Bioorganic & Medicinal Chemistry Letters 26 (2016) 351–354

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Isolation of bioactive biphenyl compounds from the twigs of *Chaenomeles sinensis*

Chung Sub Kim [a], Lalita Subedi [b,c], Oh Kil Kwon [a], Sun Yeou Kim [b,c], Eui-Ju Yeo [d], Sang Un Choi [e], Kang Ro Lee [a,*]

[a] Natural Products Laboratory, School of Pharmacy, Sungkyunkwan University, 2066 Seobu-ro, Jangan-gu, Suwon 16419, Republic of Korea
[b] Gachon Institute of Pharmaceutical Science, Gachon University, 191 Hambakmoero, Yeonsu-gu, Incheon 21936, Republic of Korea
[c] College of Pharmacy, Gachon University, 191 Hambakmoero, Yeonsu-gu, Incheon 21936, Republic of Korea
[d] Department of Biochemistry, College of Medicine, Gachon University, 191 Hambakmoero, Yeonsu-gu, Incheon 51936, Republic of Korea
[e] Korea Research Institute of Chemical Technology, Daejeon 34114, Republic of Korea

ARTICLE INFO

Article history:
Received 10 October 2015
Revised 26 November 2015
Accepted 4 December 2015
Available online 7 December 2015

Keywords:
Chaenomeles sinensis
Biphenyl compounds
Cytotoxicity
Anti-inflammation
NGF-potentiation

ABSTRACT

Investigation of the MeOH extract of *Chaenomeles sinensis* twigs resulted in the isolation of seven biphenyl compounds (**1**–**7**) including a new compound, chaenomin (**1**). The chemical structures of the isolated compounds were elucidated by extensive NMR data (^{1}H and ^{13}C NMR, ^{1}H–^{1}H COSY, HSQC and HMBC), specific optical rotation, and chemical reaction. Compounds **2** and **6** showed potent cytotoxic activities against four cancer cell lines (A549, SK-OV-3, SK-MEL-2, and HCT15), and compound **7** exhibited potent anti-neuroinflammatory and NGF-potentiating activity.

Chaenomeles sinensis Koehne is widely distributed in Korea, Japan, and China, and belongs to the Rosaceae family. The fruits of this woody tree have long been used in Korean traditional medicine for the treatment of various disorders such as cough, the common cold, diarrhea, inflammatory diseases, and dry beriberi, as well as to make tea, jam, and liquor.[1–3] Several triterpenes, flavonoids, lignans, sesquiterpenes, and simple phenolic compounds have been isolated from *C. sinensis*, and some of these compounds and the extract of *C. sinensis* have been shown to display antioxidant, antiviral, antidiabetic, antiacetylcholinesterase, antihyperglycemic, and antihyperlipidemic activities.[3–6]

In our continuing search for biological constituents from Korean medicinal plants, we recently reported lignan glycosides and oxylipins from the twigs of *C. sinensis* with anti-inflammatory and/or neuroprotective properties.[3,7] In the process of searching for new compounds from this source, we further isolated a new biphenyl compound (**1**), along with six known ones (**2**–**7**) (Fig. 1). The chemical structures of the isolated compounds were elucidated by extensive NMR data (^{1}H and ^{13}C NMR, ^{1}H–^{1}H COSY, HSQC, and HMBC), specific optical rotation, and chemical reaction. The isolated compounds (**1**–**7**) were evaluated for their cytotoxic, anti-neuroinflammatory, and NGF-potentiating activities. Herein, we describe the isolation and structural elucidation of bioactive constituents from *C. sinensis* and their biological activities.

Twigs of *C. sinensis* (7.0 kg) were extracted with 80% aqueous MeOH and the extract was partitioned with *n*-hexane, $CHCl_3$, EtOAc, and *n*-BuOH. The *n*-hexane (3 g) and $CHCl_3$ (15 g) layers were successively subjected to chromatography over a silica gel column, Lobar-A column, and preparative HPLC, to give one new biphenyl compound, chaenomin (**1**), together with six known biphenyl compounds (**2**–**6**). The known compounds were identified as berbekorin A (**2**),[8] aucuparin (**3**),[9] 2′-hydroxyaucuparin (**4**),[9] 2′-methoxyaucuparin (**5**),[10] 2′,4′-dimethoxyaucuparin (**6**),[11] and ε-cotonefuran (**7**)[12] by comparison with previously published spectroscopic data. Compound **6** was isolated for the first time from a natural source, although previously synthesized.[11]

Compound **1** was obtained as a colorless gum, whose molecular formula was determined to be $C_{26}H_{30}O_{10}$ from the positive-ion HRFABMS data at *m*/*z* 484.1732 $[M-H_2O]^+$ (calcd for $C_{26}H_{28}O_9$ 484.1733). The IR spectrum of **1** showed absorption bands at 3382 and 1453 cm^{-1}, which were indicative of hydroxyl group and aromatic ring, respectively. The UV maxima at 278, 267, and 230 nm indicated that **1** possessed aromatic ring. The ^{1}H NMR spectrum of **1** (Table 1) showed the presence of a 1,2,4-trisubstituted aromatic ring [δ_H 7.26 (1H, d, J = 8.8 Hz), 6.58 (1H, dd,

* Corresponding author. Tel.: +82 31 290 7710; fax: +82 31 290 7730.
E-mail address: krlee@skku.edu (K.R. Lee).

http://dx.doi.org/10.1016/j.bmcl.2015.12.004
0960-894X/

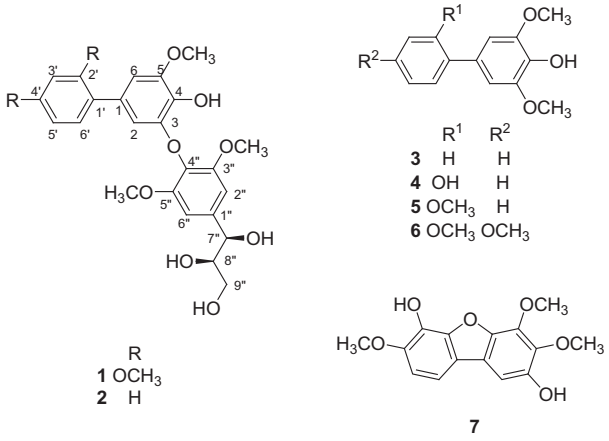

Figure 1. Structures of **1**–**7** from the twigs of *C. sinensis*.

J = 8.8, 2.1 Hz), and 6.58 (1H, d, J = 2.1 Hz)], an asymmetric 1,3,4,5-tetrasubstituted aromatic ring [δ_H 6.86 (1H, d, J = 1.7 Hz) and 6.74 (1H, d, J = 1.7 Hz)], a symmetric 1,3,4,5-tetrasubstituted aromatic ring [δ_H 6.72 (2H, s)], three oxygenated methylenes and methines [δ_H 5.02 (1H, d, J = 8.3 Hz), 4.07 (1H, ddd, J = 8.3, 3.5, 2.8 Hz), 3.93 (1H, dd, J = 12.7, 2.8 Hz), and 3.60 (1H, dd, J = 12.7, 3.5 Hz)], and five methoxy groups [δ_H 3.95 (3H, s), 3.94 (6H, s), 3.87 (3H, s), and 3.84 (3H, s)]. The ^{13}C NMR spectrum of **1** displayed 26 carbon signals including 18 aromatic carbons (from δ_C 160.2 to 99.0), three oxygenated carbons (δ_C 78.4, 76.5, and 61.6), and five methoxy carbons [δ_C 56.4 (×2), 56.2, 55.6, and 55.5]. These NMR spectra of **1** were relatively similar to those of berbekorin A (**2**),[8] except for the signals of a 1,3,4-trisubstituted aromatic ring of **1** [δ_H 7.26 (1H, d, J = 8.8 Hz), 6.58 (1H, dd, J = 8.8, 2.1 Hz), and 6.58 (1H, d, J = 2.1 Hz); δ_C 160.2, 157.4, 131.0, 123.1, 104.6, and 99.0] instead of the 1-monosubstituted aromatic ring of **2** [δ_H 7.56 (2H, dd, J = 8.0, 1.5 Hz), 7.42 (2H, td, J = 8.0, 1.5 Hz), and 7.30 (1H, tt, J = 8.0, 1.5 Hz); δ_C 141.0, 128.9 (×2), 127.3, and 127.0 (×2)], and for the presence of two more methoxy groups of **1** [δ_H 3.87 (3H, s), and 3.84 (3H, s); δ_C 55.6, and 55.5].

The location of the two methoxy groups was determined to be C-2′ and C-4′ through the HMBC cross-peaks between 2′-OCH$_3$/C-2′, 4′-OCH$_3$/C-4′, H-3′/C-1′, C-2′, and C-5′, H-5′/C-1′, C-3′, and C-4′, H-6′/C-1′, C-2′, and C-4′, and the ^{1}H–^{1}H COSY correlation of H-5′/H-6′ (Fig. 2). The additional ^{1}H–^{1}H COSY and HMBC data (Fig. 2) corroborated two partial structures, a biphenyl (unit A) and a phenylpropanoid (unit B). There were two possible linkages between units A and B, possessing 3-*O*-4″ or 4-*O*-4″ connectivity.

Since there was no observed HMBC or NOESY correlation between units A and B, methylation of **1** was performed by treatment with CH$_3$I, to yield the methyl ether **1a** (Fig. 3). The ^{1}H NMR spectrum of **1a** displayed two different signals of H-2 [δ_H 6.87 (1H, d, J = 1.9 Hz)] and H-6 [δ_H 6.75 (1H, d, J = 1.9 Hz)], indicating that the junction between units A and B was at C-3 and C-4″, because in the case possessing 4-*O*-4″ connectivity (**i**), the same chemical shifts of H-2 and H-6 were expected in the ^{1}H NMR spectrum of its methyl ether (**ia**) (Fig. 3).[11] The relative configuration between C-7″ and C-8″ was confirmed as *threo* by chemical shift difference of C-7″ and C-8″ and coupling constant. The chemical shift difference of C-7″ and C-8″ in **1** showed a relatively large value ($\Delta\delta_{C8''-C7''}$ = 2.1 ppm, in CD$_3$OD, Table 1), which was well in accordance with that of *threo*-arylglycerol moieties ($\Delta\delta_{C8''-C7''}$ > 2.0 ppm), not the *erythro*-form ($\Delta\delta_{C8''-C7''}$ < 1.0 ppm).[8] In addition, relatively large coupling constant between H-7″ and H-8″ (8.3 Hz) corroborated the configuration between C-7″ and C-8″ as *threo* (3.5–4.5 Hz, *erythro*-form; 7.5–8.0 Hz, *threo*-form).[8,13] The absolute configuration was determined as 7″*R* and 8″*R* by the negative optical rotation {$[\alpha]_D^{25}$ −20.1 (*c* 0.20, CHCl$_3$)}.[8] Thus, the structure of **1** was elucidated to be (7″*R*,8″*R*)-5,2′,4′-trimethoxy-3-syringylglyceryl-biphenyl, named chaenomin.[14]

Isolated compounds **1**–**7** were evaluated for their cytotoxic activity by determining their inhibitory effects on four cancer cell lines including A549, SK-OV-3, SK-MEL-2, and HCT15 using the SRB assay. Compounds **2** and **6** showed potent cytotoxic activities against A549, SK-OV-3, SK-MEL-2, and HCT15 cell lines with IC$_{50}$ values ranging from 1.96 to 7.84 μM (Table 2). In particular, the effect of compound **2** against the HCT15 cell line (IC$_{50}$ 1.96 μM) was better than etoposide, the positive control (IC$_{50}$ 2.95 μM). Compounds **1** and **7** displayed moderate inhibitory effects on four (A549, SK-OV-3, SK-MEL-2, and HCT15) and two (SK-OV-3 and SK-MEL-2) cancer cell lines, respectively, with IC$_{50}$ values of 12.73–29.46 μM, whereas the other compounds (**3**–**5**) were inactive (IC$_{50}$ > 30 μM). Although the chemical structures of biphenyl compounds **3**–**6** are relatively similar, only compound **6** showed a strong cytotoxic activity, suggesting that presence of a methoxy group at C-4′ may play a role in their inhibitory effects on cancer cell lines. In contrast, compound **1**, possessing a methoxy group at C-4′, was less active than compound **2**, which has no methoxy group at C-4′. The major structural difference between compounds **1**/**2** and **3**–**6** is the presence of syringylglycerol at C-3, which significantly affected their cytotoxicity.

Table 1
^{1}H (700 MHz) and ^{13}C (175 MHz) NMR data of compound **1** (δ in ppm, J values in parentheses)

Position	**1**		
	δ_H (in CDCl$_3$)	δ_C (in CDCl$_3$)	δ_C (in CD$_3$OD)
1		130.9	132.1
2	6.86, d (1.7)	111.1	112.1
3		143.9	145.4
4		131.9	133.4
5		148.2	149.6
6	6.74, d (1.7)	106.1	107.7
1′		123.1	124.6
2′		157.4	159.0
3′	6.58, d (2.1)	104.6	106.2
4′		160.2	161.9
5′	6.58, dd (8.8, 2.1)	99.0	100.2
6′	7.26, d (8.8)	131.0	132.5
1″		127.4	128.9
2″/6″	6.72, s	104.2	106.1
3″/5″		147.3	149.6
4″		135.3	137.4
7″	5.02, d (8.3)	76.5	78.1
8″	4.07, ddd (8.3, 3.5, 2.8)	78.4	80.2
9″a	3.93, dd (12.7, 2.8)	61.6	62.3
9″b	3.60, dd (12.7, 3.5)		
5-OCH$_3$	3.95, s	56.2	56.9
2′-OCH$_3$	3.84, s	55.6	56.2
4′-OCH$_3$	3.87, s	55.5	56.0
3″,5″-OCH$_3$	3.94, s	56.4	57.0

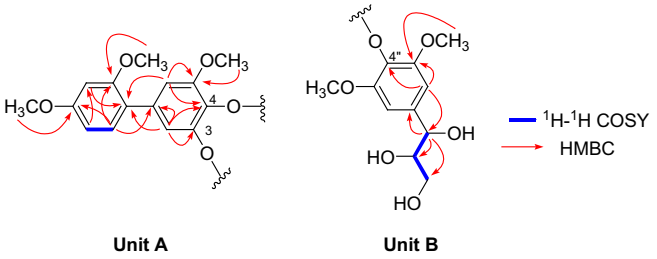

Figure 2. ^{1}H–^{1}H COSY and HMBC correlations of **1**.

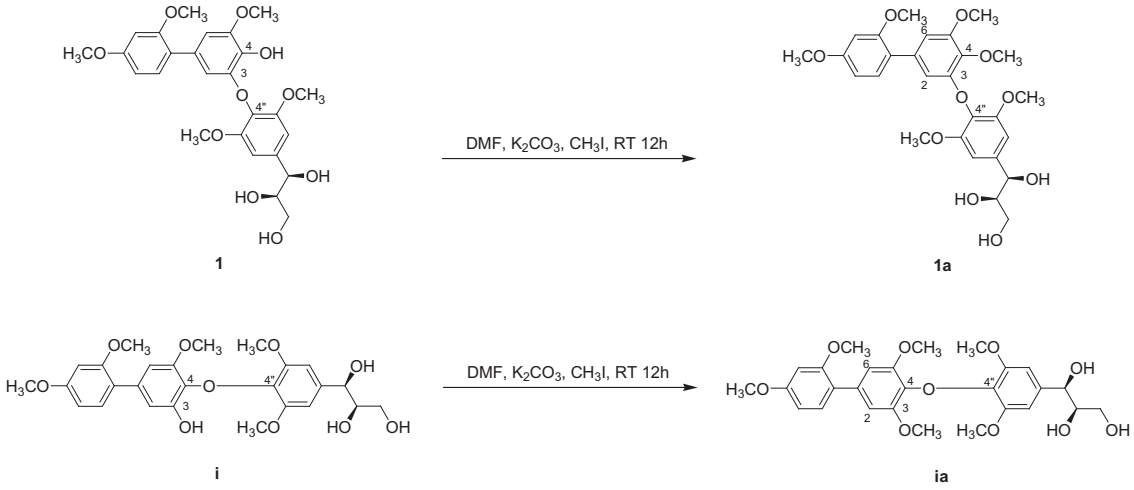

Figure 3. Methylation of the two possible structures of **1**.

Table 2
Cytotoxicity of compounds **1**, **2**, **6**, and **7** against four cultured human cancer cell lines in the SRB bioassay

Compd	IC_{50}[a] (μM)			
	A549	SK-OV-3	SK-MEL-2	HCT15
1	12.73	29.46	16.31	27.29
2	4.12	4.03	4.49	1.96
6	5.18	7.84	6.94	7.81
7	>30.0	19.28	23.51	>30.0
Etoposide[b]	1.74	1.96	1.33	2.95

[a] 50% inhibitory concentration; the concentration of compound that caused a 50% inhibition in cell growth.
[b] Etoposide as a positive control.

We also evaluated the anti-neuroinflammatory activities of the isolated compounds (**1**–**7**) by measuring NO inhibition in LPS-stimulated murine microglia BV2 cells (Table 3). Compound **7** exhibited potent inhibition of NO production with an IC_{50} value of 15.78 μM, which displayed more activity than the positive control, N^G-monomethyl-L-arginine (L-NMMA, IC_{50} 20.53 μM). Previous data showed that the protective effect of usnic acid on LPS-induced acute lung injury (ALI) in mice might relate to the suppression of excessive inflammatory responses and oxidative stress in lung tissue.[15] Our result supports fact that usnic acid, a dibenzofuran derivative found in lichen has been shown to possess anti-inflammatory activity. Compounds **1**–**6** also showed moderate NO inhibitory activity with IC_{50} values ranging from 28.09 to 50.15 μM, and there were no cytotoxic effects of any of these compounds, as assessed by a cell viability assay, at concentrations up to 20 μM. Interestingly, although the structures of **3**–**5** are very similar except for the type of substituent at C-2′ (–H, –OH, and –OCH_3, respectively), compound **5** showed the most activity, and compound **4** was more active than compound **3**. These data suggest that methoxy and hydroxy groups at C-2′ may be important for the inhibition of NO production in BV2 cells, and a previous paper supports this hypothesis.[8] We suggest that the presence of the syringylglycerol moiety at C-3 does not have an effect on the NO inhibitory activity, by comparing the activity data of **1** (IC_{50} 48.37 μM) and **2** (IC_{50} 49.29 μM), syringylglycerol linked forms of **6** and **3**, respectively, with those of **6** (IC_{50} 39.64 μM) and **3** (IC_{50} 50.15 μM).

The NGF-potentiating activities of the isolated compounds (**1**–**7**) were evaluated by determining their effects on NGF secretion in C6 cells (Table 4). Of the tested compounds (**1**–**7**) at 20 μM, compound **7** was potent stimulant of NGF release (186.49 ± 4.56%), while compounds **1**–**6** showed moderate activities, with NGF secretion levels of 125.76 ± 4.52–164.56 ± 4.98%.

In conclusion, seven biphenyl compounds (**1**–**7**) including a new compound (**1**) were isolated from the twigs of *C. sinensis*. Among the isolates (**1**–**7**), compounds **2** and **6** showed significant cytotoxic activity against four cancer cell lines (A549, SK-OV-3, SK-MEL-2, and HCT15), and compound **7** exhibited potent anti-neuroinflammatory and NGF-potentiating activities.

Table 3
Inhibitory effect of compounds **1**–**7** on NO production in LPS-activated BV-2 cells

Compd	IC_{50}[a] (μM)	Cell viability[b] (%)
1	48.37	132.10 ± 6.23
2	49.29	116.13 ± 0.67
3	50.15	126.19 ± 3.47
4	38.06	117.68 ± 0.80
5	28.09	137.60 ± 2.69
6	39.64	129.05 ± 6.60
7	15.78	197.60 ± 4.23
L-NMMA[c]	20.53	100.42 ± 5.16

[a] IC_{50} value of each compound was defined as the concentration (μM) that caused 50% inhibition of NO production in LPS-activated BV-2 cells.
[b] Cell viability after treatment with 20 μM of each compound was determined by the MTT assay and is expressed as a percentage (%). Results are the mean of three independent experiments, and the data are expressed as the mean ± SD.
[c] L-NMMA as a positive control.

Table 4
Effects of compounds **1**–**7** on NGF secretion in C6 Cells

Compd	NGF secretion (%)[a]	Cell viability (%)[b]
1	125.81 ± 5.07	115.15 ± 2.30
2	155.02 ± 2.19	119.21 ± 2.45
3	125.76 ± 4.52	116.96 ± 0.11
4	137.33 ± 1.08	118.53 ± 1.24
5	152.86 ± 0.53	117.97 ± 3.56
6	164.56 ± 4.98	119.40 ± 5.78
7	186.49 ± 4.56	112.94 ± 3.14
6-Shogaol[c]	158.18 ± 6.56	109.83 ± 4.64

[a] C6 cells were treated with 20 μM of the compounds. After 24 h, the content of NGF secreted into C6-conditioned media was measured by ELISA. The level of secreted NGF is expressed as a percentage of the untreated control (set as 100%). Data are the mean ± SD of three independent experiments performed in triplicate.
[b] Cell viability after treatment with 20 μM of each compound was determined by the MTT assay and is expressed as a percentage (%). Results are the mean of three independent experiments, and the data are expressed as the mean ± SD.
[c] 6-Shogaol as a positive control.

Acknowledgements

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2013R1A1A2A10005315). We are thankful to the Korea Basic Science Institute (KBSI) for the MS spectra measurements.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.12.004.

References and notes

1. Lee, M. H.; Son, Y. K.; Han, Y. N. *Arch. Pharm. Res.* **2002**, *25*, 842.
2. Kim, H. K.; Jeon, W. K.; Ko, B. S. *Nat. Prod. Sci.* **2000**, *6*, 79.
3. Kim, C. S.; Kwon, O. W.; Kim, S. Y.; Choi, S. U.; Kim, K. H.; Lee, K. R. *Lipids* **2014**, *49*, 1151.
4. Sawai-Kuroda, R.; Kikuchi, S.; Shimizu, Y. K.; Sasaki, Y.; Kuroda, K.; Tanaka, T.; Yamamoto, T.; Sakurai, K.; Shimizu, K. *J. Ethnopharmacol.* **2013**, *146*, 866.
5. Lee, M. H.; Han, Y. N. *Planta Med.* **2003**, *69*, 327.
6. Oku, H.; Ueda, Y.; Ishiguro, K. *Biol. Pharm. Bull.* **2003**, *26*, 1031.
7. Kim, C. S.; Subedi, L.; Kim, S. Y.; Choi, S. U.; Kim, K. H.; Lee, K. R. *J. Nat. Prod.* **2015**, *78*, 1174.
8. Kim, K. H.; Choi, S. U.; Ha, S. K.; Kim, S. Y.; Lee, K. R. *J. Nat. Prod.* **2009**, *72*, 2061.
9. Hüttner, C.; Beuerle, T.; Scharnhop, H.; Ernst, L.; Beerhues, L. *J. Agric. Food Chem.* **2010**, *58*, 11977.
10. Kokubun, T.; Harborne, J. B.; Eagles, J.; Waterman, P. G. *Phytochemistry* **1995**, *40*, 57.
11. Morimoto, K.; Sakamoto, K.; Ohnishi, Y.; Miyamoto, T.; Ito, M.; Dohi, T.; Kita, Y. *Chem. Eur. J.* **2013**, *19*, 8726.
12. Kokubun, T.; Harborne, J. B.; Eagles, J.; Waterman, P. G. *Phytochemistry* **1995**, *38*, 57.
13. Kim, K. H.; Ha, S. K.; Choi, S. U.; Kim, S. Y.; Lee, K. R. *Planta Med.* **2013**, *79*, 361.
14. Chaenomin (**1**): Colorless gum; $[\alpha]_D^{25}$ −20.1 (*c* 0.20, $CHCl_3$); IR (KBr) ν_{max} 3382, 2948, 2833, 1726, 1666, 1453, 1244, 1033, 697 cm^{-1}; UV (CH_3OD) λ_{max} (log ε) 278 (5.21), 267 (5.10), 230 (5.33) nm; ^1H and ^{13}C NMR, see Table 1; HRFABMS (positive-ion mode) *m*/*z* 484.1732 $[M-H_2O]^+$ (calcd for $C_{26}H_{28}O_9$ 484.1733).
15. Su, Z. Q.; Mo, Z. Z.; Liao, J. B.; Feng, X. X.; Liang, Y. Z.; Zhang, X.; Liu, Y. H.; Chen, X. Y.; Chen, Z. W.; Su, Z. R.; Lai, X. P. *Int. Immunopharmacol.* **2014**, *22*, 371.