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Identifying Analogues Of 2-Deoxyglucose, Alpha-D-Glucose and Beta-D-Glucose-6-Phosphate as Potential Inhibitors of Human Hexokinase II for the Development of Anti-Dengue Therapeutics

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ABSTRACT

The human hexokinase isoform II (HKII) is one of the important enzymes for dengue virus (DENV) replication and thus has been suggested as a potential therapeutic target for DENV drug development. In this work, compounds were identified using Ultrafast Shape Recognition with CREDO Atom Types (USRCAT) by utilizing both HKII's substrate and product; alpha-D-glucose (GLC) and beta-D-glucose-6-phosphate (BG6), as well as a known HKII's inhibitor, 2-deoxyglucose (2DG), as the query molecules. The analogues of the three query molecules were subsequently docked against the HKII's crystal structure (PDB ID: 2NZT) by using Auto Dock 4 program on Chain B, where the active sites and strong bonds were located. Among the top-ranked compounds, Compound 4 (ZINC26898487), which was the most similar to 2DG, showed the best binding energy (-7.63 kcal/mol) and contained two H bonds. Compound 9 (ZINC16930948), an analogue of GLC emerged as the best inhibitor candidate because it had six H bonds. Similarly, among the molecules similar to BG6, Compound 14 (ZINC4403351) had been suggested as a potential inhibitor because it contained four strong H bonds. All compounds were predicted to be non-toxic, based on Toxicity Estimation Software Tool (TEST) analysis. By providing these valuable findings, this study has paved the way for the discovery of compounds that should be further tested for the development of anti-dengue drugs.

Keywords: 2-deoxyglucose, alpha-D-glucose, beta-D-glucose-6-phosphate, Human Hexokinase II (HK2), ligand-based screening, structure-based screening, toxicity test

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INTRODUCTION

The world is currently experiencing a steady increase in the number of dengue-related cases, and the prevalence of this disease remains high (World Health Organization, 2018). Approximately 390 million people are infected with dengue virus every year, resulting in around 25,000 annual deaths (World Mosquito Program, 2018). At present, this disease is registered in more than 128 countries worldwide, with the greatest burden imposed on the South-East Asian, Eastern Mediterranean and South American countries (World Health Organization & World Mosquito Program, 2018). In Western Pacific region the greatest dengue incidence was noted in Malaysia, Vietnam and Philippines, with 41,443 cases (65 deaths), 52,482 cases (3 deaths) and 59,139 cases (237 deaths), respectively as of week 16, 2019 (World Health Organization, 2019).

Mosquito-borne dengue virus is responsible for the intense feverish dengue disease, whereby female *Aedes aegypti* or *Aedes albopictus* mosquito acts as a vector for virus transmission to humans via a single bite (Wichapong et al., 2013). Dengue virus is a single-stranded RNA virus belongs to Flaviviridae family, which exhibits four serotype types; DENV-1, DENV-2, DENV-3, and DENV-4 (Diamond & Pierson, 2015). The severity of the dengue disease is determined by sequential attacks by different serotypes.

Previously, it has been established that DENV-1 is more virulent compared to DENV-2 based on the clinical manifestation, hematological parameters and genotypic variation of limited dengue cases (Fried et al., 2010; Yung et al., 2015) . However, at present, DENV-2 is considered the most virulent serotype based on the clinical data and laboratory test reports (Idrees et al., 2012; Vinodkumar et al., 2013; Mudin, 2015; Yung et al., 2015; Wijewickrama et al., 2018). According to the published data, the percentage of DENV-2 serotypes present in patients' blood ranges from 96.02% (Idrees et al., 2012) to 92.4% (Wijewickrama et al., 2018), while 29.2% DENV-2 serotype was also reported from blood samples of 72 patients (Vinodkumar et al., 2013). However, although serotypes share approximately 65% of the genomic character, they remain difficult to distinguish (Yung et al., 2015; Mirza et al., 2016).

Unfortunately, at present, there is no effective vaccines or specific drugs that can be integrated into the global dengue prevention and control strategy (World Health Organization, 2018). However, as a part of their recent clinical study, Godói and research team (2008) produced the first dengue vaccine, Dengvaxia (CYD-TDV), which was registered in Mexico, by Sanofi Pasteur. However, at present, the vaccine has drawbacks since it cannot provide long-term protection against all four serotypes, and it is only effective for children aged 2−16 years (Godoi et al., 2008). It has also been established that sequential infections with different serotypes increase the chances of developing severe disease, which limits the use of vaccination as an early step in dengue prevention. As noted above, no well-developed antiviral drugs are presently available, which could cure this disease.

Thus, the treatment mostly relies on rehydration therapy. Typically, rehydration therapy consists of consuming high protein, low fat, non-oily, and non-spicy diet and drinking lot of water, as this has been found to increase appetite and improve nutritional status, while also elevating the platelet count, thus improving the patient's immunity (Mishra et al., 2017). However, dengue patients can suffer from wide range of health complications, such as compromised food digestion, low nutritional status, and immunity reduction, all of which can benefit from rehydration therapy. While these measures can alleviate dengue symptoms, there is an urgent need to discover new potentially effective drugs that may serve as the first line of treatment for dengue fever. As viruses are inanimate entities and do not possess metabolic machinery, they rely on their host for survival and replication (Wick et al., 1956; Schreyer & Blundell, 2012; Fontaine et al., 2015). During DENV infection, notable glucose consumption was observed by Fontaine and colleagues, who also noted that absorption of exogenous glucose had a significant impact on DENV replication. This consumption process mainly occurred in the glycolytic pathway (Fontaine et al., 2015). The authors further observed that, the expression of hexokinase isoform II (HKII) was significantly higher in the virus-affected cell, compared to the mock-infected cell. It is also important to highlight that hexokinase is the first rate-limiting enzyme of glycolytic pathway and HKII is found to play a key role of aerobic glycolysis (Gershon et al., 2013). In this pathway, with hexokinase assistance, glucose is converted to glucose-6-phosphate (G6P). G6P is produced by the phosphorylation of glucose, where hexokinase enzyme catalyzes this reaction by consumption of adenosine triphosphate (ATP). The reason behind glucose phosphorylation is that phosphorylated glucose cannot easily cross the cell membrane since negative phosphate group is attached with glucose, whereby phosphorylation traps glucose in the cell (Lunt & Heiden, 2011; Pelliccia et al., 2017). As mentioned above, viruses are lacking of metabolic function, thus they take the biosynthetic element and energy from host cell through the modulation of human's metabolic pathway. Several experiments have been conducted to observe the alteration of host cell metabolic pathway during viral infection, for example on vaccinia virus (VACV), human cytomegalovirus (HCMV) and hepatitis C virus (Munger et al., 2006; Roe et al., 2011; Fontaine et al., 2014). All these viruses caused extensive reprogramming of central carbon metabolism during infection, and similar to tumor and cancer cell, HCMV-infected cell absorbed glucose through glucose transporter 4 (GLUT4) and partially breakdown the glucose carbon through TCA cycle. Subsequently, fatty acid would be synthesized, which is crucial for HCMV replication (Yu et al., 2011). As a substitute, VACV virus may need another carbon source glutamine for the production of virion through maintenance of TCA cycle (Fontaine et al., 2014). During DENV infection, the infected cells increase the up-regulation of glucose through GLUT1 and GLUT4, as well as HKII, where later on glucose is diverted to cytoplasm through glycolytic pathway and TCA cycle, that generates a bulk of ATP and fatty acid biosynthesis required for viral

protein synthesis as well as proliferation. Glucose consumption of DENV is thus measured by highly significant HKII expression level. Pelliccia and colleagues (2017) had recently shown that DENV replication and glycolysis were positively correlated, suggesting that the inhibitory treatment of glucose competitor, 2-deoxyglucose (2DG), could significantly hamper DENV replication. Wick and colleagues (1956) established that 2DG was an analogue of glucose, in which two hydroxyl groups were replaced by hydrogen, which inhibited the production of glucose-6-phosphate and eventually hampered the glycolytic pathway (Godoi et al., 2008). Later, 2DG has been suggested by several work as a glycolytic inhibitor in cancer, tumor, as well as dengue virus replication (Ganapathy-Kanniappan & Geschwind, 2013; Kaushik et al., 2015; Fontaine et al., 2015; Savic et al., 2016). However, 2DG has shown toxic effects when introduced to patients (Zhang et al., 2014). Hence, it is important to identify new compounds similar to 2DG that would not produce such toxicity effects, as these could be valuable candidates for anti-dengue drug development. This was the goal of the present investigation, as a part of which the inhibition activity of 2DG was considered. Apart from 2-deoxyglucose (2DG), both alpha-D-glucose (GLC) and beta-D-glucose-6-phosphate (BG6) serve as interesting reference molecules for rational drug design where GLC, as the substrate for HKII-catalyzed reaction, is an anomer of glucose-6-phosphate.

In this work, we report the identification of small molecules similar to 2DG, GLC, and BG6, which have the potentials to be developed into safe and effective anti-DENV drugs. A ligand-based drug design experiment was conducted by screening similar compounds that were analogues of the substrates and product of human HKII, namely alpha-D-glucose (GLC), and beta-D-glucose-6-phosphate (BG6) as well as 2DG, which was the known inhibitor of HKII. Subsequently, a series of docking and scoring experiments was conducted, following a structure-based drug design screening based on the crystal structure of human HKII, before these compounds were being tested in virtual toxicity test.

MATERIALS AND METHODS

Ligand-Based Screening

As previously noted, 2DG, GLC and BG6 were used as query molecules for the initial ligand-based pharmacophore screening (Figure 1). The 3D structures of 2DG, GLC and BG6 (with PubChem ID CHEBI: 10822 ID CHEBI: 17925 and ID CHEBI: 17719, as well as molecular formula $C_6H_{12}O_5$ $C_6H_{12}O_6$ and $C_6H_{13}O_9P$), were downloaded in structure data file (SDF) format from PubChem (https://pubchem.ncbi.nlm.nih.gov/) to screen for similar compounds by using USRCAT (Ultrafast Shape Recognition with CREDO Atom Types) available at http://usr.marseille.inserm.fr/. We retrieved a total of 300 compounds and their ZINC IDs, where each of the query molecule (2DG, GLC and BG6) had 100 similar hits from the USRCAT program, whereas the 2D structure, hydrogen bond donor, hydrogen

bond acceptor, molecular weight, and logP value of every hit can be found in the ZINC (http:/zinc.docking.org/) database. The predicted similarity of the molecules was based on the scoring range 0 < score < 1, where a compound with higher score indicated greater similarity towards its respective query molecule. Based on the similarity score, the top 40 compounds for each of the query molecules (2DG, GLC, and BG6) were chosen, thus a total 120 compounds were selected for further docking studies.

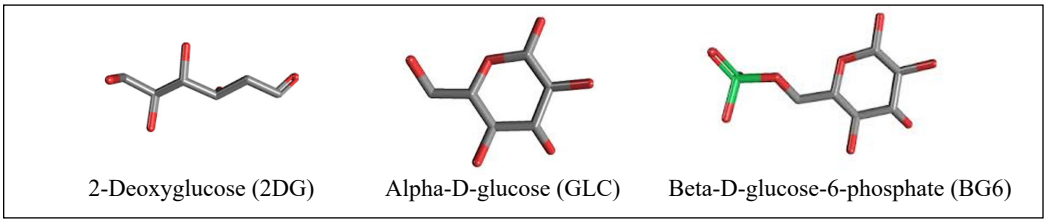


Figure 1. The 3-dimensional structure of 2-deoxyglucose (2DG), alpha-D-glucose (GLC), and beta-D-glucose-6-phosphate (BG6) molecule used in the ligand-based virtual screening analysis (PubChem ID CHEBI: 17925, ID CHEBI: 10822 and ID CHEBI: 17719). The grey, red, and green color indicate carbon, oxygen, and phosphate atoms, respectively

Structure-Based Screening

For the present study, the crystal structure of human HKII was retrieved from the Protein Data Bank (PDB ID: 2NZT) website https://www.rcsb.org/. The protein structure was prepared for molecular docking by protein optimization and energy minimization, which was performed by removing ligands from the protein structure using SPDBV-Swiss-PdbViewer software. Molecular docking was conducted by using two different software tools, namely Auto Dock 4 and Cygwin program, whereby Auto Dock 4.2 was used to dock each compound into the target protein and Cygwin program assisted in performing the docking procedure by using genetic and Lamacrkian algorithm (Morris, et al., 2010). We prepared a grid box that encompassed the binding site of the protein, where ligands were docked sequentially. The grid box covered the predicted catalytic protein residues, where the box size was (40 × 40 × 40 Å) and the box center coordinates (x = 13.062; y = 13.881; z = 7.683), on the C terminal of the protein's chain B. Cygwin was used for the docking algorithm, in which grid log and docking log files were generated. The binding energy (kcal/mol), derived from the docking log file, indicated the strength of the ligand-receptor binding, which was subsequently analyzed by using PyMol software.

Toxicity Test

Toxicity estimation software (TEST) version 4.2.1 was used to determine the toxicity of the top-ranked six compounds, following docking analysis. The protein data file (pdb file) format was converted into a molecular data file (MDL mol) through Open Bable software. We used Quantitative Structure Activity Relationship (QSARs) methodologies to predict

toxicity measures from physical characteristics, based on the compounds' structure. Selected compounds were tested using different test end-points, such as lethal dose LC50, bioconcentration factor (BCF), developmental toxicity, and mutagenicity.

RESULTS AND DISCUSSION

Ligand-Based Screening Analysis

Ligand-based virtual screening has been proven as an effective tool for discovering novel chemical platforms in compound libraries (Vyas et al., 2008; Gao et al., 2010; Lavecchia & Giovanni., 2013). In the present study, a search was carried out by using both HKII's substrate and product (GLC and BG6, respectively), as well as known inhibitors of HKII (2DG) as the query molecules, to search for similar candidates by using USRCAT program. USRCAT is a user-friendly, cost-effective and time-effective web based program which is performed for large scale ligand-base screening. The USRCAT was developed as a part of USR algorithm to assist users in their search for desired compounds similar to the query molecules, that is able to screen more than 50 million of 3D conformers per second (Schreyer & Blundell, 2012). The latest version of USRCAT is capable of differentiating not only the 3D shapes, but also the distribution of atom types pertinent for query molecule recognition (aromatic, hydrogen bond donor, hydrogen bond acceptor, and hydrophobic atoms). As a part of the current investigation, 23.1 million molecules were screened and a total of 300 most similar compounds were obtained from USRCAT for all of the reference molecules (2DG, GLC, and BG6). Thus, specifically, each query has 100 similar compounds aligned into the hits.csv file along with similarity scores, physicochemical properties, and the vendor's information. In this work, similarity was evaluated based on the scoring function range from 0-1, where zero and one indicates minimum and maximum similarity, respectively. As mentioned above, the USRCAT score zero indicates the least similar compounds, while those closer to one reflects a much closer resemblance towards the reference molecule. Based on these findings, a total 120 compounds out of 300 similar hits for each query molecule were chosen and their physiological properties were obtained from the ZINC database. The ZINC database contains 727,842 purchasable compounds from various suppliers (ChemBridge, ChemDiv, Ryan, Asinex, Sigma-Aldrich, Maybridge, Specs, Comgenex and Otava), 494,915 of which are Lipinski subjacents. Based on the similarity scores, the six top-ranked compounds that resembled each of the query molecule are shown in Table 1-3, as their ranking scores ranged from 0.76 to 0.79, 0.85 to 0.88 and 0.84 to 0.86 for 2DG, GLC and BG6 respectively. All six compounds, which are analogues of 2DG, GLC, and BG6 which have been selected for the subsequent studies obeyed the Lipinski's Rule of Five in terms of their physiological parameters; hydrogen bond donors > 5, hydrogen bond acceptor > 10, molecular weight under > 500g/mol, partition coefficient logP > 5 with no violation (Lipinski et al., 1997). The mentioned parameters

related to the Lipinski's rule of five are presented in Tables 1 to 3. Compounds 4 to 21, which are similar conformers of 2DG possess MlogP values ranging from -1.73 to -2.34, and drug-likeness from -0.92 to -1.21 (Table 1). Moreover, the top six similar hits of GLC, compounds 8 to 26 have MlogP values from -3.12 to -3.04 and drug-likeness from -1.21 to -0.15 (Table 2). Drug-likeness is the preliminary concept of drug design and its value is usually estimated from the compound's molecular structure that is standardized between -0.4 and 5.6, whereby it indicates whether the compound has drug properties or not. As our findings were optimized with respect to the drug-likeness parameter and the adherence to

Table 1
Physicochemical properties and similarity scores for 2DG and its analogues, as obtained from USRCAT

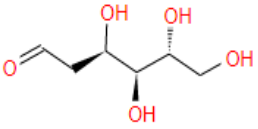
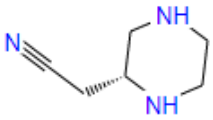
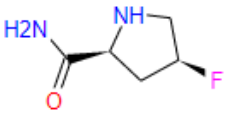
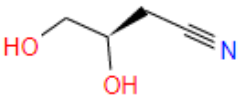
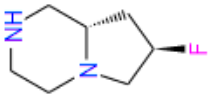
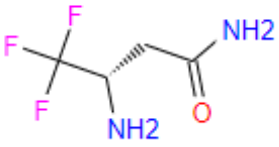
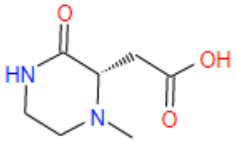
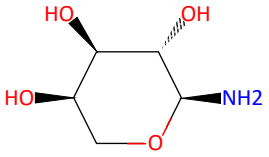
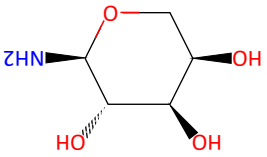
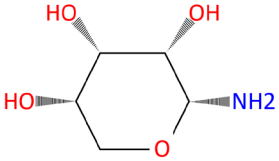
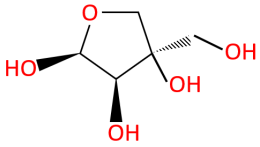
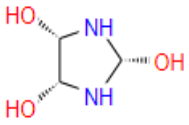
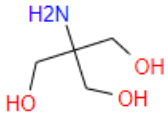
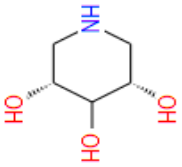
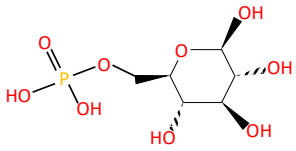
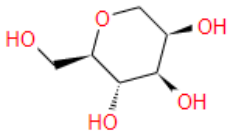
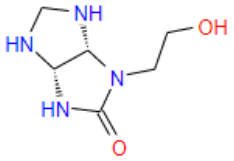
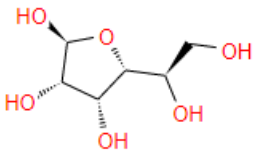
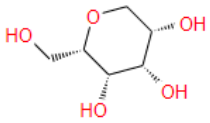
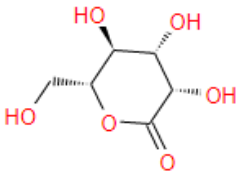
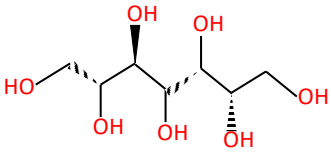
Compound number	2D structure	Similarity score	Number of HBD/HBA	Molecular weight (g/mol)	MlogP	Drug-likeness
2DG		N/A	4/5	164.157	-1.20	-0.50
4		0.79	2/3	125.175	-1.73	-0.92
6		0.78	3/3	132.138	-1.66	-0.67
8		0.78	2/3	101.105	-1.44	-1.39
18		0.76	2/2	145.201	-1.64	-1.16
19		0.76	4/3	156.107	-1.34	-1.19
21		0.76	2/5	172.184	-2.34	-1.21

Table 2
Physicochemical properties and similarity scores for GLC and its analogues, as obtained from USRCAT

Compound number	2D structure	Similarity score	Number of HBD/HBA	Molecular weight (g/mol)	MlogP	Drug-likeness
GLC		N/A	5/6	180.156	-2.11	-0.20
8		0.88	5/5	149.146	-3.12	-1.21
9		0.88	5/5	149.146	-3.12	-1.21
12		0.87	4/5	150.13	-2.38	-0.15
18		0.86	5/4	134.155	-2.77	-1.45
25		0.85	6/4	122.144	-2.20	-0.70
26		0.85	5/5	120.108	-3.04	-1.02

the Lipinski's Rule of Five, most of the compounds fulfilled all the criteria. The drug-like properties of BG6 corresponded to the MlogP values between -2.57 and -4.26, and drug-likeness between -0.92 and 0.12 (Table 3). It is noteworthy to note that, although the same scores were obtained from USRCAT program, these compounds are structurally different and contain different atom types. The rule of five provides insights for the analysis of the

Table 3
Physicochemical properties and similarity scores for BG6 and its analogues, as obtained from USRCAT

Compound number	2D structure	Similarity score	Number of HBD/HBA	Molecular weight (g/mol)	MlogP	Drug-likeness
BG6		N/A	2/2	152.132	-2.07	-0.42
2		0.86	4/5	146.157	-2.57	-0.92
3		0.86	4/6	172.188	-2.68	-1.10
12		0.85	5/6	180.156	-3.05	-0.63
14		0.85	4/5	164.157	-2.57	-0.92
21		0.84	4/6	178.14	-3.45	-0.18
23		0.84	7/7	212.198	-4.26	0.12

molecular properties and structural features of every compound, which is important for drug pharmacokinetics in human body, including their absorption, distribution, metabolism and excretion (ADME) (Lipinski et al., 1997).

Computational methods are effectively used in pharmaceutical research to improve drug discovery process. Ligand-based screening is one of the crucial parts of *in silico* drug design and several novel compounds have been invented through this process. Hence, considering the importance of computational drug design method, it was adopted in the present study to discover the potential HKII inhibitors. HKII is an important drug target for designing novel compounds for dengue treatment. Numerous successful ligand-based screening investigations have been performed, aiming to identify potential inhibitors against dengue drug targets. In extant studies, dengue virus encoded non-structural protein 3(NS3) was used a as drug target, and two known inhibitors, namely suramin and ivermectin have been successfully identified through virtual screening and *in vitro* analysis, where researchers used LOPAC compound library for inhibitor selection as well as vero-B and vero-E6 cell for *in vitro* assay (Mastrangelo et al., 2012; Basavannacharya & Vasudevan, 2014). It is noteworthy to note that most of the research conducted to search for anti-dengue drugs have been conducted against viral replication proteins or viral envelop proteins, but our research focus is on human protein as a drug target. Another fruitful ligand-based work has also been done using Ultra-Fast Shape Recognition with Atom Type (UFSRAT) to identify similar hits of S-adenosyl-L-methionine (SAH) and ribavirin triphosphate (RTP), in which those were used against drug target NS5 methyltransferase (MTase). In their work, Lim and colleagues (2011) had identified 500 compounds that exhibited the highest similarity to SAH and RTP, which were subsequently used in docking analysis (Lim et al., 2011). Nevertheless, UFSRAT is also a powerful web program, but it shows complexity in selecting the best compounds without the 3D structure of the drug target. In our study, only the 3D structure of the reference molecule has been used for screening and a total 120 compounds were chosen for further docking analysis. Ligand-based methods not only yield effective results pertinent to dengue research, but are also beneficial in cancer or tumor studies. For instance, wild-type p^{53} protein is responsible for inducing cancer, but its function is inhibited when in complex with human murine double minute 2 (MDM2), which is why p^{53}-MDM2 is an important drug target (Patil et al., 2014). Basically, ligand-based screening is a very convenient process to discover drug-like molecules within a short period of time. There are numerous web-based programs available to implement this process, whereas in this work, an on line program, USRCAT has been utilized, which is incorporated with ZINC database. Lipinski's Rule of Five functions as a filter to choose drug-like compounds based on their molecular property, including absorption, distribution, metabolism and excretion. Hence, considering the importance of computational drug design method, it was adopted in the present study to discover the potential HKII inhibitors. Findings yielded have shown selected compounds, based on the similarity score and drug-like criteria.

Structure-Based Screening Analysis

Structure-based virtual screening is an effective process that has become instrumental in the fast and cost-effective lead discovery and optimization. The goal of this process is to understand the disease at a molecular level and apply the knowledge of three-dimensional structure of the biological target (Kroemer, 2007; Lionta et al., 2014). Structure-based screening refers to the protein-ligand binding interaction, where the protein-ligand binding mode can be predicted through docking studies, which consist of two-step calculation involving the free binding energy of protein-ligand complex, and the conformational space exploration of ligand and target molecules (Reddy et al., 2007; Lavecchia & Giovanni, 2013). Prior to the emergence of the docking method, the structure was typically validated by redocking experiments, whereby the ligand was extracted from protein−ligand complexes to minimize the conformation and was subsequently docked back into the protein to validate the docking location (Cosconati et al., 2010).

In the present study, a successful docking site was determined by Root Mean Square Deviation (RMSD) value from known conformation, whereby a good docking site is generally indicated by an RMSD ≤ 2 Å (Nissink et al., 2002; Vyas et al., 2008).According to the RMSD value, the most accurate sites were selected by applying a scoring and ranking function. In this research, the 3D structure of human HKII (PDB ID: 2NZT) was retrieved from Protein Data Bank, in complex with alpha-D-glucose (GLC) and beta-D-glucose (BG6), which were removed prior to performing the docking experiment. Protein energy minimization is essential to set a protein structure in energetically-favorable state (reduction in the relaxing bond lengths, angles, and non-bond interactions), which provides more accurate docking results. Protein optimization and energy minimization were performed in the present study to avoid the complexity associated with protein−ligand docking. After protein preparation, the docking procedure was applied for 120 compounds that are identified from ligand-based screening. Each compound had been successfully docked into the catalytic site, which was validated through re-docking process as well as RMSD value. As mentioned, HKII structure in complex with the known ligands BG6 and GLC was utilized to validate the docking process, by extracting these two ligands, re-docking the ligands into HKII, prior to docking the virtually-screened compounds into the catalytic site of HKII. After completion, successful docking had been observed, where in the catalytic residues, the dock poses of the compounds were similar to the reference molecule and the RMSD value was reasonable (Figure 2, 3 and 4).

In molecular docking, the grid box size and the coordinates of its center have to be validated, in order to ensure that the ligands bind to the binding pocket in the correct conformation. The grid box (Box size: 40 × 40 × 40 Å) and the box center coordinates (x = 13.062; y = 13.881; z = 7.683) were designed, whereby all binding modes were created within this dimension for the most favorable bindings. The Human HKII (PDB ID: 2NZT) is

a homodimer protein consisting of Chain A and B with catalytic C-terminal and N-terminal domains. In this work, we used C-terminal domain of Chain B for docking because several extant studies had revealed that the C-terminal (residues 476−917) contained more capable catalytic sites (Printz et al., 1997; Bianchi et al., 1999). In the present study, Thr620, Lys621, Glu629, Asn656, Asp657, Phe623, Ser893, Asn683, Gln739, and Glu742 (Figure 2, 3 and 4) catalytic residues were predicted as the most favorable sites for docking the compounds. The docking process involved 120 compounds, with the selected residue of catalytic sites for each of the query molecule (2DG, GLC, and BG6). All the lead molecules successfully docked into the catalytic site of human HKII protein. In this study, the six top-ranked compounds (4, 18, 6, 8, 19 and 21), which are analogues of 2DG had the binding energy ranging from -7.63 kcal/mol to -4.98 kcal/mol, whereas the binding energy (kcal/mol) of 2DG itself is -7.40 kcal/mol (Table 4). Among all conformers of 2DG, compound 4 (ZINC2689487) poses good binding energy (-7.63 kcal/mol) with two H bonds attached with residues Ser893 and Gln739, that closely resembles the query molecule 2DG (-7.40 kcal/mol), which poses six H-bonds; four attached with Ser893 ,one with Asn656 and another one is Thr620 (Figure 2B and 2A, respectively). It can be suggested that compound 4 (ZINC2689487) was the best findings among similar analogues of 2DG and might be a potential inhibitor of HKII. Furthermore, compounds 8, 9, 25, 26, 18 and 12, which are the analogues of GLC exhibited binding energy ranged from -8.87 kcal/mol to -7.16 kcal/mol, whereas GLC itself poses a binding energy of -6.40 kcal/mol (Table 5). Compound 9 (ZINC16930948) had been chosen as the best analogue based on highest binding affinity value (-8.50 kcal/mol) with six H bonds, four of which are formed with Thr620, Asn683, Asp657 and Glu742, and the remaining two with Asn656 (Figure 3B), when compared to the minimum binding energy of GLC in HKII (-6.40 kcal/mol) (Table 5), and it forms up to two H bonds with Glu742 and Asn656 (Figure 3A). The best compound among GLC analogues was Compound 9 (ZINC16930948), which might be a potential inhibitor of HKII.

On the other hand, similar hits of BG6 (compounds 23,3,2,21,12 and 14) exhibited binding energy ranged from -9.16 kcal/mol to -7.09 kcal/mol, as shown in Table 6. It can be noted that Compound 14(ZINC4403351) exhibited relatively good binding energy (-7.09 kcal/mol) with four H bonds, three of which were formed with Glu629 and one with Asn656 (Figure 4B), compared to binding energy of BG6 (-5.02kcal/mol) with one H bond with Phe623 (Figure 4A). Compound 14 (ZINC4403351) thus had been suggested for further inhibition analysis, due to having good number of H bonds, as well relatively good binding affinity, compared to BG6 itself and all other analogues of BG6.

Docking sites were considered successful based on the value of three parameters. First, re-docking experiments were conducted, by setting the RMSD = 2 Å for all docked sites, as this had been considered as a threshold value. Secondly, predicted catalytic residues were present in the protein structure, before estimating the minimum binding energy and including the number of H bonds with the selected residues into the catalytic sites of the

protein. The catalytic sites of human HKII were determined based on the selected residues. In this case, the RMSD value of each dock site was 2 Å for every selected compound that was similar to each query molecules (2DG, GLC and BG6), and it was determined by known protein−ligand dock sites retrieved from the PDB file. The substrate and product (GLC and BG6) in complex with the protein were used as the primary lead for introducing these ligand derivatives. According to the findings yielded by other studies that have been conducted in the search for antiviral activity of potential inhibitors against NS2B/NS3 target protein *via* the docking method, the search was conducted using three query molecule such as mycophenolic acid (MPA), ribavirin and panduratin, (PubChem ID446541, ID37542 and ID6483648 respectively), which are the most promising known inhibitors (Parida & Yadav, 2014). However, in their study, Parida and Yadav (2014) utilized these three different query molecules, and the findings suggested that panduratin's derivatives were better inhibitors compared to mycophenolic acid and ribavirin derivatives, based on the docking score using PatchDock online server, which helped in the prediction of protein-ligand interaction and H-bond analysis results. The selected panduratin derivative's (compound 1) PatchDock score is 3698 and it contains four H bonds. In contrast, our results indicate that compound 4 (ZINC26898487), compound 9 (ZINC16930948) and compound 14 (ZINC4403351), the analogues of 2DG, GLC and BG6 respectively, are the most potential inhibitors, and should be subjected to further *in vitro* inhibition analysis based on their binding energy and H-bond analysis findings. Indeed, based on a comparison between the similarity hits and the query molecules, compound 4, the analogue of 2DG has good similarity score (0.79), binding energy (-7.63kcal/mol) and contains two H bonds (Tables 1 and 4) (Figure 2B). Meanwhile, Compound 9 and Compound 14 were chosen (Table 2, 3, 5 and 6) because of good similarity score (0.88) and (0.85), binding energy (-8.50 kcal/mol) with six H bonds (Figure 3B), and binding affinity (-7.09 kcal/mol) with four H bonds (Figure 4B), which are analogues of GLC and BG6, respectively. In this case, although other compounds also show relatively good similarity scores and binding affinities, but since these compounds lacked H bonds, they were not suggested for further inhibition studies.

Table 4
The top six molecules, which are analogues of 2DG that were ranked according to their binding energy. The binding energy of 2DG is also shown

Compound	ZINC ID	Binding energy (Kcal/mol)
2DG	2512351	-7.40
4	26898487	-7.63
18	39957300	-6.95
6	71257594	-5.97
8	2564305	-5.07
19	19795285	-5.07
21	1926703	-4.98

Table 5
The top six molecules, which are analogues of GLC that were ranked according to their binding energy. The binding energy of GLC is also shown

Compound	ZINC ID	Binding energy (Kcal/mol)
GLC	1456321	-6.40
8	35644927	-8.87
9	16930948	-8.50
25	896695	-8.33
26	15938179	-7.85
18	14658010	-7.78
12	14854290	-7.16

Table 6
The top six molecules, which are analogues of BG6 that were ranked according to their binding energy. The binding energy of BG6 is also shown

Compound	ZINC ID	Binding energy (Kcal/mol)
BG6	3875374	-5.02
23	17952732	-9.16
3	58951086	-7.69
2	5851505	-7.53
21	2539703	-7.28
12	34027427	-7.14
14	4403351	-7.16

In the previous work, *in silico* search for inhibitors against nonstructural protein 3 (NS3) by high- throughput virtual screening showed that, in descending order, ZINC95518765, ZINC44921800, ZINC71917414, ZINC39500661, and ZINC36681949 had the greatest binding capability with NS3, as well as the highest binding energies of -7.55, -7.36, -8.04, -8.41, and -9.18 kcal/mol, respectively. They also exhibited binding interaction with three catalytically important residues (His51, Asp75 and Ser135) of NS3 protein (Mirza et al., 2016). It should be noted that NS3 is a non-structural protein that assists in viral replication. In their computational study, Lim and colleagues (2011) searched for NS5 methyaltransferase (MTase) inhibitors by docking, reporting that compounds SPH1-103-799, SPH1-101-102 and 28SPH1-115-917 had the greatest binding affinity (-11.4 kcal/mol, -11.4 kcal/mol and -10.0 kcal/mol, respectively) (Lim et al., 2011). In the present study, Auto Dock Vina and EDULISS (EDinburg University Ligand Selection System) were used for structure-based and ligand-based screening, respectively. NS5 is a non-structural viral protein located on the dengue virus surface that assists in virus replication. Hence, it has been proposed as a drug target, whereby ribavirin triphosphate (RTP) and the S-adenosyl-L-methionine (SAM) act as query molecules. Four-stage computational HTS was used to find a small molecule against the envelope protein (E protein) of dengue virus (Wang et

al., 2011). Next, 23 top-ranked compounds were selected based on the E protein binding site from three NIC libraries, which were tested for antiviral activity in biological assay. The compound PO2 was found to inhibit viral reproduction. Extensive research on the dengue virus has been conducted through the docking process (Wang et al., 2011; Yang et al., 2013; Behnam et al., 2014; Sing et al., 2015; Kouretova et al., 2017; Halim et al., 2017; Byrd et al., 2018). All the above mentioned literature reported inhibitors which were developed based on the viral protein itself. On contrary, our findings were based on human protein as a drug target. This structure-based process provides good assumption of protein-ligand binding affinity as well as assisting in choosing the best compound for further inhibition studies. Antiviral drug development relies on the appropriate compound selection through relevant *in silico* studies.

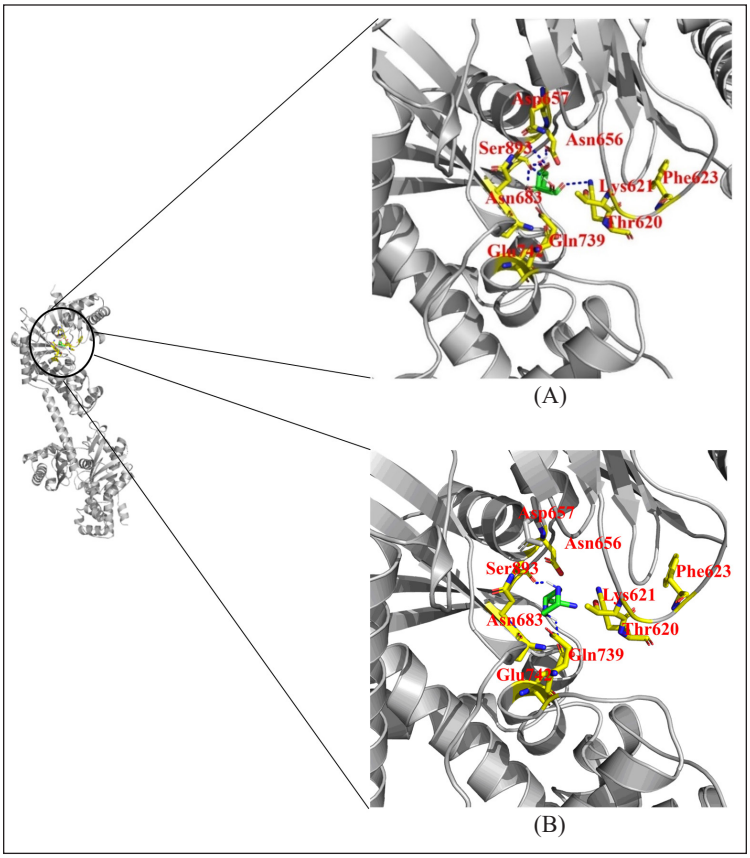


Figure 2. The interactions between 2DG and compound 4 with the residues involved in the catalytic site (A) HKII in complex with 2DG (green and red color indicates carbon and oxygen atom respectively of 2DG), which shows six H bonds; four with Ser893, one with Asn657 and another one formed with Thr620. (B) HKII in complex with compound 4 (green and blue color indicate carbon and nitrogen atom respectively of compound 4), which shows two H bonds formed with Ser893 and Gln739. Both interactions include the same catalytic residue (yellow and blue color indicates carbon and nitrogen atom of active sites residues) surrounding the ligand, while the blue lines indicate H bond.

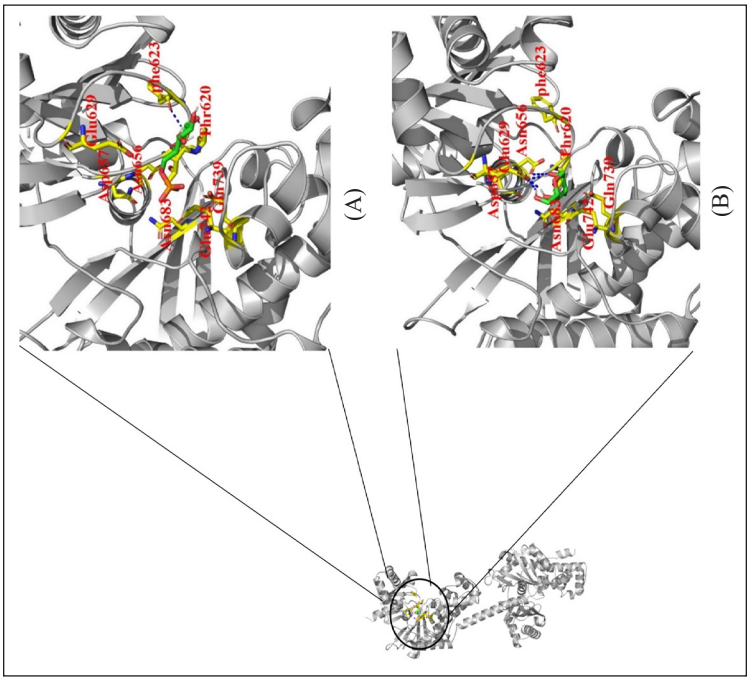


Figure 4. The interactions between BG6 and compound 14 with the residues involved in the catalytic site (A) HKII in complex with BG6 (green, red and orange color indicates carbon, oxygen and phosphorus atom of BG6), showing amino acid residues involved in Chain B with one H bond with Phe623. (B) HKII in complex with compound 14 (green and red color indicates carbon and oxygen atom respectively of compound 14), showing amino acid residues involved in Chain B with four H bonds (three formed with Glu629 and one with Asn656). Yellow and blue area indicate the carbon and nitrogen atoms of the particular amino acid residues, surrounding the ligand, while the blue lines indicate H bond.

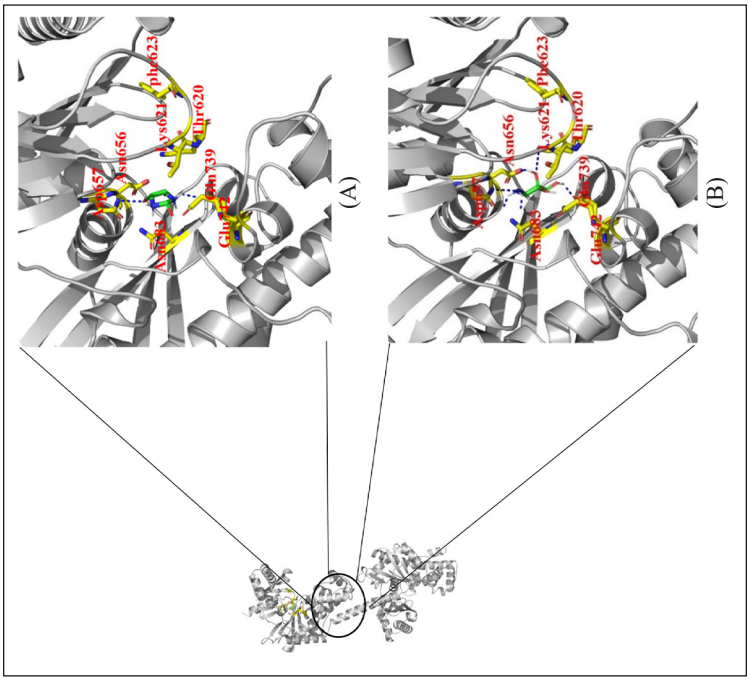


Figure 3. The interactions between GLC and compound 9 with the residues involved in the catalytic site (A) HKII in complex with GLC (green and blue color indicates carbon and nitrogen atom of GLC), which shows two H bonds formed with Glu742 and Asn656, respectively. (B) HKII in complex with compound 9 (green and red color indicate carbon and oxygen atom respectively of compound 9), which shows with six H bonds, each with Thr620, Asn683, Asp657 and Glu742 and another two H bonds formed with Asn656 (yellow and blue color indicates carbon and nitrogen atom of active sites residues) surrounding the ligand, while the blue lines indicate H bond.

Toxicity Test

Toxicity is proven essential in examining compounds for potential inclusion into drug development. The aim of toxicity studies is to ensure safety of the chemical compounds before they can be used as drugs or during clinical trials, as well as to determine the toxic effects of test substances (Arome & Chinedu, 2014). In several experiments, different types of toxicity tests (acute toxicity, sub-acute toxicity, and chronic toxicity studies) have been conducted to characterize the possible toxic effects of drugs that can range from minor to critical (Whitby et al., 2005; Ruiz et al., 2012). In this study, all compounds were assessed for toxicity using Toxicity Estimation Software Tool (TEST) and the results are reported in Table 7, 8, and 9, respectively. The TEST software utilizes the consensus method to determine the values of different compounds by using different tests end-point analysis approaches, where LD50 and LC50 values are exhibited for each compound (Tables 7-9). A smaller value indicates more toxic compound and vice versa. According to the data analysis results obtained in the present study, most of the compounds that were similar to 2DG were toxic, where only compound 4 (ZINC26898487) was non-toxic. In addition, the toxicity of the six top-ranked analogues of GLC showed that compound 9 (ZINC16930948) was found non-toxic, while the data for Compound 26 and 18 were not available (Table 8). Moreover, the top six analogues of BG6 were estimated for toxicity, and the best compound (compound 14, ZINC4403351) was confirmed as non-toxic. These toxicity results indicated that compound 4 (analogue of 2DG), compound 9 (analogue of GLC) and compound 14 (analogue of BG6) were non-toxic, and could be used in further *in vitro* HK2 inhibition studies. It should also be noted that all examined compounds were negative for mutagenicity. As already mentioned previously, 2DG is a known inhibitor of

Table 7
Estimation of toxicity values for the top six compounds similar to 2DG

Toxicity endpoint	Predicted value					
	Compound 4	Compound 18	Compound 6	Compound 8	Compound 19	Compound 21
Fathead minnow LC_{50}	606.12	N/A	609.12	535.21	404.59	N/A
Daphnia magna LC_{50}	45.87	N/A	45.87	1545.73	18.42	N/A
T.pyriformis IGC_{50}	1372.67	N/A	N/A	12725.59	N/A	N/A
Oral rat LD_{50}	1499.81	N/A	53.60	198.04	2496.78	N/A
Bioaccumulation factor	0.85	N/A	0.85	1.12	0.39	N/A
Developmental Toxicity	**Non-toxic**	N/A	Toxic	Toxic	Toxic	N/A
Mutagenicity	Negative	N/A	Negative	Negative	Negative	N/A

HKII, but it has shown toxic effect in *in-vitro* analysis (Fontaine et al., 2015), which gives an urge to search for compounds that can be developed into potent HKII inhibitor, which are non-toxic and safe to be administered.

Table 8
Estimation of toxicity values for the top six compounds similar to GLC

Toxicity endpoint	Predicted value					
	Compound 23	Compound 3	Compound 2	Compound 21	Compound 12	Compound 14
Fathead minnow LC_{50}	31166.04	2071.30	32728.60	8441.25	18386.86	32728.60
Daphnia magna LC_{50}	15038.30	474.16	9881.54	11182.63	14457.48	9881.54
T.pyriformis IGC_{50}	99007.65	N/A	34586.97	N/A	8704.83	34586.97
Oral rat LD_{50}	20804.48	2920.90	14201.45	13792.45	21288.21	14201.99
Bioaccumulation factor	0.31	0.41	0.28	N/A	0.22	0.28
Developmental Toxicity	Non-toxic	Non-toxic	Non-toxic	Non-toxic	Non-toxic	**Non-toxic**
Mutagenicity	Negative	Negative	Negative	Negative	Negative	Negative

Table 9
Estimation of toxicity values for the top six compounds similar to BG6

Toxicity endpoint	Predicted value					
	Compound 8	Compound 9	Compound 25	Compound 26	Compound 18	Compound 12
Fathead minnow LC_{50}	12144.34	12144.34	40759.57	N/A	N/A	40759.57
Daphnia magna LC_{50}	2350.97	2350.97	3596.98	N/A	N/A	3596.98
T.pyriformis IGC_{50}	6446.10	6446.10	15497.48	N/A	N/A	15497.48
Oral rat LD_{50}	9716.11	9716.11	12710.41	N/A	N/A	12710.41
Bioaccumulation factor	0.25	0.25	0.38	N/A	N/A	0.38
Developmental Toxicity	Non-toxic	**Non-toxic**	Toxic	N/A	N/A	Non-toxic
Mutagenicity	Negative	Negative	Negative	N/A	N/A	Negative

CONCLUSION

In conclusion, compounds similar to 2DG, GLC, and BG6 had been successfully identified by using ligand-based screening program. Six top-ranked compounds were then obtained for each query molecule, following the docking study, based on their binding energy and number of H bonds. Compound 9, which is an analogue of GLC, emerged as a good inhibitor, due to its best binding energy (-8.50 kcal/mol), and six H bonds (four of which are formed with Thr620, Asn683,cAsp657 and Glu742, respectively, and the remaining two with Asn656), as well as its predicted non-toxicity. Compound 14, which was most similar to BG6, had also been suggested as a potential inhibitor, because of its good binding energy (-7.09 kcal/mol), four H bonds formed with Glu629 and Asn656, negative mutagenicity, and its predicted non-toxicity. Finally, Compound 4, an analogue of 2DG, was suggested as a potential inhibitor of HKII, based on its good binding energy (-7.63 kcal/mol), with two H bond formed with Ser893 and Gln739, as well as its non-toxicity. The limitation of this studies is the utilization of only one compound library for screening the analogues of the query molecules, and through molecular docking, the interaction of the ligand and protein is analyzed in rigid condition, which might not be fully accurate. It was thus suggested that further molecular dynamics simulation studies could be conducted, where this analysis strengthens the choice of compounds to be utilized in the subsequent analysis. The selected compounds from this studies will be tested with purified recombinant HKII for inhibition analysis and subsequently will be tested against human fibroblast cells infected with DENV-2, to observe their effects in depleting DENV replication. In sum, Compound 4, Compound 9, and Compound 14 have great potentials as potent inhibitors of HKII, based on virtual screening and toxicity studies, and should be further tested in the future *in vivo* and *in vitro* inhibition studies.

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