11-Keto-boswellic acid derived amides and monodesMosidic saponins induce apoptosis in breast and cervical cancers cells

ARTICLE *in* EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY · JULY 2015

Impact Factor: 3.45 · DOI: 10.1016/j.ejmech.2015.06.003

5 AUTHORS, INCLUDING:

René Csuk

Martin Luther University Halle-Wittenberg

212 PUBLICATIONS **1,922** CITATIONS

SEE PROFILE

Ahmed Al-Harrasi

University of Nizwa

206 PUBLICATIONS **609** CITATIONS

SEE PROFILE

European Journal of Medicinal Chemistry 100 (2015) 98–105

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Research paper

11-Keto-boswellic acid derived amides and monodesmosidic saponins induce apoptosis in breast and cervical cancers cells

René Csuk [a, *], Anja Barthel-Niesen [a], Alexander Barthel [a], Renate Schäfer [a], Ahmed Al-Harrasi [b]

[a] Martin-Luther-University Halle-Wittenberg, Organic Chemistry, Kurt-Mothes-Str. 2, D-06120, Halle (Saale), Germany
[b] University of Nizwa, Chair of Oman's Medicinal Plants and Marine Natural Products, P.O. Box 33, PC 616, Birkat Al-Mauz, Nizwa, Oman

ARTICLE INFO

Article history:
Received 25 April 2015
Received in revised form
31 May 2015
Accepted 1 June 2015
Available online 4 June 2015

Keywords:
Boswellic acids
Frankincense
Tumor cells
SRB assay
Apoptosis

ABSTRACT

Beta-boswellic acids are considered the main bioactive components of frankincense. Their potential to act as cytotoxic agents, as well as that of their derivatives remained unexploited so far. In this study we were able to prepare derivatives of 11-keto-β-boswellic acid (KBA) that showed lower IC50 values as determined by a sulphorhodamine B (SRB) assay using several different human tumour cell lines. Monodesmosidic saponins of KBA are as cytotoxic as 3-acetyl-KBA. The presence of a free hydroxyl group at position C-3 seems to lower cytotoxicity while the presence of an amide function at C-24 improves cytotoxicity. The most active compound of this series gave IC_{50} values as low as 4.5 μM. Cell death proceeded mainly via apoptosis.

1. Introduction

The importance of the development of plant medicines as a key to global health is evident: over one billion people lack access to health care systems. In this context, the study of traditional medicines has been a neglected aspect of global health care for many years [1]. One of these traditional remedies is olibanum. Olibanum (frankincense) is a resin obtained from trees of the genus *Boswellia* in the family *Burseraceae* and has been used in traditional remedies for decades [2]. This precious material has been traded on the Arabian peninsula for more than 5000 years, and it has been used in the traditional medicines of India, Africa, China but also in Western countries [3]. Frankincense has been used to treat fevers and dysentery [4,5], but also – among other applications [6–10] - as an antiseptic [4,5], antihelmintic [6] and as an antitumor agent [11–14].

The resin contains different types of secondary metabolites, such as di-, sesqui- and triterpenes but also essential oils [2,15]. Among all of these, triterpenoids [16] are the most abundant, and many studies have shown among other effects the cytotoxic and antitumor properties of triterpenoids from frankincense.

Frankincense has been used and examined from various perspectives for millennia. The chemical investigations of the resin started as early as 1788 by Baer [17], and in 1898, β-boswellic acid (**1**, Fig. 1) was first isolated [18,19]. Boswellic acids and derivatives are among the major bioactive compounds of frankincense. Compared to other triterpenoic acids, such as betulinic, ursolic, oleanolic, glycyrrhetinic or maslinic acid, β-boswellic acids received less attention probably due their limited availability and high costs of the resin. Several years ago, J. Jauch et al. [20,21] improved older extraction and purification techniques [22,23] significantly; this allowed a more convenient isolation of 3-acetyl-11-keto-β-boswellic acid (**AKBA**, **2**), together with 11-keto-β-boswellic acid (**KBA**, **3**) and 3-acetyl-β-boswellic acid (**ABA**, **4**).

Recently boswellic acids and derivatives came into the focus of scientific interest due to their antitumor activity, and several compounds have been synthesized showing promising activity [10,24,25]. One major drawback of most of these compounds, however, is their poor solubility in aqueous systems. This limits their solubilization and the formulation for biological tests as well as for medicinal applications [26,27]. Therefore, we set out to synthesize a few derivatives either by transformation of the carboxylic group C-24 into amino acid/amino alcohol derived amides

* Corresponding author. Bereich Organische Chemie, Martin-Luther Universität Halle-Wittenberg, Kurt-Mothes-Str. 2, D-06120, Halle (Saale), Germany.
E-mail address: rene.csuk@chemie.uni-halle.de (R. Csuk).

http://dx.doi.org/10.1016/j.ejmech.2015.06.003
0223-5234/

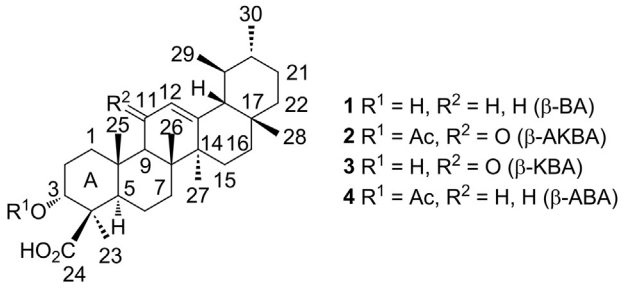


Fig. 1. Structure and ring numbering for β-boswellic acid (**1**, β-**BA**), AKBA (**2**), KBA (**3**) and ABA (**4**).

or by converting **KBA** into monodesmosidic saponins (using the hydroxyl group at C-3), and to test these derivatives for their cytotoxic activity employing several human tumor cell lines. We previously reported [28–32] a higher cytotoxicity for amino acid/amino alcohol derived amides of glycyrrhetinic acid compared to the parent compound, and a betulinic acid derived saponin [33] is regarded [34] as a very promising compound for advanced biological testing.

Triterpenoid glycosides, saponins, are known for their increased hydrosolubility [27,35]compared to their parent aglyca, and they are widely spread throughout the plant kingdom [36]. While there are many saponins derived from triterpenoids such as betulinic, glycyrrhetinic and other triterpenoic acids, to the best of our knowledge, there have been no reports for boswellic acid glycosides.

2. Results and discussion

2.1. Chemistry

Frankincense was purchased from different local suppliers; extraction of the finely grounded resin followed by oxidation and acetylation [20] allowed the convenient isolation of **AKBA** on a larger preparative scale. Due to incomplete oxidation, 3-acetyl-β-boswellic acid (**ABA**, **4**) was obtained as a side product.

Reaction of **2** with oxalyl chloride and ethyl glycinate (Scheme 1) afforded **5** in 88% yield, while the reaction of **2** with oxalyl chloride and methyl 3-aminopropionate furnished **6** in 64%. Under similar conditions dimethylglutamate yielded **7**. This compound is characterized in its ^{13}C NMR spectrum by the presence of two additional signals for carbonyl groups at $\delta = 173.4$ and 172.4 ppm for the dimethylester; the carbonyl group of the α,β-unsaturated system at C-11 was detected at $\delta = 190.0$ ppm. Under similar conditions, the amino alcohol derivatives **8** and **9** were obtained from **AKBA** and **KBA** respectively in good yields.

KBA (**3**) served also as a precursor for the synthesis of the monodesmosidic saponins. Thus, **KBA** was transformed (Scheme 2) into the corresponding benzylester **10** [25,37]. Glycosylation of **10** with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl trichloracetimidate or 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl trichloracetimidate [38,39] in the presence of $BF_3 \cdot Et_2O$ [40] at −25 °C gave a 52% yield of **11** and 48% of **12**, respectively. Lower yields, however, were achieved with trimethylsilyl trifluormethanesulfonate [40] as a catalyst under various conditions; this parallels our previous findings for the synthesis of glycyrrhetinic acid derived saponins [41]. Similar yields were obtained when Königs-Knorr conditions were applied for the synthesis of these saponins.

The anomeric configuration of the glycosides was deduced from their ^1H NMR spectra. For *gluco*-configured **11** a coupling constant $J_{H-1',H-2'} = 8.0$ Hz was detected being typical for a β-D-configuration of the anomeric center. For the galactosyl derivative **12** the signal for the anomeric proton H−1′ was detected as a dublet at $\delta = 4.46$ ppm ($J_{H-1',H-2'} = 8.0$ Hz) – hence further supports the presence of a β-configuration at the anomeric center.

Debenzylation of **11** with ammonium formate in methanol in the presence of Pd/C [42–44] gave a 79% yield of **13**; similarly, from **12** debenzylated **14** was obtained. Both compounds were deacetylated under Zemplén conditions to afford **15** and **16** in almost quantitative yield.

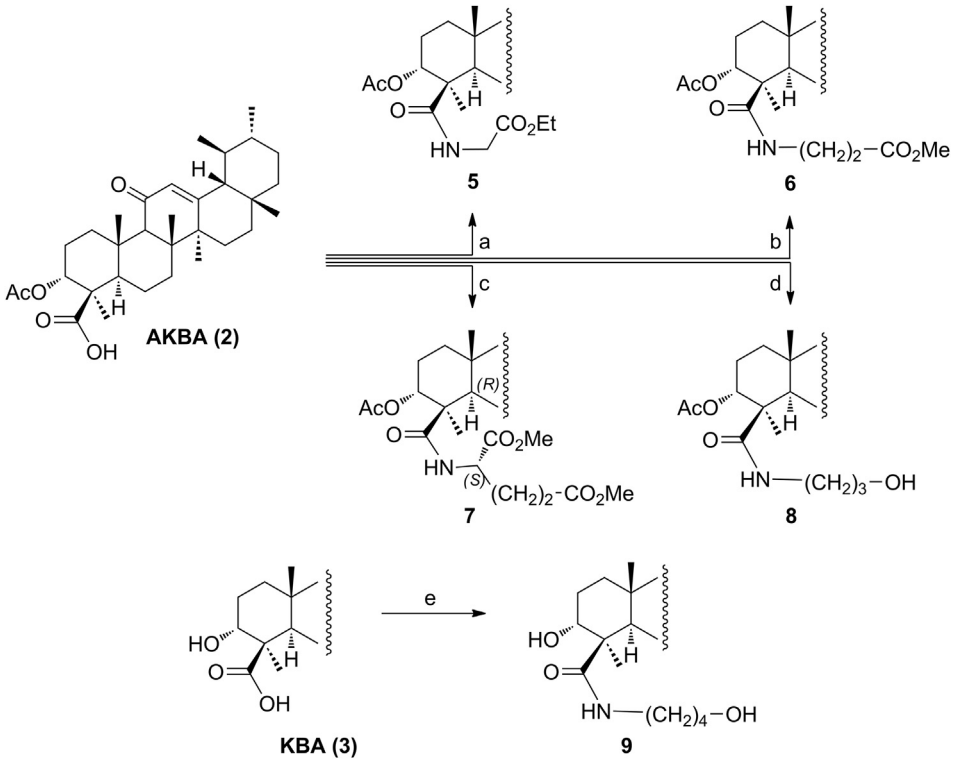
2.2. Biology

The in vitro cytotoxic activity of triterpenes **5**–**9** and of the saponins **15** and **16** was evaluated against a panel of different human tumor cell lines using a photometric sulforhodamine B assay (SRB) [45]. The results of these assays are summarized in Table 1. **AKBA** and **KBA** were used as positive controls in these assays. Compounds with IC_{50} values >100 μM were considered inactive. As a result, lowest cytotoxicity was established for **KBA**. Acetylation at position C-3 (→ **AKBA**) improved cytotoxicity significantly; depending on the human tumor cell line ratios $IC_{50,KBA}/IC_{50,AKBA} = 2.06$ (MCF-7, human breast adenocarcinoma) to 3.97 (A431, human epithelial cervical carcinoma) were determined. Loss of the protecting group at C-3 led to a lowered cytotoxicity – thus **9** always showed higher IC_{50} values than acetylated **8**. Transformation of the carboxyl group C-24 of **AKBA** into an amino acid derived amide did not significantly alter the cytotoxicity of the compounds. These derivatives showed the same IC_{50} values as **AKBA**. Glycosidation at position C-3 led to compounds **15** and **16**: while *gluco*-configured **R52** showed slightly improved IC_{50} values (as compared to **AKBA**), slightly increased IC_{50} values were obtained for *galacto*-configured **16**. The best result in this series of compounds was obtained for hydroxypropyl substituted **8**. For this compound in MCF-7 human breast cancer cells an IC_{50} value was as low as 4.5 μM.

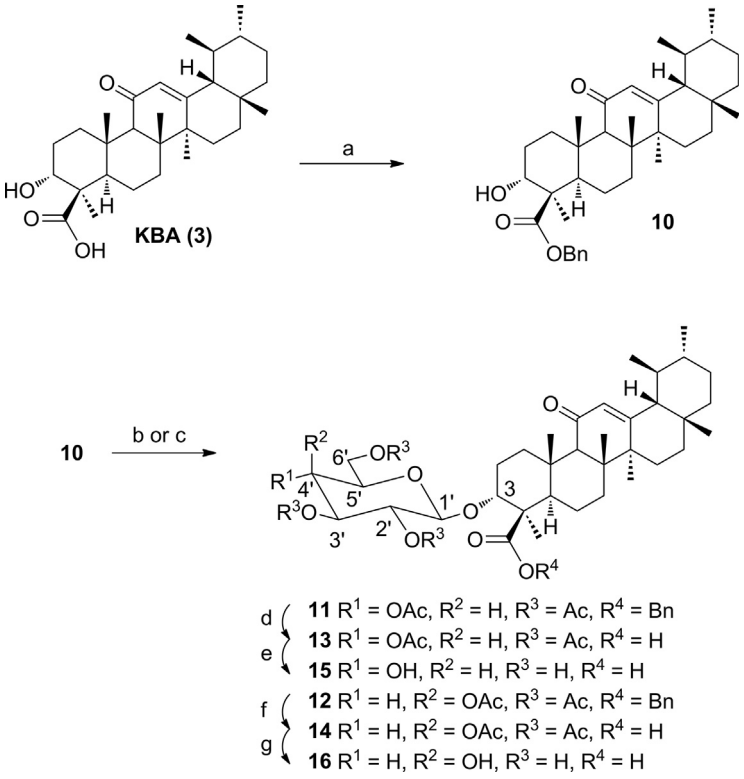
Several triterpenoids induce apoptosis, and to evaluate the anticancer activity of our most active compound, compound **8** was tested for an induction of apoptosis using a DNA-laddering assay. During apoptosis, endonucleases cleave DNA into smaller fragments that were stained and finally detected by gel electrophoresis as "ladders". Thus, floating A2780 cancer cells (obtained after treatment with IC_{90}-concentrations of **8** for 24 h) were analyzed by DNA gel electrophoresis, and the typical DNA ladders were found (Fig. 2). In addition, a trypan blue dye exclusion test was performed to distinguish between cells having died by apoptosis or by necrosis. As depicted in Fig. 2, the presence of an intact cell membrane in A2780 human ovarian cancer cells in most of the cells (having been treated with an IC_{90} concentration of **8** for 24 h) confirmed that this compound is able to trigger apoptosis.

3. Conclusion

Here we prepared several amide derivatives and monodesmosidic saponins from **KBA**. All synthesized compounds showed increased cytotoxicity towards a broad panel of human tumor cell lines. The presence of a free hydroxyl group at position C-3 seems to lower cytotoxicity while the presence of an amide function at C-24 improves cytotoxicity. Monodesmosidic saponins of **KBA** are as cytotoxic as **AKBA**. The most cytotoxic compound of this series gave IC_{50} values as low as 4.5 μM and the cell death proceeded mainly by apoptosis as demonstrated by DNA laddering experiments and a trypan blue dye exclusion test.



Scheme 1. Synthesis of derivatives **5**–**9**: a) $(COCl)_2$, DCM, 25 °C, 30 min; ethyl glycinate hydrochloride, NEt_3, 25 °C, 12 h, 88%; b) $(COCl)_2$, DCM, 25 °C, 30 min; methyl 3-aminopropionate hydrochloride, NEt_3, 25 °C, 12 h, 64%; c) $(COCl)_2$, DCM, 25 °C, 30 min; dimethyl L-glutamate, pyridine, 25 °C, 12 h, 44%; d) $(COCl)_2$, DCM, 25 °C, 30 min; 3-aminopropanol, pyridine, 25 °C, 12 h, 86%; e) $(COCl)_2$, DCM, 25 °C, 30 min; 4-aminobutanol, pyridine, 25 °C, 12 h, 78%.



Scheme 2. Synthesis of monodesmosidic saponins **15** and **16**: a) ref [25,37]; b) 2,3,4,6-tetra-*O*-acetyl-α-D-*gluco*-pyranosyl trichloroacetimidate, DCM, $BF_3 \cdot Et_2O$, −25 °C, 3 days, 52%; c) 2,3,4,6-tetra-*O*-acetyl-α-D-*galacto*-pyranosyl trichloroacetimidate, DCM, $BF_3 \cdot Et_2O$, −25 °C, 3 days, 48%; d) $NH_4^+ HCO_2^-$, Pd/C, MeOH, reflux, 5 h, 79%; e) MeOH, NaOMe (catal.), 25 °C, 5 h, 91%; f) $NH_4^+ HCO_2^-$, Pd/C, MeOH, reflux, 5 h, 61%; e) MeOH, NaOMe (catal.), 25 °C, 5 h, 96%.

Table 1
Cytotoxicity of β-KBA, β-AKBA and several derivatives (IC_{50} values in μM from SRB assays after 96 h of treatment; the values are averaged from three independent experiments performed each in triplicate; confidence interval CI = 95%). The cell lines are human cancer cell lines: 518A2 (melanoma), A431 (epithelial carcinoma), DLD1 (colorectal adenocarcinoma), HT29 (colorectal adenocarcinoma), HCT8 (ileocecal adenocarcinoma), SW480 (colorectal adenocarcinoma), 8505C (thyroid carcinoma), A549 (alveolar basal epithelial adenocarcinoma), FaDu (hypopharyngeal squamous carcinoma), A253 (submandibular carcinoma), A2780 (cis-platin resistant ovarian cancer).

Compound/Cell line	AKBA	KBA	5	6	7	8	9	15	16
518A2	20.5 ± 1.1	48.5 ± 1.3	16.1 ± 0.9	17.6 ± 0.7	15.2 ± 1.6	14.0 ± 1.7	>100	11.0 ± 0.8	14.8 ± 1.1
A431	19.7 ± 1.4	78.3 ± 1.3	19.7 ± 2.0	13.8 ± 1.9	11.1 ± 1.1	7.7 ± 0.9	18.5 ± 2.1	9.3 ± 1.2	11.4 ± 1.1
DLD1	20.9 ± 1.9	57.7 ± 1.7	24.4 ± 1.5	17.7 ± 1.9	13.9 ± 1.7	11.0 ± 0.9	85.6 ± 3.1	19.7 ± 1.4	20.5 ± 1.2
HT29	19.4 ± 1.1	71.3 ± 2.8	14.0 ± 1.6	14.8 ± 2.2	13.6 ± 1.6	10.7 ± 1.9	43.9 ± 0.7	17.1 ± 0.5	19.5 ± 1.8
HCT8	17.5 ± 1.3	44.6 ± 2.5	17.9 ± 2.0	12.8 ± 1.7	12.4 ± 1.7	8.1 ± 0.4	16.1 ± 0.6	17.2 ± 1.4	19.3 ± 2.4
SW480	27.2 ± 2.6	67.9 ± 3.7	16.8 ± 2.3	16.8 ± 1.7	15.2 ± 1.1	15.9 ± 2.3	20.6 ± 1.7	15.1 ± 0.9	17.4 ± 1.5
8505C	17.9 ± 1.4	39.7 ± 2.6	14.8 ± 2.0	11.2 ± 1.1	10.4 ± 1.2	9.0 ± 0.4	15.5 ± 1.1	19.9 ± 2.3	21.5 ± 2.5
A549	24.6 ± 2.4	82.1 ± 4.3	17.8 ± 2.0	16.4 ± 1.9	10.8 ± 1.1	8.5 ± 0.7	>100 ± 1.3	18.7 ± 2.3	19.4 ± 1.1
FaDu	21.6 ± 1.8	56.9 ± 3.5	18.9 ± 2.1	16.0 ± 1.7	11.4 ± 0.9	14.9 ± 1.3	>100	17.1 ± 2.0	20.0 ± 2.3
MCF-7	17.4 ± 1.7	35.8 ± 3.2	15.7 ± 2.7	11.3 ± 1.1	5.5 ± 0.2	4.5 ± 0.3	18.4 ± 1.1	18.2 ± 1.3	19.7 ± 2.4
A253	16.9 ± 1.5	45.9 ± 3.8	15.1 ± 1.1	14.9 ± 1.2	11.6 ± 1.1	7.2 ± 0.6	21.1 ± 1.9	16.9 ± 2.5	18.4 ± 1.7
A2780	14.4 ± 2.0	37.9 ± 3.6	18.3 ± 1.3	13.1 ± 1.7	11.9 ± 2.0	5.9 ± 0.3	13.7 ± 1.9	17.2 ± 1.9	19.5 ± 2.1

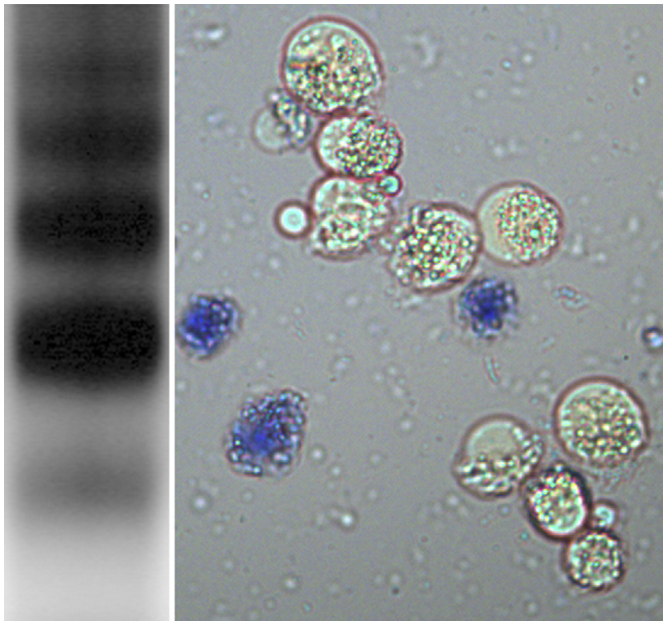


Fig. 2. DNA laddering experiments and trypan blue assays: A2780 cells were treated with IC_{90} concentrations of **8** for 24 h: left: result from the DNA gel electrophoresis (showing a typical DNA ladder); right: trypan blue dye exclusion assay (blue colored cells have a disrupted cell membrane). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Experimental part

4.1. General – chemistry

Melting points are uncorrected (*Leica* hot stage microscope), NMR spectra were recorded using the Varian spectrometers Gemini 2000 or Unity 500 (δ given in ppm, J in Hz, internal Me_4Si), MS spectra were taken on a Finnigan MAT LCQ 7000 (electrospray, voltage 4.5 kV, sheath gas nitrogen) instrument. The optical rotation was measured on a Perkin–Elmer polarimeter at 20 °C; TLC was performed on silica gel (Merck 5554); elemental analyses were performed on a Vario EL (CHNS). The solvents were dried according to usual procedures. Frankincense was obtained from different commercial suppliers in bulk quantities; compounds **AKBA** and KBA were isolated from frankincense, 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate, 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate [38,39] and benzyl (3α) 3-hydroxy-11-oxours-12-en-24-oate (**10**) [25] were prepared as previously reported. The purity of the compounds were determined by HPLC and found to be >98%.

4.2. General – biological screening

4.2.1. Cytotoxicity assay

This assay was performed as previously described [46–48]. The IC_{50} was estimated by linear regression between the value before and after the 50% line is crossed in a dose–response curve.

4.2.2. Apoptosis test – DNA laddering and trypan blue staining

These assays were performed as described previously [25].

4.3. Ethyl N-[(3α)-3-(acetyloxy)-11,24-dioxours-12-en-24-yl]-glycidate (**5**)

A solution of **AKBA** (100 mg, 0.2 mmol) and oxalyl chloride (50 mg, 0.4 mmol) in dry DCM (10 mL) was stirred at 25 °C for 30 min. The solvents were removed under diminished pressure, and the residue was dissolved in dry DCM (15 mL). After cooling to 0 °C, triethylamine (50 mg, 0.5 mmol) and ethyl glycinate (hydrochloride, 28 mg, 0.2 mmol) were added, and the mixture was stirred at 25 °C for 12 h. The solvents were removed under diminished pressure, and the residue was subjected to column chromatography (silica gel, hexane/ethyl acetate, 95:5 → 90:10 → 80:20) to yield **5** (105 mg, 88%) as a colorless solid; m.p. 221–224 °C; $[\alpha]_D = 75.9°$ ($c = 4.86$, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 6.07$ (t, $J = 4.6$ Hz, 1H, N*H*), 5.52 (s, 1H, H-12), 5.32 (dd, $J = 2.5$, 2.9 Hz, 1H, H-3), 4.19 (q, $J = 7.1$ Hz, 2H, H-35), 4.00 (d, $J = 4.6$ Hz, 2H, H-33), 2.51 (ddd, $J = 13.3$ Hz, 3.3, 3.3 Hz, 1H, H-1b), 2.39 (s, 1H, H-9), 2.29 (m, 1H, H-2a), 2.07 (ddd, 1H, H-16a, $J = 13.7$ Hz, 5.0, 13.7 Hz), 2.05 (s, 3H, H-32), 1.88 (m, 1H, H-6a), 1.80 (ddd, 1H, H-15a, $J = 13.7$ Hz, 5.0 Hz, 13.7 Hz), 1.74 (m, 1H, H-6b), 1.71 (ddd, 1H, H-7a, $J = 12.9$ Hz, 3.7 Hz, 12.9 Hz), 1.61 (m, 1H, H-2b), 1.52 (d, 1H, H-18, $J = 11.2$ Hz), 1.49 (m, 1H, H-7b), 1.46 (m, 1H, H-22b), 1.42 (m, 2H, H-21), 1.39 (m, 1H, H-19), 1.38 (dd, 1H, H-5, $J = 2.1$ Hz, 12.9 Hz), 1.32 (s, 3H, H-27), 1.30 (m, 1H, H-22a), 1.26 (t, 3H, H-36, $J = 7.1$ Hz), 1.20 (m, 1H, H-1a, 1H, H-15b), 1.18 (s, 3H, H-26), 1.16 (s, 3H, H-23), 1.08 (s, 3H, H-25), 0.99 (ddd, 1H, H-16b, $J = 13.7$ Hz, 2.0 Hz, 2.5 Hz), 0.94 (m, 1H, H-20), 0.92 (s, 3H, H-30), 0.79 (s, 3H, H-28), 0.77 (d, 3H, H-29, $J = 6.6$ Hz) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 199.3$ (C-11), 182.5 (C-24), 170.5 (C-31), 165.0 (C-13), 137.9 (C-34), 130.5 (C-12), 70.6 (C-3), 60.6 (C-35), 60.5 (C-9), 59.1 (C-18), 48.9 (C-5), 47.3 (C-4), 45.2 (C-8), 43.9 (C-14), 41.0 (C-22), 39.4 (C-19), 39.4 (C-20), 38.2 (C-33), 37.6 (C-10), 34.0 (C-1), 34.0 (C-17), 33.0 (C-7), 31.0 (C-21), 28.9 (C-28), 27.6 (C-16), 27.3 (C-15),

26.3 (C-2), 24.4 (C-23), 21.2 (C-32), 21.2 (C-30), 20.6 (C-27), 18.9 (C-6), 18.5 (C-26), 17.5 (C-29), 14.3 (C-36), 13.3 (C-25) ppm; MS (ESI, methanol) $C_{36}H_{55}NO_6$: *m/z* = 598.3 ([M+H]$^+$, 100%), 620.5 ([M+Na]$^+$, 75%); analysis calcd for $C_{36}H_{55}NO_6$ (597.83): C 72.33, H 9.27, N 2.34; found: C 72.11, H 9.32, N 2.21.

4.4. Methyl N-[(3α)-3-(acetyloxy)-11,24-dioxours-12-en-24-yl]-β-alaninate (**6**)

Following the procedure given for **5**, from **AKBA** (200 mg, 0.4 mmol), oxalyl chloride (76 mg, 0.6 mmol), methyl 3-aminopropionate (hydrochloride, 56 mg, 0.4 mmol) and triethylamine (101 mg 1.0 mmol) followed by column chromatography (silica gel, hexane/EtOAc, 98:2, 4:1) **6** (153 mg, 64%) was obtained as a colorless solid; m.p. 204–206 °C; $[\alpha]_D = 77.1°$ (*c* = 3.56, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.16 (*t*, 1H, N*H*, *J* = 5.9 Hz), 5.53 (*s*, 1H, H-12), 5.31 (*dd*, *J* = 2.7, 2.7 Hz, 1H, H-3), 3.66 (*s*, 3H, H-36), 3.53 (*m*, 1H, H-33), 3.43 (*m*, 1H, H-33), 2.56 (*m*, 1H, H-34), 2.50 (*m*, 1H, H-1b, 1H, H-34), 2.39 (*s*, 1H, H-9), 2.28 (*m*, 1H, H-2a), 2.08 (*ddd*, *J* = 13.7, 4.9, 13.7 Hz, 1H, H-16a), 2.05 (*s*, 3H, H-32), 1.87 (*ddd*, *J* = 13.7, *J* = 4.9, 13.7 Hz, 1H, H-15a), 1.74 (*m*, 2H, H-6), 1.68 (*m*, 1H, H-7a), 1.59 (*m*, 1H, H-2b), 1.52 (*dd*, *J* = 11.3, 1.2 Hz, 1H, H-18), 1.48 (*m*, 1H, H-7b), 1.46 (*m*, 1H, H-22b), 1.44 (*m*, 2H, H-21), 1.39 (*m*, 1H, H-19), 1.36 (*m*, 1H, H-5), 1.34 (*m*, 1H, H-22a), 1.32 (*s*, 3H, H-27), 1.20 (*m*, 2H, H-1a, H-15b), 1.16 (*s*, 3H, H-26), 1.10 (*s*, 3H, H-23), 1.05 (*s*, 3H, H-25), 1.00 (*ddd*, *J* = 13.7, 2.1, 2.7 Hz, 1H, H-16b), 0.93 (*m*, 1H, H-20), 0.92 (*s*, 3H, H-30), 0.80 (*s*, 3H, H-28), 0.78 (*d*, *J* = 6.4 Hz, 3H, H-29) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.0 (C-11), 175.3 (C-24), 173.6 (C-34), 170.1 (C-31), 164.7 (C-13), 130.5 (C-12), 73.6 (C-3), 60.3 (C-9), 59.0 (C-18), 51.8 (C-36), 50.2 (C-5), 46.6 (C-4), 45.0 (C-8), 43.7 (C-14), 40.9 (C-22), 39.3 (C-19), 39.2 (C-20), 37.5 (C-10), 34.9 (C-1), 34.8 (C-33), 33.9 (C-17), 33.1 (C-7), 33.0 (C-34), 30.9 (C-21), 28.8 (C-28), 27.5 (C-16), 27.2 (C-15), 24.7 (C-23), 23.8 (C-2), 21.3 (C-30), 21.1 (C-32), 20.5 (C-27), 19.1 (C-6), 18.2 (C-26), 17.4 (C-29), 13.2 (C-25) ppm; MS (ESI, methanol) $C_{36}H_{55}NO_6$: *m/z* = 598.3 ([M+H]$^+$, 90%), 620.5 ([M+Na]$^+$, 40%); analysis calcd for $C_{36}H_{55}NO_6$ (597.82): C 72.33, H 9.27, N 2.34; found: C 72.09, H 9.41, N 2.19.

4.5. Dimethyl N-[(3α)-3-(acetyloxy)-11,24-dioxours-12-en-24-yl]-L-glutamate (**7**)

Following the procedure given for **5**, from **AKBA** (200 mg, 0.4 mmol), oxalyl chloride (76 mg, 0.6 mmol), dimethyl L-glutamate (593 mg, 4.0 mmol) and dry pyridine (3 mL) followed by column chromatography (hexane/EtOAc, 7:3) **7** (125 mg, 44%) was obtained as a colorless solid; m.p. 93–95 °C; $[\alpha]_D = 79.7°$ (*c* = 4.2, $CHCl_3$); ^{1}H (400 MHz, $CDCl_3$): δ = 6.29 (*d*, *J* = 7.5 Hz, 1H, N*H*), 5.52 (*s*, 1H, H-12), 5.32 (*dd*, *J* = 2.5, 2.9 Hz, 1H, H-3), 4.63 (*ddd*, *J* = 5.0, 7.5, 7.5 Hz, 1H, NCH), 3.71 + 3.65 (*s*, 6H, 2 OC*H*$_3$), 2.50 (*ddd*, *J* = 13.3, 3.3 Hz, 3.3 Hz, 1H, H-1b), 2.39 (*s*, 1H, H-9), 2.19 (*m*, 1H, H-2a), 2.15 (*m*, 2H, H-34), 2.10 (*m*, 2H, H-35), 2.08 (*ddd*, *J* = 13.7, 5.0, 13.7 Hz, 1H, H-16a), 2.06 (*s*, 3H, H-32), 1.94 (*m*, 1H, H-6b), 1.88 (*ddd*, *J* = 13.7, 5.0, 13.7 Hz, 1H, H-15a), 1.78 (*m*, 1H, H-6a), 1.71 (*ddd*, *J* = 12.5, 3.7, 12.5 Hz, 1H, H-7a), 1.58 (*m*, 1H, H-2b), 1.52 (*dd*, *J* = 11.2, *J* = 1.2 Hz, 1H, H-18), 1.47 (*m*, 2H, H-22), 1.45 (*m*, 2H, H-21), 1.40 (*m*, 1H, H-7b), 1.39 (*m*, 1H, H-19), 1.39 (*dd*, *J* = 1.7, 12.5 Hz, 1H, H-5), 1.33 (*s*, 3H, H-27), 1.20 (*s*, 3H, H-26), 1.18 (*m*, 1H, H-1a, 1H, H-15b), 1.14 (*s*, 3H, H-23), 1.05 (*s*, 3H, H-25), 1.01 (*ddd*, *J* = 13.3, *J* = 2.1, 2.5 Hz, 1H, H-16b), 0.93 (*s*, 3H, H-30), 0.92 (*m*, 1H, H-20), 0.80 (*s*, 3H, H-28), 0.78 (*d*, *J* = 6.6 Hz, 3H, H-29) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.0 (C-11), 175.4 (C-24), 173.4 + 172.4 (C-36 + C-38), 170.1 (C-31), 164.6 (C-13), 130.5 (C-12), 73.5 (C-3), 60.3 (C-9), 59.0 (C-18), 52.5 + 51.8 (C-37 + C-39), 51.6 (C-33), 50.4 (C-5), 46.7 (C-4), 45.0 (C-8), 43.7 (C-14), 40.9 (C-22), 39.3 (C-19), 39.2 (C-20), 37.4 (C-10), 34.8 (C-1), 33.9 (C-17), 33.1 (C-7), 30.9 (C-21), 30.0 (C-34), 28.8 (C-28), 27.5 (C-16), 27.2 (C-15), 27.2 (C-35), 24.4 (C-23), 23.8 (C-2), 21.3 (C-30), 21.1 (C-32), 20.5 (C-27), 19.0 (C-6), 18.2 (C-26), 17.4 (C-29), 13.1 (C-25) ppm; MS (ESI, methanol) $C_{39}H_{59}NO_8$: *m/z* = 670.2 ([M+H]$^+$, 100%), 692.3 ([M+Na]$^+$, 33%), 1361.1 ([M_2Na]$^+$, 30%); analysis calcd for $C_{39}H_{59}NO_8$ (669.89): C 69.92, H 8.88, N 2.09; found: C 69.71, H 8.97, N 1.96.

4.6. (3α)-24-[(3-hydroxypropyl)amino]-11,24-dioxours-12-en-3-yl]-acetate (**8**)

Following the procedure given for **5**, from **AKBA** (200 mg, 0.4 mmol), oxalyl chloride (76 mg, 0.6 mmol), 3-aminopropanol (300 mg, 4.0 mmol) and dry pyrdine (3 mL) followed by chromatography (silica gel, EtOAc/methanol, 95:5) **8** (195 mg, 86%) was obtained as an off-white solid; m.p. = 207–209 °C; $[\alpha]_D = 72.6°$ (*c* = 5.82, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.03 (*t*, *J* = 5.8 Hz, 1H, N*H*), 5.53 (*s*, 1H, H-12), 5.28 (*dd*, *J* = 2.5, 2.9 Hz, 1H, H-3), 3.64 (*t*, *J* = 5.6 Hz, 2H, H-35), 3.45 (*m*, 1H, H-33), 3.33 (*m*, 1H, H-33), 2.52 (*ddd*, *J* = 13.3 Hz, 2.9, 3.7 Hz, 1H, H-1b), 2.39 (*s*, 1H, H-9), 2.26 (*m*, 1H, H-2a), 2.08 (*ddd*, *J* = 13.7 Hz, 5.0, 13.7 Hz, 1H, H-16a), 2.06 (*s*, 3H, H-32), 1.87 (*ddd*, *J* = 13.7, 5.0, 13.7 Hz, 1H, H-15a), 1.82 (*m*, 1H, H-6a), 1.74 (*m*, 1H, H-6b), 1.69 (*m*, 1H, H-7a, 2H, H-34), 1.59 (*m*, 1H, H-2b), 1.52 (*d*, *J* = 10.8 Hz, 1H, H-18), 1.47 (*m*, 1H, H-7b, 1H, H-22b), 1.42 (*m*, 2H, H-21), 1.39 (*m*, 1H, H-19), 1.37 (*dd*, *J* = 2.1, 12.9 Hz, 1H, H-5), 1.33 (*s*, 3H, H-27), 1.29 (*m*, 1H, H-22a), 1.19 (*m*, 1H, H-1a, 1H, H-15b), 1.18 (*s*, 3H, H-26), 1.14 (*s*, 3H, H-23), 1.12 (*s*, 3H, H-25), 1.00 (*ddd*, *J* = 13.7, 2.1, 2.5 Hz, 1H, H-16b), 0.93 (*s*, 3H, H-30), 0.92 (*m*, 1H, H-20), 0.80 (*s*, 3H, H-28), 0.78 (*d*, *J* = 6.2 Hz, 3H, H-29) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.0 (C-11), 176.2 (C-24), 170.2 (C-31), 164.7 (C-13), 130.5 (C-12), 73.4 (C-3), 60.3 (C-9), 60.2 (C-35), 59.0 (C-18), 50.4 (C-5), 46.7 (C-4), 45.0 (C-8), 43.7 (C-14), 40.9 (C-22), 39.3 (C-19), 39.2 (C-20), 37.4 (C-33), 36.9 (C-10), 34.8 (C-1), 33.9 (C-17), 33.1 (C-7), 31.8 (C-34), 30.9 (C-21), 28.8 (C-28), 27.5 (C-16), 27.2 (C-15), 24.9 (C-23), 23.9 (C-2), 21.3 (C-30), 21.1 (C-32), 20.5 (C-27), 19.4 (C-6), 18.3 (C-26), 17.4 (C-29), 13.4 (C-25) ppm; MS (ESI, methanol) $C_{35}H_{55}NO_5$: *m/z* = 570.3 ([M+H]$^+$, 100%), 592.4 ([M+Na]$^+$, 20%); analysis calcd for $C_{35}H_{55}NO_5$ (569.81): C 73.77, H 9.73, N 2.46; found: C 73.51, H 9.98, N 2.27.

4.7. (3α) 3-hydroxy-N-(4hydroxybutyl)-11-oxours-12-en-24-amide (**9**)

Following the procedure given for **5**, from **KBA** (188 mg, 0.4 mmol), oxalyl chloride (76 mg, 0.6 mmol), 4-aminobutanol (360 mg, 4.0 mmol) and dry pyridine (3 mL) followed by column chromatography (silica gel, EtOAc/methanol, 95:5) **9** (168 mg, 78%) was obtained as a colorless solid; m.p. 239–241 °C; $[\alpha]_D = 123.5°$ (*c* = 5.46, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.68 (*t*, 1H, N*H*, *J* = 5.8 Hz), 5.52 (*s*, 1H, H-12), 4.08 (*dd*, *J* = 2.5, 2.9 Hz, 1H, H-3), 3.65 (*t*, *J* = 6.0 Hz, 2H, H-34), 3.25 (*m*, 2H, H-31), 2.47 (*ddd*, *J* = 13.3, *J* = 3.3, 4.2 Hz, 1H, H-1b), 2.41 (*s*, 1H, H-9), 2.37 (*m*, 1H, H-2a), 2.07 (*ddd*, *J* = 13.7, *J* = 5.0, 13.7 Hz, 1H, H-16a), 1.85 (*ddd*, *J* = 13.7, 5.0, 13.7 Hz, 1H, H-15a), 1.76 (*m*, 2H, H-6), 1.72 (*m*, 1H, H-7a), 1.58 (*m*, 2H, H-21, 2H, H-32), 1.55 (*m*, 1H, H-2b), 1.51 (*dd*, *J* = 10.8, 1.2 Hz, 1H, H-18), 1.48 (*m*, 1H, H-7b, 1H, H-22b), 1.46 (*dd*, *J* = 2.1, 9.1 Hz, 1H, H-5), 1.44 (*m*, 2H, H-33), 1.39 (*m*, 1H, H-19), 1.31 (*m*, 1H, H-1a), 1.29 (*s*, 3H, H-27), 1.28 (*m*, 1H, H-22a), 1.23 (*s*, 3H, H-23), 1.19 (*m*, 1H, H-15b), 1.16 (*s*, 3H, H-26), 1.10 (*s*, 3H, H-25), 1.00 (*ddd*, *J* = 13.3, *J* = 2.1, 2.5 Hz, 1H, H-16b), 0.92 (*s*, 3H, H-30), 0.91 (*m*, 1H, H-20), 0.80 (*s*, 3H, H-28), 0.77 (*d*, *J* = 6.6 Hz, 3H, H-29) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.3 (C-11), 176.5 (C-24), 164.7 (C-13), 130.5 (C-12), 70.7 (C-3), 62.3 (C-34), 60.4 (C-9), 59.0 (C-18), 48.7 (C-5), 47.3 (C-4), 45.0 (C-8), 43.8 (C-14), 40.9 (C-22), 39.3 (C-19), 39.2 (C-20), 39.2 (C-31), 37.5 (C-10), 34.2 (C-1), 33.9 (C-17), 33.2 (C-7), 32.8 (C-33), 30.9 (C-21), 28.8 (C-28), 27.5 (C-16), 27.1 (C-15), 26.6 (C-2), 25.9 (C-32), 25.2 (C-23), 21.1 (C-30), 20.5 (C-27), 19.6 (C-6), 18.3 (C-26), 17.4 (C-29), 13.5

(C-25) ppm; MS (ESI, methanol) $C_{34}H_{55}NO_4$: $m/z = 542.4$ ($[M+H]^+$, 100%; analysis calcd for $C_{34}H_{55}NO_4$ (541.80): C 75.37, H 10.23, N 2.59; found: C 75.19, H 10.39, N 2.33.

4.8. Benzyl (3α)-3-(2,3,4,6-tetra-O-acetyl-β-D-gluco-pyranosyloxy)-11-oxours-12-en-24-oate (**11**)

To a solution of **10** (1.53 g, 2.72 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-*gluco*-pyranosyl trichloroacetimidate (1.50 g, 3.04 mmol) in dry DCM (10 mL), $BF_3 \cdot Et_2O$ (350 μL, 1.2 mmol) was very slowly added at −25 °C, and stirring at −25 °C was continued for 3 days. The reaction was quenched by the addition of $NaHCO_3$ (250 mL). Usual aqueous work-up followed by column chromatography (silica gel, hexane/ether/1% AcOH, 50:50), gave **11** (1.40 g, 52%) as a colorless solid; m.p. 84–86 °C; $[\alpha]_D = 44.7°$ ($c = 0.30$, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.35$ (*m*, 5H, benzyl), 5.52 (*s*, 1H, H-12), 5.25–5.10 (*m*, 2H, H-31, H-3′), 5.11–4.95 (*m*, 3H, H-31, H-2′, H-4′), 4.53 (*d*, $J = 8.0$ Hz, 1H, H-1′), 4.25–4.12 (*dd*, $J = 4.0$, 12.0 Hz, 2H, H-6′), 4.00 (*s*, 1H, H-3), 3.67–3.57 (*m*, 1H, H-5′), 2.41 (*d*, $J = 12.0$ Hz, 1H, H-1b), 2.28 (*s*, 1H, H-9), 2.10–2.02 (*m*, 2H, H-16a, H-2a), 2.00 (*s*, 3H, acetyl), 1.99 (*s*, 3H, acetyl), 1.96 (*s*, 3H, acetyl), 1.95 (*s*, 3H, acetyl), 1.90–1.82 (*m*, 1H, H-15b), 1.78–1.61 (*m*, 3H, H-6, H-7a), 1.55–1.45 (*m*, 2H, H-2a, H-18), 1.46–1.32 (*m*, 4H, H-7b, H-19, H-21b, H-22a), 1.30 (*bs*, 5H, H-5, H-22b, H-27), 1.25 (*bs*, 4H, H-21a, H-23), 1.20–1.14 (*m*, 1H, H-15a), 1.11 (*s*, 3H, H-26), 1.05–0.95 (*m*, 2H, H-1a, H-16b), 0.93 (*s*, 3H, H-25), 0.91 (*bs*, 4H, H-20, H-30), 0.82 (*s*, 3H, H-28), 0.77 (*d*, $J = 4.0$ Hz, 3H, H-29) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 199.6$ (C-11), 176.3 (C-24), 170.5 (acetyl), 170.3 (acetyl), 169.4 (acetyl), 169.2 (acetyl), 165.1 (C-13), 136.7 (C-32), 130.3 (C-12), 128.5 (aromat), 128.2 (aromat), 128.0 (aromat), 98.1 (C-1′), 77.8 (C-3), 72.5 (C-3′), 71.1 (C-5′), 71.5 (C-2′), 68.8 (C-4′), 66.2 (C-31), 62.4 (C-6′), 60.3 (C-9), 59.1 (C-18), 49.1 (C-5), 47.2 (C-4), 45.5 (C-8), 43.7 (C-14), 40.6 (C-22), 39.0 (C-19 + C-20), 37.2 (C-10), 34.2 (C-17), 33.4 (C-1), 32.5 (C-7), 30.9 (C-21), 28.8 (C-28), 27.3 (C-16), 27.0 (C-15), 24.1 (C-23), 22.1 (C-2), 21.3 (C-30), 20.5 (acetyl), 20.4 (acetyl), 20.3 (acetyl), 20.2 (acetyl), 20.1 (acetyl), 18.7 (C-6), 18.1 (C-26), 17.4 (C-29), 13.4 (C-25); MS (ESI, methanol) $C_{51}H_{70}O_{13}$: $m/z = 891.4$ ($[M+H]^+$, 100%), 913.4 ($[M+Na]^+$, 40%); analysis calcd for $C_{51}H_{70}O_{13}$ (891.09): C 68.74, H 7.92; found: C 68.58, H 8.04.

4.9. Benzyl (3α)-3-(2,3,4,6-tetra-O-acetyl-β-D-galacto-pyranosyloxy)-11-oxours-12-en-24-oate (**12**)

Following the procedure given for **11**, from **10** (1.21 g, 2.16 mmol), 2,3,4,6-tetra-*O*-acetyl-α-D-*galacto*-pyranosyl trichloroacetimidate (1.24 g, 2.52 mmol) followed by column chromatography (silica gel, hexane/ether/1% AcOH, 50:50), **12** (0.92 g, 48%) was obtained as a colorless solid; m.p. 116–118 °C; $[\alpha]_D = 41.8°$ ($c = 1.20$, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.40–7.29$ (*m*, 5H, benzyl), 5.50 (*s*, 1H, H-12), 5.38 (*d*, $J = 3.0$ Hz, 1H, H-4′), 5.21–5.15 (*m*, 2H, H-31, H-2′), 5.05–5.00 (*m*, 2H, H-31, H-3′), 4.45 (*d*, $J = 8.0$ Hz, 1H, H-1′), 4.25–4.07 (*dd*, $J = 4.0$, 12.0 Hz, 2H, H-6′), 4.03 (*s*, 1H, H-3), 3.85 (*t*, $J = 6.7$ Hz, 1H, H-5′), 2.40 (*d*, $J = 12.0$ Hz, 1H, H-1b), 2.30 (*s*, 1H, H-9), 2.15 (*s*, 3H, acetyl), 2.10–2.00 (*m*, 2H, H-16a, H-2b), 1.99 (*s*, 3H, acetyl), 1.98 (*s*, 3H, acetyl), 1.95 (*s*, 3H, acetyl), 1.85 (*dd*, $J = 4.0$, 12.0 Hz, 1H, H-15b), 1.84–1.69 (*m*, 2H, H-6b, H-6a), 1.63 (*dd*, $J = 4.0$, 12.0 Hz, 1H, H-7a), 1.60–1.46 (*m*, 2H, H-2a, H-18), 1.45–1.42 (*m*, 2H, H-22a, H-21b), 1.41–1.36 (*m*, 3H, H-5, H-19, H-7b), 1.33 (*bs*, 3H, H-27), 1.29 (*bs*, 4H, H-23, H-22b), 1.25–1.15 (*m*, 2H, H-15a, H-21a), 1.07 (*s*, 3H, H-26), 1.06–0.98 (*m*, 2H, H-1a, H-16b), 0.95 (*s*, 3H, H-25), 0.94 (*bs*, 4H, H-20, H-30), 0.81 (*s*, 3H, H-28), 0.77 (*d*, $J = 4.0$ Hz, 3H, H-29) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 199.5$ (C-11), 176.4 (C-24), 170.5 (acetyl), 170.4 (acetyl), 170.1 (acetyl), 169.3 (acetyl), 165.1 (C-13), 135.7 (C-32), 130.1 (C-12), 128.6 (benzyl), 128.5 (benzyl), 128.4 (benzyl), 98.7 (C-1′), 77.2 (C-3), 71.0 (C-3′), 70.8 (C-5′), 69.2 (C-2′), 67.2 (C-4′), 66.7 (C-31), 61.3 (C-6′), 60.5 (C-9), 59.1 (C-18), 49.3 (C-5), 47.5 (C-4), 45.0 (C-8), 44.1 (C-14), 41.1 (C-22), 39.4 (C-19 + C-20), 37.0 (C-10), 34.0 (C-17 + C-1), 33.1 (C-7), 31.0 (C-21), 28.6 (C-28), 27.3 (C-16), 27.2 (C-15), 24.5 (C-23), 22.3 (C-2), 21.3 (C-30), 21.1 (acetyl), 20.9 (2 x acetyl), 20.7 (acetyl), 20.4 (C-27), 19.1 (C-6), 18.2 (C-26), 17.4 (C-29), 13.4 (C-25) ppm; MS (ESI, methanol) $C_{51}H_{70}O_{13}$: $m/z = 891.5$ ($[M+H]^+$, 100%), 913.5 ($[M+Na]^+$, 50%); analysis calcd for $C_{51}H_{70}O_{13}$ (891.09): C 68.74, H 7.92; found: C 68.50, H 8.12.

4.10. (3α) 3-(2,3,4,6-tetra-O-acetyl-β-D-gluco-pyranosyloxy)-11-oxours-12-en-24-oic acid (**13**)

To a solution of **11** (0.2 g, 0.22 mmol) in methanol (25 mL) containing Pd/C (0.2 g), ammonium formate (70 mg, 1.13 mmol) was added, and the mixture was heated under reflux for 5 h. The catalyst was filtered off (Celite), the solvent was removed, and the residue subjected to column chromatography (silica gel, hexane/ether 50:50, containing 1% AcOH) to yield **13** (140 mg, 79%) as a colorless solid; m.p. 148–150 °C; $[\alpha]_D = 50.8°$ ($c = 0.30$, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 5.56$ (*s*, 1H, H-12), 5.20 (*t*, $J = 8.0$ Hz, 1H, H-3′), 5.10–4.95 (*m*, 2H, H-4′ + H-2′), 4.52 (*d*, $J = 8.0$ Hz, 1H, H-1′), 4.25–4.10 (*dd*, $J = 4.0$, 12.0 Hz, 2H, H-6′), 3.95 (*s*, 1H, H-3), 3.65–3.60 (*m*, 1H, H-5′), 2.45 (*d*, $J = 12.0$ Hz, 1H, H-1b), 2.30 (*s*, 1H, H-9), 2.13–2.04 (*m*, 5H, H-16a, H-2b, + acetyl), 2.04 (*s*, 3H, acetyl), 1.95 (*s*, 3H, acetyl), 1.94 (*s*, 3H, acetyl), 1.90–1.75 (m, 2H, H-15b, H-6b), 1.72–1.67 (m, 2H, H-6a, H-7a), 1.58–1.45 (*m*, 2H, H-2a, H-18), 1.47–1.36 (*m*, 4H, H-22a, H-21b, H-19, H-7b), 1.34–1.30 (*bs*, 4H, H-5, H-27), 1.29 (*bs*, 2H, H-21a, H-22b), 1.25–1.20 (*m*, 4H, H-23, H-15a), 1.15 (*s*, 3H, H-26), 1.12 (*s*, 3H, H-25), 1.05–0.95 (*m*, 2H, H-1a, H-16b), 0.94 (*bs*, 4H, H-20, H-30), 0.80 (*s*, 3H, H-28), 0.75 (*d*, $J = 8.0$ Hz, 3H, H-29) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 199.8$ (C-11), 182.2 (C-24), 170.8 (acetyl), 170.4 (acetyl), 169.4 (acetyl), 169.0 (acetyl), 165.2 (C-13), 130.5 (C-12), 98.0 (C-1′), 77.1 (C-3), 72.7 (C-3′), 71.4 (C-5′), 71.2 (C-2′), 68.9 (C-4′), 62.0 (C-6′), 60.3 (C-9), 59.0 (C-18), 49.2 (C-5), 46.7 (C-4), 45.0 (C-8), 43.9 (C-14), 40.8 (C-22), 39.0 (C-19 + C-20), 37.2 (C-10), 34.1 (C-17), 33.5 (C-1), 32.7 (C-7), 30.6 (C-21), 28.9 (C-28), 27.5 (C-16), 27.3 (C-15), 24.4 (C-23), 22.1 (C-2), 21.0 (C-30), 20.7 (acetyl), 20.6 (acetyl), 20.5 (acetyl), 20.4 (acetyl), 20.3 (C-27), 18.8 (C-6), 18.3 (C-26), 17.5 (C-29), 13.1 (C-25) ppm; MS (ESI, methanol) $C_{44}H_{64}O_{13}$: $m/z = 823.4$ ($[M+Na]^+$, 100%); analysis calcd for $C_{44}H_{64}O_{13}$ (800.97): C 65.98, H 8.05; found: C 65.74, H 8.19.

4.11. (3α) 3-(2,3,4,6-tetra-O-acetyl-β-D-galacto-pyranosyloxy)-11-oxours-12-en-24-oic acid (**14**)

Following the procedure given for **11**, from **12** (500 mg, 0.56 mmol), Pd/C (10%, 500 mg), ammonium formate (200 mg), **14** (271 mg, 61%) was obtained as a colorless solid; m.p. 164–167 °C; $[\alpha]_D = 43.9°$ ($c = 0.10$, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 5.58$ (*s*, 1H, H-12), 5.39 (*d*, $J = 4.0$ Hz, 1H, H-4′), 5.20 (t, $J = 8.0$ Hz, 1H, H-2′), 5.03 (*dd*, $J = 4.0$, 12.0 Hz, 1H, H-3′), 4.45 (*d*, $J = 8.0$ Hz, 1H, H-1′), 4.19–4.05 (*m*, 2H, H-6′), 3.97 (*s*, 1H, H-3), 3.83 (*t*, $J = 8.0$ Hz, 1H, H-5′), 2.46 (*d*, $J = 12.0$ Hz, 1H, H-1b), 2.35 (*s*, 1H, H-9), 2.15 (*s*, 3H, acetyl), 2.11 (*bs*, 2H, H-16a, H-2b), 2.01 (*s*, 3H, acetyl), 1.97 (*s*, 3H, acetyl), 1.94 (*s*, 3H, acetyl), 1.90–1.80 (*m*, 2H, H-15b, H-6b), 1.75–1.65 (*m*, 2H, H-6a, H-7b), 1.58–1.51 (*m*, 2H, H-1a, H-18), 1.48-1.38 (*m*, 5H, H-22a, H-21b, H-19, H-7a, H-5), 1.36 (*s*, 3H, H-27), 1.34 (*s*, 3H, H-23), 1.31–1.20 (*m*, 3H, H-22b, H-21a, H-15a), 1.18 (*s*, 3H, H-26), 1.11 (*s*, 3H, H-25), 1.08–1.00 (*m*, 2H, H-1a, H-16b), 0.95 (*bs*, 4H, H-30, H-20), 0.80 (*s*, 3H, H-28), 0.78 (*d*, $J = 8.0$ Hz, 3H, H-29) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 199.8$ (C-11), 181.5 (C-24), 170.3 (C-10′, acetyl), 170.2 (acetyl), 170.0 (acetyl), 169.1 (acetyl), 165.1 (C-13), 130.5 (C-12), 98.9 (C-1′)0.77.0 (C-3), 70.7 (C-3′), 70.4 (C-5′ -), 69.0 (C-2′), 67.3 (C-4′), 61.0 (C-6′), 60.6 (C-9), 59.0 (C-18), 48.8 (C-5), 46.7

(C-4), 45.2 (C-8), 43.7 (C-14), 40.7 (C-22), 39.0 (C-19 + C-20), 37.1 (C-10), 34.1 (C-17), 33.6 (C-1), 32.7 (C-7), 30.8 (C-21), 28.8 (C-28), 27.4 (C-16), 27.1 (C-15), 24.5 (C-23), 22.0 (C-2), 21.2 (C-30), 20.7 (2 x acetyl), 20.5 (acetyl + C-27), 20.2 (acetyl), 18.6 (C-6), 18.5 (C-26), 17.2 (C-29), 13.2 (C-25) ppm; MS (ESI, methanol) $C_{44}H_{64}O_{13}$: $m/z = 823.2$ ($[M+Na]^+$, 100%); analysis calcd for $C_{44}H_{64}O_{13}$ (800.97): C 65.98, H 8.05; found: C 65.83, H 8.24.

4.12. (3α) 3-(β-D-gluco-pyranosyloxy)-11-oxours-12-en-24-oic acid (**15**)

Zemplén deacetylation at 25 °C for 5 h of **13** (100 mg, 0.12 mmol) in dry methanol (5 mL) containing catal. amounts of sodium methoxide followed by neutralization (Amberlyst IR $120H^+$) and usual workup afforded **15** (90 mg, 91%) as a colorless amorphous solid; $[\alpha]_D = 82.3°$ ($c = 0.1$, MeOH); ^{1}H NMR (500 MHz, CD_3OD) = 5.52 (*s*, 1H, H-12), 4.31 (*d*, $J = 10.0$ Hz, 1H, H-1′), 4.05 (*s*, 1H. H-3), 3.82 (*dd*, $J = 5.0$, 14.8 Hz, 1H, H-6′)), 3.67 (*dd*, $J = 5.0$ Hz, 14.8 Hz, 1H, H-6′), 3.34 (*t*, $J = 10.0$ Hz, 1H, H-3′), 3.31 (*m*, 1H, H-4′), 3.24 (*m*, 1H, H-5′), 3.16 (*t*, $J = 10.0$ Hz, 1H, H-2′), 2.61 (*s*, 1H, H-9), 2.33 (*d*, $J = 15.0$ Hz, 1H, H-1b), 2.17 (*dt*, $J = 5.0$, 15.0 Hz, 1H, H-16a), 2.04 (*dt*, $J = 5.0$, 15.0 Hz, 1H, H-2b), 1.99–1.86 (*m*, 2H, H-15b, H-6b), 1.77–1.66 (*m*, 3H, H-6a, H-7a, H-2a), 1.60 (*d*, $J = 15.0$ Hz, 1H, H-18) 0.1.51 (*d*, $J = 15.0$ Hz, 1H, H-5), 1.52–1.45 (*m*, 5H, H-22a, H-1a, H-19, H-7b, H-21b), 1.40 (*s*, 3H, H-27), 1.38–1.34 (*m*, 2H, H-22b, H-21a), 1.30 (*s*, 3H, H-23), 1.29 (*m*, 1H, H-15a), 1.19 (*s*, 3H, H-26), 1.15 (*s*, 3H, H-25), 1.02 (*d*, $J = 15.0$ Hz, 1H, H-16b), 0.98 (*bs*, 4H, H-20, H-30), 0.86 (s, 3H, H-28), 0.80 (*d*, $J = 5.0$ Hz, 3H, H-29) ppm; ^{13}C NMR (125 MHz, CD_3OD): δ = 202.6 (C-11), 181.0 (C-24), 167.7 (C-13), 131.1 (C-12), 101.7 (C-1′), 78.4 (C-3′), 77.7 (C-5′), 77.6 (C-3), 75.0 (C-2′), 71.8 (C-4′), 62.8 (C-6′), 61.6 (C-9), 60.7 (C-18), 50.4 (C-5), 48.0 (C-4), 46.6 (C-8), 45.4 (C-14), 42.0 (C-22), 40.5 (C-19 + C-20), 38.7 (C-10), 35.0 (C-17), 34.8 (C-1), 34.1 (C-7), 32.1 (C-21), 29.4 (C-28), 28.8 (C-16), 28.5 (C-15), 25.4 (C-23), 22.5 (C-2), 21.4 (C-30), 20.8 (C-27), 20.3 (C-6), 19.1 (C-26), 17.7 (C-29), 14.2 (C-25) ppm; MS (ESI, methanol) $C_{36}H_{56}O_9$: $m/z = 655.4$ ($[M+Na]^+$, 100%); analysis calcd for $C_{36}H_{56}O_9$ (632.82): C 68.32, H 8.92; found: C 68.09, H 9.05.

4.13. (3α) 3-(β-D-galacto-pyranosyloxy)-11-oxours-12-en-24-oic acid (**16**)

Following the procedure given for **15**, from **14** (125 mg, 0.16 mmol) **16** (98 mg, 96%) was obtained as a colorless solid; m.p. 268–269 °C; $[\alpha]_D = 50.1°$ ($c = 0.75$, MeOH); ^{1}H NMR (500 MHz, CD_3OD): $\delta = 5.49$ (*s*, 1H, H-12), 4.26 (*d*, $J = 4.0$ Hz, 1H, H-1′), 4.12 (*s*, 1H, H-3), 3.82 (*d*, $J = 4.0$ Hz, 1H, H-4′), 3.70 (*m*, 2H, H-6′), 3.52–3.45 (*m*, 3H, H-2′, H-3′, H-5′), 2.59 (*s*, 1H, H-9), 2.32 (*d*, $J = 8.0$ Hz, 1H, H-1b), 2.20–2.10 (*m*, 2H, H-2b, H-16a), 2.08–1.98 (*m*, 1H, H-6b), 1.97–1.91 (*m*, 1H, H-15b), 1.78-1-71 (*m*, 1H, H-6a), 1.70–1.63 (*m*, 2H, H-7a, H-2a), 1.57 (*d*, $J = 12.0$ Hz, 1H, H-18), 1.52–1.40 (*m*, 6H, H-22a, H-1a, H-19, H-21b, H-7b, H-5), 1.39 (*s*, 3H, H-27), 1.37–1.34 (*m*, 2H, H-21a, H-22b), 1.29–1.24 (*m*, 1H, H-15a), 1.25 (*bs*, 3H, H-23), 1.17 (*s*, 6H, H-25 + H-26), 1.06–1.01 (*m*, 1H, H-16b), 0.97 (*bs*, 4H, H-20 + H-30), 0.84 (*s*, 3H, H-28), 0.80 (*d*, $J = 8.0$ Hz, 3H, H-29) ppm; ^{13}C NMR (125 MHz, CD_3OD): $\delta = 202.8$ (C-11), 183.5 (C-24), 167.7 (C-13), 131.0 (C-12), 102.2 (C-1′), 79.1 (C-3), 76.3 (C-5′), 75.2 (C-3′), 72.6 (C-2′), 70.6 (C-4′), 62.6 (C-6′), 61.9 (C-9), 60.7 (C-18), 51.1 (C-5), 49.1 (C-4), 46.4 (C-8), 45.3 (C-14), 42.0 (C-22), 40.6 (C-20), 40.5 (C-19), 38.7 (C-10), 35.4 (C-1), 35.0 (C-17), 34.1 (C-7), 32.0 (C-21), 29.4 (C-28), 28.5 (C-16), 28.2 (C-15), 25.7 (C-23), 23.1 (C-2), 21.4 (C-30), 21.1 (C-27), 20.4 (C-6), 19.0 (C-26), 17.8 (C-29), 14.5 (C-25) ppm; MS (ESI, methanol) $C_{36}H_{56}O_9$: $m/z = 655.5$ ($[M+Na]^+$, 100%); analysis calcd for $C_{36}H_{56}O_9$ (632.82): C 68.32, H 8.92; found: C 68.17, H 9.11.

Acknowledgments

Thanks are due to Dr. R. Kluge for measuring the ESI-MS spectra, and to Dr. D. Ströhl for numerous NMR spectra. The cell lines were kindly provided by Dr. Thomas Müller (Dept. of Haematology/Oncology, Martin-Luther Universität Halle-Wittenberg). Support by the "Gründerwerkstatt – Biowissenschaften"is gratefully acknowledged. The authors would like to thank The Oman Research Council (TRC) for the generous support through the project ORG/HSS/14/004.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.06.003.

References

[1] G.A. Cordell, M.D. Colvard, Natural products and traditional medicine: turning on a paradigm, J. Nat. Prod. 75 (2012) 514–525.
[2] M. Verhoff, S. Seitz, M. Paul, S.M. Noha, J. Jauch, D. Schuster, O. Werz, Tetra- and pentacyclic triterpene acids from the ancient anti-inflammatory remedy frankincense as inhibitors of microsomal prostaglandin E-2 synthase-1, J. Nat. Prod. 77 (2014) 1445–1451.
[3] A. Sultana, K. Ur Rahman, A.R. Padmaja, S. Ur Rahman, Boswellia serrata Roxb. a traditional herb with versatile pharmacological activity: a review, Int. J. Pharm. Sci. Res. 4 (2013) 2106–2117.
[4] T. Shen, H.-X. Lou, Bioactive constituents of myrrh and frankincense, two simultaneously prescribed gum resins in chinese traditional medicine, Chem. Biodivers. 5 (2008) 540–553.
[5] H.P.T. Ammon, Boswellic acids in chronic inflammatory diseases, Planta Med. 72 (2006) 1100–1116.
[6] N. Bansal, S. Mehan, S. Kalra, D. Khanna, Boswellia serrata-frankincense (a jesus gifted herb); an updated pharmacological profile, Pharmacologia 4 (2013) 457–463.
[7] D. Poeckel, L. Tausch, S. George, J. Jauch, O. Werz, 3-O-Acetyl-11-keto-boswellic acid decreases basal intracellular Ca^{2+} levels and inhibits agonist-induced Ca^{2+} mobilization and mitogen-activated protein kinase activation in human monocytic cells, J. Pharmacol. Exp. Ther. 316 (2006) 224–232.
[8] D. Poeckel, O. Werz, Boswellic acids: biological actions and molecular targets, Curr. Med. Chem. 13 (2006) 3359–3369.
[9] S. Golbabaei, R. Bazl, S. Golestanian, F. Nabati, Z.B. Omrany, B. Yousefi, R. Hajiaghaee, S. Rezazadeh, M. Amanlou, Urease inhibitory activities of beta-boswellic acid derivatives, Daru J. Pharm. Sci. 21 (2013), http://dx.doi.org/10.1186/2008-2231-21-2.
[10] Z. Du, Z. Liu, Z. Ning, Y. Liu, Z. Song, C. Wang, A. Lu, Prospects of boswellic acids as potential pharmaceutics, Planta Med. 81 (2015) 259–271.
[11] H.-P. Liu, Z.-H. Gao, S.-X. Cui, Y. Wang, B.-Y. Li, H.-X. Lou, X.-J. Qu, Chemoprevention of intestinal adenomatous polyposis by acetyl-11-keto-beta-boswellic acid in APCMin/+ mice, Int. J. Cancer 132 (2013) 2667–2681.
[12] J.-J. Liu, W.C. Toy, S. Liu, A. Cheng, B.K. Lim, T. Subramaniam, C.F. Sum, S.C. Lim, Acetyl-keto-β-boswellic acid induces lipolysis in mature adipocytes, Biochem. Biophys. Res. Commun. 431 (2013) 192–196.
[13] S. Kapoor, Boswellic acid and its inhibitory effect on tumor growth in systemic malignancies: an emerging concept in oncology, Future Oncol. 9 (2013) 627–628.
[14] A. Moussaieff, R. Mechoulam, Boswellia resin: from religious ceremonies to medical uses; a review of in-vitro, in-vivo and clinical trials, J. Pharm. Pharmacol. 61 (2009) 1281–1293.
[15] M. Paul, G. Bruning, J. Weihrather, J. Jauch, Qualitative and quantitative analysis of 17 different types of tetra- and pentacyclic triterpenic acids in boswellia papyrifera by a semi-automatic homomodal 2D HPLC method, Chromatographia 74 (2011) 29–40.
[16] A. Petronelli, G. Pannitteri, U. Testa, Triterpenoids as new promising anticancer drugs, Anti-Cancer Drug 20 (2009) 880–892.
[17] J.E. Baer, Experimenta Chemica Cum Gummiresinis Nonnullis Instituta, PhD thesis, 1788. Erlangen.
[18] A. Tschirch, O. von Halbey, Untersuchungen über die Sekrete: Über das Olibanum, Schweiz. Wchschr. Pharm. 36 (1898) 466–476.
[19] A. Tschirch, O. von Halbey, Untersuchungen über die Sekrete. 28: Über das Olibanum, Arch. Pharm. 236 (1898) 487–503.
[20] J. Jauch, J. Bergmann, An efficient method for the large-scale preparation of 3-O-acetyl-11-oxo-beta-boswellic acid and other boswellic acids, Eur. J. Org. Chem. (2003) 4752–4756.
[21] J. Jauch, Simple Method for the Synthesis of Boswellic Acids and Derivatives Thereof, WO 02/085921A2, 31.10.2002.
[22] A. Winterstein, G. Stein, Untersuchungen in der Saponinreihe. X. Mitteilung, Zur Kenntnis der Mono-oxy-triterpensäuren, Hoppe-Seyler's Z. Physiol. Chem. 208 (1932) 9–25.

[23] S. Corsano, C. Iavarone, Isolation of 3-acetyl-11-hydroxy-β-bosvellic acid from incense, Gazz. Chim. Ital. 94 (1964) 328–339.
[24] R. Csuk, A. Niesen-Barthel, A. Barthel, R. Kluge, D. Ströhl, Synthesis of an antitumor active endoperoxide from 11-keto-beta-boswellic acid, Eur. J. Med. Chem. 45 (2010) 3840–3843.
[25] R. Csuk, A. Niesen-Barthel, R. Schaefer, A. Barthel, A. Al-Harrasi, Synthesis and antitumor activity of ring A modified 11-keto-β-boswellic acid derivatives, Eur. J. Med. Chem. 92 (2015) 700–711.
[26] C. Soica, C. Trandafirescu, C. Danciu, D. Muntean, C. Dehelean, G. Simu, New improved drug delivery technologies for pentacyclic triterpenes: a review, Protein Pept. Lett. 21 (2014) 1137–1145.
[27] C. Gauthier, J. Legault, M. Lebrun, P. Dufour, A. Pichette, Glycosidation of lupane-type triterpenoids as potent in vitro cytotoxic agents, Bioorg. Med. Chem. 14 (2006) 6713–6725.
[28] R. Csuk, S. Schwarz, B. Siewert, R. Kluge, D. Ströhl, Synthesis and cytotoxic activity of methyl glycyrrhetinate esterified with amino acids, Z. Naturforsch. B 67 (2012) 731–746.
[29] R. Csuk, S. Schwarz, B. Siewert, R. Kluge, D. Ströhl, Conversions at C-30 of glycyrrhetinic acid and their impact on antitumor activity, Arch. Pharm. 345 (2012) 223–230.
[30] R. Csuk, S. Schwarz, R. Kluge, D. Ströhl, Improvement of the cytotoxicity and tumor selectivity of glycyrrhetinic acid by derivatization with bifunctional aminoacids, Arch. Pharm. 344 (2011) 505–513.
[31] R. Csuk, S. Schwarz, R. Kluge, D. Ströhl, Synthesis and biological activity of some antitumor active derivatives from glycyrrhetinic acid, Eur. J. Med. Chem. 45 (2010) 5718–5723.
[32] S. Schwarz, R. Csuk, Synthesis and antitumour activity of glycyrrhetinic acid derivatives, Bioorgan. Med. Chem. 18 (2010) 7458–7474.
[33] H. Kommera, G.N. Kaluderovic, M. Bette, J. Kalbitz, P. Fuchs, S. Fulda, W. Mier, R. Paschke, In vitro anticancer studies of α- and β–glucopyranose betulin anomers, Chem. Biol. Interact. 185 (2010) 128–136.
[34] R. Csuk, Betulinic acid and its derivatives: a patent review (2008-2013), Expert Opin. Ther. Pat. 24 (2014) 913–923.
[35] D. Thibeault, C. Gauthier, J. Legault, J. Bouchard, P. Dufour, A. Pichette, Synthesis and structure-activity relationship study of cytotoxic germanicane- and lupane-type 3β-O-monodesmosidic saponins starting from betulin, Bioorg. Med. Chem. 15 (2007) 6144–6157.
[36] S.G. Sparg, M.E. Light, J. Van Staden, Biological activities and distribution of plant saponins, J. Ethnopharmacol. 94 (2004) 219–243.
[37] R. Csuk, A. Barthel-Niesen, D. Stroehl, R. Kluge, C. Wagner, A. Al-Harrasi, Oxidative and reductive transformations of 11-keto-β-boswellic acid, Tetrahedron 71 (2015) 2025–2034.
[38] M.R.E. Aly, E.-S.I. Ibrahim, E.-S.H. El Ashry, R.R. Schmidt, Synthesis of lacto-N-neotetraose and lacto-N-tetraose using the dimethylmaleoyl group as amino protective group, Carbohydr. Res. 316 (1999) 121–132.
[39] R.R. Schmidt, R. Klaeger, Glycosylimidates. Part 15. Short synthesis of cerebrosides, Angew. Chem. 97 (1985) 60–61.
[40] R.R. Schmidt, W. Kinzy, Anomeric-oxygen activation for glycoside synthesis: the trichloroacetimidate method, Adv. Carbohydr. Chem. Biochem. 50 (1994), 21–123.
[41] S. Schwarz, B. Siewert, N.M. Xavier, A.R. Jesus, A.P. Rauter, R. Csuk, A “natural” approach: synthesis and cytoxicity of monodesmosidic glycyrrhetinic acid glycosides, Eur. J. Med. Chem. 72 (2014) 78–83.
[42] M.K. Anwer, A.F. Spatola, C.D. Bossinger, E. Flanigan, R.C. Liu, D.B. Olsen, D. Stevenson, Quantitative removal of a pentadecapeptide acth fragment analog from a merrifield resin using ammonium formate catalytic transfer hydrogenation - synthesis of [Asp25,Ala26,Gly27,Gln30]-Acth-(25-39)-Oh, J. Org. Chem. 48 (1983) 3503–3507.
[43] M.K. Anwer, A.F. Spatola, A rapid non-acidolytic method for deprotection and removal of peptides from solid-phase resins - applications of ammonium formate catalytic transfer hydrogenation, Tetrahedron Lett. 22 (1981) 4369–4372.
[44] M.K. Anwer, A.F. Spatola, An advantageous method for the rapid removal of hydrogenolysable protecting groups under ambient conditions - synthesis of Leucine-Enkephalin, Synthesis (1980) 929–932.
[45] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. Mcmahon, D. Vistica, J.T. Warren, H. Bokesch, S. Kenney, M.R. Boyd, New colorimetric cytotoxicity assay for anticancer-drug screening, J. Natl. Cancer Inst. 82 (1990) 1107–1112.
[46] B. Siewert, R. Csuk, Membrane damaging activity of a maslinic acid analog, Eur. J. Med. Chem. 74 (2014) 1–6.
[47] B. Siewert, J. Wiemann, A. Kowitsch, R. Csuk, The chemical and biological potential of C ring modified triterpenoids, Eur. J. Med. Chem. 72 (2014) 84–101.
[48] B. Siewert, E. Pianowski, A. Obernauer, R. Csuk, Towards cytotoxic and selective derivatives of maslinic acid, Bioorgan. Med. Chem. 22 (2014) 594–615.