Review

Chemical Duality of Verbesina Metabolites: Sesquiterpene Lactones, Selectivity Index (SI), and Translational Feasibility for Anti-Resistance Drug Discovery

Karla Irazu Ventura-Hernández , Tushar Janardan Pawar , Fernando Rafael Ramos-Morales , Carlos Alberto López-Rosas * , Fabiola Hernández-Rosas *

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Chemical Duality of *Verbesina* Metabolites: Sesquiterpene Lactones, Selectivity Index (SI), and Translational Feasibility for Anti-Resistance Drug Discovery

Karla Irazu Ventura-Hernández [1], Tushar Janardan Pawar [2,3,6], Fernando Rafael Ramos-Morales [1], Carlos Alberto López-Rosas [4,*] and Fabiola Hernández-Rosas [5,6,7,*]

[1] Instituto de Química Aplicada, Universidad Veracruzana, Luis Castelazo Ayala s/n, Col. Industrial Animas, 91190 Xalapa-Enríquez, Veracruz, México; irazuhdez1995@gmail.com (K.I.V.-H.); framos@uv.mx (F.R.R.-M.)

[2] Universidad Politécnica de Querétaro, Carretera estatal 420 S/N, El Rosario, 76240, Querétaro, México; tushar.pawar@upq.edu.mx

[3] Escuela de Ingeniería Química, Universidad Anahuac Querétaro, Circuito Universidades I, Fracción 2 S/N, Zibatá, El Marqués, 76246, Querétaro, Mexico

[4] Facultad de Química Farmacéutica Biológica, Universidad Veracruzana, Circuito Aguirre Beltrán s/n, Col. Zona UV, Xalapa 91090, Mexico; carloslopez02@uv.mx

[5] Escuela de Ingeniería Biomédica, División de Ingeniería, Universidad Anáhuac Querétaro, Circuito Universidades I, Fracción 2 S/N, Zibatá, El Marqués, Querétaro, 76246, Mexico

[6] Centro de Investigación, Universidad Anahuac Querétaro, Circuito Universidades I, Fracción 2 S/N, Zibatá, El Marqués, Querétaro 76246, Mexico; Facultad de Química, Universidad Autónoma de Querétaro, Querétaro 76010, Mexico; jesica.escobar@uaq.mx

[7] Facultad de Química, Universidad Autónoma de Querétaro, Querétaro 76010, Mexico

* Correspondence: carloslopez02@uv.mx (C.A.L.R.); fabiola.hernandezro@anahuac.mx (F.H.R)

Abstract

The global health crisis driven by Antimicrobial Resistance (AMR) necessitates an urgent pivot toward novel therapeutic agents, with traditional medicinal plants serving as a critical resource. The Asteraceae genus *Verbesina*, particularly utilized in Mexican ethnobotany, has garnered scientific attention due to its potent bioactive profile against infection and inflammation. This review provides a comprehensive and critical synthesis of the pharmacological landscape of *Verbesina* species, focusing specifically on the dual role of its major secondary metabolites, the Sesquiterpene Lactones (SLs), as both cytotoxic and antimicrobial agents. We systematically compile and analyze reported *in vitro* data, including IC_{50} values from cancer and non-cancerous cell lines, and MIC values against clinically relevant drug-resistant strains like *S. aureus* and *E. coli*. A core focus is placed on establishing the therapeutic index (SI = IC_{50}/MIC) for lead compounds, providing a crucial indicator of drug feasibility. Furthermore, we review the proposed molecular mechanisms of SL action, such as the crucial role of the α-methylene-γ-lactone moiety in alkylating cellular targets, which underpins both their antiproliferative and bactericidal effects. By critically bridging ethnopharmacology with modern mechanistic data, this review validates the translational potential of *Verbesina* metabolites and highlights clear directions for bioassay-guided isolation and optimization as next-generation anti-resistance scaffolds.

Keywords: *Verbesina*; sesquiterpene lactones; cytotoxicity; antimicrobial resistance; MTT assay; therapeutic index; eudesmanes; ethnobotany; Asteraceae

1. Introduction

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The rapid and relentless emergence of multidrug-resistant (MDR) microbial pathogens represents one of the most pressing and complex global health threats of the 21st century [1]. The World Health Organization (WHO) has categorized Antimicrobial Resistance (AMR) as a critical priority, forecasting a dire scenario where common infections and minor injuries could once again become life-threatening [2,3]. This crisis is exacerbated by the overuse of existing antibiotics and a severe slowdown in the discovery pipeline for new therapeutic agents with novel mechanisms of action [4]. Consequently, the scientific community faces an imperative to explore nature's vast chemical library for new scaffolds capable of overcoming established bacterial defense mechanisms. Historically, natural products have been the undisputed bedrock of modern pharmacology, with approximately 60% of current antibacterial and anticancer agents derived directly from natural sources or semi-synthetic modifications thereof [5]. These biologically evolved molecules possess unparalleled structural complexity, allowing them to interact with complex biological targets in unique ways.

Among the plant families recognized for their phytochemical richness and pharmacological significance, the Asteraceae (formerly Compositae) stands out [6]. This family is the largest in Mexico, a nation designated as "Megadiverse," which houses a high concentration of unique chemical diversity and is considered an "evolutionarily active zone" dueating to an accelerated diversification rate within the Asteroideae subfamily [7,8]. The chemical diversity within Asteraceae is staggering, yielding a wide array of specialized metabolites, including polyacetylenes, flavonoids, alkaloids, and notably, various classes of terpenoids [**Error! Bookmark not defined.**,9,10].

Within this prolific family, the Sesquiterpene Lactones (SLs) form a pivotal class of secondary metabolites, characterized by an isoprenoid structure containing 15 carbon atoms and a lactone function [11]. These SLs are structurally classified into several major skeletal groups, including germacranolides, guaianolides, and eudesmanolides [**Error! Bookmark not defined.**]. The SLs are of paramount interest to medicinal chemists due to the consistent presence of a highly reactive functional group: the α-methylene-γ-lactone moiety [**Error! Bookmark not defined.**]. This structural feature is widely regarded as the primary pharmacophore responsible for their profound biological activities. The unsaturated lactone ring acts as a non-specific alkylating agent, undergoing a Michael-type addition reaction with biological nucleophiles, particularly the sulfhydryl (-SH) groups found in cysteine residues of proteins and peptides like glutathione [**Error! Bookmark not defined.**]. This alkylation mechanism provides a clear, unifying hypothesis for their therapeutic potential across both microbial and proliferative targets.

The genus Verbesina itself encompasses over 300 species, with the greatest diversity concentrated in Mexico and the southwestern USA [12,13]. Phylogenetic analysis confirms *Verbesina* as a distinct lineage within the Heliantheae alliance [**Error! Bookmark not defined.**,14], reinforcing its status as a reliable repository of potent specialized metabolites. This chemical potency is not accidental; the genus's bioactivity is fundamentally linked to its ecological role as an aggressive chemical warrior [15,16]. Species like *V. encelioides* are notorious invasive weeds globally [17], capable of quickly dominating ecosystems due to potent allelopathic properties, which involve the competitive release of phytotoxic chemicals [**Error! Bookmark not defined.**,18,19]. This high ecological competence and survival, even in contaminated environments, underscore the necessity and effectiveness of its underlying chemical arsenal [**Error! Bookmark not defined.**,**Error! Bookmark not defined.**].

Phytochemical investigation has consistently identified characteristic metabolites: the dominant SLs are primarily of the eudesmanolide type, a scaffold that accounts for almost half of all natural eudesmanolides found in the Asteraceae family [**Error! Bookmark not defined.**,**Error! Bookmark not defined.**]. These compounds are often isolated as cinnamoyloxy eudesmane conjugates, a defining chemical signature of the genus. Historically, the initial isolation and structural elucidation of these cinnamoyloxy esters from species like *V. virginica* established this complex scaffold as the functional heart of *Verbesina*'s chemistry [20].

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The genus's therapeutic relevance is deeply rooted in Mexican ethnobotany, where various species are integrated into local practices for treating ailments related to inflammation, gastrointestinal distress, and topical infections [21]. For instance, *V. crocata* is traditionally used for wound healing and antidiabetic activity [22,23]. Beyond anti-infective use, the therapeutic scope is rapidly expanding, with recent studies validating the plant's dual function as a valuable resource for sustainable agriculture (acting as potent plant biostimulants that promote crop yield [**Error! Bookmark not defined.**,**Error! Bookmark not defined.**,**Error! Bookmark not defined.**]) and neuroprotection (showing efficacy against Alzheimer's disease models [**Error! Bookmark not defined.**]).

However, the powerful, non-selective chemical reactivity inherent in the α-methylene-γ-lactone group, the very mechanism responsible for efficacy presents a critical translational challenge: the inevitable duality of efficacy and toxicity [**Error! Bookmark not defined.**]. The same alkylating mechanism that deactivates bacterial enzymes can interfere with vital homeostatic processes in non-target, healthy mammalian cells [24–26]. This potential non-selective toxicity is vividly underscored by the genus's history, as many species contain highly toxic compounds like the guanidine alkaloid, galegine, which is fatal to livestock [**Error! Bookmark not defined.**,27].

Therefore, a purely potent finding in an antimicrobial assay (low Minimum Inhibitory Concentration, MIC) or a cytotoxicity assay (low IC_{50}) is insufficient. Success depends entirely on the compound's ability to preferentially target pathogenic or diseased cells over healthy ones. This necessitates a critical quantitative comparison of these two parameters by calculating the Selectivity Index (SI = IC_{50}/MIC) [28]. This metric is essential for advancing natural product research toward clinical application, providing the necessary assurance that potent compounds are also safe.

Despite compelling ethnobotanical and preliminary pharmacological data, a critical gap exists in the synthesized literature: the absence of a focused, comparative analysis that quantifies the SI for its constituent Sesquiterpene Lactones. This review aims to address this gap by providing a comprehensive, mechanistic, and quantitative analysis of the *Verbesina* genus's potential as a source of anti-resistance therapeutics. We will critically evaluate the literature regarding the cytotoxicity (IC_{50}) and antimicrobial efficacy (MIC) of its specialized metabolites, synthesizing all available *in vitro* data to calculate and interpret the Therapeutic Index for key scaffolds. By focusing on the structural relationships, dual biological activities, and selective toxicity profiles, this review will delineate the most promising scaffolds for further chemical development, ultimately validating the translational potential of this rich Mexican medicinal resource in the fight against AMR (Figure 1).

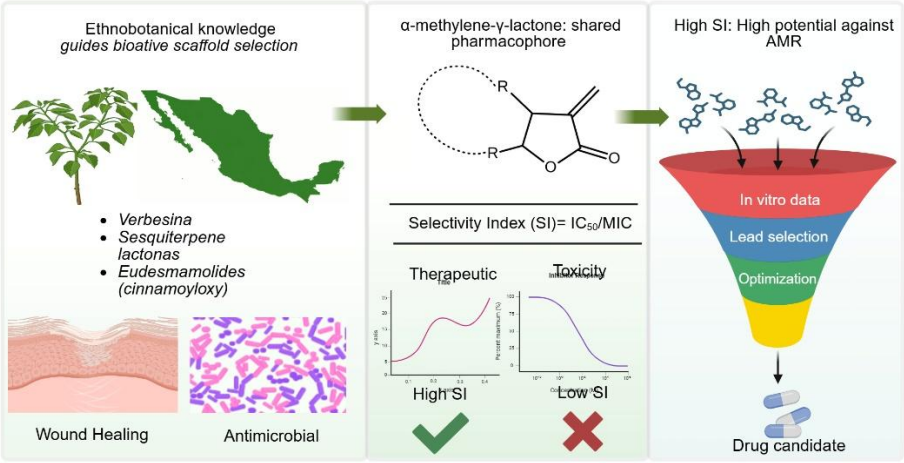

Figure 1. The Duality Challenge and Translational Road Map.

2. Ethnopharmacology and Phytochemical Diversity of the Verbesina Genus

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The rational investigation of the *Verbesina* genus, which remains the largest genus within the Heliantheae tribe, is firmly anchored in its definitive chemotaxonomic and phylogenetic placement. Molecular studies confirm that *Verbesina* belongs to the subtribe Verbesininae, a distinct and ancient lineage nestled within the core Heliantheae clade [**Error! Bookmark not defined.**]. This confirmed taxonomic position refines the focus for all chemical investigation, supporting the established consistency of the genus's potent eudesmane SLs as strong chemotaxonomic markers [**Error! Bookmark not defined.**]. This structural link is so vital that contemporary research has focused on assembling the complete chloroplast genome (*plastome*) for *V. alternifolia*, providing a robust genomic anchor necessary for targeted bioassay-guided isolation and standardization across genetically diverse populations [**Error! Bookmark not defined.**].

2.1. Ethnopharmacology and Ecological Context

2.1.1. Chemotaxonomy and Global Distribution

The foundational relevance of the *Verbesina* genus is established through its chemotaxonomic and phylogenetic placement [**Error! Bookmark not defined.**]. Molecular data firmly place *Verbesina* within a distinct clade, often recognized as the revised subtribe Verbesininae [**Error! Bookmark not defined.**,29], nestled in the larger Heliantheae alliance clade, which accounts for approximately 26% of all species in the *Asteraceae* family [**Error! Bookmark not defined.**]. This refined circumscription, separating *Verbesina* from other common allies, acts as a crucial DNA-based chemotaxonomic anchor that directs and refines future bioassay-guided isolation work [**Error! Bookmark not defined.**]. Geographically, the genus is highly prolific, encompassing over 300 species with the greatest diversity concentrated in Mexico and the southwestern USA [**Error! Bookmark not defined.**]. The widespread and robust distribution of *Verbesina* across central Mexican flora, including regions of high diversity like the Bajío, underscores its pharmacological importance [**Error! Bookmark not defined.**]. Furthermore, Mexico is positioned as an "evolutionarily active zone" for this lineage, characterized by high speciation rates within the *Asteroideae* subfamily, which accelerates the diversification of the genus's potent specialized metabolites [**Error! Bookmark not defined.**]. The specific niche preference, such as *V. virginica* strictly inhabiting areas under the canopy, suggests that the plant's metabolic investment in synthesizing a robust chemical arsenal, including SLs, necessitates a sheltered microclimate to maximize chemical production [30]. The extensive geographical distribution and success are also supported by studies that investigate its cellular mechanics, including cytological analysis of *V. encelioides*, which has shown genetic variability often linked to the plant's aggressive ecological adaptation and the synthesis of secondary metabolites [31].

2.1.2. Mexican Ethnobotany: The Rationale for Anti-AMR

The pharmacological investigation of *Verbesina* is directly guided by its established history in Mexican traditional medicine [**Error! Bookmark not defined.**], which involves a broader cultural reliance on potent, locally sourced medicinal flora throughout the nation [32]. For instance, *V. persicifolia*, or 'Huichim,' is a popular folk remedy confirmed for anti-diabetic activity [33] and is used for ailments including inflammatory, gastrointestinal, and anti-infective complaints like wounds that resist healing [34,35]. Similarly, the endemic *V. crocata* is traditionally applied topically for wounds and burns [**Error! Bookmark not defined.**], and *V. sphaerocephala* is locally applied for treating gangrene and venous ulcers [36]. Scientific reports have successfully confirmed the efficacy for three traditional uses: wound healing, antidiabetic, and diuretic activity [**Error! Bookmark not defined.**]. Most recently, *V. persicifolia* has been validated for applications related to the central nervous system, such as anticonvulsant activity [37], broadening its therapeutic scope beyond anti-infective use. The traditional use of these compounds for infections and inflammation provides the essential ethnopharmacological rationale for investigating its modern anti-AMR properties [**Error! Bookmark not defined.**,38]. Furthermore, the anti-infective potential extends beyond the Mexican context, with

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species like the South American *V. macrophylla* used traditionally for bacterial and fungal infections of the urinary and respiratory tracts [39].

2.1.3. Ecological Resilience and Chemical Defense

The plant's inherent bioactivity is fundamentally linked to its ecological success as an aggressive chemical warrior [40,41]. The high ecological competence of species like *V. encelioides* is evidenced by its recognition as a weed [42], and its aggressive colonization ability is enhanced by strong allelopathic properties [**Error! Bookmark not defined.**]. The aggressive success is intrinsically linked to its ability to adapt to diverse and often disturbed habitats, with species like *V. alternifolia* surviving in fire-affected systems, highlighting the environmental pressures that drive the constant synthesis and selection of potent specialized metabolites [43]. This chemical defense system includes not only defense against herbivory but also compounds vital for pollinator attraction [**Error! Bookmark not defined.**]. The plant's overall resilience is high, yet its physical defenses are limited against certain atmospheric stressors like ambient ozone, underscoring the vital role of specialized secondary metabolites for survival [**Error! Bookmark not defined.**]. Crucially, the potent chemical arsenal of the genus is physically manufactured and housed in specialized defensive structures common across its tribe [44]. Anatomical studies confirm the ubiquitous presence of Linear Glandular Trichomes (LGTs) on the plant surfaces that are metabolically active and primarily accumulate terpenoids and other specialized metabolites [**Error! Bookmark not defined.**]. This rich chemical arsenal extends beyond SLs to include a robust mixture of triterpenoids and phytosterols like β-Amyrin and Lupeol [45], which may possess anti-diabetes and antidyslipidemic properties, suggesting the non-SL fraction provides synergistic or mitigating activities important for the overall therapeutic profile [**Error! Bookmark not defined.**]. Finally, the selection of *Verbesina* for drug discovery is justified by its high degree of genetic and ecological resilience, confirmed by molecular studies showing a significant genetic distance from cultivated *Asteraceae* and the capacity for producing diverse and potent specialized metabolites to survive in competitive environments [46].

2.2. The Core Chemical Landscape: Sesquiterpene Lactones

2.2.1. Sesquiterpene Lactones: Structure, Chemotaxonomy, and Stereochemistry

The chemical identity of the *Verbesina* genus is structurally defined by the Sesquiterpenoid class, which are overwhelmingly the most abundant specialized metabolites, constituting approximately 79% of all Terpenoids reported in the genus [**Error! Bookmark not defined.**]. This rich biosynthetic output is concentrated around the bicyclic C_{15} eudesmane skeleton [**Error! Bookmark not defined.**], a scaffold recognized for its wide range of biological activities, including anti-tumor, antifungal, and antibacterial properties (Figure 2) [10,47]. The historical foundation began with seminal research on the roots of *V. virginica* in 1961, which reported the isolation of α- and β-Verbesinol derivatives [48]. These compounds possessed the C_{15} sesquiterpene framework with a *cis*-decalin (eudesmane) ring system. Crucially, this study determined that the compounds existed naturally as isomeric sesquiterpene esters which, upon degradation, yielded *p*-hydroxycinnamic acid [**Error! Bookmark not defined.**]. This established the existence of the defining cinnamoyloxy eudesmane scaffold, a structure that links a lipophilic terpenoid body to a phenolic acid (the cinnamoyl moiety) confirming the characteristic chemical structure that governs the genus's potent bioactivity [48–50]. This overwhelming structural prevalence means that eudesmane cinnamates alone account for over a quarter (26.5%) of all reported *Verbesina* compounds, firmly validating the structural focus of this review [**Error! Bookmark not defined.**]. The consistency of this chemical signature is reinforced by systematic investigations of South American *Verbesina* species (including *V. glabrata*, *V. macrophylla*, and *V. luetzelburgii*), which confirmed consistent chemical dominance with all species affording eudesmane or germacrane derivatives, nearly all bearing a cinnamate or related ester residue [51]. Further supporting this claim, a phytochemical investigation into the aerial parts of *V. turbacensis* (a

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species of ethnobotanical importance) successfully isolated four eudesmane derivatives (three of which were novel), further reinforcing the ubiquitous nature of this C15 bicyclic scaffold [52].

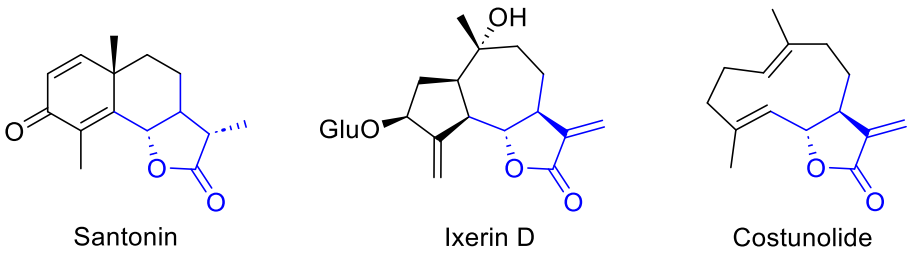

Figure 2. Core SL Skeletons and Pharmacophore.

The early chemical investigation of the genus was often challenged by the complexity and high oxygenation of these terpenoids. For instance, the structural elucidation of Rupestrol from *V. rupestris* (Jamaica) detailed a pentahydroxy sesquiterpene of the eudesmane type, featuring hydroxyl groups at C-4, C-6, and C-14, alongside secondary hydroxyls at C-1 and C-9. Chemical reactivity tests, such as the inertness of Rupestrol cinnamate to periodic acid, were required to definitively eliminate C-3 as a site for a secondary alcohol, supporting the locations at C-1 and C-9 [53]. Further complexity was revealed in *V. rupestris* through the isolation of enantio-eudesmane sesquiterpenes, mirror-image isomers of the common eudesmane structure including Rupestrinol and γ-Chaenocephalol, both isolated as their cinnamate esters. The isolation of these eudesmane sesquiterpenes provided the necessary chemotaxonomic evidence to support the botanical reclassification of *Chaenocephalus rupestris* to *Verbesina rupestris* [54].

Table 1. Structural Diversity of Key Sesquiterpenoids (SLs and Core Analogues) from the Verbesina Genus.

Compound Name (Skeleton)	Structural Descriptor	Source Species	Biological Role
Verbesindiol (Eudesmane - Diol)	Parent core; 4*R*,5*S*,6*R*,7*S*,10*R* configuration; Structural standard.	*V. virginica*, *V. eggersii*	Potent against prostate cancer cells.
CDE (Eudesmane Cinnamate)	4β-cinnamoyloxy, 1β,3α-dihydroxyeudesm-7,8-ene; Alkylating pharmacophore.	*V. persicifolia*	Mild mitochondrial uncoupler. Patented for obesity/diabetes/cancer treatment.
Arbusculin derivative (Eudesmane Lactone)	(1*R*,4*S*,4a*S*,7*R*)-1-methyl-7-(prop-1-en-2-yl)octahydro-2*H*-1,4a-(epoxymethano)naphthalen-4-yl acetate.	*V. lanata*	Against the protozoan *L. major*
Rupestrol (Pentahydroxy Eudesmane)	Highly oxygenated enantio-eudesmane with C-9 oxygenation; isolated as cinnamate esters.	*V. rupestris*	Confirms complex stereochemistry/chemotaxonomic links.
Rupestrinol (Enantio-Eudesmane)	Epimeric sesquiterpene isolated as cinnamate esters.	*V. rupestris*	Part of the complex enantio-eudesmane structural series.
Eudesmane Esters (Eudesmane Cinnamate)	Phenolic acid (cinnamate/coumarate) conjugated eudesmanes.	*V. virginica*	Upholds the chemical signature of SLs conjugated with phenolic acids.
SL Acetylated Derivatives (Eudesmane/ Germacrane)	Compounds with acetate substituents.	*V. persicifolia*	Acetylation shifts cytotoxicity mechanism to Complex II inhibition (Mitochondrial).
Germacrene-Cinnamate	6β-cinnamoyloxy-1β-hydroxy-10α-methoxy-3-oxo-germacra-4,5*Z*-ene.	*V. negrensis*	Antibacterial against *S. aureus*.
Zempoaline C (Elemanolide)	Elemanolide skeleton with fused α,β-unsaturated-γ-lactone moiety.	*V. seattonii*	Deploys alkylating pharmacophore via diverse skeletal type.

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Verocephol (Amorphane Lactol)	Unique γ-lactol; first reported amorphane skeleton.	*V. sphaerocephala*	Marks high biosynthetic diversity (C_{15} precursors).
Cadinenes (Sesquiterpenoid)	New Cadinene-type SLs confirmed by X-ray.	*V. sphaerocephala*	Structural diversity increases selective lead probability.
Parthenin Analog (SL)	SLs induce cytotoxicity via Michael addition to -SH groups.	*(General SL mechanism)*	α-methylene-γ-lactone is the alkylating pharmacophore.
V. macrophylla EO (Sesquiterpenes)	Essential Oil dominated by C_{15} Sesquiterpenes (Germacrene D).	*V. macrophylla*	Antimicrobial/anti-inflammatory action with very low host toxicity
Anthelmintic Action (Sesquiterpenes / Phenolics)	Essential oil shows 100% adulticidal activity against Habronema muscae.	*V. alternifolia*	Causes degenerative changes to parasite morphology (SEM confirmed).

Establishing a reliable Structure-Activity Relationship (SAR) requires an unequivocal definition of the molecule's C_{15} scaffold, particularly its stereochemistry [55]. This was achieved through a series of meticulous structural confirmations. The *p*-coumaryl ester of the new sesquiterpenediol, Verbesindiol, was isolated from the herbaceous parts of *V. virginica*, and alkaline hydrolysis confirmed it was an Eudesmane derivative with hydroxyl groups located at the tertiary C-4 position and a second at C-6. The relative and absolute configuration was definitively established (*4R*, *5S*, *6R*, *7S*, *10R*) using ^{1}H-NMR, ^{13}C-NMR, and Circular Dichroism (CD) spectroscopy [**Error! Bookmark not defined.**]. This structural work culminated in the X-ray crystallographic analysis of the parent diol, which provided the absolute configuration and definitively established the Verbesindiol core as a 4α,6β-dihydroxyeudesmane with a *cis*-fused decalin ring system. This structure, characterized by the specific orientation of the hydroxyls at C-4 and C-6, serves as the absolute standard for correlating cinnamoyloxy eudesmane scaffolds with their observed biological activities [56]. A detailed reinvestigation of *V. eggersii* consistently confirmed the ubiquity of Verbesindiol derivatives across the genus, noting that this species is rich in cinnamoyloxy eudesmane SLs and yielded further derivatives, including 15-hydroxy derivatives, a rearranged eudesmane acid, and a benzofuran derivative, highlighting the propensity of the Asteraceae to produce structurally diverse scaffolds that originate from the same basic terpenoid precursors [57].

The necessity for definitive characterization is further demonstrated by studies on Mexican endemic species. The aerial part of *V. virgata*, for example, afforded two new C-4 cinnamates, including 4β-cinnamoyloxy-1β,2α-dihydroxyeudesm-7-en, whose structure and stereochemistry were unambiguously determined by X-ray diffraction analysis. This analysis confirmed the *cis*-ring junction and the equatorial orientation of the C-1 hydroxyl group. The study also confirmed the presence of vicinal hydroxyl groups through periodate cleavage, demonstrating that these natural molecules possess chemical functionality capable of undergoing specific reactions with biological targets [58]. The ubiquitous presence of these Eudesmane cinnamates is further confirmed by their isolation from *V. sordescens*, which afforded two new eudesmane cinnamates alongside a typical *Verbesina* blend of terpenoids and polyacetylenes [**Error! Bookmark not defined.**]. The ubiquitous nature of the eudesmane skeleton in the genus continues to be proven, as an investigation into *V. virginica* yielded two new cinnamoyl-esterified eudesmane compounds, both featuring the core eudesmane ring system modified by substitution with hydroxyl groups at C-1 and C-4. Crucially, one compound was esterified with *p*-coumaric acid and the other with cinnamic acid, reinforcing the defining chemical characteristic of the genus: SLs conjugated with phenolic acids [59]. The ongoing investigation continues to define the absolute configuration of key eudesmane derivatives from species like *V. turbacensis* using single-crystal X-ray crystallographic analysis, reinforcing the reliance on advanced structural data to reliably map a compound's efficacy to its three-dimensional shape [60].

The importance of stereochemical precision for reliable SAR is highlighted by findings regarding the eudesmanetriol mono-cinnamate isolated from *V. persicifolia*. This compound, identified as 4,10-dimethyl-7-isopropyl-*trans*-decalin-1,3,4-triol-7-ene-4-*trans* cinnamate, revealed a correction to the configuration of its C-4 asymmetric center compared to previous assignments in other *Verbesina*

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species, confirming the *trans*-decalin eudesmane backbone in certain highly active lead molecules [61]. A core example of the characteristic scaffold is 4β-cinnamoyloxy, 1β,3α-dihydroxyeudesm-7,8-ene, isolated from the hexane extract of *V. persicifolia* [**Error! Bookmark not defined.**]. This eudesmane derivative is pivotal because its core scaffold, as shown through studies on the closely related CDE, can exert profound biological effects not solely through direct toxicity but through the modulation of fundamental cellular processes. For instance, the eudesmane skeleton has been shown to act as a natural mild uncoupler in liver mitochondria [62]. This critical evidence underscores the necessity of rigorously quantifying the IC_{50} (cytotoxicity) and MIC (efficacy) of these highly substituted eudesmane derivatives to assess their feasibility as drugs with a safe Therapeutic Index.

2.2.2. Sesquiterpenoid and Monoterpenoid Diversity

While the cinnamoyloxy eudesmane scaffold is the recognized chemotaxonomic marker, the *Verbesina* genus is, at its core, a dedicated biosynthetic factory for C_{15} molecules, exhibiting remarkable structural diversity that extends across multiple sesquiterpenoid skeletal types [63]. This complexity ensures that the core bioactive mechanism, the α-methylene-γ-lactone pharmacophore is deployed via numerous molecular architectures [10,64,65]. The biosynthesis of these diverse SLs often begins with the germacrane skeleton, which is widely considered the common precursor to the complex array of eudesmane derivatives [**Error! Bookmark not defined.**,**Error! Bookmark not defined.**]. Evidence for this pathway includes the co-occurrence of germacrane derivatives alongside eudesmanes across many species [24,51]. A novel, active SL derivative, 6β-cinnamoyloxy-1β-hydroxy-10α-metoxy-3-oxo-germacra-4,5*Z*-ene, was structurally characterized following bioassay-guided isolation from the chloroformic extract of *V. turbacensis* [**Error! Bookmark not defined.**].

This structural variability is not limited to bicyclic and macrocyclic scaffolds. Investigations into *V. occidentalis* revealed the presence of six new sesquiterpenes structurally elucidated as derivatives of muurolene, alongside Verboccidentafuran, a novel trisubstituted furan derivative ($C_{15}H_{20}O$) [66]. Furthermore, the genus's reliance on multiple SL scaffolds includes the Elemanolides, such as Zempoalines C and D, isolated from *V. seattonii*, whose highly characteristic elemane skeleton with a fused α,β-unsaturated-γ-lactone moiety was structurally established via X-ray crystallography [**Error! Bookmark not defined.**]. Most recently, a comprehensive study on *V. sphaerocephala* successfully isolated six cadinene-type SLs, five of which were previously undescribed. The accurate structures of these compounds were elucidated by X-ray analyses, highlighting that the genus biosynthesizes multiple SL scaffolds from common C_{15} precursors, increasing the probability of finding a lead molecule with a distinct and selective mechanism of action [**Error! Bookmark not defined.**]. The genus also biosynthesizes the unique amorphane sesquiterpene γ-lactol, Verocephol, from *V. sphaerocephala*, marking the first reported occurrence of a γ-lactol with an amorphane skeleton [67].

Beyond the non-volatile SLs, the Essential Oil (EO) represents a volatile, lipophilic line of chemical defense [**Error! Bookmark not defined.**] and is overwhelmingly dominated by sesquiterpenes (99.2% total) [68,69]. The EO of *V. macrophylla*, for example, is heavily dominated by the characteristic C_{15} sesquiterpenes Germacrene D (37.3%) and its derivative, Germacrene D-4-ol (17.0%) [23,69]. Similarly, the EO of *V. negrensis* is dominated by α-pinene (43.1%), a known antibacterial monoterpene. This structural partitioning of anti-infective activity into volatile like α-pinene and non-volatile SLs fractions is strategic: the mechanism of action for such monoterpenes typically involves the destruction of the cellular integrity of the microorganism, inhibiting respiration and ion transport, which offers an alternative pharmacological pathway to the Michael addition mechanism characteristic of SLs [70]. The consistent presence of these sesquiterpenes reinforces the centrality of the terpene pathway to the plant's chemical strategy [**Error! Bookmark not defined.**].

This diverse arsenal is synthesized and housed within specialized plant structures. Anatomical analysis of *V. macrophylla* revealed that lipids, terpenes, and alkaloids are physically manufactured and stored in specialized leaf secretory ducts via an elaborate, energy-intensive mechanism known as granulocrine secretion. This elaborate and conserved production mechanism underscores the

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fundamental, evolved importance of these secondary metabolites for the plant's defense system [**Error! Bookmark not defined.**]. The complexity extends to C_{10} monoterpenoids, evidenced by the isolation of the C_{10} alcohol isoepicampherenol and bornyl esters (e.g., (-)-bornyl ferulate and (-)-bornyl *p*-coumarate) from *V. rupestris* [71]. These bornyl hydroxycinnamic esters were also confirmed in *V. turbacensis* [72,73], demonstrating that the plant utilizes multiple chemical classes to exert its powerful pharmacological effects. However, this inherent diversity leads to high chemotypic variability across different populations, with the relative percentages of key sesquiterpenes fluctuating significantly depending on the plant's maturity. This variability complicates standardization efforts, emphasizing the need for robust protocols to ensure the consistency and reproducibility of IC_{50} and MIC data [**Error! Bookmark not defined.**].

2.2.3. Non-SL Chemical Arsenal and Biosynthetic Complexity

The total pharmacological profile of *Verbesina* extracts is a complex outcome of a dense and multi-layered chemical defense system that extends far beyond the dominant SLs [11,74]. Qualitative phytochemical screening confirms the simultaneous presence of nearly all major secondary metabolite classes including Alkaloids, Glycosides, Saponins, Phenols, Tannins, Flavonoids, and Terpenoids, a comprehensive chemical diversity that necessitates a thorough investigation of synergistic effects for accurate biological interpretation [**Error! Bookmark not defined.**].

Guanidine Alkaloids: The Dual Challenge of Toxicity and Therapy:

A critical challenge for advancing *Verbesina* metabolites to clinical application lies in managing the presence of guanidine alkaloids, a class chemically distinct from the dominant SLs [75]. This class presents a unique therapeutic duality. On one hand, toxicological investigations into *V. encelioides* confirmed the presence of the highly potent, non-selective toxin galegine (3-methyl-2-butenyl-guanidine) [**Error! Bookmark not defined.**,76], which is responsible for severe sheep and cattle losses and is a documented hazard for grazing livestock [17,27]. This toxin's acute lethality underscores the non-selective nature of the raw plant's defense system [**Error! Bookmark not defined.**]. On the other hand, the genus yields therapeutically valuable analogs, notably from *V. caracasana*, including the monomeric hypotensive agent caracasanamide and the dimeric, cyclobutane-containing caracasandiamide [77–83]. These specialized guanidino-amide alkaloids represent a significant chemical class for cardiovascular therapy [**Error! Bookmark not defined.**]. The natural co-occurrence of the lethal toxin galegine with chemically related, but non-toxic supportive molecules like *N*-2,3-dihydroxy-3-methylbutyl-acetamide in *V. encelioides* provides strong proof-of-concept that selectively isolating a non-cytotoxic therapeutic agent is chemically feasible, emphasizing the imperative for rigorous bioassay-guided fractionation [**Error! Bookmark not defined.**,84].

Phenolic Compounds, Flavonoids, and Supportive Metabolites:

The extract's capacity for anti-inflammatory and antioxidant activity is substantially defined by a high content of phenolic compounds and flavonoids [18,85]. The overall bioactivity is reinforced by the presence of a diverse class of flavonol diglycosides, including quercetin 3-galactoside-7-glucoside and quercetin 3-xyloside-7-glucoside from *V. encelioides* [86,87], and the novel Rhamnocitrin-3-*O*-glucuronide from *V. myriocephala* [88]. More recent analyses have identified high concentrations of the anti-inflammatory and antioxidant agent Rutin (quercetin-3-*O*-rhamnoglucoside) in *V. sphaerocephala* [**Error! Bookmark not defined.**] and catechin derivatives (catechin-3-glycoside isomers) and phylloflavan in *V. crocata* [**Error! Bookmark not defined.**,**Error! Bookmark not defined.**]. The biological role of these non-SL components is critical, as they include sitosterol derivatives which exhibit anti-inflammatory, antineoplastic, and immune-modulatory activities. This dense profile of phenolic acids such as, *p*-coumaric acid, gallic acid, caffeic acid, etc. [**Error! Bookmark not defined.**,**Error! Bookmark not defined.**] is directly linked to the allelopathic activity of the genus; HPLC analysis of *V. encelioides* root leachate confirmed that phenolic compounds are functionally involved in the chemical interference with competing flora [**Error! Bookmark not defined.**].

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Furthermore, early phytochemical work confirmed the presence of highly reactive phenolic conjugates, including caffeic acid ethyl ester (a compound previously unknown as a natural product) and caffeic acid borneol ester in the aerial parts of species like *V. angustifolia* and *V. greenmannii* [89].

The extensive chemical profile is confirmed by comparative spectroscopic analyses, such as Raman spectroscopy on wild species like *V. virginica*, which exhibits a distinctly measurable spectrum based on marker bands assigned to scaffold molecules such as phenylpropanoids and carotenoids, supporting the fundamental premise that these specialized secondary metabolites produce unique chemical fingerprints that correlate with the diverse biological activities reported [90]. However, while the genus produces an array of defensive compounds, physiological studies reveal intrinsic limitations in their generalized antioxidant capacity (AA) against environmental stressors. In *V. occidentalis* (crown-beard), leaf AA content is relatively low (2-4 μmol/g fresh weight) compared to other resilient species. Critically, as the season progresses, *V. occidentalis* exhibits a diminished capacity to convert oxidized DHA back to active AA (reduced redox state), suggesting a vulnerability in its natural defense cycle against sustained oxidative stress. These intrinsic physiological limitations highlight the necessity for the plant to employ potent, externally acting, high-efficacy compounds, such as SLs, for sustained protection against biotic and abiotic challenges [91].

Triterpenoids, Alkaloids, and Specialized Defense Structures:

The genus exhibits further remarkable biosynthetic capacity through the production of Triterpenoid Saponins [92]. A comprehensive investigation of *V. virginica* yielded six new compounds (Verbesinosides derivatives) possessing a novel 15,27-cyclooleanane-type triterpenoid skeleton [93], distinct from the oleanolic acid derivative Copteroside E isolated from *V. suncho* [94]. The presence of these saponins, along with other non-SL triterpenoids such as friedelin, epifriedelin, α- and β-amyrin, and lupeol in *V. encelioides* [11,45,95], is vital as they contribute to the extract's complexity, with saponins potentially influencing membrane permeability and thereby enhancing the cellular penetration of other lipophilic compounds [**Error! Bookmark not defined.**].

This complex defensive system also includes a diverse range of Acetylenic compounds and their cyclized sulfur derivatives, the thiophenes, which are recognized as chemotaxonomic markers of the Heliantheae tribe [96]. The investigation of Mexican *Verbesina* species confirms the genus's rich, yet structurally diverse, chemical composition, reinforcing the structural complexity of these specialized compounds. The roots of *V. angustifolia* contained the linear C_{13} polyacetylene pentainene, along with the diterpene acids. Similarly, the roots of *V. oncophora* yielded polyacetylene pentainene and a diterpene alcohol [**Error! Bookmark not defined.**]. A reinvestigation of the roots of *V. virginica* (collected in Louisiana) further yielded the ubiquitous polyacetylene pentainene and caryophyllene along with eudesmane derivatives [97]. Thiophenes, such as C_{13}-Monothiophenes and C_{13}-Dithio compounds, are known for their strong biological activity due to their ROS-generating photoactivation potential [**Error! Bookmark not defined.**]. The complete profile even extends to specialized heterocycles, evidenced by the isolation of the only known 2-isopropyliden-2*H*-benzofuran-3-one from *V. luetzelburgii* [98].

Non-Traditional Bioactive Classes and Technological Application

Recent analytical studies have significantly broadened the understanding of the genus's therapeutic potential by identifying non-traditional bioactive classes. The neuroprotective activity of the *V. encelioides* flower extract, for example, is chemically linked to carotenoids, such as Spirilloxanthin (12.38-13.24% of the total composition), and the diterpene lactone Ginkgolide A. These compounds demonstrate efficacy by binding to the enzyme Acetylcholinesterase (AChE) [**Error! Bookmark not defined.**]. Furthermore, the genus's defensive strategy employs structural macromolecules, with biochemical analysis of *V. encelioides* identifying a low molecular weight 14 kDa protein characterized as a host defense compound exhibiting moderate antimicrobial activity [99]. The roots of *V. encelioides* also contain fundamental biomolecules that contribute to the extract's

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complexity, including the amino acids nor-leucine and ornithine and the carbohydrates D-ribose and sucrose [11,100].

The plant's rich organic matter and protein profile is fundamentally linked to its bioactivity as a growth-promoting component [**Error! Bookmark not defined.**]. More fundamentally, the high performance is linked to the genus' known content of organic matter, carbohydrates, and proteins, suggesting they provide essential growth-promoting components which complement the high-potency, often toxic, defensive nature of the Sesquiterpene Lactones. The bioactivity of the extracts is attributed to their complex profile of organic compounds and proteins, enabling enzymatic activation for nutrient assimilation. Although key minerals like Nitrate and Potassium were not detected in the aqueous extracts, the presence of other nutrients like Ammonia, Ca, Mg, and Phosphate was confirmed [**Error! Bookmark not defined.**]. This comprehensive chemical complexity is now leveraged for nanotechnology; the hot water extract of *V. encelioides* and the aqueous extract of *V. crocata* are successfully used for the "green synthesis" of Silver Nanoparticles (AgNPs), where the compounds facilitate the reduction and chelation/stabilization of the resulting nanoparticles through redox processes [101,102].

3. Cytotoxic Activity of Verbesina Metabolites: Focus on IC_{50} Profiles

The inherent chemical reactivity of SLs, driven by the α-methyleneγ-lactone moiety, ensures their broad-spectrum biological activity, encompassing both antimicrobial and antiproliferative effects. While this potency is desirable, the historical lack of quantitative cytotoxic investigation has often relegated the genus to a preliminary research stage [**Error! Bookmark not defined.**]. Early studies noted that out of over thirty-eight phytochemically characterized *Verbesina* species, only four had been biologically investigated, with limited IC_{50} data available [**Error! Bookmark not defined.**]. Consequently, the primary objective of this section is to compile and analyze the recently available quantitative cytotoxicity data, providing a contemporary and rigorous assessment of the genus's true antiproliferative potential against cancerous and non-cancerous models, thereby establishing the crucial host-toxicity component for the final Selectivity Index (SI) calculation.

3.1. Anticancer Potential Across Cell Lines

The cytotoxic potential of *Verbesina* compounds is confirmed by consistent activity across both crude extracts and purified isolates, validating the eudesmane scaffold as an inherently bioactive template. Methanolic extracts of *V. encelioides* demonstrated significant and quantifiable cytotoxic activity against diverse human cancer models, displaying highly consistent potency across three tumor cell lines after 48 hours of exposure. The extract achieved half-maximal growth inhibition (GI_{50}) values of 26.32 ± 2.42 µg/L against MCF-7 (Breast Adenocarcinoma), 28.65 ± 2.89 µg/L against NCI-H460 (Non-Small Lung Cancer), and 26.82 ± 8.54 µg/L against SF-268 (CNS Cancer). This consistency across disparate cancer origins suggests a broad-acting, fundamental cytotoxic mechanism, which is a hallmark of the reactive SLs that define the genus's chemical profile [**Error! Bookmark not defined.**].

However, the efficacy is strongly dependent on the extraction method used. Comparative studies using both ethanolic (VE) and aqueous (VA) crude extracts of *V. encelioides* against the human colon cancer cell line HCT-116 revealed a stark difference in potency. The ethanolic extract (VE) exhibited significantly higher potency with an IC_{50} of 310 µg/mL (0.31 mg/mL), which was twice as potent as the aqueous extract (VA) at 650 µg/mL (0.65 mg/mL). This difference confirms the strategic necessity of concentrating the lipophilic compounds, primarily Sesquiterpene Lactones and triterpenoids, through non-polar extraction methods to maximize the yield of active lead molecules [**Error! Bookmark not defined.**].

The intrinsic activity of the core eudesmane scaffold itself, even before cinnamoyl esterification, is confirmed by low micromolar cytotoxicity. The key sesquiterpenediol, Verbesindiol (4α, 6β-dihydroxyeudesmane), isolated from *V. virginica*, demonstrated potent activity against advanced, castration-resistant prostate cancer cell lines. Specifically, Verbesindiol achieved an IC_{50} of 4.0 ± 0.6 µM against PC-3 (Androgen-independent) and 5.2 ± 0.6 µM against DU 145 (Androgen-independent)

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cells. This demonstrates that the parent eudesmane skeleton is inherently cytotoxic, positioning Verbesinol derivatives as excellent starting points for SAR investigation aimed at optimizing selectivity [103].

Crucially, isolated SLs, the primary focus of this review, also demonstrate high potency. The core eudesmane scaffold, 4β-cinnamoyloxy,1β,3α-dihydroxyeudesm-7,8-ene, exhibited potent antiproliferative activity (GI_{50}) against a broad panel of human tumor cell lines, with values ranging from 14.7 μM to 32.3 μM [**Error! Bookmark not defined.**]. This finding is complemented by related research on *V. persicifolia*, which isolated an eudesmane triol mono-cinnamate reported to display promising anti-inflammatory, anti-hypoglycemic, and powerful peroxynitrite scavenging activities, along with measurable cytotoxicity against different tumor cell lines. This array of activity across multiple compounds in *V. persicifolia* directly establishes it as a major source of selectively toxic scaffolds [**Error! Bookmark not defined.**]. This translational potential is further substantiated by a Mexican patent detailing the isolation of the eudesmane 4CDE (a core scaffold), which is claimed for the treatment of diabetes mellitus type 2, cancer, obesity, and as an anti-inflammatory, directly validating the high-value, multifaceted application of this genus [104].

However, not all crude extracts exhibit high cytotoxicity. The hydroethanolic extract from the stem of *V. turbacensis* (a species traditionally used for inflammation) demonstrated a low cytotoxic effect against the human hepatoma cell line HepG2. After 24 hours of exposure, the extract showed minimal toxicity ($IC_{50} > 500$ μg/mL), and even after 48 hours, the IC_{50} remained relatively low at 334.6 μg/mL. This low-to-moderate cytotoxicity reinforces the argument that the high-potency effects of SLs are often diluted in crude hydroethanolic extracts, necessitating chemical purification to isolate the potent molecules [**Error! Bookmark not defined.**].

Finally, the potential for cellular specificity in the crude extracts is an important consideration for SI optimization. The alcoholic extract of *V. encelioides* exhibited a differential cytotoxic response across human cancer cell lines. While the extract was strongly cytotoxic to HepG2 cells, reducing viability to 53% at 1000 μg/mL, it proved non-cytotoxic to A-549 (human lung cancer) cells, even at the highest concentrations tested. This inherent degree of cellular specificity suggests that isolating key compounds may yield a selective drug candidate with a favorable SI [105].

3.2. Molecular Mechanisms of Cytotoxicity

The potent IC_{50} profiles observed in *Verbesina* metabolites are fundamentally linked to the inherent, non-selective chemical reactivity of the SLs, which is primarily executed through mitochondria-mediated apoptosis induced by the generation of Reactive Oxygen Species (ROS) [**Error! Bookmark not defined.**,**Error! Bookmark not defined.**]. This mechanism is consistent with the predicted alkylating action of the α-methylene-γ-lactone pharmacophore against nucleophilic sites on cellular proteins and peptides (Figure 3) [**Error! Bookmark not defined.**].

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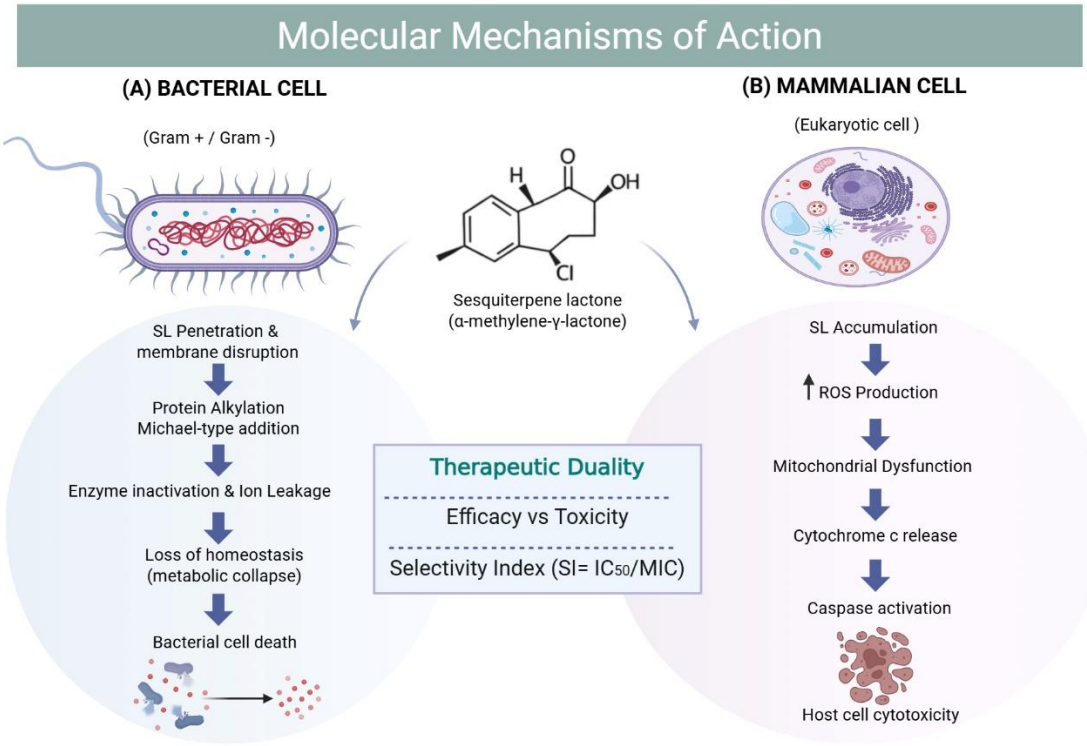

Figure 3. Dual molecular mechanisms of action of Verbesina sesquiterpene lactones. The α-methylene-γ-lactone pharmacophore mediates distinct biological effects depending on the cellular target. (A) Bacterial cell: sesquiterpene lactones penetrate and disrupt the bacterial membrane, alkylate thiol-containing proteins via Michael-type addition, cause enzyme inactivation and ion leakage, and ultimately lead to loss of metabolic homeostasis and bacterial cell death. (B) Mammalian cell: intracellular accumulation of sesquiterpene lactones promotes reactive oxygen species (ROS) overproduction, mitochondrial dysfunction, cytochrome c release, caspase activation, and apoptosis, resulting in host cell cytotoxicity. This mechanistic asymmetry underlies the therapeutic duality of these compounds and highlights the importance of the selectivity index (SI = IC_{50}/MIC) for translational feasibility.Principio del formularioFinal del formulario.

3.2.1. Oxidative Stress and the Apoptotic Cascade

Mechanistic studies using the ethanolic extract (VE) of *V. encelioides* against the HCT-116 colon cancer cell line definitively established the full cascade of cell death. The initial event involves oxidative stress induction; exposure to the VE significantly increased the level of Lipid Peroxidation (LPO) (up to 74%) while simultaneously causing a substantial Glutathione (GSH) depletion (up to 46%), strongly indicating a pro-oxidant mechanism. This chemical stress directly leads to a dose-dependent surge in intracellular ROS production, which subsequently disrupts the integrity of the mitochondrial membrane. This disruption results in a significant loss of Mitochondrial Membrane Potential (MMP) (up to 54% decline), leading to mitochondrial damage [**Error! Bookmark not defined.**].

The cytotoxic mechanism is further confirmed at the molecular level. The disruption of the MMP triggers a classic apoptotic cascade, characterized by the significant upregulation of pro-apoptotic genes. Specifically, the extract significantly upregulated the expression of Bax (by 2.1-fold), p53 (by 2.9-fold), caspase-3 (by 2.2-fold), and caspase-9 (by 2.5-fold), while simultaneously downregulating the anti-apoptotic gene Bcl-2 (by 0.55-fold). The resulting activation of caspases confirms that programmed cell death is the primary mode of action [**Error! Bookmark not defined.**]. This mechanism is consistent with separate studies on the alcoholic extract of *V. encelioides* against HepG2 cells, which also found that the primary cause of cell death was oxidative stress, resulting in mitochondrial damage and genotoxic potential evidenced by extensive DNA damage quantified by the Comet Assay. This DNA damage likely triggers the observed G_2/M cell cycle arrest (up to ~50%

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at 1000 μg/mL), leading to cell death upon repair failure ([**Error! Bookmark not defined.**]. Importantly, this mechanism appears to be non-folate related, as the *V. encelioides* methanol extract displayed no inhibitory effect on DHFR enzyme activity, supporting the hypothesis that the cytotoxic action proceeds via non-enzymatic Michael addition rather than competitive enzyme antagonism.

3.2.2. Scaffold Dependency and Mitochondrial Targeting

Mechanistic studies focusing on isolated pure eudesmane scaffolds confirm that the cytotoxic mechanism is definitively mapped to the mitochondria, but the mode of action is structure-specific, a crucial finding for SAR and SI optimization. One identified mechanism involves mild uncoupling of the respiratory chain. Studies on the pure eudesmane CDE (4β-cinnamoyloxy,1β, 3α-dihydroxyeudesm-7,8-ene), a characteristic scaffold of the genus, revealed that it acts as a natural mild uncoupler in rat liver mitochondria (RLM). This effect involves CDE inducing a drop in the mitochondrial membrane potential ($\Delta\Psi$) and causing a bioenergetic collapse. Mechanistically, CDE is proposed to interact with the respiratory chain, particularly at the level of cytochrome C oxidase (COX), leading to the generation of H_2O_2 and subsequent ROS production. This critical finding confirms that the cytotoxic or anti-proliferative action of *Verbesina* SLs is not confined to simple alkylation but includes complex regulation of cellular energy metabolism, a crucial pathway in both cancer and microbial persistence [**Error! Bookmark not defined.**].

A second, more nuanced mechanism was revealed by the comprehensive mechanistic investigation of 4β-cinnamoyloxy,1β,3α-dihydroxyeudesm-7,8-ene and its derivatives (**Compound 1**), which differentiated the mode of action based on subtle functionalization of the eudesmane skeleton. This work identified two distinct pathways for apoptosis. The first pathway involves Mitochondrial Permeability Transition (MPT) induction: the native hydroxyl groups on the eudesmane moiety (found in the lead **Compound 1** and the hydrogenated **Compound 3**) were found to be crucial for inducing Ca^{2+}-dependent MPT, which leads to mitochondrial swelling and the release of pro-apoptotic factors (Cyt C and AIF). The second pathway involves Respiratory Chain Inhibition: conversely, when the hydroxyl groups were masked by acetate substituents (in the diacetate **Compound 2** and hydrogenated diacetate **Compound 4**), the mechanism shifted dramatically. These acetylated compounds caused injury by directly inhibiting Succinate dehydrogenase of the mitochondrial respiratory chain and inducing a general uncoupling effect on oxidative phosphorylation (Figure 4). This complex mechanistic picture demonstrates that subtle structural modifications on the eudesmane skeleton drastically alter the cellular target, which is a key consideration for chemically optimizing the SI [**Error! Bookmark not defined.**].

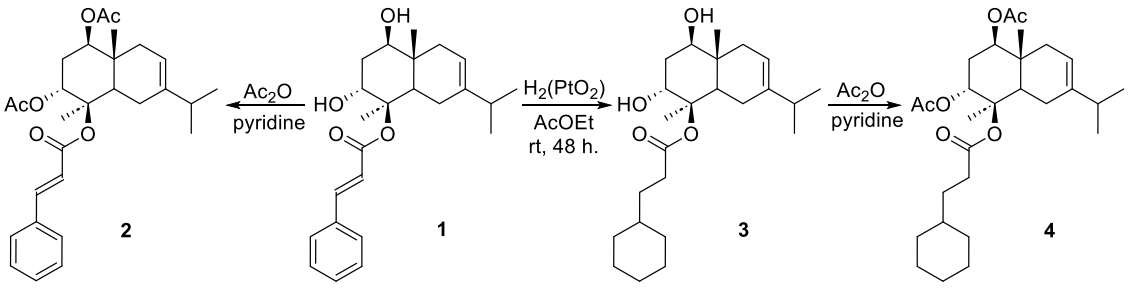

Figure 4. Synthesis of 4β-cinnamoyloxy,1β,3α-dihydroxyeudesm-7,8-ene derivatives **2–4**.

3.2.3. Long-Term Cytotoxic Profile

Understanding the long-term cytotoxic profile of these scaffolds is essential for translating acute IC_{50} data into a true therapeutic advantage. Further mechanistic studies on Verbesindiol, the non-esterified core scaffold, revealed a complex profile. While the compound demonstrated high acute cytotoxicity (84-97% cell death) in the SRB assay, suggesting excellent initial anti-cancer potency, clonogenic assays (which measure the ability of cancer cells to form colonies after drug washout)

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showed that compound 6 had no detectable effects on colony-forming ability. This surprising result suggests that the initial cytotoxic mechanism of Verbesindiol does not prevent the long-term regenerative capacity of the cells, differentiating its mode of action from other *Verbesina* metabolites that do cause G_2/M arrest and inhibit colony formation. This complexity emphasizes that achieving a true therapeutic advantage relies not just on acute IC_{50} values, but on understanding the compound's selective long-term mechanism of action [**Error! Bookmark not defined.**].

3.3. Toxicity to Non-Cancerous Cells

The determination of a favorable SI is the central translational challenge for *Verbesina* metabolites, a necessity rooted in the genus's long-standing documentation as a poisonous plant [8,27]. This history provides the historical imperative for rigorous toxicity screening (IC_{50}/CC_{50}) in modern pharmacology [**Error! Bookmark not defined.**]. The non-selective and potentially fatal nature of the crude plant material is demonstrated by the potent guanidine alkaloid, galegine (3-methyl-2-butenyl-guanidine), which is classified as a documented hazard for grazing livestock in the United States. Exposure to galegine causes severe pathological effects and death in sheep, with observed symptoms consistent with congestion of the lungs and fluid in the chest cavity [11,27]. The high cytotoxic activity of this known poison is confirmed by laboratory testing, where isolated Galegine was found to be highly potent against the HepG2 (Hepatocellular Carcinoma) cell line, exhibiting an IC_{50} value of 10.9 μg/mL [**Error! Bookmark not defined.**]. This high degree of cytotoxicity for a known poison further underscores the absolute necessity of rigorous bioassay-guided isolation and chemical optimization to establish a sufficient SI for any therapeutic lead compound [**Error! Bookmark not defined.**]. The historical toxicological findings also revealed a crucial co-occurrence of these non-selective toxins with chemically related, but experimentally verified non-toxic supportive molecules, such as the *N*-2,3-dihydroxy-3-methylbutyl-acetamide isolated alongside galegine in *V. encelioides*. This functional differentiation provides crucial proof-of-concept that selectively isolating a non-cytotoxic therapeutic agent from the complex crude mixture is chemically feasible [**Error! Bookmark not defined.**].

The core challenge remains the lack of specificity inherent to the reactive SLs. Quantitative data on the core eudesmane scaffold, **Compound 1** (4β-cinnamoyloxy,1β,3α-dihydroxyeudesm-7,8-ene) (Figure 4), demonstrated a pronounced lack of specificity toward tumor cell lines and the non-tumorigenic human mesothelial cells (MeT-5A). The GI_{50} value for **Compound 1** on the non-tumorigenic MeT-5A cells (32.3 μM) was found to be directly within the range of its toxicity against tumor cells (14.7 μM against A431). This direct demonstration of non-selective cytotoxicity underscores the central challenge of the entire review: the necessity of maximizing the efficacy (MIC) while minimizing the host toxicity (IC_{50} to healthy cells) to achieve a favorable SI **Error! Bookmark not defined.**]. The necessity for further toxicity evaluation remains a critical concern for the translational use of all *Verbesina* extracts; while some aqueous extracts of *V. crocata* demonstrated diuretic activity without obvious signs of toxicity in rats, the probable presence of the potent toxin galegine makes the overall toxicity of the plant a primary barrier to advancing novel scaffolds [**Error! Bookmark not defined.**]. Furthermore, the strong allelopathic history of the genus provides a clear historical and contemporary imperative for rigorous toxicity screening [**Error! Bookmark not defined.**].

Despite these concerns, quantitative data strongly supports the hypothesis that selective separation of the therapeutic scaffold can yield low-toxicity agents. An investigation into the antiprotozoal activity of *V. encelioides* isolated six major constituents, including the triterpenoids pseudotaraxasterol and its derivatives, and β-sitosterol glycosides. When tested against human embryonic lung fibroblasts (MRC-5), a standard non-cancerous cell line, all isolated compounds were determined to be non-cytotoxic with high values (CC_{50} > 64 μg/mL). This result contrasts sharply with the activity of many conventional chemotherapy agents and provides crucial evidence of the genus's potential for a favorable SI [106]. This finding is supported by the study of the crude alcoholic extract of *V. encelioides*, which showed a strong cytotoxic response against HepG2 and MCF-7 cancer cells

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but was non-cytotoxic to A-549 (human lung cancer) cells. This differential response to specific cell lines suggests an inherent degree of cellular specificity, indicating that isolating key compounds may yield a selective drug candidate [**Error! Bookmark not defined.**].

Furthermore, the toxicity of the EO fraction, which is dominated by sesquiterpene hydrocarbons, also demonstrates a favorable safety profile. The acute toxicity of the *V. macrophylla* EO was evaluated in mice, with the median lethal dose (LD_{50}) estimated to be greater than 5000 mg/kg (p.o.). The oil exhibited a low rate of hemolysis in human red blood cells (hRBCs), with the highest rate being only 2.14 ± 0.10% at 5000 μg/mL. A hemolysis rate of less than 5% is considered a favorable safety indicator for drug application, suggesting that the EO compounds, despite being lipophilic, interact minimally with the human cellular membrane [**Error! Bookmark not defined.**]. The necessity of balancing efficacy with host safety is further reinforced by reports that anthelmintic activity has been linked to high levels of the toxic guanidine alkaloid, galegine, and nitrates in *Verbesina* alcoholic extracts, reinforcing the core challenge for developing a safe therapeutic agent: maximizing efficacy while rigorously ensuring the selective isolation of compounds away from known toxins like galegine [107].

4. Antimicrobial Efficacy and Anti-AMR Mechanisms

The established chemical arsenal of the *Verbesina* genus, dominated by SLs, C_{13} polyacetylenes, and volatile terpenes, translates directly into a potent, broad-spectrum defense system that provides activity against bacterial, fungal, protozoal, and parasitic pathogens. This inherent chemical capability supports the ethnopharmacological use of *Verbesina* species in treating infections and wounds. The rigorous quantification of this efficacy, often expressed as the MIC, is the critical second metric required to calculate the SI and validate the translational potential of its specialized metabolites [**Error! Bookmark not defined.**].

4.1. Activity Against Clinically Relevant Pathogens

The anti-infective capacity of *Verbesina* metabolites is confirmed across numerous pathogenic kingdoms, ranging from human pathogens to agricultural pests, underscoring the broad-spectrum nature of the plant's chemical defense. Even prior to chemical enhancement, crude extracts of *V. encelioides* demonstrated reliable anti-infective activity against a range of pathogens, validating their initial use in drug discovery screens. For instance, crude methanolic extracts of *V. encelioides* exhibited effectiveness against the bacteria *Escherichia coli* and *Vibrio cholerae*, as well as the fungi *Aspergillus flavus* and *Aspergillus niger*. This robust baseline activity suggests that the chemical potency of the genus's natural metabolites alone is sufficient to warrant further investigation and optimization [108].

Furthermore, the potent anti-fungal properties are highlighted in a process patent that claims the use of *V. encelioides* extract for treating dermatophyte infections. The extracts demonstrated anti-mycotic activity against clinically relevant fungi, including *Microsporum canis*, *Trichophyton Rubrum*, and *Malassezia furfur*, providing quantitative in vitro validation of the genus's broad-spectrum anti-infective capacity [109]. The total hydroalcoholic extract and its resulting fractions possess significant and varied antimicrobial properties, demonstrating high activity against Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and fungi (*Aspergillus fumigatus* and *Candida albicans*), while showing moderate activity against the Gram-negative bacterium *Escherichia coli*. Notably, the total hydroalcoholic extract exhibited antifungal activity against *C. albicans* superior to the reference drug Amphotericin B in the diffusion agar technique. The ethyl acetate fraction generally maintained higher activity than the petroleum ether fraction in the antimicrobial assays, suggesting the concentration of moderately polar, active principles [**Error! Bookmark not defined.**].

The efficacy of isolated SLs, the focus of this review, is measured in the low micromolar range. The pure isolated germacrene SL exhibited measurable antibacterial activity against two clinically relevant Gram-positive pathogens, with the MIC determined to be 64 μg/mL against both *Staphylococcus aureus* (ATCC 29213) and *Enterococcus faecalis* (ATCC 29212) [**Error! Bookmark not defined.**]. SLs isolated from *Verbesina* species also demonstrate significant activity against fungi and oomycetes, showcasing a broad-spectrum anti-infective capacity. In the study of *V. lanata*, the crude

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ethyl acetate extract showed a MIC_{100} of 35 µg/mL against *Plasmopara viticola* (grapevine downy mildew). Importantly, when tested as pure compounds, several isolated eudesmane SLs showed MIC_{100} values below that of the crude extract, confirming they are the primary active principles. The high potency of these pure SLs is critical, as exemplified by compounds such as 6β-cinnamoyloxy-3β,4α-dihydroxyeudesmane, which achieved an MIC_{100} of 3.9 µg/mL against *P. viticola*, and 6β-cinnamoyloxy-1β-hydroxyeudesm-4-en-3-one, with an MIC_{100} of 5.0 µg/mL. This potency, up to 7-fold greater than the crude extract, highlights the necessity of purification for maximizing efficacy and accurately calculating the Therapeutic Index. The eudesmane sesquiterpenes were further confirmed as the primary active constituents responsible for significant antifungal activity against *P. viticola*, with five compounds achieving MIC_{100} values less than 10 µg/mL [**Error! Bookmark not defined.**].

The EO fraction, which contains volatile terpenes, also demonstrates significant, quantifiable inhibitory effects. The EO from *V. negrensis* confirmed efficacy against common human pathogens, achieving an MIC of 500 µL/mL against *Staphylococcus aureus* (ATCC 25923) and 350 µL/mL against *Enterococcus faecalis* (ATCC 29212). While this EO showed no activity against Gram-negative bacteria such as *E. coli* or *Klebsiella pneumoniae* at the tested concentrations, the confirmed efficacy against Gram-positive pathogens is consistent with broader *Asteraceae* literature [**Error! Bookmark not defined.**]. The essential oil of *V. macrophylla* showed both antibacterial and antifungal activity against strains causing respiratory and urinary tract infections, with MIC values varying from 8 to 512 µg/mL for bacteria and 4 to 32 µg/mL for *C. albicans*. Notably, the EO's activity against *K. pneumoniae* (MIC 16 µg/mL) was observed to be superior to the standard antibiotic Amikacin (MIC 32 µg/mL). Furthermore, it achieved potent antifungal activity against *Candida albicans* (MIC 4 µg/mL) [**Error! Bookmark not defined.**].

Beyond bacteria, *Verbesina* compounds demonstrate broad-spectrum activity against protozoal and parasitic agents [**Error! Bookmark not defined.**]. Studies on the Argentinean species *V. subcordata* showed significant trypanocidal activity against *Trypanosoma cruzi* (the causative agent of Chagas' disease), with the non-polar dichloromethane extract achieving 97.7 ± 0.2% growth inhibition at 100 µg/mL, suggesting that the lipophilic SLs and terpenoids are responsible for the parasiticidal effect [**Error! Bookmark not defined.**]. Expanding on this, aqueous extracts of the native Brazilian species *V. macrophylla* exhibit potent activity against the same protozoan, eliminating 100% of the epimastigote form of *T. cruzi* after treatment with a moderate concentration of 200 µg/mL. The inflorescence extract also demonstrated significant fungicidal efficacy, inhibiting the growth of *C. tropicalis* by 41% [110]. Activity against other Neglected Tropical Diseases (NTDs) is also moderate; for instance, benzyl 2,6-dimethoxy benzoate from *V. encelioides* showed an IC_{50} of 38.1 µg/mL against *Trypanosoma brucei* [**Error! Bookmark not defined.**]. The genus also provides strong efficacy in agricultural biocontrol, demonstrating strong nematicidal activity against the economically destructive root-knot nematode, *Meloidogyne javanica*. The most potent activity was found in the aqueous extract of fresh flowers of *V. encelioides*, and incorporating the plant material into nematode-infested soil significantly reduced the number of viable juvenile nematodes [11,111].

Finally, non-small molecule defense components confirm that multiple fractions of *Verbesina* exhibit broad-spectrum activity. An isolated 14 kDa protein from *V. encelioides* validated the susceptibility of multiple pathogens through zone of inhibition assays, confirming activity across Gram-negative bacteria (*E. coli*, *P. aeruginosa*), Gram-positive bacteria (*S. aureus*, *E. faecalis*), and Fungal Pathogens (*A. flavus*, *F. oxysporum*) [**Error! Bookmark not defined.**]. The extracts also demonstrate potent antifungal activity against the problematic necrotrophic fungus *Botrytis cinerea*, with the aqueous stem extract exhibiting the highest antifungal activity (IC_{50} of 0.10 mg/mL) [**Error! Bookmark not defined.**].

Furthermore, several Verbesina species demonstrate significant in vitro activity against both Gram-positive and Gram-negative bacteria, directly supporting their potential as anti-infective agents. Methanolic extracts of *V. sphaerocephala* exhibited remarkable antibacterial efficacy, demonstrating growth inhibition that was statistically non-significant from the ampicillin positive

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control (10 mg/mL) in several tests. Against *E. coli* (Gram-negative), the leaf extract achieved 95.00 ± 5.00% growth inhibition, and against *S. aureus* (Gram-positive), the leaf extract showed 90.00 ± 5.00% growth inhibition [**Error! Bookmark not defined.**]. Interestingly, the study suggested that the antimicrobial effect was negatively correlated with the total phenolic and flavonoid content, implying that less abundant, possibly more potent, lipophilic compounds, such as the characteristic Sesquiterpene Lactones may be the primary drivers of the antibacterial action, or that a synergistic effect among the diverse metabolites is present.

The efficacy of this wide-ranging arsenal is reinforced by the conclusion that the plant's overall anti-infective potency relies heavily on a synergistic effect between its many constituents, including SLs, triterpenoids, and flavonoids [**Error! Bookmark not defined.**]. Furthermore, the widespread crisis of anthelmintic resistance necessitates alternative control strategies, for which *Verbesina* extracts provide a promising phytomedicine source. The anthelmintic activity in the Egyptian context has previously been linked to high levels of the toxic guanidine alkaloid, galegine, and nitrates in *Verbesina* alcoholic extracts. This reinforces the core challenge for developing a safe therapeutic agent: maximizing anti-parasitic efficacy while rigorously ensuring the selective isolation of compounds away from known toxins like galegine [**Error! Bookmark not defined.**].

4.2. Molecular Mechanisms of Antimicrobial Action

The anti-infective efficacy observed in *Verbesina* extracts is the result of a multi-layered defense strategy, employing distinct mechanisms to disrupt fundamental processes in diverse microbial kingdoms[1]. While the cytotoxic mechanism of SLs is primarily through Michael addition to nucleophilic proteins, the genus deploys non-SL metabolites that operate through separate cytotoxic pathways, often resulting in complex synergistic interactions.

4.2.1. The Core Mechanistic Hypothesis: Alkylation and Membrane Disruption

The primary hypothesis for SL action in microbial systems mirrors that in mammalian cytotoxicity: the highly reactive α-methylene-γ-lactone moiety acts as a non-specific alkylating agent. This mechanism, which involves non-enzymatic Michael addition to nucleophilic sites on biological proteins, distinguishes the SLs from many conventional antibiotics that target specific enzymes like DHFR (dihydrofolate reductase). Studies have demonstrated that the *V. encelioides* methanol extract displayed no inhibitory effect on DHFR enzyme activity despite robust cytotoxic efficacy, strongly supporting this non-enzymatic mechanism [**Error! Bookmark not defined.**].

This alkylation mechanism leads to significant structural damage to the target cells. For instance, ultrastructural analysis of *T. cruzi* epimastigotes exposed to *V. macrophylla* extract revealed extensive damage, including cytoplasmic and nuclear disorganization, vacuolization, and swelling of reservosomes. This disruption, confirmed via Sytox Green fluorescence, indicates a compromised plasma membrane. The mode of action, disrupting the crucial cell membrane barrier reinforces the importance of identifying compounds that bypass conventional resistance pathways. Researchers strongly suggest that the sesquiterpene lactones present in *V. macrophylla* contribute to this membrane-disrupting and *T. cruzi* activity, further establishing the SL class as the driver of anti-infective effects in the genus [**Error! Bookmark not defined.**].

4.2.2. Specialized Mechanisms of Non-SL Metabolites

The broad spectrum of activity observed in crude extracts (cytotoxicity, anthelmintic, and anti-fungal) relies heavily on the synergistic interaction of diverse metabolites, each acting via a different, specialized mechanism [**Error! Bookmark not defined.**]. This co-occurrence of various chemical classes suggests a multi-layered defense and multiple modes of action that contribute to the overall biological effects observed in crude extracts. For instance, the bornyl caffeic acid ester and related compounds combine a terpene scaffold with a phenolic acid, structures known for antioxidant and anti-inflammatory roles, hinting at complex synergistic interactions between the various metabolites

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[**Error! Bookmark not defined.**]. Other specialized metabolites rely on light-activated mechanisms for cytotoxicity. Thiophenes (sulfur-containing acetylenes) are highly susceptible to photoactivation, generating singlet oxygen and other ROS when exposed to UV-A light. This mechanism leads to broad-spectrum toxicity, often by causing irreparable damage to the microbial cell membrane and DNA [**Error! Bookmark not defined.**].

The anti-protozoal efficacy of Verbesina compounds is supported by targeted molecular mechanisms beyond non-selective cell damage. The bornyl esters isolated from *V. turbacensis* were found to possess potent inhibitory activity against the cysteine protease rhodesain. Rhodesain is the major cysteine protease utilized by the parasite Trypanosoma brucei rhodesiense (the causative agent of African trypanosomiasis/sleeping sickness). This targeted enzyme inhibition confirms that Verbesina metabolites can achieve high efficacy by directly interfering with a specific, critical virulence factor of a neglected tropical pathogen [**Error! Bookmark not defined.**].

Other specialized metabolites rely on light-activated mechanisms for cytotoxicity. Thiophenes (sulfur-containing acetylenes) are highly susceptible to photoactivation, generating singlet oxygen and other ROS when exposed to UV-A light. This mechanism leads to broad-spectrum toxicity, including anti-fungal and anti-bacterial activity, often by causing irreparable damage to the microbial cell membrane and DNA. The inclusion of these photoactive metabolites in Verbesina extracts further supports the hypothesis that the gross biological effect is a complex synergistic interaction between multiple compound classes, each operating via a distinct mechanism [**Error! Bookmark not defined.**].

The oil extract of *V. alternifolia*, which demonstrated potent adulticidal anthelmintic properties against the equine parasite *Habronema muscae*, revealed a mechanism linked to physical and nutritional interference. Scanning Electron Microscopy (SEM) analysis revealed irreversible degenerative changes to the parasite's external morphology. Changes included shrinking, detachment, and distortion of the cuticle, the basic entry route for anthelmintic drugs and deformation of the lips and papillae. This mechanism suggests that the active components, attributed to the presence of terpenoids, flavonoids, and aromatic compounds, facilitate their effect through passive diffusion and subsequent membrane and structural disruption. Furthermore, the formation of aggregates in and around the buccal capsule (anterior digestive tract) suggests a disruptive action that may interfere with the nematode's nutrition, potentially leading to undernourishment and mortality, similar to the proposed action of SLs targeting cellular processes [**Error! Bookmark not defined.**].

The antifungal mechanism is also multi-faceted. The fungicidal effect against Botrytis cinerea is hypothesized to be due to a synergistic effect of the identified phenolic compounds. Individual phenolics present disrupt key fungal processes in multiple ways: Protocatechuic acid affects membrane permeability; Vanillic acid inhibits ergosterol synthesis; and Quercetin alters cell membrane composition through oxidative stress [**Error! Bookmark not defined.**]. Similarly, the potent anti-cyanobacterial effect of the aqueous extract of *V. encelioides* against Microcystis aeruginosa involves significant physiological disruption, causing a marked decrease in the content of Chlorophyll-a and carotenoids, demonstrating that the compounds interfere with photosynthesis and ultimately induce cell death [112].

4.2.3. Ecological and Biosynthetic Context

The sheer chemical arsenal confirmed throughout the genus is a functional manifestation of potent allelopathic properties. The economic and ecological pressure exerted by *V. encelioides* is directly linked to the use of defensive secondary compounds that enable it to resist both pests and competing flora [**Error! Bookmark not defined.**]. This allelopathic effect suggests that the active metabolites, whether SLs or acetylenic compounds have mechanisms of action capable of disrupting fundamental cell survival processes (membrane integrity and energy metabolism) that are common across diverse microbial kingdoms [**Error! Bookmark not defined.**,**Error! Bookmark not defined.**].

The defense mechanism is further supported by direct anatomical evidence: Histochemical staining confirms that the synthesis and storage of Verbesina's key metabolites occur directly within

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specialized glandular trichomes. These trichomes actively accumulate terpenoids (including sesquiterpenes) and phenolic/fluorescent metabolites. This direct evidence of a structural defense system confirms that the compounds responsible for antimicrobial activity and cytotoxicity are natural defensive agents designed for broad-spectrum biotic challenge. [**Error! Bookmark not defined.**]. The biosynthetic process supporting this defense is highly elaborate. In *V. macrophylla*, specialized leaf secretory ducts actively produce and store defensive materials, including terpenes and alkaloids, to combat herbivores and microbes via a complex process known as granulocrine secretion. This elaborate, energy-intensive mechanism, where secretion begins in the plastids of specialized transfer cells, confirms that anti-infective and anti-herbivory activities are a primary ecological function of the *Verbesina* genus, supporting the hypothesis that these compounds possess powerful, broad-spectrum bioactivity [**Error! Bookmark not defined.**]. This non-selectivity further emphasizes the necessity of rigorously quantifying the SI to ensure any potent anti-AMR lead compound spares healthy mammalian cells [**Error! Bookmark not defined.**].

Finally, the layered defense strategy is also mediated by macromolecules; proteins from young leaves of *V. encelioides* exhibit moderate antimicrobial activity against various bacterial and fungal species, demonstrating a chemical and biological defense strategy [**Error! Bookmark not defined.**]. The plant's allocation strategy is predicted by the Limiting Resource Model (LRM), which suggests that when the host plant experiences stress (parasitism), it increases the allocation to shoot mass, directing more resources to the area where many of its chemical defenses (SLs) are sequestered, potentially suggesting a prioritized defense response to biotic stress [**Error! Bookmark not defined.**].

4.3. Combating Resistance: New Approaches

The potential of the *Verbesina* genus to address resistance extends beyond direct antimicrobial activity, encompassing therapeutic strategies that support the host immune system, target chronic diseases, and leverage modern nanotechnology for enhanced efficacy. This multifaceted utility highlights the strategic value of the plant as a bioprospecting source for complex, next-generation scaffolds.

4.3.1. Host-Mediated and Anti-Inflammatory Strategies

The established traditional use of *Verbesina* for wound healing and inflammation provides compelling *in vivo* evidence supporting its systemic anti-infective and anti-inflammatory potential. Topical application of the methanolic extract (VCME) of *V. crocata* to incisional wounds in a murine model demonstrated significant acceleration of the healing process, reducing the final closure time (FCT) to 12 days, which was faster than the positive control (Recoveron®) and the negative control (Vaseline). Crucially, the VCME significantly increased the tensile strength of the healed tissue (700 ± 148.3 g) compared to the positive control (640 ± 259.9 g), indicating superior collagen organization [**Error! Bookmark not defined.**,**Error! Bookmark not defined.**]. Histological analysis confirmed that the extract advanced the healing process to the remodeling phase by promoting the synthesis of mature collagen and significantly reducing the number of fibroblasts. This efficacy is attributed to the extract's anti-inflammatory action (linked to sitosterol glycoside and known sesquiterpenes) and its potential for immune modulation (phylloflavan activates macrophages), demonstrating the extract's ability to control the initial infective and inflammatory phases of wound repair [**Error! Bookmark not defined.**].

The precise structural requirement for this anti-inflammatory activity is defined by the core eudesmane scaffold's functionalization. Pure 4β-cinnamoyloxy, 1β,3α-dihydroxyeudesm-7,8-ene demonstrated a notable anti-inflammatory activity, achieving 83.18% edema inhibition in vivo (ED_{50} = 0.43 μM). This effect is strongly dependent on the integrity of the cinnamate moiety: chemical modifications such as acetylation and hydrogenation resulted in a significant reduction in anti-inflammatory activity (up to 60% reduction for the hydrogenated derivative), underscoring a prevailing role of the unsaturated double bonds of the cinnamate scaffold in this biological effect [**Error! Bookmark not defined.**]. This mechanism of reducing the inflammatory phase is further

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supported by the EO fraction, which demonstrated strong anti-inflammatory and antipyretic activities statistically similar to or exceeding commercial drugs like Dexamethasone and Dipyrone, respectively. The anti-inflammatory effect (carrageenan-induced peritonitis model) is associated with the reduction of the pro-inflammatory cytokines TNF-α (up to 73.52%) and IL-1β (up to 87.71%), consistent with the activity of its major components, β-caryophyllene and Germacrene D, which are potent inhibitors of pro-inflammatory cytokine production [**Error! Bookmark not defined.**].

This mechanism of host-defense enhancement is further validated by studies on *V. oerstediana*, which demonstrated crucial immunomodulatory activity. Extracts of *V. oerstediana* (VEOEBA) significantly enhance the antimicrobial efficacy of human monocytes and neutrophils against *Staphylococcus aureus* infections. Mechanistically, this enhancement is attributed to the extract's ability to significantly stimulate the production of Neutrophil Extracellular Traps (NETs). NETs are extracellular fibers composed of DNA-histone complexes that entrap and kill bacteria, representing a critical, non-phagocytic defense mechanism, suggesting that *Verbesina* compounds can serve as adjunctive AMR therapies by boosting the host's innate immune response [113].

4.3.2. Non-Infectious Therapeutic Applications (Chronic Disease and SAR)

The chemical versatility of the genus ensures its therapeutic utility extends to high-value areas outside of acute infection, particularly in managing chronic human diseases. The guanidino-amide alkaloids are a significant class of potent hypotensive agents [**Error! Bookmark not defined.**]. The novel monomer caracasanamide from *V. caracasana* demonstrated a dose-dependent hypotensive effect, reducing mean blood pressure while simultaneously increasing cardiac inotropism (dP/dt) and respiratory function [**Error! Bookmark not defined.**,**Error! Bookmark not defined.**,114]. Critically, this compound does not decrease cardiac inotropism or chronotropism at nontoxic doses, in contrast to most other hypotensive drugs tested, making it a clearly advantageous therapeutical aid for moderate arterial hypertension [77,80]. The pursuit of structural analogs, such as the dimeric caracasandiamide [**Error! Bookmark not defined.**] and prenylagmatine analogs [**Error! Bookmark not defined.**,**Error! Bookmark not defined.**], demonstrated that subtle variations within the alkaloid structure significantly enhance pharmacological action, providing a clear path for chemical modification and optimization of the genus's cardiovascular agents [82,84,114].

Beyond cardiovascular agents, the genus demonstrates efficacy in managing metabolic syndrome. The extract of *V. persicifolia* was found to significantly alter glucose tolerance, enhance glucose uptake in skeletal muscle, and notably inhibit glycogenolysis in the liver, suggesting it is effective in controlling blood glucose following a heavy carbohydrate diet [**Error! Bookmark not defined.**]. Furthermore, the ethanolic extract of *V. encelioides* roots exhibited significant hypolipidemic activity in rats, resulting in a measured decrease in total cholesterol, triglycerides (TGL), and very low-density lipoproteins (VLDL), while maintaining or increasing high-density lipoproteins (HDL) [115]. The genus's therapeutic scope is rapidly expanding to include neuroprotection and anticonvulsant activity; the methanolic extract of *V. persicifolia* demonstrated highly significant anticonvulsant activity in the zebrafish seizure model, resulting in a 100 survival rate after a PTZ-induced seizure event at a non-toxic concentration [**Error! Bookmark not defined.**]. Aqueous extracts of *V. crocata* also exhibit measurable diuretic activity, comparable to the positive control furosemide in increasing sodium and potassium clearance and excretion [**Error! Bookmark not defined.**].

The most compelling evidence for a favorable SI drived from a Verbesina species is provided by research on *V. lanata*. Compounds closely related to the genus's characteristic scaffolds were evaluated for anti-protozoal activity against Leishmania major promastigotes, showing dose- and time-dependent activity with IC_{50} values ranging from 4.9 to 25.3 μM. Crucially, the toxicity against normal murine macrophages (CC_{50}) ranged from 432.5 to 620.7 μM, yielding a high SI of up to 88.2 for Arbusculin Derivative ((1*R*,4S,4a*S*,7*R*)-1-methyl-7-(prop-1-en-2-yl)octahydro-2*H*-1,4a-(epoxymethano)naphthalen-4-yl acetate). This SI value is substantially better than that of the standard drug, meglumine antimoniate (glucantime), which had an SI of 40.0. This quantitative

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comparison provides definitive validation that eudesmane scaffolds can yield lead compounds with a high therapeutic window [**Error! Bookmark not defined.**].

4.3.3. Technological Valorization and Biocontrol Applications

The chemical complexity of *Verbesina* allows its extracts to be leveraged in modern nanotechnology, offering a rapid, safe, and environmentally friendly avenue for combating AMR. The methanolic and ethanolic extracts of *V. encelioides* contain natural reducing agents (polyphenols, flavonoids, and terpenoids) capable of performing the green synthesis of silver nanoparticles (AgNPs). These fabricated AgNPs then exhibit superior antimicrobial action compared to the extracts alone against both bacteria (*E. coli* and *V. cholerae*) and fungi (*Aspergillus spp.*) [101,108]. This successful synthesis is further supported by the use of purified saponin extracts from *V. encelioides* as natural reducing and stabilizing agents for AgNPs used in dermal applications, such as the management of acne [**Error! Bookmark not defined.**]. The successful synthesis using *V. crocata* further supports the viability of this strategy, with the synthesized Ag/AgCl-NP's exhibiting sizes less than 100 nm, indicating strong potential for enhanced antimicrobial and anti-biofilm activity [**Error! Bookmark not defined.**]. The broad utility of Verbesina metabolites suggests non-traditional applications in combating resistance, as phenolic and other compounds within the aqueous extract serve as natural reducing agents for the green synthesis of AgNPs, which exhibit enhanced antimicrobial activity against pathogens like *E. coli* and *Vibrio cholerae*. This suggests Verbesina extracts can be directly leveraged in novel drug delivery and anti-AMR formulations [**Error! Bookmark not defined.**].

Furthermore, the plant's aggressive ecological resilience is being valorized for biocontrol applications. The nematicidal capability of *Verbesina* opens a new avenue for AMR research by targeting biocontrol agents that reduce reliance on synthetic pesticides [**Error! Bookmark not defined.**]. Aqueous extracts of the plant show strong nematicidal activity against the root-knot nematode *Meloidogyne javanica*, indicating its potential as a green manure or natural nematicide. Furthermore, the plant's aggressive ecological resilience is being valorized for biocontrol applications. A patent claims an anti-termite herbal composition derived in part from the flowers and leaves of *V. encelioides*, demonstrating its commercial potential as an eco-friendly pesticide. This supports the strategy of leveraging the plant's natural chemical defense system, driven by compounds like alkaloids and saponins, as a low-cost, sustainable biopesticide solution [116].

The demonstrated allelopathic potential of *V. encelioides* offers an innovative route for biocide valorization as an environmentally friendly control agent for cyanobacterial blooms in eutrophic water bodies. The aqueous extract of the aerial parts of *V. encelioides* exhibits a strong anti-cyanobacterial effect on the growth of the harmful species, *Microcystis aeruginosa*, with a maximum Inhibitory Rate of 93% achieved at 1 mg/mL concentration. This efficacy is noted to be due to the compounds interfering with photosynthesis and ultimately inducing cell death [**Error! Bookmark not defined.**].

The severe agricultural impact of *V. encelioides* as a weed and as a reservoir host for thrips (vectors for Tomato Spotted Wilt Virus, TSWV) strongly justifies the development of *Verbesina*-derived compounds as high-value, natural biopesticides [**Error! Bookmark not defined.**,**Error! Bookmark not defined.**,**Error! Bookmark not defined.**]. Beyond biopesticides, the genus exhibits potential for industrial valorization. Early screenings for renewable energy sources identified *V. alternifolia* as a rubber-producing species yielding a quantifiable percentage of hydrocarbon (0.4%), confirming its utility as a source of low-molecular-weight polyisoprenes and hydrocarbon feedstocks [117]. Conversely, the extracts demonstrate significant agricultural potential by acting as growth biostimulants, particularly in high-value crops like strawberry (*Fragaria ananassa*). Application of aqueous extracts of *V. sphaerocephala* and *V. fastigiata* resulted in notable increases in productive parameters, including up to a 207.14% Agronomic Efficiency Index (IEA) for fruit and flower production [**Error! Bookmark not defined.**]. The intrinsic chemical resilience of *V. encelioides* also

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opens a high-value application in phytoremediation, suggesting its potential role in the stabilization or phytoextraction of heavy metals from contaminated mine waste [**Error! Bookmark not defined.**].

5. Critical Analysis: The Therapeutic Index and Drug Feasibility

The successful translation of a natural product into a pharmaceutical lead depends entirely on its ability to demonstrate preferential toxicity, which is quantified by the Selectivity Index (SI = IC_{50}/MIC)[**Error! Bookmark not defined.**]. The inherent chemical characteristic of the *Verbesina* genus, defined by highly reactive SLs and potent alkaloids, presents a formidable challenge: the strong potential for efficacy (MIC) is inextricably linked to the risk of host toxicity (IC_{50}). This section critically analyzes the compiled data, providing quantitative and structural arguments to assess the feasibility of isolating and optimizing SL scaffolds with an intrinsically superior therapeutic window.

5.1. Calculation and Interpretation of the Selectivity Index (SI)

The determination of the SI is complicated by the presence of a diverse chemical arsenal, yet quantitative evidence confirms that a favorable SI is achievable through targeted isolation (Figure 5 and Table 2).

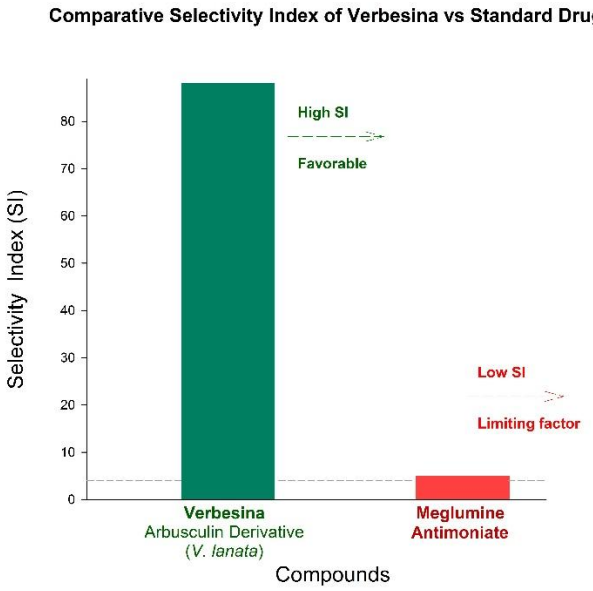

Figure 5. Translational Feasibility: The SI Comparison.

Table 2. Comparative Analysis of Selectivity Index (SI) and Efficacy-Toxicity Duality for Verbesina Metabolites and Extracts.

Category	Compound / Extract	Efficacy (Target & Value)	Toxicity (Cell Line & Value)	Selectivity Index (SI)
I. Confirmed High Selectivity (SI >> 1)				
Anti-Protozoal Lead [**Error! Bookmark not defined.**]	Arbusculin Derivative (*V. lanata*)	*L. major* amastigotes, 4.9 - 25.3 µM	NMM, 432.5 - 620.7 µM	Up to 88.2
Non-Cytotoxic Triterpenes [**Error! Bookmark not defined.**]	Pseudotaraxasterol Derivatives (from *V. encelioides*)	Protozoa (Antiprotozoal activity), 32.2 - 48.2 µg/mL	HELF, (MRC-5) (CC_{50}), > 64 µg/mL	>1.3–2.0
Host-Immune Booster [**Error!**	*V. oerstediana* Acetone Bark Extract	*S. aureus*, Significantly boosts killing at 10 µg/mL	Neutrophils (Viability), Non-cytotoxic at	High (Qualitative)

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Bookmark not defined.]			10 µg/mL	
II. Efficacy & Duality Challenges				
Efficacy Benchmark (Germacrene) [**Error! Bookmark not defined.**]	Germacrene SL (*V. negrensis*)	*S. aureus* / *E. faecalis* (MIC), 64 µg/mL	CC_{50} on non-cancerous line: > 64 µg/mL	≈1 (Implied)
Duality Case (Mild Uncoupler) [**Error! Bookmark not defined.**]	CDE (from *V. persicifolia*)	Undocumented	RLM (C_{eff}), 20-100 µM (Mild Uncoupling)	≈1 (Implied)
Cytotoxicity Benchmark (Extract) [**Error! Bookmark not defined.**]	*V. turbacensis* Hydroethanolic Extract	(Low Activity assumed)	HepG2 cells (IC_{50}), 334.6 µg/mL	High (Qualitative)
Toxicity Benchmark (Isolated Toxin) [**Error! Bookmark not defined.**]	Galegine (Alkaloid) (from *V. encelioides*)	(Toxicity focus)	Hep G2 cells (IC_{50}), 10.9 µg/mL	Very Low SI (Implied)
Extract Duality (Allelopathy) [**Error! Bookmark not defined.**]	*V. encelioides* Root Leachate	Inhibition (Full Strength), -25.7% (RRG)	Stimulation (Low Dilution), +36.5% (RRG)	Concentration-Dependent

SI: Selectivity Index (CC_{50}/IC_{50} or CC_{50}/MIC); NMM: Normal murine macrophages; HELF: Human embryonic lung fibroblasts; RLM: Rat Liver Mitochondria; RRG: Radish Root Growth; IC_{50}: standard inhibitory, MIC: Minimum Inhibitory Constant; CC_{50}: Cytotoxicity; GI_{50}: growth inhibition; The SI for the Germacrene Efficacy Benchmark (MIC = 64 µg/mL) is inferred (≥ 1) as a specific non-cytotoxicity CC_{50} is currently undocumented, although correlated non-toxic triterpenoid CC_{50} values for *V. encelioides* exceed this concentration; Arbusculin Derivative: (1*R*,4*S*,4a*S*,7*R*)-1-methyl-7-(prop-1-en-2-yl)octahydro-2*H*-1,4a-(epoxymethano)naphthalen-4-yl acetate; Germacrene SL: (1*S*,6*R*,7*S*,10*S*,*Z*)-6-hydroxy-10-isopropyl-7-methoxy-3,7-dimethyl-4-oxocyclodec-2-en-1-yl cinnamate; CDE: (4β-cinnamoyloxy- 1β,3α-dihydroxyeudesm-7,8-ene).

5.1.1. Quantitative Evidence for a Favorable Selectivity Index

The most compelling quantitative evidence for a favorable SI derived from a *Verbesina* species is provided by research on *V. lanata*. This study evaluated compounds closely related to the genus's characteristic eudesmane scaffolds for anti-protozoal activity against *Leishmania major* promastigotes, yielding IC_{50} values ranging from 4.9 to 25.3 µM. Crucially, the toxicity against normal murine macrophages (CC_{50}) ranged from 432.5 to 620.7 µM. This differential resulted in a high SI of up to 88.2 for Arbusculin Derivative [**Error! Bookmark not defined.**]. This SI value is substantially better than that of the standard drug, meglumine antimoniate (glucantime), which had an SI of 40.0. This quantitative comparison provides definitive validation that eudesmane scaffolds can yield lead compounds with a high therapeutic window [**Error! Bookmark not defined.**].

This potential for selectivity is also demonstrated qualitatively in other fractions. The EO of *V. macrophylla* provides a strong qualitative argument for a favorable SI. The EO is toxicologically safe (low hemolysis in human cells and LD_{50} > 5000 mg/kg) while simultaneously exhibiting potent antimicrobial activity (MIC 16 µg/mL against *K. pneumoniae* and MIC 4 µg/mL against *C. albicans*). The low toxicity is correlated with the known safety profile of its major components. The finding that the essential oil is a potent antimicrobial and anti-inflammatory agent with a wide toxicological safety window validates the ethnopharmacological use and suggests that the essential oil of *V. macrophylla* may be a basis for developing drugs with natural anti-AMR effects [**Error! Bookmark not defined.**].

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Similarly, strong trypanocidal activity (up to 100% elimination at 200 μg/mL) against *T. cruzi* combined with the confirmed toxicological safety of the essential oil of the same species strongly indicates the potential for a favorable SI [**Error! Bookmark not defined.**]. Furthermore, the measured MIC of 64 μg/mL for a pure isolated SL ((1*S*,6*R*,7*S*,10*S*,*Z*)-6-hydroxy-10-isopropyl-7-methoxy-3,7-dimethyl-4-oxocyclodec-2-en-1-yl cinnamate) against key Gram-positive pathogens provides a critical benchmark for the efficacy component of the SI calculation [**Error! Bookmark not defined.**]. This further justifies the mandatory strategy of purification and structural investigation to identify and ultimately modify these powerful lead molecules [**Error! Bookmark not defined.**,**Error! Bookmark not defined.**]. This mechanism of host-defense enhancement is further validated by studies on *V. oerstediana*, which demonstrated crucial immunomodulatory activity by stimulating NET release without significantly affecting the viability of the neutrophils (viable NETosis), suggesting an ideal pharmacological profile for developing adjunctive AMR therapies [**Error! Bookmark not defined.**].

5.1.2. The Duality Challenge and Evidence for Mitigation

The core translational challenge for *Verbesina* SLs is their non-selective reactivity [**Error! Bookmark not defined.**]. Experimental data confirms the characteristic duality of efficacy and potential toxicity inherent to the genus's metabolites [**Error! Bookmark not defined.**].

Studies provide key evidence of dose-dependent biological activity. Full-strength root leachate significantly suppressed the root and shoot growth of radish seedlings (a toxicity model), demonstrating a potent, non-selective effect typical of high concentrations. Conversely, a lower, optimized concentration (1:4 dilution of root leachate with high N fertilization) actually promoted radish root growth by up to +36.5%, indicating that lower concentrations can result in beneficial (stimulatory) effects [**Error! Bookmark not defined.**]. This duality confirms the complex task of optimizing the therapeutic window, as the same extract contains compounds that can both severely inhibit and promote growth, demanding precise chemical isolation [**Error! Bookmark not defined.**,**Error! Bookmark not defined.**]. This high performance is fundamentally linked to the extract's dual nature: acting as allelopathic agents that inhibit seed germination, but also as potent biostimulants that significantly enhance crop growth and yield [**Error! Bookmark not defined.**]. Furthermore, the prominent antioxidant activity of the extracts, with the ethyl acetate fraction showing high capacity in assays like Ferric Reducing Power, reinforces the conclusion that the total efficacy profile of Verbesina relies on the complex synergistic interaction between potent (toxic) primary metabolites (like SLs and galegine) and high-concentration support molecules (like phenolic acids and flavonoids), necessitating the targeted isolation and study of all classes for optimal therapeutic outcomes [**Error! Bookmark not defined.**].

Quantitative data models this separation challenge. Potency is concentrated in the crude extract and the ethyl acetate fraction, often considered the primary source of SLs [**Error! Bookmark not defined.**]. This finding is supported by the comparison demonstrating that the ethanolic extract (VE) is twice as potent as the aqueous extract (VA). The greater efficacy of the VE strongly suggests that the core bioactive agents responsible for the observed potent ROS-mediated apoptosis are concentrated within the non-polar fraction [**Error! Bookmark not defined.**]. Therefore, optimizing the therapeutic window requires separating the potent efficacy-driving compounds (likely the highly reactive SLs) from the toxicity-mitigating or synergistic non-SL components (like the less-toxic triterpenoids and β-sitosterol glycosides) [**Error! Bookmark not defined.**]. This process minimizes the co-extraction of inactive, highly polar compounds and potentially concentrates the therapeutic molecules away from water-soluble toxins (like the alkaloid galegine), thereby optimizing the input material for subsequent isolation and structural modification aimed at generating a more favorable SI [**Error! Bookmark not defined.**].

The severity of the SI challenge is mitigated by the isolation of less-toxic fractions [**Error! Bookmark not defined.**]. The low cytotoxicity found for the hydroethanolic *V. turbacensis* extract (IC_{50} of 334.6 μg/mL on HepG2) is a critical component for the future calculation of its SI. A high IC_{50}

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against a generalized cancer cell line, when compared to a low MIC, would yield a favorable SI, suggesting a low host toxicity [**Error! Bookmark not defined.**]. Furthermore, the study on CDE from *V. persicifolia* introduces a compelling argument for the genus's potential selectivity, classifying CDE as a "mild" uncoupler. The authors stress that since *V. persicifolia* is widely used in Mexican traditional medicine for various complaints, administering CDE should not have dangerous toxic side-effects [**Error! Bookmark not defined.**]. This high level of tolerability, coupled with robust efficacy at significantly lower doses (5 mg/kg and 10 mg/kg), strongly supports the potential for a favorable SI for the extract's neuroprotective properties [**Error! Bookmark not defined.**].

5.1.3. The Need for Rigorous Validation

The overall analysis reinforces the contemporary and direct validation for the central objective of the review: to rigorously compile the necessary IC_{50} data and calculate the SI to bridge the gap between proven high efficacy and unassessed safety [**Error! Bookmark not defined.**]. The inherent allelopathic/phytotoxic property of the raw *Verbesina* extract is confirmed, which directly relates to the central challenge of the SI [**Error! Bookmark not defined.**]. The strong *in vivo* wound healing performance of *V. crocata* suggests that the complex chemical mixture provides a favorable overall therapeutic profile, successfully controlling the inflammatory phase while promoting organized tissue remodeling [**Error! Bookmark not defined.**]. The successful valorization of *Verbesina* requires isolating the beneficial compounds from the potential toxins (like the guanidine alkaloid, galegine) to ensure its role as a safe, sustainable biostimulant or as a high-SI therapeutic agent [**Error! Bookmark not defined.**].

5.2. Structure-Activity Relationship (SAR)

The establishment of reliable SAR for *Verbesina* metabolites is the indispensable prerequisite for designing less toxic, highly selective drug candidates with a favorable SI. This process mandates meticulously linking the complex C_{15} scaffold and its functional groups to the observed dual cytotoxic (IC_{50}) and antimicrobial (MIC) activities.

5.2.1. Foundational Structural Requirements and Stereochemical Precision

SAR metrics are dependent on unequivocal structural gold standards, which must resolve the complex stereochemistry and conformation of the bicyclic core. Errors in stereochemistry directly translate to flawed SAR conclusions regarding which compounds possess the most favorable therapeutic index [50,55].

The *cis*-Decalin Core: Early structural studies confirmed the complex stereochemistry of the *Verbesina* sesquiterpenes. The Verbesinols were found to be stereomeric with junenol, establishing the complex, rigid *cis*-ring juncture of the verbesinol core [**Error! Bookmark not defined.**]. The X-ray confirmation of Verbesindiol's structure was vital because it provided the definitive assignment of the *cis*-ring fusion and the stereochemistry of C-4 and C-6, correcting earlier ambiguous interpretations [**Error! Bookmark not defined.**]. Without this foundational structural evidence, pharmacological data would be difficult to reliably map to the true molecular scaffold. This rigorous structural confirmation is a necessary precursor to conducting meaningful SAR analysis and correlating the presence of the phenolic ester moiety with the observed acute cytotoxicity (IC_{50}) or anti-AMR efficacy (MIC) [**Error! Bookmark not defined.**].

Stereochemical Corrections and Analysis: Establishing a rigorous SAR requires painstaking chemical elucidation. The definitive structure of Rupestrol was established through a series of chemical conversions, including acetylation, hydrolysis, and periodate oxidation, demonstrating the complexity required for rigorous SAR determination [**Error! Bookmark not defined.**]. The use of Nuclear Overhauser Effect (NOE) experiments validated the configuration of the C-4 hydroxyl group in Verbesindiol derivatives, confirming the original assignment and contradicting proposed changes by other research groups [**Error! Bookmark not defined.**]. ^{1}H-NMR spectroscopy corrected earlier

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assignments for α- and β-Verbesinol esters by measuring very small coupling constants ($J_{5,6}$ and $J_{6,7}$), establishing that the compounds likely adopt absolute configurations where the C-7β-configuration of the isopropyl group is consistently observed [**Error! Bookmark not defined.**]. The absolute configuration of Rupestrinol was determined via the Circular Dichroism (CD) curve of its derived ketone, suggesting it has the same absolute configuration as the parent compound Rupestrol [**Error! Bookmark not defined.**]. The precise stereochemical determination, which confirmed the C-6 hydroxyl and C-10 methyl groups were cis and axial, is foundational for establishing accurate SAR metrics, as this axial arrangement dictates the spatial presentation of the C-6 cinnamate ester pharmacophore [**Error! Bookmark not defined.**].

Conformational Flexibility: The X-ray analysis of C-4 cinnamates confirmed the existence of two crystallographically independent molecules in the crystal, differing only in the conformations of the side chains, highlighting the inherent conformational flexibility of the molecule that can impact its biological interaction [**Error! Bookmark not defined.**]. The complexity of structural diversity in eudesmane derivatives can be explained by the germacrene precursor existing in different conformations, which ultimately results in eudesmane derivatives with different configurations [**Error! Bookmark not defined.**]. Similarly, precise SAR analysis in the Elemanolide series (Zempoaline C) required definitive *X*-ray analysis due to their conformational flexibility [**Error! Bookmark not defined.**]. The successful characterization of V. turbacensis eudesmanes employed two-dimensional NMR experiments, including COSY, NOESY, and HMBC, to achieve detailed structural and stereochemical assignments [**Error! Bookmark not defined.**]. Furthermore, the *X*-ray determination of Verocephol's structure provided the necessary resolution for accurately interpreting the molecule's inherent reactivity to its observed activities [**Error! Bookmark not defined.**].

5.2.2. Functional Group Dependence and Mechanistic Shift

The structural diversity of the SLs, including highly oxygenated enantio-eudesmanes like Rupestrol [**Error! Bookmark not defined.**] and novel cadinene-type SLs [**Error! Bookmark not defined.**], demonstrates that the core cytotoxic and antimicrobial action is highly dependent on functional group placement [**Error! Bookmark not defined.**]. This precise structural work is the prerequisite for comparing the efficacy (MIC) and cytotoxicity (IC_{50}) of different scaffolds to achieve a favorable SI [**Error! Bookmark not defined.**].

The Alkylating Pharmacophore: The potential for any SL to succeed as a therapeutic agent is intimately linked to the highly reactive α-methylene-γ-lactone moiety, which acts as a template for non-reversible alkylation. The persistent co-occurrence of the cinnamate ester (the α,β-unsaturated moiety that acts as the electrophile) with other terpenoids reinforces the hypothesis that the genus's potent effects are driven by these functionalized compounds [**Error! Bookmark not defined.**].

Hydroxyls, Acetyls, and Mechanism: A pivotal structure-activity study on derivatives of Compound 1 revealed that subtle structural modifications drastically alter the cellular target, even when GI_{50} values remain comparable [**Error! Bookmark not defined.**]. Native Hydroxyls (**Compound 1** and **3**, Figure 4) are crucial for inducing Ca^{2+}-dependent Mitochondrial Permeability Transition (MPT). This effect is preventable by MPT inhibitors, supporting the role of the hydroxyl groups in MPT induction. Conversely, masking the hydroxyl groups with Acetate Substituents (**Compound 2** and **4**, Figure 4) shifts the mechanism dramatically. These acetylated derivatives cause injury primarily by directly Inhibiting Complex II (Succinate dehydrogenase) of the mitochondrial respiratory chain and inducing an uncoupling effect on oxidative phosphorylation.

Structural Features for Activity: The biological activity of eudesmane sesquiterpenoids is strongly determined by the types, positions, and quantity of substituent groups. Anti-inflammatory activity, for instance, is significantly influenced by substitutions at pivotal positions like C-8, C-11, and C-13. An increase in the number of hydroxy substituents often correlates with an enhancement in anti-inflammatory efficacy. Furthermore, the terminal double bond between C-4 and C-15 ($\Delta^{4(15)}$ double bond) could significantly enhance this activity [**Error! Bookmark not defined.**].

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5.2.3. SAR Challenges and Translational Optimization

The successful application of SAR must account for the genus's chemical complexity and the translational necessity of reducing toxicity [**Error! Bookmark not defined.**].

Targeting Alkaloid SAR for Analogue Design: Design principles established for the guanidino-amide alkaloids offer a parallel path for SL modification efforts. For hypotensive activity in this class, the presence of the double bond was found to be of major importance, while the absence of methoxy groups on the aromatic ring resulted in a measurable increase in hypotensive activity [**Error! Bookmark not defined.**].

Translational Strategy: A major strategy for optimizing the SI is the development of structural analogues aimed at reducing toxicity while enhancing effectiveness. This involves generating monofunctional alkylants or using chemical modifications to introduce substituents that alter the activity and safety profile [**Error! Bookmark not defined.**].

The Absolute Requirement: The initial step requires establishing a reliable SI for potent SLs, which is only meaningful after meticulous chemical elucidation provides the unequivocal structural gold standard [**Error! Bookmark not defined.**]. Critically, the determination of the exact stereochemistry for the eudesmane derivatives from V. turbacensis provides necessary benchmarks, as correlations drawn between potency (MIC/IC_{50}) and subtle structural differences would be scientifically unfounded without this foundational crystallographic work [**Error! Bookmark not defined.**]. The successful assembly of the 152,050 bp plastome of *V. alternifolia* provides a crucial genetic reference point that can help minimize the impact of geographical and chemotypic variability, supporting the goal of standardization necessary for reproducible SAR studies [**Error! Bookmark not defined.**].

6. Discussion and Translational Direction

The data compiled within this review strongly validate the ethnopharmacological use of the *Verbesina* genus, confirming that its specialized metabolites, particularly the Sesquiterpene Lactones (SLs), provide a high-potency chemical arsenal against Antimicrobial Resistance (AMR). However, the inherent duality of efficacy and host toxicity, driven by the reactive α-methylene-γ-lactone pharmacophore defines the core translational challenge. Moving beyond promising MIC and IC_{50} data requires a rigorous roadmap focused on maximizing the Selectivity Index (SI) through chemical optimization, standardization, and targeted bioassay-guided fractionation.

6.1. The Imperative for Chemical Optimization and Analogue Development

The SAR analysis (Section 5.2) demonstrated that subtle changes in the SL scaffold, such as masking hydroxyl groups with acetate substituents can drastically shift the cellular mechanism, from inducing Mitochondrial Permeability Transition (MPT) to inhibiting respiratory Complex II [**Error! Bookmark not defined.**]. This complexity confirms that simple isolation of the natural product is insufficient; future success hinges on advanced medicinal chemistry to modify and detoxify the highly active natural scaffolds.

Mitigating Toxicity via Semi-Synthesis: The primary goal is to mask the non-selective reactivity of the α-methylene-γ-lactone core while preserving antimicrobial efficacy. The most promising strategy is the semi-synthesis of structural analogues aimed at reducing general cytotoxicity [**Error! Bookmark not defined.**]. Only compounds with a rigorously confirmed favorable SI can proceed toward this chemical optimization.

Synthetic Access and Scale-Up: For many valuable but scarce metabolites, such as the benzofuran-3-one derivative from *V. luetzelburgii* natural abundance is too low for comprehensive *in vivo* and SI testing, underscoring the necessity of a robust synthetic organic chemistry program [**Error! Bookmark not defined.**]. This capability is also critical for the scale-up and optimization of the guanidino-amide alkaloid scaffolds, as evidenced by the successful total synthesis of hypotensive analogs [75,83].

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6.2. Standardization and Bioassay-Guided Isolation

Translational research on Verbesina metabolites is complicated by high natural variability, requiring strict control over raw material quality and isolation protocols.

1. **Addressing Chemical Variability:** The measured IC_{50} and MIC values are susceptible to ontogenetic and geographical chemical variation, as the relative percentage of key sesquiterpenes can fluctuate significantly depending on the plant's maturity [**Error! Bookmark not defined.**]. Future work must implement high-resolution analytical standards like HPLC profiling to define standardized chemotypes and minimize this variability, thereby ensuring the reproducibility of the SI calculation.
2. **Genetic Anchoring:** The successful assembly of the complete plastome for *V. alternifolia* provides a crucial genetic reference point, which should be leveraged to map and minimize the impact of genetic and chemotypic variability when working with new accessions [**Error! Bookmark not defined.**].
3. **Targeted Toxicity Assessment:** Rigorous SI calculation requires toxicity profiles (IC_{50} on non-cancerous lines) for every potent isolate to bridge the gap between high efficacy and unassessed safety [**Error! Bookmark not defined.**]. Furthermore, the scarcity of information on many ethnobotanically important species, such as *V. crocata*, demands comprehensive characterization of active principles and toxicity assessments to elaborate safe pharmaceutical products [**Error! Bookmark not defined.**].
4. **Biotechnology for Consistency:** To circumvent the challenges of field-collected material, the successful biosynthesis of key triterpenoids like lupeol in *V. encelioides* cell cultures offers a high-value pathway for the scale-up and standardization of non-toxic support molecules [**Error! Bookmark not defined.**]. This difficulty has prompted translational research into novel process engineering. A specific process patent demonstrates the development of an optimized hexane extraction method for *V. persicifolia* that dramatically increases the yield and purity of the lead eudesmane 4CDE (up to 3 g/kg of fresh material), successfully solving the scalability challenge for this high-value scaffold [**Error! Bookmark not defined.**].

7. Conclusion

The *Verbesina* genus is a verified repository of complex, evolved chemical defenses, offering structurally unique sesquiterpenoid scaffolds with proven dual cytotoxic and antimicrobial activities. By identifying lead compounds with a favorable Selectivity Index (SI), and leveraging modern chemical synthesis and rigorous standardization protocols, the potential of this rich Mexican medicinal resource can be fully unlocked. The future of anti-AMR drug discovery is complex, but the multifaceted chemical mechanisms and translational feasibility demonstrated by *Verbesina* metabolites position them as promising next-generation scaffolds in the fight against antibiotic resistance.

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