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Design and synthesis of thiourea derivatives with sulfur-containing heterocyclic scaffolds as potential tyrosinase inhibitors

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ABSTRACT

Tyrosinase is a key enzyme during the production of melanins in plants and animals. A class of novel *N*-aryl-*N*′-substituted phenylthiourea derivatives (**3a**–**i**, **6a**–**k**) were designed, synthesized and their inhibitory effects on the diphenolase activity of mushroom tyrosinase were evaluated. The results showed some 4,5,6,7-tetrahydro-2-[[(phenylamino)thioxomethyl]amino]-benzo[*b*]thiophene-3-carboxylic acid derivatives (**3a**–**i**) exhibited moderate inhibitory potency on diphenolase activity of tyrosinase. When the scaffold of 4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylic acid was replaced with 2-(1,3,4-thiadiazol-2-yl)thio acetic acid, the inhibitory activity of compounds (**6a**–**k**) against tyrosinase was improved obviously; especially, the inhibitory activity of compound **6h** (IC_{50} = 6.13 μM) is significantly higher than kojic acid (IC_{50} = 33.3 μM). Moreover, the analysis on inhibition mechanism revealed that compound **6h** might plays the role as a noncompetitive inhibitor.

1. Introduction

Tyrosinase (polyphenol oxidase, EC 1.14.18.1),[1] a multifunctional copper-containing polyphenol oxidative enzyme widely distributed in plants, microorganisms, fungi and animals, catalyzes two types of oxidative reactions: hydroxylation of monophenols to o-diphenols, which are oxidized to the corresponding *o*-quinones.[2] Quinones can polymerize non-enzymatically to melanin as the most important natural biopolymer responsible of pigmentation and the color and patterns of mammalian skin. It had also been reported that the tyrosinase might be relevant to melanoma.[3] In agriculture areas, tyrosinase is responsible for the undesired enzymatic browning of fruits and vegetables that take place during senescence or damage at the time of post-harvest handling. The browning pigments lead to organoleptic and nutritional modifications, thus depreciating the quality of the food product.[4]

Tyrosinase inhibitors were widely used in agriculture and food industry, as well as in medicinal and cosmetic products due to preventing oxidization and decreasing the excessive accumulation of pigmentation.[5] Many tyrosinase inhibitors are widely used in cosmetic products for whitening and depigmentation after sunburn. Moreover, tyrosinase inhibitors could be used in the control of the molting process of insect.[6] With the increasing concern on health issues, tyrosinase was also used as a main target in research of skin-whitening products. As a commonly used inhibitor, kojic acid has been banned as cosmetic ingredient in many countries because of its serious side effects.[6] However, as a phenolic compound, kojic acid (Scheme 1, a) is unstable under aerobic condition, especially in solutions. Hence, more efforts should be made in the development of effective and stable novel tyrosinase inhibitors with lower side effects.

A given structure of two copper ions in the active center of tyrosinase and a lipophilic long-narrow gorge near to the active center provided ideal models to develop original tyrosinase inhibitor. Over the past decades, a broad spectrum of natural and synthetic compounds, such as Resveratrol (Scheme 1, b),[7] chalcone derivatives (Scheme 1, c),[8] kojic acid and derivatives,[9] thiocarbonyl-containing benzylidene derivatives (Scheme 1, d),[10] thiosemicarbazone analogues (Scheme 1, e)[11] and phenylthiourea derivatives (Scheme 1, f),[12] have been described to inhibit tyrosinase.

Most of those compounds have inherent function in chelating metal ions which plays a critical role in the inhibition of tyrosinase. As be well known, sulfur-containing moieties, such as thiocarbonyl in thioureas or thiosemicarbazones, sulfur atoms on heterocycles, can chelate transition metal ions.[11f,g,i] In the present work, initially, we designed and synthesized a serial of novel *N*-aryl-*N*′-substituted phenyl thiourea derivatives (Scheme 1, **3a**–**i**) in order to discover efficient tyrosinase inhibitors. Thiophene scaffold was introduced into the structure because sulphur-containing heterocycles were

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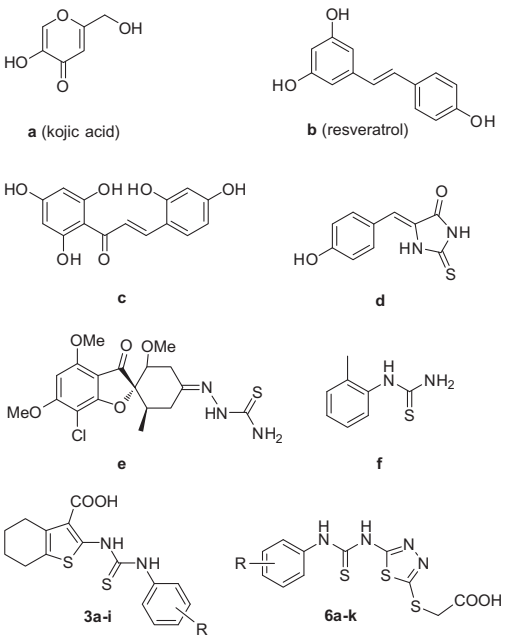
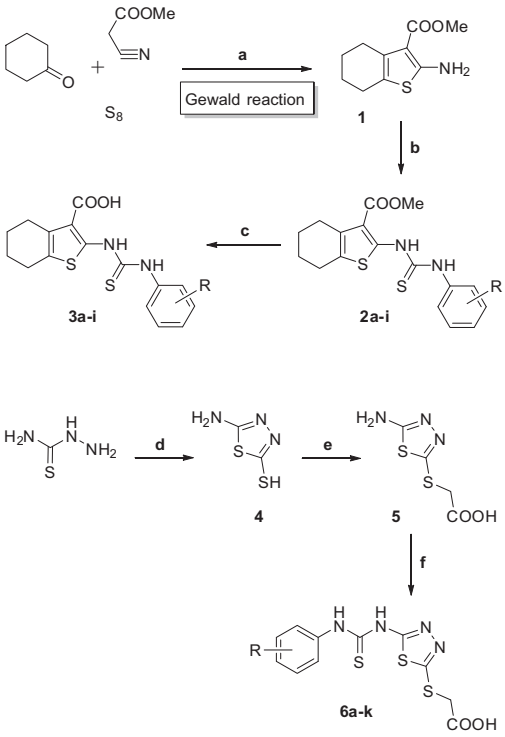


Scheme 1.

expected to be effective in chelating with copper ion. At the beginning of this work, 4,5,6,7-tetrahydro-2-[[(substitutedphenylamino) thioxomethyl]amino]-benzo[*b*]thiophene-3-carboxylate derivatives (**2a–i**) were synthesized by the designed route (Scheme 2). Unfortunately, it was very difficult to dissolve these compounds in aqueous buffer solution containing enzyme due to their poor solubility so the activity of compounds **2a–i** could not be evaluated.



Scheme 2. Synthesis of compounds **3a–i**, **6a–k**. Reagents and conditions: (a) morpholine, EtOH, reflux; (b) aryl isothiocyanates, ethanol, reflux; (c) ethanol, 20% NaOH, reflux; (d) CS_2, NaOH, ethanol, reflux; (e) $ClCH_2COOH$, NaOH, H_2O, 60 °C; (f) aryl isothiocyanates, Et_3N, DMF, CH_3CN, 50 °C.

Considering carboxylic acids have relatively better solubility in aqueous phase, moreover, some carboxylic acids themselves were reported showed inhibitory effect on tyrosinase.[13] Hence, carboxylates **2** were then transformed to corresponding carboxylic acids **3** by hydrolysis in alkaline solution.

To our delight, some of these compounds exhibit inhibitory effect on tyrosinase, although the activities were lower than kojic acid. Encouraged by these results, we decided to extend the structural diversity by the replacement of the thiophene scaffold of **3** with 1,3,4-thiadiazol containing thioether-type side chain. Subsequently, *N*-phenyl-*N*′-[5-(carboxymethylthio)-1,3,4-thiadiazol-2-yl] thiourea derivatives (**6a–k**) were synthesized as represented in Scheme 2. In comparison with **3**, the inhibitory activities of compounds **6** against tyrosinase were distinctly enhanced. To explore the inhibition mechanism of these compounds, the inhibitory kinetics of compound **6h** was also studied in detail.

2. Experimental section

2.1. General

Melting points were measured on BUCHI B-450 melting point apparatus. ^1H and ^{13}C NMR spectra were recorded on a Bruker AM-400 (400 MHz) spectrometer. Mass spectral analyses were performed on an Agilent mass spectrometer under electron spray ionization. Mushroom tyrosinase (EC 1.14.18.1) was purchased from Worthington Biochemical Corporation. All other reagents were commercial products with analytical grade purity and used as received.

2.2. Chemistry

2.2.1. Synthesis of compounds 1, 2a–i and 3a–i

Compound **1** was prepared through standard Gewald reaction.[14] 4.4 g (0.05 mol) of morpholine was added dropwise into a stirred solution of cyclohexanone (4.91 g, 0.05 mol), methyl cyanoacetate (4.95 g, 0.05 mol) and sulfur (1.92 g, 0.06 mol) in 35 mL ethanol. On completion, the mixture was refluxed for further 3 h. After cooling to room temperature, the precipitate was separated by filtration and recrystallized from ethanol to give **1** as pale yellow powders (8.6 g, 82% yield), mp 128.2–129.4 °C (lit.[15] 128–130 °C), MS (GC–MS): *m/z*, 211 $[M+H]^+$, 179, 151, 125, 91, 77, 65, 53.

To the stirred solution of compound **1** (0.22 g, 2 mmol) in 3 mL absolute ethanol, 2 mmol of aryl isothiocyanate was added and refluxed for 13–16 h under the atmosphere of nitrogen. The solvent was removed by rotary evaporation and the crude product was washed with cold ethanol, dried, and recrystallized from ethanol to afford compounds **2a–i**.

10 mL of aqueous NaOH solution (20% wt) was added into the stirred solution of compounds **2a–i** in ethanol (5 mL). The resulting mixture was refluxed for 2 h. Subsequently, the solvent was removed by rotary evaporation, and the residue was added 20 mL of water followed by extracted with dichloromethane (10 mL). The water layer was neutralized with diluted hydrochloric acid to afford crude product as a precipitate which was then collected by filtration. Recrystallization from ethanol gave purified compounds **3a–i**.

2.2.1.1. 4,5,6,7-Tetrahydro-2-[[(phenylamino)thioxomethyl] amino]-benzo[*b*]thiophene-3-carboxylic acid (3a). Yield 86%, white solid, mp 241.3–241.7 °C. ^1H NMR (400 MHz, DMSO-d_6) δ: 7.48–7.44 (m, 2H, Ar-H), 7.40–7.37 (m, 1H, Ar-H), 7.21 (d, J = 7.6 Hz, 2H, Ar-H), 2.73–2.68 (m, 4H, 2 × CH_2), 1.79–1.71 (m, 4H, 2 × CH_2) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ: 179.72, 162.12, 154.50, 144.53, 136.37, 134.23, 134.13, 133.54, 133.22,