

Glycyrrhizin and Glycyrrhetic Acid: Scaffolds to Promising New Pharmacologically Active Compounds

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O ácido glicirrizínico (GL), também conhecido como glicirrizina, é uma saponina triterpênica, um produto natural encontrado na raiz de *Glycyrrhiza glabra* L. (“licquorice” ou “alcaçuz”), utilizada mundialmente como edulcorante e também na medicina tradicional do Oriente. Este artigo de revisão enfoca os novos compostos sintetizados usando GL ou sua aglicona, o ácido glicirretínico (GLA), como materiais de partida e as atividades farmacológicas descritas para os mesmos e seus derivados.

Glycyrrhizinic acid (GL), also known as glycyrrhizin, is a triterpene saponin, a natural product found on the root of *Glycyrrhiza glabra* L. (“licquorice”), used worldwide as sweetener and in the traditional eastern medicines. This review is focused on a series of new derivatives synthesized using GL and its aglycon, glycyrrhetic acid (GLA), as starting materials, the pharmacological activities described for those compounds, as well as new activities reported for GL and GLA themselves.

Keywords: glycyrrhizin, glycyrrhetic acid, glycyrrhetic acid, licquorice, organic synthesis, pharmacological activity

1. Introduction

Glycyrrhizinic acid or glycyrrhizin (GL) (**1**), Figure 1 is a triterpene saponin found in the root of the Leguminosae *Glycyrrhiza glabra* L., commonly known as “licquorice” or ‘licorice’ and is used worldwide as a natural sweetener and flavoring additive,¹ as well as in the traditional

Chinese, Indian and Tibetan medicines.² GL, along with its aglycon, glycyrrhetic or glycyrrhetic acid (GLA), also known as “enoxolone” (**2**), are the most studied and abundant compounds from this plant and are commercially obtained from the crude extract of the licquorice root. As a sweetener, GL is reported to be 30-50 times sweeter than sucrose.¹⁻³

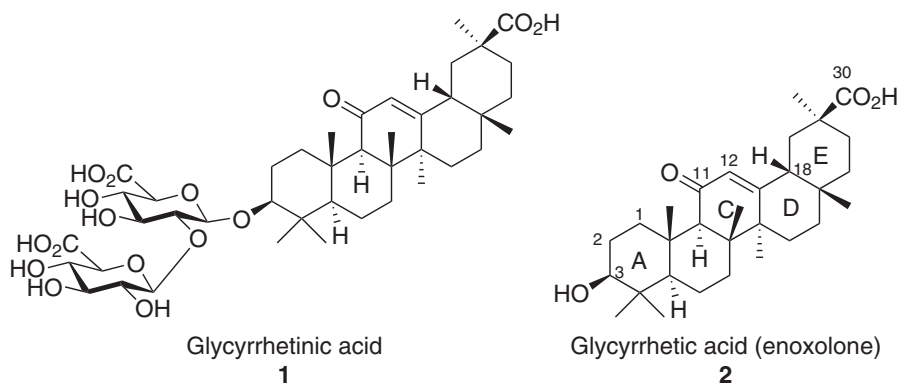


Figure 1. Chemical structures of glycyrrhizinic and glycyrrhetic acid.

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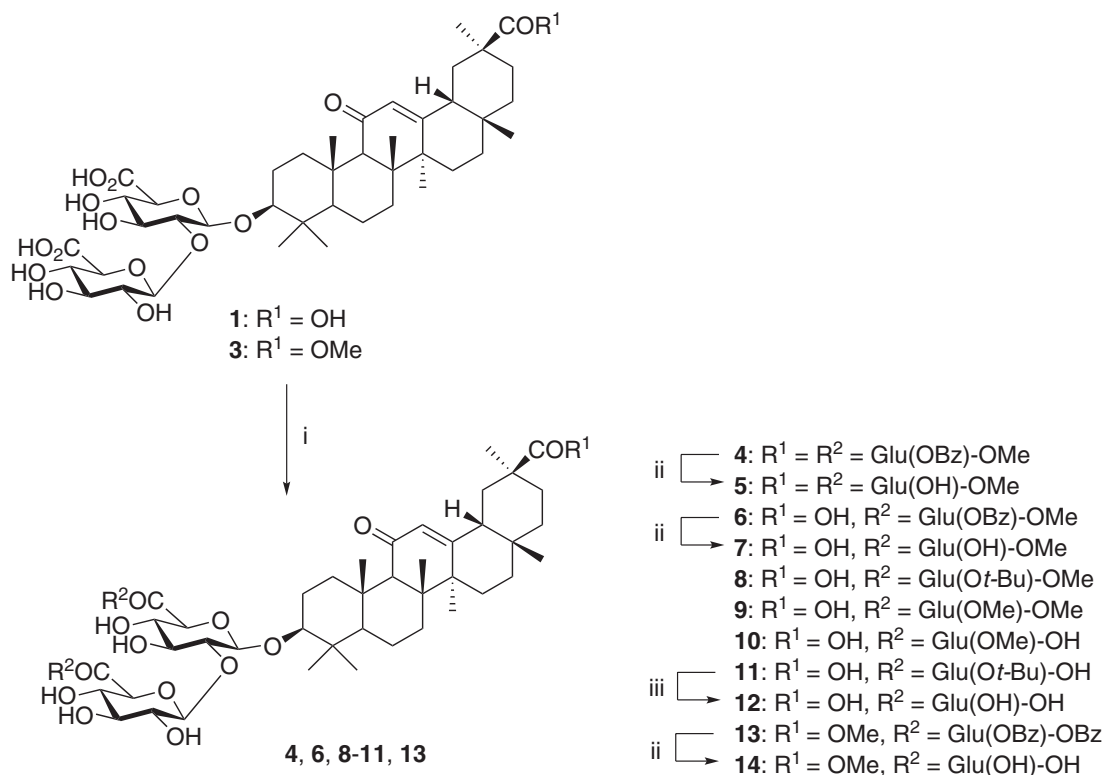
GL, its aglycon and liquorice extracts are known for its wide range of biological activities, such as anti-inflammatory, anti-allergic, anti-arthritic, antioxidant, hypolipidaemic, antiviral and antitumoral, as well as presenting hepato and immunoprotective effects in humans and animals.^{3,4} Both GL and its aglycon have been employed in the past decades as starting materials for the chemical synthesis of new derivatives, many of them with interesting biological activities. Some reviews have been published reporting new GL and GLA derivatives, as well as their activities.^{1,3} The current review is focused, therefore, in recent data on the syntheses and the synthetic strategies employed for GL and GLA derivatives, published from 2003 onwards, the pharmacological activities of these synthesized derivatives, and new pharmacological activities reported for both GL and GLA.

2. Chemical Modifications of GL

2.1. Immunomodulating derivatives

Guided by previous results indicating that GL-aminoacid or GL-peptide conjugates can exert immunomodulating activities,^{3,5} Kondratenko *et al.*⁶ have prepared some GL

derivatives containing residues of L-glutamic acid and its α -, γ - and α,γ -diesters (**4-14**, Scheme 1) using **1** or its 30-methyl ester **3** as starting materials, employing dicyclohexylcarbodiimide (DCC) as coupling promotor. The deprotected derivatives **5**, **7** and **14** were obtained from selective hydrogenolysis of the protected esters **4**, **6** and **13**, respectively, with Pd/C and **12** was obtained with removal of the tertbutyl ester group with trifluoroacetic acid TFA. Compounds **5** and **12**, when administered intraperitoneally in mice, had opposite effects in the production of antibody-forming cells (AFC), when compared with control (isotonic saline solution) animals. Compound **5** increased the number of AFCs, while **12** lowered the AFC count, indicating that **5** may exert an immunostimulating effect, while **12** may act as an immunosuppressor. Although only these two compounds were tested in this assay, a brief comparison of **5** (a compound with its three carboxylic groups esterified with glutamic acid methyl ester) and **12** (esterified only at the glycosyl carboxylic groups) points to the importance of modifications at the C-30 carboxylic group to this activity. Moreover, compound **13**, when orally administered in mice, increased serum levels of hemagglutinin when compared with control animals, indicating that **13** can exert an immunostimulating activity.



Conditions: i) DCC, HOBt or HONSu, dioxane or THF, 0-5 °C, 2-4 h; then L-glutamic acid or esters (R²), Et₃N, 22-24 °C, 24 h; ii) 10% Pd/C, AcOH 75%, r.t., 24 h; iii) TFA, 20-22 °C, 30 min.

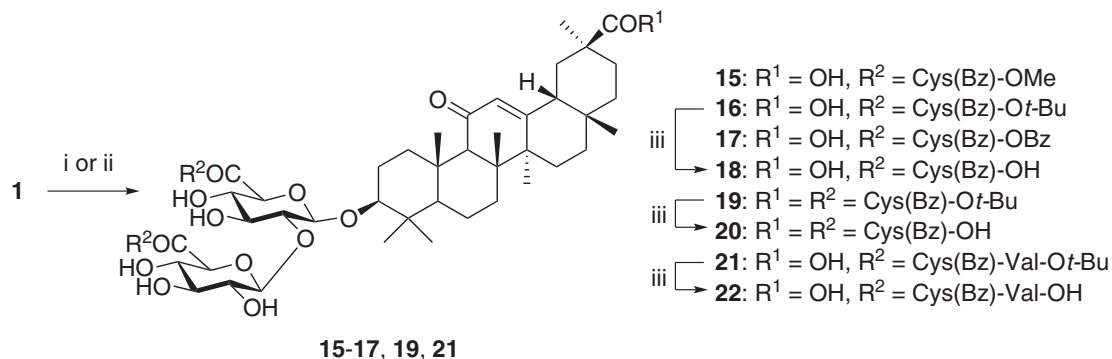
Scheme 1.

On the search of new immunomodulating GA-aminoacid conjugates, the same research group synthesized GL-cysteine derivatives **15-22** (Scheme 2) employing DCC/*N*-hydroxysuccinimide (HOSu) or Woodward's reagent K as coupling agents. Chemoselectivity for the glycosidic carboxylic acids was achieved by performing the DCC coupling at 0-5 °C. At 20-22 °C, the three carboxylic acid groups of GL were coupled. Compounds **18** and **22**, administered intraperitoneally, presented an immunostimulating activity in mice, increasing the number of antibody-producing cells when compared to control (isotonic saline solution) animals.⁷ Another work from the same group yielded GL-aminoacid ester derivatives **22-32** (Scheme 3) in a similar fashion. Compounds **23**, **26** and **29**, administered intraperitoneally, presented *in vivo* immunomodulating activity, increasing the serum level of agglutinins and hemolysins when compared to control (isotonic saline solution) animals.⁸ Although other compounds in both series were not tested, the chemical modifications performed may indicate that the esterification

of glucosyl carboxylic groups of GL with aminoacid derivatives such as Cys(Bz)OH, Cys(Bz)-Val(OH), glycine ethyl ester, alanine butyl ester or valine butyl ester, with the C-30 carboxyl group either unmodified or esterified with methyl groups lead to immunostimulating compounds. In one case, esterification of the three carboxyl groups of GL with glutamic acid methyl ester lead to an immunostimulating compound (**5**), whereas the derivative with only the glucosyl acid groups esterified (**12**) lead to an immunosuppressor compound, which indicates that the carboxyl group of this aminoacid derivative is important for the interaction of the compound with its molecular target.

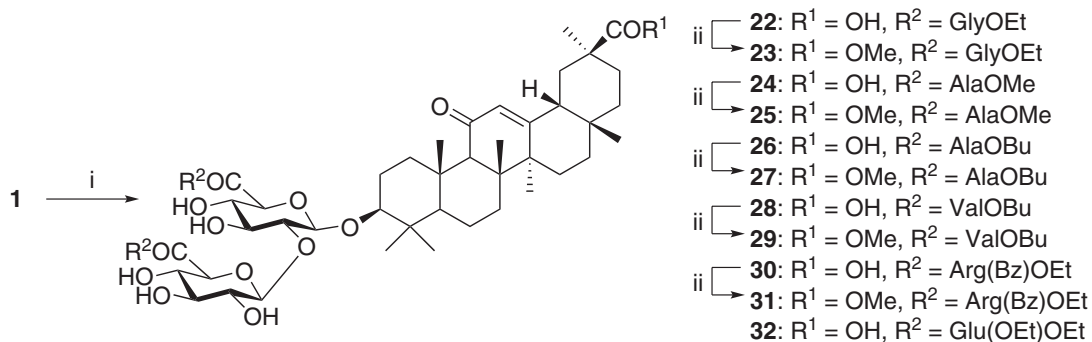
2.2. Antiviral derivatives

As previously mentioned, GL was reported as an antiviral compound, being moderately active against HIV and herpes simplex virus.^{3,4} Exploring the chemical versatility of this saponin, Baltina Jr. *et al.*⁹ employed the technique described in Schemes 1-3 to obtain GL-lysine conjugate



Conditions: i) DCC, HOSu, THF, 0-5 °C or 20-22 °C, 2 h; then cysteine ester (R²), Et₃N, 0-5 °C, 2 h, then 22-24 °C, 18 h; ii) Woodward's reagent K, Et₃N, 0-5 °C, 1.5 h, then 20-22 °C, 1.5 h, then cysteine ester (R²), Et₃N, 0-5 °C, 2 h, then 22-24 °C, 24 h; iii) TFA, 20-22 °C, 30 min.

Scheme 2.



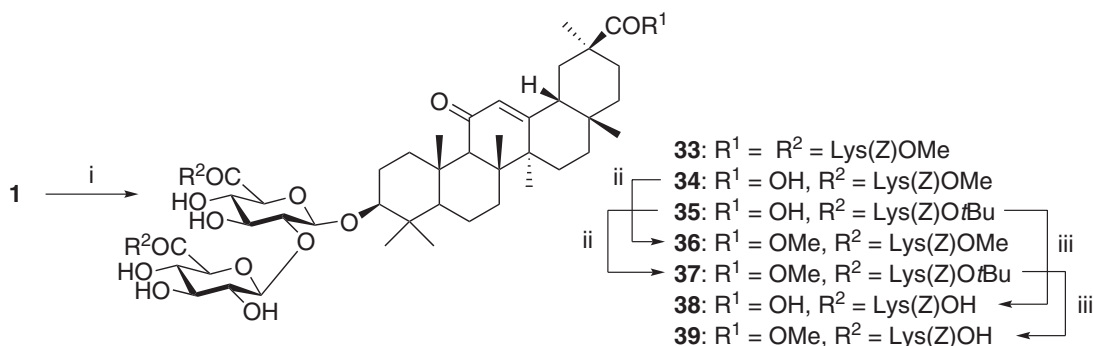
Conditions: i) DCC, HOSu, THF, 0-5 °C, 3 h; then aminoacid ester (R²), Et₃N, 0-5 °C, 1 h, then 22-24 °C, 20-22 h; ii) CH₂N₂, MeOH/dioxane, 0-5 °C.

Scheme 3.

compounds **33-39** (Scheme 4). Compound **39** was tested against HIV strains in MT-4 cell culture assay and showed moderate anti-HIV activity ($IC_{50} = 0.145 \mu\text{M}$), somewhat greater than its precursor GL ($IC_{50} = 0.304 \mu\text{M}$).

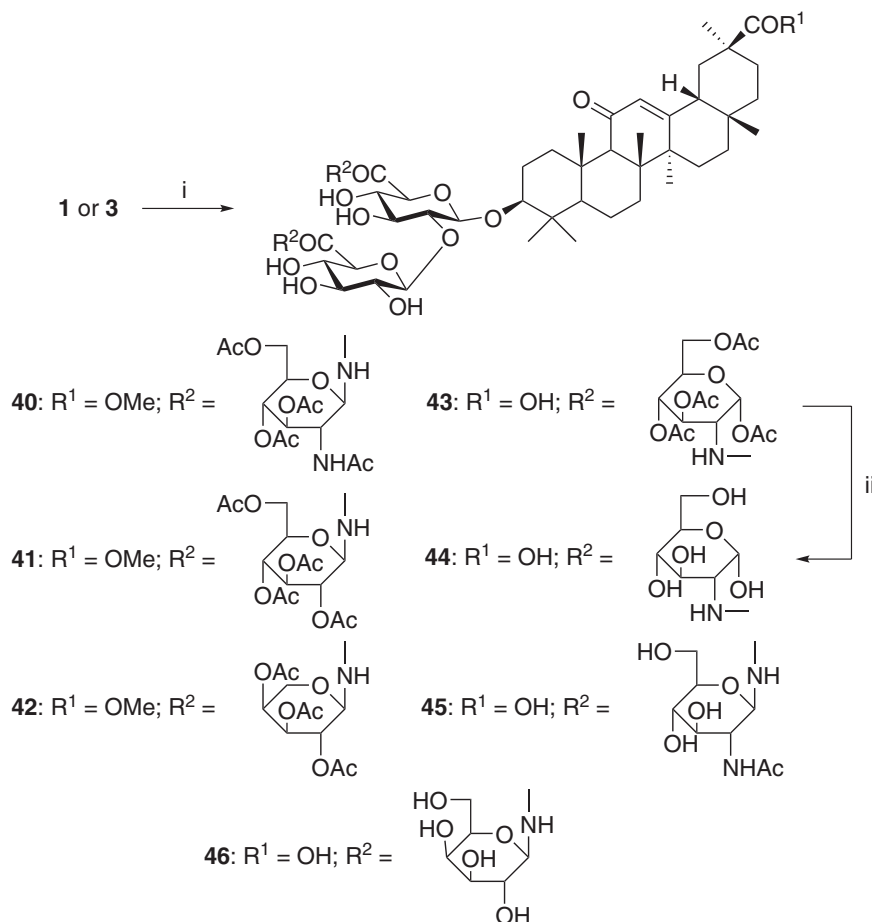
Kondratenko *et al.*¹⁰ synthesized GL-aminosugar amide conjugates **40-46** employing DCC as coupling

agent and some aminosugars selectively at the glucoside carboxyl groups (Scheme 5). Compound **45** presented *in vitro* antiviral activity against herpes simplex virus 1 (HSV-1) ($IC_{50} = 0.0032 \mu\text{M}$) and compounds **45** and **46** presented moderate *in vitro* antiviral activity against HIV-1 ($IC_{50} = 0.35$ and $0.09 \mu\text{M}$, respectively), lower than the



Conditions: i) DCC, HOBT or HOSu, dioxane or THF, 0-5 °C, 3 h; then lysine ester (R^2), Et_3N , 0-5 °C, 1 h, then 22-24 °C, 20-22 h; ii) CH_2N_2 , MeOH/dioxane, 0-5 °C; iii) TFA, 20-22 °C, 30 min.

Scheme 4.



Conditions: i) DCC, sugar, DMF/pyridine, 0-5 °C, 1 h, then 20-22 °C, 20 h;
ii) 1% KOH in MeOH, CH_2Cl_2 , 20-22 °C, 1h.

Scheme 5.

standard drug Azidothymidine (AZT) ($IC_{50} = 0.014 \mu\text{M}$), suggesting that the presence of more polar hydroxyl groups in **46**, instead of the *N*-acetyl group from **45**, is important for the anti-HIV activity of these derivatives.

Considering SARS-Coronavirus, Hoefer *et al.*¹¹ synthesized and tested the derivatives **18**, **45**, as well as GL derivatives **47–59** and GLA derivatives **60** and **61** (Figure 2). Glycoderivatives **55–58** presented the higher *in vitro* antiviral activity against this virus ($IC_{50} = 8 \pm 2$, 50 ± 10 , 5 ± 3 and $16 \pm 1 \mu\text{M}$, respectively), greater than GL itself ($365 \pm 12 \mu\text{M}$). Derivatives **47**, **48** and **49** were also active ($IC_{50} = 40 \pm 13$, 35 ± 7 and $139 \pm 20 \mu\text{M}$), while **57–61** were inactive ($IC_{50} > 1000 \mu\text{M}$), indicating that, for aminoacid-GL conjugates, the free 30-COOH group is important for this activity. Carbohydrate-free compounds, such as **2**, **60** and **61**, were also inactive ($IC_{50} > 1000 \mu\text{M}$), indicating that the glycosyl moiety is important to the antiviral activity of the derivatives, probably because Coronaviruses are highly glycosylated, especially in the spike proteins, that project from the surface of the viral envelope. So, according to the authors, the glycosyl moiety in the active derivatives is important for interactions with these and other glycosylated proteins

in the viral surface. Compounds **55–58** were also found to be cytotoxic against VERO cell cultures.

2.3. Anti-allergic derivatives

Employing *Streptococcus* LJ-22 bacteria as biocatalysts, Park *et al.*¹² synthesized the glycyrrhetic acid-3-*O*- β -D-glucuronide (GAMG, **62**). This monoglucuronide presented greater *in vitro* anti-allergic activity (measured by the inhibition of β -hexosaminidase release from RLB-2H3 cells) than GL and comparable with GLA ($IC_{50} = 0.28$, 0.37 and 0.23 nM , respectively), although the molecular basis for this action was not further explored. It seems that, although the authors considered that the three compounds presented anti-allergic activities, the carbohydrate portion of the molecule isn't essential for this activity, paving the way for new anti-allergic derivatives with simpler molecular patterns.

2.4. Anti-ulcer derivatives

Alternatively, the obtaining of glycoderivates of GL was also accessed for anti-ulcer activity. Starting from

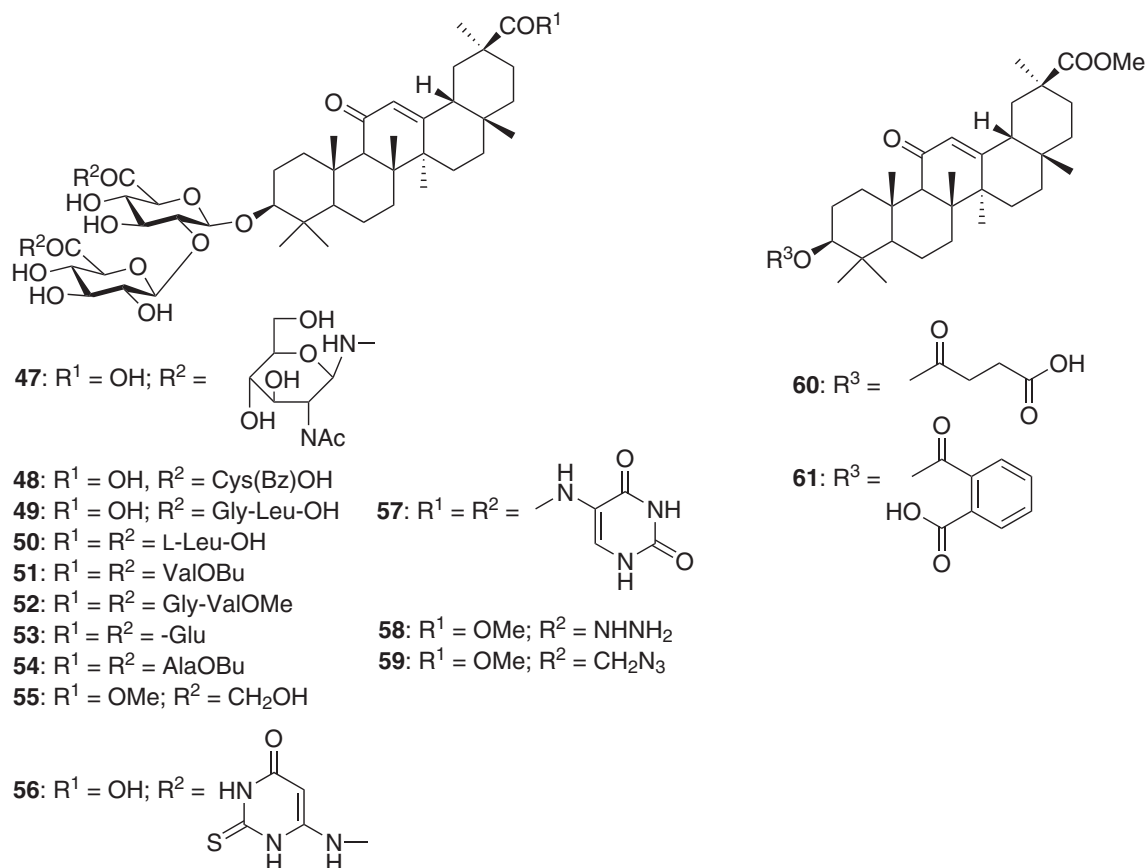


Figure 2. GL and GLA derivatives tested against SARS-Coronavirus.

penta-acetyl GL (**63**) or penta-acetyl GL 30-methyl ester (**64**), Kondratenko *et al.*¹³ synthesized new saponin compounds with monossacharide residues of glucose and galactose through ester bonds (**65-69**, Scheme 6). Compound **69**, in the model of experimental rat stomach lesions induced by acetylsalicylic acid, expressed anti-ulcer effects at the dose of 25 mg kg⁻¹ (orally administered), against 100 mg kg⁻¹ of the standard drug carbenxolone or 50 mg kg⁻¹ of GL. According to Williams *et al.*,⁴ in their evaluation of the pharmacological data associated with the use of GL as a food additive, GL is poorly absorbed orally, being metabolized in the gastrointestinal tract (by bacterial β -glucuronidases) to GLA (**2**) and in a lesser extent to GAMG (**62**), which is further metabolized to GLA. It is possible, therefore, that the greater anti-ulcer effect of those derivatives (when comparing their activities against GLA and GAMG) are a combination of the intrinsic anti-ulcer activity of the same along with a prodrug effect related to the probable metabolite GLA.

Such gastroprotective effects of GL derivatives (**1**, **2** and the per-acetylated GAMG (**62**) derivative **70**) was also observed by Krause *et al.*¹⁴ employing as a model 29 strains of the bacteria *Helicobacter pylori*, known to contribute to a wide spectrum of gastroduodenal diseases, such as gastric and duodenal ulcers. Therefore, compound **2** was found as the most potent compound in the assay ($MIC_{50/90} = 50/100$ mg mL⁻¹), followed by **70** ($MIC_{50/90} = 400/ > 400$ mg mL⁻¹) and GL ($MIC_{50/90} > 400$ mg mL⁻¹). According to the authors, this anti-*H. pylori* activity accounts for the anti-ulcer effect of **1** and **2**.

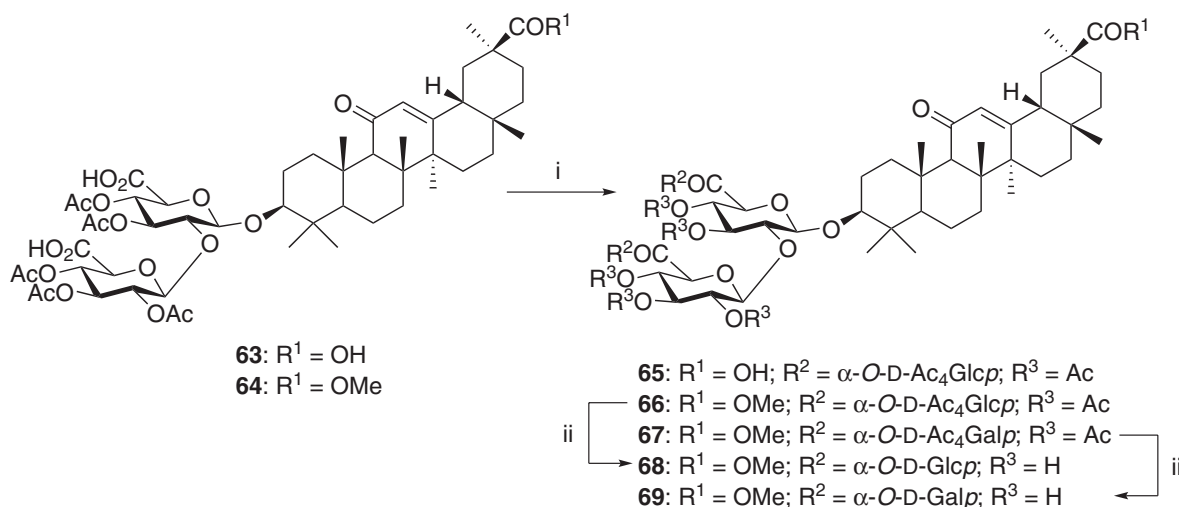
3. Chemical Modifications of GLA 2

3.1. Antiviral derivatives

Moronic and betulinic acid (**71** and **72**), Figure 3, are naturally-occurring triterpenoids, known for its antiviral properties, specially against HIV and herpes simplex virus. Some derivatives of both acids have anti-HIV activities as well.¹⁵ Guided by these active betulinic and moronic acid derivatives, Yu *et al.*¹⁵ synthesized two derivatives from GLA, **74** and **75** (Scheme 7), along with other triterpene aglycons. Those two compounds, when tested against *in vitro* HIV strains, showed no antiviral activity. According to Hoever *et al.*,¹¹ the glycosyl moiety present in GL seems to be important for its interaction with highly glycosylated viral surface proteins, and GLA derivatives without glycosyl substituents exerted no antiviral activity against coronaviruses. Based on such proposal, it seems that the absence of glycosyl substituents in the GLA derivatives **74** and **75** may be responsible for its lack of anti-HIV activity as well.

3.2. Cytotoxic and antiproliferative derivatives

Chintharlapalli *et al.*¹⁶ inspired by some reports of cytotoxic activity of GLA and some oleane-related triterpenes that exerted its cytotoxic activity by acting as peroxisome proliferator-activated γ -receptor (PPAR- γ) agonists, synthesized 1,12-dienes (**77-80**, Scheme 8) from 3-keto-18 β -GLA methyl ester (**76**) and 3-keto-18 α -GLA methyl ester (**76'**). Only cyanoderivatives **79** and **80**



Conditions: i) Molecular sieve (4A), Ag₂CO₃, 2,3,4,6-tetra-O-acetyl- α -D-glucopyranosyl or galactopyranosyl bromide, CH₂Cl₂, reflux, 6 h; ii) 1% KOH in MeOH, CH₂Cl₂, r. t.

Scheme 6.

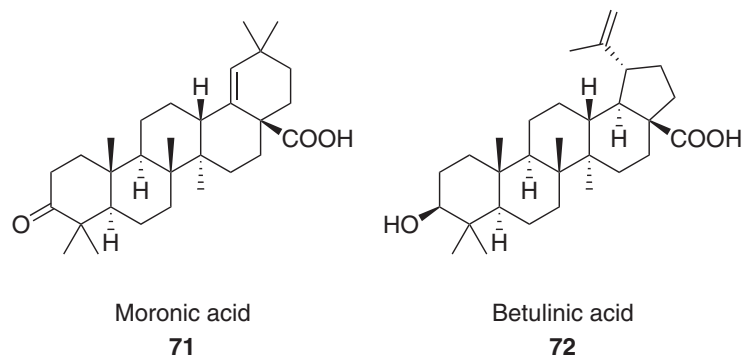
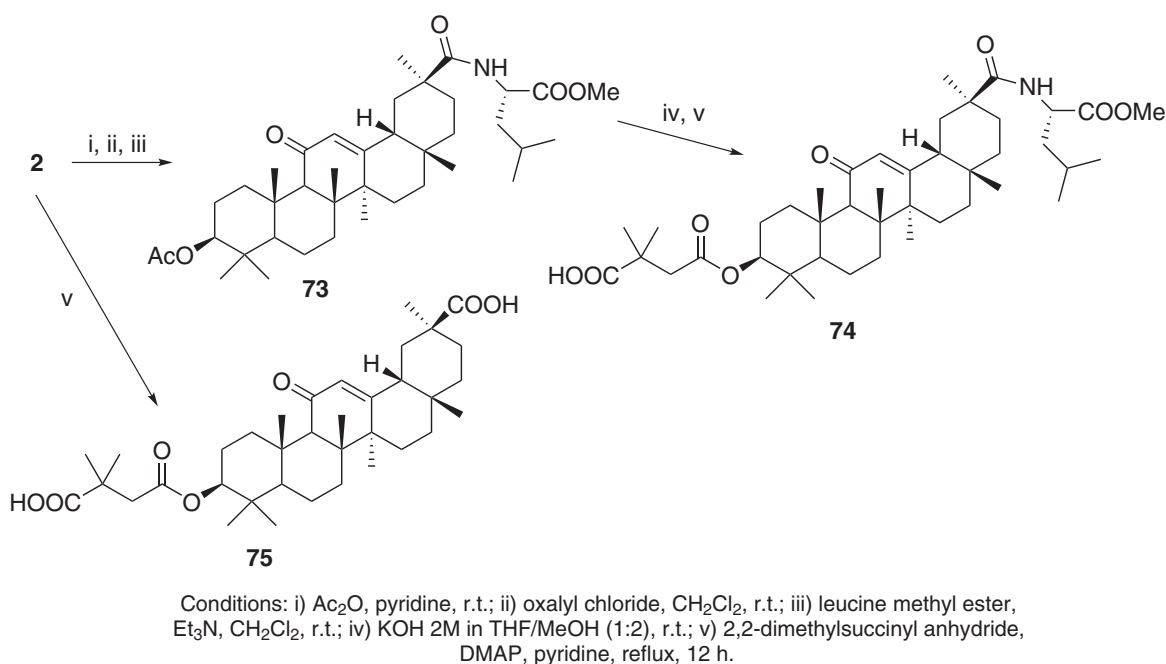


Figure 3. Chemical structures of moronic (**71**) and betulinic (**72**) acids.



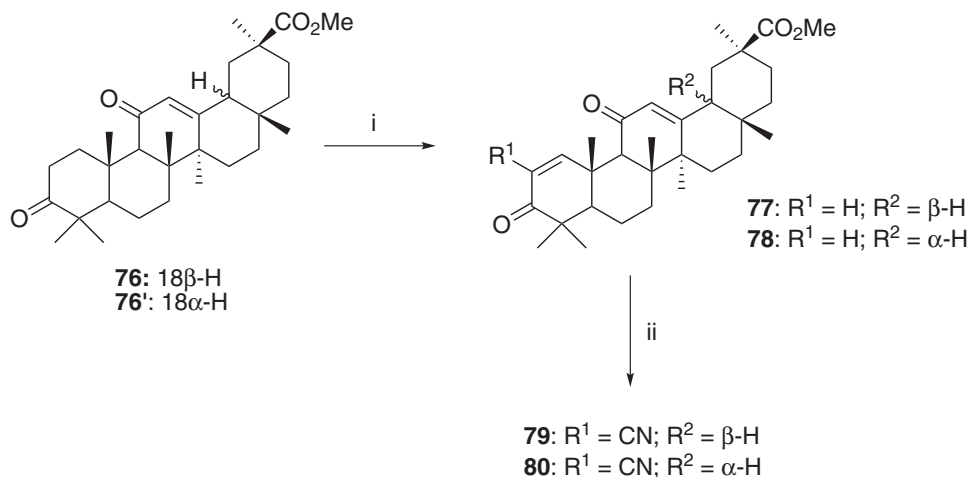
Scheme 7.

presented *in vitro* activity as PPAR- γ agonists, leading the researchers to conclude that both the 30-methyl ester and the cyano group are essential for this activity, and that the stereochemistry at the C-18 position, although responsible for conformational differences between the **79** and **80**, is not important for the activity.

In order to improve the cytotoxic activity reported for GLA through increasing of its lipophilic profiles, a series of oximes (**82-91**) were synthesized from 3-keto GLA (**81**) by Liu *et al.*¹⁷ (Scheme 9). Some of the compounds exhibited *in vitro* cytotoxic and apoptosis induction activity against HL-60 cells. The data presented by the authors indicates that the replacement of the hydroxyl group of **2** with carbonyl or oxime groups does not influence the antiproliferative activity, but compounds with alkoximino groups at C-3 and

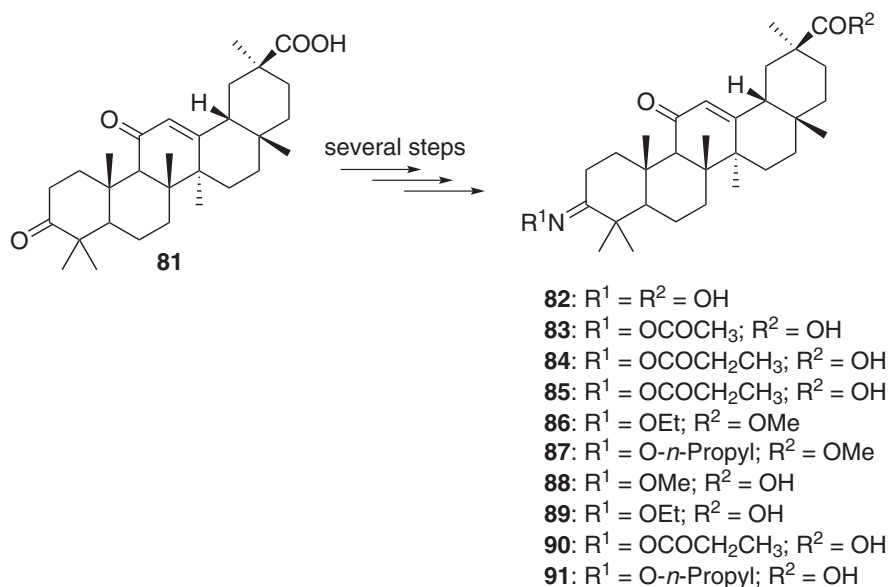
free C-30 acid groups presented greater antiproliferative activity and apoptosis induction. Moreover, C-30 esterified compounds with alkylated C-3 oxime groups lack apoptosis induction activity, while retain the antiproliferative activity, probably because the cytotoxic and the apoptosis induction effects are being modulated through different pathways.

Previous studies with some oleanane-derived triterpenes, such as the oleanoic acid derivatives CDDO and its methyl ester CDDO-Me¹⁸ indicated that 1-en-3-one derivatives with electronegative 2-substituent groups are highly cytotoxic, and the same effect was observed with GLA derivatives, such as **79** (Scheme 8).¹⁶ Based on such pharmacophoric group, Chadalapaka *et al.*¹⁹ synthesized analogs of **77** (**79** and **92-95**, Scheme 10) and other analogues with rearrangements in the C ring



Conditions: i) 2-iodoxybenzoic acid, DMSO, 85 °C, 21 h; ii) 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, benzene, reflux, 6 h.

Scheme 8.

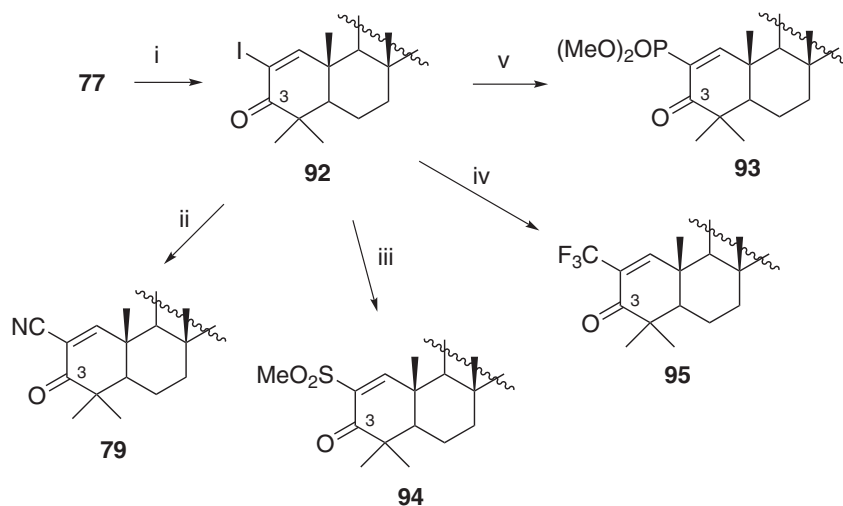


Scheme 9.

of GLA (**99-101**, Scheme 11), inserting along with the cyano group other electronegative groups. The cyano derivatives (with or without C-ring rearrangements) and the 2-trifluoro derivative **95** were the most active in *in vitro* anti-proliferative assays against four cancer cell lines (253JB-V and KU7 bladder cancer cells and Panc-1 and Panc-28 pancreatic cancer cells). These results seem to agree with the findings of Chintharlapalli *et al.*,¹⁶ where the cyano groups at C-2 were also important for the antiproliferative activity. It seems that, along with the electronegativity required at C-2, the size of this substituent group has a steric limit for a good interaction with the target receptor, which could explain the reason

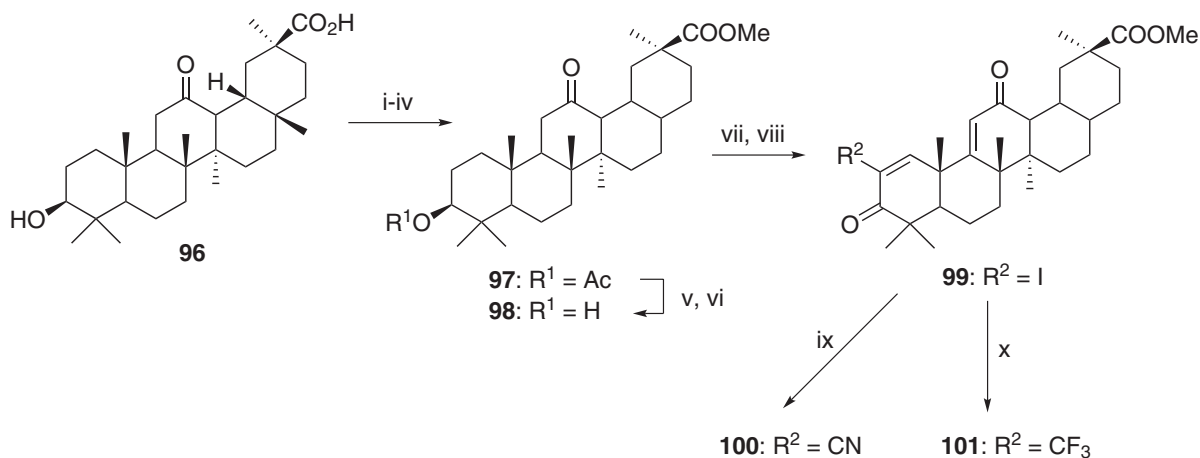
why more bulky substituents such as the methanesulfonyl and dimethylphosphonyl groups were inactive.

Paclitaxel (**102**, Scheme 13) is a natural product isolated originally from the barks of the Pacific Yew tree *Taxus brevifolia*, and is used in cancer therapy acting as a mitotic inhibitor. GLA (**2**) is also reported as presenting antitumor activity,^{3,4} and the conjugation of two antitumor agents with different mechanisms of action is reported to possibly increase the potency of both compounds and/or reduce its side effects and drug-resistance development.²⁰ Guided by previous reports of the antitumoral activity of conjugates such as paclitaxel-camptothecin, paclitaxel-epipodophyllotoxin



Conditions: i) I₂/Pyridine, THF, reflux; ii) CuCN, N-methylpyrrolidone (NMP), 130 °C, 2 h; iii) NaSO₂CH₃, CuI, DMSO, 120-125 °C, 20 h; iv) FSO₂CF₂COOCH₃, CuI, DMF/HMPT, 70 °C, 20 h; v) dimethylphosphite, Cs₂CO₃, *N,N*-dimethylethylenediamine, toluene, 95-100 °C, 25 h.

Scheme 10.



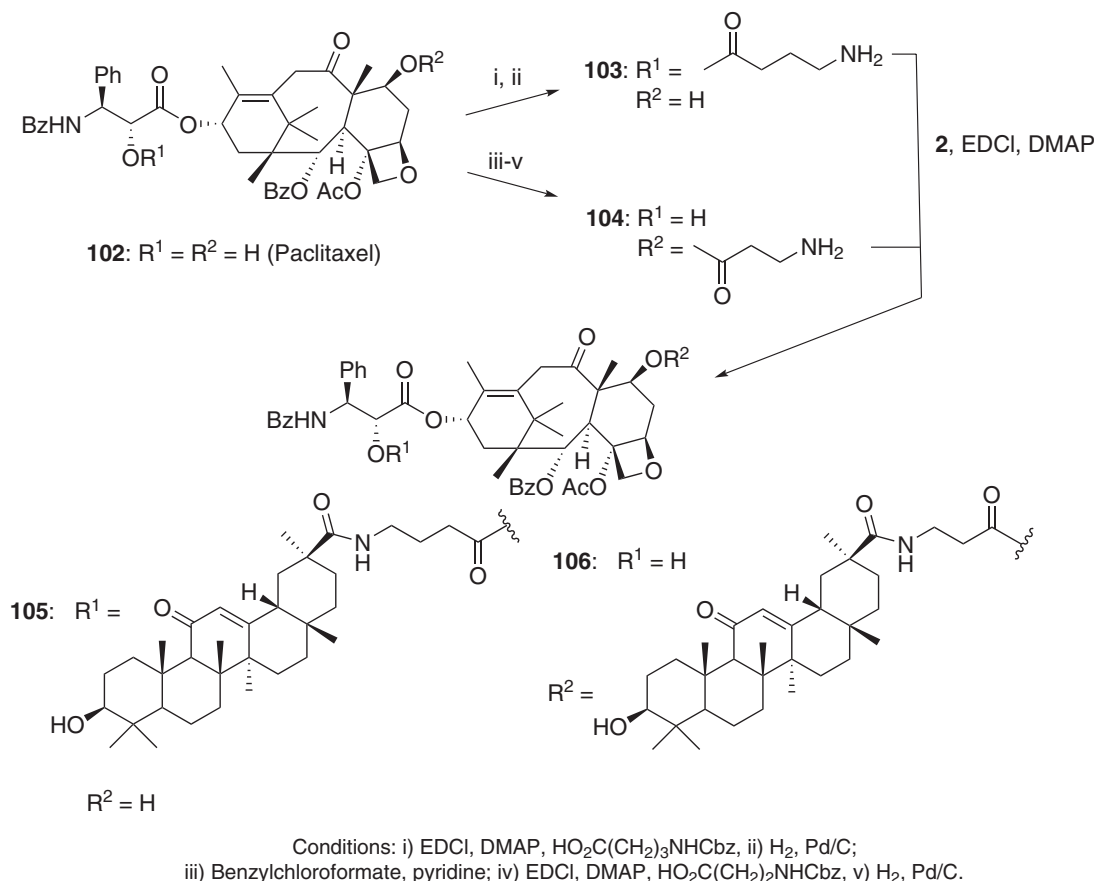
Conditions: i) H₂/PtO₂, AcOH; ii) Ac₂O/pyridine, DMAP; iii) *m*-CPBA, CH₂Cl₂; iv) BF₃·Et₂O, CH₂Cl₂; v) Br₂/HBr in AcOH; vi) KOH, MeOH; vii) 4-iodoxybenzoic acid, DMSO; viii) I₂/Pyridine, THF, reflux; ix) CuCN, N-methylpyrrolidone (NMP), 130 °C, 2 h; x) FSO₂CF₂COOCH₃, CuI, DMF/HMPT, 70 °C, 20 h.

Scheme 11.

and camptothecin-epipodophyllotoxin, Nakagawa-Goto *et al.*²⁰ synthesized new hybrid paclitaxel-conjugated compounds with several diverse scaffolds, including two amide derivatives coupled with GLA, **105** and **106** (Scheme 12). Those two compounds presented moderate to weak cytotoxic activities against 6 cancer cell lines (**105**: IC₅₀ ranging from 45 to 103 nM; **106**: IC₅₀ ranging from 147 to 652 μM) and, although less active than paclitaxel itself (IC₅₀ ranging from 1.0 to 55 nM), they

can serve as prototypes for new hybrid compounds with other active antitumoral molecules.

The same research group also studied the dehydrozingerone (DZ, **107**, Scheme 13), a phenolic natural product reported as presenting anti-oxidant, anti-inflammatory and anti-tumor properties. GLA (**2**) was coupled with DZ derivatives (Scheme 14) leading to eleven GLA-DZ conjugates **108-118**. Compounds **108**, **109** and **110** presented *in vitro* cytotoxic effects against some



Scheme 12.

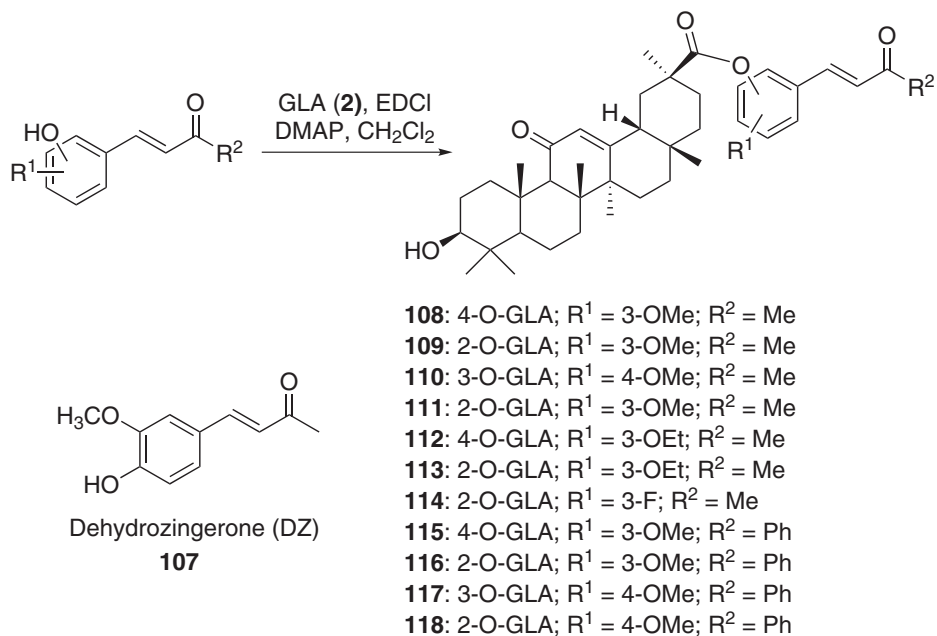
cancer cell lines, being either GLA or DZ alone inactive at those assays.²¹ The researchers found that replacing methoxy (**108-110**) with ethoxy (**112** and **113**) or fluor (**114**) substituents diminished the cytotoxic activity of derivatives, while replacing the methyl (R^2) groups with phenyl groups in compounds **115-118** abolished the activity.

3.3. Anti-inflammatory derivatives

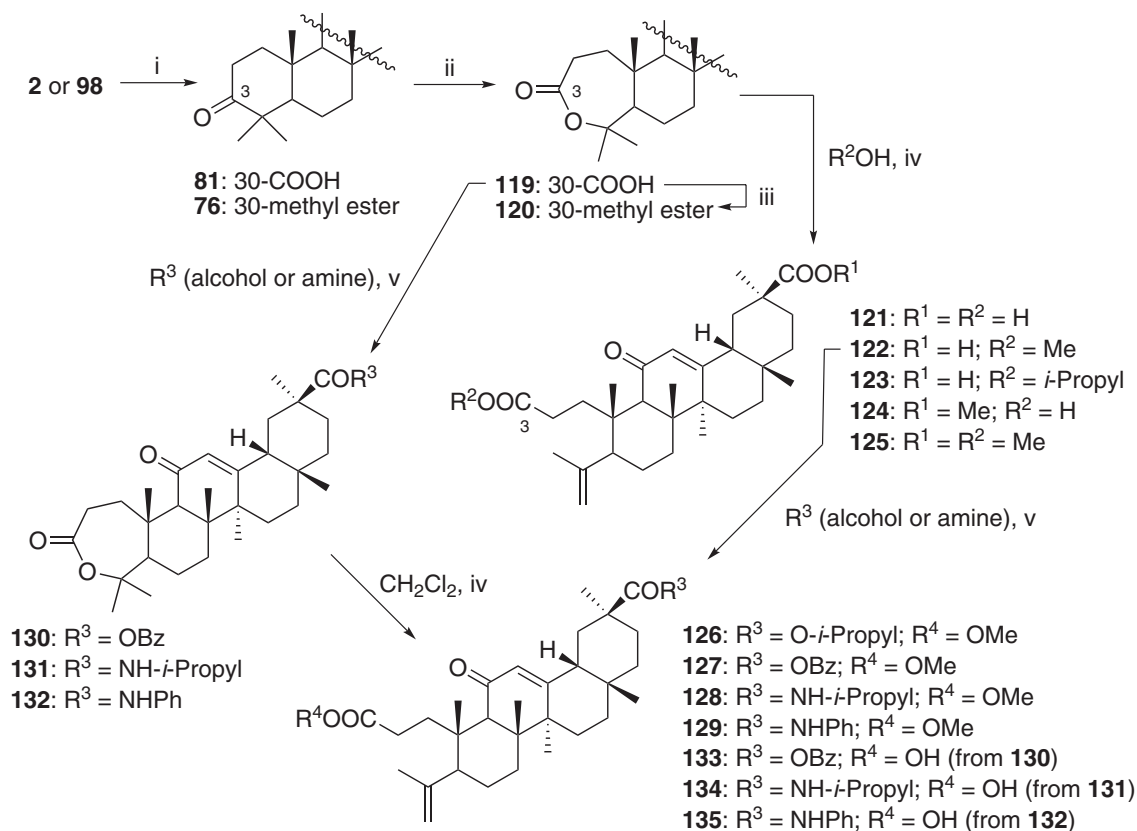
Searching for *in vitro* anti-inflammatory and anti-oxidant activities, Maitraie *et al.*²² synthesized two 7-member lactones (**119** and **120**) from the Bayer-Villiger oxidation of the 3-keto derivatives **81** and **76** respectively, starting with **2** or the methyl ester **98**. Both oxidation protocols did not affect the double bond at C-12 (Scheme 14). From these lactones, using two different synthetic pathways, they were able to obtain a series of derivatives (**126-129** and **133-135**). Compounds **120**, **121** and **134** inhibited superoxide anion generation in rat neutrophils, while compounds **98**, **124**, **127**, **129**, **133** and **135** inhibited tumor necrosis factor- α (TNF- α) formation in RAW 264.7 cells in response to lipopolysaccharide (LPS). In this series of compounds, lipophilic group substituted at C-3 or C-30 and/or the

presence of a carbonyl group at the ring A enhanced the anti-inflammatory activity.

One of the most studied mechanisms of action of GL and GLA is their anti-inflammatory activity, attributed mostly to the inhibition of 11 β -hydroxysteroid dehydrogenase (11 β -HSD), an enzyme responsible for the metabolism of the anti-inflammatory glucocorticoid hormones cortisol (hydrocortisone) and its inactive form cortisone. The variant 11 β -HSD-1 converts cortisone into cortisol, while the variant 11 β -HSD-2 does the opposite, converting cortisol into cortisone, inactivating this hormone. GL and GLA are known to inhibit primarily 11 β -HSD-2, presenting an indirect anti-inflammatory effect by increasing the levels of cortisol.²⁻⁴ In this context, Su *et al.*²³ employing **2** as starting material for several chemical modifications, obtained a series of 11- and 30- modified GLA derivatives (**136-140**, Scheme 15). Compound **136** presented the higher rat 11 β -HSD inhibitory activity of the series. Also, the authors found that the introduction of the 11 α -methyl-11 β -hydroxyl induces selectivity to the isoform 1 of the enzyme, which can be related to the role of HSD isoform 1 in converting the 11-hydroxyl group of cortisol to the 11-ketone group of cortisone. Compound **137**



Scheme 13.



Conditions: i) CrO₃, DMF, r.t., 12 h; ii) *m*-CPBA, CH₂Cl₂, r.t., 12 h; iii) EDCI, DMAP, CH₂Cl₂, MeOH, r.t., 6-8 h; iv) *p*-TsOH, r.t., 6-8 h; v) EDCI, DMAP, CH₂Cl₂, amine or alcohol, r.t., 24 h.

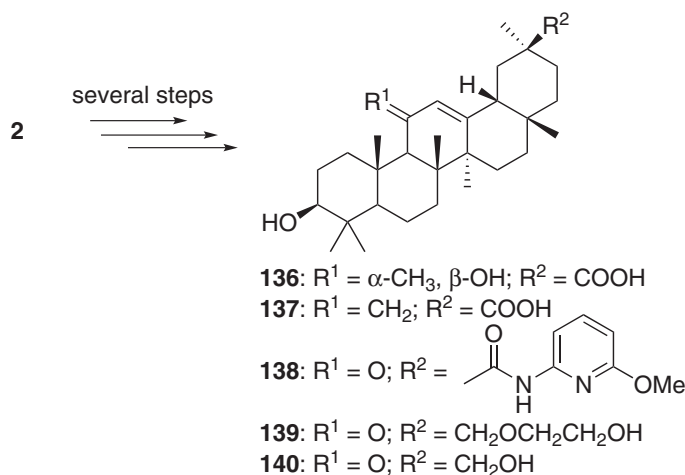
Scheme 14.

was the less active of the series, indicating that the presence of an oxygen atom (either as an alcohol or ketone), at C-11 position is important for the interaction with the rat 11 β -HSD enzyme, probably because the C-11 oxo group is also present at the enzyme substrate cortisol. On the other hand, compound **139** also presented selectivity to the isoform 1 of the enzyme, indicating that a larger side chain at C-30 can be detrimental to the interaction of the compound with the rat 11 β -HSD² enzyme.

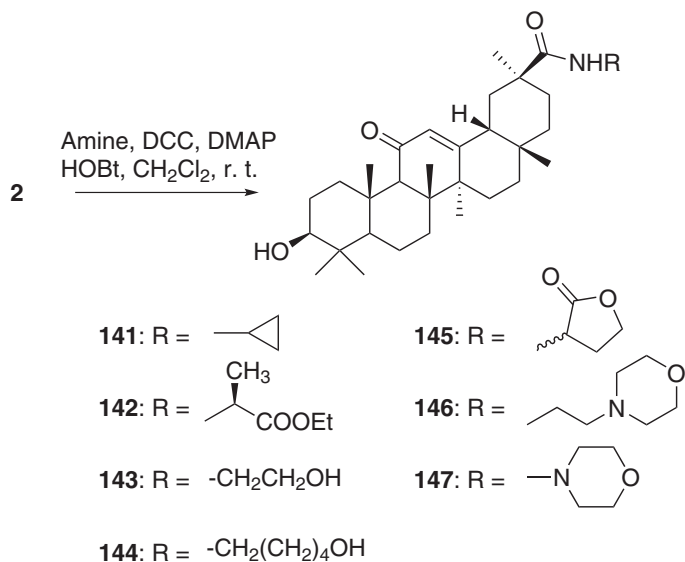
Vicker *et al.*²⁴ synthesized 7 GLA-derived amides *via* the DCC-promoted coupling (**141-147**, Scheme 16). Amide **142** inhibited (*in vitro*) the human 11 β -HSD-2 enzyme, presenting little activity against the 11 β -HSD-1 variant. The side chain of **142** was designed to mimic the side chain of cortisol/cortisone, while the keto group of the glucocorticoid hormone was replaced by the bioisostere amide group.

3.4. Proteasome inhibitors

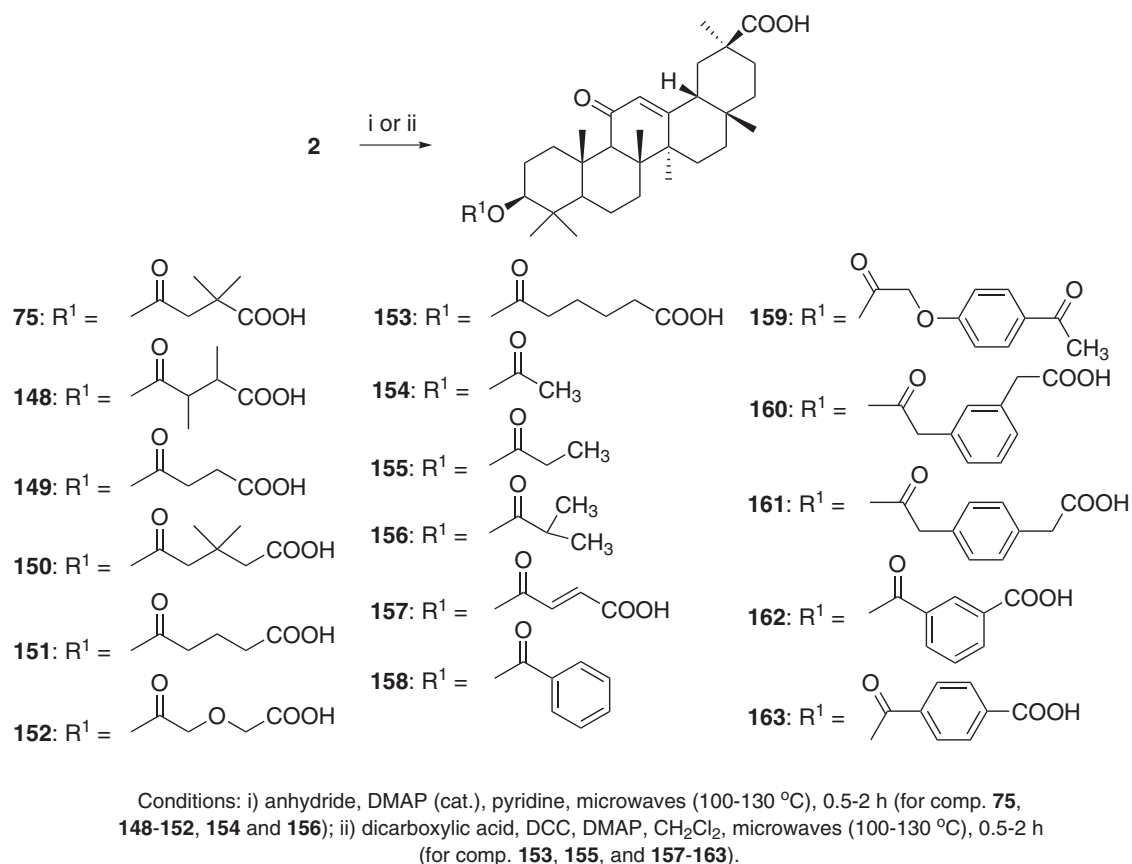
Based on previous results indicating that some betulinic acid derivatives regulated the activity of the human 20S proteasome-ubiquitin complex, important in cell-stress responses and cell cycle regulations, Huang *et al.*²⁵ synthesized a series of C-3 ester derivatives employing anhydrides or dicarboxylic acids (Scheme 17) and some amides employing aminoacids (Scheme 18). Compounds **162** (IC₅₀ = 0.22 μ M), **153** (IC₅₀ = 0.29 μ M), **163** (IC₅₀ = 0.33 μ M), **157** (IC₅₀ = 0.35 μ M) and **75** (IC₅₀ = 0.87 μ M) were the most active of the series, inhibiting *in vitro* the chemotrypsin-like activity of the human 20S proteasome complex with more potency than GLA (**2**, IC₅₀ = 22.3 μ M). The researchers concluded that free carboxylate groups seemed to increase the activity of the compounds, although not required for the



Scheme 15.



Scheme 16.

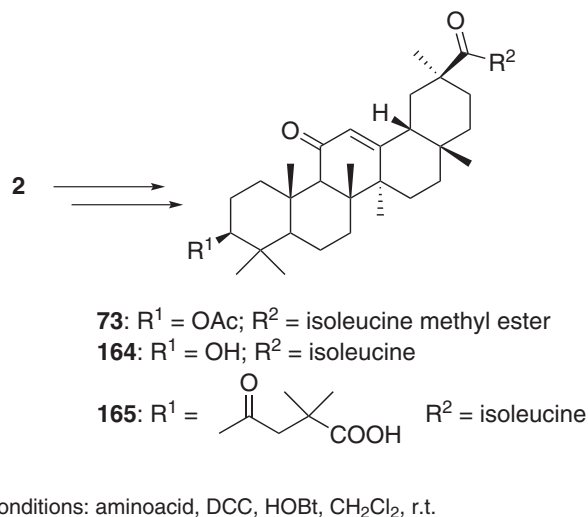


Scheme 17.

compounds to present this activity (**156** has an IC₅₀ = 1.92 μM and **154** and **155** were inactive in this assay, with IC₅₀ > 40 μM). Additionally, the presence of an aromatic ring and the position of the carboxylate group in that ring do not appear to be relevant for this activity (comparing the aromatic derivative **162**, the aliphatic derivative **153** and the unsaturated derivative **157**). Further modifications at C-30 appeared to diminish the proteasome activity.

3.5. Gene silencers

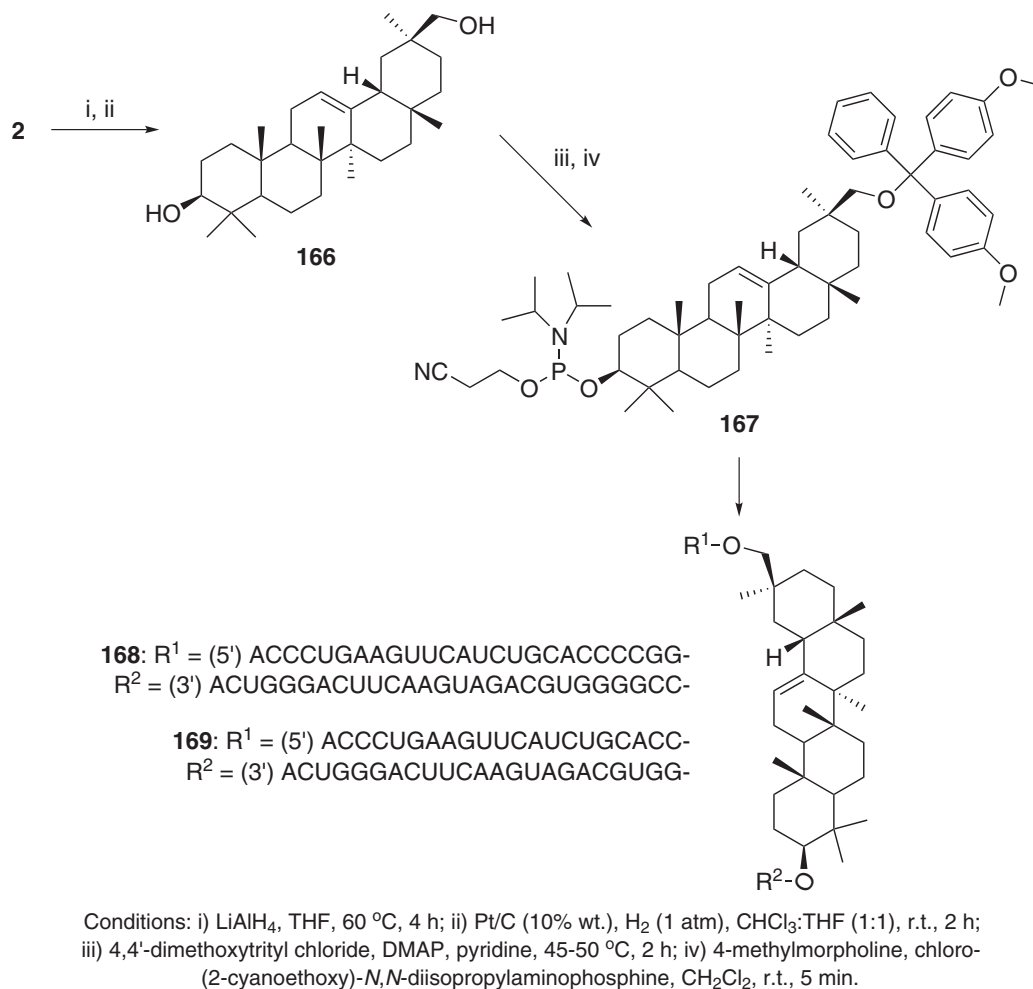
Short hairpin RNA (shRNA) are modified small interfering RNA (siRNA) molecules, capable of suppress gene expression of targeted genes with better properties (stability, cellular uptake and target specificity) than siRNA. Bang and Kim, starting from **2**, synthesized the 11-desoxo-3,30-diol **166** which was selectively protected leading to **167**. This protected diol was used as a hairpin loop for two shRNAs, **168** and **169**, that acted as gene silencers against the EGFP gene, a gene that codifies the Green Fluorescent Protein (GFP) and is used as a template to measure the activity of siRNAs (Scheme 19), indicating that the GLA derivative **166** can act as a hairpin loop for the syntheses of other shRNAs targeting different and clinically relevant genes.²⁶



Scheme 18.

3.6. Antioxidant derivatives

Ablise *et al.*²⁷ reduced **2** in order to obtain a 11- and 30-diol (Scheme 20, conditions not shown in reference) as a mixture of epimers 11α and 11β (**170** and **171**, respectively) that were reacted as a mixture in order to



Scheme 19.

generate the 11-deoxo compound **173** or separately, leading to the conjugated dienes **172** and **174**, respectively. The synthesized compounds presented *in vitro* antioxidant activity in a rat microsomal cytochrome P₄₅₀/NADPH system, being **170** and **171** the most active, inhibiting in 50 and 51% (respectively) the formation of reactive oxygen species, followed by **172** (41%) and **174** (44%) indicating, according with the authors, that chemical reduction of the 11-keto and 30-carboxylic acid groups lead to compounds with greater antioxidant activity.

3.7. Strategies for new GLA derivatives

Some syntheses reported at the literature were performed in order to explore the chemical versatility of GLA and, even without pharmacological activities associated to them yet, may constitute as valuable information to the medicinal chemists as they pinpoint new chemical strategies for obtaining new bioactive GLA derivatives. The following syntheses are classified by the GLA rings being modified.

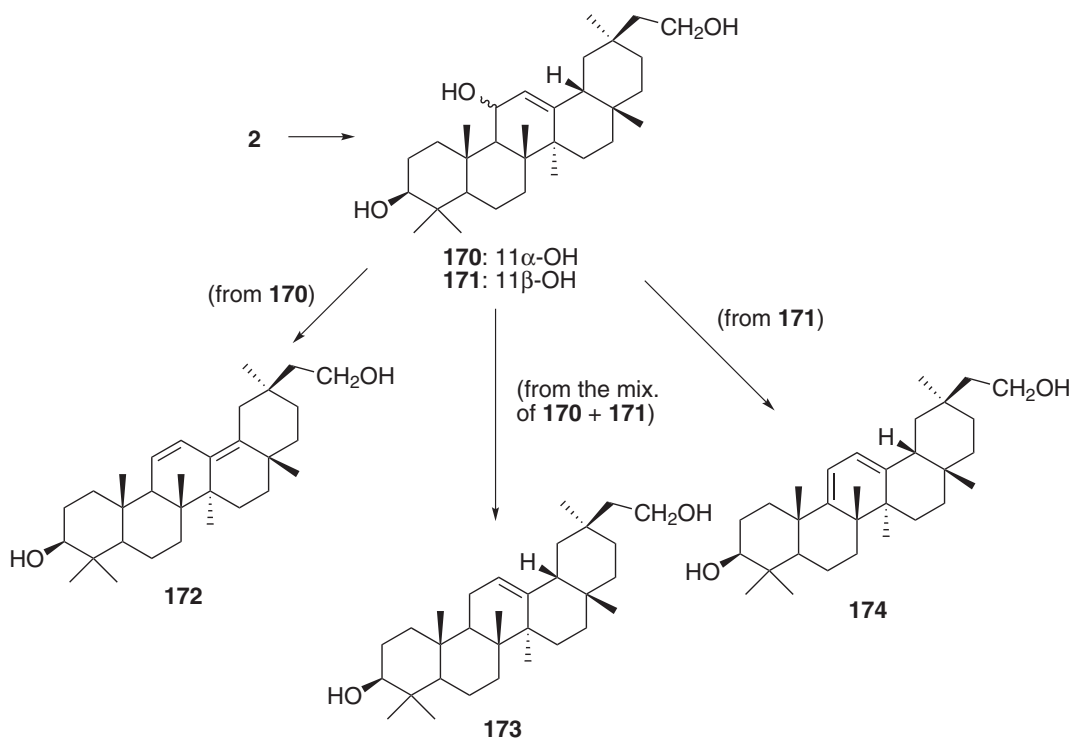
3.7.1. Modifications at rings A and C

Mikhailova *et al.*²⁸ performed the ozonolysis reaction of 11-desoxo-GLA (**137**), obtaining the 3,12-dione derivative **175** using CH₂Cl₂ as solvent or the 12-one derivative **96** using a mixture of CH₂Cl₂:MeOH as solvent (Scheme 21).

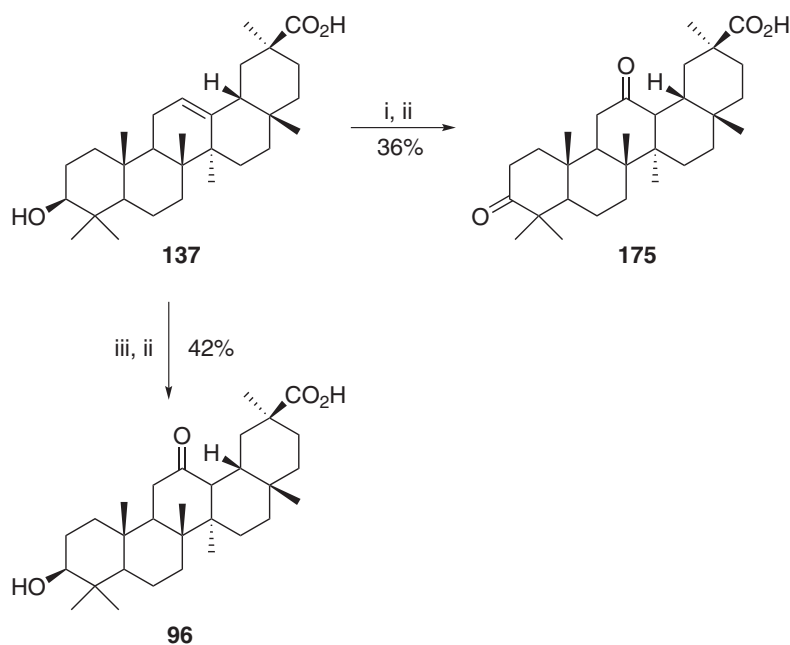
The same research group employed the 30-methyl ester of **137** (**176**) as starting material for the synthesis of *A-nor*-derivatives through the Wagner-Meerwein rearrangement. Depending on the reaction temperature, two different products were obtained (**177** and **179**), as shown in Scheme 22. Partial ozonolysis of **177** gave the epoxide **178** as product, and oxidation of **179** with RuO₄ gave **180** and **181** as a mixture (2:1) of products, while partial ozonolysis of **179** gave only **180** as product.²⁹

3.7.2. Modifications at rings A and E

Employing 18- α (**182**) or 18- β (**149**) hemisuccinyl esters of GLA, Kondratenko *et al.*³⁰ synthesized new glycopyranosyl amides employing an aminosugar



Scheme 20.

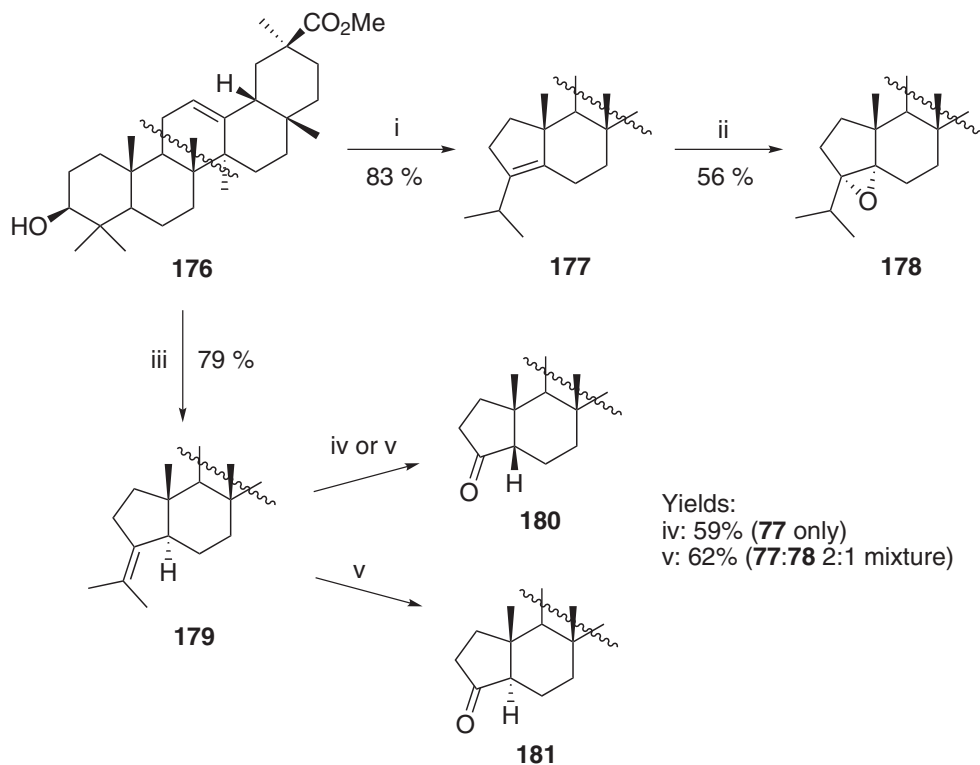


Conditions: i) O₃, CH₂Cl₂, -60 °C; ii) Zn, AcOH, 0 °C, 1 h;
 iii) O₃, CH₂Cl₂:MeOH (5:1), -60 °C.

Scheme 21.

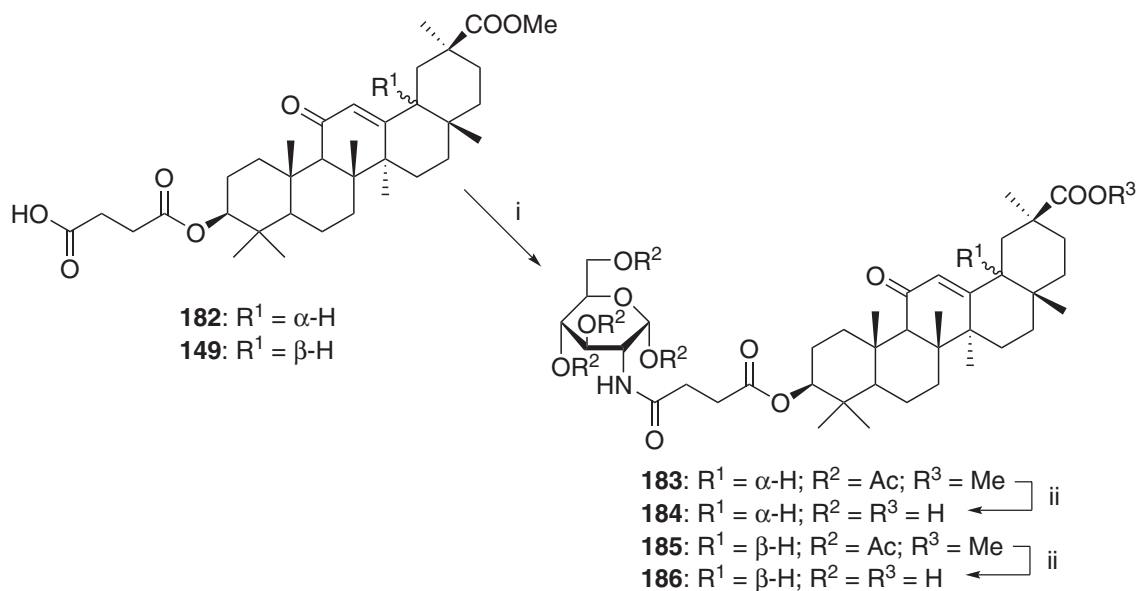
derivative and DCC as coupling promoter (Scheme 23), leading to the respective amides **183** and **185**, which were reacted with KOH in order to generate the un-protected derivatives **184** and **186**, respectively.

Mustafina *et al.*³¹ further expanded this family of compounds employing the hemiptalyl and hemisuccinyl esters of GLA (**61** and **149**, respectively) leading to the *N*-glycoconjugates **187-192** (Scheme 24).



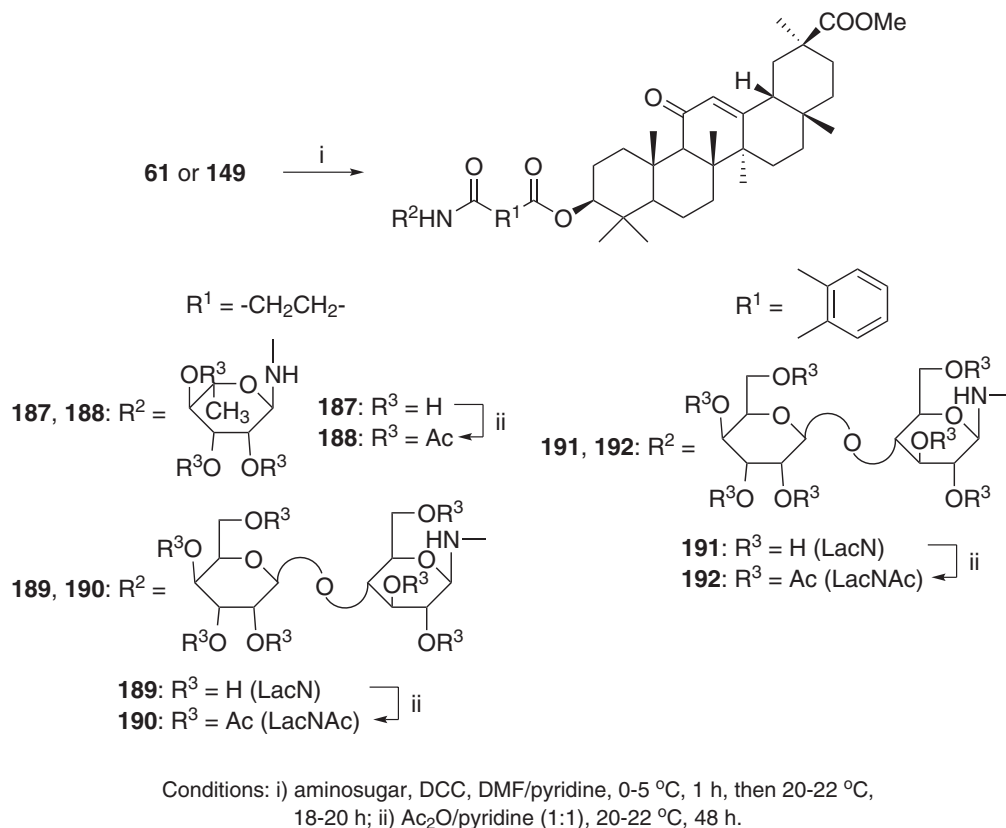
Conditions: i) PCl_5 , benzene:toluene (1:1), 5-10 °C, 1 h; ii) O_3 , CH_2Cl_2 , -60 °C, then Zn, AcOH, r.t., 1.5 h; iii) PCl_5 , benzene:toluene (1:1), -10-0 °C, 1 h; iv) O_3 , CH_2Cl_2 , -60 °C, then Zn, AcOH, r.t., 1 h; v) NaIO_4 , $\text{RuCl}_3 \cdot 3\text{H}_2\text{O}$, $\text{CCl}_4:\text{CH}_3\text{CN}:\text{H}_2\text{O}$ (1:1:1.5), 20-22 °C, 3 days.

Scheme 22.



Conditions: i) 2-amino-1,3,4,6-tetra-*O*-acetyl-2-deoxy- α -D-glycopyranose, DCC, DMF/pyridine, 0-5 °C, 1 h, then 22-24 °C, 20 h; ii) KOH 1% in MeOH, 20-22 °C.

Scheme 23.



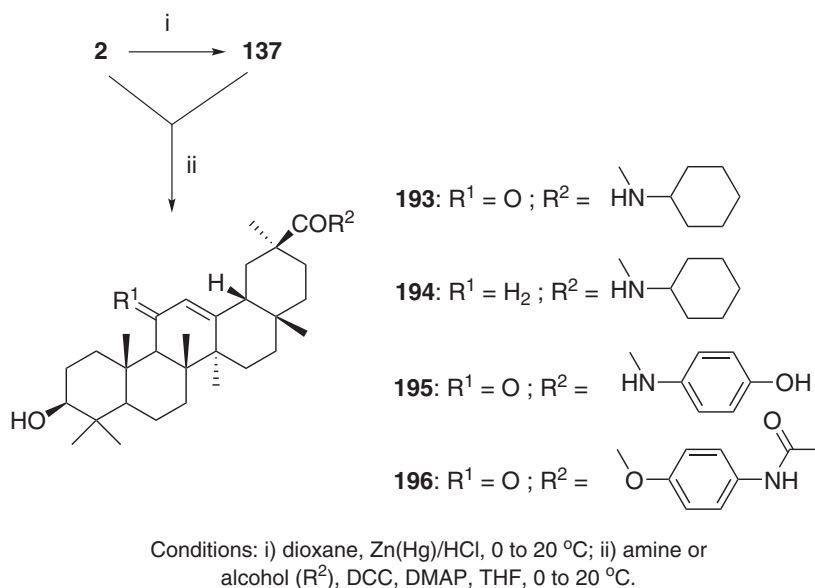
Scheme 24.

3.7.3. Modifications at rings C and E

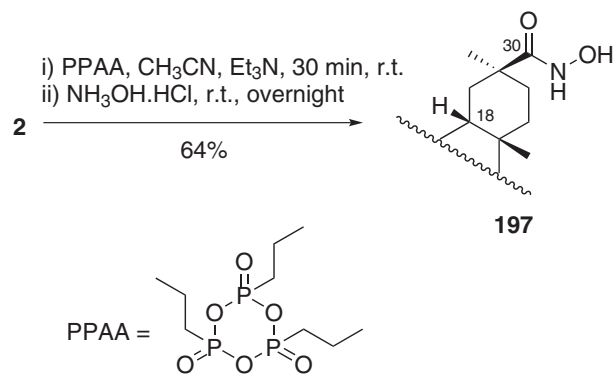
Yong *et al.*³² employed **2** or its 11-deoxy derivative **137** (obtained through the Clemmensen reduction of **2**) to synthesize a series of 4 derivatives (**193-196**, Scheme 25).

3.7.4. Modifications at ring E

Ech-Chalad *et al.*³³ employed the cyclic phosphonic anhydride PPAA as a carboxylic acid activating agent for hydroxamation reactions in a series of carboxylic acids,



Scheme 25.

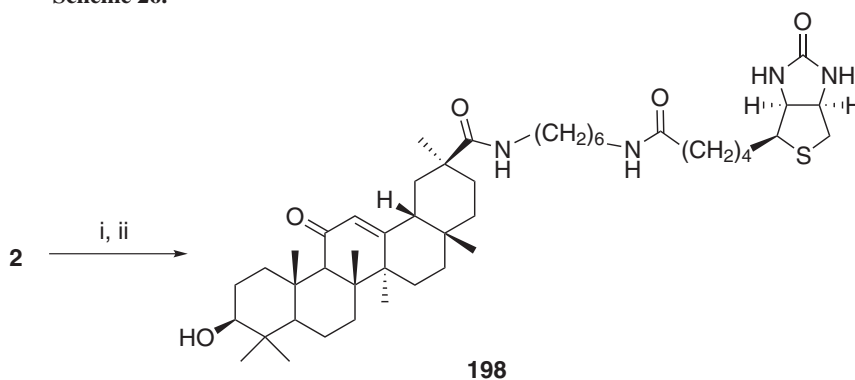


Scheme 26.

including GLA (**2**) (Scheme 26), obtaining selectively the 30-hydroxamic acid **197**.

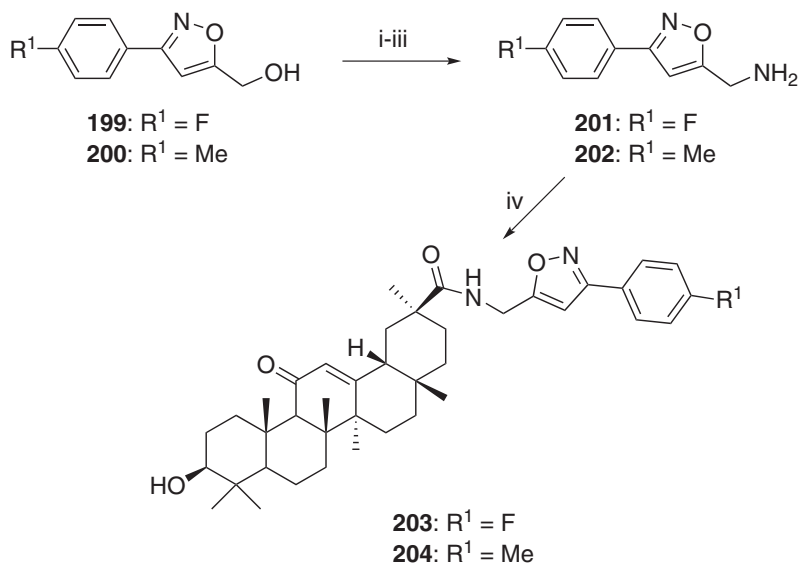
Two sequential couplings involving GLA (**2**), 1,6-hexanediamine and biotin (Scheme 27) generated the GLA-hexanediamine-biotin conjugate **198**, reported by Shi *et al.*³⁴

Liu *et al.*³⁵ synthesized novel GLA-isoxazole derivatives **203** and **204** via DCC-promoted coupling of the isoxazole amines **201** and **202**, respectively (Scheme 28).



Conditions: i) DCC, *N*-hydroxysuccinimide (NHS), CH₂Cl₂:DMF (5:1), r.t., 24 h, then 1,6-hexanediamine, r.t., overnight; ii) biotin, DCC, NHS, CH₂Cl₂:DMF (5:1), r.t., 28 h.

Scheme 27.



Conditions: i) MsCl, Et₃N; ii) NaN₃; iii) Zn, NH₄Cl, EtOH/H₂O; iv) GLA (**2**), DCC, DMAP, THF, 0 °C to r.t.

Scheme 28.

4. New Pharmacological Activities Reported for GL and GLA

In the period covered by this review, some new pharmacological activities were reported for GL (**1**), such as: antiviral (against flavivirus³⁶, HIV³⁷ and SARS-coronavirus³⁸) antiallergic,³⁹ antithrombotic in experimental models in rats,^{40,41} capable of lowering hepatocellular carcinogenesis rates in patients with interferon-resistant active chronic hepatitis C,⁴² presenting a protective *in vitro* effect in gastric epithelial cells against H₂O₂-induced cell death⁴³ and as an inhibitor of influenza A virus uptake into human lung cells.⁴⁴ GL and GLA (**2**) were also reported as possessing a protective effect on acute cholestasis induced by α -naphthyl isocyanate in rats⁴⁵ and GLA was reported as an apoptosis inductor in human hepatoma, promyelotic leukemia and stomach cancer cells,⁴⁶ as an antihyperglycemic agent in rats with streptozocin-induced diabetes,⁴⁷ and as presenting antiviral activity against herpes simplex virus 1 (HSV-1).⁴⁸

Although several interesting biological activities are known for these two compounds, few molecular targets are described for them. GL (**1**) is described as an inhibitor of the high-mobility group box 1 protein (HMGB1), a signaling molecule involved in acute and chronic inflammation. GL inhibits the chemoattractant and mitogenic activities of HMGB1, but not its intranuclear DNA-binding function.⁴⁹ GL is also described as an inhibitor of thrombin, a protease involved in the blood clotting cascade.⁴⁰ As mentioned before, the anti-inflammatory activity of GL and GLA is accounted for the inhibition of the β -hydroxysteroid dehydrogenase enzyme 2 (β -HSD-2), an enzyme responsible for the conversion of the glucocorticoid hormone cortisol in its active form cortisone.^{3,4}

5. Concluding Remarks

Several different synthetic strategies and some pharmacological activities were highlighted in this review, employing GL or its aglycon, GLA, mainly from research groups in Russia, Korea and Japan. As for the synthetic strategies, most of the reactions described consisted of functionalizations and/or chemical derivatizations of the functional groups already present in both compounds, and can be summarized as: (i) amide or ester synthesis involving the carboxyl groups at C-30 (GL or GLA) or both of the glucuronic acids (GLA); (ii) ester synthesis, oxidation or elimination of the hydroxyl group at C-3; (iii) further functionalization of the 3-keto derivative; (iv) reduction of the ketone group at C-11 and/or reduction of the carboxyl

group at C-30; and (v) insaturation of rings A and/or C and further modification of the same. Such chemical strategies are straightforward and easily applicable to these compounds and other related triterpenes. Also, in some of the described reactions it is possible to observe some chemoselectivity (ex.: Schemes 2 and 3) between similar functional groups in the compounds.

It is noteworthy that GL and GLA, as well as its derivatives described in this review, presented a plethora of pharmacological activities, such as: antiviral, immunomodulating (inducing or suppressing immune response), antiallergic, anti-ulcer, antibacterial (against *H. pylori*), as PPAR- γ agonists, anti-inflammatory, human 20S proteasome-ubiquitin complex inhibitors, cytotoxic and antiproliferative, antioxidant and 11 β -HSD-1 and 2 inhibitors, some with selectivity for one of the subtypes of the enzyme. Such broad range of activities is, at first, undesirable in the choice or identification of a new lead compound. However, the pharmacological activities presented by the discussed derivatives may be associated to specific pharmacophoric groups, such as the cytotoxic activity being related to the presence of a double bond between C-1 and C-2 and a cyano group in C-2 and the presence of glycosyl substituents conjugated with aminoacids and/or other carbohydrates at C-3 leading to derivatives with antiviral activity. With additional studies, new pharmacophoric groups may unravel guides to selectively explore GL and GLA as scaffolds for diverse sets of pharmacological activities. Alternatively, as multifunctional compounds can be conceived as molecules with improved efficacy,⁵⁰ some activities described above can be conjugated in a synergic way in order to achieve a better pharmacological profile, such as the antiviral and the immunostimulating activities of some GLA-amino acid conjugates, shown in Schemes 1 through 4. Ultimately, GL and GLA may be considered for its several pharmacological activities as privileged structures,⁵¹ and potentially useful as scaffold for the design of new pharmacologically active compounds.



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