EVALUATION OF THE CYTOTOXIC ACTIVITY OF A GLYCYRRHETINIC ACID DERIVATIVE IN DIFFERENT STANDARD CANCER CELL LINES

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ABSTRACT

Glycyrrhetinic acid is a pentacyclic triterpenoid aglycone derived from licorice and is widely recognized for its anti-inflammatory, hepatoprotective, antiviral, antioxidant, and anticancer properties. In recent years, considerable attention has been directed toward the synthesis of semisynthetic GA derivatives and the evaluation of their cytotoxic activity against standard human cancer cell lines. The present article aims to analyze the cytotoxic activity of glycyrrhetinic acid derivatives in accordance with the IMRAD structure, using only three original studies published in reputable peer-reviewed journals with real and verifiable DOI numbers. The analysis includes papers published in Molecules (2016), Biological and Pharmaceutical Bulletin (2020), and Molecules (2023). These studies collectively describe the cytotoxic profile of GA derivatives in MCF-7, MDA-MB-231, HeLa, SGC-7901, BEL-7402, A549, B16, and LO2 cell lines. The reviewed evidence demonstrates that structural modification of the GA scaffold, particularly at the C-3, C-30, and ring A positions, can markedly enhance cytotoxic potency. In the 2016 study, selected derivatives exhibited IC50 values in the low micromolar range against breast cancer cell lines; in the 2020 study, several compounds showed cytotoxic effects comparable to or stronger than gefitinib and doxorubicin; and in the 2023 study, the most active derivative induced ROS-mediated mitochondrial apoptosis, S-phase cell-cycle arrest, and activation of the caspase cascade in HeLa cells. Taken together, these findings indicate that glycyrrhetinic acid derivatives represent a promising scaffold for anticancer drug development. Nevertheless, further studies are required to clarify selectivity toward malignant cells,

toxicity toward normal cells, pharmacokinetic behavior, and translational in vivo efficacy..

Introduction. Cancer remains one of the most serious public health challenges worldwide. Despite substantial progress in chemotherapy, targeted therapy, and immunotherapy, the treatment of many malignancies is still limited by drug resistance, systemic toxicity, poor selectivity, and tumor recurrence. Therefore, the search for novel antitumor agents based on natural products continues to be a major focus of medicinal chemistry and experimental pharmacology.

Pentacyclic triterpenoids have attracted considerable attention as potential anticancer agents because of their broad spectrum of biological activities, including growth inhibition, apoptosis induction, modulation of oxidative stress, and interference with signaling pathways involved in tumor progression. Among them, glycyrrhetinic acid (GA), the aglycone metabolite of glycyrrhizin from licorice (Glycyrrhiza spp.), is one of the most intensively investigated compounds. GA has been reported to possess anti-inflammatory, hepatoprotective, antiviral, antioxidant, and antitumor effects. However, the native GA molecule often displays only moderate cytotoxic activity, which has stimulated efforts to improve its potency through rational chemical modification.

The GA scaffold contains several positions suitable for structural optimization, most notably the hydroxyl group at C-3, the carboxyl group at C-30, and functional regions within ring A. Chemical transformation at these sites by esterification, amidation, introduction of amino fragments, heterocyclic moieties, or other pharmacophoric groups may alter lipophilicity, cellular uptake, target binding, and ultimately cytotoxic efficacy. As a result, a large number of GA derivatives have been synthesized and screened in cancer cell-based models over the past decade.

In vitro cytotoxicity screening is a critical early-stage strategy for the identification of new anticancer candidates. Standard human tumor cell lines such as MCF-7 and MDA-MB-231 (breast cancer), HeLa (cervical carcinoma), A549 (lung adenocarcinoma), SGC-7901 (gastric cancer), BEL-7402 (hepatocellular carcinoma), and B16 (melanoma) are frequently used to assess the antitumor potential of novel compounds. These models allow comparative evaluation of cytotoxicity, potency, and preliminary selectivity.

Although numerous publications have addressed glycyrrhetinic acid derivatives, there remains a practical need for a concise, structured IMRAD-style analytical article based strictly on original experimental papers with reliable DOI records. Such a synthesis is particularly useful for planning further experimental work, designing dissertation studies, and identifying the most promising structural motifs for future optimization.

Aim of the Study. To evaluate and summarize the cytotoxic activity of glycyrrhetinic acid derivatives in different standard cancer cell lines based on three original research articles with verified DOI numbers, and to identify the most active derivatives, their IC50 values, cell line-specific activity patterns, and principal mechanistic features.

Materials and Methods. This article was prepared as an analytical literature-based study focused on the cytotoxic activity of glycyrrhetinic acid derivatives. Only original experimental papers were included in the analysis.

The following inclusion criteria were applied: (1) the study had to investigate glycyrrhetinic acid or its derivatives; (2) cytotoxic activity had to be evaluated in vitro using standard cancer cell lines; (3) the publication had to be an original experimental paper rather than a review; (4) the article had to be published in a reputable peer-reviewed journal; and (5) the paper had to contain a real and verifiable DOI number.

Based on these criteria, the following three studies were selected for analysis:

1) Li Y., Feng L., Song Z.-F., Li H.-B., Huai Q.-Y. Synthesis and Anticancer Activities of Glycyrrhetinic Acid Derivatives. Molecules. 2016;21(2):199. DOI: 10.3390/molecules21020199.

2) Zheng Q.-X., Wang R., Xu Y., et al. Design, Preparation and Studies Regarding Cytotoxic Properties of Glycyrrhetinic Acid Derivatives. Biological and Pharmaceutical Bulletin. 2020;43(1):102–109. DOI: 10.1248/bpb.b19-00615.

3) Chen J., Xu Y., Yang Y., et al. Evaluation of the Anticancer Activity and Mechanism Studies of Glycyrrhetic Acid Derivatives toward HeLa Cells. Molecules. 2023;28(7):3164. DOI: 10.3390/molecules28073164.

For each article, the following parameters were extracted and comparatively analyzed: study objective, number and type of synthesized derivatives, cancer cell lines employed, cytotoxicity assay used, most active compounds and their IC50 values, reference drugs, selectivity toward normal cells, and mechanistic findings.

The collected information was organized both by cell line and by structure–activity relationships in order to identify the most promising trends in the optimization of the glycyrrhetinic acid scaffold.

Results. The three selected papers collectively provide a focused yet informative picture of the cytotoxic potential of glycyrrhetinic acid derivatives. The 2016 Molecules paper emphasizes broad synthetic diversification and screening in breast cancer models; the 2020 Biological and Pharmaceutical Bulletin paper investigates newly designed derivatives with enhanced cytotoxicity against HeLa and MDA-MB-231 cells; and the 2023 Molecules paper extends the analysis by exploring the mechanism of action of active derivatives in HeLa cells.

In the 2016 Molecules study, Li and co-authors synthesized 40 glycyrrhetinic acid derivatives and evaluated their cytotoxic activity in MCF-7 and MDA-MB-231 human breast cancer cell lines using the MTT assay. The most notable finding was the outstanding activity of compound 42, which displayed IC50 values of 1.88 ± 0.20 μM in MCF-7 cells and 1.37 ± 0.18 μM in MDA-MB-231 cells. These values indicate a marked improvement over the parent GA scaffold and highlight the feasibility of using rational derivatization to enhance anticancer potency.

Besides compound 42, several additional derivatives in the 2016 study exhibited moderate to strong growth-inhibitory effects, supporting the conclusion that structural tailoring of GA can significantly influence cytotoxic performance. Importantly, the pronounced activity in MDA-MB-231 cells is of particular relevance because this line represents a triple-negative breast cancer model, which is clinically associated with aggressive behavior and limited therapeutic options.

In the 2020 Biological and Pharmaceutical Bulletin study, Zheng and colleagues synthesized 18 GA derivatives and evaluated them against MDA-MB-231 and HeLa cells using the MTT method. The resulting cytotoxicity profile showed considerable variability among the compounds, indicating that relatively small structural modifications may substantially alter biological activity. Several derivatives exhibited cytotoxic effects comparable to, or even stronger than, the reference drugs gefitinib and doxorubicin under the tested conditions.

Among the compounds described in the 2020 study, compound 6g deserves particular attention. The authors demonstrated that 6g induced concentration-dependent apoptosis in MDA-MB-231 cells: approximately 7%, 10%, and 44% apoptotic cells were observed at 5, 10, and 20 μM, respectively. This finding suggests that the antitumor effect of this derivative is not limited to simple growth suppression but is closely associated with activation of programmed cell death pathways.

The 2023 Molecules study by Chen and co-authors provided additional mechanistic insight. In this work, a series of glycyrrhetinic acid derivatives designated 3a–3f was synthesized and evaluated in SGC-7901, BEL-7402, A549, HeLa, B16, and normal LO2 cell lines. Among these compounds, derivative 3a demonstrated the highest activity in HeLa cells, with an IC50 value of 11.4 ± 0.2 μM.

A major strength of the 2023 study lies in its mechanistic investigation. Compound 3a increased intracellular reactive oxygen species (ROS) levels, reduced glutathione (GSH) content, elevated intracellular Ca2+ concentration, and decreased mitochondrial membrane potential in HeLa cells. In addition, activation of caspase-9 and caspase-3, together with cleavage of PARP, was observed. Cell-cycle analysis further showed S-phase arrest after treatment with 3a. Collectively, these data indicate that the cytotoxic effect of the compound is mediated by ROS-associated mitochondrial dysfunction and apoptosis signaling.

When considered together, the three articles show that glycyrrhetinic acid derivatives can exert cytotoxic effects through multiple complementary mechanisms, including inhibition of cell proliferation, induction of apoptosis, promotion of oxidative stress, disruption of mitochondrial function, and interference with cell-cycle progression.

Table 1. Summary of the cytotoxic activity of glycyrrhetinic acid derivatives in the three selected studies

Study	Cell lines	Number of derivatives	Most active compound	Main findings	DOI
Li et al., 2016 Molecules	MCF-7, MDA-MB-231	40	Compound 42	MCF-7: IC50 1.88±0.20 μM; MDA-MB-231: IC50 1.37±0.18 μM	10.3390/molecules21020199
Zheng et al., 2020 Biological and	MDA-MB-231, HeLa	18	Compound 6g	Several derivatives were comparable to or stronger than gefitinib/doxorubici	10.1248/bpb.b19-00615

Pharmaceutical Bulletin				n; 6g induced apoptosis in MDA-MB-231 cells	
Chen et al., 2023 Molecules	SGC-7901, BEL-7402, A549, HeLa, B16, LO2	6 (3a–3f)	Compound 3a	HeLa: IC50 11.4±0.2 μM; ROS↑, GSH↓, Ca2+↑, MMP↓, caspase-3/9 activation, S-phase arrest	10.3390/molecules28073164

Discussion. The combined analysis of these three studies clearly demonstrates that the cytotoxic activity of glycyrrhetinic acid derivatives is strongly dependent on structural modification of the parent scaffold. Across all three papers, the native GA framework served as the starting platform, while functionalization at the C-3 and C-30 positions, as well as modifications in ring A, emerged as the principal strategies for enhancing biological activity.

The 2016 Molecules paper is particularly valuable from a medicinal chemistry perspective because it examined a relatively large series of compounds. The synthesis of 40 derivatives enabled a broader preliminary structure–activity relationship (SAR) assessment. The exceptionally low IC50 values of compound 42 against both MCF-7 and MDA-MB-231 cells suggest that properly selected substituents can convert the moderate cytotoxicity of the parent GA molecule into highly potent activity. The result obtained in MDA-MB-231 cells is especially noteworthy because triple-negative breast cancer is one of the most therapeutically challenging breast cancer subtypes.

The 2020 Biological and Pharmaceutical Bulletin paper adds an important comparative pharmacological dimension. The observation that some GA derivatives were as active as, or more active than, gefitinib and doxorubicin suggests that the GA scaffold can compete with established anticancer agents under certain in vitro conditions. More importantly, the study showed that cytotoxicity was accompanied by apoptosis induction, particularly for compound 6g. This is a relevant mechanistic feature because apoptosis induction is often associated with more predictable and therapeutically meaningful antitumor effects than nonspecific cytotoxicity alone.

The 2023 Molecules paper further strengthens the case for glycyrrhetinic acid derivatives by demonstrating that their cytotoxic activity can be mechanistically linked to ROS-mediated mitochondrial apoptosis. Increased ROS generation may overwhelm the antioxidant defense system of tumor cells, resulting in depletion of GSH, mitochondrial dysfunction, and activation of the intrinsic apoptotic pathway. The observed activation of caspase-9 and caspase-3, together with PARP cleavage and S-phase arrest, indicates that compound 3a interferes with several interconnected cellular processes essential for cancer cell survival.

A particularly useful feature of the 2023 study is the inclusion of normal LO2 cells. The presence of a nonmalignant cell line is highly important when evaluating new cytotoxic agents, because antitumor potency alone is insufficient for further development unless accompanied by acceptable selectivity. Although

the available information from the three selected studies does not fully resolve the selectivity issue, it highlights the importance of including normal cell models in future screening strategies.

From a broader SAR standpoint, the reviewed studies suggest several common trends. First, derivatization of GA with aromatic, amino, or heterocyclic fragments often improves cytotoxic potency. Second, modification of the carboxyl and hydroxyl functionalities appears to be a productive route for tuning both activity and physicochemical properties. Third, compounds capable of disturbing redox balance and mitochondrial homeostasis tend to exhibit stronger cytotoxic effects, indicating that oxidative stress-related pathways may represent one of the central mechanisms of action of active GA derivatives.

The cancer cell lines used in the reviewed studies also provide insight into the potential spectrum of antitumor activity. MCF-7 and MDA-MB-231 appear to be highly informative models for breast cancer-related screening, while HeLa cells serve not only as a cytotoxicity model but also as a suitable system for mechanistic investigation. The inclusion of A549, BEL-7402, SGC-7901, and B16 in the 2023 study suggests that GA derivatives may possess broader activity beyond breast and cervical cancer, although the depth of evidence varies across cell lines.

Several limitations should be acknowledged. The three studies differ in the number of compounds synthesized, the concentration ranges used, the choice of reference drugs, and the extent of mechanistic analysis. Consequently, direct comparison of IC50 values across studies should be performed with caution. In addition, only a limited number of normal cell lines were included, and most findings remain confined to the in vitro level. In vivo efficacy, bioavailability, metabolism, and safety still need to be systematically investigated before GA derivatives can be considered serious translational anticancer candidates.

Despite these limitations, the overall evidence remains compelling. Glycyrrhetinic acid derivatives clearly represent a promising family of semisynthetic anticancer agents, especially for breast and cervical cancer models. Future studies should prioritize expanded screening across a wider panel of standard cancer cell lines, rigorous selectivity assessment against normal cells, deeper mechanistic profiling, and in vivo validation of the most active derivatives.

Practical Significance. The present analysis systematizes available experimental evidence on the cytotoxic activity of glycyrrhetinic acid derivatives and may serve as a useful framework for future laboratory and dissertation-based research.

First, it identifies structural modification patterns that are associated with enhanced cytotoxicity and therefore may guide the design of new derivatives. Second, it highlights the most informative standard cancer cell lines for preliminary screening, particularly MCF-7, MDA-MB-231, and HeLa. Third, it emphasizes the importance of mechanistic endpoints such as ROS production, mitochondrial membrane potential, caspase-3/9 activation, PARP cleavage, and cell-cycle arrest when evaluating promising GA-based compounds.

Accordingly, this article may be used as a methodological and conceptual basis for planning further studies on the pharmacology of glycyrrhetinic acid derivatives, including the design of new compounds and the development of original experimental manuscripts.

Conclusion. Based on the analysis of three original studies published in reputable journals with verified DOI numbers, glycyrrhetinic acid derivatives demonstrate significant cytotoxic activity in a range of standard cancer cell lines. The 2016

Molecules study showed that selected derivatives possess very strong activity in breast cancer cells, with low micromolar IC50 values. The 2020 Biological and Pharmaceutical Bulletin study demonstrated that certain compounds may approach or exceed the cytotoxic performance of established anticancer drugs and are capable of inducing apoptosis. The 2023 Molecules study further revealed that the activity of glycyrrhetinic acid derivatives in HeLa cells is associated with ROS-mediated mitochondrial apoptosis, caspase activation, and cell-cycle disruption. Overall, glycyrrhetinic acid derivatives represent a promising molecular platform for anticancer drug discovery. Future research should focus on broader cancer cell-line panels, careful selectivity assessment against normal cells, pharmacokinetic characterization, and in vivo validation of the most active compounds.

References:

1. Li Y., Feng L., Song Z.-F., Li H.-B., Huai Q.-Y. Synthesis and Anticancer Activities of Glycyrrhetinic Acid Derivatives. Molecules. 2016;21(2):199. DOI: 10.3390/molecules21020199.
2. Zheng Q.-X., Wang R., Xu Y., He C.-X., Zhao C.-Y., Wang Z.-F., Zhang R., Dehaen W., Li H.-J., Huai Q.-Y. Design, Preparation and Studies Regarding Cytotoxic Properties of Glycyrrhetinic Acid Derivatives. Biological and Pharmaceutical Bulletin. 2020;43(1):102–109. DOI: 10.1248/bpb.b19-00615.
3. Chen J., Xu Y., Yang Y., Yao X., Fu Y., Wang Y., Liu Y., Wang X. Evaluation of the Anticancer Activity and Mechanism Studies of Glycyrrhetic Acid Derivatives toward HeLa Cells. Molecules. 2023;28(7):3164. DOI: 10.3390/molecules28073164.