1-(2,4-Dihydroxyphenyl)-3-(2,4-dimethoxy-3-methylphenyl)propane, a Novel Tyrosinase Inhibitor with Strong Depigmenting Effects

Alexandre Nesterov,*[,a] Jifu Zhao,[a,#] David Minter,[b] Carmen Hertel,[a] Wenwen Ma,[a] Padmapriya Abeysinghe,[a] Mei Hong,[a] and Qi Jia[a]

[a] *Unigen Pharmaceuticals, Inc.; 2660 Willamette Drive NE, Lacey, WA 98516, U.S.A.: and* [b] *Chemistry Department, Texas Christian University; Fort Worth, TX 76129, U.S.A.*

Received May 7, 2008; accepted June 4, 2008; published online June 9, 2008

A series of diarylpropane compounds was isolated by screening a plant extract library for inhibitors of mushroom tyrosinase. The most potent compound, 1-(2,4-dihydroxyphenyl)-3-(2,4-dimethoxy-3-methylphenyl)-propane (UP302: CAS# 869743-37-3), was found in the medicinal plant *Dianella ensifolia*. Synthetic and plant-derived versions of UP302 inhibited mushroom tyrosinase with similar potencies. UP302 inhibited mushroom tyrosinase with K_i=0.3 μM, in a competitive and reversible fashion. UP302 was 22 times more potent than Kojic acid in inhibiting murine tyrosinase, with IC_{50} values of 12 and 273 μM respectively. Experiments on mouse melanoma cells B16-F1 and on human primary melanocytes demonstrated that UP302 inhibits melanin formation with IC_{50} values of 15 and 8 μM respectively. Long-term treatment of cultured melanocytes with up to 62 μM of UP302 revealed no detectable cytotoxicity. In a reconstructed skin model (MelanoDerm™) topical application of 0.1% UP302 resulted in significant skin lightening and decrease of melanin production without effects on cell viability, melanocyte morphology or overall tissue histology. In conclusion, UP302 is a novel tyrosinase inhibitor that suppresses melanin production in both cultured melanocytes and reconstructed skin with high potency and without adverse side effects.

Key words tyrosinase; inhibitor; competitive; skin; melanin; depigmenting

Abnormal pigmentation is related to a variety of cosmetic and clinical conditions including melasma, lentigo, age spots, inflammatory hypermelanosis and trauma-induced hyperpigmentation.[1)] A number of studies have been devoted to producing safe and efficient depigmenting agents. However, many popular depigmenting compounds either lack potency or produce undesirable side effects.

Skin color depends on the type and quantity of melanin, a natural pigment produced by melanocytes. The major rate-limiting step in melanin biosynthesis involves the enzyme tyrosinase [EC 1.14.18.1]. Mutations that eliminate activity of the tyrosinase gene result in albinism.[2)] Tyrosinase, a copper-containing binuclear enzyme catalyzes three steps of melanin biosynthesis: the hydroxylation of tyrosine to 3,4-dihydroxyphenylalanine (DOPA), oxidation of DOPA to DOPAquinone, and oxidation of 5,6-dihydroxyindole to indolequinone. Because of its key role in melanogenesis, tyrosinase is an attractive target in the search for various kinds of depigmenting agents.[3—6)]

This study is a report of the discovery and characterization of a novel tyrosinase inhibitor: 1-(2,4-dihydroxyphenyl)-3-(2,4-dimethoxy-3-methylphenyl)propane (coded as UP302), isolated from a medicinal plant. UP302 inhibited tyrosinase with nanomolar potency and exhibited strong skin-whitening effects.

Results

Identification of UP302 A screening of 1144 plant extracts identified 20 extracts that inhibited DOPA oxidase activity of mushroom tyrosinase. Three compounds were isolated and identified from two different families of plants, Moraceae and Liliacea (Fig. 1). All three compounds shared a common diarylpropane structure, revealing the existence of a new class of tyrosinase inhibitors. Compound **3**, isolated from *Dianella ensifolia* (Liliacea) exhibited the highest potency with IC_{50} value equal to 0.24 μM. This compound, 1-(2,4-dihydroxyphenyl)-3-(2,4-dimethoxy-3-methylphenyl) propane (CAS# 869743-37-3) was coded as UP302, reflecting the molecular mass of this agent (302 Da).

Enzyme Kinetics Studies As UP302 is not a very abundant compound in nature (0.01—0.05% of plant dry weight), an organic synthesis procedure was developed to produce enough of this compound for further studies. Figure 2 demonstrates that synthetic UP302 is equally capable of inhibiting mushroom tyrosinase as its plant-derived counterpart. Therefore, all the following studies were performed

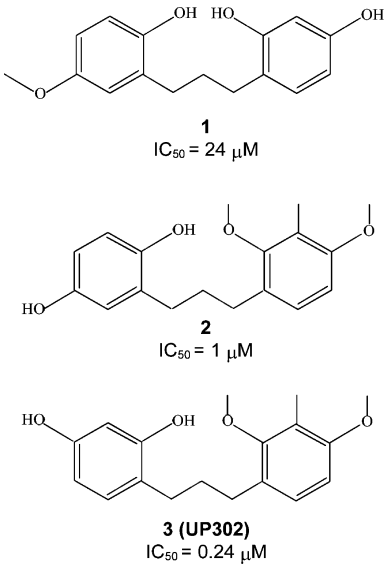

Fig. 1. Molecular Structures of Plant-Derived Diarylpropane Tyrosinase Inhibitors

* To whom correspondence should be addressed. e-mail: ANesterov@UnigenUSA.com
# Equal contribution with first author.

using synthetic UP302.

The potency of UP302 was compared with Kojic acid, a commonly used tyrosinase inhibitor. K_i values of both compounds were determined using L-DOPA as a mushroom tyrosinase substrate (Fig. 3). A series of substrate–velocity curves were taken at different concentrations of the inhibitors. K_i values were computed from global (shared) non-linear regression fits. UP302 (K_i=0.3 μM) is about 15 times more potent than Kojic acid (K_i=4.3 μM) in inhibiting mushroom tyrosinase.

Lineweaver–Burk plots were generated to determine the mechanism of inhibition (Fig. 3). The Y-axis intercepts are the same in the presence and in the absence of UP302, indicating competitive nature of inhibition.

Mushroom tyrosinase pre-treated with UP302 was passed through a series of de-salting columns. Removal of UP302 restored up to 85% of the original enzyme activity (Fig. 4), demonstrating reversible mechanism of inhibition.

Mammalian Tyrosinase Studies The ability of UP302 to inhibit mammalian DOPA oxidase activity was tested in cell-free system. Homogenates of murine melanoma cells, B16-F1, were treated with different concentrations of either UP302 or Kojic acid and incubated in the presence of L-DOPA. UP302 (IC_{50}=12 μM) was 22 times more potent than Kojic acid (IC_{50}=273 μM) in inhibiting DOPA oxidase activity of murine tyrosinase (Fig. 5).

Cultured Melanocytes Studies The effects of UP302

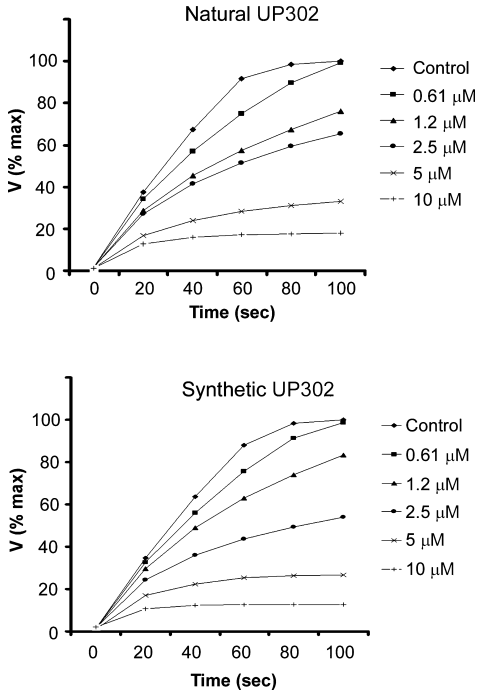

Fig. 2. Inhibition of Mushroom Tyrosinase by Naturally-Produced and Synthetic UP302

The ability of natural (plant-derived) and synthetic UP302 to inhibit purified mushroom tyrosinase was measured using L-DOPA (1 mM) as the substrate. The velocities were determined by the change in absorbance at 490 nm and expressed as % of maximal observed velocity.

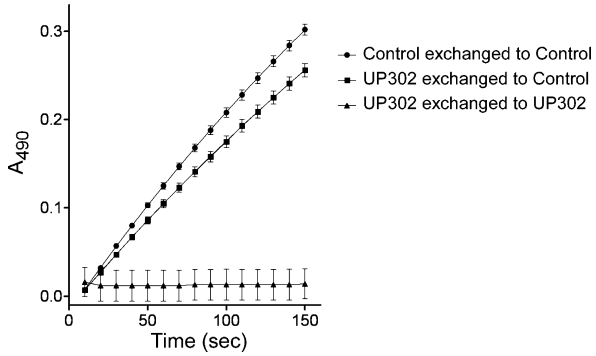

Fig. 4. Reversibility of Mushroom Tyrosinase Inhibition by UP302

Where indicated mushroom tyrosinase was pre-treated with 10 μM UP302. Sample buffers were then exchanged by passing through gel-filtration columns equilibrated with either control assay buffer (UP302 exchanged to control) or assay buffer containing UP302 (UP302 exchanged to UP302). For positive control the un-inhibited enzyme was passed through a column equilibrated with the assay buffer without UP302 (control exchanged to control). Tyrosinase activity of samples was measured using 1 mM L-DOPA as the substrate. DOPA oxidase activity was determined by the change in absorbance at 490 nm and expressed as % of the activity in un-inhibited homogenates.

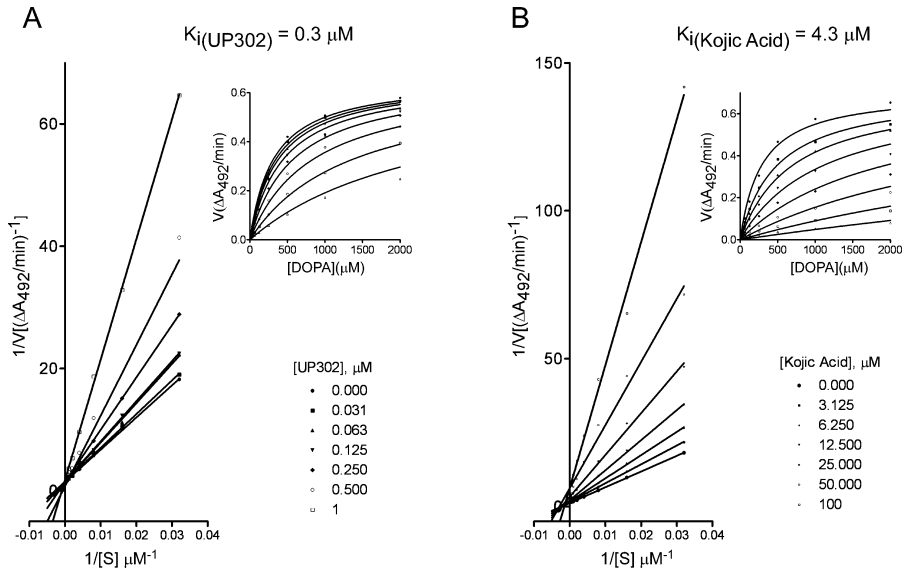

Fig. 3. Kinetics of Mushroom Tyrosinase Inhibition by UP302 and Kojic Acid

A series of substrate–velocity plots were taken at increasing concentrations of L-DOPA as a substrate in the presence of UP302 (A) or Kojic Acid (B). The curves were fit using a non-linear regression method. K_i values were calculated from global (shared) fit using GraphPad Prism software. Lineweaver–Burk plots for the inhibition of mushroom tyrosinase by UP302 or Kojic acid.

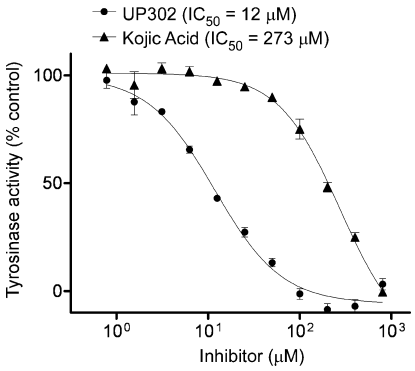

Fig. 5. The Effects of UP302 on DOPA Oxidase Activity in B16-F1 Cells Homogenates

Homogenates of B16-F1 cells were incubated with 1 mM of L-DOPA in the presence of different concentrations of either UP302 or Kojic acid.

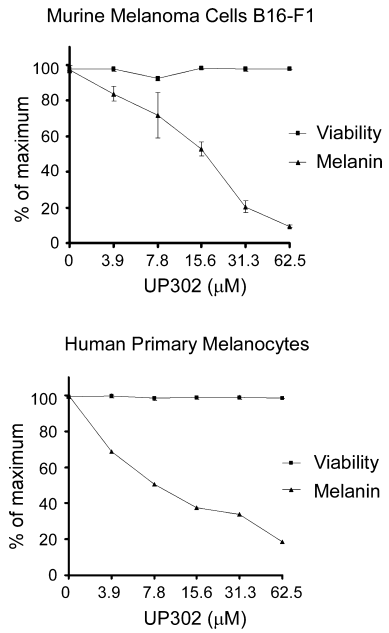

Fig. 6. The Effects of UP302 on Melanin Production and Viability of B16-F1 Cells and Primary Human Melanocytes

Mouse melanoma cells B16-F1 and primary human melanocytes were treated with increasing concentrations of UP302 for 72 h. At the end of treatment cell viability was assessed using a tetrazolium conversion assay (MTT). Production of melanin was measured by taking absorbance of culture media (B16-F1 cells), or by extracting intracellular melanin from pooled cells with KOH (primary human melanocytes) as outlined in Experimental.

on intact cells were investigated using murine melanoma cells, B16-F1, and primary human epidermal melanocytes (Fig. 6). The cells were treated with different concentrations of UP302 for 72 h. Extracellular melanin was measured directly by collecting the tissue culture supernatants and taking absorbance at 490 nm. The intracellular melanin was measured using a modified procedure of Siegrist and Eberle.[7] UP302 suppressed melanin production with IC_{50} values around 15 μM for B16-F1 cells and 8 μM for primary human melanocytes (Fig. 6).

Viability of cells was measured using standard tetrazolium reduction assays that are based on redox potential of live cells. As demonstrated in Fig. 6, UP302 has no detectable effect on cell viability at concentrations up to 62 μM. Treatment of primary human melanocytes with UP302 (62 μM) for up to

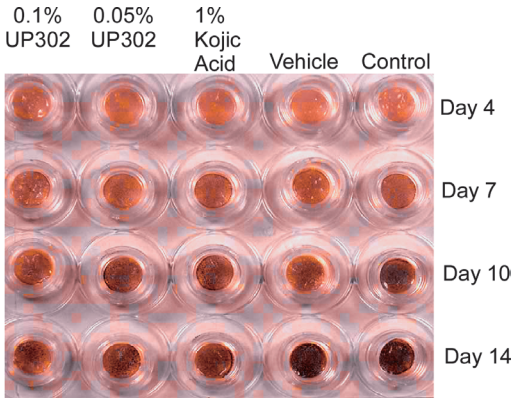

Fig. 7. The Effects of UP302 and Kojic Acid on Melanin Production in Reconstructed Skin Tissue

Samples of reconstructed skin were treated for up to 14 d with indicated concentrations of UP302, Kojic acid, or vehicle (80% propylene glycol). At the end of treatment tissues were fixed in formalin. The samples were photographed altogether at the end of the 14 d experiment.

Table 1. Inhibition of Melanogenesis in the Reconstructed Human Skin Model

Treatment (14 d)	Melanin content		Cell viability (% control)
	μg/sample	% control (mean)± standard deviation	
0.05% UP302, #1	35.8	57±13.4	Not determined
0.05% UP302, #2	25.6		
0.1% UP302, #1	34.1	64±1.3	78
0.1% UP302, #2	35.1		
1% Kojic acid, #1	35.2	66±2	Not determined
1% Kojic acid, #2	36.7		
Vehicle, #1	50.7	94±0.8	80
Vehicle, #2	51.3		
Untreated control, #1	57.2	100±8.5	100
Untreated control, #2	50.7		

Tissue samples (in duplicates) were treated for 14 d with indicated amounts of UP302, Kojic acid, or vehicle. #1 and #2 indicate parallel samples.

6 d also did not reveal any cytotoxicity (data not shown).

Reconstructed Skin Studies The potential skin-whitening properties of UP302 were further explored in a reconstructed skin model (MEL-300-B). The model consists of normal, human-derived epidermal keratinocytes and melanocytes, which have been co-cultured to form a multilayered, highly differentiated human epidermis.[8] In this study the melanocytes were obtained from a highly pigmented donor. The reconstructed skin was grown at the air–liquid interface, making it possible to mimic topical application of skin whitening agents.

Different concentrations of UP302, Kojic acid, or 80% propylene glycol (the vehicle for UP302) were applied topically on the reconstructed skin and tissues were monitored for up to 14 d post-application. Photographs of skin specimens (Fig. 7) show significantly reduced pigmentation in UP302-treated tissues.

Quantification of melanin content (Table 1) indicated that by the end of 14 d treatment 0.1% UP302 inhibited melanin production as efficiently as 1% Kojic acid. Cell viability assays based on tetrazolium reduction demonstrated that the safety profile of UP302 was comparable to the vehicle control (Table 1). These treatments had no adverse effects on

melanocyte morphology or overall tissue histology.

Discussion

Despite the fact that a number of tyrosinase inhibitors have been reported in literature,[3,5,9] skin-whitening activities have only been confirmed for relatively few compounds. Among the factors that preclude the clinical use of seemingly potent tyrosinase inhibitors are toxicity, limited solubility, and low stability in formulations.[6] In addition, in human skin the stratum corneum and superficial epidermis can efficiently prevent tyrosinase inhibitors from reaching melanocytes.[10] Tyrosinase inhibitors with proven efficacy in skin systems include competitive inhibitors: aloesin,[11] arbutin,[11] azelaic acid,[12] deoxyarbutin,[13] hydroquinone,[10] and kojic acid,[14] as well as non-competitive inhibitors: ellagic acid,[15] glabridin,[16] haginin A,[17] and oxyresveratrol.[18] With exception of deoxyarbutin and glabridin, these compounds suppress skin pigmentation at relatively high concentrations, from 1 to 20%. Topically applied UP302 exhibited significant inhibition of skin pigmentation at concentration as low as 0.05%. In comparison, two of the most potent depigmenting agents, deoxyarbutin[13] and glabridin[16] suppressed skin pigmentation at concentrations of 0.3% and 0.5%, respectively. UP302 inhibits mushroom tyrosinase with nanomolar potency, and murine tyrosinase with low micromolar potency. Among the above mentioned tyrosinase inhibitors, only deoxyarbutin (IC_{50} for murine tyrosinase in the range of 5—10 μM) and oxyresveratrol (K_i for mushroom tyrosinase equal to 0.43 μM, and IC_{50} for murine tyrosinase equal to 52.7 μM) exhibited comparable efficiencies.

UP302 may have a better safety profile than commonly used depigmenting compounds, hydroquinone and kojic acid. Hydroquinone can induce contact dermatitis and permanent skin depigmentation.[12] Because of its adverse effects hydroquinone has been banned in Europe and Asia and a ban is under consideration in the U.S.A.[10] Kojic acid can also induce contact dermatitis[19] and was shown to be mutagenic both in an Ames assay and in Chinese hamster ovary cells.[20,21] UP302 has been isolated from *Dianella ensifolia* (Liliacea), a plant used in traditional Chinese medicine for topical applications.[22] Our data obtained on cultured melanocytes and reconstructed skin model demonstrated that UP302 does not alter cell viability, melanocyte morphology, and skin histology. In addition, UP302 was found negative in Ames mutagenesis test (data not shown).

Although the exact mechanism of action for UP302 is not clear, comparison of compounds **2** and **3** (Fig. 1) indicates that the presence of the resorcinol ring is significant for tyrosinase inhibitory activity. The involvement of the resorcinol ring is also suggested by the results reported for the related compound, 4-*n*-butylresorcinol.[4] UP302 and 4-*n*-butylresorcinol share the same pronyl resorcinol moiety, but 4-*n*-butylresorcinol lacks the second aryl ring. By comparing published data on 4-*n*-butylresorcinol with our experimental results on UP302, both compounds appear to inhibit murine tyrosinase with similar potencies *in vitro*. However, 4-*n*-butylresorcinol seems to be several fold less potent than UP302 on intact murine melanocytes.[4] As such, it is conceivable that resorcinol moiety might be responsible for the inhibitor-enzyme interactions, whereas the second aryl ring of UP302 may facilitate the inhibitory effects in cell-based systems. Direct side-by-side comparison of these compounds would be required to confirm this hypothesis.

In summary, UP302 is highly potent and safe natural skin whitening agent with potential use for cosmetic or clinical applications.

Experimental

Reagents Mushroom tyrosinase isolated from *Agaricus bisporus* 50000 U (Cat# T3824-50KU), Kojic Acid (Cat# 3125-5G), and 3,4-dihydroxyphenilalanine (L-DOPA) (Cat# 37830), were purchased from Sigma. Alpha-MSH (Cat# H-1075.0005) was purchased from Peninsula Laboratories (Bachem). Low protein binding filters (Tuffryn), 0.2 μm pore size were purchased from PALL Life Sciences (Cat# PN4454). CellTiter96 Aqueous One Solution (Cat# G3581) was purchased from Promega.

Discovery and Development of UP302 A total of 1144 plant extracts were screened for their ability to inhibit mushroom tyrosinase. The primary screen identified 20 extracts with >80% tyrosinase inhibition threshold. UP302 was isolated from the methanol extract of *Dianella ensifolia* (Liliacea) by a series of assay-guided chromatography procedures. The molecular structure of UP302 was established by the elucidation of MS and NMR (^1H, ^{13}C, HMQC and HMBC) spectra. As it occurs in nature, UP302 is about 0.02% in *Dianella ensifolia* and is quite difficult and costly to purify from the plant. UP302 used in this study was produced synthetically. The purities of both isolated and synthetic UP302 samples were analyzed by HPLC method with chromatogram purity higher than 98%.

Tissue Culture B16-F1 cells were purchased from ATCC (American Type Culture Collection), ATCC # CRL-63223. B16-F1 were grown in phenol-red free DMEM/F12 (Gibco 11039) supplemented with 10% heat-inactivated fetal calf serum (Gibco) and 50 units/ml penicillin and 50 μg/ml of streptomycin at 37 °C in a humidified atmosphere of 95% air and 5% CO_2.

Moderately pigmented neonatal human epidermal melanocyte cells were obtained from Cascade Biologics, Portland Oregon. The cells were subcultured according the supplier's protocol. Briefly, the cells were grown in Medium 254, supplemented with 10% heat inactivated fetal calf serum (Gibco 26400-044), and human melanocyte growth supplement (Cascade Biologics, S-016-5) at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. Subculturing was done as recommended by Cascade Biologics every 2 to 3 d using Trypsin/EDTA (Cascade Biologics, R-001-100) and Trypsin neutralizer (Cascade Biologics, R-002-100) for detaching the cells.

Mushroom Tyrosinase Assay Both UP302 and Kojic acid were dissolved in DMSO at 400 mM. Different concentrations of 4× stock solutions were prepared in the Assay Buffer, 100 mM Na-Phosphate, pH 6.8. To assure solubility of UP302, the assay buffer was supplemented with 4% DMSO. Tyrosinase substrate (L-DOPA) was freshly prepared as 2× stock solutions in the assay buffer. 50000 U of mushroom tyrosinase was dissolved in 5 ml of the assay buffer (10000 U/ml), aliquoted and stored at −70 °C. For experiments the enzyme was freshly diluted in the assay buffer to 400 U/ml (4× stock solution 100 μl of 2× substrate were pre-mixed with 50 μl of 4× test compounds in flat-bottom 96 well plates. Assays were performed using TECAN Genius Pro plate reader equipped with automatic injectors. Reactions were initiated by injecting 50 μl of the enzyme (20 U/reaction) and sample absorption was monitored at 492 nm, at 20 s intervals. As the reaction was linear for at least 60 s, absorbance values taken at 40 s were used for further calculations. K_i values were calculated from shared non-linear regression fits of substrate–velocity curves taken at different concentrations of the inhibitors. The data was analyzed using GraphPad Prism software.

For reversibility studies mushroom tyrosinase was diluted in the assay buffer at 6666 U/ml and mixed with equal volumes of the tyrosinase assay buffer containing either 20 μM UP302 and 10% DMSO (inhibited enzyme), or 10% DMSO without UP302 (uninhibited enzyme). Sixty microliters of the inhibited enzyme was sequentially passed through a series of four Micro Bio-Spin™ P-6 de-salting columns (Bio-Rad) equilibrated with tyrosinase assay buffer containing either 10 μM UP302 and 5% DMSO, or 5% DMSO without UP302 (restored enzyme). For a positive control, 60 μl of the uninhibited enzyme was passed through columns equilibrated with tyrosinase assay buffer containing 5% DMSO but no UP302.

DOPA Oxidase Assay in B16 Cell Homogenates B16-F1 cells were grown for two passages (4 d) in the presence of 250 μM of dibutyryl-cAMP. Nine confluent T-75 flasks (approximately 10^8 cells) were harvested into Versene solution. The cells were washed twice with PBS and twice with tyrosinase assay buffer (100 mM Na-Phosphate, pH 6.8). The cells were re-suspended in 10 ml of the tyrosinase assay buffer, and lysed by sonication (3 times×10 s×20 watts). The lysates were aliquoted, and stored at −70 °C.

DOPA oxidase assays were performed in 96-well plates. Fifty microliters of lysates were mixed with 50 μl of tyrosinase assay buffer and 50 μl of 4× test compounds (formulated in the assay buffer containing 40% DMSO). Reactions were initiated by injecting 50 μl of 4 mM L-DOPA, incubated for 6.6 h at 37 °C and sample absorptions were taken at 490 nm.

Melanin Production by Intact Murine Melanoma Cells For cell-based experiments 6 μl of 400 mM UP302 in DMSO was mixed with 1.2 ml of cell growth media containing 2 nM alpha-MSH, giving 2 mM UP302 in 0.5% DMSO (2× stock solution). This solution was spun for 1 h at 20000 ***g***. The supernatant was collected and filter-sterilized through 0.2 μm Tuffryn filters. This formulation was further serially diluted with sterile cell growth media containing 2 nM alpha-MSH and 0.5% DMSO.

B16-F1 cells were seeded at a density of 40000 cells/well in a 100 μl medium in 96-well microtest tissue culture plates (Falcon 353072). After 24 h, 100 μl of growth media containing 2× test compounds, 2 nM alpha-MSH and 0.5% DMSO was added to the wells in quadruplicate. At the time of absorbance reading (typically, 72 h) 200 μl of supernatants was removed from the cell plates and transferred to another flat-bottom plate. The cells were immediately replenished with 100 μl of fresh media for cell viability measurement. The absorbance of the 200 μl of cell supernatants was measured at 490 nm using a Victor 2 plate reader.

Cell viability was measured using CellTiter 96 Aqueous One Reagent (Promega). Twenty microliters of the reagent was added to the wells containing 100 μl of fresh media. These plates were placed in the CO_2 incubator for 1—1.5 h and then absorbance was read at 490 nm wavelength.

Melanin Production by Intact Human Epidermal Melanocytes UP302 was formulated as outlined for Murine Melanoma Assays (see above), except that 2× compounds were diluted in the complete medium 254 instead of DMEM, and 5 ml of the 2× compound were used for each assay point. Human epidermal melanocytes were seed in T-25 flasks at 1.2×10^6 cells/flask in 5 ml of growth medium. After 24 h 5 ml of 2× test compounds prepared in growth medium were added to the cells. The cells were harvested after 72 h.

Intracellular melanin was measured by a method modified from Siegrist and Eberle.[7] Briefly, each T-25 flask was rinsed with 2 ml of Versene solution and cells were detached in 2 ml of trypsin/EDTA. The cells were harvested by centrifugation, cell pellets were resuspended in 1.0 ml of H_2O, and lysed by two cycles of freezing and thawing. The cells were centrifuged at 20000 ***g*** for 5 min, the pellets were washed three times with 1 ml of 5% trichloroacetic acid, twice with a cold mixture of ethanol/ethyl ether (3 : 1), once with cold ethyl ether, and air-dried. Dry pellets were resuspended in 200 μl of 0.85 N KOH, and dissolved for 10 min at 100 °C water bath for 10 min. The dissolved material (200 μl) was transferred to the clear-bottom 96 well plates and absorbance was read at 490 nm.

To determine cell viability, in parallel experiments the cells were grown in 96-well microtest tissue culture plates. Viability was measured as described for Murine Melanoma Assays.

Reconstructed Skin Model The effects of UP302 and Kojic acid on the *in vitro* skin model (MEL-300-B) were studied at MatTek Corp. (Ashland, MA, U.S.A.) according to manufacturer's specifications. Briefly, normal human epidermal keratinocytes and normal human melanocytes derived from dark skin donors were co-cultured on a surface of collagen-coated membrane to generate several cell layers. The apical surfaces of the reconstructed skin (9 mm in diameter) were then exposed to air whereas the bottom surfaces remained in contact with 5 ml of maintenance medium. The following test compounds were applied to the apical surface of the tissues: 10 μl of 0.05% or 10 μl 0.1% UP302 in 80% Propylene Glycol, 10 μl of 80% propylene glycol, 25 μl of 1% Kojic acid in H_2O, or 25 μl of H_2O (negative control). The tissues were maintained for up to 14 d. At indicated time points the samples were fixed in 10% formalin and photographed simultaneously at the end of the experiment. Afterwards the tissues were processed, cut and stained for histological analysis. Duplicate samples were also frozen for melanin analysis (day 14). Melanin was measured as described by Bessou-Touya *et al*.[23] Cell viability was determined by standard tetrazolim reduction assay.

Acknowledgements We wish to thank Dr. Raymond Boissy (University of Cincinnati) and Lidia Brownell (Unigen Pharmaceuticals) for their helpful discussions about these studies. Our special thanks to Linnea Butler for expert technical assistance.

References

1) Nordlund J. J., "The Pigmentary System: Physiology and Pathophysiology," Oxford University Press, New York, 1998.
2) Giebel L. B., Strunk K. M., King R. A., Hanifin J. M., Spritz R. A., *Proc. Natl. Acad. Sci. U.S.A.*, **87**, 3255—3258 (1990).
3) Briganti S., Camera E., Picardo M., *Pigment Cell Res.*, **16**, 101—110 (2003).
4) Kim D. S., Kim S. Y., Park S. H., Choi Y. G., Kwon S. B., Kim M. K., Na J. I., Youn S. W., Park K. C., *Biol. Pharm. Bull.*, **28**, 2216—2219 (2005).
5) Parvez S., Kang M., Chung H. S., Bae H., *Phytother. Res.*, **21**, 805—816 (2007).
6) Solano F., Briganti S., Picardo M., Ghanem G., *Pigment Cell Res.*, **19**, 550—571 (2006).
7) Siegrist W., Eberle A. N., *Anal. Biochem.*, **159**, 191—197 (1986).
8) Yoon T. J., Lei T. C., Yamaguchi Y., Batzer J., Wolber R., Hearing V. J., *Anal. Biochem.*, **318**, 260—269 (2003).
9) Kim Y. J., Uyama H., *Cell. Mol. Life Sci.*, **62**, 1707—1723 (2005).
10) Draelos Z. D., *Dermatol. Ther.*, **20**, 308—313 (2007).
11) Choi S., Lee S. K., Kim J. E., Chung M. H., Park Y. I., *Clin. Exp. Dermatol.*, **27**, 513—515 (2002).
12) Nikolaou V., Stratigos A. J., Katsambas A. D., *J. Cosmet. Dermatol.*, **5**, 303—308 (2006).
13) Boissy R. E., Visscher M., DeLong M. A., *Exp. Dermatol.*, **14**, 601—608 (2005).
14) Lim J. T., *Dermatol. Surg.*, **25**, 282—284 (1999).
15) Shimogaki H., Tanaka Y., Tamai H., Masuda M., *Int. J. Cosmet. Sci.*, **22**, 291—303 (2000).
16) Yokota T., Nishio H., Kubota Y., Mizoguchi M., *Pigment Cell Res.*, **11**, 355—361 (1998).
17) Kim J. H., Baek S. H., Kim D. H., Choi T. Y., Yoon T. J., Hwang J. S., Kim M. R., Kwon H. J., Lee C. H., *J. Invest. Dermatol.*, **128**, 1227—1235 (2008).
18) Lee K. T., Lee K. S., Jeong J. H., Jo B. K., Heo M. Y., Kim H. P., *J. Cosmet. Sci.*, **54**, 133—142 (2003).
19) Nakagawa M., Kawai K., *Contact Dermatitis*, **32**, 9—13 (1995).
20) Shibuya T., Murota T., Sakamoto K., Iwahara S., Ikeno M., *J. Toxicol. Sci.*, **7**, 255—262 (1982).
21) Wei C. I., Huang T. S., Fernando S. Y., Chung K. T., *Toxicol. Lett.*, **59**, 213—220 (1991).
22) "Dictionary of Traditional Chinese Medicine," 1st ed., Jiang Shu Medical School, 1977.
23) Bessou-Touya S., Picardo M., Maresca V., Surleve-Bazeille J. E., Pain C., Taieb A., *J. Invest. Dermatol.*, **111**, 1103—1108 (1998).