Introduction

Several glycosidases catalyze the cleavage of glycosidic bonds in oligosaccharides or glycoconjugates and release glucose from the non-reducing end of the oligosaccharide chain. \(\alpha\)-glucosidase (EC 3.2.1.20; \(\alpha\)-glucosidase glucohydrolase) is an enzyme that catalyzes the cleavage of glycosidic bond in maltose [1]. Inhibition of the enzyme helps to absorb less glucose and suppresses digestion of carbohydrates since the carbohydrates are not hydrolyzed to glucose molecules [2]. Moreover, glycosidase inhibitors have proven useful to reduce postprandial hyperglycemia by preventing the digestion of carbohydrates, being effective for the treatment of type II diabetes and obesity [3-5].

Glycosidic derivatives are potential therapeutic agents for the treatment of disorders such as human immunodeficiency virus (HIV) infection, as well as diabetes, Gaucher’s disease, metastatic cancer, and lysosomal storage diseases, and can disrupt glycoprotein processing through direct-site irreversible glucosidase inhibition [6-8]. These derivatives have a profound role to play on this process because they mimic the disaccharide unit which is cleaved during protein processing through direct-site irreversible glucosidase inhibition [6-8]. These derivatives have a profound role to play on this process because they mimic the disaccharide unit which is cleaved during protein processing through direct-site irreversible glucosidase inhibition [6-8]. These derivatives have a profound role to play on this process because they mimic the disaccharide unit which is cleaved during protein processing through direct-site irreversible glucosidase inhibition [6-8]. These derivatives have a profound role to play on this process because they mimic the disaccharide unit which is cleaved during protein processing through direct-site irreversible glucosidase inhibition [6-8]. These derivatives have a profound role to play on this process because they mimic the disaccharide unit which is cleaved during protein processing through direct-site irreversible glucosidase inhibition [6-8]. These derivatives have a profound role to play on this process because they mimic the disaccharide unit which is cleaved during protein processing through direct-site irreversible glucosidase inhibition [6-8]. These derivatives have a profound role to play on this process because they mimic the disaccharide unit which is cleaved during protein processing through direct-site irreversible glucosidase inhibition [6-8]. These derivatives have a profound role to play on this process because they mimic the disaccharide unit which is cleaved during protein processing through direct-site irreversible glucosidase inhibition [6-8]. These derivatives have a profound role to play on this process because they mimic the disaccharide unit which is cleaved during protein processing through direct-site irreversible glucosidase inhibition [6-8]. These derivatives have a profound role to play on this process because they mimic the disaccharide unit which is cleaved during protein processing through direct-site irreversible glucosidase inhibition [6-8]. These derivatives have a profound role to play on this process because they mimic the disaccharide unit which is cleaved during protein processing through direct-site irreversible glucosidase inhibition [6-8]. These derivatives have a profound role to play on this process because they mimic the disaccharide unit which is cleaved during protein processing through direct-site irreversible glucosidase inhibition [6-8]. These derivatives have a profound role to play on this process because they mimic the disaccharide unit which is cleaved during protein processing through direct-site irreversible glucosidase inhibition [6-8].
very difficult because it has many loops in active site. Thus, here, new approach was introduced to solve this problem. Combined molecular modeling studies including molecular docking and molecular dynamics (MD) simulations were carried out to investigate structural rationales for the inhibitory activities of the stilbene derivatives, especially for compounds 6 and 12 (Figure 1). The compound 12 has two fluorine atoms on the C ring, while compound 6 has hydrogen atoms instead of fluorine. This subtle structural difference of the 12 with 6 makes much difference in binding affinities. Hence, to find out the proper reason for this, the MD simulations were performed two times for two different purposes: i) for adjustment of protein structure with the most active molecule, compound 12, and ii) for refinement of final docking poses. Based on these results, finally we have developed a reasonable pharmacophore model using receptor-ligand pharmacophore generation method.

Results/Discussion

Structure generation and validation of S. cerevisiae α-glucosidase

The α-glucosidase from S. cerevisiae was used in biological testing of the inhibitors for present study. The 3D structure of the protein is required to investigate the binding mode of stilbene derivatives within the α-glucosidase structure. The homology modeling of the protein has already been reported in several publications [12–14]. To construct the 3D structure of the α-glucosidase, homology modeling method was used like that of the previous studies. The structure of oligo-1,6-glucosidase from B. cereus (PDB ID: 1UOK) [15] was selected as template and the sequence alignment between α-glucosidase and the template was carried out using ClustalW2 package [16] (Figure 2A). According to this alignment, the α-glucosidase shares around 38.0% sequence identity and 62.0% sequence similarity with the template.

The 3D structure of α-glucosidase was generated by Build homology models protocol which implements MODELER program available in Discovery Studio [DS] 3.0 software [17]. The homology model was built by omitting the first 8 residues at the N-terminal region, since no sequence similarity was found for N-terminal residues from the sequence alignment. Figure 2B and 2C show the modeled structure of α-glucosidase compared with X-ray crystal structure of the template representing the three domains: the N-terminal, the subdomain, and the C-terminal domain. The catalytic triad residues (Asp199, Glu255, and Asp329) are found in the N-terminal domain of the template protein [15] while the catalytic triad in the α-glucosidase is formed by Asp214, Glu276 and Asp349 residues, respectively. The substrate binding site is located in the cleft between the N-terminal domain and the subdomain. Two His residues near to center of catalytic triad, His103 and His328 of oligo-1,6-glucosidase which may be required for substrate binding are also conserved in α-glucosidase (His111 and His348, respectively) [18].

The final structure of α-glucosidase generated from homology modeling was evaluated by two programs namely PROCHECK [19] and ProSA [20] to check the stereochemical quality. The ramachandran plot obtained by PROCHECK program showed that 87.5% of residues of the final 3D structure lied in most favored regions better than that of the X-ray crystal structure of the template which has 86.3% residues (Figure 2D). The ProSA z-score value of the final model structure is −8.66 and the plot indicates that the overall model quality is within the range of scores typically found for proteins of similar size (Figure 2E).

Initial molecular docking results and validation

In order to gain insight into the most probable binding modes of the stilbene derivatives within the active site of α-glucosidase, the molecular docking simulations of the reported derivatives ([11]), Figure 3) were performed with the modeled structure of α-glucosidase using CDocker program [21].

For validating the CDocker docking protocol, the crystal structure of isomaltase from S. cerevisiae (PDB ID: 3MAA) co-crystallized with the α-D-glucose, which is part of inhibitor maltose, was used for additional homology modeling and docking simulation. Although the 2D structure of maltose is not similar with the further docked compounds, the sequence identity (39.6%) of this isomaltase enzyme with the template from B. cereus is similar to that (38%) of the modeled enzyme with the template (Figure S1A). Hence, we selected this different enzyme for validation process. To reproduce same protocol, homology modeling of the isomaltase was carried out using the template from B. cereus and then the modeled isomaltase was compared with its own crystal structure (Figure S1B). The root mean square deviation (RMSD) value between the homology model and crystal structure of isomaltase is 0.22 nm indicating that the homology model of α-glucosidase built by the template was validated. Subsequently, the docking simulation was performed based on the modeled isomaltase with the α-D-glucose. We compared the crystallographic conformation & position of α-D-glucose in the X-ray structure of the complex to its poses obtained by docking (Figure 4A). The hydrogen bond interactions of α-D-glucose with active site residues His112, Glu277, His351, Asp352, and Arg442 in the crystal structure also appeared in the docked poses of α-D-glucose. The root mean square deviation (RMSD) between the crystal and docked structure is 0.11 nm. This validation has proven that our docking protocol was reasonable in investigating the binding conformation accurately.

All the derivatives were well docked into the active site of the modeled structure. Due to the same scaffold of the derivatives,
binding modes of the derivatives were almost same each other.
From the binding mode comparison between compounds 6 and 12, same hydrogen bond, π, and hydrophobic interactions were observed with only few different interactions (Figures 4B to 4D). Moreover, the negative CDOCKER energy score (34.31) of compound 6 is similar to the score (36.1) of compound 12. These results indicate that the docking simulation is not enough to explain the activity difference between compounds 6 and 12.
Thus, molecular dynamics (MD) simulation was implemented to better understand this difference. To do this, an improved binding mode of the derivatives was required. Hence the initial docking pose of compound 12 which is the most active one was subjected into MD simulation during 20 ns.

Figure 2. Sequence alignment and homology modeling for S. cerevisiae α-glucosidase using a template B. cereus oligo-1,6-glucosidase. (A) Sequence alignment of S. cerevisiae α-glucosidase (represented as YEAST) with B. cereus oligo-1,6-glucosidase (1UOK). Sequence identities are denoted by asterisks (*), conservative substitutions by colons (:), and semi-conservative substitutions by dots (.). The catalytic residues are indicated in a red box. Comparative view of the homology modeled structure S. cerevisiae α-glucosidase (B), the template structure of B. cereus oligo-1,6-glucosidase (PDB ID: 1UOK) (C) with the conserved catalytic residues represented as sticks. The N-terminal, subdomain, and C-terminal domains are shown in blue, orange, and yellow, respectively. (D) Ramachandran plot of the ϕ/ψ distribution of the homology model as obtained by PROCHECK. (E) Z-score plot for our modeled structure shows that the score is within the range of scores typically found for native proteins of similar size.
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Figure 3. 2D chemical structures of stilbene derivatives with experimental binding affinity value such as Ki and IC50.
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Selection of the best adjusted structure using interaction energy and negative CDOCKER energy

Four 20 ns MD simulations (Apo, first, second, and third trials) were carried out to obtain a reasonable structure adjusted in compound 12-bound state. These three trial simulations are started from the same system with same conditions to approach global minimum conformation of the complex. The Cα RMSD analysis showed that all systems were well stabilized at around 0.3 nm (Figure 5A). Although the third trial system has relatively higher Cα RMSD than the other systems, the RMSD of compound 12 is well maintained after 15 ns. The value of RMSD for compound 12 is measured, after superimposition of ligand conformations in initial and each time step. In first and second trial systems, compound 12 is also well stabilized (Figure 5B).

Based on the RMSD results, the closest frame to average structure during the last 5 ns was selected as the representative structure of each trial. From the comparison of initial docked structure and the three representative structures, similar binding mode was observed but average poses mostly different from each other (Figure 5C to 5F). Among these different three local minima, global minimum conformation, the best adjusted conformation for compound 12, was selected by computing and comparing averaged interaction energies (sum of columnic and van der Waals energies) in the last 5 ns of simulation (Table 1). Some publications reported that correlation of the interaction energy with binding affinity can be observed positively [22,23]. The lowest averaged interaction energy (-253.253 kJ/mol) was shown in the second trial system compared to the other systems. Hence, we concluded that the adjusted protein structure in the second trial system is the closest to the global minimum conformation.

In order to check whether the lowest energy structure is more reasonable for binding of stilbene derivatives than the homology modeled one, negative CDOCKER energies were also compared after conducting a several molecular docking simulations of compound 12 with the representative protein conformations (Figure 6). As expected, the lowest interaction energy value of -73.0613 was observed in the docked pose of compound 12 in second trial system compared to the other systems. Hence, we concluded that the adjusted protein structure in the second trial system is the closest to the global minimum conformation.

Three trial MD simulations of initial docked compound 6-bound state were also conducted to check whether the homology modeled structure which is in Apo state is reasonable to bind the...
derivatives. As clearly shown in compound 6-bound trial systems as well as compound 12-bound trial systems, the results of MD simulation with compound 6 were deviated from the proper binding region indicating that inappropriate starting conformation of the protein was used. But, after using the best adjusted conformation in the compound 12-bound state, the structure of

Table 1. Averaged interaction energy of compound 12 obtained from MD simulation.

| System     | Interaction Energy (kJ/mol) | Van der Waals Energy (kJ/mol) | Electrostatic Energy (kJ/mol) |
|------------|-----------------------------|-------------------------------|-------------------------------|
| Comp12_1st | –235.876                    | –168.048                     | –67.8278                     |
| Comp12_2nd | –253.253                    | –202.956                     | –50.2964                     |
| Comp12_3rd | –203.842                    | –159.364                     | –44.4776                     |

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compound 6 was maintained stably during the simulation time (Figure 7A). From these results, we can suppose that the best adjusted conformation by the most active compound was required to find a more reasonable binding mode of the derivatives at least in this system.

These comparative analyses suggested that the adjusted protein conformation in second trial system is the suitable structure rather than the other ones. Thus, the lowest energy protein structure was used for molecular docking simulation of the derivatives.

Molecular docking and molecular dynamics simulations with the adjusted protein conformation

In order to find out the most reasonable binding mode, molecular docking and MD simulations of the derivatives were performed with the adjusted protein structure having lowest energy conformation. Initial docking poses of derivatives in adjusted protein structure were well overlaid in same binding mode showing only subtle difference in the C rings (Figure S3).

Table 2. Interaction energy and negative CDOCKER energy of compound 12 obtained from molecular docking simulation.

| System            | Interaction Energy (kJ/mol) | Van der Waals Energy (kJ/mol) | Electrostatic Energy (kJ/mol) | –CDOCKER Energy (kJ/mol) | –CDOCKER Interaction Energy (kJ/mol) |
|-------------------|-----------------------------|-------------------------------|-------------------------------|--------------------------|-------------------------------------|
| Initial (CDOCKER) | –59.2922                    | –41.7959                      | –17.4963                      | 36.098                   | 47.5829                             |
| Apo               | –66.9361                    | –43.52                        | –23.4162                      | 39.1709                  | 51.3061                             |
| Comp12_1st       | –59.0961                    | –43.039                       | –16.0571                      | 35.7489                  | 49.0586                             |
| Comp12_2nd       | –73.0613                    | –48.1349                      | –24.9264                      | 44.1057                  | 57.4398                             |
| Comp12_3rd       | –57.3609                    | –38.5771                      | –18.7839                      | 31.1857                  | 44.715                              |

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These poses have lower scores than the first docking results. But, to obtain more refined poses, MD simulations were conducted. Hence eight 1.5 ns MD simulations were carried out and analyzed (Figure 7). Unlike the simulation results of the homology modeled structure in Apo state, all the structures are well converged in similar binding mode (Figure 7B). The Ca RMSDs of the systems also showed that the deviation (around 0.15 nm) of the structure from initial one was much lower than previous MD simulations (around 0.30 nm) at the same time (Figure 7A). In addition, calculated interaction energies were maintained stably for almost compounds (Figure 7C). But, the electrostatic energy of compound 10 was highly unstable compared to the other system (Figure S2). These results indicate that the structures except for compound 10-bound state are maintained stably during the simulation time due to the adjusted protein conformation.

In order to evaluate whether the binding mode is reasonable, correlation was calculated between experimental Kᵢ value and the interaction energy obtained from the eight 1.5 ns MD simulations. As a result, the correlation coefficient value was 0.89 (Figure 7D). This means that there’s a positive linear correlation between calculated interaction energy and experimental Kᵢ value (Table 3). Thus, the binding mode of the derivatives with the protein is suitable to use receptor-ligand pharmacophore model generation.

Comparison of final docking poses refined by MD simulation

When the final binding modes of the derivatives were compared, those of the two compounds 6 and 12 on behalf of eight derivatives were focused on to analyze the differences of their binding affinities because the compound 12 is the most active molecule and the structural difference of the 12 with 6 makes much difference in binding affinities. The structural difference is that compound 12 has two fluorine atoms on the C ring, while compound 6 has hydrogen atoms instead of fluorine. Due to this subtle difference, the binding affinity of 12 is 3 folds higher than of 6. To analyze the reasons for this, the binding modes of the compounds were compared using the 1.5 ns snapshot. Several differences were observed in hydrogen bonding interaction but the

Table 3. Correlation between exp. Kᵢ and calculated energy.

| System  | Experimental Kᵢ (μM) | Interaction Energy (kJ/mol) |
|---------|----------------------|----------------------------|
| Comp6   | 10.6                 | -245.265                   |
| Comp7   | 10.5                 | -252.126                   |
| Comp11  | 7.2                  | -281.353                   |
| Comp12  | 3.2                  | -287.378                   |
| Comp13  | 4.6                  | -292.982                   |
| Comp14  | 17                   | -226.249                   |
| Comp16  | 9.4                  | -233.907                   |

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Figure 7. Results of MD simulations of stilbene derivatives starting with docked structure in the second trial system. (A) Ca RMSD plot of different stilbene derivative-bound systems. (B) Overlapped structure of compound 6 (orange), compound 7 (red), compound 10 (bluish green), compound 11 (violet), compound 12 (green), compound 13 (sky blue), compound 14 (light blue), and compound 16 (light violet). (C) Interaction energy plot of all systems during the 1.5 ns simulation time. (D) Correlation graph between experimental Kᵢ value and interaction energy. doi:10.1371/journal.pone.0085827.g007
other interactions are similar with each other. Hence, to find out the clear reason of the activity difference and to obtain more refined binding mode of the compounds, two MD simulations of compounds 6 and 12 bound systems were extended to 10 ns. To quantitatively compare binding mode difference between 1.5 ns and 10 ns simulations, final snapshot of 1.5 ns and representative structure (9,046 ps) of 10 ns simulations were superimposed by protein and then RMSD of protein (0.15 nm) and compound 12 (0.09 nm) was calculated and compared between two simulations. From the result, we found that the binding modes obtained from the two simulations are similar with each other. But, we wanted to obtain more refined structure for further analysis and pharmacophore model generation. Hence, the closest frame to average structure during the last 2 ns was selected as representative structure.

Many different interacting points are observed upon comparison of the refined docking conformations for 6 and 12 by analyzing through monitor command in DS 3.0. Whereas the compound 12 was found to have four hydrogen bonding interactions with Glu276, Val303, Thr307, and His348, the compound 6 having no fluorine atom has formed three hydrogen bonds with Glu276, Ser306, and Arg312 (Table 4). The time occupancies of hydrogen bonds for compound 6 are relatively higher than those for compound 12. But, one of the hydroxyl groups of the A ring in both compounds formed strong hydrogen bond interaction with Glu276 which is one of catalytic triad residues (Figure 8). The number of interacting residues involved in charge or polar interaction in 12 was higher than in 6. In π interactions comparison, same π-sigma and π-π interactions in both compounds were formed with Glu304 and Phe311, respectively.

In order to provide another clear reason of the activity difference in terms of dynamic behavior, distances of π-sigma interaction between Glu304 and each compound were measured and compared during the simulation time (Figure 9). The distance should be less than 0.5 nm to form a π-sigma interaction [24]. Some cases showed that involvement of π-sigma interaction play an important role in protein-ligand interaction [25–27]. Whereas the distance between γ-carbon of Glu304 and compound 12 was maintained stable, the distance in case of compound 6 was deviated out of the range. This indicated that the π-sigma interaction could also be one of the key interactions to explain the activity difference in terms of dynamic behavior.

To find out the effect of π-sigma interaction, interaction energy difference of the several snapshots with and without π-sigma interaction was calculated. To compare mostly similar frames excepting the π-sigma interaction only, several 1 ps different snapshots were selected and then differences of interaction energy between each two snapshots were calculated. For example, the difference between 9,784 ps (~307.62) and 9,785 ps (~280.93) snapshots was 26.69 kJ/mol and each distance of interaction between Glu304 and each compound were measured and compared during the simulation time (Figure 9). The distance difference in terms of dynamic behavior, differences of the several snapshots with and without π-sigma interaction was calculated and compared between two simulations. From the result, we found that the binding modes obtained from the two simulations are similar with each other. But, we wanted to obtain more refined structure for further analysis and pharmacophore model generation. Hence, the closest frame to average structure during the last 2 ns was selected as representative structure.

Conclusions

The main purposes of this study were to find out the most suitable binding conformations of stilbene derivatives and to explain the differences of binding affinity and then finally to develop a receptor-ligand pharmacophore model. We constructed the homology modeled structure of S. cerevisiae α-glucosidase with human α-amylase. Although the overall sequence identity (17.4%) and similarity (34.1%) of modeled yeast and human enzymes are in low level, sequence identity (33.3%) and similarity (52.4%) of interaction residues for compound 12 is much higher than those of the rest sequences (Figure S4). The catalytic triad residues Asp214, Glu276, and Asp349 and substrate binding residues His111 and His348 are identical between human and yeast. These results suggested that our pharmacophore model is also useful for human α-amylase which is the main pharmaceutical target for stibene derivatives.
Especially, \( \pi \)-sigma interaction of Glu304 with each compound could also be one of the key reasons to explain the activity difference in terms of dynamic behavior. Finally, a proper four featured pharmacophore model was generated using the validated compound 12-bound structure obtained from combining approach of docking and MD simulation. Interestingly, we also obtained a good agreement between the experimental Ki and the calculated fit values. These results will be helpful for understanding the relationship between binding mode and bioactivity of the stilbene derivatives and then for designing better inhibitor.

### Methods

**Homology modeling**

The 3D structure of \( S.\text{cerevisiae} \) \( \alpha \)-glucosidase was built by homology modeling method. The crystal structure of \( B.\text{cerus} \) oligo-1,6-glucosidase (PDB ID: 1UOK, 2.00 Å resolution) was used as template. Sequence alignment between \( S.\text{cerevisiae} \) \( \alpha \)-glucosidase and the template was carried out using ClustalW2 package in EMBL-EBI (www.ebi.ac.uk). The Build homology models protocol available in Discovery Studio (DS) 3.0 software [17] was used to create 3D structure of \( S.\text{cerevisiae} \) \( \alpha \)-glucosidase sequence based on an alignment with template. The final structure

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**Figure 8. Binding modes of compound 6 (orange) and compound 12 (green) obtained from 10 ns MD simulation.** (A) Hydrogen bonding interactions (light blue line) of compound 6 with Glu276, Ser308, and Arg312 are displayed with \( \pi \)-interacting residues (orange line). (B) Interactions of compound 12 with four hydrogen bonding residues including Glu276, Val303, Thr307, and His348 are represented with \( \pi \)-interacting residues: Glu304 for \( \pi \)-sigma and Phe311 for \( \pi \)-\( \pi \) interactions. 2D interaction diagram of compound 6 (C) and compound 12 (D) with representing charged (pink plate), \( \pi \) (orange line), and hydrophobic (light blue plate) interacting residues.

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**Table 4. Hydrogen bonding and hydrophobic contacting residues between protein and compound.**

| Ligand | Hydrogen bonding residues (Time occupancy during the last 2 ns) | Residues involved in charge or polar interaction | Hydrophobic contacting residues |
|--------|---------------------------------------------------------------|-------------------------------------------------|-------------------------------|
| Compound 6 | Glu276 (99.95%), Ser308 (80.95%), Arg312 (97.65%) | Arg212, Asp214, Pro309, Phe311 | Phe157, Phe158, Phe177, His239, Pro240, Phe300, Val303, Glu304, Thr307, Asp349, Gln350 |
| Compound 12 | Glu276 (99.75%), Val303 (26.6%), Thr307 (67.2%), His348 (57.35%) | Tyr71, Arg212, Asp214, Glu304, Pro300, Phe311, Gln350 | Phe157, Phe158, His239, Pro240, His279, Ser308, Pro309, Arg312, Asn347, Asp349 |

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was validated by PROCHECK [19] for the evaluation of ramachandran plot and Protein Structure Analysis (ProSA) [20] from ProSA-web.

Molecular docking simulation

For molecular modeling study, we mainly used two different programs such as CDOCKER and GROMACS for the respective purpose, i) to generate a docking pose and ii) to refine the pose within a solvated system. CHARMM force field in CDOCKER program is only used in active site region, but the Amber force field in GROMACS is used for whole system including protein, ligand, water, and ions. The CDOCKER [21] which has a significant advantage in full ligand flexibility including bonds, angles, and dihedrals is a CHARMM based docking tool to predict putative geometry of a protein-ligand complex. The CDOCKER docking simulations were performed to evaluate the binding mode of stilbene derivatives within active site of homology modeled α-glucosidase. The centroid point was generated at the center of the catalytic triad which consists of Asp214, Glu276, and Asp349 in the protein and the active site defined as 15 Å around it. Hundred ligand conformations were generated from each initial ligand structure through high temperature (1,000 K) MD simulation (1,000 steps), followed by random rotations. The conformations were then translated into the defined active site. Then candidate poses were created by dynamics based simulated annealing refinement. In the refinement, the temperature is heated up to 700 K for 2,000 steps and then cooled to 300K for 5,000 steps. Out of top 20 docked poses, a docking pose with the highest negative CDOCKER energy was only used for comparison. The interaction energy (including van der Waals and electrostatics) was calculated after docking process. The docking methodology was validated with co-crystallized α-D-glucose, part of maltose, which is competitive inhibitor of the Saccharomyces cerevisiae isomaltase (PDB ID: 3A4A) by comparing the initial binding conformation in crystal structure and docked pose obtained from docking simulation of the α-D-glucose into the homology model of isomaltase structure. Docking modes and binding interactions were analyzed by 2D diagram visualization and monitor command in DS 3.0 software [17].

Molecular dynamics simulation

Totally, 12 MD simulations were performed using the GROMACS program (version 4.5.3) [28,29] with AMBER03 [30] force field. The initial structure was immersed in an orthorhombic water box (1 nm thickness) and the net charge was neutralized by the addition of Na⁺ counterions. The long range electrostatic interactions were calculated by the particle mesh Ewald (PME) method [31]. In entire system, protein alone consists of 9,293 atoms and is made up of approximately 80,000 atoms which include about 23,000 water molecules. The general Amber force field (GAFF) [32] parameter was used for the compounds and the atomic partial charges were calculated by the semi-empirical quantum chemistry program SQM [33] via ANTECHAMBER 1.5 [34] and ACPYPE web portal (http://www.ambient الأدلة-ac.uk/ccp4/ software/acpype/). The systems were subjected to a steepest descent energy minimization process to remove possible bad contacts from initial structures until a tolerance of 1,000 kJ/mol. During the system equilibration process the heavy atoms were restrained and the solvent molecules with the counterions were allowed to move during the 100 ps under NPT conditions at 300 K. Bonds between heavy atoms and corresponding hydrogen atoms were constrained to their equilibrium bond lengths using
the LINCS algorithm [35]. The equilibrated structures were used to perform the production runs. A constant temperature and pressure for the whole system (300 K and 1 bar) are achieved with the V-rescale thermostat [36] and Parrinello-Rahman [37] barostat. The time step for the simulations was set to 2 fs and the coordinate data were written to the file every pico second (ps). All the analyses of the MD simulations were carried out by GROMACS, DS 3.0, and VMD software. To analyze the protein-compound interactions for the final MD simulation result, monitor command in DS3.0 was used. Each threshold for hydrogen bond distance and D-H-A angle range is set to about 3.0 Å and from about 90 to 180 degrees, respectively. VMD analysis tool [38] was also used to calculate the hydrogen bond occupancy (%) with same distance and angle range thresholds used in DS3.0.

**Pharmacophore generation and validation**

The representative structure taken from final 10 ns MD simulation of compound 12-bound system was used to generate the receptor-ligand pharmacophore model finding the pharmacophoric features in the active site and important for ligand binding. Four to six features (default) in the receptor-ligand pharmacophore generation algorithm were chosen to extract useful pharmacophores of reasonable size from all the receptor-ligand interactions. Receptor-ligand pharmacophore generation was carried out by pharmacophore generation tools in DS 3.0 software with default parameters for further use in the screening for new lead derivatives. This protocol generates selective pharmacophore models from the features corresponding to the receptor-ligand interactions. Based on the generated pharmacophore model, scale fit values of the stilbene urea derivatives were calculated by ligand pharmacophore mapping tools implemented in DS. For calculating the fit value, conformation of each compound in the final snapshot of 1.5 ns MD simulation was used. Fitting method was set to flexible which is slightly modified to better fit into the pharmacophore model. As validation of the generated pharmacophore model, the correlation analysis was conducted between the scale fit value and the Kᵢ value.

**Table 5. Correlation between exp. Kᵢ and scale fit value.**

| System | Time, ns | Exp. Kᵢ (µM) | Scale fit value |
|--------|----------|----------------|-----------------|
| Comp6  | 10 (1.5) | 10.6           | 0.93 (0.95)*    |
| Comp7  | 1.5      | 10.5           | 0.89            |
| Comp10 | 1.5      | 12.1           | 0.76            |
| Comp11 | 1.5      | 7.2            | 0.91            |
| Comp12 | 10 (1.5) | 3.2            | 0.97 (0.95)*    |
| Comp13 | 1.5      | 4.6            | 0.88            |
| Comp14 | 1.5      | 17             | 0.49            |
| Comp16 | 1.5      | 9.4            | 0.73            |

*Scale fit value of each conformation for compounds 6 and 12 after 1.5 ns.
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Supporting Information

Figure S1 Sequence alignment and homology modeling structure of S. cerevisiae isomaltase using a template B. cereus oligo-1,6-glucosidase. (A) Sequence alignment of S. cerevisiae isomaltase (represented as 3AA4) with oligo-1,6-glucosidase (1UOK). The catalytic residues are indicated in a red box. (B) Comparative view of the homology modeled structure of S. cerevisiae isomaltase constructed by the template with its own crystal structure (PDB ID: 3AA4). The conserved catalytic residues are represented as sticks. The N-terminal, subdomain, and C-terminal domains for the homology model are shown in blue, orange, and yellow, respectively. The crystal structure of isomaltase is colored by black.

Figure S2 Electrostatic energy plot of all systems during the 1.5 ns simulation time. Energy values for all the other compounds are represented as transparent colors to highlight the energy values for compound 10.

Figure S3 Docking poses of stilbene derivatives in adjusted protein structure of the second trial system, which is the lowest energy conformation, with interacting residues which are highlighted by violet sticks.

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