Thai Journal of Pharmaceutical Sciences (TJPS)

40(1), January-March 2016:1-53

Journal homepage: http://www.tjps.pharm.chula.ac.th

Molecular docking study of tyrosinase inhibitors using ArgusLab 4.0.1: A comparative study

Prasan Tanguenyongwatana[1*] and Nathjanan Jongkon[2]

[1] *College of Oriental Medicine, Rangsit University, Pathumthani, 12000, Thailand.*
[2] *Department of Social and Applied Science, College of Industrial Technology, King Mongkut's University of Technology North Bangkok, Bangkok, 10800, Thailand.*

ARTICLE INFO

Article history:
Received: 10 October 2015
Revised: 6 December 2015
Accepted: 30 January 2016

Available online: 30 January 2016

Keywords:
Molecular docking
Tyrosinase inhibitor
ArgusLab,
AutoDock
AutoDock Vina

ABSTRACT

We conducted a docking study with ArgusLab 4.0.1, a free molecular docking software, on tyrosinase inhibitors comparing with AutoDock 4 and AutoDock Vina. In this study, hydroxyl substituted 2-phenyl-naphthalenes (a group of modified structure based on oxyresveratrol and resveratrol) were docking with tyrosinase enzyme (pdb entry: 3NQ1) with genetic algorithm (GA) in ArgusLab, AutoDock 4 and AutoDock Vina, respectively. The binding energies were correlated with the inhibitory concentrations (IC_{50}) and ArgusLab performed the best linear correlation coefficient of 0.8865 while AutoDock 4 and AutoDock Vina obtained 0.6849 and 0.7805, respectively. From the results, all inhibitors stayed near the entrance of the active site to prevent the substrate binding and showed no interaction with copper atoms in the enzyme. In this study, we found that ArgusLab is an easy to use program with high-speed calculation and has an accessible user-interface even by beginners in molecular docking.

1. Introduction

Molecular modeling has become a well-established tool in pharmaceutical research especially in structure-based drug design. It has created a good opportunity for assisting researchers in finding of new therapeutic agents [1]. Docking is a method which small molecules are docked into a pocket or active site where drugs could possibly bind and the binding affinities are predicted using simplified free energy calculation methods. The preferred orientation of one molecule to a second when bound to target enzyme to form a stable complex was chosen by researcher [2] and based on their energetically favorable conformations. Normally most of programs capable for executing this job are highly in cost payware. However, there are some non-commercial free-wares for docking study and the most renowned program is AutoDock. The Scripps Research Institute has launched many versions and platforms of AutoDock. For Windows operating system, sometimes AutoDock has some inconsistency working with different versions of Windows operating system and it is not friendly for those who are new to computer-aided drug design. In 2004, ArgusLab was created by Mark Thompson who now works in Medio System Inc. Seattle, USA [3]. ArgusLab is free distributed molecular docking software in Windows platform. This program is developed as molecular modeling software. It provides us with molecular building analyses, the

**Corresponding author at:* College of Oriental Medicine, Rangsit University, Pathumthani, 12000, Thailand
Email: prtang2000@yahoo.com (P Tanguenyongwata)

ability to perform various molecular calculations, molecular docking and molecular structure visualization capabilities. ArgusLab is an easy to use program and has an accessible user-interface even by beginners in molecular docking. For new researchers in molecular docking, ArgusLab provides a fast and robust method of a binding site optimization which means the program can locate binding site automatically which make the docking process fast while AutoDock 4.0 the user need to know and specify the co-ordinates of the binding site for quickly calculation. Furthermore, ArgusLab program does not need to do blind docking which normally need a lot of time for calculation and sometime obtains the wrong binding site. Many researchers used ArgusLab to perform their molecular docking researches [4-6].

Tyrosinase (EC 1.14.18.1) is a multifunctional copper-containing enzyme which plays a key role in melanin biosynthesis by catalyzing the oxidation of phenol to o-quinone observed in the early stage of various browning phenomena in nature [7]. Our objectives were to carry out ArgusLab 4.0.1 to study modified tyrosinase inhibitors and showed that this program could be used as an alternative program for molecular docking which usually performed on renowned free docking program, AutoDock 4 and AutoDock Vina.

2. Materials and Methods

2.1 Software and PDB file

To examine the binding orientation of subs trates with tyrosinase enzyme, we selected ArgusLab 4.0.1 (www.arguslab.com), AutoDock 4 and AutoDock Vina (autodock.scripps.edu) for this study. For tyrosinase protein, a crystal structure of tyrosinase from Bacillus magaterium (PDB ID: 3NQ1) [8] was retrieved from RCSB Protein Data Bank. Structure and anti-tyrosinase activity data were obtained from Song *et al.* [9,10] and Likhitwitayawuid *et al*. [11] Kojic acid was used as a positive control compound. For Windows operating system, ArgusLab can perform on Windows 7 and Windows 8.1.

2.1.1 Preparation of the ligand structures

The ligands 2D structures of hydroquinone, kojic acid, resveratrol, oxyresveratrol, HS-1713, HS-1784, HS-1791, HS-1792, and HS-1793 were drawn in ChemSketch program (http://www.acdlabs.com/resources/freeware/chemsketch/).The ligands 2D structures were then converted to 3D structures with molecular mechanic optimising by Avogadro free package [12] (http://avogadro.cc/wiki/Main_Page).

2.1.2 Docking studies

The tyrosinase crystal structure (PDB ID: 3NQ1) was downloaded into ArgusLab program and binding site was made by choosing “Make binding site for this protein” option. The ligand, kojic acid or other inhibitors were chosen, centered and added hydrogens. After that, the ligands were allowed to run using GA algorithm and AScore scoring function. To observe the precision of the docking condition and package, the docking condition was search and re-docking kojic acid to 3NQ1 to reproduced co-crystal bound to the tyrosinase enzyme that had a root mean square deviation (RMSD) value of 1.36 Å. In ArgusLab 4.0.1 program, there are two options for docking algorithm which are GA (Genetic Algorithm) dock and Argusdock(shape-based search algorithm). We chose GA dock only to compare with AutoDock 4 and AutoDock Vina programs. For GA parameters of ArgusLab, population size 50, grid resolution 0.35 Å, binding site box size (17.137 × 18.5 × 16.5 Å), maximum generation 1,000, crossover rate 0.8, mutation rate 0.2, elitism 5 and dock engine used Lamarckian Genetic Algorithm. Docking calculation type was set to “Dock” and “Flexible” ligand docking mode and used for each docking run. In this study, three docking programs were compared with the tyrosinase inhibition activity. The best free energy of binding values would be obtained in log files. The poses were chosen by compared with kojic acid orientation. Discovery Studio Visualizer 4.0 (accelrys.com) was used to perform for all figures. For AutoDock 4 and AutoDock Vina docking parameters and the computational procedures, we followed a similar approach to our previous reported [13].

2.1.3 Anti-tyrosinase inhibition data

A new family of hydroxyl-substituted phenyl-naphthalenes which were modified from oxyresveratrol and resveratrol was synthesized for finding more potent tyrosinase inhibitors [9] as structures showed in Fig. 1.

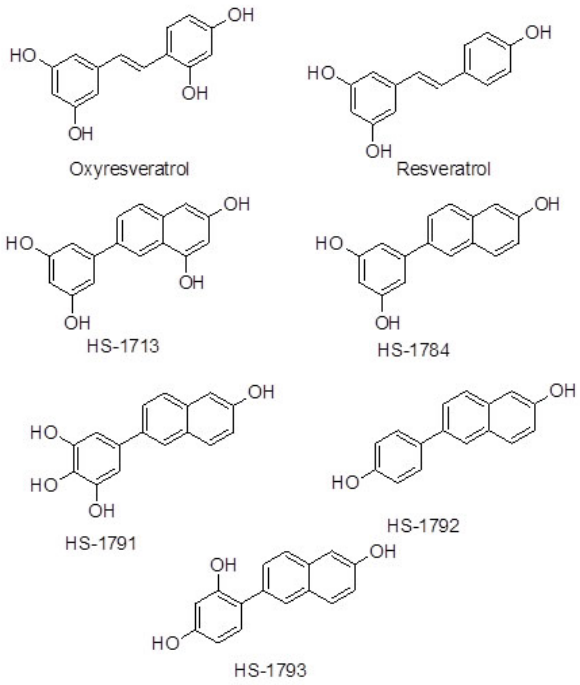

Figure 1 Structures of oxyresveratrol, resveratrol and hydroxyl-substituted phenylnaphthalenes derivatives

The tyrosinase inhibition activities of proposed compound and the molecular docking results with ArgusLab 4.0.1, AutoDock 4 and AutoDock Vina were shown in table 1. For the IC50 of resveratrol from Song *et al*. [9] result, it looked unusual that the tyrosinase inhibition activity of resveratrol was lower than kojic acid. We searched for other literatures to confirm the result and we found Song *et al*. [10] reported the more reasonable IC_{50} for resveratrol. In addition, the IC_{50} of oxyresveratrol which was obtained from Likhitwitayawuid *et al*. [11] was also included into Table 1.

Table 1 Binding energy of tyrosinase inhibitors from ArgusLab, AutoDock 4 and AutoDockVina.

Name of compound	IC_{50} (μM)	Binding energy (Kcal/mol) ArgusLab	Binding energy (Kcal/mol) AutoDock	Binding energy (Kcal/mol) Vina
Hydroquinone	33.48[a]	-4.98	-3.82	-3.80
Kojic acid	38.24[a]	-4.28	-3.57	-4.40
Resveratrol	26.63[b]	-7.87	-5.83	-5.40
Oxyresveratrol	12.70[c]	-8.45	-5.96	-5.96
HS-1713	0.49[a]	-9.59	-6.34	-6.34
HS-1784	16.52[a]	-7.40	-6.27	-6.30
HS-1791	2.95[a]	-10.51	-10.51	-6.50
HS-1792	6.40[a]	-9.54	-6.40	-6.20
HS-1793	0.034[a]	-9.46	-6.06	-6.20

[a] IC_{50} from Song et al.[9]
[b] IC_{50} from Song et al.[10]
[c] IC_{50} from Likhitwitayawuid *et al*. [11]

3. Results and Discussion

There are several docking programs that have been launched to study ligand-protein interactions. We used these 3 programs of ArgusLab, AutoDock and AutoDockVina on this study because they are convenient for users who are familiar with the Windows operating system. Some of them are also friendly to new users for computer-aided drug designing. ArgusLab and AutoDock programs use Lamarckian genetic algorithm protocol to do docking while Vina use genetic algorithm combined with particle swarm optimization which make the speed of calculation faster than AutoDock 4.0. They are stand-alone programs that are not depend on remote server and can do the docking experiments anytime with obtaining quick results.

From the study, the docking results with ArgusLab 4.0.1, AutoDock 4 and AutoDock Vina were shown in table 1. The linear correlations between tyrosinase inhibitory concentrations (IC_{50}) were depicted in figure 2-4. ArgusLab docking result showed a good linear coefficient of 0.8865 while AutoDock 4 and AutoDock Vina programs obtained 0.6849 and 0.7805, respectively.

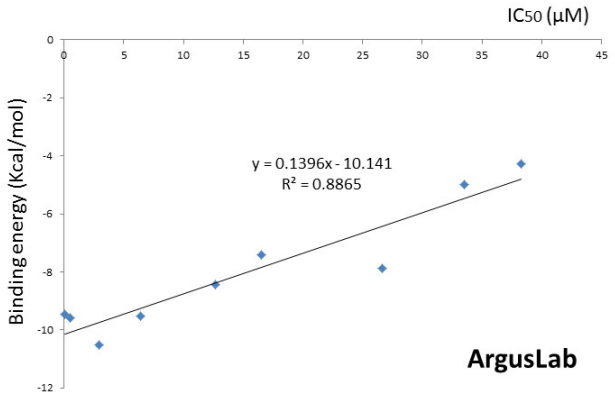

Figure 2 Linear correlation between binding energy from ArgusLab program and IC_{50}

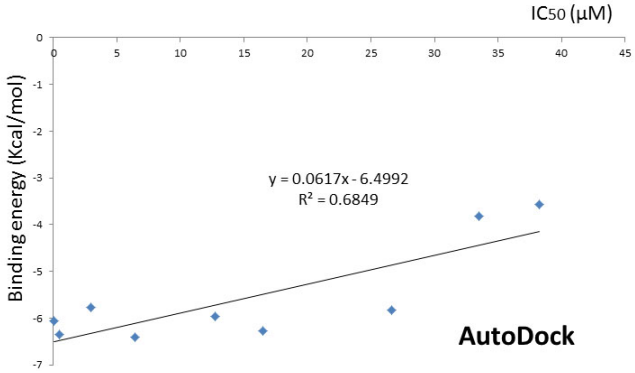

Figure 3. Linear correlation between binding energy from AutoDock program and IC_{50}

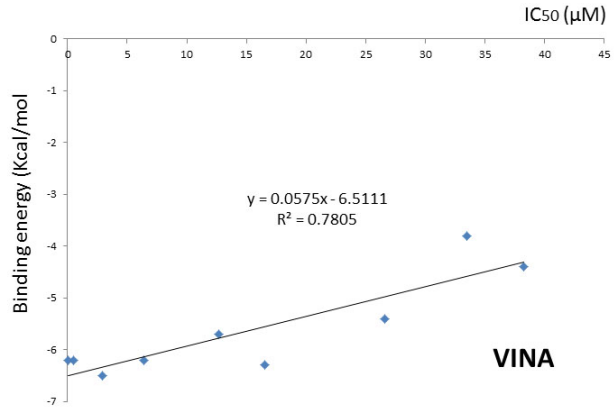

Figure 4 Linear correlation between binding energy from AutoDock Vina program and IC_{50}

X-ray crystal structure of kojic acid in 3NQ1 enzyme showed interactions with amino acids in active site which were Gly200, Pro201 and Arg209 (Fig. 5). For the best active derivative in the group, HS-1973, the orientation

was also close to kojic acid and the ligand had interactions with Gly200, Pro201 Asn205 and Val217 (Fig. 6).

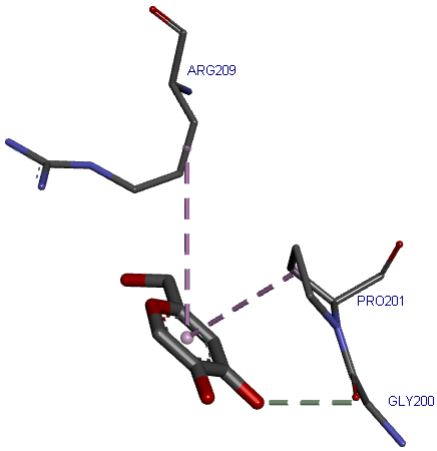

Figure 5 Kojic acid interacted with Gly200, Pro201 and Arg209

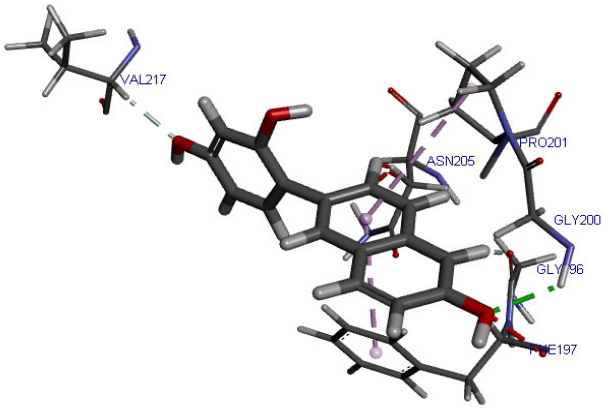

Figure 6 HS-1973 interacted with Gly200, Pro201 Asn205 and Val217

From Table 1, entry 5 and 7 showed contradiction data as HS-1791 show lower inhibitory activity but better binding energy than HS-1713. This case can be explained that the docking method work in silico environment which is different from in vivo, so sometime the inhibitory activity result is not linear correlate with the binding energy. A ligand with having lower inhibitory activity is not always having the lowest energy. In addition, the IC_{50} data of the entry 5, 8 and 9 were significantly different up to 100 folds more potent but the docking results (docking energy) were similar. These kinds of results may occur as the same above reason. For the enzyme binding in vivo, there are many parameters concern with the ligand-enzyme binding such as water in the binding pocket which can make the results different from that of the in silico.

Goldfeder *et al*. [14] published x-ray crystal structure of tyrosinase substrate-binding mode as shown in Fig. 7. In this Figure, tyrosine, a substrate of enzyme, has bonding with CuA at a distance of 1.9 Å (PDB ID: 4P6R). We can compare with kojic acid in Fig. 8 which stayed in the entrance of the active site to prevent tyrosine or L-Dopa to enter the site. HS-1973 was also bind to the tyrosinase enzyme in the same orientation as kojic acid but had phenyl ring aiming into the active site cavity (Fig. 9).

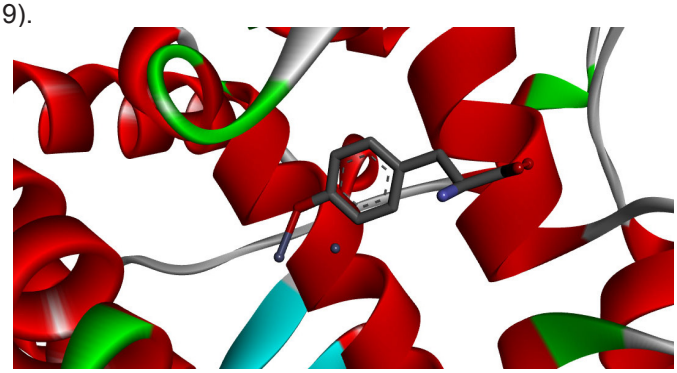

Figure 7 Tyrosine in tyrosinase active site bonding with CuA

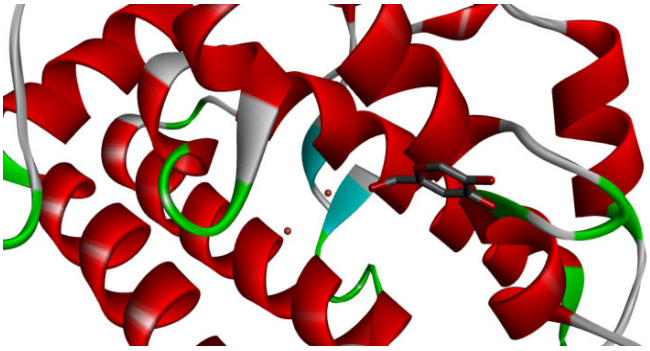

Figure 8 Kojic acid in tyrosinase active site

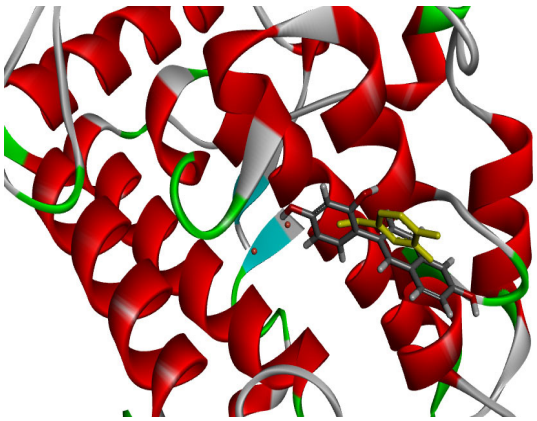

Figure 9 Kojic acid (yellow) and HS-1973 in tyrosinase active site

One issue concerned with the different parameters between different docking programs such as grid box size for ArgusLab (17.137 × 18.5 × 16.5 Å) versus AutoDock (60 × 60 × 60 Å) is that these may cause variation of the result. Mostly, free programs are designed for non-professional users and some of them can change grid resolution but some program is designed for automatically calculated grid resolution (ArgusLab). This means that different docking programs have different protocols. For the ArgusLab which is designed in the different way from AutoDock. If we use the same grid size, we will obtain not reliable results.

When we calculated the correlation of binding energy between ArgusLab and AutoDock4 results with Pearson correlation method, we obtained a good correlation coefficient (r) up to 0.870 (N = 9, $p<0.01$). In addition,

the correlation of binding energy between ArgusLab and AutoDock Vina results also gained a good correlation coefficient (r) up to 0.900 (N = 9, $p<0.01$). This information shows that ArgusLab is a reliable program for docking small molecule with high efficiency, time saving and at no cost.

4. Conclusion

We tested ability of ArgusLab 4.0.1 for docking between tyrosinase enzyme and tyrosinase inhibitors comparing with AutoDock 4 and AutoDockVina programs. ArgusLab performed high-speed calculation and easily accessible with Windows operating system. We obtained the best correlation coefficient with ArgusLab. All inhibitors stayed near the entrance of the active site and had no interaction with copper atoms in the enzyme. In addition, ArgusLab also has database docking supporting SDF file as ligand database. This function initiates our plan to do future researches on virtual screening with other groups of compounds and targets such as anti-inflammatory agents from Zingiberaceae plants with cyclooxygenase-2 enzyme.

Acknowledgments

This work was supported by College of Oriental Medicine, Rangsit University, Thailand.

References

1. Cohen NC. Guidebook on molecular modeling in drug design,1stEdn. Academicpress.Xiii, 1996.
2. Kitchen DB, Decornez H, Furr JR, and Bajorath J. Docking and scoring in virtual screening for drug discovery: methods and applications. Nature.Rev. Drug. Disc. 2004;3:935-949.
3. Thompson MA. Molecular docking using ArgusLab, an efficient shape-based search algorithm and the AScoring function. ACS meeting Philadelphia 172, CINF 42, PA. 2004.
4. Hafeez A, Saify ZS, Naz A, Yasmin F, and Akhtar N. Molecular docking study on the interaction of riboflavin (Vitamin B2) and cyanocobalamine (Vitamin B12) coenzyme.J. Comp. Med. Volume 2013, Article ID 312183: 5 pages.
5. Naz A, Bano K, Bano F, Ghafoor NA, and Akhtar N. Conformational analysis (geometry optimization) of nucleosidic antitumor antibiotic showdomycin by ArgusLab 4 software. Pak. J. Pharm. Sci. 2009; 22(1):78-82.
6. Oda A, and Takahashi O. Validation of ArgusLab efficiencies for binding free energy calculations. Chem-Bio. Inform. J. 2009, 9:52-61.
7. Vontzalidou A, Zoidis G, Chaita E, Makropoulou M, Aligiannis N, Lambrinidis G, Mikro E, and Skaltsounis A. Design, synthesis and molecular simulation of dihydrostilbene derivatives as potent tyrosinase inhibitors. Bioorg. Med. Chem. Lett. 2012; 22: 5523-5526.
8. Sendovski M, Kanteev M, Shuster VB, N. Adir, and Fisherman A. Crystal structure of tyrosinase from Bacillus magaterium in complex with inhibitor kojic acid. J. Mol. Biol. 2011;405:227-237.
9. Song S, Lee H, Jin Y, Ha YM, Bae S, Chung HY, and Suh H. Synthesis of hydroxyl-substituted phenylnaphthalenes as inhibitors of tyrosinase. Bioorg. Med. Chem. Lett. 2007;17: 461-464.
10. Song YM, Young MH, Kim J, Chung KW, Uehara Y, Lee KJ, Chun P, Byun Y, Chung HY, and Moon HR. Synthesis of novel azo-resveratrol, azo-oxyresveratrol and their derivatives as potent tyrosinase inhibitors.Bioorg. Med. Chem. Lett. 2012;22:7451-7455.
11. Likhitwitayawuid K, Sornsute A, Sritularak B, and Ploypradith P. Chemical transformations of oxyresveratrol (trans-2,4,3',5'-tetrahydroxystilbene) into a potent tyrosinase inhibitor and a strong cytotoxic agent. Bioorg. Med. Chem.Lett. 2006;6: 5650-5653.
12. Hanwell MD, Curtis DE, Lonie DC, Vandermeersch T, Zurek E, and Hutchinson GR. Avogadro: an advanced semantic chemical editor, visualization, and analysis platform. J. Cheminformatics. 2012;4: 17.
13 Jongkon N, and Tangyuenyongwatana P. Molecular binding modes of diarylheptanoids from Curcuma comosa on the ER-β receptor.Thai. J. Pharm.Sci. 2014;38: 82-89.
14 Goldfeder M, Kanteev M, Adir N, Fishman A. Determination of tyrosinase substrate-binding modes reveals mechanistics between type-3 copper proteins. Nat.Commun. 2014;5: 4505-4509.