Novel A-ring cleaved glycyrrhetinic acid derivatives: Synthesis and evaluation of antiproliferative activity

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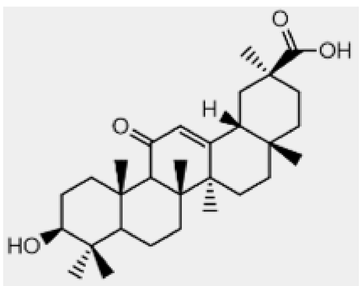
INTRODUCTION



Glycyrrhetinic acid (**GA**)

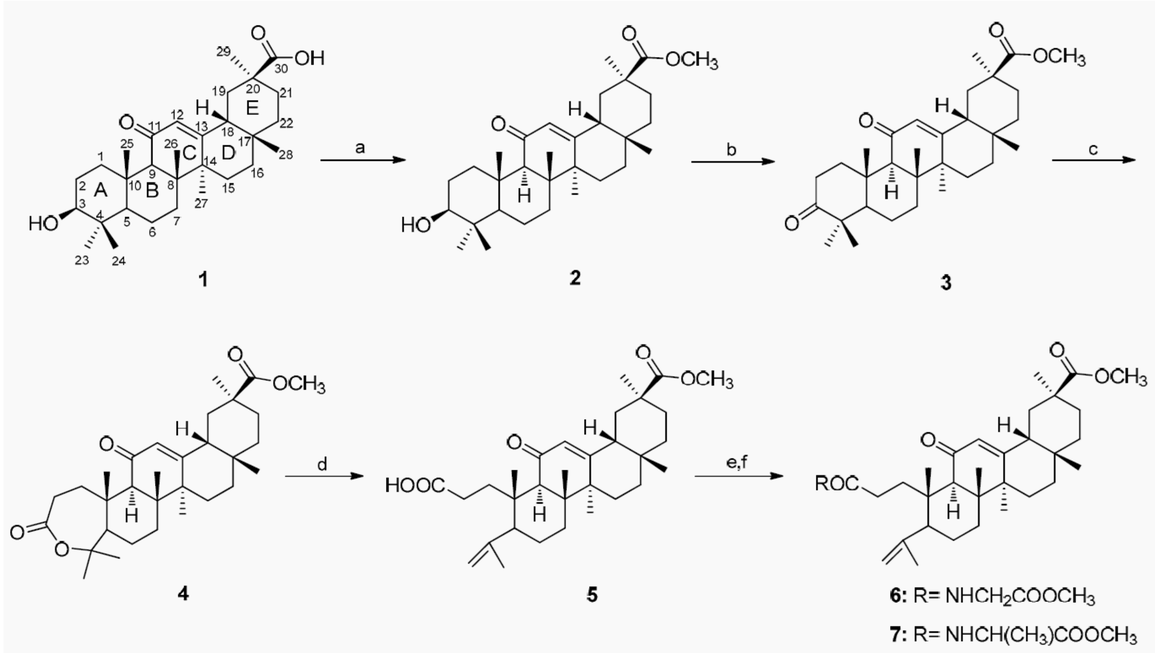
Glycyrrhetinic acid (GA) is the hydrolyzed metabolite of glycyrrhizin, a major pentacyclic triterpenoid saponin obtained from the roots of licorice, that has been shown to inhibit tumor initiation and proliferation in several cancer cell lines[1,2]. Nevertheless, it lacks potency and selectivity as an antitumor agent[3]. Many derivatizations have been performed in order to enhance its potency, but the cleavage of the ring A is still poorly explored. On the other hand, is well known that the conjugation of an amino acid moiety to pentacyclic triterpenoids improves their cytotoxicity and their selectivity towards tumour cells.

Objective

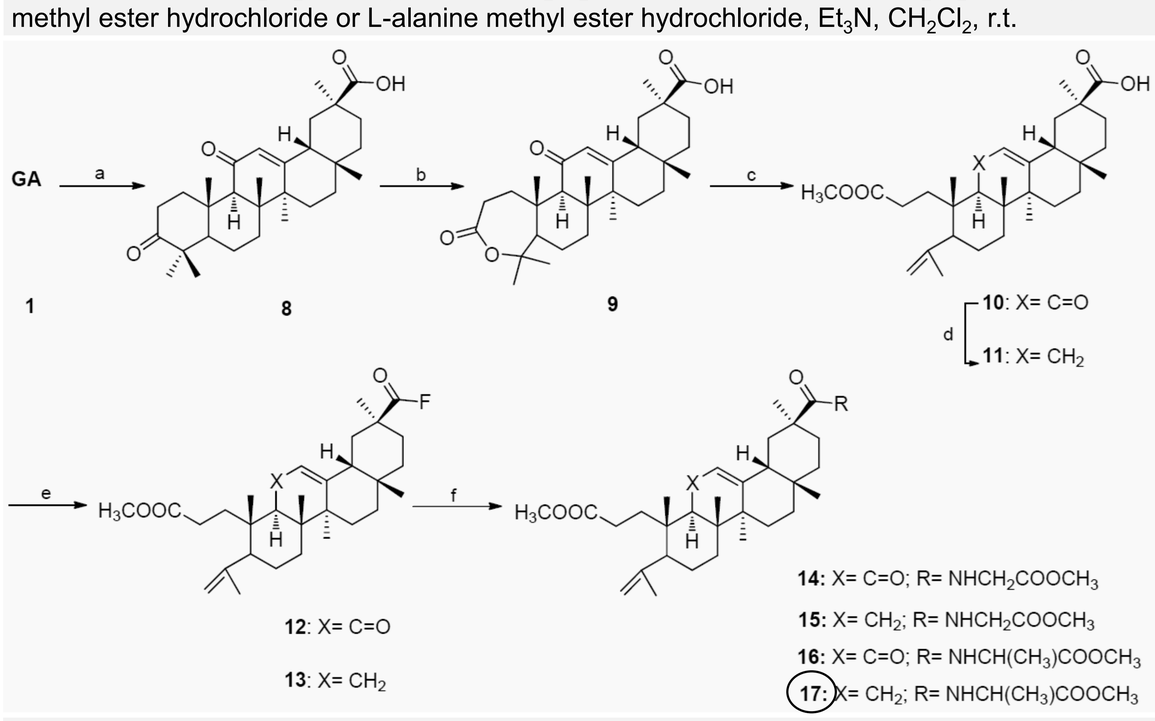
Synthesis of a library of novel GA derivatives via the opening of its ring A along with the coupling with an amino acid and evaluation of their antiproliferative activity against human cancer cell lines, as well as against a non-tumour cell line (BJ). Additionally, we also pretend to elucidate the preliminary mechanisms of action of the most active compounds.

METHODS AND RESULTS

Chemistry



Scheme 1. Reagents and conditions: a) CH_3I, K_2CO_3, DMF, r.t.; b) Jones reagent, acetone, 0 ºC; c) *m*-CPBA, CH_2Cl_2, r.t.; d) *p*-TSA, CH_2Cl_2, r.t.; e) Deoxo-Fluor®, CH_2Cl_2, r.t.; f) glycine methyl ester hydrochloride or L-alanine methyl ester hydrochloride, Et_3N, CH_2Cl_2, r.t.



Scheme 2. Reagents and conditions: a) Jones reagent, acetone, 0 ºC; b) *m*-CPBA, CH_2Cl_2, r.t.; c) MeOH, *p*-TSA, CH_2Cl_2, r.t.; d) zinc dust, conc. HCl, dioxane, r.t.; e) Deoxo-Fluor®, CH_2Cl_2, r.t.; f) glycine methyl ester hydrochloride or L-alanine methyl ester hydrochloride, Et_3N, CH_2Cl_2, r.t.

Biology

Antiproliferative Activity

Table 1. Antiproliferative activities of GA **1**, its derivatives **14-17,** and cisplatin against several cancer cell lines and the human nontumoral BJ cell line

Compound	Cell line (IC_{50}, μM)							
	Jurkat	MOLT-4	MIAPaca2	MCF7	HeLa	A375	HepG2	BJ
1	105.6 ± 5.0	95.5 ± 3.9	101.6 ± 1.6	97.8 ± 3.9	107.2 ± 2.5	112.2 ± 2.6	125.1 ± 9.1	165.0 ± 7.1
14	13.3 ± 1.1	23.5 ± 0.8	32.5 ± 3.2	28.8 ± 0.7	34.2 ± 2.4	30.0 ± 2.2	34.7 ± 1.1	N.D.
15	12.5 ± 0.5	18.9 ± 1.6	20.2 ± 1.2	24.8 ± 1.3	22.2 ± 0.3	18.8 ± 1.1	25.4 ± 1.3	N.D.
16	9.6 ± 0.4	19.1 ± 1.3	22.6 ± 0.6	23.8 ± 1.6	19.1 ± 0.5	17.0 ± 1.1	25.7 ± 0.8	N.D.
17	6.1 ± 0.2	15.3 ± 0.7	11.8 ± 1.1	21.6 ± 0.6	13.0 ± 0.5	11.3 ± 0.4	16.0 ± 0.3	> 100
Cisplatin	1.9	1.4	5.0 ± 1.0	19.1 ± 4.5	2.3 ± 0.3	3.1 ± 1.0	2.9	10.1 ± 2.0

Analysis of Cell Cycle Distribution and Apoptosis (compound 17)

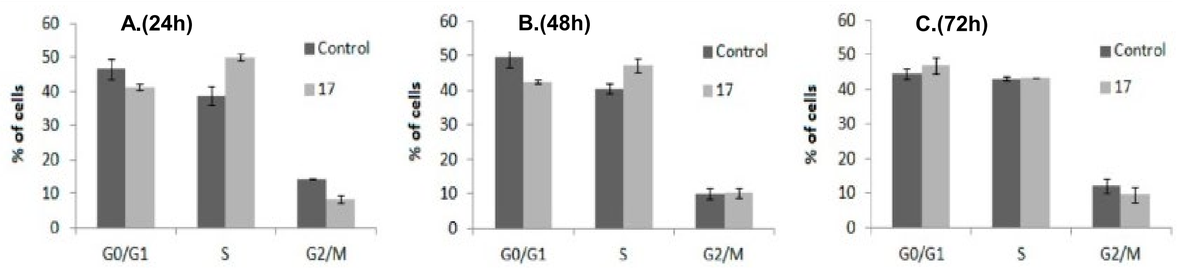


Figure 1. Effect of compound **17** on cell cycle distribution. Cell cycle analysis of Jurkat cells untreated (Control) or treated with 6.1 μM for 24 h (**A**), 48 h (**B**), and 72 h (**C**)

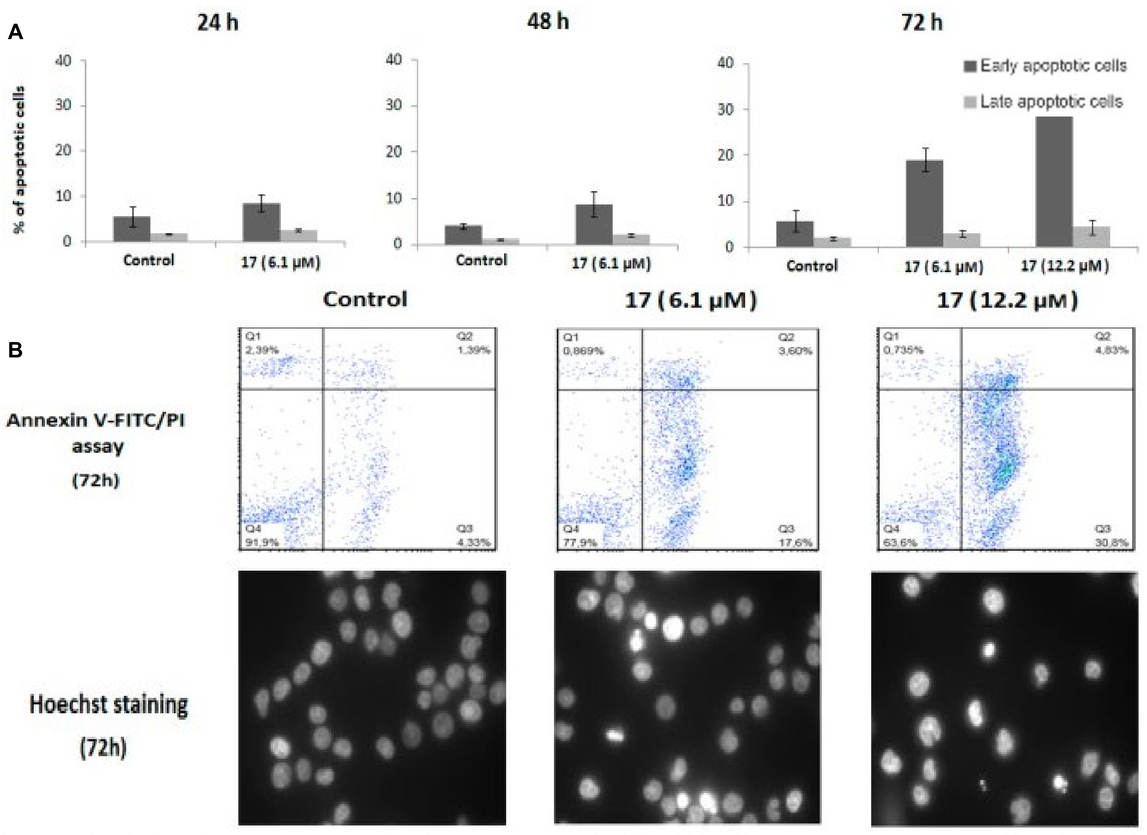


Figure 2. Induction of apoptosis by compound **17**. (**A**) Flow cytometry quantification of apoptosis in Jurkat cells untreated (Control) or treated with compound **17** at specified concentrations for 24, 48 and 72h. (**B**) *Upper panel*: Representative dot plots of Annexin V-FITC/PI assays of Jurkat cells untreated (Control) or treated with compound **17** at specified concentrations for 72 h. *Lower panel*: Representative fluorescence microscopic images of Jurkat cells untreated (Control) or treated with compound **17** at specified concentrations for 72h

CONCLUSIONS

We synthesized a series of new **GA** derivatives via the opening of its ring A along with the coupling of an amino acid. Antiproliferative activity assays in a panel of nine human cancer cell lines showed that the most potent compound **17** was 5 to 17-fold more active than **GA 1**. The study of selectivity revealed that this new derivative was up to 10 times more selective towards malignant cells than its parental compound. Preliminary mechanism investigation indicated that compound **17** may act through arresting cell cycle progression at the S phase and inducing apoptosis[4].

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