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A newly synthesized, potent tyrosinase inhibitor: 5-(6-Hydroxy-2-naphthyl)-1,2,3-benzenetriol

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ABSTRACT

In searching for new agents with a depigmenting effect, we synthesized a derivative of resveratrol, 5-(6-hydroxy-2-naphthyl)-1,2,3-benzenetriol (5HNB) with a potent tyrosinase inhibitory activity. 5HNB inhibited mushroom tyrosinase with an IC_{50} value of 2.95 μM, which is more potent than the well-known anti-tyrosinase activity of kojic acid (IC_{50} = 38.24). The results of the enzymatic inhibition kinetics by Lineweaver–Burk analysis indicated 5HNB inhibits tyrosinase non-competitively when L-tyrosine was used as the substrate. Based on the strong inhibitory action of 5HNB, it is expected that 5HNB can suppress melanin production in which tyrosinase plays the essential role. Our expectation was confirmed by the experimentations with B16 melanoma cells in which 5HNB inhibited melanin production. We propose that 5HNB might have skin-whitening effects as well as therapeutic potential for treating skin pigmentation disorders.

Tyrosinase inhibitors have become increasingly important in medication,[1] cosmetic industry[2] and food industry[3] due to decreasing the excessive accumulation of pigmentation resulting from the enzyme action.[4–7] Although a number of tyrosinase inhibitors are reported from both natural and synthetic sources, only a few of them are used as skin-whitening agents, primarily due to various safety concerns. For example, linoleic acid, hinokitiol, kojic acid, all naturally occurring hydroquinones, and catechols inhibit enzyme activity but also exhibit harmful side effects.[1] Thus, melanin synthesis inhibitors may help treating localized hyper-pigmentation in humans. Epidermal and dermal hyper-pigmentation can be dependent either on an increased number of melanocytes or on melanogenic enzyme activity.[8]

Tyrosinase (monophenol monooxygenase, EC 1.14.18.1), a multifunctional type-3 copper-containing metalloenzyme, is a key enzyme in the undesirable browning of fruits and vegetables and in the coloring of skin, hair, and eyes in animals.[9] The tyrosinase is a rate-limiting enzyme, catalyzing step in melanin biosynthesis, which causes abnormal accumulation of melanin pigments.[10] This enzyme plays a role in the oxidation from L-tyrosine to 3,4-dihydroxyphenylalanine (L-DOPA) and from DOPA to DOPA quinone, which is the initial step in melanin synthesis.[11] Because of its key role in melanogenesis, tyrosinase is an attractive target in the search for various kinds of depigmenting agents.[12–15] Thus, the present study has focused on tyrosinase activity and melanin production.

The previous study showed 3,4-dihydroxyacetophenone and 4,4′-dihydroxybiphenyl have strong anti-tyrosinase that inhibits melanogenesis.[16,17] Recently, our laboratory reported that naphthalene-type resveratrol analogs, 6-(3-hydroxyphenyl)-2-naphthol and 4-(6-hydroxy-2-naphtayl-1,3-bezendiol inhibited tyrosinase activity and reduced melanin in B16 cells, which indicated that these compounds could be depigmentation reagents. Furthermore, some hydroxystilbene compounds, including oxyresveratrol and resveratrol, can also inhibit tyrosinase activity.[11,18]

We have therefore searched for easily accessible biological substances, including polyphenolic compounds, that potently inhibited tyrosinase activity and could potentially prevent abnormal pigmentation.[19,20] Therefore, we synthesized a series of novel resveratrol derivatives successfully through our constant efforts, including 5-(6-hydroxy-2-naphthyl)-1,2,3-benzenetriol (5HNB), a polyphenolic compound, to identify tyrosinase inhibitors. The synthesis of compounds including 5HNB has been described in our previous report.[21]

Here we report the characterization and evaluation of new synthesized reveratrol analog, 5HNB. We investigated the inhibitory effects of 5HNB on tyrosinase activity and melanin production in

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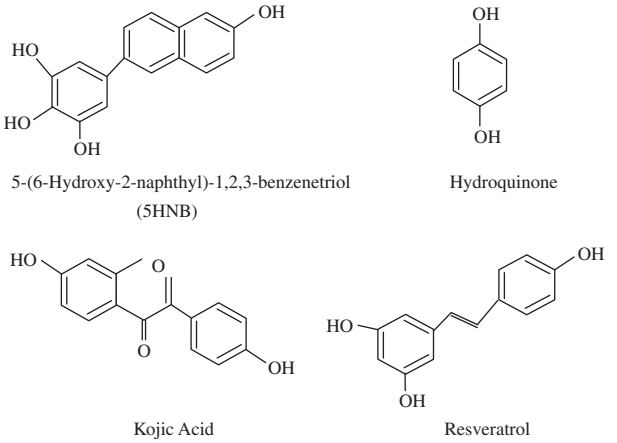

Figure 1. Comparison of chemical structures of 5-(6-hydroxy-2-naphthyl)-1,2,3-benzenetriol (5HNB), hydroquinone, kojic acid, and resveratrol.

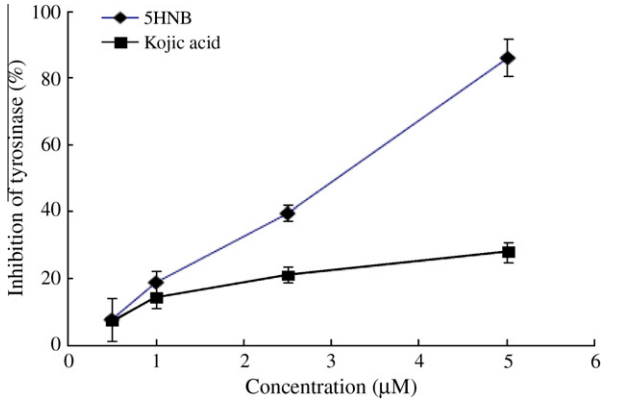

Figure 2. Dose-dependent inhibitory effects of 5HNB (diamond shape) on tyrosinase activity. Tyrosinase activity was measured using L-tyrosine as the substrate. Values are mean ± S.E. of $n = 3$ determinations.

Table 1
Inhibitory effects of hydroquinone, kojic acid, resveratrol, and 5-(6-hydroxy-2-naphthyl)-1,2,3-benzenetriol (5HNB) on mushroom tyrosinase activity

Compounds	IC_{50} (μM)[a,b]
Hydroquinone	33.48 (±1.70)
Kojic acid	38.24* (±1.47)
Resveratrol	55.61 (±2.77)
5HNB	2.95** (±1.26)

[a] IC_{50}: refers to the concentration of compound that caused 50% inhibition.
[b] Values are means of three experiments, standard deviation is given in parentheses.
* $P < 0.05$, compared to the hydroquinone.
** $P < 0.01$, compared to the hydroquinone.

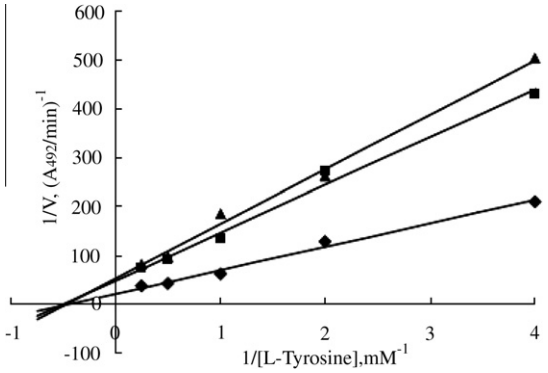

Figure 3. Lineweaver–Burk plot of 5HNB on mushroom tyrosinase. Data were obtained as mean values of $1/V$, inverse of the increase of absorbance at a wavelength 492 nm per min (ΔA_{492}/min), of three independent tests with different concentrations of L-tyrosine as a substrate. Inhibitors of the enzyme were 5HNB 0.5 μM (triangle), 0.125 μM (rectangle), and 5HNB (diamond shape). The modified Michaelis–Menten equation is: $1/V_{max} = 1/K_m\ (1 + [I]/K_i)$. V denotes the velocity of the reaction, S for L-tyrosine concentration, K_i for inhibitor constant.

the B16 murine melanoma cell line. Furthermore, we report here on the tyrosinase inhibitory potential of 5HNB for use in the treatment of tyrosinase-related skin disorders.

5HNB (Fig. 1) dose-dependently inhibited mushroom tyrosinase by 7.53 ± 6.43% at 0.5 μM, 18.66 ± 3.16% at 1 μM, 39.38 ± 2.46% at 2.5 μM, and 86.12 ± 5.47% at 5 μM, as monitored by a spectrophotometric assay (Fig. 2). 5HNB thus inhibited the oxidation of L-DOPA by mushroom tyrosinase with an IC_{50} value of 2.95 μM.

Kojic acid, a well-known tyrosinase inhibitor, one of the most popular tyrosinase inhibitors and it has been widely used as whitening.[22] Using hydroquinone, kojic acid, and resveratrol as positive controls, these inhibitors exhibited IC_{50} values of 33.48, 38.24, and 55.61 μM, respectively (Fig. 2 and Table 1). Therefore, 5HNB was a more potent inhibitor of tyrosinase activity than kojic acid.

We next measured the inhibitory mechanism of 5HNB on mushroom tyrosinase for oxidation of L-DOPA was determined from Lineweaver–Burk double-reciprocal plots. Figure 3 showed the double-reciprocal plots of the enzyme inhibited by 5HNB. The result displayed that the plot of $1/V$ versus $1/[S]$ gave three straight lines with different slopes, but intersected on the horizontal axis. Accompanying the increase of the concentration of compound, the value of V_{max} descended but the values of K_m remained the same, which suggested that 5HNB was a non-competitive inhibitor of the tyrosinase.

5HNB was not toxic to B16 melanoma, producing cell viability values of 98.32%, 98.08%, and 95.14% at 25, 50, and 100 μM, respectively, after 24 h (Fig. 4A). We next tested whether 5HNB could inhibit melanin production in B16 melanoma cells after treatment with α-MSH. 5HNB decreased melanin content to 50.67% at 25 μM, 45.83% at 100 μM, and 34.67% at 400 μM compared to control (the α-MSH-treated group at 100%) (Fig. 4B). Similarly, 5HNB reduced tyrosinase activity to 26.18% at 25 μM, 14.09% at 100 μM, and 2.05% at 400 μM compared to controls (Fig. 4C).

In the present study, we found that 5HNB clearly reduced both tyrosinase activity and melanin synthesis in a dose-dependent manner. During our search for potent tyrosinase inhibitors, we found that 5HNB was more effective for tyrosinase and melanin synthesis than kojic acid and resveratrol (Table 1). A kinetics study of 5HNB inhibition against mushroom tyrosinase showed that it behaves as a non-competitive inhibitor of the enzyme with L-tyrosine as the substrate.

It would be interesting to know something about the mode of the interaction between 5HNB and tyrosinase. 5HNB shows non-competitive inhibition of tyrosinase, based on Michaelis–Menten kinetics (Fig. 3), implying that 5HNB binds to the enzyme at a site other than the substrate-binding site. Thus, it allosterically reduces the maximum rate of the catalytic reaction (i.e., V_{max}) without affecting the apparent binding affinity of the enzyme for the substrate. This 5HNB binding could change the shape or the structure of tyrosinase, reducing substrate binding to the enzyme and inhibiting the enzymatic reaction.

Considering cytotoxicity, the cell permeability of 5HNB would be important to determine. Although we have no quantitative information on cell permeability, 5HNB can inhibit tyrosinase

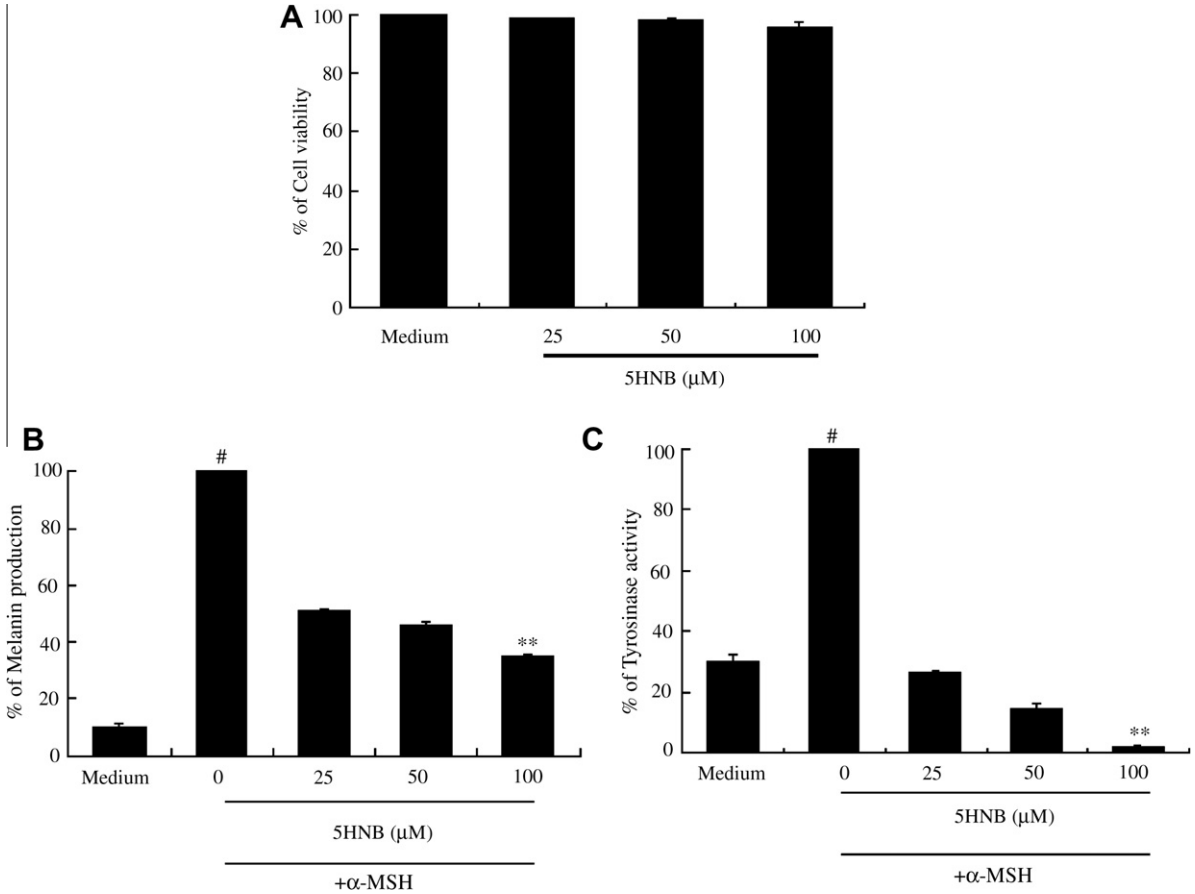

Figure 4. The effect of 5HNB on cell viability, melanin production, and tyrosinase activity in B16 melanoma cells. Cells were treated with 5HNB (25–100 μM) and were examined by the MTT assay (A). Melanin content was measured at 405 nm (B). The cells were harvested and tyrosinase activity was measured (C). Data are expressed as % of control, and each column represents the mean ± S.E. of three determinations. This result is representative of three experiments that gave similar results. $^{\#}P$ <0.01 versus control, $^{**}P$ <0.01 versus the group treated by α-MSH alone.

and melanin synthesis in B16 melanoma cells, indicating sufficient membrane permeability. In addition, 5HNB shows negligible cytotoxicity (Fig. 4A). 5HNB could dose-dependently inhibit tyrosinase activity and melanin synthesis in B16 melanoma cells pretreated with α-MSH (Fig. 4B and C). These results indicate that 5HNB, a naphthalene-type resveratrol analogue, may be able to inhibit skin tyrosinase and reduce melanogenesis, and potentially have anti-proliferative and apoptosis-inducing effects in leukemia cells as other phenolic compounds.[19]

5HNB is a newly synthesized, potent tyrosinase inhibitor that suppresses tyrosinase activity and total melanin content without adversely affecting cell viability. These data suggest that 5HNB may be a potential skin-lightening agent. Further investigations on the safety and efficacy properties of this compound are underway in our laboratory.

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Supplementary data

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