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Diarylheptanoids from *Curcuma kwangsiensis* and their inhibitory activity on nitric oxide production in lipopolysaccharide-activated macrophages

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ABSTRACT

Eleven diarylheptanoids (**1**–**11**) were isolated from rhizomes of *Curcuma kwangsiensis*, together with seven known compounds. Their structures were elucidated by 1D and 2D NMR, circular dichroism (CD), and accurate mass measurements. Inhibitory effects of the isolated compounds on nitric oxide production in lipopolysaccaride-activated macrophages were evaluated. Compounds **1**, **2**, and **3** showed strong inhibitory activity on NO production with IC_{50} values of 3.13, 2.81 and 2.41 μM, respectively.

Curcuma kwangsiensis S.G. Lee et C.F. Ling, a perennial herbaceous plant of Zingiberaceae, spreads widely in the southwest of China, including Guangxi, Sichuan, Guangdong and Yunnan Provinces. The dried rhizome of *C. kwangsiensis* is an important crude drug, frequently listed in prescriptions of traditional Chinese medicine for the treatment of stomach trouble and 'Oketsu',[1] various syndromes caused by the obstruction of blood circulation, such as arthralgia, psychataxia, and dysmenorhea. Major secondary metabolites of genus of *Curcuma* have been reported to be rich in diarylheptanoids and sesquiterpenoids.[2–5] These substances showed various bioactivities, such as estrogenic,[6] vasorelaxant,[7] heptoprotective,[8] and antifungal activities.[9]

Our previous paper reported the isolation and characterization of eighteen diarylheptanoids from EtOH extracts of *C. kwangsiensis*.[10] In a continued search for bioactive constituents from this plant, eleven new diarylheptanoids (**1**–**11**), together with seven known ones (**7a**, **9a**, **12**–**16**) were obtained (Fig. 1). This Letter describes the isolation and structural elucidation of these new compounds, and reports the inhibitory effect of the compounds on NO production in LPS-activated macrophages.

Dried rhizomes of *C. kwangsiensis* were extracted with EtOH–H_2O (7:3, v/v) and fractionated with cyclohexane, EtOAc, and *n*-BuOH. From these extracts and by using combined chromatographic separations, eleven new and seven known compounds were isolated.[11] Their strctures were elucidated using physicochemical and spectroscopic methods. Interestingly, all isolated diarylheptanoids possessed the 3, 5-dihydroxyheptane moiety that is different from our previous studies on this plant.[10]

Compound **1**,[12] $[\alpha]_D^{25}$ = +0.20 (*c* 0.1, MeOH), was isolated as a yellowish oil. The HR-ESI-MS spectrum exhibited a unique $[M+Na]^+$ ion peak at *m/z* 355.1514 corresponding to the molecular formula of $C_{19}H_{24}O_5$ (calcd. for $[M+Na]^+$: 355.1516). The ^1H NMR spectrum of **1** revealed a 1,3,4-trisubstituted aromatic ring [δ_H 6.67 (1H, d, *J* = 8.4 Hz), 6.63 (1H, d, *J* = 2.0 Hz), and 6.50 (1H, dd, *J* = 8.4, 2.0 Hz)] and a 1,4-disubstituted aromatic ring [δ_H 6.69 (2H, d, *J* = 8.2 Hz), 7.00 (2H, d, *J* = 8.2 Hz)]. The deduction was supported by its ^{13}C NMR data, the two oxygenated aromatic rings were evidenced from the resonances at δ_C 156.5, 146.3, 144.3, 135.4, 134.6, 130.4 (×2), 120.8, 116.7, 116.4, and 116.3 (×2). ^1H NMR, ^{13}C NMR and IR spectra of **1** were similar to those of the known compound **9a**,[13] which had been obtained previously from *Tacca chantrieri*. However, distinctive differences in the ^{13}C NMR spectrum were observed between these molecules. The chemical shifts of C-2, C-4, and C-6 of **1** were upfield 0.3, 0.6, and 0.5 ppm, respectively, while those of C-3 and C-5 were downfield 2.4 ppm in the ^{13}C NMR spectrum of **1** (Fig. 1). These facts suggested that **1** is a stereoisomer of **9a**. Since **9a** was optically active ($[\alpha]_D^{25} = +1.7$, *c* 0.12, MeOH) while **1** was optically inactive, which suggested that the two hydroxyl groups in C-3 and C-5 were of the syn type.[14,15] Furthermore, the observed COSY and HMBC correlations of **1** are in agreement with the elucidated structure of **9a**. On the basis of these data, compound **1** was established as *rel*-(3*R*,5*S*)-3,5-dihydroxy-1-(3,4-dihydroxyphenyl)-7-(4-hydroxyphenyl) heptane.

Compound **2**,[16] $[\alpha]_D^{25} = +0.4$ (*c* 0.1, MeOH), was isolated as a yellowish oil. The HR-ESI-MS spectrum exhibited a unique $[M+Na]^+$

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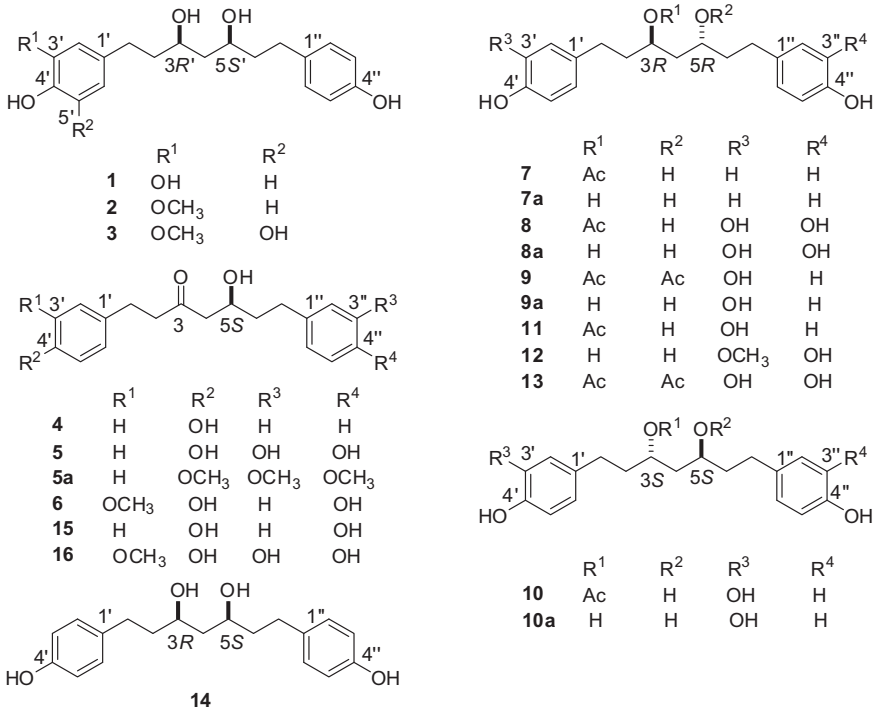

Figure 1. Structures of compounds **1**–**16**, **5a**, **7a**, **8a**, **9a**, and **10a**.

ion peak at *m/z* 369.1671 corresponding to the molecular formula of $C_{20}H_{26}O_5$ (calcd. for $[M+Na]^+$: 369.1672). The ^1H NMR spectrum of **2** revealed a 1,3,4-trisubstituted aromatic ring [δ_H 6.75 (1H, br s), 6.68 (1H, d, J = 7.7 Hz), and 6.61 (1H, br d, J = 7.7 Hz)] and a 1,4-disubstituted aromatic ring [δ_H 6.69 (2H, d, J = 8.2 Hz), 6.99 (2H, d, J = 8.2 Hz)]. The deduction was supported by its ^{13}C NMR data, the two oxygenated aromatic rings were evidenced from the resonances at δ_C 156.5, 149.0, 145.6, 135.4, 134.6, 130.4 (×2), 121.9, 116.3 (×3), and 113.3. ^1H NMR, ^{13}C NMR and IR spectra of **2** were similar to those of the known compound (3*R*,5*R*)-3,5-dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)heptane.[13] However, distinctive differences in the ^{13}C NMR spectrum were observed between these molecules. The chemical shifts of C-2, C-4, and C-6 of **2** were upfield 0.4, 0.6, and 0.2 ppm, respectively, while those of C-3 and C-5 were downfield 2.3 ppm in the ^{13}C NMR spectrum of **2** (Fig. 1). These facts suggested that **2** is a stereoisomer of the known compound. In addition, **2** was optically inactive, which suggested that the two hydroxyl groups in C-3 and C-5 were also of the syn type. On the basis of the 1D and 2D (COSY, HSQC, and HMBC) NMR data (Table 1), the structure of **2** was determined as *rel*-(3*R*,5*S*)-3,5-dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-heptane.

Compound **3**,[16] a yellowish oil, showed the molecular formula $C_{20}H_{26}O_6$, as determined by HR-ESI-MS *m/z* 363.1802 $[M+H]^+$ (calcd. for $C_{20}H_{27}O_6$, 363.1802) and NMR data. The ^1H NMR spectrum of **3** revealed a 1,3,4,5-tetrasubstituted benzene ring [δ_H 6.35 (2H, d, J = 2.2 Hz, H-2′ and H-6′)] and a 1,4-disubstituted aromatic protons [δ_H 6.71 (2H, d, J = 8.5 Hz), 7.03 (2H, d, J = 8.5 Hz)]. The ^{13}C NMR spectrum displayed 20 signals consistent with seven methylene [δ_C 32.0, 32.8, 40.8, 41.0, 45.0, and 71.0 (×2)], one methoxyl (δ_C 56.7), and 12 aromatic ring carbons, and suggesting a diarylheptanoid structure. The ^1H NMR spectrum exhibited some similarities between **3** and **2**, except that the H-5′ signal of aromatic ring was absent in **3**, being replaced by an OH group. Furthermore, the ^{13}C NMR spectrum showed a significant downfield shift for C-5′ (δ_C 146.6) compared to that of **2**. This deduction was supported by HSQC and HMBC spectra. The HMBC correlation of H-1 (δ_H 2.52, 2.62) with C-1′ (δ_C 134.7), C-2′ (δ_C 104.9), and C-6′ (δ_C 110.0), and H-7 (δ_H 2.57, 2.67) with C-1″ (δ_C 134.6), C-2″ and C-6″ (δ_C 130.4 × 2) confirmed that the 1,3,4,5-tetrasubstituted phenyl moiety was connected to C-1 and the 1,4-disubstituted phenyl moiety was connected to C-7. Additionally, **3** was optically inactive, suggesting that the relative configuration at C-3 and C-5 was also of the syn type. This deduction was supported by its ^1H and ^{13}C NMR data in the non-aromatic region (Table 1). On the basis of the 1D and 2D (COSY, HSQC, and HMBC) NMR data, the structure of **3** was determined as *rel*-(3*R*,5*S*)-3,5-dihydroxy-1-(3-methoxy-4,5-dihydroxyphenyl)-7-(4-hydroxyphenyl)heptane.

Compound **4**,[16] was obtained as a yellowish oil. The molecular formula of **4** was determined to be $C_{19}H_{22}O_3$ on the basis of HR-ESI-MS (*m/z* 316.1904 $[M+NH_4]^+$) and NMR data (Table 2). Analysis of the 1D and 2D (HMQC, HMBC) NMR spectroscopic data, the planar structure of **4** was assigned as 5-hydroxy-1-(4-hydroxyphenyl)-7-phenyl-3-heptanone.[2] The absolute configuration of the hydroxyl group at C-5 was determined by application of the circular dichroism (CD) spectrum. The absolute configuration of hydroxyl group in analogous compounds showing negative Cotton effect associated with the carbonyl $n \rightarrow \pi^*$ transition in the region of about 300 nm was determined to be 5*S*, and those showing positive Cotton effect was determined as 5*R* using CD spectra in $CHCl_3$.[17] Thus, the negative Cotton effect at 297.0 nm ($\Delta\varepsilon$ −0.050, in $CHCl_3$) in the CD spectrum suggested the *S* configuration at C-5 in compound 4, and the structure of **4** was determined (5*S*)-5-hydroxy-1-(4-hydroxyphenyl)-7-phenyl-3-heptanone.

Compound **5**,[18] was obtained as a yellowish oil. The molecular formula of **5** was determined to be $C_{19}H_{22}O_5$ on the basis of HR-ESI-MS (*m/z* 353.1358 $[M+Na]^+$) and NMR data (Table 2). Analysis of the 1D and 2D (HMQC, HMBC) NMR spectroscopic data identified the planar structure of **5** as 5-hydroxy-1-(4-hydroxyphenyl)-7-(3,4-dihydroxyphenyl)-3-heptanone.[19] Due to being less soluble in $CHCl_3$, **5** was methylated to give the corresponding trimethyl derivative **5a** (Fig. 1), with trimethylsilyldiazomethane in MeOH.[20] The CD spectrum of **5a** in $CHCl_3$ exhibited a negative Cotton effect

Table 1
^{1}H and ^{13}C NMR spectroscopic data for compounds **1**–**3** in CD_3OD

No.	**1**		**2**		**3**	
	^{1}H[a]	^{13}C[b]	^{1}H[a]	^{13}C[b]	^{1}H[a]	^{13}C[b]
1a	2.61 (1H, m)	32.2	2.65 (1H, m)[c]	32.5	2.62 (1H, m)	32.8
1b	2.51 (1H, m)		2.56 (1H, m,)[c]		2.52 (1H, m)	
2	1.68 (2H, m)[c]	41.0[e]	1.70 (2H, m)[c]	40.9[e]	1.74 (2H, m)[c]	41.0[e]
3	3.79 (1H, m)	71.1	3.75 (1H, m)	71.0	3.78 (1H, m)	71.0
4	1.60 (2H, m)	45.0	1.61 (2H, m)	45.0	1.63 (2H, m)	45.0
5	3.79 (1H, m)	71.1	3.79 (1H, m)	71.0	3.78 (1H, m)	71.0
6	1.68 (2H, m)[c]	40.9[e]	1.70 (2H, m)[c]	41.0[e]	1.67 (2H, m)[c]	40.8[e]
7a	2.64 (1H, m)	32.0	2.64 (1H, m)[c]	32.0	2.67 (1H, m)	32.0
7b	2.54 (1H, m)		2.55 (1H, m)[c]		2.57 (1H, m)	
1′		135.4		135.4		134.7
2′	6.63 (1H, d, 2.0)	116.7	6.75 (1H, br s)	113.3	6.35 (1H, d, 2.2)	104.9
3′		146.3		149.0		149.7
4′		144.3		145.6		133.2
5′	6.67 (1H, d, 8.4)	116.4	6.68 (1H, d, 7.7)	116.3		146.6
6′	6.50 (1H, dd, 8.4, 2.0)	120.8	6.61 (1H, br d, 7.7)	121.9	6.35 (1H, d, 2.2)	110.0
1″		134.6		134.6		134.6
2″	7.00 (1H, d, 8.2)[d]	130.4	6.99 (1H, d, 8.2)[d]	130.4	7.03 (1H, d, 8.5)[d]	130.4
3″	6.69 (1H, d, 8.2)[d]	116.3	6.69 (1H, d, 8.2)[d]	116.3	6.71 (1H, d, 8.5)[d]	116.2
4″		156.5		156.5		156.5
5″	6.69 (1H, d, 8.2)[d]	116.3	6.69 (1H, d, 8.2)[d]	116.3	6.71 (1H, d, 8.5)[d]	116.2
6″	7.00 (1H, d, 8.2)[d]	130.4	6.99 (1H, d, 8.2)[d]	130.4	7.03 (1H, d, 8.5)[d]	130.4
3′-OCH_3			3.81 (3H, s)	56.5	3.82 (3H, s)	56.7

[a] 600 MHz for ^{1}H NMR; the coupling constants (*J*) are in parentheses and reported in Hz; chemical shifts are given in ppm.
[b] 150 MHz for ^{13}C NMR.
[c] Overlapped signal.
[d] Pseudo-doublet.
[e] Assignments may be interchangeable within the same column.

Table 2
^{1}H and ^{13}C NMR spectroscopic data for compounds **4**–**6** in CD_3OD

No.	**4**		**5**		**6**	
	^{1}H[a]	^{13}C[b]	^{1}H[a]	^{13}C[c]	^{1}H[a]	^{13}C[c]
1	2.74 (2H, m)[d]	29.9	2.74 (2H, m)[d]	29.9	2.72 (2H, m)[d]	30.4
2	2.72 (2H, m)	46.6	2.73 (2H, m)[d]	46.5	2.71 (2H, m)[d]	46.5
3		211.9		212.2		212.3
4a	2.52 (1H, dd,4.3,15.9)	51.4	2.53 (1H, m)	51.4	2.48 (1H, m)	51.4
4b	2.57 (1H, m)		2.57 (1H, m)		2.59 (1H, m)	
5	4.02 (1H, m)	68.4	4.01 (1H, m)	68.5	3.97 (1H, m)	68.4
6	1.70 (2H, m)	40.4	1.66 (2H, m)	40.6	1.62 (2H, m)	40.6
7a	2.60 (1H, m)	33.0	2.51 (1H, m)	32.3	2.56 (1H, m)	32.0
7b	2.73 (1H, m)[d]		2.61 (1H, m)		2.60 (1H, m)	
1′		133.4		133.4		134.3
2′	6.98 (1H, d, 8.2)[e]	130.4	7.00 (1H, d, 8.4)	130.4	6.62 (1H, d, 1.9)	113.2
3′	6.67 (1H, d, 8.2)[e]	116.3	6.68 (1H, d, 8.4)	116.3		149.0
4′		156.9		156.7		145.8
5′	6.67 (1H, d, 8.2)[e]	116.3	6.68 (1H, d, 8.4)	116.3	6.66 (1H, d, 8.1)	116.3
6′	6.98 (1H, d, 8.2)[e]	130.4	7.00 (1H, d, 8.4)	130.4	6.50 (1H, dd, 1.9,8.1)	121.8
1″		143.5		135.1		134.2
2″	7.17 (1H, d, 7.4)[e]	129.6	6.71 (1H, d, 1.9)	116.7	6.95 (1H, d, 8.4)[e]	130.5
3″	7.24 (1H, t, 7.4)	129.5		146.3	6.66 (1H, d, 8.4) [e]	116.3
4″	7.13 (1H, t, 7.4)	126.9		144.4		156.4
5″	7.24 (1H, t, 7.4)	129.5	6.66 (1H, d, 8.0)	116.5	6.66 (1H, d, 8.4)[e]	116.3
6″	7.17 (1H, d, 7.4)[e]	129.6	6.57 (1H, dd, 1.9,8.0)	120.8	6.95 (1H, d, 8.4)[e]	130.5
3′-OCH_3					3.77 (3H, s)	56.5

[a] 600 MHz for ^{1}H NMR; the coupling constants (*J*) are in parentheses and reported in Hz; chemical shifts are given in ppm.
[b] 75 MHz for ^{13}C NMR.
[c] 150 MHz for ^{13}C NMR.
[d] Overlapped signal.
[e] Pseudo-doublet.

(292.6 nm), similar to **4**. The structure of **5** were therefore formulated as (5*S*)-5-hydroxy-1-(4-hydroxyphenyl)-7-(3,4-dihydroxyphenyl)-3-heptanone.

Compound **6**,[21] was obtained as a yellowish oil. The molecular formula of **6** was determined to be $C_{20}H_{24}O_5$ on the basis of HR-ESI-MS (*m*/*z* 367.1523 $[M+Na]^+$) and NMR data. Analysis of the NMR spectra, the planar structure of **6** was assigned as 5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-3-heptanone.[22] The CD spectrum of **6** in $CHCl_3$ exhibited a negative Cotton effect (296.8 nm). The structure of **6** were therefore formulated as (5*S*)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-3-heptanone.

Compound **7**,[23] was obtained as a colorless oil and gave the molecular formula $C_{21}H_{26}O_5$, on the basis of the HR-ESI-MS *m*/*z*

381.1675 [M+Na]$^+$ (calcd for $C_{21}H_{26}O_5Na$, 381.1672). The ^1H and ^{13}C NMR data of **7** in the aromatic regions were similar with those of the known compound **7a** (Fig. 1),[13] suggesting that **7** also possessed two 1,4-disubstituted benzene rings. However, distinctive differences in the ^{13}C NMR spectrum were observed between these molecules. The chemical shifts of C-2, and C-4 of **7** were upfield 3.3 and 2.6 ppm, respectively, while that of C-3 was downfield 4.3 ppm; meanwhile, an acetyl carbon signal at δ_C 21.3 and 173.3 was present in the ^{13}C NMR spectrum of **7**. Notably, the HMBC correlations of methyl protons δ_H 2.05 with the ester carbonyl carbon (δ_C 173.3) and C-3 (δ_C 73.0), and H-3 (δ_H 5.13) with the ester carbonyl carbon (δ_C 173.3) were observed, respectively. These facts suggested that **7** was the C-3 acetylated derivative of **7a**.

In order to determine the absolute configuration at C-3 and C-5, compound **7** was deacetylated with HCl/MeOH gave the corresponding 3-deacetyl derivative **7a**,[24] by comparing their ^1H and ^{13}C NMR spectroscopic data and optical rotation with the reported in the literature.[13] Therefore, compound **7** was assigned as (3*R*,5*R*)-3-acetoxy-5-hydroxy-1,7-bis(4-hydroxyphenyl)heptane.

Compound **8**,[25] was obtained as a yellowish oil. The HR-ESI-MS of **8** showed a [M+Na]$^+$ ion peak at *m/z* 413.1578 (calcd for $C_{21}H_{26}O_7Na$, 413.1571), consistent with the molecular formula $C_{21}H_{26}O_7$. The ^1H NMR spectrum of **8** showed the presence of two 1,3,4-trisubstituted benzene rings [δ_H 6.67 (1H, d, J = 8.0 Hz), 6.49 (1H, dd, J = 8.0, 3.1 Hz), and 6.59 (1H, d, J = 3.1 Hz); δ_H 6.66 (1H, d, J = 8.0 Hz), 6.46 (1H, dd, J = 8.0, 3.1 Hz), and 6.61 (1H, d, J = 3.1 Hz)]. The ^{13}C NMR spectrum displayed 21 signals consistent with seven methylene (δ_C 32.1, 32.4, 38.1, 41.1, 43.1, 68.2 and 73.0), one acetoxy (δ_C 21.2 and 173.2), and 12 aromatic ring carbons, and suggesting an acetylated diarylheptanoid structure. This deduction was supported by COSY, HSQC and HMBC spectra (Table 3). The attachment of 3,4-dihydroxyphenyl moieties at C-1 and C-7, respectively, were confirmed by the HMBC correlations of H-1 with C-1′, C-2′, and C-6′, H-2 with C-1′, H-2′/H-6′ with C-1, H-7 with C-1″, C-2″, and C-6″, H-6 with C-1″, H-2″/H-6″ with C-7. Meanwhile, **8** was hydrolyzed with HCl/MeOH to give **8a** (Fig. 1),[24] by comparing their ^1H and ^{13}C NMR spectroscopic data and optical rotation with the reported in the literature.[13] Therefore, **8** was assigned as (3*R*,5*R*)-3-acetoxy-5-hydroxy-1,7-bis(3,4-dihydroxyphenyl)heptane.

Compound **9**,[25] was obtained as a yellowish oil. The molecular formula $C_{23}H_{28}O_7$ ([M+Na]$^+$ 439.1731, calcd for 439.1727), was determined by HR-ESI-MS. The ^1H NMR spectrum of **9** displayed a 1,3,4-trisubstituted benzene ring [δ_H 6.68 (1H, d, J = 8.0 Hz), 6.61 (1H, d, J = 2.0 Hz), and 6.48 (1H, dd, J = 8.0, 2.0 Hz)] and a 1,4-disubstituted benzene ring [δ_H 6.70 (2H, d, J = 8.5 Hz) and 6.99 (2H, d, J = 8.5 Hz)]. The ^{13}C NMR spectrum showed the presence of eleven non-aromatic carbons in **9**; five methylenes (δ_C 31.9, 32.1, 37.8, 37.9, and 39.6), two acetoxy [δ_C 21.2 (×2), 172.88, and 172.90], suggesting a diarylheptanoid structure. The ^{13}C NMR data of **9** were similar to that of the known compound **9a** (Fig. 1), except that two acetyl units was present in **9**. Meanwhile, the chemical shifts of C-2, C-4, and C-6 of **9** were upfield 3.5, 6.0, and 3.5 ppm, respectively, while that of C-3 and C-5 were downfield 2.9 ppm in the ^{13}C NMR data of **9**. These facts suggested that **9** is a diacetylated derivative of **9a**. Deacetylation of **9** with HCl/MeOH gave the corresponding 3, 5-dihydroxyl derivative **9a** which was identified as (3*R*,5*R*)-dihydroxy-1-(3,4-dihydroxyphenyl)-7-(4-hydroxyphenyl)heptane,[24] by comparing their ^1H and ^{13}C NMR spectroscopic data and optical rotation with the reported in the literature.[13]

Finally, the assignment of all protons and carbons were made unambiguously by COSY, HMQC and HMBC NMR experiments (Table 3). On the basis of the above evidence, **9** was determined as (3*R*,5*R*)-3,5-diacetoxy-1-(3,4-dihydroxyphenyl)-7-(4-hydroxyphenyl)heptane.

Compounds 10 and **11**,[25] were obtained as a mixture, in a yellowish oil form, and could not be further separated in achiral stationary phase. The HR-ESI-MS spectrum exhibited a unique [M+Na]$^+$ ion peak at *m/z* 397.1618 corresponding to the molecular formula $C_{21}H_{26}O_6$ (calcd. for $C_{21}H_{26}O_6Na$, 397.1622). The 1D and 2D (HMQC, HMBC) NMR spectra of the mixture demonstrated two sets of similar resonances due to diarylheptanoid moieties, but with not identical intensities in the ^1H NMR spectrum (a ratio of **10** and **11**, about 1:2). Detailed analysis of the ^1H NMR spectrum in the aromatic region revealed that two sets of 1,3,4-trisubstituted aromatic protons [δ_H 6.65 (1H, d, J = 8.2 Hz), 6.48 (1H, dd, J = 8.2, 2.0 Hz), and 6.60 (1H, d, J = 2.0 Hz) weakly, and δ_H 6.64 (1H, d, J = 8.2 Hz), 6.46 (1H, dd, J = 8.2, 2.0 Hz), and 6.58 (1H, d, J = 2.0 Hz) intensely, respectively] and two sets of 1,4-disubstituted aromatic protons [δ_H 6.66 (2H, d, J = 8.4 Hz) and 6.96 (2H, d, J = 8.4 Hz) weakly, and at δ_H 6.67 (2H, d, J = 8.4 Hz) and 6.98 (2H, d, J = 8.4 Hz) intensely, respectively]. Careful investigation of the ^{13}C NMR data of **10** and **11** in the nonaromatic region revealed that **10** and **11** possessed also the same monoacetylated 3,5-dihydroxyheptane moiety as **7** and **8** in comparison with those of **7** and **8**.

In the HMBC spectrum, long range correlations were observed between the proton and carbon pairs of **10** and **11**, example, HMBC correlations from H-1 to C-2, C-3, C-1′, C-2′, and C-6′, from H-7 to C-5, C-6, C-1″, C-2″, and C-6″, and from H-3 to C-1, C-2, C-4, C-5, and ester carbonyl carbon, respectively, indicating 1,3,4-trisubstituted phenyl moiety was connected to C-1, 1,4-disubstituted phenyl moiety was connected to C-7, and the acetoxyl group was connected to C-3, respectively. Therefore, all the spectroscopic data disclosed that **10** and **11** are a pair of stereoisomers, possessing the same planar structure 3-acetoxy-5-hydroxy-1-(3,4-dihydroxyphenyl)-7-(4-hydroxyphenyl)heptane. The mixture was hydrolyzed with HCl/MeOH to give deacetyl mixture (**10a** and **11a**), however, whose ^1H and ^{13}C NMR spectra displayed only a set of resonances that were identical to those of **9a**, especially, at C-3 and C-5 (δ_C 68.2 in **9a** and deacetyl mixture, unlike δ_C 71.1 in **1**) in the ^{13}C NMR spectrum. These facts suggested that the two hydroxyl groups at C-3 and C-5 in **10** and **11** were of the anti type.[13,14] In additon, the deacetyl hydrolyzate prepared from mixture of **10** and **11**,[24] exhibited positive optical rotation in MeOH, and **11a** derived from **11** should predominate in the enantiomeric mixture of **10a** and **11a**, and consequently, **11a** rather than **10a** is the same as **9a** according to the optical rotation ($[\alpha]_D^{25} = +1.7$, *c* 0.12, MeOH).[13] Therefore, the pair of diarylheptanoid enantiomers were identified as (3*S*,5*S*)-3-acetoxy-5-hydroxy-1-(3,4-dihydroxyphenyl)-7-(4-hydroxyphenyl)heptane (**10**) and (3*R*,5*R*)-3-acetoxy-5-hydroxy-1-(3,4-dihydroxyphenyl)-7-(4-hydroxyphenyl)heptane (**11**), respectively.(HPLC chromatogram of the enantioseparation of mixtures of **10** and **11** see Supplementary data.)

In addition to eleven new diarylheptanoids (**1**–**11**), seven known ones, (3*R*,5*R*)-3,5-dihydroxy-1,7-bis(4-hydroxyphenyl)heptane (**7a**),[13] (3*R*,5*R*)-3,5-dihydroxy-1-(3,4-dihydroxyphenyl)-7-(4-hydroxyphenyl)heptane (**9a**),[13] (3*R*,5*R*)-3,5-dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(3,4-dihydroxyphenyl)-heptane (**12**),[13] (3 *R*,5*R*)-3,5-diacetoxy-1,7-bis(3,4-dihydroxyphenyl)-heptane (**13**),[22] (3*R*,5*S*)-3,5-dihydroxy-1,7-bis(4-hydroxyphenyl)-heptane (**14**),[26] (5*S*)-5-hydroxy-1,7-bis(4-hydroxyphenyl)heptan-3-one (**15**),[27] and (5*S*)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(3,4-dihydroxyphenyl)heptan-3-one (**16**),[15] were isolated and identified by comparison of their spectroscopic data with those reported in the literature.

All isolated compounds were evaluated for their inhibitory effects on NO production induced by LPS in macrophages (Table 4). Cell viability was determined by the MTT method to find whether inhibition of NO production was due to cytotoxicity of the test compounds.[28] As shown in Table 4, indomethacin (IC_{50} 12.96 ± 1.16 μM) and hydrocortisone (IC_{50} 40.64 ± 3.22 μM) were used as

Table 3
^{1}H and ^{13}C NMR spectroscopic data for compounds 7–**11** in CD_3OD

No.	**7**		**8**		**9**		**10**		**11**	
	^{1}H[a]	^{13}C[b]	^{1}H[a]	^{13}C[b]	^{1}H[a]	^{13}C[b]	^{1}H[a]	^{13}C[b]	^{1}H[a]	^{13}C[b]
1	2.59 (2H, m)[g]	32.2	2.48 (2H, m)[g]	32.1	2.46 (2H, t, 7.9)	32.1	2.45 (2H, m)[g]	31.9	2.45 (2H, m)[g]	32.1[c]
2	1.88 (2H, m)	38.1	1.81 (2H, m)	38.1	1.78 (2H, m)[g]	37.8[c]	1.80 (2H, m)	38.2	1.80 (2H, m)	38.1
3	5.13 (1H, m)	73.0	5.08 (1H, m)	73.0	4.93 (1H, m)	71.6[d]	5.06 (1H, m)	73.0	5.06 (1H, m)	73.1
4a	1.78 (1H, ddd, 13.1, 10.3, 3.0)	43.1	1.71 (1H, m)	43.1	1.82 (2H, m)	39.6	1.71 (1H, m)	43.1	1.71 (1H, m)	43.1
4b	1.68 (1H, ddd, 13.1, 9.2, 3.0)		1.59 (1H, m)				1.58 (1H, m)		1.58 (1H, m)	
5	3.58 (1H, m)	68.2	3.34 (1H, m)	68.2	4.93 (1H, m)	71.7[d]	3.51 (1H, m)	68.2	3.51 (1H, m)	68.2
6	1.71 (2H, m)	41.2	1.64 (2H, m)	41.1	1.78 (2H, m)[g]	37.9[c]	1.64 (2H, m)	41.1	1.64 (2H, m)	41.2
7a	2.70 (1H, m)	31.9	2.58 (1H, ddd, 14.1, 8.8, 5.8)	32.4	2.51 (2H, t, 8.0)	31.9	2.62 (1H, m)	32.4	2.62 (1H, m)	32.2[c]
7b	2.60 (1H, m)[g]		2.48 (1H, m)[g]				2.49 (1H, m)[g]		2.49 (1H, m)[g]	
1′		133.9		134.7		134.5		135.2		134.7
2′	7.06 (1H, d, 8.4)[f]	130.4[c]	6.59 (1H, d, 3.1)	116.6[c]	6.61 (1H, d, 2.0)	116.6	6.60 (1H, d, 2.0)	116.4	6.58 (1H, d, 2.0)	116.5
3′	6.76 (1H, d, 8.4)[f]	116.2[d]		146.2		146.3		146.3		146.3
4′		156.5[e]		144.3[d]		144.5		144.3		144.5
5′	6.76 (1H, d, 8.4)[f]	116.2[d]	6.67 (1H, d, 8.0)	116.40[e]	6.68 (1H, d, 8.0)	116.5	6.65 (1H, d, 8.2)	116.7	6.64 (1H, d, 8.2)	116.6
6′	7.06 (1H, d, 8.4) [f]	130.4 [c]	6.49 (1H, dd, 8.0, 3.1)	120.7	6.48 (1H, dd, 8.0, 2.0)	120.7	6.48 (1H, dd, 8.2, 2.0)	120.8	6.46 (1H, dd, 8.2, 2.0)	120.7
1″		134.4		135.2		133.7		133.9		134.4
2″	7.04 (1H, d, 8.5)[f]	130.5[c]	6.61 (1H, d, 3.1)	116.7[c]	6.99 (1H, d, 8.5)[f]	130.4	6.96 (1H, d, 8.4)[f]	130.4	6.98 (1H, d, 8.4)[f]	130.5
3″	6.75 (1H, d, 8.5)[f]	116.3[d]		146.2	6.70 (1H, d, 8.5)[f]	116.3	6.66 (1H, d, 8.4)[f]	116.3	6.67 (1H, d, 8.4)[f]	116.2
4″		156.6[e]		144.4[d]		156.7		156.6		156.5
5″	6.75 (1H, d, 8.5)[f]	116.3[d]	6.66 (1H, d, 8.0)	116.44[e]	6.70 (1H, d, 8.5)[f]	116.3	6.66 (1H, d, 8.4)[f]	116.3	6.67 (1H, d, 8.4)[f]	116.2
6″	7.04 (1H, d, 8.5)[f]	130.5[c]	6.46 (1H, dd, 8.0, 3.1)	120.8	6.99 (1H, d, 8.5)[f]	130.4	6.96 (1H, d, 8.4)[f]	130.4	6.98 (1H, d, 8.4)[f]	130.5
3-OAc	2.05 (3H, s)	21.3	1.99 (3H, s)	21.2	1.978 (3H, s)[c]	21.2	1.972 (3H, s)	21.3	1.970 (3H, s)	21.3
		173.3		173.2		172.88[e]		173.3		173.3
5-OAc					1.981 (3H, S)[c]	21.2				
						172.90[e]				

[a] 600 MHz for ^{1}H NMR; the coupling constants (*J*) are in parentheses and reported in Hz; chemical shifts are given in ppm.
[b] 150 MHz for ^{13}C NMR.
[c,d,e] Assignments may be interchangeable within the same column.
[f] Pseudo-doublet.
[g] Overlapped signal.

Table 4
Inhibitory effect of compounds isolated from *Curcuma kwangsiensis* on NO production induced by LPS in macrophages[a]

Compound	IC_{50} ± SD (μM)	Compound	IC_{50} ± SD (μM)
1[c]	3.13 ± 0.33	**7a**	>100
2	2.81 ± 0.26	**9a**	11.49 ± 1.03
3[c]	2.41 ± 0.18	**12**	25.79 ± 2.15
4	75.71 ± 6.46	**13**[c]	7.98 ± 0.66
5	82.84 ± 6.83	**14**	>100
6	37.51 ± 2.78	**15**	46.37 ± 4.22
7	32.00 ± 3.28	**16**[c]	9.37 ± 0.89
8[c]	35.50 ± 3.41	Indomethacin[b]	12.96 ± 1.16
9	10.67 ± 1.02	Hydrocortisone[b]	40.64 ± 3.22
10,**11**[c]	9.52 ± 0.87		

[a] NO concentration of control group: 3.24 ± 0.21 μM. NO concentration of LPS-treated group: 33.46 ± 2.13 μM.
[b] Positive control.
[c] Cytotoxicity (100 μM). Other compounds show no cytotoxicity.

positive controls. Compounds **1**, **2**, and **3** showed strong inhibitory activity on NO production with IC_{50} values of 3.13, 2.81 and 2.41 μM, respectively. From the structural features of the diarylheptanoid skeleton, it was found that the stereochemistry of C-3 and C-5 could influence the inhibitory effects on NO production, and these compounds with two syn type of hydroxyl groups in C-3 and C-5 exhibited conspicuously inhibitory activity (e.g., **1**, **2**, **3**).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.07.012.

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11. The rhizomes of *C. kwangsiensis* (10 kg) were cut into approximately 2 cm pieces and repeatedly (×3) extracted with EtOH–H_2O (7:3, v/v, 100 L) for 2 h. The combined extracts were concentrated in vacuo, suspended in H_2O, and partitioned successively with cyclohexane, EtOAc, and *n*-BuOH. The EtOAc extract (65 g) was subjected to silica gel CC with $CHCl_3$–MeOH (100:1 to 0:100) to obtain nine fractions (E1–E9), which were combined according to TLC analysis. Fraction E3 (6 g) was chromatographed on a Sephadex LH-20 column with $CHCl_3$–MeOH (1:1) to give three subfractions (E31–E33). Fraction E32 (2.1 g) was purified by ODS open column chromatography [MeOH–H_2O (1:9 to 9:1)], and resolution of fraction E323 by RP-HPLC with MeOH–H_2O (3:2) to afford compound **9a** (t_R 26.8 min, 16.5 mg) and compound **3** (t_R 34.2 min, 40.6 mg). Fraction E5 (8.1 g) was subjected to ODS CC [MeOH–H_2O (2:8 to 9:1)], and subfaction E52 was separated by RP-HPLC with MeOH–H_2O (1:1) to afford compound 5 (t_R 19.8 min, 21.5 mg), compound **15** (t_R 27.1 min, 16.5 mg), and compound **4** (t_R 27.2 min, 10.6 mg). Fraction E8 (5.2 g) was subjected to a Sephadex LH-20 column [$CHCl_3$–MeOH (1:1)] to give four subfractions (E81–E84). Fraction E82 was purified by RP-HPLC [MeOH–H_2O (1:1)] to obtain compound 16 (t_R 23.3 min, 22.5 mg) and compound **6** (t_R 28.2 min, 19.7 mg). Fraction E83 was purified by RP-HPLC [MeOH–H_2O (4:6)] to obtain compound **1** (t_R 31.3 min, 36.5 mg), compound 12 (t_R 35.6 min, 26.1 mg) and compound 2 (t_R 40.2 min, 12.7 mg). The *n*-BuOH extract (82 g) was subjected to silica gel CC with $CHCl_3$–MeOH (100:2 to 0:100) to obtain eight fractions (B1–B8). Fraction B3 (15.8 g) was then subjected to Sephadex LH-20 ($CHCl_3$–MeOH, 1:1) and separated by RP-HPLC with MeOH–H_2O (7:13) to afford compound **7** (t_R 35.1 min, 11.8 mg), compound **8** (t_R 38.6 min, 13.4 mg), compound **7a** (t_R 41.5 min, 28.1 mg), and compound **14** (t_R 45.3 min, 33.2 mg). Fraction B4 (12.4 g) was purified by ODS open CC [MeOH–H_2O (1:4 to 9:1)], and fraction B43 by RP-HPLC with MeOH–H_2O (3:7) to afford the mixture of **10** and **11** (t_R 34.2 min, 40.6 mg), compound **13** (t_R 39.1 min, 14.2 mg) and compound **9** (t_R 42.5 min, 35.2 mg).
12. *rel*-(3*R*,5*S*)-3,5-Dihydroxy-1-(3,4-dihydroxyphenyl)-7-(4-hydroxyphenyl)-heptane (1): yellowish oil; $[\alpha]_D^{25}$: +0.2 (*c* 0.1, MeOH); UV λ_{max} (MeOH) nm (log ε): 280.6 (3.65); IR (KBr) ν_{max} cm^{-1}: 3319, 2941, 1610, 1514, 1449, 1367, 1239, 1112, 826; for ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz), spectroscopic data, see Table 1; HR-ESI-MS *m/z* 355.1514 (calcd. for $C_{19}H_{24}O_5Na$, 355.1516).
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21. (5*S*)-5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-3-heptanone (6): yellowish oil; $[\alpha]_D^{25}$: +12.6 (*c* 0.1, MeOH); UV λ_{max} (MeOH) nm (log ε): 282.3 (3.76); IR (KBr) ν_{max} cm^{-1}: 3414, 2938, 1694, 1605, 1515, 1451, 1371, 1273, 1152, 1117, 1033, 958, 815; CD ($CHCl_3$) λ_{max} (Δε): 296.8 (−0.047); for ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz), spectroscopic data, see Table 2; HR-ESI-MS *m/z* 367.1523 (calcd. for $C_{20}H_{24}O_5Na$, 367.1516).
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24. Hydrolysis of **7**, **8**, **9**, and mixtures of **10** and **11**: To a solution of **7** (6.0 mg) in MeOH (1.5 mL), a drop of analytical grade HCl was added. The resulting mixture was stirred at room temperature overnight and dried under vacuum. The residue was purified by preparative TLC ($CHCl_3$–MeOH, 5:1, R_f = 0.42) and yielded 7a (4.3 mg). Following the above procedure, the corresponding deacetyl derivatives (**8a**, **9a**, and mixtures of **10a** and **11a**) were prepared from **8**, **9**, and mixtures of **10** and **11**, respectively.
25. (3*R*,5*R*)-3-Acetoxy-5-hydroxy-1,7-bis(3,4-dihydroxyphenyl)heptane (**8**): yellowish oil; $[\alpha]_D^{25}$: +18.1 (*c* 0.1, MeOH); UV λ_{max} (MeOH) nm (log ε): 283.2 (3.64); IR (KBr) ν_{max} cm^{-1}: 3336, 2943, 1708, 1605, 1524, 1445, 1376, 1281, 1114, 1023, 958, 813; for ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz), spectroscopic data, see Table 3; HR-ESI-MS *m/z* 413.1578 (calcd. for $C_{21}H_{26}O_7Na$, 413.1571). (3*R*,5*R*)-3,5-diacetoxy-1-(3,4-dihydroxyphenyl)-7-(4-hydroxyphenyl)heptane (**9**): yellowish oil; $[\alpha]_D^{25}$: +17.2 (*c* 0.1, MeOH); UV λ_{max}

(MeOH) nm (log ε): 280.6 (3.48); IR (KBr) ν_{max} cm^{-1}: 3372, 2936, 1708, 1611, 1515, 1444, 1376, 1265, 1114, 1026, 957, 827; for ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz), spectroscopic data, see Table 3; HR-ESI-MS *m/z* 439.1731 (calcd. for $C_{23}H_{28}O_7Na$, 439.1727). (3*S*,5*S*)- and (3*R*,5*R*)-3-acetoxy-5-hydroxy-1-(3,4-dihydroxyphenyl)-7-(4-hydroxyphenyl)heptane (**10**) and (**11**): yellowish oil; UV λ_{max} (MeOH) nm (log ε): 280.8 (3.55); IR (KBr) ν_{max} cm^{-1}: 3320, 2942, 1708, 1610, 1514, 1444, 1376, 1261, 1113, 1024, 957, 819; for ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz), spectroscopic data, see Table 3; HR-ESI-MS *m/z* 397.1618 (calcd. for $C_{21}H_{26}O_6Na$, 397.1622).

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28. *Determination of NO production and cell viability assay:* The nitrite concentration in the medium was measured as an indicator of NO production according to the Griess reaction. Briefly, RAW264.7 cells were seeded into 96-well tissue culture plates at a density of 1×10^5 cells/well, and stimulated with 1 μg/mL of LPS in the presence or absence of compounds. After incubation at 37 °C for 24 h, 100 μL of cell-free supernatant was mixed with 100 μL of Griess containing equal volumes of 2% (w/v) sulfanilamide in 5% (w/v) phosphoric acid and 0.2% (w/v) of *N*-(1-naphthyl)ethylenediamine solution to determine nitrite production. Absorbance was measured in a microplate reader at 550 nm against a calibration curve with sodium nitrite standards. Experiments were performed in triplicate, and data are expressed as the mean ± SD of three independent experiments.[10,29,30]
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