http://dx.doi.org/10.5012/bkcs.2012.33.9.3079

Arginase II Inhibitory Activity of Phenolic Compounds from *Saururus chinensis*

Chae Jin Lim,[a] To Dao Cuong,[a] Tran Manh Hung,[†] Sungwoo Ryoo,[‡] Jeong Hyung Lee,[‡] Eun-Hee Kim,[§] Mi Hee Woo,[a] Jae Sue Choi,[#] and Byung Sun Min[*]

College of Pharmacy, Catholic University of Daegu, Gyeongsan, Gyeongbuk 712-702, Korea. [*]E-mail: bsmin@cu.ac.kr
[†]Faculty of Medicine and Pharmacy, Duy Tan University, Danang City, Vietnam
[‡]College of Natural Science, Kangwon National University, Kangwon 200-701, Korea
[§]Korea Basic Science Institute, Daejeon 305-806, Korea
[#]Pukyoung National University, Busan 608-737, Korea
Received April 1, 2012, Accepted May 26, 2012

Key Words : *Saururus chinensis*, Saururaceae, Lignan, Arginase

The endothelium plays a central role in overall vascular homeostasis by regulating vasoreactivity, platelet activation, leukocyte adhesion, and smooth muscle cell proliferation and migration. Endothelial nitric oxide (NO), an important vasoprotective molecule, is a major modulator of these effects, and impaired NO signaling associated with endothelial function is considered as an early marker of the atherogenic process.[1] Arginase competitively inhibits nitric oxide synthase (NOS) by using the common substrate L-arginine.[2-4] Arginase is present in two isoforms as arginase I, the hepatic isoform, and arginase II, the extrahepatic isoform, each of which is encoded by a distinct gene. Arginase activation/upregulation results in arginase/NOS imbalance, decrease NO production, and contributes to endothelial dysfunction in a number of pathophysiological processes such as aging,[2] diabetes,[5] hypertension,[6,7] and atherosclerosis.[8,9] Additionally, arginase enhances production of reactive oxygen species by eNOS. Arginase inhibition in hypercholesterolemic (ApoE(-/-)) mice or arginase II deletion (ArgII(-/-)) mice restores endothelial vasorelaxant function, reduces vascular stiffness, and markedly reduces atherosclerotic plaque burden. Furthermore, arginase activation contributes to vascular changes including polyamine-dependent vascular smooth muscle cell proliferation and collagen synthesis. Collectively, arginase may play a key role in the prevention and treatment of atherosclerotic vascular disease.[10,11]

Saururus chinensis Baill. belongs to the family Saururaceae and is an endemic species in Korea. As a traditional medicine, *S. chinensis* has been used to treat beriberi, pneumonia, edema, urination, jaundice, and gonorrhea.[12] This plant has been investigated extensively, resulting in the isolation of various lignans (sauchinone, saucerneol, manassantin A, and manassantin B), flavonoids (rutin, hyperoside, quercitrin and quercetin) and alkaloid (aristolactam BII).[13-15] Many bioactive compounds have been reported from the extract of *S. chinensis* and shown strong antioxidant activity,[16,17] and its extract has been shown to induce apoptosis through the activation of caspase-3 in prostate and breast cancer cells,[18] and to reduce lipid peroxide levels in rats fed a high-fat diet,[19] and in rats with carbon tetrachloride-induced hepatic fibrosis.[20] Saucerneol D, a lignan constituent of *S. chinensis,* efficiently inhibited melanin production,[21] and showed antioxidant and antiasthmatic effects in a mouse model of airway inflammation.[22] In our continuing investigation to discover inhibitors of arginase II, further fractionation of the EtOAc-soluble fraction resulted in the isolation of a new compound (**1**), along with nine known compounds (**2-10**). This study describes the isolation, structural elucidation of these isolates and their inhibitory arginase II activity.

The MeOH extract of the aerial part of *S. chinensis* was partitioned into *n*-hexane-, EtOAc-, and *n*-BuOH-soluble fractions and a H_2O layer. Chromatographic purification of the EtOAc-soluble fraction led to the isolation of ten compounds (**1-10**) (Fig. 1). The structures of known compounds were identified as sauchinone (**2**),[23] di-*O*-methyltetrahydrofuriguaiacin B (**3**),[24] henricine (**4**),[25] saucerneol K (**5**),[26] *meso*-dihydroguaiaretic acid (**6**),[16] guaiacin (**7**),[27] (7*S*,8*R*)-4-hydroxy-3,7-dimethoxy-1',2',3',4',5',6',7'-heptanorlign-8'-one (**8**),[28] (*E*)-7-(4-hydroxy-3-methoxyphenyl)-7-methylbut-8-en-9-one (**9**),[29] and licarin A (**10**),[30] by comparing their physiochemical and spectroscopic data with those reported in the literature.

Compound **1** was isolated as white amorphous powder, with the molecular formula $C_{20}H_{20}O_7$, as determined by the HR-EIMS at *m/z* 372.1209 for the $[M]^+$ ion (calcd. for $C_{20}H_{20}O_7$, 372.1209). The optical rotation value is −9.5 (*c* = 0.37, $CHCl_3$) and the UV spectrum exhibited λ_{max} at 244 and 297 nm ($CHCl_3$). The ^1H NMR spectra showed the presence of two aromatic protons (δ 6.43 and 7.00) and two methylenedioxy groups, one (^1H δ 5.97, 5.94; ^{13}C δ 101.8) attached to an aromatic ring and the other (^1H δ 5.67; ^{13}C δ 98.7) attached to aliphatic carbons. Furthermore, the ^1H NMR signals at δ 1.25 (d, 7.2 Hz) and 0.60 (d, 7.6 Hz) showed two methyl groups, each coupled to a vicinal proton. In addition, four methine proton signals at [δ 2.52 (H-8), 2.53 (H-1', 2.62 (H-6'), and 1.84 (H-8')], and one olefinic proton signal at [δ 5.56 (H-3')] was also observed in the ^1H NMR spectrum. The ^{13}C NMR signals at δ 143.8, 144.4, and 148.6 indicated that, in addition to a methylenedioxy group connected to aromatic ring, there is an additional oxygen attached to the

[a]These authors contributed equally to this work.

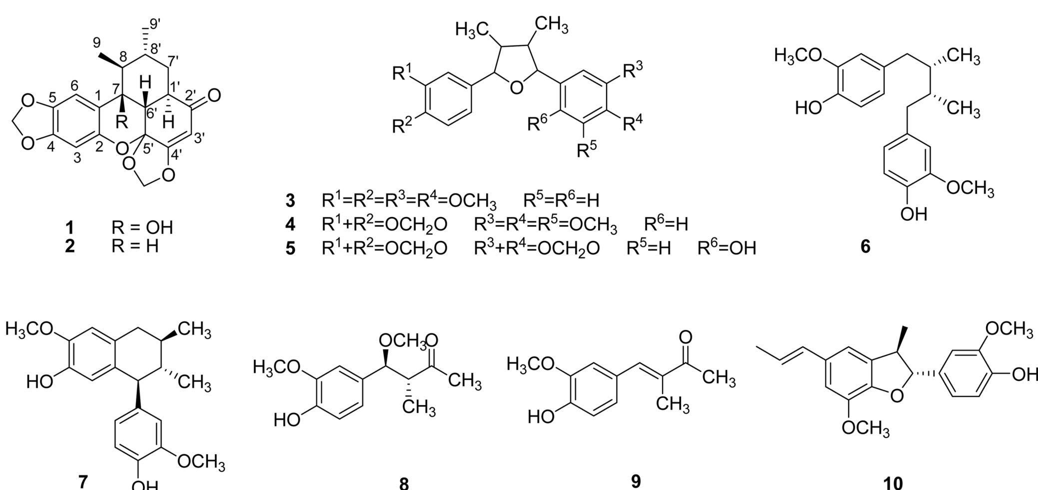

Figure 1. Chemical structure of isolated compounds (**1-10**).

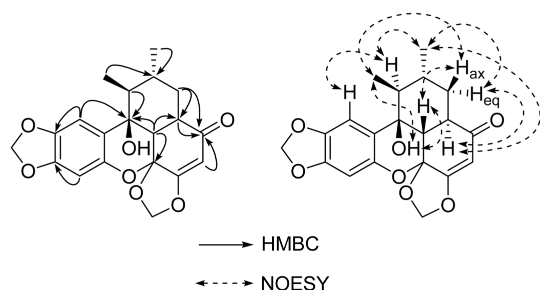

Figure 2. Selected HMBC correlations (H→C) and NOESY spectra of compound **1**.

aromatic ring, and the signal at δ 198.6 was attributed to the carbonyl group of an enone, while the ^{13}C NMR signal at δ 168.1 indicated the presence of an oxygen atom attached to the β-carbon of an enone. Comparison of ^{1}H and ^{13}C NMR spectral data between **1** and sauchinone (**2**) suggested that compound **1** was similar with **2** previously isolated from *Saururus chinensis*.[23] However, the methine proton at position C-7 of sauchinone (**2**) was substituted by a hydroxyl group at [δ_H 5.55 (1H, br s, 7-OH), δ_C 68.9], and it was further confirmed by HMBC spectrum. The HMBC spec-

Table 1. ^{1}H NMR and ^{13}C NMR spectroscopic data for compound **1** and **2**

Position	1					2	
	δ_H (ppm)^a	δ_H (ppm)^b	δ_C (ppm)^c	HMBC	NOESY	δ_H (ppm)^b	δ_C (ppm)^c
1			119.1				115.8
2			144.4				145.0
3	6.64 (s)	6.43 (s)	99.3	C-1, C-2, C-4, C-5		6.40 (s)	99.3
4			143.8				143.3
5			148.7				146.7
6	7.36 (s)	7.00 (s)	106.0	C-1, C-2, C-4, C-7		6.85 (s)	106.6
7			68.9			3.05 (d, 4.8)	35.1
8	2.68 (br q, 7.2)	2.52 (m)	40.2	C-7, C-9, C-7', C-9'		2.45 (m)	34.8
9	1.46 (d, 7.2)	1.25 (d, 7.2)	16.8	C-7, C-8, C-8'	7-OH, 7'-H$_{ax}$	1.22 (d, 7.6)	21.3
1'	2.70 (td, 3.6, 12.6)	2.53 (td, 4.0, 14.2)	40.5	C-7, C-2', C-3', C-6'	9'-CH$_3$, 7'-H$_{eq}$	2.55 (td, 2.8, 12.0)	37.6
2'			198.6				199.7
3'	5.73 (s)	5.56 (s)	100.7	C-1', C-2', C-4', C-5'		5.52 (s)	101.4
4'			168.1				168.7
5'			100.9				100.4
6'	2.90 (d, 12.6)	2.62 (d, 14.2)	44.7	C-7, C-8, C-1', C-2'	7-OH, 9-CH$_3$, 7'-H$_{ax}$	2.50 (d, 5.6)	37.6
7'	2.04 (m)	2.05 (m)	24.4	C-8', C-9', C1', C-2'	9'-CH$_3$, 1'-H	1.93 (m)	25.3
	1.93 (td, 4.5, 13.5)	1.74 (td, 5.2, 14.0)			9-CH$_3$, 6'-H	1.65 (m)	
8′	1.96 (m)	1.84 (m)	34.9	C-8, C-9, C-7', C-9'		1.90 (m)	33.5
9'	0.61 (d, 8.1 Hz)	0.60 (d, 7.6 Hz)	20.4	C-8, C-7', C-8'	7'-H$_{eq}$	0.73 (d, 7.6 Hz)	20.9
4,5-OCH$_2$O	5.97 (s), 5.94 (s)	5.97 (d, 1.2), 5.94 (d, 1.2)	101.8	C-4, C-5		5.93 (s), 5.89 (s)	101.4
4′,5'-OCH$_2$O	5.81 (s), 5.77 (s)	5.67 (s)	98.7	C-4', C-5′		5.68 (s), 5.62 (s)	98.7
7-OH	5.55 (br s)				9-CH$_3$, 6'-H		

[a] ^{1}H NMR (900 MHz in pyridine-d_5, δ values) spectroscopic data. [b] ^{1}H NMR (400 MHz in CDCl$_3$, δ values) spectroscopic data. [c] ^{13}C NMR (100 MHz in CDCl$_3$, δ values) spectroscopic data

Table 2. Arginase II inhibitory activity of compounds **1-10**

Compound	IC_{50} value (μM)[a]
1	89.6 ± 5.8
2	61.4 ± 5.4
3	> 200
4	> 200
5	> 200
6	> 200
7	> 200
8	> 200
9	> 200
10	> 200
PG[b]	1.0 ± 0.1

[a] The inhibitory effects are represented as the molar concentration (μM) giving 50% inhibition (IC_{50}) relative to the vehicle control. These data represent the average values of three repeated experiments. [b] Piceatannol-3'-*O*-*b*-D-glucopyranoside (PG) was used as positive control[1]

trum showed the long-range correlation between proton signals at δ_H 7.00 (H-6), 2.52 (H-8), 1.25 (H-9), 2.53 (H-1'), and 2.62 (H-6') and carbon signal at δ_C 68.9, and then these correlations suggested that hydroxyl group was located at C-7 (Fig. 2). From COSY, NOESY, and HMBC data, we concluded that **1** is a derivative of sauchinone (**2**) (Table 1 and Fig. 2). From the NOESY spectrum (measured in pyridine-d_5), the proton at δ_H 5.55 (7-OH) displayed NOEs with the methyl protons signal at δ_H 1.46 (9-CH_3) and 2.90 (H-6'), and the methine resonance at δ_H 2.70 (H-1') displayed NOEs with the methyl protons of δ_H 0.61 (9'-CH_3) and 1.93 (7'-H_{eq}). Furthermore, the methine proton signal at δ_H 2.90 (H-6') also displayed NOEs with the methyl proton signals at δ_H 1.46 (9-CH_3), 5.55 (7-OH), and 2.04 (7'-H_{ax}). Hence, the configurations of 7-OH, 6'-H, and 1'-H are a *cis-trans* form by comparison with the *cis-trans* form in sauchinone (**2**). Based on the above analysis, the structure of compound **1** was elucidated as 7-hydroxysauchinone.

Arginase II activity is upregulated in atherosclerosis-prone mice and is associated with impaired endothelial NO production, endothelial dysfunction, vascular stiffness, and ultimately, aortic plaque development. Conversely, inhibiting endothelial arginase or deleting the arginase II gene enhances NO production, restores endothelial function and aortic compliance, and reduces plaque burden. Therefore, arginase II represents a novel target for preventing and treating of atherosclerotic vascular disease.[8] In the present study, we screened the isolated compounds for anti-arginase II activity. Incubating compounds **1** and **2** from kidney lysates significantly inhibited arginase II activity with IC_{50} values of 89.6 and 61.4 μM (Table 2), respectively, whereas the other compounds were apparently inactive.

Experimental

General Experimental Procedure. Optical rotations were measured with a JASCO DIP 370 digital polarimeter. UV spectra were taken in MeOH using a Thermo spectrometer. The nuclear magnetic resonance (NMR) spectra were obtained on Varian Unity Inova 400 MHz spectrometer. Silica gel (Merck, 63-200 μm particle size), RP-18 (Merck, 75 μm particle size) were used for column chromatography. TLC was carried out using Merck silica gel 60 F_{254} and RP-18 F_{254} plates. HPLC was carried out using a Water system (515 pump) with a UV detector (486 Tunable Absorbance) and an YMC Pak ODS-A column (20 × 250 mm, 5 μm particle size, YMC Co., Ltd., Japan) and HPLC solvents were from Burdick & Jackson, USA.

Plant Material. The dried aerial part of *S. chinensis* was purchased from a local folk medicine market named "Yak-ryoung-si" in Daegu, Korea, in May 2010. Botanical identification was performed by Prof. Byung-Sun Min, and the voucher specimen CUD-1384 was deposited at the herbarium of the College of Pharmacy, Catholic University of Daegu, Korea.

Extraction and Isolation. The dried aerial part of *S. chinensis* (12 kg) was extracted three times with MeOH at room temperature for seven days and then MeOH extract (1.5 kg) was suspended in hot-water (4 L) and partitioned with *n*-hexane (4 L × 3), ethyl acetate (4 L × 3), and *n*-butanol (4 L × 3), successively. The resulting fractions were concentrated *in vacuo* to give the hexane-soluble fraction (400.8 g), EtOAc-soluble fraction (584.93 g), and BuOH-soluble fraction (314.2 g), respectively. By the activity-guided fractionation, the EtOAc soluble fraction (584.93 g) was chromatographed on a silica gel column chromatography eluting with a gradient of $CHCl_3$-MeOH (50:1 → 5:1) to afford fifteen fractions (Fr. 1~15). Fraction 7 (80 g) was subjected on a silica gel column chromatography eluting with a gradient of *n*-hexane-acetone (5:1 → 0:1) to afford three subfractions (Fr. 7-1~7-3). Subfraction 7-1 (20 g) was subjected on a silica gel column chromatography eluting with a gradient of *n*-hexane-EtOAc (10:1 → 1:1) to afford compounds **2** (120 mg), **3** (32.8 mg), and **4** (25 mg). Subfraction 7-2 (25 g) was subjected to silica gel column chromatography with a gradient of *n*-hexane-EtOAc (5:1 → 1:1) to afford seven subfractions (Fr. 7-2-1~7-2-7). Subfraction 7-2-5 (450 mg) was chromatographed over a RP-18 gel eluting with a gradient of MeOH-H_2O (3:1 → 6:1) to afford compounds **8** (12.3 mg), **9** (8 mg), **6** (34.4 mg), and **7** (17 mg). Subfraction 7-2-2 was chromatographed by MPLC on a ODS column using MeOH-H_2O (3:1) and purified by preparative HPLC on a RP-18 column using MeOH-H_2O (72:28 → 74:26) to yield compound **10** (8 mg). Subfraction 7-2-6 was chromatographed by MPLC on a ODS column using MeOH-H_2O (3:1) followed by re-crystallization to yield compound **1** (10.9 mg) and **5** (7.2 mg), respectively.

7-Hydroxysauchinone (1): white amorphous powder; $[\alpha]_D^{25}$ −9.5 (*c* 0.37, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm: 244, 297; HR-EIMS *m/e*: 372.1209 $[M]^+$ (calcd. for $C_{20}H_{20}O_7$); ^1H NMR (900 MHz in pyridine-d_5 and 400 MHz in $CDCl_3$) and ^{13}C NMR (100 MHz in $CDCl_3$) spectroscopic data, see Table 1.

Arginase II: Arginase II solution was prepared from kidney lysates of anesthetized C57BL/6 mice.

Arginase Activity. Tissue lysates of kidney were prepared

using lysis buffer (50 mM Tris-HCl, pH7.5, 0.1 mM EDTA and protease inhibitors) by homogenization at 4 °C followed by centrifugation for 20 min at 14,000 × g at 4 °C. Briefly, aortic lysates were added to Tris-HCl. The hydrolysis reaction of L-arginine by Arg was performed by incubating the mixture containing activated Arg and was stopped by adding acid solution. For calorimetric determination of urea, α-isonitrosopropiophenone was added, and the mixture was heated at 100 °C for 45 minutes. After placing the sample in the dark for 10 minutes at room temperature, the urea concentration was determined spectrophotometrically by the absorbance at 550 nm.[31]

Acknowledgments. This research was supported by the National Research Foundation of Korea Grant funded by the Korean Government (MEST) (KRF-2009-0084675). We are grateful to the Korean Basic Science Institute (KBSI) for supplying the NMR spectra.

References and Notes

1. Woo, A. N.; Min, B. S.; Ryoo, S. W. *Exp. Mol. Med.* **2010**, *42*, 524.
2. Berkowitz, D. E.; White, R.; Li, D.; Minhas, K. M.; Cernetich, A.; Kim, S.; Burke, S.; Shoukas, A. A.; Nyhan, D.; Champion, H. C.; Hare, J. M. *Circulation* **2003**, *108*, 2000.
3. Simon, A.; Plies, L.; Habermeier, A.; Martine, U.; Reining, M.; Closs, E. I. *Circ. Res.* **2003**, *93*, 813.
4. Holowatz, L. A.; Thompson, C. S.; Kenney, W. L. *J. Physiol.* **2006**, *574*, 573.
5. Bivalaqua, T. J.; Hellstrom, W. J.; Kadowitz, P. J.; Champion, H. C. *Biochem. Biophys. Res. Commun.* **2001**, *283*, 923.
6. Demougeot, C.; Prigent-Tessier, A.; Marie, C.; Berthelot, A. *J. Hypertens.* **2005**, *25*, 971.
7. Johnson, F. K.; Johnson, R. A.; Peyton, K. J.; Durante, W. *Am. J. Physiol. Regul Integr. Comp. Physiol.* **2005**, *288*, 1057.
8. Ryoo, S.; Gupta, G.; Benjo, A.; Lim, H. K.; Camara, A.; Sikka, G.; Lim, H. K.; Sohi, J.; Santhanam, L.; Soucy, K.; Tuday, E.; Baraban, E.; Ilies, M.; Gerstenblith, G.; Nyhan, D.; Shoukas, A.; Christianson, D. W.; Alp, N. J.; Champion, H. C.; Huso, D.; Berkowitz, D. E. *Circ. Res.* **2008**, *102*, 923.
9. Ryoo, S.; Lemmon, C. A.; Soucy, K. G.; Gupta, G.; White, A. R.; Nyhan, D.; Shoukas, A.; Romer, L. H.; Berkowitz, D. E. *Circ. Res.* **2006**, *99*, 951.
10. Ryoo, S.; Berkowitz, D. E.; Lim, H. K. *Korean. J. Anesthesiol.* **2011**, *61*, 3.
11. Ryoo, S.; Bhunia, A.; Chang, F.; Shoukas, A.; Berkowitz, D. E.; Romer, L. H. *Atherosclerosis* **2011**, *214*, 279.
12. Chung, B. S.; Shin, M. G. *Dictionary of Korean Folk Medicine*; Young Lim Sa: Seoul, 1990; pp 813-814.
13. Kim, S. N.; Kim, M. H.; Kim, Y. S.; Ryu, S. Y.; Min, Y. K.; Kim, S. H. *Phytother. Res*. **2009**, *23*, 185.
14. Sung, S. H. *Fitoterapia* **2006**, *277*, 487.
15. Kim, S. R.; Sung, S. H.; Kang, S. Y.; Koo, K. A.; Kim, S. H.; Ma, C. J.; Lee, H. S.; Park, M. J.; Kim, Y. C. *Planta Med.* **2004**, *70*, 391.
16. Lee, W. S.; Baek, Y. I.; Kim, J. R.; Cho, K. H.; Sok, D. E.; Jeong, T. S. *Bioorg. Med. Chem. Lett*. **2004**, *14*, 5623.
17. Choi, C. W.; Kim, S. C.; Hwang, S. S.; Choi, B. K.; Ahn, H. J.; Lee, M. Y.; Park, S. H.; Kim, S. K. *Plant Sci*. **2002**, *163*, 1161.
18. Kim, H. Y.; Choi, T. W.; Kim, H. J.; Kim, S. M.; Park, K. R.; Jang, H. J.; Lee, E. H.; Kim, C. Y.; Jung, S. H.; Shim, B. S.; Ahn, K. S. *Phytomedicine* **2011**, *18*, 567.
19. Yu, M. H.; Im, H. G.; Lee, J.; Bo, M. H.; Kim, H. J.; Kim, S. K.; Chung, S. K.; Lee, I. S. *Nat. Prod. Res*. **2008**, *22*, 275.
20. Wang, L.; Cheng, D.; Wang, H.; Di, L.; Zhou, X.; Xu, T.; Yang, X.; Liu, Y. *J. Ethnopharmacol*. **2009**, *126*, 487.
21. Yun, J. Y.; Roh, E.; Son, J. K.; Lee, S. H.; Seo, C. S.; Hwang, B. Y.; Han, S. B.; Kim, Y. *Arch. Pharm. Res*. **2011**, *34*, 1339.
22. Jung, J. Y.; Lee, K. Y.; Lee, M. Y.; Jung, D.; Cho, E. S.; Son, H. Y. *Int. Immunopharmacol*. **2011**, *11*, 698.
23. Sung, S. H.; Kim, Y. C. *J. Nat. Prod*. **2000**, *63*, 1019.
24. Hwang, B. Y.; Lee, J. H.; Nam, J. B.; Hong, Y. S.; Lee, J. J. *Phytochemistry* **2003**, *4*, 765.
25. Li, L. N.; Xue, Hong. *Planta Med.* **1986**, *6*, 493.
26. Chang, S. S.; Zheng, M. S.; Li, Y.; Jang, Y. D.; Chang, H. W.; Son, J. K. *Bull. Korean Chem. Soc.* **2009**, *30*, 716.
27. Kwon, H. S.; Kim, M. J.; Jeong, H. J.; Yang, M. S.; Park, K. H.; Jeong, T. S.; Lee, W. S. *Bioorg. Med. Chem. Lett*. **2008**, *18*, 194.
28. Cheng, W.; Zhu, C.; Xu, W.; Fan, X.; Yang, Y.; Li, Y.; Chen, X.; Wang, W.; Shi, J. *J. Nat. Prod.* **2009**, *72*, 2145.
29. Suri, K. A.; Sood, R. P.; Suri, O. P.; Atal, C. K.; Singh, G. B. *Indian J. Pharmaceutical Sci.* **1981**, *43*, 226.
30. Pereira, A. C.; Megalhaes, L. G.; Januario, A. H.; Pauletti, P. M.; Cunha, W. R.; Bastos J. K.; Nanayakkara, D. N. P.; Silva, M. L. A. *J. Chromatography A* **2011**, *1218*, 7051.
31. White, A. R.; Ryoo, S.; Li, D.; Champion, H. C.; Steppan, J.; Wang, D.; Nyhan, D.; Shoukas, A. A.; Hare, J. M.; Berkowitz, D. E. *Hypertension* **2006**, *47*, 245.