



RESEARCH ARTICLE

In silico studies for the identification of potential thiazolidine-2,4-diones as α -amylase inhibitors

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ABSTRACT

Type 2 diabetes, which is characterized by hyperglycemia, is a chronic endocrine metabolic condition. α -amylase inhibitors may have a major therapeutic impact in type 2 diabetic mellitus. In the present study, virtual screening database preparation by R-group enumeration, virtual screening, docking study, and pharmacokinetics analysis was performed by taking α -amylase as a target. The results highlight the important binding features of novel thiazolidine-2,4-dione compounds as α -amylase inhibitors. An R-group enumeration study was performed for the generation of novel thiazolidine-2,4-dione derivatives (6250 compounds). These derivatives further proceed for virtual screening study using Lipinski rule of 5, high-throughput virtual screening, standard precision, and extra precision (XP) screening filters. Only the top 4 compounds were selected after the XP docking process. The molecular docking study of virtual screening hits showed that compound 1 showed a good binding score of -9.129 kcal/mole on α -amylase enzyme (Protein Data Bank ID-3BAY). The present study may be used for the further development of potential compounds against type 2 diabetes.

KEY WORDS: Structure-based pharmacophore, Thiazolidine-2,4-dione, Virtual screening, α -amylase

INTRODUCTION

Type 2 diabetes, which is characterized by hyperglycemia, is a chronic endocrine metabolic condition.^[1] It is most common and is caused by abnormal insulin activity.^[2] It causes a variety of long-term issues.^[3] Despite medical improvements, type 2 diabetes remains one of the leading causes of death in adults.^[4]

Delaying carbohydrate digestion helps to reduce the effects of hyperglycemia. The hydrolysis of carbohydrates involves several enzymes that include human pancreatic α -amylase (HPA) and α -glucosidase.^[5,6] The effects of both inhibitors on blood glucose levels are shown in Figure 1. According to the investigation, three catalytic residues (ASP197, GLU233, and ASP300) are present

in an eight-stranded parallel beta-barrel in domain A (1–99, 169–404).^[7] ASP197 operates as a nucleophile in carbohydrate hydrolysis, whereas GLU233 and ASP300 may act as acid/base catalysts.^[5,7] As a result, one of the scientifically validated approaches for managing type 2 diabetes is to suppress HPA using a ligand, which lowers postprandial blood glucose levels.^[7,8]

Doctors employed a wide range of oral anti-diabetic drugs, including acarbose and miglitol, to treat type 2 diabetes.^[9] However, unfavorable and dangerous side effects such as gas, bloating, and diarrhea are becoming apparent.^[10] As a result, there is an urgent need for effective, low-cost, and non-toxic anti-diabetic drugs.^[7]

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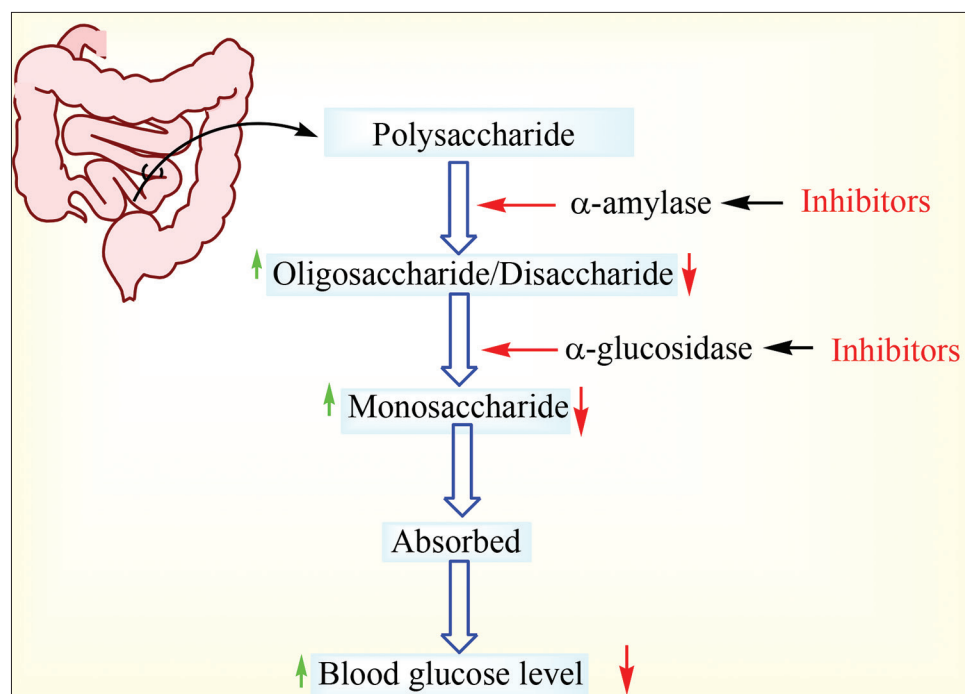


Figure 1: Flowchart describing the effect of α -amylase inhibitors as anti-diabetic agents.

Ancient human beings relied on remedies from nature to treat diabetes.^[11,12] As a result, natural products obtained from these sources may be a fascinating approach to type 2 diabetes management.^[13] To find a powerful HPA inhibitor, research is intensifying.^[7,14] The *in vitro* investigation demonstrates that many natural compounds have α -amylase inhibitory efficacy.^[15,16] Various *in vitro* studies on natural compounds have been conducted up to this point, but an effective HPA inhibitor remains lacking.^[7,17] As a result, structure-based molecular modeling was used to find a putative HPA inhibitor from natural compounds.^[18] Molecular docking, MM/GBSA binding energy estimates, molecular dynamics simulations, and absorption, distribution, metabolism, excretion, and toxicity studies are all part of the modeling process.^[19]

The current computational investigation includes the identification of a novel thiazolidine-2,4-dione as a possible HPA inhibitor. The research includes pharmacophore development, validation of pharmacophores, R-group enumeration, virtual screening, and docking investigations.

MATERIALS AND METHODS

Virtual screening database preparation by R-group enumeration

R-group enumeration study has been performed to generate compounds library based on the core moiety. The core moiety of thiazolidine-2,4-dione has been taken with the selection of possible site for substitution using SAR of

previously published database.^[20] Using R-group creator, fragments were generated on the pharmacophore. The selected positions on the core scaffold were substituted with fragments to the generation of novel compounds.

Molecular docking analysis

Docking investigations were carried out on the crystal structure of α -amylase (PDB ID-3BAY) at a resolution of 1.99Å which was obtained from the Protein Data Bank.^[21] The protein preparation wizard module in Maestro was used to add missing hydrogens and missing bond ordering. The Empirical pKa Prediction (Epik) method was used for generating ionization and tautomeric states at pH 7.0 $\pm$ 2.0. Using PROPKA at a pH of 7.0, hydrogen bond optimization of the protonated residues was carried out by refining. The resulting structure was subsequently subjected to restricted minimization using the OPLS 2005 force field until the heavy atoms were within 0.3Å relative mean standard deviation (RMSD). Then, the receptor grid was generated using the receptor grid generation tool found in the Schrödinger Glide module. Based on the provided coordinates (x-11.22, y-16.93, z-51.03), a grid with a default size of 10 $\times$ 10 $\times$ 10Å was produced.^[22]

Virtual screening study

A virtual screening of the enumerated database of 6250 thiazolidine-2,4-dione compounds was carried out to generate hit compounds for the development of α -amylase receptor antagonists. The enumerated database of known

3D structures was optimized in LigPrep utilizing the OPLS 2005 force field. The recovered compounds from this stage were screened further utilizing a virtual screening methodology. A well-known compound Acarbose was added to the database as a reference compound. Compounds recovered from the R-group enumeration were docked in the active site of the α -amylase receptor utilizing the high-throughput virtual screening (HTVS) approach and flexible docking mode in Glide. Compounds obtained in this manner were re-docked using the standard precision (SP) approach to acquire higher precision. The procedure was repeated by re-docking the compounds in the active site of α -amylase receptors using the extra precision (XP) approach. The resultant compounds were screened using another filter, that is, Lipinski's rule of five. Other properties of the compounds obtained after passing through these filters were evaluated, including polar surface area (PSA), percentage absorption, CNS score, and receptor-ligand interactions. Finally, the compounds that survived all of the filtration steps were reported in the present study.^[23] The flowchart of the virtual screening step of lead identification is shown in Figure 2.

Absorption, distribution, metabolism, and excretion (ADME) analysis

In docking study, all of the synthesized compounds showed good stability and lower binding energy. The Qikprop program of Schrodinger was used to perform additional ADME prediction analysis on four chemicals from the mentioned library. The LigPrep output file of

four compounds was sent to the Qikprop tool for ADME predictions to discover drug-likeness features.^[24]

RESULTS AND DISCUSSION

Result of R-group enumeration

Novel compounds were generated by R-group enumeration tools. The compounds have been produced by substituting various groups on different positions of the core moiety. Only four positions were chosen for the generation of the library, each with a different functional group. The R-group was chosen based on data from our prior research paper. The graphical representation of all groups is shown in Figure 3. Only three features were chosen for the generation of the library with various chemical structures. For the generation of compounds library different hydrogen bond acceptor groups (25) and various hydrogen bond donor groups (10) were substituted on R1 position. R2 (25) substitution was used with electronic withdrawing groups. A total of 6250 compounds were produced.

Result of structure-based virtual screening

These compounds were screened further using a virtual screening procedure that included the Lipinski Rule of Five, which tested 5238 compounds. These compounds were subsequently screened using HTVS, and the top 10% of about 523 were chosen for the following investigation. The following phase was SP, where only the top 20 scoring compounds were maintained for the following

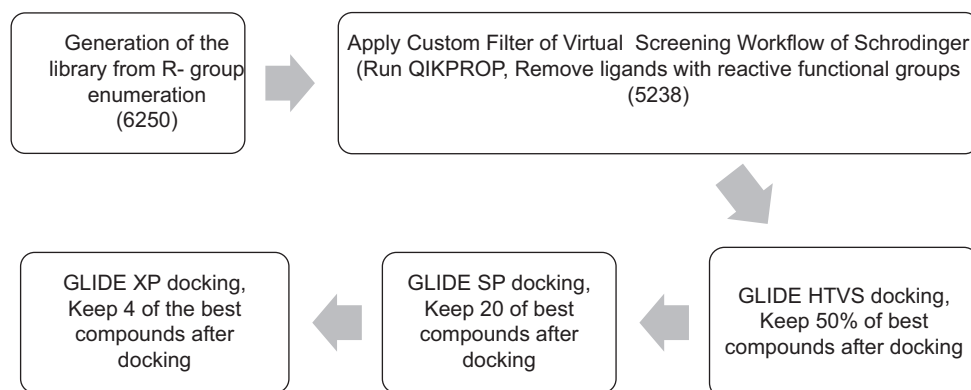


Figure 2: The flowchart shows various steps involve in the virtual screening study.

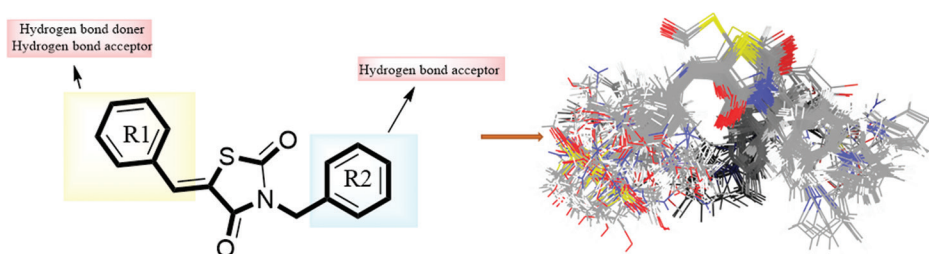


Figure 3: Features and position selected for R-group enumeration.

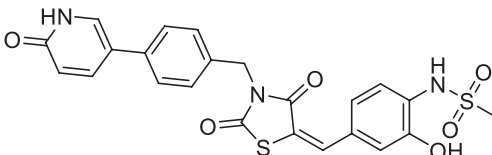
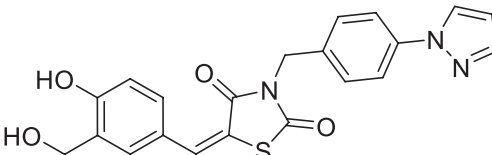
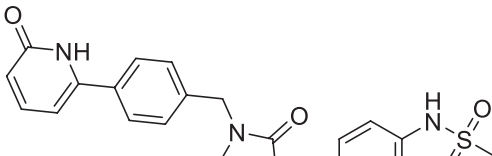
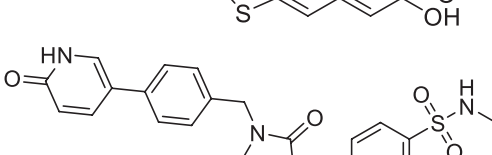
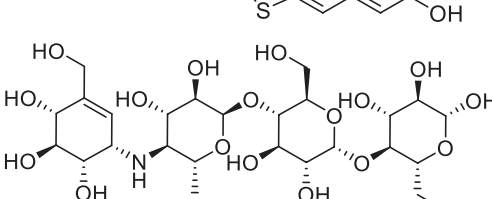
step. The final step involves XP docking, in which only four compounds were chosen based on docking scores and binding interactions.

Molecular docking

Docking's study found that the most profitable ligand-protein interactions are hydrogen bonds, pi-pi stacking, and hydrophobic interactions between virtual screening hits (VS hits) and active site amino acids. In addition,

strong hydrogen bond donor interactions with HIS 201, LYS 200, and THR 163 amino acids were detected for VS hits. The docking score of the cocrystallized ligand (acarbose) is -10.189 Kcal/mol. The docking score of VS hits ranged from -9.022 to -7.281 , showing stronger activity against the α -amylase enzyme. The docking score, interaction with amino acid, and predicate activity are shown in Table 1. The interaction maps of most score compound with the cocrystallized ligand are shown in Figure 4.

Table 1: The docking score of VS hits and standard drug with amino acid residues interaction

Compounds	Structure	Docking score	Interaction with amino acid residues
1		-9.022	HIS 201, LYS 200, and THR 163
2		-7.728	HIS 201
3		-7.399	HIS 201, LYS 200, ARG 195, and THR 163
4		-7.218	HIS 201, LYS 200, and THR 163
Acarbose		-10.189	GLU 233, LYS 200, ARG 195, HIE 305, ASP 300, and THR 163

VS hits: Virtual screening hits

Table 2: ADME of all VS hits generated by Qikprop

Compounds	MW	HBD	HBA	Log P	QPPCaco	QPlogBB	QPlogKhsa	% HOA	PSA
1	497.54	2	9.75	2.346	16.886	-3.043	0.112	62.654	167.818
2	407.443	2	6.45	3.461	231.862	-1.542	0.321	89.542	106.626
3	497.54	3	10.75	1.961	16.909	-3.119	-0.031	60.408	168.424
4	497.54	3	10.75	1.81	12.191	-3.242	-0.035	56.982	170.226

MW: Molecular weight of the molecule range between 130.0 and 725.0 Da. HBD: Hydrogen bond donor range between 0.0 and 6.0. HBA: Hydrogen bond acceptor range between 2.0 and 20.0. Log P: Log *P* range between 2.0 and 6.5. QPPCaco: QPPCaco range between <25 for poor to >500 for greater. QPlogBB: QPlogBB range between -3.0 and 1.2 . QPlogKhsa: QPlogKhsa range between -1.5 and 1.5 . % HOA: % human oral absorption. PSA: PSA ranges between 7.0 and 200.0, ADME: Absorption, distribution, metabolism, and excretion

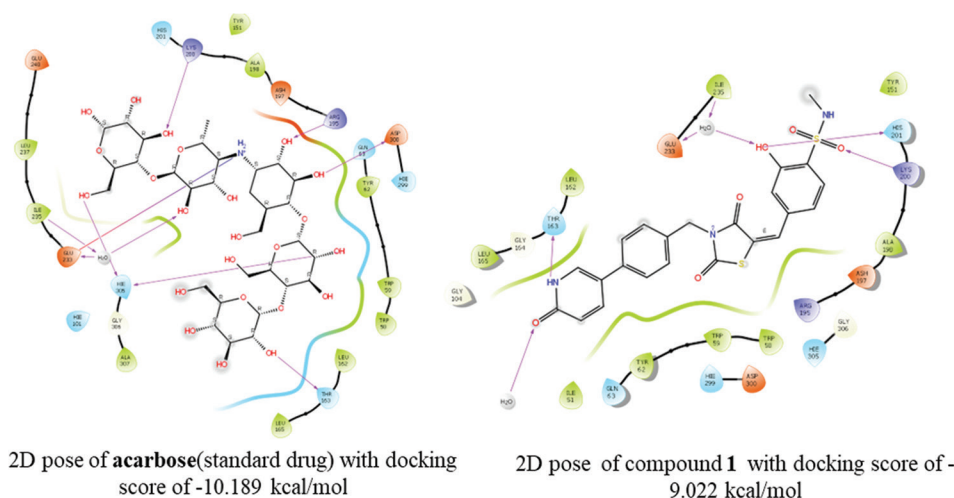


Figure 4: The two-dimensional-interaction maps of top-scored compound (1) with the cocrystallized ligand (acarbose) on α -amylase (Protein Data Bank ID-3BAY).

Prediction of ADME

The QikProp module was used to investigate all of the ADME parameters, and the findings are shown in Table 2. For the selection of compounds, the Lipinski rule of five was used. The current investigation determines human oral absorption (% HOA), prediction of binding to human serum albumin (QPlogKhsa), and Van der Waals surface area of polar nitrogen and oxygen atoms (PSA). Surprisingly, all of these predictions were within the permitted range.

CONCLUSION

α -amylase enzyme is crucial in the management of postprandial hyperglycemia. As a result, effective α -amylase inhibitors may be of substantial therapeutic value in type 2 diabetes. The findings of this study emphasize the essential binding properties of novel thiazolidine-2,4-dione compounds as α -amylase inhibitors. R-group enumeration investigation was carried out to generate novel thiazolidine-2,4-dione derivatives (6250 molecules). These derivatives are then subjected to virtual screening testing using the Lipinski rule of 5, HTVS, SP, and XP screening filters. After the XP docking process, only the top four molecules were chosen. The molecular docking research of VS hits revealed that compound 1 had an excellent binding score of -9.129 kcal/mole on the α -amylase enzyme (PDB ID-3BAY). The present study may be used to further develop novel anti-type 2 diabetes drugs.

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