Prenylflavones from *Psoralea corylifolia* Inhibit Nitric Oxide Synthase Expression through the Inhibition of I- κ B- α Degradation in Activated Microglial Cells

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The overproduction of nitric oxide (NO) by inducible nitric oxide synthase (iNOS) switches the function of NO from a physiological neuromodulator to a neurotoxic effector in central nervous system (CNS) after brain injury. From the methanol extracts of *Psoralea corylifolia*, we purified two inhibitors of NO production in lipopolysaccharide (LPS)-activated microglia by activity guided purification along with two inactive compounds. The active compounds were identified as a chromenoflavanone [7,8-dihydro-8-(4-hydroxyphenyl)-2,2-dimethyl-2H,6H-benzo-(1,2-b:5,4-b')dipyran-6-one] (1) and 4-hydroxyonchocarpin (2). And the inactive two compounds were identified as bavachinin (3) and bavachalcone (4) by spectral analysis. The compound 2 was isolated first time from this plant. Compounds 1 and 2 inhibited the production of NO in LPS-activated microglia in a dose dependent manner (IC_{50} 's were 11.4, 10.2 μ M, respectively). They also suppressed the expression of protein and mRNA of iNOS in LPS-activated microglial cells at 10 μ M as observed in Western blot analysis and RT-PCR experiment. Furthermore they inhibited the degradation of I- κ B- α in activated microglia. These results imply that compounds 1 and 2 can be lead compounds for the development of neuroprotective drug with the inhibitory activity of NO overproduction by activated microglial cells.

Key words prenylflavone; nitric oxide; microglia; *Psoralea corylifolia*

Microglia, the immune regulatory and inflammatory cells of the central nervous system (CNS), can be activated in response to a variety of neurodegenerative and neuroinflammatory conditions. Activation of microglia results in the production of proinflammatory materials such as tumor necrosis factor- α (TNF- α), interleukin-1 and 6, reactive oxygen species (ROS), and nitric oxide (NO) in response to injury.^{1–4)} Large amounts of NO produced by inducible nitric oxide synthase (iNOS) can rapidly react with superoxide anion radical ($\cdot O_2^-$) to produce peroxynitrite anion ($ONOO^-$).^{5,6)} These reactive nitrogen species might be involved in the pathogenesis of various neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and ischemia.⁷⁾ As a neuromodulator in CNS, NO participates in brain development, pain perception, memory, neuronal plasticity and behavior.⁸⁾ The excessive amounts of NO has been observed in damaged tissues associated with type I diabetes, arthritis, or nephritis,⁹⁾ suggesting that deregulation of iNOS expression is linked to many diseases. The expression and activity of iNOS are increased in chronic diseases, such as inflammation or cancer.¹⁰⁾ Generally the iNOS along with the release of NO by microglia contributes to progress neurodegeneration and aggravate diseases. Therefore, the inhibitors of NO production may have beneficial therapeutic effects in the treatment of diverse diseases.

In order to find new iNOS inhibitors from medicinal plants, we have screened inhibitory activity of NO production in lipopolysaccharide (LPS)-activated BV-2 mouse microglial cells. The methanol extract of *Psoralea corylifolia* L. (Fabaceae) showed remarkable inhibitory effects on NO production. The seeds of *P. corylifolia* have been used traditionally for the treatment of stomachic, deobstruent, anthelmintic, diuretic, vitiligo and certain skin diseases.^{11,12)} Several reports revealed that its seeds contain antimutagenic coumarins, antioxidants, antibacterial and antiplatelet flav-

onoids.^{13–16)} This article describes the identification of two prenylflavonoids from *Psoralea corylifolia* and their inhibitory activity of NO production in LPS-activated microglial cells.

MATERIALS AND METHODS

Plant Materials The dried seeds of *Psoralea corylifolia* were purchased from the Kyungdong oriental drug market in Seoul, Korea and authenticated by Prof. T. H. Kim at the College of Pharmacy, Sookmyung Women's University. A voucher specimen (No. SPH 04005) was deposited in the herbarium of the Sookmyung Women's University. The plant materials (540 g) were extracted three times with MeOH and the combined extracts (130 g) were successively partitioned with *n*-hexane, EtOAc and BuOH.

The EtOAc soluble fraction (53 g) was subjected to silica gel column chromatography eluting with an *n*-hexane–EtOAc gradient system (50:1–1:5) to afford active fractions A₁ (0.8 g) and A₂ (3.6 g). Repeated silica gel column chromatography of fraction A₁ with a gradient elution of *n*-hexane–EtOAc (30:1–1:1) to give an active sub-fraction A₁₋₁ (402 mg), which was further purified by preparative MPLC (RP-C18 column, 80% MeOH as eluent) to yield compounds 2 (22 mg) and 4 (44 mg). The chromatography of active fraction A₂ on silica gel with a gradient elution of CH₂Cl₂–EtOAc (70:1–1:1) to give an active sub-fraction A₂₋₂ (0.8 g), which was further purified by preparative MPLC (RP-C18 column, 80% MeOH as eluent) to yield compounds 1 (78 mg) and 3 (173 mg).

The purity of compounds 1–4 were evaluated by HPLC analysis (μ -Bondapak C18 column, 3.9 $\times$ 300 mm; 1.0 ml/min; UV 254 nm) eluting as a single peak for compounds 1 and 3 at 8 and 11 min (80% MeOH as eluent), for compounds 2 and 4 at 11 and 15 min (85% MeOH as eluent), re-

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spectively.

A Chromenoflavanone [7,8-Dihydro-8-(4-hydroxyphenyl)-2,2-dimethyl-2H,6H-benzo-(1,2-b:5,4-b')dipyran-6-one] (**1**): Yellow amorphous powder. $[\alpha]_D^{25} -1.9^\circ$ ($c=1.5$, CHCl_3). FAB-MS m/z : 323 $[\text{M}+\text{H}]^+$. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ : 1.44 (6H, s, H_3 -7'', 8''), 2.77 (1H, dd, $J=2.8$, 17.2 Hz, H_a -3), 3.04 (1H, dd, $J=13.2$, 17.2 Hz, H_b -3), 5.36 (1H, dd, $J=2.8$, 13.2 Hz, H-2), 5.61 (1H, d, $J=10.0$ Hz, H-5''), 6.32 (1H, d, $J=10.0$ Hz, H-4''), 6.37 (1H, s, H-8), 6.87 (2H, d, $J=8.4$ Hz, H-3', 5'), 7.32 (2H, d, $J=8.4$ Hz, H-2', 6'), 7.55 (1H, s, H-5). $^{13}\text{C-NMR}$ (100 MHz, CDCl_3) δ : 28.4 (q, C-7'', 8''), 43.9 (t, C-3), 77.9 (s, C-6''), 79.6 (d, C-2), 104.3 (d, C-8), 114.6 (s, C-10), 115.5 (d, C-3', 5'), 116.0 (s, C-6), 121.0 (d, C-4''), 124.6 (d, C-5), 127.8 (d, C-2', 6'), 129.6 (s, C-1'), 129.6 (d, C-5''), 157.2 (s, C-4'), 160.5 (s, C-9), 163.4 (s, C-7), 191.7 (s, C-4).

4-Hydroxylonchocarpin (**2**): Yellow amorphous powder. FAB-MS m/z : 323 $[\text{M}+\text{H}]^+$. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ : 1.47 (6H, s, H_3 -7'', 8''), 5.59 (1H, d, $J=10.4$ Hz, H-5''), 6.38 (1H, d, $J=8.8$ Hz, H-5), 6.75 (1H, d, $J=10.4$ Hz, H-4''), 6.88 (2H, d, $J=8.4$ Hz, H-3', 5'), 7.43 (1H, d, $J=15.2$ Hz, H- β), 7.56 (2H, d, $J=8.4$ Hz, H-2', 6'), 7.71 (1H, d, $J=8.8$ Hz, H-6), 7.83 (1H, d, $J=15.2$ Hz, H- α). $^{13}\text{C-NMR}$ (100 MHz, CDCl_3) δ : 28.4 (q, C-7'', 8''), 77.8 (s, C-6''), 108.3 (d, C-5), 109.4 (s, C-1), 114.1 (s, C-3), 115.9 (d, C-4''), 116.0 (d, C-3', 5'), 117.9 (d, C- β), 127.8 (s, C-1'), 128.1 (d, C-5''), 130.6 (d, C-6, 2', 6'), 144.1 (d, C- α), 158.0 (s, C-4'), 159.7 (s, C-2), 160.9 (s, C-4), 192.1 (s, C=O).

Bavachinin (**3**): Yellow amorphous powder. $[\alpha]_D^{25} -19^\circ$ ($c=1.4$, CHCl_3). FAB-MS m/z : 339 $[\text{M}+\text{H}]^+$. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ : 1.69 (3H, s, H_3 -5''), 1.74 (3H, s, H_3 -4''), 2.78 (1H, dd, $J=2.8$, 16.8 Hz, H_a -3), 3.04 (1H, dd, $J=13.2$, 16.8 Hz, H_b -3), 3.24 (2H, d, $J=7.2$ Hz, H_2 -1''), 3.84 (3H, s, 7-OMe), 5.27 (1H, t, $J=7.2$ Hz, H-2''), 5.38 (1H, dd, $J=2.8$, 13.2 Hz, H-2), 6.44 (1H, s, H-8), 6.90 (2H, d, $J=8.4$ Hz, H-3', 5'), 7.34 (2H, d, $J=8.4$ Hz, H-2', 6'), 7.68 (1H, s, H-5). $^{13}\text{C-NMR}$ (100 MHz, CDCl_3) δ : 17.7 (q, C-5''), 25.8 (q, C-4''), 27.8 (t, C-1'), 44.0 (t, C-3), 55.8 (q, 7-OMe), 79.8 (d, C-2), 98.8 (d, C-8), 113.8 (s, C-10), 115.7 (d, C-3', 5'), 121.7 (d, C-2''), 124.9 (s, C-6), 127.1 (d, C-5), 127.9 (d, C-2', 6'), 130.8 (s, C-1'), 133.1 (s, C-3''), 156.3 (s, C-4'), 162.4 (s, C-9), 164.3 (s, C-7), 191.5 (s, C-4).

Bavachalcone (**4**): Yellow amorphous powder. FAB-MS m/z : 339 $[\text{M}+\text{H}]^+$. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ : 1.74 (3H, s, H_3 -5''), 1.77 (3H, s, H_3 -4''), 3.26 (2H, d, $J=7.2$ Hz, H_2 -1''), 3.87 (3H, s, 4-OMe), 5.28 (1H, t, $J=7.2$ Hz, H-2''), 6.44 (1H, s, H-3), 6.90 (2H, d, $J=8.0$ Hz, H-3', 5'), 7.43 (1H, d, $J=15.2$ Hz, H- β), 7.56 (2H, d, $J=8.0$ Hz, H-2', 6'), 7.59 (1H, s, H-6), 7.83 (1H, d, $J=15.2$ Hz, H- α). $^{13}\text{C-NMR}$ (100 MHz, CDCl_3) δ : 18.1 (q, C-5''), 26.0 (q, C-4''), 28.2 (t, C-1''), 55.9 (q, 4-OMe), 99.6 (d, C-3), 113.5 (s, C-1), 116.2 (d, C-3', 5'), 118.3 (d, C- β), 121.9 (s, C-5), 122.5 (d, C-2''), 127.9 (s, C-1'), 129.9 (d, C-6), 130.7 (d, C-2', 6'), 133.2 (s, C-3''), 144.2 (d, C- α), 158.3 (s, C-4'), 164.4 (s, C-4), 165.5 (s, C-2), 192.1 (s, C=O).

Cell Culture Murine microglial cell line (BV-2) was obtained from American Type Culture Collection (Rockville, MD, U.S.A.). Cells were cultured in DMEM containing 10% fetal bovine serum, 2 mM glutamine, 1 mM pyruvate, penicillin (100 U/ml) and streptomycin (10 $\mu\text{g}/\text{ml}$). BV-2 cells were seeded at 1×10^5 cells/ml in 48 well plates and were activated by incubation in medium containing LPS (0.1 $\mu\text{g}/\text{ml}$,

Escherichia coli, 0127:B8, Sigma, St. Louis, MO, U.S.A.) and various concentrations of test compounds dissolved in DMSO (0.1% final concentration in media). The supernatant was collected as a source of secreted NO.

Nitrite Assay Cells were incubated with medium containing LPS (0.1 $\mu\text{g}/\text{ml}$) in the presence or absence of various concentrations of test compounds for 20 h. NO released from BV-2 cells was assessed by determination of NO_2^- concentration in culture supernatant based on the Griess reaction.¹⁷⁾ Briefly, samples (100 μl) of culture media were incubated with an 150 μl of Griess reagent (1% sulfanilamide, 0.1% naphthylethylenediamine in 2.5% phosphoric acid solution) at room temperature for 10 min in 96-well microplate. Absorbance at 540 nm was read using an ELISA plate reader. Standard calibration curves were prepared using sodium nitrite as standard.

Western Blot Analysis The cells were rinsed with phosphate buffered saline and lysed by boiling with lysis buffer (1% SDS, 1.0 mM sod. vanadate, 10 mM Tris, pH 7.4) for 5 min. Total protein (10 μg) of cell lysates was applied on SDS-polyacrylamide gels and transferred to PVDF membrane by the standard method. The membrane was probed with antibody for anti-mouse iNOS (Transduction Laboratories, Lexington, KY, U.S.A.), anti-I- κB - α (Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.) and anti- β -actin (Sigma, St. Louis, MO, U.S.A.). The Western blot was visualized using an enhanced chemiluminescence detection kit (Amersham Bioscience, Piscataway, NJ, U.S.A.) according to the manufacturer's instruction.

Reverse Transcription and Polymerase Chain Reaction (RT-PCR) BV-2 cells were treated with and without test compounds together with LPS (0.1 $\mu\text{g}/\text{ml}$) for 4 h. The whole RNA was isolated with TRIzol[®] reagent (Invitrogen, Carlsbad, CA, U.S.A.). Each RNA extract (2 μg) was reverse-transcribed into cDNA using superscript II reverse transcriptase (Invitrogen, Carlsbad, CA U.S.A.). PCR was performed in 25 μl of a solution containing Go Taq[™] DNA polymerase (Promega, Madison, WI, U.S.A.), iNOS primer, β -actin primer, and RT products. The sense and antisense oligonucleotides for iNOS were 5'-CCCTTCCGAAGTTTCTGGC-AGCAGC-3' and 5'-GGCTGTCAGAGCCTCGTGGCTTTGG-3', respectively. The sense and antisense primers for β -actin were 5'-TGTGATGGTGGGAATGGGTCAG-3' and 5'-TTTGATGTCACGCACGATTTC-3', respectively. After preincubation for 3 min at 94 °C, 26 cycles of amplification (94 °C for 1 min, 64 °C for 1 min and 72 °C for 1 min) were performed and β -actin was used for an internal control. Each PCR reaction was analyzed by 1% agarose gel electrophoresis in Tris acetate-EDTA buffer and stained with ethidium bromide.

Statistics The results were expressed as mean $\pm$ S.D. of three experiments. One-way ANOVA followed by Dunnett's *t*-test and Student's *t*-test were used for statistical analysis of the results, and a *p* value of <0.05 was considered to indicate a significant difference.

RESULTS

The MeOH extracts of *P. corylifolia* were subjected to sequential solvent fractionations with increment of polarity to yield *n*-hexane, EtOAc and BuOH soluble fractions. For the

activity-guided purification, BV-2 cells were stimulated with 0.1 $\mu\text{g/ml}$ of LPS in the presence of test samples for 20 h, and the amounts of NO released into culture media were determined by the Griess method.¹⁷⁾ The treatment of EtOAc soluble fraction (30 $\mu\text{g/ml}$) into the cells reduced the production of NO to $0.9 \pm 0.3 \mu\text{M}$, while that of LPS control was $28.1 \pm 0.9 \mu\text{M}$. Compounds **1**–**4** were isolated from the EtOAc soluble fraction of *P. corylifolia* through the several steps of silica gel column chromatography and preparative reverse phase MPLC. The purity of the purified compounds was assessed by reverse phase HPLC analysis and NMR spectrum.

From the NMR spectra of **1** and **3**, we found overall spectral pattern of typical flavanone. An ABX spin system assignable to H₂-3 and H-2 of flavanone were found in ¹H-NMR spectrum, and one oxymethine (C-2), one methylene (C-3) and one carbonyl (C-4) carbons were found in ¹³C-NMR spectrum. Compounds **2** and **4** were recognized as chalcone derivatives by a set of *trans*-olefinic protons at δ 7.83 and 7.43 (each, d, $J=15.2\text{ Hz}$) at α and β positions. Four protons of AA'BB' spin systems were found by the *para*-disubstituted ring-B in compounds **1**–**4**. The presence of *gem*-dimethylpyran ring in **1** and **2** were confirmed by the signals of *cis*-ethylenic protons (δ 6.32–6.75 and 5.61–5.59), two methyl protons (δ 1.44–1.47), and one quaternary oxy carbon (δ 77.9–77.8) in ¹H- and ¹³C-NMR spectrum, respectively. The signals of the isoprenyl (γ,γ' -dimethylallyl) groups in **3** and **4** were clearly observed in NMR spectra.¹⁸⁾ The position of isoprenyl groups of **3** and **4** were confirmed by the correlation between H-1'' and *ipso* and two *ortho* carbons of ring A in HMBC spectrum. The *gem*-dimethylpyran moiety of **1** was linked to the C-6 by the correlation between H-4'' (δ 6.32) and C-5, 6, 7 (δ 124.6, 116.0, 163.4) in HMBC spectrum. The *gem*-dimethylpyran moiety of **2** was linked to the C-3 by the correlation between the H-4'' (δ 6.75) and C-2 (δ 159.7) in HMBC spectrum. On the basis of above evidences and comparison of the reported spectral data,^{15,18)} the structures were identified as a chromenoflavanone [7,8-dihydro-8-(4-hydroxyphenyl)-2,2-dimethyl-2H,6H-benzo-(1,2-*b*:5,4-*b'*)dipyrans-6-one] (**1**), 4-hydroxylonchocarpin (**2**), bavachinin (**3**) and bavachalcone (**4**) (Fig. 1). Compound **2** reported from *Lonchocarpus neuroscapha* BENTH¹⁹⁾ was isolated for the first time from this plant. Copies of original spectra are obtainable from the author of correspondence.

As shown in Fig. 2, compounds **1** and **2** inhibited LPS-induced NO production in a dose dependent manner. The concentrations required for inhibiting the production of NO by 50% (IC₅₀ value) was calculated on the basis of concentrations of nitrite released into the culture media. The IC₅₀ values of active compounds **1** and **2** were 11.4, 10.2 μM , respectively. Under the experimental conditions described above, no cytotoxic effects were observed at the concentrations for the nitrite assay. Compound **3** showed a significant cytotoxicity against microglia at 10 μM ($15.3 \pm 2.5\%$ viability) compared with untreated control group) in MTT assay.²⁰⁾

Western blot analysis was performed to clarify the underlying mechanism of **1** and **2** for the NO production in LPS-activated microglia (Fig. 3). The cytosol of vehicle treated cells did not show detectable iNOS protein. The induction of iNOS protein was maximized by 18 h stimulation of BV-2

Fig. 1. Chemical Structures of Compounds **1**–**4** Isolated from *Psoralea corylifolia*

Fig. 2. Effect of Compounds **1** and **2** on the Production of Nitric Oxide in LPS-Activated Microglia

Cells were treated with LPS (0.1 $\mu\text{g/ml}$) for 20 h in the presence of compounds **1** and **2** followed by the nitrite assay using Griess reagent. The results are the means $\pm$ S.D. of three experiments. Values with asterisk represent significant ($p < 0.05$) differences of NO production compared with LPS/media control.

Fig. 3. Effects of Compounds **1** and **2** on the Expression of iNOS Protein in LPS-Activated Microglia

BV-2 cells were treated for 20 h with compound **1** or **2** (10, 15, 20 μM) during LPS (0.1 $\mu\text{g/ml}$) activation. Cell lysates were prepared and the protein levels of iNOS and β -actin were determined by Western blotting (lower panel). The blots were subjected to densitometric analysis (upper panel). Densitometric units were normalized to β -actin. The blot is the representative results of three separate experiments. Values represent mean $\pm$ S.D. for three independent experiments. Values with asterisk represent significant ($p < 0.05$) differences compared with LPS/media control.

Fig. 4. Effects of Compounds **1** and **2** on the Expression of mRNA in LPS-Activated Microglia

BV-2 cells were treated for 4 h with compound **1** or **2** (10, 20 μ M) during LPS (0.1 μ g/ml) activation. The mRNA levels of iNOS and β -actin were determined by RT-PCR from total RNA extracts (lower panel). The relative intensity of iNOS to β -actin bands was measured by densitometric analysis (upper panel). Densitometric units were normalized to β -actin. The gel shown is the representative results of three separate experiments. Values represent mean $\pm$ S.D. for three independent experiments. Values with asterisk represent significant ($p < 0.05$) differences compared with LPS/media control.

cells with 0.1 μ g/ml of LPS (data not shown). The treatment of **1** and **2** (10, 15, 20 μ M) decreased the expression of iNOS protein of microglia dose dependently.

The results of RT-PCR analysis also showed that compounds **1** and **2** significantly inhibited the expression of iNOS mRNA in LPS-activated microglia. The amounts of mRNA expression were quantitated from the volume intensity of the bands corresponding to iNOS (456 bp) and β -actin mRNA (540 bp) in agarose gels. We determined the degree of inhibition using the intensity ratio of iNOS to β -actin for the adjustment of minor differences in RT efficiency among the samples. Compounds **1** and **2** showed significant suppression of mRNA expression at 10 μ M (Fig. 4).

To elucidate the further mechanism of **1** and **2** for the inhibition of iNOS in microglia, we determined the effects of **1** and **2** on the level of I- κ B- α in LPS-activated microglia. The gene expression of iNOS can be modulated by nuclear factor- κ B (NF- κ B)²¹ that can be activated by the degradation of inhibitory- κ B (I- κ B). The I- κ B- α was fully degraded by 30 min incubation of LPS (0.1 μ g/ml), and regenerated gradually afterwards (Fig. 5A). As shown in Fig. 5B, pretreatment of **1** and **2** suppressed the degradation of I- κ B- α protein in LPS-induced microglia.

DISCUSSION

NO participates in the regulation of diverse physiological processes in CNS. But, the excessive NO acts as neurotoxic mediator and results in neurological diseases.⁸ The activated microglia is the main source of NO production in CNS injury and inflammation condition. In activated microglial/neuronal cell cocultures, NO is the principal source of neurotoxic free radical through the oxidative stress and activation of caspases.^{22,23} The expression of iNOS can be induced by β -

Fig. 5. Effects of Compounds **1** and **2** on I- κ B- α Degradation in LPS-Activated Microglia

(A) Cells were treated with LPS (0.1 μ g/ml) for the indicated times. (B) The cells were pretreated with compound **1** or **2** for 30 min and incubated with LPS (0.1 μ g/ml) for 30 min. The protein levels of I- κ B- α were determined by Western blotting.

amyloid in neuronal cell culture systems,^{24–26} and the over expression of iNOS was reported in the brains of Alzheimer's disease patients.^{27,28}

Thus, the inhibition of NO production would provide the therapeutic strategy for the treatment of oxidative neuronal diseases.⁸ In the course of our search for NO inhibitors from medicinal plants, we purified two inhibitors (**1**, **2**) of LPS-induced NO production in microglia from *Psoralea corylifolia* together with two inactive compounds (**3**, **4**). Compounds **2** and **4** have chalcone skeleton, and **1** and **3** are dihydro flavone derivatives. In the structures of **3** and **4**, one prenyl group was linked to 6-position of A-ring, while **1** and **2** has D-ring (*gem*-dimethylpyran) that was formed by the cyclization of prenyl groups. Interestingly, compounds with *gem*-dimethylpyran moiety (**1**, **2**) showed inhibitory activity of NO production, while **3** showed cytotoxicity against microglia. There are several bioactive structures with *gem*-dimethylpyran that showed cytotoxicity against cancer cells¹⁸ or inhibitory activity of NO production in activated murine macrophages.²⁹ The difference in the skeleton between flavone and chalcone did not show a big difference in activity potency. A meroterpenoid, bakuchiol from *Psoralea corylifolia* was reported as an inhibitor of iNOS in LPS-activated murine macrophages.³⁰

In order to reveal the mechanism for the inhibition of NO production, we analyzed the effects of **1** and **2** on the expression level of protein and mRNA of iNOS in LPS-activated microglia. Like the most of the flavonoids as iNOS inhibitors,^{31,32} **1** and **2** suppressed the expression of iNOS protein and mRNA dose dependently. Since the activation of NF- κ B has been known to be important for iNOS induction,²¹ we examined the effects of **1** and **2** on the I- κ B- α . The NF- κ B can be activated by the degradation of I- κ B through the phosphorylation³³ by the LPS activation of cells. Inhibitors **1** and **2** derived from *P. corylifolia* regenerated the degraded I- κ B- α , resulting in the inhibition of iNOS expression. These activities could be extrapolated to the inhibition of other inflammatory mediators such as cyclooxygenase-2 and TNF- α whose levels are also regulated by NF- κ B pathway. The further activity mechanism and the permeability of blood-brain barrier of the active compounds should be investigated for the successful drug development for CNS.

Microglia are resident macrophage-like cells that play a

role in host defense and tissue repair in CNS.³⁴⁾ However, activated microglia play a pivotal role in the amplification of inflammation and mediation of cellular degeneration.³⁵⁾ NO released from activated microglia participates in the process of neuronal cell death through DNA damage, mitochondrial disruption and induction of apoptosis. Therefore, the inhibitors of iNOS expression may have beneficial effects in the treatment of neuro-inflammatory diseases.

In summary, two prenylflavonoids, a chromenoflavanone [7,8-dihydro-8-(4-hydroxyphenyl)-2,2-dimethyl-2H,6H-benzo-(1,2-*b*:5,4-*b'*)dipyrans-6-one] (**1**) and 4-hydroxylonchocarpin (**2**) were identified from *Psoralea corylifolia* as new inhibitors of NO production in LPS-activated microglia. They exerted their activity through the inhibition of I- κ B- α degradation and subsequent suppression of protein and mRNA expression of iNOS. These results imply that *Psoralea corylifolia* may have beneficial effects in the treatment of NO-mediated neuro-inflammatory diseases through the inhibition of overproduction of NO by prenylflavonoids.

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REFERENCES

- Pawate S., Shen Q., Fan F., Bhat N. R., *J. Neurosci. Res.*, **77**, 540—551 (2004).
- Tamatani M., Che Y. H., Matsuzaki H., Ogawa S., Okado H., Miyake S., Mizuno T., Tohyama M., *J. Biol. Chem.*, **274**, 8531—8538 (1999).
- Bishop A., Anderson J. E., *Toxicology*, **208**, 193—205 (2005).
- Bruce A. J., Boling W., Kindy M. S., Peschon J., Kraemer P. J., Carpenter M. K., Holtsberg F. W., Mattson M. P., *Nat. Med.*, **2**, 788—794 (1996).
- Wang J. Y., Shum A. Y., Ho Y. J., Wang J. Y., *J. Neurosci. Res.*, **72**, 508—519 (2003).
- Beckman J. S., Beckman T. W., Chen J., Marshall P. A., Freeman B. A., *Proc. Natl. Acad. Sci. U.S.A.*, **87**, 1620—1624 (1990).
- McGeer P. L., McGeer E. G., *Brain Res. Brain Res. Rev.*, **21**, 195—218 (1995).
- Chabrier P. E., Demerle-Pallardy C., Auguet M., *Cell Mol. Life Sci.*, **55**, 1029—1035 (1999).
- Nathan C., *FASEB J.*, **6**, 3051—3064 (1992).
- Maeda H., Akaike T., *Biochem.*, **63**, 854—865 (1998).
- Kotiyal J. P., Sharma D. P., *Bull. Medico-Ethnobotanic. Res.*, **13**, 209—223 (1992).
- Zhu Y. P., "Chinese Material Medica: Chemistry, Pharmacology and Applications," Harwood Academic Publishers, Amsterdam, 1998, pp. 609—612.
- Wall M. E., Wani M. C., Manikumar G., Hughes T. J., Taylor H., McGivney R., Warner J., *J. Nat. Prod.*, **51**, 1148—1152 (1988).
- Guo J., Weng X., Wu H., Li Q., Bi K., *Food Chem.*, **91**, 287—292 (2005).
- Yin S., Fan C. Q., Wang Y., Dong L., Yue J. M., *Bioorg. Med. Chem.*, **12**, 4387—4392 (2004).
- Tsai W. J., Hsin W. C., Chen C. C., *J. Nat. Prod.*, **59**, 671—672 (1996).
- Green L. C., Wagner D. A., Glogowski J., Skipper P. L., Wishnok J. S., Tannenbaum S. R., *Anal. Biochem.*, **126**, 131—138 (1982).
- Fang N., Casida J. E., *J. Nat. Prod.*, **62**, 205—210 (1999).
- Goncalves de Lima O., de Mello J. F., de Barros Coelho J. S., de Andrade Lyra F. D., Machado de Albuquerque M., Marini-Bettolo G. B., Delle Monache G., Delle Monache F., *Farmaco [Sci.]*, **30**, 326—342 (1975).
- Mosmann T., *J. Immunol. Methods*, **65**, 55—63 (1983).
- Kim Y. M., Lee B. S., Yi K. Y., Paik S. G., *Biochem. Biophys. Res. Commun.*, **236**, 655—660 (1997).
- Chao C. C., Hu S., Molitor T. W., Shaskan E. G., Peterson P. K., *J. Immunol.*, **149**, 2736—2741 (1992).
- Kang Y. C., Kim P. K., Choi B. M., Chung H. T., Ha K. S., Kwon Y. G., Kim Y. M., *In Vivo*, **18**, 367—376 (2004).
- Combs C. K., Karlo J. C., Kao S. C., Landreth G. E., *J. Neurosci.*, **21**, 1179—1188 (2001).
- Goodwin J. L., Uemura E., Cunnick J. E., *Brain Res.*, **692**, 207—214 (1995).
- Ii M., Sunamoto M., Ohnishi K., Ichimori Y., *Brain Res.*, **720**, 93—100 (1996).
- Heneka M. T., Wiesinger H., Dumitrescu-Ozimek L., Riederer P., Feinstein D. L., Klockgether T., *J. Neuropathol. Exp. Neurol.*, **60**, 906—916 (2001).
- Lee S. C., Zhao M. L., Hirano A., Dickson D. W., *J. Neuropathol. Exp. Neurol.*, **58**, 1163—1169 (1999).
- Ryu J.-H., Ahn H., Lee H. J., *Fitoterapia*, **74**, 350—354 (2003).
- Pae H. O., Cho H., Oh G. S., Kim N. Y., Song E. K., Kim Y. C., Yun Y. G., Kang C. L., Kim J. D., Kim J. M., Chung H. T., *Int. Immunopharmacol.*, **1**, 1849—1855 (2001).
- Rao Y. K., Fang S. H., Tzeng Y. M., *Biol. Pharm. Bull.*, **28**, 912—915 (2005).
- Daikonya A., Katsuki S., Kitanaka S., *Chem. Pharm. Bull.*, **52**, 1326—1329 (2004).
- Lee J., Hur J., Lee P., Kim J. Y., Cho N., Lee M. S., Kim S. Y., Kim H., Suk K., *J. Biol. Chem.*, **276**, 32956—32965 (2001).
- Perry V. H., Gordon S., *Trends Neurosci.*, **11**, 273—277 (1988).
- Gonzalez-Scarano F., Baltuch G., *Annu. Rev. Neurosci.*, **22**, 219—240 (1999).