Structures of New Phenylbutanoids and Nitric Oxide Production Inhibitors from the Rhizomes of *Zingiber cassumunar*

Seikou NAKAMURA,[a] Junko IWAMI,[a] Hisashi MATSUDA,[a] Hiroko WAKAYAMA,[a] Yutana PONGPIRIYADACHA,[b] and Masayuki YOSHIKAWA*,[a]

[a] *Kyoto Pharmaceutical University; Misasagi, Yamashina-ku, Kyoto 607–8412, Japan: and* [b] *Faculty of Science and Technology, Rajamangala University of Technology Srivijaya; Thungyai, Nakhonsithammarat 80240, Thailand.*

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The methanolic (MeOH) extract from the rhizomes of *Zingiber cassumunar* showed nitric oxide (NO) production inhibitory effects induced by lipopolysaccharide (LPS) in mouse peritoneal macrophages. From the MeOH extract, six new phenylbutanoids, phlains I—VI, were isolated together with 16 known constituents. The structures of new phenylbutanoids were determined on the basis of physicochemical and chemical evidence. In addition, the inhibitory effects of the principal constituents on the NO production were examined. Among them, phlain III (IC_{50}=24 μM), (*E*)-1-(3,4-dimethoxyphenyl)buta-1,3-diene (69 μM), (*E*)-1-(2,4,5-trimethoxyphenyl)buta-1,3-diene (83 μM), and cassumunaquinone 1 (47 μM) were found to show the inhibitory effects.

Key words *Zingiber cassumunar*; phlain; phenylbutanoid; nitric oxide production inhibitor; Zingiberaceae

The Zingiberaceae plant, *Zingiber cassumunar* (*Z. cassumunar*), is widely distributed in the Southeast Asian countries and was called as "phlai" in Thailand. The rhizomes of this plant are used as a spice and also used for treatment of asthma, bronchitis, gastrointestinal distress, *etc.*, in traditional Thai medicine. Recently, several chemical and biological studies on *Z. cassumunar* have been reported.[1—5] For example, several phenylbutanoids isolated from *Z. cassumunar* were reported to possess P-glycoprotein inhibitory,[1] cyclooxygenase-2 inhibitory,[4] and anti-inflammatory[5] effects. During the course of our characterization studies on Zingiberaceae natural medicines,[6—16] the MeOH extract from the rhizomes of *Z. cassumunar* was found to show inhibitory effects on lipopolysaccharide (LPS)-induced nitric oxide (NO) production in mouse peritoneal macrophages. From the MeOH extract, six new phenylbutanoids named phlains I—VI were isolated together with 16 known constituents. Furthermore, we examined the inhibitory effects of principal constituents on NO production induced by LPS in mouse peritoneal macrophages. In this paper, we describe the isolation and structure elucidation of the new constituents (**1**—**6**) and the NO production inhibitory effects of principal constituents from the rhizomes of *Z. cassumunar*.

The rhizomes of *Z. cassumunar* were extracted with MeOH. The MeOH extract (24.1% from the rhizomes) was partitioned into an EtOAc–H_2O (1 : 1, v/v) mixture to furnish an EtOAc-soluble fraction (16.7%) and an aqueous phase (7.4%). The MeOH extract (IC_{50}=11 μg/ml) and the EtOAc-

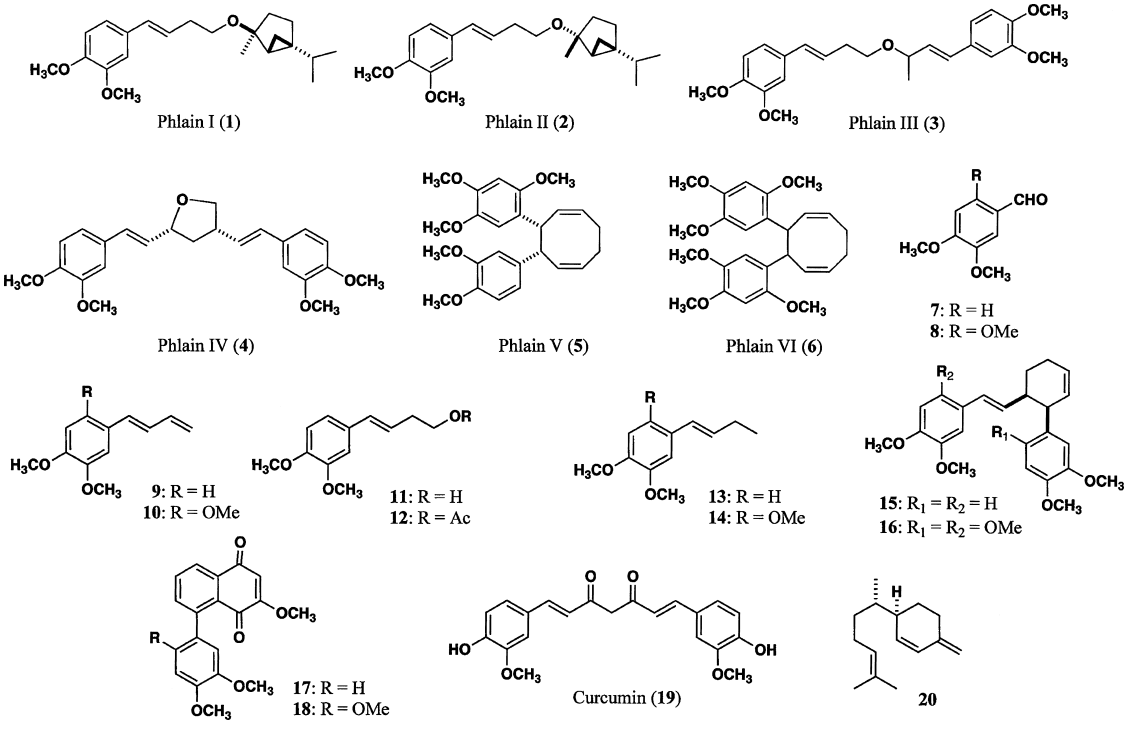

Chart 1. Structures of New Constituents from the Rhizomes of *Zingiber cassumunar*

* To whom correspondence should be addressed. e-mail: myoshika@mb.kyoto-phu.ac.jp

soluble fraction (IC_{50}=10 μg/ml) were found to show NO production inhibitory effects, while the H_2O-soluble fraction did not show the effect. The EtOAc-soluble fraction was subjected to normal-phase and reversed-phase column chromatographies, and finally HPLC to give phlains I (**1**, 0.024% from the rhizomes), II (**2**, 0.0079%), III (**3**, 0.0098%), IV (**4**, 0.0017%), V (**5**, 0.013%), and VI (**6**, 0.045%) together with 16 known compounds, 3,4-dimethoxybenzaldehyde (**7**, 0.027%),[17] 2,4,5-trimethoxybenzaldehyde (**8**, 0.010%),[17] (*E*)-1-(3,4-dimethoxyphenyl)buta-1,3-diene (**9**, 2.17%),[18] (*E*)-1-(2,4,5-trimethoxyphenyl)buta-1,3-diene (**10**, 0.20%),[18] (*E*)-4-(3,4-dimethoxyphenyl)but-3-en-1-ol (**11**, 0.75%),[19] (*E*)-4-(3,4-dimethoxyphenyl)but-3-enyl acetate (**12**, 0.58%),[20] (*E*)-1-(3,4-dimethoxyphenyl)but-1-ene (**13**, 0.066%),[21] (*E*)-1-(2,4,5-trimethoxyphenyl)but-1-ene (**14**, 0.035%),[21] (±)-*cis*-3-(3,4-dimethoxyphenyl)-4-[(*E*)-3,4-dimethoxystyryl]cyclohex-1-ene (**15**, 0.62%),[21] (±)-*cis*-3-(2,4,5-trimethoxyphenyl)-4-[(*E*)-2,4,5-trimethoxystyryl]cyclohex-1-ene (**16**, 0.0049%),[21] cassumunaquinone 1 (**17**, 0.0077%),[19] cassumunaquinone 2 (**18**, 0.0083%),[19] curcumin (**19**, 0.22%),[22] (−)-β-sesquiphellandrene (**20**, 0.17%),[23] vanillic acid (0.071%),[24] and β-sitosterol (0.073%).[25]

Structures of Phlains I—VI (1—6) Phlains I (**1**) and II (**2**) were obtained as a pale yellow oil with negative optical rotation (**1**: $[\alpha]_D^{26}$ −10.4°; **2**: $[\alpha]_D^{24}$ −5.1° in $CHCl_3$), respectively. The IR spectra of **1** and **2** showed absorption bands due to an aromatic ring (**1**: 1603, 1514 cm^{-1}; **2**: 1600, 1509 cm^{-1}) and an ether function (**1** and **2**: 1030 cm^{-1}). The UV spectra of **1** and **2** also indicated an absorption band due to an aromatic ring (**1**: 261 nm; **2**: 264 nm). The common molecular formula $C_{22}H_{32}O_3$ of **1** and **2** was determined from EI-MS (*m/z* 344 $[M]^+$) and by high-resolution (HR) EI-MS measurement. The ^{1}H- ($CDCl_3$) and ^{13}C-NMR (Table 1) spectra of **1** and **2**, which were assigned by various NMR experiments,[26] showed signals assignable to five methyls [**1**: δ 0.88, 0.92 (3H each, both d, *J*=6.8 Hz, H_3-9″, 10″, interchangeable), 1.29 (3H, s, H_3-7″), 3.87, 3.89 (3H each, both s, CH_3O-4′, 3′); **2**: δ 0.87, 0.96 (3H each, both d, *J*=6.8 Hz, H_3-9″, 10″, interchangeable), 1.25 (3H, s, H_3-7″), 3.87, 3.89 (3H each, both s, CH_3O-4′, 3′)], two olefinic protons [**1**: δ 6.12 (1H, td, *J*=6.9, 15.8 Hz, H-3), 6.39 (1H, d, *J*=15.8 Hz, H-4) **2**: δ 6.14 (1H, td, *J*=6.8, 15.8 Hz, H-3), 6.38 (1H, d, *J*=15.8 Hz, H-4)], and three *ortho*- and *meta*-coupled aromatic protons [**1**: δ 6.80 (1H, d, *J*=8.2 Hz, H-5′), 6.88 (1H, dd, *J*=2.1, 8.2 Hz, H-6′), 6.91 (1H, d, *J*=2.1 Hz, H-2′) **2**: δ 6.80 (1H, d, *J*=8.2 Hz, H-5′), 6.86 (1H, dd, *J*=1.6, 8.2 Hz, H-6′), 6.91 (1H, d, *J*=1.6 Hz, H-2′)]. The proton and carbon signals due to the phenylbutenol moieties (C-1—C-4, C-1′—C6′) in the ^{1}H- and ^{13}C-NMR spectra of **1** and **2** were superimposable on those of (*E*)-4-(3,4-dimethoxyphenyl)but-3-en-1-ol (**11**),[19] whereas the signals (C-1″—C-10″) due to the monoterpene moieties were very similar to those of sabinene hydrate.[27,28] As shown in Fig. 1, the double quantum filter correlation spectroscopy (DQF COSY) experiment on **1** and **2** indicated the presence of partial structures written in bold lines, and in the heteronuclear multiple bond connectivity spectroscopy (HMBC) experiment, long-range correlations were observed between the following protons and carbons: H-1 and C-3, 1″; H-2 and C-4; H-3 and C-1; H-4 and C-2, 2′, 6′; H-2′ and C-4; H-5′ and C-3′; H-6′ and C-4, 4′; H-2″ and C-6″; H-3″ and C-5″; H-5″ and C-1″, 2″, 3″; H-7″ and C-1″, 2″, 6″; H-8″ and C-5″; 3′-OC$\underline{H}_3$ and C-3′; 4′-OC$\underline{H}_3$ and C-4′. On the basis of these evidence, the planar structures of **1** and **2** were characterized to be (*E*)-4-(3,4-dimethoxyphenyl)but-3-en-1-ol conjugated with sabinene hydrate. The relative stereostructures of **1** and **2** were characterized by nuclear Overhauser enhancement spectroscopy (NOESY) experiment, which showed NOE correlations between the following proton pairs [**1**: H-2″ and H-3″α, H_3-7″; H-3″β and H-6″β; H-6″α and H_3-7″; **2**: H-2″ and H-3″α; H-3″β and H_3-7″]. Consequently, phlains I (**1**) and II (**2**) were determined to be the β- and α-stereoisomers at the 1″-position of the sabinene hydrate as shown.

Phlain III (**3**), obtained as a pale yellow oil with positive optical rotation ($[\alpha]_D^{20}$ +5.8° in $CHCl_3$), showed absorption bands due to an aromatic ring (1610, 1508 cm^{-1}) and an ether function (1028 cm^{-1}) in the IR spectrum. The HR-EI-MS analysis revealed the molecular formula of **3** to be $C_{24}H_{30}O_5$. The ^{1}H- ($CDCl_3$) and ^{13}C-NMR (Table 1) spectra[26] of **3** showed signals assignable to five methyls [δ 1.35

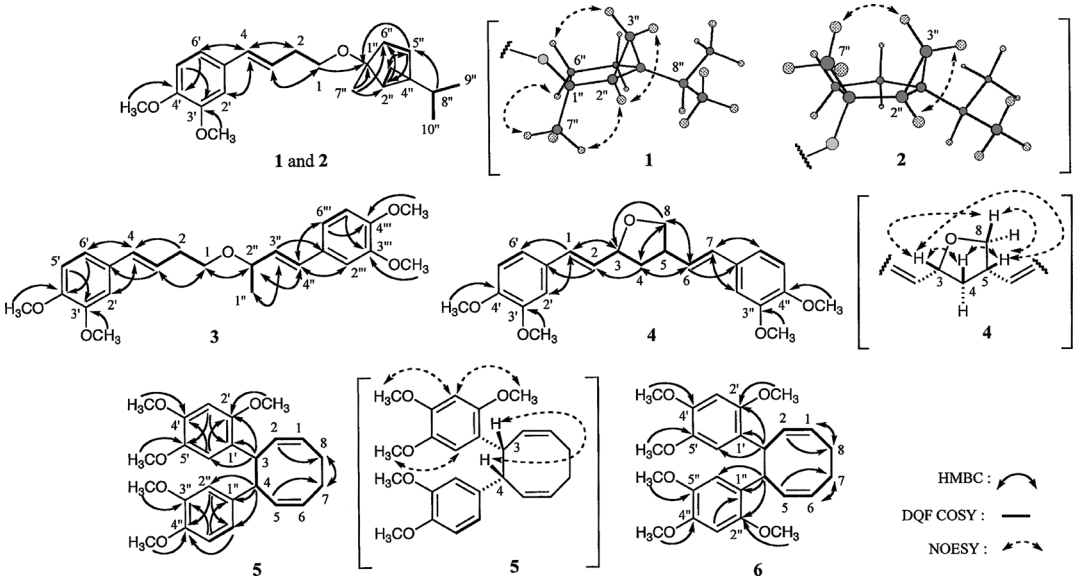

Fig. 1. Selected HMBC and NOE Correlations

(3H, d, J=6.9 Hz, H_3-1″), 3.86, 3.87, 3.88, 3.88 (3H each, all s, C$\underline{H}_3$O-3′, 4′, 3‴, 4‴, interchangeable)], a methylene bearing an oxygen function [δ 3.48 (1H, td like, J=6.9, 8.9 Hz, H-1a), 3.60 (1H, td like, J=4.8, 8.9 Hz, H-1b)], a methine bearing an oxygen function [δ 4.02 (1H, qd, J=6.9, 7.6 Hz, H-2″)], four olefinic protons [δ 5.99 (1H, dd, J=7.6, 15.8 Hz, H-3″), 6.10 (1H, td, J=6.8, 15.8 Hz, H-3), 6.39 (1H, d, J=15.8 Hz, H-4), 6.46 (1H, d, J=15.8 Hz, H-4″)], and six *ortho*- and *meta*-coupled aromatic protons [δ 6.79 (1H, d, J=8.2 Hz, H-5′), 6.81 (1H, d, J=8.3 Hz, H-5‴), 6.86 (1H, dd, J=2.0, 8.2 Hz, H-6′), 6.90 (1H, d, J=2.1 Hz, H-2‴), 6.91 (1H, dd, J=2.1, 8.3 Hz, H-6‴), 6.94 (1H, d, J=2.0 Hz, H-2′)]. As shown in Fig. 1, the DQF COSY experiment indicated the presence of partial structures written in bold lines, and in the HMBC experiment, long-range correlations were observed between the following protons and carbons: H-1 and C-3, 2″; H-2 and C-4; H-3 and C-1, 1′; H-4 and C-2′, 6′; H-2′ and C-4; H-5′ and C-3′; H-6′ and C-4, 4′; H-1″ and C-3″; H-2″ and C-4″; H-3″ and C-1‴; H-4″ and C-2″, 2‴, 6‴; H-2‴ and C-4″; H-5‴ and C-3‴; H-6‴ and C-4″, 4‴. Consequently, the dimeric phenylbutene structure of phlain III (**3**) was determined to be as shown.[29]

Phlain IV (**4**), obtained as a pale yellow oil with positive optical rotation ($[\alpha]_D^{24}$ +8.3° in $CHCl_3$), showed absorption bands due to an aromatic ring (1601, 1516 cm^{-1}) and an ether function (1026 cm^{-1}) in the IR spectrum. The HR-EI-MS analysis revealed the molecular formula of **4** to be $C_{24}H_{28}O_5$. The ^{1}H- ($CDCl_3$) and ^{13}C-NMR (Table 1) spectra[26] of **4** showed signals assignable to four methoxy groups [δ 3.88, 3.88, 3.89, 3.89 (3H each, all s, C$\underline{H}_3$O-3′, 4′, 3″, 4″, interchangeable)], a methylene bearing an oxygen function [δ 3.72 (1H, dd-like, J=8.9, 8.9 Hz, H-8α), 4.07 (1H, dd-like, J=8.3, 8.9 Hz, H-8β)], a methine bearing an oxygen function [δ 4.57 (1H, m, H-3)], four olefinic protons [δ 6.03 (1H, dd, J=8.2, 15.8 Hz, H-6), 6.13 (1H, dd, J=6.8, 15.8 Hz, H-2), 6.40 (1H, d, J=15.8 Hz, H-7), 6.56 (1H, d, J=15.8 Hz, H-1)], and six *ortho*- and *meta*-coupled aromatic protons [δ 6.80, 6.81 (1H each, both d, J=8.2 Hz, H-5′, 5″, interchangeable), 6.91 (1H, d-like, J=1.5 Hz, H-2″), 6.92 (2H, dd-like, J=1.5, 8.2 Hz, H-6′, 6″), 6.97 (1H, d-like, J=1.5 Hz, H-2′)]. The DQF COSY experiment indicated the presence of partial structures written in bold lines, and in the HMBC experiment, long-range correlations were observed between the following protons and carbons: H-1 and C-3, 2′, 6′; H-2 and C-1′; H-3 and C-1; H-4 and C-2, 6, 8; H-6 and C-4, 8, 1″; H-7 and C-2″, 6″; H-8 and C-3, 4, 6, so that the planar structure of a dimeric phenylbutene **4** was clarified as shown in Fig. 1. Next, the stereostructure of the hydrofuran part in **4** were characterized by NOESY experiment, which showed NOE correlations between the following proton pairs: H-3 and H-4β, H-5, H-8β; H-4β and H-5; H-5 and H-8β. Consequently, the structure of phlain IV was determined as shown.

Phlain V (**5**), obtained as a colorless oil, showed absorption bands due to an aromatic ring (287 nm; 1608, 1510 cm^{-1}) in the UV and IR spectra. The HR-EI-MS analysis revealed the molecular formula of **5** to be $C_{25}H_{30}O_5$. The ^{1}H- ($CDCl_3$) and ^{13}C-NMR (Table 1) spectra[26] of **5** showed signals assignable to five methoxy groups [δ 3.57, 3.60, 3.67, 3.81, 3.85 (3H each, all s, C$\underline{H}_3$O-5′, 2′, 3″, 4′, 4″)], four olefinic protons [δ 5.52 (1H, ddd like, J=2.2, 5.5, 11.6 Hz, H-2), 5.63 (1H, ddd like, J=1.6, 5.5, 12.0 Hz, H-5), 5.70 (1H, m, H-1), 5.76 (1H, m, H-6)], and five aromatic protons [δ 6.20 (1H, s, H-6′), 6.32 (1H, d, J=1.6 Hz, H-2″), 6.38 (1H, dd, J=1.6, 8.2 Hz, H-6″), 6.43 (1H, s, H-3′), 6.65 (1H, d, J=8.2 Hz, H-5″)]. As shown in Fig. 1, the DQF COSY experiment on **5** indicated the presence of partial structures written in bold lines, and in the HMBC experiment, long-range correlations were observed between the following protons and carbons: H-2 and C-8; H-3 and C-1′, 2′, 6′; H-4 and C-1″, 2″, 6″; H-5 and C-7; H-7 and C-8; H-8 and C-7; H-3′ and C-1′, 5′; H-6′ and C-2′, 4′; H-2″ and C-4″, 6″; H-5″ and C-1″, 3″; H-6″ and C-4″; 2′-OC$\underline{H}_3$ and C-2′; 4′-OC$\underline{H}_3$ and C-4′, 5′-OC$\underline{H}_3$ and C-5′; 3″-OC$\underline{H}_3$ and C-3″; 4″-OC$\underline{H}_3$ and C-4″. On the basis of these evidence, the planar structure of **5** was characterized. Next, the difference NOESY experiment of **5** showed NOE correlation between H-3 and H-4 (Fig. 1), so that the stereostructure of the 3- and 4-positions on **5** was determined to be *syn* form. Consequently, phlain V (**5**) was determined to be the dimmer of phenylbutene as shown.[30]

Phlain VI (**6**), obtained as a colorless oil, showed absorption bands due to an aromatic ring (291 nm; 1609, 1530 cm^{-1}) in the UV and IR spectrum. The HR-EI-MS analysis revealed the molecular formula of **6** to be $C_{26}H_{32}O_6$. The ^{1}H-

Table 1. ^{13}C-NMR (150 MHz) Data for **1**—**6** ($CDCl_3$)

Position	**1**	**2**	**3**	**4**	**5**	**6**
1	63.5	61.9	68.0	130.9	128.1	128.1
2	34.3	34.5	33.7	128.1	131.3	132.4
3	125.7	125.8	125.2	80.8	39.5	39.9
4	130.8	130.8	131.2	40.5	50.7	39.9
5				44.2	133.1	132.4
6				128.5	127.8	128.1
7				130.3	27.8	28.4
8				72.9	28.9	28.4
1′	131.0	131.0	130.8	129.8	122.8	123.4
2′	108.5	108.4	108.6	108.8	151.0	151.4
3′	148.9	148.9	148.4[a]	148.6[a]	97.1	96.9
4′	148.3	148.3	148.8[a]	148.9[a]	147.6[a]	147.4[a]
5′	111.1	111.1	111.1	111.1[b]	142.2[a]	142.1[a]
6′	118.9	119.0	119.0	119.8	113.8	113.3
2′-OMe					56.5	56.4[b]
3′-OMe	55.8	55.8	55.8[b]	55.8[c]		
4′-OMe	55.9	55.9	55.8[b]	55.8[c]	55.9[b]	56.1[b]
5′-OMe					56.3[b]	56.3[b]
1″	87.3	85.0	21.8	130.2	135.5	123.4
2″	30.3	31.5	76.8	108.5	112.8	151.4
3″	11.9	12.4	129.9	149.0[a]	147.8	96.9
4″	33.2	34.6	130.8	149.1[a]	147.3	147.4[a]
5″	24.8	26.7		111.2[b]	110.1	142.1[a]
6″	34.1	33.4		119.8	121.8	113.3
7″	24.3	21.4				
8″	32.8	32.3				
9″	19.7[a]	20.1[a]				
10″	19.5[a]	20.0[a]				
2″-OMe						56.4[b]
3″-OMe				55.9[c]	55.6	
4″-OMe				55.9[c]	56.1	56.1[b]
5″-OMe						56.3[b]
1‴			129.7			
2‴			108.8			
3‴			149.0[a]			
4‴			149.0[a]			
5‴			111.1			
6‴			119.7			
3‴-OMe			55.8[b]			
4‴-OMe			55.9[b]			

a—*c*) May be interchangeable within the same column.

Table 2. Inhibitory Effects of Constituents on NO Production Induced by LPS in Mouse Peritoneal Macrophages

	0 μM	3 μM	10 μM	30 μM	100 μM	IC_{50} (μM)
Phlain I (**1**)	0.0±5.2	—	23.3±6.8*	60.5±3.1**	99.7±0.1**[a)]	24
Phlain III (**3**)	0.0±0.9	—	20.7±2.1**	57.5±2.4**	96.8±0.5**	24
Phlain VI (**6**)	0.0±1.5	—	5.0±5.3	33.6±1.1**	100.3±0.1**[a)]	50
3,4-Dimethoxybenzaldehyde (**7**)	0.0±2.2	—	7.3±2.5	10.3±2.1**	6.1±1.9	—
2,4,5-Trimethoxybenzaldehyde (**8**)	0.0±2.5	—	−0.6±1.7	1.8±0.7	2.3±1.4	—
(*E*)-1-(3,4-Dimethoxyphenyl)buta-1,3-diene (**9**)	0.0±2.9	—	21.4±1.0*	32.9±2.1**	64.2±1.4**	69
(*E*)-1-(2,4,5-Trimethoxyphenyl)buta-1,3-diene (**10**)	0.0±1.7	—	9.2±2.3	22.9±5.5**	57.7±2.6**	83
(*E*)-4-(3,4-Dimethoxyphenyl)but-3-en-1-ol (**11**)	0.0±1.7	—	9.8±2.4*	15.9±2.5**	35.0±1.3**	—
(*E*)-4-(3,4-Dimethoxyphenyl)but-3-enyl acetate (**12**)	0.0±2.8	—	23.9±7.0	−17.4±22.3	32.3±2.2	—
(*E*)-1-(3,4-Dimethoxyphenyl)but-1-ene (**13**)	0.0±0.9	—	0.9±3.2	11.1±5.3**	28.9±2.1**	—
(*E*)-1-(2,4,5-Trimethoxyphenyl)but-1-ene (**14**)	0.0±2.4	—	−7.4±4.6**	7.9±4.5**	31.6±1.3**	—
15	0.0±2.6	—	15.6±2.9*	49.2±5.5**	98.8±0.6**[a)]	31
Cassumunaquinone 1 (**17**)	0.0±4.2	—	20.0±13.6	39.3±3.4**	96.8±0.2**	47
Crucumin (**19**)[9)]	0.0±2.1	10.0±1.2**	36.7±3.0**	94.3±1.9**	99.5±0.0**[a)]	11
(−)-β-Sesquiphellandrene (**20**)	0.0±3.3	—	2.7±7.1	34.3±7.5	92.3±0.9**[a)]	52
Vanillic acid	0.0±2.2	—	0.4±3.4	0.6±2.6	0.5±0.1	—
β-Sitosterol	0.0±2.7	—	0.7±2.0	3.3±1.4	12.2±0.9**	—
Caffeic acid phenethyl ester (CAPE)	0.0±5.1	10.3±1.0	30.5±3.0*	94.5±0.9**	100.1±0.1**[a)]	16

Each value represents the mean±S.E.M. (n=4). Significantly different from the control, * $p<0.05$, ** $p<0.01$. *a*) Cytotoxic effect was observed.

($CDCl_3$) and ^{13}C-NMR (Table 1) spectra[26)] of **6** showed signals assignable to six methoxy groups [δ 3.49 (6H, s, C$\underline{H}_3$O-2′, 2″), 3.63, 3.83 (6H each, both s, C$\underline{H}_3$O-4′,4″, 5′,5″, interchangeable)], four olefinic protons [δ 5.56 (2H, ddd like, J=1.5, 4.9, 11.6 Hz, H-2, 5), 5.73 (2H, m, H-1, 6)], and four aromatic protons [δ 6.33 (2H, s, H-6′, 6″), 6.36 (2H, s, H-3′, 3″)]. Since these evidence and the examination of the DQF COSY and HMBC data on **6** (Fig. 1), phlain VI (**6**) was determined to be the dimmer of phenylbutene as shown.[29,30)]

Inhibitory Effects on NO Production Induced by LPS in Mouse Peritoneal Macrophages The inorganic free radical NO has been implicated in physiologic and pathologic processes, such as vasodilation, nonspecific host defense, ischemia-reperfusion injury, and chronic or acute inflammation. NO is produced by the oxidation of L-arginine by NO synthase (NOS). In the family of NOS, inducible NOS is specifically involved in pathologic aspects with the overproduction of NO and can be expressed in response to proinflammatory agents such as interleukin-1β, tumor necrosis factor-α, and LPS in various cell types including macrophages, endothelial cells, and smooth muscle cells. As a part of our studies to characterize the bioactive constituents of natural medicines, we have investigated various NO production inhibitors, phenylpropanoids,[6—8)] diarylheptanoids,[9,31,32)] *etc.* As a continuing of these studies, the inhibitory effects of the principal constituents from the rhizomes of *Z. cassumunar* on NO production induced by LPS in mouse peritoneal macrophages were examined (Table 2). Among them, phenylbutanoids, phlain III (**3**, IC_{50}=24 μM), (*E*)-1-(3,4-dimethoxyphenyl)buta-1,3-diene (**9**, IC_{50}=69 μM), (*E*)-1-(2,4,5-trimethoxyphenyl)buta-1,3-diene (**10**, IC_{50}=83 μM), and cassumunaquinone 1 (**17**, IC_{50}=47 μM) inhibited the production of NO without cytotoxic effects in the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. In particular, the effect of phlain III (**3**) was nearly equivalent to those of curcumin (**19**, IC_{50}=11 μM), which was already known to display NO inhibitory effect,[9)] and an inhibitor of nuclear factor (NF)-κB activation, caffeic acid phenethyl ester (CAPE, IC_{50}=16 μM). On the other hand, (*E*)-4-phenylbut-3-en-1-ol derivatives, **11** and **12**, (*E*)-1-phenylbut-1-ene derivatives, **13** and **14**, showed weak effects (>100 μM). With regard to structural requirements of phenylbutanoids **9**—**14** for the activity, the terminal olefins of the side chain moieties on **9** and **10** were suggested to be important.

Experimental

General Experimental Procedures The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter (l=5 cm); UV spectra, Shimadzu UV-1600 spectrometer; IR spectra, Shimadzu FTIR-8100 spectrometer; EI-MS and HR-EI-MS, JEOL JMS-GCMATE mass spectrometer; ^{1}H-NMR spectra, JEOL EX-270 (270 MHz), JNM-LA500 (500 MHz), and JEOL ECA-600K (600 MHz) spectrometers; ^{13}C-NMR spectra, JEOL EX-270 (68 MHz), JNM-LA500 (125 MHz), and JEOL ECA-600K (150 MHz) spectrometers with tetramethylsilane as an internal standard; HPLC detector, Shimadzu RID-6A refractive index detector; and HPLC column, YMC-Pack ODS-A (YMC, Inc., 250×4.6 mm i.d.) and (250×20 mm i.d.) columns were used for analytical and preparative purposes, respectively. The following experimental materials were used for chromatography: normal-phase silica gel column chromatography, Silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150—350 mesh); reversed-phase silica gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100—200 mesh); TLC, precoated TLC plates with Silica gel $60F_{254}$ (Merck, 0.25 mm) (ordinary phase) and Silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reversed phase); reversed-phase HPTLC, precoated TLC plates with Silica gel RP-18 WF_{254S} (Merck, 0.25 mm); and detection was achieved by spraying with 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4 followed by heating.

Plant Material The rhizomes of *Zingiber cassumunar* were cultivated in Nakhon Si Thammarat of Thailand in 2007, and identified by one of authors (Y. P.). A voucher specimen is on file in our laboratory.

Extraction and Isolation The rhizomes of *Zingiber cassumunar* (1.4 kg) were extracted three times with MeOH under reflux for 3 h. Evaporation of the solvent under reduced pressure provided a MeOH extract (340.0 g, 24.1% from the rhizomes). The aliquot (277.7 g) from the extract was partitioned into an EtOAc–H_2O (1 : 1, v/v) mixture to furnish an EtOAc-soluble fraction (192.9 g, 16.7%) and an aqueous phase (84.8 g, 7.4%). The aliquot (80.9 g) from the EtOAc fraction was subjected to ordinary-phase silica gel column chromatography [2.4 kg, *n*-hexane–EtOAc (20 : 1→5 : 1→2 : 1→1 : 1, v/v)→EtOAc→MeOH] to give 6 fractions [Fr. 1 (3.2 g), Fr. 2 (15.5 g), Fr. 3 (5.1 g), Fr. 4 (28.6 g), Fr. 5 (10.0 g), Fr. 6 (16.6 g)]. The aliquot (600 mg) of fraction 1 was subjected to reversed-phase silica gel column chromatography [18 g, MeOH–H_2O (70 : 30→90 : 10, v/v)→MeOH] to give 8 fractions {Fr. 1-1, Fr. 1-2 (157 mg), Fr. 1-3, Fr. 1-4, Fr. 1-5, Fr. 1-6 [=**20** (152 mg, 0.17%)], Fr. 1-7, Fr. 1-8}. Fraction 1-2 (157 mg) was purified by

HPLC [MeOH–H_2O (70 : 30, v/v)] to give **13** (61 mg, 0.066%). The aliquot (7.8 g) of fraction 2 was subjected to reversed-phase silica gel column chromatography [234 g, MeOH–H_2O (70 : 30, v/v)→MeOH] to give 3 fractions [Fr. 2-1, Fr. 2-2 [=(**9**, 5.300 g, 2.17%)], Fr. 2-3 (528 mg)]. The aliquot (295 mg) of fraction 2-3 was purified by HPLC [MeOH–H_2O (80 : 20, v/v)] to give phlain II (**2**, 11 mg, 0.0079%) and **15** (21 mg, 0.016%). The aliquot (2.5 g) of fraction 3 was subjected to reversed-phase silica gel column chromatography [75 g, MeOH–H_2O (70 : 30→90 : 10, v/v)→MeOH] to give 8 fractions {Fr. 3-1 (78 mg), Fr. 3-2 (968 mg), Fr. 3-3 (111 mg), Fr. 3-4 (345 mg), Fr. 3-5, Fr. 3-6, Fr. 3-7, Fr. 3-8 [=β-sitosterol (167 mg, 0.073%)]}. The fraction 3-1 (78 mg) was purified by HPLC [MeOH–H_2O (50 : 50, v/v)] to give **8** (0.5 mg, 0.00022%). The aliquot (484 mg) of fraction 3-2 was purified by HPLC [MeOH–H_2O (70 : 30, v/v)] to give **10** (231 mg, 0.20%) and **14** (42 mg, 0.035%). The aliquot (56 mg) of fraction 3-3 was purified by HPLC [MeOH–H_2O (80 : 20, v/v)] to give **16** (5.6 mg, 0.0049%). The aliquot (203 mg) of fraction 3-4 was purified by HPLC [MeOH–H_2O (90 : 10, v/v)] to give phlain I (**1**, 33 mg, 0.024%). The aliquot (14.2 g) of fraction 4 was subjected to reversed-phase silica gel column chromatography [426 g, MeOH–H_2O (50 : 50→70 : 30→80 : 20, v/v)→MeOH] to give 9 fractions [Fr. 4-1 [= vanillic acid (172 mg, 0.071%)], Fr. 4-2 (183 mg), Fr. 4-3 (126 mg), Fr. 4-4, Fr. 4-5 [=**12** (1.401 g, 0.58%)], Fr. 4-6, Fr. 4-7 (283 mg), Fr. 4-8 (10.008 g), Fr. 4-9]. The aliquot (93 mg) of fraction 4-2 was purified by HPLC [MeOH–H_2O (40 : 60, v/v)] to give **7** (33 mg, 0.027%) and **8** (13 mg, 0.010%). The aliquot (126 mg) of fraction 4-3 was purified by HPLC [MeOH–H_2O (40 : 60, v/v)] to give **11** (1.1 mg, 0.00046%). The aliquot (283 mg) of fraction 4-7 was purified by HPLC [MeOH–H_2O (70 : 30, v/v)] to give phlain III (**3**, 23.0 mg, 0.0098%), phlain IV (**4**, 3.9 mg, 0.0017%), phlain V (**5**, 30.4 mg, 0.013%), and phlain VI (**6**, 107 mg, 0.045%). The aliquot (450 mg) of fraction 4-8 was purified by HPLC [MeOH–H_2O (70 : 30, v/v)] to give **15** (65 mg, 0.60%). The aliquot (5.0 g) of fraction 5 was subjected to reversed-phase silica gel column chromatography [150 g, MeOH–H_2O (60 : 40→70 : 30, v/v)→MeOH] to give 4 fractions [Fr. 5-1 (2.7 g), Fr. 5-2 (497 mg), Fr. 5-3 (1.4 g), Fr. 5-4]. The aliquot (700 mg) of fraction 5-1 was purified by HPLC [MeOH–H_2O (47 : 53, v/v)] to give **11** (461 mg, 0.73%). The aliquot (297 mg) of fraction 5-2 was purified by HPLC [MeOH–H_2O (60 : 40, v/v)] to give **11** (14 mg, 0.010%), **17** (11 mg, 0.0077%), and **18** (12 mg, 0.0083%). The aliquot (700 mg) of fraction 5-3 was purified by HPLC [MeOH–H_2O (70 : 30, v/v)] to give curcumin (**19**, 249 mg, 0.21%). The aliquot (8.3 g) of fraction 6 was subjected to ordinary-phase silica gel column chromatography [249 g, *n*-hexane–EtOAc (2 : 1→1 : 1→1 : 2→1 : 5, v/v)→EtOAc→MeOH] to give 6 fractions [Fr. 6-1, Fr. 6-2, Fr. 6-3 (156 mg), Fr. 6-4 (647 mg), Fr. 6-5, Fr. 6-6]. The aliquot (78 mg) of fraction 6-3 was subjected to reversed-phase silica gel column chromatography [4.7 g, MeOH–H_2O (50 : 50→70 : 30, v/v)→MeOH] to give **11** (7.7 mg, 0.0063%) and curcumin (**19**, 6.5 mg, 0.0053%). The fraction 6-4 (647 mg) was subjected to reversed-phase silica gel column chromatography [19.4 g, MeOH–H_2O (20 : 80→40 : 60→60 : 40→80 : 20, v/v)→MeOH] to give Fr. 6-4-1, Fr. 6-4-2, Fr. 6-4-3, Fr. 6-4-4 (44 mg), Fr. 6-4-5 [=**18** (51 mg, 0.020%)], Fr. 6-4-6 (288 mg), Fr. 6-4-7, Fr. 6-4-8, Fr. 6-4-9. The fraction 6-4-4 (44 mg) was further purified by HPLC [MeOH–H_2O (40 : 60, v/v)] to give **11** (6.1 mg, 0.0025%). The fraction 6-4-6 (288 mg) was further purified by HPLC [MeOH–H_2O (68 : 32, v/v)] to give curcumin (**19**, 4.5 mg, 0.0019%).

Phlain I (**1**): Pale yellow oil; $[\alpha]_D^{26}$ −10.4° (*c*=1.66, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 261 (3.49) nm; IR (film) ν_{max} 2930, 1603, 1514, 1030 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 0.34 (1H, dd, *J*=4.1, 8.2 Hz, H-3″a), 0.68 (1H, dd, *J*=4.1, 4.1 Hz, H-3″b), 0.88, 0.92 (3H each, both d, *J*=6.8 Hz, H_3-9″, 10″, interchangeable), 1.04 (1H, dd, *J*=4.1, 8.2 Hz, H-2″), 1.29 (3H, s, H_3-7″), 1.31 (1H, m, H-8″), 1.42 (1H, m, H-6″b), 1.52 (1H, m, H-6″a), 3.55 (2H, m, H-1), 3.87, 3.89 (3H each, both s, C$\underline{H}_3$O-4′, 3′), 6.12 (1H, td, *J*=6.9, 15.8 Hz, H-3), 6.39 (1H, d, *J*=15.8 Hz, H-4), 6.80 (1H, d, *J*=8.2 Hz, H-5′), 6.88 (1H, dd, *J*=2.1, 8.2 Hz, H-6′), 6.91 (1H, d, *J*=2.1 Hz, H-2′); ^{13}C-NMR data see Table 1; EI-MS *m*/*z* 344 $[M]^+$; HR-EI-MS *m*/*z*: 344.2347 (Calcd for $C_{22}H_{32}O_3$ $[M]^+$, 344.2351).

Phlain II (**2**): Pale yellow oil; $[\alpha]_D^{24}$ −5.1° (*c*=0.88, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 264 (4.14) nm; IR (film) ν_{max} 2962, 1600, 1509, 1030 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 0.18 (1H, dd, *J*=4.2, 4.2 Hz, H-3″β), 0.36 (1H, dd, *J*=4.2, 8.2 Hz, H-3″α), 0.87, 0.96 (3H each, both d, *J*=6.8 Hz, H_3-9″, 10″, interchangeable), 1.19 (1H, dd, *J*=4.2, 8.2 Hz, H-2″), 1.25 (3H, s, H_3-7″), 1.43 (1H, m, H-8″), 3.50 (2H, m, H-1), 3.87, 3.89 (3H each, both s, C$\underline{H}_3$O-4′, 3′), 6.14 (1H, td, *J*=6.8, 15.8 Hz, H-3), 6.38 (1H, d, *J*=15.8 Hz, H-4), 6.80 (1H, d, *J*=8.2 Hz, H-5′), 6.86 (1H, dd, *J*=1.6, 8.2 Hz, H-6′), 6.91 (1H, d, *J*=1.6 Hz, H-2′); ^{13}C-NMR data see Table 1; EI-MS *m*/*z* 344 $[M]^+$; HR-EI-MS *m*/*z*: 344.2344 (Calcd for $C_{22}H_{32}O_3$ $[M]^+$, 344.2351).

Phlain III (**3**): Pale yellow oil; $[\alpha]_D^{20}$ +5.8° (*c*=0.86, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 268 (4.24) nm; IR (film) ν_{max} 2950, 1610, 1508, 1028 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 1.35 (3H, d, *J*=6.9 Hz, H_3-1″), 3.48 (1H, td like, *J*=6.9, 8.9 Hz, H-1a), 3.60 (1H, td like, *J*=4.8, 8.9 Hz, H-1b), 3.86, 3.87, 3.88, 3.88 (3H each, all s, C$\underline{H}_3$O-3′, 4′, 3‴, 4‴, interchangeable), 4.02 (1H, qd, *J*=6.9, 7.6 Hz, H-2″), 5.99 (1H, dd, *J*=7.6, 15.8 Hz, H-3″), 6.10 (1H, td, *J*=6.8, 15.8 Hz, H-3), 6.39 (1H, d, *J*=15.8 Hz, H-4), 6.46 (1H, d, *J*=15.8 Hz, H-4″), 6.79 (1H, d, *J*=8.2 Hz, H-5′), 6.81 (1H, d, *J*=8.3 Hz, H-5‴), 6.86 (1H, dd, *J*=2.0, 8.2 Hz, H-6′), 6.90 (1H, d, *J*=2.1 Hz, H-2‴), 6.91 (1H, dd, *J*=2.1, 8.3 Hz, H-6‴), 6.94 (1H, d, *J*=2.0 Hz, H-2′); ^{13}C-NMR data see Table 1; EI-MS *m*/*z* 398 $[M]^+$; HR-EI-MS *m*/*z*: 398.2085 (Calcd for $C_{24}H_{30}O_5$ $[M]^+$, 398.2093).

Phlain IV (**4**): Pale yellow oil; $[\alpha]_D^{24}$ +8.3° (*c*=0.21, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 271 (4.20) nm; IR (film) ν_{max} 2939, 1601, 1516, 1026 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 1.70 (1H, m, H-4α), 2.38 (1H, m, H-4β), 3.15 (1H, m, H-5), 3.72 (1H, dd-like, *J*=8.9, 8.9 Hz, H-8a), 3.88, 3.88, 3.89, 3.89 (3H each, all s, C$\underline{H}_3$O-3′, 4′, 3″, 4″, interchangeable), 4.07 (1H, dd-like, *J*=8.3, 8.9 Hz, H-8β), 4.57 (1H, m, H-3), 6.03 (1H, dd, *J*=8.2, 15.8 Hz, H-6), 6.13 (1H, dd, *J*=6.8, 15.8 Hz, H-2), 6.40 (1H, d, *J*=15.8 Hz, H-7), 6.56 (1H, d, *J*=15.8 Hz, H-1), 6.80, 6.81 (1H each, both d, *J*=8.2 Hz, H-5′, 5″, interchangeable), 6.91 (1H, d-like, *J*=1.5 Hz, H-2″), 6.92 (2H, dd-like, *J*=1.5, 8.2 Hz, H-6′, 6″), 6.97 (1H, d-like, *J*=1.5 Hz, H-2′); ^{13}C-NMR data see Table 1; EI-MS *m*/*z* 396 $[M]^+$; HR-EI-MS *m*/*z*: 396.1941 (Calcd for $C_{24}H_{28}O_5$ $[M]^+$, 396.1937).

Phlain V (**5**): Colorless oil; UV ($CHCl_3$) λ_{max} (log ε) 287 (3.72) nm; IR (film) ν_{max} 2934, 1608, 1510 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 3.57, 3.60, 3.67, 3.81, 3.85 (3H each, all s, C$\underline{H}_3$O-5′, 2′, 3″, 4′, 4″), 4.10 (1H, dd, *J*=5.5, 5.5 Hz, H-4), 5.13 (1H, dd, *J*=5.5, 5.5 Hz, H-3), 5.52 (1H, ddd like, *J*=2.2, 5.5, 11.6 Hz, H-2), 5.63 (1H, ddd like, *J*=1.6, 5.5, 12.0 Hz, H-5), 5.70 (1H, m, H-1), 5.76 (1H, m, H-6), 6.20 (1H, s, H-6′), 6.32 (1H, d, *J*=1.6 Hz, H-2″), 6.38 (1H, dd, *J*=1.6, 8.2 Hz, H-6″), 6.43 (1H, s, H-3′), 6.65 (1H, d, *J*=8.2 Hz, H-5″); ^{13}C-NMR data see Table 1; EI-MS *m*/*z* 410 $[M]^+$; HR-EI-MS *m*/*z*: 410.2095 (Calcd for $C_{25}H_{30}O_5$ $[M]^+$, 410.2093).

Phlain VI (**6**): Colorless oil; UV ($CHCl_3$) λ_{max} (log ε) 291 (4.30) nm; IR (film) ν_{max} 2936, 1609, 1530 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 3.49 (6H, s, C$\underline{H}_3$O-2′, 2″), 3.63, 3.83 (6H each, both s, C$\underline{H}_3$O-4′,4″, 5′,5″, interchangeable), 4.93 (2H, d, *J*=4.9 Hz, H-3, 4), 5.56 (2H, ddd like, *J*=1.5, 4.9, 11.6 Hz, H-2, 5), 5.73 (2H, m, H-1, 6), 6.33 (2H, s, H-6′, 6″), 6.36 (2H, s, H-3′, 3″); ^{13}C-NMR data see Table 1; EI-MS *m*/*z* 440 $[M]^+$; HR-EI-MS *m*/*z*: 440.2204 (Calcd for $C_{26}H_{32}O_6$ $[M]^+$, 440.2199).

NO Production from LPS-Stimulated Macrophages Inhibitory effects on the NO production by mouse macrophages were evaluated using the method reported previously.[33] Briefly, TGC-induced peritoneal exudate cells (5×10^5 cells/well) were collected from the peritoneal cavities of male ddY mice and were suspended in 100 μl of RPMI 1640 supplemented with 5% fetal calf serum (FCS), penicillin (100 units/ml) and streptomycin (100 μg/ml), and pre-cultured in 96-well microplates at 37 °C in 5% CO_2 in air for 1 h. Nonadherent cells were removed by washing with PBS, and the adherent cells were cultured in 200 μl of fresh medium containing 10 μg/ml LPS and various concentrations of test compound for 20 h. NO production in each well was assessed by measuring the accumulation of nitrite (NO_2^-) in the culture medium using Griess reagent. Cytotoxicity was determined by the MTT colorimetric assay, after 20 h incubation with test compounds. Each test compound was dissolved in dimethyl sulfoxide (DMSO), and the solution was added to the medium (final DMSO concentration was 0.5%). Inhibition (%) was calculated using the following formula and IC_{50} was determined graphically (n=4).

$$\text{inhibition (\%)} = [(A-B)/(A-C)]\times 100$$

$A-C$: NO_2^- concentration (μM)

[*A*: LPS (+), sample (−); *B*: LPS (+), sample (+); *C*: LPS (−), sample (−)]

Statistics Values are expressed as mean±S.E.M. One-way analysis of variance followed by Dunnett's test was used for statistical analysis.

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