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Molecular docking studies of potent inhibitors of tyrosinase and α-glucosidase

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Abstract Copper containing tyrosinase enzyme is responsible for melanin biosynthesis in human. Anomalous growth of this enzyme causes hyper-pigmentation related disorders. Melanoma-specific anticarcinogenic activity has also been associated with this enzyme. Recently reported metabolites of tibolone exhibited significant inhibitory activities against both tyrosinase and α-glucosidase enzymes. Molecular docking studies of these enzymes with those metabolites have been the focus of this study. It is comprehensively studied that the inhibition of α-glucosidase is crucial for glycemic control. The active site similarity between tyrosinase and α-glucosidase has also been observed. GOLD is utilized to investigate the conformation and binding affinities of newly discovered inhibitors. In both enzymes, metal ions seem to play an important role in establishing the interaction within the cavity of active sites. Results obtained by recent study are not only consistent with the experimental findings but also provide a deeper insight into the structural attributes and overall molecular interactions.

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Keywords Alpha-glucosidase · Binding sites · Inhibitors · Ligand interaction · Molecular docking · Tyrosinase

Introduction

Tyrosinase is a copper containing enzyme which belongs to oxidase super family protein. Tyrosinase enzyme is found in vegetables, fruits, and mushrooms. This enzyme catalyzes the main reaction of melanin biosynthesis; however, its abnormal accumulation is responsible for hyper-pigmentation related disorders (Priestly, 1993). The reactions catalyzed by this enzyme, i.e., the hydroxylation of a monophenol and the conversion of an *o*-diphenol to the corresponding *o*-quinone (Sanchez-Ferrer *et al.*, 1995) lead to melanin production which plays a vital protective role against skin photo-carcinogenesis (Priestly, 1993). In the formation of neuromelanin in the human brain, tyrosinase may play a part and could be principal to dopamine neurotoxicity. There are some findings of its contribution to Parkinson's disease (Xu *et al.*, 1997) and Melanoma-specific anticarcinogenic activity reported in literature (Prezioso *et al.*, 1992). A large number of compounds have been reported as tyrosinase inhibitors. Abnormalities in the skin pigmentation, like marks, discoloration, and defects are associated with disturbance of this enzyme in mammals (Oetting, 2000). There found some evidence about the linkage of this enzyme with neurodegenerative diseases (Xu *et al.*, 1998; Asanuma *et al.*, 2003).

Another enzyme which has remained the focus of this study is α-glucosidase. In the metabolism of carbohydrates, glucosidases catalyze the last stride by means of hydrolysis of glucosidic bond in oligosaccharides. Depending on the number of monosaccharides, glycosidases are responsible for the

catalytic breakdown of glycosidic bond (Kimura *et al.,* 2004). Among the glucosidases, the most comprehensively studied are α- and β-glucosidase, they catalyze the hydrolysis of the glucosidic bonds at the site of cleavage of the terminal glucose by means of α- and β-linkages at the anomeric center. The difference between these two glucosidases is the positioning of their two carboxylic acid side chains during the course of catalysis (Heightman and Vasella, 1999). Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia and research on natural products revealed that some of them have hypoglycemic effect (Konno *et al.*, 2001; Hong *et al.*, 2007; Kubo *et al.*, 1994; Lo *et al.*, 2008; Manohar *et al.*, 2002; Horio and Ohtsuru, 2001). This lowering of blood sugar level is due to the fact that they naturally contain a α-glucosidase inhibitor (Konno *et al.*, 2001). It is well-understood that the breakdown of carbohydrates in the small intestine can impediment by the use of α-glucosidase inhibitors; and thus lessen the postprandial increase of blood glucose levels in animals and humans (Scheen, 2003; Matsuo *et al.*, 1992; Sudhir and Mohan, 2002). So, these inhibitors play a vital role in glycemic control. Enzyme inhibition studies have become a major focus in this connection and these enzymes have drawn the attention of researchers from both experimental and computational fields (Van de Laar *et al.*, 2006; Barroso *et al.*, 1999; Kim and Uyama, 2005; Khatib *et al.*, 2005; Parvaiz *et al.*, 2007). Concurrent advancements in the field of computational chemistry have provided reliable tool to have a deeper insight into a variety of system of interest (Azam *et al.*, 2009a, b, c, 2010). In this connection molecular docking studies of α-glucosidase and tyrosinase with twenty inhibitors have been focus of this study. The activity data for these inhibitors is recently published (Choudhary *et al.*, 2010).

The program utilized for molecular docking in this study is GOLD (Jones *et al.*, 1997). A comprehensive review of this package (Bissantz *et al.*, 2000; Toledo-Sherman and Chem, 2002) is beyond the scope of this article. A large number of conformations are generated for the small molecule as it happens internally in the GOLD docking program. Each conformation is positioned in the active site in a variety of orientations known as pose. Many poses are selected and ranked by the scoring function, to determine the best overall pose. The scoring function incorporated in GOLD uses classical molecular mechanics force field. GOLD uses a genetic algorithm on the whole molecule and on H-bond donors in the protein to determine the conformation and orientation of the ligand. In this study we have selected 20 diverse co-crystal structures from the Protein Data Bank (PDB) (Berman *et al.*, 2000). We show that GOLD can reproduce the bound conformations of each of the endogenous ligands with good accuracy. Additively the 20 inhibitors were then docked into the active sites of α-glucosidase and tyrosinase enzymes.

Method

Molecular docking studies were carried out to understand the binding modes of both the proteins with 20 inhibitors using the GOLD (Genetic optimization for Ligand Docking) (Jones *et al.*, 1997). This algorithm allows partial flexibility of the protein and full flexibility of the ligand and at the same time had been validated and successfully tested on a data set of over 300 complexes extracted from the PDB (GOLD, 2005; Nissink *et al.*, 2002).

Initially, the proteins were considered without ligand for the purpose of docking studies. The proteins (PDB ID: 1WX2 for tyrosinase and 2ZQO for α-glucosidase) were minimized up to a gradient of 0.01 kcal/(mol Å). The energy-minimized structures were used for further docking analysis. In the GOLD docking program, the default parameters were: population size (100); selection-pressure (1.1); number of operations (10,000); number of islands (1); niche size (2); and operator weights for migrate (0), mutate (100), and crossover (100) were applied. The active site was defined within 10 Å and the ligand-binding interactions were analyzed using scoring functions: GOLD score (GS) based on a molecular mechanics-like scoring function (35) in our analyses for both complexes. The structures of 20 compounds were built using Gaussian and energy minimization was performed using the steepest descent algorithm with a convergence gradient value of 0.001 kcal/(mol Å) in order to obtain the optimized structures of the compounds for molecular modeling studies. In addition, GOLD generates hydrophobic fitting points in the protein cavity onto which ligand CH groups are mapped. In this study, the binding site was defined as a spherical region which encompasses all protein atoms within 10.0 Å of each crystallographic ligand atom. Default settings were used for all calculations.

Before screening the compounds, the docking protocol was validated. 2ZQO protein of α-glucosidase with bound ligand was docked into its binding pocket to obtain the docked pose and the RMSD of all heavy atoms between the docked and crystal conformations was 2.04 Å indicating that the parameters for docking simulation were good in reproducing the X-ray crystal structure. Owing to unavailability of proper co-crystal ligand, the redocking procedure in case of tyrosinase was avoided.

Results and discussion

Molecular docking studies were performed with the default parameter of GOLD. The program also provides

parameters for copper as per the requirement of this system of interest, i.e., metal ion Cu in tyrosinase enzyme.

Docking analysis of tyrosinase (PDB ID: 1WX2)

For docking analysis of tyrosinase, PDB ID: 1WX2 downloaded from the protein data bank. The visual inspection of protein was carefully done using VMD software. The optimized twenty inhibitors were then docked into the binding sites. GOLD provided ten conformations of each twenty inhibitors. The top ranked conformations were selected for further studies and visual inference (see Fig. 1).

Compound 04 was ranked highest among all studied inhibitors (see Table 1) and it was the second most active compound among known inhibitors. The most active compound 09 was ranked as second that showed good agreement between the docking and experimental results. These two compounds were studied in detail in order to extract useful information about the newly discovered inhibitors conformations in the active pockets of the tyrosinase enzyme. The protein–ligand interaction diagrams were taken by using MOE Ligand interaction module of MOE.

The best ranked compound 04 is shown in the Fig. 2a. This compound was docked deeply into the active site region making interactions with the residues Arg55, His97, and metal ions Cu. There are two Cu ions present in the vicinity of the active site. Two interactions of ligand with the residues Arg55 and His97 were found with the hydroxyl group of cyclopentyl moiety of ligand. The docked compound 04 was surrounded by the residues namely, Ile42, Asp45, Tyr98, Glu182, Trp184, and Asn191.

Table 1 GOLD scores of active compounds against tyrosinase enzyme

S. no.	Compounds	pIC50 values	GOLD scores
01	04	5.13	−150.45
02	05	4.29	−166.33
03	06	5.09	−228.81
04	08	3.92	−228.27
05	09	5.29	−153.89
06	10	5.15	−205.15
07	12	4.44	−283.81
08	16	4.60	−295.94

The most active compound against tyrosinase enzymes was 09; it came up with second place on docking score. It can be seen clearly in Fig. 2b that this compound also docked deeply inside the binding region of protein making interaction with the residue Glu182, the solvent contact with the ligand is covering almost every portion of compound 09. Two Copper ions are forming some contact with the carbonyl group of cyclohexane and with six (His38, His54, His63, His190, His194, His216) residues of active site of tyrosinase enzyme. Acidic residue Glu182 of active site showed interactions with the hydroxyl group of cyclopentyl moiety of the compound. Other residues that found in the vicinity of compound 09 were Phe59, His97, and Tyr98.

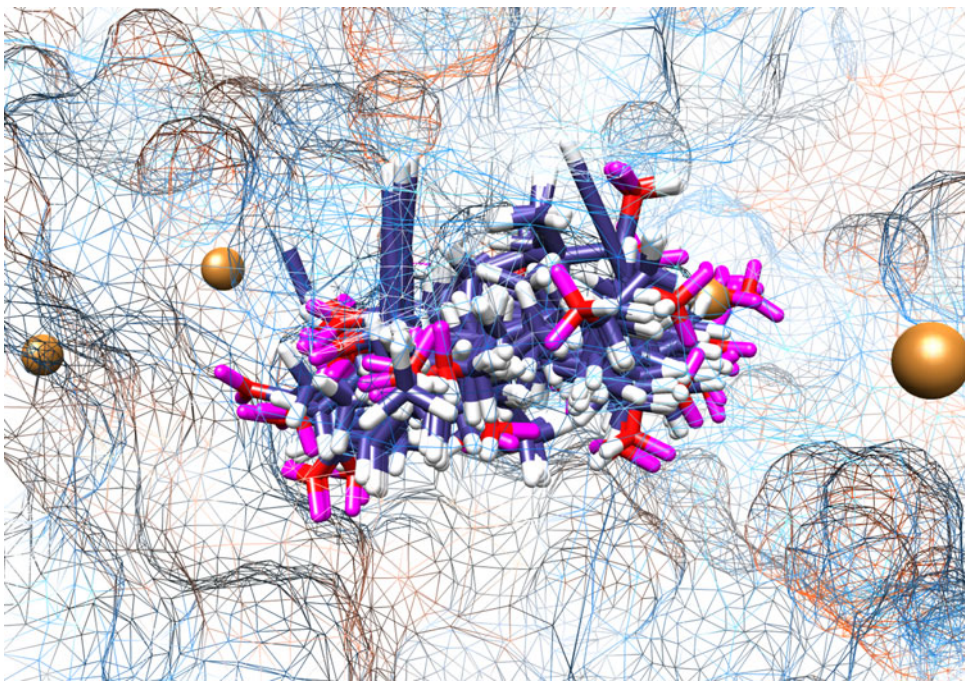

Fig. 1 Docked pose of 20 inhibitors inside the binding pocket of tyrosinase enzyme. Protein (PDB ID: 1WX2) is depicted in mesh style of CHIMERA (Pettersen *et al.*, 2004)

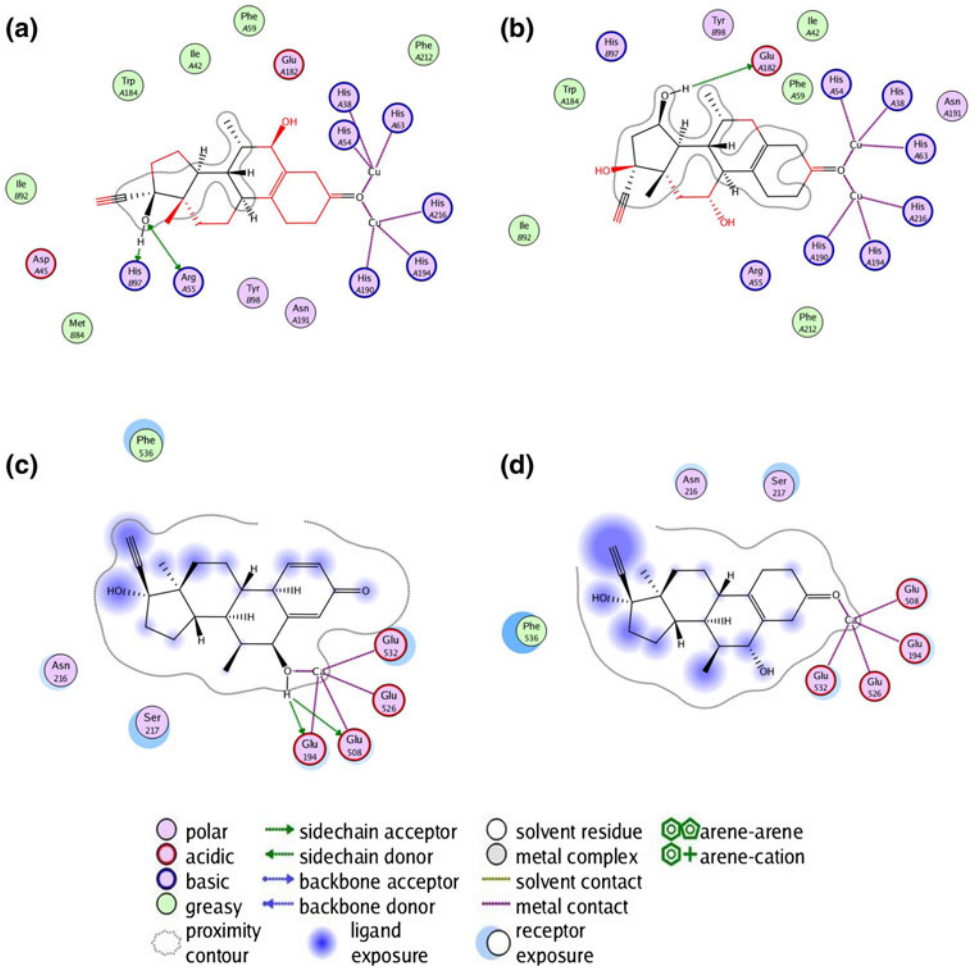

Fig. 2 The 2D depiction of the docked conformation of most active compounds **a** compound 04 and **b** compound 09 within the binding pocket of tyrosinase enzyme (PDB: 1WX2), where as **c** compound 15 and **d** compound 04 within the binding site of the α-glucosidase enzyme (PDB: 2ZQ0). 2D depiction is rendered from MOE ligand interaction (Molecular Operating Environment, 2008)

Correlation coefficient between the activities and docking scores were determined for all inhibitors, and the maximum cross validated correlation coefficient value was found to be 0.41 (see Fig. 4a). The value is relatively low as compared to the usual acceptable cross validated correlation coefficient but no further improvements were observed by applying different options. The best correlation coefficient was found between compounds 04, 05, and 09. Among the three highly ranked compounds the excellent correlation of 0.94 is noted. This indicated the inherent deficiency of docking and scoring function and might be the reason of poor overall correlation coefficient between docking scores and biological activities. Such behavior reflects that the utilization of scoring as a primary criterion is not well-guaranteed in all cases.

Docking analysis of α-glucosidase (PDB ID: 2ZQ0)

Docking studies were also performed for the same inhibitors (as in case of tyrosinase enzyme) utilizing α-glucosidase enzyme. PDB ID: 2ZQ0 was downloaded from PDB and properly visualized by using VMD in order to make sure there is no discrepancy in the PDB file. Here the co-crystal ligand was detached from the active site and re-docked using GOLD into the binding pocket to calculate the rmsd value. The rmsd value was found to be 2.04 Å that showed the extent of overlapping of docked pose and actual pose of the co-crystal ligand.

The optimized 20 inhibitors, which were utilized in docking studies for tyrosinase, again utilized for α-glucosidase docking studies. All ligands were docked into the active site of α-glucosidase enzyme. After docking the program yielded ten conformations for each ligand based upon the GOLD scoring function. The top ranked conformation of each ligand was selected and further evaluated. It can be seen that all the ligands were docked inside the cavity of active site of the targeted protein (see Fig. 3).

The compound 04 was reported most active against α-glucosidase enzyme, while the compound 15 was on third spot. These two compounds were studied in depth to extract the useful information about the inhibition of α-glucosidase enzyme and conformation of ligands. The

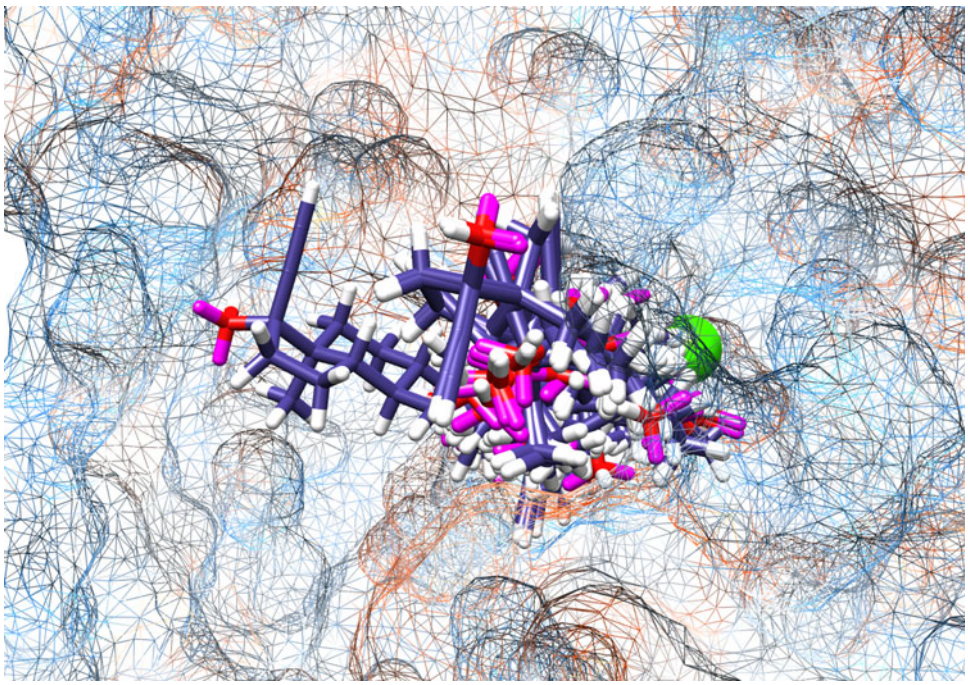

Fig. 3 Docked pose of 20 inhibitors inside the binding pocket of α-glucosidase enzyme. Protein (PDB ID: 2ZQ0) is depicted in mesh style of CHIMERA (Pettersen *et al.*, 2004)

diagrammatic sketch depicting the interaction of ligand with the protein was obtained by using MOE software (Molecular Operating Environment, 2008).

The compound 15 was ranked first on the basis of GOLD score (see Table 2) and it can be seen that it is docked firmly onto the active site of protein (see Fig. 2c). Some very prominent interactions between the compound 15 with the active site residue Glu194, Glu508, and the metal ion Calcium were observed. The solvent contact is found all over this ligand. The other residues that were found in the locality of this docked ligand were Ser217, Phe401, Glu526, Glu532, Tyr533, and Phe536.

The compound 04 came up with second top ranked among all other inhibitors according to docking GOLD score. This ligand was docked deeply into the active sac of the enzyme, making exposed to the residues Asn216, Phe401, Tyr533, and Phe536 (see Fig. 2d). It can be seen from Fig. 2d that most of the part of ligand is exposed to the active site of enzyme, and solvent has engulfed the ligand from every side. Two residues Ser217 and Val471 were also found in the vicinity of the docked conformation of ligand 04.

In order to evaluate the connection between experimentally determined activities of compounds and the docking scores, correlation coefficients were calculated and found as acceptable with the value of 0.6 (see Fig. 4b). Furthermore, the active site similarity between the two enzymes has also been noticed by aligning them. The superimposition indicates the overlapping of crucial residues of the active site. The metal ion calcium of α-glucosidase and copper ions of tyrosinase within the vicinity of active sites are lying under the cutoff of 6 Å (see Fig. 5). These findings suggest the resemblance of active sites of both the enzymes. Molecular dynamics simulation studies will be carried out as the next step of this study in order to depict the interaction of molecules with respect to the dynamics of the system.

Table 2 GOLD scores of active compounds against α-glucosidase enzyme

S. no	Compounds	pIC50 values	GOLD scores
01	04	3.65	35.03
02	06	3.46	32.60
03	07	3.64	31.00
04	08	3.06	29.18
05	12	3.19	31.90
06	15	3.47	35.56

Conclusion

In order to further understand the binding modes and activity trend, the molecular docking studies were conducted on a series of recently identified novel dual inhibitors of tyrosinase and α-glucosidase. The docking analysis resulted in identification of important ligand's interaction with respect to the binding site. The reliability of current docking protocol is established by re-docking the co-crystal ligand of α-glucosidase back into the binding pocket. The active compounds 04 and 15 depicted

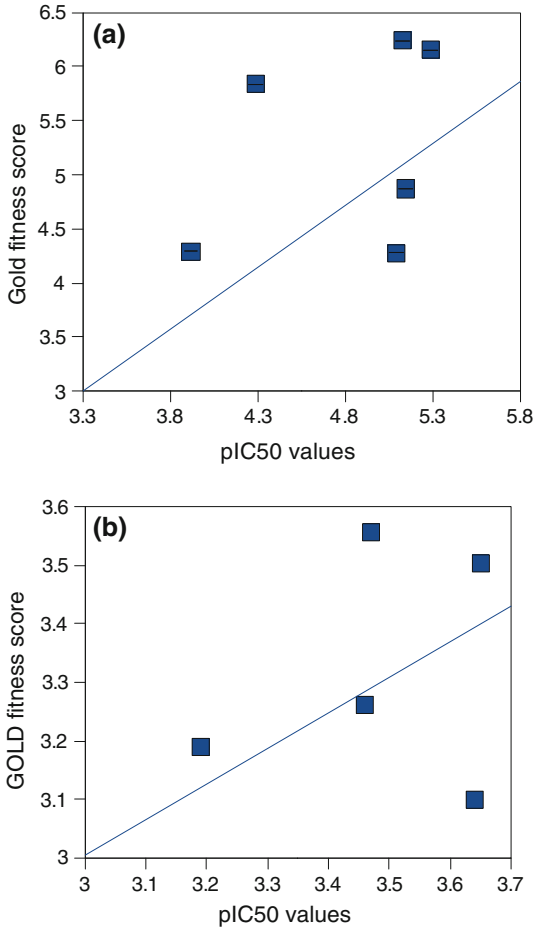

Fig. 4 a: Correlation graph between GOLD fitness score and pIC50 values of tyrosinase inhibitors. b: Correlation graph between GOLD fitness score and pIC50 values of α-glucosidase inhibitors

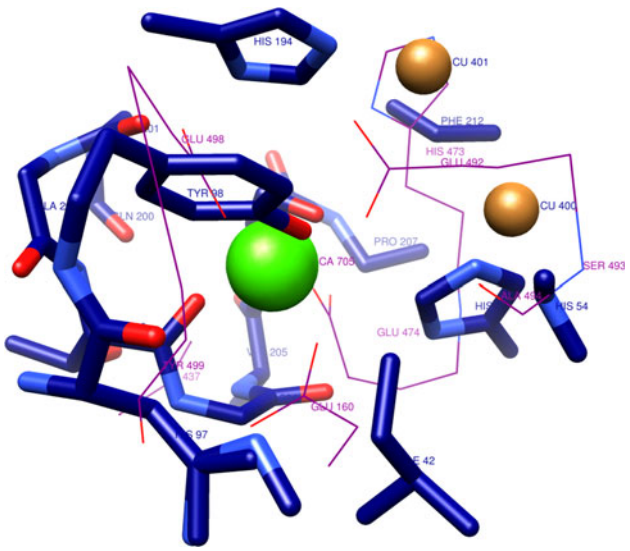

Fig. 5 Superimposition of active sites of tyrosinase (represented in *stick*) and α-glucosidase (represented in *lines*) enzymes using CHIMERA (Pettersen *et al.*, 2004)

significant interactions with the crucial residues of binding site. Furthermore, the correlation between scores and activity trends of top active compounds against tyrosinase was found satisfactory and the same is true with the case of α-glucosidase. This indicates the significance of the molecular mechanics-based scoring function in describing the biological trends of the studied compounds. The set docking and scoring protocol against α-glucosidase and tyrosinase in this study could be utilized for further computational studies against both enzymes. In follow up studies the results described here may give rise to the identification of other series of new and more potent inhibitors of tyrosinase and α-glucosidase.

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