Planta Medica

Journal of Medicinal Plant and Natural Product Research

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Rüdigerstraße 14
D-70469 Stuttgart
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Thieme Publishers
333 Seventh Avenue
New York, NY 10001, USA
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Bioactive γ-Lactones from the Fermented Broth of *Neosartorya* sp.

Authors

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Key words
- *Neosartorya*
- Ascomycete
- mycelium
- neosartolactone
- iNOS

Abstract

Two γ-lactone derivatives, namely neosartolactone (**1**) and its 7-methyl ester analogue (**2**), have been isolated from the ethyl acetate extract of the fermented broth of *Neosartorya* sp. isolated in Taiwan. Structural elucidations of compounds **1** and **2** were achieved on the basis of spectroscopic analysis. Although they had been obtained via the chemical modification of avenaciolide isolated from *Aspergillus avenaceus* several decades ago, this is the first report to describe them from a natural resource with detailed spectroscopic interpretations. The effects of **1** and **2** on the inhibition of NO production in lipopolysaccharide (LPS)-activated murine macrophages were further evaluated. Compounds **1** and **2** significantly inhibited NO production with the IC_{50} values of 12.2 ± 1.5 and 11.4 ± 1.0 µM, respectively; but displayed cytotoxicity at considerably higher concentrations than 50 µM.

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Introduction

Nitric oxide (NO), formed from L-arginine by NO synthase (NOS), is a key physiological mediator in immune functions, blood vessel dilatation and neurotransmission [1]. Among three identified isoforms of NOS, the inducible form NOS (iNOS) is upregulated in response to inflammatory challenge, such as bacterial lipopolysaccharide (LPS) or cytokines [2]. Excessive NO produced by iNOS is associated with the pathophysiology of almost all inflammatory diseases. Therefore, mechanisms controlling iNOS expression and NO production are of special interest to counteract the inflammatory process [3]. To date, NO production induced by LPS through iNOS expression in RAW 264.7 cells, a mouse macrophage cell line, has successfully reflected the degree of inflammation and provided a measure for assessing the effect of test drugs on the inflammatory process [4]. Recent evidence has shown that a health food made from the fermented broth of an indigenous fungus, *Antrodia camphorata*, can exert significant anti-inflammatory activity by suppressing NO production through reducing iNOS expression in activated macrophages [5]. This finding suggested that fungi could probably be important sources for the monitoring of iNOS inhibitory activity. In our preliminary screening of fungus strains for iNOS inhibitory activity, the ethyl acetate layer of the fermented broth of *Neosartorya* sp. has been demonstrated to exhibit such bioactivity albeit together with some cell toxicity in LPS-activated RAW 264.7 cells. Based on the above considerations, the fungus may contain some bioactive entities worthy of being investigated. Therefore, a series of extraction, separation, purification, and structural elucidation processes was undertaken, and that has led to the isolation and identification of two rare γ-lactone derivatives, **1** and **2**. This report deals with the isolation and identification of **1** and **2** (**Fig. 1**) as well as their iNOS inhibitory activities, by investigating the induction of iNOS mRNA expression and NO accumulation, the index of catalytic activity of iNOS, in RAW 264.7 cells.

* Drs. S.-S. Yang and G.-J. Wang contributed equally to this work.

received Dec. 11, 2009
revised April 2, 2010
accepted April 9, 2010

Bibliography
DOI http://dx.doi.org/10.1055/s-0030-1249907
Published online May 5, 2010
Planta Med 2010; 76: 1–5
© Georg Thieme Verlag KG Stuttgart · New York ·
ISSN 0032-0943

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Table 1 ^{1}H- and ^{13}C-NMR spectroscopic data ($CDCl_3$, 500 MHz) for compounds **1** and **2** [δ in ppm, mult. (*J* in Hz)].

Position	1			2		
	$^{13}C^{a}$	^{1}H	HMBC (H → C)	$^{13}C^{a}$	^{1}H	HMBC (H → C)
2	85.2 d	4.12 m	5, 1′, 2′	82.4 d	4.49 m	3, 4, 5, 6, 2′
3	44.1 d	3.55 m	5, 7, 8, 1′	47.1 d	3.36 m	2, 4, 5, 6, 7, 8, 1′
4	74.3 d	5.05 d (8.5)	2, 5, 6	68.8 d	4.58 d (8.3)	2, 3, 5, 6
5	169.8 s			175.8 s		
6	134.6 s			135.4 s		
7	167.5 s			167.1 s		
8a	126.3 t	5.87 d (2.0)	3, 7	128.3 t	5.85 s	3, 6, 7
8b		6.46 d (2.0)	3, 6, 7		6.44 s	3, 6, 7
9				52.4 q	3.76 s	7
1′	36.0 t	1.79 m	2, 3, 2′, 3′	34.9 t	1.66 m	2, 3, 2′, 3′
2′a	24.8 t	1.46 m	2, 1′, 3′, 4′	25.4 t	1.38 m	3′, 4′
2′b					1.45 m	2, 1′, 3′, 4′
3′	29.3 t	1.22 ~ 1.39 m	–[b]	29.3 t	1.19 ~ 1.37 m	–[b]
4′	29.1 t	1.22 ~ 1.39 m	–[b]	29.2 t	1.19 ~ 1.37 m	–[b]
5′	29.1 t	1.22 ~ 1.39 m	–[b]	29.1 t	1.19 ~ 1.37 m	–[b]
6′	31.7 t	1.22 ~ 1.39 m	–[b]	31.8 t	1.19 ~ 1.37 m	–[b]
7′	22.6 t	1.22 ~ 1.39 m	–[b]	22.6 t	1.19 ~ 1.37 m	–[b]
8′	14.0 q	0.88 t (6.8)	6′, 7′	14.0 q	0.85 t (7.0)	6′, 7′

[a] Multiplicities were obtained from DEPT experiments; [b] Signals overlapped

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Materials and Methods

General experimental procedures

Optical rotations were measured on a JASCO P-1020 polarimeter. ^{1}H- and ^{13}C-NMR spectra were acquired on a Bruker DMX-500 SB spectrometer. Low resolution mass spectra were obtained using a VG Platform Electrospray ESI/MS. IR spectra were recorded on a JASCO FT/IR 4100 spectrometer. UV spectra were measured on a Thermo Helios α spectrophotometer.

Fermentation of *Neosartorya* sp.

Neosartorya sp. (strain No. 325 from Taiwan), collected, isolated and identified by one of us (YMJ), was deposited at the Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan. The mycelium of #325 was inoculated into 1-L Erlenmeyer flasks, each containing 10 g Bacto™ Malt Extract (Becton Dickinson) and 500 mL deionized water. The fermentation was conducted at 25–30 °C for 30 days.

Extraction and isolation

The filtered fermented broth (108 L) of *Neosartorya* sp. was partitioned three times with 50 L recycled ethyl acetate, then concentrated under vacuum to dryness (0.6 g). Subsequently, this residue was redissolved in 25 mL of MeOH, and applied onto a Sephadex LH-20 column (3 cm i. d. × 65 cm) eluted by MeOH with a flow rate of 2.5 mL/min. Each subfraction (25 mL) collected was checked for its composition by TLC using EtOAc/acetic acid/H_2O (85 : 10 : 10, v/v/v) for development, and observation under UV 254 nm was used in the detection of compounds with similar chromophores. Subsequently, subfractions were combined into six portions I – VI. The portion III (subfr. 11–14) was purified by HPLC on a semipreparative normal-phase column (Hypersil-100 silica, 10 × 250 mm; Thermo Electron Corporation) with *n*-hexane/EtOAc (3 : 1, v/v) as eluent, 2 mL/min, which afforded **1** (26.0 mg, t_R = 19.3 min) and **2** (28.7 mg, t_R = 24.3 min).

Neosartolactone (**1**): white amorphous powder; $[\alpha]_D^{24}$: − 28.0 (*c* 0.65, CH_2Cl_2); UV (MeOH): λ_{max} (log ε) = 208 (4.0) nm; IR (ZnSe): ν_{max} = 3477, 2955, 2920, 2852, 1779, 1720, 1635, 1439, 1287, 1263, 1196, 1161, 1130, 1088 cm^{-1}; ^{1}H-NMR data: see **Table 1**; ^{13}C-NMR data: see **Table 1**; ESI-MS: *m*/*z* = 307 [M + Na]$^+$; HR-ESI-MS: *m*/*z* = 307.1519 [M + Na]$^+$; calcd. for $C_{15}H_{24}O_5 + Na^+$: 307.1521.

Neosartolactone 7-methyl ester (**2**): white amorphous powder; $[\alpha]_D^{24}$: + 32.5 (*c* 0.65, CH_2Cl_2); UV (MeOH): λ_{max} (log ε) = 208 (4.0) nm; IR (ZnSe): ν_{max} = 3476, 2920, 2852, 1779, 1721, 1642, 1456, 1287, 1263, 1161, 1130, 1090 cm^{-1}; ^{1}H-NMR data: see **Table 1**; ^{13}C-NMR data: see **Table 1**; ESI-MS: *m*/*z* = 321 [M + Na]$^+$; HR-ESI-MS: *m*/*z* = 321.1675 [M + Na]$^+$; calcd. for $C_{16}H_{26}O_5 + Na^+$: 321.1678.

Mosher esters of 2

The Mosher esters of **2** were obtained by using a modification of the Mosher method [6]. To a CH_2Cl_2 solution of **2** (1.0 mg) was added 4-dimethylaminopyridine (100 µg), triethylamine (10 µL) and (*S*)-(+)-MTPACl (10 µL)/(*R*)-(−)-MTPACl (10 µL) (Sigma-Aldrich Chemical Co.) at room temperature, and stirring was continued overnight. After addition of *N*,*N*-dimethyl-1,3-propanediamine (5 µL) and evaporation of the solvent, the residue was passed through a silica gel column (*n*-hexane/EtOAc, 4 : 1) to afford the (*R*)- (**2a**, 0.9 mg) and (*S*)-MTPA (**2b**, 0.5 mg) esters of **2**, respectively.

Nitrite measurement and cell viability assay

The methods were essentially the same as those reported previously [7]. To assess the effects on LPS-induced NO production, crude extracts of fermented broth of *Neosartorya* sp., compounds **1** and **2** (purity > 98 % as checked by their ^{1}H-NMR), two positive controls N^{ω}-nitro-L-arginine (L-NNA, a nonselective NOS inhibitor) and aminoguanidine (a specific inhibitor of iNOS) or vehicle (0.1 %, DMSO) were added in the presence of LPS (200 ng/mL) to the RAW 264.7 cells. Both inhibitors were purchased from Sigma-Aldrich Chemical Co. and the purity of each compound was more than 98 %.

Statistical analyses

Comparisons of the concentration and treatment effects were made using ANOVA, followed by post hoc comparisons using Newman-Keuls test as appropriate. The average IC_{50} was determined by data fitting with GraFit (Erithacus Software).

Supporting information

The original spectra for **1** are available as Supporting Information.

Results and Discussion

The fermented broth of *Neosartorya* sp. was partitioned initially using EtOAc to give a light yellow residue, which was subjected to Sephadex LH-20 column separation followed by HPLC purification to afford **1** and **2** (▶ **Fig. 1**).

Neosartolactone (**1**), a white powder, had the molecular formula $C_{15}H_{24}O_5$ as deduced from ^{13}C-NMR and HR-ESI-MS data. Its IR spectrum showed absorptions of two carbonyls at 1779 and 1720 cm^{-1}, indicating the presence of a γ-lactone carbonyl moiety and an acid carbonyl functionality, respectively. The absorptions at 3477 and 1635 cm^{-1} attributed to the respective hydroxy and olefinic groups were also observed. Analysis of the ^{1}H-, ^{13}C-NMR and HSQC spectra of **1** revealed one terminal methyl [δ_H = 0.88 (3H, t, *J* = 6.8 Hz, H_3-8′) and δ_C = 14.0 (C-8′)], an alkyl chain signal [δ_H = 1.22–1.79 (14H, m, H_2-7′ – H_2-1′) and δ_C = 22.6–36.0 (C-7′ – C-1′)], one methine [δ_H = 3.55 (1H, m, H-3) and δ_C = 44.1 (C-3)], two carbinoyl protons [δ_H = 4.12 (1H, m, H-2) and δ_C = 85.2 (C-2); δ_H = 5.05 (1H, d, *J* = 8.5 Hz, H-4) and δ_C = 74.3 (C-4)] and one exomethylene [δ_H = 5.87 (1H, d, *J* = 2.0 Hz, H-8a), 6.46 (1H, d, *J* = 2.0 Hz, H-8b) and δ_C = 126.3 (C-8)] (▶ **Table 1**). The COSY spectrum of **1** indicated the following spin systems: the hydrogen at δ_H = 4.12 (1H, m, H-2) was coupled to the alkyl chain end methylene at δ_H = 1.79 (2H, m, H_2-1′). The resonance at δ_H = 4.12 (H-2) was also coupled to the methine at δ_H = 3.55 (1H, m, H-3), which was in turn coupled to the carbinoyl methine at δ_H = 5.05 (1H, d, *J* = 8.5 Hz, H-4). In the HMBC spectrum of **1**, the exomethylene protons at δ_H = 5.78 and 6.46 (H_2-8) correlated with C-3 (δ_C = 44.1), C-6 (δ_C = 134.6) and C-7 (δ_C = 167.5), the carbinoyl proton at δ_H = 5.05 (H-4) correlated with C-2 (δ_C = 85.2), C-5 (δ_C = 169.8) and C-6 (δ_C = 134.6), indicating that an acrylic acid residue was attached at C-3. All above assignments were consistent with a γ-lactone moiety bearing an aliphatic substituent, an acrylic acid and a hydroxy group at its C-2, C-3 and C-4, respectively. In the NOESY spectrum of **1**, key mutual correlations were detected as follows: H-2/H-8a, H-3/H-1′ and H-4/H-1′ (▶ **Fig. 2**), indicating that the relative configurations of the alkyl chain attached at C-2, the acrylic acid attached at C-3 and the hydroxy group attached at C-4 were, respectively, β-, α- and α-oriented to fit the distinguishing features in the NOESY spectrum. Accordingly, **1** was characterized as the shown structure, and named as neosartolactone. Although **1** has been obtained previously from the alkaline hydrolysis of avenaciolide isolated from *Aspergillus avenaceus* [8], this is the first time for **1** to be isolated from *Neosartorya* sp., the sexual state of an *Aspergillus* sp.

Compound **2** possessed spectroscopic data closely comparable to those of **1** except that the OH-7 functionality in **1** was replaced with a methoxy group in **2**. Its ^{1}H-NMR (▶ **Table 1**) exhibited one signal for an additional methoxy singlet at δ_H = 3.76 (3H, s, H_3-9) when compared with that of **1**. In the HMBC spectrum of **2**, the methoxy protons at δ_H = 3.76 (3H, s, H_3-9) correlated with C-7 (δ_C = 167.1), indicating the location of the methoxy group was at C-7. Thus, **2** was assigned as neosartolactone 7-methyl ester, which had been obtained previously from the alkaline hydrolysis and methanolysis of avenaciolide [8]. In this research, whether **2** was produced by the enzyme system (e.g., *S*-adenosyl-L-methionine-mediated methylation) in the *Neosatorya* sp. or was just an artifact still remains to be further studied.

The absolute configurations at C-2, C-3 and C-4 of both **1** and **2** were elucidated by application of a modified Mosher method to compound **2**, but not compound **1**, since **1** was prone to lactonize. Compound **2** was treated with (*S*)-(+)- and (*R*)-(−)-MTPACl to afford the (*R*)- and (*S*)-MTPA esters (**2a** and **2b**, respectively). The Δδ values obtained from the ^{1}H chemical shifts of both Mosher esters are shown in ▶ **Fig. 3**. The Δδ values of the H_2-1′, H-2, H-3, H_a − 8 and H_b − 8 are all positive in sign. Unambiguously, the ab-

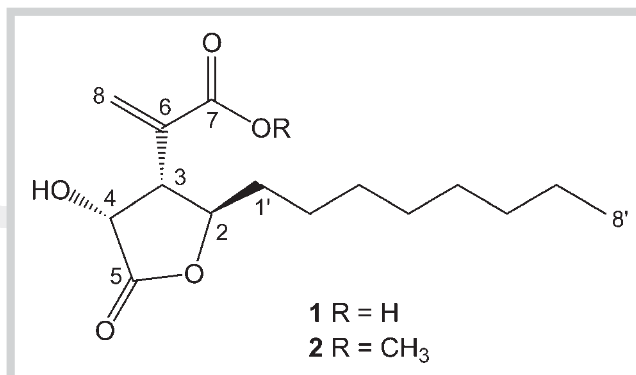

Fig. 1 Chemical structures of compounds **1** and **2** identified in this report.

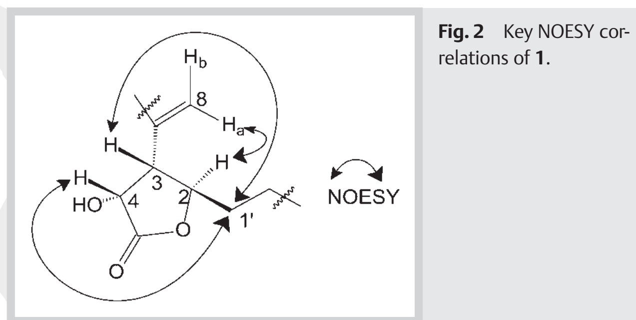

Fig. 2 Key NOESY correlations of **1**.

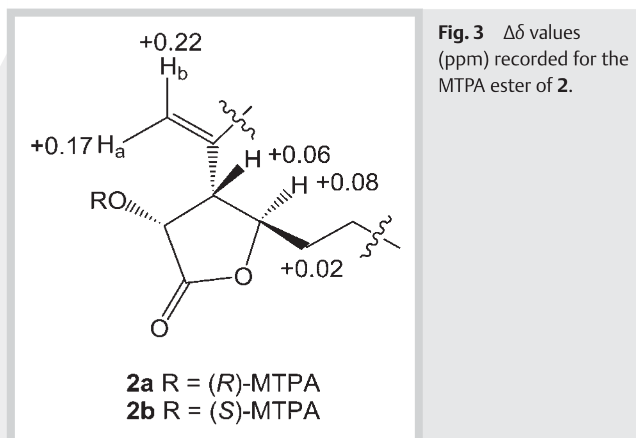

Fig. 3 Δδ values (ppm) recorded for the MTPA ester of **2**.

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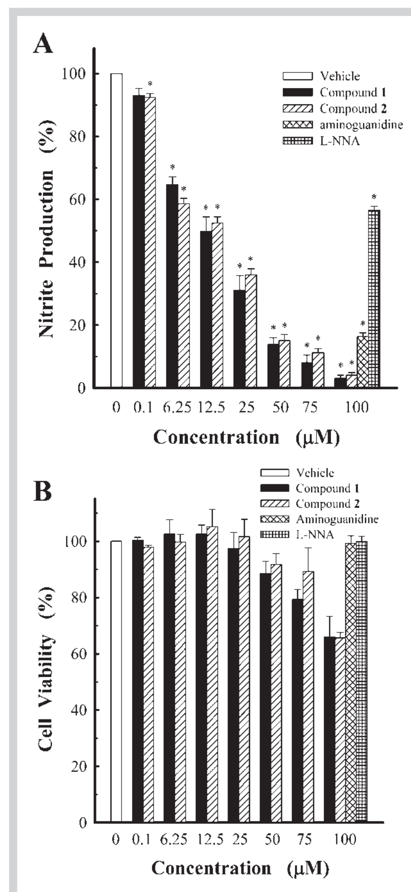

Fig. 4 The effects of the compounds **1** and **2** isolated from *Neosartorya* sp. (0.1–100 μM) on nitrite production (**A**) and cell viability (**B**) in LPS-activated RAW 264.7 cells. Vehicle representing 100% is equal to 57.6 ± 1.4 μM of nitrite produced in the medium per well. N = 6 in each group, * p < 0.05 when compared with vehicle-treated cell.

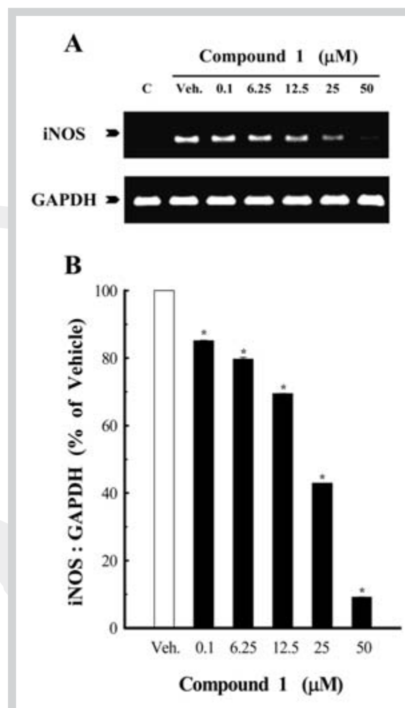

Fig. 5 Effects of compound **1** on LPS-induced iNOS mRNA expression. One of three representative RT-PCR experiments is shown (**A**). Results were generated as integrated intensity units by densitometry and expressed as percentage of vehicle treatment (**B**). When no S. E. is shown, it was smaller than the symbol for the mean. * P < 0.05 when compared with vehicle-treated cells.

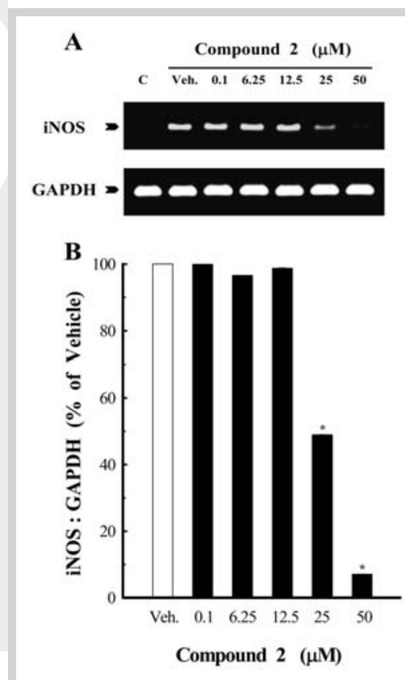

Fig. 6 Effects of compound **2** on LPS-induced iNOS mRNA expression. One of three representative RT-PCR experiments is shown (**A**). Results were generated as integrated intensity units by densitometry and expressed as percentage of vehicle treatment (**B**). When no S. E. is shown, it was smaller than the symbol for the mean. * P < 0.05 when compared with vehicle-treated cells.

solute configurations of C-2, C-3 and C-4 were determined to be *R*, *S* and *R*, respectively.

During infection and inflammation, the overexpression of iNOS may be intimately involved in the pathogenesis of many diseases and play a key role in the regulation of immune responses. At the site of an acute inflammatory reaction, all the conditions were met for the generation of NO and for a role of this compound as an inflammatory mediator. To investigate the anti-inflammatory effects of the γ-lactone derivatives from *Neosartorya* sp., **1** and **2** were tested with regard to their activities on iNOS and cell viability in LPS-induced RAW 264.7 cells, a murine macrophage-like cell line. When LPS (200 ng/mL) was added to RAW 264.7 cells, NO production was dramatically increased to 54–60 μM for the 24-h incubation period. Vehicle did not affect the NO production induced by LPS. As shown in **Fig. 4 A**, when compounds **1** and **2** (0.1–100 μM) were incubated together with LPS for 24 h, these extracts significantly attenuated NO production in a concentration-related manner, with IC_{50} values of 12.2 ± 1.5 and 11.4 ± 1.0 μM, respectively. Under the same conditions, the positive controls aminoguanidine, a selective iNOS inhibitor, and L-NNA, a nonselective NOS inhibitor, exhibited an inhibition of 83.6 ± 1.2% (IC_{50} = 27.5 ± 0.4 μM) and 43.5 ± 1.3% (IC_{50} = 145.2 ± 16.7 μM), respectively, similar to our previous report [9,10].

To investigate this suppression mechanism of the compounds, the mRNA expression of iNOS was further analyzed. The levels of iNOS and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA expression were measured by semiquantitative RT-PCR in activated RAW 264.7 cells. According to our preliminary RT-PCR experiments, the most increases of iNOS mRNA expression were obtained after LPS stimulation for 6 h. As shown in **Fig. 5** and **Fig. 6**, in the resting RAW 264.7 macrophages, the expression of iNOS mRNA was hardly detectable while it was dramatically induced in cultures treated with LPS at 6 h of incubation period. Compounds **1** and **2** suppressed the LPS-induced iNOS mRNA levels in a concentration-dependent manner without affecting mRNA expression for GAPDH, a house-keeping gene product. In general, the results are consistent with the profile of the inhibitory effect of both compounds on NO release.

The inhibitory effect on iNOS mRNA expression could account, at least in part, for the suppression of NO production.

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The Alamar blue assay was used to examine whether the amount of the test specimens used in this study caused cell damage. Our results reveal that compounds **1** and **2** showed an impairment of cell viability at considerably higher concentrations than 50 µM. The reductions in mitochondrial reductase activity by compound **1**, at the concentrations of 75 µM and 100 µM, were noted as 20.6 ± 3.5 % and 33.9 ± 3.2 %, respectively (**Fig. 4 B**). Compound **2** showed a similar interference, with 65.7 ± 1.89 % survival, of macrophages at the concentration of 100 µM used. Our results indicated that both positive controls aminoguanidine (100 µM) and L-NNA (100 µM) had no adverse effects on the growth of RAW 264.7 macrophages in the presence of LPS (200 ng/mL) (**Fig. 4 B**).

The anti-inflammatory effect of *Neosartorya* sp. is related to modulation of iNOS expression which could be linked to the presence of the derived active compounds **1** and **2**. Both compounds were able to inhibit the iNOS-mediated NO signaling pathway upon LPS stimulation. Such an effect could contribute to protection against the endotoxin-induced response in activated macrophages by modulating the expression and activity of iNOS.

Acknowledgements

This research was supported mainly by the grant (98CGH-TMU-08) to T. H. L. and by the grants to G. J. W. (NSC 96-2320-B-077-003-MY3 and 98-DBCMR-03).

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References

1 *Hibbs Jr JB, Taintor RR, Vavrin Z.* Macrophage cytotoxicity: role for L-arginine deiminase and imino nitrogen oxidation to nitrite. Science 1987; 235: 473–476
2 *Jiang JS, Shih CM, Wang SH, Chen TT, Lin CN, Ko WC.* Mechanisms of suppression of nitric oxide production by 3-*O*-methylquercetin in RAW 264.7 cells. J Ethnopharmacol 2006; 103: 281–287
3 *Kleinert H, Pautz A, Linker K, Schwarz PM.* Regulation of the expression of inducible nitric oxide synthase. Eur J Pharmacol 2004; 500: 255–266
4 *Cho H, Yun CW, Park WK, Kong JY, Kim KS, Park Y, Lee S, Kim BK.* Modulation of the acvtivity of pro-inflammatory enzymes, COX-2 and iNOS, by chrysin derivatives. Pharmacol Res 2004; 49: 37–43
5 *Yang SS, Wang GJ, Wang SY, Lin YY, Kuo YH, Lee TH.* New constituents with iNOS inhibitory activity from mycelium of *Antrodia camphorata*. Planta Med 2009; 75: 512–516
6 *Kubota T, Tsuda M, Kobayashi J.* Absolute stereochemistry of amphidinolide C. Org Lett 2001; 3: 1363–1366
7 *Wang GJ, Chen YM, Wang TM, Lee CK, Chen KJ, Lee TH.* Flavonoids with iNOS inhibitory activity from *Pogonatherum crinitum*. J Ethnopharmacol 2008; 118: 71–78
8 *Brookes D, Tidd BK, Turner WB.* Avenaciolide, an antifungal lactone from *Aspergillus avenaceus*. J Chem Soc 1963; 68: 5385–5391
9 *Wang GJ, Chen SM, Chen WC, Chang YM, Lee TH.* Selective inducible nitric oxide synthase suppression by new bracteanolides from *Murdannia bracteata*. J Ethnopharmacol 2007; 112: 221–227
10 *Lee TH, Huang NK, Lai TC, Yang ATY, Wang WJ.* Anemonin, from *Clematis crassifolia*, potent and selective inducible nitric oxide synthase inhibitor. J Ethnopharmacol 2008; 116: 518–527

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