European Journal of Medicinal Chemistry 209 (2021) 112920

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Cytotoxic triterpenoid–safirinium conjugates target the endoplasmic reticulum

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ARTICLE INFO

Article history:
Received 9 August 2020
Received in revised form
4 October 2020
Accepted 4 October 2020
Available online 8 October 2020

Keywords:
Betulinic acid
Safirinium
Cytotoxicity
Endoplasmic reticulum

ABSTRACT

Safirinium P and Q fluorescence labels were synthesized and conjugated with spacered triterpenoic acids to access hybrid structures. While the parent safirinium compounds were not cytotoxic at all, many triterpenoid safirinium P and Q conjugates showed moderate cytotoxicity. An exception, however, was safirinium P derived compound **30** holding low $EC_{50} = 5.4$ μM (for A375 cells) to $EC_{50} = 7.5$ μM (for FaDu cells) as well as $EC_{50} = 6.6$ μM for non-malignant fibroblasts NIH 3T3. Fluorescence imaging showed that the safirinium core structures cannot enter the cells (not even after a prolonged incubation time of 24 h), while the conjugates (as exemplified for **30**) are accumulating in the endoplasmic reticulum but not in the mitochondria. The development of safirinium–hybrids targeting the endoplasmic reticulum can be regarded as a promising strategy in the development of cytotoxic agents.

1. Introduction

Cancer is still the second most leading cause of death. Especially some types of cancer come with more aggressive phenotypes than others like prostate and breast cancer. Furthermore hypoxic tumors lead to an acidification of the tumor microenvironment, which further increases resistance, invasion and proliferation of the tumor [1]. Recently, mitochondrial targeted anti–cancer drugs („mitocans“) have gotten more and more attention as these compounds trigger cell death signals from the mitochondria, where tumor cells – even tumor cells not responding to death signals due to DNA damage – trigger apoptosis [2–9]. This has already been shown for analogs of vitamin E [2–9] as well as for triterpenoids [10–13] and steroids [14]. For the latter compounds this was mainly achieved by attaching lipophilic cations (such as rhodamine B [10–13], a BODIPY–moiety [15], a triphenylphosphonium residue [16–18], an ammonium salt [19,20]or derivatives of malachite green [15]) to the triterpenoid or steroidal skeleton [5,21].

However, the presence of a cation is not sufficient to achieve good cytotoxicity in tumor cells. For example, distal *N*–oxides and quaternary ammonium salts of triterpenoids [19,20] are significantly less cytotoxic than, e.g. benzylamides [19–23]. Depending on the spacer used, rhodamine B–triterpene derivatives are obviously only cytotoxic if an “open” (fluorescent) form is present. If rhodamine B, on the other hand, is present in a closed/non–fluorescent lactone or lactam form, the corresponding conjugates are practically non–cytotoxic [11]. Conjugates holding the rhodamine B residue directly attached to the basic skeleton of the triterpene (e.g. as an ester) are cytotoxic but usually not very selective [12,13].

We are particularly interested in the development of cytotoxic triterpene–conjugates carrying a permanent positively charged moiety; these compounds should also enable to act as fluorescence molecular probe [24,25]. This latter property is also associated with the ability to observe the uptake and intracellular distribution of the molecules in a more directly way. Thus, valuable information on molecular mechanisms can be gained quite easily.

Some time ago, Saczewski et al. succeeded in synthesizing so–called „safirinium–hybrids“ by tandem Mannich–electrophilic amination reactions starting from pro–fluorogenic isoxazolinones and secondary amines, such as quinolones or short peptides. The former hybrids showed higher lipophilicity and the latter were able to stain spores of *Bacillus subtilis* [24–31].

The latter observation, that safirinium hybrids are able to be well transported into cells, led us to investigate the cytotoxic potential of safirinium–triterpene hybrids. Hybridization is often associated with the ability to counterbalance the known problem (efficacy, affinity, solubility, unwanted side effects, …) with the other hybrid part. Lack of solubility in biological systems is a

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https://doi.org/10.1016/j.ejmech.2020.112920
0223-5234/

notorious problem often associated with triterpenes. This problem, however, might be solved by binding to a cationic residue, such as a safirinium moiety. Hence, we became interested to deduce whether triterpene–safirinium hybrids would also be as efficient as rhodamine B conjugates in triggering cell death.

Betulinic acid (**BA**, Fig. 1), for example, is a triterpene, that holds promising in vitro and in vivo antitumor activity in melanoma, neuro–ectodermal tumors and glioma cell lines [32–35]. It targets the mitochondrial permeability transition pore [36]. It also downregulates Bcl–2, which promotes apoptosis as well as VEGFR and NF–kB, which are both closely related to angiogenesis and the proliferation of tumors [37].

Based on this strategy, we set out to synthesize different triterpenoid–safirinium conjugates, and to examine their cytotoxicity and selectivity for different human tumor cell lines.

2. Results and discussion

2.1. Synthesis

The synthesis of the safirinium P derivatives (Scheme 1) was straightforward and followed the synthetic scheme previously outlined by Saczewski et al. [30,31] Compound **1** was obtained in an almost quantitative yield from the reaction of ethyl cyanoacetate with hydroxylamine following the procedure reported by Lee [38]. Compound **2** is a precursor for the safirinium P fluorophores and was obtained in 73% yield from the reaction of **1** with acetylacetone in the presence of piperidine followed by a precipitation of the product with hydrochloric acid. A major advantage of these reactions is that no time–consuming purifications are necessary at all.

The synthesis of the safirinium P derivatives (Scheme 1) proceeded also nicely as reported, and compounds **3**–**13** were obtained after precipitation from acetone in yields ranging between 43% and 91%, except for compound **7** (57%).

The synthesis of the safirinium Q derivatives (Scheme 2) followed the previously reported strategy.

As far as the synthesis of acetylated triterpenoic acid holding an ethylenediamine or a piperazinyl spacer is concerned, the corresponding triterpenoic acid (**BA**, **OA**, **UA**, **PA**; Fig. 1) was acetylated yielding compounds **17**–**20** (Scheme 3); these were activated with oxalyl chloride followed by the addition of the amine. Thus, compounds **21**–**28** were obtained in good yields. These spacers were chosen in analogy to our previous studies on spacered triterpenoid hybrids [10–14].

The safirinium P fluorophores were activated with oxalyl chloride followed by their reaction with piperazinyl–spacered 3–*O*–acetyl–betulinic acid (**21**) to yield derivatives **29**–**31** (Scheme 4).

Albeit all of these reactions proceeded well (as observed by TLC), the isolated yields of pure products were low. These compounds tend to "stick" to the silica gel used for chromatographic purification. Even elution with MeOH was incomplete. While upon addition of AcOH to the eluent reasonably high recovery rates were obtained, this procedure inevitably led to the formation of impurities in the product. Hence, yield was sacrificed for purity, and most of the compounds were eluted from the column with MeOH.

The synthesis of the safirinium Q derivatives proceeded in an analogous manner (Scheme 5) and yielded compounds **32**–**39**, respectively.

As exemplified for the safirinium–betulinic acid hybrids, their UV/Vis spectra are characterized by maxima at $\lambda_{\mu ax} = 253–254$ nm (with log $\varepsilon = 3.92–4.07$) and $\lambda_{max} = 352–354$ nm (with log $\varepsilon = 3.62–3.82$). These values are in perfect agreement with previously published data for analogs.

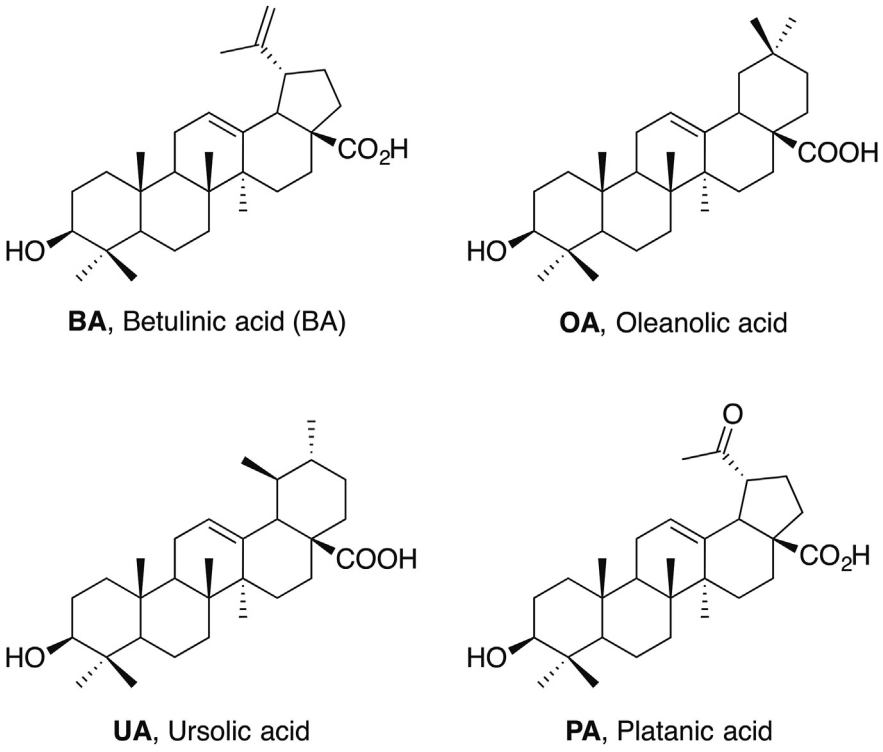
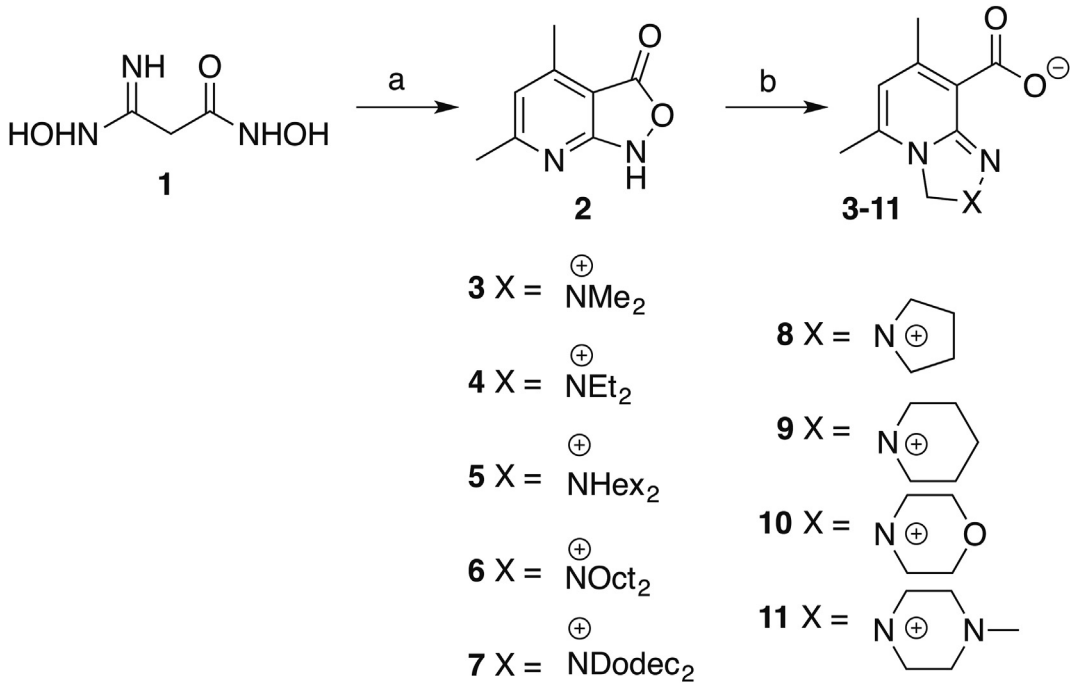
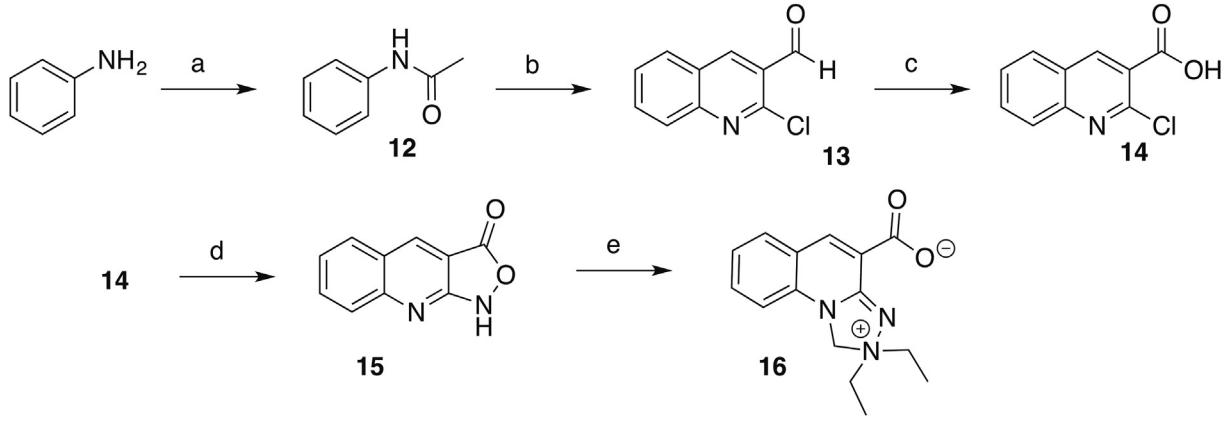


Fig. 1. Structure of triterpenoic acids: **BA** (betulinic acid), **OA** (oleanolic acid), **UA** (ursolic acid), and **PA** (platanic acid).



Scheme 1. Synthesis of safirinium P derivatives **3**–**11**: a) 1. acetylacetone, piperidine, H_2O, 100 °C, 15 min; 2. 4M HCl, 73%; b) formaldehyde, corresponding amine, MeOH, 4 h, 45 °C, 2 h.



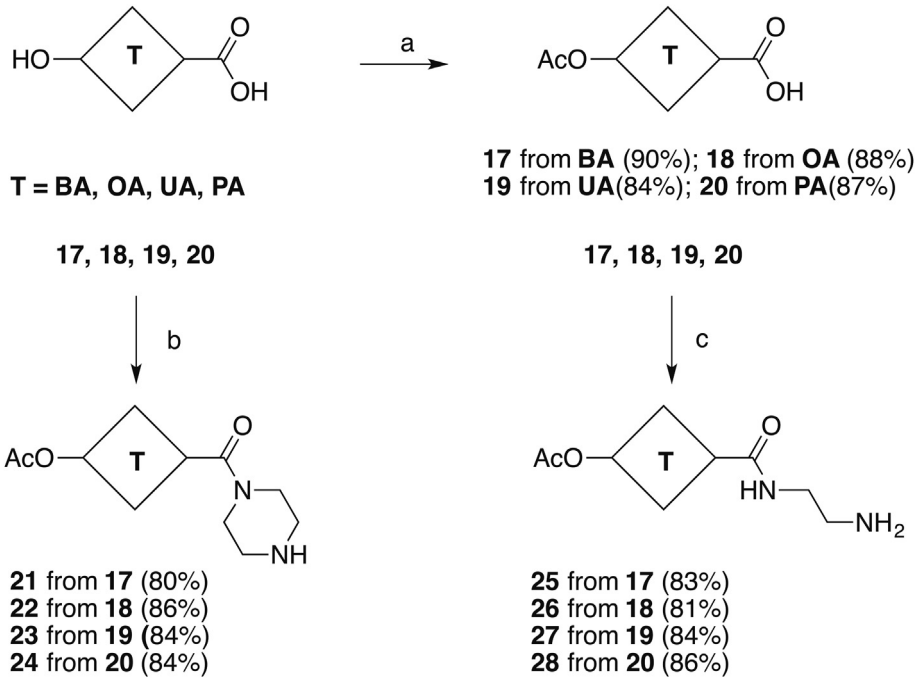
Scheme 2. Synthesis of the safirinium Q derivative **16**: a) Ac_2O, acetic acid, reflux, 1 h, 78%; b) 1. DMF, phosphoryl chloride, inert atmosphere, 0 °C → 25 °C, 4 h 2. **17**, 25 °C → reflux. 24 h, 70%; c) *n*–butanol, NaH_2PO_4, $NaClO_2$, H_2O, r.t, 2 h, 87%; d) 1. HOSA, dry DMF, 25 °C, 12 h, 2. TEA, H_2O, 25 °C, 12 h, 81%; e) diethylamine, formaldehyde (aqu., 37%), MeOH, 25 °C, 92%.

2.2. Biological evaluation

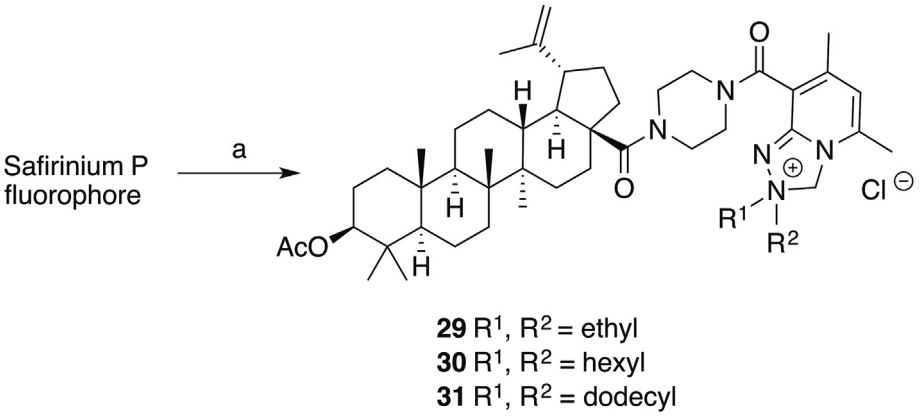
The compounds were subjected to sulforhodamine B assays to determine their cytotoxicity employing several human tumor cell lines and non–malignant fibroblasts (NIH 3T3). The results from these assays are summarized in Table 1.

The results from these assays showed safirinium P derivatives **3**–**11** as not cytotoxic up to a concentration of 30 μM, thus paralleling both previous findings as well as the also insignificant cytotoxicity of rhodamine B (Rho). Extra fluorescence imaging experiments revealed that these compounds are not taken up by the cells; this explains their non–cytotoxicity. Upon derivatization with triterpenoids, however, a slight increase in cytotoxicity was observed. Especially, betulinic acid derived **30** showed good cytotoxicity ranging between EC_{50} = 4.6 μM (for A2780 ovarian carcinoma cells) to EC_{50} = 7.5 μM (for HT29 colorectal adenocarcinoma cells). An unselective cytotoxicity, however, was also observed for this compound inasmuch as for non–malignant fibroblasts (NIH 3T3) an EC_{50} = 6.6 μM was noted. Most of the other safirinium hybrids were only of moderate cytotoxicity and selectivity was – by and large – missing at all. Quite on the contrary, compounds **35** and **38** were even more cytotoxic for the fibroblasts than for the tumor cells incorporated in this study. For comparison, human non–malignant HEK293 were incubated with representative compounds **30**–**32** and **36** showing these compounds also to be cytotoxic for these cells.

These results were somewhat surprising inasmuch as we expected the safirinium conjugates to act likewise the rhodamine B mitocans of previous studies. Therefore, a closer investigation of **30** was called for. Fluorescence imaging experiments showed **30** not to enter the mitochondria (within the limits of detection) but to accumulate in the endoplasmic reticulum (ER). Control



Scheme 3. Synthesis of compounds **17**–**28**: a) Ac_2O, pyridine, DMAP (cat.), 12 h, yields: **17** (90%), **18** (88%), **19** (84%), **20** (87%) b) 1. $(COCl)_2$, DCM, DMF, 0 °C → 25 °C, 4 h 2. piperazine, DCM, 0 °C → r.t. 2 h, yields: **21** (80%), **22** (86%), **23** (84%), **24** (84%); c) $(COCl)_2$, DCM, DMF, 0 °C → 25 °C, 4 h 2. ethylenediamine, DCM, 0 °C → 25 °C, 2 h, yields: **25** (83%), **26** (81%), **27** (84%), **28** (86%).



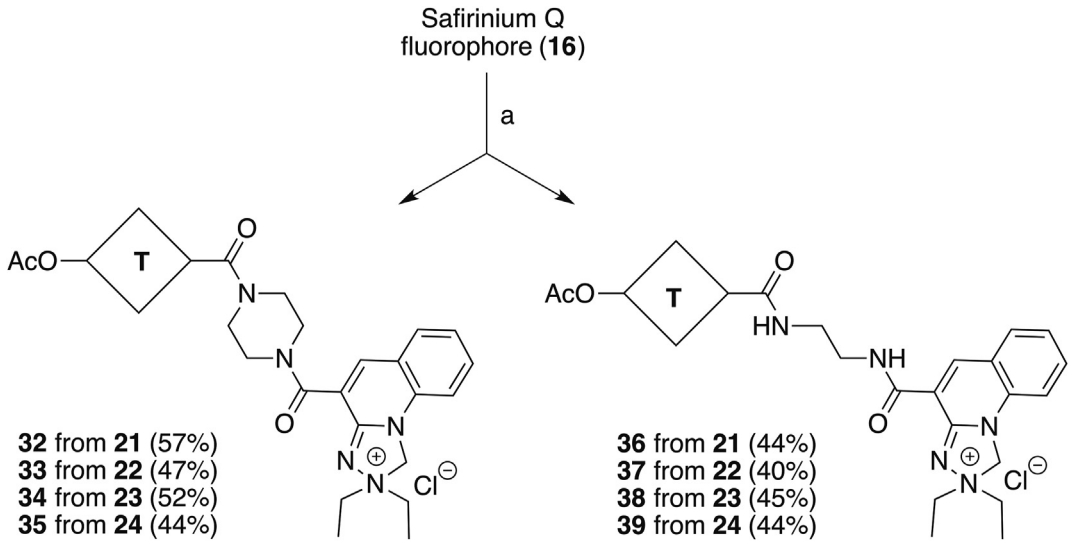
Scheme 4. Synthesis of betulinic acid derived safirinium hybrids **29**–**31**: a) 1. $(COCl)_2$, DCM, DMF 0 °C → 25 °C, 2 h, 2. **21**, NEt_3, 0 °C → 25 °C, yields. **29** (57%), **30** (39%), **31** (40%).

experiments were performed employing an established ER–Tracker (for the accumulation of **30** in the ER), and a Mito-Tracker (to exclude an accumulation in the mitochondria). Representative images are depicted in Fig. 2.

3. Conclusion

The syntheses of the safirinium fluorescence labels was straightforward following known procedures to allow an access to the safirinium P derivatives **3**–**11** and safirinium Q derivative **16** in good to excellent yields. The conjugation reaction with spacered triterpenoic acid derivatives **21**–**28** proceeded well applying standard coupling procedures; main obstacle of this reaction was the purification of the hybrid compounds due to their high affinity to silica gel during chromatographic purification. The safirinium compounds were not cytotoxic at all while many triterpenoid safirinium P and Q conjugates showed only moderate cytotoxicity. An exception was safirinium P derived compound **30** holding low EC_{50} = 5.4 μM (for A375 cells) to EC_{50} = 7.5 μM (for FaDu cells). However, safirinium Q hybrids **36** and **39** were even more cytotoxic for non–malignant NIH 3T3 fibroblasts than for the human tumor cell lines included in this study; the same cytotoxicity was also observed for representative compounds **30**–**32** and **36** and HEK293 cells. Fluorescence imaging showed that the safirinium core structures **3**–**11** and **16** cannot enter the cells (not even after a prolonged incubation time of 24 h), while the conjugates (as exemplified for **30**) are accumulating in the endoplasmic reticulum but not in the mitochondria. This finding might explain their diminished cytotoxic activity for human tumor cell lines as compared to rhodamine B analogs that enter the mitochondria.

While many cytostatics trigger an induced cell death via the activation of mitochondrial apoptosis, an increasing number of studies already indicated a pivotal role of drugs targeting the endoplasmic reticulum. Further development of the safirinium–triterpene hybrids can be seen as an encouraging strategy in the development of cytotoxic agents.



Scheme 5. a) 1. $(COCl)_2$, DCM, DMF, 0 °C → 25 °C, 2 h, 2. NEt_3, 0 °C → 25 °C, yields: **32** (57%), **33** (47%), **34** (52%), **35** (44%), **36** (44%), **37** (40%), **38** (45%), **39** (44%).

Table 1
Cytotoxicity of compounds **3**–**11** and rhodamine B (**Rho**) (EC50 values in μM from SRB assays after 72 h of treatment, the values are averaged from three independent experiments performed each in triplicate, confidence interval CI = 95%; mean ± standard mean error). Human cancer cell lines: A375 (epithelial melanoma), HT29 (colorectal adenocarcinoma), MCF−7 (breast adenocarcinoma), A2780 (ovarian carcinoma), FaDu (squamous cell carcinoma), non−malignant: NIH 3T3 (mouse fibroblasts), HEK293 (human embryonic kidney cells); n.d. not determined; doxorubicin (**DX**) and staurosporine (**ST**) were used as positive controls.

	A375	HT29	MCF−7	A2780	FaDu	NIH3T3	HEK293
Rho	>30	>30	>30	>30	>30	>30	>30
3–**11**	>30	>30	>30	>30	>30	>30	n.d.
29	26.2 ± 1.3	>30	26.3 ± 2.1	21.6 ± 2.4	>30	22.4 ± 2.2	n.d.
30	5.4 ± 0.1	7.5 ± 0.3	4.9 ± 0.3	4.6 ± 0.3	6.0 ± 0.3	6.6 ± 0.3	3.0 ± 0.3
31	16.8 ± 1.0	24.9 ± 1.7	15.7 ± 1.2	11.0 ± 1.2	10.7 ± 1.0	>30	13.3 ± 0.7
32	20.8 ± 1.7	27.0 ± 2.0	18.1 ± 1.0	14.1 ± 2.0	28.2 ± 2.3	>30	16.5 ± 0.8
33	21.4 ± 0.7	26.2 ± 0.7	19.8 ± 1.2	21.4 ± 1.9	18.1 ± 1.8	18.4 ± 2.0	n.d.
34	21.4 ± 1.4	>30	10.2 ± 1.1	9.0 ± 1.2	18.3 ± 1.1	>30	n.d.
35	15.0 ± 1.2	22.2 ± 0.9	13.2 ± 0.7	10.9 ± 1.8	13.3 ± 0.9	3.0 ± 0.6	n.d.
36	18.6 ± 2.1	26.8 ± 0.6	17.6 ± 1.5	18.3 ± 2.8	12.3 ± 1.3	17.4 ± 1.7	13.6 ± 1.0
37	19.0 ± 2.0	25.0 ± 1.0	21.8 ± 1.7	24.2 ± 2.6	27.1 ± 2.0	28.5 ± 1.1	n.d.
38	13.8 ± 0.8	22.1 ± 0.8	11.7 ± 0.7	11.2 ± 1.4	10.2 ± 0.1	3.6 ± 1.0	n.d.
39	16.6 ± 2.1	>30	15.7 ± 1.4	20.8 ± 1.9	16.1 ± 1.3	21.3 ± 1.3	n.d.
DX	n.d.	0.9 ± 0.01	1.1 ± 0.3	0.01 ± 0.01	n.d.	0.4 ± 0.07	n.d.
ST	n.d.	0.2 ± 0.02	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.05	0.008 ± 0.001	n.d.

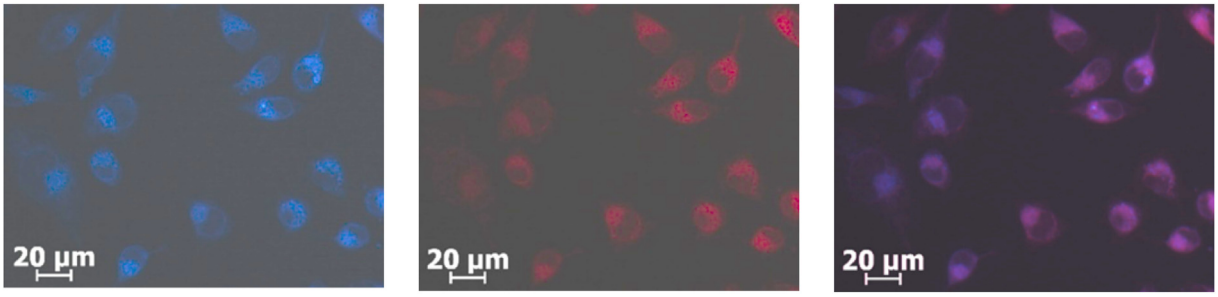


Fig. 2. Fluorescence imaging of living A375 epithelial carcinoma cells incubated with: compound **30** (left, 12 h, 5.4 μM), ER−Tracker (center) and merged pictures (right).

4. Experimental

4.1. General

Equipment and general methods have been used as previously described [10–15]. All reactions were performed under an argon; solvents were dried according to usual procedures. The triterpenoic acid were obtained from "Betulinines" (Stříbrná Skalice, Czech Republic) and used as received. The ^{1}H and ^{13}C NMR of all new compounds have been depicted in the Supplementary Materials file as well as a description of the SRB assay and the staining experiments.

4.2. Syntheses

4.2.1. 3–Amino–N–hydroxy–3–(hydroxyimino)propanamide (**1**)

Following the procedure as described by Lee [38], a mixture of ethyl cyanoacetate (10.0 g, 88.4 mmol) and hydroxylamine (50% in water, 12.9 g, 194 mmol, 2.2 eq) in EtOH (10 ml) was stirred at room temperature for 30 min. External cooling was necessary to keep the temperature constant. Upon cooling to 10 °C, crystals formed and were collected by filtration, washed with ice cold ethanol and dried in high vacuum to afford **1** as a colorless solid being used without further purification.

4.2.2. 4,6–Dimethylisoxazolo[3,4–b]pyridin–3(1H)–one (**2**)

A solution of **1** (20.0 g, 0.15 mol), acetylacetone (15.5 mL, 0.15 mol) and piperidine (14.8 mL, 0.15 mol) in water (250 mL) was heated under reflux for 30 min [15]. After cooling to room temperature, the reaction mixture was acidified with 2M HCl, and the resultant yellow precipitate was filtered off and washed with water (2 × 50 mL); yield 17.97 g (73%); m.p. 203 °C (lit [31].: m.p. 204–207 °C);

4.2.3. General procedure (GPA) for the preparation of safirinium P derivatives **3**–**11**

A solution of **2** (2.0 g, 12.2 mmol), the corresponding amine (13.4 mmol) and 37% aqueous formaldehyde (1.0 mL, 13.4 mmol) in MeOH (40 mL) was heated at 40 °C for 3 h. The resulting reaction mixture was concentrated under reduced pressure, and the solid residue was precipitated from acetone.

4.2.4. 2,2,5,7–Tetramethyl–2,3–dihydro–1h–imidazol[1,2–a] pyridin–4–ium–8–carboxylate (**3**)

Following GPA; yield: 89%; m.p. 227–230 °C (lit [29].: 228–230 °C); IR (ATR): ν = 3031w, 2964w, 2882w, 1635s, 1594vs, 1549vs, 1519vs, 1499m, 1459m, 1401w, 1382s, 1371m, 1344s, 1288w, 1241w, 1228w, 1213w, 1181m, 1119w, 1068w, 1047w, 1034w, 798s, 706m, 611s, 604 s cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD): δ = 6.02 (d, J = 1.2 Hz, 1H, 6–H), 5.76 (s, 2H, 3–H), 3.43 (s, 6H, 13–H + 13′–H), 2.30 (d, J = 1.0 Hz, 3H, 11–H), 2.21 (s, 3H, 10–H) ppm; ^{13}C NMR (125 MHz, CD_3OD): δ = 170.0 (C-12), 155.5 (C-5), 146.7 (C-7), 139.5 (C-9), 118.0 (C-8), 110.4 (C-6), 77.3 (C-3), 55.6 (C-13 + C-13‘), 18.5 (C-10), 16.9 (C-11) ppm; MS (ESI, MeOH): m/z (%) = 222.1 ([M+H]$^+$, 100%), 244.1 ([M+Na]$^+$, 18%); analysis calcd for $C_{11}H_{15}N_3O_2$ (221.26): C 59.21, H 6.83, N 18.99; found: C 58.97, H 7.01, N 18.77.

4.2.5. 2,2–Diethyl–5,7–dimethyl–2,3–1h–imidazol[1,2–a] pyridin–4–ium–8–carboxylate (**4**)

Following GPA; yield: 91%; m.p. 189–191 °C (lit [30].: 200–203 °C); IR (ATR): ν = 3367w, 3201w, 2973w, 1632s, 1586vs, 1555vs, 1528s, 1456m, 1438m, 1431m, 1407m, 1398m, 1383s, 1373s, 1353s, 1305m, 1247w, 1225m, 1193m, 1185m, 1166m, 1144w, 1121w, 1113w, 1073w, 1043w, 1031m, 802s, 784m, 752m, 687s, 669m, 618vs, 606vs, 577s, 561s, 530m, 510 s cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD): δ = 5.99 (d, J = 1.1 Hz, 1H, 6–H), 5.70 (s, 2H, 3–H), 3.62 (qq, J = 12.8, 7.1 Hz, 4H, 13–H + 13‘–H), 2.31 (d, J = 1.0 Hz, 3H, 11–H), 2.20 (s, 3H, 10–H), 1.38 (t, J = 7.1 Hz, 6H, 14–H + 14‘–H) ppm; ^{13}C NMR (101 MHz, CD_3OD): δ = 170.2 (C-12), 155.9 (C-5), 146.2 (C-7), 139.1 (C-9), 118.2 (C-8), 110.1 (C-6), 71.9 (C-3), 61.2 (C-13 + C-13‘), 18.4 (C-10), 16.8 (C-11), 7.0 (C-14 + C-14‘) ppm; MS (ESI, MeOH): m/z (%) = 250.1 ([M+H]$^+$, 100%), 272.0 ([M+Na]$^+$, 4%); analysis calcd for $C_{13}H_{19}N_3O_2$ (249.31): C 62.63, H 7.68, N 16.85; found: C 62.47, H 7.90, N 16.52.

4.2.6. 2,2–Dihexyl–5,7–dimethyl–2,3–dihydro–1h–imidazol [1,2–a]pyridin–4–ium–8–carboxylate (**5**)

Following GPA; yield: 82%; m.p. 134–135 °C; IR (ATR): ν = 3376w, 3004w, 2947m, 2927m, 2867w, 1667m, 1635s, 1587vs, 1560s, 1531s, 1485w, 1468m, 1439w, 1433w, 1398m, 1373m, 1356m, 1302w, 1225w, 1211w, 1187m, 1143m, 1055w, 1042w, 1032w, 802m, 729m, 616m, 605 s cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD): δ = 5.99 (d, J = 1.3 Hz, 1H, 6–H), 5.71 (s, 2H, 3–H), 3.53 (dddd, J = 31.0, 12.6, 10.7, 5.9 Hz, 4H, 13–H + 13–H’), 2.30 (d, J = 1.0 Hz, 3H, 11–H), 2.20 (s, 3H, 10–H), 1.86–1.75 (m, 4H, 14–H + 14‘–H), 1.41–1.30 (m, 12H, 15–17–H + 15‘–17‘–H), 0.91 (t, J = 6.9 Hz, 6H, 18–H + 18‘–H) ppm; ^{13}C NMR (125 MHz, CD_3OD): δ = 170.1 (C-12), 155.7 (C-5), 146.3 (C-7), 139.1 (C-9), 118.1 (C-8), 110.1 (C-6), 72.9 (C-3), 66.3 (C-13 + C-13‘), 31.1 (C-16 + C-16‘), 25.6 (C-15 + C-15‘), 22.2 (C-17 + C-17‘), 22.1 (C-14 + C-14‘), 18.4 (C-10), 16.8 (C-11), 12.9 (C-18 + C-18‘) ppm; MS (ESI, MeOH): m/z (%) = 362.3 ([M+H]$^+$, 100%), 384.2 ([M+Na]$^+$, 8%); analysis calcd for $C_{21}H_{35}N_3O_2$ (361.53): C 69.77, H 9.76, N 11.62; found: C 69.50, H 9.96, N 11.57.

4.2.7. 5,7–Dimethyl–2,2–dioctyl–2,3–dihydro–1h–imidazol [1,2–a]pyridin–4–ium–8–carboxylate (**6**)

Following GPA; yield: 83%; m.p. 131 °C; IR (ATR): ν = 3386w, 3002w, 2952m, 2926m, 2854m, 1664m, 1635s, 1587vs, 1560s, 1530s, 1484m, 1468m, 1432w, 1398m, 1375m, 1354m, 1306w, 1218w, 1184m, 1043w, 1032w, 802m, 725m, 614m, 605 m cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD): δ = 5.99 (d, J = 1.1 Hz, 1H, 6–H), 5.70 (s, 2H, 3–H), 3.61–3.43 (m, 4H, 13–H + 13‘–H), 2.30 (d, J = 1.0 Hz, 3H, 11–H), 2.20 (s, 3H, 10–H), 1.84–1.76 (m, 4H, 14–H + 14‘–H), 1.40–1.25 (m, 21H, 15–H + 15‘–H + 16–H + 16‘–H + 17–H + 17‘–H + 18–H + 18‘–H + 19–H + 19‘–H), 0.93–0.87 (m, 6H, 20–H + 20‘–H) ppm; ^{13}C NMR (125 MHz, CD_3OD) δ = 170.1 (C-12), 155.7 (C-5), 146.3 (–7), 139.1 (C-9), 118.1 (C-8), 110.1 (C-6), 72.9 (C-3), 66.3 (C-13 + C-13‘), 31.5 (C-16 + C-16‘), 28.9 (C-18 + C-18‘ + C-15 + C-15‘), 26.0 (C-17 + C-17‘), 22.2 (C-19 + C-19‘ + C-14 + C-14‘), 18.4 (C-10), 16.8 (C-11), 13.0 (C-20 + C-20‘) ppm; MS (ESI, MeOH): m/z (%) = 418.4 ([M+H]$^+$, 100%), 440.2 ([M+Na]$^+$, 6%); analysis calcd for $C_{25}H_{43}N_3O_2$ (417.64): C 71.90, H 10.38, N 10.06; found: C 71.64, H 10.60, N 9.84.

4.2.8. 2,2–Didodecyl–5,7–dimethyl–2,3–dihydro–1h–imidazol [1,2–a]pyridin–4–ium–8–carboxylate (**7**)

Following GPA but precipitation from diethyl ether; yield: 57%; m.p. 142–145 °C; IR (ATR): ν = 3362w, 2956m, 2917vs, 2874w, 2849s, 1635s, 1583vs, 1485w, 1466m, 1446m, 1435w, 1403m, 1375s, 1347m, 1235w, 1192m, 809m, 720m, 631m, 601 s cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD): δ = 5.98 (d, J = 1.2 Hz, 1H, 6–H), 5.70 (s, 2H, 3–H), 3.63–3.42 (m, 4H, 13–H + 13‘–H), 2.29 (d, J = 1.0 Hz, 3H, 11–H), 2.20 (s, 3H, 10–H), 1.85–1.74 (m, 4H, 14–H + 14‘–H), 1.40–1.25 (m, 36H, 15–23–H + 15‘–23‘–H), 0.88 (t, J = 7.0z Hz, 6H, 24–H + 24‘–H) ppm; ^{13}C NMR (101 MHz, CD_3OD): δ = 170.1 (C-12), 155.6 (C-5), 146.3 (C-7), 139.1 (C-9), 118.1 (C-8), 110.1 (C-6), 73.0 (C-3), 66.2 (C-13 + C-13‘), 31.7 (C-22 + C-22‘), 29.4–28.9 (C-16–C–21 + C-16‘– C-21‘), 26.0 (C-15 + C-15‘), 22.3 (C-23 + C-23‘), 22.2 (C-14 + C-14‘), 18.4 (C-10), 16.9 (C-11), 13.0 (C-24 + C-24‘) ppm; MS (ESI, MeOH): m/z (%) = 530.4 ([M+H]$^+$, 100%), 552.3 ([M+Na]$^+$, 2%); analysis calcd for $C_{33}H_{59}N_3O_2$ (529.85): C 74.81, H 11.22, N 7.93; found: C 74.73, H 11.02, N 7.68.

4.2.9. 5’,7’–Dimethyl–3’H–spiro[pyrrolidine–1,2’–[1,2,4]triazolo [4,3–a]pyridin]–1–ium–8′–carboxylate (**8**)

Following GPA; yield: 76%; m.p. 141–144 °C; IR (ATR): ν = 3386w, 3024w, 2963w, 1634m, 1591vs, 1558s, 1520m, 1478w, 1457w, 1434m, 1376s, 1345m, 1228w, 1188w, 1051w, 798m, 779m, 605m, 595 m cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD): δ = 5.99 (d, J = 1.2 Hz, 1H, 6–H), 5.83 (s, 2H, 3–H), 3.88–3.75 (m, 4H, 13–H + 13‘–H), 2.40–2.32 (m, 2H, 14–H), 2.30 (d, J = 1.0 Hz, 3H, 11–H), 2.24–2.20 (m, 2H, 14‘–H), 2.20 (s, 3H, 10–H) ppm; ^{13}C NMR (125 MHz, CD_3OD): δ = 170.2 (C-12), 155.5 (C-5), 146.3 (C-7), 139.4

(C-9), 118.0 (C-8), 110.1 (C-6), 74.6 (C-3), 68.2 (C-13 + C-13'), 21.4 (C-14 + C-14'), 18.4 (C-10), 16.9 (C-11) ppm; MS (ESI, MeOH): *m/z* (%) = 248.2 ($[M+H]^+$, 100%), 270.1 ($[M+Na]^+$, 4%); analysis calcd for $C_{13}H_{17}N_3O_2$ (247.30): C 63.14, H 6.93, N 16.99; found: C 62.85, H 7.14, N 16.73.

4.2.10. 5',7'–Dimethyl–3'H–spiro[piperidine–1,2'–[1,2,4]triazolo [4,3–a]pyridin]–1–ium–8'–carboxylate (**9**)

Following GPA; yield: 82%; m.p. 167–171 °C; IR (ATR): ν = 3383m, 3003w, 2957w, 2858w, 1634s, 1580s, 1564vs, 1558vs, 1525s, 1482w, 1450m, 1436m, 1381m, 1372s, 1350m, 1305w, 1277w, 1266w, 1232w, 1193m, 1170w, 1029w, 805m, 697m, 667m, 622m, 595s, 478 m cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD): δ = 5.98 (d, *J* = 1.2 Hz, 1H, 6–H), 5.71 (s, 2H, 3–H), 3.63 (dddd, *J* = 35.4, 12.2, 7.8, 3.7 Hz, 4H, 13–H + 13'–H), 2.30 (d, *J* = 1.0 Hz, 3H, 11–H), 2.20 (s, 3H, 10–H), 2.19–2.14 (m, 2H, H-14), 1.91–1.83 (m, 2H, H-14'), 1.73–1.67 (m, 2H, H-15) ppm; ^{13}C NMR (125 MHz, CD_3OD): δ = 170.2 (C-12), 155.3 (C-5), 146.3 (C-7), 139.5 (C-9), 118.3 (C-8), 110.1 (C-6), 75.0 (C-3), 65.2 (C-13), 20.8 (C-14), 20.4 (C-15), 18.4 (C-10), 16.8 (C-11) ppm; MS (ESI, MeOH): *m/z* (%) = 262.2 ($[M+H]^+$, 100%), 284.1 ($[M+Na]^+$, 4%); analysis calcd for $C_{14}H_{19}N_3O_2$ (261.33): C 64.35, H 7.33, N 16.08; found: C 64.17, H 7.51, N 15.88.

4.2.11. 5',7'–Dimethyl–3'H–spiro[morpholine–4,2'–[1,2,4]triazolo [4,3–a]pyridin]–4–ium–8'–carboxylate (**10**)

Following GPA; yield: 71%; m.p. 166–168 °C; IR (ATR): ν = 3350w, 3267w, 2982w, 1632m, 1587vs, 1556s, 1514s, 1476m, 1452m, 1437m, 1427m, 1418m, 1404w, 1375s, 1348s, 1320w, 1311w, 1274w, 1250w, 1233w, 1222w, 1195s, 1138w, 1120m, 1109m, 1089w, 1073m, 845m, 803m, 775m, 689m, 659m, 602s, 559m, 530m, 513 m cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD): δ = 6.04 (d, *J* = 1.2 Hz, 1H, 6–H), 5.79 (s, 2H, 3–H), 4.30 (ddd, *J* = 13.1, 8.9, 2.6 Hz, 2H, 14–H), 3.93 (dt, *J* = 13.2, 3.7 Hz, 2H, 14'–H), 3.78 (ddd, *J* = 12.3, 8.9, 3.3 Hz, 2H, 13–H), 3.69–3.61 (m, 2H, 13'–H), 2.31 (d, *J* = 1.0 Hz, 3H, 11–H), 2.21 (s, 3H, 10–H) ppm; ^{13}C NMR (125 MHz, CD_3OD): δ = 170.1 (C-12), 155.6 (C-5), 146.8 (C-7), 139.5 (C-9), 118.3 (C-8), 110.5 (C-6), 64.0 (C-14 + C-14'), 61.6 (C-13 + C-13'), 18.4 (C-10), 16.8 (C-11) ppm; MS (ESI, MeOH): *m/z* (%) = 264.2 ($[M+H]^+$, 100%), 286.1 ($[M+Na]^+$, 8%); analysis calcd for $C_{13}H_{17}N_3O_3$ (263.30): C 59.30, H 6.51, N 15.96; found: C 59.12, H 6.70, N 15.73.

4.2.12. 4,5',7'–Trimethyl–3'H–spiro[piperazine–1,2'–[1,2,4] triazolo[4,3–a]pyridin]–1–ium–8'–carboxylate (**11**)

Following GPA; yield: 43%; m.p. 187–191 °C; IR (ATR): ν = 3358m, 3175w, 2996w, 2954w, 2942w, 2855w, 2811w, 2785w, 1634s, 1586vs, 1561vs, 1531s, 1456m, 1449m, 1433m, 1407m, 1379s, 1355s, 1335m, 1314w, 1291m, 1260w, 1233w, 1222m, 1191s, 1171w, 1152m, 1129m, 1115w, 1098w, 1078w, 970m, 805m, 790s, 779m, 695m, 660m, 605m, 597s, 562 m cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD): δ = 6.01 (d, *J* = 1.1 Hz, 1H, 6–H), 5.77 (s, 2H, 3–H), 3.79–3.72 (m, 2H, 13–H), 3.72–3.65 (m, 2H, 13'–H), 3.10–3.02 (m, 2H, 14–H), 2.81–2.71 (m, 2H, 14'–H), 2.39 (s, 3H, 15–H), 2.31 (d, *J* = 1.0 Hz, 3H, 11–H), 2.20 (s, 3H, 10–H) ppm; ^{13}C NMR (125 MHz, CD_3OD): δ = 170.1 (C-12), 155.5 (C-5), 146.5 (C-7), 139.5 (C-9), 118.3 (C-8), 110.3 (C-6), 64.0 (C-13 + C-13'), 49.0 (C-14 + C-14'), 43.9 (C-15), 18.4 (C-10), 16.9 (C-11) ppm; MS (ESI, MeOH): *m/z* (%) = 277.2 ($[M+H]^+$, 100%), 299.1 ($[M+Na]^+$, 4%); analysis calcd for $C_{14}H_{20}N_4O_2$ (276.34): C 60.85, H 7.30, N 20.28; found: C 60.61, H 7.43, N 20.11.

4.2.13. Acetanilide (**12**)

Acetanilide (**12**) was prepared by usual acetylation of aniline with acetic anhydride in 78% yield; m.p. 116–118 °C (lit [39].: 116–117 °C); MS (ESI, MeOH): *m/z* (%) 136.1 ($[M+H]^+$, 100%), 158.0 ($[M+Na]^+$, 48%).

4.2.14. 2–Chloro–3–quinolinecarboxaldehyde (**13**)

To a mixture of *N, N*–dimethylformaldehyde (9.6 mL, 0.125 mol) and phosphoryl chloride (32 mL, 0.35 mol) at 0 °C, **1** (6.8 g, 0.05 mol) was added, and the resulting mixture was heated under reflux for 24 h. Usual aqueous workup gave a pale–yellow precipitate, that was filtered off, washed with water (2 × 50 mL) and dried to afford **13** (6.7 g, 70%) as a pale yellow solid; m.p. 152–154 °C (lit [40].: 150–151 °C); R_F = 0.69 (toluene/ethyl acetate/formic acid/*n*–heptane, 80:26:5:10); IR (ATR): ν = 3044vw, 2941vw, 2873w, 1687vs, 1579m, 1046s, 761s, 487s cm–1; UV–Vis (MeOH): λ_{max} (log ε) = 234 (5.50), 319 (4.53) nm; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 10.56 (s, 1H, 9'–H), 8.75 (s, 1H, 4–H), 8.07 (d, *J* = 8.5 Hz, 1H, 5–H), 7.98 (d, *J* = 8.2 Hz, 1H, 8–H), 7.93–7.84 (m, 1H, 7–H), 7.69–7.61 (m, 1H, 6–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 150.3 (C-2), 149.8 (C–8a), 140.4 (C-4), 133.7 (C-7), 129.9 (C-8), 128.8 (C-5), 128.3 (C-6), 126.7 (C–4a), 126.6 (C-3) ppm; MS (ESI, MeOH): *m/z* (%) 192.1 ($[M+H]^+$, 28%), 194.1 ($[M+H]^+$, 10%), 224.1 ($[M + H + MeOH]^+$, 100%), 226.1 ($[M + H + MeOH]^+$, 34%).

4.2.15. Chloroquinoline–3–carboxylic acid (**14**)

To a solution of **2** (0.9 g, 4.68 mmol) in *n*–butanol (90 mL), a solution of sodium dihydrogen orthophosphate (5.56 g, 35.61 mmol) and sodium chlorite (4.29 g, 47.47 mmol) in water (40 mL) was added, and the mixture was stirred at 25 °C for 2 h. The mixture was concentrated under reduced pressure, diluted with water (70 mL), and ethyl acetate (50 mL) and sodium carbonate (5.0 g) were added. The aqueous layer was separated, acidified with 2M HCl and **3** (0.85 g, 87%) was obtained as an off–white solid; m.p. 192–194 °C (lit [41].: 200 °C decomp.); IR (ATR): ν = 2940w, 1729m, 1566m, 1449m, 1190s, 1023m, 789s, 747vs, 572w cm–1; UV–Vis (DMSO): λ_{max} (log ε) = 258 (4.83), 287 (4.82) nm; ^{1}H NMR (400 MHz, DMSO–d_6): δ = 13.76 (s, 1H, OH), 8.93 (s, 1H, 4–H), 8.17 (d, *J* = 8.2 Hz, 1H, 8–H), 8.00 (d, *J* = 8.4 Hz, 1H, 5–H), 7.92 (ddd, *J* = 8.4, 6.8, 1.5 Hz, 1H, 7–H), 7.72 (ddd, *J* = 8.1, 6.8, 1.3 Hz, 1H, 6–H) ppm; ^{13}C NMR (100 MHz, DMSO–d_6): δ = 165.7 (C-9), 147.3 (C–8a), 146.4 (C-2), 141.2 (C-4), 132.7 (C-5), 128.9 (C-7), 128.0 (C-6), 127.6 (C-8), 125.9 (C–4a), 125.4 (C-3) ppm; MS (ESI, MeOH): *m/z* (%) 208.1 ($[M+H]^+$, 100%), 210.1 $[M+H]^+$, 34%).

4.2.16. Isoxazolo[3,4–b]quinolin–3(1H)–one (**15**)

A solution of **3** (1000 mg, 4.8 mmol) and hydroxyamine–*O*–sulfonic acid (2172 mg, 19.2 mmol) in dry DMF (5 mL) was stirred at 25 °C for 12 h. The solid was filtered off, washed with water (3 × 3 mL), and triethylamine (2.1 mL, 15.0 mmol) and water (10 mL) were added. After stirring at 25 °C for 12 h, the precipitate was filtered off and washed with water (3 × 3 mL) to yield **4** (726 mg, 81%) as a red solid; m.p. 247–250 °C (lit [30].: 261–264 °C); IR (ATR): ν = 2899w, 1725m, 1566m, 1449m, 1250m, 1190s, 1022s, 941w, 788vs, 747vs 572 m cm^{-1}; UV–Vis (DMSO): λ_{max} (log ε) = 259 (4.65), 313 (4.93), 482 (4.87) nm; ^{1}H NMR (400 MHz, DMSO–d_6): δ = 12.55 (s, 1H, NH), 8.71 (s, 1H, 4–H), 7.89 (dd, *J* = 8.0, 1.3 Hz, 1H, 5–H), 7.70 (ddd, *J* = 8.6, 7.2, 1.5 Hz, 1H, 8–H), 7.39 (d, *J* = 8.4 Hz, 1H, 7–H), 7.28–7.20 (m, 1H, 6–H) ppm; ^{13}C NMR (100 MHz, DMSO–d_6): δ = 168.6 (C-9), 155.2 (C-2), 143.3 (C-4), 141.0 (C–8a), 135.6 (C-8), 132.2 (C-5), 122.7 (C-6), 119.7 (C–4a), 116.8 (C-7), 112.5 (C-3) ppm; MS (ESI, MeOH): *m/z* (%) 187.1 ($[M+H]^+$, 100%); analysis calcd for $C_{10}H_6N_2O_2$ (186.17): C 64.52, H 3.25, N 15.05; found: C 64.31, H 3.48, N 14.81.

4.2.17. 2,2–Diethyl–1,2–dihydro–[1,2,4]triazolo[4,3–a] quinolin–2–ium 4–carboxylate (**16**)

A solution of **4** (514 mg, 2.76 mmol), diethylamine (0.31 mL, 3.00 mmol) and formaldehyde (37%, 0.22 mL, 2.76 mmol) in MeOH (10 mL) was stirred at 25 °C for 8 h (during this timr, the color of the mixture turned from red to slightly yellow–green). The solvent was

removed under reduced pressure, and the solid was washed with acetone (3 × 2 mL) to yield **16** (780 mg, 92%; m.p. 202–204 °C (lit [30].: 202–208 °C); IR (ATR): ν = 2961w, 1751m, 1663s, 1614m, 1487m, 1321s, 1052s, 778vs, 603s, 460vs cm^{-1}; UV–Vis (DMSO): λ_{max} (log ε) = 257 (5.50), 364 (4.94) nm; ^{1}H NMR (400 MHz, DMSO–d_6): δ = 7.90 (s, 1H, 4–H), 7.74–7.68 (m, 1H, 5–H), 7.60–7.51 (m, 1H, 7–H), 7.26–7.18 (m, 1H, 6–H), 7.09 (d, J = 8.2 Hz, 1H, 8–H), 5.77 (s, 2H, 10–H), 3.78–3.58 (m, 4H, 13–H + 13'–H), 1.27 (t, J = 7.1 Hz, 5H, 14–H + 14′–H) ppm; ^{13}C NMR (100 MHz, DMSO–d_6): δ = 164.0 (C-9), 155.5 (C-2), 137.4 (C-4), 134.1 (C–8a), 131.1 (C-7), 129.4 (C-5), 127.1 (C–4a), 123.2 (C-6), 121.8 (C-3), 114.0 (C-8), 71.1 (C-10), 60.7 (C-13 + C-13′), 8.3 (C-14 + C-14′) ppm; MS (ESI, MeOH): m/z (%) 272.13 ([M+H]$^+$, 100%); analysis calcd for $C_{15}H_{17}N_3O_2$ (271.32): C 66.40, H 6.32, N 15.49; found: C 66.19, H 6.50, N 15.25.

*4.2.18. (3β) 3–O–acetyl–betulinic acid (**17**)*

Acetylation of betulinic acid (**BA**) 20.0 g, 43.7 mmol) with acetic anhydride in the presence of DMAP (cat.) as previously reported [34] gave **17** (90%) as a colorless solid; m.p. 285–290 °C (lit [42].: 287–289 °C).

*4.2.19. (3β) 3–O–acetyl–oleanolic acid (**18**)*

Acetylation of oleanolic acid (**OA**), with acetic anhydride in the presence of DMAP (cat.) as previously reported [15] gave **18** (88%) as a colorless solid; m.p. 265–267 °C (lit [43].: 264–265 °C).

*4.2.20. (3β) 3–O–acetyl–ursolic acid (**19**)*

Acetylation of ursolic acid (**UA**) with acetic anhydride in the presence of DMAP (cat.) as previously reported gave **19** (84%) as a colorless solid; m.p. 280–283 °C (lit [15].: 281–283 °C).

*4.2.21. 4.2.19 (3β) 3–O–acetyl–platanic acid (**20**)*

Acetylation of platanic acid (**PA**) with acetic anhydride in the presence of DMAP (cat.) as previously reported gave **20** (87%) as a colorless solid; m.p. 280–283 °C (lit [11].: 280–283 °C).

*4.2.22. (3β) 3–O–acetyl–betulinic acid piperazinyl amide (**21**)*

Reaction of **17** (18.0 g, 36.1 mmol) in dry DCM (220 mL) with oxalyl chloride (15.2 mL, 177.2 mmol) followed by the addition of dry piperazine (15.6 g, 181.1 mmol) as previously reported gave **21** (15.6 g, 80%) as an off–white solid; m.p. 179–181 °C (lit [11].: 177–181 °C).

*4.2.23. (3β) 3–O–acetyl–oleanolic acid piperazinyl amide (**22**)*

Reaction of **18** with oxalyl chloride followed by the addition of dry piperazine as previously reported gave **22** (86%) as an off–white solid; m.p. 173–175 °C (lit [15].: 173–175 °C).

*4.2.24. (3β) 3–O–acetyl–ursolic acid piperazinyl amide (**23**)*

Reaction of **19** with oxalyl chloride followed by the addition of dry piperazine as previously reported gave **23** (84%) as an off–white solid; m.p. 186–188 °C (lit [15].: 187–188 °C).

*4.2.25. (3β) 3–O–acetyl–platanic acid piperazinyl amide (**24**)*

Reaction of **20** with oxalyl chloride followed by the addition of dry piperazine as previously reported gave **24** (84%) as an off–white solid; m.p. 118–121 °C (lit [11].: 115–125 °C).

*4.2.26. (3β) 3–O–acetyl–betulinic acid 2–aminoethylamide (**25**)*

Reaction of **17** (1.68 g, 3.4 mmol) in dry DCM (20 mL) with oxalyl chloride (1.5 mL, 17.5 mmol) followed by the addition of ethylenediamine (1.2 mL, 18.0 mmol) as previously reported gave **25** (1.4 g, 83%) as an off–white solid; m.p. 140–142 °C (lit [15].: 142 °C).

*4.2.27. (3β) 3–O–acetyl–oleanolic acid 2–aminoethylamide (**26**)*

Reaction of **18** with oxalyl chloride followed by the addition of ethylenediamine as previously reported gave **26** (81%) as an off–white solid; m.p. 190–193 °C (lit [15].: 190.5 °C).

*4.2.28. (3β) 3–O–acetyl–ursolic acid 2–aminoethylamide (**27**)*

Reaction of **19** with oxalyl chloride followed by the addition of ethylenediamine as previously reported gave **27** (84%) as an off–white solid; m.p. 196–198 °C (lit [15].: 198 °C).

*4.2.29. (3β) 3–O–acetyl–platanic acid 2–aminoethylamide (**28**)*

Reaction of **20** (1.68 g, 3.4 mmol) with oxalyl chloride followed by the addition of ethylenediamine as previously reported gave **28** (86%) as an off–white solid; m.p. 230–233 °C (lit [44].: 230–234 °C).

4.2.30. General procedure (GPB) for the synthesis of the conjugates

The safirinium dye (1.9 mmol) in dry DCM (20 mL) was activated with oxalyl chloride (0.4 mL, 4.7 mmol) followed by the addition of the corresponding acetylated triterpenoid amide (1.9 mmol) and triethylamine (0.56 mL, 4.0 mmol). After stirring at 25 °C for 12 h followed by usual aqueous workup and column chromatography (silica gel, $CHCl_3$/MeOH → MeOH) the conjugates were obtained.

*4.2.31. (3β) 3–Acetyloxy–28–{4–[(2,2–diethyl–5,7–dimethyl–2,3–dihydro[1,2,4]triazolo[4,3–a]pyridin–2–ium–8–yl)carbonyl]–1–piperazinyl}–28–oxolup–20(29)–ene chloride (**29**)*

Following GPB **29** (0.42 g, 57%) was obtained as a pale yellow solid; m.p. 212 °C; R_F = 0.3 (silica gel, $CHCl_3$/MeOH, 9:1); IR (ATR): ν = 2943m, 2867m, 1729m, 1632s, 1566m, 1535m, 1447m, 1392m, 1371m, 1315w, 1282m, 1245s, 1188m, 1107m, 1065m, 1027m, 999s; ^{1}H NMR (500 MHz, CD_3OD): δ = 6.22 (s,1H, 45–H), 5.93–5.89 (m, 2H, 42–H), 4.75 (s, 1H, 29–H_a), 4.64 (s, 1H, 29–H_b), 4.50–4.47 (m, 1H, 3–H), 3.84–3.73 (m, 5H, 49–$H_{a,b}$ + 49'$H_{a,b}$ + 12–H_b) 3.01–2.89 (m, 2H, 19–H + 13–H), 2.46 (s, 3H, 48–H), 2.26–2.23 (m, 1H, 16–H_a), 2.22 (s, 3H, 47–H), 2.12–2.09 (m, 1H, 22–H_a), 2.07 (s, 3H, 32–H), 1.92–1.84 (m, 2H, 21–H), 1.81–1.77 (m, 2H, 1–H_a + 12–H_a), 1.75 (s, 3H, 30–H) 1.73–1.66 (m, 4H, 18–H + 16–H_a + 2–H), 1.61–1.53 (m, 2H, 6–H_a + 22–H_b), 1.51–1.36 (m, 12H, 6–H_b + 11–H_a + 7–H + H-15–H_a + 9–H + 50–H + 50'–H), 1.34–1.25 (m, 2H, 11–H_b + 15–H_b), 1.07 (s, 3H, 27–H), 1.07–1.03 (m, 2H, 12–H_b + 1–H_b), 1.01 (s, 3H, 26–H), 0.95 (s, 3H, 25–H), 0.91 (s, 3H, 23–H), 0.90 (s, 3H, 24–H 0.89–0.87 (m, 1H, 5–H) ppm; ^{13}C NMR (125 MHz, CD_3OD): δ = 176.0 (C-28), 172.8 (C-31), 165.6 (C-37), 156.4 (C-44), 152.7 (C-20), 152.4 (C-46), 144.3 (C-39), 112.3 (C-38),111.7 (C-45), 110.0 (C-29), 82.5 (C-3), 74.0 (C-42), 62.6 (C-49 + C-49'), 56.9 (C-5), 56.1 (C-17), 53.9 (C-18), 52.1 (C-9), 47.3 (C-19), 43.0 (C-14), 42.0 (C-8), 39.6 (C-1), 38.9 (C-4), 38.4 (C-13), 38.3 (C-10), 37.0 (C-22), 35.5 (C-7), 33.4 (C-16), 32.4 (C-21), 31.0 (C-15), 28.5 (C-24), 26.9 (C-12), 24.7 (C-2), 22.3 (C-11), 21.2 (C-32), 19.8 (C-30), 19.4 (C-47), 19.3 (C-6), 18.7 (C-48), 17.0 (C-23), 16.8 (C-25), 16.7 (C-26), 15.1 (C-27), 8.7 (C-50), 8.3 (C-50') ppm; MS (ESI, MeOH): m/z (%) = 798.6 ([M – Cl]$^+$, 100%); analysis calcd for $C_{49}H_{76}ClN_5O_4$ (834.63): C 70.52, H 9.18, N 8.39; found: C 70.37, H 9.31, N 8.21.

*4.2.32. (3β) 3–Acetyloxy–28–{4–[(2,2–dihexyl–5,7–dimethyl–2,3–dihydro[1,2,4]triazolo[4,3–a]pyridin–2–ium–8–yl)carbonyl]–1–piperazinyl}–28–oxolup–20(29)–ene chloride (**30**)*

Following GPB **30** (0.32 g, 39%) was obtained as an yellowish solid; m.p. 189 °C; R_F = 0.6 (silica gel, $CHCl_3$/MeOH, 9:1); IR (ATR): ν = 2934m, 2868m, 1731m, 1632s, 1565m, 1535m, 1459m, 1371m, 1315m, 1282m, 1245s, 1184s, 1138m, 1108m, 1064m, 1027m, 999s; 1H NMR (500 MHz, CD_3OD): δ = 6.21 (m, 1H, 45–H), 5.94–5.84 (m,

2H, 42−$H_{a,b}$), 4.76 (s, 1H, 29−H_a), 4.64 (s, 1H, 29−H_b), 4.49−4.47 (m, 1H, 3−H), 3.72−3.66 (m, 4H, 49−$H_{a,b}$ + 49'−$H_{a,b}$), 3.03−2.89 (m, 2H, 19−H + 13−H), 2.44 (s, 3H, 48−H), 2.27−2.19 (m, 4H, 16−H_a + 47−H), 2.12−2.05 (m, 4H, 22H_a + 32−H), 1.88−1.69 (m, 9H, 21−H_a + 50−H_a + 50'−H_a + 12−H_a + 1−H_a + 18−H + 16−H_b + 2−H), 1.62−1.25 (m, 26H, 6−$H_{a,b}$ + H-22$_b$ + 11−H_a + 15−$H_{a,b}$ + 9−H + 7−H + 51−H + 51'−H + 52−H 52'−H + 53−H + 53'−H + 12−Hb + 21−Hb + 50−H_b + −50'−H_b + 11−H_b) 1.09−1.05 (m, 4H, 27−H + 1−H_b), 1.02 (s, 3H, 26−H), 1.00−0.96 (m, 6H, 54−H + 54'−H), 0.95 (s, 3H, 25−H), 0.91 (d, J = 3.5 Hz, 6H, 23−H + 24−H) ppm; ^{13}C NMR (125 MHz, CD_3OD): δ = 176.0 (C-28), 172.81 (C-31),165.6 (C-37), 156.2 (C-44), 152.7 (C-20), 152.4 (C-46), 144.2 (C-39), 112.3 (C-38), 111.7 (C-45), 110.1 (C-29), 82.4 (C-3), 75.0 (C-42), 68.0 (C-49+ C-49'), 56.9 (C-5), 56.2 (C-17), 56.1 (C-14), 53.9 (C-18), 52.1 (C-9), 47.3 (C-19), 42.0 (C-8), 39.6 (C-1), 38.9 (C-4), 38.4 (C-13), 38.3 (C-10), 37.0 (C-22), 35.5 (C-7), 33.4 (C-16), 32.4 (C-21), 28.5 (C-24), 27.1 (C-51 + C-51' + C-52 + C-52' + C-53 + C-53'), 26.9 (C-12), 24.7 (C-2), 23.5 (C-50), 22.3 (C-11), 21.2 (C-32), 19.8 (C-30), 19.4 (C-47), 19.3 (C-6), 18.6 (C-48), 17.0 (C-23), 16.8 (C-25), 16.7 (C-26), 15.2 (C-27), 14.4 (C-54), 14.3 (C-54') ppm; MS (ESI, MeOH): m/z (%) = 910.4 ([M − Cl]$^+$, 100%); analysis calcd for $C_{57}H_{92}ClN_5O_4$ (946.84): C 72.31, H 9.79, N 7.40; found: C 72.15, H 9.97, N 7.31.

4.2.33. (3β) 3−Acetyloxy−28−{4−[(2,2−didodecyl−5,7−dimethyl−2,3−dihydro[1,2,4]triazolo [4,3−a] pyridin−2−ium−8−yl)carbonyl]−1−piperazinyl}−28−oxolup−20(29)−ene chloride (**31**)

Following GPB **31** (390 mg, 40%) was obtained as a yellow solid; m.p. 158 °C; R_F = 0.2 (silica gel, $CHCl_3$/MeOH, 95:5); IR (ATR): ν = 2923s, 2853m, 1732m, 1627s, 1567m, 1530m, 1465m, 1405m, 1371m, 1283m, 1245s, 1186s, 1133w, 1107w, 1065w, 1029m, 999 s cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.89 (s, 1H, 45−H), 4.71 (s, 1H, 29−H_a), 4.58 (s, 1H, 29−H_b), 4.47−4.44 (m, 1H, 3−H), 3.99−3.86 (m, 2H, 49−$H_{a,b}$), 3.79−3.74 (m, 1H, 42−H_a), 3.66−3.61 (m, 2H, 49−H_b + 49'−H_b), 3.47−3.39 (m, 1H, 42−H_b), 2.94 (m, 1H, 19−H), 2.81 (m, 1H, 13−H), 2.51 (s, 3H, 48−H), 2.12 (s, 3H, 47−H), 2.02 (s, 4H, 16−H_a + 32−H), 1.92−1.83 (m, 2H, 22−H_a+21−H_a), 1.73−1.66 (m, 7H, 59−H_a + 59′−H_a + 12−H_a + 30−H + 1−H_a), 1.60−1.55 (m, 4H, 2−H + 16−H_b + 18−H), 1.50−1.46 (m, 1H, 6−H_a), 1.42−1.29 (m, 15H, 22−H_b + 11−H + 21−H_b + 6−H_b + 7−H + 50−H + 50−H' + 51−H + 51'−H), 1.27−1.21 (m, 33H, 59−H_b + 59'−H_b + 9−H + 58−H + 58'−H + 15−H + 52−H−57−H + 52'−H−57'−H), 0.95−0.94 (m, 4H, 12−H_b + 27−H), 0.92−0.90 (d, J = 4.2 Hz, 3H, 26−H), 0.88−0.86 (m, 6H, 60−H + 60'−H), 0.84−0.81 (m, 9H, 25−H + 24−H + 23−H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 174.0 (C-28), 171.1 (C-31), 163.4 (C-37), 155.1 (C-44), 151.0 (C-20), 150.7 (C-46), 142.7 (C-39), 111.6 (C-38), 110.7 (C-45), 109.6 (C-29), 81.1 (C-3), 67.5 (C-49 + C-49'), 67.2 (C-42), 55.7 (C-5), 54.8 (C-17), 54.8 (C-14), 52.8 (C-18), 50.9 (C-9), 45.8 (C-19), 40.8 (C-8), 38.6 (C-1), 37.9 (C-4), 37.3 (C-10), 37.1 (C-13), 36.1 (C-22), 34.5 (C-7), 32.7 (C-16), 32.1 (C-58 + C-58'), 31.4 (C-21), 30.0 (C-15), 30.0−29.3 (C-52−C−57 + C-52'−C−57'), 28.1 (C-24), 26.4 (C-50 + C-50' + C-51 + C-51'), 25.7 (C-12), 23.8 (C-2), 22.8 (C-59 + C-59'), 21.4 (C-32), 21.3 (C-11), 19.7 (C-30), 19.62 (C-48), 19.60 (C-47), 18.3 (C-6), 16.6 (C-23), 16.4 (C-25), 16.2 (C-26), 14.7 (C-27), 14.2 (C-60 + C-60') ppm; MS (ESI, MeOH): m/z (%) = 1079.0 ([M − Cl]$^+$, 90%); analysis calcd for $C_{69}H_{116}ClN_5O_4$ (1115.17): C 74.32, H 10.49, N 6.28; found: C 74.03, H 10.71, N 6.05.

4.2.34. (3β) 3−Acetyloxy−28−{4−[(2,2−diethyl−1,2−dihydro [1,2,4]triazolo[4,3−a]quinolin−2−ium−4−yl)carbonyl]−1−piperazinyl}−28−oxalupa−12,20(29)−diene chloride (**32**)

Following GPB **32** (450 mg, 57%) was obtained as a yellow solid; m.p. 215−218 °C; IR (ATR): ν = 3373br, 2941m, 2869m, 2095m, 1731s, 1624s, 1575m, 1453m, 1243s, 980s, 751m, 583 m cm^{-1}; UV−Vis (MeOH): λ_{max} (log ε) = 246 (5.54), 287 (4.93), 371 (4.84) nm; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.76 (s, 1H, 37−H), 7.66 (t, J = 7.7 Hz, 1H, 41−H), 7.58 (d, J = 7.3 Hz, 1H, 39−H), 7.51 (d, J = 8.6 Hz, 1H, 42−H), 7.30 (t, J = 7.6 Hz, 1H, 40−H), 6.50 (s, 2H, 44−H), 4.70 (s, 1H, 29−H_a), 4.57 (s, 1H, 29−H_b), 4.48−4.40 (m, 1H, 3−H), 4.39−4.26 (m, 2H, 46−H), 3.91−3.57 (m, 6H, 46'−H + 34−H), 3.43 (s, 4H, 33−H), 2.98−2.89 (m, 1H, 19−H), 2.85−2.75 (m, 1H, 13−H), 2.08−2.00 (m, 1H, 16−H_a), 2.01 (s, 3H, 32−H), 1.95−1.76 (m, 2H, 22−H_a + 21−H_a), 1.66 (s, 3H, 30−H), 1.74−1.52 (m, 6H, 12−H_a + 1−H_a + 2−H + 16−H_b + 18−H), 1.43 (t, J = 6.9 Hz, 6H, 47−H + 47′−H), 1.51−1.28 (m, 8H, 6−H + 22−H_b + 11−H_a + 21−H_b + 7−H + 15−H), 1.28−1.12 (m, 3H, 9−H + 11−H_b + 15−H_b), 0.94 (s, 3H, 27−H), 0.91 (s, 3H, 26−H), 1.00−0.88 (m, 2H, 1−H_b + 12−H_b), 0.82 (s, 3H, 25−H), 0.81 (s, 3H, 24−H), 0.81 (s, 3H, 23−H), 0.79−0.74 (m, 1H, 5−H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 174.2 (C-28), 171.1 (C-31), 163.1 (C-35), 154.0 (C-45), 151.0 (C-20), 140.7 (C-37), 134.2 (C-36), 134.2 (C-41), 129.9 (C-39), 124.6 (C-40), 120.3 (C-43) 118.4 (C-38), 115.1 (C-42), 109.6 (C-29), 81.0 (C-3), 72.8 (C-44), 62.6 (C-46 + C-46′), 55.6 (C-5), 54.8 (C-17), 52.7 (C-18), 50.8 (C-9), 47.5 (C-33), 45.7 (C-19), 42.5 (C-34), 42.0 (C1−4), 40.8 (C-8), 38.5 (C-1), 37.9 (C-4), 37.3 (C-10), 37.0 (C-13), 36.1 (C-22), 34.5 (C-7), 32.7 (C-16), 31.4 (C-21), 30.0 (C-15), 28.0 (C-24), 25.7 (C-12), 23.8 (C-2), 21.4 (C-1), 21.2 (C-32), 19.7 (C-30), 18.3 (C-6), 16.6 (C-23), 16.4 (C-25), 16.3 (C-26), 14.8 (C-27), 8.8 (C-47 + C-47′) ppm; MS (ESI, MeOH): m/z (%) 820.4 ([M − Cl]$^+$, 100%); analysis calcd for $C_{51}H_{72}ClN_5O_4$ (854.62): C 71.68H 8.49, N 8.19; found: C 71.42, H 8.67, N 8.06.

4.2.35. (3β) 3−Acetyloxy−28−{4−[(2,2−diethyl−1,2−dihydro [1,2,4]triazolo[4,3−a]quinolin−2−ium−4−yl)carbonyl]−1−piperazinyl}−28−oxo−olean−12−ene chloride (**33**)

Following GPB **33** (389 mg, 47%) was obtained as a yellow solid; m.p. 220−224 °C; IR (ATR): ν = 3369br, 2942s, 2096m, 1731s, 1625s, 1466m, 1391m, 1243vs, 1004s, 750m, 458 m cm^{-1}; UV−Vis (MeOH): λ_{max} (log ε) = 246 (5.57), 287 (4.98), 373 (4.87) nm; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.76 (s, 1H, 37−H), 7.65 (t, J = 7.7 Hz, 1H, 41−H), 7.58 (d, J = 7.2 Hz, 1H, 39−H), 7.50 (d, J = 8.1 Hz, 1H, 42−H), 7.30 (t, J = 7.6 Hz, 1H, 40−H), 6.49 (s, 2H, 44−H), 5.25 (dd, J = 3.3, 3.3 Hz, 1H, 12−H), 4.50−4.43 (m, 1H, 3−H), 4.39−4.27 (m, 2H, 46−H), 3.92−3.57 (m, 6H, 46′−H + 33−H + 34−H), 3.13−2.99 (m, 1H, 18−H), 2.19−2.09 (m, 1H, 16−H_a), 2.02 (s, 3H, 32−H), 1.96−1.79 (m, 2H, 11−H_a + 2−Ha), 1.75−1.46 (m, 11H, 7−H + 19−H_a + 2−H + 15−H_a + 11−H_b + 16−H_b + 1−H_a + 9−H + 6−H_a), 1.42 (t, J = 6.7 Hz, 6H, 47−H), 1.46−1.29 (m, 3H, 22−H_a + 21−H_a + 6−H_b), 1.27−1.15 (m, 3H, 22−H_b + 21−H_b + 19−H_b), 1.12 (s, 3H, 27−H), 1.10−0.95 (m, 2H, 15−H_b + 1−H_b), 0.91 (s, 3H, 29−H), 0.90 (s, 3H, 25−H), 0.89 (s, 3H, 30−H), 0.84 (s, 3H, 24−H), 0.83 (s, 3H, 23−H), 0.83−0.78 (m, 1H, 5−H), 0.69 (s, 3H, 26−H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 175.8 (C-28), 171.1 (C-31), 163.1 (C-35), 154.0 (C-45), 144.5 (C-13), 140.7 (C-37), 134.2 (C-36), 134.1 (C-41), 129.9 (C-39), 124.6 (C-40), 121.9 (C-12), 120.3 (C-43), 118.4 (C-38), 115.0 (C-42), 81.0 (C-3), 72.9 (C-44), 62.6 (C-46), 62.5 (C-46′), 55.5 (C-5), 47.7 (C-9), 47.7 (C-17), 46.4 (C-19), 45.4 (C-34), 43.7 (C-18), 42.5 (C-33), 42.0 (C-14), 39.3 (C-8), 38.2 (C-1), 37.8 (C-4), 37.1 (C-10), 34.1(C-21), 33.1 (C-30), 33.0 (C-22), 30.5 (C-20), 30.2 (C-7), 28.1 (C-23), 28.0 (C-15), 26.0 (C-27), 24.1 (C-29), 23.6 (C-11), 23.5 (C-2), 22.9 (C-16), 21.4 (C-32), 18.3 (C-6), 17.1 (C-26), 16.8 (C-24), 15.5 (C-25), 8.8 (C-47 + C-47′) ppm; MS (ESI, MeOH): m/z (%) 820.2 ([M − Cl]$^+$, 100%); analysis calcd for $C_{51}H_{74}ClN_5O_4$ (856.63): C 71.51, H 8.71, N 8.18; found: C 71.32, H 8.96, N 8.01.

*4.2.36. (3β) 3–Acetyloxy–28–{4–[(2,2–diethyl–1,2–dihydro [1,2,4]triazolo[4,3–a]quinolin–2–ium–4–yl)carbonyl]– 1–piperazinyl}–28–oxo–urs–12–ene chloride (**34**)*

Following GPB **34** (408 mg, 52%) was obtained as a yellow solid; m.p. 230–234 °C; IR (ATR): ν = 3373br, 2941m, 2096w, 1731m, 1624s, 1576m, 1368m, 1243vs, 1045m, 950m, 751m, 582w cm^{-1}; UV–Vis (MeOH): λ_{max} (log ε) = 246 (5.37), 287 (4.79), 371 (4.67) nm; ^{1}H NMR (500 MHz, CDCl3): δ = 7.76 (s, 1H, 37–H), 7.66 (t, J = 7.8 Hz, 1H, 41–H), 7.58 (d, J = 7.2 Hz, 1H, 39–H), 7.51 (d, J = 8.2 Hz, 1H, 42–H), 7.30 (t, J = 7.6 Hz, 1H, 40–H), 6.50 (s, 2H, 44–H), 5.22–5.18 (m, 1H, 12–H), 4.51–4.44 (m, 1H, 3–H), 4.38–4.26 (m, 2H, 46–H), 3.90–3.53 (m, 4H, 46′–H + 34–H), 3.49–3.36 (m, 2H, 33–H), 2.48–2.33 (m, 1H, 18–H), 2.23–2.09 (m, 1H, 16–H_a), 2.02 (s, 3H, 32–H), 1.96–1.83 (m, 2H, 11–H), 1.82–1.55 (m, 9H, 22–H + 16–H_b + 21–H + 1–H_a + 2–H + 15–H_a), 1.55–1.37 (m, 4H, 9–H + 6–H_a + 7–H_a + 19–H), 1.42 (t, J = 6.7 Hz, 6H, 47–H + 47′–H), 1.38–1.15 (m, 2H, 6–H_b + 7–H_b), 1.14–0.96 (m, 3H, 15–H_b + 1–H_b + 20–H), 1.06 (s, 3H, 27–H), 0.93 (d, J = 6.1 Hz, 3H, 30–H), 0.91 (s, 3H, 25–H), 0.88 (d, J = 6.4 Hz, 6H, 29–H), 0.85 (s, 3H, 24–H), 0.83 (s, 3H, 23–H), 0.83–0.79 (m, 1H, 5–H), 0.71 (s, 3H, 26–H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 176.0 (C-28), 171.1 (C-31), 163.1 (C-35), 154.0 (C-45), 138.5 (C-13), 140.7 (C-37), 134.2 (C-41), 134.1 (C-36), 129.9 (C-39), 125.5 (C-12), 124.6 (C-40), 120.3 (C-43), 118.4 (C-38), 115.1 (C-42), 81.0 (C-3), 72.9 (C-44), 62.6 (C-46 + C-46′), 55.4 (C-5), 55.3 (C-18), 47.7 (C-17), 47.6 (C-9), 47.3 (C-33), 42.4 (C-34), 42.2 (C-14), 39.6 (C-19), 39.3 (C-8), 38.8 (C-20), 38.3 (C-1), 37.8 (C-4), 37.0 (C-10), 34.6 (C-22), 33.1 (C-7), 30.5 (C-21), 28.3 (C-15), 28.2 (C-24), 23.8 (C-27), 23.6 (C-11), 23.5 (C-16), 23.4 (C-2), 21.4 (C-32), 21.3 (C-30), 18.3 (C-6), 17.5 (C-29), 17.1 (C-26), 16.8 (C-23), 15.6 (C-25), 8.8 (C-47 + C-47′) ppm; MS (ESI, MeOH): m/z (%) 820.2 ([M − Cl]$^+$, 100%); analysis calcd for $C_{51}H_{74}ClN_5O_4$ (856.63): C 71.51, H 8.71, N 8.18; found: C 71.26, H 8.90, N 8.03.

*4.2.37. (3β) 3–Acetyloxy–28–{4–[(2,2–diethyl–1,2–dihydro [1,2,4]triazolo[4,3–a]quinolin–2–ium–4–yl)carbonyl]– 1–piperazinyl}–20,28–dioxo–30–norlupa–12–ene chloride (**35**)*

Following GPB **35** (348 mg, 44%) was obtained as a yellow solid; m.p. 236–240 °C; IR (ATR): ν = 3373br, 2941m, 2871m, 2096m, 1731s, 1624s, 1452m, 1242vs, 1170m, 1028m, 980s, 751m, 582 m cm^{-1}; UV–Vis (MeOH): λ_{max} (log ε) = 246 (5.67), 288 (5.09), 374 (4.93) nm; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.76 (s, 1H, 36–H), 7.69–7.62 (m, 1H, 40–H), 7.58 (d, J = 7.8 Hz, 1H, 38–H), 7.54–7.49 (m, 1H, 41–H), 7.30 (t, J = 7.5 Hz, 1H, 39–H), 6.50 (s, 2H, 43–H), 4.47–4.40 (m, 1H, 3–H), 4.40–4.21 (m, 2H, 45–H), 4.07–3.33 (m, 6H, 45′–H + 33–H + 32–H), 3.19 (dd, J = 11.2, 7.3 Hz, 1H, 19–H), 2.62 (dd, J = 12.1, 8.5 Hz, 1H, 13–H), 2.14 (s, 3H, 29–H), 2.12–2.00 (m, 2H, 18–H + 16–H_a), 2.01 (s, 3H, 31–H), 1.97–1.76 (m, 2H, 22–H_a + 21–Ha), 1.72–1.47 (m, 7H, 1–H_a + 16–H_b+ 2–H + 22–H_b + 21–H_b + 6–H_a), 1.43 (t, 6H, 46–H + 46′–H), 1.45–1.13 (m, 8H, 11–H + 7–H + 6–H_b + 15–H + 9–H), 1.07–0.87 (m, 3H, 12–H + 1–H_b), 0.96 (s, 3H, 27–H), 0.89 (s, 3H, 26–H), 0.82 (s, 3H, 25–H), 0.82 (s, 3H, 24–H), 0.81 (s, 3H, 23–H), 0.80–0.73 (m, 1H, 5–H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 212.7 (C-20), 174.1 (C-28), 171.0 (C-30), 163.1 (C-34), 154.0 (C-44), 140.8 (C-36), 134.3 (C-40), 134.2 (C-35), 129.9 (C-38), 124.6 (C-39), 120.3 (C-42), 118.3 (C-37), 115.1 (C-41), 80.9 (C-3), 72.9 (C-43), 62.5 (C-45 + C-45′), 55.6 (C-5), 54.7 (C-17), 52.6 (C-18), 50.7 (C-9), 50.0 (C-19), 47.5 (C-32), 42.5 (C-33), 41.9 (C-14), 40.7 (C-8), 38.5 (C-1), 37.9 (C-4), 37.3 (C-10), 36.1 (C-13), 35.8 (C-22), 34.3 (C-7), 32.3 (C-16), 30.4 (C-29), 30.0 (C-15), 28.8 (C-21), 28.0 (C-24), 27.5 (C-12), 23.8 (C-2), 21.4 (C-31), 21.3 (C-11), 18.3 (C-6), 16.6 (C-23), 16.3 (C-25), 16.2 (C-26), 14.8 (C-27), 8.8 (C-46 + C-46′) ppm; MS (ESI, MeOH): m/z (%) 822.2 ([M − Cl]$^+$, 100%); analysis calcd for $C_{50}H_{70}ClN_5O_5$ (856.59): C 70.11, H 8.24, N 8.18; found: C 69.87, H 8.47, N 8.02.

*4.2.38. (3β) 3–Acetyloxy–28–[2–{[(2,2–diethyl–1,2–dihydro [1,2,4]triazolo[4,3–a]quinolin–2–ium–4–yl)carbonyl]amino} ethyl)amino]–28–oxolupa–12,20(29)–diene chloride (**36**)*

Following GPB **36** (316 mg, 44%) was obtained as a yellow solid; m.p. 200–204 °C; IR (ATR): ν = 3321br, 2942m, 2095w, 1731m, 1661m, 1474m, 1243m, 1172m, 1035s, 980m, 750w, 582 m cm^{-1}; UV–Vis (MeOH): λ_{max} (log ε) = 206 (5.26), 248 (5.13), 296 (4.66), 388 (4.36) nm; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.75 (s, 1H, 37–H), 8.41–8.25 (m, 1H, NH), 7.81–7.71 (m, 2H, 39–H + 41–H), 7.63–7.50 (m, 1H, 42–H), 7.39 (t, J = 7.6 Hz, 1H, 40–H), 6.70–6.25 (m, 3H, NH + 44–H), 4.65 (s, 1H, 29–H_a), 4.56 (s, 1H, 29–H_b), 4.54–4.37 (m, 3H, 46–H + 3–H), 4.03–3.84 (m, 2H, 46′–H), 3.74–3.35 (m, 4H, 33–H + 34–H), 3.15–2.98 (m, 1H, 19–H), 2.41–2.27 (m, 1H, 13–H), 2.02 (s, 3H, 32–H), 2.06–1.94 (m, 1H, 16–H_a), 1.92–1.69 (m, 2H, 21–H_a + 1–H_a), 1.64 (s, 3H, 30–H), 1.68–1.45 (m, 6H, 12–H_a + 22–H_a + 2–H + 16–H_b + 18–H), 1.41 (t, J = 7.3 Hz, 3H, 47–H + 47′–H), 1.44–1.18 (m, 7H, 22–H_b + 11–H_a + 15–H_a + 21–H_b + 6–H_a + 7–H_a + 9–H), 1.16–1.03 (m, 4H, 7–H_b + 11–H_b + 6–H_b + 15–H_b), 0.89 (s, 3H, 27–H), 1.02–0.81 (m, 2H, 12–H_b + 1–H_b), 0.78 (s, 3H, 24–H), 0.74 (s, 3H, 23–H), 0.74 (s, 3H, 26–H), 0.72–0.58 (m, 1H, 5–H), 0.64 (s, 3H, 25–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 177.2 (C-28), 171.0 (C-31), 161.5 (C-35), 154.5 (C-45), 150.6 (C-20), 146.1 (C-37), 135.3 (C-41), 134.4 (C-36), 131.1 (C-39), 125.1 (C-40), 120.4 (C-43), 114.9 (C-42), 114.4 (C-38), 109.6 (C-29), 80.8 (C-3), 72.3 (C-44), 63.2 (C-46 + C-46′), 55.7 (C-17), 55.3 (C-5), 50.3 (C-9), 50.0 (C-18), 46.9 (C-19), 40.6 (C-14), 40.4 (C-34), 39.7 (C-33), 38.4 (C-1), 38.3 (C-22), 37.7 (C-13), 37.7 (C-10), 37.0 (C-8), 34.2 (C-7), 33.5 (C-16), 30.8 (C-21), 29.5 (C-15), 27.9 (C-24), 25.4 (C-12), 23.6 (C-2), 21.3 (C-32), 20.9 (C-11), 19.3 (C-30), 17.9 (C-6), 16.5 (C-23), 16.2 (C-25), 16.1 (C-26), 14.5 (C-27), 8.8 (C-47), 8.6 (C-47′) ppm; MS (ESI, MeOH): m/z (%) 794.2 ([M − Cl]$^+$, 100%); analysis calcd for $C_{49}H_{70}ClN_5O_4$ (828.58): C 71.03, H 8.52, N 8.45; found: C 70.76, H 8.69, N 8.26.

*4.2.39. (3β) 3–Acetyloxy–28–[2–{[(2,2–diethyl–1,2–dihydro [1,2,4]triazolo[4,3–a]quinolin–2–ium–4–yl)carbonyl]amino} ethyl)amino]–28–oxo–olean–12–ene chloride (**37**)*

Following GPB **37** (314 mg, 40%) was obtained as a yellow solid; m.p. 210–214 °C; IR (ATR): ν = 3334br, 2941m, 2097w, 1731m, 1661m, 1574m,1366m, 1243m, 1027m, 750m, 583w cm−1; UV–Vis (MeOH): λ_{max} (log ε) = 213 (5.64), 248 (5.50), 296 (5.00), 387 (4.73) nm; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.72 (s, 1H, 37–H), 8.42–8.37 (m, 1H, NH), 7.77–7.69 (m, 2H, 39–H + 41–H), 7.56 (d, J = 8.3 Hz, 1H, 42–H), 7.36 (t, J = 7.6 Hz, 1H, 40–H), 6.59–6.47 (m, 2H, 44–H), 6.44–6.37 (m, 1H, NH), 5.39 (s, 1H, 12–H), 4.53–4.37 (m, 3H, 46–H + 3–H), 4.00–3.80 (m, 1H, 46′–H), 3.73–3.42 (m, 2H, 33–H), 3.32–3.16 (m, 2H, 34–H), 2.60–2.48 (m, 1H, 18–H), 2.02 (s, 3H, 31–H), 1.97–1.83 (m, 3H, 16–H_a + 11–H), 1.77–1.65 (m, 1H, 19–H_a), 1.62–1.50 (m, 6H, 16–H_b + 22–H_a + 2–H + 1–H_a + 9–H), 1.50–1.37 (m, 3H, 15–H_a + 6–H_a + 7–H_a), 1.42 (t, J = 6.9 Hz, 6H, 47–H + 47′–H), 1.36–1.22 (m, 3H, 6–H_b + 21–H_a + 22–H_b), 1.21–1.15 (m, 2H, 7–H + 19–H_b), 1.12 (s, 3H), 1.11–0.94 (m, 3H, 21–H_a + 1–H_a + 15–H_b), 0.87 (s, 3H, 30–H), 0.85 (s, 3H, 25–H), 0.84 (s, 3H, 29–H), 0.82 (s, 3H, 23–H), 0.81 (s, 3H, 24–H), 0.80–0.76 (m, 1H, 5–H), 0.71 (s, 3H, 26–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 179.2 (C-28), 171.1 (C-31), 161.5 (C-35), 154.6 (C-45), 146.0 (C-37), 144.6 (C-13), 135.3 (C-39), 134.6 (C-36), 131.1 (C-41), 125.0 (C-40), 123.0 (C-12), 120.5 (C-43), 115.1 (C-42), 114.4 (C-38), 80.9 (C-3), 72.2 (C-44), 63.3 (C-46), 63.1 (C-46‘), 55.2 (C-5), 47.5 (C-9), 46.7 (C-19), 46.4 (C-17), 42.1 (C-14), 42.0 (C-18), 40.5 (C-33), 39.8 (C-34), 39.4 (C-8), 38.2 (C-1), 37.8 (C-4), 36.9 (C-10), 34.2 (C-21), 33.0 (C-30), 32.9 (C-22), 32.4 (C-7), 30.8 (C-20), 28.1 (C-23), 27.4 (C-15), 25.8 (C-27), 23.9 (C-16), 23.7 (C-29), 23.6 (C-11), 23.6 (C-2), 21.4 (C-32), 18.2 (C-6), 17.0 (C-26), 16.8 (C-24), 15.5 (C-25), 8.9 (C-47 + C-47‘) ppm; MS (ESI, MeOH): m/z (%) 794.1 ([M − Cl]$^+$, 100%); analysis

calcd for $C_{49}H_{72}ClN_5O_4$ (829.53): C 70.86, H 8.74, N 8.43; found: C 70.69, H 8.91, N 8.25.

*4.2.40. (3β) 3−Acetyloxy−28−[2−{[(2,2−diethyl−1,2−dihydro [1,2,4]triazolo[4,3−a]quinolin−2−ium−4−yl)carbonyl]amino} ethyl)amino]−28−oxo−urs−12−ene chloride (**38**)*

Following GPB **38** (341 mg, 45%) was obtained as a yellow solid; m.p. 198−202 °C; IR (ATR): ν = 3321br, 2940m, 2095m, 1731m, 1456m, 1367m, 1243vs, 1147w, 1027m, 982m, 750w, 582w cm^{-1}; UV−Vis (MeOH): λ_{max} (log ε) = 248 (5.54), 297 (5.04), 387 (4.79) nm; ^1H NMR (400 MHz, $CDCl_3$): δ = 8.71 (s, 1H, 37−H), 8.43−8.35 (m, 1H, NH), 7.72 (t, J = 7.0 Hz, 2H, 39−H + 41−H), 7.53 (d, J = 8.5 Hz, 1H, 42−H), 7.35 (t, J = 7.6 Hz, 1H, 40−H), 6.54 (s, 2H, 44−H), 6.40−6.31 (m, 1H, NH), 5.33−5.29 (m, 1H, 12−H), 4.52−4.40 (m, 2H, 46−H + 3−H), 3.98−3.84 (m, 2H), 3.67−3.45 (m, 2H, 33−H), 3.32−3.20 (m, 2H, 34−H), 2.01 (s, 3H, 32−H), 2.07−1.81 (m, 3H, 2−H + 18−H), 1.77−1.50 (m, 6H, 22−H_a + 11−H + 15−H_a + 1−H_a +9−H), 1.49−1.16 (m, 8H, 7−H + 6−H + 19−H + 21−H + 22−H_b), 1.44 (t, J = 6.8 Hz, 6H, 47−H + 47'−H, 1.05 (s, 3H, 27−H), 1.06−0.95 (m, 2H, 1−H_b + 15−H_b), 0.90 (s, 3H, 30−H), 0.86 (s, 3H, 25−H), 0.84 (s, 3H, 29−H), 0.82 (s, 3H, 24−H), 0.80 (s, 3H, 23−H), 0.71 (s, 3H, 26−H), 0.74−0.64 (m, 1H, 5−H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 179.0 (C-28), 170.9 (C-31), 161.2 (C-35), 154.4 (C-45), 145.8 (C-37), 139.4 (C-13), 135.2 (C-41), 134.4 (C-36), 131.0 (C-39), 125.7 (C-12), 124.9 (C-40), 120.4 (C-43), 114.9 (C-42), 114.4 (C-38), 80.7 (C-3), 72.1 (C-44), 63.1 (C-46), 63.0 (C-46′), 55.1 (C-5), 53.5 (C-18), 47.7 (C-17), 47.4 (C-9), 42.3 (C-14), 40.4 (C-33), 39.6 (C-19), 39.5 (C-8), 39.3 (C-34), 39.1 (C-20), 38.2 (C-1), 37.6 (C-4), 37.4 (C-22), 36.8 (C-10), 32.6 (C-7), 30.8 (C-21), 28.0 (C-24), 27.8 (C-15), 23.5 (C-11), 23.5 (C-16), 23.3 (C-2), 23.2 (C-27), 21.3 (C-32), 21.2 (C-30), 18.1 (C-6), 17.3 (C-29), 16.9 (C-26), 16.7 (C-23), 15.5 (C-25), 8.8 (C-47), 8.8 (C-47′) ppm; MS (ESI, MeOH): m/z (%) 794.2 ([M − Cl]$^+$, 100%); analysis calcd for $C_{49}H_{72}ClN_5O_4$ (829.53): C 70.86, H 8.74, N 8.43; found: C 70.58, H 8.93, N 8.21.

*4.2.41. (3β) 3−Acetyloxy−28−[2−{[(2,2−diethyl−1,2−dihydro [1,2,4]triazolo[4,3−a]quinolin−2−ium−4−yl)carbonyl]amino} ethyl)amino]−20,28−dioxo−30−norlupan−12−ene chloride (**39**)*

Following GPB **39** (335 mg, 44%) was obtained as a yellow solid; m.p. 196−200 °C; IR (ATR): ν = 3321br, 2941m, 2095m, 1731s, 1547m, 1243vs, 1167m, 1028m, 980m, 750w, 583 cm^{-1}; UV−Vis (MeOH): λ_{max} (log ε) = 248 (5.76), 297 (5.27), 385 (5.02) nm; ^1H NMR (500 MHz, $CDCl_3$): δ = 8.72 (s, 1H, 36−H), 8.30 (t, J = 5.3 Hz, 1H, NH), 7.77−7.70 (m, 2H, 38−H + 40−H), 7.54 (d, J = 8.1 Hz, 1H, 41−H), 7.37 (t, J = 7.6 Hz, 1H, 39−H), 6.70−6.64 (m, 1H, NH), 6.60 (d, J = 10.0 Hz, 1H, 43−H_a), 6.47 (d, J = 10.1 Hz, 1H, 43−H_b), 4.49−4.35 (m, 3H, 3−H + 45−H), 3.98−3.88 (m, 2H, 45′−H), 3.71−3.48 (m, 2H, 32−H), 3.45−3.29 (m, 3H, 33−H + 18−H), 2.19−2.08 (m, 1H, 13−H), 2.11 (s, 3H, 29−H), 2.06−2.00 (m, 2H, 16−H_a + 19−H), 2.00 (s, 3H, 31−H), 1.98−1.90 (m, 1H, 21−H_a), 1.80−1.73 (m, 1H, 22−H_a), 1.61−1.42 (m, 5H, 1−H_a + 16−H_b + 2−H + 22−H_b), 1.46 (q, J = 6.9 Hz, 6H, 46−H + 46′−H), 1.42−1.19 (m, 6H, 21−H_b + 11−H_a + 15−H_a + 6−H_a + 7−H_a + 9−H), 1.19−0.96 (m, 6H, 11−H_b + 6−H_b + 7−H_b + 15−H_b + 12−H), 0.92 (s, 3H, 27−H), 0.95−0.85 (m, 1H, 1−H_b), 0.76 (s, 3H, 24−H), 0.73 (s, 3H, 26−H), 0.72 (s, 3H, 23−H), 0.71−0.64 (m, 1H, 5−H), 0.67 (s, 3H, 25−H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 212.7 (C-20), 176.9 (C-28), 171.0 (C-30), 161.8 (C-34), 154.7 (C-44), 146.2 (C-36), 135.2 (C-40), 134.5 (C-35), 131.2 (C-38), 125.2 (C-39), 120.5 (C-42), 115.0 (C-41), 114.4 (C-37), 80.8 (C-3), 72.4 (C-43), 63.2 (C-45), 63.1 (C-45′), 55.6 (C-17), 55.4 (C-5), 51.3 (C-18), 50.3 (C-9), 50.1 (C-19), 42.3 (C-8), 40.7 (C-14), 40.1 (C-32), 40.1 (C-33), 38.4 (C-1), 38.1 (C-22), 37.8 (C-4), 37.1 (C-10), 36.9 (C-11), 34.2 (C-7), 32.8 (C-16), 30.1 (C-29), 29.6 (C-15), 28.6 (C-21), 28.0 (C-24), 27.2 (C-12), 23.7 (C-2), 21.4 (C-31), 21.0 (C-11), 18.0 (C-6), 16.6 (C-23 + C-26), 16.2 (C-25), 14.7 (C-27), 8.9 (C-46), 8.9 (C-46′) ppm; MS (ESI, MeOH): m/z (%) 796.2 ([M − Cl]$^+$, 100%); analysis calcd for $C_{48}H_{66}ClN_5O_5$ (830.55): C 69.42, H 8.25, N 8.43; found: C 69.27, H 8.41, N 8.32.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We like to thank Dr. D. Ströhl, Y. Schiller and S. Ludwig for the NMR spectra and the late Dr. R. Kluge for measuring the MS spectra; some spectra were recorded by Th. Schmidt. Many thanks are also due to V. Simon and M. Schneider for taking the IR spectra. The microanalyses have been determined by S. Ludwig and M. Schneider. The cell lines have been provided by the Department of Oncology (Martin−Luther−University Halle Wittenberg).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2020.112920.

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