Journal of Science Innovations and Nature of Earth

International, Double-Blind, Quarterly, Peer-Reviewed, Refereed, Edited and Open Access Research Journal
Journal homepage: https://jsiane.com/index.php/files

Withanolide Scaffold in Drug Discovery: A Review of Structural Modifications, Structure-Activity Relationships, and Pharmacological Targets

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DOI- https://doi.org/10.59436/jsiane.v6i2.8.2583-2093

ARTICLE INFO

Article history:
Received 22 March 2026
Received in revised form
23 April 2026
Accepted 23 April 2026
Available online 25 May 2026

Keywords:
withanolides; structure–activity relationships; covalent drug discovery; Michael acceptor; steroidal lactone; Withania somnifera

ABSTRACT

Withanolides are C-28 polyoxygenated steroidal lactones that are characterised by a pair of C2-C3 enones and an α,β-unsaturated d-lactone held in fixed space geometry by a rigid ergostane tetracyclic core. The architecture allows specific covalent interaction with nucleophilic cysteine targets in the protein of interest of mechanobiological interest and has been utilised in a polypharmacology profile of anticancer, anti-inflammatory, neuroprotective, and immunomodulatory activities. A pre-existing SAR dataset of significant chemical depth in more than 1,200 natural analogues spread out in 15 genera in the Solanaceae provides a basis that is further extended by emerging semi-synthetic literature. This review critically discusses the medicinal chemistry-based analysis of withanolide scaffold modification as structural changes at the A-ring enone, lactone ring, peripheral hydroxyl positions and C-17 side chain translate into defined biological outcomes at molecular targets of validated structure requirements that are defined as pharmacophoric elements. Cytotoxic, anti-inflammatory and neuroprotective phages, with their structural separability being a key feature, are is the new opportunities in chemoproteomics-mediated target discovery, biosynthetic engineering and targeted covalent drug design.

Introduction

1.1 Privileged Scaffold of steroids in Medicinal Chemistry: Withanolides - A 'privileged structure' is a term used to define a molecular scaffold with the ability to produce strong and selective ligands against numerous biological targets following specific structural changes (Atanasov *et al*., 2021). The ergostane withanolide skeleton series meets the criteria of this definition. On such a scaffold, C-22 and C-26 are oxidised to form a d-lactone ring; the scaffold supports structural modifications that then participate in a broad range of biological activity, such as anticancer, anti-inflammatory, neuroprotective, or immunomodulatory effects (Bashir *et al*., 2023; Singh *et al*., 2022; Tewari *et al*., 2022).

The distinguishing feature of withanolides among other classes of bioactive steroids is that withanolides have an electrophilic structure. The structure is characterised by cardiac glycosides. as C-17 butenolide and sugar residues that bind the drug to the inhibition of Na+/K+-ATPase as a major effect. Plant hormones derived from cholestane (brassinosteroids) that have a typical 6-ketone and 7-oxalactone backbone do not have any comparable electrophilic reactivity. The structure of the withanolide scaffold, on the contrary, usually shows a dual Michael acceptor structure: an α,β-unsaturated carbonyl in the C-2/C-3 position in ring A and an electrophilic centre in the α,β-unsaturated lactone of the side chain, which is frequently complemented by a 5β,6β-epoxide (Singh *et al*., 2022). Closely related to these spatially localised electrophiles are the so-called sanctioned vibrations, where the nucleophilic protein residues are covalently engaged by these spatially separated electrophiles, with their geometry pre-orientated by the rigid steroidal backbone to react on mechanistically distinct targets. This is a multi-electrophile scaffold, which is the chemical backbone upon which withanolide polypharmacology is built, and it is why this type of scaffold has elicited serious attention in covalent drug design

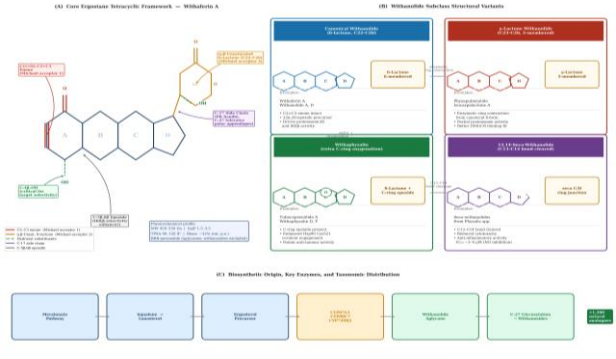

Fig. 1: Scaffold Architecture and Subclass Classification

1.2 Historical Isolation, Ethnopharmacological Context, and Taxonomic Distribution - In 1965, *Withania somnifera* was the first source of withaferin A, which marked the beginning of withanolide chemistry as a science (Bashir *et al*., 2023; Singh *et al*., 2022). A long-standing canonical use of the plant in Ayurvedic medicine was the use of Rasayana drug prescriptions as a rejuvenation and stress adaptation agent and as a promoter of physical and mental health (Bashir *et al*., 2023; Speers *et al*., 2021). This conferred an ethnomedicinal usage with a unifying pharmacological hypothesis and directed further studies on cancer, neurostress decay, and stress-related illness (Bashir *et al*., 2023; Lerose *et al*., 2024; Tewari *et al*., 2022).

There are numerous plants that produce withanolides that lie well beyond Withania. At least 15 genera of Solanaceae have been reported to contain the class, including Physalis, Datura, Nicandra, Jaborosa, Acnistus, and Vassobia (Lerose *et al*., 2024; Singh *et al*., 2022). Structurally novel analogues of Physalis angulata, still under active phytochemical investigation, for example, have caused configurational revisions of previously characterised compounds or prompted redefinition of compounds that have been described previously (Wang *et al*., 2025; Zhang *et al*., 2024). In W. somnifera itself, only differences in A-ring oxidation state, B-ring pattern of epoxidation, lactone ring size, and substitution of the side chain result in more than 300-400 structurally defined withanosides; the family-wide total is more than 1,200 natural analogues (Bashir *et al*., 2023; Singh *et al*., 2022). This chemical variety in actuality is a pre-marked dataset to SAR analysis.

1.3 Scope and Objectives

The majority of published withanolide reviews to date focus on the subject either plant-centred or disease-centred: either surveying the pharmacology of W. somnifera as a medicinal plant (Bashir *et al*., 2023; Speers *et al*., 2021; Tewari *et al*., 2022) or individual leads of withaferin A and withanolide A (singleness, not systematic) in anticancer effects (Singh *et al*., 2022; Tewari *et al*., 2023). The classical method of review that poses the question of how biological outcomes depend on structural features has not been the leading theme of recent literature.

This gap is handled in the present review based on three objectives. First, a critical analysis of the structural implications of changing the ergostane-lactone core with a focus on the two Michael acceptor moieties, the epoxide form, and the oxygenation pattern as peripheral oxygenation (Singh *et al*., 2022). Second, to develop a single structure-activity model of withanolide analogues of natural and synthetic SAR to biology (oncology, neurodegeneration, inflammation, metabolic disease, and infectious disease) and industrial (including polymer) applications (Bashir *et al*., 2023; Singh *et al*., 2022; Tewari *et al*., 2022). Third, to put into perspective the pharmacokinetic information and the concepts of translational compatibility and optimisation of the compounds withanosides and withanolides, referencing quantitative PK findings of withanosides and withanolides, which have been reported in literature (Modi *et al*., 2022; Singh *et al*., 2022). The inclusion criteria are structurally defined natural and synthetic withanolides, quantitative SAR studies using cellular or animal models and pharmacokinetic studies directly meeting withanolide drug-likeness.

2.0 Scaffold Architecture, Classification, and Physicochemical Properties

2.1 Skeletal Architecture and Structural Subclass Classification

The withanolide skeleton is a tetracyclic A/B/C/D skeleton based on steroids and with a C-17 side chain that ends in a lactone. C-22/C-26 oxidation yields a six-membered δ-lactone; C-23/C-26 oxidation yields a five-membered γ-lactone. The difference spatially reacts to the α,β-unsaturation of the cleaving carbonyl, affecting the way the electrophilic centre targets the proteins (Bailly, 2024). Diversification of the core via a tailoring process facilitated by P450 encompasses withaphysalins and physalins that feature dense oxygenation and bicyclic lactones, with the D-ring rearrangement in perulactones as exemplified by peruranolides A-D of Physalis peruviana and the clearing of C-13/C-14 bonds to form 13,14-seco-withanolides. The isolation work on Physalis angulata var. villosa was the expansion method of membership (Wang *et al*., 2025). Each subclass maintains the steroidal topology, estimating local geometry and giving unique pharmacophore presentations.

2.2 Key Pharmacophoric Elements

Most SAR of withanolide can be explained by five features. The main covalent cysteine Michael acceptor in ring A is involved in kinases, chaperones and transcription factor covalent cysteine alkylation (Bailly, 2024; Atteeq, 2022). The d-lactone, an α,β-unsaturated lactone containing a cyclic structure but not an open-chain structure, are intrinsically more reactive to the Michael reaction than an open-chain derivative (Mayer *et al*., 2021), allowing the spatially isolated cysteines of a protein to react one after the other (Bailly, 2024). Hydrogen bond contact-donating ring B oxygenations at C-4β, C-5β, and C-6α provide anti-HIV transcription potent activity in a series of Physalis nicandroides withanolide productions (Taddeo *et al*., 2021). The C-22/C-26 bridge limits the geometry of warheads with respect to the steroid core–δ-to-γ conversion effects, which are found to be selectively adjustable (Bailly, 2024; Mayer *et al*., 2021). C-12, C-14, C-20 and C-27 peripheral substituents adjust the lipophilicity and permeability and not the covalent reactivity (Silva *et al*., 2025; Li *et al*., 2022).

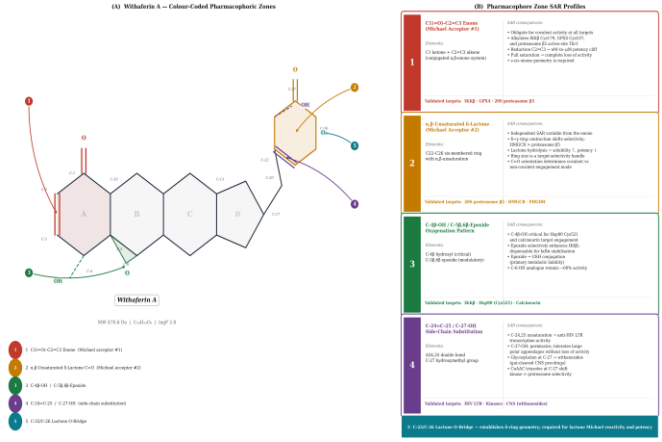

Fig. 2: Annotated Pharmacophore Map of Withaferin A

2.3 Physicochemical Profile and Drug-Likeness- Silica withaferin A, withanolide A/B, and psychrophilic lipophilic withanone display in silico with MW 450-550 Da, logP 1.5-3.5, and TPSA 90-120 Å² (Silva *et al*., 2025). The majority of the analogues are approaching the Lipinski MW ceiling; this is to a certain extent compensated by tetracyclic conformational rigidity. The ability of BBBs to permit certain aglycones, like withaferin A, to enter the cell and not glycosidic withanosides, is dependent on the compounds themselves: C-27 modifications allow CNS penetration to be controlled (Silva *et al*., 2025). According to UHPLC-MS/MS profiling, there is a difference in the oral uptake of aglycone and glycoside forms (Modi *et al*., 2022). It has three metabolic liabilities that prevail: the hydrolysis of lactones eliminates the electrophilic warhead; the 5β,6β-epoxide is conjugated with glutathione; and CYP3A4 is the first-line predicted clearance enzyme (Silva *et al*., 2025). Each is an architectural feature of lead optimisation.

2.4 Biosynthetic Origin and Structural Diversity

Withanolides are products of the mevalonate pathway that uses a sterol precursor which has been modified by a conserved Solanaceae biosynthetic gene group. The sequential oxidations to make the d-lactones are done by CYP87G1 (C-22 hydroxylase), CYP88C7 (C-1 hydroxylase), and CYP749B2 (C-26 oxidase); the subsequent P450s and glycosyltransferases develop subclass oxygenation diversity and withanosides (Hakim *et al*., 2025). Single enzymes have been confirmed by their expression in an assayable form in yeast and Nicotiana benthamiana and can be found as aglycone intermediates (Iani *et al*., 2025). Directing certain tailoring steps selectively (mutation or redirection of the C-5/C-6 epoxidase or redirection of C-24/C-25 oxidation) would access pharmacophoric variants unavailable through semipharmacological methods, resulting in access to targeted SAR libraries.

3.0 Semisynthetic Modifications and Structure–Activity Relationships

3.1 Ring-A Enone Modifications- NF-κB, IKKβ, and proteasome assays are maximally inactive at levels of the C2-C3 enone reduced in analogues of 17β-hydroxywithanolide (Wijeratne *et al*., 2021; Bailly, 2024). This retention is an indication of retention of the d-lactone warhead and the non-covalent binding contacts even in instances where the primary Michael acceptor is damaged. Complete A-ring saturation (elimination of the 1-oxo-2-ene motif) – eliminating covalent cysteine alkylation results in inactive products in cancer and inflammation models (Wijeratne *et al*., 2021; Bailly, 2024). Not only does epoxidation of the enone generate analogues with altered covalent kinetics, but also, in several series with narrowed target selectivity, the balance of reactivity decreases, but selectivity towards specific residues increases (Bailly, 2024). The overall picture of the SAR is clear, as the C2-C3 enone cannot be compromised in terms of proteasome and IKKβ inhibition, but such selective endpoints as anti-inflammation can still accept attenuated A-ring electrophilicity.

3.2 Lactone Ring Modifications

The conversion of the d-lactone to a hydroxy acid increases the aqueous solubility and formulation compatibility, which invariably leads to a decrease in potency, which confirms that the intact electrophilic lactone also contributes to target engagement in addition to solubility effects (Che *et al*., 2024; Wijeratne *et al*., 2021). Carbonyl geometry remains maintained, and the hydrolysis liability is addressed in lactam replacement, but this has not been systematically studied in the withanolide series and needs further research (Che *et al*., 2024). The structural explanation of the variations in selectivity profiles between the series is the biosynthetic precedent of d-to-g ring contraction, which has recently been described as an enzymatic cascade of deletion of atoms in related g-alkylidenebutenolide natural products (Li *et al*., 2025). Lactone geometry is thus a variable of SAR, which can be discontinued with respect to A-ring electronics.

3.3 Hydroxyl Group Functionalisation

C-4β-OH was found to be an essential contact of binding: synthetic C-4β-OH inhibitors always affect antiproliferative efficacy, either through its deletion or reversal (Wijeratne *et al*., 2021). C-27-OH does not act in the same way; it is permissive. Early acetylation and pumpkin spice functionalisation in the late stages at this site regulate selectivity and pharmacokinetic traits devoid of core activity (Che *et al*., 2024; Wijeratne *et al*., 2021). The extremity positioning of polar groups at the C-27 stage enhance solubility in aqueous environments and, in some analogues, promote exposure in vivo (Che *et al*., 2024). The naturally occurring withanosides, C-27 glycosides of withanolide aglycones, prove that this is also a biosynthetic strategy: C-27 glycosylation is a natural method of optimising solubility and stability (Narayanan & Nagegowda, 2024).

3.4 Side-Chain Modifications at C-17, C-20, and C-27

C-20 is antiproliferative constrained: alkylation of this bulky site is antiproliferative and reactive to the 17β-hydroxywithanolide series, with females accompanying an unexplored binding pocket at the d-lactone junction (Wijeratne *et al*., 2021). C-27 is tolerant to polar appendages; amine and amide creation enhance the solubility yet do not disrupt the activity (Che *et al*., 2024; Wijeratne *et al*., 2021). Triazole conjugates at C-27 increased selectivity of CuAAC, resulting in different kinase inhibition patterns in comparison to the original withanolide, which supported their use as a selectivity handle in target bias without affecting the dual Michael acceptor core (Wijeratne *et al*., 2021).

Table 1. Representative Withanolide Structural Classes and Pharmacological Activities

Compound / Class	Plant Source	Key Structural Feature(s)	Primary Activity / Target	Reference
Withaferin A (WA)	*Withania somnifera*	C1(=O)–C2=C3 enone; C5β,6β-epoxide; δ-lactone (ring E)	Proteasome β5 inhibition; IKKβ (Cys179) and GPX4 covalent alkylation; HSP90 C-terminal binding	Atteeq, 2022; Bailly, 2024
Ring A/B-modified 17β-hydroxywithanolide analogues	W. somnifera (semisynthetic)	C3-OH variation; ring A/B saturation; 17β-OH retained	Antiproliferative (prostate, renal, melanoma); immunotherapy potentiation	Wijeratne *et al*., 2021
HIV-inhibitory withanolides	Physalis nicandroides	Variable C-4β-OH; C-20/22 unsaturation; epoxide ±	HIV transcription inhibition (LTR-luciferase reporter)	Taddeo *et al*., 2021
Tubocapsenolide A	Tubocapsicum anomalum	α,β-Unsaturated δ-lactone; no C5β,6β-epoxide	HSP90 C-terminal Cys521 covalent engagement; anti-tumour	Zhu *et al*., 2021
Semisynthetic library (divergent platform)	W. somnifera (total synthesis base)	Late-stage C–H functionalisation at C-4, C-17, C-27	Divergent target profiles across ring-modified analogues	Che *et al*., 2024

Note. WA = Withaferin A; GPX4 = glutathione peroxidase 4; HSP90 = heat-shock protein 90; IKKβ = IκB kinase β; LTR = long terminal repeat. Plant names italicised in original manuscript. References: Atteeq (2022) Front. Pharmacol.; Bailly (2024) Biochem. Pharmacol.; Wijeratne *et al*. (2021) J. Nat. Prod.; Taddeo *et al*. (2021) J. Nat. Prod.; Zhu *et al*. (2021) Pharmacol. Res.; Che *et al*. (2024) Sci. Adv.

3.5 Scaffold Hybridization

Curcumin conjugates and chalcone tethers have been described, and they almost all exhibit cytotoxicity in the cancer cell lines (Che *et al*., 2024; Bailly, 2024). The recurring weakness is mechanistic: experiments generally do not have a dual-target validation and therefore do not provide covalent cyperimethrin permiocrine ping of the withanolide warhead nor any orthogonal engagement activity of the conjugated pharmacophore. Compounding permeability and metabolic stability concerns Street and permiocrine molecular weights are routinely more than 650 Da. Recruitment dual-mechanism confirmation should be the minimum reporting requirement in any future claim of a hybrid withanolide.

3.6 Glycosidic Withanolides and Prodrug Design

Withanosides IV and V represent C-27-glycosylated withanolides with less acute cytotoxicity compared to their aglycones (Narayanan and Nagegowda, 2024). Gut glycosidases to cut off the sugar residue and restore the active aglycone set a precedent of such natural prodrugs (Che *et al*., 2024). The hypothetical proposal that the selective intratumoral unmasking of the dual Michael acceptor scaffold could be achieved by the mechanism of differential glycosidase expression of tumour tissue has not been addressed in a study of withanolide-specific prodrugs. Another, little-studied but still logically relevant area of tumour-targeted delivery is engineered glycosidase-activated withanolide prodrugs.

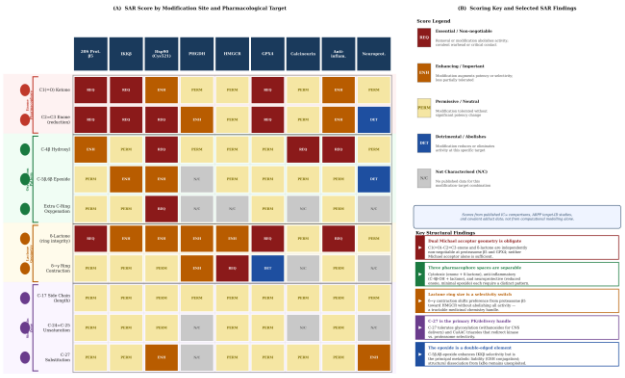

Fig. 3: SAR Heat Map across Modification Sites

4.0 Structure–Activity Relationships at Defined Pharmacological Targets

4.1 Anticancer Targets

4.1.1 Proteasome (β5 Subunit) Inhibition- The active site of the 20S proteasome is covalently blocked by withaferin A and δ-lactone withanolides via covalent groups of the C2-C3 enone, and the δ-lactone recognises the active site non-covalently (Atteeq, 2022; Bailly, 2024). A several-fold increase in activity is mediated by the canine enzyme transferrin enone saturation proportional to nanomolar to micromolar conversion; γ-lactone analogues remain active, which shows that electrophilicity and lactone geometry have an independent contribution (Wijeratne *et al*., 2021; Singh *et al*., 2022). This is the best characterised withanolide-target interaction that is yet to be surpassed in mechanistic rigour in the entire class.

4.1.2 NF-κB Pathway: IKKβ Inhibition and IκBα Stabilization

Cys179 of IKKβ potency (Tewari *et al*., 2022; Singh *et al*., 2022). Such a structural dissociation between pairs of nodes of one pathway is not used in the analogue design – a mechanistically biassed product may be produced by epoxide-selective modification without any obligatory utilisation of two-node interactions.

4.1.3 Hsp90 Chaperone Complex Disruption

Tubocapsenolide A, which is a withanolide-type steroidal lactone, is found in the covalently modified Cys521 of the Hsp90 middle domain (Zhu *et al*., 2021). C-4α-OH and the A-ring enone are the minimal pharmacophore, and C-27 chemical modifications are well tolerated, with non-significant reductions in potency (Bailly, 2024; Bashir *et al*., 2023). Weak proteasome inhibitor analogues are able to preserve Hsp90-disrupting activity and confirm that selective covalent confrontation can occur within this scaffold regardless of the shared pharmacophoric circumstances.

4.1.4 Oncometabolic Enzyme Inhibition: PHGDH, HMGCR, and KGA

PHGDH is bound by ixocarpalactone A at an allosteric location, validated by DARTS and microscale thermophoresis; and withangulatin A controls the cys295 site of the same enzyme by both reversible allosteric and covalent interaction by ABPP and LC-MS. Both of these enzymatic mechanisms are accessible on a single target (Silva *et al*., 2025). The affinity of physapubenolide to HMGCR is stronger than lovastatin by surface plasmon resonance; KGA is directly interacting with physapubescin (Silva *et al*., 2025; Singh *et al*., 2022). Each of the three targets has biochemical validation, but there is no systematic analogue-level SAR follow-up of each of them – the most obvious gap to date in the field.

4.1.5 GPX4 Inhibition and Ferroptosis

Instead, Withaferin A chemically reacts with Cys107 of GPX4 to induce ferroptotic cell death using a different pathway than caspase-dependent cell death (Silva *et al*., 2025; Bailly, 2024). The interaction with GPX4 and IKKβ both involves the intact enone, whereas no structural selectors of the two different targets could be determined – an unsolved selectivity issue to any GPX4-directed analogue campaign out of this scaffold.

4.2 Anti-inflammatory Activity

It is essential to COX-2-inhibited C-3-OH; the effects of acetylation or deacetylation are always less potent (Singh *et al*., 2022; Tewari *et al*., 2022). iNOS suppression needs lactone integrity, and A-ring alteration is tolerated better in this case than in cytotoxicity assays – a convergent pharmacophore change which has a direct impact on selectivity engineering. The most educative fact is the seco-withanolides: these are the compounds that do not have a complete A/B ring junction, which inhibit the production of NO with approximately the IC50 of 3-4 μm (Che *et al*., 2024). The steroidal fused core, thus, is not crucial to anti-inflammatory action, and structurally different, non-toxic pharmacophore space is provably available.

4.3 Neuroprotective Activity- The Withanoside IV and Withinolide A stimulate the growth of neurites using a putative NGF-mimetic mechanism in PC-12 cells; the exact saccharide involved is still unknown (Lerose *et al*., 2024; Bashir *et al*., 2023). Neuroprotective-active analogues are similar in MW (less than 450 Da), have permeability and differ from cytotoxic withanolides structurally (Lerose *et al*., 2024). No specific SAR initiative has been mentioned around this pharmacophore space, even though the structural foundation of one has been obvious.

4.4 Immunomodulatory and Antimicrobial Activity- Withinolide D also inhibits T-cell activation by the mechanism of calcineurin inhibition; the C-ring hydroxylation pattern defines the process of immunosuppressive or cytotoxic selectivity in this series of proteins (Bashir *et al*., 2023; Singh *et al*., 2022). C-3-ketone and C-6-epoxide are selectivity determinants between mammalian and microbial cell targets in antileishmanial and antimicrobial activities (Taddeo *et al*., 2021; Singh *et al*., 2022), although the mechanistic resolution method is much lower in comparison to the validated mammalian enzyme targets. The central covalent warhead is the A-ring enone, as was identified in all targets examined. Electrophilicity and ring rigidity lowering preserve anti-inflammatory and neuroprotective activities and support the notion that cytotoxic, anti-inflammatory, and neuroprotective pharmacophores of the withanolide fold-back are structurally isolable and available separately to facilitate selective optimisation.

Table 2. Validated Molecular Targets, Engagement Modes, and Assay Evidence

Target	Engagement Mode	Key Residue (s)	Representative Compound	Assay / Evidence	Reference
Proteasome β5	Covalent (irreversible)	Thr1 (N-terminal)	Withaferin A	Proteasome activity assay; ubiquitinated substrate accumulation	Atteeq, 2022; Bailly, 2024
IKKβ	Covalent	Cys179 (activation loop)	Withaferin A	Kinase inhibition; IκBα stabilisation; NF-κB reporter	Bailly, 2024
HSP90 (C-terminal)	Covalent	Cys521	Tubocapsenolide A; Withaferin A	ABPP; thermal shift; client protein degradation	Zhu *et al*., 2021; Bailly, 2024
GPX4	Covalent	Cys46 / Cys107	Withaferin A	ABPP; ferroptosis induction; lipid peroxidation assay	Bailly, 2024
Vimentin	Covalent	Cys328	Withaferin A	Fluorescent probe pull-down;	Bailly, 2024

				ABPP; filament disassembly imaging	
Multiple Cys targets (promiscuous)	Covalent (electrophile)	Proteome-wide Cys	Withaferin A (broad)	Quantitative isoTOP-ABPP; GSH competition; thiol reactivity profiling	Bailly, 2024; Mayer & Ofial, 2021

Note. ABPP = activity-based protein profiling; Cys = cysteine; GPX4 = glutathione peroxidase 4; GSH = glutathione; HSP90 = heat-shock protein 90; IKKβ = IκB kinase β; isoTOP-ABPP = isotopic tandem orthogonal proteolysis ABPP. References: Atteeq (2022) Front. Pharmacol.; Bailly (2024) Biochem. Pharmacol.; Zhu *et al.* (2021) Pharmacol. Res.; Mayer & Ofial (2021) Angew. Chem. Int. Ed.

5.0 Mechanisms of Target Engagement- Interaction of withanolide-type Michael acceptors with targets is an indication of selective covalent chemistry, non-covalent recognition, and purposeful polypharmacology, as opposed to random alkylation of thiols in cells.

5.1 Covalent Interaction through Michael addition- With physiological conditions, the main covalent reaction is the addition of thiol to an α,β-unsaturated carbon system, which forms a stable C-S bond with protein cysteines (Bailly, 2024; Yan *et al*., 2024). Michael acceptor-cysteine reactions modelled by QM/MM display that thiol addition follows a concerted proton transfer/nucleophilic attack reaction to an enolate form with solvent-assisted tautomerisation as the rate-limiting step instead of a two-step base-catalysed reaction (Yan *et al*., 2024). Intrinsic electrophilicity is not sufficient to determine covalent selectivity because chemical competition experiments with chemoproteomics and site-resolved LC-MS/MS mapping indicate that binding-site geometry and local cysteine microenvironment control the preferential labelling of proteins, which has been shown to be true in systematic studies of cysteine-reactive covalent probes of varying size (Kim *et al*., 2025; Petri *et al*., 2025). In the case of withanolides in particular, withaferin A also displays ABPP profiling of withaferin A in all three classes of kinases, chaperones, and metabolic enzymes, indicating that the biological effects are not a result of random thiol alkylation of proteins (Bailly, 2024). Both spatially separated cysteines on a protein (or translationally orientated cysteines on a substrate) can be sequentially or parallelly engaged with the use of the dual Michael acceptor architecture (A-ring enone and α,β-unsaturated δ-lactone). ABBP competition, pulled-down, and LC-MS/MS direct covalent adduct detection are always required to establish the relationship of each cysteine modification to a specific phenotype (Mons *et al*., 2023).

5.2 Non-Covalent Target Interactions- All interactions withanolide targets are not always covalent. The ability to bind HMGCR with physapubenolide through surface plasmon resonance with KD values of 1/T indicates that classical non-covalent interaction is available in this scaffold (Silva *et al*., 2025). Hydrogen bonds between C-4-OH or C-27-OH and the polar residues along with hydrophobic burial of the rigid tetracyclic steroidal core in non-polar binding pockets and pi-stacking or dipolar contacts between the lactone and enone carbonyls are all stabilising interactions in such complexes (Bailly, 2024; Silva *et al*., 2025). Producing other allosteric interactions like the one of ixocarpalactone A with PHGDH, non-covalent pre-organisation of the ligand inside the binding site is a requirement to engage with the target productively, confirmed by DARTS and microscale thermophoresis (Silva *et al*., 2025). The binding of docking is only interpretative when orthogonal biophysical techniques like ITC, SPR, or MST are used to support the results; to a greater extent, the docking-derived binding can be enhanced by molecular dynamics simulations but is still requires experimental validation (Mons *et al*., 2023; Valdes-Albuernes *et al*., 2024).

5.3 Multi-Target Pharmacology: Mechanistic Implications- Polypharmacology is a cystinome. A cystinome is obtained by promiscuous interactions (Stefan and Rafehi, 2023; Abdelsayed, 2025). The concept of multi-target engagement is able to conquer redundancy of pathways and acquired resistance, whereas detrimental outcomes can be observed in case anti-targets occupy the same ligandable cysteine environment as the target proteins (Ryszkiewicz *et al*., 2025; Doostmohammadi *et al*., 2024). ABPP hitlists, docking output, and omics data are employed together in networks of compound-target-pathways and direct the structural adjustments that preferably engage the target of interest and have minimal anti-target liability, which will be achieved using network pharmacology and AI technologies (Abdelsayed, 2025; Cichonska *et al*., 2024; Bi *et al*., 2025). Such computational models are effective organisational aids; however, they cannot replace a direct experimental validation with covalent site mapping, kinetic characterisation and direct functional target engagement assays, which are capable of deciding individual engagements as therapeutic or intoxicating phenotypes (Bailly, 2024; Kim *et al*., 2025; Yan *et al*., 2024).

6.0 Translational Barriers

Although there has been a great deal of preclinical work and a number of early clinical trials, not one of the withanolides has been able to pass Phase II. Such a deficiency is always explained by a lack of unmet pharmacokinetics, safety characterisation, and disciplined lead optimisation issues as opposed to a lack of biological rationale (Devabattula *et al*., 2023; Zhang *et al*., 2024; Singh *et al*., 2022).

6.1 Pharmacokinetics and Metabolic Stability

Oral withaferin A has about 32% bioavailability in rats at 10 mg/kg and is quickly cleared in intestine-liver perfusion because extensive first-pass clearance occurs before the systemic exposure is achieved (Modi *et al*., 2022; Silva *et al*., 2025). The oxidation and Phase II conjugation at the lactone and epoxide sites through CYP3A4 are the main metabolic liabilities (Modi *et al*., 2022; Bailly, 2024). These structures also act as toxicophores to produce reactive intermediates which provoke breakage of metabolic stability and off-target covalent overload (Devabattula *et al*., 2023; Bailly, 2024). The intestinal absorption and plasma exposure have been enhanced in the animal models through the use of nanoformulation methodologies such as the PLGA nanoparticles, liposomes, and cyclodextrin inclusion complexes (Alqahtani *et al*., 2021; Sarabia-Vallejo *et al*., 2023; Raza *et al*., 2021). Such systems are still at the exploratory level and do not meet the manufacturing uniformity, stability, and regulatory reports needed in the submissions of IND (Devabattula *et al*., 2023; Singh *et al*., 2022).

6.2 Selectivity, Safety and Structural Alerts

The 5β,6β-epoxide and the dual Michael acceptor architecture are known structural red flags of non-specific cysteine alkylation and liability to adverse drug reactions (Bailly, 2024; Silva *et al*., 2025). ADMET profiling detecting cardiotoxicity and hepatotoxicity signals in the chosen analogues In silico Ashwagandha extract use has revealed itself to cause liver toxicity in strongly dosed studies in humans (Devabattula *et al*., 2023; Silva *et al*., 2025). Systematic hERG channel and cardiac safety counter-screening, a typical attrition filter used in covalent drug programmes, has never been utilised in a systematic manner in any of the withanolide analogue series (Sun *et al*., 2022; Miyashita *et al*., 2024). None of the published programs have available a specific off-target cysteine counter-screen or specific therapeutic window in normal and disease tissue.

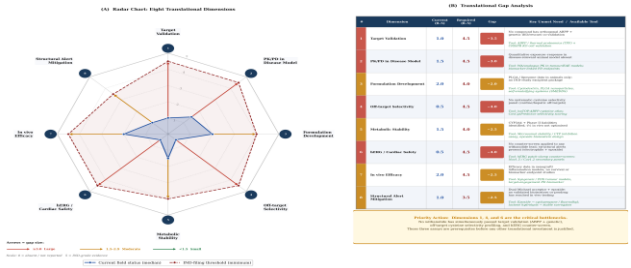

Fig. 5: Translational Gap Radar Chart

6.3 The Missing Development Architecture- The reason why withinolides in preclinical development have not been advanced to a single programme is not due to poor biology but four translational assets (a validated primary target with chemoproteomics and genetic support; a PK/PD relationship measured in disease-relevant models correlating drug exposure with target modulation and T endpoints) (Devabattula *et al*., 2022). The instruments to overcome all the barriers are available, including predictive computational cysteine selectivity, simulation pipelines of the ADMET and the engineering of nanoformulations (Reimer *et al*., 2025; Chehelgerdi *et al*., 2023; Sarabia-Vallejo *et al*., 2023). This is lacking in favour of a sustained, purpose-orientated medicinal chemistry initiative based on a single, or a few, best-in-class targets instead of comprehensive, perpetual floribundance exploration of the natural and semisynthetic withanolide space with only limited safety information (Zhang *et al*., 2024; Devabattula *et al*., 2023).

Table 3. Translational Barriers, Current Status, and Mitigation Strategies

Barrier	Molecular Basis	Current Status	Mitigation Strategy	Reference
Metabolic instability	C5β,6β-epoxide ring-opening by GSH; CYP3A4 side-chain oxidation	Liabilities characterised; no analogue with improved in vivo t½ reported	Cyclopropane/fluoroalkyl epoxide bioisosteres; microsomal stability-guided optimisation	Mayer & Ofial, 2021; Devabattula *et al*., 2023
Poor oral bioavailability	Aqueous solubility <1 µg/mL; rapid first-pass catabolism	PLGA/liposome data in animals; no IND-ready oral excipient package	Cyclodextrin complexes; PLGA nanoparticles; SMEDDS formulations	Raza *et al*., 2021; Sarabia-Vallejo *et al*., 2023
Off-target cysteine reactivity	Dual Michael acceptor engages multiple proteome cysteines	No systematic cysteine selectivity panel applied to any withanolide lead	isoTOP-ABPP cysteine atlas; CovCysPredictor selectivity scoring	Boike *et al*., 2022; Reimer *et al*., 2025
Absent orthogonal target validation	No compound co-validated by ABPP and genetic (CRISPR KO/rescue) evidence	Nominations based on biochemical/cell assays; CETSA/TPP rarely applied to withanolides	ABPP + CETSA or TPP combined; CRISPR KO/rescue confirmation	Tu *et al*., 2023; Song, 2025
Drug supply and scalability	Low natural abundance; complex scaffold requires multistep synthesis	Divergent synthesis demonstrated; biosynthetic gene cluster identified in Solanaceae	Metabolic engineering of host plants; scalable divergent synthesis; plant cell culture	Che *et al*., 2024; Hakim *et al*., 2025; Ahmad *et al*., 2024

Note. ABPP = activity-based protein profiling; CETSA = cellular thermal shift assay; CYP3A4 = cytochrome P450 3A4; GSH = glutathione; IND = investigational new drug; PLGA = poly(lactic-co-glycolic acid); SMEDDS = self-microemulsifying drug delivery system; t½ = half-life; TPP = thermal proteome profiling. References: Mayer & Ofial (2021) Angew. Chem. Int. Ed.; Devabattula *et al*. (2023) Planta Med.; Raza *et al*. (2021) Int. J. Pharm.; Sarabia-Vallejo *et al*. (2023) Pharmaceutics; Boike *et al*. (2022) Nat. Rev. Drug Discov.; Reimer *et al*. (2025) J. Chem. Inf. Model.; Tu *et al*. (2023) Phytomedicine; Song (2025) Int. J. Mol. Sci.; Che *et al*. (2024) Sci. Adv.; Hakim *et al*. (2025) Nat. Commun.; Ahmad *et al*. (2024) Plants

7.0 Future Directions

7.1 Target Discovery through chemoproteomics- ABPP, DARTS, CETSA, and thermal proteome profiling can be recognised as an established system of tools that allow unbiased target identification of natural products and have directly facilitated target designation of a range of plant metabolites, such as withanolides (Chen *et al*., 2022; Song, 2025; Tu *et al*., 2023). MPlexed TPP and TMT or data-independent acquisition quantification could be systematically applied to previously unexplored subgroups of the validated target, including physalins, ixocarpalactones, and seco-withanolides (George *et al*., 2023; Koudelka *et al*., 2025; Peters-Clarke *et al*., 2024). These orthogonal pathways to target validation, coupling chemoproteomic hit lists to PROTAC-mediated protein clearance as a functional readout, offer another orthogonal avenue to target validation.

7.2 Optimization of AI and Computational SAR- It is demonstrated that graph neural networks that are trained on withanolide and steroidal lactone sets have the ability to learn non-linear SAR and ADMET profiles which classical regression could not (Kensert *et al*., 2024; Wu *et al*., 2023). Diffusion architectures and transformers are generative models, which allow systematic exploration of withanolide-inspired chemical space beyond natural product space, after accounting for synthesizability constraints, which can immediately be used to address the gaps of pharmacophores identified in Sections 3 and 4 (Anstine and Isayev, 2023; Gao *et al*., 2025). Silico warhead reactivity screening can be used to design calibrated cysteine electrophilicity pre-screening designs on systematic covalent warhead kinase analyses (Zhao & Bourne, 2024).

7.3 Targeted Protein Degradation- Reported covalent interactions with oncoproteins set withanolides as prospective warheads in PROTAC and molecular glue foldamers, and existing E3-ligase recruitment chemistry to finish bifunctional degrader architectures can be used to execute this (Bekes *et al*., 2022; Li and Crews, 2022; Dale *et al*., 2021; Bond and Crews, 2021). Intramolecular bivalent glue systems go further to form productive ternary assemblies of degraders, extending withanolide targeting transcription factors and other targets that cannot be targeted with the direct occupancy (Hsia *et al*., 2023; Eladl, 2025).

7.4 Biosynthetic Engineering and Versatile Availability- The development of analogue libraries is limited by variability of sup gives, giving an opportunity to support heterologous production platforms producing SAR-critical analogues at desired concentrations and qualities (Hakim *et al*., 2025; Ahmad *et al*., 2024). The optimisation of metabolic fluxes under the guidance of machine learning and CRISPR-guided gene editing will be needed to complete AI-based SAR and degrader campaigns with semisynthetic pathways to unnatural variants (Ahmad *et al*., 2024; Hakim *et al*., 2025).

Conclusion

Withinolides hold a strategically advantageous role in the natural product drug discovery. The constrained dual Michael acceptor geometry, ergostane tetracyclic core and pre-organised structure of hydrogen-bond donors and acceptors in over 1,200 natural analogues make up a chemical framework whose pharmacological richness remains undervalued. This review has followed the manner individual structural elements are converted to specific biological effects: the C2-C3 enone is the non-negotiable covalent warhead of proteasome, IKK-beta and GPX4 inhibition; the δ-lactone is an independent regulator of potency and target selectivity; peripheral hydroxylation is the major determinant of target preference at Hsp90, calcineurin and the anti-inflammatory vs. cytotoxic drug target paradigm; and the C-17 side chain is the main handle to tune pharmacokinetics. The main medicinal chemistry discovery of this work is the structural separability of these pharmacophoric elements: cytotoxic, anti-inflammatory and neuroprotective functions are not inseparable characteristics but design goals that can be attained independently. It is programmatic against this proven biology on which the translational deficit is founded. None of the withanolides have progressed using the sequential steps of chemoproteomic target validation, quantitative PK/PD characterisation, formulation development and structured off-target profiling, leading to a plausible IND-enabling package. The facilitating technologies, which include activity-based protein profiling, thermal proteome profiling, AI-guided analogue design, and biosynthetic gene cluster engineering, are now of a mature nature. Belonging to the field, however, is not about increased biological activity but rather about having the intellectual discipline that a handful of best-characterised candidates should be pursued with the intensity that the quality of the underlying science requires.

References

Abdelsayed, M. (2025). AI-driven polypharmacology in small-molecule drug discovery. International Journal of Molecular Sciences, 26. https://doi.org/10.3390/ijms26146996

Ahmad, Z., Ganie, I. H., Firdaus, F., Ramakrishnan, M., Shahzad, A., & Ding, Y. (2024). Enhancing withanolide production in the Withania species: Advances in in vitro culture and synthetic biology approaches. Plants, 13. https://doi.org/10.3390/plants13152171

Alqahtani, M. S., Kazi, M., Alsenaidy, M. A., & Ahmad, M. Z. (2021). Advances in oral drug delivery. Frontiers in Pharmacology, 12. https://doi.org/10.3389/fphar.2021.618411

Anstine, D. M., & Isayev, O. (2023). Generative models as an emerging paradigm in the chemical sciences. Journal of the American Chemical Society, 145, 8736–8750. https://doi.org/10.1021/jacs.2c13467

Atanasov, A. G., Zotchev, S. B., Dirsch, V. M., & Supuran, C. T. (2021). Natural products in drug discovery: Advances and opportunities. Nature Reviews Drug Discovery, 20, 200–216. https://doi.org/10.1038/s41573-020-00114-z

Atteeq, M. (2022). Evaluating anticancer properties of Withaferin A—a potent phytochemical. Frontiers in Pharmacology, 13, 975320. https://doi.org/10.3389/fphar.2022.975320

Bailly, C. (2024). Covalent binding of withanolides to cysteines of protein targets. Biochemical Pharmacology, 116405. https://doi.org/10.1016/j.bcp.2024.116405

Bashir, A., Nabi, M., Tabassum, N., Afzal, S., & Ayoub, M. (2023). An updated review on phytochemistry and molecular targets of *Withania somnifera* (L.) Dunal (Ashwagandha). Frontiers in Pharmacology, 14, 1049334. https://doi.org/10.3389/fphar.2023.1049334

Békés, M., Langley, D. R., & Crews, C. M. (2022). PROTAC targeted protein degraders: The past is prologue. Nature Reviews Drug Discovery, 21, 181–200. https://doi.org/10.1038/s41573-021-00371-6

Bi, X., Wang, Y., Wang, J., & Liu, C. (2025). Machine learning for multi-target drug discovery: Challenges and opportunities in systems pharmacology. Pharmaceutics, 17. https://doi.org/10.3390/pharmaceutics17091186

Boike, L., Henning, N. J., & Nomura, D. K. (2022). Advances in covalent drug discovery. Nature Reviews Drug Discovery, 21, 881–898. https://doi.org/10.1038/s41573-022-00542-z

Bond, M. J., & Crews, C. M. (2021). Proteolysis targeting chimeras (PROTACs) come of age: Entering the third decade of targeted protein degradation. RSC Chemical Biology, 2, 725–742. https://doi.org/10.1039/d1cb00011j

Che, W., Wojitas, L., Shan, C., & Lopchuk, J. M. (2024). Divergent synthesis of complex withanolides enabled by a scalable route and late-stage functionalization. Science Advances, 10. https://doi.org/10.1126/sciadv.adp9375

Chehelgerdi, M., Chehelgerdi, M., Allela, O. Q. B., Pecho, R. D. C., Jayasankar, N., Rao, D. P., Thamaraikani, T., Vasanthan, M., Polák, V., Lakshmaiya, N., Saadh, M. J., Amajd, A., Abo-Zaid, M. A., Castillo-Acobo, R. Y., Ismail, A. H., Amin, A. H., & Akhavan-Sigari, R. (2023). Progressing nanotechnology to improve targeted cancer treatment: Overcoming hurdles in its clinical implementation. Molecular Cancer, 22. https://doi.org/10.1186/s12943-023-01865-0

Chen, F., Li, C., Cao, H., Zhang, H., Lu, C., Li, R., Zhu, Z., Chen, L., & Zhao, Y. (2022). Identification of adenylate kinase 5 as a protein target of ginsenosides in brain tissues using mass spectrometry-based DARTS and CETSA techniques. Journal of Agricultural and Food Chemistry. https://doi.org/10.1021/acs.jafc.1c07819

Cichońska, A., Ravikumar, B., & Rahman, R. (2024). AI for targeted polypharmacology: The next frontier in drug discovery. Current Opinion in Structural Biology, 84, 102771. https://doi.org/10.1016/j.sbi.2023.102771

Dale, B., Cheng, M., Park, K.-S., Kaniskan, H. Ü., Xiong, Y., & Jin, J. (2021). Advancing targeted protein degradation for cancer therapy. Nature Reviews Cancer, 21, 638–654. https://doi.org/10.1038/s41568-021-00365-x

Devabattula, G., Panda, B., Yadav, R., & Godugu, C. (2023). The potential pharmacological effects of natural product Withaferin A in cancer: Opportunities and challenges for clinical translation. Planta Medica. https://doi.org/10.1055/a-2289-9600

Doostmohammadi, A., Jooya, H., Ghorbanian, K., Gohari, S., & Dadashpour, M. (2024). Potentials and future perspectives of multi-target drugs in cancer treatment: The next generation anti-cancer agents. Cell Communication and Signaling, 22. https://doi.org/10.1186/s12964-024-01607-9

Eladl, O. (2025). Molecular glues and PROTACs in targeted protein degradation: Mechanisms, advances, and therapeutic potential. Biochemical Pharmacology, 117297. https://doi.org/10.1016/j.bcp.2025.117297

Gao, W., Luo, S., & Coley, C. W. (2025). Generative AI for navigating synthesizable chemical space. Proceedings of the National Academy of Sciences, 122. https://doi.org/10.1073/pnas.2415665122

George, A., Sidgwick, F. R., Watt, J. E., Martin, M. P., Trost, M., Marín-Rubio, J. L., & Dueñas, M. E. (2023). Comparison of quantitative mass spectrometric methods for drug target identification by thermal proteome profiling. Journal of Proteome Research, 22, 2629–2640. https://doi.org/10.1021/acs.jproteome.3c00111

Hakim, S., Choudhary, N., Malhotra, K., Peng, J., Bültemeier, A., Arafa, A., Friedhoff, R., Bauer, M., Eikenberg, J., Witte, C., Herde, M., Heretsch, P., Pucker, B., & Franke, J. (2025). Phylogenomics and metabolic engineering reveal a conserved gene cluster in Solanaceae plants for withanolide biosynthesis. Nature Communications, 16. https://doi.org/10.1038/s41467-025-61686-1

Hsia, O., Hinterndorfer, M., Cowan, A. D., Iso, K., Ishida, T., Sundaramoorthy, R., Nakasone, M. A., Imrichová, H., Schätz, C., Rukavina, A., Husnjak, K., Wegner, M., Correa-Sáez, A., Craigon, C., Casement, R., Maniaci, C., Testa, A., Kaulich, M., Dikić, I., … Ciulli, A. (2023). Targeted protein

degradation via intramolecular bivalent glues. Nature, 627, 204–211. https://doi.org/10.1038/s41586-024-07089-6

Kensert, A., Desmet, G., & Cabooter, D. (2024). A hands-on tutorial on quantitative structure-activity relationships using fully expressive graph neural networks. Analytica Chimica Acta, 1331, 343046. https://doi.org/10.1016/j.aca.2024.343046

Kim, G. Y., Grams, R. J., & Hsu, K.-L. (2025). Advancing covalent ligand and drug discovery beyond cysteine. Chemical Reviews, 125, 6653–6684. https://doi.org/10.1021/acs.chemrev.5c00001

Koudelka, T., Bassot, C., & Piazza, I. (2025). Benchmarking of quantitative proteomics workflows for limited proteolysis mass spectrometry. Molecular and Cellular Proteomics, 24. https://doi.org/10.1016/j.mcpro.2025.100945

Lerose, V., Ponticelli, M., Benedetto, N., Carlucci, V., Lela, L., Tzvetkov, N. T., & Milella, L. (2024). *Withania somnifera* (L.) Dunal, a potential source of phytochemicals for treating neurodegenerative diseases: A systematic review. Plants, 13(6), 771. https://doi.org/10.3390/plants13060771

Li, K., & Crews, C. M. (2022). PROTACs: Past, present and future. Chemical Society Reviews. https://doi.org/10.1039/d2cs00193d

Li, Y., *et al*. (2022). Peruranolides A–D: New withanolide-type steroids from Physalis peruviana. Natural Product Research.

Li, Y., *et al*. (2025). Biosynthetic ring contraction in withanolide-type steroidal lactones. Organic Letters.

Mayer, R. J., & Ofial, A. R. (2021). Nucleophilic reactivities of glutathione and related thiols toward Michael acceptors. Angewandte Chemie International Edition, 60, 2966–2970. https://doi.org/10.1002/anie.202012347

Misakyan, M., Wijeratne, E. M. K., Issa, M., Xu, Y., Monteillier, A., Gunatilaka, A. A. L., & Cuendet, M. (2021). Structure-activity relationships of withanolides as antiproliferative agents for multiple myeloma: Comparison of activity in 2D models and a 3D coculture model. Journal of Natural Products, 84(8), 2321–2335. https://doi.org/10.1021/acs.jnatprod.1c00446

Miyashita, Y., Moriya, T., Kato, T., Kawasaki, M., Yasuda, S., Adachi, N., Suzuki, K., Ogasawara, S., Saito, T., Senda, T., & Murata, T. (2024). Improved higher resolution cryo-EM structures reveal the binding modes of hERG channel inhibitors. Structure. https://doi.org/10.1016/j.str.2024.08.021

Modi, S., Tiwari, A., Ghule, C., Pawar, S., Saste, G., Jagtap, S., Singh, R., Deshmukh, A., Girme, A., & Hingorani, L. (2022). Pharmacokinetic study of withanosides and withanolides from *Withania somnifera* using ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). Molecules, 27(5), 1476. https://doi.org/10.3390/molecules27051476

Mons, E., Kim, R. Q., & Mulder, M. P. C. (2023). Technologies for direct detection of covalent protein–drug adducts. Pharmaceuticals, 16. https://doi.org/10.3390/ph16040547

Narayanan, S., & Nagegowda, D. A. (2024). Biosynthesis, transport, and accumulation of withanolides in *Withania somnifera*. Plant Cell Reports. https://doi.org/10.1007/s00299-024-03165-3

Peters-Clarke, T. M., Liang, Y., Mertz, K., Lee, K.-J., Westphall, M. S., Hinkle, J. D., McAlister, G. C., Syka, J. E. P., Kelly, R. T., & Coon, J. J. (2024). Boosting the sensitivity of quantitative single-cell proteomics with infrared-tandem mass tags. Journal of Proteome Research. https://doi.org/10.1021/acs.jproteome.4c00076

Petri, L., Gabizon, R., Ferenczy, G. G., Péczka, N., Egyed, A., Ábrányi-Balogh, P., Takács, T., & Keserű, G. M. (2025). Size-dependent target engagement of covalent probes. Journal of Medicinal Chemistry, 68, 6616–6632. https://doi.org/10.1021/acs.jmedchem.5c00017

Raza, A., Miles, J. J., Sime, F. B., Ross, B. P., Roberts, J. A., Popat, A., Kumeria, T., & Falconer, J. R. (2021). PLGA encapsulated γ-cyclodextrin–meropenem inclusion complex formulation for oral delivery. International Journal of Pharmaceutics, 120280. https://doi.org/10.1016/j.ijpharm.2021.120280

Reimer, B., Awoonor-Williams, E., Golosov, A., & Hornak, V. (2025). CovCysPredictor: Predicting selective covalently modifiable cysteines using protein structure and interpretable machine learning. Journal of Chemical Information and Modeling. https://doi.org/10.1021/acs.jcim.4c01281

Ryszkiewicz, P., Malinowska, B., & Schlicker, E. (2025). Polypharmacology: New drugs in 2023–2024. Pharmacological Reports, 77, 543–560. https://doi.org/10.1007/s43440-025-00715-8

Sarabia-Vallejo, Á., Caja, M. M., Olives, A. I., Martín, M. A., & Menéndez, J. C. (2023). Cyclodextrin inclusion complexes for improved drug bioavailability and activity: Synthetic and analytical aspects. Pharmaceutics, 15. https://doi.org/10.3390/pharmaceutics15092345

Silva, G. W. D. S. E., Marques, A., & Sampaio, A. (2025). Anticancer effects of withanolides: In silico prediction of pharmacological properties. Molecules, 30, 2457. https://doi.org/10.3390/molecules30112457

Singh, A., Raza, A., Amin, S., Damodaran, C., & Sharma, A. (2022). Recent advances in the chemistry and therapeutic evaluation of naturally occurring and synthetic withanolides. Molecules, 27(3), 886. https://doi.org/10.3390/molecules27030886

Smith, E., Vishwakarma, D., Sun, S., Moorhouse, A. D., Tuveson, D. A., & Moses, J. E. (2025). Click chemistry for natural product-inspired covalent drug discovery. Drug Discovery Today, 104500. https://doi.org/10.1016/j.drudis.2025.104500

Song, J. (2025). Applications of the cellular thermal shift assay to drug discovery in natural products: A review. International Journal of Molecular Sciences, 26. https://doi.org/10.3390/ijms26093940

Stefan, S. M., & Rafehi, M. (2023). Medicinal polypharmacology: Exploration and exploitation of the polypharmacolome in modern drug development. Drug Development Research, 85. https://doi.org/10.1002/ddr.22125

Sun, D., Gao, W., Hu, H., & Zhou, S. (2022). Why 90% of clinical drug development fails and how to improve it? Acta Pharmaceutica Sinica B, 12, 3049–3062. https://doi.org/10.1016/j.apsb.2022.02.002

Taddeo, V. A., Núñez, M. J., Beltrán, M., Castillo, U., Menjívar, J., Jiménez, I., Alcamí, J., Bedoya, L. M., & Bazzocchi, I. L. (2021). Withanolide-type steroids from Physalis nicandroides inhibit HIV transcription. Journal of Natural Products, 84, 2717–2726. https://doi.org/10.1021/acs.jnatprod.1c00637

Tewari, D., Chander, V., Dhyani, S., Sahu, S., Gupta, P., Patni, P., Kalick, L. S., & Bishayee, A. (2022). *Withania somnifera* (L.) Dunal: Phytochemistry, structure-activity relationship, and anticancer potential. Phytomedicine, 98, 153949. https://doi.org/10.1016/j.phymed.2022.153949

Tu, Y., Tan, L., Tao, H., & Li, Y. (2023). CETSA and thermal proteome profiling strategies for target identification and drug discovery of natural products. Phytomedicine, 116, 154862. https://doi.org/10.1016/j.phymed.2023.154862

Valdés-Albuernes, J. L., Díaz-Pico, E., Alfaro, S., & Caballero, J. (2024). Modeling of noncovalent inhibitors of the papain-like protease (PLpro) from SARS-CoV-2 considering the protein flexibility by using molecular dynamics and cross-docking. Frontiers in Molecular Biosciences, 11. https://doi.org/10.3389/fmolb.2024.1374364

Wang, Y.-F., Zhang, W.-D., *et al*. (2025). Withanolide-type steroids from Physalis angulata. Phytochemistry.

Wijeratne, E. M. K., Xu, Y., Liu, M., Inácio, M. C., Brooks, A. D., Tewary, P., Sayers, T. J., & Gunatilaka, A. A. L. (2021). Ring A/B-modified 17β-hydroxywithanolide analogues as antiproliferative agents for prostate cancer and potentiators of immunotherapy for renal carcinoma and melanoma. Journal of Natural Products. https://doi.org/10.1021/acs.jnatprod.1c00724

Wu, Z., Wang, J., Du, H., Jiang, D., Kang, Y., Li, D., Pan, P., Deng, Y., Cao, D., Hsieh, C.-Y., & Hou, T. (2023). Chemistry-intuitive explanation of graph neural networks for molecular property prediction with substructure masking. Nature Communications, 14. https://doi.org/10.1038/s41467-023-38192-3

Yan, X., Qu, C., Li, Q., Zhu, L., Tong, H., Liu, H., Qin, O., & Yao, X. (2024). Multiscale calculations reveal new insights into the reaction mechanism between KRASG12C and α,β-unsaturated carbonyl of covalent inhibitors. Computational and Structural Biotechnology Journal, 23, 1408–1417. https://doi.org/10.1016/j.csbj.2024.03.027

Zhang, Q., Yuan, Y., Cao, S., Kang, N., & Qiu, F. (2024). Withanolides: Promising candidates for cancer therapy. Phytotherapy Research, 38, 1104–1158. https://doi.org/10.1002/ptr.8090

Zhao, Z., & Bourne, P. E. (2024). Exploring extended warheads toward developing cysteine-targeted covalent kinase inhibitors. Journal of Chemical Information and Modeling, 64, 9517–9527. https://doi.org/10.1021/acs.jcim.4c00890

Zhu, D., Li, S., Chen, C., Wang, S., Zhu, J., Kong, L., & Luo, J. (2021). Tubocapsenolide A targets C-terminal cysteine residues of HSP90 to exert the anti-tumor effect. Pharmacological Research, 105523. https://doi.org/10.1016/j.phrs.2021.105523