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Evaluating The Effects of Chitin and Its Analogues with Improved Solubility on Human Hexokinase Isoform 2 for Dengue Treatment via Virtual Screening Analyses

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ABSTRACT. Dengue fever, a vector-borne disease caused by dengue virus (DENV) is currently endemic in over 100 countries. Despite being pronounced as one of the WHO's ten threats to global health in 2019, there are currently no antiviral therapies for dengue fever available in the market. Hence, finding the cure for dengue is still currently under pursuit. Chitin, the most abundant biopolymer after cellulose was shown to be one of the potential inhibitors of human hexokinase isoform 2 (HK2), which can subsequently impair DENV upon their replication in human bodies. However, the insolubility characteristics of chitin may cause issues in the subsequent *in vitro* and *in vivo* analyses. This project aims to screen for analogues of chitin with improved solubility and high binding affinity when interacting with HK2 *via* an in silico approach. In this study, ligand-based screening was conducted to find the analogues of chitin, while solubility prediction was performed to predict the solubility of the analogue compounds. Subsequently, structure-based screening was conducted via molecular docking to observe the binding affinity of the analogues with HK2. As a result, a compound known as β-N-acetyl-D-galactosamine was identified as an analogue of chitin with a similarity score of 99.47%. The compound also possessed high solubility (higher than 0.06 mg/ml) and negative LogP value (-3.22), which indicates a higher preferential solubility in water. In addition to that, toxicity prediction test exhibited that β-N-acetyl-D-galactosamine falls under class 6 of toxicity, indicating that it is a non-toxic compound. Thus, a potential drug that may treat dengue fever and safe to be administered to patients can be potentially developed by using this compound.

Keywords: Chitin, chitin analogues, dengue fever, dengue treatment, hexokinase isoform 2

INTRODUCTION

Dengue fever is a viral disease carried by female *Aedes aegypti* mosquito that affects more than 100 nations, with Malaysia having the highest prevalence among infectious diseases (Mashudi et al., 2022). Dengue fever is caused by dengue virus (DENV), a mosquito-transmitted RNA virus that consists of four different but closely related serotypes, which are DEN-1, DEN-2, DEN-3, and DEN-4 (Back & Lundkvist, 2013). If an individual is infected with one serotype, it will render lifetime immunity to that particular serotype but not to others (Reich et al., 2013). The World Health Organization (WHO) has classified dengue as one of the top ten global health hazards in 2019. Dengue affects around 3.9 billion people in 129 countries, with an estimated 96 million symptomatic cases and 40,000 deaths per year (Ahmad Zamzuri et al., 2022). The highest number of infection cases was recorded in 2023, with over 6.5 million cases along with more than 7,300 dengue-related fatalities recorded, according to the WHO. However, despite this condition, there are currently no specific antiviral therapies available on the market for this disease. Due to the fact that the disease is caused by four viruses known as DEN serotypes 1-4, there is a growing public health need for an effective dengue prevention approach.

Currently, the only authorized live attenuated tetravalent dengue vaccines in the market are Sanofi Pasteur's dengue vaccine which is Dengvaxia® (CYD-TDV) (Thomas & Yoon, 2019) and Qdenga® (TAK-003) (Angelin et al., 2023). Dengvaxia® vaccine has been approved for use by the Food and Drug Administration (FDA) on 1st May 2019 and has been registered in 20 dengue-endemic countries (Thomas & Yoon, 2019). However, despite its effectiveness in treating DENV, Dengvaxia® has significant limits in its usage. Dengvaxia® is not licensed for treatment in

individuals who were not previously infected with a dengue virus serotype or those whose medical history is uncertain (Thomas & Yoon, 2019). Those who have never been previously infected are at a greater risk of acquiring severe dengue disease if they are vaccinated and subsequently infected with the dengue virus (Thomas & Yoon, 2019). Contrary to Dengvaxia®, Qdenga® vaccine is able to be administered to dengue-unexposed individuals (Angelin et al., 2023; Freedman, 2023). Qdenga® has been approved for use in 2022 for individuals aged ≥ 4 years old (Angelin et al., 2023; Freedman, 2023). To this date, this vaccine has demonstrated excellent effectiveness against DENV by a clinical test of individuals aged 4 to 16 years old in endemic locations (Angelin et al., 2023). Nevertheless, since this vaccine is still new in the market, there is still a lack of information about its effectiveness on individuals aged 16 and above. Consequently, the discovery of novel drugs is still highly pertinent and necessary in the battle against dengue infection.

Viruses, including DENV, rely on the energy and biosynthetic building blocks given by host cellular metabolism for their replication since they do not have their own metabolism. DENV exploits multiple metabolic pathways during its replication in infected cells, including glycolysis, fatty acid oxidation, the anaplerotic role of glucose, and the oxidation of glutamine (Fernandes-Siqueira et al., 2018). During DENV infection, central carbon metabolism, particularly glycolysis, undergoes substantial changes (Fontaine et al., 2015). Pharmacological suppression of the glycolytic pathway drastically reduced DENV RNA synthesis and infectious virion production, proving that glycolysis is necessary during DENV infection (Fontaine et al., 2015). Thus, glycolysis can be used to monitor viral activity in humans after being infected.

Glycolysis is an energy-producing mechanism that results in the cleavage of a hexose (glucose) to a triose (pyruvate) (Bender, 2013). The initial stage in glycolysis is the breakdown of glucose into glucose-6-phosphate (G6P), which is catalyzed by the hexokinase isoform 2 (HK2) enzyme. According to Fontaine et al. (2015), during DENV infection, glucose consumption is increased, and eliminating exogenous glucose from DENV-infected cells has a substantial effect on viral replication (Fontaine et al., 2015). Furthermore, DENV-infected cells exhibit higher expression of both glucose transporter 1 and HK2. HK2 has been recognized as an important therapeutic target due to its role in viral multiplication and survival in host cells. It was also shown that HK2 enhanced glucose consumption in infected cells since it is a key enzyme in the glycolytic pathway, paving the groundwork for the enzyme to be a target in antiviral therapy (Tanbin et al., 2020). DENV stimulates the glycolytic pathway, hence hindering glycolysis is significant in reducing the infection of DENV (Fontaine et al., 2015). Thus, this study focuses specifically on the glycolytic pathway owing to the pivotal function of HK2 within the pathway rendering it an intriguing therapeutic target.

Based on previous virtual analyses research, chitin, the second most abundant organic polymer in nature, has been identified as a potential inhibitor that may inhibit the activities of HK2 and hence limits DENV replication in the human's glycolysis. However, despite being one of the potential HK2 inhibitors, chitin has a restriction in its solubility. Because of the strong inter- and intramolecular hydrogen bonding, chitin is insoluble in most organic solvents such as water and diluted acids, limiting its usage in various industries. Furthermore, most solvents used to dissolve chitin are toxic and corrosive such as Dimethyl Sulfoxide (DMSO) (Rouhani Shirvan et al., 2019). Because of these limitations, potential antiviral therapies that utilize chitin as the inhibitor of HK2 cannot be developed. Due to these factors, it is critical to identify molecules that mimic chitin but possess improved solubility, so that these compounds may be proposed to replace chitin in the context of novel drug development.

In this study, potential HK2 inhibitors based on chitin as the query molecule were screened by using ligand-based screening, solubility prediction, and structure-based screening. The purpose of ligand-based screening is to find the analogue compounds by using chitin as the query molecule. From the analogue compounds, five compounds that have the highest similarity to chitin were chosen for solubility predictions and structure-based screening. In the solubility prediction method, the aqueous solubility of the analogue compounds as well as the partition coefficient (LogP) were determined by using ChemAxon's solubility predictor. Subsequently, structure-based screening is done by molecular docking between the analogue compounds and HK2. Molecular docking between the two compounds aims to predict the ideal interaction of a ligand with a target protein, resulting in enzyme-ligand complexes, and applies scoring functions to quantify the protein-ligand complex's binding affinity (Yang et al., 2022). Then, the binding energy of the analogue compounds with the target protein was then used to select the potential inhibitor. In addition to that, toxicity tests were also conducted to observe the toxicity of the potential compound so that it can be further used for novel drug development.

EXPERIMENTAL SECTION

Ligand-Based Screening

In ligand-based screening, Rchemcpp web-based program was utilized in order to find the analogue compounds by using chitin as the query molecule. Rchemcpp is a web-based software for identifying structural analogues in databases such as ChEMBL and Drugbank. Rchemcpp implements the best-performing similarity functions, i.e., molecular

kernels, as structural similarity measures. Molecule kernels have outperformed other similarity measures and are currently governing machine learning problems (Klambauer et al., 2015). The first step of ligand-based screening was to load the SD file of the query molecule into the program. Subsequently, the list of analogues based on the chosen databases were generated by the Rchemcpp software. In this software, the similarity score was rated according to the compounds with similar structures to chitin, and the top ten compounds from the results out of 500 were then analysed in the solubility prediction study.

Solubility Prediction

Solubility prediction studies were carried out in this study to predict the solubility of the analogue compounds in aqueous solution. ChemAxon's solubility predictor program is the primary tool employed in this investigation. The solubility predictor in ChemAxon can predict a compound's aqueous solubility at a given pH, as well as its intrinsic solubility and partition coefficient (LogP) (Szisz, 2023). It can generate quantitative findings, such as solubility in logS, mg/mL, or mol/L. The first step in predicting solubility was to load the SD files of the analogue compounds into the program. Subsequently, the program produced the compounds' 2D structure. The solubility was then computed using the solubility calculator, and the results were generated together with the graph to estimate the solubility at different pH levels. In addition, the software automatically generated the solubility category whether the compound has high solubility (higher than 0.06 mg/ml) or low solubility (lower than 0.06 mg/ml).

Molecular Docking

Structure-based virtual screening is a computational method used in the early-stage drug development research to search for novel bioactive compounds against a specific therapeutic target in a chemical compound library (Li & Shah, 2017). In this study, molecular docking, which is part of the structure-based virtual screening method was conducted by using InstaDock software (Mohammad et al., 2021). The initial step in structure-based screening was to obtain the protein of interest, which is HK2 (PDB ID: 2NZT), from the Protein Data Bank (PDB) website. Meanwhile, the analogue compounds' SD files were retrieved from the PubChem website (https://pubchem.ncbi.nlm.nih.gov/). This procedure used InstaDock software to perform molecular docking of the analogue molecules with HK2. InstaDock is a Python front-end graphical user interface for virtual high-throughput screening based on molecular docking in a single step (Mohammad et al., 2021). The analogous compounds' SD files were retrieved from the PubChem website. The results were analyzed based on the binding free energy (kcal/mol) and torsional energy.

Toxicity Test

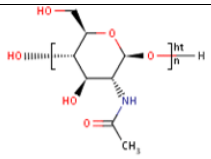
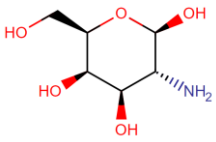
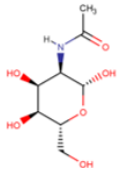
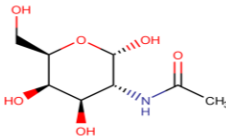
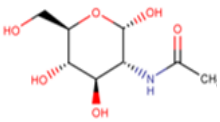
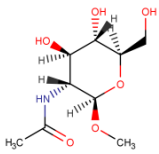
One of the crucial parameters in novel drug development is the toxicity test. Toxicity testing is critical in discovering possible detrimental effects produced by chemical compounds. In general, persistent chemical exposure causes genotoxicity, carcinogenicity, immunotoxicity, and reproductive and developmental toxicity in humans (Gupta et al., 2022). The toxicity test prediction in this study is done by the Toxicity Estimation Software Tool (TEST) from the United States Environmental Protection Agency (EPA). This software utilized the Quantitative Structure Activity Relationship (QSAR) methodologies which are mathematical models that predict toxicity based on the physical properties of chemical structures (or also known as molecular descriptors) (Gatnik & Worth, 2010; Noga et al., 2023; Vegosen & Martin, 2020). To predict the toxicity of the compound, the SD file of the best compound was deposited into the software and the oral rat LD_{50} for the compound was generated by the software.

RESULTS AND DISCUSSION

Ligand-Based Screening

Ligand-based virtual screening is a significant approach in discovering the analogues of the query molecule by performing quick screening of enormous compound databases (Sharma, 2023). The approach is based on the idea that compounds with identical structures are likely to have comparable biological functions. Based on the ligand-based screening from Rchemcpp web-based programme, a total of 500 compounds were generated based on chitin, which is the query molecule. From the 500 compounds, the five best compounds were chosen for the docking analyses. The list of compounds from ChEMBL and DrugBank is shown in **Table 1**. From **Table 1**, it can be seen that compounds with high similarity scores are β-N-acetyl-D-galactosamine, N-Acetyl-D-Allosamine, N-Acetyl-2-Deoxy-2-Amino-Galactose, and 2-(Acetylamino)-2-Deoxy-a-D-Glucopyranose with 99.47% (0.9947). The closer the similarity score to 1, the higher the similarity of the compound to the query molecule. Rchemcpp web-based programme works by utilising molecule kernels which is a function that converts the similarity of two chemical compounds into a numerical value. The structural similarity measurement from Rchemcpp frequently relies on the number of substructures that appear in both query and analogue molecules (Klambauer et al., 2015). Hence, compounds that appear to have a high similarity score might exhibit almost similar characteristics such as chemical structure and molecular formula as chitin (Klambauer et al., 2015). Furthermore, all of the compounds listed feature eight carbon atoms bound with hydrogen, oxygen, and nitrogen atoms, which is identical to chitin. This explains why the indicated compounds have a larger percentage of similarities compared to other tested compounds.

Table 1. The list of analogue compounds generated from ChEMBL and DrugBank databases

No	Ligand Name	Chemical Structure	Molecular Formula	Similarity Score	Database
1	Chitin (query molecule)		$(C_8H_{13}O_5N)_n$	N/A	N/A
2	β-N-acetyl-D-galactosamine		$C_8H_{15}NO_6$	0.9947	ChEMBL
3	N-Acetyl-D-Allosamine		$C_8H_{15}NO_6$	0.9947	DrugBank
4	N-Acetyl-2-Deoxy-2-Amino-Galactose		$C_8H_{15}NO_6$	0.9947	DrugBank
5	2-(Acetylamino)-2-Deoxy-a-D-Glucopyranose		$C_8H_{15}NO_6$	0.9947	DrugBank
6	Alpha-Methyl-N-Acetyl-D-Glucosamine		$C_9H_{17}NO_6$	0.9894	DrugBank

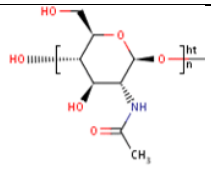
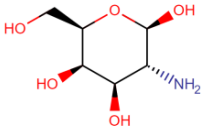
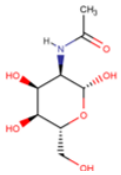
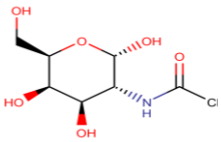
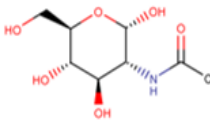
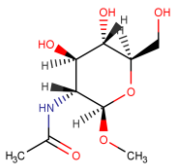
Solubility Prediction

Solubility is an important criterion in drug design and development since it has a direct impact on bioavailability and therapeutic effectiveness. Solubility is the phenomenon of a solute dissolving in a solvent to form a homogenous molecular dispersion, which must occur to achieve the target concentration of the drug in the systemic circulation and activate the intended pharmacological reaction (Deokate et al., 2023). The main issue in developing formulations for novel compounds and generic development is low water solubility. The low solubility of drugs may influence the drug's absorption in the gastrointestinal tract, its dissolving rate, degree of absorption, and bioavailability (Gohil et al., 2023; Jagtap et al., 2023).

Since chitin is insoluble in most organic solvents, it is important to find a compound that can mimic chitin with high solubility. Hence, in this study, the solubility prediction of the five best compounds from ligand-based screening is done by using ChemAxon's solubility predictor software. As seen in **Table 2**, the five best compounds chosen from ChEMBL and DrugBank databases shown to have high solubility (higher than 0.06 mg/ml) in aqueous solution. This is shown at pH 7.4 which is the physiological properties in the body.

Furthermore, one of the important parameters in determining the compound's solubility characteristics is the partition coefficient or also denoted as LogP. LogP is a critical parameter that indicates how well the drug will be absorbed, transported, and dispersed in the body, and also how it should be made and dosed. A medication with low water solubility but high lipophilicity (high positive LogP) will have poor bioavailability (Tredwell & Gouverneur, 2012). Highly lipophilic medications tend to become poorly soluble in aqueous environments, such as the gastrointestinal system, resulting in limited absorption into the blood vessels (Klimoszek et al., 2024; Wardecki et al., 2023). As shown in **Table 2**, all compounds exhibit a negative LogP, indicating a preference for solubility in water. This indicates that all of the compounds chosen are hydrophilic and have a high affinity for the aqueous phase (Tredwell & Gouverneur, 2012).

Table 2. Solubility of analogue compounds from ChEMBL and DrugBank databases

No	Ligand Name	Chemical Structure	Molecular Formula	Solubility at pH 7.4	Solubility Category	LogP
1	Chitin (query molecule)		$(C_8H_{13}O_5N)_n$	N/A	N/A	N/A
2	β-N-acetyl-D-galactosamine		$C_8H_{15}NO_6$	-0.17 logS	254.0 mg/mL [High (higher than 0.06 mg/ml)]	-3.22
3	N-Acetyl-D-Allosamine		$C_8H_{15}NO_6$	-0.17 logS	254.0 mg/ml [High (higher than 0.06 mg/ml)]	-3.22
4	N-Acetyl-2-Deoxy-2-Amino-Galactose		$C_8H_{15}NO_6$	-0.17 logS	High (higher than 0.06 mg/ml)	-3.22
5	2-(Acetylamino)-2-Deoxy-a-D-Glucopyranose		$C_8H_{15}NO_6$	-0.17 logS	High (higher than 0.06 mg/ml)	-3.22
6	Alpha-Methyl-N-Acetyl-D-Glucosamine		$C_9H_{17}NO_6$	0.05 logS	High (higher than 0.06 mg/ml)	-2.58

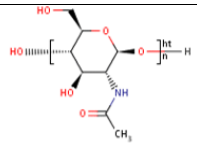
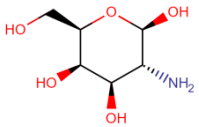
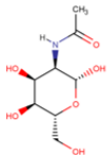
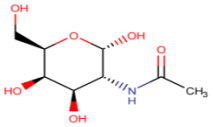
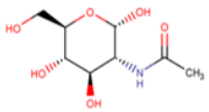
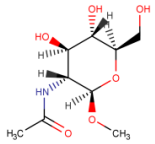
Molecular Docking

Structure-based virtual screening is accomplished by applying molecular docking *via* InstaDock software, in which a ligand and a protein are docked together and result in several docked poses. This docking pose is assessed using a specific scoring function which is binding affinity. The binding affinity principle involves the interaction of small ligands in the active site of the macromolecular target influenced through multiple non-covalent interactions including van der Waals forces, hydrogen bonds, and electrostatic interactions. Besides estimating the binding affinity, molecular docking is also performed to predict the optimal docking pose of the ligand to the target's active site. This is critical when developing compounds that can efficiently alter the biological activity of the target (Li et al., 2015; Pantsar & Poso, 2018).

In this study, the ligand used is from the ligand-based screening in **Table 1** which is the analogues of chitin, meanwhile, the protein used is HK2 (PDB ID: 2NZT). From the screening, the compound with the highest negative binding affinity as seen in **Table 3** is 2-(Acetylamino)-2-Deoxy-a-D-Glucopyranose (compound 5), which was subjected to docking analysis and yielded a binding affinity value of -6.6 kcal mol^{-1} towards the receptor HK2. This is because, a greater negative score implies a stronger binding of the ligand towards the receptor (Owoloye et al., 2022). Protein-ligand binding occurs only when the change in the system's Gibbs free energy (ΔG) is negative, hence explaining the negative of the binding affinity (Du et al., 2016).

Nonetheless, upon comparing the torsional energies of all the compounds in **Table 3**, β-N-acetyl-D-galactosamine (compound 2) is shown to possess a lower torsional energy than other compounds. Torsional energy is described as the amount of energy necessary to overcome torsional strain or the energy difference between eclipsed and staggered conformations. Low torsional energy is more favourable since the binding stability of the compound is high. Thus, β-N-acetyl-D-galactosamine (compound 2) is an excellent compound since it has a low torsional energy of 0.6226, which is even lower than chitin. The docked pose is shown in **Figure 1**.

Table 3. Solubility of analogue compounds from ChEMBL and DrugBank databases

No	Ligand Name	Chemical Structure	Molecular Formula	Binding affinity (kcal/mol)	Torsional energy
1	Chitin (query molecule)		$(C_8H_{13}O_5N)_n$	-6.1	0.9339
2	β-N-acetyl-D-galactosamine		$C_8H_{15}NO_6$	-6.3	0.6226
3	N-Acetyl-D-Allosamine		$C_8H_{15}NO_6$	-6.2	1.8678
4	N-Acetyl-2-Deoxy-2-Amino-Galactose		$C_8H_{15}NO_6$	-6.1	1.8678
5	2-(Acetylamino)-2-Deoxy-a-D-Glucopyranose		$C_8H_{15}NO_6$	-6.6	1.8678
6	Alpha-Methyl-N-Acetyl-D-Glucosamine		$C_9H_{17}NO_6$	-6.3	1.8678

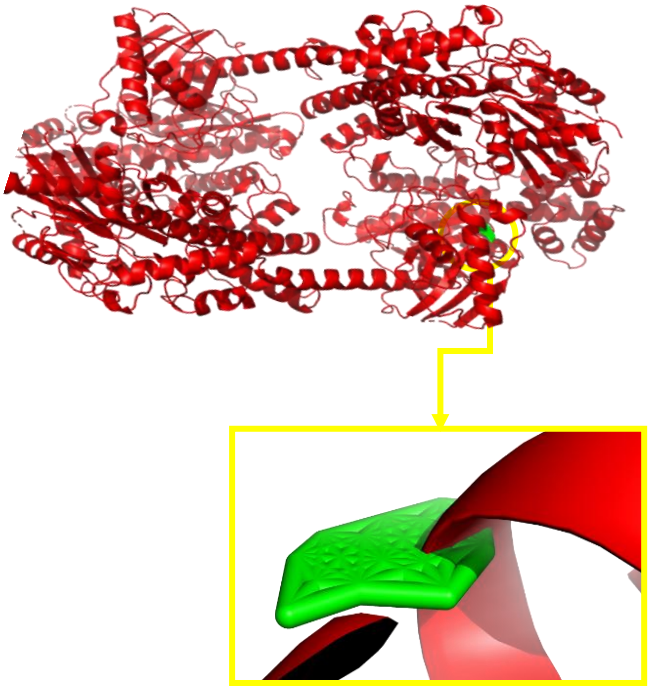


Figure 1. The post-docking visualization of β-N-acetyl-D-galactosamine (green colour) with HK2 (red colour) with a binding affinity of -6.3 kcal/mol

Toxicity Test

Toxicity prediction plays an important role in drug development since it aids in predicting the rate of success and safety of novel therapeutic drugs. According to Amorim et al. (2024), approximately 90% of drugs are unable to make it through clinical trials due to the discovery of unanticipated toxicity issues during the process. Traditional toxicity prediction relied on animal testing which caused limitations including costly, long trial durations, and unethical (Liu et al., 2022; Rajpoot et al., 2022; Xu et al., 2020). With the advancement of technology, the usage of machine learning (ML) and artificial intelligence (AI) has appeared as a potential approach to predict the toxicity of drugs, adopting various techniques including deep learning, neural networks, and quantitative structure-activity relationship (QSAR) (Malani et al., 2023).

In this research, the toxicity test prediction was done by using the Toxicity Estimation Software Tool (TEST). TEST software helps users to estimate toxicity by utilizing quantitative structure-activity relationships (QSAR) methodologies which consist of the consensus method, hierarchical method, single-model method, group contribution method, and nearest neighbour method (Noga et al., 2023). However, in this research, only the consensus method is chosen since it has been shown as the best method, compared to others (Noga et al., 2023).

For toxicity test prediction, the chosen compound is β-N-acetyl-D-galactosamine since it has the best result from ligand-based screening, solubility prediction, and structure-based screening. Hence, the result of predicted oral rat LD_{50} (median lethal dose) which is the chemical dosage in mg/kg body weight that, when consumed orally, will cause death in 50% of rats is shown in **Table 4**. The reason for choosing the said properties is because oral rat LD_{50} is more common and relevant in finding toxicity for mammals since the drug is going to be administered to patients. From the result, it is shown that β-N-acetyl-D-galactosamine falls under toxicity class 6 since the predicted LD_{50} of the compound is 6494.63 mg/kg, hence showing that this compound is practically non-toxic and can be developed as a drug with suitable doses.

The toxicity classes are as stated below:

- Class 1: Fatal if swallowed ($LD_{50} \leq 5$)
- Class 2: Fatal if swallowed ($5 < LD_{50} \leq 50$)
- Class 3: Toxic if swallowed ($50 < LD_{50} \leq 300$)
- Class 4: Harmful if swallowed ($300 < LD_{50} \leq 2000$)
- Class 5: May be harmful if swallowed ($2000 < LD_{50} \leq 5000$)
- Class 6: Non-toxic ($LD_{50} > 5000$)

Comparative Analysis of Currently Available Dengue Drugs and β-N-Acetyl-D-Galactosamine

Although there is no approved antiviral therapy for dengue, several substances and therapeutic approaches are currently being explored. Few small molecule antivirals are currently undergoing clinical trials to evaluate their efficacy towards the virus. Among them are efavirenz, tipranavir, dasabuvir, and 7D (Chauhan et al., 2024). Efavirenz, tipranavir, and dasabuvir target the virus's envelope and non-structural proteins, as well as host factors like the PF4-CXCR3 axis (Chauhan et al., 2024).

Dengue fever has been identified to have a substantial effect on various organ systems, with the liver being the most frequently affected (Samanta & Sharma, 2015). Comparing β-N-acetyl-D-galactosamine to small molecule compound, β-N-acetyl-D-galactosamine is a promising compound in drug development due to its capacity to improve the transportation and effectiveness of antisense oligonucleotides (ASO), as well as play a role in glycosylation processes (Alexander et al., 2019). This is done by targeting the asialoglycoprotein receptor (ASGPR) in the liver cell, facilitating more effective absorption of the ASOs by the liver, enabling them to perform their therapeutic actions more efficiently (Alexander et al., 2019).

Furthermore, the preparation of β-N-acetyl-D-galactosamine from N-acetyl-D-glucosamine can be performed through a series of chemical reactions which entails selective protection of hydroxy groups (Ito et al., 2004). This approach enables the efficient production of the compound, which is critical for its usage in drug development. β-N-acetyl-D-galactosamine's structural features also aid in its function as a suitable candidate for DENV drug, especially its conformation and the presence of the N-acetyl group, offering stability and functionality in biological systems, thus rendering it vital for its role in drug administration and therapeutic applications (Lakshmanan et al., 2001). In addition, the β-N-acetyl-D-galactosamine role focuses on its biological action, specifically immunological regulation and glycosyltransferase inhibition (Tomaska & Parish, 1981). This is due to the fact that galactosamine can strongly inhibit the secondary IgG antibody response, a component of the body's immune system that aids in the fight against infections (Tomaska & Parish, 1981).

Safety Consideration for β-N-Acetyl-D-Galactosamine

β-N-acetyl-D-galactosamine is a compound that is highly promising in the field of drug development, particularly due to its high solubility and ability to target HK2. Nevertheless, considerations of long-term

Table 4. Predicted LD_{50} toxicity from TEST software

Properties	Results
Predicted oral rat LD_{50}	6494.63 mg/kg
Predicted toxicity class	Class 6

toxicity in humans of this compound must be taken in order for a novel drug to be developed and undergo clinical trial.

There are few studies that have shown that β-N-acetyl-D-galactosamine can be metabolized in the liver hence causing hepatocellular injury through a process known as "lethal synthesis", particularly when administered in a high dosage (London et al., 1990; MacNicoll et al., 1978). This phrase refers to the biochemical activation of certain chemicals that cause cellular harm. This happens during the phosphorylation of galactosamine by enzymes that participate in glucose and galactose metabolism. This metabolic change can result in the generation of toxic substances that are not generally seen in untreated tissue, eventually leading to liver cell death (London et al., 1990). This is then proved by a study from Tsutsui et al. (1997) where they investigate the effect of high doses of galactosamine in mice hepatocytes in vivo. From their study, high dosages of galactosamine have been demonstrated to produce apoptosis and necrosis in mouse hepatocytes, with visible cellular degradation and increased hepatic enzyme activity, suggesting liver injury (Tsutsui et al., 1997).

In addition to that, a study has also shown variability in response to galactosamine-induced hepatoxicity in male Sparague-Dawley rats (Coen et al., 2012). Certain subjects exhibit adaptive responses following re-exposure, whilst others demonstrate elevated sensitivity. This variability is associated with alterations in metabolic profiles and cytokine levels, indicating that individual susceptibility contributes to detrimental outcomes (Coen et al., 2012). Certain individuals may possess a metabolic or immune system tendency that renders them more susceptible to liver injury, whilst others may exhibit more resilience (Coen et al., 2012).

Nevertheless, presently, there are no available studies examining the long-term effects and potential off-target reactions associated with this compound. While the studies give insights into the mechanisms and hepatoxicity of β-N-acetyl-D-galactosamine, it does not explicitly explore the consequences in humans. The findings are based on animal models, which are frequently used to anticipate potential human reactions, yet further studies involving humans are required. Therefore, it is crucial to closely monitor its dosage to ensure safety and effectiveness of drugs produced by this compound.

Formulation and Delivery Optimisation of β-N-Acetyl-D-Galactosamine

The enhanced solubility of β-N-acetyl-D-galactosamine from this study renders it a viable option for therapeutic purposes. Nevertheless, appropriate formulation and delivery methods are essential for its clinical implementation. Optimizing the formulation and administration of β-N-acetyl-D-galactosamine as a therapeutic agent necessitates a comprehensive strategy that includes solubility and stability studies, formulation development, and targeted delivery system assurance. This subsection aims to propose strategies and efforts to improve its formulation and delivery.

Drug solubility is the utmost crucial parameter to be considered in novel drug development. Since β-N-acetyl-D-galactosamine has been shown to have improved solubility in aqueous solution via virtual screening, the solubility and stability studies are still important in understanding the solubility profile of this compound. Detailed solubility studies, including examining this compound under different pH conditions may provide details on the absorption of the compound in different parts of the body such as the stomach (acidic) and intestines (neutral to basic). This is due to the fact that the solubility of drugs can be affected by the pH of the surroundings and the presence of amphiphiles in the digestive tract (Dunn et al., 2019). Experimental approaches include generating saturated solutions and altering pH with titrants to test solubility at various pH levels would aid in comprehending the solubility of compounds with acidic or basic groups (Stuart & Box, 2005).

Furthermore, various formulation strategies can be employed for the formulation development of β-N-acetyl-D-galactosamine. This includes strategies such as encapsulation in nanoparticles (Biswal et al., 2024), liposomes (Mhaske & Raskar, 2024), or dendrimers (Patil et al., 2023) that are promising to improve the compound's solubility and stability. Other than that, the utilisation of excipients in drugs can also help in improving solubility and bioavailability while maintaining therapeutic efficacy (Patel et al., 2020).

In the context of drug delivery, developing targeted delivery systems of β-N-acetyl-D-galactosamine towards DENV-infected cells is crucial in enhancing drug delivery specificity and efficacy while minimizing systemic side effects. Systems-based methodologies, including as proteomics and phage display libraries, can discern target molecules and ligands that contribute to the specificity of GalNAc delivery systems. These strategies facilitate the development of carriers that concentrate on targeted areas, such as HK2, by interacting with particular cell surface receptors (Myerson et al., 2015).

CONCLUSIONS

Based on the findings of this study, β-N-acetyl-D-galactosamine, an analogue compound of chitin with 99.47% similarities, is the best ligand that may well bind to HK2, with a high solubility in aqueous solution and the highest binding affinity with HK2 of -6.3 kcal/mol. Furthermore, β-N-acetyl-D-galactosamine is also non-toxic where it possesses an oral LD_{50} of 6494.63 mg/kg by TEST software, which shows that novel drug development from this compound is possible to be administered to patients.

Additional assessments of β-N-acetyl-D-galactosamine as powerful HK2 inhibitors are needed in the future to develop potential anti-dengue therapies since virtual screening can only be utilised as a prediction of the compound's interaction. Hence, subsequent laboratory analyses are needed in order to validate the results from the virtual screening. Although translating the in silico finding of β-N-acetyl-D-galactosamine to in vitro and in vivo testing anticipates a few challenges, especially with the complex synthesis of the compound, the potential for successful implementation remains feasible. With careful strategies and planning, navigating the hurdles would yield a successful outcome. Furthermore, since this study only focuses on targeting HK2 and not specifically on different types of DENV, further studies such as dengue model studies, in vitro and in vivo assays, and many more.

In addition, concurrent hepatotoxicity is an important barrier that must be resolved in its drug discovery. The advancement of β-N-acetyl-D-galactosamine as a prospective drug necessitates a thorough study to alleviate its toxicological consequences such as altering the compound in order to reduce its toxicity or discovering methods to safeguard non-target cells from its detrimental effects. Overall, this study has identified promising compounds that may inhibit HK2 which may also be a potential compound for the novel drug development of dengue virus.

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