Inhibition Properties and Thermodynamic Changes of Binding of *p*-perazine-bis and *p*-peridine Dithiocarbamate Sodium Salts to Mushroom Tyrosinase

Amin, Ehsan•; Saboury, Ali Akbar*+

Institute of Biochemistry and Biophysics, University of Tehran, Tehran, I.R. IRAN

Mansuri-Torshizi, Hassan

Department of Chemistry, University of Sistan & Bluchestan, Zahedan, I.R. IRAN

Zolghadri, Samaneh*+

Department of Biology, Jahrom Branch, Islamic Azad University, Jahrom, I.R. IRAN

ABSTRACT: *A mono- and a bi-functional dithiocarbamates as sodium salts were obtained by treating p-peridine or p-perazine in aceton-water mixture with CS_2 in the presence of NaOH. These anionic water soluble compounds have been characterized by elemental analysis, IR and ^1H NMR spectroscopic studies. Both compounds (p-peridine (**I**) and p-perazine-bis dithiocarbamate (**II**) sodium salts) were examined for inhibition of mushroom tyrosinase (MT) activity. The results showed that they inhibit MT competitively. K_I values of two compounds at 27 ℃ are 2 and 4 μM. Therefore, the compound (**I**) is more potent than (**II**). They chelate active site of tyrosinase via electrostatic interactions. These conclusions are proved by obtained thermodynamic parameters and fluorescence studies. Extrinsic fluorescence studies disprove any tertiary structure changes of MT. Major enthalpy changes in binding of compound (**II**) in comparison to (**I**) show that including two carbamate tails in such compounds disturb balancing of hydrophobic interactions with vicinity of active site of enzyme.*

KEYWORDS: *Inhibition; Thermodynamic changes; Dithiocarbamate sodium salts; Mushroom tyrosinase.*

INTRODUCTION

Tyrosinase is a copper-containing enzyme that catalyzes specially the first step in melanin biosynthesis [1-3]. The enzyme catalyzes dioxygen reduction (oxidase activity) and activation for incorporation into organic substrates

* *To whom correspondence should be addressed.*

+ E-mail: Saboury@ut.ac.ir ; szjahromi@yahoo.com

• Other Address: Institute of Neural and Sensory Physiology, Medical Faculty of the HeinrichHeine University, Düsseldorf, GERMANY

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(oxygenase activity) by two anti-ferromagnetically coupled coppers in its active site [4]. Each atom of the binuclear copper cluster is ligated to three histidines and makes enzyme able to bind to molecular oxygen [5]. Naturally, L-tyrosine and L-DOPA (L-3,4-dihydroxyphenylalanine) are substrates of oxygenase (monophenolase) and oxidase (diphenolase) activities, respectively [6-7]. Oxidation of phenolic compounds to the corresponding reactive quinones and a cascade of oxidative condensation cause further non-enzymatic polymerization and are responsible for melanin formations [8-10]. Melanins are heterogeneous biopolymers with a complex structure and color ranging from yellow to black [2]. Melanin pigments fulfill various physiological roles in different organisms such as Fruits, fungi [11], vegetables, invertebrates, and mammals. Browning of exposed to air, wounded tissue, browning occurring during post-harvest storage [12], fungi spore formations [8], wound healing, cuticular hardening (sclerotization), photo-protection of the skin, diseases of melanin disorders like oculocutaneous albinism and vitiligo [13] and so many other are wide range of melanogenesis importance [14-17].

In the food and cosmetic industries and in the treatment of some skin disorders, the activity of tyrosinase and subsequent generation of melanins is undesirable [18]. So, inhibiting tyrosinase that catalysis the rate limiting of melanin formation involves the importance of attention [19]. Thus, prevention of browning of fruits and vegetables and production of skin-whitening agents leads researchers to study and design inhibitors of tyrosinase. Up to now a wide range of inhibitors from different sources are known and studied as inhibiting activities of tyrosinase. Plant polyphenols (flovonoids are one of the best-studied groups) and bioactive phytochemicals Aldehydes, fungal metabolites (kojic acid is a good example), derivatives and modified natural compounds (such as gallic acid alkyl esters) and finally a large number of synthetic compound have been reported to date [20-29]. Among numerous groups of tyrosinase inhibitors sulfur-containing compound are one well-studied groups due to chelating ability of thiols. Cysteine [30], thiosemicarbazide derivatives [31-32], n-aklyl xhanthates [33-34], n-alkyl dithiocarbamates [35], hydroxyl-phenylthiourea [36], tetrathiotungstate [37], benzyl and p-xylidine-bis dithiocarbamates are recent interesting inhibitors [38].

In compounds of the type RR′SSNa or RHNCSSNa, the –NCSS- represent the dithiocarbamate functional group and R represents non-dithiocarbamate parts. There is much interest in the potential activity of these compounds usage in agriculture and their effects on human health [39]. Other biological effects of these agents have been described in the literature such as Thyroid peroxidase inhibition; modification of redox regulation in the cell [40]; and metal transport in membranes [41]. In most of these cases, -NCSS- group react with metal atoms of metal containing enzymes and thus blocking their catalytic activity [42]. Moreover, metal dithiocarbamates have diversified application in the field of rubber chemistry, as vulcanization accelerators and antioxidants [43]. They have gained acceptance as fungicides, insecticides and rodent repellents [42].

Thus we thought of interest to extend the study of such a particular dithiocarbamate as enzyme inhibitor. However, we still have little understanding of how the structure-activity relationships and inhibitory mechanisms affect the inhibitory activities when modifying dithiocarbamates by increasing the number of dithiocarbamate functional groups. In following our group‘s researches, in this paper we have selected two structurally related compounds bearing one and two dithiocarbamate functional groups. *P*-peridine is mono-functional compound (**I**) and *p*-perazine-bis is bi-functional one (**II**) (Fig. 1). They may exhibit many technical advantages, such as binding capacity and better settling performance with mushroom tyrosinase. Because, to enhance the binding ability, it is desirable to increase the number of dithiocarbamate functional groups in a molecule. Our study helped further clarify the relationship between modifiers and tyrosinase and understand effects on inhibitory activity introduced by dithiocarbamates by increasing the number of dithiocarbamate functional groups, which may provide a promising route to obtain novel and highly potent tyrosinase inhibitors.

EXPERIMENTAL SECTION

Mushroom tyrosinase (MT; EC 1.14.18.1; specific activity 5370 units/mg), L-3, 4- dihydroxyphenylalanine (L-DOPA) and 1-anilinonaphthalene-8-sulfonate (ANS) were purchased from Sigma. *P*-peridine, *p*-perazine, carbon disulfide and sodium hydroxide were bought

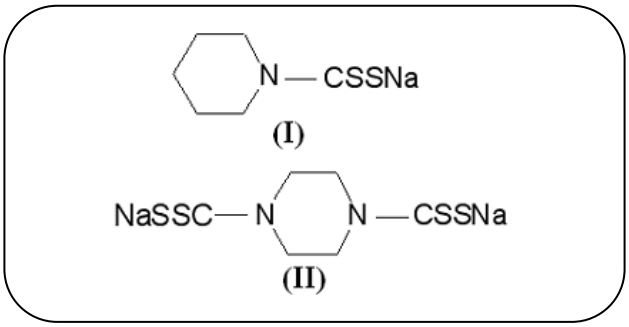

Fig. 1: Structure of the used p-peridine dithiocarbamate sodium salt (I) and p-perazine-bis (dithiocarbamate) sodium salt (II).

from Merck (Germany). High purity solvents were used as received from Merck. Phosphate buffer (50 mM, pH 6.8) was used throughout kinetic measurements. All buffers were prepared with water purified by a Milli Q water purification system.

Microchemical analysis of carbon, hydrogen, and nitrogen was done on CHN Rapid Herause. Melting points were measured on a Unimelt capillary melting point apparatus and are reported uncorrected.

^{1}H NMR spectra were recorded on a Brucker DRX-500 Avance spectrophotometer at 500MHz in DMSO-d_6 using sodium-3-trimethylpropionate as an internal reference. ^{1}HNMR data are expressed in part per million (ppm) and are reported as chemical shift position (δH), multiplicity (s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, sb=singlet broad) and assignment. Infrared spectra were obtained on a Nicolet 5-DXB FT-IR spectrophotometer in rang 4000-400 cm^{-1} in KBr pellets.

Synthesis of p-peridine dithiocarbamate sodium salt NaSSCN<C_5H_{10} (I)

This compound was prepared by the method described by *Marcheselli* and *Preti* [44] with some modifications.

5ml (50mmol) of p-peridine was dissolved in 40ml acetone and chilled. To this, a chilled solution of 2g (50mmol) sodium hydroxide in 10 mL water was mixed with constant stirring. The mixed solution was treated with an ice cold solution of 10mL (excess) carbon disulphide in 10ml acetone keeping the temperature of reaction mixture below 5°C. Stirring continued for one hour at 5°C and another four hours at room temperature. It was then filtered and the solvent evaporated under reduced pressure to complete dryness. Recrystallization was carried out by stirring the crude product in 40mL acetone and filtering the undissolved particles out. 35mL n-hexane was added to the filtrate and then left in a refrigerator overnight. The desired product was collected by filtration as microcrystalline and washed with a small amount of n-hexane and vacuum dried. Yield was 7.2g (79%). Analysis calculated for $C_6H_{10}NS_2Na$ (183): C, 39.34; H, 5.46; N, 7.65%. Found: C, 39.35; H, 5.41; N, 7.66%. Solid state IR spectroscopy of the above compound showed two characteristic bands at 1472 and 967 cm^{-1} assigned to υ(N-CSS) and υ(SCS) modes respectively. ^{1}H NMR (500MHz, DMSO-d6, ppm) 1.56(m, a para -CH_2-), 1.42(m, two meta -CH_2-), 4.27(m, two ortho -CH_2-).

Synthesis of p-perazine-bis (dithiocarbamate) sodium salt NaSSC-N<C_4H_8>N-CSSNa (II)

This compound was synthesized by following the method as described for (I) except that *p*-perazine (2.16g, 25 mmol) was used instead of *p*-peridine. The yield was 4.5g, 64% and analysis calculated for $C_6H_8N_2S_4Na_2$ (282): C, 25.53; H, 2.84; N, 9.93%. Found: C, 25.50; H, 2.82; N, 9.91%. Solid state IR spectroscopy of the above compound showed two characteristic bands at 1462 and 1000 cm^{-1} assigned to υ(N-CSS) and υ(SCS) modes respectively [38]. ^{1}H NMR (500MHz, DMSO-d6, ppm): 4.22 (s, C_4H_8).

Tyrosinase kinetic assay

The spectrophotometric assay for the diphenolase activity of tyrosinase was performed according to the method that was reported in the literature with slight modification [45]. L-DOPA is the substrate of MT that produces a very unstable *o*-quinone that evolves into a stable product (dopachrome) through a first-order reaction. A kinetic analysis showed that the experimentally measurable velocity of the diphenolase activity can be directly monitored by measuring the accumulation of stable product generated [46]. So, rates of dopachrome formation were measured at 475 nm (ε = 3700 M^{-1} cm^{-1}) in the first two minutes by using a Cary spectrophotometer, 100 Biomodel, with jacketed cell holders. The reaction media is one milliliter in 50 mM phosphate buffer (pH 6.8). The final concentration of mushroom tyrosinase was 6.52 µg/mL (35U per assay) for the catecholase activity. The freshly prepared enzyme,

substrate, and dithiocabamates **I** and **II** were used in this work. The reaction was carried out under constant temperatures of 27 and 37°C. Substrate addition followed incubation of the enzyme with different concentrations of synthetic inhibitors. Catecholase activity was carried out using six different fixed concentrations of L-DOPA (0.15, 0.22, 0.30, 0.40, 0.55 and 0.70 mM) in different fixed concentrations of the inhibitors: **I** (0, 0.9, 1.7 and 3.7 μM at 27°C – 0, 1.5, 2.5, 5.0 and 8.0 μM at 37°C) and **II** (0, 1, 2, 4 and 6 μM at 27°C – 0, 6, 12, 22 and 34 μM at 37°C). All experiments were done in triplicate assays.

Intrinsic and extrinsic fluorescence studies

The fluorescence intensities were recorded using a Hitachi spectrofluorimeter, MPF-4 model, equipped with a thermostatically controlled cuvette compartment. The intrinsic emission of protein was seen at the excitation wavelength of 280 nm. 0.17 mg/mL was the concentration of MT in per experiment. Emissions of MT were measured in different concentrations of inhibitors in the same ratio in kinetics experiments. After each addition of inhibitors, the sample volume and the enzyme concentration were kept constant by adding portions of the buffer solution. The extrinsic emission of ANS labeled protein (with 50-concentration ratio) was monitored at the excitation wavelength of 385 nm. The experiments were repeated in the presence of different concentrations of inhibitors, too. Also, maximum extrinsic fluorescence intensity (530 nm) versus inhibitor concentrations was plotted. All graphs here were smoothing using Sigma Plot software [47].

RESULTS AND DSCUSSION

Inhibition types and binding constants of (I) and (II)

Additions of both *p*-peridine (**I**) and *p*-perazine-bis (**II**) dithiocarbamate sodium salts in reaction mixtures of MT and L-DOPA at 27°C have shown potent inhibitory effects of these compounds. In order to understanding of type of inhibitions, it has been described the dependence of reaction rates on different concentrations of inhibitors in several fixed substrate concentrations by Lineweaver-Burk plots [48].

Double reciprocal plots (1/V versus 1/[S], V is initial velocity and [S] is substrate concentration) in the presence of (**I**) and (**II**) are shown in Figs. 2 and 3 respectively. Both figures dedicate the same manners: a series of straight lines that intersect each other in the axis of 1/V. It means that the velocity of the reaction in infinity concentration of substrate is independent of inhibitor (V_{max} = constant). It can be concluded competitive inhibition for these two compounds. Afterwards, comparison of inhibition 's potencies is needed. Following Lineweaver-Burk equation of competitive inhibition can lead to determination of inhibition constant (K_I):

$$\frac{1}{V} = \frac{K'_m}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}} \quad (1)$$

V_{max} and K'_m are maximum velocity and Michaelis-Menten constant in the presence of an inhibitor, respectively. The slope of Equation (1) is a linear function of inhibitor concentrations as follows [48,49]:

$$\text{Slope} = \frac{K_m}{V_{max}} \left(1 + \frac{[I]}{K_I} \right) \quad (2)$$

As can be seen, the y-intercept of this secondary equation leads to K_I value. Secondary plots for lineweaver-Burk plots of (**I**) and (**II**) are shown in insects of Figs. 2 and 3 which corresponds to K_I values of *2* and *4* μM, respectively at 27°C (shown in Table 1). By comparing the K_I values of different inhibitors it has been found out that these ligands are powerful inhibitors comparing to the previous inhibitors. It has been reported that arbutin is one of the tyrosinase inhibitors (K_I=2.98 mM), which has been used widely in the cosmetic industry. As it is shown our ligands have the small Ki value. So we could synthesize ligand derivatives with smaller K_I to inhibit the tyrosinase [50].

A prerequisite for a deeper understanding of the molecular basis of binding of ligands to the enzyme and its interactions is a thorough characterization and quantification of the energetics governing complex formation [51]. An observed free energy ΔG can be the same for interaction with positive entropy ΔS and enthalpy ΔH (binding dominated by hydrophobic effect) and interaction with negative ΔS and ΔH (when specific interactions dominate). Moreover, interacting systems tend to compensate enthalpic and entropic contributions to ΔG, making binding free energy relative insensitive to changes in the molecular details of the interactions process [52]. Thus, consideration of ΔH and

Table 1: Inhibition constants and thermodynamic changes of P-peridine (I) and perazine-bis (II) dithiocarbamates bindings.

Compound	Temp. (°C)	K_I (μM)	K_a (M^{-1}) × 10^5	ΔG° (kJ/mol)	ΔH° (kJ/mol)	TΔS° (kJ/mol)
(**I**)	27	2.0	5	-33	-92	-59
	37	6.6	1.52	-31		-61
(**II**)	27	4.0	2.5	-31	-139	-108
	37	24.1	0.41	-27		-112

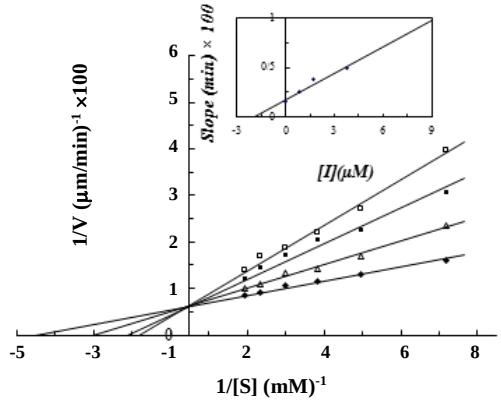

Fig. 2: Inhibition of MT by (I): Lineweaver–Burk plot of reciprocal of initial velocities versus reciprocal of six fixed substrate concentrations in absence (◆) and presence of 0.9 μM (Δ), 1.7 μM (■), 3.7 μM (□), of p-peridine (I) in temperature 27℃. Insect: secondary replots of the Lineweaver–Burk plot, slope versus various concentrations of (I).

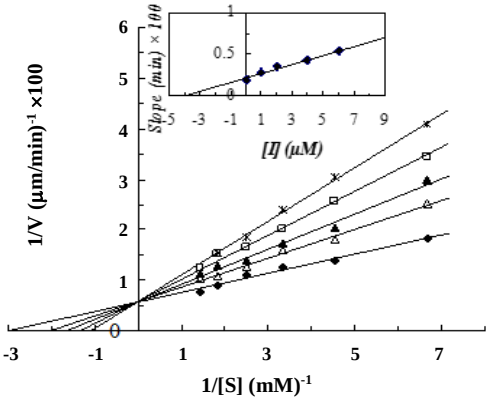

Fig. 3: Inhibition of MT by (II): Lineweaver–Burk plot of reciprocal of initial velocities versus reciprocal of six fixed substrate concentrations in the absence (◆) and presence of 1 μM (Δ), 2 μM (■), 4 μM (□) and 6 μM (*), of p-perazine-bis (II) in temperature 27°C. Insect: secondary replots of the Lineweaver–Burk plot, slope versus various concentrations of (II).

ΔS are crucial for a detailed understanding of the free energy of binding. The association constant K_a (=1/K_I) is related to the Gibbs free energy ΔG by the well-known relation:

$$AG^\circ = -RT\ln K_a \qquad (3)$$

Where R is the universal gas constant and T is the temperature on the Kelvin scale. On the other hand, equilibrium constant K_a for a process is related to the standard enthalpy changes and absolute temperature. Familiar van't Hoff equation describes this relation as follows:

$$\frac{\delta \ln K_a}{\delta\left(\frac{1}{T}\right)} = -\frac{\Delta H^\circ}{R} \qquad (4)$$

Where K_a is the association constant. Therefore, measuring K_I and so on calculating K_a can be leaded the approaching for estimating ΔH. Also, ΔG is composed of an enthalpy term (ΔH) and an entropy term (ΔS), related by another fundamental equation:

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \qquad (5)$$

In order to gain thermodynamic parameters inhibition experiments have been repeated at 37°C. Double reciprocal Linweaver-Burk and secondary plots of dithiocarbamates (**I**) and (**II**) are shown in Figs. 4 and 5, respectively. Inhibition constant of compounds (**I**) and (**II**) are 6.6 and 24.1 μM at 37°C, respectively. Also, thermodynamic parameters were calculated and summarized in Table 1.

Intrinsic Fluorescence spectra

Tryptophan fluorescence has been frequently examined as an intrinsic aromatic fluorophore in tyrosinase molecules to obtain information about the conformational changes. The interaction of compounds (**I**) and (**II**) with tyrosinase and the subsequent

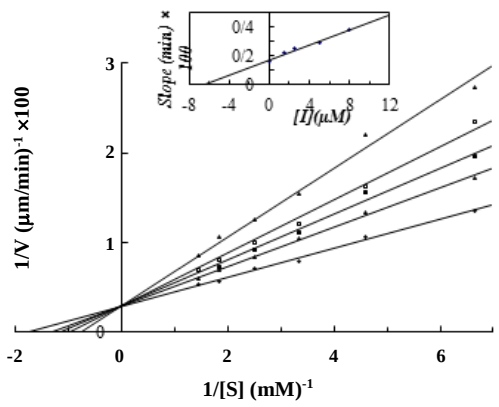

Fig. 4: Inhibition of MT by (I): Lineweaver–Burk plot of reciprocal of initial velocities versus reciprocal of six fixed substrate concentrations in the absence (◆) and presence of 1.5 µM (Δ), 2.5 µM (■), 5 µM (□) and 8 µM (*), of p-peridine (I) in temperature 37°C. Insect: secondary replots of the Lineweaver–Burk plot, slope versus various concentrations of (I).

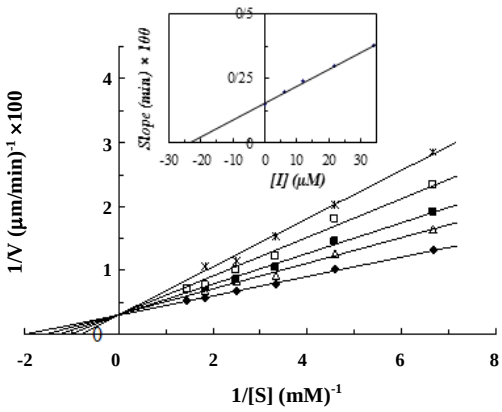

Fig. 5: Inhibition of MT by (II): Lineweaver–Burk plot of reciprocal of initial velocities versus reciprocal of six fixed substrate concentrations in the absence (◆) and presence of 6 µM (Δ), 12 µM (■), 22 µM (□) and 34 µM (*), of p-perazine-bis (II) in temperature 37°C. Insect: secondary replots of the Lineweaver–Burk plot, slope versus various concentrations of (II).

conformational alterations are evaluated by measuring the intensity of emission spectra of the tyrosinase with same ratio concentrations of compound (**I**) and (**II**), which exhibited the inhibitory activity. The tyrosinase has a strong fluorescence emission with a peak at 340 nm on excitation at 290 nm where 94% of light is absorbed by tryptophan residues. Emission intensities are recorded from a range of 295-400 nm. Figs. 6 and 7 showed that the addition of compounds (**I**) and (**II**) caused a dramatic change in the fluorescence emission spectra. In this study, the fluorescence intensities of the emission peaks decreased considerably with an increasing concentration of compounds (**I**) and (**II**). Although quenching casued the decline in the fluorescence intensity there is no significant peak shift with the accumulation of compounds (**I**) and (**II**) which indicated that the polarity environment of tryptophan was not altered.

Extrinsic Fluorescence spectra

In order to remove any doubt about alterations of tertiary structure of MT, emissions of ANS binding to MT in presence of different concentrations of inhibitors were carried out. If factors such as temperature, ligands and etc caused any tertiary structure changes, hydrophobic core of protein can be exposed to ANS molecules in solution and binding with them make ANS molecules fluorophore [53]. In the native form, surface of tyrosinase is predominantly hydrophilic [54]. Fig. 8 shows the plotting of maximum extrinsic fluorescence intensity (530 nm) versus inhibitor concentrations. It can be seen that ANS emissions after inhibitor addition are not very different from before that.

CONCLUSIONS

This paper reported the effects of two novel dithiocarbamate inhibitors on the oxidation of L-DOPA by MT. The results showed that *p*-peridine dithiocarbamate sodium salt (**I**) and *p*-perazine-bis (dithiocarbamate) sodium salt (**II**) were both potent tyrosinase inhibitors. The two compounds due to negatively charged heads are able to chelate copper ions in the active site of MT. K_I values indicated that the (**I**) structure can bind in comparison to (**II**) more tightly to active site. So compound (**I**) can inhibit oxidation of L-DOPA more strongly than (**II**). Competitive inhibition behaviors of two compounds confirm chelating of active site as inhibitor and substrate competing binding to enzyme. In recent publication we have pointed that bi-functional dithiocarbamates can inhibit tyrosinase more strongly than mono-functional one[27]. Nonetheless, in this paper it was showed that mono-functional one is more potent. It can be supposed that *p*-perazine-bis (dithiocarbamate) sodium salt due to short tails cannot be curved to chelate two Cu atoms in the active site. The raising of the temperature has brought about an increase in the K_I value during the catecholase inhibition by both

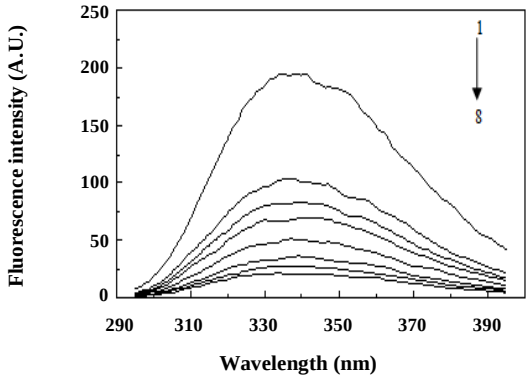

Fig. 6: Emission spectra of tyrosinase showing the quenching effect of increasing the concentration of (I) in temperature 27℃. (Emissions are in absence of (I) and in presence of concentrations: 0.5, 1, 2, 3, 4, 5 and 6 µM).

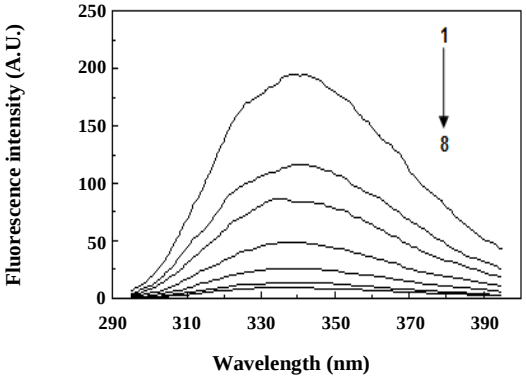

Fig. 7: Emission spectra of tyrosinase showing the quenching effect of increasing the concentration of (II) in temperature 27℃. (Emissions are in absence of (I) and in presence of concentrations: 0.5, 1, 2, 3, 4, 5 and 6 µM).

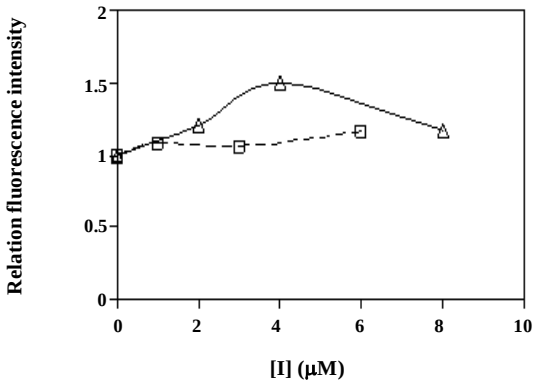

Fig. 8: The ANS-binding fluorescence changes of tyrosinase in the absence and presence of different concentrations of (I) and (II).

compounds (**I**) and (**II**) which is mostly enthalpy driven ($\Delta H < 0$). Therefore, electrostatic interactions are predominant forces in the binding process. These interactions are more vulnerable in the compound (**II**). It has been showed that numerous MT inhibitors are compounds which one end is hydrophilic, and the other end is hydrophobic. This conformation will aid the hydrophilic end of inhibitor interact with the tyrosinase active center and the hydrophobic end of inhibitor interacts with the hydrophobic vicinity of the tyrosinase active center. Balance interactions of these two parts are very sensible in inhibition potent. In our investigation it seems that the second tail in *p*-perazine-bis dithiocarbamate disturbs this balance.

In addition, the intrinsic and extrinsic fluorescence of the MT is very sensitive to its micro environment [54]. Extrinsic fluorescence studies disprove any tertiary structure changes of MT. Nevertheless, it has monitored strong intrinsic fluorescence quenching. So it can be proved that compounds enter the active site. Additional research including toxicity is necessary to develop novel and highly potent tyrosinase inhibitors.

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REFERENCES

[1] Decker H., Tuczek F., Tyrosinase/Catecholoxidase Activity of Hemocyanins: Structural Basis and Molecular mechanism, *Trends. Biochem. Sci*., **25**(8): 392-397 (2000).

[2] Goding C.R., Melanocytes: the New Black, *Int. J. Biochem. Cell Biol.*, **39**(2): 275-9 (2007).

[3] Garcia-Borron J.C., Solano F., Molecular Anatomy of Tyrosinase and Its Related Proteins: Beyond the Histidine-Bound Metal Catalytic Center, *Pigment. Cell. Res.*, **15**(3): 162-73 (2002).

[4] Holm R.H., Kennepohl P., Solomon E.I., Structural and Functional Aspects of Metal Sites in Biology. *Chem. Rev.*, **96**(7): 2239-2314(1996).

[5] Matoba Y., Kumagai T., Yamamoto A., Yoshitsu H., Sugiyama M. Crystallographic Evidence That the Dinuclear Copper Center of Tyrosinase Is Flexible during Catalysis, *J. Biol. Chem.*, **281**(13): 8981-8990 (2006).

[6] Fenoll L. G., Rodriguez-Lopez J. N., Garcia-Sevilla F., Garcia-Ruiz P. A., Varon R., Garcia-Canovas F., Tudela J., Analysis and Interpretation of the Action Mechanism of Mushroom Tyrosinase on Monophenols and Diphenols Generating Highly Unstable o-quinones, *Biochim. Biophys. Acta*., **1548**(1): 1-22 (2001).

[7] Molina F. G., Munoz J. L., Varon R., Lopez J. N., Canovas F. G., Tudela J., An Approximate Analytical Solution to the Lag Period of Monophenolase Activity of Tyrosinase, *Int. J. Biochem. Cell Biol.*, **39**(1): 238-52(2007).

[8] Halaouli S., Asther M., Sigoillot J. C., Hamdi M., Lomascolo A., Fungal Tyrosinases: New Prospects in molecular characteristics, bioengineering and Biotechnological Applications, *J. Appl. Microbiol.*, **100** (2): 219-32 (2006).

[9] Kubo I., Nihei K., Shimizu K., Oxidation Products of Quercetin Catalyzed by Mushroom Tyrosinase, *Bioorg. Med. Chem.*, **12**(20): 5343-7 (2004).

[10] Mayer A.M., Polyphenol Oxidases in Plants and Fungi: Going Places? A Review, *Phytochemistry.*, **67**(21): 2318-31 (2006).

[11] Jacobson E.S., Pathogenic Roles for Fungal Melanins, *Clin. Microbiol. Rev.*, **13**(4): 708-17(2000).

[12] Artes F., Castaner M., Gil M. I., Review: Enzymatic Browning in Minimally Processed Fruit and Vegetables, *Food Sci. Technol. Int.*, **4**: 377-89 (1998).

[13] Niu C., Aisa HA., Upregulation of Melanogenesis and Tyrosinase Activity: Potential Agents for Vitiligo. *Molecules.*, **22**(8): 1303- (2017)

[14] Spritz R.A., The Genetics of Generalized Vitiligo and Associated Autoimmune Diseases, *J. Dermatol. Sci.*, **41**(1): 3-10 (2006).

[15] Asanuma M., Miyazaki I., Ogawa N., Dopamine- or L-DOPA-Induced Neurotoxicity: the Role of Dopamine Quinone Formation and Tyrosinase in a Model of Parkinson's Disease, *Neurotox. Res.*, **5**(3): 165-76 (2003).

[16] Briganti S., Camera E., Picardo M., Chemical and Instrumental Approaches to Treat Hyperpigmentation, *Pigment. Cell. Res.*, **16**(2): 101-110 (2003).

[17] Sugumaran M., Barek H., Critical Analysis of the Melanogenic Pathway in Insects and Higher Animals, *Int. J. Mol.*, **17**(10):1753-77 (2016).

[18] Garcia-Molina F., Hiner A. N., Fenoll L. G., Rodriguez-Lopez J. N., Garcia-Ruiz P. A., Garcia-Canovas F., Tudela J., Mushroom Tyrosinase: Catalase Activity, Inhibition, and Suicide Inactivation, *J. Agric. Food. Chem.*, **53**(9): 3702-9 (2005).

[19] Kim Y. J., Uyama H., Tyrosinase Inhibitors from Natural and Synthetic Sources: Structure, Inhibition Mechanism and Perspective for the Future, *Cell. Mol. Life Sci.*, **62**(15): 1707-23 (2005).

[20] Taherkhani M., Chemical Investigation and Protective Effects of Bioactive Phytochemicals from Artemisia Ciniformis, *Iran. J. Chem. Chem. Eng. (IJCCE)*, **35** (2): 15-26 (2016)

[21] Kwong H.C., Chidan Kumar C.S., Mah S.H., Chia T.S., Quah C.K., Loh Z.H., et al. Novel Biphenyl ester Derivatives as Tyrosinase Inhibitors: Synthesis, Crystallographic, Spectral Analysis and Molecular Docking studies, *Plose One.*, **12** (2): 1-18 (2017)

[22] Tang J., Liu J., Wu F., Molecular Docking Studies and Biological Evaluation of 1,3,4-Thiadiazole Derivatives Bearing Schiff Base Moieties as Tyrosinase Inhibitors, *Bioorg .Chem.*, **69**: 29-36 (2016)

[23] Wang R., Chai W.M., Yang Q., Wei M.K., Peng Y., 2-(4-Fluorophenyl)-quinazolin-4(3H)-one as a Novel Tyrosinase Inhibitor: Synthesis, Inhibitory Activity, and Mechanism, *Bioorg . Med. Chem.*, **24**(19):4620-4625 (2016)

[24] Zhao D.Y., Zhang M.X., Dong X.W., Hu Y.Z., Dai X.Y., Wei X., Hider R.C., Zhang J.C., Zhou T., Design and Synthesis of Novel Hydroxypyridinone Derivatives as Potential Tyrosinase Inhibitors, *Bioorg. Med. Chem. Lett.*, **26**(13): 3103-3108 (2016)

[25] Son S., Kim H., Yun H.Y., Kim D.H., Ullah S., Kim S.J., Kim Y.J., Kim M.S, Yoo J.W., Chun P., Moon H.R., (E)-2-Cyano-3-(substituted phenyl)acrylamide Analogs as Potent Inhibitors of Tyrosinase: A Linear Betaphenyl- Alpha, Beta-Unsaturated Carbonyl Scaffold, *Bioorg. Med. Chem.*, **23**(24):7728-7734 (2015)

[26] Xie W., Zhang J., Ma X., Yang W., Zhou Y., Tang X., Zou Y., Li H., He J., Xie S., Zhao Y., Liu F., Synthesis and Biological Evaluation of Kojic Acid Derivatives Containing 1,2,4-triazole as Potent Tyrosinase Inhibitors, *Chem. Bio. Drug. Des.*, **86**(5):1087-92 (2015).

[27] Chen Z., Cai D., Mou D., Yan Q., Sun Y., Pan W., Wan Y., Song H., Yi W., Design, Synthesis and Biological Evaluation of Hydroxy- or Methoxy-Substituted 5-benzylidene (thio) Barbiturates as Novel Tyrosinase Inhibitors, *Bioorg. Med. Chem.*, **22**(13): 3279-3284 (2014).

[28] Ha Y.M., Park Y.J., Kim J.A., Park D., Park J.Y, Lee H.J, Lee J.Y., Moon H.R., Chung H.Y., Design and Synthesis of 5-(substituted benzylidene) thiazolidine-2,4-dione Derivatives as Novel Tyrosinase Inhibitors, *Eur. J. Med. Chem.*, **49**: 245-52 (2012).

[29] Rho H.S., Baek H.S., Ahn S.M., Kim M.K., Ghimeray A.K., Cho, D.H.,Hwang J.S., Synthesis and Biological Evaluation of Kojyl Thioether Derivatives as Tyrosinase Inhibitors, *Bull. Korean Chem.*, **31**(8): 2375-2378 (2010).

[30] Ali H.M., El-Gizawy A.M., El-Bassiouny R.E., Saleh M.A., The Role of Various Amino Acids in Enzymatic Browning Process in Potato Tubers, and Identifying the Browning Products, *Food. Chem.*, **192**: 879-85 (2016).

[31] Liu J., Cao R., Yi W., Ma C., Wan Y., Zhou B., Ma L., Song H., A Class of Potent Tyrosinase Inhibitors: Alkylidenethiosemicarbazide Compounds, *Eur. J. Med. Chem.*, **44** (4): 1773-8 (2009).

[32] Liu J., Yi W., Wan Y., Ma L., Song H., 1-(1-Arylethylidene)thiosemicarbazide Derivatives: A New Class of Tyrosinase Inhibitors, *Biorg. Med. Chem.*, **16**(3): 1096-102 (2008).

[33] Alijanianzadeh M., Saboury A.A., Temperature Dependence of Activation and Inhibition of Mushroom Tyrosinase by Ethyl Xanthate, *Bull. Korean. Chem. Soc.*, **28**(5): 758-62 (2007).

[34] Alijanianzadeh M., Saboury A. A., Mansuri-Torshizi H., Haghbeen K., Moosavi-Movahedi A.A., The Inhibitory Effect of Some New Synthesized Xanthates on Mushroom Tyrosinase activities, *J. Enzyme Inhib. Med. Chem.***, 22** (2): 239-46 (2007).

[35] Gheibi N., Saboury A.A., Mansuri-Torshizi H., Haghbeen K., Moosavi-Movahedi A.A., The Inhibition Effect of Some n-Alkyl Dithiocarbamates on Mushroom Tyrosinase, *J. Enzyme Inhib. Med. Chem.***, 20** (4), 393-9 (2005).

[36] Criton M, Mellay-Hamon V. L., Analogues of N-hydroxy-N'-phenylthiourea and N-hydroxy-N'-Phenylurea as Inhibitors of Tyrosinase and Melanin Formation, *Bioorg. Med. Chem. Lett.*, **18**(12): 3607-3610 (2008)

[37] Park K.H., Lee J.R., Hahn H.S., Kim Y.H., Bae C.D., Yang J.M., Oh S., Bae Y. J., Kim D. E., Hahn M., Inhibitory Effect of Ammonium Tetrathiotungstate on Tyrosinase and Its kinetic Mechanism, *J. Chem. Pharm. Bull. (Tokyo).*, **54**(9):1266-70. (2006)

[38] Amin E., Saboury A. A., Mansoori-Torshizi H., Moosavi-Movahedi A. A., Potent Inhibitory Effects of Benzyl and p-xylidine-bis Dithiocarbamate Sodium Salts on Activities of Mushroom Tyrosinase, *J. Enzyme Inhib. Med. Chem.*, **25**(2): 272–281 (2009)

[39] Farkhanda S., Amin B., Marcel G., Michal D., Karla F., Dickde V., Bushra M. Synthesis, Characterization, Antibacterial and Cytotoxic Activity of New Palladium(II) Complexes with Dithiocarbamate Ligands: X-Ray Structure of Bis(dibenzyl-1-S:S′-dithiocarbamato)Pd(II), *J. Organomet. Chem.*, **692**(14): 3019-3026 (2007).

[40] Sanchez-Cortes S., Vasina M., Francioso O., Garcia-Ramos J.V., Raman and Surface-Enhanced Raman Spectroscopy of Dithiocarbamate Fungicides *Vib. Spectrosc.*, **17**(2): 133-134 (1998) .

[41] Faraglia G., Sitran S., Montagner D., Pyrrolidine Dithiocarbamates of Pd(II). *Inorg. Chim. Acta.*, **358**: 971-80 (2005).

[42] Kim I., Kim C. H., Kim J. H., Lee J., Choi J. J., Chen Z., Lee M G., Chung K.C., Hsu C.Y., Ahn Y.S., Pyrrolidine Dithiocarbamate and Zinc Inhibit Proteasome-Dependent Proteolysis, *Exp. Cell. Res.*, **298**(1): 229-38 (2004).

[43] Ronconi L., Maccato C., Barreca D., Saini R., Zancato M., Fregona D., Gold(III) Dithiocarbamate Derivatives of N-Methylglycine: An Experimental and Theoretical Investigation, *Polyhedron.*, **24**(4): 521-31 (2005).

[44] Marcheselli L., Preti C., Tagliazucchi M., Cherchi V., Sindellari L., Furlani A., Pepaioannou A., Scarcia V., Synthesis, Characterization and Evaluation of Biological Activity of Palladium (II) and Platinum (II) Complexes with Dithiocarbamic Acids and Their Derivatives as Lgands, *Eur. J. Med. Chem.***, 28**(4): 347-52 (1993).

[45] Jimenez M., Chazarra S., Escribano J., Cabanes J., Garcia-Carmona F., Competitive Inhibition of Mushroom Tyrosinase by 4-Substituted Benzaldehydes. *J. Agric. Food. Chem.*, **49** (8): 4060-3 (2001).

[46] García-Carmona F., García-Cánovas F., Iborra J.L., Lozano J.A., Kinetic Study of the Pathway of Melanizationn between l-dopa and Dopachrome. *Biochim. Biophys. Acta*., **717** (1): 124-131 (1982).

[47] Han H.Y., Zou H.C., Jeon J.Y., Wang, Y.-J., Xu W.A., Yang J.M., Park Y.D., The Inhibition Kinetics and Thermodynamic Changes of Tyrosinase via the Zinc Ion, *Biochim. Biophys. Acta*., **1774** (7): 822-7 (2007).

[48] Saboury A.A., Enzyme Inhibition and Activation: A General Theory, *J. Iran. Chem. Soc.*, **6**(2): 219-29 (2009).

[49] Segel I.H., "Enzyme Kinetics: Behavior and Analysis of Rapid Equilibrium and Steady State Enzyme Systems", New York: Wiley-Interscience., 1975 (1975).

[50] Song K.K., Qiu L., Huang H., Chen Q.X., The Inhibitory Effect of Tyrosinase by Arbutin as Cosmetic Additive, *Journal of Xiamen University (Natural Science)*, **42** (6):791–794 (2003).

[51] Perozzo R., Folkers G., Scapozza L., Thermodynamics of Protein-Ligand Interactions: History, Presence, and Future Aspects, *J. Recept. Signal Transduct.*, **24**(1-2): 1-52 (2004).

[52] Eftink M. R., Anusiem A. C., Biltonen R. L., Enthalpy-Entropy Compensation and Heat Capacity Changes for Protein-Ligand Interactions: General Thermodynamic Models and Data for the Binding of Nucleotides to Ribonuclease A, *Biochemistry (Mosc)***.,** **22**(16): 3884-96 (1983).

[53] Gasymov O.K., Glasgow B., ANS Fluorescence: Potential to Augment the Identification of the External Binding Sites of Proteins, *J. Biochim. Biophys. Acta***.,** **1774**(3): 403-11 (2007).

[54] Beltramini M., Lerch K., Fluorescence Properties of Neurospora Tyrosinase, *Biochem. J*., **205**(1): 173-80 (1982).