An Apigenin-Derived Xanthine Oxidase Inhibitor from *Palhinhaea cernua*

Rui H. Jiao, Hui M. Ge, Da H. Shi, and Ren X. Tan*

Institute of Functional Biomolecules, State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing 210093, People's Republic of China

Received January 24, 2006

Fractionation of the ethanol extract of *Palhinhaea cernua* afforded a new (**1**) and two known *p*-coumaroylated apigenin glycosides (**2**, **3**) in addition to dillenetin, rhamnazin, α-onocerin, β-sitosterol, and (*E*)-2-hydroxy-5-methoxycinnamic acid. The structure of compound **1** was elucidated as apigenin-4′-*O*-(2″-*O*-*p*-coumaroyl)-β-D-glucopyranoside by a combination of its spectroscopic data. The new glycoside **1** inhibits xanthine oxidase (IC_{50}: 23.95 ± 0.43 μM) in a competitive−noncompetitive manner with K_i and K_I values of 14.35 and 93.68 μM, whereas compounds **2** and **3** were inactive. The distribution and significance of acylated flavonoid glycosides are discussed.

The club moss *Palhinhaea cernua* has been used for several centuries as a traditional Chinese medicine to treat rheumatism, whooping coughs, hepatitis, and nephrolith.[1,2] It has also been described as being substantially effective in attenuating joint pains.[3] Pathogenically, the discomfort or sharp pain in joints is frequently generated by gout, a typical late-stage symptom of hyperuricaemia resulting from the overproduction and/or insufficient excretion of uric acid.[4] Biochemically, xanthine oxidase (XO) is a key enzyme that is involved in the production of this acidic metabolite from purine-containing food stuff. This explains why inhibition of the enzyme is an ideal strategy for reducing the serum level of uric acid. Furthermore, hypercuricaemia is demonstrated to be correlated with cardiovascular events such as stroke and coronary heart disease,[5,6] although uric acid is a natural scavenger of peroxynitrite in experimental allergic encephalomyelitis and multiple sclerosis.[4,7] In view of the folk usage of the title plant, we hypothesized that it could contain XO inhibitor(s). This assumption was first reinforced by the XO inhibitory effect of its ethanol extract tested as detailed elsewhere.[8] This encouraged us to characterize the XO inhibitor(s) from the plant extract. In addition, the title moss was taxonomically ambiguous and was believed to be *Lycopodium cernuum* long before it was thus named. An extra motivation of the present phytochemical work was therefore to acquire the chemotaxonomic evidence that may rationalize its reclassification as a *Palhinhaea* species.

Eight constituents were obtained by repeated chromatography of the EtOH extract of *P. cernua*. The first was identified as β-sitosterol by direct comparison (TLC, MS, and ^{1}H NMR) with the authentic material available in our laboratory. The identification of α-onocerin,[9] dillenetin,[10] rhamnazin,[11,12] and (*E*)-2-hydroxy-5-methoxycinnamic acid[13] was evidenced from the ^{1}H and ^{13}C NMR and MS data.[9] Previously, α-onocerin was shown to be acetylcholinesterase inhibitory,[14] and dillenetin was shown to be antiosteoporosis[15] and antioxidant.[16] Rhamnazin was anti-inflammatory,[17] antibacterial,[17] lipid peroxidation inhibitory,[18] and antioxidant.[19]

Compound **1** was obtained as light yellow amorphous powder. Its flavone framework was indicated both by the typical UV curve peaking at 222, 273, 275, and 314 nm and by its IR absorption bands of hydroxyl (3249−3500 cm^{-1}), carbonyl (1714 and 1659 cm^{-1}), and phenyl groups (1586, 1510, and 1455 cm^{-1}). In the HRESIMS spectrum of compound **1**, the protonated molecular ion ($[M + H]^+$) at *m*/*z* 579.1200 demonstrated that its molecular formula was $C_{30}H_{26}O_{12}$ (calc for $C_{30}H_{27}O_{12}$, 579.1194), consistent with its ^{13}C NMR data (Table 1, Supporting Information). The subsequent scrutiny of its ^{1}H and ^{13}C NMR and DEPT spectra highlighted the presence of a 5,7,4′-trisubstituted flavone framework, a *trans*-*p*-coumaroyl group, and a β-D-glucopyranosyl residue. This assumption was proved to be correct by its COSY, HMQC, and HMBC spectra, which allowed the exact assignment of all proton and carbon signals (Table 1). The β-D-glucopyranosyl moiety was located at C-4′ by the HMBC correlation between C-4′ and H-1″ (Figure 1). This linkage was reinforced by NOE experiments. Irradiation of H-1″ at δ 5.39 generated the anticipated NOE enhancements of H-3′/5′ at δ 7.20 and of H-2″ at δ 5.17, respectively. The *trans*-*p*-coumaroyl group was demonstrated to be bonded to C-2″ by the HMBC correlation between H-2″ and C-9‴ at δ 166.5 (Figure 1) and by the magnitude of the H-2″ signal being close to that (δ 5.09) of 2″-acylated apigenin 4′-*O*-β-D-glucoside.[20] The β-D-glucopyanose nature of **1** was evidenced from the splitting pattern of saccharide methine proton signals and the chemical shift of sugar carbon resonance lines.[20−22] As anticipated, acid hydrolysis of **1** with 10% aqueous methanolic HCl liberated D-glucose ($[\alpha]_D^{20} = +24.9$ (*c* 0.05, MeOH), which was identified

Table 1. ^{1}H and ^{13}C NMR Data of **1** and **2** (acetone-d_6)

	1		**2**	
position	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
2	164.2, qC		163.8	
3	105.0, CH	6.68 (s)	104.7	6.60 (s)
4	182.7, qC		182.6	
5	158.7, qC		158.2	
6	94.7, CH	6.54 (d, 2.1)	94.9	6.50 (d, 2.1)
7	164.8, CH		165.1	
8	99.6, CH	6.25 (d, 2.1)	100.4	6.26 (d, 2.1)
9	163.2, qC		162.3	
10	105.2, qC		104.7	
1′	126.0, qC		124.9	
2′	128.9, CH	7.99 (d, 8.7)	129.0	7.96 (d, 8.7)
6′	128.9, CH	7.99 (d, 8.7)	129.0	7.96 (d, 8.7)
3′	117.6, CH	7.20 (d, 8.7)	117.4	7.25 (d, 8.7)
5′	117.6, CH	7.20 (d, 8.7)	117.4	7.25 (d, 8.7)
4′	161.0, qC		160.8	
1″	99.4, CH	5.39 (d, 8.1)	99.8	5.16 (d, 8.0)
2″	74.1, CH	5.17 (dd, 9.4, 8.1)	70.8	3.50 (t, 9.0)
3″	75.6, CH	3.82 (t, 9.0)	73.9	3.60 (t, 9.0)
4″	71.2, CH	3.61 (t, 9.0)	77.2	3.56 (t, 9.0)
5″	78.1, CH	3.71 m	74.6	3.89 m
6″	62.2, CH_2	3.96 (dd, 12.1, 1.6)	64.1	4.56 (br d, 11.9)
		3.76 (dd, 12.1, 5.8)		4.34 (dd, 11.9, 6.3)
1‴	126.8, qC		125.8	
2‴	130.9, CH	7.54 (d, 8.5)	131.2	7.53 (d, 8.4)
6‴	130.9, CH	7.54 (d, 8.5)	131.2	7.53 (d, 8.4)
3‴	116.5, CH	6.88 (d, 8.5)	116.6	6.86 (d, 8.4)
5‴	116.5, CH	6.88 (d, 8.5)	116.6	6.86 (d, 8.4)
4‴	160.5, qC		160.8	
7‴	145.8, CH	7.66 (br d, 15.9)	145.8	7.62 (br d, 15.9)
8‴	115.2, CH	6.37 (br d, 15.9)	114.8	6.38 (br d, 15.9)
9‴	166.5, qC		167.3	

* To whom correspondence should be addressed. Tel: +86-25-8359 2945. Fax: +86-25-8330 2728. E-mail: rxtan@nju.edu.cn.

10.1021/np060038a CCC: $33.50
Published on Web 06/27/2006

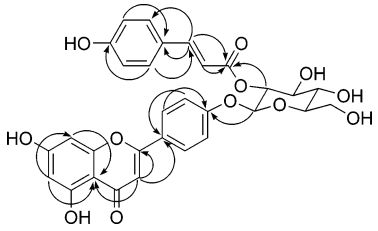
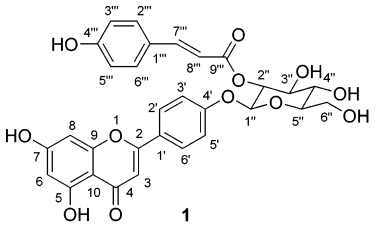
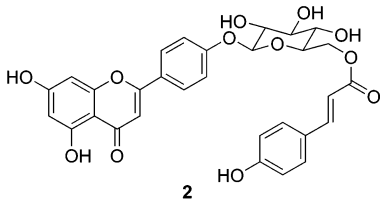


Figure 1. Key HMBC correlations of **1**.

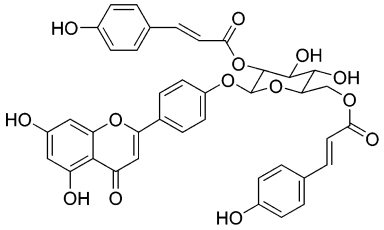
by paper chromatographic and optical rotation comparisons with the authentic material ($[\alpha]_D^{20} = +26.7$ (*c* 0.05, MeOH). Thus, the structure of compound **1** was apigenin-4′-*O*-(2″-*O*-*p*-coumaroyl)-*β*-D-glucopyranoside.



1



2



3

The ESIMS and ^{1}H and ^{13}C NMR spectra of **2** suggested that it was closely related to **1**. All ^{1}H and ^{13}C NMR signals of **2** were assigned by its COSY, HMQC, and HMBC spectra (Table 1). In the ^{1}H NMR spectrum of **2**, the upfield H-2″ signal and the downfield shifted 6″-hydroxymethyl resonances led to its identification as apigenin-4′-*O*-(6″-*O*-*p*-coumaroyl)-*β*-D-glucopyranoside, previously characterized from *Thymus serpyllum* (Labiatae) by a combination of chemical transformations and equivocally assigned ^{1}H NMR spectroscopic data.[21] The exactly allocated ^{1}H and ^{13}C NMR data of **2** are shown in Table 1. The spectroscopic data (ESIMS, ^{1}H and ^{13}C NMR) of **3** indicated that it was apigenin-4′-*O*-(2″,6″-di-*O*-*p*-coumaroyl)-*β*-D-glucopyranoside, first isolated from the leaves of *Lycopodium clavatum*[20] and later found to be an inhibitor of *Candida albicans*-secreted aspartic proteases.[22]

Compounds **1**−**3** were separately evaluated for XO inhibitory activity. The new glycoside **1** was substantially active against the enzyme with an IC_{50} value of 23.95 ± 0.43 μM, comparable to that (9.82 ± 0.18 μM) of the positive control allopurinol, a drug clinically prescribed in clinic for gout treatment. To understand the mode of the enzyme inhibition, the Lineweaver−Burk plot of **1** (Figure 2) was established, suggesting that its inhibition of XO was in a competitive−noncompetitive mode, with K_i and K_I values of 14.35 and 93.68 μM, respectively. This discerned mixed-type inhibition highlighted that glycoside **1** could be bound to both the free enzyme and the enzyme−substrate complex.[23] However, glycosides **2** and **3** were inactive against the enzyme (IC_{50} > 100 μM). This suggested that the XO inhibition of acylated apigenin-4′-*O*-*β*-D-glucopyranosides **1**−**3** depends more strictly on the acylation pattern. Among XO inhibitory phytochemicals, polyphenols attracted more attention because of their remarkable activity. For example, some stilbenoids such as resveratrol[24] and flavonoids such as apigenin[25] all exhibited pronounced inhibitions of the enzyme. Biochemically, the enzyme inhibition is associated with the hydrogen bonding of phenolic hydroxyls and/or carbonyls of the substrates with the amide carbonyls and/or amino groups rich in the peptide chain of the enzyme. Therefore, the striking difference of **1**−**3** in XO inhibition could be rationalized by the presence of 6″-*O*-*p*-coumaroyl groups that may interfere with the interaction (e.g., hydrogen bonding) between the substrate and the enzyme, although all possessed a flavone-based 2,3-double bond and 5,7-dihydroxyls previously ascertained to be essential for the XO inhibition of flavonoids.[26]

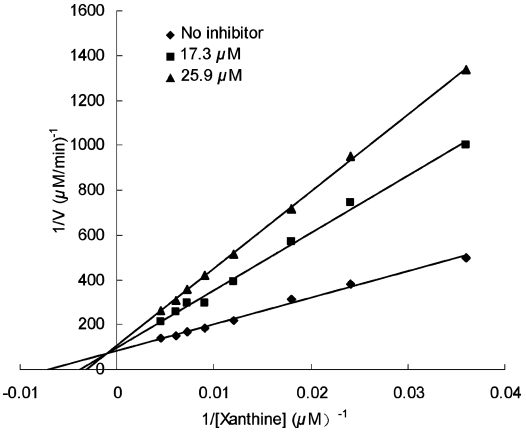


Figure 2. Linweaver−Burk plot of XO inhibition of **1** with various concentrations of xanthine. Lineweaver−Burk transformed data were plotted and followed by linear regression of the points. Data represent the average of triplicate experiments.

The present paper describes the characterization of a new XO inhibitor (**1**) from *P. cernua* as apigenin-4′-*O*-(2″-*O*-*p*-coumaroyl)-*β*-D-glucopyranoside. This enzyme inhibitory phytochemical and its congeners (**2** and **3**) were accompanied by other constituents including phytosterol, triterpene, flavonols, and phenolic acid. These types of constituents were previously characterized also from representatives of the genus *Lycopodium*.[22] However, alkaloids of phlegmarane, cernuane, and quinolizidine types, also common in the phytochemically investigated *Lycopodium* species,[27] were not detected in the extract of the title moss. Although more confirmation is needed, this observation could be accepted as the chemotaxonomic evidence supporting the reclassification of the title plant as a *Palhinhaea* species.

Acylated flavone glycosides have been characterized from a wide array of plants such as *Allium tuberosum*,[28] *Brassica napus*,[29] *Chrysanthemum morifolium*,[30] *Eruca sativa*,[31] *Medicago sativa*,[32] *Veronica thymoides* subsp. *pseudocinerea*,[33] and *Wasabia japonica*.[34] However, plants belonging to *Lycopodium* and its related genera usually produce flavone glycosides with one or two *p*-coumaroyl groups linked to the saccharide moieties. Furthermore, *p*-coumaroylated flavone glycosides were also detected in *Marrubium* (Labiatae)[35] and *Tagetes* species (Asteraceae).[36] Biochemicaly, coumaroylated flavone glycosides play supportive roles in flower pigmentation if the acylation pattern could allow the stable chelation with metal ions as discerned with African lilies,[37] *Commelina communis*,[38] and *Pisum sativum* var. *alaska*.[39] The above observation, along with the described recognition of **1** as a new XO inhibitor, highlighted the fact that acylated flavone glycosides in plants are worthy of multidisciplinary attention.

Experimental Section

General Experimental Procedures. Melting points were measured on an XT-4 apparatus and are uncorrected. Optical rotation was determined in MeOH on a WXG-4 disk polarimeter; IR spectra in KBr disks were acquired on a Nexus 870 FT-IR spectrometer. The UV spectra were recorded on a Hitachi U-3000 spectrophotometer. NMR spectra were acquired on a Bruker DRX-500 NMR spectrometer using TMS as internal standard. The ESIMS and HRESIMS spectra were recorded on a Mariner Mass 5304 instrument. Silica gel (200−300 mesh) for column chromatography and silica GF_{254} for TLC were produced by Qingdao Marine Chemical Company, China. Sephadex LH-20 was purchased from Pharmacia Biotech, Sweden. All chemicals used in the study were of analytical grade. Xanthine oxidase from buttermilk, xanthine, and allopurinol were purchased from Sigma-Aldrich Co. Other reagents used in the study were of analytical grade.

Plant Material. The whole plant of *P. cernua* was collected in July 2003 in Yunnan, China, and later identified by Prof. L. X. Zhang. A voucher specimen was deposited in the herbarium of Nanjing University, Nanjing, China.

Extraction and isolation. The air-dried plant material (2 kg) was pulverized and extracted with 95% EtOH (18 L × 5) at room temperature to give approximately 300 g of residue, which was successively partitioned with EtOAc, 1% HCl, and *n*-butanol, respectively. The EtOAc extract was concentrated in vacuo to give a gum (71 g), which was subjected to column chromatography on silica gel (350 g, 200−300 mesh) eluting with a $CHCl_3$/MeOH gradient (100% $CHCl_3$ to 100% MeOH) to afford 11 fractions (LB1: 17.6 g, LB2: 7.9 g, LB3-1: 13.6 g, LB3-2: 3.8 g, LB4: 10.66 g, LB4-5: 2.1 g, LB5: 5.3 g, LB6: 3.7 g, LB7: 3.1 g, LB8: 1.4 g; LB9: 0.9 g). The XO inhibitory fraction (fraction LB4) was further separated on a Sephadex LH-20 column with $CHCl_3$/MeOH (1:1) to give five fractions (LB4-1−LB4-5). The bioactive fraction LB4-3 (1.71 g) was rechromatographed on a silica gel column eluted with $CHCl_3$/MeOH (100:1 → 100:16) to afford four subfractions (LB4-3-1−LB4-3-4). Gel filtration of the most active one (LB4-3-4, 0.61 g) over Sephadex LH-20 in MeOH gave **1** (31.8 mg) and **2** (11.2 mg). Chromatography of LB4-5 over Sephadex LH-20 with MeOH afforded **3** (613.1 mg). Fraction LB1 (17.6 g) was further separated over a silica gel column (300 g, 200−300 mesh) with a $CHCl_3$/MeOH gradient to yield α-onocerin (34 mg) and *β*-sitosterol (9.2 mg). Fraction LB2 (7.9 g) was rechromatographed on a silica gel column (150 g, 200−300 mesh) eluted with $CHCl_3$/MeOH mixtures (100:0 → 20:1) and was then followed by repeated gel filtration over Sephadex LH-20 ($CHCl_3$/MeOH, 1:1) to furnish (*E*)-2-hydroxy-5-methoxycinnamic acid (15.4 mg). Repeated chromatography of fraction LB3-1 (13.6 g) on a silica gel column (300 g, 200−300 mesh, $CHCl_3$/MeOH, 100:0 → 10:1) followed by gel filtration over Sephadex LH-20 in MeOH afforded dillenetin (16.1 mg) and rhamnazin (14.9 mg).

Apigenin-4′-*O*-(2″-*O*-*p*-coumaroyl)-*β*-D-glucopyranoside (1): light yellow amorphous powder; $[\alpha]_D^{20}$ +0.17 (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ϵ) 222 (2.89) (sh), 273 (2.87), 275 (2.86), and 314 (3.00); IR ν_{max}^{KBr} (cm^{-1}) 3361.8, 3249.4, 2946.7, 2899.5, 1714.0, 1704.9, 1659.0, 1622.5, 1607.9, 1586.4, 1576.8, 1510.0, 1455.0, 1434.7, 1394.2 cm^{-1}; HRESI MS *m*/*z* 579.1200 $[M + H]^+$, calcd for $C_{30}H_{27}O_{12}$, 579.1194; ^1H (500 MHz) and ^{13}C NMR (125 MHz) data, see Table 1.

Acid Hydrolysis. A solution of **1** (14 mg) in 10% aqueous methanoic HCl was refluxed for 8 h. After cooling, the reaction mixture was dried with a stream of N_2 to yield a residue that was dissolved in MeOH (1 mL) and subsequently purified by preparative HPLC (acetonitrile/H_2O, 60:15) to yield glucose (t_R 5.09 min, $[\alpha]_D^{20}$ +24.9 (*c* 0.05, MeOH)).

Assay for XO Inhibitory Activity. The XO activity with xanthine as the substrate was measured at 25 °C, according to the protocol of Kong and others.[4] The reaction mixture contained 650 μL of 50 mM K_2HPO_4 buffer (pH 7.8), 200 μL of 84.8 μg/mL xanthine in 50 mM K_2HPO_4 buffer, and 50 μL of the various concentrations of tested compounds, which were dissolved in DMSO. The reaction was started by addition of 100 μL of XO (25 mU/mL) and was monitored for 6 min at 295 nm; the XO activity was expressed as micromoles of uric acid per minute.

Acknowledgment. The work was co-financed by grants for R.X.T. from NSFC (Projects 20432030 and 30570010) and MOE (Grant No. 104195).

Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Zhang, X. C.; Zhang, L. B. In *Flora Reipublicae Popularis Sinicae*; Zhang, X. C., Ed.; Science Press: Beijing, 2004; Vol. 6(3), pp 69−72.
(2) Xiao, P. G. *Modern Chinese Material Medica*; Chemistry and Industry Press: Beijing, 2002; Vol. 3, pp 135−139.
(3) Jiangsu College of New Medicine. *A Comprehensive Dictionary of the Traditional Chinese Medicines*; 1996; pp 2394−2395.
(4) Kong, L. D.; Zhang, Y.; Pan, X.; Tan, R. X.; Cheng, C. H. K. *Cell Mol. Life Sci.* **2000**, *57*, 500−505.
(5) Alderman, M. H.; Cohen, H.; Madhavan, S.; Kivlighn, S. *Hypertension* **1999**, *34*, 144−150.
(6) Longo-Mbenza, B.; Luila, E. L.; Mbete, P.; Vita, E. K. *Int. J. Cardiol.* **1999**, *71*, 17−22.
(7) Hooper, D. C.; Spitsin, S.; Kean, R. B.; Champion, J. M.; Dickson, G. M. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 675−680.
(8) Kong, L. D.; Cai, Y.; Huang, W. W.; Cheng, C. H. K.; Tan, R. X. *J. Ethnopharmacol.* **2000**, *73*, 199−207.
(9) Pauli, G. F. *Planta Med.* **2000**, *66*, 299−302.
(10) Simonsen, H. T.; Adsersen, A.; Smitt, U. W.; Strasberg, D.; Jaroszewski, J. W. *Biochem. Syst. Ecol.* **2003**, *31*, 327−330.
(11) Paya, M.; Manez, S.; Villar, A. *Z. Naturforsch. C* **1986**, *41*, 976−978.
(12) Martín-Cordero, C.; López-Lázaro, M.; Gil-Serrano, A.; Rodriguez-Carvajal, M. A.; Ayuso-González, M. J. *Phytochemistry* **1999**, *51*, 1129−1131.
(13) Santavy, F.; Walterova, D.; Hruban, L. *Collect. Czech. Chem. C* **1972**, *37*, 1825−1850.
(14) Orhan, I.; Terzioglu, S.; Sener, B. *Planta Med.* **2003**, *69*, 265−267.
(15) Kim, C. S.; Ha, H. K.; Song, K. Y. W.O. Patent 2,002,017,909, 2002, p 37.
(16) Kwon, Y. S.; Kim, C. M. *Arch. Pharm. Res.* **2003**, *26*, 535−539.
(17) Martini, N. D.; Katerere, D. R. P.; Eloff, J. N. *J. Ethnopharmacol.* **2004**, *93*, 207−212.
(18) Yun, B. S.; Lee, I. K.; Kim, J. P.; Chung, S. H.; Shim, G. S.; Yoo, I. D. *Arch. Pharm. Res.* **2000**, *23*, 147−150.
(19) Lee, I. K.; Yun, B. S.; Kim, J. P.; Chung, S. H.; Shim, G. S.; Yoo, I. D. *Saengyak Hakhoechi*. **1998**, *29*, 163−168.
(20) Ansari, F. R.; Ansari, W. H.; Rahman, W.; Seligman, O.; Chari, V. M.; Wagner, H.; Osterdahl, B. G. *Planta Med.* **1979**, *36*, 196−199.
(21) Washington, J. S.; Saxena, V. K. *J. Inst. Chem. (India)* **1985**, *57* (4), 153−155.
(22) Zhang, Z. Z.; ElSohly, H. N.; Jacob, M. R.; Pasco, D. S.; Walker, L. A.; Clark, A. M. *J. Nat.* Prod. **2002**, *65*, 979−985, and related references therein.
(23) Palmer, T. *Understanding Enzymes*, 3rd ed.; Ellis Horwood: London, 1991; pp 139−165.
(24) Zhou, C. X.; Kong, L. D.; Ye, W. C.; Cheng, C. H. K.; Tan, R. X. *Planta Med.* **2001**, *67*, 158−161.
(25) Van Hoorn, D. E. C.; Nijveldt, R. J.; Van Leeuwen, P. A. M.; Hofman, Z.; M'Rabet, L.; De Bont, D. B. A.; Van Norren, K. *Eur. J. Pharmacol.* **2002**, *451*, 111−118.
(26) Borges, F.; Fernandes, E.; Roleira, F. *Curr. Med. Chem.* **2002**, *9*, 195−217.
(27) Morita, H.; Hirasawa, Y.; Shinzato, T.; Kobayashi, J. *Tetrahedron* **2004**, *60*, 7015−7023.
(28) Yoshida, T.; Saito, T.; Kadoya, S. *Chem. Pharm. Bull.* **1987**, *35*, 97−107.
(29) Baumert, A.; Milkowski, C.; Schmidt, J.; Nimtz, M.; Wray, V.; Strack, D. *Phytochemistry* **2005**, *66*, 1334−1345.
(30) Lee, J. S.; Kim, H. J.; Lee, Y. S. *Planta Med.* **2003**, *69*, 859−861.
(31) Weckerle, B.; Michel, K.; Balázs, B.; Schreier, P.; Tóth, G. *Phytochemistry* **2001**, *57*, 547−551.
(32) Stochmal, A.; Simonet, A. M.; Macias, F. A.; Oliveira, M. A.; Abreu, J. M.; Nash, R.; Oleszek, W. *Phytochemistry* **2001**, *57*, 1223−1226.
(33) Saracoglu, I.; Varel, M.; Harput, U. S.; Nagatsu, A. *Phytochemistry* **2004**, *65*, 2379−2385.
(34) Hosoya, T.; Yun, Y. S.; Kunugi, A. *Tetrahedron* **2005**, *61*, 7037−7044.
(35) Karioti, A.; Skaltsa, H.; Heilmann, J.; Sticher, O. *Phytochemistry* **2003**, *64*, 655−660.
(36) Parejo, I.; Bastida, J.; Viladomat, F.; Codina, C. *Phytochemistry* **2005**, *66*, 2356−2362.
(37) Bloor, S. J.; Falshaw, R. *Phytochemistry* **2000**, *53*, 575−579.
(38) Kondo, T.; Yoshida, K.; Nakagawa, A.; Kawai, T.; Tamura, H.; Goto, T. *Nature* **1992**, *358*, 515−518.
(39) Bottomley, W.; Smith, H.; Galston, A. W. *Nature* **1965**, *207*, 1311−1312.

NP060038A