Design, synthesis, and antimelanogenic effects of (2-substituted phenyl-1,3-dithiolan-4-yl)methanol derivatives

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Abstract: The authors designed and synthesized 17 (2-substituted phenyl-1,3-dithiolan-4-yl)methanol (PDTM) derivatives to find a new chemical scaffold, showing excellent tyrosinase-inhibitory activity. Their tyrosinase-inhibitory activities were evaluated against mushroom tyrosinase at 50 μM, and five of the PDTM derivatives (PDTM3, PDTM7–PDTM9, and PDTM13) were found to inhibit mushroom tyrosinase more than kojic acid or arbutin, the positive controls. Of seventeen PDTMs, PDTM3 (half-maximal inhibitory concentration 13.94±1.76 μM), with a 2,4-dihydroxyphenyl moiety, exhibited greatest inhibitory effects (kojic acid half-maximal inhibitory concentration 18.86±2.14 μM). Interestingly, PDTM compounds with no hydroxyl group, PDTM7–PDTM9, also had stronger inhibitory activities than kojic acid. In silico studies of interactions between tyrosinase and the five PDTMs suggested their binding affinities were closely related to their tyrosinase-inhibitory activities. Cell-based experiments performed using B16F10 mouse-skin melanoma cells showed that PDTM3 effectively inhibited melanogenesis and cellular tyrosinase activity. A cell-viability study conducted using B16F10 cells indicated that the antimelanogenic effect of PDTM3 was not attributable to its cytotoxicity. Kinetic studies showed PDTM3 competitively inhibited tyrosinase, indicating binding to the tyrosinase-active site. We found that PDTM3 with a new chemical scaffold could be a promising candidate for skin-whitening agents, and that the 1,3-dithiolane ring could be used as a chemical scaffold for potent tyrosinase inhibition.

Keywords: tyrosinase inhibitor, melanogenesis, 1,3-dithiolane, PDTM

Introduction

For the past few decades, tyrosinase inhibitors have been of considerable interest, due to the key role played by tyrosinase in melanogenesis. Melanin is produced using a combination of enzymatically catalyzed and chemical reactions. The biosynthetic pathway responsible for melanin production was initially elucidated by Raper¹ and Mason² and recently modified by Cooksey et al³ and Schallreuter et al.⁴ Melanogenesis is initiated by the enzyme tyrosinase, which catalyses the first two oxidative steps in the melanin biosynthetic pathway: the oxidations of L-tyrosine to L-dopa followed by L-dopa to L-dopaquinone (Figure 1). These two steps are also the rate-determining steps in melanin biosynthesis, because physiological pH can continue spontaneously subsequent steps.⁵ The dopaquinone produced in the second step is converted to cysteinyl-dopa by glutathione or cysteine, and finally to pheomelanin, which is responsible for yellow to red colors of mammal skin, or to dopachrome by auto-oxidation and finally eumelanin, which is responsible for brown/black colors of mammal skin (Figure 1).

Melanin protects human skin from harmful ultraviolet radiation, and also determines
phenotypic appearance. On the other hand, excessive melanin accumulation in skin can cause hyperpigmentation-associated diseases and cosmetic problems, such as freckles, melasma, and senile lentigines.

Tyrosinase is widely distributed in bacteria, fungi, insects, plants, and animals, including humans, and is responsible for the colors of human skin and hair and the undesirable browning of fruit and vegetables. Undesirable enzymatic browning and diseases associated with hyperpigmentation in skin have encouraged scientists to seek novel potent tyrosinase inhibitors for use as skin-whitening and antibrowning agents. Many tyrosinase inhibitors have been discovered to date, but relatively few have been approved as skin-whitening materials, due to safety concerns or weak whitening effects.

In our previous studies, on the basis of structures of $L$-tyrosine and $L$-dopa, natural substrates of tyrosinase, we designed two potential tyrosinase inhibitors with a thiazolidine ring (MHY384 and MHY794; Figure 1) and synthesized them by condensation of an appropriate benzaldehyde and $L$-cysteine or cysteamine hydrochloride. These two compounds were identified to be potent competitive tyrosinase inhibitors and to effectively reduce melanogenesis in HRM2 hairless mice. In addition to directly inhibiting tyrosinase activity, MHY384 inhibited tyrosinase expression by suppressing the cyclic adenosine monophosphate–PKA and NO-induced cyclic guanosine monophosphate–PKG pathways, and MHY794 also suppressed tyrosinase expression induced by NO-mediated melanogenesis signaling. These positive results and the fact that divalent $\text{S}^2$ is a classical isostere of divalent $\text{NH}^-$ encouraged us to synthesize derivatives with a dithiolane ring as a surrogate for the thiazolidine ring commonly existing in MHY384 and MHY794, in order to find novel tyrosinase inhibitors.

In the present study, as part of our ongoing efforts to find a new and strong chemical scaffold for inhibition of tyrosinase and develop new tyrosinase inhibitors, we synthesized a class of structurally novel (2-substituted phenyl-1,3-dithiolan-4-yl) methanol (PDTM) derivatives (Figure 1) and investigated their antimelanogenic effects using mushroom tyrosinase and cell-based assays. In this study, kojic acid and arbutin were used as a positive control for tyrosinase-inhibitory activity, because kojic acid is one of the most commonly used positive controls for evaluation of tyrosinase inhibition and arbutin is clinically used as a whitening agent.

**Materials and methods**

**Materials**

Unless otherwise noted, all commercially available reagents—$L$-tyrosine (code T3754), $L$-dopa (code PHR1271), kojic acid (code K3125), MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl$H$-tetrazolium bromide) (code M2128), PMSF (phenylmethylsulfonyl fluoride) (code P7626), Triton X-100 (4-(1,1,3,3-tetramethylbutyl)phenylpolyethylene glycol) (code T8787), potassium hydrogen phosphate (code P9666), potassium dihydrogen phosphate (code PHR1330), 2,3-dimercapto-1-propanol (code 64046), 1,4-dioxane (code 296309), sulfuric acid (code 339741), and benzaldehydes—were purchased from Sigma-Aldrich (St Louis, MO, USA). Fetal bovine serum, Dulbecco’s Modified Eagle’s Medium, phosphate-buffered saline (PBS), penicillin, streptomycin,
and trypsin were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Mushroom tyrosinase (code T3824) and \( \alpha \)-melanocyte-stimulating hormone (\( \alpha \)-MSH; code M4135) were also purchased from Sigma-Aldrich. \(^1\)H and \(^{13}\)C nuclear magnetic resonance (NMR) spectra were recorded on Varian Unity Inova 400 and Varian Unity AS 500 instruments (Agilent Technologies, Santa Clara, CA, USA). Low-resolution mass spectrometry (MS) and high-resolution MS data were obtained on an Expression CMS (Advion, Ithaca, NY, USA) and a 6530 Accurate Mass quadrupole time-of-flight liquid-chromatography mass spectrometer (Agilent), respectively. Synthesis of PDTM1–PDTM17 was accomplished in our laboratory.

**General procedure for synthesis of PDTM1–PDTM17**

To a stirred solution of 2,3-dimercapto-1-propanol (100 mg, 0.80 mmol) in 1,4-dioxane (1 mL) were added a solution of sulfuric acid (0.1 equivalence) in 1,4-dioxane (1 mL) and an appropriate benzaldehyde (1.1 equivalence) subsequently. After being stirred at room temperature for 10 minutes, the reaction mixture was stirred at 70°C for 40 minutes to 1 hour. The mixture was then partitioned between ethyl acetate and water, and the organic layer was dried over anhydrous MgSO\(_4\), filtered, and evaporated. The residue obtained was purified using silica-gel column chromatography to give pure PDTM products: PDTM1–PDTM17.\(^1\) Structural characterization (\(^1\)H and \(^{13}\)C NMR and mass data of all PDTMs and 1-D and 2-D NMR spectra of some PDTMs) of synthesized compounds is provided in 

**Inhibition of mushroom tyrosinase by PDTM1–PDTM17**

The inhibitory effects of PDTM analogues on mushroom tyrosinase were explored with minor modifications, as described previously in our work.\(^1\) Each compound (10 \( \mu \)L, final concentration 50 \( \mu \)M) was mixed with substrate solution (170 \( \mu \)L), prepared from 14.7 mM potassium phosphate buffer (pH 6.5) and 293 \( \mu \)M L-tyrosine solution (1:1, v/v), in each well of a 96-well plate. To each well, mushroom tyrosinase solution (20 \( \mu \)L, 1,000 U/mL) was added and incubated for 30 minutes at 37°C. The in silico docking simulation of protein–ligand was performed with AutoDock Vina using a systematic search technique and the 3-D structure of *Agaricus bisporus* tyrosinase (Protein Data Bank ID 2Y9X).\(^16\) In the crystal structure of tyrosinase, the binding site of L-tyrosine was used as a docking pocket. Simulation results were obtained from docking between tyrosinase and synthetic compounds (PDTM3, PDTM7, PDTM8, PDTM9, and PDTM13) or kojic acid. Before performing docking simulation with the compounds, 2-D structures of compounds were transformed into 3-D structures, charges of compounds were determined, and hydrogen atoms were inserted using ChemOffice (http://www.cambridgesoft.com). LigandScout 3.1.2 was used for the prediction of possible interactions between ligands and proteins.
and tyrosinase and the identification of pharmacophores. Docking-simulation images of 17 PDTMs are provided in Supplementary materials.

**Cell culture**

Murine melanoma B16F10 cells were cultured in Dulbecco’s Modified Eagle’s Medium with penicillin–streptomycin (100 IU/50 μg/mL) and 10% heat-inactivated fetal bovine serum in a humidified atmosphere containing 5% CO₂ at 37°C. B16F10 cells were cultured in 24-well plates for cell viability (MTT) assay, a melanin-content assay, and tyrosinase-activity assay. All experiments were performed at least three times to ensure reproducibility.

**Cell-viability assay**

MTT assays were performed in B16F10 cells for cell-viability determination, as previously described. Cells seeded at a density of 5×10⁴ cells/well in a 24-well plate were allowed to adhere at 37°C for 24 hours in a 5% humidified CO₂ atmosphere. On the following day, the cells were exposed to diverse concentrations of PDTM3 (0, 5, 10, or 25 μM) and incubated for 24 hours under the same conditions. To each well, MTT stock solution (0.5 mg/mL) was added and the plate incubated at 37°C for 2 hours. Formazan crystals isolated after removing supernatants were dissolved in dimethyl sulfoxide–ethanol (200 μL, 1:1) and then moved to a 96-well plate. The optical density of each well was measured at 570 nm by an enzyme-linked immunosorbent assay reader. All experiments were performed in triplicate.

**Determination of melanogenesis level in B16F10 cells**

Melanin-content assays with minor modifications were used in B16F10 cells for the inhibitory effects of PDTM3 on melanogenesis. Cells seeded at a density of 5×10⁴ cells/well in a 24-well plate were allowed to adhere at 37°C in a humidified atmosphere containing 5% CO₂ overnight. The following day, the cells were exposed to α-MSH (1 μM) and PDTM3 (0, 5, 10, or 25 μM) or kojic acid (25 μM), and the plate was incubated for 24 hours under the same conditions. After being washed twice with PBS, the cells were lysed with 100 μL lysis buffer containing 0.1 mM PMSF (5 μL), 50 mM PBS (90 μL, pH 6.8), and 1% Triton X-100 (5 μL) and frozen at −80°C for 30 minutes. Lysates were thawed and centrifuged at 12,000 g for 30 minutes at 4°C and supernatants (80 μL) were combined with 10 mM L-dopa (20 μL) in a 96-well plate, which was then incubated for 30 minutes at 37°C. Optical densities were calculated at 500 nm, and the inhibitory activities of tyrosinase were determined thus:

\[
\text{Inhibition} (\%) = 100 \times \frac{[A - B] - [C - D]}{[A - B]} \tag{2}
\]

where B and A are the optical densities of the blank before and after incubation, respectively, and D and C are the optical densities of the test compound before and after incubation, respectively.

**DPPH radical scavenging-activity assay**

The DPPH scavenging activity of PDTM derivatives was analyzed as described in a previous study, with minor modifications. DPPH methanol solution (0.2 mM, 180 μL) was mixed with a dimethyl sulfoxide solution (20 μL) of each compound (10 mM) in each well of a 96-well plate and then left at room temperature in the dark for 30 minutes. The absorbance of the resulting solutions was measured at 517 nm using a microplate reader. Scavenging capacity of each PDTM compound was compared to that of L-ascorbic acid, a positive control. All experiments were independently conducted in triplicate.

**Statistical analysis**

One way analysis of variance followed by Dunnett’s test was used to determine whether group means differed significantly from those of controls. Welch’s unpaired t-test was used to determine whether the effects of PDTM3 and kojic acid were significantly different. Statistical analysis was performed using GraphPad (GraphPad Software, La Jolla, CA, USA). All results are indicated as means ± standard error of three independent experiments. Two sided P-values of <0.05 were considered statistically significant.
**Results and discussion**

**Preparation of PDTM1–PDTM17**

PDTM1–PDTM17 were synthesized as shown in Scheme 1. Heating 2,3-dimercapto-1-propanol and numerous suitably substituted benzaldehydes (1–17) in 1,4-dioxane in the presence of sulfuric acid afforded the desired PDTM products as solids or sticky oil. The structures of the final products were confirmed by $^1$H and $^{13}$C NMR, correlation spectroscopy, heteronuclear single-quantum correlation spectroscopy, heteronuclear multiple-bond correlation spectroscopy, and low- and high-resolution MS. The presence of the singlet corresponding to the –SCH(Ph)S– proton at $\delta$=6.09–5.56 ppm in $^1$H NMR spectra confirmed the formation of a dithiolane ring between benzaldehydes and 2,3-mercapto-1-propanol. In $^1$H NMR spectroscopic data of PDTMs, the 2-H peak appeared more downfield than the peaks of protons attached to sp$^3$ carbon atoms, and the 4-H exomethylene and 5-H$_2$ peaks were observed more upfield in named order. In $^{13}$C NMR spectroscopic data, apart from peaks from the phenyl ring, the carbon peak of exomethylene (~64 ppm) was most downfield, followed in order by 4-C (~58 ppm), 2-C (~55.3 ppm), and 5-C (~41 ppm). Four products – PDTM6, PDTM12, PDTM13, and PDTM15 – were each obtained as a single racemate, whereas the others were obtained as

![Scheme 1 Structures of (2-substituted phenyl-1,3-dithiolan-4-yl)methanol (PDTM) derivatives, kojic acid, and arbutin, and reagents and conditions. Notes: a, $\text{H}_2\text{SO}_4$ in 1,4-dioxane at 70°C. t-Bu, tertiary butyl (group).](image-url)
a mixture of two racemates ([2R,4R]/[2S,4S] and [2R,4S]/
[2S,4R], 1:1–1:1.5), the ratios of which were calculated using
\(^1\)H NMR spectroscopic data. The phenomenon in the product
formation could not be explained simply by steric, electronic,
and/or stereoelectronic effects. The tyrosinase-inhibition
assay was performed without separation of the racemates.

**Inhibitory effects of PDTM1–PDTM17 on mushroom tyrosinase**

The potentials of the 17 synthesized products, PDTM1–
PDTM17, to inhibit mushroom tyrosinase activity were
examined using kojic acid\(^{22}\) and arbutin\(^{23}\) as positive
controls. Inhibitions were determined using 293 \(\mu M\) L-tyrosine
as the substrate and synthetic compounds at a concentration
of 50 \(\mu M\). Tyrosinase inhibitions by kojic acid and arbutin
were determined at 50 and 500 \(\mu M\), respectively.

Three compounds – PDTM11 (3,4,5-trimethoxyphenyl),
PDTM15 (4-hydroxy-3-methylphenyl), and PDTM17
(3,5-di-\(R\)-butyl-4-hydroxymethyl) – inhibited tyrosinase
to the same extent as kojic acid and inhibited it more than
arbutin (Table 1). Five PDTM derivatives – PDTM3 (2,4-
dihydroxyphenyl), PDTM7 (4-methoxyphenyl), PDTM8 (3,4-
dimethoxyphenyl), PDTM9 (2,4-dimethoxyphenyl), and
PDTM13 (3-bromo-4-hydroxyphenyl) – inhibited tyrosi-
nsinase more potently than kojic acid. IC\(_{50}\) values of these
compounds were examined: 13.94±1.76 \(\mu M\) (PDTM3),
16.47±2.36 \(\mu M\) (PDTM7), 16.97±2.99 \(\mu M\) (PDTM8),
27.85±1.47 \(\mu M\) (PDTM9), and 15.57±3.31 \(\mu M\) (PDTM13).
The low IC\(_{50}\) values of PDTM3, PDTM7, PDTM8, and
PDTM13 indicated that potency was stronger than that of
tojic acid (18.86±2.14 \(\mu M\)) and arbutin (381.26±9.01 \(\mu M\)),
which were used as positive controls. Interestingly, this result
corresponds with our previous finding that many analogues with
a 2,4-dihydroxyphenyl moiety possess higher tyrosinase-
inhibitory activity than kojic acid.\(^{10,13,24–32}\)

The remaining PDTM derivatives had no (PDTM1 and
PDTM2) or low inhibitory effect (PDTM4, PDTM5, PDTM6,
PDTM10, PDTM12, PDTM14, and PDTM16) compared with
kojic acid. According to our accumulated structure-activity
relationship data, analogues with at least one hydroxyl group
were more capable of inhibiting tyrosinase. Somewhat sur-
prisingly, four PDTM derivatives with no hydroxyl group
inhibited tyrosinase activity as much or more effectively than
kojic acid. These were PDTM7 (4-methoxyphenyl), PDTM8
(3,4-dimethoxyphenyl), PDTM9 (2,4-dimethoxyphenyl), and
PDTM11 (3,4,5-trimethoxyphenyl). Compounds (PDTM1–2,
PDTM4–5, and PDTM12) with only a 4-hydroxyl group on
the phenyl ring or an alkoxy or hydroxyl group at position 3
with a 4-hydroxyl group had low or no activity, whereas com-
ounds (PDTM13–PDTM17) with an alkyl or bromo group
at position 3 with a 4-hydroxyl group showed moderate–high
tyrosinase-inhibitory activity. These structure-activity
relationship results support the hypothesis that the tyrosinase-
inhibitory activity of compounds with a 4-hydroxyl group is
greatly affected by the type of 3-substituent.

**Mode of mushroom tyrosinase inhibition by PDTM3**

Since PDTM3 inhibited mushroom tyrosinase most, a
Lineweaver–Burk plot was used to determine the nature
of its inhibitory effect. As depicted in Figure 2, a double-
reciprocal plot was obtained, and all lines with different
slopes intersected the y-axis at the same point. Therefore,
K\(_m\) (Michaelis constant) values increased gradually with
concentration of PDTM3. Detailed kinetic-parameter values
are shown in Table 2. On the other hand, maximum reaction

"Table 1: Substitution patterns and tyrosinase-inhibitory activities by the synthesized compounds PDTM1–PDTM17, kojic acid, and arbutin"
of tyrosinase compared to kojic acid (-5.4 kcal/mol), and PDTM3 demonstrated greatest affinity. This result indicated that the strong inhibitory effects of these five PDTM derivatives were attributable to their binding affinity with tyrosinase, and showed a close relationship between binding affinity, as determined by docking simulation, and tyrosinase inhibition.

LigandScout 3.1.2 was used to identify tyrosinase amino acid residues that interact with PDTM analogues. As shown in Figure 3B and C, the 4-hydroxyl group on the phenyl ring of PDTM3 interacted with His85 of tyrosinase through hydrogen bonding and the phenyl ring interacted with Val283 and Ala286 through hydrophobic interactions and with His263 through π–π stacking interaction. Hydrogen bonding between the alcoholic hydroxyl group and amino acid residues of tyrosinase was detected only for PDTM7 and PDTM8. However, the alcoholic hydroxyl group of both PDTM analogues interacted with different amino acid residues, eg, PDTM7 interacted with His244 and Asn260, whereas PDTM8 interacted with Gly281. Furthermore, Val283 of tyrosinase was involved in hydrophobic interactions with all five analogues.

Cell-viability assays
The possible cytotoxicity of PDTM3, the most potent tyrosinase inhibitor, was examined by determining B16F10 cell (a melanoma cell line) viability using MTT assays. After 24 hours of treatment with 5, 10, or 25 μM of PDTM3, cell viability was 99.9%, 98.4%, and 97.4% versus untreated controls (Figure 4). This result indicates that PDTM3 at a concentration of ≤25 μM is not cytotoxic.

Melanin-content assays
B16F10 cells were used to evaluate the depigmenting activities of the PDTM analogues. PDTM3 was chosen for the cell-based experiments, due to its potent mushroom tyrosinase-inhibitory effect and its lack of toxicity to B16F10 cells. PDTM3 was evaluated for its inhibitory effect against α-MSH-stimulated melanogenesis in B16F10 cells by determining melanin levels in the cells. Melanin levels were significantly reduced after cells were cotreated with PDTM3 and α-MSH compared with cells treated with α-MSH alone. Over the concentration range 0–25 μM, PDTM3 exhibited a significant and dose-dependent antimelanogenic effect. Furthermore, at a concentration of only 10 μM, PDTM3 showed higher inhibitory potency than kojic acid at 25 μM, as depicted in Figure 5. These results indicated the antimelanogenic effect of PDTM3 in B16F10 cells was not related to cytotoxicity.
A

<table>
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<tr>
<td></td>
<td>(2R,4R)/(2S,4S)</td>
</tr>
<tr>
<td>PDTM3</td>
<td>–6.0/–5.8 kcal/mol</td>
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<tr>
<td>PDTM7</td>
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</tr>
<tr>
<td>Kojic acid</td>
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B

Figure 3 Docking simulation of PDTM derivatives and kojic acid with tyrosinase.

Notes: (A) Tyrosinase docking scores of the five PDTM analogues and kojic acid. (B) Docking result between (2S,4S)-PDTM3 and mushroom tyrosinase. (C) The pharmacophore model obtained by the LigandScout 3.1.2 program indicated possible hydrophobic (yellow), hydrogen-bonding (green arrow), and π–π stacking (violet arrow) interactions between the amino acid residues of tyrosinase and the ligands tested.

Abbreviation: PDTM, (2-substituted phenyl-1,3-dithiolan-4-yl)methanol.

C

Figure 4 Effect of PDTM3 on B16F10 cell viability.

Notes: Cell viability after treatment with PDTM3 at different concentrations for 24 hours presented as mean percentage viability against untreated controls. All experiments were independently conducted in triplicate. Bars represent standard error of the mean.

Abbreviation: PDTM, (2-substituted phenyl-1,3-dithiolan-4-yl)methanol.

Tyrosinase-activity assay in B16F10 cells

The inhibitory effect of PDTM3 on cellular tyrosinase activity was examined in B16F10 cells prestimulated with α-MSH. PDTM3 dose dependently inhibited tyrosinase activity over the concentration range 0–25 μM (Figure 6). Furthermore, these results matched melanin-content results well, which suggested that the antimelano-genic effect of PDTM3 was attributable to the inhibition of tyrosinase.

Effect on DPPH radical scavenging activity

The effect of PDTM derivatives on DPPH radical scavenging activity was investigated. PDTM4 exhibited similar DPPH radical scavenging activity to L-ascorbic acid and PDTM6, PDTM16, and PDTM17 also showed significantly potent activity, as shown in Figure 7. However, PDTM3, showing
the greatest tyrosinase-inhibitory activity, exerted only moderate DPPH radical scavenging potency.

We showed through this study that the 1,3-dithiolane ring is a promising chemical scaffold that can inhibit the activity of tyrosinase and that PDTM3 bearing the scaffold not only inhibits the tyrosinase enzyme itself but also has antimelanogenic effects in a cell-based system. The limitation of this study – the activity test of PDTMs without separation of the racemates – will be overcome after finding methods of separating them in the future.

Conclusion

In this work, a variety of PDTM analogues were synthesized and evaluated for their effects on melanogenesis and tyrosinase activity in B16F10 cells. Eight analogues exhibited as much or more potent inhibition against mushroom tyrosinase than kojic acid, and PDTM3 had the greatest inhibitory activity. In B16F10 cells, PDTM3 significantly inhibited melanin biosynthesis in a dose-dependent manner and tyrosinase activity over the concentration range 0–25 μM with no cytotoxic effect. At a concentration of 10 μM, PDTM3 reduced melanin biosynthesis and tyrosinase activity significantly more than kojic acid at 25 μM. In view of our observation that many PDTM analogues showed potent tyrosinase-inhibitory activities, we suggest that 1,3-dithiolane be considered an appropriate scaffold for tyrosinase-inhibitory activity. Now, we are looking for separation conditions of each PDTM mixture of two racemates, and the separation conditions and antimelanogenic effect of each single racemate will be reported in due course.

Acknowledgment

This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIP; grant number 2009-0083538).
Disclosure
The authors report no conflicts of interest in this work.

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