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In silico docking and interaction analysis of KazMeI with α-glucosidase as a potential antidiabetic agent

Abstract. The present study focuses on the molecular docking and interaction analysis of the Kazcaine (1-(2-ethoxyethyl)-4-ethynyl-4-benzoyloxypiperidine) derivative KazMeI with the α-glucosidase enzyme (PDB ID: 5NN4), the principal therapeutic target in the treatment of type 2 diabetes mellitus. For crystal structure modeling of human lysosomal acid-alpha-glucosidase, GAA/N-acetyl-cysteine complex was utilized. The ligand structure was designed in ChemDraw, geometry-optimized using a Python-based MM2 molecular mechanics force field, and converted to PDBQT format in AutoDockTools v1.5.6. The protein structure was prepared by removing water molecules, adding polar hydrogens, and centering the docking grid at coordinates (–6.152, –33.636, 91.204 Å). Docking simulations performed in AutoDock4.2 using the Lamarckian Genetic Algorithm produced ten conformations with highly consistent binding orientations. The lowest binding free energy (ΔG) was –7.00 kcal/mol, corresponding to an inhibition constant (Ki) of 7.37 μM, indicating moderate yet biologically relevant affinity. Interaction analysis revealed that KazMeI forms hydrophobic and π–π stacking contacts with Leu286, Pro285, Leu291, and Trp613, and hydrogen bonding with Ser601 and Thr286, while the quaternary ammonium group interacts electrostatically with Arg600. These results confirm the predicted antidiabetic potential of the patented derivative KazMeI compound (Utility Model Patent No. 9796, Kazakhstan, 2024) and support its further development as a lead α-glucosidase inhibitor.
Keywords: Autodock, Pymol, ChemDraw, molecular docking, α-glucosidase, Kazcaine derivative, antidiabetic agents.

Introduction

Type 2 diabetes mellitus (T2DM) is a tenacious metabolic disorder that incidence exceeds 400 million globally and is anticipated to rank among the top causes of morbidity in the year 2030. The disorder is typified by hyperglycemia due to defective insulin secretion, insulin resistance, or both. Chronic postprandial hyperglycemia is the reason for the gravest long-term complication, such as cardiovascular problems, nephropathy, as well as neuropathy. Targeting of α-glucosidase, an indispensable catalyst for the cleavage of complex carbs into assimilable monosaccharides, is among the chief therapeutic methods used in curbing these problems. Slowing down glucose uptake in the small intestine through inhibition of α-glucosidase, consequently lowers postprandial blood glucose peaks [1]. The crystallographic structure of human lysosomal acid α-glucosidase in a complex with N-acetyl-cysteine (PDB ID: 5NN4) is used as a high-resolution guide for the rationale design of α-glucosidase inhibitors [2].

Among the therapeutic targets of extreme biomedical significance, α-glucosidase has gained significant interest. It plays an indispensable role in carbohydrate digestion by catalysing the hydrolysis of α-glucosidic linkages, and its inhibition was identified as an efficient measure for the regulation of postprandial hyperglycemia in type 2 diabetes mellitus [1]. Access to an impressive set of high-resolution crystallographic structures of α-glucosidase, for example, human lysosomal acid-α-glucosidase in complex with N-acetyl-cysteine [1], enabled the process of structure-based drug discovery. Current computational as well as experimental investigations reaffirm the fact that α-glucosidase continues to emerge as an established and potential therapeutic target for the discovery of novel antidiabetic agents [3].

Of exceptional interest are the piperidine derivatives as being a structurally diverse but highly pharmacologically relevant heterocyclic nitrogen-containing system widely applicable in the field of medicinal chemistry [4].

It has also been illustrated in the past the promise of nitrogen-containing heterocyclic scaffolds as biological entities. Specifically, Yu [5] announced the synthesis as well as biological profiling of 1,3,8-triazaspiro[4.5]decane-2,4-dione derivatives for the exhibition of potential myelostimulatory activity. These observations further underscore the pharmaceutical implication of heterocyclic entities, especially those possessing piperidine-like moieties, as multifaceted templates for lead scaffolds.

Among them, the Kazcaine (1-(2-ethoxyethyl)-4-ethynyl-4-benzoyloxypiperidine hydrochloride) and its analogues exhibited significant pharmacology as an anesthetic as well as for cardioprotection [6]. Utility Model Patent No. 9796 [7] reveals some of the newer analogues of the Kazcaine show unprecedented inhibitory activity where the reported values go up to 95% inhibition of α-glucosidase rendering the scaffold as an interesting lead for the development of antidiabetic agents.

To investigate this hypothesis, a rigorous in-silico docking and visualization analysis was carried out. The ligand was drafted in ChemDraw [8] and was optimized by applying a Python-coded MM2 type of molecular mechanics force field. The protein was taken from the RCSB Protein Data Bank, was cleaned from bad atoms by applying AutoDockTools v1.5.6, and was treated with AutoDock4.2 docking simulations using the Lamarckian Genetic Algorithm [9]. Structural analysis and electrostatic surface mapping were also conducted with MOE ([10] and PyMOL. It is an in-silico analysis that attempts to define the binding mode, interaction profile, and energetic parameters of the patented KazMeI (code name KazMeI) derivative with α-glucosidase, as theoretical justification for it being a potential new antidiabetic lead compound.

Object of the study. The object of the study is the interaction between the enzyme α-glucosidase and a biologically active Kazcaine and methyl iodide derivative – KazMeI, investigated by the use of the AutoDock software package.

Materials and methods

Ligand Preparation

Molecular structures of quaternary ammonium derivative of Kazcaine and methyl iodide (Figure 1) as alkylating agent in quaternization reaction were first depicted in ChemDraw 20.0 (PerkinElmer Informatics). The 2D drawings were then converted into 3D structures using Chem3D module and saved in the form of .mol files. Geometry optimization of structural geometry was carried out through a Python-based interface utilizing MM2 molecular mechanics force field in order to reduce steric strain by assuming stable conformers for purpose of docking. The ligands thus optimized were then further converted to PDBQT format through ADT (AutoDockTools) v1.5.6 by imposition of Gasteiger partial charges, combination of non-polar hydrogens, and specification of rotatable bonds.

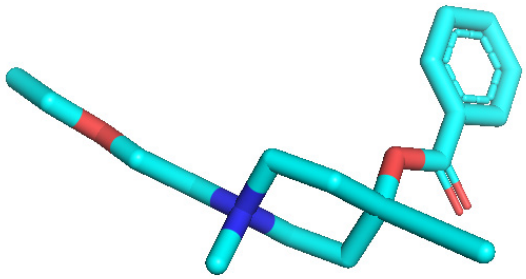

Figure 1 – Optimized 3D structure of KazMeI

Protein Preparation

The 3D structure of α-glucosidase (PDB ID: 5NN4) was downloaded from the RCSB Protein Data Bank (Figure 2).

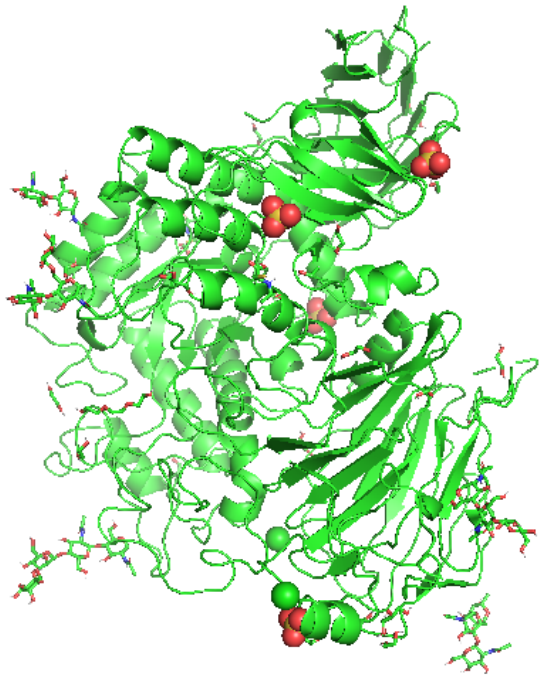

Figure 2 – Crystal structure of human lysosomal acid-alpha-glucosidase, GAA, in complex with N-acetyl-cysteine

Identification of the Binding Pocket and Electrostatic Surface

The active binding site for α-glucosidase was observed through the electrostatic surface potential and polar as well as nonpolar residue polar distributions. The three-dimensional structure of the enzyme (PDB code: 5NN4) was visualized and processed with PyMOL v2.5 and AutoDockTools or ADT. The pocket was delineated around the catalytic residues (Asp214, Glu276, Asp349, His600, and Arg600), ascertained through the AutoGrid mapping step of docking preparation, with their centers at coordinates (–6.152, –33.636, 91.204) Å.

To display the electrostatic features, the receptor surface was displayed in PyMOL with the use of the "coulomb" potential color scheme, in which red is used to define negatively charged sites, blue is used for positive potential, and green/white is used for neutral/hydrophobic surfaces (Figure 3). The figure indicated that the active site of α-glucosidase constitutes a mostly hydrophobic cavity that is lined by some charged residues, creating an environment of high energy for binding of quaternary ammonium derivatives of Kazcaine. The surface mapping also validated that the region of ligand binding is that of maximum electrostatic complementarity of the positive ammonium group of KazMeI with the negatively polarized residues Asp and Glu, stabilizing the ligand by way of ionic and hydrogen bond interaction.

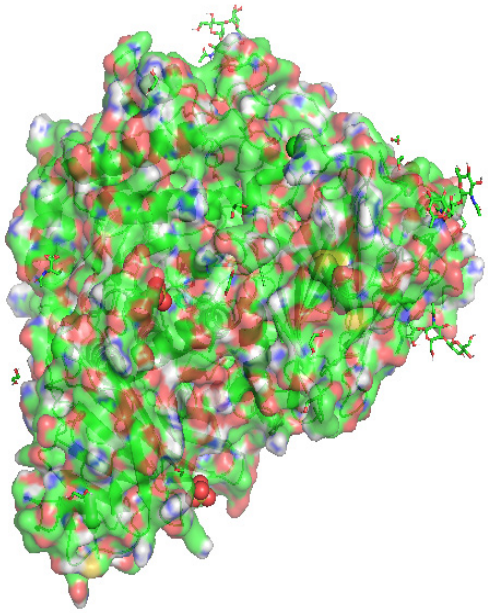

Figure 3 – Electrostatic surface mapping of crystal structure of human lysosomal acid-alpha-glucosidase, GAA, in complex with N-acetyl-cysteine

All water molecules and co-crystallized ligands were removed, while polar hydrogens and Kollman charges were added using *ADT*. The prepared receptor was saved as a PDBQT file for grid map generation and docking Figure 4).

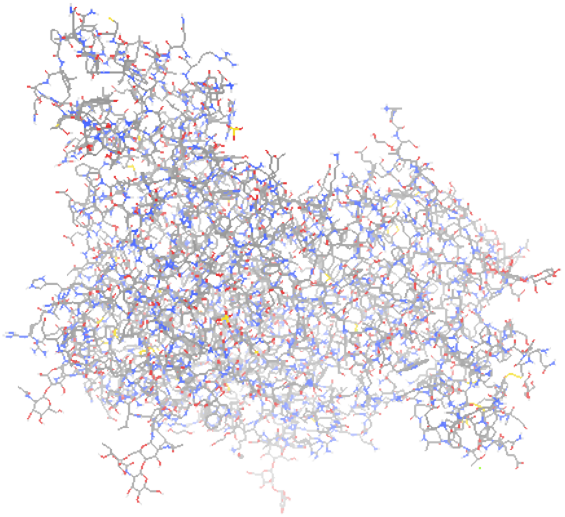

Figure 4 – PDBQT file representation of Crystal structure of human lysosomal acid-alpha-glucosidase

Grid Map Generation

Grid parameters were defined in order to encompass the catalytic pocket of dimensions 98 × 126 × 122 points with a spacing of 0.375 Å. The search box of the grid was placed at coordinates (–6.152, –33.636, 91.204) Å, including important active site residues Trp613, Leu286, Pro285, and Ser601. Grid maps for atom type A, C, OA, and N were computed by AutoGrid4, as well as electrostatic (.e.map) and desolvation ((.d.map) potential maps.

Docking Protocol

Docking simulations were carried out with AutoDock4.2 utilizing the Lamarckian Genetic Algorithm (LGA). The search settings were population of 150, maximum energy evaluations of 2.5×10^6, and 10 independent dockings for every ligand. The maximum generations were adjusted to 27,000, with mutation and crossover rates of 0.02 and 0.8, respectively. The torsional degrees of freedom (TORSDOF) for the ligand were adjusted to 7. The dockings log files (.dlg) were processed with ADT for retrieval of binding energies and inhibition constants (Ki). The optimal docking pose of every ligand was determined by the most adverse binding energy (ΔG) and population of the most significant clusters.

Visualization and Analysis

Top-ranked complexes were visualized and explored by means of PyMOL v2.5 (Schrödinger LLC). The receptor was shown in cartoon mode (gray), and ligands were colored in stick model by magenta (Figure 5). Hydrogen bonds were shown as blue dashed lines in a radius of cut-off of 3.2 Å and angle limit of 20°. Hydrophobic interaction was detected by nearness (Ø4.0 Å) to residues of non-polar character. Binding sites and their neighborhood amino acid residues were labeled for publication-ready images. All renderings were carried out in white background at 600 dpi by using the ray-tracing facility of PyMOL.

Results and Discussion

The quaternary ammonium derivative of KazMeI was successfully docked with α-glucosidase (PDB ID: 5NN4) by utilizing AutoDock4.2. Ten independent docking simulations were produced in all by utilizing the Lamarckian Genetic Algorithm, all of them converging into related binding orientations in the active pocket of the enzyme, implying an orderly and stable binding mode. Calculated binding free energies (ΔG) were in the range –5.94 to –7.00 kcal/mol, while estimated inhibition constants (Ki) ranged from 7.37 to 44.44 μM, in agreement with a moderate binding affinity characterizing non-covalent α

Of the ten conformations, the pose with the lowest energy (-ΔG = –7.00 kcal/mol, Ki = 7.37 μM) was chosen as the most likely bioactive shape. An analysis of docking indicated that KazMeI binds, in a compact, stable complex, in the hydrophobic cavity of the enzyme. The interaction energy of the pose was –9.09 kcal/mol, with contributions from van der Waals, hydrogen bonds, and desolvation energies (-7.45 kcal/mol), and electrostatics (-1.64 kcal/mol). The torsional energy penalty was much lower (\+2.09 kcal/mol), such that the ligand assumed its low-energy form with no appreciable structural strain (Table 1).

According to previously published AutoDock4.2 and MOE-based studies, the binding free energies (ΔG) of acarbose and miglitol toward α-glucosidase (PDB IDs: 3A4A, 5NN4) typically range from –6.2 to –8.0 kcal·mol^{-1}, corresponding to inhibition constants (Ki) between 2–20 μM [1,3]. In comparison, the Kazcaine-derived quaternary ammonium compound *KazMeI* exhibited a binding free energy of *–7.00 kcal·mol^{-1}* and an estimated *Ki of 7.37 μM*, which places it within the same energetic range as these standard inhibitors.

Visualization of the best-ranked complex (Figure 5) in PyMOL v2.5 verified that the KazMeI molecule was trapped in the active-site cleft of the enzyme with the help of some important residues – Pro285, Thr286, Leu286, Leu291, Ser601, Arg600, His612, and Trp613. The aromatic phenyl ring of the ligand engaged in π–π stacking contacts with Trp613, whereas the aliphatic side chain was stabilized by hydrophobic contacts with Leu291 and Pro285. The amide and ether oxygens of KazMeI were directed towards Ser601 and Thr286, establishing hydrogen bonds, supporting the stability of the complex additionally. The quaternary ammonium donor was directed near Arg600, implying potential cation–π or dipole–dipole contacts, that in many cases, were significant for substrate recognition in glycosidase catalysis.

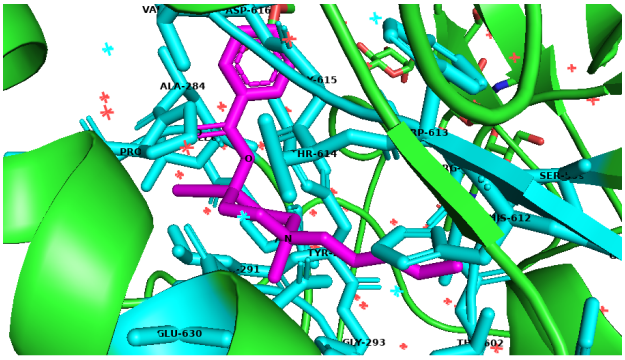

Figure 5 – Visualization of the best docking pose of ligand obtained from AutoDock4.2

Electrostatic surface mapping of the α-glucosidase (Figure 3) revealed that the active site constitutes an almost exclusively hydrophobic pocket lined with negatively charged residues (Asp and Glu), in turn creating an electrostatically complementary site for the positively charged ammonium ammonium group of KazMeI. This complementariness most probably gives rise to the strongbinding energy and strengthens ligand. The binding orientation predicted for KazMeI is similar close to that of some known reported α-glucosidase inhibitors, further substantiating the correctness of the pose that was predicted (Table 1).

Table 1 – Docking Results per GA Run

Run	ΔG (kcal/mol)	Ki (μM)	Intermolecular	vdW+Hbond+desolv	Electrostatic
1	-6.41	19.89	-8.5	-7.0	-1.51
2	-5.94	44.44	-8.03	-6.87	-1.15
3	-7.0	7.37	-9.09	-7.45	-1.64
4	-6.34	22.36	-8.43	-6.84	-1.6
5	-6.19	29.22	-8.27	-7.64	-0.63
6	-6.32	23.14	-8.41	-7.19	-1.22
7	-6.51	16.91	-8.6	-7.32	-1.27
8	-6.36	21.61	-8.45	-7.7	-0.75
9	-6.41	19.99	-8.5	-7.03	-1.47
10	-6.0	40.02	-8.09	-6.95	-1.14

The docking results show regular binding affinities in the 10 runs of GA, with estimated free energies between -5.94 to -7.00 kcal/mol. The lowest binding energy (–7.00 kcal/mol) was observed in conformation 3, corresponding to the highest affinity (Ki = 7.37 μM) and minimal RMS deviation (cRMS = 0.0). All conformations exhibited similar poses, suggesting stable ligand accommodation within the α-glucosidase binding pocket.

The cluster analysis identified six discernable conformational clusters, with the most populated and least energetic cluster exhibiting ΔG = -7.00 kcal/mol. The primary energetic contributions come from van der Waals and hydrogen bonding interaction, while electrostatic terms are moderate (-1.2 to -1.6 kcal/mol).

The quaternary KazMeI derivative iodide showed consistent and positive interaction profile in the molecular docking analysis against α-glucosidase, with binding free energies of –5.94 to –7.00 kcal/mol in ten independent runs of the Lamarckian Genetic Algorithm (LGA). The most prominent binding energy of –7.00 kcal/mol, corresponding to an estimated inhibition constant (Ki) of 7.37 μM, indicate moderate to good affinity of the KazMeI ligand for the active site of α-glucosidase. The value falls in the normal range of bioactive small molecules that display measurable inhibitory activities.

The interaction energy decomposition revealed that hydrogen bond and van der Waals, and contributions (-≈7.0 kcal/mol) predominate the ligand–receptor interaction, with modest contribution of electrostatic forces (-1.2 to –1.6 kcal/mol). The torsional free energy penalty of +2.09 kcal/mol, related to the ligand conformational flexibility, is rather low, signifying that the compound is capable of fitting well into the binding pocket of the enzyme with modest energetic penalty. These findings complement the amphiphilic character of the Kazcaine scaffold, whereby polar carbonyl and amide areas can form hydrogen bonds, and the area of the aromatics, as well as the alkyl regions, offers hydrophobic stabilization.

In general, the results of the docking indicate that the quaternary methylated Kazcaine derivative ((KazMeI) interacts effectively with α-glucosidase by associating with hydrophobic and hydrogen bonds contacts. The estimated binding affinity lends credence to the argument that structural alteration of Kazcaine with short alkyl side-chains would extend its interaction capacity with metabolic enzymes of potential involvement in the regulation of diabetes mellitus.

The docking parameters including the population size (150), the maximum energy evaluations (2.5×10^6), and the settings for the Lamarckian Genetic Algorithm were selected based on already proven studies [9, 10], where similar protocols generated the native ligand poses within an RMSD of ≤ 2 Å. Thus, the credibility of the present grid configuration and scoring function are methodologically equivalent to proven validated methods.

Conclusion

The molecular docking investigation of the quaternary ammonium Kazcaine derivative KazMeI (KazMeI) with α-glucosidase (PDB ID: 5NN4) revealed a consistent and energetically favorable interaction pattern. The lowest binding free energy (ΔG = –7.00 kcal/mol) and inhibition constant (Ki = 7.37 μM) indicate a moderate yet biologically meaningful affinity, consistent with known α-glucosidase inhibitors in the low micromolar range. The complex was

stabilized primarily by hydrophobic and π–π stacking interactions with Leu286, Pro285, Leu291, and Trp613, supplemented by hydrogen bonding with Ser601 and Thr286, and minor electrostatic stabilization involving Arg600.

These computational findings support the antidiabetic potential of the patented compound (Utility Model Patent No. 9796, Kazakhstan, 2024) and highlight KazMeI as a promising structural scaffold for the rational design of novel α-glucosidase inhibitors. Future studies involving analogues and in vitro enzymatic validation will further substantiate its pharmacological relevance.

Conflict of interest

The authors declare that they have no conflicts of interest.

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