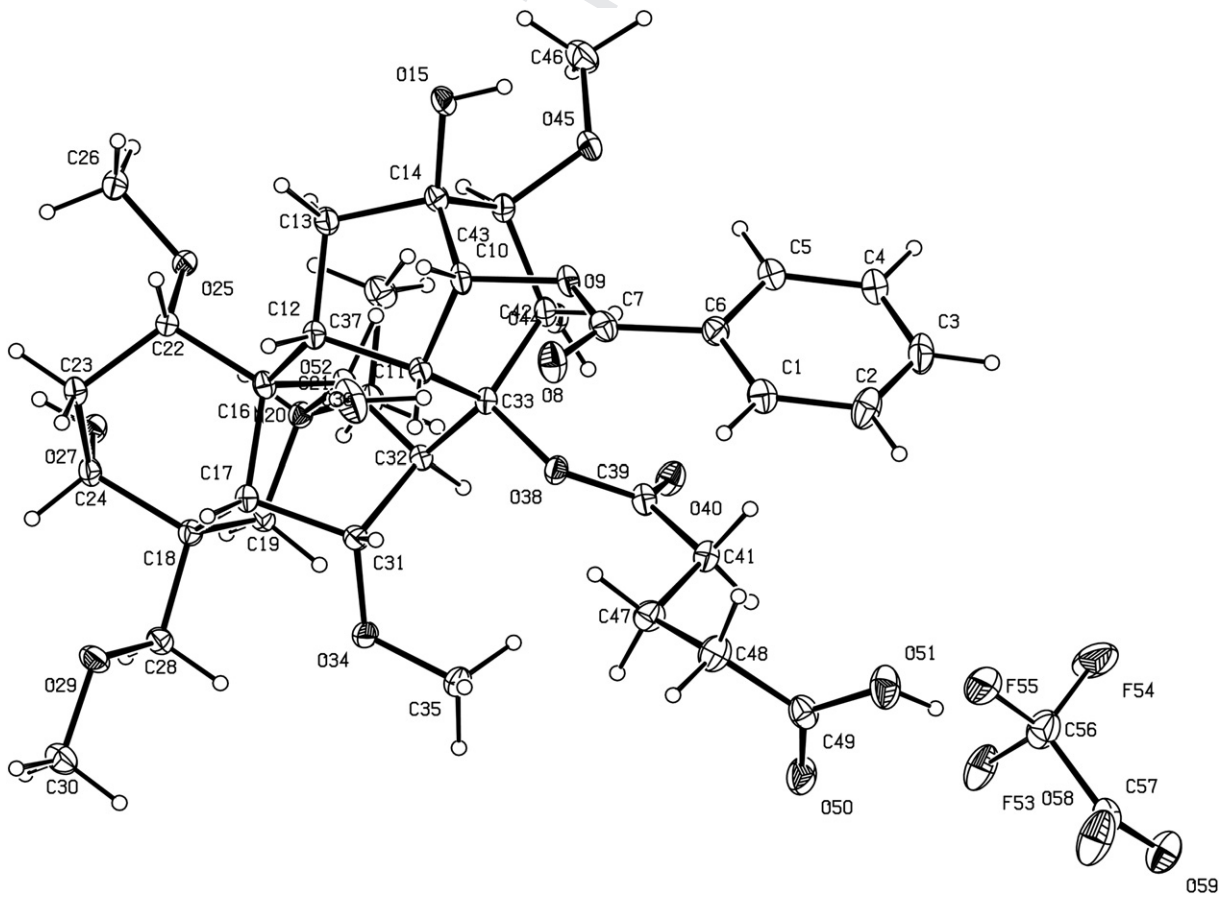


Fig. 1. The ORTEP drawing of mono-[*O*-(14-benzoylaconine-8-yl)]succinate 1d as trifluoroacetate salt with thermal ellipsoids at 20% level.

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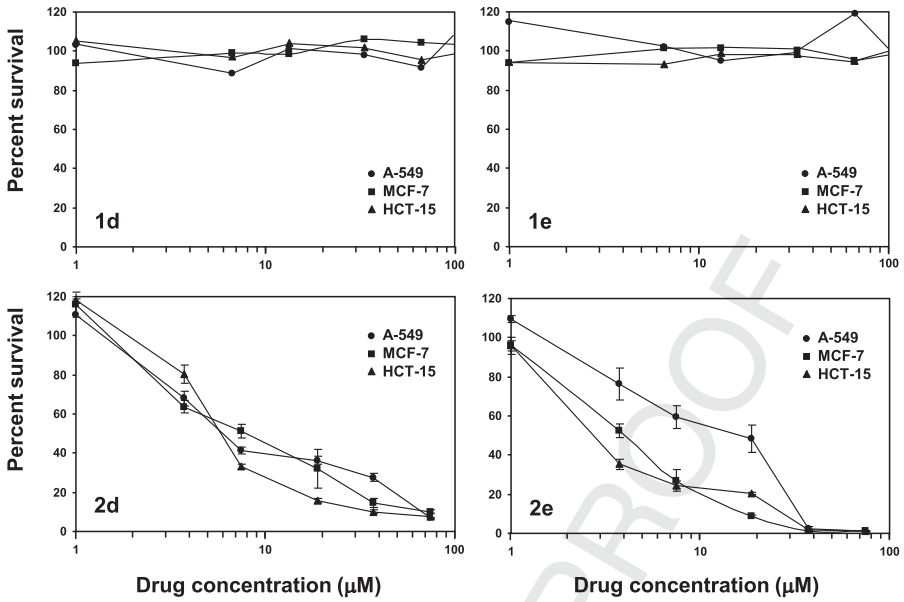

Fig. 2. *In vitro* cytotoxicity of compounds **1d**, **1e**, **2d** and **2e** against three human tumour cell lines: A-549; MCF-7; HCT-15. A representative experiment made in triplicate is shown.

of the target cancer cell may have a strict structural requirement. Finally, this difference of *in vitro* cytotoxic behaviour between mono- and bis-[*O*-(14-benzoylaconine-8-yl)]esters prompted us to select the latter for further *in vivo* studies performed with breast cancer (MCF-7) and colon cancer (HCT-15) cells.

Among compounds **2a**–**f**, the bis-[*O*-(14-benzoylaconine-8-yl)] esters **2d**–**f** linked by a C-7 to C-9 moiety, exhibited the best antiproliferative activity on the human tumour cell lines (IC_{50} from ~~3.70 to 22 μM~~) in comparison with their C-4 to C-6 linked analogues **2a**–**c** (IC_{50} > 60 μM). ~~Hence, **2d** was found as the most active compound on the A-549 cell line (IC_{50} = 3.76 μM). The replacement of the pimelate (**2d**) linker by a suberate (**2e**) or an azelate one (**2f**) led to a slight decrease in the antiproliferative activity (IC_{50} = 18.7 and 19.1 μM, respectively). In the MCF-7 cell line, the diester **2e**, bearing a suberate linker, was found more potent than its pimelate analogue **2d** with a 3.70 μM versus 7.5 μM IC_{50}. In the HCT-15 colon cancer cell line, the two di-esters **2d, 2e**, bearing a pimelate or suberate linker, were found equally active with 3.70–3.76 μM IC_{50}. In addition, the bis-[*O*-(14-benzoylaconine-8-yl)]ester **2f** was found less potent (IC_{50} = 22 μM) against this colon cancer line.~~

The *in vitro* activity of the diester compounds **2d**–**f** was encouraging enough to test the activity of **2e** on the same human tumour models growing *in vivo* in immunodeficient mice. Antitumour activity was clearly detected at the dose of 10 mg/kg, a dose largely below the maximum tolerated dose (15 mg/kg). Thus, obtaining an antitumour activity before any optimisation of the dose to be injected, or of the tumour models to be tested, appears very promising and prompted us to patent new hemisynthetic bis [*O*-(14-benzoylaconine-8-yl)]esters [15].

4. Conclusion

In the present report, we described the synthesis of new series of mono-[*O*-(14-benzoylaconine-8-yl)]esters **1a**–**h** and bis-[*O*-(14-benzoylaconine-8-yl)]esters **2a**–**f** through a transesterification carried out on the 8-acetyl function of the aconitine moiety. Their antiproliferative activity on the human cancer cell lines A-549, MCF-7 and HCT-15 shows that the bis-[*O*-(14-benzoylaconine-8-yl)]esters **2a**–**f** are more active than their mono-[*O*-(14-benzoylaconine-8-yl)]esters counterparts **1a**–**h**, indicating that

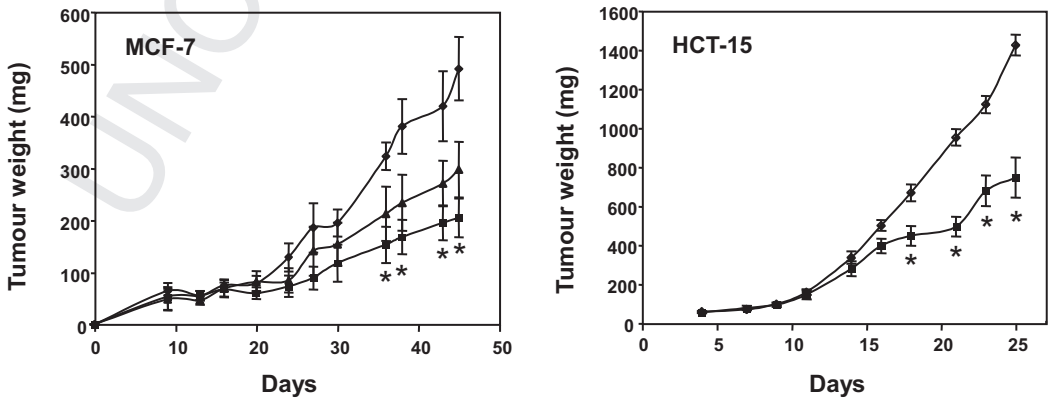

Fig. 3. *In vivo* antitumour activity of bis[*O*-(14-benzoylaconine-8-yl)]suberate 2e on MCF-7 and HCT-15 tumour models grown in immunodeficient mice. Two schedules were used for MCF-7 tumours (see Table 2). Tumour weights are means ± s.e.m. of 8–10 individual values. Each asterisk indicates a significant difference between treated and control animals at the $p < 0.01$ level.

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Table 1
In vitro cytotoxicity of bis[*O*-(14-benzoylaconine-8-yl)]esters **2a**–**f** against human tumour cell lines.

Compound		IC_{50} (μM)		
		A-549	MCF-7	HCT-15
bis-[*O*-(14-benzoylaconine-8-yl)]-succinate	**2a**	>60	>60	>60
bis-[*O*-(14-benzoylaconine-8-yl)]-glutarate	**2b**	>60	>60	>60
bis-[*O*-(14-benzoylaconine-8-yl)]-adipate	**2c**	>60	>60	>60
bis-[*O*-(14-benzoylaconine-8-yl)]-pimelate	**2d**	9.50 ± 3.21	7.56 ± 0.84	4.64 ± 1.53
bis-[*O*-(14-benzoylaconine-8-yl)]-suberate	**2e**	7.53 ± 3.08	6.90 ± 1.62	4.01 ± 0.51
bis-[*O*-(14-benzoylaconine-8-yl)]-azelate	**2f**	19.5	16.9	28.0

IC_{50} values are means ± s.e.m. of 2–4 experiments.

the binding site of the target cancer cell may have a strict structural requirement. Furthermore, compounds **2d**–**f** linked by a pimelate, a suberate or an azelate moiety are the most active with IC_{50} ranging from ~~3.7 to 22~~ ~~μM~~, enlightening the influence of the length of the alkyl linker.

Based on these preliminary results, further studies to enlarge the biological evaluation of the most active bis-[*O*-(14-benzoylaconine-8-yl)]esters are in progress in order to address its mechanism of action. This series can be considered as a promising lead for future rational design of novel antiproliferative agents.

5. Experimental procedures

5.1. Chemistry

Commercially available reagents were used as received without additional purification. Melting points were determined with an SM-LUX-POL Leitz hot-stage microscope and are uncorrected. IR spectra were recorded on a BRUKER IFS-25 spectrophotometer. NMR spectra were recorded with tetramethylsilane as an internal standard using a BRUKER AVANCE III/600 spectrometer (2D-COSY, HSQC and HMBC). Splitting patterns have been designated as follows: s = singlet; bs = broad singlet; d = doublet; *t* = triplet; *q* = quartet; qt = quintuplet; m = multiplet. Analytical TLC was carried out on 0.25 precoated silica gel plates (POLYGRAM SIL G/UV_{254}) with visualisation by irradiation with a UV lamp. The conditions used for HPLC purification were the following: Column: Ultrasep ES 10, RP 18 6.0 μm reversed phase, C18 column, 250 × 10 mm (Bischoff, Germany). Solvents: A: trifluoroacetic acid/water 1/1000; B: trifluoroacetic acid/acetonitrile 1/1000. Elution conditions: injection of a volume of 0.5 mL; gradient from 25% of B to 50% in 50 min, followed by 100% of B during 10 min. Absorbance was monitored at 234 nm. High-resolution ESI mass measurements were performed on an Applied Biosystems QStar mass spectrometer equipped with an electrospray source in positive mode.

5.1.1. General procedure for synthesis of mono-[*O*-(14-benzoylaconine-8-yl)]esters (1) and bis[*O*-(14-benzoylaconine-8-yl)]esters (**2**)

Aconitine (200 mg, 0.30 mmol) and aliphatic diacid (0.15 mmol) in DMF (5 mL) were heated at 80 °C under stirring during 7 h, then stirred at room temperature overnight. The solvent was then removed under reduced pressure at a temperature lower than 70 °C. The oily residue was purified by semi-preparative high pressure liquid chromatography to give mono-[*O*-(14-benzoylaconine-8-yl)]esters **1a**–**g** and bis[*O*-(14-benzoylaconine-8-yl)]esters **2a**–**f**. Flash chromatography on silica gel was used to isolate the mono-[*O*-(14-benzoylaconine-8-yl)]ester **1h**.

*5.1.1.1. 8-O-(14-Benzoylaconine)succinate (**1a**).* White powder (39%); t_R = 7.8 min; ^{1}H NMR spectrum (400.13 MHz, $CDCl_3$) δ: 8.09 (m, 1H, NH^+), 8.03 (d, 2H, *J* = 7.7 Hz, H-2′/H-6′), 7.64 (*t*, 1H, *J* = 7.3 Hz, H-4′), 7.51 (*t*, 2H, *J* = 7.6 Hz, H-3′/H-5′), 4.97 (d, 1H, *J* = 4.4 Hz, H-14), 4.55 (d, 1H, *J* = 4.8 Hz, H-15), 4.32 (br s, 1H, H-3), 4.18 (d, 1H, *J* = 6.0 Hz, H-6), 3.81 (s, 3H, 16−OCH_3), 3.69 (m, 1H, H-19β), 3.54 (br s, 1H, H-1), 3.47 (m, 1H, 18β), 3.41 (s, 3H, 1−OCH_3), 3.39 (s, 1H, H-17), 3.31 (s, 3H, 18−OCH_3), 3.29 (m, 4H, N−CH_2−CH_3, H-16, H-18α), 3.27 (s, 3H), 6(−OCH_3), 3.21 (m, 1H, H-19α), 3.02 (br s, 1H, H-5), 2.97 (m, 1H, H-9), 2.52 (d, 1H, *J* = 5.9 Hz, H-7), 2.42 (m, 1H, H-2β), 2.40 (br d, 1H, H-10), 2.34 (br d, 3H, H-12β, 8−CO_2−$(CH_2)2''$), 1.88 (br d, 1H, H-12α), 1.55 (m, 3H, 8−CO_2−$(CH_2)1''$, H-2α), 1.45 (br s, 3H, N−CH_2−CH_3). ^{13}C NMR ($CDCl_3$) δ: 175.0 (COOH), 173.90 (8-COO), 166.0 (C-7′), 134.16 (C-4′), 129.86 (C-2′/C-6′), 129.50 (C-1′), 129.16 (C3′/C-5′), 90.48 (C-8), 89.92 (C-16), 81.99 (C-6), 79.87 (C-1), 78.72 (C-15), 78.58 (C-14), 76.23 (C-18), 74.06 (C-13), 69.98 (C-3), 63.72 (C-17), 61.73 (16-OCH_3), 59.22 (18-OCH_3), 58.06 (6-OCH_3), 55.40 (1-OCH_3), 50.42 (C-19), 50.41 (C-11), 50.40 (N−CH_2−CH_3), 44.95 (C-4), 43.48 (C-4), 43.27 (C-9), 42.78 (C-7), 39.84 (C-10), 35.09 (C-12), 29.42 (C-2), 29.19 (C-1″, C-2″), 10.81 (N−CH_2−CH_3). HRMS $[M]^+$ $C_{36}H_{49}NO_{13}$ requires 703.78, found 704.3.

*5.1.1.2. 8-O-(14-Benzoylaconine)glutarate (**1b**).* White powder (37%); t_R = 15.3 min; ^{1}H NMR spectrum (400.13 MHz, $CDCl_3$) δ: 8.18 (m, 1H, NH^+), 8.02 (d, 2H, *J* = 5.12 Hz, H-2′/H-6′), 7.64 (*t*, 1H, *J* = 5.0 Hz, H-4′), 7.51 (*t*, 2H, *J* = 5.06 Hz, H-3′/H-5′), 4.93 (d, 1H, *J* = 3.0 Hz, H-14), 4.53 (d, 1H, *J* = 3.5 Hz, H-15), 4.30 (br s, 1H, H-3), 4.16 (d, 1H, *J* = 4.08 Hz, H-6), 3.82 (s, 3H, 16-OCH_3), 3.70 (m, 1H, H-19β), 3.52 (br s, 1H, H-1), 3.50 (m, 1H, H-18β), 3.40 (s, 3H, 1-OCH_3), 3.36 (s, 1H, H-17), 3.30 (s, 3H, 18-OCH_3), 3.29 (m, 2H, N−CH_2−CH_3), 3.28 (s, 1H, H-16), 3.27 (s, 1H, H-18α), 3.25 (s, 3H, 6-OCH_3), 3.19 (m, 1H, H-19α), 2.97 (m, 2H, H-5, H-9), 2.59 (d, 1H, *J* = 3.8 Hz, H-7), 2.42 (br d, 1H, H-2β), 2.39 (br d, 1H, H-10), 2.36 (br d, 1H, H-12β), 2.07 (m, 1H, 8−CO_2−$(CH_2)3''$β), 2.0 (m, 2H, 8−CO_2−$(CH_2)1''$β, 8−CO_2−$(CH_2)3''$α),1.92 (d, 1H, *J* = 6.64 Hz, H-12α), 1.54 (m, 2H, 8−CO_2−$(CH_2)1''$α, 8−CO_2−$(CH_2)2''$β), 1.46 (br s, 3H, N−CH_2−CH_3), 1.44 (m, 2H, H-2α, 8−CO_2−$(CH_2)2''$α). ^{13}C NMR ($CDCl_3$) δ: 175.0 (COOH), 174.42 (8-COO), 165.68 (C-7′), 133.91 (C-4′), 129.63 (C-2′/C-6′), 129.07 (C-1′), 129.95 (C3′/C-5′),

Table 2
In vivo evaluation of antitumour efficacy of bis[*O*-(14-benzoylaconine-8-yl)]suberate (**2e**).

Tumour	Treatment schedule	Weight loss %	Tumour weight (mg)		*p* value	%T/C
			Control	Treated		
HCT-15	Q3D × 5, st. Day 7	9.34	1425 ± 166	776 ± 358	0.00001	49
MCF-7	Q7D × 4, st. Day 13	0	491 ± 137	297 ± 142	0.0394	56
	Q4D × 5, st. Day 9	0		205 ± 99	0.0022	36

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89.88 (C-8), 89.60 (C-16), 82.11 (C-6), 79.64 (C-1), 78.56 (C-15), 78.07 (C-14), 75.63 (C-18), 73.82 (C-13), 63.61 (C-3), 63.09 (C-17), 61.66 (16-OCH_3), 59.03 (18-OCH_3), 58.90 (6-OCH_3), 55.15 (1-OCH_3), 50.33 (C-19), 50.19 (C-11), 50.11 (N—CH_2—CH_3), 46.76 (C-4), 44.75 (C-5), 43.17 (C-9), 41.87 (C-7), 39.75 (C-10), 34.85 (C-12), 33.22 (C-1″), 32.30 (C-3″), 29.27 (C-2), 19.08 (C-2″), 10.70 (N—CH_2—CH_3). HRMS $[M]^+$ $C_{37}H_{51}NO_{13}$ requires 717.81, found 718.3.

*5.1.1.3. 8-O-(14-Benzoylaconine)adipate (**1c**).* White powder (42%); t_R = 5.65 min; ^{1}H NMR spectrum (400.13 MHz, $CDCl_3$) δ: 8.23 (m, 1H, NH^+), 8.0 (d, 2H, *J* = 7.2 Hz, H-2′/H-6′), 7.63 (*t*, 1H, *J* = 7.4 Hz, H-4′), 7.49 (*t*, 2H, *J* = 7.6 Hz, H-3′/H-5′), 4.94 (d, 1H, *J* = 4.8 Hz, H-14), 4.55 (d, 1H, *J* = 5.2 Hz, H-15), 4.30 (br s, 1H, H-3), 4.15 (d, 1H, *J* = 6.0 Hz, H-6), 3.80 (s, 3H, 16-OCH_3), 3.75 (m, 1H, H-19β), 3.53 (m, 1H, H-18β), 3.5 (br s, 1H, H-1), 3.39 (s, 3H, 1-OCH_3), 3.34 (s, 1H, H-17), 3.29 (s, 3H, 18-OCH_3), 3.28 (m, 1H, 18α), 3.26 (br s, 2H, N—CH_2—CH_3), 3.25 (s, 1H, H-16), 3.24 (s, 3H, 6-OCH_3), 3.19 (m, 1H, H-19α), 2.96 (m, 1H, H-9), 2.94 (s, 1H, H-5), 2.59 (d, 1H, *J* = 6.4 Hz, H-7), 2.43 (br d, 1H, H-2β), 2.38 (d, 1H, *J* = 4.4 Hz, H-10), 2.34 (br d, 1H, H-12β), 2.13 (*t*, 2H, *J* = 6.4 Hz, 8—CO_2—$(CH_2)4''$), 1.92 (d, 1H, *J* = 9.6 Hz, H-12α), 1.87 (m, 1H, 8—CO_2—$(CH_2)1''\beta$), 1.56 (m, 1H, 8—CO_2—$(CH_2)1''\alpha$), 1.52 (m, 1H, H-2α), 1.46 (*t*, 3H, *J* = 6.8 Hz, N—CH_2—CH_3), 1.28 (m, 3H, 8—CO_2—$(CH_2)2''\beta$, 3″), 1.16 (m, 1H, 8—CO_2—$(CH_2)2''\alpha$). ^{13}C NMR ($CDCl_3$) δ: 177.70 (COOH), 174.97 (8-COO), 165.89 (C-7′), 134.2 (C-4′), 130.2 (C-2′/C-6′), 129.87 (C-1′), 129.15 (C3′/C-5′), 92.18 (C-16), 90.07 (C-8) 83.5 (C-6), 82.52 (C-1), 79.92 (C-15), 78.85 (C-14), 75.83 (C-18), 74.09 (C-13), 69.86 (C-3), 63.19 (C-17), 61.88 (16-OCH_3), 59.26 (18-OCH_3), 59.15 (6-OCH3), 55.35 (1-OCH_3), 50.64 (C-19), 50.48 (C-11), 50.35 (N—CH_2—CH_3), 46.01 (C-4), 45.07 (C-5), 43.55 (C-9), 42.0 (C-7), 40.12 (C-10), 35.12 (C-12), 34.29 (C-1″), 33.0 (C-4″), 29.79 (C-2), 24.04 (C-3″, C-2″), 8.83 (N—CH_2—CH_3). HRMS $[M]^+$ $C_{38}H_{53}NO_{13}$ requires 731.84, found 732.4.

*5.1.1.4. 8-O-(14-Benzoylaconine)pimelate (**1d**).* White powder (43%); t_R = 13.34 min; mp 107 °C. ^{1}H NMR spectrum (400.13 MHz, $CDCl_3$) δ: 8.15 (br s, 1H, NH+), 7.99 (d, 2H, *J* = 7.6 Hz, H-2′/H-6′), 7.62 (*t*, 1H, *J* = 7.4 Hz, H-4′), 7.48 (*t*, 2H, *J* = 7.4 Hz, H-3′/H-5′), 4.92 (d, 1H, *J* = 4.92 Hz, H-14), 4.50 (d, 1H, *J* = 5.2 Hz, H-15), 4.28 (br s, 1H, H-3), 4.14 (d, 1H, *J* = 5.6 Hz, H-6), 3.74 (m, 1H, H-19β), 3.80 (s, 3H, 16-OCH_3), 3.51 (m, 1H, H-18β), 3.5 (s, 1H, H-1), 3.38 (s, 3H, 1-OCH_3), 3.34 (s, 1H, H-17), 3.29 (s, 3H, 18-OCH_3), 3.26 (s, 1H, 18α), 3.25 (s, 1H, H-16), 3.22 (s, 3H, 6-OCH_3), 3.18 (m, 2H, N—CH_2—CH_3), 3.13 (m, 1H, H-19α), 2.96 (m, 1H, H-9), 2.94 (s, 1H, H-5), 2.56 (d, 1H, *J* = 5.2 Hz, H-7), 2.41 (m, 1H, H-2β), 2.38 (br s, 1H, H-12β), 2.35 (m, 1H, H-10), 2.23 (br s, 1H, H-12α), 1.85 (*q*, 2H, *J* = 8.4 Hz, 8—CO_2—$(CH_2)5''$), 1.53 (br d, 1H, H-2α), 1.44 (br s, 3H, N—CH_2—CH_3), 1.38 (m, 2H, 8—CO_2—$(CH_2)1''$), 1.31 (m, 2H, 8—CO_2—$(CH_2)2''$), 1.23 (*q*, 1H, *J* = 7.0 Hz, 8—CO_2—$(CH_2)4''\beta$), 1.06 (*q*, 1H, *J* = 6.6 Hz, 8—CO_2—$(CH_2)4''\alpha$), 0.96 (*t*, 2H, *J* = 6.0 Hz, 8—CO_2—$(CH_2)3''$). ^{13}C NMR ($CDCl_3$) δ: 177.76 (COOH), 175.21 (8-COO), 165.91 (C-7′), 134.19 (C-4′), 129.85 (C-2′/C-6′), 129.29 (C-1′), 129.13 (C3′/C-5′), 89.93 (C-16), 89.76 (C-8), 82.27 (C-6), 79.81 (C-1), 78.77 (C-15), 78.29 (C-14), 75.95 (C-18), 74.06 (C-13), 69.78 (C-3), 63.46 (C-17), 61.80 (16-OCH_3), 59.21 (18-OCH_3), 59.03 (6-OCH_3), 55.32 (1-OCH_3), 50.50 (C-19), 50.40 (C-11), 50.36 (N—CH_2—CH_3), 44.95 (C-5), 43.36 (C-9), 43.10 (C-4), 42.11 (C-7), 39.84 (C-10), 35.04 (C-12), 34.37 (C-1″), 33.94 (C-5″), 29.46 (C-2), 28.29 (C-3″), 24.34 (C-2″), 23.89 (C-4″), 10.86 (N—CH_2—CH_3). HRMS $[M]^+$ $C_{39}H_{55}NO_{13}$ requires 745.86, found 746.4.

*5.1.1.5. 8-O-(14-Benzoylaconine)suberate (**1e**).* White powder (40%); t_R = 10.03 min; ^{1}H NMR spectrum (400.13 MHz, $CDCl_3$) δ: 8.20 (br s, 1H, NH^+), 7.99 (d, 2H, *J* = 7.14 Hz, H-2′/H-6′), 7.62 (*t*, 1H, *J* = 7.41 Hz, H-4′), 7.48 (*t*, 2H, *J* = 7.62 Hz, H-3′/H-5′), 4.90 (d, 1H, *J* = 4.9 Hz, H-14), 4.50 (d, 1H, *J* = 5.39 Hz, H-15), 4.29 (br s, 1H, H-3), 4.14 (d, 1H, *J* = 5.99 Hz, H-6), 3.75 (m, 1H, H-19β), 3.80 (s, 3H, 16-OCH_3), 3.52 (s, 1H, H-18β), 3.49 (s, 1H, H-1), 3.38 (s, 3H, 1-OCH_3), 3.33 (s, 1H, H-17), 3.29 (s, 3H, 18-OCH_3), 3.26 (s, 1H, 18α), 3.24 (s, 1H, H-16), 3.23 (s, 3H, 6-OCH_3), 3.20 (m, 2H, N—CH_2—CH_3), 3.15 (m, 1H, H-19α), 2.96 (br s, 1H, H-9), 2.93 (s, 1H, H-5), 2.58 (d, 1H, *J* = 6.05 Hz, H-7), 2.44 (br s, 1H, H-2β), 2.37 (br s, 1H, H-12β), 2.34 (d, 1H, *J* = 6.23 Hz, H-10), 1.99 (*q*, 2H, *J* = 7.2 Hz, 8—CO_2—$(CH_2)5''$), 1.91 (m, 1H, H-12α), 1.82 (m, 1H, 8—CO_2—$(CH_2)1''\beta$), 1.55 (m, 2H, 8—CO_2—$(CH_2)2''$), 1.50 (m, 1H, 8—CO_2—$(CH_2)1''\alpha$), 1.45 (*t*, 3H, *J* = 7.02 Hz, N—CH_2—CH_3), 1.40 (br s, 1H, H-2α), 1.09 (*q*, 2H, *J* = 7.5 Hz, 8—CO_2—$(CH_2)4''$), 0.92 (*q*, 2H, *J* = 7.35 Hz, 8—CO_2—$(CH_2)3''$). ^{13}C NMR ($CDCl_3$) δ: 177.66 (COOH), 175.11 (8-COO), 165.66 (C-7′), 133.79 (C-4′), 129.62 (C-2′/C-6′), 129.09 (C-1′), 128.84 (C3′/C-5′), 89.68 (C-16), 89.54 (C-8), 82.18 (C-6), 79.60 (C-1), 78.54 (C-15), 78.09 (C-14), 75.51 (C-18), 73.77 (C-13), 69.57 (C-3), 62.87 (C-17), 61.63 (16-OCH_3), 59.0 (18-OCH_3), 58.84 (6-OCH_3), 55.10 (1-OCH_3), 50.33 (C-19), 50.22 (C-11), 50.04 (N—CH_2—CH_3), 44.72 (C-5), 43.19 (C-9), 43.13 (C-4), 41.61 (C-7), 39.79 (C-10), 34.76 (C-12), 34.25 (C-1″), 33.58 (C-6″), 29.27 (C-2), 28.29 (C-4″), 28.23 (C-3″), 24.21 (C-2″), 23.78 (C-5″), 10.69 (N—CH_2—CH_3). HRMS $[M]^+$ $C_{40}H_{57}NO_{13}$ requires 759.89, found 760.4.

*5.1.1.6. 8-O-(14-Benzoylaconine)azelate (**1f**).* White powder (44%); t_R = 15.99 min; ^{1}H NMR spectrum (400.13 MHz, $CDCl_3$) δ: 8.16 (br s, 1H, NH+), 7.98 (d, 2H, *J* = 7.2 Hz, H-2′/H-6′), 7.6 (*t*, 1H, *J* = 7.5 Hz, H-4′), 7.46 (*t*, 2H, *J* = 7.6 Hz, H-3′/H-5′), 4.89 (d, 1H, *J* = 4.8 Hz, H-14), 4.49 (d, 1H, *J* = 5.3 Hz, H-15), 4.26 (br s, 1H, H-3), 4.13 (d, 1H, *J* = 5.85 Hz, H-6), 3.78 (s, 3H, 16-OCH_3), 3.73—4.02 (m, 1H, H-19β), 3.50 (s, 1H, H-18β), 3.47 (s, 1H, H-1), 3.36 (s, 3H, 1-OCH_3), 3.32 (s, 1H, H-17), 3.27 (s, 3H, 18-OCH_3), 3.25 (s, 1H, 18α), 3.23 (s, 1H, H-16), 3.21 (s, 3H, 6-OCH_3), 3.20 (m, 2H, N—CH_2—CH_3), 3.14 (m, 1H, H-19α), 2.95 (br s, 1H, H-9), 2.92 (s, 1H, H-5), 2.56 (d, 1H, *J* = 6.25 Hz, H-7), 2.41 (br s, 1H, H-2β), 2.36 (br s, 1H, H-12β), 2.34 (d, 1H, *J* = 6.73, H-10), 2.29 (*t*, 2H, *J* = 7.5 Hz, 8—CO_2—$(CH_2)7''$), 1.90 (m, 1H, H-12α), 1.78 (*t*, 1H, *J* = 7.6 Hz, 8—CO_2—$(CH_2)1''\beta$), 1.54 (*q*, 2H, *J* = 7.4 Hz, 8—CO_2—$(CH_2)2''$), 1.47 (m, 1H, 8—CO_2—$(CH_2)1''\alpha$), 1.44 (*t*, 3H, *J* = 7.1 Hz, N—CH_2—CH_3), 1.20 (m, 4H, 8—CO_2—$(CH_2)5'',6''$), 1.05 (*q*, 2H, *J* = 7.1 Hz, 8—CO_2—$(CH_2)4''$), 0.92 (*q*, 2H, *J* = 6.9 Hz, 8—CO_2—$(CH_2)3''$). ^{13}C NMR ($CDCl_3$) δ: 177.91 (8-COOH), 175.22 (8-COO″), 165.47 (C-7′), 133.87 (C-4′), 129.60 (C-2′/C-6′), 129.07 (C-1′), 128.82 (C3′/C-5′), 89.66 (C-16), 89.50 (C-8), 82.15 (C-6), 79.59 (C-1), 78.53 (C-15), 78.07 (C-14), 75.53 (C-18), 73.77 (C-13), 69/59 (C-3), 62.97 (C-17), 61.58 (16-OCH_3), 58.99 (18-OCH_3), 58.84 (6-OCH_3), 55.08 (1-OCH_3), 50.33 (C-19), 50.14 (C-11), 50.07 (N—CH_2—CH_3), 44.72 (C-5), 43.19 (C-9), 43.10 (C-4), 41.60 (C-7), 39.75 (C-10), 34.77 (C-12), 34.27 (C-1″), 33.81 (C-7″), 29.94 (C-3″), 29.23 (C-2), 28.44 (C-5″), 28.24 (C-4″), 24.38 (C-2″), 23.88 (C-6″), 10.67 (N—CH_2—CH_3). HRMS $[M]^+$ $C_{41}H_{59}NO_{13}$ requires 773.92, found 774.5.

*5.1.1.7. 8-O-(14-Benzoylaconine)sebacate (**1g**).* White powder (33%); t_R = 11.12 min; ^{1}H NMR spectrum (400.13 MHz, $CDCl_3$) δ: 8.14 (m, 1H, NH^+), 7.99 (d, 2H, *J* = 7.2 Hz, H-2′/H-6′), 7.61 (*t*, 1H, *J* = 7.4 Hz, H-4′), 7.47 (*t*, 2H, *J* = 7.6 Hz, H-3′/H-5′), 4.91 (d, 1H, *J* = 5.1 Hz, H-14), 4.51 (d, 1H, *J* = 5.1 Hz, H-15), 4.26—4.32 (m, 1H, H-3), 4.15 (d, 1H, *J* = 5.7 Hz, H-6), 3.79 (s, 3H, 16-OCH_3), 3.88—4.02 (m, 1H, H-19β), 3.51—3.54 (m, 1H, H-18β), 3.46—3.50 (m, 1H, H-1), 3.38 (s, 3H, 1-OCH_3), 3.32 (s, 1H, H-17), 3.29 (s, 3H, 18-OCH_3), 3.25—3.28 (m, 1H, 18α), 3.22—3.25 (m, 1H, H-16), 3.23 (s, 3H, 6-OCH_3), 3.22—3.25 (m, 2H, N—CH_2—CH_3), 3.11—3.20 (m, 1H, H-19α), 2.95 (*t*, 1H, *J* = 5.5 Hz, H-9), 2.93 (s, 1H, H-5), 2.60 (d, 1H, *J* = 5.7 Hz, H-7), 2.38—2.46 (m, 1H, H-2β), 2.33—2.38 (m, 2H, H-10,12β), 2.30 (*t*, 2H, *J* = 7.6 Hz, 8—CO_2—$(CH_2)8''$), 1.94 (br d, 1H, *J* = 9.6 Hz, H-12α), 1.82 (*q*, 1H, *J* = 8.25 Hz, 8—CO_2—$(CH_2)1''\beta$), 1.58—1.66 (m, 2H, 8—CO_2—$(CH_2)2''$), 1.50—1.58 (m, 1H, 8—CO_2—$(CH_2)1''\alpha$), 1.44 (*t*, 3H, *J* = 6.92 Hz, N—CH_2—CH_3), 1.13—1.34 (m, 7H, 8—CO_2—$(CH_2)3''$, 4″, 6″, 7″β), 0.86—1.12 (m, 3H, 8—CO_2—$(CH_2)5''$, 7″α). ^{13}C NMR ($CDCl_3$) δ: 176.65 (8-COOH), 175.28 (8-COO″), 165.72 (C-7′), 133.56 (C-4′),

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129.47 (C-2′/C-6′), 129.03 (C-1′), 128.66 (C3′/C-5′), 89.60 (C-16), 89.41 (C-8), 82.18 (C-6), 79.51 (C-1), 78.41 (C-15), 78.0 (C-14), 75.4 (C-18), 77.06 (C-13), 73.68 (C-3), 62.73 (C-17), 61.25 (16-OCH_3), 58.87 (18-OCH_3), 58.76 (6-OCH_3), 54.89 (1-OCH_3), 50.31 (C-19), 49.87 (C-11), 49.93 (N−CH_2−CH_3), 44.71 (C-5), 43.21 (C-4), 43.03 (C-9), 41.35 (C-7), 39.79 (C-10), 34.63 (C-12), 34.07 (C-1″), 33.85 (C-8″), 28.83 (C-2), 28.82 (C-6″), 28.76 (C-3″), 28.61 (C-4″), 28.4 (C-5″), 24.50 (C-2″), 23.68 (C-7″), 10.61 (N−CH_2−CH_3). HRMS $[M]^+$ $C_{42}H_{61}NO_{13}$ requires 787.95, found 787.8.

*5.1.1.8. 8-O-(14-benzoylaconine)undecanoate (**1h**).* White powder (34%); ^{1}H NMR spectrum (400.13 MHz, $CDCl_3$) δ: 8.16 (m, 1H, NH^+), 7.99 (d, 2H, J = 7.2 Hz, H-2′/H-6′), 7.61 (*t*, 1H, J = 7.32 Hz, H-4′), 7.47 (*t*, 2H, J = 7.6 Hz, H-3′/H-5′), 4.90 (d, 1H, J = 4.79 Hz, H-14), 4.51 (d, 1H, J = 4.50 Hz, H-15), 4.28 (br s, 1H, H-3), 4.14 (d, 1H, J = 6.20 Hz, H-6), 3.79 (s, 3H, 16-OCH_3), 3.71 (m, 1H, H-19β), 3.52 (s, 1H, H-18β), 3.49 (s, 1H, H-1), 3.38 (s, 3H, 1-OCH_3), 3.33 (s, 1H, H-17), 3.27 (s, 3H, 18-OCH_3), 3.26 (s, 1H, 18α), 3.24 (s, 1H, H-16), 3.23 (s, 3H, 6-OCH_3), 3.20 (m, 2H, N−CH_2−CH_3), 3.17 (m, 1H, H-19α), 2.96 (br s, 1H, H-9), 2.93 (s, 1H, H-5), 2.57 (d, 1H, J = 6.0 Hz, H-7), 2.43 (br s, 1H, H-2β), 2.37 (m, 2H, H-10,12β), 2.34 (*t*, 2H, J = 6.93 Hz, 8−CO_2−(CH_2)9″), 1.89 (m, 1H, H-12α), 1.80 (*t*, 1H, J = 8.0 Hz, 8−CO_2−(CH_2)1″β), 1.61 (*q*, 2H, J = 7.4 Hz, 8−CO_2−(CH_2)2″), 1.53 (m, 1H, 8−CO_2−(CH_2)1″α), 1.45 (m, 3H, N−CH_2−CH_3), 1.20 (m, 8H, 8−CO_2−(CH_2)5″, 6″, 7″, 8″), 1.02 (*q*, 2H, J = 6.95 Hz, 8−CO_2−(CH_2)4″), 0.90 (*q*, 2H, J = 7.18 Hz, 8−CO_2−(CH_2)3″). ^{13}C NMR ($CDCl_3$) δ: 177.05 (8-COOH), 175.25 (8-COO″), 165.66 (C-7′), 133.66 (C-4′), 129.62 (C-2′/C-6′), 129.15 (C-1′), 128.8 (C3′/C-5′), 89.82 (C-16), 89.58 (C-8), 82.31 (C-6), 79.74 (C-1), 78.67 (C-15), 78.20 (C-14), 75.64 (C-18), 73.89 (C-13), 69.47 (C-3), 63.07 (C-17), 61.66 (16-OCH_3), 59.08 (18-OCH_3), 58.92 (6-OCH_3), 55.17 (1-OCH_3), 50.45 (C-19), 50.36 (C-11), 50.20 (N−CH_2−CH_3), 44.88 (C-5), 43.32 (C-4), 43.31 (C-9), 41.79 (C-7), 39.93 (C-10), 34.99 (C-12), 34.85 (C-1″), 34.45 (C-9″), 29.50 (C-2), 28.99 (C-4″), 28.92 (C-5″), 28.86 (C-6″), 28.84 (C-7″), 28.64 (C-3″), 24.09 (C-2″), 23.74 (C-8″), 10.73 (N−CH_2−CH_3). HRMS $[M]^+$ $C_{43}H_{63}NO_{13}$ requires 801.97, found 802.1.

*5.1.1.9. Bis[O-(14-benzoylaconin-8-yl)]succinate (**2a**).* White powder (18%); t_R = 21.3 min; ^{1}H NMR spectrum (400.13 MHz, $CDCl_3$) δ: 8.11 (m, 1H, NH^+), 7.98 (d, 4H, J = 7.4 Hz, H-2′/H-6′), 7.61 (*t*, 2H, J = 7.4 Hz, H-4′), 7.45 (*t*, 4H, J = 7.7 Hz, H-3′/H-5′), 4.92 (d, 2H, J = 4.7 Hz, H-14), 4.42 (d, 2H, J = 5.3 Hz, H-15), 4.31 (br s, 2H, H-3), 4.11 (d, 2H, J = 6.1 Hz, H-6), 3.81 (s, 6H, 16-OCH_3), 3.66 (m, 2H, H-19β), 3.53 (br s, 2H, H-1), 3.48 (m, 2H, H-18β), 3.41 (s, 6H, 1-OCH_3), 3.35 (br s, 2H, H-17), 3.32 (s, 6H, 18-OCH_3), 3.30 (m, 4H, 18α, 19α), 3.26 (br s, 4H, N−CH_2−CH_3), 3.25 (s, 2H, H-16), 3.21 (s, 6H, 6-OCH_3), 2.91 (br s, 2H, H-5), 2.89 (s, 2H, H-9), 2.54 (d, 2H, J = 6.4 Hz, H-7), 2.44 (br s, 2H, H-2β), 2.39 (br s, 2H, H-10), 2.38 (br d, 2H, H-12β), 1.89 (s, 2H, H-12α), 1.85 (br d, 4H, 8−CO_2−(CH_2)1″β, 8−CO_2−(CH_2)2″β), 1.51 (m, 2H, H-2α), 1.45 (m, 6H, N−CH_2−CH_3), 1.10 (m, 4H, 8−CO_2−(CH_2)1″α, 8−CO_2−(CH_2)2″α). ^{13}C NMR ($CDCl_3$) δ: 172.99 (8-COO, 8-COO″), 165.66 (C-7′), 133.88 (C-4′), 129.84 (C-2′/C-6′), 129.34 (C-1′), 128.99 (C3′/C-5′), 90.67 (C-8), 89.98 (C-16), 81.99 (C-6), 79.83 (C-1), 78.48 (C-15), 78.33 (C-14), 76.18 (C-18), 73.98 (C-13), 70.0 (C-3), 63.49 (C-17), 61.94 (16-OCH_3), 59.25 (18-OCH_3), 58.89 (6-OCH_3), 55.36 (1-OCH_3), 50.38 (C-19), 50.37 (C-11), 50.36 (N−CH_2−CH_3), 44.84 (C-9), 43.63 (C-4), 43.45 (C-5), 42.67 (C-7), 39.85 (C-10), 35.08 (C-12), 29.35 (C-2), 28.19 (C-1″, C-2″), 10.86 (N−CH_2−CH_3). HRMS $[M]^+$ $C_{68}H_{92}N_2O_{22}$ requires 1289.48, found 1289.5.

*5.1.1.10. Bis[O-(14-benzoylaconin-8-yl)]glutarate (**2b**).* White powder (22%); t_R = 25.13 min; ^{1}H NMR spectrum (400.13 MHz, $CDCl_3$) δ: 8.27 (m, 1H, NH^+), 7.98 (d, 4H, J = 5.04 Hz, H-2′/H-6′), 7.59 (*t*, 2H, J = 4.88 Hz, H-4′), 7.45 (*t*, 4H, J = 5.06 Hz, H-3′/H-5′), 4.92 (d, 2H, J = 2.96 Hz, H-14), 4.47 (d, 2H, J = 3.32 Hz, H-15), 4.32 (br s, 2H, H-3), 4.12 (d, 2H, J = 3.68 Hz, H-6), 3.92 (m, 2H, H-19β), 3.82 (s, 6H, 16-OCH_3), 3.53 (d, 2H, J = 5.16 Hz, H-18β), 3.51 (br s, 2H, H-1), 3.41 (s, 6H, 1-OCH_3), 3.33 (m, 2H, H-17), 3.32 (s, 6H, 18-OCH_3), 3.30 (m, 2H, 18α), 3.27 (br s, 4H, N−CH_2−CH_3), 3.26 (s, 2H, H-16), 3.21 (s, 6H, 6-OCH_3), 3.15 (m, 2H, H-19α), 2.93 (m, 2H, H-9), 2.88 (br s, 2H, H-5), 2.62 (d, 2H, J = 3.76, H-7), 2.44 (d, 2H, J = 11.0 Hz, H-2β), 2.38 (br s, 2H, H-10), 2.35 (d, 2H, J = 8.32 Hz, H-12β), 1.95 (d, 2H, J = 7.24 Hz, H-12α), 1.56 (m, 4H, 8−CO_2−(CH_2)1″β, 8−CO_2−(CH_2)3″β), 1.48 (m, 6H, N−CH_2−CH_3), 1.45 (m, 2H, H-2α), 1.25 (m, 4H, 8−CO_2−(CH_2)1″α, 8−CO_2−(CH_2)3″α), 1.05 (m, 4H, 8−CO_2−(CH_2)2″). ^{13}C NMR ($CDCl_3$) δ: 173.68 (8-COO, 8-COO″), 165.50 (C-7′), 133.62 (C-4′), 129.63 (C-2′/C-6′), 129.29 (C-1′), 128.77 (C3′/C-5′), 90.20 (C-8), 89.85 (C-16), 82.34 (C-6), 79.61 (C-1), 78.53 (C-15), 78.20 (C-14), 75.55 (C-18), 73.80 (C-13), 69.63 (C-3), 62.80 (C-17), 61.78 (16-OCH_3), 59.06 (18-OCH_3), 58.75 (6-OCH_3), 55.06 (1-OCH_3), 50.46 (C-19), 50.37 (C-11), 50.07 (N−CH_2−CH_3), 44.78 (C-5), 43.33 (C-9), 43.22 (C-4), 41.67 (C-7), 39.90 (C-10), 34.76 (C-12), 33.96 (C-1″, C-3″), 29.32 (C-2), 18.63 (C-2″), 10.74 (N−CH_2−CH_3). HRMS $[M]^+$ $C_{69}H_{94}N_2O_{22}$ requires 1303.51, found 1303.3.

*5.1.1.11. Bis[O-(14-benzoylaconin-8-yl)]adipate (**2c**).* White powder (24%); t_R = 16.53 min; ^{1}H NMR spectrum (400.13 MHz, $CDCl_3$) δ: 8.27 (m, 1H, NH^+), 8.01 (d, 4H, J = 5.0 Hz, H-2′/H-6′), 7.58 (*t*, 2H, J = 5.0 Hz, H-4′), 7.47 (*t*, 4H, J = 5.1 Hz, H-3′/H-5′), 4.91 (d, 2H, J = 3.12 Hz, H-14), 4.49 (d, 2H, J = 3.52 Hz, H-15), 4.33 (br s, 2H, H-3), 4.15 (d, 2H, J = 3.96 Hz, H-6), 3.86 (m, 2H, H-19β), 3.82 (s, 6H, 16-OCH_3), 3.54 (m, 2H, H-18β), 3.52 (br s, 2H, H-1), 3.41 (s, 6H, 1-OCH_3), 3.35 (s, 2H, H-17), 3.33 (s, 6H, 18-OCH_3), 3.30 (m, 2H, 18α), 3.28 (br s, 4H, N−CH_2−CH_3), 3.27 (s, 2H, H-16), 3.23 (s, 6H, 6-OCH_3), 3.21 (m, 2H, H-19α), 2.95 (m, 2H, H-9), 2.93 (br s, 2H, H-5), 2.62 (d, 2H, J = 4.0 Hz, H-7), 2.44 (d, 2H, J = 10.5 Hz, H-2β), 2.39 (br s, 2H, H-10), 2.36 (d, 2H, J = 8.24 Hz, H-12β), 1.94 (d, 2H, J = 7.4 Hz, H-12α), 1.68 (m, 4H, 8−CO_2−(CH_2)1″β, 8−CO_2−(CH_2)4″β), 1.48 (m, 6H, N−CH_2−CH_3), 1.47 (m, 2H, H-2α), 1.35 (m, 4H, 8−CO_2−(CH_2)1″α, 8−CO_2−(CH_2)4″α), 0.91 (m, 4H, 8−CO_2−(CH_2)2″β, 8−CO_2−(CH_2)3″β), 0.75 (m, 4H, 8−CO_2−(CH_2)2″α, 8−CO_2−(CH_2)3″α). ^{13}C NMR ($CDCl_3$) δ: 174.2 (8-COO, 8-COO″), 165.52 (C-7′), 133.59 (C-4′), 129.67 (C-2′/C-6′), 129.28 (C-1′), 128.82 (C3′/C-5′), 89.91 (C-8), 89.84 (C-16), 82.29 (C-6), 79.62 (C-1), 78.56 (C-15), 78.21 (C-14), 75.59 (C-18), 73.82 (C-13), 69.71 (C-3), 62.91 (C-17), 61.78 (16-OCH_3), 59.06 (18-OCH_3), 58.79 (6-OCH_3), 55.10 (1-OCH_3), 50.42 (C-19), 50.30 (C-11), 50.11 (N−CH_2−CH_3), 44.77 (C-5), 43.28 (C-9), 43.22 (C-4), 41.73 (C-7), 39.87 (C-10), 34.79 (C-12), 33.80 (C-1″, C-4″), 29.31 (C-2), 23.11 (C-2″, C-3″), 10; 73 (N−CH_2−CH_3). HRMS $[M]^+$ $C_{70}H_{96}N_2O_{22}$ requires 1317.53, found 1317.3.

*5.1.1.12. Bis[O-(14-benzoylaconine-8-yl)]pimelate (**2d**).* White powder (25%); t_R = 27.21 min; mp 151 °C. ^{1}H NMR spectrum (400.13 MHz, $CDCl_3$) δ: 8.1 (d, 4H, J = 7.6 Hz, H-2′/H-6′), 7.71 (*t*, 2H, J = 7.4 Hz, H-4′), 7.58 (*t*, 4H, J = 7.6, H-3′/H-5′), 5.04 (d, 2H, J = 4.4 Hz, H-14), 4.63 (d, 2H, J = 5.2 Hz, H-15), 4.44 (br s, 2H, H-3), 4.27 (br d, 2H, H-6), 3.92 (m, 2H, H-19β), 3.95 (s, 6H, 16-OCH_3), 3.67 (s, 2H, H-18β), 3.64 (m, 2H, H-1), 3.53 (s, 6H, 1-OCH_3), 3.48 (s, 2H, H-17), 3.47 (s, 6H, 18-OCH_3), 3.41 (s, 2H, 18α), 3.39 (br d, 2H, H-16), 3.36 (s, 6H, 6-OCH_3), 3.31 (m, 2H, H-19α), 3.33 (m, 4H, N−CH_2−CH_3), 3.08 (m, 2H, H-9), 3.06 (s, 2H, H-5), 2.74 (br d, 2H, H-7), 2.58 (br s, 2H, H-2β), 2.51 (br d, 2H, H-12β), 2.49 (m, 2H, H-10), 2.07 (d, 2H, J = 10.0 Hz, H-12α), 1.87 (m, 4H, 8−CO_2−(CH_2)1″b, 8−CO_2−(CH_2)6″b), 1.60 (m, 12H, 8−CO_2−(CH_2)1″α, 8−CO_2−(CH_2)5″α, N−CH_2−CH_3, H-2α), 1.12 (m, 4H, 8−CO_2−(CH_2)2″β, 8−CO_2−(CH_2)4″β), 0.95 (m, 4H, 8−CO_2−(CH_2)2″α, 8−CO_2−(CH_2)4″α), 0.72 (quint, 2H, J = 7.2 Hz, 8−CO_2−(CH_2)3″). ^{13}C NMR ($CDCl_3$) δ: 174.9 (8-COO, 8-COO″), 165.76 (C-7′), 133.8 (C-4′), 129.9 (C-2′/C-6′), 129.5 (C-1′), 129 (C3′/C-5′), 90 (C-16), 89.98 (C-8), 82.48 (C-6), 79.83 (C-1), 78.12 (C-15), 78.45 (C-14), 75.79 (C-18), 74.02 (C-13), 69.9 (C-3), 63.12 (C-17), 62.01 (16-OCH_3), 59.3 (18-OCH_3), 59.05

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(6-OCH_3), 55.36 (1-OCH_3), 50.63 (C-19), 50.42 (C-11), 50.38 (N−CH_2−CH_3), 45.0 (C-5), 43.42 (C-4), 43.47 (C-9), 41.91 (C-7), 40.09 (C-10), 35.01 (C-12), 34.27 (C-1″, C-5″), 29.49 (C-2″, C-4″), 28.02 (C-2), 23.86 (C-3″), 10; 99 (N−CH_2−CH_3). HRMS $[M]^+$ $C_{71}H_{98}N_2O_{22}$ requires 1331.56, found 1331.2.

*5.1.1.13. 3Bis[O-(14-benzoylaconine-8-yl)]suberate (**2e**).* White powder (24%); t_R = 28.91 min; mp 153 °C. ^{1}H NMR spectrum (400.13 MHz, $CDCl_3$) δ: 8.00 (d, 4H, *J* = 7.8 Hz, H-2′/H-6′), 7.58 (*t*, 2H, *J* = 7.32 Hz, H-4′), 7.46 (*t*, 4H, *J* = 7.66 Hz, H-3′/H-5′), 5.04 (d, 2H, *J* = 4.9 Hz, H-14), 4.64 (d, 2H, *J* = 5.36 Hz, H-15), 4.43 (br s, 2H, H-3), 4.28 (d, 2H, *J* = 6.1 Hz, H-6), 3.97 (d, 2H, *J* = 12.96 Hz, H-19b), 3.94 (s, 6H, 16-OCH_3), 3.67 (s, 2H, H-18b), 3.64 (m, 2H, H-1), 3.53 (s, 6H, 1-OCH_3), 3.47 (s, 2H, H-17), 3.43 (s, 6H, 18-OCH_3), 3.41 (s, 2H, 18a), 3.39 (d, 2H, *J* = 5.4 Hz, H-16), 3.36 (s, 6H, 6-OCH_3), 3.31 (d, 2H, *J* = 12.96 Hz, H-19a), 3.30 (m, 4H, N−CH_2−CH_3), 3.10 (m, 2H, H-9), 3.07 (s, 2H, H-5), 2.74 (d, 2H, *J* = 6.1 Hz, H-7), 2.58 (br s, 2H, H-2b), 2.51 (br d, 2H, H-12b), 2.49 (m, 2H, H-10), 2.07 (d, 2H, *J* = 9.9 Hz, H-12a), 1.94 (m, 2H, 8−CO_2−(CH_2)1″b, 8−CO_2−(CH_2)6″b), 1.63 (m, 2H, 8−CO_2−(CH_2)1″a, 8−CO_2−(CH_2)6″a), 1.60 (*t*, 6H, *J* = 6.98 Hz, N−CH_2−CH_3), 1.56 (br d, 2H, H-2a), 1.24 (m, 2H, 8−CO_2−(CH_2)2″b, 8−CO_2−(CH_2)5″b), 1.09 (m, 2H, 8−CO_2−(CH_2)2″a, 8−CO_2−(CH_2)5″a), 0.83 (m, 4H, 8−CO_2−(CH_2)3″, 8−CO_2−(CH_2)4″). ^{13}C NMR ($CDCl_3$) δ: 175.1 (8-COO, 8-COO″), 165.8 (C-7′), 133.8 (C-4′), 129.9 (C-2′/C-6′), 129.5 (C-1′), 129 (C3′/C-5′), 90 (C-16), 89.97 (C-8), 82.51 (C-6), 79.82 (C-1), 78.82 (C-15), 78.40 (C-14), 75.75 (C-18), 74.01 (C-13), 70 (C-3), 63.13 (C-17), 61.95 (16-OCH_3), 59.26 (18-OCH_3), 59.01 (6-OCH_3), 55.29 (1-OCH_3), 50.64 (C-19), 50.51 (C-11), 50.28 (N−CH_2−CH_3), 45.0 (C-5), 43.43 (C-4), 43.40 (C-9), 41.87 (C-7), 40.06 (C-10), 35.0 (C-12), 34.49 (C-1″, C-6″), 29.50 (C-2), 28.54 (C-3″, C-4″), 24.05 (C-2″, C-5″), 10; 94 (N−CH_2−CH_3). HRMS $[M]^+$ $C_{72}H_{100}N_2O_{22}$ requires 1345.59, found 1345.68.

*5.1.1.14. Bis[O-(14-benzoylaconine-8-yl)]azelate (**2f**).* White powder (24%); t_R = 32.24 min; ^{1}H NMR spectrum (400.13 MHz, $CDCl_3$) δ: 8.03 (d, 4H, *J* = 7.2 Hz, H-2′/H-6′), 7.56 (*t*, 2H, *J* = 7.4 Hz, H-4′), 7.45 (*t*, 4H, *J* = 7.2 Hz, H-3′/H-5′), 5.09 (d, 2H, *J* = 5.0 Hz, H-14), 4.51 (d, 2H, *J* = 5.2 Hz, H-15), 4.30 (br s, 2H, H-3), 4.12 (d, 2H, *J* = 6.1 Hz, H-6), 4.06 (d, 2H, *J* = 11.91 Hz, H-19b), 3.79 (s, 6H, 16-OCH_3), 3.52 (s, 2H, H-18b), 3.47 (m, 2H, H-1), 3.36 (s, 6H, 1-OCH_3), 3.29 (s, 2H, H-17), 3.27 (s, 6H, 18-OCH_3), 3.24 (s, 2H, 18a), 3.22 (br s, 2H, H-16), 3.19 (s, 6H, 6-OCH_3), 3.11 (d, 2H, *J* = 11.9 Hz, H-19a), 3.16 (m, 4H, N−CH_2−CH_3), 2.90 (m, 2H, H-9), 2.86 (s, 2H, H-5), 2.55 (d, 2H, *J* = 6.1 Hz, H-7), 2.34 (br s, 2H, H-2b), 2.28 (br d, 2H, H-12b), 2.24 (m, 2H, H-10), 2.02 (br d, 2H, H-12a), 1.82 (m, 2H, 8−CO_2−(CH_2)1″b, 8−CO_2−(CH_2)7″b), 1.50 (m, 2H, 8−CO_2−(CH_2)1″a, 8−CO_2−(CH_2)7″a), 1.42 (*t*, 6H, *J* = 7.01 Hz, N−CH_2−CH_3), 1.35 (br s, 2H, H-2a), 1.13 (m, 4H, 8−CO_2−(CH_2)2″b, 8−CO_2−(CH_2)3″b, 8−CO_2−(CH_2)5″b, 8−CO_2−(CH_2)6″b), 1.04 (m, 4H, 8−CO_2−(CH_2)2″a, 8−CO_2−(CH_2)3″a, 8−CO_2−(CH_2)5″a, 8−CO_2−(CH_2)6″a), 0.87 (m, 2H, 8−CO_2−(CH_2)4″). ^{13}C NMR ($CDCl_3$) δ: 175.1 (8-COO, 8-COO″), 166 (C-7′), 134 (C-4′), 130 (C-2′/C-6′), 129.7 (C-1′), 129.3 (C3′/C-5′), 90.3 (C-16), 90.1 (C-8), 82.9 (C-6), 80.1 (C-1), 79.0 (C-15), 78.7 (C-14), 76.0 (C-18), 74.3 (C-13), 70.3 (C-3), 63.4 (C-17), 60.9 (16-OCH_3), 59.5 (18-OCH_3), 59.06 (6-OCH_3), 55.6 (1-OCH_3), 50.7 (C-19), 50.51 (C-11), 50.50 (N−CH_2−CH_3), 45.4 (C-5), 43.7 (C-4), 43.40 (C-9), 41.9 (C-7), 40.5 (C-10), 35.3 (C-12), 34.49 (C-1″,C-7″), 28.8 (C-2″, C-6″), 26.54 (C-3″, C-5″), 24.05 (C-4″), 11 (N−CH_2−CH_3). HRMS $[M]^+$ $C_{73}H_{102}N_2O_{22}$ requires 1359.61, found 1359.5.

5.2. X-ray data

The structure of compound **1d** has been established by X-ray crystallography (Fig. 1). Colorless single crystal (0.12 × 0.10 × 0.10 mm^3) of **1d** was obtained by slow evaporation from methanol/chloroform/trifluoroacetic acid (20/80/0.5) solution: monoclinic, space group P21, *a* = 10.3646 (9) Å, *b* = 14.7961 (10) Å, *c* = 13.6373 (9) Å, α = 90°, β = 110.022 (5)°, γ = 90°, *V* = 1965.0 (3) $Å^3$, *Z* = 2, δ (calcd) = 1.436 Mg m^{-3}, FW = 849.83 for $C_{37}H_{53}NO_{14}.CF_3$-COOH, *F*(000) = 900. The data were collected on a Rigaku R-axis rapid diffractometer equipped with micro-focus rotating anode Cu−K radiation (λ = 1.5418 Å) mode at 133 (2) K. In the range of 6.58° < (< 72.03°, a total of 27,953 reflections were collected, of which 7064 were independent (R_{int} = 0.0834). The structure was solved by direct methods with SHELXS-97 [16]. Non-hydrogen atoms were refined by full-matrix least-squares techniques on F^2 with anisotropic thermal parameters, using SHELXL-97 [17]. Full crystallographic results have been deposited at the Cambridge Crystallographic Data Centre (CCDC-857072), UK, as supplementary Material [18].

5.3. In vitro testing

5.3.1. Tumour cell lines

The human tumour cell lines A-549 (lung cancer), MCF-7 (breast cancer) and HCT-15 (colon cancers), were obtained from the Developmental Therapeutics Program (DTP) of the National Cancer Institute (NCI) (Rockville, MD, USA). Cells were routinely grown with RPMI 1640 medium supplemented with 10% foetal calf serum, both obtained from Biochrom AG (Berlin, Germany). They were grown on Petri dishes (Nunc, Denmark) at 37 °C in a humidified atmosphere containing 5% CO_2. Cells were replicated every 4–5 days and the medium changed once in-between.

5.3.2. Cytotoxicity

Cytotoxicity was evaluated on exponentially growing cells grown for 24 h in the presence of the compounds to be tested. These were dissolved in distilled water, the same solvent being used as a control. All samples were first sterilised using polycarbonate 0.22 μm membrane filters (Millipore, Molsheim, France). Briefly, 1000 cells were seeded in 96-well plates with 200 μL of complete medium; 24 h later, the medium was supplemented with a series of concentrations of the compounds to be tested and the contact maintained for 24 h. The cells were then allowed to re-grow for 48 h; viable and metabolically active cells were quantitatively estimated by coloration with MTT (1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan, Sigma−Aldrich Chimie, Saint-Quentin-Fallavier, France) [19]. The amount of substance allowing 50% decrease in cell numbers is indicative of the cytotoxicity and represents the IC_{50} of the tested compound.

5.4. In vivo testing

5.4.1. Animals

Experiments were performed in accordance with the European Community Standards on the Care and Use of Laboratory Animals and approved by the Animal Care and Use Committee of University Bordeaux Segalen. All animals were bred and housed under specific pathogen-free conditions and provided food and water *ad libitum*. Eight-week old immunodeficient mice (NSG nomenclature: NOD.Cg-$Prkdc^{scid}$ $Il2rg^{tmw}$/SzJ jax mice 005557), males for the HCT-15 tumour model and females for the MCF-7 tumour model, weighing 19–25 g, were used for the experiments. Each experimental group contained 8–10 animals. For the MCF-7 tumour model, mice received 17β-oestradiol (Sigma−Aldrich Chimie, Saint-Quentin-Fallavier, France) previously dissolved in ethanol (4.25 mg/mL), which was added to the drinking water at a final concentration of 8.5 μg/mL.

5.4.2. Drug testing

For drug testing, 1.5 × 10^6 human tumour cells in culture medium were inoculated subcutaneously into the right flank of the

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mice (day 0). When the tumour reached 4 × 4 mm in average, animals were randomly divided into control and test groups, consisting of at least 8 mice each, and the treatment was started. Compound **2e** (bis[*O*-(14-benzoylaconine-8-yl)]suberate) in 0.9% solution of NaCl was injected i.v. at the dose of 10 mg/kg. This dose was chosen after independent evaluation of the maximum tolerated dose of the compound in mice (15 mg/kg) by Phycher (Cestas, France), a contract research organisation dedicated to toxicology and agreed for regulatory evaluations. Two treatment schedules were chosen for MCF-7 tumour cells: administration of five doses every 4 days, and weekly administration of four doses. The mice weighed three times a week, and only one for HCT-15 cells: administration of five doses every 3 days, starting one week after tumour cell graft.

The length (L) and width (W) of the tumour were measured by calipers three times a week and the tumour volume was calculated as follows: $T\,(mg) = (L \times W^2)/2$. Tumour regression was determined as $\%\ T/C = \Delta T/\Delta C \times 100$ where ΔT is the difference between the mean tumour volumes of the treated group at the end (T_{final}) and at the start (T_{start}) of the treatment, and ΔC is the difference between the mean tumour volumes of the control group at the end (C_{final}) at the start (C_{start}) of the treatment. According to the National Cancer Institute standards, a $T/C < 42\%$ is considered as the minimum level for activity [20]. At the end of experiments, animals were sacrificed by cervical dislocation. Tumours were extracted and frozen in liquid nitrogen for further immuno-histological investigations. The tumour volumes of treated and control groups were compared using bilateral Student's test.

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