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Anti-inflammatory Properties of Yellow and Orange Pigments from *Monascus purpureus* NTU 568

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ABSTRACT: The *Monascus* species has been used in foods for thousands of years in China. In this study, 10 azaphilone pigments, including four yellow and six orange pigments, were isolated from the fermented rice and dioscorea of *Monascus purpureus* NTU 568. By employing lipopolysaccharide (LPS)-stimulated murine macrophage RAW 264.7 cells, we determined the inhibitory activities of these pigments on nitric oxide (NO) production. As a result, four orange pigments, monaphilols A−D, showed the highest activities (IC_{50} = 1.0−3.8 μM), compared with the other two orange pigments, monascorubrin (IC_{50} > 40 μM) and rubropunctatin (IC_{50} = 21.2 μM), and the four yellow pigments ankaflavin (IC_{50} = 21.8 μM), monascin (IC_{50} = 29.1 μM), monaphilone A (IC_{50} = 19.3 μM), and monaphilone B (IC_{50} = 22.6 μM). Using Western blot and ELISA kits, we found that treatments with 30 μM of the yellow pigments and 5 μM of the orange pigments could down-regulate the protein expression of inducible nitric oxide synthase (iNOS) and suppress the production of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6). We also used two animal experiments to evaluate the anti-inflammatory effects of these pigments. In a 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced ear edema model, eight of these pigments (0.5 mg/ear) could prevent ear edema against TPA administrations on the ears of BALB/c mice. In an LPS-injection mice model, several of these pigments (10 mg/kg) could inhibit the NO, TNF-α, IL-1β, and IL-6 levels in the plasma of BALB/c mice. As concluded from the *in vitro* and *in vivo* studies, six azaphilonoid pigments, namely, ankaflavin, monaphilone A, and monaphilols A−D, showed high potential to be developed into chemopreventive foods or drugs against inflammation-associated diseases.

KEYWORDS: *Monascus purpureus, azaphilonoid pigment, anti-inflammation, lipopolysaccharide, 12-O-tetradecanoylphorbol-13-acetate*

INTRODUCTION

Monascus species have been used as food additives in Asian countries for thousands of years. Recently, *Monascus*-fermented products, such as red mold rice (RMR) and red mold dioscorea (RMD), were reported to block or retard the development of some diseases. For example, they could decrease cholesterol level and thus lower cardiovascular diseases,[1] reduce injuries from diabetes,[2,3] inhibit carcinogenesis[4] or tumor progression,[5] and improve the learning ability in patients with Alzheimer's disease.[6,7] Similarly, much evidence has also revealed that chronic inflammation is the key event for cardiovascular diseases,[8] diabetes,[9,10] cancers,[11,12] and Alzheimer's disease.[13,14] These coincidences highlight that the chemoprotective functions of RMR and RMD possibly result from their anti-inflammatory properties.

The anti-inflammatory functions of *Monascus* sp. are contributed by their azaphilonoid pigments. In 1994, azaphilonoid pigments from *M. anka* were demonstrated to inhibit 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced ear edema in mice.[15] In 2005, *Monascus* pigments from *M. pilosus* were also tested on a TPA-induced ear edema model.[16] Compared with non-azaphilonoid pigments, the inhibitory effects of azaphilonoid pigments on TPA-induced ear edema were much stronger. In total, five azaphilonoid pigments, including two yellow, two orange, and one red pigment, were proven to exhibit anti-inflammatory activities. Recently, a variety of new azaphilonoid pigments, named monasnicotinates A−D, were isolated from *M. pilosus* BCRC 38093 and showed inhibitory effects against nitric oxide (NO) production in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells.[17]

In our research group, we found that ethanol extracts of rice or dioscorea fermented from *M. purpureus* NTU 568 could down-regulate the productions of NO, PEG2, and pro-inflammatory cytokines in a 7′,12′-dimethylbenz[*a*]anthracene (DMBA)-stimulation hamster model.[18,19] From the fermented products of *M. purpureus* NTU 568, we have identified a series of new azaphilonoid pigments with inhibitory effects against nitric oxide production in LPS-stimulated RAW 264.7 cells.[20−23] Here, we report the structures and anti-inflammatory activities of 10 azaphilonoid pigments isolated from *M. purpureus* NTU 568, including four yellow and six orange pigments. For these purposes, we obtained these pigments on a large scale and evaluated their anti-inflammatory properties for *in vitro* and *in vivo* experiments.

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MATERIALS AND METHODS

General Experimental Procedures. Nuclear magnetic resonance (NMR) spectra were generated on a Bruker NMR unit (Unity Plus 400 MHz; Bruker BioSpin, Rheinstetten, Germany) using d_4-methanol as the solvent. High-performance liquid chromatography (HPLC) separations were performed on a Shimadzu LC-6AD series apparatus with an SPD-6AV UV detector, equipped with a 250 × 20 mm i.d. preparative Cosmosil AR-II column (Nacalai Tesque, Inc., Kyoto, Japan).

Materials. Methanol and acetonitrile (HPLC grade) and acetone and methanol (analytical grade) were purchased from ECHO (Miaoli, Taiwan). Fetal bovine serum (FBS), Dulbecco's minimum essential medium (DMEM), and phosphate-buffered saline (PBS) were purchased from Biological Industries (Kibbutz Beit-Haemek, North District, Israel). Other chemicals such as LPS (from *Escherichia coli* O55:B5), TPA, and dimethyl sulfoxide were obtained from Sigma (St. Louis, MO, USA). The powders of RMR and RMD were generously provided by SunWay Biotech Co., Ltd. (Taipei, Taiwan). The QC quality of commercial RMR and RMD provided by SunWay Biotech Co. was described as follows: monascin, >3 mg/g; ankaflavin, >1.1 mg/g; citrinin, <2 ppm; total plate count, $<5 \times 10^4$ CFU/g; coliform, $<10^3$ CFU/g; *E. coli*, negative; mold and yeast, <100 CFU/g; *Staphylococcus*, negative; *Salmonella*, negative; moisture, <10%.

Extraction of *Monascus* Pigments. The procedures for the extraction and isolation of *Monascus* pigments of RMR and RMD were adapted and modified from the methods published by our group.[20,23] Briefly described, RMR (5 kg) and RMD (30 kg) were extracted respectively with methanol (50 L, 50 °C) and acetone (150 L, 40 °C), three times each. The methanol extracts of RMR and the acetone extracts of RMD were further chromatographed on several types of columns, including silica gel (Kieselgel 60), Sephadex LH-20, and HPLC. All of the pure substances were purified twice by HPLC at the final step, and the purities of compounds (>98%) were checked by photodiode array detector. Four yellow pigments, namely, ankaflavin (AK), monascin (MS), and monaphilones A and B (MA and MB), were harvested from RMR, and six orange pigments, namely, rubropunctatin (RPT), monascorubrin (MCB), and monaphilols A–D (MPA–MPD), were harvested from RMD. The structures of these 10 azaphilonoid pigments are shown in Figure 1. The structural elucidations of these compounds were determined by NMR, mass, infrared, and ultraviolet spectroscopies.

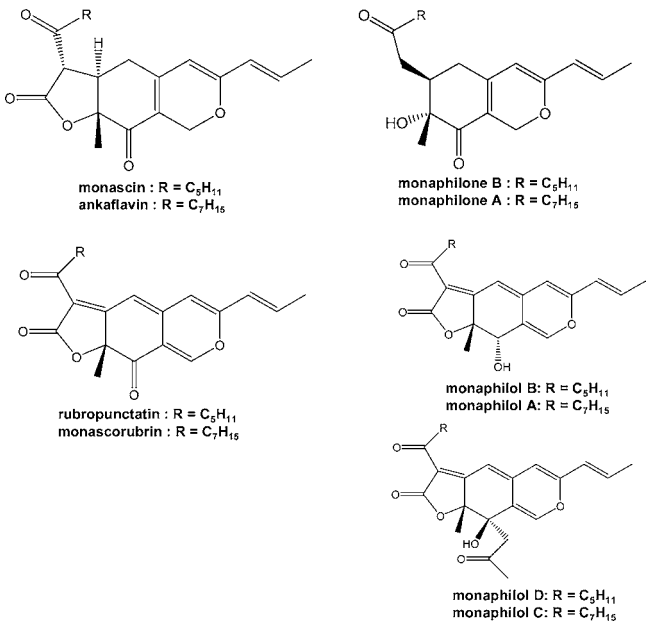

Figure 1. Structures of azaphilonoid pigments.

Cell Lines and Culture. The murine macrophage cell line RAW 264.7 was obtained from Bioresource Collection and Research Center (Hsinchu, Taiwan). The cells were cultured in DMEM (5% FBS) in a humidified incubator with 5% CO_2 at 37 °C. DMSO was used to prepare stock solutions (20 000 μg/mL) of the pigments and then diluted to appropriate concentrations in the cell model.

Assay of Nitrite Production. RAW 264.7 cells (2×10^5 per well) were seeded and maintained with 500 μL of DMEM in 24-well plates at 37 °C. After 24 h, the cells were cotreated with LPS (1 μg/mL) and test agents (0.625, 2.5, 10, 20, and 40 μM) dissolved in DMEM. After 24 h of incubation, determination of the nitrite levels in the supernatants was performed using a Griess reagent kit (Promega, Madison, WI, USA), as adapted from reported methods.[24]

Western Blot Analysis. The procedures were modified from published literature.[25] Cells (about 8×10^6 RAW 264.7) were seeded with 10 mL of medium in a 75 cm^2 flask. After 12 h, the medium was replaced with 10 mL of test agents (yellow pigments, 30 μM; orange pigments, 5 μM) dissolved in fresh medium. After 24 h of incubation, the cells were harvested and extracted with RIPA lysis buffer (Millipore, Bellerica, MA, USA) containing 1% protease inhibitor (Sigma, St. Louis, MO, USA). The cell lysates were further separated by polyacrylamide gel electrophoresis (10% sodium dodecyl sulfate) and analyzed with primary antibodies, including β-actin murine monoclonal antibody (Epitomics, Burlingame, CA, USA) and inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX-2) murine polyclonal antibody (Cayman Chemical, Ann Arbor, MI, USA). A goat anti-mouse secondary IgG (Jackson ImmunoResearch, West Grove, PA, USA) was further added. Finally, the detection was performed using the Western Lightning chemiluminescence reagent (PerkinElmer Life Sciences, Waltham, MA, USA).

Cytokine Assay. RAW 264.7 cells (2×10^5 per well) were seeded and maintained with 500 μL of DMEM in 24-well plates at 37 °C. After 12 h, cells were treated with LPS (1 μg/mL) and test agents (yellow pigments, 30 μM; orange pigments, 5 μM) dissolved in 10 mL of DMEM. After 24 h of incubation, the cells were harvested and tested by using a colorimetric assay kit (eBioscience, San Diego, CA, USA). Cytokines, including interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α), were determined according to the manufacturer's protocols.

Animal Experiments. The procedures were modified from published literature.[16,26] Male Balb/cByJNarl mice (8 weeks old, n = 3) were purchased from the National Animal Institute (Taipei, Taiwan). The animals were stimulated with inflammatory agents by two methods: LPS injection (2 mg/kg, intraperitoneal injection) or TPA induction (1 μg/ear, scribbled on the both sides of ear). Mice were grouped randomly for (a) vehicle control (PBS or acetone); (b) inflammatory agents (LPS or TPA); (c) sample (inflammatory agents combined with test agents); and (d) positive control (inflammatory agents combined with quercetin or indomethacin). Sample administration was performed at 0.5 h before LPS injection or TPA induction. In the TPA-induction model, we chose acetone as vehicle for drug administrations on the ears of mice because it was a fast evaporating and not a toxic solvent. In the LPS-injection model, mice were sacrificed at 12 h after LPS injection and further evaluated for the alteration of serum NO levels and pro-inflammatory cytokines. In the TPA-induced ear edema model, mice were sacrificed at 4 h after TPA administrations and further evaluated for the changes of ear biopsy weight.

Statistical Analysis. Data are presented as the mean ± standard deviation (n = 3). The statistical comparisons were performed by one-way analysis of variance (ANOVA) with Duncan's test. The significant differences ($p < 0.05$) were presented as different letters for each group.

RESULTS

Anti-NO Activities of Azaphilonoid Pigments from RMR and RMD. In this study, we performed large-scale preparations of four yellow pigments (from the fermented rice) and six orange pigments (from the fermented dioscorea) of *M.*

purpureus NTU 568. The 10 pigments were structural analogues, and all belonged to the azaphilone family. In Table 1, we show the anti-NO activities of these pigments. The

Table 1. Anti-NO Activity of Azaphilonoid Pigments on LPS-Stimulated RAW 264.7 Cells[a]

	IC_{50}	
compound	μg/mL	μM
ankaflavin	8.4 ± 0.3	21.8 ± 0.9
monascin	10.4 ± 0.9	29.1 ± 2.6
monaphilone A	7.1 ± 0.4	19.3 ± 1.1
monaphilone B	7.5 ± 0.3	22.6 ± 0.8
monascorubrin	>15	>40
rubropunctatin	7.5 ± 0.7	21.2 ± 1.9
monaphilol A	0.4 ± 0.0	1.0 ± 0.3
monaphilol B	1.3 ± 0.1	3.8 ± 0.1
monaphilol C	1.2 ± 0.1	2.8 ± 0.2
monaphilol D	0.7 ± 0.0	1.7 ± 0.1

[a]Quercetin (IC_{50} = 13.2 ± 1.2 μM) was used as the positive control.

four yellow pigments, AK (IC_{50} = 21.8 μM), MS (IC_{50} = 29.1 μM), MA (IC_{50} = 19.3 μM), and MB (IC_{50} = 22.6 μM), and one orange pigment, RPT (IC_{50} = 21.2 μM), showed moderate anti-NO activities. The four orange pigments, MPA, MPB, MPC, and MPD, showed high anti-NO activities (IC_{50} = 1.0–3.8 μM). However, MCB showed low anti-NO activity (IC_{50} > 40 μM). To a certain extent, the anti-NO activities of the orange pigments were stronger than those of the yellow pigments. Thus, we decided to use different concentrations (30 μM for yellow pigments; 5 μM for orange pigments) to explore the correlations between the anti-NO activities and other anti-inflammatory parameters. In Figure 2, the anti-NO activities of the yellow and orange pigments had been screened in separate groups. Comparing among the yellow pigments, AK, MA, MS, and MB possessed significantly ($p < 0.05$) higher anti-NO activities than the LPS group. As for the orange pigments, MPA, MPB, MPC, MPD, MCB, and RPT possessed significantly ($p < 0.05$) higher anti-NO activities than the LPS group.

Inhibition of iNOS/COX-2 Expression by Azaphilonoid Pigments. Using Western blotting, the anti-inflammatory effects of the azaphilonoid pigments were evaluated on the protein level, namely, the expression of iNOS and COX-2. As shown in Figure 3, all of the yellow pigments could suppress LPS-induced iNOS expression significantly ($p < 0.05$), but only three of them (MS, MA, and MB) could down-regulate LPS-induced COX-2 expression significantly ($p < 0.05$). Comparing among the yellow pigments, MA and MB suppressed more iNOS expression (significant difference, $p < 0.05$) than AK and MS. As shown in Figure 4, all of the orange pigments could suppress LPS-induced iNOS expression significantly ($p < 0.05$), but none of them could down-regulate LPS-induced COX-2 expression. Comparing among the orange pigments, MPD suppressed more iNOS expression (significant difference, $p < 0.05$) than MPA, MPB, MPC, MCB, and RPT. The results suggested that the azaphilonoid pigments worked like iNOS inhibitors, but not COX-2 inhibitors. Our data also revealed the correlations between the inhibition on iNOS expression and NO production.

Inhibition of Inflammatory Cytokine Production by Azaphilonoid Pigments. Using ELISA kits, the anti-inflammatory properties of the azaphilonoid pigments were

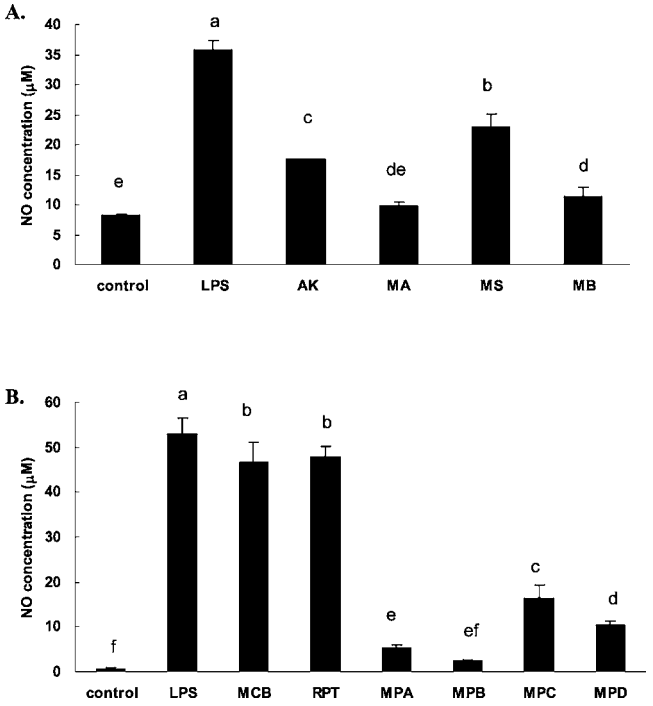

Figure 2. Azaphilonoid pigments suppressed lipopolysaccharide (LPS)-induced NO production in RAW 264.7 cells. (A) Cells were treated with LPS (1 μg/mL) or in combination with yellow pigments (30 μM) for 24 h. (B) Cells were treated with LPS (1 μg/mL) or in combination with tested agents (5 μM) for 24 h. Different letters indicate statistically significant differences between the means ($p < 0.05$) for each group. AK: ankaflavin; MA: monaphilone A; MS: monascin; MB: monaphilone B; MCB: monascorubrin; RPT: rubropunctatin; MPA: monaphilol A; MPB: monaphilol B; MPC: monaphilol C; MPD: monaphilol D.

explored on the cytokines TNF-α, IL-6, and IL-1β. As evident in Figure 5, most of the yellow pigments could suppress cytokine production significantly ($p < 0.05$) more than the LPS group, but MS could not down-regulate the IL-1β production significantly. Comparing among the yellow pigments, AK and MA suppressed more TNF-α production (significant difference, $p < 0.05$) than MS and MB. In the case of IL-6 production, MA suppressed more production (significant difference, $p < 0.05$) than AK, MS, and MB. With regard to IL-1β production, MA suppressed more production (significant difference, $p < 0.05$) than AK and MB. In Figure 6, most of the orange pigments are shown to suppress cytokine production significantly ($p < 0.05$) more than the LPS group, but MCB and RPT could not down-regulate IL-1β production significantly. Comparing among the orange pigments, MPA, MPB, MPC, and MPD suppressed more TNF-α production than MCB and RPT. In the case of IL-6 production, MPA, MPB, MPC, and MPD suppressed more production than MCB and RPT. With regard to IL-1β production, MPD suppressed more production than MPA, MPB, and MPC. The findings implied that the yellow and orange pigments were suitable agents to reduce the production of inflammatory cytokines on LPS-stimulated RAW 264.7 cells. Therefore, our aims turned to animal experiments in the subsequent steps.

Inhibition of TPA-Induced Ear Edema by Azaphilonoid Pigments. Using a TPA-induced ear edema model, the anti-inflammatory effects of the azaphilonoid pigments were evaluated on mice. At a dosage of 0.5 mg/ear, AK, MA, MS,

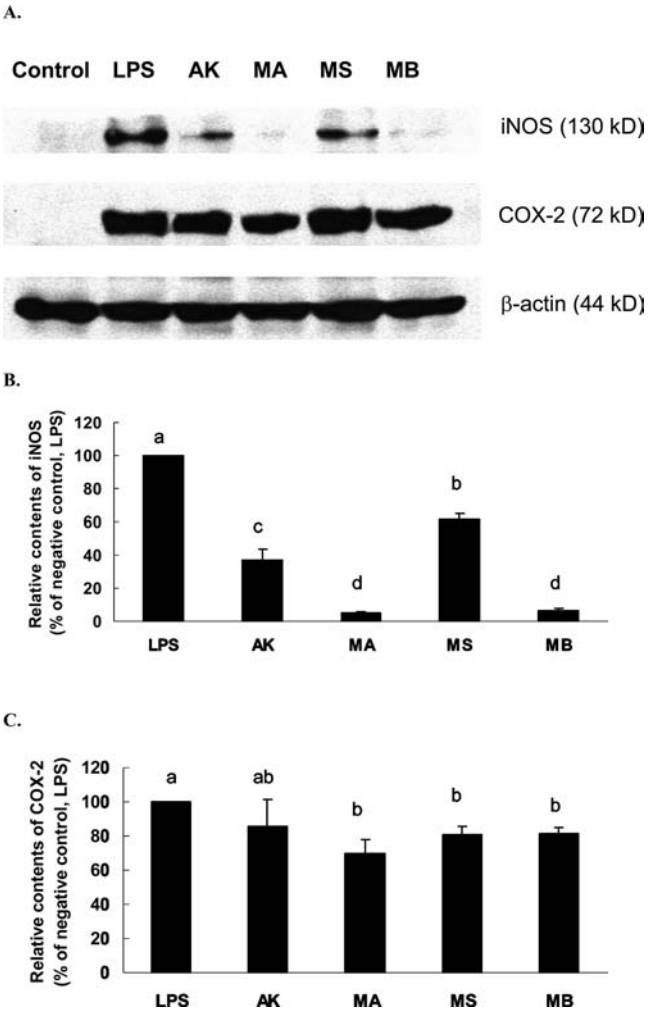

Figure 3. (A) Yellow pigments suppressed LPS-induced inflammatory enzyme expression in RAW 264.7 cells. Cells were treated with LPS (1 μg/mL) or in combination with tested agents (30 μM). After 24 h, cells were harvested and determined for iNOS and COX-2 expression. (B) Quantification of iNOS and (C) quantification of COX-2. Different letters indicate statistically significant differences between the means ($p < 0.05$) for each group. AK: ankaflavin; MA: monaphilone A; MS: monascin; MB: monaphilone B.

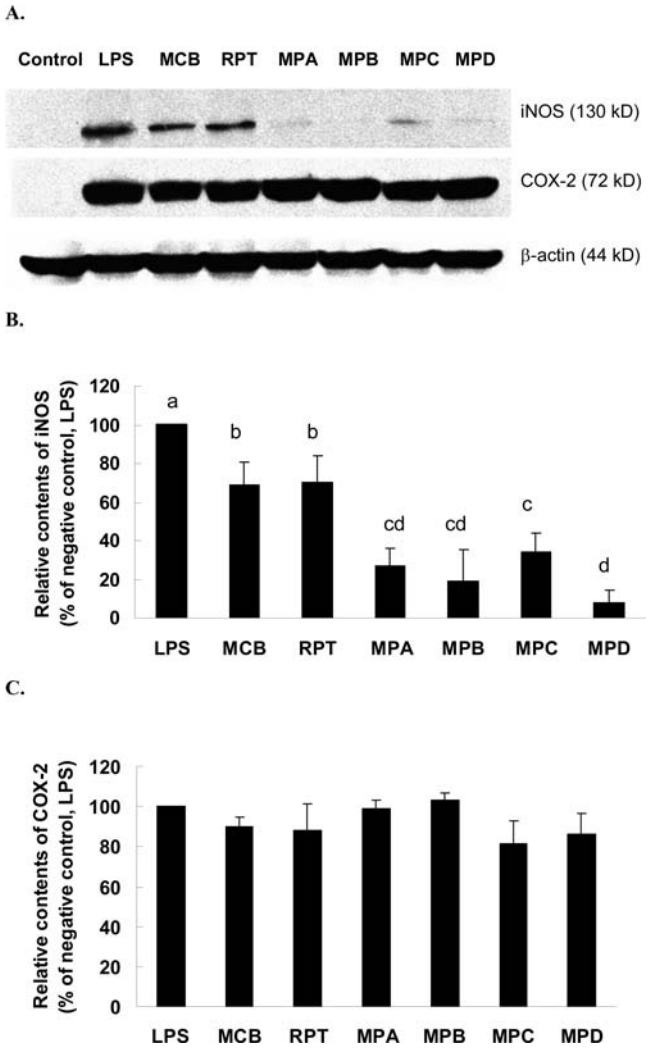

Figure 4. (A) Orange pigments suppressed LPS-induced inflammatory enzyme expression in RAW 264.7 cells. Cells were treated with LPS (1 μg/mL) or in combination with tested agents (5 μM). After 24 h, cells were harvested and determined for iNOS and COX-2 expression. Different letters indicate statistically significant differences between the means ($p < 0.05$) for each group. MCB: monascorubrin; RPT: rubropunctatin; MPA: monaphilol A; MPB: monaphilol B; MPC: monaphilol C; MPD: monaphilol D.

MB, MPA, MPB, MPC, MPD, and positive control indomethacin inhibited ear edema significantly ($p < 0.05$) compared with the TPA group, but MCB and RPT did not (Figure 7). The results indicated that all four yellow pigments and four orange pigments (MPA, MPB, MPC, and MPD) were excellent chemoprotective agents against inflammatory responses that occurred on the body surface.

Inhibition of LPS-Induced Inflammation by Azaphilonoid Pigments. Using LPS-injected mice, the anti-inflammatory potentials of azaphilonoid pigments were evaluated according to the NO and cytokines (TNF-α, IL-6, and IL-1β) found in the blood. At a dosage of 10 mg/kg, AK, MA, MS, MB, MPA, MPB, MPC, and MPD inhibited serum NO levels significantly ($p < 0.05$) compared with the LPS group, but MCB and RPT did not (Figure 8). However, the inhibition of cytokines by the azaphilonoid pigments was more complicated (Figure 9). All four yellow pigments and five orange pigments (RPT, MPA, MPB, MPC, and MPD) could reduce TNF-α production significantly ($p < 0.05$), but MCB could not. In the case of IL-6, all the azaphilonoid pigments showed significant inhibitory abilities ($p < 0.05$). With regard to IL-1β, only six of the azaphilonoid pigments (AK, MA, MPA, MPB, MPC, and MPD) showed inhibitory abilities (significant difference, $p < 0.05$). Generally speaking, two yellow pigments (AK and MA) and four orange pigments (MPA, MPB, MPC, and MPD) were the most effective chemoprotective agents against the inflammatory responses in the blood.

DISCUSSION

RMR and RMD fermented by *M. purpureus* NTU 568 are recognized to contain various azaphilonoid pigments, which are associated with antitumor or anti-inflammatory properties.[20−23] In this study, we prepared 10 azaphilonoid pigments from RMR and RMD. With the help of *in vitro* and *in vivo* experiments, we further investigated the structure−activity relationship (SAR) for the anti-inflammatory effects by these azaphilonoid pigments. In the case of the yellow pigments, alterations of

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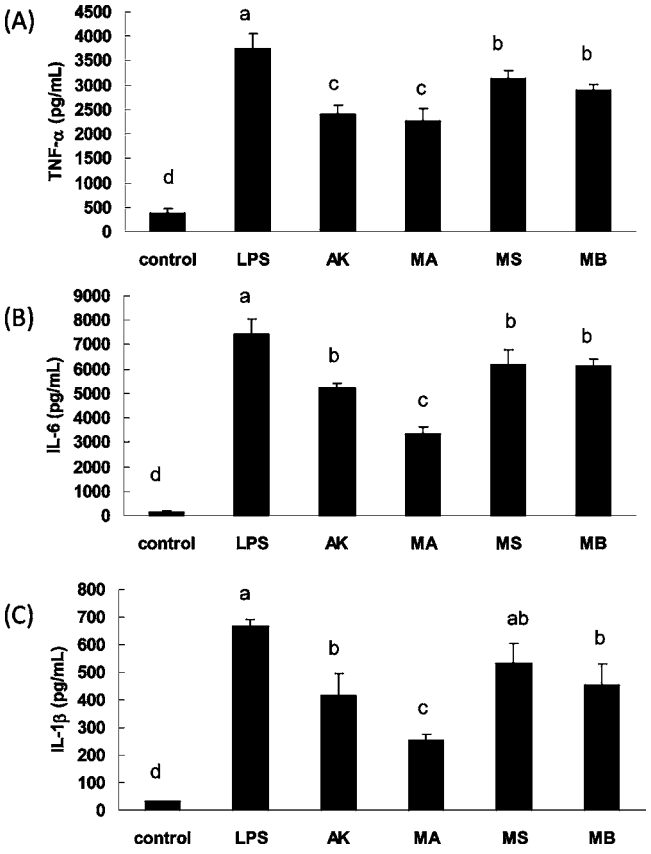

Figure 5. Yellow pigments suppressed LPS-induced inflammatory cytokines in RAW 264.7 cells. Cells were treated with LPS (1 μg/mL) or in combination with tested agents (30 μM). After 24 h, cells were harvested and determined for (A) TNF-α, (B) IL-6, and (C) IL-1β. Different letters indicate statistically significant differences between the means ($p < 0.05$) for each group. AK: ankaflavin; MA: monaphilone A; MS: monascin; MB: monaphilone B.

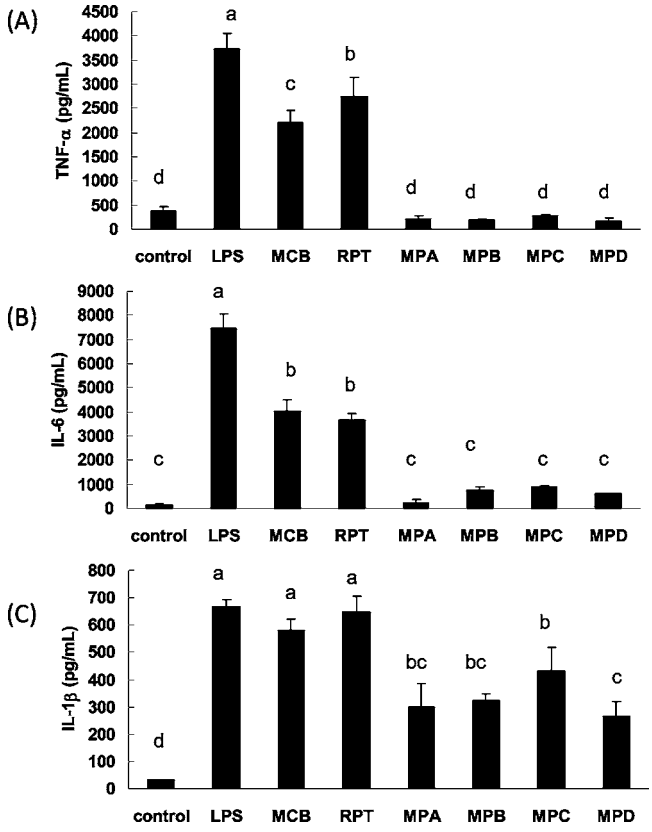

Figure 6. Orange pigments suppressed LPS-induced inflammatory cytokines in RAW 264.7 cells. Cells were treated with LPS (1 μg/mL) or in combination with tested agents (5 μM). After 24 h, cells were harvested and determined for (A) TNF-α, (B) IL-6, and (C) IL-1β. Different letters indicate statistically significant differences between the means ($p < 0.05$) for each group. MCB: monascorubrin; RPT: rubropunctatin; MPA: monaphilol A; MPB: monaphilol B; MPC: monaphilol C; MPD: monaphilol D.

the alkyl side chain and the γ-lactone ring could affect the anti-inflammatory activities slightly. For example, AK and MA (R = C_7H_{15}) possessed higher anti-inflammatory activities than MS and MB (R = C_5H_{11}). Moreover, MA (lacking the γ-lactone ring) possessed higher anti-inflammatory activity than AK. However, the alterations that occurred on C-8 affected the anti-inflammatory activities more obviously in the case of the orange pigments. For example, MPA, MPB, MPC, and MPD (a hydroxyl group on C-8) possessed much higher anti-inflammatory activities than MCB and RPT (a ketone group on C-8). From the SAR studies, we successfully identified key structures that could enhance the anti-inflammatory activities of azaphilonoid pigments.

In our *in vitro* designs, we selected LPS as a chemical challenge to stimulate RAW 264.7 cells. Upon analyzing the iNOS/COX-2 expression, we found that two yellow pigments (AK and MA) and four orange pigments (MPA, MPB, MPC, and MPD) were effective chemoprotective agents against LPS-induced inflammation. According to the findings, we suggested that the down-regulation of excess iNOS protein expression was likely to be a common event exerted by azaphilonoid pigments. The findings were also similar to our published literature, which claims that AK and MA could inhibit the expression of iNOS, but not COX-2.[22]

In our *in vivo* designs, two yellow pigments (AK and MA) and four orange pigments (MPA, MPB, MPC, and MPD) showed good anti-inflammatory effects on TPA- or LPS-stimulated mice. The six azaphilonoid pigments were all

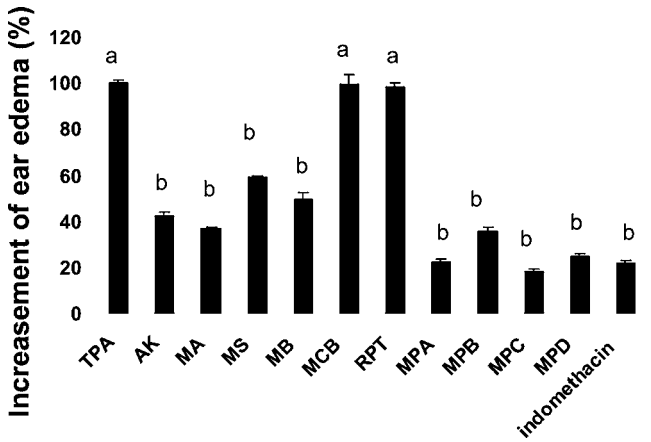

Figure 7. Inhibitory effect of *Monascus*-fermented pigments on 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced ear edema. The samples (0.5 mg/ear) were topically applied about 30 min before the TPA treatment. Different letters indicate statistically significant differences between the means ($p < 0.05$) for each group. AK: ankaflavin; MA: monaphilone A; MS: monascin; MB: monaphilone B; MCB: monascorubrin; RPT: rubropunctatin; MPA: monaphilol A; MPB: monaphilol B; MPC: monaphilol C; MPD: monaphilol D.

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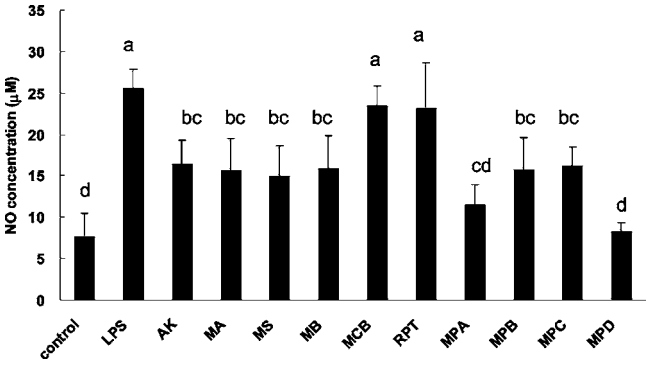

Figure 8. *Monascus*-fermented pigments suppressed plasma NO levels in LPS-injected mice. The compounds (10 mg/kg) were injected 30 min before the LPS (2 mg/kg) treatment. Different letters indicate statistically significant differences between the means ($p < 0.05$) for each group. AK: ankaflavin; MA: monaphilone A; MS: monascin; MB: monaphilone B; MCB: monascorubrin; RPT: rubropunctatin; MPA: monaphilol A; MPB: monaphilol B; MPC: monaphilol C; MPD: monaphilol D.

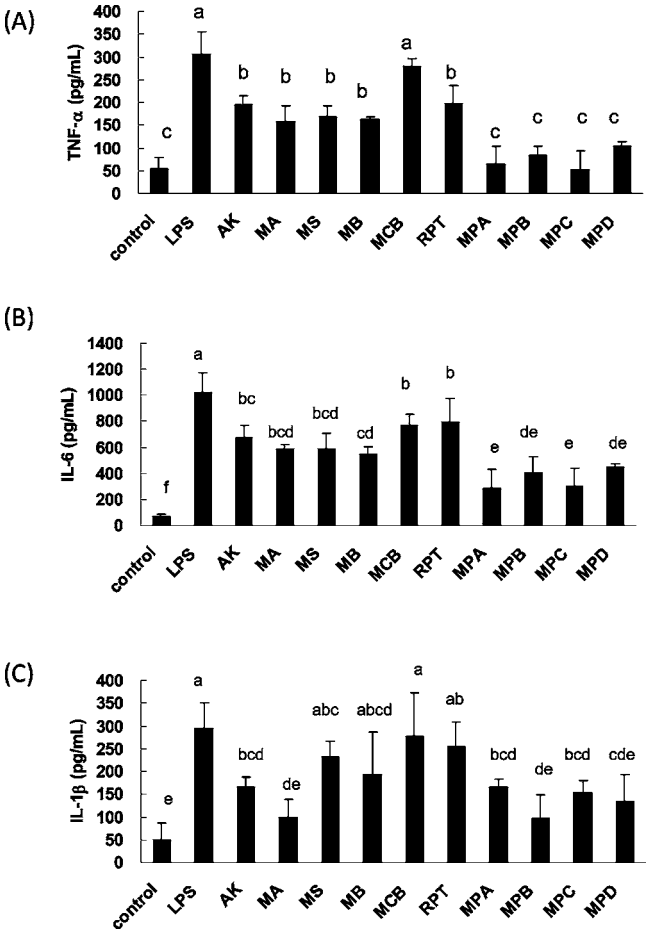

Figure 9. *Monascus*-fermented pigments suppressed the plasma inflammatory cytokine level in LPS-injected mice. The compounds (10 mg/kg) were injected 30 min before the LPS (2 mg/kg) treatment. Different letters indicate statistically significant differences between the means ($p < 0.05$) for each group. AK: ankaflavin; MA: monaphilone A; MS: monascin; MB: monaphilone B; MCB: monascorubrin; RPT: rubropunctatin; MPA: monaphilol A; MPB: monaphilol B; MPC: monaphilol C; MPD: monaphilol D.

effective inhibitors against ear edema, serum NO, and inflammatory cytokines. According to the findings, we suggest that these pigments are capable of preventing anti-inflammatory responses that occur outside or inside the body. The findings were also consistent with our published literature, which claims that RMR and RMD can reduce inflammatory cytokines on DMBA/TPA-stimulated mice[18,19] or STZ-induced diabetic rats.[2,3]

In this study, four orange pigments (MPA–MPD) were found to possess the highest anti-inflammatory activities in mice. In our previous studies,[20,23] however, the optimal yields of yellow pigments (from RMR) were almost 6-fold higher than those of orange pigments (from RMD). Briefly described, the RMR was found to contain 15.2 mg/kg of AK, 19.9 mg/kg of MS, 2.0 mg/kg of MA, and 3.0 mg/g of MB.[20] As for the RMD, it contained only 2.0–3.0 mg/kg of MCB or RPT and an even lesser amount of MPA–D (0.3–1.1 mg/kg).[23] We hope that the yields of MPA–D can be enhanced by some adjustments of the fermentation process. Otherwise, with respect to industrial yields from *M. purpureus* NTU 568, AK and MA are better choices for development into anti-inflammatory drugs or foods than any of the other orange pigments.

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Notes

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