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ORIGINAL ARTICLE / ÖZGÜN MAKALE

TOWARDS UNDERSTANDING NATURAL ALPHA-GLUCOSIDASE INHIBITORS: A COMPUTATIONAL STUDY

DOĞAL ALFA-GLUKOSİDAZ İNHİBİTÖRLERİNİ ANLAMAYA DOĞRU: HESAPLAMALI BİR ÇALIŞMA

Muhammed Tilahun MUHAMMED[1]*, Nesli AKSOY[1], Aybüke KIRILMAZ[1], Enise TÜRKMEN[1]

[1]Suleyman Demirel University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Isparta, 32260, Türkiye

ABSTRACT

Objective: *Diabetes mellitus is a metabolic disorder affecting hundreds of millions of people around the world. It is characterized by hyperglycemia caused by impaired glucose homeostasis that results from insufficient insulin production or insulin resistance. There are clinically available α-glucosidase inhibitor drugs that are used to decrease postprandial blood glucose level. However, these drugs have side effects that necessitated the discovery of new α-glucosidase inhibitors with less side effects and high potency. The interest in the use of natural products to deal with diabetes has been increasing. Therefore, the potential of natural α-glucosidase inhibitors to inhibit the enzyme was investigated through computational methods.*

Material and Method: *The binding potential of selected natural α-glucosidase inhibitors was investigated through molecular docking. Thereafter, the stability of the complexes with the highest binding potential were assessed through molecular dynamics (MD) simulation.*

Result and Discussion: *The molecular docking demonstrated that compound 2 had better binding potential than the standard drug, acarbose. Compound 7 had comparable binding potential to the standard drug. Furthermore, all the tested compounds exhibited a reasonable binding potential towards the enzyme but were weaker than the standard drug. The MD simulation demonstrated that compounds 2 and 7 gave complexes with similar stability to the standard drug. The overall computational results revealed that the natural inhibitors investigated had the ability to bind to the enzyme and formed stable complexes. Therefore, these compounds could be potential α-glucosidase inhibitors for clinical use. For this reason, further in vitro investigations on compounds with the highest binding potential is recommended.*

Keywords: *Diabetes, α-glucosidase, MD simulation, molecular docking, natural inhibitors*

ÖZ

Amaç: *Şeker hastalığı dünya çapında yüz milyonlarca insanı etkileyen metabolik bir hastalıktır. Hastalık yetersiz insülin üretimi veya insülin direncinden kaynaklanan bozulmuş glukoz homeostazisinin neden olduğu hiperglisemi ile karakterizedir. Yemek sonrası kan şekeri seviyesini düşürmek amacıyla klinikte kullanılan α-glukosidaz inhibitörü ilaçlar bulunmaktadır. Ancak bu ilaçların yan etkileri olduğundan daha az yan etkili ve yüksek etkinliği olan yeni α-glukosidaz inhibitörlerinin keşfedilmesine ihtiyaç duyulmaktadır. Şeker hastalığıyla mücadelede doğal kaynaklı ürünlerin kullanımına olan ilgi giderek artmaktadır. Bu nedenle doğal kaynaklı α-*

* **Corresponding Author / Sorumlu Yazar:** Muhammed Tilahun Muhammed
e-mail / e-posta: muh.tila@gmail.com, **Phone / Tel.:** +902462110360

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glukosidaz inhibitörlerinin enzimi inhibe etme potansiyelleri hesaplamalı yöntemlerle araştırılmıştır.

Gereç ve Yöntem: *Seçilmiş doğal kaynaklı α-glukosidaz inhibitörlerinin bağlanma potansiyeli, moleküler doking yoluyla araştırılmıştır. Daha sonra, en yüksek bağlanma potansiyeline sahip komplekslerin stabilitesi moleküler dinamik (MD) simülasyonu yoluyla değerlendirilmiştir.*

Sonuç ve Tartışma: *Moleküler doking çalışması bileşik **2**'nin standart ilaç olan akarbozdan daha iyi bağlanma potansiyeline sahip olduğunu göstermiştir. Bileşik 7 ise standart ilaca benzer bağlanma potansiyeline sahipti. Ayrıca, test edilen bileşiklerin hepsi enzime karşı makul bağlanma potansiyeli sergilemelerine rağmen standart ilaçtan daha zayıf bağlandığı görülmüştür. MD simülasyonu da bileşik 2 ve 7'nin standart ilaca benzer stabiliteye sahip kompleksler verdiğini göstermiştir. Hesaplama yöntemlerin sonuçları araştırılan doğal kaynaklı inhibitörlerin enzime bağlanma yeteneğine sahip olduğunu ve stabil kompleksler oluşturduğunu ortaya çıkarmıştır. Bu nedenle bu bileşikler klinik kullanım için potansiyel α-glukosidaz inhibitörleri olabilir. Bu yüzden en yüksek bağlanma potansiyeline sahip bileşikler üzerinde daha fazla in vitro araştırma yapılması tavsiye edilir.*

Anahtar Kelimeler: *Diyabet, doğal inhibitörler, α-glukosidaz, MD simülasyon, moleküler doking*

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized mainly by hyperglycemia [1]. According to the International Diabetes Federation Diabetes figures, DM affected 537 million people in 2021 and also the ninth cause of death. The projection made estimated that the number of affected people will rise to 643 million by 2030 and it is expected to be the seventh cause of death worldwide by then [2]. Insulin plays a crucial role in regulating blood glucose level. It takes part in glucose uptake and transport, glycogen, fatty acid, and protein synthesis. Insufficient production of insulin or resistance to insulin impairs the glucose homeostasis that leads to hyperglycemia eventually [3]. Chronic hyperglycemia can lead to long-term complications like nerve damage, cardiovascular disease, and kidney failure [4].

There are three categories of DM: Type 1 DM, type 2 DM, and gestational DM. Among these categories, type 2 DM comprises approximately %90-95 of the cases [5]. The type 2 category is manifested mainly by insulin resistance and to some extent insulin secretion impairment [6]. The insensitivity of the related targets to insulin can lead to a decreased glucose uptake in the skeletal muscle cells and an increased glucose formation in the liver [6]. As a result, most of the drugs that are used to treat type 2 DM decrease glucose absorption, hinder hepatic gluconeogenesis, and slow down renal glucose reabsorption [7]. These types of drugs played a prominent role in attenuating blood glucose level [8]. However, there are various side effects of such drugs. Hence, there is a need for antidiabetic drugs with high efficacy and low side effects [9].

Alpha-glucosidase inhibitors are typical examples that delay intestinal glucose absorption and thus decrease postprandial blood glucose level (Figure 1) [10]. Acarbose, miglitol, and voglibose are α-glucosidase inhibitors that are available in the pharmaceutical market. The molecular structure of these drugs is similar to carbohydrates (Figure 2). As a result, these drugs can bind to the carbohydrate binding site of α-glucosidase. The resulting complexes have higher affinity than the carbohydrate complex. Consequently, a delay in carbohydrate digestion and absorption that leads to a decrease in postprandial hyperglycemia is observed. However, these drugs have adverse effects like flatulence, severe stomach discomfort, and allergic responses [11]. Therefore, there is need of new α-glucosidase inhibitors with high potency and less adverse effects.

There have been efforts to develop bioactive compounds to alleviate diabetic conditions together with the efforts to synthesize new active compounds. The interest in the use of natural products to prevent and treat type 2 DM has been increasing. Natural products have been utilized to prevent and treat various medical disorders, including DM, throughout history [2]. For instance, one of the approved antidiabetic drugs, metformin, was originally isolated from *Galega officinalis* [12]. In the light of reported findings, potential α-glucosidase inhibitor compounds from natural origin were selected and their inhibiting potential was investigated through computational methods. For this end, akebonoic acid (1), alaternin (2), morusin (3), mulberrofuran K (4), procyanidin A2 (5), psoralidin (6), rhaponticin (7),

and taxumariene F (8) compounds were selected based on the literature review performed to dig out the potency of natural α-glucosidase inhibitors (Figure 3) [9].

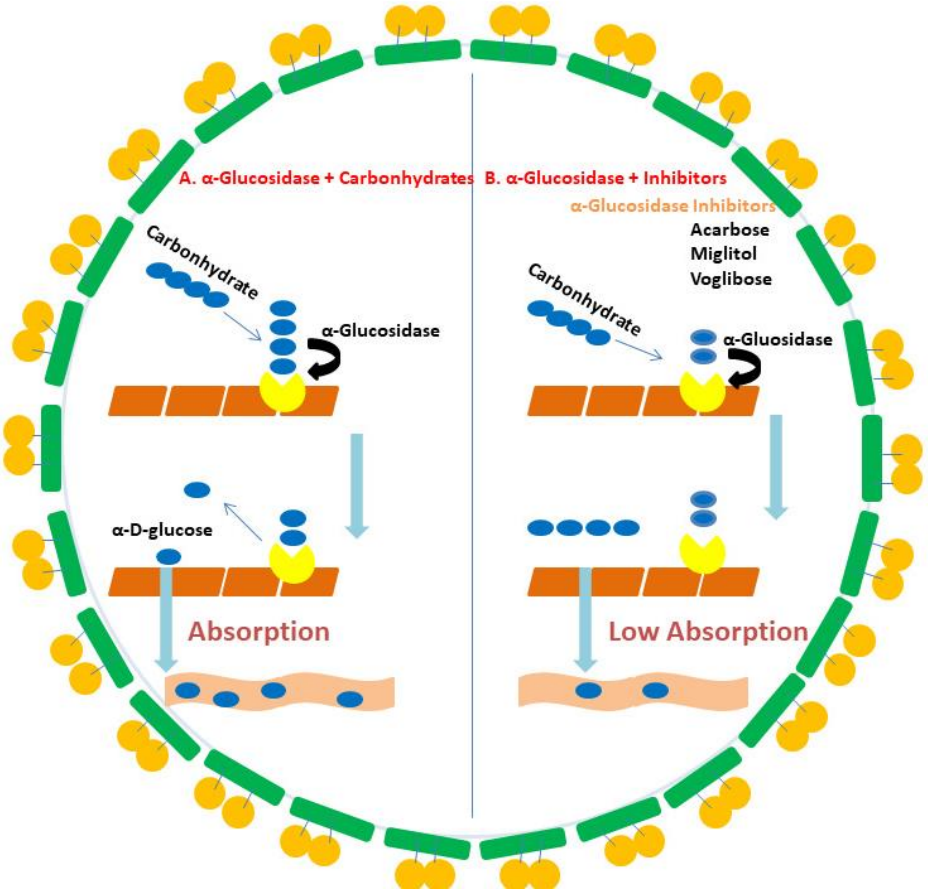

Figure 1. α-Glucosidase inhibition mechanism and its effect

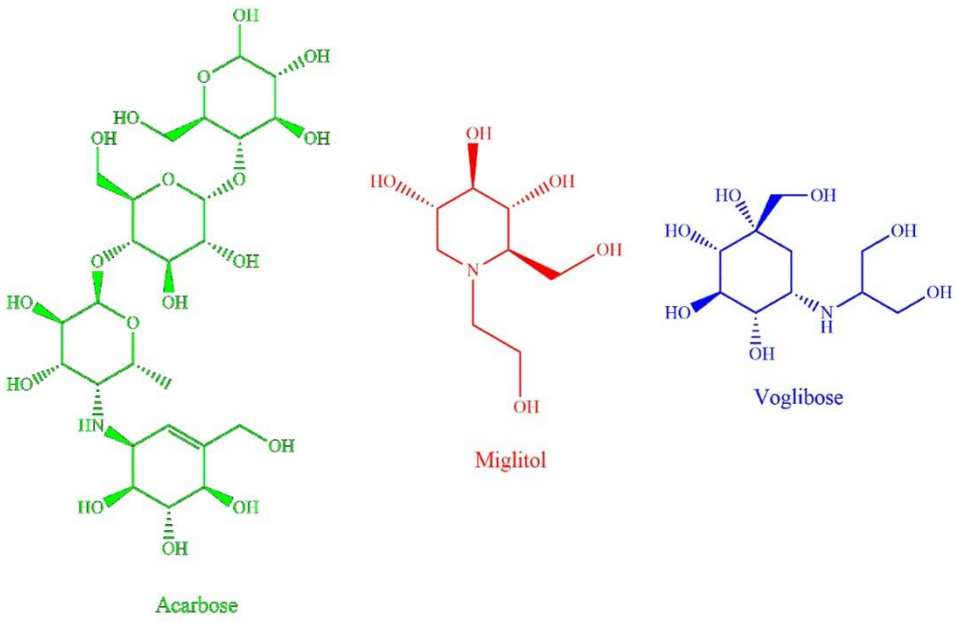

Figure 2. α-Glucosidase inhibitors that are clinically available

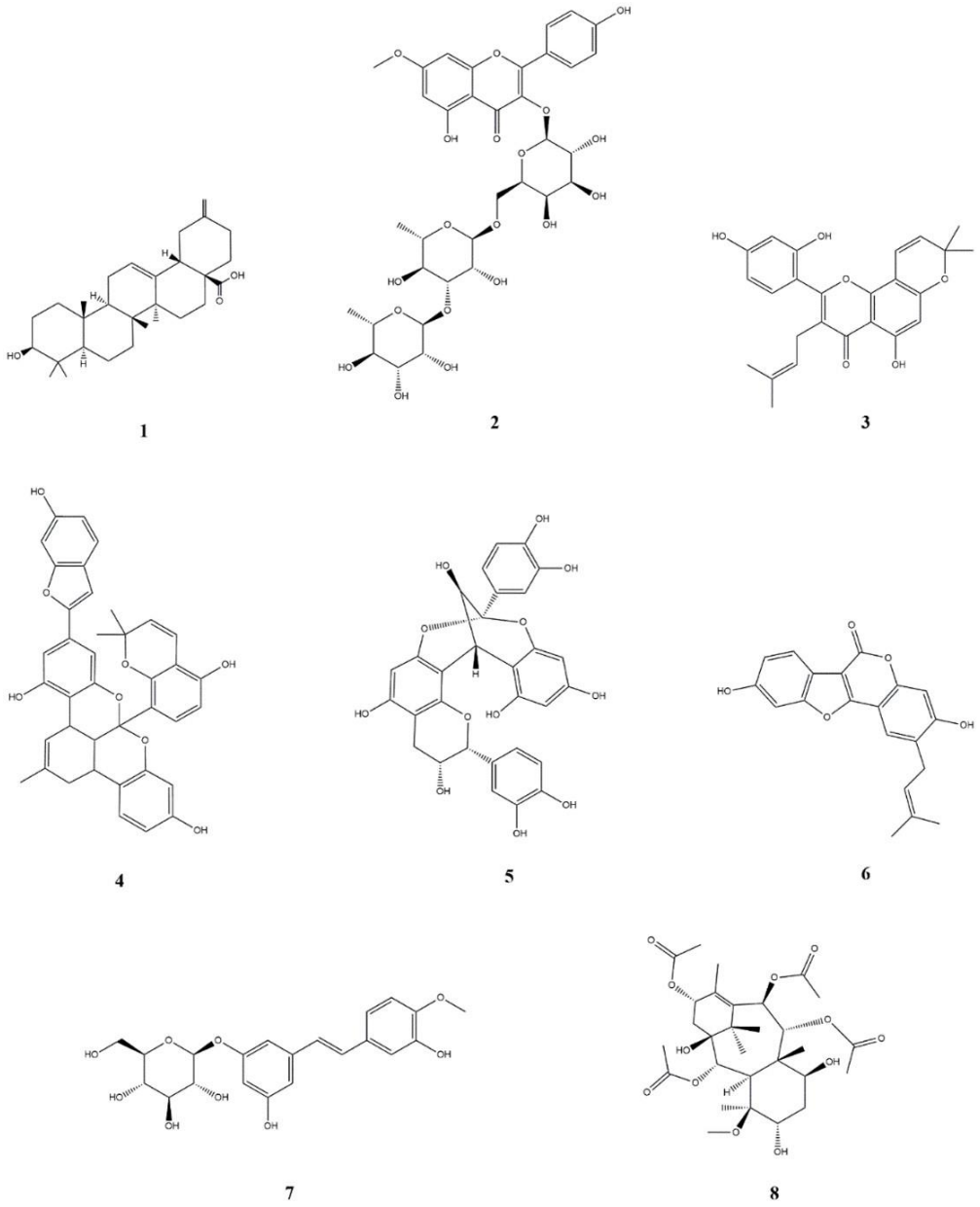

Figure 3. Natural α-glucosidase inhibitor compounds

Computational methods have been applied to minimize the cost and time required for drug design and discovery process [13]. Among such methods, molecular docking is employed to elucidate the mechanism of binding for drug candidate molecules [14,15]. Then, molecular dynamics (MD) simulation is undertaken to measure the stability level of the resulting target-compound complexes obtained from the docking.

In this study, natural α-glucosidase inhibitors were selected. The potential of these compounds to bind to α-glucosidase was investigated through computational methods. Molecular docking of these compounds to the enzyme crystal structure showed that the compounds had good binding potential, compounds **2** and **7** having the highest binding potential. The complexes of compounds **2** and **7** with the enzyme procured from the docking were tested for their stability via MD simulation. The two compounds had stability similar to the standard drug, acarbose. Compound **7** gave better stability profile according to some parameters.

MATERIAL AND METHOD

Molecular Docking

Molecular docking of the most active natural α-glucosidase inhibitors was done on crystal structure retrieved from the protein data bank (PDB). The crystal structure had a resolution of 1.55 Å and had a ligand complexed with it (PDB code: 5ZCE) [16]. The molecular docking was done with AutoDock Vina as described in previous studies [17,18].

MD Simulation

MD simulation of the natural α-glucosidase inhibitors with the highest binding potential to the enzyme was performed by using the respective complexes retrieved from the docking. MD simulations were done by using GROMACS (GROningen MAchine for Chemical Simulations) package as described in previous studies [19,20]. Then, root mean square deviation (RMSD), root mean square fluctuation (RMSF), Rg (radius of gyration), and ligand hydrogen bond plots were drawn through qtgrace and analyzed accordingly.

RESULT AND DISCUSSION

Molecular Docking

The interaction of the selected natural α-glucosidase inhibitor compounds to the crystal structure was investigated through molecular docking. Before docking the natural compounds to the crystal structure, the process was validated by redocking the ligand complexed in the crystal structure, alpha-maltotetraose. The bound ligand interacted to the structure very well. The ligand interacted to the structure with ten conventional hydrogen bonds (Asp60(2), His203, Gln256(2), Asp327(2), Asp382(2), Arg411) and two carbon hydrogen bonds (Phe163, Asp327) with the enzyme (Figure 4). The previous experimental study revealed similar conventional hydrogen bonding points detected in this study except with Asp382. The number of hydrogen bonds reported by the experimental study and detected in this study were similar [16]. Hence, the findings in the docking of the bound ligand to the crystal structure in this study were found to be similar to the previous experimental findings.

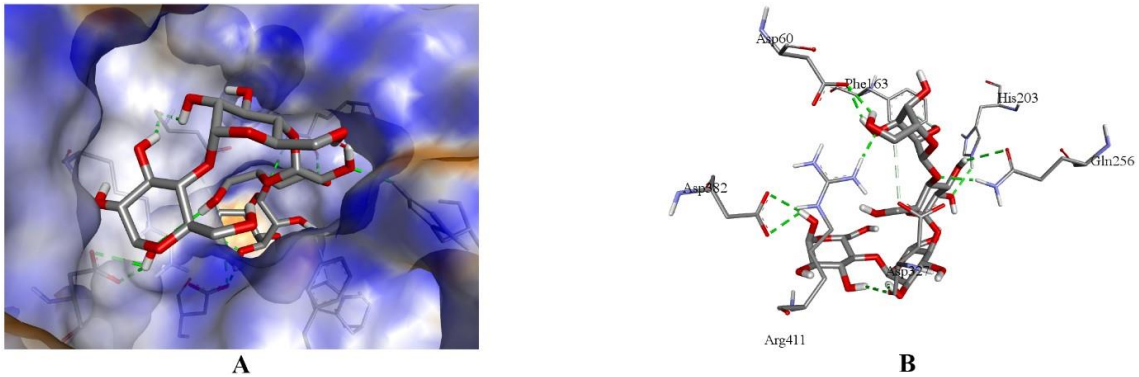

Figure 4. Binding mode of alpha-maltotetraose: A) its binding pose in the binding site, B) its 3D binding

In addition to docking to the bound ligand, the docking process was checked by docking the reference drug, acarbose, with the PDB structure. Acarbose interacted with the structure very well through seven conventional hydrogen bonds (Ile143(2), Asn258(3), Phe282, Gly286) and a carbon-hydrogen bond (Gly384) (Table 1, Figure 5). This has implicated that the natural compounds could also exhibit a reasonable interaction that fit their activity with the structure. The interaction residues of acarbose had also similarities with the crystallographic analysis ones. In this regard, the hydrogen bonding interactions with Asn258 and Phe282 were observed in the previous experimental study and

the docking study [16]. The standard drug had an interaction that fit its activity *in vitro* with the enzyme but less than with that of the complexed ligand inside the structure utilized. The standard ligands had an interaction that justified their activity with the enzyme. In addition to this, the complexed ligand had a high level of interaction similarity to the experimental structure analysis. The overall results implied that the docking process would result in a reliable interaction profile. Together with this, the stability of the complexes obtained from the docking was assessed through MD simulation.

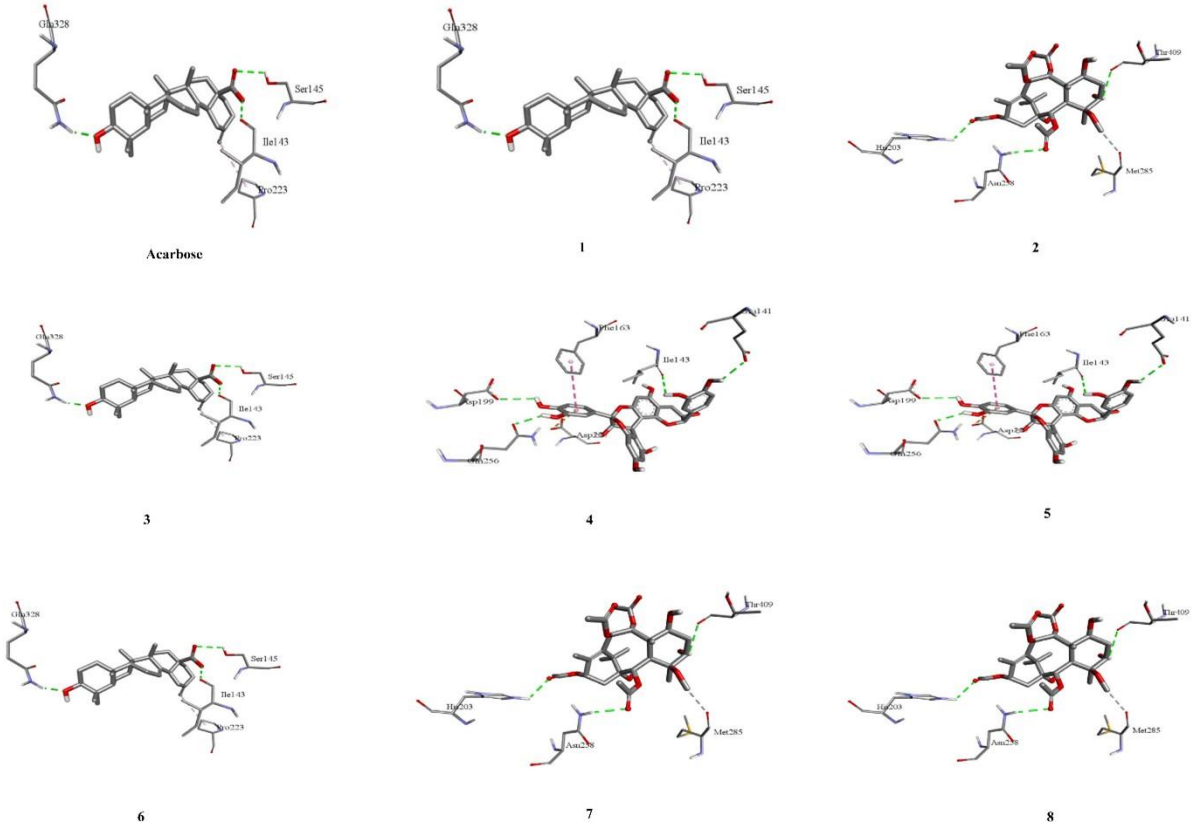

Figure 5. Binding profile of the natural inhibitors with the α-glucosidase crystal structure. In the figure color representation is; green-conventional hydrogen bonds, pale pink-alkyl/pi-alkyl, very pale green-carbon hydrogen bond, magenta-pi-sigma, yellow-pi-ion, and pink-pi-ion

The natural α-glucosidase inhibitors had interactions with the crystal structure. They interacted with at least three conventional hydrogen bonds and more other types of interactions. A previous crystallographic study revealed that interactions at Asp60, Tyr63, His103, Arg197, Asp199, His203, Gln256, Asn258, Phe282, Met285, His326, Asp327, Gln328, and Arg411 residues were important in the binding of ligands to the enzyme and stabilizing them inside the binding site [16]. In this study, compounds **2** and **7** exhibited the highest binding to the enzyme. Compound **2** had better binding than the standard drug, acarbose, as it formed more non-hydrogen bonds with the enzyme. Compound **7** had less number of conventional hydrogen bonds in relative to the reference drug but had more other types of interactions (Figure 5, Table 1). In addition to this, both of them had lower binding energy than the reference drug that implicated a better binding affinity for them (Table 1). The compounds with better binding potential had similar binding profile with the previous experimental study. Compound **2** had common binding at His203, Asn258, Phe282, and Arg411 amino acid residues. Similarly, compound **7** had common binding at Phe163, Asp199, His203, Gln256, Asn258, Phe282, and Asp327 residues (Table 1, Figure 5). All of its interaction residues except at Ala200 were similar to the previous experimental interaction revealed [16]. Therefore, it had high level of interaction residue similarity with the previous experimental findings. Furthermore, all the natural α-glucosidase inhibitors had at least one common

interaction residue with the experimental residue ones. This has implicated that the computational study findings were similar to the previous experimental finding [16]. The computational study demonstrated compounds **2** and **7** could bind to the enzyme and thus inhibit it at a comparable even better strength level than the reference drug. This premise was further assessed via MD simulation.

Table 1. Binding points of the ligands with the crystal structure

Ligands	Binding energy (kcal/mol)	Conventional hydrogen bonding residues	Other interaction residues
1	-8.4	Ile143, Ser145, Gln328	Pro223[a]
2	-9.0	Ile143(2), Ser145, Asn258, Gly384, Thr409, Arg411	Ile143(2)[a], Ile143[b], His203[a], Phe282[a]
3	-8.5	Asp327, Gln328, Asp382(2)	Phe163[a], Gly384[b], Met385[a], Tyr388[c]
4	-10.1	Ile143, Gln256, Phe282	Glu141[d], Ile143[a], Pro223(2)[a]
5	-9.4	Glu141, Ile143, Asp199, Gln256	Phe163[e], Asp327[d]
6	-9.4	Asn258, Thr409, Arg411	Tyr63[c], Phe163[c], Phe282(2)[e], Arg411[a]
7	-8.1	His203, Gln256, Asn258(2), Phe282, Asp327	Phe163[a], Asp199[b], Ala200[a], Asp327[d]
8	-7.1	His203, Asn258, Thr409	Met285[b]
Acarbose	-7.6	Ile143(2), Asn258(3), Phe282, Gly286	Gly384[b]

[a]Alkyl/pi-alkyl, [b]carbon-hydrogen bond, [c]pi-sigma, [d]pi-ion, [e]pi-pi

MD Simulation

The stability of the binding of acarbose, 2, and 7 to the enzyme was assessed through MD simulation. The MD simulation revealed that the three compounds had complexes with moderate stability as the plots flipped at some points. There was no significant difference in the stability of complexes of these compounds (Figure 6). As RMSD is used to measure fluctuation of a structure during the simulation period, the RMSD plots of the compounds with the highest binding potential and the standard drug were drawn [21]. At 29 ns, compound 2 exhibited high fluctuation that might be a sign of high movement for the compound inside the binding site. Thereafter, it got some level of stability but at a higher RMSD value. Similarly, acarbose had fluctuation at 42 ns and sustained relative stability then at a higher value. Compound **7** also had high fluctuation at 67 ns and then attained relative stability (Figure 6).

The RMSF plots of the compound containing complexes were drawn to evaluate changes in the enzyme structure amino acid residues [21]. RMSF value of complexes bearing the compounds was similar. Significant RMSF fluctuations were observed in 200-233, 283-300, and 370-413 residue intervals for the three complexes (Figure 6). Rg plots of the compound containing complexes were drawn to understand the effect of compound binding on the overall secondary structure of the enzyme [22]. Rg values of the complexes were similar to each other. Especially in the first 27 ns, they depicted similar Rg value. Thereafter, compound 7 had the lowest Rg value up to 70 ns that implied the highest compactness for it. After 70 ns, the complexes had varying Rg values, acarbose having the highest Rg value in this time interval (Figure 6). The role of intermolecular hydrogen bonding between the crystal structure and compounds was assessed by drawing ligand hydrogen bonds during the simulation period [22]. Acarbose had various number of hydrogen bonds during the simulation period. Though it formed up to seven hydrogen bonds, the dominant number of hydrogen bonds were two and three hydrogen bonds. Similarly, compound 2 had various number of hydrogen bonds up to seven during the simulation period. However, two and three hydrogen bonds were observed predominantly for it. Compound 7 also formed up to six hydrogen bonds with predominantly two and three hydrogen bonds during the simulation period (Figure 6). The maximum number of hydrogen bonds in the molecular docking for the compounds were met by the MD simulation. Together with this, the predominant number of

hydrogen bonds obtained from the MD simulation was less than that of the molecular docking. In short, the MD simulation revealed that compounds 2 and 7 gave complexes with similar stability to the standard drug.

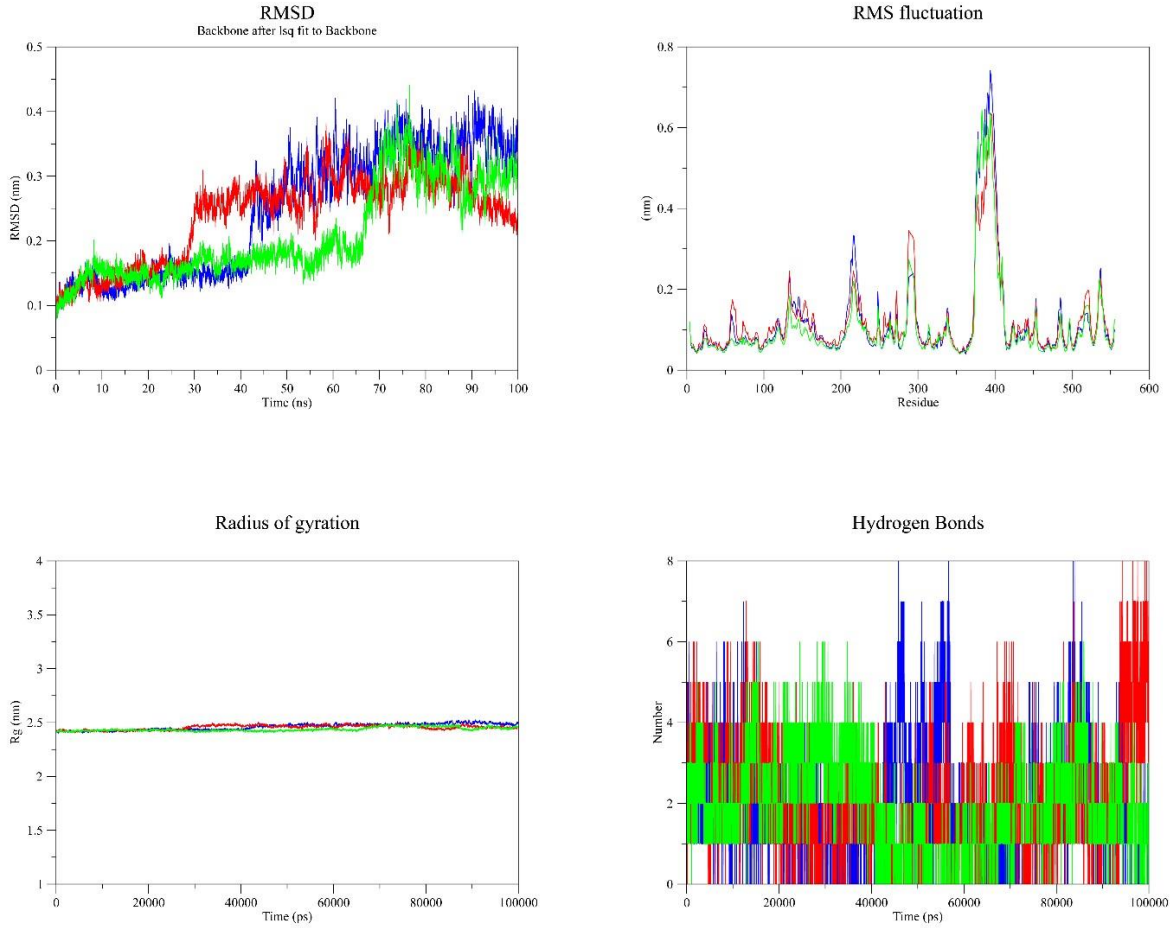

Figure 6. RMSD, RMSF, Rg, and number ligand hydrogen bonds plots obtained from the MD simulation (5ZCE-Acarbose in blue, 5ZCE-**2** in red, 5ZCE-**7** in green)

The potential of natural compounds in fighting DM is getting attention. For this, end, potential natural α-glucosidase inhibitors that could be used in DM treatment were selected by exploring the literature available. The potential of these compounds to inhibit α-glucosidase was investigated through molecular docking and MD simulation. For this end, molecular docking of the selected compounds towards the crystal structure of α-glucosidase was performed. The docking results demonstrated that the compounds had a reasonable interaction potential with the enzyme. Especially, compounds 2 and 7 had interactions similar to the standard drug. Compound 2 interacted better than the standard drug and compound 7 interacted at a comparable level. In general, the complexes procured from the docking were stable with some unexpected movements at a point. Compound 7 had relatively better stability in some time intervals in relative to compound 2 and the standard drug. In short, compounds 2 and 7 could bind to the enzyme and form a stable complex. Therefore, they have the potential to inhibit the enzyme.

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AUTHOR CONTRIBUTIONS

Concept: M.T.M.; Design: M.T.M., N.A., A.K., E.T.; Control: M.T.M.; Sources: M.T.M., N.A., A.K., E.T.; Materials: M.T.M., N.A., A.K., E.T.; Data Collection and/or Processing: N.A., A.K., E.T.; Analysis and/or Interpretation: M.T.M.; Literature Review: M.T.M., N.A., A.K., E.T.; Manuscript Writing: M.T.M.; Critical Review: M.T.M., N.A., A.K., E.T.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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