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In Silico Approach for Designing Potent Inhibitors against Tyrosinase

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In recent years regulation of the enzymatic activity of tyrosinase has been the main focus of investigation due to its potential applications in medicine, agriculture and cosmetics. In the present study, Eighteen derivatives of 3-hydroxypyridine-4-one scaffold were subjected to molecular docking studies to investigating the mode of interaction of the compounds with tyrosinase active site. We applied Autodock tools 4.2, in order to set up the docking runs and predict the inhibitors binding free energy. The final product of molecular docking was clustered to specify the binding free energy and optimal docking energy conformation that is investigated as the best docked structure. Among the total of molecules tested, it was proved that Ligands 3, and 10 have the lowest binding free energy. The docked conformation revealed that these compounds could form metal-ligand interaction with The Cu^{2+} ions in the active site. These insilico results can thus serve as a template for further studies invitro and invivo.

Key words: In Silico Approach, Docking, Kojic acid, Tyrosinase.

Melanin synthesis is initiated from tyrosine by tyrosinase. Although melanin is essential for protecting skin against UV irradiation damage, abnormal melanin production can lead to hyperpigmentation disorders[1-3]. Browning in crops is unfavorable and decreases the commercial value of the products[4, 5]. Therefore, tyrosinase inhibitors have potential applications in medicine, cosmetics and agriculture.

The most intensively studied inhibitor of tyrosinase, KA, was discovered in Japan by Saito in 1907 and is produced by various fungies[6]. KA chelates transition metal ions such as Cu^{2+} and Fe^{3+} and is an effective free radicals scavenger. This inhibitor is currently applied as a food additive to prevent enzymatic browning of fruits. KA is also added to cosmeceuticals such as skin-lightening agents. However, its use has been limited, for its cytotoxicity, the skin irritation caused by its use in cosmetics and also its instability during storage[7, 8]. Therefore, the development of novel, potent, non-toxic and stable kojic acid derivatives as tyrosinase inhibitors is of great importance.

Docking technique is a structure-based virtual screening that predicts the preferred binding orientation of the ligand to the desired receptor in a stable complex with respect to experiment[9]. This technique searches over many possible interactions in order to identify a set of ligand poses that represent local minimum-energy positions of the ligand. It calculates a binding energy that can be used to accurately rank-order different ligands relative to experimentally measured binding affinities[10].

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In the present study, Eighteen derivatives of 3-hydroxypyridine-4-one scaffold were subjected to molecular docking studies. The preparation of some of these compounds is reported previously by this group[11, 12]. These compounds are very close analogues of kojic acid having structural elements which provides them the ability to chelate copper ions and set up interactions with key residues located in the active site. Based on the *in vitro* evaluations some of these compounds had tyrosinase inhibition activity and were considered in the design of the study compounds[12].

MATERIALS AND METHODS

Protein and Ligand Structure Preparation

The crystal structure of tyrosinase from *Agaricus bisporus* (2Y9X) was chosen as the protein model for the present study. Co-crystallized ligands (tropolone), all chains except chain A, two holmium ions and water molecules of crystallization were removed from the complex using Discovery StudioVisualizer[13]. All missing hydrogens were added and after determining the Kolman united atom charges, non-polar hydrogens were merged to their corresponding carbons using Autodock tools[14].

The structural detailes of the compounds subjected to molecular docking simulations are provided in Table 1. All 2D structures of compounds were built using ChemDraw program, then were transferred into Hyperchem 8.0 software and energy minimized. These optimized structures were used as inputs of the AutoDock tools. Then the partial charges of atoms were calculated using the Gasteiger-Marsili procedure implemented in the AutoDock tools package. Non-polar hydrogens of the compounds were merged and then rotatable bonds were assigned.

Docking Procedure

The AutoGrid program performs pre-calculations for the docking of a ligand to a set of grids that describe the effect that the protein has on point charges. The effect of these forces on the ligand is then analyzed by the AutoDock program**.** Using Autogrid as a part of the Auto dock package, desolvation parameters and electrostatic interactions were assigned to each protein atom.

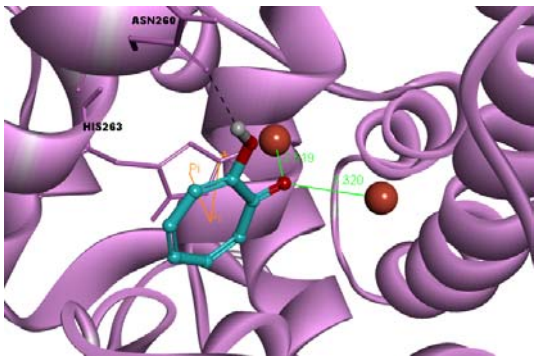


Fig. 1. Redocking results of tropolone in the active site of tyrosinase. This figure was prepared using the Accelrys discovery studio visualizer program.

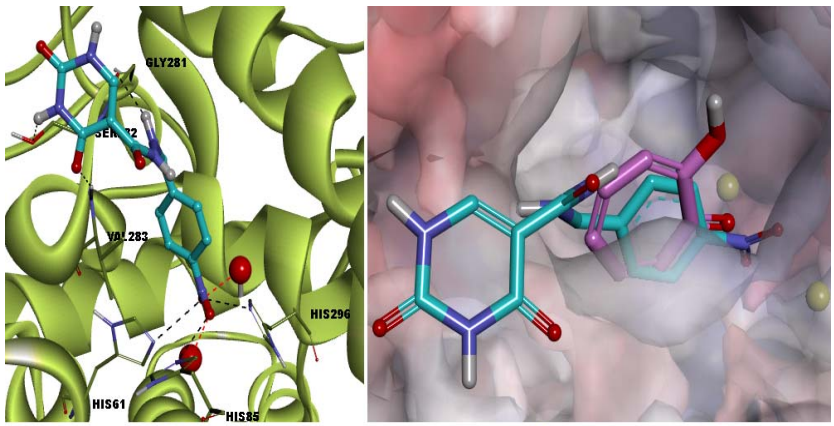


Fig. 2. A. Binding model of compound **3** for the best docked pose in the tyrosinase active site. **B.** Superimposition of the best docking poses for compound **3** (cyane) with tropolone (violet) in the active site of tyrosinase. Divalent Cu^{2+} ions are shown as yellow sphere.

The grid points were set as 40 × 40 × 40 with the spacing valued at 0.375 to the catalytic site of the tyrosinase while the grid center was placed between the two metal ions located in the active site. The Lamarckian Genetic Algorithm (LGA) approach was selected as the search algorithm for the global optimum binding position search among the three different search algorithms offered by AutoDock 4.2. The resulting docking poses were analyzed in AutoDockTools, DS Visualizer 3.5 and Ligplot softwares[15].

RESULTS

Tropolone was redocked into the active site of tyrosinase for the method validation in the first step of docking procedure. The binding energy of the best bound conformation of tropolone was -3.11 kcal/mol. It interacts with HIS263 through ð–ð and cation-ð interactions and its OH group forms a hydrogen-bond with Asn260 (Figure 1).

After validation of the docking protocol, the 3D structures of the study compounds were docked into the tyrosinase active site. The estimated free binding energy values ($ÄG_{bind}$) of the docked positions, intermolecular energy, electrostatic energy, total internal energy and torsional energy of these inhibitors into the active site are listed in Table 2. The favorable interactions with the key amino acid residues at the active site of the enzyme are presented Tables 3.

DISCUSSION

The binding mode of the study compounds against tyrosinase was investigated by performing molecular docking smulations. There is no X-ray crystallography data available for the three-dimensional structure of human tyrosinase[16]. Thus, the 3D structure of *Agaricus bisporus* mushroom tyrosinase was used for the docking study. Possible H-bonding, ð-sigma, cation-ð and metal interactions assessed using the Discovery StudioVisualizer program. Ligplot software predicted the possible hydrophobic interactions between the docked ligands and the catalytic site residues. In agreement with binding energies, Ligands **3** and **10** displayed good docking results. Among the all studied compounds, the best docking result was obtained for compound **3** that showed a high inhibitory potency. In fact, this compound had the most negative $ÄG_{bind}$ (-16.43 Kcal/mol) that indicated favorable interactions and tight binding with the key amino acid residues at active site of tyrosinase. The His85, His61, His296, Val283, Ser282 and Gly281 residues of tyrosinase were the sites for hydrogen bonding interactions with compound **3**. The docked conformation revealed that the NO_2 group of compound **3**, which resided 2.10-2.14 Å adjacent to the di-copper nucleus, could form metal-ligand interaction. The interaction mode of compound **3** with the binding region of tyrosinase revealed that

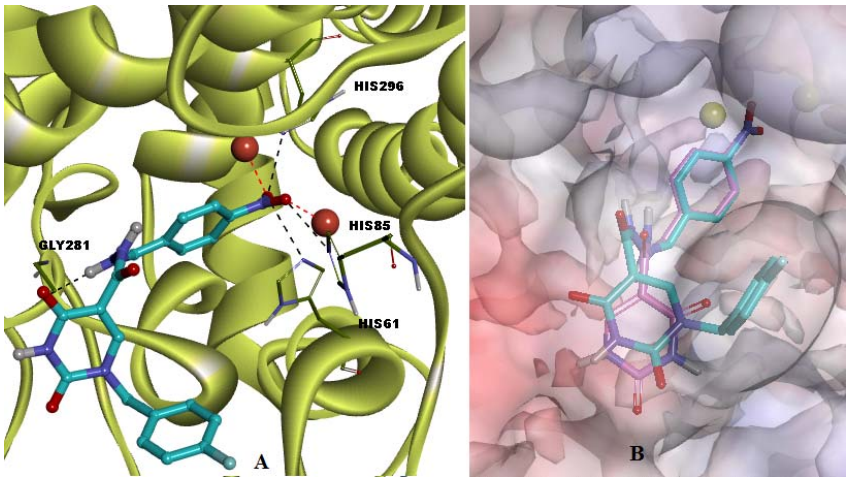


Fig. 3. A. Binding model of the compound **10** for the best docked pose in the tyrosinase active site.
B. The superimposed structures of compound **10** (cyan) with compound **3** (violet) in the active site of tyrosinase. Divalent Cu^{2+} ions are shown as yellow sphere.

Table 1. Structural details of the studied compounds

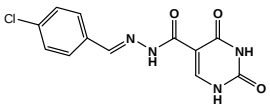
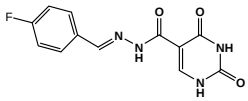
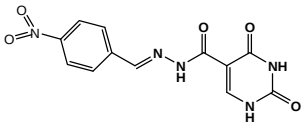
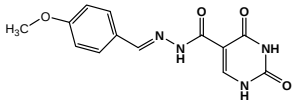
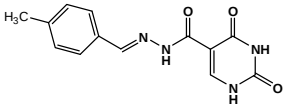
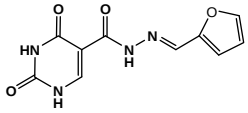
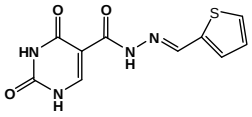
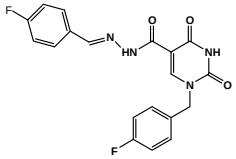
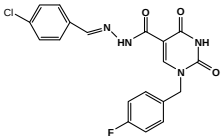
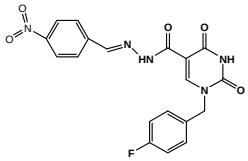
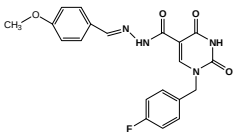
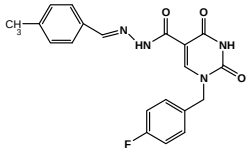
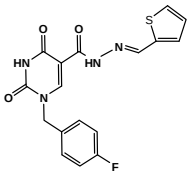
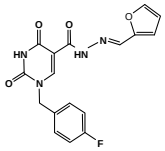
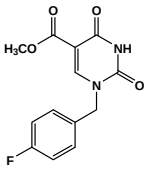
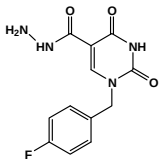
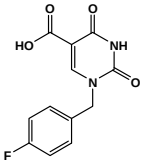
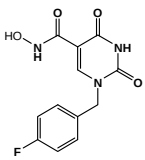
Compound	Structure	Compound	Structure
1		2	
3		4	
5		6	
7		8	
9		10	
11		12	
13		14	
15		16	
17		18	

Table 2. Docking results of kojic acid derivatives docked into tyrosinase active site. The values are expressed in kcal/mol

Compound	Binding energy	Intermol energy	Electrostatic energy	Total internalenergy	Torsional energy
1	-4.5	-5.32	-1.07	0.03	0.82
2	-4.41	-5.23	-0.58	0.03	0.82
3	-16.43	-17.53	-13.52	0.01	1.1
4	-6.63	-7.73	-0.88	0.01	1.1
5	-3.75	-4.57	-0.04	0.02	0.82
6	-3.22	-4.04	-0.49	-0.09	0.82
7	-3.60	-4.42	-0.1	-0.05	0.82
8	-3.83	-5.21	-0.93	-0.41	1.37
9	-3.84	-5.21	-0.46	-0.41	1.37
10	-14.97	-16.62	-13.96	-0.01	1.65
11	-6.87	-8.51	-0.67	-0.07	1.65
12	-3.26	-4.63	-0.77	-0.41	1.37
13	-3.54	-4.91	-0.92	-0.34	1.37
14	-3.36	-4.73	-0.99	0.18	1.37
15	-3.79	-4.89	-0.37	-0.06	1.1
16	-2.17	-3.27	-1.29	0.43	1.1
17	-11.21	-12.31	-14.38	0.57	1.1
18	-2.25	-3.35	-1.94	0.12	1.1
Tropolone	-3.11	-3.38	-1.51	-0.03	0.27

this molecule makes hydrophobic interactions with Ser282, His61, His259, His263, Met280, Ala286, Phe264, Phe292, Pro277 and Pro284. A comparison of the binding modes of this compound and tropolone reveals that hydrophobic interactions with Ser282, His61, His259, His263, Ala286 and Met280 residues are the same for compounds **3** and tropolone. Instead of the hydrophobic interactions with Val283 and Gly281 in tropolone, compound **3** binds to these residues through hydrogen bonding. The binding mode of this compound for the best pose in the tyrosinase active site is shown in Figure 2 A. The superimposition of this compound with tropolone is illustrated in Figure 2 B. $\ddot{A}G_{bind}$ for Compound **10** was -14.97 kcal/mol. Similar to compound **3,** this compound have an NO_2 moiety. The obtained binding mode of compound **10** suggests that it forms hydrogen bond with His85, His61,His296 and Gly281(Figure 3A). This compound also makes hydrophobic contacts with Gly281, His61, His259, Val283, Ser282, His94, His263, Phe264, Phe292, and Phe90. These interactions are almost the same as the interactions of compound **3** with the tyrosinase binding site. Superimposition of compound **10** and compound **3** implies that the main scaffolds and the substituents have almost the same orientations (Figure 3B).

The docking results for these compound are in accordance with the docking results reported by others in terms of the amino residues involved in interaction with the inhibitor molecule[16-20].

CONCLUSION

In the resent study, we employed computational approaches, such as molecular docking to estimate the binding free energy of Eighteen inhibitors with tyrosinase. Among the all studied compounds, the best docking result was obtained for compound **3**. NO_2 group in this Compound binds to both of Cu^{2+} ions located inside the active site of the enzyme.

ACKNOWLEDGMENT

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Table 3. Interactions between the study compounds and tyrosinase active site residues

Compound	Interaction with Cu^{+2}	Interaction with amino acid residues		
		H-bonding	Pi interactions	Hydrophobic
1	-	Val_{283}, Ser_{282}, Gly_{281}	-	Met_{280}, Ser_{282}, His_{61}, $His_{259,}His_{263}$, Ala_{286} , $Phe_{264,}$ $Phe_{284,}$ Pro_{277}
2	-	Val_{283}, Ser_{282}, Gly_{281}	-	Met_{280}, $His_{259,}His_{263}$, Ala_{286}, $Phe_{264,}$ $Phe_{284,}$ Pro_{277}
3	N=O... Cu^{2+} (2.107) [a]N=O... Cu^{2+} (2.146)	His_{85}, His_{61}, His_{296}, Val_{283}, Ser_{282}, Gly_{281}	-	Ser_{282}, His_{61}, $His_{259,}His_{263}$, $Met_{280,}$ Ala_{286}, $Phe_{264,}$ $Phe_{292,}$ Pro_{277}, Pro_{284}
4	-	Val_{283} ,Ser_{282}, Gly_{281}	-	Ser_{282}, His_{61}, $His_{259,}His_{263}$, $Met_{280,}$ Ala_{286}, $Phe_{264,}$ $Phe_{90,}$ Pro_{277}, Pro_{284}, His_{85}
5		Val_{283}, Ser_{282}, Gly_{281}	-	His_{61}, $His_{259,}His_{263}$, $Met_{280,}$ Ala_{286}, $Phe_{264,}$ Pro_{277}, His_{85}
6	-	Gly_{281}, Met_{280}, Asn_{260}	-	Met_{280}, Ser_{282}, His_{85}, $His_{259,}$ Val_{283}, His_{263}, $Arg_{268,}$ Ala_{286}, $Phe_{264,}$ Pro_{277}
7	-	Val_{283}, Ser_{282}, Gly_{281}	-	His_{61}, His_{263}, $Met_{280,}$ Ala_{286}, $Phe_{264,}$ Pro_{277}, Pro_{284}
8	-	Val_{283}, His_{244}, Arg_{268}, Gly_{281}	-	Ser_{282}, His_{244}, $His_{259,}$ His_{263}, $Arg_{268,}$ Asn_{260}, Phe_{264}
9	-	Val_{283}, His_{244}, Arg_{268}, Gly_{281}	His_{263}(sigma- ð)	Ser_{282}, His_{244}, $His_{259,}$ His_{263},$Ala_{286,}$ Asn_{260}, Met_{280}, Phe_{264}
10	N=O... Cu^{2+} (2.135) N=O... Cu^{2+} (2.141)	His_{85}, His_{61}, His_{296}, Gly_{281}	-	Gly_{281}, His_{61}, His_{259}, Val_{283}, Ser_{282}, His_{94}, His_{263}, Phe_{264}, Phe_{292}, Phe_{90}
11	-	His_{244}, His_{85}, Gly_{281}	-	Val_{283}, His_{85}, His_{259}, $Ala_{286,}$ His_{263}, His_{61}, Thr_{84}, Thr_{24}, Asn_{260}, Asn_{81}, Glu_{322}, Cys_{83}
12	-	Val_{283}, Ser_{282}, Gly_{281}	-	Ser_{282}, His_{263}, Val_{283}, $Ala_{286,}$ Asn_{260}, Pro_{277}, Pro_{284}, Phe_{264}
13	-	Val_{283}, Gly_{281}	-	His_{61}, His_{259}, Val_{283}, Ser_{282}, His_{263}, Phe_{264}, Pro_{284}, Ala_{286}
14	-	Val_{283}, Asn_{260}, His_{244}, Gly_{281}	-	His_{85}, His_{259}, Val_{248}, Ser_{282}, His_{263}, $Phe_{264,}$ Gly_{281}, Ala_{286}
15	-	Gly_{281}, Val_{283}, Ser_{282}, Arg_{268}	-	His_{263}, $Phe_{284,}$ Gly_{281}, Asn_{260}, Phe_{264}, Pro_{277}
16	C=O... Cu^{2+} (2.426)	Glu_{256}, Ser_{282}, Met_{280}, Asn_{260}	His_{263}(sigma- ð)	Ser_{282}, His_{61}, $His_{259,}$ His_{263}, Ala_{286}, $Phe_{264,}$ His_{85}, Val_{248}, His_{244}, Gly_{281}, Asn_{260}, Glu_{256}
17	C=O... Cu^{2+} (2.062)	His_{244}, His_{85}	His_{263}(sigma- ð)	His_{94}, His_{259}, Val_{283}, Ser_{282}, His_{244}, Phe_{292}, Ala_{286}, Gly_{281}, Asn_{260}, Glu_{256}
18	C=O... Cu^{2+} (2.325)	His_{244}, Asn_{260}, His_{263}, Glu_{256}	-	His_{85}, His_{259}, Val_{283}, Ser_{282}, His_{259}, Phe_{264}, Ala_{286}, Gly_{281}, Asn_{260}, Phe_{90}
Tropolone	C=O... Cu^{2+} (2.519)	Asn_{260}	His_{263}(cation-ð) (ð -ð)	Gly_{281}, His_{259}, Val_{283}, Ser_{282}, His_{61}, His_{263}, Ala_{286}, Met_{280}

[a] The value in parenthesis is the distance between donor atom and Cu^{2+} ion expressed in angstrom

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