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Synthesis and biological evaluation of novel hydroxybenzaldehyde-based kojic acid analogues as inhibitors of mushroom tyrosinase

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ABSTRACT

Two series of novel kojic acid analogues (**4a**–**j**) and (**5a**–**d**) were designed and synthesized, and their mushroom tyrosinase inhibitory activities was evaluated. The result indicated that all the synthesized derivatives exhibited excellent tyrosinase inhibitory properties having IC_{50} values in the range of 1.35 ± 2.15–17.50 ± 2.75 μM, whereas standard inhibitor kojic acid have IC_{50} values 20.00 ± 1.08 μM. Specifically, 5-phenyl-3-[5-hydroxy-4-pyrone-2-yl-methylmercap-to]-4-(2,4-dihydroxyl-benzylamino)-1,2,4-triazole (**4f**) exhibited the most potent tyrosinase inhibitory activity with IC_{50} value of 1.35 ± 2.15 μM. The kinetic studies of the compound (**4f**) demonstrated that the inhibitory effects of the compound on the tyrosinase were belonging to competitive inhibitors. Meanwhile, the structure-activity relationship was discussed.

Introduction

Tyrosinase (EC 1.14.18.1) is copper-containing metalloenzyme that are widespread among microorganisms, plants, and animals. They catalyze the *ortho*-hydroxylation of monophenols, which leads to the corresponding o-diphenols, as well as the oxidation of catechols, which leads to the corresponding o-quinones,[1] under many conditions, these then react further to result in the formation of various pigments.[2] Although the melanin production in human skin is a major defense mechanism against UV light, excessive accumulations of epidermal pigmentation can cause various hyperpigmentation disorders. Therefore, the regulation of melanin synthesis via the inhibition of tyrosinase is an important research topic.[3] In clinical usage, tyrosinase inhibitors are used for treatments of dermatological disorders related to melanin hyperaccumulation and are essential in cosmetics for depigmentation,[4] such as age spots and freckle, caused by the accumulation of an excessive level of epidermal pigmentation.[5] Inhibition of tyrosinase is equally important commercially. In most fruits and vegetables, the enzyme is responsible for undesired browning that takes place during senescence or damage during post-harvest handling, leading to faster degradation and shorter shelf life.[6] Taking into account the key role of tyrosinase in melanin production, many tyrosinase inhibitors have been found application in cosmetics and pharmaceutical products.[7,8] A large number of tyrosinase inhibitors have been reported but only a few are used because of their limitations with regards to cytotoxicity, selectivity and stability. Thus, it is great need of developing new tyrosinase inhibitors without causing adverse reactions.

So far, a number of kojic acid and benzaldehyde analogues have been reported as mushroom tyrosinase inhibitors,[9,10] such as Rho et al. reported that Kojyl thioether 2-((4-Hydroxyphenylthio) methyl)-5-hydroxy-4*H*-pyran-4-one inhibited mushroom tyrosinase with an IC_{50} of 0.54 μM which is better kojic acid (IC_{50} = 48.52 μM),[11] Li et al. reported that chlorobenzaldehyde thiosemicarbazones had inhibitory activity on tyrosinase.[12] Previously, we have also reported the synthesis of 1,2,4-triazoles-containing kojic acid derivatives by reactions of 5-hydroxy-2-chloromethyl-4H-pyran-4-one with 5-substituted-3-mercapto-4-amino-1,2,4-triazole,[13] and most of them showed significant tyrosinase inhibitory activity. As a part of our continuous project aimed at searching for new effective mushroom tyrosinase inhibitors, we hybridized the kojic acid with Hydroxybenzaldehyde by a linker 1,2,4-triazole to form 5-substituted-3-[5-hydroxy-4-pyrone-2-yl-methylmercapto]-4-aryl-methylamino-1,2,4-triazole **(4)** and 5-substituted-3-[5-hydroxy-4-pyrone-2-yl-methylmercapto]-4-arylmethyleneamino-1,2,4-triazole **(5)**.

The synthetic procedure employed to obtain the target compounds **4a**–**j** and **5a**–**d** was depicted in Scheme 1. The intermediate

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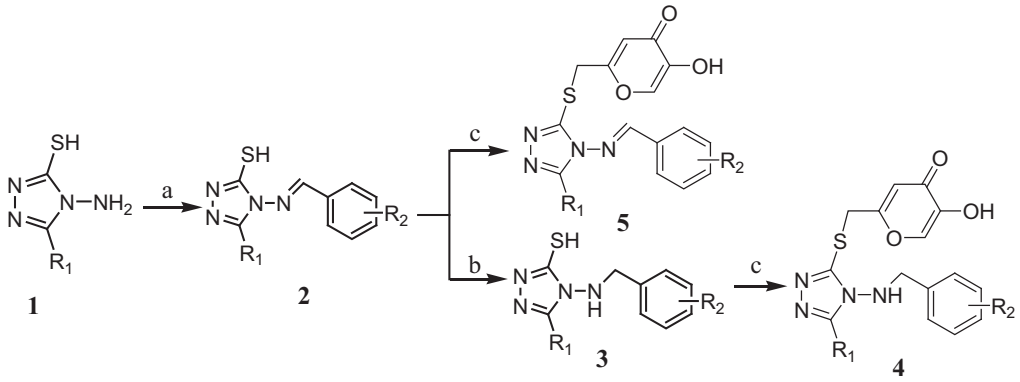

Scheme 1. General synthesis of compounds (**4a–j**; **5a–d**). Reagents and conditions: (a) substituted benzaldehyde/NaOAc, AcOH; (b) NaBH4, stir 1 h; (c) 5-hydroxy-2-chloromethyl-4H-pyran-4-one, Et3N/EtOH, reflux 0.5 h.

Schiff bases **2** were prepared using 5-substituted-3-mercapto-4-amino-1,2,4-triazoles **1** were reacted with Hydroxylbenzaldehyde by condensation reaction with TsOH as catalyst in anhydrous ethanol, and the compound **2** was reduced to amine **3** using $NaBH_4$ in methanol. And then, the compound **3** was reacted with 5-hydroxy-2-chloromethyl-4H-pyran-4-one[13] by nucleophilic substitution reaction in DMF medium in the presence of Et_3N to give 5-substituted-3-[5-hydroxy-4-pyrone-2-yl-methylmercapto]-4-arylmethylamino-1,2,4-triazole **4** with good yields**.** Similarly, the compound **2** was reacted with 5-hydroxy-2-chloromethyl-4H-pyran-4-one to obtain 5-substituted-3-[5-hydroxy-4-pyrone-2-yl-me-thylmercapto]-4-arylmethyleneamino-1,2,4-triazole **5**. The structures of all the newly synthesized compounds **4a–j** and **5a–d** were confirmed by ^1H NMR, ^{13}C NMR, HRMS, and IR spectral analysis.

For evaluating the tyrosinase inhibitory activity, all the synthesized compounds were subjected to tyrosinase inhibition assay with l-DOPA as substrate using kojic acid as the positive control, according to a method described by Kubo[14] with some slight modifications. Fig. 1 showed that the remaining enzyme activity rapidly decreased with the increasing concentrations of compound 4f.

The IC_{50} values of the titled compounds **4a–j** and **5a–d** against tyrosinase were summarized in Table 1. From our preliminary investigation, we found that all of the synthesized compounds exhibited potent inhibitory effect on mushroom tyrosinase with

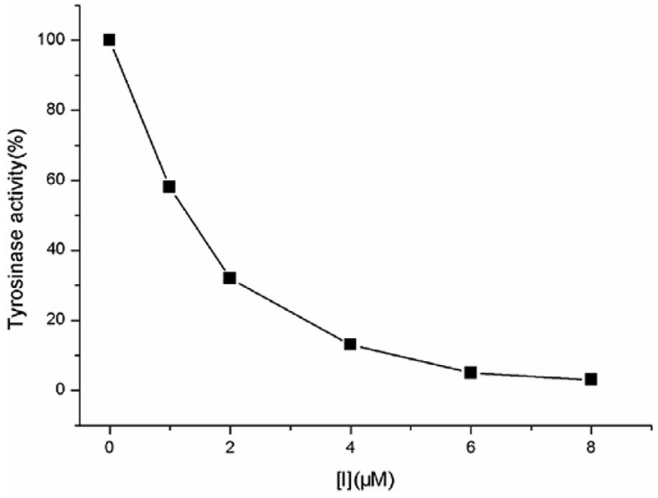

Fig. 1. Dose-dependent inhibitory effects of compound **4f** on tyrosinase activity. Tyrosinase activity was measured using l-DOPA as the substrate.

Table 1
Tyrosinase inhibitory activities of the synthesized compounds.

Compounds	R_1	R_2	IC_{50} (±SD, μmol/L) [a]
4a	CH_3	2,4-di-OH	5.80 ± 0.28
4b	CH_3	2-OH	10.20 ± 0.56
4c	CH_2CH_3	2-OH	7.60 ± 1.50
4d	H	2-OH	5.85 ± 0.60
4e	CH_3	3-OH	12.5 ± 1.08
4f	Ph	2,4-di-OH	1.35 ± 2.15
4g	4-CH_3Ph	2-OH	3.80 ± 0.78
4h	3-ClPh	2-OH	2.50 ± 0.89
4i	4-ClPh	2-OH	1.71 ± 1.56
4j	Ph	2-OH	1.50 ± 1.25
5a	2-ClPh	3,4-di-OH	5.20 ± 0.69
5b	H	4-OH	8.54 ± 2.38
5c	H	2,4-di-OH	9.60 ± 1.78
5d	H	3-CH_3O-4-OH	17.50 ± 2.75
Kojic acid[b]			20.00 ± 1.08

[a] IC50 values: the concentration of the inhibitor required to produce 50% inhibition of tyrosinase, results are the mean ± SD from three independent experiments.
[b] Standard inhibitors of the tyrosinase.

IC_{50} values ranged from 1.35 ± 2.15 to 17.50 ± 2.75 μM. They displayed higher inhibitory activities on tyrosinase in vitro than the reference standard inhibitor kojic acid. When the Schiff bases were reduced to obtain the amines **4**, by comparing two classes of compounds no clear superiority could be observed, but when the aryl groups were introduced to 5-position in triazole ring, it can be observed that the inhibitory activities of the compounds **4** were more potent than the compounds **5**, such as compounds **4f**, **4g**, **4h**, **4i**, **4j**. Among the compounds (**4a–j**) and (**5a–d**), compounds **4a**, **4d**, **4f**, **4g**, **4h**, **4i**, **4j** and **5a** demonstrated the most potent inhibitory activities. In particular, compound **4f** exhibited greater inhibitory activities with IC_{50} 1.35 ± 2.15 μM. From the inhibitory activities on mushroom tyrosinase, preliminary structure-activity relationships of the synthesized compounds were achieved. In general, compared with compounds (**4a–j**) and (**5a–d**), the aromatic substituents on 1,2,4-triazole ring had great effect on the inhibitory activities, for example, the compounds **4f**, **4g**, **4h**, **4i**, **4j and 5a** displayed the more potent inhibitory activities than the compounds with no substituent and aliphatic hydrocarbon on 1,2,4-triazole ring (**4a**, **4b**, **4c**, **4d**, **4e**, **5b**, **5c**, and **5d)**. But when the substituents (such as methyl, chlorine) were introduced to benzene ring in 5-position, we found that the inhibitory activities of these compounds (**4g**, **4h**, **4i**) were less than compound **4f** and **4j**. Among compounds **5a–d**, comparing the activities of the compound **5b** and **5d** it can be observed that the introduction of methoxyl group to position 3 of benzene ring on aromatic aldehyde decreased the activity.

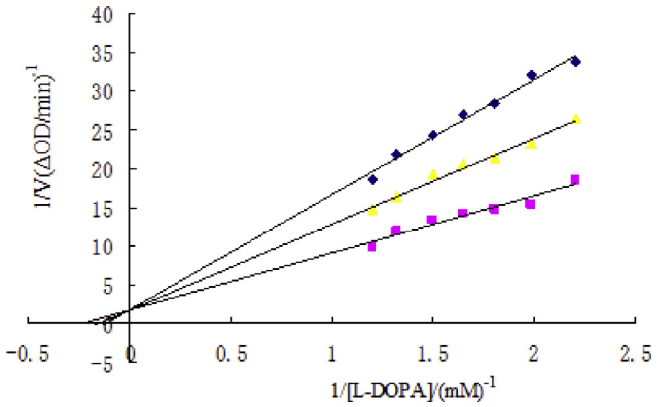

Fig. 2. Lineweaver–Burk plots for the inhibition of Mushroom tyrosinase by compound **4f**. Concentrations of compound **4f** for curves were (■) 0 μM, (▲) 0.6 μM and (◆) 1.2 μM respectively.

Among the tested target compounds, 5-phenyl-3-[5-hydroxy-4-pyrone-2-yl-methylmercap-to]-4-(2,4-dihydroxyl-benzylamino)-1,2,4-triazole **4f** showed the highest inhibitory activity, therefore, we carried out the kinetic analysis of **4f** for tyrosinase inhibition using l-DOPA as a substrate. The Lineweaver-Burk plots for the inhibition of tyrosinase by **4f** were obtained with variable concentrations of **4f** and the substrate (Fig. 2). The intersection of these lines on the vertical axis indicated that **4f** was a competitive inhibitor of tyrosinase with respect to l-DOPA as a substrate, thus, we speculated that this type of compound could enter into the active center of tyrosinase.

In summary, we designed and synthesized two series of new kojic acid analogues 5-substituted-3-[5-hydroxy-4-pyrone-2-yl-methylmercapto]-4-arylmethylamino-1,2,4-triazole **(4)** and 5-substituted-3-[5-hydroxy-4-pyrone-2-yl-methylmercapto]-4-arylmethyleneamino-1,2,4-triazole **(5)**, and their activity as tyrosinase inhibitors has been examined. The results displayed that all the synthesized compounds exhibited higher inhibitory activity on tyrosinase than kojic acid (**1**). Among the compounds synthesized, compound **4f** showed the strongest inhibitory activity with IC_{50} value of 1.35 ± 2.15 μM. Kinetic study revealed that compound **4f** acted as a competitive inhibitor of mushroom tyrosinase. Preliminary structure activity relationships analysis indicated that aromatic substituents on the triazole ring might play an important role in determining their inhibitory activities, but the substituent on the benzene ring in 5-position was somewhat unfavorable to their biological activity. Further investigations into the structure-activity relationships of these compounds are currently in progress.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.12.027.

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