

Gomisin A as a multi-target therapeutic agent: Pharmacological mechanisms, pharmacokinetics and network pharmacology (Review)

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Abstract. Gomisin A (GA; C₂₃H₂₈O₇) is a principal bioactive dibenzocyclooctadiene lignan derived from *Schisandra chinensis* (Turcz.) Baill. (Wu Wei Zi), a herb traditionally used in Chinese medicine for its adaptogenic, hepatoprotective and detoxifying properties, particularly in treating fatigue, chronic cough and liver disorders. GA exhibits multi-target potential against a spectrum of complex diseases. The present study aimed to assess the pharmacological profile, mechanisms, pharmacokinetics (PK) and computational docking of GA, emphasizing its potential as a multi-target agent. A comprehensive literature search was performed in the PubMed and China National Knowledge Infrastructure database platforms. All mechanistic findings were appraised and stratified by supporting evidence strength, complemented by network pharmacology screening, molecular docking

and molecular dynamics (MD) assessment of representative target complexes. GA exhibits polypharmacological properties, including hepatoprotective, anticancer, cardiovascular-modulating, neuroprotective and anti-inflammatory/antioxidant effects, with hepatoprotection representing the most robustly supported preclinical indication. GA modulates key signaling pathways such as the PI3K/AKT/mTOR, MAPK and ErbB receptor family pathways. Network pharmacology analysis identified STAT3, EGFR and mTOR as high-priority candidate targets. Molecular docking predicted moderate-to-strong binding affinity across nine hub proteins, with that of EGFR reaching -7.7 kcal/mol. MD simulations further supported the conformational plausibility of the GA-EGFR, GA-GSK3 β and GA-STAT3 complexes. PK data identified limited oral bioavailability as a key translational bottleneck. GA holds promise as a multi-target natural product. Advancing GA toward clinical translation requires addressing PK limitations through advanced delivery systems, alongside orthogonal validation of its predicted targets and preclinical and clinical evaluation.

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Abbreviations: APAP, N-acetyl-para-aminophenol; CYP3A4, cytochrome P450 family 3 subfamily A member 4; ERBB2, erb-b2 receptor tyrosine kinase 2; GA, gomisin A; MD, molecular dynamics; PAINS, pan-assay interference compounds; P-gp, P-glycoprotein; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α ; PK, pharmacokinetics; PXR, pregnane X receptor

Key words: GA, traditional Chinese medicine, pharmacological activity, PK, molecular docking, bioinformatics analysis

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1. Introduction

Complex chronic diseases such as cancer, metabolic disorders, cardiovascular diseases (CVDs) and neurodegenerative

conditions continue to pose formidable global health challenges. These multifactorial illnesses are typically driven by a combination of genetic predisposition, environmental factors and lifestyle, and are notable contributors to worldwide morbidity and mortality (1). Noncommunicable diseases caused at least 43 million deaths in 2021, accounting for 75% of non-pandemic-related deaths worldwide (2). Despite advances in modern medicine, effective therapeutic strategies, particularly those that are both efficacious and safe for long-term use, remain an unmet clinical need for a number of these conditions. The limitations of single-target drugs, coupled with drug resistance and adverse effects, have spurred the search for novel multi-target agents derived from nature.

Natural products are a cornerstone in drug discovery; >60% of approved small-molecule drugs are inspired by or derived from natural sources (3,4). Although the overall number of novel molecular entities approved annually fluctuates, natural product-based drugs have maintained a steady trajectory, highlighting their enduring relevance and chemical diversity (3). Traditional Chinese medicine (TCM) uses multicomponent herbal preparations, whose constituents may act on multiple biological pathways relevant to complex diseases (3).

Schisandra chinensis (Turcz.) Baill. is used in TCM. Its dried ripe fruits are constituents of herbal preparations investigated for liver disorder and neurobehavioral effects (5,6). These traditional applications motivate investigation of the biological activities of its individual constituents.

Gomisin A (GA), also known as schisandrol B or schizandrol B, is a dibenzocyclooctadiene lignan isolated from *S. chinensis*. The identification of GA as a notable bioactive constituent has bridged the gap between traditional medicinal use of *S. chinensis* and modern pharmacological research. Preliminary and growing evidence indicates that GA exhibits bioactivity, including hepatoprotective, anticancer, anti-inflammatory, antioxidant, neuroprotective and cardiovascular-modulating effects (7-10). However, the current research landscape remains fragmented. Although individual studies have reported diverse biological effects of GA, these findings have not yet been evaluated within a unified framework that considers pharmacological evidence, pharmacokinetic (PK) behavior, and computational analyses. Furthermore, a coherent framework linking the diverse activities of GA with specific molecular targets and pathways is needed to guide future research and development.

Therefore, the present review aims to provide a holistic assessment of GA, its pharmacological profile, PK behavior and associated challenges, and to integrate novel network pharmacology and molecular docking analyses to predict and elucidate its polypharmacological mechanisms. The present review also aims to assess the potential of GA as a promising multi-target lead compound, identify existing research gaps and provide priorities for future translational studies. Fig. 1 summarizes the reported hepatoprotective (7,11,12), anticancer (8,13), cardiovascular (9,14), neurological (10,15) and anti-inflammatory/antioxidant effects (16,17) of GA, together with PK and safety constraints (18,19), as well as candidate target network and docking results generated in the present study; these computational interactions remain hypothesis-generating.

2. Review approach

Literature search strategy. The present review used PubMed (<https://pubmed.ncbi.nlm.nih.gov/>) and the China National Knowledge Infrastructure database (cnki.net/). The search terms included GA, Schisandrol B, and combinations with hepatoprotective, anticancer, cardiovascular, neuroprotective, anti-inflammatory, antioxidant, PK and network pharmacology key words. Studies using purified GA monomers were prioritized for mechanistic analysis. Studies of well-characterized *Schisandra*-derived extracts or multicomponent preparations were retained as supportive evidence where relevant, but their effects were not attributed specifically to GA unless its independent contribution could be established.

Hierarchy of mechanistic evidence. To avoid equally weighting conclusions of varying reliability, each mechanistic claim was assigned to one of four evidence tiers (Table I): Tier A, direct target engagement shown by both a biophysical or cellular binding assay [surface plasmon resonance (SPR), cellular thermal shift assay (CETSA), drug affinity responsive target stability assay (DARTS) or co-crystallography] and a functional dependency experiment (knockdown, knockout or rescue), representing the strongest level of mechanistic support; tier B, target or pathway dependency is supported by loss- or gain-of-function studies (small interfering and short hairpin RNA knockdown, clustered regularly interspaced short palindromic repeats knockout, selective pharmacological inhibition or overexpression rescue) but lacking experimentally demonstrated direct target binding or cellular target engagement. Computational docking predictions, when available, were not considered direct binding evidence; tier C, pathway association includes findings inferred from reporter activity, changes in gene or protein expression, phosphorylation status, nuclear translocation, subcellular localization, or downstream biomarkers without formal perturbation demonstrating that the pathway is required for the phenotype; and tier D, computational prediction encompassing network pharmacology targets, molecular docking poses and molecular dynamics (MD) simulations, none of which have been experimentally validated.

Additional dimensions of evidence maturity. Beyond the mechanistic hierarchy, several contextual factors shape overall confidence in a pharmacological claim. These include the pathophysiological relevance of the disease model to human disease, independent replication across laboratories, whether the study used purified monomers or crude extract, whether effective concentrations or doses fall within a physiologically achievable range, whether dosing was prophylactic or therapeutic, the adequacy of selectivity and vehicle controls, the availability of paired PK data for PK/pharmacodynamic (PD) alignment, and the existence of any human or clinical data. These dimensions yield a more balanced assessment of translational potential than a single positive experiment alone.

Application of the evidence framework. Applying this appraisal across therapeutic areas places the activities of GA into three broad categories of translational maturity. Well-established preclinical activity is represented by hepatoprotection, the most

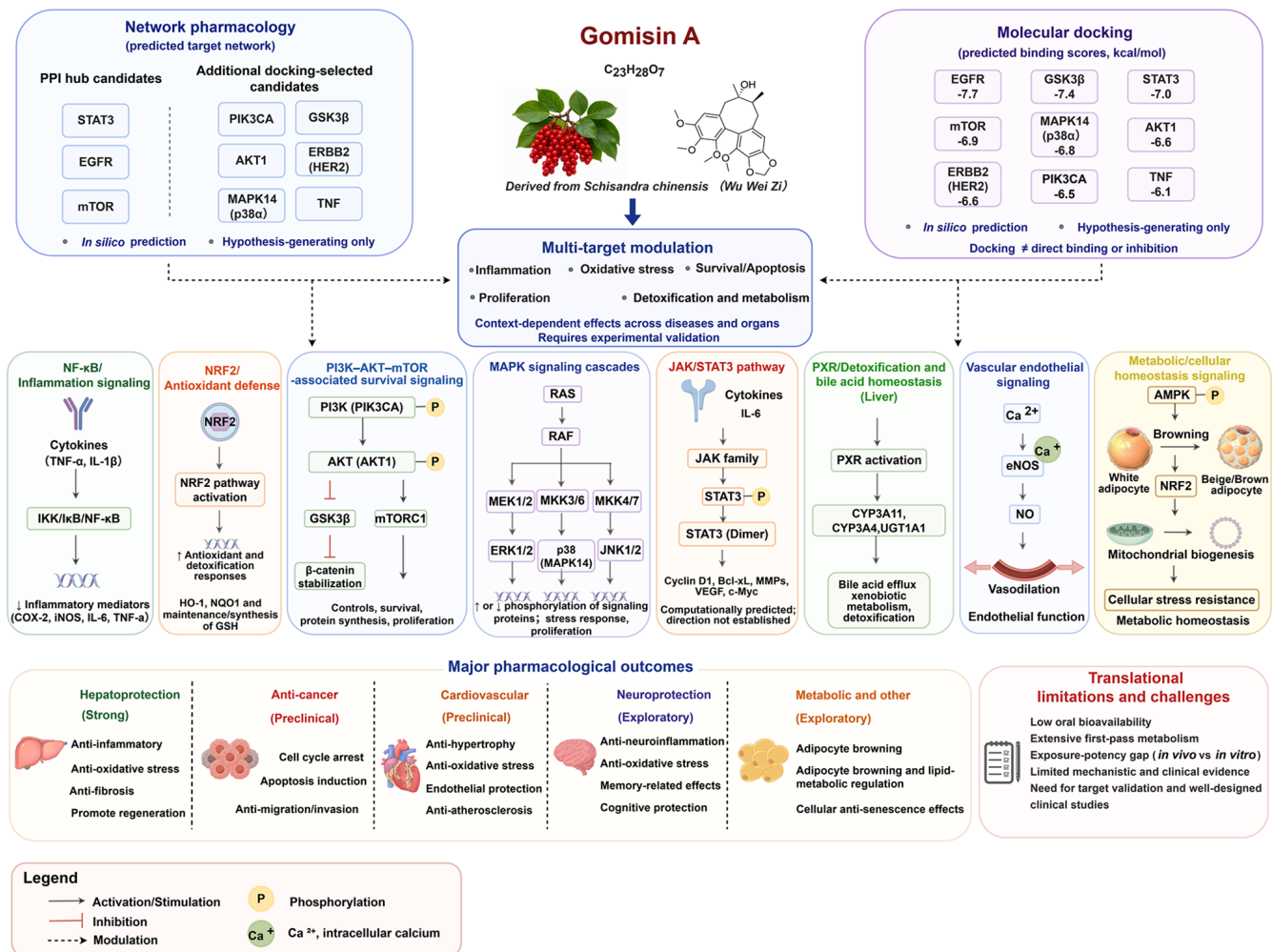


Figure 1. Pharmacological activities, putative molecular mechanisms and translational challenges of GA. GA, gomisin A.

thoroughly studied area, with replicated findings across acute injury, cholestasis, fibrosis and regeneration models (7,11,12, 20-22). Promising but preclinical activity includes anticancer and cardiovascular effects; both have substantial *in vitro* data but more limited *in vivo* validation, with most mechanisms remaining at the associative level (tier C) and little PK/PD alignment to support translatability (8,9,13,14,23-25). Exploratory observations include neuroprotection, anti-obesity, renoprotection, erectile function, antimicrobial and immunomodulatory effects (largely single-center, single-model reports with small sample sizes and thin mechanistic grounding, regarded as preliminary) (10,15,26-29).

Within this framework, examples of tier C evidence include NF- κ B suppression (7,16), nuclear factor erythroid 2-related factor 2 (NRF2) nuclear translocation and antioxidant response element reporter activation (17), and MAPK- and apoptosis-associated protein changes (8,26), where pathway-associated changes were reported without experimental demonstration of pathway necessity.

Network pharmacology analysis. To investigate the potential molecular mechanisms underlying the effects of GA, the present study used the Swiss Target Prediction platform (<https://www.swisstargetprediction.ch/>) to identify its candidate target genes in *Homo sapiens*. Only targets with a

non-zero prediction probability were retained for subsequent analysis, yielding 100 predicted candidate targets. This computational workflow was designed to prioritize candidate targets for experimental validation, rather than to confirm functional target engagement. The present study constructed a protein-protein interaction (PPI) network for these target genes using the STRING database (version 12.0; string-db.org/). Subsequently, to identify the core components of the PPI network, Cytoscape (version 3.10.4; <https://cytoscape.org/>) was used for network screening.

To explore potential associations between predicted GA targets and disease-associated genes while reducing non-specific background noise, disease-associated genes were retrieved separately for each disease entity from the GeneCards database (<https://www.genecards.org/>). A GeneCards Relevance Score ≥ 10 was applied as a predefined retrieval cutoff and the resulting gene sets were used for descriptive overlap analysis. The disease entities included ALI, hepatic fibrosis, cholestasis, liver regeneration, HC, CRC, OC, prostate cancer, breast cancer, retinoblastoma and metastatic melanoma. For each disease, the number of retained GeneCards entries and genes overlapping with the 100 predicted GA targets were summarized descriptively (Table SI). Because the GeneCards-derived disease sets were not restricted to one predefined set of human genes eligible to be counted in every

Table I. Hierarchy of mechanistic evidence used for critical appraisal.

Tier	Definition	Criteria	Representative examples
A (direct target engagement)	Direct physical binding confirmed + functional dependency verified	Biophysical or cellular binding assay (SPR, CETSA, DARTS or co-crystallography) and functional rescue/perturbation confirming target dependency	None identified among the studies included in the present review
B (target or pathway dependency)	Functional dependency confirmed, lacking experimentally demonstrated direct target binding or cell target engagement	Loss- or gain-of-function studies (siRNA/shRNA knockdown, CRISPR knockout, selective inhibitor blockade or overexpression rescue); no direct binding data	PXR-dependent anticholestatic protection: Loss of the major protective effects in PXR-null mice, supported by reporter transactivation and PXR target-gene induction
C (pathway association)	Pathway involvement inferred from biomarker changes, no dependency test	Evidence from expression levels, phosphorylation status, subcellular localization, reporter activity or downstream biomarker shifts; no formal perturbation to confirm causality	NF- κ B suppression; NRF2 nuclear translocation and ARE reporter activation; p38/ERK/JNK and PI3K/AKT phosphorylation changes; BCL-2/BAX and caspase-associated changes; downstream target gene expression changes in the absence of dependency testing
D (computational prediction)	<i>In silico</i> prediction only, no experimental validation	Targets predicted by network pharmacology, binding poses predicted by molecular docking and complex behavior assessed by molecular dynamics simulations	EGFR, STAT3 and mTOR docking binding modes

SPR, surface plasmon resonance; CETSA, cellular thermal shift assay; DARTS, drug affinity responsive target stability; siRNA, small interfering RNA; shRNA, short hairpin RNA; PXR, pregnane X receptor; ARE, antioxidant response element.

disease and target analysis, no formal enrichment significance was inferred from these overlaps. The overlap analysis was used to provide descriptive disease-context information for the predicted GA targets and should not be interpreted as evidence of target specificity, causal disease mechanisms or direct GA target engagement. The disease overlap analysis was restricted to well-defined disease entities (liver disease and individual cancer types) with notable pharmacological evidence. Cardiovascular and neurological effects were not subjected to analogous GeneCards overlap analysis because these encompass heterogeneous phenotypes and exploratory indications, and their mechanistic interpretation is based primarily on experimental literature and pathway-level evidence.

Gene Ontology (GO; geneontology.org/) and Kyoto Encyclopedia of Genes and Genomes (KEGG; kegg.jp/) enrichment analyses were performed on the 100 candidate target genes using the STRING platform, with the human whole genome as the background set and false discovery rate (FDR) correction applied for multiple testing. The 100 predicted GA targets and the full KEGG enrichment results

are provided in Tables SII and SIII, respectively. Only terms with an FDR <0.05 were considered.

Target prioritization. Key targets for experimental validation were identified as follows: i) Core hub proteins derived from the PPI network prioritized via topological indices (STAT3, EGFR and mTOR); ii) central nodes within significantly enriched KEGG pathways, including PIK3CA and AKT1 in the PI3K-Akt signaling pathway and MAPK14 in the MAPK signaling pathway; iii) molecules concurrently identified in network pharmacology and pathway enrichment analyses (ERBB2); and iv) proteins with experimental biological relevance associated with GA responses, including TNF (10,16), or pathway-associated relevance, including GSK3 β (30-33), while recognizing that these studies do not establish direct GA binding.

Molecular docking methods. Protein information for the target structures was obtained from UniProt (<https://www.uniprot.org/>). Molecular docking of GA (PubChem Compound ID: 634470) with these proteins was performed using

AutoDock Vina 1.1.2 software (34). These proteins were preprocessed in PyMOL 2.4 (pymol.org/; removal of water molecules and unnecessary ligands, and addition of hydrogen atoms). PDBQT files for docking simulations were generated using AutoDock Tools 1.5.6 (ccsb.scripps.edu/mgltools/). The docking results were set to output the top nine optimal docking poses. For each target, the top-ranked pose with the lowest Vina score was selected for interaction analysis. Finally, Protein-Ligand Interaction Profiler (version 3.0.0; plip-tool.biotech.tu-dresden.de/plip-web/plip) (35) and PyMOL 2.4 software were used to visualize the docking results to display predicted ligand-receptor contacts. According to the predicted docking score classification criteria from previous studies (36,37), a predicted docking score < -7.0 kcal/mol was assigned to the more favorable-score category; a value ≥ -7.0 and < -5.5 kcal/mol was assigned to the intermediate-score category; and a value ≥ -5.5 and ≤ -4.25 kcal/mol was assigned to the less favorable-score category. These categories do not establish binding affinity or specificity. These criteria were used only for descriptive comparison of the predicted docking scores of GA across the target proteins.

All simulations were performed in GROMACS 2020.6 (38). Protein topologies were generated using the AMBER99SB-ILDN force field, the ILDN modification of ff99SB described by Lindorff-Larsen *et al* (39); (<https://doi.org/10.1002/prot.22711>) and GA ligand parameters were derived from the General Amber Force Field using Sobotop 1.0 (sobereva.com/soft/Sobotop/). Each protein-ligand complex was solvated in a truncated octahedral transferable intermolecular potential with 3 points water box with a 1.0-nm minimum distance between the solute and the box boundary, and Na^+/Cl^- counterions were added to neutralize the net system charge (40). Energy minimization was conducted via 2,500 steps of steepest descent followed by 2,500 steps of conjugate gradient. Systems were equilibrated for 100 psec under the constant number-volume-temperature ensemble at 298.15 K and 100 psec under the constant number-pressure-temperature (NPT) ensemble at 298.15 K and 101.325 kPa. Production simulations were run for 100-nsec under NPT conditions with periodic boundary conditions and a 2-fsec integration time step (41). Long-range electrostatic interactions were treated with the particle mesh Ewald method and short-range electrostatic and van der Waals cutoffs were set to 1.0 nm. Trajectory frames were saved every 10 psec for analyses of the RMSD, root-mean-square fluctuation (RMSF), radius of gyration (Rg), intermolecular hydrogen bonds and solvent-accessible surface area (SASA) (42-44).

PAINS structural alert screening. Structural alert screening was performed using the RDKit FilterCatalog module (RDKit version 2025.09.4; <https://www.rdkit.org/>). The stereochemically defined structure of GA was evaluated against the combined RDKit PAINS catalog, comprising the PAINS_A, PAINS_B and PAINS_C rule sets.

3. Chemical identity and physicochemical properties of GA

GA is a stereochemically defined dibenzocyclooctadiene lignan with the molecular formula $\text{C}_{23}\text{H}_{28}\text{O}_7$ and a molecular weight of 416.47 g/mol (45). The scaffold of GA contains two aromatic rings connected through an eight-membered

Table II. Physicochemical properties and structural characteristics of gomisin A.

Property	Description
Chemical class	Dibenzocyclooctadiene lignan
Molecular formula	$\text{C}_{23}\text{H}_{28}\text{O}_7$
Molecular weight	416.47 g/mol
Calculated cLogP	3.60
Topological polar surface area	75.61 $\text{\AA}^2$
Hydrogen bond donors/acceptors	1/7
Rotatable bonds	4
PAINS/structural alerts	0 ^a

^aNone detected in the FilterCatalog of RDKit, an open-source cheminformatics toolkit; absence of an alert does not exclude non-specific assay behavior. cLogP, calculated logarithm of the octanol/water partition coefficient; PAINS, pan-assay interference compounds.

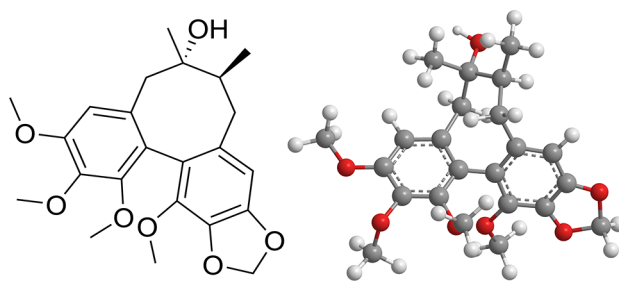


Figure 2. Chemical structure and three-dimensional conformation of gomisin A.

carbocyclic framework, one methylenedioxy group, four methoxy substituents, two methyl groups and one non-phenolic hydroxyl group (Fig. 2) (46). The primary physicochemical properties of GA are summarized in Table II.

Using the stereochemically defined structure and descriptors calculated with RDKit, an open-source cheminformatics toolkit (rdkit.org/), GA has a calculated molecular weight of 416.47 g/mol, calculated octanol-water partition coefficient (cLogP) of 3.60, topological polar surface area of 75.61 $\text{\AA}^2$, one hydrogen bond donor, seven hydrogen bond acceptors and four rotatable bonds. These properties are consistent with a moderately lipophilic small molecule that may cross biological membranes, but they do not predict oral bioavailability because first-pass metabolism and transporter interactions are substantial.

A structural-alert screen using the RDKit FilterCatalog found no matches to pan-assay interference compounds (PAINS) A, B, or C filters. This negative result decreases concern about classic PAINS substructures but does not establish assay specificity. High-micromolar lipophilic compounds cause colloidal aggregation, membrane perturbation, fluorescence or absorbance interference, redox effects and non-specific protein binding. PAINS filters are best used as flags for experimental follow-up rather than as proof of validity or invalidity (47,48).

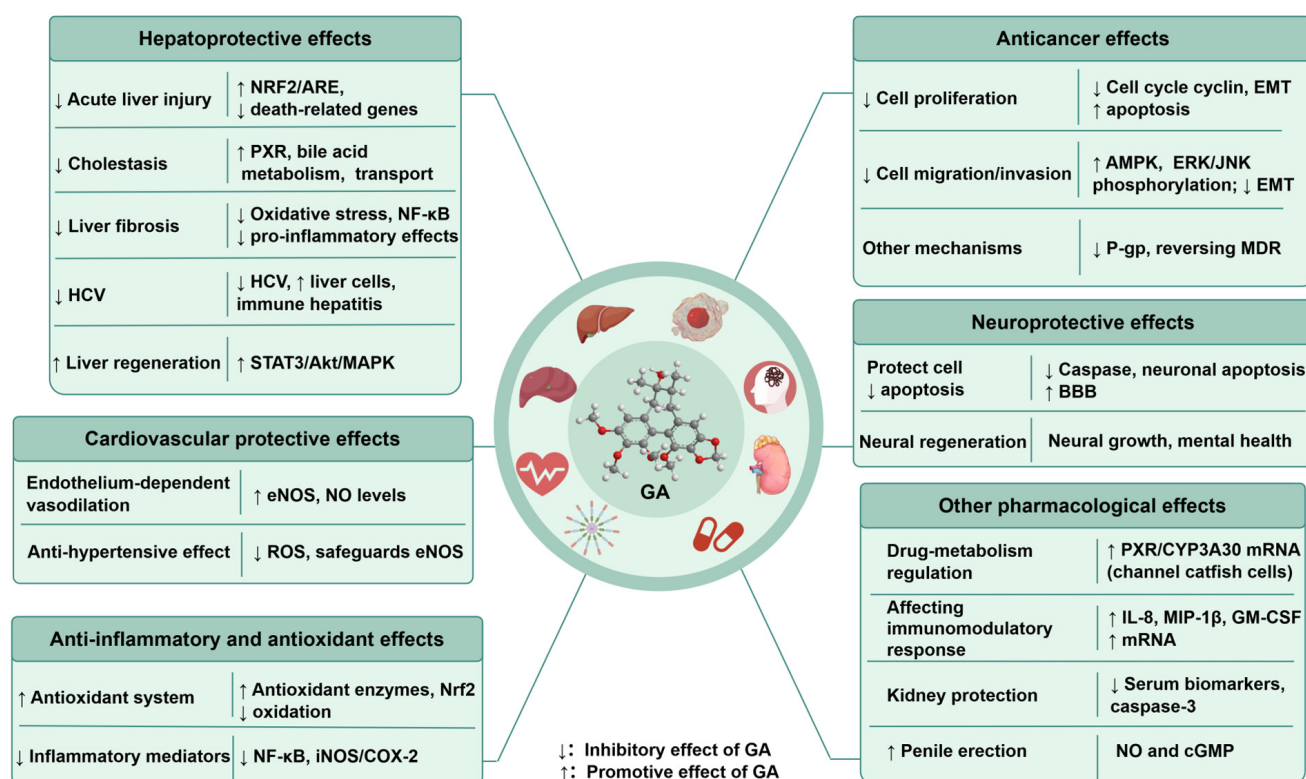


Figure 3. Overview of the reported pharmacological activities of GA. GA, gomisin A; PXR, pregnane X receptor; HCV, hepatitis C virus; iNOS, inducible nitric oxide synthase; P-gp, P-glycoprotein; MDR, multidrug resistance; EMT, epithelial-mesenchymal transition; BBB, blood-brain barrier; ROS, reactive oxygen species; eNOS, endothelial nitric oxide synthase; MIP, macrophage inflammatory protein; GM-CSF, granulocyte-macrophage colony-stimulating factor; ARE, antioxidant response element; COX-2, cyclooxygenase-2.

4. Pharmacological effects of GA

As a constituent of *S. chinensis*, GA has preclinical hepatoprotective (7,11,12), anticancer and chemosensitizing (8,13,49), cardiovascular (9,14,24,25), neuroprotective (10,15), and anti-inflammatory and antioxidant effects (16,17) (Fig. 3).

To determine the molecular basis underlying the reported hepatoprotective, anticancer, cardiovascular, neurological, anti-inflammatory and antioxidant effects, the present review identified nine core targets of GA [EGFR, erb-b2 receptor tyrosine kinase 2 (ERBB2), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α (PIK3CA), AKT1, mTOR, MAPK14, GSK3 β , STAT3 and TNF] via network pharmacology screening, prioritized by molecular docking. These computational predictions were integrated into a unified mechanistic interpretation of the activity of GA in the pathological context. The nine targets were prioritized by their acknowledged key position in the pathophysiology of the disease in question. Second, consideration was given to whether the direction of their pathway regulation can directly account for the experimental phenotypes reported in the literature. Third, selection was informed by their tissue and cell-type expression specificity. A target not elaborated upon does not mean it is uninvolved in that process but that it serves a secondary or auxiliary role and is therefore not treated as a main line of mechanistic explanation. This variable weighting of targets across disease contexts reflects the tissue specificity and pathway preference inherent to the multi-target pharmacological profile of GA.

Hepatoprotection: Best-supported preclinical domain.

The liver is a key metabolic organ that serves a pivotal role in numerous physiological processes, including nutrient metabolism, lipid and cholesterol homeostasis, and the degradation of xenobiotic compounds (50). In recent years, the number of individuals with liver disease in the world has increased annually (51). Therefore, the search for drugs to treat liver disease is a topic of concern. Numerous studies indicate that GA may exert a protective effect on hepatic disorder (7,11,12,20).

Alleviation of acute LI (ALI). The occurrence of ALI is attributable to various etiologies, encompassing viral infection, substance abuse (alcohol or drugs) and exposure to toxic agents (52). LI can be divided into four categories: Chemically, immune-, drug- and alcohol-induced LI. Experimental ALI is induced with hepatotoxic agents (53), including carbon tetrachloride (CCl_4), acetaminophen [N-acetyl-para-aminophenol (APAP)], D-galactosamine (D-GalN) and lipopolysaccharide (LPS) (Fig. 4). These models encompass the major experimental settings in which GA-mediated hepatoprotection has been mechanistically investigated (7,54,55).

CCl_4 . CCl_4 is one of the most widely used hepatotoxic agents and the mouse CCl_4 -induced ALI model is a classic paradigm of oxidative liver damage research (7,26,56). GA reduces hepatic malondialdehyde (MDA) content and restores glutathione (GSH) peroxidase and superoxide dismutase activities, supporting an antioxidant contribution to GA-mediated protection. These biochemical changes are accompanied by marked decreases in serum aspartate aminotransferase (AST) and alanine aminotransferase

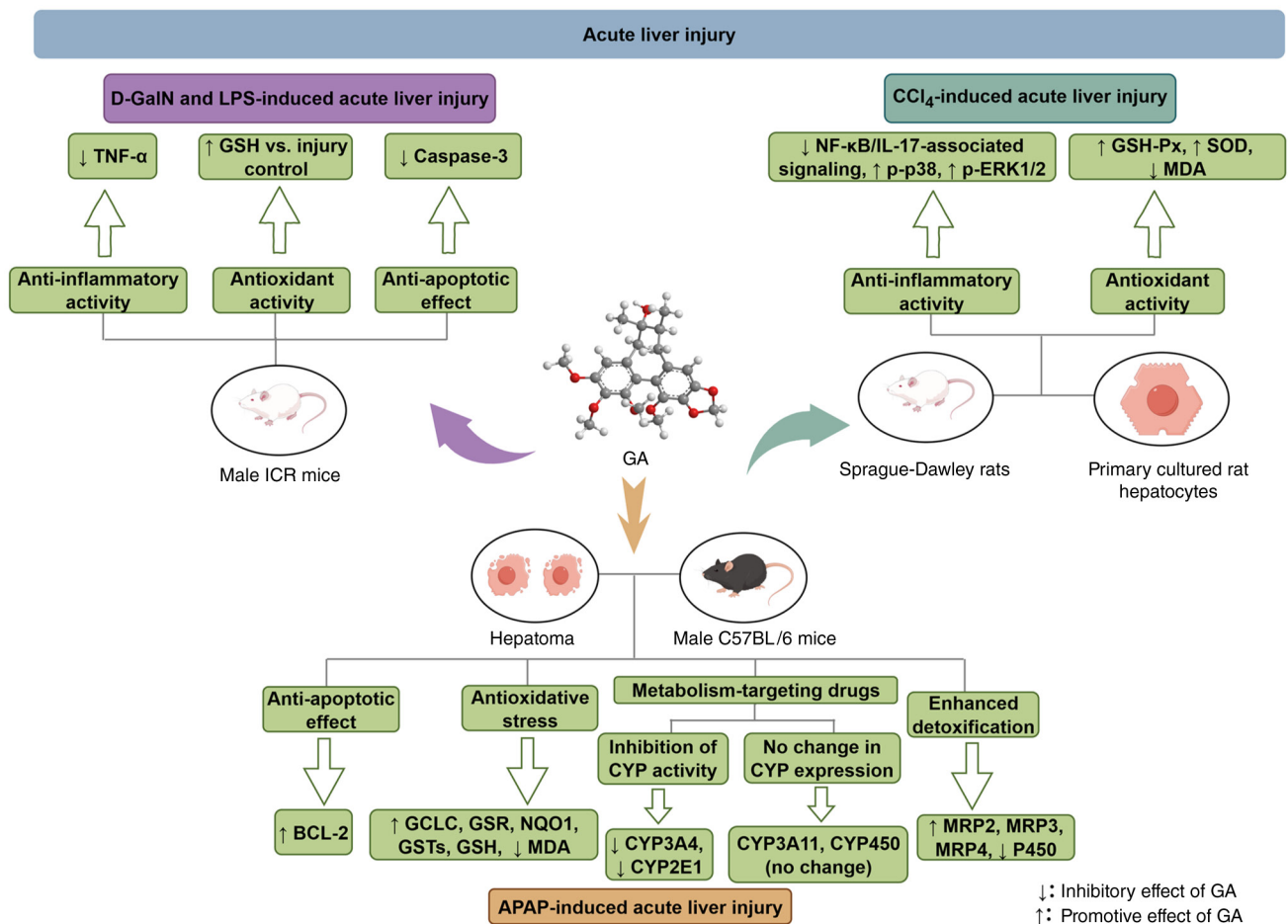


Figure 4. Effects of GA in experimental models of acute liver injury. D-GalN, D-galactosamine; LPS, lipopolysaccharide; GSH-Px, glutathione peroxidase; ICR, Institute of Cancer Research mouse stock; SOD, superoxide dismutase; MDA, malondialdehyde; GCLC, glutamate-cysteine ligase catalytic subunit; GSR, glutathione reductase; NQO1, NAD(P)H quinone dehydrogenase 1; GST, glutathione S-transferase; GA, gomisin A; CYP, cytochrome P450; MRP, multidrug resistance-associated protein; APAP, acetaminophen.

(ALT) levels, indicating overall protection against hepatocellular damage (7,26). The anti-inflammatory component of this protection is associated with NF- κ B suppression (7,54). GA downregulates NF- κ B and IL-17 signaling, with corresponding decreases in inducible nitric oxide (NO) synthase (iNOS) and cyclooxygenase-2 (COX-2) expression in injured liver tissue (54). NF- κ B inhibition may contribute to the reduction in early fibrotic changes, given the well-established link between inflammatory signaling and hepatic fibrogenic activation (7,57,58).

In the rat liver, GA increased ERK1/2 and p38 phosphorylation relative to CCl₄-treated controls; JNK did not show the same clearly documented increase (26). These changes coincide with upregulated BAX levels and decreased caspase-3 levels, suggesting that MAPK signaling shapes the apoptotic response to CCl₄ injury (26). The net direction of this regulation is not straightforward because both pro-apoptotic (BAX induction) and anti-apoptotic (caspase-3 suppression) features are observed. Microarray data demonstrate that GA-induced liver repair is associated with TGF- β -dependent transcriptional changes, including the upregulation of cell cycle genes and downregulation of cell death-associated transcripts, consistent with a pro-regenerative gene expression program (59).

Taken together, GA counteracts CCl₄ hepatotoxicity through overlapping antioxidant, anti-inflammatory, apoptosis-regulating and pro-repair pathways. These pathway alterations should therefore be interpreted as associated biological responses rather than experimentally confirmed direct molecular targets. The data suggest a combined decrease in oxidative and inflammatory burden, together with repair-associated transcriptional changes (7,59). Inconsistencies, such as increased BAX levels together with reduced active caspase-3 levels in the CCl₄ study (26), caution against assigning a simple anti-apoptotic mechanism from single protein measurements. Most CCl₄ studies do not use target-selective antagonists, gene deletion or rescue experiments; consequently, NF- κ B, MAPKs, TGF- β -associated genes and apoptosis proteins are pathway-associated readouts rather than established direct GA targets (7,26,54,59).

APAP. APAP is one of the most widely used analgesics and antipyretics worldwide. Although safe at therapeutic doses, overdose causes severe hepatotoxicity and remains a notable cause of acute liver failure globally (60). APAP undergoes bioactivation by cytochrome P450 (CYP) enzymes, principally CYP2E1, CYP3A4 and CYP1A2, to form the reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI), which depletes hepatic GSH levels and triggers mitochondrial dysfunction

and oxidative stress (61). GA intervenes at several points along this cascade. Upstream, it inhibits CYP3A4 activity and decreases CYP-mediated APAP bioactivation (62,63). Further downstream, GA activates the NRF2/antioxidant responsive element (ARE) pathway and upregulates NRF2-driven detoxification genes, thereby enhancing cell antioxidant capacity and limiting GSH depletion in APAP-challenged animals (17). At the regenerative stage, GA modulates p53, p21, cyclin D1 (CCND1), proliferating cell nuclear antigen and BCL-2 expression to promote hepatocyte proliferation and tissue repair (11). Functionally, these effects translate into decreased serum ALT and AST activity, diminished hepatic MDA formation and preserved mitochondrial GSH pools in a dose-dependent manner (11).

In summary, GA protects against APAP hepatotoxicity through decreased metabolic activation, enhanced antioxidant defense and accelerated liver regeneration. These findings are compatible with antioxidant, metabolic and regenerative contributions, but the relative importance of each mechanism remains uncertain because intrahepatic exposure, NAPQI-protein adducts and target-dependency interventions have not been consistently measured across studies (11,17,63). In addition, numerous experiments use pretreatment schedules, which demonstrate prophylactic activity rather than therapeutic efficacy against established injury (11,17,63).

D-GalN and LPS. D-GalN is selectively taken up by hepatocytes, where it depletes intracellular uridine nucleotide pools, and impairs RNA and protein synthesis. When co-administered with low-dose LPS, it triggers fulminant hepatic failure that recapitulates the metabolic and inflammatory features of acute liver failure in humans (64). The resulting lesion is driven by two parallel pathways. On the inflammatory side, NF- κ B nuclear translocation increases, driving transcriptional upregulation of iNOS, TNF- α , IFN- γ and multiple ILs (IL-1 β , IL-6, IL-12 and IL-18). On the antioxidant side, NRF2 and its downstream targets NAD(P)H quinone dehydrogenase 1, heme oxygenase-1 (HO-1) and GSH S-transferase α are concurrently suppressed, further tilting the balance toward oxidative damage (65). In this model, GA reduces serum ALT levels and preserves hepatic GSH content relative to D-GalN/LPS-treated controls, while also attenuating mitochondrial edema. These effects are attributable to reduced oxidative stress and suppression of TNF- α and caspase-3 activity, suggesting combined anti-inflammatory and anti-apoptotic actions (20). GA can inhibit the expression of TNF- α itself by downregulating pathways such as the NF- κ B pathway (16), and decrease inflammatory responses in experimental asthma and activated microglia (10,66). Thus, experimental data support decreased inflammatory mediator production associated with NF- κ B suppression (10,16); direct GA-TNF binding remains a computational hypothesis. In summary, GA protects against D-GalN/LPS-induced LI through concurrent anti-inflammatory, antioxidant and anti-apoptotic mechanisms.

In conclusion, across CCl₄-, APAP- and D-GalN/LPS-induced injury, the most reproducible phenotype is the attenuation of hepatocellular damage. GA decreases serum aminotransferase activity, histological necrosis, lipid peroxidation and mitochondrial injury, while improving antioxidant indices (7,11,17,20,26,54,59,63). The convergence of these

endpoints across distinct injury models supports hepatoprotective pharmacology. It does not, however, establish one universal molecular target, because the initiating toxic mechanisms differ between CCl₄ and APAP bioactivation and GalN/LPS-driven inflammatory apoptosis. Taken together, ALI studies provide moderate-to-strong evidence for a hepatoprotective phenotype and low-to-moderate evidence for most individual signaling mechanisms. Future work should use post-injury dosing, exposure-matched concentrations, APAP-protein adduct measurements and pathway-specific loss-of-function designs.

Cholestatic LI and PXR dependency. Cholestatic liver disease, characterized by impaired hepatic bile formation or biliary bile flow (67), is induced by genetic immune defects, toxins and physiological dysregulation that damage bile ducts, leading to bile retention, hepatic injury, inflammation, fibrosis and progression to cirrhosis or liver malignancy (68).

In the lithocholic acid (LCA)-induced cholestatic injury model, GA decreases mortality and improves both histological and biochemical markers of damage. Serum lactate dehydrogenase, ALT, AST, alkaline phosphatase, total bile acids and total bilirubin levels are all lowered and TUNEL-positive cells and hepatocyte membrane pore formation are decreased (69,70).

The protective mechanism converges on PXR activation, which drives two parallel downstream programs. On the inflammatory and cell death side, PXR signaling suppresses NF- κ B activity, which reduces NLR family pyrin domain containing 3 (NLRP3) inflammasome assembly; PXR also downregulates FoxO1, decreasing apoptotic protease-activating factor 1 (APAF-1)-dependent atypical inflammasome activation (12,70). On the detoxification side, PXR upregulates bile acid metabolism and efflux machinery, including the prototypical target genes CYP3A11 and uridine diphosphate glucuronosyltransferase 1A1, accelerating metabolic conversion and fecal clearance of toxic bile acids (12,70). Direct activation of human PXR with the corresponding upregulation of its target genes has also been demonstrated (70).

In summary, GA prevents LCA-induced cholestatic LI primarily through PXR-mediated dual effects: Suppression of inflammasome-driven injury, and enhancement of bile acid detoxification and excretion. PXR is the best-supported functionally required mediator of GA-induced anticholestatic effects. Reporter transactivation, induction of PXR target genes and the marked loss of the anticholestatic phenotype in *Pxr*-null mice support PXR dependency (12). However, the aforementioned PXR studies provide functional rather than experimentally demonstrated direct physical GA-PXR binding evidence (12,69,70). Therefore, the PXR mechanism should be classified as tier B. The evidence suggests PXR-dependent protection but does not prove that GA directly binds to PXR or directly targets each downstream component of the proposed NF- κ B/NLRP3 and FoxO1/APAF-1 pathways. Translational limitations remain substantial. The protective doses used in rodent studies are high, in the order of 100 mg/kg twice daily in representative protocols (12,71). Rodent and human PXR differ in ligand selectivity, so activity in mouse models does not guarantee equivalent potency at the human receptor (12). Clinical efficacy cannot currently be determined because controlled human studies remain unavailable. Furthermore, PXR activation upregulates the expression of CYP enzymes

and drug transporters (72), which carries clinically meaningful drug-interaction risk.

Improvement of liver fibrosis. Liver fibrosis develops when chronic or repeated hepatocellular injury drives excessive accumulation of the extracellular matrix (ECM), predominantly collagen. Without intervention, it progresses to cirrhosis and, in certain patients, hepatocellular carcinoma (57). Two signaling axes are key to fibrogenesis. The TGF- β 1/Smad pathway is the canonical driver: TGF- β 1 activates hepatic stellate cells (HSCs) via Smad proteins, stimulating ECM secretion and scar formation (73). The NF- κ B pathway acts in parallel by sustaining the inflammatory milieu that fuels fibrogenic progression (58).

Surfeit locus protein 4 (Surf4), an endoplasmic reticulum (ER) cargo receptor involved in ER-to-Golgi trafficking, has been identified as a potential pro-fibrotic regulator: Surf4 facilitates the secretion of serum amyloid A1 (SAA1) from hepatocytes. SAA1 promotes HSC activation, at least in part through toll-like receptor 2 signaling (74). Hepatocyte-specific Surf4 deletion markedly attenuates CCl₄-induced liver fibrosis in mice, whereas increased Surf4 and SAA1 expression are observed in human fibrotic liver samples (74). These findings support the Surf4-SAA1-HSC axis as a potential pathogenic pathway contributing to liver fibrosis (74).

In CCl₄-induced fibrotic mice, combined treatment with GA and wedelolactone, a plant-derived coumarin, reverses established fibrosis, potentially by inhibiting TGF- β 1 and NF- κ B pathway activity and decreasing cytoglobin expression, a marker of HSC activation and the fibrogenic response (21).

To the best of our knowledge, evidence supporting the antifibrotic activity of GA remains limited. In CCl₄-induced fibrotic mice, GA monotherapy shows antifibrotic activity; however, its combination with wedelolactone produces greater improvements than either monotherapy (21). However, superiority of the combination treatment alone does not establish pharmacological synergy; formal interaction analyses were not reported. Changes in cytoglobin and fibrosis-associated markers, together with decreased TGF- β 1/Smad and NF- κ B signaling, support the antifibrotic activity of the treatment regimen but do not establish a GA-specific molecular target (21). Thus, the combination treatment shows promising antifibrotic activity in mice but the confirmation of pharmacological synergy and clarification of GA-specific mechanisms require formal combination analysis, independent replication and target-dependency experiments.

Regulation of hepatitis C virus (HCV). Chronic HC is a global public health burden, with ~71 million individuals living with HCV worldwide and substantial risk of progression to cirrhosis and hepatocellular carcinoma (75). GA exerts dose-dependent anti-HCV activity *in vitro*, putatively through the suppression of host membrane proteins involved in viral binding or entry (5,76). However, the specific host proteins involved have not been definitively identified. The evidence base remains small and largely preclinical (5,76), and unsupported by contemporary antiviral target validation or PK exposure studies. Neither the relevant host factor, target dependency, resistance profile nor comparative activity against modern direct-acting antivirals have been established. These findings are best framed as preliminary antiviral pharmacology: Exploratory observations insufficient to position

GA as an antiviral candidate without renewed testing in state-of-the-art HCV systems.

Promotion of liver regeneration. The liver possesses notable regenerative capacity, restoring mass and function following injury or partial hepatectomy through tightly controlled hepatocyte proliferation (77,78). GA accelerates this process through two converging mechanisms. First, it increases the hepatic levels of key growth factors and cytokines, such as hepatocyte growth factor (HGF), EGF and IL-6, thereby activating the STAT3, AKT and MAPK cascades that drive hepatocyte proliferation. This pathway is associated with faster functional recovery than vehicle treatment following partial hepatectomy in mice (22). Second, GA-mediated PXR activation enhances bile acid detoxification and efflux, decreasing cholestatic stress and creating a more permissive environment for regeneration (12). Although these regeneration signals are biologically coherent, existing data represent pathway associations rather than established direct targets; the contribution of STAT3, AKT, and MAPK signaling to GA-mediated regeneration has not been established because pathway inhibition or genetic validation studies remain limited. Furthermore, whether the pathway activations reflect direct kinase targeting by GA or are downstream consequences of improved tissue viability and decreased bile acid toxicity remains unclear. Temporal profiling, pathway blockade experiments and target-engagement assays are needed to distinguish these possibilities. Finally, enhanced growth signaling may benefit post-resection recovery, but may result in undesirable effects in premalignant or chronically injured livers (77).

CYP3A4-mediated drug interactions and metabolic dysfunction-associated steatotic liver disease (MASLD) therapy. CYP3A4 is most abundantly expressed in the liver and is also present in the human prostate, breast, gastrointestinal tract, colon and small intestine tissue. In total, ~38 structural classes of substrates, totaling >150 drugs, are metabolized by CYP3A4, accounting for ~50% of all medicines metabolized. Therefore, CYP3A4 is among the most clinically important P450 enzymes in drug metabolism and drug-interaction risk assessment (79). The interaction between GA and CYP3A4 is multifaceted. GA has also been developed as an isoform-selective fluorescent probe substrate for CYP3A4 in microsomes and living cells (80). This analytical application should be separated from hepatoprotection. GA inhibits CYP3A activity (62), whereas PXR activation may increase the expression of CYP3A and transporters over a longer time scale (12). Therefore, the net effect may depend on dose, exposure duration, species and disease state, creating a risk of altered exposure to co-administered CYP3A substrates.

Beyond toxin-, cholestasis- and inflammation-driven models of LI, MASLD represents a major metabolic liver disorder; a meta-analysis using the earlier NAFLD definition estimated global prevalence at 32.4% (81). MASLD is also a relatively underexplored therapeutic context for GA. Hepatic steatosis, lipotoxicity and oxidative stress are key features of MASLD pathogenesis, whereas peroxisome proliferator-activated receptor α (PPAR α) is key in regulating hepatic fatty acid oxidation and lipid homeostasis. In transgenic hamsters, expression of the *Caenorhabditis elegans* ω -3 fatty-acid desaturase gene *fat-1* promotes the nuclear localization of PPAR α , accompanied by improvements in dyslipidemia,

hepatic steatosis and atherosclerotic lesions. These findings suggest that PPAR α serves as a molecular link between hepatic lipid metabolism and cardiovascular metabolic homeostasis (82). Although direct evidence regarding the effects of GA in MASLD remains limited, its proprotein convertase subtilisin/kexin type 9 (PCSK9)-mediated low-density lipoprotein (LDL)-lowering effect, PXR-dependent bile acid regulation, and broad NRF2/NF- κ B-modulated antioxidant and anti-inflammatory activity collectively support mechanistic plausibility (55,70). The involvement of PPAR α or other lipid-sensing nuclear receptors in GA-associated metabolic regulation requires further experimental clarification; therefore, MASLD represents a high-potential, mechanistically coherent direction for future hepatoprotective investigation.

Critical assessment and translational considerations. LI models provide a general picture of the dose range, translational potential and possible mechanisms of GA (7,11,20). Rodent hepatoprotection has been reported with oral GA pretreatment in CCl $_4$ -, APAP- and D-GalN/LPS-associated injury, although dose and dosing frequency differ across models (7,11,20). A rat tissue-distribution study found preferential GA accumulation in the liver and kidneys after oral administration (18). This may make liver disease a more practical therapeutic setting for GA than indications that depend on sustained exposure in peripheral tissues, such as cancer or CVD (7,11,12,18,20).

Nonetheless, studies employ pretreatment schedules (7,11,17), so the data support prophylactic protection rather than therapeutic reversal of established injury. In fibrosis, combination of GA and wedelolactone is more effective than monotherapy (21). Differences in GA purity, vehicle and dosing frequency across laboratories further complicate cross-study dose-response comparisons. On balance, hepatoprotection is the best-supported pharmacological activity of GA, but meaningful clinical translation requires post-injury treatment protocols, precise dose-response characterization and direct measurement of the hepatic tissue concentration.

Mechanistic insights from network pharmacology. Network pharmacology and molecular docking data provide a multi-target framework that may account for the breadth of this hepatoprotective phenotype, organized into three signaling tiers. At the most upstream receptor tier, EGFR (predicted affinity score, -7.7 kcal/mol) is a membrane tyrosine kinase whose inhibition would simultaneously dampen both PI3K/AKT and MAPK signaling, potentially decreasing proliferation of activated HSCs, a prediction consistent with reported anti-fibrotic trends across liver and vascular injury models (26,83), while modulating hepatocyte proliferation during regeneration. At the intermediate kinase tier, four nodes are central: The PI3K/AKT/mTOR pathway (affinity, -6.5, -6.6 and -6.9 kcal/mol, respectively), where PIK3CA shows moderate binding consistent with the regulatory (rather than complete inhibitory) modulation of PI3K/AKT signaling observed in ALI (54); MAPK14 may constitute a link between the computational network and the inflammatory and apoptosis-associated responses observed in LI; in the CCl $_4$ model, GA suppresses NF- κ B/IL-17 signaling and decreases iNOS and COX-2 expression, whereas separate experiments have demonstrated increased hepatic p38 and ERK phosphorylation relative to CCl $_4$ -treated controls (26,54); GSK3 β exhibits a predicted affinity of -7.4 kcal/mol and may

represent an upstream regulatory candidate for the antioxidant branch of the hepatoprotective response. This hypothesis is consistent with activation of NRF2/ARE signaling, preservation of hepatic GSH pools, decreased MDA accumulation and improvement of other antioxidant indices in APAP- and D-GalN/LPS-associated injury models (11,17,20). To the best of our knowledge, however, whether GSK3 β contributes to GA-mediated NRF2 regulation has not been experimentally established. Experimentally, increased HGF, EGF and IL-6 levels and the activation of STAT3, AKT and MAPK signaling have been associated with GA-promoted liver regeneration following partial hepatectomy or transplantation (22). Consistent with these experimental findings, STAT3 (predicted docking score, -7.0 kcal/mol) participates in IL-6-dependent regenerative transcription and is associated with GA-promoted liver regeneration (22,84). At the downstream cytokine tier, TNF (-6.1 kcal/mol) is predicted to interact with GA at the trimer interface, adding a potential protein-level layer of suppression on top of the transcriptional inhibition via NF- κ B signaling (16). In line with these docking-derived hypotheses, the 100-nsec trajectories support the dynamic plausibility of the GA-EGFR and GA-GSK3 β complexes, whereas the GA-STAT3 system undergoes a longer relaxation period and exhibits greater conformational mobility (Fig. 5A). These findings provide structural support for candidate interactions but do not establish direct target engagement in hepatocytes.

These computational data suggest GA targets across receptor, kinase, transcription factor and cytokine levels, consistent with the integrated anti-inflammatory, antioxidant, antifibrotic and pro-regenerative phenotype observed experimentally (16,17,21,22). GA exhibits preclinical promise across a spectrum of liver disease models (Table III; Fig. 6) (11,12,17,20,22,85).

Anticancer activity: Promising phenotypes with a substantial exposure gap. Cancer is a severe disease that endangers human health. Cancer results from cells in local tissue losing normal proliferation regulation at the genetic level under the influence of carcinogenic factors, leading to abnormal clonal proliferation and the formation of neoplastic lesions (86). GA has notable anticancer effects across a broad spectrum of malignancies, encompassing colorectal, ovarian, breast and human prostate cancer, as well as metastatic melanoma (8,13,23,87). Experimental evidence indicates that the anticancer activity of GA involves multiple complementary mechanisms, including the inhibition of cell proliferation, induction of cell cycle arrest and cell death, suppression of cell migration and invasion, and modulation of chemosensitivity and drug resistance (Figs. 7 and 8) (23,49).

Inhibition of cell proliferation. Cancer cells are not controlled by the normal regulatory system. Abnormal growth and proliferation are notable characteristics of cancer (88), making the inhibition of tumor cell proliferation a potential strategy for cancer treatment. Induction of cell cycle arrest and promotion of apoptosis effectively limit the proliferation of cancer cells (8,23).

Induction of cell cycle arrest. Cell cycle arrest can directly inhibit cellular proliferation, rendering cell cycle regulation pivotal in preventing cancerous development (89). The cell cycle, comprising G $_0$ /G $_1$, S, G $_2$ and M phases, is key for cell viability and is regulated by a series of molecular mechanisms.

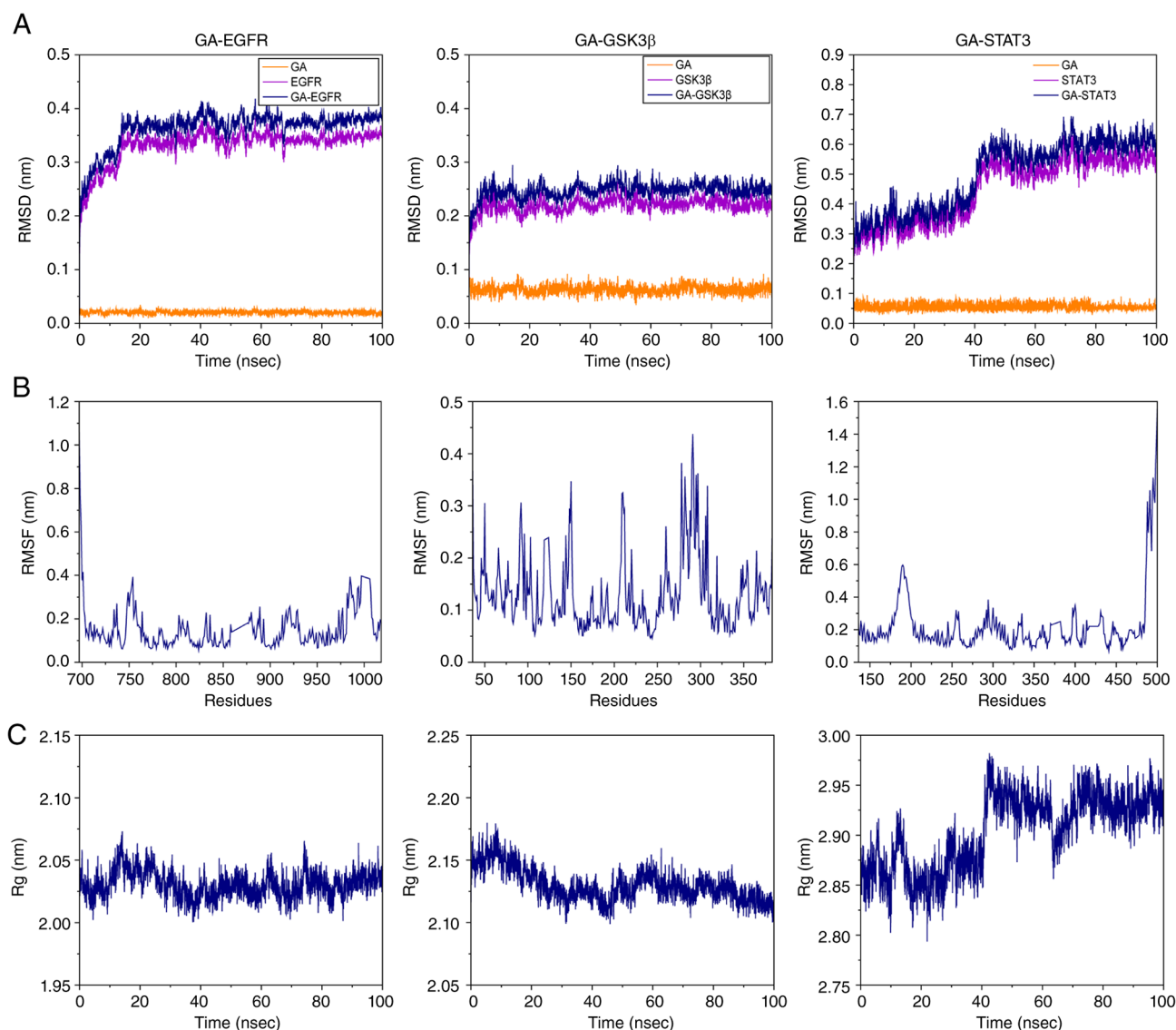


Figure 5. MD analysis of representative GA-target complexes. (A) RMSD profiles of the GA-EGFR, GA-GSK3 β and GA-STAT3 complexes during 100-nsec MD simulations. (B) RMSF profiles of the complexes. (C) Rg profiles over the simulation trajectories. GA, gomisin A; MD, molecular dynamics; RMSD, root-mean-square deviation; RMSF, root-mean-square fluctuation; Rg, radius of gyration.

These cell cycle stages are controlled by cyclins, CDKs and CDK inhibitors (90). GA consistently induces G₀/G₁ phase arrest in CRC and melanoma cells. The central effector mechanism is downregulation of the CCND1-CDK4 complex, which reduces retinoblastoma protein phosphorylation and blocks progression through the G₁/S restriction point. This phenotype has been documented in both colorectal cancer (CRC) (8) and melanoma cells (23).

Two upstream signaling pathways converge on this key cell cycle effect. First, GA suppresses STAT1 expression, which in turn reduces CCND1 transcription and reinforces G₁ arrest (91). Second, it activates AMP-activated protein kinase (AMPK) and MAPK signaling, increasing the phosphorylation of AMPK, ERK and JNK in melanoma cells, leading to dose-dependent decreases in CCND1 and CDK4 levels at both the mRNA and protein levels (23). In ovarian cancer (OC) cells, GA serves primarily as a paclitaxel (PTX) sensitizer. By reducing intracellular reactive oxygen species (ROS) levels, GA enhances the antiproliferative and cell cycle-arresting effects

of PTX, accompanied by decreased expression of CDK4 and cyclin B1. This combination improved antitumor efficacy in both cell-based assays and an OC mouse model (13). The effect is somewhat counterintuitive as reduced ROS levels would typically be expected to attenuate chemotherapeutic toxicity, and likely reflects the selective suppression of pro-survival ROS signaling rather than global antioxidant activity (89).

In summary, GA drives G₀/G₁ arrest in CRC and melanoma via STAT1- and AMPK/MAPK-mediated downregulation of CCND1-CDK4 expression, whereas in OC, it primarily serves as a PTX sensitizer acting through ROS and PI3K/AKT signaling.

Induction of cell death. Apoptosis is a regulated form of programmed cell death. Apoptosis is necessary for successful organogenesis during embryonic development and for maintaining normal tissue homeostasis (92). Insufficient apoptosis may contribute to uncontrolled cell proliferation, including in cancer (92). Inducing apoptosis is a major mechanism of numerous anticancer agents such as PTX (90). GA is reported to

Table III. Hepatoprotective effects of gomisin A in experimental liver disease.

Condition	Model	Biochemical effects	Antioxidant effects	Histological effects	(Refs.)
Acute liver injury	CCl ₄ -induced mice	Decreased ALT and AST	Increased GSH-Px and SOD; decreased MDA	Reduced hepatocyte injury	(54)
Acute liver injury	CCl ₄ -treated SD rats	Decreased ALT, AST and ALP	Decreased lipid peroxidation; increased antioxidant defenses	Decreased hepatocellular necrosis and central-vein dilation	(26)
Acute liver injury	CCl ₄ -treated SD rats	Decreased serum liver injury markers	Restoration of oxidative stress-related pathways and antioxidant responses	Reduced CCl ₄ -induced liver damage	(59)
Acute liver injury	APAP-treated mice	Decreased ALT and AST	Preserved GSH; decreased MDA	Decreased hepatic necrosis	(11,17,63)
APAP bioactivation	Mouse liver	Reduced CYP-mediated APAP bioactivation	Not applicable	Not applicable	(17,63)
Drug metabolism	CYP3A4 cells	Inhibition of CYP3A4 activity	Not applicable	Not applicable	(62)
Antioxidant response	HepG2 cells	NRF2/ARE reporter activation; induction of protective genes	Activation of antioxidant defenses	Not applicable	(17)
Acute liver injury	D-GalN/LPS-treated mice	Decreased ALT and AST	Preserved GSH	Decreased hepatocyte necrosis and hemorrhage	(20)
Cholestasis	LCA-treated mice	Decreased liver injury markers and bile-acid burden	Not specifically evaluated	Reduced liver necrosis and inflammatory/neutrophil infiltration	(12,69,70)
Cholestatic mechanism	HepG2 and 293T cells	PXR transactivation and downstream gene regulation	Not specifically evaluated	Not applicable	(12,70)
Liver fibrosis	BDL-induced fibrosis in mice; GA alone and with wedelolactone	Decreased liver injury markers; combination treatment more effective	Modulation of oxidative stress-related pathways associated with fibrosis improvement	Reduced hepatocyte injury and collagen deposition	(21)
Liver fibrosis	CCl ₄ -induced fibrosis in mice; GA + wedelolactone	Decreased liver injury markers	Modulation of oxidative stress-related pathways associated with fibrosis improvement	Reduced hepatic injury and fibrosis	(21)
Hepatocyte injury	APAP-treated L02 cells	Improved cell viability	Not specifically evaluated	Not applicable	(21)
Hepatitis C	MOLT-4 cells	Inhibition of HCV infection	Not applicable	Not applicable	(5)
Liver regeneration	Partial hepatectomy in mice	Decreased TBA	Not specifically evaluated	Increased liver-to-body weight ratio	(22)

GPT, glutamic pyruvic transaminase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBA, total bile acid; TBIL, total bilirubin; LDH, lactate dehydrogenase; GT, glutamyltransferase; DBIL, direct bilirubin; GSH-Px, glutathione peroxidase; SOD, superoxide dismutase; MDA, malondialdehyde; APAP, N-acetyl-para-aminophenol; GalN, galactosamine; LPS, lipopolysaccharide; LCA, lithocholic acid; SD, Sprague Dawley; BDL, bile duct ligation; CYP, cytochrome P450; NRF2, nuclear factor erythroid 2-related factor 2; ARE, antioxidant response element; PXR, pregnane X receptor; HCV, hepatitis C virus.

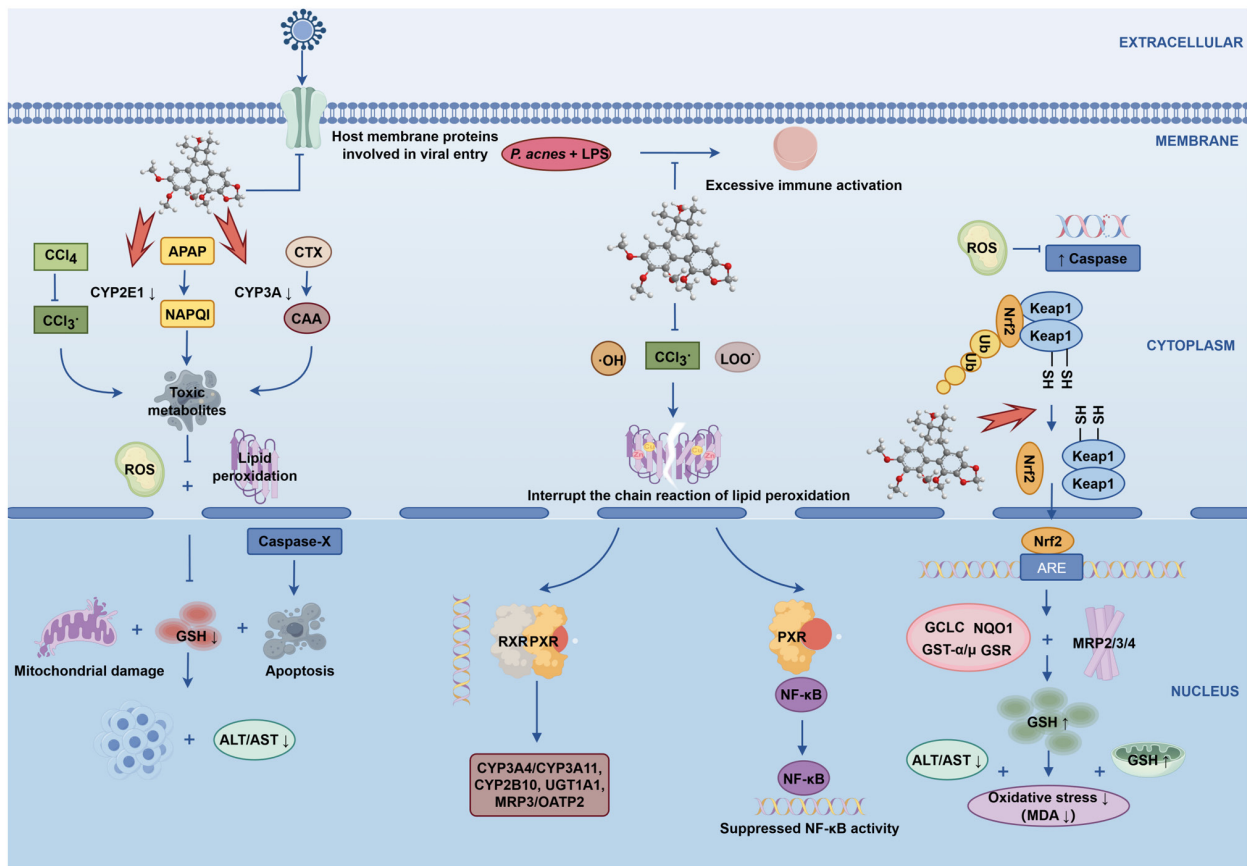


Figure 6. Proposed integrated mechanisms underlying the hepatoprotective effects of gomisin A. *P. acnes*, *Propionibacterium acnes*; LPS, lipopolysaccharide; APAP, acetaminophen; CYP, cytochrome P450; CTX, cyclophosphamide; NAPQI, N-acetyl-p-benzoquinone imine; CAA, chloroacetaldehyde; ROS, reactive oxygen species; RXR, retinoid X receptor; PXR, pregnane X receptor; Ub, ubiquitin; GSH, glutathione; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GCLC, glutamate-cysteine ligase catalytic subunit; NQO1, NAD(P)H quinone oxidoreductase 1; GST, glutathione S-transferase; GSR, glutathione reductase; MRP, multidrug resistance-associated protein; UGT1A1, uridine diphosphate glucuronosyltransferase 1A1; OATP2, organic anion-transporting polypeptide 2; Keap1, Kelch-like ECH-associated protein 1; ARE, antioxidant response element; MDA, malondialdehyde.

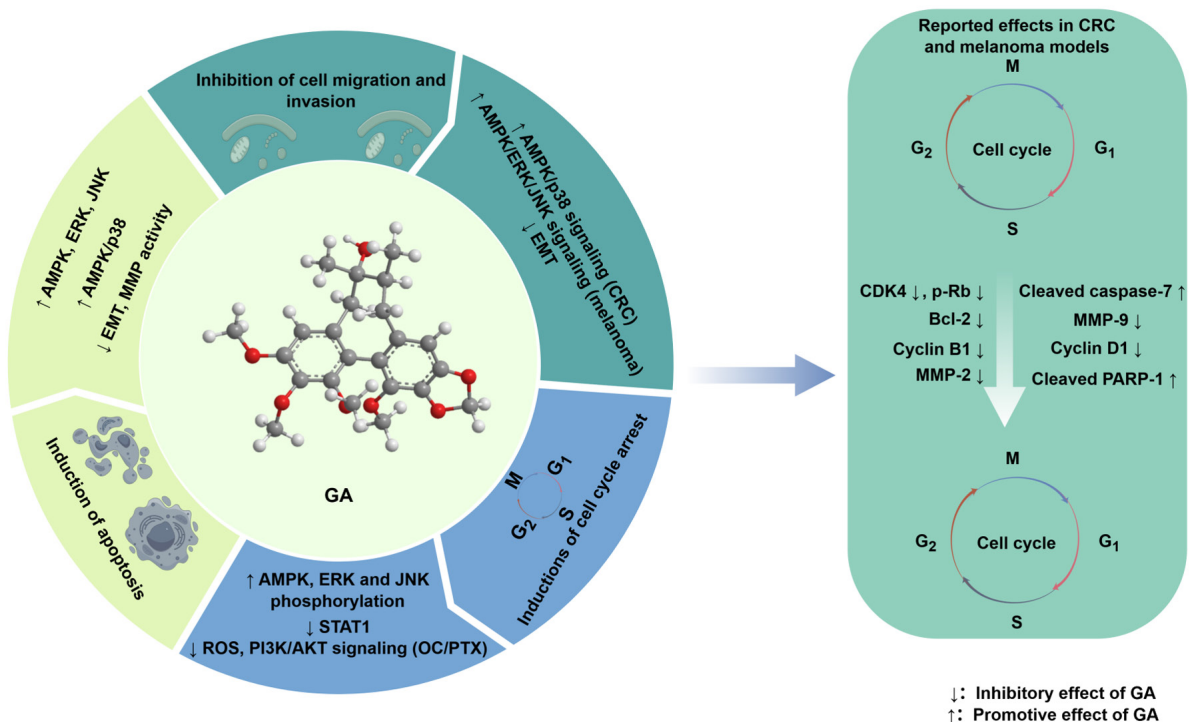


Figure 7. Major cellular and molecular mechanisms associated with the anticancer activity of GA. GA, gomisin A; ROS, reactive oxygen species; EMT, epithelial-mesenchymal transition; p-Rb, phosphorylated retinoblastoma protein; AMPK, AMP-activated protein kinase; PARP-1, poly (ADP-ribose) polymerase 1.

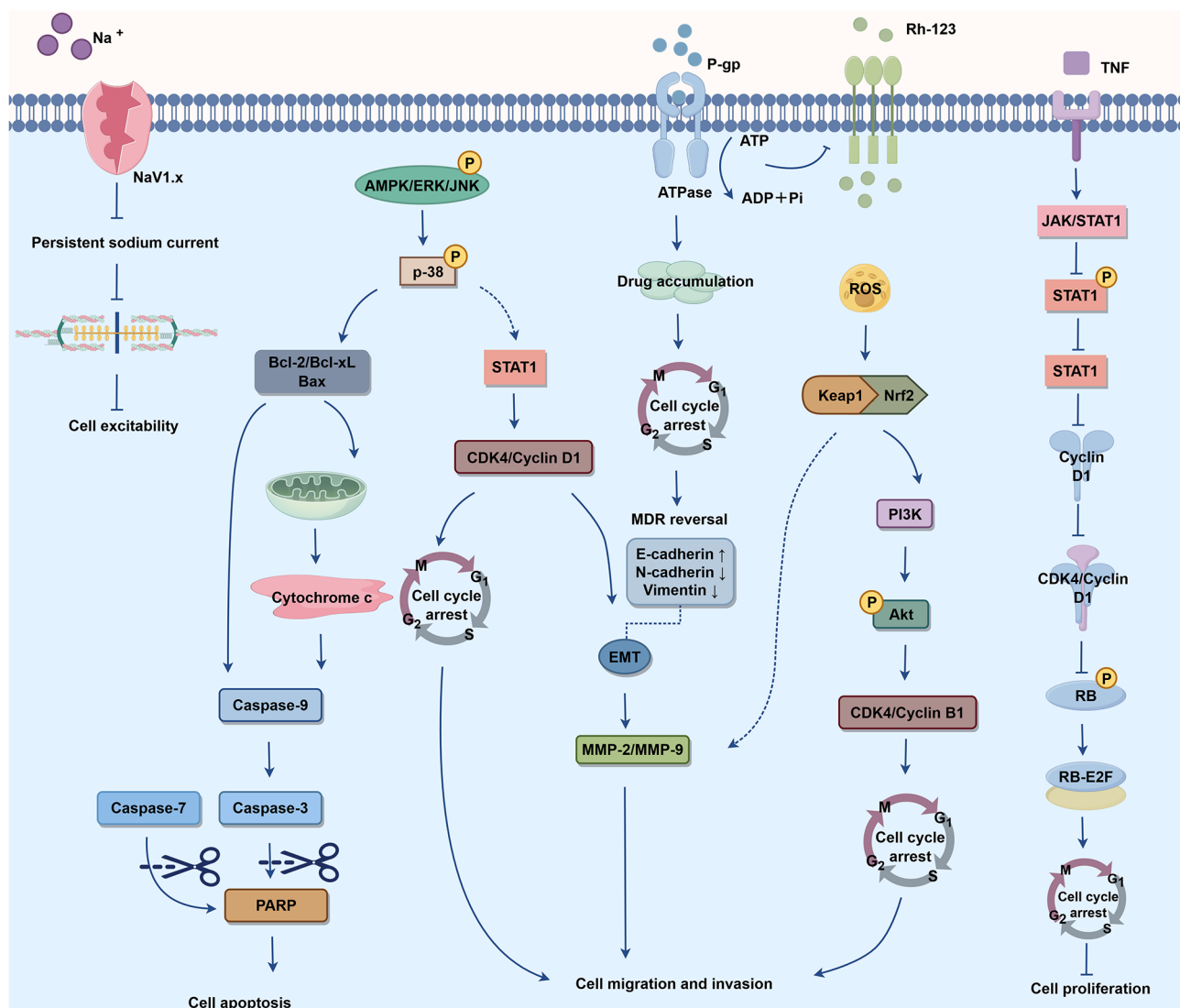


Figure 8. Major cellular pathways implicated in the anticancer activity of gomisin A. NaV1.x, voltage-gated sodium channel family 1; P-gp, P-glycoprotein; Rh, rhodamine; Pi, inorganic phosphate; MDR, multidrug resistance; EMT, epithelial-mesenchymal transition; ROS, reactive oxygen species; RB-E2F, retinoblastoma protein-E2F transcription factor pathway; AMPK, AMP-activated protein kinase; P, phosphorylation; PARP, poly (ADP-ribose) polymerase; Keap1, Kelch-like ECH-associated protein 1.

promote apoptosis primarily through mitochondria-dependent mechanisms, with an additional calcium-dependent cytotoxic mechanism identified in prostate cancer cells (8,87).

GA shifts the BCL-2 family balance toward mitochondrial outer membrane permeabilization. GA upregulates expression of the pro-apoptotic protein BAX, while downregulating that of anti-apoptotic BCL-2 and BCL-xL, leading to the activation of caspase-9, caspase-3 and PARP cleavage in CRC cells (8). Cleavage of caspase-7, as an additional executioner caspase, has also been documented in HCT-116 colon cancer cells (93).

AMPK and MAPK activation are implicated upstream of both canonical pathways. In CRC cells, GA increases AMPK and p38 phosphorylation, which feeds into the mitochondrial apoptosis cascade (8). In melanoma cells, GA activates AMPK, ERK and JNK, all converging on caspase-3 and PARP cleavage (23). An additional calcium-dependent cytotoxic mechanism has been reported in prostate cancer cells. GA induces Ca^{2+} influx via protein kinase C-dependent and store-operated pathways, together with phospholipase

C-mediated Ca^{2+} release from the ER. This calcium mobilization promotes cell death independently of the canonical caspase pathways (87).

In conclusion, GA promotes cancer cell death through at least two experimentally reported mechanisms: Mitochondria/caspase-associated apoptosis in CRC and melanoma models and a calcium-dependent cytotoxic mechanism in prostate cancer cells.

Inhibition of cell migration and invasion. Cancer cell invasion involves migration through tissue barriers and extracellular matrix (94). Therefore, inhibiting the migration and invasion of tumor cells serves a significant role in suppressing tumor progression. GA suppresses migration and invasion in both CRC and melanoma cells, primarily through inhibition of epithelial-mesenchymal transition (EMT). GA reverses the EMT marker profile by upregulating E-cadherin expression while downregulating that of N-cadherin, vimentin, snail and twist, and it decreases MMP activity (8,23). In summary, EMT reversal and MMP inhibition account for the reduced

migratory and invasive capacity observed across GA-sensitive cancer cell lines.

Chemosensitization and resistance modulation. Beyond direct cytotoxicity, GA enhances the activity of conventional chemotherapeutics through several distinct mechanisms. GA reverses multidrug resistance (MDR) via the non-competitive inhibition of two major ATP-binding cassette transporters. GA alters substrate interactions with P-glycoprotein (P-gp) and induces conformational changes that impair transport function, restoring chemosensitivity in HepG2-DR cells (49). GA also inhibits breast cancer resistance protein (BCRP)-mediated transport in a non-competitive manner, thus broadening its MDR-reversal spectrum (95).

Beyond efflux pumps, GA inhibits drug-metabolizing enzymes, such as CYP3A4 and CYP2C8, which increases systemic exposure to co-administered chemotherapeutics. For PTX, the dual inhibition of intestinal P-gp and CYP3A levels notably improves oral bioavailability by reducing both efflux and first-pass metabolism (96). Similarly, evidence from *Schisandra*-derived preparations suggests lignan-mediated modulation of CYP3A4 may increase bosutinib exposure, although the specific contribution of purified GA has not been established (97). In summary, GA enhances chemotherapeutic efficacy through transporter-mediated MDR reversal and metabolic enzyme inhibition that boosts drug bioavailability.

Critical assessment and translational considerations. The aforementioned anticancer phenotypes are well established at the cellular level, including in CRC, melanoma, OC and prostate cancer (8,13,23,87). For anticancer activity, the core bottleneck is that the concentrations needed for efficacy in cell culture are higher than what oral dosing delivers in circulation. This concentration dependence follows a consistent pattern. At the high range tested *in vitro* (25–100 μ M), GA acts as a direct cytotoxic agent, suppressing viability, driving G₀/G₁ arrest and triggering apoptosis. At lower concentrations (5–20 μ M), the effects shift toward targeting metastatic phenotypes, reversing EMT, dampening MMP activity, and reducing migration and invasion. Corresponding *in vivo* doses range from 2 to 50 mg/kg, with CRC lung metastasis studies typically using 50 mg/kg orally or intraperitoneally (8,23). Oral GA exposure varies with species, dose and preparation; human extract data may not predict the exposure achieved by purified GA in rodents (18,98). Low rat oral bioavailability has been reported (~20.8% in one study), but the relative contributions of intestinal absorption and presystemic metabolism remain incompletely resolved (99,100). Dose-matched measurements of unbound plasma and tumor GA are therefore required to quantify the exposure gap.

Exposure cannot be inferred from dose alone. Human extract data and certain rodent preparation studies report low plasma GA concentrations, whereas purified-compound and extract exposures vary substantially by formulation (18,98). Effective cell concentrations should therefore be compared with measured unbound plasma and tumor concentrations in the same dosing regimen, rather than assuming a universal three-order-of-magnitude gap.

Unless GA concentrates selectively in tumor tissue, active metabolites accumulate or formulation strategies drive local high exposure, the G₀/G₁ arrest and apoptosis phenotypes observed *in vitro* are better treated as mechanistic readouts

and screening tools than as effects readily reproducible *in vivo* by conventional oral dosing and do not directly support oral GA as a clinical anticancer agent. The MDR reversal and chemosensitization activity, by contrast, translate more plausibly. Intestinal transporter and enzyme inhibition may alter co-administered drug exposure, but the required local GA concentrations need to be measured (96,101). Framing GA as a chemosensitizer or bioavailability enhancer therefore fits its PK profile better than positioning it as a standalone cytotoxic drug. Current *in vivo* cancer studies of GA combinations remain restricted mainly to CRC/metastasis and OC models (8,13), and additional animal studies are needed to determine whether these effects extend beyond the currently studied settings.

Mechanistic insights from network pharmacology. The present network pharmacology and molecular docking analyses suggested the anticancer effects of GA involved multiple receptor-associated, intracellular signaling and transcriptional targets. The experimental phenotypes used for this comparison derive from CRC, melanoma and OC studies (8,13,23). These computational findings should be viewed primarily as a framework for generating testable hypotheses rather than as evidence of direct target inhibition.

Among the predicted membrane-associated targets, EGFR and ERBB2 are docking candidates. GA is predicted to adopt putative ATP-pocket poses in the present docking analysis; these predictions do not establish ATP-competitive inhibition (102). Potential modulation of ERBB signaling may influence pathways involved in cell proliferation, survival and EMT (103). However, the reported effects of GA on G₀/G₁ arrest, CCND1-CDK4 downregulation and EMT inhibition have not been experimentally attributed to EGFR or ERBB2 inhibition (8,23).

PIK3CA, AKT1 and mTOR have been identified as candidate targets, with docking scores of -6.5, -6.6 and -6.9 kcal/mol, respectively. These predictions suggest GA interacts with components of the PI3K/AKT/mTOR pathway. In OC cells, GA enhances PTX sensitivity while decreasing intracellular ROS levels and decreasing PI3K/AKT-related signaling (13). However, current evidence does not establish direct binding between GA and AKT1 or other components of this pathway.

MAPK14/p38 α is a docking candidate. Experimental studies support the involvement of MAPK signaling in GA-associated cancer-cell apoptosis and growth inhibition, as GA increases AMPK and p38 phosphorylation in CRC cells, and activates AMPK, ERK and JNK signaling in melanoma cells (8,23). These findings contradict uniform MAPK inhibition and suggest the context-dependent modulation of MAPK signaling.

STAT3 is another candidate target, with a docking score of -7.0 kcal/mol, and a potential STAT3 interaction region was suggested by the docking pose. Although modulation of STAT3 may affect proliferation- and survival-associated transcription, STAT1 expression is decreased during GA- and TNF- α -induced G₁ arrest in HeLa cells (91). Thus, the data support the potential involvement of STAT family signaling but do not establish STAT3 as a direct molecular target of GA.

Outside this receptor-kinase-transcription factor network, inhibition of drug transporters is a more directly supported and mechanistically independent anticancer

mechanism (49,95). GA non-competitively inhibits P-gp and BCRP-mediated drug efflux (49,95) and increases PTX exposure through combined effects on intestinal P-gp and CYP3A (96); evidence from *Schisandra*-derived preparations suggests a potential CYP3A4-mediated interaction with bosutinib, although a GA-specific contribution has not been established (97). Thus, the anticancer profile of GA comprises a predicted three-tiered receptor-kinase-transcription factor cascade (ERBB/PI3K/MAPK/STAT signaling network) and a separately validated transporter- and metabolism-based chemosensitization mechanism (49,95,96). The present MD simulations assessed the structural behavior of selected predicted complexes; they do not establish modulation of downstream signaling.

For EGFR, STAT3 and mTOR, the present computational analyses provide tier D evidence; cancer studies provide tier C support for downstream pathway changes (8,13,23). Network pharmacology and molecular docking analyses prioritize these as core targets, placing them at tier D. Downstream experimental observations, including CCND1-CDK4 down-regulation, BCL-2 family shifts and caspase activation, are directionally consistent with receptor-kinase-transcription factor cascade modulation and provide tier C associative support (8,23). However, EGFR remains a candidate target rather than an experimentally validated target of GA because direct kinase inhibition, genetic dependency, and cellular target-engagement studies have not been demonstrated.

In summary, GA exerts a variety of anticancer effects and may become a potential antineoplastic drug. Despite this, the studies summarized here remain preclinical (8,13,23), and clinical trials are needed to verify its safety and effectiveness.

Effects on CVDs: Early but mechanistically diverse evidence. CVD caused an estimated 19.8 million deaths worldwide in 2022, ~32% of all deaths, imposing a substantial public health and economic burden (104,105). The CVD literature on GA spans several experimental levels, including cultured vascular cells, isolated vessel preparations, a mouse hypertension model and multicomponent herbal mixtures (9,14,24,25,106). These models address different layers of biological organization and do not provide equivalent evidence for clinical cardiovascular efficacy.

In angiotensin II-infused mice, continuous GA infusion at 2 or 10 $\mu\text{g/kg/min}$ attenuates blood pressure elevation, preserves plasma and vascular NO indices, restores endothelial NOS (eNOS) phosphorylation, and decreases vascular ROS generation (9). This *in vivo* blood pressure phenotype, paired with concordant vascular measurements, provides evidence for an NO/oxidative stress mechanism. A notable limitation is that the aforementioned study used continuous parenteral infusion rather than oral administration, bypassing absorption and first-pass metabolism; oral efficacy at physiologically relevant exposures therefore remains unconfirmed.

At the cell and isolated vessel level, the most fully characterized mechanistic effect is vasorelaxation, which operates through both endothelium-dependent and -independent pathways. Endothelium-dependent relaxation is driven by Ca^{2+} -mediated eNOS activation and translocation to the cytoplasm, leading to increased NO production (14), with partial contribution from myosin light chain dephosphorylation (24). In endothelium-denuded vessels, GA acts via the

RhoA/Rho-associated coiled-coil-containing protein kinase pathway to decrease vascular contractility (25).

GA suppresses both mRNA and protein expression of PCSK9 (55). Because PCSK9 typically reduces LDL receptor density and impairs LDL-cholesterol (LDL-C) clearance, decreased PCSK9 expression provides a hypothesis for improving LDL-C clearance, but does not demonstrate LDL-C lowering by GA *in vivo* (55). For vascular fibrosis, GA inhibits TGF- β 1-driven fibrogenic signaling in vascular smooth muscle cells (VSMCs) through a Smad-independent mechanism. GA suppresses inhibitor of NF- κ B kinase α/β activation and I κ B α phosphorylation, thereby inhibiting the NF- κ B component of the TGF- β 1 response that contributes to vascular fibrotic disease (83). Antithrombotic effects have been reported for a *S. chinensis* + *Morus alba* L. mixture in which GA serves as a marker compound. The mixture decreases platelet activation and thrombus formation, associated with the decreased phosphorylation of MAPK (ERK and JNK) and AKT in platelet signaling pathways (106). However, whether this activity is attributable to GA alone or reflects additive or synergistic effects with other components remains unresolved.

In summary, GA shows promising and mechanistically diverse cardiovascular activity in rodent and cell-based models (9,14,24,25); the evidence base remains early, however, with most data from small animal and *in vitro* studies and no large-animal or clinical validation. The antithrombotic data rely on mixture formulations rather than on purified GA, which limits mechanistic interpretation (106). The schematic also includes PCSK9 expression in cultured cells (55) and TGF- β 1-associated vascular fibrotic signaling (83). Further studies should establish dose-response associations, confirm target engagement and assess translational potential (Fig. 9).

Critical assessment and translational considerations. The cardiovascular evidence includes continuous GA infusion in mice and experiments in vascular cells and isolated rat vessels (9,14,24,25). The aforementioned studies did not measure unbound GA concentrations at the vascular site of action alongside efficacy; therefore, it remains unclear whether the effective concentrations can be achieved by oral dosing. The continuous-infusion hypertension experiment also bypassed oral absorption and first-pass metabolism (9). Pharmacological inhibitor experiments support an NO/eNOS contribution to vasorelaxation (14,24), but direct GA target engagement and durable oral efficacy remain unresolved. CYP3A and P-gp inhibition create a potential for interactions with co-administered drugs that requires exposure-based evaluation (49,62).

Mechanistic insights from network pharmacology. The present network pharmacology and molecular docking analyses provided hypotheses for experimental observations concerning endothelial function (9,14), VSMC fibrotic responses (83), PCSK9 expression (55) and platelet activation by a multicomponent preparation (106). Among the predicted targets, PIK3CA, AKT1, GSK3 β and MAPK14 have emerged as major candidate nodes. AKT1 and GSK3 β form a functionally connected regulatory module, with predicted docking scores of -6.6 and -7.4 kcal/mol, respectively. AKT is an established upstream regulator of GSK3 β and suppresses its kinase activity via phosphorylation at Ser9 (107). However, these docking results indicate only a potential interaction with

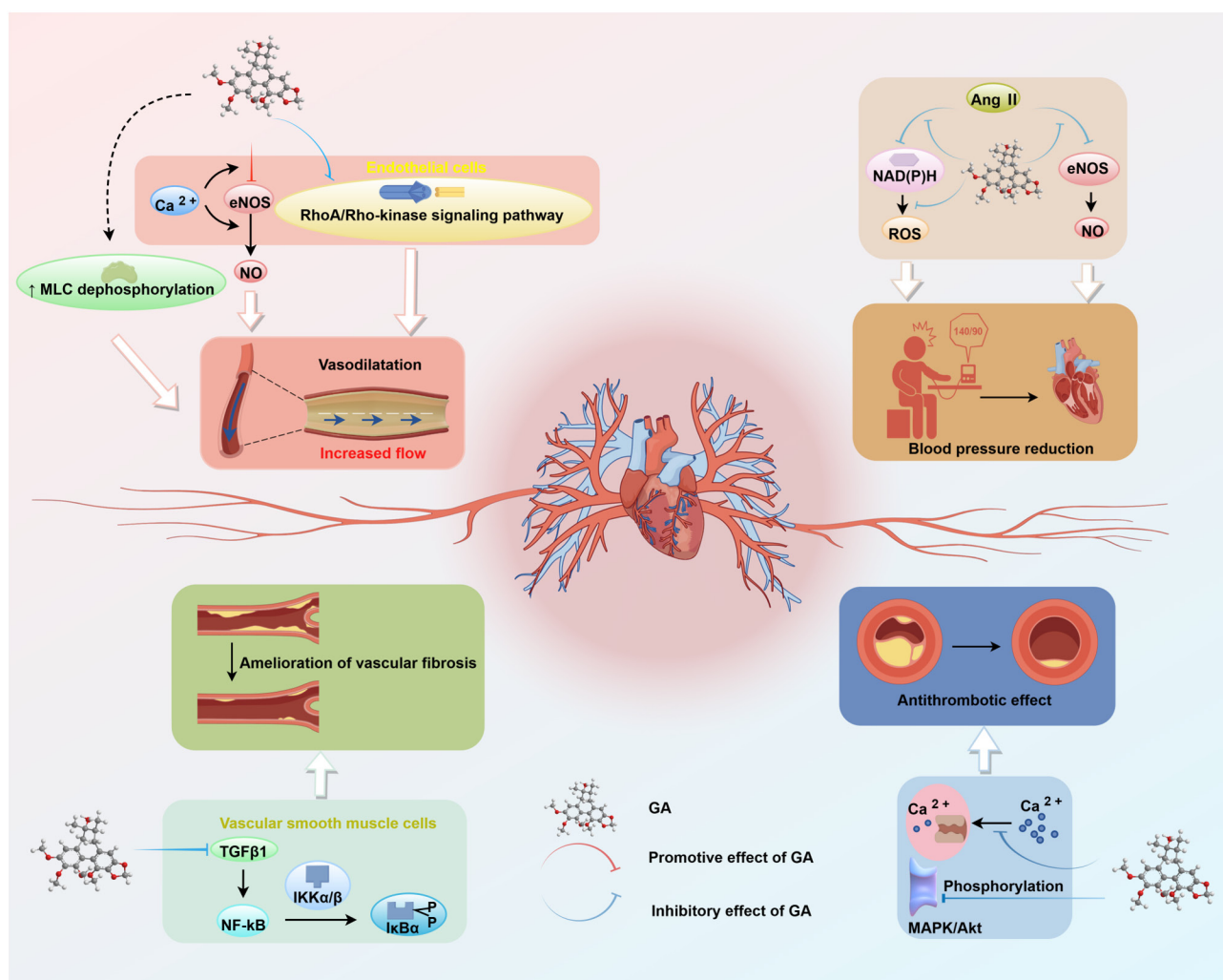


Figure 9. Proposed mechanisms of the cardiovascular effects of GA. GA, gomisin A; eNOS, endothelial nitric oxide synthase; MLC, myosin light chain; P, phosphorylation; Ang, angiotensin; ROS, reactive oxygen species.

GA and do not establish whether either kinase is activated or inhibited.

AKT1 is also a well-characterized upstream kinase of eNOS. Activation of the AKT1/eNOS pathway promotes phosphorylation of the activating Ser1177-equivalent residue and enhances NO production, providing a plausible mechanistic basis for the endothelium-dependent vasorelaxation observed in experimental models (14,24,108). eNOS phosphorylation is not exclusively dependent on PI3K/AKT signaling, as AMPK-mediated phosphorylation at Ser1177 has also been reported in response to thrombin and histamine (109).

GSK3β is implicated in the regulation of VSMC proliferation, migration and phenotypic remodeling, although the direction of its effects may be context-dependent. For example, increased GSK3β expression is associated with decreased angiotensin II-induced VSMC proliferation and migration; circular UBE4B RNA sequesters microRNA-199a-5p, thereby increasing GSK3β expression (110). These findings support a role for GSK3β-associated signaling in vascular remodeling but do not confirm that inhibition of GSK3β is responsible for the anti-fibrotic effects attributed to GA. Similarly, GSK3β-associated signaling is associated with PCSK9-induced metabolic injury, but current evidence does

not identify GSK3β as an upstream regulator of PCSK9 expression (111). Therefore, the contribution of this pathway to decreased PCSK9 and LDL-C levels remains speculative (111).

MAPK14, which encodes p38α, exhibits a predicted affinity score of -6.8 kcal/mol and may represent a parallel node linking vascular inflammation, fibrotic signaling and platelet activation. This prediction is consistent with the involvement of p38α in platelet-mediated inflammatory and thrombotic responses and with reports that decreased MAPK- and AKT-associated phosphorylation accompanies decreased platelet activation and thrombus formation (106,112). The aforementioned antithrombotic evidence was obtained using a multicomponent *S. chinensis-Morus alba* preparation rather than purified GA and (106) did not directly demonstrate inhibition of MAPK14 by GA.

PIK3CA, with a predicted binding affinity of -6.5 kcal/mol, is an upstream component of the canonical PI3K/AKT/eNOS pathway. The inclusion of PIK3CA in the predicted target network supports the potential involvement of this signaling pathway, although the direction and functional consequences of the interaction remain to be experimentally determined. Taken together, the predicted target profile is broadly compatible with the antihypertensive, lipid-lowering,

anti-fibrotic and vasorelaxant effects reported in preclinical studies (9,14,24,25,55,83). However, these associations should be regarded as hypothesis-generating; direct target-engagement assay, kinase activity measurements, pathway-specific intervention studies and validation using purified GA are required to establish causal associations.

Overall, the cardiovascular evidence supports vasorelaxant and antihypertensive pharmacology in preclinical systems, with moderate phenotypical and low-to-moderate target-level confidence. However, clinical efficacy has not yet been evaluated in controlled human studies.

Neurological activity: Exploratory evidence and unresolved brain exposure. GA exerts a range of neuropharmacological effects in preclinical models, spanning anti-neuroinflammatory, cognition-enhancing, anxiolytic, sedative-hypnotic and analgesic activity (6,10,15,19,113). A notable PK advantage is that GA penetrates the blood-brain barrier (BBB) (18,114,115), establishing feasibility for central nervous system (CNS) targeting.

Reported central effects include anti-neuroinflammatory and neuroprotective activity (10,116). In LPS-activated microglial cells, GA suppresses NF- κ B and MAPK signaling, decreasing pro-inflammatory output (10). In a 3-nitropropionic acid model of Huntington's disease, *S. chinensis* stem extract protected against striatal toxicity through combined antioxidant and anti-inflammatory actions (117). A separate study examined Schisandra lignans, including GA, in serum/glucose-deprived SH-SY5Y cells; this is an *in vitro* deprivation model, not an *in vivo* ischemic-stroke experiment (116).

In terms of cognitive function, GA inhibits acetylcholinesterase (AChE) activity in a dose-dependent manner *in vitro* (45,118) and reverses scopolamine-induced memory impairment *in vivo* (15). These findings align with enhancement of cholinergic neurotransmission, a classical symptomatic strategy for cognitive impairment (15). Mood- and sleep-associated effects have also been reported, although largely from multi-component herbal preparations (6,114,115,119). In the Suanzaoren-Wuweizi herb-pair, GA has been identified as a putative active constituent modulating serotonin and dopamine signaling to mediate anxiolytic effects (6). Antidepressant-associated observations of *S. chinensis* preparations do not establish an antidepressant mechanism of purified GA (119). Sedative-hypnotic activity is hypothesized to involve binding to neurotransmitter receptors in the hypothalamus following BBB penetration (114,115). To the best of our knowledge, whether purified GA alone reproduces these mood-associated effects has not been tested. On the analgesic side, GA inhibits capsaicin-induced transient receptor potential vanilloid 1 (TRPV1) channel currents in a concentration-dependent, reversible and non-competitive manner, independent of voltage. This action may alleviate pain hypersensitivity and neuropathic pain driven by excessive TRPV1 activation (113).

Critical assessment and translational considerations. The key translational bottleneck for neuroprotective applications lies in uncertainty regarding target exposure within the brain. Brain homogenate and microdialysis studies have measured GA-related brain exposure after Schisandra preparations (18,115). However, unbound brain exposure

matched to the efficacy regimen and target potency remains insufficiently defined. If brain exposure is in the same order of magnitude as plasma levels, a notable gap would separate achievable concentrations from the micromolar levels required to inhibit kinases such as GSK3 β and STAT3, calling into question the physiological relevance of kinase-mediated mechanisms. A second key limitation is the mismatch between model duration and clinical disease course. The majority of studies employ acute injury models with short-term treatment regimens (15,116,117), which differ substantially from the progressive, chronic pathology of neurodegenerative conditions such as Alzheimer's and Huntington's disease. Efficacy in acute settings does not guarantee that long-term intervention would slow disease progression.

Among neuropharmacological effects, AChE is supported by a direct enzyme assay (118). To the best of our knowledge, its *in vitro* inhibitory concentration has not been matched with unbound brain GA exposure, therefore superior translational feasibility over predicted kinase mechanisms cannot yet be established. Because unbound brain drug concentrations remain undefined, findings across Alzheimer's disease, Huntington's disease, depression, anxiety, insomnia and neuropathic pain should be framed as exploratory preclinical observations rather than evidence of broad neuropsychiatric therapeutic potential. Future studies should establish quantitative brain exposure measurements via microdialysis or brain homogenate assays to provide a firmer basis for evaluating the druggability of each proposed mechanism.

Mechanistic insights from network pharmacology. Network pharmacology analysis has identified GSK3 β as a prominent candidate hub within the putative neuropharmacological target network of GA in the present analysis. GSK3 β is biologically relevant to processes implicated in neurological disease, including tau hyperphosphorylation, amyloid- β (A β) accumulation and microglia-associated neuroinflammation (30-32). Modulation of the PI3K/AKT/GSK3 β pathway is associated with decreased tau hyperphosphorylation in experimental models of Alzheimer's disease (30), decreased accumulation of A β and pyroglutamate-modified A β (31), and attenuation of microglia-mediated inflammation and neuronal apoptosis following ischemic stroke (32). Although these studies did not examine GA directly, they support the biological relevance of GSK3 β -associated signaling to the memory-enhancing (15) and neuroprotective (116) effects reported for GA in experimental models (15,116).

The predicted GA-GSK3 β complex was evaluated using a 100-nsec MD simulation. The protein backbone root-mean-square deviation (RMSD) profile supported the relative conformational stability of the simulated complex (Fig. 5A). However, neither molecular docking nor MD analysis establishes direct target engagement or determines whether GA activates or inhibits GSK3 β . Biochemical kinase assays and cell target engagement studies are required before GSK3 β can be regarded as a functionally validated target of GA.

PIK3CA and AKT1, which are upstream of GSK3 β in the canonical PI3K/AKT signaling pathway, were identified as docking candidates. Activated AKT inhibits GSK3 β through phosphorylation at Ser9, thereby promoting neuronal survival under several forms of cell stress (33). This signaling association

provides a plausible framework for interpreting the anti-apoptotic effects reported for GA in serum/glucose-deprived neuronal cells and memory-impairment (15,116). Nevertheless, the predicted interactions with PIK3CA, AKT1 and GSK3 β do not establish the direction of pathway regulation by GA, and activation of the PI3K/AKT/GSK3 β pathway remains to be demonstrated experimentally.

STAT3 and MAPK14, also known as p38 α , are additional inflammation-associated candidate nodes, with predicted binding affinities of -7.0 and -6.8 kcal/mol, respectively. Both signaling proteins participate in the regulation of inflammatory gene expression, cell stress responses and survival in neurons and glial cells (10,120). Their inclusion in the predicted network is compatible with the decreased NF- κ B signaling and pro-inflammatory cytokine production observed in LPS-stimulated microglial models treated with GA (10,117). However, this does not demonstrate direct inhibition of STAT3 or MAPK14, because decreased inflammatory signaling may arise through upstream or indirect mechanisms. TNF may be more appropriately regarded as a downstream inflammatory mediator within this network, consistent with its decreased levels in the microglial inflammatory response (10); accordingly, TNF should be considered a candidate downstream mediator rather than a validated direct target of GA, as direct binding and functional neutralization studies are lacking.

Detection of GA in brain samples following administration provides evidence of CNS exposure but does not demonstrate that GA binds GSK3 β , PIK3CA, AKT1, STAT3 or MAPK14 in the brain (18,114,115). The strength of this conclusion depends on whether the detected species is unchanged GA or a metabolite, the integrity of the BBB in the experimental model and whether the measured brain concentrations are sufficient to produce pharmacological effects.

Taken together, the computational findings identified GSK3 β as a potential point of convergence between PI3K/AKT-dependent survival signaling and pathological processes involving tau phosphorylation, A β accumulation and neuroinflammation. STAT3 and MAPK14 may constitute parallel inflammation-associated components of the same predicted network. These associations provide a mechanistic framework that is broadly consistent with the reported effects of GA on cognition, neuroinflammation, ischemic neuronal injury and mutant huntingtin-associated toxicity (10,15,116,117). They should nevertheless be considered hypothesis-generating until confirmed by direct binding assays, measurements of kinase activity and phosphorylation, pathway-specific pharmacological or genetic intervention and studies conducted at pharmacologically relevant brain concentrations.

GA exhibits a broad spectrum of neuropharmacological activity in preclinical models. The evidence base remains largely exploratory overall. Anti-neuroinflammatory effects are supported by microglial experiments (10), while AChE inhibition and TRPV1 channel modulation rest on more direct functional assays (113,118). Anxiolytic and antidepressant findings rely on mixture studies and require confirmation using the purified compound (6,119). The neurological evidence remains largely at the phenotype-association level, as pathway-specific inhibition, genetic validation, and clinical efficacy studies are currently lacking. Brain detection supports CNS accessibility, but efficacy-matched unbound brain exposure remains

a translational gap (18,115). Future studies prioritizing brain concentration measurement and target validation are essential to determine the true therapeutic potential of GA in neurological disorders (Fig. 10) (15,19,116,117).

Anti-inflammatory and antioxidant effects. GA exhibits both anti-inflammatory and antioxidant activity in preclinical models, operating via partially overlapping NF- κ B and NRF2 pathways (10,16,17).

The anti-inflammatory effect centers on suppression of receptor-interacting protein 2 (RIP2) and NF- κ B activation, which decreases the expression of downstream inflammatory mediators, including COX-2, iNOS, IL-6, TNF- α and NO (16). In an asthma model, GA similarly acts via NF- κ B inhibition to reduce serum IgE levels, lower IL-4 and IL-5 concentrations in bronchoalveolar lavage fluid, and ameliorate lung tissue pathology (66). In human diploid fibroblasts, GA-associated changes in MAPK/NF- κ B signaling accompanied reduced cellular-senescence markers and improved mitochondrial biogenesis; these findings concern cellular senescence, not demonstrated slowing of organismal aging (121).

In terms of antioxidant activity, GA drives cell defense primarily through NRF2 nuclear translocation, which upregulates downstream cytoprotective genes, including HO-1. This decreases ROS production and senescence-associated β -galactosidase (SA- β -gal) activity, while enhancing autophagy and mitochondrial biogenesis. In stress-induced premature senescence, these effects improve mitochondrial function and modulate aging-related molecular markers (121). Under high-glucose conditions, GA upregulates HO-1 and preserves mitochondrial homeostasis to support osteoblast differentiation, with potential relevance for diabetic osteoporosis and implant failure (122). Capillary electrophoresis profiling has identified GA as one of the primary antioxidant lignans in *Schisandra* extracts, consistent with the established association between total lignan content and overall antioxidant capacity (123,124).

Anti-inflammatory and antioxidant activities represent the most common properties of GA across all therapeutic indications. NF- κ B suppression, decreased ROS levels and NRF2 activation have been observed in APAP liver injury, activated macrophages and microglia (10,16,17). This broad activity profile supports the pleiotropic character of GA, but also raises questions about mechanistic specificity. It is unclear whether the anti-inflammatory effect derives from selective inhibition of specific kinases or from non-specific membrane effects and radical scavenging common to lipophilic molecules tested at high concentrations.

Critical assessment and translational considerations. If GA operates through a polypharmacological mode of partial multi-target inhibition rather than complete single-target blockade, the overall effect threshold may fall below the concentration required for full inhibition of any individual kinase, which would leave a window for oral translational feasibility. The converse is also true, however. A weak multi-target mode of action would be difficult to verify through single target knockdown approaches, increasing the technical challenge of mechanistic studies. Anti-inflammatory research relies on transcriptional and protein-level endpoint readouts, with little direct kinase activity data available, making it

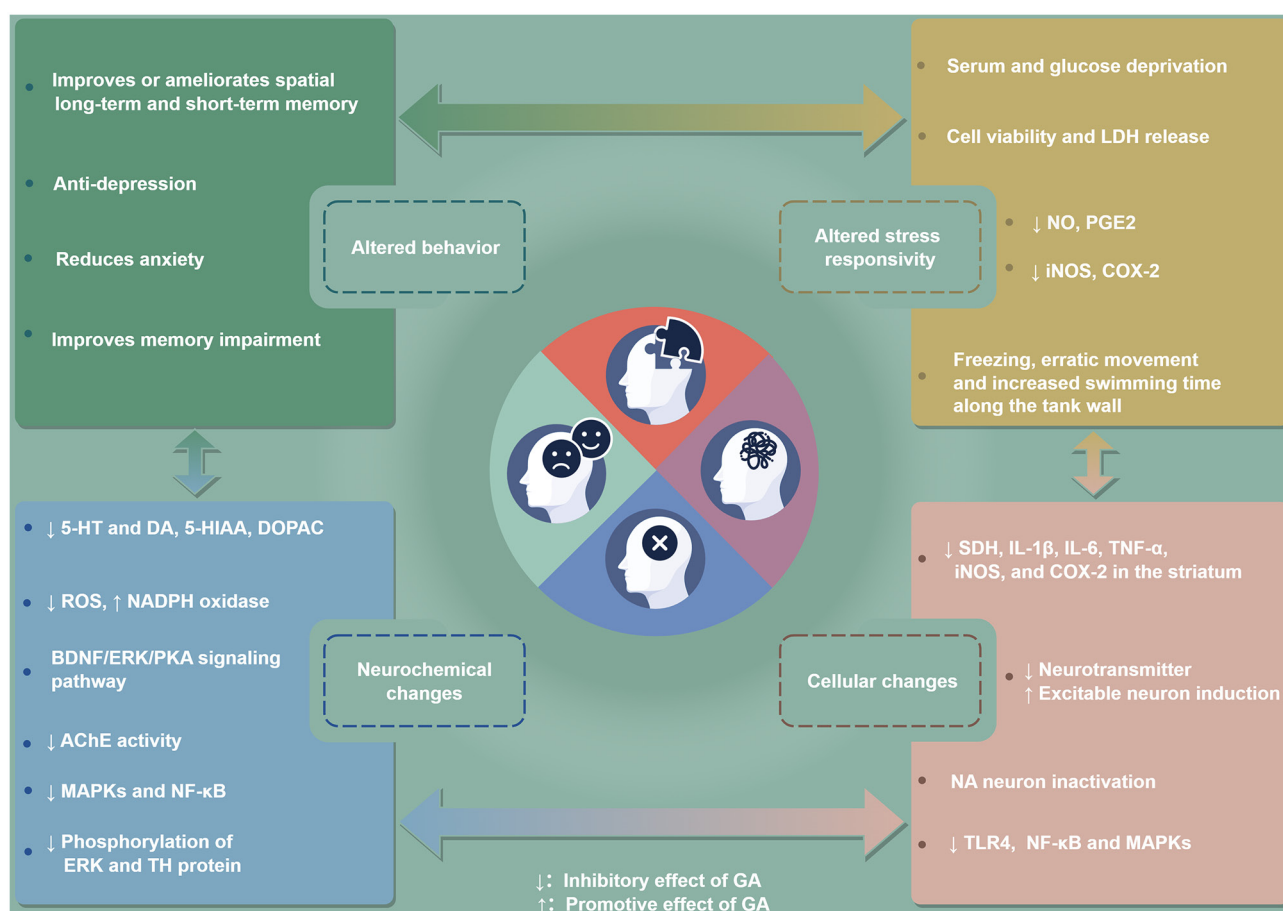


Figure 10. Reported neuropharmacological effects and associated mechanisms of GA. GA, gomisin A; LDH, lactate dehydrogenase; PGE, prostaglandin E; iNOS, inducible nitric oxide synthase; COX, cyclooxygenase; SDH, succinate dehydrogenase; NA, noradrenaline; TLR, toll-like receptor; 5-HT, 5-hydroxytryptamine; DA, dopamine; 5-HIAA, 5-hydroxyindoleacetic acid; DOPAC, 3,4-dihydroxyphenylacetic acid; ROS, reactive oxygen species; BDNF, brain-derived neurotrophic factor; AChE, acetylcholinesterase; PKA, protein kinase A; TH, tyrosine hydroxylase.

difficult to pinpoint the action of GA to specific nodes within signaling cascades (10,16,66).

NF- κ B suppression is supported by evidence at the boundary between tiers B and C. NF- κ B suppression is consistently replicated across multiple disease models (7,10,16), with decreased p65 nuclear translocation, attenuated I κ B α degradation, and coordinated decreases in downstream COX-2, iNOS, IL-6 and TNF- α levels. The coherence across independent laboratories and injury models raises confidence above simple pathway association. However, to the best of our knowledge, p65 overexpression or pathway-rescue experiments to establish strict causal dependency have not been reported.

Mechanistic insights from network pharmacology. In the present molecular docking analysis, the anti-inflammatory and antioxidant activity of GA may be interpreted within two conceptually distinguishable but potentially interconnected signaling modules: A MAPK14/NF- κ B-associated inflammatory module and a GSK3 β /NRF2-associated antioxidant module. These assignments provide a framework for integrating the computational predictions with previously reported biological effects but do not establish direct regulation of the proposed targets by GA (10,16,17).

MAPK14, which encodes p38 α , has emerged as a prominent inflammation-associated candidate, with a predicted binding affinity of -6.8 kcal/mol. A potential interaction

between GA and MAPK14 may provide a structural hypothesis for the recurrent involvement of MAPK and NF- κ B signaling in experimental studies (10,16,66). GA suppresses RIP2/NF- κ B activation, and decreases the production or expression of COX-2, iNOS, IL-6, TNF- α and NO (16). In an experimental model of asthma, attenuation of NF- κ B signaling was accompanied by decreased IgE, IL-4 and IL-5 levels, and improved pulmonary histopathology (66). GA also decreases NF- κ B/MAPK-associated inflammatory responses in activated microglia (10,117), while modulation of MAPK and NF- κ B signaling is implicated in its anti-senescence activity (121). Together, these findings show pathway-level consistency with the MAPK14 prediction. However, they do not demonstrate direct binding to MAPK14 or inhibition of its kinase activity by GA.

TNF, with a predicted binding affinity of -6.1 kcal/mol, has also been identified within the inflammation-associated target network. Although small molecules can, in principle, modulate TNF activity by interacting with the trimer interface and altering receptor-competent conformations, the docking prediction alone is insufficient to establish a mechanism for GA. TNF should be regarded primarily as a downstream inflammatory mediator and a tentative direct binding candidate rather than a validated molecular target. Direct interference with TNF trimer stability, receptor

binding or biological activity remains to be examined experimentally (16,66).

STAT3 is a docking candidate that may constitute an additional immunoregulatory component of the network. STAT3 participates in both pro-inflammatory and anti-inflammatory signaling, depending on the upstream cytokine, cell type and biological context (120). Consequently, the functional role and direction of any interaction between GA and STAT3 cannot be inferred from docking analysis alone.

The antioxidant module is centered computationally on GSK3 β , which showed a predicted binding affinity of -7.4 kcal/mol. GSK3 β is a recognized regulator of NRF2 stability and nuclear localization, providing a biologically plausible connection between the computational prediction and the antioxidant responses reported for GA (125). These responses include increased NRF2 nuclear accumulation and HO-1 expression, decreased ROS production and SA- β -gal activity and improvements in autophagic activity and mitochondrial biogenesis (17,121,122). Activation of the NRF2/ARE pathway has also been observed in APAP-associated LI (17), whereas HO-1 induction and preservation of mitochondrial homeostasis have been reported under high-glucose conditions (122). Nevertheless, these findings do not establish GSK3 β as the direct mediator of NRF2 activation by GA because the changes may arise through Kelch-like ECH-associated protein 1-dependent regulation or other oxidative stress-responsive pathways (126).

Analytical studies have associated GA with the antioxidant activity of *Schisandra*-derived preparations (123,124). However, the extent to which GA independently accounts for the activity of multicomponent preparations requires confirmation using purified compounds and pharmacologically relevant concentrations.

Taken together, the predicted MAPK14/NF- κ B- and GSK3 β /NRF2-associated modules provide a working model for integrating the anti-inflammatory and antioxidant effects of GA. The two modules are unlikely to operate in isolation given the extensive crosstalk between inflammatory and redox-sensitive signaling pathways (127). Accordingly, the proposed network should be regarded as hypothesis-generating. Direct kinase assays, biophysical target engagement measurements, genetic loss-of-function approaches and pathway rescue experiments will be necessary to distinguish primary molecular interactions from downstream or compensatory signaling changes.

Other pharmacological activities. GA exhibits a range of additional pharmacological activities in preclinical models (26-28), although most remain at an early exploratory stage. In terms of renal protection, GA modulates MAPK signaling and apoptosis pathways to reduce pathological kidney damage (26). GA increases phosphorylation of p38 and JNK, while decreasing ERK phosphorylation, and lowers active caspase-3 levels, while shifting the BCL-2/BAX balance toward cell survival (26). In drug metabolism and PK interactions, GA acts on two key pathways. GA induces PXR gene expression, which upregulates renal CYP3A30 and alters the expression profile of drug-metabolizing enzymes, with implications for drug-drug interactions and tolerance development (12,128). Separately, GA inhibits P-gp-mediated efflux, which increases the blood concentration, area under the curve

(AUC) and oral bioavailability of the immunosuppressant tacrolimus in a dose-dependent manner. This P-gp inhibition raises the potential of using GA as a bioavailability enhancer to decrease tacrolimus dosing and associated costs (101).

Anti-obesity activity has been documented in both animal and cells (27). GA decreases weight gain, epididymal white adipose tissue mass and liver weight, while increasing serum high-density lipoprotein-cholesterol levels (27). GA inhibits adipogenesis across multiple cell types and promotes fat browning through AMPK activation, shifting the balance between adipose tissue browning and whitening to suppress weight gain (27). An extract of *S. chinensis* fruits acts against *Vibrio parahaemolyticus* by disrupting cell membrane integrity, suppressing polysaccharide intercellular adhesin synthesis, decreasing extracellular DNA secretion and lowering biofilm metabolic activity. The extract inhibits biofilm formation and disrupts established biofilms but does not establish GA as the responsible antimicrobial constituent (129). For erectile function, GA relaxes penile corpus cavernosum smooth muscle through both NO-cGMP and cAMP signaling pathways. GA also enhances sildenafil-induced relaxation, which may offer a supplemental option for patients who respond incompletely to standard erectile dysfunction treatment (28,130). Immunomodulatory effects have been observed in THP-1 monocytic leukemia cells, where GA induces release of IL-8, macrophage inflammatory protein (MIP)-1 β and granulocyte-macrophage colony-stimulating factor (GM-CSF), suggesting modulation of innate immune responses (29). Finally, an electrophysiological study shows that GA inhibits sodium currents in pituitary GH3 and pancreatic INS-1 cells, affecting both peak amplitude and current sustainability. This provides a preliminary electrophysiological basis for potential applications in hyperprolactinemia and prolactinoma (131).

Critical assessment and translational considerations. Studies across the aforementioned diverse endpoints typically remain at a single-model, single-laboratory, small-sample exploratory stage with overall low evidence strength (26-29,131). The most robust finding is combined P-gp/CYP3A-mediated enhancement of tacrolimus bioavailability. This effect is supported by direct transporter functional assays and rat PK data (101), but clinical dosing utility and safety of purified GA have not been established. Anti-obesity effects have been observed at both cell and animal levels, but the causal chain between AMPK activation and fat browning has not been confirmed through inhibitor or knockdown experiments. Renal protection data remain at the level of pathway biomarkers and histopathology, lacking long-term follow-up of gold standard renal function endpoints such as creatinine clearance and urinary protein. To the best of our knowledge, erectile function improvement has been validated in isolated tissue strip experiments, with no *in vivo* functional data available. Antibacterial and immunomodulatory studies are preliminary, with both reproducibility and specificity yet to be established.

Mechanistic insights from network pharmacology. Network pharmacology may also place less extensively studied pharmacological effects of GA within a broader mechanistic context. In relation to its anti-obesity activity, GSK3 β and AKT1 were identified as potential components of a metabolism-associated target network. This prediction is

broadly consistent with the report that GA suppresses adipogenesis and promotes adipose tissue browning in association with activation of AMPK signaling (27). However, to the best of our knowledge, the evidence does not establish a continuous AKT1/GSK3 β /AMPK signaling pathway, nor has it been determined whether pharmacological inhibition or genetic depletion of GSK3 β or AKT1 modifies the metabolic response to GA. The association between these predicted targets and AMPK-associated adipose browning therefore remains to be clarified.

The reported renoprotective effects of GA also show potential pathway-level relevance to the predicted MAPK14 node (26). Hwang *et al* (26) described differential regulation of MAPK signaling, involving changes in p38, JNK and ERK activity (26). Because these kinases exert distinct and context-dependent effects on tubular cell stress, apoptosis, inflammation and repair, the pattern cannot be attributed solely to direct regulation of MAPK14 (26). The phosphorylation measurements reported by Hwang *et al* (26) do not distinguish direct action on p38 from an indirect response to altered cell stress. Direct engagement and functional modulation of MAPK14 therefore remain unconfirmed. A separate diabetic rat study reported modest renal improvement with GA alone and greater improvement with GA plus metformin, associated with AGE/RAGE and NF- κ B modulation; this does not independently establish a GA-MAPK14 mechanism (132).

For erectile function, the predicted PIK3CA and AKT1 nodes provide a potential upstream link to NO-dependent signaling and cavernosal smooth muscle relaxation. These findings are compatible with modulation of endothelial and cyclic nucleotide-dependent smooth muscle pathways. However, the contribution of PIK3CA, AKT1 or eNOS signaling to GA-mediated erectile effects has not been experimentally established, and additional mechanisms acting directly within cavernosal smooth muscle cannot be excluded.

The immunomodulatory findings require a different interpretation. MAPK14 and STAT3 are candidate signaling nodes that may influence cytokine production and monocyte activation (29). Because these mediators are upregulated rather than uniformly suppressed, this response should not be interpreted as evidence of inhibition of MAPK14 or STAT3. Instead, it may reflect a context-dependent immunostimulatory or cell activation phenotype. The docking results indicate potential in monocyte activation and cytokine regulation, but neither the direction of their regulation nor the broader immunological consequences have been established at present. Taken together, these findings extend the predicted target network of GA to metabolic regulation, renal stress response, cavernosal relaxation and immune cell signaling. Nevertheless, the mechanistic links remain largely inferential. Target-specific inhibition or knockdown, phosphorylation and activity measurements, and pathway-rescue experiments will be required to determine whether the predicted nodes are necessary for the corresponding pharmacological effects.

GA exerts a range of pharmacological activities, covering renal protection, drug metabolism regulation, anti-obesity, antibacterial, immunomodulatory and endocrine modulatory effects, as well as improvements in erectile function (26-29,101,131). These pharmacological findings enrich the overall pharmacological profile of GA, yet they are limited

in depth and reliability to support clinical translation. Studies are confined to exploratory preclinical research (26-29,131), with uneven mechanistic exploration and inadequate *in vivo* experimental verification. The present network analysis prioritized candidate proteins that may be relevant to these phenotypes; it does not demonstrate that these proteins mediate the reported effects. Nevertheless, such associations are primarily hypothetical. Experimental evidence primarily validates the participation of signaling pathways, rather than the direct interaction of GA with key targets.

GA is a dibenzocyclooctadiene lignan from *S. chinensis* with a broad spectrum of preclinical pharmacological activity. Its best-supported indication is hepatoprotection, with consistent efficacy across chemical, cholestatic and fibrotic LI models. Anticancer activity is well-documented at the cell level but faces a significant *in vitro-in vivo* concentration gap, positioning GA more plausibly as a chemosensitizer than a single-agent cytotoxic therapy. Cardiovascular and neurological effects show promising phenotypic signals but require target validation and quantitative exposure data. Anti-inflammatory and antioxidant activity represents a shared, foundational property across all disease contexts, potentially operating through a combination of NF- κ B suppression and NRF2 activation. Additional activities, such as renal protection, anti-obesity, antibiofilm, erectile function, immunomodulation and endocrine modulation effects, have been reported but remain exploratory. Across all indications, the evidence base is predominantly preclinical, with no controlled clinical efficacy studies of purified GA. Network pharmacology and molecular docking studies suggest a multi-target profile centered on EGFR, GSK3 β , STAT3, MAPK14 and mTOR, but these computational predictions require orthogonal validation through binding assays, target dependency studies and structural biology approaches. The absence of classical PAINS alerts decreases concern regarding recognized PAINS chemotypes but does not establish target specificity or polypharmacology, and non-specific assay interference remains possible. Quantitative tissue distribution studies are needed to establish actual target exposure, particularly in the brain and tumor compartments. Pathway-specific rescue experiments are essential to distinguish direct target engagement from downstream correlative changes. Oral formulation optimization to improve the ~20% oral bioavailability would also advance translational prospects. Early-phase clinical trials in the most feasible indications, such as hepatoprotection or chemosensitization, are needed to move beyond the current preclinical evidence base.

5. PK characteristics of GA

PK research provides a scientific basis for drug development, and new drug safety, pharmacology and toxicology assessments. PK studies of GA are conducted using ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS), ultra-fast LC-MS/MS, UHPLC-quadrupole time-of-flight MS, LC-MS/MS, UHPLC-electrospray ionization MS and HPLC for the analysis of rat plasma samples (18,98-100,114,133-138). Table IV provides a summary of the PK characteristics of GA reported in humans and experimental models. Cross-study comparisons must remain cautious because certain reports examined purified GA, whereas others measured GA following

Table IV. Pharmacokinetic characteristics of GA reported in humans and experimental models.

Authors, year	Model/source	Route and dose	Cmax	Tmax	AUC	Elimination/distribution	Critical interpretation	(Refs.)
Kim <i>et al</i> , 2017	Human	Oral <i>Schisandrae fructus</i> extract powder; 0.36 mg GA-equivalent	8.98±0.79 ng/ml	0.30±0.10 h	AUC0-24, 23.5±4.8 ng·h/ml	t _{1/2} , 3.94±0.65 h; Vd/F, 94.35±8.35 liter; CL/F, 19.80±2.63 l/h	Limited human evidence; Tmax is 0.30±0.10 h and must not be confused with t _{1/2} .	(98)
Kim <i>et al</i> , 2014	Male SD rats	IV; 30 mg/kg	Not stated	Not applicable	21.7±8.9 µg·min/ml	t _{1/2} , 78±27 min; CL, 37.3±18 ml/min/kg	Provides IV reference for bioavailability calculation	(99)
Kim <i>et al</i> , 2014	Male SD rats	Oral; 150 mg/kg	Not stated	Not stated	22.5±9.4 µg·min/ml	t _{1/2} , 195±172 min; absolute F, 20.8%	Low oral bioavailability despite evidence compatible with gastrointestinal absorption; substantial first-pass loss is likely	(99)
Sun <i>et al</i> , 2013	Male Wistar rats	Oral; dose not stated	Not stated	2.93±1.36 h	AUCinf, 38.31±17.93 ng·h/ml	t _{1/2} , 5.19±4.40 h	Low exposure	(134)
Wei <i>et al</i> , 2013	Male Sprague-Dawley rats; normal vs. insomnic	Oral <i>S. chinensis</i> extract; 7.5 g/kg	Normal: 141.95±45.06 µg/l; insomnic: 225.2±53.95 µg/l	Normal: 1.75±0.27 h; insomnic: 1.83±0.26 h	AUC0-t: 1305±441.6 vs. 1935±558.2 µg h/l; AUC0-∞: 1347±457.9 vs. 2059±542.0 µg·h/l (normal vs. insomnic)	t _{1/2} : 6.40±0.50 vs. 8.07±1.68 h; CLz: 13.52±5.555 vs. 8.349±2.292 l/kg/h (normal vs. insomnic)	GA exposure was higher in insomnic rats (Cmax + ~58.6%; AUC0-∞ + ~52.9%), with prolonged t _{1/2} and reduced clearance; Tmax was essentially unchanged.	(114)
Xu <i>et al</i> , 2013	Male vs. female Sprague-Dawley rats	Oral <i>S. chinensis</i> extract, 20 mg/kg (≈1.2 mg/kg GA)	Male: 4.59±1.92 ng/ml; female: 45.22±14.82 ng/ml	Male: 0.3±0.2 h; female: 0.6±0.3 h	Male: 2.37±0.82 ng·h/ml; female: 71.62±21.32 ng·h/ml	Male: t _{1/2} , 0.3±0.1 h; female, 1.4±0.2 h	Marked sex difference, potentially associated with lower CYP activity and metabolite excretion in females	(136)
Zhang <i>et al</i> , 2021	Rats	Oral; dose not stated	29.76±4.45 ng/ml	0.28±0.07 h	AUC0-t, 69.67±9.41; AUC0-∞, 122.61±44.35 ng·h/ml	t _{1/2} , 17.88±5.19 h; MRT0-t, 8.47±0.52 h	The reported values describe exposure; they do not establish poor absorption or lower exposure without a defined comparator	(137)

Table IV. Continued.

Authors, year	Model/source	Route and dose	C _{max}	T _{max}	AUC	Elimination/distribution	Critical interpretation	(Refs.)
Yang <i>et al.</i> , 2015	Normal vs. fibrotic male Wistar rats	Oral; 15 g/kg; GA-containing preparation	Not applicable	Not applicable	GA/SOB AUC0-t in fibrotic rats= 295.08% of the normal-rat value (P<0.05)	Decreased clearance in fibrosis	Pathological state of liver fibrosis may enhance the absorption of GA and decrease its CL/F	(133)
Zhan <i>et al.</i> , 2014	Male Sprague-Dawley rats (293±28 g)	IV Shengmai injection, 10.8 ml/kg (equivalent to 7.10 µg/kg GA)	Not applicable	Not applicable	Not applicable	Rapid distribution from blood into most tissues; very low unchanged biliary, urinary and fecal excretion	Tissue-distribution/excretion study. The 7.10 µg/kg value is the GA-equivalent dose within Shengmai injection.	(135)
Qin <i>et al.</i> , 2016	Male Sprague-Dawley rats	Oral Wuzhi tablet, 0.25 g/kg (≈0.223 mg/kg GA based on 0.89±0.10 mg/g)	Not applicable	Not applicable	Not applicable	Tissue concentrations were highest at 0.25 h; GA concentration was higher in small intestine than liver; undetectable in tissues at 24 h.	The 0.25-h value is a tissue sampling/distribution observation, not plasma T _{max} . Tissue data should not be reported as plasma AUC/T _{max} .	(138)
Madgula <i>et al.</i> , 2008	Caco-2 cell monolayer	50, 100 and 200 µM	Not applicable	Not applicable	Not applicable	Efflux ratio, ~1	Quantitative analysis of the bidirectional transport characteristics of GA in a Caco-2 cell monolayer model	(100)

GA, gomisin A; SD, Sprague Dawley; C_{max}, maximum measured plasma concentration; T_{max}, time to C_{max}; t_{1/2}, half-life; Vd/F, apparent volume of distribution after extravascular dosing; CL/F, apparent clearance after extravascular dosing; IV, intravenous; AUC, area under the plasma concentration-time curve; AUC_{inf} and AUC_{0-∞}, AUC extrapolated from time zero to infinity; AUC_{0-t}, AUC from time zero to the last measured time; CYP, cytochrome P450; MRT_{0-t}, mean residence time from time zero to the last measured time; F, absolute bioavailability.

administration of *Schisandra* extracts or multi-component preparations (18,98,99,133,134). The dose, formulation and analytical treatment of metabolites are not uniform.

Three recurring PK considerations emerge across the aforementioned studies, although their strength has not been compared statistically. First, oral systemic exposure is low-to-moderate and highly variable. The reported rat absolute bioavailability of 20.8% suggests notable pre-systemic loss (99). A Caco-2 efflux ratio close to 1, calculated as secretory divided by absorptive apparent permeability, indicates similar transport in both directions under those assay conditions (100), and thus, poor oral exposure should not be attributed to failed intestinal permeation. Intestinal and hepatic metabolism, formulation and dose-dependent solubility are plausible contributors.

Second, sex and disease state influence exposure. Female rats exhibit an ~10-fold higher maximum plasma concentration (C_{max}) and ~30-fold higher AUC than male rats at the same GA-equivalent dose of *S. chinensis* extract (133). Liver fibrosis and the experimental insomnia model used in rats alter absorption or clearance (114,133). These findings do not support one universal dosing scheme and indicate that disease-mediated changes in CYP activity, hepatic blood flow, transport and protein binding require direct measurement.

Third, the plasma concentration of unchanged GA is an incomplete representation of pharmacologically relevant exposure. GA distributes rapidly into tissue, while unchanged excretion is low and metabolism is extensive (135). However, unbound tissue concentrations and the pharmacological activity of metabolites are largely unknown. A low plasma C_{max} does not exclude liver or intestinal activity, but limits claims for tissues unless tissue exposure is measured.

Formulation approaches, including natural deep eutectic solvents and hydrogel carriers, may improve apparent solubility or delivery (139). These are proof-of-concept technologies, not established solutions. Future studies should define the solubility-vs. metabolism-limited components of oral exposure, then select a formulation that raises the unbound target site concentration without disproportionately increasing CYP or transporter interactions.

6. Safety, toxicity, drug interactions and assay-interference liability

The safety database for purified GA is less developed than the efficacy literature. A historical mouse study reported acute median lethal dose values of 777 mg/kg orally, 390 mg/kg intraperitoneally and 500 mg/kg subcutaneously (19). When the dosage is increased, GA induces acute toxic symptoms, including somnolence, ataxia, hypothermia and muscle relaxation, and may cause respiratory depression and death (19). These data provide only an acute toxicity estimate (19). They do not substitute for modern repeat-dose toxicology, toxicokinetics, histopathology, genotoxicity, reproductive and developmental toxicity, carcinogenicity, immunotoxicity or cardiovascular safety assessment. Current safety evidence is summarized in Table V.

The central-depressant findings are relevant because sedative and hypnotic effects are also potential therapeutic activities (19). A dose-related continuum may exist between

sleep promotion and adverse psychomotor or respiratory depression. Brain exposure, electroencephalography, motor coordination and interaction with sedatives should therefore be included in safety studies.

Drug interactions are a translational liability. GA inhibits CYP3A activity, including time- and NADPH-dependent inhibition, whereas longer exposure may induce CYP3A expression through PXR-associated regulation (12,62,140). In the rat cyclophosphamide study, altered CYP3A-mediated metabolism reduced formation of chloroacetaldehyde, illustrating the potential for schedule-dependent drug interactions (140). In total, six dibenzocyclooctadiene lignans from *S. chinensis* have been screened for inhibitory effects on nine CYP450 (CYP) and six uridine diphosphate glucuronosyl-transferase (UGT) enzymes using human liver microsomes, demonstrating that the time-dependent inhibition of GA against CYP2C8, CYP2C19 and CYP3A4 is attributed to reactive carbene metabolites, whereas these lignans exhibit only mild UGT inhibition, which facilitates the prediction of herb-drug interactions between *Schisandra* lignans and P450-cleared co-administered drugs (141). These findings collectively underscore the need to consider CYP-mediated GA-drug interactions when using *Schisandra* preparations concomitantly with CYP-metabolized medications.

P-gp and BCRP inhibition broadens the interaction risk (49,95). Increases in PTX or tacrolimus exposure (96,101) may increase both pharmacological effects and concentration-dependent toxicity, and require safety monitoring rather than being treated as an unqualified dosing benefit. Tacrolimus has a narrow therapeutic index; uncontrolled elevation increases nephrotoxicity, neurotoxicity and the infection risk (142). Similarly, higher anticancer drug exposure may increase toxicity. The direction and magnitude of interaction may also change with repeated administration if PXR-mediated enzyme induction competes with acute inhibition (72).

For cell assays conducted at micromolar concentrations, aggregation, non-specific membrane effects and general cytotoxicity are plausible. Recommended controls include detergent-sensitive aggregation testing, dynamic light scattering (DLS) where appropriate, orthogonal readouts, counterscreens against unrelated proteins, evaluation of fluorescence/absorbance interference, measurement of the free concentration in culture medium and demonstration of a pharmacological window relative to non-malignant or primary cells (47,143,144).

7. In silico target prioritization and docking-based hypotheses

The constructed PPI network contains 88 nodes and 654 edges (Fig. 11A). Core hub targets were screened by integrating three topological parameters: Degree, betweenness centrality and closeness centrality, with the top 10% ranked nodes defined as core hubs. STAT3, EGFR and mTOR ranked highest across all three parameters and were identified as core candidate targets (Fig. 11B). High network centrality reflects the topological importance of these proteins in the interaction network, and does not equate to biological causality or confirm that GA exerts its pharmacological effects through these targets. These hubs should be prioritized as candidates for subsequent mechanistic validation.

Table V. Safety profile and potential liabilities of GA.

Liability	Available evidence	Implications	Limitations	(Refs.)
Acute toxicity	Mouse LD50: oral, 777 mg/kg; intraperitoneal, 390 mg/kg; subcutaneous, 500 mg/kg	Route-dependent acute toxicity; therapeutic margin undefined	Historical non-GLP study; no repeat-dose data or toxicokinetics	(19)
Central nervous system	Somnolence, ataxia, hypothermia, muscle relaxation and respiratory depression at toxic exposure	Sedation and psychomotor or respiratory depression may be dose-limiting	No exposure-response margin, combination studies or human data	(19)
CYP3A	Competitive, time- and NADPH-dependent inhibition; later CYP3A upregulation in rats	Bidirectional, schedule-dependent interactions with CYP3A substrates	Clinical magnitude and chronic net effect unknown	(140)
CYP2C8/ CYP2C19/ UGTs	Time-dependent CYP2C8, CYP2C19 and CYP3A4 inhibition; limited UGT inhibition	Potential interactions with P450-cleared drugs	No quantitative IVIVE or clinical validation	(141)
P-gp/BCRP	Transport inhibition; increased paclitaxel and tacrolimus exposure in rats	May reverse resistance but increase substrate toxicity	CYP3A contribution and repeated-dose effects unresolved	(49,95, 96,101)
Genotoxicity, reproductive/developmental toxicity, carcinogenicity	No adequate purified GA studies	Critical development gap	Standard regulatory battery required	
Cardiovascular safety	Vasorelaxation and Na ⁺ /ERG-mediated K ⁺ current inhibition	Hypotension; risk of impaired cardiac electrical conduction or delayed ventricular repolarization cannot be excluded	Non-cardiac cell data; no hERG, telemetry or QT assessment	(9,14,24, 25,131)
Assay interference	No PAINS alerts detected; micromolar to high-micromolar testing is common	Classical PAINS risk is low; non-specific effects possible	No aggregation, optical-interference or orthogonal counterscreens	(47,143, 144)

LD50, median lethal dose; GLP, Good Laboratory Practice; CYP, cytochrome P450; UGT, UDP-glucuronosyltransferase; IVIVE, *in vitro-in vivo* extrapolation; P-gp, P-glycoprotein; BCRP, breast cancer resistance protein; GA, gomisin A; hERG, human ether-à-go-go-related gene; QT, electrocardiographic interval from the onset of the QRS complex to the end of the T wave; PAINS, pan-assay interference compounds; P450, cytochrome P450.

GO enrichment analysis indicated that the predicted target set was enriched for the biological process terms ‘phosphorylation’ and ‘protein autophosphorylation’. Cellular-component annotations included ‘plasma membrane’, ‘membrane raft’ and ‘receptor complex’, while molecular-function annotations included ‘ATP binding’ and ‘protein kinase activity’ (Fig. 12A and C). These are annotations of predicted target proteins, not measurements of GA localization or evidence that GA activates or inhibits the annotated functions. KEGG terms included ‘pathways in cancer’, ‘EGFR tyrosine kinase inhibitor resistance’, ‘PI3K-Akt signaling pathway’, ‘MAPK signaling pathway’ and ‘ErbB signaling pathway’

(Fig. 12B and D; Table SIII). The enrichment profiles describe the functional context of predicted targets and do not establish pathway regulation by GA *in vivo*.

Structure-based assessment of prioritized GA-target interactions. The selected proteins have pharmacological research relevance, including receptor-kinase, PI3K/mTOR, STAT3 and p38 signaling studies (103,145-148). Their biological activities align with the known hepatoprotective, anticancer, anti-inflammatory and neuroprotective effects of GA (7,8,10). Such congruence strengthens the credibility of the predicted multi-target network, although it does not confirm direct binding between GA and these proteins.

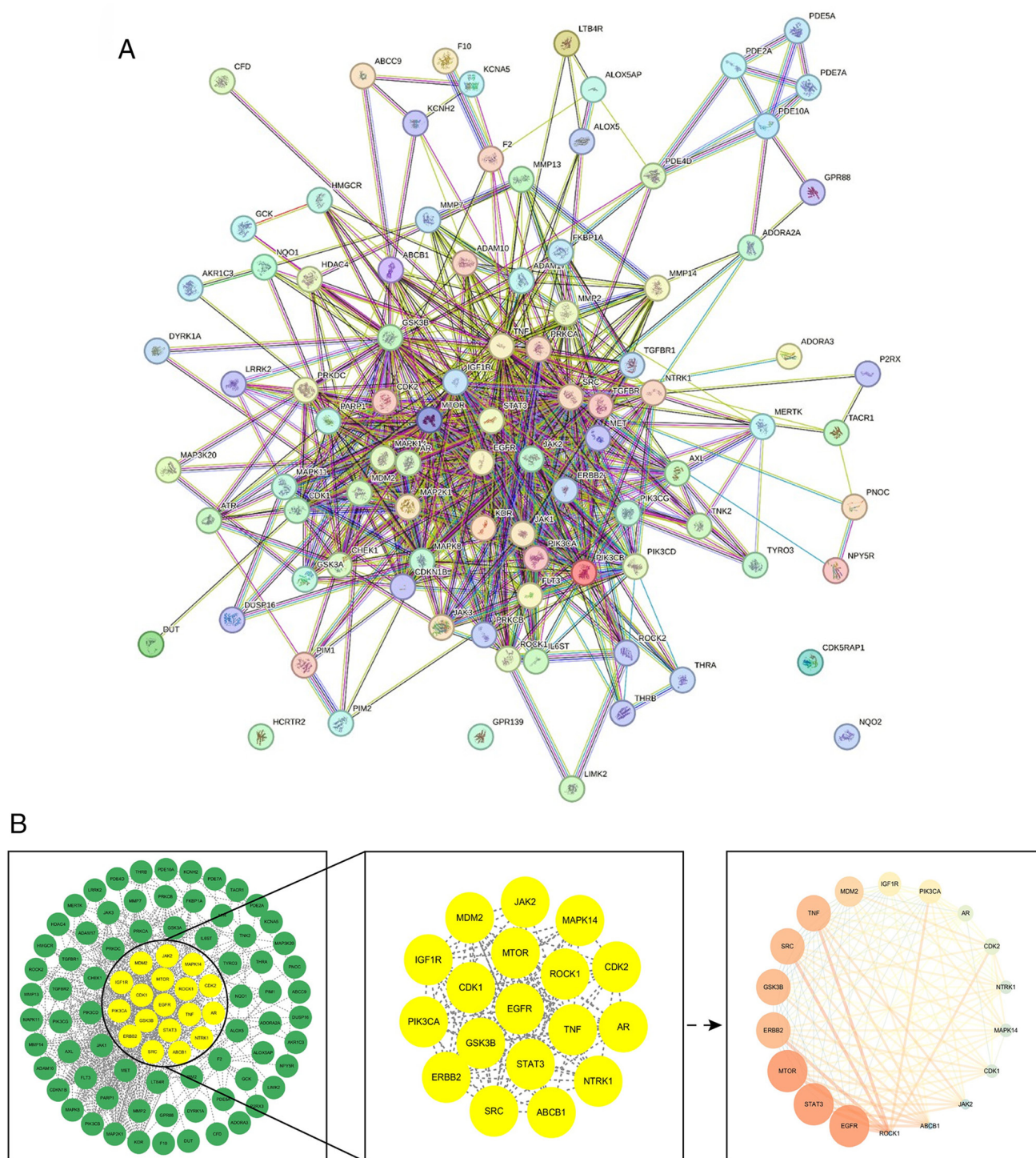


Figure 11. Network pharmacology analysis of predicted GA targets. (A) Protein-protein interaction network constructed from predicted targets of GA. (B) Topological screening and visualization of candidate hub proteins. GA, gomisin A.

Based on the aforementioned criteria, nine proteins, including STAT3, EGFR, mTOR, TNF, MAPK14, PIK3CA, AKT1, ERBB2 and GSK3 β , were selected for molecular docking validation. Further structural simulation aimed to characterize binding modes between GA and these hub molecules (Fig. 13).

GA yielded favorable docking poses across the nine selected proteins. (-7.7 for EGFR, -7.4 for GSK3 β , -7.0 for STAT3, -6.9 for mTOR, -6.8 for MAPK14, -6.6 for AKT1

and ERBB2, -6.5 for PIK3CA, and -6.1 kcal/mol for TNF); representative poses are shown in Fig. 13. These calculations generate structural hypotheses and prioritize candidates for biochemical follow-up; they do not constitute evidence of functional target engagement.

EGFR, GSK3 β and STAT3 were selected for MD simulation using a combined criterion that considered docking score, network priority, disease relevance and target-class representation. EGFR and GSK3 β showed the most favorable

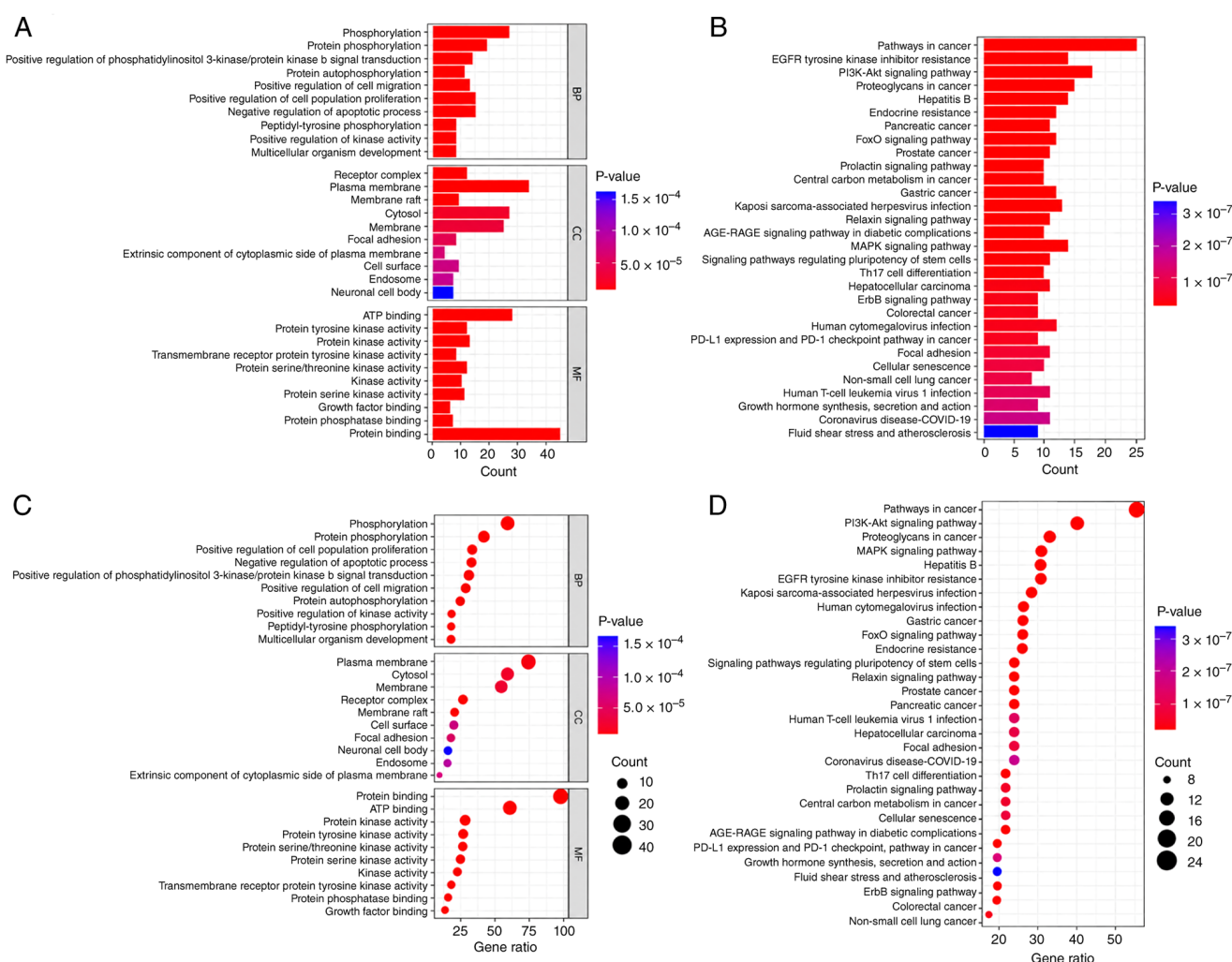


Figure 12. Gene Ontology and pathway enrichment analyses of predicted gomisin A targets. Enriched (A) Gene Ontology terms and (B) Kyoto Encyclopedia of Genes and Genomes pathways. Bubble plot of enriched (C) Gene Ontology terms and (D) Kyoto Encyclopedia of Genes and Genomes pathways. AGE-RAGE, advanced glycation end-products-receptor for AGE system; BP, biological process; CC, cellular component; COVID-19, coronavirus disease 2019; MF, molecular function; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; Th17, T helper 17.

docking scores, whereas STAT3 ranked among the principal network hubs and represented a non-kinase regulatory target with broad relevance to the reported pharmacological effects of GA (22,77,145). The 100-nsec simulations were used to examine the dynamic behavior of these docking-derived complexes under explicit solvent conditions.

To elucidate the characteristics of these interactions, analysis of the specific binding modes and potential pharmacological implications between GA and each key target was performed, based on the strength of the predicted docking score and functional relevance.

Structural interaction characteristics of major hub targets.

i) EGFR. EGFR is a key transmembrane protein that serves a central role in maintaining cell homeostasis by regulating signaling pathways related to cell survival, migration and proliferation (103). The abnormal activation and/or overexpression of EGFR are closely associated with the development and progression of solid tumors, such as breast and non-small cell lung cancer and CRC (149,150).

Molecular docking generated a putative GA-EGFR pose (Fig. 13A). The polar and hydrophobic contacts are docking-derived observations and do not establish

ATP-competitive inhibition or downstream MAPK/PI3K regulation.

ii) GSK3 β . The GSK3 β protein is a multifunctional serine/threonine kinase that serves as a key regulatory node in signaling pathways, including the Wnt/ β -catenin, insulin and PI3K/AKT pathways (151). In metabolism, GSK3 β is involved in regulating glycogen synthesis, glucose homeostasis and insulin signaling. Aberrant activity of GSK3 β is associated with metabolic conditions such as diabetes and obesity (152). Within the nervous system, excessive activation of GSK3 β promotes hyperphosphorylation of tau protein, accumulation of A β and neuroinflammation, constituting one of the core pathological mechanisms in neurodegenerative diseases such as Alzheimer's disease (153). GSK3 β is regarded as a pivotal hub target linking metabolic disorders and neurodegenerative processes. The present docking analysis generated a favorable GA-GSK3 β pose (Fig. 13B). Binding mode analysis indicated that GA stably occupied the ATP-binding pocket through its rigid skeleton, suggesting a putative inhibitory potential against GSK3 β kinase activity. This high-affinity ATP-pocket engagement structurally supports GSK3 β as a core multi-system regulatory target of GA.

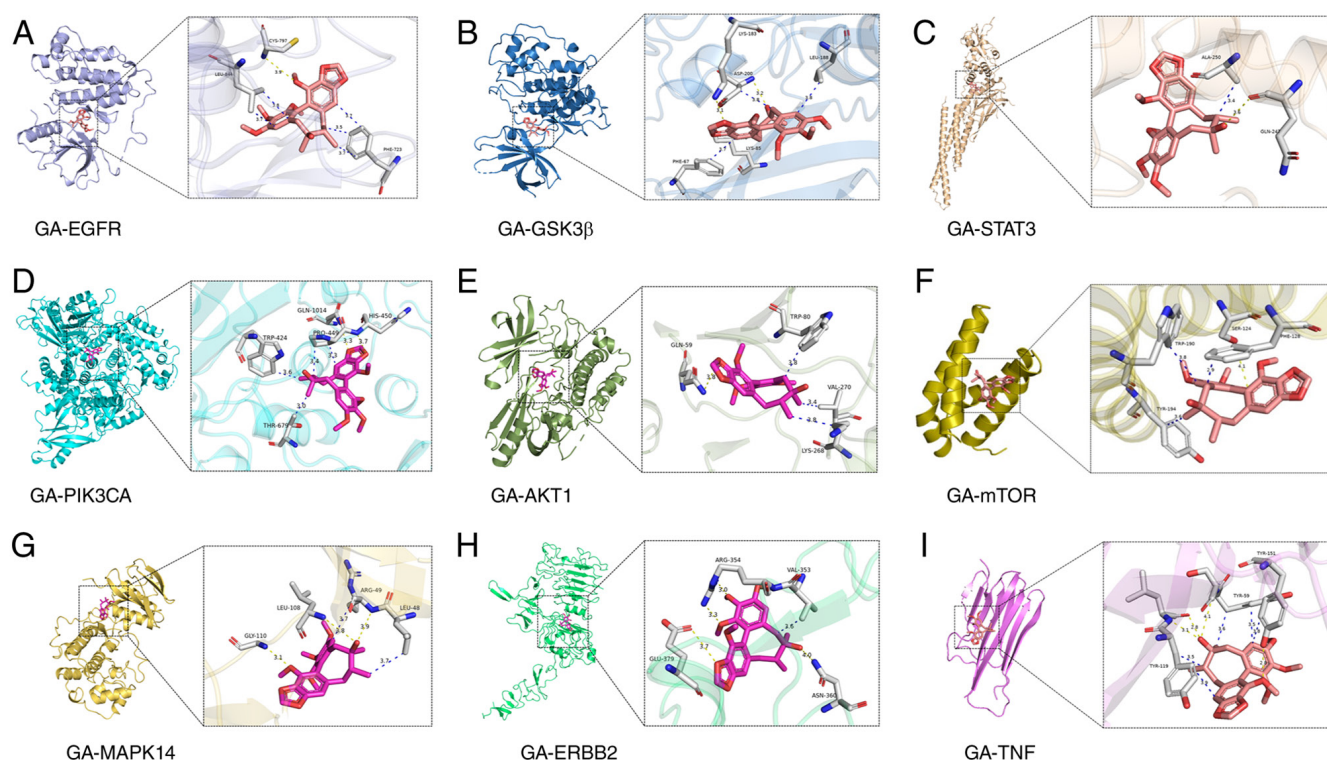


Figure 13. Predicted binding modes of GA with nine prioritized candidate targets. Representative molecular docking poses are shown for GA with (A) EGFR, (B) GSK3 β , (C) STAT3, (D) PIK3CA, (E) AKT1, (F) mTOR, (G) MAPK14, (H) ERBB2 and (I) TNF. ERBB2, erb-b2 receptor tyrosine kinase 2; GA, gomisin A; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α .

iii) STAT3. STAT3 is a key member of the STAT protein family and serves a central regulatory role in tumorigenesis and progression (154). By activating target genes such as CCND1 and BCL-xL, it promotes tumor cell proliferation, inhibits apoptosis, and drives angiogenesis and invasion processes (145). Furthermore, the STAT3 signaling pathway is involved in tumor immune evasion: It can suppress the presentation of tumor-associated antigens and promote the activation of immunosuppressive cells such as regulatory T and myeloid-derived suppressor cells, thereby weakening antitumor immune responses (155). Given its key roles in tumor progression and the regulation of the immune micro-environment, STAT3 has become a prominent antitumor target (145,155).

The present molecular docking analysis generated a predicted GA-STAT3 binding pose. The docking results suggested potential interactions between GA and the modeled STAT3 region; however, the exact binding domain and the functional consequences for STAT3 activity require further structural and experimental validation.

iv) PI3K/AKT/mTOR. The PI3K/AKT/mTOR pathway is a key pathway regulating cell proliferation, survival and metabolism, and can be activated by growth factors through receptor tyrosine kinases, among other mechanisms (146). Signal transduction in this pathway begins with the activation of PI3K, which catalyzes the production of phosphatidylinositol (3,4,5)-trisphosphate (PIP3). PIP3 recruits and activates AKT (also known as protein kinase B). AKT, serving as a key hub, phosphorylates various downstream target proteins, including mTOR (156). mTOR forms two complexes, mTOR complex 1 (mTORC1) and mTORC2, with mTORC1 serving a

central role in responding to growth signals and nutrient status by directly regulating protein synthesis by phosphorylating substrates such as ribosomal protein S6 kinase 1 and eukaryotic translation initiation factor 4E-binding protein 1 (147). Aberrant activation of this pathway is prevalent in various diseases, including cancer, making it a notable therapeutic target (146,147). GA yielded similar docking scores for three nodal proteins along this axis, PIK3CA, AKT1 and mTOR, with Vina scores of -6.5, -6.6 and -6.9 kcal/mol, respectively. These results provide structural support for a potential multi-node interaction pattern within the PI3K/AKT/mTOR pathway, although the direction and magnitude of any functional effects cannot be inferred from docking alone. For PIK3CA, the predicted pose was located within the ATP-binding pocket and involved a network of hydrogen bond interactions. For AKT1, the predicted interaction included cation- π and hydrophobic contacts. Notably, mTOR showed a relatively favorable docking score, accompanied by a hydrogen bond network and hydrophobic contacts. The broadly similar docking scores across the three proteins support the structural plausibility of potential interactions at multiple nodes of the pathway, with mTOR prioritized because it was also identified as a core PPI-network hub, rather than because its small score difference establishes dominant inhibition.

v) MAPK14. Among the key targets highlighted by KEGG pathway analysis, prior literature and network pharmacology, the MAPK signaling pathway is a classic cell signal transduction pathway. Its key mechanism involves transmitting extracellular signals to the nucleus through a series of kinase cascades, thereby regulating gene expression (148). As a key member of the MAPK family, MAPK14 (p38 α) serves a key

role in pathophysiological processes such as inflammation, stress responses and apoptosis (148).

The predicted GA-MAPK14 pose included a hinge region hydrogen bond network and extensive hydrophobic interactions within the ATP-binding pocket. These findings provide structural support for putative GA-mediated modulation of the MAPK signaling pathway via interaction with MAPK14.

vi) ERBB2. ERBB2 is a crucial member of the EGFR family. Its aberrant activation or overexpression is associated with the progression and poor prognosis of malignant tumors, such as breast and gastric cancer (157), making it one of the key targets in tumor-targeted therapy.

Molecular docking generated a putative GA-ERBB2 pose. GA forms a single hydrogen bond between its C-4' hydroxyl group and Cys-805 in the hinge region. Due to partial occlusion of the active pocket entrance by the gatekeeper residue Thr798, the cyclooctadiene skeleton of GA undergoes a conformational adjustment (twist-boat angle decreased to 32°) to accommodate the narrower channel, resulting in a smaller hydrophobic contact area (~240 Å²) and weaker water displacement compared with EGFR. This compromised binding mode is consistent with its lower affinity relative to EGFR and suggests GA may co-target ERBB family receptors.

vii) TNF. TNF is a key cytokine in inflammatory responses, regulating acute and chronic inflammation, autoimmune disease, and the tumor microenvironment (158). Its excessive production is associated with the pathological processes of rheumatoid arthritis, inflammatory bowel disease and cancer-associated inflammation (158). Molecular docking indicated that GA has binding potential to TNF. GA may insert its dibenzocyclooctadiene skeleton into a continuous hydrophobic groove formed by Tyr59, Tyr119 and Tyr151 on the TNF surface, establishing multiple π - π stacking interactions. This structural feature implies GA may directly interfere with TNF trimerization or receptor binding, representing an additional protein-level anti-inflammatory mechanism.

MD assessment of representative GA-target complexes: Conformational stability and binding interface analysis. The protein-ligand systems reached conformational plateaus during the latter portions of the trajectories, supporting the conformational plausibility of the docking-derived complexes. The GSK3 β complex equilibrated most rapidly, with backbone RMSD reaching a stable plateau after ~10 nsec. The EGFR system reached equilibrium at ~20 nsec. The STAT3 complex required the longest adjustment period, with backbone fluctuations stabilizing only near 80 nsec, indicating greater structural adjustment in the simulated STAT3 construct. At equilibrium, the backbone RMSD values were 0.25 for GSK3 β , 0.38 for EGFR and 0.63 nm for STAT3. The higher STAT3 RMSD cannot by itself identify the responsible domain motion or establish ligand retention within a specific binding groove. Rg analysis supported global structural integrity. After initial relaxation, Rg values remained steady across all three complexes (2.11 for GSK3 β , 2.04 for EGFR and 2.93 nm for STAT3) with no persistent up- or downward drift, supporting the maintenance of overall protein compactness, with no sustained global structural expansion observed (Fig. 5).

Residue-level flexibility and binding interface behavior were evaluated using RMSF and intermolecular hydrogen bond analyses. For all three complexes, residue fluctuations were

generally lower around the predicted binding interfaces than in solvent-exposed loop regions, although ligand-free control simulations are required to determine whether this pattern was specifically associated with GA binding. Intermolecular hydrogen bonds formed and broke dynamically throughout the trajectories, with no hydrogen bond and intermittent higher-count peaks; the EGFR complex generally shows fewer hydrogen bonds during the latter part of the trajectory (Fig. 14A). Intermolecular hydrogen bonds were intermittently maintained throughout the trajectory, although their occupancy and donor-acceptor identities varied over time. These observations indicated that polar interactions between GA and the predicted binding sites were dynamically retained during the simulation, while their specific contribution to complex stability cannot be established from hydrogen bond counts alone.

Total SASA decreased during the early relaxation phase of all simulations and stabilized in later trajectory segments, consistent with decreased overall solvent exposure as the systems adjusted from their rigid docking starting conformations. This aligns with the expected pattern of hydrophobic residue burial at the protein-ligand interface, although interface-specific SASA calculations are required to formally attribute the changes to ligand binding rather than global protein compaction (Fig. 14B).

Taken together, the 100-nsec simulation provided multi-dimensional support for the conformational plausibility of the top-ranked docking poses. Across all three complexes, GA maintained its core binding orientation within the cognate pocket over the production phase, with preserved global protein folds, stable interface core rigidity, persistent polar interactions and SASA trends consistent with hydrophobic packing. These findings extend static docking predictions into a dynamic solvated context and reinforce the computational plausibility of the proposed multi-target interaction profile of GA. These trajectory-level findings provide a dynamic consistency check for the selected docking poses but do not determine whether GA binds these proteins selectively in biochemical or cell settings.

Specificity assessment of the predicted multi-target profile: PAINS structural alerts and remaining uncertainties. GA was predicted to interact with a broad set of proteins, including kinases, such as EGFR, GSK3 β , mTOR, MAPK14, AKT1, PIK3CA and ERBB2, the transcription factor STAT3 and the cytokine TNF, raising an important question regarding target specificity. Specifically, it remains necessary to distinguish genuine polypharmacology from frequent hitter behavior or assay interference. PAINS alerts are substructural patterns originally associated with compounds that repeatedly produce apparent activity in high-throughput screening assays. However, a PAINS match is a warning requiring further investigation rather than definitive evidence that a compound is artifactual; conversely, the absence of a PAINS match does not establish assay specificity (47,143).

No PAINS substructure match was returned for GA. This indicates that GA does not contain any of the classical PAINS motifs represented in the applied RDKit catalogs.

The negative RDKit result does not demonstrate that GA is free from assay interference and does not exclude mechanisms that are not encoded by the applied structural rules.

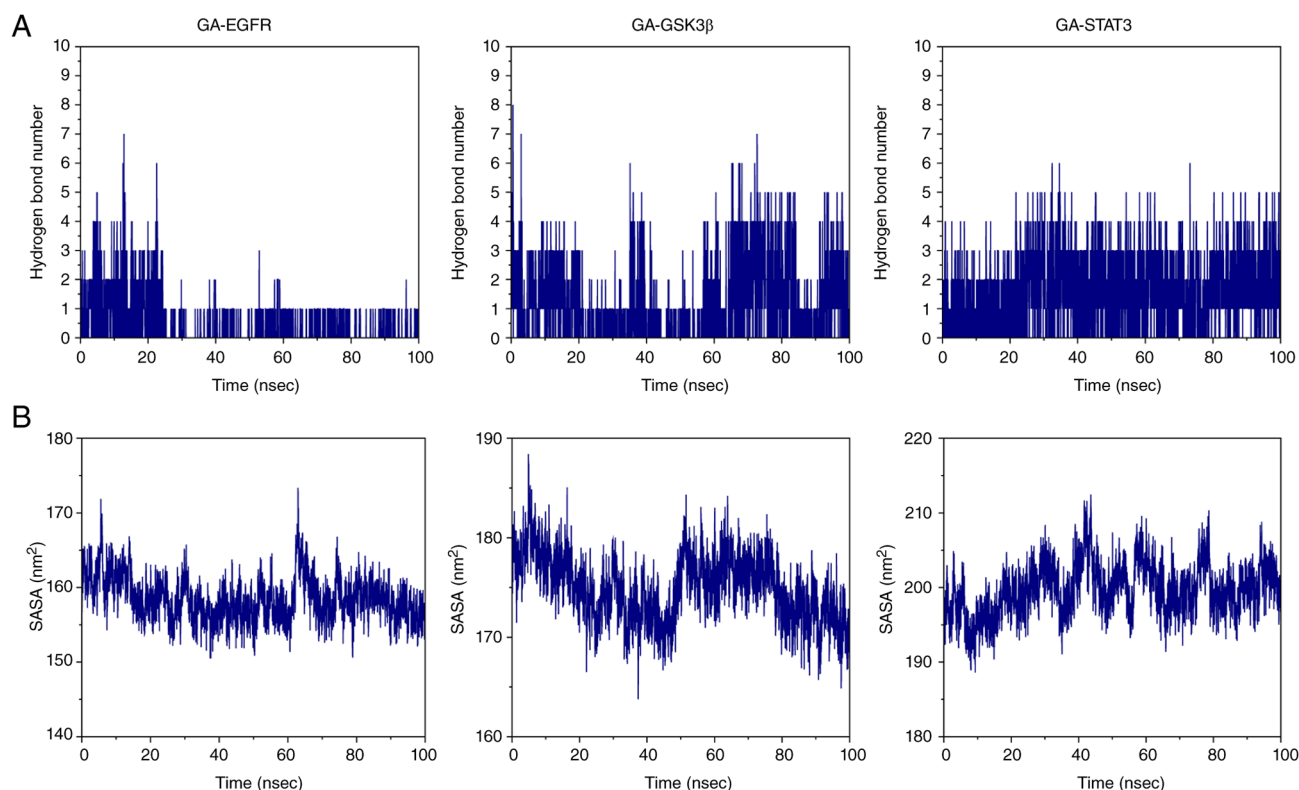


Figure 14. Hydrogen bond and solvent-accessibility analyses of representative GA-target complexes. (A) Intermolecular hydrogen bond counts for GA-EGFR, GA-GSK3 β and GA-STAT3 complexes during 100-nsec molecular dynamics simulations. (B) SASA profiles of complexes over the simulation trajectories. GA, gomisin A; SASA, solvent-accessible surface area.

In particular, colloidal aggregation, non-specific membrane partitioning, fluorescence or absorbance interference, redox-associated effects and non-specific protein binding at high compound concentrations cannot be assessed by PAINS substructure matching alone (47,143,144). Therefore, the present results decrease concern regarding classical PAINS chemotypes but do not establish that GA is assay-specific or non-promiscuous (47,143,144).

i) Structural features compatible with, but not diagnostic of, polypharmacology. The dibenzocyclooctadiene scaffold of GA possesses a moderately lipophilic and conformationally adaptable framework (46). Its ether oxygen atoms and non-phenolic hydroxyl group support polar interactions, whereas its aromatic and carbocyclic framework may accommodate hydrophobic regions within protein-binding pockets. The possible contacts described here are interpretations of the structure and the present docking poses, not evidence of experimentally verified polypharmacology. Most of the computationally prioritized proteins are kinases with evolutionarily associated ATP-binding pockets. Consequently, similar docking orientations across EGFR, GSK3 β , mTOR, MAPK14, AKT1, PIK3CA and ERBB2 may partly reflect conservation of kinase pocket architecture and the preferences of the docking scoring function, rather than experimentally verified engagement of these proteins (34,103). The Vina docking scores of -6.1 to -7.7 kcal/mol should be used for relative pose and target prioritization only. They should not be interpreted as experimentally measured binding free energies or converted directly into micromolar binding potency.

The MD trajectories provide an additional dynamic consistency check for the GA-EGFR, GA-GSK3 β and GA-STAT3 docking poses. However, trajectory persistence does not demonstrate biochemical selectivity, direct target engagement or functional inhibition. Furthermore, these findings cannot be extrapolated to mTOR, MAPK14, AKT1, PIK3CA, ERBB2 or TNF, which were not subjected to MD simulation.

ii) Caveats and recommended orthogonal validation. GA is moderately lipophilic, with a calculated cLogP of ~ 3.6 , and cell effects such as CRC apoptosis and melanoma growth suppression occur at micromolar or high-micromolar concentrations (8,23). Under these conditions, non-specific membrane partitioning, colloidal aggregation or general cytotoxicity remains plausible in the absence of a PAINS alert. A tiered program of orthogonal validation is therefore recommended, proceeding from biochemical binding through cell target engagement to functional dependency and structural confirmation.

Biochemical-level validation should be pursued. For kinase candidates among the top predicted targets, including EGFR, GSK3 β and mTOR, recombinant kinase assays should measure direct effects on catalytic activity and derive IC₅₀ values at physiologically relevant ATP concentrations. Parallel biophysical binding measurements using SPR, biolayer interferometry, microscale thermophoresis or isothermal titration calorimetry (ITC) may confirm direct physical binding and provide equilibrium dissociation constants together with association and dissociation kinetics.

CETSA test target stabilization in cells, while DARTS can test ligand-associated protease protection in an appropriate protein preparation (159,160). These methods bridge

recombinant biochemistry and the intracellular environment, where off-target binding, metabolic conversion and restricted compartmental access may modify the effective interactome (159,160).

Functional dependency validation is critical. Target knock-down, knockout, point mutation, overexpression or rescue experiments are required to determine whether the reported cell phenotypes depend on EGFR, GSK3 β , STAT3 or other predicted proteins. Attenuation or loss of the GA response following target disruption is the strongest available preclinical evidence that a candidate target mediates the observed phenotype.

Co-crystallography or cryo-electron microscopy of GA in complex with its highest-confidence targets would definitively resolve the binding mode and distinguish specific ligand-protein interactions from non-specific hydrophobic associations. This level of validation sets the long-term standard for target confirmation.

Before target-specific assays are interpreted, basic counter-screens for artifactual activity are key. Aggregation-sensitive controls include parallel testing with and without a low concentration of non-ionic detergent, such as ~0.01% Triton X-100, as well as BSA or other decoy protein additions. DLS may be used where technically applicable to determine whether GA forms colloidal particles under the assay conditions. Additional controls should include fluorescence and absorbance interference testing, orthogonal assay formats, counterscreens against unrelated proteins, concentration-response analysis and parallel assessment of cytotoxicity in non-malignant or primary cells. Detergent and BSA concentrations should be optimized for each assay because these additives may alter target activity or decrease the free concentration of GA (144).

In summary, the molecular docking and network pharmacology support target prioritization and hypothesis generation. They do not constitute target confirmation or evidence that GA directly engages these proteins in a functional setting. Taken together, the absence of an RDKit PAINS match argues against the presence of classical PAINS substructures in GA, but does not establish genuine polypharmacology. The present network pharmacology, docking and MD findings support a set of structurally plausible and biologically relevant candidate interactions. Until these interactions are confirmed through orthogonal biochemical, cell target engagement and functional dependency experiments, EGFR, GSK3 β , STAT3, mTOR and the other predicted proteins should be regarded as computationally prioritized candidate targets rather than established direct targets of GA.

8. Discussion

The pathogenesis of complex chronic diseases relies on dynamic crosstalk across multiple signaling pathways and cell networks, a number of which remain incompletely characterized (1). Treatment limitations differ by liver disease and cancer type; incomplete responses, resistance and adverse effects are relevant in selected settings (57,157). By contrast, traditional Chinese herbal medicines, as natural active drugs, boast advantages such as abundant resources, high safety, low toxicity, low production cost and suitability for long-term use (161). Furthermore, the complex composition of herbal

medicines enables them to exert combined therapeutic activities through binding with multiple targets. Additionally, due to the unique structures and pharmacological actions of herbal medicines, individual constituents exhibit several preclinical activities, as illustrated by GA-associated apoptosis and transporter inhibition (8,49). Therefore, developing therapeutic agents from traditional herbs has become a promising direction for treating complex diseases such as liver disease, cancer, CVD, and neurodegenerative and inflammatory disorders.

Hepatoprotection is the best-supported preclinical activity, with consistent efficacy replicated across multiple independent laboratories and injury models, including CCl₄-, APAP-, D-GalN/LPS- and LCA-induced cholestasis (7,11,12,20). The incorporated studies on the Surf4-SAA1 paracrine axis and PPAR α nuclear localization extend the mechanistic discussion of liver fibrosis and MASLD beyond the canonical TGF- β /Smad and NF- κ B pathways to hepatocyte secretome biology and nuclear receptor subcellular trafficking, providing biological context rather than evidence that GA directly acts on Surf4 or PPAR α (74,82). Beyond shared antioxidant and anti-inflammatory mechanisms, PXR-mediated bile acid regulation is the most strongly supported functionally dependent mechanism in the GA literature. Genetic evidence from *Pxr*-null mice supports tier B classification, but direct physical binding of GA to PXR has not been established (12).

Anticancer and cardiovascular activity form a second tier of promising but less mature evidence. Both are supported by substantial cell and *in vivo* phenotypic data, with plausible mechanistic links to cell cycle regulation, apoptosis and endothelial function (8,9,13,14,24,25). However, a notable gap separates effective *in vitro* concentrations from physiologically achievable concentrations during *in vivo* exposure, and most pathway-level observations remain associative rather than formally confirmed as target-dependent.

Neuroprotection, anti-inflammatory and antioxidant effects, renal protection, anti-obesity activity, and other exploratory endpoints constitute a third tier of preliminary observations. Most derive from single-center, single-model reports with limited mechanistic depth and should be regarded as hypothesis-generating leads rather than translation-ready findings (10,15,26,27,29).

GA faces well-documented PK challenges, including limited oral bioavailability, rapid systemic clearance, a large volume of distribution and predominantly non-renal elimination (18,99,135). Pathological states may alter its absorption, metabolism and tissue exposure (114,133). While formulation strategies, such as natural deep eutectic solvents, have shown early potential to improve absorption and bioavailability (139), these approaches remain at the proof-of-concept stage (18,99,114,133).

Translational development is constrained by two poorly addressed risks: Limited safety data and clinically meaningful drug-interaction liability. Available acute toxicity data provide only an estimate of tolerability and modern repeat-dose, genotoxicity, reproductive and carcinogenicity studies are lacking. The central depressant effects of GA also suggest a dose continuum between desired sedative-hypnotic activity and adverse CNS depression, which requires formal exposure-response characterization (19). GA exerts bidirectional regulatory effects on CYP3A4, and inhibits P-gp and BCRP transporters (12,49,62,95,140). When

co-administered with narrow therapeutic index agents such as tacrolimus or PTX, it may increase systemic exposure and elevate the adverse event risk (96,101,142). This drug-herb interaction potential must be prioritized in safety assessment and clinical development planning.

Network pharmacology and molecular docking analyses provide a computational framework to generate hypotheses about the molecular basis of GA-mediated pharmacological actions, rather than definitive mechanistic proof. Network pharmacology screening identified 100 candidate targets with non-zero prediction probability and generated a PPI network, with STAT3, EGFR and mTOR ranking as core topological hubs. These candidate targets align directionally with the hepatoprotective and anticancer phenotypes of GA (8,11,12). Several methodological limitations should be noted. Disease gene sets derived from GeneCards cover a broad functional scope, which may overestimate the biological relevance of target-disease overlaps. Network centrality reflects topological importance rather than causal involvement and docking affinities in the -6 to -8 kcal/mol range only indicate moderate binding potential. All computational findings fall under tier D of the evidence hierarchy and require orthogonal experimental validation before definitive mechanistic conclusions can be drawn.

In summary, GA is a prototypical multi-target natural product whose most prominent pharmacological activity (hepatoprotection, anticancer, neuroprotection and anti-inflammation/antioxidant effects) are primarily attributable to its concerted modulation of key signaling hubs, including PI3K/AKT/mTOR, MAPK and NF- κ B, and targets, such as STAT3, EGFR and GSK3 β . These targets and pathways intersect to regulate cell survival, death, inflammation and metabolism, forming an integrated network that underlies GA-mediated polypharmacology and its ability to address multifactorial diseases. Its historical application in liver disorders corresponds to robust hepatoprotective and antifibrotic effects, while its role as an adaptogen is consistent with observed neuroprotective, anti-inflammatory and antioxidant properties (5,10,16,17,21). Nonetheless, the evidence base remains predominantly preclinical and phenotype-driven (7,8,10,17). Incomplete target validation, suboptimal PK properties, and insufficient safety and drug-interaction data represent the principal barriers to clinical translation. Future research should prioritize rigorous target validation and focused translational development.

Building on validated target mechanisms, future studies should further prioritize developing advanced delivery systems such as nanoformulations and prodrugs to improve PK properties, conducting rigorous proof-of-concept clinical trials in well-supported indications such as chemotherapy-induced LI and metabolic dysfunction-associated fatty liver disease, applying systems pharmacology and multi-omics to delineate its comprehensive target network and combined modes of action, and systematically assessing drug-herb interactions to ensure clinical safety. These efforts may bridge traditional knowledge and modern science, advancing GA toward a scientifically grounded, multi-target therapeutic agent.

To conclude, GA, a natural TCM monomer with extensive application prospects, may propel the modernization of TCM through thorough research and development. Rigorous scientific investigation, clinical trials and precise elucidation of disease-alleviating targets may establish GA as a paradigm

for TCM integration into international markets. This will not only foster the standardization and global acceptance of TCM but may furnish robust evidentiary support for the scientific inquiry and clinical use of traditional remedies.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

JX conceived the study and wrote the manuscript. CZ, JZ and SS performed the computational analyses. QS and SZ analyzed and interpreted data. YZ and PC conceived the study and revised the manuscript. All authors have read and approved the final manuscript. JX and PC confirm the authenticity of all the raw data.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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