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# Assessment of Anti-tyrosinase and Antioxidant Activities along with Molecular Docking Studies, and in silico ADME of Some 3-Hydroxypyridin-4-one Derivatives

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## Abstract

Tyrosinase is an essential enzyme in melanin production, which plays an important role in the browning of plants and vegetables and skin diseases in humans. Therefore, the design and synthesis of tyrosinase inhibitors are important in the food industry and in treating melanin-related skin diseases. In this study, the tyrosinase inhibitory activity and the antioxidant capacity of a series of 3-hydroxypyridin-4-one derivatives that have been synthesized in the Department of Medicinal Chemistry at the Faculty of Pharmacy, Shiraz University of Medical Sciences, were assessed. The antioxidant activities were evaluated using the 1,1-diphenyl-2-picrylhydrazine (DPPH) radical scavenging method. The biological results indicated that these derivatives exhibit mild anti-tyrosinase and antioxidant properties. Docking studies were also conducted, and the results showed that the compounds have suitable binding free energy and interactions with the active site of the tyrosinase enzyme, which were not consistent with the biological results. Finally, the investigation of the pharmacokinetic characteristics and drug-likeness of the derivatives showed that they have the potential for oral bioavailability.

*Keywords*: 3-Hydroxypyridin-4-one, Tyrosinase inhibitor, Free radical scavenging, ADME.

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## 1. Introduction

Tyrosinase is a copper-containing metalloenzyme that catalyzes the oxidation of monophenols and diphenols to orthoquinone in melanin synthesis (1). Melanogenesis causes fruits and vegetables to turn brown and reduces their nutritional value, as well as causing skin diseases in humans (2). These cases emphasize the significance of inhibiting the tyrosinase enzyme. In the past decades, kojic acid has been used as a potent tyrosinase inhibitor (3). However, despite its high inhibitory potential, this compound can cause side effects such as irritation, rash, dermatitis, itching, and pain, which can be bothersome for patients (4). This has led researchers to search for new tyrosinase inhibitors with lower side effects (5). Researchers have focused on designing compounds that are structurally similar to kojic acid (6). A class of compounds that have recently been widely studied for their inhibitory properties are hydroxypyridinones derivatives, which have high structural similarity with kojic acid due to hydroxy and carbonyl groups (7). Hydroxypyridinones can

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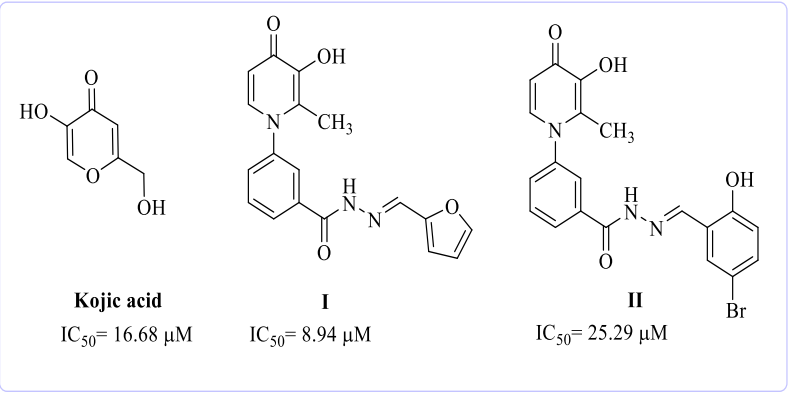



Figure 1. The structures of kojic acid and previously synthesized potent tyrosinase inhibitors.

act as chelators due to their unique structures (8), which enables these compounds to interact with copper atoms in the active site of tyrosinase (9).

Stilbene-hydroxypyridinone hybrids (10), hydroxypyridinone-L-phenylalanine conjugates (11), hydroxypyridinone derivatives containing an oxime ether moiety (12), chitosan oligosaccharide-hydroxypyridinone conjugates (13), and chalcone-hydroxypyridinone hybrids (14) are some of the hydroxypyridinones compounds which previously studied as anti-tyrosinase agents. In recent studies, our research group also used 3-hydroxypyridin-4-ones, including acylhydrazone attached to substituted phenyl or heterocycles, and achieved favorable results. For example, for the derivative containing furan (compound I), IC50 was 8.94 ± 0.52 µM (15), and in another study, for the derivative containing 2-hydroxy 4-bromophenyl (compound II), the IC50 was 25.29 µM (Figure 1) (16).

The current study evaluated the anti-tyrosinase and antioxidant activity of a series of 3-hydroxypyridin-4-one derivatives to establish the structure-activity relationship. The studied compounds (1-10) were previously synthesized in the Department of Medicinal Chemistry at the Faculty of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran (17, 18). Additionally, molecular docking studies were conducted to comprehend these compounds' binding poses and interactions in the enzyme's active site. Furthermore, the physicochemical properties of these compounds were assessed.

## 2. Material and methods

### *2.1. Enzymatic assay for measurement of tyrosinase inhibition*

The inhibitory activity of the studied compounds against the tyrosinase enzyme, using L-Dopa as the substrate, was determined based on previously reported methods (19). In this procedure, compounds (1-10) were initially diluted and then introduced into 96-well microplates containing tyrosinase dissolved in a phosphate buffer (pH 6.8). Subsequently, L-Dopa was added to the mixture, and the microplates were incubated for 20 min. During this process, L-Dopa is oxidized to dopachrome, and the absorbance at a wavelength of 475 nm is measured. The inhibition percentage of the tested compounds was determined using the following equation:

% Inhibition= (Acontrol −Asample /Acontrol) *100

### *2.2. Enzymatic assay for measurement of antioxidant*

The antioxidant capacity of the tested compounds was evaluated using 1,1-diphenyl-2-picrylhydrazine (DPPH) radical scavenging, following a previously published method (20). Compounds (1-10) were introduced to the DPPH solution. If they possess antioxidant properties, the solution's color transitions from purple to colorless. The absorption of the compounds was then assessed at a wavelength of 517 nm. The percentage of DPPH radical inhibition activity was determined as follows:

DPPH radical scavenging, %= (A0-As/A0) *100

where A0 is the absorbance of the DPPH solution and the same amount of sample absorbance. In this study, ascorbic acid was used as a positive control.

### *2.3. Molecular docking study*

A molecular docking study was performed

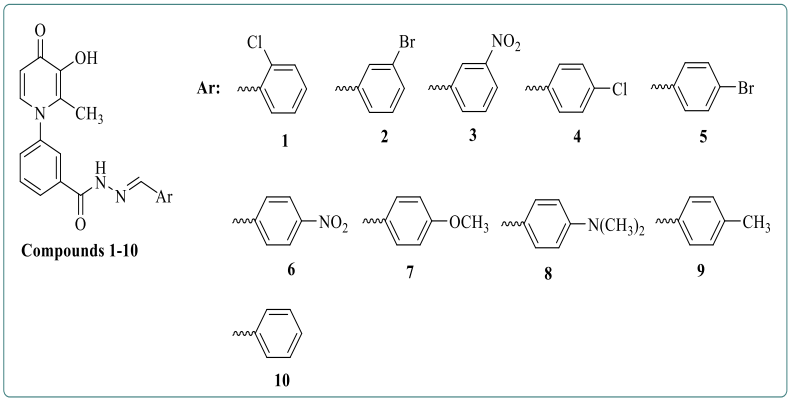



Figure 2. The structures of the studied compounds (1-10).

using AutoDock 4.2 and AutoDock Tools 1.5.4 (ADT). Initially, the three-dimensional structure of the tyrosinase enzyme in complex with tropolone was extracted with a PDB code of 2y9x from the website (http://www.rcsb.org) (21). Subsequently, water, ions, and non-polar hydrogens were eliminated, polar hydrogens were added, and Gasteiger charges were computed and saved in pdbqt format. The structures of the studied compounds were drawn, minimized using ChemBio 3D, and converted to pdbqt format. For the docking study, a grid box of 40 × 40 × 40 was selected, and the docking was configured to 100 exhaustiveness (22). The binding pose and interactions of the studied compounds were visualized using the Discovery Studio 2016 client.

### *2.4. In silico ADME profile*

The ADME profile, including absorption, distribution, metabolism, and excretion, was determined using the SwissADME online software (http://www.swissadme.ch/) (23). To assess drug-likeness, parameters such as molecular weight (MW), number of hydrogen bond acceptors (nHBA), number of hydrogen bond donors (nHBD), number of rotatable bonds (nRB), and topological polar surface area (TPSA) were examined (24). Skin permeability (log Kp) was also analyzed to determine the compounds' penetration into the skin (25).

## 3. Results and discussion

### *3.1. Anti-tyrosinase activity*

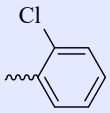
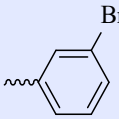
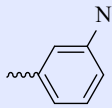
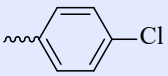
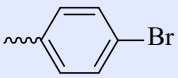
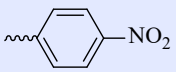
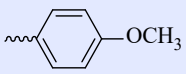
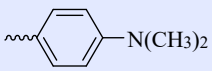
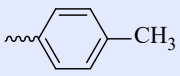
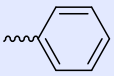
The anti-tyrosinase activity of previously synthesized compounds (1-10) whose structures were confirmed by 1H-NMR, 13C-NMR, IR, and Mass spectroscopy, was evaluated (Figure 2) (17, 26).

The results of anti-tyrosinase activity of compounds (1-10) with electron-donating and electron-withdrawing substitutions at ortho, meta, and para positions of the phenyl ring are provided in Table 1. As shown in Table 1, for compounds with halogen substitutions such as chlorine (Cl) and bromine (Br), changing the position of these substitutions from meta position to para position did not significantly change the inhibitory activity. Although, for compounds with chlorine substitution, shifting the chlorine group from ortho (1) to para (4) position slightly increased the inhibitory activity (IC50= 64.58 µM). Furthermore, the anti-tyrosinase activities of studied compounds with electron-donating substitutions such as methoxy (7) and methyl (9) were not considerable. While compound 8 with a dimethylamine substitution at the para position of the phenyl ring showed an IC50 value of 76.81 µM.

### *3.2. Free radical scavenging activity*

The radical scavenging activity of the studied compounds (1-10) was evaluated using the 1,1-diphenyl-2-picrylhydrazine (DPPH) method, and the obtained results are shown in Table 2. From Table 2, it is evident that among the tested compounds (1-10), compounds 1 and 10, with 2-Cl and -H substitutions, showed the highest inhibition percentage of 43.57 (EC50=379.00 µM) and 43.12 (EC50= 206.03 µM), respectively. These values are weaker compared to the quercetin, reference compound, which showed an inhibition percentage of 74.33 and an EC50 of 9.4 µM. Other compounds did not show significant antioxidant effects.

Table 1. The Anti-tyrosinase activity of studied compounds (1-10) and kojic acid.

| ID | Ar | MP (ºC) | Inhibition%[a] ±SEM[b] | IC50 (µM)[c] |
|---|---|---|---|---|
| 1 |  | 158-159 | 15.15±2.11 | >100 |
| 2 |  | 258-259 | 17.58±2.85 | >100 |
| 3 |  | 275-276 | 27.84±3.17 | >100 |
| 4 |  | 219-220 | 42.76±4.12 | 64.58 |
| 5 |  | 174-175 | 17.37±6.45 | >100 |
| 6 |  | 290-291 | 17.47±2.53 | >100 |
| 7 |  | 281-282 | 30.38±10.15 | 76.81 |
| 8 |  | 201-202 | 40.22±13.22 | >100 |
| 9 |  | 244-245 | 37.47±7.72 | >100 |
| 10 |  | 278-289 | 14.72±3.80 | >100 |
| *kojic acid* | ------- | ------- | 62.67±7.88 | 16.68 |

[a]Values for tested compounds and kojic acid were measured at 50 µM.
[b]Values for 3 repetitions of the experiment.
[c]50% inhibitory concentration (IC50).

### *3.3. Molecular docking study*

Molecular docking studies were performed for all tested compounds (1-10) against tyrosinase enzyme with PDB ID: 2y9x (Figure 3a) (16). At the binding site of the tyrosinase enzyme, His85, His61, His94, His259, His263, and His296 residues play a key role in the activity of the enzyme, which interacts with two copper atoms in the active site (Figure 3b) (27). To validate the docking process, re-docking was performed, resulting in an RMSD of 0.5 (Figure 4).

The binding free energies of the residues involved in hydrogen bonds and π interactions are presented in Table 3. As can be seen, the binding free energy of the compounds ranges from -7.4 to -8.6 kcal mol-1, indicating that the binding free energies are close to each other. Among the studied compounds, 2, 4, and 8 have the highest binding free energy.

The molecular docking results indicated that all the studied compounds interact with key residues such as His296, His61, His263, His259, and His85. Consequently, all the compounds are situated in the binding pocket of tyrosinase. Although the molecular docking results indicated the appropriate interactions of the studied compounds with the active site of the tyrosinase enzyme, these compounds did not show acceptable inhibitory ac-

Table 2. The radical scavenging activity of the studied compounds (1-10) and quercetin.

| ID | DPPH (%) | EC50(µM) | Compound | DPPH (%) | EC50(µM) |
|---|---|---|---|---|---|
| 1 | 43.57±2.25 | 379.00 | 6 | 33.64±0.75 | >400 |
| 2 | 33.94±1.05 | >400 | 7 | 33.79±1.5 | 299.70 |
| 3 | 29.88±2.4 | >400 | 8 | 26.27±3.0 | >400 |
| 4 | 31.98±1.8 | >400 | 9 | 31.68±0.3 | 316.89 |
| 5 | 30.93±2.85 | 326.45 | 10 | 43.12±0.3 | 206.03 |
| quercetin | 74.33±1.68 | 9.4 | | | |

tivity. For example, compound 2 (m-Br) with the highest binding free energy and having suitable interactions did not show tyrosinase inhibitory activity. However, it can be seen that derivatives 4 (p-Cl) and 8 (p-N(CH3)2), which have a higher binding energy than the other compounds, their tyrosinase inhibitory effect is slightly improved compared to other compounds, although it is not proportional to the good interactions that these derivatives have formed in the active site. Generally, there is a lack of correlation between the studied compounds' biological activities and docking results. One reason for the inconsistency between docking and experimental results is that the docking software treats the protein as rigid rather than flexible, leading to inaccurate docking outcomes. Also, molecular docking alone cannot confirm biological activity. Therefore, further experimental studies should be conducted to validate the predicted binding interactions, such as binding assays, biochemical assays, or in vivo experiments. Accordingly, these findings could be valuable in guiding the design of compounds for future studies.

### *3.4. In silico ADME properties*

*In silico* ADME properties of the studied compounds were obtained using the SwissADME online software (28). The physicochemical characteristics of these compounds are provided in Table 4. To ensure oral bioavailability, the compounds must meet specific parameters: MW ≤ 500 Da, log P < 5, nHBD ≤ 5, nHBA ≤ 10, and Topological Polar Surface Area (TPSA) ≤ 140 Å (29). As shown in Table 3, all compounds (1-10) exhibit favorable bioavailability and drug-likeness properties. The

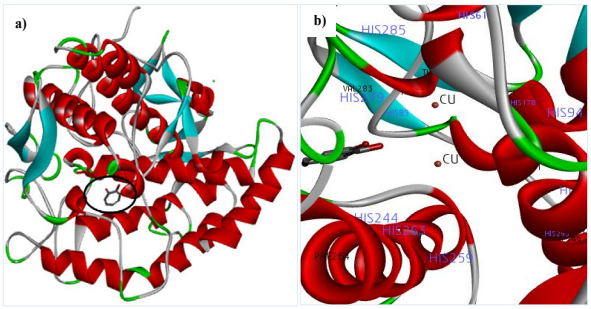



Figure 3. a) Crystal structure of tyrosinase from Agaricus bisporus complexed with tropolone (PDBID: 2y9x) and b) the active site of tyrosinase enzyme.

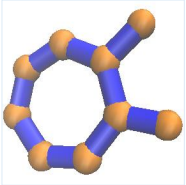


Figure 4. The superimposition of the docked mode (blue) and co-crystal (orange) of tropolone (0TR) in the active site of 2y9x.

Table 3. Interactions and free binding energies of all compounds (1-10) in the active site of 2y9x.

| ID | Binding free energy (kcal mol-1) | Hydrogen bonding | π interaction |
|---|---|---|---|
| 1 | -7.9 | His 296, His 61 | His 244, His 263, Val 248, Val 283, Ala 286, His 259, His 85 |
| 2 | -8.6 | His 296, His 61 | Val 283, His 85, ala 286, His 259, Phe 90 |
| 3 | -8.0 | His 85 | His 85, His 263, His 296, His 61, Val 283, Ala 286, His 259, Phe 90 |
| 4 | -8.4 | His 296, His 61, Asn 81, Cys 83 | His 85, His 263, Val 283, Ala 286, His 259, Phe 90 |
| 5 | -8.2 | His 296, His 61, Asn 81 | His 85, His 263, Val 283, Pro 284, His 259, Phe 90 |
| 6 | -7.7 | His 296, His 61 | His 85, His 263, Val 283, Ala 286, His 259, Phe 90 |
| 7 | -8.0 | His 61 | His 85, His 263, His 61, Val 283, Ala 286, His 259, Phe 90 |
| 8 | -8.3 | His 296, His 61 | His 85, His 263, Val 283, Ala 286, His 259, Phe 90 |
| 9 | -8.1 | His 296, His 61, Glu 256 | His 85, His 263, Val 283, Ala 286, His 259, Phe 90, Asn 260 |
| 10 | -7.4 | His 61 | His 85, His 263, Val 283, Ala 286, His 259, Phe 90, Asn 260, Glu 322, Arg 321 |

permeability coefficient (kp) indicates the extent of chemical penetration into the skin. The Log kp ranges from -8.0 to -1.0, suggesting the potential of various compounds to penetrate the skin (30). The Log kp values for the studied compounds fall within the range of -6.32 to -7.25. The more negative Log kp value for these compounds indicates a lower potential for skin permeation.

## 5. Conclusion

In the present study, several 3-hydroxypyridin-4-one derivatives with different electron-donating and electron-withdrawing substituents in the meta and para positions of the phenyl ring were investigated for their anti-tyrosinase and antioxidant activities. The biological results indicated that the studied compounds exhibited moderate to weak anti-tyrosinase and antioxidant effects. Subsequently, molecular docking studies of the studied compounds on the tyrosinase enzyme with PDBID: 2y9x were conducted. The results revealed that the compounds demonstrated good binding free energy and suitable interactions with the key amino acids in the active site of the tyrosinase enzyme. However, the docking results did not align with the obtained biological results. Finally, to assess drug-likeness and bioavailability, the pharmacokinetic properties of the tested compounds ADME study were performed, indicating that they possess drug-like properties. Overall, the findings suggest that improved biological outcomes could be achieved through better molecular design and substitutions.

## Conflict of Interest

The authors declare no conflict of interest.

Table 4. *In silico* ADME of (1-10) derivatives.

| ID | M.W (g/mol) | nRB | nHBA | nHBD | TPSA (Å2) | Log p | Lipinski Rule/violation | log kp (cm/s) |
|---|---|---|---|---|---|---|---|---|
| 1 | 380.82 | 5 | 3 | 2 | 71.33 | 2.38 | 0 | -6.49 |
| 2 | 425.28 | 5 | 3 | 2 | 71.33 | 2.48 | 0 | -6.71 |
| 3 | 391.38 | 6 | 5 | 2 | 117.15 | 0.9 | 0 | -7.25 |
| 4 | 380.82 | 5 | 3 | 2 | 71.33 | 2.38 | 0 | -6.32 |
| 5 | 425.28 | 5 | 3 | 2 | 71.33 | 2.48 | 0 | -6.54 |
| 6 | 391.38 | 6 | 5 | 2 | 117.15 | 0.9 | 0 | -7.25 |
| 7 | 376.41 | 6 | 4 | 2 | 80.56 | 1.56 | 0 | -6.80 |
| 8 | 389.45 | 6 | 3 | 2 | 74.57 | 1.78 | 0 | -6.73 |
| 9 | 360.41 | 5 | 3 | 2 | 71.33 | 2.11 | 0 | -6.38 |
| 10 | 346.38 | 5 | 3 | 2 | 71.33 | 1.89 | 0 | -6.72 |

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