An Overview of Structurally Modified Glycyrrhetinic Acid Derivatives as Antitumor Agents

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Abstract: Glycyrrhetinic Acid (GA), a triterpenoid aglycone component of the natural product glycyrrhizinic acid, was found to possess remarkable anti-proliferative and apoptosis-inducing activity in various cancer cell lines. Though GA was not as active as other triterpenes, such as betulinic acid and oleanolic acid, it could trigger apoptosis in tumor cells and it can be obtained easily and cheaply, which has stimulated scientific interest in using GA as a scaffold to synthesize new antitumor agents. The structural modifications of GA reported in recent decades can be divided into four groups, which include structural modifications on ring-A, ring-C, ring-E and multiple ring modifications. The lack of a comprehensive and recent review on this topic prompted us to gather more new information. This overview is dedicated to summarizing and updating the structural modification of GA to improve its antitumor activity published between 2005 and 2016. We reviewed a total of 210 GA derivatives that we encountered and compiled the most active GA derivatives along with their activity profile in different series. Furthermore, the structure activity relationships of these derivatives are briefly discussed. The included information is expected to be of benefit to further studies of structural modifications of GA to enhance its antitumor activity.

Keywords: glycyrrhetinic acid; overview; structural modification; antitumor

1. Introduction

Natural products have played a highly significant role in the medicine discovery and development processes and many useful medicines were developed from plant sources [1]. This was particularly evident in the area of cancer treatment, where over 60% of current antitumor drugs, such as vinblastine, etoposide and paclitaxel, originated from Nature [2].

Glycyrrhetinic acid (GA, Figure 1) is a triterpenoid aglycone component of the natural product glycyrrhizinic acid (GL), which is abundant in licorice root [3]. GA was proved to possess a variety of remarkable biological activities, including anti-inflammatory [4,5], antiviral [6,7], hepatoprotective [8,9], and antitumor properties [10,11]. GA is highly regarded for its remarkable antitumor activities, whereby it shows significant cytotoxic activity against a broad variety of different cell types in vitro, for example non-small cell lung cancer cells [11], pituitary adenoma cells [12], human hepatocellular carcinoma cells [13], prostate cancer cells [14] and glioblastoma cells [15]. It also exhibits noteworthy activity in various experimental cancer models in vivo [16,17], and it is known to trigger apoptosis in tumor cell lines [14,18,19]. Some experimental reports have indicated that GA triggered
apoptosis via the mitochondrial pathway through the collapse of mitochondrial membrane potential, the accumulation of the cytosolic cytochrome c and the activation of caspase-9 and caspase-3 [19,20].

![Structure of glycyrrhetinic acid.](image)

**Figure 1.** Structure of glycyrrhetinic acid.

The remarkable antitumor activity of GA has been the focus of researchers worldwide. However, because GA can inhibit type 2 11ß-hydroxysteroid dehydrogenase (11ß-HSD2), administrating GA at a high dose for a long time often causes pseudoaldosteronism, which is characterized by hypertension, hypokalemia and other adverse clinical effects [21–23]. Studies on using GA as a scaffold to develop new low-toxicity and high-effectivity antitumor agents have attracted much attention, and a number of structural modifications of GA were carried out and some reports of novel GA derivatives as antitumor agents have been published [24–26]. This overview is dedicated to summarizing and updating four aspects of the structural modification of GA leading to antitumor agents published between 2005 and 2016, including modifications at the ring-A, ring-C, ring-E and multiple ring modification. We have compiled the most active GA derivatives along with their activity profile in different series. Furthermore, the structure activity relationships of these derivatives are briefly discussed.

## 2. Four Aspects of the Structural Modifications of Glycyrrhetinic Acid

In the past few years, plenty of researchers around the world have designed and synthesized series of GA derivatives as potential antitumor agents. Most reports about the chemical and structural modifications of GA were focused on the specific functional groups of the A, C, and E rings, as these three rings contain three functional groups which are the most suitable for modification: a hydroxyl group at C-3 in ring-A, an α,β-unsaturated carbonyl function located in ring-C at C-11 and a carboxyl group at C-30 on ring-E. Meanwhile, studies on the skeleton ring architecture modification of this pentacyclic triterpene are increasing too, hence, the modifications of GA to produce novel antitumor agents can be classified into four styles, including structural modifications at ring-A, at ring-C, at ring-E and at multiple ring modifications.

### 2.1. Structural Modifications on Ring-A

#### 2.1.1. Structural Modifications at the C3-OH in Ring-A

The structural modifications at the C3-OH group of GA are very common. For example, it could be converted into an oxime group, a carbonyl group and a 3-oxo group. However, in order to change the polarity pattern or improve the antitumor activity of GA, the C-30 carboxyl group was often esterified too.

It was reported that changing the polarity pattern of GA might be an advantage in obtaining better cytotoxicity. Based on this, different C-3 amino alkyl derivatives of GA (compounds 4–11, Scheme 1,
were synthesized by Csuk et al. [27]. The antitumor activity of these derivatives was tested in a panel of 15 human cancer cell lines by a SRB assay. In the SRB assay, all of the amino compounds 4–11 showed significantly improved activity compared with GA. Among them, it could be observed that a diaminohexyl chain with seven carbon atoms was the most active derivative, about 60 times more so than GA. The antitumor activity was changed with the change of the carbon number. The results also showed that the esterification at C-30 (compound 3, Scheme 1) could improve the antitumor efficacy compared with compound 2. The same result could be found from previous findings and parallel results [28–32]. Besides, the introduction of nitrogen-containing substituents to the ring-A seemed to improve the anti-proliferative effect of GA derivatives. The cytotoxicity (IC50 values in μmol) of 1–11 in a panel of various cancer cell lines is summarized in Table 1.

![Scheme 1](image-url)

**Scheme 1.** Synthesis of the GA amino alkyl derivatives 1–11. Reagents and conditions: (a) K2CO3, CH3I, DMF, 24 h, 25 °C; (b) ClCH2COCl, Et3N, THF (or CH2Cl2), 25 °C, 12 h; (c) H2N-(CH2)n-NH2, DMF, K2CO3, 12 h, 25 °C.

| Cell Lines | GA | 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 | 7.60 |
| A253      | 80.78 | 19.42 | 25.94 | 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-11    | 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     | 80.59 | 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 | 28.07 | 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 | 25.22 | 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 | 3.40 | 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 |

Similarly, in order to change the polarity pattern of GA, Schwarz et al. [33] prepared a series of novel derivatives 12–32 by introducing an extra amino group into C-3 and esterifying at C-30 (Scheme 2). These derivatives showed a higher antitumor activity and a better selectivity towards tumor cells compared with GA on 15 different human tumor cell lines and mouse embryonic fibroblasts.
(NiH3T3). Compound 24 substituted with glycine and esterified with an i-propyl moiety was the most active compound. As discussed above for antitumor activity, in this case, the esterification at C-30 also resulted in improved activity against tumor cell lines compared with GA. The most active compound among the C-30 ester derivatives was the benzyl ester (compound 14) showing IC50 value between 6.15–23.82 μM. The decrease of the IC50 value paralleled the size and lipophilic character of the alkyl chain of the esters. From the SAR of these compounds, it was concluded that the introduction of an extra amino acid moiety at C3-OH or an alkyl group at C30-COOH could enhance the antitumor activity. There seemed to be no effect by adding a stereogenic center in the side chain according to the results. Besides, the amines and their respective ammonium salts might be considered bioequivalent in biological activity. The cytotoxicity (IC50 values in μM) of 12–32 in a panel of various cancer cell lines is summarized in Table 2.

**Scheme 2.** Synthesis of the GA amino acid derivatives 12–32. **Reagents and conditions:** (a) K2CO3, alkyl halides, DMF, 24 h, 25 °C; (b) These compounds were synthesized by DCC mediated esterification of N-Boc protected amino acids followed by their deportation using TFA in dry DCM (for the amines) or by treating them with dry HCl gas in DCM (for the ammonium hydrochlorides).

**Table 2.** Cytotoxicity (IC50 values in μM) of 12–32 in a panel of various cancer cell lines.

| Compound | 8505C | A253 | A2780 | A549 | DLD-1 | LIPO | Average |
|----------|-------|------|-------|------|-------|------|---------|
| GA       | 86.50 ± 4.20 | 80.78 ± 4.04 | 74.57 ± 3.73 | 82.76 ± 4.14 | 81.21 ± 4.06 | 81.44 ± 4.07 | 81.4 ± 4.07 |
| 12       | 24.58 ± 1.23 | 25.04 ± 1.25 | 26.96 ± 1.35 | 22.74 ± 1.14 | 28.14 ± 1.41 | 27.66 ± 1.38 | 24.39 ± 1.22 |
| 13       | 14.24 ± 0.71 | 15.76 ± 0.79 | 14.41 ± 0.72 | 27.61 ± 1.38 | 15.93 ± 0.80 | 19.21 ± 0.96 |
| 14       | 18.65 ± 0.71 | 19.67 ± 0.79 | 18.41 ± 0.72 | 22.69 ± 1.13 | 19.55 ± 0.80 | 23.76 ± 0.96 | 17.36 ± 0.89 |
| 15       | >30        | >30        | >30        | >30        | >30        | >30        | >30        |
| 16       | >30        | >30        | >30        | >30        | >30        | >30        | >30        |
| 17       | >30        | >30        | >30        | >30        | >30        | >30        | >30        |
| 18       | 7.45 ± 0.37 | 6.26 ± 0.31 | 7.99 ± 0.30 | 6.42 ± 0.32 | 8.59 ± 0.43 | 7.54 ± 0.38 | 7.04 ± 0.35 |
| 19       | 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 |
| 20       | 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 |
| 21       | 5.32 ± 0.27 | 5.39 ± 0.18 | 3.90 ± 0.20 | 5.39 ± 0.27 | 5.61 ± 0.28 | 4.32 ± 0.22 | 4.69 ± 0.23 |
| 22       | 3.87 ± 0.22 | 3.23 ± 0.12 | 2.59 ± 0.13 | 3.43 ± 0.17 | 3.72 ± 0.19 | 2.74 ± 0.14 | 3.11 ± 0.16 |
| 23       | 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 |
| 24       | 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 |
| 25       | 3.49 ± 0.17 | 3.51 ± 0.18 | 2.08 ± 0.10 | 3.43 ± 0.17 | 5.54 ± 0.28 | 3.55 ± 0.19 | 3.60 ± 0.18 |
| 26       | 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 |
| 27       | 4.79 ± 0.24 | 5.03 ± 0.25 | 5.54 ± 0.18 | 5.07 ± 0.25 | 5.45 ± 0.23 | 4.81 ± 0.24 | 4.63 ± 0.23 |
| 28       | 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 |
| 29       | 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 |
| 30       | >30        | >30        | >30        | >30        | >30        | >30        | >30        |
| 31       | >30        | >30        | >30        | >30        | >30        | >30        | >30        |
| 32       | >30        | >30        | >30        | >30        | >30        | >30        | >30        |

In subsequent research Csuk et al. conducted another study in a similar manner, producing a series of derivatives 33–44 substituted with aspartic and glutamic acid (Scheme 3) [34]. The glutamic acid derivative 36 with a benzyl-protected side chain was the most active derivative among this series,
showing an IC₅₀ value between 1.27–2.33 μM. Meanwhile, compound 36 displayed an extraordinary selectivity (Mean F = 23) in comparison with other compounds. The derivatives carrying a free amino group and an unprotected carboxylic group such as compounds 39 and 40 turned out to be inactive (IC₅₀ > 100 μM). The cytotoxicity (IC₅₀ values in μM) of 33–40, 43, 44 in a panel of various cancer cell lines is summarized in Table 3.

| Cell Lines | 35 | 36 | 37 | 38 | 39 | 40 | 43 | 44 |
|------------|----|----|----|----|----|----|----|----|
| 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 ± 0.45 | 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 | 30.47 ± 1.52 | 53.07 ± 2.65 |
| 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 | 31.59 ± 1.58 | 60.96 ± 3.05 |
| SW1736     | 16.68 ± 0.83 | 2.33 ± 0.12 | 17.13 ± 0.86 | 19.24 ± 0.96 | >100 | >100 | 40.62 ± 2.03 | 54.77 ± 2.74 |
| Average    | 10.88 ± 0.54 | 1.69 ± 0.08 | 17.06 ± 0.85 | 17.90 ± 0.90 | >100 | >100 | 30.93 ± 1.55 | 46.77 ± 2.34 |
| NIH3T3     | 14.74 ± 0.74 | 39.09 ± 1.95 | 23.09 ± 1.15 | 24.42 ± 1.22 | >100 | >100 | 31.59 ± 1.58 | 60.96 ± 3.05 |
| F          | 1.38 | 23.13 | 1.35 | 1.36 | 1.36 | 0.55 | 0.72 |

As mentioned, introduction an extra amino group into C-3 and esterification at C-30 could improve the antitumor activity of GA derivatives. To further increase the cytotoxicity and improve the selectivity, some other amino acid derivatives of glycyrrhetinic acid 45–59 (Scheme 4) were designed and synthesized in a similar way by Csuk et al. [35]. The derivatives possessing short side chains like the alanyloxy or sarcosyloxy moiety, turned out to exhibit higher cytotoxic activity, for example, compound 46 showed IC₅₀ values between 1.83 and 3.42 μM. However compounds with a more lipophilic side chains, such as compound 50, 51 showed decreased cytotoxic effects compared with GA–Me in the SRB assay. These results indicated that the structure of the amino acid side chain
affected the cytotoxicity most. The cytotoxicity (IC₅₀ values in μM) of 45–59 on a panel of various cancer cell lines is summarized in Table 4.

Table 4. Cytotoxicity (IC₅₀ values in μM) of 45–59 in a panel of various cancer cell lines.

| Compound | 8505C | A253 | A2780 | A549 | DLD-1 | LIPO | MCF-7 |
|----------|-------|------|-------|------|-------|------|-------|
| 45       | 2.92  | 2.26 | 2.24  | 2.26 | 3.35  | 3.56 | 2.25  |
| 46       | 2.50  | 2.46 | 1.83  | 2.13 | 3.42  | 2.50 | 2.49  |
| 47       | 9.62  | 5.56 | 4.58  | 6.91 | 11.64 | 7.96 | 5.49  |
| 48       | 16.93 | 6.41 | 5.50  | 9.94 | 8.70  | 16.15| 4.60  |
| 49       | 11.47 | 7.48 | 12.56 | 14.48| 12.45 | 22.32| 6.06  |
| 50       | >30   | >30  | >30   | >30  | >30   | >30  | >30   |
| 51       | >30   | >30  | >30   | >30  | >30   | >30  | >30   |
| 52       | >30   | >30  | >30   | >30  | >30   | >30  | >30   |
| 53       | 3.47  | 3.41 | 2.13  | 3.39 | 3.41  | 3.54 | 2.73  |
| 54       | 3.52  | 3.52 | 2.48  | 3.38 | 4.49  | 4.54 | 3.40  |
| 55       | 5.48  | 4.05 | 4.94  | 5.43 | 6.27  | 5.95 | 4.03  |
| 56       | 4.02  | 3.76 | 4.06  | 3.88 | 4.38  | 4.02 | 2.46  |
| 57       | 2.89  | 4.04 | 2.59  | 2.35 | 1.48  | 0.80 | 3.01  |
| 58       | 2.49  | 2.21 | 1.98  | 2.53 | 3.01  | 2.70 | 1.55  |
| 59       | 2.40  | 2.43 | 1.58  | 2.43 | 2.27  | 2.51 | 1.75  |

It was reported that the introduction of an extra hydrophilic sugar moiety into betulinic acid could increase its cytotoxicity [36]. Inspired by this, Schwarz et al. [37] prepared some GA glycoside structural analogues 60–66 (Scheme 5) utilizing methyl glycyrrhetinate (compound 1, Scheme 1) as starting material.

Scheme 4. Synthesis of the GA–Me (GA methyl ester) amino ester derivatives 45–59. Reagents and conditions: (a) Boc-amino acids, DCM, DMAP, DCC, 12 h, 25 °C; (b) TFA in DCM, 12 h, 25 °C, or HCl (gas) in DCM, 12 h, 25 °C.

Scheme 5. Synthesis of the GA glycosides derivatives 60–66. Reagents and conditions: (a) Sugar trichloroacetimidate, TMSOTf, DCM, −70 °C–25 °C, 2 h.

Their antitumor activity was evaluated in a SRB assay on various tumor cell lines. These derivatizations did not result in increased cytotoxicity, with the exception of compound 64 which...
showed IC\textsubscript{50} values as low as 9.48 \(\mu\)M on breast carcinoma MCF-7 cells, which was twice the activity of GA-Me. It seemed that there was no correlation between the monosaccharide structure and the cytotoxicity, and similar results could also be found in [36,38,39]. The cytotoxicity (IC\textsubscript{50} values in \(\mu\)M) of 60–66 in a panel of various cancer cell lines is summarized in Table 5.

Table 5. Cytotoxicity (IC\textsubscript{50} values in \(\mu\)M) of 60–66 in a panel of various cancer cell lines (NA = not active).

| Cell Lines | 60 | 61 | 62 | 63 | 64 | 65 | 66 | GA-Me |
|------------|----|----|----|----|----|----|----|------|
| SW1736     | NA | NA | NA | 23.87 ± 1.3 | 11.18 ± 0.9 | 21.38 ± 1.9 | NA | 34.87 ± 1.2 |
| MCF-7      | NA | 16.7 ± 1.4 | 19.60 ± 1.4 | NA | 9.48 ± 1.4 | 20.11 ± 1.3 | NA | 22.14 ± 0.9 |
| LIPO       | NA | NA | NA | 28.45 ± 2.1 | NA | 23.23 ± 1.3 | NA | 20.47 ± 1.1 |
| DLD-1      | NA | NA | NA | NA | 23.18 ± 1.7 | NA | 26.12 ± 1.0 |
| A253       | NA | NA | NA | 27.25 ± 1.8 | 13.16 ± 0.9 | 19.70 ± 1.4 | NA | 19.42 ± 1.1 |
| 8505C      | NA | NA | NA | NA | 21.97 ± 0.6 | 22.77 ± 1.4 | NA | 26.07 ± 1.3 |
| 518A2      | NA | NA | NA | 28.92 ± 2.0 | 25.95 ± 0.8 | 23.26 ± 1.2 | NA | 27.54 ± 1.0 |
| NIH3T3     | NA | NA | NA | NA | 23.45 ± 0.1 | NA | 22.81 ± 0.6 |

Lai et al. [40] designed and synthesized a series of novel furan-based nitric oxide (NO)-releasing derivatives of GA 68–74 (Scheme 6) as antitumor agents. According to the MTT assay results, compounds 68–74 displayed increased anti-HCC (HepG2, BEL-7402) activity (IC\textsubscript{50} 2.90–36.52 \(\mu\)M on HepG2, IC\textsubscript{50} 2.94–19.92 \(\mu\)M on BEL-7402) compared with GA (IC\textsubscript{50} > 50 \(\mu\)M on HepG2, BEL-7402). The most active compound was 74, showing IC\textsubscript{50} values as low as 2.90 \(\mu\)M, 2.94 \(\mu\)M on HepG2 and BEL-7402, respectively. These findings might provide more information for the design of new chemotherapeutic reagents for the intervention on human HCC in the clinic. The cytotoxicity (IC\textsubscript{50} values in \(\mu\)M) of 68–74 in a panel of various cancer cell lines is summarized in Table 6.

Scheme 6. Synthesis of the GA furan-based nitric oxide (NO)-releasing derivatives 67–74. Reagents and conditions: (a) CH\textsubscript{3}OH, p-TSA; (b) succinic anhydride, DMAP, dry DCM, 15 h; (c) phenylsulfonyl furans, DCC, DMAP, dry DCM, 24 h.

Table 6. Cytotoxicity (IC\textsubscript{50} values in \(\mu\)M) of 68–74 in a panel of various cancer cell lines.

| Cell Lines | GA | 68 | 69 | 70 | 71 | 72 | 73 | 74 |
|------------|----|----|----|----|----|----|----|----|
| HepG2      | >50| 18.18| 13.41| 26.03| 36.52| 15.67| 7.90| 2.90|
| BEL-7402   | >50| 7.85| 9.22| 6.03| 8.20| 19.92| 7.37| 2.94|

After forming long chains with ester bonds at C-3, Kumar Yadav et al. [41] found the GA-1, GA-2 and GA-3 (Figure 2) expressed significant antitumor activity against the human lung cancer cell line
A-549 with pred. log IC$_{50}$ = 1.182, 1.044, 1.274 µM according to the quantitative structure-activity relationship (QSAR) model. The cytotoxicity (IC$_{50}$ values in µM) of GA-1, GA-2 and GA-3 on A-549 is summarized in Table 7.

Table 7. Cytotoxicity (IC$_{50}$ values in µM) of GA-1, GA-2 and GA-3 in A-549.

| Cell Lines | GA-1 | GA-2 | GA-3 |
|------------|------|------|------|
| A549       | 1.182| 1.044| 1.274|

2.1.2. Structural Modifications at the Skeleton of Ring-A

Previous studies revealed that some triterpenoid derivatives which contained a 2-cyano-1-en-3-one functionality on ring-A, such as the oleanoic acid derivatives CDDO (Figure 3) and its methyl ester CDDO-Me (Figure 3), exerted potent cytotoxic activity in various cancer cell lines [42,43]. Similar results were also obtained with GA and betulinic acid derivatives containing a 2-cyano-1-en-3-one function, for example β-CDODA-Me [44,45] (Figure 3). Inspired by this, Chadalapaka et al. [31] synthesized some β-CDODA-Me analogs 75–79 (Scheme 7) with different electronegative 2-substituents including iodo, cyano, trifluoromethyl, dimethylphosphonyl and methanesulfonyl groups. The cell culture studies showed that the anti-proliferative activity of methyl derivative (β-CDODA-Me) on bladder and pancreatic cancer cells was more potent than that of the free acid (β-CDODA). This was consistent with a previous report [46]. Among the derivatives, 2-cyano and 2-trifluoromethyl ones showed the highest anti-proliferation activity. However, compound 79 and compound 77 were relatively inactive, showing higher IC$_{50}$ values ranging from 3.34 to 11.97 µM than the corresponding 2-cyano and 2-trifluoromethyl derivatives on the four cell lines. It could be seen that their relative potencies were dependent on the cell context: 2-trifluoromethyl derivative (compound 78) (IC$_{50}$ 0.38 µM in KU7, IC$_{50}$ 0.82 µM in Panc-1, IC$_{50}$ 1.14 µM in Panc-28) was more active than β-CDODA-Me (IC$_{50}$ 1.59 µM in KU7, IC$_{50}$ 1.22 µM in Panc-1, IC$_{50}$ 1.80 µM in Panc-28), whereas β-CDODA-Me was more active in 253JB-V cells, showing IC$_{50}$ values as low as 0.25 µM, lower than that of the compound 78 (IC$_{50}$ 0.67 µM). The results provided a new way for the structural modifications of GA. The cytotoxicity (IC$_{50}$ values in µM) of 76–79 in a panel of various cancer cell lines is summarized in Table 8.

Figure 2. Structures of GA-1, GA-2 and GA-3.

Figure 3. Structures of CDDO, CDDO-Me, β-CDODA and β-CDODA-Me.
The preliminary pharmacological study showed compound GA as precursor and synthesized a series of derivatives, which was summarized in Table 9. Deoxidized GA derivatives 80–82 and oxidized GA derivatives 83–85 did not show any significant antitumor activity. Acetylated GA derivatives 86 and 97 were relatively active, showing IC50 < 20 μM in several tested cancer cell lines. The cytotoxicity (IC50 values in μM) of 80–95, 97 in a panel of various cancer cell lines is summarized in Table 9.

In order to alter the lipophilicity of GA, several functional modifications were carried out at the C-2 and/or C-3 positions in ring-A by Csuk et al. [46] and a series of derivatives 80–97 (Scheme 8) were obtained. Their cytotoxicity was investigated on eight different human tumor cell lines. According to the SRB assays, most of the derivatives showed lower antitumor activity than GA. Acetylated GA derivatives 80–82 did not show any significant antitumor activity. Deoxidized GA derivatives 86 and 97 were relatively active, showing IC50 < 20 μM in several tested cancer cell lines. The cytotoxicity (IC50 values in μM) of 80–95, 97 in a panel of various cancer cell lines is summarized in Table 9.

In the search of new GA derivatives as antitumor agents, Jun et al. [47] employed GA as precursor and synthesized a series of GA derivatives 98–112 (Scheme 9) with major changes to ring-A. The preliminary pharmacological study showed compound 98, 100, 101, 105, 106, 110 with hydroxyl

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**Scheme 7.** Synthesis of the GA 2-substituted derivatives 75–79. Reagents and conditions: (a) CH2N2, Et2O, 0 °C; (b) IBX, DMSO, 21 h, 80–85 °C; (c) iodine, pyridine, tetrahydrofuran; (d) CuCN, NMP, 2 h, 130 °C; (e) CH3SO3Na, CuI, DMSO, 20 h, 120–125 °C; (f) CuI, methyl-2,2-difluoro-2- (fluorosulfonyl) acetate, DMF/HMPT, 20 h, 70 °C; (g) dimethyl phosphite, Cs2CO3, N,N-dimethylethylenediamine, toluene, 26 h, 95–100 °C.

**Table 8.** Cytotoxicity (IC50 values in μM) of 76–79 and β-CDODA-Me in a panel of various cancer cell lines.

| Compound | 253JB-V | KU7 | Panc-1 | Panc-28 |
|----------|---------|-----|--------|---------|
| 76       | 2.67    | 3.04| 4.08   | 12.75   |
| 77       | 11.97   | 3.34| 7.69   | 9.75    |
| 78       | 0.67    | 0.38| 0.82   | 1.14    |
| 79       | 7.90    | 3.73| 6.11   | 8.14    |
| β-CDODA-Me | 0.25  | 1.59| 1.22   | 1.80    |
groups displayed some cytotoxicity on HepG-2. The derivative 105 with two hydroxyl groups at C-2 and C-3 displayed more potent activity than GA showing IC\textsubscript{50} as low as 0.22 \( \mu \)M on HepG-2.

It seemed that the number and location of hydroxyl groups in ring-A had an important influence on the antitumor activity of GA derivatives. The cytotoxicity (IC\textsubscript{50} values in \( \mu \)M) of 98–112 on HepG-2 os summarized in Table 10.

Scheme 8. Synthesis of the C-2 and C-3 modified GA derivatives 80–97. Reagents and conditions: (a) AcCl, pyridine, CH\(_2\)Cl\(_2\), 2 h, 25 °C; (b) Jones reagent, 20–60 min, 25 °C; (c) KOH, hydrazine, ethylene glycol, 24 h, 200 °C; (d) periodic acid, DMSO, 3 days, −50 °C; (e) HOAc, p-TsOH, 24 h, 80 °C; (f) MeSO\(_2\)Cl, pyridine (or Et\(_3\)N for 15), 1–70 h, 25 °C; (g) for 92: K\(_2\)CO\(_3\), DMF, 24 h, 120 °C; for 93: Bu\(_4\)NF, DMF, 4 days, 102 °C; for 94: PPh\(_3\), 3,3-dimethylglutarimide, DEAD, THF, 24 h, 25 °C; (h) m-CPBA, CH\(_2\)Cl\(_2\), 20 h, 25 °C; (i) 1,1′-thiocarbonyldiimidazole, 1,2-dichloroethane, 70 h, 100 °C; (j) Bu\(_3\)SnH, AIBN (cat.), toluene, 40 h, 115 °C.
Table 9. Cytotoxicity (IC\textsubscript{50} values in \(\mu\text{M}\)) of 80–95, 97 in a panel of various cancer cell lines.

| Compound | 518A2 | 8505C | A2780 | A549 | DLD-1 | LIPO | MCF-7 | SW1736 |
|----------|-------|-------|-------|------|-------|------|-------|--------|
| 80–85    | >30   | >30   | >30   | >30  | >30   | >30  | >30   | >30    |
| 86       | 18.33 | 19.28 | 26.83 | >30  | >30   | 28.74| 21.87 | 16.56  |
| 87       | 29.82 | 27.69 | 14.84 | 26.62| 29.56 | 24.80| 28.68 | 27.00  |
| 88       | >30   | >30   | >30   | >30  | >30   | >30  | >30   | >30    |
| 89       | >30   | >30   | 14.95 | >30  | >30   | >30  | >30   | >30    |
| 90–92    | >30   | >30   | >30   | >30  | >30   | >30  | >30   | >30    |
| 93       | >30   | >30   | 23.69 | 24.30| >30   | 25.52| >30   | 16.98  |
| 94, 95   | >30   | >30   | >30   | >30  | >30   | >30  | >30   | >30    |
| 97       | 23.69 | 24.30 | 10.39 | >30  | >30   | >30  | >30   | >30    |

Scheme 9. Synthesis of ring A modified GA derivatives 98–112. Reagents and conditions: (a) Jones’ reagent; (b) HCO\textsubscript{2}Et, NaOMe; (c) NaOMe, \(\text{H}_2\text{O}_2\); (d) \(\text{t-ButOK/}\text{t-ButOH, n-ButONO}\); (e) NaBH\textsubscript{4}; (f) \(\text{p-TsCl}\); (g) \(\text{CH}_3\text{I, K}_2\text{CO}_3\); (h) LiBr, \(\text{Li}_2\text{CO}_3\); (i) \(\text{m-CPBA, K}_2\text{CO}_3\); (j) \(\text{HClO}_4\); (k) KOH; (l) \(\text{m-CPBA, NaHCO}_3\); (m) NaOMe; (n) NH\textsubscript{2}OH·HCl; (o) p-TsCl, DMAP.
2.2. Structural Modifications on Ring-C

The studies on structural modifications at ring-C were mainly focused on the carbonyl function located at C-11. According to Fiore and Salvi [48,49], a ketone group at position C-11 was the primary cause for the apoptotic activity of GA derivatives, but the research conducted by Csuk et al. [50] showed that there was no direct relation between the presence of the C-11 ketone group and the apoptotic activity of the compounds. Also, esterification at C-30 was important, as mentioned above. Six compounds (Scheme 10) were tested in a SRB assay for cytotoxicity screening on 12 tumor cell lines and mouse embryonic fibroblasts (NIH3T3) which showed that GA and compound 113 nearly had the same activity on tumor cells, but after esterification at C-30, compounds 1 and 114 showed a relatively high cytotoxicity against the tested tumor cell lines. For the fibroblasts and most of the tumor cell lines, the toxicity of compound 114 was reduced, while the cytotoxic effect on the tumor cells of compounds 12 and 115 was similar to their effect on NIH3T3 cells. However, according to Lin et al. [51], when GA was converted into 11-DOGA, it showed higher toxicity toward gastric cancer cells both in vivo and in vitro, so the relation between the existence of the C-11 ketone group and the apoptotic activity should be further studied. The cytotoxicity (IC50 values in µM) of 1, 12, 113–115 in a panel of various cancer cell lines is summarized in Table 11.

Table 10. Cytotoxicity (IC50 values in µM) of 98–112 in a panel of various cancer cell lines.

| Cell Lines | 98  | 99  | 100 | 101 | 102 | 103 | 104 | 105 |
|------------|-----|-----|-----|-----|-----|-----|-----|-----|
| HepG-2     | 61.70 | >100 | 71.83 | 47.12 | >100 | >100 | >100 | 0.22 |
|            | 106  | 107  | 108  | 109  | 110 | 111 | 112 |     |
| HepG-2     | 59.98 | >100 | >100 | >100 | 88.68 | >100 | >100 |     |

Scheme 10. Synthesis of ring C modified GA derivatives 113–115. Reagents and conditions: (a) Zinc dust, conc. HCl, dioxane, 25 °C, 24 h; (b) MeI, K2CO3, DMF, 25 °C, 24 h; (c) BH3-THF, THF, citric acid, 25 °C, 20 h; (d) EtI, K2CO3, DMF, 25 °C, 24 h; (e) BH3-THF, THF, Na2CO3, 25 °C, 4 days.

Table 11. Cytotoxicity (IC50 values in µM) of 1, 12, 113–115 in a panel of various cancer cell lines.

| Cell Lines | GA  | 113 | 1  | 114 | 12  | 115 |
|------------|-----|-----|----|-----|-----|-----|
| 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 | 25.23 | 51.52 |
| A431       | 79.58 | 86.13 | 25.28 | 33.88 | 24.58 | 52.80 |
| 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 |
Table 11. Cont.

| Cell Lines | GA 113 | 1 | 114 | 12 | 115 |
|------------|--------|---|-----|----|-----|
| HCT-116    | 78.83  | 22.10 | 31.82 | 21.58 | 47.78 |
| HCT-8      | 78.85  | 24.36 | 31.34 | 43.42 | 44.32 |
| HT-29      | 80.09  | 27.54 | 34.81 | 22.14 | 44.42 |
| LIPO       | 81.44  | 20.47 | 34.87 | 27.66 | 52.80 |
| MCF-7      | 84.70  | 22.14 | 34.37 | 18.61 | 48.97 |
| SW1736     | 76.93  | 34.87 | 32.35 | 13.37 | 45.48 |
| NIH3T3     | 18.52  | 22.81 | 42.22 | 23.66 | 43.16 |

2.3. Structural Modifications on Ring-E

The C-30 position in GA has been widely exploited and hundreds of derivatives have been reported in the literature. To increase the antitumor activity of GA and to obtain potent cytostatic compounds, Lallemand et al. [52] synthesized a series of GA amide derivatives 116–130 (Scheme 11) by coupling GA with various amines. The antitumor activity screening showed that compound 127 appeared to be the most potent one, with single-digit micro molarity IC50 values in a panel of eight cancer cell lines. Further pharmacokinetic studies by the same group suggested that compound 127 was rapidly distributed (t1/2 dist of ~3 min) but slowly eliminated (t1/2 elim = ~77 min). This study was helpful in producing this kind of GA antitumor derivatives.

Scheme 11. Synthesis of ring E modified GA derivatives 116–130. Reagents and conditions: (a) 1. DCC, HOBt, DIPEA, DMF, r.t., 30 min; 2. R1NH2, r.t., overnight; (b) 1. DCC, HOBt, DIPEA, DMF, r.t., 30 min; 2. H2N(CH2)2NHBoc, r.t., overnight; (c) TFA, DCM, 0 °C, 3 h; (d) DMAP, RCOCl, DCM; (e) THF, RNCO, r.t., 20 h; (f) THF, RNCS, r.t., 20 h; (g) Jones reagent, acetone, 0 °C, 45 min.
Similarly, Shi et al. [53] synthesized biotinylated GA (BGA) by introducing biotin into the C-30 carboxyl of GA, and evaluated its antitumor effects on mouse B16 melanoma cells and BEL 7402 cells. The result showed that the biotin group in BGA had no influence on the antitumor effects of GA. The cytotoxicity (IC$_{50}$ values in µM) of 116–130 in a panel of various cancer cell lines is summarized in Table 12.

**Table 12.** Cytotoxicity (IC$_{50}$ values in µM) of 116–130 in a panel of various cancer cell lines.

| Compound | A549 | SKMEL | T98G | HS683 | U373 | PC3 | MCF7 | 816F10 |
|----------|------|-------|------|--------|------|-----|------|--------|
| GA       | >100 | 92    | 85   | 84     | 83   | 80  | 76   | 37     |
| 116      | 52   | >100  | 91   | 59     | 43   | 34  | 34   | 37     |
| 117      | 40   | >100  | >100 | 57     | 75   | 43  | 38   | 31     |
| 118      | 33   | 82    | 46   | 56     | 42   | 33  | 31   | 32     |
| 119      | 43   | 60    | 73   | 63     | 57   | 41  | 37   | 48     |
| 120      | 31   | >100  | >100 | 58     | 32   | 31  | 31   | 59     |
| 121      | 47   | 49    | 62   | 38     | 55   | 53  | 28   | 36     |
| 122      | 63   | 42    | 77   | 58     | 75   | 72  | 46   | 31     |
| 123      | 37   | 38    | 54   | 36     | 37   | 47  | 30   | 31     |
| 124      | 68   | 35    | 77   | 67     | 76   | 72  | 27   | 31     |
| 125      | 28   | 37    | 35   | 31     | 29   | 30  | 25   | 28     |
| 126      | 29   | 49    | 30   | 28     | 30   | 32  | 28   | 31     |
| 127      | 7    | 9     | 12   | 6      | 6    | 8   | 4    | 4      |
| 128      | 29   | 65    | 71   | 42     | 42   | 46  | 42   | 41     |
| 129      | 31   | 38    | 25   | 8      | 29   | 9   | 30   | 34     |
| 130      | 38   | 33    | 35   | 36     | 35   | 39  | 30   | 33     |

Guided by previous results indicating that incorporation of a stable nitroxyl radical or amino acids into antitumor molecules could increase their activity and decrease their toxicity [34,54,55], Liu et al. [56] designed and synthesized a series of GA derivatives 131–140 (Scheme 12) by introducing a nitroxyl functionality and amino acid segments into GA.

![Scheme 12. Synthesis of ring E modified GA derivatives 131–155. Reagents and conditions: (a) (i) amino acid methyl ester EDCI/HOBt/Et$_3$N, DMF; (ii) 4N NaOH THF/MeOH; (b) EDCI/HOBt/Et$_3$N DMF, r.t., overnight; (c) EDCI/HOBt/Et$_3$N DMF, r.t., overnight.](image-url)
The in vitro cytotoxicity screening showed that compounds 131–140 with only various free amino acids at C-30 showed no significant cytotoxicity (GI_{50} > 70 \mu M). However, incorporation of a piperidine (compounds 141–150) or pyrroline (compounds 151–155) nitroxyl radical at the terminus of the C-30 side chains could significantly enhance the cytotoxic effects. Among the new derivatives, compound 150 with a tryptophan amino moiety and a piperidine nitroxyl radical showed the greatest cytotoxicity (GI_{50} 13.7–15.0 \mu M), five-fold more potent than GA. These results suggested that the incorporation of a nitroxyl functionality and amino acid segments into the C-30 carboxyl group of GA might contribute to improve its cytotoxicity. The cytotoxicity (GI_{50} values in \mu M) of 141–155 in a panel of various cancer cell lines is summarized in Table 13.

**Table 13.** Cytotoxicity (GI_{50} values in \mu M) of 141–155 in a panel of various cancer cell lines.

| Compound | A549 | DU145 | KB | Kbvin |
|----------|------|-------|----|-------|
| GA       | 61.2 ± 2.33 | 64.9 ± 0.505 | 61.2 ± 0.118 | 62.3 ± 1.41 |
| 141      | >70  | >70   | >70 | >70   |
| 142      | >70  | >70   | >70 | >70   |
| 143      | 19.4 ± 0.909 | 19.3 ± 0.292 | 14.6 ± 0.448 | 14.9 ± 0.471 |
| 144      | 34.2 ± 1.88 | 28.9 ± 0.921 | 17.5 ± 0.927 | 18.6 ± 0.931 |
| 145      | 23.3 ± 0.304 | 21.7 ± 0.402 | 16.9 ± 0.501 | 19.2 ± 0.497 |
| 146      | 44.0 ± 0.057 | 45.5 ± 0.666 | 39.9 ± 0.618 | 47.6 ± 1.06  |
| 147      | 18.3 ± 0.373 | 17.4 ± 0.619 | 15.3 ± 0.469 | 19.5 ± 1.33  |
| 148      | >70  | >70   | >70 | >70   |
| 149      | 19.6 ± 1.60 | 22.0 ± 0.546 | 16.0 ± 0.368 | 17.0 ± 0.377 |
| 150      | 15.0 ± 0.689 | 15.0 ± 0.363 | 14.2 ± 0.670 | 13.7 ± 1.25  |
| 151      | 46.7 ± 1.90 | 46.2 ± 0.697 | 45.5 ± 1.04  | 46.9 ± 0.230 |
| 152      | 46.1 ± 0.653 | 45.2 ± 1.27 | 41.3 ± 0.346 | 44.2 ± 0.280 |
| 153      | 19.0 ± 1.13 | 22.5 ± 0.606 | 17.8 ± 0.193 | 16.6 ± 0.591 |
| 154      | 34.5 ± 0.187 | 39.5 ± 1.05 | 30.7 ± 0.480 | 27.3 ± 0.338 |
| 155      | 41.5 ± 1.83 | 43.2 ± 1.61 | 38.4 ± 1.15  | 38.5 ± 0.956 |

Inspired by previous studies indicating that esterification of glycyrrhetinic acid (GA) with dehydrozingerone (DZ) resulted in a novel cytotoxic GA–DZ conjugate, Tatsuzaki et al. [57] synthesized a series of triterpenoid—dehydrozingerone derivatives by combining DZ analogs with different triterpenoids, such as oleanolic acid (OA), ursolic acid (UA), glycyrrhetinic acid (GA).

The in vitro antitumor assay indicated that most of the GA–DZ conjugates 156–166 (Scheme 13) showed significant antitumor activity. In particular, compounds 156–158 exhibited prominent cytotoxicity against LN-Cap, 1A9, and KB cells with ED_{50} values of 0.6, 0.8 and 0.9 \mu M. However, similar conjugates between DZ and OA or UA were inactive suggesting that the GA component was critical for activity. The cytotoxicity (ED_{50} values in \mu M) of 156–166 in a panel of various cancer cell lines is summarized in Table 14.

**Scheme 13.** Syntheses of GA–DZ derivatives 156–166. Reagents and conditions: (a) \( \text{R}_2 \), 1N NaOH (for \( R_2 = \text{Me} \)), 5N KOH (for \( R_2 = \text{Ph} \)); (b) GA, EDCI, DMAP, CH_2Cl_2.
Scheme 15 exhibited high cytotoxic activity. In particular, compound 156 showed no cytotoxic activity (IC<sub>50</sub> = 1.88 µM) on SW1736 cells (IC<sub>50</sub> = 1.88 µM), while compound 175 esterified at C-30 and etherified at C-3 almost showed no cytotoxic activity (IC<sub>50</sub> > 30 µM) against seven tested human tumor cell lines. This suggested that not only the type of the chemical bonding but also the position of substituent groups affects the antitumor activity. This study greatly enriched the modification strategy of the carbonyl group. The cytotoxicity (IC<sub>50</sub> values in µM) of 167–169 in a panel of various cancer cell lines is summarized in Table 15.

**Table 14. Cytotoxicity (ED<sub>50</sub> values in µM) of 156–166 in a panel of various cancer cell lines.**

| Compound | KB | KB-VIN | A549 | 1A9 | HCT-8 | ZR-751 | PC-3 | DU-145 | LN-Cap |
|----------|----|--------|------|-----|-------|--------|------|--------|--------|
| GA       | >21| >21    | NA   | >21 | 19.5  | NA     | >21  | >21    | >21    |
| DZ       | NA | NA     | >52  | 33.9| >52   | >52    | >52  | 51     |        |
| 156      | 1.6| 2.5    | 2    | 0.9 | 1.7   | 2.8    | 1.4  | 3.1    | 0.6    |
| 157      | 0.8| 2.8    | 2.2  | 0.8 | 1.9   | 3      | 1.1  | 3.6    | 2.8    |
| 158      | 0.9| 1.9    | 2.8  | 1.6 | 2     | 1.9    | 2.8  | 9.9    | 6.5    |
| 159      | 6.2| >15    | 15.5 | 5.9 | 2.6   | >15    | 7.4  | >15    | 1.9    |
| 160      | 1.8| 1.7    | 1.7  | 1.1 | 2.7   | 5.2    | 3.3  | 5.8    | 1.1    |
| 161      | 2.9| 13.2   | 3    | 1.8 | 4.9   | 8.8    | 3.5  | >15    | 6.8    |
| 162      | 3  | 8.7    | 3.2  | 1.3 | 2.2   | 2.7    | 1.6  | 2.7    | 4.4    |
| 163      | NA | NA     | >14  | >14 | >14   | NA     | >14  | >14    | >14    |
| 164      | 9.9| NA     | >14  | 13.3| >14   | >14    | 14.1 | >14    | 14.1   |
| 165      | NA | NA     | NA   | >14 | >14   | NA     | 14.1 | >14    | 14.1   |
| 166      | >14| >14    | NA   | >14 | NA    | >14    | NA   | 13     | >14    |

In the search of new GA derivatives as antitumor agents, Csuk et al. [58] performed some variations at C-30 of GA, including esterification, the formation of amides and a nitrile. The antitumor evaluation showed the amide derivatives like compounds 167–169 (Scheme 14) showed no cytotoxic activity at 30 µM concentration, but nearly all the ester derivatives like compounds 170, 172–174 (Scheme 15) exhibited high cytotoxic activity. In particular, compound 172 exhibited potent cytotoxic activity on SW1736 cells (IC<sub>50</sub> = 1.88 µM), while compound 175 esterified at C-30 and etherified at C-3 almost showed no cytotoxic activity (IC<sub>50</sub> > 30 µM) against seven tested human tumor cell lines. This suggested that not only the type of the chemical bonding but also the position of substituent groups affects the antitumor activity. This study greatly enriched the modification strategy of the carbonyl group. The cytotoxicity (IC<sub>50</sub> values in µM) of 167–175 in a panel of various cancer cell lines is summarized in Table 15.

**Scheme 14. Synthesis of the GA amide derivatives 167–169. Reagents and conditions: (a) K<sub>2</sub>CO<sub>3</sub>, diamine, DMF, 25 °C, 20 h; (b) Boc<sub>2</sub>O, Et<sub>3</sub>N, MeOH, 25 °C, 20 h.**

**Scheme 15. Synthesis of the GA ester derivatives 170–175. Reagents and conditions: (a) K<sub>2</sub>CO<sub>3</sub>, alkyl halide, DMF, 25 °C, 20 h.**
which the 30-carboxyl group was modified by ferulic acid analogs and the 3-hydroxyl group was

cytotoxicity against normal cells (hTERT-RPE1 cells).

higher antitumor activity than

coupled with amino acids. The MTT assay results showed that most of the derivatives exhibited much

the activity decreased. Meanwhile, they also found that when there were nitrate moieties at C-3 and

carbon chain was lengthened, while when the carbon chain length of the linking group was 5,

as the carbon chain was lengthened, while when the carbon chain length of the linking group was 5,

the activity decreased. Meanwhile, they also found that when there were nitrate moieties at C-3 and

C-30 simultaneously; the antitumor activity of the compounds was enhanced.

2.4. Structural Modifications of Multiple Rings

In an attempt to improve the pharmacological activity of GA, structural modification at multiple

rings has been reported. Structural modifications of multiple rings in GA has focused on the A, C,

and E rings, especially at A and E ring. Shen et al. [59,60] reported syntheses and antitumor activity

of some GA derivatives by simultaneously modifying the C-3 hydroxyl group and the C-30 carboxyl

group in GA. They found when the carbon chain of the linking group was 2 to 4, the activity increased

as the carbon chain was lengthened, while when the carbon chain length of the linking group was 5,

the activity decreased. Meanwhile, they also found that when there were nitrate moieties at C-3 and

C-30 simultaneously; the antitumor activity of the compounds was enhanced.

Starting from GA, Li et al. [61] synthesized a series of GA derivatives 176–199 (Scheme 16) in

which the 30-carboxyl group was modified by ferulic acid analogs and the 3-hydroxyl group was
coupled with amino acids. The MTT assay results showed that most of the derivatives exhibited much
higher antitumor activity than GA against cancer cell lines (MCF-7 cells, MDA-MB-231) and lower
cytotoxicity against normal cells (hTERT-RPE1 cells).

| Compound | 518A2 | 8505C | A253 | A549 | DLD-1 | Lipo | SW1736 |
|----------|-------|-------|------|------|-------|------|--------|
| GA       | 83.92 | 86.50 | 80.78| 82.76| 81.21 | 81.44| 76.93  |
| 167      | >30   | >30   | >30  | >30  | >30   | >30  | >30    |
| 168      | >30   | >30   | >30  | >30  | >30   | >30  | >30    |
| 169      | >30   | >30   | >30  | >30  | >30   | >30  | >30    |
| 170      | 15.19 | 15.59 | 15.89| 20.27| 22.98 | 15.46| 19.87  |
| 171      | 28.99 | >30   | >30  | >30  | >30   | >30  | >30    |
| 172      | 21.00 | 8.82  | 10.97| 4.28 | 23.09 | 11.47| 1.88   |
| 173      | 14.91 | 11.61 | 13.57| 19.16| 14.88 | 12.77| 16.36  |
| 174      | 15.33 | 15.59 | 15.89| 20.27| 22.98 | 15.46| 19.87  |
| 175      | >30   | >30   | >30  | >30  | >30   | >30  | >30    |

2.4. Structural Modifications of Multiple Rings

In an attempt to improve the pharmacological activity of GA, structural modification at multiple rings has been reported. Structural modifications of multiple rings in GA has focused on the A, C, and E rings, especially at A and E ring. Shen et al. [59,60] reported syntheses and antitumor activity of some GA derivatives by simultaneously modifying the C-3 hydroxyl group and the C-30 carboxyl group in GA. They found when the carbon chain of the linking group was 2 to 4, the activity increased as the carbon chain was lengthened, while when the carbon chain length of the linking group was 5, the activity decreased. Meanwhile, they also found that when there were nitrate moieties at C-3 and C-30 simultaneously; the antitumor activity of the compounds was enhanced.

Starting from GA, Li et al. [61] synthesized a series of GA derivatives 176–199 (Scheme 16) in which the 30-carboxyl group was modified by ferulic acid analogs and the 3-hydroxyl group was coupled with amino acids. The MTT assay results showed that most of the derivatives exhibited much higher antitumor activity than GA against cancer cell lines (MCF-7 cells, MDA-MB-231) and lower cytotoxicity against normal cells (hTERT-RPE1 cells).

**Scheme 16.** Synthesis of multiple rings modified GA derivatives 176–199. **Reagents and conditions:**

(a) ferulic acid analogs, EDCI, DMAP, CH₂Cl₂, r.t.; (b) Boc-L-methionine or Boc-L-selenomethionine, EDCI, DMAP, CH₂Cl₂, r.t.; (c) HCl (gas) in CH₂Cl₂, r.t.
Among the derivatives, compound 193 was the most active one (IC$_{50}$ 1.88 ± 0.20 µM for MCF-7; IC$_{50}$ 1.37 ± 0.18 µM for MDA-MB-231). The results displayed that introduction of a lipophilic fragment or amino acid groups into C-3 and C-30 might increase the antitumor activity. The cytotoxicity (IC$_{50}$ values in µM) of 176–199 in a panel of various cancer cell lines is summarized in Table 16.

### Table 16. Cytotoxicity (IC$_{50}$ values in µM) of 176–199 in a panel of various cancer cell lines.

| Compound | MCF-7       | MDA-MB-231 | hTERT-RPE1 |
|----------|-------------|------------|------------|
| GA       | 75.66 ± 1.52| 87.00 ± 1.73| 63.41 ± 1.07 |
| 176      | 13.64 ± 0.93| 5.03 ± 0.82 | 17.32 ± 1.21 |
| 177      | 22.46 ± 1.26| 8.14 ± 0.76 | 22.80 ± 0.97 |
| 178      | 20.29 ± 1.47| 14.38 ± 0.52| 29.63 ± 1.16 |
| 179      | 24.45 ± 1.36| 14.46 ± 0.58| 28.41 ± 0.87 |
| 180      | 8.54 ± 0.67 | 7.31 ± 0.16 | 18.59 ± 0.54 |
| 181      | 19.27 ± 1.01| 9.41 ± 1.03 | 21.11 ± 0.73 |
| 182      | 14.90 ± 0.75| 20.84 ± 1.20| 24.09 ± 0.88 |
| 183      | 19.30 ± 0.98| 23.15 ± 1.07| 22.88 ± 0.68 |
| 184      | 6.00 ± 0.43 | 3.52 ± 0.61 | 10.36 ± 0.80 |
| 185      | 1.88 ± 0.20 | 1.37 ± 0.18 | 4.93 ± 0.36  |
| 186      | 8.62 ± 0.23 | 5.36 ± 0.44 | 16.28 ± 0.51 |
| 187      | 8.45 ± 0.32 | 3.49 ± 0.61 | 12.33 ± 0.46 |
| 188      | 7.24 ± 0.30 | 6.43 ± 0.84 | 8.48 ± 0.73  |
| 189      | 6.02 ± 0.35 | 6.27 ± 0.24 | 6.33 ± 0.19  |
| 190      | 2.65 ± 0.12 | 2.31 ± 0.65 | 5.65 ± 1.02  |
| 191      | 2.42 ± 0.23 | 1.86 ± 0.29 | 7.08 ± 0.73  |

In order to further improve the antitumor activity of GA, Song et al. [62] designed and synthesized a series of novel GA derivatives by modifying the structure at the C-3 hydroxyl or C-11 carbonyl or C-30 carboxyl.

The biological activity evaluation showed that compound 203 (Scheme 17) exhibited the most promising antitumor activity against tumor cell lines MDA-MB-231 cells, DU-145 cells and Hep-G2 cells (IC$_{50}$ 10.01 µM for HepG2, 11.96 µM for DU-145 and 17.8 µM for MDA-MB-231), which was much better than starting material GA (IC$_{50}$ values of 74.35, 69.40, 72.65 µM, respectively). What’s more, compound 200 with linker $n = 2$ and compound 205 with linker $n = 4$ also showed higher antitumor activity than GA on all tested tumor cell lines. But other compound, such as 201, 202, 204, showed weak anti-proliferative effect due to their poor solubility. The cytotoxicity (IC$_{50}$ values in µM) of 200–206, 209, 210 in a panel of various cancer cell lines is summarized in Table 17.

### Table 17. Cytotoxicity (IC$_{50}$ values in µM) of 200–206, 209 and 210 in a panel of various cancer cell lines.

| Compound | HepG2       | DU-145      | MDA-MB-231 |
|----------|-------------|-------------|------------|
| GA       | 74.35 ± 2.03| 69.40 ± 2.37| 72.65 ± 1.67 |
| 200      | >100        | 21.59 ± 3.22| 24.66 ± 2.71 |
| 201      | >100        | >100        | 89.40 ± 2.85 |
| 202      | >100        | >100        | >100        |
| 203      | 10.01 ± 2.29| 11.96 ± 1.42| 17.80 ± 1.76 |
| 204      | >100        | >100        | 79.3 ± 2.34 |
| 205      | 36.37 ± 1.89| >100        | 40.65 ± 2.11 |
| 206      | >100        | >100        | >100        |
| 209      | >100        | >100        | >100        |
| 210      | >100        | >100        | >100        |
The hydroxyl at the C-3 position seems to be critical in maintaining the cytotoxicity. The A ring skeleton plays an important role in eliciting antitumor activity. A cyano or trifluoromethyl substituent at C-2 position of GA improved the cytotoxicity. Expansion of ring A did not make a major difference in the cytotoxicity, but the number and location of hydroxyl groups in the A-ring has an important influence on the antitumor activity.

3. Conclusions

Glycyrrhetinic Acid was found to possess remarkable anti-proliferative and apoptosis-inducing activity against various cancer cell lines. A number of structural modifications of GA were carried out to synthesize new potential antitumor agents. As for the many synthetic strategies reported in this review, they can be summarized as follows: (i) introduction of aminoalkyl, amino acid, sugar and other groups into the hydroxyl group at C-3 by esterification; (ii) oxidation or elimination of the hydroxyl group at C-3, introduction of functional groups at C-2, opening or increasing the number of atoms of ring-A; (iii) elimination of the C-11 ketone group in ring-C; (iv) esterification or amidation of the carboxyl group at C-30 in ring-E; (v) esterification at the C-3 hydroxyl group and C-30 carboxyl group simultaneously.

To some extent, the reported GA derivatives and their biological activity confirmed that there are many factors affecting the antitumor activity, such as the kind, quantity and position of substituents, and the type of chemical bonding. The published studies of GA derivatives as the antitumor agents have provided us much useful information which was as follows and is summarized in Figure 4:

1. The hydroxyl at the C-3 position seems to be critical in maintaining the cytotoxicity. The introduction of an extra amino acid or a nitrogen-containing substituent was found to be beneficial to increase the cytotoxicity, but the acetylation or oxidation of the hydroxyl group at the C-3 position resulted in a decreased anti-proliferative activity.
2. The A ring skeleton plays an important role in eliciting antitumor activity. A cyano or trifluoromethyl substituent at C-2 position of GA improved the cytotoxicity. Expansion of ring A did not make a major difference in the cytotoxicity, but the number and location of hydroxyl groups in the A-ring has an important influence on the antitumor activity.
3. The C-11 keto group of C ring seems to show no direct relation with cytotoxicity.
4. The C-30 carboxyl group is essential for cytotoxicity. Esterification at the C-30 carboxylic acid could improve the antitumor efficacy.

5. Esterification at the C-3 hydroxyl group and C-30 carboxyl group simultaneously increased the antitumor activity.

**Figure 4.** Structure-activity relationships of GA.

The chemical methods for the structural modifications of GA are efficient but the strategies were long and complicated and often involve harsh reaction conditions, therefore, in the future studies structure-activity relationships should be a prerequisite and focused on obtaining highly effective and low-toxicity antitumor derivatives of GA.

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**Conflicts of Interest:** The authors declare no conflict of interest.

**Abbreviations**

| Abbreviation | Full Name |
|--------------|-----------|
| DCC          | Dicyclohexylcarbodiimide |
| DCM          | Dichloromethane |
| DEAD         | Diethyl azodicarboxylate |
| DMAP         | 4-Dimethylaminopyridine |
| DMF          | N,N-Dimethylformamide |
| DMSO         | Dimethylsulfoxide |
| EDCI         | 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride |
| HMPT         | Hexamethylphosphoryl triamide |
| HOBr         | 1-Hydroxybenzotriazole |
| IBX          | 2-Iodoxybenzoic acid |
| m-CPBA       | m-Chloroperbenzoic acid |
| NMP          | N-Methylpyrrolidone |
| p-TSA        | p-Toluenesulfonic acid |
| TFA          | Trifluoroacetic acid |
| THF          | Tetrahydrofuran |
| TMSOTf       | Trimethylsilyl trifluoro methanesulfonate |
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