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De novo tyrosinase inhibitor: 4-(6,7-Dihydro-5*H*-indeno[5,6-*d*] thiazol-2-yl)benzene-1,3-diol (MHY1556)

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ABSTRACT

In this study, we have synthesized and studied de novo tyrosinase inhibitor, MHY1556, which showed significantly better efficacy than other pre-existing tyrosinase inhibitors in vitro experiments. The IC_{50} value of MHY1556 was 0.50 μM which was significantly lower than that of kojic acid (IC_{50} = 53.95 μM), which is a well-known tyrosinase inhibitor and was used as a positive control in this study. We predicted the tertiary structure of tyrosinase, simulated the docking with compound MHY1556 and confirmed that the compound strongly interacts with mushroom tyrosinase residues. Substitutions with a hydroxy group at both R1 and R3 of the phenyl ring indicated that these groups play a major role in the high binding affinity to tyrosinase, especially through the hydrogen bonding interaction of the hydroxyl group at R1 with GLY281. In addition, MHY1556 showed concentration-dependent inhibitory effects in melanin content assay where B16F10 melanoma cells were treated with α-melanocyte stimulating hormone (α-MSH), and also there is no significant cytotoxicity of this compound in cell viability assay conducted in B16F10 melanoma cells. The tyrosinase activity assay results with MHY1556 also support its potent inhibitory effects. Therefore, our data strongly suggest MHY1556 suppresses the melanogenesis via a tyrosinase inhibitory effect.

Human skin color is primarily determined by the production of melanin, dark-colored pigments, found in the basal layer of the epidermis.[1] The amount of melanin production varies in different ethnic groups and determines their skin color.[1] Melanin has some protective effects on skin. In response to sun exposure the body naturally produces more melanin in the skin and the skin color darkens.[1–3] Melanin is located in the outermost layer of skin and absorbs UV radiation, which prevents UV radiation from causing direct and indirect DNA damage to the skin.[1–3] However, overproduction of melanin is unwanted and there are several skin-whitening products that are commercially available for cosmetic purposes to obtain lighter skin color.[2,4] They can also be used for medical purposes.[2,4] A number of dermatological problems are characterized by overproduction and accumulation of melanin in skin.[5,6] Such hyperpigmentation disorders include Café au lait macules, ephelides (freckles), solar lentigo (age spots), and melisma.[5]

Most of skin-whitening products target melanogenesis, and tyrosinase inhibitors are the most commonly used agents.[2,3] Upon exposure of the skin to UV lights, melanogenesis is greatly increased by the activation of tyrosinase.[2] Tyrosinase is a key enzyme that plays a key role in production of melanin.[1–3] It catalyses the hydroxylation of L-tyrosine to form L-3,4-dihydroxyphenylalanine (L-DOPA) and the oxidation of L-DOPA to DOPA quinone.[7]

Most melanin-biosynthesis inhibitors are phenolic compounds with a hydroxyl group, which are structurally similar to tyrosinase substrate, tyrosine or L-DOPA.[8] Acting as a rate-determining enzyme in melanogenesis, tyrosinase is often used as a main target in research of skin-whitening products. As shown in Figure 1, L-tyrosine, L-DOPA, and dopaquinone are tyrosinase substrates and/or products. These compounds all possess one or more hydroxyl group, indicating that this group may play an important role in binding to tyrosinase at the active site.

Currently, there are a number of tyrosinase inhibitors including ascorbic acid, kojic acid, and polyphenol compounds such as

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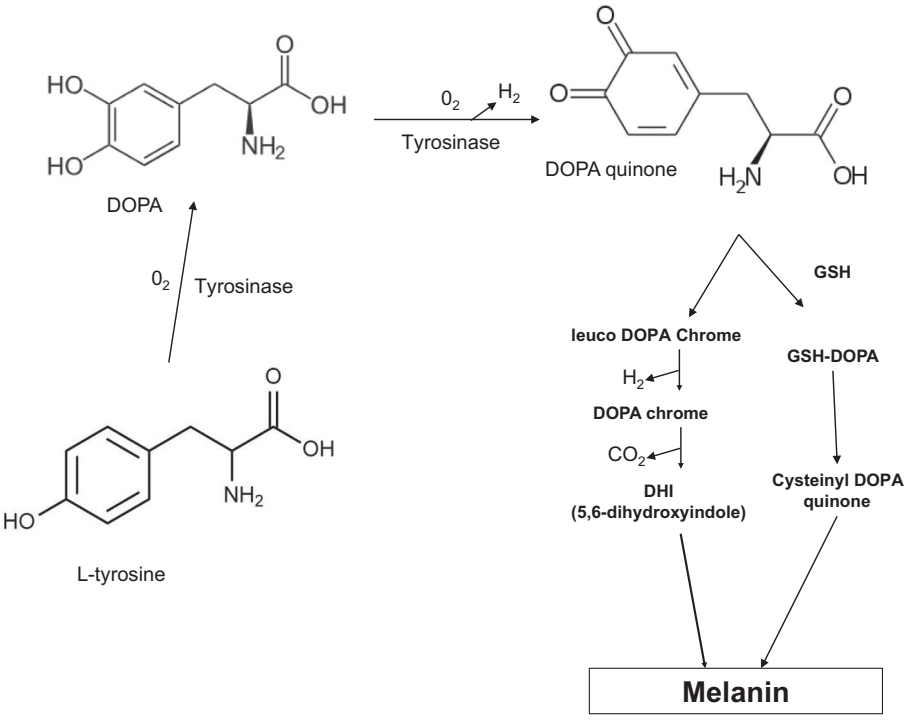


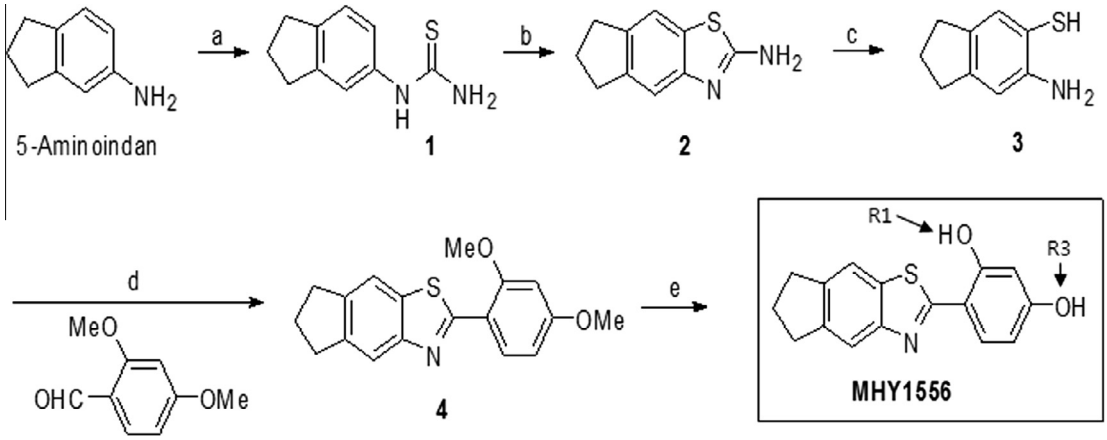
Figure 1. Biosynthetic pathway for melanin production.

flavonols and coumarins.[9] Several phenolic compounds which showed significant tyrosinase inhibitory effects have been introduced by our laboratory as well.[10,11]

As our continuous efforts to find potent tyrosinase inhibitors, 4-(6,7-dihydro-5*H*-indeno[5,6-*d*]thiazol-2-yl)benzene-1,3-diol (MHY1556) with a hydroxyl group at both R1 and R3 of the phenyl ring was designed. It was envisioned that compound **3** would be an appropriate key intermediate for the preparation of MHY1556. Compound **3** was prepared according to the procedures reported by Kajino et al. with minor modification (Scheme 1).[12] Direct coupling reaction of compound **3** with 2,4-dihydroxybenzaldehyde in the presence of $Na_2S_2S_5$ as an oxidizing agent just afforded the corresponding imine derivative instead of the cyclic product such as benzothiazolidine or benzothiazole analog, whereas condensation of compound **3** with 2,4-dimethoxybenzaldehyde in the presence of $Na_2S_2S_5$ produced the corresponding cyclized and oxidized benzothiazole product, compound **4** in 58% yield. Therefore, we tried to synthesize MHY1556, our target compound, from compound **4** via O-demethylation (Scheme 1). Treatment of compound **4** with BBr_3 as a Lewis acid gave MHY1556 in a moderate yield. The structures of MHY1556 and compound **4** were determined by ^1H and ^{13}C NMR and low and high mass spectral analysis (Scheme 1).

In this study, we investigated in greater detail the bioactivities of MHY1556 in vitro. As shown in Table 1, kojic acid and MHY1556 were found to inhibit mushroom tyrosinase activity in a concentration-dependent manner. The data showed that MHY1556 (IC_{50} = 0.50 μM) is a more potent inhibitor of mushroom tyrosinase than kojic acid (IC_{50} = 53.95 μM), which was used as a reference compound (Table 1).

Through a modeling of docking, we were able to detail the tertiary structure of mushroom tyrosinase and simulate its docking with MHY1556, supporting the hypothesis that MHY1556 interacts



Scheme 1. Synthesis of MHY1556. Reagents and conditions: (a) NH_4SCN, benzoyl chloride, acetone, 2 N-NaOH; (b) $-Br_2$, AcOH, 25% NH_4OH; (c) KOH, 2-methoxyethanol, H_2O; (d) $Na_2S_2O_5$, DMF; (e) BBr_3, CH_2Cl_2.

Table 1
Comparing IC_{50} values of MHY1556 and kojic acid

Sample	Conc (μM)	Tyrosinase inhibition[a] (%)	IC_{50}[b] (μM)
Kojic acid	10	5.44 ± 2.50	53.95 ± 2.45
	20	12.81 ± 5.08	
	30	12.71 ± 6.12	
	40	37.73 ± 5.40	
	50	48.15 ± 1.44	
MHY1556	0.1	22.21 ± 4.31	0.50 ± 0.05
	0.5	56.52 ± 0.43	
	0.8	68.34 ± 0.33	
	1	73.63 ± 0.96	
	2	83.55 ± 0.34	

[a] Values represent means ± S.E. of three experiments.
[b] 50% Inhibitory concentration (IC_{50}).

Table 2
Amino acid via docking simulation between tyrosinase and active compound

Amino acid (*Z* <3)	Compound	
HIS61	Kojic acid	MHY1556
HIS85	Kojic acid	MHY1556
PHE90	Kojic acid	MHY1556
HIS94	—	MHY1556
HIS244	Kojic acid	—
GLU256	Kojic acid	—
HIS259	Kojic acid	MHY1556
ASN260	Kojic acid	MHY1556
HIS263	Kojic acid	MHY1556
PHE264	Kojic acid	MHY1556
ARG268	—	MHY1556
HIS279	Kojic acid	—
MET280	Kojic acid	MHY1556
GLY281	Kojic acid	MHY1556
SER282	Kojic acid	MHY1556
VAL283	Kojic acid	MHY1556
ALA286	Kojic acid	MHY1556
PHE292	—	MHY1556
HIS296	—	MHY1556
CU400	Kojic acid	MHY1556
CU401	Kojic acid	MHY1556

with residues in the active site of tyrosinase. In our previous study, we checked the *Z*-score of the 3D structure to validate the predicted structural model using QMEAN server (3A). The docking simulation was successful with significant scores. The binding energy of MHY1556, as determined by AutoDock4.2 analysis, was −7.03 kcal/mol and that of kojic acid was −4.20 kcal/mol (Fig. 2). We searched for tyrosinase residues that would bind to MHY1556. The most important residues were predicted to interact with MHY1556 by AutoDock4.2 analysis, as shown in Table 2. Additionally, we searched for hydrogen bonding interactions between tyrosinase and MHY1556 or kojic acid. The Met280 and Gly281 residues of the tyrosinase were responsible for the hydrogen bonding interactions with kojic acid and MHY1556, respectively (Fig. 3). Probably, these residues might function as key determinants for the efficacy of each inhibitor and have an important effect on the binding affinity.

To evaluate the cytotoxic effect of MHY1556, we used the murine B16F10 melanoma cell line (B16F10 cells). The results of cell viability assay using an MTT kit are shown in Figure 4. At the treatment doses of 2, 5, 10, 20 and 40 μM of MHY1556, cell viability results were recorded as 97.87%, 94.98%, 98.11%, 95.6%, and 88.42%, respectively. These results indicate that MHY1556 is not cytotoxic to B16F10 cells at the concentrations used in the following melanin content assay.

To evaluate the inhibitory effect of MHY1556 on melanin production, we measured the melanin content of B16F10 cells after treating with MHY1556. As shown in Figure 5A, the melanin content in the cells treated with MHY1556 in the presence of α-MSH decreased concentration-dependently, showing 120.71% at 0.05 μM, 100.85% at 0.1 μM, 94.49% at 0.2 μM, and 88.85% at 2 μM, compared to the control group treated with α-MSH alone (163.78%). MHY1556 showed a strong inhibitory effect on

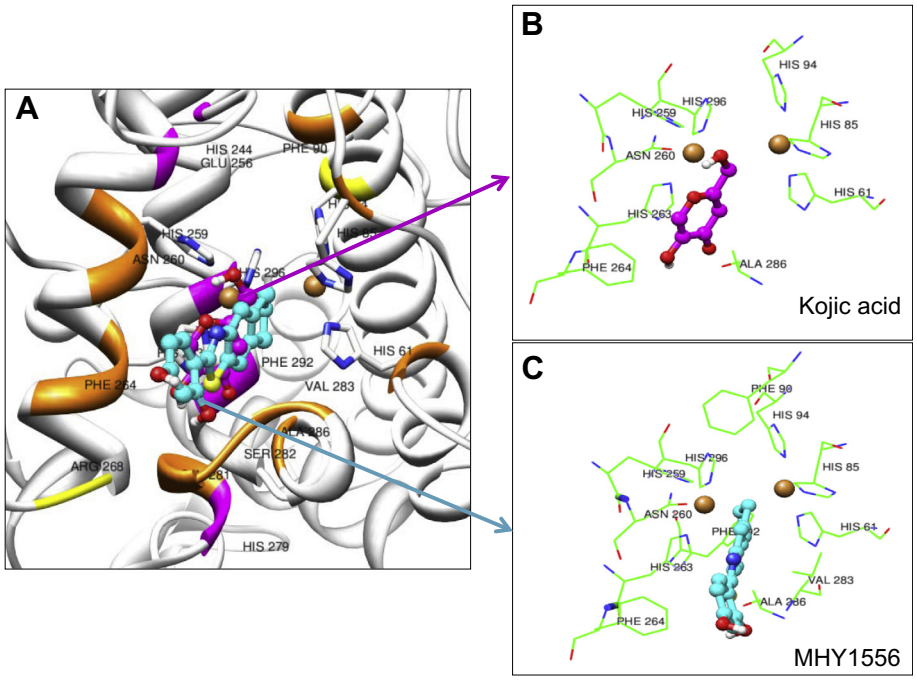


Figure 2. Computational structure prediction for mushroom tyrosinase and docking simulation with MHY1556 and kojic acid. Predicted 3D structure of mushroom tyrosinase (A). The boxes indicate kojic acid and MHY1556 binding sites with tyrosinase residues. Kojic acid (B), which was used as a control compound, is shown in magenta and MHY1556 (C) is shown in navy.

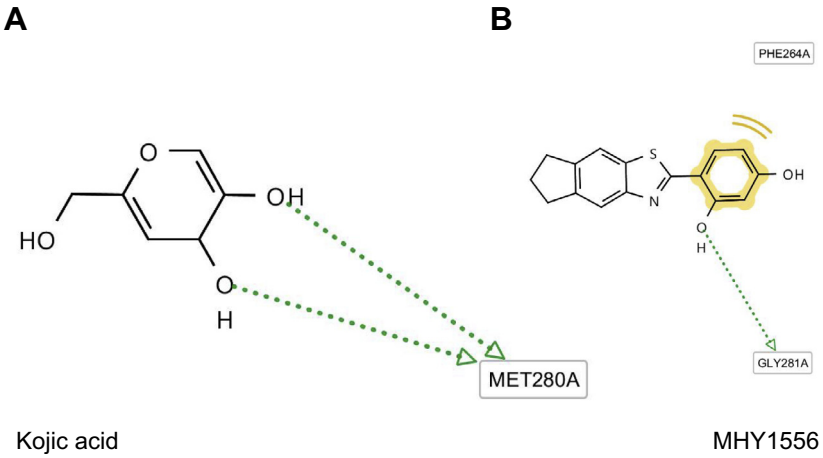


Figure 3. Possible hydrogen bonding interactions between tyrosinase residues and MHY1556 or kojic acid. We searched for hydrogen binding interactions between tyrosinase and kojic acid (A) or MHY1556 (B) in the simulated docked structures, the green lines indicate hydrogen bonding interactions.

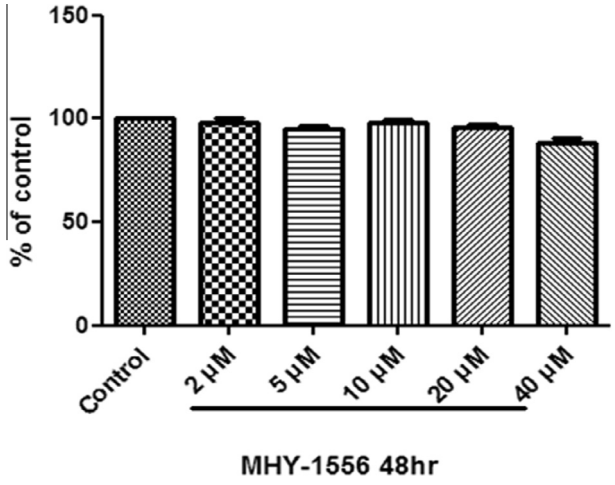


Figure 4. Effect of MHY1556 on B16F10 cell viability. Cells were treated with varying doses of MHY1556 (2–40 μM) for 48 h and examined using an MTT assay. Data are expressed as a percentage of the control.

melanogenesis at concentrations that did not adversely affect the cell viability. Tyrosinase activity assay was performed to measure the inhibitory effect of MHY1556 on B16F10 cells. As shown in Figure 5B, MHY1556 concentration-dependently decreased the tyrosinase activity by 165.73% at 0.05 μM, 133.14% at 0.1 μM, and 108.92% at 0.2 μM, compared to the control group treated with α-MSH alone (205.70%). The inhibitory effect of MHY1556 was much more potent than kojic acid; the inhibition of MHY1556 at 0.1 μM was superior to that of kojic aicd at 30 μM.

In this study, we synthesized, from 5-aminoindan, de novo tyrosinase inhibitor, MHY1556, which showed a potent inhibitory effect on melanogenesis. MHY1556 was found to be approximately 108-fold more potent than kojic acid which is often used as a positive reference compound. Docking simulation with MHY1556 confirmed that the compound binds strongly to mushroom tyrosinase and also suggested that Gly281 plays a key role in determination of binding affinity of MHY1556 through hydrogen bonding interaction. Therefore, docking simulation results imply that MHY1556 binds to the active site of tyrosinase, resulting in inhibiting tyrosinase activity. Due to the highly potent

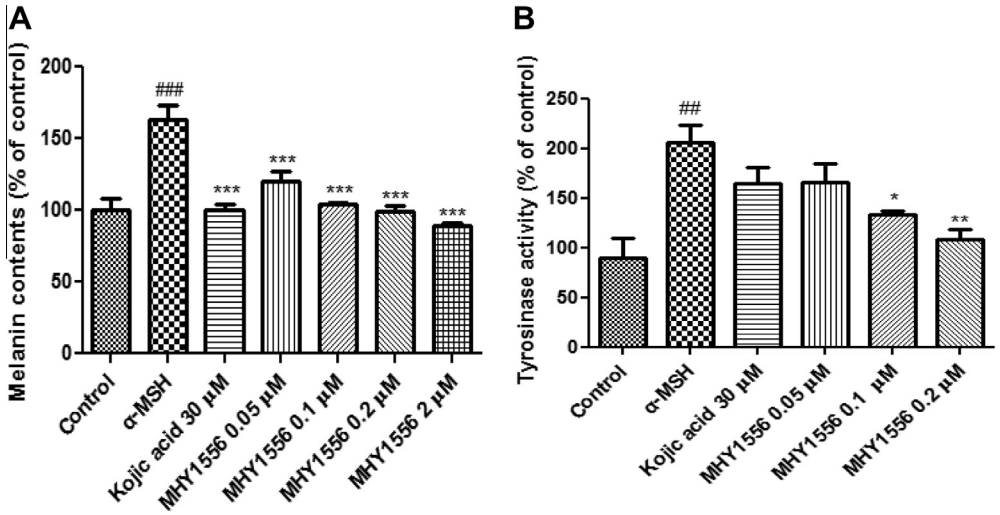


Figure 5. Inhibitory effect of kojic acid and MHY1556 after treatment with 100 nM α-MSH in B16F10 cells 5A; Melanin contents were measured at 405 nM. Values represent the mean ± S.E. of three experiments. Data are expressed as % of control. $^{###}p$ <0.001 compared to the untreated control, $^{***}p$ <0.001 compared to the group treated with 100 nM α-MSH. (B) Effect of the active compounds on tyrosinase activity: in the presence of 100 nM α-MSH, B16F10 cells were treated with varying doses of kojic acid (30 μM) or MHY1556 (0.05–0.2 μM) for 24 h. Results are expressed as the percentage of control and each column represents the mean ± S.E. of three determinations. $^{###}p$ <0.001 compared to the untreated control, $^{**}p$ <0.01 and $^{***}p$ <0.001 compared to the group treated with 100 nM α-MSH.

inhibitory effect of MHY1556 on mushroom tyrosinase, further experiments were conducted to evaluate the efficacy of MHY1556 on B16F10 cells. The cell viability assay showed that MHY1556 was non-cytotoxic to B16F10 cells at the concentrations tested in the experiments of tyrosinase activity and melanin content. Considering the cell viability assay result, MHY1556 effectively inhibited tyrosinase activity and melanogenesis without adversely affecting cell viability. These results provide the strongest evidence that the newly synthesized compound, MHY1556 with a hydroxyl group at both R1 and R3 of the phenyl ring might serve as a potent tyrosinase inhibitor. Based on these findings, we suggest MHY1556 could be potentially utilized as a new skin-whitening agent.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.05.029.

References and notes

1. Brenner, M.; Hearing, V. *J. Photochem. Photobiol.* **2008**, *84*, 539.
2. Parvez, S.; Kang, M.; Chung, H. S.; Bae, H. *Phytother. Res.* **2007**, *21*, 805.
3. Kim, Y. J.; Uyama, H. *Cell. Mol. Life Sci.* **2005**, *62*, 1707.
4. Gillbro, J. M.; Olsson, M. J. *Int. J. Cosmet. Sci.* **2011**, *33*, 210.
5. Briganti, S.; Camera, E.; Picardo, M. *Pigment Cell Res.* **2003**, *16*, 101.
6. Brown, D. A. *Biology* **2001**, *63*, 148.
7. Cooksey, C. J.; Garratt, P. J.; Land, E. J.; Pavel, S.; Ramsden, C. A.; Riley, P. A.; Smit, N. P. *J. Biol. Chem.* **1997**, *272*, 26226.
8. Passi, S.; Nazzaro-Porro, M. *Br. J. Dermatol.* **1981**, *104*, 659.
9. Chang, T. S. *Int. J. Mol. Sci.* **2009**, *10*, 2440.
10. Ha, Y. M.; Kim, J. A.; Park, Y. J.; Park, D.; Kim, J. M.; Chung, K. W.; Lee, E. K.; Park, J. Y.; Lee, J. Y.; Lee, H. J.; Yoon, J. H.; Moon, H. R.; Chung, H. Y. *Biochim. Biophys. Acta* **2011**, *1810*, 612.
11. Chung, K. W.; Park, Y. J.; Choi, Y. J.; Park, M. H.; Ha, Y. M.; Uehara, Y.; Yoon, J. H.; Chun, P.; Moon, H. R.; Chung, H. Y. *Biochim. Biophys. Acta* **2012**, *1820*, 962.
12. Kajino, M.; Mizuno, K.; Tawada, H.; Shibouta, Y.; Nishikawa, K.; Meguro, K. *Chem. Pharm. Bull.* **1991**, *39*, 2888.