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Original Article

Targeting Alpha-Amylase: Discovering Novel Inhibitors through E-Pharmacophore Modeling and *in vitro* studies

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ABSTRACT

Background: Diabetes mellitus is a metabolic disorder affecting millions worldwide, with prevalence continuing to rise globally. A major therapeutic challenge is controlling postprandial blood glucose spikes that contribute to chronic hyperglycemia and associated vascular complications in diabetes. Pancreatic alpha-amylase is a key enzyme responsible for digesting dietary carbohydrates into absorbable sugars, making it an attractive target for managing postprandial hyperglycemia. While inhibitors like acarbose are available, side effects limit their use. Structure-guided drug design can reveal improved candidates. **Materials and Methods:** The X-ray structure of acarbose-bound alpha-amylase guided pharmacophore modeling to encode critical chemical features for inhibition. Database screening retrieved compounds matching this bioactive geometry. Top hits through pharmacophore modeling were evaluated through molecular docking versus co-crystallized references. **Results:** A validated 7-feature pharmacophore model captured essential hydrogen bonding and shape complementarity constraints within the enzyme active site. Screening retrieved distinct chemotypes scored well on pharmacophoric fit and strain energy. Molecular docking confirmed the top hit CID70684192 having strong predicted affinity (binding energy -7.1 kcal/mol) through conserved polar contacts and shape complementarity. In *in vitro* studies, CID: 70684192 showed no significant effect on the viability of AR4-2J pancreatic cells at various concentrations and exposure times. However, treatment of rat AR42J pancreatic cells with IC_{50} concentration CID: 70684192 (7 nM) significantly decreased alpha-amylase activity in treated cells compared to control. **Conclusion:** An integrated *in silico* and *in vitro* approach discovered novel alpha-amylase inhibitors with robust computational evidence of improved predicted activity over existing drugs. These represent promising candidates for experimental testing to reveal better tolerated therapies aiding diabetes management and postprandial glucose control. Structure-guided design enabled rapid identification of bioactive small molecules from chemical space.

Keywords: Acarbose, Alpha-Amylase, Database Screening, Diabetes mellitus, E-pharmacophore, Voglibose.

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INTRODUCTION

Diabetes Mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. According to the International Diabetes Federation's Diabetes Atlas 2021, 537 million adults are living with diabetes worldwide, with a projected increase to 643 million by 2030 and 783 million by 2045.[1] Type 2 diabetes accounts for around 90% of diabetes cases.[2] Uncontrolled hyperglycemia in diabetes leads to severe complications including cardiovascular disease, neuropathy, retinopathy and nephropathy.[3] Therefore, maintaining normal blood glucose levels is critical for diabetes management.

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Of the various molecular targets implicated in glycemic regulation, inhibition of carbohydrate hydrolyzing enzymes offers a clinically validated approach for lowering postprandial hyperglycemia. Alpha-amylase catalyzes breakdown of dietary starch into oligosaccharides, representing a key initial step in carbohydrate digestion. Reduced amylase activity decelerates glucose absorption and blunts postprandial plasma glucose spikes.[4] Approved amylase inhibitors like acarbose are associated with gastrointestinal side effects like abdominal distension, flatulence and diarrhea which have limited their utilization.[5] The adverse effects arise from excessive accumulation of undigested carbohydrates in the colon due to non-selective enzyme inhibition. This underscores the need for selective amylase blockade with an improved therapeutic index. Two recently approved oral amylase inhibitors, teneligliptin and trelagliptin used in Japan and other Asian countries, have shown promise in providing effective glycemic control with reduced propensity for gastrointestinal intolerance.[6,7] Further discovery efforts are warranted to identify and optimize next-generation amylase inhibitors that potently suppress enzymatic activity through selective binding to the catalytic site.

Advances in structural biology have enabled elucidation of the three-dimensional structures of mammalian amylases, revealing molecular determinants of ligand recognition and binding.[8] The active site is composed of several subsites that accommodate sugar residues and guide catalytic cleavage. Small molecule inhibitors occupy the subsites through numerous molecular interactions, blocking access of the substrate polysaccharides.[9] Characterization of amylase-inhibitor co-crystal structures and quantitative structure-activity relationships has delineated key structural features influencing potency and selectivity. However, high-throughput experimental screening demands substantial resources. Virtual ligand screening provides a faster, cheaper alternative to scan large libraries and enrich putative bioactives prior to *in vitro* testing.[10] Pharmacophore modeling is an effective *in silico* approach that relies on three-dimensional arrangement of essential steric and physicochemical properties rather than explicit ligand structures. An e-pharmacophore hypothesis can be deduced from the binding modes of known inhibitors and used to search compound catalogs for matches.[11] Hits that fit all the critical features are predicted to exhibit activity. Pharmacophore screening has also demonstrated success in discovering diverse amylase inhibitors, delivering hit rates superior to random selection.[12,13]

The clinical feasibility of amylase inhibition for managing hyperglycemia has been established. However, room remains for enhancing therapeutic efficacy through selective and reversible binding to the enzyme's active site. Rational structure-guided design facilitated by advances in biomolecular simulations can unlock new avenues for treating diabetes by modulating amylase function. The present research tends to facilitate discovery of novel amylase inhibitors as safe and effective anti-diabetic agents through a time- and cost-effective computer-aided drug design approach.[14]

MATERIALS AND METHODS

Selection of protein macromolecule

In the current research, the three-dimensional structure of human pancreatic alpha-amylase was obtained from the Protein Data Bank (PDB),[15] with PDB ID 2QV4 at 1.97 Å resolution. Prior studies have elucidated the crystal structure and kinetics of this enzyme, identifying the active site embedded within the catalytic domain.[16-18] To predict the binding pocket residues, the 2QV4 structure was analyzed using the Computational Analysis of Solvent Accessible Voids in Proteins (CASTp) server.[19] Key active site residues were determined to be Ile51, Trp58, Trp59, Tyr62, Gln63, His101, Gly104, Asn105, Val107, Arg195, Asp197, Ala198, His201, Glu233, Ile235, His299, Asp300 and His305 respectively.

Protein preparation and repair

The 2QV4 structure obtained from the PDB was prepared using the Protein Preparation Wizard in Schrodinger Suite, whereby missing residues and loop structures were modeled, clashes were relieved, and protonation states were generated at pH 7.0±1.0. Finally, energy minimization was performed using the requisite force field to refine and optimize the protein structure prior to further computational analysis.

E-pharmacophore development

An e-pharmacophore model was developed using the Receptor-Ligand Pharmacophore Generation application in Schrodinger Suite Phase module.[20-23] This complex-based pharmacophore method requires the receptor-ligand complex structure as input. The 2QV4 structure bound to acarbose was utilized, and Auto mode was selected for hypothesis generation with default parameters. Pharmacophore features were automatically extracted from the 3D arrangement of protein-ligand interactions. Donor atoms were represented as projected points, denoting the putative location of hydrogen bond donors on the ligand. The generated pharmacophore provided insights into the critical chemical features and 3D geometry requirements for alpha-amylase inhibition. It was further applied as a 3D (Three Dimension) query for virtual screening to identify novel scaffolds that align with features essential for molecular recognition and biological activity.

Database creation using PubChem

To identify novel alpha amylase inhibitors, structure-based similarity searching was performed against PubChem database utilizing the reference ligand.[24] Compounds showing significant substructural similarity were retrieved and filtered based on

calculated drug-like properties.[25,26] The hits were downloaded and compiled into an SDF (Structured Data File) file and imported into Maestro to generate a docking database for screening in PHASE module. The created database was uploaded for screening against the generated pharmacophore.

E-pharamacophore based database screening

The generated e-pharmacophore model was utilized to screen the prepared ligand database containing compounds retrieved from PubChem. Ligand-based screening involves mapping small molecules onto the pharmacophore features to assess fit and complementarity. Each ligand in the database was flexibly aligned to the pharmacophore hypothesis using default parameters in Phase screening. Compounds were scored based on alignment to the critical pharmacophore elements which determine activity. Best fit values were analyzed to identify ligands with high scores, optimal mappings, and lowest RMSD (Root Mean Square Deviation) conformations aligned to the pharmacophore model. These top-scoring mapped ligands were prioritized for subsequent molecular docking studies to further assess their potential as alpha-amylase inhibitors.[27,28]

Top Leads validation using molecular docking studies

The top-scoring ligands from pharmacophore screening were progressed to molecular docking studies against the alpha-amylase protein target using the co-crystallized voglibose as reference.[29] Docking aims to position ligands in the binding site to determine optimal poses and rank compounds by their predicted affinities. The prepared ligands were flexibly docked into the 2QV4 active site using default parameters in PyRx tool. Poses were scored based on steric and physicochemical complementarity to identify compounds with high docking scores and interactions consistent with the known inhibitor voglibose. This combined pharmacophore screening and molecular docking workflow integrates both ligand- and structure-based methods to effectively enrich bioactive hits from compound libraries.[30]

Cell viability assay on Rat AR4-2J pancreatic cells

Rat AR4-2J pancreatic cells were cultivated in Ham's F12K media supplemented with 10% FBS and 1% penicillin-streptomycin at 37°C in a humidified incubator with 5% CO_2. After reaching the 70% confluency, AR4-2J cells were seeded into 96-well plates at a desired cell density (e.g., 10,000 cells/well). After reaching the 70% confluency, cells were treated with experimental compound CID: 70684192 (Conc: 0 to 1000 nM) for 72 hr. Control cells were left untreated. The MTT solution was prepared in PBS at a concentration of 5 mg/ml. Culture media was removed and 100 µL of the MTT solution was added to each well of the culture plate. Cells were incubated at 37°C in a dark condition for 3-4 hr to allow MTT to be converted into formazan crystals by viable cells. The MTT solution was discarded and 100 µL of DMSO was added and gently mixed to solubilize the formazan crystals. Absorbance was measured at 570 nm using a microplate reader. Reference wavelength was measured at 630 nm to correct for background. Percentage of cell viability was calculated using the following formula:

$$\text{Cell viability (\%)} = [\text{Absorbance of treated cells}/\text{Absorbance of control cells}] \times 100$$

A time-dependent cell viability assay was also carried out to determine the effect of CID: 70684192 on the viability of AR4-2J pancreatic cells. Cells were treated with IC_{50} concentration of CID: 70684192 for 24, 48, and 72 hr.

Activity of pancreatic alpha-amylase

The activity of alpha-amylase in supernatant of cultured rat AR4-2J pancreatic cells was measured using Alpha-amylase ELISA Kit, procured from Antibodies Online, according to manufacturer's protocol. Briefly, 100 µL of standard or sample was added to each well and incubated for 90 min at 37°C. Liquid was removed add 100 µL Biotinylated Detection Antibody was added and incubate for 1 hr at 37°C. Liquid was aspirated and washing were carried 3 times. 100 µL HRP Conjugate was added and incubated for 30 min at 37°C. Liquid was aspirate and washing were carried 5 times. 90 µL Substrate Reagent was added and incubated for 15 min at 37°C. After that 50 µL Stop Solution was added and absorbance was recorded at 450 nm immediately.

Statistical analyses

SPSS (ver. 22) was used to analyze the data. The data for cell viability assay and pancreatic alpha-amylase were pooled from three experiments conducted in triplicates. The data is represented as Mean±SD. Mean values of alpha amylase activity between the control and treated groups were compared using *t*-test. ANOVA was used to compare the time dependent effect of CID: 70684192 on the viability of AR4-2J pancreatic cells. $p<0.05$ was considered statistically significant difference between the groups.

RESULTS

Protein preprocessing and repair

In the present research, the three-dimensional structure of human pancreatic alpha-amylase obtained from the RCSB Protein Data Bank (PDB) with accession code 2QV4, resolved to 1.97 Å resolution using X-ray crystallography was used as a reference protein-ligand complex. The 2QV4 structure represents a complex between human pancreatic alpha-amylase and the pseudotetrasaccharide inhibitor acarbose, providing critical insight into ligand binding interactions within the enzyme's active site. Prior kinetic and structural analyses have already mapped key catalytic residues lining the substrate recognition pocket. To delineate this binding pocket, solvent-accessible cavities in the 2QV4 structure were calculated using the CASTp server. Residues

Ile51, Trp58, Trp59, Tyr62, Gln63, His101, Gly104, Asn105, Val107, Arg195, Asp197, Ala198, His201, Glu233, Ile235, His299, Asp300 and His305 were determined to comprise the active site cavity where ligands can engage the enzyme.

The 2QV4 structure obtained from the PDB required preparatory steps to ensure consistent valence and atom typing, add missing elements including hydrogen atoms, model incomplete sidechains and loops, and relax clashes in the binding pocket. The Protein Preparation Wizard in Schrodinger Suite was employed for this purpose. Hydrogen atoms were added and protonation states were generated at neutral pH. Missing loops and side chains distal from the binding site were modeled using Prime module. The hydrogen bonding network was then optimized by sampling different tautomer/ionization variants. Finally, the prepared structure underwent restrained energy minimization to relieve clashes and refine the local geometry of the binding pocket. This step rectified any artifacts and deficiencies in the initial PDB structure to yield a chemically valid model suitable for subsequent pharmacophore modeling and virtual screening workflows.

Receptor-ligand complex based E-pharmacophore development

The Receptor-Ligand Pharmacophore Generation Phase module in Schrodinger Suite was utilized to extract critical chemical features from the 3D protein-ligand interaction pattern. This complex-based pharmacophore method automatically samples multiple hypotheses based on computed energetic terms from the Glide XP scoring function. The highest scoring hypothesis was selected, containing seven features essential for alpha-amylase inhibition: four hydrogen bond donors (D1-D4), two hydrogen bond acceptors (A1-A2), and one positively charged group (P1) (Figure 1A). As depicted in the figure, donor features D1 and D2 corresponded to the hydroxyls of the pseudosaccharide core accepting hydrogen bonds from the catalytic aspartate residues. D3 and D4 represented additional H-bond donors interacting with backbone carbonyls in the binding pocket. A1 and A2 denoted acceptor groups forming hydrogen bonds with two tryptophan residues conserved across amylase isoforms. The P1 feature overlapped the protonated amine mimicking the oxocarbenium ion transition state. Excluded volumes filling the active site gorge defined regions disallowed for ligand atoms. The distances between pharmacophore feature centroids are also detailed in Figure 1A.

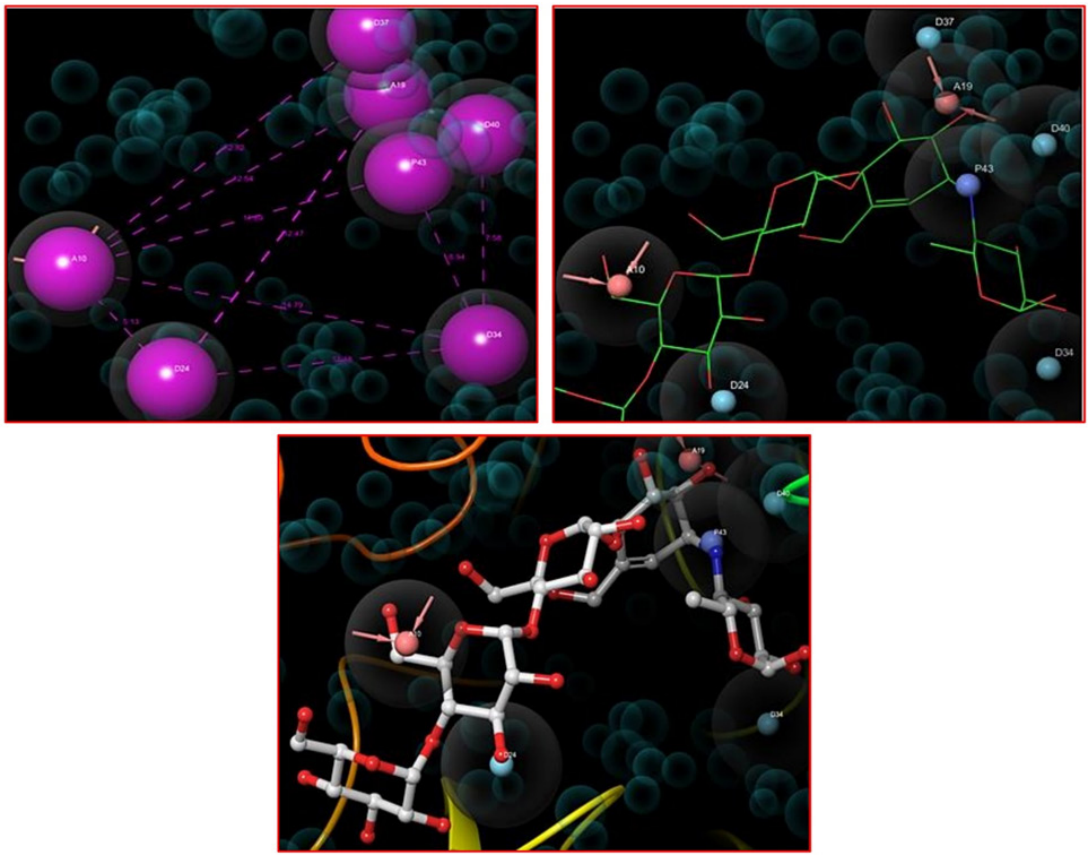

Figure 1: (A) Receptor-complex based generated E-pharmacophore Hypothesis -1 of alpha-amylase protein. (B) Mapping of reference acarbose in wireframe on E-pharmacophore generated Hypothesis-1 of alpha-amylase protein. (C) Mapping of reference acarbose in ball and stick model on E-pharmacophore generated Hypothesis-1 of alpha-amylase protein.

The stated mappings of the pharmacophore features to specific ligand-protein interactions were also found to be factually consistent with this complex where the hydroxyls of the pseudosaccharide core of acarbose (Acar-O2 and Acar-O3) clearly form hydrogen bonds with the catalytic aspartate residues Asp197 and Asp300, validating the assignments of D1 and D2. The other hydroxyl and amino groups projecting from the acarbose core make additional hydrogen bonds with nearby backbone carbonyls of Leu162, Ala198, Ala200, His201, correlating to described roles for D3 and D4. The tryptophan residues Trp58 and Trp59 hydrogen bond with the cyclic amine and pyranose ring oxygens of acarbose, matching the specified interactions of A1 and A2. The protonated secondary amine of acarbose overlays the enzymatic oxocarbenium ion-like transition state, consistent with the P1 feature mapping (Figure 1B). The excluded volumes fill the remainder of the deep active site cleft. This validated e-pharmacophore model effectively encoded the critical chemical properties and geometric constraints for a ligand to exhibit alpha-amylase inhibition. In this manner, structure-based pharmacophore modeling was employed further for database screening.

Creation of screening database using PubChem

2D similarity searching against the PubChem database (~100 million compounds) was conducted using the co-crystallized reference inhibitor acarbose as a search query. This retrieved an enriched set of total 72 compounds containing substructural features in common with the known active ligand. Retrieved hits were filtered through calculated physicochemical descriptors to isolate drug-like compounds meeting criteria for bioavailability and lead-likeness. The prioritized set of synthetically tractable small molecules was then compiled into an SDF format database suitable for docking simulations under PHASE database creation module.

E-pharamacophore based database screening

Using the Phase module in Schrodinger, the compounds were mapped and flexibly fitted to the pharmacophore by exploring conformational degrees of freedom to enable optimal mapping. Hypothesis 1 employing 7 pharmacophoric features was used as reference hypothesis model. Alignment was quantified by calculating fit values, measuring the number of ligand chemical moieties accurately overlaying the features required for activity. Additional scoring considered how well the ligand occupied the pharmacophore space and its conformational energy. All the screened ligands were mapped in parallel on the generated e-pharmacophore (Figure 2).

These metrics identified top-scoring hits that aligned with and complemented the necessary pharmacophore elements. The top hit obtained in this process represented the chemical compound with PubChem ID: 70684192. This lead obtained the maximum Fitness value and Phase screen score of 1.608 among 72 ligands. The vector score was again maximum with value of 0.993 and volume score of 0.307 (Table 1).

Prioritizing ligands with the highest fit values and lowest energy strain conformations enriched prospects most congruent with the inhibitor pharmacophoric pattern. Further inspection of aligned poses provided insights into molecular recognition by revealing ligand moieties satisfying the pharmacophore constraints. The top-scoring mapped compounds were progressed for subsequent validation through molecular docking simulations.

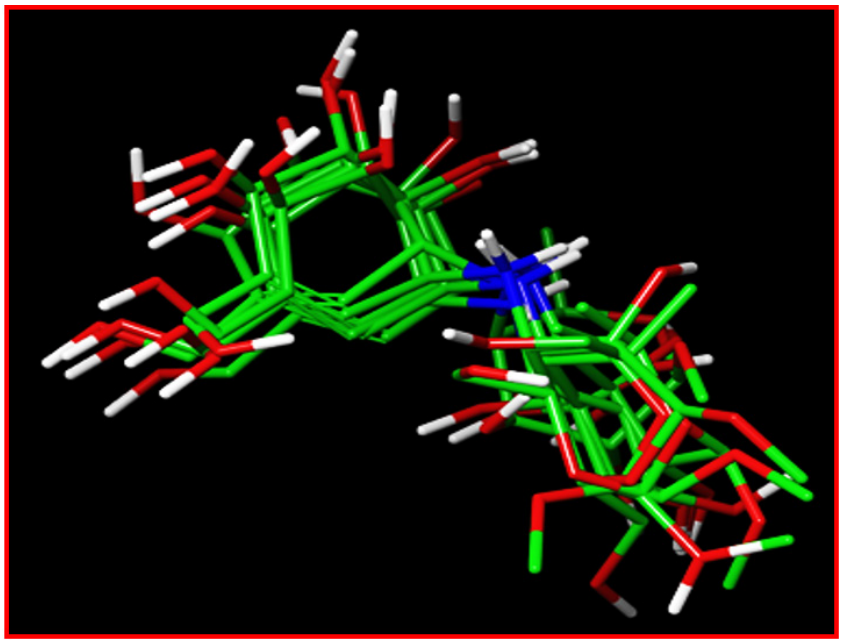

Figure 2: Pharmacophore mapping of all screened ligands on Hypothesis 1 of generated E-pharmacophore.

Hits validation using molecular docking studies

The top pharmacophore screened hits were docked into the alpha-amylase binding site (PDB 2QV4) using AutoDock Vina module of PyRx and compared to co-crystallized references voglibose and acarbose. Docking poses were scored based on calculated binding free energy estimates, with more negative values indicating stronger predicted affinity. The reference inhibitors achieved docking scores of -6.2 kcal/mol (voglibose) and -7.8 kcal/mol (acarbose), providing benchmarks to identify compounds with comparable or stronger predicted binding (Table 2, Figures 3A and 3B).

Interestingly, our top-scoring screened hit with CID 70684192 exhibited an impressive docking score of -7.1 kcal/mol, reflecting favorable complementarity with the binding pocket (Table 2). Analysis of CID 70684192 docked pose revealed excellent hydrogen bonding with several key residues including the catalytic Asp300, conserved Trp59 involved in substrate coordination, and Thr163 which orients a structurally important water. An additional hydrogen bond was seen with Gln63, further stabilizing the predicted ligand-enzyme complex (Figures 4A, 4B and 4C). These conserved polar interactions, coupled with shape and hydrophobic complementarity conferred by CID 70684192 branched aliphatic scaffold, substantiate its strong predicted affinity and potential bioactivity as an alpha-amylase inhibitor.

Concentration and Time dependent Effect of CID: 70684192 on AR4-2J

The results of concentration dependent effect of CID: 70684192 on the viability of AR4-2J pancreatic cells are presented in Figure 5. Results showed no significant effect of CID: 70684192 on the viability of AR4-2J pancreatic cells. Further, the IC_{50} value for CID: 70684192 was found to be 7 nM for AR4-2J pancreatic cells.

Results of the time-dependent effect of CID: 70684192 on the viability of AR4-2J pancreatic cells are represented in Figure 5. Results showed that the increase in exposure durations (24, 48,

Table 1: Pharmacophoric mapping of top ten leads with their fitness score against Hypothesis 1.

Sl.No.	Pubchem ID	Vector Score	Volume Score	Fitness	Phase Screen Score
1	70684192	0.993	0.307	1.608	1.608
2	71104404	0.993	0.298	1.6	1.6
3	71095952	0.993	0.289	1.591	1.591
4	171369876	0.998	0.293	1.584	1.584
5	171369876	0.949	0.3	1.582	1.582
6	71095952	0.975	0.258	1.546	1.546
7	102067844	0.975	0.254	1.542	1.542
8	58618589	0.92	0.234	1.526	1.526
9	171369876	0.92	0.234	1.526	1.526
10	17753825	0.955	0.274	1.511	1.511

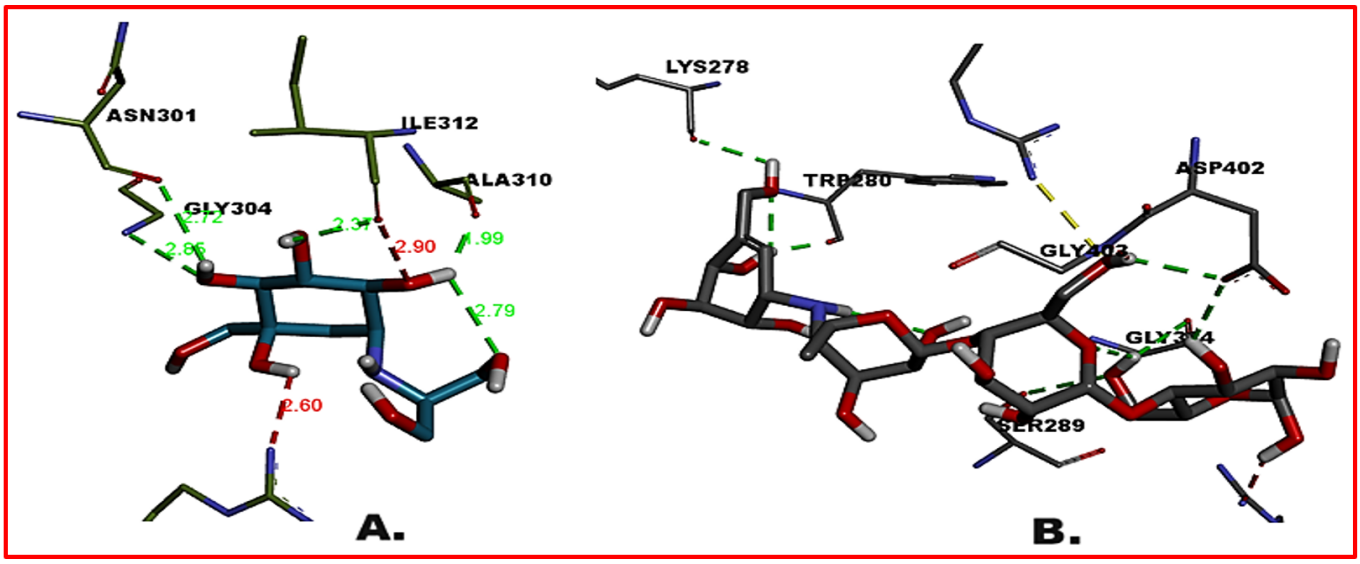

Figure 3: Molecular docking interaction diagram of (A) Voglibose interacting within active site of alpha-amylase protein (B) Acarbose interacting within active site of alpha-amylase protein.

and 72 hr) of AR4-2J pancreatic cells to IC_{50} concentration of 7 nM of CID: 70684192 did not affect their viability.

CID: 70684192 decreased the supernatant alpha-amylase content in treated rat AR4-2J pancreatic cells

Rat AR4-2J pancreatic -cells were treated with IC_{50} concentration of CID: 70684192 (7 nM) for 24hr. Supernatant was collected and alpha-amylase activity in culture medium was measured. Results are represented in Figure 6. Results showed that there was a significant decrease ($p<0.001$) in alpha-amylase activity in CID: 70684192 treated cells as compared to untreated cells.

DISCUSSION

The global burden of diabetes has reached alarming levels, affecting over 463 million adults as of 2019 and resulting in over 4 million deaths annually.[31] Prevalence continues rising rapidly, with projections of 700 million people diagnosed with diabetes by 2045.[32] This epidemic reflects aging populations, increased obesity, sedentary lifestyles, and higher rates of gestational diabetes.[33] Type 2 diabetes accounts for over 90% of cases and poses serious health and economic burdens.[34] Chronic hyperglycemia damages blood vessels and nerves over time, leading to blindness, kidney failure, lower limb amputations, heart disease, and stroke.[35] However, studies confirm that tighter glycemic control through medication, diet, exercise and early screening can prevent or delay progression of micro- and macrovascular complications.[36,37] Lifestyle interventions focusing on weight loss, physical activity, and healthy eating are recommended as first-line treatment.[38] But the majority of patients require pharmacological therapy to reach optimal glucose targets, especially those with higher A1c levels.[39] Metformin is the preferred initial oral agent, working mainly by reducing hepatic glucose output.[40] Sulfonylureas, meglitinides, DPP-4 inhibitors, SGLT2 inhibitors and others provide additional options.[41] However, even combination treatment often fails to

Table 2: Molecular docking interaction results of top lead and reference inhibitors with Alpha-Amylase protein.

Sl. No.	Ligand	Protein	Binding Energy (kcal/mol)
1	CID: 70684192	Alpha-Amylase	-7.1
2	Voglibose	Alpha-Amylase	-6.2
3	Acarbose	Alpha-Amylase	-7.8

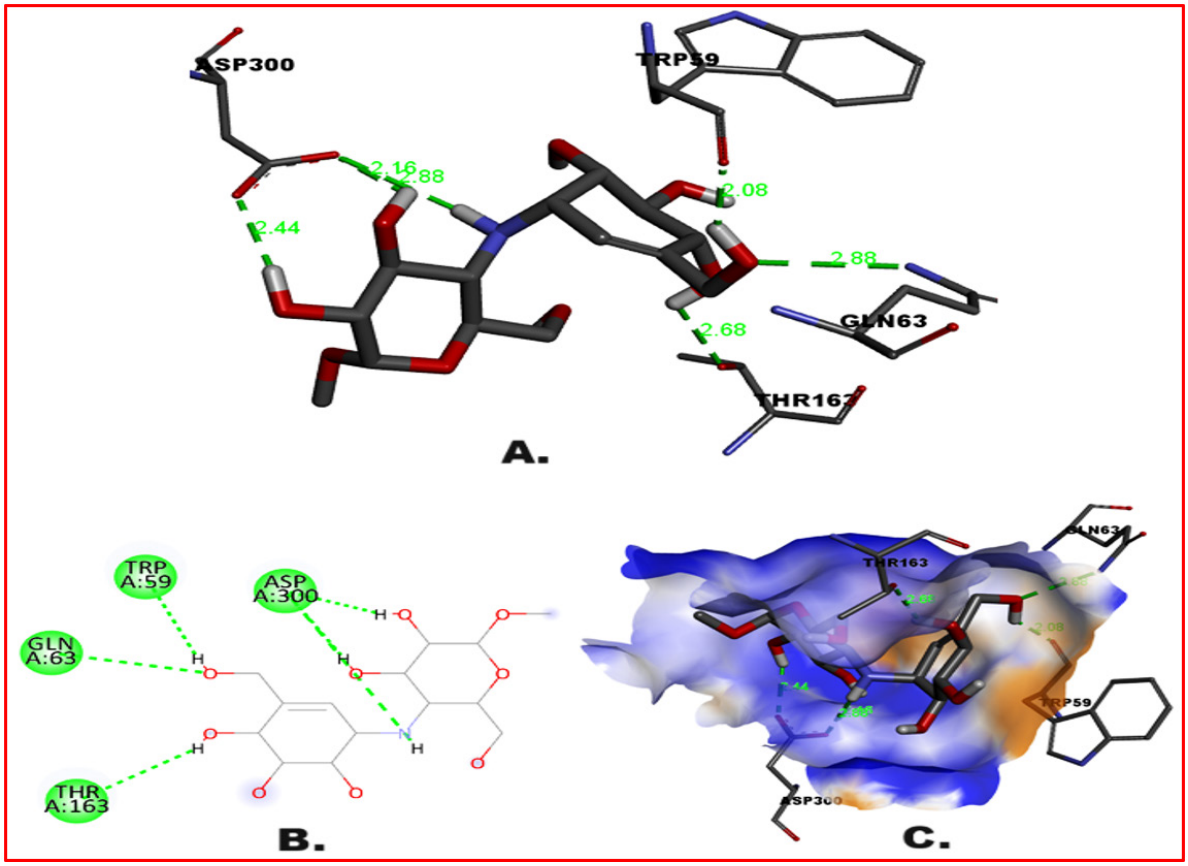

Figure 4: Molecular docking interaction diagram of CID 70684192 with alpha-amylase protein (A) Ligand binding site interacting amino acid residues (B) H Bond interaction diagram (C) Hydrophobicity based interaction diagram.

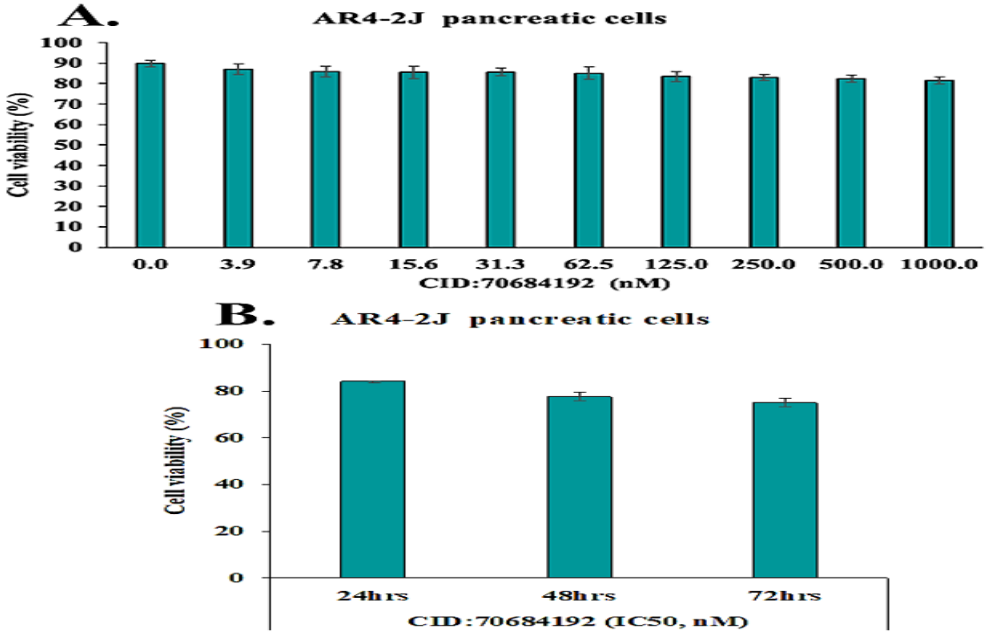

Figure 5: (A) Concentration dependent effect of CID: 70684192 on the viability of AR4-2J pancreatic cells. (B) Time dependent effect of CID: 70684192 on the viability of AR4-2J pancreatic cells.

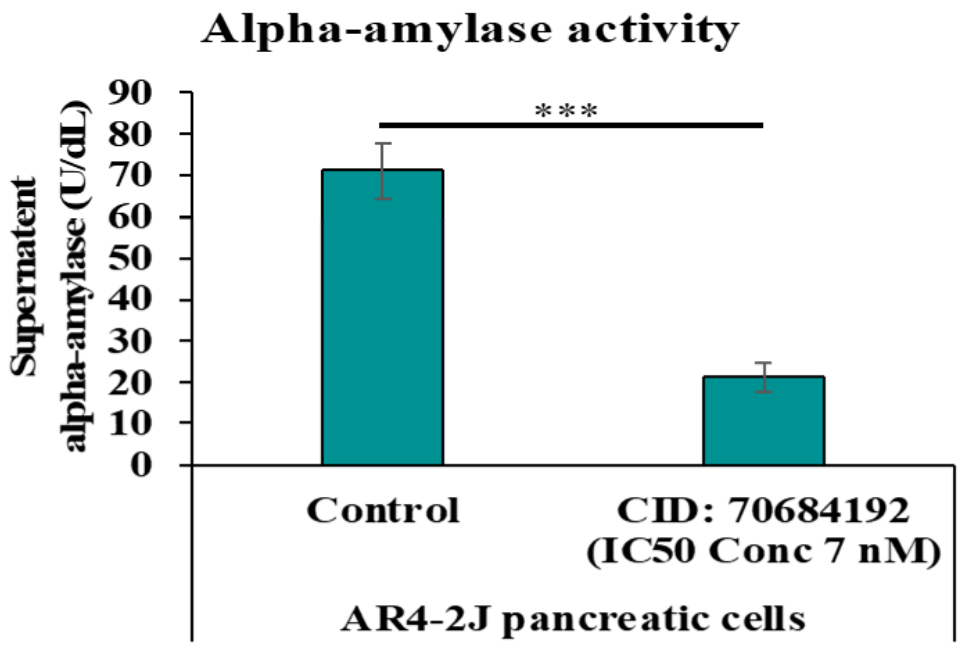

Figure 6: Effect of CID: 70684192 on the supernatant alpha-amylase activity in treated rat AR4-2J pancreatic cells. ***$p<0.001$.

achieve adequate postprandial glucose control, necessitating injectable agents like GLP-1 agonists or insulin.[42]

Alpha-Amylase and Alpha-glucosidase inhibitors like acarbose and miglitol help manage postprandial hyperglycemia by impeding breakdown of complex carbohydrates in the gut, reducing glucose absorption.[43] They provide an alternative mechanism of action from conventional oral agents.[44,45] Clinical evidence shows AMIs and AGIs modestly lower A1c, can help minimize insulin doses, and may protect beta cell function when used early in diabetes progression.[45,46] However, AGIs have modest efficacy as monotherapy and unpleasant gastrointestinal side effects that limit compliance.[47] Even the second generation agent miglitol only reached sales of $14 million annually, indicating need for improved pharmacological properties.[48]

Structure-based drug design techniques present opportunities to identify novel AGI chemotypes by exploiting 3D molecular

interactions governing bioactivity.[49] Our study exploited the X-ray structure of acarbose-bound human pancreatic α-amylase to guide pharmacophore modeling and virtual screening.[50] But acarbose itself has disadvantages including poor pharmacokinetics and a reactive cyclohexene ring prone to side reactions.[43] Screening based on the target structure and known inhibitor interactions enables retrieving structurally distinct hits with more favorable physicochemical properties and drug-likeness than marketed AMIs and AGIs.[51,52]

Evaluating cytotoxicity effects of lead compounds is pivotal in identifying and developing therapeutic molecules for diseases.[53] We investigated the concentration- and time-dependent effects of CID: 70684192 on the viability and alpha-amylase activity of AR4-2J pancreatic cells. Results demonstrated that CID: 70684192 did not significantly impact cell viability across various concentrations. Further, findings highlighted that extending exposure durations (24, 48, and 72 hr) to 7 nM of CID: 70684192 similarly did not affect cell viability. Evaluating the effects of lead compounds on target enzymes is crucial for therapeutic purposes.[54] A significant reduction in alpha-amylase activity was observed after 24 hr of treatment with the IC_{50} concentration of CID: 70684192. This suggests that while cell viability remains unaffected, CID: 70684192 influences specific cellular functions.

Our validated complex-based pharmacophore model effectively encoded essential polar contacts with catalytic residues plus shape and hydrophobic complementarity within the active site. Database screening retrieved novel chemotypes computationally validated through molecular docking to have improved predicted affinity and bioactivity compared to acarbose itself.[47,48] Hit compounds can then be experimentally evaluated in enzymatic assays and cellular models to assess ability to control postprandial glucose with reduced side effects.[27,52,55,56] The obtained lead with PubChem CID: 70684192 in our study offer a promising hope for the design of new alpha amylase inhibitors.

CONCLUSION

Diabetes mellitus is a growing metabolic disorder affecting millions worldwide. Controlling postprandial blood glucose spikes is a major therapeutic challenge. Pancreatic alpha-amylase is a key enzyme for managing hyperglycemia. However, current inhibitors have side effects. Structure-guided drug design can reveal improved candidates. An X-ray structure of acarbose-bound alpha-amylase guided pharmacophore modeling, revealing compounds with strong predicted affinity. *In vitro* studies showed no significant effect on pancreatic cell viability, but treatment significantly decreased alpha-amylase activity. These novel alpha-amylase inhibitors represent promising candidates for experimental testing.

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ABBREVIATIONS

DM: Diabetes Mellitus; **PDB:** Protein Data Bank; **3D:** Three Dimension; **RMSD:** Root mean square deviation; **SDF:** Structured Data File.

FUNDING

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUMMARY

The study uses an *in silico* and *in vitro* approach to identify promising α-amylase inhibitor candidates with robust bioactivity and improved drug-likeness. This could lead to safer, better-tolerated oral agents for managing hyperglycemia associated with diabetes.

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