Food Chemistry

Letter to the editor about “Evaluation of inhibitory effects of some novel phenolic derivatives on the mushroom tyrosinase activity" paper.

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Abstract:	Recently, Mahdavi et al. published an article in Food Chemistry entitled “Evaluation of inhibitory effects of some novel phenolic derivatives on the mushroom tyrosinase activity: Insights from spectroscopic analyses, molecular docking and in vitro assays” (Mahdavi et al., 2022). The article deals with the synthesis of some novel phenolic derivatives and their ability to inhibit the enzyme mushroom tyrosinase. This work described the spectroscopic studies of fluorescence quenching and molecular docking and the authors selected the most potent compound as inhibitor (Table 1, compound 1b, raspberry ketone) and performed cytotoxicity and hemolysis studies on it. This work was available online from April 9, 2022.In this letter, it can be concluded that compounds having an aromatic ring with a free hydroxyl group with free ortho positions can act as alternative substrates to the physiological substrates of tyrosinase and form reactive o-quinones that can change the redox status of melanocytes by consuming thiol groups and, furthermore, the polymerization of these quinones can cause cytotoxicity due to the formation of hydrogen peroxide.In view of what has been said among the possible tyrosinase inhibitors, aromatic molecules with free hydroxyls and free ortho positions should be avoided, that is, avoid compounds that apparently inhibit but that generate very reactive quinones that can give adverse effects such as cytotoxicity and leukoderma.
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Editor-in-Chief
Dr. Paul Finglas,
Quadram Institute Bioscience, NR4 7UA,
Norwich, United Kingdom.

Murcia, 17th May 2022

Dear Professor Paul Finglas,

Please find enclosed the letter entitled "*Considerations about the evaluation of inhibitory effects of some novel phenolic derivatives on the mushroom tyrosinase activity*" for consideration and possible publication as an original research article in "Food Chemistry".

The study of tyrosinase inhibitors must be done carefully because some possible inhibitors behave as alternative substrates of the enzyme. As these compounds are used in food, agriculture and cosmetics, the aspect mentioned above is important since if the inhibitor is an alternative substrate, it can give adverse reactions such as melasma, post-inflammatory hyperpigmentation, cytotoxicity and leukoderma.

We suggest the following possible referees for the manuscript:

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The authors have no conflict of interest to declare.

Thank you, in advance, for your attention and courtesy. I look forward to hearing from you.

Sincerely yours
Jose Munoz-Munoz

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Letter to the Editor-In-Chief of Food Chemistry

Dear Professor Paul Finglas,

Recently, Mahdavi et al. published an article in Food Chemistry entitled "Evaluation of inhibitory effects of some novel phenolic derivatives on the mushroom tyrosinase activity: Insights from spectroscopic analyses, molecular docking and *in vitro* assays" (Mahdavi et al., 2022). The article deals with the synthesis of some novel phenolic derivatives and their ability to inhibit the enzyme mushroom tyrosinase. This work described the spectroscopic studies of fluorescence quenching and molecular docking and the authors selected the most potent compound as inhibitor (Table 1, compound 1b, raspberry ketone) and performed cytotoxicity and hemolysis studies on it. This work was available online from April 9, 2022.

Our group has been working for many years with tyrosinases from different sources (apple, pear, artichoke, strawberry, avocado) (Espín et al., 1998) but especially with mushroom tyrosinase (Fenoll et al., 2001). We have studied the kinetic mechanism of the enzyme, both in its action on monophenols and *o*-diphenols (Sánchez-Ferrer et al., 1995). We recently published a review on the action of monophenols on the kinetics of tyrosinase (García-Molina et al., 2022), and we also addressed studies on enzyme inhibition (Zolghadri et al., 2019). The compounds synthesized by (Mahdavi et al., 2022) are monophenols and are shown in Table 1. Of these compounds (Table 1), (2a, 2b and 3b) behave as true inhibitors, but compounds (1a and 1b) are alternative substrates to L-tyrosine.

The problem with the study of these compounds as tyrosinase inhibitors is that it has been experimentally shown that both 4-bromophenol (1a) (Yamazaki & Itoh, 2003) and 4-(4-Hydroxyphenyl)butan-2-one (1b) (Ito et al., 2017) have been described as enzyme substrates. Thus, the inhibition assays, which do not take the above into account, have an inappropriate experimental design, since the authors preincubate the enzyme with the possible inhibitors under study for 20 minutes, during which time the enzyme attacks these two alternative substrates (1a or 1b, respectively). The mechanism of action of the enzyme on

L-dopa and an alternative substrate is shown in Figure 1. Compound 1b is a better substrate than 1a, since despite the fact that ($\delta_4^{1b} = 154\ \mathrm{ppm} \cong \delta_4^{1a} = 154.33\ \mathrm{ppm}$) (Lin et al., 2011), the Kd value of binding to the oxy-tyrosinase form (García-Molina et al., 2022) is 0.75 mM versus 1.75 mM for 1b and 1a, respectively, therefore it takes higher concentration of compound 1b than 1a to achieve, according to the authors, the maximum inhibition (Mahdavi et al., 2022). The assay follows the measurement of dopachrome formation at 475 nm for 18 min, the enzyme in the presence of 2 mM L-dopa consumes the alternative substrates, especially 1b, but the product is an *o*-quinone (Ito et al., 2017) and does not provide absorbance at 475 nm. The best experimental design would consist of preincubating L-dopa and the inhibitor and starting the reaction by adding the enzyme.

From docking studies of compounds 1a and 1b to oxy-tyrosinase (Figs. 2A and 2B), the dissociation constant of these ligands and the distance of the peroxide oxygen in the oxy-tyrosinase form to the ortho position of the monophenol are determined (see Figs. 2A and 2B) (García-Molina et al., 2022), thus demonstrating the possibility that compound 1a and 1b really have of being substrates, but the affinity of compound 1b is greater than that of 1a , as indicated in Figs. 2A and 2B. The hydrophobicity of compound 1b makes the Kd value lower than in the case of compound 1a.

The value of the chemical displacement of the carbon that supports the hydroxyl is related to the nucleophilic power of the oxygen from the phenolic hydroxyl to the copper atom (Espín et al., 2000). If we compare the chemical shifts of these two compounds with the physiological substrate of the mammalian enzyme, L-tyrosine $(\delta_4 = 158.86\ \mathrm{ppm})$, it turns out that both compounds could be better substrates for the enzyme than L-tyrosine.

Therefore, it can be concluded that compounds having an aromatic ring with a free hydroxyl group with free ortho positions can act as alternative substrates to the physiological substrates of tyrosinase and form reactive *o*-quinones that can change the redox status of melanocytes by consuming thiol groups and, furthermore, the polymerization of these quinones can cause cytotoxicity due to the formation of hydrogen peroxide.

In view of what has been said among the possible tyrosinase inhibitors, aromatic molecules with free hydroxyls and free ortho positions should be avoided, that is, avoid compounds that apparently inhibit but that generate very reactive quinones that can give adverse effects such as cytotoxicity and leukoderma.

Thank you in advance for the consideration of this letter.

Yours sincerely.

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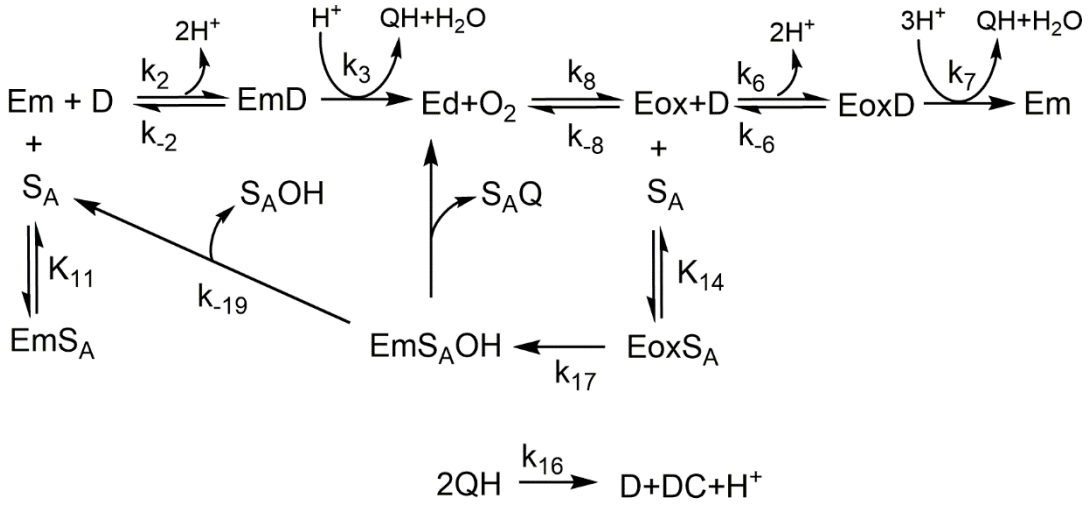


Fig. 1. Mechanism of action of tyrosinase on L-dopa (D) in the presence of an alternative substrate (monophenol). Where: S_A is the alternative substrate, S_AOH is the hydroxylated alternative substrate, S_AQ is the *o*-quinone of the hydroxylated alternative substrate, EmS_A is the complex of Em with S_A, EmS_AOH is the complex of Em and hydroxylated S_A, Em is meta-tyrosinase, Ed is deoxy-tyrosinase and Eox is oxy-tyrosinase.

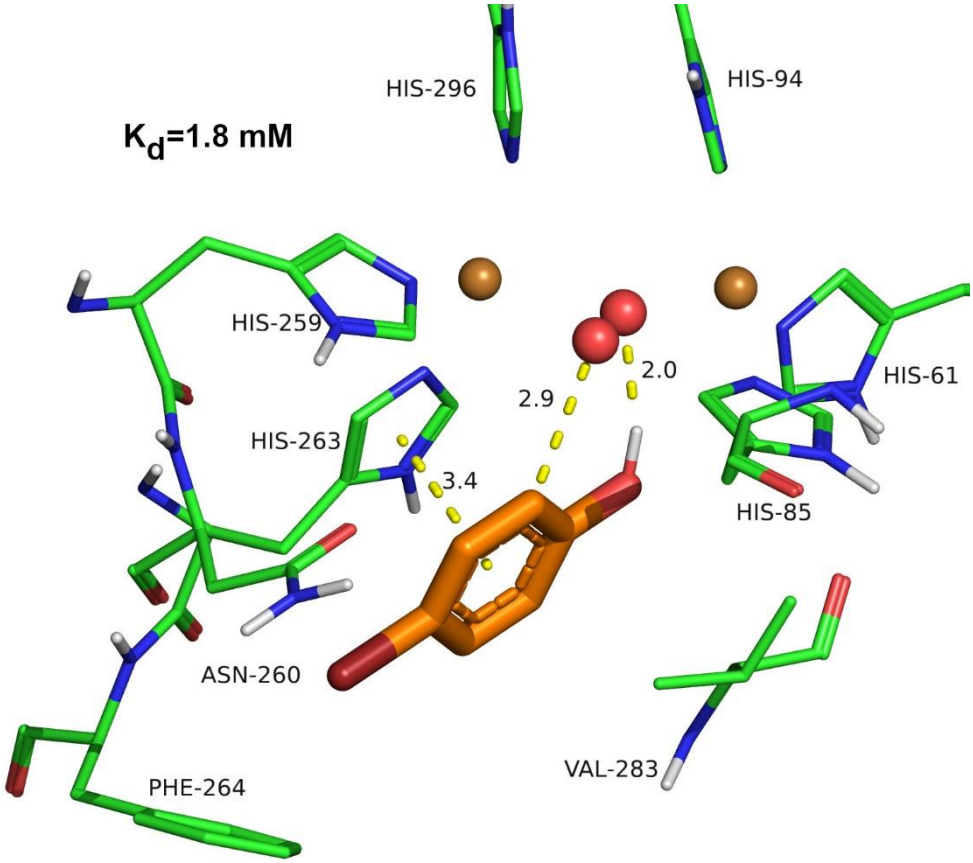


Fig. 2A. Molecular docking of 4-bromophenol (1a) to oxy-tyrosinase. The atom colors are as follows: oxygen = red, nitrogen = blue, copper = brown, white = hydrogen and bromine = dark red. Ligands are shown in thick sticks with carbon atoms in orange and tyrosinase residues in thin sticks with carbon atoms in green. The distances (Å) from the peroxide group to the ortho carbon and to the phenolic hydrogen atom, and from the aromatic ring of the ligand to the aromatic ring of H263 residue are shown in yellow dashed lines.

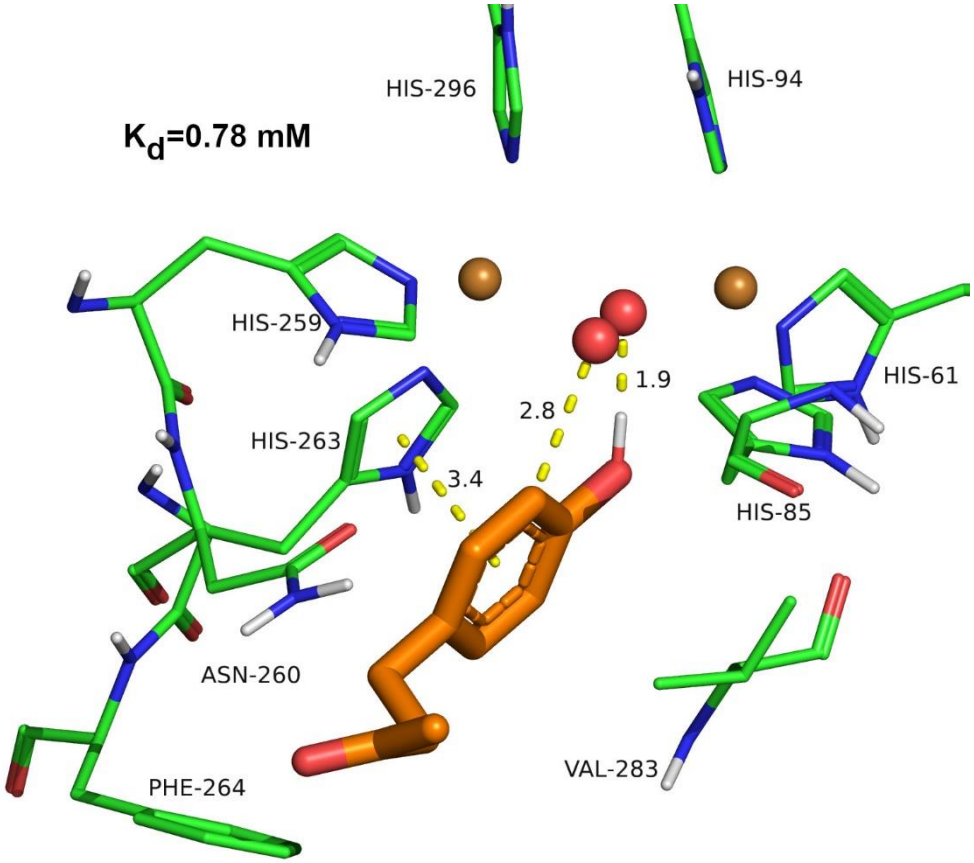
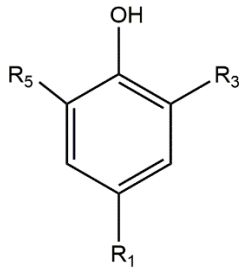


Fig. 2B. Molecular docking of 4-(4-hydroxyphenyl)butan-2-one (1b) to oxy-tyrosinase. Color scheme is as in Figure 1A.

198

Table 1. The structural characteristics of phenolic compounds 1a, 2a, 1b, 2b, 3b and L-tyrosine.

	R_1 = Br	R_3 = H	R_5 = H	4-Bromophenol (1a)*
	R_1 = Br	R_3 = *t*-Bu	R_5 = H	2-t-butyl-4-bromophenol (2a)*
	R_1 = C_4H_7O	R_3 = H	R_5 = H	4-(4-hydroxyphenyl)butan-2-one (1b)*
	R_1 = C_4H_7O	R_3 = *t*-Bu	R_5 = H	4-(3-t-butyl-4-hydroxyphenyl)butan-2-one (2b)*
	R_1 = C_4H_7O	R_3 = *t*-Bu	R_5 = *t*-Bu	4-(3,5-di-t-butyl-4-hydroxyphenyl)butan-2-one (3b)*
	R_1 = $C_3H_6O_2N$	R_3 = H	R_5 = H	L-tyrosine

*Mahdavi et al., 2022

199
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