Structure-Activity Relationship Analyses of Glycyrrhetinic Acid Derivatives as Anticancer Agents

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Abstract: Cancer cell resistance to kinase inhibitors and targeted agents, acquisition of a multidrug-resistant (MDR) phenotype and/or intrinsic resistance to apoptosis prevent effective treatment in about 50% of solid cancers in adults, and the percentage is even higher in children. Glycyrrhetinic acid (GA) and some of its derivatives may offer hope in combating cancer types associated with poor prognoses. Some GA derivatives are indeed able to target both the proteasome and peroxisome proliferator-activated receptors (PPARs), two proteins that play major roles in cancer cell biology but are not related to MDR and/or apoptosis-related resistance phenotypes.

Keywords: Glycyrrhetinic acid derivatives, cancer, proteasome inhibitors, PPAR, structure-activity relationship.

INTRODUCTION

What Are the Major Limitations in Combating Cancer at the Clinical Level?

The number of cancer-related deaths is increasing worldwide, and more than 90% of cancer patients die from tumor metastases because metastatic cancer cells are intrinsically resistant to apoptosis and therefore unresponsive to a large majority of currently available apoptosis-inducing anticancer drugs [1,2]. Many cancer types also display intrinsic resistance to proapoptotic stimuli even before metastasizing, such as non-small cell lung cancer (NSCLC) [3,4], melanoma [5,6], pancreatic cancer [7], esophageal cancer [8,9] and gliomas [10-12]. In addition to intrinsic resistance to proapoptotic stimuli, many cancers develop chemoresistance during chronic treatments by acquiring a multidrug-resistant (MDR) phenotype characterized by decreased drug accumulation in cancer cells due to enhanced drug efflux [13,14].

One solution to apoptosis resistance and/or a MDR phenotype entails the complementation of cytotoxic therapeutic regimens with cytostatic agents, such as drugs targeting specific protein tyrosine kinases (PTK) or membrane receptors [15-17]. However, it is already apparent that most cancers can escape the inhibition of any single kinase [15-17]. Thus, cancer cell resistance to kinase inhibitors and targeted agents, acquisition of an MDR phenotype and/or intrinsic resistance to apoptosis prevent effective cancer treatment. As detailed in the current review, GA and its derivatives may offer hope in combating cancer types associated with poor prognoses.

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Historical Overview of the Anticancer Potential of Glycyrrhetinic Acid and Its Derivatives

GA is the main constituent of *Glycyrrhiza glabra*, which has long been used as an antitussive, an anti-inflammatory, an antiulcer treatment, an antiallergic, an immunotropic, and/or a hypolipidemic agent [18]. The *in vitro* growth inhibiting activity of GA was reported about three decades ago in mouse [19] and human [20, 21] cancer cell lines. More recently, GA-induced *in vivo* activity has also been reported in various experimental cancer models [22-24].

The cytostatic effects of GA occur by decreasing cancer cell proliferation, with an accumulation of the cells in the G1 phase of the cell cycle and a concomitant decrease of cells in the proliferating S phase [23,25,26]. The cytostatic effects become cytotoxic when cell cycle arrest persists for long durations [25]. Many data from the literature link GA-induced cytotoxic effects in cancer cells to proapoptotic stimuli [22,25]. Some GA derivatives display higher proapoptotic effects than GA itself [27-30], some of which are attributable to activation of the non-steroidal anti-inflammatory drug-activated gene- (NAG-1) related proapoptotic protein [31]. The induction of apoptosis by GA relies, at least partly, on modifications of mitochondrial membrane permeability that result in cytochrome c release and caspase-3 activation [32,33]. Furthermore, GA also down-regulates H-Ras activity in cancer cells [34]. Lastly, GA disrupts F-actin extensions and down-regulates β-actin protein in cancer cells, inducing significant cytostatic (anti-proliferative) and anti-migratory effects in these cancer cells through the disruption of the actin cytoskeleton [35].

In addition, GA also inhibits efflux pumps, such as P-glycoprotein and MRP-1, and the inhibition of these drug resistance pumps increases the efficacy of various chemotherapeutic agents [36]. Cytotoxic compounds have also

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been grafted onto the GA chemical structure to improve anticancer activity [37,38].

Why Glycyrrhetinic Acid Derivatives Represent a Promising Family of Anticancer Agents?

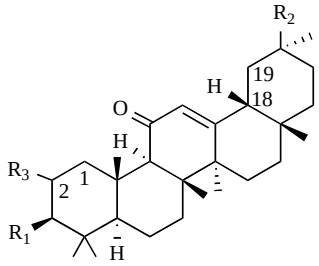
The fact that many cancers, including gliomas, melanomas, NSCLCs, esophageal cancers, pancreatic cancers and metastatic cancers, exhibit varying degrees of resistance to proapoptotic stimuli offers a potential explanation for their poor prognoses. Moreover, several reports of the anticancer effects of GA highlight its proapoptotic effects. Thus, why focus on a compound that exerts proapoptotic effects as a potential treatment for poor-prognosis cancers that are more or less resistant to proapoptotic stimuli? As discussed hereafter, it has been recently emphasized that GA and several of its derivatives inhibit proteasome activity. Moreover, some GA derivatives also activate proliferating peroxisome-activated receptors (PPARs). Notably, the proteasome and PPARs represent major targets of novel anticancer agents. Lastly, GA also displays marked anti-angiogenic effects [39].

SAR ANALYSES OF GLYCYRRHETINIC ACID AND ITS DERIVATIVES AS ANTICANCER AGENTS

The first synthesized GA derivatives enabled preliminary structure-activity relationship (SAR) analysis in terms of anticancer activity (Table **1**; Fig. **1**). Moreover, Terasawa *et al*. [40] showed that the elimination of the 11-oxo functional group led to a loss of anticancer activity. In contrast, the replacement of the carboxylic acid by a hydroxymethyl group significantly increased the *in vitro* anticancer activity (Table **1**; entry 2 versus 1). The removal of the hydroxyl group at the C-3 position, which decreases the polarity of the steroid scaffold, resulted in an increased anti-proliferative activity. Addition of oxyimino, acyloxyimino and alkoxyimino groups at the C-3 position or an ester at the C-30 position improved the anticancer efficacy of GA, mainly through the activation of apoptosis (Table **1**; entry 4 versus 1) [29]. The induction of apoptosis in cancer cells by GA derivatives containing an esterified C-30 carboxyl group and an alkylated C-3 oxime was later confirmed (Table **1**) [29]. The anti-proliferative activity in cancer cells of several 2-substituted GA derivatives was evaluated using comparative analyses with respect to ursolic, oleanolic, boswelic and betulinic acids [41]. It appears that the most effective compound in terms of *in vitro* anticancer activity are compounds containing a cyano C-2, oxidated C-3 and an esterified C-30 carboxyl group (Table **1**; entry 6) [27,42]. Additional SAR analyses underscored the importance of the alcohol functional group at C-3 and its oxidized form (Table **1**; entries 6-8) [30,43].

Several research groups have grafted cytotoxic drugs on a GA scaffold and analyzed the *in vitro* anticancer activity of the hybrid compounds (Table **2**). Taxol was thus added to the GA structure thanks to an amido link with an alkyl spacer at two different hydroxyl group of taxol (Table **2**;

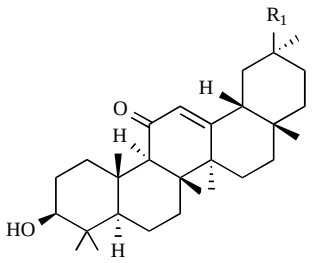
Table 1. SAR Analyses of GA Derivatives in Terms of *In Vitro* Cancer Cell Growth Inhibition



Entry	Scaffold	R1	R2	R3	IC_{50} range (μM)*	References
1	A	OH	COOH	H	63.2 ± 3.5 on HL-60 cells	[28]
2	A	OH	CH_2OH	H	80% inhibition on HeLa cells	[39]
3	A	oxyimino	$COOCH_3$	H	63.0 ± 5.8 on HL-60 cells	[28]
4	A	alkoxyimino	$COOCH_3$	H	19.0 ± 0.8 to 57.7 ± 5.5 on HL-60 cells	
5	A	acyloxyimino	$COOCH_3$	H	58.8 ± 3.0 on HL-60 cells	
6	B	oxo	$COOCH_3$	CN	0.2 - 0.5 on SW480 and HT-29 cells	[26]
7	B	OH	$COOCH_3$	CN	0.6 on KB-3-1 cells	[42]
8	C	oxo	$COOCH_3$	CN	0.9 - 7.7 on KB-3-1, A549, A431, HL-60, MCF-7, T47D and HT1080 cells	[29,42]

A. 18β-glycyrrhetinic acid derivatives.
B. 1,2 dehydro-18β-glycyrrhetinic acid derivatives.
C. 1,2,18,19 tetradehydro-glycyrrhetinic acid derivatives.
*IC_{50} range concentrations are related to the cell growth inhibition induced by the compounds described in Table 1 on different cell lines including leukemia (HL-60), uterine cancer (HeLa), colon cancer (SW480, HT-29), epidermoid carcinoma (KB-3-1), lung carcinoma (A549), skin carcinoma (A431), breast carcinoma (MCF-7, T47D) and fibrosarcoma (HT1080) cell lines.

Table 2. SAR Analyses of GA Conjugates in Terms of *In Vitro* Cancer Cell Growth Inhibitory Activity



Entry		IC_{50} value (μM)	Cell lines*	References
1	R1 = COOH (GA)	> 20	1A9, A549, MCF-7, LNCap, PC-3, DU-145, KB cell lines	[36]
2	R1 = COOH (GA)	> 20	KB, A549, 1A9, HCT-8, ZR-751, PC-3, DU-145, LN-Cap cell lines	[30]
3	R1 = spacer - 2'-taxol	< 0.1	1A9, A549, MCF-7, LNCap, PC-3, DU-145, KB cell lines	[36]
4	R1 = spacer - 7-taxol	< 1		
5	taxol	< 0.01		
6	R1 = dehydrozingerone	≤ 3	KB, A549, 1A9, HCT-8, ZR-751, PC-3, DU-145, LN-Cap cell lines	[30]
7	dehydrozingerone	> 30		
8	biotin	> 150	B16, BEL7402 cell lines	[43]

entries 3-4). *In vitro* pharmacological evaluation revealed that the hybrid compounds displayed greater *in vitro* anticancer activity than GA alone but not greater than taxol alone (Table **2**; entries 3-4 versus 1 and 5) [37]. The same strategy was applied to the dehydrozingerone compound generating an esterified C-30 bond (Table **2**; entry 6 versus 2 and 7). Moving the methoxy group of dehydrozingerone to different positions on the ring showed that the best cytotoxic activity was obtained when the methoxy group was ortho to the GA [31]. Coupling biotin to GA did not improve the *in vitro* anticancer activity (Table **2**; entry 8) [44].

From all these data, it appears that chemical modifications at C-2, C-3 and C-30 positions could increase GA anticancer activity, at least *in vitro*.

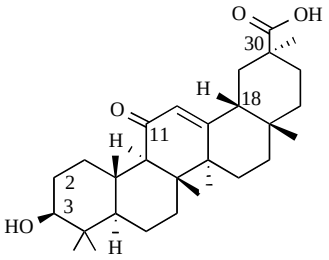


Fig. (1). Preferred positions of 18β-glycyrrhetinic acid for the synthesis of novel GA derivatives (Table **1**) or conjugates (Table **2**) with improved *in vitro* anticancer activity.

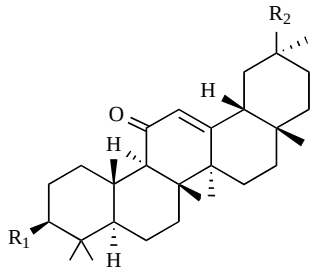
GLYCYRRHETINIC ACID DERIVATIVES AS PROTEASOME INHIBITORS: SAR ANALYSES

Proteasomes are large, multicatalytic proteinase complexes located in the cytosol and the nucleus of eukaryotic cells [45,46]. The ubiquitin-proteasome system is responsible for the degradation of most intracellular proteins and therefore plays an essential regulatory role in critical cellular processes including cell cycle progression, proliferation, differentiation, angiogenesis and apoptosis [45,46]. As emphasized by Chen and Dou [45], in addition to its involvement in normal cellular functions and homeostasis, proteasomal activity contributes to the pathology of several disorders, including inflammation, neurodegeneration and cancer [45,46]. Chen and Dou [45] further report that human cancer cells possess elevated levels of proteasome activity and are more sensitive to proteasome inhibitors than normal cells, indicating that inhibition of the ubiquitin-proteasome system could be used as a novel approach for cancer therapies. The recent approval of bortezomib, a synthetic proteasome inhibitor, for the treatment of relapsed multiple myeloma has paved the way for the discovery of drugs targeting the proteasome, ubiquitinating and deubiquitinating enzymes and of novel ways to administer them [46]. To date, various synthetic and natural products have been reported to inhibit the components of the ubiquitin-proteasome system [46], including traditional Chinese medicines [47]. The new proteasome inhibitors that are now under development include peptide boronic acid analogs MLN9708 and CEP-18770, peptide epoxyketones carfilzomib and PR-047, and NPI-0052, a β-lactone compound [48]. Targeting the ubiquitin-proteasome system using proteasome inhibitors reduces cell proliferation and induces apoptosis in solid and hematologic malignancies through multiple mechanisms, including stabilization of cell cycle regulators and proapoptotic factors, stimulation of bone morphogenetic protein signaling, inhibition of protein translation, and sensitization to ligand-induced apoptosis [49]. Proteasome inhibition bypasses, at least partly, the re-

sistance of cancer cells to apoptosis because it activates macroautophagy, a compensatory protein degradation system, and other pro-survival signaling pathways [49]. Inhibition of these auto-protective responses sensitizes cancer cells to the anti-proliferative effects of proteasome inhibitors [49].

Along these lines, GA and its derivatives represent proteasome inhibitor candidates as detailed in Table **3** [50] and graphically illustrated in Fig. (**2**).

Table 3. SAR Analyses of GA Derivatives that Inhibit Proteasome Activity



R1	R2	IC_{50} on chymotrypsine like related site (μM)
OH	COOH	> 20
alkylester	COOH	0.3 - 40
alkylester	alkylamide	3 - 40
phenylester	COOH	0.22 - 2

Reference: [49].

It must also be emphasized that compounds capable of inducing the proteasomal degradation of oncogenic products may also act as anticancer agents. This is the case for instance for compounds that induce proteasome-dependent degradation of retinoic acid receptors [51], E6 proteins of human papillomavirus types 11 and 18 [52], c-Fos [53] and H-Ras [54].

GLYCYRRHETINIC ACID DERIVATIVES AS PPAR ACTIVATORS VERSUS PPAR INHIBITORS: SAR ANALYSES

In their review, Penna *et al*. [55] report that the biological activity of peroxisome proliferators (PPs) is mediated by a class of receptors, known as PPARs (PP-activated receptors), which belong to the nuclear receptor superfamily. The PPAR subfamily is composed of three members (PPARα, PPARβ, PPARγ) that differ in their cell and tissue distribution as well as in their target genes [56,57]. Two PPARs, PPARα and PPARγ, are expressed by tumor and endothelial cells [55,56,58,59]. Upon ligand binding, PPARs dimerize with retinoid receptors, translocate to the nucleus, bind to specific PP-responsive promoter elements on target genes and transactivate gene transcription [55,57-59]. Several processes are regulated by PPARs, such as mitochondrial and peroxisomal fatty acid uptake and beta-oxidation, inflammation, intracellular lipid trafficking, cell proliferation and death. In addition, PPARs have been proposed to act as tumor suppressors or as tumor promoters, depending on the circumstances [55]. Thus, depending on cancer type, anticancer activity can be achieved by stimulating tumor-suppressing PPARs and/or inhibiting tumor-promoting PPARs.

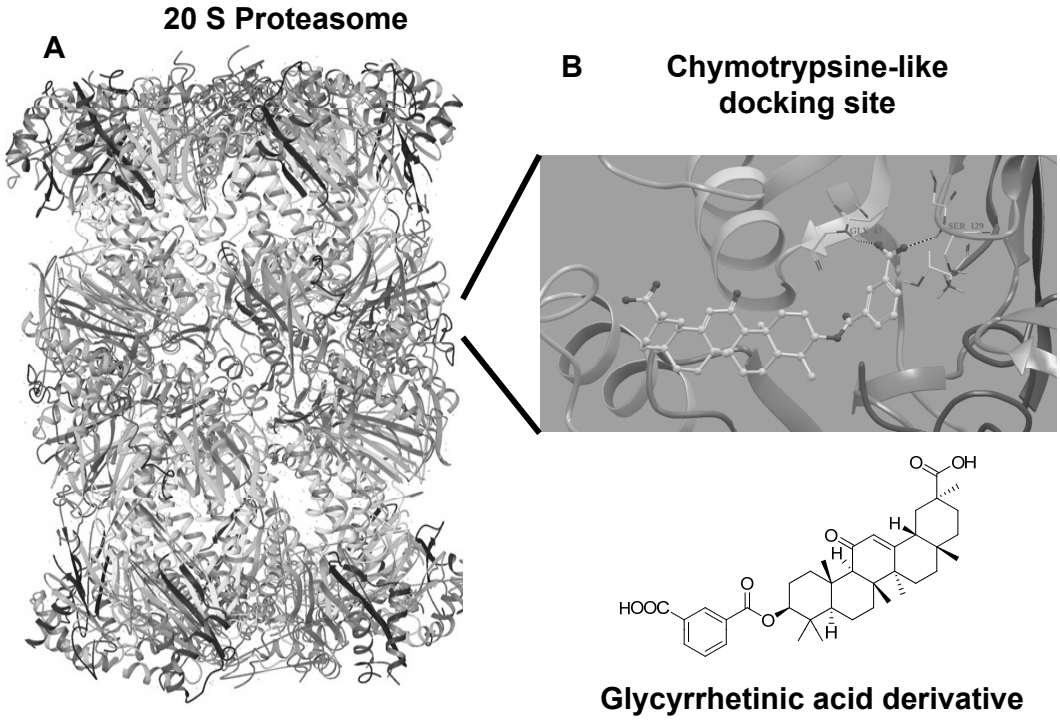
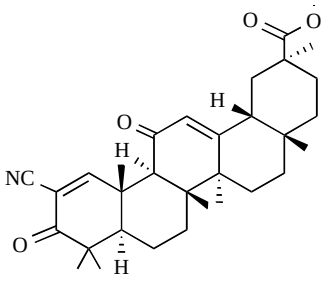


Fig. (2). (**A**) Ribbon representation of the 20S proteasome crystallographic structure (PDB code: 3HYE). (**B**) Docked position of a glycyrrhetinic acid derivative in the chymotrypsin-like site of the 3HYE structure. Two hydrogen bonds are formed with GLY47 and SER129.

Table 4. SAR Analyses for GA Derivatives that Activate PPAR-γ



Chintharlapalli S *et al*. [26] demonstrate PPAR-γ activation in colon cancer cells with 1 - 5 μM range.
Jutooru I *et al.* [37] demonstrate PPAR-γ activation in pancreatic cancer cells with 2.5 - 7.5 μM range.

Given that PPAR ligands are currently used clinically as hypolipidemic drugs with excellent tolerance and limited toxicity, PPARα activation might offer a novel and potentially low-toxic approach to treating tumor-associated angiogenesis and cancer [56]. In addition, PPARγ is expressed in cancer cells [27,28], and PPARγ ligands, including endogenous prostaglandins, and synthetic thiazolidinediones (TZDs) induce apoptosis in cancer cells [58]. Thus, PPARγ ligands have potential for both chemoprevention and chemotherapy of several types of cancer, either as single agents or as adjuncts to other antitumor agents [58].

As discussed above, GA and its derivatives target PPARs [27,28,38]. This finding opens a new avenue to combat cancers associated with poor prognoses, *i.e.*, those cancers that display various levels of resistance to proapoptotic stimuli, as detailed below.

As recently reviewed by Bundscherer *et al*. [60], tumor cells depend on and are able to modulate the tumor stroma, establishing a permissive and supportive environment of their own. Targeting the tumor stroma has evolved as a novel concept that has attracted the attention of cancer researchers focusing on the treatment of metastatic cancer [60]. The novel paradigm is that modulating the stroma may not cure the cancer, but it may make the cancer more manageable for longer periods of time by prohibiting growth beyond a certain mass [60]. Some well-established drugs, primarily designed for non-oncologic diseases, have revealed antitumor activity *via* the modulation of nuclear receptors, including stroma modulation, that stimulate pleiotropic biological effects. PPAR agonists, particularly thiazolidinedione derivatives such as pioglitazone and ciglitazone, are promising examples as they exert both direct antitumor actions and broad spectrum anti-stromal, antiangiogenic and immunomodulating activities [60].

At least two reports have already emphasized that GA derivatives target PPARs (Table **4**). Fig. (**3**) illustrates the molecular interactions between PPARs and GA.

It is interesting to note that GA and some of its derivatives display higher toxic effects in cancer than in normal cells [61, 62].

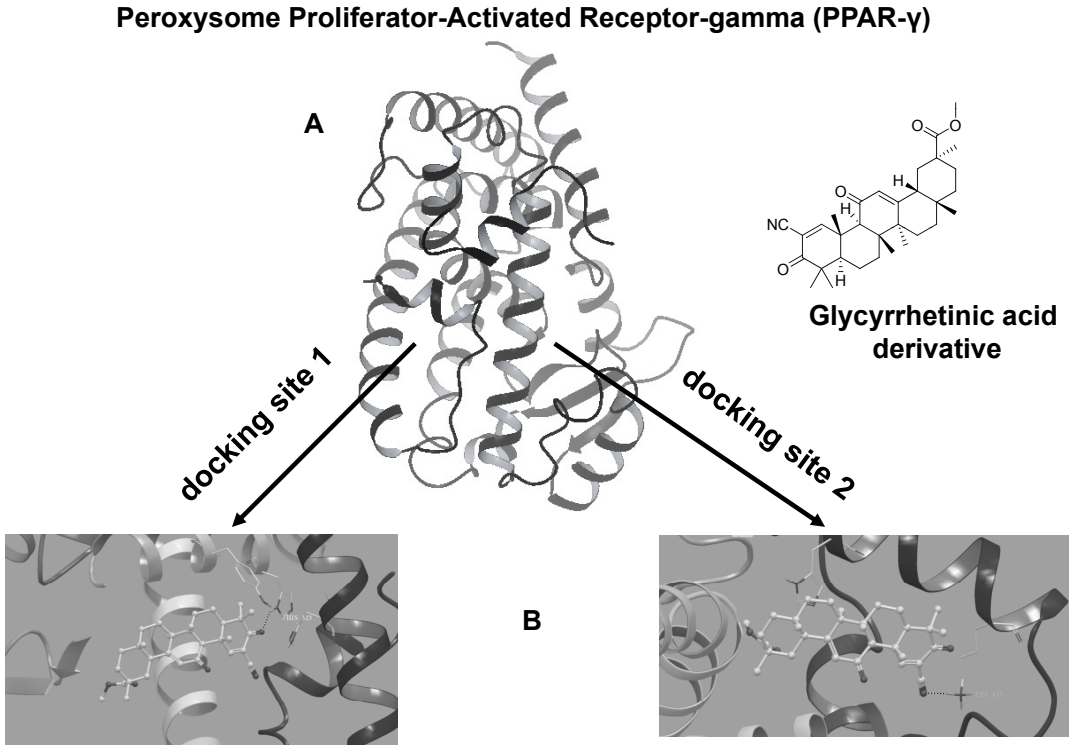


Fig. (3). (**A**) Ribbon representation of the PPARγ crystallographic structure (PDB code: 2HFP). (**B**) Docked positions of a glycyrrhetinic acid derivative in two sites of the HFP structure. In site 1, one hydrogen bond is formed with HIS323. In site 2, one hydrogen bond is formed with LYS457.

CONCLUSIONS

Because certain GA derivatives target both the proteasome and PPARs, two major targets in cancer cell biology that are independent of MDR and/or apoptosis-related resistance phenotypes, these compounds offer some hope for combating cancer types associated with poor prognoses.

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