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DESIGN AND DFT STUDY OF NOVEL SQ109 DERIVATIVES AS POTENTIAL INHIBITORS OF MMPL3 PROTEIN USING IN SILICO TECHNIQUES FOR THE TREATMENT OF MULTI-DRUGRESISTANT TUBERCULOSIS

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ABSTRACT:
Background: The increasing incidence of multidrug-resistant tuberculosis highlight the vital need for novel anti-tubercular agents that act through novel molecular mechanisms. Mycobacterial membrane MmpL3, crucial transporter involved in mycolic acid transport and mycobacterial cell-wall biosynthesis, has gained prominence as a promising therapeutic target.
Method: Seven novel SQ109 derivative (SS1-SS7) were rationally designed and evaluated using an integrated in silico approach. Molecular docking studies were performed to investigate their binding affinity and interaction patterns within the active binding site of MmpL3. The molecular interactions of the designed derivatives with key amino acid residues were analyzed to assess their binding stability. Density functional theory (DFT) calculations were executed for the lead compounds to explore its electronic properties, molecular stability and chemical reactivity of these compounds.
Results: The designed derivatives, SS2 manifested the highest docking score, signifying a favorable binding affinity against MmpL3 compared with the comparative compound SQ109. Associated analysis showed that SS2 established substantial hydrophobic and hydrogen-bonding interaction with key amino acid target site residue within the binding site, which may promote its enhanced binding stability. DFT analysis of SS2 explained established a suitable HOMO–LUMO energy gap, Promising ionization potential, and a low electrophilicity index, showing good molecular stability and potentially reduced chemical reactivity.
Discussion: The computational results indicate the designed derivative of SS2 could bind efficiently within the MmpL3 binding pocket through significant hydrophobic and hydrogen-bonding interactions, signifying its potential as a promising SQ109-derived anti-tubercular compound. The DFT analysis also show favourable electronic stability and sensible chemical reactivity, further supporting SS2 as a potential lead candidate for advance investigation.
Conclusion: The comprehensive computational findings identify the SS2 as a exhibiting lead compound targeting MmpL3 and provide a rational basis for its further investigate as a potential anti-tubercular agent against MDR-TB. Experimental validation, including in vitro antimicrobial evaluation and further pharmacological studies, is warranted to confirm its therapeutic potential.

KEYWORDS: MmpL3 protein, SQ109 derivatives, molecular docking, density functional theory, in-silico drug design, HOMO-LUMO, anti-tubercular medicines, multidrug-resistant tuberculosis.

INTRODUCTION

Tuberculosis remains one of the primary causes of mortality infectious diseases in worldwide, placing a significantly burden on universal healthcare system[1].The condition has been further infuriated by the surfacing of multi drug resistant tuberculosis (MDR-TB), which is categorized by resistance to first line anti-tuberculosis drugs such as Isoniazid and Rifampicin. This resistance drastically complicates treatment regimen, prolongs therapy duration of the drug and reduces overall success rate [2,3] of the treatment.Accordingly, there is an urgent need to developed the novel beneficial agents with better efficacy against tuberculosis drug-resistant strains. The improvement of drug resistance in Mycobacterium Tuberculosis is a complex incident involving multiple mechanism together with genetic mutations in the drug targets and activation of the efflux pump systems and metabolic alteration that reduce inter-cellular drug attention [4,5].There factors individually add to decreased drug efficacy and emphasize the necessity of identify new molecular targets competent of overcoming active resistance pathway. In the middle of promising targets, the MmpL3 transporter has gained important consideration due to its essential role in the translocation of trehalosemonomycolate, a

key precursor in mycolic acid biosynthesis required for maintaining the structural integrity of the mycobacterial cell wall[5].Inhibition of Mmpl3 disrupt cell wall legislative body, eventually leading to bacterial cell death, in that way establishing it as a validate and promise target for anti-tubercular drug discovery [6,7]. SQ109, ethylenediamine-based compound, has been recognized as a potent inhibitor of the MmpL3 transporter and has progress into clinical evaluation due to its notable anti-mycobacterial activity[8,9].However, in spite of its therapeutic potential, cenvinced limitation such as restrained binding affinity and the opportunity of resistance development impose further structural optimization to improve its pharmacological performance[9].In this context, rational modification of the SQ109 scaffold afford an effective strategy to improve binding relations and biological activity. Recent advancement in computational drug discovery have appreciably transformed the drug development process by enable rapid screening, optimization, and prophecy of potential therapeutic candidates while reducing experimental cost and time [10].Molecular docking has emerge as a widely used technique to forecast ligand - protein interactions, appraise binding affinity and recognize key residue involved in binding within the active site of target proteins [11]. In particular, Glide based docking approach offer high accuracy in predict binding conformations and interaction energies [12].In addition to docking studies, DFT study (Density Functional Theory) provides precious insights into the electronic structure, molecular stability, and chemical reactivity of compounds at the quantum level [17,18]. Parameter such as the highest engaged molecular orbital (HOMO), Lowest unoccupied molecular orbital (LUMO), energy gap, ionization potential, and electrophilicity index are essential for sympathetic the pharmacodynamic behavior of drug candidates. Furthermore, global reactivity descriptors imitative from Koopmans' theorem facilitate forecast of molecular stability and reactivity, which are significant for rational drug design [18,21] More ever, estimate of drug-likeness and pharmacokinetic properties plays a crucial role in the achievement of drug candidates. Computational approach such as Lipinski's rule of five and ADMET prediction tools are extensively employed to consider absorption, distribution, metabolism, excretion and toxicity profiles, thereby rising the likelihood of clinical success [15,16].

In the present study, a series of novel SQ109 derivatives (SS1 - SS7) were realistically designed through structural modification involving the integration of a bicyclo[2.2.1] heptene moiety to improve binding affinity and pharmacological impending. These derivatives were systematically appraise against the MmpL3 protein using an incorporated in silico move towards combining molecular docking and Density Functional Theory (DFT). The primary objective of this study is to ascertain structure activity relationships and identify potential lead compound with enhanced binding efficiency, electronic stability and beneficial potential for the treatment of multi-drug-resistance tuberculosis.

MATERIALS AND METHODS

Computational Environment and Software Suite

All computational molecular studies, ligand preparation, protein processing, and docking simulations were performed using the Schrödinger software suite [22]. The computational experiments were executed on a high-performance workstation equipped with an Intel Core i7 processor and 16 GB of RAM, running on a 64-bit Windows operating system. The OPLS4 (Optimized Potentials for Liquid Simulations 4) force field was uniformly applied across all preparation and docking phases [14]. OPLS4 was certain due to its improved parameterization for assorted chemical spaces, contribution superior precision in modeling conformational preference and non-covalent interactions compare to its predecessors.

Ligand Preparation

The structural coordinate of the standard reference drug SQ109 and the considered derivatives (denoted as SS1 through SS7), which feature the N1-((E)-3,7-dimethylocta-2,6-dien-1-yl)-N2-((1S,4R)-4-methylbicyclo[2.2.1]heptan-2-yl)ethane-1,2-diamine core scaffold, were sketched utilize the 2D Sketcher tool natively incorporated within the Maestro graphical user boundary. To certify the chemical precision and biological significance of the ligands before docking, they were process using the LigPrep module [22]. LigPrep was configured to achieve a series of standardization steps to engender accurate 3D atomic coordinates. First, the 2D structures were transformed into 3D geometries. The target pH for the generation of promising ionization state was set to 7.0 ± 2.0 to mimic the physiological upbringing of the mycobacterial cell wrapping. This ionization state generation was executed using the Epik program, which utilizes Hammett and Taft empirical rules in conjunction with semi-empirical pKa calculations to accurately predict the dominant protonation states. For the designed derivatives containing the specifically defined (1S,4R)-4-methylbicyclo[2.2.1]heptan-2-yl stereocenter, the "retain specified chiralities" option was enforced to prevent the generation of biologically irrelevant enantiomers or diastereomers. However, for any undefined chiral centers, a maximum of 32 stereoisomers per ligand were allowed to be generated. Finally, the generated 3D conformers were subjected to energy minimization using the OPLS4 force field to relieve any inherent steric clashes and to obtain the lowest-energy conformation. Desalting was also performed to remove any unassociated counter-ions or water molecules present in the drawn structures.[13]

Protein Retrieval and Preparation

The high-resolution of X-ray crystallographic structure mycobacterium MmpL3 transporter protein co-crystallized with the inhibitor of SQ109 (PDB ID: 6AJG, Resolution: 2.90 Å), (Fig. 1) was retrieved from protein data bank[5]. Specified that MmpL3 is a complex trans-membrane protein and raw PDB files habitually contain crystallographic artifacts, missing atoms, and incorrect bond orders, the structure was thoroughly refined using the Protein Preparation Wizard module in Maestro. The grounding process was alienated into three primary phase: pre-processing, optimization, and minimization. During pre-processing, crystallographic water molecules situated ahead of a 3.0 Å radius from the co-

crystallized SQ109 ligand were delete, as they typically do not contribute in considerable water-mediated bridge interactions and can synthetically impede the binding pocket. Bond briefing were give, missing hydrogen atoms were complementary, and zero-order bonds to active metals were fashioned. Disulfide bonds were also generating where spatially suitable. Following pre-processing, the hydrogen bond network of the protein complex was optimized. The PROPKA algorithm was engaged to predict and disperse the optimal protonation state of ionizable amino acid residues (such as Histidine, Aspartate, and Glutamate) at a physiological pH of 7.4 [24]. This step is predominantly crucial for MmpL3, as its mechanism of action relies deeply on proton translocation driven by precise Aspartate-Tyrosine pairs (e.g., Asp256 and Tyr646) within the transmembrane channel. Flipping of the terminal amide groups of Asparagine and Glutamine, as well as the imidazole ring of Histidine residue, was scientifically sampled to maximize the stability of the hydrogen-bonding network and minimize steric clash. Finally, a self-possessed energy minimization of the entire protein-ligand complex was perform using the OPLS4 force field. The minimization was allowed to advance until the root-mean-square divergence (RMSD) of the heavy atoms converge to a threshold of 0.30 Å, thereby relaxing the protein structure without conflicting significantly from the experimentally unwavering crystallographic coordinates.

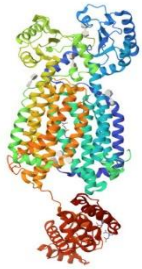

Fig. 1. 3D structure of protein 6AJG

Receptor Grid Generation

To define the energetic site architecture and constraint for the docking algorithm, a receptor grid was generate utilize the Receptor Grid Generation tool in the Glide (Grid-Based Ligand Docking with Energetics) module.[12] The grid box which proceed the spatial boundaries within which the docking algorithms search for most advantageous ligand orientations, was cantered exactingly on the centroid of the native co-crystallized ligand, SQ109.

A bound box of dimensions 20 Å × 20 Å × 20 Å was conventional to include the entire lipophilic transmembrane hollow space of the MmpL3 protein. To precisely replicate the induced-fit nature of ligand binding and to permit for minor structural accommodation of the bulky bicyclo heptane moiety of the premeditated derivatives, the van der Waals radii of the non-polar receptor atoms (atoms with a partial atomic charge of less than 0.25) were scale down by a factor of 1.0 [23]. This softening of the non-polar intermolecular potential allow for minor spatial overlap, successfully compensating for the rigid-receptor estimate inherent in model docking methodologies. No further positional, hydrogen bond, or hydrophobic constraint were useful, allowing the algorithm an impartial search of the chemical space within the distinct pocket.

Molecular Docking Simulation and Scoring

Molecular docking of the carefully prepared SQ109 criterion and the SS1-SS7 derivatives was approved out using the Glide Extra Precision (XP) module [12]. Glide employs a hierarchical series of filters to scientifically search for the best ligand-receptor conformations, orientations, and positions. Originally, a thorough conformational search of the ligand is conduct, followed by a preliminary screening to quickly eliminate poses that demonstrate severe steric clashes. Existing poses are then approved through a Monte Carlo-based modification process to optimize their direction within the grid box. The finalized poses were scored and ranked using the empirical GlideScore scoring function, which integrate manifold physical and chemical parameters, including sympathetic van der Waals interactions (Glide evdw), Coulombic electrostatic energies (Glide ecoul), lipophilic rewards (Glide lipo), penalties for freezing rotatable bonds (Glide erotb), and hydrogen bond networking. To appraise both the thermodynamic favourability and the conformational stability of the pose, the Glide Emodel score was also computed. To systematically assess the drug-like potential of the derivatives, numerous precise metrics were calculated and tabulated. These integrated the total Docking Score, Glide Energy (a combination of Evdw and Ecoul), and Ligand Efficiency (LE). Ligand efficiency is defined as the binding energy normalized by the number of heavy atoms, provided that a crucial metric to establish whether the addition of the bulkier bicycloheptane moiety in the SS series actually improves the specific binding connections per atom when compared to the adamantane ring of the SQ109 standard. The consequential docking conformations were visually analyzed utilizing the Maestro 2D Ligand interface Diagram and the 3D Workspace viewer to map critical hydrophobic contacts, hydrogen bonding dynamics, and steric occupancies within the MmpL3 channel.

Density Functional Theory (DFT) Studies

Computational Details and Software Platform

To expand deeper quantum mechanical insights into the electronic structure, molecular stability, and intrinsic reactivity of the lead considered compound SS2 (N1-((E)-3,7-dimethylocta-2,6-dien-1-yl)-N2-((1S,4R)-4-methylbicyclo[2.2.1]heptan-2-yl)ethane-1,2-diamine), Density Functional Theory (DFT) calculations were perform. All quantum chemical computations were executed using the Gaussian 09W software package [23]. The input coordinates

for the DFT study were derived from the most stable, lowest-energy conformational pose of SS2 obtained from the preceding molecular docking simulations. GaussView 5.0 (or a similar graphical user interface) was utilized for the visualization of the optimized molecular geometry, molecular orbital spatial distributions, and atomic charge mapping.

Geometry Optimization and Level of Theory

The full, unrestricted geometry optimization of the SS2 compound was conceded out in the gas phase to locate the adjacent local minimum on its potential energy surface (PES). The calculations were performed employing the hybrid Becke's three-parameter exchange functional in combination with the Lee-Yang-Parr correlation functional (B3LYP) [19, 20]. The B3LYP functional was particular due to its well-established consistency in precisely predicting the structural and electronic properties of organic molecules with a favourable balance between computational cost and accuracy. The optimization was coupled with the 3-21G split-valence basis set. This basis set allows for the expansion of molecular orbitals using two sizes of basis functions for each valence atomic orbital, providing sufficient flexibility for the electrons to adjust their spatial distribution in the varied chemical environment of the SS2 molecule. The geometry optimization process was carried out without any symmetry constraints (using the default Berny algorithm) until the maximum and root-mean-square (RMS) forces and displacements converged to the standard Gaussian threshold limits, ensuring that the final structure represents a true stationary point on the PES.

Frontier Molecular Orbital (FMO) Analysis and Global Reactivity Descriptors

Following the flourishing geometry optimization of the lead compound, Frontier Molecular Orbital (FMO) analysis was conduct to evaluate the molecule's kinetic constancy and chemical reactivity. The energies of the Highest Occupied Molecular Orbital and the Lowest Unoccupied Molecular Orbital were extract directly from the Gaussian .LOG output file. The HOMO dictates the electron-donating capacity of the molecule, while LUMO dictates its electron-accepting ability.

The energy gap between these frontier orbitals (ΔE = ELUMO – EHOMO) was calculated, serving as critical indicator of molecular polarizability and chemical hardness. A large Highest Occupied Molecular Orbital and the Lowest Unoccupied Molecular Orbital energy gap typically imply high kinetic stability and low chemical reactivity, whereas a narrow gap characterizes a highly reactive, soft molecule. To quantitatively identify the reactive profile of SS2, these orbital energies were additional utilized to compute Koopmans'-theorem-based global reactivity descriptors [18,21].

The global reactivity parameters were calculated using Eqs. (1) to (6).

$$\text{Electron affinity } (A) = -E_{\text{LUMO}} \quad (1)$$

$$\text{Chemical hardness } (\eta) = \frac{E_{\text{LUMO}} - E_{\text{HOMO}}}{2} \quad (2)$$

$$\text{Chemical softness } (S) = \frac{1}{2\eta} \quad (3)$$

$$\text{Electronegativity } (\chi) = -\frac{E_{\text{HOMO}} + E_{\text{LUMO}}}{2} \quad (4)$$

$$\text{Chemical potential } (\mu) = -\chi = \frac{E_{\text{HOMO}} + E_{\text{LUMO}}}{2} \quad (5)$$

$$\text{Electrophilicity index } (\omega) = \frac{\chi^2}{2\eta} \quad (6)$$

These mathematical descriptors provide a holistic view of the molecule's propensity to participate in charge-transfer interactions within the biological receptor (MmpL3) pocket.

Mulliken Population Analysis

To recognize the distribution of electron density diagonally the individual atoms of the SS2 framework, a Mulliken Population Analysis was perform as part of the primary B3LYP/3-21G computational work. The Mulliken atomic charges provide insight into the localized electrostatic potential and dipole moments transversely the compound. Mapping these partial charges permit for the identification of the most nucleophilic and electrophilic centers within the molecular scaffold, which directly correlate with the compound's ability to form precise non-covalent interactions (e.g., the critical hydrogen bonds recognized in the molecular docking analysis).

RESULTS AND DISCUSSION

Molecular Docking Analysis

The molecular docking study was approved out to investigate the binding affinity and interaction behavior of the designed derivatives (SS1–SS7) in similarity with the standard inhibitor SQ109 against the MmpL3 transporter protein. The docking scores exposed important variation amongst the compounds, signifying differences in their binding potential within the active site. In all the derivatives, SS2 exhibit the most sympathetic docking score according to standard (−8.165 kcal/mol), which is obviously better than the standard SQ109 (−7.186 kcal/mol), then specially demonstrating a stronger binding affinity toward the MmpL3 protein. Different designed compounds SS3 (−7.009 kcal/mol), SS5 (−6.957 kcal/mol), and SS4 (−6.929 kcal/mol) shows docking scores similar to SQ109, and then indicating moderate binding capabilities. In distinguish, SS1 (−5.537 kcal/mol) and SS6 (−4.384 kcal/mol) recognized relatively weaker binding affinity, signifying suboptimal interaction within the binding pocket. Furthermore, the reference drug Ethambutol exhibit a significantly lower docking score (−1.727 kcal/mol), highlighting the superior binding possible of the designed derivatives.(Table 1)

Table1. Molecular docking results of SQ109, Ethambutol, and designed derivatives (SS1–SS7) against the MmpL3 protein, showing docking score, Glide energy, Emodel, and ligand efficiency.

S. No.	Compound	Docking Score (kcal/mol)	Glide Energy (kcal/mol)	Emodel	Ligand Efficiency
1	SQ109	-7.186	-29.373	-58.224	-0.299
2	Ethambutol	-1.727	-14.462	-24.392	-0.123
3	SS1	-5.537	-31.845	-52.118	-0.241
4	SS2	-8.165	-38.672	-76.004	-0.327
5	SS3	-7.009	-40.552	-69.213	-0.319
6	SS4	-6.929	-35.871	-67.382	-0.305
7	SS5	-6.957	-42.705	-75.103	-0.316
8	SS6	-4.384	-33.298	-48.567	-0.210
9	SS7	-6.442	-39.185	-63.741	-0.298

Glide Energy and Binding Stability

The Glide energy standards provided further insight into the stability of the ligand–protein complexes by secretarial for van der Waals and electrostatic interactions. The compound SS5 basically displayed the most favourable Glide energy (−42.705 kcal/mol), followed by the designed derivative SS3 (−40.552 kcal/mol) and SS7 (−39.185 kcal/mol), signifying strong and stable interactions within the binding cavity. Especially, all designed derivatives exhibited more favourable Glide energy standards compared to SQ109 (−29.373 kcal/mol), symptomatic of that the structural modifications introduced in the SS series to enhanced their interaction strength with the special protein. These conclusion indicate that the designed derivatives are better accommodate within the hydrophobic transmembrane region of MmpL3, resulting in enhanced stabilization of the ligand–protein complex.

E model Analysis

The E model score, which combines different energetic components together with GlideScore, Coulombic interactions, van der Waals interactions, and internal strain energy, was used to estimate the stability and quality of the docked poses. Along with all compounds, SS2 exhibited the most favourable E model value (−76.004), follow closely by SS5 (−75.103). These standards are significantly better than that of SQ109 (−58.224), demonstrating that SS2 and SS5 adopt highly stable conformations within the binding site. The enhanced E model scores propose that these compounds realize an optimal balance between binding interactions and conformational strain, allowing them to fit extra effectively within the active site of the MmpL3 transporter.

Ligand Efficiency

Ligand efficiency investigation was performed to assess the binding efficiency of each designed compound relative to its molecular size. The results showed that SS2 exhibit the highest ligand efficiency (−0.327), follow by SS3 (−0.319) and SS5 (−0.316), all of which were superior to SQ109 (−0.299). This indicate that the designed derivatives, principally SS2, are capable of achieve stronger binding interactions per heavy atom. The improved ligand efficiency suggest that the incorporation of the bicyclo[2.2.1]heptane moiety contribute positively to binding optimization without unfairly increasing molecular bulk, thereby striking overall drug-like properties.

Binding Interaction Analysis

A detailed analysis of ligand-protein interactions revealed significant differences between the standard drug and the designed derivatives (Fig. 2-3). The reference compound SQ109 did not exhibit any hydrogen bond interactions, indicating that its binding is primarily governed by hydrophobic interactions within the transmembrane cavity. In contrast, the designed derivatives demonstrated enhanced interaction profiles involving hydrogen bonding and electrostatic interactions. SS2 formed two hydrogen bonds with the GLN40 residue through its nitrogen atom, which likely contributes to its superior binding affinity and docking score. Similarly, SS3 formed a single hydrogen bond with GLN40,(Fig.4) resulting in a comparable but slightly reduced binding affinity relative to SS2.

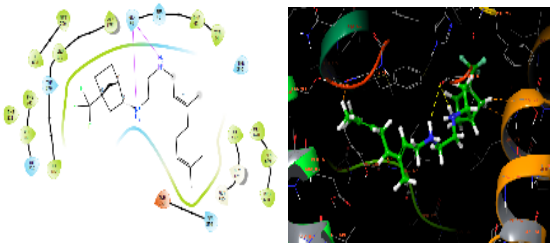

Fig. 2. 2D and 3D ligand interaction diagram of compound SS2

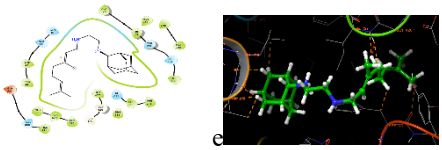

Fig. 3. 2D and 3D ligand interaction diagram of SQ109

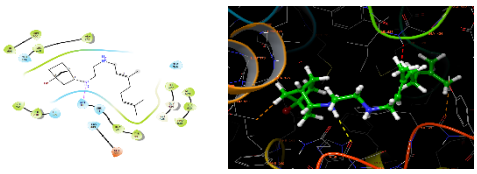

Fig 4. 2D and 3D ligand interaction diagram of compound SS3

Among all compounds, SS5 exhibited the most extensive interaction network, forming four hydrogen bonds with ARG63, ASP64, GLN40, and ASP144. In addition to these hydrogen bonds, SS5 also established two salt bridge interactions with ASP144, which significantly enhance the stability of the ligand–protein complex. The presence of these strong electrostatic interactions, along with multiple hydrogen bonds, explains the highly favorable Glide energy and Emodel values observed for SS5. These findings suggest that the ability to form multiple polar interactions plays a crucial role in improving binding stability within the MmpL3 active site.

Structure-Activity Relationship (SAR) Insights

The docking results provide valuable insights into the structure–activity relationship of the designed compounds. It was observed that the presence of functional groups capable of forming hydrogen bonds with key residues, particularly GLN40, significantly improves binding affinity. Compounds such as SS2 and SS5, which exhibit multiple hydrogen bonding interactions, showed superior docking performance. Additionally, the formation of salt bridge interactions, as observed in SS5 with ASP144, further enhances binding stability, highlighting the importance of electrostatic interactions in ligand recognition. The incorporation of the bicyclo[2.2.1]heptane moiety appears to improve the hydrophobic compatibility of the molecules within the transmembrane cavity while allowing proper orientation for polar interactions. In comparison to SQ109, which lacks hydrogen bonding interactions, the designed derivatives demonstrate a more diverse and stronger interaction profile, suggesting an improved binding mechanism.

Lead Compound Identification and Overall Interpretation

Based on the comprehensive analysis of docking score, Glide energy, Emodel, ligand efficiency, and interaction profile, SS2 emerges as the most promising lead compound due to its highest binding affinity, superior ligand efficiency, and stable hydrogen bonding interactions. SS5 also represents a strong candidate owing to its extensive hydrogen bonding network and additional salt bridge interactions, which contribute to enhanced binding stability. Overall, the results show that the rational design strategy working in this study successfully enhanced the binding characteristics of the derivatives compare to the standard drug SQ109. The improved interaction profiles and binding affinities of the designed compounds recommend their potential as effective inhibitors of the MmpL3 transporter, warrant further examination through advanced computational studies and experimental validation study.

Density Functional Theory (DFT) Analysis

To obtain a profound understanding of the intrinsic electronic structure, molecular stability, and reactive behavior of the most potent designed MmpL3 inhibitor, SS2, quantum chemical calculations were performed using Density Functional Theory (DFT). The calculations were executed at the B3LYP/3-21G level of theory, applying an unconstrained ground-state geometry optimization. The derived quantum chemical parameters and global reactivity descriptors provide critical insights into the pharmacodynamic profile of the compound, bridging its structural features with its exceptional docking performance. The computed parameters are summarized in (Table 2)

Table 2: Quantum Chemical Parameters and Global Reactivity Descriptors of Compound SS2 (Calculated at B3LYP/3-21G)

Parameter	Symbol	Formula	Value
Total Energy	ETOTAL	-	-1146.6586 A.U.
Dipole Moment	μ	-	2.8337 Debye
HOMO Energy	EHOMO	--	-5.38 eV
LUMO Energy	ELUMO	--	-0.82 eV
Energy Gap	ΔEgap	ELUMO −EHOMO	6.20 eV
Ionization Potential	I	−EHOMO	5.38 eV

Electron Affinity	A	−ELUMO	-0.82 eV
Electronegativity	χ	(I+A)/2	2.27 eV
Chemical Hardness	η	(I−A)/2	3.10 eV
Chemical Softness	S	1/(2η)	0.1611 eV-1
Electrophilicity Index	ω	χ2/(2η)	0.83 eV

Frontier Molecular Orbital (FMO) Analysis and Kinetic Stability

The Frontier Molecular Orbital (FMO) theory represents a foundation in computational pharmacology for predicting the reactive pathway and kinetic stability of drug molecule. The biological interface of a drug with its receptor is frequently modulated by the spatial distribution and energy of its Highest Occupied Molecular Orbital and Lowest Unoccupied Molecular Orbital. The HOMO energy define the molecule's capacity to donate electrons to vacant orbitals of the target protein, while the LUMO energy reflects its capacity to accept electrons. For designed compound SS2, (Fig. 5) the compute EHOMO is -5.3835 eV and the ELUMO is 0.8256 eV. The absolute energy difference between these frontier orbital shows the HOMO-LUMO energy gap (ΔE), which was calculated to be remarkably wide at 6.2091 eV. In the context of drug design, a wide energy gap is highly desirable. It indicates a high degree of kinetic inertness and excitation energy, meaning that SS2 strongly resists spontaneous intramolecular charge transfer or unprovoked electron excitation. Consequently, the molecule exhibits exceptional thermodynamic stability, ensuring that its critical pharmacophoric elements (specifically the geranyl chain and the bicyclo[2.2.1]heptane ring) remain chemically intact within the aggressive metabolic environment of the host and the mycobacterial cell envelope prior to reaching the MmpL3 target.5.2. Global Reactivity Descriptors and Toxicity Prediction to translate the FMO energies into quantitative predictors of chemical behavior, global reactivity descriptors were derived based on Koopmans' theorem. Ionization Potential (I) and Electron Affinity (A): The Ionization Potential of SS2 is strongly positive at 5.3835 eV, signifying that extracting an electron from the optimized structure requires substantial energy input. Furthermore, the Electron Affinity is negative (-0.8256 eV), dictating that the addition of an external electron to the molecular system is an energetically disfavored process. Together, these values confirm that SS2 possesses a highly robust redox profile, making it highly resistant to both oxidative degradation by cytochrome P450 enzymes and reductive stress in vivo. Chemical Hardness (η) and Softness (S): The global chemical hardness of SS2 is 3.1045 eV, paired with a very low chemical softness of 0.1611 Ev-1. According to Pearson's Maximum Hardness Principle, molecules with elevated hardness values are intrinsically less polarizable, less reactive, and more chemically stable. From a toxicological perspective, the high "hardness" of SS2 strongly suggests that it will interact with its biological target through highly specific, non-covalent, electrostatic and hydrophobic interactions (as seen in the molecular docking phase) rather than indiscriminately reacting with off-target macromolecules. Electronegativity (χ) and Electrophilicity Index (ω): The electronegativity of SS2 is 2.2790 eV, representing a moderate baseline tendency to attract electrons. Crucially, the global electrophilicity index (ω)—a powerful metric for assessing the toxicity associated with covalent drug binding—was calculated at a exceptionally low 0.8365 eV. Molecules with elevated electrophilicity frequently act as reactive Michael acceptors, covalently modify off-target biological nucleophiles such as glutathione, DNA or the sulfhydryl groups of necessary host proteins, leading to strict hepatotoxicity or mutagenicity. The low electrophilicity index of design SS2 structurally classify it as a weak, safe electrophile, effectively eliminating concerns regarding non-specific covalent toxicity.5.3. with Conformational Thermodynamics and Electrostatic Topography. The unconstrained geometry optimization confirmed that the 3D topology of SS2 converges to a deep local minimum with a total ground-state energy of -1146.6586 A.U. This substantial negative energy value indicates an absence of severe internal steric strain, further supporting the rational incorporation of the bulky (1S,4R)-4-methylbicyclo[2.2.1]heptan-2-yl moiety without destabilizing the molecular framework. Finally, the designed Dipole Moment (μ) of 2.8337 Debye is a critical parameter for accepting the compound's mechanism of action. This moderate dipole moment shows a pronounced, restricted asymmetry in the electron density crosswise the molecule. This electrostatic topography efficiently grants SS2 amphiphilic characteristics: the central ethylenediamine nitrogen atoms create a polarized, electron-rich core, while the generous geranyl and bicycloheptane hydrocarbon chains preserve deep lipophilicity. This precise distribution of electron density is necessary for MmpL3 inhibition; the lipophilic regions permit the drug to flawlessly partition into and traverse the highly hydrophobic mycobacterial membrane, Although the 2.83 Debye polar center precisely aligns the molecule to form the powerful, directional electrostatic hydrogen bonds essential to arrest the proton-translocation channel.

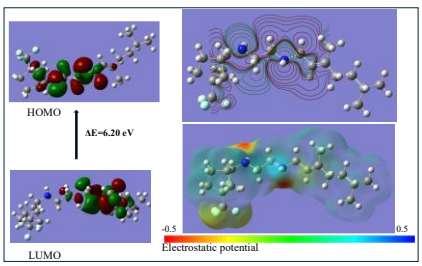

Fig. 5. Density Functional Theory (DFT) analysis of the lead compound SS2. The figure illustrates the optimized ground-state molecular geometry alongside the spatial distributions of the Frontier Molecular Orbitals, including

the Highest Occupied Molecular Orbital (HOMO) and the Lowest Unoccupied Molecular Orbital (LUMO), computed at the B3LYP/3-21G level of theory.

CONCLUSION

Surprisingly, computational techniques prove powerful when crafting new versions of SQ109 targeting the MmpL3 target in tough TB strains. Of the tested molecules, SS2 stands out due to stronger binding, smarter ligand use, along with consistent molecular contacts - beating SQ109 itself. Rather than guesswork, quantum calculations confirm its resilience, showing balanced electron behavior and minimal risk for harmful effects.The data point toward SS2 as a potential starting point for new drugs targeting tuberculosis. Still, models built by computers need real-world testing to back them up. Next steps might explore how it behaves in lab cultures and live organisms, check its toxicity profile, and run detailed motion-based calculations. Taken together, the research lays solid groundwork for better molecules that block MmpL3 in stubborn forms of TB.

Study limitation

One of the main limitations of this study is that the conclusion is based only on computational approaches. Although molecular docking and DFT studies give useful insight into binding interactions and molecular properties, they cannot completely predict the actual biological activity, toxicity, pharmacokinetic and cellular permeability of SS2. Consequently, further in vitro antimycobacterial and cytotoxicity studies, follow by in vivo evaluation, are essential to confirm its potential as an anti-tubercular agent against MDR-TB.

List of abbreviation

S. No.	Abbreviation	Full form
1	SQ109	N-geranyl-N'-(2-adamantyl)ethane-1,2-diamine
2	MmpL3	Mycobacterial Membrane Protein Large 3
3	DFT	Density functional theory
4	MDR-TB	multi drug resistant tuberculosis
5	HOMO	Highest Occupied Molecular Orbital
6	LUMO	Lowest Unoccupied Molecular Orbital
7	FMO	Frontier Molecular Orbital
8	SAR	Structure- Activity Relationship
9	RMS	root-mean-square
10	OPLS4	Optimized Potentials for Liquid Simulations 4
11	RMSD	root-mean-square divergence
12	LE	Ligand Efficiency
13	SS1-SS7	(N1-((E)-3,7-dimethylocta-2,6-dien-1-yl)-N2-((1S,4R)-4-methylbicyclo[2.2.1]heptan-2-yl)ethane-1,2-diamine)

Consent for Publication - Not applicable

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