PREPARATION OF SELECTED SEMISYNTHETIC GLYCYRRHETINIC ACID DERIVATIVES FOR IN VITRO AND IN VIVO STUDIES AND SELECTION OF APPROPRIATE EXPERIMENTAL MODELS

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The preparation of semisynthetic derivatives is only one side of the research problem. The second, equally important side is experimental model selection.

ABSTRACT

Glycyrrhetinic acid is one of the most widely investigated pentacyclic triterpenoid scaffolds in contemporary natural-product-based medicinal chemistry. Its parent molecule already exhibits anti-inflammatory, antiviral, hepatoprotective, antimicrobial, and antitumor effects, but its intrinsic potency and pharmacokinetic behavior are often insufficient for direct translational development. For this reason, a large body of work has focused on semisynthetic modification of GA in order to generate derivatives with improved biological performance. However, the value of these derivatives depends not only on successful chemical preparation but also on the rational selection of in vitro and in vivo experimental models that can reveal cytotoxic, anti-inflammatory, anti-metastatic, and tumor-microenvironment-modulating activity. The aim of the present article was to analyze the preparation of selected semisynthetic GA derivatives and the principles of choosing appropriate in vitro and in vivo pharmacological models using six original experimental papers published in reputable journals with real and verifiable DOI numbers. The selected studies included ring A–modified antitumor derivatives, C-2/C-3/C-11/C-30 modified anti-inflammatory derivatives, soloxolone methyl and its epoxide/amide analogues, and multifunctional derivatives combining antimicrobial, anti-inflammatory, and antitumor properties. Across the six studies, several recurrent experimental strategies emerged. First, the most informative in vitro models were standard tumor cell lines such as HCT-116, A549, MCF-7, MDA-MB-231, U87, and B16, together with inflammatory macrophage systems and normal-cell comparators. Second, the most frequently used in vivo

models were TPA-induced ear edema, carrageenan- or histamine-induced inflammation, xenograft and ascites tumor models, murine melanoma metastasis, and glioblastoma xenografts. Third, derivative selection was guided by a stepwise pipeline in which synthesis and structural confirmation were followed by in vitro screening, mechanism-oriented cellular assays, and finally disease-relevant in vivo validation. The comparative analysis shows that the choice of model must be linked to the expected pharmacodynamic direction of each derivative class: anti-inflammatory derivatives require acute inflammation models and cytokine readouts, whereas antitumor derivatives demand cytotoxicity panels, apoptosis assays, and tumor-growth or metastasis models. Accordingly, semisynthetic GA derivatives should be studied not in a generic manner but through a structure-driven model-selection strategy. Such an approach is particularly relevant for dissertation-level work, where the objective is not merely to report biological activity, but to justify why a specific derivative is tested in a specific experimental system and how in vitro findings are translated into in vivo pharmacodynamic evidence.

Introduction. Natural triterpenoids remain one of the richest sources of lead structures in medicinal chemistry because they combine high structural complexity with broad pharmacological potential. Among them, glycyrrhetinic acid (GA), the aglycone metabolite of glycyrrhizin from licorice, has attracted sustained interest owing to its anti-inflammatory, antioxidant, hepatoprotective, antiviral, and antitumor properties. Nevertheless, the parent GA scaffold often displays only moderate potency, limited selectivity, and suboptimal physicochemical behavior, which has stimulated extensive research into semisynthetic GA derivatives. Through rational modification at chemically accessible positions such as C-2, C-3, C-11, C-30, and ring A, medicinal chemists have generated derivatives with markedly different biological profiles, ranging from antimicrobial and anti-inflammatory activity to apoptosis-inducing and anti-metastatic antitumor effects.

The preparation of semisynthetic derivatives is only one side of the research problem. The second, equally important side is experimental model selection. A newly synthesized derivative can appear highly promising or disappointingly inactive depending on which biological model is chosen for its evaluation. A derivative designed to suppress inflammatory cytokines may show little relevance in a generic tumor-cell panel if its principal mechanism is immunomodulation. Conversely, a strongly pro-apoptotic ring A derivative may require cancer-cell models, mitochondrial assays, and xenograft studies rather than topical inflammation models. Therefore, the pharmacological evaluation of GA derivatives must be understood as a chain of interconnected decisions: which derivative class to prepare, which in vitro model to

use for first-pass screening, which mechanistic assays to perform, and which in vivo model can best validate the expected biological direction.

This issue is highly relevant for dissertation planning and for the design of original experimental work on glycyrrhetinic acid derivatives. In practice, researchers do not merely ask whether a derivative is “active”; they must decide whether it is active in the right model, whether the chosen model reflects the intended pharmacodynamic endpoint, and whether in vitro observations are sufficiently strong to justify in vivo testing. The available literature already provides valuable examples of how this decision-making process can be organized. Some studies use acute inflammatory models to validate derivatives that were first identified through macrophage cytokine assays. Others use cytotoxicity panels and apoptosis assays to prioritize derivatives for xenograft or metastasis experiments. Soloxolone-based derivatives, in particular, illustrate how a semisynthetic GA branch can be advanced through multiple levels of testing, from cell viability and ROS-dependent apoptosis to tumor growth, metastasis, and microenvironment remodeling in animals.

The present article addresses this methodological and pharmacological problem by focusing on the preparation of selected semisynthetic GA derivatives and the rationale for choosing in vitro and in vivo models for their evaluation. Rather than reviewing all biological effects of glycyrrhetinic acid, the paper examines six original experimental studies from reputable journals that explicitly combine semisynthetic derivative preparation with biological testing. These papers were selected because they allow a comparative analysis of three interrelated questions: (1) which structural modifications of GA were introduced and why; (2) which in vitro models were chosen to characterize the resulting derivatives; and (3) which in vivo models were used to confirm pharmacodynamic relevance. In this sense, the article is designed not only as a review of results but also as a practical framework for model selection in future work on glycyrrhetinic acid derivatives.

Aim of the Study. To analyze the preparation of selected semisynthetic glycyrrhetinic acid derivatives and to determine the principles for choosing appropriate in vitro and in vivo experimental models for their pharmacological evaluation, using six original peer-reviewed studies with verified DOI numbers as the analytical basis.

Materials and Methods. This article was prepared as an analytical review in IMRAD format based exclusively on original experimental publications. The inclusion criteria were defined to match the specific theme of the paper: (1) the study had to involve semisynthetic derivatives of glycyrrhetinic acid or 18β-glycyrrhetinic acid; (2) the paper had to describe the preparation or synthesis of those derivatives; (3) at least one in vitro biological model had to be used for primary evaluation; (4) at least one in vivo model had to be included or the paper had to contain an explicit bridge from in vitro findings to animal pharmacodynamics; (5) the article had to be published in a peer-reviewed journal and have a real, verifiable DOI number. Reviews, editorials, and papers limited solely to synthetic chemistry without biological testing were excluded.

Six original studies fulfilled these criteria and were selected as the core literature set for the present analysis:

1) Huang M., Gong P., Wang Y., Xie X., Ma Z., Xu Q., Liu D., Jing Y., Zhao L. Synthesis and antitumor effects of novel 18β-glycyrrhetinic acid derivatives featuring an exocyclic α,β-

unsaturated carbonyl moiety in ring A. Bioorganic Chemistry. 2020;103:104187. DOI: 10.1016/j.bioorg.2020.104187.

2) Yang Y., Zhu Q., Zhong Y., Cui X., Jiang Z., Wu P., Zheng X., Zhang K., Zhao S. Synthesis, anti-microbial and anti-inflammatory activities of 18β-glycyrrhetinic acid derivatives. Bioorganic Chemistry. 2020;101:103985. DOI: 10.1016/j.bioorg.2020.103985.

3) Markov A.V., Sen’kova A.V., Zenkova M.A., Logashenko E.B. Novel Glycyrrhetinic Acid Derivative Soloxolone Methyl Inhibits the Inflammatory Response and Tumor Growth in vivo. Molecular Biology. 2018;52(2):306–313. DOI: 10.7868/S0026898418020143.

4) Tu B., Liang J., Ou Y., Zhang X., Zheng W., Wu R., Gan L., Li D., Lu Y., Wu J., Hong W.-D., Zhang K., Wu P., Jin J., Wong W.-L. Novel 18β-glycyrrhetinic acid derivatives as a Two-in-One agent with potent antimicrobial and anti-inflammatory activity. Bioorganic Chemistry. 2022;122:105714. DOI: 10.1016/j.bioorg.2022.105714.

5) Salomatina O.V., Sen’kova A.V., Moralev A.D., Savin I.A., Komarova N.I., Salakhutdinov N.F., Zenkova M.A., Markov A.V. Novel Epoxides of Soloxolone Methyl: An Effect of the Formation of Oxirane Ring and Stereoisomerism on Cytotoxic Profile, Anti-Metastatic and Anti-Inflammatory Activities In Vitro and In Vivo. International Journal of Molecular Sciences. 2022;23(11):6214. DOI: 10.3390/ijms23116214.

6) Markov A.V., Ilyina A.A., Salomatina O.V., Sen’kova A.V., Okhina A.A., Rogachev A.D., Salakhutdinov N.F., Zenkova M.A. Novel Soloxolone Amides as Potent Anti-Glioblastoma Candidates: Design, Synthesis, In Silico Analysis and Biological Activities In Vitro and In Vivo. Pharmaceuticals. 2022;15(5):603. DOI: 10.3390/ph15050603.

For each study, the following variables were extracted and compared: derivative class and key synthetic modification; intended pharmacological direction; in vitro models used for screening; mechanistic cell-based assays; in vivo model selection and principal endpoints; and the translational logic linking synthesis, in vitro activity, and in vivo testing. The analysis was performed comparatively rather than chronologically. In other words, the six studies were treated as case examples for model-selection strategy rather than merely summarized one by one.

Results. The six selected studies collectively show that preparation of semisynthetic glycyrrhetinic acid derivatives follows a highly rational medicinal-chemistry logic in which structural modification and model selection are tightly linked. Although all derivatives are based on the GA scaffold, the biological models chosen for their evaluation differ substantially according to the pharmacodynamic objective of the project. Broadly, the studies can be divided into two major streams: derivatives intended primarily for anti-inflammatory activity and derivatives intended primarily for antitumor or anti-metastatic activity. A third, hybrid stream includes derivatives with dual or multi-target profiles, such as soloxolone methyl and its analogues.

1. Preparation of semisynthetic derivatives and the logic of structural modification

The first recurrent pattern across the literature is that the semisynthetic strategy is not random. Huang et al. designed ring A–modified derivatives containing an exocyclic α,β-unsaturated carbonyl moiety. This was a deliberate attempt to enhance electrophilic reactivity and apoptosis-inducing antitumor potential. Yang et al. and Tu et al. modified 18β-glycyrrhetinic acid mainly around C-2, C-3, C-11, and related positions in order to improve anti-inflammatory and antimicrobial performance. Markov and co-workers developed soloxolone methyl, a cyano

enone-bearing derivative of 18βH-glycyrrhetinic acid, and subsequently expanded this branch into epoxides and amides. In each case, the chemical transformation created a derivative family with a predicted pharmacodynamic direction. This point is essential: model selection begins not with biology but with chemistry. Once the scaffold has been modified in a way expected to promote apoptosis, redox stress, cytokine suppression, or microenvironment modulation, the biological model must be chosen to match that expectation.

2. Selection of in vitro models for anti-inflammatory derivatives.

The anti-inflammatory studies demonstrate a clear principle of model choice. Yang et al. (2020) synthesized thirteen GA derivatives and initially evaluated them for anti-microbial activity, but the anti-inflammatory branch of the work was validated in a TPA-induced mouse ear edema model after the identification of promising candidates. The cellular logic behind this choice was cytokine-centered: compound 10 was selected because its profile suggested anti-inflammatory potential, and in vivo testing focused on edema and inflammatory mediator expression. The authors then measured TNF-α, IL-1β, and p65, thereby linking the animal model to the expected NF-κB-related mechanism.

Tu et al. (2022) followed a more elaborate version of the same logic. Their derivatives were first characterized in vitro in LPS-stimulated inflammatory systems, with attention to nitric oxide production and cytokine release. Only after this mechanistic anti-inflammatory profile was established did the authors proceed to a TPA-induced ear inflammation model in mice. In that in vivo setting, the lead compounds GA-O-02 and GA-O-06 demonstrated robust anti-inflammatory activity. Importantly, the in vitro panel was not generic; it was selected to mirror the inflammatory pathways expected to drive the animal phenotype. This makes the study a particularly useful example for dissertation planning: if the derivative is intended as an anti-inflammatory agent, macrophage-based cytokine and NO models are more informative than unrelated tumor-cell panels.

3. Selection of in vitro models for antitumor derivatives.

The antitumor-oriented studies used a different logic. Huang et al. (2020) tested their ring A derivatives against tumor cell lines such as HCT-116 and related cancer models because the structural design aimed at apoptosis induction and tumor suppression. Here, the in vitro stage served as a potency and mechanism filter. Cytotoxicity assays were used to identify the most active derivatives, and mechanistic assays focused on apoptosis-related biomarkers such as c-Flip, Noxa, HDAC3, and histone H3 acetylation. Only compounds with convincing in vitro antitumor signatures—especially 5c and 5l—were advanced to in vivo tumor testing.

The 2022 Pharmaceuticals study by Markov et al. applied a similarly targeted strategy to glioblastoma. Soloxolone amides were not screened in a broad, unfocused manner; rather, they were evaluated in a glioblastoma-relevant in vitro context that included U87 cells and mechanistic assays linked to apoptosis and oxidative stress. This is a crucial methodological lesson. When a derivative is being positioned for a specific oncological indication, model selection should not stop at “cancer cells in general.” Instead, the in vitro model should reflect the intended disease context as closely as possible. In the case of soloxolone tryptamide (compound 12), the in vitro findings were strong enough to justify subsequent testing in a U87 xenograft model, creating a coherent in vitro–in vivo research trajectory.

4. Hybrid in vitro strategies: cytotoxicity plus inflammation plus metastasis.

The soloxolone series illustrates the most sophisticated model-selection strategy among the six papers. Soloxolone methyl itself was investigated by Markov et al. (2018) in the context of both inflammation and tumor growth. This already suggests that the derivative had a hybrid pharmacological profile. Later, Salomatina et al. (2022) prepared epoxide analogues of soloxolone methyl and evaluated them in vitro not only for cytotoxicity but also for their effects on inflammatory macrophage behavior. The reason is straightforward: the authors expected the derivatives to influence both tumor progression and inflammation. Consequently, their in vivo model selection also had a dual structure, including metastatic melanoma and carrageenan-induced peritonitis.

This study is especially instructive because it shows that a single derivative family may require several complementary in vitro models before animal testing. Cytotoxicity assays alone would not have been sufficient to justify the anti-inflammatory arm of the project, while macrophage NO assays alone would not have captured the anti-metastatic potential. By combining these approaches, the authors created a much stronger rationale for selecting both metastatic and inflammatory animal models.

5. Selection of in vivo models according to pharmacodynamic objective.

A major result of the comparative analysis is that the in vivo models used in GA-derivative research can be grouped into a practical framework:

• For anti-inflammatory derivatives, acute murine models such as TPA-induced ear edema, carrageenan-induced inflammation, histamine-induced edema, and peritonitis are the preferred first-line in vivo systems. These models are suitable when the derivative is expected to suppress cytokine production, vascular permeability, leukocyte infiltration, or NF-κB-related signaling.

• For broad antitumor derivatives, xenograft or transplantable tumor models are appropriate once in vitro cytotoxicity and apoptosis data indicate strong activity. Huang et al. used tumor-bearing mice to validate compounds 5c and 5l, while Markov et al. used the U87 glioblastoma xenograft model for soloxolone amides.

• For derivatives suspected of affecting metastasis or epithelial–mesenchymal transition, specialized metastasis models such as B16 melanoma dissemination are more informative than simple subcutaneous tumor-volume models. Salomatina et al. chose this route because their derivatives showed a biological profile consistent with anti-metastatic potential.

• For multifunctional derivatives, it is methodologically sound to use more than one in vivo model. Soloxolone methyl and its epoxide derivatives are the clearest examples: the same chemical branch was tested in both inflammatory and tumor-related settings because the pharmacodynamic hypothesis demanded it.

6. Stepwise decision pipeline from synthesis to in vivo validation.

Across all six studies, a stepwise experimental pipeline can be reconstructed. First, the derivative is synthesized and structurally confirmed by standard chemical methods. Second, a primary in vitro screen is selected according to the expected activity direction: inflammatory-cell assays for anti-inflammatory derivatives, tumor-cell viability panels for antitumor derivatives, or a combined panel for multifunctional compounds. Third, mechanistic assays are added to refine candidate selection. These may include ROS measurements, apoptosis markers, cytokine quantification, NF-κB signaling, mitochondrial dysfunction, migration, clonogenicity, or macrophage mediator production. Fourth, only the most convincing compounds are

advanced to in vivo models that match the intended pharmacodynamic endpoint. Finally, in vivo biomarker or histological analysis is used to confirm that the animal phenotype is supported by a plausible mechanism rather than a purely descriptive efficacy signal.

This stepwise logic is perhaps the most important practical result of the present review. It suggests that successful work on semisynthetic glycyrrhetinic acid derivatives requires not only competent synthetic chemistry but also disciplined model selection. A derivative should not be moved into animal studies simply because it exists; it should be moved because the preceding in vitro data indicate that a specific in vivo model is biologically justified.

Discussion. The six selected studies make it clear that the research value of semisynthetic glycyrrhetinic acid derivatives depends on the integration of chemistry and pharmacology. In other words, derivative preparation and experimental model selection are not separate stages but mutually defining components of one research strategy. The best papers in this field do not merely synthesize a large number of analogues and then test them indiscriminately. Instead, they start from a structural hypothesis, choose an in vitro system capable of revealing the expected mechanism, and then use an in vivo model that meaningfully extends the cellular findings.

One of the most important lessons concerns the distinction between anti-inflammatory and antitumor development routes. Anti-inflammatory derivatives such as those described by Yang et al. and Tu et al. are best approached through a cytokine-centered model hierarchy. At the in vitro level, macrophage or inflammatory mediator assays allow the investigator to determine whether the derivative actually affects NO production, TNF-α, IL-1β, IL-6, or related pathways. If this profile is promising, acute inflammatory animal models such as TPA ear edema or carrageenan-induced inflammation become appropriate because they directly test the derivative’s ability to suppress tissue-level inflammation. This route is scientifically coherent because the cellular and animal models interrogate the same pharmacodynamic axis.

Antitumor derivatives require a different hierarchy. The ring A derivatives reported by Huang et al. and the soloxolone amides described by Markov et al. were designed to enhance cytotoxicity, apoptosis induction, and tumor suppression. Accordingly, their in vitro evaluation focused on tumor-cell lines, cell viability, and apoptosis-related signaling. Only after potent activity and a plausible mechanism were demonstrated were the lead compounds taken into animal models. This sequence is critical. Without strong in vitro evidence of tumor-selective or mechanism-based activity, moving directly into xenograft studies would be inefficient and scientifically weak. Thus, the antitumor route emphasizes cell-line selection, potency ranking, apoptosis or ROS assays, and then disease-relevant tumor models.

The hybrid soloxolone branch shows why a rigid separation between anti-inflammatory and antitumor research can be misleading. Soloxolone methyl, epoxides, and amides repeatedly display activities that cross pharmacological boundaries: suppression of inflammation, inhibition of tumor growth, anti-metastatic effects, and microenvironment remodeling. This means that model selection for such derivatives must be broader and more hypothesis-driven. If a derivative appears to affect both macrophage mediators and tumor-cell survival, it is reasonable to design a two-arm evaluation strategy in which both inflammatory and tumor models are used. Salomatina et al. provide an excellent example of this logic by combining cytotoxicity assays, macrophage-based inflammatory evaluation, metastatic melanoma models, and peritonitis models in a single derivative study.

From the standpoint of dissertation planning, the reviewed literature suggests a practical framework for selecting models for newly prepared semisynthetic GA derivatives. First, the chemical modification should be classified by likely pharmacodynamic direction: pro-apoptotic antitumor, anti-inflammatory, anti-metastatic, or multifunctional. Second, the first-pass in vitro model should be chosen to match that direction. For example, if the derivative contains electrophilic ring A motifs similar to those in Huang’s study, colorectal or breast cancer cell lines and apoptosis assays may be the best starting point. If the derivative resembles the anti-inflammatory derivatives of Yang or Tu, macrophage cytokine systems and NO assays may be more appropriate. Third, the in vivo model should be selected only after the in vitro results have identified a clear lead compound and a plausible mechanism. Fourth, whenever possible, the animal experiment should include biomarker analysis—cytokines, apoptosis proteins, histology, vascular changes, or oxidative-stress markers—so that the in vivo effect can be mechanistically interpreted.

Another important point is that model selection is also a matter of experimental economy and ethical justification. In vivo studies are time-consuming, expensive, and ethically sensitive. The six reviewed papers implicitly show that the strongest justification for animal testing comes from a stepwise pipeline in which synthesis is followed by targeted in vitro screening and only the most promising compounds are advanced. This is particularly relevant for early-stage dissertation projects, where resources may be limited and the number of synthesized derivatives may be high. A rational screening funnel helps avoid the common problem of generating many analogues but lacking a clear basis for choosing which one deserves in vivo validation.

At the same time, the literature also reveals some limitations that future work should address. Many studies rely on acute inflammatory models rather than chronic disease models, and several antitumor papers remain focused on relatively short-term xenograft outcomes. Comparative pharmacokinetic data are not always available, and selectivity toward normal cells is sometimes insufficiently explored before animal testing. These limitations do not undermine the value of the existing studies, but they do indicate where dissertation-level projects can contribute original improvement. For example, a new GA derivative project could deliberately incorporate normal-cell comparators, repeated-dose tolerability, or more detailed histopathological and molecular analysis in vivo.

Overall, the central message of the current literature is straightforward: semisynthetic glycyrrhetinic acid derivatives should be studied through a structure-guided experimental strategy. Chemistry determines the likely pharmacodynamic direction; that direction determines the most informative in vitro models; and only the strongest, mechanistically supported in vitro candidates should be taken into carefully chosen in vivo systems. This integrated view is the most reliable way to transform a semisynthetic GA derivative from a synthetic product into a pharmacologically interpretable lead compound.

Conclusion. The preparation of selected semisynthetic glycyrrhetinic acid derivatives and the selection of appropriate in vitro and in vivo models should be regarded as a single integrated research process rather than two independent tasks. Analysis of six original studies with verified DOI numbers shows that successful GA-derivative research follows a consistent pattern: rational structural modification of the parent scaffold, targeted in vitro screening according to the expected pharmacodynamic direction, mechanism-oriented cellular assays,

and in vivo validation in a model that matches the biological hypothesis. Anti-inflammatory derivatives are most appropriately studied in macrophage-based inflammatory systems and acute edema models, whereas antitumor derivatives require tumor-cell panels, apoptosis/ROS assays, and xenograft or metastasis models. Multifunctional derivatives such as soloxolone analogues may justify combined inflammatory and tumor-related model systems. For dissertation and preclinical work, this structure-driven model-selection strategy provides the strongest methodological foundation for studying glycyrrhetinic acid derivatives and for identifying which semisynthetic compounds are genuinely promising for further pharmacological development.

Table 1. Comparative overview of selected semisynthetic glycyrrhetinic acid derivatives and the experimental models used for their evaluation

Study	Derivative class / key modification	Main in vitro model(s)	Main in vivo model(s)	Pharmacodynamic direction	Why these models fit the derivative	Journal	DOI
Huang et al., 2020	Ring A GA derivatives with exocyclic α,β-unsaturated carbonyl moiety (lead 5c/5l)	Tumor cell lines (e.g., HCT-116), apoptosis-related cellular assays	Tumor-bearing mice / in vivo antitumor evaluation	Antitumor, apoptosis-inducing	Electrophilic ring A design predicted direct tumor-cell toxicity; cancer-cell panels and xenograft-style validation were therefore appropriate	Bioorganic Chemistry	10.1016/j.bioorg.2020.104187
Yang et al., 2020	C-11 reduction, C-3 oxidation, C-2 condensation derivatives (lead compound 10)	Anti-microbial testing plus inflammation-related biomarker assessment	TPA-induced mouse ear edema	Anti-inflammatory / antimicrobial	Derivative series was designed to suppress inflammatory mediators; ear edema with TNF-α/IL-1β/p65 readouts directly matched this aim	Bioorganic Chemistry	10.1016/j.bioorg.2020.103985

Markov et al., 2018	Soloxolone methyl (cyanoenone GA derivative)	Inflammation/tumor-oriented preclinical profiling	Carrageenan- and histamine-induced inflammation; Krebs-2 ascites carcinoma	Dual anti-inflammatory + antitumor	Hybrid pharmacology justified both inflammation and tumor models rather than a single-model approach	Molecular Biology	10.7868/S0026898418020143
Tu et al., 2022	C-2–oriented multifunctional GA derivatives (GA-O-02, GA-O-06)	LPS-related inflammatory cell systems; NO/cytokine assays	TPA-induced mouse ear inflammation	Anti-inflammatory with antimicrobial co-profile	Macrophage/inflammatory-cell assays identified cytokine-active leads; the ear model then validated tissue-level anti-inflammatory action	Bioorganic Chemistry	10.1016/j.bioorg.2022.105714
Salomatina et al., 2022	Epoxides of soloxolone methyl (αO-SM, βO-SM)	Cytotoxicity assays + inflammatory macrophage assays	Murine melanoma metastasis; carrageenan-induced peritonitis	Anti-metastatic + anti-inflammatory	Dual mechanism required both tumor-related and inflammatory models to capture the full pharmacodynamic profile	International Journal of Molecular Sciences	10.3390/ijms23116214
Markov et al., 2022	Soloxolone amides; soloxolone tryptamide (compound 12)	U87 glioblastoma cells; apoptosis / ROS-related assays	U87 glioblastoma xenograft	Antitumor / anti-glioblastoma	Disease-oriented glioblastoma cell model provided a focused bridge to the xenograft setting	Pharmaceuticals	10.3390/ph15050603

Practical model-selection framework derived from the six studies

1. Define the derivative’s expected pharmacodynamic direction from its chemical design before choosing biological models.

2. Use inflammation-centered in vitro models (NO, cytokines, NF-κB, macrophage assays) for anti-inflammatory derivatives.

3. Use tumor-cell viability, apoptosis, ROS, migration, and clonogenicity assays for antitumor derivatives.

4. Advance only the most active and mechanistically supported derivatives to in vivo testing.

5. Match the animal model to the biological hypothesis: ear edema/peritonitis for inflammation, xenograft/ascites/metastasis models for tumor-related activity.

6. Include biomarker analysis in vivo whenever possible so that efficacy is linked to mechanism rather than described only phenomenologically

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