Synthese und Antitumoraktivität von Glycyrrhetinsäurederivaten

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- meinen Eltern und Großeltern

Henrietta Lacks

August 01, 1920 - October 04, 1951

In loving memory of a phenomenal woman, wife and mother who touched the lives of many.

Here lies Henrietta Lacks (HeLa). Her immortal cells will continue to help mankind forever.

Eternal Love and Admiration, From Your Family

Grabstein in Clover, Virginia, USA

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Inhaltsverzeichnis

Abkürzungsverzeichnis

11β-HSD2	11β-Hydroxysteroiddehydrogenase
AIF	Apoptose induzierender Faktor
Apaf-1	apoptotic protease activating factor-1
Bcl-2	B-cell lymphoma 2
bzw.	beziehungsweise
ca.	zirka
CDODA-Me	2-Cyano-3,11-dioxo-18-olean-1,12-dien-30-säuremethylester
cFLIP	caspase-8 (FLICE)-like inhibitory protein
cMOAT	canalicular multispecific organic anion transporter 1
DNS	Desoxyribonukleinsäure
F	Selektivitätsfaktor ($F = IC_{50}$[NiH3T3] / IC_{50}[Tumorzelllinie])
GA	Glycyrrhetinsäure
GJC	gap junction communication
GM	Glycyrrhetinsäure-monoglucuronid
GZ	Glycyrrhizinsäure
H2N2	Subtyp des Influenzavirus A_2
HBsAg	hepatitis B surface antigen
HIV	Human Immundefizienz Virus
IC_{50}	mittlere inhibierende Konzentration
ID_{50}	mittlere inhibierende Dosis
i.d.R.	in der Regel
LANA	latency-associated nuclear antigen
Mcl-1	myeloid cell leukemia sequence 1
MPT	mitochondrial permeability transition
MR	Mineralokortikoidrezeptor
mTOR	mammalian target of rapamycin
NAG-1	NSAID activated gene 1
PGE_2	Prostaglandin-E_2
$PGF_{2\alpha}$	Prostaglandin-$F_{2\alpha}$
PPARγ	Peroxisome proliferator-activated receptor γ
ROS	reaktive Sauerstoffspezies (reactive oxygen species)

SRB	Sulforhodamin-B
v. Chr.	vor Christus
VEGF	vascular endothelial growth factor
VEGFR-2	vascular endothelial growth factor receptor 2
XIAP	X-linked inhibitor of apoptosis protein
z.B.	zum Beispiel

1. Einleitung

Das Prinzip ist relativ einfach. Man nehme eine Pflanze der einheimischen Flora, welche möglichst in ausreichender Menge erhältlich ist. Aus dieser isoliere man anschließend die Inhaltsstoffe - i.d.R. die Sekundärmetabolite - und bestimme deren Struktur. Folgend führe man Dutzende von biologischen Assays durch, um wenigstens bei einer Komponente eine pharmakologisch relevante Wirkung zu finden. Gegebenenfalls wird die Substanz noch modifiziert, um die Wirkung zu steigern oder Nebenwirkungen zu minimieren.

Abgesehen davon, dass dieses Vorgehen sehr zeit- und kostenintensiv ist, sind die Erfolgsaussichten eher gering. Die Auswahl an Pflanzen ist riesig und die Anzahl der Inhaltsstoffe nicht minder klein. Aus diesem Grund ist eine Art Vorauswahl bzw. ein gerichtetes Suchen wünschenswert.

Beleuchtet man den Ursprung vieler heute gängiger Medikamente, so lassen sich zwei Dinge feststellen. Zum Einen folgen sie in der Tat dem Vorbild der in der Flora vorkommenden Sekundärmetaboliten. Zum Anderen war die Auswahl dieser Inhaltsstoffe nicht ganz so zufällig, wie es vielleicht den Anschein haben könnte. Weltbekannte Arzneimittel - wie beispielsweise Aspirin® oder Morphin - lassen sich auf pflanzliche Bestandteile zurückführen oder sind in der genutzten Form isolierbar.

Nach wie vor stellt sich die Frage, wie man ausgerechnet jene Pflanzen findet, die solche Inhaltsstoffe mit pharmakologischem Potential in sich tragen. Ein häufiger Ansatzpunkt findet sich in der Naturheilkunde aktueller oder vergangener Kulturen. Das Gros dieser Rezepturen besteht aus Pflanzenextrakten, welche auf mehrere Arten verwendet werden: in Form von Salben, Tinkturen oder Tees. Ausgehend von einer ersten Erwähnung der Pflanze in Quellen des Altertums, durchstreift sie meist mehrere Epochen mit einer mehr oder weniger stark ausgeprägten Beachtung. In der Neuzeit wird schließlich das alte Wissen wieder aufgegriffen und unter dem Gesichtspunkt der modernen Wissenschaft beleuchtet. Am Ende steht ein Wirkstoff, welcher eventuell seinen Ursprung vor mehreren hundert oder tausend Jahren hat.

Viele Arzneimittel haben demnach eine Geschichte und die Geschichte der Glycyrrhetinsäure als Bestandteil des Süßholzes beginnt vor über 3000 Jahren.

1.1 Süßholz

1.1.1 Blick in die Geschichte

Die ersten Aufzeichnungen zum Süßholz wurden im Mittelmeerraum gefunden. Der Grund liegt in der damaligen territorialen Verbreitung der Pflanze. Angeblich fanden sich Teile des Süßholzes als Grabbeilage des ägyptischen Pharaos Tut-ench-Amon[1] (Abb. 1). Ob daraus eine erste Verwendung des Süßholzes im Altertum abgeleitet werden kann, darin sind sich die Berichte uneins. Während Shibata mit dem Fund eine verbreitete Verwendung in Ägypten erkennt[2], sieht Putscher in der Beilage eine Exklusivität. Sie vermutet eher ein exotisches Geschenk als ein Zeichen für die allgemeine Verbreitung und Verwendung im Volk[3].

Abb. 1 *Tut-ench-amun[4]*

Die vermutlich erste verbriefte Erwähnung über die Verwendung der Süßholzwurzel findet sich in Mesopotamien. Auf zwei Rezeptlisten des 6. Jahrhunderts v. Chr. steht mit "šūšu" ein Nachweis über Mixturen, welche das Süßholz beinhaltet[5]. Theophrastos von Eresos (372 - 387 v. Chr.) liefert ein weiteres Zeugnis über die Kenntnis der Pflanze und deren mögliche Anwendung: als Tonikum u.a. gegen Husten, Asthma und Geschwüren[6]. Ferner sollte es bei Lungentuberkulose helfen sowie harntreibend wirken[7]. Andere Quellen geben sogar Hinweise, dass zur damaligen Zeit verschiedene Teile neben den Wurzeln verwendet wurden. Beispielsweise schreibt Thompson von der Nutzung der Blätter und der Samen[8].

Von dem Volk der Skythen - einem ostiranischen Nomadenvolk - ist bekannt, dass sie insbesondere die durstlöschende Wirkung der Pflanze schätzten. Laut den Überlieferungen sollen Krieger bis zu

12 Tage ohne Wasseraufnahme ausgekommen sein, lediglich durch Ernährung mit Stutenmilchkäse und Süßholz[6, 7]. Weitere Hinweise auf diese beeindruckende Wirkung finden sich auch in Quellen der damaligen Zeit, wie z.B. bei Plinius dem Älteren[9].
Zur Zeit der Griechen wurde dem Süßholz folgende Wirkungen nachgesagt[10]:

- gegen Asthma, Husten, Brustbeschwerden, Durstgefühl, als Stärkungsmittel allgemein[6]
- äußerlich zur Wundheilung[2, 6]
- zur Einnahme bei Halsentzündungen, Leberbeschwerden, Nierenleiden und Blasensteinen[2]

Die Verwendung der Pflanze wurde im römischen Reich fortgesetzt. Die Römer besaßen im Gegensatz zu vielen Eroberern der Weltgeschichte einen klugen Wesenszug. Anstatt die eroberten Völker mitsamt ihrer Kultur quasi auszulöschen, übernahmen sie Teile des Wissens. So fand auch die Süßholzwurzel Einzug in die römische Medizin bzw. ihre Rezepturen. Neben vielen Völkern des östlichen Mittelmeerraums, war insbesondere der griechische Einfluss maßgeblich. Dementsprechend zeigen sich Parallelen zwischen römischen und griechischen Erfahrungen mit der Pflanze. Zusätzlich zu den vorherigen Anführungen zur Wirkung der Pflanze werden noch die folgenden Punkte erwähnt[11, 12]:

- als Augensalbe bei Pterygium (in Pulverform)
- zur Einnahme bei Fußschmerzen

In einem anderen Teil der Welt - genauer in China - hat die Süßholzwurzel eine ähnlich lange Geschichte. Obwohl es sich dabei um andere Arten handelt, zeigen sich viele Parallelen zur Verwendung der Pflanze im Mittelmeerraum. Die getrocknete Wurzel sowie deren pulverisierte Form waren fester Bestandteil der chinesischen Pharmakologie, während der eingetrocknete Extrakt weniger genutzt wurde. Neben einigen in Europa beschriebenen Wirkungen, wie z.B. gegen Augenkrankheiten oder Leberbeschwerden, wurde erstmals auch von einer negativen Auswirkung bei größerem Genuss der Wurzel berichtet: die Induzierung von Wassersucht[13]. Über die rein medizinische Bedeutung hinaus, stand die Pflanze im Ruf die Muskeln zu stärken sowie das Hautbild zu verbessern[2, 14]. Man sprach gar von einem verjüngten Leben durch regelmäßigen Genuss[7].
Einige Kilometer weiter, im Gebiet des heutigen Indiens, war man ebenfalls von der positiven Wirkung des Süßholzes überzeugt. Laut Lucas glaubte man hier sogar an eine Erhöhung der sexuellen Spannkraft, reiche man die Süßholzwurzel in einem Getränk mit Milch und Zucker[1].
Richten wir jedoch den Blick wieder auf Europa. Im Mittelalter fand die Pflanze den Weg in

nördlichere Gefilde. Erst als Rezeptur, später auch in Kultur, zog die Süßholzwurzel bis hinauf in das Gebiet des heutigen Deutschlands bzw. Englands[12]. Die Weiterverbreitung der Pflanze zeigt sich an der Beobachtung, dass sie 1562 zum ersten Mal in England kultiviert werden konnte. Anfänglich wurde das Süßholz noch aufgrund seiner Süße verwendet, z.B. als Zuckerersatz in Kaugummis oder in Tabak[15]. Die pharmakologischen Wirkungen wurden aber auch hier erkannt und beschrieben, wobei sie sich mit den bisherigen Erkenntnissen gleichen[16-18].

Die Geschichte des Süßholzes auf deutschem Raum geht auf die Zeit der vorletzten Jahrtausendwende zurück. HILDEGARD VON BINGEN nannte in ihren Schriften den Extrakt des Süßholzes „Succus Liquiricus“, wovon sich der später im Deutschen gebräuchliche Name der „Lakritze“ ableitet[7]. Die Pflanze und ihre Wirkungen waren gleichfalls in Deutschland lange vor den ersten Anbauten bekannt. Entsprechend der Berichte aus anderen Kulturen nutzte man im deutschen Raum das Süßholz ebenfalls gemäß den überlieferten Rezepten und Anwendungsbereichen. Im 16. Jahrhundert kultivierten Benedektinermönche die Pflanze im deutschen Raum, wodurch zum ersten Mal der Eigenbedarf gedeckt und somit von ernsthaftem großflächigerem Anbau gesprochen werden konnte[7].

In den folgenden Jahrhunderten begegnet einem das Süßholz an verschiedenen Stellen der Geschichte. So soll z.B. Napoleon Bonaparte (Abb. 2) stets getrocknete Süßholwurzel mit sich geführt haben, da er häufig an Magenverstimmungen litt und die beruhigende Wirkung der Wurzel schätzte[7]. Die Indianer kannten die Pflanze von den ersten europäischen Siedler, welche die Pflanze mitbrachten. Sie nutzten das Süßholz hauptsächlich als Zuckerersatz bzw. als Mittel gegen Diabetes[19]. HOUSEMAN berichtet Mitte des letzten Jahrhunderts, dass der Rückstand der Süßholzextrakte seit 1906 in Form einer Suspension als Löschschaum verwendet wurde[20].

Abb. 2 *Paul Delaroche (1797-1856), Napoleon Fontaineblau, Öl auf Leinwand*[21]

Mit der Herausbildung der Naturwissenschaften nach unserem heutigen Verständnis, wandelte sich der Blick auf die Naturheilkunde und damit auch auf die Süßholzwurzel. Man entfernte sich von der Vorstellung die Pflanze bzw. ihre Extrakte *per se* als Arznei anzusehen. Vielmehr galt es die Inhaltsstoffe zu extrahieren, zu identifizieren und gemäß ihrer Wirkung einzuordnen.

1.1.2 pharmakologisch relevante Inhaltsstoffe

Der Extrakt der Süßholzwurzel wird in der Geschichte häufig als wirksamster Bestandteil der Pflanze beschrieben. Er ist ein Gemisch aus mehreren Inhaltsstoffen. Um ihn zu gewinnen, soll ein kurzer Blick auf die Kultivierung und eine Möglichkeit der Verarbeitung der Pflanze geworfen werden[22-24]. Das Süßholz wächst in der Natur selten weiter entfernt als 45 Meter vom Wasser und gedeiht am besten auf sandigem Untergrund. Nach einem erfolgreichen Anwachsen ist die Pflanze relativ robust, trotz ihrer Unbeständigkeit gegenüber Frost. Sie kann im Frühjahr oder im Herbst angepflanzt werden und wird üblicherweise im Herbst geerntet. Die Wurzel wird von der übrigen Pflanze befreit und über einen Zeitraum von 10 Monaten getrocknet. Nachdem sie auf diesem Wege 90 % ihres Wassergehaltes verloren hat, wird die Wurzel unter einem Mühlstein zu einem Brei gemahlen und dieser in Wasser aufgekocht. Der Extrakt kann nun vollständig eingedampft und als goldbraunes Pulver weiterverarbeitet werden. Alternativ verbleibt ein Teil des Wassers und die noch warme, dickflüssige Masse wird gerollt oder gepresst. Das dabei entstehende Produkt wird zu Lakritzstangen weiterverarbeitet oder direkt verzehrt. In vielen Nahrungsmitteln wird die gewonnene Lakritze als Geschmacksverstärker oder Aromastoff verwendet. Als Beispiele seien Süßigkeiten, Kaugummi, Getränke oder Backerzeugnisse genannt[25].

Der Vollständigkeit halber ist zu erwähnen, dass bisher vom Süßholz als eine einzige Pflanze gesprochen wurde. Natürlich existieren weltweit mehr als nur eine Art. Dies erklärt zum Teil die regionalspezifischen Anwendungen, da jede Variante der Pflanze eine andere Zusammensetzung ihrer Inhaltsstoffe besitzt. In der nachfolgenden Tabelle sind einige Arten des Süßholzes inklusive des Verbreitungsgebietes aufgeführt[2, 26]:

Art	Vorkommen
Glycyrrhiza uralensis FISCHER	Nordosten China, Ostrussland
Glycyrrhiza glabra L. *typica*	Spanien, Italien, Südeuropa
Glycyrrhiza glabra L. *violacea*	Türkei, Iran
Glycyrrhiza glabra L. *glandulifera*	China, Russland, Zentralasien
Glycyrrhiza inflata	China
Glycyrrhiza eurycarpa	China, Russland, Zentralasien
Glycyrrhiza aspera	China

Tab. 1 *Verbreitung des Süßholzes*

Im Folgenden sind die Inhaltsstoffe des Süßholzextraktes aufgelistet. Die Liste stellt jedoch keinen Anspruch auf Vollständigkeit, vielmehr werden die pharmakologisch wichtigsten Stoffe benannt.

Einige Substanzen konnten nur aus einigen Arten isoliert werden oder werden bisher als nicht sonderlich relevant angesehen. Sie sind nachfolgend unter den Stoffklasse zusammengefasst:

- Glycyrrhizin (als freie Säure und in Form ihrer Salze)[2, 7, 27]
- Flavonoide[2] (Likurasid[7, 28], Liquiritin[28-32], Liquiritigenin[28, 31, 33, 34], Isoliquiritin[28, 32], Isoliquiritigenin[31, 34-36], Ononin[32, 36], Formononentin[34, 36], Licochalcon A[28, 32], Glabridin[37-39])
- Cumarine (Umbelliferon[7, 40], Glycyrol[41, 42], Glycyrin[41], Glycycumarin[41])
- verschiedene Saponine des Oleanoltyps[43-45]
- Phenolsäuren[46] (Ferulasäure[7])
- estrogene Steroide (Estradiol[7])
- Methylsalicylat[7]

Die drei Wirkstoffgruppen, welche mengenmäßig am häufigsten im Süßholzextrakt zu finden sind, werden nachfolgend näher beleuchtet.

1.1.2.1 Cumarine

Betrachten wir zunächst die genannten Derivate des Cumarins. Die vier Substanzen leiten sich jeweils strukturell vom Cumarin ab:

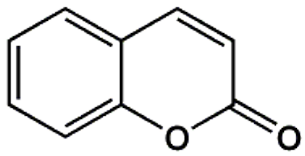


Abb. 3 *Struktur des Cumarins*

In einigen Studien zeigen diese eine biologische Aktivität. Für Glycyrol, Glycyrin und Glycycumarin konnte eine antibakterielle Wirkung nachgewiesen werden[41]. Weiterhin besitzt Glycyrol eine entzündungshemmende[42] sowie eine immunsupressive[47] Wirkung. Es wird sogar eine apoptotische Wirkung vermutet[48]. Dem Umbelliferon wiederum wird eine choleretische Wirkung zugesprochen[40].

1.1.2.2 Flavonoide

Dass Flavonoide im Süßholz zu finden sind, mag niemanden verwundern. Sie gehören zu den wichtigsten Sekundärmetaboliten der höheren Pflanzen und werden logischerweise auch im Süßholz gebildet.

Prinzipiell bestehen Flavonoide aus zwei aromatischen Ringen, welche über eine C3-Brücke miteinander verknüpft sind. Die jeweilige Verknüpfung bestimmt die genauere Einteilung nach Untergruppen. Nach HÄNSEL und STICHER gibt es Chalkone, Flavanone, Flavone, Isoflavone, Flavanonole, Flavonole, Flavandiole, Flavanole und Anthocyanidine[49]. Den Flavonoiden konnte bisher nahezu das gesamte Spektrum an biologischen Aktivitäten nachgewiesen werden: u.a. eine antivirale[50, 51], antikanzerogene[52, 53] oder antiallergische Wirkung[54, 55].

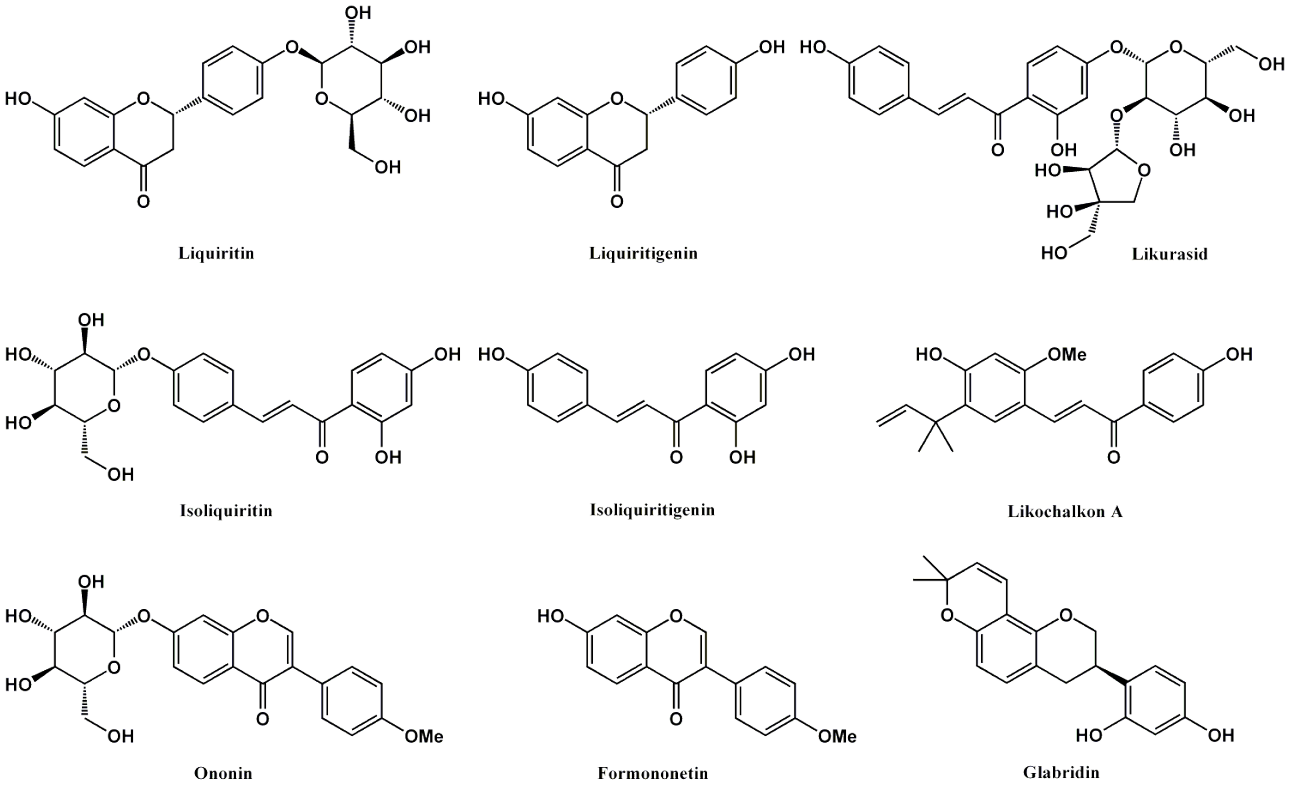


Abb. 4 *Auswahl von Flavonoiden des Süßholzes*

Die in Süßholz enthaltenen Flavonoide (Abb. 4) bilden hierbei keine Ausnahme. Den Substanzen Likurasid, Isoliquiritigenin, Isoliquiritin und Licochalkon A verfügen über eine tyrosinasehemmende und somit der Bräunung entgegenwirkende Eigenschaft[28, 36]. Über Liquiritin wiederum finden sich erfolgreiche Studien bezüglich einer nervenschützenden Wirkung[30]. Des Weiteren konnte gezeigt werden, dass die Substanz im Tierversuch als Antioxidans agieren kann

sowie einem Antidepressivum ähnliche Eigenschaften aufweist[29, 30]. Eine Studie von JAYAPRAKASAM *et al.* untersuchte die Flavonoide im Süßholz auf eine antiasthmatische Wirkung, welche *in vitro* nachgewiesen wurden[31]. KIM *et al.* attestierten dem Liquiritigenin eine zellschützende Eigenschaft in Bezug auf Leberzellen[33]. KWON *et al.* wiederum wiesen für das Isoliquiritigenin sowohl eine entzündungshemmende als auch eine antiatherosklerotische Wirkung nach[36]. Das Glabridin vermag antimykotisch zu wirken[39]. Weiterhin zeigten sich in einem Test mit Osteoplasten antiapoptotische Eigenschaften der Substanz[38].

Die Flavonoide des Süßholzes besitzen demnach eine Fülle von pharmakologisch relevanten Wirkungen. Unter Betrachtung des historischen Hintergrunds, kann man erste Querverbindungen ziehen zwischen der belegten Wirkung einzelner Komponenten und der verbrieften Anwendung in alten Quellen.

Ein Großteil der Wirkungen des Süßholzes wird jedoch dem Hauptbestandteil der Wurzel zugeschrieben, welcher bis zu 24 % des Trockengewichts ausmacht[56]: die Glycyrrhetinsäure.

1.2 Glycyrrhetinsäure

1.2.1 Glycyrrhetinsäure oder Glycyrrhizinsäure ?

Die Glycyrrhetinsäure ist eine Triterpensäure und kommt im Süßholz als Glycyrrhizinsäure vor, einem Glykosid mit zwei Molekülen Glucuronsäure (Abb. 5).

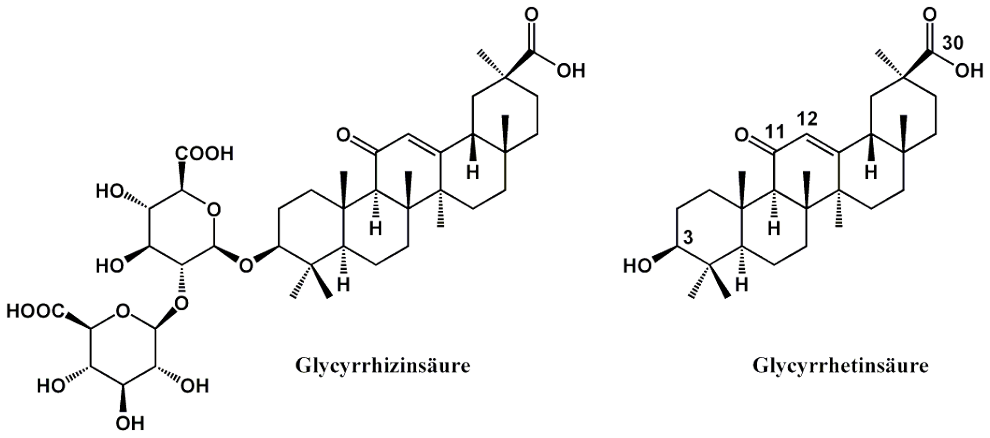


Abb. 5. *Struktur des Saponins Glycyrrhizinsäure und dem Triterpen Glycyrrhetinsäure*

Es ist zunächst zu klären, ob die Glycyrrhetinsäure oder die Glycyrrhizinsäure der wirksame Bestandteil ist. Um diese Frage einigermaßen beantworten zu können, muss man die Studien zur Bioaktivität in den letzten Jahrzehnten sehr kritisch betrachten. Nachdem anfänglich stets von

Wirkungen des Extraktes gesprochen wird, wandelte sich die Betrachtungsweise durch die Isolation der einzelnen Inhaltsstoffe. Es lag nun der Fokus einige Zeit auf der Glycyrrhizinsäure. In neueren Studien wird allerdings eher das Aglykon als der eigentliche Wirkstoff betrachtet: die Glycyrrhetinsäure. Der Grund liegt hauptsächlich auf dem Unterschied zwischen *in vitro*- und *in vivo*-Vorgängen. Studien außerhalb eines Organismus besitzen im Vergleich zu Studien im Organismus stets den Nachteil, dass sie die „Umwelt" des Studienziels außer acht lassen. In vielen Fällen unterscheiden sich die Ergebnisse maßgeblich. Glycyrrhetinsäure oder Glycyrrhizinsäure - diese Frage ist ein Beispiel dafür.

Ein Wirkstoff entfaltet seinen Einfluss meist in der Form, in welcher er nach Gabe im Plasma oder am jeweiligen Ziel im Organismus vorliegt. In unserem Fall ist es entscheidend zu wissen, ob eventuell eine der Säuren vermehrt zu finden ist oder beide nebeneinander detektierbar sind. Sowohl tierische als auch humane Untersuchungen kamen auf das gleiche Ergebnis. Während in Tiertests erst bei hohen Dosen eine gewisse Menge an Glycyrrhizinsäure bestimmt werden konnte, war dies im humanen System nicht mehr möglich. Allerdings waren in beiden Systemen - tierisch und human - Mengen an Glycyrrhetinsäure detektierbar, obwohl diese nicht verabreicht wurde[57-60]. Dies deutet sehr stark darauf hin, dass *in vivo* die Glycyrrhizinsäure innerhalb von Stunden hydrolysiert wird und der eigentliche Wirkstoff die Glycyrrhetinsäure ist.

Es ist prinzipiell nachvollziehbar, dass es im menschlichen Körper zu einer Hydrolye kommt. Wichtig war jedoch herauszufinden, wo diese hauptsächlich stattfindet. Arbeiten von u.a. Hattori *et al.*[61] konnten zeigen, dass insbesondere die Bakterienstämme *Eubacterium sp.* (GHL-Stamm), *Ruminococcus sp.* PO1-3 sowie *Clostridium innocuum* ES2406 Glycyrrhizinsäure hydrolysieren können[61-63]. Die drei Stämme leben in der menschlichen Darmflora und besitzen eine spezielle β-D-Glucuronidase, welche für diese Hydrolyse verantwortlich ist. Dass es sich um eine spezielle Form handelt, konnte Akao *et al.* zeigen. Die gemeine Form des Enzyms - isoliert z.B. aus *Escherichia coli* - ist nicht in der Lage, die Hydrolyse zu bewirken[62].

Im Weiteren unterliegt ein Teil der freigewordenen Glycyrrhetinsäure einer Epimerisierung (Abb. 6). Diese läuft unter Bildung der in Position 3 oxidierten Spezies ab, welche in einem weiteren Schritt zum 3α-Produkt umgesetzt wird. Die Fähigkeit, Glycyrrhizinsäure in einem ersten Schritt zu hydrolysieren und anschließend zu epimerisieren, ist nur in einem Zusammenspiel der genannten Bakterienstämme möglich. Bei der Durchführung der Experimente mit isolierten Stämmen konnten nur Teilprozesse der geschilderten Umsetzung beobachtet werden[61].

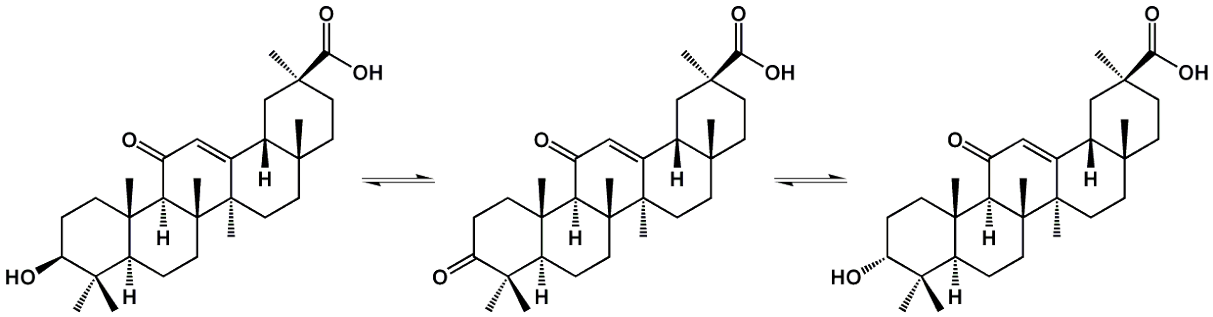


Abb. 6. Epimerisierung der Glycyrrhetinsäure durch Bakterienstämme der Darmflora

Glycyrrhizinsäure kann weiterhin vom Körper leicht ausgeschieden werden. Durch die erhöhte Polarität der zwei Glucoronsäurereste ist die Hydrophilie insgesamt deutlich höher als die der Glycyrrhetinsäure. Aus diesem Grund kann die aufgenommene Glycyrrhizinsäure - welche nicht einer Transformation der Bakterienflora unterlag - z.B. via Anionentransporter (cMOAT) von der Leber über die Galle ausgeschieden werden[64]. Allerdings ergibt sich aus dieser Tatsache ein Problem. Da die Galle schlussendlich den Darmtrakt passiert, wird ein Teil der Glycyrrhizinsäure wieder in den Organismus aufgenommen - hydrolysiert als Glycyrrhetinsäure.

Vor einem ähnlichen Problem steht der Organismus bei der Eliminierung der Glycyrrhetinsäure. Um diese über den gleichen Weg ausscheiden zu können, muss zunächst die Substanz in eine hydrophilere Form überführt werden. Es entstehen dabei u.a. das 3-*O*-hydrogensulfat, das 3-*O*-monoglucuronid sowie das 30-monoglucuronid[65]. Diese Verbindungen können nun analog zur Glycyrrhizinsäure rasch per Galle ausgeschieden werden. Als Transporter wird wiederum der cMOAT vermutet[66]. Jedoch gibt es Bakterienstämme, welche die gebildeten Glycyrrhetinsäurederivate in der Galle hydrolysieren können[67-70]. Da die freiwerdende Glycyrrhetinsäure erneut aufgenommen werden kann, ist die Ausscheidung der Substanz aus dem Organismus insgesamt verzögert (Abb. 7).

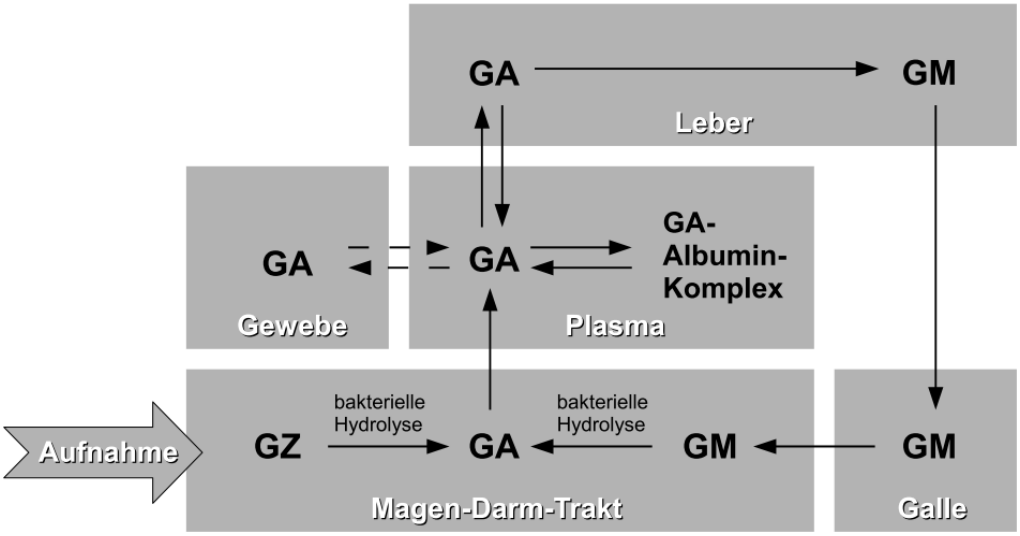


***Abb.** 7 Schema des Kreislaufes der Glycyrrhetinsäure im humanen System[57]: Glycyrrhizinsäure (GZ) wird aufgenommen und im Magen-Darm-Trakt durch die Bakterienflora zu Glycyrrhetinsäure (GA) hydrolisiert. Die Glycyrrhetinsäure gelangt ins Plasma, wo sie als Albuminkomplex vorliegt. Während ein geringer Teil ins Gewebe übergeht, wird die Mehrheit von der Leber aufgenommen und dort zu hydrophileren Derivaten metabolisiert: z.B. Glycyrrhetinsäure-monoglucuronid (GM). Das GM gelangt über die Gallenflüssigkeit wieder in den Magen-Darm-Trakt, wo ein Teil erneut einer hydrolytischen Spaltung unterliegt und erneut ins Plasma resorbiert wird.*

Aus pharmakologischer Sicht lassen sich somit zwei Schlüsse ziehen. Erstens ist die Glycyrrhetinsäure der entscheidende *in vivo*-Wirkstoff. Zweitens ist zu beachten, dass es zu einer teilweisen Wiederaufnahme der Glycyrrhetinsäure im Organismus kommt. Diese durchläuft sogar mehrere abklingende Zyklen. Es mag nicht allzu relevant erscheinen, jedoch ist zu bedenken, dass es bezüglich der verwendeten Dosenmengen sehr wichtig ist.

1.2.2 pharmakologische Wirkung der Glycyrrhetinsäure

Das Süßholz wurde über Jahrhunderte geschätzt. Dies ist vielen historischen Quellen entnehmbar; allerdings bescheinigten einige Berichte auch Nebenwirkungen. In Studien aus den Niederlanden sowie Neuseeland wurden jene bei regelmäßigem Lakritzgenuss beobachtet: Kopfschmerzen, Müdigkeit, Durstgefühl, Ödemen und Diurese. Des Weiteren konnte eine reversible Erhöhung des systolischen Blutdrucks festgestellt werden, der durch einen Abfall des Kaliumspiegels verursacht worden war[7]. Auch in neueren Untersuchungen wird ein Zusammenhang zwischen Lakritzgenuss bzw. der Aufnahme von Glycyrrhetinsäure und Bluthochdruck manifestiert[71].

1.2.2.1 mineralcorticoider Effekt

Zunächst wird der Weg der Glycyrrhetinsäure beleuchtet, welcher nach der Aufnahme über den Magen-Darm-Trakt folgt. Dies soll dazu dienen, einige Wirkungen besser verstehen zu können. Glycyrrhetinsäure hemmt die 11β-HSD2 in der Niere, welche die Umsetzung von aktivem Cortisol in das nicht aktive Cortison bewirkt[72, 73]. Analog dem Aldosterol bindet aktives Cortisol am Mineralcorticoidrezeptor (MR), dabei ist die Konzentration des Cortisols im Plasma ungefähr 100-fach höher als die des Aldosterons. Ein aktivierter MR erhöht die Transkription von Genen, welche z.B. für den Natrium/Kalium-Transport zuständig sind. Kommt es nun zu einer Hemmung der 11β-HSD2, so wird das Cortisol nicht mehr inaktiviert und bindet wie das Aldosteron am MR. Die Transkription wird erhöht und es resultieren: eine übermäßigen Aufnahme von Natriumionen in den Nierentubuli (Hypernatriämie), eine Ausschüttung von Kaliumionen in den Urin (Hypokaliämie) sowie die Erhöhung des Blutdrucks durch vermehrte Wasseraufnahme (Hypertension)[74]. Das zurückgehaltene Wasser[73] kann ebenfalls als Erklärung für die Ödembildung bei erhöhtem Lakritzgenuss dienen.

1.2.2.2 Wirkung gegen Geschwüre

Ein weiterer Effekt - der dem Süßholz zugeschrieben wurde - war die Abhilfe bei Geschwüren, insbesondere im Bereich des Magens. Glycyrrhetinsäure hemmt die zwei Enzyme 15-Hydroxyprostaglandindehydrogenase sowie Δ^{13}-Prostaglandinreduktase, die PGE_2 und $PGF_{2\alpha}$ in ihre inaktivierte Spezies überführen. PGE_2 und $PGF_{2\alpha}$ sind Prostaglandine, welche eine Gruppe der Gewebshormone darstellen. Sie sind an entzündlichen Vorgängen im menschlichen Körper beteiligt. Wird nun der Abbau dieser beiden Hormone gehemmt, so häufen sich diese am Entzündungsort an. Dadurch steigt die Sekretproduktion im Bereich der Schleimhäute und neue Zellen werden vermehrt gebildet. Dies unterstützt in der Gesamtheit die Selbstheilung der Schleimhäute[73].

1.2.2.3 entzündungshemmender Effekt

Die entzündungshemmende Eigenschaft der Glycyrrhetinsäure ist schon in den ältesten Überlieferungen beschrieben. Eine mögliche Erklärung ist, dass die Glycyrrhetinsäure den Abbau von Glucocorticoiden hindert. Da diese u.a. entzündungshemmend sind, wird die Wirkung der Glucocorticoide verstärkt[75, 76]. Des weiteren gibt es eine zweite Erklärung, die den entzündungshemmenden Effekt erklärt: die Substanz greift in den klassischen Weg der

Komplementkaskade ein. KROES *et al.* konnten zeigen, dass die Glycyrrhetinsäure den C2-Komplementfaktor hemmt. In der Studie wurde weiterhin ersichtlich, dass die 3-β-Hydroxy-Funktion unerlässlich für die Aktivität ist; das Epimere hemmte den Faktor in geringerem Ausmaß[77].

1.2.2.4 antivirale Wirkung

Ein weiterer pharmakologischer Aspekt der Glycyrrhetinsäure ist ihre Wirkung gegen Viren. Dies konnte schon für verschiedene Virentypen *in vitro* als auch *in vivo* belegt werden. UTSUNOMIYA *et al.* zeigten in einer Studie mit Influenza Typ A_2 (H_2N_2) infizierten Mäusen, dass die Gabe von Glycyrrhetinsäure die Überlebensrate signifikant steigerte. Sie beobachteten dabei keinen direkten Einfluss auf den viralen Fortpflanzungszyklus. Vielmehr vermuteten sie, dass die Substanz die Produktion von Gamma-Interferonen durch T-Zellen fördert[78]. Da die Gamma-Interferone aktivierend auf Makrophagen wirken, ist eine höhere Immunantwort gegeben. ABE *et al.* deuteten dies schon in einer früheren Studie für Glycyrrhetinsäure an[79]. *In vitro*-Studien zeigten, dass Glycyrrhizinsäure das Wachstum der Viren beeinflusst, insbesondere im späten Stadium der Virenreplikation[80].

Ähnlich erfolgreich waren Studien mit Mäusen, welche mit Herpes-simplex-Enzephalitis infiziert waren. Verglichen mit einer Kontrollgruppe, konnte auch hier die Überlebensrate auf das 2,5-fache gesteigert werden. SEKIZAWA *et al.* sahen als Grund eine um 50 Prozent verringerte Virenreplikation[81]. Unter Laborbedingungen zeigte sich im Versuch mit Varizella-Zoster-Viren - Auslöser für Windpocken und Gürtelrose - dass Glycyrrhetinsäure ebenfalls die Replikation hemmt. Für den Selektivitätsindex wurde ein Wert von 30 bestimmt[82]. Der Selektivitätsindex gibt hierbei das Verhältnis vom ID_{50}-Wert für die DNS-Synthese der Wirtszelle zum ID_{50}-Wert der Virenreplikation an. Als Vergleich sei der Selektivitätsindex für das bekannte Mittel Aciclovir mit 600 gegeben[83]. Eine kombinierte Gabe von Glycyrrhetinsäure mit Lactoferrin zeigte einen synergetischen Effekt. Die Hemmung des Herpes simplex Virus-1 konnte dadurch gesteigert werden[84]. Untersuchungen mit dem Epstein-Barr-Virus wiesen nach, dass Glycyrrhetinsäure ebenfalls die Replikation dieser Viren hemmt. Mechanistische Studien von LIN deuteten auf eine Beeinflussung des Replikationszyklus in einem der ersten Schritte. Der bestimmte Selektivitätsindex für diese Virenart beträgt 120 und ist somit viermal höher als für das Varizella-Zoster-Virus[85].

Für das Zytomegalievirus - ein weiterer Vertreter der Herpesviren - konnten NUMAZAKI *et al.* gleichfalls eine hemmende Wirkung zeigen. Es wurde vermutet, dass Glycyrrhetinsäure die

Verbreitung von infizierten zu nicht infizierten Zelle verhindert[86]. Viele der Herpesviren sind sogenannte latente Viren, dass heißt nach einmaliger Infektion verbleiben die Viren ein Leben lang im Organismus. Sie sind in Zeiten ohne akute Krankheitssymptome in den Zellen vor dem Immunsystem versteckt, in Form von einigen Genen. Es wird vermehrt LANA gebildet, welches das p53-Protein in seiner Aktivität einschränkt. Damit ist der natürliche Selbstschutzmechanismus unterbunden und die Zelle bleibt mit ihrer latenten Vireninformation am Leben. Glycyrrhetinsäure verringert die Produktion von LANA, wodurch es zu einer Reaktivierung des p53-Proteins kommt. Der Mechanismus zur Initiierung der Apoptose wird wieder in Gang gesetzt[87].

Das humane Immundefizienz Virus - kurz HIV - beschäftigt die Wissenschaft nach wie vor ohne Unterlass. Aus diesem Grund gab es auch hier Studien, welche einen Effekt der Glycyrrhetinsäure auf diesen Virus untersuchten[88, 89]. Ito *et al.* konnten mittels radioaktiv markierten HIV-Partikeln nachweisen, dass die Substanz in der Lage ist, das Eindringen der Viren in T-Zellen des Typs MT-4 teilweise zu verhindern. Weiterhin wurden geklonte T-Zellen vom Typ Molt-4 verwendet, welche nach Infektion mit HI-Viren Riesenzellen bilden. Die Ausbildung ist somit ein makroskopisch leicht zu verfolgendes Zeichen für eine Infektion mit HI-Viren. Es zeigte sich, dass Glycyrrhetinsäure die Ausbildung dieser Riesenzellen bei einer Konzentration von 0,6 mM vollständig unterdrücken konnte.

Die Proteinkinase C scheint ein wichtiges Enzym zu sein, damit das Virus an die Zelle binden kann. Glycyrrhetinsäure hemmt dieses Enzym. Damit wäre eine Erklärung geliefert, warum die Substanz gegen HI-Viren wirkt; so vermuteten Ito *et al.*[90, 91]. Sie setzt weiterhin die Fluidität der Zellmembran herab, was möglicherweise das Eindringen der Viren erschwert[92]. Wie schon bei den Herpesviren hinderte Glycyrrhetinsäure eine Zell-zu-Zell-Infektion[93].

Zuletzt soll die Wirkung auf Hepatitis-Viren betrachtet werden. In Japan wird der Extrakt der Süßholzwurzel schon seit über 20 Jahren bei chronischer Leberentzündung verwendet[94]. Der Verdacht liegt somit nahe, dass Glycyrrhetinsäure auch gegenüber dieser Virenart aktiv ist. Bei Patienten mit chronischer Leberentzündung sind die Aktivitäten der Aspartat-Aminotransferase sowie der Alanin-Aminotransferase erhöht. Die Gabe von Glycyrrhetinsäure senkt diese Werte[95]. Eine verminderte Ausschüttung der beiden Enzyme resultiert aus einer geringeren Aktivität an Phospholipase A_2, verursacht durch Glycyrrhetinsäure[96]. Weiterhin konnte eine antioxidative Wirkung festgestellt werden, auf deren Grundlage eine leberschützende Funktion der Substanz vermutet wird[97, 98]. Takahara *et al.* wiederum zeigten, dass Glycyrrhetinsäure die Freisetzung des HBsAg verhindert, indem es den intrazellulären Transport des Antigens hemmt[99]. Analog zu Untersuchungen mit dem HI-Virus setzte die Substanz die Fluidität der Zellmembran herab. Die Endozytose von Hepatitis-A-Viren wurde dadurch erschwert[100].

Neben den aufgeführten Studien gibt es noch weitere mit anderen Virenstämmen, z.B. gegen Flaviviren[101] oder dem Erreger der Newcastle-Krankheit[102]. Daruf wird jedoch nicht weiter eingegangen.

1.2.2.5 Untersuchungen zur antikanzerogenen Wirkung

In einer der ersten Studien zur Zytotoxizität stellten Logemann *et al.* im Jahre 1960 fest, dass Glycyrrhetinsäure Leukämiezellen des Typs L1210 im Wachstum hindert[103]. Dies war ein Startschuss für viele Untersuchungen auf diesem Gebiet. Abe *et al.* lieferten einen ersten Hinweis darauf, wie die Substanz wirkt. Ihre Untersuchungen deuteten auf eine Hemmung des Überganges von der G_1- zur S-Phase im Zellzyklus[104]. Die Zellen verbleiben somit in einer permanenten Ruhephase.

Seitdem sind viele Studien zur Wirkungsweise der Glycyrrhetinsäure durchgeführt wurden. Es kristallisierten sich dabei erste Prinzipien heraus, jedoch sind nach wie vor viele Mechanismen ungeklärt. Im Mittelpunkt steht dabei der Apoptose induzierende Effekt der Glycyrrhetinsäure, welcher mittlerweile in vielen Studien belegt wurde[105]. Der nachfolgende Abschnitt soll eine Übersicht über die Vorgänge in der Zelle geben, die zur Apoptose führen - dem entzündungsfreien Tod der Zelle. Es wird dabei auf eine detaillierte Aufschlüsselung aller an der Apoptose beteiligten Prozesse verzichtet. Das Hauptaugenmerk liegt auf dem Teil, welcher durch die Glycyrrhetinsäure beeinflusst wird (Abb. 8).

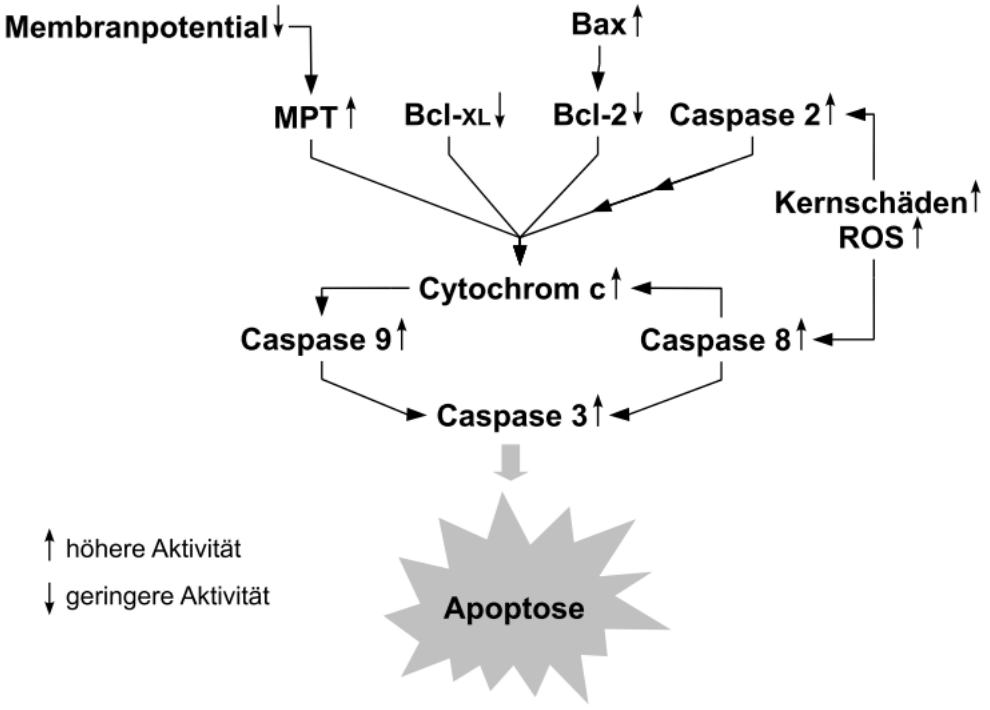


Abb. 8 *vereinfachtes Schema zur apoptotischen Wirkung der Glycyrrhetinsäure*

Ein Auslöser der Apoptose ist das Enzym Caspase 3. Sie ist eine von drei Effektorcaspasen - neben Caspasen 6 und Caspase 7. Lee *et al.* stellten eine direkte Aktivierung der Caspase 3 fest[106]. Andere Studien führten hingegen Caspase 8 an[107, 108]. Da Caspase 8 selbst die Caspase 3 beeinflusst, kann deren Aktivierung auch auf eine Initiierung von Caspase 8 zurückgehen. Auch die Zersetzung des Aktins und somit des Zytoskeletts sind eher eine Folge der Caspasenaktivierung, denn einer direkte Wirkung der Glycyrrhetinsäure[109, 110].

Neben dem direkten Einfluss auf die Caspasen wurde nachgewiesen, dass auch andere Proteine durch die Glycyrrhetinsäure beeinflusst werden können: z.B. die Proteine der Bcl-2-Familie. Es existieren anti- und pro-apoptotische Vertreter. Im Zusammenhang mit der Glycyrrhetinsäure werden Bcl-2 und Bcl-XL als anti-apoptotische sowie Bax und Bak als pro-apoptotische Proteine in der Literatur erwähnt. Beim Einfluss auf die Proteine Bax und Bak gibt es widersprüchliche Aussagen. Einmal bleiben sie beide unbeeinflusst[107], beim nächsten Mal erhöht sich die Aktivität von Bax im Test[106]. Bcl-2 sowie Bcl-XL werden durch die Glycyrrhetinsäure gehemmt[106, 107, 111]. In der Gesamtheit bewirkt dies eine vermehrte Ausschüttung von Cytochrom c - einem pro-apoptotischen Faktor im Mitochondrium - ins Zytosol. Dieses bindet an den Apaf-1 und der gebildete Komplex aktiviert Caspase 9, welche wiederum die apoptotische Kaskade über Caspase 3 in Gang setzt[112].

Das Cytochrom c ist ein Schlüsselglied zwischen Vorgängen im Mitochondrium und einer ausgelösten Apoptose. Änderungen des Membranpotentials beispielsweise führen zu einer erleichterten Diffusion von Substanzen durch die Membran. Nach einer Studie von Salvi *et al.* setzt Glycyrrhetinsäure das Membranpotential der Mitochondrien herab. Das Ergebnis ist eine MPT und es kommt somit zur Freisetzung von Cytochrom c und AIF[106, 113, 114]. Laut Battaglia *et al.* ist die Änderung des Membranpotentials jedoch konzentrationsäbhängig. In Experimenten mit Rattenherzmitochondrien zeigte sich, dass bei einer Glycyrrhetinsäurenkonzentration von unter 7,5 µM die Substanz gar vor MPT und oxidativem Stress schützt. Erst bei einer größeren Menge kam es zum MPT und der Freisetzung von Cytochrom c. Begründet wurde dies damit, dass Glycyrrhetinsäure den Transport von Calciumionen ins Mitochondrium hemmt. Andererseits kommt es zu Wechselwirkungen mit dem Eisen-Schwefel-Zentrum des Komplex I im Mitochondrium. In deren Ergebnis steht die Öffnung der MPT-Poren. Diese entgegengesetzten Effekte haben ihren Umkehrpunkt bei 7,5 µM, bei höheren Konzentrationen kommt es zum MPT[115]. Weitere Apoptose auslösende Faktoren können u.a. die Produktion von ROS oder Schäden an der DNS sein. Diese Faktoren aktivieren entweder direkt die Caspase 8 oder sorgen - über Aktivierung von Caspase 2 - für eine Ausschüttung von Cytochrom c[116]. ROS sind weitere vermutete Auslöser für eine induzierte Apoptose durch Glycyrrhetinsäure[106, 114, 115, 117]. Dabei steht

vor allem die Keto-Funktion in Position 11 im Fokus der Betrachtungen. FIORE at al. vermuteten, dass diese bei der Erzeugung von Hydroxylradikalen beteiligt ist. Die gebildeten Hydroxylradikale oxidieren Thiolgruppen, was schließlich eine Öffnung der MPT-Poren bewirkt[114].

Wie die Glycyrrhetinsäure im Endeffekt eine Apoptose induziert, ist also nach wie vor nicht vollständig geklärt. Man weiß z.B. nicht, ob die Substanz über externe oder interne Rezeptoren fungiert. Viele der aufgeführten Vorgänge lassen vermuten, dass sie in der Zelle wirksam wird. Allerdings existieren Studien, die eine Reversibilität zeigen: z.B. das erneute Wachstum von Tumoren[104] oder die Blockierung der GJC[118]. Da dieses reversible Verhalten bei einfachem Waschen der Zellen beobachtbar war, spricht dieser Umstand eher für eine externe Wirkung der Glycyrrhetinsäure.

1.2.2.6 wichtige Derivate

Im Gegensatz zu anderen Vertretern der Triterpensäuren, besitzt Glycyrrhetinsäure eine vergleichsweise geringe Zytotoxizität. Für Betulinsäure bzw. Oleanolsäure wurden beispielsweise IC_{50}-Werte von 10 bis 30 µM bestimmt[119], während Glycyrrhetinsäure Werte von über 60 µM liefert[120]. Aus diesem Grund war nicht nur das mechanistische Verständnis eine Fragestellung vieler Studien. In der Steigerung der Aktivität besteht ebenso großes Interesse. In der Literatur finden sich zwei strukturelle Änderungen, welche sehr erfolgreich waren.

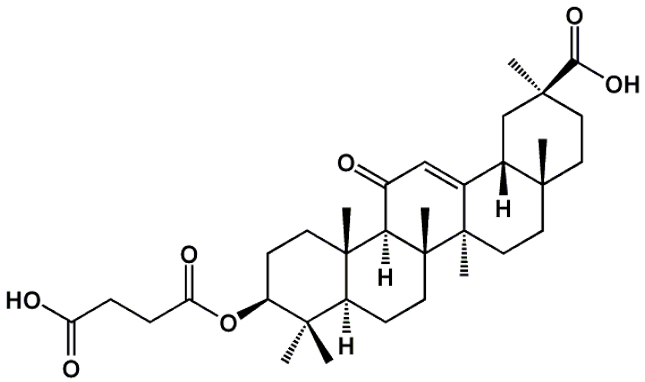


Abb. 9 *Struktur von Carbenoxolon*

Eine ist das Carbenoxolon (Abb. 9). Es wurde erstmals 1957 von Biorex Laboratories Ltd. synthetisiert[121]. Das 3-*O*-Hemisuccinat der Glycyrrhetinsäure - meist auch als Dinatriumsalz verwendet - ist deutlich besser wasserlöslich als seine Ausgangssubstanz[122]. Eigentlich als Mittel gegen Geschwüre entwickelt[121], zeigte es auch gegen Krebszellen einen Effekt. Ähnlich der Ausgangssubstanz induzierte Carbenoxolon eine Herabsetzung des mitochondrialen

Membranpotentials sowie die Oxidation von Pyridin- und Thiolnukleotiden. Durch Interaktion mit Komplex I und III kommt es zu einer vermehrten Ausschüttung von Cytochrom c, welches die Caspasen-Kaskaden auslöst. Eine verstärkte Produktion von Wasserstoffperoxid war ebenfalls beobachtbar. Salvi *et al.* vermuteten hier einen gleichen Mechanismus wie bei der Glycyrrhetinsäure[123]. Der Effekt konnte sowohl in hohen - 20 bis 200 µM - als auch in niedrigen Konzentrationsbereichen - 10 µM - beobachtet werden[123, 124]. Uyama *et al.* bestimmten in HSC-Mauszellen eine reduzierte DNS-Produktion sowie eine geringere Ausschüttung von Kollagen α1, welches an der Bildung von Fibrillen beteiligt ist[125].

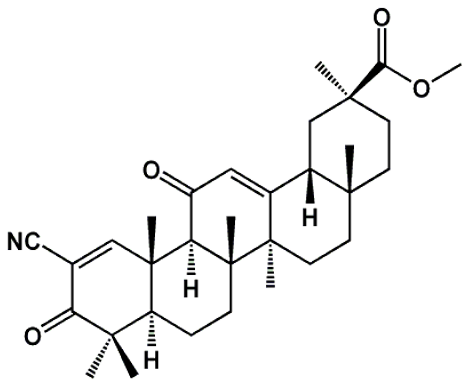


Abb. 10 *Struktur von CDODA-Me*

Das zweite Derivat ist der 2-Cyano-3,11-dioxo-18-olean-1,12-dien-30-säuremethylester (CDODA-Me, Abb. 10). Dieser zeigte eine deutlich höhere Zytotoxizität gegenüber Tumorzellen. Der IC_{50}-Wert beträgt 1,48 µM (HL-60) und ist somit wesentlich niedriger als 56,87 µM für die Glycyrrhetinsäure der selben Zelllinie. Für die Wirkung des Esters werden verschiedene Ursachen vermutet:

- Hemmung von miR-27a[126], wodurch die Zellen letztendlich in der G_2/M-Phase verbleiben
- Hemmung der anti-apoptotischen Proteine c-FLIP, XIAP und Mcl-1[127]
- Hemmung VEGF/VEGFR2 sowie mTOR/S6K[128]
- Aktivierung von Kinasen[129], PPARγ[130] oder pro-apoptotischem NAG-1[131]
- Verringerung des Membranpotentials der Mitochondrien[127]

Alle Punkte sollen letztendlich die Apoptose in den Zellen induzieren. Song *et al.* variierten das Derivat in der Stellung der Ketogruppe; die Doppelbindung befand sich hier an Position 11 und die Ketofunktion an Position 12. Die Aktivität konnte damit nochmals gesteigert werden: von 1,48 µM auf 0,36 µM bei HL-60 Zellen. Ebenfalls konnte eine höhere Effektivität bei der Induktion der

Apoptose festgestellt werden. Die Caspasen 8 und 9 werden vom neuen Derivat zehnfach besser aktiviert[127]. Logashenko *et al.* bestätigten die Wirkungssteigerung an mehreren Krebszelltypen[132]. Eine ähnliche Verbesserung erreichten Gao at el. ohne die Stellung der Ketogruppe zu verändern. Sie substituierten dazu mit Piperidinringen an Position 30 und erzielten im Maximum einen IC_{50}-Wert von 0,8 µM bei HL-60 Zellen[133]. Chadalapaka *et al.* wiederum experimentierten mit unterschiedlichen Substituenten an Position 2. Hier stachen der Nitril- und der Trichlormethylsubstituent heraus: sie lieferten IC_{50}-Werte von 0,25 µM bis 1,80 µM für die verwendeten Zelllinien[134].

Neben den zwei wichtigsten Derivaten gibt es noch zwei weitere strukturelle Änderungen an der Glycyrrhetinsäure. Lai *et al.* substituierten mit Furoxanen mittels eines Spacers an Position 3 und 30. Die Aktivität konnte dadurch bis auf einen IC_{50}-Wert von 0,25 µM bei BEL-7402 Leberkrebszellen gesteigert werden. Hingegen zeigten die Derivate auf LO2-Leberzellen kaum einen Effekt[135]. Eine Studie zu substituierten Oximen an Position 3 lieferte keine bemerkenswerte Verbesserung der Aktivität[120].

2. Zielstellung

Das Interesse an der Glycyrrhetinsäure ist nach wie vor ungebrochen. Insbesondere auf dem Gebiet der antikanzerogenen Forschung existieren inzwischen einige Derivate, die - verglichen mit dem Ausgangsstoff - eine deutlich höhere Zytotoxizität aufweisen. Weiterhin geben viele Studien einen Hinweis auf eine apoptotische Wirkung. Die Anzahl möglicher struktureller Variationen an Glycyrrhetinsäure ist jedoch überschaubar, was einen Nachteil zur Ableitung von Struktur-Aktivitäts-Beziehung darstellt.

Diese Arbeit soll helfen einen Beitrag dazu zu leisten, Auswirkungen struktureller Variationen auf die Zytotoxizität zu bestimmen. Aus diesem Grund sollen an verschiedenen Positionen des Moleküls Änderungen vorgenommen werden. Dabei werden zwei Hauptziele verfolgt. Zum Einen soll die Polarität und damit auch die Lipophilie des Gesamtmoleküls bzw. in einem Bereich des Moleküls verändert werden. Zum Anderen ist geplant am Grundgerüst des konjugierten Ringsystems Variationen vorzunehmen.

Viele Studien berichteten, dass die Bildung von ROS ein wesentlicher Grund für die Induzierung der Apotoptose durch Glycyrrhetinsäure ist. Der Zusammenhang zwischen Präsenz der Ketofunktion und einer hohen Zytotoxizität bzw. apoptotischen Wirkung soll ebenso geprüft werden.

Die Quantifizierung der Zytotoxizität wird dabei mittels Sulforhodamin-B-Assay (SRB-Assay) auf bis zu 15 humane Krebszelllinien und einer embryonale Mausfibroblasten (NiH3T3) bestimmt. Da neben der Zytotoxizität auch die apoptotoische Wirkung bedeutend ist, wird ebenfalls kontrolliert, ob diese von den synthetisierten Derivaten induziert wird. Dazu dienen Trypan-Blue-, DNA-Laddering- sowie Acridine-Orange/Ethidium-Bromid-Test.

3. Der Dissertation zugrunde liegende Veröffentlichungen und Manuskripte

3.1 Synthesis and antitumor activity of glycyrrhetinic acid derivatives *(Anhang A1)*

Schwarz, S.; Csuk, R. *Bioorg. Med. Chem.* **2010**, *18*, 7458-7474.

Abstract:

Glycyrrhetinic acid (GA) is one of many interesting triterpenoic acids showing anticancerogenic potential. GA is known to trigger apoptosis in tumour cell lines, although GA has a low cytotoxicity. In our study we were able to prepare derivatives of GA that show lowered the IC_{50} values as determined by a sulforhodamine B (SRB) assay using 15 different human tumour cell lines. Thus, combining an ester group combined with the presence of an amino acid moiety led to a ca. 60-fold improved antitumor activity. Experiments on mouse embryonic fibroblasts (NiH3T3) revealed that these compounds showed a better selectivity for tumour cells compared to the parent compound GA. An apoptotic effect of some of these compounds was determined using an acridine orange/ethidium bromide (AO/EB) test and DNA laddering experiments.

3.2 Synthesis and biological activity of some antitumor active derivatives from glycyrrhetinic acid *(Anhang A2)*

Csuk, R.; Schwarz, S.; Kluge, R.; Ströhl, D. *Eur. J. Med. Chem.* **2010**, *45(12)*, 5718-5723.

Abstract:

Aminoalkyl substituted derivatives were synthesized starting from glycyrrhetinic acid methyl ester and screened for antitumor activity in a panel of 15 human cancer cell lines by an SRB assay. The most compound 7 possesses an aminohexyl side chain, induces apoptosis and shows IC_{50} values of 0.6 - 3.0 mM.

3.3 Synthesis and antitumor activity of ring A-modified glycyrrhetinic acid derivatives *(Anhang A3)*

Csuk, R.; Schwarz, S.; Siewert, B.; Kluge, R.; Ströhl, D. *Z. Naturforsch.* **2011**, *66b*, 521-532.

Abstract:

The pentacyclic triterpene glycyrrhetinic acid is an interesting natural product exhibiting various biological activities. Especially its ability to induce apoptosis in tumor cells is of high scientific interest. In this study we altered the lipophilicity in ring A by derivatization at positions C-2 and C-3. The consequences of these variations on the cytotoxicity were investigated applying a colorimetric sulforhodamine B assay using 8 different human tumor cell lines. An acridine orange/ethidium bromide (AO/EB) test and a trypan blue test were used to determine the extent of apoptotic activity of some of these compounds.

3.4 Improvement of the Cytotoxicity and Tumor Selectivity of Glycyrrhetinic Acid by Derivatization with Bifunctional Amino Acids *(Anhang A4)*

Csuk, R.; Schwarz, S.; Kluge, R.; Ströhl, D. *Arch. Pharm. Chem. Life Sci.* **2011**, **im Druck**.

Abstract:

Various glutamyl and aspartyl substituents were selected for the synthesis of C(3) esters of GA methyl ester to improve the poor cytotoxicity of GA. A short (3-5 steps) synthesis was elaborated. Compound (5) having a glutamyl substituent with a benzyl protected side chain showed up to 67-fold higher cytoxicity and an up to 140-fold better selectivity towards tumor cells than parent GA.

3.5 Does one keto group matter ? Structure-activity relationships of glycyrrhetinic acid derivatives modified at position 11 *(Anhang A5)*

Csuk, R.; Schwarz, S.; Kluge, R.; Ströhl, D. *Arch. Pharm. (Weinheim)*, **eingereicht**.

Abstract:

Several triterpenoic acids display a remarkable cytotoxicity on tumor cells. Glycyrrhetinic acid - the main content of licorice root - possesses an apoptotic effect on tumor cells. Previous studies pointed out the presence of a keto group at position C-11 in glycyrrhetinic acid derivatives as the main reason for its apoptotic activity. Several pairs of derivatives were synthesized differing only at position C-11. These compounds were tested in a sulforhodamine B colorimetric assay for cytotoxicity screening on 12 tumor cell lines and mouse embryonic fibroblasts (NiH3T3). Our results show there is no direct relation between the existence of the C-11 keto group and the apoptotic activity of the compounds.

3.6 Synthesis and antitumor activity of ring A modified glycyrrhetinic acid derivatives *(Anhang A6)*

Csuk, R.; Schwarz, S.; Siewert, B.; Kluge, R.; Ströhl, D. *Bioorg. Med. Chem.*, **eingereicht**.

Abstract:

Triterpenoic acids show many pharmacological effects, among them an antiinflammatory or an antitumor activity. One of these, glycyrrhetinic acid (**1**) is of interest because of its antitumor profile. Glycyrrhetinic acid is not only cytotoxic but also triggers apoptosis in various human tumor cell lines. To improve the cytotoxicity of parent **1** we set out to synthesize new derivatives of it - differing in structure and lipophilicity. These compounds were tested in a sulforhodamine B assay for cytotoxicity, and screened for their ability to induce apoptosis using an acridine orange/ethidium bromide assay and trypan blue staining. The most active compound, **34**, a benzyl glycyrrhetinate holding an extra 3-N-(3-aminopropyl)glycyl substituent showed IC_{50} between 1.96 - 5.14 μM for five human cancer cell lines and triggers apoptosis in 80 % of the cells.

3.7 Conversions at C-30 of glycyrrhetinic acid and their impact on antitumor activity *(Anhang A7)*

Csuk, R.; Schwarz, S.; Siewert, B.; Kluge, R.; Ströhl, D. *Arch. Pharm. (Weinheim)*, **eingereicht**.

Abstract:

The extracts of the roots of licorice have been used in traditional and folk medicine to treat a broad variety of maladies. The main ingredient of these extracts is glycyrrhicinic acid. Its aglycon, glycyrrhetinic acid, has many biological activities, among them a pronounced cytotoxicity against tumor cells. In this study we varied glycyrrhetinic acid at position C-30 to get “simple” derivatives, for example esters, amides and a nitrile. The influence of these changes on the cytotoxic activity is noteworthy and was determined by a colorimetric sulphorhodamine B test using 7 human tumor cell lines and mouse embryonic fibroblasts (NiH3T3) for comparison. A trypan blue test as well as an acridine orange/ethidium bromide test was used to discover the ability of the compounds to induce apoptosis.

3.8 Manuskript in Bearbeitung: "A Natural Approach: Synthesis and antitumor activity of Glycyrrhetinic Acid glycosides" *(Anhang A8)*

Schwarz, S.; Siewert, B.; Csuk, R.; Xavier, N., M.; Jesus, A., R.; Rauter, A., P.
Manuskript in Bearbeitung.

Abstract:

There are a couple of pentacyclic triterpenoic acids showing antitumour activity; best-known examples are betulinic acid and oleanic acid. Another interesting representative of this class of compounds, glycyrrhetinic acid (**GA**) is not as active as betulinic acid, but **GA** is still in the focus of scientific interest. It triggers apoptosis in tumour cells and is easy to obtain from the extracts of licorice roots. The incorporation of an extra hydrophilic moiety in the molecule might increase the cytotoxicity of the compounds. Hence, a series of compounds was synthesized possessing an additional hexose and a pentose moiety (D and L) in position 3, paralleling **GA** natural occurrence occurs as glycyrrhizinic acid - a glycoside consisting of one molecule of **GA** and two molecules of glucuronic acid.

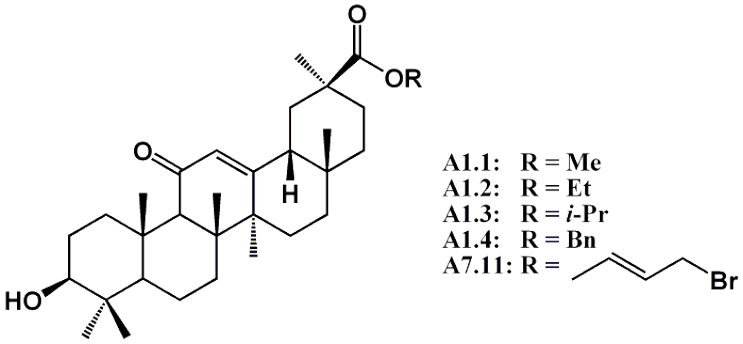
4. Zusammenfassung

Im Rahmen dieser Arbeit wurden mehrere Variationen an Glycyrrhetinsäure vorgenommen. Die Derivate unterschieden sich dabei in vielerlei Hinsicht, sei es am Oleanan-Grundgerüst oder in der Lipophilie. Alle dargestellten Derivate wurden vollständig charakterisiert und ihre Struktur eindeutig bestimmt. Die Änderungen der Zytotoxizität wurden anhand eines Sulforhodamin-B-Assays dokumentiert; dafür standen verschiedene Zelllinien zur Verfügung: bis zu 15 humane Tumorzelllinien sowie embryonale Mausfibroblasten (NiH3T3). Ausgewählte Verbindungen - in der Regel die Aktivsten - wurden auf eine apoptotische Wirkung geprüft. Dabei dienten DNA-Laddering, Trypan-Blue- bzw. Acridin Orange/Ethidium Bromid-Test als Methoden. Für die Referenz Glycyrrhetinsäure wurden zunächst IC_{50}-Werte bestimmt und mittels der genannten Tests die apoptotische Wirkung nachgewiesen sowie deren Grad ermittelt. Die IC_{50}-Werte betragen 74,57 bis 86,80 µM bezüglich den Tumorzelllinien und IC_{50} = 18,52 µM bezüglich der Fibroblasten. Die Selektivität (F) ergibt sich aus dem Quotienten vom IC_{50}-Wert hinsichtlich der Fibroblasten und dem IC_{50}-Wert hinsichtlich der Tumorzelllinien. Er beträgt für die Glycyrrhetinsäure F = 0,21 bis 0,25. Der Grad der Apoptose wurde zu 73,73 % bestimmt.

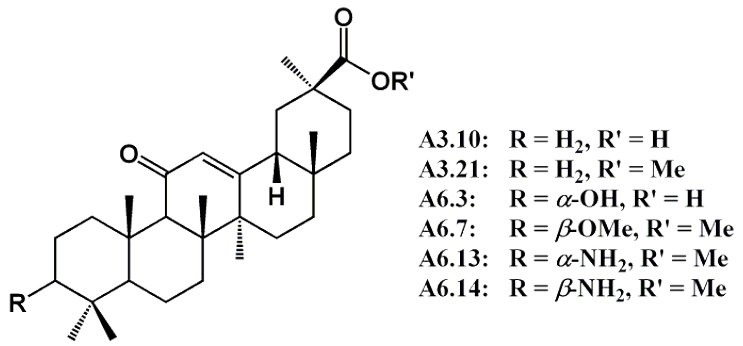
Die Ergebnisse lassen sich in folgende Bereiche gliedern:

1. Variationen an den drei funktionellen Gruppen des Moleküls
2. strukturelle Änderungen am Oleanan-Grundgerüst
3. Beeinflussung der Lipophilie des Moleküls

Eine Fragestellung der Arbeit war, ob die früher postulierte, entscheidende Rolle der 11-Keto-Funktion für die Zytotoxizität bestätigt werden kann. Dafür wurden Paare von Derivaten mit reduzierter bzw. intakter Ketofunktion verglichen (Anhang A5). Die ermittelten IC_{50}-Werte lassen den Schluss zu, dass die Ketofunktion nicht der entscheidende Bestandteil für die apoptotische Wirkung der Glycyrrhetinsäure ist. Fiore et al. vermuteten dies aufgrund einer nachgewiesenen Produktion von ROS[114]. Beim Vergleich der Aktivitäten der reduzierten Derivate mit den Referenzen zeigt sich jedoch, dass die desoxygenierten Verbindungen gleich oder etwas weniger aktiv sind. Wäre die Ketofunktion der entscheidende Faktor, so müsste die Abnahme der Aktivität bedeutend größer sein. Sie scheint somit nicht direkt an der Entwicklung von ROS beteiligt zu sein oder ihre Bedeutung bei der induzierten Apoptose ist geringer als bisher angenommen. Die geringen Änderungen der Zytotoxizität in diesem Vergleich könnten allein aus der neuen Struktur von Ring C resultieren.



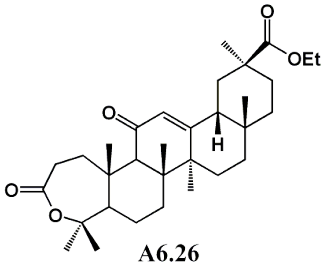
Neben der Ketofunktion wurde der Einfluss der Position 30 untersucht (Anhang A1 und A7). Dabei konnten verschiedene Ester und Amide sowie das Nitril dargestellt werden. Prinzipiell ist festzustellen, dass Veresterungen bzw. lipophile Endgruppen die Zytotoxizität steigert. So sinkt der IC_{50}-Wert mit Erhöhung des lipophilen Restes in der Reihe: Methyl- (**A1.1**†), Ethyl-(**A1.2**), *iso*-Propyl- (**A1.3**) und Benzylrest (**A1.4**). Der Benzylester (**A1.4**) besitzt eine maximale Aktivität von IC_{50} = 2,74 µM (F = 7,67) hinsichtlich SW1736-Zellen. Er ist damit 28-fach zytotoxischer als die Glycyrrhetinsäure bei etwa gleichbleibender Aktivität bezüglich der Mausfibroblasten (18,52 µM für Glycyrrhetinsäure zu 21,02 µM für **A1.4**). Während die Amide keine nennenswerten IC_{50}-Werte unter 30 µM liefern, erhöhen eingeführte, ungesättigte Alkylketten - mit oder ohne Halogensubstituent - ebenfalls die Aktivität. Das Derivat **A7.11** besitzt hierbei die höchste Zytotoxizität: IC_{50} = 1,88 µM auf SW1736-Zellen.



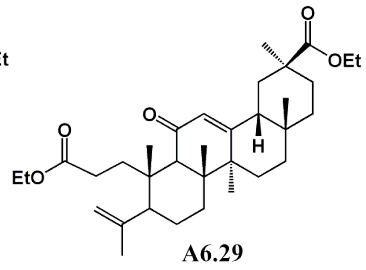
Im Gegensatz zu den Positionen 11 und 30 ist eine generelle Aussage zur Bedeutung der Position 3 nicht möglich (Anhang A3 und A6). Das 3-Epimer der Glycyrrhetinsäure **A6.3** zeigt keine Aktivität unter 30 µM. Die Desoxigenierung an Position 3 liefert gegensätzliche Aussagen für die Derivate **A3.10** und **A3.21**. Im Vergleich zur Glycyrrhetinsäure ist bei **A3.10** eine Aktivitätssteigerung zu beobachten, während **A3.21** für nahezu jede Zelllinie weniger aktiv ist als der Methylester **A1.1**.

† Bezeichnung nach folgendem Muster: A1.1 = Anhang 1.Substanz 1

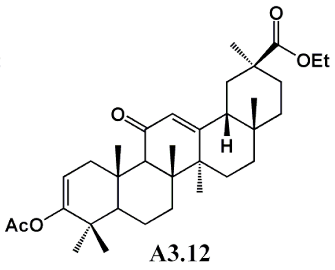
Des Weiteren wurden Derivatisierungen an Position 3 unternommen, um die Bedeutung dieser näher zu untersuchen. Dazu wurde oxidiert, mit Acetyl- sowie Methansulfonyl-Resten substituiert, methyliert und das Oxim bzw. Amin dargestellt (Anhang A3 und A6). Zunächst zeigen weder die Acetyl- bzw. Methansolfunyl- noch die oxidierten Derivate Aktivitäten, die höher waren als jene der Referenzsubstanzen. Die Amine und das Oxim waren jedoch aktiver als der entsprechende Methylester **A1.1**. Insbesondere die Amine lieferten IC_{50}-Werte von 2,33 bis 3,42 µM und einen apoptotischen Grad von 64,88 % (**A6.13**) bzw. 74,21 % (**A6.14**). Das 3-Methoxy-Derivat **A6.7** konnte ebenfalls dargestellt werden; dieses war jedoch nicht unter den Bedingungen des SRB-Assays löslich.



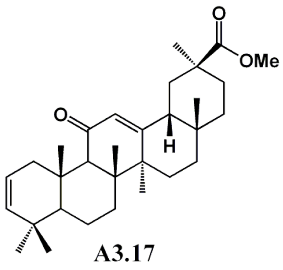
A6.26



A6.29

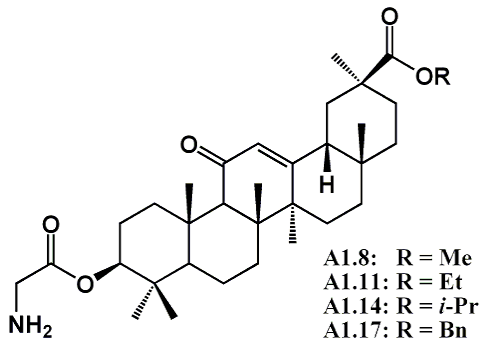


A3.12

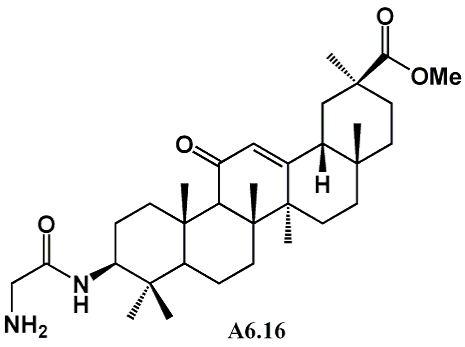


A3.17

Weitere Variationen am Ring A waren die Öffnung des Ringsystems (Anhang A6) sowie die Einführung von sp^2-hybridisierter Zentren an den Positionen 1 bis 3 (Anhang A3). Abgesehen von wenigen Ausnahmen, ist dabei keine Verbesserung der Aktivität zu sehen. Von den geöffneten Derivaten sind lediglich **A6.26** und **A6.29** aktiver als der entsprechende Ester und dabei auch nur bei einzelnen Zelllinien. Analog dazu zeigen die Substanzen mit eingeführten sp^2-hybridisierter Zentren kaum höhere Zytotoxizitäten. Ausnahmen bilden beispielsweise die Substanzen **A3.12** oder **A3.17**. Verglichen mit dem Methylester **A1.1** ist die Aktivität allerdings maximal doppelt so hoch (13,24 µM für **A3.12** zu 34,82 µM für **A1.1** auf SW1736-Zellen).



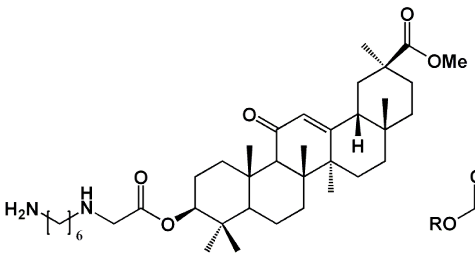
A1.8: R = Me
A1.11: R = Et
A1.14: R = *i*-Pr
A1.17: R = Bn



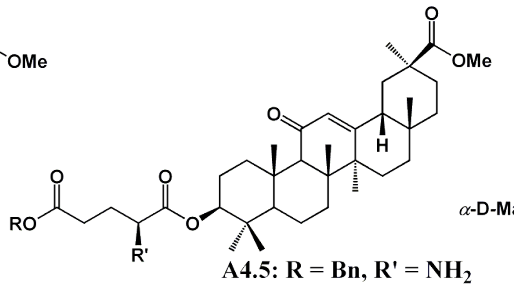
A6.16

Mit dem Ziel die Lipophilie des Moleküls maßgeblich zu beeinflussen, wurden verschiedene Substituenten ins Molekül eingeführt. Es ist zu beobachten, dass insbesondere Aminogruppen am Ring A die Zytotoxizität steigern (Anhang A1 und A6). Die Art der Substitution - sei es direkt am Ringsystem oder über einen Spacer - scheint dabei keine bedeutende Rolle zu spielen. Sowohl die 3-Amino-Verbindungen **A6.13** und **A6.14** (IC_{50} = 2,32 bis 3,42 µM) als auch z.B. die 3-Glycinderivate **A1.8**, **A1.11**, **A1.14** und **A1.17** (IC_{50} = 2,01 bis 8,59 µM) besitzen ähnliche Aktivitäten. Das Amid **A6.16** ist allerdings weniger aktiv als das entsprechende unsubstituierte Amin **A6.13**. Weiterhin hat die Stereoisomerie des substituierten Alanyl-Restes keinen Einfluss auf die Aktivität. Der steigernde Effekt der eingeführten Aminogruppen auf die Zytotoxizität verschwindet jedoch, wenn die Carboxylgruppe an Position 30 nicht verestert vorliegt. Die Hydrochloride sind verglichen mit den freien Aminen gleich aktiv, allerdings wesentlich hydrophiler. Aus diesem Grund wären sie für eventuelle *in vivo*-Tests interessant, weil dadurch eine höhere Bioverfügbarkeit erreicht werden kann.

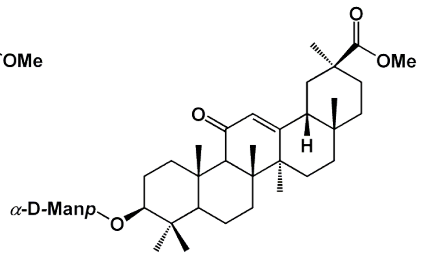
Unter Berücksichtigung des Konzepts, dass Aminogruppen die Zytotoxizität erhöhen können, wurde auf zwei Wegen die Aktivität weiter gesteigert.



A2.7



A4.5: R = Bn, R' = NH_2
A4.9: R = H, R' = $NH_3^+Cl^-$



A8.5

Zum Einen erhöht sich die Zytotoxizität durch das Einführen einer Diaminokette (Anhang A2). Es zeigt sich, dass die Aktivität von der Kettenlänge des Substituenten abhängt. Die maximale Aktivität ist bei einer Länge von 6 Kohlenstoffatomen erreicht: die IC_{50}-Werte liegen dabei zwischen 0,59 und 2,25 µM. Im Vergleich zum entsprechenden Methylester **A1.1** ist das Derivat **A2.7** bis zu 47-fach, verglichen mit Glycyrrhetinsäure bis zu 136-fach zytotoxischer.

Darüber hinaus wurde die Aktivität durch Substitution mit bifunktionalen Aminosäuren ebenfalls gesteigert (Anhang A4). Das Derivat **A4.5** zeigt IC_{50}-Werte von 1,27 bis 2,33 µM und ist damit bis zu 67-fach aktiver als die Glycyrrhetinsäure und bis zu 141-fach selektiver (F = 30,78). Der apoptotische Grad beträgt 86,10 %. Das entsprechende ungeschützte Derivat **A4.9** besitzt wiederum einen IC_{50}-Wert von über 100 µM und ist somit inaktiv, was mit der vorherigen Beobachtungen konform geht.

Ein weiterer Versuch lipophile Substituenten einzuführen, war die Synthese von Glykosiden an Position 3 (Anhang A8). Obwohl dies dem natürlichen Vorbild folgt, zeigen sich keine Steigerungen in der Aktivität. Bis auf das Mannosid **A8.5** besitzt keine der Substanzen eine höhere Zytotoxizität als die Referenz **A1.1**.

Zusammenfassend ist damit zu sagen, dass generelle Aussagen zum Einfluss der strukturellen Änderungen auf die Zytotoxizität gemacht werden konnten. Dabei erwies sich die 11-Keto-Funktion als die funktionelle Gruppe mit dem geringsten Einfluss auf die Aktivität. Sowohl die Steigerung der Zytotoxizität als auch der Selektivität gelang an mehreren Beispielen, z.B. **A4.5** (IC_{50} = 1,27 µM auf MCF-7-Zellen) oder **A2.7** (IC_{50} = 0,59 µM auf HT-29-Zellen). Die Selektivität konnte auf bis zu F = 30,78 erhöht werden (**A4.5**). Im Vergleich mit beispielsweise dem Cisplatin (IC_{50} = 1,33 µM auf FaDu-Zellen)[136], zeigt sich das Potential dieser Verbindungen in Konkurrenz zu gängigen Antitumor-Wirkstoffen.

Die aktivsten Substanzen stellen somit ein Ziel für weitere biochemische Untersuchungen zur Wirkungsweise in der Zelle dar, da sie leicht und preisgünstig synthetisiert werden können. Um eine medizinische Nutzung voranzutreiben, wäre es nun notwendig, die stark zytotoxischen Substanzen *in vivo*-Tests zu unterziehen.

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Manuskripte

Anhang A1

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Synthesis and antitumour activity of glycyrrhetinic acid derivatives

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ABSTRACT

Glycyrrhetinic acid (GA) is one of many interesting triterpenoic acids showing anticancerogenic potential. GA is known to trigger apoptosis in tumour cell lines, although GA has a low cytotoxicity. In our study we were able to prepare derivatives of GA that show lowered the IC_{50} values as determined by a sulforhodamine B (SRB) assay using 15 different human tumour cell lines. Thus, combining an ester group combined with the presence of an amino acid moiety led to a ca. 60-fold improved antitumor activity. Experiments on mouse embryonic fibroblasts (NiH3T3) revealed that these compounds showed a better selectivity for tumour cells compared to the parent compound GA. An apoptotic effect of some of these compounds was determined using an acridine orange/ethidium bromide (AO/EB) test and DNA laddering experiments.

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1. Introduction

Glycyrrhetinic acid (GA) is not the most potent antitumour active triterpenoic acid; several other triterpenes, for example, betulinic acid,[1–3] are better known for their antitumour activity. GA, however, is still of special interest because of its apoptotic effect on to tumour cells. In addition, the extract of the licorice roots contains up to 24% GA—mainly as glycosidic glycyrrhizic acid.[4] Therefore, compared to other triterpenoic acids, GA can be obtained cheaper and in a higher amount than, for example, betulinic acid (2.44%,[5] 3.3%[6]) or oleanoic acid (3.1%[5]). Improving the antitumour activity of GA without forfeiting its apoptotic effect is one of the big challenges when dealing with the derivatization of GA. We thought that changing the polarity pattern of the molecule might be of some interest; introducing an extra amino group (for gain of polarity) and preparing esters of GA (for gain of lipophilicity) seemed appropriate.

Thus, we synthesized 25 derivatives of GA—esterified at C(30) and varied by coupling with an amino acid at C(3)—and compared their biological activity (IC_{50} values) using a sulforhodamine B assay (SRB assay) with up to 15 human tumour cell lines and mouse embryonic fibroblasts NiH3T3; extra acridine orange/ethidium bromide tests (AO/EB) were performed to test for an apoptotic behaviour.

2. Results

2.1. Influence of an ester moiety

Different esters of GA differing in the alcoholic part (Fig. 1) were prepared by reacting GA with alkyl halides in DMF in the presence of potassium carbonate.[7] We were able to synthesize the *tert*-butyl ester, too. However, this compound is unstable under the conditions of the SRB assay test and was excluded from this study.

In the SRB assay the esters showed an improved activity on to tumour cell lines (Fig. 2, left and Table 1). The most active substance was the benzyl ester showing IC_{50} values between 2.7–25.2 μM. The toxicity of these compounds for the mouse embryonic fibroblasts remained almost constant throughout this series (Fig. 2, right) except for the *i*-propyl ester that shows a lowered activity; the decrease in the IC_{50} values parallels the size and lipophilic character of the alkyl chain of the ester.

As far as GA is concerned, we found a more than four times higher toxicity on mouse embryonic fibroblasts than on tumour cell lines. A maximum of selectivity towards the tumour cells, however, was established for compound **4**; this derivative is approx. eight times more toxic for the SW1736 thyroid carcinoma (IC_{50} = 2.74 μM) than for the fibroblasts (IC_{50} = 21.02 μM).

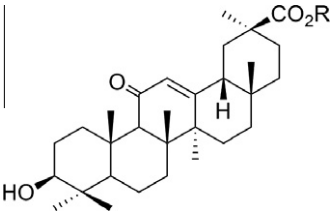

Figure 1. Esters of GA: **1**: R = Me; **2** R = Et; **3**: R = *i*-Pr; **4**: R = Bn.

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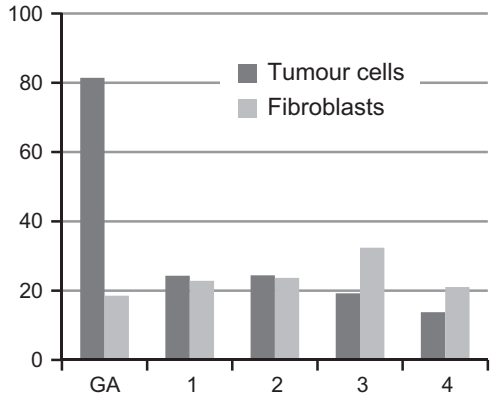

Figure 2. IC_{50} values (in μM) for GA and its esters **1**–**4** for tumour cells (averaged, 15 cell lines) and mouse fibroblastic cells (NiH3T3).

2.2. Amino acid derivatives of GA

In this series of compounds we introduced a polar proton acceptor group in position C(3). These compounds were synthesized by DCC mediated esterifications of *N*-Boc protected amino acids (glycine, D-alanine and β-alanine) followed by their deprotection using TFA in dry DCM[8] (for the amines) or by treating them with dry HCl gas in DCM (for the ammonium hydrochlorides).

For all esters (Tables 2 and 3 and Fig. 4), activity increases when C(3) is substituted with one of the three amino acids. In this series, the glycine substituted *i*-propyl ester was shown the most active compound; the ethyl ester, however, is the most active compound when a L-alaninyl or a β-alaninyl substituent was introduced. Interestingly enough, in this series of compounds the derivatives of β-alanine show the highest activity except for **14**. On average, the lowest IC_{50} values were obtained for **16** (substituted with *i*-propyl and β-alanine). All derivatives holding a free carboxylic group in position C(30) gave IC_{50} values >30 μM and were excluded from further investigations.

From the selected amino acids, the derivatives of β-alanine show the highest activity. The sole exception is derivative **14** (av 2.49 μM), substituted with glycine and esterified with *i*-propyl. The lowest average of the IC_{50} value we determined was for substance **16**, substituted with *i*-propyl and β-alanine. All derivatives having a free carboxyl group in position C(30) gave IC_{50} values higher than 30 μM; thus, they were excluded from further investigations.

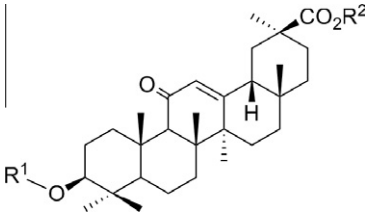

Figure 3. Amino acid derivatives of GA and of GA esters.

A priori, a dependency between biological activity and a counter-ion cannot be ruled out. Thus, to find out that the biological data obtained from amines and their respective ammonium hydrochlorides are comparable, we checked whether there is a difference in their cytotoxic activity. As a representative example, the methyl esters of the amino acid derivatives were investigated; the results from the SRB assay are compiled in Table 4.

These results suggest there is no significant difference in the biological activity between amines and their respective ammonium salts.

As far as the *N*-Boc protected derivatives are concerned, three derivatives (**23**–**25**) were tested in the SRB assay; all of them gave an IC_{50} >30 μmol.

2.3. Influence of a stereogenic centre

To work out if the stereogenic centre introduced in the amino acid derivatives of GA has any influence on the biological activity several derivatives containing a D-alaninyl residue were prepared and compared (SRB assay, 15 different tumour cell lines) with their respective L-alaninyl diastereomers. The data for this series are summarized in Table 5. In summary, the effect of the stereogenic centre is small and varies with the cell lines.

2.4. Apoptotic study using the AO/EB test and DNA laddering

As previously shown,[9–12] GA induces apoptosis. To prove whether this feature remains or is lost on derivatization, we tested four compounds for an induction of apoptosis in an AO/EB test. Figure 5 (left) depicts a section of A549 cells after being treated with GA. As expected, the cells showed apoptotic behaviour; this can easily be seen from the green fluorescence of the nucleus. The more active compounds **8**–**10** and **25** were investigated in the same way. In all of these samples, apoptotic cells were detected. Although this assay doesn't allow to quantify the ratio

Table 1
Results from the SRB assay for esters of glycyrrhetinic acid (results given in μM)

Derivative/cell line	GA	1	2	3	4
518A2	83.92 ± 4.20	27.54 ± 1.38	25.23 ± 1.26	18.15 ± 0.91	18.19 ± 0.91
8505C	86.50 ± 4.33	26.07 ± 1.30	24.58 ± 1.23	14.24 ± 0.71	8.10 ± 0.41
A253	80.78 ± 4.04	19.42 ± 0.97	25.04 ± 1.25	15.76 ± 0.79	10.67 ± 0.54
A2780	74.57 ± 3.73	25.54 ± 1.28	26.96 ± 1.35	24.95 ± 1.25	20.32 ± 1.02
A431	79.58 ± 3.99	25.28 ± 1.26	23.45 ± 1.17	32.01 ± 1.60	23.58 ± 1.18
A549	82.76 ± 4.14	23.50 ± 1.18	22.74 ± 1.14	14.41 ± 0.72	6.15 ± 0.31
DLD-1	81.21 ± 4.06	26.12 ± 1.31	28.14 ± 1.41	27.61 ± 1.38	22.69 ± 1.13
FaDu	84.55 ± 4.23	23.41 ± 1.17	23.76 ± 1.19	14.42 ± 0.72	5.48 ± 0.27
HCT-116	78.83 ± 3.94	22.10 ± 1.11	21.58 ± 1.08	26.44 ± 1.32	20.57 ± 1.03
HCT-8	78.85 ± 3.94	24.36 ± 1.22	43.42 ± 2.17	24.12 ± 1.21	25.23 ± 1.26
HT-29	80.09 ± 4.00	27.54 ± 1.38	22.14 ± 1.11	15.97 ± 0.80	11.48 ± 0.57
Lipo	81.44 ± 4.07	20.47 ± 1.02	27.66 ± 1.38	15.93 ± 0.80	11.54 ± 0.58
MCF-7	84.70 ± 4.24	22.14 ± 1.11	18.61 ± 0.93	16.04 ± 0.80	13.49 ± 0.67
SW1736	76.93 ± 3.85	34.87 ± 1.74	13.37 ± 0.67	12.77 ± 0.64	2.74 ± 0.14
SW480	86.80 ± 4.34	16.08 ± 0.80	19.10 ± 0.96	15.33 ± 0.77	6.17 ± 0.31
Average	81.43 ± 4.07	24.30 ± 1.22	24.39 ± 1.22	19.21 ± 0.96	13.76 ± 0.69
NiH3T3	18.52 ± 0.93	22.81 ± 1.14	23.66 ± 1.18	32.37 ± 1.62	21.02 ± 1.05

Table 2
Modifications of GA at positions C(3) and C(30)

Compd	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
R^1	Gly·HCl	L-Ala·HCl	β-Ala·HCl	Gly	L-Ala	β-Ala	Gly	L-Ala	β-Ala
R^2	H	H	H	Me	Me	Me	Et	Et	Et
	14	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**
R^1	Gly	L-Ala	β-Ala	Gly	L-Ala	β-Ala	Gly·HCl	L-Ala·HCl	β-Ala·HCl
R^2	*i*-Pr	*i*-Pr	*i*-Pr	Bn	Bn	Bn	Me	Me	Me

Table 3
IC_{50} values (in μM) for GA derivatives substituted with amino acids in position C(3) and esterified in C(30) (Fig. 3)

Cell lines/derivatives	8505C	A253	A2780	A549	DLD-1	Lipo	Average
5	>30	>30	>30	>30	>30	>30	>30
6	>30	>30	>30	>30	>30	>30	>30
7	>30	>30	>30	>30	>30	>30	>30
8	7.45 ± 0.37	6.26 ± 0.31	5.99 ± 0.30	6.42 ± 0.32	8.59 ± 0.43	7.54 ± 0.38	7.04 ± 0.35
9	4.31 ± 0.22	3.61 ± 0.18	2.98 ± 0.15	2.77 ± 0.14	4.49 ± 0.22	4.30 ± 0.22	3.74 ± 0.19
10	2.55 ± 0.13	2.50 ± 0.13	1.72 ± 0.09	2.40 ± 0.12	2.51 ± 0.13	2.52 ± 0.13	2.37 ± 0.12
11	5.32 ± 0.27	3.59 ± 0.18	3.90 ± 0.20	5.39 ± 0.27	5.61 ± 0.28	4.32 ± 0.22	4.69 ± 0.23
12	3.87 ± 0.19	2.33 ± 0.12	2.59 ± 0.13	3.43 ± 0.17	3.72 ± 0.19	2.74 ± 0.14	3.11 ± 0.16
13	2.32 ± 0.12	2.23 ± 0.11	1.77 ± 0.09	2.18 ± 0.11	2.74 ± 0.14	2.38 ± 0.12	2.27 ± 0.11
14	2.76 ± 0.14	2.01 ± 0.10	2.24 ± 0.11	2.65 ± 0.13	2.54 ± 0.13	2.74 ± 0.14	2.49 ± 0.12
15	3.49 ± 0.17	3.51 ± 0.18	2.08 ± 0.10	3.43 ± 0.17	5.54 ± 0.28	3.53 ± 0.18	3.60 ± 0.18
16	1.96 ± 0.10	2.68 ± 0.13	1.31 ± 0.07	1.78 ± 0.09	3.52 ± 0.18	3.49 ± 0.17	2.46 ± 0.12
17	4.79 ± 0.24	5.03 ± 0.25	3.54 ± 0.18	5.07 ± 0.25	4.54 ± 0.23	4.81 ± 0.24	4.63 ± 0.23
18	3.10 ± 0.16	3.49 ± 0.17	2.85 ± 0.14	3.51 ± 0.18	5.02 ± 0.25	3.57 ± 0.18	3.59 ± 0.18
19	3.19 ± 0.16	3.05 ± 0.15	1.73 ± 0.09	2.76 ± 0.14	4.54 ± 0.23	3.25 ± 0.16	3.09 ± 0.15

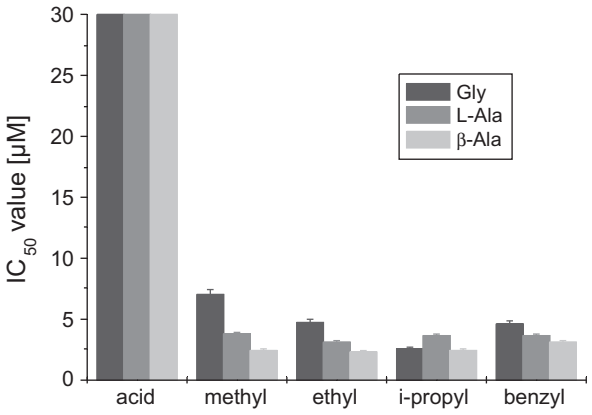

Figure 4. IC_{50} values (in μM) for the amino acid modified GA derivatives (averaged values, six tumour cell lines).

between apoptotic and necrotic cells, the ability of the compounds to trigger apoptosis is displayed unambiguously. Figure 5 (left to right) show sections from the samples showing the characteristic green fluorescent nucleus.

During apoptosis DNA is cleaved[13–15] into smaller fragments by endonucleases. These fragments are observable by gel electrophoresis as ladders. Thus, the floating cells (obtained after treatment with IC_{90}-concentrations for 24 h) were analyzed by DNA gel electrophoresis and the typical DNA ladders were observed. Some of these results are depicted in Figure 6.

3. Conclusion

In this study we were able to synthesize simple derivatives of GA; these compounds, however, showed an increased cytotoxicity for various human cancer cell lines. For several of these derivatives the selectivity index was increased, too. These results point out there is an increasing activity with increasing lipophilicity for GA esters. This tendency, however, is not found for derivatives substituted with an extra amino acid moiety. An unsubstituted carboxylic group in C(30) led to inactive (IC_{50} >30 μM) compounds; the same is true for *N*-Boc protected amino acid derivatives. The presence of an added stereogenic centre in the side chain seems not important for activity. Previously, numerous modifications[16] in the ring skeleton of GA have been reported. With respect to ring A modifications, most approaches have focused on the synthesis of C2 and/or C-3 modified derivatives. Some C-3 ester[17] and a

Table 4
SRB data for selected amines and their hydrochlorides (IC_{50} in μM)

Derivative/cell line	**9** amine	**21** hydrochloride	**8** amine	**20** hydrochloride	**10** amine	**22** hydrochloride
518A2	3.54 ± 0.18	3.56 ± 0.18	6.65 ± 0.33	5.82 ± 0.29	2.50 ± 0.13	2.51 ± 0.13
8505C	4.31 ± 0.22	3.58 ± 0.18	7.45 ± 0.37	5.71 ± 0.29	2.55 ± 0.13	2.51 ± 0.13
A253	3.61 ± 0.18	3.50 ± 0.18	6.26 ± 0.31	5.50 ± 0.28	2.50 ± 0.13	2.51 ± 0.13
A2780	2.98 ± 0.15	2.31 ± 0.12	5.99 ± 0.30	4.32 ± 0.22	1.72 ± 0.09	1.56 ± 0.08
A549	2.77 ± 0.14	3.52 ± 0.18	6.42 ± 0.32	5.54 ± 0.28	2.40 ± 0.12	1.61 ± 0.08
DLD-1	4.49 ± 0.22	3.61 ± 0.18	8.59 ± 0.43	6.42 ± 0.32	2.51 ± 0.13	3.52 ± 0.18
Lipo	4.30 ± 0.22	3.52 ± 0.18	7.54 ± 0.38	5.92 ± 0.30	2.52 ± 0.13	3.55 ± 0.18
MCF-7	3.54 ± 0.18	3.89 ± 0.19	7.10 ± 0.36	5.73 ± 0.29	2.50 ± 0.13	2.43 ± 0.12
SW1736	3.22 ± 0.16	3.98 ± 0.20	6.33 ± 0.32	5.62 ± 0.28	1.82 ± 0.09	2.48 ± 0.12
Average	3.64 ± 0.18	3.50 ± 0.18	6.93 ± 0.35	5.62 ± 0.28	2.34 ± 0.12	2.52 ± 0.13

Table 5
Comparison of L- and D-alanyl substituted derivatives (cytotoxicity, IC_{50} values in μM using 15 human tumour cell lines (error: 5%)

Derivative/cell line	**9** (L-form)	**23** (D-form)	**12** (L-form)	**24** (D-form)	**15** (L-form)	**25** (D-form)
518A2	3.54 ± 0.18	2.26 ± 0.11	2.72 ± 0.14	2.76 ± 0.14	5.09 ± 0.25	5.48 ± 0.27
8505C	4.31 ± 0.22	2.92 ± 0.15	3.87 ± 0.19	4.43 ± 0.22	3.49 ± 0.17	3.51 ± 0.18
A253	3.61 ± 0.18	2.26 ± 0.11	2.33 ± 0.12	2.55 ± 0.13	3.51 ± 0.18	3.50 ± 0.18
A2780	2.98 ± 0.15	2.24 ± 0.11	2.59 ± 0.13	3.26 ± 0.16	2.08 ± 0.10	1.70 ± 0.09
A431	3.48 ± 0.17	2.36 ± 0.12	2.27 ± 0.11	2.76 ± 0.14	3.49 ± 0.17	3.11 ± 0.16
A549	2.77 ± 0.14	2.26 ± 0.11	3.43 ± 0.17	4.35 ± 0.22	3.43 ± 0.17	3.17 ± 0.16
DLD-1	4.49 ± 0.22	3.35 ± 0.17	3.72 ± 0.19	4.49 ± 0.22	5.54 ± 0.28	5.67 ± 0.28
FaDu	4.52 ± 0.23	2.25 ± 0.11	4.25 ± 0.21	3.90 ± 0.20	5.39 ± 0.27	5.48 ± 0.27
HCT-116	2.74 ± 0.14	2.25 ± 0.11	2.82 ± 0.14	4.25 ± 0.21	5.03 ± 0.25	4.82 ± 0.24
HCT-8	2.80 ± 0.14	2.26 ± 0.11	2.21 ± 0.11	2.42 ± 0.12	5.13 ± 0.26	3.32 ± 0.17
HT-29	2.87 ± 0.14	2.80 ± 0.14	2.50 ± 0.13	4.38 ± 0.22	4.60 ± 0.23	3.47 ± 0.17
Lipo	4.30 ± 0.22	3.56 ± 0.18	2.74 ± 0.14	4.13 ± 0.21	3.53 ± 0.18	4.73 ± 0.24
MCF-7	3.54 ± 0.18	2.25 ± 0.11	3.55 ± 0.18	3.55 ± 0.18	3.52 ± 0.18	3.52 ± 0.18
SW1736	3.22 ± 0.16	2.25 ± 0.11	2.92 ± 0.15	4.11 ± 0.21	3.54 ± 0.18	3.46 ± 0.17
SW480	3.52 ± 0.18	3.52 ± 0.18	2.79 ± 0.14	3.32 ± 0.17	3.50 ± 0.18	3.47 ± 0.17
Average	3.51 ± 0.18	2.59 ± 0.13	2.98 ± 0.15	3.64 ± 0.18	4.06 ± 0.20	3.89 ± 0.19

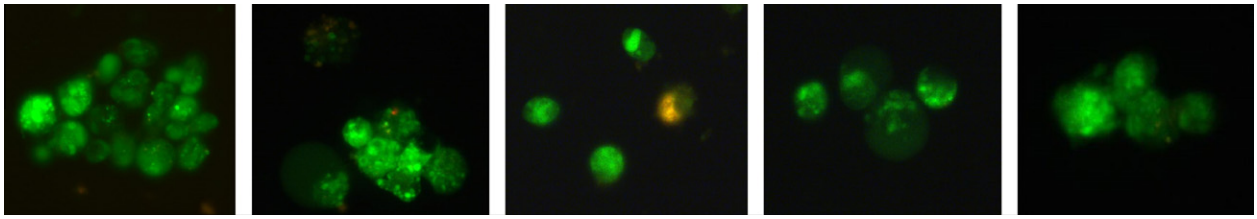

Figure 5. Results from the AO/EB test; treatment of A549 cells with (from left to right): GA (90 μM), **8** (8 μM), **9** (3.5 μM), **10** (3 μM) and **25** (3 μM).

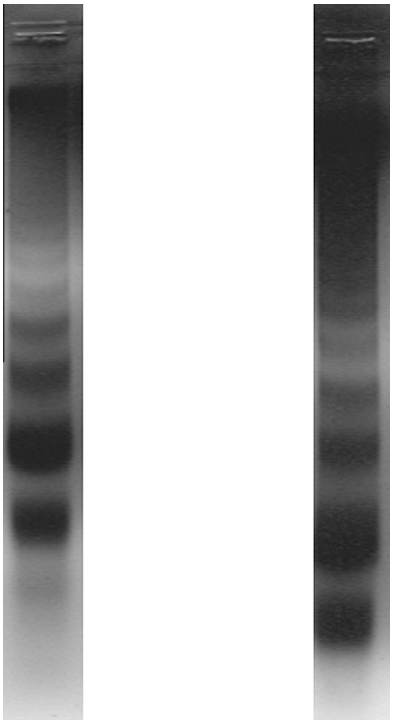

Figure 6. DNA laddering for the ovarian cancer cell line A2780 after treatment with IC_{90}-concentrations for 24 h; left trace: GA; right trace: compound **10**.

2-cyano-3,11-dioxo derivative [18–20] showed a cytotoxicity being comparable to the bioactivity observed for our compounds.

As demonstrated by fluorescence microscopy and DNA laddering experiments, the cytotoxicity of the compounds results from apoptotic processes. In summary, nearly of the GA derivatives showed a higher activity than GA and a better selectivity towards tumour cells.

4. Experimental

4.1. Biology

4.1.1. Cell lines and culture conditions

The cell lines 518A2, 8505C, A253, A2780, A431, A549, DLD-1, FaDu, HCT-116, HCT-8, HT-29, LIPO, MCF-7, NiH3T3, SW1736 and SW480 were included in this study. Cultures were maintained as monolayer in RPMI 1640 (PAA Laboratories, Pasching, Germany) supplemented with 10% heat inactivated foetal bovine serum (Biochrom AG, Berlin, Germany) and penicillin/streptomycin (PAA Laboratories) at 37 °C in a humidified atmosphere of 5% CO_2/95% air.

4.1.2. Cytotoxicity assay[21]

The cytotoxicity of the compounds was evaluated using the sulforhodamine B (SRB) (Sigma–Aldrich) microculture colorimetric assay. In short, exponentially growing cells were seeded into 96-well plates on day 0 at the appropriate cell densities to prevent confluence of the cells during the period of experiment. After 24 h, the cells were treated with serial dilutions of the compounds (0–100 μM) for 96 h. The final concentration of DMSO or DMF solvent never exceeded 0.5%, which was non-toxic to the cells. The percentages of surviving cells relative to untreated controls were determined 96 h after the beginning of drug exposure. After a 96 h treatment, the supernatant medium from the 96-well plates was thrown away and the cells were fixed with 10% TCA. For a thorough fixation, the plates were allowed to rest at 4 °C. After fixation, the cells were washed in a strip washer. The washing was done five times with water using alternate dispensing and aspiration procedures. Afterwards the plates were dyed with

100 μl of 0.4% SRB (sulforhodamine B) for about 20 min. The plates were washed with 1% acetic acid to remove the excess of the dye and allowed to air dry overnight. 100 μl of 10 mM Tris base solution were added to each well and absorbance was measured at 570 nm (using a 96-well plate reader, Tecan Spectra, Crailsheim, Germany). The IC_{50} was estimated by linear regression between the value before and after the 50% line is crossed in a dose–response curve.

4.1.3. Apoptosis test—acridine orange/ethidium bromide (AO/EB)[22]

Apoptotic cell death was analysed by acridine orange/ethidium bromide dye using fluorescence microscopy on A549 cells. Therefore approx. 500,000 cells were seeded in cell culture flasks and were allowed to grow for 24 h. The medium was removed and the substance loaded medium was added. After 24–48 h, the supernatant medium was collected and centrifuged; the pellet was suspended in phosphate-buffer saline (PBS) and centrifuged again. The liquid was removed, the pellet was suspended in PBS. After mixing the suspension with a solution of AO/EB, analysis was performed under a fluorescence microscope. While a green fluorescence shows apoptosis, a red coloured nucleus indicates necrotic cells.

4.1.4. Apoptosis test—DNA laddering

The DNA fragmentation assay was performed as described[23] previously.

4.2. Chemistry

4.2.1. General

Used reagents were bought from commercial suppliers without any further purification. NMR spectra were measured on VARIAN Gemini 200, Gemini 2000 or Unity 500 spectrometers at 27 °C with trimethylsilane as an internal standard, δ are given in ppm and J in Hertz. Mass spectra were taken on a FINNIGAN MAT TSQ 7000 (electronspray, voltage 4.5 kV, sheath gas nitrogen) instrument. IR spectra are recorded on a Perkin–Elmer FT-IR spectrometer Spectrum 1000, optical rotations on a Perkin–Elmer 341 polarimeter (1 cm micro cell) and UV–vis spectra on a Perkin–Elmer unit, Lambda 14. Melting points were measured with a LEICA hot stage microscope and are uncorrected. Elemental analysis was done on a Foss-Heraeus Vario EL unit. TLC was performed on silica gel (Merck 5554, detection by UV absorption). Solvents were dried before use according to usual procedures.

4.2.2. General procedure for the protection of the amino acids[24]

The amino acid (1 equiv) was dissolved in 1:1mixture of a potassium hydroxide solution (2 M in water) and 1,4-dioxane. Di *tert*-butyl dicarbonate (1.2 equiv) was added and the mixture allowed to stir at room temperature for 12 h. The solvent was removed under reduced pressure and ethyl acetate was added. After washing with aq sodium hydrogensulfate (10% in water), the mixture was extracted 3× with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, filtered and the solvent was evaporated. The crude protected amino acid was used without any further purification; for biological testing an analytic sample was obtained by chromatography.

4.2.3. General procedure for esterifications in position C(3) (method A)

The starting material (1 equiv) was dissolved in dry DCM, DMAP (20 mg, 0.16 mmol) and the protected amino acid (1.2 equiv) were added. After addition of DCC (1.2 equiv) the mixture was stirred at room temperature for 12 h, filtered and the filtrate was washed with water and brine, dried over sodium sulfate, filtered and the solvent was evaporated. Purification was performed by flash chromatography (SiO_2, hexane/ethyl acetate 8:2).

4.2.4. General procedure for deprotection (method B)

To a solution of the Boc-protected compound in dry DCM, trifluoroacetic acid (1 ml per 10 ml DCM) was added. The mixture was allowed to stir at room temperature for 12 h. After completion of the reaction (as monitored by TLC) the solution was washed with a satd aq sodium hydrogen carbonate. The aqueous layer was extracted with DCM, the combined organic extracts were washed with brine, dried over sodium sulfate, filtrated and evaporated to yield the amine.

4.2.5. General procedure for deprotection (method C)[25]

The solution of the benzyl ester (1 equiv) in a mixture of MeOH and THF (1:2) was purged with argon for 3 min, followed by the addition of ammonium formate (5 equiv). Palladium on activated coal (10%; 100 mg per mmol) was added and the mixture was stirred at room temperature for 12–14 h. The solvents were removed under reduced pressure and the residue was dissolved in DCM. Usual aqueous work-up followed by chromatography (SiO_2, hexane/ethyl acetate 1:1) yielded the product.

4.2.6. General procedure for deprotection (method D)

The Boc-protected compound was dissolved in dry DCM. After saturation with dry hydrogen chloride gas for 15 min stirring at room temperature was continued for 12 h. After completion of the reaction (as monitored by TLC), the solvent was removed under reduced pressure. The residue was washed with ethyl acetate until no parent substance could be detected; analytical samples were obtained by re-crystallization.

4.2.6.1. Methyl 3β-hydroxy-11-oxo-olean-12-en-30-oate (1). To a solution of **GA** (31.00 g, 65.9 mmol) in dry DMF (150 ml) potassium carbonate (15.34 g, 111.0 mmol) was added. After 30 min of stirring at room temperatures, iodomethane (4.94 ml, 79.0 mmol) was added and the mixture was stirred for an additional 2 h. The solvents were evaporated and the crude residue was dissolved in a mixture of DCM (300 ml) and hydrochloric acid (50 ml, 1.0 M). The aqueous layer was extracted with DCM (3 × 50 ml), the combined organic layers were washed with brine (50 ml), dried (Na_2SO_4), filtered and the solvent was evaporated. Recrystallization from MeOH yielded **1** (29.09 g, 91.1%) as a colourless solid; mp 254–258 °C (lit. 254–258 °C,[26] 254 °C[27]); R_f = 0.48 (hexane/ethyl acetate 7:3); $[\alpha]_D^{20}$ = 141.18 (c 0.48, $CHCl_3$); UV–vis (MeOH): λ_{max} (log ε) = 270 nm (4.15); MS (ESI): m/z (%) = 485.5 ($[M+H]^+$, 55), 507.5 ($[M+Na]^+$, 12), 539.1 ($[M+Na+MeOH]^+$, 100); IR (KBr): ν = 3614 (br), 2970 (s), 2955 (s), 2875 (m), 1726 (s), 1659 (s), 1466 (m), 1450 (m), 1364 (w), 1216 (m), 1189 (m), 1136 (w), 1085 (w), 1040 (w), 992 (w) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 5.65 (s, 1H, H-12), 3.67 (s, 3H, $COOCH_3$), 3.21 (dd, 1H, H-3, J = 10.8, 5.7 Hz), 2.78 (ddd, 1H, H-1, J = 13.3, 3.3, 3.37 Hz), 2.32 (s, 1H, H-9), 2.06 (dd, 1H, H-18, J = 13.3, 3.87 Hz), 2.00 (m, 1H, H-15), 1.95 (m, 1H, H-21), 1.90 (dd, 1H, H-19, J = 13.8, 3.97 Hz), 1.83 (ddd, 1H, H-16, J = 14.3, 14.3, 5.27 Hz), 1.65 (m, 1H, H-2), 1.62 (m, 1H, H-7), 1.58 (m, 1H, H-2′), 1.57 (m, 1H, H-19′), 1.57 (m, 1H, H-6), 1.43 (m, 1H, H-6′), 1.38 (m, 1H, H-7′), 1.36 (m, 1H, H-22), 1.34 (s, 3H, H-27), 1.30 (m, 1H, H-22′), 1.28 (m, 1H, H-21′), 1.17 (m, 1H, H-16′), 1.13 (s, 3H, H-28), 1.12 (s, 3H, H-25), 1.11 (s, 3H, H-26), 1.00 (m, 1H, H-15′), 0.99 (s, 3H, H-23), 0.96 (m, 1H, H-1′), 0.79 (s, 3H, H-24), 0.79 (s, 3H, H-29), 0.68 (m, 1H, H-5); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 176.8 (C-30), 169.0 (C-13), 128.6 (C-12), 78.8 (C-3), 61.9 (C-9), 55.1 (C-5), 51.8 ($COOCH_3$), 48.5 (C-18), 45.5 (C-8), 44.1 (C-20), 43.3 (C-14), 41.2

(C-19), 39.2 (C-1), 39.2 (C-4), 37.8 (C-22), 37.2 (C-10), 32.9 (C-7), 31.9 (C-17), 31.2 (C-21), 28.6 (C-29), 28.4 (C-23), 28.2 (C-28), 27.4 (C-2), 26.6 (C-16), 26.6 (C-15), 23.5 (C-27), 18.8 (C-26), 17.6 (C-6), 16.4 (C-25), 15.6 (C-24).

4.2.6.2. Ethyl 3β-hydroxy-11-oxo-olean-12-en-30-oate (2). Following the procedure given for **1**, compound **2** was obtained from GA and iodoethane as a white solid (1.75 g, 82%); mp 220–224 °C (lit. 93–94 °C[7]); R_f = 0.40 (hexane/ethyl acetate 7:3); $[\alpha]_D^{20}$ = 144.35 (*c* 0.48, $CHCl_3$); UV–vis (MeOH): λ_{max} (log ε) = 267 nm (3.90); MS (ESI): *m/z* (%) = 499.5 ($[M+H]^+$, 90), 521.4 ($[M+Na]^+$, 13), 552.9 ($[M+Na+MeOH]^+$, 100); IR (KBr): *ν* = 3543 (br), 2939 (s), 2869 (m), 1726 (s), 1651 (s), 1616 (m), 1455 (m), 1389 (m), 1364 (w), 1330 (m), 1312 (w), 1278 (w), 1257 (m), 1210 (m), 1172 (s), 1135 (w), 1086 (m), 1042 (m), 1028 (m), 994 (m), 920 (w), 880 (w), 767 (w), 704 (w), 671 (w), 604 (w) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 5.61 (s, 1H, H-12), 4.16 (dq, 1H, methylene-*H*H′, *J* = 10.8, 7.17 Hz), 4.10 (dq, 1H, methylene-H*H*′, *J* = 10.8, 7.17 Hz), 3.19 (dd, 1H, H-3, *J* = 11.1, 5.17 Hz), 2.76 (ddd, 1H, H-1, *J* = 13.5, 3.6, 3.67 Hz), 2.31 (s, 1H, H-9), 2.07 (ddd, 1H, H-18, *J* = 12.5, 4.1, 1.17 Hz), 2.00 (ddd, 1H, H-15, *J* = 13.7, 13.7, 4.57 Hz), 1.96 (m, 1H, H-21), 1.88 (ddd, 1H, H-19, *J* = 13.5, 4.2, 2.87 Hz), 1.80 (ddd, 1H, H-16, *J* = 13.7, 13.7, 4.37 Hz), 1.64 (m, 1H, H-2), 1.61 (m, 1H, H-7), 1.58 (m, 1H, H-2′), 1.57 (m, 1H, H-19′), 1.56 (m, 1H, H-6), 1.42 (ddd, 1H, H-6′, *J* = 12.4, 3.1, 3.17 Hz), 1.38 (m, 1H, H-7′), 1.35 (m, 1H, H-22), 1.34 (s, 3H, H-27), 1.28 (m, 1H, H-22′), 1.26 (m, 1H, H-21′), 1.23 (t, 3H, ethyl, *J* = 7.17 Hz), 1.15 (m, 1H, H-16′, *J* = 13.7, 4.5, 2.77 Hz), 1.11 (s, 3H, H-28), 1.11 (s, 3H, H-25), 1.10 (s, 3H, H-26), 0.99 (m, 1H, H-15′), 0.97 (s, 3H, H-23), 0.95 (ddd, 1H, H-1′, *J* = 10.4, 4.4, 4.47 Hz), 0.78 (s, 3H, H-24), 0.78 (s, 3H, H-29), 0.67 (dd, 1H, H-5, *J* = 11.5, 1.67 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.2 (C-11), 176.3 (C-30), 169.2 (C-13), 128.5 (C-12), 78.7 (C-3), 61.8 (C-9), 60.3 (ethylene), 54.9 (C-5), 48.4 (C-18), 45.4 (C-8), 43.8 (C-20), 43.2 (C-14), 41.1 (C-19), 39.1 (C-1), 39.1 (C-4), 37.7 (C-22), 37.1 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-29), 28.3 (C-23), 28.1 (C-28), 27.3 (C-2), 26.5 (C-16), 26.4 (C-15), 23.4 (C-27), 18.7 (C-26), 17.5 (C-6), 16.3 (C-25), 15.5 (C-24), 14.3 (methyl).

4.2.6.3. *i*-Propyl 3β-hydroxy-11-oxo-olean-12-en-30-oate (3). Compound **3** was obtained from GA and *i*-propyl iodide as a white solid (3.48 g, 59%); mp 209–213 °C (lit. 238 °C (dec)[7]); R_f = 0.50 (hexane/ethyl acetate 7:3); $[\alpha]_D^{20}$ = 145.82 (*c* 0.37, $CHCl_3$); UV–vis (MeOH): λ_{max} (log ε) = 250 nm (4.09); MS (ESI): *m/z* (%) = 513.5 ($[M+H]^+$, 68), 567.0 ($[M+MeOH+Na]^+$, 92), 1025.3 ($[2M+H]^+$, 80), 1047.3 ($[2M+Na]^+$, 100); IR (KBr): *ν* = 3521 (br), 2980 (s), 2946 (s), 2856 (s), 1720 (s), 1642 (s), 1612 (m), 1457 (s), 1388 (s), 1327 (m), 1309 (m), 1278 (m), 1255 (m), 1210 (s), 1174 (s), 1135 (m), 1106 (s), 1084 (m), 1047 (m), 1028 (m), 996 (m), 981 (m), 948 (w), 917 (m), 878 (w), 848 (s), 769 (w), 715 (w), 702 (w), 688 (w), 673 (w), 636 (w), 591 (w), 541 (w), 475 (w), 424 (w) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 5.60 (s, 1H, H-12), 5.00 (qq, 1H, *i*-Pr-CH, *J* = 6.2, 6.27 Hz), 3.20 (dd, 1H, H-3, *J* = 10.9, 5.57 Hz), 2.76 (ddd, 1H, H-1, *J* = 13.3, 3.6, 3.6), 2.32 (s, 1H, H-9), 2.07 (dd, 1H, H-18, *J* = 13.4, 3.47 Hz), 2.02 (ddd, 1H, H-15, *J* = 13.7, 13.7, 4.67 Hz), 1.97 (m, 1H, H-21), 1.87 (m, 1H, H-19), 1.80 (ddd, 1H, H-16, *J* = 13.6, 13.6, 4.57 Hz), 1.67 (m, 1H, H-2), 1.64 (m, 1H, H-7), 1.62 (m, 1H, H-2′), 1.58 (dd, 1H, H-19′, *J* = 13.1, 13.17 Hz), 1.57 (m, 1H, H-6), 1.45 (m, 1H, H-6′), 1.40 (m, 1H, H-7′), 1.34 (m, 1H, H-22), 1.34 (s, 3H, H-27), 1.28 (m, 1H, H-21′), 1.27 (m, 1H, H-22′), 1.23 (d, 3H, *i*-Pr-CH_3, *J* = 6.27 Hz), 1.20 (d, 3H, *i*-Pr-CH_3, *J* = 6.27 Hz), 1.16 (m, 1H, H-16′), 1.11 (s, 3H, H-28), 1.10 (s, 6H, H-25 and H-26), 0.99 (m, 1H, H-15′), 0.98 (s, 3H, H-23), 0.95 (m, 1H, H-1′), 0.78 (s, 3H, H-24), 0.77 (s, 3H, H-29), 0.67 (m, 1H, H-5); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.2 (C-11), 175.8 (C-30), 169.4 (C-13), 128.5 (C-12), 78.8 (C-3), 67.4 (*i*-Pr-CH), 61.8 (C-9), 54.9 (C-5), 48.4 (C-18), 45.4 (C-8), 43.7 (C-20), 43.2 (C-14), 41.1 (C-19), 39.1 (C-1), 39.1 (C-4), 37.7 (C-22), 37.1 (C-10), 32.8 (C-7), 31.8 (C-17), 31.1 (C-21), 28.6 (C-29), 28.2 (C-23), 28.1 (C-28), 27.3 (C-2), 26.5 (C-16), 26.4 (C-15), 23.4 (C-27), 21.9 (*i*-Pr-CH_3), 21.7 (*i*-Pr-CH_3), 18.7 (C-26), 17.5 (C-6), 16.3 (C-25), 15.6 (C-24).

4.2.6.4. Benzyl 3β-hydroxy-11-oxo-olean-12-en-30-oate (4). Compound **4** was obtained from GA and benzyl bromide as a white solid (8.61 g, 73%); mp 134–137 °C (lit. 125–126 °C,[7] 129–30 °C,[28] 131–133 °C[29]); R_f = 0.45 (hexane/ethyl acetate 7:3); $[\alpha]_D^{20}$ = 136.41 (*c* 0.25, $CHCl_3$); UV–vis (MeOH): λ_{max} (log ε) = 207 nm (4.08), 249 nm (4.12); MS (ESI): *m/z* (%) = 561.5 ($[M+H]^+$, 56), 583.4 ($[M+Na]^+$, 26), 863.9 ($[3M+2Na]^{2+}$, 10), 1121.2 ($[2M+H]^+$, 54), 1143.3 ($[2M+Na]^+$, 100); IR (KBr): *ν* = 3432 (br), 2932 (s), 2870 (m), 1725 (s), 1654 (s), 1616 (w), 1467 (m), 1455 (m), 1387 (m), 1356 (w), 1323 (w), 1280 (w), 1257 (w), 1215 (m), 1188 (m), 1150 (s), 1084 (m), 1038 (m), 995 (m), 954 (w), 917 (w), 880 (w), 821 (w), 754 (w), 699 (m), 674 (w), 607 (w), 543 (w) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.39–7.25 (m, 5H, H-Ar), 5.53 (s, 1H, H-12), 5.18 (d, 1H, Bn-C*H*H′, *J* = 12.37 Hz), 5.07 (d, 1H, Bn-CH*H*′, *J* = 12.47 Hz), 3.20 (dd, 1H, H-3, *J* = 11.1, 5.47 Hz), 2.77 (ddd, 1H, H-1, *J* = 13.2, 3.7, 3.77 Hz), 2.30 (s, 1H, H-9), 2.02 (m, 1H, H-18), 2.00 (m, 1H, H-15), 1.95 (m, 1H, H-21), 1.91 (dd, 1H, H-19, *J* = 13.5, 3.9, 2.67 Hz), 1.79 (ddd, 1H, H-16, *J* = 13.4, 13.4, 4.57 Hz), 1.66 (m, 1H, H-2), 1.62 (m, 1H, H-7), 1.60 (m, 1H, H-2′), 1.59 (dd, 1H, H-19′, *J* = 13.1, 13.17 Hz), 1.57 (m, 1H, H-6), 1.45 (m, 1H, H-6′), 1.40 (m, 1H, H-7′), 1.34 (m, 1H, H-22), 1.33 (s, 3H, H-27), 1.29 (m, 1H, H-21′), 1.28 (m, 1H, H-22′), 1.15 (m, 1H, H-16′), 1.14 (s, 3H, H-28), 1.12 (s, 3H, H-25), 1.09 (s, 3H, H-26), 0.99 (m, 1H, H-15′), 0.98 (s, 3H, H-23), 0.97 (m, 1H, H-1′), 0.78 (s, 3H, H-24), 0.71 (s, 3H, H-29), 0.67 (m, 1H, H-5); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.1 (C-11), 176.2 (C-30), 168.9 (C-13), 136.1 (C_{ar}), 128.6 (C_{ar}), 128.6 (C_{ar}), 128.4 (C-12), 128.3 (C_{ar}), 128.2 (C_{ar}), 128.2 (C_{ar}), 78.7 (C-3), 66.2 (Bn-CH_2), 61.8 (C-9), 54.9 (C-5), 48.2 (C-18), 45.3 (C-8), 44.0 (C-20), 43.1 (C-14), 41.1 (C-19), 39.1 (C-1), 39.1 (C-4), 37.6 (C-22), 37.1 (C-10), 32.7 (C-7), 31.7 (C-17), 31.1 (C-21), 28.4 (C-29), 28.3 (C-23), 28.1 (C-28), 27.3 (C-2), 26.4 (C-16), 26.4 (C-15), 23.3 (C-27), 18.7 (C-26), 17.5 (C-6), 16.3 (C-25), 15.5 (C-24).

4.2.6.5. 3β-(Glycyl)-11-oxo-olean-12-en-30-oic acid hydrochloride (5). Obtained from **26** by method D as a colourless powder; yield: 210 mg, 63%; mp 300–302 °C (decomp.); R_f = 0.69 (MeOH); $[\alpha]_D^{20}$ = 82.99 (*c* 0.50, MeOH); UV–vis (MeOH): λ_{max} (log ε) = 249 nm (3.93); MS (ESI): *m/z* (%) = 528.2 ($[M+H]^+$, 100); IR (KBr): *ν* = 3420 (br), 2951 (s), 1746 (s), 1656 (s), 1466 (s), 1389 (s), 1324 (m), 1245 (s), 1086 (s), 987 (m), 909 (m), 819 (w), 749 (w), 670 (w), 589 (w), 542 (w), 462 (w) cm^{-1}; ^1H NMR (400 MHz, MeOH-d_4): δ = 5.59 (s, 1H, H-12), 4.68 (dd, 1H, H-3, *J* = 12.0, 4.67 Hz), 3.84 (dd, 2H, Gly-CH_2, *J* = 23.3, 17.17 Hz), 2.79 (ddd, 1H, H-1, *J* = 13.7, 3.6, 3.67 Hz), 2.50 (s, 1H, H-9), 2.20 (m, 1H, H-18), 2.15 (ddd, 1H, H-15, *J* = 13.3, 13.3, 4.67 Hz), 1.95 (m, 1H, H-21), 1.88 (m, 1H, H-16), 1.84 (m, 1H, H-19), 1.79 (m, 1H, H-2), 1.75 (m, 1H, H-7), 1.71 (dd, 1H, H-19′, *J* = 13.4, 13.47 Hz), 1.67 (m, 1H, H-2′), 1.64 (m, 1H, H-6), 1.53 (m, 1H, H-6′), 1.49 (m, 1H, H-7′), 1.47 (m, 1H, H-22), 1.43 (s, 3H, H-27), 1.39 (m, 1H, H-21′), 1.25 (m, 1H, H-22′), 1.23 (m, 1H, H-16′), 1.17 (s, 3H, H-25), 1.16 (s, 3H, H-28), 1.15 (s, 3H, H-28), 1.12 (m, 1H, H-1′), 1.04 (m, 1H, H-15′), 0.94 (s, 3H, H-23), 0.93 (s, 3H, H-24), 0.91 (m, 1H, H-5), 0.83 (s, 3H, H-29); ^{13}C NMR (125 MHz, MeOH-d_4): δ = 200.9 (C-11), 178.9 (C-30), 171.6 (Gly-COO), 167.0 (C-13), 127.4 (C-12), 83.2 (C-3), 61.4 (C-9), 54.6 (C-5), 48.5 (C-18), 45.3 (C-8), 43.2 (C-20), 41.0 (C-14), 39.7 (C-19), 38.2 (Gly-CH_2), 37.7 (C-1 and C-4), 37.6 (C-22), 36.8 (C-10), 32.2 (C-7), 31.5 (C-17), 30.6 (C-21), 27.8 (C-29), 27.3 (C-23), 27.0 (C-28), 26.2 (C-16), 25.9 (C-15), 23.0 (C-2), 22.4 (C-27), 17.8 (C-26), 17.0 (C-6), 15.6 (C-24), 15.5

(C-25). Anal. Calcd for $C_{32}H_{50}ClNO_5$ (564.20): C, 68.12; H, 8.93; Cl, 6.28; N, 2.48. Found: C, 68.01; H, 9.17; Cl, 6.13; N, 2.41.

4.2.6.6. 3β-(L-Alanyl)-11-oxo-olean-12-en-30-oic acid hydrochloride (6). Obtained from **27** by method D as a colourless powder; yield: 50 mg, 51%; mp 309–311 °C (decomp.); R_f = 0.75 (MeOH); $[\alpha]_D^{20}$ = 103.68 (*c* 0.58, MeOH); UV–vis (MeOH): λ_{max} (log ε) = 249 nm (4.01); MS (ESI): *m/z* (%) = 542.1 ($[M+H]^+$, 100); IR (KBr): ν = 3421 (br), 2962 (s), 2642 (m), 2585 (m), 2512 (w), 2012 (w), 1737 (s), 1707 (s), 1651 (s), 1604 (s), 1518 (m), 1456 (s), 1388 (s), 1364 (m), 1326 (m), 1290 (s), 1262 (s), 1213 (s), 1163 (s), 1111 (s), 1049 (m), 1021 (m), 984 (m), 958 (m), 913 (m), 885 (w), 869 (w), 804 (w), 771 (w), 748 (w), 700 (w), 683 (w), 672 (w), 589 (w), 542 (w) cm^{-1}; ^1H NMR (400 MHz, MeOH-d_4): δ = 5.59 (s, 1H, H-12), 4.67 (dd, 1H, H-3, *J* = 12.0, 4.57 Hz), 4.09 (q, 1H, Ala-CH, *J* = 7.27 Hz), 2.79 (ddd, 1H, H-1, *J* = 13.6, 3.4, 3.47 Hz), 2.50 (s, 1H, H-9), 2.21 (m, 1H, H-18), 2.15 (ddd, 1H, H-15, *J* = 13.7, 13.7, 4.37 Hz), 1.94 (m, 1H, H-21), 1.89 (m, 1H, H-16), 1.84 (m, 1H, H-19), 1.78 (m, 1H, H-2), 1.75 (m, 1H, H-7), 1.71 (dd, 1H, H-19′, *J* = 13.2, 13.27 Hz), 1.67 (m, 1H, H-2′), 1.63 (m, 1H, H-6), 1.56 (d, 3H, Ala-CH_3, *J* = 7.37 Hz), 1.53 (m, 1H, H-6′), 1.49 (m, 1H, H-7′), 1.47 (m, 1H, H-22), 1.43 (s, 3H, H-27), 1.40 (m, 1H, H-21′), 1.25 (m, 1H, H-22′), 1.24 (m, 1H, H-16′), 1.18 (s, 3H, H-25), 1.17 (s, 3H, H-28), 1.15 (s, 3H, H-28), 1.13 (m, 1H, H-1′), 1.05 (m, 1H, H-15′), 0.95 (s, 3H, H-23), 0.93 (s, 3H, H-24), 0.92 (m, 1H, H-5), 0.83 (s, 3H, H-29); ^{13}C NMR (125 MHz, MeOH-d_4): δ = 200.9 (C-11), 178.9 (C-30), 171.6 (Ala-COO), 169.4 (C-13), 127.4 (C-12), 83.2 (C-3), 61.4 (C-9), 54.5 (C-5), 48.7 (Ala-CH), 48.5 (C-18), 45.3 (C-8), 43.2 (C-20), 41.0 (C-14), 38.2 (C-19), 37.9 (C-1), 37.6 (C-4), 37.6 (C-22), 36.8 (C-10), 32.2 (C-7), 31.5 (C-17), 30.6 (C-21), 27.8 (C-29), 27.3 (C-23), 27.1 (C-28), 26.2 (C-16), 25.9 (C-15), 23.0 (C-2), 22.4 (C-27), 17.8 (C-26), 16.9 (C-6), 15.7 (C-24), 15.5 (C-25), 15.0 (Ala-CH_3). Anal. Calcd for $C_{33}H_{52}ClNO_5$ (578.22): C, 68.55; H, 9.06; Cl, 6.13; N, 2.42. Found: C, 68.29; H, 9.21; Cl, 6.24; N, 2.34.

4.2.6.7. 3β-(β-Alanyl)-11-oxo-olean-12-en-30-oic acid hydrochloride (7). Obtained from **28** by method D as a colourless powder; yield: 100 mg, 39%; mp 305–309 °C (decomp.); R_f = 0.48 (MeOH); $[\alpha]_D^{20}$ = 117.25 (*c* 0.51, MeOH); UV–vis (MeOH): λ_{max} (log ε) = 250 nm (4.08); MS (ESI): *m/z* (%) = 542.3 ($[M+H]^+$, 100); IR (KBr): ν = 3355 (br), 3258 (m), 2957 (s), 2363 (w), 1724 (s), 1652 (s), 1582 (m), 1527 (m), 1466 (s), 1389 (s), 1365 (s), 1325 (m), 1306 (m), 1278 (m), 1233 (s), 1165 (s), 1105 (s), 1061 (w), 1022 (m), 983 (s), 918 (w), 883 (w), 850 (w), 750 (w), 700 (w), 683 (w), 669 (w), 589 (w), 541 (w) cm^{-1}; ^1H NMR (500 MHz, MeOH-d_4): δ = 5.57 (s, 1H, H-12), 4.57 (dd, 1H, H-3, *J* = 11.7, 5.07 Hz), 3.18 (t, 2H, Ala-CH_2NH_2, *J* = 6.77 Hz), 2.74 (m, 1H, H-1), 2.73 (m, 2H, Ala-CH_2COO), 2.47 (s, 1H, H-9), 2.19 (m, 1H, H-18), 2.13 (ddd, 1H, H-15, *J* = 13.6, 13.6, 4.27 Hz), 1.92 (m, 1H, H-21), 1.87 (m, 1H, H-16), 1.82 (m, 1H, H-19), 1.75 (m, 1H, H-7), 1.72 (m, 1H, H-2), 1.69 (dd, 1H, H-19′, *J* = 13.2, 13.27 Hz), 1.64 (m, 1H, H-2′), 1.60 (m, 1H, H-6), 1.51 (m, 1H, H-6′), 1.44 (m, 1H, H-7′), 1.41 (s, 3H, H-27), 1.40 (m, 3H, H-22 and H-21′ and H-22′), 1.23 (m, 1H, H-16′), 1.15 (s, 6H, H-25 and H-28), 1.13 (s, 3H, H-26), 1.09 (m, 1H, H-1′), 1.03 (m, 1H, H-15′), 0.90 (s, 6H, H-23 and H-24), 0.87 (m, 1H, H-5), 0.82 (s, 3H, H-29); ^{13}C NMR (125 MHz, MeOH-d_4): δ = 200.9 (C-11), 179.0 (C-30), 171.6 (Ala-COO), 170.6 (C-13), 127.4 (C-12), 81.7 (C-3), 61.5 (C-9), 54.7 (C-5), 48.5 (C-18), 45.3 (C-8), 43.5 (C-20), 43.2 (C-14), 41.0 (C-19), 38.3 (C-1), 37.7 (C-4), 37.6 (C-22), 36.8 (C-10), 35.0 (Ala-CH_2NH_2), 32.2 (C-7), 31.5 (C-17), 30.9 (Ala-CH_2COO), 30.6 (C-21), 27.8 (C-29), 27.3 (C-28), 27.1 (C-23), 26.2 (C-16), 25.9 (C-15), 23.0 (C-2), 22.4 (C-27), 17.8 (C-26), 17.0 (C-6), 15.7 (C-24), 15.5 (C-25). Anal. Calcd for $C_{33}H_{52}ClNO_5$ (578.22): C, 68.55; H, 9.06; Cl, 6.13; N, 2.42. Found: C, 68.43; H, 9.22; Cl, 6.24; N, 2.19.

4.2.6.8. Methyl 3β-(glycyl)-11-oxo-olean-12-en-30-oate (8). Obtained from **29** by method B as a colourless powder; yield: 200 mg, 79%; mp 280–282 °C; R_f = 0.57 (DCM/MeOH 9:1); $[\alpha]_D^{20}$ = 123.02 (*c* 0.43, $CHCl_3$); UV–vis (MeOH): λ_{max} (log ε) = 249 nm (4.08); MS (ESI): *m/z* (%) = 542.2 ($[2M+2H]^{2+}$, 10); IR (KBr): ν = 3382 (br), 2966 (s), 2873 (m), 2856 (m), 2362 (w), 1743 (s), 1728 (s), 1651 (s), 1467 (m), 1454 (m), 1390 (m), 1361 (w), 1320 (w), 1278 (w), 1248 (w), 1217 (s), 1190 (m), 1155 (m), 1086 (m), 1049 (w), 1023 (w), 984 (w), 971 (m), 921 (m), 880 (w), 869 (s), 770 (w), 748 (w), 702 (w), 668 (w), 591 (w), 543 (w) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 5.63 (s, 1H, H-12), 4.55 (dd, 1H, H-3, *J* = 11.6, 4.67 Hz), 3.66 (s, 3H, OMe), 3.41 (s, 2H, Gly-CH_2), 2.79 (ddd, 1H, H-1, *J* = 13.7, 3.6, 3.67 Hz), 2.33 (s, 1H, H-9), 2.05 (m, 1H, H-18), 2.00 (m, 1H, H-15), 1.96 (m, 1H, H-21), 1.89 (ddd, 1H, H-19, *J* = 13.3, 3.7, 2.57 Hz), 1.79 (ddd, 1H, H-16, *J* = 14.2, 14.2, 4.67 Hz), 1.69 (m, 1H, H-2), 1.64 (m, 1H, H-7), 1.61 (m, 1H, H-2′), 1.58 (dd, 1H, H-19′, *J* = 13.4, 13.47 Hz), 1.56 (m, 1H, H-6), 1.45 (m, 1H, H-6′), 1.40 (m, 1H, H-7′), 1.37 (m, 1H, H-22), 1.33 (s, 3H, H-27), 1.32 (m, 1H, H-22′), 1.28 (m, 1H, H-21′), 1.15 (m, 1H, H-16′), 1.13 (s, 3H, H-25), 1.12 (s, 3H, H-26), 1.09 (s, 3H, H-28), 1.03 (ddd, 1H, H-1′, *J* = 13.3, 13.3, 3.87 Hz), 0.98 (m, 1H, H-15′), 0.85 (s, 6H, H-23 and H-24), 0.78 (m, 1H, H-5), 0.77 (s, 3H, H-29); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 176.9 (C-30), 173.9 (Gly-COO), 169.2 (C-13), 128.5 (C-12), 81.4 (C-3), 61.7 (C-9), 55.0 (C-5), 51.7 (OMe), 48.4 (C-18), 45.4 (C-8), 44.0 (C-20), 44.0 (Gly-CH_2), 43.2 (C-14), 41.1 (C-19), 38.7 (C-1), 38.1 (C-4), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-29), 28.3 (C-23), 28.1 (C-28), 26.4 (C-16), 26.4 (C-15), 23.6 (C-2), 23.3 (C-27), 18.6 (C-26), 17.3 (C-6), 16.7 (C-24), 16.4 (C-25). Anal. Calcd for $C_{33}H_{51}NO_5$ (541.76): C, 73.16; H, 9.49; N, 2.59. Found: C, 73.02; H, 9.57; N, 2.41.

4.2.6.9. Methyl 3β-(L-alanyl)-11-oxo-olean-12-en-30-oate (9). Obtained from **30** by method B as a colourless powder; yield: 200 mg, 91%; mp 264–267 °C; R_f = 0.59 (DCM/MeOH 9:1); $[\alpha]_D^{20}$ = 137.30 (*c* 0.35, $CHCl_3$); UV–vis (MeOH): λ_{max} (log ε) = 249 nm (4.07); MS (ESI): *m/z* (%) = 556.1 ($[M+H]^+$, 100); IR (KBr): ν = 3442 (br), 2958 (s), 2874 (m), 1731 (s), 1652 (s), 1616 (m), 1457 (m), 1389 (m), 1362 (w), 1318 (m), 1278 (w), 1248 (m), 1217 (s), 1191 (s), 1154 (s), 1087 (m), 1064 (w), 1050 (w), 1022 (w), 986 (m), 973 (w), 984 (w), 918 (w), 880 (w), 868 (w), 825 (w), 770 (w), 685 (w), 671 (w), 590 (w), 544 (w) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 5.64 (s, 1H, H-12), 4.54 (dd, 1H, H-3, *J* = 11.9, 4.67 Hz), 3.66 (s, 3H, OMe), 3.55 (q, 1H, Ala-CH, *J* = 7.27 Hz), 2.79 (ddd, 1H, H-1, *J* = 13.3, 3.6, 3.67 Hz), 2.34 (s, 1H, H-9), 2.06 (m, 1H, H-18), 2.00 (m, 1H, H-15), 1.97 (m, 1H, H-21), 1.90 (ddd, 1H, H-19, *J* = 13.8, 4.1, 2.67 Hz), 1.80 (ddd, 1H, H-16, *J* = 13.9, 13.9, 4.77 Hz), 1.70 (m, 1H, H-2), 1.63 (m, 1H, H-7), 1.60 (m, 1H, H-2′), 1.59 (dd, 1H, H-19′, *J* = 13.4, 13.47 Hz), 1.56 (m, 1H, H-6), 1.44 (m, 1H, H-6′), 1.39 (m, 1H, H-7′), 1.35 (m, 1H, H-22), 1.35 (m, 3H, Ala-CH_3), 1.34 (s, 3H, H-27), 1.29 (m, 2H, H-22′ and H-21′), 1.16 (m, 1H, H-16′), 1.14 (s, 3H, H-25), 1.12 (s, 3H, H-28), 1.10 (s, 3H, H-26), 1.04 (ddd, 1H, H-1′, *J* = 13.8, 13.8, 3.67 Hz), 0.99 (m, 1H, H-15′), 0.87 (s, 3H, H-23), 0.85 (s, 3H, H-24), 0.79 (m, 1H, H-5), 0.78 (s, 3H, H-29); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 176.9 (C-30), 175.9 (Ala-COO), 169.2 (C-13), 128.5 (C-12), 81.2 (C-3), 61.7 (C-9), 55.0 (C-5), 51.8 (OMe), 50.3 (Ala-CH), 48.4 (C-18), 45.4 (C-8), 44.0 (C-20), 43.2 (C-14), 41.1 (C-19), 38.7 (C-1), 38.2 (C-4), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-29), 28.3 (C-23), 28.1 (C-28), 26.5 (C-16), 26.4 (C-15), 23.6 (C-2), 23.3 (C-27), 20.6 (Ala-CH_3), 18.7 (C-26), 17.3 (C-6), 16.7 (C-24), 16.4 (C-25). Anal. Calcd for $C_{34}H_{53}NO_5$ (555.79): C, 73.47; H, 9.61; N, 2.52. Found: C, 73.28; H, 9.81; N, 2.34.

4.2.6.10. Methyl 3β-(β-alanyl)-11-oxo-olean-12-en-30-oate (10). Obtained from **31** by method B as a colourless powder;

yield: 370 mg, 95%; mp 267–270 °C; R_f = 0.21 (DCM/MeOH 9:1); $[\alpha]_D^{20}$ = 121.76 (*c* 0.48, $CHCl_3$); UV–vis (MeOH): λ_{max} (log ε) = 250 nm (4.07); MS (ESI): *m/z* (%) = 556.2 ($[M+H]^+$, 100); IR (KBr): ν = 3380 (br), 2960 (s), 2874 (m), 1726 (s), 1652 (s), 1465 (m), 1388 (m), 1368 (m), 1318 (m), 1278 (w), 1250 (m), 1218 (s), 1188 (s), 1160 (s), 1087 (m), 1070 (w), 1023 (w), 986 (m), 948 (w), 918 (w), 880 (w), 853 (w), 824 (w), 770 (w), 721 (w), 685 (w), 669 (w), 590 (w), 543 (w) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.60 (s, 1H, H-12), 4.49 (dd, 1H, H-3, *J* = 11.8, 4.77 Hz), 3.62 (s, 3H, OMe), 2.94 (m, 2H, Ala-CH_2NH_2), 2.74 (ddd, 1H, H-1, *J* = 13.8, 3.5, 3.57 Hz), 2.42 (t, 2H, Ala-CH_2COO, *J* = 6.37 Hz), 2.30 (s, 1H, H-9), 2.02 (dd, 1H, H-18, *J* = 13.8, 3.77 Hz), 1.96 (ddd, 1H, H-15, *J* = 13.7, 13.7, 4.57 Hz), 1.92 (m, 1H, H-21), 1.86 (ddd, 1H, H-19, *J* = 13.8, 4.2, 3.07 Hz), 1.76 (ddd, 1H, H-16, *J* = 13.8, 13.8, 4.57 Hz), 1.66 (ddd, 1H, H-2, *J* = 13.4, 13.4, 3.57 Hz), 1.60 (m, 1H, H-7), 1.56 (m, 1H, H-2′), 1.55 (dd, 1H, H-19′, *J* = 13.6, 13.67 Hz), 1.52 (m, 1H, H-6), 1.40 (ddd, 1H, H-6′, *J* = 12.8, 12.8, 3.27 Hz), 1.35 (m, 1H, H-7′), 1.31 (m, 1H, H-22), 1.30 (s, 3H, H-27), 1.25 (m, 2H, H-22′ and H-21′), 1.11 (m, 1H, H-16′), 1.10 (s, 3H, H-25), 1.08 (s, 3H, H-28), 1.06 (s, 3H, H-26), 0.99 (ddd, 1H, H-1′, *J* = 13.6, 13.6, 3.67 Hz), 0.95 (m, 1H, H-15′), 0.82 (s, 6H, H-23 and H-24), 0.75 (m, 1H, H-5), 0.74 (s, 3H, H-29); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 176.9 (C-30), 172.4 (Ala-COO), 169.2 (C-13), 128.5 (C-12), 80.7 (C-3), 61.7 (C-9), 55.0 (C-5), 51.7 (OMe), 48.4 (C-18), 45.4 (C-8), 44.0 (C-20), 43.2 (C-14), 41.1 (C-19), 38.7 (C-1), 38.0 (C-4 and Ala-CH_2COO), 37.9 (Ala-CH_2NH_2), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-29), 28.3 (C-28), 28.1 (C-23), 26.4 (C-16), 26.4 (C-15), 23.6 (C-2), 23.3 (C-27), 18.6 (C-26), 17.4 (C-6), 16.7 (C-24), 16.4 (C-25). Anal. Calcd for $C_{34}H_{53}NO_5$ (555.79): C, 73.47; H, 9.61; N, 2.52. Found: C, 73.32; H, 9.83; N, 2.37.

4.2.6.11. Ethyl 3β-(glycyl)-11-oxo-olean-12-en-30-oate (11). Obtained from **32** by method B as a colourless powder; yield: 145 mg, 97%; mp 204–206 °C; R_f = 0.27 (DCM/MeOH 9:1); $[\alpha]_D^{20}$ = 126.20 (*c* 0.57, $CHCl_3$); UV–vis (MeOH): λ_{max} (log ε) = 249 nm (4.05); MS (ESI): *m/z* (%) = 556.3 ($[2M+2H]^{2+}$, 100), 834.3 ($[3M+2H]^{2+}$, 10), 1111.8 ($[2M+H]^+$, 8); IR (KBr): ν = 3366 (br), 3216 (w), 2958 (s), 2874 (m), 1742 (s), 1713 (s), 1651 (s), 1616 (w), 1464 (m), 1390 (m), 1377 (w), 1363 (w), 1321 (m), 1278 (w), 1249 (w), 1217 (s), 1175 (s), 1114 (w), 1087 (w), 1032 (w), 986 (m), 970 (m), 948 (w), 919 (w), 901 (w), 879 (w), 770 (w), 751 (w), 669 (w), 589 (w), 544 (w) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.64 (s, 1H, H-12), 4.57 (dd, 1H, H-3, *J* = 11.8, 4.87 Hz), 4.18 (dq, 1H, COOC*H*H′, *J* = 10.8, 7.17 Hz), 4.11 (dq, 1H, COOCH*H*′, *J* = 10.8, 7.17 Hz), 3.41 (s, 2H, Gly-CH_2), 2.81 (ddd, 1H, H-1, *J* = 13.7, 3.6, 3.67 Hz), 2.36 (s, 1H, H-9), 2.10 (ddd, 1H, H-18, *J* = 13.4, 4.0, 0.87 Hz), 2.02 (ddd, 1H, H-15, *J* = 13.6, 13.6, 4.47 Hz), 1.98 (m, 1H, H-21), 1.92 (ddd, 1H, H-19, *J* = 13.6, 4.1, 2.87 Hz), 1.82 (ddd, 1H, H-16, *J* = 13.6, 13.6, 4.57 Hz), 1.71 (m, 1H, H-2), 1.68 (m, 1H, H-7), 1.66 (m, 1H, H-2′), 1.60 (dd, 1H, H-19′, *J* = 13.7, 13.77 Hz), 1.57 (m, 1H, H-6), 1.44 (m, 1H, H-6′), 1.40 (m, 1H, H-7′), 1.38 (m, 1H, H-22), 1.36 (s, 3H, H-27), 1.32 (m, 1H, H-22′), 1.31 (m, 1H, H-21′), 1.25 (t, 3H, $COOCH_2CH_3$, *J* = 7.17 Hz), 1.17 (m, 1H, H-16′), 1.15 (s, 3H, H-25), 1.13 (s, 3H, H-28), 1.12 (s, 3H, H-26), 1.06 (ddd, 1H, H-1′, *J* = 13.7, 13.7, 3.67 Hz), 1.01 (m, 1H, H-15′), 0.87 (s, 6H, H-23 and H-24), 0.81 (m, 1H, H-5), 0.79 (s, 3H, H-29); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 176.3 (C-30), 174.1 (Gly-COO), 169.4 (C-13), 128.4 (C-12), 81.3 (C-3), 61.7 (C-9), 60.3 ($COOCH_2$), 55.0 (C-5), 48.4 (C-18), 45.4 (C-8), 44.1 (Gly-CNH), 43.8 (C-20), 43.2 (C-14), 41.0 (C-19), 38.7 (C-1), 38.1 (C-4), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-29), 28.3 (C-23), 28.1 (C-28), 26.5 (C-16), 26.4 (C-15), 23.6 (C-2), 23.3 (C-27), 18.6 (C-26), 17.3 (C-6), 16.7 (C-24), 16.4 (C-25), 14.3 ($COOCH_2CH_3$). Anal. Calcd for $C_{34}H_{53}NO_5$ (555.79): C, 73.47; H, 9.61; N, 2.52. Found: C, 73.51; H, 9.84; N, 2.28.

4.2.6.12. Ethyl 3β-(L-alanyl)-11-oxo-olean-12-en-30-oate (12). Obtained from **33** by method B as a colourless powder; yield: 297 mg, 92%; mp 201–205 °C; R_f = 0.27 (DCM/MeOH 9:1); $[\alpha]_D^{20}$ = 131.56 (*c* 0.52, $CHCl_3$); UV–vis (MeOH): λ_{max} (log ε) = 250 nm (4.09); MS (ESI): *m/z* (%) = 570.4 ($[M+H]^+$, 100), 855.4 ($[[3M+2H]]^{2+}$, 10), 1139.9 ($[2M+H]^+$, 6); IR (KBr): ν = 3425 (br), 2960 (s), 2874 (m), 1728 (s), 1654 (s), 1617 (w), 1456 (m), 1389 (m), 1324 (w), 1279 (w), 1247 (w), 1217 (s), 1176 (s), 1086 (w), 1075 (w), 1031 (w), 985 (w), 972 (w), 948 (w), 917 (w), 866 (w), 768 (w), 685 (w), 589 (w), 544 (w) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.62 (s, 1H, H-12), 4.54 (dd, 1H, H-3, *J* = 11.6, 4.67 Hz), 4.17 (dq, 1H, COOC*H*H′, *J* = 10.8, 7.17 Hz), 4.10 (dq, 1H, COOCH*H*′, *J* = 10.8, 7.17 Hz), 3.52 (q, 1H, Ala-C*H*NH_2, *J* = 7.17 Hz), 2.80 (ddd, 1H, H-1, *J* = 13.7, 3.7, 3.77 Hz), 2.35 (s, 1H, H-9), 2.08 (dd, 1H, H-18, *J* = 13.7, 3.77 Hz), 2.01 (ddd, 1H, H-15, *J* = 13.3, 13.3, 4.27 Hz), 1.97 (m, 1H, H-21), 1.90 (ddd, 1H, H-19, *J* = 13.7, 4.2, 2.97 Hz), 1.81 (ddd, 1H, H-16, *J* = 13.7, 13.7, 4.67 Hz), 1.70 (m, 1H, H-2), 1.64 (m, 1H, H-7), 1.60 (m, 1H, H-2′), 1.59 (dd, 1H, H-19′, *J* = 13.3, 13.37 Hz), 1.57 (m, 1H, H-6), 1.45 (m, 1H, H-6′), 1.40 (m, 1H, H-7′), 1.37 (m, 3H, H-27), 1.35 (s, 3H, H-27), 1.34 (d, 3H, Ala-CH_3, *J* = 7.17 Hz), 1.31 (m, 1H, H-22′), 1.29 (m, 1H, H-21′), 1.24 (t, 3H, $COOCH_2CH_3$, *J* = 7.17 Hz), 1.17 (m, 1H, H-16′), 1.15 (s, 3H, H-25), 1.12 (s, 3H, H-28), 1.10 (s, 3H, H-26), 1.04 (ddd, 1H, H-1′, *J* = 13.7, 13.7, 3.77 Hz), 1.00 (m, 1H, H-15′), 0.88 (s, 3H, H-23), 0.86 (s, 3H, H-24), 0.80 (m, 1H, H-5), 0.78 (s, 3H, H-29); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 176.4 (C-30 and Ala-COO), 169.4 (C-13), 128.4 (C-12), 81.1 (C-3), 61.7 (C-9), 60.3 ($COOCH_2$), 55.0 (C-5), 50.3 (Ala-CNH), 48.4 (C-18), 45.4 (C-8), 43.8 (C-20), 43.2 (C-14), 41.1 (C-19), 38.7 (C-1), 38.2 (C-4), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-29), 28.3 (C-23), 28.1 (C-28), 26.5 (C-16), 26.4 (C-15), 23.6 (C-2), 23.3 (C-27), 20.8 (Ala-CH_3), 18.7 (C-26), 17.4 (C-6), 16.7 (C-24), 16.4 (C-25), 14.3 ($COOCH_2CH_3$). Anal. Calcd for $C_{35}H_{55}NO_5$ (569.81): C, 73.77; H, 9.73; N, 2.46. Found: C, 73.51; H, 9.84; N, 2.22.

4.2.6.13. Ethyl 3β-(β-alanyl)-11-oxo-olean-12-en-30-oate (13). Obtained from **34** by method B as a colourless powder; yield: 127 mg, 93%; mp 181–184 °C; R_f = 0.12 (DCM/MeOH 9:1); $[\alpha]_D^{20}$ = 125.66 (*c* 0.51, $CHCl_3$); UV–vis (MeOH): λ_{max} (log ε) = 250 nm (4.14); MS (ESI): *m/z* (%) = 570.3 ($[2M+2H]^{2+}$, 100); IR (KBr): ν = 3434 (br), 2958 (s), 2873 (m), 1727 (s), 1653 (s), 1465 (m), 1389 (m), 1362 (w), 1324 (m), 1278 (w), 1248 (m), 1218 (s), 1174 (s), 1087 (m), 1021 (w), 985 (m), 878 (w), 769 (w), 684 (w), 669 (w), 589 (w), 543 (w) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.64 (s, 1H, H-12), 4.55 (dd, 1H, H-3, *J* = 11.8, 4.87 Hz), 4.18 (dq, 1H, COOC*H*H′, *J* = 10.8, 7.17 Hz), 4.11 (dq, 1H, COOCH*H*′, *J* = 10.8, 7.17 Hz), 2.98 (m, 2H, Ala-CH_2NH_2), 2.80 (ddd, 1H, H-1, *J* = 13.7, 3.7, 3.77 Hz), 2.46 (t, 2H, Ala-CH_2COO, *J* = 6.67 Hz), 2.36 (s, 1H, H-9), 2.09 (dd, 1H, H-18, *J* = 13.5, 3.67 Hz), 2.02 (ddd, 1H, H-15, *J* = 13.5, 13.5, 4.47 Hz), 1.98 (m, 1H, H-21), 1.92 (ddd, 1H, H-19, *J* = 13.6, 3.8, 2.97 Hz), 1.82 (ddd, 1H, H-16, *J* = 13.7, 13.7, 4.77 Hz), 1.71 (m, 1H, H-2), 1.67 (m, 1H, H-7), 1.60 (dd, 1H, H-19′, *J* = 13.6, 13.67 Hz), 1.60 (m, 1H, H-2′), 1.58 (m, 1H, H-6), 1.46 (m, 1H, H-6′), 1.41 (m, 1H, H-7′), 1.38 (m, 1H, H-22), 1.36 (s, 3H, H-27), 1.32 (m, 1H, H-22′), 1.31 (m, 1H, H-21′), 1.25 (t, 3H, $COOCH_2CH_3$, *J* = 7.17 Hz), 1.17 (m, 1H, H-16′), 1.16 (s, 3H, H-25), 1.13 (s, 3H, H-28), 1.12 (s, 3H, H-26), 1.05 (ddd, 1H, H-1′, *J* = 13.5, 13.5, 3.57 Hz), 1.01 (m, 1H, H-15′), 0.88 (s, 6H, H-23 and H-24), 0.80 (m, 1H, H-5), 0.79 (s, 3H, H-29); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 176.4 (C-30), 172.4 (Ala-COO), 169.3 (C-13), 128.4 (C-12), 80.7 (C-3), 61.7 (C-9), 60.3 ($COOCH_2$), 55.0

(C-5), 48.4 (C-18), 45.4 (C-8), 43.8 (C-20), 43.2 (C-14), 41.0 (C-19), 38.7 (C-1), 38.4 (Ala-CH_2COO), 38.0 (C-4 and Ala-CH_2NH_2), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-29), 28.3 (C-28), 28.1 (C-23), 26.5 (C-16), 26.4 (C-15), 23.6 (C-2), 23.3 (C-27), 18.7 (C-26), 17.4 (C-6), 16.7 (C-24), 16.4 (C-25), 14.3 ($COOCH_2CH_3$). Anal. Calcd for $C_{35}H_{55}NO_5$ (569.81): C, 73.77; H, 9.73; N, 2.46. Found: C, 73.62; H, 9.91; N, 2.32.

4.2.6.14. *i*-Propyl 3β-(glycyl)-11-oxo-olean-12-en-30-oate (14). Obtained from **35** by method B as a colourless powder; yield: 120 mg, 46%; mp 265–268 °C; R_f = 0.57 (DCM/MeOH 9:1); $[\alpha]_D^{20}$ = 122.07 (*c* 0.34, $CHCl_3$); UV–vis (MeOH): λ_{max} (log ε) = 250 nm (4.09); MS (ESI): *m/z* (%) = 570.3 ($[M+H]^+$, 100), 855.3 ($[3M+2H]^{2+}$, 6); IR (KBr): *v* = 3404 (br), 2977 (s), 2858 (m), 1735 (s), 1655 (s), 1466 (m), 1389 (m), 1328 (w), 1311 (w), 1218 (s), 1179 (s), 1144 (m), 1108 (m), 1086 (m), 1020 (w), 974 (w), 917 (w), 849 (w), 669 (w), 543 (w) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 5.60 (s, 1H, H-12), 5.01 (qq, 1H, *i*-Pr-CH, *J* = 6.2, 6.27 Hz), 4.56 (dd, 1H, H-3, *J* = 11.6, 4.97 Hz), 3.42 (s, 2H, Gly-CH_2), 2.79 (ddd, 1H, H-1, *J* = 13.7, 3.5, 3.57 Hz), 2.34 (s, 1H, H-9), 2.08 (dd, 1H, H-18, *J* = 13.5, 3.77 Hz), 2.01 (ddd, 1H, H-15, *J* = 13.3, 4.4, 4.47 Hz), 1.96 (m, 1H, H-21), 1.89 (ddd, 1H, H-19, *J* = 13.2, 3.6, 2.97 Hz), 1.80 (ddd, 1H, H-16, *J* = 14.2, 14.2, 4.77 Hz), 1.70 (m, 1H, H-2), 1.65 (m, 1H, H-7), 1.62 (m, 1H, H-2′), 1.58 (dd, 1H, H-19′, *J* = 13.3, 13.37 Hz), 1.56 (m, 1H, H-6), 1.46 (m, 1H, H-6′), 1.41 (m, 1H, H-7′), 1.36 (m, 1H, H-22), 1.34 (s, 3H, H-27), 1.33 (m, 1H, H-22′), 1.27 (m, 1H, H-21′), 1.23 (d, 3H, *i*-Pr-CH_3, *J* = 6.27 Hz), 1.20 (d, 3H, *i*-Pr-CH_3, *J* = 6.27 Hz), 1.16 (m, 1H, H-16′), 1.14 (s, 3H, H-25), 1.10 (s, 6H, H-28 and H-26), 1.04 (ddd, 1H, H-1′, *J* = 13.5, 13.5, 3.67 Hz), 0.99 (m, 1H, H-15′), 0.85 (s, 6H, H-23 and H-24), 0.79 (m, 1H, H-5), 0.77 (s, 3H, H-29); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 175.8 (C-30), 173.9 (Gly-COO), 169.5 (C-13), 128.4 (C-12), 81.4 (C-3), 67.3 (*i*-Pr-CH), 61.7 (C-9), 55.0 (C-5), 48.4 (C-18), 45.4 (C-8), 44.0 (C-20), 43.7 (Gly-CH_2), 43.2 (C-14), 41.0 (C-19), 38.7 (C-1), 38.1 (C-4), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.6 (C-29), 28.2 (C-23), 28.1 (C-28), 26.5 (C-16), 26.4 (C-15), 23.6 (C-2), 23.3 (C-27), 21.9 (*i*-Pr-CH_3), 21.7 (*i*-Pr-CH_3), 18.6 (C-26), 17.3 (C-6), 16.7 (C-24), 16.4 (C-25). Anal. Calcd for $C_{35}H_{55}NO_5$ (569.81): C, 73.77; H, 9.73; N, 2.46. Found: C, 73.56; H, 9.93; N, 2.34.

4.2.6.15. *i*-Propyl 3β-(L-alanyl)-11-oxo-olean-12-en-30-oate (15). Obtained from **36** by method B as a colourless powder; yield: 160 mg, 96%; mp 249–252 °C; R_f = 0.56 (DCM/MeOH 9:1); $[\alpha]_D^{20}$ = 114.82 (*c* 0.32, $CHCl_3$); UV–vis (MeOH): λ_{max} (log ε) = 249 nm (4.10); MS (ESI): *m/z* (%) = 584.3 ($[M+H]^+$, 100); IR (KBr): *v* = 3426 (br), 2976 (s), 2874 (s), 1725 (s), 1657 (s), 1619 (m), 1466 (m), 1387 (m), 1374 (m), 1325 (m), 1280 (m), 1258 (m), 1216 (s), 1174 (s), 1142 (m), 1109 (s), 1086 (m), 1048 (w), 1021 (w), 983 (m), 948 (w), 916 (w), 880 (w), 847 (w), 770 (w), 686 (w), 671 (w), 589 (w), 544 (w) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 5.61 (s, 1H, H-12), 5.02 (qq, 1H, *i*-Pr-CH, *J* = 6.2, 6.27 Hz), 4.51 (dd, 1H, H-3, *J* = 11.8, 4.67 Hz), 3.57 (q, 1H, Ala-CH, *J* = 7.17 Hz), 2.79 (ddd, 1H, H-1, *J* = 13.5, 3.5, 3.57 Hz), 2.35 (s, 1H, H-9), 2.09 (dd, 1H, H-18, *J* = 13.5, 3.17 Hz), 2.01 (m, 1H, H-15), 1.96 (m, 1H, H-21), 1.89 (m, 1H, H-19), 1.80 (m, 1H, H-16), 1.71 (m, 1H, H-2), 1.64 (m, 1H, H-7), 1.60 (m, 1H, H-2′), 1.58 (dd, 1H, H-19′, *J* = 13.5, 13.57 Hz), 1.56 (m, 1H, H-6), 1.44 (m, 1H, H-6′), 1.41 (m, 1H, H-7′), 1.36 (m, 1H, H-22), 1.35 (s, 3H, H-27), 1.31 (d, 3H, Ala-CH_3, *J* = 7.07 Hz), 1.31 (m, 1H, H-22′), 1.30 (m, 1H, H-21′), 1.23 (d, 3H, *i*-Pr-CH_3, *J* = 6.27 Hz), 1.20 (d, 3H, *i*-Pr-CH_3, *J* = 6.27 Hz), 1.18 (m, 1H, H-16′), 1.15 (s, 3H, H-25), 1.11 (s, 6H, H-28 and H-26), 1.04 (m, 1H, H-1′), 0.99 (m, 1H, H-15′), 0.88 (s, 3H, H-23), 0.86 (s, 3H, H-24), 0.79 (m, 1H, H-5), 0.78 (s, 3H, H-29); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 176.2 (C-30), 175.8 (Ala-COO), 169.5 (C-13), 128.4 (C-12), 81.3 (C-3), 67.4 (*i*-Pr-CH), 61.7 (C-9), 55.0 (C-5), 50.3 (Ala-CH), 48.4 (C-18), 45.4 (C-8), 43.7 (C-20), 43.2 (C-14), 41.1 (C-19), 38.7 (C-1), 38.2 (C-4), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.6 (C-29), 28.2 (C-23), 28.1 (C-28), 26.5 (C-16), 26.4 (C-15), 23.6 (C-2), 23.3 (C-27), 21.9 (*i*-Pr-CH_3), 21.7 (*i*-Pr-CH_3), 20.5 (Ala-CH_3), 18.7 (C-26), 17.4 (C-6), 16.7 (C-24), 16.4 (C-25). Anal. Calcd for $C_{36}H_{57}NO_7$ (583.84): C, 74.06; H, 9.84; N, 2.40. Found: C, 73.99; H, 10.01; N, 2.26.

4.2.6.16. *i*-Propyl 3β-(β-alanyl)-11-oxo-olean-12-en-30-oate (16). Obtained from **37** by method B as a colourless powder; yield: 200 mg, 76%; mp 266–269 °C (decomp.); R_f = 0.19 (DCM/MeOH 9:1); $[\alpha]_D^{20}$ = 120.18 (*c* 0.36, $CHCl_3$); UV–vis (MeOH): λ_{max} (log ε) = 250 nm (4.08); MS (ESI): *m/z* (%) = 584.9 ($[2M+2H]^{2+}$, 100); IR (KBr): *v* = 3388 (br), 2956 (s), 1722 (s), 1655 (s), 1466 (m), 1387 (m), 1218 (s), 1173 (m), 1141 (m), 1111 (m), 1085 (m), 1022 (w), 987 (w), 917 (w), 878 (w), 830 (w), 670 (w), 545 (w) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.60 (s, 1H, H-12), 5.01 (qq, 1H, *i*-Pr-CH, *J* = 6.2, 6.27 Hz), 4.53 (dd, 1H, H-3, *J* = 11.7, 4.57 Hz), 2.98 (m, 2H, Ala-CH_2NH_2), 2.78 (ddd, 1H, H-1, *J* = 13.2, 3.3, 3.37 Hz), 2.47 (t, 2H, Ala-CH_2COO, *J* = 5.47 Hz), 2.34 (s, 1H, H-9), 2.08 (dd, 1H, H-18, *J* = 13.3, 3.37 Hz), 2.01 (ddd, 1H, H-15, *J* = 13.7, 13.7, 4.77 Hz), 1.96 (m, 1H, H-21), 1.89 (ddd, 1H, H-19, *J* = 13.3, 3.7, 2.57 Hz), 1.80 (ddd, 1H, H-16, *J* = 14.1, 14.1, 4.77 Hz), 1.71 (m, 1H, H-2), 1.66 (m, 1H, H-7), 1.60 (m, 1H, H-2′), 1.58 (dd, 1H, H-19′, *J* = 13.5, 13.57 Hz), 1.56 (m, 1H, H-6), 1.44 (m, 1H, H-6′), 1.38 (m, 1H, H-7′), 1.35 (m, 1H, H-22), 1.34 (s, 3H, H-27), 1.34 (m, 1H, H-22′), 1.32 (m, 1H, H-21′), 1.23 (d, 3H, *i*-Pr-CH_3, *J* = 6.27 Hz), 1.20 (d, 3H, *i*-Pr-CH_3, *J* = 6.27 Hz), 1.16 (m, 1H, H-16′), 1.14 (s, 3H, H-25), 1.10 (s, 6H, H-28 and H-26), 1.03 (m, 1H, H-1′), 0.99 (m, 1H, H-15′), 0.86 (s, 6H, H-23 and H-24), 0.79 (m, 1H, H-5), 0.77 (s, 3H, H-29); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 175.8 (C-30), 172.3 (Ala-COO), 169.4 (C-13), 128.4 (C-12), 80.8 (C-3), 67.3 (*i*-Pr-CH), 61.7 (C-9), 55.0 (C-5), 48.4 (C-18), 45.4 (C-8), 43.7 (C-20), 43.2 (C-14), 41.0 (C-19), 38.7 (C-1), 38.5 (Ala-CH_2COO), 38.0 (C-4 and Ala-CH_2NH_2), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.6 (C-29), 28.2 (C-28), 28.1 (C-23), 26.5 (C-16), 26.4 (C-15), 23.6 (C-2), 23.3 (C-27), 21.9 (*i*-Pr-CH_3), 21.7 (*i*-Pr-CH_3), 18.6 (C-26), 17.4 (C-6), 16.7 (C-24), 16.3 (C-25). Anal. Calcd for $C_{36}H_{57}NO_5$ (583.84): C, 74.33; H, 9.95; N, 2.34. Found: C, 74.21; H, 10.07; N, 2.28.

4.2.6.17. Benzyl 3β-(glycyl)-11-oxo-olean-12-en-30-oate (17). Obtained from **38** by method B as a colourless powder; yield: 200 mg, 89%; mp 186–190 °C; R_f = 0.58 (DCM/MeOH 9:1); $[\alpha]_D^{20}$ = 123.22 (*c* 0.56, $CHCl_3$); UV–vis (MeOH): λ_{max} (log ε) = 249 nm (4.05); MS (ESI): *m/z* (%) = 618.3 ($[2M+2H]^{2+}$, 100), 927.3 ($[3M+3H]^{2+}$, 5); IR (KBr): *v* = 3393 (br), 2951 (s), 2874 (m), 1731 (s), 1655 (s), 1618 (w), 1498 (w), 1456 (m), 1388 (m), 1365 (m), 1324 (m), 1214 (s), 1147 (s), 1085 (m), 1049 (w), 1028 (w), 985 (m), 916 (w), 880 (w), 767 (w), 748 (w), 697 (w), 670 (w), 586 (w), 542 (w) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.38–7.28 (m, 5H, H-Ar), 5.53 (s, 1H, H-12), 5.18 (d, 1H, Bn-C*H*H′, *J* = 12.67 Hz), 5.07 (d, 1H, Bn-H*H*′, *J* = 12.17 Hz), 4.56 (dd, 1H, H-3, *J* = 11.5, 4.77 Hz), 3.41 (s, 2H, Gly-CH_2), 2.79 (ddd, 1H, H-1, *J* = 13.6, 3.6, 3.67 Hz), 2.32 (s, 1H, H-9), 2.02 (m, 1H, H-18), 2.00 (m, 1H, H-15), 1.97 (m, 1H, H-21), 1.92 (dd, 1H, H-19, *J* = 13.0, 3.8, 2.97 Hz), 1.79 (ddd, 1H, H-16, *J* = 14.0, 14.0, 4.57 Hz), 1.70 (m, 1H, H-2), 1.65 (m, 1H, H-7), 1.62 (m, 1H, H-2′), 1.60 (dd, 1H, H-19′, *J* = 13.2, 13.27 Hz), 1.56 (m, 1H, H-6), 1.45 (m, 1H, H-6′), 1.40 (m, 1H, H-7′), 1.35 (m, 1H, H-22), 1.33 (s, 3H, H-27), 1.30 (m, 1H, H-21′), 1.28 (m, 1H, H-22′), 1.15 (m, 1H, H-16′), 1.14 (s, 6H, H-28 and H-25), 1.09 (s, 3H, H-26), 1.04 (m, 1H, H-1′), 0.98 (m, 1H, H-15′), 0.86 (s, 6H, H-23 and H-24), 0.78 (m, 1H, H-5), 0.71 (s, 3H, H-29); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.9 (C-11),

176.2 (C-30), 174.0 (Gly-COO), 169.1 (C-13), 136.1 (C_{ar}), 128.6 (C-12), 128.6 (C_{ar}), 128.4 (C_{ar}), 128.3 (C_{ar}), 128.2 (C_{ar}), 128.2 (C_{ar}), 81.3 (C-3), 66.2 (Bn-CH_2), 61.6 (C-9), 55.0 (C-5), 48.2 (C-18), 45.3 (C-8), 44.1 (Gly-CH_2), 44.0 (C-20), 43.2 (C-14), 41.0 (C-19), 38.7 (C-1), 38.1 (C-4), 37.6 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.2 (C-21), 28.4 (C-29), 28.3 (C-23), 28.1 (C-28), 26.4 (C-16), 26.4 (C-15), 23.6 (C-2), 23.3 (C-27), 18.6 (C-26), 17.4 (C-6), 16.7 (C-24), 16.4 (C-25). Anal. Calcd for $C_{39}H_{55}NO_5$ (617.86): C, 75.81; H, 8.97; N, 2.27. Found: C, 75.77; H, 9.12; N, 2.16.

4.2.6.18. Benzyl 3β-(L-alanyl)-11-oxo-olean-12-en-30-oate (18). Obtained from **39** by method B as a colourless powder; yield: 330 mg, 98%; mp 200–203 °C; R_f = 0.58 (DCM/MeOH 9:1); $[\alpha]_D^{20}$ = 128.39 (*c* 0.52, $CHCl_3$); UV–vis (MeOH): λ_{max} (log ε) = 207 nm (4.08), 249 nm (4.10); MS (ESI): *m/z* (%) = 632.2 ($[2M+2H]^{2+}$, 100), 948.2 ($[3M+2H]^{2+}$, 10), 1263.7 ($[2M+H]^+$, 4); IR (KBr): *ν* = 3369 (br), 2958 (s), 2874 (m), 1728 (s), 1652 (s), 1616 (w), 1498 (w), 1455 (m), 1389 (m), 1368 (w), 1324 (m), 1278 (w), 1263 (w), 1214 (s), 1190 (s), 1146 (s), 1085 (m), 1063 (m), 1050 (w), 1029 (m), 972 (w), 916 (w), 890 (w), 868 (w), 850 (w), 768 (w), 742 (m), 696 (w), 613 (w), 587 (w), 544 (w), 471 (w) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.38–7.28 (m, 5H, H-Ar), 5.52 (s, 1H, H-12), 5.18 (d, 1H, Bn-C*H*H′, *J* = 12.57 Hz), 5.06 (d, 1H, Bn-CH*H*′, *J* = 12.37 Hz), 4.53 (dd, 1H, H-3, *J* = 11.7, 4.87 Hz), 3.52 (q, 1H, Ala-CH, *J* = 7.17 Hz), 2.79 (ddd, 1H, H-1, *J* = 13.6, 3.4, 3.47 Hz), 2.32 (s, 1H, H-9), 2.01 (m, 1H, H-18), 1.99 (m, 1H, H-15), 1.96 (m, 1H, H-21), 1.92 (m, 1H, H-19), 1.78 (ddd, 1H, H-16, *J* = 13.7, 13.7, 4.67 Hz), 1.70 (m, 1H, H-2), 1.65 (m, 1H,H-7), 1.62 (m, 1H, H-2′), 1.59 (dd, 1H, H-19′, *J* = 13.3, 13.37 Hz), 1.57 (m, 1H, H-6), 1.44 (m, 1H, H-6′), 1.37 (m, 1H, H-7′), 1.34 (m, 1H, H-22), 1.34 (m, 3H, Ala-CH_3), 1.33 (s, 3H, H-27), 1.29 (m, 2H, H-22′ and H-21′), 1.15 (m, 1H, H-16′), 1.14 (s, 6H, H-25 and H-28), 1.09 (s, 3H, H-26), 1.03 (ddd, 1H, H-1′, *J* = 13.3, 13.3, 3.37 Hz), 0.97 (m, 1H, H-15′), 0.87 (s, 3H, H-23), 0.85 (s, 3H, H-24), 0.79 (m, 1H, H-5), 0.71 (s, 3H, H-29); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 176.3 (C-30), 176.2 (Ala-COO), 169.0 (C-13), 136.1 (C_{ar}), 128.6 (C_{ar}), 128.6 (C_{ar}), 128.4 (C-12), 128.3 (C_{ar}), 128.2 (C_{ar}), 128.2 (C_{ar}), 81.1 (C-3), 66.2 (Bn-CH_2), 61.6 (C-9), 55.0 (C-5), 50.4 (Ala-CH), 48.2 (C-18), 45.3 (C-8), 44.0 (C-20), 43.2 (C-14), 41.1 (C-19), 38.7 (C-1), 38.2 (C-4), 37.6 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.2 (C-21), 28.4 (C-29), 28.3 (C-23), 28.1 (C-28), 26.4 (C-16), 26.4 (C-15), 23.6 (C-2), 23.3 (C-27), 20.8 (Ala-CH_3), 18.7 (C-26), 17.3 (C-6), 16.7 (C-24), 16.4 (C-25). Anal. Calcd for $C_{40}H_{57}NO_5$ (631.88): C, 76.03; H, 9.09; N, 2.22. Found: C, 75.87; H, 9.12; N, 2.17.

4.2.6.19. Benzyl 3β-(β-alanyl)-11-oxo-olean-12-en-30-oate (19). Obtained from **40** by method B as a colourless powder; yield: 150 mg, 91%; mp 233–236 °C (decomp.); R_f = 0.20 (DCM/MeOH 9:1); $[\alpha]_D^{20}$ = 114.31 (*c* 0.59, $CHCl_3$); UV–vis (MeOH): λ_{max} (log ε) = 207 nm (4.02), 250 nm (4.08); MS (ESI): *m/z* (%) = 632.3 ($[M+H]^+$, 100); IR (KBr): *ν* = 3424 (br), 2949 (s), 2872 (m), 1727 (s), 1657 (s), 1456 (m), 1387 (m), 1367 (m), 1324 (m), 1279 (m), 1248 (m), 1213 (s), 1148 (s), 1085 (m), 1048 (w), 1023 (w), 986 (m), 915 (w), 880 (w), 830 (w), 754 (w), 698 (m), 670 (w), 586 (w), 543 (w) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.38–7.28 (m, 5H, H-Ar), 5.52 (s, 1H, H-12), 5.18 (d, 1H, Bn-C*H*H′, *J* = 12.27 Hz), 5.06 (d, 1H, Bn-C*H*H′, *J* = 12.27 Hz), 4.54 (dd, 1H, H-3, *J* = 11.7, 5.17 Hz), 3.10 (m, 2H, Ala-CH_2NH_2), 2.79 (ddd, 1H, H-1, *J* = 13.7, 3.7, 3.77 Hz), 2.61 (m, 2H, Ala-CH_2COO), 2.32 (s, 1H, H-9), 2.01 (m 1H, H-18), 1.99 (m, 1H, H-15), 1.97 (m, 1H, H-21), 1.92 (ddd, 1H, H-19, *J* = 13.3, 3.7, 2.97 Hz), 1.78 (ddd, 1H, H-16, *J* = 13.7, 13.7, 4.27 Hz), 1.70 (m, 1H, H-2), 1.65 (m, 1H, H-7), 1.60 (m, 1H, H-2′), 1.60 (dd, 1H, H-19′, *J* = 13.5, 13.57 Hz), 1.56 (m, 1H, H-6), 1.44 (m, 1H, H-6′), 1.38 (m, 1H, H-7′), 1.34 (m, 1H, H-22), 1.33 (s, 3H, H-27), 1.30 (m, 1H, H-22′), 1.27 (m, 1H, H-21′), 1.16 (m, 1H, H-16′), 1.14 (s, 6H, H-25 and H-28), 1.09 (s, 3H, H-26), 1.02 (ddd, 1H, H-1′, *J* = 13.3, 13.3, 3.77 Hz), 0.97 (m, 1H, H-15′), 0.86 (s, 6H, H-23 and H-24), 0.78 (m, 1H, H-5), 0.71 (s, 3H, H-29); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.9 (C-11), 176.2 (C-30), 172.0 (Ala-COO), 169.1 (C-13), 136.1 (C_{ar}), 128.6 (C_{ar}), 128.5 (C-12), 128.6 (C_{ar}), 128.3 (C_{ar}), 128.2 (C_{ar}), 128.2 (C_{ar}), 81.4 (C-3), 66.2 (Bn-CH_2), 61.6 (C-9), 55.0 (C-5), 48.2 (C-18), 45.3 (C-8), 44.0 (C-20), 43.2 (C-14), 41.1 (C-19), 38.8 (C-1), 38.7 (Ala-CH_2COO), 38.1 (C-4), 38.1 (Ala-CH_2NH_2), 37.6 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.2 (C-21), 28.4 (C-29), 28.3 (C-23), 28.1 (C-28), 26.5 (C-16), 26.4 (C-15), 23.6 (C-2), 23.3 (C-27), 18.7 (C-26), 17.4 (C-6), 16.7 (C-24), 16.4 (C-25). Anal. Calcd for $C_{40}H_{57}NO_5$ (631.88): C, 76.03; H, 9.09; N, 2.22. Found: C, 75.79; H, 9.21; N, 2.11.

4.2.6.20. Methyl 3β-(glycyl)-11-oxo-olean-12-en-30-oate hydrochloride (20). Obtained from **29** by method D as a colourless powder; yield:130 mg, 48%; mp 288–292 °C (decomp.); R_f = 0.54 (MeOH); $[\alpha]_D^{20}$ = 130.16 (*c* 0.39, CH_2Cl_2); UV–vis (MeOH): λ_{max} (log ε) = 249 nm (4.03); MS (ESI): *m/z* (%) = 542.3 ($[2M+2H]^{2+}$, 100), 812.9 ($[3M+2H]^{2+}$, 12), 1083.1 ($[2M+H]^+$, 6); IR (KBr): *ν* = 3439 (br), 2951 (s), 1731 (s), 1657 (s), 1618 (m), 1466 (s), 1432 (m), 1389 (m), 1323 (m), 1244 (s), 1155 (s), 1087 (m), 1049 (m), 987 (m), 911 (w), 880 (w), 825 (w), 770 (w), 686 (w), 670 (s), 590 (w), 544 (w), 501 (w) cm^{-1}; ^1H NMR (400 MHz, MeOH-d_4): δ = 5.57 (s, 1H, H-12), 4.68 (dd, 1H, H-3, *J* = 11.9, 4.77 Hz), 3.84 (dd, 2H, Gly-CH_2, *J* = 26.5, 7.07 Hz), 3.69 (s, 3H, OMe), 2.79 (ddd, 1H, H-1, *J* = 13.8, 3.7, 3.77 Hz), 2.49 (s, 1H, H-9), 2.14 (m, 1H, H-15), 2.12 (dd, 1H, H-18, *J* = 13.3, 4.17 Hz), 1.96 (m, 1H, H-21), 1.88 (ddd, 1H, H-16, *J* = 13.7, 13.7, 4.37 Hz), 1.86 (m, 1H, H-19), 1.81 (m, 1H, H-2), 1.77 (m, 1H, H-7), 1.73 (dd, 1H, H-19′, *J* = 13.3, 13.37 Hz), 1.67 (m, 1H, H-2′), 1.64 (m, 1H, H-6), 1.53 (m, 1H, H-6′), 1.47 (m, 1H, H-7′), 1.44 (m, 1H, H-22), 1.43 (s, 3H, H-27), 1.41 (m, 1H, H-21′), 1.28 (m, 1H, H-22′), 1.24 (m, 1H, H-16′), 1.17 (s, 3H, H-25), 1.14 (s, 6H, H-26 and H-28), 1.12 (m, 1H, H-1′), 1.04 (m, 1H, H-15′), 0.94 (s, 3H, H-23), 0.93 (s, 3H, H-24), 0.91 (m, 1H, H-5), 0.82 (s, 3H, H-29); ^{13}C NMR (125 MHz, MeOH-d_4): δ = 202.2 (C-11), 178.6 (C-30), 172.7 (Gly-COO), 168.4 (C-13), 128.9 (C-12), 84.6 (C-3), 62.9 (C-9), 56.0 (C-5), 52.3 (OMe), 49.9 (C-18), 46.7 (C-8), 44.7 (C-20), 44.6 (C-14), 42.4 (C-19), 41.1 (Gly-CH_2), 39.6 (C-1), 39.2 (C-4), 39.0 (C-22), 38.2 (C-10), 33.6 (C-7), 33.0 (C-17), 32.0 (C-21), 29.1 (C-29), 28.5 (C-23), 28.5 (C-28), 27.6 (C-16), 27.3 (C-15), 24.4 (C-2), 23.8 (C-27), 19.3 (C-26), 18.4 (C-6), 17.1 (C-24), 16.9 (C-25). Anal. Calcd for $C_{33}H_{52}ClNO_5$ (578.22): C, 68.55; H, 9.06; Cl, 6.13; N, 2.42. Found: C, 68.49; H, 9.27; Cl, 6.27; N, 2.13.

4.2.6.21. Methyl 3β-(L-alanyl)-11-oxo-olean-12-en-30-oate hydrochloride (21). Obtained from **30** by method D as a colourless powder; yield: 180 mg, 56%; mp 288–292 °C (decomp.); R_f = 0.61 (MeOH); $[\alpha]_D^{20}$ = 125.80 (*c* 0.36, MeOH); UV–vis (MeOH): λ_{max} (log ε) = 249 nm (4.02); MS (ESI): *m/z* (%) = 556.2 ($[M+H]^+$, 100), 833.8 ($[3M+2H]^{2+}$, 10); IR (KBr): *ν* = 3441 (br), 2951 (s), 2362 (w), 1743 (s), 1730 (s), 1661 (s), 1622 (w), 1517 (m), 1466 (m), 1387 (m), 1322 (w), 1257 (m), 1217 (m), 1191 (m), 1153 (m), 1122 (m), 1086 (w), 1049 (s), 985 (m), 914 (w), 867 (m), 823 (w), 769 (w), 670 (w), 546 (w) cm^{-1}; ^1H NMR (400 MHz, MeOH-d_4): δ = 5.58 (s, 1H, H-12), 4.67 (dd, 1H, H-3, *J* = 11.7, 5.07 Hz), 4.10 (q, 1H, Ala-CH, *J* = 7.47 Hz), 3.69 (s, 3H, OMe), 2.80 (ddd, 1H, H-1, *J* = 13.3, 3.3, 3.37 Hz), 2.50 (s, 1H, H-9), 2.16 (m, 1H, H-15), 2.13 (m, 1H, H-18), 1.97 (m, 1H, H-21), 1.90 (m, 1H, H-16), 1.84 (m, 1H, H-19), 1.81 (m, 1H, H-2), 1.77 (m, 1H, H-7), 1.72 (dd, 1H, H-19′, *J* = 13.2, 13.27 Hz), 1.68 (m, 1H, H-2′), 1.66 (m, 1H, H-6), 1.51 (m, 1H, H-6′), 1.47 (m, 1H, H-7′), 1.41 (m, 1H, H-22), 1.43 (s, 3H, H-27), 1.44 (m, 1H, H-21′), 1.56 (d, 3H, Ala-CH_3, *J* = 7.37 Hz), 1.29 (m, 1H, H-22′), 1.25 (m, 1H, H-16′),

1.18 (s, 3H, H-25), 1.15 (s, 6H, H-26 and H-28), 1.12 (m, 1H, H-1′), 1.04 (m, 1H, H-15′), 0.95 (s, 3H, H-24), 0.93 (s, 3H, H-23), 0.92 (m, 1H, H-5), 0.82 (s, 3H, H-29); ^{13}C NMR (125 MHz, MeOH-d_4): δ = 200.8 (C-11), 177.2 (C-30), 171.3 (Ala-COO), 169.4 (C-13), 127.5 (C-12), 83.2 (C-3), 61.4 (C-9), 54.5 (C-5), 50.9 (OMe), 48.5 (Ala-CH), 48.1 (C-18), 45.3 (C-8), 43.9 (C-20), 43.2 (C-14), 40.9 (C-19), 38.2 (C-1), 37.9 (C-4), 37.5 (C-22), 36.8 (C-10), 32.2 (C-7), 31.5 (C-17), 30.6 (C-21), 27.7 (C-29), 27.1 (C-23), 27.1 (C-28), 26.1 (C-16), 25.9 (C-15), 23.0 (C-2), 22.4 (C-27), 17.8 (C-26), 17.0 (C-6), 15.7 (C-24), 15.5 (C-25), 15.0 (Ala-CH_3). Anal. Calcd for $C_{34}H_{54}ClNO_5$ (592.25): C, 68.95; H, 9.19; Cl, 5.99; N, 2.37. Found: C, 68.68; H, 9.25; Cl, 6.09; N, 2.22.

4.2.6.22. Methyl 3β-(β-alanyl)-11-oxo-olean-12-en-30-oate hydrochloride (22). Obtained from **31** by method D as a colourless powder; yield: 160 mg, 76%; mp 282–285 °C (decomp.); R_f = 0.19 (MeOH); $[\alpha]_D^{20}$ = 128.78 (*c* 0.45, MeOH); UV–vis (MeOH): λ_{max} (log ε) = 247 nm (4.17); MS (ESI): *m/z* (%) = 556.2 ([M+H]$^+$, 100), 834.3 ([3M+2H]$^{2+}$, 10), 1111.1 ([2M+H]$^+$, 4); IR (KBr): ν = 3437 (br), 2951 (s), 2034 (w), 1729 (s), 1660 (s), 1619 (m), 1465 (s), 1387 (m), 1323 (m), 1279 (m), 1218 (s), 1154 (s), 1108 (m), 1087 (m), 1049 (w), 1013 (w), 989 (s), 916 (w), 862 (w), 824 (w), 788 (w), 770 (w), 670 (w), 590 (w), 544 (w) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.56 (s, 1H, H-12), 4.57 (dd, 1H, H-3, *J* = 11.8, 4.77 Hz), 3.67 (s, 3H, OMe), 3.18 (m, 2H, Ala-CH_2NH_2), 2.74 (m, 1H, H-1), 2.72 (m, 2H, Ala-*C*H$_2$COO), 2.47 (s, 1H, H-9), 2.13 (m, 1H, H-15), 2.11 (m, 1H, H-18), 1.95 (m, 1H, H-21), 1.86 (m, 1H, H-16), 1.84 (m, 1H, H-19), 1.73 (m, 1H, H-2), 1.71 (dd, 1H, H-19′, *J* = 13.3, 13.37 Hz), 1.66 (m, 1H, H-7), 1.63 (m, 1H, H-2′), 1.61 (m, 1H, H-6), 1.51 (m, 1H, H-6′), 1.45 (m, 1H, H-7′), 1.41 (s, 3H, H-27), 1.40 (m, 1H, H-22), 1.28 (m, 1H, H-21′), 1.25 (m, 1H, H-22′), 1.23 (m, 1H, H-16′), 1.15 (s, 3H, H-25), 1.13 (s, 6H, H-28 and H-26), 1.09 (m, 1H, H-1′), 1.03 (m, 1H, H-15′), 0.90 (s, 6H, H-23 and H-24), 0.88 (m, 1H, H-5), 0.80 (s, 3H, H-29); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.9 (C-11), 177.2 (C-30), 171.3 (Ala-COO), 170.6 (C-13), 127.5 (C-12), 81.8 (C-3), 61.5 (C-9), 54.7 (C-5), 50.9 (OMe), 48.5 (C-18), 45.3 (C-8), 43.9 (C-20), 43.2 (C-14), 40.9 (C-19), 38.3 (C-1), 37.7 (C-4), 37.5 (C-22), 36.8 (C-10), 35.0 (Ala-CH_2NH_2), 32.2 (C-7), 31.5 (C-17), 30.9 (Ala-*C*H$_2$COO), 30.6 (C-21), 27.7 (C-29), 27.1 (C-28), 27.1 (C-23), 26.1 (C-16), 25.9 (C-15), 23.0 (C-2), 22.4 (C-27), 17.8 (C-26), 17.0 (C-6), 15.7 (C-24), 15.5 (C-25). Anal. Calcd for $C_{34}H_{54}ClNO_5$ (592.25): C, 68.95; H, 9.19; Cl, 5.99; N, 2.37. Found: C, 68.87; H, 9.32; Cl, 6.12; N, 2.24.

4.2.6.23. Methyl 3β-(D-alanyl)-11-oxo-olean-12-en-30-oate (23). Obtained from **41** by method B as a colourless powder; yield: 220 mg, 76%; mp 243–247 °C; R_f = 0.58 (DCM/MeOH 9:1); $[\alpha]_D^{20}$ = 123.94 (*c* 0.28, $CHCl_3$); UV–vis (MeOH): λ_{max} (log ε) = 250 nm (4.08); MS (ESI): *m/z* (%) = 556.7 ([2M+2H]$^{2+}$, 100); IR (KBr): ν = 3387 (br), 2958 (s), 2874 (m), 1731 (s), 1652 (s), 1455 (m), 1389 (m), 1323 (w), 1279 (w), 1248 (m), 1218 (m), 1190 (m), 1160 (m), 1087 (w), 1050 (w), 1022 (w), 985 (w), 973 (m), 917 (w), 867 (w), 825 (w), 770 (w), 669 (w), 590 (w), 544 (w) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.64 (s, 1H, H-12), 4.51 (dd, 1H, H-3, *J* = 11.6, 4.87 Hz), 3.66 (s, 3H, OMe), 3.53 (q, 1H, Ala-CH, *J* = 7.07 Hz), 2.79 (ddd, 1H, H-1, *J* = 13.7, 3.8, 3.87 Hz), 2.34 (s, 1H, H-9), 2.06 (dd, 1H, H-18, *J* = 13.3, 3.37 Hz), 2.00 (m, 1H, H-15), 1.97 (m, 1H, H-21), 1.90 (m, 1H, H-19), 1.80 (ddd, 1H, H-16, *J* = 13.8, 13.8, 4.57 Hz), 1.70 (m, 1H, H-2), 1.63 (m, 1H, H-7), 1.59 (m, 1H, H-2′), 1.59 (dd, 1H, H-19′, *J* = 13.6, 13.67 Hz), 1.56 (m, 1H, H-6), 1.44 (m, 1H, H-6′), 1.39 (m, 1H, H-7′), 1.36 (m, 1H, H-22), 1.34 (s, 3H, H-27), 1.32 (d, 3H, Ala-CH3, *J* = 7.07 Hz), 1.29 (m, 2H, H-22′ and H-21′), 1.16 (m, 1H, H-16′), 1.14 (s, 3H, H-25), 1.12 (s, 3H, H-28), 1.10 (s, 3H, H-26), 1.04 (ddd, 1H, H-1′, *J* = 13.5, 13.5, 4.17 Hz), 0.99 (m, 1H, H-15′), 0.87 (s, 3H, H-23), 0.86 (s, 3H, H-24), 0.79 (m, 1H, H-5), 0.78 (s, 3H, H-29); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 176.9 (C-30), 176.1 (Ala-COO), 169.2 (C-13), 128.5 (C-12), 81.1 (C-3), 61.7 (C-9), 55.0 (C-5), 51.7 (OMe), 50.2 (Ala-CH), 48.4 (C-18), 45.4 (C-8), 44.0 (C-20), 43.2 (C-14), 41.1 (C-19), 38.7 (C-1), 38.1 (C-4), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-29), 28.3 (C-23), 28.1 (C-28), 26.4 (C-16), 26.4 (C-15), 23.4 (C-2), 23.3 (C-27), 20.6 (Ala-CH_3), 18.6 (C-26), 17.3 (C-6), 16.7 (C-24), 16.4 (C-25). Anal. Calcd for $C_{34}H_{53}NO_5$ (555.79): C, 73.47; H, 9.61; N, 2.52. Found: C, 73.41; H, 9.77; N, 2.49.

4.2.6.24. Ethyl 3β-(D-alanyl)-11-oxo-olean-12-en-30-oate (24). Obtained from **42** by method B as a colourless powder; yield: 240 mg, 86%; mp 236–239 °C; R_f = 0.58 (DCM/MeOH 9:1); $[\alpha]_D^{20}$ = 108.78 (*c* 0.33, $CHCl_3$); UV–vis (MeOH): λ_{max} (log ε) = 249 nm (4.06); MS (ESI): *m/z* (%) = 570.3 ([M+H]$^+$, 100), 854.8 ([3M+2Na]$^{2+}$, 10), 1139.2 ([2M+H]$^+$, 4); IR (KBr): ν = 3387 (br), 2958 (s), 2874 (m), 1725 (s), 1653 (s), 1626 (m), 1454 (m), 1389 (m), 1323 (w), 1279 (w), 1246 (m), 1216 (s), 1177 (s), 1087 (m), 1049 (w), 1022 (w), 984 (w), 972 (w), 916 (w), 878 (w), 841 (w), 755 (w), 670 (w), 544 (w) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.62 (s, 1H, H-12), 4.51 (dd, 1H, H-3, *J* = 11.6, 4.77 Hz), 4.16 (dq, 1H, COOC*H*H′, *J* = 10.8, 7.17 Hz), 4.11 (dq, 1H, COOCH*H*′, *J* = 10.8, 7.17 Hz), 3.54 (q, 1H, Ala-CH, *J* = 7.17 Hz), 2.79 (ddd, 1H, H-1, *J* = 13.8, 3.5, 3.57 Hz), 2.34 (s, 1H, H-9), 2.08 (dd, 1H, H-18, *J* = 13.3, 3.37 Hz), 2.01 (ddd, 1H, H-15, *J* = 13.8, 13.8, 4.57 Hz), 1.97 (m, 1H, H-21), 1.90 (m, 1H, H-19), 1.80 (ddd, 1H, H-16, *J* = 13.6, 13.6, 4.67 Hz), 1.71 (m, 1H, H-2), 1.66 (m, 1H, H-7), 1.60 (m, 1H, H-2′), 1.59 (dd, 1H, H-19′, *J* = 13.4, 13.47 Hz), 1.56 (m, 1H, H-6), 1.44 (m, 1H, H-6′), 1.39 (m, 1H, H-7′), 1.36 (m, 1H, H-22), 1.34 (s, 3H, H-27), 1.32 (d, 3H, Ala-CH_3, *J* = 7.17 Hz), 1.29 (m, 1H, H-22′), 1.27 (m, 1H, H-21′), 1.24 (t, 3H, Et-CH_3, *J* = 7.17 Hz), 1.16 (m, 1H, H-16′), 1.14 (s, 3H, H-25), 1.12 (s, 3H, H-28), 1.10 (s, 3H, H-26), 1.04 (m, 1H, H-1′), 0.99 (m, 1H, H-15′), 0.87 (s, 3H, H-24), 0.86 (s, 3H, H-24), 0.80 (m, 1H, H-5), 0.78 (s, 3H, H-29); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 176.3 (C-30), 176.1 (Ala-COO), 169.4 (C-13), 128.4 (C-12), 81.2 (C-3), 61.7 (C-9), 60.3 (Et-CH_2), 55.0 (C-5), 50.2 (Ala-CH), 48.4 (C-18), 45.4 (C-8), 43.8 (C-20), 43.2 (C-14), 41.1 (C-19), 38.7 (C-1), 38.1 (C-4), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-29), 28.3 (C-23), 28.1 (C-28), 26.5 (C-16), 26.4 (C-15), 23.4 (C-2), 23.3 (C-27), 20.7 (Ala-CH_3), 18.7 (C-26), 17.3 (C-6), 16.7 (C-24), 16.4 (C-25), 14.3 (Et-CH_3). Anal. Calcd for $C_{35}H_{55}NO_5$ (569.81): C, 73.77; H, 9.73; N, 2.46. Found: C, 73.56; H, 9.87; N, 2.42.

4.2.6.25. *i*-Propyl 3β-(D-alanyl)-11-oxo-olean-12-en-30-oate (25). Obtained from **43** by method B as a colourless powder; yield: 200 mg, 90%; mp 263–266 °C; R_f = 0.59 (DCM/MeOH 9:1); $[\alpha]_D^{20}$ = 113.51 (*c* 0.24, $CHCl_3$); UV–vis (MeOH): λ_{max} (log ε) = 250 nm (4.07); MS (ESI): *m/z* (%) = 584.1 ([M+H]$^+$, 100), 876.3 ([3M+3Na]$^{2+}$, 8), 1167.3 ([2M+H]$^+$, 4); IR (KBr): ν = 3420 (br), 2971 (s), 2875 (s), 1721 (s), 1652 (s), 1455 (m), 1388 (m), 1313 (m), 1279 (m), 1217 (s), 1180 (s), 1142 (m), 1108 (s), 1086 (m), 1021 (w), 986 (m), 972 (m), 917 (m), 879 (w), 848 (w), 770 (w), 686 (w), 545 (w) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.60 (s, 1H, H-12), 5.01 (qq, 1H, *i*-Pr-CH, *J* = 6.4, 6.47 Hz), 4.51 (dd, 1H, H-3, *J* = 11.7, 4.57 Hz), 3.53 (q, 1H, Ala-CH, *J* = 7.17 Hz), 2.79 (ddd, 1H, H-1, *J* = 13.6, 3.5, 3.57 Hz), 2.34 (s, 1H, H-9), 2.08 (dd, 1H, H-18, *J* = 13.2, 3.37 Hz), 2.01 (dd, 1H, H-15, *J* = 13.7, 4.67 Hz), 1.96 (m, 1H, H-21), 1.89 (m, 1H, H-19), 1.80 (ddd, 1H, H-16, *J* = 13.6, 13.6, 4.57 Hz), 1.71 (m, 1H, H-2), 1.64 (m, 1H, H-7), 1.60 (m, 1H, H-2′), 1.58 (dd, 1H, H-19′, *J* = 13.2, 13.27 Hz), 1.56 (m, 1H, H-6), 1.44 (m, 1H, H-6′), 1.41 (m, 1H, H-7′), 1.36 (m, 1H, H-22), 1.34 (s, 3H, H-27), 1.32 (d, 3H, Ala-CH_3, *J* = 7.07 Hz), 1.31 (m, 1H, H-22′), 1.30 (m, 1H, H-21′), 1.23 (d, 3H, *i*-Pr-CH_3, *J* = 6.27 Hz), 1.20 (d, 3H,

i-Pr-CH_3, J = 6.27 Hz), 1.18 (m, 1H, H-16′), 1.14 (s, 3H, H-25), 1.10 (s, 6H, H-28 and H-26), 1.04 (ddd, 1H, H-1′, J = 13.1, 13.1, 3.27 Hz), 0.99 (m, 1H, H-15′), 0.87 (s, 3H, H-23), 0.86 (s, 3H, H-24), 0.79 (m, 1H, H-5), 0.77 (s, 3H, H-29); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 176.2 (C-30), 175.8 (Ala-COO), 169.5 (C-13), 128.4 (C-12), 81.1 (C-3), 67.3 (*i*-Pr-CH), 61.7 (C-9), 55.0 (C-5), 50.2 (Ala-CH), 48.4 (C-18), 45.4 (C-8), 43.7 (C-20), 43.2 (C-14), 41.0 (C-19), 38.7 (C-1), 38.1 (C-4), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.6 (C-29), 28.2 (C-23), 28.1 (C-28), 26.5 (C-16), 26.4 (C-15), 23.4 (C-2), 23.3 (C-27), 21.9 (*i*-Pr-CH_3), 21.7 (*i*-Pr-CH_3), 20.7 (Ala-CH_3), 18.7 (C-26), 17.3 (C-6), 16.7 (C-24), 16.4 (C-25). Anal. Calcd for $C_{36}H_{57}NO_5$ (583.84): C, 74.06; H, 9.84; N, 2.40. Found: C, 73.99; H, 9.95; N, 2.35.

4.2.6.26. 3β-(Boc-glycyl)-11-oxo-olean-12-en-30-oic acid (26). Obtained from **38** by method C as a colourless powder; yield: 320 mg, 58%; mp 288–290 °C (decomp.); R_f = 0.14 (hexane/ethyl acetate 7:3); $[\alpha]_D^{20}$ = 115.49 (c 0.43, $CHCl_3$); UV–vis (MeOH): λ_{max} (log ε) = 250 nm (4.06); MS (ESI): m/z (%) = 650.4 ([M+Na]$^+$, 100), 964.2 ([3M+2Na]$^{2+}$, 14), 1278.1 ([2M+Na]$^+$, 18); IR (KBr): ν = 3295 (br), 2973 (s), 2874 (m), 1752 (s), 1719 (s), 1651 (s), 1514 (m), 1456 (m), 1390 (s), 1368 (s), 1281 (m), 1258 (m), 1217 (s), 1164 (s), 1088 (w), 1051 (m), 1028 (m), 984 (m), 948 (w), 916 (w), 883 (w), 867 (w), 777 (w), 754 (w), 700 (w), 671 (w), 590 (w), 558 (w), 442 (w) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.68 (s, 1H, H-12), 4.98 (m, 1H, Gly-NH), 4.57 (dd, 1H, H-3, J = 11.6, 4.87 Hz), 3.88 (m, 2H, Gly-CH_2), 2.79 (ddd, 1H, H-1, J = 13.7, 3.3, 3.37 Hz), 2.34 (s, 1H, H-9), 2.17 (dd, 1H, H-18, J = 12.9, 4.27 Hz), 2.01 (ddd, 1H, H-15, J = 13.3, 13.3, 4.27 Hz), 1.98 (m, 1H, H-21), 1.91 (m, 1H, H-19), 1.81 (ddd, 1H, H-16, J = 13.3, 13.3, 3.77 Hz), 1.72 (m, 1H, H-2), 1.68 (m, 1H, H-7), 1.63 (m, 1H, H-2′), 1.61 (dd, 1H, H-19′, J = 13.7, 13.77 Hz), 1.57 (m, 1H, H-6), 1.43 (s, 9H, Boc-CH_3), 1.42 (m, 1H, H-6′), 1.39 (m, 1H, H-7′), 1.38 (m, 1H, H-22), 1.37 (s, 3H, H-27), 1.36 (m, 1H, H-22′), 1.32 (m, 1H, H-21′), 1.20 (s, 3H, H-28), 1.19 (m, 1H, H-16′), 1.14 (s, 3H, H-25), 1.11 (s, 3H, H-26), 1.03 (m, 1H, H-1′), 1.01 (m, 1H, H-15′), 0.86 (s, 6H, H-23 and H-24), 0.81 (s, 3H, H-29), 0.78 (m, 1H, H-5); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.1 (C-11), 180.6 (C-30), 170.1 (Gly-COO), 169.4 (C-13), 155.6 (Boc-COO), 128.5 (C-12), 82.0 (C-3), 77.3 (Boc-*quart*.-C), 61.7 (C-9), 55.0 (C-5), 48.2 (C-18), 45.4 (C-8), 43.7 (C-20), 43.2 (C-14), 42.7 (Gly-CH_2), 40.9 (C-19), 38.7 (C-1), 38.1 (C-4), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.9 (C-17), 31.0 (C-21), 28.5 (C-29), 28.4 (C-23), 28.3 (Boc-CH_3), 28.1 (C-28), 26.5 (C-16), 26.4 (C-15), 23.5 (C-2), 23.4 (C-27), 18.7 (C-26), 17.4 (C-6), 16.6 (C-24), 16.4 (C-25). Anal. Calcd for $C_{37}H_{57}NO_7$ (627.85): C, 70.78; H, 9.15; N, 2.23. Found: C, 70.75; H, 9.25; N, 2.12.

4.2.6.27. 3β-(Boc-L-alanyl)-11-oxo-olean-12-en-30-oic acid (27). Obtained from **39** by method C as a colourless powder; yield: 220 mg, 66%; mp 231–234 °C (decomp.); R_f = 0.71 (hexane/ethyl acetate 7:3); $[\alpha]_D^{20}$ = 95.89 (c 0.45, $CHCl_3$); UV–vis (MeOH): λ_{max} (log ε) = 250 nm (4.81); MS (ESI): m/z (%) = 642.2 ([M+H]$^+$, 4), 664.4 ([M+Na]$^+$, 100), 985.3 ([3M+2Na]$^+$, 22); IR (KBr): ν = 3383 (br), 2976 (s), 2876 (m), 1719 (s), 1661 (s), 1499 (m), 1455 (s), 1390 (s), 1368 (s), 1306 (m), 1259 (m), 1214 (s), 1167 (s), 1087 (m), 1066 (m), 1023 (m), 973 (m), 916 (w), 881 (w), 865 (w), 754 (w), 699 (s), 668 (w), 588 (w), 541 (w), 464 (w) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.69 (s, 1H, H-12), 5.06 (m, 1H, Ala-NH), 4.55 (dd, 1H, H-3, J = 11.6, 4.67 Hz), 4.28 (m, 1H, Ala-CH), 2.80 (ddd, 1H, H-1, J = 13.7, 3.4, 3.47 Hz), 2.35 (s, 1H, H-9), 2.17 (dd, 1H, H-18, J = 13.3, 3.37 Hz), 2.02 (m, 1H, H-15), 1.98 (m, 1H, H-21), 1.92 (m, 1H, H-19), 1.82 (m, 1H, H-16), 1.71 (m, 1H, H-2), 1.65 (m, 1H, H-7), 1.62 (m, 1H, H-2′), 1.61 (dd, 1H, H-19′, J = 13.4, 13.47 Hz), 1.57 (m, 1H, H-6), 1.43 (m, 1H, H-6′), 1.42 (s, 9H, Boc-CH_3), 1.40 (m, 1H, H-7′), 1.38 (d, 3H, Ala-CH_3, J = 7.17 Hz), 1.36 (m, 1H, H-22), 1.35 (s, 3H, H-27), 1.33 (m, 2H, H-22′ and H-21′), 1.21 (s, 3H, H-28), 1.17 (m, 1H, H-16′), 1.15 (s, 3H, H-25), 1.11 (s, 3H, H-26), 1.03 (m, 1H, H-1′), 1.01 (m, 1H, H-15′), 0.87 (s, 3H, H-24), 0.85 (s, 3H, H-23), 0.82 (s, 3H, H-29), 0.79 (m, 1H, H-5); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.2 (C-11), 181.0 (C-30), 173.0 (Ala-COO), 169.4 (C-13), 155.0 (Boc-COO), 128.4 (C-12), 81.7 (C-3), 77.9 (Boc-*quart*.-C), 61.6 (C-9), 55.0 (C-5), 49.5 (Ala-CH), 48.2 (C-18), 45.4 (C-8), 43.7 (C-20), 43.2 (C-14), 40.9 (C-19), 38.7 (C-1), 38.2 (C-4), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 30.9 (C-21), 28.5 (C-29), 28.4 (C-23), 28.3 (Boc-CH_3), 28.0 (C-28), 26.5 (C-16), 26.4 (C-15), 23.5 (C-2), 23.3 (C-27), 18.9 (Ala-CH_3), 18.7 (C-26), 17.3 (C-6), 16.7 (C-24), 16.4 (C-25). Anal. Calcd for $C_{38}H_{59}NO_7$ (641.88): C, 71.10; H, 9.26; N, 2.18. Found: C, 71.00; H, 9.31; N, 2.12.

4.2.6.28. 3β-(Boc-β-alanyl)-11-oxo-olean-12-en-30-oic acid (28). Obtained from **40** by method C as a colourless powder; yield: 190 mg, 52%; mp 227–231 °C (decomp.); R_f = 0.09 (hexane/ethyl acetate 7:3); $[\alpha]_D^{20}$ = 113.35 (c 0.21, $CHCl_3$); UV–vis (MeOH): λ_{max} (log ε) = 250 nm (4.08); MS (ESI): m/z (%) = 664.4 ([M+Na]$^+$, 100), 985.3 ([3M+2Na]$^{2+}$, 8), 1305.1 ([2M+Na]$^+$, 20); IR (KBr): ν = 3356 (br), 2968 (s), 1739 (s), 1703 (s), 1653 (s), 1528 (m), 1452 (m), 1390 (m), 1368 (m), 1326 (m), 1302 (m), 1257 (m), 1210 (m), 1179 (s), 1072 (w), 1022 (w), 984 (w), 915 (m), 880 (w), 860 (w), 754 (w), 542 (w) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.69 (s, 1H, H-12), 4.96 (m, 1H, Ala-NH), 4.52 (dd, 1H, H-3, J = 11.7, 4.67 Hz), 3.37 (dt, 2H, Ala-CH_2NH_2, J = 5.8, 5.87 Hz), 2.79 (ddd, 1H, H-1, J = 13.8, 3.3, 3.37 Hz), 2.51 (m, 2H, Ala-CH_2COO), 2.35 (s, 1H, H-9), 2.17 (dd, 1H, H-18, J = 12.8, 3.77 Hz), 2.01 (m, 1H, H-15), 1.98 (m, 1H, H-21), 1.92 (ddd, 1H, H-19, J = 13.8, 4.1, 2.47 Hz), 1.82 (m, 1H, H-16), 1.71 (m, 1H, H-2), 1.66 (m, 1H, H-7), 1.63 (m, 1H, H-2′), 1.61 (dd, 1H, H-19′, J = 13.2, 13.27 Hz), 1.57 (m, 1H, H-6), 1.45 (m, 1H, H-6′), 1.42 (s, 9H, Boc-CH_3), 1.41 (m, 1H, H-7′), 1.39 (m, 1H, H-22), 1.35 (s, 3H, H-27), 1.33 (m, 1H, H-22′), 1.31 (m, 1H, H-21′), 1.21 (s, 3H, H-28), 1.20 (m, 1H, H-16′), 1.15 (s, 3H, H-25), 1.11 (s, 3H, H-26), 1.03 (m, 1H, H-1′), 1.01 (m, 1H, H-15′), 0.86 (s, 3H, H-24), 0.85 (s, 3H, H-23), 0.82 (s, 3H, H-29), 0.78 (m, 1H, H-5); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.2 (C-11), 180.6 (C-30), 172.1 (Ala-COO), 169.3 (C-13), 155.6 (Boc-COO), 128.5 (C-12), 81.1 (C-3), 77.3 (Boc-*quart*.-C), 61.7 (C-9), 55.0 (C-5), 48.2 (C-18), 45.4 (C-8), 43.7 (C-20), 43.2 (C-14), 40.9 (C-19), 38.8 (C-1), 38.1 (C-4), 37.7 (C-22), 36.9 (C-10), 36.4 (Ala-CH_2NH_2), 34.7 (Ala-CH_2COO), 32.7 (C-7), 31.9 (C-17), 31.0 (C-21), 28.5 (C-29), 28.4 (C-23 and Boc-CH_3), 28.1 (C-28), 26.5 (C-16), 26.5 (C-15), 23.6 (C-2), 23.4 (C-27), 18.7 (C-26), 17.4 (C-6), 16.7 (C-24), 16.4 (C-25). Anal. Calcd for $C_{38}H_{59}NO_7$ (641.88): C, 71.10; H, 9.26; N, 2.18. Found: C, 71.02; H, 9.33; N, 2.05.

4.2.6.29. Methyl 3β-(Boc-glycyl)-11-oxo-olean-12-en-30-oate (29). Obtained from **1** and Boc-Gly by method A as a colourless powder; yield: 450 mg, 96%; mp 251–254 °C (decomp.); R_f = 0.58 (hexane/ethyl acetate 7:3); $[\alpha]_D^{20}$ = 92.72 (c 0.29, MeOH); UV–vis (MeOH): λ_{max} (log ε) = 249 nm (4.03); MS (ESI): m/z (%) = 641.9 ([M+H]$^+$, 12), 659.0 ([M+NH$_4$]$^+$, 12), 664.3 ([M+Na]$^+$, 100); IR (KBr): ν = 3397 (br), 2969 (s), 2874 (m), 1754 (s), 1724 (s), 1651 (s), 1522 (s), 1464 (m), 1390 (s), 1366 (s), 1348 (m), 1319 (m), 1248 (m), 1215 (s), 1164 (s), 1106 (m), 1087 (m), 1053 (m), 1024 (m), 985 (m), 948 (w), 916 (w), 881 (w), 868 (w), 825 (w), 756 (s), 686 (w), 667 (w), 590 (w), 551 (w), 537 (w) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.63 (s, 1H, H-12), 4.98 (m, 1H, Gly-NH), 4.56 (dd, 1H, H-3, J = 11.6, 5.07 Hz), 3.87 (d, 2H, Gly-CH_2, J = 5.07 Hz), 3.66 (s, 3H, OMe), 2.79 (ddd, 1H, H-1, J = 13.7, 3.6, 3.67 Hz), 2.33 (s, 1H, H-9), 2.05 (m, 1H, H-18), 2.00 (m, 1H, H-15), 1.96 (m, 1H, H-21), 1.90 (ddd, 1H, H-19, J = 13.7, 3.7,

2.57 Hz), 1.80 (ddd, 1H, H-16, *J* = 13.7, 13.7, 4.77 Hz), 1.72 (m, 1H, H-2), 1.66 (m, 1H, H-7), 1.63 (m, 1H, H-2′), 1.58 (dd, 1H, H-19′, *J* = 13.7, 13.77 Hz), 1.56 (m, 1H, H-6), 1.43 (m, 1H, H-6′), 1.42 (s, 9H, Boc-CH_3), 1.39 (m, 1H, H-7′), 1.37 (m, 1H, H-22), 1.33 (s, 3H, H-27), 1.32 (m, 1H, H-22′), 1.28 (m, 1H, H-21′), 1.16 (m, 1H, H-16′), 1.13 (s, 3H, H-25), 1.12 (s, 3H, H-26), 1.10 (s, 3H, H-28), 1.02 (ddd, 1H, H-1′, *J* = 13.3, 13.3, 3.77 Hz), 0.99 (m, 1H, H-15′), 0.85 (s, 6H, H-23 and H-24), 0.78 (s, 3H, H-29), 0.77 (m, 1H, H-5); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.9 (C-11), 176.9 (C-30), 170.1 (Gly-COO), 169.2 (C-13), 155.6 (Boc-COO), 128.5 (C-12), 81.9 (C-3), 77.8 (Boc-*quart*.-C), 61.6 (C-9), 55.0 (C-5), 51.7 (OMe), 48.4 (C-18), 45.4 (C-8), 44.0 (C-20), 43.2 (C-14), 42.6 (Gly-CH_2), 41.1 (C-19), 38.7 (C-1), 38.1 (C-4), 37.7 (C-22), 36.9 (C-10), 32.6 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-29), 28.3 (C-23 and Boc-CH_3), 28.0 (C-28), 26.4 (C-16), 26.4 (C-15), 23.5 (C-2), 23.3 (C-27), 18.6 (C-26), 17.3 (C-6), 16.6 (C-24), 16.4 (C-25). Anal. Calcd for $C_{38}H_{59}NO_7$ (641.88): C, 71.10; H, 9.26; N, 2.18. Found: C, 71.08; H, 9.35; N, 2.11.

4.2.6.30. Methyl 3β-(Boc-L-alanyl)-11-oxo-olean-12-en-30-oate (30). Obtained from **1** and Boc-L-Ala by method A as a colourless powder; yield: 400 mg, 72%; mp 249–252 °C (decomp.); R_f = 0.68 (hexane/ethyl acetate 7:3); $[\alpha]_D^{20}$ = 98.76 (*c* 0.43, $CHCl_3$); UV–vis (MeOH): λ_{max} (log ε) = 249 nm (4.08); MS (ESI): *m/z* (%) = 655.9 ($[M+H]^+$, 14), 673.1 ($M+NH_4$, 16), 678.3 ($[M+Na]^+$, 100); IR (KBr): ν = 3393 (br), 2972 (s), 2931 (s), 2874 (m), 1746 (s), 1722 (s), 1706 (s), 1652 (s), 1514 (s), 1455 (s), 1390 (m), 1365 (m), 1336 (s), 1280 (w), 1248 (m), 1216 (s), 1165 (s), 1094 (m), 1052 (s), 1029 (m), 985 (m), 970 (m), 917 (w), 882 (w), 866 (w), 850 (w), 827 (w), 780 (w), 772 (w), 686 (w), 670 (w), 590 (w), 538 (w) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.64 (s, 1H, H-12), 5.04 (m, 1H, Ala-NH), 4.54 (dd, 1H, H-3, *J* = 11.6, 4.67 Hz), 4.28 (m, 1H, Ala-CH), 3.66 (s, 3H, OMe), 2.80 (ddd, 1H, H-1, *J* = 13.5, 3.4, 3.47 Hz), 2.34 (s, 1H, H-9), 2.06 (dd, 1H, H-18, *J* = 13.7, 3.77 Hz), 2.00 (m, 1H, H-15), 1.97 (m, 1H, H-21), 1.90 (ddd, 1H, H-19, *J* = 13.7, 3.7, 2.47 Hz), 1.80 (ddd, 1H, H-16, *J* = 13.8, 13.8, 4.77 Hz), 1.72 (m, 1H, H-2), 1.63 (m, 1H, H-7), 1.60 (m, 1H, H-2′), 1.59 (dd, 1H, H-19′, *J* = 13.4, 13.47 Hz), 1.56 (m, 1H, H-6), 1.44 (m, 1H, H-6′), 1.42 (s, 9H, Boc-CH_3), 1.41 (m, 1H, H-7′), 1.38 (d, 3H, Ala-CH_3, *J* = 7.27 Hz), 1.37 (m, 1H, H-22), 1.34 (s, 3H, H-27), 1.29 (m, 2H, H-22′ and H-21′), 1.16 (m, 1H, H-16′), 1.14 (s, 3H, H-25), 1.13 (s, 3H, H-28), 1.10 (s, 3H, H-26), 1.03 (ddd, 1H, H-1′, *J* = 13.7, 13.7, 3.87 Hz), 0.99 (m, 1H, H-15′), 0.87 (s, 3H, H-23), 0.85 (s, 3H, H-24), 0.79 (m, 1H, H-5), 0.78 (s, 3H, H-29); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 176.9 (C-30), 173.0 (Ala-COO), 169.2 (C-13), 155.0 (Boc-COO), 128.5 (C-12), 81.7 (C-3), 79.6 (Boc-*quart*.-C), 61.7 (C-9), 55.0 (C-5), 51.7 (OMe), 49.5 (Ala-CH), 48.4 (C-18), 45.4 (C-8), 44.0 (C-20), 43.2 (C-14), 41.1 (C-19), 38.7 (C-1), 38.2 (C-4), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-29), 28.3 (Boc-CH_3), 28.3 (C-23), 28.0 (C-28), 26.4 (C-16), 26.4 (C-15), 23.5 (C-2), 23.3 (C-27), 18.9 (Ala-CH_3), 18.7 (C-26), 17.3 (C-6), 16.7 (C-24), 16.4 (C-25). Anal. Calcd for $C_{39}H_{61}NO_7$ (655.90): C, 71.42; H, 9.37; N, 2.14. Found: C, 71.18; H, 9.42; N, 2.08.

4.2.6.31. Methyl 3β-(Boc-β-alanyl)-11-oxo-olean-12-en-30-oate (31). Obtained from **1** and Boc-β-Ala by method A as a colourless powder; yield: 460 mg, 88%; mp 188–191 °C (decomp.); R_f = 0.58 (hexane/ethyl acetate 7:3); $[\alpha]_D^{20}$ = 105.52 (*c* 0.28, $CHCl_3$); UV–vis (MeOH): λ_{max} (log ε) = 249 nm (4.08); MS (ESI): *m/z* (%) = 656.2 ($[M+H]^+$, 15), 678.3 ($[M+Na]^+$, 100); IR (KBr): ν = 3406 (br), 2973 (s), 1731 (s), 1660 (m), 1508 (m), 1456 (m), 1390 (m), 1366 (m), 1249 (m), 1216 (m), 1170 (s), 1086 (w), 986 (w), 880 (w), 755 (w) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.65 (s, 1H, H-12), 4.94 (m, 1H, Ala-NH), 4.52 (dd, 1H, H-3, *J* = 11.7, 4.77 Hz), 3.67 (s, 3H, OMe), 3.37 (dt, 2H, Ala-CH_2NH_2, *J* = 5.8, 5.87 Hz), 2.79 (ddd, 1H, H-1, *J* = 13.7, 3.7, 3.77 Hz), 2.51 (m, 2H, Ala-CH_2COO), 2.34 (s, 1H, H-9), 2.06 (m, 1H, H-18), 2.01 (m, 1H, H-15), 1.97 (m, 1H, H-21), 1.91 (m, 1H, H-19), 1.81 (ddd, 1H, H-16, *J* = 13.2, 13.2, 4.27 Hz), 1.71 (m, 1H, H-2), 1.66 (m, 1H, H-7), 1.62 (m, 1H, H-2′), 1.59 (dd, 1H, H-19′, *J* = 13.3, 13.37 Hz), 1.56 (m, 1H, H-6), 1.44 (m, 1H, H-6′), 1.41 (s, 9H, Boc-CH_3), 1.40 (m, 1H, H-7′), 1.38 (m, 1H, H-22), 1.35 (s, 3H, H-27), 1.31 (m, 1H, H-22′), 1.28 (m, 1H, H-21′), 1.16 (m, 1H, H-16′), 1.14 (s, 3H, H-25), 1.13 (s, 3H, H-26), 1.11 (s, 3H, H-28), 1.03 (m, 1H, H-1′), 0.99 (m, 1H, H-15′), 0.86 (s, 3H, H-24), 0.85 (s, 3H, H-23), 0.79 (s, 3H, H-29), 0.78 (m, 1H, H-5); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 176.9 (C-30), 172.3 (Ala-COO), 169.2 (C-13), 156.3 (Boc-COO), 128.5 (C-12), 81.1 (C-3), 79.6 (Boc-*quart*.-C), 61.7 (C-9), 55.0 (C-5), 51.8 (OMe), 48.4 (C-18), 45.4 (C-8), 44.0 (C-20), 43.2 (C-14), 41.1 (C-19), 38.8 (C-1), 38.1 (C-4), 37.7 (C-22), 36.9 (C-10), 36.4 (Ala-CH_2NH_2), 34.7 (Ala-CH_2COO), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-29), 28.4 (Boc-CH_3), 28.3 (C-28), 28.1 (C-23), 26.4 (C-16), 26.4 (C-15), 23.6 (C-2), 23.3 (C-27), 18.7 (C-26), 17.4 (C-6), 16.7 (C-24), 16.4 (C-25). Anal. Calcd for $C_{39}H_{61}NO_7$ (655.90): C, 71.42; H, 9.37; N, 2.14. Found: C, 71.19; H, 9.52; N, 1.99.

4.2.6.32. Ethyl 3β-(Boc-L-glycyl)-11-oxo-olean-12-en-30-oate (32). Obtained from **2** and Boc-Gly by method A as a colourless powder; yield: 372 mg, 71%; mp 227–229 °C (decomp.); R_f = 0.56 (hexane/ethyl acetate 7:3); $[\alpha]_D^{20}$ = 108.70 (*c* 0.40, $CHCl_3$); UV–vis (MeOH): λ_{max} (log ε) = 249 nm (4.10); MS (ESI): *m/z* (%) = 656.3 ($[M+H]^+$, 7), 667.3 ($[2M+H+Na]^{2+}$, 19), 678.4 ($[M+Na]^+$, 100), 1006.5 ($[3M+2Na]^{2+}$, 34), 1333.8 ($[2M+Na]^+$, 60); IR (KBr): ν = 3407 (br), 2978 (s), 1724 (s), 1659 (s), 1508 (w), 1456 (m), 1390 (m), 1366 (m), 1249 (m), 1215 (s), 1172 (s), 1086 (w), 1053 (w), 1022 (w), 985 (w), 867 (w), 769 (w) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.64 (s, 1H, H-12), 4.99 (t, 1H, Gly-NH, *J* = 4.97 Hz), 4.58 (dd, 1H, H-3, *J* = 11.7, 4.8 7 Hz), 4.18 (dq, 1H, COOC*H*H′, *J* = 10.7, 7.27 Hz), 4.12 (dq, 1H, COOCH*H*′, *J* = 10.7, 7.27 Hz), 3.89 (d, 2H, Gly-CH_2NH, *J* = 4.97 Hz), 2.81 (ddd, 1H, H-1, *J* = 13.7, 3.7, 3.77 Hz), 2.35 (s, 1H, H-9), 2.09 (dd, 1H, H-18, *J* = 13.3, 3.37 Hz), 2.02 (ddd, 1H, H-16, *J* = 13.6, 13.6, 4.57 Hz), 1.98 (m, 1H, H-21), 1.92 (ddd, 1H, H-19, *J* = 13.7, 4.0, 2.67 Hz), 1.82 (ddd, 1H, H-16, *J* = 13.7, 13.7, 4.57 Hz), 1.72 (m, 1H, H-2), 1.65 (m, 1H, H-7), 1.63 (m, 1H, H-2′), 1.60 (dd, 1H, H-19′, *J* = 1.34, 13.47 Hz), 1.58 (m, 1H, H-6), 1.45 (m, 1H, H-6′), 1.44 (s, 9H, Boc-CH_3), 1.42 (m, 1H, H-7′), 1.38 (m, 1H, H-22), 1.35 (s, 3H, H-27), 1.32 (m, 1H, H-22′), 1.30 (m, 1H, H-21′), 1.25 (t, 3H, $COOCH_2CH_3$, *J* = 7.27 Hz), 1.17 (m, 1H, H-16′), 1.15 (s, 3H, H-25), 1.13 (s, 3H, H-28), 1.12 (s, 3H, H-26), 1.04 (ddd, 1H, H-1′, *J* = 13.6, 13.6, 3.47 Hz), 1.01 (m, 1H, H-15′), 0.87 (s, 6H, H-23 and H-24), 0.79 (s, 3H, H-29), 0.79 (m, 1H, H-5); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 176.3 (C-30), 170.1 (Gly-COO), 169.4 (C-13), 156.2 (Boc-COO), 128.4 (C-12), 82.0 (C-3), 79.7 (Boc-*quart*.-C), 61.7 (C-9), 60.3 ($COOCH_2$), 55.0 (C-5), 48.4 (C-18), 45.4 (C-8), 43.8 (C-20), 43.2 (C-14), 42.6 (Gly-CNH), 41.0 (C-19), 38.7 (C-1), 38.1 (C-10), 37.7 (C-22), 36.9 (C-4), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-29), 28.3 (3× Boc-CH_3 and C-23), 28.0 (C-28), 26.5 (C-16), 26.4 (C-15), 23.5 (C-2), 23.3 (C-27), 18.6 (C-26), 17.3 (C-6), 16.6 (C-24), 16.4 (C-25), 14.3 ($COOCH_2CH_3$). Anal. Calcd for $C_{39}H_{61}NO_7$ (655.90): C, 71.42; H, 9.37; N, 2.14. Found: C, 71.27; H, 9.52; N, 2.05.

4.2.6.33. Ethyl 3β-(Boc-L-alanyl)-11-oxo-olean-12-en-30-oate (33). Obtained from **2** and Boc-L-Ala by method A as a colourless powder; yield: 589 mg, 95%; mp 202–205 °C (decomp.); R_f = 0.66 (hexane/ethyl acetate 7:3); $[\alpha]_D^{20}$ = 99.75 (*c* 0.61, $CHCl_3$); UV–vis (MeOH): λ_{max} (log ε) = 249 nm (4.09); MS (ESI): *m/z* (%) = 670.3 ($[M+H]^+$, 23), 629.4 ($[M+Na]^+$, 100), 1027.3 ($[3M+2Na]^{2+}$, 28), 1339.2 ($[2M+H]^+$, 22), 1362.2 ($[2M+Na]^+$, 26); IR (KBr): ν = 3397 (br), 2972 (s), 2874 (m), 1746 (s), 1720 (s), 1652 (s), 1512 (m),

1456 (m), 1390 (m), 1367 (m), 1336 (m), 1280 (w), 1248 (w), 1216 (s), 1169 (s), 1087 (w), 1051 (m), 1029 (m), 984 (w), 970 (w), 917 (w), 881 (w), 865 (w), 770 (w), 670 (w), 589 (w), 538 (w) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.63 (s, 1H, H-12), 5.06 (d, 1H, Ala-N*H*), 4.55 (dd, 1H, H-3, *J* = 11.9, 4.77 Hz), 4.29 (m, 1H, Ala-C*H*NH), 4.17 (dq, 1H, COOC*H*H′, *J* = 10.8, 7.27 Hz), 4.11 (dq, 1H, COOCH*H*′, *J* = 10.8, 7.27 Hz), 2.81 (ddd, 1H, H-1, *J* = 13.7, 3.5, 3.57 Hz), 2.35 (s, 1H, H-9), 2.09 (dd, 1H, H-18, *J* = 13.1, 3.77 Hz), 2.02 (ddd, 1H, H-15, *J* = 13.4, 13.4, 4.67 Hz), 1.98 (m, 1H, H-21), 1.91 (ddd, 1H, H-19, *J* = 13.6, 4.1, 2.77 Hz), 1.81 (ddd, 1H, H-16, *J* = 13.4, 13.4, 4.77 Hz), 1.72 (m, 1H, H-2), 1.61 (m, 1H, H-2′), 1.60 (dd, 1H, H-19′, *J* = 13.6, 13.67 Hz), 1.45 (m, 1H, H-7), 1.43 (s, 9H, Boc-CH_3), 1.41 (m, 1H, H-7′), 1.40 (m, 1H, H-22), 1.39 (d, 3H, Ala-CH_3, *J* = 7.27 Hz), 1.38 (m, 1H, H-6), 1.37 (m, 1H, H-6′), 1.35 (s, 3H, H-27), 1.34 (m, 1H, H-22′), 1.30 (m, 1H, H-21′), 1.25 (t, 3H, $COOCH_2CH_3$, *J* = 7.27 Hz), 1.17 (m, 1H, H-16′), 1.15 (s, 3H, H-25), 1.13 (s, 3H, H-28), 1.11 (s, 3H, H-26), 1.04 (ddd, 1H, H-1′, *J* = 13.7, 13.7, 3.47 Hz), 1.00 (m, 1H, H-15′), 0.88 (s, 3H, H-24), 0.86 (s, 3H, H-23), 0.80 (m, 1H, H-5), 0.79 (s, 3H, H-29); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.9 (C-11), 176.3 (C-30), 173.0 (Ala-COO), 169.3 (C-13), 155.0 (Boc-COO), 128.4 (C-12), 81.7 (C-3), 79.6 (Boc-*quart*.-C), 61.7 (C-9), 60.3 ($COOCH_2$), 55.0 (C-5), 49.5 (Ala-CNH), 48.4 (C-18), 45.4 (C-8), 43.8 (C-20), 43.2 (C-14), 41.0 (C-19), 38.7 (C-1), 38.2 (C-4), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-29), 28.3 (3× Boc-CH_3), 28.2 (C-28), 28.0 (C-23), 26.5 (C-16), 26.4 (C-15), 23.5 (C-2), 23.3 (C-27), 18.9 (Ala-CH_3), 18.6 (C-26), 17.3 (C-6), 16.7 (C-24), 16.3 (C-25), 14.3 ($COOCH_2CH_3$). Anal. Calcd for $C_{40}H_{63}NO_7$ (669.93): C, 71.71; H, 9.48; N, 2.09. Found: C, 71.51; H, 9.53; N, 1.87.

4.2.6.34. Ethyl 3β-(Boc-β-alanyl)-11-oxo-olean-12-en-30-oate (34). Obtained from **2** and Boc-β-Ala by method A as a colourless powder; yield: 225 mg, 55%; mp 79–82 °C; R_f = 0.59 (hexane/ethyl acetate 7:3); $[\alpha]_D^{20}$ = 108.65 (*c* 0.55, $CHCl_3$); UV–vis (MeOH): λ_{max} (log ε) = 250 nm (4.07); MS (ESI): *m/z* (%) = 670.3 ($[M+H]^+$, 42), 692.5 ($[M+Na]^+$, 100), 1024.9 ($[3M+K+H]^{2+}$, 30), 1027.5 ($[3M+2Na]^{2+}$, 34), 1362.3 ($[2M+Na]^+$, 50); IR (KBr): ν = 3421 (br), 2975 (s), 1727 (s), 1660 (m), 1508 (w), 1457 (w), 1389 (m), 1366 (m), 1325 (w), 1249 (m), 1215 (m), 1174 (s), 1086 (w), 1020 (w), 985 (w), 862 (w), 770 (w), 543 (w) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.64 (s, 1H, H-12), 4.96 (br, 1H, Ala-NH), 4.54 (dd, 1H, H-3, *J* = 11.7, 4.87 Hz), 4.18 (dq, 1H, COOC*H*H′, *J* = 10.7, 7.27 Hz), 4.12 (dq, 1H, COOCH*H*′, *J* = 10.7, 7.27 Hz), 3.39 (dt, 2H, Ala-NHCH_2, *J* = 5.9, 5.97 Hz), 2.81 (ddd, 1H, H-1, *J* = 13.7, 3.7, 3.77 Hz), 2.52 (m, 2H, CH_2COO), 2.36 (s, 1H, H-9), 2.10 (dd, 1H, H-18, *J* = 13.7, 3.87 Hz), 2.03 (ddd, 1H, H-15, *J* = 13.6, 13.6, 4.57 Hz), 1.99 (m, 1H, H-21), 1.92 (ddd, 1H, H-19, *J* = 13.7, 4.0, 2.77 Hz), 1.82 (ddd, 1H, H-16, *J* = 13.4, 13.4, 4.97 Hz), 1.71 (m, 1H, H-2), 1.68 (m, 1H, H-7), 1.60 (dd, 1H, H-19′, *J* = 13.7, 13.77 Hz), 1.58 (m, 1H, H-2′), 1.57 (m, 1H, H-6), 1.46 (m, 1H, H-6′), 1.43 (s, 9H, Boc-CH_3), 1.40 (m, 1H, H-7′), 1.38 (m, 1H, H-22), 1.36 (s, 3H, H-27), 1.33 (m, 1H, H-22′), 1.31 (m, 1H, H-21′), 1.26 (t, 3H, $COOCH_2CH_3$, *J* = 7.17 Hz), 1.17 (m, 1H, H-16′), 1.15 (s, 3H, H-25), 1.14 (s, 3H, H-28), 1.12 (s, 3H, H-26), 1.04 (ddd, 1H, H-1′, *J* = 13.7, 13.7, 3.77 Hz), 1.01 (m, 1H, H-15′), 0.87 (s, 3H, H-23), 0.86 (s, 3H, H-24), 0.80 (s, 3H, H-29), 0.79 (m, 1H, H-5); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 176.3 (C-30), 172.7 (Ala-COO), 169.3 (C-13), 156.9 (Boc-COO), 128.4 (C-12), 81.1 (C-3), 79.7 (Boc-*quart*.-C), 61.7 (C-9), 60.3 ($COOCH_2$), 55.0 (C-5), 48.4 (C-18), 45.4 (C-8), 43.8 (C-20), 43.2 (C-14), 41.0 (C-19), 38.7 (C-1 and CH_2COO), 38.0 (C-4 and CH_2NH), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-29), 28.4 (Boc-CH_3), 28.3 (C-23), 28.1 (C-28), 26.5 (C-16), 26.4 (C-15), 23.6 (C-2), 23.3 (C-27), 18.7 (C-26), 17.4 (C-6), 16.7 (C-24), 16.4 (C-25), 14.3 ($COOCH_2CH_3$). Anal. Calcd for $C_{40}H_{63}NO_7$ (669.93): C, 71.71; H, 9.48; N, 2.09. Found: C, 71.52; H, 9.56; N, 2.01.

4.2.6.35. *i*-Propyl 3β-(Boc-β-glycyl)-11-oxo-olean-12-en-30-oate (35). Obtained from **3** and Boc-Gly by method A as a colourless powder; yield: 410 mg, 96%; mp 220–222 °C (decomp.); R_f = 0.69 (hexane/ethyl acetate 7:3); $[\alpha]_D^{20}$ = 90.23 (*c* 0.50, $CHCl_3$); UV–vis (MeOH): λ_{max} (log ε) = 250 nm (4.07); MS (ESI): *m/z* (%) = 692.3 ($[M+Na]^+$, 100), 1027.3 ($[3M+2Na]^{2+}$, 14), 1361.2 ($[2M+Na]^+$, 12); IR (KBr): ν = 3405 (br), 2978 (s), 2875 (m), 1723 (s), 1661 (s), 1509 (m), 1456 (m), 1388 (m), 1367 (m), 1281 (m), 1250 (m), 1216 (s), 1172 (s), 1108 (m), 1086 (w), 1054 (w), 1028 (w), 985 (m), 948 (w), 916 (w), 866 (w), 770 (w), 588 (w) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 5.61 (s, 1H, H-12), 5.02 (qq, 1H, *i*-Pr-CH, *J* = 6.2, 6.27 Hz), 4.98 (m, 1H, Gly-NH), 4.56 (dd, 1H, H-3, *J* = 11.5, 4.47 Hz), 3.88 (d, 2H, Gly-CH_2, *J* = 4.97 Hz), 2.79 (ddd, 1H, H-1, *J* = 13.7, 3.6, 3.67 Hz), 2.34 (s, 1H, H-9), 2.09 (dd, 1H, H-18, *J* = 13.3, 3.37 Hz), 2.01 (ddd, 1H, H-15, *J* = 13.3, 5.0, 5.07 Hz), 1.96 (m, 1H, H-21), 1.89 (ddd, 1H, H-19), 1.80 (ddd, 1H, H-16, *J* = 13.7, 13.7, 4.27 Hz), 1.73 (m, 1H, H-2), 1.64 (m, 1H, H-7), 1.60 (m, 1H, H-2′), 1.58 (dd, 1H, H-19′, *J* = 13.5, 13.57 Hz), 1.57 (m, 1H, H-6), 1.44 (m, 1H, H-6′), 1.43 (s, 9H, Boc-CH_3), 1.41 (m, 1H, H-7′), 1.36 (m, 1H, H-22), 1.34 (s, 3H, H-27), 1.32 (m, 1H, H-22′), 1.26 (m, 1H, H-21′), 1.23 (d, 3H, *i*-Pr-CH_3, *J* = 6.27 Hz), 1.20 (d, 3H, *i*-Pr-CH_3, *J* = 6.27 Hz), 1.16 (m, 1H, H-16′), 1.14 (s, 3H, H-25), 1.11 (s, 6H, H-28 and H-26), 1.03 (ddd, 1H, H-1′, *J* = 13.7, 13.7, 3.77 Hz), 0.99 (m, 1H, H-15′), 0.86 (s, 6H, H-23 and H-24), 0.79 (m, 1H, H-5), 0.78 (s, 3H, H-29); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 175.8 (C-30), 170.1 (Gly-COO), 169.5 (C-13), 155.7 (Boc-COO), 128.4 (C-12), 82.0 (C-3), 79.9 (Boc-*quart*.-C), 67.4 (*i*-Pr-CH), 61.7 (C-9), 55.0 (C-5), 48.4 (C-18), 45.4 (C-8), 43.7 (C-20), 43.2 (C-14), 42.7 (Gly-CH_2), 41.0 (C-19), 38.7 (C-1), 38.1 (C-4), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.6 (C-29), 28.3 (Boc-CH_3), 28.2 (C-23), 28.1 (C-28), 26.5 (C-16), 26.4 (C-15), 23.5 (C-2), 23.3 (C-27), 21.9 (*i*-Pr-CH_3), 21.7 (*i*-Pr-CH_3), 18.7 (C-26), 17.4 (C-6), 16.6 (C-24), 16.4 (C-25). Anal. Calcd for $C_{40}H_{63}NO_7$ (669.93): C, 71.71; H, 9.48; N, 2.09. Found: C, 71.56; H, 9.64; N, 1.94.

4.2.6.36. *i*-Propyl 3β-(Boc-L-alanyl)-11-oxo-olean-12-en-30-oate (36). Obtained from **3** and Boc-L-Ala by method A as a colourless powder; yield: 280 mg, 75%; mp 224–228 °C (decomp.); R_f = 0.59 (hexane/ethyl acetate 7:3); $[\alpha]_D^{20}$ = 93.61 (*c* 0.29, $CHCl_3$); UV–vis (MeOH): λ_{max} (log ε) = 249 nm (4.10); MS (ESI): *m/z* (%) = 684.0 ($[M+H]^+$, 7), 706.4 ($[M+Na]^+$, 100), 1048.3 ($[3M+2Na]^{2+}$, 6), 1389.2 ($[2M+Na]^+$, 18); IR (KBr): ν = 3443 (br), 3388 (m), 2978 (s), 2876 (m), 1724 (s), 1656 (s), 1618 (w), 1496 (m), 1454 (s), 1368 (s), 1354 (m), 1311 (m), 1280 (m), 1248 (m), 1216 (s), 1170 (s), 1110 (m), 1087 (m), 1053 (m), 1022 (m), 972 (w), 916 (w), 879 (w), 863 (w), 770 (w), 671 (w), 466 (w) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 5.60 (s, 1H, H-12), 5.04 (m, 1H, Ala-NH), 5.01 (qq, 1H, *i*-Pr-CH, *J* = 6.2, 6.27 Hz), 4.54 (dd, 1H, H-3, *J* = 11.7, 4.87 Hz), 4.28 (m, 1H, Ala-CH), 2.79 (ddd, 1H, H-1, *J* = 13.7, 3.4, 3.47 Hz), 2.34 (s, 1H, H-9), 2.08 (dd, 1H, H-18, *J* = 13.3, 3.37 Hz), 2.01 (dd, 1H, H-15, *J* = 13.7, 4.67 Hz), 1.96 (m, 1H, H-21), 1.89 (ddd, 1H, H-19, *J* = 13.6, 4.2, 2.37 Hz), 1.80 (ddd, 1H, H-16, *J* = 13.7, 13.7, 4.37 Hz), 1.72 (m, 1H, H-2), 1.64 (m, 1H, H-7), 1.60 (m, 1H, H-2′), 1.58 (dd, 1H, H-19′, *J* = 13.6, 13.67 Hz), 1.56 (m, 1H, H-6), 1.44 (m, 1H, H-6′), 1.42 (s, 9H, Boc-CH_3), 1.40 (m, 1H, H-7′), 1.38 (d, 3H, Ala-CH_3, *J* = 7.27 Hz), 1.35 (m, 1H, H-22), 1.34 (s, 3H, H-27), 1.31 (m, 2H, H-22′ and H-21′), 1.23 (d, 3H, *i*-Pr-CH_3, *J* = 6.27 Hz), 1.20 (d, 3H, *i*-Pr-CH_3, *J* = 6.27 Hz), 1.16 (m, 1H, H-16′), 1.14 (s, 3H, H-25), 1.10 (s, 6H, H-28 and H-26), 1.03 (m, 1H, H-1′), 0.99 (m, 1H, H-15′), 0.87 (s, 3H, H-23), 0.85 (s, 3H, H-24), 0.79 (m, 1H, H-5), 0.77 (s, 3H, H-29); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 175.8 (C-30), 173.0 (Ala-COO), 169.4 (C-13), 155.0 (Boc-COO), 128.4 (C-12), 81.7 (C-3), 79.6 (Boc-*quart*.-C), 67.3 (*i*-Pr-CH), 61.7 (C-9), 55.0 (C-5), 49.5 (Ala-CH), 48.4 (C-18), 45.4 (C-8), 43.7 (C-20), 43.2 (C-14), 41.0 (C-19), 38.7 (C-1), 38.2 (C-4), 37.7 (C-22), 36.9

(C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.6 (C-29), 28.3 (Boc-CH_3), 28.2 (C-23), 28.0 (C-28), 26.5 (C-16), 26.4 (C-15), 23.5 (C-2), 23.3 (C-27), 21.9 (*i*-Pr-CH_3), 21.7 (*i*-Pr-CH_3), 18.8 (Ala-CH_3), 18.7 (C-26), 17.3 (C-6), 16.7 (C-24), 16.3 (C-25). Anal. Calcd for $C_{41}H_{65}NO_7$ (683.96): C, 72.00; H, 9.58; N, 2.05. Found: C, 71.88; H, 9.71; N, 1.96.

4.2.6.37. *i*-Propyl 3β-(Boc-β-alanyl)-11-oxo-olean-12-en-30-oate (37). Obtained from **3** and Boc-β-Ala by method A as a colourless powder; yield: 400 mg, 97%; mp 114–116 °C (decomp.); R_f = 0.44 (hexane/ethyl acetate 7:3); $[\alpha]_D^{20}$ = 98.00 (*c* 0.50, $CHCl_3$); UV–vis (MeOH): λ_{max} (log ε) = 250 nm (4.05); MS (ESI): *m/z* (%) = 684.2 ($[M+H]^+$, 22), 706.3 ($[M+Na]^+$, 100), 1048 ($[3M+2Na]^{2+}$, 28), 1390.2 ($[2M+Na]^+$, 30); IR (KBr): ν = 3405 (br), 2977 (s), 1724 (s), 1660 (s), 1509 (m), 1456 (m), 1389 (m), 1366 (m), 1326 (w), 1280 (m), 1250 (m), 1217 (m), 1174 (s), 1109 (m), 1086 (w), 1020 (w), 985 (w), 879 (w), 770 (w), 543 (w) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 5.60 (s, 1H, H-12), 5.02 (qq, 1H, *i*-Pr-CH, *J* = 6.2, 6.27 Hz), 4.95 (m, 1H, Ala-NH), 4.52 (dd, 1H, H-3, *J* = 11.7, 5.07 Hz), 3.37 (dt, 2H, Ala-CH_2NH_2, *J* = 5.8, 5.87 Hz), 2.79 (ddd, 1H, H-1, *J* = 13.7, 3.4, 3.47 Hz), 2.47 (t, 2H, Ala-CH_2COO, *J* = 6.27 Hz), 2.34 (s, 1H, H-9), 2.09 (dd, 1H, H-18, *J* = 12.9, 3.77 Hz), 2.01 (ddd, 1H, H-15, *J* = 13.6, 13.6, 4.57 Hz), 1.96 (m, 1H, H-21), 1.89 (ddd, 1H, H-19, *J* = 13.3, 3.3, 2.57 Hz), 1.80 (ddd, 1H, H-16, *J* = 13.8, 13.8, 4.77 Hz), 1.71 (ddd, 1H, H-2, *J* = 13.3, 13.3, 3.37 Hz), 1.66 (m, 1H, H-7), 1.59 (m, 1H, H-2′), 1.58 (dd, 1H, H-19′, *J* = 13.5, 13.57 Hz), 1.55 (m, 1H, H-6), 1.44 (m, 1H, H-6′), 1.41 (s, 9H, Boc-CH_3), 1.38 (m, 1H, H-7′), 1.37 (m, 1H, H-22), 1.35 (s, 3H, H-27), 1.33 (m, 1H, H-22′), 1.27 (m, 1H, H-21′), 1.23 (d, 3H, *i*-Pr-CH_3, *J* = 6.27 Hz), 1.20 (d, 3H, *i*-Pr-CH_3, *J* = 6.27 Hz), 1.19 (m, 1H, H-16′), 1.14 (s, 3H, H-25), 1.10 (s, 6H, H-28 and H-26), 1.03 (ddd, 1H, H-1′, *J* = 13.5, 13.5, 3.57 Hz), 0.99 (m, 1H, H-15′), 0.86 (s, 3H, H-24), 0.85 (s, 3H, H-23), 0.79 (m, 1H, H-5), 0.78 (s, 3H, H-29); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 175.8 (C-30), 172.1 (Ala-COO), 169.5 (C-13), 156.6 (Boc-COO), 128.4 (C-12), 81.1 (C-3), 79.5 (Boc-*quart.*-C), 67.3 (*i*-Pr-CH), 61.7 (C-9), 55.0 (C-5), 48.4 (C-18), 45.4 (C-8), 43.7 (C-20), 43.2 (C-14), 41.0 (C-19), 38.7 (C-1), 38.0 (C-4), 37.7 (C-22), 36.9 (C-10), 36.3 (Ala-CH_2NH_2), 34.9 (Ala-CH_2COO), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.6 (C-29), 28.4 (Boc-CH_3), 28.2 (C-28), 28.1 (C-23), 26.5 (C-16), 26.4 (C-15), 23.6 (C-2), 23.3 (C-27), 21.9 (*i*-Pr-CH_3), 21.7 (*i*-Pr-CH_3), 18.7 (C-26), 17.4 (C-6), 16.7 (C-24), 16.4 (C-25). Anal. Calcd for $C_{41}H_{65}NO_7$ (683.96): C, 72.00; H, 9.58; N, 2.05. Found: C, 71.86; H, 9.63; N, 1.85.

4.2.6.38. Benzyl 3β-(Boc-glycyl)-11-oxo-olean-12-en-30-oate (38). Obtained from **4** and Boc-Gly by method A as a colourless powder; yield: 120 mg, 47%; mp 186–189 °C (decomp.); R_f = 0.55 (hexane/ethyl acetate 7:3); $[\alpha]_D^{20}$ = 107.04 (*c* 0.51, $CHCl_3$); UV–vis (MeOH): λ_{max} (log ε) = 205 (4.03), 250 nm (4.05); MS (ESI): *m/z* (%) = 740.4 ($[M+Na]^+$, 100), 1099.3 ($[3M+2Na]^{2+}$, 17.3), 1458.2 ($[2M+Na]^+$, 20.2); IR (KBr): ν = 3398 (br), 2973 (s), 2873 (m), 1727 (s), 1660 (s), 1619 (w), 1499 (m), 1456 (m), 1388 (m), 1366 (s), 1280 (m), 1249 (m), 1213 (s), 1165 (s), 1085 (m), 1052 (w), 1028 (w), 984 (m), 915 (w), 881 (w), 866 (w), 768 (w), 752 (w), 698 (w), 670 (w), 586 (w), 464 (w) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.40–7.31 (m, 5H, H-Ar), 5.54 (s, 1H, H-12), 5.20 (d, 1H, Bn-C*H*H′, *J* = 12.57 Hz), 5.08 (d, 1H, Bn-C*H*H′, *J* = 12.57 Hz), 4.98 (m, 1H, Gly-NH), 4.58 (dd, 1H, H-3, *J* = 11.7, 4.77 Hz), 3.90 (d, 2H, Gly-CH_2, *J* = 5.17 Hz), 2.81 (ddd, 1H, H-1, *J* = 13.6, 3.3, 3.37 Hz), 2.33 (s, 1H, H-9), 2.03 (m 1H, H-18), 2.02 (m, 1H, H-15), 2.01 (m, 1H, H-21), 1.93 (m, 1H, H-19), 1.80 (ddd, 1H, H-16, *J* = 13.9, 13.9, 4.87 Hz), 1.73 (m, 1H, H-2), 1.65 (m, 1H, H-7), 1.62 (m, 1H, H-2′), 1.61 (dd, 1H, H-19′, *J* = 13.6, 13.6), 1.57 (m, 1H, H-6), 1.45 (s, 9H, Boc-CH_3), 1.43 (m, 1H, H-6′), 1.41 (m, 1H, H-7′), 1.38 (m, 1H, H-22), 1.34 (s, 3H, H-27), 1.32 (m, 1H, H-21′), 1.29 (m, 1H, H-22′), 1.16 (s, 3H, H-25), 1.16 (s, 3H, H-28), 1.14 (m, 1H, H-16′), 1.10 (s, 3H, H-26), 1.04 (ddd, 1H, H-1′, *J* = 13.3, 13.3, 3.67 Hz), 0.99 (m, 1H, H-15′), 0.87 (s, 6H, H-24 and H-23), 0.79 (m, 1H, H-5), 0.73 (s, 3H, H-29); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.9 (C-11), 176.2 (C-30), 170.0 (Gly-COO), 169.1 (C-13), 155.7 (Boc-COO), 136.1 (C_{ar}), 128.6 (C_{ar}), 128.6 (C_{ar}), 128.4 (C-12), 128.3 (C_{ar}), 128.2 (C_{ar}), 128.2 (C_{ar}), 82.0 (C-3), 77.2 (Boc-*quart.*-C), 66.2 (Bn-CH_2), 61.6 (C-9), 55.0 (C-5), 48.2 (C-18), 45.3 (C-8), 44.0 (C-20), 43.2 (C-14), 42.8 (Gly-CH_2), 41.1 (C-19), 38.7 (C-1), 38.1 (C-4), 37.6 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.2 (C-21), 28.4 (C-29), 28.3 (Boc-CH_3), 28.3 (C-23), 28.1 (C-28), 26.5 (C-16), 26.4 (C-15), 23.5 (C-2), 23.3 (C-27), 18.7 (C-26), 17.4 (C-6), 16.6 (C-24), 16.4 (C-25). Anal. Calcd for $C_{44}H_{63}NO_7$ (717.97): C, 73.61; H, 8.84; N, 1.95. Found: C, 73.52; H, 8.69; N, 1.85.

4.2.6.39. Benzyl 3β-(Boc-L-alanyl)-11-oxo-olean-12-en-30-oate (39). Obtained from **4** and Boc-L-Ala by method A as a colourless powder; yield: 430 mg, 46%; mp 199–203 °C (decomp.); R_f = 0.59 (hexane/ethyl acetate 7:3); $[\alpha]_D^{20}$ = 101.74 (*c* 0.24, $CHCl_3$); UV–vis (MeOH): λ_{max} (log ε) = 206 nm (4.16), 250 nm (4.16); MS (ESI): *m/z* (%) = 754.4 ($[M+Na]^+$, 100), 1120.3 ($[3M+2Na]^{2+}$, 8), 1485.2 ($[2M+Na]^+$, 14); IR (KBr): ν = 3440 (br), 2969 (s), 2876 (m), 1731 (s), 1715 (s), 1654 (s), 1496 (m), 1455 (m), 1398 (m), 1366 (m), 1215 (s), 1162 (s), 1086 (m), 1050 (m), 1026 (m), 986 (w), 970 (w), 914 (w), 879 (s), 861 (w), 768 (w), 745 (w), 733 (w), 697 (w), 670 (w), 586 (w), 475 (w) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.38–7.28 (m, 5H, H-Ar), 5.53 (s, 1H, H-12), 5.18 (d, 1H, Bn-C*H*H′, *J* = 12.47 Hz), 5.07 (d, 1H, Bn-CH*H*′, *J* = 12.47 Hz), 5.04 (m, 1H, Ala-NH), 4.54 (dd, 1H, H-3, *J* = 11.8, 4.77 Hz), 4.28 (m, 1H, Ala-CH), 2.80 (ddd, 1H, H-1, *J* = 13.7, 3.3, 3.37 Hz), 2.32 (s, 1H, H-9), 2.02 (m, 1H, H-18), 2.00 (m, 1H, H-15), 1.97 (m, 1H, H-21), 1.92 (m, 1H, H-19), 1.79 (ddd, 1H, H-16, *J* = 13.7, 13.7, 4.27 Hz), 1.72 (m, 1H, H-2), 1.65 (m, 1H, H-7), 1.61 (m, 1H, H-2′), 1.60 (dd, 1H, H-19′, *J* = 13.4, 13.47 Hz), 1.57 (m, 1H, H-6), 1.45 (m, 1H, H-6′), 1.43 (s, 9H, Boc-CH_3), 1.41 (m, 1H, H-7′), 1.38 (m, 1H, H-22), 1.38 (d, 3H, Ala-CH_3, *J* = 7.0), 1.33 (s, 3H, H-27), 1.29 (m, 2H, H-22′ and H-21′), 1.16 (m, 1H, H-16′), 1.14 (s, 6H, H-25 and H-28), 1.09 (s, 3H, H-26), 1.03 (m, 1H, H-1′), 0.98 (m, 1H, H-15′), 0.87 (s, 3H, H-23), 0.85 (s, 3H, H-24), 0.78 (m, 1H, H-5), 0.72 (s, 3H, H-29); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.9 (C-11), 176.2 (C-30), 173.0 (Ala-COO), 169.0 (C-13), 155.0 (Boc-COO), 136.1 (C_{ar}), 128.6 (C_{ar}), 128.6 (C_{ar}), 128.4 (C-12), 128.3 (C_{ar}), 128.2 (C_{ar}), 128.2 (C_{ar}), 81.7 (C-3), 78.3 (Boc-*quart.*-C), 66.2 (Bn-CH_2), 61.6 (C-9), 55.0 (C-5), 49.6 (Ala-CH), 48.2 (C-18), 45.3 (C-8), 44.0 (C-20), 43.1 (C-14), 41.0 (C-19), 38.7 (C-1), 38.2 (C-4), 37.6 (C-22), 36.9 (C-10), 32.7 (C-7), 31.7 (C-17), 31.1 (C-21), 28.4 (C-29), 28.3 (Boc-CH_3), 28.2 (C-23), 28.0 (C-28), 26.4 (C-16), 26.4 (C-15), 23.5 (C-2), 23.3 (C-27), 18.9 (Ala-CH_3), 18.6 (C-26), 17.3 (C-6), 16.7 (C-24), 16.4 (C-25). Anal. Calcd for $C_{45}H_{65}NO_7$ (732.00): C, 73.84; H, 8.95; N, 1.91. Found: C, 73.77; H, 9.01; N, 1.78.

4.2.6.40. Benzyl 3β-(Boc-β-alanyl)-11-oxo-olean-12-en-30-oate (40). Obtained from **4** and Boc-β-Ala by method A as a colourless powder; yield: 600 mg, 66%; mp 176–179 °C (decomp.); R_f = 0.36 (hexane/ethyl acetate 7:3); $[\alpha]_D^{20}$ = 132.62 (*c* 0.34, MeOH); UV–vis (MeOH): λ_{max} (log ε) = 207 nm (4.06), 250 nm (4.10); MS (ESI): *m/z* (%) = 732.3 ($[M+H]^+$, 14), 754.4 ($[M+Na]^+$, 100), 763.3 ($[M+MeOH]^+$, 6), 770.3 ($[M+K]^+$, 20); IR (KBr): ν = 3436 (br), 2964 (s), 2873 (m), 1725 (s), 1654 (s), 1498 (m), 1456 (m), 1390 (m), 1365 (m), 1331 (m), 1256 (m), 1213 (m), 1170 (s), 1146 (s), 1076 (w), 1063 (w), 1022 (w), 984 (w), 915 (w), 878 (s), 756 (w), 699 (w), 545 (w) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.38–7.28 (m, 5H, H-Ar), 5.52 (s, 1H, H-12), 5.18 (d, 1H, Bn-C*H*H′, *J* = 12.57 Hz), 5.06 (d, 1H, Bn-C*H*H′, *J* = 12.57 Hz), 4.94 (m, 1H, Ala-NH), 4.52 (dd, 1H, H-3, *J* = 11.7, 5.27 Hz), 3.37 (dt, 2H,

Ala-CH_2NH_2, J = 5.8, 5.87 Hz), 2.78 (ddd, 1H, H-1, J = 13.8, 3.8, 3.87 Hz), 2.50 (m, 2H, Ala-CH_2COO), 2.32 (s, 1H, H-9), 2.02 (m 1H, H-18), 1.97 (m, 1H, H-15), 1.96 (m, 1H, H-21), 1.92 (m, 1H, H-19), 1.78 (ddd, 1H, H-16, J = 13.9, 13.9, 4.17 Hz), 1.71 (m, 1H, H-2), 1.64 (m, 1H, H-7), 1.60 (m, 1H, H-2′), 1.59 (dd, 1H, H-19′, J = 13.6, 13.67 Hz), 1.55 (m, 1H, H-6), 1.44 (m, 1H, H-6′), 1.41 (s, 9H, Boc-CH_3), 1.38 (m, 1H, H-7′), 1.33 (m, 1H, H-22), 1.33 (s, 3H, H-27), 1.31 (m, 1H, H-21′), 1.28 (m, 1H, H-22′), 1.15 (m, 1H, H-16′), 1.14 (s, 6H, H-25 and H-28), 1.09 (s, 3H, H-26), 1.02 (m 1H, H-1′), 0.97 (m, 1H, H-15′), 0.86 (s, 3H, H-24), (s, 3H, H-23), 0.77 (m, 1H, H-5), 0.71 (s, 3H, H-29); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.9 (C-11), 176.1 (C-30), 172.1 (Ala-COO), 169.0 (C-13), 155.7 (Boc-COO), 136.1 (C_{ar}), 128.6 (C_{ar}), 128.6 (C_{ar}), 128.4 (C-12), 128.3 (C_{ar}), 128.2 (C_{ar}), 128.2 (C_{ar}), 81.1 (C-3), 77.3 (Boc-*quart*.-C), 66.2 (Bn-CH_2), 61.6 (C-9), 55.0 (C-5), 48.2 (C-18), 45.3 (C-8), 44.0 (C-20), 43.2 (C-14), 41.0 (C-19), 38.8 (C-1), 38.0 (C-4), 37.6 (C-22), 36.9 (C-10), 36.5 (Ala-CH_2COO), 34.9 (Ala-COO), 32.7 (C-7), 31.7 (C-17), 31.1 (C-21), 28.4 (C-29), 28.4 (Boc-CH_3), 28.2 (C-23), 28.1 (C-28), 26.4 (C-16), 26.4 (C-15), 23.6 (C-2), 23.3 (C-27), 18.6 (C-26), 17.4 (C-6), 16.7 (C-24), 16.4 (C-25). Anal. Calcd for $C_{45}H_{65}NO_7$ (732.00): C, 73.84; H, 8.95; N, 1.91. Found: C, 73.74; H, 9.11; N, 1.69.

4.2.6.41. Methyl 3β-(Boc-D-alanyl)-11-oxo-olean-12-en-30-oate (41). Obtained from **1** and Boc-D-Ala by method A as a colourless powder; yield: 450 mg, 97%; mp 205–208 °C (decomp.); R_f = 0.74 (hexane/ethyl acetate 7:3); $[\alpha]_D^{20}$ = 82.74 (c 0.41, $CHCl_3$); UV–vis (MeOH): λ_{max} (log ε) = 249 nm (3.97); MS (ESI): m/z (%) = 656.0 ($[M+H]^+$, 8), 678.3 ($[M+Na]^+$, 100), 1006.3 ($[3M+2Na]^{2+}$, 12), 1334.1 ($[2M+Na]^+$, 14); IR (KBr): ν = 3371 (br), 2950 (s), 2874 (m), 1734 (s), 1718 (s), 1650 (s), 1509 (m), 1455 (m), 1388 (m), 1366 (m), 1306 (m), 1248 (m), 1216 (s), 1168 (s), 1088 (w), 1074 (m), 1049 (w), 1023 (w), 986 (w), 916 (w), 882 (w), 866 (w), 851 (w), 834 (w), 754 (s), 666 (w), 601 (w) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.64 (s, 1H, H-12), 5.03 (m, 1H, Ala-NH), 4.53 (dd, 1H, H-3, J = 11.6, 4.47 Hz), 4.27 (m, 1H, Ala-CH), 3.67 (s, 3H, OMe), 2.80 (ddd, 1H, H-1, J = 13.7, 3.8, 3.87 Hz), 2.34 (s, 1H, H-9), 2.06 (dd, 1H, H-18, J = 13.7, 3.77 Hz), 2.00 (m, 1H, H-15), 1.97 (m, 1H, H-21), 1.90 (ddd, 1H, H-19, J = 13.7, 3.3, 2.97 Hz), 1.80 (ddd, 1H, H-16, J = 13.7, 13.7, 4.67 Hz), 1.70 (m, 1H, H-2), 1.64 (m, 1H, H-7), 1.61 (m, 1H, H-2′), 1.59 (dd, 1H, H-19′, J = 13.7, 13.77 Hz), 1.57 (m, 1H, H-6), 1.44 (m, 1H, H-6′), 1.42 (s, 9H, Boc-CH_3), 1.41 (m, 1H, H-7′), 1.37 (m, 1H, H-22), 1.35 (d, 3H, Ala-CH_3, J = 7.17 Hz), 1.34 (s, 3H, H-27), 1.29 (m, 2H, H-22′ and H-21′), 1.16 (m, 1H, H-16′), 1.14 (s, 3H, H-25), 1.13 (s, 3H, H-28), 1.10 (s, 3H, H-26), 1.03 (ddd, 1H, H-1′, J = 13.7, 13.7, 3.77 Hz), 1.00 (m, 1H, H-15′), 0.87 (s, 6H, H-23 and H-24), 0.79 (m, 1H, H-5), 0.78 (s, 3H, H-29); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 176.9 (C-30), 172.0 (Ala-COO), 169.3 (C-13), 155.0 (Boc-COO), 128.5 (C-12), 81.7 (C-3), 78.4 (Boc-*quart*.-C), 61.7 (C-9), 55.0 (C-5), 51.8 (OMe), 50.6 (Ala-CH), 48.4 (C-18), 45.4 (C-8), 44.0 (C-20), 43.2 (C-14), 41.1 (C-19), 38.7 (C-1), 38.1 (C-4), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-29), 28.3 (C-23 and Boc-CH_3), 28.0 (C-28), 26.5 (C-16), 26.4 (C-15), 23.4 (C-2), 23.3 (C-27), 18.8 (Ala-CH_3), 18.7 (C-26), 17.3 (C-6), 16.6 (C-24), 16.4 (C-25). Anal. Calcd for $C_{39}H_{61}NO_7$ (655.90): C, 71.42; H, 9.37; N, 2.14. Found: C, 71.28; H, 9.54; N, 1.95.

4.2.6.42. Ethyl 3β-(Boc-D-alanyl)-11-oxo-olean-12-en-30-oate (42). Obtained from **2** and Boc-D-Ala by method A as a colourless powder; yield: 440 mg, 98%; mp 175–178 °C (decomp.); R_f = 0.54 (hexane/ethyl acetate 7:3); $[\alpha]_D^{20}$ = 90.56 (c 0.29, $CHCl_3$); UV–vis (MeOH): λ_{max} (log ε) = 250 nm (4.03); MS (ESI): m/z (%) = 670.2 ($[M+H]^+$, 6), 692.4 ($[M+Na]^+$, 100), 1027.3 ($[3M+2Na]^{2+}$, 14) 1339.3 ($[2M+H]^+$, 8), 1361.3 ($[2M+Na]^+$, 24); IR (KBr): ν = 3366 (br), 2979 (s), 2873 (m), 1715 (s), 1698 (s), 1649 (s), 1625 (m), 1575 (w), 1514 (m), 1454 (m), 1389 (m), 1366 (m), 1313 (m), 1246 (m), 1215 (s), 1168 (s), 1088 (m), 1074 (m), 1049 (m), 1024 (m), 966 (w), 916 (w), 880 (w), 865 (w), 753 (s), 666 (w), 602 (w) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.65 (s, 1H, H-12), 5.03 (m, 1H, Ala-NH), 4.52 (dd, 1H, H-3, J = 11.6, 4.67 Hz), 4.30 (m, 1H, Ala-CH), 4.16 (dq, 1H, COOC*H*H′, J = 10.8, 7.17 Hz), 4.11 (dq, 1H, COOCH*H*′, J = 10.8, 7.17 Hz), 2.80 (ddd, 1H, H-1, J = 13.7, 3.8, 3.87 Hz), 2.34 (s, 1H, H-9), 2.08 (dd, 1H, H-18, J = 13.6, 3.77 Hz), 2.01 (m, 1H, H-15), 1.97 (m, 1H, H-21), 1.90 (m, 1H, H-19), 1.81 (m, 1H, H-16), 1.71 (m, 1H, H-2), 1.66 (m, 1H, H-7), 1.60 (m, 1H, H-2′), 1.59 (dd, 1H, H-19′, J = 13.3, 13.37 Hz), 1.56 (m, 1H, H-6), 1.44 (m, 1H, H-6′), 1.41 (s, 9H, Boc-CH3), 1.39 (m, 1H, H-7′), 1.36 (m, 1H, H-22), 1.35 (s, 3H, H-27), 1.35 (d, 3H, Ala-CH_3, J = 7.17 Hz), 1.29 (m, 1H, H-22′), 1.27 (m, 1H, H-21′), 1.24 (t, 3H, Et-CH_3, J = 7.17 Hz), 1.16 (m, 1H, H-16′), 1.14 (s, 3H, H-25), 1.12 (s, 3H, H-28), 1.10 (s, 3H, H-26), 1.04 (m, 1H, H-1′), 0.99 (m, 1H, H-15′), 0.86 (s, 3H, H-23), 0.86 (s, 3H, H-24), 0.80 (m, 1H, H-5), 0.79 (s, 3H, H-29); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 176.3 (C-30), 171.8 (Ala-COO), 169.4 (C-13), 155.0 (Boc-COO), 128.4 (C-12), 81.6 (C-3), 78.1 (Boc-*quart*.-C), 61.7 (C-9), 60.3 (Et-CH_2), 55.0 (C-5), 50.8 (Ala-CH), 48.4 (C-18), 45.4 (C-8), 43.8 (C-20), 43.2 (C-14), 41.0 (C-19), 38.6 (C-1), 38.1 (C-4), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-29), 28.3 (C-23), 28.0 (C-28), 26.5 (C-16), 26.4 (C-15), 23.3 (C-2), 23.3 (C-27), 18.8 (Ala-CH_3), 18.6 (C-26), 17.3 (C-6), 16.6 (C-24), 16.4 (C-25), 14.3 (Et-CH_3). Anal. Calcd for $C_{40}H_{63}NO_7$ (669.93): C, 71.71; H, 9.48; N, 2.09. Found: C, 71.52; H, 9.63; N, 1.86.

4.2.6.43. *i*-Propyl 3β-(Boc-D-alanyl)-11-oxo-olean-12-en-30-oate (43). Obtained from **3** and Boc-D-Ala by method A as a colourless powder; yield: 380 mg, 84%; mp 178–181 °C (decomp.); R_f = 0.55 (hexane/ethyl acetate 7:3); $[\alpha]_D^{20}$ = 101.37 (c 0.39, $CHCl_3$); UV–vis (MeOH): λ_{max} (log ε) = 250 nm (4.04); MS (ESI): m/z (%) = 684.1 ($[M+H]^+$, 10), 706.3 ($[M+Na]^+$, 100), 1048.3 ($[3M+2Na]^{2+}$, 8), 1390.1 ($[2M+Na]^+$, 13); IR (KBr): ν = 3382 (br), 2978 (s), 2876 (m), 1723 (s), 1661 (s), 1621 (w), 1499 (m), 1455 (s), 1389 (s), 1367 (s), 1312 (m), 1249 (m), 1216 (s), 1171 (s), 1109 (s), 1087 (m), 1086 (m), 1022 (m), 973 (m), 916 (w), 880 (w), 864 (w), 770 (w), 669 (w), 539 (w), 464 (w) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.60 (s, 1H, H-12), 5.04 (m, 1H, Ala-NH), 5.01 (qq, 1H, *i*-Pr-CH, J = 6.5, 6.57 Hz), 4.53 (dd, 1H, H-3, J = 11.7, 4.67 Hz), 4.27 (m, 1H, Ala-CH), 2.79 (ddd, 1H, H-1, J = 13.7, 3.5, 3.57 Hz), 2.34 (s, 1H, H-9), 2.09 (dd, 1H, H-18, J = 13.3, 3.77 Hz), 2.01 (dd, 1H, H-15, J = 13.3, 5.07 Hz), 1.96 (m, 1H, H-21), 1.89 (ddd, 1H, H-19, J = 13.7, 4.2, 2.57 Hz), 1.80 (ddd, 1H, H-16, J = 13.8, 13.8, 4.57 Hz), 1.70 (m, 1H, H-2), 1.66 (m, 1H, H-7), 1.60 (m, 1H, H-2′), 1.58 (dd, 1H, H-19′, J = 13.2, 13.27 Hz), 1.56 (m, 1H, H-6), 1.44 (m, 1H, H-6′), 1.42 (s, 9H, Boc-CH_3), 1.38 (m, 1H, H-7′), 1.36 (m, 1H, H-22), 1.35 (m, 3H, Ala-CH_3), 1.34 (s, 3H, H-27), 1.33 (m, 1H, H-22′), 1.31 (m, 1H, H-21′), 1.23 (d, 3H, *i*-Pr-CH_3, J = 6.3), 1.20 (d, 3H, *i*-Pr-CH_3, J = 6.37 Hz), 1.19 (m, 1H, H-16′), 1.14 (s, 3H, H-25), 1.11 (s, 6H, H-28 and H-26), 1.03 (ddd, 1H, H-1′, J = 13.3, 13.3, 3.37 Hz), 0.99 (m, 1H, H-15′), 0.87 (s, 6H, H-23 and H-24), 0.79 (m, 1H, H-5), 0.78 (s, 3H, H-29); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 175.8 (C-30), 171.2 (Ala-COO), 169.5 (C-13), 156.7 (Boc-COO), 128.4 (C-12), 81.6 (C-3), 79.2 (Boc-*quart*.-C), 67.3 (*i*-Pr-CH), 61.7 (C-9), 55.0 (C-5), 49.3 (Ala-CH), 48.4 (C-18), 45.4 (C-8), 43.7 (C-20), 43.2 (C-14), 41.0 (C-19), 38.6 (C-1), 38.1 (C-4), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.6 (C-29), 28.3 (Boc-CH_3), 28.2 (C-23), 28.0 (C-28), 26.5 (C-16), 26.4 (C-15), 23.3 (C-2), 23.3 (C-27), 21.9 (*i*-Pr-CH_3), 21.7 (*i*-Pr-CH_3), 18.8 (Ala-CH_3), 18.6 (C-26), 17.3 (C-6), 16.6 (C-24), 16.3 (C-25). Anal. Calcd for $C_{41}H_{65}NO_7$ (683.96): C, 72.00; H, 9.58; N, 2.05. Found: C, 71.89; H, 9.71; N, 1.94.

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Anhang A2

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Original article

Synthesis and biological activity of some antitumor active derivatives from glycyrrhetinic acid

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ABSTRACT

Aminoalkyl substituted derivatives were synthesized starting from glycyrrhetinic acid methyl ester and screened for antitumor activity in a panel of 15 human cancer cell lines by an SRB assay. The most compound **7** possesses an aminohexyl side chain, induces apoptosis and shows IC_{50} values of 0.6–3.0 μM.
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1. Introduction

Glycyrrhetinic acid (GA) got into the focus of scientific interest at least for its antitumor activity; on-going investigations for the last two decades confirmed an apoptotic effect of GA on tumor cells [1–4]. Its vast abundance in natural sources is another important argument in the search of GA derived antitumor agents. Thus, the extract of licorice contains up to 15% of the glycoside glycyrrhizin [5,6] whereas other antitumor active triterpenoids, e.g. betulinic acid (2.4%) [7] or oleanoic acid (ca. 3.1%) [7,8] are more difficult to obtain from their natural sources. One of our goals was increasing the activity of GA by "simple" transformations—but retaining the advantages residing in the parent compound GA.

2. Chemistry

Preliminary results indicated that changing of the polarity pattern of GA might be of some advantage in obtaining better cytotoxicity; hence, preparing an ester of GA (for gain in lipophility) and introducing an extra amino group (for gain in polarity) seemed appropriate. Starting from GA, the methyl ester **1** was prepared [9] in 91% yield by reacting GA with methyl iodide and potassium carbonate (Scheme 1). GA as well as its ester **1** were converted into the corresponding chloroacetyl derivatives **2** and **3**, respectively. Reaction of **3** with a variety of α,ω-alkanediamines (Scheme 2) furnished compounds **4**–**11**.

3. Results

The compounds **1**–**11** were tested for antitumor activity in a panel of 15 human cancer cell lines using a sulforhodamine B assay (SRB). The results from these tests are summarized in Table 1.

Screening of compounds **2** and **3** in the SRB assay revealed that a free carboxylic group in position C(30) results in a lower inhibition of cell growth compared to the corresponding esters. Esterification results in an approx. 3-fold higher cytotoxicity. Additional chloroacetylation afforded an analogue **3** showing a ca. 10-fold higher cytotoxicity than the parent compound GA.

In the SRB assay all of the amino compounds **4**–**11** showed significantly improved IC_{50} values compared to GA. Compound **7** carrying a diaminohexyl chain was the most active derivative showing IC_{50} values between 0.59 and 3.0 μM. This corresponds—in average—to an approx. 60 times higher cytotoxicity than GA. In addition, the length of the alkyl chain seems to be of importance (Fig. 1). As depicted in Fig. 1, compound **7** inhibits the growth of the tumor cells best. Our findings parallel previous results [10–13] showing that a methyl esterification of the C-30 carboxylic group significantly improves the cytotoxic activities of GA. Nitrogen-containing substituents directly attached to the A-ring or close by seem to improve [10,14] the antiproliferative effect of GA derivatives.

Supposing an extrinsic mechanism of apoptotic action [15] one might reason that these compounds interact with a membrane

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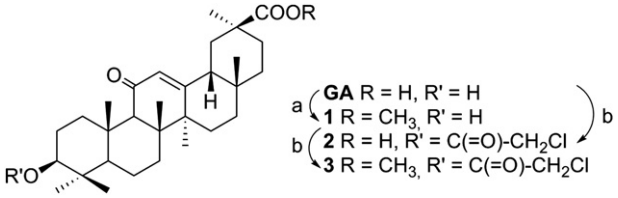


Scheme 1. Synthesis of the chloroacetyl derivatives: reagents and conditions: (a) K_2CO_3, CH_3I, DMF, 24 h, 25 °C, 91%; (b) Cl–CH_2COCl, Et_3N, THF (or CH_2Cl_2), 25 °C, 12 h, 45% (for **2**) and 86.9% (for **3**).

located receptor that belongs to the TNF family. The data presented in Fig. 1 seem to support this assumption since an optimized length of the aminoalkyl side chain should fit into the binding pocket of the receptor best. But these assumptions, however, cannot explain the lowered cytotoxicity associated with compounds **1**–**3**; especially **3** show a high cytotoxicity but differs in its structure significantly from **4**–**11**. Hence, explaining our findings by evoking an intrinsic mechanism of action [1,16] seems more likely.

As shown by an extra dye exclusion test (trypan blue) and DNA laddering experiments (by gel electrophoresis) compound **7** triggers apoptosis. Quite recently, Yamaguchi et al. [17] were able to show that GA exhibited a tumor cell-selective toxicity through H-Ras down-regulation but also induces actin disruption [18]. In addition, GA suppresses [19] colon carcinogenesis. Further studies concerning the apoptotic mechanism are presently under investigation in our laboratory.

4. Conclusions

In this study we have synthesized aminoalkyl modified GA methyl esters; screening for antitumor activity in a panel of 14 human cancer cell lines by an SRB assay showed for the most active compound **7** an IC_{50} values between 0.6 and 3.0 μM. Cell death is triggered by apoptosis as shown by a dye-exclusion test and DNA-laddering experiments.

5. Experimental

5.1. General

Melting points are uncorrected (*Leica* hot stage microscope),-NMR spectra were recorded using the Varian spectrometers Gemini 200, Gemini 2000 or Unity 500 (δ given in ppm, J in Hz, internal Me_4Si), IR spectra (film or KBr pellet) on a Perkin–Elmer FT-IR spectrometer Spectrum 1000, MS spectra were taken on an Intectra GmbH AMD 402 (electron impact, 70 eV) or on a Finnigan MAT TSQ 7000 (electrospray, voltage 4.5 kV, sheath gas nitrogen) instrument. TLC was performed on silica gel (Merck 5554, detection by UV absorption). The solvents were dried according to usual procedures.

5.2. Cell lines and culture conditions

The cell lines 518A2, 8505C, A253, A2780, A431, A549, DLD-1, FaDu, HCT-116, HCT-8, HT-29, LIPO, MCF-7, SW1736, SW480 were included in this study. All these cell lines were kindly provided by Dr. Thomas Müller, Department of Hematology/Oncology, Martin-Luther-University Halle-Wittenberg, Halle (Saale), Germany.

Cultures were maintained as monolayer in RPMI 1640 (PAA Laboratories, Pasching, Germany) supplemented with 10% heat inactivated fetal bovine serum (Biochrom AG, Berlin, Germany) and penicillin/streptomycin (PAA Laboratories) at 37 °C in a humidified atmosphere of 5% CO_2/95% air.

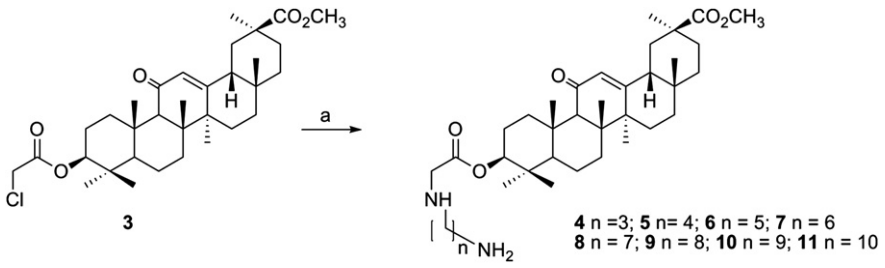
5.3. Cytotoxicity assay

The cytotoxic activities of our compounds were evaluated using the sulforhodamine B (SRB) [20] (Sigma–Aldrich) microculture colorimetric assay. In short, exponentially growing cells were seeded into 96-well plates on day 0 at the appropriate cell densities to prevent confluence of the cells during the period of experiment. After 24 h, the cells were treated with serial dilutions of the compounds (0–30 μM) for 96 h. The final concentration of DMSO or DMF as a solvent never exceeded 0.5%, which was shown to be non-toxic to the cells. The percentages of surviving cells compared to untreated controls were determined 96 h after the beginning of drug exposure. After 96 h of treatment, the supernatant medium from the 96-well plates was discarded and the cells were fixed with 10% TCA. For a thorough fixation, the plates were allowed to stand at 4 °C. After fixation, the cells were washed in a strip washer for four times with water using alternate dispensing and aspiration procedures. The plates were dyed with 100 μl of 0.4% SRB (sulforhodamine B) for about 20 min. After dying, the plates were washed with 1% acetic acid to remove the dye and allowed to air dry overnight. Then 100 μl of 10 mM Tris base solution were added to each well and absorbance was measured at 570 nm using a 96-well plate reader (Tecan Spectra, Crailsheim, Germany). The IC 50 was calculated from the semi-logarithmic dose-response curves.

5.4. Apoptosis tests [21]

5.4.1. Dye exclusion test

Apoptotic cell death was analyzed by trypan blue dye (Sigma Aldrich, Germany) on A431 and A2780 cell lines. The cell culture flasks with 70–80% confluence were treated with IC 90 doses of the compounds for 24 h. After treatment, the supernatant medium with floating cells was collected and centrifuged to collect the dead and apoptotic cells. The cell pellet was suspended in serum free media. Equal amounts of cell suspension and trypan blue were mixed and analyzed by microscope. The viable cells exclude the dye and are colourless and cells whose cell membrane was destroyed are turning into blue. When there were more colourless cells than colored, death is characterised as apoptotic.



Scheme 2. Synthesis of the aminoalkyl derivatives: reagents and conditions: (a) H_2N-$(CH_2)_n$-NH_2, DMF, K_2CO_3, 12 h, 25 °C, 21–73%.

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Table 1
Cytotoxicity (IC_{50} values in μmol) for **1**–**11** in a panel of various cancer cell lines.

	GA[a]	1	2	3	4	5	6	7	8	9	10	11
518A2	83.92	27.54	25.43	5.24	3.79	2.55	2.02	1.09	1.27	3.49	3.12	4.33
8505C	86.50	26.07	26.08	15.86	3.37	2.12	1.78	1.68	2.13	3.35	6.18	7.6
A253	80.78	19.42	25.54	6.19	3.64	2.56	2.27	1.12	1.74	3.01	4.65	5.48
A2780	74.57	25.54	23.77	6.01	4.39	2.43	2.00	1.36	1.14	2.80	3.30	3.63
A549	82.76	23.50	24.80	8.39	5.15	3.31	2.52	1.59	2.21	4.08	2.23	5.16
DLD-1	81.21	26.12	17.36	6.13	4.39	2.66	2.40	0.91	1.25	3.96	4.50	5.53
FADU	84.55	23.41	23.56	12.44	5.57	3.51	3.30	1.78	2.20	4.26	5.54	5.65
HCT-116	78.83	22.10	14.41	5.13	4.30	2.41	2.19	1.17	1.70	3.53	3.44	3.86
HCT-8	78.85	24.36	13.39	3.97	2.37	1.51	1.38	0.62	0.89	2.92	2.42	4.07
HT-29	80.09	27.54	16.91	5.34	2.90	1.69	1.28	0.59	0.86	2.76	2.06	2.73
LIPO	81.44	20.47	25.39	14.55	3.89	2.57	1.93	1.59	1.44	4.36	5.48	6.93
MCF-7	84.70	22.14	25.11	6.69	3.55	2.45	1.79	1.17	0.98	3.89	3.33	2.68
SW1736	76.93	34.87	16.42	3.14	6.05	3.30	2.69	1.61	2.24	4.09	3.30	3.73
SW480	86.80	16.08	25.91	8.92	3.68	2.54	1.91	2.25	2.24	3.93	5.74	4.73

[a] From SRB assays after 96 h of treatment; the values are averaged from at least 3 independent experiments; variation ± 5%.

5.4.2. DNA Fragmentation assay

Determination of apoptotic cell death was performed by DNA gel electrophoresis. Briefly, A431 and A2780 were treated with respective IC 90 doses of the compounds for 24 h. Floating cells (induced by drug exposure) were collected, washed with PBS and lysed with lysis buffer (100 mM Tris-HCL Ph 8.0; 20 mM EDTA; 0.8% SDS) (all from Sigma–Aldrich). Then they are treated with RNAse A at 37 °C for 2 h and proteinase K at 50 °C (both from Roche Diagnostics chemical company, Mannheim, Germany). DNA laddering was observed by running the samples on 2% agarose gel followed by ethidium bromide (Sigma–Aldrich) staining.

*5.5. Methyl 3β-hydroxy-11-oxo-olean-12-en-30-oate (**1**)*

To a solution of GA (31.00 g, 65.9 mmol) in dry DMF (150 ml), potassium carbonate (15.34 g, 111.0 mmol) was added. After 30 min of stirring 25 °C, iodomethane (4.94 ml, 79.0 mmol) was added and stirring continued for 2 h. The solvents were evaporated and the crude residue was dissolved in a mixture of dichloromethane (300 ml) and hydrochloric acid (50 ml, 1.0 M). The aqueous layer was extracted with dichloromethane (3 × 50 ml), the combined organic layers were washed with brine (50 ml), dried (Na_2SO_4), filtered and the solvent was evaporated. Re-crystallization from methanol yielded **1** (29.09 g, 91.1%) as a colourless crystalline solid. Mp 254–258 °C (lit. 254–258 °C [9]); $R_F = 0.48$ (hexane/ethyl acetate 7:3); $[\alpha]_D^{20} = 141.18°$ ($c = 0.48$, $CHCl_3$); UV–vis (methanol): λ_{max} (log ε) = 270 nm (4.15); IR (KBr): ν = 3614*br*, 2970*s*, 2955*s*, 2875*m*, 1726*s*, 1659*s*, 1466*m*, 1450*m*, 1364*w*, 1216*m*, 1189*m*, 1136*w*, 1085*w*, 1040*w*, 992*w* cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 5.65 (*s*, 1H, H-12), 3.67 (*s*, 3H, $COOCH_3$), 3.21 (*dd*, 1H, H-3, *J* = 10.8, 5.7 Hz), 2.78 (*ddd*, 1H, H-1, *J* = 13.3, 3.3, 3.3 Hz), 2.32 (*s*, 1H, H-9), 2.06 (*dd*, 1H, H-18, *J* = 13.3, 3.8 Hz), 2.00 (*m*, 1H, H-15), 1.95 (*m*, 1H, H-21), 1.90 (*dd*, 1H, H-19, *J* = 13.8, 3.9 Hz), 1.83 (*ddd*, 1H, H-16, *J* = 14.3, 14.3, 5.2 Hz), 1.65 (*m*, 1H, H-2), 1.62 (*m*, 1H, H-7), 1.58 (*m*, 1H, H-2′), 1.57 (*m*, 1H, H-19′), 1.57 (*m*, 1H, H-6), 1.43 (*m*, 1H, H-6′), 1.38 (*m*, 1H, H-7′), 1.36 (*m*, 1H, H-22), 1.34 (*s*, 3H, H-27), 1.30 (*m*, 1H, H-22′), 1.28 (*m*, 1H, H-21′), 1.17 (*m*, 1H, H-16′), 1.13 (*s*, 3H, H-28), 1.12 (*s*, 3H, H-25), 1.11 (*s*, 3H, H-26), 1.00 (*m*, 1H, H-15′), 0.99 (*s*, 3H, H-23), 0.96 (*m*, 1H, H-1′), 0.79 (*s*, 3H, H-24), 0.79 (*s*, 3H, H-29), 0.68 (*m*, 1H, H-5) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 176.8 (C-30), 169.0 (C-13), 128.6 (C-12), 78.8 (C-3), 61.9 (C-9), 55.1 (C-5), 51.8 ($COOCH_3$), 48.5 (C-18), 45.5 (C-14), 44.1 (C-20), 43.3 (C-8), 41.2 (C-19), 39.2 (C-1), 39.2 (C-4), 37.8 (C-22), 37.2 (C-10), 32.9 (C-7), 31.9 (C-17), 31.2 (C-21), 28.6 (C-29), 28.4 (C-23), 28.2 (C-28), 27.4 (C-2), 26.6 (C-16), 26.6 (C-15), 23.5 (C-27), 18.8 (C-26), 17.6 (C-6), 16.4 (C-25), 15.6 (C-24) ppm; MS (ESI): *m/z* (%) = 485.5 ($[M+H]^+$, 55), 507.5 ($[M+Na]^+$, 12), 539.1 ($[M+Na+MeOH]^+$, 100).

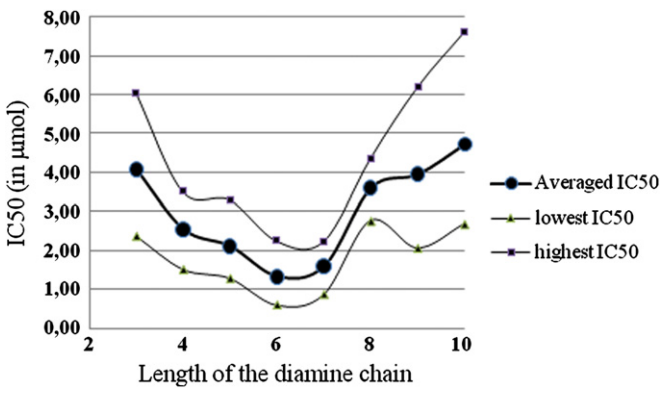

Fig. 1. IC50 values (μM) as a function of the alkyl chain length; lowest, highest and an averaged value (mean of 14 cell lines) are given.

*5.6. 3β-(Chloroacetyl)-11-oxo-olean-12-en-30-oic acid (**2**)*

GA (0.76 g, 1.62 mmol) was dissolved in mixture of dry THF (15 ml) and dry triethylamine (1 ml). Chloro-acetylchloride (142 μl, 1.78 mmol) was added dropwise and the solution was stirred at 25 °C for 12 h. The solvent was removed and the residue dissolved in dichloromethane (15 ml). After washing with hydrochloric acid (10 ml, 1.0 M), the aqueous layer was extracted with dichloromethane (3 × 10 ml), followed by washing the combined organic layers with brine (10 ml). They were dried (Na_2SO_4), filtered and evaporated. The crude residue was subjected to chromatography (silica gel, hexane/ethyl acetate 7:3) to afford **2** (0.40 g, 45.3%) as colourless crystals. Mp 259–262 °C; $R_F = 0.20$ (hexane/ethyl acetate 7:3); $[\alpha]_D^{20} = 117.21°$ ($c = 0.5$, CHCl3); UV–vis (methanol): λ_{max} (log ε) = 267 nm (4.01); IR (KBr): ν = 3385*br*, 2960*s*, 1738*s*, 1652*s*, 1466*w*, 1387*m*, 1326*m*, 1214*m*, 985*w*, 789*w* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.69 (*s*, 1H, H-12), 4.59 (*dd*, 1H, H-3, *J* = 11.8, 4.9Hz), 4.06 (*d*, 1H, $CHH'Cl$, *J* = 16.8 Hz), 4.02 (*d*, 1H, $CHH'Cl$, *J* = 16.8 Hz), 2.82 (*ddd*, 1H, H-1, *J* = 13.7, 3.6, 3.6 Hz), 2.35 (*s*, 1H, H-9), 2.17 (*dd*, 1H, H-18, *J* = 13.4, 3.8 Hz), 2.02 (*ddd*, 1H, H-15, *J* = 13.4, 13.4, 4.5 Hz), 1.98 (*m*, 1H, H-21), 1.92 (*dd*, 1H, H-19, *J* = 13.8, 3.9 Hz), 1.82 (*ddd*, 1H, H-16, *J* = 13.5, 13.5, 4.5 Hz), 1.72 (*m*, 1H, H-2), 1.66 (*m*, 1H, H-7), 1.63 (*m*, 1H, H-2′), 1.61 (*dd*, 1H, H-19′, *J* = 13.6 Hz), 1.57 (*m*, 1H, H-6), 1.44 (*m*, 1H, H-6′), 1.41 (*m*, 1H, H-7′), 1.39 (*m*, 1H, H-22), 1.36 (*s*, 3H, H-27), 1.31 (*m*, 1H, H-22′), 1.28 (*m*, 1H, H-21′), 1.21 (*s*, 3H, H-28), 1.19 (*m*, 1H, H-16′), 1.15 (*s*, 3H, H-25), 1.12 (*s*, 3H, H-26), 1.05 (*m*, 1H, H-15′), 0.97 (*m*, 1H, H-1′), 0.89 (*s*, 6H, H-23, H-24), 0.82 (*s*, 3H, H-29), 0.80 (*m*, 1H, H-5) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 181.2 (C-30), 169.3 (C-13), 167.1 (CH_2ClCOO), 128.5 (C-12), 83.1 (C-3), 61.7 (C-9), 55.0 (C-5), 48.3 (C-18), 45.5 (C-14), 43.8

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(C-20), 43.3 (C-8), 41.2 ($\underline{C}H_2ClCOO$), 40.9 (C-19), 38.7 (C-1), 38.3 (C-4), 37.7 (C-22), 37.0 (C-10), 32.7 (C-7), 31.9 (C-17), 31.0 (C-21), 28.6 (C-29), 28.4 (C-23), 28.1 (C-28), 26.5 (C-16), 26.5 (C-15), 23.5 (C-2), 23.4 (C-27), 18.8 (C-26), 17.4 (C-6), 16.7 (C-24), 16.4 (C-25) ppm; MS (ESI): *m/z* (%) = 547.6 ($[M + H]^+$, 31), 569.4 ($[M + Na]^+$, 100), 601.1 ($[M + Na + MeOH]^+$, 32); anal. calcd for $C_{32}H_{47}ClO_5$ (547.17): C, 70.24; H, 8.66; Cl, 6.48; found: C, 70.11; H, 8.78; Cl, 6.31.

5.7. Methyl 3β-(chloroacetyl)-11-oxo-olean-12-en-30-oate (**3**)

Compound **1** (2.00 g, 4.1 mmol) was dissolved in dry dichloromethane (20 ml) and mixed with dry triethylamine (1 ml). Chloroacetylchloride (360 μl, 4.5 mmol) was added dropwise and stirring at 25 °C continued for 12 h. After completion of the reaction (as monitored by TLC), the mixture was washed with hydrochloric acid (10 ml, 1.0 M). The aqueous layer was extracted with dichloromethane (3 × 10 ml), the combined organic layers were washed with brine (15 ml), dried (Na_2SO_4), filtered and the solvents were evaporated. The residue was subjected to chromatography (silica gel, hexane/ethyl acetate 9:1) to afford **3** (2.30 g, 86.9%) as colourless crystals. Mp 243–246 °C; R_F = 0.77 (hexane/ethyl acetate 7:3); $[\alpha]_D^{20}$ = 115.53° (*c* = 0.31, $CHCl_3$); UV–vis (methanol): λ_{max} (log ε) = 267 nm (4.08); IR (KBr): ν = 3432*br*, 2951*s*, 1732*s*, 1645*m*, 1540*m*, 1463*s*, 1371*s*, 1331*m*, 1250*s*, 1210*s*, 1179*s*, 1062*m*, 1025*m*, 978*m*, 945*w*, 888*m*, 831*m*, 778*w*, 749*w* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.65 (*s*, 1H, H-12), 4.58 (*dd*, 1H, H-3, *J* = 11.6, 4.8 Hz), 4.05 (*d*, 1H, $C\underline{H}H'Cl$, *J* = 17.1 Hz), 4.01 (*d*, 1H, $CH\underline{H}'Cl$, *J* = 17.1 Hz), 3.67 (*s*, 3H, $COOC\underline{H}_3$), 2.81 (*ddd*, 1H, H-1, *J* = 13.7, 3.6, 3.6 Hz), 2.34 (*s*, 1H, H-9), 2.06 (*dd*, 1H, H-18, *J* = 13.5, 3.5 Hz), 2.01 (*m*, 1H, H-15), 1.97 (*m*, 1H, H-21), 1.90 (*ddd*, 1H, H-19, *J* = 13.5, 4.1, 2.7 Hz), 1.80 (*ddd*, 1H, H-16, *J* = 13.7, 13.7, 4.4 Hz), 1.73 (*ddd*, 1H, H-2, *J* = 11.8, 3.3, 1.5 Hz), 1.65 (*m*, 1H, H-7), 1.63 (*m*, 1H, H-2′), 1.59 (*dd*, 1H, H-19′, *J* = 13.5 Hz), 1.57 (*m*, 1H, H-6), 1.45 (*m*, 1H, H-6′), 1.40 (*m*, 1H, H-7′), 1.37 (*m*, 1H, H-22), 1.34 (*s*, 3H, H-27), 1.29 (*m*, 1H, H-22′), 1.28 (*m*, 1H, H-21′), 1.16 (*m*, 1H, H-16′), 1.14 (*s*, 3H, H-28), 1.13 (*s*, 3H, H-25), 1.10 (*s*, 3H, H-26), 1.04 (*ddd*, 1H, H-1′, *J* = 13.4, 13.4, 3.5 Hz), 0.99 (*m*, 1H, H-15′), 0.88 (*s*, 6H, H-23, H-24), 0.79 (*m*, 1H, H-5), 0.78 (*s*, 3H, H-29) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.1 (C-11), 176.9 (C-30), 169.5 (C-13), 167.1 ($CH_2Cl\underline{C}OO$), 128.4 (C-12), 83.0 (C-3), 61.6 (C-9), 55.0 (C-5), 51.8 ($COO\underline{C}H_3$), 48.4 (C-18), 45.4 (C-14), 44.0 (C-20), 43.2 (C-8), 41.2 ($\underline{C}H_2ClCOO$), 41.1 (C-19), 38.7 (C-1), 38.2 (C-4), 37.7 (C-22), 36.9 (C-10), 32.6 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-29), 28.3 (C-23), 28.0 (C-28), 26.4 (C-16), 26.4 (C-15), 23.4 (C-2), 23.3 (C-27), 18.6 (C-26), 17.3 (C-6), 16.6 (C-24), 16.4 (C-25) ppm; MS (ESI): *m/z* (%) = 561.4 ($[M + H]^+$, 80), 583.3 ($[M + Na]^+$, 70), 614.8 ($[M + Na + MeOH]^+$, 100), 1121.1 ([cluster 2M + H]$^+$, 36), 1143.1 (cluster $[2M + Na]^+$, 72); anal. calcd for $C_{33}H_{49}ClO_5$ (561.19): C, 70.63; H, 8.80; found: C, 70.51; H, 8.96.

5.8. General procedure for the preparation of the amines

To a solution of **3** (1 eq.) in dry DMF (15 ml per eq.), potassium carbonate (2 eq.) and the respective 1,ω-alkandiamine (2 eq.) was added. After 12 h of stirring at 25 °C, the solvent was removed under reduced pressure and the crude residue was dissolved in a mixture of dichloromethane and water. The aqueous layer was extracted with dichloromethane, the combined organic layers were washed with brine, dried (Na_2SO_4), filtered and evaporated. The remaining residue was subjected to chromatography (silica gel, loaded with methanol, unloaded with methanol/diethylamine 9:1) to afford the final products (**4**–**11**).

5.8.1. Methyl 3β-3-{[N-(3-aminopropyl)glycyl]oxy}-11-oxo-olean-12-en-30-oate (**4**)

Following the general procedure, **4** was obtained from 1,3-propanediamine; yield: 140 mg, 27.0%; mp 191–195 °C; R_F = 0.71 (MeOH/diethylamine 9:1); $[\alpha]_D^{20}$ = 93.49° (*c* = 0.43, $CHCl_3$); UV–vis (methanol): λ_{max} (log ε) = 267 nm (4.03); IR (KBr): ν = 3432*br*, 2934*s*, 1731*s*, 1660*s*, 1464*m*, 1387*m*, 1322*m*, 1217*m*, 1154*m*, 1086*w*, 1022*w*, 985*m*, 880*w*, 769*w* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.64 (*s*, 1H, H-12), 4.56 (*m*, 1H, H-3), 3.66 (*s*, 3H, $COOC\underline{H}_3$), 3.38 (*s*, 2H, $C\underline{H}_2COO$), 2.90 (*t*, 2H, chain-3, *J* = 6.3 Hz), 2.79 (*ddd*, 1H, H-1, *J* = 13.5, 3.2, 3.2 Hz), 2.71 (*t*, 2H, chain-1, *J* = 6.4 Hz), 2.34 (*s*, 1H, H-9), 2.06 (*m*, 1H, H-18), 2.01 (*m*, 1H, H-15), 1.97 (*m*, 1H, H-21), 1.90 (*ddd*, 1H, H-19, *J* = 13.6, 3.6, 2.1 Hz), 1.80 (*m*, 1H, H-16), 1.71, (*m*, 1H, H-2), 1.70 (*m*, 2H, chain-2), 1.64 (*m*, 1H, H-7), 1.61 (*m*, 1H, H-2′), 1.59 (*dd*, 1H, H-19′, *J* = 12.4, 12.4 Hz), 1.54 (*m*, 1H, H-6), 1.46 (*m*, 1H, H-6′), 1.41 (*m*, 1H, H-7′), 1.38 (*m*, 1H, H-22), 1.34 (*s*, 3H, H-27), 1.30 (*m*, 1H, H-22′), 1.28 (*m*, 1H, H-21′), 1.19 (*m*, 1H, H-16′), 1.14 (*s*, 3H, H-25), 1.13 (*s*, 3H, H-23), 1.10 (*s*, 3H, H-26), 1.04 (*ddd*, 1H, H-1′, *J* = 13.1, 13.1, 3.7 Hz), 0.99 (*m*, 1H, H-15′), 0.85 (*s*, 6H, H-24, H-28), 0.79 (*m*, 1H, H-5), 0.78 (*s*, 3H, H-29) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 176.9 (C-30), 172.4 ($CH_2\underline{C}OO$), 169.2 (C-13), 128.5 (C-12), 81.4 (C-3), 61.7 (C-9), 55.0 (C-5), 51.7 ($COO\underline{C}H_3$), 50.9 ($\underline{C}H_2COO$), 48.4 (C-18), 47.8 (chain-1), 45.4 (C-14), 44.0 (C-20), 43.2 (C-8), 41.1 (C-19), 40.6 (chain-3), 38.7 (C-1), 38.1 (C-4), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.3 (chain-2), 31.1 (C-21), 28.5 (C-29), 28.3 (C-23), 28.1 (C-28), 26.4 (C-16), 26.4 (C-15), 23.6 (C-2), 23.3 (C-27), 18.7 (C-26), 17.4 (C-6), 16.7 (C-24), 16.4 (C-25) ppm; MS (ESI): *m/z* (%) = 599.3 ($[M + H]^+$, 100), 625.3 ($[2M + Na + MeOH]^{2+}$, 95); anal. calcd for $C_{36}H_{58}N_2O_5$ (598.86): C, 72.20; H, 9.76; N, 4.68; found: C, 72.06; H, 9.84; N, 4.51.

5.8.2. Methyl 3β-3-{[N-(4-aminobutyl)glycyl]oxy}-11-oxo-olean-12-en-30-oate (**5**)

Following the general procedure, **5** was obtained from 1,4-butanediamine; yield: 120 mg, 38.5%; mp 219–222 °C; R_F = 0.71 (MeOH/diethylamine 9:1); $[\alpha]_D^{20}$ = 82.38° (*c* = 0.32, $CHCl_3$); UV–vis (methanol): λ_{max} (log ε) = 267 nm (4.01); IR (KBr): ν = 3423*br*, 2949*s*, 1730*s*, 1660*s*, 1465*m*, 1388*m*, 1217*m*, 1153*m*, 1086*w*, 986*w*, 769*w* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.65 (*s*, 1H, H-12), 4.56 (*m*, 1H, H-3), 3.67 (*s*, 3H, $COOC\underline{H}_3$), 3.38 (*s*, 2H, $C\underline{H}_2COO$), 2.88 (*m*, 2H, chain-4), 2.80 (*ddd*, 1H, H-1, *J* = 13.5, 3.2, 3.2 Hz), 2.66 (*t*, 2H, chain-1, *J* = 6.2 Hz), 2.34 (*s*, 1H, H-9), 2.06 (*m*, 1H, H-18), 2.01 (*m*, 1H, H-15), 1.97 (*m*, 1H, H-21), 1.90 (*m*, 1H, H-19), 1.81 (*m*, 1H, H-16), 1.72 (*m*, 2H, chain-3), 1.69 (*m*, 1H, H-2), 1.64 (*m*, 1H, H-7), 1.63 (*m*, 2H, chain-2), 1.62 (*m*, 1H, H-2′), 1.59 (*dd*, 1H, H-19′, *J* = 13.6, 13.6 Hz), 1.54 (*m*, 1H, H-6), 1.46 (*m*, 1H, H-6′), 1.41 (*m*, 1H, H-7′), 1.38 (*m*, 1H, H-22), 1.35 (*s*, 3H, H-27), 1.31 (*m*, 1H, H-22′), 1.28 (*m*, 1H, H-21′), 1.19 (*m*, 1H, H-16′), 1.14 (*s*, 3H, H-25), 1.13 (*s*, 3H, H-23), 1.11 (*s*, 3H, H-26), 1.02 (*ddd*, 1H, H-1′, *J* = 13.1, 13.1, 3.7 Hz), 0.99 (*m*, 1H, H-15′), 0.86 (*s*, 6H, H-24, H-28), 0.80 (*m*, 1H, H-5), 0.79 (*s*, 3H, H-29) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 176.9 (C-30), 172.4 ($CH_2\underline{C}OO$), 169.2 (C-13), 128.5 (C-12), 81.2 (C-3), 61.7 (C-9), 55.0 (C-5), 51.8 ($COO\underline{C}H_3$), 51.1 ($\underline{C}H_2COO$), 48.9 (chain-1), 48.4 (C-18), 45.4 (C-14), 44.0 (C-20), 43.2 (C-8), 41.1 (C-19), 40.7 (chain-4), 38.7 (C-1), 38.1 (C-4), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-29), 28.3 (C-23), 28.1 (C-28), 28.2 (chain-3), 27.8 (chain-2), 26.4 (C-16), 26.4 (C-15), 23.6 (C-2), 23.3 (C-27), 18.7 (C-26), 17.4 (C-6), 16.7 (C-24), 16.4 (C-25) ppm; MS (ESI): *m/z* (%) = 613.5 ($[M + H]^+$, 100); anal. calcd for $C_{37}H_{60}N_2O_5$ (612.88): C, 72.51; H, 9.87; N, 4.57; found: C, 72.37; H, 9.98; N, 4.34.

5.8.3. Methyl 3β-3-{[N-(5-aminopentyl)glycyl]oxy}-11-oxo-olean-12-en-30-oate (**6**)

Following the general procedure, **6** was obtained from 1,5-pentanediamine; yield: 110 mg, 21.2%; mp 214–216 °C; R_F = 0.71 (MeOH/diethylamine 9:1); $[\alpha]_D^{20}$ = 94.52° (*c* = 0.28, $CHCl_3$); UV–vis (methanol): λ_{max} (log ε) = 267 nm (3.98); IR (KBr): ν = 3432*br*, 2934*s*, 1731*s*, 1660*s*, 1464*m*, 1387*m*, 1322*w*, 1217*s*, 1154*m*, 1086*w*, 1022*w*, 985*w*, 880*w*, 769*w* cm^{-1}; ^1H NMR (500 MHz,

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$CDCl_3$): $\delta = 5.64$ (*s*, 1H, H-12), 4.56 (*dd*, 1H, H-3, $J = 11.5$, 4.5 Hz), 3.66 (*s*, 3H, $COOCH_3$), 3.36 (*s*, 2H, CH_2COO), 2.79 (*ddd*, 1H, H-1, $J = 13.7$, 3.6, 3.6 Hz), 2.72 (*t*, 2H, chain-5, $J = 5.4$ Hz), 2.59 (*m*, 2H, chain-1), 2.34 (*s*, 1H, H-9), 2.06 (*m*, 1H, H-18), 2.00 (*m*, 1H, H-15), 1.97 (*m*, 1H, H-21), 1.90 (*m*, 1H, H-19), 1.80 (*m*, 1H, H-16), 1.68 (*m*, 1H, H-2), 1.62 (*m*, 1H, H-7), 1.59 (*dd*, 1H, H-19′, $J = 13.7$, 13.7 Hz), 1.58 (*m*, 1H, H-2′), 1.53 (*m*, 1H, H-6), 1.49 (*m*, 4H, chain-2, chain-4), 1.41 (*m*, 1H, H-6′), 1.38 (*m*, 1H, H-7′), 1.37 (*m*, 1H, H-22), 1.34 (*s*, 3H, H-27), 1.30 (*m*, 1H, H-22′), 1.28 (*m*, 1H, H-21′), 1.18 (*m*, 1H, H-16′), 1.14 (*s*, 3H, H-25), 1.12 (*s*, 3H, H-23), 1.10 (*s*, 3H, H-26), 1.02 (*m*, 1H, H-1′), 1.01 (*m*, 2H, chain-3), 0.98 (*m*, 1H, H-15′), 0.85 (*s*, 6H, H-24, H-28), 0.79 (*m*, 1H, H-5), 0.78 (*s*, 3H, H-29) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 200.0$ (C-11), 176.9 (C-30), 172.4 (CH_2COO), 169.2 (C-13), 128.5 (C-12), 81.2 (C-3), 61.7 (C-9), 55.0 (C-5), 51.7 ($COOCH_3$), 51.1 (CH_2COO), 49.4 (chain-1), 48.4 (C-18), 45.4 (C-14), 44.0 (C-20), 43.2 (C-8), 41.6 (chain-5), 41.1 (C-19), 38.7 (C-1), 38.1 (C-4), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 29.7 (chain-2), 29.7 (chain-4), 28.5 (C-29), 28.3 (C-23), 28.1 (C-28), 26.4 (C-16), 26.4 (C-15), 24.4 (chain-3), 23.6 (C-2), 23.3 (C-27), 18.6 (C-26), 17.4 (C-6), 16.7 (C-24), 16.4 (C-25) ppm; MS (ESI): *m/z* (%) = 627.4 ($[M + H]^+$, 100), 653.3 ($[2M + Na + MeOH]^{2+}$, 24); anal. calcd for $C_{38}H_{62}N_2O_5$ (626.91): C, 72.80; H, 9.97; N, 4.47; found: C, 72.63; H, 10.11; N, 4.26.

5.8.4. Methyl 3β-3-{[N-(6-aminohexyl)glycyl]oxy}-11-oxo-olean-12-en-30-oate (**7**)

Following the general procedure, **7** was obtained from 1,6-hexanediamine; yield: 260 mg, 47.9%; mp 141–144 °C; $R_F = 0.71$ (MeOH/diethylamine 9:1); $[\alpha]_D^{20} = 108.05°$ ($c = 0.54$, $CHCl_3$); UV–vis (methanol): λ_{max} (log ε) = 266 nm (4.00); IR (KBr): ν = 3422*br*, 2931*s*, 2857*s*, 1732*s*, 1659*s*, 1464*m*, 1388*m*, 1322*m*, 1280*m*, 1216*s*, 1154*s*, 1086*m*, 1021*w*, 985*m*, 917*w*, 879*w*, 769*w*, 670*w* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): $\delta = 5.64$ (*s*, 1H, H-12), 4.57 (*dd*, 1H, H-3, $J = 11.6$, 4.7 Hz), 3.67 (*s*, 3H, $COOCH_3$), 3.37 (*s*, 2H, CH_2COO), 2.79 (*ddd*, 1H, H-1, $J = 13.7$, 3.5, 3.5 Hz), 2.66 (*t*, 2H, chain-6, $J = 7.0$ Hz), 2.58 (*t*, 2H, chain-1, $J = 7.2$ Hz), 2.34 (*s*, 1H, H-9), 2.06 (*m*, 1H, H-18), 2.01 (*m*, 1H, H-15), 1.97 (*m*, 1H, H-21), 1.87 (*m*, 1H, H-19), 1.82 (*m*, 1H, H-16), 1.70 (*m*, 1H, H-2), 1.63 (*m*, 1H, H-7), 1.62 (*m*, 1H, H-2′), 1.59 (*dd*, 1H, H-19′, $J = 13.5$, 13.5 Hz), 1.55 (*m*, 1H, H-6), 1.48 (*m*, 2H, chain-3), 1.45 (*m*, 2H, chain-5), 1.42 (*m*, 1H, H-6′), 1.40 (*m*, 1H, H-7′), 1.38 (*m*, 1H, H-22), 1.34 (*s*, 3H, H-27), 1.33–1.27 (*m*, 6H, H-21′, H-22′, chain-2, chain-4), 1.19 (*m*, 1H, H-16′), 1.14 (*s*, 3H, H-25), 1.13 (*s*, 3H, H-23), 1.11 (*s*, 3H, H-26), 1.03 (*m*, 1H, H-1′), 0.98 (*m*, 1H, H-15′), 0.86 (*s*, 6H, H-24, H-28), 0.79 (*m*, 1H, H-5), 0.78 (*s*, 3H, H-29) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 200.2$ (C-11), 177.1 (C-30), 172.3 (CH_2COO), 169.1 (C-13), 128.5 (C-12), 81.2 (C-3), 61.8 (C-9), 55.2 (C-5), 51.9 ($COOCH_3$), 51.4 (CH_2COO), 49.7 (chain-1), 48.6 (C-18), 45.5 (C-14), 44.2 (C-20), 43.3 (C-8), 42.3 (chain-6), 41.3 (C-19), 38.9 (C-1), 38.3 (C-4), 37.9 (C-22), 37.1 (C-10), 33.9 (chain-5), 32.9 (C-7), 32.0 (C-17), 31.3 (C-21), 30.2 (chain-2), 28.7 (C-29), 28.5 (C-23), 28.3 (C-28), 27.3 (chain-3), 27.0 (chain-4), 26.7 (C-16), 26.7 (C-15), 23.8 (C-2), 23.5 (C-27), 18.9 (C-26), 17.6 (C-6), 16.9 (C-24), 16.6 (C-25) ppm; MS (ESI): *m/z* (%) = 641.5 ($[M + H]^+$, 100), 667.4 ($[2M + Na + MeOH]^{2+}$, 54); anal. calcd for $C_{39}H_{64}N_2O_5$ (640.94): C, 73.08; H, 10.06; N, 4.37; found: C, 72.95; H, 10.18; N, 4.26.

5.8.5. Methyl 3β-3-{[N-(7-aminoheptyl)glycyl]oxy}-11-oxo-olean-12-en-30-oate (**9**)

Following the general procedure, **9** was obtained from 1,7-heptanediamine; yield: 160 mg, 30.0%; mp 130–132 °C; $R_F = 0.71$ (MeOH/diethylamine 9:1); $[\alpha]_D^{20} = 97.66°$ ($c = 0.34$, $CHCl_3$); UV–vis (methanol): λ_{max} (log ε) = 266 nm (4.00); IR (KBr): ν = 3421br, 2929s, 2856s, 1732s, 1655s, 1464m, 1386m, 1322m, 1216s, 1154m, 1086w, 1022w, 985m, 879w, 770 (w) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): $\delta = 5.64$ (*s*, 1H, H-12), 4.57 (*dd*, 1H, H-3, $J = 11.7$, 4.6), 3.66 (*s*, 3H, $COOCH_3$), 3.36 (*s*, 2H, CH_2COO), 2.79 (*ddd*, 1H, H-1, $J = 13.6$, 3.2, 3.2), 2.65 (t, 2H, chain-7, $J = 7.1$), 2.57 (t, 2H, chain-1, $J = 7.2$), 2.34 (*s*, 1H, H-9), 2.06 (*m*, 1H, H-18), 2.00 (*m*, 1H, H-15), 1.97 (*m*, 1H, H-21), 1.90 (*m*, 1H, H-19), 1.80 (*m*, 1H, H-16), 1.70 (*m*, 1H, H-2), 1.63 (*m*, 1H, H-7), 1.62 (*m*, 1H, H-2′), 1.59 (*dd*, 1H, H-19′, $J = 13.1$, 13.1), 1.55 (*m*, 1H, H-6), 1.47 (*m*, 2H, chain-3), 1.44 (*m*, 2H, chain-6), 1.43 (*m*, 1H, H-6′), 1.40 (*m*, 1H, H-7′), 1.38 (*m*, 1H, H-22), 1.34 (*s*, 3H, H-27), 1.31–1.28 (*m*, 8H, H-21′, H-22′, chain-2, chain-4, chain-5), 1.20 (*m*, 1H, H-16′), 1.14 (*s*, 3H, H-25), 1.13 (*s*, 3H, H-23), 1.10 (*s*, 3H, H-26), 1.03 (*m*, 1H, H-1′), 0.98 (*m*, 1H, H-15′), 0.86 (*s*, 6H, H-24, H-28), 0.79 (*m*, 1H, H-5), 0.78 (*s*, 3H, H-29); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 200.0$ (C-11), 176.9 (C-30), 172.4 (CH_2COO), 169.2 (C-13), 128.5 (C-12), 81.1 (C-3), 61.7 (C-9), 55.0 (C-5), 51.7 ($COOCH_3$), 51.2 (CH_2COO), 49.6 (chain-1), 48.4 (C-18), 45.4 (C-14), 44.0 (C-20), 43.2 (C-8), 42.2 (chain-7), 41.1 (C-19), 38.7 (C-1), 38.1 (C-4), 37.7 (C-22), 36.9 (C-10), 33.8 (chain-6), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 30.0 (chain-3), 29.3 (chain-4), 28.5 (C-29), 28.3 (C-23), 28.1 (C-28), 27.2 (chain-2), 26.8 (chain-5), 26.5 (C-16), 26.4 (C-15), 23.6 (C-2), 23.3 (C-27), 18.7 (C-26), 17.4 (C-6), 16.7 (C-24), 16.4 (C-25); MS (ESI): *m/z* (%) = 328.6 ($[M+2H]^{2+}$, 9), 655.5 ($[M + H]^+$, 100); anal. calcd for $C_{40}H_{66}N_2O_5$ (654.96): C, 73.35; H, 10.16; N, 4.28; found: C, 73.22; H, 10.29; N, 4.11.

5.8.6. Methyl 3β-3-{[N-(8-aminooctyl)glycyl]oxy}-11-oxo-olean-12-en-30-oate (**9**)

Following the general procedure, **9** was obtained from 1,8-octanediamine; yield: 300 mg, 45.2%; mp 249–252 °C; $R_F = 0.71$ (MeOH/diethylamine 9:1); $[\alpha]_D^{20} = 97.05°$ ($c = 0.63$, $CHCl_3$); UV–vis (methanol): λ_{max} (log ε) = 266 nm (4.00); IR (KBr): ν = 3384br, 2928s, 2856s, 1732s, 1655s, 1465m, 1387m, 1216s, 1154m, 1086w, 1049w, 985m, 880w, 770 (w) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): $\delta = 5.64$ (*s*, 1H, H-12), 4.57 (*dd*, 1H, H-3, $J = 11.7$, 4.8), 3.67 (*s*, 3H, $COOCH_3$), 3.37 (*s*, 2H, CH_2COO), 2.79 (*ddd*, 1H, H-1, $J = 13.7$, 3.4, 3.4), 2.65 (t, 2H, chain-8, $J = 7.0$), 2.57 (t, 2H, chain-1, $J = 7.2$), 2.34 (*s*, 1H, H-9), 2.06 (*m*, 1H, H-18), 2.01 (*m*, 1H, H-15), 1.97 (*m*, 1H, H-21), 1.90 (*m*, 1H, H-19), 1.82 (*m*, 1H, H-16), 1.70 (*m*, 1H, H-2), 1.64 (*m*, 1H, H-7), 1.62 (*m*, 1H, H-2′), 1.59 (*dd*, 1H, H-19′, $J = 13.2$, 13.2), 1.54 (*m*, 1H, H-6), 1.47 (*m*, 2H, chain-7), 1.44 (*m*, 1H, H-6′), 1.41 (*m*, 1H, H-7′), 1.38 (*m*, 1H, H-22), 1.34 (*s*, 3H, H-27), 1.30 (*m*, 1H, H-21′), 1.29–1.26 (*m*, 11H, H-22′, chain-2, chain-3, chain-4, chain-5, chain-6), 1.18 (*m*, 1H, H-16′), 1.14 (*s*, 3H, H-25), 1.13 (*s*, 3H, H-23), 1.10 (*s*, 3H, H-26), 1.01 (*m*, 1H, H-1′), 0.98 (*m*, 1H, H-15′), 0.86 (*s*, 6H, H-24, H-28), 0.80 (*m*, 1H, H-5), 0.78 (*s*, 3H, H-29); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 200.0$ (C-11), 176.9 (C-30), 172.4 (CH_2COO), 169.2 (C-13), 128.5 (C-12), 81.1 (C-3), 61.7 (C-9), 55.0 (C-5), 51.7 ($COOCH_3$), 51.2 (CH_2COO), 49.6 (chain-1), 48.4 (C-18), 45.4 (C-14), 44.0 (C-20), 43.2 (C-8), 41.1 (C-19), 39.1 (chain-8), 38.7 (C-1), 38.1 (C-4), 37.7 (C-22), 36.9 (C-10), 33.7 (chain-7), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 30.0 (chain-3), 29.5 (chain-5), 29.4 (chain-4), 28.5 (C-29), 28.3 (C-23), 28.1 (C-28), 27.2 (chain-2), 26.8 (chain-6), 26.5 (C-16), 26.4 (C-15), 23.6 (C-2), 23.3 (C-27), 18.7 (C-26), 17.4 (C-6), 16.7 (C-24), 16.4 (C-25); MS (ESI): *m/z* (%) = 335.6 ($[M+2H]^{2+}$, 100), 669.4 ($[M + H]^+$, 84); anal. calcd for $C_{41}H_{68}N_2O_5$ (668.99): C, 73.61; H, 10.25; N, 4.19; found: C, 73.42; H, 10.39; N, 4.09.

5.8.7. Methyl 3β-3-{[N-(9-aminononyl)glycyl]oxy}-11-oxo-olean-12-en-30-oate (**10**)

Following the general procedure, **10** was obtained from 1,9-nonanediamine; yield: 410 mg, 72.6%; mp 93–97 °C; $R_F = 0.71$ (MeOH/diethylamine 9:1); $[\alpha]_D^{20} = 53.17°$ ($c = 0.48$, MeO); UV–vis (methanol): λ_{max} (log ε) = 266 nm (3.77); IR (KBr): ν = 3326br, 2925s, 2853s, 2153w, 1732 m, 1652 m, 1571s, 1489s, 1386s, 1339 m, 1312s, 1223 m, 1156 m, 986w, 878w, 817w, 770w, 746w, 720w, 686 (w) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): $\delta = 5.64$ (*s*, 1H, H-12), 4.56 (*dd*, 1H, H-3, $J = 11.8$, 4.7), 3.66 (*s*, 3H, $COOCH_3$), 3.36 (*s*, 2H, CH_2COO), 2.79 (*ddd*, 1H, H-1, $J = 13.9$, 3.4, 3.4), 2.65 (t, 2H, chain-9,

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$J = 7.0$), 2.56 (t, 2H, chain-1, $J = 7.2$), 2.34 (*s*, 1H, H-9), 2.06 (*m*, 1H, H-18), 2.00 (*m*, 1H, H-15), 1.97 (*m*, 1H, H-21), 1.92 (*m*, 1H, H-19), 1.80 (*m*, 1H, H-16), 1.70 (*m*, 1H, H-2), 1.64 (*m*, 1H, H-7), 1.62 (*m*, 1H, H-2′), 1.58 (*dd*, 1H, H-19′, $J = 13.5, 13.5$), 1.53 (*m*, 1H, H-6), 1.48 (*m*, 2H, chain-8), 1.43 (*m*, 1H, H-6′), 1.40 (*m*, 1H, H-7′), 1.38 (*m*, 1H, H-22), 1.34 (*s*, 3H, H-27), 1.30 (*m*, 1H, H-21′), 1.28–1.25 (*m*, 13H, H-22′, chain-2, chain-3, chain-4, chain-5, chain-6, chain-7), 1.19 (*m*, 1H, H-16′), 1.14 (*s*, 3H, H-25), 1.12 (*s*, 3H, H-23), 1.10 (*s*, 3H, H-26), 1.02 (*m*, 1H, H-1′), 0.98 (*m*, 1H, H-15′), 0.85 (*s*, 6H, H-24, H-28), 0.79 (*m*, 1H, H-5), 0.78 (*s*, 3H, H-29); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 200.0$ (C-11), 176.9 (C-30), 172.4 ($CH_2\underline{C}OO$), 169.2 (C-13), 128.5 (C-12), 81.1 (C-3), 61.7 (C-9), 55.0 (C-5), 51.8 ($COO\underline{C}H_3$), 51.2 ($\underline{C}H_2COO$), 49.6 (chain-1), 48.4 (C-18), 45.4 (C-14), 44.0 (C-20), 43.2 (C-8), 42.2 (chain-9), 41.1 (C-19), 38.7 (C-1), 38.1 (C-4), 37.7 (C-22), 36.9 (C-10), 33.8 (chain-8), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 30.1 (chain-3), 29.6 (chain-6), 29.5 (chain-5), 29.4 (chain-4), 28.5 (C-29), 28.3 (C-23), 28.1 (C-28), 27.2 (chain-2), 26.9 (chain-7), 26.5 (C-16), 26.4 (C-15), 23.6 (C-2), 23.3 (C-27), 18.7 (C-26), 17.4 (C-6), 16.7 (C-24), 16.4 (C-25); MS (ESI): *m/z* (%) = 342.6 ($[M+2H]^{2+}$, 100), 683.4 ($[M + H]^+$, 40); anal. calcd for $C_{42}H_{70}N_2O_5$ (638.02): C, 73.86; H, 10.33; N, 4.10; found: C, 73.65; H, 10.54; N, 4.03.

5.8.8. Methyl 3β-3-{[N-(10-aminodecl)glycyl]oxy}-11-oxo-olean-12-en-30-oate (**11**)

Following the general procedure, **11** was obtained from 1,10-decanediamine; yield: 290 mg, 41.6%; mp 82–85 °C; $R_F = 0.71$ (MeOH/diethylamine 9:1); $[\alpha]_D^{20} = 65.77°$ ($c = 0.45$, MeOH); UV–vis (methanol): λ_{max} (log ε) = 266 nm (3.83); IR (KBr): ν = 3336br, 2923s, 2852s, 1732s, 1652s, 1577m, 1466s, 1387m, 1317m, 1217m, 1154m, 1086w, 987w, 878w, 818w, 721 (w) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 5.64$ (*s*, 1H, H-12), 4.57 (*dd*, 1H, H-3, $J = 11.6, 4.8$), 3.67 (*s*, 3H, $COOC\underline{H}_3$), 3.37 (*s*, 2H, $C\underline{H}_2COO$), 2.79 (*ddd*, 1H, H-1, $J = 13.3, 3.7, 3.7$), 2.65 (t, 2H, chain-10, $J = 7.0$), 2.57 (t, 2H, chain-1, $J = 7.2$), 2.34 (*s*, 1H, H-9), 2.06 (*m*, 1H, H-18), 2.00 (*m*, 1H, H-15), 1.97 (*m*, 1H, H-21), 1.90 (*m*, 1H, H-19), 1.82 (*m*, 1H, H-16), 1.70 (*m*, 1H, H-2), 1.63 (*m*, 1H, H-7), 1.62 (*m*, 1H, H-2′), 1.59 (*dd*, 1H, H-19′, $J = 13.7, 13.7$), 1.55 (*m*, 1H, H-6), 1.47 (*m*, 2H, chain-9), 1.43 (*m*, 1H, H-6′), 1.40 (*m*, 1H, H-7′), 1.38 (*m*, 1H, H-22), 1.34 (*s*, 3H, H-27), 1.30 (*m*, 1H, H-21′), 1.29–1.24 (*m*, 15H, H-22′, chain-2, chain-3, chain-4, chain-5, chain-6, chain-7, chain-8), 1.20 (*m*, 1H, H-16′), 1.14 (*s*, 3H, H-25), 1.13 (*s*, 3H, H-23), 1.10 (*s*, 3H, H-26), 1.02 (*m*, 1H, H-1′), 0.98 (*m*, 1H, H-15′), 0.86 (*s*, 6H, H-24, H-28), 0.80 (*m*, 1H, H-5), 0.78 (*s*, 3H, H-29); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 200.0$ (C-11), 176.9 (C-30), 172.5 ($CH_2\underline{C}OO$), 169.2 (C-13), 128.5 (C-12), 81.0 (C-3), 61.7 (C-9), 55.0 (C-5), 51.8 ($COO\underline{C}H_3$), 51.3 ($\underline{C}H_2COO$), 49.7 (chain-1), 48.4 (C-18), 45.4 (C-14), 44.0 (C-20), 43.2 (C-8), 42.3 (chain-10), 41.1 (C-19), 38.7 (C-1), 38.1 (C-4), 37.7 (C-22), 36.9 (C-10), 33.8 (chain-9), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 30.1 (chain-3), 29.5 (chain-7), 29.5 (chain-5), 29.5 (chain-6), 29.5 (chain-4), 28.5 (C-29), 28.3 (C-23), 28.1 (C-28), 27.2 (cahin-2), 26.9 (chain-8), 26.5 (C-16), 26.4 (C-15), 23.6 (C-2), 23.3 (C-27), 18.7 (C-26), 17.4 (C-6), 16.7 (C-24), 16.4 (C-25); MS (ESI): *m/z* (%) = 349.5 ($[M+2H]^{2+}$, 100), 697.4 ($[M + H]^+$, 22); anal. calcd for $C_{43}H_{72}ClO_5$ (697.04): C, 74.09; H, 10.41; N, 4.02; found: C, 73.86; H, 10.61; N, 3.95.

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Anhang A3

Synthesis and Antitumor Activity of Ring A-modified Glycyrrhetinic Acid Derivatives

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The pentacyclic triterpene glycyrrhetinic acid is an interesting natural product exhibiting various biological activities. Especially its ability to induce apoptosis in tumor cells is of high scientific interest. In this study we altered the lipophilicity in ring A by derivatization at positions C-2 and C-3. The consequences of these variations on the cytotoxicity were investigated applying a colorimetric sulforhodamine B assay using 8 different human tumor cell lines. An acridine orange/ethidium bromide (AO/EB) test and a trypan blue test were used to determine the extent of apoptotic activity of some of these compounds.

Key words: Glycyrrhetinic Acid, Antitumor Activity, Apoptosis

Introduction

Glycyrrhetinic acid (**1**, Fig. 1) belongs to the group of triterpenoic acids; this group of natural products shows some interesting biological activities. Most prominent members are betulinic and oleanoic acid. Both compounds were shown to be cytotoxic towards tumor cell lines in *in-vitro* [1 – 3] as well as in *in vivo* tests [4, 5]. Although **1** has a lower cytotoxicity than betulinic acid, **1** shows a similar apoptotic behavior [6 – 9]. Another advantage of **1** is its occurrence in well accessible plants; **1** can be isolated in high yields from the roots of licorice [10, 11].

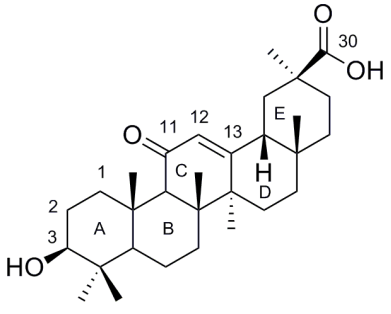

Fig. 1. Structure of glycyrrhetinic acid (**1**).

The parent structure of **1** displays three functionalities best suited for modifications: a carboxyl group in ring E at C-30, an α,β-unsaturated carbonyl function located in ring C and a hydroxyl group in ring A at position C-3. In this study several functional modifications were performed at C-2 and/or C-3 in ring A. The principal aim was to alter the lipophilicity of the molecule by these modifications and to investigate them by a sulforhodamine B assay to determine their IC_{50} values for 8 human tumor cell lines. To affirm an assumed apoptotic way of action, additional trypan blue and acridine orange/ethidium bromide (AO/EB) tests were performed.

Results and Discussion

The methyl and ethyl esters of **1** (**2** and **3**, Scheme 1) were synthesized by known procedures [9, 12]; compounds **4** – **6**, being acetylated in position O-3, were synthesized in high yields by acetylating **1** or the esters **2** and **3** with acetyl chloride in the presence of pyridine.

Jones oxidation [13] of **1**, **2** or **3** at C-2 (Scheme 2) gave ketones **7** – **9**. Compound **9** is characterized in its ^{13}C NMR spectrum by a signal at δ = 216.1 ppm (C=O). The reaction of **9** with periodic acid in DMSO [14] afforded the 3-keto-2-enol **11** as the main product; in its ^{1}H NMR spectrum 1-H is detected at δ = 7.16 ppm. In the corresponding ^{13}C NMR spectrum carbons C-1, C-2, and C-3 are found at δ = 130.9, 143.3 and 198.8 ppm, respectively. While the reaction of **9** with glacial acetic acid in the presence of *p*-tolu-

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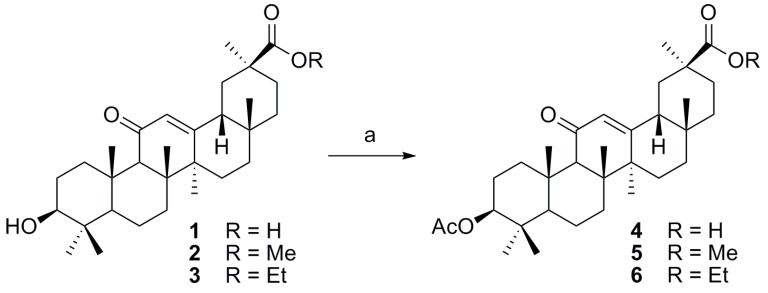
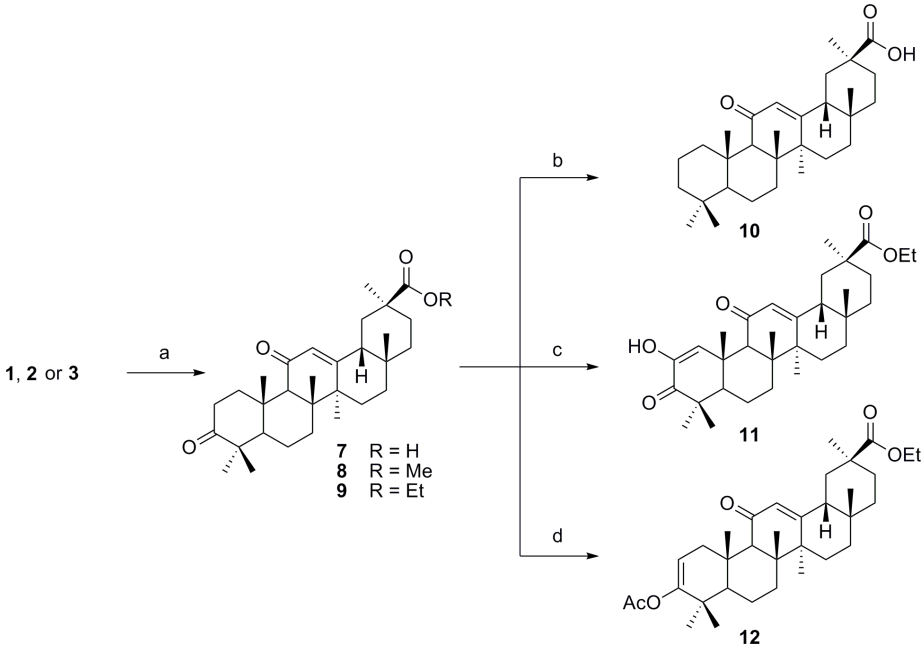


Scheme 1. a) AcCl, pyridine, CH_2Cl_2, 25 °C, 2 h, 86 – 91 %.



Scheme 2. a) Jones reagent, 25 °C, 20 – 60 min, 78 – 86 %; b) KOH, hydrazine, ethylene glycol, 200 °C, 24 h, 45 %; c) periodic acid, DMSO, 50 °C, 3 d, 15 %; d) HOAc, *p*-TsOH, 80 °C, 24 h, 59 %.

enesulfonic acid gave **12**, the reaction of **9** applying Wolff-Kishner conditions [15] afforded **10**.

The reaction of **1**, **2** or **3** (Scheme 3) with methanesulfonyl chloride in dry pyridine or triethylamine gave the corresponding mesylates **13** – **15** in excellent yields.

An elimination reaction, however, occurred upon heating of **3** or of the mesylates **13** or **14** in dry DMF in the presence of a base, and products **16** – **18** were obtained although the yields of these reactions never exceeded 50 %. Applying Mitsunobu conditions [16], however, gave **18** in 90 % isolated yield. The 2,3-epoxide **19** was obtained from **18** by reaction with *m*-CPBA in dichloromethane. Reaction of **2** with 1,1′-thiocarbonyl-diimidazole [13] gave **20** whose reaction with AIBN gave **21** in 18 % yield.

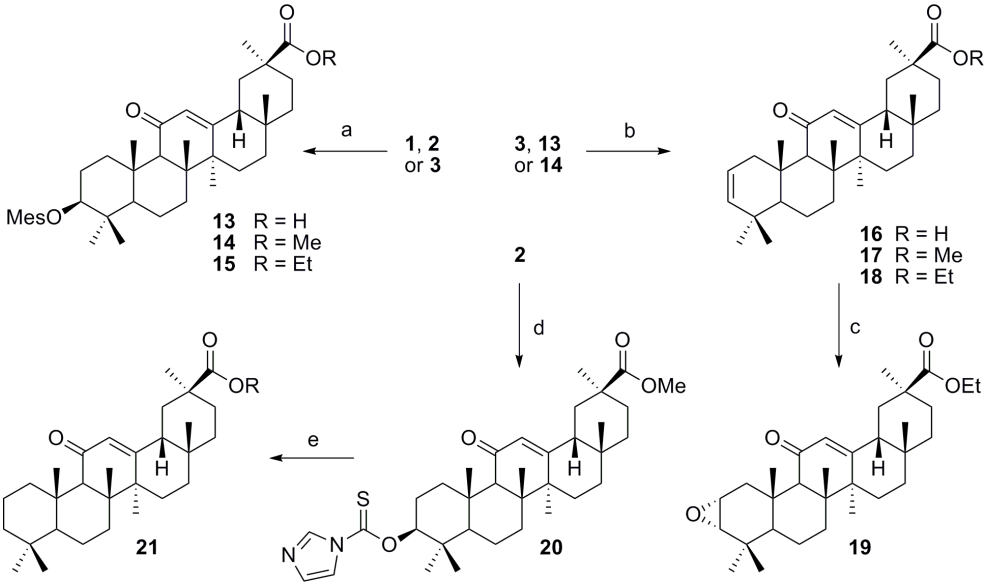
Thus, these variations in ring A afforded compounds differing significantly in lipophilicity compared to parent **1**. Although most of these compounds showed higher IC_{50} values than **1** in the corresponding SRB assays, a few of them gave IC_{50} values $< 30\ \mu$M. All acetates **4** – **6** and oxidized compounds **7** – **9** did not show any significant antitumor activity. With a few exceptions (*e. g.* **17** on A2780 cells), the mesylates **13** – **15** and deoxygenated compounds **16** – **18**, showed a similar behavior. Deoxidized **10** and **21** gave comparable results in this assay; for A2780 ovarian cancer cells, however, **21** showed a significantly higher activity than **10**. The derivatives **12** and **19** are almost inactive within the limits of our test (30 μM) whereas the cytotoxicity of **11** is comparable to that of **3**. Interestingly enough, **12** is cytotoxic especially against SW1736 human thyroid cancer cells (Table 1).

Two of these derivatives (**11** and **15**) were selected as suitable candidates to investigate their ability to induce apoptosis in A549 lung carcinoma cells using an

	518A2	8505C	A2780	A549	DLD-1	Lipo	MCF7	SW1736
1[a]	83.92	86.50	74.57	82.76	81.21	81.44	84.70	76.93
2[a]	27.54	26.07	25.54	23.50	26.12	20.47	22.14	34.82
3[a]	25.23	24.58	26.96	22.74	28.14	27.66	18.61	13.37
4 – 9	> 30	> 30	> 30	> 30	> 30	> 30	> 30	> 30
10	18.33	19.28	28.83	> 30	> 30	28.74	21.87	16.56
11	29.82	27.69	14.84	26.62	29.56	24.80	28.68	27.00
12	> 30	> 30	> 30	> 30	> 30	> 30	> 30	13.24
13	> 30	29.42	> 30	> 30	> 30	> 30	> 30	29.40
14 – 16	> 30	> 30	> 30	> 30	> 30	> 30	> 30	> 30
17	> 30	> 30	14.95	> 30	> 30	> 30	> 30	19.14
18, 19	> 30	> 30	> 30	> 30	> 30	> 30	> 30	> 30
21	23.69	24.30	10.39	> 30	> 30	25.52	> 30	16.98

Table 1. Biological activity (IC_{50} in μM) of derivatives of **1** (error: ±5 %).

[a] Data from previous studies: see ref. [9].



Scheme 3. a) $MeSO_2Cl$, pyridine (or Et_3N for **15**), 25 °C, 1 – 70 h, 94 – 99 %; b) for **16**: K_2CO_3, DMF, 120 °C, 24 h, 44 %; for **17**: Bu_4NF, DMF, 102 °C, 4 d, 51 %; for **18**: PPh_3, 3,3-dimethyl glutarimide, DEAD, THF, 25 °C, 24 h, 82 %; c) *m*-CPBA, CH_2Cl_2, 25 °C, 20 h, 77 %; d) 1,1′-thiocarbonyl-diimidazole, 1,2-dichloroethane, 100 °C, 70 h, 70 %; e) Bu_3SnH, AIBN (cat.), toluene, 115 °C, 40 h, 18 %.

acridine orange/ethidium bromide (AO/EB) and a colorimetric trypan blue test. A quantitative trypan blue test gave evidence that 82 % of the dead cells previously treated with compound **11** (35 μM) had undergone apoptosis, compared to only 47 % of the cells treated with **15** (40 μM).

In this study we were able to synthesize a series of glycyrrhetinic acid derivatives differing in ring A. In summary, acetylated derivatives exhibited a lower cytotoxicity than the parent compound. This is in excellent agreement with previous findings for the corresponding betulinic or ursolic acid derivatives [17 – 19]. A similar trend was observed for oxidized derivatives **7 – 9** when compared to the corresponding analogs in the ursolic or oleanoic acid series [19], but a different behavior was established [20, 21] for betulinic acid derivatives.

Usually esters of triterpenoic acids are more cytotoxic than the corresponding acids [9] but this seems to be not necessarily true for all derivatives of **1**. Apoptotic behavior, however, is retained by-and-large in this series of compounds despite the changes in the substitution pattern in ring A.

Experimental Section

General methods

Reagents were bought from commercial suppliers and used without any further purification. NMR spectra were measured on Varian Gemini 200, Gemini 2000 or Unity 500

spectrometers at 27 °C with tetramethylsilane as an internal standard, δ values are given in ppm and J in Hz. Mass spectra were taken on a Finnigan MAT TSQ 7000 instrument (electron spray, voltage 4.5 kV, sheath gas nitrogen). IR spectra were recorded on a Perkin-Elmer FT-IR spectrometer Spectrum 1000, optical rotations on a Perkin-Elmer 341 polarimeter (1 cm micro cell, 20 °C), and UV/Vis spectra on a Perkin-Elmer unit, Lambda 14. Melting points were measured with a Leica hot stage microscope and are uncorrected. Elemental analysis was done on a Foss-Heraeus Vario EL unit. TLC was performed on silica gel (Merck 5554, detection by UV absorption). Solvents were dried before use according to usual procedures. Derivatives **2** and **3** were synthesized by known procedures [9, 12].

Cell lines and culture conditions

The cell lines 518A2, 8505C, A2780, A549, DLD-1, Lipo, MCF-7 and SW1736 were included in this study. Cultures were maintained as monolayers in RPMI 1640 (PAA Laboratories, Pasching, Germany) supplemented with 10 % heat inactivated fetal bovine serum (Biochrom AG, Berlin, Germany) and penicillin / streptomycin (PAA Laboratories) at 37 °C in a humidified atmosphere of 5 % CO_2 / 95 % air.

Cytotoxicity assay [22]

The cytotoxicity of the compounds was evaluated using the sulforhodamine-B (SRB) (Sigma Aldrich) microculture colorimetric assay. In short, exponentially growing cells were seeded into 96-well plates on day 0 at the appropriate cell densities to prevent confluence of the cells during the period of experiment. After 24 h, the cells were treated with serial dilutions of the compounds (0 – 100 μM) for 96 h. The final concentration of DMSO or DMF solvent never exceeded 0.5 %, which was non-toxic to the cells. The percentages of surviving cells relative to untreated controls were determined 96 h after the beginning of drug exposure. After a 96 h treatment, the supernatant medium from the 96 well plates was discarded, and the cells were fixed with 10 % TCA. For a thorough fixation, the plates were allowed to rest at 4 °C. After fixation, the cells were washed in a strip washer. The washing was done four times with water using alternate dispensing and aspiration procedures. The plates were then dyed with 100 μL of 0.4 % SRB (sulforhodamine B) for about 20 min. After dying the plates were washed with 1 % acetic acid to remove the excess of the dye and allowed to air-dry overnight. 100 μL of 10 mM Tris base solution was added to each well, and the absorbance was measured at 570 nm (using a 96 well plate reader, Tecan Spectra, Crailsheim, Germany). The IC_{50} was estimated by linear regression between the value before and after the 50 % line is crossed in a dose-response curve.

Apoptosis test – acridine orange/ethidium bromide (AO/EB)

Apoptotic cell death was analyzed by acridine orange/ethidium bromide dye using fluorescence microscopy on A549 cells. Approx. 500 000 cells were seeded in cell culture flasks and were allowed to grow for 24 h. The medium was removed, and the loaded medium was added. After 24 – 48 h, the supernatant medium was collected and centrifuged; the pellet was suspended in phosphate-buffer saline (PBS) and centrifuged again. The liquid was removed, and the pellet was suspended in PBS. After mixing the suspension with a solution of AO/EB, analysis was performed under a fluorescence microscope. While a green fluorescence shows apoptosis, a red colored nucleus indicates necrotic cells.

Apoptosis test – trypan blue cell counting

Approx. 500 000 cells (A549) were seeded in cell culture flasks and allowed to grow for 1 d. After removing the medium, the loaded medium was introduced, and the flasks were incubated for about 24 – 48 h. The supernatant medium was collected and centrifuged, and the cell pellet was suspended in PBS and centrifuged again. Equal amounts of a Trypan blue solution (0.4 % in phosphate buffer saline, pH = 7.2) and a suspension of the pellet in PBS were mixed and put on chamber slides (invitrogen™). An automatic cell counter (invitrogen™ countess® automated cell counter) was used for counting the cells, differentiating between cells with and without an intact cell membrane.

*(3β)-3-(Acetyloxy)-11-oxo-olean-12-en-30-oic acid (**4**)*

Compound **1** (1.01 g, 2.2 mmol) was dissolved in dry dichloromethane (50 mL) containing dry pyridine (5 mL). Acetyl chloride (0.28 mL, 3.94 mmol) was added, and the mixture was stirred at r. t. for 40 h. Aqueous workup and extraction with $CHCl_3$ (3 × 30 mL) gave crude **4** whose recrystallization from methanol yielded **4** (1.01 g, 91 %) as colorless crystals. M. p. 171 – 174 °C (lit.: 175 – 177 °C [23], 310 – 313 °C [24]). – R_f = 0.50 (hexane / ethyl acetate 7 : 3). – $[\alpha]_D$ = 143.67° (c = 0.44, $CHCl_3$). – UV/Vis (methanol): $\lambda_{max}(\log \varepsilon)$ = 250 nm (4.10). – IR (KBr): ν = 3424br, 2958m, 1870m, 1729s, 1706s, 1646s, 1451w, 1383m, 1329w, 1276m, 1259m, 1210w, 1144m, 1090w, 1031w cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 5.69 (s, 1 H, 12-H), 4.49 (dd, 1 H, J = 11.6, 4.6 Hz, 3-H), 2.77 (ddd, 1 H, J = 13.7, 3.3, 3.3 Hz, 1-H), 2.34 (s, 1 H, 9-H), 2.17 (dd, 1 H, J = 14.1, 3.7 Hz, 18-H), 2.02 (s, 3 H, Ac-Me), 2.01 (m, 1 H, 15-H), 1.97 (m, 1 H, 21-H), 1.91 (m, 1 H, 19-H), 1.81 (ddd, 1 H, J = 13.7, 13.7, 4.2 Hz, 16-H), 1.69 (ddd, 1 H, J = 13.3, 13.3, 3.3 Hz, 2-H), 1.67 (m, 1 H, 7-H), 1.64 (m, 1 H, 2′-H), 1.61 (m, 1 H, 19′-H), 1.59 (m, 1 H, 6-H), 1.43 (m, 1 H, 6′-H), 1.41 (m, 1 H, 22-H), 1.39 (m, 1 H, 22′-H), 1.36 (m, 1 H, 7′-H), 1.34 (s, 3 H, 27-H), 1.30 (m, 1 H, 21′-H), 1.20 (s,

3 H, 29-H), 1.18 (m, 1 H, 16′-H), 1.14 (s, 3 H, 25-H), 1.10 (s, 3 H, 26-H), 1.04 (m, 1 H, 1′-H), 1.00 (m, 1 H, 15′-H), 0.85 (s, 6 H, 23-H and 24-H), 0.81 (s, 3 H, 28-H), 0.78 (m, 1 H, 5-H). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.3 (C-11), 181.8 (C-30), 171.0 (Ac-COO), 169.4 (C-13), 128.4 (C-12), 80.6 (C-3), 61.7 (C-9), 55.0 (C-5), 48.2 (C-18), 45.4 (C-8), 43.8 (C-20), 43.2 (C-14), 40.8 (C-19), 38.8 (C-1), 38.0 (C-4), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 30.9 (C-21), 28.5 (C-28), 28.4 (C-29), 28.0 (C-23), 26.5 (C-16), 26.4 (C-15), 23.5 (C-2), 23.3 (C-27), 21.3 (Ac-Me), 18.7 (C-26), 17.4 (C-6), 16.7 (C-24), 16.4 (C-25). – MS (ESI): m/z (%) = 513.5 (100) $[M+H]^+$, 535.5 (60) $[M+Na]^+$, 567.0 (69) $[M+MeOH+Na]^+$.

*Methyl (3β)-3-(acetyloxy)-11-oxo-olean-12-en-30-oate (**5**)*

Compound **2** (360 mg, 0.75 mmol) was dissolved in a mixture of dry dichloromethane (30 mL) and dry pyridine (2 mL), and acetylchloride (1.1 ml, 1.5 mmol) was added. After 2 h of stirring at r. t., the mixture was washed with water (30 mL). The aqueous layer was extracted with $CHCl_3$ (3 × 20 mL), and the extracts were dried (Na_2SO_4), filtered and evaporated to dryness. Recrystallization from methanol yielded **5** (340 mg, 86 %) as colorless crystals. M. p. 297 – 300 °C (lit.: 282 – 284 °C [24]). – R_f = 0.73 (hexane / ethyl acetate 7 : 3). – $[\alpha]_D$ = 145.37° (c = 0.50, $CHCl_3$). – UV/Vis (methanol): $\lambda_{max}(\log\varepsilon)$ = 249 nm (4.07). – IR (KBr): ν = 3448br, 2951s, 2875 m, 2361w, 1734s, 1654s, 1618w, 1465m, 1390m, 1366m, 1318w, 1247s, 1216m, 1190m, 1155m, 1087w, 1028m, 987m cm^{-1}. – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.66 (s, 1 H, 12-H), 4.52 (dd, 1 H, J = 11.6, 4.8 Hz, 3-H), 3.69 (s, 3 H, Me), 2.80 (ddd, 1 H, J = 13.7, 3.6, 3.6 Hz, 1-H), 2.36 (s, 1 H, 9-H), 2.08 (m, 1 H, 18-H), 2.05 (s, 3 H, Ac-Me), 2.03 (m 1 H, 15-H), 1.99 (m, 1 H, 21-H), 1.93 (ddd, 1 H, J = 13.4, 3.8, 2.6 Hz, 19-H), 1.82 (ddd, 1 H, J = 13.7, 13.7, 4.7 Hz, 16-H), 1.71 (m, 1 H, 2-H), 1.68 (m, 1 H, 7-H), 1.63 (m, 1 H, 2′-H), 1.61 (dd, 1 H, J = 13.4, 13.4 Hz, 19′-H), 1.58 (m, 1 H, 6-H), 1.46 (m, 1 H, 6′-H), 1.43 (m, 1 H, 7′-H), 1.39 (m, 1 H, 22-H), 1.36 (s, 3 H, 27-H), 1.31 (m, 2 H, 22′-H and 21′-H), 1.18 (m, 1 H, 16′-H), 1.16 (s, 3 H, 25-H), 1.15 (s, 3 H, 29-H), 1.13 (s, 3 H, 26-H), 1.06 (m, 1 H, 1′-H), 1.01 (m, 1 H, 15′-H), 0.88 (s, 6 H, 24-H and 23-H), 0.81 (s, 3 H, 28-H), 0.80 (m, 1 H, 5-H). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 176.9 (C-30), 171.0 (Ac-COO), 169.2 (C-13), 128.5 (C-12), 80.6 (C-3), 61.7 (C-9), 55.0 (C-5), 51.8 (Me), 48.4 (C-18), 45.4 (C-8), 44.0 (C-20), 43.2 (C-14), 41.1 (C-19), 38.8 (C-1), 38.0 (C-4), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-28), 28.3 (C-29), 28.0 (C-23), 26.5 (C-16), 26.4 (C-15), 23.6 (C-2), 23.3 (C-27), 21.3 (Ac-Me), 18.7 (C-26), 17.4 (C-6), 16.7 (C-24), 16.4 (C-25). – MS (ESI): m/z (%) = 527.5 (24) $[M+H]^+$, 549.5 (20) $[M+Na]^+$, 581.0 (100) $[M+MeOH+Na]^+$, 812.3 (22) $[3\ M+2Na]^{2+}$, 1053.1 (12) $[2M+H]^+$, 1075.3 (44) $[2M+Na]^+$.

*Ethyl (3β)-3-(acetyloxy)-11-oxo-olean-12-en-30-oate (**6**)*

To a solution of **3** (215 mg, 0.43 mmol) in dry dichloromethane (50 mL) and dry pyridine (5 mL), acetyl chloride (0.8 mL, 1.1 mmol) was added and the mixture stirred for 2 h at r. t. Aqueous workup followed by extraction with $CHCl_3$ (3 × 20 mL), and recrystallization from methanol gave **6** (210 mg, 90 %) as colorless crystals. M. p. 217 – 220 °C; R_f = 0.70 (hexane / ethyl acetate 7 : 3). – $[\alpha]_D$ = 135.14° (c = 0.40, $CHCl_3$). – UV/Vis (methanol): $\lambda_{max}(\log\varepsilon)$ = 250 nm (4.10). – IR (KBr): ν = 3442br, 2958s, 2874m, 1731s, 1654s, 1466w, 1390m, 1365m, 1316w, 1246s, 1215m, 1174m, 1152m, 1086w, 1027m, 1000w cm^{-1}. – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.64 (s, 1 H, 12-H), 4.51 (dd, 1 H, J = 11.6, 4.6 Hz, 3-H), 4.18 (dq, 1 H, J = 10.7, 7.2 Hz, Et-C*H*H′), 4.11 (dq, 1 H, J = 10.7, 7.2 Hz, Et-CH*H*′), 2.79 (ddd, 1 H, J = 13.5, 3.4, 3.4 Hz, 1-H), 2.35 (s, 1 H, 9-H), 2.09 (ddd, 1 H, J = 13.2, 4.1, 1.0 Hz, 18-H), 2.04 (s, 3 H, Ac-Me), 2.02 (ddd, 1 H, J = 13.5, 13.5, 4.7 Hz, 15-H), 1.98 (m, 1 H, 21-H), 1.92 (ddd, 1 H, J = 13.5, 4.1, 2.8 Hz, 19-H), 1.82 (ddd, 1 H, J = 13.5, 13.5, 4.7 Hz, 16-H), 1.71 (m, 1 H, 2-H), 1.68 (m, 1 H, 7-H), 1.62 (m, 1 H, 2′-H), 1.60 (dd, 1 H, J = 13.5, 13.5 Hz, 19′-H), 1.58 (m, 1 H, 6-H), 1.45 (m, 1 H, 6′-H), 1.40 (m, 1 H, 7′-H), 1.38 (m, 1 H, 22-H), 1.35 (s, 3 H, 27-H), 1.32 (m, 1 H, 22′-H), 1.31 (m, 1 H, 21′-H), 1.25 (t, 3 H, J = 7.2 Hz, Me), 1.17 (m, 1 H, 16′-H), 1.15 (s, 3 H, 25-H), 1.13 (s, 3 H, 29-H), 1.12 (s, 3 H, 26-H), 1.05 (ddd, 1 H, J = 13.5, 13.5, 3.8 Hz, 1-H′), 1.01 (m, 1 H, 15′-H), 0.87 (s, 6 H, 24-H and 23-H), 0.79 (s, 3 H, 28-H), 0.79 (m, 1 H, 5-H). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 176.4 (C-30), 171.0 (Ac-COO), 169.3 (C-13), 128.4 (C-12), 80.6 (C-3), 61.7 (C-9), 60.3 (Et-CH_2), 55.0 (C-5), 48.4 (C-18), 45.4 (C-8), 43.8 (C-20), 43.2 (C-14), 41.0 (C-19), 38.8 (C-1), 38.0 (C-4), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-28), 28.3 (C-29), 28.0 (C-23), 26.5 (C-16), 26.4 (C-15), 23.6 (C-2), 23.3 (C-27), 21.3 (Ac-Me), 18.7 (C-26), 17.4 (C-6), 16.6 (C-24), 16.4 (C-25), 14.3 (Me). – MS (ESI): m/z (%) = 541.6 (100) $[M+H]^+$, 563.5 (27) $[M+Na]^+$, 595.1 (59) $[M+MeOH+Na]^+$, 1081.4 (72) ($[2M+H]^+$, 1103.4 (76) $[2M+Na]^+$, 1119.2 (56) $[2M+K]^+$. – $C_{34}H_{52}O_5$ (540.77): calcd. C 75.51, H 9.69; found C 75.27, H 9.81.

*3,11-Dioxo-olean-12-en-30-oic acid (**7**)*

To a solution of **1** (600 mg, 1.3 mmol) in acetone (100 mL), CrO_3 (150 mg, 1.5 mmol) in sulfuric acid (1 M, 15 mL) was added. The mixture was stirred at r. t. for 1 h, followed by the addition of ethanol (50 mL). The precipitate was filtered off, and the filtrate was evaporated to dryness. Recrystallization from methanol afforded **7** (470 mg, 78 %) as colorless crystals. M. p. 308 – 311 °C (lit.: > 310 °C [13], 311 – 313 °C [24], 308 – 310 °C [25]). – R_f = 0.48 (hexane / ethyl acetate 7 : 3). – $[\alpha]_D$ = 172.84° (c = 0.49, $CHCl_3$). – UV/Vis (methanol): $\lambda_{max}(\log\varepsilon)$ =

250 nm (4.09). – IR (KBr): ν = 3435br, 2965s, 1728s, 1683s, 1645s, 1457w, 1386m, 1367w, 1347w, 1328w, 1279w, 1250w, 1206m, 1144s, 1110w, 1087m, 1028w cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 5.72 (s, 1 H, 12-H), 2.94 (ddd, 1 H, J = 13.3, 7.1, 4.2 Hz, 1-H), 2.61 (ddd, 1 H, J = 15.8, 7.1, 4.2 Hz, 2-H), 2.42 (s, 1 H, 9-H), 2.34 (ddd, 1 H, J = 15.8, 6.2, 4.2 Hz, 2′-H), 2.20 (dd, 1 H, J = 13.7, 3.3 Hz, 18-H), 2.02 (ddd, 1 H, J = 14.1, 14.1, 4.6 Hz, 15-H), 1.99 (m, 1 H, 21-H), 1.92 (ddd, 1 H, J = 13.7, 4.2, 2.5 Hz, 19-H), 1.84 (ddd, 1 H, J = 4.2, 13.7, 13.7 Hz, 16-H), 1.68 (ddd, 1 H, J = 11.5, 11.5, 5.5 Hz, 7-H), 1.60 (dd, 1 H, J = 13.7, 13.7 Hz, 19′-H), 1.55 (m, 1 H, 6-H), 1.51 (m, 1 H, 6′-H), 1.44 (m, 1 H, 7′-H), 1.42 (m, 1 H, 22-H), 1.41 (m, 1 H, 1′-H), 1.39 (m, 1 H, 22′-H), 1.36 (s, 3 H, 27-H), 1.35 (m, 1 H, 21′-H), 1.30 (m, 1 H, 5-H), 1.25 (s, 3 H, 25-H), 1.21 (s, 3 H, 29-H), 1.19 (m, 1 H, 16′-H), 1.15 (s, 3 H, 26-H), 1.08 (s, 3 H, 23-H), 1.04 (s, 3 H, 24-H), 1.02 (m, 1 H, 15′-H), 0.83 (s, 3 H, 28-H). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 217.2 (C-3), 199.6 (C-11), 181.9 (C-30), 169.8 (C-13), 128.4 (C-12), 61.0 (C-9), 55.4 (C-5), 48.2 (C-18), 47.7 (C-4), 45.3 (C-8), 43.8 (C-20), 43.3 (C-14), 40.9 (C-19), 39.7 (C-1), 37.7 (C-22), 36.7 (C-10), 34.2 (C-2), 32.1 (C-7), 31.9 (C-17), 30.9 (C-21), 28.5 (C-28), 28.4 (C-29), 26.5 (C-15), 26.4 (C-23), 26.4 (C-16), 23.3 (C-27), 21.4 (C-24), 18.8 (C-6), 18.5 (C-26), 15.6 (C-25). – MS (ESI): m/z (%) = 469.5 (40) $[M+H]^+$, 491.5 (3) $[M+Na]^+$, 522.9 (44) $[M+MeOH+Na]^+$, 937.3 (38) $[2M+H]^+$, 959.3 (100) $[2M+Na]^+$.

*Methyl 3,11-dioxo-olean-12-en-30-oate (**8**)*

A mixture of **7** (1.80 g, 3.84 mmol) and potassium carbonate (4.30 g, 31.1 mmol) in dry DMF (80 mL) was stirred at r. t. for 20 min. Iodomethane (3 mL, 48.2 mmol) was added, and stirring was continued for an additional 24 h. The solvent was removed under reduced pressure and the residue suspended in water (40 mL). The suspension was extracted with dichloromethane (3 × 30 mL), and the combined extracts were washed with brine (20 mL), dried (Na_2SO_4), filtered and evaporated. Recrystallization from methanol gave **8** (1.60 g, 86 %) as colorless crystals. M. p. 244 – 246 °C (lit.: 242 – 243 °C [24], 248 – 250 °C [25]). – R_f = 0.60 (hexane / ethyl acetate 7 : 3). – $[\alpha]_D$ = 172.95° (c = 0.31, $CHCl_3$). – UV/Vis (methanol): $\lambda_{max}(\log \varepsilon)$ = 249 nm (4.00). – IR (KBr): ν = 3428br, 2962s, 2874s, 1725s, 1706s, 1655s, 1616m, 1540w, 1457m, 1427m, 1386m, 1366m, 1318m, 1280m, 1245m, 1220s, 1181s, 1153s, 1110m, 1087m, 1048w, 1029m, 997m, 986m cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 5.70 (s, 1 H, 12-H), 3.69 (s, 3 H, Me), 2.97 (ddd, 1 H, J = 13.6, 7.1, 4.1 Hz, 1-H), 2.64 (ddd, 1 H, J = 18.3, 11.2, 7.1 Hz, 2-H), 2.44 (s, 1 H, 9-H), 2.36 (ddd, 1 H, J = 15.8, 6.5, 4.0 Hz, 2′-H), 2.11 (dd, 1 H, J = 13.4, 3.8 Hz, 18-H), 2.04 (ddd, 1 H, J = 13.7, 13.7, 4.3 Hz, 15-H), 2.00 (m, 1 H, 21-H), 1.93 (m, 1 H, J = 13.5, 4.2, 2.7 Hz, 19-H), 1.85 (ddd, 1 H, J = 13.4, 13.4, 4.1 Hz, 16-H), 1.68 (m, 1 H, 7-H), 1.61 (dd, 1 H, J = 13.5, 13.5 Hz, 19′-H), 1.58 (m, 1 H, 6-H), 1.55 (m, 1 H, 6′-H), 1.46 (m, 1 H, J = 12.7, 3.1, 3.1 Hz, 7′-H), 1.43 (m, 1 H, 1′-H), 1.39 (m, 1 H, 22-H), 1.37 (s, 3 H, 27-H), 1.32 (m, 2 H, 21′-H and 22′-H), 1.28 (m, 1 H, 5-H), 1.27 (s, 3 H, 25-H), 1.21 (m, 1 H, 16′-H), 1.17 (s, 3 H, 26-H), 1.15 (s, 3 H, 29-H), 1.11 (s, 3 H, 23-H), 1.07 (s, 3 H, 24-H), 1.03 (m, 1 H, 15′-H), 0.82 (s, 3 H, 28-H). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 217.0 (C-3), 199.3 (C-11), 176.8 (C-30), 169.6 (C-13), 128.5 (C-12), 61.1 (C-9), 55.5 (C-5), 51.8 (Me), 48.5 (C-18), 47.8 (C-4), 45.3 (C-8), 43.8 (C-20), 43.4 (C-14), 41.3 (C-19), 39.8 (C-1), 37.8 (C-22), 36.8 (C-10), 34.3 (C-2), 32.2 (C-7), 31.9 (C-17), 31.1 (C-21), 28.6 (C-28), 28.4 (C-29), 26.6 (C-23), 26.5 (C-15), 26.5 (C-16), 23.4 (C-27), 21.5 (C-24), 18.9 (C-6), 18.6 (C-26), 15.7 (C-25). – MS (ESI): m/z (%) = 483.5 (66) $[M+H]^+$, 505.4 (6) $[M+Na]^+$, 523.2 (16) $[M+Na+H_2O]^+$, 537.1 (98) $[M+Na+MeOH]^+$, 746.4 (26) $[3M+2Na]^{2+}$, 965.3 (44) $[2M+H]^+$, 987.3 (100) $[2M+Na]^+$.

*Ethyl 3,11-dioxo-olean-12-en-30-oate (**9**)*

Compound **3** (1.03 g, 2.1 mmol) was dissolved in acetone (180 mL), followed by the addition of CrO_3 (227 mg, 2.3 mmol) in diluted sulfuric acid (15 mL). The solution was stirred at r. t. for 70 min. Ethanol (50 mL) was added, and the precipitate was filtered off. The filtrate was evaporated to dryness, and recrystallization from methanol gave **9** (820 mg, 80 %) as colorless crystals. M. p. 138 – 142 °C; R_f = 0.63 (hexane / ethyl acetate 7 : 3). – $[\alpha]_D$ = 165.96° (c = 0.52, $CHCl_3$). – UV/Vis (methanol): $\lambda_{max}(\log \varepsilon)$ = 249 nm (4.11). – IR (KBr): ν = 3427br, 2974s, 2945s, 2866m, 1723s, 1703s, 1654s, 1616w, 1466m, 1387m, 1365w, 1324w, 1280w, 1258w, 1219m, 1175s, 1155m, 1110w, 1087m, 1030w cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 5.66 (s, 1 H, 12-H), 4.16 (dq, 1 H, J = 10.8, 7.1 Hz, Et-C*H*H′), 4.10 (dq, 1 H, J = 10.8, 7.1 Hz, Et-CH*H*′), 2.94 (ddd, 1 H, J = 13.7, 6.6, 4.2 Hz, 1-H), 2.61 (ddd, 1 H, J = 15.8, 11.2, 7.1 Hz, 2-H), 2.41 (s, 1 H, 9-H), 2.33 (ddd, 1 H, J = 15.8, 6.6, 4.2 Hz, 2′-H), 2.11 (dd, 1 H, J = 13.3, 3.7 Hz, 18-H), 2.01 (ddd, 1 H, J = 13.7, 13.7, 4.2 Hz, 15-H), 1.97 (m, 1 H, 21-H), 1.90 (m, 1 H, 19-H), 1.82 (ddd, 1 H, J = 13.7, 13.7, 4.2 Hz, 16-H), 1.65 (m, 1 H, 7-H), 1.58 (dd, 1 H, J = 13.3, 13.3 Hz, 19′-H), 1.53 (m, 1 H, 6-H), 1.51 (m, 1 H, 6′-H), 1.44 (m, 1 H, 7′-H), 1.39 (m, 1 H, 1′-H), 1.36 (m, 1 H, 22-H), 1.35 (s, 3 H, 27-H), 1.32 (m, 1 H, 22′-H), 1.30 (m, 1 H, 21′-H), 1.26 (m, 1 H, 5-H), 1.24 (s, 3 H, 25-H), 1.18 (m, 1 H, 16′-H), 1.14 (s, 3 H, 26-H), 1.12 (s, 3 H, 29-H), 1.08 (s, 3 H, 23-H), 1.04 (s, 3 H, 24-H), 1.01 (m, 1 H, 15′-H), 0.79 (s, 3 H, 28-H). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 216.1 (C-3), 199.4 (C-11), 176.3 (C-30), 169.8 (C-13), 128.4 (C-12), 61.1 (C-9), 60.3 (Et-CH_2), 55.4 (C-5), 48.4 (C-18), 47.8 (C-4), 45.2 (C-8), 43.8 (C-20), 43.3 (C-14), 41.1 (C-19), 39.7 (C-1), 37.7 (C-22), 36.7 (C-10), 34.2 (C-2), 32.1 (C-7), 31.8 (C-17), 31.1 (C-21), 28.6 (C-28), 28.3 (C-29), 26.5

(C-23), 26.4 (C-15), 26.4 (C-16), 23.3 (C-27), 21.4 (C-24), 18.8 (C-6), 18.5 (C-26), 15.6 (C-25), 14.3 (Et-Me). – MS (ESI): m/z (%) = 497.5 (100) $[M+H]^+$, 519.4 (3) $[M+Na]^+$, 551.0 (45) $[M+MeOH+Na]^+$, 767.9 (6) $[3M+2Na+H]^{2+}$, 993.3 (74) $[2M+H]^+$, 1015.3 (52) $[2M+Na]^+$. – $C_{30}H_{44}O_4$ (468.67): calcd. C 76.88, H 9.46; found C 76.66, H 9.63.

*11-Oxo-olean-12-en-30-oic acid (**10**)*

To a mixture of **9** (797 mg, 1.6 mmol) and potassium hydroxide (778 mg, 13.9 mmol) in dry diethylene glycol (20 mL), hydrazine hydrate (80 %, 1.2 mL, 18.4 mmol) was added dropwise. After 24 h of stirring at 200 °C the mixture was cooled, water (50 mL) was added, and the mixture was extracted with dichloromethane (3 × 20 mL). The combined organic layers were dried (Na_2SO_4), filtered and evaporated to dryness. Purification by chromatography (silica gel, dichloromethane / methanol / ammonia 90 : 10 : 1) gave **10** (330 mg, 45 %) as a colorless powder. M. p. 288 – 292 °C (lit.: 298 – 300 °C [13]). – R_f = 0.60 (hexane / ethyl acetate 7 : 3). – $[\alpha]_D$ = 99.56° (c = 0.47, $CHCl_3$). – UV/Vis (methanol): $\lambda_{max}(\log\varepsilon)$ = 248 nm (4.00). – IR (KBr): ν = 3425br, 2947s, 1729m, 1701s, 1662s, 1459m, 1387m, 1366w, 1216w, 1113w, 1035w cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 5.58 (d, 1 H, J = 1.7 Hz, 12-H), 2.60 (m, 1 H, 1-H), 2.30 (s, 1 H, 9-H), 2.23 (ddd, 1 H, J = 12.5, 2.9, 2.1 Hz, 18-H), 1.96 (m, 2 H, 15-H and 16-H), 1.70 (m, 1 H, 21-H), 1.65 (m, 1 H, 7-H), 1.58 (m, 1 H, 21′-H), 1.50 (m, 1 H, 22-H), 1.48 (m, 1 H, 6-H), 1.47 (m, 1 H, 16′-H), 1.46 (m, 1 H, 6′-H), 1.45 (m, 1 H, 19-H), 1.44 (m, 1 H, 7′-H), 1.43 (m, 1 H, 22′-H), 1.39 (m, 1 H, 3-H), 1.38 (m, 1 H, 19′-H), 1.36 (m, 1 H, 2-H), 1.34 (s, 3 H, 29-H), 1.32 (m, 1 H, 2′-H), 1.25 (m, 1 H, 15′-H), 1.25 (s, 3 H, 27-H), 1.19 (s, 3 H, 25-H), 1.15 (m, 1 H, 3-H′), 1.13 (s, 3 H, 26-H), 0.85 (s, 3 H, 23-H), 0.82 (s, 3 H, 28-H), 0.71 (s, 3 H, 24-H), 0.70 (m, 1 H, 5-H). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.6 (C-11), 183.1 (C-30), 165.6 (C-13), 124.1 (C-12), 60.8 (C-9), 55.8 (C-5), 45.0 (C-8), 44.1 (C-20), 42.3 (C-14), 42.0 (C-3), 40.9 (C-1), 40.4 (C-18), 37.6 (C-22), 37.1 (C-10), 35.9 (C-19), 35.5 (C-4), 33.8 (C-7), 33.5 (C-23), 33.3 (C-17), 31.6 (C-21), 28.3 (C-16), 26.6 (C-15), 21.9 (C-28), 20.7 (C-29), 20.5 (C-27), 18.6 (C-26), 18.4 (C-2), 17.8 (C-6), 16.4 (C-25), 16.0 (C-24). – MS (ESI): m/z (%) = 455.5 (16) ($[M+H]^+$, 495.2 (6) $[M+H_2O+Na]^+$, 509.1 (10) $[M+MeOH+Na]^+$, 909.4 (100) $[2M+H]^+$, 931.4 (40) $[2M+Na]^+$, 947.3 (6) $[2M+K]^+$.

*Ethyl 2-hydroxy-3,11-dioxo-olean-1,12-dien-30-oate (**11**)*

To a solution of **9** (1.07 g, 2.10 mmol) in dry DMSO (25 mL), periodic acid (580 mg, 3.3 mmol) was added. The mixture was stirred at 50 °C for 3 d and then poured into water (30 mL). The aqueous layer was extracted with dichloromethane (3 × 20 mL), and the combined extracts were dried (Na_2SO_4), filtered and evaporated. Purification by chromatography (silica gel, chloroform / ether 9 : 1) gave **11** (160 mg, 15 %) as a slightly yellow powder. M. p. 140 – 144 °C (decomp.). – R_f = 0.23 (hexane / ethyl acetate 7 : 3). – $[\alpha]_D$ = 193.38° (c = 0.30, $CHCl_3$). – UV/Vis (methanol): $\lambda_{max}(\log\varepsilon)$ = 253 nm (4.12). – IR (KBr): ν = 3438br, 2977s, 2361w, 1726s, 1660s, 1465m, 1387m, 1314w, 1282w, 1217m, 1153m, 1087w, 1058m, 1030w, 879m, 756s cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 7.16 (s, 1 H, 1-H), 5.73 (s, 1 H, 12-H), 4.19 (dq, 1 H, J = 10.8, 7.2 Hz, Et-C*H*H′), 4.15 (dq, 1 H, J = 10.8, 7.2 Hz, Et-CH*H*′), 2.73 (s, 1 H, 9-H), 2.15 (dd, 1 H, J = 13.0, 4.1 Hz, 18-H), 2.05 (ddd, 1 H, J = 13.4, 13.4, 4.1 Hz, 15-H), 2.01 (m, 1 H, 21-H), 1.93 (ddd, 1 H, J = 13.5, 4.0, 2.7 Hz, 19-H), 1.85 (ddd, 1 H, J = 13.5, 13.5, 4.5 Hz, 16-H), 1.73 (m, 1 H, 7-H), 1.61 (dd, 1 H, J = 13.5, 13.5 Hz, 19′-H), 1.60 (m, 1 H, 6-H), 1.59 (m, 1 H, 5-H), 1.49 (m, 1 H, 6′-H), 1.48 (s, 3 H, 26-H), 1.43 (m, 1 H, 7′-H), 1.39 (m, 1 H, 22-H), 1.38 (s, 3 H, 27-H), 1.34 (m, 2 H, 22′-H and 21′-H), 1.28 (t, 3 H, J = 7.1 Hz, Me), 1.24 (s, 3 H, 29-H), 1.19 (m, 1 H, 16′-H), 1.18 (s, 3 H, 25-H), 1.15 (s, 3 H, 23-H), 1.15 (s, 3 H, 24-H), 1.04 (m, 1 H, 15′-H), 0.83 (s, 3 H, 28-H). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.4 (C-11), 198.8 (C-3), 176.3 (C-30), 170.3 (C-13), 143.3 (C-2), 130.9 (C-1), 128.1 (C-12), 60.3 (Et-CH_2), 56.9 (C-9), 53.2 (C-5), 48.5 (C-18), 44.0 (C-4), 45.5 (C-8), 43.8 (C-20), 43.5 (C-14), 41.1 (C-19), 37.8 (C-22), 37.7 (C-10), 32.2 (C-7), 31.8 (C-17), 31.1 (C-21), 28.6 (C-28), 28.3 (C-23), 26.9 (C-29), 26.5 (C-15), 26.3 (C-16), 23.4 (C-27), 21.7 (C-24), 20.8 (C-26), 19.0 (C-25), 18.0 (C-6), 14.3 (Et-Me). – MS (ESI): m/z (%) = 511.3 (100) $[M+H]^+$, 533.3 (18) $[M+Na]^+$, 564.7 (46) $[M+MeOH+Na]^+$. – $C_{32}H_{46}O_5$ (510.70): calcd. C 75.26, H 9.08; found C 75.98, H 9.24.

*Ethyl (3β)-3-(acetyloxy)-11-oxo-olean-2,12-dien-30-oate (**12**)*

Compound **9** (990 mg, 1.99 mmol) was dissolved in glacial acetic acid (15 mL) and heated to 80 °C. *p*-TsOH (40 mg, 0.23 mmol) was added, and stirring was continued for an additional 24 h. The mixture was cooled and diluted with dichloromethane (30 mL). After washing with water (20 mL), the aqueous layer was extracted with dichloromethane (3 × 30 mL), and the extracts were dried (Na_2SO_4), filtered and evaporated to dryness. Chromatographic purification (silica gel, chloroform / ether 9 : 1) afforded **12** (630 mg, 59 %) as a colorless powder. M. p. 225 – 228 °C; R_f = 0.73 (hexane / ethyl acetate 7 : 3). – $[\alpha]_D$ = 191.71° (c = 0.36, $CHCl_3$). – UV/Vis (methanol): $\lambda_{max}(\log\varepsilon)$ = 249 nm (4.08). – IR (KBr): ν = 3431br, 2977s, 2874s, 2840m, 1751s, 1725s, 1657s, 1623m, 1457m, 1388s, 1364s, 1325m, 1281m, 1259s, 1217s, 1160s, 1101m, 1083s, 1030m, 980m cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 5.67 (s, 1 H, 12-H), 5.16 (dd, 1 H, J = 6.8, 1.9 Hz, 2-H), 4.17 (dq, 1 H, J = 10.8, 7.1 Hz, Et-C*H*H′), 4.13 (dq, 1 H, J = 10.8, 7.1 Hz, Et-CH*H*′), 3.20 (dd, 1 H, J = 17.4, 6.8 Hz, 1-H), 2.42 (s, 1 H, 9-H), 2.14 (s, 3 H, Ac-Me), 2.12 (m, 1 H,

18-H), 2.03 (ddd, 1 H, J = 13.6, 13.6, 4.7 Hz, 15-H), 1.98 (m, 1 H, 21-H), 1.92 (ddd, 1 H, J = 13.6, 4.2, 2.5 Hz, 19-H), 1.85 (m, 1 H, 1′-H), 1.83 (ddd, 1 H, J = 13.0, 13.0, 5.0 Hz, 16-H), 1.67 (m, 1 H, 7-H), 1.60 (dd, 1 H, J = 13.5, 13.5 Hz, 19′-H), 1.53 (m, 1 H, 6-H), 1.47 (m, 1 H, 6′-H), 1.44 (m, 1 H, 7′-H), 1.37 (m, 1 H, 22-H), 1.35 (s, 3 H, 27-H), 1.32 (m, 2 H, 21′-H and 22′-H), 1.26 (t, 3 H, J = 7.1 Hz, Me), 1.22 (s, 3 H, 25-H), 1.21 (m, 1 H, 16′-H), 1.15 (s, 3 H, 26-H), 1.13 (s, 3 H, 29-H), 1.03 (s, 3 H, 23-H), 1.01 (m, 1 H, 15′-H), 0.95 (s, 3 H, 23-H), 0.81 (s, 3 H, 28-H). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.6 (C-11), 176.3 (C-30), 169.8 (Ac-COO), 169.6 (C-13), 151.3 (C-3), 128.5 (C-12), 112.4 (C-2), 60.3 (C-9), 60.3 (Et-CH_2), 52.5 (C-5), 48.4 (C-18), 45.0 (C-8), 43.8 (C-20), 43.3 (C-14), 41.2 (C-19), 40.1 (C-1), 37.7 (C-22), 37.4 (C-4), 36.1 (C-10), 31.8 (C-7), 31.8 (C-17), 31.1 (C-21), 28.6 (C-28), 28.3 (C-29), 27.8 (C-23), 26.5 (C-16), 26.4 (C-15), 23.2 (C-27), 21.0 (Ac-Me), 19.8 (C-24), 18.6 (C-6), 18.2 (C-26), 16.0 (C-25), 14.3 (Et-Me). – MS (ESI): m/z (%) = 539.5 (88) $[M+H]^+$, 593.0 (100) $[M+Na+MeOH]^+$, 831.5 (10) $[3M+2Na+H]^+$, 1099.2 (42) $[2M+Na]^+$. – $C_{34}H_{50}O_5$ (538.76): calcd. C 75.80, H 9.35; found C 75.62, H 9.55.

*(3β)-3[(Methylsulfonyl)oxy]-11-oxo-olean-12-en-30-oic acid (**13**)*

Compound **1** (527 mg, 1.2 mmol) was dissolved in dry pyridine (15 mL), methanesulfonyl chloride (120 μL, 1.6 mmol) was added, and the mixture was stirred at r. t. for 70 h. Usual workup gave **13** (600 mg, 99 %) as a slightly yellow powder. M. p. 156 – 158 °C; R_f = 0.37 (hexane / ethyl acetate 7 : 3). – $[\alpha]_D$ = 118.89° (c = 0.48, $CHCl_3$). – UV/Vis (methanol): $\lambda_{max}(\log\varepsilon)$ = 250 nm (4.13). – IR (KBr): ν = 3427br, 2955s, 1792w, 1707m, 1653s, 1467m, 1389m, 1334s, 1263w, 1209m, 1172s, 1088w, 1016w, 984w, 932m, 914s, 881m, 836w, 753m cm^{-1}. – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.69 (s, 1 H, 12-H), 4.36 (dd, 1 H, J = 11.9, 4.9 Hz, 3-H), 3.00 (s, 3 H, Mes-Me), 2.85 (ddd, 1 H, J = 13.9, 3.2, 3.2 Hz, 1-H), 2.32 (s, 1 H, 9-H), 2.18 (dd, 1 H, J = 13.6, 3.5 Hz, 18-H), 2.03 (ddd, 1 H, J = 13.4, 13.4, 4.3 Hz, 15-H), 1.99 (m, 1 H, 21-H), 1.94 (m, 1 H, 19-H), 1.87 (m, 1 H, 2-H), 1.82 (ddd, 1 H, J = 13.3, 13.3, 4.0 Hz, 16-H), 1.65 (m, 1 H, 2-H), 1.64 (m, 1 H, 7-H), 1.60 (dd, 1 H, J = 13.6, 13.6, 19′-H), 1.59 (m, 1 H, 2′-H), 1.48 (m, 1 H, 22-H), 1.45 (m, 1 H, 6-H), 1.41 (m, 1 H, 6′-H), 1.40 (m, 1 H, 22′-H), 1.35 (s, 3 H, 27-H), 1.20 (m, 1 H, 7′-H), 1.19 (m, 1 H, 21′-H), 1.15 (s, 3 H, 29-H), 1.14 (m, 1 H, 16′-H), 1.11 (s, 3 H, 25-H), 1.03 (s, 3 H, 26-H), 1.02 (m, 2 H, 1′-H and 15′-H), 0.87 (s, 6 H, 23-H and 24-H), 0.82 (s, 3 H, 28-H), 0.79 (m, 1 H, 5-H). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 181.7 (C-30), 169.7 (C-13), 128.3 (C-12), 90.2 (C-3), 61.5 (C-9), 55.2 (C-5), 48.2 (C-18), 45.4 (C-8), 43.8 (C-20), 43.2 (C-14), 40.8 (C-19), 39.0 (C-4), 39.0 (C-1), 38.8 (Mes-Me), 37.7 (C-22), 36.7 (C-10), 32.6 (C-7), 31.8 (C-17), 30.9 (C-21), 28.5 (C-28), 28.4 (C-29), 28.2 (C-23), 26.4 (C-16), 26.3 (C-15), 25.0 (C-2), 23.3 (C-27), 18.6 (C-26), 17.5 (C-6), 16.4 (C-24), 16.3 (C-25). – MS (ESI): m/z (%) = 549.5 (20) $[M+H]^+$, 571.2 (100) $[M+Na]^+$, 845.9 (24) $[3M+2Na]^{2+}$, 1097.2 (38) $[2M+H]^+$, 1119.7 (62) $[2M+Na]^+$. – $C_{32}H_{50}O_6S$ (562.80): calcd. C 68.29, H 8.95; found C 69.99, H 9.01.

*Methyl (3β)-3[(methylsulfonyl)oxy]-11-oxo-olean-12-en-30-oate (**14**)*

Following the procedure given for the preparation of **13**, from **2** (522 mg, 1.1 mmol), dry pyridine (15 mL) and methanesulfonyl chloride (126 μL, 1.1 mmol) **14** (623 mg, 96 %) was obtained as a colorless powder. M. p. 165 – 168 °C (lit.: 169 – 170 °C [26]). – R_f = 0.50 (hexane / ethyl acetate 7 : 3). – $[\alpha]_D$ = 123.20° (c = 0.57, $CHCl_3$). – UV/Vis (methanol): $\lambda_{max}(\log\varepsilon)$ = 250 nm (4.06). – IR (KBr): ν = 3428br, 2953s, 2874m, 1724s, 1651s, 1618w, 1467m, 1413w, 1389m, 1358s, 1325m, 1277w, 1264w, 1247w, 1222m, 1192m, 1174s, 1089w, 1030w, 931m, 879s, 838m cm^{-1}. – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.66 (s, 1 H, 12-H), 4.35 (dd, 1 H, J = 11.8, 5.0 Hz, 3-H), 3.67 (s, 3 H, Me) 3.00 (s, 3 H, Mes-Me), 2.85 (ddd, 1 H, J = 13.9, 3.2, 3.2 Hz, 1-H), 2.33 (s, 1 H, 9-H), 2.06 (dd, 1 H, J = 13.6, 3.5 Hz, 18-H), 2.02 (ddd, 1 H, J = 13.4, 13.4, 4.3 Hz, 15-H), 1.99 (m, 1 H, 21-H), 1.94 (m, 1 H, 19-H), 1.87 (m, 1 H, 2-H), 1.82 (ddd, 1 H, J = 13.3, 13.3, 4.0 Hz, 16-H), 1.65 (m, 1 H, 2-H), 1.64 (m, 1 H, 7-H), 1.60 (dd, 1 H, J = 13.6, 13.6 Hz, 19′-H), 1.59 (m, 1 H, 2′-H), 1.48 (m, 1 H, 22-H), 1.46 (m, 1 H, 6-H), 1.43 (m, 1 H, 6′-H), 1.40 (m, 1 H, 22′-H), 1.34 (s, 3 H, 27-H), 1.20 (m, 1 H, 7′-H), 1.19 (m, 1 H, 21′-H), 1.15 (s, 3 H, 29-H), 1.13 (m, 1 H, 16′-H), 1.11 (s, 3 H, 25-H), 1.03 (s, 3 H, 26-H), 1.02 (m, 2 H, 1′-H and 15-H), 0.87 (s, 6 H, 23-H and 24-H), 0.79 (s, 3 H, 28-H), 0.79 (m, 1 H, 5-H). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.5 (C-11), 176.7 (C-30), 169.2 (C-13), 128.4 (C-12), 90.2 (C-3), 61.7 (C-9), 55.5 (C-5), 51.8 (Me), 48.6 (C-18), 45.5 (C-8), 44.2 (C-20), 43.4 (C-14), 41.3 (C-19), 39.2 (Mes-Me), 39.0 (C-4), 39.0 (C-1), 37.9 (C-22), 37.0 (C-10), 32.8 (C-7), 32.0 (C-17), 31.3 (C-21), 28.7 (C-28), 28.4 (C-29), 28.4 (C-23), 26.7 (C-16), 26.6 (C-15), 25.2 (C-2), 23.5 (C-27), 18.9 (C-26), 17.8 (C-6), 16.5 (C-24), 16.5 (C-25). – MS (ESI): m/z (%) = 563.5 (38) $[M+H]^+$, 585.1 (100) $[M+Na]^+$, 601.1 (7) $[M+K]^+$, 863.8 (6) $[3M+K+2H]^{2+}$, 866.3 (14) $[3M+2Na]^{2+}$, 1125.1 (32) $[2M+H]^+$, 1147.1 (68) $[2M+Na]^+$, 1162.9 (10) $[2M+K]^+$.

*Ethyl (3β)-3[(methylsulfonyl)oxy]-11-oxo-olean-12-en-30-oate (**15**)*

Following the procedure given for the preparation of **13**, from **3** (1.41 g, 2.8 mmol), triethylamine (1.5 mL, 10.8 mmol) and methanesulfonyl chloride (340 μL, 4.4 mmol) **15** (1.50 g, 94 %) was obtained as a colorless powder. M. p. 172 – 175 °C; R_f = 0.51 (hexane / ethyl ac-

etate 7 : 3). – $[\alpha]_D$ = 129.76° (c = 0.48, $CHCl_3$). – UV/Vis (methanol): $\lambda_{max}(\log\varepsilon)$ = 250 nm (4.04). – IR (KBr): ν = 3422br, 2958s, 2875m, 1718s, 1652s, 1466w, 1389m, 1361s, 1276w, 1247w, 1219m, 1175s, 1155m, 1088w, 1031w, 931m, 913s, 880m cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 5.64 (s, 1 H, 12-H), 4.37 (dd, 1 H, J = 11.8, 4.9 Hz, 3-H), 4.18 (dq, 1 H, J = 10.8, 7.1 Hz, Et-C*H*H′), 4.11 (dq, 1 H, J = 10.8, 7.1 Hz, Et-CH*H*'), 3.01 (s, 3 H, Mes-Me), 2.86 (ddd, 1 H, J = 13.9, 3.7, 3.7 Hz, 1-H), 2.33 (s, 1 H, 9-H), 2.10 (ddd, 1 H, J = 13.7, 4.2, 1.1 Hz, 18-H), 2.02 (ddd, 1 H, J = 13.9, 13.9, 4.2 Hz, 15-H), 1.99 (m, 1 H, 21-H), 1.97 (m, 1 H, 22-H), 1.92 (m, 1 H, 19-H), 1.89 (m, 1 H, 22′-H), 1.82 (ddd, 1 H, J = 13.9, 13.9, 4.9 Hz, 16-H), 1.66 (m, 1 H, 7-H), 1.60 (dd, 1 H, J = 13.5, 13.5 Hz, 19′-H), 1.60 (m, 1 H, 6-H), 1.47 (m, 1 H, 6′-H), 1.41 (ddd, 1 H, J = 12.8, 2.7, 2.7 Hz, 7′-H), 1.38 (m, 1 H, 22-H), 1.35 (s, 3 H, 27-H), 1.32 (m, 1 H, 22′-H), 1.31 (m, 1 H, 21′-H), 1.25 (t, 3 H, J = 7.1 Hz, Me), 1.17 (m, 1 H, 16′-H), 1.16 (s, 3 H, 25-H), 1.13 (s, 3 H, 29-H), 1.12 (s, 3 H, 26-H), 1.04 (s, 3 H, 23-H), 1.03 (m, 1 H, 1′-H), 0.97 (m, 1 H, 15′-H), 0.88 (s, 3 H, 24-H), 0.80 (m, 1 H, 5-H), 0.80 (s, 3 H, 28-H). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.7 (C-11), 176.3 (C-30), 169.5 (C-13), 128.4 (C-12), 90.1 (C-3), 61.6 (C-9), 60.3 (Et-CH_2), 55.3 (C-5), 48.4 (C-18), 45.3 (C-8), 43.8 (C-20), 43.2 (C-14), 41.1 (C-19), 39.0 (Mes-Me), 38.8 (C-1 and C-4), 37.7 (C-22), 36.7 (C-10), 32.6 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-28), 28.3 (C-29), 28.2 (C-23), 26.5 (C-16), 26.4 (C-15), 25.0 (C-2), 23.3 (C-27), 18.6 (C-26), 17.6 (C-6), 16.3 (C-24), 16.3 (C-25), 14.3 (Me). – MS (ESI): m/z (%) = 577.4 (50) $[M+H]^+$, 599.1 (100) $[M+Na]^+$, 615.1 (4) $[M+K]^+$, 887.8 (6) $[3\,M+2Na]^{2+}$, 1153.1 (18) $[2M+H]^+$, 1176.0 (17) $[2M+Na]^+$, 1191.1 (2) $[2M+K]^+$. – $C_{33}H_{52}O_6S$ (576.83): calcd. C 75.26, H 9.08; found C 75.06, H 9.23.

*11-Oxo-olean-2,12-dien-30-oic acid (**16**)*

A mixture of **13** (450 mg, 1.0 mmol) and potassium carbonate (152 mg, 1.1 mmol) in dry DMF (10 mL) was stirred at 120 °C for 24 h. After removal of the solvent, aqueous work-up followed by extraction with $CHCl_3$ (3 × 15 mL) and chromatography (silica gel, hexane / ethyl acetate 8 : 2), **16** (200 mg, 44 %) was obtained as colorless crystals. M. p. 292 – 295 °C (lit.: 290 – 296 °C [13]). – R_f = 0.50 (hexane / ethyl acetate 7 : 3). – $[\alpha]_D$ = 209.79° (c = 0.66, $CHCl_3$). – UV/Vis (methanol): $\lambda_{max}(\log\varepsilon)$ = 250 nm (4.08). – IR (KBr): ν = 3432br, 2950s, 2362w, 1699s, 1656s, 1458m, 1386m, 1361w, 1328m, 1282w, 1260m, 1232m, 1209m, 1176m, 1088w, 1020w cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 5.74 (s, 1 H, 12-H), 5.42 (ddd, 1 H, J = 9.8, 6.1, 1.7, 2-H), 5.36 (dd, 1 H, J = 9.9, 2.1 Hz, 3-H), 3.04 (dd, 1 H, J = 17.5, 6.1 Hz, 1-H), 2.42 (s, 1 H, 9-H), 2.20 (dd, 1 H, J = 12.8, 3.5 Hz, 18-H), 2.03 (m, 1 H, 15-H), 2.00 (m, 1 H, 21-H), 1.94 (ddd, 1 H, J = 13.4, 4.1, 2.6 Hz, 19-H), 1.84 (ddd, 1 H, J = 13.7, 13.7, 4.1 Hz, 16-H), 1.68 (ddd, H, J = 12.7, 3.7, 3.7 Hz, 7-H), 1.62 (dd, 1 H, J = 13.4, 13.4 Hz, 19′-H), 1.56 (m, 1 H, 6-H), 1.49 (m, 1 H, 6′-H), 1.44 (m, 1 H, 22-H), 1.42 (m, 1 H, 7′-H), 1.40 (m, 1 H, 22′-H), 1.36 (s, 3 H, 27-H), 1.22 (s, 3 H, 29-H), 1.22 (m, 1 H, 16′-H), 1.22 (m, 1 H, 21′-H), 1.16 (s, 3 H, 25-H), 1.16 (s, 3 H, 26-H), 1.11 (dd, 1 H, J = 11.9, 2.4 Hz, 5-H), 1.03 (m, 1 H, 15′-H), 0.96 (s, 3 H, 23-H), 0.91 (s, 3 H, 24-H), 0.85 (s, 3 H, 28-H). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.4 (C-11), 181.9 (C-30), 169.6 (C-13), 137.0 (C-3), 128.6 (C-12), 121.9 (C-2), 60.5 (C-9), 51.8 (C-5), 48.2 (C-18), 45.4 (C-14), 43.8 (C-20), 43.3 (C-8), 41.5 (C-1), 40.9 (C-19), 37.7 (C-22), 36.2 (C-4), 34.3 (C-17), 31.9 (C-7), 31.9 (C-23), 31.8 (C-10), 30.9 (C-21), 28.6 (C-28), 28.4 (C-29), 26.5 (C-15), 26.4 (C-16), 23.3 (C-27), 23.0 (C-24), 18.7 (C-6), 18.3 (C-26), 16.1 (C-25). – MS (ESI): m/z (%) = 453.5 (35) $[M+H]^+$, 475.4 (8) $[M+Na]^+$, 507.0 (100) $[M+MeOH+Na]^+$, 905.3 (18) $[2M+H]^+$, 927.3 (40) $[2M+Na]^+$.

*Methyl 11-oxo-olean-2,12-dien-30-oate (**17**)*

To a solution of **14** (219 mg, 0.39 mmol) in dry DMF (10 mL), tetrabutyl ammonium fluoride trihydrate (163 mg, 0.39 mmol) was added. After 4 d of stirring at between 100 – 105 °C, the solvent was removed under reduced pressure, and the residue was subjected to chromatography (silica gel, hexane / ethyl acetate 8 : 2) to yield **16** (92 mg, 51 %) as a colorless powder. M. p. 220 – 222 °C (lit.: 224 – 228 °C [13]). – R_f = 0.81 (hexane / ethyl acetate 7 : 3). – $[\alpha]_D$ = 209.85° (c = 0.33, $CHCl_3$). – UV/Vis (methanol): $\lambda_{max}(\log\varepsilon)$ = 248 nm (4.06). – IR (KBr): ν = 3438br, 2956s, 1728s, 1655s, 1617w, 1465m, 1385m, 1360w, 1328w, 1279w, 1259w, 1217m, 1156s, 1089w, 1029w cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 5.67 (s, 1 H, 12-H), 5.42 (ddd, 1 H, J = 10.0, 5.8, 1.7 Hz, 2-H), 5.35 (dd, 1 H, J = 10.0, 2.1 Hz, 3-H), 3.67 (s, 1 H, Me), 3.03 (dd, 1 H, J = 17.4, 5.8 Hz, 1-H), 2.40 (s, 1 H, 9-H), 2.08 (ddd, 1 H, J = 13.7, 3.7, 1.3 Hz, 18-H), 2.01 (ddd, 1 H, J = 13.7, 13.7, 4.2 Hz, 15-H), 1.98 (m, 1 H, 21-H), 1.91 (ddd, 1 H, J = 13.7, 4.2, 2.5 Hz, 19-H), 1.82 (ddd, 1 H, J = 13.3, 13.3, 4.2 Hz, 16-H), 1.68 (ddd, 1 H, J = 12.0, 12.0, 3.7 Hz, 7-H), 1.64 (m, 1 H, 1′-H), 1.60 (dd, 1 H, J = 13.7, 13.7 Hz, 19′-H), 1.53 (m, 1 H, 6-H), 1.43 (m, 1 H, 6′-H), 1.41 (m, 1 H, 7′-H), 1.38 (m, 1 H, 22-H), 1.35 (s, 3 H, 27-H), 1.30 (m, 2 H, 22′-H and 21′-H), 1.22 (m, 1 H, 16′-H), 1.15 (s, 3 H, 25-H), 1.14 (s, 3 H, 26-H), 1.13 (s, 3 H, 29-H), 1.10 (m, 1 H, 5-H), 1.00 (m, 1 H, 15′-H), 0.95 (s, 3 H, 23-H), 0.90 (s, 3 H, 24-H), 0.80 (s, 3 H, 28-H). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.8 (C-11), 176.8 (C-30), 169.1 (C-13), 137.0 (C-3), 128.7 (C-12), 122.0 (C-2), 60.6 (C-9), 52.0 (Me), 51.8 (C-5), 48.6 (C-18), 45.5 (C-14), 44.2 (C-20), 43.4 (C-8), 41.7 (C-1), 41.4 (C-19), 38.0 (C-22), 36.4 (C-9), 34.5 (C-10), 32.1 (C-7), 32.0 (C-23), 32.0 (C-17), 31.3 (C-21), 28.7 (C-28), 28.5 (C-29), 26.7 (C-16), 26.7 (C-15), 23.5 (C-27), 23.2 (C-24), 18.9 (C-6), 18.54 (C-26), 16.3 (C-25). – MS (ESI): m/z (%) =

467.5 (100) $[M+H]^+$, 521.0 (73) $[M+MeOH+Na]^+$, 933.2 (86) $[2M+H]^+$, 955.3 (48) $[2M+Na]^+$.

*Ethyl 11-oxo-olean-2,12-dien-30-oate (**18**)*

A mixture of **3** (1.31 g, 2.6 mmol), triphenyl phosphane (2.78 g, 10.6 mmol) and 3,3-dimethyl glutarimide (1.49 g, 10.6 mmol) in dry THF (25 mL) was cooled to 0 °C. Under continuous stirring, DEAD (1.65 ml, 10.4 mmol) was added dropwise, and stirring was continued at r. t. for 24 h. After concentration to dryness, the residue was subjected to chromatography (silica gel, chloroform / ether 9 : 1) to yield **18** (1.02 g, 82 %) as colorless crystals. M. p. 138 – 142 °C; R_f = 0.87 (hexane / ethyl acetate 7 : 3). – $[\alpha]_D$ = 216.97° (c = 0.33, $CHCl_3$). – UV/Vis (methanol): $\lambda_{max}(\log\varepsilon)$ = 249 nm (4.02). – IR (KBr): ν = 3422br, 2960s, 2872s, 1723s, 1648s, 1612w, 1458m, 1386m, 1360w, 1348w, 1328w, 1310w, 1277w, 1256m, 1210m, 1169s, 1134m, 1088w, 1062w, 1031m cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 5.67 (s, 1 H, 12-H), 5.43 (ddd, 1 H, J = 10.1, 6.1, 1.7 Hz, 2-H), 5.37 (dd, 1 H, J = 10.1, 2.3, 3-H), 4.19 (dq, 1 H, J = 10.8, 7.2 Hz, Et-C*H*H′), 4.12 (dq, 1 H, J = 10.8, 7.2 Hz, Et-CH*H*′), 3.04 (dd, 1 H, J = 17.5, 6.0 Hz, 1-H), 2.41 (s, 1 H, 9-H), 2.11 (dd, 1 H, J = 12.8, 4.3 Hz, 18-H), 2.03 (ddd, 1 H, J = 13.4, 13.4, 4.7 Hz, 15-H), 1.99 (m, 1 H, 21-H), 1.92 (ddd, 1 H, J = 13.9, 4.1, 2.9 Hz, 19-H), 1.83 (ddd, 1 H, J = 13.6, 13.6, 4.3 Hz, 16-H), 1.70 (m, 1 H, 7-H), 1.65 (m, 1 H, 1′-H), 1.61 (dd, 1 H, J = 13.5, 13.5 Hz, 19′-H), 1.56 (m, 1 H, 6-H), 1.48 (ddd, 1 H, J = 12.5, 12.5, 3.2 Hz, 6′-H), 1.43 (m, 1 H, 7′-H), 1.39 (m, 1 H, 22-H), 1.33 (m, 1 H, 21′-H), 1.30 (m, 1 H, 22′-H), 1.36 (s, 3 H, 27-H), 1.26 (t, 3 H, J = 7.2 Hz, Me), 1.21 (ddd, 1 H, J = 13.9, 4.4, 2.4 Hz, 16-H′), 1.16 (s, 3 H, 25-H), 1.16 (s, 3 H, 26-H), 1.14 (s, 3 H, 29-H), 1.12 (m, 1 H, 5-H), 1.02 (m, 1 H, 15′-H), 0.96 (s, 3 H, 23-H), 0.91 (s, 3 H, 24-H), 0.82 (s, 3 H, 28-H). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.1 (C-11), 176.4 (C-30), 169.4 (C-13), 137.0 (C-3), 128.6 (C-12), 121.9 (C-2), 60.5 (C-9), 60.3 (Et-CH_2), 51.8 (C-5), 48.4 (C-18), 45.3 (C-14), 43.8 (C-20), 43.3 (C-8), 41.5 (C-1), 41.2 (C-19), 37.7 (C-22), 36.2 (C-4), 34.3 (C-10), 31.9 (C-7), 31.9 (C-23), 31.8 (C-17), 31.1 (C-21), 28.6 (C-28), 28.3 (C-29), 26.5 (C-16), 26.5 (C-15), 23.3 (C-27), 23.0 (C-24), 18.7 (C-6), 18.3 (C-26), 16.1 (C-25), 14.3 (Me). – MS (ESI): m/z (%) = 481.5 (100) $[M+H]^+$, 503.3 (7) $[M+Na]^+$, 534.9 (50) $[M+MeOH+Na]^+$, 961.3 (66) $[2M+H]^+$, 983.4 (54) $[2M+Na]^+$, 999.2 (4) $[2M+K]^+$. – $C_{32}H_{48}O_3$ (480.72): calcd. C 79.95, H 10.06; found C 79.68, H 10.18.

*Ethyl (2α,3α)-2,3-epoxy-11-oxo-olean-12-en-30-oate (**19**)*

Compound **18** (1.01 g, 2.1 mmol) was dissolved in dry dichloromethane (20 mL), *m*-CPBA (1.14 g, 4.68 mmol) was added, and the mixture was stirred at r. t. for 20 h. An aq. solution of potassium hydrogensulfate (satd., 10 mL) was added, the aqueous layer was extracted with dichloromethane (3 × 15 mL), and the combined organic layers were dried (Na_2SO_4), filtered and evaporated to dryness. Chromatographic purification (silica gel, chloroform / ether 9 : 1) afforded **19** (806 mg, 77 %) as a colorless powder. M. p. 191 – 193 °C; R_f = 0.71 (hexane / ethyl acetate 7 : 3). – $[\alpha]_D$ = 143.65° (c = 0.48, $CHCl_3$). – UV/Vis (methanol): $\lambda_{max}(\log\varepsilon)$ = 251 nm (4.09). – IR (KBr): ν = 3416br, 2978s, 2955s, 1736s, 1718s, 1645s, 1614w, 1458m, 1385m, 1314m, 1301m, 1285m, 1260m, 1222s, 1163s, 1113m, 1091m, 1039m, 1014w cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 5.63 (s, 1 H, 12-H), 4.16 (dq, 1 H, J = 10.8, 7.1 Hz, Et-C*H*H′), 4.10 (dq, 1 H, J = 10.8, 7.1 Hz, Et-CH*H*′), 3.19 (dd, 1 H, J = 6.6, 3.7 Hz, 2-H), 3.13 (dd, 1 H, J = 14.9, 6.6 Hz, 1-H), 2.79 (d, 1 H, J = 3.7 Hz, 3-H), 2.29 (s, 1 H, 9-H), 2.09 (dd, 1 H, J = 13.3, 4.2 Hz, 18-H), 1.99 (ddd, 1 H, J = 13.3, 13.3, 4.6 Hz, 15-H), 1.96 (m, 1 H, 21-H), 1.90 (ddd, 1 H, J = 13.7, 4.2, 2.9 Hz, 19-H), 1.78 (ddd, 1 H, J = 13.7, 13.7, 5.0 Hz, 16-H), 1.61 (m, 1 H, 7-H), 1.57 (dd, 1 H, J = 13.7, 13.7 Hz, 19′-H), 1.48 (m, 1 H, 6-H), 1.39 (m, 1 H, 21-H), 1.37 (m, 1 H, 6′-H), 1.35 (m, 1 H, 22-H), 1.33 (m, 1 H, 1′-H), 1.30 (s, 3 H, 27-H), 1.29 (m, 1 H, 22′-H), 1.27 (m, 1 H, 21′-H), 1.24 (t, 1 H, J = 7.1 Hz, Me), 1.17 (m, 1 H, 16′-H), 1.13 (s, 3 H, 26-H), 1.11 (s, 3 H, 28-H), 1.09 (s, 3 H, 23-H), 1.07 (s, 3 H, 25-H), 1.02 (s, 3 H, 24-H), 0.93 (m, 1 H, 15′-H), 0.92 (dd, 1 H, J = 11.6, 2.9 Hz, 5-H), 0.78 (s, 3 H, 29-H). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.7 (C-11), 176.3 (C-30), 169.7 (C-13), 128.5 (C-12), 61.3 (C-3), 60.4 (C-9), 60.3 (Et-CH_2), 52.6 (C-2), 48.4 (C-18), 46.6 (C-5), 45.1 (C-8), 43.8 (C-20), 43.3 (C-14), 41.1 (C-19), 40.6 (C-1), 37.7 (C-22), 35.9 (C-4), 32.6 (C-10), 31.9 (C-7), 31.8 (C-17), 31.1 (C-21), 28.6 (C-28), 28.3 (C-29), 28.2 (C-23), 26.4 (C-16), 26.4 (C-15), 23.2 (C-27), 22.0 (C-24), 18.3 (C-26), 17.9 (C-6), 17.9 (C-25), 14.3 (Me). – MS (ESI): m/z (%) = 497.6 (92) $[M+H]^+$, 519.4 (10) $[M+Na]^+$, 551.0 (62) $[M+MeOH+Na]^+$, 767.4 (6) $[3M+2Na]^{2+}$, 993.3 (94) $[2M+H]^+$, 1015.4 (100) $[2M+Na]^+$, 1031.3 (12) $[2M+K]^+$. – $C_{32}H_{48}O_4$ (496.72): calcd. C 77.38, H 9.74; found C 77.26, H 9.92.

*Methyl (3β)-3[(1H-imidazol-1-yl)carbonothioyl)oxy]-11-oxo-olean-12-en-30-oate (**20**)*

Compound **2** (230 mg, 0.48 mmol) and 1,1′-thiocarbonyl-diimidazole (90 %, 235 mg, 1.06 mmol) were dissolved in dry 1,2-dichloroethane (3.5 mL). The mixture was stirred at 100 °C under Ar for 70 h. After addition of cold hydrochloric acid (1 M, 10 mL), the aqueous layer was extracted with dichloromethane (3 × 10 mL), the extracts were washed with an aq. solution of sodium hydrogencarbonate, water and brine (10 mL each), dried (Na_2SO_4) and evaporated to dryness yielding **20** (200 mg, 70 %) as a colorless powder. M. p. 245 – 247 °C (lit.: 242 – 243 °C [13]). – R_f = 0.46 (hexane / ethyl acetate = 7 : 3). – $[\alpha]_D$ = 142.33° (c = 0.35, $CHCl_3$). – UV/Vis (methanol): $\lambda_{max}(\log\varepsilon)$ = 208 nm (4.38),

252 nm (4.26). – IR (KBr): ν = 3439br, 3155w, 3113w, 2945s, 2863m, 1728s, 1659s, 1622m, 1531m, 1500m, 1464s, 1387s, 1349s, 1327s, 1292s, 1234s, 1153s, 1110s, 1088m, 1038m, 1008m, 974s, 923m, 896m, 828m, 750m, cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 8.35 (s, 1 H, NCHN), 7.62 (m, 1 H, CSNC*H*CHN), 7.04 (s, 1 H, CSNCHC*H*N), 5.68 (s, 1 H, 12-H), 5.25 (dd, 1 H, *J* = 11.8, 4.9, 3-H), 3.68 (s, 3 H, Me), 2.92 (ddd, 1 H, *J* = 13.7, 3.4, 3.4 Hz, 1-H), 2.39 (s, 1 H, 9-H), 2.09 (dd, 1 H, *J* = 13.3, 3.8 Hz, 18-H), 2.02 (ddd, 1 H, *J* = 13.7, 13.7, 4.6 Hz, 15-H), 1.99 (m, 1 H, 21-H), 1.95 (m, 1 H, 2-H), 1.92 (m, 1 H, 19-H), 1.84 (m, 1 H, 16-H), 1.82 (m, 1 H, 2′-H), 1.68 (m, 1 H, 7-H), 1.64 (m, 1 H, 6-H), 1.61 (dd, 1 H, *J* = 13.3, 13.3 Hz, 19′-H), 1.60 (m, 1 H, 6′-H), 1.49 (m, 1 H, 7′), 1.44 (m, 1 H, 22-H), 1.38 (m, 1 H, 22′-H), 1.37 (s, 3 H, 27-H), 1.35 (m, 1 H, 21′-H), 1.20 (s, 3 H, 25-H), 1.19 (m, 1 H, 16-H), 1.14 (s, 3 H, 29-H), 1.14 (s, 3 H, 26-H), 1.11 (ddd, 1 H, *J* = 13.7, 13.7, 3.4 Hz, 1′-H), 1.03 (s, 3 H, 24-H), 1.02 (m, 1 H, 16′-H), 0.97 (s, 3 H, 23-H), 0.90 (dd, 1 H, *J* = 11.8, 1.5 Hz, 5-H), 0.80 (s, 3 H, 28-H). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.7 (C-11), 183.8 (CS), 176.9 (C-30), 169.4 (C-13), 130.3 (NCHN), 128.4 (C-12), 124.2 (CSNCH*C*HN), 117.8 (CSN*C*HCHN), 91.4 (C-3), 61.5 (C-9), 55.0 (C-5), 51.8 (Me), 48.4 (C-18), 45.4 (C-8), 44.0 (C-20), 43.2 (C-14), 41.1 (C-19), 38.8 (C-4), 38.5 (C-1), 37.7 (C-22), 36.9 (C-10), 32.6 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-28), 28.3 (C-29), 28.1 (C-23), 26.5 (C-16), 26.4 (C-15), 23.4 (C-27), 22.3 (C-2), 18.7 (C-26), 17.5 (C-24), 17.3 (C-6), 16.4 (C-25). – MS (ESI): m/z = 595.1 (100) $[M+H]^+$, 617.2 (28) $[M+Na]^+$, 892.7 (14) $[3M+2H]^{2+}$, 903.6 (10) $[3M+Na+H]^{2+}$, 914.7 (7) $[3M+2Na]^{2+}$, 1189.0 (14) $[2M+H]^+$, 1200.4 (10) $[4M+Na+H]^{2+}$.

*Methyl 11-oxo-olean-12-en-30-oate (**21**)*

To a solution of **20** (200 mg, 0.3 mmol) in dry toluene (15 mL), tributyltin hydride (0.45 ml, 2.2 mmol) and catalytic amounts of AIBN were added. The mixture was stirred at 115 °C for 40 h and concentrated under reduced pressure. The residue was subjected to chromatography (silica gel, hexane / ethyl acetate 9 : 1) to afford **21** (30 mg, 18 %) as a colorless powder. M. p. 215 – 218 °C (lit.: 222 – 223 °C [13]). – R_f = 0.80 (hexane / ethyl acetate = 7 : 3). – $[\alpha]_D$ = 103.98° (*c* = 0.28, $CHCl_3$). – UV/Vis (methanol): $\lambda_{max}(\log\varepsilon)$ = 249 nm (4.02). – IR (KBr): ν = 3435br, 2955s, 2284w, 1728s, 1655s, 1618w, 1465m, 1432w, 1386m, 1317w, 1278m, 1217m, 1188m, 1153m, 1087w, 1074w, 1030w cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 5.58 (s, 1 H, 12-H), 3.62 (s, 3 H, Me), 2.65 (ddd, 1 H, *J* = 13.0, 4.7, 3.0 Hz, 1-H), 2.32 (s, 1 H, 9-H), 2.00 (m, 1 H, 18-H), 1.97 (m, 1 H, 15-H), 1.94 (m, 1 H, 7-H), 1.85 (ddd, 1 H, *J* = 13.6, 4.2, 2.7 Hz, 19-H), 1.75 (ddd, 1 H, *J* = 13.4, 13.4, 4.3 Hz, 16-H), 1.62 (m, 1 H, 6-H), 1.59 (m, 1 H, 21-H), 1.55 (dd, 1 H, *J* = 13.6, 13.6 Hz, 19′-H), 1.50 (m, 2 H, 2-H and 2′-H), 1.34 (m, 1 H, 21-H), 1.32 (m, 1 H, 22-H), 1.30 (m, 1 H, 3-H), 1.29 (m, 1 H, 6′-H), 1.28 (m, 1 H, 7′-H), 1.27 (m, 1 H, 22′-H), 1.31 (s, 3 H, 27-H), 1.11 (m, 1 H, 16′-H), 1.09 (m, 1 H, 3-H′), 1.08 (s, 6 H, 25-H and 29-H), 1.06 (s, 3 H, 26-H), 0.94 (m, 1 H, 15′-H), 0.80 (s, 3 H, 23-H), 0.77 (s, 3 H, 24-H), 0.77 (m, 1 H, 1′-H), 0.74 (s, 3 H, 28-H), 0.66 (dd, 1 H, *J* = 12.3, 2.2 Hz, 5-H). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.6 (C-11), 176.9 (C-30), 168.8 (C-13), 128.6 (C-12), 61.9 (C-9), 55.7 (C-5), 51.7 (Me), 48.4 (C-18), 45.6 (C-8), 44.0 (C-20), 43.2 (C-14), 42.0 (C-3), 41.1 (C-19), 40.9 (C-1), 37.8 (C-22), 37.4 (C-10), 33.5 (C-23), 33.4 (C-4), 32.8 (C-7), 31.8 (C-17), 31.2 (C-21), 28.5 (C-28), 28.3 (C-29), 26.5 (C-16), 26.4 (C-15), 23.4 (C-27), 21.8 (C-24), 18.8 (C-26), 18.4 (C-2), 17.7 (C-6), 16.3 (C-25). – MS (ESI): m/z (%) = 469.5 (72) $[M+H]^+$, 522.9 (98) $[M+MeOH+Na]^+$, 585.5 (20) $[M+MeOH+Na]^+$, 937.3 (46) $[2M+H]^+$, 959.3 (100) $[2M+Na]^+$, 975.2 (4) $[2M+K]^+$.

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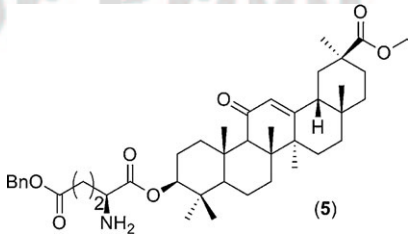
Anhang A4

Full Paper

Improvement of the Cytotoxicity and Tumor Selectivity of Glycyrrhetinic Acid by Derivatization with Bifunctional Aminoacids

R. Csuk, S. Schwarz, R. Kluge, and D. Ströhl



Various glutamyl and aspartyl substituents were selected for the synthesis of C(3) esters of GA methyl ester to improve the poor cytotoxicity of GA. A short (3-5 steps) synthesis was elaborated. Compound (**5**) having a glutamyl substituent with a benzyl protected side chain showed up to 67-fold higher cytoxicity and an up to 140-fold better selectivity towards tumor cells than parent GA. **1**

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Full Paper

Improvement of the Cytotoxicity and Tumor Selectivity of Glycyrrhetinic Acid by Derivatization with Bifunctional Aminoacids

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Glycyrrhetinic acid (GA) is a major ingredient of the dried extract of licorice roots; its antitumor activity is low compared to other members of the triterpenoic family. For example, oleanolic acid, betulin or betulinic acid are more cytotoxic with a pronounced activity for tumor cells. GA, however, is easily to earn, cheap and shows apoptotic effects on tumor cells – like the other triterpenoic acids. These facts bring GA and derivatives in the focus of our scientific interest. Here we tried to improve the poor cytotoxicity of GA by simple derivatization. Thus, we selected various glutamyl and aspartyl substituents for the synthesis of C(3) esters of GA methyl ester. A short (3-5 steps) synthesis was elaborated that allowed to access more effective compounds. One compound, methyl 3β 3-(*O*-benzyl-L-glutamyl)-11-oxo-olean-12-en-30-oate (**5**), having a glutamyl substituent with a benzyl protected side chain showed up to 67-fold higher cytoxicity and an up to 140-fold better selectivity towards tumor cells than parent GA. All compounds were evaluated by a sulforhodamine B assay as well as by a trypan blue test and extra acridine orange/ethidium bromide tests for apoptosis.

Keywords: Antitumor activity / Apoptosis / Glycyrrhetinic acid / Mouse embryonic fibroblasts

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Introduction

Searching for an effective antitumor drug does not necessarily mean to look for the most cytotoxic molecules. Although, a high toxicity is compulsory, other features – among them the ability to induce apoptosis and a high selectivity between tumor cells and normal cells – are important.

Glycyrrhetinic acid (GA), a triterpenoic acid has a proven [1–5] cytotoxic effect on tumor cells. It is also known [1–3, 6] to induce apoptosis. Because of the high content of GA (up to 24%) found in the roots of licorice (*Glycyrrhiza glabra*) [7, 8], GA is still in the focus of scientific interest. However, the cytotoxicity of GA is significantly lower [4] than those of other triterpenoic acids (for example betulinic acid, oleanolic acid, ursolic acid). In addition, GA displayed only a poor selectivity [6] towards tumor cells. Hence, our main aim was to increase the cytotoxicity and to improve the selectivity. From preliminary screenings, aspartyl and glutamyl substituted derivatives of GA methyl ester (**1**) appeared most promising. Thus, these substances were evaluated in a sulforhodamine B assay (SRB) as well as by acridine orange/ethidium bromide (AO/EB) and trypan blue tests.

Results and discussion

Four pairs of different aspartic and glutamic acid substituted GA derivatives (see Scheme 1)were investigated in a SRB assay to determine their cytotoxicity on various tumor cell lines. The protected compounds **8** and **9** did not show any significant activity on the cells in the applied range of concentrations. Derivatives **6** and **7**, however, still protected by a N-Boc group, showed lower IC_{50} values on eight tumor cell lines (cf. Table 1) than the methyl ester [6] of GA. The tumor-to-control selectivity (as estimated dividing the IC_{50} for NiH3T3 (mouse embryonic fibroblasts) by an IC_{50} value (averaged for all tumor cell lines) increased from 1.09 (for **1**) to 1.35 (for **5**) and 1.36 (for **6**).

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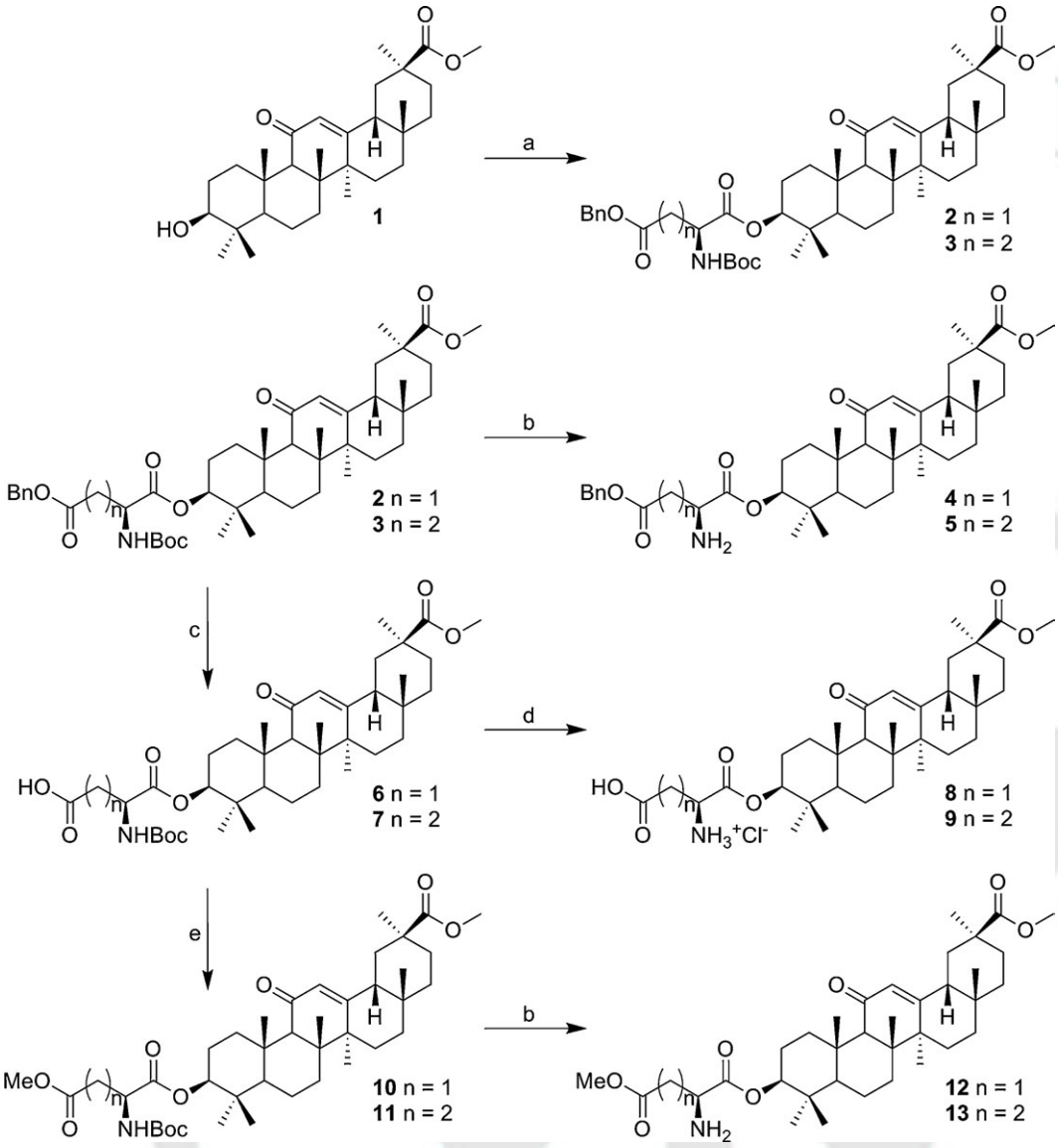

Scheme 1. Reagents and conditions: a) DCC, DMAP, Boc-Asp(OBzl)OH or Boc-Glu(OBzl)OH, DCM, 12 h, 25°C; b) TFA, DCM, 12 h, 25°C; $NH_4^+HCO_2^-$, Pd/C (10%), THF/MeOH, 12 h, 25°C; d) HCl (gas), DCM, 12 h, 25°C; e) CH_3I, K_2CO_3, DMF, 2 h, 25°C.

Compounds **4** and **5** as well as compounds **12** and **13** still carry a free amino group but a protected side chain. The two methyl esters **12** and **13** showed an insignificant activity, except for compound **12** on the human breast adenocarcinoma cell line MCF-7. Selectivity switches: again, except for the MCF-7 cells, the IC_{50} value for NiH3T3 cells is lower than for the human tumor cell lines, therefore resulting in a selectivity index of 0.55 for **12** and 0.72 for **13**.

The use of a benzyl group to protect the side chain led to compounds showing the best results in the SRB assay. The aspartic acid derivative **4** showed the same selectivity (1.35) as the Boc-protected compound **6** (with a free side chain) but an IC_{50} of 15-19 μM. For MCF-7 cells, compound **4** showed the highest activity (IC_{50} = 7.35 μM), hence doubling the selectivity towards the tumor cell line. Compound **5**, being the most active derivative of this series gave IC_{50} values between 1.27-2.33 μM. Compared to other compounds, **5** displayed an extraordinary selectivity $F = 23$ in average. For A253 human head and neck carcinoma cells a 30 times lower IC_{50} value for the tumor cells than for the NiH3T3 fibroblasts is observed.

To rule out an alleged apoptotic behavior, an AO/EB test was performed. For all of the compounds, except for **8** and **9** (that did not show a significant activity in the SRB assay) a sufficient number of cells undergoing apoptosis were

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Table 1. Results of the SRB-assay on various tumor cell lines and mouse embryonic fibroblasts (NiH3T3): Results (IC_{50}) are given in μM; F indicates the quotient of the averaged IC_{50} value (tumor cells) divided by the IC_{50} (mouse fibroblasts).

substance/cell line	4	5	6	7	8	9	12	13
518A2	10.90 ± 0.55	1.75 ± 0.09	17.19 ± 0.86	17.94 ± 0.90	>100	>100	39.24 ± 1.96	47.72 ± 2.39
8505C	12.97 ± 0.65	1.76 ± 0.09	15.82 ± 0.79	17.00 ± 0.85	>100	>100	45.36 ± 2.27	61.57 ± 3.08
A253	7.99 ± 0.40	1.28 ± 0.06	15.07 ± 0.75	13.80 ± 0.69	>100	>100	30.47 ± 1.52	53.07 ± 2.65
A2780	8.84 ± 0.44	1.65 ± 0.08	17.29 ± 0.86	18.24 ± 0.91	>100	>100	22.44 ± 1.12	29.19 ± 1.46
A549	10.94 ± 0.55	1.77 ± 0.09	19.82 ± 0.99	21.20 ± 1.06	>100	>100	31.59 ± 1.58	60.96 ± 3.05
Lipo	11.35 ± 0.57	1.74 ± 0.09	16.67 ± 0.83	18.78 ± 0.94	>100	>100	40.62 ± 2.03	54.77 ± 2.74
MCF-7	7.35 ± 0.36	1.27 ± 0.06	17.47 ± 0.87	16.96 ± 0.85	>100	>100	16.89 ± 0.84	29.26 ± 1.46
SW1736	16.68 ± 0.83	2.33 ± 0.12	17.13 ± 0.86	19.24 ± 0.96	>100	>100	20.85 ± 1.04	38.50 ± 1.93
average	10.88 ± 0.54	1.69 ± 0.08	17.06 ± 0.85	17.90 ± 0.90	>100	>100	30.93 ± 1.55	46.88 ± 2.34
NiH3T3	14.74 ± 0.74	39.09 ± 1.95	23.09 ± 1.15	24.42 ± 1.22	>100	>100	16.89 ± 0.84	33.63 ± 1.68
F	1.35	23.13	1.35	1.36			0.55	0.72

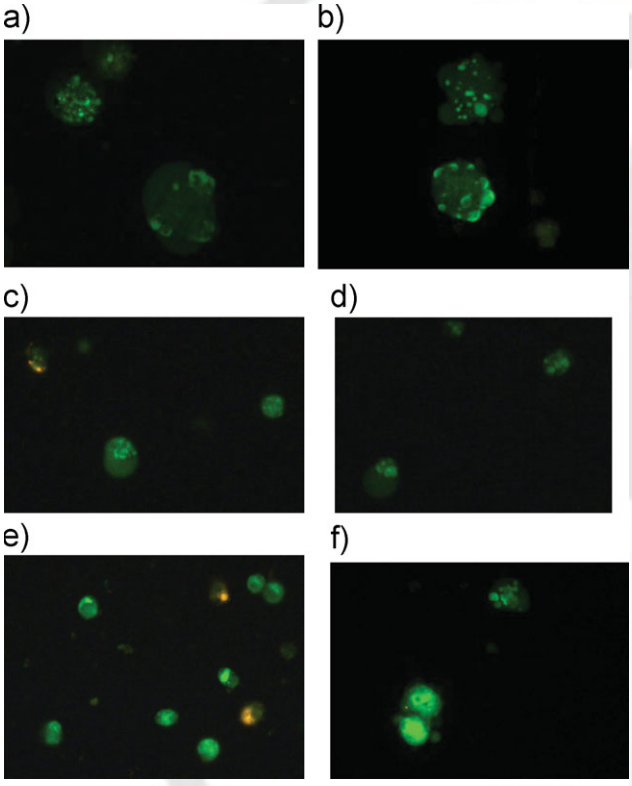

Figure 1. Results from the AO/EB test: treatment of A549 cells (from left to right): **4** (15 μM), **5** (3 μM), **6** (25 μM), **7** (25 μM), **12** (35 μM) and **13** (80 μM).

detected as depicted in Fig. 1. In this assay apoptotic cells appear in a green color with bright fluorescent parts whereas necrotic cells exhibit an orange color.

To determine the extent of apoptosis, A549 lung carcinoma cells were incubated with our compounds at a concentration $>IC_{50}$, a trypan blue test was performed and quantification of apoptosis was done by using an automated cell counter (Table 2). In this test, GA has a default value of approx. 74%; all of our compounds showed levels of apoptosis between 65-86%.

In this study a number of derivatives of GA methyl ester (**1**) were synthesized. For compound **5** a IC_{50} = 1.27 μM for MCF-7 cells was established; the toxicity of **5** for A549 cells (IC_{50} = 1.77 μM) is higher than that of betulinic acid (IC_{50} = 10.3 μM [9] or 11.9 μM [10], oleanolic acid (IC_{50} = 27 μM [9], betulin [IC_{50} = 3.8 μM [9]) or parent glycyrrhetinic acid (IC_{50} = 63.2 μM [2] or IC_{50} = 82.8 μM [6]). In addition, previously for derivatives of GA similar to CDDO [11] a significantly lower activity on various tumor cells has been reported.

A comparison of the aspartyl and glutamyl substituted derivatives revealed an interesting behavior. For the Boc-protected compounds having an unprotected side chain (compounds **6** and **7**) no significant difference in the IC_{50} value is observed. Both compounds show IC_{50} values of circa 17-18 μM with a slightly higher value for NiH3T3 mouse fibroblasts (23.09 μM and 24.42 μM, respectively). The deprotected derivatives **8** and **9**, however, did not show any activity

Table 2. Apoptotic effect of GA derivatives in A549 cells in % (± standard error, 6 experiments), cells were treated with **GA** (90 μM), **4** (15 μM), **5** (3 μM), **6** (30 μM), **7** (30 μM), **12** (40 μM) and **13** (80 μM).

Compound	GA	4	5	6	7	12	13
Apoptosis [%]	73.73 ± 1.40	80.53 ± 2.93	86.10 ± 2.98	65.50 ± 3.08	82.63 ± 3.36	76.76 ± 3.20	79.44 ± 2.17

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at concentrations <100 μM at all. They are neither cytotoxic for tumor cells nor for the mouse embryonic fibroblasts.

Pairs of side chain protected derivatives show anomalous activity with respect to the protecting group. The methyl esters **12** and **13** show higher IC_{50} values than compounds **6** and **7** – except for **12** on MCF-7 cells. Furthermore, the aspartyl substituent (in **12**) seems to be more effective than a glutamyl moiety. For **12** a lower IC_{50} value is found for each of the cell lines including the fibroblasts. The selectivity of both compounds, however, is worse than for all other compounds.

The benzyl esters **4** and **5** are the most effective cytotoxic compounds of this study. As indicated in a previous investigation [6], combining a free amino group and an unprotected carboxylic group creates inactive compounds – as exemplified for **8** and **9**. The derivatives **6** and **7** are more active than **12** and **13**; obviously an unprotected amino group is not that important for retaining cytotoxicity as previously assumed. Changing a methyl ester to a benzyl ester (as exemplified in **3** and **4**) increases activity. Hence, a free amino group seems necessary for gaining cytotoxicity; a benzyl group seems to add to stability under biological conditions and to higher lipophilicity.

Glutamyl substituted **5** is more active than aspartyl substituted **4**. The reason for this behavior remains unclear up to now and has to be subject of further investigations. The selectivities of these compounds are better than for parent GA but lower [12] as for betulinic acid. Derivative **5**, however, combines a higher cytotoxicity and an outstanding selectivity of approx. 30 (for MCF-7 cells). Derivatization does not affect apoptosis as deduced from the AO/EB test.

In conclusion, we were able to increase the cytotoxicity of GA by introducing glutamyl and aspartyl substituents. Compound **5** showed an $IC_{50} = 1.27$ μM for MCF-7 cells (67-fold better than GA) and a tumor-to-control selectivity of approx. 30.8 (for MCF-7 vs. NiH3T3; 140-fold better than GA). Hence, for MCF-7 cells, compound **5** is more effective but also more selective than gold standard betulinic acid.

Experimental

General

Melting points are uncorrected (Leica hot stage microscope), NMR spectra were recorded using the Varian spectrometers Gemini 200, Gemini 2000 or Unity 500 (δ given in ppm, J in Hz, internal Me_4Si), optical rotations were obtained using a Perkin-Elmer 341 polarimeter (1 cm micro cell, 25°C), IR spectra (film or KBr pellet) on a Perkin-Elmer FT-IR spectrometer Spectrum 1000, MS spectra were taken on an Intectra GmbH AMD 402 (electron impact, 70 eV) or on a Finnigan MAT TSQ 7000 (electrospray, voltage 4.5 kV, sheath gas nitrogen) instrument. TLC was performed on silica gel (Merck 5554, detection by UV absorption). The solvents were dried according to usual procedures.

Cell lines and culture conditions

The cell lines 518A2, 8505C, A253, A2780, A549, LIPO, MCF-7, NiH3T3, and SW1736 were included in this study. Cultures were maintained as monolayer in RPMI 1640 (PAA Laboratories, Pasching, Germany) supplemented with 10% heat inactivated fetal bovine serum (Biochrom AG, Berlin, Germany) and penicillin/streptomycin (PAA Laboratories) at 37°C in a humidified atmosphere of 5% CO_2/95% air.

Cytotoxicity Assay [13]

The cytotoxicity of the compounds was evaluated using the sulforhodamine B (SRB) (Sigma Aldrich) microculture colorimetric assay. In short, exponentially growing cells were seeded into 96-well plates on day 0 at the appropriate cell densities to prevent confluence of the cells during the period of experiment. After 24 h, the cells were treated with serial dilutions of the compounds (0-100 μM) for 96 h. The final concentration of DMSO or DMF solvent never exceeded 0.5% which was non-toxic to the cells. The percentages of surviving cells relative to untreated controls were determined 96 h after the beginning of drug exposure. After a 96 h treatment, the supernatant medium from the 96 well plates was thrown away and the cells were fixed with 10% TCA. For a thorough fixation, the plates were allowed to rest at 4°C. After fixation, the cells were washed in a strip washer. The washing was done four times with water using alternate dispensing and aspiration procedures. The plates were then dyed with 100 μl of 0.4% SRB (sulforhodamine B) for about 20 min. After dying, the plates were washed with 1% acetic acid to remove the excess of the dye and allowed to air dry overnight. 100 μl of 10 mM Tris base solution were added to each well and absorbance was measured at ν =570 nm (using a 96 well plate reader, Tecan Spectra, Crailsheim, Germany). The IC_{50} was estimated by linear regression between the value before and after the 50% line is crossed in a dose-response curve.

Apoptosis Test – Acridine Orange/Ethidium Bromide (AO/EB)

Apoptotic cell death was analysed by acridine orange/ethidium bromide dye using fluorescence microscopy on A549 cells. Therefore approx. 500 000 cells were seeded in cell culture flasks and were allowed to grow for 24 h. The medium was removed and the substance loaded medium was added. After 24-48 h, the supernatant medium was collected and centrifuged; the pellet was suspended in phosphate-buffer saline (PBS) and centrifuged again. The liquid was removed and the pellet was suspended in PBS. After mixing the suspension with a solution of AO/EB, analysis was performed under a fluorescence microscope. While a green fluorescence shows apoptosis, a red colored nucleus indicates necrotic cells.

Apoptosis test – trypan blue cell counting

Approx. 500 000 cells (A549) were seeded in cell culture flasks and were allowed to grow for 1 day. After removing of the medium, the substance loaded medium was introduced and the flasks were incubated for about 24-48 h. The supernatant medium was collected and centrifuged; cell pellet was suspended in PBS and centrifuged again. Equal amounts of trypan blue solution (0.4% in phosphate-buffer saline, pH 7.2) and suspension of the pellet in PBS were mixed and put on chamber slides (Invitrogen™). Automatic cell counter (Invitrogen™ countess® automated cell counter) was used for counting the cells,

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differing between cells with an intact cell membrane and cells without.

Apoptosis test - trypan blue cell counting

Approx. 500 000 cells (A549) were seeded in cell culture flasks and were allowed to grow for 1 day. After removing of the medium, the substance loaded medium was introduced and the flasks were incubated for about 24-48 h. The supernatant medium was collected and centrifuged; cell pellet was suspended in PBS and centrifuged again. Equal amounts of trypan blue solution (0.4% in phosphate-buffer saline, pH 7.2) and suspension of the pellet in PBS were mixed and put on chamber slides (Invitrogen™). Automatic cell counter (Invitrogen™ countess® automated cell counter) was used for counting the cells, differing between cells with an intact cell membrane and cells without.

General procedure for the esterifications in position C(3) (method A)

The starting material (1 eq.) was dissolved in dry DCM (15 ml), DMAP (20 mg, 0.16 mmol) and the protected amino acid (1.2 eq.) were added. After addition of DCC (1.2 eq.) the mixture was stirred at room temperature for 12 h, filtered. The filtrate was washed with water and brine (15 ml each), dried over sodium sulfate, filtered and the solvent was evaporated. Purification was performed by flash chromatography (SiO_2, hexane/ethyl acetate 8:2).

General procedure for deprotection (method B)

To a solution of the Boc-protected compound in dry DCM (10 ml/mmol), trifluoroacetic acid (1 ml/10 ml DCM) was added. The mixture was allowed to stir at room temperature for 12 h. After completion of the reaction (as monitored by tlc), the solution was washed with a satd. aq. solution of sodium hydrogen carbonate (20 ml). The aqueous layer was extracted with DCM (3 × 25 ml), the combined organic extracts were washed with brine (20 ml), dried over sodium sulfate, filtrated and evaporated to yield the amine.

General procedure for deprotection (method C) [14]

The solution of the benzyl ester (1 eq.) in a mixture of MeOH and THF (1:2, 50 ml/mmol) was purged with argon for 3 min, followed by the addition of ammonium formate (5 eq.). Palladium on activated coal (10%; 100 mg/mmol) was added and the mixture was stirred at room temperature for 12-14 h. The solvents were removed under reduced pressure and the residue was dissolved in DCM (25 ml). Usual aqueous work-up followed by chromatography (SiO_2, hexane/ethyl acetate 1:1) yielded the product.

General procedure for the deprotection (method D)

The Boc-protected compound was dissolved in dry DCM (10 ml/mmol). After saturation with dry hydrogen chloride gas for 15 min stirring at room temperature was continued for 12 h. After completion of the reaction (as monitored by tlc), the solvent was removed under reduced pressure. The residue was washed with ethyl acetate until no parent substance could be detected; analytical samples were obtained by re-crystallization.

General procedure for the esterification (method E)

To a solution of the starting material (1 eq.) in dry DMF (15 ml/mmol), finely grounded potassium carbonate (2 eq.) was added. After 30 min of stirring at room temperature, iodomethane (1.1 eq.) was added and the mixture was stirred for an additional 2 h. The solvents were evaporated and the crude residue was dissolved in a mixture of DCM (30 ml) and aq. hydrochloric acid (1.0 M). The aqueous layer was extracted with DCM (3 × 25 ml), the combined organic layers were washed with brine, dried (Na_2SO_4), filtered and the solvent was evaporated. Purification by flash chromatography (SiO_2, hexane/ethyl acetate 8:2) yielded the ester.

*Methyl 3β 3-(Boc-O-benzyl-L-asparagyl)-11-oxo-olean-12-en-30-oate (**2**)*

Obtained from **1** (530 mg, 1.1 mmol) and Boc-L-Asp(OBzl)OH (410 mg, 1.27 mmol) by method A as a colorless powder; yield: 720 mg, 83%; mp 74-76°C; $R_F = 0.71$ (hexane/ethyl acetate 7:3); $[\alpha]_D = 77.68°$ ($c = 0.27$, $CHCl_3$); UV-VIS (methanol): λ_{max} (log ε) = 206 nm (4.08), 249 nm (3.95); IR (KBr): ν = 3439 (br), 2976 (m), 2361 (w), 1731 (s), 1659 (m), 1499 (m), 1456 (m), 1389 (m), 1367 (m), 1217 (s), 1166 (s), 1048 (w), 1025 (w), 986 (w), 863 (w), 752 (w), 698 (w) cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 7.38-7.31 (m, 5H, H-Ar), 5.67 (s, 1H, H-12), 5.51 (d, 1H, NH, J = 8.9 Hz), 5.11 (s, 2H, Bzl-CH_2), 4.56 (m, 2H, Asp-CH), 4.55 (dd, 1H, H-3, J = 11.5, 4.9 Hz), 3.70 (s, 3H, OMe), 3.05 (ddd, 1H, Asp-C*H*H', J = 15.9, 15.9, 3.9 Hz), 2.87 (ddd, 1H, Asp-CH*H*', J = 17.1, 4.7, 1.9 Hz), 2.81 (ddd, 1H, H-1, J = 13.7, 3.5, 3.5 Hz), 2.35 (s, 1H, H-9), 2.09 (dd, 1H, H-18, J = 14.0, 3.6 Hz), 2.03 (m, 1H, H-15), 2.00 (m, 1H, H-21), 1.93 (ddd, 1H, H-19, J = 13.7, 3.6, 2.4 Hz), 1.83 (ddd, 1H, H-16, J = 13.7, 13.7, 4.3 Hz), 1.70 (m, 1H, H-2), 1.66 (m, 1H, H-7), 1.63 (m, 1H, H-2'), 1.59 (dd, 1H, H-19', J = 13.5, 13.5 Hz), 1.57 (m, 1H, H-6), 1.44 (s, 9H, Boc-CH_3), 1.42 (m, 1H, H-6'), 1.39 (m, 1H, H-7'), 1.38 (m, 1H, H-22), 1.37 (s, 3H, H-27), 1.32 (m, 1H, H-22'), 1.32 (m, 1H, H-21'), 1.18 (m, 1H, H-16'), 1.15 (s, 3H, H-25), 1.15 (s, 3H, H-29), 1.13 (s, 3H, H-26), 1.03 (m, 1H, H-1'), 1.02 (m, 1H, H-15'), 0.84 (s, 3H, H-24), 0.81 (s, 3H, H-23), 0.81 (s, 3H, H-28), 0.78 (m, 1H, H-5) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 176.9 (C-30), 170.9 (Asp-COOBzl), 170.7 (Asp-COO), 169.2 (C-13), 155.4 (Boc-COO),135.7 (C_{ar}), 128.6 (C_{ar}), 128.6 (C_{ar}), 128.5 (C_{ar}), 128.4 (C-12), 128.3 (C_{ar}), 128.3 (C_{ar}), 82.3 (C-3), 80.0 (Boc-*quart.*-C), 66.8 (Bzl-CH_2), 61.7 (C-9), 55.0 (C-5), 51.8 (OMe), 50.2 (Asp-$CHNH_2$), 48.4 (C-18), 45.4 (C-8), 44.0 (C-20), 43.2 (C-14), 41.1 (C-19), 38.7 (C-1), 38.1 (C-4), 37.7 (C-22), 36.9 (C-10), 36.8 (Asp-CH_2), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-28), 28.3 (C-29), 28.3 (Boc-CH_3), 27.9 (C-23), 26.5 (C-16), 26.4 (C-15), 23.4 (C-2), 23.3 (C-27), 18.7 (C-26), 17.3 (C-6), 16.7 (C-24), 16.3 (C-25) ppm; MS (ESI, MeOH): m/z (%) = 790.1 ($[M + H]^+$, 10), 812.3 ($[M + Na]^+$, 100); anal. calcd. for $C_{47}H_{67}O_9$ (790.03): C, 71.45; H, 8.55; N, 1.77; found: C, 71.32; H, 8.67; N, 1.59.

*Methyl 3β 3-(Boc-O-benzyl-L-glutamyl)-11-oxo-olean-12-en-30-oate (**3**)*

Obtained from **1** (560 mg, 1.16 mmol) and Boc-L-Glu(OBzl)OH (440 mg, 1.28 mmol) by method A as a colorless powder; yield: 680 mg, 73%; mp 139-143°C; $R_F = 0.41$ (hexane/ethyl acetate 7:3); $[\alpha]_D = 75.92°$ ($c = 0.28$, $CHCl_3$); UV-VIS (methanol): λ_{max} (log ε) = 205 nm (4.30), 249 nm (4.18); IR (KBr): ν = 3386 (br), 2950 (s), 1732 (s), 1659 (m), 1500 (m), 1455 (m), 1390 (m), 1367 (m), 1257 (m), 1216 (m), 1165 (s), 1086 (w), 1049 (w), 1027 (w), 986 (w), 751 (w), 698 (w) cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 7.39-7.29 (m, 5H, H-Ar), 5.67 (s, 1H, H-12), 5.12 (s, 2H, Bzl-CH_2), 5.10 (m, 1H, Glu-NH), 4.56 (dd, 1 H, H-3, J = 11.6, 4.7 Hz), 4.33 (m, 1 H, Glu-

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CHN), 3.69 (s, 3H, OMe), 2.82 (ddd, 1H, H-1, J = 13.8, 3.4, 3.4 Hz), 2.51 (m, 1H, Glu-C*H*H'COOBzl), 2.44 (m, 1H, Glu-CH*H*'COOBzl), 2.35 (s, 1H, H-9), 2.24 (m, 1H, Glu-C*H*H'CHN), 2.08 (dd, 1H, H-18, J = 13.7, 3.5 Hz), 2.03 (m, 1H, H-15), 1.99 (m, 1H, H-21), 1.96 (m, 1H, Glu-CH*H*'CHN), 1.92 (m, 1H, H-19), 1.83 (ddd, 1H, H-16, J = 13.9, 13.9, 4.4 Hz), 1.73 (m, 1H, H-2), 1.66 (m, 1H, H-7), 1.63 (m, 1H, H-2'), 1.61 (dd, 1H, H-19', J = 13.3, 13.3 Hz), 1.58 (m, 1H, H-6), 1.48 (m, 1H, H-6'), 1.43 (s, 9H, Boc-CH_3), 1.41 (m, 1H, H-7'), 1.39 (m, 1H, H-22), 1.36 (s, 3H, H-27), 1.31 (m, 2H, H-22' and H-21'), 1.18 (m, 1H, H-16'), 1.16 (s, 3H, H-25), 1.15 (s, 3H, H-29), 1.13 (s, 3H, H-26), 1.08 (m, 1H, H-1'), 1.02 (m, 1H, H-15'), 0.88 (s, 3H, H-24), 0.86 (s, 3H, H-23), 0.81 (s, 3H, H-28), 0.79 (m, 1H, H-5) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 199.9 (C-11), 176.9 (C-30), 172.6 (Glu-COOBzl), 171.9 (Glu-COO), 169.2 (C-13), 155.4 (Boc-COO), 135.8 ($C_{ar.}$), 128.5 ($C_{ar.}$), 128.5 ($C_{ar.}$), 128.5 (C-12), 128.2 ($C_{ar.}$), 128.2 ($C_{ar.}$), 128.2 ($C_{ar.}$), 82.2 (C-3), 79.9 (Boc-*quart.*-C), 61.7 (C-9), 55.0 (C-5), 53.3 (Glu-CHNH), 51.8 (OMe), 48.4 (C-18), 45.4 (C-8), 44.0 (C-20), 43.2 (C-14), 41.1 (C-19), 38.7 (C-1), 38.1 (C-4), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 30.4 (Glu-CH_2COOBzl), 28.5 (C-28), 28.3 (C-29), 28.3 (Boc-CH_3), 28.1 (C-23), 27.9 (Glu-CH_2CHNH), 26.5 (C-16), 26.4 (C-15), 23.5 (C-2), 23.3 (C-27), 18.7 (C-26), 17.3 (C-6), 16.8 (C-24), 16.4 (C-25) ppm; MS (ESI, MeOH): m/z (%) = 804.1 ($[M + H]^+$, 8), 826.3 ($[M + Na]^+$, 100); anal. calcd. for $C_{48}H_{69}NO_9$ (804.06): C, 71.70; H, 8.65; N, 1.74; found: C, 71.56; H, 8.81; N, 1.62.

*Methyl 3β 3-(O-benzyl-L-asparagyl)-11-oxo-olean-12-en-30-oate (**4**)*

Obtained from **2** (150 mg, 0.19 mmol) and TFA (1 ml, 13.0 mmol) by method B as a colorless powder; yield: 120 mg, 73%; mp 172-179°C; R_F = 0.78 (dichloromethane/methanol 9:1); $[\alpha]_D$ = 86.22° (c = 0.35, $CHCl_3$); UV-VIS (methanol): λ_{max} (log ε) = 207 nm (4.18), 249 nm (4.08); IR (KBr): ν = 3414 (br), 2960 (s), 1728 (s), 1652 (s), 1616 (m), 1456 (m), 1390 (s), 1354 (m), 1324 (m), 1264 (s), 1216 (s), 1172 (s), 1086 (m), 1028 (m), 985 (m), 971 (m), 910 (w), 869 (w), 826 (w), 752 (m), 699 (m), 589 (w), 546 (w), 508 (w) cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 7.36-7.29 (m, 5H, H-Ar), 5.64 (s, 1H, H-12), 5.12 (s, 2H, Bzl-CH_2), 4.55 (dd, 1H, H-3, J = 11.7, 4.9 Hz), 3.82 (dd, 2H, Asp-CH, J = 5.5, 5.5 Hz), 3.67 (s, 3H, OMe), 2.88 (dd, 1H, Asp-C*H*H', J = 16.5, 4.6 Hz), 2.80 (m, 1H, H-1), 2.76 (m, 1H, Asp-CH*H*'), 2.33 (s, 1H, H-9), 2.06 (m, 1H, H-18), 2.00 (m, 1H, H-15), 1.97 (m, 1H, H-21), 1.90 (ddd, 1H, H-19, J = 13.4, 3.6, 2.8 Hz), 1.80 (ddd, 1H, H-16, J = 13.7, 13.7, 4.3 Hz), 1.67 (m, 1H, H-2), 1.65 (m, 1H, H-7), 1.62 (m, 1H, H-2'), 1.59 (dd, 1H, H-19', J = 13.5, 13.5 Hz), 1.55 (m, 1H, H-6), 1.42 (m, 1H, H-6'), 1.39 (m, 1H, H-7'), 1.36 (m, 1H, H-22), 1.34 (s, 3H, H-27), 1.29 (m, 1H, H-22'), 1.29 (m, 1H, H-21'), 1.16 (m, 1H, H-16'), 1.13 (s, 3H, H-25), 1.13 (s, 3H, H-29), 1.10 (s, 3H, H-26), 1.02 (ddd, 1H, H-1', J = 14.1, 14.1, 3.7 Hz), 1.00 (m, 1H, H-15'), 0.83 (s, 3H, H-24), 0.81 (s, 3H, H-23), 0.78 (s, 3H, H-28), 0.77 (m, 1H, H-5) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 199.9 (C-11), 176.9 (C-30), 171.0 (Asp-COO), 171.0 (Asp-COOBzl), 169.2 (C-13), 135.5 (C_{ar}), 128.6 (C_{ar}), 128.6 (C_{ar}), 128.5 (C-12), 128.3 (C_{ar}), 128.3 (C_{ar}), 128.2 (C_{ar}), 81.9 (C-3), 66.7 (Bzl-CH_2), 61.6 (C-9), 55.0 (C-5), 51.7 (OMe), 51.2 (Asp-$CHNH_2$), 48.4 (C-18), 45.4 (C-8), 44.0 (C-20), 43.2 (C-14), 41.1 (C-19), 38.9 (Asp-CH_2), 38.7 (C-1), 38.1 (C-4), 37.7 (C-22), 36.9 (C-10), 32.6 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-28), 28.3 (C-29), 28.0 (C-23), 26.4 (C-16), 26.4 (C-15), 23.5 (C-2), 23.3 (C-27), 18.6 (C-26), 17.3 (C-6), 16.8 (C-24), 16.3 (C-25) ppm; MS (ESI, MeOH): m/z (%) = 690.2 ($[M + H]^+$, 100); anal. calcd. for $C_{42}H_{59}NO_7$ (689.92): C, 73.12; H, 8.62; N, 2.03; found: C, 73.02; H, 8.72; N, 1.87.

*Methyl 3β 3-(O-benzyl-L-glutamyl)-11-oxo-olean-12-en-30-oate (**5**)*

Obtained from **3** (150 mg, 0.19 mmol) and TFA (1 ml, 13 mmol) by method B as a colorless powder; yield: 90 mg, 64%; mp 282-288°C (dec.); R_F = 0.74 (dichloromethane/methanol 9:1); $[\alpha]_D$ = 100.51° (c = 0.66, $CHCl_3$); UV-VIS (methanol): λ_{max} (log ε) = 249 nm (3.99); IR (KBr): ν = 3398 (br), 2935 (s), 1729 (s), 1660 (s), 1464 (m), 1387 (m), 1322 (m), 1280 (m), 1217 (s), 1152 (s), 1084 (m), 1024 (m), 986 (m), 916 (w), 870 (w), 822 (w), 768 (w), 737 (w), 699 (m), 590 (w), 543 (w), 481 (w) cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 7.39-7.29 (m, 5H, H-Ar), 5.67 (s, 1H, H-12), 5.12 (s, 2H, Bzl-CH_2), 4.57 (dd, 1H, H-3, J = 11.8, 4.7 Hz), 4.22 (m, 1H, Glu-CHN), 3.67 (s, 3H, OMe), 2.81 (ddd, 1H, H-1, J = 13.6, 3.7, 3.7 Hz), 2.57 (m, 1H, Glu-C*H*H'COOBzl), 2.47 (m, 1H, Glu-CH*H*'COOBzl), 2.33 (s, 1H, H-9), 2.21 (m, 1H, Glu-C*H*H'CHN), 2.07 (m, 1H, H-18), 2.01 (m, 1H, H-15), 1.97 (m, 1H, H-21), 1.97 (m, 1H, Glu-CH*H*'CHN), 1.90 (ddd, 1H, H-19, J = 13.7, 4.2, 2.2 Hz), 1.81 (ddd, 1H, H-16, J = 14.0, 14.0, 4.2 Hz), 1.72 (m, 1H, H-2), 1.66 (m, 1H, H-7), 1.61 (m, 1H, H-2'), 1.59 (dd, 1H, H-19', J = 13.4, 13.4 Hz), 1.57 (m, 1H, H-6), 1.45 (m, 1H, H-6'), 1.40 (m, 1H, H-7'), 1.38 (m, 1H, H-22), 1.34 (s, 3H, H-27), 1.29 (m, 2H, H-22' and H-21'), 1.16 (m, 1H, H-16'), 1.15 (s, 3H, H-25), 1.13 (s, 3H, H-29), 1.11 (s, 3H, H-26), 1.06 (m, 1H, H-1'), 1.00 (m, 1H, H-15'), 0.86 (s, 3H, H-24), 0.86 (s, 3H, H-23), 0.78 (s, 3H, H-28), 0.77 (m, 1H, H-5) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 199.9 (C-11), 177.4 (Glu-COOBzl), 176.9 (C-30), 171.6 (Glu-COO), 169.2 (C-13), 135.8 ($C_{ar.}$), 128.6 ($C_{ar.}$), 128.6 ($C_{ar.}$), 128.5 (C-12), 128.2 ($C_{ar.}$), 128.2 ($C_{ar.}$), 128.2 ($C_{ar.}$), 82.2 (C-3), 61.7 (C-9), 55.0 (C-5), 53.6 (Glu-CHNH), 51.7 (OMe), 48.4 (C-18), 45.3 (C-8), 44.0 (C-20), 43.2 (C-14), 41.1 (C-19), 38.6 (C-1), 38.2 (C-4), 37.7 (C-22), 36.9 (C-10), 32.6 (C-7), 31.8 (C-17), 31.1 (C-21), 30.5 (Glu-CH_2COOBzl), 29.3 (Glu-CH_2CHNH), 28.5 (C-28), 28.3 (C-29), 28.1 (C-23), 26.5 (C-16), 26.4 (C-15), 23.5 (C-2), 23.4 (C-27), 18.7 (C-26), 17.3 (C-6), 16.8 (C-24), 16.4 (C-25) ppm; MS (ESI, MeOH): m/z (%) = 704.3 ($[M + H]^+$, 100); anal. calcd. for $C_{43}H_{61}NO_7$ (703.95): C, 73.37; H, 8.73; N, 1.99; found: C, 73.22; H, 8.99; N, 1.85.

*Methyl 3β 3-(Boc-L-asparagyl)-11-oxo-olean-12-en-30-oate (**6**)*

Obtained from **2** (390 mg, 0.49 mmol) by method C as a colorless powder; yield: 310 mg, 90%; mp 213-215°C; R_F = 0.22 (hexane/ethyl acetate 1:1); $[\alpha]_D$ = 68.90° (c = 0.90, $CHCl_3$); UV-VIS (methanol): λ_{max} (log ε) = 249 nm (4.10); IR (KBr): ν = 3366 (br), 2974 (s), 2874 (m), 1732 (s), 1654 (s), 1521 (s), 1456 (m), 1392 (s), 1369 (s), 1288 (m), 1216 (s), 1166 (s), 1087 (m), 1043 (m), 1025 (m), 986 (m), 971 (m), 919 (w), 903 (w), 882 (w), 867 (w), 824 (w), 770 (w), 670 (w), 541 (w) cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 5.65 (s, 1H, H-12), 5.47 (m, 1H, NH), 4.55 (m, 2H, H-3 and Asp-CH), 3.67 (s, 3H, OMe), 3.05 (m, 1H, Asp-C*H*H'), 2.87 (m, 1H, Asp-CH*H*'), 2.81 (m, 1H, H-1), 2.33 (s, 1H, H-9), 2.06 (m, 1H, H-18), 1.99 (m, 1H, H-15), 1.97 (m, 1H, H-21), 1.93 (m, 1H, H-19), 1.82 (m, 1H, H-16), 1.72 (m, 1H, H-2), 1.68 (m, 1H, H-2'), 1.63 (m, 1H, H-7), 1.59 (dd, 1H, H-19', J = 13.7, 13.7 Hz), 1.55 (m, 1H, H-6), 1.43 (s, 9H, Boc-CH_3), 1.42 (m, 1H, H-6'), 1.40 (m, 1H, H-7'), 1.37 (m, 1H, H-22), 1.34 (s, 3H, H-27), 1.30 (m, 1H, H-22'), 1.28 (m, 1H, H-21'), 1.18 (m, 1H, H-16'), 1.14 (s, 3H, H-25), 1.13 (s, 3H, H-29), 1.10 (s, 3H, H-26), 1.03 (m, 1H, H-1'), 0.98 (m, 1H, H-15'), 0.85 (s, 3H, H-24), 0.84 (s, 3H, H-23), 0.78 (s, 3H, H-28), 0.77 (m, 1H, H-5) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 200.1 (C-11), 176.9 (C-30), 176.9 (Asp-COOH), 170.8 (Asp-COO), 169.4 (C-13), 155.5 (Boc-COO), 128.5 (C-12), 82.7 (C-3), 80.2 (Boc-*quart.*-C), 61.7 (C-9), 55.0 (C-5), 51.8 (OMe), 50.2 (Asp-$CHNH_2$), 48.4 (C-18),

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45.4 (C-8), 44.0 (C-20), 43.2 (C-14), 41.1 (C-19), 38.7 (C-1), 38.1 (C-4), 37.7 (C-22), 36.9 (C-10), 36.5 (Asp-CH_2), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-28), 28.3 (C-29), 28.3 (Boc-CH_3), 27.8 (C-23), 26.5 (C-16), 26.4 (C-15), 23.4 (C-2), 23.3 (C-27), 18.7 (C-26), 17.3 (C-6), 16.7 (C-24), 16.4 (C-25) ppm; MS (ESI, MeOH): m/z (%) = 722.4 ($[M + Na]^+$, 100), 1072.3 ($[3\ M + 2\ Na]^{2+}$, 11); anal. calcd. for $C_{40}H_{61}NO_9$ (699.91): C, 68.64; H, 8.78; N, 2.00; found: C, 68.49; H, 8.87; N, 1.77.

*Methyl 3β 3-(Boc-L-glutamyl)-11-oxo-olean-12-en-30-oate (**7**)*

Obtained from **3** (210 mg, 0.26 mmol) by method C as a colorless powder; yield: 190 mg, 96%; mp 212-214°C; R_F = 0.13 (hexane/ethyl acetate 1:1); $[\alpha]_D$ = 123.60° (c = 0.54, MeOH); UV-vis (methanol): λ_{max} (log ε) = 250 nm (4.13); IR (KBr): ν = 3259 (br), 2956 (s), 1724 (s), 1652 (s), 1582 (w), 1466 (m), 1389 (m), 1365 (m), 1322 (w), 1233 (s), 1164 (m), 1105 (m), 1022 (w), 982 (m), 919 (w), 850 (w), 750 (w), 682 (w), 589 (w), 541 (w) cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 5.64 (s, 1H, H-12), 5.14 (d, 1H, Glu-NH, J = 7.5 Hz), 4.53 (dd, 1H, H-3, J = 11.8, 4.7 Hz), 4.31 (m, 1H, Glu-CHN), 3.67 (s, 3H, OMe), 2.79 (ddd, 1H, H-1, J = 13.5, 3.4, 3.4 Hz), 2.46 (m, 1H, Glu-C*H*H'COOMe), 2.42 (m, 1H, Glu-CH*H*'COOMe), 2.32 (s, 1H, H-9), 2.20 (m, 1H, Glu-C*H*H'CHN), 2.06 (dd, 1H, H-18, J = 13.5, 3.3 Hz), 1.99 (ddd, 1H, H-15, J = 13.7, 13.7, 4.3 Hz), 1.96 (m, 1H, H-21), 1.94 (m, 1H, Glu-CH*H*'CHN), 1.89 (m, 1H, H-19), 1.79 (ddd, 1H, H-16, J = 13.8, 13.8, 4.4 Hz), 1.72 (m, 1H, H-2), 1.66 (m, 1H, H-7), 1.61 (m, 1H, H-2'), 1.61 (dd, 1H, H-19', J = 13.6, 13.6 Hz), 1.56 (m, 1H, H-6), 1.45 (m, 1H, H-6'), 1.41 (s, 9H, Boc-CH_3), 1.38 (m, 1H, H-7'), 1.37 (m, 1H, H-22), 1.33 (s, 3H, H-27), 1.28 (m, 2H, H-22' and H-21'), 1.15 (m, 1H, H-16'), 1.13 (s, 3H, H-25), 1.12 (s, 3H, H-29), 1.09 (s, 3H, H-26), 1.04 (m, 1H, H-1'), 0.98 (m, 1H, H-15'), 0.86 (s, 3H, H-24), 0.84 (s, 3H, H-23), 0.77 (s, 3H, H-28), 0.76 (m, 1H, H-5) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 177.2 (Glu-COOH), 176.9 (C-30), 171.8 (Glu-*C*OO), 169.3 (C-13), 155.6 (Boc-COO), 128.4 (C-12), 82.4 (C-3), 80.1 (Boc-*quart.*-C), 61.6 (C-9), 55.0 (C-5), 53.1 (Glu-CHNH), 51.8 (OMe), 48.4 (C-18), 45.4 (C-8), 44.0 (C-20), 43.2 (C-14), 41.1 (C-19), 38.7 (C-1), 38.1 (C-4), 37.7 (C-22), 36.9 (C-10), 32.6 (C-7), 31.8 (C-17), 31.1 (C-21), 30.1 (Glu-CH_2COO), 28.5 (C-28), 28.3 (C-29), 28.3 (Boc-CH_3), 28.1 (C-23), 28.0 (Glu-CH_2CHNH), 26.4 (C-16), 26.4 (C-15), 23.5 (C-2), 23.3 (C-27), 18.7 (C-26), 17.3 (C-6), 16.7 (C-24), 16.4 (C-25) ppm; MS (ESI, MeOH): m/z (%) = 628.3 ($[M + H]^+$, 100); anal. calcd. for $C_{41}H_{63}NO_9$ (713.94): C, 68.97; H, 8.89; N, 1.96: found: C, 68.81; H, 9.02; N, 1.81.

*Methyl 3β 3-(L-asparagyl)-11-oxo-olean-12-en-30-oate hydrochloride (**8**)*

Obtained from **6** 340 mg, 0.49 mmol) by method D as a colorless powder; yield: 280 mg, 91%; mp 272-276°C (dec.); R_F = 0.66 (methanol); $[\alpha]_D$ = 122.88° (c = 0.44, MeOH); UV-VIS (methanol): λ_{max} (log ε) = 250 nm (4.07); IR (KBr): ν = 3439 (br), 2952 (s), 2362 (w), 1732 (s), 1660 (s), 1520 (m), 1466 (m), 1387 (m), 1322 (m), 1255 (s), 1217 (s), 1153 (m), 1085 (m), 1049 (w), 987 (w), 946 (w), 901 (w), 878 (w), 823 (w), 800 (w), 688 (w), 670 (w), 638 (w), 545 (w) cm^{-1}; ^{1}H-NMR (500 MHz, MeOH-d_4): δ = 5.58 (s, 1H, H-12), 4.67 (dd, 1H, H-3, J = 11.7, 4.7 Hz), 4.32 (dd, 1H, Asp-CHN, J = 4.9, 4.9 Hz), 3.70 (s, 3H, OMe), 3.07 (dd, 1H, Asp-C*H*H', J = 18.2, 5.5 Hz), 2.97 (dd, 1H, Asp-CH*H*', J = 18.2, 4.5 Hz), 2.80 (ddd, 1H, H-1, J = 13.7, 3.7, 3.7 Hz), 2.50 (s, 1H, H-9), 2.15 (m, 1H, H-15), 2.13 (m, 1H, H-18), 1.97 (m, 1H, H-21), 1.89 (m, 1H, H-19), 1.87 (m, 1H, H-16), 1.80 (m, 1H, H-2), 1.77 (m, 1H, H-7), 1.74 (dd, 1H, H-19', J = 13.4, 13.4 Hz), 1.72 (m, 1H, H-2'), 1.65 (m, 1H, H-6), 1.54 (m, 1H, H-6'), 1.50 (m, 1H, H-7'), 1.47 (m, 1H, H-22), 1.44 (s, 3H, H-27), 1.41 (m, 2H, H-22' and H-21'), 1.29 (m, 1H, H-16'), 1.18 (s, 3H, H-25), 1.15 (s, 3H, H-29), 1.15 (s, 3H, H-26), 1.13 (m, 1H, H-1'), 1.05 (m, 1H, H-15'), 0.94 (s, 3H, H-23), 0.93 (s, 3H, H-24), 0.91 (m, 1H, H-5), 0.83 (s, 3H, H-28) ppm; ^{13}C-NMR (125 MHz, MeOH-d_4): δ = 202.0 (C-11), 178.5 (C-30), 172.6 (Asp-COOMe), 172.5 (Asp-COO), 169.0 (C-13), 128.8 (C-12), 85.3 (C-3), 62.9 (C-9), 56.1 (C-5), 52.4 (OMe), 50.9 (Asp-CHNH), 49.9 (C-18), 46.8 (C-8), 45.4 (C-20), 44.7 (C-14), 42.5 (C-19), 39.7 (C-1), 39.3 (C-22), 39.1 (C-4), 38.3 (C-10), 34.8 (Asp-CH_2), 33.7 (C-7), 33.0 (C-17), 32.1 (C-21), 29.2 (C-28), 28.6 (C-23), 28.6 (C-29), 27.7 (C-16), 27.5 (C-15), 24.5 (C-2), 23.9 (C-27), 19.4 (C-26), 18.5 (C-6), 17.3 (C-24), 17.0 (C-25) ppm; MS (ESI, MeOH): m/z (%) = 600.4 ($[M + H]^+$, 100); anal. calcd. for $C_{35}H_{54}NO_7Cl$ (636.26): C, 66.07; H, 8.55; N, 2.20; Cl, 5.57; found: C, 65.86; H, 8.73; N, 2.11; Cl, 5.64.

*Methyl 3β 3-(L-glutamyl)-11-oxo-olean-12-en-30-oate hydrochloride (**9**)*

Obtained from **7** (560 mg, 0.78 mmol) by method D as a colorless powder; yield: 470 mg, 93%; mp 236-239°C (dec.); R_F = 0.68 (methanol); $[\alpha]_D$ = 125.68° (c = 0.56, methanol); UV-VIS (methanol): λ_{max} (log ε) = 250 nm (4.11); IR (KBr): ν = 3438 (br), 2950 (s), 1732 (s), 1660 (s), 1619 (m), 1509 (m), 1465 (s), 1387 (s), 1355 (m), 1323 (m), 1279 (s), 1218 (s), 1153 (s), 1086 (m), 1048 (w), 987 (m), 948 (m), 914 (w), 868 (w), 823 (w), 769 (w), 748 (w), 714 (w), 701 (w), 672 (w), 619 (w), 590 (w), 546 (w), 505 (w), 456 (w) cm^{-1}; ^{1}H-NMR (500 MHz, MeOH-d_4): δ = 5.59 (s, 1H, H-12), 4.69 (dd, 1H, H-3, J = 11.7, 4.7 Hz), 4.15 (dd, 1H, Glu-CHN, J = 6.4, 6.4 Hz), 3.70 (s, 3H, OMe), 2.81 (ddd, 1H, H-1, J = 13.7, 3.4, 3.4 Hz), 2.60 (m, 1H, Glu-C*H*H'COOMe), 2.54 (m, 1H, Glu-CH*H*'COOMe), 2.51 (s, 1H, H-9), 2.28 (m, 1H, Glu-C*H*H'CHN), 2.17 (m, 1H, Glu-CH*H*'CHN), 2.16 (m, 1H, H-15), 2.13 (m, 1H, H-18), 1.97 (m, 1H, H-21), 1.89 (m, 1H, H-19), 1.87 (m, 1H, H-16), 1.79 (m, 1H, H-2), 1.76 (m, 1H, H-7), 1.74 (dd, 1H, H-19', J = 13.5, 13.5 Hz), 1.72 (m, 1H, H-2'), 1.66 (m, 1H, H-6), 1.55 (m, 1H, H-6'), 1.48 (m, 1H, H-7'), 1.46 (m, 1H, H-22), 1.44 (s, 3H, H-27), 1.41 (m, 2H, H-22' and H-21'), 1.29 (m, 1H, H-16'), 1.19 (s, 3H, H-25), 1.16 (s, 3H, H-29), 1.15 (s, 3H, H-26), 1.13 (m, 1H, H-1'), 1.05 (m, 1H, H-15'), 0.97 (s, 3H, H-23), 0.96 (s, 3H, H-24), 0.93 (m, 1H, H-5), 0.83 (s, 3H, H-28) ppm; ^{13}C-NMR (125 MHz, MeOH-d_4): δ = 202.2 (C-11), 178.6 (C-30), 175.2 (Glu-COOMe), 172.8 (Glu-COO), 170.2 (C-13), 128.9 (C-12), 85.2 (C-3), 62.9 (C-9), 56.0 (C-5), 53.7 (Glu-CHNH), 52.3 (OMe), 49.9 (C-18), 46.7 (C-8), 45.3 (C-20), 44.7 (C-14), 42.4 (C-19), 39.6 (C-1), 39.2 (C-22), 39.0 (C-4), 38.2 (C-10), 33.6 (C-7), 33.0 (C-17), 32.0 (C-21), 30.4 (Glu-CH_2COOMe), 29.1 (C-28), 28.7 (C-23), 28.5 (C-29), 27.6 (C-16), 27.3 (C-15), 26.8 (Glu-CH_2CHNH), 24.4 (C-2), 23.8 (C-27), 19.3 (C-26), 18.4 (C-6), 17.3 (C-24), 16.9 (C-25) ppm; MS (ESI, MeOH): m/z (%) = 614.3 ($[M + H]^+$, 100), 921.1 ($[3\ M + 2\ H]^{2+}$, 4); anal. calcd. for $C_{36}H_{56}NO_7Cl$ (650.29): C, 66.49; H, 8.68; N, 2.15; Cl, 5.45; found: C, 66.28; H, 8.85; N, 2.01; Cl, 5.67.

*Methyl 3β 3-(Boc-O-methyl-L-asparagyl)-11-oxo-olean-12-en-30-oate (**10**)*

Obtained from **6** (250 mg, 0.36 mmol) by method E as a colorless powder; yield: 240 mg, 93%; mp 213-217°C (dec.); R_F = 0.52 (hexane/ethyl acetate 7:3); $[\alpha]_D$ = 99.89° (c = 0.54, $CHCl_3$); UV-VIS (methanol): λ_{max} (log ε) = 250 nm (4.07); IR (KBr): ν = 3423 (br), 2954 (s), 2875 (m), 1747 (s), 1709 (s), 1652 (s), 1615 (w), 1506 (m), 1455 (m), 1433 (m), 1390 (m), 1364 (m), 1338 (m), 1287 (m), 1217 (s), 1162 (s), 1086 (m), 1052 (m), 1003 (m), 986 (m), 957 (w), 916 (w), 879 (w), 866 (m), 826 (w), 771 (w), 670 (w), 590 (w), 540 (w) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.66 (s, 1H,

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H-12), 5.50 (d, 1H, NH, $J = 8.6$ Hz), 4.56 (m, 2H, H-3 and Asp-CH), 3.69 (s, 3H, OMe), 3.68 (s, 3H, Asp-OMe), 3.03 (dd, 1H, Asp-C*H*H', $J = 17.1$, 3.9 Hz), 2.83 (m, 1H, Asp-CH*H*'), 2.80 (m, 1H, H-1), 2.35 (s, 1H, H-9), 2.08 (dd, 1H, H-18, $J = 13.4$, 3.3 Hz), 2.03 (m, 1H, H-15), 1.99 (m, 1H, H-21), 1.92 (ddd, 1H, H-19, $J = 13.8$, 4.0, 2.6 Hz), 1.82 (ddd, 1H, H-16, $J = 13.6$, 13.6, 4.1 Hz), 1.73 (m, 1H, H-2), 1.67 (m, 1H, H-2'), 1.63 (m, 1H, H-7), 1.60 (dd, 1H, H-19', $J = 13.5$, 13.5 Hz), 1.58 (m, 1H, H-6), 1.45 (s, 9H, Boc-CH_3), 1.43 (m, 1H, H-6'), 1.41 (m, 1H, H-7'), 1.38 (m, 1H, H-22), 1.36 (s, 3H, H-27), 1.31 (m, 1H, H-22'), 1.31 (m, 1H, H-21'), 1.18 (m, 1H, H-16'), 1.16 (s, 3H, H-25), 1.15 (s, 3H, H-29), 1.12 (s, 3H, H-26), 1.06 (m, 1H, H-1'), 1.02 (m, 1H, H-15'), 0.87 (s, 3H, H-24), 0.85 (s, 3H, H-23), 0.80 (m, 1H, H-5), 0.79 (s, 3H, H-28) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$): $\delta = 199.9$ (C-11), 176.9 (C-30), 171.5 (Asp-COOMe), 170.7 (Asp-COO), 169.2 (C-13), 155.4 (Boc-COO), 128.5 (C-12), 82.4 (C-3), 79.9 (Boc-*quart.*-C), 61.6 (C-9), 55.0 (C-5), 51.9 (Asp-OMe), 51.7 (OMe), 50.2 (Asp-$CHNH_2$), 48.4 (C-18), 45.4 (C-8), 44.0 (C-20), 43.2 (C-14), 41.1 (C-19), 38.7 (C-1), 38.1 (C-4), 37.7 (C-22), 36.9 (C-10), 36.6 (Asp-CH_2), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-28), 28.3 (C-29), 27.8 (C-23), 26.5 (C-16), 26.4 (C-15), 23.4 (C-2), 23.3 (C-27), 18.7 (C-26), 17.3 (C-6), 16.7 (C-24), 16.3 (C-25) ppm; MS (ESI, MeOH): *m/z* (%) = 714.2 ($[M + H]^+$, 5), 736.4 ($[M + Na]^+$, 100), 1093.3 ($[3\ M + 2\ Na]^{2+}$, 12), 1450.0 ($[2\ M + Na]^+$, 22); anal. calcd. for $C_{41}H_{63}NO_9$ (713.94): C, 68.97; H, 8.89; N, 1.96; found: C, 68.76; H, 9.04; N, 1.79.

*Methyl 3β 3-(Boc-O-methyl-L-glutamyl)-11-oxo-olean-12-en-30-oate (**11**)*

Obtained from **7** (500 mg, 0.70 mmol) by method E as a colorless powder; yield: 390 mg, 77%; mp 202-205°C; $R_F = 0.38$ (hexane/ethyl acetate 7:3); $[\alpha]_D = 95.04°$ ($c = 0.66$, $CHCl_3$); UV-VIS (methanol): λ_{max} (log ε) = 250 nm (4.10); IR (KBr): $\nu = 3381$ (br), 2951 (s), 2874 (m), 1748 (s), 1726 (s), 1711 (s), 1652 (s), 1509 (m), 1453 (m), 1390 (m), 1366 (m), 1354 (m), 1326 (m), 1264 (m), 1216 (s), 1163 (s), 1087 (w), 1076 (w), 1050 (w), 1026 (m), 984 (m), 918 (w), 882 (w), 825 (w), 772 (w), 538 (w) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): $\delta = 5.67$ (s, 1H, H-12), 5.09 (d, 1H, Glu-NH, $J = 7.9$ Hz), 4.56 (dd, 1H, H-3, $J = 11.6$, 4.8 Hz), 4.32 (m, 1H, Glu-CHN), 3.69 (s, 3H, Glu-OMe), 3.68 (s, 3H, OMe), 2.82 (ddd, 1H, H-1, $J = 13.7$, 3.4, 3.4 Hz), 2.45 (m, 1H, Glu-C*H*H'COOMe), 2.39 (m, 1H, Glu-CH*H*'COOMe), 2.36 (s, 1H, H-9), 2.23 (m, 1H, Glu-C*H*H'CHN), 2.08 (dd, 1H, H-18, $J = 13.7$, 3.5 Hz), 2.03 (m, 1 H, H-15), 1.99 (m, 1H, H-21), 1.96 (m, 1H, Glu-CH*H*'CHN), 1.92 (ddd, 1H, H-19, $J = 13.3$, 3.3, 2.3 Hz), 1.83 (ddd, 1H, H-16, $J = 13.7$, 13.7, 4.2 Hz), 1.74 (m, 1H, H-2), 1.66 (m, 1H, H-7), 1.63 (m, 1H, H-2'), 1.61 (dd, 1H, H-19', $J = 13.5$, 13.5 Hz), 1.57 (m, 1H, H-6), 1.46 (m, 1H, H-6'), 1.44 (s, 9H, Boc-CH_3), 1.42 (m, 1H, H-7'), 1.39 (m, 1H, H-22), 1.36 (s, 3H, H-27), 1.31 (m, 2H, H-22' and H-21'), 1.18 (m, 1H, H-16'), 1.17 (s, 3H, H-25), 1.15 (s, 3H, H-29), 1.13 (s, 3H, H-26), 1.07 (m, 1H, H-1'), 1.02 (m, 1H, H-15'), 0.90 (s, 3H, H-24), 0.88 (s, 3H, H-23), 0.81 (s, 3H, H-28), 0.80 (m, 1H, H-5) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$): $\delta = 199.9$ (C-11), 173.2 (Glu-COOMe), 176.9 (C-30), 171.6 (Glu-COO), 169.2 (C-13), 155.3 (Boc-COO), 128.5 (C-12), 82.2 (C-3), 79.9 (Boc-*quart.*-C), 61.7 (C-9), 55.0 (C-5), 53.3 (Glu-CHNH), 51.8 (OMe), 48.4 (C-18), 45.4 (C-8), 44.0 (C-20), 43.2 (C-14), 41.1 (C-19), 38.7 (C-1), 38.1 (C-4), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 30.2 (Glu-CH_2COOMe), 28.5 (C-28), 28.3 (C-29), 28.3 (Boc-CH_3), 28.1 (C-23), 28.0 (Glu-CH_2CHNH), 26.5 (C-16), 26.4 (C-15), 23.6 (C-2), 23.3 (C-27), 18.7 (C-26), 17.3 (C-6), 16.8 (C-24), 16.4 (C-25) ppm; MS (ESI, MeOH): *m/z* (%) = 750.4 ($[M + Na]^+$, 100), 1114.3 ($[3\ M + 2\ Na]^{2+}$, 22), 1478.1 ($[2\ M + Na]^+$, 24); anal. calcd. for $C_{42}H_{65}NO_9$ (727.97): C, 69.30; H, 9.00; N, 1.92; found: C, 69.11; H, 9.24; N, 1.68.

*Methyl 3β 3-(O-methyl-L-asparagyl)-11-oxo-olean-12-en-30-oate (**12**)*

Obtained from **10** (550 mg, 0.79 mmol) by method B as a colorless powder; yield: 360 mg, 83%; mp 226-229°C (dec.); $R_F = 0.73$ (dichloromethane/methanol 9:1); $[\alpha]_D = 111.72°$ ($c = 0.47$, $CHCl_3$); UV-VIS (methanol): λ_{max} (log ε) = 249 nm (3.62); IR (KBr): $\nu = 3365$ (br), 2962 (m), 2874 (m), 1739 (s), 1727 (s), 1652 (s), 1614 (w), 1438 (m), 1389 (m), 1318 (w), 1264 (m), 1218 (m), 1186 (m), 1161 (m), 1086 (w), 1026 (m), 988 (w), 959 (w), 904 (w), 879 (w), 851 (w), 826 (w), 771 (w), 670 (w), 590 (w), 543 (w) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): $\delta = 5.67$ (s, 1H, H-12), 4.58 (dd, 1H, H-3, $J = 11.6$, 4.9 Hz), 3.80 (dd, 2H, Asp-CH, $J = 7.1$, 4.7 Hz), 3.70 (s, 3H, OMe), 3.69 (s, 3H, Asp-OMe), 2.83 (m, 1H, Asp-C*H*H'), 2.81 (m, 1H, H-1), 2.72 (dd, 1H, Asp-CH*H*', $J = 16.5$, 7.2 Hz), 2.36 (s, 1H, H-9), 2.08 (dd, 1H, H-18, $J = 12.8$, 3.6), 2.03 (m, 1H, H-15), 1.99 (m, 1H, H-21), 1.92 (ddd, 1H, H-19, $J = 13.5$, 3.9, 2.7 Hz), 1.82 (ddd, 1H, H-16, $J = 13.6$, 13.6, 4.2 Hz), 1.73 (m, 1H, H-2), 1.69 (m, 1H, H-7), 1.63 (m, 1H, H-2'), 1.61 (dd, 1H, H-19', $J = 13.5$, 13.5 Hz), 1.57 (m, 1H, H-6), 1.48 (m, 1H, H-6'), 1.43 (m, 1H, H-7'), 1.40 (m, 1H, H-22), 1.36 (s, 3H, H-27), 1.31 (m, 1H, H-22'), 1.31 (m, 1H, H-21'), 1.18 (m, 1H, H-16'), 1.16 (s, 3H, H-25), 1.15 (s, 3H, H-29), 1.13 (s, 3H, H-26), 1.06 (m, 1H, H-1'), 1.01 (m, 1H, H-15'), 0.87 (s, 3H, H-24), 0.87 (s, 3H, H-23), 0.81 (m, 1H, H-5), 0.80 (s, 3H, H-28) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$): $\delta = 199.9$ (C-11), 176.9 (C-30), 173.9 (Asp-COO), 171.6 (Asp-COOMe), 169.2 (C-13), 128.5 (C-12), 81.8 (C-3), 61.6 (C-9), 55.0 (C-5), 51.8 (Asp-OMe), 51.7 (OMe), 51.5 (Asp-CH), 48.4 (C-18), 45.4 (C-8), 44.0 (C-20), 43.2 (C-14), 41.1 (C-19), 38.7 (C-1), 38.6 (Asp-CH_2), 38.1 (C-4), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-28), 28.3 (C-29), 28.0 (C-23), 26.5 (C-16), 26.4 (C-15), 23.5 (C-2), 23.3 (C-27), 18.7 (C-26), 17.3 (C-6), 16.7 (C-24), 16.3 (C-25) ppm; MS (ESI, MeOH): *m/z* (%) = 614.2 ($[M + H]^+$, 100), 636.3 ($[M + Na]^+$, 4); anal. calcd. for $C_{36}H_{55}NO_7$ (613.82): C, 70.44; H, 9.03; N, 2.28; found: C, 70.25; H, 9.19; N, 2.14.

*Methyl 3β 3-(O-methyl-L-glutamyl)-11-oxo-olean-12-en-30-oate (**13**)*

Obtained from **11** (320 mg, 0.44 mmol) by method B as a colorless powder; yield: 260 mg, 94%; mp 288-290°C (dec.); $R_F = 0.73$ (dichloromethane/methanol 9:1); $[\alpha]_D = 123.60°$ ($c = 0.54$, methanol); UV-VIS (methanol): λ_{max} (log ε) = 250 nm (4.13); IR (KBr): $\nu = 3259$ (br), 2956 (s), 1724 (s), 1652 (s), 1582 (w), 1466 (m), 1389 (m), 1365 (m), 1322 (w), 1233 (s), 1164 (m), 1105 (m), 1022 (w), 982 (m), 919 (w), 850 (w), 750 (w), 682 (w), 589 (w), 541 (w) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): $\delta = 5.66$ (s, 1H, H-12), 4.56 (dd, 1H, H-3, $J = 11.8$, 4.7 Hz), 3.67 (s, 3H, Glu-OMe), 3.66 (s, 3H, OMe), 3.43 (dd, 1H, Glu-CHN, $J = 8.4$, 4.7 Hz), 2.80 (ddd, 1H, H-1, $J = 13.7$, 3.3, 3.3 Hz), 2.47 (dd, 2H, Glu-CH_2COOMe, $J = 7.6$, 7.2 Hz), 2.34 (s, 1H, H-9), 2.11 (m, 1H, Glu-C*H*H'CHN), 2.06 (m, 1H, H-18), 2.01 (m, 1H, H-15), 1.97 (m, 1H, H-21), 1.90 (ddd, 1H, H-19, $J = 13.7$, 3.3, 2.5 Hz), 1.83 (m, 1H, Glu-CH*H*'CHN), 1.79 (m, 1H, H-16), 1.70 (m, 1H, H-2), 1.65 (m, 1H, H-7), 1.61 (m, 1H, H-2'), 1.59 (dd, 1H, H-19', $J = 13.3$, 13.3 Hz), 1.55 (m, 1H, H-6), 1.45 (m, 1H, H-6'), 1.40 (m, 1H, H-7'), 1.35 (m, 1H, H-22), 1.34 (s, 3H, H-27), 1.29 (m, 2H, H-22' and H-21'), 1.16 (m, 1H, H-16'), 1.14 (s, 3H, H-25), 1.13 (s, 3H, H-29), 1.11 (s, 3H, H-26), 1.04 (m, 1H, H-1'), 0.99 (m, 1H, H-15'), 0.88 (s, 3H, H-24), 0.86 (s, 3H, H-23), 0.80 (m, 1H, H-5), 0.78 (s, 3H, H-28) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$): $\delta = 200.0$ (C-11), 176.9 (C-30), 173.6 (Glu-COOMe), 171.6 (Glu-COO), 169.2 (C-13), 128.5 (C-12), 81.5 (C-3), 61.7 (C-9), 55.0 (C-5), 54.1 (Glu-CHNH), 51.7 (OMe), 51.6 (Glu-OMe), 48.4 (C-18), 45.4 (C-8), 44.0 (C-20), 43.2 (C-14), 41.1 (C-19), 38.7 (C-1), 38.1 (C-4), 37.7 (C-22), 36.9 (C-10), 32.7

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(C-7), 31.8 (C-17), 31.1 (C-21), 30.5 (Glu-CH_2COOMe), 29.6 (Glu-CH_2CHNH), 28.5 (C-28), 28.3 (C-29), 28.1 (C-23), 26.5 (C-16), 26.4 (C-15), 23.6 (C-2), 23.3 (C-27), 18.7 (C-26), 17.3 (C-6), 16.8 (C-24), 16.4 (C-25) ppm; MS (ESI, MeOH): m/z (%) = 628.3 ($[M + H]^+$, 100); anal. calcd. for $C_{37}H_{57}NO_7$ (627.85): C, 70.78; H, 9.15; N, 2.23; found: C, 70.59; H, 9.25; N, 2.07

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The authors have declared no conflict of interest.

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Anhang A5

Does one keto group matter? Structure-activity relationships of glycyrrhetinic acid derivatives modified at position C-11.

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Glycyrrhetinic Acid Derivatives

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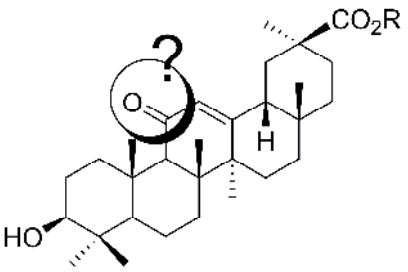
Keywords: *glycyrrhetinic acid; apoptosis; antitumor activity; mouse embryonic fibroblasts*

Graphical abstract

It remains disputable whether the C11 keto group in glycyrrhetinic acid derivatives is the main reason for their apoptotic effect.

Insert Graphic “graphical abstract”



Summary

Several triterpenoic acids display a remarkable cytotoxicity on tumor cells. Glycyrrhetinic acid - the main content of licorice root - possesses an apoptotic effect on tumor cells. Previous studies pointed out the presence of a keto group at position C-11 in glycyrrhetinic acid derivatives as the main reason for its apoptotic activity. Several pairs of derivatives were synthesized differing only at position C-11. These compounds were tested in a sulforhodamine B colorimetric assay for cytotoxicity screening on 12 tumor cell lines and mouse embryonic fibroblasts (NiH3T3). Our results show there is no direct relation between the existence of the C-11 keto group and the apoptotic activity of the compounds.

Introduction

Known as antitumor agents for several years, triterpenoic acids are still in the focus of scientific interest. Betulinic acid, oleanoic acid, ursolic acid, boswellic acid or glycyrrhetinic acid are well-known species of this class. They have a proven cytotoxicity on tumor cells [1-4] by triggering apoptosis [4-8]. All of these triterpenes consist of five condensed cycloalkyl rings which give them a high degree in molecular likeness. Having a hydroxyl group on ring A and a carboxyl group on ring E in common, glycyrrhetinic acid (**1**, Scheme 1), however, differs because of the presence of an α,β-unsaturated keto group in position C-11 on ring C. Previous studies pointed out the importance of both A and C ring substitutions.

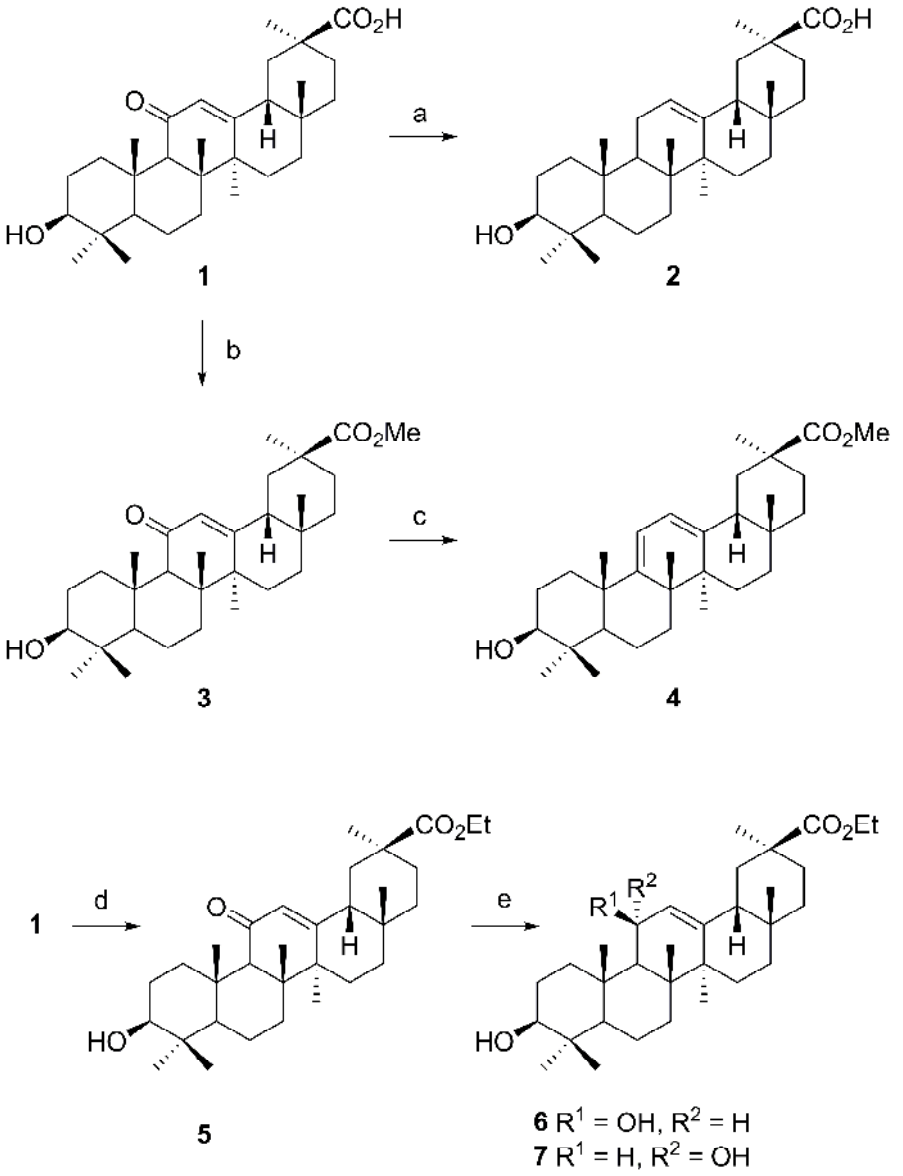
Results and Discussion

For glycyrrhetinic acid (**1**) several references suggested the keto-group in position C-11 is the major reason for the cytotoxic and apoptotic effects [9, 10]. Based on a probable keto-enol tautomerism this keto group was proposed to be involved in a mechanism producing an oxygen radical. Among others, Fiore et al. [9] deduced that this should impel mitochondrial permeability transition finally leading to cell death.

We examined three different pairs of derivatives to explore the influence of a keto group in position C-11. In a first try, we chose **1** and its deoxygenated analogue **2** (Scheme 1) for comparison. To remove the keto group, **1** was subjected to a *Clemmensen*-reduction with zinc

dust and concd. HCl in 1,4-dioxane at room temperature to afford **2** [11]. A second pair of compounds consisted of the respective methyl ester **3** and the diene **4**. Following the procedure of Su et al. [12], **1** was easily formed into the ester **3** by adding methyl iodide and potassium carbonate to a solution of **1** in dry DMF. The reaction of **3** with borane in THF followed by acidic workup provided **4**. Finally, a third couple consisted of the ethyl ester **5** and the products of its reduction (borane/THF, basic work-up) **6** and **7**, respectively.

Insert Scheme 1



Scheme 1. Reagents and conditions: a) zinc dust, conc. HCl, dioxane, 25°C, 24 h; b) MeI, K_2CO_3, DMF, 25°C, 24 h; c) BH_3-THF, THF, citric acid, 25°C, 20 h; d) EtI, K_2CO_3, DMF, 25°C, 24 h; e) BH_3-THF, THF, Na_2CO_3, 25°C, 4 d.

To show a correlation between the biological activity of the compounds and the presence or the absence of the keto group a paired comparison was carried out. A sulforhodamine assay (SRB) [13] was conducted on 12 different tumor cell lines as well as on a mouse embryonic fibroblast cell line (NiH3T3). Our first pair, **1** and **2** showed in a SRB-assay almost the same activity on tumor cells, i.e. the averaged IC_{50} (from 12 cell lines, Table 1) was 80.78 μM and 80.99 μM, respectively. For the methyl ester **3** a slightly lower cytotoxicity (22.81 μM) on mouse embryonic fibroblast NiH3T3 cells was established; the parent compound **1** displayed an activity of 18.52 μM.

However, there are different effects to be established between the non-tumorous cell line and the tumor cell lines as suggested by the IC_{50}-values. Compounds **3** and **4** possess a comparable high cytotoxicity for the tumor cell lines. Compared to ester **3**, the deoxygenated compound **4** shows a decreased toxicity for the fibroblasts and most of the tumor cell lines.

In accord with these results, a similar behaviour was found for the third pair of compounds - **5** and **6**. Their cytotoxic effect on the tumor cells is similar to their effect on NiH3T3 cells. Unfortunately, we were not able to test compound **7** in an SRB-assay; compound **7** undergoes under screening conditions an elimination reaction leading to the formation of **4**. As shown by NMR, significant deterioration of **7** advances quickly in less than one day.

Insert Table 1

Table 1: Results of SRB-assay: The values (IC_{50} in μM) for melanoma (518A2), zervic cancer (A431), lung carcinoma (A549), ovarian cancer (A2780), colon cancer (DLD-1, HCT-8, HCT-116, HT-29), anaplastic thyroid cancer (8505C, SW-1736), mamma carcinoma (MCF-7) and liposarcoma were obtained by an SRB-assay after 96 h of treatment and are the average from at least three independent experiments; standard error 5%.

	compound					
	1	**2**	**3**	**4**	**5**	**6**
518A2	83.92	71.49	27.54	34.54	25.23	51.52
8505C	86.50	78.52	26.07	33.88	24.58	52.80
A2780	74.57	62.78	25.54	23.58	26.96	57.01

A431	79.58	86.13	25.28	33.55	23.45	46.55
A549	82.76	79.13	23.50	31.59	22.74	48.97
DLD-1	81.21	90.50	26.12	31.73	28.14	52.80
HCT-116	78.83	87.70	22.10	31.82	21.58	47.78
HCT-8	78.85	88.76	24.36	31.34	43.42	44.32
HT-29	80.09	90.30	27.54	23.89	22.14	44.32
LIPO	81.44	73.88	20.47	34.81	27.66	52.80
MCF-7	84.70	90.19	22.14	34.37	18.61	48.97
SW1736	76.93	72.47	34.87	32.35	13.37	45.48
NIH 3T3	18.52	68.70	22.81	42.22	23.66	43.16

In Acridine Orange/Ethidium Bromide (AO/EB) tests (Fig. 1), apoptotic cells are easily distinguished from necrotic. In addition, trypan blue staining followed by an automated cell counting allows quantification of apoptotic behaviour.

Insert Fig. 1

Fig 1. Results from the AO/EB test: Treatment of A549 cells with (from left to right): **1** (90 μM) and **2** (90 μM), **3** (35 μM) and **4** (35 μM), **5** (60 μM) and **6** (60 μM).

As displayed in Fig. 1, in the AO/EB tests apoptotic cells are stained in a green colour with bright spots whereas necrotic cells appear in an orange-red colour. The results from these experiments are summarized in Table 2.

Table 2. Apoptotic effect of GA derivatives in A549 cells (in %, standard error 5 %, 6 independent experiments); cells were treated with **1** (90 μM), **2** (90 μM), **3** (35 μM), **4** (35 μM), **5** (60 μM) or **6** (60 μM).

Compound	**1**	**2**	**3**	**4**	**5**	**6**
Apoptosis [%]	73.73 ±1.40	76.43 ±1.78	70.64 ±0.74	66.93 ±5.80	44.92 ±4.13	80.62 ±2.67

Insert Table 2

Investigations by Fiore et al. [9] for **1** and by Salvi et al. [10] for the antitumor active drug carbenoxolone suggest the presence of a keto group at C-11 was the main reason for the cytotoxicity of these compounds on liver cells. Pairs of compounds, **1** and **2** as well as **3** and **4** showed insignificant differences in the extent of apoptosis. For compounds **5** and **6**, however, a significant difference is encountered (Δ% = ca. 35%). Interestingly enough, treatment of the cells with **6** (with a missing carbonyl group) results in the formation of more apoptotic cells compared to compound **5**.

In addition, our results show that **1** has a much stronger activity on fibroblasts than on tumor cells. This effect is lost after removing of the 11-keto group. Upon removal of this group, the cytotoxicity on tumor cell lines and on fibroblasts becomes comparably high. Except for glycyrrhetinic acid (**1**), all the compounds holding an 11-keto group show similar IC_{50} values both for the tumor cell lines and for the fibroblast cells. One would expect a significant loss of cytotoxicity after removal of the keto group; however, the activity remains unaltered. The differences in the activity of the compounds **5** and **6** might be based on the presence of an extra hydroxyl group. Introducing such a polar group creates a different polarity pattern in the molecule. Decreasing the number of free polar groups in the molecule, for example, by esterification, increases the cytotoxicity of the compounds. Therefore, the presence of an extra hydroxyl group at position C-11 would be responsible for the drop of activity.

In summary, it is disputable whether the C-11 keto group is the main reason for the apoptotic effect of glycyrrhetinic acid and derivatives

Experimental

General

Melting points are uncorrected (*Leica* hot stage microscope), NMR spectra were recorded using the Varian spectrometers Gemini 200, Gemini 2000 or Unity 500 (δ given in ppm, *J* in Hz, internal Me_4Si), optical rotations were obtained using a Perkin-Elmer 341 polarimeter (1 cm micro cell, 25 °C), IR spectra (film or KBr pellet) on a Perkin-Elmer FT-IR spectrometer Spectrum 1000, MS spectra were taken on an Intectra GmbH AMD 402 (electron impact, 70

eV) or on a Finnigan MAT TSQ 7000 (electrospray, voltage 4.5 kV, sheath gas nitrogen) instrument. TLC was performed on silica gel (Merck 5554, detection by UV absorption). The solvents were dried according to usual procedures.

Cell lines and Culture Conditions

The cell lines 518A2, 8505C, A253, A2780, A431, A549, DLD-1, FaDu, HCT-116, HCT-8, HT-29, LIPO, MCF-7, SW1736, and SW480 were included in this study. Cultures were maintained as monolayer in RPMI 1640 (PAA Laboratories, Pasching, Germany) supplemented with 10% heat inactivated fetal bovine serum (Biochrom AG, Berlin, Germany) and penicillin / streptomycin (PAA Laboratories) at 37 °C in a humidified atmosphere of 5% CO_2/ 95% air.

Cytotoxicity Assay

The cytotoxicity of the compounds was evaluated using the sulforhodamine-B (SRB) (Sigma Aldrich) microculture colorimetric assay. In short, exponentially growing cells were seeded into 96-well plates on day 0 at the appropriate cell densities to prevent confluence of the cells during the period of experiment. After 24h, the cells were treated with serial dilutions of the compounds (0-300 µM) for 96 h. The final concentration of DMSO or DMF solvent never exceeded 0.5%, which was non-toxic to the cells. The percentages of surviving cells relative to untreated controls were determined 96 h after the beginning of drug exposure. After a 96 h treatment, the supernatant medium from the 96 well plates was thrown away and the cells were fixed with 10% TCA. For a thorough fixation, the plates were allowed to rest at 4 °C. After fixation, the cells were washed in a strip washer. The washing was done four times with water using alternate dispensing and aspiration procedures. The plates were then dyed with 100 µl of 0.4% SRB (sulforhodamine B) for about 20 min. After dying the plates were washed with 1% acetic acid to remove the excess of the dye and allowed to air dry overnight. 100 µl of 10 mM Tris base solution were added to each well and absorbance was measured at 570 nm (using a 96 well plate reader, Tecan Spectra, Crailsheim, Germany). The IC_{50} was estimated from the semi-logarithmic dose-response curves.

Apoptosis test - dye exclusion test

Apoptotic cell death was analysed by trypan blue dye (Sigma Aldrich, Germany) on A431 and A2780 cell lines. The cell culture flasks with 70% to 80% confluence were treated with IC_{90} does of the compounds for 24h. The supernatant medium with floating cells was collected

after treatment and centrifuged to collect dead and apoptotic cells. This pellet was re-suspended in serum free media. Equal amounts of cell suspension and trypan blue were mixed and analysed under a microscope. Viable cells exclude the dye and appear colourless whereas cells whose cell membrane is destroyed are stained in blue colour. If there are more colourless cells than stained cells, then death of the cells can be characterised as apoptotic.

Apoptosis test – Acridine Orange/Ethidium Bromide (AO/EB)

Apoptotic cell death was analysed by acridine orange/ethidium bromide dye using fluorescence microscopy on A549 cells. Therefore approx. 500.000 cells were seeded in cell culture flasks and were allowed to grow for 24 hours. The medium was removed and the substance loaded medium was added. After 24-48 h, the supernatant medium was collected and centrifuged; the pellet was suspended in phosphate-buffer saline (PBS) and centrifuged again. The liquid was removed and the pellet was suspended in PBS. After mixing the suspension with a solution of AO/EB, analysis was performed under a fluorescence microscope. While a green fluorescence shows apoptosis, a red coloured nucleus indicates necrotic cells.

Apoptosis test - Trypan blue cell counting

Approx. 500,000 cells (A549) were seeded in cell culture flasks and were allowed to grow for 1 day. After removing of the medium, the substance loaded medium was introduced and the flasks were incubated for about 24-48 hours. The supernatant medium was collected and centrifuged; cell pellet was suspended in PBS and centrifuged again. Equal amounts of trypan blue solution (0.4 % in phosphate-buffer saline, pH 7.2) and suspension of the pellet in PBS were mixed and put on chamber slides (Invitrogen™). Automatic cell counter (Invitrogen™ countess® automated cell counter) was used for counting the cells, differing between cells with an intact cell membrane and cells without.

3β-Hydroxy-olean-12-en-30-acid (**2**)

To a mixture of **1** (490 mg, 1.04 mmol) and zinc dust (160 mg, 2.45 mmol) in 1,4-dioxane (15 ml), conc. HCl was slowly added until the reaction of the zinc was complete. After one day of stirring at 25 °C, the solvent was evaporated and the residue subjected to chromatography (silical gel, hexane/ethyl acetate 7:3). Recrystallization from acetic acid gave **2** (180 mg, 38%) as colourless needles; mp 308-313 °C (lit. 323-325 °C [15], 322-325 °C [16]); R_f = 0.77 (ethyl acetate/hexane 1:1); $[\alpha]_D$ = 116.97° (*c* 0.34, methanol).

Methyl 3β-hydroxy-11-oxo-olean-12-en-30-oate (**3**)

A solution of **1** (31.00 g, 65.9 mmol) in dry DMF (150 ml) containing potassium carbonate (15.34 g, 111.0 mmol) was stirred at room temperature for 30 min. Iodomethane (4.94 ml, 79.0 mmol) was added and the mixture was further stirred for 2 h. The solvents were evaporated and the crude residue was dissolved in a mixture of dichloromethane (300 ml) and hydrochloric acid (50 ml, 1.0 M). The aqueous layer was extracted with dichloromethane (3 x 50 ml), the combined organic layers were washed with brine (50 ml), dried (Na_2SO_4), filtered and evaporated. Recrystallization from methanol yielded **3** (29.09 g, 91.1 %) as a colourless crystalline solid; mp 254-258 °C (lit. 254-258 °C [12], 254-256 °C [17], 248-250°C [18,19]); R_f = 0.48 (hexane/ethyl acetate 7:3); $[\alpha]_D$ = 141.18° (*c* 0.48, $CHCl_3$). UV-vis (methanol): λ_{max} (log ε) = 270 nm (4.15); MS (ESI): *m/z* (%) = 485.5 ($[M+H]^+$, 55), 507.5 ($[M+Na]^+$, 12), 539.1 ($[M+Na+MeOH]^+$, 100); IR (KBr): ν = 3614*br*, 2970*s*, 2955*s*, 2875*m*, 1726*s*, 1659*s*, 1466*m*, 1450*m*, 1364*w*, 1216*m*, 1189*m*, 1136*w*, 1085*w*, 1040*w*, 992*w* cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 5.65 (*s*, 1 H, H-12), 3.67 (*s*, 3 H, methyl), 3.21 (*dd*, 1 H, H-3, *J* = 10.8, 5.7 Hz), 2.78 (*ddd*, 1 H, H-1, *J* = 13.3, 3.3, 3.3 Hz), 2.32 (*s*, 1 H, H-9), 2.06 (*dd*, 1 H, H-18, *J* = 13.3, 3.8 Hz), 2.00 (*m*, 1 H, H-15), 1.95 (*m*, 1 H, H-21), 1.90 (*dd*, 1 H, H-19, *J* = 13.8, 3.9 Hz), 1.83 (*ddd*, 1 H, H-16, *J* = 14.3, 14.3, 5.2 Hz), 1.65 (*m*, 1 H, H-2), 1.62 (*m*, 1 H, H-7), 1.58 (*m*, 1 H, H-2'), 1.57 (*m*, 1 H, H-19'), 1.57 (*m*, 1 H, H-6), 1.43 (*m*, 1 H, H-6'), 1.38 (*m*, 1 H, H-7'), 1.36 (*m*, 1 H, H-22), 1.34 (*s*, 3 H, H-27), 1.30 (*m*, 1 H, H-22'), 1.28 (*m*, 1 H, H-21'), 1.17 (*m*, 1 H, H-16'), 1.13 (*s*, 3 H, H-28), 1.12 (*s*, 3 H, H-25), 1.11 (*s*, 3 H, H-26), 1.00 (*m*, 1 H, H-15'), 0.99 (*s*, 3 H, H-23), 0.96 (*m*, 1 H, H-1'), 0.79 (*s*, 3 H, H-24), 0.79 (*s*, 3 H, H-29), 0.68 (*m*, 1 H, H-5); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 176.8 (C-30), 169.0 (C-13), 128.6 (C-12), 78.8 (C-3), 61.9 (C-9), 55.1 (C-5), 51.8 (methyl), 48.5 (C-18), 45.5 (C-14), 44.1 (C-20), 43.3 (C-8), 41.2 (C-19), 39.2 (C-1), 39.2 (C-4), 37.8 (C-22), 37.2 (C-10), 32.9 (C-7), 31.9 (C-17), 31.2 (C-21), 28.6 (C-29), 28.4 (C-23), 28.2 (C-28), 27.4 (C-2), 26.6 (C-16), 26.6 (C-15), 23.5 (C-27), 18.8 (C-26), 17.6 (C-6), 16.4 (C-25), 15.6 (C-24).

Methyl 3β-hydroxy-oleana-9(11),12-dien-30-oate (**4**)

To a solution of **3** (240 mg, 0.49 mmol) in dry THF (10 ml), borane (1 M in THF, 7 ml, 7 mmol) was slowly added. After 20 h of stirring at 25 °C, ethyl acetate (25 ml) was added and the organic layer was washed with a solution of citric acid (10%), a saturated solution of sodium hydrogen carbonate and water. After drying (Na_2SO_4), filtration and evaporation, the residue was subjected to chromatography (silical gel, hexane/ethyl acetate 7:3) to yield **4** (162 mg, 71%) as colourless crystals. Mp 207-210 °C (lit. 243-245 °C [20]); R_f = 0.60

(hexane/ethyl acetate 7:3); $[\alpha]_D$ = 296.70° (*c* 0.50, $CHCl_3$).

Ethyl 3β-hydroxy-11-oxo-olean-12-en-30-oate (**5**)

Following the procedure given for **3**, **5** was obtained from **1** and iodoethane (4.0 ml, 49.5 mmol) as a white crystalline solid (1.75 g, 82%). Mp 220-224 °C (lit. 93-94 °C [12]); R_f = 0.40 (hexane/ethyl acetate 7:3); $[\alpha]_D$ = 144.35° (*c* 0.48, $CHCl_3$); UV-vis (methanol): λ_{max} (log ε) = 267 nm (3.90); MS (ESI): *m/z* (%) = 499.5 ($[M+H]^+$, 90), 521.4 ($[M+Na]^+$, 13), 552.9 ($[M+Na+MeOH]^+$, 100); IR (KBr): ν = 3543*br*, 2939*s*, 2869*m*, 1726*s*, 1651*s*, 1616*m*, 1455*m*, 1389*m*, 1364*w*, 1330*m*, 1312*w*, 1278*w*, 1257*m*, 1210*m*, 1172*s*, 1135*w*, 1086*m*, 1042*m*, 1028*m*, 994*m*, 920*w*, 880*w*, 767*w*, 704*w*, 671*w*, 604*w* cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 5.61 (*s*, 1 H, H-12), 4.16 (*dq*, 1 H, methylene-HH', *J* = 10.8 Hz, 7.1), 4.10 (*dq*, 1 H, methylene-HH', *J* = 10.8, 7.1 Hz), 3.19 (*dd*, 1 H, H-3, *J* = 11.1, 5.1 Hz), 2.76 (*ddd*, 1 H, H-1, *J* = 13.5, 3.6, 3.6 Hz), 2.31 (*s*, 1 H, H-9), 2.07 (*ddd*, 1 H, H-18, *J* = 12.5, 4.1, 1.1 Hz), 2.00 (*ddd*, 1 H, H-15, *J* = 13.7, 13.7, 4.5 Hz), 1.96 (*m*, 1 H, H-21), 1.88 (*ddd*, 1 H, H-19, *J* = 13.5, 4.2, 2.8 Hz), 1.80 (*ddd*, 1 H, H-16, *J* = 13.7, 13.7, 4.3 Hz), 1.64 (*m*, 1 H, H-2), 1.61 (*m*, 1 H, H-7), 1.58 (*m*, 1 H, H-2'), 1.57 (*m*, 1 H, H-19'), 1.56 (*m*, 1 H, H-6), 1.42 (*ddd*, 1 H, H-6', *J* = 12.4, 3.1, 3.1 Hz), 1.38 (*m*, 1 H, H-7'), 1.35 (*m*, 1 H, H-22), 1.34 (*s*, 3 H, H-27), 1.28 (*m*, 1 H, H-22'), 1.26 (*m*, 1 H, H-21'), 1.23 (*t*, 3 H, ethyl, *J* = 7.1 Hz), 1.15 (*m*, 1 H, H-16', *J* = 13.7, 4.5, 2.7 Hz), 1.11 (*s*, 3 H, H-28), 1.11 (*s*, 3 H, H-25), 1.10 (*s*, 3 H, H-26), 0.99 (*m*, 1 H, H-15'), 0.97 (*s*, 3 H, H-23), 0.95 (*ddd*, 1 H, H-1', *J* = 10.4, 4.4, 4.4 Hz), 0.78 (*s*, 3 H, H-24), 0.78 (*s*, 3 H, H-29), 0.67 (*dd*, 1 H, H-5, *J* = 11.5, 1.6 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.2 (C-11), 176.3 (C-30), 169.2 (C-13), 128.5 (C-12), 78.7 (C-3), 61.8 (C-9), 60.3 (ethylene), 54.9 (C-5), 48.4 (C-18), 45.4 (C-14), 43.8 (C-20), 43.2 (C-8), 41.1 (C-19), 39.1 (C-1), 39.1 (C-4), 37.7 (C-22), 37.1 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-29), 28.3 (C-23), 28.1 (C-28), 27.3 (C-2), 26.5 (C-16), 26.4 (C-15), 23.4 (C-27), 18.7 (C-26), 17.5 (C-6), 16.3 (C-25), 15.5 (C-24), 14.3 (methyl); anal. calcd for $C_{32}H_{50}O_4$ (498.74): C, 77.06; H, 10.10; found: C, 76.82; H, 10.21.

Ethyl 3β,11β-dihydroxy-olean-12-en-30-oate (6) and ethyl 3β,11α-dihydroxy-olean-12-en-30-oate (**7**)

Compound **2** (2.00 g, 4.02 mmol) was dissolved in anhydrous THF (80 ml). After addition of borane (1 M in THF, 12 ml, 12 mmol), the mixture was stirred for 4 days at 25 °C. The mixture was poured into a saturated solution of sodium bicarbonate (50 ml) and extracted with chloroform. The combined organic layers were concentrated in vacuo; the residue was purified by chromatography (silica gel, hexane/ethyl acetate 7:3) to yield **6** and **7**.

Data for **6:** colourless solid (1.18 g, 59%); mp 157-160 °C; R_f = 0.38 (hexane/ethyl acetate 7:3); $[\alpha]_D$ = 112.38° (*c* 0.55, $CHCl_3$); UV-vis (methanol): λ_{max} (log ε) = 298 nm (3.44); MS (ESI): *m/z* (%) = 523.4 ($[M+Na]^+$, 27), 554.9 ($[M+Na+MeOH]^+$, 100); IR (KBr): ν = 3440*br*, 2932*s*, 1728*s*, 1636*w*, 1465*m*, 1383*m*, 1313*m*, 1218*m*, 1173*s*, 1086*m*, 1029*m*, 996*w*, 755*w* cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 5.37 (*d*, 1 H, H-12, *J* = 4.0 Hz), 4.33 (*dd*, 1 H, H-11, *J* = 4.4, 4.4 Hz), 4.16 (*dq*, 1 H, methylene-HH', *J* = 10.8, 7.1 Hz), 4.13 (*dq*, 1 H, methylene-HH', *J* = 10.8, 7.1 Hz), 3.23 (*dd*, 1 H, H-3, *J* = 11.1, 5.0 Hz), 2.05 (*ddd*, 1 H, H-1, *J* = 13.0, 3.4, 3.4 Hz), 2.00 (*dd*, 1 H, H-18, *J* = 13.6, 4.0 Hz), 1.95 (*m*, 1 H, H-21), 1.93 (*m*, 1 H, H-15), 1.87 (*ddd*, 1 H, H-19, *J* = 13.6, 4.3, 2.7 Hz), 1.80 (*ddd*, 1 H, H-16, *J* = 13.6, 13.6, 4.6 Hz), 1.72 (*m*, 1 H, H-2), 1.68 (*m*, 1 H, H-2'), 1.61 (*dd*, 1 H, H-19', *J* = 13.6, 13.6 Hz), 1.59 (*m*, 1 H, H-7), 1.55 (*m*, 1 H, H-6), 1.52 (*ddd*, 1 H, H-6', *J* = 12.4, 3.1, 3.1 Hz), 1.40 (*s*, 1 H, H-9), 1.40 (*s*, 3 H, H-25), 1.34 (*m*, 1 H, H-22), 1.32 (*m*, 1 H, H-22'), 1.30 (*m*, 1 H, H-7'), 1.28 (*m*, 1 H, H-21'), 1.26 (*t*, 3 H, ethyl, *J* = 7.1 Hz), 1.22 (*s*, 3 H, H-26), 1.19 (*m*, 1 H, H-1'), 1.12 (*s*, 3 H, H-28), 1.09 (*s*, 3 H, H-27), 1.05 (*ddd*, 1 H, H-16', *J* = 13.6, 4.2, 2.4 Hz), 0.99 (*s*, 3 H, H-23), 0.91 (*ddd*, 1 H, H-15', *J* = 13.6, 4.3, 2.0 Hz), 0.82 (*s*, 3 H, H-24), 0.80 (*s*, 3 H, H-29), 0.70 (*dd*, 1 H, H-5, *J* = 10.7, 2.7 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 176.9 (C-30), 147.8 (C-13), 126.2 (C-12), 79.0 (C-3), 66.7 (C-11), 60.1 (ethylene), 55.7 (C-5), 52.5 (C-9), 47.9 (C-18), 44.0 (C-20), 42.7 (C-8), 42.3 (C-19), 39.5 (C-14), 38.7 (C-1), 38.7 (C-22), 38.3 (C-10), 38.2 (C-4), 33.2 (C-7), 31.7 (C-17), 31.2 (C-21), 28.4 (C-23), 28.4 (C-29), 28.3 (C-28), 27.0 (C-2), 26.8 (C-15), 26.5 (C-16), 25.2 (C-27), 19.3 (C-26), 18.7 (C-6), 18.1 (C-25), 15.7 (C-24), 14.3 (methyl); anal. calcd for $C_{33}H_{45}O_4$ (514.78): C, 76.99; H, 10.57; found:C,76.73; h, 11.07.

Data for **7**: White crystalline solid (167 mg, 8%); mp 161-165 °C; R_f = 0.21 (hexane/ethyl acetate 7:3); [α] = 82.74° (*c* 0.38, $CHCl_3$); UV-vis (methanol): λ_{max} (log ε) = 224 nm (3.85); MS (ESI): *m/z* (%) = 523.5 ($[M+Na]^+$, 100); IR (KBr): ν = 3405*br*, 2945*s*, 1727*m*, 1715*m*, 1618*w*, 1466*m*, 1390*w*, 1314*w*, 1218*m*, 1152*m*, 1109*w*, 1085*w*, 1040*w*, 858*w*, 620*w* cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ = 5.11 (*d*, 1 H, H-12, *J* = 3.3 Hz), 4.12 (*dq*, 1 H, methylene-HH', *J* = 10.8, 7.1 Hz), 4.05 (*dq*, 1 H, methylene-HH', *J* = 10.8, 7.1 Hz), 3.96 (*dd*, 1 H, H-11, *J* = 7.5, 3.3 Hz), 2.97 (*m*, 1 H, H-3), 2.05 (*ddd*, 1 H, H-1, *J* = 14.1, 3.1, 3.1 Hz), 1.97 (*m*, 1 H, H-15), 1.77 (*m*, 1 H, H-21), 1.77 (*ddd*, 1 H, H-19, *J* = 13.6, 4.3, 2.7 Hz), 1.76 (*m*, 1 H, H-18), 1.57 (*ddd*, 1 H, H-16, *J* = 13.6, 13.6, 4.6 Hz), 1.57 (*dd*, 1 H, H-19', *J* = 13.6, 13.6 Hz), 1.49 (*m*, 1 H, H-6), 1.48 (*d*, 1 H, H-9, *J* = 7.9 Hz), 1.44 (*m*, 1 H, H-2), 1.40 (*m*, 1 H, H-2'), 1.39 (*m*, 1 H, H-7), 1.33 (*m*, 1 H, H-21'), 1.30 (*m*, 1 H, H-6'), 1.28 (*m*, 1 H, H-22), 1.20 (*m*, 1 H, H-7'), 1.18 (*t*, 3 H, ethyl, *J* = 7.1 Hz), 1.17 (*s*, 3 H, H-27), 1.16 (*m*, 1 H, H-22'), 1.10 (*m*, 1 H, H-1'),

1.07 (*s*, 3 H, H-28), 0.95 (*s*, 3 H, H-25), 0.93 (*m*, 1 H, H-26), 0.92 (*s*, 3 H, H-16'), 0.89 (*s*, 3 H, H-23), 0.83 (*m*, 1 H, H-15'), 0.72 (*s*, 3 H, H-29), 0.68 (*s*, 3 H, H-24), 0.67 (*dd*, 1 H, H-5); ^{13}C NMR (125 MHz, DMSO-d_6): δ = 175.9 (C-30), 144.6 (C-13), 127.5 (C-12), 76.7 (C-3), 65.4 (C-11), 59.6 (ethylene), 55.0 (C-5), 54.7 (C-9), 47.0 (C-18), 43.4 (C-20), 42.6 (C-8), 41.7 (C-19), 41.0 (C-14), 40.3 (C-1), 38.7 (C-10), 37.8 (C-22), 37.6 (C-4), 32.9 (C-7), 31.4 (C-17), 30.5 (C-21), 28.3 (C-23), 28.1 (C-29), 27.9 (C-28), 27.2 (C-2), 26.3 (C-15), 25.8 (C-16), 25.2 (C-27), 18.1 (C-6), 17.8 (C-26), 16.5 (C-25), 16.0 (C-24), 14.2 (methyl); anal. calcd for $C_{33}H_{45}O_4$ (514.78): C, 76.99; H, 10.57; found: C, 76.78; H, 10.69

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Captions:

Fig. 1. Results from the AO/EB test: Treatment of A549 cells with (from left to right): **1** (90 μM) and **2** (90 μM), **3** (35 μM) and **4** (35 μM), **5** (60 μM) and **6** (60 μM).

Scheme 1. Reagents and conditions: a) zinc dust, conc. HCl, dioxane, 25°C, 24 h; b) MeI, K_2CO_3, DMF, 25°C, 24 h; c) BH_3-THF, THF, citric acid, 25°C, 20 h; d) EtI, K_2CO_3, DMF, 25°C, 24 h; e) BH_3-THF, THF, Na_2CO_3, 25°C, 4 d.

Table 1

Table 1: Results of SRB-assay: The values for melanoma (518A2), zervic cancer (A431), lung carcinoma (A549), ovarian cancer (A2780), colon cancer (DLD-1, HCT-8, HCT-116, HT-29), anaplastic thyroid cancer (8505C, SW-1736), mamma carcinoma (MCF-7) and liposarcoma were obtained by an SRB-assay after 96 h of treatment and are the average from at least three independent experiments; standard error 5%.

	compound					
	1	**2**	**3**	**4**	**5**	**6**
518A2	83.92	71.49	27.54	34.54	25.23	51.52
8505C	86.50	78.52	26.07	33.88	24.58	52.80
A2780	74.57	62.78	25.54	23.58	26.96	57.01
A431	79.58	86.13	25.28	33.55	23.45	46.55
A549	82.76	79.13	23.50	31.59	22.74	48.97
DLD-1	81.21	90.50	26.12	31.73	28.14	52.80
HCT-116	78.83	87.70	22.10	31.82	21.58	47.78
HCT-8	78.85	88.76	24.36	31.34	43.42	44.32
HT-29	80.09	90.30	27.54	23.89	22.14	44.32
LIPO	81.44	73.88	20.47	34.81	27.66	52.80
MCF-7	84.70	90.19	22.14	34.37	18.61	48.97
SW1736	76.93	72.47	34.87	32.35	13.37	45.48
NIH 3T3	18.52	68.70	22.81	42.22	23.66	43.16

Table 2

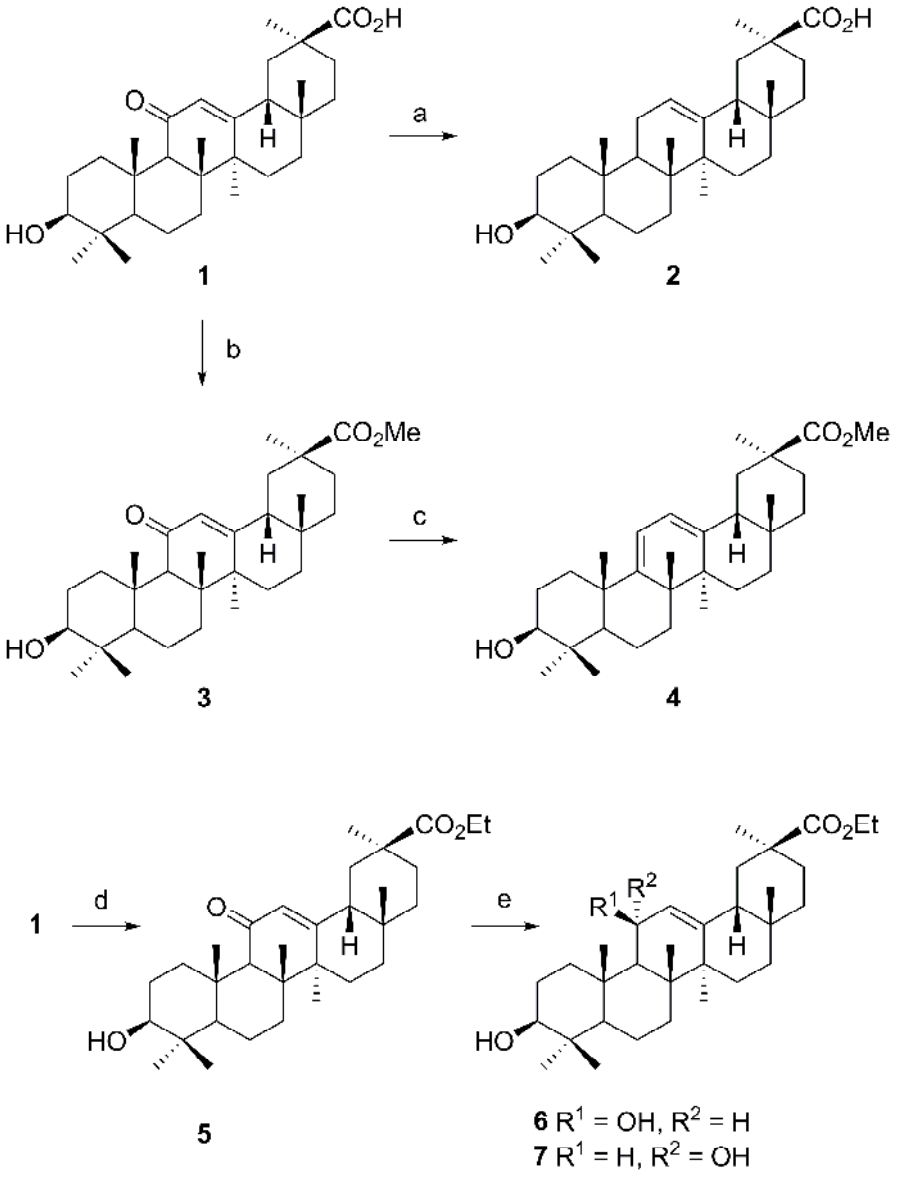
Table 2. Apoptotic effect of GA derivatives in A549 cells (in %, standard error 5 %, 6 independent experiments); cells were treated with **1** (90 μM), **2** (90 μM), **3** (35 μM), **4** (35 μM), **5** (60 μM) or **6** (60 μM).

Compound	**1**	**2**	**3**	**4**	**5**	**6**
Apoptosis [%]	73.73 ±1.40	76.43 ±1.78	70.64 ±0.74	66.93 ±5.80	44.92 ±4.13	80.62 ±2.67

Fig. 1:

Fig 1. Results from the AO/EB test: Treatment of A549 cells with (from left to right): **1** (90 μM) and **2** (90 μM), **3** (35 μM) and **4** (35 μM), **5** (60 μM) and **6** (60 μM).

Scheme1:



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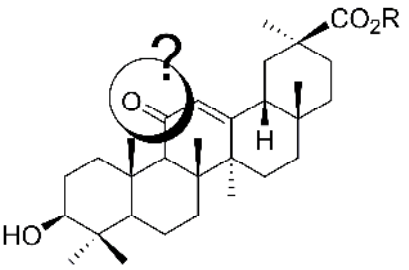

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Action Links		Synthesis and antitumor activity of ring A modified glycyrrhetinic acid derivatives	Jun 08, 2011	Jun 08, 2011	Submitted to Journal
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Anhang 6

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Manuscript Number:

Title: Synthesis and antitumor activity of ring A modified glycyrrhetinic acid derivatives

Article Type: Article

Keywords: antitumor activity; glycyrrhetinic acid; cytotoxicity

Corresponding Author: Prof. Rene Csuk, Dr

Corresponding Author's Institution: Martin-Luther-Universität Halle-Wittenberg

First Author: Rene Csuk, Dr

Order of Authors: Rene Csuk, Dr; Stefan Schwarz; Bianka Siewert; Dieter Ströhl, Dr; Ralph Kluge, Dr

Abstract: Triterpenoic acids show many pharmacological effects, among them an antiinflammatory or an antitumor activity. One of these, glycyrrhetinic acid (1) is of interest because of its antitumor profile. Glycyrrhetinic acid is not only cytotoxic but also triggers apoptosis in various human tumor cell lines. To improve the cytotoxicity of parent 1 we set out to synthesize new derivatives of it - differing in structure and lipophilicity. These compounds were tested in a sulforhodamine B assay for cytotoxicity, and screened for their ability to induce apoptosis using an acridine orange/ethidium bromide assay and trypan blue staining. The most active compound, 34, a benzyl glycyrrhetinate holding an extra 3-N-(3-aminopropyl)glycyl substituent showed IC50 between 1.96 - 5.14 μm for five human cancer cell lines and triggers apoptosis in 80 % of the cells.

*Manuscript
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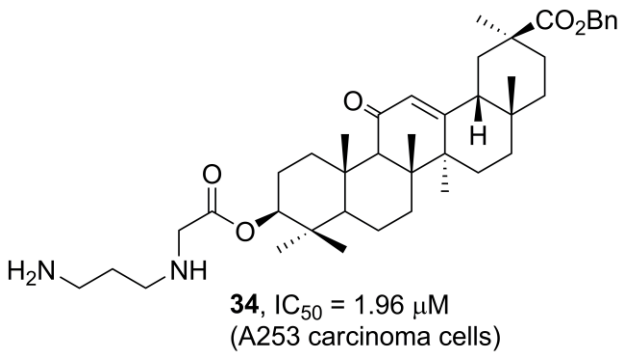
Graphical abstract



Synthesis and antitumor activity of ring A modified glycyrrhetinic acid derivatives

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ABSTRACT

Triterpenoic acids show many pharmacological effects, among them an antiinflammatory or an antitumor activity. One of these, glycyrrhetinic acid (**1**) is of interest because of its antitumor profile. Glycyrrhetinic acid is not only cytotoxic but also triggers apoptosis in various human tumor cell lines. To improve the cytotoxicity of parent **1** we set out to synthesize new derivatives of it – differing in structure and lipophilicity. These compounds were tested in a sulforhodamine B assay for cytotoxicity, and screened for their ability to induce apoptosis using an acridine orange/ethidium bromide assay and trypan blue staining. The most active compound, **34**, a benzyl glycyrrhetinate holding an extra 3-N-(3-aminopropyl)glycyl substituent showed IC_{50}

between 1.96 – 5.14 μm for five human cancer cell lines and triggers apoptosis in 80 % of the cells.

KEYWORDS:

Antitumor activity, glycyrrhetinic acid, cytotoxicity,

1. Introduction

While betulinic acid and oleanoic acid and their derivatives have already been studied extensively, there are still some gaps in the knowledge about glycyrrhetinic acid (**1**). Compound **1** shows a modest cytotoxicity [1, 2] but is able to trigger apoptosis in tumor cells [3-5]. Also, **1** can be accessed easily from the roots of licorice in high yields of up to 24 % [6]. Nevertheless, the cytotoxicity of **1** needs to be improved: in a previous study we were able to determine the IC_{50} value of **1** for 15 different human tumor cells lines, and for all tumor cell lines IC_{50} > 80 μM [7] were measured; this is poor compared to betulinic acid (IC_{50} = 7-14 μM [8]).

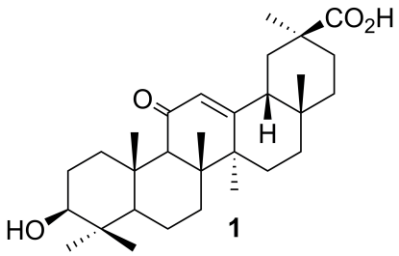
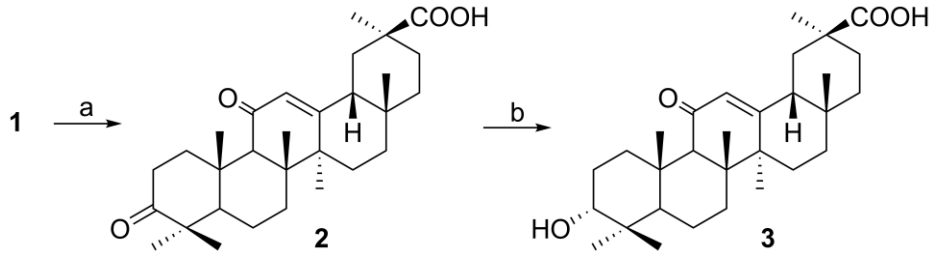


Fig. 1. Structure of glycyrrhetinic acid (**1**)

Results and Discussion

To improve the cytotoxicity of **1** it seems necessary to change the molecule at various positions. Our focus in this study was on ring A, especially positions O-3 and C-2 seemed of interest; this should lead to compounds displaying an altered pattern of lipophilicity compared to parent **1**. IC_{50} values were determined in sulforhodamine B assays as a measure of the cytotoxicity using different human tumor cell lines. Additional trypan blue staining experiments as well as acridine orange/ethidium bromide assays on human alveolar basal epithelial cells A549 were used to determine their ability of inducing apoptosis in this cancer cell line.

To determine whether a configurational inversion at position C-3 has an influence on the cytotoxicity of **1**, the corresponding α-epimer was prepared in a two-step synthesis [9] as depicted in Scheme 1. Thus, oxidation of **1** using CrO_3/acetone provided ketone **2** [3] whose stereoselective reduction [9] with L-selectride [10] at – 75 °C gave the 3-*epi* derivative **3**.



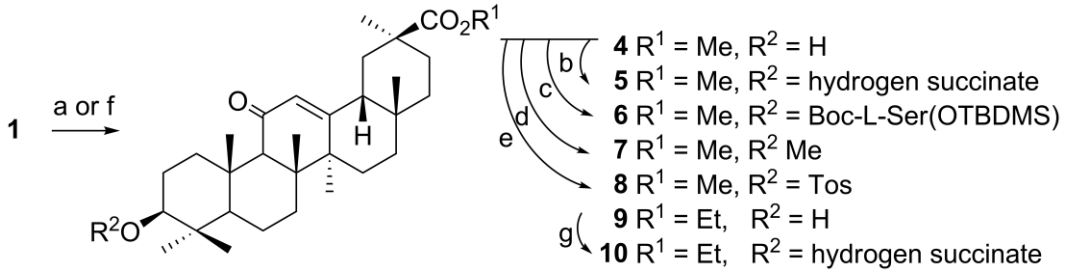
Scheme 1. Inversion of configuration at C-3: a) CrO_3, acetone, 25 °C, 1 h, 78 %; b) L-selectride, THF, -75 °C, 2 h, 61 %.

Screening of the compounds **1-3** in a SRB assay using 11 different human cancer cell lines (Table 1) revealed that no improvement of the antitumor activity is connected with these structural changes.

	518A2	**A253**	**A431**	**A549**	**DLD-1**	**HCT-116**	**HCT-8**	**HT-29**	**Lipo**	**MCF-7**	**SW 1736**
1*	83.92	80.78	79.58	82.76	81.21	78.33	78.85	80.09	81.44	84.70	76.93
2	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30
3	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30

Table 1. Results from the SRB assay for compounds **1-3** (IC_{50} given in μM, three independent experiments each, errors +/- 7 %); * data from a previous study [7].

To alter the lipophilicity of the molecule and to improve bioavailability, the carboxylic acid at position C-30 was transformed into different esters either by alkylation with alkyl iodides [7, 11] in the presence of finely grounded K_2CO_3 in DMF (→ **4** [12]) or via a DCC mediated coupling in DCM (→ **6**). The hydrogen succinate **5** was obtained from **1** using succinic anhydride in pyridine [13], and the ether **7** was obtained from **4** with sodium hydride/methyl iodide in THF [11]. The tosylate **8** [14] of methyl glycyrrhetinate (**4**) was accessed by tosylation of **4**.



Scheme 2. Derivatization of **1**; reagents and conditions: a) K_2CO_3/MeI, DMF, 25 °C, 2 h [7, 11]; b) succinic anhydride, pyridine, 85 °C, 48 h, 64 %; c) Boc-L-Ser(OTBTMS)-OH, DCC, DMAP, DCM, 25 °C, 20 h, 22 %; d) NaH (60 % in mineral oil), MeI, THF, reflux, 30 min, 34 %; e) *p*-TsCl, pyridine, 0 °C, 4 h, 50 %; K_2CO_3/EtI, DMF, 25 °C, 2 h [7, 11]; g) succinic anhydride, pyridine, 85 °C, 48 h, 86 %.

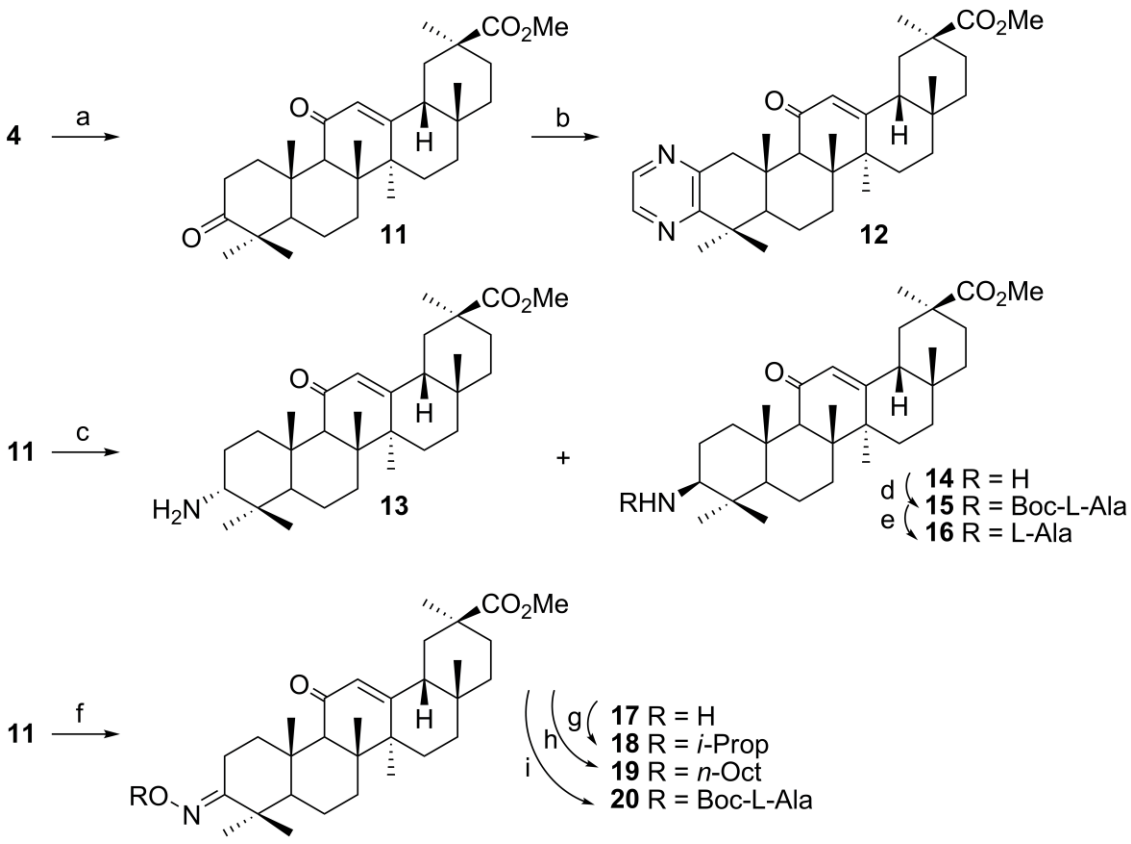
These functionalizations, however, did not improve cytotoxicity. Except for compounds **6** (IC_{50} = 28.59 μM on A253 cells) and **7** (IC_{50} = 28.99 μM in 518A2 cells), all IC_{50} values were above 30 μM. For the methyl ether **7** an IC_{50} could not be determined because of its insolubility in solvents usually used in biological tests (DMSO, DMF or MeOH).

	518A2	8505C	A253	A549	DLD-1	Lipo
4*	27.54	26.07	19.42	23.50	26.12	20.47
5	>30	>30	>30	>30	>30	>30
6	>30	>30	28.59	>30	>30	>30
8	>30	>30	>30	>30	>30	>30
9*	25.23	24.58	25.04	22.74	28.14	27.66
10	28.99	>30	>30	>30	>30	>30

Table 2. Results from the SRB assay for compounds **4**-**10** (IC_{50} given in μM, three independent experiments each, errors +/- 7 %); * data from a previous study [7].

To improve cytotoxicity, we considered the synthesis of derivatives possessing an extra nitrogen-containing moiety. Thus, compound **4** was oxidized at O-3 by a Jones oxidation [9] to yield **11** (Scheme 3). A derivative possessing an anellated pyrazine ring at positions C-2 and C-3 (of ring A) was obtained from the reaction of ketone **11** with ethylenediamine and sulfur in morpholine at 130 °C [15]. The corresponding 3-amino epimers resulted from a reductive amination of **11** with ammonium acetate in ethanol [16] followed by reducing the imine using sodium cyanoborohydride [17] in a one-pot reaction. Both epimers (**13** [18] and **14** [19]) were obtained from this reduction with the β-epimer **14** being the major product under these conditions.

In addition, compound **14** served as a starting material for several derivatives: Steglich esterification of **14** with Boc-L-Ala furnished **15** whose deprotection with trifluoroacetic acid in DCM yielded amine **16**. The synthesis of the oximes **17**-**19** started from **11**. Thus, reaction of **11** with hydroxylamine hydrochloride in pyridine followed by an acid work-up furnished **17**; the corresponding alkylated products **18**-**20** were obtained from the alkylation of **17** with an alkyl halide in THF. Compound **20** was obtained from **17**; its deprotection did not result in the formation of a free amine but a seconitrile, hence paralleling previous findings of Askam and Bradley for an analogous tosylate [20].



Scheme 3. Derivatization of **4** or **11**; reagents and conditions: a) CrO_3, acetone, 25 °C, 20 min [9]; b) S, ethylendiamine, morpholine, 130 °C, 4 h, 39 %; c) $H_3CCOONH_4$, MeOH, 25 °C, 10 min followed by $NaBH_3CN$, MeOH, 25 °C, 24 h yielding **13** (20 %) and **14** (52 %); d) Boc-L-Ala, DCC, DMAP, DCM, 25 °C, 16 h, 57 %; F_3CO_2H, DCM, 25 °C, 1 h, 60 %; f) $H_2NOH.HCl$, pyridine, 60 °C, 3 h, 80 %; i-PropI, KOH, THF, reflux, 24 h, 37 %; Oct-Br, KOH, THF, reflux, 24 h, 29 %; Boc-L-Ala, DCC, DMAP, DCM, 25 °C, 16 h, 48 %.

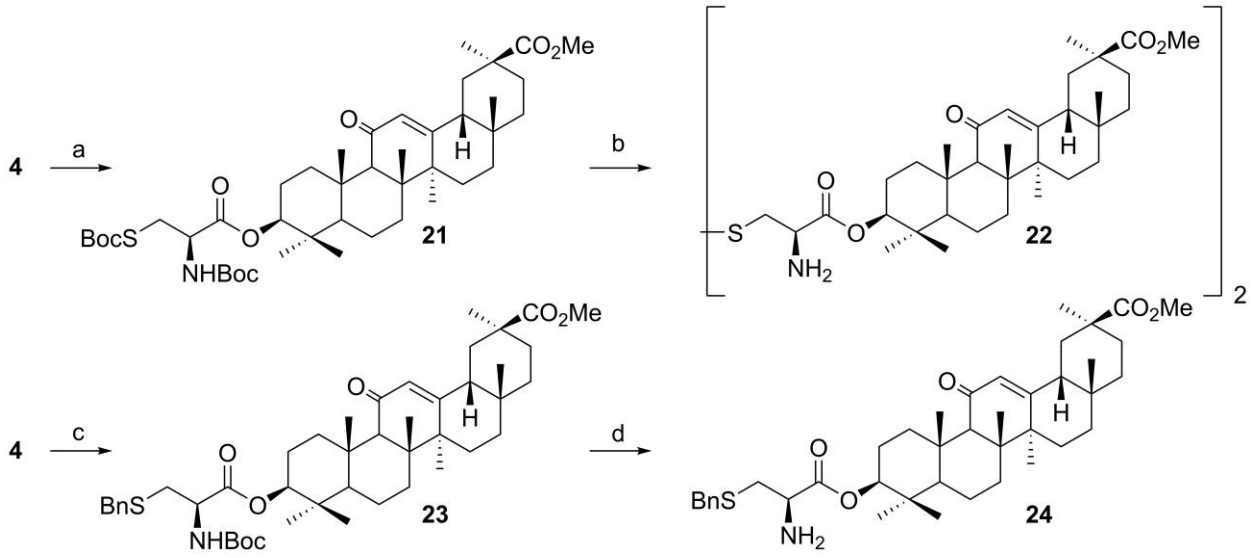
Whereas the pyrazine derivative **12** gave IC_{50} > 30 μM (Table 3), the amines **13** and **14** showed an excellent cytotoxicity for the tumor cell lines being about 10 times higher than the activity of parent compound **4**.

For the oxime **17** IC_{50} values of 12-19 μM were determined; for the human ovarian tumor cell line A2780 a low IC_{50} = 6.18 μM was determined. Incorporation of alkyl chains in oxime **17**, however, led to a decrease of cytotoxicity.

	518A2	8505C	A253	A2780	A549	DLD-1	Lipo	MCF-7	SW1736
4*	27.54	26.07	19.42	25.54	23.50	26.12	20.47	22.14	>30
11	>30	>30	>30	>30	>30	>30	>30	>30	29.09
12	>30	>30	>30	>30	>30	>30	>30	>30	>30
13	2.74	2.33	2.44	3.42	3.33	3.21	3.30	2.55	2.84
14	2.52	2.45	2.36	3.32	2.51	2.56	2.74	2.47	2.62
16	5.55	4.46	4.46	5.39	5.38	6.44	5.82	5.08	5.25
17	18.93	17.18	13.56	6.18	17.40	10.46	18.34	15.95	13.00
18	>30	>30	>30	>30	>30	>30	>30	>30	>30
19	>30	>30	>30	19.73	>30	>30	>30	26.35	17.77
20	16.49	15.47	14.12	12.03	15.83	19.10	18.99	15.17	14.61

Table 3. Results from the SRB assay for compounds **4**, **11-20** (IC_{50} given in μM, three independent experiments each, errors +/- 7 %); * data from a previous study [7].

Another option to modify the lipophilicity and/or polarity pattern was accomplished by the incorporation of a thiol moiety. While a direct sulfurization at position C-11 with Lawesson's reagent [21] failed under a variety of different conditions, dimeric compound **22** was obtained from **4** by a DCC mediated coupling reaction of a bis-Boc protected cysteine followed by the removal of the Boc group with trifluoroacetic acid in DCM. A selective deprotection of the amino group in **23** was achieved in 86 % resulting in compound **24** possessing a free amino group as well as a protected side chain.



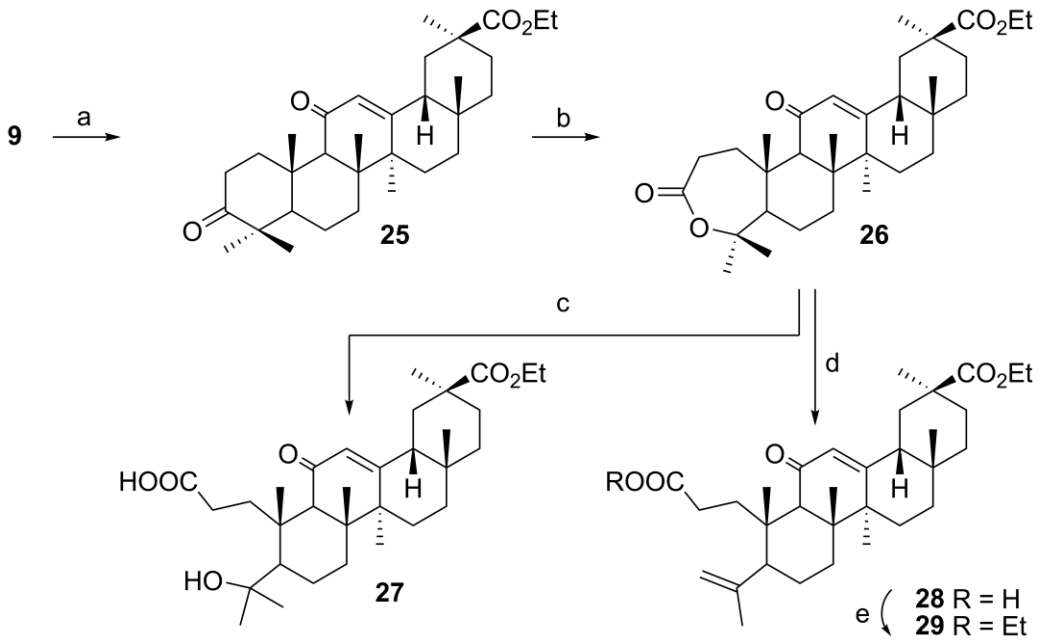
Scheme 4. Derivatization of **4** leading to sulfur-containing derivatives **22-24**; reagents and conditions: a) Boc-L-Cys(SBoc)-OH, DCC, DMAP, DCM, 25 °C, 16 h, 93 %; b) F_3CCO_2 H, DCM, 25 °C, 24 h, 43 and 86 %; c) Boc-L-Cys(SBn)-OH, DCC, DMAP, DCM, 25 °C, 16 h, 57 %; F_3CCO_2H, DCM, 25 °C, 24 h, 86 %.

The cytotoxicity of compounds **21-24** (Table 4) was determined in SRB assays, and their activity was similar to **4**, except for compounds **22** and **24** being about twice as active as their parent compound **4**.

	518A2	**8505C**	**A253**	**A549**	**DLD-1**	**Lipo**	**SW1736**
4*	27.54	26.07	19.42	23.50	26.12	20.47	>30
21	>30	>30	>30	>30	>30	>30	>30
22	24.54	15.10	19.07	18.75	>30	22.60	16.82
23	>30	>30	>30	>30	>30	>30	>30
24	16.78	15.45	15.84	17.90	>30	16.72	15.69

Table 4. Results from the SRB assay for compounds **4**, **21-24** (IC_{50} given in μM, three independent experiments each, errors +/- 7 %); * data from a previous study [7].

Ring opening reactions concerning ring A (Scheme 5) started from compound **25** [22] (obtained from **9**), and ring A was expanded by an insertion of oxygen using a Baeyer-Villiger oxidation [23] to yield **26**. Ring opening under basic conditions (KOH/EtOH) yielded the hydroxyl-substituted acid **27** whereas an elimination reaction occurred when **26** was treated with hydrochloric acid in EtOH, and **28** was obtained. Prolonged heating under reflux led to the formation of ethyl ester **29**.



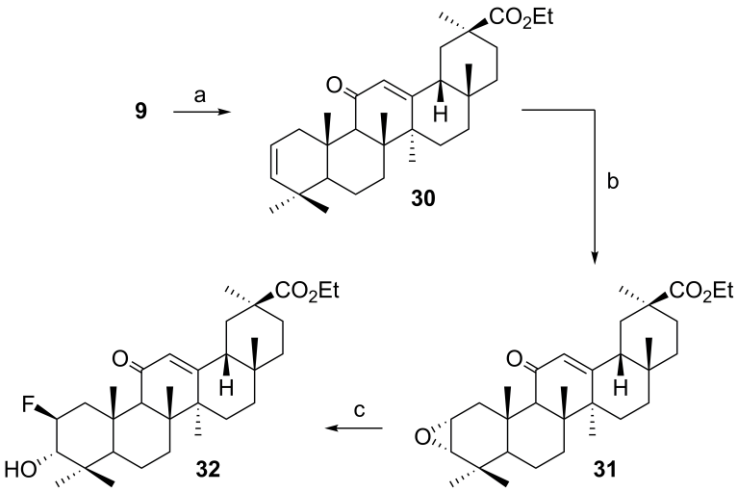
Scheme 5. Ring A modifications; reactions and conditions: a) CrO_3, acetone, 25 °C, 20 min [9]; b) *m*CPBA, $CHCl_3$, 55 °C, 24 h, 81 %; c) NaOH, EtOH, 25 °C, 24 h, 20 %; d) HCl, EtOH, 25 °C, 5 min, 96 %; e) HCl, EtOH, reflux, 30 min, 95 %.

Except for derivative **29**, none of these compounds showed increased cytotoxicity (Table 5). The IC_{50} values of all compounds were higher than that of parent compound **9**. Compounds **26** and **29**, however, exhibited lower IC_{50} values for A2780 cells (IC_{50} = 15.00 μM for **26** and IC_{50} = 5.89 μM for **29**) as well as for 518A2 cells (IC_{50} = 9.28 μM for **29**).

	518A2	**8505C**	**A253**	**A2780**	**A549**	**DLD-1**	**Lipo**	**MCF-7**	**SW1736**
9*	25.23	24.58	25.04	26.96	22.74	28.14	27.66	18.61	13.37
25	>30	>30	>30	>30	>30	>30	>30	>30	>30
26	>30	>30	28.73	15.00	>30	>30	>30	20.58	12.71
27	>30	>30	>30	>30	>30	>30	>30	>30	>30
28	>30	>30	>30	>30	>30	>30	>30	>30	>30
29	9.28	>30	>30	5.89	>30	>30	>30	25.42	26.74

Table 5. Results from the SRB assay for compounds **9**, **25-29** (IC_{50} given in μM, three independent experiments each, errors +/- 7 %); * data from a previous study [7].

Fluorine's special properties (small size, high electronegativity) contribute to its importance in medicinal chemistry. The effects of fluorine substitution on the biological behavior of biologically active molecules have been used effectively in the development of new drugs. To introduce a fluorine substituent on ring A, epoxide **31** [24, 25] was reacted with Olah's reagent [26] and **32** was obtained. Nucleophilic ring opening of the epoxide occurred at the less hindered carbon C-2 and followed Fürst-Plattner's [27] rule. Compound **32** is characterized in its ^{1}H NMR spectrum by a chemical shift of H-2 at δ = 4.64 ppm showing $J_{\text{H-1, H-2}}$ = 5.8 Hz, $J_{\text{H-2, H-3}}$ = 6.4 Hz and $J_{\text{H-2, F}}$ = 49.7 Hz.



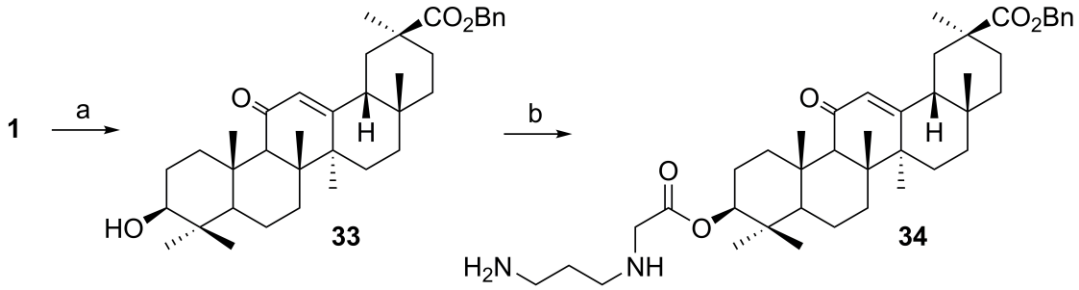
Scheme 6. Fluorination at position C-2; reactions and conditions: a) PPh_3, 3,3-dimethyl glutarimide, DEAD,THF, 25 °C, 24 h, 82 %; b) *m*-CPBA, CH_2Cl_2, 25 °C, 20 h, 77 %; c) Olah's reagent, 25 °C, 5 h, 19 %.

None of these modifications, however, led to compounds of higher cytotoxicity (Table 6) than parent compound **9**.

	518A2	**A253**	**A431**	**A549**	**DLD-1**	**HCT-116**	**HT-29**	**Lipo**	**MCF-7**
9*	25.23	25.04	23.45	22.74	28.14	21.58	22.14	27.66	18.61
30	>30	>30	>30	>30	>30	>30	>30	>30	>30
31	>30	>30	>30	>30	>30	>30	>30	>30	>30
32	>30	>30	>30	>30	>30	>30	24.72	>30	>30

Table 6. Results from the SRB assay for compounds **9**, **30**-**32** (IC_{50} given in μM, three independent experiments each, errors +/- 7 %); * data from a previous study [7].

Better results were obtained when benzylester **33** (obtained from **1**, benzyl chloride and potassium carbonate [7, 11]) was transformed into the aminopropyl derivative **34**.



Scheme 7. Synthesis of compound **34**; reactions and conditions: a) benzyl chloride, K_2CO_3, DMF, 25 °C, 2 h [7, 11]; b) chloroacetyl chloride, DCM, 25 °C, 24 h, then diaminopropane, K_2CO_3, DMF, 25 °C, 2 h, 82 %.

The IC_{50} values of compound **34** were about 3-5 times better than those of its parent compound **33**.

	518A2	**8505C**	**A253**	**A549**	**DLD-1**	**Lipo**
33*	18.19	8.10	10.67	6.15	22.69	11.54
34	5.14	2.07	1.96	4.74	4.96	2.99

Table 7. Results from the SRB assay for compounds **33** and **34** (IC_{50} given in μM, three independent experiments each, errors +/- 7 %); * data from a previous study [7].

Glycyrrhetinic acid is known for its ability to trigger apoptosis. To determine the extent of apoptosis, trypan blue staining and counting experiments were performed the results of which are summarized in Table 8. For parent glycyrrhetinic acid an extent of ca. 74 % was determined. Most of the compounds gave a positive result in these experiments except for compounds **17** and **22**.

compound	1	13	14	16	17	20	22	24	32	34
apoptosis [%]	73.73 ± 1.40	64.88 ± 3.60	74.21 ± 1.85	56.77 ± 4.56	32.43 ± 3.40	68.54 ± 2.40	40.91 ± 3.43	60.64 ± 3.16	80.32 ± 1.68	80.57 ± 3.23

Table 8. Apoptotic effect [in %] of derivatives of **1** on A549 cells (+/- standard error, 6 experiments each); cells were treated with **1** (90 μM), **13** (4 μM), **14** (4 μM), **16** (8 μM), **17** (20 μM), **22** (20 μM), **24** (20 μM), **32** (60 μM), and **34** (8 μM), respectively.

Additional AO/EB tests support these results. In this test, green fluorescent cells were found, hence indicating an apoptotic behavior of the compounds. On principle, an AO/EB assay doesn't allow quantification of the extent of apoptosis but confirms the results from the trypan blue staining experiments.

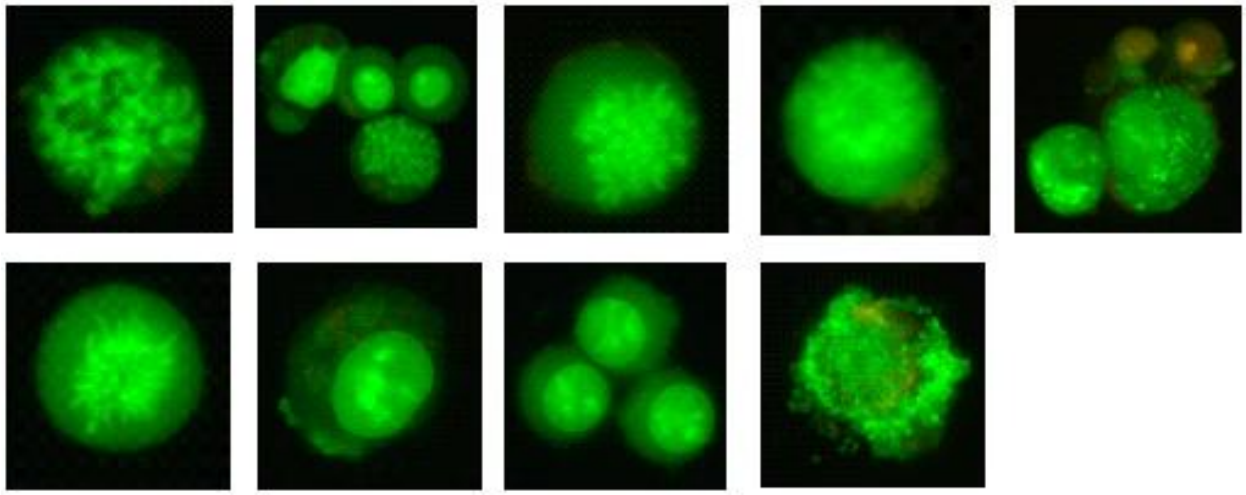


Fig. 2. Results from the AO/EB tests: A549 cells treated with (left to right, upper row then lower row): **13** (4 μM), **14** (4 μM), **16** (8 μM), **17** (20 μM), **20** (20 μM), **22** (20 μM), **24** (20 μM), **32** (60 μM), **34** (8 μM).

3. Conclusions

Herein we synthesized 25 derivatives of **1** differing in lipophilicity and structure at ring A, and screened them for their cytotoxicity. Neither configurational inversion at position C-2 nor structural modifications at position C-3 led to higher cytotoxicity. The introduction of at least one nitrogen containing substituent, however, was quite promising. Thus, derivatives possessing a primary amino group (**13**-**15**) showed the highest activities whereas substitution (**12**, **17**-**20**) decreased cytotoxicity. A similar behavior was established for compounds **21**-**24**. In addition, introduction of sulfur decreased cytotoxicity.

Structural modification of ring A, i.e. expanding (→ **26**) or ring opening (→ **27**-**29**) by and large decreased cytotoxicity, except for **29** on A2780 cells. It seems that the presence of an intact ring A is essential for cytotoxicity.

Attachment of a diaminoalkyl moiety at position 3 of a lipophilic ester of glycyrrhetinic acid, has an increasing effect on the cytotoxicity and was most rewarding: compound **34** exhibits IC_{50} values between 1.96-5.14 μM. Hence, compound **34** was the most active compound of this study, thus indicating a feasible route for the development of glycyrrhetinic acid derivatives showing promising cytotoxicity.

4. Experimental

4.1. Chemistry

Melting points are uncorrected (*Leica* hot stage microscope), NMR spectra were recorded using the Varian spectrometers Gemini 200, Gemini 2000 or Unity 500 (δ given in ppm, *J* in Hz, internal Me_4Si), IR spectra (film or KBr pellet) on a Perkin-Elmer FT-IR spectrometer Spectrum 1000, MS spectra were taken on a Intectra GmbH AMD 402 (electron impact, 70 eV) instrument; for elemental analysis a Foss-Heraeus Vario EL instrument was used; TLC was performed on silica gel (Merck 5554, detection by UV absorption or by treatment with a

solution of 10% sulfuric acid, ammonium molybdate and cerium(IV) sulfate) followed by gentle heating. The solvents were dried according to usual procedures.

4.2. Biology

4.2.1. Cell lines and Culture Conditions

Cultures of the cell lines were maintained as monolayer in RPMI 1640 (PAA Laboratories, Pasching, Germany) supplemented with 10% heat inactivated fetal bovine serum (Biochrom AG, Berlin, Germany) and penicillin / streptomycin (PAA Laboratories) at 37 °C in a humidified atmosphere of 5% CO_2/ 95% air.

4.2.2. Cytotoxicity Assay [28]

The cytotoxicity of the compounds was evaluated using the sulforhodamine-B (SRB) (Sigma Aldrich) microculture colorimetric assay. In short, exponentially growing cells were seeded into 96-well plates on day 0 at the appropriate cell densities to prevent confluence of the cells during the period of experiment. After 24h, the cells were treated with serial dilutions of the compounds (0-100 µM) for 96 h. The final concentration of DMSO or DMF solvent never exceeded 0.5%, which was non-toxic to the cells. The percentages of surviving cells relative to untreated controls were determined 96 h after the beginning of drug exposure. After a 96 h treatment, the supernatant medium from the 96 well plates was thrown away and the cells were fixed with 10% TCA. For a thorough fixation, the plates were allowed to rest at 4 °C. After fixation, the cells were washed in a strip washer. The washing was done four times with water using alternate dispensing and aspiration procedures. The plates were then dyed with 100 µl of 0.4% SRB (sulforhodamine B) for about 20 min. After dying the plates were washed with 1% acetic acid to remove the excess of the dye and allowed to air dry overnight. 100 µl of 10 mM Tris base solution were added to each well and absorbance was measured at 570 nm (using a 96 well plate reader, Tecan Spectra, Crailsheim, Germany). The IC_{50} was estimated by linear regression between the value before and after the 50% line is crossed in a dose-response curve.

4.2.3. Apoptosis test – Acridine Orange/Ethidium Bromide (AO/EB) [29]

Apoptotic cell death was analysed by acridine orange/ethidium bromide dye using fluorescence microscopy on A549 cells. Therefore, approx. 500,000 cells were seeded in cell culture flasks and were allowed to grow for 24 hours. The medium was removed and the substance loaded medium was added. After 24-48 hours, the supernatant medium was collected and centrifuged; the pellet was suspended in phosphate-buffer saline (PBS) and centrifuged again. The liquid was removed, and the pellet was suspended in PBS. After mixing the suspension with a solution of AO/EB, analysis was performed under a fluorescence microscope. While a green fluorescence shows apoptosis, a red coloured nucleus indicates necrotic cells.

4.2.4. Apoptosis test - Trypan blue cell counting

Approx. 500,000 cells (A549) were seeded in cell culture flasks and were allowed to grow for 1 day. After removing of the medium, the substance loaded medium was introduced and the flasks were incubated for about 24-48 hours. The supernatant medium was collected and centrifuged; cell pellet was suspended in PBS and centrifuged again. Equal amounts of Trypan blue solution (0.4 % in phosphate-buffer saline, pH 7.2) and suspension of the pellet in PBS were mixed and put on chamber slides (invitrogen™). Automatic cell counter (invitrogen™ countess® automated cell counter) was used for counting the cells, differing between cells with an intact cell membrane and cells without.

4.2.5. Compounds

4.2.5.1. (3α)-3-Hydroxy-11-oxo-olean-12-en-30-oic acid (3) [9]

To a solution of **2** (450 mg, 0.96 mmol) in dry THF (30 ml) at -75 °C, L-Selectride (1 M in THF, 10 ml, 10 mmol) was added, and the mixture was stirred at -75 °C for 2 h. After warming to room temperature, hydrochloric acid (1 M) was added until the pH = 2. The aqueous layer was extracted with chloroform (3 x 15 ml), the combined extracts were washed with water (20 ml), dried (Na_2SO_4) and evaporated. Recrystalisation from methanol afforded **3** (170 mg, 38 %) as colorless crystals; mp 308-310 °C (lit. > 325 °C [24]); R_f = 0.32 (hexane/ethyl acetate 7:3); $[\alpha]_D$

= + 114.66° (*c* 0.31, $CHCl_3$); UV-vis (methanol): λ_{max} (log ε) = 250 nm (3.95); IR (KBr): ν = 3424*br*, 2960*s*, 1717*m*, 1645*s*, 1458*w*, 1386*m*, 1328*w*, 1253*w*, 1208*w*, 1159*m*, 1088*w*, 1062*w*, 1028*w*, 1003*w* cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 12.11 (*s*, 1 H, COOH), 5.38 (*s*, 1 H, H-12), 4.17 (*d*, 1 H, OH, *J* = 4.3 Hz), 3.15 (*d*, 1 H, H-3, *J* = 3.5 Hz), 2.37 (*s*, 1 H, H-9), 2.27 (*ddd*, 1 H, H-1, *J* = 13.1, 3.5, 3.5 Hz), 2.08 (*m*, 1 H, H-15), 2.05 (*m*, 1 H, H-18), 1.85 (*m*, 1 H, H-2), 1.78 (*m*, 1 H, H-21), 1.71 (*m*, 1 H, H-16), 1.64 (*m*, 1 H, H-19), 1.62 (*m*, 1 H, H-7), 1.37 (*m*, 1 H, H-6), 1.35 (*s*, 3 H, H-27), 1.34 (*m*, 1 H, H-21'), 1.32 (*m*, 1 H, H-22), 1.31 (*m*, 1 H, H-2'), 1.30 (*m*, 1 H, H-7'), 1.29 (*m*, 1 H, H-1'), 1.27 (*m*, 1 H, H-6'), 1.25 (*m*, 1 H, H-22'), 1.18 (*m*, 1 H, H-5), 1.13 (*m*, 1 H, H-16'), 1.08 (*s*, 3 H, H-29), 1.02 (*s*, 6 H, H-26 and H-25), 0.94 (*m*, 1 H, H-15'), 0.82 (*s*, 3 H, H-28), 0.75 (*s*, 3 H, H-24), 0.74 (*s*, 3 H, H-23) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ = 199.1 (C11), 177.6 (C30), 169.5 (C13), 127.3 (C12), 73.5 (C3), 61.1 (C9), 48.0 (C18), 47.5 (C5), 45.0 (C8), 43.0 (C20), 42.9 (C14), 40.6 (C19), 37.5 (C22), 37.1 (C10), 36.7 (C4), 33.0 (C1), 32.1 (C7), 31.5 (C17), 30.3 (C21), 28.7 (C28), 28.3 (C23), 27.8 (C29), 25.9 (C16), 25.8 (C15), 25.1 (C2), 23.1 (C27), 22.2 (C24), 18.3 (C26), 16.9 (C6), 16.1 (C25) ppm; MS (ESI): *m*/*z* (%) = 471.5 ($[M+H]^+$, 70), 493.5 ($[M+Na]^+$, 25), 525.1 ($[M+MeOH+Na]^+$, 100).

4.2.5.2. Methyl (3 β) 3-hydroxy-11-oxo-olean-12-en-30 oate (4)

Compound **4** was prepared according to [12].

4.2.5.3. 4-{[(3 β)-30-Methoxy-11,30-dioxoolean-12-en-3-yl]oxy}4-oxo-butanoic acid (5) [13]

To a solution of **4** (960 mg, 1.98 mmol) in dry pyridine (20 ml), succinic anhydride (430 mg, 4.3 mmol) was added, and the mixture was stirred at 85 °C for 2 d. The solvent was removed, DCM (30 ml) added, and the reaction was washed with hydrochloric acid (1 M, 20 ml), extracted with DCM (3 x 20 ml), and the combined organic layers were dried (Na_2SO_4), filtered and evaporated. The residue was subjected to chromatography (silica gel, hexane/ethyl acetate 1:1) to afford **5** (740 mg, 64 %) as a colorless powder; mp 260-263 °C (lit. 262-264 °C [13]); R_f = 0.14 (hexane/ethyl acetate 1:1); $[\alpha]_D$ = + 154.55° (*c* 0.21, $CHCl_3$) (lit. + 156 ± 2° (*c* 0.05, $CHCl_3$) [13]); UV-vis (methanol): λ_{max} (log ε) = 249 nm (4.09); IR (KBr): *v* = 3433*br*, 2952*s*, 2875*m*, 1731*s*, 1654*s*, 1465*m*, 1388*m*, 1363*m*, 1329*w*, 1280*m*, 1217*s*, 1167*s*, 1087*w*, 1049*w*, 1021*w*, 989*m* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.67 (*s*, 1 H, H-12), 4.55 (*dd*, 1 H, H-3, *J* = 11.6,

4.8 Hz), 3.69 (*s*, 3 H, CH_3), 2.80 (*ddd*, 1 H, H-1, *J* = 13.7, 3.3, 3.3 Hz), 2.69 (*m*, 2 H, chain-γ-CH_2), 2.64 (*m*, 2 H, chain-β-CH_2), 2.36 (*s*, 1 H, H-9), 2.08 (*dd*, 1 H, H-18, *J* = 13.9, 3.5 Hz), 2.03 (*m*, 1 H, H-15), 1.99 (*m*, 1 H, H-21), 1.93 (*ddd*, 1 H, H-19, *J* = 13.5, 3.8, 2.6 Hz), 1.82 (*ddd*, 1 H, H-16, *J* = 13.3, 13.3, 4.2 Hz), 1.71 (*m*, 1 H, H-2), 1.66 (*m*, 1 H, H-7), 1.62 (*m*, 1 H, H-2'), 1.61 (*dd*, 1 H, H-19', *J* = 13.6, 13.6 Hz), 1.58 (*m*, 1 H, H-6), 1.48 (*m*, 1 H, H-6'), 1.42 (*m*, 1 H, H-7'), 1.40 (*m*, 1 H, H-22), 1.36 (*s*, 3 H, H-27), 1.31 (*m*, 1 H, H-22'), 1.31 (*m*, 1 H, H-21'), 1.18 (*m*, 1 H, H-16'), 1.16 (*s*, 3 H, H-25), 1.15 (*s*, 3 H, H-29), 1.13 (*s*, 3 H, H-26), 1.08 (*m*, 1 H, H-1'), 1.02 (*m*, 1 H, H-15'), 0.88 (*s*, 3 H, H-24), 0.87 (*s*, 3 H, H-23), 0.81 (*s*, 3 H, H-28), 0.79 (*m*, 1 H, H-5) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.1 (C-11), 177.1 (chain-δ-COOH), 176.9 (C-30), 171.8 (chain-α-COO), 169.3 (C-13), 128.5 (C-12), 81.2 (C-3), 61.7 (C-9), 55.0 (C-5), 51.7 (OCH_3), 48.4 (C-18), 45.4 (C-8), 44.0 (C-20), 43.2 (C-14), 41.1 (C-19), 38.7 (C-1), 38.1 (C-4), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 29.3 (chain-γ-CH_2), 28.9 (chain-β-CH_2), 28.5 (C-28), 28.3 (C-29), 28.0 (C-23), 26.4 (C-16), 26.4 (C-15), 23.5 (C-2), 23.3 (C-27), 18.7 (C-26), 17.3 (C-6), 16.7 (C-24), 16.4 (C-25) ppm; MS (ESI): *m*/*z* (%) = 585.4 ($[M+H]^+$, 12), 607.5 ($[M+Na]^+$, 54), 899.4 ($[3M+2Na]^{2+}$, 88), 1169.2 ($[2M+H]^+$, 18), 1191.6 ($[2M+Na]^+$, 100), 583.2 ($[M-H]^-$, 32), 629.0 ($[M+HCO_2]^-$, 96), 1167.0 ($[2M-H]^-$, 100).

4.2.5.4. Methyl (3β) 3-({N-(*tert*-butoxycarbonyl9)-O-[*tert*-butyl(dimethyl)silyl]-L-seryl}oxy)-11-oxoolean-12-en-30-oate (6)

Compound **4** (410 mg, 0.85 mmol), Boc-L-Ser(OTBDMS)-OH (320 mg, 1.00 mmol) and DMAP (30 mg, 0.25 mmol) were dissolved in dry DCM (25 ml). DCC (210 mg, 1.02 mmol) was added and the solution was stirred at 25 °C for 20 h. The precipitate was filtered off and the filtrate evaporated. Purification via chromatography (silica gel, $CHCl_3$/ether 9:1) yielded **6** (150 mg, 22 %) as a colorless powder; mp 120-123 °C (decomp.); R_f = 0.68 (hexane/ethyl acetate 7:3); $[\alpha]_D$ = + 74.12° (*c* 0.57, $CHCl_3$); UV-vis (methanol): λ_{max} (log ε) = 249 nm (4.05); IR (KBr): ν = 3448*br*, 2952*s*, 2858*s*, 1732*s*, 1662*s*, 1498*s*, 1465*m*, 1389*m*, 1367*m*, 1257*m*, 1216*s*, 1167*s*, 1115*s*, 1062*m*, 986*m*, 837*w*, 779*m*, 723*m* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.66 (*s*, 1 H, H-12), 5.34 (*d*, 1 H, NH, *J* = 8.1 Hz), 4.59 (*dd*, 1 H, H-3, *J* = 11.7, 4.8 Hz), 4.30 (*m*, 1 H, Ser-CH), 4.07 (*dd*, 1 H, Ser-C*H*H', *J* = 10.0, 2.1 Hz), 3.87 (*dd*, 1 H, Ser-CH*H'*, *J* = 10.0, 2.1 Hz), 3.69 (*s*, 3 H, OCH_3), 2.81 (*ddd*, 1 H, H-1, *J* = 13.6, 3.5, 3.5 Hz), 2.36 (*s*, 1 H, H-9), 2.08 (*dd*, 1 H, H-18, *J*

= 13.3, 3.7 Hz), 2.03 (*m*, 1 H, H-15), 1.99 (*m*, 1 H, H-21), 1.93 (*ddd*, 1 H, H-19, *J* = 13.5, 4.2, 2.7 Hz), 1.82 (*ddd*, 1 H, H-16, *J* = 13.7, 13.7, 4.4 Hz), 1.73 (*m*, 1 H, H-2), 1.66 (*m*, 1 H, H-7), 1.63 (*m*, 1 H, H-2'), 1.61 (*dd*, 1 H, H-19', *J* = 13.6, 13.6 Hz), 1.57 (*m*, 1 H, H-6), 1.48 (*m*, 1 H, H-6'), 1.45 (*s*, 9 H, Boc-CH_3), 1.43 (*m*, 1 H, H-7'), 1.40 (*m*, 1 H, H-22), 1.36 (*s*, 3 H, H-27), 1.31 (*m*, 1 H, H-22'), 1.31 (*m*, 1 H, H-21'), 1.18 (*m*, 1 H, H-16'), 1.16 (*s*, 3 H, H-25), 1.15 (*s*, 3 H, H-29), 1.13 (*s*, 3 H, H-26), 1.06 (*m*, 1 H, H-1'), 1.01 (*m*, 1 H, H-15'), 0.90 (*s*, 3 H, H-24), 0.89 (*s*, 3 H, H-23), 0.86 (*s*, 9 H, TBDMS-CH_3), 0.80 (*s*, 3 H, H-28), 0.80 (*m*, 1 H, H-5), 0.04 (*s*, 3 H, Si-CH_3), 0.02 (*s*, 3 H, Si-CH_3) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 176.9 (C-30), 170.4 (Ser-COO), 169.2 (C-13), 155.2 (Boc-COO), 128.5 (C-12), 81.8 (C-3), 79.6 (Boc-*quart.*-C), 63.8 (Ser-CH_2), 61.7 (C-9), 55.9 (Ser-CH), 55.0 (C-5), 51.7 (OCH_3), 48.4 (C-18), 45.4 (C-8), 44.0 (C-20), 43.2 (C-14), 41.1 (C-19), 38.7 (C-1), 38.2 (C-4), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-28), 28.4 (Boc-CH_3), 28.4 (C-29), 28.0 (C-23), 26.5 (C-16), 26.4 (C-15), 25.8 (TBDMS-CH_3), 23.6 (C-2), 23.3 (C-27), 18.7 (C-26), 18.2 (TBDMS-*quart.*-C), 17.3 (C-6), 16.7 (C-24), 16.3 (C-25), -5.5 (Si-CH_3), -5.6 (Si-CH_3) ppm; ^{29}Si NMR (100 MHz, $CDCl_3$): δ = 21.4 (TBDMS-Si) ppm; MS (ESI): *m*/*z* (%) = 786.2 ($[M+H]^+$, 11), 808.4 ($[M+Na]^+$, 100), 824.3 ($[M+K]^+$, 6), 1201.1 ($[3M+2Na]^{2+}$, 8), 1593.1 ($[2M+Na]^+$, 16); analysis for $C_{45}H_{75}NO_8Si$ (786.2): C, 68.75; H, 9.62, N, 1.78; found; C, 68.65; H, 9.81; N, 1.63.

4.2.5.5. Methyl (3β)- 3-(methoxy)-11-oxo-olean-12-en-30-oate (7)

To a solution of **4** (200 mg, 0.41 mmol) in dry THF (10 ml), sodium hydride (60 % in mineral oil, 30 mg, 0.75 mmol) was added, and the mixture was refluxed for 30 min. Methyl iodide was added and refluxing was continued for another 30 min. After cooling to 25 °C, water (10 ml) was added dropwise, and the mixture was extracted with DCM (3x 10 ml). The organic layers were washed with brine (20 ml), dried (Na_2SO_4), filtered and evaporated. The residue was subjected to chromatography (silica gel, hexane/ethyl acetate 9:1) to afford **7** (70 mg, 34 %) as a colorless powder; mp > 300 °C (lit. > 300 °C [3]); R_f = 0.75 (hexane/ethyl acetate 7:3); $[\alpha]_D$ = + 160.78° (*c* 0.63, $CHCl_3$); UV-vis (methanol): λ_{max} (log ε) = 250 nm (4.02); IR (KBr): ν = 3442*br*, 2930*s*, 2872*s*, 2856*m*, 2816*m*, 1732*s*, 1654*s*, 1616*w*, 1463*m*, 1388*m*, 1358*m*, 1324*m*, 1279*w*, 1264*w*, 1246*w*, 1220*s*, 1184*m*, 1156*s*, 1101*s*, 1087*m*, 1048*w*, 1027*w* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.65 (*s*, 1 H, H-12), 3.67 (*s*, 3 H, OCH_3), 3.34 (*s*, 3 H, CH_3O), 2.81 (*ddd*, 1 H, H-1, *J* = 13.2,

3.7, 3.7 Hz), 2.65 (*dd*, 1 H, H-3, *J* = 11.6, 4.4 Hz), 2.31 (*s*, 1 H, H-9), 2.06 (*dd*, 1 H, H-18, *J* = 14.8, 3.9 Hz), 2.01 (*m*, 1 H, H-15), 1.98 (*m*, 1 H, H-21), 1.90 (*ddd*, 1 H, H-19, *J* = 13.7, 3.9, 2.5 Hz), 1.81 (*m*, 1 H, H-16), 1.78 (*m*, 1 H, H-2), 1.62 (*m*, 1 H, H-7), 1.59 (*dd*, 1 H, H-19', *J* = 13.5, 13.5 Hz), 1.55 (*m*, 1 H, H-6), 1.49 (*m*, 1 H, H-2'), 1.42 (*m*, 1 H, H-6'), 1.38 (*m*, 1 H, H-7'), 1.37 (*m*, 1 H, H-22), 1.34 (*s*, 3 H, H-27), 1.30 (*m*, 1 H, H-22'), 1.30 (*m*, 1 H, H-21'), 1.16 (*m*, 1 H, H-16'), 1.13 (*s*, 3 H, H-25), 1.13 (*s*, 3 H, H-29), 1.11 (*s*, 3 H, H-26), 1.00 (*m*, 1 H, H-15'), 0.97 (*s*, 3 H, H-23), 0.88 (*m*, 1 H, H-1'), 0.79 (*s*, 3 H, H-28), 0.77 (*s*, 3 H, H-24), 0.67 (*m*, 1 H, H-5) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.3 (C-11), 176.9 (C-30), 169.1 (C-13), 128.6 (C-12), 88.3 (C-3), 61.8 (C-9), 57.4 (CH_3O), 55.5 (C-5), 51.7 (OCH_3), 48.4 (C-18), 45.4 (C-8), 44.0 (C-20), 43.2 (C-14), 41.1 (C-19), 39.0 (C-1), 39.0 (C-4), 37.7 (C-22), 37.1 (C-10), 32.8 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-28), 28.3 (C-29), 28.1 (C-23), 26.5 (C-16), 26.4 (C-15), 23.3 (C-27), 22.0 (C-2), 18.7 (C-26), 17.4 (C-6), 16.3 (C-24), 16.3 (C-25) ppm; MS (ESI): *m*/*z* (%) = 499.5 ($[M+H]^+$, 56), 521.5 ($[M+Na]^+$, 6), 539.1 ($[M+Na+H_2O]^+$, 11), 552.9 ($[M+Na+MeOH]^+$, 100), 997.2 ($[2M+H]^+$, 20), 1019.3 ($[2M+Na]^+$, 42).

4.2.5.6. Methyl (3β)-3-{[(4-methylphenyl)sulfonyl]oxy}-11-oxolean-12-en-30-oate (8) [14]

Compound **4** (300 mg, 0.62 mmol) was dissolved in dry pyridine (10 ml) and cooled to 0° C. 4-Toluenesulfonyl chloride (160 mg, 0.81 mmol) was added, and the solution was stirred for 4 h. After usual aqueous work-up, extraction with ethyl acetate (3 x 10ml) and chromatography (silica gel, hexane/ethyl acetate 7:3) **8** (200 mg, 50 %) was obtained as a colorless powder; mp 205 – 207 °C; R_f = 0.57 (hexane/ethyl acetate 7:3); $[\alpha]_D$ = + 106.7° (*c* 0.53, $CHCl_3$); UV-vis (methanol): λ_{max} (log ε) = 196 (4.58), 228 (4.16), 249 nm (4.02); IR (KBr): ν = 3439*br*, 2950*s*, 2869*s*, 1920*w*, 1731*s*, 1659*s*, 1621*m*, 1598*w*, 1466*s*, 1386*m*, 1338*s*, 1293*m*, 1279*m*, 1261*m*, 1246*m*, 1216*s*, 1189*s*, 1169*s*, 1098*m*, 1088*m*, 1048*w*, 1030*w*, 1018*w*, 983*m*, 943*s*, 930*s*, 913*s*, 881*s*, 840*m*, 808*m*, 794*m*, 768*w*, 714*w*, 703*w*, 690*s*, 629*w*, 610*w*, 582*w*, 559*m*, 547*m*, 538*m*, 475*w* cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 7.76 (*d*, 2 H, aromatic-H2, *J* = 8.1 Hz), 7.28 (*d*, 2 H, aromatic-H3, *J* = 8.0 Hz), 5.60 (*s*, 1 H, H-12), 4.23 (*dd*, 1 H, H-3, *J* = 12.1, 4.6 Hz), 3.64 (*s*, 3 H, CH_3), 2.73 (*ddd*, 1 H, H-1, *J* = 13.6, 3.8, 3.8 Hz), 2.40 (*s*, 3 H, aromatic-CH_3), 2.26 (*s*, 1 H, H-9), 2.04 (*dd*, 1 H, H-18, *J* = 13.2, 3.3 Hz), 2.00 (*m*, 1 H, H-15), 1.95 (*m*, 1 H, H-21), 1.87 (*m*, 1 H, H-19), 1.78 (*m*, 1 H, H-2), 1.77 (*m*, 1 H, H-16), 1.61 (*m*, 1 H, H-7), 1.58 (*m*, 1 H, H-2'), 1.56

(*dd*, 1 H, H-19', *J* = 13.4, 13.4 Hz), 1.54 (*m*, 1 H, H-6), 1.41 (*m*, 1 H, H-6'), 1.39 (*m*, 1 H, H-7'), 1.36 (*m*, 1 H, H-22), 1.31 (*s*, 3 H, H-27), 1.26 (*m*, 2 H, H22' and H21'), 1.14 (*m*, 1 H, H-16'), 1.11 (*s*, 3 H, H-29), 1.06 (*s*, 3 H, H-25), 1.05 (*s*, 3 H, H-26), 0.97 (*m*, 1 H, H-15'), 0.90 (*ddd*, 1 H, H-1', *J* = 13.7, 13.7, 2.9 Hz), 0.84 (*s*, 3 H, H-23), 0.8 (*s*, 3 H, H-24), 0.76 (*s*, 3 H, H-28), 0.70 (*d*, 1 H, H-5, *J* = 11.5, 1.5) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 199.7 (C11), 176.8 (C30), 169.4 (C13), 144.2 (aromatic-C4), 135.0 (aromatic-C1), 129.6 (aromatic-C2), 128.3 (C12), 127.5 (aromatic-C3), 90.6 (C3), 61.5 (C9), 55.2 (C5), 51.7 (OCH_3), 48.3 (C18), 45.3 (C8), 44.0 (C20), 43.1 (C14), 41.1 (C19), 38.8 (C1), 37.7 (C22), 36.7 (C10), 32.6 (C7), 31.8 (C17), 31.1 (C21), 28.5 (C28), 28.3 (C29), 27.9 (C28), 26.4 (C16), 26.3 (C15), 24.5 (C2), 23.3 (C27), 21.6 (aromatic-CH_3), 18.6 (C26), 17.5 (C6), 16.3 (C24), 16.3 (C25) ppm; MS (ESI): *m*/*z* (%) = 639.4 ($[M+H]^+$, 20), 661.1 ($[M+Na]^+$, 100), 677.1 ($[M+K]^+$, 8), 980.8 ($[3M+2Na]^{2+}$, 8), 1299.0 ($[2M+Na]^+$, 8).

4.2.5.7. Ethyl (3β)-3-hydroxy-11-oxoolean-12-en-30-oate (9)

Compound 9 was prepared according to [7].

4.2.5.8. 4-{(3β)-30-Ethoxy-11,30-dioxo-olean-12-en-3-yl]oxy}-4-oxobutanoic acid (10)

Following the procedure given for **5**, from **9** (310 mg, 0.62 mmol), succinic anhydride (60 mg, 0.62 mmol) and dry pyridine (10 ml), **10** (320 mg, 86 %) was obtained as colorless solid; mp 221-224 °C; R_f = 0.08 (hexane/ethyl acetate 1:1); $[\alpha]_D$ = + 119.54° (*c* 0.31, $CHCl_3$); UV-vis (methanol): λ_{max} (log ε) = 249 nm (4.08); IR (KBr): ν = 3430*br*, 2953*s*, 1728*s*, 1657*s*, 1466*s*, 1387*s*, 1315*m*, 1291*m*, 1215*s*, 1176*s*, 1086*m*, 1022*m*, 987*m*, 958*m* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.64 (*s*, 1 H, H-12), 4.55 (*dd*, 1 H, H-3, *J* = 11.6, 4.8 Hz), 4.19 (*dt*, 1 H, Et-C*H*H', *J* = 10.8, 7.1 Hz), 4.12 (*dt*, 1 H, Et-CH*H'*, *J* = 10.8, 7.1 Hz), 2.80 (*ddd*, 1 H, H-1, *J* = 13.7, 3.3, 3.3 Hz), 2.69 (*m*, 2 H, chain-γ-CH_2), 2.64 (*m*, 2 H, chain-β-CH_2), 2.36 (*s*, 1 H, H-9), 2.10 (*dd*, 1 H, H-18, *J* = 13.5, 3.9 Hz), 2.03 (*ddd*, 1 H, H-15, *J* = 13.5, 13.5, 4.2 Hz), 1.99 (*m*, 1 H, H-21), 1.93 (*ddd*, 1 H, H-19, *J* = 13.6, 4.0, 2.8 Hz), 1.82 (*ddd*, 1 H, H-16, *J* = 13.6, 13.6, 4.3 Hz), 1.71 (*m*, 1 H, H-2), 1.66 (*m*, 1 H, H-7), 1.63 (*m*, 1 H, H-2'), 1.61 (*dd*, 1 H, H-19', *J* = 13.5, 13.5 Hz), 1.58 (*m*, 1 H, H-6), 1.48 (*m*, 1 H, H-6'), 1.43 (*m*, 1 H, H-7'), 1.39 (*m*, 1 H, H-22), 1.36 (*s*, 3 H, H-27), 1.33 (*m*, 1 H, H-22'), 1.33 (*m*, 1 H, H-21'), 1.26 (*t*, 3 H, Et-CH_3, *J* = 7.1 Hz), 1.18 (*m*, 1 H, H-

16'), 1.16 (*s*, 3 H, H-25), 1.14 (*s*, 3 H, H-29), 1.12 (*s*, 3 H, H-26), 1.04 (*ddd*, 1 H, H-1', *J* = 13.8, 13.8, 3.6 Hz), 1.02 (*m*, 1 H, H-15'), 0.88 (*s*, 3 H, H-24), 0.87 (*s*, 3 H, H-23), 0.80 (*s*, 3 H, H-28), 0.79 (*m*, 1 H, H-5) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.1 (C-11), 177.1 (chain-δ-COOH), 176.4 (C-30), 171.8 (chain-α-COO), 169.5 (C-13), 128.4 (C-12), 81.2 (C-3), 61.7 (C-9), 60.3 (Et-CH_2), 55.0 (C-5), 48.4 (C-18), 45.4 (C-8), 43.8 (C-20), 43.2 (C-14), 41.1 (C-19), 38.7 (C-1), 38.1 (C-4), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 29.3 (chain-γ-CH_2), 28.9 (chain-β-CH_2), 28.6 (C-28), 28.3 (C-29), 28.0 (C-23), 26.5 (C-16), 26.4 (C-15), 23.5 (C-2), 23.3 (C-27), 18.7 (C-26), 17.4 (C-6), 16.7 (C-24), 16.4 (C-25), 14.3 (Et-CH_3) ppm; MS (ESI): *m*/*z* (%) = 599.5 ($[M+H]^+$, 20), 621.4 ($[M+Na]^+$, 68), 921.0 ($[3M+2Na]^{2+}$, 58), 1197.3 ($[2M+H]^+$, 22), 1219.4 ($[2M+Na]^+$, 100); analysis for $C_{36}H_{54}O_7$ (598.8): C, 72.21; H, 9.09; found: C, 72.03; H, 9.24.

4.2.5.9. Methyl 3,11-dioxoolean-12-en-30-oate (11)

Compound **11** was prepared according to [9].

4.2.5.10. Methyl (2*S*,4a*S*,6a*S*,6b*R*,14a*S*,16b*R*)-2,4a,6a,6b,9,9,14a-heptamethyl-15-oxo-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,14,14a,14b,15,16b-octadecahydrochryseno[1,2-*g*]quinoxaline-2-carboxylate (12)

Compound **11** (0.5 g, 1.00 mmol) was dissolved in morpholine (20 ml), sulfur (powder, 150 mg, 2.50 mmol) and ethylenediamine (15 ml, 2.20 mmol) was added. The mixture was stirred at 130 °C for 4 h. After cooling, the mixture was poured into ice water (50 ml), the aqueous layer was extracted with $CHCl_3$ (3 x 50 ml). The combined organic extracts were dried (Na_2SO_4), filtered and evaporated. Chromatographic purification (silica gel, chloroform/ether 7:3) gave **12** (200 mg, 39 %) as an off-white powder; mp 315 °C; R_f = 0.16 (chloroform/ether 7:3); $[a]_D$ = + 191.8° (*c* 0.36, $CHCl_3$); UV-vis (methanol): λ_{max} (log ε) = 226 (3.97), 253 (4.14) nm; IR (KBr): ν = 3432*br*, 2941*s*, 1731*s*, 1654*s*, 1451*m*, 1400*m*, 1387*w*, 1361*w*, 1323*w*, 1280*m*, 1209*m*, 1186*s*, 1153*w*, 1120*m*, 1108*m*, 1085*w*, 1063*w*, 1028*w* cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 8.38 (*d*, 1 H, pyrazine-H, *J* = 2.2 Hz), 8.30 (*d*, 1 H, pyrazine-H, *J* = 2.2 Hz), 5.75 (*s*, 1 H, H-12), 4.15 (*d*, 1 H, H-1, *J* = 17.0 Hz), 3.67 (*s*, 3 H, CH_3), 2.54 (*s*, 1 H, H-9), 2.54 (*d*, 1 H, H-1', *J* = 17.0 Hz), 2.10 (*dd*, 1 H, H-18, *J* = 13.3, 3.1 Hz), 2.03 (*ddd*, 1 H, H-15, *J* = 13.6, 13.6, 4.3 Hz), 1.96 (*m*, 1 H, H-

21), 1.93 (*m*, 1 H, H-19), 1.85 (*ddd*, 1 H, H-16, *J* = 13.7, 13.7, 4.1 Hz), 1.75 (*m*, 1 H, H-7), 1.67 (*m*, 1 H, H-6), 1.59 (*dd*, 1 H, H-19', *J* = 13.5, 13.5 Hz), 1.52 (*ddd*, 1 H, H-7', *J* = 12.6, 2.8, 2.8 Hz), 1.43 (*dd*, 1 H, H-5, *J* = 11.3, 2.1 Hz), 1.41 (*m*, 1 H, H-22), 1.38 (*m*, 2 H, H-22' and H-21'), 1.38 (*s*, 3 H, H-24), 1.31 (*s*, 3 H, H-27), 1.29 (*s*, 3 H, H-23), 1.22 (*m*, 1 H, H-16'), 1.18 (*s*, 3 H, H-26), 1.12 (*s*, 3 H, H-29), 1.10 (*s*, 3 H, H-25), 1.01 (*m*, 1 H, H-15'), 0.80 (*s*, 3 H, H-28) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 198.9 (C11), 176.8 (C30), 169.4 (C13), 158.8 (C3), 150.7 (C2), 142.1 (pyrazine), 141.7 (pyrazine), 128.6 (C12), 59.6 (C9), 53.0 (C5), 51.7 (OCH_3), 48.7 (C1), 48.4 (C18), 45.0 (C8), 44.0 (C20), 43.3 (C14), 41.2 (C19), 39.2 (C10), 37.7 (C22), 36.5 (C4), 31.8 (C23), 31.7 (C17), 31.7 (C7), 31.1 (C21), 28.5 (C28), 28.3 (C29), 26.5 (C16), 26.4 (C15), 24.2 (C27), 23.3 (C24), 19.3 (C6), 18.2 (C26), 15.8 (C25) ppm; MS (ESI): *m*/*z* (%) = 519.5 ($[M+H]^+$, 89), 572.9 ($[M+MeOH+Na]^+$, 100), 1037.2 ($[2M+H]^+$, 39); analysis for $C_{33}H_{46}N_2O_3$ (518.7): C, 76.41; H, 8.94; N, 5.40; found: C, 76.27; H, 9.11; N, 5. 32.

4.2.5.11. Methyl (3α)-3-amino-11-oxo-olean-12-en-30-oate (13) and methyl (3β)-3-amino-11-oxo-olean-12-en-30-oate (14)

To a solution of **11** (1.00 g, 2.06 mmol) in dry methanol (60 ml), ammonium acetate (1.60 g, 20.6 mmol) was added, and the mixture was stirred at 25 °C for 10 min. Sodium cyanoborohydride (130 mg, 2.06 mmol) was added, and stirring was continued for 24 h. The mixture was concentrated to 20 ml and acidified with conc. hydrochloric acid. The precipitate was filtered off and washed with water. Purification by chromatography (silica gel, chloroform/methanol 9:1) gave **13** (200 mg, 20 %) and **14** (520 mg, 52 %) each as colorless powder.

Data for **13**: mp 212 - 215 °C; R_f = 0.71 (chloroform/methanol 8:2); $[\alpha]_D$ = + 114.3° (*c* 0.35, $CDCl_3$); UV-vis (methanol): λ_{max} (log ε) = 249 (4.02) nm; IR (KBr): *ν* = 3422*br*, 2950*s*, 2361*w*, 1732*s*, 1660*s*, 1618*m*, 1519*m*, 1463*m*, 1385*m*, 1315*w*, 1280*w*, 1259*w*, 1216*m*, 1191*m*, 1155*m*, 1133*w*, 1084*w*, 1063*w*, 1031*w* cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 5.66 (*s*, 1 H, H-12), 3.69 (*s*, 3 H, CH_3), 3.03 (*m*, 1 H, H-3), 2.73 (*s*, 1 H, H-9), 2.60 (*ddd*, 1 H, H-1, *J* = 15.3, 3.4, 3.4 Hz), 2.07 (*m*, 1 H, H-18), 2.04 (*m*, 1 H, H-6), 2.02 (*m*, 1 H, H-15), 1.99 (*m*, 1 H, H-21), 1.92 (*m*, 1 H, H-19), 1.83 (*m*, 1 H, H-6'), 1.80 (*m*, 1 H, H-16), 1.78 (*m*, 1 H, H-7), 1.63 (*dd*, 1 H, H-19', *J* =

13.8, 13.8 Hz), 1.49 (*m*, 1 H, H-5), 1.44 (*m*, 1 H, H-2), 1.43 (*s*, 3 H, H-27), 1.38 (*m*, 1 H, H-2'), 1.37 (*m*, 1 H, H-1'), 1.36 (*m*, 1 H, H-22), 1.35 (*m*, 1 H, H-7'), 1.31 (*m*, 2 H, H-22' and H-21'), 1.17 (*m*, 1 H, H-16'), 1.14 (*s*, 3 H, H-29), 1.13 (*s*, 3 H, H-25), 1.09 (*s*, 3 H, H-26), 1.07 (*s*, 3 H, H-29), 1.01 (*m*, 1 H, H-15), 0.95 (*s*, 3 H, H-24), 0.80 (*s*, 3 H, H-28) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C11), 177.0 (C30), 169.1 (C13), 128.3 (C12), 60.7 (C9), 58.0 (C3), 51.8 (OCH_3), 48.4 (C18), 47.4 (C5), 45.5 (C8), 44.0 (C20), 43.5 (C14), 41.1 (C19), 37.7 (C22), 36.9 (C4), 35.7 (C10), 32.7 (C1), 32.4 (C7), 31.8 (C17), 31.2 (C21), 28.8 (C23), 28.5 (C28), 28.3 (C29), 26.5 (C16), 26.4 (C15), 24.3 (C27), 22.7 (C24), 22.1 (C6), 18.6 (C26), 17.4 (C2), 16.5 (C25) ppm; MS (ESI): *m*/*z* (%) = 484.3 ($[M+H]^+$, 100).

Data for **14**: mp 206 °C; R_f = 0.64 (chlorform/methanol 8:2); $[\alpha]_D$ = + 121.8° (*c* 0.57, $CDCl_3$); UV-vis (methanol): λ_{max} (log ε) = 249 (4.02) nm; IR (KBr): ν = 3431*br*, 2950*s*, 1731*s*, 1659*s*, 1551*s*, 1465*s*, 1387*s*, 1328*m*, 1278*m*, 1249*m*, 1217*s*, 1190*m*, 1154*s*, 1088*m*, 1014*m*, 993*m* cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 5.65 (*s*, 1 H, H-12), 3.68 (*s*, 3 H, CH_3), 2.84 (*ddd*, 1 H, H-1, *J* = 13.0, 2.9, 2.9 Hz), 2.74 (*m*, 1 H, H-3), 2.33 (*s*, 1 H, H-9), 2.07 (*dd*, 1 H, H-18, *J* = 13.4, 4.3 Hz), 2.01 (*ddd*, 1 H, H-15, *J* = 9.8, 9.8, 3.8 Hz), 1.99 (*m*, 1 H, H-21), 1.92 (*ddd*, 1 H, H-19, *J* = 13.5, 4.3, 2.8 Hz), 1.90 (*m*, 1 H, H-2), 1.81 (*m*, 1 H, H-2'), 1.78 (*m*, 1 H, H-16), 1.64 (*m*, 1 H, H-7), 1.62 (*m*, 1 H, H-6), 1.60 (*dd*, 1 H, H-19', *J* = 13.5, 13.5 Hz), 1.44 (*m*, 1 H, H-6'), 1.41 (*m*, 1 H, H-7'), 1.38 (*m*, 1 H, H-22), 1.35 (*s*, 3 H, H-27), 1.28 (*m*, 2 H, H-22' and H-21'), 1.17 (*m*, 1 H, H-16'), 1.14 (*s*, 6 H, H-25 and H-29), 1.12 (*s*, 3 H, H-24), 1.10 (*s*, 3 H, H-23), 0.99 (*m*, 1 H, H-15'), 0.96 (*m*, 1 H, H-1'), 0.94 (*s*, 3 H, H-26), 0.80 (*s*, 3 H, H-28), 0.74 (*dd*, 1 H, H-5, *J* = 11.9, 1.3 Hz) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 199.6 (C11), 176.7 (C30), 169.0 (C13), 128.3 (C12), 61.3 (C9), 59.6 (C3), 55.1 (C5), 51.5 (OCH_3), 48.2 (C18), 45.1 (C8), 43.8 (C20), 43.0 (C14), 41.0 (C19), 38.9 (C4), 37.6 (C1), 37.0 (C10), 36.7 (C22), 32.4 (C7), 31.6 (C17), 31.0 (C21), 28.3 (C29), 28.1 (C28), 28.1 (C23), 26.4 (C16), 26.4 (C15), 24.2 (C2), 23.1 (C27), 18.5 (C24), 17.3 (C6), 16.0 (C26), 15.8 (C25) ppm; MS (ESI): *m*/*z* (%) = 484.3 ($[M+H]^+$, 100);

4.2.5.12. Methyl (3β) 3-{[N-(*tert*-butoxycarbonyl)-L-alanyl]amino}-11-oxoolean-12-en-30-oate (15)

Following the procedure given for **20**, compound **15** was obtained from **14** (160 mg, 0.32 mmol), DCC (80 mg, 0.39 mmol), DMAP (10 mg, 0.08 mmol) and boc-L-alanine (80 mg, 0.42 mmol). Purification by chromatography (silica gel, hexane/ethyl acetate 3:7) and recrystallization from ethyl acetate/hexane gave **15** (120 mg, 57 %) as colorless crystals; mp 198 – 203 °C; R_f = 0.1 (hexane/ethyl acetate 7:3); $[\alpha]_D$ = + 49.6 ° (*c* 0.50, $CHCl_3$); UV-vis (methanol): λ_{max} (log ε) = 249 (3.92) nm; IR (KBr): ν = 3328*br*, 2930*s*, 2852*s*, 1731*s*, 1662*s*, 1626*s*, 1575*m*, 1534*m*, 1456*m*, 1388*m*, 1367*m*, 1313*m*, 1245*m*, 1218*m*, 1166*m* cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 6.02 (*d*, 1 H, NH), 5.59 (*s*, 1 H, H-12), 4.88 (*m*, 1 H, NH), 3.62 (*s*, 3 H, CH_3), 3.59 (*dd*, 1 H, H-3, *J* = 11.0, 4.4 Hz), 3.47 (*m*, 1 H, Ala-CH), 2.70 (*ddd*, 1 H, H-1, *J* = 13.2, 2.8, 2.8 Hz), 2.32 (*s*, 1 H, H-9), 2.01 (*dd*, 1 H, H-18, *J* = 13.7, 3.5 Hz), 1.95 (*ddd*, 1 H, H-15, *J* = 13.5, 13.5, 4.1 Hz), 1.93 (*m*, 1 H, H-21), 1.84 (*m*, 1 H, H-19), 1.75 (*ddd*, 1 H, H-16, *J* = 13.7, 13.7, 4.3 Hz), 1.62 (*m*, 1 H, H-7), 1.55 (*dd*, 1 H, H-19', *J* = 13.6, 13.6 Hz), 1.58 (*m*, 1 H, H-6), 1.56 (*m*, 1 H, H-2), 1.46 (*m*, 1 H, H-2'), 1.43 (*m*, 1 H, H-6'), 1.38 (*s*, 9 H, Boc-CH_3), 1.37 (*m*, 1 H, H-7'), 1.34 (*m*, 1 H, H-22), 1.30 (*s*, 3 H, H-27), 1.28 (*s*, 3 H, Ala-CH_3), 1.25 (*m*, 2 H, H-22' and H-21'), 1.10 (*m*, 1 H, H-16'), 1.08 (*s*, 3 H, H-29), 1.07 (*s*, 3 H, H-25), 1.05 (*s*, 3 H, H-26), 1.00 (*m*, 1 H, H-1'), 0.95 (*m*, 1 H, H-15'), 0.83 (*s*, 3 H, H-23), 0.81 (*m*, 1 H, H-5), 0.73 (*s*, 3 H, H-28), 0.72 (*s*, 3 H, H-24) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 200.2 (C11), 177.0 (C30), 172.0 (Ala-COO), 169.2 (C13), 156.7 (Boc-COO), 128.5 (C12), 80.1 (Boc-*quart.*-C), 60.7 (C9), 58.0 (C3), 51.8 (OCH_3), 48.4 (C18), 47.4 (C5), 45.4 (C8), 44.0 (C20), 43.2 (C14), 41.1 (C19), 39.6 (C1), 38.2 (C4), 37.7 (C22), 36.9 (C10), 32.7 (C7), 31.8 (C17), 31.1 (C21), 28.5 (C28), 28.4 (C29), 28.3 (C23), 28.3 (Boc-CH_3), 26.4 (C16), 26.4 (C15), 25.6 (C2), 23.3 (C27), 18.6 (C26), 17.9 (Ala-CH_3), 17.7 (C6), 16.5 (C24), 16.2 (C25) ppm; MS (ESI): *m*/*z* (%) = 655.3 ($[M+H]^+$, 32), 677.4 ($[M+Na]^+$, 100), 1004.82 ($[3M+2Na]^{2+}$, 12), 1332.2 ($[2M+Na]^+$, 25); analysis for $C_{39}H_{62}N_2O_6$ (654.9): C, 71.52; H, 9.54; N, 4.28; found: C, 71.41; H, 9.73; N, 4.13.

4.2.5.13. Methyl (3β) 3-(L-alanylamino)-11-oxo-olean-12-en-30-oate (16)

Compound **15** (100 mg, 0.15 mmol) was dissolved in DCM and trifluoroacetic acid (2 ml; 25.96 mmol) was added. After 1 h of continous stirring, a saturated solution of sodium hydrogencarbonate (10 ml) was added, and the organic layer was washed with water (20 ml) afterwards. The solvent was removed, and the residue was subjected to chromatography (silica gel, chloroform/methanol 8:2) to yield **16** (50 mg, 60 %) as a colorless powder; mp 183 – 185 °C; R_f = 0.50 (chloroform/methanol 8:2); UV-vis (methanol): λ_{max} (log ε) = 248 (3.57) nm; IR (KBr): ν = 3330*br*, 2930*s*, 2852*m*, 1730*s*, 1659*s*, 1539*s*, 1451*m*, 1385*m*, 1314*w*, 1246*m*, 1218*m*, 1154*w*, 1088*w*, 1030*w* cm^{-1}; ^{1}H-NMR (500 MHz, methanol-d_4): δ = 5.48 (*s*, 1 H, H-12), 3.60 (*s*, 3 H, CH_3), 3.50 (*dd*, 1 H, H-3, *J* = 12.6, 4.0 Hz), 3.39 (*m*, 1 H, Ala-CH), 2.67 (*ddd*, 1 H, H-1, *J* = 13.0, 3.2, 3.2 Hz), 2.42 (*s*, 1 H, H-9), 2.07 (*dd*, 1 H, H-18, *J* = 13.9, 4.3 Hz), 2.03 (*ddd*, 1 H, H-15, *J* = 13.8, 4.1, 4.1 Hz), 1.87 (*ddd*, 1 H, H-21, *J* = 13.6, 5.2, 3.0 Hz), 1.79 (*m*, 1 H, H-16), 1.77 (*m*, 1 H, H-19), 1.70 (*m*, 1 H, H-2), 1.68 (*m*, 1 H, H-7), 1.65 (*dd*, 1 H, H-19', *J* = 13.5, 13.5 Hz), 1.62 (*ddd*, 1 H, H-2', *J* = 13.9, 9.1, 3.2 Hz), 1.60 (*m*, 1 H, H-6), 1.44 (*m*, 1 H, H-6'), 1.43 (*m*, 1 H, H-7'), 1.38 (*m*, 1 H, H-22), 1.34 (*s*, 3 H, H-27), 1.31 (*m*, 2 H, H-22' and H-21'), 1.18 (*d*, 3 H, Ala-CH_3, *J* = 7.0 Hz), 1.15 (*m*, 1 H, H-16'), 1.06 (*s*, 3 H, H-25), 1.05 (*s*, 3 H, H-26), 1.05 (*s*, 3 H, H-29), 1.00 (*m*, 1 H, H-1'), 0.95 (*m*, 1 H, H-15'), 0.83 (*dd*, 1 H, H-5, *J* = 12.1, 1.2 Hz), 0.77 (*s*, 3 H, 23), 0.75 (*s*, 3 H, H-24), 0.73 (*s*, 3 H, H-28) ppm; ^{13}C-NMR (125 Hz, methanol-d_4): δ = 202.2 (C11), 178.4 (C30), 177.1 (Ala-COO), 172.4 (C13), 128.8 (C12), 63.0 (C9), 57.9 (C3), 56.7 (C5), 52.3 (Ala-CH), 51.4 (OCH_3), 49.9 (C18), 46.7 (C8), 45.3 (C20), 44.7 (C14), 42.4 (C19), 40.9 (C1), 39.6 (C4), 39.0 (C22, *C*H_2), 38.4 (C10), 33.7 (C7), 33.0 (C17), 32.0 (C21), 29.2 (C28), 29.2 (C23), 28.5 (C29), 27.6 (C16), 27.4 (C15), 26.0 (C2), 23.9 (C27), 21.8 (Ala-CH_3), 19.3 (C26), 18.9 (C6), 17.2 (C24), 16.9 (C25) ppm; MS (ESI): *m*/*z* (%) = 555.4 ($[M+H]^+$, 100); analysis for $C_{34}H_{54}N_2O_4$ (554.8): C, 73.61; H, 9.81; N, 5.05; found: C, 73.49; H, 9.99; N, 4.88.

4.2.5.14. Methyl 3-(hydroxyimino)-11-oxo-olean-12-en-30-oate (17)

To a solution of **11** (1.00 g, 2.10 mmol) in dry pyridine (10 ml), hydroxylamine hydrochloride (348 mg, 4.00 mmol) was added. The mixture was stirred at 60 °C for 3 h and acidified with hydrochloric acid (1 M, 10 ml). Usual aq. workup followed by chromatography (silica gel,

hexane/ethyl acetate 7:3) gave **17** (835 mg, 80 %) as a colorless powder; mp 283 °C (lit. 289 - 290 °C [20]); R_f = 0.66 (hexane/ethyl acetate 8:2); $[\alpha]_D$ = + 101.5° (*c* 0.68, $CHCl_3$) (lit. $[\alpha]_D$ = + 106.7° (*c* 2.0, $CHCl_3$) [20]); UV-vis (methanol): λ_{max} (log ε) = 249 nm (4.11); IR (KBr): ν = 3278*br*, 2932*s*, 2870*m*, 1729*s*, 1655*s*, 1618*w*, 1464*w*, 1387*s*, 1366*m*, 1320*s*, 1281*m*, 1248*w*, 1218*w*, 1190*w*, 1153*w*, 1087*w*, 1030*w* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.61 (*s*, 1 H, H-12), 3.62 (*s*, 3 H, CH3), 2.99 (*ddd*, 1 H, H-2, *J* = 15.5, 4.2, 4.2 Hz), 2.81 (*ddd*, 1 H, H-1, *J* = 13.2, 5.3, 3.7 Hz), 2.31 (*s*, 1 H, H-9), 2.22 (*ddd*, 1 H, H-2', *J* = 15.6, 12.9, 5.7 Hz), 2.02 (*dd*, 1 H, H-18, *J* = 13.2, 2.8 Hz), 1.95 (*m*, 1 H, H-15), 1.92 (*m*, 1 H, H-21), 1.84 (*ddd*, 1 H, H-19, *J* = 13.5, 3.7, 2.8 Hz), 1.77 (*ddd*, 1 H, H-16, *J* = 13.4, 13.3, 4.2 Hz), 1.60 (*ddd*, 1 H, H-7, *J* = 12.6, 12.6, 3.5 Hz), 1.54 (*dd*, 1 H, H-19', *J* = 13.7, 13.7 Hz), 1.54 (*m*, 1 H, H-6), 1.45 (*ddd*, 1 H, H-6', *J* = 12.5, 12.5, 2.5 Hz), 1.37 (*ddd*, 1 H, H-7', *J* = 12.5, 2.9, 2.9 Hz), 1.32 (*ddd*, 1 H, H-22, *J* = 10.0, 10.0, 3.0 Hz), 1.28 (*s*, 3 H, H-29), 1.25 (*m*, 2 H, H-21' and H-22'), 1.19 (*s*, 3 H, H-25), 1.12 (*s*, 3 H, H-23), 1.12 (*m*, 1 H, H-16'), 1.09 (*s*, 3 H, H-26), 1.08 (*s*, 3 H, H-28), 1.03 (*s*, 3 H, H-24), 0.98 (*m*, 1 H, H-1'), 0.94 (*m*, 1 H, H-15'), 0.91 (*m*, 1 H, H-5), 0.74 (*s*, 3 H, H-27) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.6 (C11), 176.9 (C30), 169.4 (C13), 167.2 (C3), 128.5 (C12), 61.3 (C9), 55.6 (C5), 51.7 (OCH_3), 48.4 (C18), 45.3 (C8), 44.0 (C20), 43.2 (C14), 41.1 (C19), 40.5 (C4), 39.0 (C1), 37.7 (C22), 37.0 (C10), 32.4 (C7), 31.8 (C17), 31.1 (C21), 28.5 (C28), 28.3 (C29), 27.1 (C23), 26.5 (C16), 26.4 (C15), 23.3 (C27), 23.2 (C24), 18.6 (C26), 18.2 (C6), 17.4 (C2), 15.7 (C25) ppm; MS (ESI): *m*/*z* (%) = 498.5 ($[M+H]^+$, 58), 520.3 ($[M+Na]^+$, 6), 552.0 ($[M+MeOH+Na]^+$, 100), 995.0 ($[2M+H]^+$, 42), 1017.3 ($[2M+H]^+$, 43).

4.2.5.15. Methyl 3-(*i*-propoxyimino)-11-oxo-olean-12-en-30-oate (18)

To a solution of **17** (500 mg, 0.60 mmol) in dry THF (20 ml), potassium hydroxide (0.16 g, 3.00 mmol) and i-propyl iodide (340 mg, 2.00 mmol) was added. After refluxing for 24 h, the mixture was cooled and poured into water (40 ml). The aqueous layer was extracted with chloroform (3 x 50 ml), the extracts were dried (Na_2SO_4), filtered and evaporated. Purification by chromatography (silica gel, chloroform/ether 8:2) afforded **18** (120 mg, 37 %) as a colorless powder; mp 145 °C; R_f = 0.77 (chloroform/ether 9:1); $[\alpha]_D$ = 105.42° (*c* 0.32, $CHCl_3$); UV-vis (methanol): λ_{max} (log ε) = 257 nm (4.26), IR (KBr): ν = 3446*s*, 2972*s*, 2870*s*, 1732*s*, 1662*s*,

1622*w*, 1458*m*, 1386*m*, 1366*w*, 1325*w*, 1281*w*, 1262*w*, 1218*m*, 1189*m*, 1153*m*, 1088*w*, 1028*w*, 963*m* cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 5.66 (*s*, 1 H, H-12), 4.26 (*qq*, 1 H, chain-CH, *J* = 6.2, 6.2 Hz), 3.67 (*s*, 3 H, CH_3), 2.89 (*ddd*, 1 H, H-2, *J* = 15.7, 5.0, 4.0 Hz), 2.76 (*ddd*, 1 H, H-1, *J* = 13.3, 5.7, 4.0 Hz), 2.36 (*s*, 1 H, H-9), 2.24 (*ddd*, 1 H, H-2', *J* = 15.6, 12.2, 5.9 Hz), 2.06 (*dd*, 1 H, H-18, *J* = 13.5, 3.3 Hz), 1.99 (*m*, 1 H, H-15), 1.97 (*m*, 1 H, H-21), 1.90 (*ddd*, 1 H, H-19, *J* = 13.5, 3.6, 2.7 Hz), 1.82 (*ddd*, 1 H, H-16, *J* = 13.6, 13.6, 4.4 Hz), 1.69 (*m*, 1 H, H-7), 1.59 (*dd*, 1 H, H-19', *J* = 13.5, 13.5 Hz), 1.56 (*m*, 1 H, H-6), 1.52 (*m*, 1 H, H-6'), 1.44 (*m*, 1 H, H-7'), 1.39 (*m*, 1 H, H-22), 1.33 (*s*, 3 H, H-27), 1.30 (*m*, 2 H, H-22' and H-21'), 1.21 (*s*, 3 H, H-25), 1.20 (*m*, 1 H, H-16'), 1.19 (*m*, 6 H, chain-CH_3), 1.16 (*s*, 3 H, H-23), 1.13 (*s*, 6 H, H-26 and H-29), 1.04 (*s*, 3 H, H-24), 1.03 (*m*, 1 H, H-1'), 1.01 (*m*, 1 H, H-5), 1.00 (*m*, 1 H, H-15'), 0.78 (*s*, 3 H, H-28) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 199.6 (C11), 176.7 (C30), 169.0 (C13), 164.5 (C3), 128.6 (C12), 74.3 (chain-CH), 61.5 (C9), 55.6 (C5), 51.8 (OCH_3), 48.6 (C18), 45.5 (C8), 44.2 (C20), 43.4 (C14), 41.3 (C19), 40.3 (C4), 39.1 (C1), 37.9 (C22), 37.1 (C10), 32.6 (C7), 32.0 (C17), 31.3 (C21), 28.7 (C28), 28.5 (C29), 27.8 (C23), 26.7 (C16), 26.7 (C15), 23.7 (C24), 23.5 (C27), 21.8 (chain-CH_3), 18.8 (C26), 18.5 (C6), 18.2 (C2), 15.8 (C25) ppm; MS (ESI): *m*/*z* (%) = 540.5 ($[M+H]^+$, 84), 593.5 ($[M+MeOH+Na]^+$, 100), 1101.3 ($[2M+Na]^+$, 40); analysis for $C_{34}H_{53}NO_4$ (539.8): C, 75.65; H, 9.90; N, 2.59; found: C, 75.47; H, 10.02; N, 2.37.

4.2.5.16. Methyl 3-[(octyloxy)imino]-11-oxo-olean-12-en-30-oate (19)

To a solution of **17** (300 mg, 0.60 mmol) in dry THF (20 ml), potassium hydroxide (0.1 g, 1.8 mmol) and n-bromooctane (230 mg, 1.20 mmol) was added. After refluxing for 24 h, the mixture was cooled and filtered. The solvent was removed and the residue was subjected to chromatography (silica gel, chloroform/ether 9:1) to yield **19** (106 mg, 29 %) as a colorless powder; mp 92 – 96 °C; R_f = 0.81 (hexane/ethyl acetate 95:5); $[\alpha]_D$ = + 81.6° (*c* 0.44, $CHCl_3$); UV-vis (methanol): λ_{max} (log ε) = 200 (4.04), 249 (4.03) nm; IR (KBr): ν = 3440*br*, 2927*m*, 2858*s*, 1731*s*, 1655*s*, 1464*s*, 1386*m*, 1363*m*, 1321*w*, 1280*w*, 1262*w*, 1217*w*, 1189*w*, 1155*m*, 1088*m*, 1050*m*, 1028*w* cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 5.64 (*s*, 1 H, CH (12)), 3.95 (*t*, 2 H, chain-1, *J* = 6.7 Hz), 3.65 (*s*, 3 H, CH_3), 2.91 (*ddd*, 1 H, H-2, *J* = 15.7, 5.2, 3.8 Hz), 2.76 (*ddd*, 1 H, H-1, *J* = 13.3, 5.8, 3.8 Hz), 2.34 (*s*, 1 H, H-9), 2.21 (*ddd*, 1 H, H-2', *J* = 15.7, 12.3, 5.8 Hz), 2.08 (*dd*, 1 H, H-18, *J* = 13.5, 3.5 Hz), 1.98 (*m*, 1 H, H-15), 1.94 (*m*, 1 H, H-21), 1.87 (*ddd*, 1 H,

H-19, *J* = 13.6, 4.0, 2.6 Hz), 1.82 (*m*, 2 H, H-16 and H-7), 1.58 (*dd*, 1 H, H-19', *J* = 13.5, 13.5 Hz), 1.63 (*m*, 2 H, chain-4), 1.57 (*m*, 2 H, chain-2), 1.41 (*m*, 1 H, H-7'), 1.36 (*m*, 1 H, H-22), 1.31 (*s*, 3 H, H-24), 1.30 – 1.28 (*m*, 9 H, H-22', H-21', chain-6, chain-5, chain-3, H-6), 1.19 (*s*, 3 H, H-25), 1.13 (*s*, 3 H, H-23), 1.11 (*s*, 3 H, H-29), 1.10 (*s*, 3 H, H-26), 1.02 (*s*, 3 H, H-27), 1.02 – 0.97 (*m*, 3 H, H-1', H-16', H-15'), 0.84 (*m*, 3 H, chain-8), 0.77 (*s*, 3 H, H-28) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.7 (C11), 176.8 (C30), 169.1 (C13), 165.1 (C3), 128.5 (C12), 73.3 (chain-1), 61.3 (C9), 55.5 (C5), 51.7 (OCH_3), 48.3 (C18), 45.2 (C8), 44.0 (C20), 43.2 (C14), 41.1 (C19), 40.1 (C4), 39.0 (C1), 37.7 (C22), 36.9 (C10), 32.8 (C7), 32.4 (C17), 31.8 (C21), 31.1 (chain-6), 29.4 (chain-2), 29.2 (chain-5), 29.1 (chain-4), 28.5 (C28), 28.2 (C29), 28.1 (chain-3), 27.4 (C23), 26.4 (C15), 26.3 (C16), 23.4 (C27), 23.3 (C24), 22.6 (C2), 18.6 (C26), 18.2 (chain-7), 17.8 (C6), 15.6 (C25), 14.1 (chain-8) ppm; MS (ESI): *m*/*z* (%) = 610.5 ($[M+H]^+$, 88), 632.5 ($[M+Na]^+$, 100), 1219.2 ($[2M+H]^+$, 20), 1241.4 ($[2M+Na]^+$, 44); analysis for $C_{39}H_{63}NO_4$ (609.9): C, 76.80; H, 10.41; N, 2.30; found: C, 76.54; H, 10.69; N, 2.11.

4.2.5.17. Methyl 3-[6-{(2L)-2-[*tert*-butoxycarbonyl)amino]propanoyl}oxy)imino]-11-oxo-olean-12-en-30 oate (20)

To a solution of **17** (250 mg, 0.50 mmol) and DMAP (10 mg, 0.08 mmol) in dry DCM (20 ml), boc-L-alanine (120 mg, 0.63 mmol) and DCC (120 mg, 0.60 mmol) were added. The mixture was stirred at 25 °C for 16 h. The solvent was removed under reduced pressure, the residue purified by chromatography (silica gel, hexane/ethyl acetate 7:3) to afford **20** (160 mg, 48 %) as a colorless powder; mp 116 – 118 °C; R_f = 0.65 (hexane/ethylacetate 7:3); $[\alpha]_D$ = + 95.5° (*c* 0.40, $CHCl_3$); UV-vis (methanol): λ_{max} (log ε) = 249 (4.11) nm; IR (KBr): ν = 3385*br*, 2979*s*, 2872*m*, 2361*w*, 1763*m*, 1727*s*, 1660*s*, 1619*w*, 1509*m*, 1456*s*, 1389*m*, 1367*m*, 1345*m*, 1249*m*, 1218*m*, 1161*m*, 1109*s*, 1064*w*, 1026*w* cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 5.67 (*s*, 1 H, H-12), 5.09 (*d*, 1 H, Ala-NH, *J* = 7.0 Hz), 4.47 (*m*, 1 H, Ala-CH), 3.67 (*s*, 3 H, CH_3), 2.88 (*m*, 1 H, H-2), 2.85 (*m*, 1 H, H-1), 2.41 (*m*, 1 H, H-2'), 2.37 (*s*, 1 H, H-9), 2.08 (*dd*, 1 H, H-18, *J* = 13.2, 3.3 Hz), 2.03 (*m*, 1 H, H-15), 1.98 (*m*, 1 H, H-21), 1.90 (*ddd*, 1 H, H-19, *J* = 13.9, 4.0, 2.7 Hz), 1.82 (*ddd*, 1 H, H-16, *J* = 13.5, 13.5, 4.5 Hz), 1.69 (*m*, 1 H, H-7), 1.64 (*m*, 1 H, H-6), 1.58 (*dd*, 1 H, H-19', *J* = 13.8, 13.8 Hz), 1.51 (*m*, 1 H, H-6'), 1.42 (*m*, 1 H, H-7'), 1.41 (*s*, 12 H, Boc-CH_3 and Ala-CH_3), 1.39 (*m*, 1 H, H-22), 1.33 (*s*, 3 H, H-27), 1.29 (*m*, 2 H, H-22' and H-21'), 1.26 (*s*, 3 H, H-23),

1.25 (*s*, 3 H, H-25), 1.18 (*m*, 1 H, H-16'), 1.14 (*s*, 3 H, H-24), 1.14 (*m*, 1 H, H-1'), 1.13 (*m*, 1 H, H-5), 1.12 (*s*, 6 H, H-26 and H-29), 1.01 (*m*, 1 H, H-15'), 0.79 (*s*, 3 H, H-28) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 199.2 (C11), 176.7 (C30), 175.8 (Ala-COO), 171.4 (C3), 169.5 (C13), 154.9 (Boc-COO), 128.4 (C12), 79.9 (Boc-*quart.*-C), 61.4 (C9), 55.6 (C5), 51.7 (OCH_3), 48.6 (C18), 47.8 (Ala-CH), 45.4 (C8) 44.2 (C20), 43.5 (C14), 41.7 (C4), 41.3 (C19), 39.4 (C1), 37.9 (C22), 37.1 (C10), 32.5 (C7), 32.0 (C17), 31.3 (C21), 28.7 (C28), 28.3 (Boc-CH_3), 28.4 (C29), 27.4 (C23), 26.7 (C16), 26.6 (C15), 23.5 (C27), 23.2 (C24), 19.9 (C2), 19.0 (Ala-CH_3), 18.8 (C26), 18.4 (C6), 16.1 (C25) ppm; MS (ESI): *m*/*z* (%) = 669.1 ($[M+H]^+$, 5), 686.2 ($[M+NH_4]^+$, 5), 691.3 ($[M+Na]^+$, 100), 1359.1 ($[2M+Na]^+$, 24); analysis for $C_{39}H_{60}N_2O_7$ (668.9): C, 70.03; H, 9.04; N, 4.19; found: C, 69.85; H, 9.25; N, 3.98.

4.2.5.18. Methyl (3β)-3-({[(2L)-(*tert*-butoxycarbonyl)amino][(*tert*-butoxycarbonyl)thio]acetyl}oxy)-11-oxoolean-12-en-30-oate (21)

Following the procedure for **20**, **21** (740 mg, 93 %) was obtained from **4** (490 mg, 1.01 mmol), Boc-L-Cys(SBoc)-OH (380 mg, 1.18 mmol), DCC (380 mg, 1.84 mmol) and DMAP (30 mg, 0.25 mmol) after purification by chromatography (silica gel, chloroform/ether 9:1) as a colorless powder; mp 130-133 °C; R_f = 0.70 (hexane/ethyl acetate 7:3); $[\alpha]_D$ = + 78.66° (*c* 0.41, $CHCl_3$); UV-vis (methanol): λ_{max} (log ε) = 250 nm (4.00); IR (KBr): ν = 3439*br*, 2978*s*, 2875*m*, 1727*s*, 1661*s*, 1500*m*, 1457*m*, 1392*m*, 1369*s*, 1249*m*, 1216*s*, 1168*s*, 1128*s*, 1087*m*, 1050*m*, 1021*m*, 986*m* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.67 (*s*, 1 H, H-12), 5.30 (*d*, 1 H, NH, *J* = 7.6 Hz), 4.58 (*dd*, 1 H, H-3, *J* = 11.8, 4.8 Hz), 4.54 (*m*, 1 H, Cys-CH), 3.69 (*s*, 3 H, OCH_3), 3.40 (*dd*, 1 H, Cys-C*H*H', *J* = 14.0, 4.0 Hz), 3.19 (*dd*, 1 H, Cys-CH*H'*, *J* = 14.0, 6.6 Hz), 2.82 (*ddd*, 1 H, H-1, *J* = 13.7, 3.2, 3.2 Hz), 2.35 (*s*, 1 H, H-9), 2.08 (*dd*, 1 H, H-18, *J* = 13.0, 3.4 Hz), 2.03 (*ddd*, 1 H, H-15, *J* = 13.4, 13.4, 4.3 Hz), 1.99 (*m*, 1 H, H-21), 1.92 (*ddd*, 1 H, H-19, *J* = 13.6, 3.5, 2.9 Hz), 1.82 (*ddd*, 1 H, H-16, *J* = 13.7, 13.7, 4.5 Hz), 1.74 (*m*, 1 H, H-2), 1.66 (*m*, 1 H, H-7), 1.62 (*m*, 1 H, H-2'), 1.61 (*dd*, 1 H, H-19', *J* = 13.5, 13.5 Hz), 1.59 (*m*, 1 H, H-6), 1.46 (*s*, 9 H, S-Boc-CH_3), 1.45 (*m*, 1 H, H-6), 1.44 (*s*, 9 H, O-Boc-CH_3), 1.43 (*m*, 1 H, H-7'), 1.39 (*m*, 1 H, H-22), 1.36 (*s*, 3 H, H-27), 1.31 (*m*, 1 H, H-22'), 1.31 (*m*, 1 H, H-21'), 1.18 (*m*, 1 H, H-16'), 1.16 (*s*, 3 H, H-25), 1.15 (*s*, 3 H, H-29), 1.13 (*s*, 3 H, H-26), 1.04 (*m*, 1 H, H-1'), 1.01 (*m*, 1 H, H-15'), 0.89 (*s*, 3 H,

H-23), 0.89 (*s*, 3 H, H-24), 0.80 (*s*, 3 H, H-28), 0.79 (*m*, 1 H, H-5) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.9 (C-11), 176.9 (C-30), 170.2 (Cys-COO), 169.2 (C-13), 168.4 (S-Boc-COO), 155.0 (O-Boc-COO), 128.5 (C-12), 85.4 (S-Boc-*quart.*-C), 82.5 (C-3), 79.8 (Boc-*quart.*-C), 61.7 (C-9), 55.0 (C-5), 53.9 (Cys-CH), 51.8 (OCH_3), 48.4 (C-18), 45.4 (C-8), 44.0 (C-20), 43.2 (C-14), 41.1 (C-19), 38.7 (C-1), 38.2 (C-4), 37.7 (C-22), 36.9 (C-10), 33.0 (Cys-CH_2), 32.7 (C-7), 31.8 (C-17), 31.2 (C-21), 28.5 (C-28), 28.3 (3x O-Boc-CH_3), 28.3 (C-29), 28.1 (3x S-Boc-CH_3), 28.1 (C-23), 26.5 (C-16), 26.4 (C-15), 23.6 (C-2), 23.4 (C-27), 18.7 (C-26), 17.4 (C-6), 16.8 (C-24), 16.4 (C-25) ppm; MS (ESI): *m*/*z* (%) = 788.2 ($[M+H]^+$, 10), 810.3 ($[M+Na]^+$, 100), 1204.5 ($[3M+2Na]^{2+}$, 6), 1597.2 ($[2M+H]^+$, 12); analysis for $C_{44}H_{69}NO_9S$ (788.1): C, 67.06; H, 8.82; N, 1.78; S, 4.07; found: C, 66.88; H, 9.01; N, 1.64; S, 3.86.

4.2.5.19. Dimethyl (3β,3'β)-3,3'-(dithiobis{[(2*S*)-2-amino-1-oxoethane-2,1-diyl]oxy}) bis(11-oxoolean-12-en-30-oate) (22)

To a solution of **21** (640 mg, 0.81 mmol) in dry DCM (15 ml), trifluoroacetic acid (4 ml, 51.92 mmol) was added, and the mixture was stirred at room temperature for 1 d. A saturated solution of $NaHCO_3$ (10 ml) was slowly added, and the mixture was extracted with DCM (3 x 10 ml). The organic layers were washed with water (20 ml) and brine (20 ml), dried (Na_2SO_4) and filtered. The solvent was removed and **22** (410 mg, 43%) was obtained as a slightly yellowish powder; mp 220-224 °C; R_f = 0.95 (chloroform/methanol 9:1); $[\alpha]_D$ = + 103.37° (*c* 0.49, $CHCl_3$); UV-vis (methanol): λ_{max} (log ε) = 249 nm (4.34); IR (KBr): ν = 3432*br*, 2950*s*, 1732*s*, 1660*s*, 1465*m*, 1388*m*, 1324*w*, 1216*s*, 1087*w* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.67 (*s*, 1 H, H-12), 4.59 (*dd*, 1 H, H-3, *J* = 11.7, 4.6 Hz), 3.87 (*m*, 1 H, Cys-CH), 3.68 (*s*, 3 H, OCH_3), 3.22 (*m*, 1 H, Cys-C*H*H'), 2.97 (*m*, 1 H, Cys-CH*H'*), 2.82 (*ddd*, 1 H, H-1, *J* = 13.7, 3.5, 3.5 Hz), 2.36 (*s*, 1 H, H-9), 2.22 (*m*, 2 H, NH_2), 2.08 (*dd*, 1 H, H-18, *J* = 13.3, 3.5 Hz), 2.02 (*ddd*, 1 H, H-15, *J* = 13.6, 13.6, 4.2 Hz), 1.99 (*m*, 1 H, H-21), 1.92 (*ddd*, 1 H, H-19, *J* = 13.7, 3.6, 2.5 Hz), 1.82 (*ddd*, 1 H, H-16, *J* = 13.5, 13.5, 4.4 Hz), 1.73 (*m*, 1 H, H-2), 1.68 (*m*, 1 H, H-7), 1.65 (*m*, 1 H, H-2'), 1.61 (*dd*, 1 H, H-19', *J* = 13.6, 13.6 Hz), 1.57 (*m*, 1 H, H-6), 1.45 (*m*, 1 H, H-6'), 1.40 (*m*, 1 H, H-7'), 1.38 (*m*, 1 H, H-22), 1.36 (*s*, 3 H, H-27), 1.31 (*m*, 1 H, H-22'), 1.31 (*m*, 1 H, H-21'), 1.18 (*m*, 1 H, H-16'), 1.16 (*s*, 3 H, H-25), 1.15 (*s*, 3 H, H-29), 1.13 (*s*, 3 H, H-26), 1.05 (*m*, 1 H, H-1'), 1.01 (*m*, 1 H, H-15'), 0.90 (*s*, 3 H, H-23), 0.90 (*s*, 3 H, H-24), 0.81 (*m*, 1 H, H-5), 0.80 (*s*, 3 H, H-

28) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.9 (C-11), 176.9 (C-30), 172.9 (Cys-COO), 169.3 (C-13), 128.5 (C-12), 82.2 (C-3), 61.7 (C-9), 55.0 (C-5), 54.0 (Cys-CH), 51.8 (OCH_3), 48.4 (C-18), 45.4 (C-8), 44.0 (C-20), 44.0 (Cys-CH_2), 43.2 (C-14), 41.1 (C-19), 38.7 (C-1), 38.1 (C-4), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-28), 28.3 (C-29), 28.3 (C-23), 26.5 (C-16), 26.4 (C-15), 23.6 (C-2), 23.4 (C-27), 18.7 (C-26), 17.4 (C-6), 16.9 (C-24), 16.4 (C-25) ppm; MS (ESI): *m*/*z* (%) = 587.4 ($[M+2H]^{2+}$, 100), 1173.3 ($[2M+2H]^{2+}$, 38); analysis for $C_{68}H_{104}N_2O_{10}S_2$ (1173.1): C, 69.59; H, 8.93; N, 2.39; S, 5.46; found: C, 69.37; H, 9.04; N, 2.21; S, 5.41.

4.2.5.20. Methyl (3β)-3-({(benzylthio)[(*tert*-butoxycarbonyl)-(2 L)-amino]acetyl}oxy)-11-oxoolean-12-en-30-oate (23)

Following the procedure given for **20**, from **4** (730 mg, 1.51 mmol), Boc-L-Cys(SBn)-OH (720 mg, 2.32 mmol), DCC (390 mg, 1.89 mmol) and DMAP (30 mg, 0.25 mmol), followed by chromatography (silica gel, hexane/ethyl acetate 95:5) **23** (670 mg, 57 %) was obtained as a colorless powder; mp 112-115 °C; R_f = 0.64 (hexane/ethyl acetate 7:3); $[\alpha]_D$ = + 57.94° (*c* 0.43, $CHCl_3$); UV-vis (methanol): λ_{max} (log ε) = 248 nm (4.01); IR (KBr): ν = 3329*br*, 2930*s*, 2852*m*, 1719*s*, 1654*s*, 1627*s*, 1576*m*, 1508*m*, 1455*m*, 1390*m*, 1367*m*, 1341*m*, 1246*m*, 1216*s*, 1167*s*, 1088*w*, 1063*w* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.32-7.20 (*m*, 5 H, H-Ar), 5.67 (*s*, 1 H, H-12), 5.29 (*d*, 1 H, NH, *J* = 7.9 Hz), 4.57 (*dd*, 1 H, H-3, *J* = 11.7, 4.8 Hz), 4.51 (*m*, 1 H, Cys-CH), 3.75 (*s*, 2 H, Bn-CH_2), 3.69 (*s*, 3 H, OCH_3), 2.88 (*m*, 1 H, Cys-C*H*H'), 2.84 (*m*, 1 H, Cys-CH*H'*), 2.82 (*m*, 1 H, H-1), 2.35 (*s*, 1 H, H-9), 2.09 (*dd*, 1 H, H-18, *J* = 13.5, 3.5 Hz), 2.03 (*m*, 1 H, H-15), 1.99 (*m*, 1 H, H-21), 1.93 (*m*, 1 H, H-19), 1.83 (*ddd*, 1 H, H-16, *J* = 13.7, 13.7, 3.7 Hz), 1.74 (*m*, 1 H, H-2), 1.72 (*m*, 1 H, H-7), 1.66 (*m*, 1 H, H-6), 1.64 (*m*, 1 H, H-2'), 1.61 (*dd*, 1 H, H-19', *J* = 13.6, 13.6 Hz), 1.58 (*m*, 1 H, H-6'), 1.46 (*s*, 9 H, Boc-CH_3), 1.43 (*m*, 1 H, H-7'), 1.38 (*m*, 1 H, H-22), 1.36 (*s*, 3 H, H-27), 1.31 (*m*, 1 H, H-22'), 1.31 (*m*, 1 H, H-21'), 1.18 (*m*, 1 H, H-16'), 1.16 (*s*, 3 H, H-25), 1.15 (*s*, 3 H, H-29), 1.13 (*s*, 3 H, H-26), 1.08 (*m*, 1 H, H-15'), 1.02 (*m*, 1 H, H-1'), 0.87 (*s*, 3 H, H-23), 0.85 (*s*, 3 H, H-24), 0.81 (*s*, 3 H, H-28), 0.79 (*m*, 1 H, H-5) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 176.9 (C-30), 170.8 (Cys-COO), 169.3 (C-13), 157.6 (Boc-COO), 137.7 (C_{ar}), 130.1 (C_{ar}), 128.9 (C_{ar}), 128.6 (C_{ar}), 128.5 (C-12), 128.4 (C_{ar}), 127.1 (C_{ar}), 82.4 (C-3), 79.7 (Boc-*quart.*-C), 61.6 (C-9), 55.0 (C-5), 53.5 (Cys-CH), 51.7 (OCH_3), 48.4

(C-18), 45.4 (C-8), 44.0 (C-20), 43.2 (C-14), 41.1 (C-19), 38.7 (C-1), 38.1 (C-4), 37.7 (C-22), 36.9 (C-10), 36.8 (Bn-CH_2), 33.7 (Cys-CH_2), 32.6 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-28), 28.3 (3 x Boc-CH_3), 28.3 (C-29), 28.1 (C-23), 26.4 (C-16), 26.4 (C-15), 23.5 (C-2), 23.3 (C-27), 18.7 (C-26), 17.3 (C-6), 16.7 (C-24), 16.3 (C-25) ppm; MS (ESI): *m*/*z* (%) = 778.1 ($[M+H]^+$, 10), 795.3 ($[M+NH_4]^+$, 11), 800.4 ($[M+Na]^+$, 100), 816.3 ($[M+K]^+$, 20), 832.7 ($[M+Na+MeOH]^+$, 13), 1189.7 ($[3M+2Na]^{2+}$, 12), 1578.3 ($[2M+H+Na]^+$, 20); analysis for $C_{46}H_{67}NO_7S$ (778.1): C, 71.01; H, 8.68; N, 1.80; S, 4.12; found: C, 70.88; H, 8.81; N, 1.55; S, 4.00.

4.2.5.21. Methyl (3β)-3-{[(2*S*)-2-amino-2-(benzylthio)acetyl]oxy}-11-oxoolean-12-en-30-oate (24)

Following the procedure given for **22**, from **23** (190 mg, 0.24 mmol) and trifluoroacetic acid (1 ml, 12.98 mmol) **24** (140 mg, 86 %) was obtained as a colorless powder; mp 128-131 °C; R_f = 0.66 (chloroform/methanol 9:1); $[\alpha]_D$ = + 62.38° (*c* 0.43, $CHCl_3$); UV-vis (methanol): λ_{max} (log ε) = 249 nm (4.31); IR (KBr): ν = 3406*br*, 2929*s*, 1732*s*, 1660*s*, 1570*w*, 1454*m*, 1387*m*, 1324*w*, 1217*s*, 1155*m*, 1087*w*, 1028*w* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 7.36-7.20 (*m*, 5 H, H-Ar), 5.66 (*s*, 1 H, H-12), 4.58 (*dd*, 1 H, H-3, *J* = 11.8, 4.7 Hz), 3.93 (*m*, 1 H, Cys-CH), 3.82 (*s*, 2 H, Bn-CH_2), 3.69 (*s*, 3 H, OCH_3), 3.02 (*dd*, 1 H, Cys-C*H*H', *J* = 13.9, 4.4 Hz), 2.91 (*dd*, 1 H, Cys-CH*H'*, *J* = 13.9, 7.1 Hz), 2.81 (*m*, 1 H, H-1), 2.34 (*s*, 1 H, H-9), 2.09 (*dd*, 1 H, H-18, *J* = 13.5, 3.7 Hz), 2.03 (*m*, 1 H, H-15), 1.99 (*m*, 1 H, H-21), 1.93 (*m*, 1 H, H-19), 1.82 (*ddd*, 1 H, H-16, *J* = 13.5, 13.5, 3.9 Hz), 1.71 (*m*, 1 H, H-2), 1.68 (*m*, 1 H, H-7), 1.66 (*m*, 1 H, H-6), 1.63 (*m*, 1 H, H-2'), 1.61 (*dd*, 1 H, H-19', *J* = 13.5, 13.5 Hz), 1.56 (*m*, 1 H, H-6'), 1.42 (*m*, 1 H, H-7'), 1.38 (*m*, 1 H, H-22), 1.36 (*s*, 3 H, H-27), 1.31 (*m*, 1 H, H-22'), 1.31 (*m*, 1 H, H-21'), 1.18 (*m*, 1 H, H-16'), 1.15 (*s*, 3 H, H-25), 1.15 (*s*, 3 H, H-29), 1.12 (*s*, 3 H, H-26), 1.09 (*m*, 1 H, H-15'), 1.02 (*m*, 1 H, H-1'), 0.85 (*s*, 3 H, H-23), 0.83 (*s*, 3 H, H-24), 0.81 (*s*, 3 H, H-28), 0.77 (*m*, 1 H, H-5) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.8 (C-11), 176.9 (C-30), 169.2 (Cys-COO), 169.2 (C-13), 137.6 (C_{ar}), 129.0 (C_{ar}), 129.0 (C_{ar}), 128.6 (C_{ar}), 128.5 (C-12), 128.5 (C_{ar}), 127.2 (C_{ar}), 83.1 (C-3), 61.6 (C-9), 55.0 (C-5), 51.7 (OCH_3), 49.2 (Cys-CH), 48.4 (C-18), 45.4 (C-8), 44.0 (C-20), 43.2 (C-14), 41.1 (C-19), 38.6 (C-1), 38.1 (C-4), 37.7 (C-22), 36.9 (C-10), 36.7 (Bn-CH_2), 33.9 (Cys-CH_2), 32.6 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-28), 28.3 (C-29), 28.2 (C-23), 26.4 (C-16), 26.4 (C-15), 23.5 (C-2), 23.4 (C-27), 18.7 (C-26), 17.3 (C-6), 16.8 (C-24), 16.3 (C-25) ppm;

MS (ESI): *m*/*z* (%) = 678.3 ($[M+H]^+$, 100), 1017.2 ($[3M+2H]^{2+}$, 6), 1355.6 ($[2M+H]^+$, 2); analysis for $C_{41}H_{59}NO_5S$ (678.0): C, 72.63; H, 8.77; N, 2.07; S, 4.73; found: C, 72.46; H, 8.88; N, 1.97; S, 4.62.

4.2.5.22. Ethyl 3,11-dioxo-olean-12-en-30-oate (25)

Compound **25** was prepared according to [9, 22].

4.2.5.23. Ethyl (7a*R*,7b*S*,9a*S*,12*S*,13a*R*,15b*S*)-5,5,7a,7b,9a,12,15b-heptamethyl-3,15-dioxo-1,2,3,5,5a,6,7,7a,7b,8,9,9a,10,11,12,13,13a,15,15a,15b-icosahydrochryseno[2,1-*c*]oxepine-12-carboxylate (26)

To a solution of **25** (2.56 g, 5.20 mmol) in chloroform (25 ml), 4-chloroperbenzoic acid (2.63 g, 15.40 mmol) was added. The mixture was stirred at 55 °C for 24 h, followed by the addition of saturated solution of $NaHCO_3$ (30 ml). The aqueous layer was extracted with DCM (3 x 30 ml), the combined extracts were dried (Na_2SO_4), filtered and evaporated. The residue was purified by chromatography (silica gel, chloroform/ether 9:1) to yield **26** (2.15 g, 81 %) as a colorless powder; mp 181-185 °C (lit. 166-171 °C [30]); R_f = 0.42 (hexane/ethyl acetate 7:3); $[\alpha]_D$ = + 159.73° (*c* 0.23, $CHCl_3$) (lit. $[\alpha]_D$ = + 189° (*c* 0.1, $CHCl_3$) [30]); UV-vis (methanol): λ_{max} (log ε) = 250 nm (4.07); IR (KBr): ν = 3432*br*, 2964*s*, 2934*s*, 2866*m*, 1728*s*, 1651*s*, 1618*w*, 1460*m*, 1386*m*, 1329*m*, 1314*m*, 1281*m*, 1251*m*, 1219*m*, 1172*s*, 1152*s*, 1115*s*, 1087*m*, 1017*m*, 980*m* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.66 (*s*, 1 H, H-12), 4.17 (*dq*, 1 H, Et-C*H*H', *J* = 10.8, 7.1 Hz), 4.10 (*dq*, 1 H, Et-CH*H'*, *J* = 10.8, 7.2 Hz), 2.69 (*m*, 1 H, H-1), 2.59 (*m*, 1 H, H-2), 2.53 (*s*, 1 H, H-9), 2.11 (*dd*, 1 H, H-18, *J* = 13.3, 3.3 Hz), 2.01 (*ddd*, 1 H, H-15, *J* = 13.7, 13.7, 4.6 Hz), 1.97 (*m*, 1 H, H-21), 1.90 (*ddd*, 1 H, H-19, *J* = 13.7, 4.2, 2.5 Hz), 1.81 (*ddd*, 1 H, H-16, *J* = 13.7, 13.7, 4.6 Hz), 1.69 (*m*, 1 H, H-7), 1.63 (*m*, 1 H, H-6), 1.58 (*dd*, 1 H, H-19', *J* = 13.7, 13.7 Hz), 1.57 (*m*, 1 H, H-5), 1.53 (*m*, 1 H, H-6'), 1.51 (*m*, 1 H, H-1'), 1.46 (*s*, 3 H, H-23), 1.44 (*m*, 1 H, H-2'), 1.43 (*s*, 3 H, H-24), 1.41 (*m*, 1 H, H-7'), 1.38 (*m*, 1 H, H-22), 1.36 (*s*, 6 H, H-25 and H-27), 1.32 (*m*, 1 H, H-22'), 1.29 (*m*, 1 H, H-21'), 1.24 (*t*, 3 H, Et-CH_3, *J* = 7.1 Hz), 1.18 (*m*, 1 H, H-16'), 1.14 (*s*, 3 H, H-26), 1.12 (*s*, 3 H, H-29), 1.01 (*m*, 1 H, H-15'), 0.79 (*s*, 3 H, H-28) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 198.8 (C11), 176.3 (C30), 175.5 (C3), 169.4 (C13), 128.6 (C12), 85.6 (C4), 61.3 (C9), 60.3 (Et-CH_2), 54.5 (C5), 48.3 (C18), 45.3 (C8), 43.8 (C20), 43.4 (C14), 41.1 (C19),

39.6 (C10), 38.7 (C1), 37.7 (C22), 32.3 (C2), 32.2 (C23), 32.0 (C7), 31.8 (C17), 31.1 (C22), 28.6 (C28), 28.3 (C29), 26.4 (C16), 26.4 (C15), 25.9 (C24), 23.1 (C27), 22.1 (C6), 18.2 (C26), 17.5 (C25), 14.3 (Et-CH_3) ppm; MS (ESI): *m*/*z* (%) = 513.4 ($[M+H]^+$, 100), 535.7 ($[M+Na]^+$, 7), 567.0 ($[M+MeOH+Na]^+$, 46).

4.2.5.24. 3-[(1*S*,4a*R*,4b*S*,6a*S*,9*S*,10a*R*)-9-(Ethoxycarbonyl)-2-(1-hydroxy-1-methylethyl)-1,4a,4b,6a,9-pentamethyl-12-oxo-1,2,3,4,4a,4b,5,6,6a,7,8,9,10,10a,12,12a-hexadecahydrochrysen-1-yl]propanoic acid (27)

Compound **26** (2.15 g, 4.19 mmol) was dissolved in ethanol (20 ml), potassium hydroxide (1.14 g, 20.32 mmol in 5 ml water) added. The mixture was stirred at room temperature for 24 h, the pH was adjusted to 7 by adding diluted hydrochloric acid, and the solvents were removed under diminished pressure. Water (20 ml) and ethyl acetate (20 ml) were added, the aqueous layer was extracted with ethyl acetate (3 x 20 ml), the combined organic layers were dried (Na_2SO_4), filtered and evaporated to yield **27** (453 mg, 20 %) as colorless crystals; mp 115-119 °C; R_f = 0.16 (hexane/ethyl acetate 7:3); $[\alpha]_D$ = + 99.01° (*c* 0.79, $CHCl_3$); UV-vis (methanol): λ_{max} (log ε) = 249 nm (4.01); IR (KBr): ν = 3432*br*, 2976*s*, 1727*s*, 1660*s*, 1560*w*, 1457*m*, 1385*s*, 1313*m*, 1247*w*, 1216*s*, 1175*s*, 1086*m*, 1030*w* cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 5.36 (*s*, 1 H, H-12), 4.11 (*dq*, 1 H, Et-C*H*H', *J* = 10.8, 7.1 Hz), 4.03 (*m*, 1 H, Et-CH*H'*), 2.69 (*s*, 1 H, H-9), 2.32 (*m*, 2 H, H-2), 2.22 (*m*, 1 H, H-1), 2.04 (*ddd*, 1 H, H-15, *J* = 13.7, 13.7, 4.2 Hz), 1.95 (*dd*, 1 H, H-18, *J* = 12.0, 5.4 Hz), 1.87 (*m*, 1 H, H-1'), 1.79 (*m*, 1 H, H-21), 1.72 (*m*, 1 H, H-19), 1.70 (*m*, 1 H, H-19'), 1.67 (*m*, 1 H, H- 16), 1.55 (*m*, 1 H, H-7), 1.37 (*m*, 1 H, H-21'), 1.35 (*m*, 2 H, H-6 and H-6'), 1.33 (*s*, 3 H, H-27), 1.31 (*m*, 1 H, H-22), 1.72 (*m*, 1 H, H-7'), 1.26 (*s*, 3 H, H-25), 1.25 (*m*, 1 H, H-5), 1.20 (*m*, 1 H, H-22'), 1.16 (*t*, 3 H, Et-CH_3, *J* = 7.1 Hz), 1.13 (*m*, 1 H, H-16'), 1.12 (*s*, 3 H, H-23), 1.08 (*s*, 3 H, H-29), 1.06 (*s*, 3 H, H-24), 1.01 (*s*, 3 H, H-26), 0.92 (*m*, 1 H, H-15'), 0.71 (*s*, 3 H, H-28) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ = 198.8 (C11), 176.3 (C30), 175.6 (C3), 168.1 (C13), 127.8 (C12), 73.6 (C4), 59.8 (Et-CH_2), 52.5 (C9), 49.9 (C5), 48.0 (C18), 44.8 (C8), 43.4 (C20), 43.3 (C14), 40.8 (C10), 40.4 (C19), 37.3 (C22), 34.8 (C2), 33.0 (C23), 31.6 (C17), 31.6 (C7), 31.3 (C1), 30.3 (C21), 28.3 (C28), 27.8 (C29), 27.6 (C24), 26.0 (C16), 25.7 (C15), 22.5 (C27), 21.2 (C6), 19.3 (C25), 18.1 (C26), 14.1 (Et-CH_3) ppm; MS (ESI): *m*/*z* (%) = 531.3

([M+H]$^+$, 100), 553.5 ([M+Na]$^+$, 73); analysis for $C_{32}H_{50}O_6$ (530.7): C, 72.42; H, 9.50; found: C, 72.35; H, 9.71.

4.2.5.25. 3-[(1*S*,4a*R*,4b*S*,6a*S*,9*S*,10a*R*)-9-(Ethoxycarbonyl)-2-isopropenyl-1,4a,4b,6a,9-pentamethyl-12-oxo-1,2,3,4,4a,4b,5,6,6a,7,8,9,10,10a,12,12a-hexadecahydrochrysen-1-yl]propanoic acid (28)

The pH of a solution of **27** (357 mg, 0.67 mmol) in ethanol (20 ml) was adjusted to 2 by adding diluted hydrochloric. The mixture was stirred at room temperature for 5 min, followed by extraction with DCM (3 x 15 ml); the organic layer was dried (Na_2SO_4), filtered and evaporated to afford **28** (331 mg, 96 %) as colorless powder; mp 256-260 °C (decomp.); R_f = 0.38 (hexane/ethyl acetate 7:3); $[\alpha]_D$ = + 99.91° (*c* 0.41, $CHCl_3$); UV-vis (methanol): λ_{max} (log ε) = 249 nm (3.99); IR (KBr): ν = 3432*br*, 2976*s*, 1727*s*, 1657*s*, 1561*s*, 1455*m*, 1386*s*, 1314*m*, 1218*m*, 1175*w*, 1087*w*, 1032*w* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.60 (*s*, 1 H, H-12), 4.84 (*s*, 1 H, H-23), 4.68 (*s*, 1 H, H-23'), 4.18 (*dq*, 1 H, Et-C*H*H', *J* = 10.8, 7.1 Hz), 4.11 (*dq*, 1 H, Et-CH*H'*, *J* = 10.8, 7.1 Hz), 2.72 (*s*, 1 H, H-9), 2.44 (*m*, 1 H, H-1), 2.15 (*m*, 1 H, H-2), 2.10 (*m*, 1 H, H-18), 2.01 (*ddd*, 1 H, H-15, *J* = 13.7, 13.7, 3.7 Hz), 1.97 (*m*, 1 H, H-2), 1.94 (*m*, 1 H, H-21), 1.92 (*m*, 1 H, H-5), 1.88 (*m*, 1 H, H-19), 1.79 (*m*, 1 H, H-6), 1.76 (*m*, 1 H, H-16), 1.73 (*s*, 3 H, H-24), 1.67 (*m*, 1 H, H-1'), 1.62 (*m*, 1 H, H-7), 1.58 (*dd*, 1 H, H-19', *J* = 13.7, 13.7 Hz), 1.39 (*m*, 1 H, H-6'), 1.37 (*m*, 1 H, H-22), 1.34 (*m*, 1 H, 7'), 1.29 (*m*, 2 H, H-22' and H-21'), 1.37 (*s*, 3 H, H-27), 1.25 (*t*, 3 H, Et-CH_3, *J* = 7.1 Hz), 1.18 (*m*, 1 H, H-16'), 1.13 (*s*, 3 H, H-26), 1.12 (*s*, 3 H, H-25), 1.08 (*s*, 3 H, H-29), 0.98 (*m*, 1 H, H-15'), 0.78 (*s*, 3 H, H-28) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.8 (C11), 181.5 (C3), 176.5 (C30), 169.8 (C13), 147.0 (C4), 128.6 (C12), 113.9 (C23), 60.4 (Et-CH_2), 52.8 (C9), 50.6 (C5), 48.2 (C18), 45.1 (C8), 43.9 (C20), 43.7 (C14), 41.0 (C19), 39.0 (C10), 37.8 (C22), 36.2 (C1), 31.8 (C17), 31.8 (C2), 31.4 (C7), 31.2 (C21), 28.6 (C28), 28.3 (C29), 26.6 (C16), 26.5 (C15), 24.0 (C6), 23.8 (C24), 23.3 (C27), 19.9 (C25), 18.8 (C26), 14.4 (Et-CH_3) ppm; MS (ESI): *m*/*z* (%) = 511.5 ([M-H]$^-$, 100), 557.2 ([M+HCO_2]$^-$, 40); analysis for $C_{32}H_{48}O_5$ (512.7): C, 74.96; H, 9.44; found: C, 74.77; H, 9.51.

4.2.5.26. Ethyl (3*S*,4a*R*,7*S*,10a*R*,10b*S*,12a*S*)-7-(3-ethoxy-3-oxopropyl)-8-isopropenyl-3,7,10a,10b,12a-pentamethyl-6-oxo-1,2,3,4,4a,6,6a,7,8,9,10,10a,10b,11,12,12a-hexadecahydrochrysene-3-carboxylate (29)

Compound **27** (543 mg, 1.02 mmol) was dissolved in ethanol (50 ml), hydrochloric acid (conc., 2 ml) was added. After refluxing for 30 min, the solvent was removed under reduced pressure, and the residue was subjected to chromatography (silica gel, hexane/ethyl acetate 8:2) to afford **29** (526 mg, 95 %) as a colorless powder; mp 101-104 °C; R_f = 0.79 (hexane/ethyl acetate 7:3); $[\alpha]_D$ = + 125.70° (*c* 0.44, $CHCl_3$); UV-vis (methanol): λ_{max} (log ε) = 250 nm (4.14); IR (KBr): ν = 3424*br*, 2977*s*, 2942*s*, 1724*s*, 1645*s*, 1611*m*, 1460*m*, 1386*m*, 1364*w*, 1330*m*, 1310*w*, 1278*m*, 1260*m*, 1213*m*, 1171*s*, 1115*w*, 1089*w*, 1064*w*, 1024*w* cm^{-1}; ^1H NMR (500 MHz, CDCl3): δ = 5.64 (*s*, 1 H, H-12), 4.87 (*dd*, 1 H, H-23, *J* = 1.3, 1.3 Hz), 4.67 (*d*, 1 H, H-23', *J* = 1.2 Hz), 4.17 (*dq*, 1 H, C^{30}-C*H*H', *J* = 10.8, 7.1 Hz), 4.10 (dq, 1 H, C^{30}-CH*H'*, *J* = 10.8, 7.1 Hz), 4.05 (*q*, 2 H, C^3-CH_2, *J* = 7.1 Hz), 2.59 (*ddd*, 1 H, H-1, *J* = 14.1, 12.0, 6.2 Hz), 2.58 (*s*, 1 H, H-9), 2.27 (*ddd*, 1 H, H-2, *J* = 14.1, 12.0, 4.2 Hz), 2.09 (*m*, 1 H, H-18), 2.03 (*m*,1 H, H-2'), 1.99 (*m*, 1 H, H-15), 1.97 (*m*, 1 H, H-21), 1.94 (*dd*, 1 H, H-5, *J* = 12.0, 2.5 Hz), 1.91 (*m*, 1 H, H-19), 1.83 (*m*, 1 H, H-6), 1.80 (*m*, 1 H, H-16), 1.74 (*s*, 3 H, H-24), 1.72 (*m*, 1 H, H-1'), 1.68 (*m*, 1 H, H-7), 1.59 (*dd*, 1 H, H-19', *J* = 13.3, 13.3 Hz), 1.42 (*m*, 1 H, H-6'), 1.37 (*m*, 1 H, H-22), 1.35 (*m*, 1 H, H-7'), 1.32 (*m*, 1 H, H-22'), 1.28 (*m*, 1 H, H-21'), 1.37 (*s*, 3 H, H-27), 1.25 (*t*, 3 H, C^{30}-CH_3, *J* = 7.1 Hz), 1.21 (*m*, 1 H, H-16'), 1.20 (*t*, 3 H, C^3-CH_3, *J* = 7.1 Hz), 1.15 (*s*, 3 H, H-26), 1.15 (*s*, 3 H, H-25), 1.12 (*s*, 3 H, H-29), 1.00 (*m*, 1 H, H-15'), 0.79 (*s*, 3 H, H-28) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.5 (C11), 176.3 (C30), 173.8 (C3), 169.4 (C13), 146.6 (C4), 128.4 (C12), 114.1 (C23), 60.3 (C^{30}-CH_2), 60.2 (C^3-CH_2), 52.8 (C9), 50.8 (C5), 48.3 (C18), 45.1 (C8), 43.8 (C20), 43.7 (C14), 41.2 (C19), 38.8 (C10), 37.7 (C22), 34.4 (C1), 31.8 (C17), 31.4 (C7), 31.1 (C21), 29.4 (C2), 28.6 (C28), 28.3 (C29), 26.5 (C16), 26.4 (C15), 23.8 (C6), 23.5 (C24), 23.3 (C27), 19.5 (C25), 18.6 (C26), 14.3 (C^{30}-CH_3), 14.2 (C^3-CH_3) ppm; MS (ESI): *m*/*z* (%) = 541.5 ($[M+H]^+$, 100), 558.3 ($[M+NH_4]^+$, 13), 563.6 ($[M+Na]^+$, 16); analysis for $C_{34}H_{52}O_5$ (540.8): C, 75.51; H, 9.69; found: C, 75.41; H, 9.82.

4.2.5.27. Ethyl 11-oxo-olean-2,12-dien-30-oate (30)

A mixture of **9** (1.31 g, 2.6 mmol), triphenyl phosphane (2.78 g, 10.6 mmol) and 3,3-dimethyl glutarimide (1.49 g, 10.6 mmol) in dry THF (25 ml) was cooled to 0°C. Under continuous stirring, DEAD (1.65 ml, 10.4 mmol) was added dropwise, and stirring was continued at 25 °C for 24 h. After concentration to dryness, the residue was subjected to chromatography (silica gel, chloroform/ether 9:1) to yield **30** (1.02 g, 82 %) as colorless crystals; mp 138-142 °C; R_f = 0.87 (hexane/ethyl acetate 7:3); $[\alpha]_D$ = 216.97° (*c* 0.33, $CHCl_3$); UV/Vis (methanol): λ_{max} (log ε) = 249 nm (4.02); IR (KBr): ν = 3422*br*, 2960*s*, 2872*s*, 1723*s*, 1648*s*, 1612*w*, 1458*m*, 1386*m*, 1360*w*, 1348*w*, 1328*w*, 1310*w*, 1277*w*, 1256*m*, 1210*m*, 1169*s*, 1134*m*, 1088*w*, 1062*w*, 1031*m* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.67 (*s*, 1 H, 12-H), 5.43 (*ddd*, 1 H, H-2, *J* = 10.1, 6.1, 1.7 Hz), 5.37 (*dd*, 1 H, H-3, *J* = 10.1, 2.3 Hz), 4.19 (*dq*, 1 H, Et-C*H*H', *J* = 10.8, 7.2 Hz), 4.12 (*dq*, 1 H, Et-CH*H'*, *J* = 10.8, 7.2 Hz), 3.04 (*dd*, 1 H, H-1, *J* = 17.5, 6.0 Hz), 2.41 (*s*, 1 H, H-9), 2.11 (*dd*, 1 H, H-18, *J* = 12.8, 4.3 Hz), 2.03 (*ddd*, 1 H, H-15, *J* = 13.4, 13.4, 4.7 Hz), 1.99 (*m*, 1 H, H-21), 1.92 (*ddd*, 1 H, H-19, *J* = 13.9, 4.1, 2.9 Hz), 1.83 (*ddd*, 1 H, H-16, *J* = 13.6, 13.6, 4.3 Hz), 1.70 (*m*, 1 H, H-7), 1.65 (*m*, 1 H, H-1'), 1.61 (*dd*, 1 H, H-19', *J* = 13.5, 13.5 Hz), 1.56 (*m*, 1 H, H-6), 1.48 (*ddd*, 1 H, H-6', *J* = 12.5, 12.5, 3.2 Hz), 1.43 (*m*, 1 H, H-7'), 1.39 (*m*, 1 H, H-22), 1.33 (*m*, 1 H, H-21'), 1.30 (*m*, 1 H, H-22'), 1.36 (*s*, 3 H, H-27), 1.26 (*t*, 3 H, Me, *J* = 7.2 Hz), 1.21 (*ddd*, 1 H, H-16', *J* = 13.9, 4.4, 2.4 Hz), 1.16 (*s*, 3 H, H-25), 1.16 (*s*, 3 H, H-26), 1.14 (*s*, 3 H, H-29), 1.12 (*m*, 1 H, H-5), 1.02 (*m*, 1 H, H-15'), 0.96 (*s*, 3 H, H-23), 0.91 (*s*, 3 H, H-24), 0.82 (s, 3 H, H-28) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.1 (C11), 176.4 (C30), 169.4 (C13), 137.0 (C3), 128.6 (C12), 121.9 (C2), 60.5 (C9), 60.3 (Et-CH_2), 51.8 (C5), 48.4 (C18), 45.3 (C14), 43.8 (C20), 43.3 (C8), 41.5 (C1), 41.2 (C19), 37.7 (C22), 36.2 (C4), 34.3 (C10), 31.9 (C7), 31.9 (C23), 31.8 (C17), 31.1 (C21), 28.6 (C28), 28.3 (C29), 26.5 (C16), 26.5 (C15), 23.3 (C27), 23.0 (C24), 18.7 (C6), 18.3 (C26), 16.1 (C25), 14.3 (Me) ppm; MS (ESI): *m/z* (%) = 481.5 ($[M+H]^+$, 100), 503.3 ($[M+Na]^+$, 7), 534.9 ($[M+MeOH+Na]^+$, 50), 961.3 ($[2M+H]^+$, 66), 983.4 ($[2M+Na]^+$, 54), 999.2 ($[2M+K]^+$, 4); analysis for $C_{32}H_{48}O_3$ (480.72): C, 79.95; H, 10.06; found: C, 79.68; H, 10.18.

4.2.5.28. Ethyl (2α, 3α)-2,3-epoxy-11-oxo-olean-12-en-31-oate (31)

Compound **30** (1.01 g, 2.1 mmol) was dissolved in dry dichloromethane (20 ml), *m*-CPBA (1.14 g, 4.68 mmol) was added, and the mixture was stirred at room temperature for 20 h. An aq.

solution of potassium hydrogensulfate (satd., 10 ml) was added, the aqueous layer extracted with dichloromethane (3 x 15 ml), and the combined organic layers were dried (Na_2SO_4), filtrated and evaporated to dryness. Chromatographic purification (silica gel, chloroform/ether 9:1) afforded **31** (806 mg, 77 %) as colorless powder; mp 191-193 °C; R_f = 0.71 (hexane/ethyl acetate 7:3); $[\alpha]_D$ = 143.65° (*c* 0.48, $CHCl_3$); UV/Vis (methanol): λ_{max} (log ε) = 251 nm (4.09); IR (KBr): ν = 3416*br*, 2978*s*, 2955*s*, 1736*s*, 1718*s*, 1645*s*, 1614*w*, 1458*m*, 1385*m*, 1314*m*, 1301*m*, 1285*m*, 1260*m*, 1222*s*, 1163*s*, 1113*m*, 1091*m*, 1039*m*, 1014*w*; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.63 (*s*, 1 H, H-12), 4.16 (*dq*, 1 H, Et-C*H*H', *J* = 10.8, 7.1 Hz), 4.10 (*dq*, 1 H, Et-CH*H*', *J* = 10.8, 7.1 Hz), 3.19 (*dd*, 1 H, H-2, *J* = 6.6, 3.7 Hz), 3.13 (*dd*, 1 H, H-1, *J* = 14.9, 6.6 Hz), 2.79 (*d*, 1 H, H-3, *J* = 3.7 Hz), 2.29 (*s*, 1 H, H-9), 2.09 (*dd*, 1 H, H-18, *J* = 13.3, 4.2 Hz), 1.99 (*ddd*, 1 H, H-15, *J* = 13.3, 13.3, 4.6 Hz), 1.96 (*m*, 1 H, H-21), 1.90 (*ddd*, 1 H, H-19, *J* = 13.7, 4.2, 2.9 Hz), 1.78 (*ddd*, 1 H, H-16, *J* = 13.7, 13.7, 5.0 Hz), 1.61 (*m*, 1 H, H-7), 1.57 (*dd*, 1 H, H-19', *J* = 13.7, 13.7 Hz), 1.48 (*m*, 1 H, H-6), 1.39 (*m*, 1 H, H-21), 1.37 (*m*, 1 H, H-6'), 1.35 (*m*, 1 H, H-22), 1.33 (*m*, 1 H, H-1'), 1.30 (*s*, 3 H, H-27), 1.29 (*m*, 1 H, H-22'), 1.27 (*m*, 1 H, H-21'), 1.24 (*t*, 1 H, *J* = 7.1 Hz, Me), 1.17 (*m*, 1 H, H-16'), 1.13 (*s*, 3 H, H-26), 1.11 (*s*, 3 H, H-28), 1.09 (*s*, 3 H, H-23), 1.07 (*s*, 3 H, H-25), 1.02 (*s*, 3 H, H-24), 0.93 (*m*, 1 H, H-15'), 0.92 (*dd*, 1 H, H-5, *J* = 11.6, 2.9 Hz), 0.78 (*s*, 3 H, H-29) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.7 (C11), 176.3 (C30), 169.7 (C13), 128.5 (C12), 61.3 (C3), 60.4 (C9), 60.3 (Et-CH_2), 52.6 (C2), 48.4 (C18), 46.6 (C5), 45.1 (C8), 43.8 (C20), 43.3 (C14), 41.1 (C19), 40.6 (C1), 37.7 (C22), 35.9 (C4), 32.6 (C10), 31.9 (C7), 31.8 (C17), 31.1 (C21), 28.6 (C28), 28.3 (C29), 28.2 (C23), 26.4 (C16), 26.4 (C15), 23.2 (C27), 22.0 (C24), 18.3 (C26), 17.9 (C6), 17.9 (C25), 14.3 (Me); MS (ESI): *m/z* (%) = 497.6 ($[M+H]^+$, 92), 519.4 ($[M+Na]^+$, 10), 551.0 ($[M+MeOH+Na]^+$, 62), 767.4 ($3M+2Na]^{2+}$, 6), 993.3 ($[2M+H]^+$, 94), 1015.4 ($[2M+Na]^+$, 100), 1031.3 ($[2M+K]^+$, 12); analysis for $C_{32}H_{48}O_4$ (496.72): C, 77.38; H, 9.74; found: C, 77.26; H, 9.92.

4.2.5.29. Ethyl (2β, 3α) 2-fluoro-3-hydroxy-11-oxo-olean-12-en-30-oate (32)

Compound **31** (619 mg, 1.25 mmol) was dissolved in dry DCM (10 ml) and Olah's-reagent [26] (1 ml, 4.35 mmol) was slowly added. The mixture was stirred at room temperature for 5 h, and crushed ice was added. The aqueous layer was extracted with DCM (3 x 20 ml), the extracts were dried (Na_2SO_4), filtered and evaporated. The residue was subjected to chromatography (silica gel, chloroform/ether 9:1) to afford **32** (120 mg, 19 %) as colorless powder; mp 152-155

°C; R_f = 0.48 (hexane/ethyl acetate = 7:3); $[\alpha]_D$ = + 113.77° (*c* 0.64, $CHCl_3$); UV-vis (methanol): λ_{max} (log ε) = 249 nm (3.98); IR (KBr): ν = 3448*br*, 2959*s*, 2873*s*, 1727*s*, 1659*s*, 1456*m*, 1389*s*, 1365*m*, 1314*m*, 1280*m*, 1246*m*, 1218*s*, 1174*s*, 1121*m*, 1089*m*, 1048*m*, 1021*s* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.67 (*s*, 1 H, H-12), 4.64 (*ddd*, 1 H, H-2, *J* = 49.7, 6.4, 5.6 Hz), 4.18 (*dq*, 1 H, Et-C*H*H', *J* = 10.8, 7.1 Hz), 4.12 (*dq*, 1 H, Et-CH*H'*, *J* = 10.8, 7.1 Hz), 3.73 (*dd*, 1 H, H-3, *J* = 8.6, 6.4 Hz), 2.91 (*ddd*, 1 H, H-1, *J* = 15.4, 15.4, 5.5 Hz), 2.43 (*s*, 1 H, H-9), 2.11 (*ddd*, 1 H, H-18, *J* = 13.5, 4.2, 1.2 Hz), 2.02 (*ddd*, 1 H, H-15, *J* = 13.6, 13.6, 4.6 Hz), 1.98 (*m*, 1 H, H-21), 1.91 (*ddd*, 1 H, H-19, *J* = 13.6, 4.3, 2.8 Hz), 1.82 (*ddd*, 1 H, H-16, *J* = 13.6, 13.6, 4.3 Hz), 1.68 (*ddd*, 1 H, H-1', *J* = 15.4, 15.4, 5.7 Hz), 1.64 (*m*, 1 H, H-7), 1.59 (*dd*, 1 H, H-19', *J* = 13.5, 13.5 Hz), 1.53 (*m*, 1 H, H-6), 1.50 (*m*, 1 H, H-6'), 1.40 (*m*, 1 H, H-7'), 1.38 (*m*, 1 H, H-22), 1.35 (*s*, 3 H, H-27), 1.33 (*m*, 2 H, H-22' and H-21'), 1.26 (*t*, 3 H, Et-CH_3, *J* = 7.1 Hz), 1.20 (*m*, 1 H, H-16'), 1.15 (*m*, 1 H, H-5), 1.13 (*s*, 3 H, H-29), 1.13 (*s*, 3 H, H-25), 1.11 (*s*, 3 H, H-26), 1.04 (*s*, 3 H, H-24), 1.03 (*m*, 1 H, H-15'), 0.98 (*s*, 3 H, H-23), 0.80 (*s*, 3 H, H-28) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.5 (C11), 176.4 (C30), 169.9 (C13), 128.3 (C12), 92.8 (*d*, C2, *J* = 172 Hz), 75.2 (*d*, C3, *J* = 21 Hz), 62.5 (C9), 60.3 (Et-CH_2), 49.1 (C5), 48.3 (C18), 45.3 (C8), 43.8 (C20), 42.5 (*d*, C1, *J* = 18 Hz), 42.2 (C14), 41.2 (C19), 37.7 (C22), 36.9 (C4), 36.8 (C10), 32.1 (C7), 31.8 (C17), 31.1 (C21), 28.6 (C28), 28.3 (C29), 26.6 (C23), 26.4 (C16), 26.4 (C15), 23.5 (C27), 22.1 (C24), 19.5 (C26), 18.4 (C25), 18.0 (C6), 14.3 (Et-CH_3) ppm; ^{19}F NMR (190 MHz, $CDCl_3$): δ = -180.9 (*m*, 1 F, F-2) ppm; MS (ESI): *m*/*z* (%) = 517.5 ($[M+H]^+$, 100), 539.5 ($[M+Na]^+$, 52), 571.1 ($[M+MeOH+Na]^+$, 22); analysis for $C_{32}H_{49}FO_4$ (516.7): C, 74.38; H, 9.56; found: C, 74.21; H, 9.74.

4.2.5.30. Benzyl (3a) 3-hydroxy-11-oxo-olean-12-en-30 oate (33)

Compound **33** was prepared according to [7, 11].

4.2.5.31. Benzyl 3β-3-([N-(3-aminopropyl)glycyl]oxy)-11-oxo-olean-12-en-30-oate (34)

To a solution of **33** (290 mg, 0.51 mmol) in dry DCM (15 ml), chloroacetyl chloride (50 μl, 0.61 mmol) was added. After stirring at 25 °C for 24 h and usual aqueous work-up, the crude product was dissolved in dry DMF (10 ml), finely grounded K_2CO_3 (700 mg, 5.07 mmol) and 1,3-diaminopropane (0.5 ml, 5.95 mmol) were added, and the mixture was stirred at 25 °C for 2h.

The solvent was removed under reduced pressure, and water (30 ml) was added. After the extraction with DCM (3 x 20 ml), the combined organic layers were washed with water (20 ml) and brine (20 ml), dried (Na_2SO_4), filtered, and the solvent was evaporated. Purification by chromatography (silica gel, load with methanol, unload with methanol/diethylamine 9:1) gave **34** (270 mg, 82 %) as a slight yellowish powder; mp 154-157 °C; R_f = 0.02 (chloroform/methanol 9:1); $[\alpha]_D$ = + 100.62° (*c* 0.49, $CHCl_3$); UV-vis (methanol): λ_{max} (log ε) = 249 nm (3.99); IR (KBr): ν = 3433*br*, 2945*s*, 1728*s*, 1660*s*, 1464*m*, 1387*m*, 1306*w*, 1279*m*, 1248*m*, 1213*s*, 1174*m*, 1145*s*, 1083*m*, 1023*w*, 985*m* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 7.38 - 7.29 (*m*, 5 H, H-Ar), 5.52 (*s*, 1 H, H-12), 5.18 (*d*, 1 H, Bn-C*H*H', *J* = 12.3 Hz), 5.07 (*d*, 1 H, Bn-CH*H'*, *J* = 12.3 Hz), 4.55 (*m*, 1 H, H-3), 3.37 (*s*, 2 H, CH_2COO), 2.78 (*m*, 1 H, H-1), 2.77 (*t*, 2 H, chain-3, *J* = 6.8 Hz), 2.67 (*t*, 2 H, chain-1, *J* = 6.8 Hz), 2.32 (*s*, 1 H, H-9), 2.02 (*m*, 1 H, H-18), 1.99 (*m*, 1 H, H-15), 1.97 (*m*, 1 H, H-21), 1.92 (*m*, 1 H, H-19), 1.78 (*m*, 1 H, H-16), 1.73 (*m*, 1 H, H-2), 1.67 (*m*, 1 H, H-7), 1.63 (*m*, 2 H, chain-2), 1.62 (*m*, 1 H, H-2'), 1.61 (*dd*, 1 H, H-19', *J* = 13.9, 13.9 Hz), 1.55 (*m*, 1 H, H-6), 1.46 (*m*, 1 H, H-6'), 1.40 (*m*, 1 H, H-7'), 1.36 (*m*, 1 H, H-22), 1.33 (*s*, 3 H, H-27), 1.29 (*m*, 1 H, H-22'), 1.29 (*m*, 1 H, H-21'), 1.16 (*m*, 1 H, H-16'), 1.14 (*s*, 6 H, H-25 & H-29), 1.09 (*s*, 3 H, H-26), 1.03 (*m*, 1 H, H-1'), 0.97 (*m*, 1 H, H-15'), 0.87 (*s*, 3 H, H-24), 0.86 (*s*, 3 H, H-23), 0.79 (*m*, 1 H, H-5), 0.71 (*s*, 3 H, H-28) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.9 (C-11), 176.2 (C-30), 172.4 (CH_2COO), 169.0 (C-13), 136.1 (C_{ar}), 128.5 (C_{ar}), 128.5 (C_{ar}), 128.4 (C-12), 128.3 (C_{ar}), 128.2 (C_{ar}), 127.8 (C_{ar}), 81.2 (C-3), 66.2 (Bn-CH_2), 61.6 (C-9), 55.0 (C-5), 51.2 (CH_2COO), 48.2 (C-18), 47.4 (chain-1), 45.3 (C-8), 44.0 (C-20), 43.2 (C-14), 41.0 (C-19), 40.3 (chain-3), 38.7 (C-1), 38.1 (C-4), 37.6 (C-22), 36.9 (C-10), 33.4 (chain-2), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.4 (C-28), 28.2 (C-29), 28.1 (C-23), 26.4 (C-16), 26.4 (C-15), 23.6 (C-2), 23.3 (C-27),18.6 (C-26), 17.4 (C-6), 16.7 (C-24), 16.4 (C-25) ppm; MS (ESI): *m*/*z* (%) = 675.5 ($[M+H]^+$, 4), 701.5 ($[M+Na]^+$, 100); analysis for $C_{42}H_{62}N_2O_5$ (675.0): C, 74.74; H, 9.26; N, 4.15; found: C, 74.57; H, 9.41; N, 4.00.

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Anhang 7

Conversions at C-30 of glycyrrhetinic acid and their impact on antitumor activity.

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Glycyrrhetinic Acid Derivatives

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Keywords: *glycyrrhetinic acid; apoptosis; antitumor activity; mouse embryonic fibroblasts*

Herrn Prof. Dr. Ernst Mutschler zum 80. Geburtstag. Ad multos annos!

Graphical abstract

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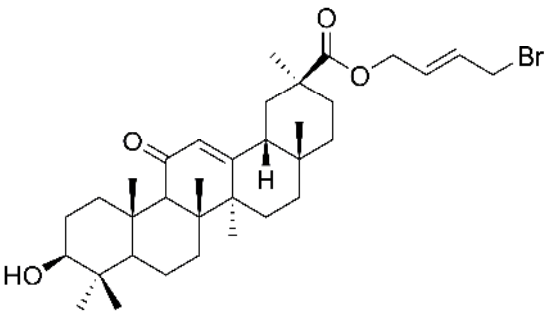


IC_{50} = 1.88 μM (SW1736; human thyroid carcinoma cell line)

Summary

The extracts of the roots of licorice have been used in traditional and folk medicine to treat a broad variety of maladies. The main ingredient of these extracts is glycyrrhicinic acid. Its aglycon, glycyrrhetinic acid, has many biological activities, among them a pronounced cytotoxicity against tumor cells. In this study we varied glycyrrhetinic acid at position C-30 to get “simple” derivatives, for example esters, amides and a nitrile. The influence of these changes on the cytotoxic activity is noteworthy and was determined by a colorimetric sulphorhodamine B test using 7 human tumor cell lines and mouse embryonic fibroblasts (NiH3T3) for comparison. A trypan blue test as well as an acridine orange/ethidium bromide test was used to discover the ability of the compounds to induce apoptosis.

Introduction

Glycyrrhetinic acid (**GA**, Fig. 1) is the main content of the extract of the licorice roots, and it is known to show many biological activities, for example antiinflammatory [1, 2] or antiviral [3, 4]. Recently, we became interested in the cytotoxicity and the antitumor effect of this compound and its analogues. Like other triterpenoic acids, as betulinic acid [5, 6] and oleanic acid [7], GA is toxic for tumors cells [8, 9] and is known to trigger apoptosis [10, 11]. Although the cytotoxicity of GA is lower than that of betulinic acid, its occurrence in natural sources is significantly higher (up to 24 % [12, 13]). This leads to a pronounced economical interest in this compound as a starting material to develop drugs.

Insert Fig. 1

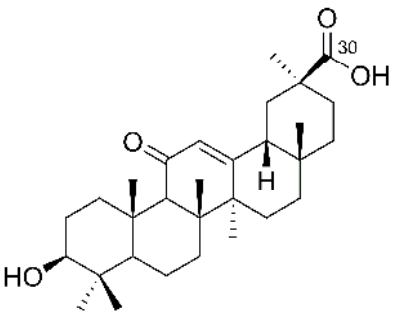


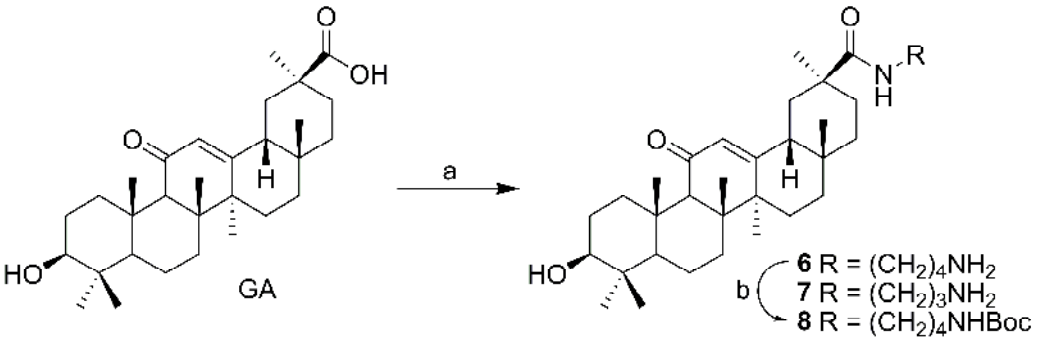
Fig. 1. Structure of glycyrrhetinic acid (GA).

Results and Discussion

Aim of this study was to perform some variations at C-30, among them esterifications, the formation of amides and of a nitrile. All derivatives were tested in a sulphorhodamine B (SRB) assay to determine IC_{50} values on 7 human tumor cell lines. The most active compounds were also tested on mouse embryonic fibroblasts (NiH3T3). To find out whether apoptosis is triggered, three of our compounds were subjected to a trypan blue test as well an acridine orange/ethidium bromide (AO/EB) test.

The esters **1** – **4** [14] as well as the compounds **5** [15] and **15** [16] were synthesized according to previously described procedures. To obtain the amides **6** and **7**, a solution of GA was stirred with an excess (Scheme 1) of the respective diamine in the presence of 1-hydroxybenzotriazole. The boc protected derivative **8** was synthesized by reacting **6** in DMF with di-*tert*.butyl dicarbonate in the presence of triethylamine.

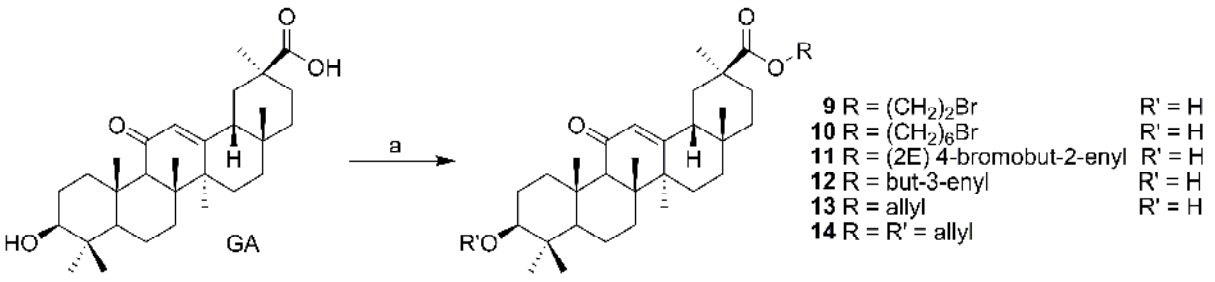
Insert Scheme 1



Scheme 1. Synthesis of the amides: (a) K_2CO_3, diamine, DMF, 25 °C, 20 h, 28 % for **6**, 54 % for **7**; (b) Boc_2O, Et_3N, MeOH, 25 °C, 20 h, 36 %.

The esters **9**-**13** (Scheme 2) were synthesized by the reaction [14] of GA with an alkyl halide/K_2CO_3 in DMF. Compound **14** was obtained as a side product by dialkylation of GA.

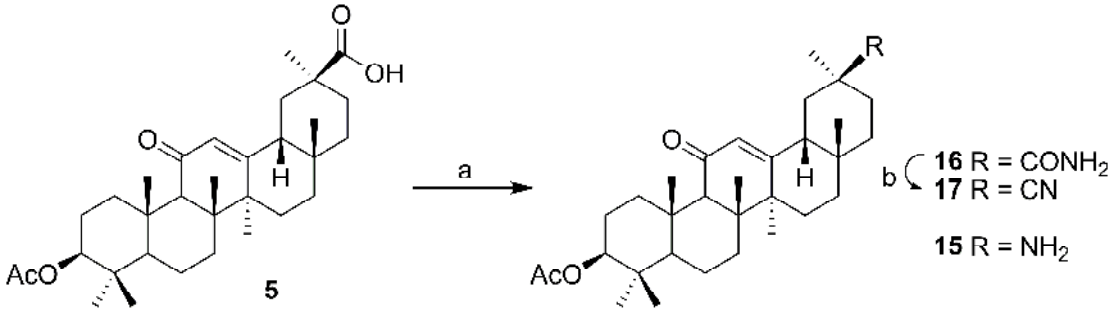
Insert Scheme 2



Scheme 2. Synthesis of the esters: (a) K_2CO_3, alkyl halide, DMF, 25 °C, 20 h, 16-79 %.

Reacting GA with oxalyl chloride in dichloromethane (Scheme 3) followed by the addition of a solution of concentrated ammonia gave the amide **16**. Refluxing of **16** in thionyl chloride yielded the nitrile **17**.

Insert Scheme 3



Scheme 3. Structure of **15** and synthesis of the nitrile **17**: (a) $(COCl)_2$, CH_2Cl_2, 25 °C, 2 h, then conc. ammonia, toluene, 4 °C, 1 h, 99 %; (b) $SOCl_2$, reflux, 4 h, 98 %.

The amides **6**-**8** and **16** did not show any cytotoxic activity at concentrations < 30 µM, whereas for the esters IC_{50} values < 30 µM were determined. The highest cytotoxicity was detected for the *trans*-bromobutenyl ester **11**. This compound showed an activity up to IC_{50} = 1.88 µM (for SW1736 cells). For all the other esters with exception of long-chain bromo-substituted **10**, higher cytotoxicities were determined than for the esters **1**-**4**. Compound **14**, being esterified and etherified, did not show any significant cytotoxic activity.

The biological activity of the amine **15** is comparable to those of the esters. Contrary, for the amide **16** as well as for the nitrile **17**, no IC_{50} values below 25 µM could be detected.

Insert Table 1

	518A2	8505C	A253	A549	DLD-1	Lipo	SW1736
GA*	83.92	86.50	80.78	82.76	81.21	81.44	76.93
1*	27.54	26.07	19.42	23.50	26.12	20.47	34.82
2*	25.23	24.58	25.04	22.74	28.14	27.66	13.37
3*	18.15	14.24	15.75	14.41	27.61	15.93	12.77
4*	18.19	8.10	10.67	6.15	22.69	11.54	2.74
5	>30	>30	>30	>30	>30	>30	>30
6	>30	>30	>30	>30	>30	>30	>30
7	>30	>30	>30	>30	>30	>30	>30
8	>30	>30	>30	>30	>30	>30	>30
9	15.19	15.59	15.89	20.27	22.98	15.46	19.87
10	28.99	>30	>30	>30	>30	>30	28.64
11	21.00	8.82	10.97	4.28	23.09	11.47	1.88
12	14.91	11.61	13.57	19.16	14.88	12.77	16.36
13	15.33	15.59	15.89	20.27	22.98	15.46	19.87
14	>30	>30	>30	>30	>30	>30	>30
15	14.91	11.61	13.57	19.16	14.88	12.77	16.36
16	>30	>30	>30	>30	>30	>30	25.65
17	>30	>30	>30	>30	>30	>30	>30

Table 1. Results of the SRB-assay on various tumor cell lines: Results (IC_{50}) are given in μM; error ± 5 %); *) data from previous studies [17].

For those derivatives displaying a higher cytotoxicity, we determined their IC_{50} values on mouse embryonic fibroblasts (NiH3T3); a comparably high cytotoxicity was found. For **12** and **15** the best selectivity can be seen on 8505C cells (1.7 times more active for tumor cells than for the fibroblasts).

Insert Table 2

	GA*	1*	2*	3*	4*	9	12	13	15
NiH3T3	18.52	22.81	23.66	32.37	21.02	19.79	19.73	19.79	19.73

Table 2. Results of the SRB-assay on mouse embryonic fibroblasts (NiH3T3): Results (IC_{50}) are given in μM; error ± 5 %); *) data from previous studies [17].

A quantitative trypan blue test showed an >80 % rate of apoptosis for compounds **11**, **12** and **15** for A549 cells. Therefore, compared with parent GA, a higher percentage of apoptotic cells were found treating the tumor cells with these GA derivatives.

Insert Table 3

Compound	**GA**	**11**	**12**	**15**
Apoptosis [%]	73.73 ± 1.40	82.77 ± 4.55	91.51 ± 0.79	88.77 ± 2.12

Table 3. Apoptotic effect of GA and derivatives in A549 cells in % (± standard error, 6 experiments), cells were treated with **11** (6 µM), **12** (25 µM) and **15** (25 µM).

The second test (Fig. 2) for apoptotic behavior was an AO/EB test. We were able to detect green fluorescent cells as depicted in fig. 2; this green fluorescence is typical for apoptotic cells.

Insert Fig. 2

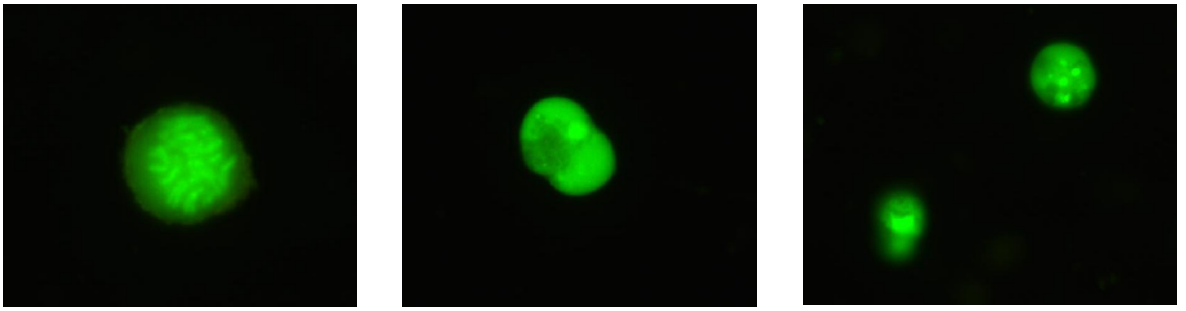


Fig. 2. Results from the AO/EB test; treatment of A549 cells with (from left to right): **11** (6 µM), **12** (25 µM) and **15** (25 µM).

In the present study a couple of GA derivatives varying at C-30 were synthesized. The substitution of this position with of a diamino chain group yielded the amides **6** and **7**. These compounds as well as boc-protected **8** displayed IC_{50} > 30 µM. Previously [18] we could present a series of GA derivatives being substituted with 1,ω-diaminoalkyl groups at position C-3. These compounds, however, showed high cytotoxic potential. Thus, introducing 1,ω-diaminoalkyl groups at C-3 has a completely different effect on the activity of GA derivatives.

This suggests that not only the presence of such a moiety [17, 18] is responsible for an increased antitumor activity but also the position of such a functional group.

As far as the esters of triterpenoic acids are concerned, the methyl esters of oleanoic acid and ursolic acid are more cytotoxic than the free acids [19, 20], whereas for betulinic acid [21] the opposite is true. The esters **1** – **4** [17] and the derivatives **9**, **11-13** exhibited a higher cytotoxicity than parent GA. For the 4-bromobut-2-enyl ester **11** a noteworthy IC_{50} value of 1.88 µM on SW1736 cells was observed. An extra ether moiety in position C-3 (as exemplified in **14**) decreased the cytotoxicity of the propenyl ester (**13**). Converting the carboxyl group into an amino function as in **15** resulted in decrease of activity; IC_{50} values > 30 µM were for the amide **16**) and the nitrile **17**. The assay on mouse embryonic fibroblasts showed a slightly improved selectivity.

In conclusion, cytotoxicity of the esters **9**, **11-13** and of the amine **15** was similar to those of the esters **1-4**. The compounds **11**, **12** and **15** triggered apoptosis as determined by trypan blue and AO/EB assays.

Experimental

General

Melting points are uncorrected (*Leica* hot stage microscope), NMR spectra were recorded using the Varian spectrometers Gemini 200, Gemini 2000 or Unity 500 (δ given in ppm, *J* in Hz, internal Me_4Si), optical rotations were obtained using a Perkin-Elmer 341 polarimeter (1 cm micro cell, 25 °C), IR spectra (film or KBr pellet) on a Perkin-Elmer FT-IR spectrometer Spectrum 1000, MS spectra were taken on an Intectra GmbH AMD 402 (electron impact, 70 eV) or on a Finnigan MAT TSQ 7000 (electrospray, voltage 4.5 kV, sheath gas nitrogen) instrument. TLC was performed on silica gel (Merck 5554, detection by UV absorption). The solvents were dried according to usual procedures.

Cell lines and Culture Conditions

The cell lines 518A2, 8505C, A253, A549, DLD-1, LIPO, NiH3T3, SW1736 were included in this study. Cultures were maintained as monolayer in RPMI 1640 (PAA Laboratories, Pasching, Germany) supplemented with 10% heat inactivated fetal bovine serum (Biochrom

AG, Berlin, Germany) and penicillin / streptomycin (PAA Laboratories) at 37 °C in a humidified atmosphere of 5% CO_2/ 95% air.

Cytotoxicity Assay [22]

The cytotoxicity of the compounds was evaluated using the sulforhodamine-B (SRB) (Sigma Aldrich) microculture colorimetric assay. In short, exponentially growing cells were seeded into 96-well plates on day 0 at the appropriate cell densities to prevent confluence of the cells during the period of experiment. After 24h, the cells were treated with serial dilutions of the compounds (0-100 µM) for 96 h. The final concentration of DMSO or DMF solvent never exceeded 0.5%, which was non-toxic to the cells. The percentages of surviving cells relative to untreated controls were determined 96 h after the beginning of drug exposure. After a 96 h treatment, the supernatant medium from the 96 well plates was thrown away and the cells were fixed with 10% TCA. For a thorough fixation, the plates were allowed to rest at 4 °C. After fixation, the cells were washed in a strip washer. The washing was done four times with water using alternate dispensing and aspiration procedures. The plates were then dyed with 100 µl of 0.4% SRB (sulforhodamine B) for about 20 min. After dying the plates were washed with 1% acetic acid to remove the excess of the dye and allowed to air dry overnight. 100 µl of 10 mM Tris base solution were added to each well and absorbance was measured at 570 nm (using a 96 well plate reader, Tecan Spectra, Crailsheim, Germany). The IC_{50} was estimated by linear regression between the value before and after the 50% line is crossed in a dose-response curve.

Apoptosis test – Acridine Orange/Ethidium Bromide (AO/EB)

Apoptotic cell death was analysed by acridine orange/ethidium bromide dye using fluorescence microscopy on A549 cells. Therefore approx. 500,000 cells were seeded in cell culture flasks and were allowed to grow for 24 hours. The medium was removed and the substance loaded medium was added. After 24-48 hours, the supernatant medium was collected and centrifuged; the pellet was suspended in phosphate-buffer saline (PBS) and centrifuged again. The liquid was removed and the pellet was suspended in PBS. After mixing the suspension with a solution of AO/EB, analysis was performed under a fluorescence microscope. While a green fluorescence shows apoptosis, a red coloured nucleus indicates necrotic cells.

Apoptosis test - Trypan blue cell counting

Approx. 500,000 cells (A549) were seeded in cell culture flasks and were allowed to grow for 1 day. After removing of the medium, the substance loaded medium was introduced and the flasks were incubated for about 24-48 hours. The supernatant medium was collected and centrifuged; the cell pellet was suspended in PBS and centrifuged again. Equal amounts of a trypan blue solution (0.4 % in phosphate-buffer saline, pH 7.2) and a suspension of the pellet in PBS were mixed and put on chamber slides (invitrogen™). Automatic cell counter (invitrogen™ countess® automated cell counter) was used for counting the cells, differing between cells with an intact cell membrane and cells without.

General procedure for synthesis of the amides (method A)

GA (1 equiv) and DCC (1.1 equiv) were dissolved in a 2:1 mixture of dry dichloromethane and dry DMF (50 ml). 1-Hydroxybenzotriazole (1.2 equiv) was added and the mixture was stirred at room temperature for 1-2 h. The diamine (2 equiv) was added and stirring was continued for 20 h. The solvents were removed under reduced pressure and the residue was dissolved in dichloromethane. After usual aqueous work-up, chromatography (silica gel, load with methanol, unload with methanol/diethylamine 9:1) yielded the product.

General procedure for the synthesis of the esters (method B)

GA (1 equiv) was dissolved in dry DMF (30 ml) and potassium carbonate (5 equiv) was added. After 30 min of stirring at room temperature, the bromide (2 equiv) was added and stirring was continued for 18 h. The solvents were removed under reduced pressure and the residue was dissolved in dichloromethane. Usual aqueous work-up followed by chromatographic purification (silica gel, hexane/ethyl acetate 7:3) afforded the product.

(3β) N-(4-Aminobutyl) 3-hydroxy-11-oxo-olean-12-en-30-amide (**6**)

Obtained from **GA** by method A as a colourless powder; yield: 160 mg, 28 %; mp 226-230 °C (decomp.); R_f = 0.07 (MeOH); $[\alpha]_D$ = 136.70° (*c* 0.60, MeOH); UV-vis (MeOH): λ_{max} (log ε) = 249 nm (4.01); IR (KBr): ν = 3366*br*, 2929*s*, 2866*s*, 1654*s*, 1534*s*, 1455*s*, 1386*s*, 1364*s*, 1327*s*, 1281*s*, 1259*s*, 1206*s*, 1137*m*, 1089*m*, 1049*s*, 995*s*, 920*m*, 880*m*, 743*w*, 673*m*, 636*m*, 542*m*, 473*m* cm^{-1}; ^1H NMR (500 MHz, CD_3OD): δ = 5.64 (*s*, 1 H, H-12), 3.18 (*dd*, 1 H, H-3, *J* = 11.7, 4.7 Hz), 2.73 (*m*, 1 H, H-1), 2.72 (*m*, 2 H, chain-4-CH_2), 2.46 (*s*, 1 H, H-9), 2.16 (*m*, 1 H, H-18), 1.97 (*m*, 1 H, H-15), 1.93 (*m*, 1 H, H-21), 1.90 (*m*, 1 H, H-19), 1.75 (*m*, 1 H, H-16), 1.72 (*m*, 1 H, H-2), 1.69 (*m*, 1 H, H-7), 1.65 (*m*, 1 H, H-2'), 1.74 (*dd*, 1 H, H-19', *J* = 13.5, 13.5 Hz), 1.57 (*m*, 1 H, H-6), 1.55 (*m*, 2 H, chain-2-CH_2), 1.55 (*m*, 2 H, chain-3-CH_2),

1.50 (*m*, 1 H, H-6'), 1.48 (*m*, 1 H, H-7'), 1.45 (*m*, 1 H, H-22), 1.44 (*s*, 3 H, H-27), 1.42 (*m*, 2 H, chain-1-CH_2), 1.41 (*m*, 2 H, H-22' and H-21'), 1.27 (*m*, 1 H, H-16'), 1.15 (*s*, 3 H, H-25), 1.15 (*s*, 3 H, H-26), 1.12 (*s*, 3 H, H-29), 1.06 (*m*, 1 H, H-1'), 1.03 (*m*, 1 H, H-15'), 1.01 (*s*, 3 H, H-23), 0.84 (*s*, 3 H, H-28), 0.81 (*s*, 3 H, H-24), 0.78 (*m*, 1 H, H-5); ^{13}C NMR (125 MHz, CD_3OD): δ = 202.4 (C-11), 178.6 (C-30), 172.4 (C-13), 128.9 (C-12), 79.2 (C-3), 63.0 (C-9), 56.0 (C-5), 49.6 (C-18), 46.5 (C-8), 44.6 (C-20), 44.4 (C-14), 42.4 (C-19), 41.7 (chain-4), 40.1 (C-1), 40.0 (C-4), 38.6 (C-22), 38.2 (C-10), 38.6 (chain-1), 33.7 (C-7), 32.7 (C-17), 31.8 (C-21), 30.2 (chain-3), 29.4 (C-29), 29.1 (C-28), 28.5 (C-23), 27.9 (chain-2), 27.7 (C-2), 27.4 (C-16), 27.3 (C-15), 23.6 (C-27), 19.1 (C-26), 18.4 (C-6), 16.8 (C-25), 16.1 (C-24); MS (ESI): *m*/*z* (%) = 541.6 ($[M+H]^+$, 100), 1081.3 ($[2M+H]^+$, 5), 1103.4 ($[2M+Na]^+$, 2); analysis for $C_{34}H_{56}N_2O_3$ (540.82): C, 75.51; H, 10.44; N, 5.18; found: C, 75.17; H, 10.67; N, 5.03.

(3β) N-(3-Aminopropyl) 3-hydroxy-11-oxo-olean-12-en-30-amide (**7**)

Obtained from **GA** by method A as a colourless powder; yield: 330 mg, 54 %; mp 228-231 °C (decomp.); R_f = 0.04 (CH_2Cl_2/MeOH 9:1); $[\alpha]_D$ = 91.80° (*c* 0.50, MeOH); UV-vis (MeOH): λ_{max} (log ε) = 250 nm (3.82); IR (KBr): ν = 3396*br*, 2926*s*, 2119*m*, 1756*s*, 1657*s*, 1577*s*, 1388*s*, 1243*s*, 1214*s*, 1040*m*, 994*m*, 880*m*, 658*w*, 455*w* cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD): δ = 5.60 (*s*, 1 H, H-12), 3.33 (*m*, 2 H, chain-1-CH_2), 3.18 (*dd*, 1 H, H-3, *J* = 11.2, 4.7 Hz), 2.91 (*t*, 2 H, chain-3-CH_2, *J* = 7.2), 2.71 (*ddd*, 1 H, H-1, *J* = 13.3, 3.3, 3.3 Hz), 2.49 (*s*, 1 H, H-9), 2.17 (*ddd*, 1 H, H-15, *J* = 13.6, 13.6, 4.4 Hz), 2.11 (*dd*, 1 H, H-18, *J* = 12.8, 3.3 Hz), 1.93 (*m*, 1 H, H-21), 1.91 (*m*, 1 H, H-16), 1.89 (*m*, 1 H, H-19), 1.85 (*m*, 2 H, chain-2-CH_2), 1.75 (*dd*, 1 H, H-19', *J* = 13.6, 13.6 Hz), 1.69 (*m*, 1 H, H-2), 1.63 (*m*, 1 H, H-7), 1.63 (*m*, 1 H, H-6), 1.53 (*m*, 1 H, H-2'), 1.49 (*m*, 1 H, H-6'), 1.48 (*m*, 1 H, H-7'), 1.45 (*m*, 1 H, H-22), 1.42 (*s*, 3 H, H-27), 1.37 (*m*, 2 H, H-22' and H-21'), 1.26 (*m*, 1 H, H-16'), 1.14 (*s*, 3 H, H-25), 1.14 (*s*, 3 H, H-26), 1.14 (*s*, 3 H, H-29), 1.07 (*m*, 1 H, H-1'), 1.01 (*m*, 1 H, H-15'), 1.00 (*s*, 3 H, H-23), 0.83 (*s*, 3 H, H-28), 0.80 (*s*, 3 H, H-24), 0.77 (*m*, 1 H, H-5); ^{13}C NMR (125 MHz, CD_3OD): δ = 201.2 (C-11), 178.5 (C-30), 171.1 (C-13), 127.6 (C-12), 77.9 (C-3), 61.7 (C-9), 54.7 (C-5), 48.5 (C-18), 45.3 (C-8), 43.4 (C-20), 43.2 (C-14), 41.0 (C-19), 38.9 (C-1), 38.8 (C-4), 37.4 (C-22), 36.9 (C-10), 36.8 (chain-3), 35.4 (chain-1), 32.4 (C-7), 31.5 (C-17), 30.4 (C-21), 28.1 (C-29), 27.8 (C-28), 27.6 (chain-2), 27.2 (C-23), 26.4 (C-2), 26.1 (C-16), 26.0 (C-15), 22.3 (C-27), 17.8 (C-26), 17.2 (C-6), 15.5 (C-25), 14.9 (C-24); MS (ESI): *m*/*z* (%) = 527.5 ($[M+H]^+$, 100); analysis for $C_{33}H_{54}N_2O_3$ (526.79): C, 75.24; H, 10.33; N, 5.32; found: C, 75.03; H, 10.58; N, 5.16.

(3β) N-(N^3-Boc-aminopropyl) 3-hydroxy-11-oxo-olean-12-en-30-amide (**8**)

To a solution of **7** (260 mg, 0.49 mmol) in dry methanol (20 ml), triethylamine (1.0 ml, 13.6 mmol) and Boc_2O (210 mg, 0.98 mmol) were added and the mixture was stirred at room temperature for 20 h. The solvent was removed and the residue was dissolved in dichloromethane (25 ml), washed with an aqu. solution of sodium hydrogensulfate (20 ml), the aqu. layer was extracted with dichloromethane (3 x 15 ml). The extracts were washed with brine (20 ml), dried (Na_2SO_4), filtered and evaporated. The residue was subjected to chromatographic purification (silica gel, hexane/ethyl acetate 1:1) to afford **8** (110 mg, 36 %) as colourless powder; mp 227-230 °C (decomp.); R_f = 0.75 (CH_2Cl_2/MeOH 9:1); $[\alpha]_D$ = 116.02° (*c* 0.50, $CHCl_3$); UV-vis (MeOH): λ_{max} (log ε) = 250 nm (4.10); IR (KBr): ν = 3370*br*, 2931*s*, 2870*s*, 1698*s*, 1657*s*, 1534*s*, 1455*s*, 1387*s*, 1365*s*, 1328*m*, 1278*s*, 1255*s*, 1207*m*, 1170*s*, 1089*m*, 1039*m*, 994*m*, 959*w*, 921*w*, 879*w*, 785*w*, 744*w*, 673*w*, 636*w*, 589*w*, 542*w*, 462*w* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 6.56 (*m*, 1 H, NH), 5.69 (*s*, 1 H, H-12), 4.93 (*m*, 1 H, CONH), 3.31 (dt, 2 H, chain-1-CH_2, *J* = 6.1, 6.0 Hz), 3.22 (*dd*, 1 H, H-3, *J* = 10.8, 5.4 Hz), 3.16 (dt, 2 H, chain-3, *J* = 6.1, 6.0 Hz), 2.77 (*ddd*, 1 H, H-1, *J* = 13.5, 3.3, 3.3 Hz), 2.33 (*s*, 1 H, H-9), 2.18 (*dd*, 1 H, H-18, *J* = 13.2, 2.9 Hz), 2.05 (*ddd*, 1 H, H-15, *J* = 13.8, 13.8, 4.1 Hz), 1.97 (*m*, 1 H, H-21), 1.85 (*m*, 1 H, H-19), 1.83 (*m*, 1 H, H-16), 1.70 (*dd*, 1 H, H-19', *J* = 13.8, 13.8 Hz), 1.67 (*m*, 1 H, H-6), 1.65 (*m*, 1 H, H-7), 1.63 (*m*, 1 H, H-2), 1.62 (*m*, 1 H, H-2'), 1.61 (*m*, 2 H, chain-2-CH_2), 1.58 (*m*, 1 H, H-6'), 1.46 (*m*, 1 H, H-7'), 1.45 (*m*, 1 H, H-22), 1.44 (*s*, 9 H, Boc-Me), 1.41 (*m*, 2 H, H-22' and H-21'), 1.38 (*s*, 3 H, H-27), 1.19 (*m*, 1 H, H-16'), 1.14 (*s*, 3 H, H-29), 1.13 (*s*, 3 H, H-25), 1.12 (*s*, 3 H, H-26), 1.02 (*m*, 1 H, H-15'), 1.00 (*s*, 3 H, H-23), 0.95 (*ddd*, 1 H, H-1', *J* = 12.5, 12.5, 3.7 Hz), 0.81 (*s*, 3 H, H-28), 0.81 (*s*, 3 H, H-24), 0.69 (*m*, 1 H, H-5); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.9 (C-11), 176.1 (C-30), 169.0 (C-13), 128.6 (C-12), 78.8 (C-3), 79.5 (Boc-*quart.*-C), 61.8 (C-9), 55.0 (C-5), 48.1 (C-18), 45.4 (C-8), 43.6 (C-20), 43.2 (C-14), 41.7 (C-19), 39.2 (C-1), 39.1 (C-4), 37.6 (C-22), 37.1 (C-10), 36.9 (chain-3), 35.5 (chain-1), 32.8 (C-7), 31.9 (C-17), 31.4 (C-21), 30.4 (chain-2), 29.7 (C-29), 28.5 (C-28), 28.4 (Boc-Me), 28.1 (C-23), 27.3 (C-2), 26.5 (C-16), 26.5 (C-15), 23.4 (C-27), 18.7 (C-26), 17.5 (C-6), 16.3 (C-25), 15.6 (C-24); MS (ESI): *m*/*z* (%) = 627.3 ($[M+H]^+$, 35), 649.4 ($[M+Na]^+$, 100), 1253.1 ($[2M+H]^+$, 4), 1275.3 ($[2M+Na]^+$, 16); analysis for $C_{38}H_{62}N_2O_5$ (626.91): C, 72.80; H, 9.97; N, 4.47; found: C, 72.61; H, 10.11; N, 4.38.

2-Bromoethyl (3β) 3-hydroxy-11-oxo-olean-12-en-30-oate (**9**)

Obtained from **GA** by method B as a colourless powder; yield: 820 mg, 63 %; mp 190-194 °C; R_f = 0.28 (hexane/ethyl acetate 7:3); $[\alpha]_D$ = 132.50° (*c* 0.52, $CHCl_3$); UV-vis (MeOH): λ_{max} (log ε) = 249 nm (4.05); IR (KBr): ν = 3546*br*, 2934*s*, 2869*s*, 2119*w*, 1729*m*, 1708*s*, 1656*s*, 1616*m*, 1466*m*, 1387*m*, 1316*m*, 1288*m*, 1246*m*, 1214*s*, 1164*s*, 1088*m*, 1045*m*, 997*m*, 981*m*, 923*w*, 878*w*, 766*w*, 742*w*, 673*w*, 590*w*, 570*w*, 543*w*, 444*w* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.71 (*s*, 1 H, H-12), 4.46 (dt, 1 H, chain-1-C*H*H', *J* = 5.8, 5.8), 4.39 (dt, 1 H, chain-1-CH*H'*, *J* = 11.8, 5.8 Hz), 3.54 (*dd*, 2 H, chain-2-CH_2, *J* = 11.8, 5.8 Hz), 3.22 (*dd*, 1 H, H-3, *J* = 11.1, 5.2 Hz), 2.79 (*ddd*, 1 H, H-1, *J* = 13.5, 3.4, 3.4 Hz), 2.34 (*s*, 1 H, H-9), 2.18 (*dd*, 1 H, H-18, *J* = 13.4, 4.0 Hz), 2.03 (*ddd*, 1 H, H-15, *J* = 13.4, 13.4, 4.5 Hz), 2.01 (*m*, 1 H, H-21), 1.94 (*ddd*, 1 H, H-19, *J* = 13.7, 4.0, 2.8 Hz), 1.83 (*ddd*, 1 H, H-16, *J* = 13.8, 13.8, 4.5 Hz), 1.67 (*m*, 1 H, H-2), 1.65 (*m*, 1 H, H-7), 1.64 (*dd*, 1 H, H-19', *J* = 13.5, 13.5 Hz), 1.63 (*m*, 1 H, H-2'), 1.62 (*m*, 2 H, chain-2-CH_2), 1.59 (*m*, 1 H, H-6), 1.49 (*m*, 1 H, H-6'), 1.41 (*m*, 1 H, H-7'), 1.39 (*m*, 1 H, H-22), 1.37 (*s*, 3 H, H-27), 1.35 (*m*, 2 H, H-22' and H-21'), 1.19 (*m*, 1 H, H-16'), 1.18 (*s*, 3 H, H-29), 1.13 (*s*, 3 H, H-25), 1.13 (*s*, 3 H, H-26), 1.02 (*m*, 1 H, H-15'), 1.00 (*s*, 3 H, H-23), 0.97 (*ddd*, 1 H, H-1', *J* = 13.0, 13.0, 4.5 Hz), 0.82 (*s*, 3 H, H-28), 0.80 (*s*, 3 H, H-24), 0.70 (*m*, 1 H, H-5); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.9 (C-11), 176.0 (C-30), 169.0 (C-13), 128.6 (C-12), 78.7 (C-3), 63.4 (chain-1), 61.8 (C-9), 54.9 (C-5), 48.2 (C-18), 45.4 (C-8), 44.1 (C-20), 43.2 (C-14), 40.9 (C-19), 39.1 (C-1), 39.1 (C-4), 37.7 (C-22), 37.1 (C-10), 32.8 (C-7), 31.8 (C-17), 31.1 (C-21), 29.0 (chain-2), 28.5 (C-28), 28.4 (C-29), 28.1 (C-23), 27.3 (C-2), 26.4 (C-16), 26.4 (C-15), 23.4 (C-27), 18.7 (C-26), 17.5 (C-6), 16.3 (C-25), 15.4 (C-24); MS (ESI): *m*/*z* (%) = 577.5 ($[M+H]^+$, 34), 599.3 ($[M+Na]^+$, 80), 630.9 ($[M+Na+MeOH]^+$, 21), 1153.0 ($[2M+H]^+$, 22), 1176.9 ($[2M+Na]^+$, 94); analysis for $C_{32}H_{49}BrO_4$ (577.63): C, 66.54; H, 8.55; found: C, 66.39; H, 8.65.

6-Bromohexyl (3β) 3-hydroxy-11-oxo-olean-12-en-30-oate (**10**)

Obtained from **GA** by method B as a colourless powder; yield: 530 mg, 79 %; mp 84-87 °C; R_f = 0.40 (hexane/ethyl acetate 7:3); $[\alpha]_D$ = 111.45° (*c* 0.71, $CHCl_3$); UV-vis (MeOH): λ_{max} (log ε) = 249 nm (4.04); IR (KBr): ν = 3447*br*, 2934*s*, 2864*s*, 1726*s*, 1657*s*, 1464*s*, 1387*s*, 1359*m*, 1327*m*, 1313*m*, 1280*m*, 1256*m*, 1210*s*, 1169*s*, 1085*m*, 1047*m*, 994*m*, 920*w*, 880*w*, 769*w*, 727*w*, 686*w*, 673*w*, 638*w*, 542*w*, 460*w* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.62 (*s*, 1 H, H-12), 4.11 (*m*, 1 H, chain-1-C*H*H'), 4.07 (*m*, 1 H, chain-1-CH*H'*), 3.41 (*t*, 2 H, chain-6-CH_2, *J* = 6.7 Hz), 3.22 (*dd*, 1 H, H-3, *J* = 11.1, 5.1 Hz), 2.78 (*ddd*, 1 H, H-1, *J* = 13.5, 3.5, 3.5 Hz), 2.33 (*s*, 1 H, H-9), 2.09 (*dd*, 1 H, H-18, *J* = 13.1, 3.2 Hz), 2.02 (*ddd*, 1 H, H-15, *J* = 13.6, 13.6, 4.3 Hz), 1.98 (*m*, 1 H, H-21), 1.91 (*m*, 1 H, H-19), 1.87 (*m*, 2 H, chain-5-CH_2), 1.82

(*ddd*, 1 H, H-16, *J* = 13.5, 13.5, 4.4 Hz), 1.66 (*m*, 1 H, H-2), 1.65 (*m*, 2 H, chain-2-CH_2), 1.65 (*m*, 1 H, H-7), 1.61 (*dd*, 1 H, H-19', *J* = 13.7, 13.7 Hz), 1.59 (*m*, 1 H, H-2'), 1.58 (*m*, 1 H, H-6), 1.47 (*m*, 2 H, chain-4-CH_2), 1.46 (*m*, 1 H, H-6'), 1.41 (*m*, 1 H, H-7'), 1.40 (*m*, 2 H, chain-3-CH_2), 1.39 (*m*, 1 H, H-22), 1.36 (*s*, 3 H, H-27), 1.31 (*m*, 2 H, H-22' and H-21'), 1.18 (*m*, 1 H, H-16'), 1.14 (*s*, 3 H, H-29), 1.13 (*s*, 3 H, H-25), 1.12 (*s*, 3 H, H-26), 1.01 (*m*, 1 H, H-15'), 1.00 (*s*, 3 H, H-23), 0.97 (*ddd*, 1 H, H-1', *J* = 13.0, 13.0, 4.2 Hz), 0.80 (*s*, 3 H, H-28), 0.80 (*s*, 3 H, H-24), 0.69 (*m*, 1 H, H-5); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.1 (C-11), 176.4 (C-30), 169.1 (C-13), 128.5 (C-12), 78.7 (C-3), 64.2 (chain-1), 61.8 (C-9), 54.9 (C-5), 48.4 (C-18), 45.4 (C-8), 44.0 (C-20), 43.2 (C-14), 41.1 (C-19), 39.1 (C-1), 39.1 (C-4), 37.7 (C-22), 37.1 (C-10), 33.7 (chain-6), 32.8 (C-7), 32.6 (chain-5), 31.8 (C-17), 31.1 (C-21), 28.6 (chain-2), 28.4 (C-28), 28.4 (C-29), 28.1 (C-23), 27.7 (chain-4), 27.3 (C-2), 26.5 (C-16), 26.4 (C-15), 25.2 (chain-3), 23.4 (C-27), 18.7 (C-26), 17.5 (C-6), 16.3 (C-25), 15.6 (C-24); MS (ESI): *m*/*z* (%) = 633.5 ($[M+H]^+$, 53), 655 ($[M+Na]^+$, 74), 686.7 ($[M+Na+MeOH]^+$, 12), 970.5 ($[3M+2Na]^{2+}$, 7), 1265.0 ($[2M+H]^+$, 16), 1287.1 ($[2M+Na]^+$, 44); analysis for $C_{36}H_{57}BrO_4$ (633.74): C, 68.23; H, 9.07; found: C, 68.00; H, 9.17.

(2E) 4-Bromobut-2-en-1-yl (3β) 3-hydroxy-11-oxo-olean-12-en-30-oate (**11**)

Obtained from **GA** by method B as a colourless powder; yield: 730 mg, 58 %; mp 114-117 °C; R_f = 0.80 (CH_2Cl_2/MeOH 9:1); $[\alpha]_D$ = 118.50° (*c* 0.68, $CHCl_3$); UV-vis (MeOH): λ_{max} (log ε) = 248 nm (4.12); IR (KBr): ν = 3440*br*, 2930*s*, 2867*s*, 1812*w*, 1728*s*, 1655*s*, 1456*m*, 1386*m*, 1314*m*, 1280*m*, 1258*m*, 1209*s*, 1152*s*, 1086*m*, 1048*m*, 981*m*, 880*w*, 768*w*, 673*w*, 600*w*, 542*w* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.98 (*m*, 1 H, chain-3-CH), 5.87 (*m*, 1 H, chain-2-CH), 5.63 (*s*, 1 H, H-12), 4.61 (*d*, 2 H, chain-1-CH_2, *J* = 4.9 Hz), 3.96 (*d*, 2 H, chain-4-CH_2, *J* = 7.3 Hz), 3.22 (*dd*, 1 H, H-3, *J* = 10.7, 5.6 Hz), 2.79 (*ddd*, 1 H, H-1, *J* =13.5, 3.4, 3.4 Hz), 2.34 (*s*, 1 H, H-9), 2.09 (*dd*, 1 H, H-18, *J* = 13.7, 3.4 Hz), 2.03 (*m*, 1 H, H-15), 2.00 (*m*, 1 H, H-21), 1.92 (*ddd*, 1 H, H-19, *J* = 13.7, 4.0, 2.7 Hz), 1.83 (*ddd*, 1 H, H-16, *J* = 13.8, 13.8, 4.4 Hz), 1.66 (*m*, 1 H, H-2), 1.65 (*m*, 1 H, H-7), 1.63 (*m*, 1 H, H-2'), 1.63 (*dd*, 1 H, H-19', *J* = 13.5, 13.5 Hz), 1.60 (*m*, 1 H, H-6), 1.44 (*m*, 1 H, H-6'), 1.43 (*m*, 1 H, H-22), 1.41 (*m*, 1 H, H-7'), 1.37 (*s*, 3 H, H-27), 1.33 (*m*, 2 H, H-22' and H-21'), 1.19 (*m*, 1 H, H-16'), 1.17 (*s*, 3 H, H-29), 1.14 (*s*, 3 H, H-25), 1.13 (*s*, 3 H, H-26), 1.02 (*m*, 1 H, H-1'), 1.01 (*s*, 3 H, H-23), 0.98 (*m*, 1 H, H-15'), 0.81 (*s*, 3 H, H-24), 0.81 (*s*, 3 H, H-28), 0.70 (*m*, 1 H, H-5); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.1 (C-11), 175.9 (C-30), 169.0 (C-13), 130.3 (chain-2), 128.9 (chain-3), 128.6 (C-12), 78.8 (C-3), 63.4 (chain-1), 61.8 (C-9), 54.9 (C-5), 48.3 (C-18), 45.4 (C-8), 44.0 (C-20), 43.2 (C-14), 41.1 (C-19), 39.1 (C-1), 39.1 (C-4), 37.7 (C-22), 37.1 (C-10),

32.8 (C-7), 31.8 (C-17), 31.2 (chain-4), 31.1 (C-21), 28.5 (C-28), 28.3 (C-29), 28.1 (C-23), 27.3 (C-2), 26.5 (C-16), 26.4 (C-15), 23.4 (C-27), 18.7 (C-26), 17.5 (C-6), 16.4 (C-25), 15.6 (C-24); MS (ESI): *m*/*z* (%) = 603.4 ($[M+H]^+$, 20), 625.4 ($[M+Na]^+$, 94), 656.8 ($[M+Na+MeOH]^+$, 6), 1229.0 ($[2M+Na]^+$, 40); analysis for $C_{34}H_{51}BrO_4$ (603.67): C, 67.65; H, 8.52; found: C, 67.57; H, 9.04.

But-3-en1-yl (3β) 3-hydroxy-11-oxo-olean-12-en-30-oate (**12**)

Obtained from **GA** by method B as a colourless powder; yield: 450 mg, 76 %; mp 181-184 °C; R_f = 0.44 (hexane/ethyl acetate 7:3); $[\alpha]_D$ = 141.52° (*c* 0.52, $CHCl_3$); UV-vis (MeOH): λ_{max} (log ε) = 250 nm (4.11); IR (KBr): ν = 3515*br*, 2975*s*, 2925*s*, 2867*s*, 1726*s*, 1644*s*, 1614*m*, 1463*m*, 1430*w*, 1389*m*, 1358*m*, 1315*m*, 1278*w*, 1253*m*, 1214*s*, 1156*s*, 1104*w*, 1088*m*, 1046*m*, 995*m*, 980*m*, 947*w*, 928*m*, 880*w*, 868*w*, 857*w*, 805*w*, 768*w*, 715*w*, 702*w*, 688*w*, 673*w*, 638*w*, 589*w*, 542*w*, 504*w* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.79 (*ddt*, 1 H, chain-3-CH, *J* = 17.0, 10.2, 6.7 Hz), 5.65 (*s*, 1 H, H-12), 5.13 (*d*, 1 H, chain-4-CH^E, *J* = 17.0 Hz), 5.10 (*d*, 1 H, chain-4-CH^Z, *J* = 10.2 Hz), 4.16 (*m*, 2 H, chain-2-CH_2), 3.22 (*dd*, 1 H, H-3, *J* = 11.0, 5.2 Hz), 2.79 (*ddd*, 1 H, H-1, *J* = 13.2, 3.0, 3.0 Hz), 2.40 (*m*, 2 H, chain-1-CH_2), 2.34 (*s*, 1 H, H-9), 2.11 (*dd*, 1 H, H-18, *J* = 13.4, 3.5 Hz), 2.02 (*ddd*, 1 H, H-15, *J* = 13.6, 13.6, 4.3 Hz), 1.98 (*m*, 1 H, H-21), 1.91 (*ddd*, 1 H, H-19, *J* = 13.4, 3.7, 3.1 Hz), 1.82 (*ddd*, 1 H, H-16, *J* = 13.6, 13.6, 4.4 Hz), 1.66 (*m*, 1 H, H-2), 1.64 (*m*, 1 H, H-7), 1.61 (*m*, 1 H, H-2'), 1.60 (*dd*, 1 H, H-19', *J* = 13.3, 13.3 Hz), 1.58 (*m*, 1 H, H-6), 1.45 (*m*, 1 H, H-6'), 1.44 (*m*, 1 H, H-7'), 1.38 (*m*, 1 H, H-22), 1.36 (*s*, 3 H, H-27), 1.32 (*m*, 2 H, H-22' and H-21'), 1.18 (*m*, 1 H, H-16'), 1.14 (*s*, 3 H, H-25), 1.14 (*s*, 3 H, H-29), 1.12 (*s*, 3 H, H-26), 1.01 (*m*, 1 H, H-1'), 1.00 (*s*, 3 H, H-23), 0.98 (*m*, 1 H, H-15'), 0.80 (*s*, 3 H, H-24), 0.80 (*s*, 3 H, H-28), 0.70 (*m*, 1 H, H-5); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.2 (C-11), 176.4 (C-30), 169.2 (C-13), 134.0 (chain-3), 128.5 (C-12), 117.4 (chain-4), 78.7 (C-3), 63.4 (chain-2), 61.8 (C-9), 54.9 (C-5), 48.2 (C-18), 45.4 (C-8), 44.0 (C-20), 43.2 (C-14), 41.0 (C-19), 39.1 (C-1), 39.1 (C-4), 37.7 (C-22), 37.1 (C-10), 33.2 (chain-1), 32.8 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-28), 28.4 (C-29), 28.1 (C-23), 27.3 (C-2), 26.5 (C-16), 26.4 (C-15), 23.4 (C-27), 18.7 (C-26), 17.5 (C-6), 16.3 (C-25), 15.5 (C-24); MS (ESI): *m*/*z* (%) = 525.5 ($[M+H]^+$, 52), 547.5 ($[M+Na]^+$, 8), 578.9 ($[M+Na+MeOH]^+$, 58), 1049.2 ($[2M+H]^+$, 82), 1071.2 ($[2M+Na]^+$, 100); analysis for $C_{34}H_{52}O_4$ (524.77): C, 77.82; H, 9.99; found: 77.72; H, 10.16.

Allyl (3β) 3-hydroxy-11-oxo-olean-12-en-30-oate (13) and allyl (3β) 3-allyloxy-11-oxo-olean-12-en-30-oate (**14**)

To a solution of **GA** (1.47 g, 3.13 mmol) in dry DMF (50 ml), potassium carbonate (4.52 g, 32.8 mmol) was added and the mixture was stirred at room temperature for 1 h. After adding allyl iodide (2.5 ml, 27.2 mmol), stirring was continued for 17 h. Brine (30 ml) was added and the aqueous layer was extracted with chloroform (3 x 20 ml). The combined extracts were dried (Na_2SO_4), filtered and evaporated. Purification by chromatography (silica gel, hexane/ethyl acetate 7:3) yielded **13** (1.20 g, 75 %) and **14** (270 mg, 16 %) as colourless powders.

Data for **13**: mp 212-214 °C (lit. 208-210 °C [23], 210-211 °C [24]); R_f = 0.49 (hexane/ethyl acetate 3:7); $[\alpha]_D$ = 144.59° (*c* 0.50, $CHCl_3$); UV-vis (MeOH): λ_{max} (log ε) = 249 nm (4.10); IR (KBr): *v* = 3523*br*, 2926*s*, 2865*s*, 1729*s*, 1641*s*, 1460*m*, 1390*m*, 1358*w*, 1328*w*, 1309*w*, 1279*w*, 1253*m*, 1210*m*, 1171*s*, 1133*w*, 1086*m*, 1044*m*, 987*m*, 938*m*, 878*w*, 768*w* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.89 (*ddt*, 1 H, chain-2-CH, *J* = 17.0, 10.4, 5.8 Hz), 5.61 (*s*, 1 H, H-12), 5.30 (*dd*, 1 H, chain-3-CH^Z, *J* = 17.0, 1.7 Hz), 5.22 (*dd*, 1 H, chain-3-CH^E, *J* = 10.4, 1.7) Hz, 4.57 (*ddd*, 2 H, chain-1-CH_2, *J* = 13.4, 5.8, 1.2 Hz), 3.20 (*dd*, 1 H, H-3, *J* = 10.8, 5.4 Hz), 2.77 (*ddd*, 1 H, H-1, *J* = 13.7, 3.3, 3.3 Hz), 2.31 (*s*, 1 H, H-9), 2.08 (*dd*, 1 H, H-18, *J* = 12.9, 3.3 Hz), 2.00 (*ddd*, 1 H, H-15, *J* = 13.7, 13.7, 4.6 Hz), 1.98 (*m*, 1 H, H-21), 1.91 (*ddd*, 1 H, H-19, *J* = 13.7, 4.2, 1.2 Hz), 1.80 (*ddd*, 1 H, H-16, *J* = 13.7, 13.7, 4.6 Hz), 1.65 (*ddd*, 1 H, H-2, *J* = 13.3, 13.3, 3.3 Hz), 1.63 (*m*, 1 H, H-7), 1.61 (*m*, 1 H, H-2'), 1.60 (*m*, 1 H, H-19'), 1.58 (*m*, 1 H, H-6), 1.43 (*m*, 1 H, H-6'), 1.38 (*m*, 1 H, H-7'), 1.36 (*m*, 1 H, H-22), 1.34 (*s*, 3 H, H-27), 1.31 (*m*, 2 H, H-22' and H-21'), 1.16 (*m*, 1 H, H-16'), 1.14 (*s*, 3 H, H-29), 1.11 (*s*, 3 H, H-25), 1.10 (*s*, 3 H, H-26), 0.99 (*m*, 1 H, H-15'), 0.98 (*s*, 3 H, H-23), 0.94 (*m*, 1 H, H-1'), 0.78 (*s*, 3 H, H-24), 0.78 (*s*, 3 H, H-28), 0.67 (*dd*, 1 H, H-5, *J* = 10.3, 1.3 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): *δ* = 200.2 (C11), 176.0 (C30), 169.1 (C13), 132.2 (chain-2), 128.5 (C12), 118.4 (chain-3), 78.7 (C3), 65.0 (chain-1), 61.8 (C9), 54.9 (C5), 48.3 (C18), 45.4 (C8), 44.0 (C20), 43.2 (C14), 41.1 (C19), 39.1 (C1), 39.1 (C4), 37.7 (C22), 37.1 (C10), 32.8 (C7), 31.8 (C17), 31.1 (C21), 28.5 (C28), 28.3 (C29), 28.1 (C23), 27.3 (C2), 26.5 (C16), 26.4 (C15), 23.4 (C27), 18.7 (C26), 17.5 (C6), 16.3 (C25), 15.6 (C24); MS (ESI): *m*/*z* (%) = 511.5 ($[M+H]^+$, 93), 533.5 ($[M+Na]^+$, 24), 564.9 ($[M+MeOH+Na]^+$, 100); analysis for $C_{33}H_{50}O_4$ (510.75): C, 77.60; H, 9.87; found: C; 77.50; H, 9.98.

Data for **14**: mp 222-226 °C; R_f = 0.85 (hexane/ethyl acetate 7:3); $[\alpha]_D$ = 122.42° (*c* 0.48, $CHCl_3$); UV-vis (MeOH): λ_{max} (log ε) = 249 nm (4.19); IR (KBr): *v* = 3434*br*, 2960*m*, 2875*m*, 1750*s*, 1728*s*, 1652*s*, 1463*m*, 1389*m*, 1364*w*, 1324*m*, 1257*s*, 1214*m*, 1154*m*, 1085*w*, 1020*w*, 985*m*, 969*m*, 928*m*, 880*w*, 790*w*, 768*w*, 542*w* cm^{-1};^1H NMR (500 MHz, $CDCl_3$): *δ* = 5.93 (*m*,

1 H, C^3-chain-2-CH), 5.92 (*m*, 1 H, C^{30}-chain-2-CH), 5.64 (*s*, 1 H, H-12), 5.35 (*ddt*, 1 H, C^3-chain-3-CH^Z, *J* = 17.2, 1.5, 1.5 Hz), 5.33 (*ddt*, 1 H, C^{30}-chain-3-CH^Z, *J* = 17.2, 1.5, 1.5 Hz), 5.26 (*ddt*, 1 H, C^3-chain-3-CH^E, *J* = 10.4, 1.3 Hz), 5.24 (*ddt*, 1 H, C^{30}-chain-3-CH^E, *J* = 10.4, 1.3 Hz), 4.61 (*m*, 2 H, C^3-chain-1-CH_2), 4.59 (*m*, 2 H, C^{30}-chain-1-CH_2), 4.35 (*dd*, 1 H, H-3, *J* = 11.4, 5.2 Hz), 2.77 (*ddd*, 1 H, H-1, *J* = 13.6, 3.5, 3.5 Hz), 2.35 (*s*, 1 H, H-9), 2.11 (*dd*, 1 H, H-18, *J* = 13.4, 3.2 Hz), 2.03 (*ddd*, 1 H, H-15, *J* = 13.5, 13.5, 4.2 Hz), 2.01 (*m*, 1 H, H-21), 1.94 (*ddd*, 1 H, H-19, *J* = 13.6, 4.1, 2.8 Hz), 1.83 (*ddd*, 1 H, H-16, *J* = 13.7, 13.7, 4.4 Hz), 1.78 (*m*, 1 H, H-2), 1.74 (*m*, 1 H, H-2'), 1.66 (*m*, 1 H, H-7), 1.62 (*dd*, 1 H, H-19', *J* = 13.6, 13.6 Hz), 1.58 (*m*, 1 H, H-6), 1.45 (*m*, 1 H, H-6'), 1.38 (*m*, 1 H, H-7'), 1.36 (*s*, 3 H, H-27), 1.36 (*m*, 1 H, H-22), 1.33 (*m*, 2 H, H-22' and H-21'), 1.18 (*m*, 1 H, H-16'), 1.16 (*s*, 3 H, H-29), 1.16 (*s*, 3 H, H-25), 1.12 (*s*, 3 H, H-26), 1.04 (*ddd*, 1 H, H-15', *J* = 13.9, 13.9, 4.2 Hz), 1.02 (*m*, 1 H, H-1'), 0.95 (*s*, 3 H, H-23), 0.90 (*s*, 3 H, H-24), 0.80 (*s*, 3 H, H-28), 0.79 (*m*, 1 H, H-5); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.9 (C-11), 176.0 (C-30), 169.2 (C-13), 132.2 (C^{30}-chain-2), 131.8 (C^3-chain-2), 128.5 (C-12), 118.7 (C^3-chain-3), 118.4 (C^{30}-chain-3), 85.2 (C-3), 68.2 (C^3-chain-1), 65.0 (C^{30}-chain-1), 61.7 (C-9), 55.0 (C-5), 48.3 (C-18), 45.4 (C-8), 44.0 (C-20), 43.2 (C-14), 41.1 (C-19), 38.7 (C-4), 38.3 (C-1), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-28), 28.3 (C-29), 27.9 (C-23), 26.5 (C-16), 26.4 (C-15), 23.5 (C-2), 23.3 (C-27), 18.7 (C-26), 17.3 (C-6), 16.6 (C-24), 16.3 (C-25); MS (ESI): *m*/*z* (%) = 595.5 ($[M+H]^+$, 74), 617.5 ($[M+Na]^+$, 100), 1189.3 ([2M+H], 78), 1211.3 ($[2M+Na]^+$, 38); analysis for $C_{36}H_{54}O_4$ (550.81): C, 78.50; H, 9.88; found: C, 78.39; H, 10.01.

(3β) 3-Acetyloxy-11-oxo-olean-12-en-30-amine (**15**)

Compound **15** was prepared according to a known procedure [16] and obtained as a colourless powder (4.90 g, 98 %); mp 230-233 °C (235-237 °C [25], 236-237 °C [16]); R_f = 0.20 (CH_2Cl_2/MeOH 9:1); $[\alpha]_D$ = 80.51° (*c* 0.63, $CHCl_3$); UV-vis (MeOH): λ_{max} (log ε) = 247 nm (3.88); IR (KBr): *ν* = 3434*br*, 2974*s*, 1732*s*, 1660*s*, 1553*s*, 1466*m*, 1391*s*, 1246*s*, 1144*w*, 1087*w*, 1030*m*, 985*m*, 881*w*, 656*w*, 615*w*, 542*w*, 453*w* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.67 (*s*, 1 H, H-12), 4.51 (*dd*, 1 H, H-3, *J* = 11.6, 4.8 Hz), 2.77 (*ddd*, 1 H, H-1, *J* = 13.2, 3.0, 3.0 Hz), 2.41 (*dd*, 1 H, H-18, *J* = 13.0, 3.0 Hz), 2.33 (*s*, 1 H, H-9), 2.04 (*s*, 3 H, Ac-Me), 2.01 (*m*, 1 H, H-15), 1.96 (*ddd*, 1 H, H-21, *J* = 13.2, 13.2, 3.5 Hz), 1.84 (*m*, 1 H, H-19), 1.71 (*m*, 1 H, H-16), 1.65 (*m*, 1 H, H-2), 1.62 (*m*, 1 H, H-7), 1.61 (*m*, 1 H, H-19'), 1.59 (*m*, 1 H, H-2'), 1.57 (*m*, 1 H, H-6), 1.48 (*m*, 1 H, H-6'), 1.42 (*m*, 1 H, H-7'), 1.41 (*m*, 1 H, H-22), 1.40 (*m*, 2 H, H-22' and H-21'), 1.34 (*s*, 3 H, H-29), 1.34 (*s*, 3 H, H-27), 1.16 (*m*, 1 H, H-16'), 1.15 (*s*, 3 H, H-25), 1.13 (*s*, 3 H, H-26), 1.04 (*m*, 1 H, H-1'), 1.02 (*m*, 1 H, H-15'), 0.92 (*s*, 3 H, H-23), 0.87

(*s*, 3 H, H-24), 0.87 (*s*, 3 H, H-28), 0.79 (*m*, 1 H, H-5); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.8 (C-11), 171.0 (Ac-COO), 167.7 (C-13), 128.7 (C-12), 80.6 (C-3), 61.7 (C-9), 55.0 (C-5), 53.0 (C-20), 45.4 (C-8), 45.1 (C-18), 44.0 (C-20), 43.1 (C-14), 41.4 (C-19), 38.8 (C-1), 38.0 (C-4), 36.9 (C-10), 34.4 (C-22), 32.6 (C-7), 31.8 (C-17), 31.5 (C-21), 28.6 (C-29), 28.0 (C-28), 27.3 (C-23), 26.2 (C-16), 26.2 (C-15), 23.5 (C-2), 23.5 (C-27), 21.3 (Ac-Me), 18.7 (C-26), 17.3 (C-6), 16.7 (C-24), 16.4 (C-25); MS (ESI): *m*/*z* (%) = 484.3 ($[M+H]^+$, 100), 725.8 ($[3M+2H]^{2+}$, 3), 967.3 ($[2M+H]^+$, 8).

(3β) 3-Acetyloxy-11-oxo-olean-12-en-30-amide (**16**)

Compound **5** (1.00 g, 1.95 mmol) was dissolved in dry dichloromethane (100 ml) and oxalylchloride (1 ml, 11.65 mmol) was added. After 2 h of stirring at room temperature, the solvent was removed under reduced pressure and the residue was dissolved in dry toluene (50 ml). At 4 °C a conc. solution of ammonia (25 ml) was added and the mixture was stirred for 1 h. The mixture was extracted with dichloromethane (3 x 25 ml), the combined extracts were washed with a saturated solution of sodium hydrogencarbonate (20 ml), water (20 ml) and brine (10 ml), dried (Na_2SO_4) and filtered. Evaporation to dryness gave **16** (1.00 g, 99 %) as a slightly brown powder; mp 270-274 °C (lit. 312-314 °C [23]); R_f = 0.36 (hexane/ethyl acetate 7:3); $[\alpha]_D$ = 119.05° (*c* 0.41, $CHCl_3$); UV-vis (MeOH): λ_{max} (log ε) = 250 nm (4.08); IR (KBr): ν = 3367*br*, 2958*m*, 2869*m*, 1733*m*, 1644*s*, 1607*m*, 1456*w*, 1389*m*, 1366*m*, 1327*w*, 1244*s*, 1208*w*, 1142*w*, 1031*w*, 1001*w*, 985*w*, 886*w*, 726*w*, 606*w* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.66 (*br*, 1 H, COONH*H*'), 5.65 (*s*, 1 H, H-12), 5.43 (*br*, 1 H, COONH*H*'), 4.48 (*dd*, 1 H, H-3, *J* = 11.6, 4.6 Hz), 2.77 (*ddd*, 1 H, H-1, *J* = 13.7, 3.7, 3.7 Hz), 2.33 (*s*, 1 H, H-9), 2.19 (*dd*, 1 H, H-18, *J* = 8.7, 8.7 Hz), 2.02 (*s*, 3 H, Ac-Me), 2.02 (*ddd*, 1 H, H-15, *J* = 12.9, 12.9, 4.2 Hz), 1.89 (*m*, 1 H, H-21), 1.81 (*ddd*, 1 H, H-16, *J* = 13.7, 13.7, 4.2 Hz), 1.72 (*m*, 1 H, H-19), 1.69 (*m*, 1 H, H-19'), 1.65 (*m*, 1 H, H-2), 1.64 (*m*, 1 H, H-7), 1.61 (*m*, 1 H, H-2'), 1.56 (*m*, 1 H, H-6), 1.47 (*m*, 1 H, H-22), 1.45 (*m*, 1 H, H-6'), 1.41 (*m*, 1 H, H-7'), 1.39 (*m*, 1 H, H-22'), 1.35 (*m*, 1 H, H-21'), 1.34 (*s*, 3 H, H-27), 1.17 (*m*, 1 H, H- 16'), 1.15 (*s*, 3 H, H-29), 1.13 (*s*, 3 H, H-25), 1.10 (*s*, 3 H, H-26), 1.20 (*m*, 1 H, H-1'), 1.01 (*m*, 1 H, H- 15'), 0.85 (*s*, 3 H, H-23), 0.85 (*s*, 3 H, H-24), 0.80 (*s*, 3 H, H-28), 0.78 (*m*, 1 H, H-5); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.9 (C11), 178.4 (C30), 171.0 (Ac-COO), 169.2 (C13), 128.5 (C12), 80.6 (C3), 61.7 (C9), 55.0 (C5), 48.1 (C18), 45.4 (C8), 43.7 (C20), 43.2 (C14), 42.0 (C19), 38.8 (C1), 38.0 (C4), 37.4 (C22), 36.9 (C10), 32.7 (C7), 31.9 (C21), 31.5 (C17), 29.5 (C29), 28.4 (C28), 28.0 (C23), 26.4 (C16), 26.4 (C15), 23.5 (C2), 23.3 (C27), 21.3 (Ac-Me), 18.7 (C26), 17.3

(C6), 16.6 (C24), 16.4 (C25); MS (ESI): *m*/*z* (%) = 512.5 ($[M+H]^+$, 100), 534.5 ($[M+Na]^+$, 47), 550.2 ($[M+K]^+$, 22).

(3β) 3-Acetyloxy-11-oxo-olean-12-en-30-nitrile (**17**)

Compound **16** (950 mg, 1.86 mmol) was dissolved in thionyl chloride (25 ml) and the solution was heated ander reflux for 4 h. The solvent was removed under reduced pressure; re-crystallisation from MeOH yielded **17** (900 mg, 98 %) as colourless crystalls; mp 206-208 °C (lit. 297-299 °C [26]); R_f = 0.61 (hexane/ethyl acetate 7:3); $[\alpha]_D$ = 150.03° (*c* 0.45, $CHCl_3$); UV-vis (MeOH): λ_{max} (log ε) = 247 nm (4.01); IR (KBr): ν = 3447*br*, 2951*s*, 2875*m*, 2228*w*, 1734*s*, 1662*s*, 1455*w*, 1385*w*, 1366*w*, 1326*w*, 1250*s*, 1211*w*, 1142*w*, 1087*w*, 1029*w*, 986*w*, 878*w*, 662*w*, 610*w* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.67 (*s*, 1 H, H-12), 4.49 (*dd*, 1 H, H-3, *J* = 11.6, 5.0), 2.76 (*ddd*, 1 H, H-1, *J* = 13.7, 3.6, 3.6 Hz), 2.34 (*dd*, 1 H, H-18, *J* = 12.9, 4.2 Hz), 2.31 (*s*, 1 H, H-9), 2.02 (*s*, 3 H, Ac-Me), 1.92 (*ddd*, 1 H, H-15, *J* = 13.3, 13.3, 4.2 Hz), 1.83 (*ddd*, 1 H, H-16, *J* = 12.7, 12.7, 4.2 Hz), 1.76 (*m*, 1 H, H-21), 1.73 (*m*, 1 H, H-19), 1.70 (*m*, 1 H, H-22), 1.68 (*m*, 2 H, H-19' and H-2), 1.63 (*m*, 1 H, H-2'), 1.60 (*m*, 1 H, H-6), 1.55 (*m*, 1 H, H-22'), 1.50 (*m*, 1 H, H-6'), 1.44 (*m*, 1 H, H-21'), 1.34 (*s*, 3 H, H-29), 1.30 (*s*, 3 H, H-27), 1.17 (*m*, 1 H, H-16'), 1.14 (*s*, 3 H, H-25), 1.12 (*s*, 3 H, H-26), 1.04 (*m*, 1 H, H-15'), 1.00 (*m*, 1 H, H-1'), 0.91 (*s*, 3 H, H-23), 0.86 (*s*, 3 H, H-28), 0.85 (*s*, 3 H, H-24), 0.77 (*dd*, 1 H, H-5, *J* = 11.6, 1.7 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.6 (C11), 171.0 (Ac-COO), 167.0 (C13), 129.0 (C12), 123.3 (C30), 80.5 (C3), 61.7 (C9), 55.0 (C5), 48.2 (C18), 45.3 (C8), 43.1 (C14), 42.3 (C19), 38.8 (C1), 38.0 (C4), 37.3 (C22), 36.9 (C10), 35.1 (C17), 32.7 (C21), 32.6 (C20), 32.0 (C7), 28.0 (C23), 28.0 (C28), 27.3 (C29), 26.3 (C16), 26.3 (C15), 23.5 (C2), 23.3 (C27), 21.3 (Ac-Me), 18.7 (C26), 17.3 (C6), 16.4 (C24), 16.3 (C25); MS (ESI): *m*/*z* (%) = 494.5 ($[M+H]^+$, 59), 516.5 ($[M+Na]^+$, 10), 548.0 ($[M+MeOH+Na]^+$, 100)

Acknowledgment

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Captions:

Fig. 1. Structure of glycyrrhetinic acid (GA).

Scheme 1. Synthesis of the amides: (a) K_2CO_3, diamine, DMF, 25 °C, 20 h, 28 % for **6**, 54 % for **7**; (b) Boc_2O, Et_3N, MeOH, 25 °C, 20 h, 36 %.

Scheme 2. Synthesis of the esters: (a) K_2CO_3, alkyl halide, DMF, 25 °C, 20 h, 16-79 %.

Scheme 3. Structure of **15** and synthesis of the nitrile **17**: (a) $(COCl)_2$, CH_2Cl_2, 25 °C, 2 h, then conc. ammonia, toluene, 4 °C, 1 h, 99 %; (b) $SOCl_2$, reflux, 4 h, 98 %.

Fig. 2. Results from the AO/EB test; treatment of A549 cells with (from left to right): **11** (6 µM), **12** (25 µM) and **15** (25 µM).

Table 1

Table 1. Results of the SRB-assay on various tumor cell lines: Results (IC_{50}) are given in μM; error ± 5 %); *) data from previous studies [17].

	518A2	**8505C**	**A253**	**A549**	**DLD-1**	**Lipo**	**SW1736**
GA*	83.92	86.50	80.78	82.76	81.21	81.44	76.93
1*	27.54	26.07	19.42	23.50	26.12	20.47	34.82
2*	25.23	24.58	25.04	22.74	28.14	27.66	13.37
3*	18.15	14.24	15.75	14.41	27.61	15.93	12.77
4*	18.19	8.10	10.67	6.15	22.69	11.54	2.74
5	>30	>30	>30	>30	>30	>30	>30
6	>30	>30	>30	>30	>30	>30	>30
7	>30	>30	>30	>30	>30	>30	>30
8	>30	>30	>30	>30	>30	>30	>30
9	15.19	15.59	15.89	20.27	22.98	15.46	19.87
10	28.99	>30	>30	>30	>30	>30	28.64
11	21.00	8.82	10.97	4.28	23.09	11.47	1.88
12	14.91	11.61	13.57	19.16	14.88	12.77	16.36
13	15.33	15.59	15.89	20.27	22.98	15.46	19.87
14	>30	>30	>30	>30	>30	>30	>30
15	14.91	11.61	13.57	19.16	14.88	12.77	16.36
16	>30	>30	>30	>30	>30	>30	25.65
17	>30	>30	>30	>30	>30	>30	>30

Table 2

Table 2. Results of the SRB-assay on mouse embryonic fibroblasts (NiH3T3): Results (IC_{50}) are given in μM; error ± 5 %); *) data from previous studies [17].

	GA*	**1***	**2***	**3***	**4***	**9**	**12**	**13**	**15**
NiH3T3	18.52	22.81	23.66	32.37	21.02	19.79	19.73	19.79	19.73

Table 3

Table 3. Apoptotic effect of GA and derivatives in A549 cells in % (± standard error, 6 experiments), cells were treated with **11** (6 µM), **12** (25 µM) and **15** (25 µM).

Compound	**GA**	**11**	**12**	**15**
Apoptosis [%]	73.73 ± 1.40	82.77 ± 4.55	91.51 ± 0.79	88.77 ± 2.12

Fig. 1

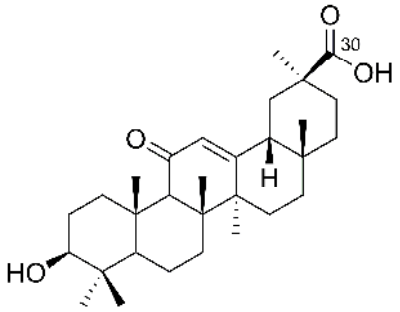
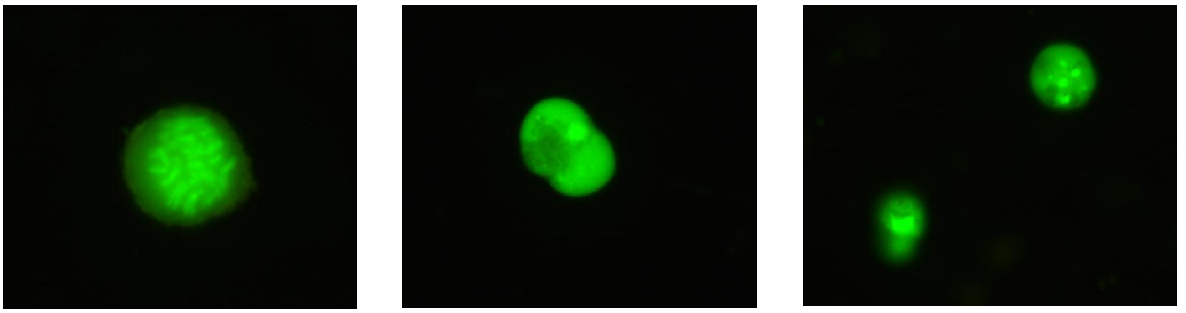
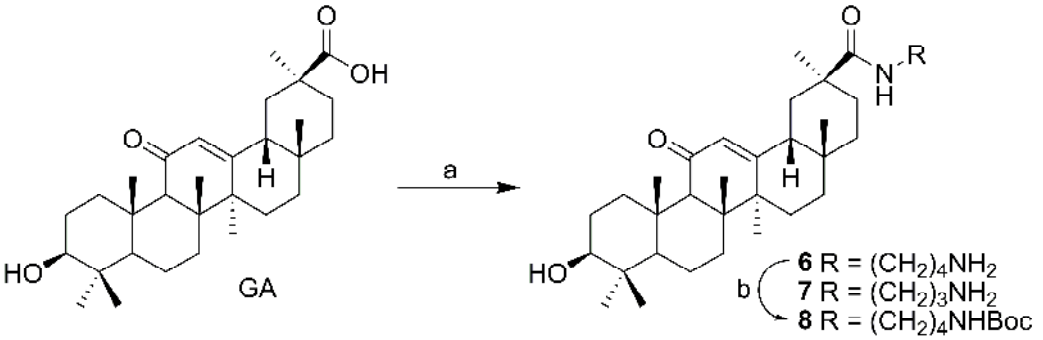


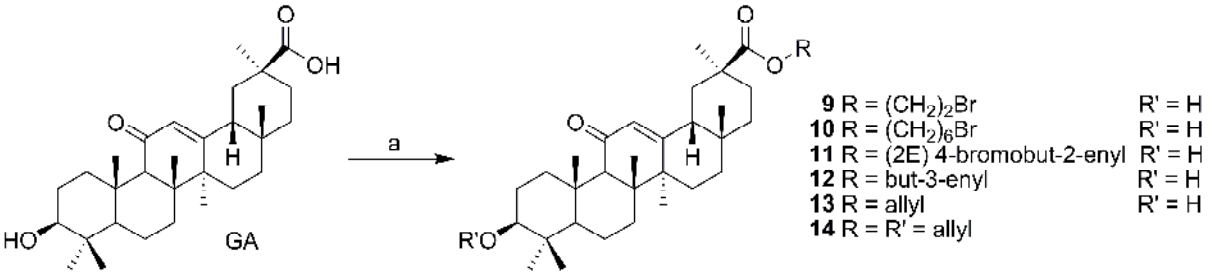
Fig. 2



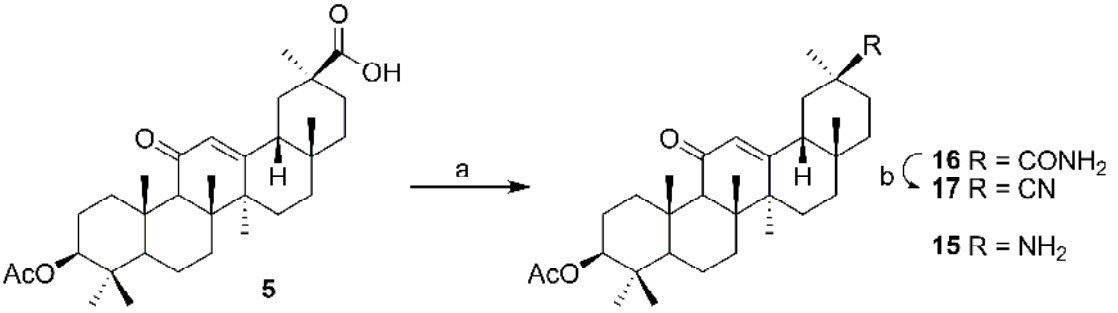
Scheme 1



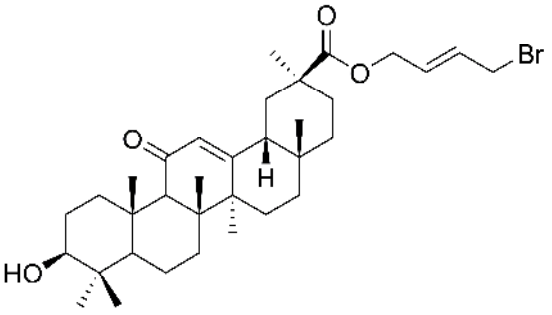
Scheme 2



Scheme 3



graphical abstract



IC_{50} = 1.88 μM (SW1736; human thyroid carcinoma cell line)

Anhang A8

„A Natural Approach: Synthesis and studies on cytotoxicity of Glycyrrhetinic Acid glycosides“

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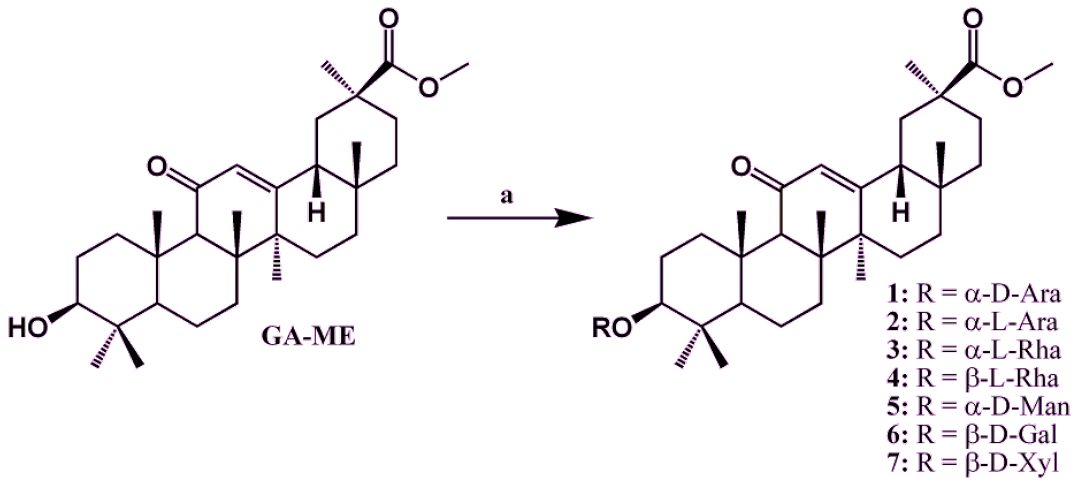
Abstract

There are a couple of pentacyclic triterpenoic acids showing antitumour activity; best-known examples are betulinic acid and oleanic acid. Another interesting representative of this class of compounds, glycyrrhetinic acid (**GA**) is not as active as betulinic acid, but **GA** is still in the focus of scientific interest. It triggers apoptosis in tumour cells and is easy to obtain from the extracts of licorice roots. The incorporation of an extra hydrophilic moiety in the molecule might increase the cytotoxicity of the compounds. Hence, a series of compounds was synthesized possessing an additional hexose and a pentose moiety (D and L) in position 3, paralleling **GA** natural occurrence occurs as glycyrrhizinic acid - a glycoside consisting of one molecule of **GA** and two molecules of glucuronic acid.



Scheme 1. Synthesis of the glycosides; reagents and conditions: (a) I: trichloro acetimidate, TMSOTf, molecular sieve 4 Å, DCM, -70 °C - rt, 2 h; II: NaOH, methanol/water/thf, rt, 30 min; 4 - 59 %.

2.1. Cytotoxicity assay

	518A2	8505C	A253	DLD-1	Lipo	MCF-7	SW1736	NiH3T3
GA-Me*	27.54	26.07	19.42	26.12	20.47	22.14	34.87	22.81
1	>30	>30	>30	>30	>30	>30	>30	>30
2	>30	>30	>30	>30	>30	16.70	>30	>30
3	>30	>30	>30	>30	>30	19.60	>30	>30
4	28.92	>30	27.25	>30	28.45	>30	23.87	>30
5	25.95	21.97	13.16	>30	>30	9.48	11.18	>30
6	23.26	22.77	19.70	23.18	23.03	20.11	21.38	23.45
7	>30	>30	>30	>30	>30	>30	>30	>30

Table 1. biological activity of GA glycosides with respect to their IC_{50} values on 7 tumour cell lines and mouse embryonic fibroblasts (NiH3T3) in µM (error: ± 10 %). *) data from previous studies[1]

2.2. Apopotosis test - AO/EB assay

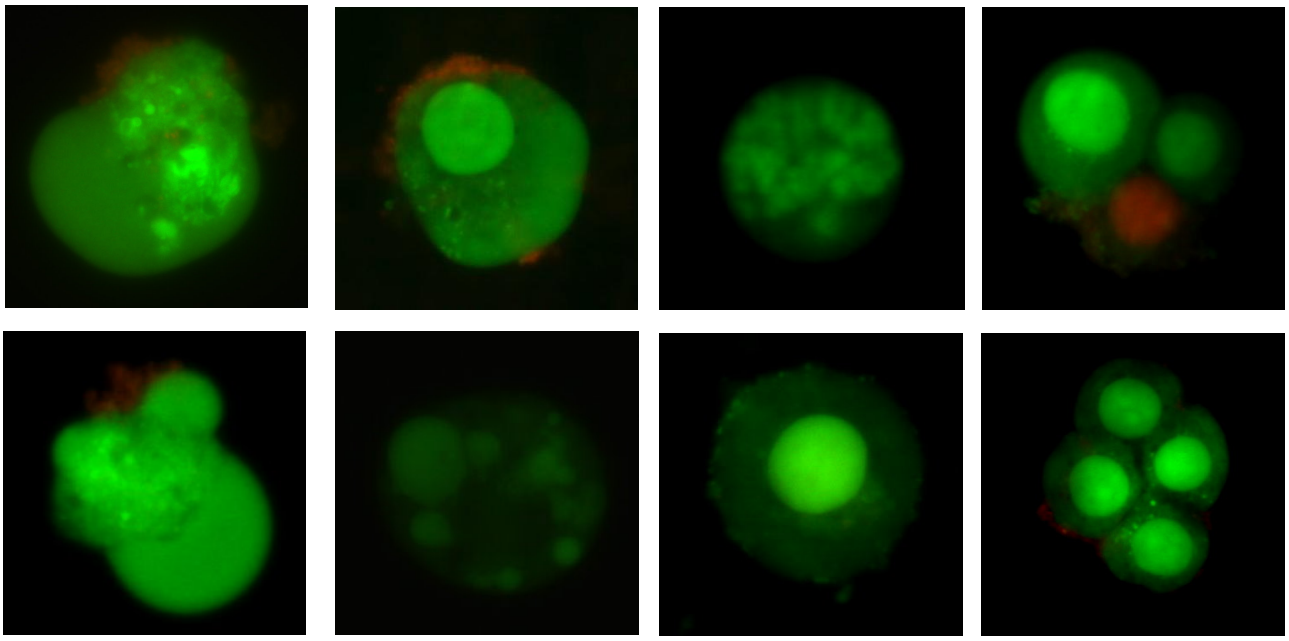


Figure 1. Results from the AO/EB test; treatment of MCF-7 cells with (from left up to right down): **GA-Me** (30 µM), **1** (40 µM), **2** (20 µM), **3** (30 µM), **4** (40 µM), **5** (12 µM), **6** (30 µM), **7** (40 µM).

4. Experimental

4.1. General

Used reagents were bought from commercial suppliers without any further purification. NMR spectra were measured on VARIAN Gemini 200, Gemini 2000 or Unity 500 spectrometers at 27 °C with trimethylsilane as an internal standard, δ are given in ppm and *J* in Hz. IR spectra are recorded on a Perkin-Elmer FT-IR spectrometer Spectrum 1000, optical rotations on a Perkin-Elmer 341 polarimeter (1 cm micro cell) and UV-vis spectra on a Perkin-Elmer unit, Lambda 14. Melting points were measured with a LEICA hot stage microscope and are uncorrected. Elemental analysis was done on a Foss-Heraeus Vario EL unit. TLC was performed on silical gel (Merck 5554, detection by UV absorption). HRMS spectra were acquired in an Apex Ultra FTICR Mass Spectrometer equipped with an Apollo II Dual ESI/MALDI ion source, from Bruker Daltonics, and a 7T actively shielded magnet from Magnex Scientific. Solvents were dried before use according to usual procedures.

The trichloro acetimidates[2] and GA-ME[1] were synthesized by well known procedures in multistep syntheses[2].

4.2. Cell lines and culture conditions

The cell lines 518A2, 8505C, A253, Lipo, MCF-7, NiH3T3, SW1736 were included in this study. Cultures were maintained as monolayer in RPMI 1640 (PAA Laboratories, Pasching, Germany) supplemented with 10% heat inactivated fetal bovine serum (Biochrom AG, Berlin, Germany) and penicillin / streptomycin (PAA Laboratories) at 37 °C in a humidified atmosphere of 5% CO_2/ 95% air.

4.3. Cytotoxicity Assay[3]

The cytotoxicity of the compounds was evaluated using the sulforhodamine-B (SRB) (Sigma Aldrich) microculture colorimetric assay. In short, exponentially growing cells were seeded into 96-well plates on day 0 at the appropriate cell densities to prevent confluence of the cells during the period of experiment. After 24h, the cells were treated with serial dilutions of the compounds (0-100 μM) for 96 h. The final concentration of DMSO or DMF solvent never exceeded 0.5%, which was non-toxic to the cells. The percentages of surviving cells relative to untreated controls were determined 96 h after the beginning of drug exposure. After a 96 h treatment, the supernatant medium from the 96 well plates was thrown away and the cells were fixed with 10% TCA. For a thorough fixation, the plates were allowed to rest at 4 °C. After fixation, the cells were washed in a

strip washer. The washing was done four times with water using alternate dispensing and aspiration procedures. The plates were then dyed with 100 µl of 0.4% SRB (sulforhodamine B) for about 20 min. After dying the plates were washed with 1% acetic acid to remove the excess of the dye and allowed to air dry overnight. 100 µl of 10 mM Tris base solution were added to each well and absorbance was measured at 570 nm (using a 96 well plate reader, Tecan Spectra, Crailsheim, Germany). The IC_{50} was estimated by linear regression between the value before and after the 50% line is crossed in a dose-response curve.

4.4. Apoptosis test – Acridine Orange/Ethidium Bromide (AO/EB)[4]

Apoptotic cell death was analysed by acridine orange/ethidium bromide dye using fluorescence microscopy on MCF-7 cells. Therefore approx. 500,000 cells were seeded in cell culture flasks and were allowed to grow for 24 hours. The medium was removed and the substance loaded medium was added. After 24-48 hours, the supernatant medium was collected and centrifuged; the pellet was suspended in phosphate-buffer saline (PBS) and centrifuged again. The liquid was removed, the pellet was suspended in PBS. After mixing the suspension with a solution of AO/EB, analysis was performed under a fluorescence microscope. While a green fluorescence shows apoptosis, a red coloured nucleus indicates necrotic cells.

4.5. General procedure for glycosylation

A mixture of trichloro acetimidate, methyl glycyrrhetinate and activated molecular sieve (4 Å) in 25 ml of dry dichloromethane was stirred for 30 30 minutes of stirring at rt, cooled (cf Table 2), and TMSOTf was added. For work-up, after 2 h the reaction mixture was quenched with triethylamine (1 eq. referred to TMSOTf), and the solvent was removed under reduced pressure. The residue was dissolved in an aq. solution of sodium hydroxide (0.25 molar in methanol/THF/water 1:2:1; 25 ml) and stirred at room temperature for another 20 minutes. The mixture was poured into dichloromethane, and the organic layer was washed with water and brine, dried ($MgSO_4$), and the solvent was removed. Purification by chromatography (silica gel, dichloromethane/methanol 9:1) afforded the glycoside.

conditions	**donor**	**GA-ME**	**TMSOTf**	**temperature**	**max. yield (rel. to sugar)**
1	1 eq.	1/3 eq.	100 µl	rt	4 %
2	1 eq.	1/3 eq.	1 eq.	-70° C	5 %
3	1 eq.	2/3 eq.	100 µl	rt	10 %

4	1 eq.	1 eq.	1 eq.	-50° C	21 %
5	1 eq.	2 eq.	1 eq.	-70 °C	59 %

Table 2. Conditions for the glycosylation.

*4.6. Methyl (3β) 3-(α-D-arabinopyranosyloxy)-11-oxo-olean-12-en-30-oate (**1**)*

Colourless powder; mp 281-283 °C; R_f = 0.22 (chloroform/methanol 19:1); [**α**] = + 127.53 (*c* 0.55, $CHCl_3$); UV-vis (methanol): λ_{max} (log ε) = 248 nm (4.12); IR (KBr): ν = 3440 (br), 2949 (s), 2874 (s), 1730 (s), 1665 (s), 1464 (m), 1388 (m), 1363 (m), 1322 (m), 1280 (w), 1249 (m), 1219 (s), 1154 (s), 1086 (s), 1002 (m), 919 (w), 868 (w), 824 (w), 785 (w), 655 (w) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.65 (s, 1H, H-12), 4.35 (d, 1H, Ara-1, *J* = 5.6), 3.94 (m, 1H, Ara-5-*H*H'), 3.92 (m, 1H, Ara-4), 3.69 (m, 1H, Ara-2), 3.68 (s, 3H, OMe), 3.67 (m, 1H, Ara-3), 3.53 (m, 1H, Ara-5-H*H'*), 3.30 (dd, 1H, H-3, *J* = 11.6, 4.5), 2.82 (ddd, 1H, H-1, *J* = 13.6, 3.1, 3.1), 2.31 (s, 1H, H-9), 2.07 (dd, 1H, H-18, *J* = 13.4, 3.3), 2.01 (m, 1H, H-15), 1.99 (m, 1H, H-21), 1.90 (m, 1H, H-19), 1.81 (ddd, 1H, H-16, *J* = 13.7, 13.7, 4.1), 1.69 (m, 1H, H-2), 1.62 (m, 1H, H-7), 1.59 (dd, 1H, H-19', *J* = 13.7, 13.7), 1.58 (m, 1H, H-6), 1.57 (m, 1H, H-2'), 1.41 (m, 1H, H-6'), 1.39 (m, 1H, H-22), 1.38 (m, 1H, H-7'), 1.33 (s, 3H, H-27), 1.30 (m, 2H, H-22' & H-21'), 1.17 (m, 1H, H-16'), 1.13 (s, 3H, H-29), 1.13 (s, 3H, H-25), 1.11 (s, 3H, H-26), 1.03 (s, 3H, H-23), 0.97 (m, 1H, H-15'), 0.91 (m, 1H, H-1'), 0.80 (s, 3H, H-24), 0.79 (s, 3H, H-28), 0.71 (m, 1H, H-5); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.3 (C-11), 176.9 (C-30), 169.4 (C-13), 128.4 (C-12), 99.4 (Ara-1), 84.0 (C-3), 72.6 (Ara-3), 71.5 (Ara-2), 67.5 (Ara-4), 64.8 (Ara-5), 61.7 (C-9), 55.4 (C-5), 51.8 (OMe), 48.3 (C-18), 45.4 (C-8), 44.0 (C-20), 43.1 (C-14), 41.0 (C-19), 38.7 (C-4), 38.4 (C-1), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-23), 28.3 (C-28), 28.3 (C-29), 26.4 (C-16), 26.3 (C-15), 23.3 (C-27), 22.6 (C2), 18.7 (C-26), 17.4 (C-6), 16.5 (C-24), 16.3 (C-25). HRMS *m/z* calcd for $C_{36}H_{56}O_8Na$ 639.3873 $[M + Na]^+$, found 639.3843.

*4.7. Methyl (3β) 3-(α-L-arabinopyranosyloxy)-11-oxo-olean-12-en-30-oate (**2**)*

Colourless powder; mp 213-216 °C; R_f = 0.21 (chloroform/methanol 19:1); [**α**] = + 102.62 (*c* 0.49, $CHCl_3$); UV-vis (methanol): λ_{max} (log ε) = 248 nm (4.03); IR (KBr): ν = 3427 (br), 2938 (s), 2870 (s), 1731 (s), 1658 (s), 1467 (m), 1387 (s), 1368 (m), 1319 (m), 1279 (m), 1247 (m), 1218 (s), 1190 (m), 1152 (s), 1086 (s), 1065 (s), 985 (s), 921 (m), 868 (w), 822 (w), 782 (w), 657 (w), 590 (w) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.65 (s, 1H, H-12), 4.32 (d, 1H, Ara-1, *J* = 6.3), 3.92 (m, 1H, Ara-4), 3.90 (m, 1H, Ara-5-*H*H'), 3.75 (dd, 1H, Ara-2, *J* = 7.8, 6.6), 3.68 (s, 3H, OMe), 3.66 (m, 1H, Ara-3), 3.51 (m, 1H, Ara-5-H*H'*), 3.15 (dd, 1H, H-3, *J* = 10.8, 5.5), 2.77 (ddd, 1H, H-1, *J* =

13.6, 3.3, 3.3), 2.31 (s, 1H, H-9), 2.06 (dd, 1H, H-18, *J* = 13.7, 3.4), 2.01 (m, 1H, H-15), 1.98 (m, 1H, H-21), 1.91 (m, 1H, H-19), 1.83 (m, 1H, H-2), 1.80 (m, 1H, H-16), 1.76 (m, 1H, H-2'), 1.63 (m, 1H, H-7), 1.58 (m, 1H, H-6), 1.59 (dd, 1H, H-19', *J* = 13.6, 13.6), 1.42 (m, 1H, H-6'), 1.39 (m, 1H, H-22), 1.38 (m, 1H, H-7'), 1.34 (s, 3H, H-27), 1.29 (m, 2H, H-22' & H-21'), 1.16 (m, 1H, H-16'), 1.13 (s, 3H, H-29), 1.12 (s, 3H, H-25), 1.10 (s, 3H, H-26), 1.00 (s, 3H, H-23), 0.99 (m, 1H, H-15'), 0.95 (m, 1H, H-1'), 0.83 (s, 3H, H-24), 0.79 (s, 3H, H-28), 0.70 (m, 1H, H-5); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.2 (C-11), 176.9 (C-30), 169.2 (C-13), 128.5 (C-12), 104.8 (Ara-1), 89.5 (C-3), 72.7 (Ara-3), 71.6 (Ara-2), 67.5 (Ara-4), 64.6 (Ara-5), 61.7 (C-9), 55.2 (C-5), 51.8 (OMe), 48.3 (C-18), 45.4 (C-8), 44.0 (C-20), 43.1 (C-14), 41.0 (C-19), 39.4 (C-4), 39.1 (C-1), 37.7 (C-22), 36.7 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-28), 28.3 (C-29), 28.1 (C-23), 26.4 (C-16), 26.4 (C-15), 25.8 (C2), 23.3 (C-27), 18.6 (C-26), 17.3 (C-6), 16.6 (C-24), 16.4 (C-25). HRMS *m/z* calcd for $C_{36}H_{56}O_8Na$ 639.3873 $[M + Na]^+$, found 639.3858.

*4.8. Methyl (3β) 3-(α-L-rhamnopyranosyloxy)-11-oxo-olean-12-en-30-oate (**3**)*

Colourless powder; mp 203-207 °C; R_f = 0.18 (chloroform/methanol 19:1); [**α**] = + 66.03 (*c* 0.40, $CHCl_3$); UV-vis (methanol): λ_{max} (log ε) = 249 nm (4.02); IR (KBr): ν = 3417 (br), 2949 (s), 1729 (s), 1657 (s), 1466 (s), 1387 (s), 1324 (m), 1218 (s), 1190 (m), 1154 (s), 1131 (s), 1052 (s), 980 (s), 916 (m), 880 (w), 835 (m), 811 (m), 769 (w), 731 (w), 662 (w), 590 (w), 546 (w), 474 (w) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.66 (s, 1H, H-12), 4.81 (m, 1H, Rha-1), 3.94 (m, 1H, Rha-3), 3.80 (m, 1H, Rha-5), 3.76 (dd, 1H, Rha-2, *J* = 9.3, 2.2), 3.68 (s, 3H, OMe), 3.45 (m, 1H, Rha-4), 3.09 (m, 1H, H-3), 2.76 (m, 1H, H-1), 2.32 (s, 1H, H-9), 2.06 (m, 1H, H-18), 2.01 (m, 1H, H-15), 1.99 (m, 1H, H-21), 1.91 (m, 1H, H-19), 1.81 (m, 1H, H-16), 1.78 (m, 1H, H-2), 1.73 (m, 1H, H-2'), 1.63 (m, 1H, H-7), 1.57 (m, 1H, H-6), 1.60 (dd, 1H, H-19', *J* = 13.4, 13.4), 1.41 (m, 1H, H-6'), 1.40 (m, 1H, H-22), 1.39 (m, 1H, H-7'), 1.35 (s, 3H, H-27), 1.30 (m, 2H, H-22' & H-21'), 1.27 (d, 3H, Rha-6, *J* = 6.2), 1.17 (m, 1H, H-16'), 1.14 (s, 3H, H-29), 1.12 (s, 3H, H-25), 1.11 (s, 3H, H-26), 1.01 (m, 1H, H-15'), 0.93 (m, 1H, H-1'), 0.92 (s, 3H, H-23), 0.79 (s, 3H, H-28), 0.78 (s, 3H, H-24), 0.69 (m, 1H, H-5); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.3 (C-11), 177.0 (C-30), 169.4 (C-13), 128.4 (C-12), 102.2 (Rha-1), 89.1 (C-3), 73.3 (Rha-4), 71.9 (Rha-2), 71.3 (Rha-3), 67.9 (Rha-5), 61.8 (C-9), 55.0 (C-5), 51.8 (OMe), 48.4 (C-18), 45.4 (C-8), 44.0 (C-20), 43.2 (C-14), 41.0 (C-19), 39.3 (C-4), 39.0 (C-1), 37.7 (C-22), 36.7 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-28), 28.3 (C-29), 28.2 (C-23), 26.4 (C-16), 26.4 (C-15), 25.3 (C2), 23.4 (C-27), 18.6 (C-26), 17.4 (C-6), 17.3 (Rha-6), 16.4 (C-24), 16.4 (C-25). HRMS *m/z* calcd for $C_{37}H_{58}O_8Na$ 653.4029 $[M + Na]^+$, found 653.4021.

*4.9. Methyl (3β) 3-(β-L-rhamnopyranosyloxy)-11-oxo-olean-12-en-30-oate (**4**)*

Colourless powder; mp 229-232 °C; R_f = 0.32 (chloroform/methanol 19:1); [**α**] = + 96.00 (*c* 0.49, $CHCl_3$); UV-vis (methanol): λ_{max} (log ε) = 248 nm (4.00); IR (KBr): ν = 3406 (br), 2947 (s), 2872 (m), 1724 (s), 1657 (s), 1620 (w), 1467 (m), 1388 (s), 1325 (m), 1247 (s), 1219 (s), 1190 (m), 1155 (s), 1049 (s), 994 (m), 878 (w), 832 (w), 798 (w), 770 (w), 668 (w), 590 (w), 543 (w) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.66 (s, 1H, H-12), 5.43 (d, 1H, Rha-1, *J* = 2.3), 4.40 (dd, 1H, Rha-2, *J* = 3.8, 2.4), 3.68 (s, 3H, OMe), 3.62 (dd, 1H, Rha-3, *J* = 9.1, 4.2), 3.40 (m, 1H, Rha-5), 3.30 (m, 1H, Rha-4), 3.29 (m, 1H, H-3), 2.78 (m, 1H, H-1), 2.31 (s, 1H, H-9), 2.07 (m, 1H, H-18), 2.01 (m, 1H, H-15), 1.99 (m, 1H, H-21), 1.91 (m, 1H, H-19), 1.82 (m, 1H, H-16), 1.79 (m, 1H, H-2), 1.71 (m, 1H, H-2'), 1.64 (m, 1H, H-6), 1.63 (m, 1H, H-7), 1.60 (dd, 1H, H-19', *J* = 13.5, 13.5), 1.42 (m, 1H, H-22), 1.38 (m, 1H, H-7'), 1.35 (s, 3H, H-27), 1.36 (m, 1H, H-6'), 1.32 (d, 3H, Rha-6, *J* = 6.0), 1.30 (m, 2H, H-22' & H-21'), 1.17 (m, 1H, H-16'), 1.14 (s, 3H, H-29), 1.13 (s, 3H, H-25), 1.11 (s, 3H, H-26), 1.01 (m, 1H, H-15'), 0.92 (s, 3H, H-23), 0.89 (m, 1H, H-1'), 0.80 (s, 3H, H-28), 0.78 (s, 3H, H-24), 0.67 (m, 1H, H-5); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.3 (C-11), 177.0 (C-30), 169.3 (C-13), 128.4 (C-12), 97.2 (Rha-1), 79.8 (C-3), 78.0 (Rha-2), 73.1 (Rha-5), 73.0 (Ara-3), 70.3 (Rha-4), 61.9 (C-9), 55.7 (C-5), 51.8 (OMe), 48.3 (C-18), 45.4 (C-8), 44.0 (C-20), 43.2 (C-14), 41.1 (C-19), 39.6 (C-4), 39.1 (C-1), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-28), 28.3 (C-29), 28.3 (C-23), 26.4 (C-16), 26.4 (C-15), 26.3 (C2), 23.4 (C-27), 18.7 (C-26), 17.6 (C-6), 17.5 (Rha-6), 16.4 (C-24), 16.4 (C-25). HRMS *m/z* calcd for $C_{37}H_{58}O_8Na$ 653.4029 $[M + Na]^+$, found 653.4025.

*4.10. Methyl (3β) 3-(α-D-mannopyranosyloxy)-11-oxo-olean-12-en-30-oate (**5**)*

Colourless powder; mp 197-199 °C; R_f = 0.16 (chloroform/methanol 19:1); [**α**] = + 61.67 (*c* 0.41, $CHCl_3$); UV-vis (methanol): λ_{max} (log ε) = 249 nm (4.30); IR (KBr): ν = 3449 (br), 2952 (s), 1717 (s), 1658 (m), 1628 (m), 1597 (m), 1454 (s), 1380 (s), 1249 (s), 1200 (s), 1155 (s), 1110 (s), 1072 (s), 1021 (s), 1005 (m), 937 (m), 914 (s), 893 (s), 840 (m), 749 (w), 723 (w), 682 (w), 545 (w), 476 (w) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.66 (s, 1H, H-12), 5.40 (m, 1H, Man-1), 4.02 (m, 1H, Man-4), 4.01 (m, 1H, Man-2), 3.94 (m, 1H, Man-6-C*H*H'), 3.85 (m, 1H, Man-5), 3.68 (s, 3H, OMe), 3.67 (m, 1H, Man-6-CH*H'*), 3.49 (m, 1H, Man-3), 3.17 (dd, 1H, H-3, *J* = 11.5, 4.5), 2.47 (m, 1H, H-1), 2.34 (s, 1H, H-9), 2.07 (m, 1H, H-18), 1.99 (m, 1H, H-15), 1.94 (m, 1H, H-21), 1.91 (m, 1H, H-19), 1.80 (m, 1H, H-16), 1.78 (m, 1H, H-2), 1.67 (m, 1H, H-7), 1.64 (m, 1H, H-2'), 1.62 (m, 1H, H-6), 1.59 (dd, 1H, H-19', *J* = 13.4, 13.4), 1.41 (m, 1H, H-6'), 1.40 (m, 1H, H-22), 1.39 (m, 1H, H-7'), 1.36 (s, 3H, H-27), 1.31 (m, 2H, H-22' & H-21'), 1.18 (m, 1H, H-16'), 1.15 (s, 3H, H-29),

1.13 (s, 3H, H-25), 1.11 (s, 3H, H-26), 1.02 (s, 3H, H-23), 0.96 (m, 1H, H-15'), 1.00 (m, 1H, H-1'), 0.85 (s, 3H, H-24), 0.78 (s, 3H, H-28), 0.74 (m, 1H, H-5); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.2 (C-11), 177.0 (C-30), 169.3 (C-13), 128.5 (C-12), 98.2 (Man-1), 78.8 (C-3), 72.2 (Man-3), 72.0 (Man-5), 70.9 (Man-2), 66.1 (Man-4), 61.8 (C-9), 61.1 (Man-6), 55.2 (C-5), 51.8 (OMe), 48.4 (C-18), 45.4 (C-8), 44.1 (C-20), 43.1 (C-14), 41.1 (C-19), 40.6 (C-1), 39.8 (C-4), 37.7 (C-22), 35.9 (C-10), 32.8 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-28), 28.3 (C-29), 28.2 (C-23), 26.4 (C-16), 26.4 (C-15), 27.3 (C2), 23.4 (C-27), 18.7 (C-26), 17.4 (C-6), 16.6 (C-24), 16.4 (C-25). HRMS *m/z* calcd for $C_{37}H_{58}O_9Na$ 669.3979 $[M + Na]^+$, found 669.3973.

*4.11. Methyl (3β) 3-(β-D-galactopyranosyloxy)-11-oxo-olean-12-en-30-oate (**6**)*

Colourless powder; mp 190-193 °C; R_f = 0.07 (chloroform/methanol 19:1); [**α**] = + 45.61 (*c* 0.54, $CHCl_3$); UV-vis (methanol): λ_{max} (log ε) = 249 nm (4.03); IR (KBr): ν = 3426 (br), 2950 (s), 1732 (s), 1661 (s), 1466 (s), 1387 (s), 1324 (m), 1280 (m), 1217 (s), 1153 (s), 1086 (s), 983 (s), 920 (w), 879 (w), 823 (w), 770 (w), 674 (w), 547 (w) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.65 (s, 1H, H-12), 4.31 (d, 1H, Gal-1, *J* = 7.0), 4.05 (m, 1H, Gal-4), 3.82 (m, 2H, Gal-6), 3.70 (m, 1H, Gal-2), 3.68 (s, 3H, OMe), 3.61 (m, 1H, Gal-3), 3.52 (m, 1H, Gal-5), 3.19 (dd, 1H, H-3, *J* = 7.6, 7.6), 2.77 (m, 1H, H-1), 2.32 (s, 1H, H-9), 2.06 (m, 1H, H-18), 2.00 (m, 1H, H-15), 1.94 (m, 1H, H-21), 1.91 (m, 1H, H-19), 1.80 (m, 1H, H-16), 1.79 (m, 1H, H-2), 1.66 (m, 1H, H-7), 1.64 (m, 1H, H-2'), 1.62 (m, 1H, H-6), 1.60 (dd, 1H, H-19', *J* = 13.6, 13.6), 1.41 (m, 1H, H-6'), 1.40 (m, 1H, H-22), 1.39 (m, 1H, H-7'), 1.34 (s, 3H, H-27), 1.30 (m, 2H, H-22' & H-21'), 1.17 (m, 1H, H-16'), 1.14 (s, 3H, H-29), 1.13 (s, 3H, H-25), 1.11 (s, 3H, H-26), 1.03 (s, 3H, H-23), 0.97 (m, 1H, H-15'), 0.87 (m, 1H, H-1'), 0.85 (s, 3H, H-24), 0.79 (s, 3H, H-28), 0.71 (m, 1H, H-5); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.2 (C-11), 176.9 (C-30), 169.2 (C-13), 128.5 (C-12), 105.5 (Gal-1), 89.7 (C-3), 74.1 (Gal-5), 73.6 (Gal-3), 72.0 (Gal-2), 68.9 (Gal-4), 61.8 (C-9), 61.7 (Gal-6), 55.2 (C-5), 51.8 (OMe), 48.4 (C-18), 45.4 (C-8), 44.0 (C-20), 43.1 (C-14), 41.0 (C-19), 39.4 (C-4), 39.1 (C-1), 37.7 (C-22), 36.8 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-28), 28.3 (C-29), 28.1 (C-23), 26.5 (C-16), 26.4 (C-15), 26.1 (C2), 23.4 (C-27), 18.7 (C-26), 17.3 (C-6), 16.7 (C-24), 16.4 (C-25). HRMS *m/z* calcd for $C_{37}H_{58}O_9Na$ 669.3979 $[M + Na]^+$, found 669.3959.

4.12. Methyl (3β9 3-(β-D-xylopyranosyloxy)-11-oxo-olean-12-en-30-oate (7)

Colourless powder; mp 289-292 °C; R_f = 0.25 (chloroform/methanol 19:1); [**α**] = + 94.13 (*c* 0.54, $CHCl_3$); UV-vis (methanol): λ_{max} (log ε) = 248 nm (4.06); IR (KBr): ν = 3423 (br), 2950 (s), 1731 (s), 1658 (s), 1620 (m), 1466 (s), 1387 (s), 1361 (m), 1279 (m), 1247 (m), 1217 (m), 1153 (s), 1045

(s), 982 (s), 922 (s), 898 (m), 878 (w), 823 (w), 769 (w), 716 (w), 661 (w), 630 (w), 590 (w), 537 (w) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.66 (s, 1H, H-12), 4.43 (d, 1H, Xyl-1, *J* = 5.4), 3.94 (dd, 1H, Xyl-5-*H*H', *J* = 11.8, 3.9), 3.71 (m, 1H, Xyl-4), 3.68 (s, 3H, OMe), 3.57 (m, 1H, Xyl-3, *J* = 7.3, 7.3), 3.48 (m, 1H, Xyl-2), 3.32 (dd, 1H, Xyl-5-H*H'*, *J* = 11.8, 8.2), 3.16 (dd, 1H, H-3, *J* = 9.7, 6.5), 2.78 (ddd, 1H, H-1, *J* = 13.6, 3.2, 3.2), 2.32 (s, 1H, H-9), 2.07 (dd, 1H, H-18, *J* = 13.5, 3.3), 2.01 (m, 1H, H-15), 1.98 (m, 1H, H-21), 1.91 (m, 1H, H-19), 1.81 (m, 1H, H-16), 1.80 (m, 1H, H-2), 1.78 (m, 1H, H-2'), 1.64 (m, 1H, H-7), 1.60 (dd, 1H, H-19', *J* = 13.7, 13.7), 1.58 (m, 1H, H-6), 1.41 (m, 1H, H-6'), 1.40 (m, 1H, H-7'), 1.39 (m, 1H, H-22), 1.35 (s, 3H, H-27), 1.30 (m, 2H, H-22' & H-21'), 1.16 (m, 1H, H-16'), 1.14 (s, 3H, H-29), 1.13 (s, 3H, H-25), 1.11 (s, 3H, H-26), 1.00 (s, 3H, H-23), 1.01 (m, 1H, H-15'), 0.96 (m, 1H, H-1'), 0.83 (s, 3H, H-24), 0.79 (s, 3H, H-28), 0.71 (m, 1H, H-5); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.1 (C-11), 176.9 (C-30), 169.3 (C-13), 128.3 (C-12), 104.7 (Xyl-1), 89.5 (C-3), 74.1 (Xyl-3), 72.3 (Xyl-2), 69.6 (Xyl-4), 63.9 (Xyl-5), 61.5 (C-9), 55.0 (C-5), 51.6 (OMe), 48.3 (C-18), 45.4 (C-8), 44.2 (C-20), 43.1 (C-14), 40.9 (C-19), 39.4 (C-4), 38.8 (C-1), 37.5 (C-22), 36.8 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-28), 28.3 (C-29), 28.0 (C-23), 26.2 (C-16), 26.2 (C-15), 25.9 (C2), 23.3 (C-27), 18.6 (C-26), 17.2 (C-6), 16.5 (C-24), 16.4 (C-25). HRMS *m/z* calcd for $C_{36}H_{56}O_8Na$ 639.3873 $[M + Na]^+$, found 639.3885.

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verwendete Krebszelllinien

I. humane Tumorzelllinien

Name	Ursprung
518A2	Melanom
8505C	Schilddrüse
A253	Kopf- und Nackenepithel
A431	Hautepithel
A549	Lunge
A2780	Eierstöcke
DLD-1	Dickdarm
FaDu	Rachen
Liposarcoma	Weichteilgewebe
HCT-116	Dickdarm
HCT-8	Dickdarm
MCF-7	Brust
HT-29	Dickdarm
SW1736	Schilddrüse
SW480	Dickdarm

II. weitere Zelllinie

Name	Ursprung
NiH3T3	embryonale Mausfibroblasten

Lebenslauf

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Mai/10 - Jul/10	Arbeitsgruppe Prof. Dr. Amélia P. Rauter, Universidade de Lisboa, Portugal

Publikationsliste

I. als Erstautor

"Synthesis and antitumor activity of glycyrrhetinic acid derivatives"; Schwarz, S.; Csuk, R. *Bioorg. Med. Chem.* **2010**, *18(21)*, 7458-7474.

"Synthesis and biological activity of some antitumor active derivatives from glycyrrhetinic acid"; Csuk, R.; Schwarz, S.; Kluge, R.; Ströhl, D. *Eur. J. Med. Chem.* **2010**, *45(12)*, 5718-5723.

"Synthesis and antitumor activity of ring A-modified glycyrrhetinic acid derivatives"; Csuk, R.; Schwarz, S.; Siewert, B.; Kluge, R.; Ströhl, D. *Z. Naturforsch.* **2011**, *66b*, 521-532.

"Improvement of the Cytotoxicity and Tumor Selectivity of Glycyrrhetinic Acid by Derivatization with Bifunctional Amino Acids"; Csuk, R.; Schwarz, S.; Kluge, R.; Ströhl, D. *Arch. Pharm. Chem. Life Sci.* **2011**, **im Druck**.

"Does one keto group matter ? Structure-activity relationships of glycyrrhetinic acid derivatives modified at position 11"; Csuk, R.; Schwarz, S.; Kluge, R.; Ströhl, D. *Arch. Pharm. (Weinheim)*, **angenommen**.

"Conversions at C-30 of glycyrrhetinic acid and their impact on antitumor activity"; Csuk, R.; Schwarz, S.; Siewert, B.; Kluge, R.; Ströhl, D. *Arch. Pharm. (Weinheim)*, **eingereicht**.

"Synthesis and antitumor activity of ring A modified glycyrrhetinic acid derivatives"; Csuk, R.; Schwarz, S.; Siewert, B.; Kluge, R.; Ströhl, D. *Bioorg. Med. Chem.*, **eingereicht**.

II. als Co-Autor

"Antitumoractive Endoperoxides from Triterpenes"; Niesen, A.; Barthel, A.; Kluge, R.; Köwitzsch, A.; Ströhl, D.; Schwarz, S.; Csuk, R. *Arch. Pharm. (Weinheim)* **2009**, *342(10)*, 569-576.

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"Synthesis, Encapsulation and Antitumor Activity of New Betulin Derivatives"; Csuk, R.; Barthel, A.; Sczepek, R.; Siewert, B.; Schwarz, S. *Arch. Pharm. (Weinheim)* **2011**, *344(1)*, 37-39.

"Synthesis and biological evaluation of antitumor-active arglabin derivatives"; Csuk, R.; Heinold, A.; Siewert, B.; Schwarz, S.; Barthel, A.; Kluge, R.; Ströhl, D. *Arch. Pharm. (Weinheim)*, **eingereicht**.

III. Tagungsbeiträge

"Antitumor active derivatives of glycyrrhetinic acid"; Csuk, R.; Schwarz, S.; Kommera, H.; Paschke, R. *Poster , XXth International Symposium on Medicinal Chemistry*, Wien, **2008**, 31.August-04.September.

"Antitumor activity of new derivatives of glycyrrhetinic acid"; Schwarz, S.; Csuk, R.; Kommera, H.; Paschke, R. *Poster n° 11732, 2nd EuCheMS Chemistry Congress*, Turin, **2008**, 16.-20. September.

"Simple ways to improve the antitumor activity of glycyrrhetinic acid"; Schwarz, S.; Csuk, R. *Poster CAN 18, Frontiers in Medicinal Chemistry*, Münster, **2010**, 14.-17. März.

"New anticancer active derivatives from glycyrrhetinic acid"; Siewert, B.; Schwarz, S.; Csuk, R. *Poster CAN 12, Frontiers in Medicinal Chemistry*, Münster, **2010**, 14.-17. März.

"Derivatives of Glycyrrhetinic acid induce apoptosis in tumor cells"; Siewert, B.; Schwarz, S.; Csuk, R. *Poster 319, 18th International Conference on Organic Synthesis*, Bergen, **2010**, 01.-06.August.

"Antitumor activity of some glycyrrhetinic acid glycosides"; Schwarz, S.; Siewert, B.; Csuk, R.; Xavier, N., M.; Jesus, A., R.; Rauter, A., P. *Poster angenommen, 16th European Carbohydrate Symposium*, Sorrento-Neapel, **2011**, 3.-7. Juli.

"Synthesis and cytotoxic activity of ring A modified glycyrrhetinic acid derivatives"; Schwarz, S.; Siewert, B.; Csuk, R. *Vortrag IUPAC448 angenommen, 43rd IUPAC World Chemistry Congress*, San Juan, **2011**, 31. Juli-5. August.

Erklärung über Autorenanteil

1. "Synthesis and antitumor activity of glycyrrhetinic acid derivatives"
Schwarz, S.; Csuk, R. *Bioorg. Med. Chem.* **2010**, *18(21)*, 7458-7474.

Sowohl die Synthese als auch die biologische Evaluierung wurde von mir vorgenommen. R. Csuk betreute die Arbeit und stand jederzeit für fachliche Diskussionen zur Verfügung.

2. "Synthesis and biological activity of some antitumor active derivatives from glycyrrhetinic acid"
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Sowohl die Synthese als auch die biologische Evaluierung wurde von mir vorgenommen. R. Kluge nahm die ESI-Massenspektren auf und wertete diese aus. Die NMR-Aufnahmen wurden unter Leitung von D. Ströhl vorgenommen. R. Csuk betreute die Arbeit und stand jederzeit für fachliche Diskussionen zur Verfügung.

3. "Synthesis and antitumor activity of ring A-modified glycyrrhetinic acid derivatives"
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Die Derivate wurden von mir synthetisiert, genauso wie die Tests zur apoptotischen Wirkung. Die Bestimmung der IC_{50}-Werte geschah unter Assistenz von B. Siewert. R. Kluge nahm die ESI-Massenspektren auf und wertete diese aus. Die NMR-Aufnahmen wurden unter Leitung von D. Ströhl vorgenommen. R. Csuk betreute die Arbeit und stand jederzeit für fachliche Diskussionen zur Verfügung.

4. "Improvement of the Cytotoxicity and Tumor Selectivity of Glycyrrhetinic Acid by Derivatization with Bifunctional Amino Acids"
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5. "Does one keto group matter ? Structure-activity relationships of glycyrrhetinic acid derivatives modified at position 11"

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6. "Synthesis and antitumor activity of ring A modified glycyrrhetinic acid derivatives"

Csuk, R.; Schwarz, S.; Siewert, B.; Kluge, R.; Ströhl, D. *Bioorg. Med. Chem.*, **eingereicht**.

Die Synthese der Derivate und die Bestimmung der IC_{50}-Werte wurden unter Assistenz von B. Siewert. durchgeführt. Die Tests zur apoptotischen Wirkung wurden von mir selbst realisiert. Die Bestimmung der IC_{50}-Werte geschah unter Assistenz von B. Siewert. R. Kluge nahm die ESI-Massenspektren auf und wertete diese aus. Die NMR-Aufnahmen wurden unter Leitung von D. Ströhl vorgenommen. R. Csuk betreute die Arbeit und stand jederzeit für fachliche Diskussionen zur Verfügung.

7. "Conversions at C-30 of glycyrrhetinic acid and their impact on antitumor activity."

Csuk, R.; Schwarz, S.; Siewert, B.; Kluge, R.; Ströhl, D. *Arch. Pharm. (Weinheim)*, **eingereicht**.

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8. Manuskript in Bearbeitung: " A Natural Approach: Synthesis and antitumor activity of Glycyrrhetinic Acid glycosides"

Schwarz, S.; Siewert, B.; Csuk, R.; Xavier, N., M.; Jesus, A., R.; Rauter, A., P.

Manuskript in Bearbeitung.

Die Synthese der Derivate wurde selbständig durchgeführt, N. M. Xavier und A. R. Jesus halfen mir bei der Einarbeitung in die Chemie der Kohlenhydrate. Die Bestimmung der IC_{50}-Werte geschah unter Assistenz von B. Siewert. Die Tests zur apoptotischen Wirkung nahm ich selbst vor. A. P. Rauter und R. Csuk betreuten die Arbeit und standen jederzeit für fachliche Diskussionen zur Verfügung.

Selbständigkeitserklärung

Ich erkläre hiermit an Eides statt, dass ich die vorliegende Arbeit selbständig und nur unter Verwendung der angegebenen Hilfsmittel und Quellen angefertigt habe.

Die Arbeit wurde bisher an keiner anderen Universität oder Hochschule vorgelegt.

Halle (Saale), 09.06.2011 Stefan Schwarz