J Herbmed Pharmacol. 2024; 13(2): 308-323.

http://www.herbmedpharmacol.com doi: 10.34172/jhp.2024.49407

Journal of Herbmed Pharmacology

Integrative computational approaches for designing novel alpha-glucosidase inhibitors based on curculigoside A derivatives: Virtual screening, molecular docking, and molecular dynamics

Aiyi Asnawi[1*], Shifa Mieldianisa[1], Widhya Aligita[1], Anne Yuliantini[1], Ellin Febrina[2]

[1]Faculty of Pharmacy, Universitas Bhakti Kencana, Jl. Soekarno-Hatta No. 754, Bandung 40617, Indonesia
[2]Faculty of Pharmacy, Universitas Padjadjaran, Jl. Raya Bandung-Sumedang km. 21, Jatinangor 45363, Indonesia

ARTICLE INFO

Article Type:
Original Article

Article History:
Received: 21 December 2023
Accepted: 5 February 2024

Keywords:
Virtual screening, Ligand-based pharmacophore modeling, Molecular docking, Molecular dynamics, Alpha-glucosidase inhibitor

ABSTRACT

Introduction: Over 422 million people worldwide suffer from diabetes, causing 1.5 million fatalities annually. The existing medications have shortcomings, including poor glucose control and adverse effects. The present study aimed to create possible alpha-glucosidase inhibitors utilizing a curculigoside A derivative ligand-based model.

Methods: A pharmacophore model was constructed utilizing the structure information of curculigoside A derivatives and PharmaGist. Subsequently, virtual screening, molecular docking, and molecular dynamics were employed to simulate the hits.

Results: From six training sets, eleven pharmacophore models were developed; the model with the highest score (18.0435) was chosen for further analysis. Using the verified pharmacophore model, 270 547 chemicals from the ZINC natural product database were subjected to virtual screening. Subsequently, molecular docking was performed on the top 1000 hits with AutoDock Wizard from PyRx. This analysis unveiled 434 hits with better binding energies than acarbose, the native ligand. Subsequently, second optimal docking configurations were evaluated with AutoDock 2.4; this process yielded the discovery of three prospective inhibitors (ZINC000150350051, ZINC000008382292, and ZINC000085595291) characterized by the most advantageous binding energies. To evaluate the stability and dynamics of these ligand-receptor complexes, Gromacs 2022 molecular dynamics simulations were executed for one hundred nanoseconds. Out of the three hits, ZINC000085595291 (Hit03) exhibited good energy components and interaction stability constantly during the simulation.

Conclusion: The integrated computational strategy identified promising alpha-glucosidase inhibitors in curculigoside A compounds, highlighting the potential of ZINC000085595291 (Hit03) as a potential diabetes therapeutic agent.

Implication for health policy/practice/research/medical education:
The integrated computational strategy identified promising alpha-glucosidase inhibitors in curculigoside A compounds, highlighting the potential of ZINC000085595291 (Hit03) as a potential diabetes therapeutic agent. Future research avenues may include experimental validation of the computationally identified compounds and further optimization of their structures for enhanced therapeutic efficacy.

Please cite this paper as: Asnawi A, Mieldianisa S, Aligita W, Yuliantini A, Febrina E. Integrative computational approaches for designing novel alpha-glucosidase inhibitors based on curculigoside A derivatives: Virtual screening, molecular docking, and molecular dynamics. J Herbmed Pharmacol. 2024;13(2):308-323. doi: 10.34172/jhp.2024.49407.

Introduction

Diabetes mellitus (DM) is a chronic condition causing hypoglycemia (1). It is categorized into three forms: type 1 diabetes mellitus (T1DM), type 2 gestational diabetes mellitus (T2DM), and additional subtypes of DM. T2DM, the most widespread, is caused by insulin resistance in pancreatic beta cells, making it a non-insulin-dependent DM, affecting 90%-95% of the population (2). DM is projected to affect 686 million people globally by 2045 (3), with Indonesia's prevalence expected to rise to 21.3

***Corresponding author**: Aiyi Asnawi,
Email: aiyi.asnawi@bku.ac.id

million by 2030. While adults are more affected, children and adolescents may also be affected, as seen in recent years (4).

An antidiabetic drug known as alpha-glucosidase inhibitor (AGI) functions by impeding the activity of the alpha-glucosidase (AG) enzyme. One of the therapeutic approaches for postprandial hyperglycemia is the inhibition of carbohydrate absorption from food in the digestive tract, particularly the intestines, via the mechanism of action of AGI. Complex polysaccharides are digested by amylase into dextrin, which is then re-hydrolyzed by AG enzyme into glucose before entering the bloodstream. Acarbose, which is an amylase and AGI enzyme, can be produced. Although acarbose has been often employed in the treatment of T2DM patients, it has been associated with a range of adverse effects (5), including gastrointestinal pain, hypoglycemia, and weight gain, which can have a negative influence on patient adherence and quality of life. Furthermore, unpleasant effects, variability in drug reactions between individuals, and inability to attain glycemic control are a few of the constraints associated with contemporary diabetes medications. The quality of life and patient adherence may be adversely affected by these side effects. Similarly, metformin is frequently associated with adverse effects including nausea, vomiting, diarrhea, and lactic acidosis (6). Furthermore, the existing diabetic medications have several drawbacks, including inadequate glucose control, side effects, and variability in drug reactions between patients (7). It is critical to investigate novel targets for diabetic medicines to expand the availability of treatment options and to develop more effective therapeutic strategies for patients with diabetes.

Active pharmaceutical compounds derived from natural substances have the potential to serve as prospective anti-T2DM medicines. Curculigosides, which are organic chemicals discovered in plants belonging to the *Curculigo* genus, have a wide range of pharmacological effects, such as anti-inflammatory and antioxidant capabilities. Their potential as AGIs has generated interest in investigating the efficacy of their derivatives in diabetes management. Curculigoside A derivatives can delay glucose absorption in the gastrointestinal tract by modifying alpha-glucosidase activity. This could effectively alleviate postprandial glucose spikes and contribute to enhanced glycemic control among people with diabetes (8).

The synthesis and inhibitory activity of curculigoside A derivatives have been studied for their potential antidiabetic effects and β-cell apoptosis (9). The synthesis process involves using imidazole as a catalyst and esterification with halogenated carboxylic acids, producing good yields. Moreover, curculigoside and polyphenol-rich ethyl acetate fraction of *Molineria latifolia* rhizome has been reported to improve glucose uptake, indicating their potential for diabetes management (10). Further research and development of these derivatives may lead to more effective and targeted treatments for diabetes.

Ligand-based drug design is a very effective and strategic methodology utilized in the realm of pharmaceutical discovery and development. It provides several notable benefits that significantly contribute to its extensive implementation. Ligand-based drug design, as opposed to structure-based approaches, which necessitate a comprehensive understanding of target protein structures, concentrates on the attributes and features of established ligands, which are compounds that exhibit binding affinity towards the target (11,12). Adopting this methodology has demonstrated its immense value in the quest for innovative treatments, providing a variety of advantages that augment efficacy and triumph in the process of medication development.

Fundamentally, structure-based drug design (SBDD) and ligand-based drug design (LBDD) can collaborate to tackle the difficult drug design process (13). SBDD designs new medications using the three-dimensional shape and structure of the protein as a guide, whereas LBDD analyzes the qualities and characteristics of known ligands that bind to the target of interest. By integrating the benefits of both approaches, it is possible to build medication research and development techniques that are more efficient and successful.

An important benefit of LBDD is that it can be utilized even in the lack of three-dimensional structures of target proteins with high resolution. LBDD techniques overcome the difficulty of acquiring crystallographic or NMR data by utilizing information regarding ligand interactions and structure-activity relationships (14). As a result, ligand-based design exhibits remarkable versatility, facilitating the investigation of an extensive array of molecular targets.

The benefit of LBDD is that it accounts for the dynamic nature of ligand-receptor interactions, including the conformational changes and flexibility of both ligands and receptors. This feature facilitates a more accurate depiction of the binding process, which is particularly significant in the context of drug design, including flexible binding sites or adapting structural modifications caused by ligand binding. Moreover, ligand-based methodologies are highly suitable for identifying ligands that possess comparable pharmacophoric characteristics, hence facilitating the investigation of the chemical environment surrounding established active substances. This process enables the enhancement of lead chemical optimization and the creation of analogues that possess superior pharmacokinetics, selectivity, and bioactivity (14).

The objective of this study was to utilize computational methods, including molecular docking, virtual screening, ligand-based pharmacophore modeling, and molecular dynamics simulations, to detect and analyze derivatives of curculigoside A that exhibit improved inhibitory activity against alpha-glucosidase. The research aims to understand the molecular interactions that happen between the different forms of "curculigoside A" and the

target enzyme by using this multidisciplinary approach. By doing so, it hopes to contribute significantly to the understanding of how to rationally develop innovative and highly effective inhibitors.

Materials and Methods

An HP Z820 WorkStation Server with 32 GB of random-access memory (RAM), an Intel Xeon E5-2667 Double Processor, a Nvidia® RTX 3060 graphics processing unit (GPU), and a dual system running Ubuntu 22.04 LTS were used for virtual screening, molecular docking, and molecular dynamics simulations.

Pharmacophore model

A systematic procedure was followed to create a ligand-based pharmacophore model for a series of curculigoside A derivatives. The chemical structures of the curculigoside A derivatives were created and converted into the appropriate *.mol2 file format for use in the PharmaGist (15). A ligand-based pharmacophore model was developed using the PharmaGist application based on similar chemical characteristics and spatial arrangements shared by the curculigoside A derivatives. Hydrogen bond donors and acceptors, aromatic rings, and other key chemical moieties that contribute to bioactivity were recognized as pharmacophoric characteristics. The developed pharmacophore model was validated and optimized to verify its dependability and predictive capability. This included testing its capacity to correctly predict the bioactivity of known ligands and fine-tuning model parameters for improved accuracy.

The ChEMBL database (https://www.ebi.ac.uk/chemb) was used to degenerate the active dataset. The alpha-glucosidase target was employed, and 605 compounds were produced. To obtain 52 compounds, these compounds were filtered using an IC_{50} value of 100 nM. Compounds representing each IC_{50} value in the range 1-100 nM were chosen from the 52 compounds, yielding 36 compounds (Table 1).

Following the optimization process with the Avogadro program, these active compounds were saved in the *.mol2 format after being stored in the compound smiling data source. A total of 36 active compounds (Table 2) were chosen with the assistance of the DecoyFinder program (https://dud.docking.org/), which was then used to produce decoys (16). The active dataset was converted using OpenBabel software from the *.smi file to the *.sdf file format (17). After that, a database that was similar to a medicine called zinc was created and used in the *.sdf format. As a consequence of this, decoys of 1332 compounds were generated, which were subsequently optimized with the Avogadro software and saved in the *.mol2 format (18).

By interpreting the final ligand-based pharmacophore model, it was possible to determine which essential characteristics were required for binding to the target receptor. The provided data was of great significance in informing the development and refinement of innovative compounds that exhibited enhanced affinity and selectivity. This ligand-based pharmacophore modeling approach, which utilized the PharmaGist application, offered valuable insights into the critical structural elements that were indispensable for the bioactivity of curculigoside A derivatives.

Pharmacophore-based virtual screening

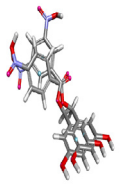
A virtual screening strategy was implemented to identify possible bioactive drugs, utilizing a ligand-based pharmacophore model produced by the PharmaGist. The selected pharmacophore model was constructed from a collection of curculigoside A derivatives. It incorporated critical structural characteristics that were required for inhibiting alpha-glucosidase, the intended biological target. The virtual screening was performed by employing the Zinc Natural Product Database (https://zinc15.docking.org/substances/subsets/natural-products/), which was an extensive collection of various natural compounds. In its infancy, the database comprised a considerable quantity of 224 205 compounds, all of which were represented in *.mol2 format.

The procedure commenced with the procurement and arrangement of chemical structures from the Zinc Natural Product Database, with particular emphasis on compounds that were categorized as natural products. Following this, virtual screening was performed on these structures against the ligand-based pharmacophore

Table 1. Active set alpha glucosidase dataset from the CheMBL database

No.	ChEMBL ID	No.	CheMBL ID
1	CHEMBL4448033	19	CHEMBL4448264
2	CHEMBL4547928	20	CHEMBL4449167
3	CHEMBL4552230	21	CHEMBL4549180
4	CHEMBL4468873	22	CHEMBL4574481
5	CHEMBL4461882	23	CHEMBL4464119
6	CHEMBL4448529	24	CHEMBL4538427
7	CHEMBL4535608	25	CHEMBL4589823
8	CHEMBL4551617	26	CHEMBL4557506
9	CHEMBL4451108	27	CHEMBL4441658
10	CHEMBL4467305	28	CHEMBL4457539
11	CHEMBL4453742	29	CHEMBL4591646
12	CHEMBL4466971	30	CHEMBL4463785
13	CHEMBL4549137	31	CHEMBL4127059
14	CHEMBL4459113	32	CHEMBL4469426
15	CHEMBL4533762	33	CHEMBL4561204
16	CHEMBL4461780	34	CHEMBL4475313
17	CHEMBL4438224	35	CHEMBL4531736
18	CHEMBL4513326	36	CHEMBL4516968

Table 2. Training set and pharmacophore model with best-pairwise of curculigoside A derivatives (9)

Entry	Ligand	IC_{50} (ppm)	Best pairwise alignments
1	3,5-dihydroxybenzyl-3,5-dinitrobenzoat	35.4250	
2	3,5-dihydroxybenzyl-4-nitrobenzoat	30.3006	
3	3,5-dihydroxybenzyl-4-chlorobenzoat	76.5167	
4	3,5-dihydroxybenzyl-4-bromobenzoat	56.7798	
5	3,5-dihydroxybenzyl-4-fluorobenzoat	48.6507	
6	4-hydroxybenzyl-4-fluorobenzoat	33.8714	

model that had been developed earlier. By considering the structural characteristics extracted from the curculigoside A derivatives, the pharmacophore model incorporated crucial attributes necessary for ligand binding to the target receptor. The outcomes of the virtual screening yielded a selection of natural product compounds that had promising potential for favorable interaction with the target site. Additional evaluations, including dynamic simulations and molecular docking, might be implemented in the future to refine the selection process and determine the stability and binding affinity of these molecules.

Molecular docking using AutoDock Wizard

The second stage of the research involved a thorough examination by molecular docking techniques on the top about 1000 hits that were identified by the ligand-based pharmacophore model and the virtual screening. All the 3D structures of hits were minimized (MMFF94) using openbabel. Using the AutoDock wizard from PyRx and the crystal structure of alpha-glucosidase as the macromolecular target, molecular docking was executed (PDB ID 2QMJ). The utilization of the AutoDock wizard in PyRx enabled the exploration of various binding modes and affinities between the chosen ligands and the alpha-glucosidase receptors; which expedited the preparation and execution of the docking simulations. The grid box size of 50×50×50 (npts) was used for capturing the crucial ligand-receptor interactions. Meanwhile, the coordinates of the grid box were modified based on the docking validation findings. The Lamarckian algorithm was employed to simulate molecular docking, employing certain docking settings such as limiting the number of energy evaluations to a brief length (250,000). The utilization of this parameter arrangement facilitated an investigation of ligand-receptor interaction in a computationally efficient manner, guaranteeing a prompt assessment of possible binding conformations. By utilizing the short maximum energy evaluation parameter, an attempt was made to achieve a harmonious coexistence of computing resources and a comprehensive investigation of ligand binding prospects. Following the molecular docking procedure, the ligand positions and binding affinities were determined by analysis.

Molecular docking using AutoDock 4.2

Following the initial molecular docking with PyRx's AutoDock wizard, the 30 best docking poses from the findings were evaluated further utilizing molecular docking with AutoDock 2.4. The macro-molecular target for the docking simulations was the alpha-glucosidase enzyme, whose crystal structure was recognized by PDB ID 2QMJ. This series of docking research was conducted using the AutoDock 2.4 program and the Lamarckian method. The grid box size of 50×50×50 (npts) was used for capturing the crucial ligand-receptor interactions. Meanwhile, the coordinates of the grid box were modified based on the docking validation findings. To ensure a more extensive investigation of the conformational space for ligand binding within the active site of alpha-glucosidase, specific docking settings were chosen, including a moderate maximum number of energy assessments (2,500,000).

Following the docking poses were determined, the optimal binding orientations and interactions between the ligands and the target receptor were evaluated. By integrating various docking tools and parameters, this iterative methodology sought to improve the precision and dependability of ligand binding forecasts. As a result, it facilitated the discovery of prospective lead compounds that possessed ideal binding properties for inhibiting alpha-glucosidase, a crucial factor in the treatment of diabetes.

Interaction analysis

The output of the molecular docking performed in the *.dlg format was used to acquire the best pose that was produced by the docking process. Discovery Studio Visualizer was utilized to visualize the findings of the molecular docking process (19). This allowed for the visualization of the interaction with the target protein as well as the hydrogen bond distance.

Molecular dynamic

After conducting molecular docking studies with AutoDock 2.4, a thorough evaluation was conducted on the interaction stability of the three optimal docking poses of the identified hits and the native ligand, Acarbose, bound to the alpha-glucosidase enzyme (PDB ID 2QMJ),

with the macromolecular target. Gromacs 2022 (20) was employed to perform molecular dynamics simulations to investigate the stability and dynamic behavior of ligand-receptor complexes over a prolonged duration of 100 ns.

The native ligand, Acarbose, and the best pose structures of the hits from the AutoDock 2.4 were produced and solvated in a suitable solvent model implemented in the simulation box. The execution of the molecular dynamics simulations was facilitated by the Gromacs software suite, which utilized Gromacs 2022's most recent force fields and algorithms to accurately simulate the intermolecular interactions. The AMBER99SB-ILDN force field was utilized to characterize the interactions between ligand and protein atoms. To preserve the neutrality of the system, the ligand-receptor complexes were solvated in an appropriate water box and counterions were supplied as necessary. Before commencing the production of molecular dynamics run, subsequent equilibration processes were executed to enable the system to attain a stable state while its energy was decreased.

Results

Pharmacophore model

The development of a ligand-based pharmacophore model entailed identifying common structural features and pharmacophoric factors required for binding to the chosen biological target associated with alpha-glucosidase inhibition. A training set of six curculigoside A derivatives was used to generate a pharmacophore model by utilizing the PharmaGist.

Eleven pharmacophore models were produced from a training set with best-pairwise curculigoside A derivatives (Table 1). The assessment criteria, which were pairwise comparisons, determined that the pharmacophore model with the highest predictive capability (with a score of 18.0435) was the most effective. Ten characteristics crucial for ligand-receptor binding interactions were incorporated into this ideal model. Two of these characteristics were ascribed to aromatic rings, underscoring the importance of π-π interactions. In addition, the model incorporated five hydrogen bond acceptor features, emphasizing the crucial role of hydrogen bonding in ligand-receptor recognition, and three hydrogen bond donor features, emphasizing the significance of donor-acceptor interactions. The combination of these characteristics in the most effective pharmacophore model not only demonstrated its capability to capture crucial ligand-receptor interactions but also offered valuable insights for the logical development of new compounds that exhibited enhanced affinity and selectivity towards the desired biological activity.

Pharmacophore model validation

The optimal pharmacophore model was validated via a rigorous evaluation employing receiver operating characteristics (ROC) parameters, with particular emphasis on enrichment factors (EF) and areas under the curves (AUC) values. The outcomes acquired from the experiment confirmed the effectiveness of the pharmacophore model in differentiating active from inactive drugs. The EF value of 36.50, which was computed, highlighted the model's substantial capability to augment the active chemicals in the screened dataset. In addition, the pharmacophore model's discriminatory capability was further validated by the AUC value of 0.710. A valid model would possess an AUC value beyond 0.5 (Figure 1). The results of this study were consistent with the standards set forth, which underscored the need for an EF value exceeding 1 and an AUC value surpassing 0.5 for a pharmacophore model to be considered legitimate. Given the validation parameters examined, the generated pharmacophore model's high EF value and AUC value demonstrated its dependability and predictive capacity. These results further substantiated the model's potential

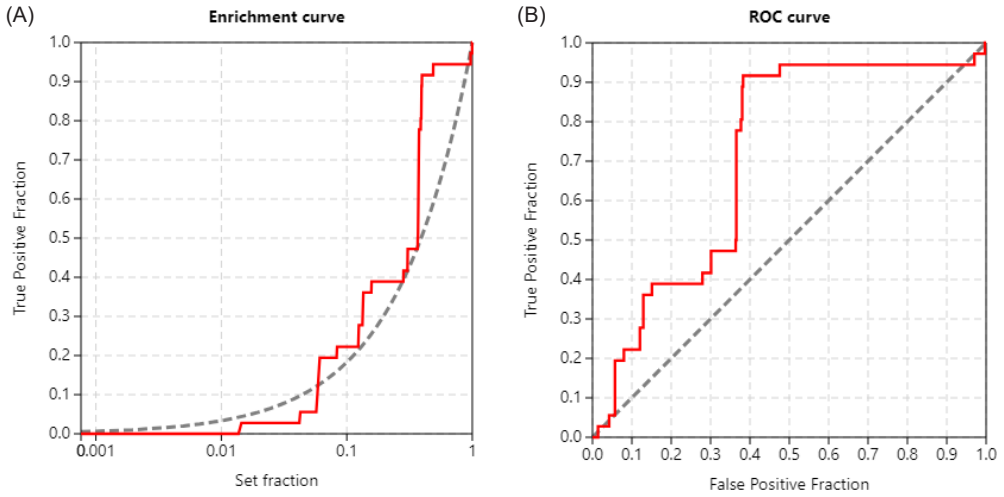


Figure 1. Pharmacophore model validation parameters. (A) Enrichment curve, and (B) Receiver operating characteristics (ROC) curve.

utility in directing the discovery of novel compounds possessing the intended biological activity.

The accuracy, sensitivity, and specificity scores were critical for assessing the performance of the model. The sensitivity, which quantified the capacity to identify active molecules, produced a substantial value of 1, suggesting that the model possessed an exceptional ability to accurately detect active ligands. The specificity of the model, which indicated its capability to identify inactive substances, was calculated to be 0.2. The accuracy value, which represented the comprehensive correctness of the model, attained a value of one hundred percent. This further validated the dependability and efficacy of the validated pharmacophore model in capturing crucial characteristics of ligand-receptor interactions. The thorough validation outcomes emphasize the model's capacity to effectively differentiate between active and inactive molecules, providing additional evidence for its usefulness in informing future drug discovery efforts.

Pharmacophore-based virtual screening

By utilizing the proven pharmacophore model, an extensive collection of 270,547 chemicals obtained from the ZINC natural product database was screened. As a result of the virtual screening process, 956 hits were identified as hits; their fit scores varied, with the highest score indicating the most favorable fit and the lowest score being 43.11. The pharmacophore hits, which were a specific type of output, were utilized as the input compound for the docking-based virtual screening phase of the research.

Validation of docking procedures

During the validation process for the docking methods utilizing AutoDock Wizard and AutoDock 4.2, significant care was taken to guarantee that the docking results were accurate and reliable. The validation was carried out by re-docking with acarbose as the native ligand into the binding site of alpha-glucosidase (PDB ID 2QMJ) as the macromolecular target. The docking experiments used acarbose as a native ligand, aligning with its crystal structure, but it's crucial as a positive control due to its anti-diabetic medication use. The purpose of the validation was to verify that the utilized grid box's dimensions and coordinates could replicate re-docking poses for the native ligand with a root means square deviation (RMSD) of no more than 2 Å. The RMSD value of 1.834 Å was noteworthy and confirmed the accuracy and effectiveness of the docking techniques. The grid box, which measured 50×50×50 (npts) in area, demonstrated the most effective proportions for capturing the crucial ligand-receptor interactions. The grid center coordinates (-21.214, -6.443, and -5.286) were ascertained for the grid box, thereby guaranteeing a precise depiction of the active site in the docking simulations. These parameters were used to re-dock the acarbose into the alpha-glucosidase binding site (PDB ID 2QMJ). The RMSD values for AutoDock Wizard and AutoDock 4.2 were 1.63 Å and 1.834 Å, respectively. By conducting this validation process, the reliability of the docking methods was ensured, which in turn inspired trust in the subsequent docking investigations that sought to find prospective AGIs.

Molecular docking using AutoDock wizard

The 956 hits, obtained from the ligand-based pharmacophore model, were subjected to a rigorous evaluation using molecular docking techniques. The objective was to give precedence to drugs that demonstrated robust binding interactions with the active site of alpha-glucosidase. The docking simulation results yielded significant knowledge regarding the possible effectiveness of the chosen hits, which assisted in the identification of lead compounds that would be examined in greater depth during the following phases of the drug discovery procedure. For these docking simulations, the AutoDock wizard from PyRx was utilized. The native ligand was acarbose, and the crystal structure of alpha-glucosidase (PDB ID 2QMJ) was utilized as the macromolecular target. The docking simulations utilized the Lamarckian technique, which was configured with certain settings, including a time constraint on the maximum number of energy evaluations (250,000). Through the implementation of this computational screening method, 434 hits were successfully found. Notably, each of these hits demonstrated a binding energy value that exceeded that of the native ligand, acarbose, by -7.75 kcal/mol (Free energy of binding). The obtained result indicates that the aforementioned hits exhibit excellent binding interactions with the alpha-glucosidase receptor.

Molecular docking using AutoDock 4.2

After doing the preliminary molecular docking using the AutoDock wizard from PyRx, the inquiry continued by subjecting the 30 most optimal docking poses derived from the outcomes to a more comprehensive assessment using AutoDock 2.4 (Table 3). The objective of the AutoDock 2.4 docking simulations was to enhance comprehension of the interactions between ligands and receptors and to provide more insight into the binding mechanisms of the chosen molecules. A more comprehensive sample of ligand conformations was possible due to the increased number of energy assessments; this enabled a precise evaluation of the ligand's stability and binding affinity within the active site of the alpha-glucosidase enzyme.

Using the crystal structure of alpha-glucosidase (PDB ID 2QMJ) as the macromolecular target and acarbose as the native ligand, AutoDock 4.2 was employed in this subsequent round of docking tests. The use of the Lamarckian algorithm-equipped AutoDock 2.4 program was crucial in the execution of this particular stage of the research. The docking settings were carefully determined, with a moderate maximum number of energy assessments (2,500,000) to provide a comprehensive investigation of

Table 3. Free energy of binding for thirty best hits into the alpha-glucosidase enzyme (PDB ID 2QMJ)

Hits	Free energy of binding (kcal/mol)	Hits	Free energy of binding (kcal/mol)
ZINC000150350051	-17.84	ZINC000085595197	-9.81
ZINC000008382292	-11.92	ZINC000085488581	-9.75
ZINC000085595291	-11.72	ZINC000085569386	-9.69
ZINC000022752881	-10.64	ZINC000253412302	-9.62
ZINC000150342254	-11.09	ZINC000085595265	-9.58
ZINC000150350019	-11.04	ZINC000085569450	-9.54
ZINC000085595209	-10.72	ZINC000085595262	-9.48
ZINC000014690589	-10.63	ZINC000097972030	-9.39
ZINC000085569403	-10.49	ZINC000140338495	-9.36
ZINC000217857326	-10.41	ZINC000085569417	-9.34
ZINC000085632439	-10.4	ZINC000103533270	-9.14
ZINC000085595224	-10.09	ZINC000245241871	-8.72
ZINC000085569391	-10.04	ZINC000034504732	-8.72
ZINC000085488555	-9.96	ZINC000098083363	-8.72
ZINC000085595243	-9.91	ZINC000002726682	-8.7
		Native ligand	-7.75

the conformational space of the ligand within the alpha-glucosidase active site. Following that, three hits with the most advantageous binding energies were selected for additional analysis. The following hits were identified as prospective lead compounds ZINC000150350051, ZINC000008382292, and ZINC000085595291 with free energy binding of -17.84, -11.92, and -11.72 kcal/mol, respectively. These compounds displayed encouraging binding affinities for alpha-glucosidase. The successful discovery of these hits was facilitated by the intentional incorporation of AutoDock 2.4 into the virtual screening pipeline, thereby propelling the investigation of innovative AGIs for diabetes treatment.

Interaction analysis hits into binding site

The active site amino acid residues ASP203, THR205, ARG526, HIS600, ASP542, and ASP327 are crucial for the native ligand (acarbose) to be stabilized and the active pouch to be formed. After the three hits (ZINC000150350051, ZINC000022752881, and ZINC000085595291) with the highest binding energies were identified using virtual screening with AutoDock Wizard, a comprehensive examination of their interactions with the alpha-glucosidase enzyme's amino acid residues was undertaken (Figure 2). The depiction of these interactions was facilitated by the Discovery Studio Visualizer tool. It was discovered that the initial hit, ZINC000150350051, formed hydrogen bonds with SER454 and engaged in hydrophobic interactions with VAL455. ZINC000022752881, the second hit, exhibited hydrophobic interactions with VAL451 and CYS458. The third hit denoted as ZINC000085595291, demonstrated a more complex interaction profile, including hydrophobic and hydrogen bonding interactions with PRO400, LEU401, and VAL455, as well as SER454, ILE402, and SER394. The comprehensive analysis of the particular amino acid residues implicated in the interactions between the ligand and receptor offered significant contributions to the knowledge base regarding the mechanisms of binding and facilitated the logical development of inhibitors for alpha-glucosidase. The application of the Discovery Studio Visualizer facilitated a clearer understanding of the molecular interactions that regulate the binding of these hits, providing more evidence for their potential as therapeutic candidates targeting alpha-glucosidase in the context of diabetes management.

Stability of the complex's interactions

After conducting molecular docking studies with AutoDock 2.4, a comprehensive evaluation was conducted on the interaction stability of the three optimal docking poses of the identified hits (ZINC000150350051, ZINC000008382292, and ZINC000085595291) with the native ligand Acarbose, which was bound to the alpha-glucosidase enzyme (PDB ID 2QMJ). Gromacs 2022 was employed to perform molecular dynamics simulations, which enabled a comprehensive investigation into the stability and dynamic characteristics of the ligand-receptor complexes during a prolonged duration of 100 ns. The simulations were executed in a physiological environment, which facilitated the dynamic fluctuations and structural alterations of the ligand-receptor complexes during the 100 ns journey. The evaluation of the ligand binding's stability and conformational integrity was conducted by examining significant metrics including RMSD, root mean square fluctuation (RMSF), and patterns of hydrogen bonding.

The utilization of this molecular dynamics' methodology

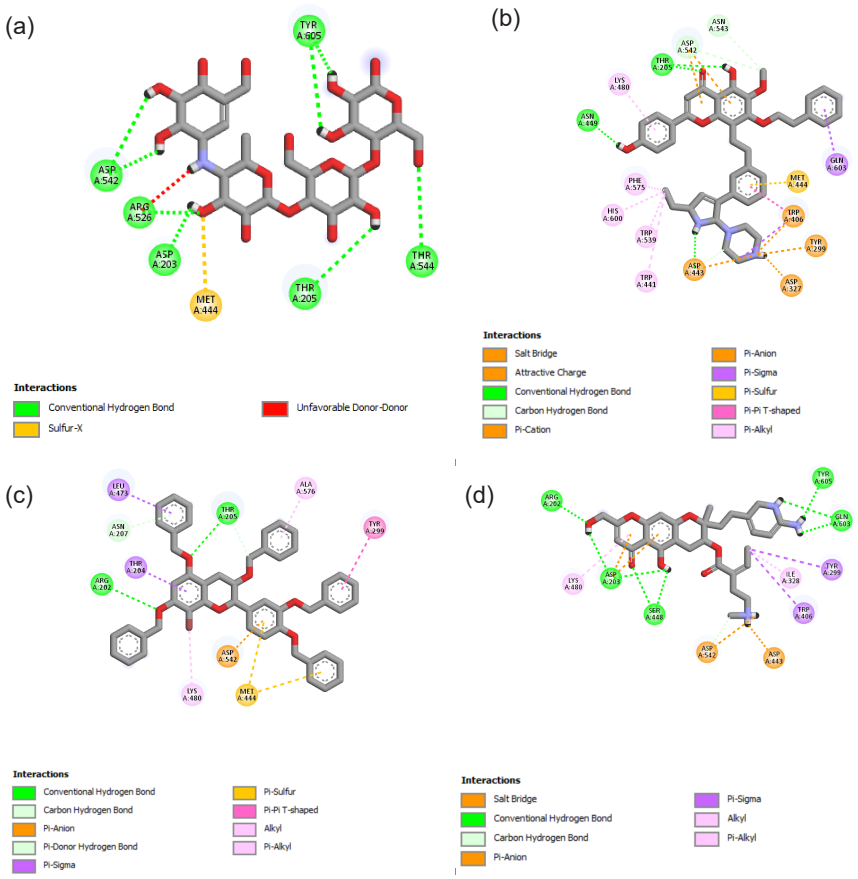


Figure 2. Molecular interaction of three top hits against alpha-glucosidase (PDB ID 2QMJ). (a) Acarbose, (b) ZINC000150350051, (c) ZINC000008382292, and (d) ZINC000085595291.

yielded valuable insights regarding the enduring characteristics of the ligand-receptor interactions. As a result, a more intricate comprehension of the binding stability and adaptability of the identified hits relative to the native ligand, Acarbose, was attained. The lead compounds with strong and prolonged interactions within the active site of alpha-glucosidase were identified with the assistance of the 100 ns molecular dynamics simulations. This information was instrumental in optimizing the development of potential AGIs for the treatment of diabetes.

The simulations were assessed by monitoring critical parameters such as gyration, solvent accessible surface area (SASA), RMSD, RMSF, and molecular mechanics generalized born surface area (MMGBSA). The aforementioned factors were vital in determining the stability of the ligand-receptor system, providing valuable information regarding the energies, fluctuations, and conformational alterations that occurred during the simulation phase. The in-depth examination of these molecular dynamics' simulations provided significant insights into the stability of the identified hits' interactions with the alpha-glucosidase enzyme. This aids in the identification of lead compounds that can be further optimized in the development of AGIs intended for the treatment of diabetes.

Root means square deviation

Using RMSD measurements, the contact stability between alpha-glucosidase and the native ligand (NL) was evaluated during a molecular dynamics simulation lasting 100 ns (Figure 3a). Insights into the temporal progression of the ligand-receptor complex's divergence from its original structure are offered by the RMSD values. At the onset of the simulation (0 ns), the RMSD of the alpha-glucosidase complex was determined to be 0.3639245 Å, but the NL complex displayed an exceptionally low RMSD of 0.0004979 Å, suggesting its initial stability. Varying degrees of variation were detected in both complexes during the simulation. Significantly, the RMSD of the alpha-glucosidase complex increased to 0.9416655 Å at 80 ns, indicating some degree of structural change. Conversely, the RMSD of the NL complex remained comparatively low at 1.1814362 Å, suggesting that it exhibited a reasonably stable conformation. The RMSD profiles provided significant contributions to the comprehension of the stability of ligand-receptor complexes over time by illuminating their dynamic behavior.

Significant trends were evident in the time evolution of RMSD values for both alpha-glucosidase and the NL

complex. With a maximum RMSD of 80 ns, the alpha-glucosidase complex underwent fluctuations, which might suggest modifications in conformation or adaptability within the binding site. On the contrary, the RMSD profile of the NL complex was more uniform, displaying oscillations that occurred within a rather small range. The RMSD values of the NL complex were significantly lower in comparison to those of the alpha-glucosidase complex, suggesting that the binding remained rather stable during the simulation period. The results of this study indicated that the native ligand and the alpha-glucosidase enzyme maintained a comparatively stable relationship, thereby establishing the native ligand's suitability as a benchmark for stable binding conformations.

A thorough evaluation of the stability of the interaction between alpha-glucosidase and ZINC000150350051 (Hit01) was performed by calculating the RMSD values throughout a molecular dynamics simulation lasting 100 ns (Figure 3b). During the initial seconds of the simulation (0 ns), the RMSD of the alpha-glucosidase complex was 0.3618751 Å. In contrast, ZINC000150350051 (Hit01) exhibited initial stability with an RMSD of 0.0004842 Å. Varying degrees of variation were detected in both complexes during the simulation. The observed significant rise in the RMSD of the alpha-glucosidase complex to 0.9507553 Å at 95 ns indicated possible alterations in conformation or flexibility within the binding site. On the other hand, ZINC000150350051 (Hit01) had a comparatively modest RMSD of 0.8937667 Å, which suggested a relatively stable interaction. The RMSD analysis offered significant insights into the dynamic characteristics of ligand-receptor complexes, facilitating a more nuanced comprehension of their temporal stability.

Distinct trends may be observed in the temporal evolution of RMSD values for both alpha-glucosidase and ZINC000150350051 (Hit01). The RMSD of the alpha-glucosidase complex exhibited variability, culminating in a maximum at 95 ns, which indicated possible alterations in structure or adaptability within the binding site. On the contrary, ZINC000150350051 (Hit01) exhibited a greater degree of stability in its RMSD profile during the duration of the simulation, suggesting a stable interaction with alpha-glucosidase. It is important to highlight that the RMSD values for ZINC000150350051 (Hit01) remained significantly lower than those of the alpha-glucosidase complex, indicating that the ligand-receptor contact was stable. The results of this study underscored the promise of ZINC000150350051 (Hit01) as a candidate for stable binding, providing valuable information for further investigations concerning the inhibition of alpha-glucosidase.

The stability of the interaction between alpha-glucosidase and ZINC000008382292 (Hit02) was assessed by performing a comprehensive analysis of RMSD values during a molecular dynamics simulation lasting 100 ns (Figure 3c). During the initial seconds of the simulation (0 ns), the RMSD of the alpha-glucosidase complex was 0.3527685 Å. In contrast, ZINC000008382292 (Hit02) exhibited initial stability with an RMSD of 0.0005183 Å. Throughout the course of the simulation, variations were detected in both complexes. An increase in the RMSD of the alpha-glucosidase complex to 0.8614435 Å at 15 ns may suggest conformational alterations or flexibility within the binding site. On the other hand, ZINC000008382292 (Hit02) had a comparatively greater RMSD of 1.0257909 Å, indicating that its interaction with alpha-glucosidase was rather adaptable. The RMSD profiles provided significant contributions to the comprehension of the stability of ligand-receptor complexes over time by shedding light on their dynamic behavior.

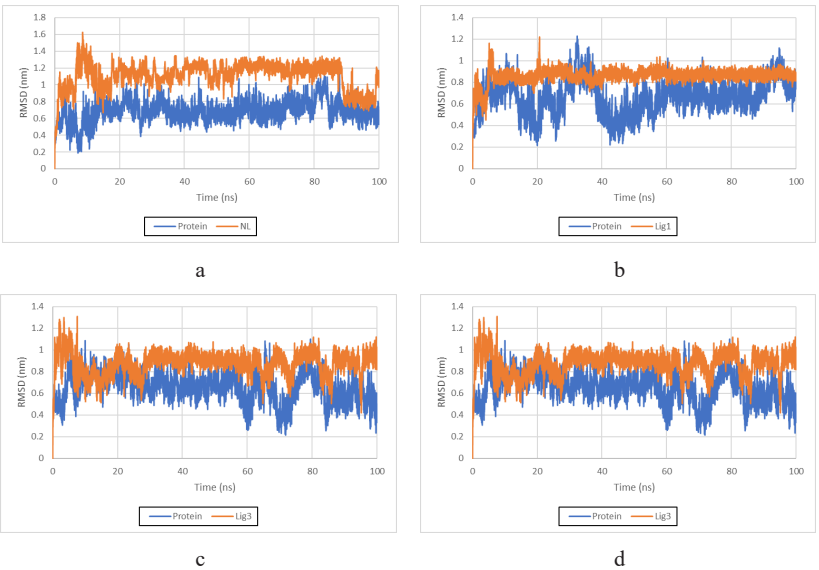


Figure 3. Variations in the alpha-glucosidase enzyme's motion during a 100 ns simulation. (a) The native ligand (acarbose) (NL), (b) ZINC000150350051 (Lig1), (c) ZINC000008382292 (Lig2), and (d) ZINC000085595291 (Lig3).

Distinct trends may be observed in the temporal evolution of RMSD values for both alpha-glucosidase and ZINC000008382292 (Hit02). The RMSD of the alpha-glucosidase complex exhibited variability, culminating in a maximum value at 85 ns, which implied possible alterations in structure or adaptability within the binding site. In contrast, ZINC000008382292 (Hit02) had a consistently elevated RMSD over the course of the simulation, suggesting that it engaged in a dynamic interaction with alpha-glucosidase. The alpha-glucosidase complex possessed lower RMSD values for ZINC000008382292 (Hit02), indicating that the ligand might investigate a wider range of conformations over the simulation time. The results of this study offer valuable information regarding the possible versatility of ZINC000008382292 (Hit02) in its interaction with alpha-glucosidase, hence enhancing its malleability.

To assess the stability of the interaction between alpha-glucosidase and ZINC000085595291 (Hit03), a thorough evaluation of RMSD values was performed throughout a molecular dynamics simulation lasting 100 ns (Figure 3d). During the initial seconds of the simulation (0 ns), the RMSD of the alpha-glucosidase complex was 0.3367476 Å. In contrast, ZINC000085595291 (Hit03) demonstrated initial stability with an RMSD of 0.0005149 Å. During the course of the simulation, both complexes underwent variations. The RMSD of the alpha-glucosidase complex reached its maximum value of 0.7778209 Å at 55 ns, suggesting the possibility of structural reorganizations or adaptability within the binding site. On the contrary, ZINC000085595291 (Hit03) exhibited a comparatively steady RMSD of 0.969072 Å for the identical time interval, indicating a more reliable and persistent interaction with alpha-glucosidase. The RMSD profiles provided invaluable information regarding the ever-changing stability of the ligand-receptor complexes by revealing their dynamic behavior.

Distinct trends can be observed in the time evolution of RMSD values for both alpha-glucosidase and ZINC000085595291 (Hit03). The RMSD of the alpha-glucosidase complex exhibited variability, culminating in a peak at 55 ns, which indicated possible alterations in conformation or adaptability within the binding site. On the other hand, the RMSD profile of ZINC000085595291 (Hit03) remained comparatively steady, suggesting that it maintained a sustained interaction with alpha-glucosidase. Significantly, the RMSD values for ZINC000085595291 (Hit03) exhibited a constant decrease in comparison to those of the alpha-glucosidase complex, so underscoring the ligand-receptor interaction's stability. The results of this study underscored the promise of ZINC000085595291 (Hit03) as a candidate for stable binding, indicating its favorable suitability as a lead molecule in subsequent research endeavors.

In brief, the evaluation of RMSD values for alpha-glucosidase complexes containing various ligands unveiled unique dynamic characteristics. Although the native ligand exhibited consistent stability, ZINC000085595291 (Hit03) showed superior qualities in terms of stability and RMSD values when compared to ZINC000150350051 (Hit01) and ZINC000008382292 (Hit02). The ligand-receptor dynamics were better understood as a result of these discoveries, which also may assist in the identification of lead compounds that can be further optimized during the development of AGIs for the treatment of diabetes.

Root means square fluctuation

By comparing the RMSF values of alpha-glucosidase complexes with those of the native ligand (NL), ZINC000150350051 (Hit01), ZINC000008382292 (Hit02), and ZINC000085595291 (Hit03) during a molecular dynamics simulation lasting 100 ns, valuable insights could be gained regarding the flexibility and interaction of these ligands with particular amino acid residues (Figure 4). The RMSF values indicate the extent to which amino acid residues deviate from their mean positions throughout the simulation.

An exhaustive examination of the RMSF values corresponding to particular amino acid residues unveiled discrepancies in the binding site's flexibility. As an example, ZINC000085595291 (Hit03) displayed the lowest RMSF value (0.2646) at residue 375, which was critical for ligand

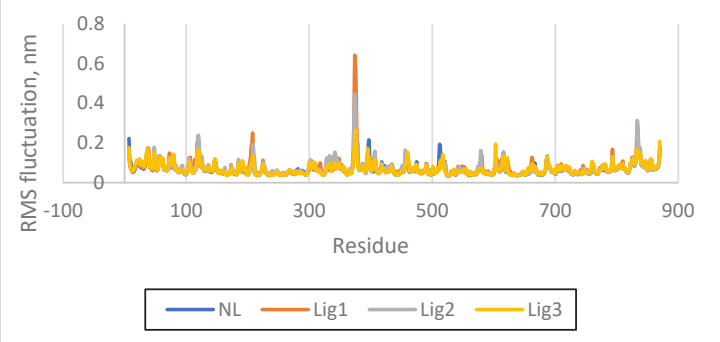


Figure 4. Variations in the orientation of amino acid residues in proximity to the binding site for the native ligand (acarbose) (NL), ZINC000150350051 (Lig1), ZINC000008382292 (Lig2), and ZINC000085595291 (Lig3).

binding, indicating a more stable association with this residue in comparison to the NL, ZINC000150350051 (Hit01), and ZINC000008382292. (Hit02). Furthermore, it was observed that ZINC000085595291 (Hit03) exhibited the least RMSF value (0.1318) at residue 461, suggesting a more restricted interaction with this particular residue. The examination of RMSF values at these particular residues underscored the potential of ZINC000085595291 (Hit03) as a ligand that formed stable and precise contacts with crucial amino acid residues located in the binding region of alpha-glucosidase.

The analysis of RMSF values across various amino acid residues provided additional insight into the dynamic characteristics shown by the alpha-glucosidase complexes. The residues, where the NL and ZINC000150350051 (Hit01) had variability in RMSF values, were those where ZINC000085595291 (Hit03) consistently exhibited lower RMSF values. This observation implies that the interaction between ZINC000085595291 and these residues is more stable and restricted. This observation is consistent with the concept that ZINC000085595291 (Hit03) can establish stable hydrogen bonds or engage in other interactions with particular amino acid residues. This feature enhances its potential as a lead chemical for inhibiting alpha-glucosidase.

In brief, the RMSF analysis offers significant insights into the interaction patterns and flexibility of alpha-glucosidase complexes involving various ligands. ZINC000085595291 (Hit03) exhibited a more stable and specific association in comparison to the NL, ZINC000150350051 (Hit01), and ZINC000008382292 due to its lower RMSF values at critical residues (Hit02). The results of this study make a valuable contribution to the process of ligand selection and prioritization in the development of AGIs for the treatment of diabetes.

Gyration

The examination of gyration, which signified the structural stability and compactness of alpha-glucosidase complexes involving the NL, ZINC000150350051 (Hit01), ZINC000008382292 (Hit02), and ZINC000085595291 (Hit03), during a molecular dynamics simulation lasting 100 ns, provided valuable information regarding the dynamic characteristics of these complexes (Figure 5). At the onset of the simulation (0 ps), the gyration values of all ligands were found to be equivalent, indicating that they all had comparable structural compactness at the outset. Nevertheless, as the simulation advanced, differences in gyration patterns became apparent. In contrast to the remaining ligands, ZINC000085595291 (Hit03) consistently exhibited reduced gyration values, suggesting a conformation that was more stable and compact. This implies that ZINC000085595291 (Hit03) maintains a structurally advantageous contact with alpha-glucosidase, which may enhance its prospects as a stable ligand in subsequent research and development.

During the 100-ns simulation, ZINC000085595291 (Hit03) maintained consistently low gyration values in comparison to the NL and alternative ligands, suggesting that its structural stability was maintained. The fluctuating gyration values of ZINC000150350051 (Hit01) and ZINC000008382292 (Hit02) during the simulation indicated that their conformations underwent dynamic alterations. The aforementioned observations were consistent with the notion that ZINC000085595291 (Hit03) established a more compact and stable complex with alpha-glucosidase. This underscored the potential of ZINC000085595291 (Hit03) as a lead compound that warranted additional research to develop AGIs for the treatment of diabetes.

In brief, throughout a simulation lasting 100 ns, the gyration analysis offered significant information about the compactness and structural stability of alpha-glucosidase complexes including various ligands. ZINC000085595291, denoted as Hit03, consistently demonstrated reduced gyration values in comparison to the NL and alternative ligands, suggesting a more stable and compact structure. The results of this study enhanced our comprehension

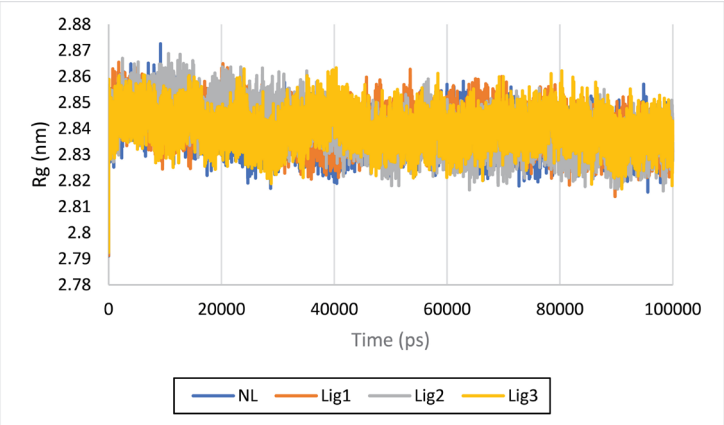


Figure 5. The compactness and structural stability of alpha-glucosidase complexes for the native ligand (acarbose) (NL), ZINC000150350051 (Lig1), ZINC000008382292 (Lig2), and (d) ZINC000085595291 (Lig3).

of the interactions between ligands and receptors and provided further support for the prioritization of ZINC000085595291 (Hit03) in the pursuit of optimizing AGIs.

Solvent accessible surface area

During a molecular dynamics simulation lasting 100 ns, the SASA analysis offered significant insights into the accessibility and exposure of alpha-glucosidase and its complexes with the NL, ZINC000150350051 (Hit01), ZINC000008382292 (Hit02), and ZINC000085595291 (Hit03), to the surrounding solvent (Figure 6).

Simultaneous solvent exposure was shown by the fact that all ligands displayed equivalent SASA values at the onset of the simulation (0 ps). As the experiment advanced, discernible patterns surfaced. In comparison to the other ligands, ZINC000085595291 (Hit03) consistently exhibited reduced SASA values, indicating a conformation that was more compact and less exposed. This showed that ZINC000085595291 (Hit03) reduced the solvent accessibility of alpha-glucosidase by forming a more stable complex with it. The decreased solvent exposure of ZINC000085595291 (Hit03) should potentially enhance its prospects for further development as a stable ligand.

During the whole 100-ns simulation, ZINC000085595291 (Hit03) maintained consistently low SASA values in comparison to the NL and other ligands. The observed consistent decrease in SASA values suggests that the interaction between ZINC000085595291 (Hit03) and alpha-glucosidase is comparatively less dynamic and more stable. On the other hand, the SASA values of ZINC000150350051 (Hit01) and ZINC000008382292 (Hit02) fluctuated, indicating that their conformations and solvent exposure underwent dynamic changes throughout the simulation. The results of this study provided additional evidence in favor of the hypothesis that ZINC000085595291 (Hit03) formed a compact and stable complex with alpha-glucosidase. This underscores the potential of ZINC000085595291 as a lead compound to be further explored in the pursuit of AGIs for the treatment of diabetes.

In brief, the SASA analysis provided valuable insights into the accessibility and solvent exposure of alpha-glucosidase complexes containing various ligands over a simulation lasting 100 ns. ZINC000085595291 (Hit03) consistently demonstrated reduced solvent-exposed conformation SASA values, suggesting a more compact structure in comparison to the NL and alternative ligands. The results of this study enhanced our comprehension of the interactions between ligands and receptors and provided further support for the prioritization of ZINC000085595291 (Hit03) in the pursuit of optimizing AGIs.

Molecular mechanics generalized born surface area

Over the course of a molecular dynamics simulation lasting 100 ns, the MMGBSA analysis offered an exhaustive comprehension of the energy constituents that influenced the stability and binding affinity of alpha-glucosidase complexes involving the NL, ZINC000150350051 (Hit01), ZINC000008382292 (Hit02), and ZINC000085595291 (Hit03) (Figure 7).

Significant variations were observed in the energy components when examining the various ligands. ZINC000008382292 (Hit02) demonstrated an unforeseen elevated positive ΔVDWAALS value, indicating a significant augmentation in van der Waals interactions. The peculiarity in question could potentially be explained by the chemical characteristics of ZINC000008382292 (Hit02) and its interactions with alpha-glucosidase. On the other hand, ZINC000085595291 (Hit03) had a negative ΔVDWAALS value, which signified favorable van der

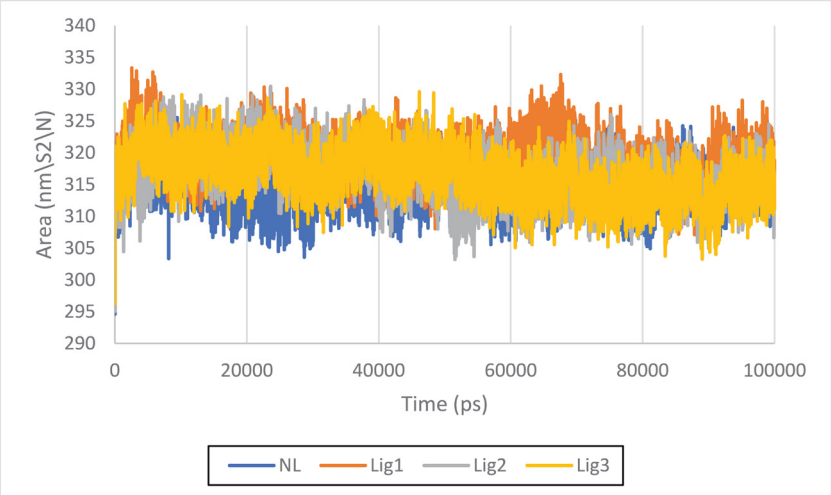


Figure 6. The surface area of solvent accessible (SASA) analysis in alpha-glucosidase and its complexes for the native ligand (acarbose) (NL), ZINC000150350051 (Lig1), ZINC000008382292 (Lig2), and (d) ZINC000085595291 (Lig3).

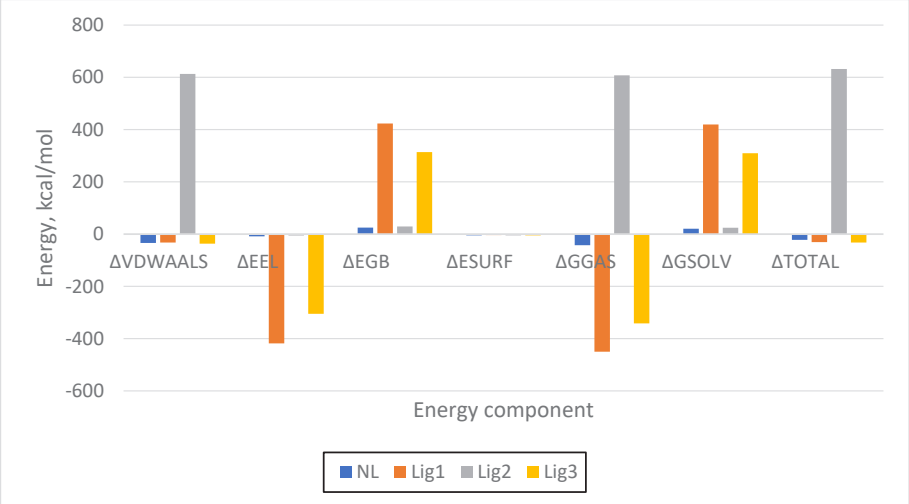


Figure 7. Molecular mechanics generalized born surface area (MMGBSA)-determined energy component of the complex for the native ligand (acarbose) (NL), ZINC000150350051 (Lig1), ZINC000008382292 (Lig2), and (d) ZINC000085595291 (Lig3).

Waals interactions and the creation of a stable complex.

The electrostatic energy (ΔEEL) is a significant factor in the interactions between ligands and receptors. ZINC000150350051 (Hit01) demonstrated a notably negative ΔEEL, which signified prominent electrostatic contacts, but ZINC000008382292 (Hit02) exhibited a substantially positive value, which implied repulsive forces. ZINC000085595291 (Hit03) exhibited a ΔEEL that was moderately negative, suggesting the presence of favorable electrostatic interactions. The collective pattern indicated that ZINC000150350051 (Hit01) and ZINC000085595291 (Hit03) established more advantageous electrostatic bonds with alpha-glucosidase, hence enhancing their prospective utility as inhibitors.

Glucose-solving free energy (GSOLV) is an essential determinant in ligand binding. ZINC000085595291 (Hit03) demonstrated a very positive ΔGSOLV value, which suggested decreased solvent accessibility and consistent solvation. On the contrary, ZINC000008382292 (Hit02) had a significantly positive value, indicating enhanced accessibility to the solvent. The results of this study are consistent with the SASA analysis, providing further evidence that ZINC000085595291 (Hit03) forms a solvent-exposed complex with alpha-glucosidase that is more stable.

The total energy (ΔTOTAL) of the ligand-receptor combination serves as an indicator of its overall stability. ZINC000008382292 (Hit02) exhibited an atypically elevated positive result, which signified an adverse interaction. In contrast, ZINC000085595291 exhibited a negative ΔTOTAL, which indicated the creation of a stable and advantageous complex. This was consistent with the patterns seen in additional energy constituents and lends credence to the idea that ZINC000085595291 (Hit03) exhibited encouraging prospects as an AGI.

In brief, the MMGBSA analysis furnished a thorough comprehension of the energy constituents that dictated the stability and affinity for binding of alpha-glucosidase complexes involving various ligands. The positive energy components regularly displayed by ZINC000085595291 (Hit03) indicated a stable and promising interaction with alpha-glucosidase. The results of this study provided significant contributions to the process of prioritizing ZINC000085595291 (Hit03) to optimize the development of AGIs for the treatment of diabetes.

Discussion

The pharmacophore model developed in this study is critical in ligand-based drug design for creating AGIs. The pharmacophore model has ten features in total, including two aromatic ring features, three hydrogen bond donor features, and five hydrogen bond acceptor features. It gives a thorough description of the critical chemical interactions required for successful inhibition of the target enzyme. It is especially significant since the pharmacophore model includes aromatic ring characteristics (21). It is well-established that aromatic rings interact with particular amino acid residues in the binding pocket of the alpha-glucosidase enzyme via π-π stacking (22). These interactions play a critical role in maintaining the stability of the ligand-receptor complex and significantly influence the overall affinity for binding. The inclusion of aromatic ring characteristics in the pharmacophore model enables the prioritization of drugs that can engage in advantageous aromatic interactions, hence augmenting the probability of effective inhibition (23).

Furthermore, the incorporation of donor and acceptor hydrogen bond characteristics into the pharmacophore model underscores the significance of these particular interactions in the establishment of stable complexes with the alpha-glucosidase enzyme (24). The establishment of hydrogen bonds between the ligand and critical amino acid

residues inside the active site enables accurate molecular recognition and binding. The process of selecting and optimizing molecules capable of forming these crucial hydrogen bonds is guided by the pharmacophore model, which ultimately enhances the effectiveness of the chosen inhibitors (25).

The pharmacophore model functions as a virtual template in ligand-based drug discovery, facilitating the process of screening, and choosing prospective drug candidates. Substrates that correspond to the predetermined characteristics of the pharmacophore are more inclined to demonstrate the intended biological activity (26). By employing this methodology, it becomes possible to logically devise innovative AGIs with an emphasis on molecular interactions that are crucial for achieving potent inhibitory effects.

Furthermore, the pharmacophore model provides a significant asset in the form of a virtual screening mechanism for compound databases. This technology aids in the detection of substances that can interact positively with the alpha-glucosidase enzyme, hence optimizing the process of drug discovery (27). Similar studies have explored the binding mode of interaction for stilbene derivatives, a new class of AGIs (28), used virtual screening to find AGIs from existing drugs, and rationally designed novel inhibitors using quantitative structure-activity relationship and structure-based virtual screening (29). These findings highlight the importance of pharmacophore models in enzyme design.

The binding sites of alpha-glucosidase have been identified as curculigoside A derivatives through molecular analysis. Molecular docking and molecular dynamics analyses were utilized in the research to determine the manner of binding and the interactions between these derivatives and the alpha-glucosidase enzyme. Certain residues, including Asp203, Asp542, Asp327, His600, and Arg526, have been discovered through the studies as being implicated in the inhibitory potential and binding interactions of the curculigoside A derivatives. Furthermore, *in vitro* investigations into the inhibition of alpha-glucosidase have been undertaken to authenticate the findings obtained *in silico* (9). The study has provided evidence that these derivatives possess the capacity to function as inhibitors of alpha-glucosidase, a target therapeutic agent in the treatment of type 2 diabetes. Significant insights have been obtained on the interaction, stability, and inhibitory activity of these drugs with the alpha-glucosidase enzyme by the utilization of molecular docking and dynamic modeling.

Molecular docking and molecular dynamics simulations are critical in the development of new AGIs (30). These techniques can be used to better understand the binding interactions between prospective inhibitors and the target enzyme, as well as to assess the stability and flexibility of the inhibitor-enzyme complex.

Variations in the flexibility of the binding site can be seen by examining the RMSF values for individual amino acid residues. For example, amino acid residues 7, 119, 208, and 461 have variable RMSF values across complexes. Notably, Lig 3 (Hit03) regularly had lower RMSF values for these residues than Lig 1 (Hit01) and Lig 2 (Hit02), indicating a more stable binding to alpha-particular glucosidase's areas. Furthermore, Lig 3 (Hit03) interacted with particular residues such as SER454, ILE402, and SER394 via hydrogen bonds and hydrophobic interactions, which supported the stable binding profile found in the RMSF analysis.

The comparison of RMSF values with the individual amino acid residues, implicated in the interaction with virtual screening hits, clarifies the relationship between flexibility and binding. For example, the interaction of ZINC000150350051 with SER454 and VAL455 involves hydrogen bonding and hydrophobic interactions, which agree with the lower RMSF values reported for these residues in Lig 3. (Hit03). These data reveal that Lig 3 (Hit03) not only has decreased overall flexibility in the binding site but also forms selective interactions with critical amino acid residues, indicating that it has the potential to be a stable and effective AGI.

By comparing binding affinity and MMGBSA values, the strength of interactions and overall stability of alpha-glucosidase complexes with various ligands may be determined with critical precision. The binding affinity serves as an indicator of the potency of the ligand-receptor interaction, whereas MMGBSA calculations provide an approximation of the free energy change linked to ligand binding, taking into account solvent effects and contributions from molecular mechanics.

The NL demonstrated an MMGBSA value of -21.69 kcal/mol and a binding affinity of -6.9 kcal/mol. On the other hand, the results obtained from virtual screening, including Hit01 (ZINC000150350051), exhibited much enhanced binding affinities (-17.84 kcal/mol), suggesting a more robust interaction with alpha-glucosidase. The MMGBSA value of -30.43 kcal/mol for Hit01 provides more evidence in favor of its favorable binding energies. The heightened affinity of Hit01 for binding alpha-glucosidase indicates its potential as a highly effective inhibitor, surpassing the performance of the native ligand.

Hit02 (ZINC000008382292) and Hit03 (ZINC000085595291) exhibited very high MMGBSA values while having lower binding affinities of -11.92 kcal/mol and -11.72 kcal/mol, respectively. Hit02 displayed an exceptional MMGBSA value of 632.13 kcal/mol, but Hit03 revealed a value of -31.93 kcal/mol. The results of this study indicate that although Hit02 exhibits a decreased affinity for binding, its overall stability inside the binding site is affected positively by solvation effects. On the other hand, Hit03 has a potentially stabilizing effect from the solvent environment, as seen by its more favorable MMGBSA value despite its lower binding affinity.

The current study used a simplified *in silico* technique

that may fail to convey the complexities of biological systems. The use of molecular dynamics simulations and computational models simplifies the dynamic nature of biological interactions, and prediction accuracy is strongly dependent on force field precision and parameterization. The ligand-based pharmacophore modeling and molecular docking methods assume that alpha-glucosidase has a static binding site. In actuality, protein structures can change shape, therefore static models may not capture the dynamic nature of binding sites.

The predicted interactions have not been experimentally validated in this study. While computational approaches provide useful information, experimental studies, such as binding tests or *in vitro* testing, are required to corroborate the identified ligands' inhibitory efficacy against alpha-glucosidase. The study's primary focus was alpha-glucosidase inhibition, and the specificity and selectivity of the discovered ligands for this target were not thoroughly investigated. Future studies should include testing the ligands against a wider range of targets to determine their specificity.

Conclusion

The *in silico* study for identifying AGI derivatives has been developed using ligand-based pharmacophore models, molecular docking, and molecular dynamics simulations. The model identified essential molecular features in curculigoside A derivatives, yielding 956 hits from 270 547 compounds. The top 956 hits were evaluated for binding energy values superior to acarbose, revealing promising compounds with binding affinities for alpha-glucosidase. The three selected hits (ZINC000150350051, ZINC000008382292, and ZINC000085595291) were evaluated over molecular dynamics for 100 ns. The promising hits, particularly ZINC000085595291, demonstrated favorable binding affinities and stable interactions throughout the simulation. The simulations revealed favorable binding affinities and stable interactions, indicating potential for further drug development for diabetes treatment.

Acknowledgments

We express our sincere gratitude to the Internal Research Funding provided by Lembaga Penelitian dan Pengabdian kepada Masyarakat (LPPM) Universitas Bhakti Kencana in 2023. This financial support has played a crucial role in facilitating and advancing our research endeavors in the field of ligand-based pharmacophore modeling, molecular docking, and molecular dynamics simulations for the identification of inhibitors targeting alpha-glucosidase.

Authors' contributions

Conceptualization: Aiyi Asnawi.
Data curation: Asnawi, Shifa Mieldianisa, Widhya Aligita, Anne Yuliantini, Ellin Febrina.
Formal analysis: Aiyi Asnawi, Shifa Mieldianisa, Widhya Aligita, Anne Yuliantini, Ellin Febrina.
Funding acquisition: Aiyi Asnawi, Anne Yuliantini, Ellin Febrina.
Investigation: Asnawi, Shifa Mieldianisa, Widhya Aligita, Anne Yuliantini, Ellin Febrina.
Methodology: Aiyi Asnawi, Widhya Aligita, Anne Yuliantini, Ellin Febrina.
Project administration: Aiyi Asnawi
Resources: Aiyi Asnawi, Ellin Febrina.
Software: Aiyi Asnawi, Shifa Mieldianisa.
Supervision: Aiyi Asnawi.
Validation: Aiyi Asnawi, Anne Yuliantini, Ellin Febrina.
Visualization: Widhya Aligita, Anne Yuliantini, Ellin Febrina.
Writing–original draft: Aiyi Asnawi, Shifa Mieldianisa, Widhya Aligita, Anne Yuliantini, Ellin Febrina.
Writing–review & editing: Aiyi Asnawi, Shifa Mieldianisa, Widhya Aligita, Anne Yuliantini, Ellin Febrina.

Conflict of interests

The authors declare that there is no conflict of interest associated with this research work.

Ethical considerations

This research adheres to ethical principles and standards, and it does not involve the use of animals or humans. The study primarily relies on computational and *in silico* methodologies, such as ligand-based pharmacophore modeling, molecular docking, and molecular dynamics simulations. Therefore, the research design eliminates the need for ethical approval related to human or animal subjects.

Funding/Support

This research was generously funded by Internal Research, LPPM Universitas Bhakti Kencana in 2023, under Grant Number 081/14.LPPM/PE.I/UBK/2023.

References

1. Mukhtar Y, Galalain A, Yunusa U. A modern overview on diabetes mellitus: a chronic endocrine disorder. Eur J Biol. 2020;5(2):1-14. doi: 10.47672/ejb.409.
2. Czech MP. Insulin action and resistance in obesity and type 2 diabetes. Nat Med. 2017;23(7):804-14. doi: 10.1038/nm.4350.
3. Wang H, Li N, Chivese T, Werfalli M, Sun H, Yuen L, et al. IDF diabetes atlas: estimation of global and regional gestational diabetes mellitus prevalence for 2021 by International Association of Diabetes in Pregnancy Study Group's Criteria. Diabetes Res Clin Pract. 2022;183:109050. doi: 10.1016/j.diabres.2021.109050.
4. Candler TP, Mahmoud O, Lynn RM, Majbar AA, Barrett TG, Shield JPH. Continuing rise of Type 2 diabetes incidence in children and young people in the UK. Diabet Med. 2018;35(6):737-44. doi: 10.1111/dme.13609.
5. Smith DL Jr, Orlandella RM, Allison DB, Norian LA. Diabetes medications as potential calorie restriction mimetics-a focus on the alpha-glucosidase inhibitor acarbose. Geroscience. 2021;43(3):1123-33. doi: 10.1007/s11357-020-00278-x.
6. Kuan IHS, Savage RL, Duffull SB, Walker RJ, Wright DFB. The association between metformin therapy and lactic acidosis. Drug Saf. 2019;42(12):1449-69. doi: 10.1007/s40264-019-00854-x.

7. Zeng Z, Huang SY, Sun T. Pharmacogenomic studies of current antidiabetic agents and potential new drug targets for precision medicine of diabetes. Diabetes Ther. 2020;11(11):2521-38. doi: 10.1007/s13300-020-00922-x.
8. Zabidi NA, Ishak NA, Hamid M, Ashari SE, Mohammad Latif MA. Inhibitory evaluation of *Curculigo latifolia* on α-glucosidase, DPP (IV) and in vitro studies in antidiabetic with molecular docking relevance to type 2 diabetes mellitus. J Enzyme Inhib Med Chem. 2021;36(1):109-21. doi: 10.1080/14756366.2020.1844680.
9. Nursamsiar, Nur S, Febrina E, Asnawi A, Syafiie S. Synthesis and inhibitory activity of curculigoside A derivatives as potential anti-diabetic agents with β-cell apoptosis. J Mol Struct. 2022;1265:133292. doi: 10.1016/j.molstruc.2022.133292.
10. Ooi DJ, Azmi NH, Imam MU, Alitheen NB, Ismail M. Curculigoside and polyphenol-rich ethyl acetate fraction of *Molineria latifolia* rhizome improved glucose uptake via potential mTOR/AKT activated GLUT4 translocation. J Food Drug Anal. 2018;26(4):1253-64. doi: 10.1016/j.jfda.2018.03.003.
11. Estrada AK, Mendez-Alvarez D, Juarez-Saldivar A, Lara-Ramirez EE, Martinez-Vazquez AV, Villalobos-Rocha JC, et al. Ligand-based and structure-based virtual screening of new sodium glucose cotransporter type 2 inhibitors. Med Chem. 2023;19(10):1049-60. doi: 10.2174/1573406419666230803122020.
12. Febrina E, Asnawi A. Lead compound discovery using pharmacophore-based models of small-molecule metabolites from human blood as inhibitor cellular entry of SARS-CoV-2. J Pharm Pharmacogn Res. 2023;11(5):810-22. doi: 10.56499/jppres23.1688_11.5.810.
13. Grinter SZ, Zou X. Challenges, applications, and recent advances of protein-ligand docking in structure-based drug design. Molecules. 2014;19(7):10150-76. doi: 10.3390/molecules190710150.
14. Acharya C, Coop A, Polli JE, Mackerell AD Jr. Recent advances in ligand-based drug design: relevance and utility of the conformationally sampled pharmacophore approach. Curr Comput Aided Drug Des. 2011;7(1):10-22. doi: 10.2174/157340911793743547.
15. Schneidman-Duhovny D, Dror O, Inbar Y, Nussinov R, Wolfson HJ. PharmaGist: a webserver for ligand-based pharmacophore detection. Nucleic Acids Res. 2008;36(Suppl 2):W223-8. doi: 10.1093/nar/gkn187.
16. Cereto-Massagué A, Guasch L, Valls C, Mulero M, Pujadas G, Garcia-Vallvé S. DecoyFinder: an easy-to-use python GUI application for building target-specific decoy sets. Bioinformatics. 2012;28(12):1661-2. doi: 10.1093/bioinformatics/bts249.
17. Yoshikawa N, Hutchison GR. Fast, efficient fragment-based coordinate generation for Open Babel. J Cheminform. 2019;11(1):49. doi: 10.1186/s13321-019-0372-5.
18. Snyder HD, Kucukkal TG. Computational chemistry activities with *Avogadro* and ORCA. J Chem Educ. 2021;98(4):1335-41. doi: 10.1021/acs.jchemed.0c00959.
19. Jejurikar BL, Rohane SH. Drug designing in discovery studio. Asian J Res Chem. 2021;14(2):135-8. doi: 10.5958/0974-4150.2021.00025.0.
20. Kutzner C, Kniep C, Cherian A, Nordstrom L, Grubmüller H, de Groot BL, et al. GROMACS in the cloud: a global supercomputer to speed up alchemical drug design. J Chem Inf Model. 2022;62(7):1691-711. doi: 10.1021/acs.jcim.2c00044.
21. Kaserer T, Beck KR, Akram M, Odermatt A, Schuster D. Pharmacophore models and pharmacophore-based virtual screening: concepts and applications exemplified on hydroxysteroid dehydrogenases. Molecules. 2015;20(12):22799-832. doi: 10.3390/molecules201219880.
22. Tang H, Huang L, Sun C, Zhao D. Exploring the structure-activity relationship and interaction mechanism of flavonoids and α-glucosidase based on experimental analysis and molecular docking studies. Food Funct. 2020;11(4):3332-50. doi: 10.1039/c9fo02806d.
23. Lanka G, Begum D, Banerjee S, Adhikari N, P Y, Ghosh B. Pharmacophore-based virtual screening, 3D QSAR, Docking, ADMET, and MD simulation studies: an in silico perspective for the identification of new potential HDAC3 inhibitors. Comput Biol Med. 2023;166:107481. doi: 10.1016/j.compbiomed.2023.107481.
24. Kothiwale SK. A Novel Knowledge Based Conformation Sampling Algorithm and Applications in Drug Discovery [dissertation]. Nashville, Tennessee: Vanderbilt University; 2016.
25. Ran T, Li W, Peng B, Xie B, Lu T, Lu S, et al. Virtual screening with a structure-based pharmacophore model to identify small-molecule inhibitors of CARM1. J Chem Inf Model. 2019;59(1):522-34. doi: 10.1021/acs.jcim.8b00610.
26. Mohammed M, Bayissa B, Getachew M, Adem F. Drug-related problems and determinants among elective surgical patients: a prospective observational study. SAGE Open Med. 2022;10:20503121221122438. doi: 10.1177/20503121221122438.
27. Tripathi NM, Bandyopadhyay A. High throughput virtual screening (HTVS) of peptide library: technological advancement in ligand discovery. Eur J Med Chem. 2022;243:114766. doi: 10.1016/j.ejmech.2022.114766.
28. Lee Y, Kim S, Kim JY, Arooj M, Kim S, Hwang S, et al. Binding mode analyses and pharmacophore model development for stilbene derivatives as a novel and competitive class of α-glucosidase inhibitors. PLoS One. 2014;9(1):e85827. doi: 10.1371/journal.pone.0085827.
29. Halim SA, Jabeen S, Khan A, Al-Harrasi A. Rational design of novel inhibitors of α-glucosidase: an application of quantitative structure activity relationship and structure-based virtual screening. Pharmaceuticals (Basel). 2021;14(5):482. doi: 10.3390/ph14050482.
30. Siregar M, Awaluddin A, Nurnahari N, Nur S, Febrina E, Asnawi A. Molecular docking and molecular dynamic simulation of the aglycone of curculigoside a and its derivatives as alpha glucosidase inhibitors. Rasayan J Chem. 2020;13(1):690-8. doi: 10.31788/rjc.2020.1315577.