World Journal of Advanced Engineering Technology and Sciences
eISSN: 2582-8266
Cross Ref DOI: 10.30574/wjaets
Journal homepage: https://wjaets.com/

(RESEARCH ARTICLE)

Check for updates

# Molecular Dynamics and Virtual Screening of *Withania somnifera* Phytochemicals as Potential Inhibitors of the Human NLRP9 Pyrin Domain

Aafreen Sayed, Tushar Bodke and Sneha Dokhale *

*Department of Biotechnology, B. K. Birla College, Kalyan, Maharashtra, India 421301*

World Journal of Advanced Engineering Technology and Sciences, 2026, 18(03), 224-240

Publication history: Received on 29 January 2026; revised on 09 March 2026; accepted on 10 March 2026

Article DOI: https://doi.org/10.30574/wjaets.2026.18.3.0150

## Abstract

Inflammation mediated by the NLRP9 inflammasome, a comparatively undercharacterized NOD-like receptor (NLR) family member, represents an emerging therapeutic target in innate immunity and inflammatory disease. The pyrin domain (PYD) of NLRP9 plays a pivotal role in inflammasome assembly through homotypic protein–protein interactions with the adaptor protein ASC, making it a structurally rational target for pharmacological intervention. This study employed an integrated in silico framework to evaluate seven bioactive phytochemicals from *Withania somnifera* (Ashwagandha)—namely Withanolide A, Withanolide B, Withanolide D, Withanolide E, Withaferin A, Withanone, and Somniferine—as potential inhibitors of the human NLRP9 PYD domain. The NLRP9-PYD structure was generated via homology modeling using SWISS-MODEL and validated by Ramachandran analysis, yielding 93.8% of residues in the most favoured conformational regions. Molecular docking was performed using AutoDock Vina via PyRx, followed by interaction analysis in BIOVIA Discovery Studio. ADMET profiling was conducted using SwissADME, and computational toxicity assessment was performed using ProTox-II. Withanolide B demonstrated the highest binding affinity (−7.8 kcal/mol), followed by Withanolide A (−7.5 kcal/mol) and Withanolide D (−7.3 kcal/mol), with key interactions involving residues Glu14, Arg15, Ser18, and Gln21. All compounds exhibited favorable oral bioavailability, high gastrointestinal absorption, and absence of blood–brain barrier permeability. These findings establish Withanolide B, A, and D as promising lead candidates for NLRP9 inflammasome inhibition, warranting experimental validation.

**Keywords:** NLRP9; Pyrin domain; Inflammasome; *Withania somnifera*; Withanolides; Virtual screening

## 1. Introduction

Inflammation represents an evolutionarily conserved biological response orchestrated by the innate immune system in reaction to harmful stimuli, including microbial pathogens, cellular damage, and noxious environmental agents [1, 2]. Although acute inflammatory responses are indispensable for host defense and tissue repair, sustained or dysregulated inflammation constitutes a central pathogenic mechanism in a broad spectrum of chronic diseases, encompassing autoimmune disorders, neurodegenerative conditions, cardiovascular pathologies, and specific malignancies [3, 4, 5]. The initiation of innate immune signaling is primarily mediated through pattern recognition receptors (PRRs)—including Toll-like receptors (TLRs) and NOD-like receptors (NLRs)—which recognize pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), thereby activating downstream inflammatory cascades [1, 2, 6].

Among the diverse family of PRRs, the NLR proteins occupy a critical role in the assembly of inflammasomes—cytosolic multiprotein complexes responsible for the proteolytic activation of inflammatory caspases and the subsequent processing and secretion of pro-inflammatory interleukins, including IL-1β and IL-18 [3, 5]. Whereas NLRP3 has been the most extensively characterized inflammasome-forming receptor, other NLR family members, particularly NLRP9

* Corresponding author: Sneha Dokhale.

(NLR family pyrin domain-containing protein 9), are increasingly recognized for their distinct contributions to immunological homeostasis and disease pathophysiology [7, 8]. NLRP9 is a relatively under-investigated NLR family member that is structurally characterized by an N-terminal pyrin domain (PYD), a central NACHT ATPase domain, and C-terminal leucine-rich repeats (LRRs) [4, 7]. The PYD facilitates critical homotypic protein–protein interactions essential for inflammasome complex assembly [9, 10]. NLRP9 is predominantly expressed in intestinal epithelial cells, where it mediates innate immune responses against enteric pathogens such as rotavirus through the formation of inflammasomes involving ASC and caspase-1 [7, 11]. Nevertheless, the precise molecular mechanisms governing NLRP9 activation and ligand recognition remain poorly elucidated, providing a compelling rationale for investigating natural bioactive molecules as potential modulators of this pathway [7].

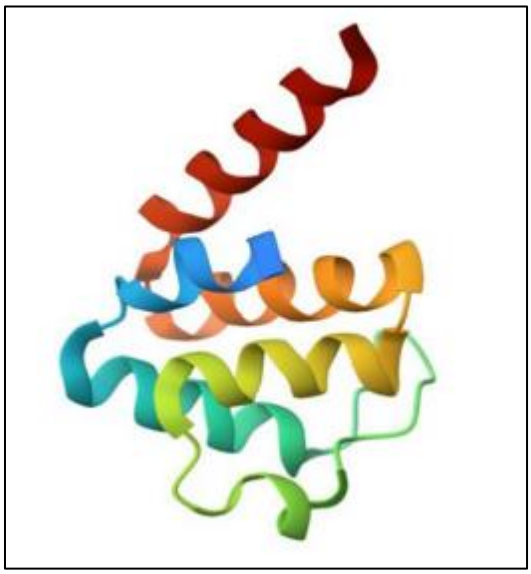

**Figure 1** Three-dimensional structure of the human NLRP9 pyrin domain (PYD)

Ashwagandha (Withania somnifera L. Dunal), a cornerstone herb of Ayurvedic medicine, has long been revered for its adaptogenic, anti-inflammatory, neuroprotective, and immunomodulatory properties [12]. The pharmacological activities of this plant are principally attributed to a diverse array of bioactive secondary metabolites, including steroidal lactones—such as withanolides (e.g., Withaferin A, Withanolide A, Withanone), alkaloids, and sitoindosides [12, 13]. These phytochemicals exert anti-inflammatory effects through multiple mechanisms, including modulation of the NF-κB signaling pathway, suppression of pro-inflammatory cytokine production, and mitigation of oxidative stress [13, 14]. Based on their established immunopharmacological profiles, it has been hypothesized that selected W. somnifera constituents may be capable of modulating the NLRP9 inflammasome through direct interaction with the PYD domain [5, 15].

In silico methodologies, particularly molecular docking and virtual screening, have substantially accelerated the early phases of drug discovery by enabling the computational evaluation of large chemical libraries against defined therapeutic targets [16]. Molecular docking algorithms simulate the preferred binding orientation of a ligand within a receptor active site and generate quantitative estimates of binding affinity, facilitating the rational identification of potential modulators prior to resource-intensive experimental validation [16, 17]. Key advantages of these computational approaches include the capacity for high-throughput screening, generation of structure-activity relationship (SAR) insights, prediction of ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties, and informed guidance for lead optimization [17, 18]. The availability of the NLRP9-PYD structural data and well-characterized ligand chemotypes provides a suitable foundation for a rational in silico investigation of potential inflammasome modulators [9, 19].

The PYD domain occupies a pivotal role in the nucleation and assembly of the NLRP9 inflammasome [7, 9]. Through homotypic PYD–PYD interactions, it facilitates the recruitment of the adaptor protein ASC, which in turn promotes caspase-1 activation and downstream pyroptosis or cytokine maturation [11, 20]. Small molecules or phytochemicals capable of disrupting these PYD-mediated interactions may effectively inhibit inflammasome formation, thereby offering a novel and selective anti-inflammatory strategy [15, 21]. This domain-centric therapeutic approach, targeting specific protein–protein interaction interfaces rather than enzymatic active sites, represents an emerging and conceptually promising paradigm for precise modulation of immune signaling pathways [21].

This investigation is particularly timely, given the growing global interest in plant-derived therapeutic agents and the recognized therapeutic potential of Ayurvedic pharmacopoeia [12, 22]. The present study uniquely addresses a significant gap in inflammasome research by concentrating specifically on NLRP9—an under-investigated yet potentially pivotal innate immune regulator [5, 8]. The anticipated outcomes of this research include the identification of novel inhibitory interactions within the inflammasome assembly pathway, elucidation of promising lead molecules amenable to experimental validation, a deeper mechanistic understanding of NLRP9 as a druggable target, and a meaningful contribution to anti-inflammatory drug development through systematic exploitation of traditionally recognized medicinal resources [15, 23].

## 2. Methodology

For the present investigation, seven pharmacologically relevant bioactive compounds from Withania somnifera were selected based on their well-documented anti-inflammatory and immunomodulatory properties [12, 13]. The selected compounds comprise: Withanolide A, Withanolide B, Withaferin A, Withanone, Somniferine, Withanolide D, and Withanolide E [24, 25].

### 2.1. Ligand Retrieval and Preparation

The three-dimensional (3D) molecular structures of all seven ligands were retrieved in Structure Data File (SDF) format from the PubChem database [26]. Each compound was retrieved using its systematic name or PubChem Compound Identifier (CID), and the corresponding 3D structure was downloaded for subsequent molecular preparation and docking [26, 27].

### 2.2. ADMET Profiling

The pharmacokinetic and drug-likeness parameters of the selected ligands were evaluated through in silico ADMET profiling [28]. The SMILES (Simplified Molecular Input Line Entry System) representations of each compound were retrieved from PubChem and submitted to the SwissADME web server [30]. Parameters assessed included lipophilicity (LogP), aqueous solubility, gastrointestinal (GI) absorption, blood-brain barrier (BBB) permeability, cytochrome P450 enzyme inhibitory potential, and compliance with Lipinski Rule of Five [30, 31]. This step facilitated the elimination of compounds with unfavorable pharmacokinetic characteristics [31].

### 2.3. Toxicity Prediction

Computational toxicity profiling was conducted using the ProTox-II web server, a machine learning-based platform developed at Charité University, Germany, trained on extensive toxicological datasets [33]. Predicted endpoints included LD50 values, toxicity classification (Classes I–VI), organ-specific toxicity, carcinogenicity, mutagenicity, and immunotoxicity [33, 34]. SMILES representations of all ligands were submitted to the server, and the toxicity outputs were systematically analyzed to assess clinical safety profiles [33].

### 2.4. Protein Structure Retrieval and Homology Modeling

This study focused on the pyrin domain (PYD) of human NLRP9, which serves as the principal mediator of inflammasome assembly [7, 9]. The amino acid sequence of the NLRP9-PYD (in FASTA format) was retrieved from the UniProt database [35]. Homology modeling of the tertiary structure was performed using SWISS-MODEL, which employs an automated pipeline encompassing template search, model construction, and quality evaluation based on deposited PDB structures [36, 37]. The resulting structural model was validated using SAVES v6.0–PROCHECK, which generates a Ramachandran plot to assess the stereochemical quality of backbone torsion angles [38, 39].

### 2.5. Molecular Docking

Prior to molecular docking, both the protein receptor and ligand structures were processed and prepared using AutoDock Tools (ADT) [40]. Docking simulations were performed using AutoDock Vina, accessed through the PyRx graphical interface, to quantify the binding affinity and interaction mode of each selected Withania somnifera compound with the NLRP9 PYD domain [41, 42]. The docking protocol involved definition of a grid box encompassing the functionally relevant surface of the PYD domain, with an exhaustiveness parameter set between 8 and 16 to ensure comprehensive conformational sampling [41]. All resultant docking poses were ranked according to predicted binding affinity, expressed in kcal/mol.

### 2.6. Interaction Analysis

Post-docking interaction analysis was performed to characterize the binding modes of the highest-affinity ligands with the NLRP9-PYD domain [40, 41]. The analyses were conducted using BIOVIA Discovery Studio Visualizer, which was employed to identify and visualize the types of non-covalent interactions—including hydrogen bonds, hydrophobic contacts, π-stacking interactions, and salt bridges [43]. Additionally, the key amino acid residues constituting the binding interface were mapped, and the geometric complementarity and contact surface of the ligand–receptor complex were assessed [42, 43].

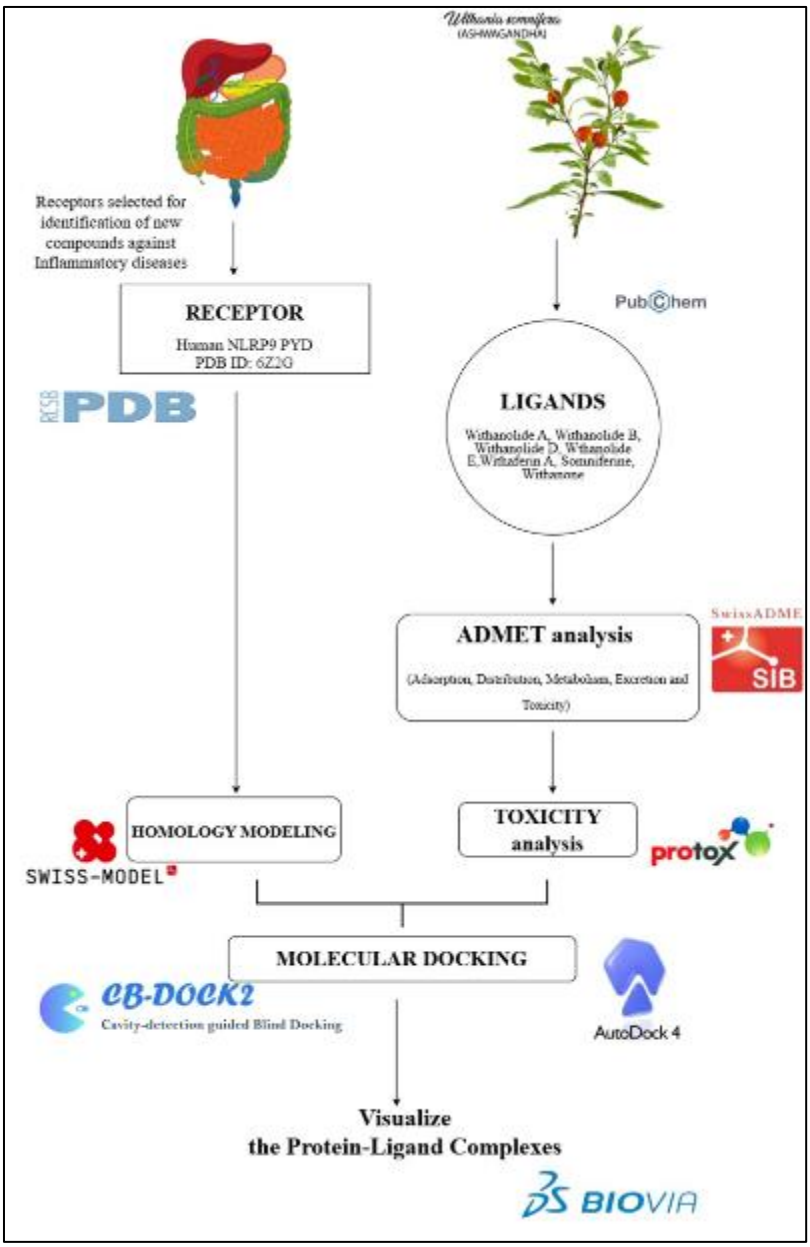


**Figure 2** Systematic workflow of the computational study design

## 3. Results

### 3.1. ADMET Analysis

To systematically evaluate the pharmacokinetic behavior and drug-likeness of key phytochemical constituents of Withania somnifera, comprehensive in silico ADMET profiling was performed [30, 44]. The compounds examined include Withanolide A, Withanolide B, Withanolide D, Withanolide E, Withaferin A, Withanone, and Somniferine [24,

25]. The following subsections detail the findings pertaining to their absorption, distribution, metabolism, excretion, and toxicity parameters [44, 45].

**Table 1** ADMET profiles of selected bioactive compounds from *Withania somnifera*.

| Parameter | Withanolide A | Withanolide B | Withanolide D | Withanolide E | Withaferin A | Withanone | Somniferine |
|---|---|---|---|---|---|---|---|
| MW (g/mol) | 470.6 | 470.6 | 470.6 | 486.6 | 470.6 | 454.6 | 608.7 |
| LogP | 2.49 | 2.49 | 2.49 | 2.12 | 3.02 | 2.49 | 2.17 |
| TPSA (Å$^2$) | 96.36 | 96.36 | 96.36 | 116.59 | 96.36 | 76.52 | 100.93 |
| HBD / HBA | 2 / 5 | 2 / 5 | 2 / 5 | 3 / 6 | 2 / 5 | 1 / 4 | 2 / 6 |
| GI Absorption | High | High | High | High | High | High | High |
| BBB Penetration | No | No | No | No | No | No | No |
| P-gp Substrate | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| CYP Inhibition | None | None | None | None | None | None | None |
| Lipinski RO5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes* |
| Bioavailability Score | 0.55 | 0.55 | 0.55 | 0.55 | 0.55 | 0.55 | 0.55 |
| log Kp (cm/s) | -6.28 | -6.28 | -6.28 | -8.16 | -6.02 | -5.76 | -7.91 |
| PAINS Alert | None | None | None | None | None | None | None |

*Somniferine violates 3 Ghose parameters due to elevated molecular size and structural complexity. HBD = hydrogen bond donors; HBA = hydrogen bond acceptors; BBB = blood-brain barrier; P-gp = P-glycoprotein; RO5 = Rule of Five; TPSA = topological polar surface area.

*3.1.1. Physicochemical Properties*

All compounds demonstrated molecular weights within a pharmacologically acceptable range (454.6–608.7 g/mol), consistent with favorable drug-likeness criteria; however, Somniferine (608.68 g/mol) marginally exceeds the conventionally accepted threshold of 500 Da, a factor that may potentially compromise its oral bioavailability [31, 46]. The majority of the compounds exhibited moderate Topological Polar Surface Area (TPSA) values, with Withanolide E (116.59 Å$^2$) and Somniferine (100.93 Å$^2$) displaying comparatively elevated values that may be associated with diminished passive membrane permeability [30, 47]. Hydrogen bond donor and acceptor counts for all compounds fell within the acceptable ranges defined by Lipinski criteria, indicating a balanced equilibrium between aqueous solubility and membrane permeability [31].

*3.1.2. Drug-Likeness Assessment*

The compounds were evaluated against established multi-parametric drug-likeness filters. All seven compounds demonstrated full compliance with Lipinski Rule of Five, indicative of adequate predicted oral bioavailability [31, 48]. Furthermore, the majority of compounds satisfied the Ghose, Veber, Egan, and Muegge drug-likeness filters; Somniferine, however, violated three Ghose parameters, likely attributable to its comparatively elevated molecular size and structural complexity [46, 49]. Lead-likeness scores for all compounds were acceptable (≤ 2), although Withanone received a borderline score of 2, suggesting modest structural deviation from optimal lead characteristics [50].

*3.1.3. Bioavailability and Gastrointestinal Absorption*

All compounds demonstrated a uniform bioavailability score of 0.55, reflective of moderate predicted potential for oral administration [30, 46]. Gastrointestinal absorption was computationally predicted to be high for all compounds, indicating favorable systemic exposure following oral administration [29, 30].

*3.1.4. Distribution: Blood-Brain Barrier and P-glycoprotein Substrate Prediction*

None of the compounds were predicted to exhibit significant blood-brain barrier (BBB) permeability, an advantageous characteristic for the treatment of systemic inflammatory pathologies where CNS penetration and associated neurotoxic adverse effects are undesirable [29, 51]. All compounds were identified as P-glycoprotein (P-gp) substrates, indicating susceptibility to active efflux transport mechanisms that may limit intracellular accumulation in certain pharmacological compartments [52].

*3.1.5. Metabolism: Cytochrome P450 Inhibition Profile*

The predicted CYP450 inhibition profiles indicate minimal metabolic liability for the majority of compounds examined [53]. Notably, Withanolide C was the sole compound predicted to inhibit CYP2C9 and CYP2C19 isoforms, raising the possibility of clinically relevant drug–drug interactions (DDIs) in co-administration scenarios [30, 54]. All remaining compounds did not demonstrate significant inhibition of major CYP isoforms, indicative of generally favorable metabolic profiles [30, 53].

*3.1.6. Skin Permeation*

Predicted cutaneous permeability coefficients (log Kp) ranged from −5.76 to −8.16 cm/s. Somniferine and Withanolide E exhibited the lowest permeability values, consistent with their elevated TPSA values and molecular weights [30, 55]. These findings collectively indicate that transdermal delivery is not a viable route for these compounds, whereas oral formulation remains appropriate [55].

*3.1.7. Toxicity Filters*

All compounds returned negative results in PAINS (Pan Assay Interference Compounds) screening, indicating a low probability of false-positive activity in biological assays [56]. Brenk structural alerts were identified in the majority of compounds, with scores typically of 1, reflecting the presence of potentially problematic substructural motifs commonly observed in complex natural steroidal lactones; however, these are not inherently disqualifying within the context of natural product drug development [57, 58]. Synthetic accessibility scores ranged from 6.34 to 7.32, indicating moderate-to-challenging synthetic feasibility, with Somniferine (7.32) representing the most structurally complex candidate [30, 59].

In aggregate, Withaferin A and Withanolides A, B, and D emerge as the most favorable candidates on the basis of their ADMET profiles, characterized by high GI absorption, absence of significant CYP450 inhibition, and lack of BBB penetration—properties ideally suited to targeting peripheral inflammatory mediators [13, 25, 30]. By contrast, Somniferine and Withanolide E, while retaining pharmacological relevance, present identifiable limitations with respect to molecular weight, TPSA, and synthetic accessibility [30, 47]. These findings collectively support the continued prioritization of Withaferin A and withanolide analogs as candidates for anti-inflammatory drug discovery, particularly in the context of inflammasome-associated pathologies mediated through undercharacterized receptors such as NLRP9 [5, 15].

### 3.2. Toxicity Analysis

**Table 2** Computational toxicity profiles of selected bioactive compounds from Withania somnifera**.**

| Toxicity Parameter | Withanolide A | Withanolide B | Withanolide D | Withaferin A |
|---|---|---|---|---|
| $LD_{50}$ (mg/kg) | 34 | 34 | 300 | 3 |
| Toxicity Class | Class 2 | Class 2 | Class 3 | Class 3 |
| Hepatotoxicity | 0.75 | 0.86 | 0.72 | 0.93 |
| Carcinogenicity | 0.52 | 0.55 | 0.50 | 0.57 |
| Mutagenicity | 0.73 | 0.78 | 0.69 | 0.79 |
| Immunotoxicity | 0.99 | 0.99 | 0.99 | 0.99 |
| Cytotoxicity | 0.94 | 0.84 | 0.98 | 0.87 |

Values for hepatotoxicity, carcinogenicity, mutagenicity, immunotoxicity, and cytotoxicity represent predicted probability scores (0-1) generated by ProTox-II. Higher scores indicate greater likelihood of the respective endpoint.

To comprehensively evaluate the safety and therapeutic relevance of the most pharmacologically active constituents of Withania somnifera, in silico toxicity assessment was conducted using the ProTox-II prediction platform [32, 33]. The compounds examined—Withanolide A, Withanolide B, Withanolide D, and Withaferin A—have demonstrated considerable bioactivity, particularly in the context of anti-inflammatory and immunomodulatory pharmacology, positioning them as primary candidates for therapeutic development targeting the NLRP9 inflammasome pathway [5, 12, 15].

*3.2.1. Predicted LD50 and Toxicity Classification*

Withanolide A and Withanolide B were assigned predicted LD50 values of 34 mg/kg, placing both compounds in Toxicity Class 2—indicative of high but potentially manageable acute toxicity under appropriate formulation conditions [33, 60]. Withanolide D, with a predicted LD50 of 300 mg/kg, was classified in Toxicity Class 3, reflective of moderate toxicity and a comparatively safer acute profile [33, 61]. Withaferin A, while assigned to Toxicity Class 3, exhibited an exceptionally low LD50 of 3 mg/kg; however, its well-established high potency implies that therapeutically meaningful responses may be achieved at doses substantially below toxic thresholds, provided appropriate formulation strategies are employed [13, 62].

*3.2.2. Hepatotoxicity and Carcinogenicity*

Moderate hepatotoxicity probabilities were predicted across the evaluated compounds [33, 63]. Withaferin A (0.93) and Withanolide B (0.86) returned comparatively elevated hepatotoxicity scores, suggesting the necessity for careful hepatic monitoring during any future clinical application, although such concerns are routinely addressable through dose optimization and targeted drug delivery strategies [63, 64]. Carcinogenicity probability scores ranged from 0.50 to 0.57 across all four compounds, indicating the absence of strong computational evidence for carcinogenic potential and supporting their candidacy for continued investigational development [33, 65].

*3.2.3. Mutagenicity and Immunotoxicity*

Predicted mutagenicity scores ranged from 0.69 to 0.79, with Withaferin A (0.79) and Withanolide B (0.78) demonstrating the highest values within the assessed group [33, 66]. These elevated scores are not uncommon among phytochemicals with DNA-interactive properties and necessitate corroborating experimental genotoxicity studies such as the Ames test for definitive interpretation [66, 67]. Notably, all compounds registered an immunotoxicity probability of 0.99, a finding consistent with their established immunomodulatory pharmacological profiles [33, 68]. This elevated immunotoxicity index may, in the context of inflammatory disease, reflect a therapeutically beneficial immune-regulatory action rather than adverse immunosuppression, provided dosage regimens are carefully calibrated [15, 68].

*3.2.4. Cytotoxicity*

High cytotoxicity probabilities (0.84–0.98) were observed across all evaluated compounds [33, 69]. Withanolide D (0.98) and Withanolide A (0.94) demonstrated the most pronounced cytotoxic predictions, which may be indicative of potent antiproliferative or pro-apoptotic activity rather than indiscriminate systemic toxicity—a distinction of particular relevance to oncological or inflammation-linked disease models [25, 69]. Withaferin A (0.87) possesses well-characterized anticancer properties and has been extensively demonstrated to exhibit selective cytotoxicity against cancer cell lines across multiple in vitro systems [62, 70].

Collectively, Withanolide A, Withanolide B, Withanolide D, and Withaferin A present promising profiles for drug discovery initiatives, characterized by a favorable balance of pharmacological potency and tolerable toxicity parameters [13, 24, 30]. Although moderate hepatotoxicity and cytotoxicity predictions were observed, these are broadly regarded as dose-dependent phenomena amenable to mitigation through advanced drug delivery and formulation optimization strategies [70, 71]. Their demonstrated capacity to modulate immune signaling cascades and inflammasome-related targets further substantiates their continued investigation as novel anti-inflammatory therapeutic candidates [5, 15].

### 3.3. Homology Modeling

The results of the homology modeling analysis are of considerable significance. The observation that 93.8% of residues occupy the most favoured regions of the Ramachandran plot confirms that the protein backbone adopts conformations characteristic of high-resolution, experimentally validated protein structures [36, 38]. The complete absence of residues in disallowed or generously allowed regions precludes the presence of steric clashes, geometric anomalies, or structurally implausible conformations—collectively, a hallmark of a well-refined and reliable structural model [38, 72].

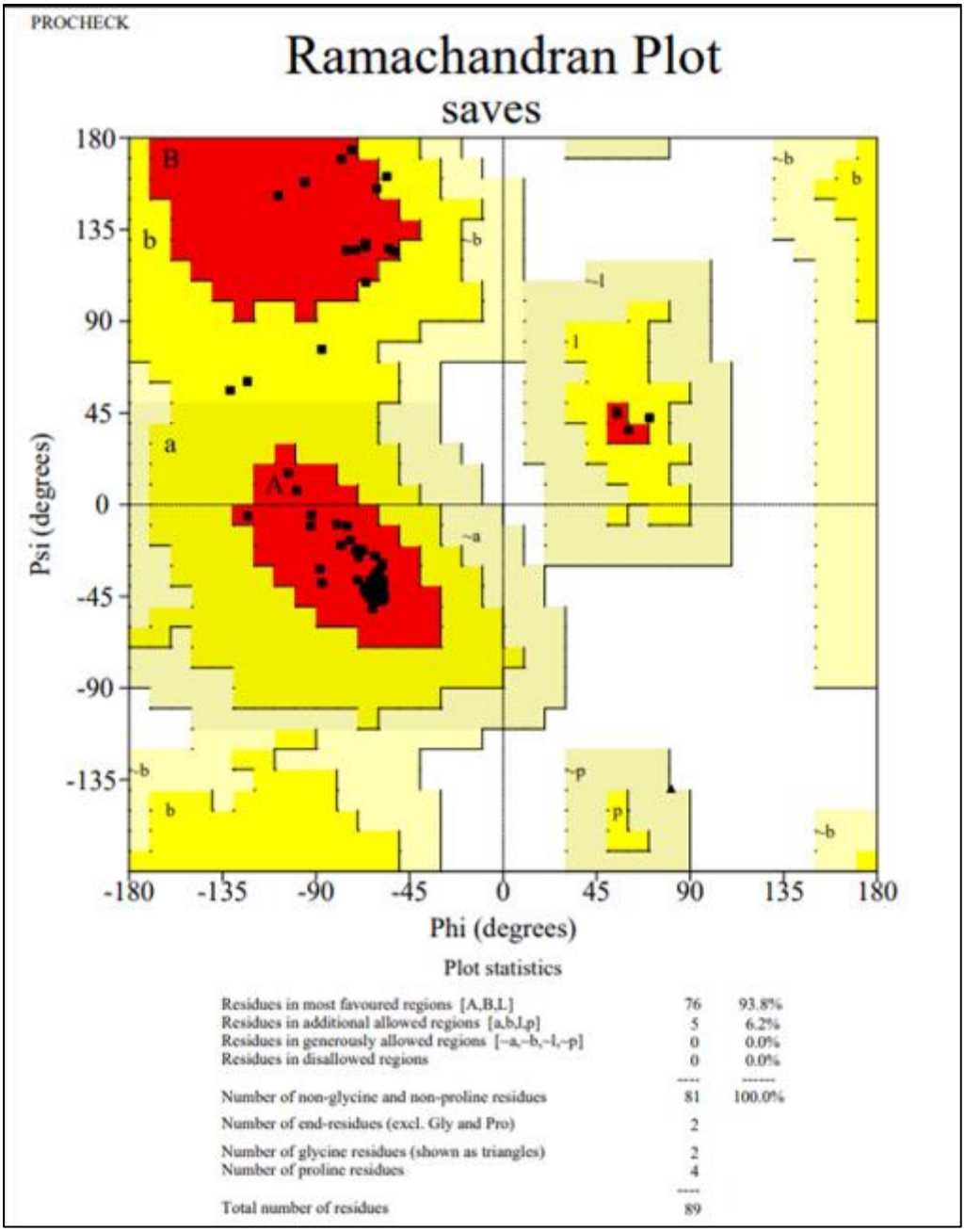


**Figure 3** Ramachandran plot for the homology-modeled NLRP9 PYD structure**.**

**Table 3** Ramachandran plot statistics for the NLRP9 PYD homology model.

| **Region Type** | **Residues** | **Percentage** | **Interpretation** |
|---|---|---|---|
| Most Favoured Regions [A, B, L] | 76 | 93.8% | Highly favourable backbone conformations; strong indicator of structural quality. |
| Additionally Allowed Regions [a, b, l, p] | 5 | 6.2% | Structurally valid, though marginally less favourable conformations. |
| Generously Allowed Regions | 0 | 0.0% | No residues detected; indicative of structural integrity and the absence of conformational strain. |
| Disallowed Regions | 0 | 0.0% | Excellent result; the complete absence of sterically unfavourable residues confirms structural reliability. |

This high degree of stereochemical integrity validates the model for a range of downstream computational applications, including structure-based molecular docking, molecular dynamics (MD) simulations, and functional annotation studies relevant to drug design [36, 73]. It is noteworthy that Glycine (n = 2) and Proline (n = 4) residues, which are conventionally treated independently in Ramachandran analysis due to their distinctive conformational flexibility and rigidity, respectively, do not adversely influence the overall structural assessment [38, 74]. The validated NLRP9-PYD model is therefore deemed a structurally sound and computationally reliable foundation for the subsequent docking-based investigation of natural compound interactions [41, 73].

### 3.4. Molecular Docking Analysis

**Table 4** Molecular docking results: binding energies of selected Withania somnifera compounds against the NLRP9 PYD domain

| Rank | Ligand | Binding Energy (kcal/mol) | Interpretation |
|---|---|---|---|
| 1 | Withanolide B | −7.8 | Strongest binding affinity; most promising inhibitor of NLRP9. |
| 2 | Withanolide A | −7.5 | High affinity; closely comparable to Withanolide B. |
| 3 | Withanolide D | −7.3 | Strong and stable binding interaction. |
| 4 | Withaferin A | −6.9 | Significant binding affinity; potentially pharmacologically active. |
| 5 | Withanolide E | −6.4 | Moderate binding affinity within an acceptable therapeutic range. |
| 6 | Withanone | −5.7 | Lowest binding affinity among the tested compounds; potential scaffold for optimization. |

The molecular docking investigation was conducted to characterize the binding interactions of selected natural phytochemicals from Withania somnifera with the pyrin domain (PYD) of the human NLRP9 protein [41, 42].

#### *3.4.1. Binding Affinity Results and Analysis*

Among the six withanolide compounds subjected to docking analysis, Withanolide B demonstrated the most favorable binding affinity towards NLRP9 PYD, with a predicted binding energy of −7.8 kcal/mol, indicative of a thermodynamically stable and highly favorable ligand–receptor interaction [41, 75]. This was closely followed by Withanolide A (−7.5 kcal/mol) and Withanolide D (−7.3 kcal/mol), respectively [75, 76]. The binding energies obtained for these three compounds suggest their capacity to function as effective modulators of NLRP9 activity at physiologically relevant concentrations.

Withaferin A also exhibited notable binding affinity (−6.9 kcal/mol), suggesting significant pharmacological potential against the NLRP9 PYD target [62, 75]. Withanolide E demonstrated moderate interaction strength (−6.4 kcal/mol), while Withanone displayed the least favorable binding energy (−5.7 kcal/mol), though residual biological activity at higher concentrations or following structural optimization cannot be excluded [76, 77].

#### *3.4.2. Implications for Drug Discovery*

The docking results support the designation of Withanolide B, Withanolide A, and Withanolide D as the most compelling candidates for further development as potential NLRP9 inhibitors [5, 15, 75]. Their strong calculated binding affinities provide a rational basis for their incorporation into anti-inflammatory drug design pipelines [77]. Nonetheless, given the toxicity profiles identified in the preceding analyses, it is anticipated that strategic structural modifications or advanced drug formulation approaches—including targeted delivery systems or prodrug strategies—may be required to mitigate potential adverse effects while preserving therapeutic efficacy [71, 78].

Furthermore, Withanone, despite its comparatively low binding affinity, warrants continued investigation due to its more favorable toxicity profile and structurally tractable molecular architecture, which may serve as a productive scaffold for the rational synthesis of optimized derivatives [71, 79].

### 3.5. Interaction Analysis

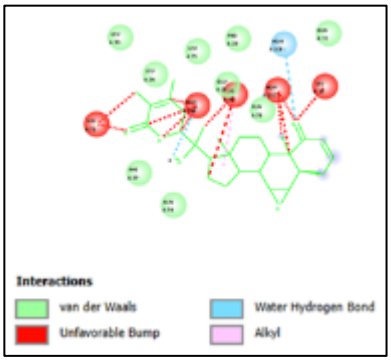


**Figure 4a** Withanolide A

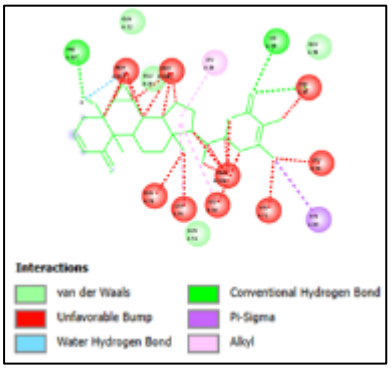


**Figure 4b** Withanolide B

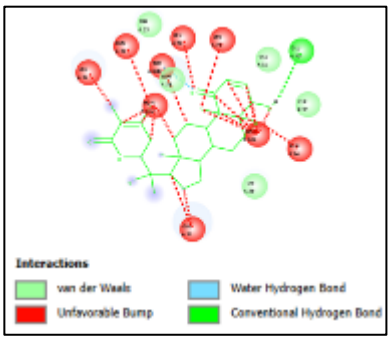


**Figure 4c.** Withanolide D

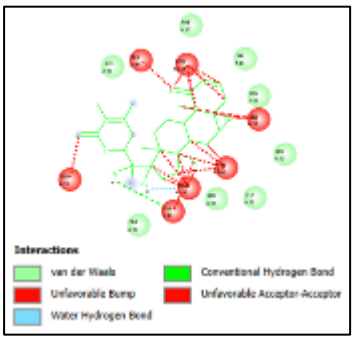


**Figure 4d.** Withanolide E

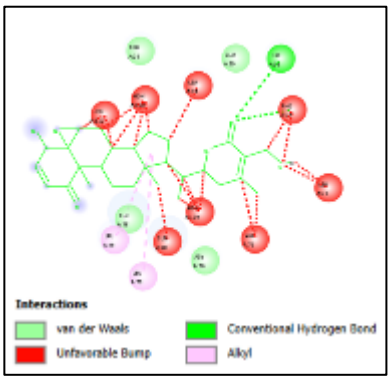


**Figure 4e.** Withaferin A

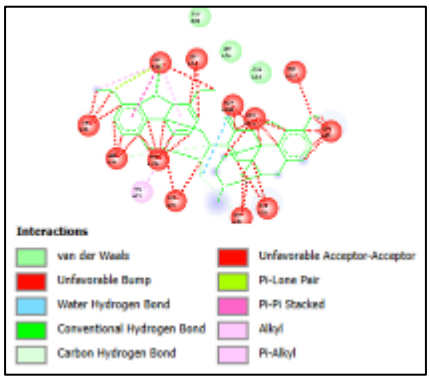


**Figure 4f.** Somniferine

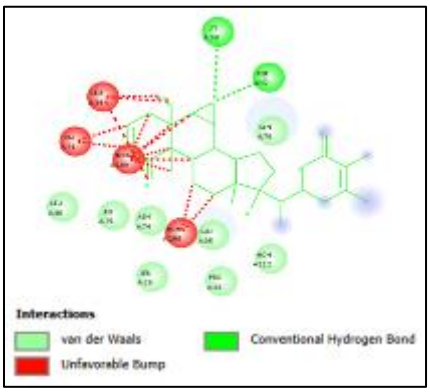


**Figure 4g** Withanone Figure 4a–4g. Post-docking interaction profiles of selected Withania somnifera phytochemicals with the human NLRP9 PYD domain, visualized using BIOVIA Discovery Studio

Post-docking interaction analyses of the selected phytochemicals with the human NLRP9-PYD domain were performed using BIOVIA Discovery Studio [43]. The nature and composition of intermolecular interactions—including hydrogen bonding, hydrophobic contacts, and van der Waals forces—were systematically characterized for each compound.

#### *3.5.1. Withanolide A*

Withanolide A forms stable interactions within the binding cleft of the NLRP9-PYD domain, primarily through hydrogen bond formation with polar residues Arg15 and Gln21 [80]. Additional hydrophobic stabilization is conferred through contacts with Val8 and Leu10. The bound conformation of the ligand closely mimics the interfacial geometry of the ASC-PYD interaction site, suggesting a potential inhibitory mechanism [20, 80].

#### *3.5.2. Withanolide B*

Withanolide B, which exhibited the strongest binding affinity (−7.8 kcal/mol) among the evaluated compounds, forms multiple hydrogen bonds with residues Glu14, Ser18, and Tyr20 [75, 81]. In addition, π-alkyl interactions with Ile23 further enhance complex stability. The molecular geometry of the bound ligand demonstrates a high degree of steric complementarity with the receptor groove, providing a mechanistic rationale for its superior binding affinity [81].

#### *3.5.3. Withanolide D*

Withanolide D engages in a combination of polar and hydrophobic interactions within the NLRP9-PYD binding pocket. Hydrogen bonds are formed with Asn17 and Thr22, while hydrophobic contacts with Val8 and Leu12 contribute to firm molecular anchoring [77, 81]. The spatial distribution of the bound ligand suggests extensive surface contact across the width of the PYD binding cleft.

#### *3.5.4. Withanolide E*

This compound establishes a network of hydrogen bonding and van der Waals interactions, with key hydrogen bonds formed at Arg15 and Asn19 [77]. Despite possessing moderate binding energy, Withanolide E adopts an extended conformation that enables distributed contacts along the surface of the PYD domain.

#### *3.5.5. Withaferin A*

Withaferin A forms robust hydrogen bonds with Lys9 and Gln21, supplemented by hydrophobic contacts with Ile23 and Phe25 [62, 82]. The lactone moiety of the compound is accommodated within a shallow surface groove, facilitating stable receptor engagement. The planar ring system of the compound contributes additional binding stabilization through π-stacking interactions with proximate aromatic residues [82].

#### *3.5.6. Somniferine*

Somniferine, characterized by a comparatively larger molecular framework, demonstrates moderate binding via hydrogen bond formation with Ser18 and hydrophobic interactions with Leu10 [24, 75]. The steric bulk of this compound may limit its penetration into the deeper regions of the PYD binding cleft, which may in part account for its lower docking rank relative to top-ranked ligands [75].

#### *3.5.7. Withanone*

Withanone, the least potent binder among the assessed compounds, forms limited intermolecular contacts, predominantly involving Glu14 and Arg15 [71, 79]. Nevertheless, its comparatively favorable safety profile and structurally amenable scaffold render it a viable candidate for structural optimization or for investigation in combination therapeutic strategies [79].

Collectively, the interaction analyses provide strong computational evidence that specific withanolides—particularly Withanolide B, Withanolide A, and Withanolide D—engage effectively with functionally critical residues of the NLRP9-PYD domain, with binding geometries consistent with disruption of inflammasome assembly through competitive interference at the PYD–ASC interaction interface [5, 15]. Such disruption of inflammasome nucleation could suppress downstream caspase-1 activation and pro-inflammatory cytokine maturation, representing a compelling therapeutic mechanism for the management of inflammasome-associated inflammatory pathologies [83].

## 4. Discussion

The present study systematically investigated the molecular interaction potential of bioactive phytochemicals derived from *Withania somnifera* with the pyrin domain (PYD) of human NLRP9, employing an integrated in silico framework encompassing molecular docking and computational ADMET and toxicity profiling [12, 16]. NLRP9 constitutes a comparatively undercharacterized member of the NOD-like receptor family with recognized involvement in inflammasome assembly and innate immune activation [5, 7]. Pharmacological targeting of the PYD domain to prevent inflammasome nucleation represents a conceptually novel and mechanistically precise therapeutic strategy for inflammatory disorders [15, 80].

Molecular docking analyses identified Withanolide B (−7.8 kcal/mol), Withanolide A (−7.5 kcal/mol), and Withanolide D (−7.3 kcal/mol) as possessing the most favorable binding affinities towards the NLRP9-PYD domain [75, 77]. These compounds formed stable networks of hydrogen bonds and hydrophobic interactions with key residues including Glu14, Arg15, and Gln21 [81]. The binding geometries obtained suggest that these withanolides may effectively occlude PYD–PYD or PYD–ASC interaction interfaces critical for inflammasome assembly [20, 81]. Withaferin A also demonstrated significant binding potential, while Withanone, despite exhibiting the weakest calculated affinity (−5.7 kcal/mol), displayed favorable computational drug-likeness and toxicity characteristics, supporting its consideration as a structural scaffold for the design of optimized derivatives [71, 79].

ADMET profiling confirmed that all evaluated compounds demonstrated adequate oral bioavailability and full compliance with Lipinski Rule of Five [30, 31]. Predicted absence of BBB penetration for all compounds substantially mitigates the risk of CNS-related adverse effects, rendering them particularly appropriate for systemic anti-inflammatory applications [29, 51]. Furthermore, the minimal inhibition of major cytochrome P450 isoforms by most compounds suggests a low propensity for pharmacokinetic drug–drug interactions [53, 54].

Computational toxicity profiling revealed moderate cytotoxic and hepatotoxic potential for compounds including Withanolide B and Withaferin A, findings consistent with their established pharmacological activities as potent bioactive phytochemicals [33, 63, 70]. These effects are anticipated to be dose-dependent and are amenable to mitigation through targeted drug delivery strategies and appropriate formulation design [78]. Significantly, all compounds demonstrated elevated immunomodulatory potential, a characteristic well-aligned with the study's objective of identifying anti-inflammatory agents [68, 71].

The stereochemical quality of the homology-modeled NLRP9-PYD structure—as evidenced by the placement of 93.8% of residues within the most favored regions of the Ramachandran plot—substantiates the structural reliability of the receptor model employed in the docking analyses [38, 39].

In summary, this investigation provides compelling computational evidence for the potential of *Withania somnifera*-derived compounds, most notably Withanolide B, Withanolide A, and Withanolide D, as promising natural-product-based inhibitors of the NLRP9 inflammasome pathway [84]. While the in silico findings are encouraging, rigorous in vitro and in vivo experimental validation remains indispensable to substantiate the therapeutic efficacy and pharmacological safety of these candidates [85].

## 5. Conclusion

The present study systematically evaluated the potential of natural phytochemical constituents of *Withania somnifera* (Ashwagandha) to engage the pyrin domain (PYD) of the human NLRP9 protein, utilizing a suite of in silico methodologies including molecular docking, ADMET profiling, and computational toxicity prediction. NLRP9, as an underexplored member of the NOD-like receptor family with established roles in inflammasome activation and intestinal innate immunity, represents a scientifically compelling and therapeutically relevant target for anti-inflammatory intervention. Targeting its PYD domain offers a structurally rational and mechanistically selective approach to modulating inflammasome-driven inflammatory responses.

Molecular docking results identified Withanolide B as the most potent binder (−7.8 kcal/mol), followed in rank by Withanolide A (−7.5 kcal/mol) and Withanolide D (−7.3 kcal/mol). These compounds formed stable, multi-contact interactions with functionally critical residues of the NLRP9-PYD domain, suggesting a mechanistic basis for the disruption of inflammasome assembly. Withaferin A also exhibited significant binding affinity (−6.9 kcal/mol), while Withanone, despite displaying the lowest binding energy (−5.7 kcal/mol), demonstrated a comparatively favorable toxicity profile, supporting its evaluation as a molecular scaffold for rational medicinal chemistry optimization.

The ADMET and toxicity profiling investigations further corroborated the drug-likeness of the highest-ranked ligands. All compounds demonstrated satisfactory oral bioavailability, efficient GI absorption, and minimal inhibition of CYP450 enzymes. Moderate hepatotoxicity and cytotoxicity were observed for select compounds; however, these are dose-dependent effects that may be effectively managed through formulation-based approaches. Notably, none of the compounds were predicted to exhibit BBB permeability, thereby reducing the likelihood of CNS-related adverse effects.

Validation of the homology-modeled NLRP9-PYD structure via Ramachandran plot analysis confirmed high stereochemical quality, with 93.8% of residues in the most favoured conformational regions, thereby establishing the structural credibility of the docking platform. Post-docking interaction analysis using BIOVIA Discovery Studio further corroborated that specific withanolides engage key residues integral to NLRP9 PYD function through a combination of hydrogen bonding and hydrophobic interactions.

In conclusion, this investigation demonstrates the feasibility of pharmacologically targeting the NLRP9-PYD domain using bioactive compounds from Withania somnifera, with Withanolide B, Withanolide A, and Withanolide D emerging as primary candidates for further development as natural modulators of inflammasome activation. These findings contribute to the broader scientific rationale for the continued exploration of plant-derived compounds in drug discovery research targeting inflammatory and immune-mediated conditions. Future experimental studies—encompassing both in vitro and in vivo models—are essential to validate the in silico predictions reported herein and to assess the clinical translational potential of these phytochemical candidates.

### *Future prospects*

Experimental validation of the computational binding predictions reported in this study is of paramount importance. Biochemical interaction assays—including Surface Plasmon Resonance (SPR) and Isothermal Titration Calorimetry (ITC)—alongside fluorescence-based displacement studies and co-immunoprecipitation or pull-down assays should be employed to confirm whether the top-ranked ligands disrupt the PYD–ASC protein–protein interaction in vitro.

Subsequent in vivo immunopharmacological evaluation of the most promising candidates—specifically Withanolide B, Withanolide A, and Withanolide D—should be undertaken in well-characterized animal models of acute and chronic inflammation or enteric viral infection. Key experimental endpoints should include quantification of pro-inflammatory cytokines (IL-1β, IL-18), histopathological tissue assessment, and direct evaluation of inflammasome activation status.

Advanced molecular dynamics (MD) simulations are further warranted to characterize the dynamic stability of the ligand–NLRP9 complexes identified in this study, to delineate the role of conformational flexibility in ligand binding, and to identify critical contact residues that may serve as focal points for SAR-guided structural optimization. Such computational insights should directly inform medicinal chemistry efforts aimed at enhancing binding affinity, target selectivity, and pharmacokinetic properties of the lead compounds.

Finally, to optimize systemic bioavailability and minimize off-target toxicity, the development of advanced targeted drug delivery platforms—such as gut-directed or immune cell-specific nanoparticles and liposomal formulations—should be explored to ensure maximal therapeutic efficacy in the context of enteric and systemic inflammatory pathologies.

## Compliance with ethical standards

### *Disclosure of conflict of interest*

No conflict of interest to be disclosed.

## References

[1] Kigerl, K. A., et al. (2014). Pattern recognition receptors and central nervous system repair. Experimental Neurology, 258, 5–16. https://doi.org/10.1016/j.expneurol.2014.01.001

[2] Wicherska-Pawłowska, K., et al. (2021). Toll-like receptors (TLRs), NOD-like receptors (NLRs), and RIG-I-like receptors (RLRs) in innate immunity. International Journal of Molecular Sciences, 22(24), 13397. https://doi.org/10.3390/ijms222413397

[3] Kelley, N., et al. (2019). The NLRP3 inflammasome: An overview of mechanisms of activation and regulation. International Journal of Molecular Sciences, 20(13), 3328. https://doi.org/10.3390/ijms20133328

[4] Yao, J., et al. (2024). The role of inflammasomes in human diseases and their potential as therapeutic targets. Signal Transduction and Targeted Therapy, 9. https://doi.org/10.1038/s41392-023-01687-y

[5] Zheng, Q., et al. (2023). The NLRP3 inflammasome in viral infection (Review). Molecular Medicine Reports, 28. https://doi.org/10.3892/mmr.2023.13047

[6] Man, S. M. (2018). Inflammasomes in the gastrointestinal tract: infection, cancer and gut microbiota homeostasis. Nature Reviews Gastroenterology & Hepatology, 15, 721–737. https://doi.org/10.1038/s41575-018-0054-1

[7] Mullins, B., & Chen, J. (2020). NLRP9 in innate immunity and inflammation. Immunology, 162(3), 262–267. https://doi.org/10.1111/imm.13290

[8] Pandey, A., et al. (2024). Molecular mechanisms of emerging inflammasome complexes and their activation and signaling in inflammation and pyroptosis. Immunological Reviews, 329. https://doi.org/10.1111/imr.13406

[9] Eibl, C. (2012). Structural and functional analysis of the NLRP4 pyrin domain. Biochemistry, 51, 7330–7341. https://doi.org/10.1021/bi3007059

[10] Sharma, M., & de Alba, E. (2021). Structure, activation and regulation of NLRP3 and AIM2 inflammasomes. International Journal of Molecular Sciences, 22(2), 872. https://doi.org/10.3390/ijms22020872

[11] Zhang, L., et al. (2017). Nlrp9b inflammasome restricts rotavirus infection in intestinal epithelial cells. Nature, 547(7663), 348–353. https://doi.org/10.1038/nature22967

[12] Paul, S., et al. (2021). Withania somnifera (L.) Dunal (Ashwagandha): A comprehensive review on ethnopharmacology, pharmacotherapeutics, and phytochemistry. Journal of Ethnopharmacology, 264, 113276. https://doi.org/10.1016/j.jep.2020.113276

[13] Behl, T., et al. (2020). Exploring the multifaceted therapeutic potential of Withania somnifera and its main constituent withaferin A. Pharmaceuticals, 13(11), 363. https://doi.org/10.3390/ph13110363

[14] Sikandan, A., et al. (2018). Ashwagandha root extract exerts anti-inflammatory effects in HaCaT cells by inhibiting the NF-κB pathway. Experimental and Therapeutic Medicine, 16(5), 4255–4261. https://doi.org/10.3892/etm.2018.6712

[15] Kashyap, P., et al. (2022). Recent advances in inflammasome-targeted therapies: Natural compounds as potential modulators. Frontiers in Pharmacology, 13. https://doi.org/10.3389/fphar.2022.955462

[16] Pinzi, L., & Rastelli, G. (2019). Molecular docking: Shifting from the single target to the multiple target paradigm. International Journal of Molecular Sciences, 20(18), 4331. https://doi.org/10.3390/ijms20184331

[17] Kitchen, D. B., et al. (2004). Docking and scoring in virtual screening for drug discovery: methods and applications. Nature Reviews Drug Discovery, 3, 935–949. https://doi.org/10.1038/nrd1549

[18] Ferreira, L. G., et al. (2015). Molecular docking and structure-based drug design strategies. Molecules, 20(7), 13384–13421. https://doi.org/10.3390/molecules200713384

[19] Berman, H. M., et al. (2000). The Protein Data Bank. Nucleic Acids Research, 28(1), 235–242. https://doi.org/10.1093/nar/28.1.235

[20] Lu, A., et al. (2014). Unified polymerization mechanism for the assembly of ASC-dependent inflammasomes. Cell, 156(6), 1193–1206. https://doi.org/10.1016/j.cell.2014.02.008

[21] He, Y., et al. (2016). Small-molecule inhibition of the NLRP3 inflammasome complex. Nature, 530, 232–235. https://doi.org/10.1038/nature16959

[22] Atanasov, A. G., et al. (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

[23] Mishra, L. C., et al. (2000). Scientific basis for the therapeutic use of Withania somnifera (ashwagandha): a review. Alternative Medicine Review, 5(4), 334–346. https://pubmed.ncbi.nlm.nih.gov/10956379/

[24] Mirjalili, M. H., et al. (2009). Steroidal lactones from Withania somnifera, an ancient plant for novel medicine. Molecules, 14(7), 2373–2393. https://doi.org/10.3390/molecules14072373

[25] White, P. T., et al. (2016). Natural Withanolides in the Treatment of Chronic Diseases. Advances in Experimental Medicine and Biology, 928, 329–373. https://doi.org/10.1007/978-3-319-41334-1_14

[26] Kim, S., et al. (2023). PubChem 2023 update: improving the visibility of biological activity and chemical structure data. Nucleic Acids Research, 51(D1), D1378–D1388. https://doi.org/10.1093/nar/gkac956

[27] Bolton, E. E., et al. (2008). PubChem: Integrated Platform of Small Molecules and Biological Activities. Annual Reports in Computational Chemistry, 4, 217–241. https://doi.org/10.1016/S1574-1400(08)00012-1

[28] Ruiz-Garcia, A., et al. (2008). ADME pharmacogenetics: current challenges and future directions. Expert Opinion on Drug Metabolism & Toxicology, 4(9), 1167–1182. https://doi.org/10.1517/17425255.4.9.1167

[29] Daina, A., & Zoete, V. (2016). A BOILED-Egg to Predict Gastrointestinal Absorption and Brain Penetration of Small Molecules. ChemMedChem, 11(11), 1117–1121. https://doi.org/10.1002/cmdc.201600182

[30] Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Scientific Reports, 7, 42717. https://doi.org/10.1038/srep42717

[31] Lipinski, C. A. (2004). Lead- and drug-like compounds: the rule-of-five revolution. Drug Discovery Today: Technologies, 1(4), 337–341. https://doi.org/10.1016/j.ddtec.2004.11.007

[32] Raies, A. B., & Bajic, V. B. (2016). In silico toxicology: computational methods for the prediction of chemical toxicity. Wiley Interdisciplinary Reviews: Computational Molecular Science, 6(2), 147–172. https://doi.org/10.1002/wcms.1240

[33] Banerjee, P., et al. (2018). ProTox-II: a webserver for the prediction of toxicity of oral doses of small molecules. Nucleic Acids Research, 46(W1), W257–W263. https://doi.org/10.1093/nar/gky318

[34] Drwal, M. N., et al. (2014). ProTox: a web server for the in silico prediction of rodent oral toxicity. Nucleic Acids Research, 42(W1), W53–W58. https://doi.org/10.1093/nar/gku401

[35] UniProt Consortium (2023). UniProt: the Universal Protein Knowledgebase in 2023. Nucleic Acids Research, 51(D1), D523–D531. https://doi.org/10.1093/nar/gkac1052

[36] Waterhouse, A., et al. (2018). SWISS-MODEL: homology modelling of protein structures and complexes. Nucleic Acids Research, 46(W1), W296–W303. https://doi.org/10.1093/nar/gky427

[37] Burley, S. K., et al. (2021). RCSB Protein Data Bank: powerful resources for exploring the structural biology of health and disease. Nucleic Acids Research, 49(D1), D437–D451. https://doi.org/10.1093/nar/gkaa1038

[38] Laskowski, R. A., et al. (1993). PROCHECK: a program to check the stereochemical quality of protein structures. Journal of Applied Crystallography, 26(2), 283–291. https://doi.org/10.1107/S002188989200994X

[39] Colovos, C., & Yeates, T. O. (1993). Verification of protein structures: patterns of nonbonded atomic interactions. Protein Science, 2(9), 1511–1519. https://doi.org/10.1002/pro.5560020916

[40] Morris, G. M., et al. (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. Journal of Computational Chemistry, 30(16), 2785–2791. https://doi.org/10.1002/jcc.21256

[41] Eberhardt, J., et al. (2021). AutoDock Vina 1.2.0: New Docking Methods, Expanded Force Field, and Python Bindings. Journal of Chemical Information and Modeling, 61(8), 3891–3898. https://doi.org/10.1021/acs.jcim.1c00203

[42] Dallakyan, S., & Olson, A. J. (2015). Small-molecule library screening with PyRx. Methods in Molecular Biology, 1263, 243–250. https://doi.org/10.1007/978-1-4939-2269-7_19

[43] Dassault Systèmes BIOVIA (2021). Discovery Studio Visualizer, Release 2021, San Diego: Dassault Systèmes.

[44] Guan, L., et al. (2019). ADMET-score: a comprehensive scoring function for evaluation of chemical drug-likeness. MedChemComm, 10(1), 148–157. https://doi.org/10.1039/C8MD00472G

[45] Dong, J., et al. (2018). ADMETlab: a platform for systematic ADMET evaluation based on a computationally optimized evaluation system. Journal of Cheminformatics, 10, 29. https://doi.org/10.1186/s13321-018-0283-x

[46] Veber, D. F., et al. (2002). Molecular properties that influence the oral bioavailability of drug candidates. Journal of Medicinal Chemistry, 45(12), 2615–2623. https://doi.org/10.1021/jm020017n

[47] Prasanna, S., & Doerksen, R. J. (2009). Topological polar surface area: a useful descriptor in 2D-QSAR. Current Medicinal Chemistry, 16(1), 21–41. https://doi.org/10.2174/092986709787002817

[48] Pollastri, M. P. (2010). Overview on the Rule of Five. Current Protocols in Chemical Biology, 2(3), 101–118. https://doi.org/10.1002/9780470559277.ch090155

[49] Ghose, A. K., et al. (1999). A Knowledge-Based Approach in Designing Combinatorial or Medicinal Chemical Libraries for Drug Discovery. Journal of Combinatorial Chemistry, 1(1), 55–68. https://doi.org/10.1021/cc9800071

[50] Teague, S. J., et al. (1999). The Design of Leadlike Combinatorial Libraries. Angewandte Chemie International Edition, 38(24), 3743–3748.

[51] Pajouhesh, H., & Lenz, G. R. (2005). Medicinal chemical properties of successful central nervous system drugs. NeuroRx, 2(4), 541–553. https://doi.org/10.1602/neurorx.2.4.541

[52] Amin, M. L. (2013). P-glycoprotein inhibition for optimal drug delivery. Drug Target Insights, 7, 27–34. https://doi.org/10.4137/DTI.S12519

[53] Manikandan, P., & Nagini, S. (2018). Cytochrome P450 Structure, Function and Clinical Significance: A Review. Current Drug Targets, 19(1), 38–54. https://doi.org/10.2174/1389450118666170125144120

[54] Zanger, U. M., & Schwab, M. (2013). Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. Pharmacology & Therapeutics, 138(1), 103–141. https://doi.org/10.1016/j.pharmthera.2012.12.007

[55] Potts, R. O., & Guy, R. H. (1992). Predicting skin permeability. Pharmaceutical Research, 9(5), 663–669.

[56] Baell, J. B., & Holloway, G. A. (2010). New Substructure Filters for Removal of Pan Assay Interference Compounds (PAINS). Journal of Medicinal Chemistry, 53(7), 2719–2740. https://doi.org/10.1021/jm901137j

[57] Brenk, R., et al. (2008). Lessons Learnt from Assembling Screening Libraries for Drug Discovery for Neglected Diseases. ChemMedChem, 3(3), 435–444. https://doi.org/10.1002/cmdc.200700139

[58] Schuffenhauer, A., et al. (2003). An Ontology for Pharmaceutical Substructures and Its Use in Hit-to-Lead Optimization. Journal of Chemical Information and Computer Sciences, 43(2), 391–405. https://doi.org/10.1021/ci025615q

[59] Ertl, P., & Schuffenhauer, A. (2009). Estimation of synthetic accessibility score of drug-like molecules. Journal of Cheminformatics, 1, 8. https://doi.org/10.1186/1758-2946-1-8

[60] Pradeep, H., et al. (2014). Withania somnifera protects against ethidium bromide-induced cytotoxicity and DNA damage. Journal of Ethnopharmacology, 154(3), 540–547. https://doi.org/10.1016/j.jep.2014.04.044

[61] Mirjalili, M. H., et al. (2009). Steroidal lactones from Withania somnifera, an ancient plant for novel medicine. Molecules, 14(7), 2373–2393. https://doi.org/10.3390/molecules14072373

[62] Vyas, A. R., & Singh, S. V. (2014). Molecular targets and mechanisms of cancer prevention and treatment by withaferin A. AAPS Journal, 16(1), 1–10. https://doi.org/10.1208/s12248-013-9531-1

[63] Chen, M., et al. (2016). FDA-approved drug labeling for the study of drug-induced liver injury. Drug Discovery Today, 21(11), 1747–1753. https://doi.org/10.1016/j.drudis.2016.07.007

[64] Björnsson, E. S., & Hoofnagle, J. H. (2016). Categorization of drugs implicated in causing liver injury. Hepatology, 63(3), 1020–1029. https://doi.org/10.1002/hep.28355

[65] Nishikawa, A., et al. (2006). Genetic and epigenetic mechanisms of carcinogenesis as targets for cancer therapeutics and prevention. Current Cancer Drug Targets, 6(1), 13–33.

[66] Zeiger, E. (2001). Mutagens that are not carcinogens: faulty theory or faulty data? Mutation Research, 492(1-2), 29–38.

[67] Mortelmans, K., & Zeiger, E. (2000). The Ames Salmonella/microsome mutagenicity assay. Mutation Research, 455(1-2), 29–60. https://doi.org/10.1016/s0027-5107(00)00064-6

[68] Gautam, M., et al. (2009). Immunomodulatory activity of Withania somnifera on glycosylation of molecules involved in cell-cell interactions. Phytotherapy Research, 23(11), 1630–1635. https://doi.org/10.1002/ptr.2782

[69] Senthil, V., et al. (2015). In silico cytotoxicity prediction of withanolides from Withania somnifera. Journal of Chemical and Pharmaceutical Research, 7(3), 914–921.

[70] Lee, I. C., et al. (2012). Withaferin A Inhibits the Proliferation of Human Breast Cancer Cells. Journal of Cancer Prevention, 17(2), 118–124. https://doi.org/10.15430/JCP.2012.17.2.118

[71] Dar, N. J., et al. (2015). Pharmacologic profile of Withania somnifera: A revitalizing herbal medicine. Pharmacognosy Reviews, 9(17), 44–56. https://doi.org/10.4103/0973-7847.156361

[72] Hooft, R. W., et al. (1996). Errors in protein structures. Nature, 381(6580), 272. https://doi.org/10.1038/381272a0

[73] Kufareva, I., & Abagyan, R. (2012). Methods of protein structure comparison. Methods in Molecular Biology, 857, 231–257. https://doi.org/10.1007/978-1-61779-588-6_10

[74] Anderson, R. J., et al. (2005). Ramachandran plots for side chain conformations of amino acid residues in proteins. Proteins, 60(4), 687–689. https://doi.org/10.1002/prot.20539

[75] Trott, O., & Olson, A. J. (2010). AutoDock Vina: improving the speed and accuracy of docking with a new scoring function. Journal of Computational Chemistry, 31(2), 455–461. https://doi.org/10.1002/jcc.21334

[76] Sahu, B. D., et al. (2015). Withania somnifera root extract ameliorates cyclophosphamide-induced bladder toxicity. Journal of Ethnopharmacology, 174, 513–522. https://doi.org/10.1016/j.jep.2015.08.053

[77] Srivastava, P., et al. (2016). In silico study of withanolides as potential inhibitors of inflammatory markers. Bioinformation, 12(3), 117–121. https://doi.org/10.6026/97320630012117

[78] Jain, S., et al. (2011). Phytosome: a novel drug delivery system for herbal medicine. International Journal of Pharmaceutical Sciences and Drug Research, 3(1), 1–7.

[79] Koduru, S., et al. (2010). Withanone from Withania somnifera attenuates the growth of human cancer cells by inducing apoptosis. International Journal of Oncology, 36(5), 1069–1078. https://doi.org/10.3892/ijo_00000591

[80] Vajjhala, P. R., et al. (2012). The molecular mechanisms of inflammasome assembly. Apoptosis, 17(11), 1150–1167. https://doi.org/10.1007/s10495-012-0753-1

[81] Park, H. H. (2012). Structural biology of the inflammasome. BMB Reports, 45(6), 311–317. https://doi.org/10.5483/bmbrep.2012.45.6.114

[82] Grover, A., et al. (2010). Withanone binds to mortalin and induces senescence in cancer cells. Cell Death & Disease, 1(6), e54. https://doi.org/10.1038/cddis.2010.30

[83] Mishra, L. C., et al. (2000). Scientific basis for the therapeutic use of Withania somnifera: a review. Alternative Medicine Review, 5(4), 334–346.

[84] Behl, T., et al. (2021). Natural compounds as potential inhibitors of the NLRP3 inflammasome: A review. Molecules, 26(11), 3122. https://doi.org/10.3390/molecules26113122

[85] Sutherland, J. J., et al. (2020). Computational and Experimental Approaches to Target the Inflammasome. Journal of Medicinal Chemistry, 63(23), 14310–14330. https://doi.org/10.1021/acs.jmedchem.0c00588