Bioorganic & Medicinal Chemistry Letters 21 (2011) 2376–2379

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

# Evaluation of dihydropyrimidin-(2*H*)-one analogues and rhodanine derivatives as tyrosinase inhibitors

Jinbing Liu [a,*], Fengyan Wu [a], Lingjuan Chen [b], Jianming Hu [a], Liangzhong Zhao [a], Changhong Chen [a], Liwang Peng [a]

[a] Department of Biology and Chemical Engineering, Shaoyang University, Shao Shui Xi Road, Shaoyang 422100, People's Republic of China
[b] College of Food Science and Engineering, Central South University of Forestry and Technology, People's Republic of China

ARTICLE INFO

*Article history:*
Received 9 December 2010
Revised 16 February 2011
Accepted 21 February 2011
Available online 26 February 2011

*Keywords:*
Dihydropyrimidin-(2*H*)-one
Rhodanine
Tyrosinase inhibitors
Inhibition mechanism

ABSTRACT

A series of dihydropyrimidin-(2*H*)-one analogues and rhodanine derivatives were synthesized and their inhibitory effects on the diphenolase activity of mushroom tyrosinase were evaluated. The results showed that some of the synthesized compounds exhibited significant inhibitory activities. Especially, compound **15** bearing a hydroxyethoxyl group at position-4 of phenyl ring exhibited most potent tyrosinase inhibitory activity with $IC_{50}$ value of 0.56 mM. The inhibition mechanism analysis of compound **15** demonstrated that the inhibitory effect of the compound on the tyrosinase was irreversible. These results suggested that such compounds might be served as lead compounds for further designing new potential tyrosinase inhibitors.



Dihydropyrimidinones (DHPMs) and their derivatives have attracted interest in medicinal chemistry, exhibiting pharmacological and therapeutic properties. In the past decades, a broad range of biological effects including antiviral, antitumor, antibacterial and anti-inflammatory activities have been described for these compounds.[1,2] Compounds containing the 2-thioxothiazolidin-4-one ring have showed a wide range of pharmacological activities, which includes antimicrobial, antiviral, and anticonvulsant effects.[3] Prominent amongst the resultant hits was a series of derivatives containing a 5-benzylidenerhodanine substructure (Fig. 1). Benzylidenerhodanines have been reported as small molecule inhibitors of numerous targets such as cyclooxygenase and 5-lipoxygenase, β-lactamase, cathepsin D, HCV NS3 protease, aldose reductase, protein mannosyl transferase and JNK-stimulating phosphatase.[4] Although, during the last years extensive studies on the pharmacology of DHPMs and rhodanine derivatives have been reported, the tyrosinase inhibitor activities of this kind of compounds have never appeared in the literature.

Tyrosinase (monophenol or *o*-diphenol, oxygen oxidoreductase, EC 1.14.18.1, syn. polyphenol oxidase), also known as polyphenol oxidase (PPO), is a copper-containing monooxygenase that is widely distributed in microorganisms, animals, and plants.[5] Tyrosinase could catalyze two distinct reactions involving molecular oxygen in the hydroxylation of monophenols to *o*-diphenols (monophenolase) and in the oxidation of *o*-diphenols to *o*-quinones (diphenolase).[6] Due to the high reactivity, quinines could polymerize spontaneously to form high molecular weight brown-pigments (melanins) or react with amino acids and proteins to enhance brown color of the pigment produced.[7,8] Previous reports confirmed that tyrosinase not only was involved in melanizing in animals, but also was one of the main causes of most fruits and vegetables quality loss during post harvest handling and processing, leading to faster degradation and shorter shelf life.[9] Recently, investigation demonstrated that various dermatological disorders, such as age spots and freckle, were caused by the accumulation of an excessive level of epidermal pigmentation.[10,11] Tyrosinase has also been linked to Parkinson's and other neurodegenerative diseases.[12] In insects, tyrosinase is uniquely associated with three different biochemical processes, including sclerotization of cuticle, defensive encapsulation and melanization of foreign organism, and wound healing.[13] These processes provide potential targets for developing safer and effective tyrosinase inhibitors as insecticides and ultimately for insect control. Thus, the development of safe and effective tyrosinase inhibitors is of great concern in the medical, agricultural, and cosmetic industries. However, only a few such as kojic acid, arbutin, tropolone, and 1-phenyl-2-thiourea

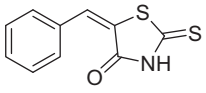



**Figure 1.** The substructure of 5-benzylidenerhodanine.

* Corresponding author. Tel.: +86 739 5431768; fax: +86 739 5431768.
*E-mail address:* xtliujb@yahoo.com.cn (J. Liu).

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.02.076

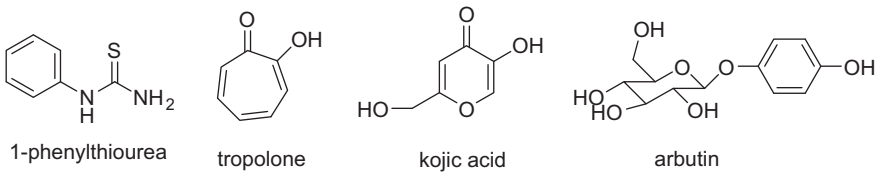



**Figure 2.** Chemical structure of known tyrosinase inhibitors.

(PTU) (Fig. 2) are used as therapeutic agents and cosmetic products.[11,14]

On the other hand, it was reported that phenyl thioureas and alkyl thioureas could exhibit weak to moderate depigmenting activity[15] and Ley and Bertram reported that benzaldoximes and benzaldehyde-*o*-alkyloximes possess higher tyrosinase inhibitory ability.[16] Tyrosinase belongs with catechol oxidase to the type-3 copper protein group. Crystal structure of phenyl thioureas bound catechol oxidase was reported. The sulfur atom of this compound binds to both copper ions in the active site of the enzyme.[8] More recently, our investigation also demonstrated that condensation products of aldehyde or ketone and thiosemicarbazide derivatives exhibited potent inhibitory activities against mushroom tyrosinase.[17,18] Stimulated by these results, in the present investigation, we synthesized a series of dihydropyrimidin-(2*H*)-ones analogues and rhodanine derivatives, and their inhibitory effects on the diphenolase activity of mushroom tyrosinase were investigated.

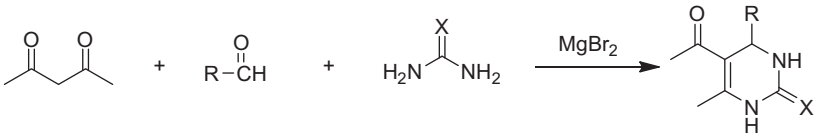
According to the general procedure shown in Scheme 1,[19,20] we carried out the preparation of 3,4-dihydropyrimidin-2-(1*H*)-ones (DHPMs) and thione analogs from the corresponding aldehydes, pentane-2,4-dione and urea or thiourea in the presence of magnesium bromide under solvent-free conditions. The synthesis is usually complete within 0.5–3 h at 50 °C or 100 °C, and the target compounds could be purified by recrystallization from 95% alcohol. The yields for these reactions are from moderate to good, and all the target compounds were characterized by IR, NMR and MS. The Schiff base of 3,4-dihydropyrimidin-2(1*H*)-one was prepared according to Scheme 2.[21] Rhodanine derivatives were synthesised for this study by means of the Knoevenagel condensation of the suitable aldehydes with 2-thioxo-4-thiazolidinone, respectively, in refluxing acetic acid in the presence of sodium acetate (Scheme 3).[22,23] The target compounds were also characterized by IR, NMR and MS.

For evaluating the tyrosinase inhibitory activity, all the synthesized compounds were subjected to tyrosinase inhibition assay with L-DOPA as substrate, according to the method reported by our groups with some slight modifications.[9,18] The tyrosinase inhibitory activities of arbutin and 4-methoxycinnamic acid were ever reported, therefore, they were selected as comparing substances. Figure 3 showed that the remaining enzyme activity rapidly decreased with the increasing concentrations of compound **15**. The $IC_{50}$ values of dihydropyrimidin-(2*H*)-one analogues and rhodanine derivatives against tyrosinase were summarized in Table 1, and $IC_{50}$ values of all these compounds were determined from logarithmic concentration–inhibition curves (at least eight points) and are given as means of three experiments .

Our results showed that compounds **7–16** exhibited potent inhibitory on mushroom tyrosinase with $IC_{50}$ values ranged from 0.56 to 25.11 mM. Some of the synthesized compounds demonstrated more potent inhibitory activities than the reference standard inhibitor arbutin. Compounds **1–6** and **17** did not show any significant activities at 50 mM concentration. The result may be related to the structure of tyrosinase contained a type-3 copper center with a coupled dinuclear copper active site in the catalytic core. Tyrosinase inhibition of compounds **7–16** depended on the competency of the sulfur atom to chelate with the dicopper nucleus in the active site, and tyrosinase would lose its catalyzing ability after forming complex.

Comparing to the tyrosinase inhibitory activities of 3,4-dihydropyrimidin-2-(1*H*)-thione analogs, compounds with hydroxyl group in the benzene ring exhibited more potent inhibitory activities. This suggested that hydroxyl group might play an important role in determining their inhibitory activities. The result was consistent with the Kim's propose that the hydroxyl groups might inhibit the tyrosinase enzyme by participating in a metal–ligand binding interaction with the dicopper nucleus.[27] The introduction of a hydroxy group in the *para* position led to appreciably better inhibitory activity than the hydroxyl group in the *ortho* position, when the substituents introduced in the *meta* position on the phenyl rings may have hindered docking of the inhibitor to tyrosinase, and led to the decrease in inhibitory activities. Interestingly, compound **10** was found to be the most potent inhibitor with an $IC_{50}$ value of 10.67 mM. These results suggested that benzene ring might cause stereohindrance for the inhibitors approaching the active site of the enzyme.

As shown in Table 1, rhodanine derivatives exhibited more potent inhibitor activities than dihydropyrimidin-(2*H*)-ones




1. R = Ph, X = O
2. R = 4-OHPh, X = O
3. R = 4-MeOPh, X = O
4. R = 2-OHPh, X = O
5. R = 4-OH-3-MeOPh, X = O
6. R = Et, X = O
7. R = 2-OHPh, X = S
8 .R = 4-OH-3-MeOPh, X = S
9. R = 4-MeOPh, X = S
10. R = Et, X = S
11. R = 4-OHPh, X = S

**Scheme 1.** Synthesis of 3,4-dihydropyrimidin-2-(1*H*)-ones (DHPMs) and thione analogs. Reagents and conditions: $MgBr_2$, 50 °C or 100 °C, 0.5–3 h.