

In-Silico Computational Study of Polyhydroxylated Nortropane Alkaloid from Kangkong (*Ipomoea aquatica*) as a Potential Alpha-Glucosidase Inhibitor for Possible Blood Sugar Reduction via Molecular Docking

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Abstract—This study, In-silico computational study of polyhydroxylated nortropane alkaloid from kangkong (*Ipomoea aquatica*) as a potential alpha-glucosidase Inhibitor for possible blood sugar reduction via molecular docking, investigated polyhydroxylated nortropane alkaloids from Kangkong (*Ipomoea aquatica*) as potential α -glucosidase inhibitors for blood sugar reduction using molecular docking. Calystegine derivatives (A3, B1, B2, B3, B4, and C1) were docked against N-terminal maltase-glucoamylase (NtMGAM), C-terminal maltase-glucoamylase (CtMGAM), and N-terminal sucrase-isomaltase (NTSI) enzymes, with acarbose as the reference inhibitor. All ligands exhibited negative binding affinities, indicating stable and energetically favorable interactions. Among the derivatives, Calystegine B4 demonstrated the highest binding affinities across all targets (−6.9 kcal/mol for NtMGAM, −6.6 kcal/mol for CtMGAM, and −6.7 kcal/mol for NTSI), followed by B1 and B3, while A3, B2, and C1 exhibited moderate to low interactions. The binding energies of the calystegines were comparable to that of acarbose. Interaction analyses revealed that calystegines bind within the catalytic sites through hydrogen bonding and van der Waals interactions, suggesting a competitive inhibition mechanism. These findings identify Calystegine B4 and B1 as the most promising candidates and support the potential application of calystegine derivatives as natural α -glucosidase inhibitors for regulating post-meal blood glucose levels.

Index Terms—*Ipomoea aquatica*, calystegine, α -glucosidase inhibitor, molecular docking, maltase-glucoamylase, sucrase-isomaltase, binding affinity.

1. Introduction

A. Background of the Study

Ipomoea aquatica, popularly known as kangkong, is a green leafy vegetable rich in biologically active compounds such as flavonoids, phenolics, and nortropane alkaloids called calystegines (Saikia, 2023; Nakade & Nasare, 2024). These alkaloids imitate sugar molecules that can fit into the enzyme's active site, possibly blocking α -glucosidase and slowing down

carbohydrate digestion. Since α -glucosidase plays an essential role in converting complex carbohydrates into glucose, its inhibition can help manage blood sugar levels after meals. Therefore, this study seeks to evaluate whether the polyhydroxylated nortropane alkaloids present in *Ipomoea aquatica* can function as alternative α -glucosidase inhibitors. Using molecular docking analysis, this research aims to compare their binding affinities with those of established commercial inhibitors such as acarbose, voglibose, and miglitol.

Southeast Asian nations have disturbingly high prevalence rates of reported diabetes cases. For instance, Malaysia reports a diabetes prevalence rate of 16.8% among adults, closely followed by Singapore at 14.2% and Brunei at 13.2% (Lim, Rajah, Lee, Wong, Tan, & Karim, 2021). Furthermore, Kunasegaran, Balasubramaniam, Arasoo, Palanisamy, & Ramadas (2021) show a concerning prevalence of Gestational Diabetes Mellitus (GDM) in Vietnam reaching 21.3% in pregnant women. In Thailand, the age- and sex-standardized prevalence of diabetes among Thai adults increases from 7.5% in 2004 to 10.1% in 2020. Only 62.8% of adults with diabetes are aware of their condition, meaning nearly 4 out of

10 cases remain undiagnosed (Aekplakorn, Chariyalertsak, Bumreraj, Assanangkornchai, Taneeapanichskul, Neelapaichit, & Patanavanich, 2025). Based on the IDF Diabetes Atlas (2025), the estimated diabetes prevalence among adults aged 20 to 79 years in the South-East Asia Region is approximately 10.8% for 2024, affecting 107 million adults, with nearly 42.7% of cases remaining undiagnosed (Magliano & Boyko, 2025). The escalating prevalence of diabetes is particularly pronounced in the Southeast Asian region, which bears a disproportionately high share of the global disease burden due to rapid urbanization, demographic shifts, and lifestyle changes (Liberty, Kurniawan, Wijaya, Soewondo, & Tahapary, 2021).

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In the Philippines, approximately 4.7 million Filipino adults are affected by diabetes, with a critical 53.5% of these cases remaining undiagnosed (Magliano & Boyko, 2025). This burden is highly visible in vulnerable populations, such as community-living older persons, who show a diabetes prevalence of 20.5% (Giron & de la Vega, 2022). Uy and Jimeno (2021) reveal that out of 5.43% prevalence of diabetes mellitus (DM), 2.64% of those cases are classified as Young-Onset Diabetes (YOD) driven by obesity, metabolic syndrome, and certain social behaviors such as smoking and drinking. Acha's (2024) cross-sectional study finds that non-adherence to Type 2 Diabetes medication at Cebu South Medical Center is at 37.7% due to socioeconomic factors, primarily medication costs and financial constraints. Meanwhile, certain scientists are testing new alternatives to combat the diabetes. Tulin, Atok, Tulin, Vergara, & Loreto (2024) demonstrate that several Philippine indigenous medicinal plants are potent natural sources of α -amylase and α -glucosidase inhibitors. This breakthrough suggests new knowledge for developing new therapeutic agents to manage diabetes mellitus.

In Davao City, the clinical urgency of diabetes is underscored by recent data from the Southern Philippines Medical Center (SPMC). Reports from July 2025 indicate that the hospital's outpatient department now evaluates approximately 100 diabetic patients every day. Medical experts at the facility, including endocrinologist Dr. Jenny Pareñas, note that a significant number of these individuals are diagnosed only after the onset of severe, irreversible complications. These advanced medical conditions frequently include kidney failure, cardiovascular disease, and vision loss, which now account for over 50 percent of the mortality rate at the institution (Lodovice, 2025). In response to this need, a pilot diabetes intervention program by Candelario, Bandala-Dingal, Misoles, & Cuajotor (2023) in the rural Southern Philippines successfully uses a culturally tailored, vernacular-based learning module to significantly improve participants' disease-related knowledge and health practices regarding diabetes and its self-management. This can improve the knowledge and self-management practices of rural Filipinos with hyperglycemia, potentially leading to better public health outcomes and reduced disease complications in underserved areas.

The research gap of this study is the lack of study on molecular docking analysis of *Ipomoea aquatica* (Kangkong) as a potential α -glucosidase inhibitor. While many studies examine flavonoids and phenolic compounds as α -glucosidase inhibitors, there is limited investigation on nortropane alkaloids, especially polyhydroxylated derivatives found in *Ipomoea aquatica*. Moreover, most molecular docking or in silico analysis related to *Ipomoea aquatica* are conducted in other countries. Specifically, there are no existing docking research studies published that investigate *Ipomoea aquatica* as a potential α -glucosidase inhibitor within the context of Davao del Norte. This gap shows the need to investigate *Ipomoea aquatica* through molecular docking analysis to explore its potential and provide a foundation for future laboratory-based studies.

This study seeks to investigate the potential of *Ipomoea*

aquatica (kangkong) as a natural α -glucosidase inhibitor using molecular docking analysis. The findings support the development of affordable, and locally accessible alternatives to synthetic antidiabetic medications. The findings also promote public awareness of the importance of scientific research in improving community health and ultimately enhance the quality of life among individuals who are affected by diabetes.

The results of this study are presented to the research panel and faculty members of the institution for evaluation, feedback, and validation. After completion, the researchers present the study at the research congress at Arriesgado College Foundation, Inc. and local research conferences, if given the opportunity. Moreover, if possible, the researchers submit the study's results to online scientific journals and research forums focused on phytochemistry and computational biology. After completion, the research is also archived in the college library, giving future students and educators can readily access it as a valuable resource. Through these dissemination efforts, the study aims to turn its findings into meaningful contributions that benefit both the academic community and society at large.

B. Statement of the Problem

This study investigates the in-silico behavior of Polyhydroxylated Nortropane Alkaloids (Calystegines) derived from *Ipomoea aquatica* (kangkong) as potential α -glucosidase inhibitors. Through molecular docking analysis, the study aims to determine their predicted binding affinity, molecular interactions, and potential inhibitory mechanism toward α -glucosidase, which may indicate their feasibility as blood sugar-lowering agents. Specifically, this study seeks to answer the following questions through molecular docking analysis:

1. How do the selected Polyhydroxylated Nortropane Alkaloid compounds interact with the active-site residues of the α -glucosidase enzyme as reflected in their binding affinity values and molecular interaction profiles?
2. Do the binding interactions of kangkong-derived Polyhydroxylated Nortropane Alkaloid compounds with α -glucosidase suggest a plausible inhibitory mechanism that could potentially interfere with the enzyme's activity related to blood sugar regulation?
3. What differences in the predicted inhibitory potential of the selected Polyhydroxylated Nortropane Alkaloid compounds derived from *Ipomoea aquatica* are described based on their molecular docking scores and binding affinity values against α -glucosidase?

C. Hypotheses

H₀₁: The selected Polyhydroxylated Nortropane Alkaloid compounds from *Ipomoea aquatica* do not form significant interactions with the active-site residues of the α -glucosidase enzyme based on molecular docking and binding affinity results.

H₀₂: The binding of kangkong-derived Polyhydroxylated Nortropane Alkaloid compounds does not suggest any plausible inhibitory mechanism that could interfere with the enzymic

activity of α -glucosidase.

H₀₃: There are no observable differences in the predicted inhibitory potential among the Polyhydroxylated Nortropane Alkaloid compounds derived from *Ipomoea aquatica* against the α -glucosidase enzyme based on molecular docking scores and binding affinity values.

D. Theoretical Framework

The Enzyme Inhibition Theory, based on the foundational Michaelis-Menten Kinetics of 1913, serves as the conceptual basis of this study. This theory proposes that the substrate (S) reacts with the enzyme (E) to form an enzyme-substrate complex (ES) which later proceeds to an irreversible step to form a product (P) and frees the enzyme for catalysis again (Farhaana, 2024). Within this framework, inhibitors are characterized as agents that bind to the enzyme to decrease its catalytic activity. Biochemical literature distinguishes between three primary types of reversible inhibition based on the interaction between the inhibitor, the enzyme, and the substrate. Competitive inhibition occurs when the inhibitor binds specifically to the active site of the enzyme, thereby preventing the substrate from binding. Noncompetitive inhibition involves the inhibitor binding to a site other than the active site, which reduces the ability of the substrate to bind to the enzyme. Finally, uncompetitive inhibition, occasionally referred to as anticompetitive inhibition, occurs when the inhibitor binds exclusively to the enzyme-substrate complex, a mechanism typically observed in reactions involving more than one substrate (Lewis & Stone, 2023).

On the foundation of this theoretical basis, the research study tackles the possibilities of the use of calystegines, which are known as the nortropane alkaloid present in the *Ipomoea aquatica* plant commonly referred to as kangkong, as the potential natural competitive inhibitors for alpha-glucosidase. This study specifically focuses on how the nature of competitive inhibitors, which are the molecules that are similar to the naturally occurring substrate and tend to compete for the active site of the enzyme, makes the use of calystegines as one of the most effective possibilities, especially because of their similarity to the sugar molecules. This potential existence of the molecules to bind to the active site of the alpha-glucosidase enzyme, as the existing synthetic compounds do, is investigate using the molecular docking study technique, which helps judge the potential of the use of the calystegines compared to the already existing competitive inhibitors.

E. Conceptual Framework

This research focuses on examining polyhydroxylated nortropane alkaloids, as prospective inhibitors of the α -glucosidase enzyme. The α -glucosidase enzyme is an enzyme responsible for breaking down carbohydrates into glucose that affects the blood sugar level after meals.

To address this, the study employs molecular docking simulations to evaluate the binding affinity and interaction patterns of polyhydroxylated nortropane alkaloids with the α -glucosidase enzyme. Through computational algorithms, this research aims to predict whether these compounds can

effectively inhibit α -glucosidase, in comparison to known standard inhibitors.

The graph illustrates how the natural compound and its predicted therapeutic effect via in silico analysis. The Independent Variables (IVs), the Polyhydroxylated Nortropane Alkaloids (Calystegines) isolated from *Ipomoea aquatica*, are computationally modeled to interact with the α -Glucosidase receptor. The Dependent Variable (DV), the resulting Binding Affinity derived from the Molecular Docking Simulation, directly determines the compounds' predicted role as α -Glucosidase Inhibitors. A favorable affinity is predicted to translate into a strong Anti-Diabetic Potential for Kangkong.

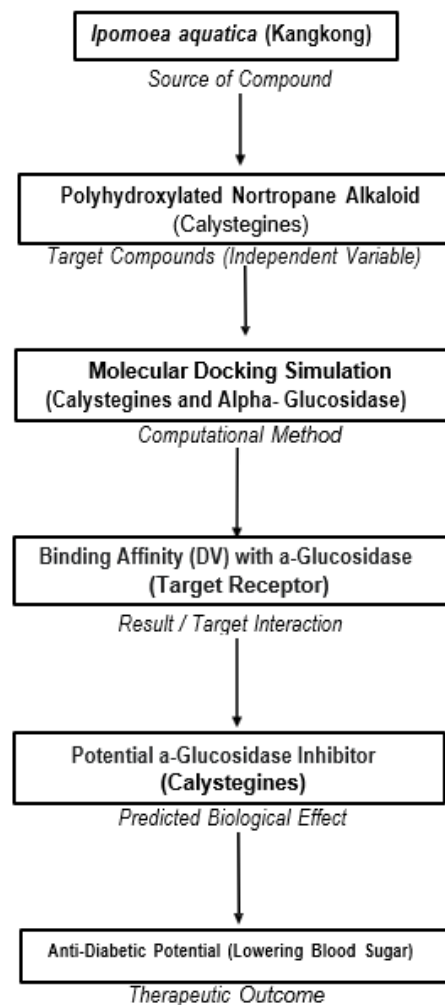


Fig. 1. Conceptual framework

F. Significance of the Study

This study emphasizes the potential of *Ipomoea aquatica* (kangkong) as a useful source of bioactive chemicals for diabetes treatment. Through a molecular docking analysis, this work explores the potential interactions between a polyhydroxylated nortropane alkaloid and glycosidase enzymes, which are highly active in the hydrolysis of carbohydrates to glucose. Inhibition of these enzymes is a proven technique for postprandial regulation of blood glucose levels, and the current study is very pertinent to the issue of control of glycemia. The result of the study will be beneficial to

the following:

Scientists. The results of the study offer a computational basis that helps as a model for future experimental verification and drug discovery research.

Pharmaceutical and medical fields. The result points to a useful inhibitory compound obtained from plants. This has important implications for the creation of less expensive and safer substitutes for synthetic anti-diabetes agents.

Community and local residents. The study emphasizes the importance of locally accessible plants like kangkong, particularly in areas with high diabetes rates. It supports the perception that functional foods made from common vegetables help people stay healthy and prevent illness in the future.

Future Researchers. This entire study not only serves as scientific validation of ethnomedicinal resources, but also provides new avenues for creating plant-based diabetes therapies, thereby improving health outcomes and lowering the disease's cost burden.

G. Scope and Delimitation

The research docked the polyhydroxylated nortropane alkaloids (calystegines), present in *Ipomoea aquatica* (kangkong) as an inhibitor of the enzyme α -glucosidase for lowering blood sugar level. Specifically, the scope of this study was limited to the molecular docking analysis of Calystegine A3, B1, B2, B3, B4, and C1 as potential inhibitors of α -glucosidase enzymes, namely the N-terminal maltase-glucoamylase, C-terminal maltase-glucoamylase, and the N-terminal domain of sucrase-isomaltase. This also included a comparison of docking results between calystegines and acarbose as the known standard of α -glucosidase inhibitors. The study was conducted from January to February, during which molecular docking simulations were performed with various consultations with verified scientists to ensure the accuracy of data and the docking process.

The study was limited to molecular docking simulations only. No laboratory work, human testing, or animal experimentation were conducted. It did not involve the actual extraction, purification, or quantification of calystegines from *Ipomoea aquatica*. Instead, this study used secondary data and compound databases, with the α -glucosidase enzyme obtained from PubChem and the Protein Data Bank serving as the docking target. Only calystegines A3, B1, B2, B3, B4, and C1 were tested as ligands; other phytochemicals from *Ipomoea aquatica* were excluded from the scope. The docking outcomes were predictive and served as preliminary evidence intended to guide future experimental studies, providing an initial indication of potential therapeutic effectiveness.

H. Definition of Terms

To facilitate the understanding of the study, different terms are defined herein.

Polyhydroxylated Nortropane Alkaloid. A naturally occurring nitrogen-containing compounds with a nortropane ring structure (a bicyclic system derived from tropane) that carry multiple hydroxyl (-OH) groups. These compounds, such as calystegines, are found in plants of the Convolvulaceae

(Herbs) and Solanaceae (Food crops) families and are known to inhibit glycosidases, making them potential candidates for antidiabetic applications (Gangopadhyay, Das, Bandyopadhyay, & Das, 2021). In this study, they are studied as possible natural α -glucosidase inhibitors that can reduce blood sugar levels.

Ipomoea aquatica (Kangkong). It is a wild edible food plant that can be found throughout Asia, Africa, and Australia. It contains a diverse chemical and biological composition and exhibits antioxidant activity. It has long been utilized in anti-diabetes treatment (Saikia, 2023). In this study, it is investigated as a naturally occurring source of polyhydroxylated nortropane alkaloids that possess possible α -glucosidase inhibitory activity in diabetes management.

α -Glycosidase. An enzyme that breaks down complex carbohydrates into glucose in the small intestine (Liu, Hao, Bian, Ge, Lu, Xie, & Zhu, 2021). In this study, it is the target enzyme studied in order to assess the inhibitory activity of polyhydroxylated nortropane alkaloids in *Ipomoea aquatica*.

Glycemic Control. It is the ideal serum glucose concentration for diabetes people. It is necessary to identify factors affecting the glycemic control of patients to prevent control and complications (Bin Rakhis, AlDuwayhis, Aleid, AlBarrak, & Aloraini, 2022). In this study, glycemic control is relevant to blood sugar control in relation to α -glucosidase inhibition by *Ipomoea aquatica* extracts.

Diabetes Mellitus. It is a common metabolic condition characterized by a deficiency in the secretion of insulin or in its effect or both (Bin Rakhis, 2022). In this study, it is a health condition that is targeted for management by means of inhibition of α -glucosidase via *Ipomoea aquatica* extracts.

Hyperglycemia. It is a symptom wherein having too much glucose (sugar) in your blood (Australia, 2025). In this study, it is the condition that α -Glycosidase inhibition aims to prevent through compounds on the *ipomea aquatica*.

Alpha-Glucosidase Inhibitors. A class of drugs used to treat type 2 diabetes alone or combined with other antidiabetic drugs (Akmal, Patel, & Wadhwa, 2024). In this study, they are used as reference compounds to compare the inhibitory activity of *Ipomoea aquatica* alkaloids.

Enzymes. It is a protein that interacts with substrate molecules to reduce the activation energy required for a chemical reaction to occur by stabilizing the transition state (Lewis & Stone, 2023). In this study, α -glucosidase is the enzyme that is targeted for inhibition using the compounds of *ipomea aquatica*.

Calystegines. A new group of polyhydroxy alkaloids with a nortropane skeleton. It is a potent glycosidase inhibitor with therapeutic potential and a constituent of food and feed with potential toxic effects (Souto, Borges, Costa, dos Santos, & da Silva, 2024). In this study, they are evaluated for their inhibitory potential against the α -glucosidase enzyme.

Simplified Molecular-Input Line-Entry System. SMILES is a line notation that describes the chemical structure using short American Standard Code for Information Interchange-ASCII strings. SMILES is like a connection table in that it identifies nodes and edges of a molecular graph. SMILES will be used for

the subsequent ligand preparations for docking.

Protein Data Bank + Partial charges (Q) + Atom types (T). The PDBQT format is a critical format in most AutoDock and AutoDock Vina software programs because it saves information like molecular structural information, atomic coordinates, partial charges, and atomic types (Wisdomlib.org). For the docking simulation to be performed, the prepared ligands, the calystegine compounds, will be saved in PDBQT format. In addition, the receptor in the target enzyme α -glucosidase will be saved in PDBQT format.

Root Mean Square Deviation. RMSD is a measure of the similarity between two molecular poses in molecular docking. If the RMSD value is low (< 2 Angstroms), a very similar docked pose to a reference pose is obtained. The RMSD also serves in the calculation for measuring the stability of a docked molecule in a simulation. Usually, the degree of similarity of two protein 3D structures is measured by the root-mean-square distance (RMSD) between equivalent atom pairs (Sapundzhi and Slavov, 2020). In this study, RMSD will be applied to assess the accuracy of the resulting predicted ligand-binding pose from the docking analysis by comparing to the known reference structure, which is Acarbose.

2. Review of Related Literature

This section outlines the studies and literature discovered by the researchers during their in-depth search for the study. This will also present the state of the art in order to better comprehend the study.

A. Polyhydroxylated Nortropane Alkaloids

Polyhydroxy alkaloids comprise a nitrogen heterocyclic ring with several hydroxyl groups attached to the ring and are soluble in water. Polyhydroxy alkaloids are also called iminosugars, azasugars, and nitrogenous glycomimetics. Polyhydroxy alkaloid comprises five different subclasses which are pyrrolidines, piperidines, pyrrolizidines, indolizidines and nor-tropane. The nortropane alkaloid is a fused bicyclic system, a combination of a five-membered pyrrolidine ring with a six-membered piperidine ring at positions α - to the nitrogen atom of each monocyclic system. Nor-tropane alkaloid calystegines has been isolated from many plants mainly from Convolvulaceae, Solanaceae, and Moraceae families. (Ismail 2024).

Calystegines are a group of recently identified polyhydroxylated nortropane alkaloids. Due to their inability to pass the blood-brain barrier, these compounds have no psychoactive effect and, until now, their occurrence and analysis in food samples has gained little research attention. (González-Gómez, Morante-Zarcero, Pérez-Quintanilla, & Sierra, 2022).

The carbohydrate-mimicry of the calystegines' structure is also noteworthy: due to their multiple hydroxyl groups and inflexible bicyclic structure, they are spatially equivalent to sugar and thus can be coordinated spatially by glycosidase enzymes a point which makes chemical structure account for biological activity (Kowalczyk, Bourebaba, Panchuk, Marycz, & Bourebaba, 2022).

Remarkably, due to their hydrophilicity and high polarity, calystegines are far less lipophilic than standard tropane esters; this is the most common cited explanation for why they cannot cross the blood-brain barrier, thus accounting for the lack of usual tropane-derived central nervous system effects (Wang, 2023).

The recent studies have proved that polyhydroxylated nortropane alkaloids called Calystegines occur naturally in various plant families, mainly in Solanaceae, Convolvulaceae, and Brassicaceae. The common food plants in which calystegines have been found according to De Nijs, Crews, Dorgelo, MacDonald, & Mulder (2023) are potatoes (*Solanum tuberosum*), eggplants (*Solanum melongena*), and bell peppers (*Capsicum annuum*).

Their occurrence in a variety of plants suggests that nortropane alkaloids have a natural biochemical role, either in defense mechanisms or metabolic regulation. However, their distribution is not uniform, with very variable concentration levels depending on both environmental conditions and plant development. According to the Laboratory of Chemistry and Food (LCI, 2022), potatoes contain calystegines A₃, B₂, and B₄, while eggplants and sweet potatoes contain almost exclusively calystegine B₂. More recently, phytochemical screenings have identified the presence of nortropane alkaloids in *Ipomoea aquatica*-water spinach, a member of the Convolvulaceae family that is closely related to other species which produce calystegines. This discovery extends the known range of edible plants that are able to produce these compounds, suggesting that they could well be distributed among plant orders far more frequently than was hitherto considered. This variation of alkaloid profiles implies a species-specific production and accumulation controlled by ecological and genetic variables. Changes in such profiles are very important from the point of view of food safety assessment and for potential pharmaceutical uses, because such substances have an effect on glucose metabolism and enzyme inhibition in mammals.

The strong polarity of the alkaloids has determined the development of modern extraction and purification technologies. Abdel-Hafez (2021) presented a systematic approach for the isolation of calystegines from the seeds of *Hyoscyamus albus*, including defatting the plant material with petroleum ether, extraction with aqueous methanol, and further purification by cation-exchange chromatography. This methodology separated the total fraction of calystegines, thus making future biological testing possible. For edible plants, methodologies have been developed, but there is still a difficulty with respect to the complexity of the matrix and possible co-extraction of sugars and other polar molecules. High-performance liquid chromatography and gas chromatography coupled with mass spectrometry are the preferred techniques for detection and quantification, owing to their great sensitivity toward trace-level analyses.

B. Phytochemical Properties of Calystegines

Calystegines are polyhydroxylated nortropane alkaloids found in a variety of solanaceous foods, including potatoes and aubergines. Calystegines' biological activity and possible

toxicity are linked to their ability to inhibit glycosidases and limit carbohydrate metabolism, which induces lysosomal storage toxicity (EFSA 2019).

There has also been more recent experimental evidence highlighting biological activity beyond enzyme inhibition. According to Kowalczyk (2022) antioxidant and cytoprotective effects on metabolic cells by calystegine-containing preparations in vitro have been reported, and it has been suggested that they can decrease intracellular reactive oxygen species and mitigate glucotoxic stress in hyperglycaemic models; these findings point towards potential metabolic-modulatory activity that is worthy of further pharmacological investigation.

According to Beckmann (2020) grifood and food safety, calystegines were found in foods containing varying levels of Solanaceous content. Their occurrence in foods led to the development of analytical methods and dereplication studies to identify and quantify correctly calystegines in food matrices of varying complexity. While these developments have been made, there are significant knowledge gaps. The biosynthetic pathway has only recently come to be elucidated, and thorough toxicological and exposure-assessment information for

humans is still lacking (Zeng, Liu, Dong, Zhang, Qiu, Zhong, & Liao, 2024). Diabetes mellitus trigger tissue and organ failure through insulin resistance, oxidative stress and chronic low-grade inflammation. Therefore, the screening formolecules that may counteract such conditions is essential in current existing therapeutic strategies, thereby the use of medicinal plant derivatives represents a promising axis in this regard (Kowalczyk et. al 2022). Alkaloids such as calystegines found in medicinal plants represent an intriguing potential for the inception of novel approaches to diabetes mellitus therapies (Behl, Gupta, Albratty, Najmi, Meraya, Alhazmi, & Bungau, 2022). Research found out that calystegines may represent potentially potent antioxidant substances, which could ameliorate the characteristic oxidative stress of cells affected by hyperglycemia and glucotoxicity in the course of severe metabolic disorders such as diabetes mellitus (Kowalczyk et al., 2022). *aHyoscyamus albus* calystegines have demonstrated great potential in promoting MSC antidiabetic potential essentially by improving overall metabolic homeostasis including insulin signaling pathways of mesenchymal stromal cells or MSCs (Bourebaba, Bourebaba, & Marycz, 2023). Research by Amer & Amer, 2025 also stated that calystegines hold promise as a potential alternative for managing metabolic conditions especially those caused by hyperglycemic conditions.

Furthermore, new research by (Zeng, 2024) have also found out the biosynthetic pathway of producing calystegines in plants belonging to the solanceae (tomato, eggplant, nightshades, etc.) which may help in producing calystegines synthetically. Research further exploring the potential of calystegines in pharmaceutical and industrial applications also found out the role of mitochondria in plants that contain calystegines further enhancing the potential of calystegines as a viable treatment to various illnesses most prominently diabetes (D'Auria & Fernie, 2024).

Additionally, research also found that calystegines (B3 & 7) possess inhibitory properties that inhibits biofilm production in *A. baumannii*, a multidrug resistant bacterium responsible for several hospital acquired infections assisted by ability to adhere to surfaces both biotic and abiotic because of its biofilm development and formation (Lokhande, Pawar, Madkaiker, Nawani, Venkateswara, & Ghosh, 2022).

C. *Ipomoea Aquatica* (Kangkong) as a Source of Polyhydroxylated Nortropane Alkaloids

Srivastava and Rauniyar (2020) stated in their journal that *Ipomea aquatica* have been known to contain calystegine B1, B2 beyond B4 along with other phytochemicals that can be extracted in the plant's roots, leaves and stem. Also, research in Europe said that a sample of *Ipomea aquatica* from Thailand found out that *Ipomea aquatica* also contains calystegine A3 (de Nijs et al., 2023). Additionally, calystegine C1 have also been found in *Ipomea aquatica* according to research by Kusumah and Pertiwi (2021).

Moreover, Saikia, Dey, Hazarika, Handique, Thakur, & Handique (2023) analyzed the biochemical composition of *Ipomea aquatica* and found out that the plant contains various phytochemicals including polyhydroxylated alkaloids (calystegines) that have bioactive properties such as α -amylase and α -glucosidase inhibition.

D. Docking Affinity Analysis

Molecular docking is emerging as a pivotal tool in drug discovery. To improve efficiency, the ligand–receptor affinity predicted by molecular docking needs to be revised by using actual affinity data (Lu, Jia, Shan, Xia, Qian, Wang, Yang, Yang, Che, Zhang, Wang, & He, 2025). Furthermore, high binding affinity (low dissociation constant K_d) indicates that a compound can create a stable complex with its target protein.

Binding Affinity is the equilibrium constant of complex formation, laws of thermodynamics relate it to the change in Gibbs free energy upon binding: The change in Gibbs free Energy of Binding is equal to $-RT$ times the natural logarithm of the Equilibrium constant, where R is the gas constant and T the absolute temperature (Annemarie & Honegger, 2020).

E. Molecular Docking

Molecular docking has become essential in the study of biochemistry. It can help in predicting a compound's binding affinity towards certain biomolecules such as enzymes or proteins. Docking can be used to discover novel ligands for a target by screening large virtual compound libraries. Docking can also provide a useful starting point for structure-based ligand optimization or for investigating a ligand's mechanism of action (Paggi, Pandit, & Dror, 2024). Moreover, Molecular docking has played a crucial role in making drug discovery faster, cheaper, and more effective. There are also approved drugs in the pharmaceutical market that are developed through the use of molecular docking (Muhammed and Aki-Yalcin, 2024). Furthermore, Molecular docking is considered to be the most widely utilized computational phenomenon in the field of computer-aided drug design (CADD). It is being utilized at the academic level as well as in pharmaceutical companies for the

lead discovery process (Raval and Ganatra, 2022) further highlighting its importance in testing new compounds that will not require in vivo experiments which are generally more expensive and time consuming.

F. ADME Evaluation

In pharmacokinetics, ADME is an abbreviation representing absorption, distribution, metabolism, and excretion. ADME screening is used to identify and optimize lead compounds in the drug discovery process. Computational models predicting ADME properties have been developed with evolving model-building technologies from a simplified relationship between ADME endpoints and physicochemical properties to machine learning, including support vector machines, random forests, and convolution neural networks (Ko, Komura, Watanabe, & Mizuguchi, 2023). In vivo ADME testing is costly, time-consuming, and puts animal lives at risk, whereas in silico ADME testing is safer, simpler, and faster (Azzam, 2023). Additionally, innovations in the form of AI and multidisciplinary cooperation remains crucial in the progress to make a more efficient and precise in vitro ADME analysis (Paul-Chima, Ugwu, & Alum, 2024).

G. Alpha-Glucosidase

The enzyme α -glucosidase helps break down carbohydrates into simple sugars, mainly glucose. It works by cutting the bonds, α -1,4-glycosidic bonds, that connect sugar units in starch and other carbohydrates. This process happens in two main steps. First, the enzyme attaches to the sugar chain and forms a temporary bond with it. Then, a water molecule breaks this bond, releasing one glucose molecule and allowing the enzyme to be reused (Zhai, Wu, Ji, Zhao, Lu, Yu, & Huang, 2022; Tagami, 2024). Two important amino acids in the enzyme, usually aspartic acid and glutamic acid, are responsible for this reaction. The shape of the enzyme, especially its barrel-like structure, helps hold the sugar in the right position so that the reaction can happen smoothly (Tagami, 2024).

The α -glucosidase enzyme plays a crucial role in carbohydrate metabolism by catalyzing the hydrolysis of terminal α -1,4-glycosidic linkages in oligosaccharides, producing glucose as the end product (Zhai et al., 2022). Structurally, α -glucosidase belongs to the glycoside hydrolase family 13 (GH13), characterized by a $(\beta/\alpha)_8$ -barrel catalytic domain that accommodates its active site (Zhai et al., 2022). According to research done by Liu et al. (2021), α -glucosidase has a flexible active pocket that allows interaction with carbohydrate substrates which explains the broad substrate choices and potential modulation through inhibitors. These structural insights help explain its broad substrate specificity and potential for modulation through inhibitors or mutations.

α -glucosidase as stated by Pan, Lu, Wei, Li, Li, & Pan (2024), is important for glucose homeostasis as the enzyme regulates the carbohydrate digestion in the small intestine. Furthermore, α -glucosidase inhibition can delay glucose absorption and reduce postprandial (after meal) hyperglycemia (Sohrabi, Binaeizadeh, Iraj, Larijani, Saedi, & Mahdavi, 2022). α -glucosidase activity is also linked to aging and age-related

diseases because of malfunction that can cause oxidative stress and imbalance in the metabolism (Zhong, Yang, Syed, Zhang, Tian, & Fu, 2024). It is important to understand the structural and functional dynamics of α -glucosidase to produce novel inhibitors and therapeutic techniques to control blood sugar levels (Liu et al., 2021). Moreover, small changes in the active site of α -glucosidase can affect the effectiveness of the enzyme inhibition (Liu et al., 2021). Also, Lam, Tran, Pham, Lai, Dang, Truong, & Tran (2024), found out that flavonoids, a group of plant compounds that are very common, can in fact slow down α -glucosidase activity by blocking the active site of the enzyme.

Studies using structural and computational analyses have deepened understanding of the α -glucosidase hydrolysis mechanism and its modulation by inhibitors. Liu et al. (2021) found that small changes in the enzyme's active site can make it work faster or slower. Lam, Tran, Pham, Lai, Dang, Truong, & Tran, (2024) conducted a study and discovered that plant compounds called flavonoids can slow down the enzyme (α -glucosidase) by blocking the spot where sugar normally attaches. Additionally, metal compounds and synthetic inhibitors can also stop α -glucosidase activity by mimicking sugar structure (Sohrabi et al., 2022; Pan et al., 2024). Furthermore, α -glucosidase's ability to break down carbohydrates depends on how well it can bind to sugar and other molecules that might inhibit it (Xu, Hileuskaya, Kraskouski, Yang, Huang, & Zhao, 2024).

Moreover, understanding how α -glucosidase breaks down carbohydrates helps scientists develop medicine to inhibit the enzyme that will help control blood sugar levels especially for people with diabetes (Lam et al., 2024; Xu et al., 2024). α -glucosidase inhibition helps in lowering sugar absorption, helping keep postprandial blood sugar levels under control (Kashtoh and Baek, 2022).

H. MGAM and SI as Human Intestinal Alpha-Glucosidase

α -glucosidase is a group of digestive enzymes located in the small intestine that catalyze the breakdown of complex carbohydrates into glucose. Because of its central role in postprandial glucose regulation, it has become a major therapeutic target in Type 2 diabetes research (Lu et al., 2023). Among the key human intestinal α -glucosidases are maltase-glucoamylase (MGAM) and sucrase-isomaltase (SI), both of which contribute significantly to carbohydrate digestion. Inhibition of these enzymes delays glucose absorption and helps reduce postprandial hyperglycemia, which explains their importance in recent pharmacological and molecular docking studies (Pan et al., 2024).

MGAM consists of two catalytic domains: the N-terminal (NtMGAM) and the C-terminal (CtMGAM), each possessing distinct but complementary substrate specificities. The N-terminal domain primarily hydrolyzes short-chain substrates such as maltose, while the C-terminal domain shows greater activity toward longer oligosaccharides. In contrast, SI is commonly studied using only its N-terminal domain, as the C-terminal domain of SI is highly homologous to the N-terminal domain of MGAM (Tannous et al., 2023). Due to this structural similarity and overlapping catalytic characteristics, many

studies prioritize NtSI to avoid redundancy and to better distinguish enzymatic interactions during inhibition assays and molecular docking analyses.

Recent molecular biology has refined the understanding of the functioning of MGAM by emphasizing the distinct yet collaborating roles of its N-terminal and C-terminal parts. Based on the statement of Stephanie Tannous, Stellbrinck Tammy, Abdullah Hoter, Hassan Y. Naim 2026, these are no longer considered as separate enzymes but as parts of a "hetero-complex" in the intestinal brush border. This arrangement helps both the N- and C-terminals to work in conjunction with Sucrase-Isomaltase, making it a very effective enzymatic machinery tuned for efficient accomplishment of the final steps of carbohydrate digestion.

The functional differentiation between the two terminals is one of the premier focuses of recent kinetic studies. As Watanabe, Tagami, Biwa, Kawasaki, Adachi, Moriya, Senda & Okuyama (2026) confirm, the C-terminal CtMGAM is the primary "glucoamylase" devoted to hydrolysis of long-chain starches and maltodextrins. A very unique regulatory mechanism, called a "substrate inhibitory brake," acts inherently to impede glucose release and therefore ensures postprandial hyperglycemic peaks. The N-terminal, however, is actually the more versatile "maltase" since it shows high efficiency against the breakup of shorter-chain oligosaccharides. This division of labor ensures that while CtMGAM initiates the breakdown of complex starch polymers, NtMGAM completes the process by releasing free glucose units.

Furthermore, Lam, Tran, Pham, Lai, Dang, Truong, Nguyen-Vo, Hoang, Mai & Tran (2024) note a trend toward the finding of "dual target" inhibitors from natural phytoconstituents. The literature indicates a possibility that since NtMGAM and CtMGAM have independent active sites and distinct binding affinities, a monotherapeutic. Recent evidence by Tannous et al. (2026) reiterated that, for effective management of Type 2 Diabetes, there is a need for "dual competitive inhibition" where both the N-terminal and C-terminal should be modulated simultaneously to achieve a balanced glycemic response.

I. Enzyme Inhibition

Postprandial hyperglycemia is the sharp increase in blood sugar levels after eating which is an early sign of glucose metabolism imbalance which is strongly linked to cardiovascular diseases and oxidative stress (Hossain, Das, Ghosh, & Sil 2020). Additionally, high post-meal blood glucose levels can damage blood vessels and can increase the risk of complications such as neuropathy and retinopathy which is why postprandial blood glucose level control is a very important part of diabetes control (Ren, Ji, & Zhu 2023). Furthermore, effective ways of reducing postprandial hyperglycemia is by slowing down the digestion of sugar through a-glucosidase and a-amylase inhibition (Pan et al., 2024).

Moreover, a-glucosidase inhibition helps slow down the rate at which carbohydrate is converted to glucose helping prevent sudden blood sugar spike after meal. Acarbose, miglitol and voglibose are widely used for this reason as they are synthetic

inhibitors of a-glucosidase (Zhong et al., 2024). Additionally, natural compounds such as flavonoids, phenolics, and peptides have also been found out to inhibit a-glucosidase activity (Dirir, Daou, Yousef, & Yousef, 2021). Furthermore, the aforementioned natural compounds not only exhibit a-glucosidase inhibition, they are also anti-inflammatory and antioxidant which helps in protecting against diabetes complications (Hossain et al., 2020; Xu et al., 2024).

In addition, targeting a-glucosidase not only reduced postprandial blood sugar levels but also help in improving lipid metabolism and reducing oxidative damage in diabetic patients (Kowalczyk et al., 2022; Xu et al., 2024). Furthermore, combining natural a-glucosidase inhibitors with synthetic treatments also help in enhancing treatment outcomes while reducing side effects (Ren et al., 2023). Lastly, a-glucosidase inhibition not only help in lowering postprandial blood sugar levels but also promise a long-term diabetes management and diabetes related complications to patients with diabetes (Alssema, Ruijgrok, Blaak, Egli, Dussort, Vinoy, and Robertson, 2021).

J. Alpha-Glucosidase Inhibitors

Alpha-Glucosidase Inhibitors (AGIs) blocks enzyme activity of a-glucosidase in the small intestine helping in lowering postprandial blood glucose levels without increasing insulin levels in people with type 2 diabetes (Kashtoh & Baek, 2022; Pan et al., 2024). To add to that, AGIs like acarbose, miglitol, and voglibose has been widely used, showing effective results in reducing postprandial hyperglycemia (Ren et al., 2023). Furthermore, studies have shown that AGIs such as voglibose, miglitol, and acarbose help in protecting against other diabetic complications such as oxidative stress and inflammation (Kowalczyk et al., 2022). Alssema et al. (2021) also revealed that a-glucosidase inhibitors significantly reduced postprandial blood sugar levels.

Dirir et al., (2021) revealed that recent research has also expanded to natural AGIs such as flavonoids, alkaloids, and phenolic compounds derived from plants have shown strong inhibitory effects to a-glucosidase in both in vivo and in vitro experiments. Additionally, flavonoids have become popular due to it's ability to react to both a-glucosidase and a-amylase while metal-based complexes and modified natural compounds was also explored to reduce side effects and enhance potency (Sohrabi et al., 2022). Meanwhile, computational and molecular docking have revealed various potential new inhibitors for a-glucosidase with better bioavailability and efficiency in binding (Liu et al., 2021).

Moreover, a-glucosidase inhibition has also been explored as a means to manage obesity and prevent age-related metabolic disorders by enhancing glucose metabolism and lipid regulation (Zhong et al., 2024). Additionally, natural inhibitors have also been studied for their antioxidants and enzyme-inhibiting properties for better overall metabolic health (Hossain et al., 2020; Dirir et al., 2021). Lastly, continuous investigations will potentially reveal new inhibitors and their mechanisms to come up with new therapeutic agents that are not only safe but effective for long-term use for diabetic patients (Sohrabi et al.,

2022).

K. Inhibitory Compounds

Interaction between α -glucosidase and inhibitors occurs mainly in the enzyme's active site which contains important amino acid residues. These inhibitors form hydrogen bonds, hydrophobic interactions, and π - π stacking interactions with the amino acid residues blocking carbohydrates from accessing the catalytic site (Halim, Jabee, Khan, & Al-Harrasi 2021). Many inhibitors for α -glucosidase mimic the natural substrate (carbohydrates) allowing the inhibitors to bind tightly to catalytic residues, therefore stabilizing the enzyme-inhibitor complex, thus preventing carbohydrate hydrolysis (Halim *et al.*, 2021; Xu *et al.*, 2024).

Additionally, natural compounds like flavonoids, polyphenols, and alkaloids can exhibit strong inhibitory properties against α -glucosidase through stable hydrogen bonding and aromatic interactions ((Liu *et al.*, 2021). Also, synthetic drugs like acarbose act as competitive inhibitors for α -glucosidase and is proven with clinical efficacy (Alssema *et al.*, 2021). Molecular docking simulations further demonstrate that natural inhibitors can induce structural changes in the enzyme, reducing its flexibility and activity (Xu *et al.*, 2024). Recent clinical assessments highlight that α -glucosidase inhibitors are increasingly recognized not only for glucose regulation but also for their potential in managing aging related metabolic diseases due to their ability to modulate oxidative stress and gut microbiota (Zhong, Yang, Syed, Zhang, Tian, & Fu, 2025). Understanding of how inhibitory compounds interact with α -glucosidase at the molecular level enables researchers to design targeted, efficient, and low toxicity inhibitors that hold promise as next-generation antidiabetic agents (Halim *et al.*, 2021).

L. α -Glucosidase Inhibition as a Therapeutic Strategy for Diabetes Management

Diabetes mellitus stands as a significant global health challenge, ranking third among causes of mortality worldwide. Despite extensive endeavors, effective treatments for diabetes remain elusive. α -glucosidase, a key enzyme in regulating glycoprotein metabolism and carbohydrate digestion, has gained considerable attention in managing diabetes. This enzyme has a vital function in decomposing complex carbohydrates in the small intestine into glucose molecules, which can be readily absorbed and utilized by the body. The inhibition of this enzyme slows down carbohydrate digestion and glucose absorption, consequently lowering postprandial hyperglycemia, which is one of the characteristic conditions of diabetes mellitus (Malik, Lodhi, Ayaz & Ullah, 2024). Additionally, modulating postprandial glucose peak levels remains paramount, as prolonged levels of elevated glucose concentrations contribute to the development of various complications of diabetes. They can be used alone or in a combination regimen along with other anti-diabetics based upon the clinical conditions of a patient. These are recommended in combination therapy in case a sole anti-diabetic doesn't work effectively and poses a low risk of adverse

effects altogether. These are quite effective in patients having a low tolerance level for blood sugar and in cases having either early or delayed type 2 diabetes because these help in regulating postprandial levels of glucose in a way that does not lead to low hypoglycemia (Bhatnagar & Mishra, 2022).

The inhibition of α -glucosidase emerged as an important therapeutic target that can decrease blood sugar levels by reducing carbohydrate digestion. However, the α -glucosidase inhibitors mainly act on hyperglycemia without directly effecting the insulin secretion. Therefore, these are considered as first line oral sugar reducing agents and used as monotherapy under mild diabetic conditions (Mushtaq, Azam, Mehreen & Naseer 2023).

Furthermore, many attempts have been made in recent years to uncover efficient α -glucosidase inhibitors from natural sources to build a physiologic functional diet or lead compound for diabetes treatment. Many phytoconstituent α -glucosidase inhibitors have been identified from plants, including alkaloids, flavonoids, anthocyanins, terpenoids, phenolic compounds, glycosides and others (Kashtoh & Baek, 2022). Moreover, approved inhibitors for these enzymes are restricted to acarbose, miglitol and voglibose. Although these inhibitors retard glucose absorption, undesirable gastrointestinal side effects impede their application. Therefore, research efforts continue to seek novel inhibitors with improved efficacy and minimal side effects. Natural products of plant origin have been a valuable source of therapeutic agents with lesser toxicity and side effects (Dirir, Daou, Yousef & Yousef, 2022).

α -glucosidase inhibitors are a class of oral medication used in treating type 2 diabetes mellitus. They can be administered alone or in combination therapy with other antidiabetic medications. They are used for patients with low blood sugar tolerance and delayed type 2 diabetes. They are convenient for patients at risk of hypoglycemia or lactic acidosis. α -glucosidase inhibitors can also be referred to as starch blockers that help to reduce postprandial blood sugar levels. These enzyme inhibitors do not directly affect insulin secretion or sensitivity, unlike other antidiabetic drugs. Instead, they work by delaying the digestion of sugars found in starchy foods by raising post-meal levels of GLP-1 and lowering HbA1c levels (Bhatnagar & Mishra, 2022).

To add to that, the enzyme α -glucosidase in the small intestine regulates blood glucose levels and stimulates the hydrolysis of oligosaccharides and polysaccharides, increasing glucose levels in the body. Inhibiting this enzyme slows glucose digestion and absorption and as a result post-prandial blood glucose levels remain low, causing decreased insulin demand (Khalid, Butt, Ahmad, Iqbal, Shamim, Nishan, Ullah, Ibrahim, Moura, Shah & Sun, 2024). The development of effective antidiabetic agents remains a critical challenge in diabetes management (Marzouk, Mahmoud, Shehab, Fawzy, Mohammed, Abdel-Razek, Khedr & Elsayed, 2025).

On the other hand, a study conducted by Talab, Ullah, Alam, Halin, Rehman, Zainab, Ali, Latif, Khan, Al-Harrasi and Ahmad (2023) examined Polyhydroquinoline derivatives as an inhibitor of α -glucosidase. Among the synthesized series, two compounds, i.e., 11 and 10, showed potent α -glucosidase

inhibitory potential higher than the standard. All the compounds were compared with standard drug “acarbose”. Furthermore, an in-silico method was used to predict their mode of binding within the active site of enzyme to understand their mechanism of inhibition. Furthermore, traditional medicinal plants with α -G inhibitory potential are commonly used to treat diabetes mellitus due to the adverse effects of currently used α -G inhibitors miglitol, acarbose, and voglibose. Various bioactive compounds derived from natural sources, including lupenone, Wilforlide A, Baicalein, Betulinic acid, Ursolic acid, Oleanolic acid, Katonic acid, Carnosol, Hypericin, Astilbin, lupeol, betulonic acid, Fagomine, Lactuaxanthin, Erythritol, GP90-1B, Procyanidins, Galangin, and vomifoliol retain α -G inhibitory potential for regulating hyperglycaemia (Kumari, Saini, Bhatnagar & Mishra, 2024).

With the increasing global prevalence of diabetes, identification of alternative enzyme inhibitors with potentially less negative side effects becomes imperative. Compounds of natural sources (such as dietary component) are more desirable due to the advantages in safety, diversity, and economy compared with the synthetic inhibitors. Food-derived non-starch polysaccharides, peptides, and lipids compounds have been determined to inhibit α -amylase and α -glucosidase activities (Li, Bai, Jin & Svensson, 2022). Additionally, a study by June Zhang, Linyan Zhang, Lain, Liang, Gaia, Kaliaperumal and Jiang (2022) examined navel orange peel extracts and identified seventeen flavonoids from the peel extract, of which 5-hydroxy-6,7,3',4'-tetramethoxyflavone, didymin and sinensetin were the most potent antioxidant, α -glucosidase

inhibitor, and glycation inhibitor, respectively.

3. Materials and Methods

A. Materials

This study was conducted purely in silico using freely available computational platforms and software tools.

Protein Data Bank (PDB) - The Protein Data Bank was the only worldwide repository of experimentally determined 3D structures of biological macromolecules and their complexes. The researchers used PDB to acquire the 3D crystal structure of the target enzyme α -glucosidase.

PubChem - PubChem was the world's largest collection of freely accessible chemical information online (Pubchem.ncbi.nlm.nih.gov). The researchers obtained the chemical structure of the ligands (Calystegine A3, B1, B2, B3, B4, and C1).

SwissADME - Free online tool for assessing small compounds' pharmacokinetics, drug-likeness, and medicine chemistry friendliness (swissadme.ch). The researchers used SwissADME to determine the ligands' capacity to be processed by human bodies through checking the ligands' Absorption, Digestion, Metabolism, and Excretion capacity.

PyMol - PyMOL provided a builder menu for drawing small chemical compounds, peptides, and nucleic acid sequences to facilitate molecular construction and editing (PyMOL.org). It was used to mimic the natural environment in the gut, where the reaction between the enzyme α -glucosidase and the ligands took place.

Autodock Tools - Predicted how small molecules, such as

Table 1
Studied compounds with corresponding authors, source websites, and reference links

Compound	Website	Author	URL
N- terminal Maltase-Glucoamylase	Protein Data Bank	Sim, L., Rose, D.R.	https://doi.org/10.220/pdb2QLY/pdb
C- terminal Maltase-Glucoamylase	Protein Data Bank	Shen, Y., Qin, X.H., Ren, L.M.	https://doi.org/10.221 0/pdb3TON/pdb
N-terminal Sucrase-Isomaltase	Protein Data Bank	Sim, L., Rose, D.R.	https://doi.org/10.221 0/pdb3LPO/pdb
Acarbose	PubChem	Not Applicable	https://pubchem.ncbi.nlm.nih.gov/compound/9811704
Calystegine A3	PubChem	Not Applicable	https://pubchem.ncbi.nlm.nih.gov/compound/183073
Calystegine B1	PubChem	Not Applicable	https://pubchem.ncbi.nlm.nih.gov/compound/164245
Calystegine B2	PubChem	Not Applicable	https://pubchem.ncbi.nlm.nih.gov/compound/124434

Table 2
Software applications used in the study and website references

Application	URL
Protein Data Bank (PDB).	https://www.rcsb.org/
PubChem	https://pubchem.ncbi.nlm.nih.gov/
SwissADME	https://www.swissadme.ch/
AutoDock Tools	https://autodocksuite.scripps.edu/adt/
AutoDock Vina	https://vina.scripps.edu/

Table 3
Application and their uses in the study

Protein Data Bank (PDB)	Protein structures of a glucosidase, were obtained from the Protein Data Bank (PDB).
PubChem	Kangkong-derived calystegine, including Calystegine A3, Calystegine B1, Calystegine B2, Calystegine B3, Calystegine B4 Calystegine C1, were retrieved from the PubChem database in SDF and SMILES formats for subsequent ligand preparation. Acarbose was also be retrieved and processed as a benchmark reference compound for comparison with Kangkong-derived ligands.
SwissADME	To assess pharmacokinetics and safety, SwissADME was used to predict absorption, distribution, metabolism, and skin permeation profiles.
Pymol	Molecular visualization software for building and editing compounds; simulated the enzyme–ligand environment in the gut.
AutoDock Tools	AutoDock Tools are employed for assigning torsions, adding hydrogens, and preparing both ligands and receptors in PDBQT format.
AutoDock Vina	Docking simulations were performed using AutoDock Vina.
Biovia Discovery Studio	Final analyses will integrate docking sources, ADME/Tox predictions, and interaction data, supported by visualization and statistical processing using Biovia Discovery Studio.

potential drugs, bind to proteins (autodocksuite.scripps.edu). The researchers used AutoDock tools to process the acquired PDB format converted via Open Babel to determine rotatable bonds and assign Gasteiger charges to the ligand structures.

Autodock Vina - A powerful and widely used computational program specifically designed for molecular docking and virtual screening (vina.scripps.edu). The researchers used Autodock Vina as the docking software to conduct the molecular docking experiment.

Biovia Discovery Studio - Provided various analysis tools to evaluate the ligand–target complex's binding free energy, binding affinity, and other properties (Dassault Systèmes). BIOVIA Discovery Studio was used together with PyMOL to visualize and analyze the molecular docking outcomes.

B. Experimentation Process

1) Research Design

The study used an in-silico experimental research design to examine how the polyhydroxylated nortropane alkaloids from *Ipomoea aquatica* interacted with the α -glucosidase enzyme. It used molecular docking analysis, a computer-based method that relied on data from online scientific databases. In-silico analysis used computer simulation to study biological processes and check many compounds quickly without doing actual lab experiments, as explained by Chang et al. (2023). According to Paggi et al. (2024), molecular docking helped predict how a ligand attached to a protein and measured the strength of the binding by examining their interactions and binding energy. In this study, these computer methods assessed the binding strength, position, and interaction behavior of *Ipomoea aquatica* alkaloids inside the active site of α -glucosidase, providing a clear picture of their potential as competitive inhibitors.

This research relied entirely on in-silico and computer-based methods to generate accurate and measurable data on ligand conformations, interaction energies, and binding behavior. These computational results allowed the researchers to systematically and efficiently assess the alkaloids' potential to inhibit the target enzyme, even without performing wet-lab experiments. By documenting every computational procedure and maintaining full transparency, the study established a strong foundation for future laboratory validation and provided early evidence of the compounds' possible effectiveness as α -glucosidase inhibitors. If followed by in vitro and in vivo testing, the findings can be further strengthened, offering more definitive proof of the alkaloids' ability to reduce blood sugar levels.

2) Concepts and Notations

The study employed molecular docking simulations to predict the binding affinity and interaction patterns of calystegines (Calystegine A3, B1, B2, B3, B4, and C1) against α -glucosidase enzymes, specifically the N-terminal maltase-glucoamylase (NT-MGAM), C-terminal maltase-glucoamylase (CT-MGAM), and the N-terminal domain of sucrase-isomaltase (NT-SI). Computational algorithms were used to identify compounds with favorable oral bioavailability and potential inhibitory activity associated with the regulation of

postprandial blood glucose levels.

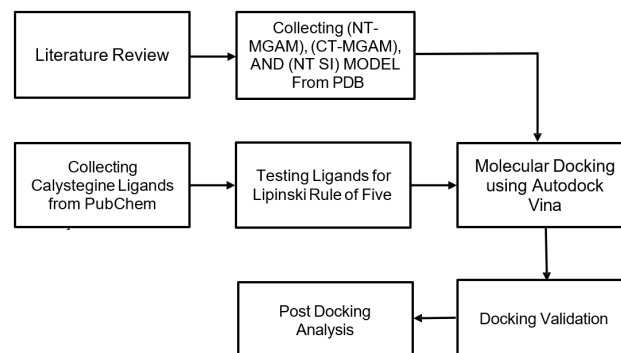


Fig. 2. Illustration of methodology

This study utilized multiple computational software programs to perform molecular docking simulations and evaluate the interactions between selected ligands and the target enzymes. The software employed included (1) AutoDock Vina v1.1.2, (2) AutoDock Tools v1.5.7, (3) PyMOL v2.6, (4) SwissADME, and (5) BIOVIA Discovery Studio Visualizer v24.1.0. Structural data were obtained from publicly accessible databases, specifically the Protein Data Bank (<https://www.rcsb.org>) and PubChem (<https://pubchem.ncbi.nlm.nih.gov>), which provided the three-dimensional (3D) structures of the target proteins and the 3D conformations of the selected calystegine ligands used in the analysis.

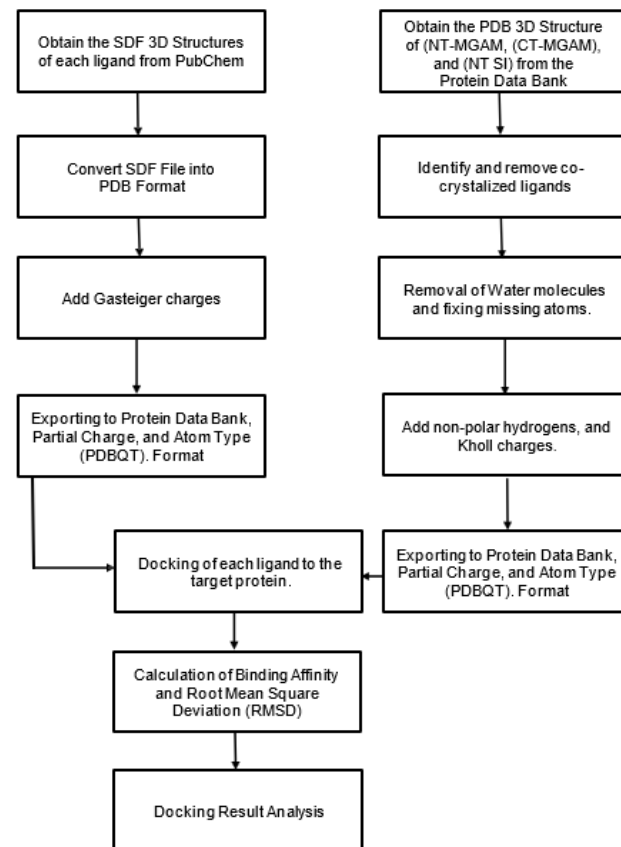


Fig. 3. Illustration of molecular docking analysis process

The molecular docking workflow was designed to evaluate the binding interactions between selected ligands and the target proteins. The preparation process involved obtaining three-dimensional structures of ligands and proteins from reliable structural databases, followed by systematic preprocessing to ensure compatibility with docking simulations. Ligand preparation included format conversion, charge assignment, and optimization, while protein preparation involved removal of co-crystallized molecules, correction of structural issues, and addition of appropriate charges and hydrogen atoms. The prepared ligand and protein structures were then converted into docking-compatible formats and subjected to molecular docking analysis. Binding affinity values and root mean square deviation (RMSD) were calculated to assess ligand–protein interactions, and the docking results were subsequently analyzed. The overall workflow of the molecular docking procedure is illustrated in Figure 3.

Phase 1: Compound Collection and Preparation

In silico docking experiments remained a cornerstone for evaluating the binding affinity of calystegines derived from *Ipomoea aquatica* to the active sites of metabolic enzymes, such as α -glucosidase and α -amylase, thereby validating their potential as natural glycemic regulators (Saikia, Dey, Hazarika, Handique, Thakur, & Handique, 2024). This included the presence of calystegines B1, B2, B3, and B4 in *Ipomoea aquatica* (Rauniyar, 2020). Furthermore, de Nijs *et al.* (2023) reported that *Ipomoea aquatica* also contained calystegine A3, while Kusumah & Pertiwi (2021) noted that it contained calystegine C1. The total number of calystegines in *Ipomoea aquatica* was as follows: A3, B1, B2, B3, B4, and C1.

Based on Libertext (2013), the first step in the molecular docking process was acquiring the three-dimensional structure of the target–ligand complex. PubChem (<https://pubchem.ncbi.nlm.nih.gov/>) was a public chemical repository that allowed both the general public and scholarly groups to access information on chemicals. It enabled various structure searches, including identification searches, 2-D and 3-D similarity searches, and substructure and superstructure searches. The chemical data from hundreds of sources were collected by this database and organized into several data collections, for instance, Substance, Compound, BioAssay, Protein, Gene, Pathway, and Patent (Kim, 2021). The PubChem database was searched to retrieve polyhydroxylated nortropane alkaloids reported in *Ipomoea aquatica*, such as Calystegine A3, Calystegine B1, Calystegine B2, Calystegine B3, Calystegine B4, and Calystegine C1 in the 3D SDF format. The 3D conformers provided by PubChem were used directly to ensure the accuracy of the molecular geometry.

Open Babel (<https://openbabel.org/docs/index.html>) was utilized for the conversion of every compound into the PDB format without the loss of 3D coordinates. The software served as a comprehensive chemical expert system that supported molecular modeling and cheminformatics by interpreting various languages of chemical data. The implementation of this chemical toolbox allowed for the search, conversion, and analysis of data across biological and chemical disciplines (O’Boyle, Banck, James, Morley, Vandermeersch, &

Hutchison, 2020). The generated PDB structures were processed within the AutoDock Tools graphical environment (<https://autodocksuite.scripps.edu/adst/>) to determine rotatable bonds and assign Gasteiger charges, which were critical for characterizing the electrostatic properties of the ligand and receptor. AutoDockTools remained a globally recognized, open-access resource that provided essential infrastructure for structure-based drug design, facilitating the precise preparation of molecular coordinates required for automated docking procedures (Goodsell, Sanner, Olson, & Forli, 2021).

The finally prepared ligands were saved in PDBQT format for docking simulations. Acarbose, a known inhibitor of α -glucosidase, was also downloaded, processed, and kept as a benchmark to compare and validate activities. Acarbose was an oligosaccharide of complicated structure, which inhibited the intestinal absorption of dietary carbohydrates and, consequently, the postprandial blood glucose and insulin increase by inhibiting the activity of several enzymes that hydrolyzed complex carbohydrates in the intestines, such as membrane-bound α -glucosidases (Akmal *et al.*, 2024).

Phase 2: ADME Prediction

The evaluation of ADME and toxicity within drug research was critically important for identifying viable therapeutic candidates. According to Ivanović, Jevtić, and Milovanović (2020), establishing qualitative and quantitative dependencies between molecular structure and pharmacokinetic properties was essential for predicting successful bioavailability. To achieve this, in silico pharmacokinetic screening was performed on each ligand using the SwissADME web tool. As detailed by Ancuceanu, Lascu, Drăgănescu, and Dinu (2025), this computational process provided rapid and cost-effective alternatives to traditional experimental testing by predicting molecular weight, lipophilicity (log P), topological polar surface area, and the number of hydrogen bond donors and acceptors.

This analysis specifically confirmed compliance with Lipinski’s Rule of Five, often called the Pfizer Rule of Five. Although initially proposed in 1997, recent literature indicated that this framework remained the most well-known standard for predicting favorable oral bioavailability (Ivanović *et al.*, 2020). The rule established that a compound was more likely to exhibit effective pharmacokinetics if it met specific thresholds: a molecular weight of 500 Daltons or less, an octanol–water partition coefficient (log P) of 5 or less, and no more than 5 hydrogen bond donors and 10 hydrogen bond acceptors. By integrating these predictions, the platform allowed for the prioritization of compounds that possessed the necessary medicinal chemistry friendliness to advance through modern drug discovery programs (Ancuceanu *et al.*, 2025).

Additionally, the ligands went via ProTox-II, which forecasted their toxicity class, values, and possible organ-specific toxicities. ProTox-II offered a freely accessible web server for in silico toxicity prediction for toxicologists, regulatory agencies, computational and medicinal chemists, and all users without login at http://tox.charite.de/protox_II. Compounds with low permeability ($\log K_p < -8.0$) or significant anticipated toxicity (ProTox class ≤ 3) were removed.

According to Banerjee, Kemmler, Dunkel, and Preissner (2024), the platform processed two-dimensional chemical structures to generate a comprehensive toxicity profile based on 61 distinct models, each accompanied by a confidence score. The output included a global toxicity radar chart and identified the three most structurally similar compounds with documented acute toxicity. The resulting ADME data were organized into descriptive tables and visualized using bar charts and radar plots, allowing for a clear assessment of the compound's pharmacokinetic properties and overall safety.

Phase 3: Protein Target Preparation

The 3D crystal structure of the α -glucosidase enzyme was retrieved from the Protein Data Bank (PDB) in .pdb format. According to Berman and Burley (2025), the Protein Data Bank (PDB), established in 1971 as the biological sciences' first open-access digital resource, served as the sole worldwide repository for experimentally determined 3D structures of biological macromolecules. The atomic-level models and metadata archived therein were produced using three primary methodologies: crystallography, nuclear magnetic resonance (NMR) spectroscopy, and electron microscopy (3DEM). To ensure the sustainable archiving and validation of structures derived from integrative or hybrid methods, PDB partners actively collaborated with international experts to develop a unified federation of data resources. In PyMOL, the α -glucosidase was cleaned by eliminating ions, water molecules, and co-crystallized ligands. PyMOL provided a builder menu for drawing small chemical compounds, peptides, and nucleic acid sequences in order to manage molecular construction and editing. The constructor menu included 3D-model cleaning techniques as well as comprehensive hydrogen and charge repair options (Rosignoli & Paiardini, 2022).

The receptor then was processed in AutoDock Tools (ADT) to add polar hydrogens, assign Gasteiger charges, and define the binding site based on the coordinates of the co-crystal ligand or active site residues. The processed receptor was saved in pdbqt format, and grid box dimensions and coordinates were recorded for docking configuration.

Phase 4: Molecular Docking

Molecular docking served as a sophisticated computational framework for predicting the noncovalent associations between biological macromolecules and small-molecule ligands. This methodology focused on identifying the optimal bound orientations and quantifying the resulting binding affinities to understand how molecules interacted at a structural level. In a practical research context, the ability to digitally screen virtual libraries of drug-like molecules was essential for identifying potential leads for further development, a process that relied on the efficiency and accuracy of modern docking engines. To establish the reliability of the docking environment, a validation protocol was performed by re-docking a known inhibitor, such as Acarbose, into its established crystal structure. The success of the docking system was confirmed only when the software replicated the experimental conformation with high precision, specifically yielding a Root Mean Square Deviation (RMSD) value of less than 2.0 Å. This verification step ensured the integrity of the search algorithm before evaluating the binding

characteristics of novel compounds (Eberhardt, Santos-Martins, Tillack, & Forli, 2021).

C. Data Collection and Analysis

1) Post-Docking Interaction Analysis

The visualization of molecular interactions was a crucial part of interpreting docking results before a final determination was made. PyMOL and BIOVIA Discovery Studio Visualizer were utilized to examine the optimal docking poses, with a focus on identifying the lowest binding energy and accurate active site orientation. According to Baroroh, Muscifa, Destiarani, Rohmatullah, and Yusuf (2022), the analysis of these interactions helped to strengthen docking findings in addition to recording the free energy of binding. The optimal docked mode for each ligand was determined by evaluating the molecular mechanism and the surface characteristics of the receptor. To fully understand the structural features that affected the strength of a bond between a ligand and its receptor, it was necessary to perform a detailed inspection of the binding interactions. This visual process ensured that the data provided a clear understanding of protein–ligand complementarity and was suitable for high-quality publication results. PyMOL automatically discovered polar interactions (hydrogen bonds) and π – π / π –cation interactions (Del Conte et al., 2023). Being an essential tool in the visualization and analysis of molecular docking results, the application of BIOVIA Discovery Studio was one of the most powerful software available at the time. Moreover, it offered various kinds of analyses to calculate the binding free energy, the binding affinity, and other properties of the ligand–target complex. Besides this, the software presented a simple-to-use interface, which allowed the user to set the appearance of images and animations as desired, thus facilitating the production of high-quality figures for publication (Baroroh et al., 2022). Two-dimensional (2D) and three-dimensional (3D) interaction diagrams were produced to analyze hydrogen bonds, hydrophobic interactions, van der Waals forces, and π – π stacking that existed between the ligands and the active-site residues.

The binding energies were normalized by ligand efficiency (the energy of binding per heavy atom), and then, the compounds were ranked based on their efficiency and interaction strength. The results were presented in tables, while the significant ligand–residue interactions were displayed to emphasize the potential inhibitory mechanisms.

2) Integrative Analysis

The bonding interactions between calystegines and the α -glucosidase enzyme were visualized using interaction networks generated by Cytoscape. As an open-source software platform, Cytoscape provided a comprehensive environment for the visualization, modeling, and analysis of complex molecular and genetic interaction networks. By integrating structural docking results and interaction profiles into a unified dataset, the platform enabled a detailed assessment of pharmacological potential. This free, community-driven resource was essential for mapping biochemical relationships and interpreting the structural data of biological complexes (Franz, Lopes, Fong, Kucera, Cheung, Siper, Huck, Dong, Sumer, and Bader, 2023).

All the final results were presented in both tabular and graphical formats, including the comparison of docking scores, pharmacokinetic parameters, and toxicity profiles. The use of this integrative method led to the generation of a comprehensive computational assessment of the polyhydroxylated nortropane alkaloids from *Ipomoea aquatica* as a source of natural α -glucosidase inhibitors for blood sugar regulation.

3) Consultation

This study was consulted with an expert, a BS Biology graduate from Indonesia with a major in Biotechnology and Bioinformatics at the University of Sam Ratulangi, was conducted to validate the research and ensure that the study was reliable and followed proper scientific procedures. During the session, the researcher presented the methodology, data, and analysis for review. The expert helped verify the biological concepts, biotechnological approaches, and bioinformatics analyses applied in the study. The scientist expertise confirmed that the methods utilized were appropriate interpretations were accurate, and the procedures aligned with accepted research standards. The consultation strengthened the credibility of the study and provided a clearer direction on how to develop the study.

4) Ethical Consideration

The research study was entirely computational and solely relied on free and publicly available resources, such as ligand structures from PubChem, protein files from the Protein Data Bank, and data from free-to-use digital applications. As an in-silico investigation, it did not involve human participants, animals, biological specimens, or any form of personal or sensitive information; thus, it complied with established ethical standards and the Data Privacy Act. All databases and software utilized were properly acknowledged, and the research team ensured accuracy at every stage, avoided data manipulation, and adhered to responsible research practices that promoted scientific transparency and workflow integrity. Ethical and secure data management was further ensured through non-disclosure measures that restricted access to internal files, computational logs, and results exclusively to authorized members, with all generated data stored in secured digital folders to minimize exposure; only essential computational outputs were shared, while raw and intermediate files were protected to prevent unauthorized access or misuse.

Beyond data security, the study demonstrated clear social value by contributing to scientific knowledge on α -glucosidase inhibition as a potential mechanism for controlling blood glucose levels, supported diabetes-related research through cost-effective and low-risk computational methods, and

provided a foundation for future laboratory and clinical investigations that may benefit communities, particularly in resource-limited settings, thereby ensuring accountability, transparency, and compliance with ethical standards governing computationally based scientific research.

4. Results, Analysis and Discussions

This study employs an in-silico molecular docking approach to evaluate the inhibitory potential of polyhydroxylated nortropane alkaloids (calystegines) derived from *Ipomoea aquatica* against the α -glucosidase enzyme. Target compounds are selected through an extensive review of related literature and prepared for docking analysis, with their pharmacokinetic properties assessed using SwissADME to evaluate drug-likeness and bioavailability. The three-dimensional crystal structure of α -glucosidase is retrieved from the Protein Data Bank and optimized using PyMOL by removing water molecules and co-crystallized ligands. Ligand and protein preparation, including the addition of polar hydrogens and assignment of charges, is performed using AutoDock Tools. Molecular docking simulations are carried out using AutoDock Vina to predict binding affinities and interaction modes, with acarbose used as the standard reference inhibitor. Post-docking analyses are conducted using Discovery Studio to examine hydrogen bonding, hydrophobic, and electrostatic interactions, allowing for the identification and evaluation of promising α -glucosidase inhibitor candidates.

A. System Information

The simulations of molecular docking are conducted on a device equipped with the following specifications: Processor: Intel(R) Core(TM) i5-8265U CPU @ 1.60GHz, 1.80 GHz, Installed RAM: 16.0 GB (15.8 GB usable), System Type: 64-bit operating system, x64-based processor, Operating System: Windows 10 Pro, Version 22H2, OS Build: 19045.6466, Windows Feature Experience Pack: 1000.22700.1041.0.

B. Ligands

The results of the molecular docking analysis are evaluated and compared with acarbose; a standard α -glucosidase inhibitor commonly used in the management of postprandial hyperglycemia. The selected ligands consist of polyhydroxylated nortropane alkaloids (calystegines) derived from *Ipomoea aquatica*, which are identified based on a comprehensive review of related literature. Detailed information for each ligand, including the International Union of Pure and Applied Chemistry (IUPAC) name, chemical formula, and compound name, is presented in Table 2. The

Table 4
Ligands and their respective IUPAC name and chemical formula

Compound	IUPAC Name	Chemical Formula
Acarbose	(2R,3R,4R,5R)-4-[(2R,3R,4R,5S,6R)-5-[(2R,3R,4S,5S,6R)-3,4-dihydroxy-6-methyl-5-[[[(1S,4R,5S,6S)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-en-1-yl]amino]oxan-2-yl]oxy-3,4-dihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-2,3,5,6-tetrahydroxyhexanal	C ₂₅ H ₄₃ NO ₁₈
Calystegine A3	(1R,2S,3R,5R)-8-azabicyclo[3.2.1]octane-1,2,3-triol	C ₇ H ₁₃ NO ₃
Calystegine B1	(1R,2S,3R,5S,6R)-8-azabicyclo[3.2.1]octane-1,2,3,6-tetrol	C ₇ H ₁₃ NO
Calystegine B2	(1R,2S,3R,4S,5R)-8-azabicyclo[3.2.1]octane-1,2,3,4-tetrol	C ₇ H ₁₃ NO ₄
Calystegine B3	(1R,2R,3R,4S,5R)-8-azabicyclo[3.2.1]octane-1,2,3,4-tetrol	C ₇ H ₁₃ NO ₄
Calystegine B4	(1R,2S,3R,4R,5R)-8-azabicyclo[3.2.1]octane-1,2,3,4-tetrol	C ₇ H ₁₃ NO ₄
Calystegine C1	(1R,2S,3R,4S,5R,6R)-8-azabicyclo[3.2.1]octane-1,2,3,4,6-pentol	C ₇ H ₁₃ NO ₅

three-dimensional SDF structures of the selected calystegines are retrieved from the PubChem database and visualized using PyMOL, as illustrated in Figure 4.

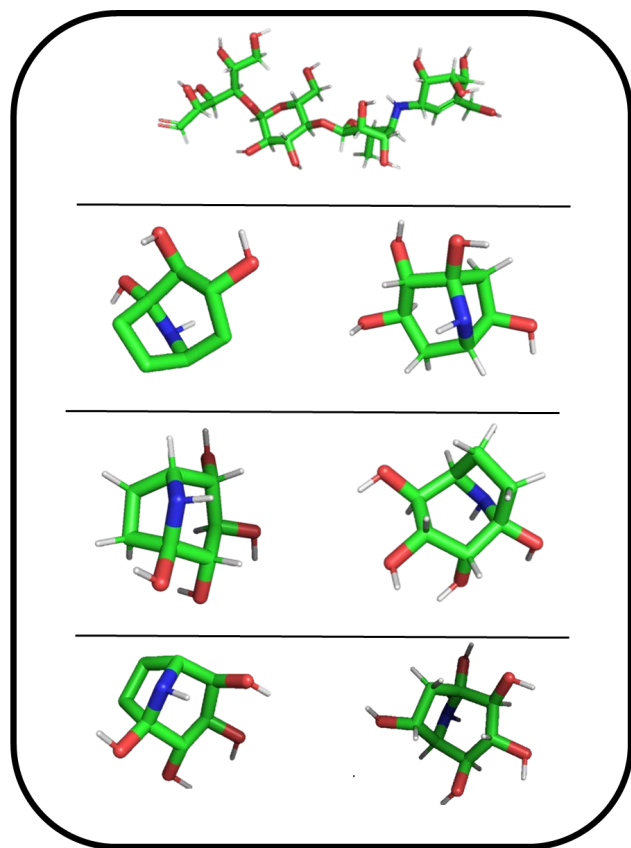


Fig. 4. The three-dimensional structures of the selected ligands

C. Lipinski Drug-likeness Test

The compounds Acarbose, Calystegine A3, Calystegine B1, Calystegine B2, Calystegine B3, Calystegine B4, and Calystegine C1 were screened for drug-likeness using the Lipinski Drug-Likeness Test via SwissADME to evaluate the ADME properties related to oral bioavailability (Riyadi et al., 2021). The assessment focused on key parameters, including molecular weight (preferably <500 Daltons), lipophilicity (mlogP between -0.4 and +5.6), hydrogen bond donors (≤ 5), hydrogen bond acceptors (≤ 10), topological polar surface area (TPSA ≤ 140 Å²), and predicted gastrointestinal (GI) absorption.

Compounds complying with Lipinski's Rule of Five with no more than two violations and exhibiting favorable ADME characteristics, such as acceptable molecular weight, balanced lipophilicity, and adequate GI absorption, were considered suitable for further molecular docking analysis.

Conversely, compounds showing high molecular weight, excessive polarity, low GI absorption, and multiple Lipinski violations were regarded as having limited oral drug-likeness. This screening ensured that only compounds with appropriate physicochemical properties and potential medicinal applicability were advanced for subsequent docking studies (Karami et al., 2022).

The analysis of the six phytochemical constituents of *Ipomoea aquatica* based on Lipinski's Rule of Five indicates that all compounds are classified as Lipinski-compliant. The molecular weights of the compounds are well below 500 g/mol, and the numbers of hydrogen bond donors and acceptors largely fall within the recommended limits. Although Calystegine C1 exhibits a hydrogen bond donor value of 6, which slightly exceeds the recommended threshold, this represents only a single Lipinski violation. Consequently, Calystegine C1 remains classified as compliant, as one violation is permissible under Lipinski's criteria. Additionally, all compounds display negative LogP values, suggesting high hydrophilicity, which may influence membrane permeability but does not constitute a violation of the rule. Overall, the results suggest that the phytochemical constituents of *Ipomoea aquatica* possess favorable drug-likeness properties and potential oral bioavailability.

Acarbose is considered non-compliant with Lipinski's Rule of Five because it is a complex oligosaccharide (a pseudo-tetrasaccharide) with high molecular weight, high polarity, and poor passive membrane permeability. Specifically, it often violates rules regarding the number of hydrogen bond donors/acceptors and molecular weight (MW > 500 Da) (Marzouk et al., 2025). However, acarbose is still widely used and effective because its therapeutic mechanism does not require it to be absorbed into the bloodstream (McIver et al., 2024).

D. Molecular Docking Binding Affinity

Molecular docking emerges as a potent instrument for lead discovery and optimization. Numerous docking systems have been developed over the past thirty years, utilizing various search algorithms and scoring functions (Mario S. Valdés-Tresanco, Mario E. Valdés-Tresanco, Pedro A. Valiente & Ernesto Moreno, 2020). AutoDock Vina is one of the most rapid and extensively utilized open-source systems for molecular docking. Furthermore, AutoDock Vina 1.2.0 accommodates the AutoDock4.2 scoring mechanism, enables simultaneous docking of multiple ligands, and includes a batch mode for docking several ligands. This procedure enhances ligand placement within the binding pocket by identifying structural ensembles exhibiting minimal binding free energy

Table 5
Lipinski drug-likeness test results

Compound	Molecular Weight (g/mol)	H-Bond Donors	H-Bond Acceptors	LogP	Evaluation
Acarbose	645.60	14	19	0.17	Non-compliant
Calystegine A3	159.18	4	4	0.87	Qualified
Calystegine B1	175.18	5	5	0.82	Qualified
Calystegine B2	175.18	5	5	0.79	Qualified
Calystegine B3	175.18	5	5	0.34	Qualified
Calystegine B4	175.18	5	5	0.56	Qualified
Calystegine C1	191.18	6	6	-1.43	Qualified

(Jerome Eberhardt, Diogo Santos-Martins, Andreas F. Tillack, Stefano Forli, 2021).

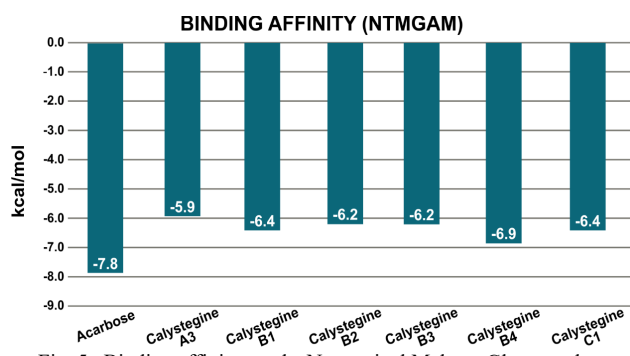


Fig. 5. Binding affinity results N- terminal Maltase-Glucoamylase

The molecular docking results revealed that all tested compounds exhibited negative binding affinity values toward the N-terminal domain of maltase-glucoamylase (NtMGAM), indicating energetically favorable and stable ligand–enzyme interactions. In docking studies, more negative binding energy values correspond to stronger binding stability within the enzyme’s active site.

Among the tested compounds, the reference inhibitor Acarbose demonstrated the strongest binding affinity (−7.8 kcal/mol), indicating the most stable interaction with NtMGAM. Among the calystegine derivatives, Calystegine B4 showed the highest binding affinity (−6.9 kcal/mol), suggesting a strong and stable interaction with the catalytic domain. This was followed by Calystegine B1 and Calystegine C1, both exhibiting binding affinities of −6.4 kcal/mol, indicating comparable interaction strength within the active site.

Meanwhile, Calystegine B2 and Calystegine B3 demonstrated similar binding affinities (−6.2 kcal/mol), suggesting moderate and stable interactions with NtMGAM. Calystegine A3 showed the lowest binding affinity (−5.9 kcal/mol) among the calystegine derivatives, indicating relatively weaker binding compared to the other compounds. Despite these variations, all calystegine derivatives exhibited binding energies close to that of acarbose.

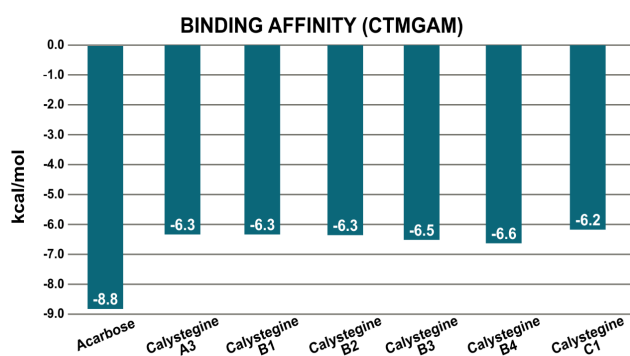


Fig. 6. Binding affinity results C- terminal Maltase-Glucoamylase

Overall, the results suggest that calystegine derivatives possess promising inhibitory potential against NtMGAM, with Calystegine B4 showing the strongest binding affinity among the tested derivatives. Although acarbose demonstrated

superior binding stability, the calystegines showed comparable interaction energies, supporting their potential as candidate inhibitors targeting the N-terminal domain of maltase-glucoamylase for postprandial glucose regulation.

The molecular docking results revealed that all tested compounds exhibited negative binding affinity values toward the C-terminal domain of maltase-glucoamylase (CtMGAM), indicating energetically favorable and stable ligand–enzyme interactions. In docking studies, more negative binding energy values correspond to stronger binding stability within the enzyme active site.

Among the tested compounds, the reference inhibitor Acarbose demonstrated the strongest binding affinity (−8.8 kcal/mol), indicating the most stable interaction with CtMGAM. Among the calystegine derivatives, Calystegine B4 showed the highest binding affinity (−6.6 kcal/mol), suggesting the strongest interaction with the catalytic domain. This was followed by Calystegine B3 (−6.5 kcal/mol), indicating slightly stronger interaction compared to the other derivatives. Meanwhile, Calystegine A3, Calystegine B1, and Calystegine B2 exhibited identical binding affinities (−6.3 kcal/mol), suggesting comparable and moderate interactions within the CtMGAM active site. Calystegine C1 showed the lowest binding affinity (−6.2 kcal/mol) among the calystegine derivatives, indicating slightly weaker binding stability compared to the other compounds. Despite these differences, all calystegine derivatives demonstrated binding energies within a narrow range.

Overall, the results suggest that calystegine derivatives possess promising inhibitory potential against CtMGAM, with Calystegine B4 showing the strongest binding affinity among the derivatives, while acarbose exhibited superior binding stability overall. These findings support the potential of calystegines as candidate inhibitors targeting the C-terminal domain of maltase-glucoamylase for postprandial glucose regulation.

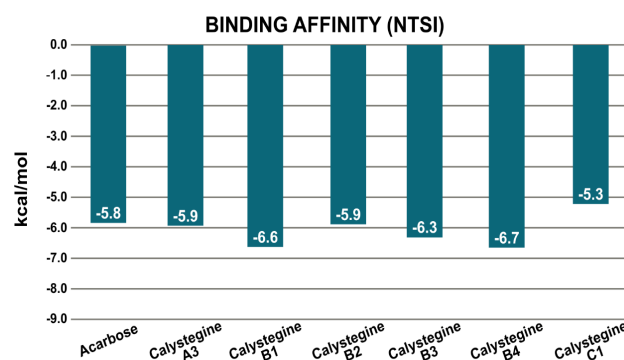


Fig. 7. Binding affinity results N-terminal domain of sucrase-isomaltase

The molecular docking results revealed that all tested compounds exhibited negative binding affinity values toward the N-terminal domain of sucrase–isomaltase (NTSI), indicating energetically favorable and stable ligand–enzyme interactions. In docking studies, more negative binding energy values correspond to stronger binding stability within the active site.

Among the tested compounds, Calystegine B4 demonstrated the strongest binding affinity (-6.7 kcal/mol), indicating the most stable interaction with the NTSI active site. This was followed by Calystegine B1 (-6.6 kcal/mol), suggesting a similarly strong interaction within the catalytic domain. Calystegine B3 exhibited a binding affinity of -6.3 kcal/mol, indicating moderate and stable binding.

Meanwhile, Calystegine A3 and Calystegine B2 showed identical binding affinities (-5.9 kcal/mol), indicating comparable moderate interactions with the enzyme. The reference inhibitor Acarbose demonstrated a binding affinity of -5.8 kcal/mol, which was slightly weaker than several of the calystegine derivatives. Calystegine C1 showed the lowest binding affinity (-5.3 kcal/mol), indicating relatively weaker binding stability compared to the other compounds.

Overall, the results suggest that calystegine derivatives exhibit promising inhibitory potential against the N-terminal domain of sucrase-isomaltase, with Calystegine B4 and Calystegine B1 showing superior binding affinities compared to the reference inhibitor acarbose. These findings indicate that calystegines may serve as potential candidates for inhibiting carbohydrate-digesting enzymes and regulating postprandial glucose levels.

E. Post Docking Analysis: N- terminal Maltase-Glucoamylase

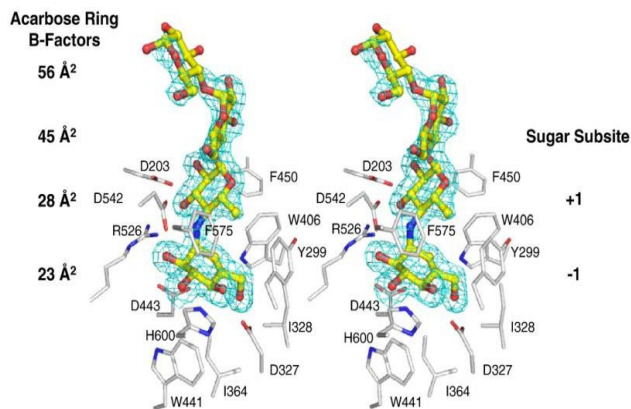


Fig. 8. Visualization of active sites in N-terminal Maltase-Glucoamylase
Credits: Lyann Sim, Roberto Quezada-Calvillo, Erwin E. Sterchi, Buford L. Nichols and David R. Rose (2008)

N-terminal subunit of human maltase-glucoamylase (ntMGAM) is a constrained pocket specifically optimized for the hydrolysis of short-chain α -1,4-linked glucose substrates. Advanced molecular dynamics (MD) simulations and adaptive steered molecular dynamics (ASMD) have identified that the catalytic machinery is anchored by the conserved residues Asp442 (the nucleophile) and Asp542 (the acid/base catalyst), which facilitate the retaining glycosyl transferase mechanism (Zhang et al., 2021). Unlike the C-terminal subunit, ntMGAM lacks extended subsites, meaning its binding affinity is primarily dictated by interactions at the subsites, where residues like Trp441, Trp406, and Phe450 provide aromatic stabilization for the glucose rings (Watanabe et al., 2026). Beyond basic binding, recent research emphasizes the specific interactive network of amino acids that dictate inhibitor selectivity. Studies mapping the unbinding pathways

of glucose-mimetic inhibitors show that Asp327 and Arg526 are critical for forming stable hydrogen bonding and salt-bridge networks that restrict substrate access to the catalytic center (Zhang et al., 2021). Furthermore, the flexibility of "Inserted Loop 1" (residues 491–499) and "Inserted Loop 2" has been shown to influence the active pocket's volume, where the spiralization of residues 497–499 can either stabilize or obstruct the binding of therapeutic inhibitors like de-O-sulfonated kotalanol (DSK) (Zhang et al., 2021). These interaction profiles clarify why ntMGAM remains the primary target for controlling the earliest stages of starch digestion and postprandial glucose release (Watanabe et al., 2026).

Molecular interaction analyses are conducted on the N-terminal domain of maltase-glucoamylase (NtMGAM) to clarify the binding outcomes of the calystegine ligands A3, B1, B2, B3, B4, and C1. A comprehensive assessment of enzyme-ligand interactions is performed using Biovia Discovery Studio, which facilitates the visualization of essential molecular characteristics, such as interacting amino acid residues, hydrogen bond formation, and the number of hydrogen bonds involved (Eberhardt et al., 2021), as outlined in Table 6. These visualizations provide profound insight into enzyme-ligand binding interactions and enhance the evaluation of each ligand's potential inhibitory efficacy against NT-MGAM.

1) Acarbose

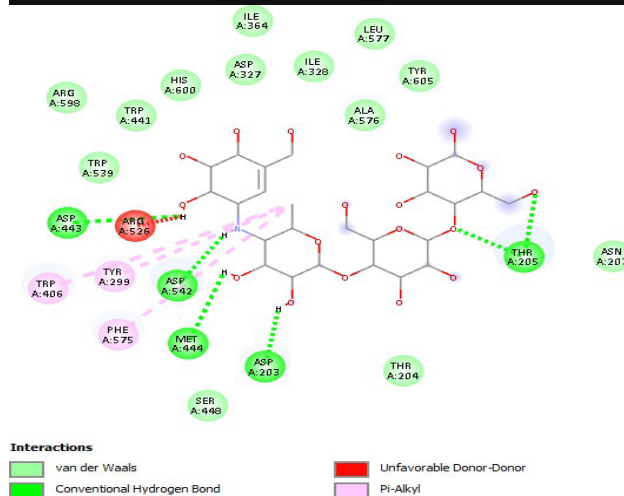
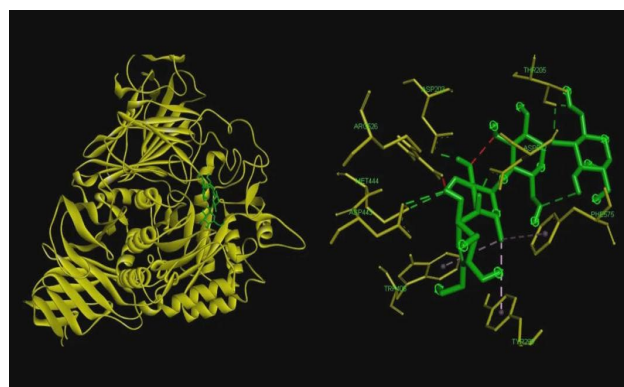


Fig. 9. Visualization of Acarbose docking simulation.

(A) Best Binding Position with target Protein (NT-MGAM), (B) Three-dimensional structure display, (C) Two-dimensional visualization of binding interactions

Credits: Jan Criss Peralta

Table 6
Simulation results of interactions between Ligands and N- terminal Maltase Glucoamylase

Chemical Compound	Binding Affinity	Interacting Amino acids	No. of Hydrogen Bonds	Hydrogen Bond Interactions
Acarbose	-7.8 kcal/mol	ASP 203, THR 205, ARG 443, MET 444, ASP 542, TYR 299, TRP 206, PHE 575, ARG 526, THR 204, ASN 207, ASP 327, ILE 328, ILE 364, TRP 441, SER 448, TRP 539, ALA 576, LEU 577, ARG 598, HIS 600, TYR 605	6	ASP 203, THR 205, ARG 443, MET 444, ASP 542
Calystegine A3	-5.9 kcal/mol	ASP 443, ASP 542, HIS 600, TYR 299, TRP 406, PHE 575, ASP 327, ILE 328, ILE 364, TRP 441, MET 444, ARG 526, TRP 539	4	ASP 443, ASP 542, HIS 600
Calystegine B1	-6.4 kcal/mol	GLU 300, GLY 302, ASP 329, MET 331, ARG 334, ASP 343, ILE 328, GLU 333, ASP 340, PHE 344	6	GLU 300, GLY 302, ASP 329, MET 331, ARG 334
Calystegine B2	-6.2 kcal/mol	ASP 327, ASP 443, ASP 542, HIS 600, TYR 299, PHE 575, ILE 364, TRP 406, TRP 441, MET 444, ARG 526, TRP 539	3	ASP 327, ASP 443, ASP 542,
Calystegine B3	-6.2 kcal/mol	ASP 327, ASP 443, ARG 526, ASP 542, HIS 600, TYR 299, TRP 406, PHE 575, ILE 328, ILE 364, TRP 441, MET 444, TRP 539, ARG 598	6	ASP 327, ASP 443, ARG 526, ASP 542, HIS 600
Calystegine B4	-6.9 kcal/mol	GLU 300, ASP 329, MET 331, ASP 340, ARG 334, GLY 302, ILE 328, ASP 332, GLU 333, ASP 343, PHE 344	6	GLU 300, ASP 329, MET 331, ASP 340
Calystegine C1	-6.4 kcal/mol	GLU 300, GLY 302, ASP 329, MET 331, ASP 343, ILE 328, GLU 333, ARG 334, ASP 340, PHE 344	6	GLU 300, GLY 302, ASP 329, MET 331, ASP 343

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#
# DOI 10.1002/jcc.21334
#
# Please see http://vina.scripps.edu for more information.
#####
WARNING: The search space volume > 27000 Angstrom^3 (See FAQ)
Detected 8 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: -1443085696
Performing search ...
0% 10 20 30 40 50 60 70 80 90 100%
|-----|-----|-----|-----|-----|-----|-----|
done.
Refining results ... done.

mode | affinity | dist from best mode
      | (kcal/mol) | rmsd l.b. | rmsd u.b.
-----|-----|-----|-----|
1      -7.8      0.000      0.000
2      -7.7      2.857      4.985
3      -7.5      1.591      2.733
4      -7.5      14.741     18.299
5      -7.0      14.290     16.452
6      -7.0      14.478     17.819
7      -7.0      14.709     16.626
8      -6.8      14.900     18.459
9      -6.8      17.676     21.527
10     -6.7      17.771     21.844
Writing output ... done.
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Acarbose exhibits a binding affinity of -7.8 kcal/mol toward the ntMGAM enzyme, indicating a strong and energetically favorable interaction within the active site. Molecular docking analysis reveals the formation of multiple conventional hydrogen bonds with key residues, including ASP 203, THR 205, ARG 443, MET 444, and ASP 542, which contribute to significant ligand stabilization and precise orientation within the catalytic pocket. The involvement of multiple Aspartic Acid residues is particularly notable, as these often serve as the catalytic machinery in glycosidases. Extensive van der Waals interactions are observed with THR 204, ASN 207, ASP 327, ILE 328, ILE 364, TRP 441, SER 448, TRP 539, ALA 576,

LEU 577, ARG 598, HIS 600, and TYR 605, further supporting favorable ligand accommodation and high shape complementarity. Additionally, Pi-Alkyl interactions with TRP 206, PHE 575, and ARG 526, provide aromatic stabilization, anchoring the core of the molecule through hydrophobic contacts.

Fig. 10. Molecular docking CMD result of acarbose
Credits: Jan Criss Peralta

However, an unfavorable donor–donor interaction is detected with ARG 526, suggesting a minor electrostatic repulsion between the ligand and the residue's side chain. Overall, these interaction patterns indicate that Calystegine C1 establishes robust and diverse stabilizing contacts within the enzyme's active site, suggesting it is a potent inhibitor of N-terminal maltase-glucoamylase.

2) Calystegine A3

Calystegine A3 has a binding affinity of -5.9 kcal/mol for the target enzyme. Molecular docking studies demonstrate the establishment of hydrogen bonds with the amino acid residues ASP 443, ASP 542, and HIS 600, while ASP 327, ILE 328, ILE 364, TRP 441, MET 444, ARG 526, and TRP 539 participate in van der Waals and hydrophobic interactions. An alkyl interaction is established with TYR 299, TRP 406, and PHE 575, enhancing hydrophobic stability inside the binding pocket. These interactions suggest that Calystegine A3 resides within the enzyme's active site and forms several molecular connections with essential residues.

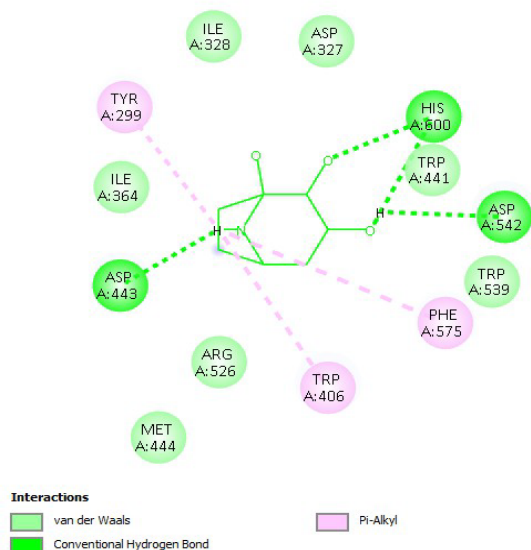


Fig. 11. Visualization of Calystegine A3 docking simulation.

(A) Best Binding Position with target Protein (NT-MGAM), (B) Three-Dimensional structure display, (C) Two-dimensional visualization of binding interactions

Credits: Dave Mejorada

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#####
# If you used AutoDock Vina in your work, please cite:
#
# O. Trott, A. J. Olson,
# AutoDock Vina: improving the speed and accuracy of docking
# with a new scoring function, efficient optimization and
# multithreading, Journal of Computational Chemistry 31 (2010)
# 455-461
#
# DOI 10.1002/jcc.21334
#
# Please see http://vina.scripps.edu for more information.
#
#####
WARNING: The search space volume > 27000 Angstrom^3 (See FAQ)
Detected 8 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: -352979656
Performing search ...
0% 10 20 30 40 50 60 70 80 90 100%
|----|----|----|----|----|----|----|----|----|----|
done.
Refining results ... done.

mode | affinity | dist from best mode
      | (kcal/mol) | rmsd l.b. | rmsd u.b.
-----+-----+-----+-----
1      | -5.9      | 0.000     | 0.000
2      | -5.8      | 12.146    | 13.490
3      | -5.7      | 34.587    | 36.093
4      | -5.6      | 27.332    | 28.253
5      | -5.5      | 27.277    | 28.293
6      | -5.4      | 12.503    | 13.660
7      | -5.4      | 12.966    | 13.984
8      | -5.3      | 27.430    | 28.401
9      | -5.3      | 13.011    | 13.484
10     | -5.1      | 12.584    | 13.655
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Fig. 12. Molecular docking CMD result of Calystegine A3

3) Calystegine B1

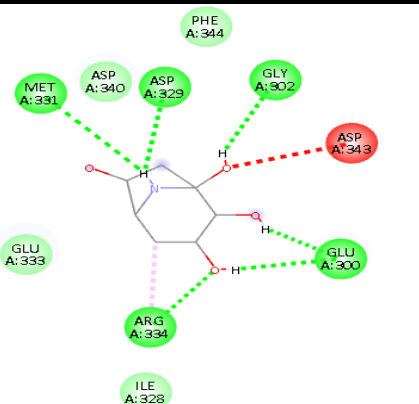
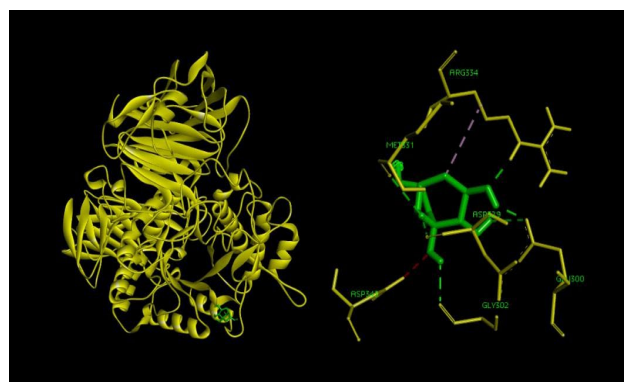


Fig. 13. Visualization of Calystegine B1 docking simulation. (A) Best Binding Position with target Protein (NT-MGAM), (B) Three-dimensional structure display, (C) Two-dimensional visualization of binding interactions

Credits: Dave Mejorada

Calystegine B1 exhibits a binding affinity of -6.4 kcal/mol for the ntMGAM enzyme, indicating a stable and spontaneous inhibitory profile. Molecular docking studies reveal that the

compound resides within the active site, anchored by a network of conventional hydrogen bonds with GLU 300, GLY 302, ASP 329, MET 331, and ARG 334. These polar connections are bolstered by van der Waals interactions with ILE 328, GLU 333, ASP 340, and PHE 344. While these interactions suggest a potent fit, the model also identifies a slight electrostatic repulsion with ASP 343. Collectively, these connections position Calystegine B1 as an effective competitive inhibitor that obstructs the catalytic machinery of ntMGAM by mimicking natural glycone substrates.

```
#
# DOI 10.1002/jcc.21334
#
# Please see http://vina.scripps.edu for more information.
#
#####

WARNING: The search space volume > 27000 Angstrom^3 (See FAQ)
Detected 8 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: -529234432
Performing search ...
0% 10 20 30 40 50 60 70 80 90 100%
|-----|-----|-----|-----|-----|
done.
Refining results ... done.

mode | affinity | dist from best mode
      | (kcal/mol) | rmsd l.b. | rmsd u.b.
-----|-----|-----|-----
1      -6.4      0.000      0.000
2      -6.2     12.553     14.036
3      -6.1     1.939      3.755
4      -5.9     1.712      2.992
5      -5.7     1.624      3.231
6      -5.7     12.395     14.427
7      -5.6     1.564      3.701
8      -5.5     1.998      2.784
9      -5.5     1.906      2.849
10     -5.4     1.903      3.335
Writing output ... done.
```

Fig. 14. Molecular docking CMD result of Calystegine B1

Credits: Dave Mejorada

4) Calystegine B2

Calystegine B2 exhibits a binding affinity of -6.2 kcal/mol toward the target enzyme, suggesting a moderate and energetically favorable interaction within the active site. Molecular docking analysis reveals the formation of several conventional hydrogen bonds with key residues, notably ASP 327, ASP 443, and ASP 542, which play an important role in anchoring the ligand and stabilizing its orientation. In addition, Pi-alkyl interactions are observed with aromatic residues TYR 299 and PHE 575, contributing to hydrophobic stabilization within the binding pocket. Several surrounding residues, including ILE 364, TRP 406, TRP 441, MET 444, ARG 526, and TRP 539, are primarily involved through van der Waals interactions, further supporting favorable ligand accommodation. While a potential electrostatic clash is noted with HIS 600 (indicated as an unfavorable donor-donor interaction), the overall interaction pattern suggests that Calystegine B2 is well positioned within the enzyme's active site and establishes multiple stabilizing contacts with residues relevant to enzymatic function.

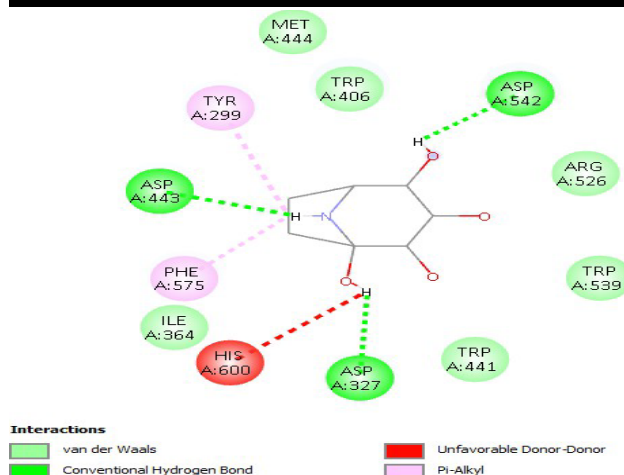
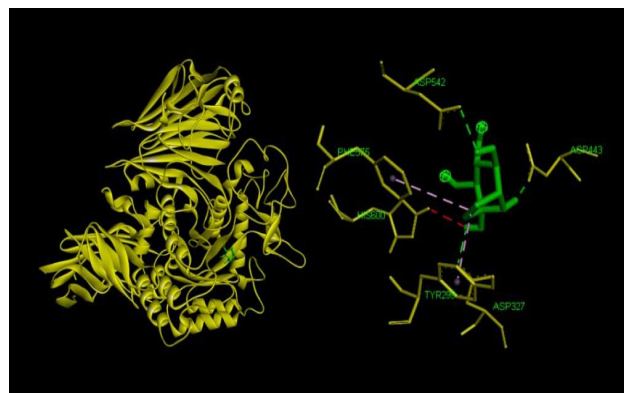


Fig. 15. Visualization of Calystegine B2 docking simulation. (A) Best Binding Position with target Protein (NT-MGAM), (B) Three-dimensional structure display, (C) Two-dimensional visualization of binding interactions

Credits: Dave Mejorada

```
#
# DOI 10.1002/jcc.21334
#
# Please see http://vina.scripps.edu for more information.
#
#####

WARNING: The search space volume > 27000 Angstrom^3 (See FAQ)
Detected 8 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: -1714363764
Performing search ...
0% 10 20 30 40 50 60 70 80 90 100%
|-----|-----|-----|-----|-----|
done.
Refining results ... done.

mode | affinity | dist from best mode
      | (kcal/mol) | rmsd l.b. | rmsd u.b.
-----|-----|-----|-----
1      -6.2      0.000      0.000
2      -6.2     12.820     14.095
3      -5.9     12.974     14.306
4      -5.9     12.730     14.112
5      -5.8     13.188     14.785
6      -5.6     12.850     14.333
7      -5.6     13.214     14.484
8      -5.5     12.679     14.307
9      -5.5     12.961     14.246
10     -5.4     13.066     14.498
Writing output ... done.
```

Fig. 16. Molecular docking CMD result of Calystegine B2

Credits: Dave Mejorada

5) Calystegine B3

The Calystegine B3 ligand exhibits a binding affinity of -6.2 kcal/mol toward the target enzyme, suggesting a moderate and

energetically favorable interaction within the active site. Molecular docking analysis reveals the formation of several conventional hydrogen bonds with key residues, notably ASP 327, ASP 443, ARG 526, ASP 542, and HIS 600, which play an important role in anchoring the ligand and stabilizing its binding orientation. In addition, π -alkyl interactions are observed with aromatic residues TYR 299, TRP 406, and PHE 575, contributing to hydrophobic stabilization within the binding pocket. Several surrounding residues, including ILE 328, ILE 364, TRP 441, MET 444, TRP 539, and ARG 598, are primarily involved through van der Waals interactions, further supporting favorable ligand accommodation in the active site. No significant unfavorable donor–donor or acceptor–acceptor interactions are detected, indicating a relatively stable binding conformation. Overall, these interaction patterns suggest that the B3 ligand is well positioned within the enzyme’s active site and establishes multiple stabilizing contacts with residues relevant to enzymatic function.

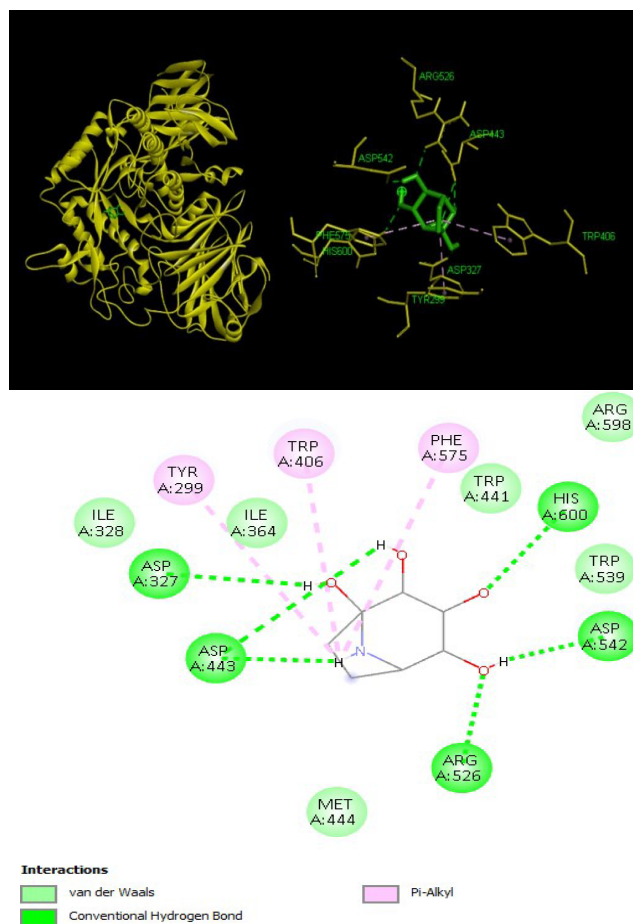


Fig. 17. Visualization of Calystegine B3 docking simulation. (A) Best Binding Position with target Protein (NT-MGAM), (B) Three-dimensional structure display, (C) Two-dimensional, (D) Visualization of binding interactions

Credits: Rea Dela Peña

```
#
# Please see http://vina.scripps.edu for more information.
#
#####

WARNING: The search space volume > 27000 Angstrom^3 (See FAQ)
Detected 8 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: 324413568
Performing search ...
0% 10 20 30 40 50 60 70 80 90 100%
|-----|-----|-----|-----|-----|-----|-----|
done.
Refining results ... done.

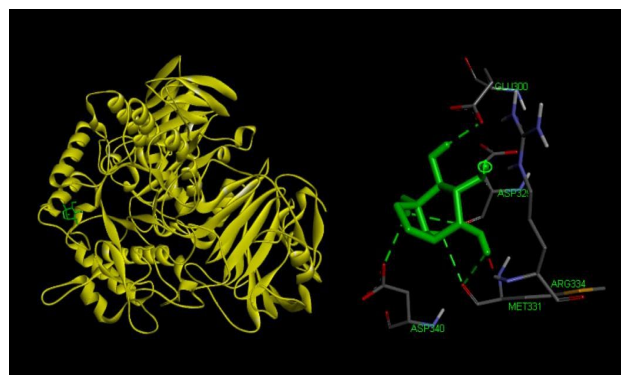
mode | affinity | dist from best mode
      | (kcal/mol) | rmsd l.b. | rmsd u.b.
-----|-----|-----|-----|
1 | -6.2 | 0.000 | 0.000
2 | -6.2 | 11.989 | 13.478
3 | -6.1 | 12.019 | 13.492
4 | -5.8 | 12.275 | 13.932
5 | -5.8 | 12.970 | 14.873
6 | -5.7 | 12.451 | 14.045
7 | -5.7 | 12.388 | 13.895
8 | -5.5 | 12.825 | 14.105
9 | -5.5 | 12.745 | 14.420
10 | -5.4 | 13.164 | 13.905
Writing output ... done.
```

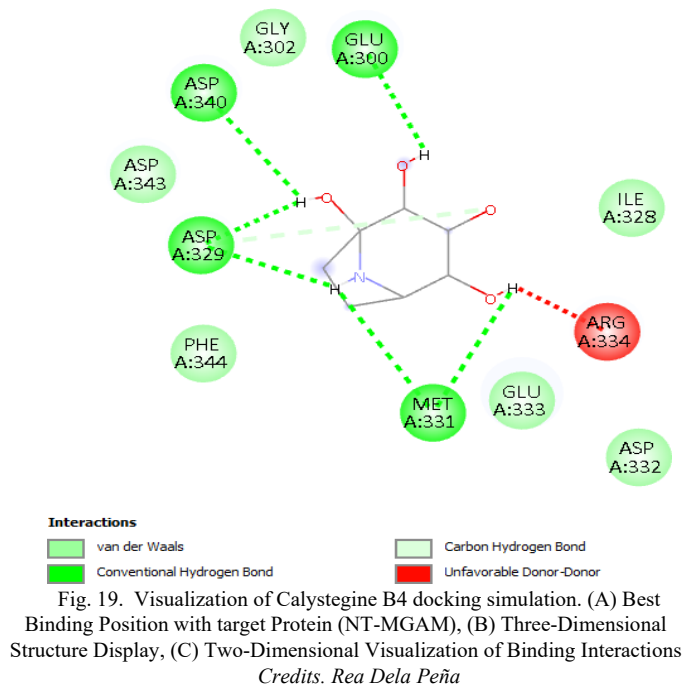
Fig. 18. Molecular docking CMD result of Calystegine B3

Credits: Rea Dela Peña

6) Calystegine B4

The Calystegine B4 ligand exhibits a binding affinity of -6.9 kcal/mol toward the target enzyme, indicating a moderate and energetically favorable interaction within the active site. Molecular docking analysis reveals the formation of several conventional hydrogen bonds with key residues, particularly GLU 300, ASP 329, MET 331, and ASP 340, which contribute to ligand stabilization and proper orientation within the binding pocket. Additional stabilization is observed through van der Waals interactions with GLY 302, ILE 328, ASP 332, GLU 333, ASP 343, and PHE 344, supporting effective ligand accommodation in the catalytic region. However, an unfavorable donor–donor interaction is detected with ARG 334, suggesting minor electrostatic repulsion within the binding environment. Overall, these interaction patterns indicate that the B4 ligand is well positioned within the active site and establishes multiple stabilizing contacts with residues relevant to enzymatic activity.





```

WARNING: The search space volume > 27000 Angstrom^3 (See FAQ)
Detected 8 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: 167249120
Performing search ...
0% 10 20 30 40 50 60 70 80 90 100%
|-----|-----|-----|-----|-----|
*****
done.
Refining results ... done.

```

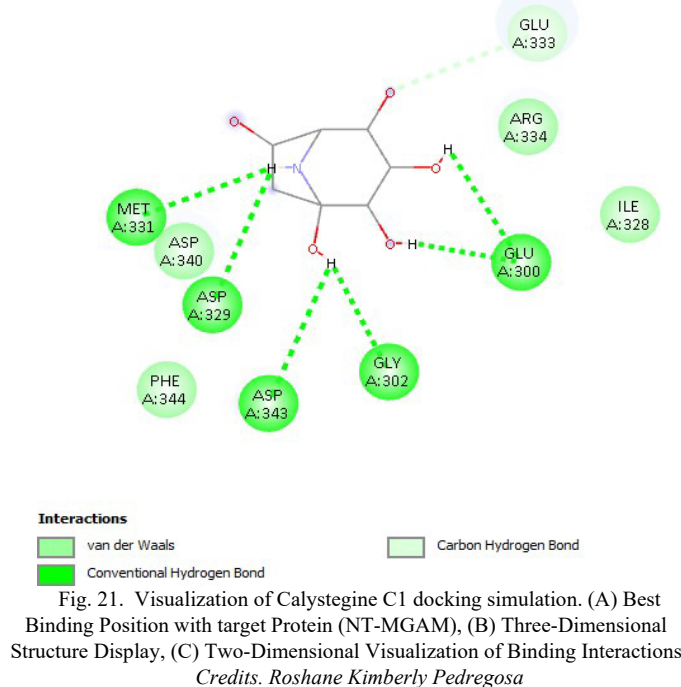
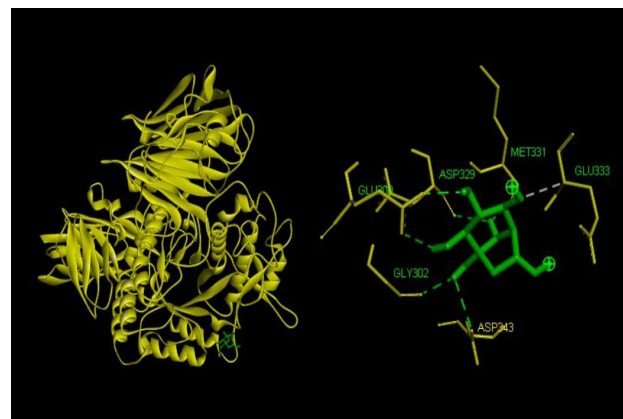
mode	affinity (kcal/mol)	dist from best mode rmsd l.b. rmsd u.b.
1	-6.9	0.000 0.000
2	-6.3	1.609 3.635
3	-5.8	1.488 2.480
4	-5.8	1.967 3.689
5	-5.8	1.923 3.492
6	-5.7	1.569 2.470
7	-5.7	2.115 3.085
8	-5.5	2.325 3.099
9	-5.4	10.836 11.935
10	-5.3	1.951 3.030

Writing output ... done.

Fig. 20. Molecular docking CMD result of Calystegine B4
Credits. Rea Dela Peña

7) Calystegine C1

The Calystegine C1 ligand demonstrates a binding affinity of -6.4 kcal/mol, representing a stable and energetically favorable binding profile. Molecular docking analysis identifies several conventional hydrogen bonds with essential residues, including GLU 300, GLY 302, ASP 329, MET 331, and ASP 343, which help anchor the ligand and stabilize its orientation. Further stabilization is provided by ILE 328, GLU 333, ARG 344, ASP 340, and PHE 344, which facilitate ligand accommodation through van der Waals interactions. Because no significant unfavorable donor-donor or acceptor-acceptor clashes are observed, the binding conformation appears highly stable. These combined interaction patterns suggest that Calystegine C1 is effectively positioned within the active site, forming multiple critical contacts with residues necessary for enzymatic activity.



```

WARNING: The search space volume > 27000 Angstrom^3 (See FAQ)
Detected 8 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: 1476792900
Performing search ...
0% 10 20 30 40 50 60 70 80 90 100%
|-----|-----|-----|-----|-----|
*****
done.
Refining results ... done.

```

mode	affinity (kcal/mol)	dist from best mode rmsd l.b. rmsd u.b.
1	-6.4	0.000 0.000
2	-6.1	12.485 14.241
3	-6.1	1.278 3.097
4	-6.1	1.927 3.749
5	-5.8	2.016 3.583
6	-5.8	1.704 3.928
7	-5.8	1.997 3.366
8	-5.8	1.603 1.653
9	-5.8	1.749 2.961
10	-5.7	1.522 3.290

Writing output ... done.

Fig. 22. Molecular docking CMD result of Calystegine C1
Credits. Roshane Kimberly Pedregosa

F. Post Docking Analysis: C-terminal Domain of Maltase-Glucoamylase

Structural and kinetic characterizations emphasize that the C-terminal subunit of human maltase-glucoamylase (ctMGAM) possesses an expansive active site optimized for the hydrolysis

of long-chain α -1,4-linked glucose oligomers. Recent comparative studies highlight that while both subunits share the same catalytic machinery, ctMGAM features extended sugar subsites that allow it to process larger maltodextrins more efficiently than the N-terminal subunit (Watanabe, Tagami, Biwa, Kawasaki, Adachi, Moriya, 2026).

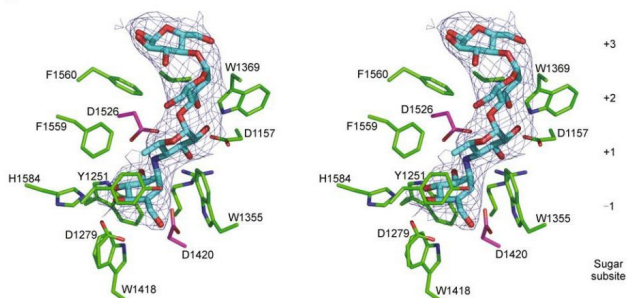


Fig. 23. Visualization of active sites in C-terminal Maltase-Glucoamylase
Credits: Limei Ren, Xiaohong Qin, Xiaofang Cao, Lele Wang, Fang Bai, Gang Bai, Yuequan Shen (2011)

The catalytic mechanism is driven by the conserved aspartic acid pair, identified in the C-terminal domain as Asp1420 (nucleophile) and Asp1526 (acid/base catalyst), which provide the structural basis for its high binding affinity toward complex starch breakdown products (Guan, Han, Li, Xu, Cui, Chen, Zhang, Chen, Shan, Wang, 2022). The interactive amino acid network within the ctMGAM pocket is further distinguished by a dense web of aromatic and polar residues that stabilize substrates. Advanced molecular dynamics simulations and cryo-EM studies show that residues such as Trp1355, Trp1369, and Phe1560 form a "hydrophobic platform" that stabilizes glucose rings via stacking, particularly as they extend into the subsite (Zhang, Wang, Han, Fu, Wang, Li, Han, 2021). Furthermore, the stability of inhibitor complexes in ctMGAM is maintained through specific salt bridges and hydrogen bonds with residues like Asp1157 and His1584, which anchor ligands in an orientation-dependent manner to prevent the final glucose-releasing step of digestion (Watanabe et al., 2026). This residue-rich environment explains ctMGAM's superior ability to sequester longer glucose chains compared to the more restricted ntMGAM pocket.

Molecular interaction analyses are conducted on the C-terminal (CT) domain of maltase-glucoamylase (MGA) to clarify the binding outcomes of the calystegine ligands A3, B1, B2, B3, B4, and C1. A thorough evaluation of enzyme-ligand interactions is performed using Biovia Discovery Studio, which enables the visualization of critical molecular features, including interacting amino acid residues, hydrogen bond formation, and the number of hydrogen bonds involved (Eberhardt et al., 2021), as illustrated in Table 7. These visualizations provide significant insight into enzyme-ligand binding interactions and enhance the assessment of each ligand's potential inhibitory effectiveness against CT-MGAM.

1) Acarbose

The ligand exhibits a binding affinity of -8.8 kcal/mol toward the target α -glucosidase enzyme, indicating strong and stable interaction within the active site. It forms several

conventional hydrogen bonds with key residues including Asp1157, Asp1370, Lys1460, His1584, and Asp1279, which ensure proper anchoring and correct orientation within the catalytic pocket. Additional stabilization is provided by electrostatic interactions, particularly attractive charge and salt bridge contacts involving Asp1157, Asp1526, and Arg1510, strengthening ligand retention inside the binding cavity. An unfavorable donor-donor interaction is observed with Arg1510, suggesting localized electrostatic repulsion within the binding environment. The complex is further stabilized by π -alkyl interactions with Trp1355 and Phe1560, along with extensive van der Waals interactions with surrounding residues such as Trp1369, Gln1158, Pro1159, Met1421, Ile1280, Ile1315, Tyr1251, Trp1418, Trp1523, and Asp1420. Overall, this interaction profile demonstrates significant stabilizing contacts within the enzyme's active region, supporting its potential inhibitory activity.

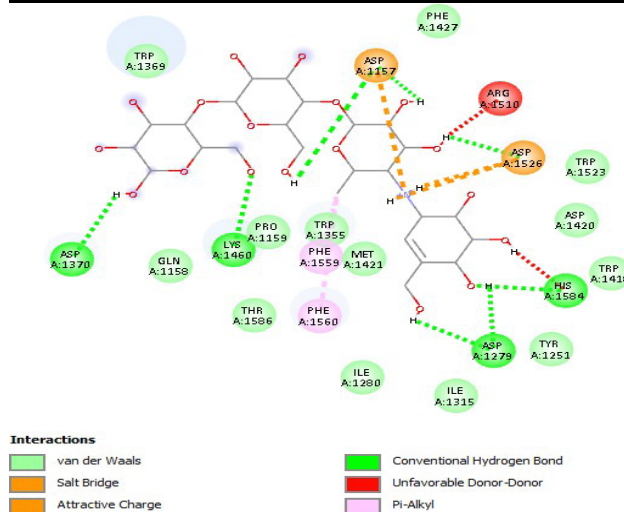
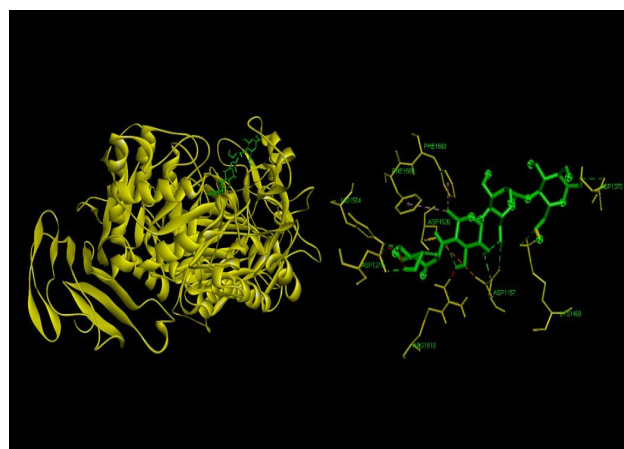


Fig. 24. Visualization of Acarbose docking simulation.
(A) Best Binding Position with target Protein (CT-MGAM), (B) Three-Dimensional Structure Display, (C) Two-Dimensional Visualization of Binding Interactions
Credits: Jan Criss Peralta

Table 7
Simulation results of interactions between Ligands and C-terminal Domain of Maltase-Glucoamylase

Chemical Compound	Binding Affinity	Interacting Amino acids	No. of Hydrogen Bonds	Hydrogen Bond Interactions
Acarbose	-8.8 kcal/mol	ASP1157, ASP1370, LYS1460, HIS1584, ASP1279, ASP1526, ARG1510, TRP1355, PHE1560, TRP1369, GLN1158, PRO1159, MET1421, ILE1280, ILE1315, TYR1251, TRP1418, TRP1523, ASP1420	5	ASP1157, ASP1370, LYS1460, HIS1584, ASP1279
Calystegine A3	-6.3 kcal/mol	ASP1420, TRP1355, PHE1559, HIS1584, TYR1251, TRP1418, ARG1510, ASP1526, MET1421, TRP1523, ASP1279, ILE1315, PHE1560	1	ASP1420
Calystegine B1	-6.3 kcal/mol	ASP1420, TRP1355, PHE1559, HIS1584, TYR1251, TRP1418, ARG1510, ASP1526, MET1421, TRP1523, ASP1279, ILE1315, PHE1560	2	ASP1420
Calystegine B2	-6.3 kcal/mol	ASP 1420, ASP 1526, TRP 1418, PHE 1559, HIS 1584, TYR 1251, ASP 1279, ILE 1580, ILE 1315, TRP 1355, MET 1421, ARG 1510, TRP 1523	2	ASP 1420, ASP 1526
Calystegine B3	-6.3 kcal/mol	ASP 1420, ARG 1510, TRP 1418, PHE 1559, HIS 1584, TYR 1251, ASP 1279, ILE 1280, ILE 1315, TRP 1355, MET 1421, TRP 1523, ASP 1526	3	ASP 1420, ARG 1510
Calystegine B4	-6.6 kcal/mol	ARG1510, ASP1526, HIS1584, TYR1251, MET1421, TRP1355, TRP1523, ARG1582, TRP1418, ASP1420, ILE1315, ASP1279, ILE1280, PHE1559	3	ARG1510, ASP1526, HIS1584
Calystegine C1	-6.2 kcal/mol	ASP1526, ASP1279, ASP1420, TRP1523, TRP1418, ARG1510, PHE1559, PHE1560, TRP1355, TYR1251, ILE1280, ILE1315, MET1421, HIS1584	2	ASP1526, ASP1279

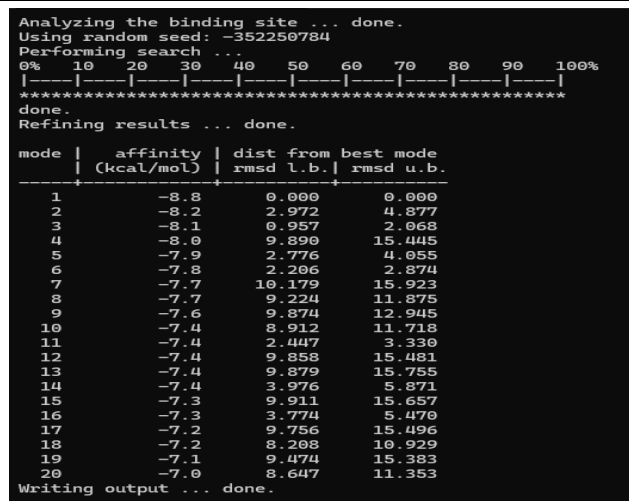


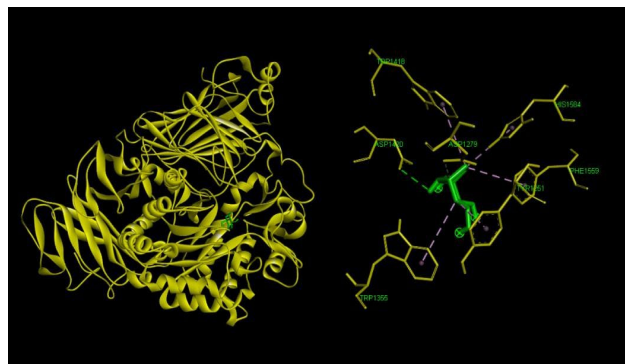
Fig. 25. Molecular docking CMD result of Acarbose
Credits: Jan Criss Peralta

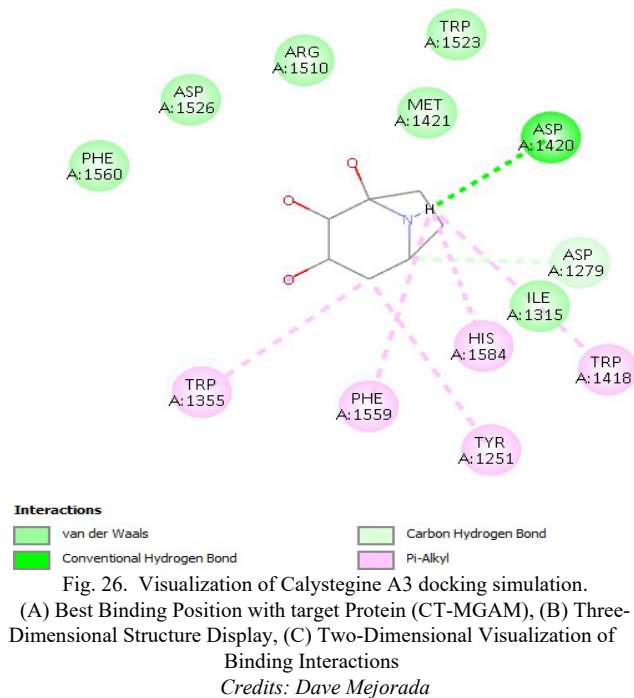
2) Calystegine A3

The ligand exhibits a binding affinity of -6.3 kcal/mol toward the target α -glucosidase enzyme, indicating moderate interaction within the active site. It forms a conventional hydrogen bond with ASP1420, which contributes to ligand anchoring and stabilization within the catalytic pocket. The complex is further stabilized by multiple π -alkyl interactions involving TRP1355, PHE1559, HIS1584, TYR1251, and TRP1418, enhancing hydrophobic contact within the binding

cavity. Additional van der Waals interactions with surrounding residues such as ARG1510, ASP1526, MET1421, TRP1523, ASP1279, ILE1315, and PHE1560 contribute to overall ligand accommodation and structural complementarity.

Collectively, these interactions support stable binding; however, the lower binding affinity compared to stronger inhibitors suggests relatively weaker stabilization within the enzyme's active region.





```
Analyzing the binding site ... done.
Using random seed: -1782516996
Performing search ...
0% 10 20 30 40 50 60 70 80 90 100%
|-----|-----|-----|-----|-----|
done.
Refining results ... done.
```

mode	affinity (kcal/mol)	dist from best mode rmsd l.b. rmsd u.b.
1	-6.3	0.000 0.000
2	-6.2	1.146 2.986
3	-6.0	26.422 27.448
4	-5.9	21.601 22.563
5	-5.7	34.802 35.987
6	-5.6	22.181 23.181
7	-5.6	21.651 22.267
8	-5.5	21.839 22.886
9	-5.5	22.301 23.226
10	-5.5	21.417 22.329
11	-5.5	21.809 22.550
12	-5.4	22.200 22.845
13	-5.4	13.679 14.764
14	-5.3	14.442 15.654
15	-5.3	32.470 33.639
16	-5.2	26.752 27.896
17	-5.2	22.037 23.090
18	-5.2	21.518 22.382
19	-5.1	29.613 30.398
20	-5.1	29.559 30.326

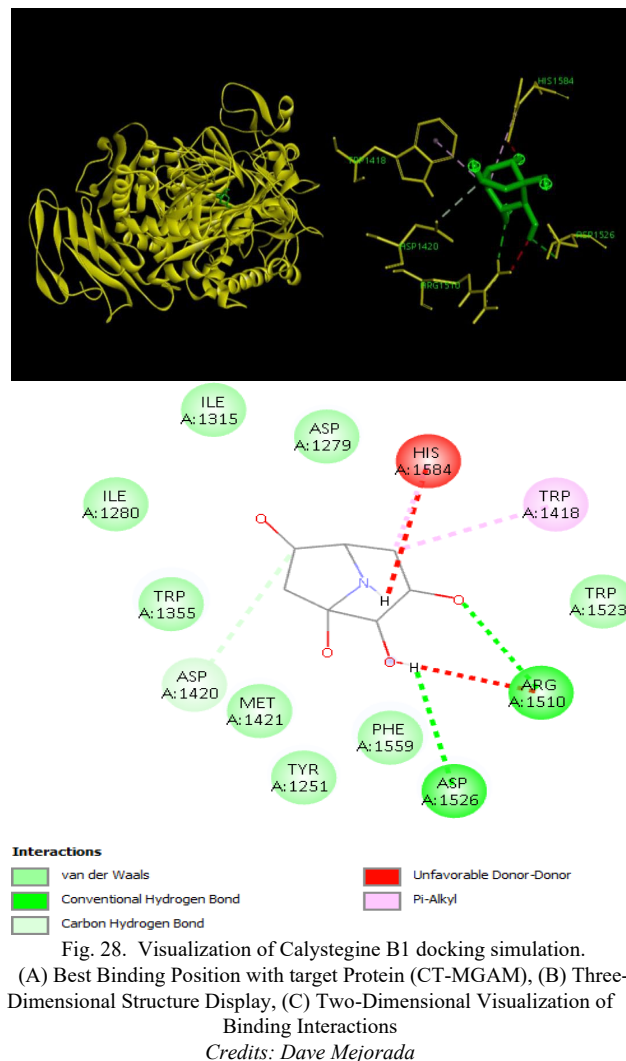
Writing output ... done.

Fig. 27. Molecular docking CMD result of Calystegine A3
Credits: Dave Mejorada

3) Calystegine B1

The ligand exhibits a binding affinity of -6.3 kcal/mol toward the target α -glucosidase enzyme, indicating moderate interaction within the active site. It forms a conventional hydrogen bond with ASP1420, which contributes to ligand anchoring and stabilization within the catalytic pocket. The complex is further stabilized by multiple π -alkyl interactions involving TRP1355, PHE1559, HIS1584, TYR1251, and TRP1418, enhancing hydrophobic contact within the binding cavity. Additional van der Waals interactions with surrounding residues such as ARG1510, ASP1526, MET1421, TRP1523, ASP1279, ILE1315, and PHE1560 contribute to overall ligand accommodation and structural complementarity.

Collectively, these interactions support stable binding; however, the lower binding affinity compared to stronger inhibitors suggests relatively weaker stabilization within the enzyme's active region.



```
Performing search ...
0% 10 20 30 40 50 60 70 80 90 100%
|-----|-----|-----|-----|-----|
done.
Refining results ... done.
```

mode	affinity (kcal/mol)	dist from best mode rmsd l.b. rmsd u.b.
1	-6.3	0.000 0.000
2	-6.3	1.510 3.632
3	-6.2	21.123 22.486
4	-6.1	26.427 27.756
5	-6.1	21.552 22.796
6	-6.0	21.079 22.169
7	-6.0	21.900 22.687
8	-6.0	1.589 3.056
9	-5.7	12.559 14.344
10	-5.6	1.389 3.415
11	-5.5	26.952 28.411
12	-5.5	21.148 22.030
13	-5.5	21.042 22.875
14	-5.4	1.662 3.929
15	-5.4	20.991 22.142
16	-5.4	32.745 33.973
17	-5.4	21.514 22.471
18	-5.4	13.076 14.647
19	-5.3	29.523 30.350
20	-5.3	32.673 33.858

Writing output ... done.

Fig. 29. Molecular docking CMD result of Calystegine B1

4) Calystegine B2

Calystegine B2 demonstrates a binding affinity of -6.3 kcal/mol, indicating a moderate interaction with the target enzyme, though slightly weaker compared to compounds with

lower (more negative) binding energies. The complex forms a key conventional hydrogen bond with ASP 1420 and ASP 1526, which plays an important role in stabilizing the ligand within the active site and maintaining proper orientation inside the catalytic pocket. Hydrophobic stabilization is evident through π -alkyl interactions involving TRP 1418, PHE 1559, and HIS 1584, strengthening nonpolar contacts within the binding cavity. Additional van der Waals interactions with residues such as TYR 1251, ASP 1279, ILE 1580, ILE 1315, TRP 1355, MET 1421, ARG 1510, and TRP 1523, contribute to structural complementarity and overall binding stabilization. Overall, the combination of hydrogen bonding, hydrophobic interactions, and van der Waals forces supports a reasonably stable ligand–protein complex within the enzyme's active region.

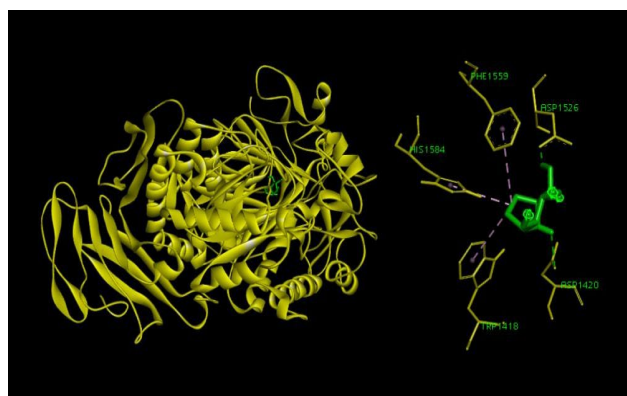


Fig. 30. Visualization of Calystegine B2 docking simulation.

(A) Best Binding Position with target Protein (CT-MGAM), (B) Three-Dimensional Structure Display, (C) Two-Dimensional Visualization of Binding Interactions

Credits: Dave Mejorada

```
Analyzing the binding site ... done.
Using random seed: 356171964
Performing search ...
0% 10 20 30 40 50 60 70 80 90 100%
|-----|-----|-----|-----|-----|-----|-----|
*****
done.
Refining results ... done.
```

mode	affinity (kcal/mol)	dist from best mode rmsd l.b. rmsd u.b.
1	-6.3	0.000 0.000
2	-6.2	1.537 2.361
3	-6.2	26.226 27.419
4	-6.0	21.289 22.527
5	-5.9	21.337 22.308
6	-5.9	21.531 22.123
7	-5.7	22.086 23.304
8	-5.7	32.189 33.432
9	-5.6	22.162 23.484
10	-5.5	21.726 22.586
11	-5.5	21.531 22.399
12	-5.5	26.693 28.020
13	-5.5	1.496 3.061
14	-5.5	12.931 14.640
15	-5.4	22.105 23.598
16	-5.4	32.179 33.553
17	-5.4	13.159 14.623
18	-5.2	32.425 33.659
19	-5.2	30.110 30.741
20	-5.1	13.673 15.496

Writing output ... done.

Fig. 31. Molecular Docking CMD Result of Calystegine B2 with target Protein (CT-MGAM)

Credits: Dave Mejorada

5) Calystegine B3

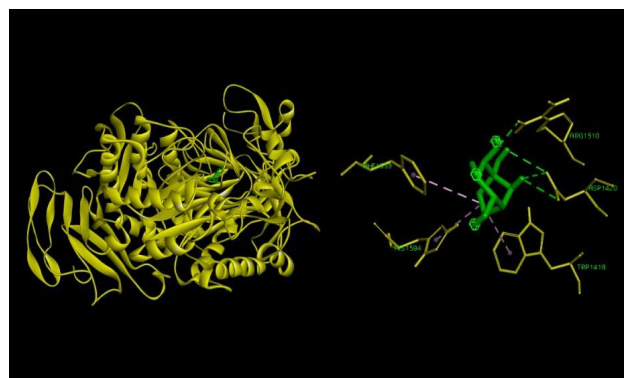


Fig. 32. Visualization of Calystegine B3 docking simulation.

(A) Best Binding Position with target Protein (CT-MGAM), (B) Three-Dimensional Structure Display, (C) Two-Dimensional Visualization of Binding Interactions

Credits: Dave Mejorada

Calystegine B3 exhibits a binding affinity of -6.3 kcal per mol, indicating a moderately stable interaction within the active site of the target enzyme. The complex is primarily stabilized by a carbon hydrogen bond with ASP 1420 and ARG 1510, which contributes to proper ligand positioning and electrostatic

support within the binding pocket. A pi-alkyl interaction with TRP 1418, PHE 1559, HIS and 1584, enhances hydrophobic stabilization, strengthening nonpolar contacts and improving ligand accommodation inside the cavity. Several van der Waals interactions involving residues such as TYR 1251, ASP 1279, ILE 1280, ILE 1315, TRP 1355, MET 1421, TRP 1523, and ASP 1526 further promote structural complementarity and compact fitting of the ligand within the enzyme active region. The overall interaction profile dominated by van der Waals forces and supported by hydrogen bonding and hydrophobic interactions suggests a reasonably stable and favorable ligand protein complex.

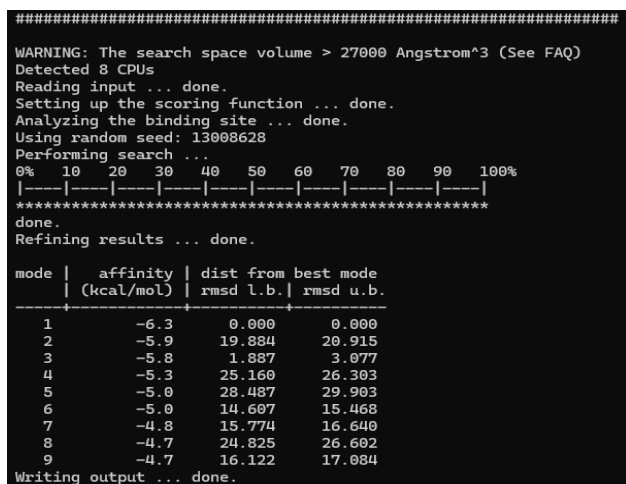


Fig. 33. Molecular Docking CMD Result of Calystegine B3 with target Protein (CT-MGAM)

Credits: Dave Mejorada

6) Calystegine B4

The ligand Calystegine B4 exhibits a binding affinity of -6.6 kcal/mol toward the target enzyme, indicating a moderately strong interaction within the active site. The ligand forms several conventional hydrogen bonds with ARG1510, ASP1526, and HIS1584, which play a significant role in stabilizing the ligand and anchoring it within the catalytic pocket. A pi-donor hydrogen bond interaction with TYR1251 further contributes to electrostatic stabilization and proper ligand orientation. Hydrophobic stabilization is supported by alkyl interactions with MET1421 and TRP1355, which enhance ligand affinity within the binding cavity. In addition, multiple van der Waals interactions involving residues TRP1523, ARG1582, TRP1418, ASP1420, ILE1315, ASP1279, ILE1280, and PHE1559 promote structural complementarity and ligand accommodation. However, an unfavorable donor-donor interaction with ARG1510 is observed, which may slightly reduce binding efficiency due to electrostatic repulsion. Overall, these interactions suggest that Calystegine B4 forms a stable and relatively stronger binding complex within the enzyme active site compared to the other ligands.

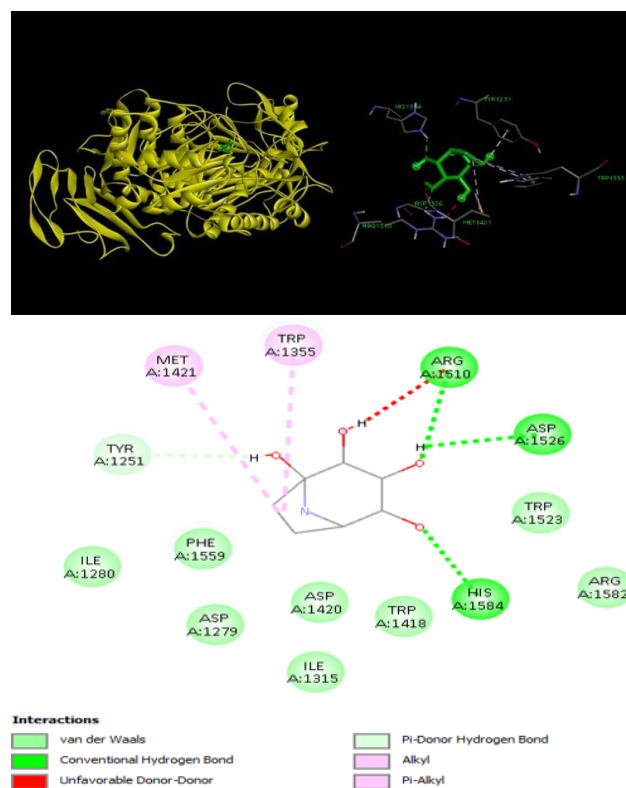


Fig. 34. Visualization of Calystegine B4 docking simulation with target Protein (CT-MGAM). (A) Best Binding Position with target Protein (CT-MGAM), (B) Three-dimensional Structure Display, (C) Two-Dimensional Visualization of Binding Interactions

Credits: Dave Mejorada

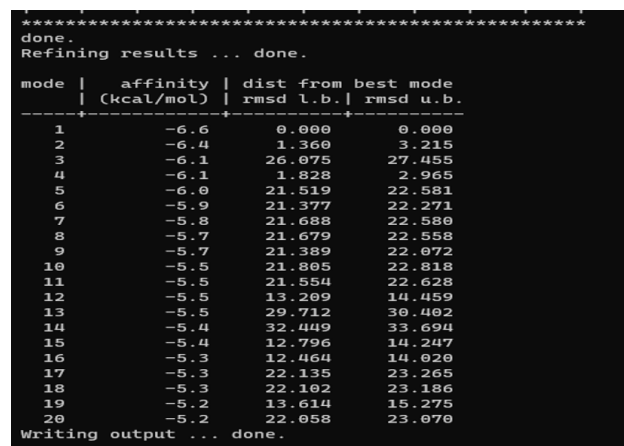


Fig. 35. Molecular Docking CMD Result of Calystegine B4 with target Protein (CT-MGAM)

Credits: Dave Mejorada

7) Calystegine C1

The ligand Calystegine C1 exhibits a binding affinity of -6.2 kcal/mol toward the target enzyme, indicating a moderate interaction within the active site. The ligand forms conventional hydrogen bonds with ASP1526 and ASP1279, which contribute to ligand anchoring and stabilization within the catalytic pocket. A carbon-hydrogen bond interaction with ASP1420 provides additional weak electrostatic support and helps maintain proper ligand orientation. The complex is further stabilized by several van der Waals interactions involving residues TRP1523, TRP1418, ARG1510, PHE1559, PHE1560, TRP1355,

TYR1251, ILE1280, ILE1315, and MET1421, which promote structural complementarity and ligand accommodation within the binding cavity. However, an unfavorable donor-donor interaction with HIS1584 is observed, which may slightly reduce binding efficiency due to electrostatic repulsion. Overall, these interactions suggest that Calystegine C1 establishes a stable and moderately strong binding within the enzyme active site.

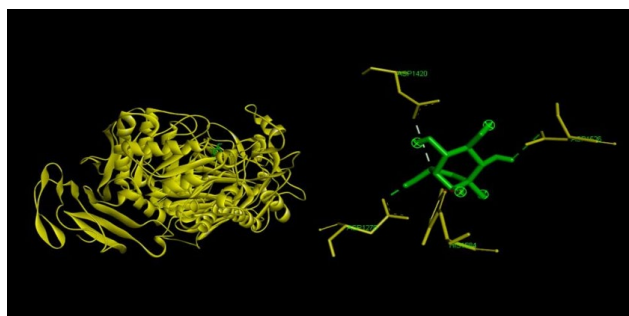


Fig. 36. Visualization of Calystegine B4 docking simulation with target Protein (CT-MGAM). (A) Best Binding Position with target Protein (CT-MGAM), (B) Three-Dimensional Structure Display, (C) Two-Dimensional Visualization of Binding Interactions

Credits: Joana Iwayan

Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: -733299456
Performing search ...
0% 10 20 30 40 50 60 70 80 90 100%

done.
Refining results ... done.

mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-6.2	0.000	0.000
2	-6.0	1.489	3.136
3	-5.7	1.616	3.354
4	-5.6	12.597	14.144
5	-5.4	1.354	3.718
6	-5.4	12.101	13.836
7	-5.4	1.480	3.280
8	-5.3	12.443	14.086
9	-5.3	14.488	15.698
10	-5.3	14.223	15.969
11	-5.2	12.395	14.423
12	-5.2	12.580	14.182
13	-5.2	13.056	14.745
14	-5.1	12.090	13.857
15	-5.1	11.970	13.748
16	-5.1	12.087	13.785
17	-5.1	4.542	6.576
18	-5.0	4.826	6.452
19	-5.0	12.366	13.931
20	-5.0	9.863	11.262

Writing output ... done.

Fig. 37. Molecular Docking CMD Result of Calystegine C1 with target Protein (CT-MGAM)

Credits: Joana Iwayan

G. Post Docking Analysis: N-terminal Domain of Sucrase-Isomaltase

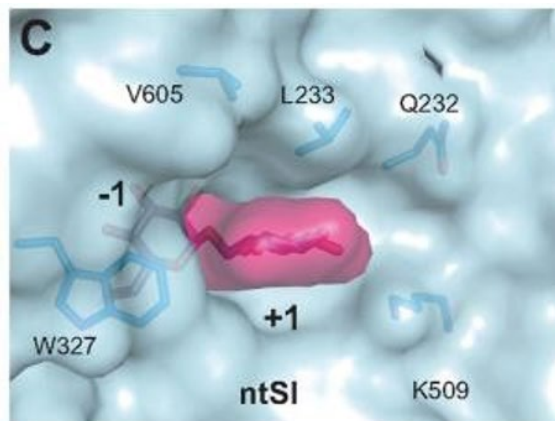


Fig. 38. Visualization of active sites in N-terminal Sucrase-Isomaltase
Credits: Lyann Sim, Carly Willemsma, Sankar Mohan, Hassan Y. Naim, B. Mario Pinto, and David R. Rose (2010)

The active site of the N-terminal subunit of human sucrase-isomaltase (ntSI) is characterized as a specialized catalytic pocket capable of accommodating both linear 1,4 and branched 1,6-glycosidic linkages. Recent theoretical and docking studies indicate that this dual-specificity is facilitated by a unique arrangement of interactive amino acids, specifically Trp327, Val605, Leu233, Gln232, and Lys509, which define the steric boundaries and chemical environment of the pocket (Martinez, Almazan, Bojorge, Flores, Baez, Valdez, 2023). Molecular dynamics (MD) simulations have shown that these residues are essential for anchoring substrates within the -1 and +1 subsites, providing a structural scaffold that stabilizes the transition state during the hydrolysis of isomaltose and other limit dextrins (Martínez et al., 2023). Further investigations into enzyme inhibition reveal that the binding affinity of therapeutic compounds and natural polyphenols is heavily dependent on specific interactions with the catalytic residues Asp231 and Asp571, alongside aromatic stabilizers like Phe604 and Trp435 (Martínez et al., 2023). Studies published in 2021 and 2022 emphasize that the flexibility of the active site—specifically a partial loss of helical structure in residues 530–580 upon ligand binding—can break key hydrogen bonds (such as the Gly570-Asn572 bond), thereby modulating the enzyme's activity (Li, Qian, Han, 2021). These insights into the interactive amino acid network are critical for the design of high-selectivity inhibitors that target ntSI without cross-reactivity with other intestinal glucosidases (Kashtoh & Baek, 2022).

To clarify the binding outcomes of calystegine A3, B1, B2, B3, B4, and C1, molecular interaction analysis is conducted on the N-terminal (NT) domain of Sucrase-Isomaltase (SI). Using Biovia Discovery Studio, which allows the visualization of important molecular features, such as interacting amino acid residues, hydrogen bond formation, and the number of hydrogen bonds involved (Eberhardt et al., 2021), a comprehensive assessment of enzyme-ligand interactions is carried out, as shown in Table 7. These illustrations enhance the evaluation of each ligand's potential inhibitory efficacy against NT-SI and provide important insights into enzyme-ligand

binding interactions.

1) Acarbose

Acarbose exhibits a binding affinity of -5.8 kcal/mol toward the target α -glucosidase enzyme, indicating moderate interaction within the active site consistent with its role as a competitive inhibitor. It forms conventional hydrogen bonds with ARG230, ASP571, and SER631, which contribute significantly to ligand anchoring and stabilization within the catalytic pocket. A carbon hydrogen bond with LEU233 further enhances binding stability through additional electrostatic support. However, an unfavorable acceptor-acceptor interaction with ASP503 is observed, which may slightly reduce interaction efficiency due to electrostatic repulsion. The complex is further stabilized by extensive van der Waals interactions involving residues such as TRP327, TRP435, PHE479, ASP231, LYS509, GLN232, LEU502, GLN481, SER508, SVAL 605, ARG555, ALA606,

ASP632, TYR634, and PHE604, promoting structural complementarity and optimal ligand accommodation within the binding cavity. Overall, these interactions support stable binding of acarbose within the enzyme's active region despite the presence of a minor unfavorable contact.

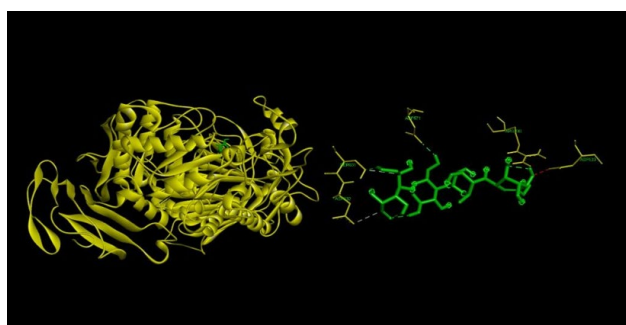


Fig. 39. Visualization of Acarbose docking simulation with target Protein (NT-SI). (A) Best Binding Position with target Protein (NT-SI), (B) Three-Dimensional Structure Display, (C) Two-Dimensional Visualization of Binding Interactions

Credits: Jan Criss Peralta

```
Analyzing the binding site ... done.
Using random seed: -1385171608
Performing search ...
0% 10 20 30 40 50 60 70 80 90 100%
*****
done.
Refining results ... done.
```

mode	affinity (kcal/mol)	dist from best mode	
		rmsd l.b.	rmsd u.b.
1	-5.8	0.000	0.000
2	-5.7	16.604	21.483
3	-5.7	2.055	10.032
4	-5.6	2.134	4.637
5	-5.5	15.443	21.411
6	-5.5	9.087	14.162
7	-5.5	10.248	12.768
8	-5.4	4.652	10.992
9	-5.4	14.600	20.239
10	-5.4	14.826	20.947
11	-5.4	14.700	19.844
12	-5.3	0.854	2.172
13	-5.3	10.148	15.541
14	-5.3	16.004	19.816
15	-5.3	14.021	16.813
16	-5.3	15.818	20.888
17	-5.3	5.121	8.087
18	-5.3	16.045	19.365
19	-5.2	12.671	19.106
20	-5.2	16.427	19.720

Writing output ... done.

Fig. 40. Molecular Docking CMD Result of Acarbose with target Protein (NT-SI)

Credits: Jan Criss Peralta

2) Calystegine A3

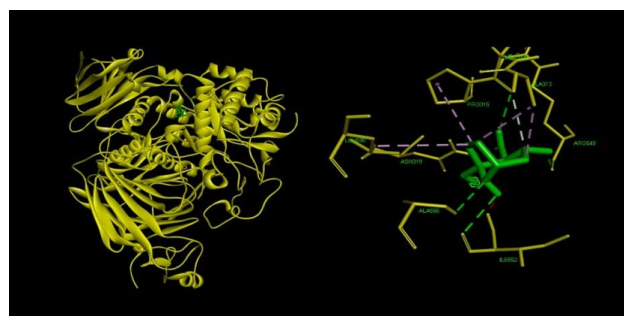


Fig. 41. Visualization of Calystegine A3 docking simulation with target Protein (NT-SI). (A) Best Binding Position with target Protein (NT-SI), (B) Three-Dimensional Structure Display, (C) Two-Dimensional Visualization of Binding Interactions

Credits: Dave Mejorada

Calystegine A3 exhibits a binding affinity of -6.2 kcal/mol toward the target α -glucosidase enzyme, indicating moderate and stable interaction within the active site. It forms conventional hydrogen bonds with ALA566, ILE552, ASN319, MET314, and ARG549, which contribute to strong ligand anchoring and proper orientation within the catalytic pocket. A carbon hydrogen bond with MET314 further enhances electrostatic stabilization. The complex is additionally

supported by alkyl interactions involving ALA313, LEU596, and PRO315, strengthening hydrophobic contacts within the binding cavity. Several van der Waals interactions with residues such as HIS564, ALA565, PHE551, SER550, and ALA316 contribute to structural complementarity and optimal ligand accommodation. However, an unfavorable donor-donor interaction is observed, which may slightly reduce binding efficiency. Overall, the combination of hydrogen bonding, hydrophobic interactions, and van der Waals forces indicates favorable and stable ligand-protein binding within the enzyme's active region.

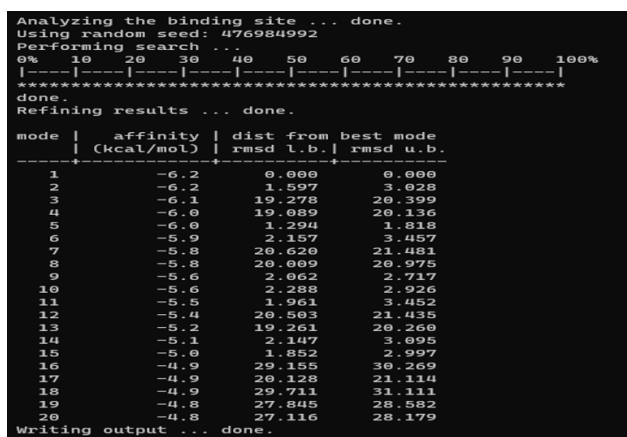


Fig. 42. Molecular Docking CMD Result of Calystegine A3 with target Protein (NT-SI)
Credits: Dave Mejorada

3) Calystegine B1

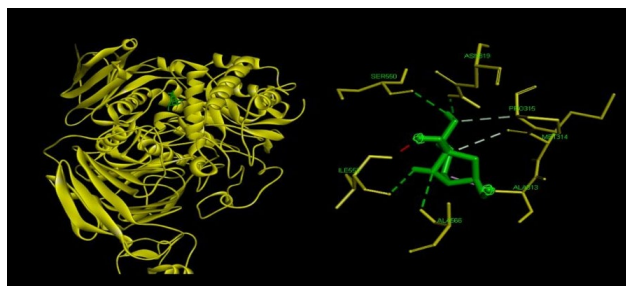


Fig. 43. Visualization of Calystegine B1 docking simulation with target Protein (NT-SI). (A) Best Binding Position with target Protein (NT-SI), (B) Three-Dimensional Structure Display, (C) Two-Dimensional Visualization of Binding Interactions
Credits: Dave Mejorada

Calystegine B1 exhibits a binding affinity of -6.6 kcal/mol, indicating a moderately strong and stable interaction within the active site of the target enzyme. The complex is primarily stabilized by multiple conventional hydrogen bonds with ILE552, ALA566, ASN319, and SER550, which ensure firm anchoring and proper ligand orientation within the catalytic pocket. A carbon hydrogen bond with MET314 further enhances electrostatic stabilization. Hydrophobic support is provided through an alkyl interaction with ALA313, while several van der Waals interactions involving ARG549, LEU596, PHE551, PRO315, ALA316, HIS564, and ALA565 contribute to structural complementarity and optimal accommodation of the ligand within the binding cavity. Although an unfavorable donor-donor interaction with ILE552 is observed, which may slightly reduce binding efficiency due to electrostatic repulsion, the overall network of hydrogen bonding, hydrophobic contacts, and van der Waals forces suggests favorable and stable ligand-protein binding within the enzyme's active region.

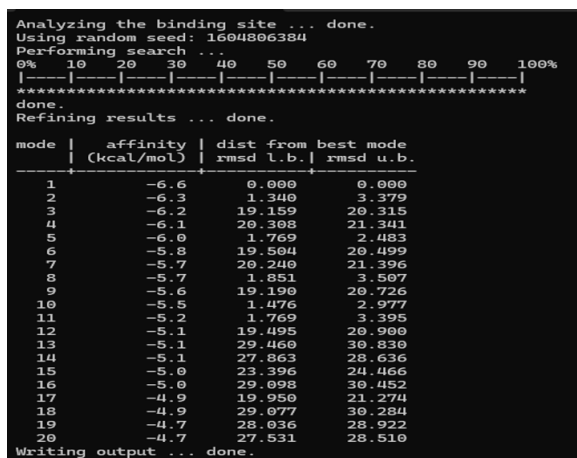


Fig. 44. Molecular Docking CMD Result of Calystegine B1 with target Protein (NT-SI)
Credits: Dave Mejorada

4) Calystegine B2

The ligand B2 exhibits a binding affinity of -5.9 kcal/mol toward the target enzyme, indicating moderate interaction within the active site. It forms a conventional hydrogen bond with ARG549, contributing to ligand anchoring and stabilization within the catalytic pocket. The complex is further supported by alkyl interactions involving ALA313, PRO315, and LEU596, which enhance hydrophobic stabilization within the binding cavity. A carbon-hydrogen bond interaction with PHE551 also contributes additional weak electrostatic support. Moreover, van der Waals interactions with residues such as SER550, MET314, VAL541, ALA316, ASN319, ARG563, HIS564, ALA565, and ALA566 promote ligand accommodation and structural complementarity. However, an unfavorable donor-donor interaction with ILE552 is observed, which may slightly reduce binding efficiency due to electrostatic repulsion. Collectively, these interactions suggest that B2 establishes stable yet moderate binding within the enzyme's active region.

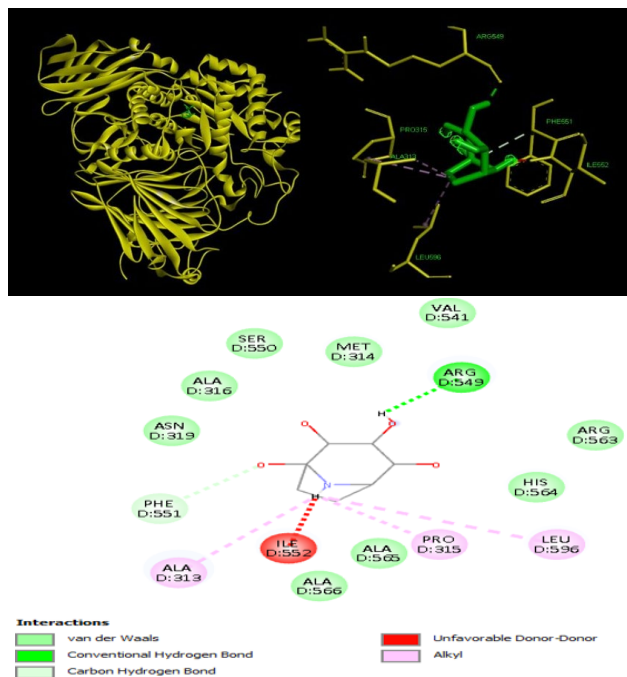


Fig. 45. Visualization of Calystegine B2 docking simulation with target Protein (NT-SI). (A) Best Binding Position with target Protein (NT-SI), (B) Three-Dimensional Structure Display, (C) Two-Dimensional Visualization of Binding Interactions
Credits: Rea Dela Peña

```
=====
WARNING: The search space volume > 27000 Angstrom^3 (See FAQ)
Detected 8 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: -617763404
Performing search ...
0% 10 20 30 40 50 60 70 80 90 100%
|-----|-----|-----|-----|-----|-----|-----|-----|
*****
done.
Refining results ... done.

mode | affinity | dist from best mode
      | (kcal/mol) | rmsd l.b. | rmsd u.b.
-----|-----|-----|-----|
1 | -5.9 | 0.000 | 0.000
2 | -5.6 | 19.862 | 20.989
3 | -5.6 | 1.629 | 2.958
4 | -5.6 | 20.306 | 21.401
5 | -5.6 | 1.509 | 3.218
6 | -5.5 | 1.433 | 3.871
7 | -5.4 | 19.235 | 20.445
8 | -5.2 | 19.861 | 20.835
9 | -5.2 | 20.432 | 21.564
Writing output ... done.
```

Fig. 46. Molecular Docking CMD Result of Calystegine B2 with target Protein (NT-SI)
Credits: Rea Dela Peña

5) Calystegine B3

The ligand Calystegine B3 exhibits a binding affinity of -6.3 kcal/mol toward the target enzyme, indicating a moderately strong interaction within the active site. The ligand forms a carbon-hydrogen bond with GLY585, which contributes to stabilization and proper positioning of the ligand within the binding pocket. Hydrophobic stabilization is further supported by an alkyl interaction with LEU569, enhancing ligand-protein affinity. In addition, multiple van der Waals interactions with residues GLU588, HIS213, LYS214, TRP581, SER582, GLN578, ASN572, PRO234, LEU241, GLU210, PHE589, THR584, and VAL212 promote structural complementarity and stable accommodation of the ligand inside the binding cavity.

However, an unfavorable acceptor-acceptor interaction with GLN211 is observed, which may slightly reduce binding efficiency due to electrostatic repulsion. Overall, these interactions suggest that Calystegine establishes a stable and moderately strong binding within the enzyme active site.

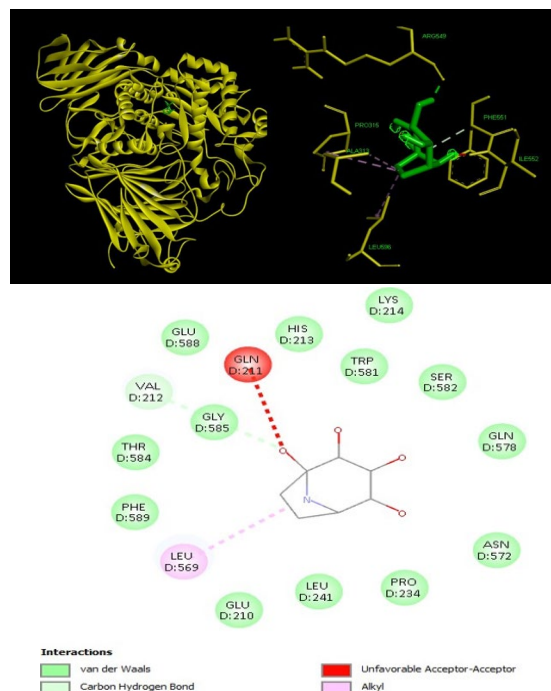


Fig. 47. Visualization of Calystegine B3 docking simulation with target Protein (NT-SI). (A) Best Binding Position with target Protein (NT-SI), (B) Three-Dimensional Structure Display, (C) Two-Dimensional Visualization of Binding Interactions
Credits: Rea Dela Peña

```
=====
WARNING: The search space volume > 27000 Angstrom^3 (See FAQ)
Detected 8 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: -617763404
Performing search ...
0% 10 20 30 40 50 60 70 80 90 100%
|-----|-----|-----|-----|-----|-----|-----|-----|
*****
done.
Refining results ... done.

mode | affinity | dist from best mode
      | (kcal/mol) | rmsd l.b. | rmsd u.b.
-----|-----|-----|-----|
1 | -5.9 | 0.000 | 0.000
2 | -5.6 | 19.862 | 20.989
3 | -5.6 | 1.629 | 2.958
4 | -5.6 | 20.306 | 21.401
5 | -5.6 | 1.509 | 3.218
6 | -5.5 | 1.433 | 3.871
7 | -5.4 | 19.235 | 20.445
8 | -5.2 | 19.861 | 20.835
9 | -5.2 | 20.432 | 21.564
Writing output ... done.
```

Fig. 48. Molecular Docking CMD Result of Calystegine B3 with target Protein (NT-SI)
Credits: Rea Dela Peña

6) Calystegine B4

The ligand B4 exhibits a binding affinity of -6.7 kcal/mol toward the target enzyme, indicating moderate and stable interaction within the active site. It forms conventional hydrogen bonds with ASN319, ILE552, ALA566, and HIS564, which contribute to ligand anchoring and stabilization within the catalytic pocket, while a carbon-hydrogen bond interaction with ALA316 provides additional electrostatic support. The

complex is further stabilized by alkyl interactions involving LEU596, PRO315, and ALA313, strengthening hydrophobic contacts within the binding cavity. Additional van der Waals interactions with residues such as SER550, PHE551, MET314, GLU538, and ALA565 enhance ligand accommodation and structural complementarity. However, an unfavorable donor–donor interaction with ARG549 is observed, which may slightly reduce binding efficiency due to electrostatic repulsion. Overall, these interactions support stable ligand–protein binding within the enzyme’s active region and indicate favorable interaction potential.

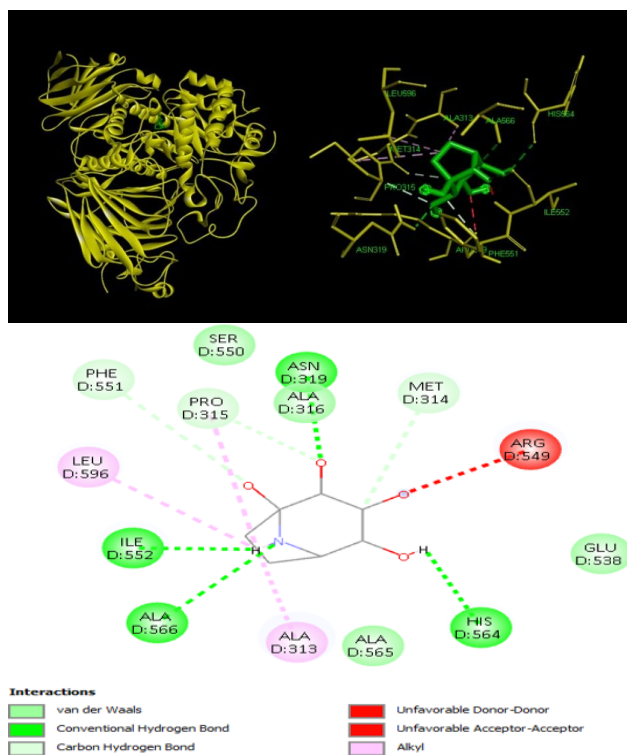


Fig. 49. Visualization of Calystegine B4 docking simulation with target Protein (NT-SI). (A) Best Binding Position with target Protein (NT-SI), (B) Three-Dimensional Structure Display, (C) Two-Dimensional Visualization of Binding Interactions

Credits: Joana Iwayan

```
#####
WARNING: The search space volume > 27000 Angstrom^3 (See FAQ)
Detected 8 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: -1882592132
Performing search ...
0% 10 20 30 40 50 60 70 80 90 100%
|-----|-----|-----|-----|-----|
*****
done.
Refining results ... done.

mode | affinity | dist from best mode
| (kcal/mol) | rmsd l.b. | rmsd u.b.
-----|-----|-----|-----|
1 | -6.7 | 0.000 | 0.000
2 | -6.1 | 1.594 | 3.752
3 | -5.1 | 29.417 | 30.151
4 | -5.1 | 19.940 | 21.075
5 | -5.1 | 27.309 | 28.302
6 | -5.0 | 29.066 | 30.507
7 | -5.0 | 29.523 | 30.976
8 | -4.9 | 27.707 | 28.724
9 | -4.9 | 29.516 | 30.911
Writing output ... done.
```

Fig. 50. Molecular Docking CMD Result of Calystegine B4 with target Protein (NT-SI)

Credits: Joana Iwayan

7) Calystegine C1

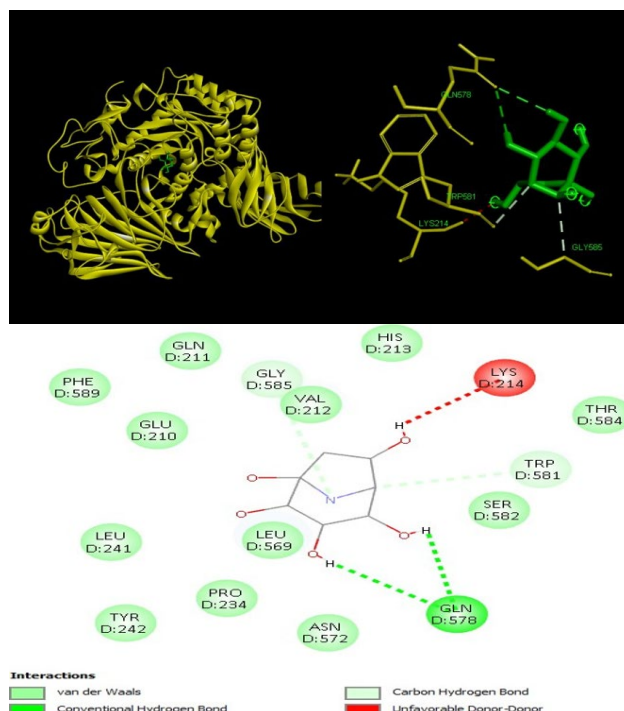


Fig. 51. Visualization of Calystegine C1 docking simulation with target Protein (NT-SI). (A) Best Binding Position with target Protein (NT-SI), (B) Three-Dimensional Structure Display, (C) Two-Dimensional Visualization of Binding Interactions

Credits: Joana Iwayan

```
# multithreading, Journal of Computational Chemistry 31 (2010) #
# 455-461 #
# DOI 10.1002/jcc.21334 #
# #
# Please see http://vina.scripps.edu for more information. #
#####
Detected 8 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: -713931136
Performing search ...
0% 10 20 30 40 50 60 70 80 90 100%
|-----|-----|-----|-----|-----|
*****
done.
Refining results ... done.

mode | affinity | dist from best mode
| (kcal/mol) | rmsd l.b. | rmsd u.b.
-----|-----|-----|-----|
1 | -5.3 | 0.000 | 0.000
2 | -5.2 | 1.463 | 3.253
3 | -5.2 | 26.714 | 28.329
4 | -4.9 | 27.034 | 28.319
5 | -4.9 | 1.558 | 3.328
6 | -4.8 | 14.365 | 15.233
7 | -4.7 | 24.441 | 26.361
8 | -4.6 | 1.312 | 3.847
9 | -4.6 | 24.346 | 26.215
Writing output ... done.
```

Fig. 52. Molecular Docking CMD Result of Calystegine B4 with target Protein (NT-SI)

Credits: Joana Iwayan

Calystegine C1 exhibits a binding affinity of -5.3 kcal/mol toward the target enzyme, indicating relatively weak to moderate interaction within the active site. It forms a two conventional hydrogen bond with GLN578, contributing to ligand anchoring and stabilization within the catalytic pocket. The complex is further supported by multiple van der Waals interactions with residues including PHE589, GLN211, GLY585, VAL212, HIS213, THR584, TRP581, SER582, LEU241, TYR242, PRO234, ASN572, GLU210, and LEU569,

which enhance ligand accommodation and structural complementarity within the binding cavity. However, an unfavorable donor–donor interaction with LYS214 is observed, which may slightly reduce binding efficiency due to electrostatic repulsion. Collectively, these interactions suggest that while Calystegine C1 is capable of establishing stabilizing contacts within the enzyme's active region, the limited hydrogen bonding and presence of an unfavorable interaction are consistent with its comparatively lower binding affinity.

H. Post Docking Discussion: N- terminal Maltase-Glucoamylase

Calystegine B4 is identified as the premier candidate among the test group, primarily due to its superior binding affinity, representing the most thermodynamically favorable interaction outside of the control. Although it does not directly engage the catalytic acid-base residue ASP 542, it achieves effective inhibition by forming a robust network of 6 hydrogen bonds with residues such as ASP 329 and ASP 340. This high level of stability suggests that B4 serves as a potent steric inhibitor, maintaining a secure and persistent presence within the enzyme's active site.

Calystegine B3 is categorized as the most biochemically accurate inhibitor, as it prioritizes specific active site docking over total energy. Despite a moderate affinity, it is the only test ligand that specifically targets ASP 542, the critical catalytic residue essential for enzyme activity. By establishing 6 hydrogen bonds and interacting with HIS 600 in the negative 1 subsite, B3 mimics the binding orientation of the Acarbose control more precisely than any other variant. This targeted interaction suggests that B3 may offer a more specific inhibitory mechanism despite its lower thermodynamic score.

The analysis places Calystegines B1 and C1 in the third position as reliable, high-stability alternatives. Both compounds exhibit a significant binding affinity and maintain a full complement of 6 hydrogen bonds, indicating a stable fit within the enzyme's acidic pocket via interactions with GLU 300 and MET 331. While these ligands provide higher binding energy than B3, they are ranked lower in this specific hierarchy because they do not directly interact with the primary catalytic machinery, focusing instead on peripheral residues such as ARG 334 within the active site architecture.

Calystegine B2 is ranked fourth because its structural stability is insufficient for effective long-term inhibition. While it successfully docks near the catalytic ASP 542 and shares a binding affinity similar to B3, it only establishes 3 hydrogen bonds. Under the established hierarchy, this limited number of interactions indicates a weak residence time within the pocket. Consequently, B2 is considered a less effective candidate, as it lacks the necessary hydrogen bond network involving residues like ASP 443 to remain securely docked against competitive substrates.

The study concludes that Calystegine A3 is the least effective inhibitor evaluated. It fails to meet the performance thresholds established by the other ligands, recording the weakest binding affinity in the group. Furthermore, it establishes only 4 hydrogen bonds, which is insufficient to provide the structural

stability and thermodynamic favorability required to compete with the Acarbose control. Due to its poor affinity and lack of significant stabilizing interactions with residues like HIS 600, A3 is less favorable for further consideration as a viable lead for enzyme inhibition.

I. Post Docking Discussion: C-terminal Maltase-Glucoamylase

Calystegine B4 emerges as the leading candidate for ctMGAM inhibition. It exhibits the highest binding affinity among the tested ligands at -6.6 kcal/mol, indicating a highly thermodynamically stable interaction second only to the Acarbose control. Unlike Acarbose, which interacts with multiple residues such as ASP 1157 and ASP 1279, Calystegine B4 effectively binds to ARG 1510, ASP 1526, and HIS 1584. The formation of three hydrogen bonds provides sufficient structural stability to maintain its secure positioning within the active site.

Calystegine B3 ranks just below the top candidate, functioning as a potent inhibitor due to its strong docking characteristics. With a binding affinity of -6.3 kcal/mol, it maintains comparable structural stability to B4 by forming three hydrogen bonds. These bonds engage residues such as ASP 1420 and ARG 1510. The interaction with ARG 1510, also targeted by the leading compound, allows B3 to anchor effectively, making it a precise and reliable option for enzyme inhibition.

Calystegines B1, B2, and A3 perform moderately in the docking analysis. Each exhibits a binding affinity of -6.3 kcal/mol, but they show lower effectiveness than B3 due to fewer hydrogen bonds. B1 and B2 form two hydrogen bonds each, while A3 forms only one. All three interact with ASP 1420, a residue also targeted by the Acarbose control, yet their limited number of chemical interactions indicates a shorter residence time within the catalytic pocket compared to the higher-ranked compounds.

The analysis concludes that Calystegine C1 is the least effective inhibitor evaluated for the ctMGAM subunit. It recorded the weakest binding affinity in the group at -6.2 kcal/mol. Although it forms 2 hydrogen bonds with ASP 1526 and ASP 1279, its lower thermodynamic score indicates it cannot compete as effectively for the active site. Under your hierarchy, it's the least promising candidate for further pharmaceutical development against this specific domain.

J. Post Docking Discussion: N-terminal Domain of Sucrase-Isomaltase

Calystegine B4 stands out as the leading compound among the tested molecules because of its strong binding affinity, indicating the most energetically favorable interaction aside from the control. Although it does not replicate all the interactions observed with acarbose, it effectively inhibits the enzyme by occupying the active site pocket and forming four hydrogen bonds with residues including ASN 319, ILE 552, ALA 566, and HIS 564. This strong interaction suggests that B4 functions as a powerful inhibitor, remaining firmly bound within the active site and preventing substrate binding and

catalysis.

Calystegine B1 follows closely behind B4, showing a binding affinity that is nearly comparable. It forms five hydrogen bonds with important residues such as ILE 552, ALA 566, ASN 319, ARG 549, and SER 550. These multiple interactions enhance its structural stability within the ntSI active site, enabling it to function as a reliable inhibitor with only a minimal difference in binding energy compared to the top-ranked compound.

The results show that Calystegine A3 follows closely behind the leading candidates. Although its binding affinity is slightly lower than the top compounds, it still demonstrates strong inhibitory potential with a notable interaction profile. It forms five hydrogen bonds with key residues such as ALA 566, ILE 552, ASN 319, MET 314, and ARG 549. These interactions indicate that A3 can effectively bind within the active site, even if its overall binding strength is slightly less than that of Calystegine B4.

Calystegine B3 falls behind the other candidates due to its limited interaction profile within the active site. While it shows a reasonable binding affinity, the analysis reveals that it does not form any hydrogen bonds. Based on the established criteria, the absence of these stabilizing interactions suggests that B3 may have a short residence time in the binding pocket, making it less effective as an inhibitor compared to compounds that can establish strong hydrogen bonding with the enzyme.

The findings indicate that Calystegines B2 and C1 show the weakest inhibitory potential against ntSI among the tested compounds. Calystegine B2 exhibited a relatively low binding affinity and formed only one hydrogen bond with ARG 549, suggesting limited structural stability within the active site. Similarly, Calystegine C1 displayed the lowest binding affinity in the group. Although C1 formed five hydrogen bonds with residues such as GLN 578, its unfavorable thermodynamic profile indicates poor competitiveness for the active site. Consequently, these compounds were deemed unlikely to serve as effective enzyme inhibitors.

1) Validation of Docking Results

The accepted metric for a well-docked pose is a root-mean-square deviation (RMSD) of ≤ 2.0 Å from the experimentally determined ligand structure (Lexa & Carlson, 2020). RMSD values at or below this threshold indicate that the predicted ligand orientation closely aligns with the crystallographic conformation, reflecting precise ligand placement within the enzyme active site and supporting the reliability of subsequent binding interaction analyses. Conversely, RMSD values exceeding 2.0 Å suggest substantial deviation from the experimental structure, which may reduce confidence in the predicted binding mode and compromise the accuracy of binding affinity estimations (Li *et al.*, 2025).

In this study, the docking protocol was validated by redocking the co-crystallized ligand into the enzyme active sites. The resulting RMSD values were 0.367 Å for NtMGAM, 0.715 Å for CtMGAM, and 1.845 Å for Nt-SI, all well below the 2.0 Å threshold. These results confirm that the docking parameters, grid configurations, and scoring function accurately reproduce the experimental ligand positions.

Therefore, this validation establishes a reliable framework for predicting the binding affinities and interaction profiles of calystegine derivatives with α -glucosidase enzymes, providing confidence in the interpretation and downstream application of the molecular docking results.

```
C4A
N4A
O4
C5
C5B
O5
C6
C6B
O6B
C7B
H2
H6
H8
H15
H17
H18
H22
Iterate: iterated over 28 atoms.
PyMOL>pair_fit docked_ligand and name C1+C2+C3+C4+C5, orig_ligand and name C1+C2+C3+C4+C5
ExecutiveRMSPairs: RMSD = 0.033 (5 to 5 atoms)
PyMOL>rms_cur docked_ligand, orig_ligand
ExecutiveRMS-Error: No atoms selected.
PyMOL>rms_cur docked_ligand, orig_ligand, matchmaker=4
Executive: RMSD = 0.367 (21 to 21 atoms)
```

Fig. 53. Molecular docking CMD result NT-MGAM
Credits: Jan Criss Peralta

```
C5B
O5
C6
C6B
O6B
C7B
H2
H3
H4
H2B
H3B
1H4A
2H4A
H6B
Iterate: iterated over 29 atoms.
PyMOL>align ctcleanpdb, CtMGAM-acarb_one_enzyme_and_ligand
Match: read scoring matrix.
Match: assigning 890 x 923 pairwise scores.
MatchAlign: aligning residues (890 vs 923)...
MatchAlign: score 4825.000
ExecutiveAlign: 7131 atoms aligned.
Executive: RMSD = 0.000 (7131 to 7131 atoms)
PyMOL>rms_cur docked_ligand, orig_ligand, matchmaker=4
Executive: RMSD = 0.715 (21 to 21 atoms)
```

Fig. 54. Molecular docking CMD result CT-MGAM
Credits: Jan Criss Peralta

```
S1V
S1Z
H1
H2
H3
H4
H5
H6
H7
H8
Iterate: iterated over 34 atoms.
PyMOL>save /Users/ADMIN/DO 4 real 5001, animate=1, buffer=2
PyMOL>super cleanCHAIN, CHAIN_D
MatchAlign: aligning residues (853 vs 853)...
MatchAlign: score 4502.734
ExecutiveAlign: 8362 atoms aligned.
Executive: RMSD = 0.000 (8362 to 8362 atoms)
PyMOL>rms_cur posepdb, orig_ligand
ExecutiveRMS-Error: No atoms selected.
PyMOL>rms_cur posepdb, orig_ligand, matchmaker=5
Executive: RMSD = 1.845 (34 to 34 atoms)
PyMOL>png c:/Users/ADMIN/Documents/THESIS 2025/docking/PROTEIN 2/ntSI/RMSD/docu/kotarmsd.png, 0, 0, -1, ray=0
ScenePNG: wrote 1806x536 pixel image to file "C:/Users/ADMIN/Documents/THESIS 2025/docking/PROTEIN 2/ntSI/RMSD/docu/kotarmsd.png".
```

Fig. 55. Molecular docking CMD result NT-SI
Credits: Jan Criss Peralta

5. Conclusions and Recommendations

This section presents the summary of findings, conclusions, and recommendations of the study based on the Statement of the Problem and the results obtained from the molecular docking results, analysis, and discussion of polyhydroxylated nortropane alkaloids derived from *Ipomoea aquatica* as potential α -glucosidase inhibitors.

A. Findings

1. The selected polyhydroxylated nortropane alkaloids (Calystegine A3, B1, B2, B3, B4, and C1) derived

from *Ipomoea aquatica* showed favorable interactions with the active-site residues of α -glucosidase-related enzymes as reflected by their negative binding affinity values and molecular interaction profiles. The compounds formed stable ligand–enzyme complexes through hydrogen bonding, hydrophobic interactions, and van der Waals forces within the catalytic pockets of NtMGAM, CtMGAM, and NTSI, indicating effective interaction with the enzyme active sites.

2. The binding interactions of the kangkong-derived polyhydroxylated nortropane alkaloids with α -glucosidase suggest a plausible inhibitory mechanism. The compounds were observed to bind within the catalytic regions of the enzymes in a manner comparable to the control inhibitor acarbose, indicating that the calystegines may act as competitive inhibitors that interfere with substrate binding and carbohydrate hydrolysis associated with blood sugar regulation.
3. Differences were observed in the predicted inhibitory potential of the polyhydroxylated nortropane alkaloid compounds based on their molecular docking scores. Calystegine B4 consistently demonstrated the strongest binding affinities across the enzyme targets, followed by Calystegine B1 and Calystegine B3, while Calystegine C1 generally exhibited lower binding affinity values. These variations indicate differences in the predicted effectiveness of the compounds as α -glucosidase inhibitors.

B. Conclusions

After the findings of the study were taken into consideration, the following conclusions were established:

1. The polyhydroxylated nortropane alkaloids derived from *Ipomoea aquatica* are capable of forming stable interactions with the active-site residues of α -glucosidase enzymes as demonstrated by their favorable binding affinity values and molecular interaction profiles.
2. The binding interactions and orientations of the calystegine compounds indicate a possible competitive inhibitory mechanism against α -glucosidase, suggesting their potential to interfere with enzyme activity related to blood sugar regulation.
3. The predicted inhibitory potential of the polyhydroxylated nortropane alkaloid compounds varies among the different derivatives, indicating that some compounds, particularly Calystegine B4 and Calystegine B1, may be more promising candidates for further antidiabetic investigation.

C. Recommendations

Based on the findings and conclusions, the following recommendations were presented:

1. Since the present study was limited to in-silico molecular docking analysis, future researchers are encouraged to conduct in-vitro enzyme inhibition

assays to validate the α -glucosidase inhibitory activity of polyhydroxylated nortropane alkaloids derived from *Ipomoea aquatica*.

2. In-vivo studies using appropriate animal models are recommended to further evaluate the antidiabetic potential, bioavailability, and safety of the identified calystegine compounds.
3. Future researchers may explore the isolation and purification of calystegines from *Ipomoea aquatica* to support experimental validation and determine appropriate dosage levels for therapeutic applications.
4. Researchers are encouraged to perform additional computational studies, such as molecular dynamics simulations and pharmacokinetic analysis, to further evaluate the stability and drug-like properties of the identified compounds.
5. Future studies may investigate other phytochemical compounds present in *Ipomoea aquatica* and evaluate their potential inhibitory activity against α -glucosidase and related carbohydrate-digesting enzymes.

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