Identification of Optimal Tyrosinase Target for Anti-tyrosinase Agents by Using Lead Finder Docking Program

Prasan Tangyuenyongwatana[1] Napa Boonma[2]

Abstract

Lead Finder, a molecular docking program in Flare Suit (Cresset-Group, UK), utilizes a genetic algorithm with three scoring functions and a unique method that sets it apart from other GA docking programs. The purpose of this study was to evaluate three tyrosinase enzymes, 3NQ1, 4P6S, and 5OAE, and dock them against a set of positive antityrosinase compounds. The docking results were collected as FL Rank Score and FL dG Score. Plot the LF Rank and LF dG Scores with antityrosinase (IC50) values to obtain linear regression and correlation coefficient values. It was found out that enzyme 5OAE gave the best fit value in LF Rank Score (y = 0.1330X – 11.13, r^2 = 0.8948) while 4P6S and 3NQ1 showed lesser values. For LF dG Scores, three enzymes exhibited modest correlation coefficients, ranging from 0.6394 to 0.7897, indicating minimal differences in the superposition of structures. In conclusion, 5OAE is a suitable enzyme for use in anti-tyrosinase docking or virtual screening experiments.

Keywords: Molecular docking, Anti-tyrosinase, Lead finder, Genetic algorithms

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Introduction

In drug discovery, the identification of a lead structure typically kicks off a medicinal chemistry program. Traditionally, lead compounds for a particular protein target are delivered via high-throughput screens (HTS) of sizable chemical libraries. Iterative efforts at analog synthesis are required to optimize the resulting small molecule leads. The early 1990s showed an acceleration of the development of faster and less expensive computational alternatives due to the high costs and low hit rates associated with HTS screening campaigns and the continuously growing number of potential drug targets. These days, early-stage drug discovery makes extensive use of virtual screening (VS) techniques to identify hits by examining chemical databases (Hughes, Rees, Kalindjian, & Philpott., 2011)

Molecular docking is a technique that examines how molecules align and conform—collectively known as their "pose"—to the binding site of a macromolecular target. Possibilities are produced by searching algorithms and then ranked using scoring methods. Over the past few decades, many pieces of software have been developed; some of the more well-known ones include AutoDock (Morris, 1998), AutoDock Vina (Trott, & Olson, 2009), GOLD (Verdonk, 2003), and FlexX (Rarey**,** 1996). Typically, a grid representation containing pre-calculated potential energies for interaction within the target binding site is used during docking calculations. This method, which essentially involves dividing the binding site, expedites docking runs. Subsequently, interactions of the electrostatic and Lennard-Jones potentials are computed at every grid point.

Lead Finder is an application program recently released from Flare Suite (www.cresset-group.com/software), for molecular docking that utilizes a variety of extra optimization steps; sampling employs an innovative genetic algorithm implementation. Lead Finder uses a collection of semi-empiric molecular mechanics functionals, separately parameterized for virtual screening, docking, and binding energy predictions, in its scoring functions. The sampling and scoring algorithms stages are similar in that they progress from quick but imprecise versions to slower but more precise ones that require more computing power (Novikov et.al., 2012). There were many reports on docking applications of this program (Zampieri et.al., 2024; Manmeet et.al., 2022; Mudi et.al., 2022).

Tyrosinase (EC 1.14.18.1) is a multifunctional, glycosylated phenol oxidase (a polyphenolase) that contains copper. It has drawn a lot of interest as an anti-melanogenic target because it is the enzyme that determines the rate at which melanogenesis proceeds. Tyrosinase has emerged as the most popular and effective target for melanogenesis inhibitors that specifically block the catalytic activity of tyrosinase because it is necessary for melanogenesis (Hwang et.al., 2022). Tyrosinase inhibitors make up the majority of skin-lightening products that are sold commercially, and several of them have shown promise for use in medicine, cosmetics, or agriculture. However, only a small number of chemicals are utilized in clinical

settings because of their lack of effectiveness or unfavorable side effects, including cancer. In both medical and cosmetic uses, arbutin, azelaic acid, hydroquinone, and kojic acid are utilized as skin-lightening agents (Zachary, Wang, & Saedi., 2020) So, we would like to have some best models of tyrosinase enzyme for virtual screening a large number of compounds to find new leads.

The primary goal of the current experiment was to dock with a known data set of anti-tyrosinase compounds against three tyrosinase enzymes (PDB IDs: 5OAE, 4P6S, and 3NQ1) to assess the efficacy of Lead Finder's sampling and scoring methodologies.

A conceptual framework

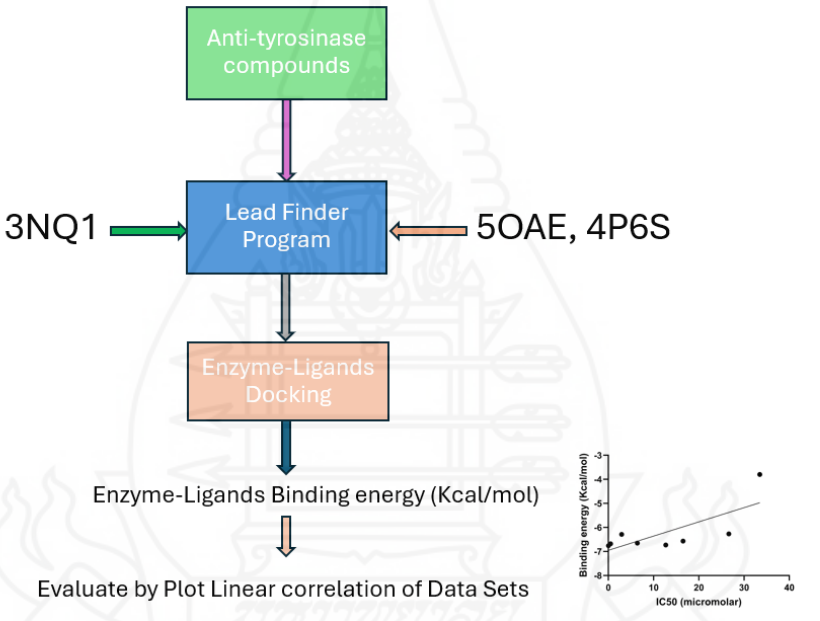

Figure 1. Conceptual framework

Materials and Methods

1. **Target enzyme for docking procedure**

From the Protein Data Bank (www.rcsb.org), three tyrosinase enzymes—3NQ1, 4P6S, and 5OAE—were chosen. Each enzyme was subjected into Lead Finder program by opening the pdb file. Next, each enzyme needs to be prepared before docking. By clicking Prepare Protein Buttom, polar hydrogen atoms or missing residues will be added; water molecules will be removed; and states for the heteroatoms will be created at pH 7.0 ± 0.5. The last stages are minimization and optimization.

2. Selection of compounds for docking research

ChemSketch (www.adclabs.com) was used to sketch the 2D structures in mol format of the positive anti-tyrosinase set, which includes HS-1713, HS-1784, HS-1791, resveratrol, and kojic acid (Prasan & Jongkon., 2016). Next, using the Avogadro program (www.avogadro.cc) to minimize energy with the MMFF94 force field, the 2D structures were transformed into 3D structures and saved in sdf format. The Lead Finder program added each compound by importing the ligand file and clicking on the Prepare ligand button. The ligand will be calculated the appropriate protonation states of the ligand based on its chemical environment, and the ligand undergo energy minimization to relive any steric clashes or unrealistic geometries.

3. Molecular docking process

Lead Finder was used in the Flare module of the Cresset software to simulate molecular docking. The docking scores were computed in a slowly but accurate mode and by centering a 6.0 Å docking grid on the crystallographic ligands. The measures utilized to determine the scoring were LF Rank, and and LF dG Score. The LF Rank is a scoring function that ranks the docked poses of ligands based on their predicted binding affinities. It helps in identifying the most promising ligand conformations from a set of docked poses. A lower rank indicates a more favorable docking pose, suggesting stronger potential interactions with the target protein. The LF dG Score represents the estimated change in Gibbs free energy (**Δ**G) associated with the binding of a ligand to its target protein. This score quantifies the stability of the ligand-protein complex. A more negative LF dG Score indicates a more thermodynamically favorable binding interaction, suggesting that the ligand is likely to bind strongly to the protein. (Mudi et.al., 2022). The LF Rank Score displays the energy ranking for its docked postures, and LF dG determines the degree of its precise binding energy to its target protein. The active ligands are indicated by higher negative ratings (Singh et al., 2022).

4. Evaluate docking results

Following the completion of the docking process, the Lead Finder program displayed the optimal binding energy between the enzyme and ligand, along with the LF Rank Score and LF dG Score. Each pose was evaluated by superimposing with the x-ray ligand (SVP) and choosing the best fit one for each pair. The versatile statistical graphing software GraphPad Prism 10 (GraphPad, USA) plotted the linear regression of pose scores with anti-tyrosianse activity, provided the correlation coefficient (r^2), and set a confidence interval at 95%.

Results

In this study, three tyrosinase enzymes—3NQ1, 4P6S, and 5OAE—have been selected (Example of 5OAE Figure 2) to investigate ligand-protein interactions. Since each of them possesses a unique anti-tyrosinase ligand, there is a possibility that one enzyme will provide the best fit between the test set's enzyme ligands. First, the redock experiments were proceeded for each enzyme: 3NQ1 redock Kojic acid to obtain RMSD = 0.212 Å, 4p6s (X-ray ligand = L-Dopa) redock to obtain RMSD = 1.624 Å, and 5OAE redock SVF to obtain RMSD = 1.159 Å. Tables 1, 2, and 3 displayed the docking results using the Lead Finder application, which showed the LF Rank Score and LF dG. Figure 3 displayed the ligands after docking with the Lead Finder overlay, which included the x-ray ligands of the enzymes. Figures 4 and 5 display examples of x-ray ligand (SVP) and test ligand HS-1713 interactions with amino acid residues in the binding site of enzyme 5OAE.

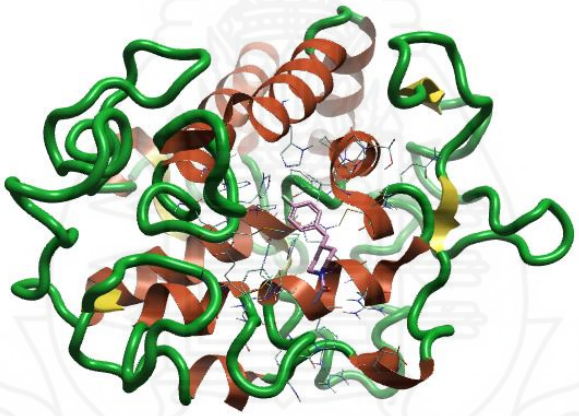

Figure 2. Enzyme tyrosinase (5OAE) with x-ray ligand SVP (purple color)

(a) (b)

Figure 3. Examples of docking pose results include (a) HS-1791 (blue color) superimposing with SVP and (b) HS-1713 superimposing with SVP.

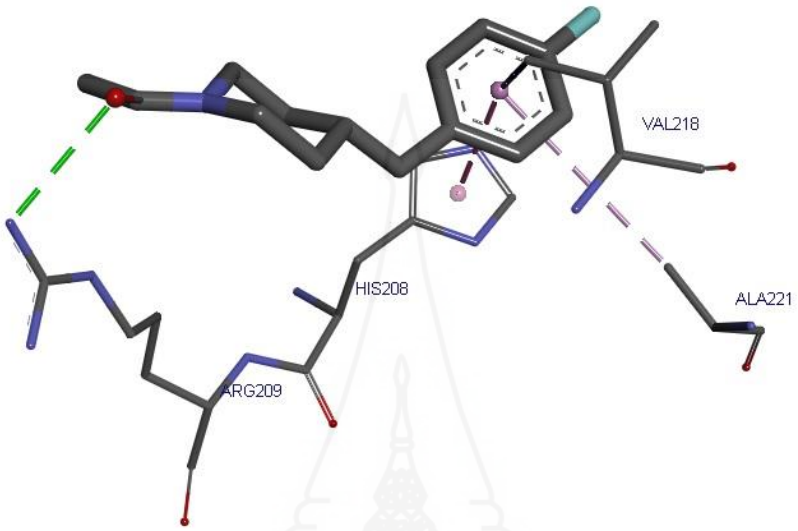

Figure 4. Ligand SVP of 5OAE enzyme interacted with amino acids HIS208, ARG209, VAL218, and ALA221.

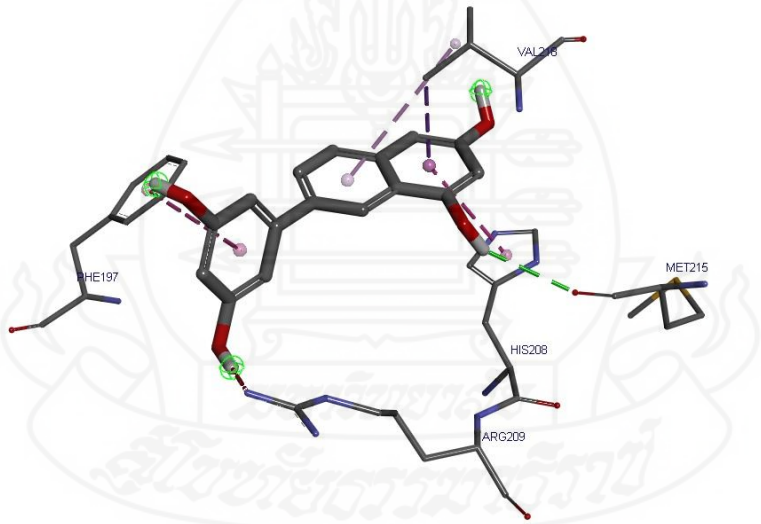

Figure 5. HS-1713 interacted with amino acids HIS208, ARG209, and VAL218.

Table 1 Lead Finder docking results of anti-tyrosinase test set with 5OAE enzymes.

Enzyme	Compounds	IC50 (μM)	LF Rank (Kcal/mol)	LF dG (Kcal/mol)
5OAE	HS-1713	0.49	-10.188	-9.894
	HS-1784	16.52	-10.136	-9.251
	HS-1791	2.95	-10.871	-9.634
	HS1792	6.4	-10.284	-7.597
	Hydroquinone	33.48	-6.001	-5.476
	Kojic acid	38.24	-5.821	-5.616
	Resveratrol	26.63	-7.986	-8.666

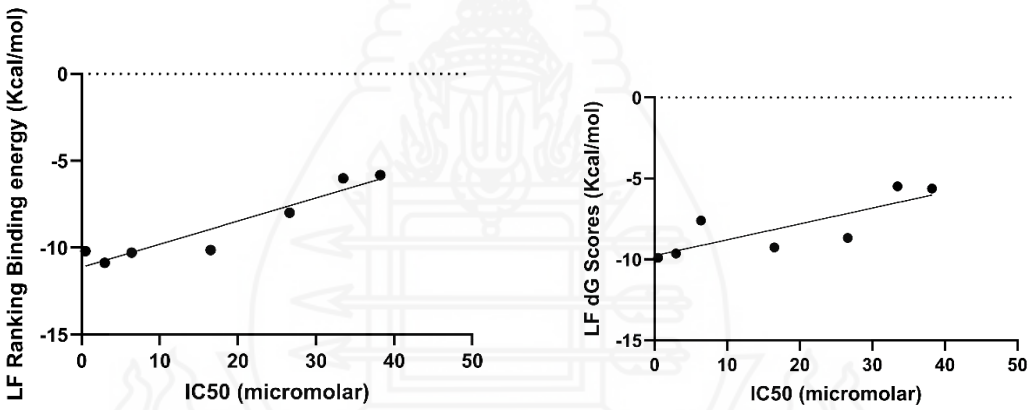

Figure 6. Garph charts of docking results (a) LF Ranking binding energy (Kcal/mol) of 5OAE was plotted with anti-tyrosinase activity (IC50, μM) with linear regression equation of y = 0.1330X – 11.13, r^2 = 0.8948 (p-value < 0.05), (b) LF dG scores (Kcal/mol) was plotted with anti-tyrosinase activity (IC50, μM) with linear regression equation of y = 0.09795X – 9.764, r^2 = 0.6528 (p-value < 0.05).

Table 2 Lead Finder docking results of anti-tyrosinase test set with 4P6S enzymes.

Enzyme	Compounds	IC50 (μM)	LF Rank (Kcal/mol)	LF dG (Kcal/mol)
4P6S	HS-1713	0.49	-8.457.	-8.324
	HS-1784	16.52	-8.699	-8.223
	HS-1791	2.95	-8.906	-8.575
	HS-1792	6.4	-9.221	-8.217
	Hydroquinone	33.48	-5.317	-5.015
	Kojic acid	38.24	-5.858	-5.334
	Resveratrol	26.63	-7.759	-7.565

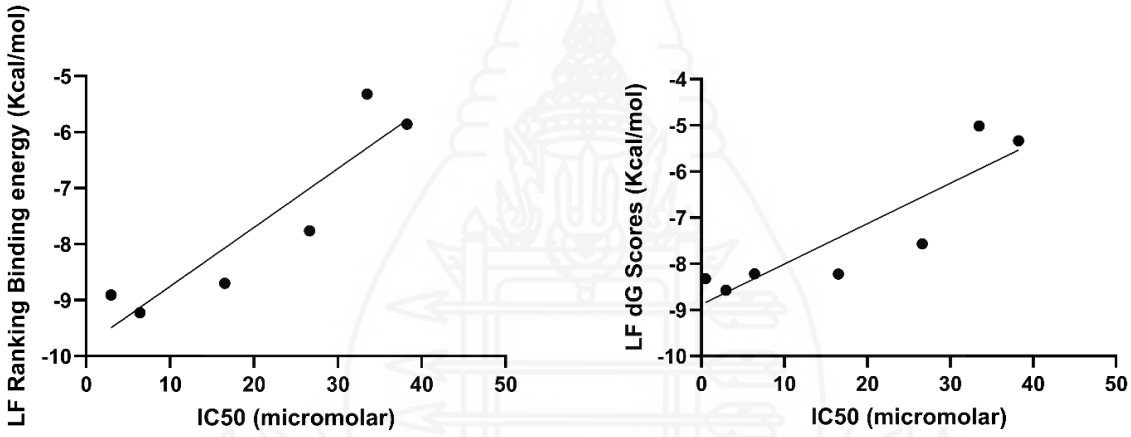

Figure 7. Garph charts of docking results (a) LF Ranking binding energy (Kcal/mol) of 4P6S was plotted with anti-tyrosinase activity (IC50, μM) with linear regression equation of y = 0.1052X – 9.805, r^2 = 0.8361 (p-value < 0.05), (b) LF dG scores (Kcal/mol) was plotted with anti-tyrosinase activity (IC50, μM) with linear regression equation of y = 0.08754X – 8.881, r^2 = 0.7897 (p-value < 0.05).

Table 3 Lead Finder docking results of anti-tyrosinase test set with 3NQ1 enzymes.

Enzyme	Compounds	IC50 (μM)	LF Rank (Kcal/mol)	LF dG (Kcal/mol)
3NQ1	HS-1713	0.49	-9.628	-8.932
	HS-1784	16.52	-9.188	-8.662
	HS-1791	2.95	-8.928	-8.864
	HS-1792	6.4	-9.212	-7.807
	Hydroquinone	33.48	-5.355	-4.676
	Kojic acid	38.24	-6.011	-5.347
	Resveratrol	26.63	-8.367	-8.477

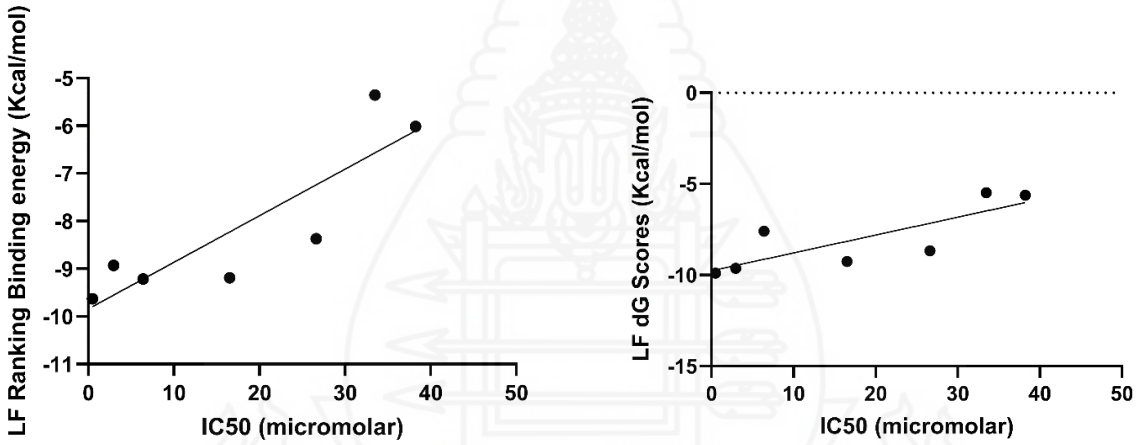

Figure 8. Garph charts of docking results (a) LF Ranking binding energy (Kcal/mol) of 3NQ1 was plotted with anti-tyrosinase activity (IC50, μM) with linear regression equation of y = 0.09775X – 9.840, r^2 = 0.7646 (p-value < 0.05), (b) LF dG scores (Kcal/mol) was plotted with anti-tyrosinase activity (IC50, μM) with linear regression equation of y = 0.09308X – 9.196, r^2 = 0.6384 (p-value < 0.05).

Table 4 Summarized of linear regression equations and correlation coefficients of docking results

Enzyme	LF Rank Linear regression equations, Correlation coefficients (r^2)	LF dG Linear regression equations, Correlation coefficients (r^2)
5OAE	y = 0.1330X – 11.13, r^2 = 0.8948	y = 0.09795X – 9.764, r^2 = 0.6528
4P6S	y = 0.1052X – 9.805, r^2 = 0.8361	y = 0.08754X – 8.881, r^2 = 0.7897
3NQ1	y = 0.09775X – 9.840, r^2 = 0.7646	y = 0.09308X – 9.196, r^2 = 0.6384

Discussion

Lead Finder, a molecular docking program in Flare Suit (Cresset-group, UK), utilizes a genetic algorithm with three scoring functions and a unique method that sets it apart from other GA docking programs (Stroganov et.al., 2008). For instance, AutoDock 4 requires an x-ray ligand to determine the ligand's center of coordination, which is then covered by a square box. After finding the target for docking, the x-ray ligand was removed before docking. On the other hand, Lead Finder needs an x-ray ligand to stay with the enzyme and create a grid box with this ligand, and the x-ray ligand does not need to be removed during the docking. In a redock experiment involving three tyrosinase enzymes (3NQ1, 4P6S, and 5OAE), two of them (4P6S and 5OAE) showed an RMSD of less than 2 Å, while 3NQ1 showed a slightly higher RMSD than 2 Å. This set of enzymes should be appropriate for further experimentation.

The LF Rank Score in Lead Finder software is used to rank ligand poses obtained during a docking run. It is a scoring function that helps in the ranking of ligand poses, with the lower (more negative) LF Rank Score indicating a higher likelihood that the docked pose reproduces the crystallographic pose. This score is part of the software's approach to accurately predict protein-ligand interactions and assess ligand binding and biological activity. "LF dG" typically stands for "Lead Finder delta G," where "delta G" refers to the change in Gibbs free energy. This is a concept used in computational chemistry and drug discovery to predict the binding affinity of a ligand to a target protein. The unit for delta G is usually kilocalories per mole (kcal/mol). A more negative delta G value indicates a stronger binding affinity between the ligand and the protein (Mudi et al., 2022). The data were plotted with IC50 to generate graphs with linear regression and correlation coefficients as summarized in table 4.

Based on the LF Rank correlation coefficient values, enzyme 5OAE excelled in this experiment, exhibiting high correlation coefficients of 0.8948 (p-value < 0.05), whereas 4P6S and 3NQ1 demonstrate lower correlation coefficients. Figure 3 shows the test compounds (HS-1791 and HS-1713) superimposed on the docking position of the 5OAE x-ray ligand. In Figures 4 and 5, the ligands and the amino acids HIS208, ARG209, VAL218, and ALA221

interact in the enzyme binding site. When examining the LF dG values, we found that three enzymes exhibited modest correlation coefficients, ranging from 0.6394 to 0.7897, indicating minimal differences in the superposition of structures. While 4P6S scored higher than the others, the differences between each enzyme were not significant. It's possible that 5-OAE did better in the docking test because it has a large x-ray ligand called 1-[4-[(4-fluorophenyl)methyl]piperidin-1-yl]ethenone that leaves a bigger binding space in the enzyme. For 4P6S and 3NQ1, the ligands, which are L-dopa and kojic acid, have smaller structure sizes that make them less suitable for binding with the test set. Overall, 5OAE is a suitable enzyme for use in anti-tyrosinase docking or virtual screening experiments.

Suggestion

This study demonstrated the usefulness of molecular docking with the Lead Finder program to find the appropriate tyrosinase enzyme for molecular docking or virtual screening. The next goal is to work on virtual screening of larger databases (public databases), such as PubChem, ZINC, and COCONUT. Another option is to enable Lead Finder to dock with other programs, such as AutoDock Vina in PyRx, which is currently one of the most popular docking programs.

This research is free of any conflict of interest.

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