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Research Note

# Molecular docking studies for discovery of plant-derived α-glucosidase inhibitors

**Tae Kyung Hyun[1], Seung Hee Eom[1] and Ju-Sung Kim[2,*]**

**[1]Division of Applied Life Science, Plant Molecular Biology and Biotechnology Research Center, Gyeongsang National University, Jinju 660-701, Republic of Korea**
**[2]College of Applied Life Sciences, the Research Institute for Subtropical Agriculture and Biotechnology, Jeju National University, Jeju 690-756, Republic of Korea**

***Corresponding author: aha2011@jejunu.ac.kr**

**Abstract**

The identification of naturally occurring α-glucosidase inhibitors has been actively pursued with the aim of developing therapeutics for the treatment of type 2 diabetes mellitus. To identify α-glucosidase inhibitors, we screened 40 natural compounds, including flavonoids and phenolics, using the structure-based molecular docking approach. The rank of each compound was determined on the basis of the binding free energy of the lowest energy cluster. Results showed that all the tested compounds exhibited a binding energy ranging from -8.2 kcal/mol to -3.6 kcal/mol, indicating the variation in antidiabetic potential of tested compounds. The top-screened compounds were rutin, quercetin, and myricetin, which exhibited higher inhibitory activities ($IC_{50}$ = 1.0 to 84.1 μg/mL) against α-glucosidase than acarbose ($IC_{50}$ = 140.5 μg/mL), a reference inhibitor. We also demonstrated that a variety of inhibitory actions (competitive and noncompetitive inhibition) exist among these compounds. Our results suggest that rutin, quercetin, and myricetin can be used to further develop potent α-glucosidase inhibitors.

**Keyword:** α-glucosidase, molecular docking, rutin, quercetin, myricetin.
**Abbreviations:** T2DM_Type 2 diabetes mellitus; pNPG_p-nitrophenyl glucopyranoside.

## Introduction

Type 2 diabetes mellitus (T2DM), also called non-insulin-dependent diabetes mellitus, is a common chronic disease that is characterized by progressive β-cell dysfunction on a background of peripheral insulin resistance and relative insulin deficiency (Zimmet et al., 2001; Lian et al., 2013). Although T2DM is prevalent mainly in adults, it currently occurs more often in children and teenagers because of an increase in obesity in children and adolescents (Kahn et al., 2006; Lian et al., 2013). Therefore, T2DM has become an increasingly important public health issue throughout the world (Rosenbloom et al., 2009). Starch is not only a storage α-glucan that comprises α-1,4-linked D-glucose main chains and α-1,6-linked branched chains, but also a carbon and energy source in various organisms, such as animals, plants, and microorganisms (Satoh et al., 2013). In humans, six enzyme activities (those of two α-amylases and four α-glucosidases) are required for the breakdown of dietary starches and sugars into glucose (Sim et al., 2010). Dietary starch is degraded to maltooligosaccharides by salivary and pancreatic α-amylases. Then, α-glucosidases hydrolyze the glycosidic linkage of maltooligosaccharides and produce glucose (Satoh et al., 2013). Given that α-amylases and α-glucosidases function as starch-degrading enzymes, the inhibition of their activities is one of the most effective approaches to controlling blood glucose levels in people with T2DM. α-glucosidase inhibitors, including acarbose and miglitol, have been developed and used as oral antidiabetic agents (Mizuno et al., 2008). Unlike other oral antidiabetic agents, α-glucosidase inhibitors act locally in the intestine rather than modulate certain biochemical processes in the body. For this reason, commercial α-glucosidase inhibitors, such as acarbose, voglibose, and miglitol, are widely used clinically to control the blood glucose levels of patients (van de Laar et al., 2005). Nevertheless, these compounds have been reported to cause side effects, such as such as flatulence, diarrhea, and abdominal pain. The most common side effects are due to the intracolonic fermentation of carbohydrates that are unabsorbed in the small bowel; such fermentation produces gas (Mizuno et al., 2008). Given this backdrop, the screening and identification of more effective and safer α-glucosidase inhibitors from natural products have been critical requirements in determining alternative prevention and treatment measures for T2DM. Structure-based computational methods, including molecular docking, have increasingly been used in the study of biomolecular structure and function, as well as in the design of structure-based rational drugs. In particular, molecular docking contributed to the development of several inhibitors and inhibitor candidates that have been advanced to clinical trials (Kufareva and Abagyan, 2008; Torktaz et al., 2013; Zhang et al., 2014), indicating that docking simulation is a useful tool for enriching a chemical library with active compounds. Appreciating the potential of this technique, we carried out structure-based molecular docking to screen natural compound-based α-glucosidase inhibitors. On the basis of an *in-vitro* assay, the types of inhibitory mechanisms that underlie the effects of selected compounds were classified into two groups: competitive and non-competitive inhibition.

## Results and Discussion

### *Screening of plant-derived α-glucosidase inhibitors using molecular docking*

Plant-derived flavonoids and phenolics are known as potential active compounds that possess a broad range of pharmaceutical properties, including antibacterial, antifungal, antiviral, and anticancer activity (Soto-Vaca et al., 2012). These compounds have also been used as templates for the development of new pharmaceuticals (Weston and Mathesius, 2013). To identify potential α-glucosidase inhibitors, we selected 40 natural compounds (flavonoids and phenolics), including gallic acid, caffeic acid, ferulic acid, and rutin, which are naturally occurring compounds present in various plants (Table 1). All the selected molecules, except rutin have no more than 5 hydrogen bond donors, not more than 10 hydrogen bond acceptors, a molecular weight under 500 dalton, and a partition co-efficient log P less than 5 (Table 1), indicating that these compounds satisfy Lipinski's rule of five. The rank of each compound was determined on the basis of the binding free energy of the lowest energy cluster. The tested compounds showed a binding energy ranging from -8.2 kcal/mol to -3.6 kcal/mol (Table 1). The top three compounds with low binding energies were rutin, quercetin, and myricetin, whereas the compound with the highest binding energy (-3.6 kcal/mol) was decannoic acid. This result indicates that rutin, quercetin, and myricetin are potential inhibitors of α-glucosidase. Rutin interacts with α-glucosidase residues of Glu322, Lys324, and Asp325, with a binding energy of -8.2 kcal/mol (Fig. 1A and Table 1). The obtained quercetin docking interaction is shown in Fig. 1B, with Asp325 and Asp521 residues having a binding energy of -8.0 kcal/mol. Furthermore, residue Lys324 supported the formation of Vander Waals interaction, thereby further stabilizing α-glucosidase-quercetin interaction (Fig. 1B). In case of myricetin, four hydrogen bonds were observed from α-glucosidase residues of Glu322, Lys324, Asp325, and Asp521, with a binding energy of -7.7 kcal/mol (Fig. 1C). Taken together, the two-dimensional diagrams of the protein-ligand interaction suggest that the difference in binding energy among the compounds is attributed to the difference in the position of the functional groups in such compounds.

### *The inhibitory effect of rutin, quercetin, and myricetin on α-glucosidase activity*

To confirm the inhibitory effects of the plant-derived flavonoids and phenolics used in *in- silico* docking analysis, we investigated the effects of rutin, quercetin, and myricetin on the activity of α-glucosidase by *in-vitro* testing. α-glucosidase inhibitory activities occurred in the order quercetin ($IC_{50}$ = 1.0±0.1 μg/mL) > myricetin ($IC_{50}$ = 3.2±0.1 μg/mL) > rutin ($IC_{50}$ = 84.1±4.1 μg/mL), indicating that these compounds exerted higher α-glucosidase inhibitory activities than did acarbose ($IC_{50}$ = 140.5±0.5 μg/mL) (Table 2). Similarly, quercetin, rutin and myricetin exhibited pharmaceutical properties, including anti-oxidant and anti-diabetic activities (Tadera et al., 2006; Yang et al., 2008; Justino and Vieira, 2010; Hussain et al., 2012; Hong et al., 2013; Hyun et al., 2013). These findings indicate that *in-silico* docking analysis serves as a valuable resource for facilitating the discovery of α-glucosidase inhibitors. However, rutin exhibited a significant binding energy (Table 1), although it showed lower α-glucosidase inhibitory activities than did quercetin and myricetin (Table 2). This difference may be due to varying inhibitory mechanisms, such as competitive and noncompetitive inhibition. Therefore, to characterize the inhibitory mechanisms of rutin, quercetin, and myricetin, the α-glucosidase inhibitory activity of each compound was analyzed using different concentrations of pNPG. As shown in Fig. 2, acarbose, quercetin, and myricetin behaved as competitive inhibitors, whereas rutin bound to the substrate-enzyme complex (pNPG-*B. stearothermophilus* α-glucosidase, in this case). This result indicates that a variety of inhibitory mechanisms occur among these compounds.

## Materials and Methods

### *Preparation of molecules and ligands for docking*

Molecular docking was performed using a crystallized yeast glucose-α-glucosidase. The 3D structure for yeast glucose-α-glucosidase (PDB code, 3A4A, Yamamoto et al., 2010) was obtained from a protein data bank (http://www.rcsb.org/). Polar hydrogens were added to a macromolecule by using AutoDock, after which the structure was saved in PDBQT file format that contains a protein structure with hydrogen in all polar residues. For ligands, the 3D structures of natural flavonoids and phenolics were searched in the PubChem database (http://pubchem.ncbi.nlm.nih.gov/). An SDF file for the 3D structure was converted into a PDB file by using Open Babel software. Forty of the natural compound structures were minimized by computing gasteiger changes and the structures were saved in PDBQT file format via AutoDock.

### *Virtual screening of α-glucosidase inhibitors*

We used the automated version of the AutoDock program (AutoDock Vina, Trott and Olson, 2010) in the structure-based virtual screening of α-glucosidase inhibitors. The selected values for grid dimensions and the center were 40×40×40 and x = 54.867, y = 8.813, and z = 14.817, respectively. The predicted binding affinity (kcal/mol), which indicates how strongly a ligand binds to a receptor, was calculated on the basis of the scoring function used in AutoDock Vina. The best molecular interaction was identified using the binding affinity score. The two-dimensional diagrams of protein-ligand interaction were analyzed using the web-based online tool, PoseView (http://poseview.zbh.uni-hamburg.de/poseview).

### *Assay for α-glucosidase inhibitory activity*

The inhibitory activity on α-glucosidase was determined following the method described by Kim et al. (2011), with minor modifications. *Bacillus stearothermophilus* α-glucosidase was pre-incubated with different compound concentrations and 0.2 M potassium phosphate buffer (pH 6.8) at 37 °C for 15 min. The reaction was initiated by the addition of 3 mM p-nitrophenyl glucopyranoside (pNPG). After incubation at 37 °C for 10 min, 750 μL of 0.1 M sodium carbonate solution was added to each mixture to stop the reaction. Then, absorbance at 405 nm was measured with a UV-visible spectrophotometer. The α-glucosidase inhibitory effects of each compound were calculated as a percentage of the control using the following formula:

Inhibition rate (%) = [1- (Abssample – Absblank) / Abscontrol] × 100, where Abssample represents the absorbance of the experimental sample, Absblank denotes the absorbance of the blank, and Abscontrol is the absorbance of the control. The measurement was carried out in triplicate.

**Table 1.** Ligand parameters for satisfying Lipinski's rule of five and their binding affinities (kcal/mol) with Saccharomyces cerevisiae α-glucosidase.

| No. | Compound | Molecular formula | Molecular weight | Log P | H-Bond Donor | H-Bond Acceptor | Remark [a] | Binding affinity (kcal.mol) |
|---|---|---|---|---|---|---|---|---|
| 1 | 1-Naphthyl butyrate | $C_{14}H_{14}O_2$ | 214.3 | 3.6 | 0 | 2 | P | -5.7 |
| 2 | 2,5-Dihydroxybenzoic acid | $C_7H_6O_4$ | 154.1 | 1.6 | 3 | 4 | P | -5.7 |
| 3 | 2-Hydroxycinnamic acid | $C_9H_8O_3$ | 164.2 | 1.5 | 2 | 3 | P | -5.5 |
| 4 | 3-Aminobutanoic acid | $C_4H_9NO_2$ | 103.1 | -3.1 | 2 | 3 | P | -3.8 |
| 5 | 3-Hydroxybenzoic acid | $C_7H_6O_3$ | 138.1 | 1.5 | 2 | 3 | P | -5.1 |
| 6 | 4-Hydroxy-3-methoxybenzoic acid | $C_8H_8O_4$ | 168.1 | 1.4 | 2 | 4 | P | -5.7 |
| 7 | 4-Hydroxybenzoic acid | C7H6O3 | 138.1 | 1.6 | 2 | 3 | P | -5.0 |
| 8 | 4-Methoxybenzoic acid | $C_8H_8O_3$ | 152.1 | 2 | 1 | 3 | P | -5.0 |
| 9 | 5-Sulfosalicylic acid | $C_7H_6O_6S$ | 218.2 | 1.3 | 3 | 6 | P | -5.6 |
| 10 | Acetylenedicarboxylic acid | $C_4H_2O_4$ | 114.1 | 0.1 | 2 | 4 | P | -5.1 |
| 11 | Alpha-humulene | $C_{15}H_{24}$ | 204.4 | 4.5 | 0 | 0 | P | -5.5 |
| 12 | Benzaldehyde | $C_7H_6O$ | 106.1 | 1.5 | 0 | 1 | P | -4.5 |
| 13 | Benzoic acid | $C_7H_6O_2$ | 122.1 | 1.9 | 1 | 2 | P | -4.8 |
| 14 | Caffeic acid | $C_9H_8O_4$ | 180.2 | 1.2 | 3 | 4 | P | -5.6 |
| 15 | Decannoic acid | $C_{10}H_{20}O_2$ | 172.3 | 4.1 | 1 | 2 | P | -3.6 |
| 16 | Dodecanedioic acid | $C_{12}H_{22}O_4$ | 230.3 | 3.2 | 2 | 4 | P | -4.1 |
| 17 | Ferulic acid | $C_{10}H_{10}O_4$ | 194.2 | 1.5 | 2 | 4 | P | -5.4 |
| 18 | Gallic acid | $C_7H_6O_5$ | 170.1 | 0.7 | 4 | 5 | P | -6.2 |
| 19 | Glutaric acid | $C_5H_8O_4$ | 132.1 | -0.3 | 2 | 4 | P | -4.3 |
| 20 | Hydroquinone | $C_6H_6O_2$ | 110.1 | 0.6 | 2 | 2 | P | -4.5 |
| 21 | Indole | $C_8H_7N$ | 117.1 | 2.1 | 1 | 0 | P | -4.9 |
| 22 | l-Glutamic acid | $C_5H_9NO_4$ | 147.1 | -3.7 | 3 | 5 | P | -4.5 |
| 23 | Myricetin | $C_{15}H_{10}O_8$ | 318.2 | 1.2 | 6 | 8 | P | -7.7 |
| 24 | Naringenin | $C_{15}H_{12}O_5$ | 272.3 | 2.4 | 3 | 5 | P | -7.3 |
| 25 | Nobiletin | $C_{21}H_{22}O_8$ | 402.4 | 3 | 0 | 8 | P | -6.1 |
| 26 | p-Coumaric acid | $C_9H_8O_3$ | 164.2 | 1.5 | 2 | 3 | P | -5.5 |
| 27 | Phenylacetic acid | $C_8H_8O_2$ | 136.1 | 1.4 | 1 | 2 | P | -5.0 |
| 28 | Phloroglucinol | C6H6O3 | 126.1 | 0.2 | 3 | 3 | P | -5.1 |
| 29 | Propionic acid | C3H6O2 | 74.1 | 0.3 | 1 | 2 | P | -4.1 |
| 30 | Protocatechuic acid | $C_7H_6O_4$ | 154.1 | 1.1 | 3 | 4 | P | -5.8 |
| 31 | p-Toluidine | C7H9N | 107.2 | 1.4 | 1 | 1 | P | -4.6 |
| 32 | Quercetin | $C_{15}H_{10}O_7$ | 302.2 | 1.5 | 5 | 7 | P | -8.0 |
| 33 | Rutin | $C_{27}H_{30}O_{16}$ | 610.5 | -1.3 | 10 | 16 | F | -8.2 |
| 34 | Scopoletin | $C_{10}H_8O_4$ | 192.2 | 1.5 | 1 | 4 | P | -5.9 |
| 35 | Suberic acid | $C_8H_{14}O_4$ | 174.2 | 1 | 2 | 4 | P | -3.9 |
| 36 | Succinic acid | $C_4H_6O_4$ | 118.1 | -0.6 | 2 | 4 | P | -4.3 |
| 37 | Syringic acid | $C_9H_{10}O_5$ | 198.2 | 1 | 2 | 5 | P | -5.3 |
| 38 | Tangeretin | $C_{20}H_{20}O_7$ | 372.4 | 3 | 0 | 7 | P | -6.1 |
| 39 | trans-3-Hydroxycinnamic acid | $C_9H_8O_3$ | 164.2 | 1.8 | 2 | 3 | P | -5.5 |
| 40 | trans-Cinnamic acid | $C_9H_8O_2$ | 148.2 | 2.1 | 1 | 2 | P | -5.1 |

[a] "P" indicates that a compound fully satisfies Lipinski's rule of five, and "F" indicates that the compound violates the rule.

**Table 2.** Inhibitory effects of rutin, quercetin, and myricetin on α-glucosidase activity.

| | $IC_{50}$ (μL/mL)[a] |
|---|---|
| Acarbose | 140.5±0.5c[b] |
| Myricetin | 3.2±0.1a |
| Quercetin | 1.0±0.1a |
| Rutin | 84.1±4.1b |

[a] $IC_{50}$: Amount required for a 50% reduction in α-glucosidase activity; each value is a mean ± standard derivation of triplicate experiments.
[b] Values followed by different letters are significantly different at $P < 0.05$ as determined by Tukey's protected least significant difference.

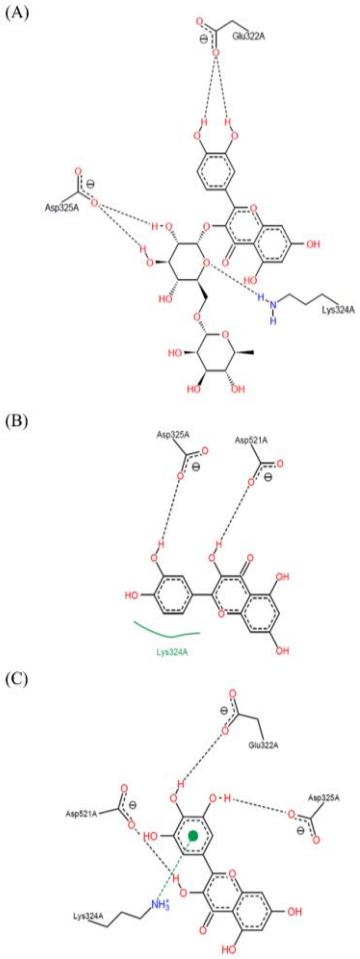


**Fig 1.** The two-dimensional representative docking interaction of α-glucosidase with rutin (A), quercetin (B), and myricetin (C). The interaction pattern is composed of hydrogen bonds, visualized as black dashed lines; π interactions, shown as green dashed lines with dots denoting the participating π systems; and hydrophobic contacts, which are represented by the residue labels and spline segments along the contacting hydrophobic ligand parts.

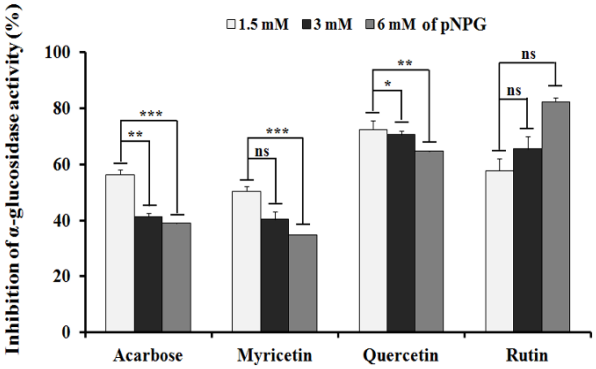


**Fig 2.** Effect of substrate concentration on the action of rutin, quercetin, and myricetin as α-glucosidase inhibitors. We used 3 μL/mL of quercetin and myricetin and 100 μL/mL of rutin and acarbose; these concentrations are close to the $IC_{50}$ values of the compounds. Different concentrations of p-nitrophenyl glucopyranoside were used as substrates. Values are the average of triplicate experiments and represented as mean±standard deviation. ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

***Statistical analysis***

All experiments were conducted for three independent replicates. The data are expressed in terms of mean and standard deviation. Significant differences between means were detected by one-way analysis of variance (ANOVA), followed by multiple comparisons using Tukey's least significant difference test. Differences were considered significant when $p < 0.05$.

## Conclusions

In this study, the structure-based computational method was used to search for plant-derived α-glucosidase inhibitors. The binding energies of the top-ranked molecules (rutin, quercetin, and myricetin) ranged from -8.2 to -7.7 kcal/mol. On the basis of *in-vitro* testing, we demonstrated that rutin, quercetin, and myricetin are potential α-glucosidase inhibitors that exert stronger inhibitory effects than does acarbose. Nonetheless, further investigations, including explorations into the specificity of inhibitors and *in-vivo* models, are needed to analyze the pharmaceutical value of plant-derived α-glucosidase inhibitors.

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