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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Pentacyclic triterpene acid conjugated with mitochondria-targeting cation F16: Synthesis and evaluation of cytotoxic activities

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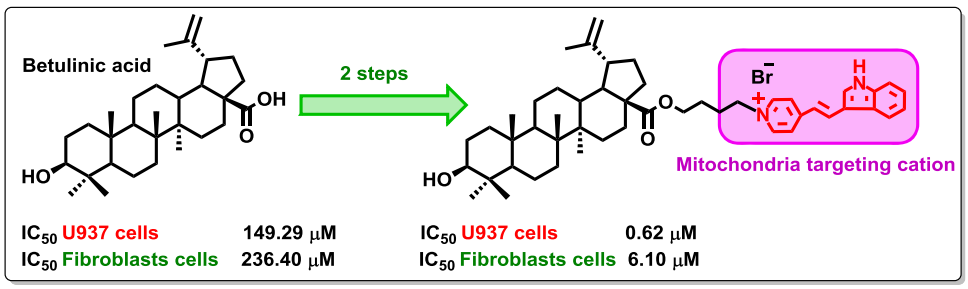
Abstract

The first representatives of F16-conjugated pentacyclic triterpenoids, betulin and betulinic, ursolic, oleanolic, and glycyrrhetic acid derivatives, were synthesized. The triterpene core was linked, at the C-3, C-28, or C-30 position, to one or two mitochondria-targeting delocalized lipophilic cations **F16** via butane or triethylene glycol spacer. The human cancer cell lines U937 (leukemic monocyte lymphoma), K562 (chronic myeloid leukemia), and Jurkat (T-lymphoblastic leukemia), and a human nonmalignant fibroblast cell line were used to evaluate the cytotoxic activities of the products. Most of the obtained conjugates showed considerable enhancement of the antitumor action in comparison with the parent betulinic acid (≈100−200-fold) and a markedly higher cytotoxic effect against tumor cell lines over healthy fibroblast cells. In the series of test compounds, **F16** conjugates with betulin and betulinic acid **6, 8**, and **11** were most selective, showing acceptable values of selectivity index (≥10).

Graphical Abstract



Keywords Pentacyclic triterpenoids · F16 · Mitochondria-targeting cations · Cytotoxicity · Photoluminescence

Introduction

Currently, mitochondria are considered as a new promising versatile target for the therapy of cancer [1, 2]. These organelles, which are present in all types of cells and organs, determine the life and death of cells, by triggering apoptosis [3, 4]. Mitochondria were found to play a key role in neoplasia and cell differentiation. It is known that mitochondria of malignant-transformed and normal cells are considerably different in structure and physicochemical features. In particular, mitochondria have a uniquely high transmembrane potential (negative inside) compared with other organelles. In addition, the transmembrane potential

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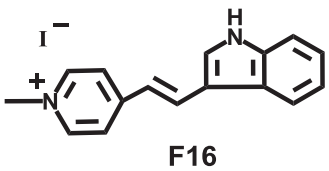
of the cells of solid tumors exceeds the potential of normal cells, which opens up good opportunities for the selective accumulation of lipophilic cationic molecules in the tumor cell mitochondria [5]. Known chemically different delocalized lipophilic cations, such as Rhodamine-123, rhodacyanines MKT-077, F16, dequalinium, and triphenylphosphonium cations can, in principle, be successfully used for the selective delivery of cytotoxic substances to tumor cell mitochondria [6, 7]. However, among these positively charged small molecules, only the triphenylphosphonium cation has been actively studied to date [7–12]. In recent years, available natural pentacyclic triterpenoids, such as betulin and betulinic, ursolic, oleanolic, and glycyrrhetic acids, have been considered as promising scaffolds for the development of novel mitochondria-targeting anticancer agents. The antitumor activity of the native triterpene acids, which was detected in vitro for various types of tumor cells (melanoma, adenocarcinoma, neuroblastoma, medulloblastoma, and glioblastoma), is successfully combined with their low systemic toxicity [13–19]. These secondary metabolites act on the mitochondria of tumor cells, thus initiating the formation of reactive oxygen species. Free radicals formed in high concentrations increase the mitochondrial membrane permeability or rupture the membranes, which results in the release of cytochrome *c*, a mediator of apoptosis, into the cytosol, caspase activation, and DNA fragmentation [20, 21]. However, because of low bioavailability of triterpenes, caused by their poor water solubility, they cannot reach the target in vivo and produce the desired therapeutic effect. Recently, we showed that betulin and betulinic and ursolic acid derivatives covalently linked to the triphenylphosphonium moiety were substantially (40–50 times) superior over their prototypes in the antitumor activity and in the ability to induce mitochondrial apoptosis pathway of cancer cells [22–24]. Excellent cytotoxic activity was also detected for cationic pentacyclic triterpenoid derivatives containing a Rhodamine B cationic residue [25]. In the light of the above considerations, in this paper, we report the rational design, synthesis, and biological evaluation of F16 cation-conjugated betulinic, ursolic, oleanolic, and glycyrrhetic acids, containing F16 moiety at the C-3, C-28, or C-30 positions of the triterpenoid core.



F16

Fig. 1 Molecular structure of E-4-(1H-indol-3-ylvinyl)-N-methylpyridinium iodide (**F16**)

Results and discussion

The recently discovered cationic compound **F16** [E-4-(1H-indol-3-ylvinyl)-N-methylpyridinium iodide], toxic to mitochondria, is selectively accumulated in the mitochondrial matrix of various tumor cells (Fig. 1) [26, 27].

High concentrations of this compound in mitochondria induce cell death associated with the cell cycle arrest, interruption of the mitochondrial respiratory chain, decrease in the intracellular ATP level, and induction of apoptosis. However, unlike the triphenylphosphonium cation, which is now widely used, **F16** has been scarcely studied as a means for delivery of biologically active agents to malignant cells [28, 29].

The synthesis of **F16**-conjugated pentacyclic triterpenoids **6–19** was started from commercially available betulin and ursolic, oleanolic, and glycyrrhetinic acids **1–5** (Figs. 2 and 3). Betulinic acid **2** was prepared by oxidation of betulin according to a known method [30]. Triterpenoids **6,**

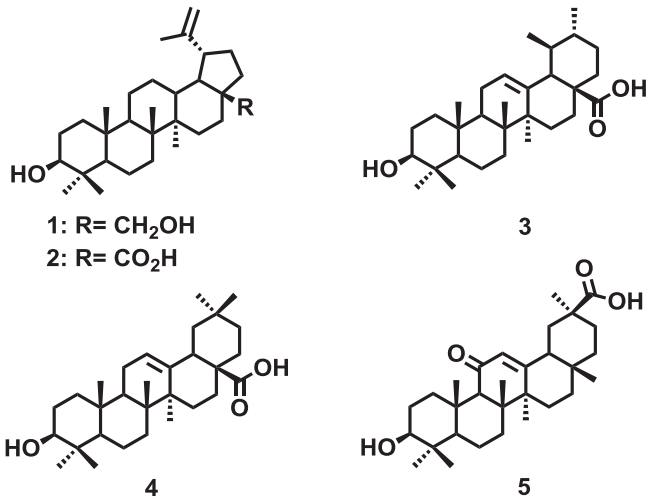


Fig. 2 Molecular structures of betulin (**1**), betulinic acid (**2**), ursolic acid (**3**), oleanolic acid (**4**), and 18β-glycyrrhetinic acid (**5**)

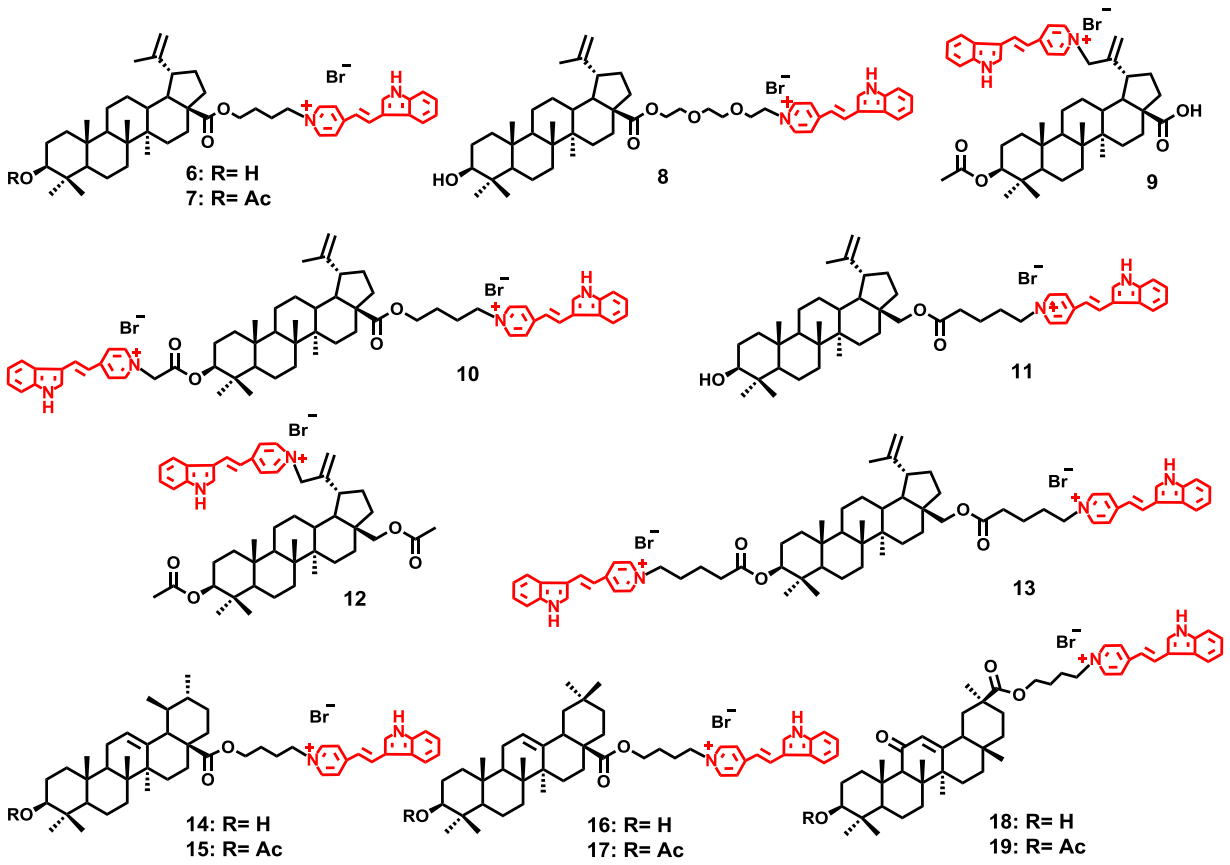


Fig. 3 Structure of conjugates of triterpenoids with (E)-4-(2-(1H-indol-3-yl)vinyl)pyridines **6–19**

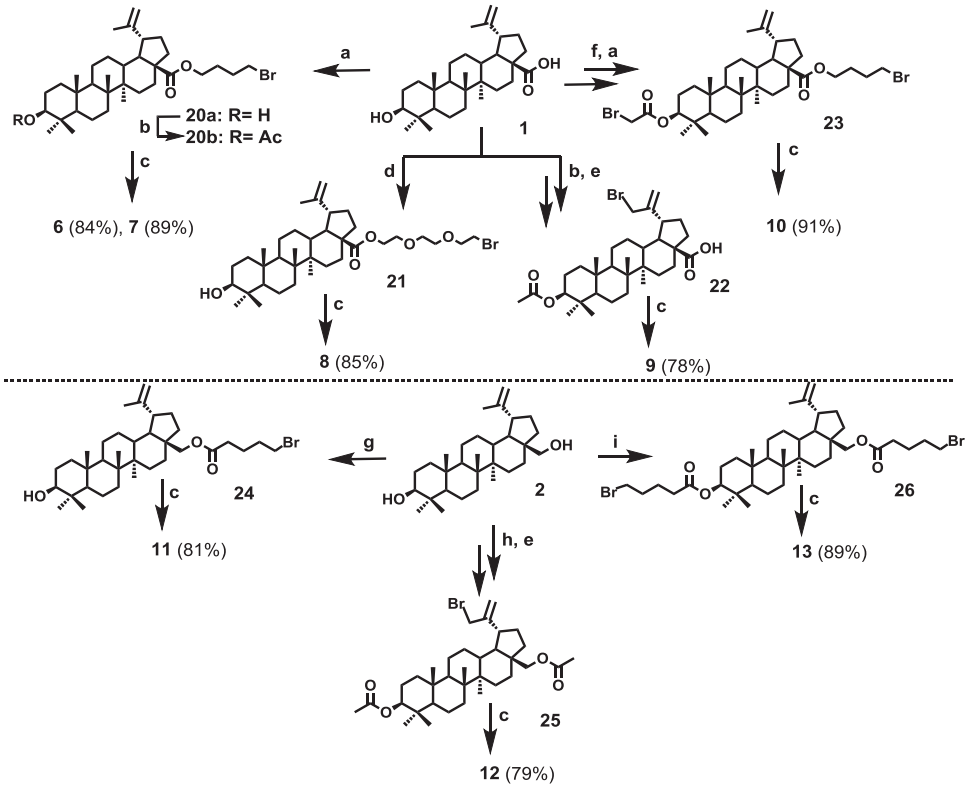
8, 14, **16**, and **18**, in which the triterpene core is linked to the cationic moiety **F16** at position C-28 via butane or triethylene glycol spacer, were prepared from bromides **20a, 21**, and **27a–29a** with the yields ranging from 79 to 91% (Schemes 1 and 2).

The required bromides were obtained by the reaction of triterpene acids **2–5** with a fourfold molar excess of 1,4-dibromobutane or 1.4-dibromotri(ethylene glycol) in DMF or in a DMF–CH_3CN solvent mixture (3:1) with addition of K_2CO_3.

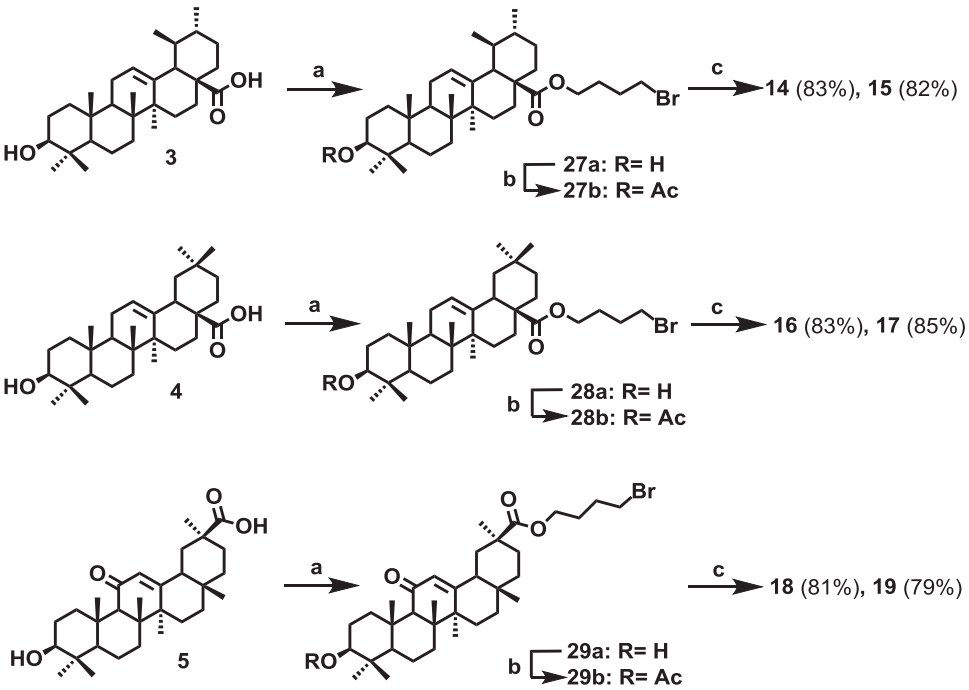
For the synthesis of conjugates **7, 15, 17**, and **19**, the 3-OH function of bromides **20a** and **27a–29a** was first acetylated with AcCl in THF in the presence of pyridine and 4-dimethylaminopyridine (DMAP) (Schemes 1 and 2).

The C-30 conjugates of lupane triterpenoids **9** and **12** with the **F16** cationic group were synthesized by a three-step procedure, starting from betulin and betulinic acid, which were acetylated and brominated under previously reported conditions [23]. Lupane triterpenoids **10** and **13** linked to two **F16** moieties were prepared from dibromides **23** and **26**. Dibromide **26** was obtained using the carbodiimide method by the reaction of betulin with a twofold molar excess of 5-bromovaleric acid in the presence of N,N′-dicyclohexylcarbodiimide (DCC) and DMAP under previously developed conditions [10]. Dibromide **23** was prepared by acylation of the 3-OH group of betulinic acid **1** with bromoacetic acid in THF in the presence of pyridine and DMAP followed by reaction of the resulting bromide with a fourfold molar excess of 1,4-dibromobutane. The resulting bromides **20a,b, 21–26**, and **27a,b–29a,b** were converted to F16-conjugated triterpenoids **6–19** (Fig. 3) by the reaction with (E)-4-(1H-indol-3-ylvinyl)pyridine in DMF at 85 °C as described previously [31].

The structures of all products were confirmed by 1D (^{1}H, ^{13}C, APT) and 2D homo- (COSY, NOESY) and heteronuclear (HSQC, HMBC) NMR experiments. The nuclear chemical shifts for the terpene core and for (E)-4-(1H-indol-3-ylvinyl)pyridine were determined by comparison with published data [23, 24, 32, 33]. In the ^{1}H NMR spectra, the presence of (E)-4-(1H-indol-3-ylvinyl) pyridinium moiety is evidenced by two characteristic doublets for the pyridine ring at 8.02 and 8.58 ppm with $J = 6.0$ Hz, two vinyl group doublets at 7.10 and 8.09 ppm with $J = 16.0$ Hz, and three characteristic multiplets for the indole moiety at 7.22–7.29, 7.48–7.88, and 8.07–8.09 ppm. The ^{13}C NMR spectra show signals for the (E)-4-(1H-indol-3-ylvinyl)pyridinium carbons in the 113.6–157.4 ppm range.



Scheme 1 Synthesis of compounds **6–13**. Reagents and conditions: **a** 1,4-dibromobutane, K_2CO_3, DMF, MeCN, 50 °C; **b** AcCl, THF, Py, DMAP, rt; **c** (E)-4-(2-(1H-indol-3-yl)vinyl)pyridine, DMF, 85 °C, Ar; **d** tri(ethylene glycol) dibromide, K_2CO_3, DMF, 50 °C; **e** NBS, CCl_4; **f** bromoacetic acid, THF, Py, DMAP, rt; **g** 5-bromovaleric acid (1 equiv), DCC, DMAP, DCM, rt; **h** AcOH, reflux; **i** 5-bromovaleric acid (2 equiv), DCC, DMAP, DCM, rt



Scheme 2 Synthesis of compounds **14–19**. Reagents and conditions: **a** 1,4-dibromobutane, K_2CO_3, DMF, MeCN, 50 °C; **b** AcCl, THF, Py, DMAP, rt; **c** (E)-4-(2-(1H-indol-3-yl)vinyl)pyridine, DMF, 85 °C, Ar

Since **F16** has good fluorescent properties with intense fluorescence in the visible region (absorbance, 436 nm; emission, 540 nm [32]), we investigated luminescence spectral properties of conjugates **6–19** in methanol at a concentration of 5 μM (Fig. 4).

It is noteworthy that the replacement of methanol solvent by DMSO did not have a significant effect on the photoluminescence (PL) spectra of the products. As shown in Fig. 4, the shape and position of the absorption and fluorescence spectra of compounds **6–19** are similar to

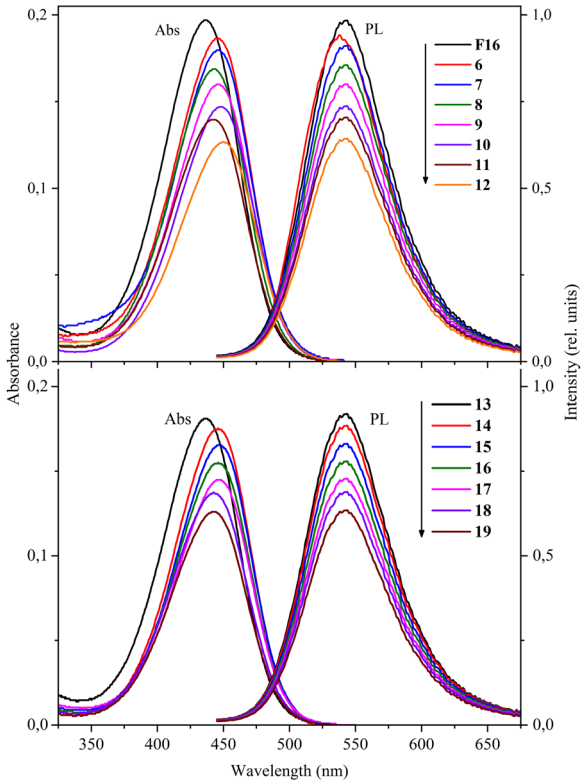


Fig. 4 Absorption and fluorescence spectrum of **F16, 6–19** (5 μM) in methanol. All spectra were registered under air atmosphere at room temperature ($T = 297$ K). Fluorolog-3, λ_{exc} 435 nm, $\Delta\lambda = 2$ nm

luminescence characteristics of **F16**. However, the covalent binding of the **F16** fluorophore moiety to the triterpenoid core induced a slight blue shift of the luminescence maximum (e.g., for compound **17**, $\lambda = 534$ nm; for **F16**, $\lambda = 542$ nm). The fluorescence quantum yield of the resulting conjugates **6–19**, determined by the reported method [34] using an ethanol solution of anthracene as the fluorescence standard ($\phi_{PL} = 0.28$), varied in the range from 17 to 40 %.

The obtained conjugates **6–19**, betulinic acid **1**, compound **F16**, and an equimolar mechanical mixture of betulinic acid **1** and **F16** (1:1, mol/mol) were tested in vitro against three tumor cell lines: U937 (leukemic monocyte lymphoma), K562 (chronic myeloid leukemia), and Jurkat (T-lymphoblastic leukemia) a human nonmalignant fibroblast cell line. The cell viability after treatment with the test compounds was studied by flow cytometry. The viability (live/dead) was assessed by staining cells with 7-AAD (7-Aminoactinomycin D) (Biolegend). Most of the conjugates **6–8** and **14–19** showed high cytotoxic activities against all of the tested cancer cell lines. These compounds were markedly (≈100−200-fold) more cytotoxic than parent betulinic acid (Table 1).

Derivatives of betulinic acid **6** and **7**, ursolic acid **14** and **15**, oleanolic acid **16** and **17**, and glycyrrhetic acid **18** and **19** exhibited antitumor activities against the Jurkat cell line, with IC_{50} being in the 0.56–0.84 μM range, while betulinic acid **1** showed IC_{50} of 81.68 μM. Among all conjugates, lupane terpenoids **6**, **8**, and **11** were most selective, with the selectivity indices being approximately 10 (defined as IC_{50} for the U937 tumor cell line versus IC_{50} for the nonmalignant fibroblasts). Contrary to our expectations, the introduction of a second (E)-4-(1H-indol-3-ylvinyl)pyridine moiety into the triterpenoid molecule did not increase the antitumor activity. The betulin and betulinic acid diconjugates **10** and **13** (IC_{50}, 4.19 and 2.06 M, U937) were less active than the corresponding mono derivatives. These results are in perfect agreement with previously published data [23]. Similarly, ditriphenylphosphonium derivatives of lupane triterpenoids did not significantly improve the antitumor effect as compared with mono-phosphonium salts. **F16**-conjugated with the betulinic acid molecule at C-30 position (compound **9**) did not increase the cytotoxic activity ($IC_{50} > 125$ μM), and the C-30 conjugate of betulin diacetate **12** displayed a moderate antitumor activity (IC_{50}, 3.04 μM, U937). These results show that the cytotoxicity of **F16**-triterpenoid conjugates may change depending on the cationic residue position in the triterpenoid core. This has been previously noted for cationic dimethylaminopiridine derivatives of lupane triterpenoids [35].

Under the conditions we used, compound **F16** did not show cytotoxicity, while betulinic acid exhibited an antitumor activity, with IC_{50} being 149 (U937), 81.7 (Jurkat), and 78.5 (K562) μM. Unlike betulinic acid and **F16** linked by a covalent bond, a mechanical mixture of these compounds did not exhibit a noticeable increase in the cytotoxic action (Table 1).

Conclusion

The paper reports the design and synthesis of 14 new pentacyclic triterpenoids, in which the lupane, ursane, or oleane triterpene core is linked at the C-3 and/or C-28 and C-30 atoms to the F16 cationic group via a butane or triethylene glycol spacer. According to in vitro bioassays with three human tumor cell lines (U937, Jurkat, K562), most of the prepared conjugates, irrespective of the triterpene core structure, showed considerably (≈100-200-fold) higher antitumor activities than the reference betulinic acid. For example, in the case of compound **6**, the IC_{50} value was 0.62 μM; for betulinic acid, IC_{50} was 149.29 μM, and for **F16**, IC_{50} was >500 μM (U937 cells). The covalent binding of two cytotoxic compounds (betulinic acid and **F16**) induced a considerable synergistic effect, unlike the use of these compounds as a mixture.

Table 1 Cytotoxic action of compounds on the U937, Jurkat, and K562 tumor cells and fibroblasts ($X \pm SE$)[a]

Compound	IC_{50} (μM)[b]			
	U937	Jurkat	K562	Fibroblasts
6	0.616 ± 0.028[a]	0.844 ± 0.034[a]	0.812 ± 0.032[a]	6.100 ± 0.220[a]
7	2.167 ± 0.073[a]	0.584 ± 0.027[a]	0.559 ± 0.025[a]	4.360 ± 0.990[a]
8	0.573 ± 0.024[a]	1.260 ± 0.042[a]	1.210 ± 0.041[a]	5.500 ± 0.340[a]
9	>125[a]	>125[a]	>125[a]	>125[a]
10	4.190 ± 0.117[a]	4.360 ± 0.122[a]	4.010 ± 0.109[a]	10.400 ± 1.230[a]
11	0.906 ± 0.037[a]	0.937 ± 0.032[a]	0.904 ± 0.033[a]	8.200 ± 0.630[a]
12	3.040 ± 0.094[a]	3.260 ± 0.091[a]	3.190 ± 0.089[a]	11.400 ± 1.030[a]
13	2.060 ± 0.079[a]	1.680 ± 0.054[a]	1.620 ± 0.051[a]	10.800 ± 0.980[a]
14	2.461 ± 0.085[a]	0.623 ± 0.031[a]	0.588 ± 0.032[a]	6.230 ± 0.850[a]
15	2.228 ± 0.076[a]	0.561 ± 0.029[a]	0.554 ± 0.028[a]	5.180 ± 0.450[a]
16	0.607 ± 0.027[a]	0.687 ± 0.034[a]	0.671 ± 0.035[a]	3.490 ± 0.560[a]
17	2.234 ± 0.081[a]	0.578 ± 0.027[a]	0.516 ± 0.029[a]	4.280 ± 0.840[a]
18	2.425 ± 0.083[a]	0.559 ± 0.024[a]	0.511 ± 0.022[a]	8.300 ± 1.190[a]
19	2.304 ± 0.078[a]	0.568 ± 0.026[a]	0.534 ± 0.026[a]	7.010 ± 0.320[a]
F16	> 500[a]	>500[a]	>500[a]	>500[a]
1	149.290 ± 4.170[a]	81.680 ± 1.820[a]	78.540 ± 1.760[a]	236.400 ± 3.600[a]
F16:1/1:1	122.170 ± 3.460[a]	91.580 ± 1.950[a]	89.150 ± 1.890[a]	280.100 ± 3.440[a]

[a]X is the average of experimental values, SE is the standard error. Each IC_{50} value ($X \pm SE$) was found from the data of three experiments performed in duplicate

[b]IC50 (μM) is the half-maximal inhibitory concentration against the tested cells

Experimental

General

IR spectra (thin films or solutions in $CHCl_3$) were obtained with the use of a Vertex 70 v spectrometer (Bruker, Karlsruhe, Germany). ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$, in MeOD or in DMSO-d_6 with Me_4Si as the internal standard on an AVANCE-500 instrument (500.13 (^1H), 125.78 MHz (^{13}C)) or on an AVANCE-400 (400.13 (^1H), 100.62 MHz (^{13}C)) (Bruker). Mass spectra of new compounds were recorded on an LCMS-2010 EV (Shimadzu) spectrometer of the UfIC RAS Center for Collective Use "Chemistry" or on a Bruker–Autoflex III spectrometer (MALDI TOF, positive ion mode, sinapic acid as the matrices) of the Collective Usage Centre "Agidel" at the Institute of Petrochemistry and Catalysis of RAS. Optical rotation was determined on a 141 polarimeter (Perkin–Elmer, Beaconsfield, UK). Specific rotation $[\alpha]_D$ is expressed in $(\text{deg mL})/(\text{g dm})^{-1}$; the concentration of the solution c is expressed in g/100 mL. Elemental analysis was carried out on a 1106 analyzer (Carlo Erba, Milan, Italy). TLC was carried out on Sorbfil plates (Sorbpolimer, Krasnodar, Russia) in chloroform–methanol, spots were visualized with anisaldehyde. Silica gel L (KSKG grade, 50–160 μm) was employed for column chromatography. All reagents and solvents were of the purest grade available, and generally were used without further treatment. The starting compounds ursolic, oleanolic, 18β-glycyrrhetinic acids and reagents: 1,4-dibromobutane, dimethylformamide, acetyl chloride, DMAP, N-bromosuccinimide, bromoacetic acid, DCC, and 5-bromovaleric acid were purchased from Acros Organics (Geel, Belgium). Betulinic acid were obtained from betulin according to the known procedures [30]. Acetates of oleanolic, ursolic, betulinic and 18β-glycyrrhetinic acids were synthesized according to the typical procedures. Synthesizes and spectral data of bromides oleanolic, ursolic, betulinic and 18β-glycyrrhetinic acids **20a,b, 21–26, 27a,b–29a,b** have been published as previously reported [10, 22, 23].

Synthesis

(E)-4-[2-(1H-indol-3-yl)-vinyl]-1-methyl-pyridinium iodide (F16)

This compound was prepared as previously reported [29]. Orange powder. Yield: 64%; mp: 273–275 °C (lit.: 277-278 °C) [32]. ^1H NMR (400 MHz, DMSO-d_6): $\delta = 11.93$ (1H, s, NH), 8.68 (2H, d, $J = 6.0$ Hz, pyridinio-H), 8.26 (1H, d, $J = 16.0$ Hz, CH = CH), 8.18–8.16 (1H, m, ArH), 8.12 (2H, d, $J = 6.0$ Hz, pyridinio-H), 8.00 (1H, s, pyrrole-H), 7.52 (1H, d, $J = 7.6$ Hz, ArH), 7.32–7.21 (3H, m, one CH = CH, two ArH), 4.18 (3H, s, N^+CH_3) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 154.6$ (pyridinio-C), 144.6 (two pyridinio-CH), 137.9 (pyrrole-C), 136.7 (one CH = CH),

132.7 (pyrrole-CH), 125.4 (pyrrole-C), 123.4 (Ar-CH), 122.1 (two pyridinio-CH), 121.6 (Ar-CH), 120.9 (Ar-CH), 117.3 (one CH = CH), 114.0 (pyrrole-C), 113.1 (ArCH), and 46.8 (N^+CH_3) ppm.

General procedure for the synthesis of the conjugates 6–19

A mixture of the corresponding bromides **20a,b, 21, 22, 24, 25, 27a,b–29a,b** (1.0 mmol), 3-[2-(4-pyridyl)vinyl]indole (200 mg, 1.0 mmol) or for dibromides **23, 26** (1.0 mmol), 3-[2-(4-pyridyl)vinyl]indole (400 mg, 2.0 mmol), and dry dimethylformamide (12 mL) was stirred at 85 °C in argon atmosphere for 12 h. Then the mixture was cooled to room temperature and evaporated under reduced pressure. The residue was chromatographed on silica gel, using CH_2Cl_2/MeOH 30:1 → 10:1, to obtain pure compounds **6–19**.

N-{4-[(3β-hydroxylup-20(29)-en-28-oyl)-butyl]-(E)-4-[2-(1H-indol-3-yl)-vinyl]}pyridinium bromide (6)

Orange powder. Yield: 84%; mp 217–219 °C (EtOH); $[\alpha]_D^{23}$ + 5.6 (*c* 0.71, CH_3OH); IR (film) ν_{max} 1718 (C = O), 2925 (OH), 3439 (NH) cm^{-1}; ^1H NMR (500 MHz, MeOD): δ = 8.58 (2H, d, *J* = 6.0 Hz, H-5′, H-9′), 8.22 (1H, d, *J* = 16.0 Hz, H-10′ or H-11′), 8.09–8.02 (3H, m, H-15′, H-6′, H-8′), 7.88 (1H, s, H-12′), 7.49–7.48 (1H, m, H-18′), 7.29–7.22 (3H, m, H-16′, H-17′, H-10′ or H-11′), 4.72, 4.61 (2H, both br s, H-29), 4.51–4.48 (2H, m, H-4′), 4.35-4.30, 4.08-4.04 (2H, both m, H-1′), 3.05-2.96 (2H, m, H-3, H-19), 2.24-0.58 (28H, m, CH, CH_2 in pentacyclic skeleton, H-2′, H-3′), 1.69 (3H, s, H-30), 0.96, 0.77, 0.75, 0.73, and 0.53 (3H each, all s, H-23–H-27) ppm; ^{13}C NMR (125 MHz, MeOD): δ = 177.6 (C-28), 157.4 (C-7′), 151.8 (C-20), 144.2 (C-5′, C-9′), 139.6 (C-19′), 139.2 (C-10′ or C-11′), 133.9 (C-12′), 126.6 (C-14′), 124.6 (C-16′), 123.4 (C-6′, C-8′), 122.9 (C-17′), 121.7 (C-15′), 117.8 (C-10′ or C-11′), 115.9 (C-13′), 113.6 (C-18′), 110.6 (C-29), 79.6 (C-3), 64.0 (C-1′), 60.6 (C-4′), 57.9 (C-17), 56.9 (C-5), 52.0 (C-9), 50.6 (C-18), 48.6 (C-19), 43.6 (C-14), 42.0 (C-8), 40.1 (C-1), 39.9 (C-4), 39.8 (C-13), 38.3 (C-10), 38.1 (C-22), 35.7 (C-7), 33.2 (C-16), 31.8 (C-21), 30.9 (C-15), 29.5 (C-2′), 28.5 (C-23), 28.0 (C-2), 26.9 (C-3′), 26.8 (C-12), 22.2 (C-11), 19.7 (C-30), 19.6 (C-6), 16.9 (C-25), 16.8 (C-24), 16.0 (C-26), and 15.2 (C-27) ppm; MS (APCI): m/z $[M\text{-}Br]^+$: 731.7 $C_{49}H_{67}N_2O_3$ (calcd. 731.5); Anal. Calcd. for $C_{49}H_{67}BrN_2O_3$: C 72.48; H 8.32. Found: 72.51; H 8.26.

N-{4-[(3β-acetoxylup-20(29)-en-28-oyl)-butyl]-(E)-4-[2-(1H-indol-3-yl)-vinyl]}pyridinium bromide (7)

Brown powder. Yield: 89%; mp 149–151 °C (EtOH); $[\alpha]_D^{21}$ −20 (*c* 0.03, $CHCl_3$); IR (film) ν_{max} 1722 (C = O), 3441 (NH) cm^{-1}; ^1H NMR (500 MHz, MeOD/$CDCl_3$): δ = 8.57–8.51 (2H, m, H-5′, H-9′), 8.15 (1H, d, *J* = 16.0 Hz, H-10′ or H-11′), 8.04–8.03 (1H, m, H-15′), 7.98-7.91 (2H, m, H-6′, H-8′), 7.84 (1H, s, H-12′), 7.49–7.48 (1H, m, H-18′), 7.23–7.25 (2H, m, H-16′, H-17′), 7.16 (1H, d, *J* = 16.0 Hz, H-10′ or H-11′), 4.72, 4.60 (2H, both br s, H-29), 4.44–4.51 (2H, m, H-4′), 4.36–4.33, 4.11–4.07 (2H, both m, H-1′), 4.30–4.23 (1H, m, H-3), 2.97–2.94 (1H, m, H-19), 2.23–0.81 (28H, m, CH, CH_2 in pentacyclic skeleton, H-2′, H-3′), 2.00 (3H, s, Me), 1.68 (3H, s, H-30), 0.96, 0.77, 0.75, 0.70, and 0.65 (3H each, all s, H-23–H-27) ppm; ^{13}C NMR (125 MHz, MeOD/$CDCl_3$): δ = 177.4 (C-28), 172.8 (C = O), 156.9 (C-7′), 151.3 (C-20), 143.8 (C-5′, C-9′), 139.2 (C-19′), 139.0 (C-10′ or C-11′), 133.8 (C-12′), 126.3 (C-14′), 124.4 (C-16′), 123.1 (C-6′, C-8′), 122.8 (C-17′), 121.4 (C-15′), 117.4 (C-10′ or C-11′), 115.5 (C-13′), 113.5 (C-18′), 110.5 (C-29), 82.3 (C-3), 63.8 (C-1′), 60.3 (C-4′), 57.7 (C-17), 56.5 (C-5), 51.5 (C-9), 50.4 (C-18), 48.6 (C-19), 43.4 (C-14), 41.8 (C-8), 39.4 (C-1), 39.3 (C-4), 38.6 (C-13), 38.0 (C-10), 37.9 (C-22), 35.3 (C-7), 33.0 (C-16), 31.5 (C-21), 30.6 (C-15), 29.2 (C-2′), 28.4 (C-23), 26.5 (C-2, C-3′), 24.4 (C-12), 21.9 (C-11), 21.4 (CH_3), 19.7 (C-30), 19.1 (C-6), 16.9 (C-25, C-24), 16.7 (C-26), and 15.3 (C-27) ppm; MS (APCI): m/z $[M\text{-}Br]^+$: 773.8 $C_{51}H_{69}N_2O_4$ (calcd. 773.5); Anal. Calcd. for $C_{51}H_{69}BrN_2O_4$: C 71.73; H 8.14. Found: 71.67; H 8.11.

N-{8′-[(3β-hydroxylup-20(29)-en-28-oyl)-3′,6′-dioxaoctan-1′-yl]-(E)-4-[2-(1H-indol-3-yl)-vinyl]}pyridinium bromide (8)

Orange powder. Yield: 85%; mp 165–167 °C (EtOH); $[\alpha]_D^{23}$ + 1.4 (*c* 0.70, CH_3OH); IR (film) ν_{max} 1721 (C = O), 2943 (OH), 3402 (NH) cm^{-1}; ^1H NMR (500 MHz, MeOD/$CDCl_3$): δ = 8.45 (2H, d, *J* = 6.0 Hz, H-5′, H-9′), 8.09 (1H, d, *J* = 16.0 Hz, H-10′ or H-11′), 8.01–7.99 (1H, m, H-15′), 7.84–7.81 (3H, m, H-6′, H-8′, H-12′), 7.49–7.48 (1H, m, H-18′), 7.27–7.26 (2H, m, H-16′, H-17′), 7.10 (1H, d, *J* = 16.0 Hz, H-10′ or H-11′), 4.66 (1H, br s, H-29), 4.57–4.53 (3H, m, H-29, H-6″), 4.22, 3.92 (4H, both br s, H-1″, H-5″), 3.65–3.62 (6H, m, H-2″, H-3″, H-4″), 3.12–3.09 (1H, m, H-3), 2.99–2.91 (1H, m, H-19), 2.19–0.62 (24H, m, CH, CH_2 in pentacyclic skeleton), 1.64 (3H, s, H-30), 0.91, 0.92, 0.86, 0.76, and 0.71 (3H each, all s, H-23–H-27) ppm; ^{13}C NMR (125 MHz, MeOD/$CDCl_3$): δ = 177.3 (C-28), 156.7 (C-7′), 151.2 (C-20), 144.1 (C-5′, C-9′), 139.0 (C-19′), 138.7 (C-10′ or C-11′), 133.5 (C-12′), 126.1 (C-14′), 124.2 (C-16′), 122.6 (C-17′), 122.5 (C-6′, C-8′), 121.2 (C-15′), 117.3 (C-10′ or C-11′), 115.3 (C-13′), 113.4 (C-18′), 110.4 (C-29), 79.3 (C-3), 71.4 (C-2″), 71.1 (C-3″), 70.1 (C-4″, C-5″), 63.7 (C-1″), 60.3 (C-6″), 57.5 (C-17), 56.4 (C-5), 51.5 (C-9), 50.2 (C-18), 48.0 (C-19), 43.2 (C-14), 41.6 (C-8), 39.7 (C-4), 39.6 (C-1), 39.2 (C-13), 38.0 (C-10), 37.8 (C-

22), 35.2 (C-7), 32.9 (C-16), 31.4 (C-21), 30.5 (C-15), 28.5 (C-23), 27.7 (C-2), 26.4 (C-12), 21.7 (C-11), 19.7 (C-30), 19.1 (C-6), 16.7 (C-25), 16.6 (C-24), 16.1 (C-26), and 15.2 (C-27) ppm; MS (APCI): m/z $[M\text{-}Br]^+$: 791.6 $C_{51}H_{71}N_2O_5$ (calcd. 791.5); Anal. Calcd. for $C_{51}H_{71}BrN_2O_5$: C 70.24; H 8.26. Found: 70.15; H 8.16.

N-[(3β-acetoxy-lup-20(29)-en-28-oic acid)-30-yl]-(E)-4-[2-(1H-indol-3-yl)-vinyl]pyridinium bromide (9)

Orange powder. Yield: 78%; mp 164–166 °C (EtOH); $[\alpha]_D^{23} + 44$ (*c* 0.78, CH_3OH); IR (film) ν_{max} 1735 (C = O), 3410 (NH) cm^{-1}; ^1H NMR (500 MHz, MeOD/$CDCl_3$): $\delta =$ 8.34–8.32 (2H, m, H-5′, H-9′), 8.12 (1H, d, $J = 16.0$ Hz, H-10′ or H-11′), 8.03–8.01 (1H, m, H-15′), 7.89–7.85 (3H, m, H-6′, H-8′, H-12′), 7.51–7.49 (1H, m, H-18′), 7.29–7.28 (2H, m, H-16′, H-17′), 7.08 (1H, d, $J = 16.0$ Hz, H-10′ or H-11′), 5.14, 4.69 (2H, both br s, H-29), 4.89 (2H, br s, H-30), 4.45–4.41 (1H, m, H-3), 3.01–2.88 (1H, m, H-19), 2.33–0.77 (24H, m, CH, CH_2 in pentacyclic skeleton), 2.06 (3H, s, Me), 0.99, 0.94, 0.85, 0.84, and 0.83 (3H each, all s, H-23–H-27) ppm; ^{13}C NMR (125 MHz, MeOD/$CDCl_3$): δ = 182.9 (C-28), 172.7 (C = O), 156.8 (C-7′), 151.3 (C-20), 143.9 (C-5′, C-9′), 139.0 (C-19′), 138.9 (C-10′ or C-11′), 133.8 (C-12′), 126.1 (C-14′), 124.3 (C-16′), 122.8 (C-6′, C-8′), 122.7 (C-17′), 121.3 (C-15′), 117.3 (C-10′ or C-11′), 115.3 (C-13′), 113.5 (C-18′), 113.1 (C-29), 82.3 (C-3), 65.2 (C-30), 57.5 (C-17), 56.4 (C-5), 51.7 (C-9), 51.4 (C-18), 48.5 (C-19), 43.8 (C-14), 41.6 (C-8), 39.3 (C-1), 39.1 (C-13), 38.6 (C-4), 37.9 (C-10), 37.8 (C-22), 35.2 (C-7), 33.2 (C-16), 30.6 (C-21), 28.5 (C-23, C-15), 28.4 (C-2), 24.4 (C-12), 22.0 (C-11), 21.5 (CH_3), 19.0 (C-6), 16.9 (C-25), 16.8 (C-24), 16.7 (C-26), and 15.2 (C-27) ppm; MS (APCI): m/z $[M\text{-}Br]^+$: 717.6 $C_{47}H_{61}N_2O_4$ (calcd. 717.5); Anal. Calcd. for $C_{47}H_{61}BrN_2O_4$: C 70.75; H 7.71. Found: 71.05; H 8.04.

N-[3β-(4-[1H-indol-3-ylvinyl]-carboxymethyl)-pyridinium bromide]-lup-20(29)-en-28-oyl-N-4-((*E*)-4-[1H-indol-3-ylvinyl]-butyl)pyridinium bromide (10)

Dark orange powder. Yield: 91%; mp 232–234 °C (EtOH); $[\alpha]_D^{23} + 32$ (*c* 0.76, CH_3OH); IR (vasel) ν_{max} 1726 (C = O), 3435 (NH) cm^{-1}; ^1H NMR (500 MHz, MeOD/$CDCl_3$): $\delta =$ 8.53, 8.45 (4H, both d, $J = 6.5$ Hz, H-5′, H-9′, H-5″, H-9″), 8.21, 8.14 (2H, both d, $J = 16.0$ Hz, H-10′ or H-11′, H-10″ or H-11″), 8.06–8.02 (2H, m, H-15′, H-15″), 7.96, 7.93 (4H, m, H-6′, H-8′, H-6″, H-8″), 7.87, 7.84 (2H, both s, H-12′, H-12″), 7.51–7.47 (2H, m, H-18′, H-18″), 7.28–7.14 (6H, m, H-16′, H-17′, H-16″, H-17″, H-10′ or H-11′, H-10″ or H-11″), 4.69, 4.58 (2H, both br s, H-29), 4.51–4.45 (5H, m, H-4′, H-4″, H-3), 4.29–4.24, 4.07–4.02 (2H, both m, H-1′), 2.99–2.87 (1H, m, H-19), 2.21–0.56 (28H, m, CH, CH_2 in pentacyclic skeleton, H-2′, H-3′), 1.65 (3H, s, H-30), 0.93, 0.72, 0.71, 0.70, 0.55 (3H each, all s, H-23–H-27) ppm; ^{13}C NMR (125 MHz, MeOD/$CDCl_3$): $\delta = 177.5$ (C-28), 167.2 (C = O), 157.8 (C-7″), 157.1 (C-7′), 151.5 (C-20), 145.1 (C-5′, C-9′), 143.9 (C-5″, C-9″), 139.8 (C-10″ or C-11″), 139.3 (C-19′, C-19″), 139.1 (C-10′ or C-11′), 134.3 (C-12″), 133.9 (C-12′), 126.4 (C-14′, C-14″), 124.5 (C-16′), 124.4 (C-16″), 123.2 (C-6′, C-8′, C-17″), 122.9 (C-17′), 122.8 (C-15″), 122.6 (C-6″, C-8″), 121.5 (C-15′), 117.6 (C-10′ or C-11′, C-10″ or C-11″), 115.7 (C-13′), 115.6 (C-13″), 113.6 (C-18′, C-18″), 110.5 (C-29), 85.6 (C-3), 63.9 (C-1′), 60.4 (C-4′, C-4″), 57.7 (C-17), 56.6 (C-5), 51.6 (C-9), 50.4 (C-18), 48.3 (C-19), 43.5 (C-14), 41.8 (C-8), 39.5 (C-13), 39.3 (C-1), 38.8 (C-4), 38.1 (C-10), 37.9 (C-22), 35.3 (C-7), 33.0 (C-16), 31.6 (C-21), 30.7 (C-15), 29.3 (C-2′), 28.4 (C-23), 26.6 (C-3′, C-12), 24.4 (C-2), 22.0 (C-11), 19.7 (C-30), 19.2 (C-6), 16.9 (C-25), 16.8 (C-24), 16.7 (C-26), and 15.2 (C-27) ppm; MS: m/z $[M\text{-}H2Br]^+$: 991.72 $C_{66}H_{80}N_4O_4$ (calcd. 991.61); Anal. Calcd. for $C_{66}H_{80}Br_2N_4O_4$: C 68.74; H 6.99. Found: 68.62; H 7.15.

N-{5-[(3β-hydroxylup-20(29)-en-28-yl)-pentanoyl]-(E)-4-[2-(1H-indol-3-yl)-vinyl]}pyridinium bromide (11)

Orange powder. Yield: 81%; mp 184–186 °C (EtOH); $[\alpha]_D^{23} + 21$ (*c* 0.64, CH_3OH); IR (film) ν_{max} 1725 (C = O), 2942 (OH), 3400 (NH) cm^{-1}; ^1H NMR (500 MHz, MeOD/$CDCl_3$): $\delta = 8.49$ (2H, d, $J = 6.5$ Hz, H-5′, H-9′), 8.11 (1H, d, $J = 16.0$ Hz, H-10′ or H-11′), 8.02–8.01 (1H, m, H-15′), 7.89 (2H, d, $J = 6.5$ Hz, H-6′, H-8′), 7.82 (1H, s, H-12′), 7.49–7.48 (1H, m, H-18′), 7.28–7.26 (2H, m, H-16′, H-17′), 7.12 (1H, d, $J = 16.0$ Hz, H-10′ or H-11′), 4.69, 4.58 (2H, both br s, H-29), 4.43–4.40 (2H, m, H-4′), 4.33, 3.86 (2H, both d, $J = 11.0$ Hz, H-28), 3.15-3.10 (1H, m, H-3), 2.48-2.40 (3H, m, H-19, H-1′), 2.24-0.58 (28H, m, CH, CH_2 in pentacyclic skeleton, H-2′, H-3′), 1.67 (3H, s, H-30), 1.01, 0.97, 0.92, 0.81, and 0.72 (3H each, all s, H-23–H-27) ppm; ^{13}C NMR (125 MHz, MeOD/$CDCl_3$): $\delta = 174.8$ (C = O), 156.7 (C-7′), 150.9 (C-20), 143.6 (C-5′, C-9′), 139.1 (C-19′), 138.8 (C-10′ or C-11′), 133.6 (C-12′), 126.1 (C-14′), 124.3 (C-16′), 123.0 (C-6′, C-8′), 122.6 (C-17′), 121.2 (C-15′), 117.3 (C-10′ or C-11′), 115.3 (C-13′), 113.4 (C-18′), 110.6 (C-29), 79.4 (C-3), 63.8 (C-28), 60.4 (C-4′), 56.4 (C-5), 51.4 (C-9), 49.7 (C-18), 48.6 (C-19), 47.4 (C-17), 43.6 (C-14), 41.8 (C-8), 39.8 (C-1), 39.7 (C-4), 38.7 (C-13), 38.0 (C-10), 35.4 (C-7), 35.2 (C-22), 34.1 (C-1′), 31.4 (C-3′), 30.6 (C-21), 30.4 (C-16), 28.6 (C-23), 28.0 (C-2), 27.8 (C-15), 26.2 (C-12), 22.3 (C-2′), 21.7 (C-11), 19.5 (C-30), 19.2 (C-6), 16.7 (C-25), 16.6 (C-24), 16.1 (C-26), and 15.4 (C-27) ppm; MS (APCI): m/z $[M\text{-}Br]^+$: 745.7 $C_{50}H_{69}N_2O_3$ (calcd. 745.5); Anal. Calcd. for $C_{50}H_{69}BrN_2O_3$: C 72.70; H 8.42. Found: C 72.65; H 8.36.

N-(3β,28-diacetoxy-lup-20(29)-en-30-yl)-(E)-4-[2-(1H-indol-3-yl)-vinyl]pyridinium bromide (12)

Orange powder. Yield: 79%; mp 219–121 °C (EtOH); $[\alpha]_D^{23} + 40.4$ (*c* 0.68, CH_3OH); IR (film) ν_{max} 1731 (C = O), 3444 (NH) cm^{-1}; ^1H NMR (500 MHz, MeOD/$CDCl_3$): $\delta =$ 8.41 (2H, d, $J = 6.5$ Hz, H-5′, H-9′), 8.16 (1H, d, $J =$ 16.0 Hz, H-10′ or H-11′), 8.06-8.04 (1H, m, H-15′), 7.94 (2H, d, $J = 6.5$ Hz, H-6′, H-8′), 7.87 (1H, s, H-12′), 7.52-7.50 (1H, m, H-18′), 7.29–7.28 (2H, m, H-16′, H-17′), 7.15 (1H, d, $J = 16.0$ Hz, H-10′ or H-11′), 5.17, 4.66 (2H, both br s, H-29), 4.94 (2H, br s, H-30), 4.45-4.42 (1H, m, H-3), 4.32, 3.79 (2H, both d, $J = 11.0$ Hz, H-28), 2.38-2.33 (1H, m, H-19), 2.11–0.79 (24H, m, CH, CH_2 in pentacyclic skeleton), 2.07, 2.04 (6H, both s, Me), 1.05, 1.03, 0.86, 0.85, and 0.84 (3H each, all s, H-23–H-27) ppm; ^{13}C NMR (125 MHz, MeOD/$CDCl_3$): $\delta = 173.1$ (C = O), 172.8 (C = O), 157.2 (C-7′), 150.8 (C-20), 144.3 (C-5′, C-9′), 139.3 (C-10′ or C-11′), 139.2 (C-19′), 134.0 (C-12′), 126.3 (C-14′), 124.4 (C-16′), 123.0 (C-6′, C-8′), 122.8 (C-17′), 121.5 (C-15′), 117.5 (C-10′ or C-11′), 115.5 (C-13′), 113.6 (C-18′), 113.2 (C-29), 82.4 (C-3), 64.6 (C-30), 63.3 (C-28), 56.5 (C-5), 51.3 (C-9), 49.5 (C-18), 48.6 (C-19), 47.5 (C-17), 43.7 (C-14), 42.0 (C-8), 39.4 (C-1), 38.7 (C-4), 38.6 (C-13), 38.1 (C-10), 35.2 (C-22), 35.1 (C-7), 30.6 (C-21), 30.5 (C-16), 28.6 (C-23), 28.0 (C-2), 27.9 (C-15), 24.6 (C-12), 21.9 (C-11), 21.5 (CH_3), 21.2 (CH_3), 19.1 (C-6), 17.1 (C-25), 16.8 (C-24), 16.6 (C-26), and 15.4 (C-27) ppm; MS (APCI): m/z $[M\text{-}Br]^+$: 745.6 $C_{49}H_{65}N_2O_4$ (calcd. 745.5); Anal. Calcd. for $C_{49}H_{65}BrN_2O_4$: C 71.25; H 7.93. Found: 71.31; H 8.09.

N,N′-{5-[(3β-pentanoyl-lup-20(29)-en-28-yl)-pentanoyl]-di-(E)-4-[2-(1H-indol-3-yl)-vinyl]}pyridinium dibromide (13)

Orange powder. Yield: 89%; mp 243–245 °C (EtOH); $[\alpha]_D^{23} + 5.7$ (*c* 0.87, CH_3OH); IR (film) ν_{max} 1726 (C = O), 3435 (NH) cm^{-1}; ^1H NMR (500 MHz, MeOD/$CDCl_3$): $\delta =$ 8.51–8.44 (4H, m, H-5′, H-9′, H-5″, H-9″), 8.09 (2H, d, $J =$ 16.0 Hz, H-10′ or H-11′, H-10″ or H-11″), 8.02–7.98 (2H, m, H-15′, H-15″), 7.89–7.84 (4H, m, H-6′, H-8′, H-6″, H-8″), 7.82 (2H, s, H-12′, H-12″), 7.51–7.45 (2H, m, H-18′, H-18″), 7.31–7.22 (4H, m, H-16′, H-17′, H-16″, H-17″), 7.11 (2H, d, $J = 16.0$ Hz, H-10′ or H-11′, H-10″ or H-11″), 4.68, 4.58 (2H, both br s, H-29), 4.44–4.39 (5H, m, H-4′, H-4″, H-3), 4.31, 3.84 (2H, both d, $J = 11.0$ Hz, H-28), 2.48-2.41 (5H, m, H-19, H-1′, H-1″), 2.01–0.74 (32H, m, CH, CH_2 in pentacyclic skeleton, H-2′, H-3′, H-2″, H-3″), 1.67 (3H, s, H-30), 0.99, 0.95, 0.82, 0.79, and 0.78 (3H each, all s, H-23–H-27) ppm; ^{13}C NMR (125 MHz, MeOD/$CDCl_3$): $\delta = 174.8$ (C = O), 174.3 (C = O), 156.7 (C-7′, C-7″), 150.9 (C-20), 143.6 (C-5′, C-9′, C-5″, C-9″), 139.1 (C-19′, C-19″), 138.7 (C-10′ or C-11′, C-10″ or C-11″), 133.6 (C-12′, C-12″), 126.2 (C-14′, C-14″), 124.3 (C-16′, C-16″), 123.0 (C-6′, C-8′, C-6″, C-8″), 122.6 (C-17′, C-17″), 121.3 (C-15′, C-15″), 117.4 (C-10′ or C-11′, C-10″ or C-11″), 115.3 (C-13′, C-13″), 113.4 (C-18′, C-18″), 110.7 (C-29), 79.1 (C-3), 63.8 (C-28), 60.4 (C-4′, C-4″), 56.4 (C-5), 51.2 (C-9), 49.7 (C-18), 48.6 (C-19), 47.4 (C-17), 43.6 (C-14), 41.8 (C-8), 39.3 (C-1), 38.6 (C-4), 38.7 (C-13), 38.0 (C-10), 35.4 (C-7), 35.1 (C-22), 34.4 (C-1″), 34.1 (C-1′), 31.4 (C-3′, C-3″), 30.6 (C-21), 30.4 (C-16), 28.6 (C-23), 28.0 (C-2), 26.1 (C-15), 24.6 (C-12), 22.4 (C-2″), 22.3 (C-2′), 21.7 (C-11), 19.5 (C-30), 19.0 (C-6), 17.1 (C-25), 16.8 (C-24), 16.6 (C-26), and 15.4 (C-27) ppm; MS: m/z $[M\text{-}H2Br]^+$: 1047.69 $C_{70}H_{88}N_4O_4$ (calcd. 1047.67); Anal. Calcd. for $C_{70}H_{88}Br_2N_4O_4$: C 69.53; H 7.34. Found: 69.43; H 7.25.

N-{4-[(3β-hydroxyurs-12-en-28-oyl)-butyl]-(E)-4-[2-(1H-indol-3-yl)-vinyl]}pyridinium bromide(14)

Dark yellow powder. Yield: 83%; mp 194–196 °C (EtOH); $[\alpha]_D^{21} + 12.5$ (*c* 0.04, DMSO); IR (film) ν_{max} 1717 (C = O), 2925 (OH), 3442 (NH) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 11.99$ (1H, s, NH), 8.84 (2H, d, $J = 6.5$ Hz, H-5′, H-9′), 8.30 (1H, d, $J = 16.0$ Hz, H-10′ or H-11′), 8.19–8.15 (3H, m, H-15′, H-6′, H-8′), 7.98 (1H, s, H-12′), 7.53–7.51 (1H, m, H-18′), 7.33–7.23 (3H, m, H-16′, H-17′, H-10′ or H-11′), 5.07 (1H, br s, H-12), 4.49–4.46 (2H, m, H-4′), 3.98-3.93 (2H, m, H-1′), 2.91–2.88 (1H, m, H-3), 2.13 (1H, d, $J = 11.0$ Hz, H-18), 1.95–0.82 (26H, m, CH, CH_2 in pentacyclic skeleton, H-2′, H-3′), 0.99, 0.91, 0.82, 0.80, 0.79, 0.57, and 0.54 (3H each, all s, H-23–H-27, H-29 and H-30) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 176.8$ (C-28), 155.3 (C-7′), 143.8 (C-5′, C-9′), 138.5 (C-19′), 138.1 (C-13), 137.3 (C-10′ or C-11′), 133.1 (C-12′), 125.3 (C-12, C-14′), 123.4 (C-16′), 122.4 (C-6′, C-8′), 121.6 (C-17′), 120.9 (C-15′), 117.2 (C-10′ or C-11′), 114.2 (C-13′), 113.1 (C-18′), 77.2 (C-3), 63.6 (C-1′), 59.1 (C-4′), 55.2 (C-5), 52.8 (C-18), 47.9 (C-17), 47.3 (C-9), 42.1 (C-14), 40.6 (C-8), 40.4 (C-19), 40.1 (C-20), 39.4 (C-1), 38.6 (C-4), 36.9 (C-22), 36.8 (C-10), 33.1 (C-7), 30.5 (C-21), 29.5 (C-2′), 28.6 (C-23), 28.4 (C-15), 27.3 (C-3′), 25.2 (C-16), 24.2 (C-11), 23.7 (C-27), 23.3 (C-2), 21.4 (C-30), 18.4 (C-6), 17.4 (C-29), 17.2 (C-26), 16.4 (C-24), and 15.6 (C-25) ppm; MS (APCI): m/z $[M\text{-}Br]^+$: 731.6 $C_{49}H_{67}N_2O_3$ (calcd. 731.5); Anal. Calcd. for $C_{49}H_{67}BrN_2O_3$: 72.28; H 8.32. Found: C 72.31; H 8.28.

N-{4-[(3β-acetoxyurs-12-en-28-oyl)-butyl]-(E)-4-[2-(1H-indol-3-yl)-vinyl]}pyridinium bromide (15)

Brown powder. Yield: 82%; mp 174–176 °C (EtOH); $[\alpha]_D^{21} + 9.5$ (*c* 0.04, CH_3OH); IR (film) ν_{max} 1719 (C = O), 3440 (NH) cm^{-1}; ^1H NMR (500 MHz, MeOD): $\delta = 8.62$ (2H, d, $J = 6.5$ Hz, H-5′, H-9′), 8.23 (1H, d, $J = 16.0$ Hz, H-10′ or H-11′), 8.10–8.04 (3H, m, H-15′, H-6′, H-8′), 7.89

(1H, s, H-12′), 7.51–7.49 (1H, m, H-18′), 7.28–7.23 (3H, m, H-16′, H-17′, H-10′ or H-11′), 5.14 (1H, br s, H-12), 4.49-4.47 (2H, m, H-4′), 4.24-4.22 (1H, m, H-3), 4.06-4.01 (2H, m, H-1′), 2.23 (1H, d, $J = 11.0$ Hz, H-18), 2.06–0.82 (26H, m, CH, CH_2 in pentacyclic skeleton, H-2′, H-3′), 1.93 (3H, s, Me), 1.06, 0.97, 0.89, 0.76, 0.73, 0.69, and 0.59 (3H each, all s, H-23–H-27, H-29 and H-30) ppm; ^{13}C NMR (125 MHz, MeOD): $\delta = 180.5$ (C-28), 172.9 (C = O), 157.4 (C-7′), 144.3 (C-5′, C-9′), 140.1 (C-19′), 139.6 (C-13), 139.3 (C-10′ or C-11′), 134.1 (C-12′), 126.7 (C-12, C-14′), 124.6 (C-16′), 123.5 (C-6′, C-8′), 122.9 (C-17′), 121.8 (C-15′), 117.9 (C-10′ or C-11′), 115.9 (C-13′), 113.6 (C-18′), 82.4 (C-3), 65.1 (C-1′), 60.8 (C-4′), 56.6 (C-5), 54.4 (C-18), 49.3 (C-17), 48.6 (C-9), 43.3 (C-14), 40.9 (C-8), 40.5 (C-19, C-20), 39.3 (C-1), 38.6 (C-4), 38.1 (C-22), 38.0 (C-10), 34.2 (C-7), 31.8 (C-21), 30.2 (C-2′), 29.1 (C-15), 28.6 (C-23), 26.5 (C-3′), 25.4 (C-16), 24.5 (C-2), 24.3 (C-11), 24.2 (C-27), 21.7 (CH_3), 21.3 (C-30), 19.3 (C-6), 18.1 (C-29), 17.8 (C-26), 17.2 (C-24), and 16.2 (C-25) ppm; MS (APCI): m/z $[M\text{-}Br]^+$: 773.6 $C_{51}H_{69}N_2O_4$ (calcd. 773.5); Anal. Calcd. for $C_{51}H_{69}BrN_2O_4$: C 71.73; H 8.14. Found: 71.68; H 8.11.

N-{4-[(3β-hydroxyolean-12-en-28-oyl)-butyl]-(E)-4-[2-(1H-indol-3-yl)-vinyl]}pyridinium bromide (16)

Orange powder. Yield: 83%; mp 184–186 °C (EtOH); $[\alpha]_D^{21} + 15$ (*c* 0.04, DMSO); IR (film) ν_{max} 1716 (C = O), 2928 (OH), 3437 (NH) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 11.99$ (1H, s, NH), 8.84 (2H, d, $J = 6.5$ Hz, H-5′, H-9′), 8.31 (1H, d, $J = 16.0$ Hz, H-10′ or H-11′), 8.19–8.15 (3H, m, H-15′, H-6′, H-8′), 7.98 (1H, s, H-12′), 7.53–7.51 (1H, m, H-18′), 7.33–7.21 (3H, m, H-16′, H-17′, H-10′ or H-11′), 5.11 (1H, br s, H-12), 4.51–4.47 (2H, m, H-4′), 4.04–3.93 (2H, m, H-1′), 2.91–2.88 (1H, m, H-3), 2.75 (1H, d, $J = 11.0$ Hz, H-18), 2.01–0.71 (26H, m, CH, CH_2 in pentacyclic skeleton, H-2′, H-3′), 1.05, 0.88, 0.87, 0.78, 0.72, 0.56, and 0.52 (3H each, all s, H-23–H-27, H-29 and H-30) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 177.1$ (C-28), 155.3 (C-7′), 143.9 (C-13), 143.7 (C-5′, C-9′), 138.1 (C-19′), 137.3 (C-10′ or C-11′), 133.1 (C-12′), 125.4 (C-14′), 123.4 (C-16′), 122.4 (C-6′, C-8′), 122.3 (C-12), 121.6 (C-17′), 120.9 (C-15′), 117.2 (C-10′ or C-11′), 114.2 (C-13′), 113.1 (C-18′), 77.2 (C-3), 63.6 (C-1′), 58.9 (C-4′), 55.2 (C-5), 47.4 (C-9), 46.5 (C-17), 45.8 (C-19), 42.9 (C-14), 41.7 (C-18), 40.2 (C-8), 39.6 (C-1), 38.7 (C-4), 36.9 (C-10), 33.6 (C-22), 33.2 (C-30), 32.8 (C-7), 32.6 (C-21), 30.8 (C-20), 29.5 (C-2′), 28.6 (C-23), 28.3 (C-15), 27.2 (C-3′), 26.1 (C-29), 25.3 (C-16), 23.9 (C-27), 23.3 (C-11), 23.1 (C-2), 18.4 (C-6), 17.2 (C-26), and 16.4 (C-24), 15.5 (C-25) ppm; MS (APCI): m/z $[M\text{-}Br]^+$: 731.6 $C_{49}H_{67}N_2O_3$ (calcd. 731.5); Anal. Calcd. for $C_{49}H_{67}BrN_2O_3$: 72.48; H 8.32. Found: C 72.51; H 8.27.

N-{4-[(3β-acetoxyolean-12-en-28-oyl)-butyl]-(E)-4-[2-(1H-indol-3-yl)-vinyl]}pyridinium bromide (17)

Brown powder. Yield: 85%; mp 162–164 °C (EtOH); $[\alpha]_D^{21} + 23.3$ (*c* 0.03, CH_3OH); IR (film) ν_{max} 1722 (C = O), 3443 (NH) cm^{-1}; ^{1}H NMR (500 MHz, MeOD): $\delta = 8.60$ (2H, d, $J = 6.5$ Hz, H-5′, H-9′), 8.21 (1H, d, $J = 16.0$ Hz, H-10′ or H-11′), 8.09–8.08 (1H, m, H-15′), 8.02 (2H, d, $J = 6.5$ Hz, H-6′, H-8′), 7.89 (1H, s, H-12′), 7.50-7.49 (1H, m, H-18′), 7.29–7.21 (3H, m, H-16′, H-17′, H-10′ or H-11′), 5.17 (1H, br s, H-12), 4.52-4.43 (2H, m, H-4′), 4.26–4.23 (1H, m, H-3), 4.09–4.02 (2H, m, H-1′), 2.86 (1H, d, $J = 11.0$ Hz, H-18), 2.07-0.71 (26H, m, CH, CH_2 in pentacyclic skeleton, H-2′, H-3′), 1.95 (3H, s, Me), 1.11, 0.95, 0.91, 0.75, 0.72, 0.69, and 0.56 (3H each, all s, H-23–H-27, H-29 and H-30) ppm; ^{13}C NMR (125 MHz, MeOD): $\delta = 179.4$ (C-28), 172.8 (C = O), 157.3 (C-7′), 145.4 (C-13), 144.2 (C-5′, C-9′), 139.6 (C-19′), 139.2 (C-10′ or C-11′), 134.1 (C-12′), 126.7 (C-14′), 124.6 (C-16′), 123.6 (C-12), 123.4 (C-6′, C-8′), 122.9 (C-17′), 121.7 (C-15′), 117.9 (C-10′ or C-11′), 115.9 (C-13′), 113.6 (C-18′), 82.4 (C-3), 64.9 (C-1′), 60.7 (C-4′), 56.6 (C-5), 48.6 (C-19), 48.2 (C-9), 47.1 (C-17), 42.9 (C-14, C-18), 40.7 (C-8), 39.2 (C-1), 38.6 (C-4), 38.1 (C-10), 34.9 (C-22), 33.9 (C-7), 33.8 (C-21), 33.7 (C-30), 31.7 (C-20), 30.2 (C-2′), 28.8 (C-15), 28.6 (C-23), 26.5 (C-3′, C-29), 24.6 (C-2), 24.3 (C-11), 24.2 (C-27, C-16), 21.3 (CH_3), 19.4 (C-6), 17.9 (C-26), 17.2 (C-24), and 16.2 (C-25) ppm; MS (APCI): m/z $[M\text{-}Br]^+$: 773.6 $C_{51}H_{69}N_2O_4$ (calcd. 773.5); Anal. Calcd. for $C_{51}H_{69}BrN_2O_4$: C 71.73; H 8.14. Found: C 71.69; H 8.11.

N-{4-[(3β-hydroxy-11-oxo-18β,20β-olean-12-en-29-oyl)-butyl]-(E)-4-[2-(1H-indol-3-yl)-vinyl]}pyridinium bromide (18)

Orange powder. Yield: 81%; mp 154–156 °C (EtOH); $[\alpha]_D^{21} + 75$ (*c* 0.04, DMSO); IR (film) ν_{max} 1721 (C = O), 2927 (OH), 3440 (NH) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 11.99$ (1H, s, NH), 8.81 (2H, d, $J = 6.5$ Hz, H-5′, H-9′), 8.27 (1H, d, $J = 16.0$ Hz, H-10′ or H-11′), 8.16–8.14 (3H, m, H-15′, H-6′, H-8′), 7.98 (1H, s, H-12′), 7.53–7.52 (1H, m, H-18′), 7.32–7.22 (3H, m, H-16′, H-17′, H-10′ or H-11′), 5.37 (1H, br s, H-12), 4.51–4.48 (2H, m, H-4′), 4.15–4.06 (2H, m, H-1′), 3.02–2.99 (1H, m, H-3), 2.59-2.56 (1H, m, H-18), 2.31 (1H, s, H-9), 2.11–0.68 (23H, m, CH, CH_2 in pentacyclic skeleton, H-2′, H-3′), 1.33, 1.11, 0.99, 0.98, 0.89, 0.73, and 0.66 (3H each, all s, H-23–H-28 and H-30) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 199.5$ (C-11), 176.2 (C-29), 169.9 (C-13), 155.1 (C-7′), 143.7 (C-5′, C-9′), 138.1 (C-19′), 137.2 (C-10′ or C-11′), 132.9 (C-12′), 127.8 (C-12), 125.4 (C-14′), 123.4 (C-16′), 122.4 (C-6′, C-8′), 121.6 (C-17′), 120.9 (C-15′), 117.3 (C-10′ or C-11′), 114.1 (C-13′), 113.1 (C-18′), 77.1 (C-3), 63.6 (C-1′), 61.6

(C-9), 58.8 (C-4′), 54.5 (C-5), 48.5 (C-14), 45.3 (C-8), 43.9 (C-20), 43.3 (C-19), 39.8 (C-18), 38.9 (C-4), 37.8 (C-1), 37.1 (C-10), 32.5 (C-7), 31.9 (C-17, C-21), 30.7 (C-2′), 28.7 (C-30), 28.6 (C-28), 28.2 (C-23), 27.8 (C-22), 27.4 (C-2), 26.5 (C-16), 26.2 (C-15), 25.4 (C-3′), 23.4 (C-27), 18.8 (C-26), 17.6 (C-6), 16.6 (C-25), and 16.4 (C-24) ppm; MS (APCI): m/z $[M\text{-}Br]^+$: 745.5 $C_{49}H_{65}N_2O_4$ (calcd. 745.5); Anal. Calcd. for $C_{49}H_{65}BrN_2O_4$: 71.25; H 7.93. Found: C 71.32; H 8.17.

N-{4-[(3β-acetoxy-11-oxo-18β,20β-olean-12-en-29-oyl)-butyl]-(E)-4-[2-(1H-indol-3-yl)-vinyl]}pyridinium bromide (19)

Dark orange powder. Yield: 79%; mp 144–146 °C (EtOH); $[\alpha]_D^{21}$ + 103.3 (*c* 0.03, $CHCl_3$); IR (film) ν_{max} 1725 (C = O), 3443 (NH) cm^{-1}; ^1H NMR (500 MHz, MeOD/$CDCl_3$): $\delta = 8.54$ (2H, d, $J = 6.5$ Hz, H-5′, H-9′), 8.14 (1H, d, $J =$ 16.0 Hz, H-10′ or H-11′), 8.05–8.03 (1H, m, H-15′), 7.95 (2H, d, $J = 6.5$ Hz, H-6′, H-8′), 7.84 (1H, s, H-12′), 7.51–7.48 (1H, m, H-18′), 7.31–7.24 (2H, m, H-16′, H-17′), 7.16 (1H, d, $J = 16.0$ Hz, H-10′ or H-11′), 5.50 (1H, br s, H-12), 4.49–4.43 (3H, m, H-4′, H-3), 4.26–4.13 (2H, m, H-1′), 2.77–2.73 (1H, m, H-18), 2.42 (1H, s, H-9), 2.08–0.83 (23H, m, CH, CH_2 in pentacyclic skeleton, H-2′, H-3′), 2.04 (3H, s, Me), 1.39, 1.17, 1.11, 1.05, 0.88, 0.86, and 0.81 (3H each, all s, H-23–H-28 and H-30) ppm; ^{13}C NMR (125 MHz, MeOD/$CDCl_3$): $\delta = 201.9$ (C-11), 177.9 (C-29), 172.8 (C = O), 172.3 (C-13), 157.0 (C-7′), 143.8 (C-5′, C-9′), 139.3 (C-19′), 138.9 (C-10′ or C-11′), 133.7 (C-12′), 128.8 (C-12), 126.3 (C-14′), 124.4 (C-16′), 123.3 (C-6′, C-8′), 122.7 (C-17′), 121.4 (C-15′), 117.6 (C-10′ or C-11′), 115.5 (C-13′), 113.5 (C-18′), 82.1 (C-3), 64.5 (C-1′), 62.9 (C-9), 60.3 (C-4′), 56.1 (C-5), 46.6 (C-14), 45.2 (C-8), 44.5 (C-20), 42.2 (C-19), 39.8 (C-18), 38.9 (C-4), 38.8 (C-1), 38.1 (C-10), 33.6 (C-7), 32.9 (C-17), 31.9 (C-21), 30.7 (C-2′), 29.3 (C-30), 29.2 (C-28), 28.8 (C-22), 28.6 (C-23), 27.5 (C-16), 27.3 (C-15), 26.6 (C-3′), 24.4 (C-2), 23.9 (C-27), 21.4 (CH_3), 19.4 (C-26), and 18.3 (C-6), 17.2 (C-25), 17.1 (C-24) ppm; MS (APCI): m/z $[M\text{-}Br]^+$: 787.5 $C_{51}H_{67}N_2O_5$ (calcd. 787.5); Anal. Calcd. for $C_{51}H_{67}BrN_2O_5$: C 70.57; H 7.78. Found: C 70.61; H 8.12.

The study of photoluminescent properties

The PL spectra were measured on a Fluorolog-3 spectrofluorimeter with a Hamamatsu P928 detector. The PL of **F16** and compounds **6–19** was studied in a methanol solution (5 μM) in a quartz cell ($l = 1$ cm), which was exposed to exciting radiation (450 W xenon lamp). The PL quantum yields (ϕ) were determined by a procedure described previously [34] by comparing the integral intensities. The 10 μM ethanol solution of anthracene was used as the fluorescence standard ($\phi_{PL} \sim 0.28$). The absorption spectra were recorded using a Shimadzu UV-1800 UV/vis spectrometer ($l = 1$ cm). The experiments were carried out at room temperature (297 K).

Cell culturing

Cells (Jurkat, K562, U937, and Fibroblasts) were purchased from Russian Cell Culture Collection (Institute of Cytology of the Russian Academy of Sciences) and cultured according to standard mammalian tissue culture protocols and sterile technique. Human cancer cell line fibroblasts was obtained from the HPA Culture Collections (UK). All cell lines used in the study were tested and shown to be free of mycoplasma and other viral contamination. Fibroblasts cell line was cultured as monolayers and maintained in Dulbecco's modified Eagle's medium (DMEM) (Gibco BRL) supplemented with 10% foetal bovine serum and 1% penicillin–streptomycin solution at 37 °C in a humidified incubator under a 5% CO_2 atmosphere. Other cell cultures were maintained in RPMI 1640 (Jurkat, K562, U937) (Gibco) supplemented with 4 mM glutamine, 10% FBS (Sigma), and 100 units/ml penicillin–streptomycin (Sigma). All types of cells were grown in an atmosphere of 5% CO_2 at 37 °C. The cells were subcultured at 2–3 days intervals. Adherent cells (Fibroblasts) were suspended using trypsin/EDTA and counted after they have reached 80% confluency. Cells were then seeded in 24 well plates at 5×10^4 cells per well and incubated overnight. Jurkat, K562, U937 cells were subcultured at 2-day intervals with a seeding density of 1×10^5 cells per 24 well plates in RPMI with 10% FBS.

Cytotoxicity study

The cell viability after treatment with the test compounds was studied by flow cytometry. The viability (live/dead) was assessed by staining cells with 7-AAD (7-Aminoactinomycin D) (Biolegend). After treatment, the cells were harvested, washed 1 or 2 times with phosphate-buffered saline (PBS), and centrifuged at 400 g for 5 min. Cell pellets were resuspended in 200 mL of flow cytometry staining buffer (PBS without Ca^{2+} and Mg^{2+}, 2.5% FBS) and stained with 5 μL of 7-AAD solution for 15 min at room temperature in the dark. The samples were acquired on a NovoCyteTM 2000 FlowCytometry System (ACEA) equipped with a 488 nm argon laser. The 7-AAD emission was collected through a 675/30 nm filter in FL4 channel.

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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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