Prenylated Flavonoid Derivatives from the Bark of Erythrina addisoniae

ARTICLE *in* JOURNAL OF NATURAL PRODUCTS · MAY 2008

Impact Factor: 3.8 · DOI: 10.1021/np070417j · Source: PubMed

Prenylated Flavonoid Derivatives from the Bark of *Erythrina addisoniae*

W. Wätjen,[†] A. K. Suckow-Schnitker,[‡,⊥] R. Rohrig,[†,⊥] A. Kulawik,[†] J. Addae-Kyereme,[§] C. W. Wright,[§] and C. M. Passreiter*[,‡]

Institute of Toxicology, Heinrich-Heine-University, P.O. Box 101007, 40001 Düsseldorf, Germany, Institute of Pharmaceutical Biology and Biotechnology, Heinrich-Heine-University, Universitätsstrasse 1, 40225 Düsseldorf, Germany, and The School of Pharmacy, University of Bradford, Richmond Road, West Yorkshire BD 7 1 DP, U.K.

Received August 10, 2007

Two new prenylated flavanones, 2*S*-3′-(2-hydroxy-3-methylbut-3-enyl)licoflavone-4′-methyl ether (**3**) and 2*S*-3′-(2-hydroxy-3-methylbut-3-enyl)abyssinone II (**4**), and four known flavanones (**1**, **2**, **5**, **6**) were isolated from the stem bark of *Erythrina addisoniae*. The structures were elucidated on the basis of their spectroscopic and physicochemical data. None of the compounds showed antioxidative properties. 4′-Methylabyssinone V (**1**) and abyssinoflavanone VII (**6**) showed moderate cytotoxic activity (IC_{50} = 5 and 3.5 μmol/L, respectively), but apoptosis (caspase-3/7-activation, nuclear fragmentation) was selectively induced by abyssinoflavanone VII (**6**).

The genus *Erythrina* (Leguminosae) comprises a group of more than 100 different trees, distributed in tropical areas all over the world.[1] Some of their extracts were used to poison darts due to their curare-like activities;[2] other species are used as medicinal plants in folk medicine of many African, Asian, and South American countries to treat different kinds of infections and inflammations.[3–6] *Erythrina addisoniae* Hutch. & Dalziel occurs in tropical areas of Ghana and other West African countries. The stem and root bark is mainly used against dysentery, hepatitis, rheumatic disorders, and pain and in some areas of Ghana additionally against swellings and cancer in folk medicine.[1,7] In spite of the various pharmacological uses of *E. addisoniae* extracts, little is known about the compounds mediating the anticancer effects. The phytochemical investigation of several *Erythrina* species afforded alkaloids and prenylated flavonoids as main constituents.[8–12] 8-Prenylnaringenin was found to be a potent inhibitor of the metabolic activation of heterocyclic amines by inhibition of CYP1A2.[13] 8-Prenylgalangin and 8-prenylchrysin are potent inhibitors of multidrug resistance mediated by P-glycoprotein and related ABC transporters and therefore promising candidates for cancer therapy.[14] Previously, we reported the enhancement of cytotoxicity by the introduction of a prenyl group at C-8 of apigenin and liquiritigenin.[15] Nkengfack et al. reported the isolation of two cytotoxic isoflavone derivatives from *E. indica*.[16]

In a recent study, isoflavonoids with inhibitory activities against tyrosine phosphatase 1B were reported from *E. addisoniae*.[17] We now report the occurrence of two new (**3** and **4**) and four known (**1**, **2**, **5**, and **6**) prenylated flavanones in the stem bark of *E. addisoniae*. The antioxidative potential, cytotoxic, and pro-apoptotic effects of the isolated compounds were tested to investigate the active principle of anticancer use of the plant extract (Figure 1).

Purification of the stem bark extract of *E. addisoniae* yielded several fractions rich in flavonoids. Further purification of fraction 2 resulted in the isolation of six compounds, **1**−**6** (see Extraction and Isolation). Compounds **1**, **2**, **5**, and **6**, previously isolated from other *Erythrina* species, were identified as 4′-methylabyssinone V, abyssinone IV, abyssinone V, and abyssinoflavanone VII, respectively.[18–21]

The two new compounds **3** and **4** were found to be derivatives of **1** and **2**, only differing in the side chain at C-3′. The ^{13}C NMR spectra of **3** displayed a total number of 25 resonances. Sixteen resonances were found at shift values similar to those of a naringenin-4′-methyl ether. The remaining nine resonances resulted from two different isoprenyl substituents attached to the B ring. The resonances found at δ 37.2, 76.1, 149.3, 110.5, and 18.1 indicated the presence of a 2-hydroxy-3-methylbut-3-enyl side chain as found in lupiniol B from *Lupinus luteus* and were confirmed by HMQC, HMBC, and COSY.[22] The remaining four resonances could be assigned to a regular isoprenyl side chain as found in **1**, **2**, and **5**. The C-5″ resonance overlapped with the C-5‴ resonance at δ 18.07, as seen from the HMQC experiment. The protons H-1″ and H-1‴ of the two different side chains at the B ring of **3** showed long-range cross resonances with the methoxylated carbon C-4′ in the HMBC spectrum, indicating that both side chains are positioned ortho to the methoxy group. The assignment of the carbon and proton resonances of 2′ and 6′ subsequently followed from this substitution pattern. All corresponding resonances in the NMR spectra of **3** are in full agreement with the proposed structure. The absolute configuration of the dihydropyranone moiety at C-2 was determined by positive and negative Cotton effects near 327 and 285 nm in the CD spectrum and its negative specific rotation.[21,23,24] Therefore, **3** was identified as the new natural product 2*S*-3′-(2-hydroxy-3-methylbut-3-enyl)licoflavone-4′-methyl ether. However, all attempts to determine the absolute configuration of C-2″ in the hydroxylated side chain failed.

The NMR spectra of compound **4** resembled those of **3**. Differences were only found due to the lack of methylation of the C-4′ hydroxy function and the absence of the resonance for the hydroxy group at C-5, replaced by an additional proton resonance at δ 7.7 (H-5, see Table 1). The A ring protons subsequently formed an ABX system, which was also found in **2**. All other resonances were in full agreement with the proposed structure. Since the Cotton effects found in the CD spectra were in accordance with the values found for **3** and **6**,[21] **4** was identified as the new natural product 2*S*-3′-(2-hydroxy-3-methylbut-3-enyl)abyssinone II.

We further analyzed the anticancer activities of compounds measuring the cytotoxic potential against a hepatoma cell line. Prenylation at C-3′ and C-5′ of the flavonoids led to an enhancement of cytotoxicity compared to the nonprenylated compound: while naringenin (**7**), isosakuranetin (**8**), and liquiritigenin (**9**) showed only weak cytotoxicity (no IC_{50} detectable up to 100 μmol/L), the corresponding C3′-/C5′-prenylated flavonoids abyssinone V (**5**), 4′-methylabyssinone V (**1**), and abyssinone IV (**2**) possess IC_{50} values of 15, 5, and 15 μmol/L, respectively (Figure 2). The introduction of an OH group in the prenyl side chain diminishes this toxic effect in the case of 2*S*-3′-(2-hydroxy-3-methylbut-3-enyl)licoflavone-4′-methyl ether (**3**) and 2*S*-3′-(2-hydroxy-3-methylbut-3-enyl)abyssi-

* Corresponding author. Phone: + 49 211 81 14172. Fax: + 49 211 81 11923. E-mail: passreit@uni-duesseldorf.de.
[†] Institute of Toxicology, Heinrich-Heine-University.
[‡] Institute of Pharmaceutical Biology and Biotechnology, Heinrich-Heine-University.
[§] University of Bradford.
[⊥] This work is part of the running Ph.D. thesis.

10.1021/np070417j CCC: $40.75
Published on Web 02/27/2008

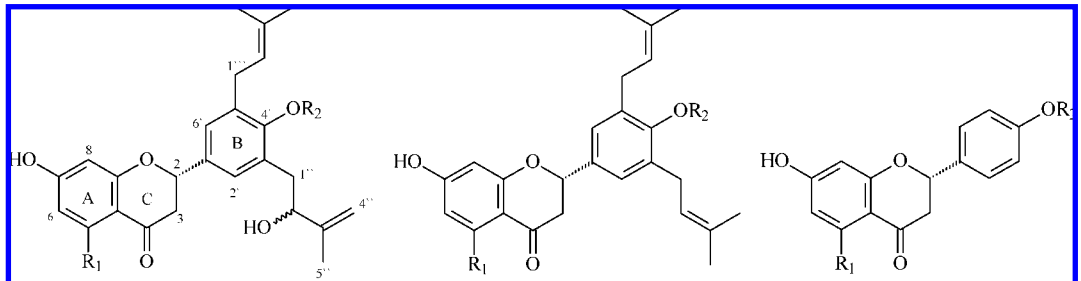

Figure 1. Chemical structures. **1**: 4′-methylabyssinone V, **2**: abyssinone IV, **3**: 2*S*-3′-(2-hydroxy-3-methylbut-3-enyl)licoflavone-4′-methyl ether, **4**: 2*S*-3′-(2-hydroxy-3-methylbut-3-enyl)abyssinone II, **5**: abyssinone V, **6**: abyssinoflavanone VII, **7**: naringenin, **8**: isosakuranetin, **9**: liquiritigenin.

Table 1. ^{1}H (500 MHz), ^{13}C (125 MHz), and HMBC NMR Data of Compounds **3** and **4** (Me_2CO-d_6)

	3			**4**		
pos.	δ_C	δ_H, m, *J* (Hz)	HMBC ($^3J_{CH}$)	δ_C	δ_H, m, *J* (Hz)	HMBC ($^3J_{CH}$)
2	79.9	5.46 dd 13.0, 3.0	2′, 6′	80.8	5.37 dd 13.0, 2.8	2′, 6′
3_{ax}	43.5	3.15 dd 17.0, 13.0		44.7	3.05 m	
3_{eq}		2.73 dd 17.0, 3.2			2.70 dd 17.0, 2.8	
4	197.0		3 (^2J)	190.6		5
5	165.3			129.4	7.70 d 8.5	
6	95.8	5.95 d 2.2	8, 5-OH, 7-OH	111.1	6.54 dd 8.5, 2.5	8, 7-OH
7	167.4			165.2		5
8	96.8	5.94 d 2.2	6, 7-OH	103.6	6.39 d 2.5	6, 7-OH
9	164.2			164.5		5
10	103.2		6, 8, 7-OH	115.2		6, 8
1′	133.8			131.7		
2′	128.3	7.23 d 2.1	2, 6′, 1″	127.5	7.17 d 2.2	2, 6′, 1″
3′	135.0			131.7		2″, 4′-OH
4′	157.8		2′, 6′, 1″, 1‴, Me	155.2		1″, 1‴
5′	135.5		2‴	129.5		2‴, 4′-OH
6′	127.3	7.34 d 2.1	2, 2′, 1‴	128.4	7.14 m	2, 2′, 1‴′
1″	37.2	2.84 m	2′	39.5	3.01 m	2′
		2.68 dd 13.2, 4.7			2.60 m	
2″	76.1	4.23 m	4″, 5″	78.1	4.39 m	4″, 5″
3″	149.3		1″	149.9		1″
4″	110.5	4.90 s	2″, 5″	110.8	5.02 s	2″, 5″
		4.73 s			4.81 s	
5″	18.1	1.79 s	2″, 4″	18.1	1.80 s	2″, 4″
1‴	29.0	3.39 d 7.3	6′	29.8	3.33 d 7.3	6′
2‴	123.9	5.30 t 7.3	4‴, 5‴	123.9	5.30 t 7.3	4‴, 5‴
3‴	132.9		1‴	131.9		1‴
4‴	25.8	1.71 s	2‴, 5‴	25.9	1.70 s	2‴, 5‴
5‴	18.1	1.71 s	2‴, 4‴	18.0	1.70 s	2‴, 4‴
5-OH		12.17 s				
7-OH		9.74 s			9.49 s	
4′-OH					9.20 s	
OMe	61.3	3.77 s				

none II (**4**), but led to enhancement of cytotoxicity in case of abyssinoflavanone VII (**6**), which possesses an IC_{50} value of 3.5 μmol/L. Only this compound induces apoptotic cell death (Figure 2). Therefore, this compound may be interesting for a potential use in cancer therapy. We further analyzed antioxidative properties of **1**−**9**, but none of these substances showed significant radical scavenging activity in the TEAC assay (Supporting Information).

Abyssinoflavanone VII (**6**) shows moderate cytotoxic activity (IC_{50} = 3.5 μmol/L) in a hepatoma cell line, and cell death is selectively mediated via activation of apoptosis (caspase-3/7-activation, nuclear fragmentation). This compound may be an active compound of *E. addisoniae* mediating the anticancer activity.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a Perkin-Elmer 241 MC polarimeter. UV spectra were determined online via a Dionex HPLC P 580. CD data were recorded in HPLC-MeOH on a Jasco J-600 spectropolarimeter. NMR spectra were measured in acetone-d_6 Uvasol (Merck) on a DRX 500 Bruker instrument operating at 500 MHz for ^{1}H and at 125 MHz for ^{13}C NMR. LC-MS were obtained using an Agilent LC HP 1100 combined with a Thermoquest Finnigan LCQ Deca mass spectrometer, an APCI ion source, and Thermoquest ESI. A Knauer Eurospher 100 C-18 (5 μm; 227 mm × 2 mm) column was eluted with HPLC-grade MeOH. For column chromatography Merck Si gel 60 was used. TLC was performed on TLC plates precoated with Si gel 60 F_{254} (Merck) and detected by anisaldehyde reagent. HPLC was performed on a Dionex P 580 equipped with an ASI-100 and STH 585 autosamplers and UVD 340 S detector. Naringenin, isosakuranetin, and liquiritigenin (purity >99%) were purchased from Extrasynthese Genay, France. All other chemicals were of analytical grade and were purchased from Sigma Deisenhofen or Merck Darmstadt. All tissue culture reagents were purchased from PAA Coelbe; cell culture dishes and multiwell plates were obtained from Falcon Heidelberg.

Plant Material. *E. addisoniae* was collected in Ghana and identified by Augustina Addae, Herbarium of the Forestry Research Institute of

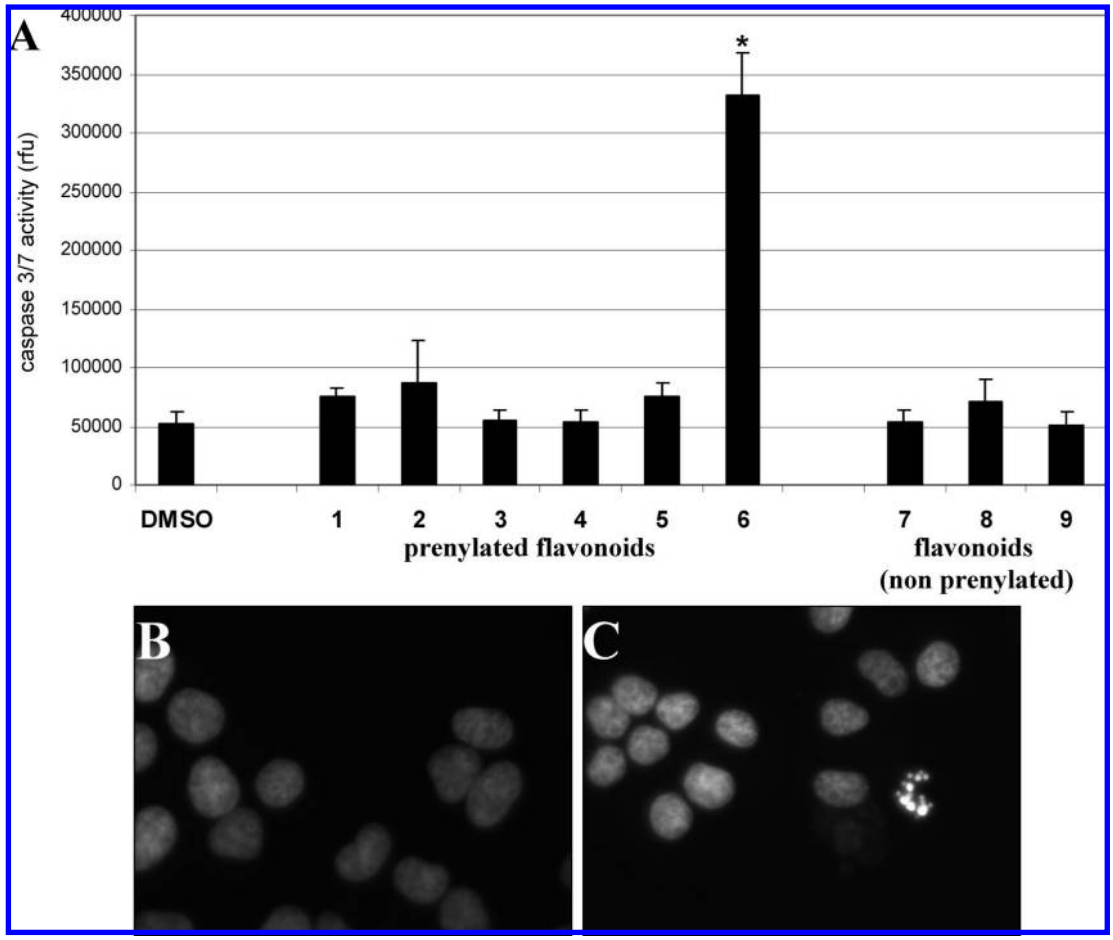

Figure 2. Induction of apoptosis in H4IIE cells. A: H4IIE cells were incubated with the compounds (10 μmol/L) for 24 h, then caspase-3/7-activity was measured using a homogeneous Apo-ONE assay (Promega). Results are expressed as increase in relative fluorescence units (rfu) for 3 h ± SEM ($n = 4$), *$p < 0.05$ vs control (DMSO). Apoptosis is further confirmed by nuclear fragmentation (Hoechst staining). B: Control cells. C: Incubation with 10 μmol/L abyssinoflavanone VII (**6**) for 24 h.

Ghana, located at Fumesua near Kumasi, where a voucher specimen (4/27/02/02) is on deposit.

Extraction and Isolation. The dried and powdered stem bark of *E. addisoniae* (500 g) was consecutively extracted with DCM and then with MeOH in a Soxhlet apparatus. After evaporation of the solvent a sticky DCM residue was obtained (75 g). The residue was then dissolved in MeOH, filtered, and dried again. Then 9.0 g of the MeOH soluble part of the DCM extract were separated by column chromatography (CC) on Sephadex LH-20 with MeOH as mobile phase. The collected eluates were combined to four fractions by monitoring with TLC (Si gel 60 F_{254}, toluene/ EtOAc, 6:4, detection anisaldehyde/ H_2SO_4). Fraction 2, rich in flavonoids, was then purified by CC on Sephadex LH-20 with MeOH, resulting in 12 subfractions. Subfraction 2.8 was purified by CC on Si gel 60 using a n-hexane/EtOAc gradient starting with 80% *n*-hexane as eluent. After this 133.42 mg of **1** were obtained in pure form. Compounds **2** (26.49 mg), **3** (5.07 mg), and **4** (3.64 mg) were purified by repeated CC on Si gel 60 with n-hexane/ EtOAc/DCM (6:3:1) from subfraction 2.9. Compounds **5** (26.18 mg) and **6** (5.64 mg) were obtained after CC of fraction 2.16 on silica gel using toluene/EtOAc, 6:4. All compounds were identified by their mass and 1D- and 2D-NMR spectra.

Cell Culture and Determination of Cytotoxicity. Metabolically active H4IIE rat hepatoma cells were grown in Dulbecco's modified Eagle's medium (4.5 g/L glucose, 2 mmol/L L-glutamine, 100 units/ mL penicillin, 100 μg/mL streptomycin, and 10% fetal bovine serum) in a humidified atmosphere (37 °C, 5% CO_2). The effect of isolated compounds on cell viability was determined using the MTT assay.[25] The cells were plated in 96-multiwell plates with 10 000 cells/well. The cells were allowed to attach for 24 h and then treated with different concentrations of the compounds for 24 h. After this treatment the medium was changed and the cells were incubated for 3 h under cell culture conditions with 0.7 mg/mL MTT. After this incubation the cells were lysed with 50% EtOH/49% H_2O/1% HOAc. The concentration of reduced MTT as a marker for cell viability was measured at 560 nm. Data are given as mean ± SEM of at least three independent experiments. The significance of changes in the test responses was assessed using a one-way ANOVA followed by LSD test (Analyze-it, Leeds, UK); differences were considered significant at $P < 0.05$.

Determination of ACD. Caspase activity was measured using the Apo-ONE homogeneous Caspase-3/7-assay (Promega) according to the manufacturers protocol. Briefly, 50 000 cells/well were plated in 96-multiwell dishes, allowed to attach for 24 h, and treated with isolated compounds for 24 h. Then 50 μL of Apo-ONE Caspase-3/7-reagent was added, and the increase in fluorescence was measured for 3 h at 37 °C using a Perkin-Elmer Wallac VICTOR 1420 multilabel counter (excitation: 485 nm, emission: 535 nm). To investigate nuclear fragmentation as a further feature of apoptotic cell death, cells were grown in 35 mm cell culture dishes for 48 h and then incubated with different concentrations of the flavonoid compounds for 24 h. Then, without medium change, nuclei were stained with 100 μmol/L Hoechst 33342 for 15 min and sealed with a glass coverslip. Cells with condensed and fragmented nuclei were photographed at 400-fold magnification using a Zeiss Axiolab fluorescence microscope (excitation: 365 nm, emission: 420 nm).

2*S*-3′-(2-Hydroxy-3-methylbut-3-enyl)licoflavone-4′-methyl ether (3): yellowish, oily residue; $[\alpha]_D^{20}$ −128 (*c* 0.15, MeOH); UV λ_{max} 232, 290, 332 nm (MeOH); HPLC t_R 26.32 min (linear gradient from 5 to 100% MeOH against nanopure H_2O with 0.1% phosphoric acid at 35 min); CD (*c* 0.2 MeOH) $[\theta]_{327}$ +1.2, $[\theta]_{285}$ −4.1; ^{1}H, ^{13}C NMR data and HMBC correlations are given in Table 1; MS 439 $[M + H]^+$.

2*S*-3′-(2-Hydroxy-3-methylbut-3-enyl)abyssinone II (4): yellowish, oily residue; $[\alpha]_D^{20}$ −47 (*c* 0.1, MeOH); UV λ_{max} 238, 276, 312 nm (MeOH); HPLC t_R 24.50 min (linear gradient from 5 to 100% MeOH against nanopure H_2O with 0.1% phosphoric acid at 35 min); CD (*c* 0.2 MeOH) $[\theta]_{330}$ +1.6, $[\theta]_{285}$ −4.4; ^{1}H, ^{13}C NMR data and HMBC correlations are given in Table 1; MS 409 $[M + H]^+$.

Acknowledgment. We thank the Deutsche Forschungsgemeinschaft (DFG graduate collegue 1427: "Food constituents as triggers of nuclear receptor-mediated intestinal signaling") for financial support and Ms. E. Müller and Mrs. S. Ohler for excellent technical assistance. We are grateful to Prof. Dr. M. Braun, Institut für Organische and Makromolekulare Chemie, Universität Düsseldorf, for recording the CD spectra.

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

References and Notes

(1) Krukoff, B. A.; Barneby, R. C. *Conspectus of Species of the Genus Erythrina*; 1974; pp 332−459.
(2) Burkill, M. *The Useful Plants of West Tropical Africa*; Royal Botanical Gardens Kew: London, 1995, Vol. 2(3), p 350.
(3) Cox, P. A. *J. Ethnopharmacol.* **1993**, *38*, 181–188.
(4) Ghosal, S.; Dutta, S. K.; Bhattacharya, S. K. *J. Pharmacol.* **1972**, *80*, 62–69.
(5) Mongelli, E.; Pampuro, S.; Coussio, J.; Salomon, H.; Ciccia, G. *J. Ethnopharmacol.* **2000**, *71*, 145–151.
(6) Saiduh, K.; Ohnah, J.; Orisadipie, A.; Olusola, A.; Wambebe, C.; Gamaniel, K. *J. Ethnopharmacol.* **2000**, *71*, 275–280.
(7) Hartwell, J. L. *Lloydia* **1970**, *33*, 103–127.
(8) Amer, M. E.; Shamma, M.; Freyer., A. J. *J. Nat. Prod.* **1991**, *54*, 329–363.
(9) Hegde, V. R.; Dai, P.; Patel, M. G.; Puar, M. S.; Das, P.; Pai, J.; Bryant, R.; Cox, P. A. *J. Nat. Prod.* **1996**, *60*, 537–539.
(10) Moriyasu, M.; Ichimaru, M.; Nishiyama, Y.; Kato, A.; Mathenge, S. G.; Juma, F. D.; Nganga, J. N. *J. Nat. Prod.* **1998**, *61*, 185–188.
(11) Na, M.; Jang, J.; Njamen, D.; Mbafor, J. T.; Fomum, Z. T.; Kim, B. Y.; Oh, W. K.; Ahn, J. S. *J. Nat. Prod.* **2006**, *69*, 1572–1576.
(12) Ichimaru, M.; Moriyasu, M.; Nishiyama, Y.; Kato, A.; Mathenge, S. G.; Juma, F. D.; Nganga, J. N. *J. Nat. Prod.* **1996**, *59*, 1113–1116.
(13) Miranda, C. L.; Yang, Y. H.; Henderson, M. C.; Stevens, J. F.; Santana-Rios, G.; Deinzer, M. L.; Buhler, D. R. *Drug Metab. Dispos.* **2000**, *28*, 1297–1302.
(14) Di Pietro, A.; Conseil, G.; Perez-Victoria, J. M.; Dayan, G.; Baubichon-Cortay, H.; Trompier, D.; Steinfels, E.; Jault, J. M.; de Wet, H.; Maitrejean, M.; Comte, G.; Boumendjel, A.; Mariotte, A. M.; Dumontet, C.; McIntosh, D. B.; Goffeau, A.; Castanys, S.; Gamarro, F.; Barron, D. *Cell. Mol. Life Sci.* **2002**, *59*, 307–322.
(15) Wätjen, W.; Weber, N.; Lou, Y.; Wang, Z.; Chovolou, Y.; Kampkötter, A.; Proksch, P.; Kahl, R. *Food Chem. Toxicol.* **2007**, *45*, 119–124.
(16) Nkengfack, A. E.; Azebaze, A. G.; Waffo, A. K.; Fomum, Z. T.; Meyer, M.; van Heerden, F. R. *Phytochemistry* **2001**, *58*, 1113–1120.
(17) Bae, E. Y.; Na, M.; Njamen, D.; Mbafor, J. T.; Fomum, Z. T.; Cui, L.; Choung, D. H.; Kim, B. Y.; Oh, W. K.; Ahn, J. S. *Planta Med.* **2006**, *72*, 945–948.
(18) Kamat, V. S.; Chuo, F. Y.; Kubo, I.; Nakanishi, K. *Heterocycles* **1981**, *15*, 1163–1170.
(19) Promsattha, R.; Mbafor, J. T.; Tempesta, M. S.; Fomum, Z. T.; Sigmoidin, F. *J. Nat. Prod.* **1989**, *52*, 1316–1318.
(20) Yenesew, A.; Irungu, B.; Derese, S.; Midiwo, J. O.; Heydenreich, M.; Peter, M. G. *Phytochemistry* **2003**, *63*, 445–448.
(21) Cui, L.; Ndinteh, D. T.; Na, M.; Thuong, P. T.; Silike-Muruumu, J.; Njamen, D.; Mbafor, J. T.; Fomum, Z. T.; Ahn, J. S.; Oh, W. K. *J. Nat. Prod.* **2007**, *70*, 1039–1042.
(22) Tahara, S.; Katagiri, Y.; Ingham, J. L.; Mizutani, J. *Phytochemistry* **1994**, *36*, 1261–1271.
(23) Yan, Y.; Kohli, A.; Koffas, M. A. *Appl. Environ. Microbiol.* **2005**, *71*, 5610–5613.
(24) Slade, D.; Ferreira, D.; Marais, J. P. *J. Phytochem.* **2005**, *66*, 2177–2215.
(25) Mosmann, T. J. *Immunol. Meth.* **1983**, *65*, 55–63.

NP070417J