International Journal of Biological Macromolecules 62 (2013) 589–595

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Review

Synthesis, characterization and biological evaluation of paeonol thiosemicarbazone analogues as mushroom tyrosinase inhibitors

Tian-Hua Zhu[a,b], Shu-Wen Cao[a], Yan-Ying Yu[b,*]

[a] State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang 330047, PR China
[b] Department of Chemistry, Nanchang University, Nanchang 330031, PR China

ARTICLE INFO

Article history:
Received 12 August 2013
Received in revised form 4 September 2013
Accepted 30 September 2013
Available online 8 October 2013

Keywords:
Paeonol thiosemicarbazone
Anti-tyrosinase
Inhibition kinetics
Copper ions chelation
Fluorescence quenching

ABSTRACT

A series of hydroxy- and methoxy-substituted paeonol thiosemicarbazone analogues were synthesized as potential tyrosinase inhibitors and their inhibitory effects on mushroom tyrosinase and inhibitory mechanism were evaluated. Paeonol thiosemicarbazone analogues have been found exhibiting more remarkable inhibition than their indexcompounds on mushroom tyrosinase. Among them, compound 2,4-dihydroxy acetophenone-4-phenyl-3-thiosemicarbazone (**d_1**) had the most potent inhibition activity with the IC_{50} value of 0.006 ± 0.001 mM, displayed as a reversible competitive inhibitor. The inhibitory ability of *o*- or *p*-substituted acetophenone thiosemicarbazones was: di-substituted acetophenone thiosemicarbazones > mono-substituted acetophenone thiosemicarbazones > non-substituted acetophenone thiosemicarbazones. Copper ions chelation assay explained that compound **d_1** exhibited competitive inhibition by forming a chelate with the copper ions at the catalytic domain of tyrosinase as well as indicate a 1.5:1 binding ratio of compound **d_1** with copper ions. In the fluorescence spectrum study, compound **d_1** behaved stronger fluorescence quenching on tyrosinase towards **d_1**–Cu^{2+} complex, inhibiting tyrosinase mainly by means of chelating the two copper ions in the active site. The newly synthesized compounds may serve as structural templates for designing and developing novel tyrosinase inhibitors.

Contents

* Corresponding author. Tel.: +86 0791 83969610.
E-mail address: yuyanying@ncu.edu.cn (Y.-Y. Yu).

0141-8130/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijbiomac.2013.09.056

1. Introduction

Tyrosinase (EC 1.14.18.1), known as polyphenol oxidase, is a widely distributed and multifunctional copper-containing metalloenzyme, which belongs to the type 3 copper protein family [1,2], structurally, with two copper ions each coordinately bonded with a distinct set of three histidine residues within the active site. It catalyzes two distinct reactions of melanin biosynthesis by hydroxylation of monophenols to *o*-diphenols and oxidation of *o*-diphenols to the corresponding *o*-quinones, which are the initial steps in the pathway. Finally, after the two reactions, a series of highly reactive quinones are produced to initiate the pigmentation and excessive activation of tyrosinase can cause various dermatological disorders, such as Parkinson and other degenerative diseases [3,4]. Tyrosinase inhibitors have become increasingly important in cosmetic [5] and medicinal industry [6], the food industry [7] and agriculture [8] to prevent hyperpigmentation.

In recent years, a large number of naturally occurring and synthetic tyrosinase inhibitors have been reported. There are many tyrosinase inhibitors, such as hydroquinone [9], ascorbic acid [10], arbutin [11], kojic acid [12], aromatic aldehydes [13,14], aromatic acids [15,16], aromatic alcohol [17,18], tropolone [19], and polyphenols [20,21]. However, some well-known whitening agents, such as hydroquinone and kojic acid, are not potent enough to put into practical use, considered as harmful agents due to their undesirable side effects such as cytotoxicity, skin cancer, dermatitis, and neurodegenerative diseases [22]. The discovery of new feasible, efficient and safe tyrosinease inhibitors is, undoubtedly, becoming an urgent concern.

In the previous studies, researchers have been identified that thiosemicarbazone and thiosemicarbazone derivatives are potential tyrosinase inhibitors, such as, naphthaldehyde thiosemicarbazones and cinnamaldehyde thiosemicarbazone [23–25]. The inhibitory effect of thiosemicarbazone derivatives on tyrosinase exhibited potent inhibitory activity, the reason being that the nitrogen and sulfur atoms of the thiosemicarbazide moiety were able to chelate the two copper ions in the active site of the enzyme and the van der Waals interactions of phenyl group with the residues lining the hydrophobic cavity contribute to the high affinity to the enzyme [26].

Paeonol (2-hydroxy-4-methoxy acetophenone, a), one of the primary bioactive components isolated from Chinese national flower-peony, is known as traditional skin-whitening agent, a potential tyrosinase inhibitor [27]. In our previous study, we defined the structural requirements of some acetophenone analogues for their inhibitory activities against tyrosinase. It has been found that replacements of *o*-hydroxy and *p*-methoxy moieties of acetophenone are more appreciable for the activity enhancement. In this paper, compounds **a**–**e** were selected as the initial structure to search new, strong, and potential tyrosinase inhibitors according to the formation of Schiff base. It was speculated that the condensation products of *o*-hydroxy- and *p*-methoxy-substituted acetophenone with thiosemicarbazide might exhibit potent tyrosinase inhibitory activity.

Therefore, the aim of the present paper is, to study the inhibitory effect of paeonol thiosemicarbazone analogues on the enzyme activity and evaluate the kinetic parameters and the inhibition mechanisms, to investigate the interaction between the inhibitors and the copper ions, and to measure the fluorescence properties of paeonol thiosemicarbazone analogues against tyrosinase and finally come up with the structure–activity relationship. We hope they can be used to provide the basis for developing novel effective tyrosinase inhibitors and searching for new whitening agents in cosmetic preparations or antibrowning agents for food products.

2. Materials and methods

2.1. Materials

Mushroom tyrosinase (EC 1.14.18.1), L-3,4-dihydroxyphenylalanine (L-DOPA), dimethyl sulfoxide (DMSO) were purchased from Sigma Chemical Co (St. Louis, MO, USA). The specific activity of the enzyme was 1881 U/mg. Paeonol (2′-hydroxy-4′-methoxy acetophenone, **a**) (see Fig. 1 for structures), acetophenone (**b**), 2′-hydroxy acetophenone (**c**), 2′,4′-dihydroxy acetophenone (**d**), and 4′-methoxy acetophenone (**e**) were obtained from J&K Chemical Co (Shanghai, China). All other reagents were of analytical grade. The water used was re-distilled and ion-free.

2.2. General procedure for synthesis of acetophenone thiosemicarbazones $\mathbf{a_1}$, $\mathbf{c_1}$, and $\mathbf{d_1}$

Compounds were prepared by the reaction of corresponding acetophenone with thiosemicarbazide in an acidic solution of ethanol, as previous described with some modifications [23]. Thiosemicarbazide (10 mmol) was dissolved in ethanol (20 mL) and stirred at 80 °C, when the thiosemicarbazide was completely dissolved, a ethanol solution of corresponding acetophenone (10 mmol) was dropwised into the reaction mixture with 0.2 g *p*-toluenesulfonic acid. The reaction mixture was refluxed for 3–5 h. Then cooled to room temperature, and evaporated of solvent under reduced pressure. The products were purified by flash chromatography (silica gel, petroleum ether/ethylacetate 4:1–6:1, v/v), detected by HPLC as one peak and identified by ESI-MS and ^{1}H NMR analyses. ESI-MS data were obtained on a Bruker ESQUIRE-LC (Germany), and NMR data were acquired on a 600 MHz NMR spectrometer (AV400) from Bruker (Germany).

2.3. General procedure for synthesis of acetophenone thiosemicarbazones $\mathbf{b_1}$, $\mathbf{e_1}$, and $\mathbf{a_2}$–$\mathbf{e_2}$

Thiosemicarbazide (10 mmol) was dissolved in ethanol (20 mL) and stirred at 80 °C, when the thiosemicarbazide was completely dissolved, a ethanol solution of corresponding acetophenone (10 mmol) was dropwised into the reaction mixture with 0.2 g *p*-toluenesulfonic acid. The reaction mixture was refluxed for 2–3 h