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Identification of potential drug target in malarial disease using molecular docking analysis

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ABSTRACT

Malaria caused by genus *Plasmodium, is* a parasite which is the main health issue for humans and about half of the population were suffered. An every year, approximately 1.2–2.7 million people died due to malaria globally. Therefore to prevent the spreading of malaria from the glob novel active drugs with specific activities are necessary. The present study aimed to identify novel drug molecule together with the bioinformatic tools for the development of active malarial drugs. As the search for latest anti malarial compound was developed, this work determined six active blends from various drug databases which possess drug-like characteristics and presents a significant anti malarial actions in *in-silico* level. Compound ID 300238, 889, 76569, 87324, 45678, and Z185397112are a few of the ligands were got from the Toss lab, Maybridge, Cambridge, Life chem, Bitter, and Examine drug databases and docked against hexokinase 1 protein (PDB: 1CZA) with high throughput practical screening (HTVS) using Glide v6.6. Amid the 6 compounds, compound no: 300238 from Toss lab has the greatest docking score of −9.889 kcal/mol targeting 1CZA protein. The active sites of Hexokinase I of protein were determine by using superimposition of the destination and template structure showed similar structural folds and active sites which were decidedly conserved. The quality of hexokinase I protein was considered to be sterically stable where the protein was prepared by utilizing the software protein preparation execute in the Schrodinger suite. Prepared proteins were evaluated using SAVES and the studies of molecular dynamics of the hexokinase, and the GROMACS were performed for protein–ligand complex. The low HOMO-LUMO energy gaps of the compound verified the greater stability of the molecule. Here, the tested drug candidates have good absorption, distribution, metabolism, and excretion (ADME) properties which were established by using QikProp, version 3.4 of Schrodinger.

1. Introduction

In designing drugs molecular docking is progressively applied as a potent tool. In pharmacy industry, it act as a crucial role in computing the orientation of the ligand specifically when it is constrain to a enzyme or protein receptor [1]. Precise designing method of protein–ligand docking is an essential phase in drug design for finding new compounds of drug [2]. The high-throughput version of docking is commonly referred as fundamental screening, targets to gather possibly guide compounds for downstream experimental testing from a million databases of compounds [3]. In recent drug discovery the shape, van der Waals, electrostatic, and interactions of coulombic and the production of hydrogen bonds are some of the essential features in docking useful for the hypothesis of the lead compound [4]. All docking procedures have a virtuous arranging mechanism which performs all promising binding modes and add all the interactions is assessed with the docking score which represent the capability of binding [5]. Auto dock, FlexX, Surflex, Glide and Gold are presence of the

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widely used commercial and non-commercial docking software's [6]. Auto dock is the high predominant software used by educational research groups as it's an open-source program, whereas others are commercial software like Gold, Glide, Surflex, and Flex, used for their speed and adeptness [7].

Today in malarial research, *In-silico* molecular docking procedures can play a significant function in the finding of new drugs having activity of anti malarial [8]. An every year, approximately 1.2–2.7 million people died due to malaria globally [9]. *Plasmodium* is an important malaria parasite [10]. The *Plasmodium falciparum* infection is dependable for the maximum mortality yearly amongst *Plasmodium* species [11]. The parasite has with an intra erythrocytic stage and a difficult life cycle being principally liable for pathology. The lack of tricarboxylic acid cycle due to this *Plasmodium*, and the limited contribution of mictochondrion in the synthesis of cellular ATP have suggested by knockout and inhibitor studies of mitochondrial proteins [12]. Particularly, the consumption of glucose by the infected erythrocytes was establish to be enlarged up to 100-fold, and ~20 to 100 times lactate level is higher when compared to that from unaffected cells [13]. These explanations proposed that glycolysis is performing a main metabolic function for the parasite all the while of the erythrocytic infection [14,15].

In glycolysis the primary step is the assemble by the main enzyme hexokinase, the role of this enzyme is from ATP it transfer γ-phosphoryl group to glucose. The influence of the glycolysis process to the malaria causing *Plasmodium* species and the investigations that an enzyme, hexokinase from *P. falciparum* is predict to distribute partial characteristics with human glucokinasesre commended that this specific enzyme effectively provide as a perfect target for various therapeutics [16]. In this research, a known 6 compounds from the different database was docked with protein hexokinase to identify its potentiality using high throughput virtual screening with the help of Glide v6.6.

2. Materials and methods

2.1. Sequence alignment and structure prediction

Hexokinase 1 protein order (Accession No: O57880) used in this study was retrieved from the database, UniProtKB (http://www.uniprot.org). Then protein BLAST search was carried out to get an appropriate pattern for homology modeling against the non-redundant PDB database (PDB, http://www.rscb.org/ pdb/). Blastp search study showed 33% order similar with the selected template (PDB ID: 1CZA) which belongs to Hexokinase I protein family. The clustalw is used to disorder the destination with the template order (http://www.ch.embnet.org/). The clustalw is a server to find the conserved remainders of the proteins. In this study, a transcriptional regulator protein, leucine-responsive regulatory protein (Lrp) was used as the template and Swiss-Model modeling server was refers to construct the protein model. Furthermore, ESPript (Easy Sequencing in PostScript) is used to form the structure-based sequence alignment (http://espript.ibcp.fr/ESPript/ESPript/) to findin the Hexokinase 1 protein of the secondary structural elements. Between Hexokinase 1 proteins with a template of the active sites residue were find that conserved and is very critical to recognize the functional resemblance of protein.

2.2. Analysis of protein and validation

The Schrodinger suite software used for the preparation of Hexokinase 1 protein. Using this software the Protein Preparation Wizard implemented. The H-atoms and the protons are added to the protein which is key to describe the accurate ionization and tautomeric states of Asp, His, Ser, Arg and Glu amino acid residues to designed the structure, denigration was carried along with the Impact Refinement module, using the OPLS-2005 force field to diminsh steric clashes that may be present in the structure. The reduction was ended when RMSD (Root Mean Square Deviation) attain the highest cutoff of 0.30 Å. Prepared proteins were evaluated using Structural Analysis Verification Server (SAVES), accessed from (http://nihserver.mbi.ucla.edu/SAVES/) server and for the estimation of the importance of the model. The side chain configuraton, chirality, the planarity of the side chain, and steric conflict of atoms in the protein were analyzed using the pro check program. The level of φ and ϕ dihedral angles of residues was defined by Ramachandran plot and occupying in the complimentary and uncomplimentary regions. The Hexokinase 1 protein structure was evaluated using the verify-3D program using scoring function. ERRAT algorithm was used to evaluate the value of the non-bonding connections between different atoms types. Furthermore, the evaluated protein was placed to Grid formation using Receptor Grid Generation panel. Based on sequence-based structure arrangement the active site of protein was identified and using the Schrodinger software complement Sitemap module implemented.

2.3. Prediction of active sites

The active site region of Hexokinase 1 protein was identified using the target and template structure superimposition which shows similar structural folds and the extremely conserved active sites are found. Hexokinase protein comprises Arg109, Thr268, Lys440, Asp102, Thr106, Asp241, Ser436, Ser472, Asp469, Gly209, and Lys201.

2.4. Virtual screening

The statistically significant Virtual Screening was placed to discover the related features available in the 2D database. The ultimate focus of this search work is to recognize the compound libraries to find the compounds with better inhibition against the Hexokinase 1 protein. To identify potential compounds the Virtual screening was approved using the extensive search of compound libraries. Initially, Glide v6.6 used for the high throughput virtual screening (HTVS) and 10% high scored particular ligands were placed to the analysis of Glide SP dock. Finally, using Glide module all the ligands obtained was subjected to XP (extra precision) docking. Default parameters are used to run the entire Glide program. An expanded search was conducted for the generation of all probable conformations. For Conjugate Gradient (CG) the Minimization cycle was used and for early step size the steepest descent reduction of a default level of 0.05 Å and for maximum step size used the level of 1.0 Å. In the confluence criteria a defaulting level of 10–7 and 0.001 kcal/mol used to minimizing, both the gradient criteria and energy change respectively. Following this, for docking studies all arrangement were considered. For the best arrangement of ligand Glide score was used. Five ligands with maximum docking score were selected for Density Functional Theory (DFT).

2.5. Molecular dynamics simulation analysis of Hexokinase 1 protein complex

Hexokinase enzyme, the protein–ligand complex was subjected to molecular dynamics studies for 30 ns using GR Oningen Machine for Chemical Simulations (GROMACS) v4.5.6 molecular dynamics package which is used consistently for protein, nucleic acid and lipid dynamics analyses. In the preliminary stage, the structure of protein was processed using water model and SPC (Simple Point Charge) GROMOS96 53a6 power field. The PRODRG

server was used for the Ligand topology [17]. It catches an explanation of a tiny molecule as PDB coordinates and using with GROMACS from it usually generates many topologies. The component cell was referred in cube shape and was merged it with SPC water molecules. The box and the solute distance were set to 1.0. The solvated system was neutralized after the supplementation of 13 chloride ions (Cl^-) at 0.15 mol/L concentration. The assembled arrangement to be permitted to lighten up via energy reduction until the high force under 1000 kcal/mol/nm with using steepest descent energy declinisation algorithm. Further, protein–ligand compound was gently equilibrated with two steps. The initial step includes the 100 ps NVT collection (constant Temperature, Number of Particles and Volume) become stable the system at 310 K and the second step, the NPT (constant Temperature, Number of Particles and Volume) equilibrium with 100 ps, highly stabilized the system's strain using 1 bar coupling reference pressure. All lengths of the bond were embraced among the LINCS (Linear Constraint Solver) method. The PME (Particles Mesh Ewald) periodic boundary and electrostatic circumstances were useful in all direction. The cut off value for van der Waals interactions and Coulomb interactions were 10Å and 9 Å, respectively. Molecular dynamics simulation was conceded out for 30 ns with 2 fs of a time period. MD (Molecular Dynamics Simulation) analysis was done by2D plotting tool of Xmgrace.

2.6. DFT and ADME properties predictions

Density functional theory was used to the least and highly active molecule and also the top screened compounds achieve from effective screening studies. The molecular electronic appearances like frontier molecular orbital density fields, electron density, (i.e. LUMO and HOMO) calculated by the DFT and molecular electrostatic map to predict the biological property and molecular material of the analyzed compounds. The hybrid DFT at B3LYP (Berke's three-parameter exchange potential and the Lee-Yang-Parr correlation functional) used to achieve the exact molecular appearances, a whole geometric characteristic escalation was conceded out using with beginning set 3-21G* level clearing up elasticity to the electrons. To analyze the energy in gaseous phase the Poisson–Boltzmann solver was used. Most of the proposed choice of drugs frequently fails in most of the clinical trials due to reduced incorporation, excretion, metabolism and distribution characters. The major feature of drug discovery is the compounds not likeliness of drug and greater property of these characters. Here, these above properties of the selected compounds were achieved by using QikProp (version 3.4) of Schrodinger.

3. Results

3.1. Sequence alignment and structure prediction

In Fig. 1 illustrated the Hexokinase 1 protein possess of 496 residues of amino acid and the structure-based order arrangement with secondary structural elements. The residues of amino acid in the derived structural elements of Hexokinase Iproteins aresemiconserved and it resemble the similar structural fold in the templates (PDB: 1CZA). The amino acid residues like as Asp16, Asp13, Arg48, Leu23, Gly56, Glu33, Gly108, Pro67 and Leu52 are generated to be conserved in the active site pockets in Hexokinase 1 proteins. The overall resulting structural essentials of Hexokinase 1 protein consists α1 (46–61), α2 (81–86), α3 (111–123), α4 (116–124), α6 (241–251), α7 (366–371), α6 (241–251), and α8 (471–486); (β1 (176–181), β2 (206–211), β3 (211–216), β4 (216–221), and β5 (286–288) and the loops regions. The modeled protein was assessed by using SAVES. Ramachandran plot (Fig. 2) assay showed 91.7% residue in the selected protein was in the most favored region, and in addition, 7.6% residues were in allowed regions. The overall value of Hexokinase I protein was well establish to be sterically highly stable.

3.2. Stability analysis of modeled hexokinase 1 protein

The stability of Hexokinase I protein was approved for 30 ns timescale. Root Mean Square Fluctuations (RMSD) is a valid process parameter to establish the stability and equilibration of targeted protein throughout the process of MD simulation. The backbone of RMSD profiles of Hexokinase protein 1 is described in Fig. 3. It depicts the average RMSD values of 0.2 to 0.4 nm and attained the highly stable value (0.4 nm) throughout the process of simulation. The complete atomic variations of the residues were analyzed using average confirmation of the protein over 30 ns MD simulation. The maximum RMSF values designate higher variation whereas the decreased values reveal lower variation during MD stimulation. The N-terminal region (Met1-Glu14) was established to fluctuate more with an average RMSF value (0.2–0.5 nm) and the three more remaining regions (Val106-Asp111, Ala82-Val87 and Ala63-Tyr72) of C-terminal region displays minimum variation with an average RMSF value of 0.2–0.3 nm. Importantly, the minimum fluctuated region Val106-Asp111 and Ala63-Tyr72 belong to the central domain of the proteins. Furthermore, the minimum potential energy trajectory of the protein was selected and retrieved for analysis which showed that models had good quality factor for advance studies.

3.3. Virtual screening

The Molecular docking studies revealed that Arg109, Asp102, and Ser436 residues were interacted frequently with the perfectly –docked ligand molecules. Among the docked ligands, Tosslab-300238, Bitter-889, Lifechem-76569, Maybridge-87324, Cambridge-45678 showed high docking score of −9.889, −8.894, −8.658,-7.326,-7.448,-6.286, respectively. Molecular docking score against Hexokinase protein were shown in Table 1.

3.4. DFT calculation and ADME properties predictions

The DFT analysis investigated the electronic characteristics of the atoms used in this study. These analyses showed the valid information about the local and global indices on the compound and their biological interest. The minimum Highest Occupied Molecular Orbital - Lowest Unoccupied Molecular Orbital (HOMO-LUMO) energy gaps obtained in the compounds and it was illustrated in Table.2. The minimum HOMO-LUMO band gap or energy gap provides greater permanence of the molecule. The ADME properties of the five important compounds were described in Table 3. Depend on the Lipinski's Rule of Five the ADME analysis was carried out. The screened compounds were predicted in the desirable range which encourages the drug capacity of the compound (Table 3).

3.5. MDS studies on potential lead molecules with Hexokinase protein

The first 6 docked molecular complexes of Hexokinase protein were located to MDS analyses for investigating the constancy in terms of RMSD and the possible communications for the suppression of the molecule was recognized at 30 ns. The five complexes in the RMSD plots were compared and described in Fig. 4. However, it is essential to identify the interaction of docked complex at the same time period (30 ns) to analyze to study the mechanism of inhibition. In the case of Hexokinase-Tosslab complex, the power of RMS deviation principles were detected an average range

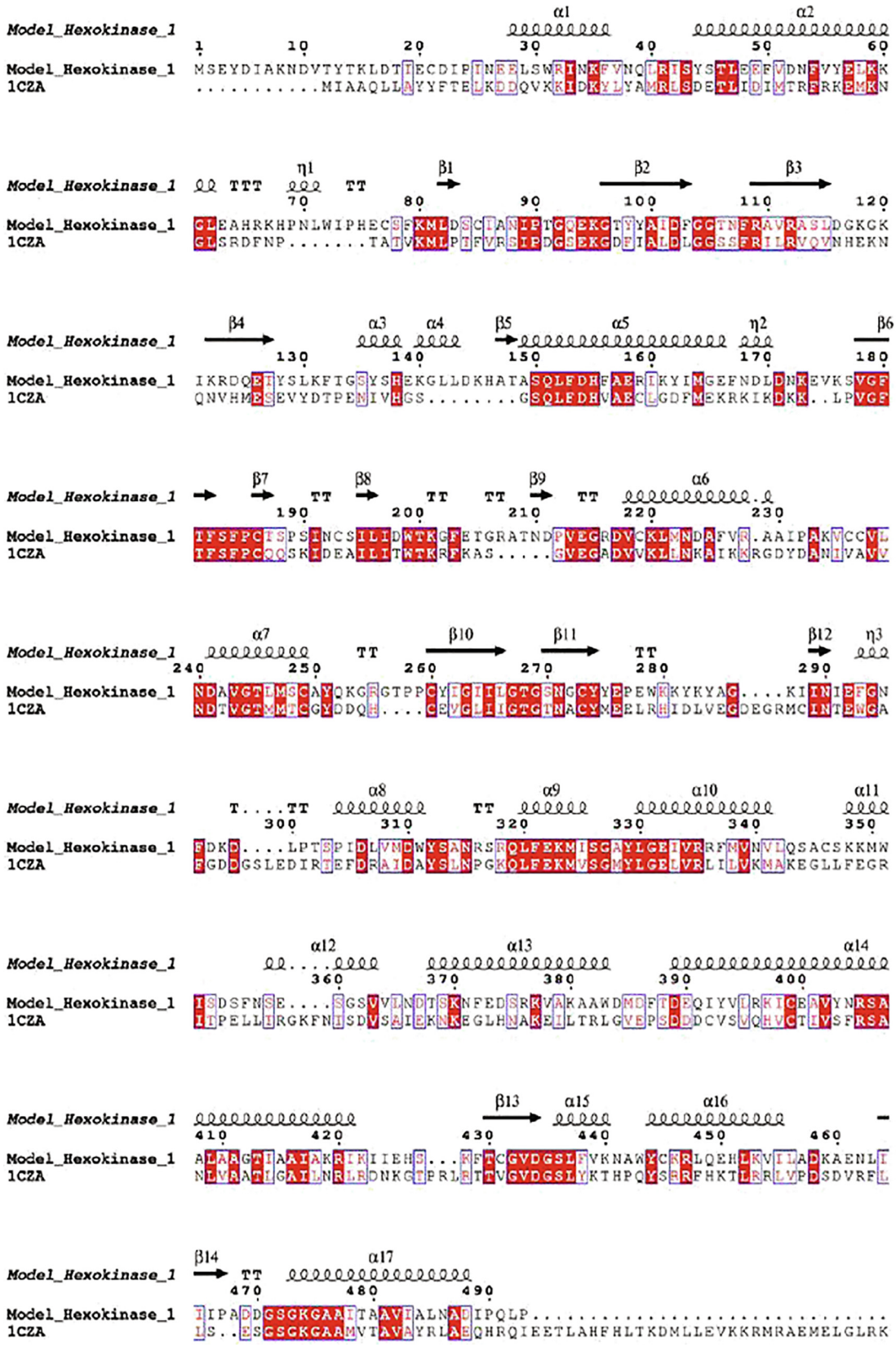

Fig. 1. Structure based sequence alignment of modeled Hexokinase 1 and Template PDB: 1CZA.

between 0.1 and 0.25 nm. It was also found that at initial 2500 ps time, the Hexokinase-Tosslab complex was highly stable at 0.1–0.15 range. Then deviation increased as 0.3 nm and attained stable conformation at 30 ns. To recognize the suppression mode of interactions, each trajectory was observed. The potential H-bond forming residues Arg 109, Asp 102, Lys 440, Gly 209, Ser 183, and Thr

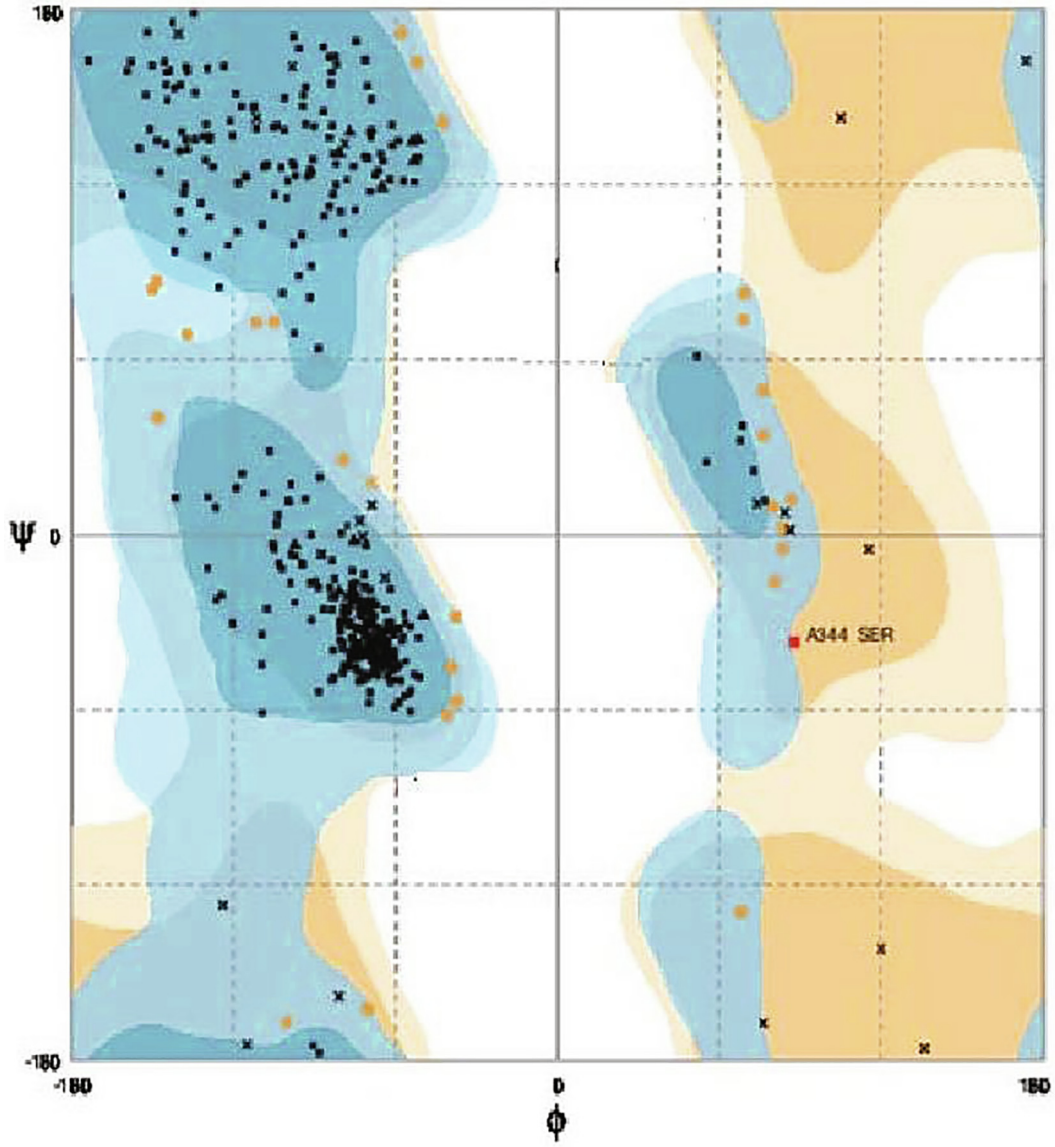

Fig. 2. Ramachandran Plot.

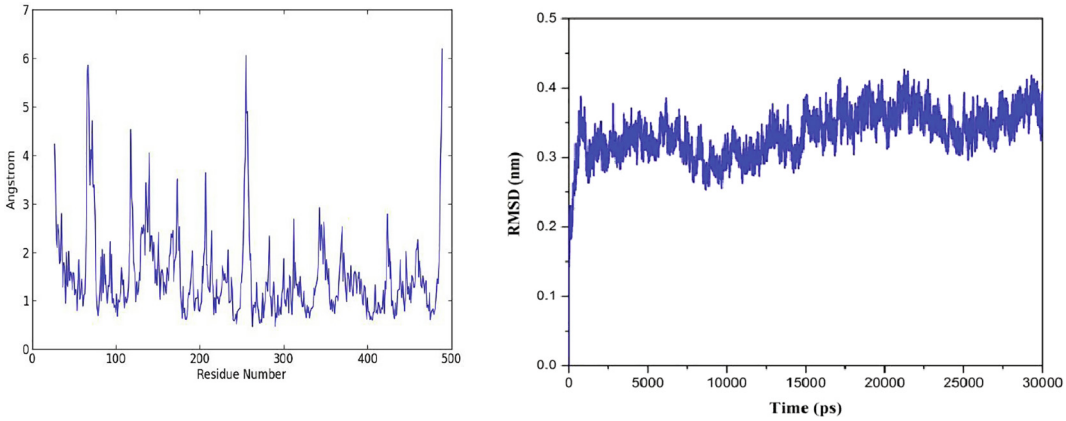

Fig. 3. RMSD and RMSF plot: Hexokinase 1 protein during 30 ns time periods.

268 were identified throughout 30 ns. In Hexokinase-Maybridge complex, the power of RMSD values establish from 0.1 to 0.25 nm and the RMSD values throughout 30 ns. The potential H-bond creating residues Arg 109, Asp 102, Lys 440, and Gly 209, were determined during 30 ns. Final 30 ns trajectory explores that only Arg 109 and Asp was interacting with ligand and also ligand

Table 1
Molecular docking score against Hexokinase protein.

S. No	Data base	Compound ID	Docking score
1	Toss lab	300238	−9.889
2	Bitter	889	−8.894
3	Lifechem	76569	−8.658
4	Maybridge	87324	−7.326
5	Cambridge	45678	−7.448
6	Enamaine	Z185397112	−6.286

not detect in the active site pocket region. In the complex of Hexokinase-Tosslab, the power of RMSD values obtained in the mean values varied initially (23 ns) and these deviation was stable in 30 ns. The potential H-bond interactions Ser 436, Asp 434, Asp 102, and Thr 106 were found in the 30 ns time periods. In Hexokinase-Cambridge complex, the backbone RMS deviation values were ranged from 0.1 to 0.25 nm and he potential H-bond interactions Ser49Arg 109, Lys 440, Gly 209, residues were determined within 30 ns time periods. To recognize the inhibitory mode of interactions, each trajectory was observed. The potential H-bond forming residues Arg 109, Asp 102, Lys 440, Gly 209, Ser 183, and Thr 268 were determined within 30 ns. These residues are catalytically significant residues and the ligand molecule inhibiting throughout 30 ns time periods.

4. Discussion

Here, we evaluated antiparasitic property of six lead molecules against *P. falciparum* erythrocyte parasitic stage and many compounds was effective against these parasites. The catalysis of Glycolysis by hexokinase enzyme has been referred an exciting potential focus as it has not been completely elucidated because

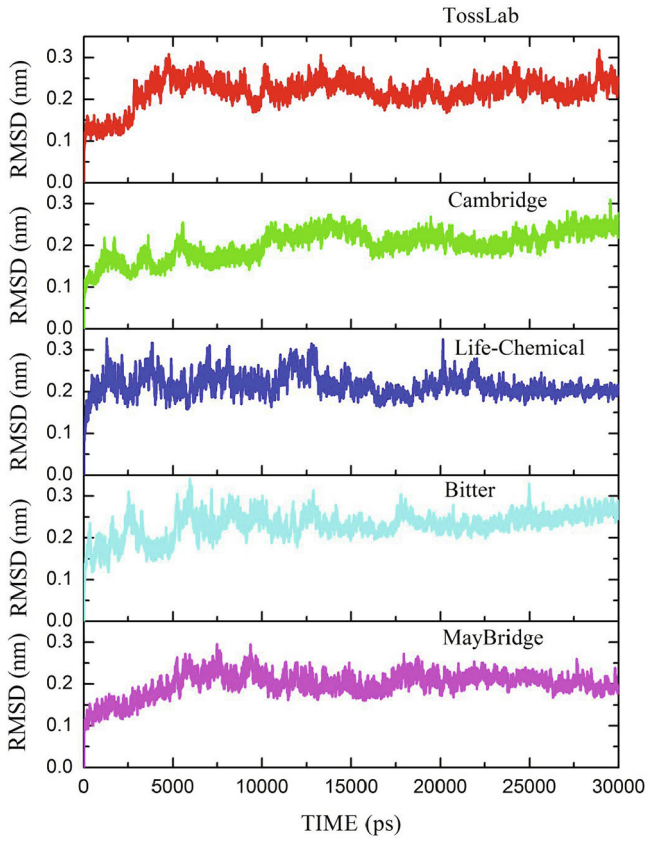

Fig. 4. RMSD analysis of five compounds from screened databases (Life chemical, Zinc, Maybridge, Enamine and Specs) complexed with Hexokinase protein at 30 ns.

Table 2
DFT analysis result for the top five identified compounds.

Compounds	HOMO (eV)	LUMO (eV)	EHOMO-ELUMO (eV)	Solv. Energy (kcal/mol)
Toss lab	−0.213341	−0.155500	−0.057841	−0.18
Bitter	0.212572	−0.063431	−0.149141	−0.05
Lifechem	−0.206448	−0.096074	−0.110374	−0.12
Maybridge	−0.21334	−0.09607	−011727	−0.32
Cambridge	−0.200609	−0.160625	−0.361234	−157.27

Table 3
ADME properties of compounds using QikProp module in the acceptable range.

S. No	Data Base/Compound ID	Molecular weight	QP log BB for brain/blood	QPlog$_s$	HERG K+	ROF (rules of five)
1.	Chembridge 7074613	358.610	−2.565	−1.768	−3.456	1
2.	LifeChem 76569	328.410	−0.643	−1.375	−5.885	1
3.	Bitter 889	318.239	−2.737	−2.535	−4.863	1
4.	Toss lab 300238	246.228	−2.014	−1.551	−2.364	1
5.	May bridge 87324	411.460	−1.165	0.095	−5.469	1
6.	Enamine Z18539711	386.256	−1.569	0.069	−3.698	1

not able to synthesize active recombinant protein in in vitro categorization [18]. The Hexokinases play important role in glycolytic enzymes which are liable for the stimulation of glucose and other hexoses [19]. Glycolysis is indispensable for *Plasmodium* parasite as it is deficiencies of the Krebs cycle to produce carbon intermediate substances and energy. For Leishmania and Trypanosoma, it is also critical though it possess Krebs cycle by aerobic fermentation. The existence of O_2 and TCA cycle in cells, they still quickly altered glucose molecule in enormous quantities to dicarboxylic and monocarboxylic acids [20,21] as phosphorylation of glucose is catalyzed by the action of hexokinase. The isozymes of Hexokinase I, II and III have high attraction for glucose and product suppression for glucose-6-phosphate. Hexokinase IV has least attraction for glucose molecule and have not any product inhibition role at physiological ranges of glucose-6-phosphate [22,23]. PI3K/AKT/mTOR-path is amajorintracellular signaling pathway is a key quality in regulating the cell cycle. It is responsively connected to cellular cancer, their proliferation, and survival. Mitochondrial hexokinase II as a main molecular governor of this improved glycolysis and the expression enlarged frequently added 100-fold of hexokinase II. Activation of the PI3K/Akt pathway, considered as regularly mutated pathways in cancer, and stimulation of PKA and PKC pathways, also generally occur in various cancers, which can demonstrate this elevated appearance of HKII [24,25,26]. The mechanism of malaria by the parasite Plasmodium falciparum quickly cause infection and lead to the clinical symptoms contributed within the blood of the crowd, with the 48-h by the process of genderless explicative cycle of the parasite eventually causing high complications and anemia due to occlusion of blood vessels [27]. Antimalarial activity of any lead molecule is achieved by inhibiting this pathway. The small molecules from various sources hinder with hexose transport, and also analogs of glucose like 2-deoxyglucose [28,29].

5. Conclusion

In conclusion, several observations sustenance that the six identified compounds from various databases such as Toss lab, Maybridge, Cambridge, Lifechem, Bitter, and Enamine were proved to be an effective therapeutic agent beside P. falciparum hexokinase. These findings with the purpose of the chemotypes recognized here might correspond to leads the future improvement of therapeutics against P. falciparum.

Declaration of Competing Interest

None declared.

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