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In Silico Evaluation of the Antituberculosis Potential of the Interaction Between Genistein and TLR2

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ABSTRACT

This study investigates the potential antituberculosis activity of genistein, a soy-derived isoflavone, through its interaction with Toll-like receptor 2 (TLR2) using in-silico methods. Tuberculosis (TB), caused by Mycobacterium tuberculosis (Mtb), remains one of the deadliest infectious diseases globally, with rising cases of multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB complicating treatment. TLR2 plays a critical role in the host immune response by recognizing Mtb cell wall components and activating signaling pathways that promote inflammation and bacterial clearance. Molecular docking studies demonstrated a strong binding affinity between genistein and TLR2, with a Vina score of -8.0 kcal/mol. Genistein interacted with key amino acid residues in the receptor's binding pocket, including ILE689, HIS697, TRP712, and ASP730. Further Absorption Distribution Metabolism Excretion (ADME) and toxicity predictions revealed that genistein undergoes extensive metabolism, generating 19 potential metabolites. Pharmacokinetic assays indicated that metabolites 7, 9, 15, 16, and 19 exhibited high Caco-2 cell line permeability, suggesting strong potential for oral bioavailability. However, metabolites 3, 5, 9, 13, 15, and 17 showed mutagenic potential in AMES tests, while metabolites 2, 5, 8, 10, 13, 15, and 17 displayed hepatotoxicity. Despite these risks, certain metabolites demonstrated favorable safety profiles, with LD50 values exceeding 2700 mg/kg. These findings suggest that genistein and its metabolites hold promise as complementary agents in TB treatment by modulating TLR2-mediated immune responses. However, further experimental validation and optimization are required to ensure efficacy and safety.

1. Introduction

Mycobacterium tuberculosis (Mtb) is the causative agent of tuberculosis (TB) and is one of the deadliest infectious diseases worldwide. Mtb causes infection by settling especially in the lungs, but it can also spread to other parts of the body in individuals with weak immune systems. By living in macrophages, this bacterium manages to evade the body's immune defense mechanisms and can remain latent for a long time. This adaptability of Mtb is supported by metabolic pathways, particularly the utilization of fatty acids and cholesterol, thus prolonging survival in host cells [1]. TB treatment is permanent, with a combination treatment usually lasting 6 months. Standard treatment regimen: It consists of antibiotics such as isoniazid, rifampicin, ethambutol, and pyrazinamide. These drugs are used together to prevent both lethality and the development of resistance. However, in cases of multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB), treatment duration may be longer and different systems (e.g., bedaquiline, linezolid) are applied. The focus is on adding new diseases and repurposing existing machinery, especially as drug resistance increases and side effects make treatment more difficult [2]. Herbal substances have an important place among alternative treatment strategies against drug-resistant Mtb strains.

Genistein is an isoflavone found in soybeans and other legumes and is known as phytoestrogen. Its chemical name is 4',5,7-trihydroxyisoflavone and it is found in high concentrations in plants such as Glycine max (soybeans), red clover, and chickpeas. Genistein has antioxidant and anti-inflammatory properties. It can be used to relieve post-menopausal symptoms by showing estrogen-like effects. It is being researched for its anticancer effects, especially on breast, prostate, and colon cancers. It has antibacterial and antiviral properties and protects against various pathogens. It strengthens the immune system and supports antioxidant defense mechanisms [4].

Genistein suppressed bacterial growth and reduced the levels of pro-inflammatory cytokines such as TNF-α and IL-6 in the treatment of osteomyelitis caused by Methicillin-Resistant *Staphylococcus aureus* (MRSA) (5). Genistein, combined with antibiotics, has shown synergistic effects against multidrug-resistant pathogens [6].

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TLR2 plays a critical role in regulating the early immune response between Mtb and the host. TLR2 recognizes pathogen-associated molecular patterns such as lipoproteins in the cell wall of Mtb, and this recognition activates signaling pathways such as NF-κB and MAPK, resulting in the secretion of pro-inflammatory cytokines (e.g. TNF-α, IL-6). This signaling increases the activation of macrophages at the site of infection and their ability to kill bacteria. However, some proteins of Mtb can weaken the immune response by suppressing TLR2 signaling, thus increasing the bacteria's chances of survival [7].

Although there is limited information in the current literature regarding the direct effects of genistein on TLR2, its modulatory effects on TLR4, TLR3, and TLR9, as well as its ability to influence inflammatory responses, provide significant clues about its potential interactions with TLR2. In this context, as TLR2 is a critical receptor that triggers immune responses against mycobacterial infections, it is hypothesized that genistein's interactions with this receptor may exhibit antituberculosis effects. In this study, the potential antituberculosis activity of genistein was investigated using in-silico methods. Molecular docking analyses were conducted to evaluate the effects of genistein on TLR2 binding sites and its potential inhibitory properties.

2. Material and Method

All computational tools and databases used in this study were employed following their standard operating guidelines and cited protocols. The protein structure of TLR2 was retrieved from the Protein Data Bank (PDB) in its most stable crystallized form, and ligand structures were sourced from PubChem in SDF format. Ligand and receptor preparations were conducted using BIOVIA Discovery Studio with default parameters for hydrogen addition and energy minimization. Docking studies were performed using CB-Dock2 with exhaustive settings (score ranking based on Vina affinity values), ensuring reproducibility. Metabolite predictions via Gloryx included both Phase I and II transformations using canonical SMILES inputs. ADME and toxicity predictions were carried out using SwissADME and ProTox-II, both of which are established and validated web-based platforms for in silico pharmacokinetic and toxicological analysis. All results were visualized and interpreted using standard visualization tools as recommended by respective software platforms.

2.1. Protein and Ligand Structure Preparation

The X-ray crystal structure of TLR2 was obtained from the Protein Data Bank (PDB). The chemical 2D structure of Genistein was obtained PubChem. BIOVIA Discovery Studio was used for Protein Preparation.

2.2. Molecular Docking Studies

The CB-Dock2 web server was used for peptide-protein docking studies. The genistein was docked to the binding site cavity by the automized system in the server. Docking calculations were performed with an exhaustive option of 10 (average accuracy). The server gives docking results as energy scores for ten positions, creates a list for the top ten positions, and names them 1 to 10 (1 is the best, 10 is the worst). For each receptor-docking study, model 1 was chosen for evaluation and visualization [8].

2.3. Phase I and Phase II Metabolite Profiling

The Gloryx web server was utilized to identify the Phase I and Phase II metabolites of genistein. Initially, the SMILES code for genistein was retrieved from PubChem. Subsequently, both Phase I and Phase II metabolism options were selected, and the SMILES code was submitted to the system [9].

2.4. ADME Properties Prediction

The SwissADME server was used to predict the ADME properties of genistein. For this purpose, the SMILES code of genistein was submitted to the system [10].

3. Results

3.1. Molecular Docking Studies

The interaction between genistein and the TLR2 protein is presented in Fig. 1. According to the analysis results, the binding score (Vina score) between genistein and TLR2 was found to be -8 kcal/mol. The specific amino acids of TLR2 that genistein may interact with are illustrated in Fig. 2.

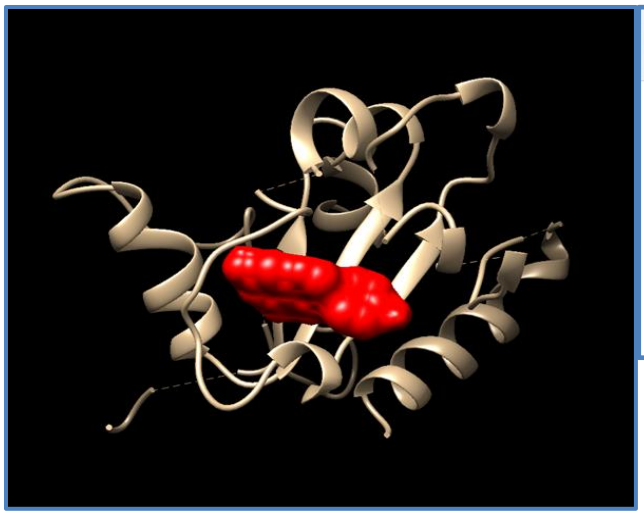

Fig. 1 Schematic view of Genistein docking with TLR2 receptor.

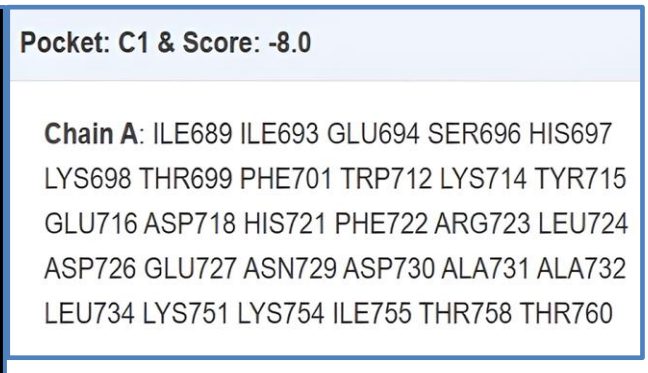

Fig. 2 Vina score of Genistein/TLR2 docking. Genistein is predicted to interact with 29 amino acids, and their respective positions are identified.

Table 1. Predicted reaction types of metabolites.

Rank	Priority	Reaction Type
1	0.984	O-glucuronidation (aromatic hydroxyl)
2	0.972	O-glucuronidation (aromatic hydroxyl)
3	0.924	Sulfation (aromatic hydroxyl)
4	0.656	Sulfation (aromatic hydroxyl)
5	0.524	Double bond reduction (aromatic)
5	0.524	Aromatic hydroxylation (para to carbon)
7	0.352	Sulfation (aromatic hydroxyl)
8	0.336	O-glucuronidation (aromatic hydroxyl)
9	0.232	Aromatic hydroxylation (para to oxygen)
10	0.232	Aromatic hydroxylation (para to oxygen)
11	0.216	Methylation (aromatic OH)
12	0.18	Aromatic hydroxylation (ortho to oxygen)
13	0.102	Oxidation of 4-substituted phenol to quinone
14	0.102	Oxidation of 4-substituted phenol to quinone
15	0.046	Oxidation of 4-substituted phenol to quinone
16	0.046	Oxidation of 4-substituted phenol to quinone
17	0.032	Aromatic hydroxylation
18	0.024	Methylation (aromatic OH)
19	0.012	Methylation (aromatic OH)

3.2. Phase I and Phase II Metabolite Profiling

According to the prediction results of genistein's Phase I and Phase II metabolism, a total of 19 metabolites are estimated to be generated. The 2D chemical structures of 9 of these potential metabolites are presented in Fig. 3. The reactions leading to the formation of these metabolites and their respective priority values are provided in Table 1. The SMILES codes of genistein and its predicted metabolites are presented in Fig. 4.

3.3. ADME Properties Prediction

The ADME properties of genistein are presented in Fig. 5, 6, 7, and 8.

The pharmacokinetic and toxicological profiles of the tested metabolites, including Caco-2 permeability assay AMES test results, hepatotoxicity, and LD50 values, provide critical insights into their potential as drug candidates.

The water solubility of the compound was predicted using three different computational models. According to the ESOL model, the compound exhibited a solubility of 5.11×10^{-2} mg/mL (1.89×10^{-4} mol/L), classifying it as soluble. The Ali model predicted a slightly lower solubility of 1.59×10^{-2} mg/mL (5.88×10^{-5} mol/L), categorizing the compound as moderately soluble. Similarly, the SILICOS-IT model estimated the solubility at 1.07×10^{-2} mg/mL (3.94×10^{-5} mol/L), also placing the compound in the moderately soluble class. These results suggest that while solubility levels vary slightly between models, the compound demonstrates at least moderate water solubility across all predictions.

3.4. Caco-2 Permeability Assay

The Caco-2 permeability assay results revealed that metabolites 7, 9, 15, 16, and 19 exhibited high permeability, indicating strong potential for intestinal absorption and oral bioavailability. Conversely, metabolites 1, 8, 12, and 18 demonstrated low permeability, which may limit their absorption unless formulation strategies are optimized. The majority of metabolites (2, 3, 4, 5, 6, 10, 11, 13, 14, and 17) displayed moderate permeability, suggesting potential but limited bioavailability under standard conditions.

3.5. AMES Test Results

The AMES test identified metabolites 3, 5, 9, 13, 15, and 17 as having potential mutagenic effects, highlighting a genotoxicity risk that may require structural modifications or further evaluations to improve safety. In contrast, metabolites 1, 2, 4, 6, 7, 8, 10, 11, 12, 14, 16, 18, and 19 returned negative results, indicating a lower likelihood of genetic toxicity and a favorable profile in terms of mutagenicity risk.

3.6. Hepatotoxicity

Hepatotoxicity analysis showed that metabolites 2, 5, 8, 10, 13, 15, and 17 have the potential to cause liver toxicity, warranting further investigation and possible dose adjustments to mitigate this risk. Metabolites 1, 3, 4, 6, 7, 9, 11, 12, 14, 16, 18, and 19 were non-hepatotoxic, suggesting greater safety concerning liver health.

3.7. LD50 Toxicity Evaluation

The LD50 values indicated that metabolite 8 had the lowest value (1500 mg/kg), suggesting higher acute toxicity, while metabolite 15 had the highest value (3100 mg/kg), indicating lower toxicity. Metabolites 7, 9, 15, 16, and 19, with LD50 values exceeding 2700 mg/kg, were classified as having low toxicity. However, metabolites 8, 10, and 11, with LD50 values at or below 1700 mg/kg, were identified as more toxic and may require additional safety assessments.

3.8. Overall Evaluation

The results demonstrate that several metabolites, particularly those with high permeability, negative mutagenicity, and low toxicity (e.g., metabolite 19), exhibit promising pharmacological profiles. However, metabolites with hepatotoxicity or high acute toxicity may require optimization in both chemical structure and formulation to balance efficacy and safety. Further studies, including in vivo testing, are necessary to validate these findings and determine clinical applicability.

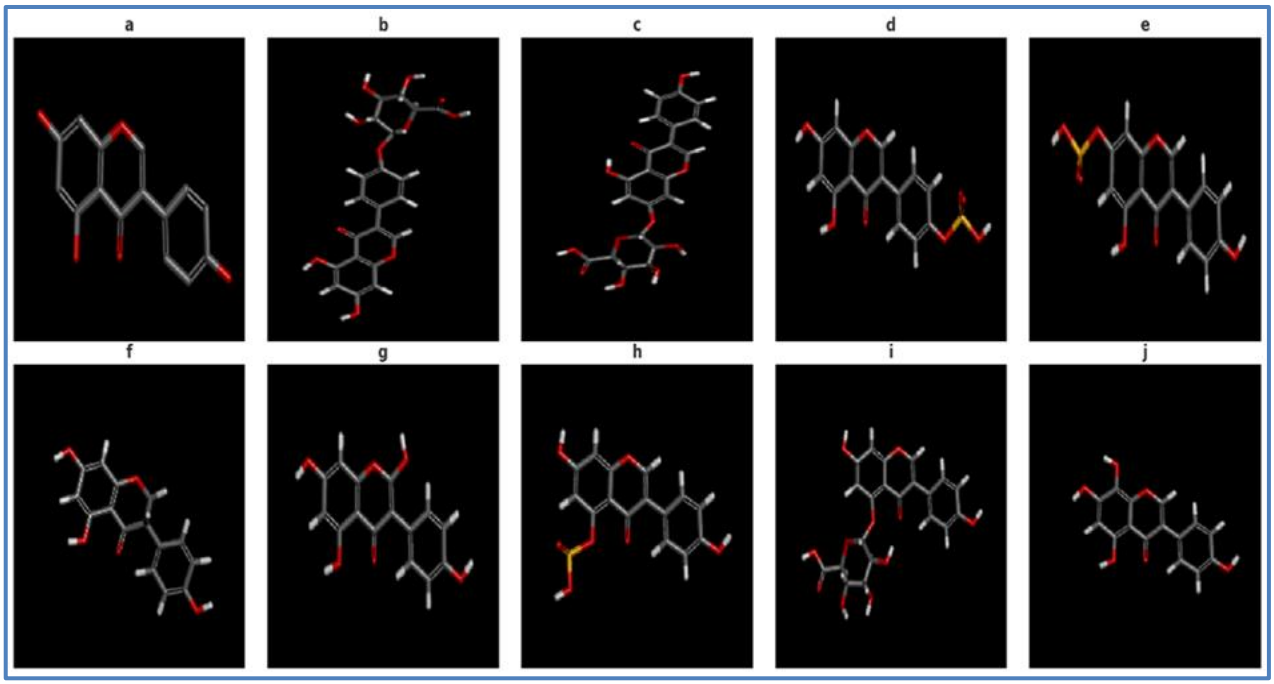

Fig. 3 2D Chemical structures of a) Genistein b-j) Metabolites from 1 to 9.

Compound	SMILES Notation
Genistein	C1=CC(=CC=C1C2=COC3=CC(=CC(=C3C2=O)O)O)O
M1	C=C1C(C(C(C)=CC)=C(\C)CC)=C(C)CC2=C1C(CC)[C@H](C)C=C2C.CCCC(CC)CC
M2	C=C3C(C1/C(C)=C(C)\C(CC)=C(C)/C1C)C(C)CC4C(C)C(C2CC(C(C)CC)C(CC)[C@H](C)C2CC)C(C)[C@@H](CC)C34
M3	CC/C3=C(C)/C2C[C@@H](C)C(/C1=C(C)/C(C)C(CC(C)CC)/C(C)=C1/C)C(C)[C@H]2C(CC)C3C
M4	CC*4C(C)/C(C)=C(C3[C@H](C)CC2C(C)C(C[C@@]1(CC)CC1)/C(C)=C(CC)\C2C3C)\C(C)C4C
M5	C=C1/C3=C(CC(C)C1C2C(C)/C(C)=C(CC)\C(C)C2C)/C(C)=C(CC)\C(C)C3CC
M6	CC/C3=C(C)/C2=C(C(C)C(/C1=C(C)/[C@H](C)C(CC)C(C)C1C)[C@H](CC)C2)\C(CC)C3C
M7	CC[C@@H](C)CC3C(C)C(CC)C(C)C2C/C(C)=C([C@@H]1C(C)C(C)C(CC)C(C)C1C)\C(C)C23
M8	C/C=C/4C(C)C(C(C)CC)CC(C/C1=C\3/C(=C(C)\C(CC)C1C)*C(C)C(c2c(C)c(C)c(CC)c(C)c2C)[C@H]3C)C4CC
M9	CC/*3=C/2CC(C)C([C@H]1/C(C)=C(C)\C(CC)C(C)C1C)C(C)C2C(CC)C(C)C3CC
M10	C=C2c1c(CC)c(CC)c(CC)c(C)c1CC(C)C2[C@H]3/C(C)=C(C)\C(CC)C(C)C3C
M11	CC/C3=C(C)/C2C/C(C)=C(C1/C(C)=C(C)\C(C[C@@H](C)C)C(C)C1C)\C(C)[C@H]2C(CC)C3C
M12	CCCC3(C)CC(C)C(CC)C2C(C)C(/C1=C(C)/C(CC)=C(CC)\C(C)C1C)C(C)CC23
M13	C=C1C(C)C(C)C(C)C(C)C1C
M14	CCc2c(C)c(CC)c1C/C(C)=C(C)\Cc1c2C
M15	C=C2*(C)C(C/C(C)=C(/C1=C(C)/C(C)=C(CC)\[C@H](C)[C@@H]1C)\[C@H](C)C)C(=C)C(CC)C2C
M16	C=C2/C(/C1=C(C)/C(C)*(CC)/C(C)=C1/C)=C(C)\Cc3c2c(=C)c(C)*(CC)c3=C
M17	CC/C3=C(C)/C2CC(C)C(C1/C(C)=C(C)\C(CC)C(C)C1CC)CC2/C(CC)=C3/C
M18	C=C(C)Cc3c(C)c(CC)c(C)c2/C=C(C)\C(C1C(C)/C(C)=C(CC)\C(C)C1C)Cc23
M19	*C(C)(C)Cc3c(C)c(CC)c2C/C(c1c(C)c(C)c(CC)c(C)c1C)=C(C)\Cc2c3C

Fig. 4 SMILES Code List for Genistein and its Metabolites M refers to Metabolites from 1 to19.

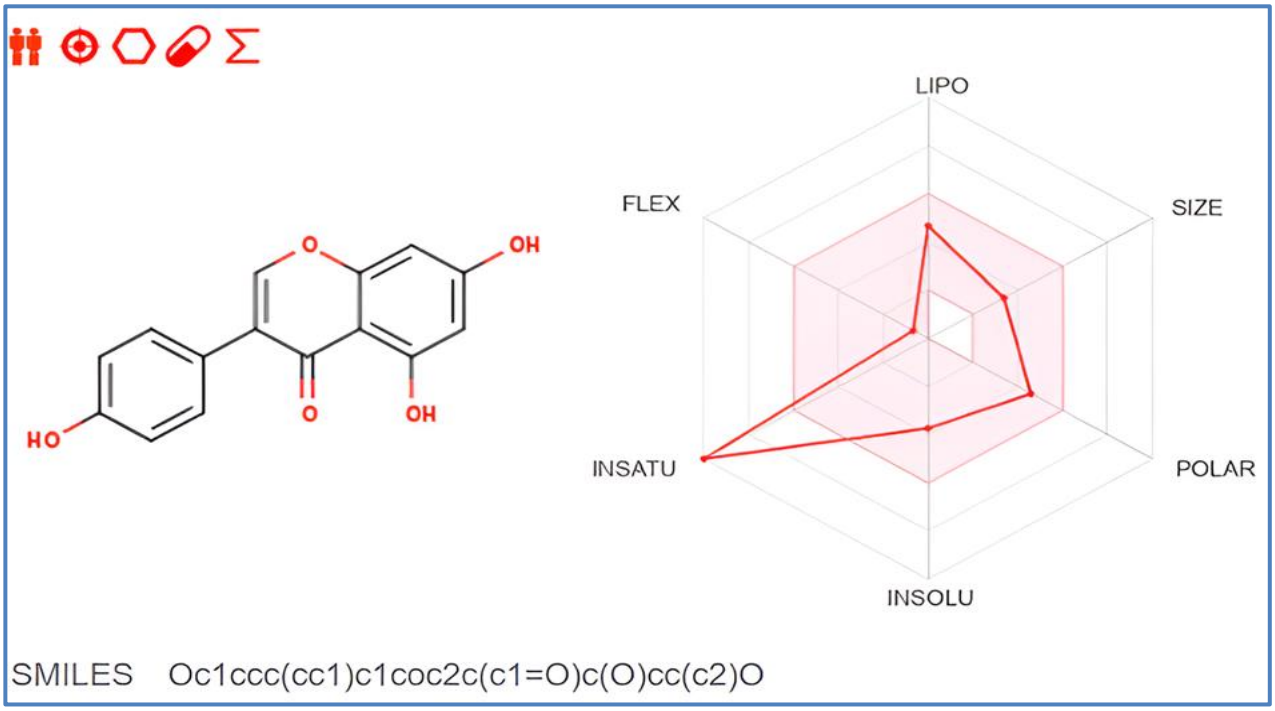

Fig. 5 Chemical Structure of Genistein.

Pharmacokinetics	
GI absorption	High
BBB permeant	No
P-gp substrate	No
CYP1A2 inhibitor	Yes
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
CYP2D6 inhibitor	Yes
CYP3A4 inhibitor	Yes
Log K_p (skin permeation)	-6.05 cm/s

Fig. 6 Pharmacokinetic Properties of Genistein.

Lipophilicity	
Log $P_{o/w}$ (iLOGP)	1.91
Log $P_{o/w}$ (XLOGP3)	2.67
Log $P_{o/w}$ (WLOGP)	2.58
Log $P_{o/w}$ (MLOGP)	0.52
Log $P_{o/w}$ (SILICOS-IT)	2.52
Consensus Log $P_{o/w}$	2.04

Fig. 7 Lipophilicity of Genistein.

Druglikeness	
Lipinski	Yes; 0 violation
Ghose	Yes
Veber	Yes
Egan	Yes
Muegge	Yes
Bioavailability Score	0.55

Fig. 8 Druglikeness Properties of Genistein.

4. Discussion

The pharmacokinetics and toxicity of genistein have been extensively investigated in both preclinical and clinical studies. It is evident that genistein, a naturally occurring isoflavone, exhibits diverse biological effects depending on dosage and exposure conditions. In rats, genistein demonstrates significant oral bioavailability differences between its aglycone and glucuronide forms, with evidence of extensive first-pass metabolism and enterohepatic circulation [11]. These findings suggest that genistein metabolism may vary across species, potentially impacting on the bioavailability of active metabolites and influencing therapeutic efficacy in humans.

Toxicologically, genistein presents a complex profile. Studies have shown that high doses can induce liver toxicity, characterized by increased oxidative stress and elevated liver enzyme biomarkers in mice [12]. However, genistein has also demonstrated protective effects against liver injury by enhancing antioxidant defenses and activating key enzymes involved in drug metabolism [13]. These dual effects highlight the importance of dose optimization in developing genistein-based therapies.

Mtb remains a significant global public health concern as the causative pathogen of TB. TB is particularly prevalent in immunocompromised individuals and in low-income regions. Current treatment protocols involve prolonged use of antibiotics; however, the increasing incidence of MDR-TB and XDR-TB poses serious challenges to effective treatment [14]. Therefore, the development of more effective and safer therapeutic agents is urgently needed. Plant-derived bioactive compounds are gaining attention as potential new therapeutic agents. Isoflavones such as genistein possess immunomodulatory and anti-inflammatory properties. Studies have shown that genistein can regulate immune pathways, including TLRs, and enhance macrophage activity, thereby supporting the host's defense against pathogens [15]. Additionally, genistein has been found to reduce oxidative stress and regulate the cell cycle, which may help mitigate pathogen-induced cellular damage. These properties suggest that compounds like genistein could play a complementary role in tuberculosis treatment. However, further clinical studies are necessary to evaluate the efficacy and safety of such bioactive compounds in combating tuberculosis.

TLR2 plays a crucial role in the immune system's recognition and response to Mtb, the pathogen responsible for TB. TLR2 recognizes various Mtb lipoproteins, such as LpqH and LprA, and activates antigen-presenting cells, including macrophages and dendritic cells, to initiate inflammatory and antibacterial responses [16]. Studies have shown that TLR2 activation promotes the production of pro-inflammatory cytokines such as IL-6 and IL-10, which help regulate early immune responses to Mtb [17].

The inhibition of TLR2 signaling can have profound effects on the progression of tuberculosis. Blocking TLR2 impairs the activation of macrophages, reducing the production of essential cytokines and antibacterial molecules, which may allow Mtb to survive and proliferate within host cells [18]. Additionally, TLR2 suppression decreases antigen presentation and MHC-II expression, critical processes for initiating adaptive immune responses, potentially exacerbating disease severity [19]. Therefore, targeting TLR2 signaling represents both an opportunity for enhancing host defense strategies and a challenge due to its dual roles in pathogen control and immune regulation.

TLR2 plays a crucial role in the innate immune system by recognizing pathogen-associated molecular patterns (PAMPs) and initiating inflammatory responses. It is predominantly involved in detecting bacterial lipoproteins and peptidoglycans, triggering downstream signaling cascades that activate pro-inflammatory cytokines. Recent research indicates that genistein, a soy isoflavone, can modulate the expression and activity of TLRs, including TLR2. For example, genistein has been shown to increase basal TLR2 expression while also attenuating inflammation induced by viral components in human endometrial cells [15]. These findings suggest that genistein may enhance immune modulation by regulating TLR-mediated pathways.

However, direct studies exploring the interaction between genistein and TLR2 in cancer and chronic inflammatory conditions remain limited. While research has demonstrated that genistein interacts with TLR4 and other TLR pathways to inhibit inflammation, particularly in microglial and macrophage models [20], data specific to TLR2 remain sparse. This highlights an opportunity for future investigations to elucidate genistein's full spectrum of effects on TLR2 signaling and its implications for immune response regulation, particularly in diseases where TLR2 is known to play a pivotal role.

The interaction between genistein and TLR2, with a Vina score of -8.0 kcal/mol, indicates a strong binding affinity, suggesting the potential for genistein to modulate TLR2's activity. Structural data (Figure 1) demonstrates genistein occupying a key binding pocket on TLR2, possibly altering receptor conformation and downstream signaling pathways. The binding site (Figure 2) is located within Pocket C1, involving crucial residues such as ILE689, HIS697, TRP712, TYR715, ARG723, ASP730, and THR758 in Chain A. These residues may play a critical role in stabilizing the interaction through hydrogen bonding, hydrophobic interactions, and electrostatic forces.

This interaction supports the hypothesis that genistein could inhibit excessive TLR2-mediated inflammation by preventing the receptor's activation or altering its ligand recognition capacity. In conditions where TLR2 is hyperactive, such as tuberculosis and chronic inflammatory diseases, this mechanism may reduce pro-inflammatory cytokine production. Consequently, genistein and similar plant-derived bioactive compounds hold promise as complementary therapeutic agents targeting pattern recognition receptors to modulate innate immune responses. Further in vitro and in vivo studies are necessary to confirm these computational predictions and explore the functional implications of genistein's binding to TLR2.

This study aimed to evaluate key pharmacokinetic and toxicological properties, including Caco-2 permeability assay, AMES test results, hepatotoxicity, and LD50 values, for a set of 19 molecules. These analyses provide critical insights into the potential absorption, distribution, metabolism, and toxicity (ADMET) profiles of the compounds, offering guidance for their further development and optimization in pharmaceutical research.

The Caco-2 permeability assay is a widely used in vitro model to predict intestinal absorption. Among the 19 molecules analyzed, 5 demonstrated high permeability (≥1.4 log Papp in 10^-6 cm/s), 10 exhibited moderate permeability (between 1.0 and 1.4), and 4 showed low permeability (≤1.0). Molecules with high permeability, such as Molecules 7, 9, 16, and 19, indicate strong potential for oral bioavailability. In contrast, the low-permeability compounds may require formulation strategies to improve absorption.

The AMES test assesses mutagenic potential, providing a key indicator of genotoxic risk. Out of the 19 molecules, 13 returned negative results, suggesting low mutagenic risk, while 6 tested positive. Molecules testing positive for mutagenicity, such as Molecule 3 and Molecule 15, may require structural modifications or additional safety evaluations to reduce their genetic toxicity risk.

Hepatotoxicity remains a critical concern in drug development, as liver damage can severely limit a drug's safety profile. The analysis revealed that 7 of the 19 molecules exhibited hepatotoxicity. These hepatotoxic compounds,

including Molecules 2, 5, and 17, may pose risks requiring targeted safety studies. Conversely, the remaining 12 molecules were deemed non-hepatotoxic, offering a more favorable safety profile.

The LD50 test provides a measure of acute toxicity, indicating the dose required to cause death in 50% of test subjects. The LD50 values for the molecules ranged from 1500 to 3100 mg/kg. Molecule 8 exhibited the lowest LD50 (1500 mg/kg), suggesting higher acute toxicity, while Molecule 15 had the highest LD50 (3100 mg/kg), indicating lower toxicity. Molecules with lower LD50 values require closer examination to ensure safe dosage levels in potential therapeutic applications.

Integrating the findings from these tests, a comprehensive picture of each molecule's pharmacokinetic and toxicological profile emerges. Molecules demonstrating both high Caco-2 permeability assay and negative AMES results, such as Molecule 19, represent promising candidates for further drug development. However, molecules concerning hepatotoxicity or low LD50 values, despite favorable permeability, highlight the importance of balancing absorption and safety profiles during the drug discovery process.

5. Conclusion

This analysis underscores the complexity of optimizing drug candidates for both efficacy and safety. While high permeability and low mutagenic potential are desirable, the presence of hepatotoxicity or high acute toxicity can impede clinical progress. Therefore, further studies focusing on structural optimization, in vivo testing, and clinical safety evaluations are essential for refining these compounds for therapeutic use.

Declaration

Author Contribution: Conceive– K.Y.; Design– K.Y.; Experimental Performance, Data Collection and Processing– K.Y.; Analysis and Interpretation– K.Y.; Literature Review– K.Y.; Writer– K.Y.; Critical Review– K.Y.

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