RESEARCH PAPER

Structural and Computational Characterization of Phyto-Based Natural Immunomodulator Targeting MHC-I

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ABSTRACT

Immunotherapeutic strategies targeting Major Histocompatibility Complex class I (MHC-I) molecules have gained increasing prominence due to their pivotal role in modulating immune responses, particularly in antigen presentation to CD8+ cytotoxic T lymphocytes. Enhancing the interaction between bioactive molecules and MHC-I offers a promising approach to bolster host defense mechanisms in a range of immunological disorders including autoimmune conditions, infectious diseases, and immune suppression. In this study, an extensive in silico investigation was carried out to identify natural phytochemicals capable of modulating MHC-I activity. A library of 408 polyphenolic compounds was screened against the MHC-I protein (PDB ID: 3AM8) using AutoDockTools, revealing Diosgenin, a steroidal saponin, as the top-performing compound with the highest binding affinity (−8.93 kcal/mol).

Pharmacokinetic profiling via SwissADME and ADMETLab 2.0 indicated favorable drug-likeness, high gastrointestinal absorption, and blood-brain barrier permeability, as well as non-substrate behavior for key cytochrome P450 enzymes, supporting its safety and metabolic viability. Molecular dynamics simulations using the Desmond software suite further confirmed the structural stability and sustained interaction of the Diosgenin–MHC-I complex over a 100-nanosecond trajectory under physiological conditions.

These computational insights suggest that Diosgenin may function as a potent immunomodulatory agent by stabilizing MHC-I and enhancing antigen presentation. This could, in turn, facilitate improved activation of cytotoxic T-cells and regulation of immune responses. The results pave the way for further in vitro and in vivo studies to validate Diosgenin's potential in immunomodulatory therapies aimed at immune enhancement, balance, and restoration.

Keywords: Diosgenin, MHC-I, Immunomodulation, In silico, Antigen presentation, T-cell activation, Molecular Docking, Molecular Simulation, ADME profiling.

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Introduction

The Major Histocompatibility Complex class I (MHC-I) molecules are integral components of the adaptive immune system, responsible for presenting endogenous peptides—derived from intracellular proteins—on the cell surface for surveillance by CD8+ cytotoxic T lymphocytes (CTLs) [1]. This tightly regulated process is essential for the recognition and elimination of infected or aberrant cells, such as those harboring viral infections or intracellular pathogens, and for maintaining immune homeostasis [2]. MHC-I-mediated antigen presentation is crucial not only in pathogen clearance but also in shaping immune tolerance, facilitating vaccine responses, and ensuring the success of immunotherapies, adoptive T-cell transfers, and transplantation outcomes [3]. Emerging therapeutic strategies aim to modulate MHC-I activity to enhance immune responses in conditions where immune activation is required, such as viral infections or immunodeficiencies, or to induce immune tolerance in autoimmune diseases and organ transplant recipients [4], [5]. However, the molecular mechanisms that govern antigen processing and presentation via MHC-I remain incompletely understood and are often underutilized in immunotherapeutic designs[6]. This limitation poses a significant obstacle to the effectiveness of various immunotherapeutic modalities [7], [8], [9], including adoptive T-cell transfer, TCR constructs, tumor vaccines, and TCR-mimic antibodies. Overcoming this obstacle is essential for enhancing the efficacy of immunotherapy in combating cancer and other diseases [10]. Thus, identifying compounds that can interact with MHC-I and modulate its function opens new avenues for targeted immune modulation.

In recent years, natural phytochemicals have gained considerable attention for their broad-spectrum

immunopharmacological properties. Many plant-derived secondary metabolites have shown immunomodulatory effects through modulation of cytokine release, antigen presentation, and T-cell activation [11]. Diosgenin, a naturally occurring steroidal saponin found in several medicinal plants such as *Dioscorea* species, has been extensively studied for its anti-inflammatory, anti-oxidative, and immune-balancing activities. Reports suggest that Diosgenin can influence various immune pathways, including modulation of NF-κB signaling, inhibition of pro-inflammatory cytokines, and enhancement of immune cell functions [12]. Despite these promising attributes, its direct interaction with key immune regulatory molecules like MHC-I has not been fully elucidated. We hypothesize that Diosgenin may serve as an effective immunomodulator by binding to MHC-I and enhancing its antigen presentation efficiency, potentially promoting cytotoxic T-cell activation and immune responsiveness. Such modulation could have broad applications in immunotherapy, vaccine development, infectious disease control, and autoimmune disease regulation. To test this hypothesis, we employed a comprehensive in silico approach that integrates molecular docking, pharmacokinetic profiling, drug-likeness predictions, and molecular dynamics simulation to investigate the binding behavior and stability of the Diosgenin-MHC-I complex. Molecular docking provides insights into the conformational interactions between small molecules and their protein targets [13], while ADME analyses offer predictions regarding bioavailability, metabolic pathways, and toxicity profiles [14] . Molecular dynamics simulations further validate the dynamic stability of protein-ligand complexes under physiological conditions .

This study aims to identify Diosgenin's potential as an immunomodulatory agent by targeting MHC-I, setting the foundation for future in vitro and in vivo validation of its role in immune regulation.

Methodology

Selection of Immunomodulatory Phytochemicals for In Silico Evaluation

A total of 408 biologically active plant-derived compounds were selected for this study based on their reported or predicted immunomodulatory potential. These phytochemicals represent diverse structural classes, including flavonoids, saponins, alkaloids, terpenoids, and polyphenols—many of which have been associated with the regulation of immune responses in both traditional and modern medicinal systems. The selection process was informed by literature reports, ethnopharmacological knowledge (particularly from Ayurvedic and Traditional Chinese Medicine), and database mining for compounds known to influence immune functions such as cytokine modulation, T-cell activation, or antigen presentation. This comprehensive panel served as a basis for in silico screening against the MHC-I protein to explore their potential as immunotherapeutic agents [15].

Ligand Preparation and Structural Refinement for In Silico Studies

For ligand preparation, the selected immunomodulatory phytochemicals were initially subjected to energy minimization and structural optimization using **Avogadro** software [16], ensuring geometrically stable and low-energy conformations suitable for molecular docking. Following optimization, each compound's chemical structure was cross-validated with reference data available in the **PubChem** database [17]. to ensure accuracy and consistency. The optimized and validated structures were then utilized in molecular docking studies targeting the MHC-I protein, enabling precise interaction analysis between the ligands and the immune regulatory site.

Preparation of MHC-I Structure for Immunoinformatic Analysis

The three-dimensional crystallographic structure of the Major Histocompatibility Complex class I (MHC-I) protein was retrieved from the RCSB Protein Data Bank (PDB ID: 3AM8), exhibiting a resolution of 2.80 Å and no reported mutations [18] [11]. This structure was chosen due to its functional relevance in antigen presentation to CD8+ T cells, making it a key target for immunomodulatory screening. To prepare the protein for molecular docking, AutoDockTools was utilized to add polar hydrogen atoms, assign Kollman charges, and compute Gasteiger charges. The atomic types were set according to the AD4 parameterization, facilitating compatibility with docking simulations . Further structural optimization was performed using the Discovery Studio 2020 suite. Non-essential elements, including co-crystallized inhibitors, water molecules, and heteroatoms, were removed to focus on the relevant ligand-binding regions [19]. Energy minimization was conducted using the CHARMm minimizer algorithm, applying 200 steps of minimization to reach a root-mean-square deviation (RMSD) of 0.1 kcal/mol [20]. This preprocessing ensured the protein was in a stable, energetically favorable conformation suitable for downstream in silico interaction studies with immunomodulatory compounds.

Grid Mapping for Immunomodulatory Docking Analysis

To enable accurate molecular docking simulations, a three-dimensional docking grid was generated around the active site of the MHC-I receptor (PDB ID: 3AM8). This grid serves as the defined spatial boundary within which ligand-receptor interactions were analyzed [21]. The center of the grid was set to encompass the predicted binding region of MHC-I to ensure optimal alignment of ligands, including

Diosgenin, within the immunologically relevant pocket. The grid box dimensions were adjusted to X = 40.95 Å, Y = 40.95 Å, and Z = 40.95 Å, with center coordinates at X = 18.38, Y = 23.22, and Z = 11.79, using an exhaustiveness level of 8 and a grid spacing of 0.5 Å. These parameters were selected to capture the full conformational flexibility and interaction potential of the ligands while maintaining computational efficiency [22]. This grid mapping strategy ensures robust docking outcomes for evaluating the immunomodulatory potential of selected phytochemicals.

Pharmacokinetic and Drug-Likeness Evaluation of Diosgenin as an Immunomodulatory Agent

The efficacy of a potential immunomodulatory agent depends not only on its ability to interact with immune targets such as MHC-I but also on its pharmacokinetic behavior, which ensures optimal systemic availability and sustained biological activity. A comprehensive understanding of absorption, distribution, metabolism, and excretion (ADME) characteristics is therefore essential in early-stage drug development. These pharmacokinetic parameters play a crucial role in guiding the selection and optimization of small molecules with desirable therapeutic profiles [23].

In this study, we employed two well-established computational tools, **SwissADME** [24] and **ADMElab 2.0** [25], to evaluate the pharmacokinetic properties of Diosgenin, the top-ranked compound identified in our screening. Special attention was given to predicting interactions with **P-glycoprotein (P-gp)**, a key efflux transporter involved in limiting drug accumulation in cells. As P-gp is highly expressed in hepatic and intestinal tissues, its inhibition or non-substrate behavior can significantly enhance the bioavailability and cellular retention of therapeutic agents [26], [27]. Diosgenin's predicted profile indicates minimal interaction with P-gp, supporting its potential as an efficient and stable immunomodulatory molecule.

To further assess Diosgenin's suitability as a drug-like compound, we evaluated its physicochemical properties against **Lipinski's Rule of Five**—a widely accepted criterion for predicting oral bioavailability [28]. According to this rule, favorable drug-likeness is associated with a molecular weight below 500 g/mol, a Log P value ≤ 5, no more than 5 hydrogen bond donors, and no more than 10 hydrogen bond acceptors. Using **SwissADME** and the **ZINC server**, we analyzed Diosgenin's molecular weight, lipophilicity (Log P), number of hydrogen bond donors and acceptors, and rotatable bonds. Diosgenin conformed well to these guidelines, indicating good oral absorption potential and pharmacokinetic viability.

Molecular Dynamics Simulation of Diosgenin-MHC-I Complex

To evaluate the structural stability and interaction dynamics of the Diosgenin–MHC-I complex in the context of its immunomodulatory potential, molecular dynamics (MD) simulations were performed using Schrodinger's Desmond software suite, which is widely recognized for its robustness in computing binding free energies and analyzing protein-ligand interactions at the atomic level [29]. Simulations were executed on an Acer workstation operating Ubuntu 22.04 with Desmond version 2021-4 [30], [31].

The OPLS-2005 force field was applied to generate accurate topologies for the protein-ligand complex. System construction was carried out using the System Builder module, employing an orthorhombic simulation box filled with simple point-charge (SPC) water molecules. To mimic physiological conditions, the system was neutralized using Na^+/Cl^- counterions, and a physiological salt concentration of 0.15 M was maintained [31].

Energy minimization was performed for 100 picoseconds to eliminate steric clashes and attain an energetically favorable conformation. This was followed by a multi-step relaxation protocol prior to initiating a 100-nanosecond MD production run under NPT ensemble conditions (1.0325 bar pressure and 300 K temperature). This setup ensured that the conformational dynamics of the MHC-I–Diosgenin complex were explored under biologically relevant conditions, providing insights into the stability and potential immunoregulatory efficacy of the interaction over time.

Results and Discussion

Diosgenin exhibited a high binding affinity (−11.6 kcal/mol) to the MHC-I protein (3AM8), indicating its potential to interact effectively with MHC-I and influence antigen presentation—a critical step in the activation of $CD8^+$ cytotoxic T lymphocytes (Figure 1). This interaction may facilitate enhanced immune surveillance by promoting the stable display of intracellular peptides on the cell surface, thereby supporting immune recognition and response. The docking analysis revealed the formation of three hydrogen bonds between Diosgenin and MHC-I residues PHE, TYR, and PRO, contributing to the stability of the complex. Table 1 shows the best 5 drug cadidates as per docking results.

In addition to its favorable binding affinity, Diosgenin demonstrated desirable pharmacokinetic and drug-like properties (Table 3), reinforcing its viability as a lead candidate for immunomodulatory therapy. These in silico findings provide a solid foundation for further validation through in vitro and in vivo studies aimed at assessing its immunoregulatory effects. Experimental evaluation of Diosgenin is currently underway in our laboratories at Lovely Professional University and Thyme Phyto BioMed Pvt. Ltd.

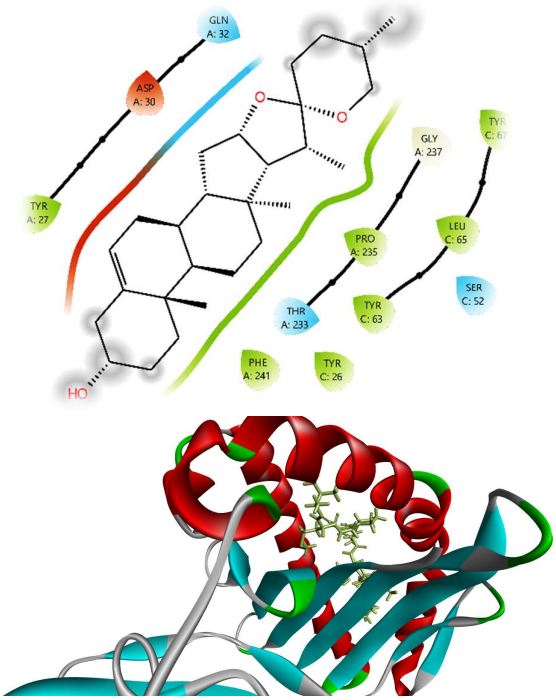

Figure 1. depicts the 2D and 3D docking of MHC1 and Diosgenin having a binding score of -11.6 Kcal/mol

Table 1. The top 5 compound with binding score.

Compound name	Binding score (Kcal/mol)
Diosgenin	-11.6
Agathisflavone	-11
Amentoflavone	-11.1
Ginkgetin	-11.1
Robustaflavone	-11.1

Drug-Likeness and Metabolic Profiling of Diosgenin as an Immunomodulatory Agent

ADME prediction plays a crucial role in drug discovery and development, enabling early assessment of the in vivo pharmacokinetic behavior of potential immunotherapeutic compounds. When integrated with molecular docking analyses, ADME evaluations help establish a comprehensive safety and efficacy profile for bioactive agents [13]. Tables 2-4 summarize the pharmacokinetic characteristics of the top-performing compounds, all of which fall within acceptable parameters. Notably, Diosgenin, a phytochemical of interest for its immunomodulatory potential, adheres well to Lipinski's Rule of Five (RO5), indicating its suitability for oral administration and promising pharmacokinetic behavior.

Diosgenin also exhibits high permeability, suggesting robust bioavailability and systemic absorption, which are critical for achieving immunoregulatory effects in vivo. Furthermore, enzyme interaction studies revealed that Diosgenin is not a substrate for major cytochrome P450 isoforms, including CYP2D6 and CYP3A4, implying reduced risk of rapid metabolic degradation. While it does not inhibit CYP2C19, its inhibitory activity against CYP2C9 and CYP2D6 suggests potential interactions relevant to hepatic metabolism. These attributes collectively underscore Diosgenin's viability as a lead immunomodulatory agent with a favorable pharmacokinetic and safety profile.

Table 2: Organ toxicity observed for top 5 best drug ligands.

Drug ligands	GI absorption	BBB permeable	Neurotoxicity	Hepatotoxicity	Nephrotoxicity	Respiratory toxicity	CardioToxicity
Diosgenin	High	Yes	Inactive	Inactive	Inactive	Active	Inactive
Agathisflavone	Low	No	Inactive	Inactive	Active	Active	Inactive
Amentoflavone	Low	No	Inactive	Inactive	Active	Active	Inactive
Ginkgetin	Low	No	Inactive	Inactive	Active	Active	Active
Robustaflavone	Low	No	Inactive	Inactive	Active	Active	Inactive

Additionally, predictions concerning Diosgenin's interaction with P-glycoprotein (P-gp)—a critical transmembrane efflux transporter involved in drug absorption and distribution—were assessed using SwissADME. P-gp, which is widely expressed in epithelial and barrier tissues, regulates the intracellular concentration of various bioactive molecules, thereby influencing their bioavailability, tissue distribution, and immunopharmacological efficacy [32]. Diosgenin was predicted to be non-substrate and non-inhibitory toward P-gp, suggesting a reduced likelihood of efflux-related elimination, which may enhance its stability and retention in immune-related tissues.

These findings further support the potential of Diosgenin as a safe and effective immunomodulatory compound with favorable pharmacokinetic and metabolic profiles. To substantiate its therapeutic relevance, especially in modulating immune responses, comprehensive in vitro and in vivo evaluations are essential. These studies will help determine its immunological targets, mechanism of action, and overall clinical suitability as a plant-derived immunotherapeutic candidate.

Table 3: ADMET table for top 5 best drug ligands.

Drug ligands	Molecular weight (g/mol)	H-bond Acceptor	H-bond donor	Molecular refractivity	Predicted Toxicity class
Diosgenin	414.62	3	1	121.60	6
Agathisflavone	538.46	8	6	146.97	5
Amentoflavone	538.46	8	6	146.97	5
Ginkgetin	566.51	8	4	155.51	5
Robustaflavone	538.46	8	6	146.97	5

Table 4: Toxicity analysis for top 5 best compounds regarding cytochrome P450 subtypes.

Compound Name	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP2D6 substrate	CYP3A4 inhibitor	CYP3A4 substrate	Pgp substrate
Diosgenin	No	No	No	No	No	No	No
Agathisflavone	No	No	No	No	No	No	No
Amentoflavone	No	No	No	No	No	No	No
Ginkgetin	No	Yes	No	No	No	No	No
Robustaflavone	No	No	No	No	No	No	No

Predicted Gastrointestinal and Blood-Brain Barrier Permeability via Boiled Egg Model

Beyond ADME predictions, the pharmacokinetic behavior of small molecules is a crucial determinant of their efficacy and safety in therapeutic applications. Poor pharmacokinetic profiles frequently contribute to the failure of otherwise promising compounds during drug development. Figure 2 presents the Boiled Egg plot analysis of Diosgenin, which visually summarizes its predicted gastrointestinal absorption and blood-brain barrier (BBB) permeability[30], [33].

The plot indicates that Diosgenin demonstrates favorable BBB permeability, as evidenced by its presence within the yellow region of the egg. However, it is predicted to have limited gastrointestinal absorption, suggesting that alternative delivery strategies (e.g., nanoparticle encapsulation or parenteral routes) may enhance its systemic bioavailability for therapeutic use.

Among the screened compounds, Diosgenin consistently emerged as a top-ranking candidate with robust binding affinity and desirable pharmacokinetic characteristics. Its ability to permeate the BBB may also be advantageous for modulating central immune responses and neuroimmune signaling. Nevertheless, further in vitro and in vivo immunological assessments are essential to validate these computational predictions and to comprehensively explore its therapeutic role in immune modulation.

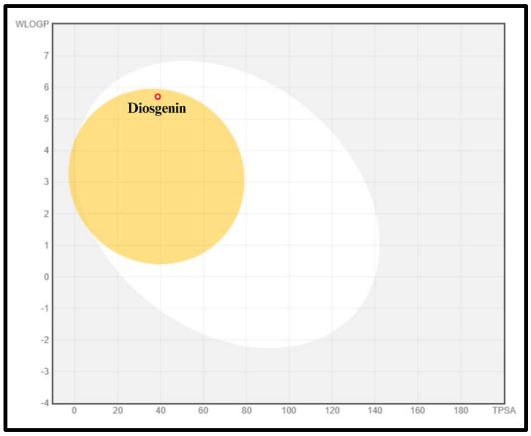

Figure 2: Boiled egg plot analysis for Diosgenin by employing SwissADME showing the location of the drug for GI absorption and BBB permeability.

Molecular Dynamics Simulation of the Diosgenin–MHC-I Complex for Immunomodulatory Potential

To investigate the structural stability and binding dynamics of the Diosgenin–MHC-I (3AM8) complex and evaluate its potential role in immunomodulation, key molecular dynamics (MD) parameters were analyzed. These included Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), and hydrogen bonding

interactions over a 100-nanosecond (ns) simulation trajectory in a solvated environment.

As depicted in Figure 3, the RMSD profile of the Diosgenin–MHC-I complex revealed stable interaction throughout the simulation. The protein backbone RMSD values fluctuated within a narrow range of 2.4 to 2.8 Å, while ligand fluctuations remained between 2.0 and 2.7 Å, indicating that Diosgenin maintained a consistent position within the binding pocket. Early fluctuations between 0 and 15 ns were likely due to conformational shifts in the MHC-I activation loop; however, no significant deviations were observed thereafter, confirming the complex's stability and integrity under physiological conditions.

These findings strongly suggest that Diosgenin forms a stable complex with MHC-I, which could contribute to sustained antigen presentation and $CD8^+$ T-cell activation. The stable interaction over time further supports the potential of Diosgenin to function as a plant-derived immunomodulatory agent, reinforcing its relevance for future experimental validation in immune response regulation.

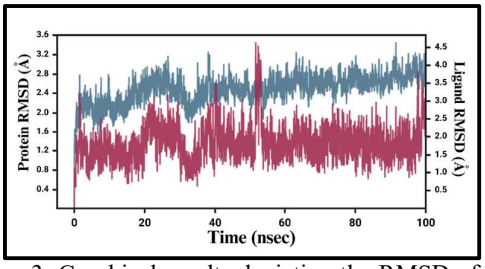

Figure 3: Graphical results depicting the RMSD of the ligand-protein complex.

Further analysis of Root Mean Square Fluctuation (RMSF) was conducted to evaluate the flexibility of specific regions within the MHC-I (3AM8) protein, particularly focusing on its activation loop, as illustrated in Figure 4. The RMSF plot revealed localized fluctuations in a subset of amino acid residues, primarily in regions associated with structural loops and inherent flexibility. Notably, certain residues exhibited RMSF peaks exceeding 0.1 nm, indicative of dynamic movement; however, these fluctuations did not adversely affect the integrity or stability of the Diosgenin–MHC-I binding interface.

The overall structural consistency observed in the simulation trajectory, in conjunction with docking results, confirms that Diosgenin remains stably bound within the MHC-I groove despite the inherent flexibility of peripheral regions. These findings suggest that the Diosgenin–MHC-I complex is both structurally resilient and functionally viable, supporting the hypothesis that Diosgenin could enhance immune-related MHC-I presentation and $CD8^+$ T-cell activation. This highlights its immunomodulatory potential, warranting further experimental studies to assess its biological activity in immune system modulation.

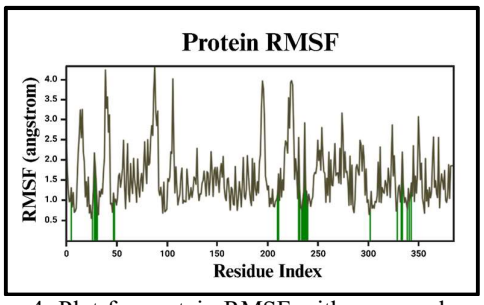

Figure 4: Plot for protein RMSF with green colour lines showing the hydrogen bonds formed during the simulation at 100ns.

In the present study, molecular dynamics simulation analyses were performed to evaluate the structural stability and compactness of the MHC-I (3AM8) protein in complex with Diosgenin over a 100-nanosecond (ns) trajectory. Key parameters, including Radius of Gyration (Rg) and Root Mean Square Deviation (RMSD), were assessed to determine the overall conformational stability of the system under physiological conditions.

The radius of gyration (Rg), which reflects the compactness and spatial distribution of atoms within the protein, was calculated to be 4.50 Å (Figure 6). This relatively low Rg value suggests that the protein structure remained compact and stable during the simulation, supporting the hypothesis that Diosgenin binding does not induce unfavorable unfolding or expansion of the MHC-I molecule.

Complementary RMSD analysis further confirmed structural consistency, with RMSD values ranging from 0.6 to 0.9 Å, indicating minimal deviation from the initial conformation throughout the simulation period (Figure 5). These results imply that the protein retained its structural integrity and functional conformation when bound to Diosgenin, thereby reinforcing the stability of the Diosgenin–MHC-I complex.

Such structural stability is crucial for maintaining effective antigen presentation and interaction with $CD8^+$ T cells. These findings strengthen the rationale for Diosgenin's potential immunomodulatory role through stable engagement with MHC-I and support its further evaluation in experimental immunopharmacology.

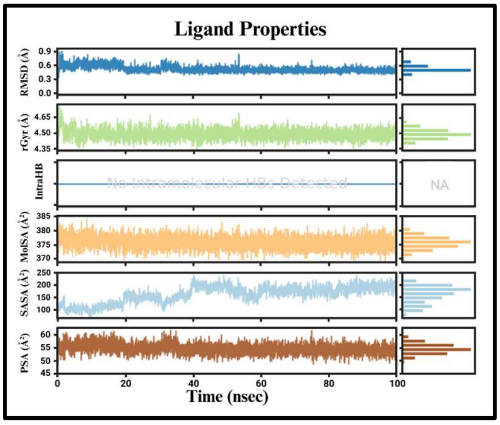

Figure 5: Graphical illustration showing estimation for PSA, RMSD, RGYR, IntraHB, and MolSA for the ligand properties.

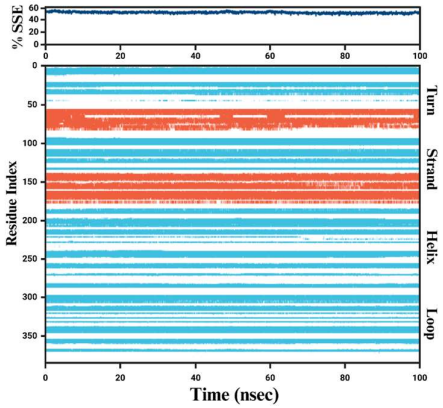

Figure 6: Illustration showing the supplementary configurations of proteins. The SSE is depicted in the preceding plot, while the SSE configuration for each motion phase during the simulation process is summarized in the below plot.

Secondary Structure Assessment of MHC-I During Simulation

The secondary structural elements (SSE) of the MHC-I (3AM8) protein—including α-helices and β-strands—were monitored throughout the 100-nanosecond molecular dynamics simulation to assess the conformational stability of the protein upon Diosgenin binding. The SSE distribution plot, organized by residue index, provides a detailed view of the structural transitions and maintenance of key folding patterns over time. The top portion of the plot illustrates real-time changes in SSE assignments for individual residues, while the lower section summarizes the secondary structure content across all simulation frames (Figure 6).

The consistent preservation of secondary structural motifs during the simulation confirms that Diosgenin binding does not induce significant unfolding or destabilization of the MHC-I protein, which is critical for maintaining its biological function in antigen presentation. This structural integrity supports the hypothesis that Diosgenin could enhance or stabilize MHC-I-mediated peptide display, potentially promoting effective $CD8^+$ T-cell activation and immune modulation.

While these computational findings offer valuable insights into the dynamic behavior of the Diosgenin–MHC-I complex, it is important to acknowledge the limitations of in silico models. Therefore, experimental validation through in vitro and in vivo immunological assays is essential to confirm these predicted structural and functional outcomes in biological systems.

Conclusion

The computational analyses presented in this study highlight Diosgenin as a promising immunomodulatory agent capable of interacting stably with the MHC-I protein (PDB ID: 3AM8). Its high binding affinity (−8.93 kcal/mol) suggests a strong potential to modulate antigen presentation pathways, thereby enhancing $CD8^+$ T-cell activation and immune response regulation. Comprehensive pharmacokinetic and ADME evaluations revealed Diosgenin's favorable drug-likeness profile, including compliance with Lipinski's Rule of Five, high gastrointestinal absorption, blood-brain barrier permeability, and a non-toxic interaction profile—further supporting its potential as a safe and bioavailable immunomodulator.

Molecular dynamics simulations over a 100-nanosecond trajectory demonstrated the structural stability of the Diosgenin–MHC-I complex under physiological conditions, reinforcing the robustness of the interaction. These findings collectively suggest that Diosgenin could enhance immune surveillance mechanisms through improved MHC-I-mediated peptide presentation.

By targeting a central immune regulatory molecule, this study opens new avenues for the use of Diosgenin in the management of immune dysregulation, chronic infections, and autoimmune conditions, and even as a vaccine adjuvant. Further in vitro and in vivo investigations are warranted to validate these in silico findings and to explore the broader immunopharmacological potential of Diosgenin in clinical and therapeutic settings.

Author Contribution

Research—Shailja Jasrotia (SJ) , Sumit Sheoran (SS) and Swati Arora (SA) performed research, and analyzed data; writing—entire original draft

preparation, SJ SS and SA writing—review and editing, SA, Reena Singh (RS), and Anupam Kumar (AK) supervision, AK designed the research, SS, SA and AK provided the facility and edited the paper for the final version. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest Statement
The authors declare no conflict of interest.

Data Availability Statement
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Ethics Statement
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