KERICE 2019

Poster ID:BPMERU005

Potential Inhibitors of Human Hexokinase II for the Development of Anti-dengue Therapeutics Identified *via In Silico* Approach

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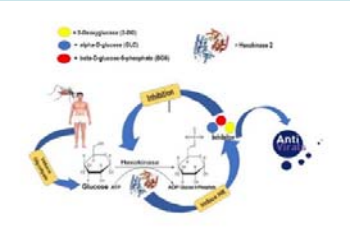
Abstract

Dengue infections are one of the most fatal diseases in the world, which is caused by dengue virus (DENV). Unfortunately until now, specific treatment for the disease has not been established. It has been reported that a glycolytic enzyme, the human hexokinase II (HKII) has a great impact in supporting viral replication in the host cell, thus the enzyme has been proposed as a drug target. The main aim of this research is to identify novel anti-dengue agents for the treatment of dengue infection through in silico screening and HKII enzymatic inhibition studies. In silico screening comprises of ligand-based and structure-based screening approaches. The former analysis was performed by using Ultrafast Shape Recognition with CREDO Atom Types (USRCAT) programme by utilizing both HKII substrates and product; alpha-D-glucose (GLC), and beta-D-glucose-6-phosphate (BG6), as well as a known inhibitor of HKII, 2- deoxyglucose (2-DG) as the query molecules. The similarity scores of the analogues of 2DG, GLC and BG6 molecules ranged from 0.75−0.80, 0.91−0.94, and 0.76−0.81 for the three query molecules, respectively. The analogues were subsequently docked against the HKII crystal structure (PDB ID: 2NZT) by using Auto Dock Vina programme on Chain A and B, where the active sites and strong bonds were located. The docking hits, which are molecules similar to GLC, BG6 and 2-DG possessed binding energy ranging from -6.1 to -6.4 kcal/mol, -6.2 to -6.8 kcal/mol and -6.9 to 7.0kcal/mol, respectively with strong H bond around the catalytic residues (Thr620, Glu629, Lys 621, Asn656, Asp657, Ser893, Asn683, Phe623 Gln739 and Glu742). Top docked-poses compounds were then used for molecular dynamics (MD) simulation. On the other hand, in-vitro studies have been conducted with the recombinant HKII, which was cloned into pET28 and pET32 vectors, followed by successful expression of HKII enzyme in Escherichia coli strain BL21 (DE3), Origami 2 (DE3) and Rosetta-gami 2 (DE3) at 18°C incubation temperature for 19 hours, with 0.5mM IPTG induction. The expressed protein was subsequently purified to homogeneity by a combination of Immobilized Metal Ion Affinity Chromatography (IMAC), size exclusion chromatography and ion-exchange chromatography. In conclusion, selected compounds from virtual screening have great potential to show inhibition effect on human HKII, which can further be developed as future anti-dengue therapeutics.

Problem Statement

Globally, approximately 390 million people are infected with dengue virus (DENV) every year, where in Malaysia 62,421 dengue cases have been reported, including 93 death cases until June 2019 (WHO,2019). Currently, no commercially-available and well-developed vaccines or specific anti-dengue drugs have been discovered, due to lack of effective drugs which may target all DENV serotypes. The process of developing new drugs also normally involve lengthy, expensive and intense effort, which hampered the initiatives.



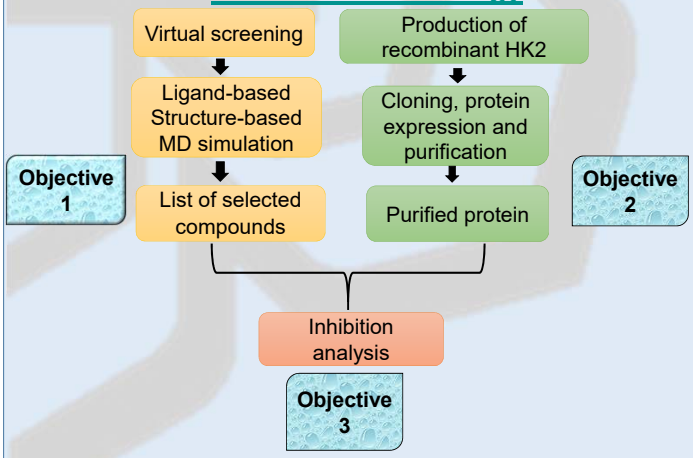
Project Objectives

The overall aim of this project is to identify novel anti-dengue agents for the treatment of dengue disease.

Specific Objectives of this Study:

1. To identify potential inhibitors of Human hexokinase II (HKII) using ligand structure-based virtual screening and molecular dynamics simulation approaches and evaluate toxic effect of selected compound.
2. To develop expression systems for recombinant HKII in bacteria and to purify HK2 enzyme using immobilized metal affinity Chromatography and gel filtration chromatography.
3. To measure the activity of purified HKII and evaluate the potency of the selected virtually-screened compounds using hexokinase enzyme inhibition assay.

Research Methodology



Findings.

1. Virtual screening (Result)

Table.1.Binding energy and similarity score of 2dg analogues

Compound	Similarity score	Binding Energy kcal/mol
2dg		-5.9
1	0.80	-6.3
4	0.79	-6.1
31	0.75	-6.4

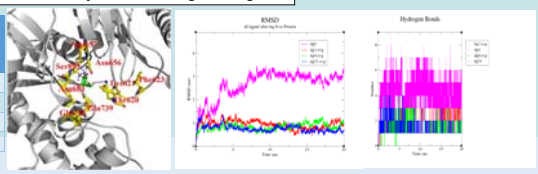


Fig1.2.3. 2dg in the dock side of protein, RMSD graph and Hbond number of 2dg and 2dg's analogues from MD simulation

Table 2. binding energy and similarity score of glc analogues

Compound	Similarity score	Binding Energy kcal/mol
glc		-6.9
10	0.94	-6.8
26	0.93	-6.2
58	0.91	-6.8

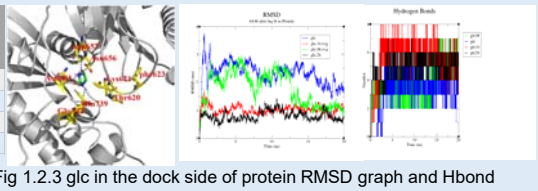


Fig 1.2.3 glc in the dock side of protein RMSD graph and Hbond number of glc and glc's analogues from MD simulation

Table 3. binding energy and similarity score of BG6 analogues

Compound	Similarity score	Binding Energy kcal/mol
BG6		-6.9
30	0.81	-7.6
36	0.81	-7.1
38	0.81	-7.0

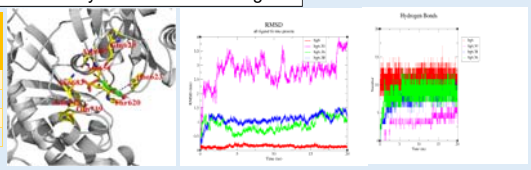


Fig 1.2.3 BG6 in the dock side of protein. RMSD graph and Hbond number of BG6 and BG6's analogues from MD simulation

2. Production of recombinant HK2 and HK2 protein expression

Cloning

HK2 gene purchased from GenScript

PCR amplification

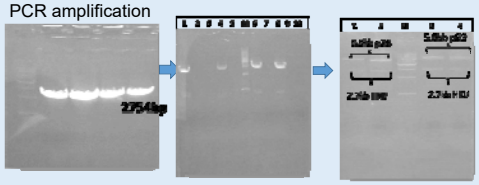


Fig.1.2.3. HK2 band purified & ligated with pETite 28b & pETite32b then Verification of transformants by PCR colony and Restriction enzyme digestion

Expression

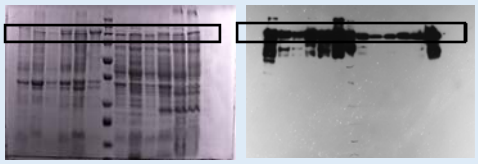


Fig1. 2. Recombinant HK2 gene was successfully expressed at 18°C temperature, with 19hr incubation time and 0.5mM IPTG concentration further it has been verified by western blotting

Conclusion

Total six compound has been successfully identified from virtual screening result based on binding energy and RMSD value and hydrogen bond number. On the contrary HK2 has been successfully expressed thus further purified HK2 protein will be tested with selected compound by inhibition assay