KERICE 2019

In Silico Screening of Inhibitors Against Bacterially Expressed *Plasmodium knowlesi* Lactate Dehydrogenase Enzyme

Poster ID:BPMERU011

NURHAINIS BINTI OGU SALIM & FAZIA ADYANI AHMAD FUAD

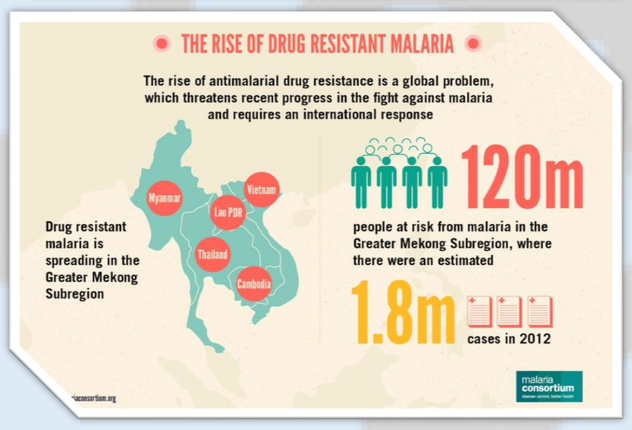
KULLIYYAH OF ENGINEERING 1994-2019

KOE LEADING THE WAY

Abstract

Malaria remains a challenge in Malaysia, due to *Plasmodium knowlesi* infection and also parasites' resistance to the current antimalarial drugs. Hence, this study is an efforts to develop novel and affordable antimalarials to overcome the issues by targeting the *Pk*-LDH enzyme for antimalarial drug development. This study, aimed to evaluate the effects of compounds obtained from virtual screening on purified recombinant lactate dehydrogenase from *P. knowlesi* (*Pk*-LDH). The *Pk*-LDH *was* expressed in bacterial system and purification was performed by using Immobilized Metal Ion Affinity Chromatography and Size Exclusion Chromatography. Potential compounds were selected using a combination of ligand-based drug design and structure-based drug design. Enzyme inhibition studies were conducted by using LDH enzymatic assay to observe the effects of virtually screened compounds on the activity of recombinant *Pk*-LDH enzyme. Subsequent SDS–PAGE analysis revealed that a fusion protein of 34 kDa size was present in the soluble fraction. The specific activity of recombinant *Pk*-LDH was found to be 475.6 U/mg, confirming the presence of active protein. All the compounds selected via virtual screening were tested on the purified *Pk*-LDH enzymatic assay and the compound namely oxalic acid showed the most active compounds with 54.12 % inhibition as compared to others and interestingly that this compound also showed significant result by docking studies with minimum binding energy of -2.59 kcal/mol.

Problem Statement



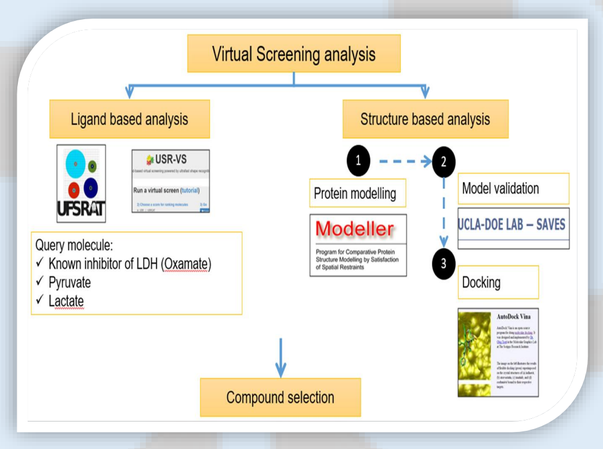
Hence, huge efforts are needed to develop novel and affordable antimalarials to overcome the effects of drug resistance

Project Objectives

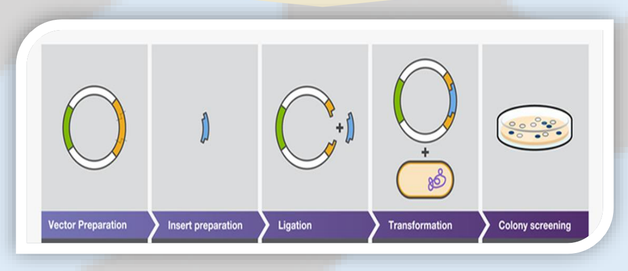
1. To identify potential inhibitors of *Pk*-LDH via virtual screening approaches
2. To develop enhanced expression for recombinant *Pk*-LDH in bacterial system and purify using chromatography method
3. To evaluate virtually-screened compounds using an LDH enzyme activity assay

Research Methodology

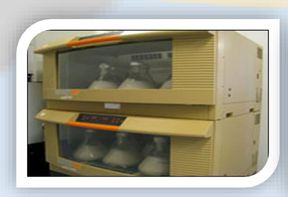
1 Compound selection



2 Production of recombinant *Pk*-LDH



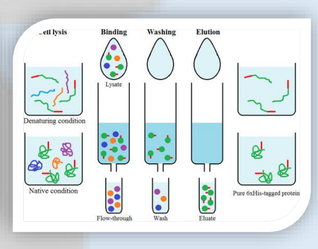
3 *Pk*-LDH expression



Incubation
Parameters:
- ✓ Competent cell
- ✓ Media
- ✓ Time
- ✓ Temperature
- ✓ IPTG concentration

4 Purification of Protein

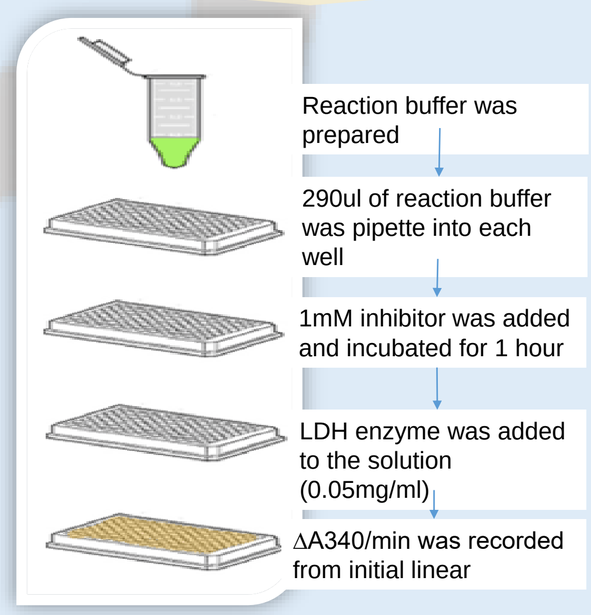
Immobilized metal affinity chromatography (IMAC)



Size exclusion chromatography (SEC)

5 Enzyme assay, enzyme kinetics

6 Inhibition analysis



Findings

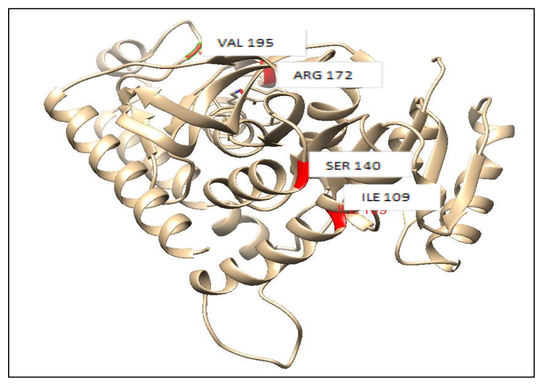


Fig 1: The model of *Pk*-LDH that was built based on the crystal structure of *Pf*-LDH (Quality factor of **92.2%**). The active sites are labelled in red

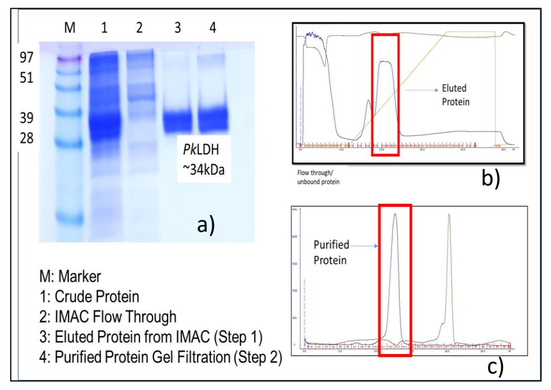
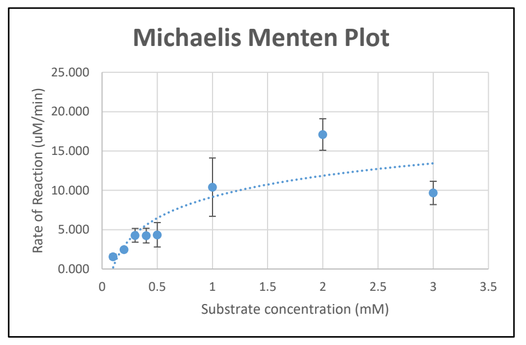


Fig 2: a) SDS–PAGE analysis revealed that a fusion protein of 34 kDa was present in the soluble fraction. b) Separation of *Pk*-LDH by IMAC. c) Pure *Pk*-LDH peak by SEC



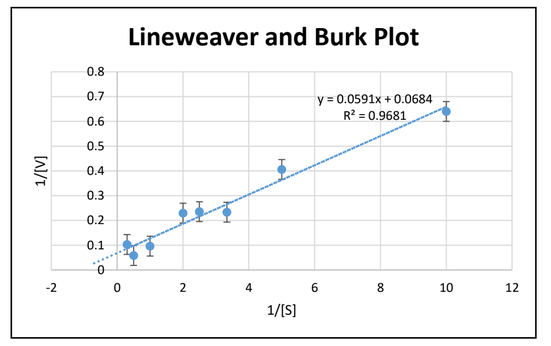
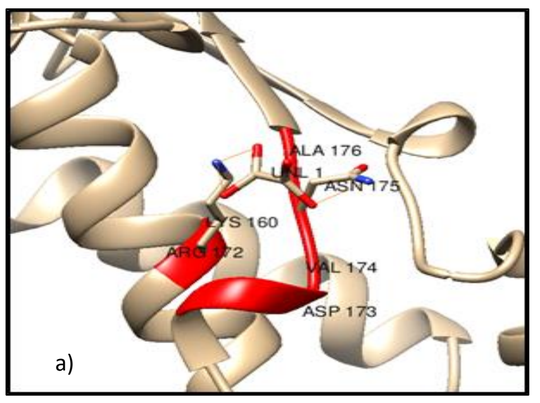


Fig 3: Enzyme kinetics of recombinant *Pk*-LDH was determined based on the a) Michaelis Menten Plot and b) Lineweaver Burk Plot. The Vmax value is 16.92 uM/min and the Km value is 1.02 mM. The specific activity of this enzyme was found to be 475.6 U/mg, confirming the presence of active protein.



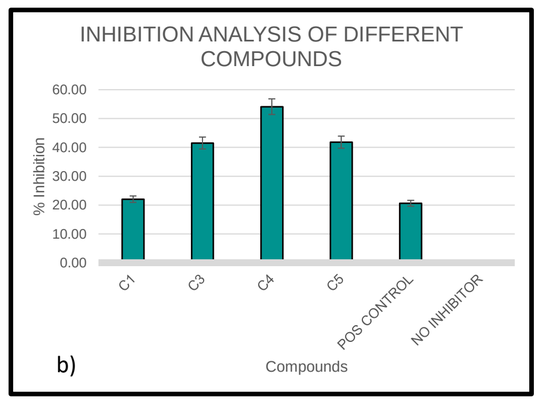


Fig. 4: a) Compound 4 is Oxalic Acid showed three H-bond formed; two H-bond formed at Lys 160 and one H-bond at Asn 175. Significant results by docking studies showed minimum binding energy of -2.59 kcal/mol. b) Compound 4 interestingly, showed to be the most active with 54.12 % inhibition determined *via* enzymatic assay, compared to others and the IC50 value determined was 0.6398mM.

Conclusion

This study has resulted in:

- ✓ **Reliable method for producing soluble *Pk*-LDH that is biologically-active**
- ✓ **Yielded promising antimalarial compounds which can be utilized in the future drug development studies, especially for infection with *P. knowlesi*.**

Acknowledgment:
This study was supported by the International Islamic University Malaysia Research Initiative Grants (RIGS15-141-0141). We would also like to thank Institute for Medical Research for facilities provided for the study.

KERICE 2019

Poster ID: BPMERU012

Virtual Screening and Molecular Docking Approaches for Identification of Natural Lead Compounds for Improved Anti-Dengue Therapeutics

Foysal Ahammad, Fazia Adyani Ahmad Fuad

Abstract

Dengue is a mosquito-borne viral disease, in which specific therapeutics have not yet been developed. During dengue virus (DENV) infection, the expression of human hexokinase II (HK2) enzyme is upregulated in infected cells. Hence, it was postulated that pharmacological inhibition of human HK2 can be a potential therapeutic approach for the development of anti-DENV drugs. The aim of the study is to identify natural lead compounds that can be developed into potent anti-DENV drugs, by using a combination of ligand and structure-based virtual screening approaches. Compounds 2-Deoxyglucose (2-DG), Metformin(MF) and Benserazide (BZ), which are known inhibitors of HK2, were used as the query molecules for dataset generation from the Ambinter (http://www.ambinter.com) natural compound library. The dataset comprising the analogues of the three molecules generated from the library were docked against the crystal structure of HK2 (PDB ID: 2NZT) on chain B, using PyRx AutoDock Vina tool. Out of 30 million compounds from Ambinter database of natural products and their derivatives, we have identified four compounds, D-Glucose hydrate, (2S,3R,4R,5S)-2,3,4,5,6-Pentahydroxyhexanal, (S)-2-Amino-3-hydroxy-N'-(2,3,4-trihydroxybenzyl)propanehydrazide hydrochloride, and (2S)-2-Amino-3-hydroxy-N',N'-bis[(2,3,4-trihydroxyphenyl)methyl]propanehydrazide with binding energy -10.2, - 9.6, - -10.2 and -9.7 kcal/mol, respectively, as potential compounds that can be tested in the subsequent inhibition studies. The compounds generated from the natural database library displayed binding energy ranging from -10.2 to - 9.6 kcal/mol, which are greater than the binding energy of the query molecules (-8.8 to - 7.1 kcal/mol). The hits bind unswervingly at the receptor site, whereas compound 2-DG and BZ were oriented towards the active sites of HK2. From the above-mentioned result, we can conclude that the study has generated a list of compounds which can be further tested in the subsequent inhibition studies of recombinant human HK2.

Keywords: Virtual Screening, Molecular Docking, Anti-Dengue Agents, Natural Compounds, Hexokinase 2 Enzyme.

Problem Statement

Dengue is endemic in more than 112 countries and each year, up to 400 million people are infected with DENV [1]. However, to date, no specific therapeutics have been developed that can treat infections caused by these pathogens. It has found that during DENV infections the human hexokinase (HK2) (Figure 1) enzymes is upregulated to support the energy and biosynthetic building blocks required for their replication [2].

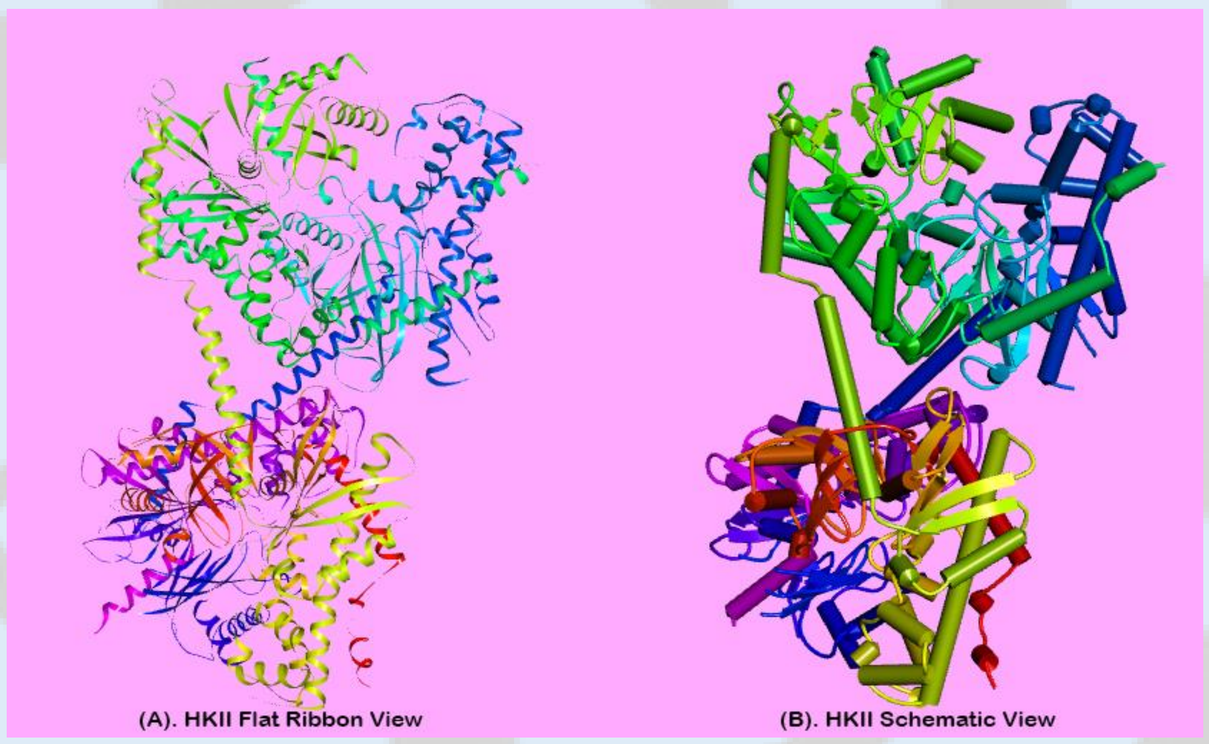


Figure 1: The crystal 3D structure of HK2 protein downloaded from the PDB (ID:2NZT). (A). Represent flat ribbon view and (B). Schematic view of HKII protein.

Project Objectives

The main objective of this study is to identify natural lead compounds *via* virtual screening and molecular docking approaches to inhibit human HK2 for the development of novel anti-dengue therapeutics.

Research Methodology

In silico virtual screening was conducted using Ambinter natural compound library (Ambinter.com), where molecular docking was performed using PyRx Autodock vina tools [3] to predict the binding affinities of newly-designed compounds with the target HK2 protein.

Findings

The analysis of 120 selected compounds, obtained by screening the natural compound database has yielded four promising compounds, as shown in Table 1, while the binding action is shown in Figure 2.

Compound Name	Energy (kcal/mol)
D-Glucose hydrate	-10.2
(2S,3R,4R,5S)-2,3,4,5,6-Pentahydroxyhexanal	- 9.6
(S)-2-Amino-3-hydroxy-N'-(2,3,4-trihydroxybenzyl)propanehydrazide hydrochloride	-10.2
(2S)-2-Amino-3-hydroxy-N',N'-bis[(2,3,4-trihydroxyphenyl)methyl]propanehydrazide	-9.7

Table 1: List of compounds selected based on their binding affinity (Kcal/mol).

Conclusion

In this study, computational methods have been used to identify several potential natural lead candidates for drug design against dengue disease. Four compounds were shortlisted, which have generated through a cycle of *in silico* studies, from virtual screening to molecular docking. The current computational results will be analyzed using *in silico* ADMET predictions tools and further evaluated by both *in vitro* and *in vivo* analyses test.

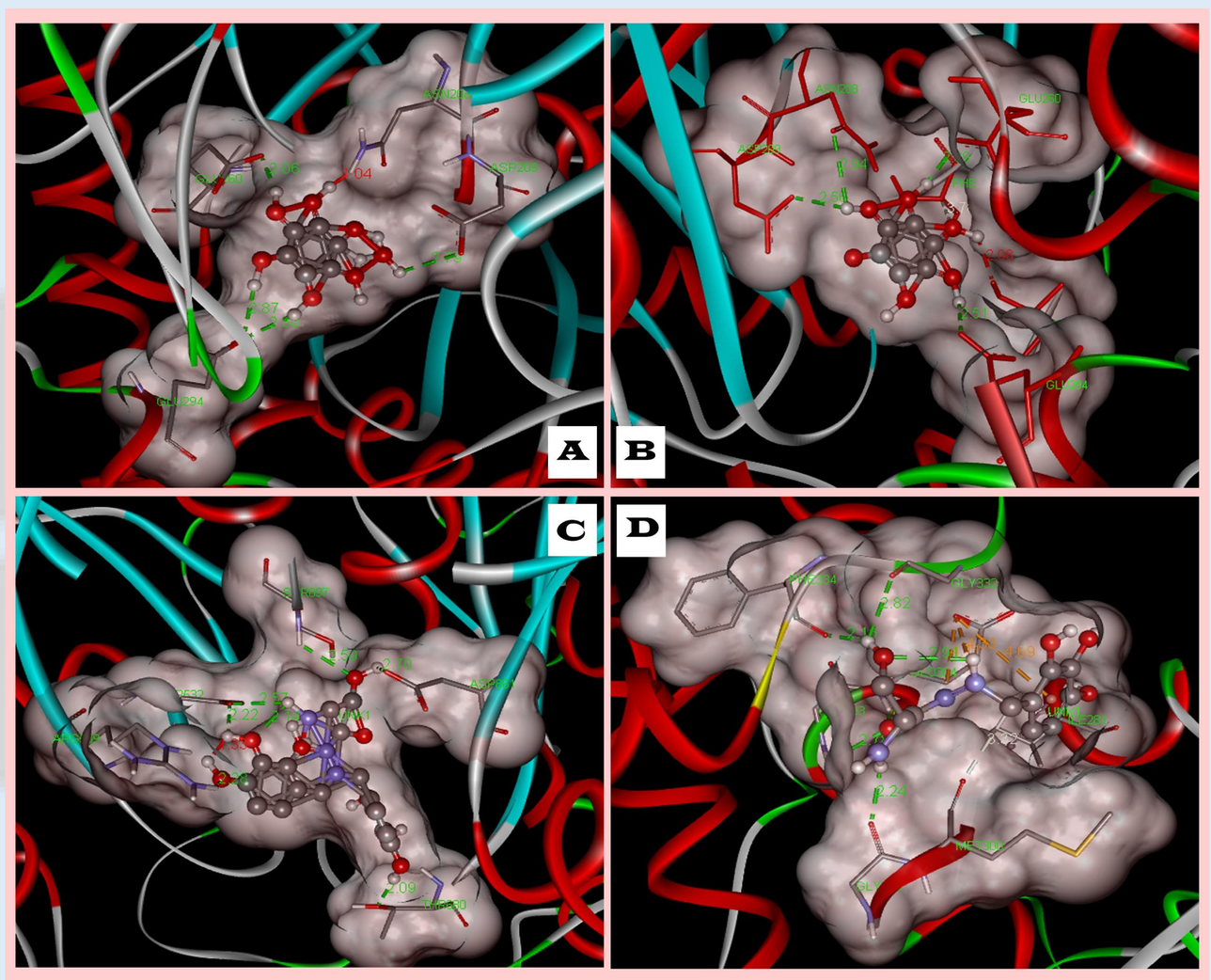


Figure 2: The binding action of selected four compounds with HK2 protein. (A) D-Glucose hydrate, (B) (2S,3R,4R,5S)-2,3,4,5,6-Pentahydroxyhexanal, (C) (2S)-2-Amino-3-hydroxy-N', N'-bis[(2,3,4-trihydroxyphenyl)methyl]propanehydrazide and (D) (S)-2-Amino-3-hydroxy-N'-(2,3,4-trihydroxybenzyl) propanehydrazide hydrochloride describe the binding activities with HKII protein.

Acknowledgement

The authors thanked the IIUM Fundamental Research Grant Scheme 2016 FRGS/1/2016/STG04/UIAM/02/1 for supporting this work.

References

[1]. Martina BE, Koraka P. (2009) Dengue virus pathogenesis: an integrated view. Clinical microbiology reviews.22(4):564-81.
[2]. Fontaine KA, Sanchez EL, Camarda R, Lagunoff M. (2015) Dengue virus induces and requires glycolysis for optimal replication. Journal of virology. 89(4):2358-66.
[3]. Wichapong K, Nueangaudom A, Pianwanit S, Sippl W, Kokpol S. (2013) Identification of potential hit compounds for Dengue virus NS2B/NS3 protease inhibitors by combining virtual screening and binding free energy calculations. Trop. Biomed. 30:388-408.

KERICE 2019

Poster ID:BPMERU005

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

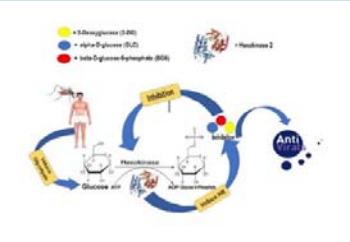
Suriyea Tanbin, Fazia Adyani Ahmad Fuad, Azzmer Azzar Abdul Hamid

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.



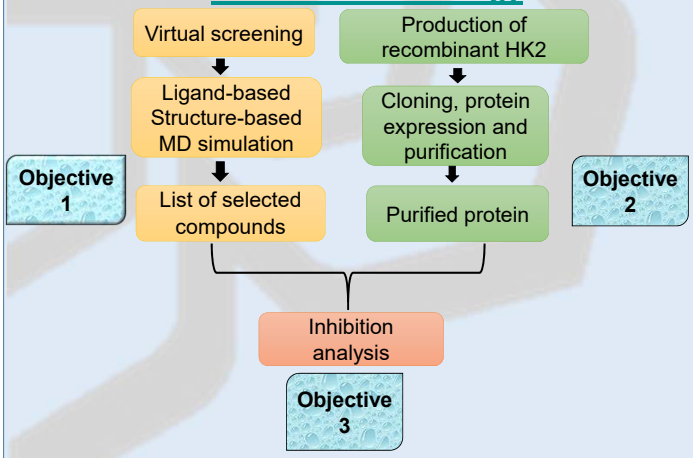
Project Objectives

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

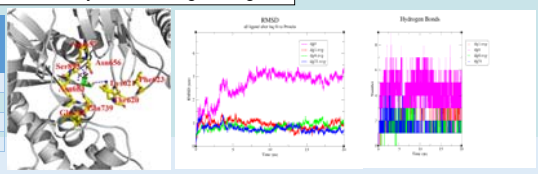


Fig1.2.3. 2dg in the dock side of protein, RMSD graph and Hbond number of 2dg and 2dg's analogues from MD simulation

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

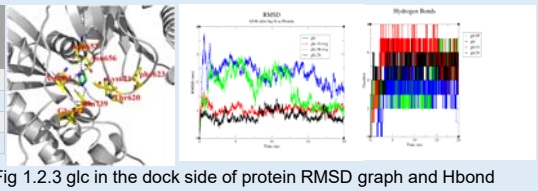


Fig 1.2.3 glc in the dock side of protein RMSD graph and Hbond number of glc and glc's analogues from MD simulation

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

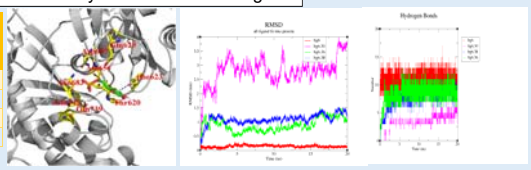


Fig 1.2.3 BG6 in the dock side of protein. RMSD graph and Hbond number of BG6 and BG6's analogues from MD simulation

2. Production of recombinant HK2 and HK2 protein expression

Cloning

HK2 gene purchased from GenScript

PCR amplification

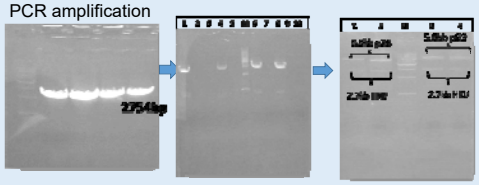


Fig.1.2.3. HK2 band purified & ligated with pETite 28b & pETite32b then Verification of transformants by PCR colony and Restriction enzyme digestion

Expression

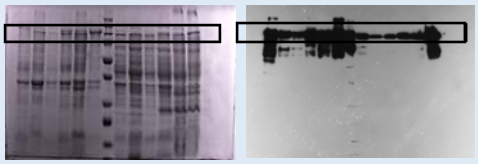


Fig1. 2. Recombinant HK2 gene was successfully expressed at 18°C temperature, with 19hr incubation time and 0.5mM IPTG concentration further it has been verified by western blotting

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