

GLAUCARUBINONE - A LEAD MOLECULE FROM *SIMAROUBA GLAUCA* AS A POTENTIAL DRUG CANDIDATE, AN *IN SILICO* STUDY

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ABSTRACT

Nature has always been a source of drug candidates. Since ancient times, people have been using plants and their metabolites for various medicinal purposes. Glaucarubinone is a quassinoid present in the family Simaroubaceae. *Simarouba glauca*, also known as Laxmitaru or Paradise tree is grouped under the family Simaroubaceae, Glaucarubinone present in *S. glauca* is known for its medicinal property. Molecular docking methods are widely used to investigate the interactions between a drug candidate and its target, and to discern the therapeutic action to design new drug candidate with enhanced activities. The information generated from docking studies helps to obtain an insight into interactions of drug candidate with amino acid in the active site of the target proteins, and to predict the binding energy of ligands to the target. By Molecular Dynamic Simulation, the flexibility and the conformational stability of target proteins-glaucarubinone complex is confirmed.

Keywords: Glaucarubinone, *Simarouba glauca*, quassinoids, triterpenoids, molecular docking, molecular dynamic simulation

INTRODUCTION

Herbal preparations have been widely used as medicines for thousands of years around the world as they are of natural origins and are assumed to have fewer side effects¹. In tropical countries, the majority of the population relies on the use of plant extracts to combat the ravages of diseases². *Simarouba glauca*, the paradise tree that belongs to the family Simaroubaceae, is widely distributed in tropical regions. *S. glauca* plant extract is noted for its bioactive compounds, which have been reported significantly for their therapeutic values.

Quassinoids are a group of tetracyclic triterpenoids present in the Simaroubaceae family. The plant has been used in folk medicine for many years. Since 1975, quassinoids are renowned for their medicinal properties with the discovery of bruceantin, a quassinoid that possess anti-leukemic activity. At present, about 150 or more quassinoids have been reported and categorized based on their structures and biological activity that has

been investigated both by *in vitro* and *in vivo* studies. Many quassinoids are noted for their inhibitory effects, such as anti-inflammatory, anti-microbial and anti-proliferative effects³. The active groups of quassinoids include glaucarubinone, holacanthone, ailanthionone, canthin-6-one, benzoquinone, glaucarubin, simarolide and melianone⁴. The structure–activity analysis of quassinoids has revealed that quassinoids possess a structure that renders a property to be a novel lead molecule for the discovery of new drug. There are five distinct groups of quassinoids based on their structural skeleton. The quassinoid glaucarubinone that is present in *S. glauca* has been found to possess a promising activity against *Plasmodium falciparum* in culture^{5,6}, cytotoxicity against several human cancer cell lines^{7,8,9} and anti-amoebic activity. The present *in silico* study aims to predict and identify the target protein for the potential drug candidate glaucarubinone.

METHODOLOGY

LIGAND PREPARATION

Ligands are small molecules that might be known to modulate the protein target by efficiently binding to the

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Table I : Target proteins and their functions

Target protein and its PDB id	Functions of the protein
Falcipain (3BPF)	Cys protease of <i>P. falciparum</i> is a key enzyme in the life cycle of <i>P. falciparum</i> . At the early trophozoite stage, it degrades hemoglobin, and at the schizont stage, it cleaves protein 4.1 and ankyrin, the cytoskeletal elements vital for the stability of red cell membrane ²⁰ .
Plasmepsin (1LF3)	Asp protease of <i>P. falciparum</i> is involved in the degradation of hemoglobin, secretory organelle protein processing for egress, invasion, and effector export ^{21,22} .
Lipoxygenase (6NCF)	Leukotrienes (LT3) biosynthesis is initiated by 5-lipoxygenase. LT3 are the mediators of the inflammatory response ²³ .
Cathepsin (1CSB)	A cysteine peptidase involved in many physiological processes, such as inflammation, parasite infection and cancer ²⁴ .
COVID 19 major protease (6LU7)	The main protease of SARS-CoV-2 is a key enzyme of coronaviruses and plays a pivotal role in mediating viral replication and transcription ²⁵ .
Histone deacetylase 3 (4A69)	Regulates gene expression by removing acetyl groups from lysine residues in histone tails, resulting in chromatin condensation ²⁶ .
α -thrombin (1D3T)	Converts soluble fibrinogen into fibrin and activates the transglutaminase factor XIII, which cross-links adjacent fibrin monomers ²⁷ .
11beta-hydroxysteroid dehydrogenase -1 (3OQ1)	11beta-hydroxysteroid dehydrogenase is a nicotinamide adenine dinucleotide phosphate (NADPH)-dependent enzyme expressed in liver and fat tissues. The enzyme is responsible for the reduction of cortisone to its active form cortisol a glucocorticoid (GC) hormone for homeostasis regulation. GC is a potent insulin-antagonistic hormone that inhibits insulin secretion ²⁸ .
Decaprenylphosphoryl-beta-D-ribose oxidase (4P8Y)	Decaprenylphosphoryl-beta-D-ribose oxidase together with decaprenylphosphoryl-D-2-keto erythropentose reductase is involved in two-step epimerization of decaprenylphosphoryl- β -D-ribofuranose (DPR) into decaprenylphosphoryl- β -D-arabinofuranose (DPA), Decaprenylphosphoryl- β -D-arabinofuranose is an essential precursor for the biosynthesis of arabinogalactan, which is a component of the mycobacterial cell wall ²⁹ .
Thioredoxin reductase from <i>E. histolytica</i> (4CCR)	Thioredoxin reductase catalyzes the reversible transfer of reducing equivalents between NADPH and thioredoxin, a small protein that plays a key metabolic function in maintaining the intracellular redox balance ³⁰ .

target. Glaucarubinone, the ligand molecule for this study, was retrieved from PubChem database¹⁰. The canonical, Smiles selected were converted to its structure in PDB format using OpenBabel version 2.4.1¹¹.

Drug likeness analysis

The drug likeness of the ligand was predicted using Lipinski filter. Lipinski rule states that an oral drug should satisfy at least four out of five rules for drug likeness such as molecular weight, hydrogen bond donor, hydrogen bond acceptor, cLogP, and molar refractive index¹². The properties were predicted by SwissADME (<http://www.swissadme.ch>), a convenient online tool for drug discovery¹³. The properties of ligands with respect to their adsorption, distribution, metabolism and excretion (ADME) were also analyzed by SwissADME. Absorption

by the gastrointestinal cells and passage into the brain are the two pharmacokinetic properties that are important to be estimated at different stages of the drug discovery.

Target preparation and docking

Molecular Docking was performed using Autodock 4.2 software. The three dimensional structure of protein molecules, namely, falcipain 2(PDB ID:3BPF), plasmepsin II (PDB ID: 1LF3), decaprenylphosphoryl-beta-D-ribose oxidase (PDB ID: 4P8Y), histone deacetylase HDAC 3 (PDB ID: 4A64), COVID-19 main protease (PDB ID:6LU7), 11beta- hydroxysteroid dehydrogenase-1(PDB ID: 3OQ1), 5-lipoxygenase activating protein (PDB ID: 6NCF), cathepsin (PDB ID: 1CS6), thioredoxin reductase from *Entamoeba histolytica* (4CCR), and human alpha thrombin (PDB ID :1D3T), were retrieved from Protein

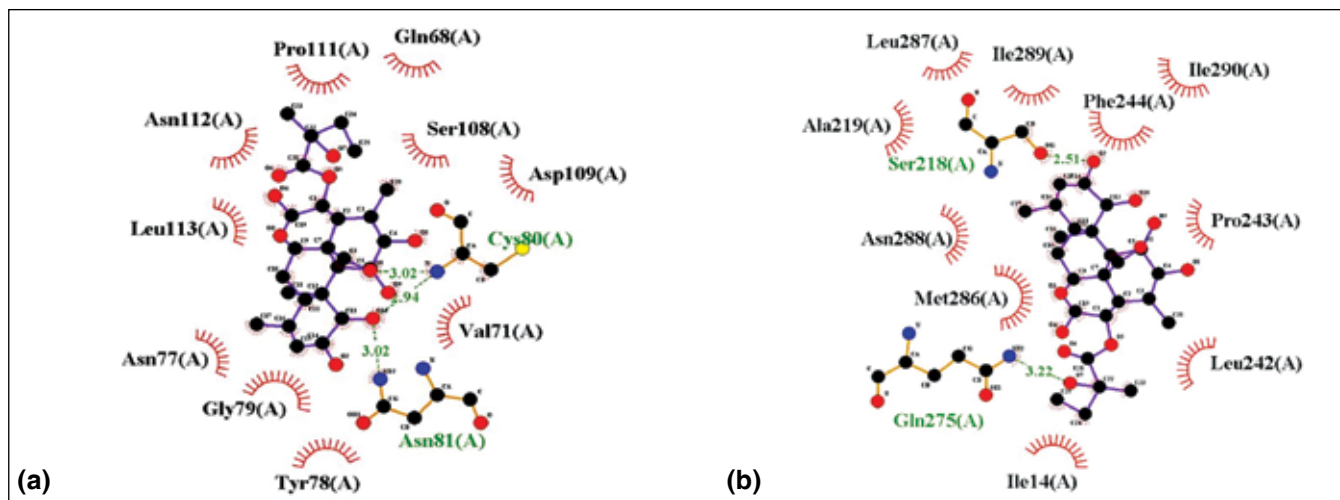


Fig. 1: Anti-plasmodial – Docking poses of glaucarubinone with a) Falcipain of *P. falciparum* b) Plasmepsins of *P. falciparum*

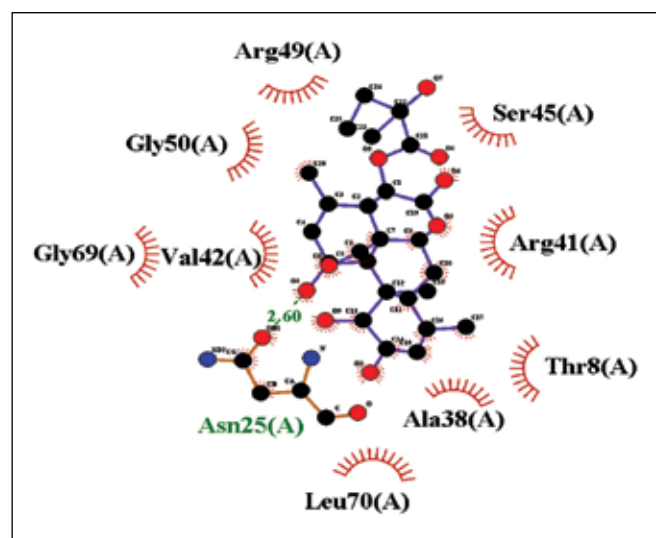


Fig. 2: Antibacterial - Docking poses of glaucarubinone with decaprenylphosphoryl-beta-D-ribose oxidase of *M. tuberculosis*

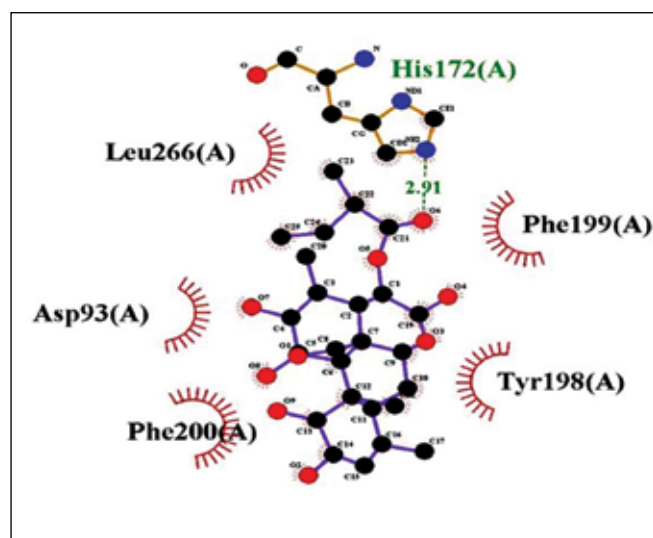


Fig. 3: Anticancer- Docking poses of glaucarubinone interactions with histone deacetylase 3

Data Bank¹⁴ (<http://www.rcsb.org/pdb>) Table I. The water molecules and heteroatoms were removed from the protein and polar hydrogen atoms and the Gasteiger charge were added. The pre-calculated grid map was obtained using Autogrid and the default parameters of 250,000 energy evaluations for optimization were selected. The energy of ligand was minimized to maintain the most stable configuration. The result of the docking is expressed in terms of binding energy (B.E), represented in units of kcal mol⁻¹, and the inhibition constant (Ki) in micro molar (μM). The poses with the lowest B.E and the most appropriate cluster were considered for visualization and further analysis. The docked complexes were viewed using PyMol version 1.3 - PyMOL Molecular graphics system¹⁵ and ligand binding sites viewed using LigPlot+

Version 2.2. LigPlot+ generates schematic diagram of protein-ligand interaction for a given PDB file¹⁶.

Molecular dynamics (MD) Simulation of Protein–Ligand Complexes

Molecular dynamic (MD) simulations are used to assess the variation in the protein conformation induced by the probable drug ligand. For evaluating the stability of protein with glaucarubinone complexes, the Molecular Dynamic (MD) simulations were performed. Molecular Dynamic simulation was performed to ascertain the protein stability, conformation and its interaction with the lead molecule. The Mol 2 format of the ligand glaucarubinone was submitted to the PRODRG 2 server (PRODRG Server

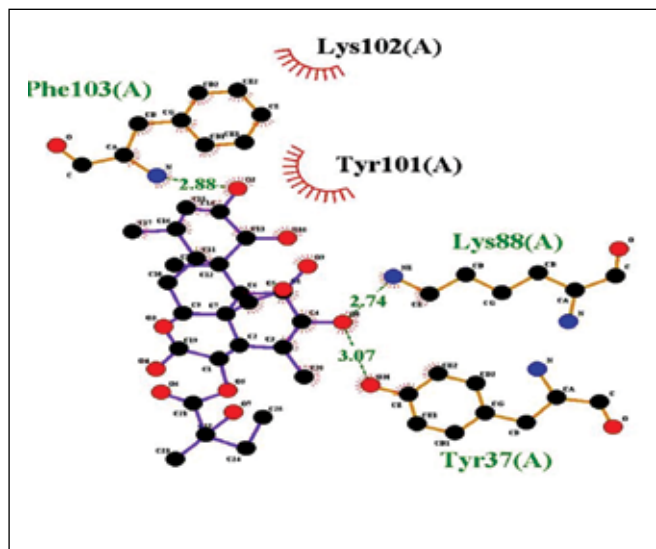


Fig. 4: Anti-viral - Docking poses of glaucarubinone with Covid 19 major protease

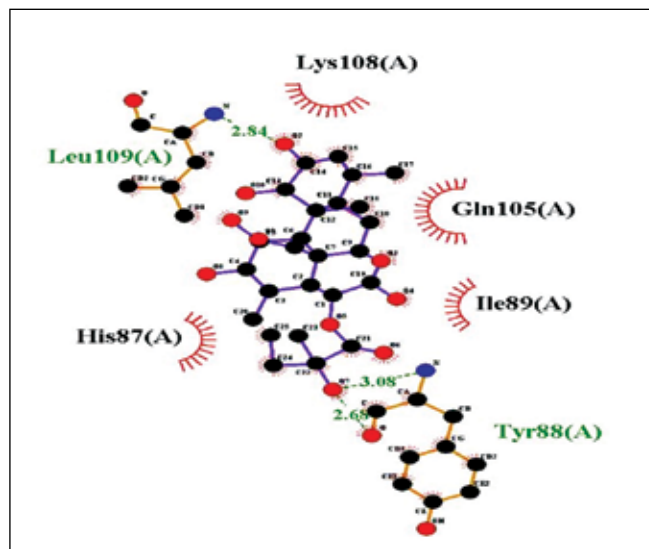


Fig. 5: Anti-diabetic - Docking poses glaucarubinone with 11beta-hydroxysteroid dehydrogenase-1

Table II: Pharmacokinetic properties of glaucarubinone

Canonical SMILES	<chem>CCC(C(=O)OC1C(=O)OC2C34C1C(C)C(O)C(C4C1(C(C2)C(=CC(=O)C1O)C)C)(OC3O)(O)C</chem>
Structure	
Formula	C ₂₅ H ₃₄ O ₁₀
Molecular weight	494.53 g mol ⁻¹
Total rotatable bonds	4
Number of H-bond acceptors	10
Number of H-bond donors	4
Solubility	Water soluble
Lipophilicity Log P _{o/w}	(iLOGP) = 2.14
GI absorption	low
BBB permeation	No
Druglikeness Lipinski rule	No violation
AMES mutagenicity	Negative
LD50	1190 mg kg ⁻¹
Toxicity	Class IV

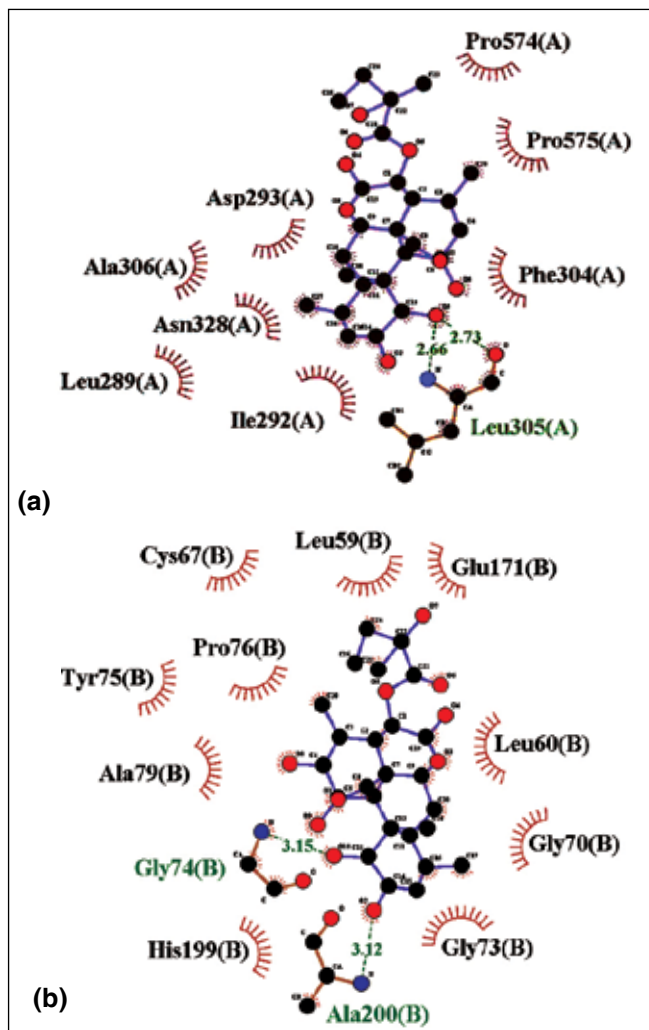


Fig. 6 : Anti-Inflammatory- Docking poses of glaucarubinone with : (a) Lipooxygenase (b) Cathepsin

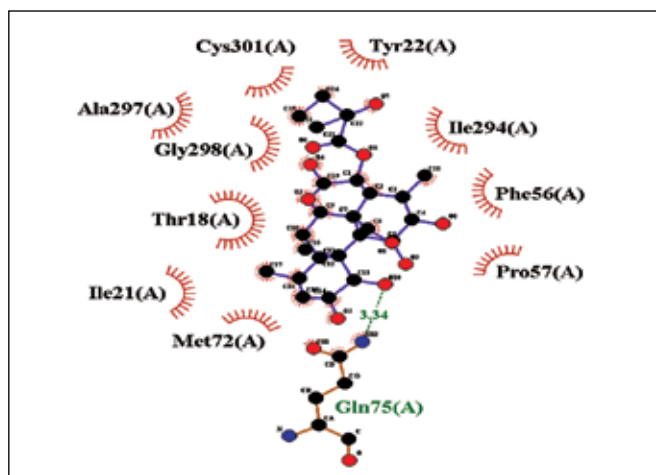


Fig. 7: Anti-amoebic - Docking poses of glaucarubinone with thioredoxin reductase from *E. histolytica*

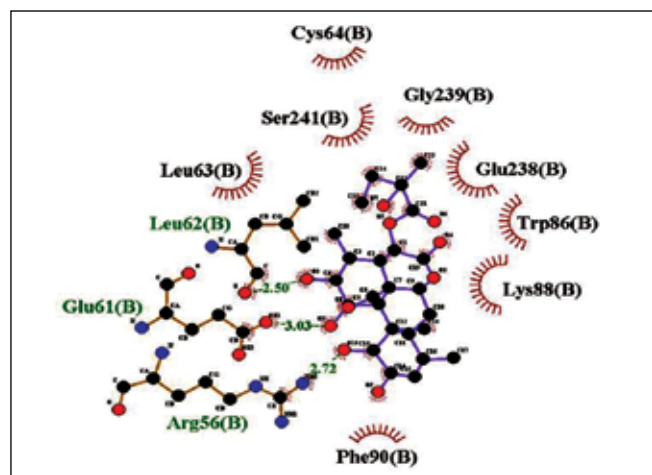


Fig. 8: Anti-coagulant - Docking poses of glaucarubinone with α -thrombin

Table III: Predicted binding affinities of glaucarubinone with various target proteins

Target protein and its PDB id	Binding energy (kcal mol ⁻¹)	K _i (μm)	Residue interacting with the target protein
Falcipain (3BPF)	- 7.35	4.10	Gln 68, Val 71, Asn 77, Tyr 78, Gly 79, Ser 108, Asp 109, Pro 111, Asn 112, Leu 113. H-bonding : Cys 80, Asn 81
Plasmepsin (1LF3)	-6.17	29.85	Ile 141, Ala 219, Pro 243, Phe 244, Met 286, Leu 287, Asn 288, Ile 290. H-bonding : Ser 218, Gln 275
Decaprenylphosphoryl-beta-D-ribose oxidase (4P8Y)	-5.11	180.59	Thr 8, Ala 38, Arg 41, Val 42, Ser 45, Arg 49, Gly 50, Leu 70. H-bonding : Asn 25
Histone deacetylase 3 (4A69)	-6.39	20.62	Asp 93, Tyr 198, Phe 199, Phe 200, Leu 266. H-bonding : His 172
COVID 19 major protease (6LU7)	-5.74	61.70	Try 101, Lys 102. H-bonding : Lys 88, Tyr 37, Phe 103
11-beta-hydroxysteroid dehydrogenase-1 (3OQ1)	-7.25	4.87	His 87, Ile 89, Gln 105, lys 108 H-bonding : Tyr 88, Leu 109
Lipoxygenase (6NCF)	-6.54	15.99	Leu 289, Asn 328, Ala 366, Pro 574, Pro 575 H-bonding : Leu 305
Cathepsin (1CSB)	-6.56	15.49	Leu 59, Leu 60, Cys 67, Gly 70, Glu 71, Gly 73, Tyr 75, Pro 76, Ala 79, His 199 H-bonding : Gly 74, Ala 200
Thioredoxin reductase from <i>E. histolytica</i> (4CCR)	-5.33	123.02	Thr 18, Ile 21, Tyr 22, Phe 56, Pro57, Met 72, Ala 297, Gly 298, Cys 301 H-bonding : Gln 75
α -thrombin (1D3T)	-5.71	64.96	Leu63, Cys 64, Lys 85, Trp 86, Phe 90, Glu 238, Gly 239, Ser 241 H-bonding : Arg 56 , Glu 61, Leu 62

Ile: Isoleucine, Met: Methionine, Pro: Proline, Ala: Alanine, Cys: Cysteine, Asp: Aspartic Acid, Glu: Glutamic Acid, Phe: Phenylalanine, Gly: Glycine, His: Histidine, Leu: Leucine, Asn: Asparagine, Gln: Glutamine, Arg: Arginine, Ser: Serine, Thr: Threonine, Val: Valine, Trp: Tryptophan, Tyr: Tyrosine

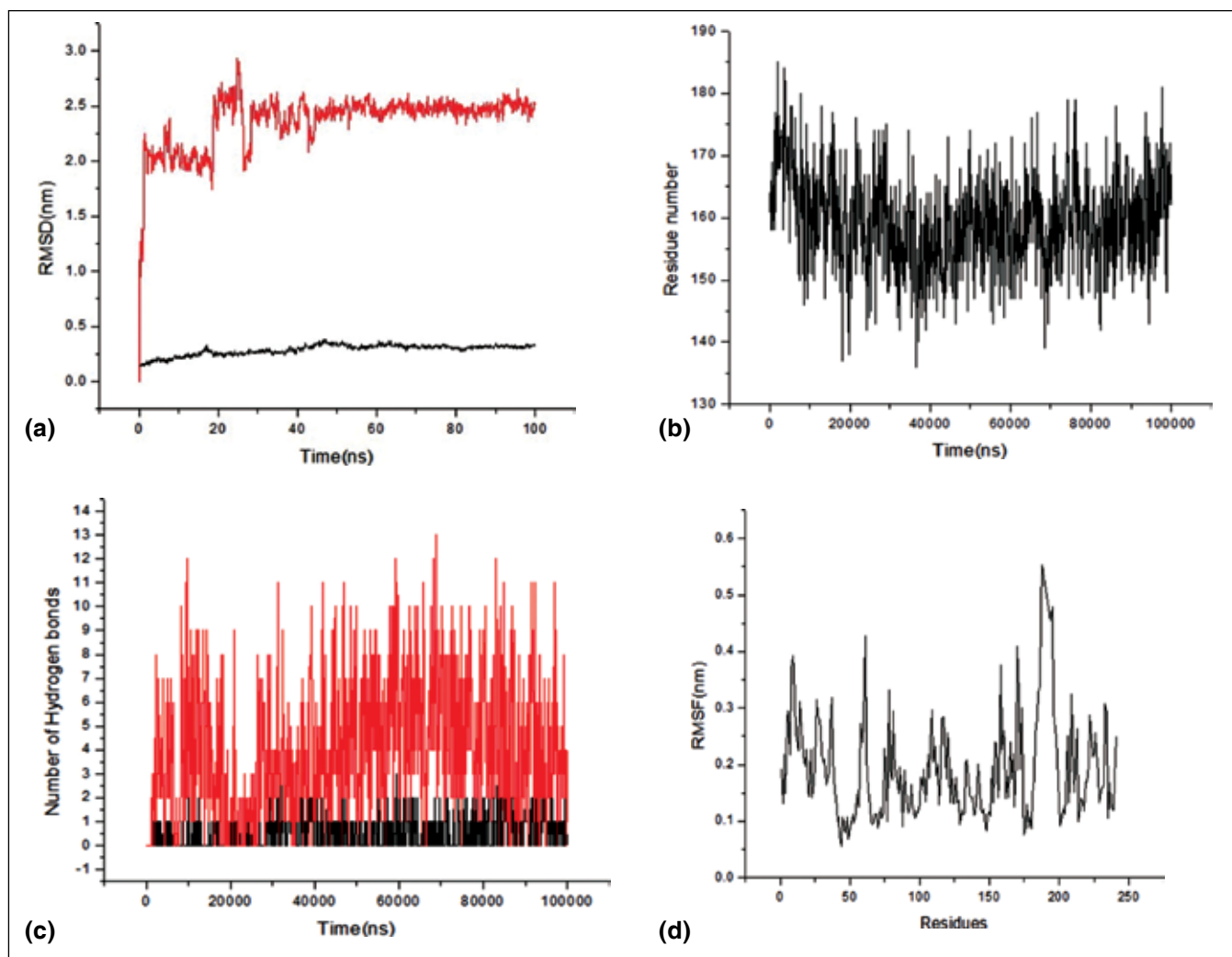


Fig. 9: Molecular dynamic simulation of glaucarubinone interaction with falcipain of *P. falciparum* a) RMSD curve of backbone (black) and complex (Red) b) Hydrogen bonding with residues c) Number of hydrogen bonds throughout the simulation(black), Pairs within 0.35 nm(red) d) RMSF curve of the complex during MD simulation

(dundee.ac.uk)¹⁷ to generate the topology file of the ligand (Prodr). The topology file was submitted to the WebGro server together with the target protein in the PDB format. For the MD simulation of the target – ligand complex Gromos96 43a1 forcefield was selected and solved with simple point charge (SPC) water model within a cubic grid box. The complex was then selected to be maintained in 0.15 M NaCl solution. Energy minimization parameter was selected as Steepest descent integrator for 5000 steps. The simulation of all the complexes was done for 100 ns under constant temperature and pressure equilibrated. The Root Mean Square Deviation (RMSD) and the Root Mean Square Fluctuation (RMSF) of each residue in the given structure and average number of H-bonds in each frame for 100 ns were analyzed¹⁸.

RESULTS AND DISCUSSION

Glaucarubinone, a quassinoid, is a secondary metabolite present in *S. glauca*³⁰. Glaucarubinone is known for its varied pharmacological properties. *In silico* Blood Brain Barrier (BBB), the molecule shows no violation of Lipinski rule and is non-mutagenic, as shown in Table II.

Table III shows the predicted binding affinities of glaucarubinone with various target proteins. Docking of glaucarubinone ligand has predicted that glaucarubinone binds to falcipain and plasmepsin of *P. falciparum*, decaprenylphosphoryl-beta-D-ribose oxidase of *M. tuberculosis*, histone deacetylase - 3, Covid 19 major protease, 11beta-hydroxysteroid dehydrogenase-1, lipoxygenase, cathepsin, *E. histolytica* thioredoxin

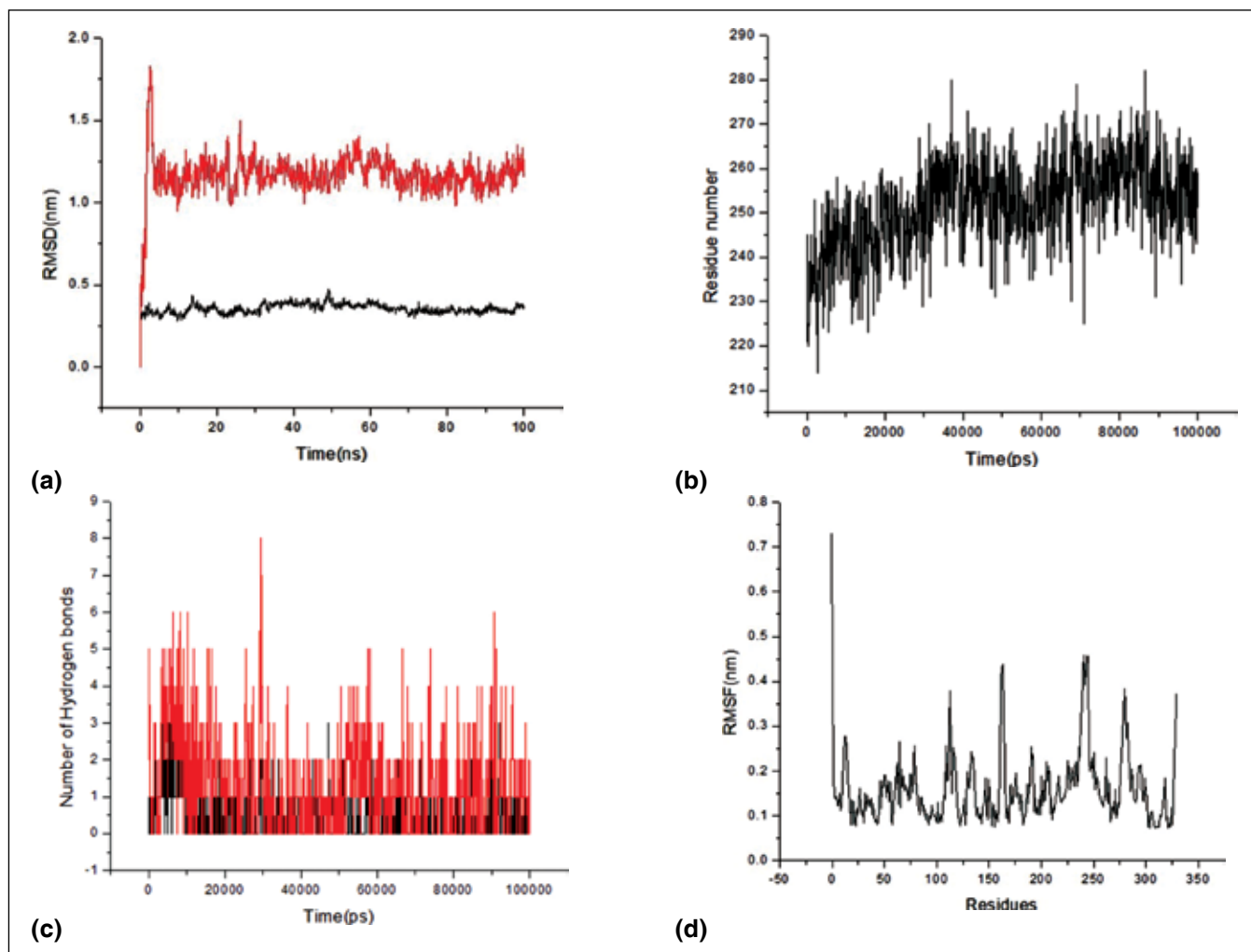


Fig. 10: Molecular dynamic simulation of glaucarubinone interaction with plasmepsin of *P. falciparum* a) RMSD curve of backbone (black) and complex (Red) b) hydrogen bonding with residues c) number of hydrogen bonds throughout the simulation(black), pairs within 0.35 nm(red) d) RMSF curve of the complex during MD simulation

reductase and α -thrombin, efficiently through hydrogen bonding and hydrophobic interaction. The predicted binding hence proves that glaucarubinone is a molecule that has diverse properties such as anti-plasmodial, anti-bacterial, anti-cancer, anti-viral, anti-diabetic, anti-inflammatory, anti-amoebic and anti-coagulant.

Anti-plasmodial

Falcipain plays a vital role in food assimilation in the malarial parasite. Inhibition of the key enzyme falcipain interrupts the nutrition source and thereby controls the parasite by starvation³¹. Glaucarubinone binds efficiently with falcipain of *P. falciparum* with the predicted binding energy - 7.35 kcal mol⁻¹ and Ki value 4.1 μ m. The predicted interactions of amino acid residues with the ligand glaucarubinone are Gln 68, Val

71, Asn 77, Tyr 78, Gly 79, Ser 108, Asp 109, Pro 111, Asn 112, Leu 113 and H-bonding with Cys 80, Asn 81 (Table III, Fig.1), glaucarubinone also binds efficiently and interacts with plasmepsin of *P. falciparum* with binding energy -6.17 kcal mol⁻¹ Ki value 29.85 μ m and the predicted interactions of amino acid residues with the ligand glaucarubinone are Ile 141, Ala 219, Pro 243, Phe 244, Met 286, Leu 287, Asn 288 and Ile 290 and H-bonding with Ser 218, Gln 275 (Table III, Fig. 1b). These predicted bindings may suggest glaucarubinone to be inferred as a pharmacophore to treat malarial infection. Glaucarubinone may be an alternate drug candidate as chloroquin resistant *P. falciparum* is common in malaria endemic area³². Molecular dynamics simulation analysis was done, to assess the structural changes and stability of the docked protein-ligand complex. The degree of 3D

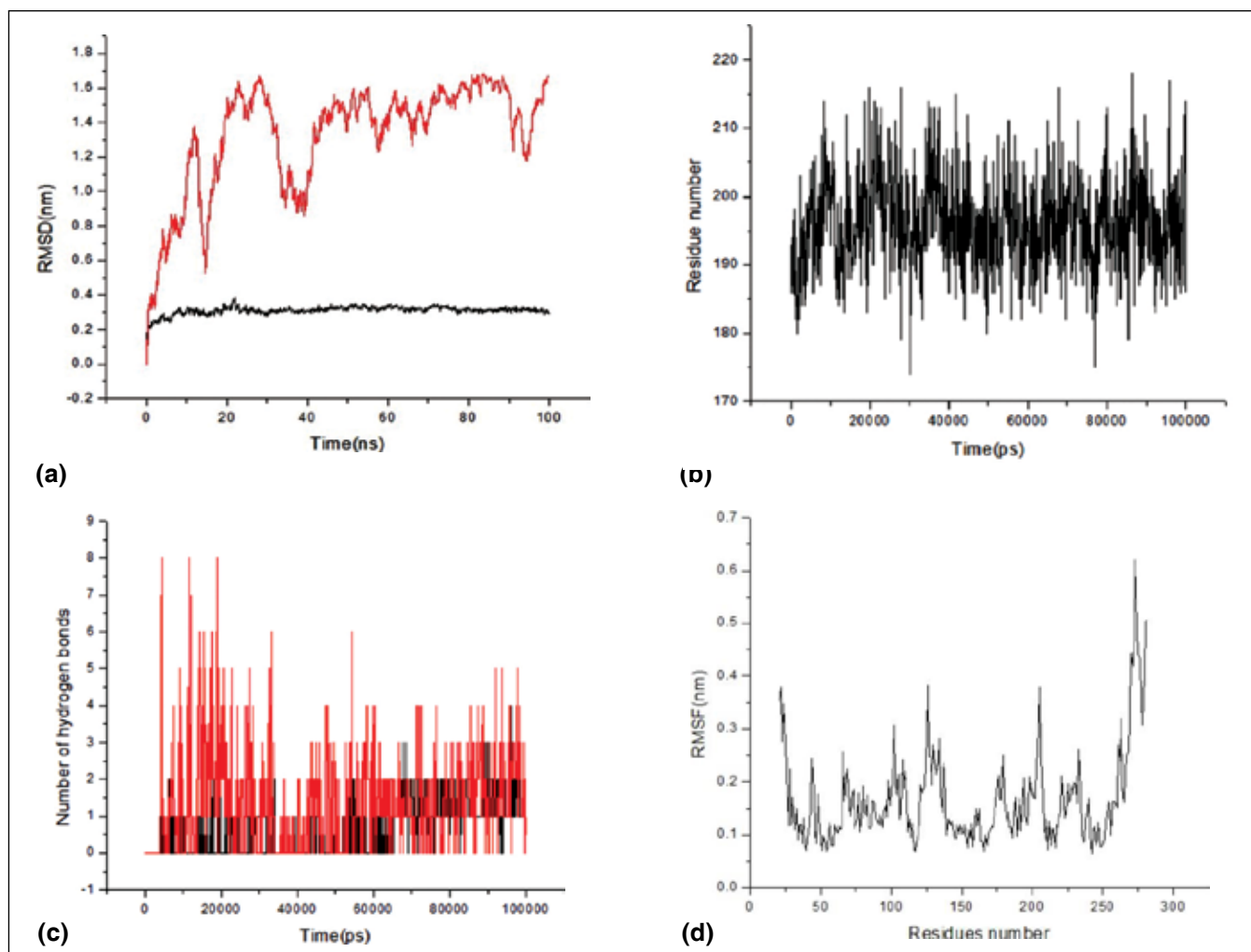


Fig. 11: Molecular dynamic simulation of glaucarubinone interaction with decaprenylphosphoryl-beta-D- ribose oxidase of *M. tuberculosis* a) RMSD curve of backbone (black) and complex (Red) b) hydrogen bonding with residues c) number of Hydrogen bonds throughout the simulation(black), pairs within 0.35 nm(red) d) RMSF curve of the complex during MD simulation

structural changes in protein backbone of falcipain and plasmepsin of *P. falciparum* and its docked complexes were assessed for its Root Mean Square Deviation (RMSD) for 100 ns simulation trajectories for falcipain-glaucarubinone complex, and plasmepsin- reductase complex of *P. falciparum*. The RMSD value of falcipain, backbone showed the stability continuously during the simulation, and the peak equilibrated at an average of 0.28 nm, also the RMSD of falcipain-glaucarubinone complex also showed a similar stability throughout the 100ns of simulation, the peak equilibrated on an average of 2.36 nm (Fig. 9). Similarly, RMSD of plasmepsin backbone showed stability throughout 100 ns of simulation; it showed an equilibrated peak at an average of 0.19 nm and interaction with glaucarubinone had the peak equilibrated at an average of 1.16 nm

(Fig. 10). Root Mean Square Fluctuation (RMSF) is yet another essential parameter for examining the conformational stability and dynamic flexibility of protein-ligand complex and to predict the steric interaction of amino acid residues with the ligand during the simulation³³. The RMSF plot of the interactions of glaucarubinone with falcipain and plasmepsin produced an assured level of fluctuations maintaining the interaction throughout 100 ns simulation process. The RMSF graph of falcipain- glaucarubinone complex and plasmepsins- glaucarubinone complex shows only minimal fluctuation maintaining the stable conformation with minimal fluctuations, by the residues Lys 61, Lys 170, Gln 187, His 194 and Lys 195 in falcipain-glaucarubinone complex and Tyr240, Ala241, Tyr243, Phe244, Asp245 in plasmepsins- glaucarubinone complex. The results

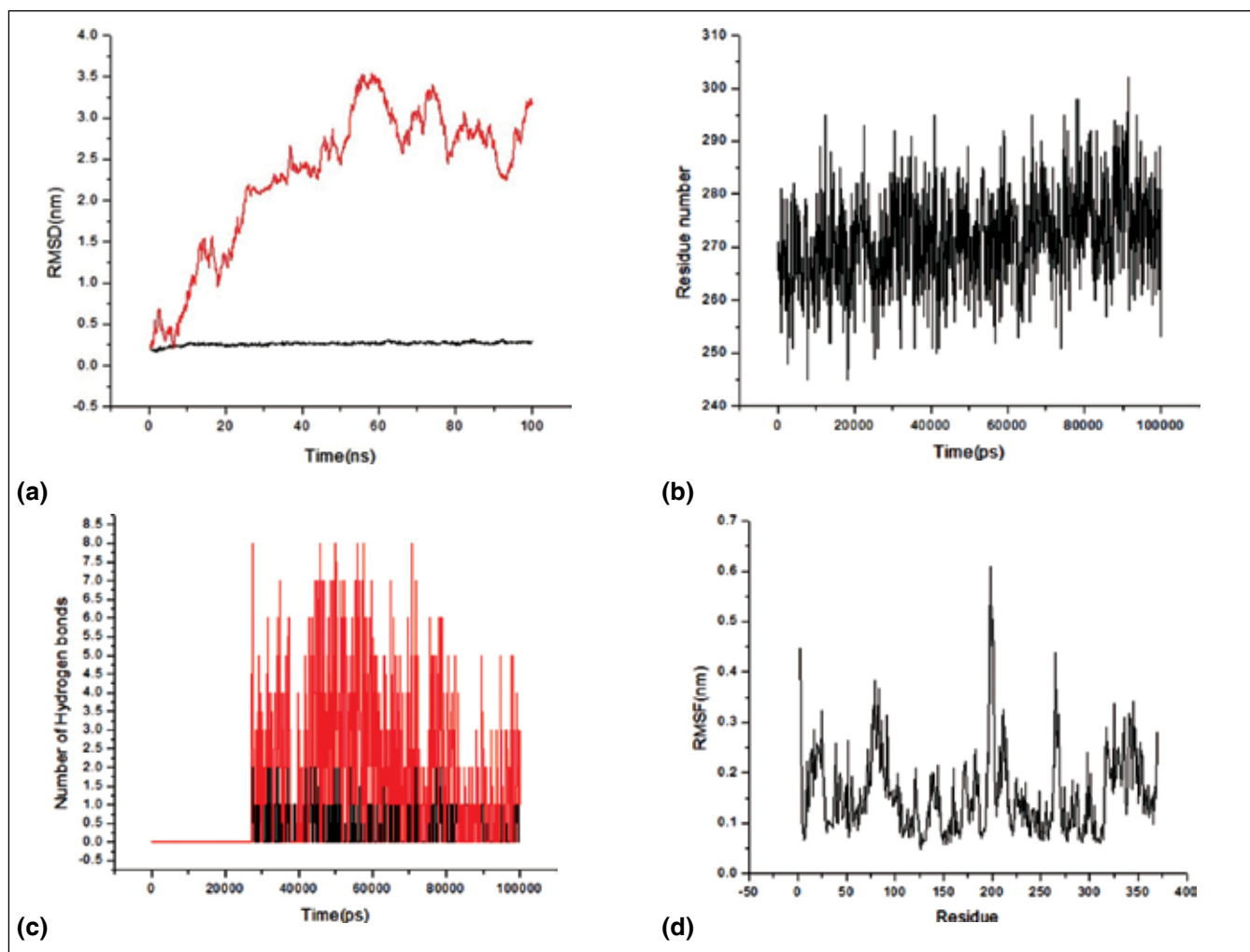


Fig. 12: Molecular dynamic simulation of glaucarubinone interaction with histone deacetylase a) RMSD curve of backbone (black) and complex (Red) b) Hydrogen bonding with residues c) Number of Hydrogen bonds throughout the simulation (black), Pairs within 0.35 nm (red) d) RMSF curve of the complex during MD simulation

reveal that binding of glaucarubinone to the target proteins falcipain and plasmepsin of *P. falciparum* generate no major effects on the flexibility of these target proteins. Glaucarubinone also shows a greater affinity to the proteins falcipain and plasmepsin by hydrogen bond formation between the targets and the glaucarubinone ligand. Glaucarubinone consistently maintains the interactions with falcipain by one hydrogen bonding and maximal of 12 residues of falcipain are placed in close proximity of 0.35 nm with the ligand glaucarubinone (Fig. 9). In the case of glaucarubinone interaction with plasmepsin, a maximum of 3 hydrogen bonds are formed between glaucarubinone and plasmepsin at several time frames and a maximum of 5 residues are consistently placed in close proximity within 0.35 nm attributing a stronger affinity towards the target protein (Fig. 10). From the results obtained, it is asserted that the glaucarubinone

interacts with falcipain and plasmepsin and the complex remains stable throughout 100 ns simulation, hence glaucarubinone, be considered as a lead molecule as a potent anti-plasmodial agent.

Anti- bacterial

Decaprenylphosphoryl-beta-D-ribose oxidase is a key enzyme involved in the biosynthesis of the precursor arabinogalactan, an essential cell wall component of mycobacteria. Inhibitor of decaprenylphosphoryl-beta-D-ribose oxidase is a drug target to control *M. tuberculosis*³⁴. Glaucarubinone interaction with decaprenylphosphoryl-beta-D-ribose oxidase of *M. tuberculosis* has been predicted, the binding energy being $-5.1 \text{ kcal mol}^{-1}$ and K_i value $9.34 \mu\text{m}$. The residues predicted to bind with the ligand glaucarubinone are Thr 8, Ala 38, Arg 41, Val 42, Ser 45, Arg 49, Gly 50 and Leu 70 and H-bonding

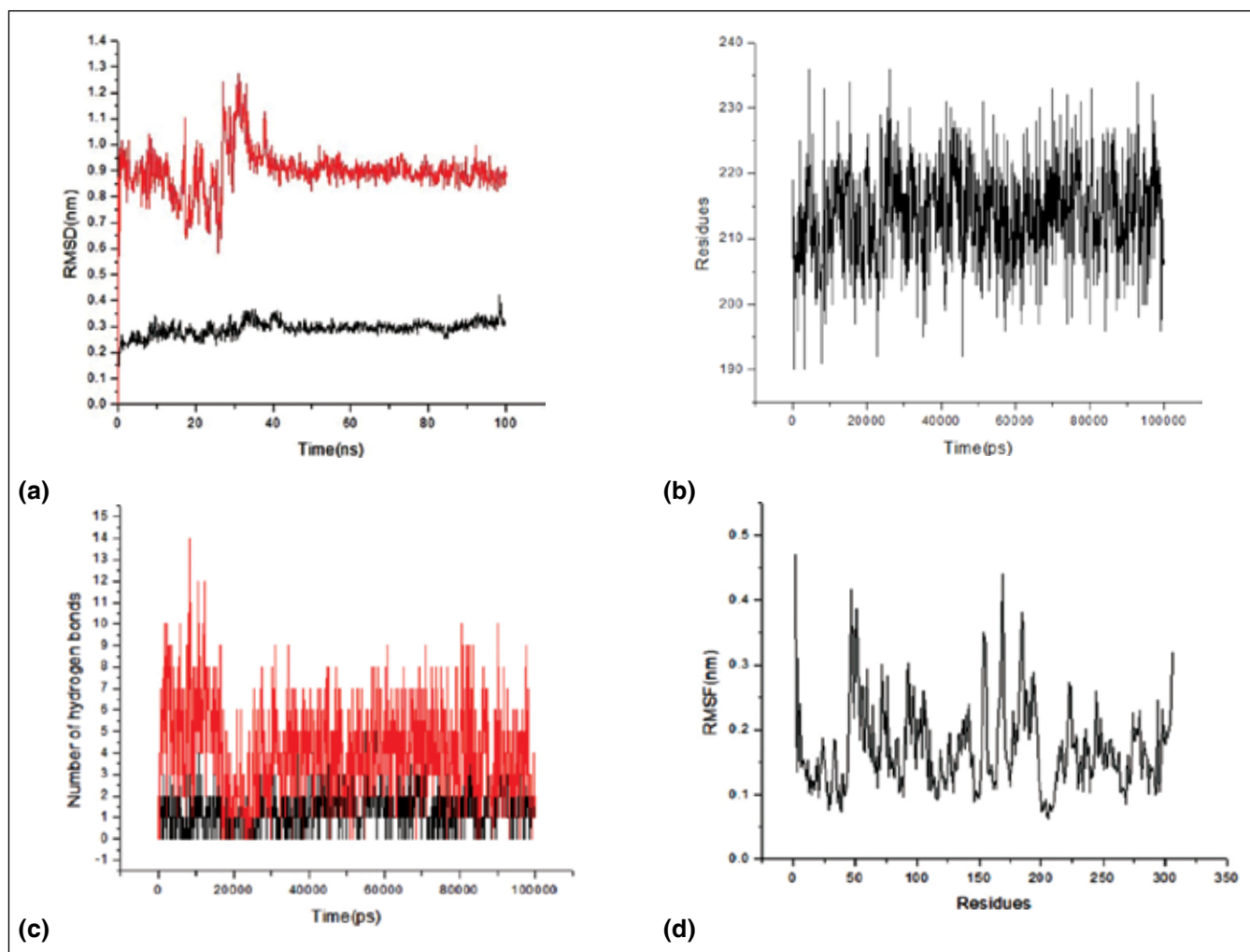


Fig. 13: Molecular dynamic simulation of glaucarubinone interaction with COVID 19 major protease a) RMSD curve of backbone (black) and complex (Red) b) hydrogen bonding with residues c) number of hydrogen bonds throughout the simulation(black), pairs within 0.35 nm(red) d) RMSF curve of the complex during MD simulation

with Asn 25 (Table III, Fig. 2). The RMSD value of decaprenylphosphoryl-beta-D-ribose oxidase confirms the structural stability of the protein throughout 100 ns simulation, the peak equilibrated at 0.31 nm; also the RMSD value of decaprenylphosphoryl-beta-D-ribose oxidase of *M. tuberculosis* with glaucarubinone showed stability throughout the 100ns of simulation, the peak equilibrated at around 1.32 nm. RMSF plot of decaprenylphosphoryl-beta-D-ribose oxidase -glaucarubinone complex reveals that the stable conformation is maintained with minimal fluctuations by the residues Leu 273, Pro 280, Gln 281 in the complex. Glaucarubinone shows a greater affinity with decaprenylphosphoryl-beta-D-ribose oxidase by forming by hydrogen bonds. Glaucarubinone consistently maintains its interaction with decaprenylphosphoryl-beta-D-ribose oxidase by 2 hydrogen bonds and maximum of 8 residues are placed in close proximity within 0.35 nm (Fig. 11).

Anti-cancer

Histone deacetylase inhibitors (HDACi), are a novel class of molecular therapeutics, and approved anticancer agents by the Food and Drug Administration³⁵. The docking results predict that glaucarubinone binds to histone deacetylase 3 efficiently with binding energy - 6.39 kcal mol⁻¹ and Ki value 20.62 μm. The residues that interact with ligand glaucarubinone are Asp 93, Tyr 198, Phe 199, Phe 200 and Leu 266 and H-bonding with His 172 (Table III, Fig. 3). The RMSD value of histone deacetylase showed stability throughout 100ns simulation, the peak equilibrated at 0.26 nm, also the RMSD of histone deacetylase-glaucarubinone complex showed a similar stability throughout the 100ns simulation, the peak equilibrated at 2.27 nm (Fig. 12). The RMSF plot of histone deacetylase -glaucarubinone complex reveals that the stable protein conformation is maintained with minimal

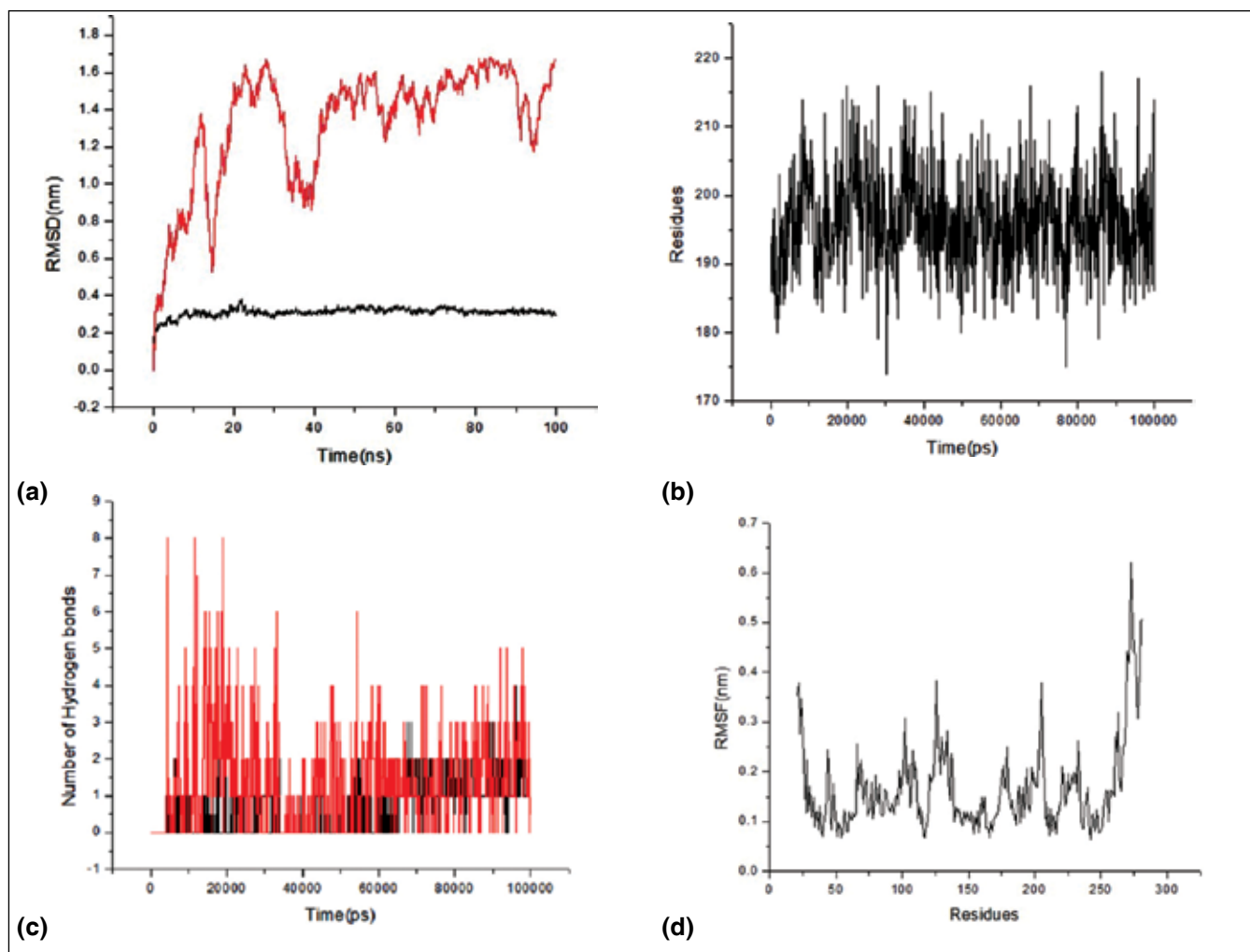


Fig. 14: Molecular dynamic simulation of glaucarubinone interaction with 11beta-hydroxysteroid dehydrogenase-1 a) RMSD curve of backbone (black) and complex (Red) b) hydrogen bonding with residues c) number of hydrogen bonds throughout the simulation(black), pairs within 0.35 nm(red) d) RMSF curve of the complex during MD simulation

fluctuations by the residues Ala 2, Asn 197, Tyr 198, Phe 199, Phe 200, Pro 201 and Arg 265 in the complex. Glaucarubinone shows affinity with histone deacetylase by forming hydrogen bonds. Glaucarubinone forms hydrogen bond only after 27ns of simulation and after 27 ns hydrogen bond formation, between histone deacetylase and glaucarubinone are consistently maintained and a maximal of 8 residues are placed in close proximity within 0.35 nm (Fig. 12).

Anti-viral

The main protease in Corona virus represents one of the antiviral drug targets³⁶. Glaucarubinone, binds adeptly to the main protease of corona virus, the binding energy and K_i value being $-5.74 \text{ kcal mol}^{-1}$ and $61.70 \mu\text{m}$, respectively. The residues that interact with

glaucarubinone are Tyr 101, Lys 102, and H-bonding with Lys 88, Tyr 37 and Phe 103 (Table III, Fig. 4). The RMSD value of main protease of Corona virus showed stability throughout 100 ns simulation, the peak equilibrated on an average of 0.26 nm, also the RMSD of the main protease -glaucarubinone complex showed a similar stability throughout the 100ns of simulation, the peak equilibrated at 0.89 (Fig. 13). The RMSF graph of the main protease -glaucarubinone complex reveals that the stable protein conformation is maintained with minimal fluctuations by the residues Met 1, Lys 47 and Val 169. Glaucarubinone shows affinity with the main protease by forming a hydrogen bond throughout the 100 ns simulation. Glaucarubinone consistently maintains the interaction with the main protease by forming a hydrogen bond throughout 100ns simulation and maximum of 12 residues are placed in close proximity within 0.35 nm (Fig. 13).

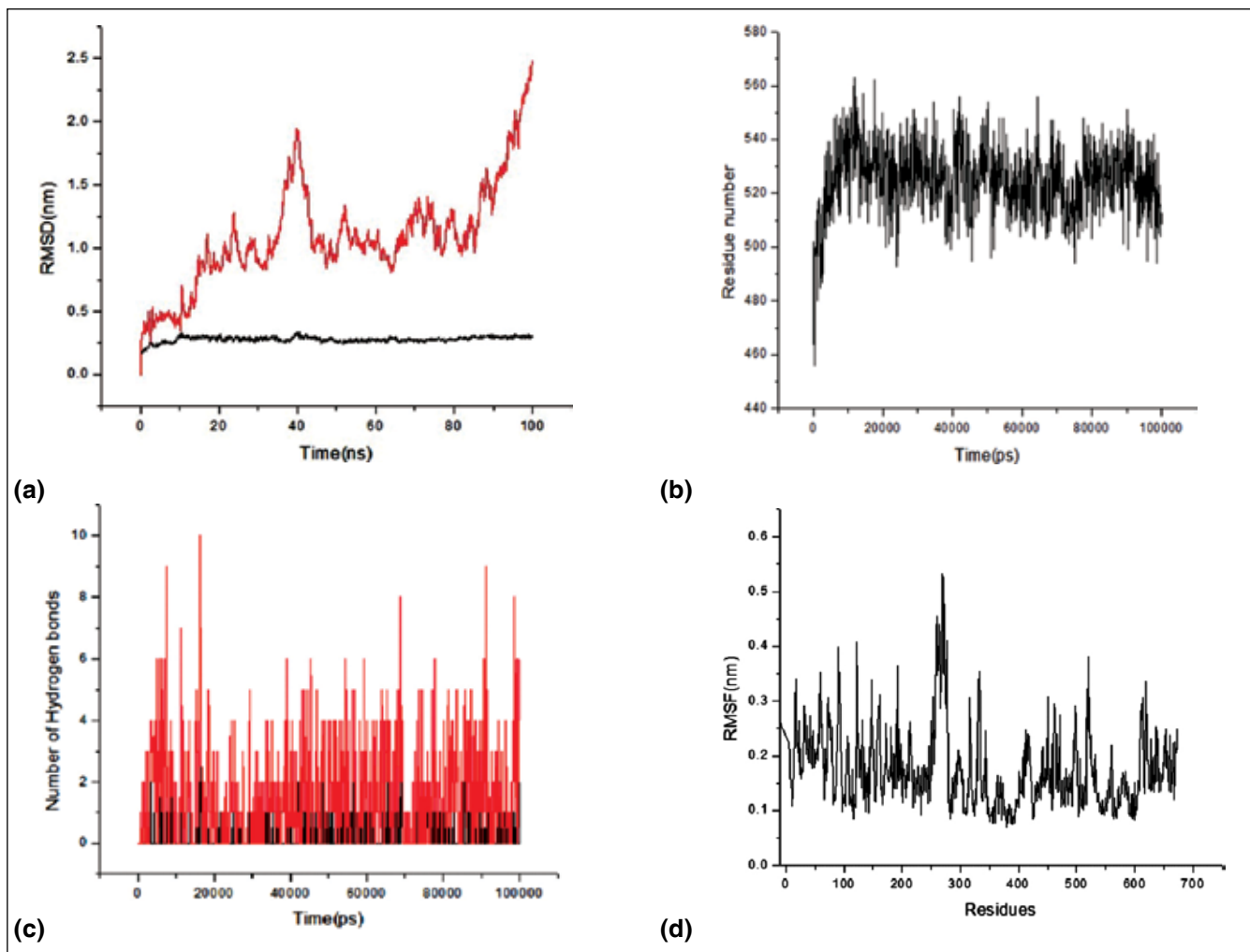


Fig. 15: Molecular dynamic simulation of glaucarubinone interaction with lipoxygenase a) RMSD curve of backbone (black) and complex (red) b) hydrogen bonding with residues c) number of hydrogen bonds throughout the simulation (red), pairs within 0.35 nm (black) d) RMSF curve of the complex during MD simulation

Anti-diabetic

In silico docking has predicted the consistent interaction of glaucarubinone to 11beta-hydroxysteroid dehydrogenase efficiently with a binding energy of $-7.25 \text{ kcal mol}^{-1}$ and K_i value $4.87 \mu\text{m}$. The residues that interact with glaucarubinone are His 87, Ile 89, Gln 105 and Lys 108 and H-bonding with Tyr 88, Leu 109 (Fig. 5). The RMSD value of 11beta hydroxysteroid dehydrogenase showed stability throughout 100 ns simulation, and the peak equilibrated at 0.31nm, also the RMSD of 11beta-hydroxysteroid dehydrogenase-glaucarubinone complex showed a similar stability throughout the 100ns of simulation, the peak equilibrated at 1.32 nm. The RMSF plot of 11beta-hydroxysteroid dehydrogenase-glaucarubinone complex reveals that the stable conformation is maintained with minimal fluctuations by

the residues Gln 253, Ser 260, Lys 261. Glaucarubinone shows affinity with 11beta-hydroxysteroid dehydrogenase by forming hydrogen bonds. Glaucarubinone maintains the hydrogen bonding intermittently throughout the 100ns simulation. Hydrogen bonds are consistently maintained and a maximal of 8 residues are placed in close proximity within 0.35 nm (Fig. 14).

Anti-inflammatory

Blocking of leukotriene production reduces the pro-inflammatory cell populations induced, recruited, and ameliorate the negative effects of inflammation²². Glaucarubinone ably interacts, with lipoxygenase with binding energy $-6.54 \text{ kcal mol}^{-1}$ and K_i value $15.99 \mu\text{m}$. The residues predicted for its interactions with glaucarubinone are Leu 289, Asn 328, Ala 366, Pro 574

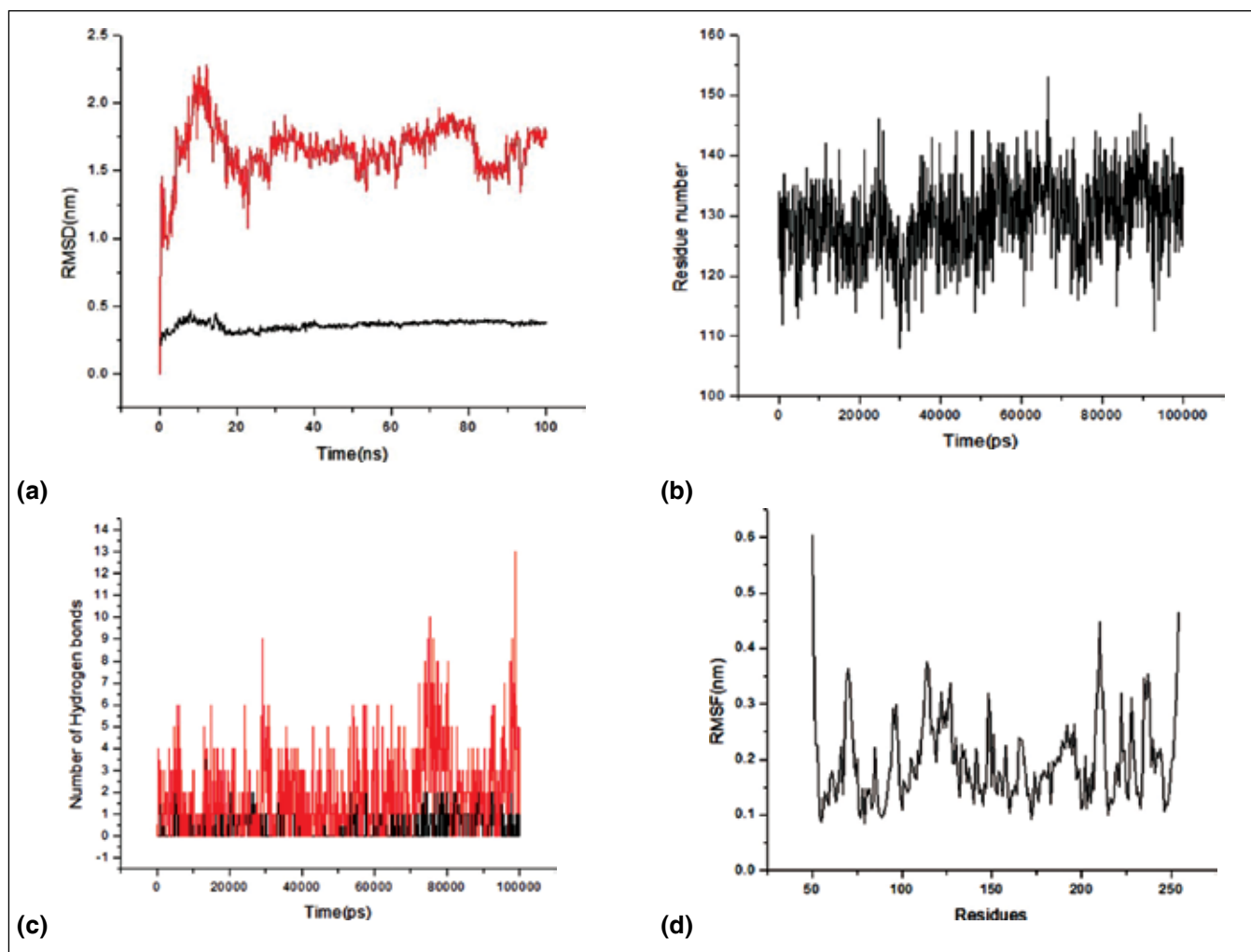


Fig. 16: Molecular dynamic simulation of glaucarubinone interaction with cathepsin a) RMSD curve of backbone (black) and complex (Red) b) Hydrogen bonding with residues c) number of hydrogen bonds throughout the simulation (red), pairs within 0.35 nm (black) d) RMSF curve of the complex during MD simulation

and Pro 575 and H-bonding with Leu 305. (Table III, Fig. 6a). The RMSD value of lipooxygenase showed stability throughout 100 ns simulation, the peak equilibrated on an average of 0.27 nm, also the RMSD value of lipooxygenase -glaucarubinone complex showed stability throughout the 100 ns of simulation, the peak equilibrated on an average of 1.09 nm (Fig. 15). The RMSF graph of lipooxygenase -glaucarubinone complex reveals that the stable protein conformation is maintained with minimal fluctuations by the residues Gln 268, Arg 269, Gln 270. Glaucarubinone shows affinity with lipooxygenase by forming by hydrogen bonds. Glaucarubinone consistently maintains the interaction with the lipooxygenase by hydrogen bonding throughout 100 ns simulation process and maximum of 10 residues are placed in close proximity within 0.35 nm (Fig. 15). 5- Lipooxygenase (5-LO) is a key enzyme in the synthesis of pro-inflammatory mediator leukotrienes

(LT3) in the host cells and prevents cancer metastasis and autoimmune diseases³⁷. Glaucarubinone is hence predicted to be an anti-inflammatory agent. Cathepsin is a key member in pathogenesis; cathepsin inhibitors can prevent the viral entry³⁸. Glaucarubinone interacts with cathepsin with predicted K_i value of 15.49 μm and binding energy -6.56 kcal mol^{-1} . The residues predicted, to interact with the ligand glaucarubinone are Leu 59, Leu 60, Cys 67, Gly 70, Glu 71, Gly 73, Tyr 75, Pro 76, Ala 79 and His 199 and H-bonding with Gly 74, Ala 200 (Fig. 6b). The RMSD value of cathepsin showed stability throughout 100 ns simulation, the peak equilibrated on an average of 0.36 nm, also the RMSD value of cathepsin -glaucarubinone complex showed a similar stability throughout 100 ns simulation, the peak equilibrated at 0.47 nm. The RMSF graph of cathepsin - glaucarubinone complex reveals that the stable protein conformation is

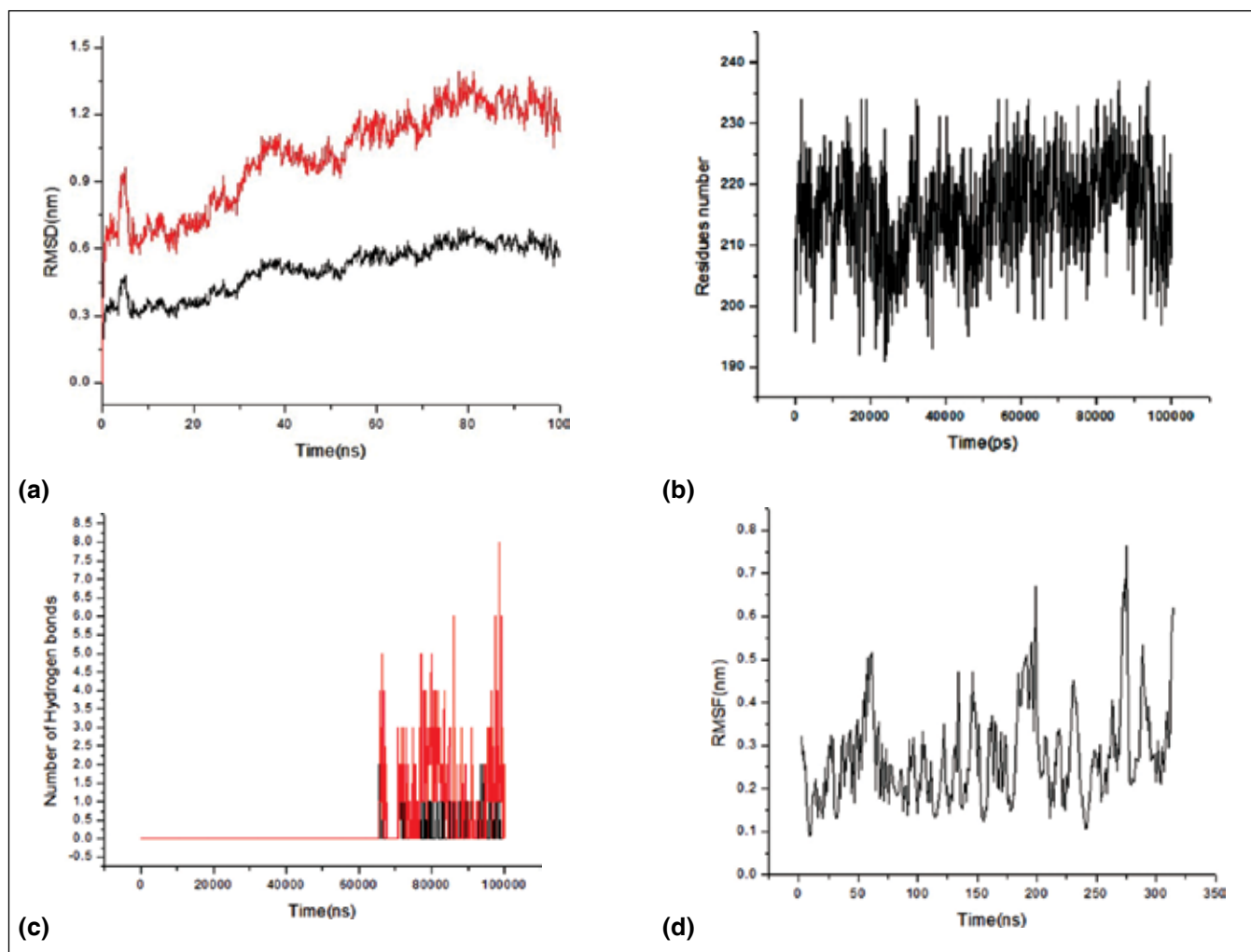


Fig. 17: Molecular dynamic simulation of glaucarubinone interaction with thioredoxin reductase of *E. histolytica*
a) RMSD curve of backbone (black) and complex (red) b) Hydrogen bonding with residues c) Number of hydrogen bonds throughout the simulation (black), pairs within 0.35 nm (red) d) RMSF curve of the complex during MD simulation

maintained with minimal fluctuations by the residues Val 50, Ile 250 and Ser 254. Glaucarubinone shows affinity with cathepsin through hydrogen bonds. Glaucarubinone consistently maintains the interaction with the cathepsin by forming a hydrogen bond during the entire simulation process and maximum of 13 residues are placed in close proximity with glaucarubinone within 0.35 nm distance (Fig. 16). Glaucarubinone may be predicted as a lead molecule to treat cancer, autoimmune diseases and viral infection as it acts as a potent anti-inflammatory agent.

Anti-amoebic

Thioredoxin reduction mechanism of action by the enzyme thioredoxin reductase in *Homo sapiens* varies from that of *E. histolytica*. This forms the basis for developing thioredoxin reductase inhibitors to treat *E. histolytica* infection³⁸. The interaction of glaucarubinone

with thioredoxin reductase from *E. histolytica* was predicted with binding energy $-5.33 \text{ kcal mol}^{-1}$ and K_i value $123.02 \mu\text{m}$. The residues that interact with glaucarubinone are Thr 18, Ile 21, Tyr 22, Phe 56, Pro 57, Met 72, Ala 297, Gly 298 and Cys 301 and H-bonding with Gln 75 (Fig. 7). The RMSD value of thioredoxin reductase showed stability throughout 100 ns simulation, the peak equilibrated at 0.50 nm, also the RMSD value of thioredoxin reductase-glaucarubinone complex also showed stability throughout the 100 ns simulation, the peak equilibrated on an average of 1.005 nm. The RMSF plot of thioredoxin reductase-glaucarubinone complex reveals that the stable protein conformation is maintained with minimal fluctuations by the residues Asn 199, Pro 272, Lys 273, Tyr 274, Ser 275 and His 314. Glaucarubinone shows affinity with thioredoxin reductase by forming hydrogen bonds. Glaucarubinone

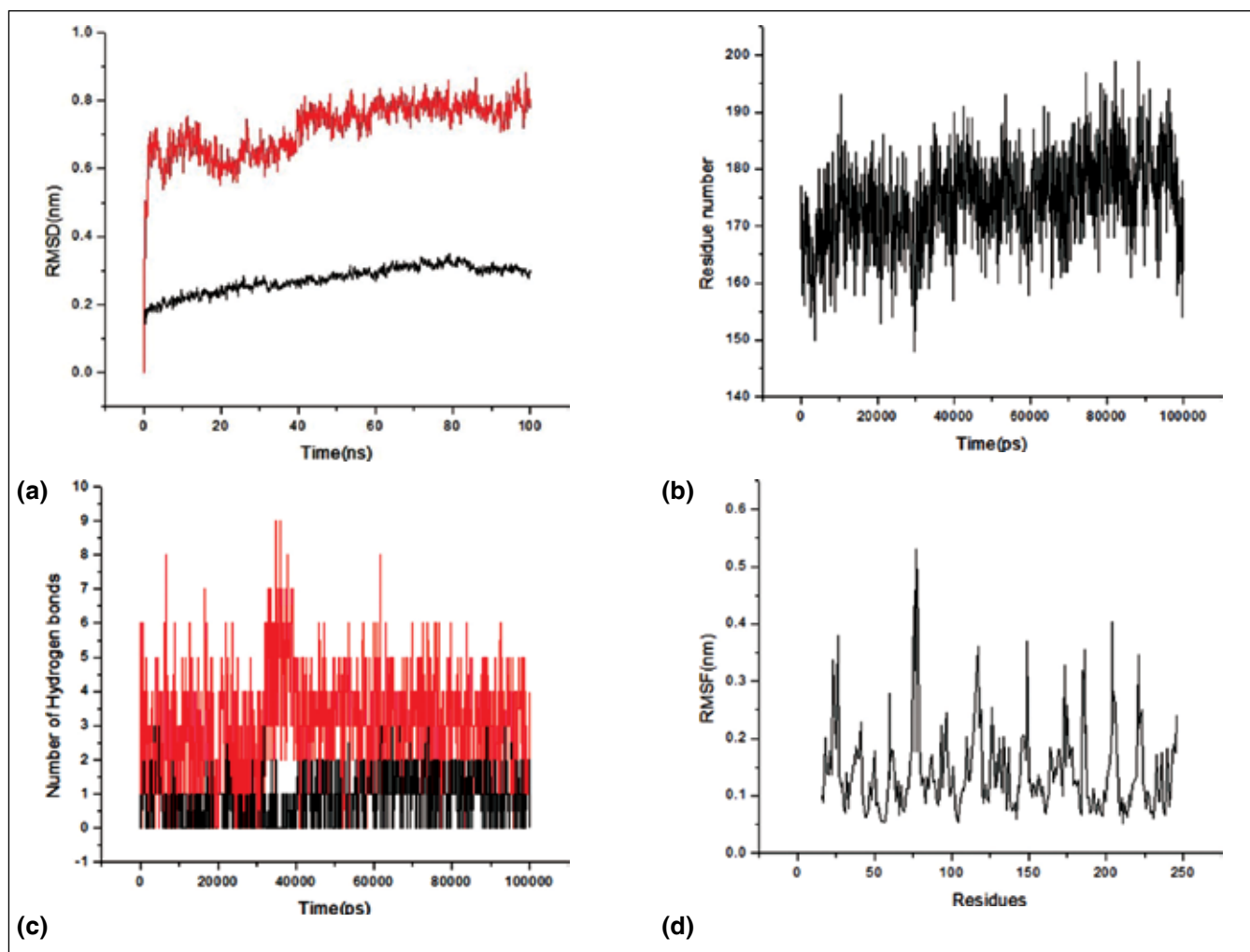


Fig. 18: Molecular dynamic simulation of glaucarubinone interaction with alpha thrombin a) RMSD curve of backbone (black) and complex (red) b) Hydrogen bonding with residues c) number of hydrogen bonds throughout the simulation (black), pairs within 0.35 nm (red) d) RMSF curve of the complex during MD simulation

shows affinity with thioredoxin reductase by forming by hydrogen bonds. Glaucarubinone forms hydrogen bond only after 70 ns of simulation and after 70 ns hydrogen bonding are maintained consistently and a maximal of 8 residues are placed in close proximity within 0.35 nm (Fig. 17).

Anti-coagulant

Alpha thrombin inhibitors are potential therapeutic agents for treating many thromboembolic disorders, such as pulmonary embolism, deep vein thrombosis, thromboembolic stroke and atrial fibrillation³⁹. Glaucarubinone has been predicted to bind to alpha thrombin efficiently with $-5.71 \text{ kcal mol}^{-1}$ binding energy and $64.93 \mu\text{m}$ as K_i value. The predicted interactions of amino acid residues with glaucarubinone are Thr

18, Ile 21, Tyr 22, Phe 56, Pro57, Met 72, Ala 297, Gly 298 and Cys 301 and H-bonding with Gln 75 (Fig. 8). The RMSD value of alpha thrombin backbone showed stability throughout the simulation, the peak equilibrated on an average of 0.18 nm, also the RMSD value of alpha thrombin -glaucarubinone complex showed a similar stability throughout the 100 ns simulation, the peak equilibrated at 0.71 nm (Fig. 18). The RMSF graph of alpha thrombin -glaucarubinone complex reveals that the stable protein conformation is maintained with minimal fluctuations by the residues Ser 76, Glu 77 and Glu 204. Glaucarubinone shows affinity with alpha thrombin by formation of hydrogen bonds. Glaucarubinone maintain the hydrogen bond throughout 100 ns simulation. Hydrogen bonds are consistently maintained and up to 9 residues are placed in close proximity within 0.35 nm (Fig.18).

CONCLUSION

S. glauca can be recognized for its bioactive compound richness in alkaloids, anthroquinones, coumarins, flavonoids, mono- and sesquiterpenes, quassinoids, steroids, and terpenes. This *in silico* study of glaucarubinone has predicted that this molecule has myriads of pharmacological properties. Further studies into this molecule can be inferred as new pharmacophores that would be useful for the betterment of human health and also to cure many diseases. The results of this present study may further be validated experimentally for further drug discovery process to combat various diseases with this miracle drug-like molecule glaucarubinone. From the above observations, it could be stated that the drug candidate molecule glaucarubinone provides a new research area for novel anti-plasmodial, anti-inflammatory, anti-viral, anti-cancerous, anti-coagulant, anti-diabetic and anti-mycobacterial agents.

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