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Metformin as a modulator of mitochondria-mediated antiviral innate immunity: Insights from molecular docking analysis

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Abstract

Metformin, a widely used antidiabetic drug, has been increasingly recognized for its immunomodulatory effects, particularly in antiviral and inflammatory responses. This study investigates the potential of metformin to modulate mitochondria-mediated antiviral innate immunity through molecular docking analysis. The study focuses on key proteins within the the stimulator of interferon gene (STING) and mitochondrial antiviral-signaling (MAVS) pathways, which play a critical role in the activation of interferon responses and the production of inflammatory cytokines. Protein-protein interaction (PPI) network analysis was performed using the STRING database, and molecular docking simulations were conducted to identify potential binding regions and affinities between metformin and target proteins. 5'-adenosine monophosphate-activated protein kinase (AMPK) was used as a reference protein to compare binding interactions. Docking results revealed that metformin exhibited moderate binding affinities (-4.5 to -5.5 kcal/mol) with key immune regulators, including MAVS, STING, interferon regulatory factors 3 (IRF3), interferon regulatory factors 7 (IRF7), TANK-binding kinase 1 (TBK1), nuclear transcription factor-κB (NF-κB), cyclic GMP-AMP synthase (cGAS), Retinoic acid-inducible gene I (RIG-I), and Melanoma Differentiation-Associated protein 5 (MDA5). Notably, metformin showed a strong interaction with AMPK (ΔG=-5.5 kcal/mol), supporting a link between its metabolic and immune-modulating effects. The ability of metformin to interact with critical components of the STING and MAVS pathways suggests that it may influence innate immune responses, particularly in viral infections and inflammatory diseases. These findings provide new insights into the immunoregulatory properties of metformin, emphasizing the need for further experimental studies to better understand its effects on inflammation.

Keywords: Metformin, stimulator of interferon gene, mitochondrial antiviral-signaling, innate immunity, molecular docking, antiviral response

Introduction

Metformin is primarily used in the treatment of type 2 diabetes (T2D); however, it has garnered attention for its potential to modulate host responses against viral and bacterial pathogens [1-3]. Due to its effects on cellular processes, metformin is considered a promising agent for enhancing host defense against various infections [4-6]. Its immunomodulatory role may be particularly beneficial in regulating the host inflammatory response [7,8].

The stimulator of interferon gene (STING) and mitochondrial antiviral-signaling (MAVS) pathways are critical components of the innate immune response, regulating antiviral defense and inflammatory signaling through the activation of downstream transcription factors [9,10]. STING is a central component of the intracellular DNA-sensing pathway, recognizing cyclic dinucleotides such as cyclic GMP-AMP (cGAMP), which are synthesized by the enzyme cyclic GMP-AMP synthase (cGAS) in response to foreign or damaged endogenous DNA and activating the signaling pathway upon binding. However, STING cannot directly bind to DNA, as it lacks a specific DNA-binding domain. Therefore, it relies on pattern recognition receptors (PRRs) such as cGAS for DNA detection. Upon sensing cytoplasmic DNA, cGAS synthesizes 2′,3′-cGAMP, which activates STING. Once activated, STING translocates from the endoplasmic reticulum to the golgi, where it triggers an immune response. This process enables the activation of the innate immune system [9,11,12]. Upon activation, STING undergoes oligomerization and recruits TANK-binding kinase 1 (TBK1). TBK1 phosphorylates and activates interferon regulatory factors 3 (IRF3), initiating a signaling cascade that

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promotes the transcription of type I interferons and the activation of antiviral defense mechanisms [13]. Additionally, the expression of inflammatory factors can be upregulated through the nuclear transcription factor-κB (NF-Kb) pathway [14].

MAVS is a critical adaptor protein located on the outer mitochondrial membrane that regulates the innate immune response against RNA viruses [15]. Upon viral recognition, it triggers the production of antiviral cytokines and interferons, supporting the immune response and contributing to the clearance of infections [16]. Upon recognition of viral RNA, the pattern-recognition receptors Retinoic acid-inducible gene I (RIG-I) and Melanoma Differentiation-Associated protein 5 (MDA5) interact with MAVS to initiate the antiviral response. MAVS activation triggers the activation of IRF3, interferon regulatory factors 7 (IRF7) and NF-κB. This process enhances the expression of antiviral genes and proinflammatory factors, leading to the production of interferons (IFNs) and IFN-stimulated genes (ISGs), which inhibit viral replication and spread [10,17,18].

The repurposing of existing drugs for different diseases offers significant advantages, as their pharmacokinetic and pharmacodynamic profiles are already well established, allowing for the acceleration of preclinical research while reducing time and costs. This study aims to investigate the interaction of metformin with the STING and MAVS signaling pathways, identifying potential binding regions within key proteins involved in these pathways and performing binding analyses through molecular docking. The findings are expected to provide insights into the potential effects of metformin on the innate immune system, contributing to further research on its immunomodulatory properties.

Material and Methods

Protein-Protein Interaction Network Analysis

In this study, protein-protein interaction (PPI) networks of the critical proteins in the STING and MAVS signaling pathways targeted for metformin were constructed using the STRING database (v12.0) to analyze molecular relationships among the selected proteins [19]. The analysis was performed with a minimum interaction score of 0.400 (medium confidence) to include both direct and indirect associations. Interaction sources considered in the network included experimental data, text mining, database annotations, co-expression, and neighborhood associations. Subsequently, to ensure high-confidence interactions, low-confidence edges were excluded, and only experimentally validated interactions were visualized separately.

Molecular Docking Analysis

The three-dimensional structures of the proteins used in this study were downloaded from the Protein Data Bank (PDB) or the AlphaFold Protein Structure Database. The proteins retrieved from PDB were as follows: 5′-adenosine monophosphate-activated protein kinase (AMPK) (PDB ID: 4EAI), cGAS (PDB ID: 8SHZ), IRF3 (PDB ID: 3QU6), MAVS (PDB ID: 2VGQ), MDA5 (PDB ID: 3GA3), NF-κB (PDB ID: 1SVC), RIG-I (PDB ID: 3LRN), STING (PDB ID: 4EMU), and TBK1 (PDB ID: 6RSU) [20]. The protein structures were obtained from the AlphaFold Protein Structure Database, with the following UniProt IDs: IRF7 (UniProt ID: Q92985). The models were downloaded in their latest available versions (3 February 2025) and used for molecular docking analysis [21,22]. The protein structures were prepared for docking using AutoDock Tools (v1.5.7). During the preparation, water molecules were removed, polar hydrogen atoms were added, and the protein structures were protonated appropriately [23].

The chemical structures of metformin (PubChem CID: 4091) and STING inhibitor SN-011 (PubChem CID: 138005721) were retrieved from the PubChem database in SDF format [24]. To prepare the ligands for docking, they were first converted to PDB format using OpenBabel (v3.1.1) [25] and subsequently subjected to energy minimization using Chimera (v1.15) [26] with the AMBER force field to obtain a low-energy conformation. The minimized structures were then converted to PDBQT format using AutoDock Tools 1.5.7, where flexible bonds were defined to allow conformational flexibility during docking simulations [23].

Docking studies were performed using AutoDock Vina (v1.1.2) to predict the optimal binding conformations of the ligands within the respective binding sites [23]. A grid spacing of 0.375 Å was applied for all docking calculations, ensuring sufficient resolution to capture precise binding interactions. Separate grid boxes were configured for each protein to fully encompass the binding pockets, with parameters including center coordinates (X, Y, Z) and dimensions (Å) detailed in Table 1.

Table 1. Grid box parameters for molecular docking simulations

Protein	Center at (X, Y, Z)	Dimension (Å)
AMPK	X: -13.639, Y: 13.423, Z: -13.224	40 Å × 40 Å × 40 Å
cGAS	X: 13.153, Y: 12.455, Z: 14.457	40 Å × 40 Å × 40 Å
IRF3	X: 21.872, Y: 12.717, Z: 21.476	40 Å × 40 Å × 40 Å
IRF7	X: -7.511, Y: 2.506, Z: -1.971	60 Å × 60 Å × 60 Å
MAVS	X: -24.354, Y: 18.271, Z: 16.39	40 Å × 40 Å × 40 Å
MDA5	X: 4.434, Y: 1.335, Z: 9.031	40 Å × 40 Å × 40 Å
NF-κB	X: 42.418, Y: 17.185, Z: 38.001	40 Å × 40 Å × 40 Å
RIG-I	X: -23.697, Y: -10.387, Z: -0.027	40 Å × 40 Å × 40 Å
STING	X: 52.143, Y: 24.212, Z: 75.058	60 Å × 60 Å × 60 Å
LKB1	X: -6.453, Y: -5.174, Z: 7.298	40 Å × 40 Å × 40 Å
TBK1	X: -4.805, Y: -49.516, Z: -3.117	60 Å × 60 Å × 60 Å

Docking parameters were optimized by considering ligand flexibility, and for each docking run, the conformation with the lowest binding energy was selected as the most favorable pose. Discovery Studio Visualizer (v21.1.0) (BIOVIA, Dassault Systèmes, Discovery Studio Visualizer, v21.1.0.20298, San Diego: Dassault Systèmes, 2021.) were used for visualization and analysis of ligand-protein interactions. The docking results were analyzed to examine the interactions of the ligand within

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the binding sites of the proteins. Binding modes such as hydrogen bonds, van der Waals forces, pi-pi, and electrostatic interactions were identified and the binding affinities (in kcal/mol) were reported. The binding pockets specific to each protein and the binding positions of the ligand were visualized in both 2D and 3D formats.

Ethical Statement

This study did not require ethical committee approval as it was conducted solely through molecular docking analysis using molecular structures obtained from publicly available databases and did not involve human participants or animal experiments.

Results

Protein-Protein Interaction (PPI) Network Analysis of Metformin-Targeted Proteins in the STING and MAVS Pathways

The interaction networks, depicted in Figures 1.a and 1.b, were generated using the STRING database to highlight molecular pathways and protein interactions relevant to potential mechanisms of action of metformin. These figures focus on key proteins involved in immune signaling and metabolic regulation. Figure 1.a shows a detailed network incorporating functional and physical protein-protein associations. Key proteins in this network include STING (STING1), cGAS, IRF3, TBK1, RIG-I (DDX58), MAVS, MDA5 (IFIH1), IRF7, NF-κB (NF-κB 1), and AMPK. The interactions were identified based on multiple sources, including text mining, experimental data, curated databases, co-expression, and neighborhood associations. The minimum interaction score was set to medium confidence (0.400), allowing the inclusion of both direct and indirect associations. Distinct clusters observed in the network suggest tightly related functional modules. For example, cGAS, STING, and IRF3 form a critical axis in innate immune signaling, potentially linking metformin to antiviral and inflammatory pathways. In contrast, Figure 1.b exclusively highlights experimentally validated protein-protein interactions, ensuring a high-confidence representation of the pathways. Key proteins include STING, CGAS, IRF3, TBK1, RIG-I, MAVS, MDA5, IRF7, and NFKB1, with AMPK placed on the periphery. Notable interactions and clusters in Figure 1.b reveal that STING, cGAS, and IRF3 demonstrate a robust connection to innate immune signaling pathways, underscoring their roles in antiviral responses and inflammation. TBK1 and MAVS cluster with RIG-I and IRF7, forming a functional module involved in signal amplification and immune activation. AMPK, while not directly connected to central hubs in this figure, indicates potential involvement in metabolic pathways such as AMPK signaling, which is crucial for energy homeostasis. LKB1 (STK11) is not present in either Figure 1.a or 1.b and was removed to reflect the current STRING-based interaction map accurately. Conversely, MAVS, MDA5, and RIG-I were emphasized in the updated interpretation as they are present in both Figures 1.a and 1.b and form critical components of the network. Figures 1.a and 1.b together provide complementary insights into the molecular pathways potentially influenced by metformin. The comprehensive network in Figure 1.a showcases a broader spectrum of interactions, while the experimentally validated network in Figure 1.b focuses on high-confidence connections.

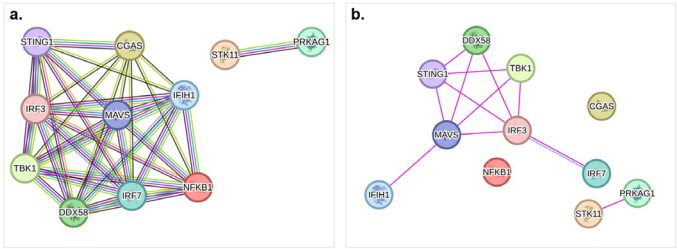

Figure 1. Protein-protein interaction networks generated using the STRING database to explore the molecular pathways and interactions potentially influenced by metformin; (**a**) illustrates both evidence-based and confidence-weighted interactions, with edges representing functional and physical associations; Active interaction sources include text mining, experimental data, databases, co-expression, and neighborhood associations, ensuring a comprehensive analysis; (**b**) focuses exclusively on experimentally validated interactions, highlighting high-confidence connections

Molecular Docking Analysis of Metformin with Key Proteins in the STING and MAVS Pathways

The molecular docking analysis revealed significant interactions between metformin and the target proteins involved in immune and inflammatory processes. The binding affinities (ΔG) ranged from -4.5 kcal/mol to -5.5 kcal/mol, indicating moderate binding strength across the analyzed proteins. IRF3, IRF7, and RIG-I each formed 4 hydrogen bonds, with binding affinities of -5.3 kcal/mol, -5.3 kcal/mol, and -4.5 kcal/mol, respectively, highlighting their potential involvement in metformin-mediated regulatory mechanisms. MAVS, with a binding affinity of -5.4 kcal/mol, formed only 1 hydrogen bond, while TBK1 formed the highest number of hydrogen bonds (7 bonds) despite a binding affinity of -4.7 kcal/mol, indicating a unique interaction profile. For NF-κB, cGAS, MDA5, and STING, the interactions were moderate, with binding affinities ranging between -4.8 kcal/mol and -4.9 kcal/mol. Specifically, NF-κB and MDA5 displayed binding affinities of -4.8 kcal/mol, each forming 2 hydrogen bonds, while cGAS exhibited a similar binding affinity of -4.8 kcal/mol, forming 3 hydrogen bonds. The interaction between metformin and STING showed a slightly stronger binding affinity of -4.9 kcal/mol, with the formation of 2 hydrogen bonds. Among the analyzed proteins, AMPK, specifically its PRKAG1 subunit (5'-AMP-activated protein kinase subunit gamma-1), demonstrated the strongest interaction with metformin, exhibiting the highest binding affinity (ΔG=-5.5 kcal/mol) and forming 4 conventional hydrogen bonds, suggesting a particularly stable and significant interaction (Table 2). This result aligns with previous findings indicating that metformin interacts with AMPK via its γ subunit [27]. Additionally, docking analysis of the STING protein with

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the SN011 inhibitor, a known STING-targeting compound reported in the literature [28], revealed a strong binding affinity of -9.4 kcal/mol. Similarly, the docking analysis of metformin with LKB1, a protein reported in the literature to interact with metformin indirectly [29], revealed a binding affinity of -4.8 kcal/mol, supporting the notion that LKB1 is not a direct target of metformin but rather mediates its effects. However, no conventional hydrogen bonds were observed in the interactions between SN011 and STING or metformin and LKB1, and thus, these results were not included in Table 2.

Table 2. Molecular docking results between metformin and target proteins

Target protein	Minimum binding affinity (Kcal/mol)	No. of conventional hydrogen bond	Hydrogen bond distance (Å)	Key structural amino acid residues
cGAS	-4.8	3	2.79	TYR483
			2.36	ASN482
			2.10	GLU336
IRF3	-5.3	4	2.24	VAL24
			2.88	GLY19
			2.47	LEU21
			2.44	VAL24
IRF7	-5.3	4	2.04	SER388
			2.99	SER386
			2.86	GLY382
			2.00	PRO383
MAVS	-5.4	1	2.50	ARG66
MDA5	-4.8	2	2.62	LEU1006
			2.40	TRP1003
RIG-I	-4.5	4	2.48	SER829
			2.19	ILE887
			2.27	SER829
			2.25	ILE887
NF-κB	-4.8	2	2.57	GLY68
			2.41	VAL115
STING	-4.9	2	2.24	ASP205
			2.61	GLY207
TBK1	-4.7	7	3.06	ARG427
			2.81	ARG427
			2.94	SER268
			2.80	LEU269
			1.88	VAL265
			3.06	VAL265
			2.44	CYS267
AMPK	-5.5	4	2.92	ALA226
			2.97	SER315
			2.34	HIS297
			2.92	SER315

The molecular interactions between metformin and target proteins involved in immune and inflammatory processes were analyzed using the docking method. The interaction analysis of metformin with the cGAS protein revealed a favorable interaction. A detailed evaluation of the binding pocket showed that metformin forms a total of three conventional hydrogen bonds, alongside other stabilizing forces, with key amino acid residues. Specifically, metformin formed hydrogen bonds with the residues TYR483 (2.79 Å), ASN482 (2.36 Å), and GLU336 (2.10 Å). These hydrogen bonds play a critical role

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in stabilizing the ligand within the binding site. In addition to these interactions, carbon-hydrogen bonds and van der Waals forces also contributed to the stabilization of the ligand-protein complex. Attractive charge interactions further enhanced the affinity and proper positioning of the ligand within the binding pocket. The 2D interaction diagram (Figure 2.a) illustrates all observed interactions, including conventional hydrogen bonds, van der Waals forces, carbon-hydrogen bonds, and charge-based interactions. The proximity and nature of these bonds suggest that metformin occupies a stable binding conformation within the active site of cGAS, potentially influencing its biological function.

The interaction analysis between metformin and IRF3 protein identified a total of four conventional hydrogen bonds between metformin and the IRF3 binding pocket. These interactions involved the amino acid residues VAL24 at a distance of 2.24 Å, GLY19 at 2.88 Å, LEU21 at 2.47 Å, and a second interaction with VAL24 at 2.44 Å. In addition to the hydrogen bonds, the ligand-protein complex was stabilized by van der Waals interactions and attractive charge interactions, which contributed to the overall binding stability. The spatial arrangement of metformin and its interaction with critical residues in the IRF3 protein are depicted in the 2D interaction diagram (Figure 2.b). These findings suggest that metformin interacts effectively with the IRF3 protein through a combination of hydrogen bonds and non-covalent forces, highlighting its potential role in modulating IRF3-related biological pathways.

The interaction analysis between metformin and the IRF7 protein revealed a total of four conventional hydrogen bonds within the IRF7 binding pocket. These hydrogen bonds involved the residues SER388 at a distance of 2.04 Å, SER386 at 2.99 Å, GLY382 at 2.86 Å, and PRO383 at 2.00 Å. In addition to the conventional hydrogen bonds, non-covalent interactions such as van der Waals forces contributed to the overall stabilization of the ligand-protein complex. Unfavorable positive-positive interactions were also observed but did not significantly disrupt the stability of the binding conformation. The 2D interaction diagram (Figure 2.c) highlights the key residues involved in the hydrogen bonding network and the spatial configuration of metformin within the binding pocket. These findings suggest that metformin can effectively bind to IRF7 through hydrogen bonding and additional non-covalent interactions, potentially influencing the biological activity of IRF7.

The interaction analysis between metformin and the MAVS protein identified a single conventional hydrogen bond within the MAVS binding pocket. This hydrogen bond involved the residue ARG66 at a distance of 2.50 Å. In addition to the hydrogen bond, non-covalent interactions such as van der Waals forces and charge-based interactions contributed to the stabilization of the ligand-protein complex. These interactions further support the stability and proper positioning of metformin in the MAVS active site. The 2D interaction diagram (Figure 2.d) illustrates the key residues involved in the hydrogen bonding network and other non-covalent interactions. These findings suggest that metformin binds effectively to MAVS through hydrogen bonding and additional stabilizing forces, potentially influencing its biological activity in MAVS-related pathways.

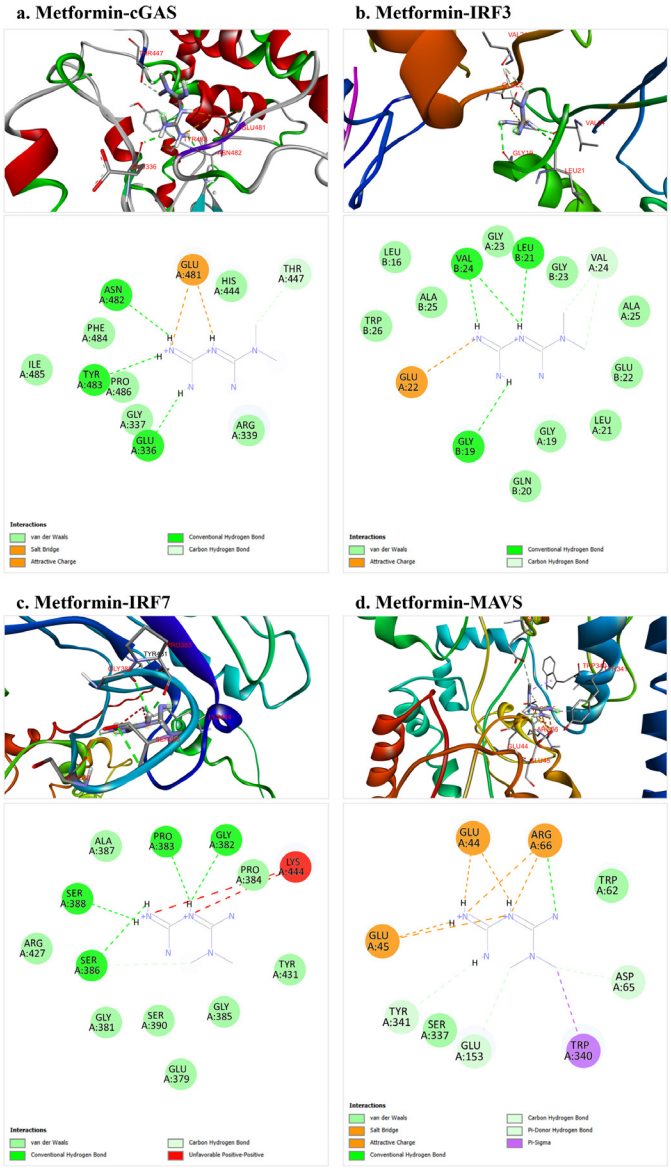

Figure 2. Molecular docking analysis of metformin with key signaling proteins; (**a**) Metformin-cGAS interaction; (**b**) Metformin-IRF3 interaction; (**c**) Metformin-IRF7 interaction; (**d**) Metformin-MAVS interaction; Interaction types include hydrogen bonds, van der Waals forces, salt bridges, and attractive charges

The interaction analysis of metformin with the MDA5 protein revealed a favorable interaction. A detailed evaluation of the binding pocket showed that metformin forms a total of two conventional hydrogen bonds, alongside other stabilizing forces, with key amino acid residues. These hydrogen bonds involved the residues LEU1006 at a distance of 2.62 Å and TRP1003 at 2.40 Å. In addition to the conventional hydrogen bonds, other stabilizing interactions, such as van der Waals forces, contributed to the overall stability of the ligand-protein complex. Unfavorable

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interactions, such as positive-positive interactions, were also observed but did not significantly impact the stability of the complex. The 2D interaction diagram (Figure 3.a) highlights the key residues involved in the hydrogen bonding network and the structural arrangement of metformin within the binding pocket. These findings suggest that metformin interacts effectively with MDA5 through hydrogen bonding and stabilizing forces, potentially influencing MDA5-related pathways.

The interaction analysis between metformin and the RIG-I protein identified a total of four conventional hydrogen bonds within the RIG-I binding pocket. These hydrogen bonds involved the residues ILE887 at distances of 2.19 Å and 2.25 Å, and SER829 at distances of 2.27 Å and 2.48 Å. In addition to the conventional hydrogen bonds, the ligand-protein complex was further stabilized by van der Waals interactions. While unfavorable donor-donor interactions were observed, they did not significantly affect the overall binding stability. The 2D interaction diagram (Figure 3.b) highlights the hydrogen bonding network and the spatial arrangement of metformin within the RIG-I binding pocket. These findings suggest that metformin effectively interacts with RIG-I through hydrogen bonding and additional stabilizing forces, which may have implications for its role in modulating RIG-I-related pathways.

The interaction analysis between metformin and the NF-κB protein revealed a favorable interaction. A detailed evaluation of the binding pocket showed that metformin forms a total of two conventional hydrogen bonds within the NF-κB binding pocket. These hydrogen bonds involved the residues GLY68 at a distance of 2.57 Å and VAL115 at a distance of 2.41 Å. In addition to the hydrogen bonds, other stabilizing interactions, such as van der Waals forces, contributed to the overall stability of the ligand-protein complex. The 2D interaction diagram (Figure 3.c) highlights the key residues involved in hydrogen bonding and the spatial arrangement of metformin within the NF-κB binding pocket. These findings suggest that metformin interacts effectively with NF-κB through hydrogen bonding and stabilizing forces, which may influence the biological activity of the protein.

The interaction analysis between metformin and the STING protein revealed a favorable interaction. A detailed evaluation of the binding pocket showed that metformin forms a total of two conventional hydrogen bonds within the STING binding pocket. These hydrogen bonds involved the residues ASP205 at a distance of 2.24 Å and GLY207 at a distance of 2.61 Å. In addition to the hydrogen bonds, charge-based interactions and van der Waals forces contributed to the overall stabilization of the ligand-protein complex. Unfavorable donor-donor interactions were observed but did not significantly disrupt the binding stability. The 2D interaction diagram (Figure 3.d) highlights the key residues involved in hydrogen bonding and the spatial configuration of metformin within the STING binding pocket. These findings suggest that metformin effectively interacts with STING through hydrogen bonding and stabilizing forces, potentially influencing STING-mediated pathways.

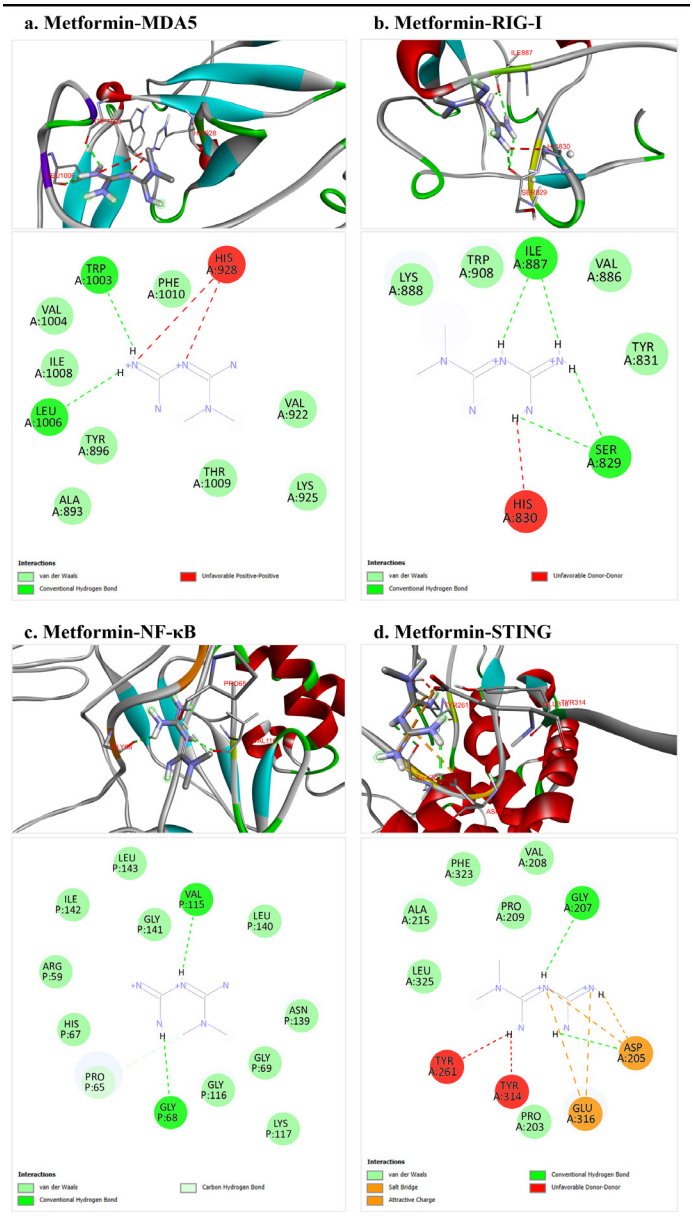

Figure 3. Molecular docking analysis of metformin with key signaling proteins; (**a**) Metformin-MDA5 interaction; (**b**) Metformin-RIG-I interaction; (**c**) Metformin-NF-κB interaction; (**d**) Metformin-STING interaction; Interaction types include hydrogen bonds, van der Waals forces, salt bridges, and attractive charges

The interaction analysis between metformin and the TBK1 protein revealed a favorable interaction. A detailed evaluation of the binding pocket showed that metformin forms a total of seven conventional hydrogen bonds with the TBK1 binding pocket. These hydrogen bonds involved the residues ARG427 at distances of 3.06 Å and 2.81 Å, SER268 at 2.94 Å, LEU269 at 2.80 Å, VAL265 at distances of 1.88 Å and 3.06 Å, and CYS267 at 2.44 Å. In addition to the hydrogen bonds, other stabilizing forces such as van der Waals interactions contributed to the overall stability of the ligand-protein complex. Unfavorable interactions, such as positive-positive interactions, were observed but did not significantly disrupt the binding stability. The 2D interaction

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diagram (Figure 4.a) highlights the key residues involved in the hydrogen bonding network and the spatial positioning of metformin within the TBK1 binding pocket. These findings suggest that metformin interacts effectively with TBK1 through hydrogen bonding and stabilizing forces, potentially influencing TBK1-mediated pathways.

The molecular interactions between metformin and AMPK, a key protein involved in metabolic and inflammatory pathways, were analyzed using docking methods. A detailed evaluation of the binding pocket showed that metformin forms a total of four conventional hydrogen bonds with AMPK. These interactions involved the amino acid residues ALA226 at a distance of 2.92 Å, SER315 at a distance of 2.97 Å, HIS297 at a distance of 2.34 Å, and a second interaction with SER315 at a distance of 2.92 Å. The docking results were visualized to further elucidate the nature of the interactions. The structural analysis demonstrated that, in addition to hydrogen bonding, other non-covalent interactions, including van der Waals forces, contributed to the overall stability of the binding conformation. Unfavorable donor-donor and positive-positive interactions were also observed, which may slightly weaken the binding but do not significantly disrupt the overall interaction stability. The visualization of the docking pose (Figure 4.b) clearly illustrates the spatial arrangement of metformin within the AMPK binding site and highlights key residues involved in hydrogen bonding.

The docking analysis of the STING protein with the SN011 inhibitor, a known STING-targeting compound reported in the literature, revealed a strong interaction. The inhibitor primarily engages with STING through pi-based hydrophobic interactions, including pi-pi stacking and T-shaped interactions, as visualized in the docking results. Notably, no conventional hydrogen bonds were identified in the interaction between SN011 and STING. However, the absence of conventional hydrogen bonding does not diminish the strength of the binding, as the pi-based interactions significantly contribute to the stability of the complex. These findings underscore the efficacy of SN011 as a STING inhibitor, as evidenced by its strong binding and hydrophobic interactions. The 2D interaction diagram (Figure 4.c) highlights the pi-pi stacking and hydrophobic interactions that stabilize the SN011-STING complex.

The docking analysis of metformin with LKB1, a known protein reported in the literature to interact with STING, revealed a stable interaction. LKB1 engages with STING primarily through electrostatic attractive charge interactions, as visualized in the docking results. Notably, no conventional hydrogen bonds were observed in the interaction between LKB1 and STING. However, the presence of strong electrostatic interactions compensates for the lack of hydrogen bonds, stabilizing the ligand-protein complex effectively. These findings demonstrate that LKB1 interacts with STING in a manner consistent with its reported activity in literature. The 2D interaction diagram (Figure 4.d) highlights the electrostatic interactions that stabilize the LKB1-STING complex.

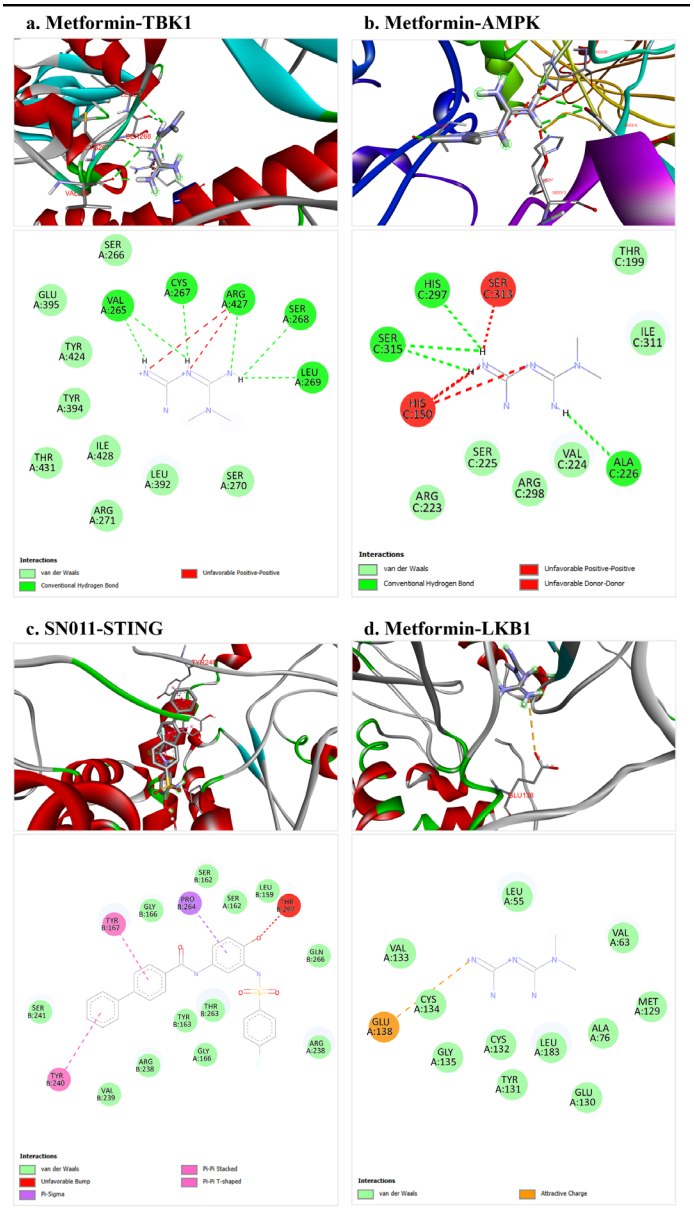

Figure 4. Molecular docking analysis of metformin and SN011 with key signaling proteins; (**a**) Metformin-TBK1 interaction; (**b**) Metformin-AMPK interaction; (**c**) SN011-STING interaction; (**d**) Metformin-LKB1 interaction; Interaction types include hydrogen bonds, van der Waals forces, salt bridges, and attractive charges, with key residues labeled in each interaction

Discussion

Metformin is widely used as the first-line pharmacological agent for the treatment of T2D worldwide. Some of the most notable clinical effects of metformin include the inhibition of autoimmune inflammation through the regulation of T-cell balance [30,31]. Metformin is a drug with anti-inflammatory properties [32]. A study demonstrated that metformin increases the expression of anti-inflammatory cytokines IL-4 and IL-10 while downregulating NF-κB translocation, thereby reducing the production of pro-inflammatory mediators such as interleukin-1β (IL-1β), IL-6, and tumor necrosis factor-alpha (TNF-α).

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Additionally, it has been shown that metformin inhibits TNF-α secretion at both the mRNA and protein levels in overweight mice [33].

Metformin induces autophagy through AMPK activation, leading to the degradation of NLRP3, a critical inflammatory mediator [34]. Another AMPK-dependent effect of metformin is its ability to disrupt the priming signal for NLRP3 inflammasome activation by blocking the Toll-like receptor 4/NF-κB signaling pathway, ultimately inhibiting the bioactive forms of IL-1β and IL-18, as well as the pyroptosis process [34].

In the context of viral infections, metformin has shown the potential to interfere with viral replication and spread by creating a less favorable intracellular environment for viral survival [35,36]. One of the primary mechanisms underlying this antiviral effect is the activation of AMPK via the effect of metformin, which shifts host cellular metabolism away from the high-energy states that many viruses utilize for their replication cycles [37,38]. Therefore, in the molecular docking analysis, we used AMPK as a reference protein as a comparative model to evaluate binding interactions and conducted the docking analysis of metformin with target proteins in the STING and MAVS pathways.

The STING and MAVS pathways play essential roles in the innate immune system, orchestrating antiviral defense and inflammatory signaling by activating downstream transcription factors [9,10]. The potential role of metformin in the STING and MAVS signaling pathways is supported by our STRING database-based interaction network and molecular docking analyses, highlighting its interactions with key proteins involved in immune signaling and inflammation. Network analysis identified essential hubs such as STING1, cGAS, IRF3, TBK1, RIG-I, MAVS, MDA5, IRF7, NFKB1, and AMPK, emphasizing their interconnected roles in antiviral and inflammatory responses. TBK1 and MAVS appear as central hubs, indicating their pivotal roles in signal transduction and immune regulation. MDA5 and RIG-I contribute to the recognition of viral RNA, linking to broader antiviral defense mechanisms. These clusters emphasize the interplay between metabolic regulation and immune response, supporting the hypothesis that metformin modulates these pathways through these interactions.

Molecular docking analyses revealed that AMPK (PRKAG1 subunit) exhibited the strongest interaction, while IRF3, IRF7, and RIG-I formed multiple hydrogen bonds, suggesting that metformin may regulate their activity. The observed interactions with MAVS, TBK1, NF-κB, cGAS, and STING further support the hypothesis that metformin is associated with key regulatory proteins that stabilize innate immune pathways. Strong binding to MDA5, along with interactions with NF-κB and RIG-I, reinforces the potential of metformin to modulate immune responses. The findings suggest that metformin modulates immune and metabolic pathways through interactions with proteins such as cGAS, STING, TBK1, and IRF3, providing a strong basis for further experimental validation. These results indicate that metformin has a strong binding potential with proteins involved in the STING and MAVS signaling pathways, which play key roles in immunity and inflammation. The identified binding distances suggest that metformin can establish close and effective interactions with these proteins. Additionally, literature evidence indicates a direct connection between metformin and AMPK, where this interaction plays a crucial role in maintaining energy homeostasis and regulating cellular metabolism [27]. One possible reason for the relatively low binding energies observed in the docking analysis is that metformin is a small and structurally simple molecule. Small molecules typically have limited contact points with protein targets that possess large surface areas, which can result in lower binding energies. In contrast, larger molecules can interact over more extensive regions of an active site of a protein, leading to stronger binding interactions. However, the low binding energies of small molecules like metformin do not necessarily indicate biologically insignificant interactions. Notably, the ability of metformin to interact with critical signaling proteins through hydrogen bonds and electrostatic interactions may underlie its biological activity [39,40]. Indeed, the binding energies of the target proteins in the STING and MAVS pathways are quite similar to the binding values reported in the literature for the interaction of metformin with AMPK. In our molecular docking study, AMPK was used as a reference protein to evaluate the binding affinity of metformin to key immune signaling components. Additionally, the number of hydrogen bonds formed with each of these proteins closely resembles the hydrogen bonding observed in the metformin-AMPK interaction. This suggests that metformin may exert potential effects on immune signaling through the STING and MAVS pathways, further supporting its role in modulating innate immune responses.

Our findings demonstrate that metformin interacts at the molecular level with key components of the STING and MAVS pathways, linking its well-established metabolic effects to potential immunomodulatory functions. Known interaction of metformin with AMPK, along with its associations with the proteins identified in our docking analysis, suggests that it may play additional regulatory roles in the immune system. In this context, further experimental studies are needed to better understand the impact of metformin on immune signaling pathways. Although these results are promising, this study represents an initial in silico investigation and should be considered as a hypothesis-generating approach. Further in vitro and in vivo studies are warranted to validate these computational findings and elucidate the exact molecular mechanisms involved.

Conclusion

Our findings indicate that metformin may exert significant immunomodulatory effects by interacting with key components of the STING and MAVS pathways. Molecular docking and network analysis suggest that metformin engages with critical proteins involved in antiviral and inflammatory responses,

including STING1, cGAS, IRF3, TBK1, RIG-I, MAVS, MDA5, IRF7, and NF-κB. These interactions highlight its potential role in regulating innate immune signaling, potentially influencing interferon production and inflammatory pathways. Although relatively low binding energy of metformin can be attributed to its small molecular structure, its ability to form multiple hydrogen bonds and electrostatic interactions with essential immune proteins suggests biological relevance. Overall, these findings support the hypothesis that metformin may play a regulatory role in immune signaling, particularly through the STING and MAVS pathways. Further experimental validation is needed to clarify its precise effects and therapeutic potential in inflammation.

Conflict of Interests
The authors declare that there is no conflict of interest in the study.

Financial Disclosure
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Ethical Approval
This study did not require ethical committee approval as it was conducted solely through molecular docking analysis using molecular structures obtained from publicly available databases and did not involve human participants or animal experiments.

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