Research Paper

Comparative Analysis of the Bonding Modes between Two Antidiabetic Drugs with β-Glucosidases from Different Species

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β-glucosidase is one of the critical enzymes to the type 2 diabetes, which belongs to a large family of glycoside hydrolases. In this article, we attempted to explore the binding modes between two drugs and β-glucosidases by comparing their bonding modes with β-glucosidases from different species. Results showed that the binding orientations and geometrical conformation of synthetic drug (miglitol) and natural product (quercetin) were all different in all active sites, which may be related to the flexibility of the molecules. Compared with the conformations obtained by density functional theory calculations at the B3LYP/6-31G* level, the docking conformations indicated that the -CH_2CH_2OH group of miglitol and the B ring of quercetin were the most critical groups to the stabilities in the active sites. Finally, protein sequence alignment was performed under default parameters. Although the sequence similarity is not very high between these β-glucosidases, the residues related to the active sites were conservative; especially the one involved in H-bond interactions between three species, namely soil metagenome, *Micrococcus antarcticus* and termite *Neotermes koshunensis*.

Key words: β-glucosidase, binding mode, docking conformation, sequence alignment D-optimal design

Diabetes is rapidly becoming an enormous health burden by decreasing quality of life all over the world. Type 2 diabetes mellitus is a metabolic disease associated with micro- and macrovascular complications such as diabetic retinopathy, diabetic neuropathy and cardiovascular diseases. One of therapeutic approaches for managing increased blood glucose is to weaken the catalytic activity of key enzymes involved in hydrolytic cleavage of dietary oligosaccharides[1], and thus the blood glucose in the diabetics and a series of possible side effects will decrease. β-glucosidase is one of the key enzymes, which belongs to a large family of glycoside hydrolases. Many of β-glucosidases can also catalyze the synthesis of poly- or oligosaccharides, which have dual activities of hydrolysis and transglycosylation[2,3].

By delaying the absorption of digested carbohydrates from the small intestine, β-glucosidase inhibitors can also attenuate postprandial blood glucose fluctuations[4-6]. There are great number of studies about the inhibiting capacity of the glucosidase[7-10]. For example, inhibitor miglitol acted as therapeutic agent with high clinical efficacy for diabetes in combination with other drugs and/or dietary changes[11,12]. Although this synthetic drug has significant role in diabetes, it subsequently leads to various gastrointestinal side effects, such as flatulence, diarrhea and abdominal discomfort[13].

There are many doubts about whether nontoxic natural flavones have the similar inhibitory features as miglitol, which is very important to the treatment of diabetes. Recent research indicated that some natural products are associated with decreasing the blood glucose of the diabetics, such as multihydroxyl flavonoid compound, quercetin[14]. Studies demonstrated that quercetin has good anti-type 2 diabetes ability based on rat experiment and yeast α-glucosidase *in vitro*[15-19]. The detailed interaction between quercetin and α-glucosidase has also been reported[20]. However, the detailed

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inhibitory mechanisms between different quercetin and β-glucosidases are rare so far[21]. Molecular docking technique is a key tool in structural molecular biology and computer-assisted drug design[22-24]. In the present work, a comparative study of the interaction between β-glucosidase and synthetic drug (miglitol) or natural product (quercetin) was undertaken by molecular docking simulation methods. For the purpose of comparison, four enzymes from different species were also chosen, which hold the focus of the present study and belong to the β-glycosidase family. The results obtained from this analysis not only revealed the interaction mechanism between β-glycosidase and inhibitors, but also provided some valuable information for drug design, pharmaceutical research and sequence analysis based on comparative studies.

MATERIALS AND METHODS

Ligand preparation:

Three dimensional coordinates of all small molecules were obtained from the PubChem database (CIDs for miglitol and quercetin are 441314 and 5280343, respectively) and their geometries were further optimized to obtain the most stable conformation by using density functional theory (DFT) calculations at the B3LYP/6-31G* level[25,26], which can be executed via Gaussian 09 program package[27]. The stable conformation was used for further docking research.

Protein preparation:

Crystal structure files of cocrystallized β-glycosidases used in this study were derived from the Protein Data Bank (PDB). The β-glycosidase from soil metagenome was noted as Sm-β-glu, β-glucosidase from *Micrococcus antarcticus* was noted as Ma-β-glu, β-glucosidase from termite *Neotermes koshunensis* was noted as Tnk-β-glu, and β-glucosidase from *Homo sapiens* was noted as Hu-β-glu. All crystallographic water molecules were removed, polar hydrogens were added, Gasteiger charges were assigned to the proteins by AutoDockTools (ADT) 1.5.6[28] and were then converted into AutoDock readable file format.

Molecular docking:

Docking and the corresponding results were carried out by using ADT 1.5.6 package and Pymol Molecular Visualization package[29,30]. The ligands were automatically prepared by detecting the flexible torsions and assigning gasteiger charges under default settings. Lamarckian genetic algorithm (LGA) was used for docking by using the following settings: maximum number of 25 000 000 energy evaluation, number of generation=27 000, mutation rate=0.02, crossover rate=0.80, elitism value of 1 and maximum number of iterations=300. The grid size was 60×60×60 Å with a spacing of 0.375 Å and was centered on the binding site showed in crystal structure of β-glucosidase cocrystallized structure.

The binding sites in these proteins were found to be the putative binding sites for the ligands based on the reference in the complex substance from the PDB database. Exact dimensions of the grid centre of Sm-β-glu, Ma-β-glu, Tnk-β-glu and Hu-β-glu were (x=34.253; y=1.006; z=-3.937), (x=17.849; y=20.669; z=24.342), (x=-26.809; y=82.413; z=13.836) and (x=-29.711; y=10.91; z=1.156), respectively. After 50 independent docking runs, the docking results were clustered with root mean square deviation tolerance of 2.0 Å. The top output poses were ranked by their calculated binding affinities.

Protein sequence analysis:

The sequences of proteins were downloaded from the National Center for Biotechnology Information (NCBI) website. Multiple sequence alignment is performed by Clustal software. The composition analysis was carried out by Lasergene software, and the sequence similarity analysis was performed by MegAlign software with Lipman-Pearson method.

RESULTS

Molecular structure and protein information:

By inhibiting the ability of the patient to break down complex carbohydrates into glucose, miglitol, is an oral antidiabetic drug[31-33]. The molecular structures of small molecules used in this study were given in fig. 1. The most stable conformation was used for further docking research after DFT calculations. β-glucosidase plays an important role in degradation of cello-oligosaccharides, which influence the liberation of glucose from cellulose[34]. Basic information of four β-glucosidases was listed in Table 1. The X-ray crystallographic structures of the β-glucosidases from soil metagenome (PDB ID: 3CMJ) at a resolution of 1.60 Å, *Micrococcus antarcticus* (PDB ID: 3W53) at a resolution of 2.20 Å, termite *Neotermes koshunensis* (PDB ID: 3VIL) at a resolution of 1.15 Å and *Homo sapiens* (PDB ID: 3VKK) at a resolution of 2.00 Å were obtained from the RCSB Protein Data Bank. All of the

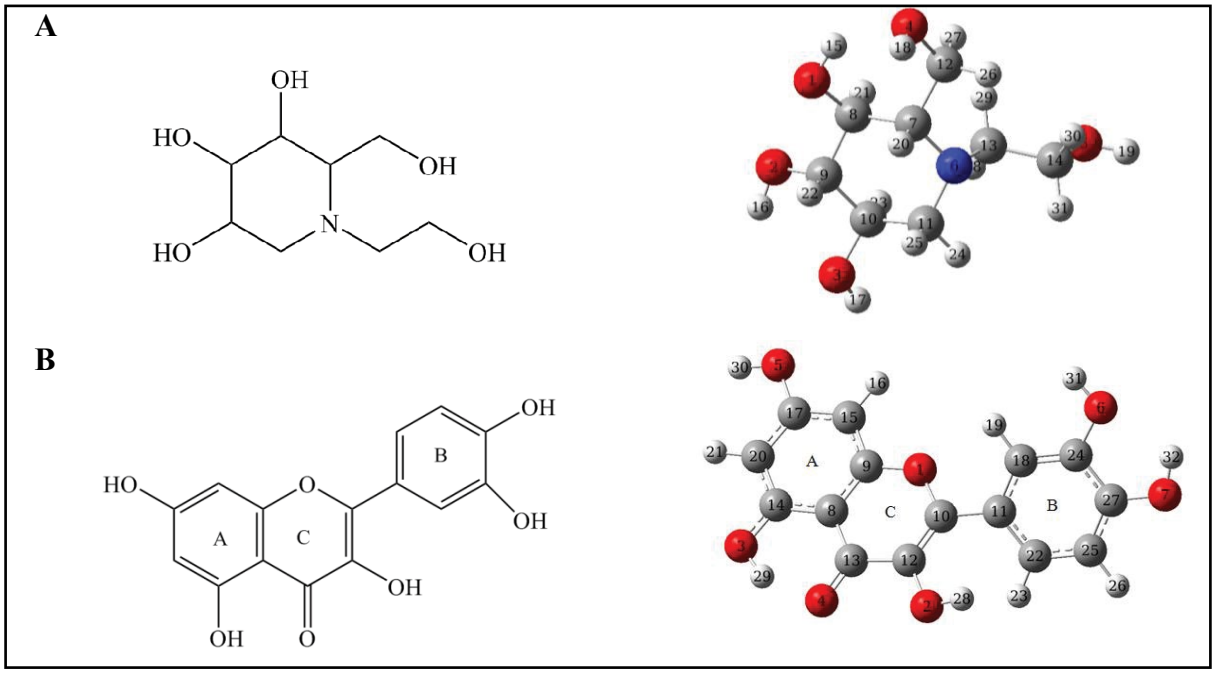

Fig. 1: The planar and optimized geometries of miglitol and quercetin.

The planar and optimized geometries of miglitol (A) and quercetin (B). All atoms are noted numerically.

TABLE 1: STRUCTURAL INFORMATION OF β-GLUCOSIDASE FOR SM-β-GLU, MA-β- GLU, TNK-β-GLU and HU-β-GLU

Name	PDB ID	Length (aa)	Amino acids composition				Organism
			S-Basic	S-Acidic	Hydrophobic	Polar	
Sm-β-glu	3CMJ	465	40	54	158	112	Soil metagenome
Ma-β-glu	3W53	506	41	67	176	113	*Micrococcus antarcticus*
Tnk-β-glu	3VIL	487	41	72	164	121	*Neotermes koshunensis*
Hu-β-glu	3VKK	469	46	57	167	125	*Homo sapiens*

protein lengths are about 500 aa. The composition ratio of Sm-β-glu, Ma-β-glu, Tnk-β-glu and Hu-β-glu are relatively similar to each other. It accounts for a large proportion for hydrophobic amino acid and polar one, sum of which own 58.06, 57.11, 58.52 and 62.26% for total number of Sm-β-glu, Ma-β-glu, Tnk-β-glu and Hu-β-glu residues, respectively. Sequence similarity analysis indicated that Hu-β-glu has similarities of 36.10, 30.70 and 45.70% with Sm-β-glu, Ma-β-glu and Tnk-β-glu, respectively.

Analysis of the binding sites of miglitol and β-glucosidases:

In order to prove the reliability of the docking process, the native ligands derived from the X-ray crystallographic structures were redocked into the active sites of the four β-glucosodases using the same parameters described in this text (fig. 2). Results indicated that the active site of the Sm-β-glu (PDB ID: 3CMJ) was composed of amino acids Gln57, His158, Trp159, Asn202, Glu203, Tyr332, Glu337, Trp360, Glu441, Trp434 and Phe450, which were consistent with the published data[35]. Similar to the information reported in PDB, amino acids surrounded the active site of the Ma-β-glu (PDB ID: 3W53) were Gln25, His125, Trp128, Glu170, Cys173, Tyr300, Trp349, Glu377, Glu431, Trp432 and Phe440. From the fig. 2, it can also be seen that amino acids located in the active site of the Tnk-β-glu (PDB ID: 3VIL) were Gln45, His148, Trp149, Thr196, Asn253, Asn335, Tyr337, Trp374, Glu402, Trp444, Glu451, Trp452, Phe460 and Phe480. These were extremely identical with the reported data[36]. Similarly, amino acids Gln17, His120, Phe121, Asn164, Gln165, Phe225, Tyr309, Trp345, Glu373, Glu424, Trp425 and Phe433 were identical with data on the Protein Data Bank about the Hu-β-glu (PDB ID: 3VKK). The values of the binding free energies of the native ligands were –6.63, –8.18, –8.91 and

–7.13 kcal/mol for Sm-β-glu, Ma-β-glu, Tnk-β-glu and Hu-β-glu, respectively. It was noticed that the docked structures derived from the molecular docking process in the text were very consistent with the original ligands located in the crystallographic complexes structures (fig. 3). Their similarities concluded by spin alignment method were 0.889, 0.976, 0.916 and 0.898 for Sm-β-glu, Ma-β-glu, Tnk-β-glu and Hu-β-glu, respectively (This value is between 0~1, the larger the value, the higher the similarity of the molecules). All of the root-mean-square deviation (RMSD) values were less than 2.0 Å, which suggested good models[37].

To explore the geometrical shape complementation, the poses of the miglitol in the binding pockets of the four β-glucosidases were shown (fig. 4). The values of the binding free energies of the miglitol

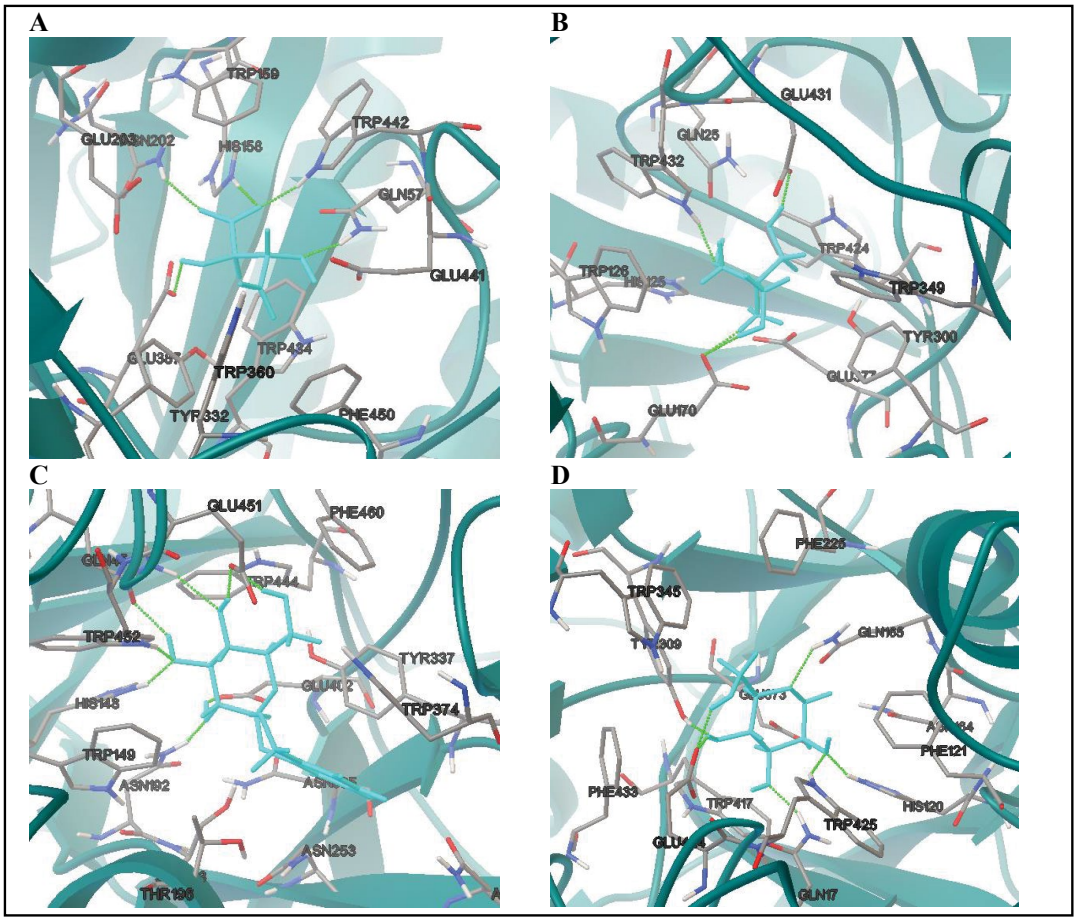

Fig. 2: The bonding active sites of native ligands (cyan).

In Sm-β-glu (A), Ma-β-glu (B), Tnk-β-glu (C) and Hu-β-glu (D), respectively. Hydrogen bonds are depicted by green dashed lines.

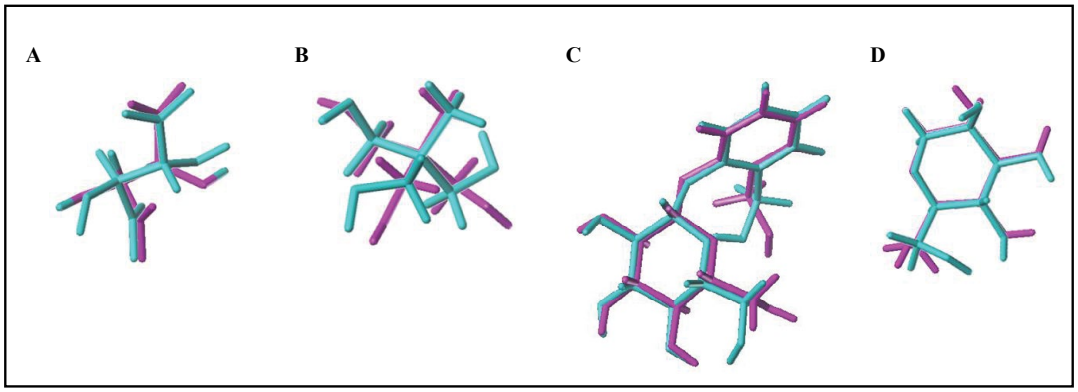

Fig. 3: The ligands derived from the X-ray crystallographic structures (purple).

Structures of 3CMJ (A), 3W53 (B), 3VIL (C), 3VKK (D) are compared with the conformations obtained from molecular docking process (cyan).

were –7.53, –6.44, –9.09 and –4.43 kcal/mol for Sm-β-glu, Ma-β-glu, Tnk-β-glu and Hu-β-glu, respectively. In the three complexes, all small molecule poses were stuck onto the walls of the pockets and embedded into the active sites, which comprised by random coil structure, showing very good geometric complementation. In order to further reveal the key residues involved in the binding sites, the detailed interactions between the molecules and β-glucosidases were analyzed.

Molecular docking results indicated that miglitol was located in a cavity formed by random coil structure of Sm-β-glu. The active site was surrounded by Gln57, His158, Trp159, Asn202, Glu203, Glu387, Glu441, Trp442 and Phe450 (fig. 5A). It was shown that there existed non-bonded interactions between the small molecule and the protein, including H-bonds bonding, van der Waals and electrostatic forces. There are eleven residues around the active site of Ma-β-glu, namely Gln25, Trp126, Glu170, His125, Cys173, Tyr300, Trp349, Trp432, Glu431, Phe440 and Trp424. The polar hydrogen atoms H19, H18, H16 and H17 could interact with nearby OE1 of acidic amino acids Glu by H-bonds except H15 (Table 2). The O4 and O1 also form two H-bonds with Asn202 (1.621 Å) and Gln57 (1.836 Å), respectively. The docking result (fig. 5B) indicated that exocyclic N-C bond rotates clockwise about 100° from the optimized stable conformation, which could lead to variation of the spatial positions for other polar hydrogens. Compared with fig. 5A,

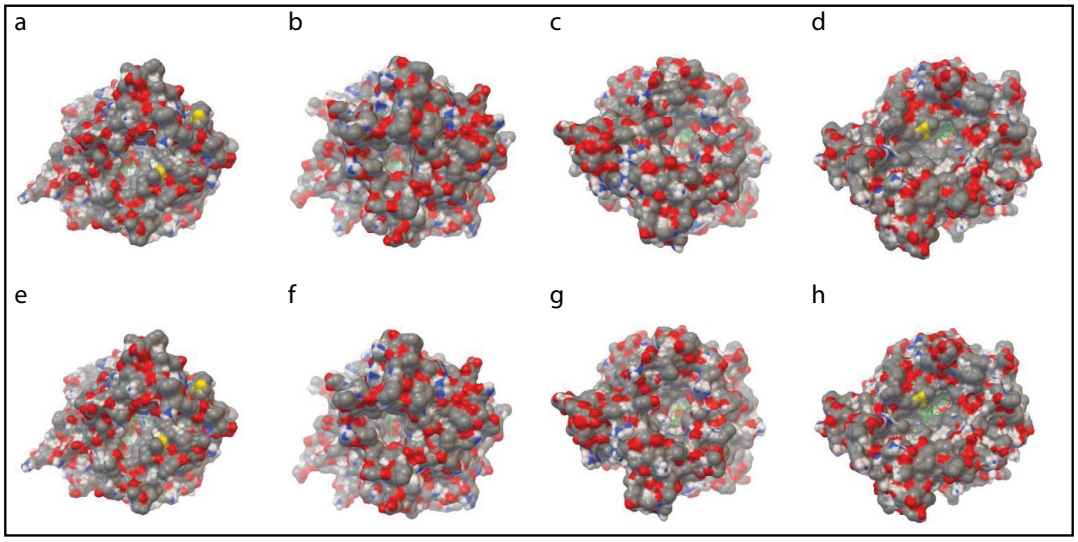

Fig. 4: The interactive sites miglitol, quercetin and β-glucosidases.
a. migltol and Sm-β-glu, b. migltol and Ma-β-glu, c. migltol and Tnk-β-glu, d. migltol and Hu-β-glu; e. quercetin and Sm-β-glu, f. quercetin and Ma-β-glu, g. quercetin and Tnk-β-glu, h. quercetin and Hu-β-glu; drug molecules are represented by fluorescent green and protein surface were painted by atom type.

TABLE 2: H-BONDS BETWEEN TWO DRUGS AND β-GLUCOSIDASE FROM SOIL METAGENOME

Ligand name	No. of H-bonds	Ligand atom	Residue name	Residue atom	Length/Å
Miglitol	1	H19	Glu203	OE1	1.777
	2	O4	Asn202	HD21	1.621
	3	H18	Glu387	OE1	2.061
	4	O1	Gln57	HE22	1.836
	5	H16	Glu441	OE1	1.71
	6	H17	Glu441	OE1	2.183
Quercetin	1	O6	Trp442	HE1	1.991
	2	O7	Gln57	HE22	1.848
	3	H32	Glu441	OE1	2.081
	4	O3	Asn262	HD21	2.095
	5	O5	Phe261	HN	2.124
	6	H30	Phe261	O	1.781

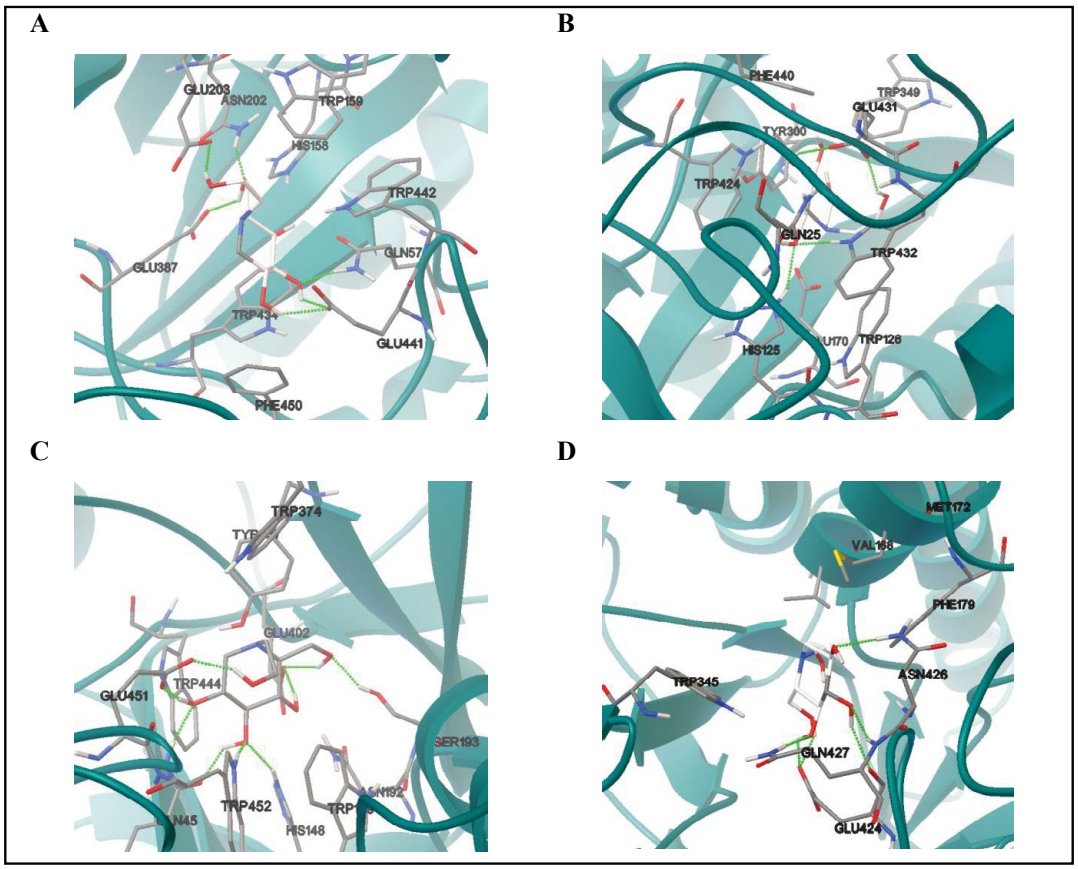

Fig. 5: The bonding active sites of miglitol.
(A) miglitol-Sm-β-glu; (B) miglitol-Ma-β-glu; (C) miglitol-Tnk-β-glu; (D) miglitol-Hu-β-glucomplexes.

obviously, it was shown that H15 can interact with acid Glu431 (2.214 Å) through H-bond (Table 3).

Results showed that the binding pocket of Tnk-β-glu was mainly formed by Gln45, His148, Trp149, Asn192, Ser193, Tyr337, Trp374, Glu402, Trp432, Trp444 and Glu451 (fig. 5C). Miglitol was stabilized by the Tnk-β-glu residues via hydrogen bonds, van der Waals and electrostatic forces. And the most number of H-bonds were observed in the four β-glu-miglitol complexes, involving residues Glu451 Ser193 Glu402 Gln45 Tyr452 and His148 (Table 4). It can be seen from fig. 5C, that the location of -CH_2CH_2OH group was greatly different from the one in fig. 5B, and other polar hydrogen atoms all changed their position accordingly. Polar hydrogen atoms were prone to interact with acid Glu by hydrogen bonds except the H_{16} atom.

The number of hydrogen bonds formed between the Hu-β-glu and miglitol molecule were the least (Table 5). Among the residues involved in the active sites, there were residues Val168 and Met172 in a small α helix, which played no significant role in the stability for both antidiabetic drugs (figs. 5D and 6D). Surrounding the binding pocket of Hu-β-glu, five residues, namely Trp345, Phe179, Glu424, Asn426 and Gln427 (fig. 5D). Miglitol interacted with adjacent residues through six H-bonds, namely Glu424, Asn426 and Gln427 (Table 5).

Analysis of quercetin and β-glucosidase binding sites:

In the four complexes (figs. 4E-H), all small molecules were also embedded into the active sites, displaying a very good geometric complementation. Compared with miglitol, the quercetin showed lower binding free energies for Sm-β-glu, Ma-β-glu, Tnk-β-glu and Hu-β-glu, which were –9.0, –8.62, –9.38 and –6.55 kcal/mol, respectively, which suggested stronger inhibitory ability. This was consistent with the reported results[38]. As for Sm-β-glu, the quercetin was fixed in the binding pocket composed of residues Gln57, His158, Asn260, Phe261, Asn262, Asn330, Tyr331, Tyr332, Trp434, Glu441 and Trp442. There were mainly hydrogen bonds and hydrophobic interactions (fig. 4A) between the complexes. The residues involved in H-bonds were mainly polar amino acids, such as Trp442, Gln57 and Asn262 (Table 2).

It was shown that residues in the binding site of Ma-

TABLE 3: H-BONDS BETWEEN TWO DRUGS AND β-GLUCOSIDASE FROM *MICROCOCCUS ANTARCTICUS*

Ligand name	No. of H-bonds	Ligand atom	Residue name	Residue atom	Length/Å
Miglitol	1	H19	Glu431	OE2	2.035
	2	O4	Trp432	HE1	2.005
	3	O4	His125	HE2	2.245
	4	H18	Gln25	OE1	2.024
	5	H15	Glu431	OE1	2.214
Quercetin	1	O6	Tyr300	HN	2.201
	2	H31	His301	O	1.999
	3	H32	His301	O	1.961
	4	H30	Glu377	OE1	2.011

TABLE 4: H-BONDS BETWEEN TWO DRUGS AND THE TNK-β-GLU

Ligand name	No. of H-bonds	Ligand atom	Residue name	Residue atom	Length/Å
Miglitol	1	H19	Glu451	OE2	1.922
	2	O4	Ser193	HG	1.904
	3	H18	Glu402	OE1	2.105
	4	H15	Glu402	OE2	1.871
	5	H16	Gln45	OE1	1.893
	6	O2	Tyr452	HE1	1.735
	7	O2	His148	HE2	2.1
	8	H17	Glu451	OE1	1.733
	9	O3	Gln45	HE21	1.918
Quercetin	1	H31	Asn255	OD1	2.151
	2	O6	Asn255	HD22	1.995
	3	H32	Asn255	OD1	1.924
	4	H28	Thr196	OG1	2.208
	5	O2	Ser193	HG	2.186
	6	H29	Glu402	OE1	2.239
	7	O3	His148	HE2	1.996
	8	H30	Glu451	OE1	1.798

TABLE 5: H-BONDS BETWEEN TWO DRUGS AND THE HU-β-GLU

Ligand name	No. of H-bonds	Ligand atom	Residue name	Residue atom	Length/Å
Migltol	1	H18	Glu424	OE2	1.712
	2	H15	Glu424	OE2	1.74
	3	O1	Gln427	HE21	1.911
	4	H16	Glu424	O	2.225
	5	O2	Gln427	HN	1.984
	6	O3	Asn426	HD21	2.11
Quercetin	1	H31	Glu424	O	2.179
	2	O6	Gln427	HN	2.057
	3	H32	Glu424	OE2	1.77
	4	O7	Gln427	HE21	1.689

β-glu-quercetin complexes was different from the one between Ma-β-glu and miglitol, whereas the ligands were all located in a cavity surrounded by random coil structure composed of Asn225, Tyr300, His301, Trp349, Glu377, Glu431 and Trp432. It could be easily observed that the most obvious variation of quercetin was the variation of the B ring compared with fig. 4A. This resulted in its H31 and H32 forming moderate

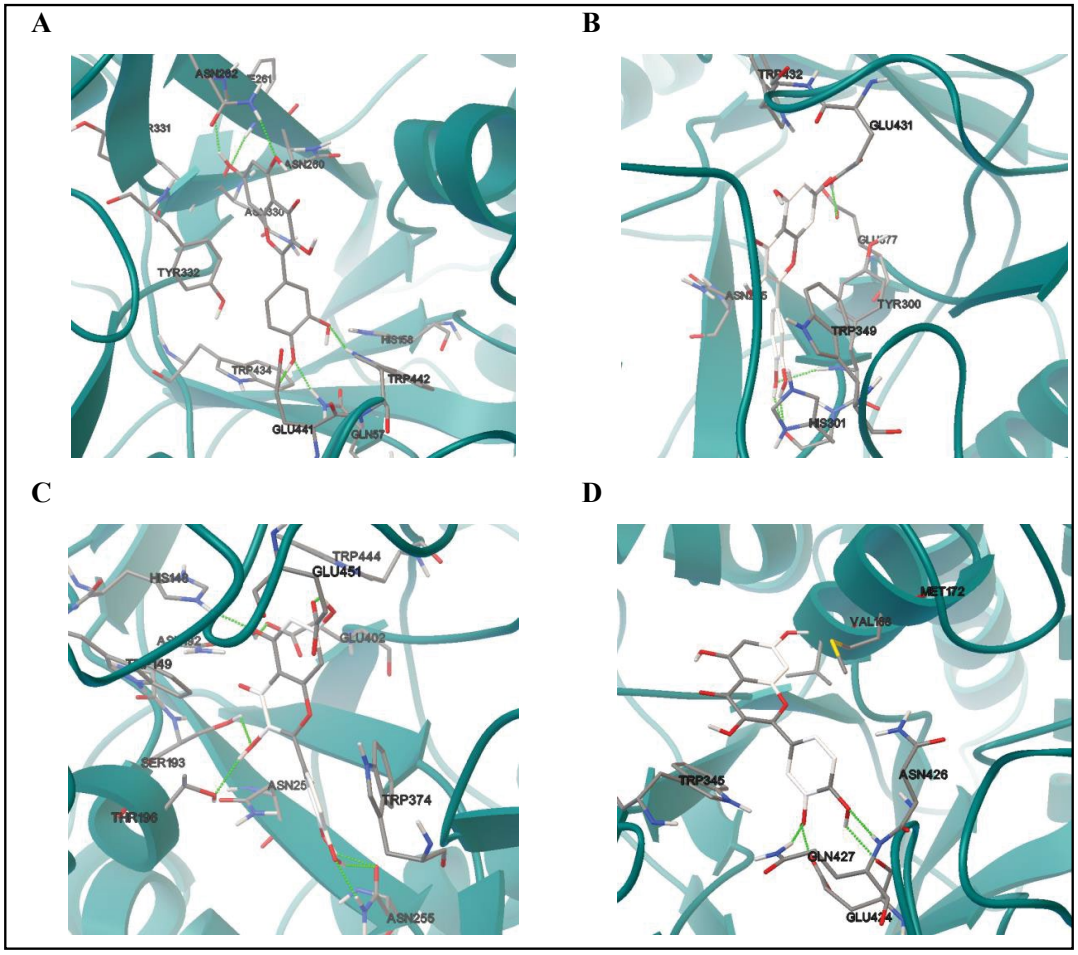

Fig. 6: The bonding active site of quercetin.
(A) quercetin -Sm-β-glu; (B) quercetin-Ma-β-glu; (C) quercetin-Tnk-β-glu; (D) quercetin-Hu-β-glucomplexes.

H-bonds with the O atom of basic His301, which had similar length of 1.999Å and 1.961Å, respectively (Table 3). The docking results of Tnk-β-glu complexes showed that quercetin and miglitol had similar binding pockets, which composed of residues Asn192, Ser193, His148, Trp149, Thr196, Asn253, Asn255, Trp374, Glu402, Trp444 and Glu451. Most of the residues involved in H-bonds interactions were identical, such as Glu451, Glu402, Ser193 and His148. Residue Asn255 played a considerably important role in the stability of Tnk-β-glu-quercetin complex, of which the OD1 and HD22 can form three H-bonds with H31, H32 and O7 of quercetin (Table 4). Quercetin remained the greatest number of H-bonds interactions with the Tnk-β-glu among all of the β-glucosidases, which might lead to the lowest binding affinity correspondingly.

However, there was the least number of H-bonds interactions when quercetin docked into the binding pocket of the Hu-β-glu. Residues composed by the H-bonds interactions were identical to miglitol, namely Glu424 and Gln427 (Table 5). Besides, there were Asn426, Val168, Met172 and Trp345 also involved in the binding site. In summary, there were two antidiabetic drugs miglitol and quercetin can form H-bonds with these β-glucosidases via some common residues, such as Glu441 and Gln57 for Sm-β-glu, Glu402, Ser193, His148 and Glu451 for Tnk-β-glu, Glu424 and Gln427 for Hu-β-glu. It can be speculated that these amino acids are extraordinarily critical to the binding of the two ligands with glucosidase enzyme, suggesting that quercetin is a good hypoglycemic agents with low IC_{50} value[39-42].

Geometrical conformation analysis of docking molecules:

In order to further analyze the inhibitory characteristics of miglitol, the conformations were listed before or after interacting with the β-glucosidases from different sources (fig. 7). Compared with the confirmation before docking, it could be seen from the figure that the most variation occurred was the $-CH_2CH_2OH$ group, which connected with N-atom via covalent

interaction. Especially the one after docking with Tnk-β-glu, -CH_2CH_2OH turned backward to the hexatomic ring, which made H1 interacting with residue Glu451 by H-bond. Actually, experimental results showed that their inhibition would be greatly increased when hydroxyl groups connected behind the miglitol ring[43]. Moreover, multiple hydroxyl groups that connected with the N atom could not be ignored[44,45].

Compared with previous docking conformation, it was shown that the –O4H18 hydroxyl group changed its geometrical conformation evidently, which might be mainly influenced by the -CH_2CH_2OH group. Compared to previous docking 61.1°, the dihedral angles of C_7—C_{12}—O_4—H_{18} were –81.7°, –1.5°, 10.2° and 43.2° while after docking with the Sm-β-glu, the Ma-β-glu, the Tnk-β-glu and the Hu-β-glu, respectively. This may lead H18 to interact with acid residue Glu by H-bonds easily in all the four β-glucosidases-miglitol complexes. From the molecular electrostatic potential, which was calculated from B3LYP optimized geometry, it could be seen that the negative (red and yellow) regions were related to electrophilic reactivity and the positive (blue) regions to nucleophilic reactivity (fig. 8A). The negative charge covers the H18 atom, which is the most reactive part in the miglitol molecule. In addition, there was a pair of H-bond between O4 and HG of Ser193 while –O4H18 group turned anticlockwise about 6.8~198.4° compared with previous docking conformations (fig. 9). Residue Ser193 (Thr196) help the miglitol stabilizing its conformation by H-bonds interactions, which was in the front (inner) of an alpha helix formed by additional nine residues from Pro194 to Ala202 (fig. 10A).

Other hydroxyls also changed their initial conformations into their better orientations, and thus were convenient for forming H-bonds with adjacent residues, such as H4 and H5. For example, H4 changed its position nearly 90°, which lead to three H-bonds interactions between H4, O4 and Gln45, Tyr452 and His148 (Table 4). Actually, multiple hydroxyl groups played a critical role in the stabilization of the miglitol at the catalytic sites of β-glucosidases[46-49].

The geometrical conformations of quercetin were also listed after interacting with the β-glucosidases from different sources (fig. 9). It can be seen that the position of B ring changed greatly, which might have some influence on variation of other hydroxyl groups. Compared with the conformation before

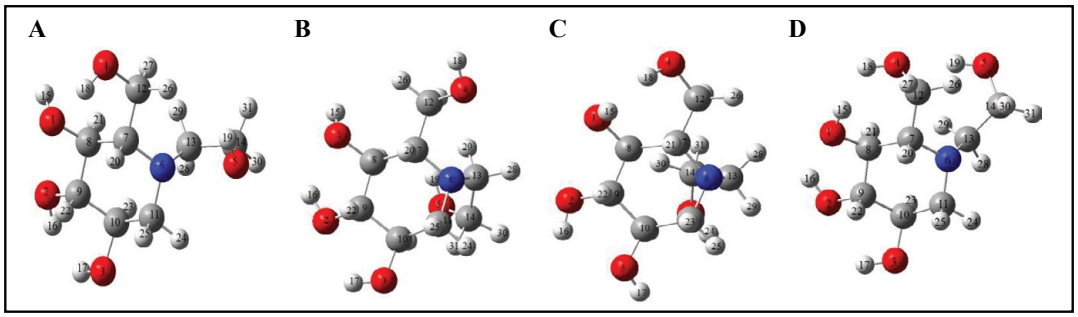

Fig. 7: The conformations of miglitol while after docking.
The conformations of miglitol while after docking with Sm-β-glu (A), Ma-β-glu (B), Tnk-β-glu (C) and Hu-β-glu (D).

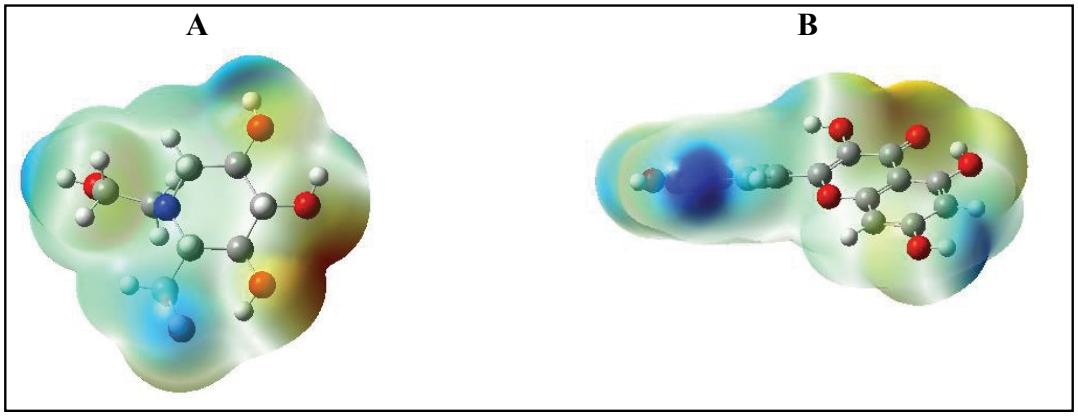

Fig. 8: Molecular electrostatic potential.
Molecular electrostatic potential of miglitol (A) and quercetin (B) calculated from B3LYP optimized geometry.

docking, the B ring of quercetin shifted clockwise by (a)→(b)→(c)→(d)→(e) order sequentially (fig. 9). However, this did not affect the H-bond numbers between surrounding amino acids and H1, O1, H2, O2 atoms, which were just located in the positive (blue) regions and acted as acceptors while forming H-bonds with other residues (fig. 8B). This was in accordance with the experiments, from which it can be speculated that most critical and significant role in the inhibition is contributed by the B ring of quercetin derivatives[50].

It can be observed, from figs. 6B-D that O4 can hardly form H-bonds with other residues except the Asn262 of the Sm-β-glu. Compared with fig. 9A, -OH3 group could remain the best geometrical conformation when rotating clockwise of about 30~45° centered by the C atom that connected with -OH_3. For example, H3 and O3 interacted with Thr196 and Ser193 by forming H-bonds via OG1 and HG, respectively. When -OH_3 group was toward ahead the B ring, the steric hindrance caused by B ring would greatly decrease. This effect was more significant while H3 was substituted by larger groups[51,52]. It was noteworthy that the B ring plays a very important role in the stability of β-glucosidases-quercetin complex. This was consistent with the α-glucosidases that the inhibition power greatly apparently increased with the replacement of hydroxyl groups of the flavones B-ring[53]. Hydrophobic interactions existed between A ring and residues Val168, which was located in a short α helix loop comprised by eleven amino acids residues from Gln165 to Asp175 (fig. 10).

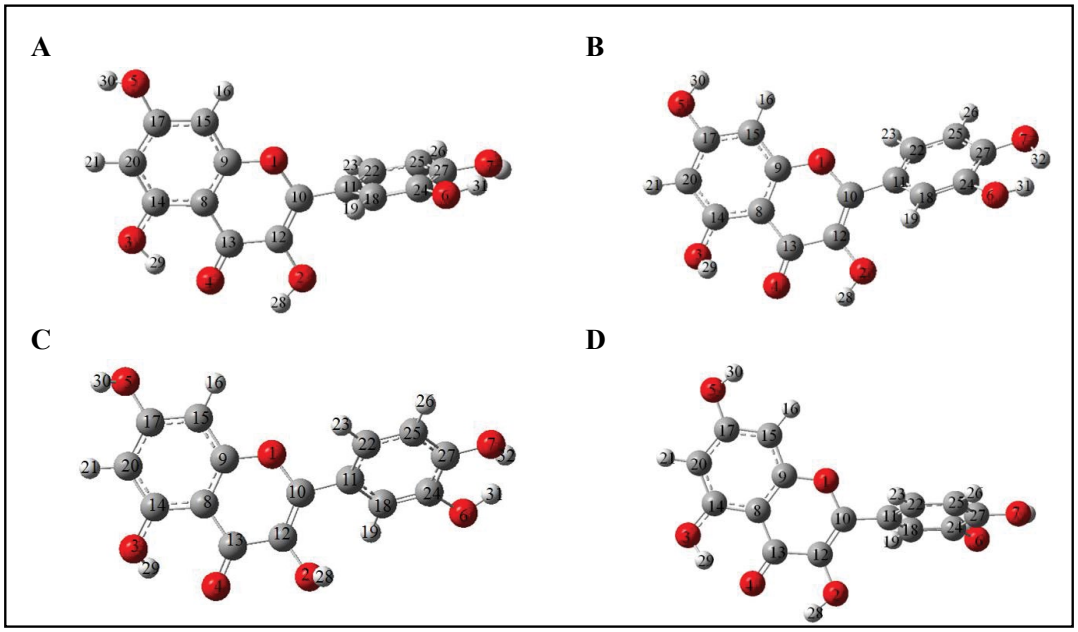

Fig. 9: The conformations of quercetin while after docking.
The conformations of quercetin while after docking with the Sm-β-glu (A), the Ma-β-glu (B),the Tnk-β-glu (C) and the Hu-β-glu (D).

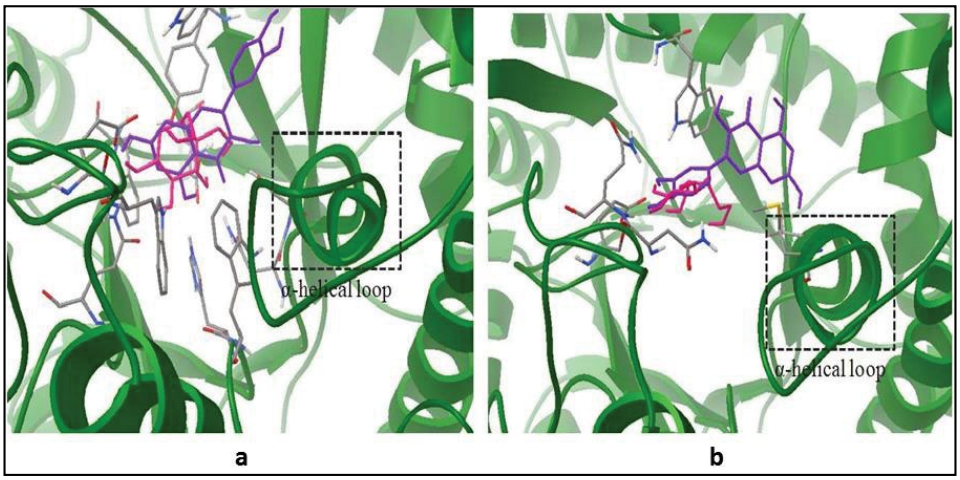

Fig. 10: The interactive sites of miglitol, quercetin Tnk-β-glu Hu-β-glu.
Tnk-β-glu (a) and Hu-β-glu (b). Miglitol is modeled by magenta sticks, and quercetin is represented by a purple stick-like models.

Sequence alignment analysis:

Finally, β-glucosidase protein sequence alignment was employed under the default settings. The results (fig. 11) revealed that although the similarities between these sequences are not very high, most of the amino acids residues related to the non-bonded interactions are conserved in the four β-glucosidases. The other three species are more conservative except the Hu-β-glu, especially the amino acids involved in forming H-bonds (indicated by blue marked letters). From the residues of the active sites, it was shown that 64.3% of the amino acids between Thk-β-glu and Sm-β-glu (Ma-β-glu) are identical, although the sequence similarities between them were only 34.9% and 33.3%, respectively. Although the sequence similarity between Thk-β-glu and Hu-β-glu was the highest (45.7%), residues that related the active sites had just only 14.3% identity.

DISCUSSION

It is critical to study the glucosidase inhibitors for further recognizing the type II diabetes. However, there have been a lot of reports about the inhibitors of α-glucosidase[54,55], the interactions between β-glucosidase and inhibitors were reported scarcely until now. In this article, molecular docking was employed to evaluate the binding modes between two drugs (synthetic and extraction from natural product) and four β-glucosidases from different species. Results showed that the binding orientations and geometrical conformation of miglitol (quercetin) were all different in all complexes, which may be related to the differences in binding affinities.

Compared with the conformations obtained at the B3LYP/6-31G* level, two molecules show different interactions in the active sites of four β-glucosidases. Compared with the optimized structures by DFT calculations, all of the conformations more or less altered after interacting with these β-glucosidases. From the two molecular electrostatic potentials, it can be seen that the negative (red and yellow) regions are related to electrophilic reactivity and the positive (blue) regions to nucleophilic reactivity. This is consistent with the molecular docking results. From the molecular structural point of view, actually, multiple hydroxyl groups are very important to the stabilization of the miglitol at the catalytic sites of β-glucosidases. In addition, hydroxyl groups of the quercetin B-ring are vital for its inhibition power. Finally, protein sequence alignment was performed under default parameters. Although the sequence similarity is not very high

Fig. 11: Amino acid sequence alignment.
Amino acid residues related to the non-bonded interactions are marked by red letters, while the blue letters denote the residues involved in H-bonds interactions.

between these β-glucosidases, most of the amino acid residues related to the active sites were conservative, especially the one involved in H-bond interactions.

Based on bioinformatics and molecular docking technology, the interactions between β-glucosidases and antidiabetic drugs are not only significant to further understand of the β-glucosidases, but also directive to design new inhibitors.

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Conflicts of interest:

There are no conflicts of interest.

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